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BIOTHERAPEUTICS AS DRUGS, ITS DELIVERY ROUTES AND IMPORTANCE OF NOVEL CARRIERS IN BIOTHERAPEUTICS

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ABSTRACT: Biopharmaceuticals or biologics are the therapeutic agents which are biomolecules (DNA, Protein, Peptide, RNA, antibodies) in nature. These are high molecular weight (300-1000000Da) molecules, which deify Lipinski rules. At present, a large portion of the market share is occupied by protein biotherapeutics. However, peptide-based therapeutics is replacing protein biotherapeutics, as the former has a broad spectrum of action, high activity at low concentration, efficiency, safety, target selectivity, etc. Currently, there are more than 130 proteins and 140 peptide-based therapeutics in the market, and around 500 peptides are in preclinical development. To release the drug at the target site with maximum efficacy and minimum side effects, the selection of a drug delivery route is very important. Some of the drug delivery routes are Oral drug delivery system (DDS), Parenteral DDS, Topical DDS, Pulmonary DDS, Nasal DDS, Brain targeted DDS, Stem cell DDS, Peptide DDS, Self-assembled peptide DDS, Rectal DDS, *etc*. In comparison to small molecules drug delivery system, there are very few delivery options for the biotherapeutics due to the difference in the physicochemical properties. Currently, some novel carriers like Cubosomes, Phytosomes, Virosomes, Exosomes, *etc.*, are being explored as a potential drug delivery system for biotherapeutics. So- in this review, we describe the characteristics, usage of various novel carriers in the DDS for the delivery of drugs, especially biotherapeutics.

INTRODUCTION: Biotherapeutics are playing an emerging role in human drug options for treating various diseases. Biopharmaceuticals or biologics are the therapeutic agents which are biomolecules (DNA, Protein, Peptide, RNA, antibodies) in nature. These are high molecular weight (300-1000000Da) molecules, which deify Lipinski rule [1]. Currently, these are showing better effects than small molecules because of its high specificity and potency.

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Unlike small molecules, biotherapeutics have a unique set of disadvantages like aggregation at high concentration levels, denaturation by pH, temperature, enzymes, shear stress, mechanical forces, and electrostatic forces [2]. Even though biotherapeutics are a chain of amino acids, the number of amino acids and complexity in secondary structure decide whether they are placed under peptide (<50 amino acids)or protein (>50 amino acids) biotherapeutics.

Further, protein therapeutics is classified based upon their biological functions. Eg: Vaccines, cytokines, growth factors, hormones, interferon's, antibodies [3]. At present, a large portion of the market share is occupied by protein biotherapeutics. However, peptide therapeutics is placing the protein biotherapeutics, as the former

has a broad spectrum of action, high efficiency, safety, target selectivity [4]. Currently, there are more than 130 proteins, 140 peptide-based therapeutics in the market and around 500 peptides are in preclinical development. European pharmacopeia has framed list of several biopharmaceuticals which includes erythropoietin, somatotropin, somato-statin, choriogonadotropin, follitropin, urofollitropin, gonadorelin, oxytoxin, insulin, glucagon, urokinase, hyaluronidase, pepsin, tuberculin, albumin, interferon solutions, blood factors (like VII, VIII, IX, XI), prothrombin complex, calcitonin, alteplase, heparin [5] and those were shown in **Fig. 1, 2.** Recombinant proteins are obtained from the microbial fermentation process [6,] and recently these peptide drugs are much focused on replacement therapies, which act as supplements for delivery of biomolecules. Some of the other peptides are ACTH (derived from a natural source) and oxytoxin, vasopressin, leuprolide acetate (synthetic source) [7].

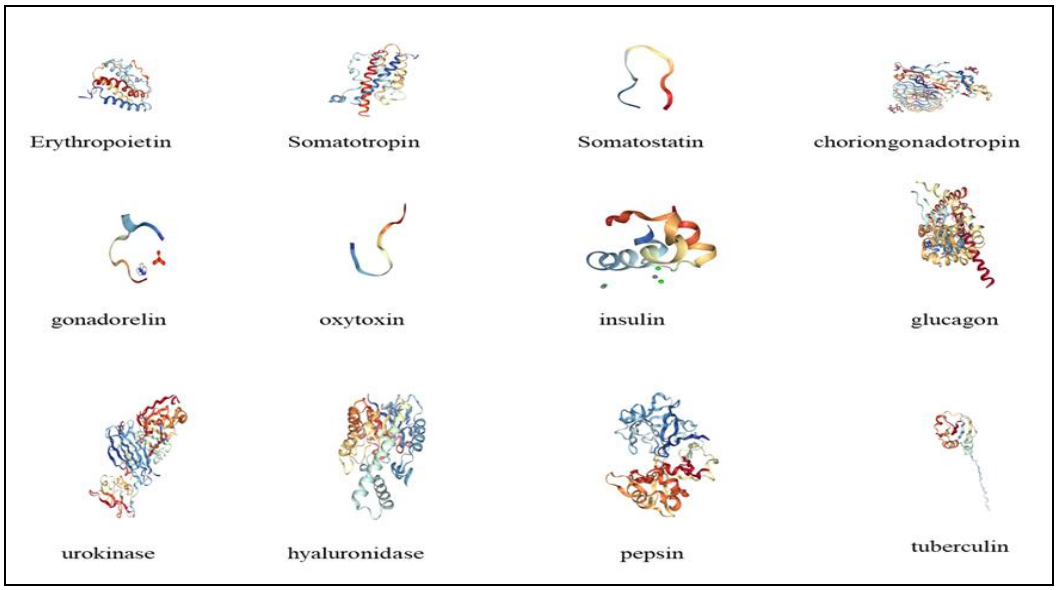

FIG. 1: LIST OF BIOPHARMACEUTICALS AS PER EUROPEAN PHARMACOPEIA

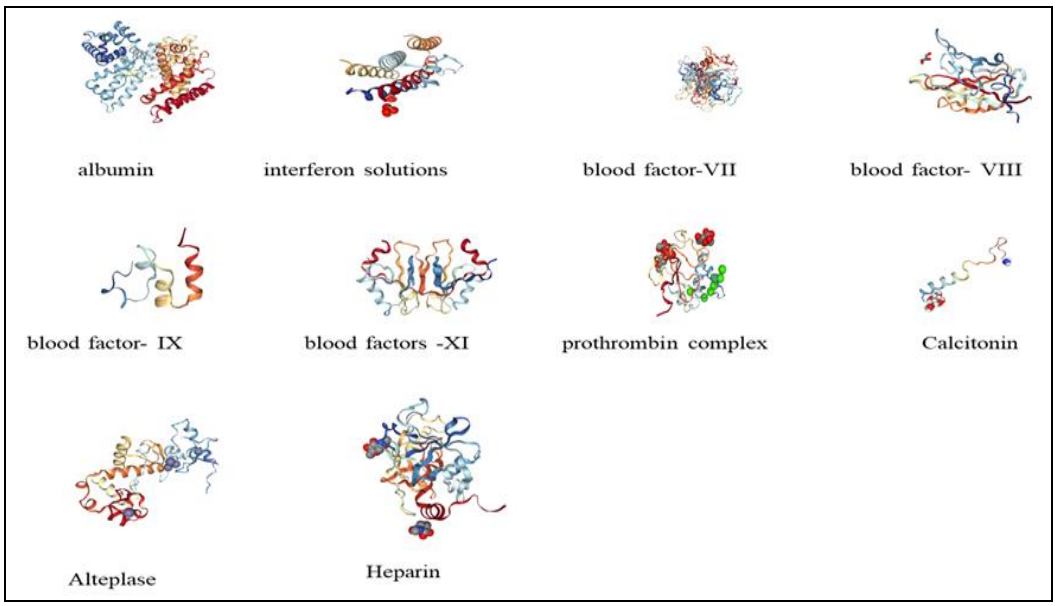

FIG. 2: LIST OF BIOPHARMACEUTICALS AS PER EUROPEAN PHARMACOPEIA

Not only the development, drug delivery of novel biotherapeutics also plays a crucial role in treating various diseases, and there are many examples to show its importance.

Some biotherapeutics have failed to produce sufficient efficacy mainly because of their unsatisfactory pharmacokinetic properties, which include poor bioavailability at the target site, long

term stability, immunogenicity, short plasma half-life, poor penetration across biological membranes.

To release the drug from the delivery system, selection of drug delivery route/method of administration is very important because it plays a vital role to deliver the drug to the target site with maximum efficacy by reducing maximum side effects and due to their high molecular weight and complexity, biotherapeutics cannot be given through orally by typical formulations (because of the presence of barriers, enzyme degradation, *etc*. Hence modifications have to be done to promote oral delivery [8].

Rather than oral route, there are many drug delivery routes to deliver biotherapeutics, and they are Oral drug delivery system (DDS), Parenteral DDS, Topical DDS, Pulmonary DDS, Nasal DDS, Transdermal DDS, Brain targeted DDS, Stem cell DDS, Peptide DDS, Nanoscale DDS, Implants, Self-assembled peptide DDS, Lipid-based DDS, Buccal DDS, Rectal DDS, *etc*. These are the various drug delivery systems that are used to deliver the drugs to the targeted site to some extent, but due to the presence of carriers, they promote the drug to reach its target site with much efficiency, selectivity with fewer doses, less toxic by providing maximum bioavailability. So, in this review- we highlight various novel carriers used in the drug delivery system for the delivery of drugs, especially biotherapeutics.

Drug Delivery System: The drug delivery system is defined as a system/ device/ formulation, which helps to deliver the drug or therapeutic agent into the body to show its therapeutic activity. It improves the efficacy, safety by controlling the rate of delivery or decay, time, and place of release of drugs in the body.

The sequence of the drug delivery process includes administration of the dosage form, release of the Active Pharmaceutical Ingredient (API) from the dosage form, and transport of API across the biological membrane through the blood to the site of action/ target site [9].

Aims of DDS Development: Drug delivery technologies give priority to improving the efficacy and safety of medicines as well as commercial pharmaceutical development.

The following are the critical points:

- Improvements in drug safety, efficacy, compliance.
- Chronopharmacological benefits.
- Reduction of cost of drug development.
- Shelf Life extension.
- Reduction of risk of failure in new product development.

1. **Oral Route:** The oral route is the best route to deliver most of the drugs as it has advantages like patient compliance, easy to administer, etc. [10, 11]. Most of the biotherapeutics are macromolecules, hydrophilic, and these biotherapeutics cannot be given through the oral route because of its poor bioavailability and get degraded by enzymes or oxidation, deamination, hydrolysis, *etc*. Biotherapeutics poor bioavailability is due to barriers like "mucus layer, enzymatic action and cellular mechanisms" [12]. Mucus consists of mucin glycoprotein, enzymes, electrolytes, water, etc. It acts as a lubricant and is responsible for protecting intestinal epithelium [13]. Due to the presence of the mucin glycoprotein, the mucus layer acts as a cohesive and adhesive which captures and prevents the external particles (pathogens) from entering inside the host. It also hinders, restricts diffusion and absorption of the drug, which ultimately decreases the bioavailability of the drug. The theory behind mucus adhesion is "interaction force generated by carboxyl group and the negative charge of sulfuric acid of the oligosaccharides, followed by the high-density hydrophobic region and mucinous protein fibers on the mucin chain [13]. However, mucus and glycocalyx layers are the first barriers that restrict the penetration of biotherapeutics and ultimately leading to less bioavailability.

When biotherapeutics are administered through the oral route, they get degraded in the Gastro-Intestinal Tract (GIT) due to the presence of the proteolytic enzymes (proteases) and drastic changes in the pH. Irrespective of the enzymes, rapid pH changes are also responsible for the degradation of ingested biotherapeutics into smaller peptides. Eg: when the peptides move from the stomach to duodenum, the pH changes from 2.0 to 8.0. Due to the change in pH, they get precipitated

because of a change in isoelectric point [14]. Transcellular and paracellular are the two significant pathways that affect the delivery of biotherapeutics across the epithelium. In the transcellular pathway, biotherapeutics cross across the epithelia through the intracellular transfer to the bloodstream. At this stage, the biotherapeutics transfer is through the uptake mechanism. The paracellular path involves the transfer of biotherapeutics through the space present between the adjacent cells. Some of the factors which affect the absorption of the biotherapeutics are molecular weight, size, structure, solubility, lipophilicity, partition coefficient, aggregation, etc. and also it has the approaches to improve the oral delivery which includes targeted delivery, colon targeting, chemical alteration in structure, prodrug approach, methylation, prodrug approach, vehicles, bio-adhesives, permeation enhancer's *etc*. One of the examples is that; P- glycoprotein (P-gp: efflux protein) acts as a barrier. In such a case, polymeric nanoparticles may play a positive role in the delivery of drugs.

The science behind this is, the P-gp protein cannot recognize the nanoparticles (NP). If the drug is formulated as nanoparticles, they can cross the barriers easily, which leads to an excellent activity. It includes specific mechanisms and pathways, and they are nanoparticles transport mechanism, paracellular, transcellular pathways and trans-cytosis by healthy enterocytes and M cell-mediated phagocytosis. To overcome all these barriers, novel approaches are being implemented for oral drug delivery, which includes Polymeric NP, solid lipid NP *etc*. Ashaben *et al.,* stated that insulin loaded chitosan nanoparticles would increase the oral bioavailability of the insulin. To prove it, he had conjugated chitosan nanoparticles with synthetic CRTLTVRKC peptide. The peptide significantly enhanced epithelial targeting and translocation of chitosan nanoparticles by interacting with transmembrane protein stabilim-2.

2. Parenteral DDS: In the Parenteral drug delivery system, drug is injected directly into the body through veins or muscle or lower layers of skin. Parenteral drug delivery includes Intravenous, intramuscular, subcutaneous, and intraperitoneal. It is the most appropriate route to deliver the drugs if rapid administration or quick onset of action of the drug is needed But in recent days, formulation scientist are much aspired to formulate parenteral by using the carriers like nanoparticles which has more advantages (like stability, better bioavailability, rapid action, *etc.*,) than the conventional parenteral [15]. Hulie *et al.*, developed nanoparticles (NP) for the Angiopep- 2 peptides. These functionalized NPs showed a remarkable potential for Blood-Brain barrier (BBB) (angiopep-2) penetration and binding to neuroglial cells (EGFP-EGF1). In addition, these dual functionalized NPs generated significantly greater brain accumulation compared to unmodified NPs. These targeting potentials of angiopep-2 peptide and EGFP-EGF1 protein is a novel, promising formulation for the neuroglial treatment [16].

3. Topical DDS: The topical drug delivery system is the system that delivers the drug to the target site via the skin. The main reason for the selection of topical DDS is to overcome the disadvantages of the parenteral route. If the drug is administered through the parenteral route, these show short *in vivo* half-life and rapid clearance from the human being due to interference of proteases and peptidases in the blood, which degrades the biotherapeutic activity [17]. Currently, topical DDS had shown its importance in delivering biotherapeutics by reducing systemic side effects and increasing therapeutic activity. So, to overcome all these issues, nanocarriers came into existence, which had shown good biocompatibility, enhanced activity with decreased side effects, etc.

4. Pulmonary DDS: It is the non-invasive route that improves the absorption of biotherapeutics, and also it has the potential to enter into the lungs. The pulmonary route has an absorptive surface area, avoiding first-pass metabolism, requires less dose when compared to oral or Parenteral, low enzymatic activity, *etc*. Physiology states that central airway epithelium contains ciliated columnar cells that have tight intercellular junctions leading to limitation of transport of "*biotherapeutics."* In this case, the diffusion rate is directly proportional to the concentration gradient and lipid solubility. But diffusion may be influenced by molecular size and ionization capabilities of the biotherapeutics, and also it is having some disadvantages like Particle wet ability, aggregation, crystallinity, polymorphism, and

susceptibility towards enzymatic degradation. So, to avoid all this, techniques have been implemented to deliver the drugs to the systemic circulation via lungs, and they include dry powders, inhalers, controlled release pulmonary drug delivery systems, etc. Apart from this, there are novel carriers for the delivery of drugs through the pulmonary route, and one of the examples is Alpha 1-antitrypsin-loaded PLGA NPs. It is used to treat respiratory diseases. Alpha 1-antitrypsin-loaded PLGA NPs is having a spherical shape with a size of 100-1000 nm, entrapment efficiency of around 90%, and releases 60% of the drug around 8 h. This example is stating the usage of various polymers for the delivery of certain drugs; it has been proven that the entrapment efficiency and delivery of the drug are increased.

5. Nasal DDS: Nasal DDS is the drug delivery system where the drug can be delivered through the nasal route, which treats nasal congestion, rhinitis, sinusitis, etc. and delivery of drugs through the nasal route depends on the physicochemical properties of the nasal mucosa and delivery device which had shown its importance in delivering the biotherapeutics. Biotherapeutics can be given through the nasal route, which is non-invasive and thereby overcoming disadvantages associated with the oral route. They include large surface area, highly vascularized mucosa, porous endothelial membrane, lower enzymatic activity relative to GIT, and avoidance of the first-pass metabolism. But equal size distribution is a great challenge for the formulation of scientist [18].

Nasal DDS serves the drug to enter into the brain *via the* nose. The main issue of brain targeting is the barrier present in the brain, which is termed as blood-brain barrier (BBB). So, nasal DDS helps the drug to enter into the brain via the nose by avoiding the blood-brain barrier (BBB). The main aim of this route is to bypass the blood-brain barrier (BBB) and first-pass metabolism. Direct delivery of drugs from the systemic circulation to the brain is much difficult because of BBB. So, the intranasal route is selected for the delivery of a drug to the brain. The nasal cavity contains nasal vestibular, respiratory, and olfactory regions. Among three regions, the olfactory region is the major route that contributes to the delivery of the drug through intranasal administration, and also it is having a porous endothelial membrane structure, high blood flow, and short half-life; it provides rapid brain absorption. Several polymeric NPs have been investigated for direct and sustained release of biotherapeutics into the brain by intranasal administration. As the molecular weight is directly proportional to membrane permeation, peptide faces low membrane permeability. So, eventually, the bioavailability also gets decreases (*i.e.,* 0.5-5%). To prevent permeation bioavailability problems, enhancers came into existence. In some cases, there is an issue in the absorption of the peptide, which cannot be absorbed due to several reasons. So, studies had been done to improve the absorption by using bile salts, surfactants, phosphatidylcholine, cyclodextrin, cell-penetrating peptides, *etc*.

Apart from all these, drug carrier systems like liposomes, emulsions, Nanoemulsions, micro-particles, Niosomes are used to deliver the drug by enhancing the bioavailability [9, 17]. Some of the examples are stated in **Table 1**. Cheng *et al*., demonstrated that neurotoxin-1 could be delivered by intranasal administration by using polylactic acid nanoparticles (NT-I-NPs). When NT-I is formulated alone, the Tmax had shown around 145 min, but when NT-I is formulated with NPs , the results states that Tmax values are observed at 65 and 95 min respectively, and absolute bioavailability is produced around 160- 196%. This result concludes that brain delivery via nasal route could improve the activity of NT-I.

6. Brain targeted DDS: Brain targeted DDS is one of the most important target sites to deliver the drug to show its maximum activity. All types of drugs cannot enter into the brain very efficiently. Hence, formulation scientists are trying hard to overcome this issue. Regarding this, peptide-based delivery vectors are playing an emerging role by using receptor-mediated transcytosis, adsorptive-mediated transcytosis, and the paracellular route. If the formulation scientist is capable of formulating the drug to cross BBB, many CNS disorders could be treated, and peptides also can be delivered through this route. Researchers had found ligands to deliver peptides which can specifically bind to BBB, and some of them are: transferrin receptor (TfR), insulin receptor, low- density lipoprotein receptor-related protein, nicotinic acetylcholine

receptor, growth factor receptor, adenosine triphosphate- binding cassette transporter, P-Glycoprotein, glucose transporter, concentrative nucleoside transporter, etc. and they try to increase the drug concentration. Even though they have increased the drug concentration in the brain, but the problem is that they lack in the selective distribution of the drug. So, according to this concept, scientists have established dual targeting delivery by modifying nanoparticles with 2 ligands among them; one is to penetrate BBB, and the other is to target the specific target site in the brain. There are other transporters that transport the peptides to the brain, and they are polypeptide transporters (PepT1, Pept2), oligopeptide transporters like glutathione transporter. PepT1 acts as peptidomimetic oral drug delivery, which had shown good bioavailability than the normal delivery [19, 20, 21].

Glutathione is the endogenous tripeptide which is having antioxidant properties that can cross the BBB through glutathione transporter. By using glutathione as a transporter, the brain targeted nano-drug delivery system has been implemented. Based on glutathione, G- technology has been implemented, which is well established, patented, and approved by FDA that had proved enhanced activity to target the brain. To prove this, many attempts had been done by using glutathione-modified PEGylated liposomes for improved brain targeting for opioid peptides. Rather than this, peptides have been delivered to promote neurogenesis by using PLGA nanoparticles. Tandem peptide-based nanoparticles have shown improved delivery of siRNAs to the site of injury and target the injured neuron upon systemic circulation. In this, nanoparticles have shown its own identify in delivering the peptides through brain targeting [22]. Some of the examples are listed in **Table 1.**

7. Stem Cell Drug Delivery System: Stem cell drug delivery system is the cell- mediated drug delivery system in which they use living cells to deliver exogenous therapeutic agent to treat human diseases and Stem cells carrying drug-loaded nanoparticles have been developed with potential activity. One of the examples is that; by using stem cells, the nuclear localization signaling peptide was integrated with DNA to transport genetic material into the nucleus, and therefore, they have shown good results. Hence, stem cells have the potency to deliver the peptides with much efficiency. So, researchers need to focus on delivering peptides through stem cell delivery [23].

8. Peptide-based Drug Delivery System: The peptide drug delivery system is considered as one of the important non-viral-based delivery systems, and it delivers the drugs to the target site. It delivers the peptides with better efficacy due to the development of a new way, *i.e.*, self-assembly of nanoscale materials with a hydrogel network. This self-assembly peptide-based drug delivery contains amino acids, which contributes beneficial activity to deliver peptides such as biocompatibility, biodegradability, flexible responsiveness, patient-specific therapy, disease-specific therapy, *etc.* and most of the drug deliveries are formulated as nanoparticles which control biodistribution, enhance efficacy, reduces toxicity [24, 25, 26].

These are the various delivery systems used to deliver the peptides to increase the efficacy/ absorption/ bioavailability of the peptides, but some of the novel carriers are to be used for better activity because of usage of biotherapeutics is increasing day by day due to its immense role. The formulation scientists are more focused on delivery systems to deliver the peptide, but they are lacking in improving the activity of bio-therapeutics. So, Scientists need to explore specific drug delivery to deliver the peptides by using the novel carrier.

Novel Carriers: Novel carriers are the substances that deliver the drugs to target with increased selectivity, effectiveness, safety, and release of the drug into the systemic circulation. Novel carriers are also known as vesicular drug delivery systems (VDDS) [27].

Advantages:

- They improve pharmacokinetic properties (like ADME), Enhancing water solubility, bio-availability.
- Used to strengthen membrane permeability.
- They are biodegradable and get metabolized.
- Stable for a long period.

- Enhances the activity of the drug by bypassing some barriers.
- Target specific.
- Less toxic compared to standard vehicles.
- Patient compliance.

Disadvantages:

- Difficult to scale up.
- Not applicable to hydrophilic drugs.

Some of the novel carriers are cubosomes, Exosomes, Phytosomes, Virosomes, Transferosomes, Aquasomes, Iscoms, and Ethosomes, *etc*. Those are the various carriers which is having independent activity with different categories.

Cubosomes: Cubosomes are distinct, sub-micron and a type of nanocarriers which is considered as bicontinuous cubic phase liquid crystals, and these are self-assembled liquid crystalline particles. The colloidal dispersions of bicontinuous cubic phase liquid crystals in water using surfactant results in nanostructures referred as cubosomes and having size range of 100- 300 nm. Due to bicontinuous cubic liquid crystalline phase, cubosomes act as high solid, viscous, optically clear.

In the structure of cubosomes (shown in **Fig. 3**), surfactant was assembled into bilayers which are having 3 dimensions (that's why it was called as cubic), periodic, resembles "honeycomb" containing bis-continuous domains of water and lipid. Cubosomes are prepared by using polymers, lipids, and surfactant and these are having large interfacial areas which can provide complex diffusion pathways for sustain release. These are thermodynamically stable in lamellar, hexagonal and bicontinuous cubic phases, and that's why some times cubosomes also termed as thermodynamically stable bicontinuous cubic liquid crystalline phase. It is having differential properties than the liposomal formulation, and they are differing in solubility.

In cubosomal formulations, these are soluble in water and hydrophilic, lipophilic, and amphiphilic drugs can be entrapped by using cubosomes. These are manufactured by using top-down technique and bottom-up technique and characterized by gel permeation chromatography, ultrafiltration technique, HPLC, UV spectrophotometer, photon correlation spectroscopy, polarized light microscopy, dynamic light scattering, X-ray scattering, transmission electron microscopy(TEM) [28, 29].

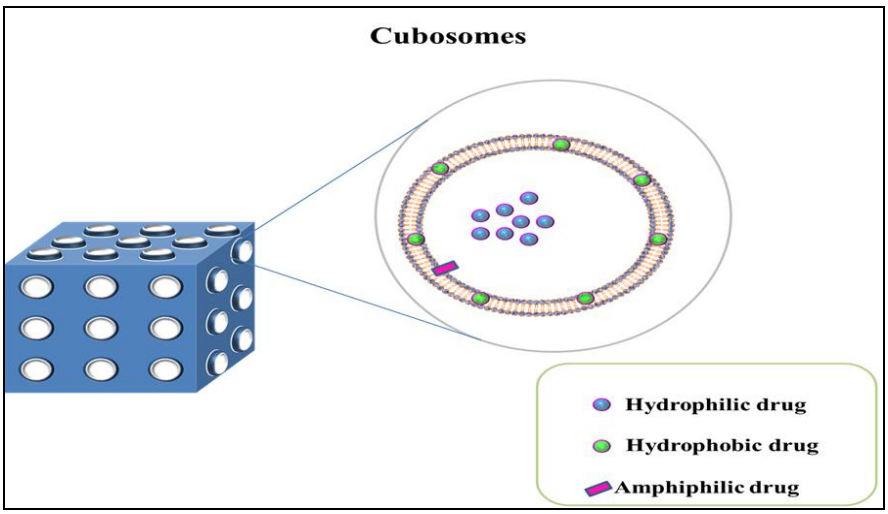

FIG. 3: STRUCTURE OF CUBOSOME

Some of the cubosomal preparations are formulated as oral, ophthalmic, topical, I.V, and intranasal, but the data regarding oral delivery is not completely investigated, and these are more focused on oncology drugs, transdermal delivery, proteins, etc. According to boge, he had formulated cubosomes by using LL-37 peptide for topical delivery.

This LL-37 cubosomal topical formulation has shown the ability to deliver effectively [30, 31]. Formulation scientists had focused more on delivering antimicrobial, anticancer peptides, antifungal, poorly soluble drugs, etc., through cubosomes. But the extensive framework has to be done to get better results, which are not clear yet.

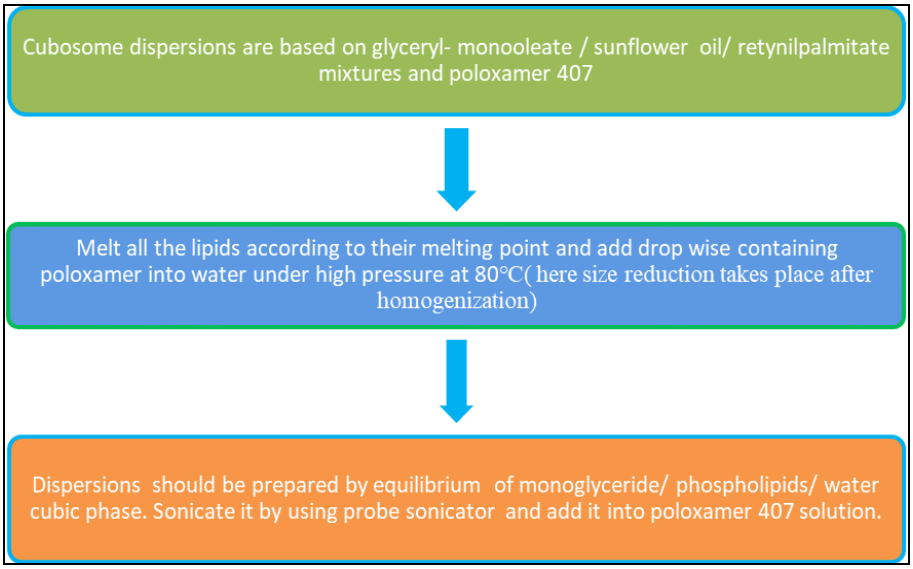

FIG. 4: GENERAL METHOD OF PREPARATION OF CUBOSOMES

Applications:

- ✓ Hydrophilic/Lipophilic/ Amphiphilic drugs can be formulated.
- ✓ Due to its small pore size, it is more compatible for control release formulations.
- ✓ Protects drug from physical and enzymatic degradation.
- ✓ Targeted release of bioactive drugs.
- ✓ High loading capacity due to its high internal surface area.
- ✓ Easy method of preparation.

Disadvantage:

1. Due to high viscous, large scale production is difficult.

Exosomes: Exosomes are the naturally occurring extracellular vesicles with 50-150 nm size that contains selected RNA or protein which can be effectively deliver drug to its target site. These are derived from the multivesicular body, and when multivesicular body fuses with the plasma membrane, it releases the drug into extracellular environment, which contains many biomolecules (like proteins, peptides, carbohydrates, lipids and nucleic acids) and protects mRNAs, regulatory microRNAs *etc*. It is extensively used as an alternative delivery for chemotherapeutics and biopharmaceuticals. These can be manufactured by electroporation, transfection with commercial reagents and with donor cells.

Exosomes have the ability to cross barriers cytoplasmic membrane, BBB, *etc*. So, the drugs which cannot cross BBB can be formulated as exosomes and can be delivered which shows better efficacy. Liu states that fusion of target protein with a constitutive protein of exosomes (*e. g*: CD63), the protein gets fused into exosomes as mediated by constitutive protein expression. This improves specific target protein loading into exosomes, which shows better efficacy. Many proteins/ peptides are loaded into exosomes, and some of them are CD63, TSG101, ARRDC, Palmitoylated, tdTomato, Lactadherin C1C2 domain, EGF VIII, PDGFR TM domain, HIV-1 Nef (mut), VSVG, LAMP2B, LAMP1, ALIX-1, CD9, CD81, HSP70, HSP90, MHC, $SCAMP_S$, ApoE, WW tag, *etc.* and all these exosomes can be characterized by using transmission electron microscopy (TEM), nanosight particle size analyzer and markers [32, 33, 34, 35].

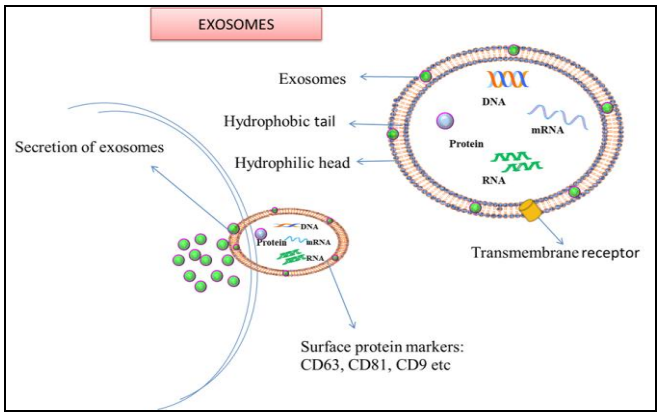

FIG. 5: STRUCTURE OF EXOSOMES

Phytosomes: Herbal based drugs are recently catching up with chemotherapy based drugs as they are providing better efficacy or answer to some of the diseases. Most of the herbal-based drugs consist of water-soluble such as phenolics, glycosides, flavonoids *etc*.,. But effectiveness was poor when taken orally/ topically. The herbal drugs were increasing day by day because of its better activity towards many diseases. So, many approaches have been conducted to improve the bioavailability of herbal drugs that includes the inclusion of solubility, bioavailability enhancer, structure modification, and entrapment of lipophilic carriers but the bioavailability percentage didn't meet its target. So, the researcher had opted phytosomal formulations for the delivery of poorly soluble drugs. Phytosome technology was developed to incorporate the herbal drugs into phospholipids to produce a lipid-compatible molecule complex with better bio-availability than traditional drug formulation. Phytosomes are the novel carriers having aqueous soluble active constituents of plants with phospholipids which were prepared by a molecular complex of lipid and phytoconstituents. Phytosomes are the unique vesicular systems because it contains bioactive compound as their core formulation along with phospholipids and these differ from liposomes and also having more advantage like permeability, bioavailability. Phytosomes contains water-soluble head and two oil-soluble tails shown in **Fig. 6**. In this, lipid-soluble tail interacts with the phosphate head of phospholipids through hydrogen bonds and polar interaction. Because of their solubility in lipids and water, these phospholipids act as the surfactant/ emulsifier. Due to the presence of emulsifiers, phospholipids combine with plant extract, which ultimately enhances the bioavailability of lipophilic drugs [36, 37, 38]. Preparation of Phytosomes includes two steps and that are 1. Preparation of thin layer of phospholipids mixture and 2. Hydration of lipid film and these phytosomes are characterized by membrane permeation, size, entrapment efficiency (in %), purity, NMR (Nuclear Magnetic Resonance), thermal gravimetric analysis (TGA), Differential Scanning calorimetry (DSC).

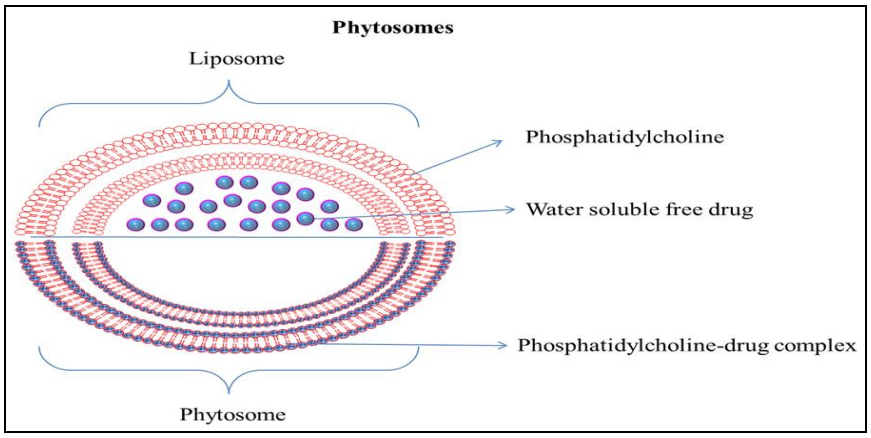

FIG. 6: STRUCTURE OF PHYTOSOME

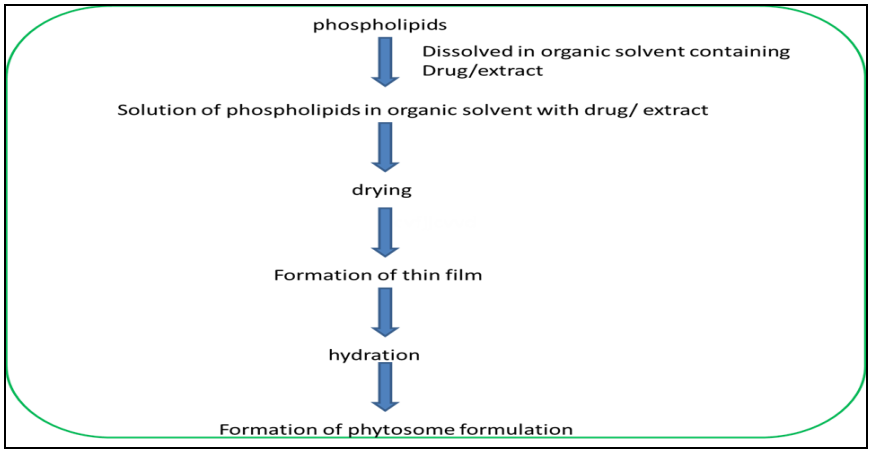

FIG. 7: GENERAL METHOD OF PREPARATION OF PHYTOSOMES

Advantages:

1. It properly delivers the drug to the target site.
2. Absorption of active constituents is increased even in small doses also.
3. Bioavailability gets enhanced.
4. Entrapment efficiency also gets enhanced.
5. Formulation is easy.
6. Liver targeting is improved by increasing the solubility in bile salts *etc*.

Naveet Nagpal stated that Phytosomes are having gastro-protective properties (due to the presence of phosphatidylcholine), which protects the active constituent of plants from gastric enzymes and intestinal bacteria. Based on this, Phytosomes can be used to formulate the enteric dosage of biotherapeutics [39].

Virosomes: Virosomes are the carriers that are spherical in shape with size 150nm composed of phospholipid bilayers in which lipid content is derived from viral components, which are approved for humans due to its safety profile and tolerance. Virosomes are prepared by enveloped viruses such as influenza, herpes simplex, human immunodeficiency, rubella, yellow fever, *etc.*, and these are derived from influenza containing constituents from the influenza virus envelope, hemagglutinin (HA), and neuraminidase (NA) that are incorporated into the phospholipids bilayers. HA and NA act as a carrier and adjuvant, and moreover, HA also responsible for rapid cellular uptake by endocytosis [40, 41].

Characteristics of Virosomes:

- Virosomes regenerate influenza virus envelops in which nucleocapsid is removed and incorporated by drug/ antigen/ genes *etc.*
- Virosomes act as a guard that protects from proteolytic degradation, pH, *etc.*
- These are biodegradable, biocompatible, non-antoimmunogenic, and nontoxic.
- These act as a carrier for cancer vaccines.
- Virosomes delivers the drug to the target site and improves its bioavailability. So, these had become an emerging trend for targeting drug delivery [42, 43].

Structure of Virosomes: Virosomes mainly contains Immune stimulating Regenerate Influenza (IRIVs), which are having phosphotidyl choline and phospholipids. A phospholipid is obtained from the influenza virus, which provides hemagglutinin and neuraminidase glycoprotein. Hemagglutinin and neuraminidase glycoprotein enables efficient vesicle uptake by the activation of the immune system. The mode of action of IRIVs is dependent on the influenza hemagglutinin, which is intercalated into the phospholipid bilayers, and hemagglutinin is the major influenza antigen that is involved in receptor binding and membrane fusion [44].

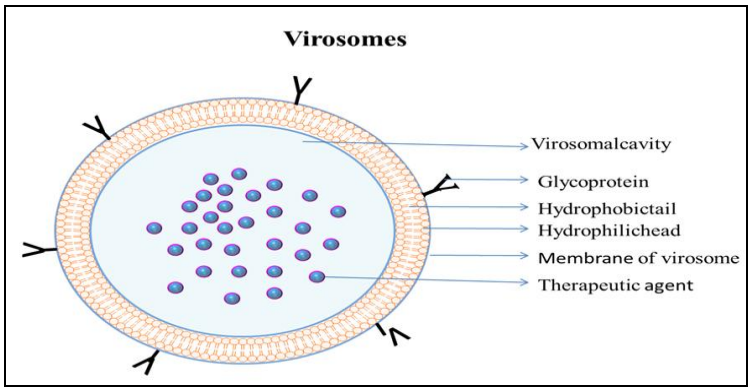

FIG. 8: STRUCTURE OF VIROSOME

Virosomes can be administered through intravenous, intramuscular, subcutaneous, intraarterial, inhalable, topical, and oral and transdermal, and these can be characterized by surface morphology, vesicle shape, size dispersion, surface charge, surface pH, electrical surface potential, lamellarity, drug efficiency, pyrogenicity, *etc.* As Virosomes can deliver maximum drugs, a scientist needs to be focused on the delivery of biotherapeutics too.

TABLE 1: LIST OF DRUGS USED TO TREAT VARIOUS DISEASES WITH INFLUENCE OF POLYMERS: [6, 7, 14, 21, 22, 26, 35, 36, 37, 38, 39, 44, 45]

Amoxicillin	PLGA Microspheres	Sustained Drug Release
Rifampicin	PLGA nanoparticles	Sustained drug release
Rifampicin	polybutylcyanoacrylate nanoparticles	They potentiated the *in-vitro* and *in-vivo* activity of rifampin and ciprofloxacin against *Mycobacterium avium*
Gentamycin	PLGA nanoparticles	Used to treat brucellosis
Rifampicin, isoniazid, pyrazinamide and ethambutol	Poly-lactide-co-glycolide (PLG) nanoparticles	They have shown enhanced bioavailability and pharmacodynamic properties
Lactoferrin	Lactoferrin-modified PEGPLGA NPs	It has efficacy to treat Parkinson's disease
Urocortin	Urocortin-loaded NPs	To treat striatum lesions
Angiopep-2 peptide and EGFP-EGF1	PEG-PCL NPs	To penetrate into BBB
Insulin	Transdermal electroporation of insulin-loaded nanocarriers	They have shown potential effect
Insulin	Insulin loaded polyalkylcyanoacryate nanocapsules	Reduction of glycemia
Insulin and calcitonin	polyisobutylcyanoacrylate nanoparticles	Achieved high plasma concentration
Human growth hormone	Human Growth hormone(hGH)-Glutathione microparticles	3 times increase in glutathione loaded microparticles
Insulin	Insulin loaded Chitosan-N-acetyl-L-cysteine (CS-NAC) polymeric nanoparticles	To deliver insulin through the nasal route
Glutathione	Glutathione coated brain targeted nano drug delivery system	Glutathione ligand binds to the transporter which subsequently increases the number of prodrug/ nano drug delivery system at the interface
DPK-060	Cubosomes	Antimicrobial peptide for topical delivery
5- fluorouracil	Cubosomes	Hydrophilic anticancer drug used for liver treatment.
Flurbiprofen	Cubosomes	Flurbiprofen cubosomal formulation to treat less ocular irritation
LL-37	Cubosomes	Antimicrobial peptide for topical delivery
Adriamycin	Exosomes	To treat Anti cancer (pancreatic cancer)
Cefixime	Phytosomes	Antibiotic used for urinary tract infections

CONCLUSION: The current review discusses the recent advances and presents an updated summary of novel drug delivery systems. Novel Drug delivery systems are now aiming to improve efficacy, increasing the selectivity and bio-availability in addition to reducing the toxicity issues.

We believe that novel delivery systems will not only increase the effectiveness of lead molecules but also provide an opportunity to reinvestigate earlier discarded lead molecules due to poor pharmacokinetic properties, Eg. Poor solubility, half-life, rapid clearance. Novel delivery systems like Cubosomes, Exosomes, and Phytosomes are relatively new and rapidly developing science to deliver biotherapeutics in a controlled manner at specifically targeted sites in the treatment of various diseases. Even though the novel drug delivery system is with high levels of uncertainties, we believe that they might be able to use in enhancing the solubility, biodegradable, bio-compatible, readily available, renewable, low toxicity, absorption, bioavailability, and controlled-release of drugs of biotherapeutics. Even though regulatory mechanisms for the use of these novel carriers are still a subject of discussion, there is no doubt about these novel molecules revolutionize the way we administer biotherapeutics.

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