Lipospheres: Emerging Carriers in the Delivery of Proteins and Peptides

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ABSTRACT: Currently, drug delivery technologies for protein and peptide delivery mainly rely on biodegradable polymers. However, protein stability during release from these systems can be critical due to physical and chemical instabilities. Lipospheres are solid microparticles composed of fat core stabilized by phospholipids layer represent an alternative carrier for the delivery of highly challenging, labile and unstable substances. This review highlights various aspects of lipospheres like physicochemical characteristics and stability for better clinical utility with a wider spectrum of proteins and peptides.

KEYWORDS: Lipospheres; protein delivery; lipid matrix; lipid carrier

Introduction

In recent years, numerous formulation technologies have been designed to protect the incorporated drug substance from the environmental conditions with controlled release. Colloidal delivery systems (CDS) have gained the most attention to deliver the drugs in the body with improved loading, controlled release, targeting, feasibility of large scale production, increased physical and chemical storage stability and minimum overall costs (Westesen, 2000).

With the advancement in biotechnology and genetic engineering, growing number of potential peptides and protein drugs have been identified. The total global market for protein drugs was $47.4 billion in 2006 and by the end of 2011 expected to reach $55.7 billion with an average annual growth rate (AAGR) of 3.3% (Talukder, 2007). However, many of them require special formulation technologies to overcome problems such as poor solubility, drug instability in biological milieu (i.e. short half life), poor bioavailability, high-protein binding and potentially strong side effects that require drug enrichment at the site of action. Thus, suitable delivery systems are crucial to preserve the protein stability and improved administration frequencies to lessen the burden on the patients.

Colloidal delivery system that have been investigated for this purpose are o/w emulsions, liposomes, microparticles and nanoparticles based on synthetic polymers or natural macromolecules (Rawat et al., 2006). Polymeric systems are associated with potential toxicological problems shifting the focus on lipid based carrier systems (Rawat et al., 2008). Lipid carriers comprise liposomes (Crommelin et al., 1997), multivesicular liposomes (Mantipragada, 2002), cubic phase gels (Shah et al., 2001), hollow lipid microparticles (Bot et al., 2000), lipid microcylinders (Meilander et al., 2001), and lipid microparticles (Reithmeier et al., 2001). These vehicles are composed of physiological lipids such as phospholipids, cholesterol, cholesterolesters and triglycerides.

The toxicological risk is much lower in these carriers due to biological origin of the carrier material but these conventional lipid carriers show some drawbacks relating to physicochemical instabilities.

Liposomes have the tendency to fuse releasing drug from the vesicles (Lentz et al., 1987).

These are unstable in the vascular system due to lipid exchange with lipoproteins (Allen, 1981).

The reproducibility of manufacture in terms of vesicle size and properties is limited, complicating large scale production.

The high mobility of incorporated drug molecules causes a faster release requiring frequent administration (Magenheim et al., 1993).

Thus there was the need to develop a system which combines the advantages of CDS and avoids their disadvantages which were primarily based on the liquid crystalline state of dispersed lipid phase.

Lipospheres

Lipospheres represent a new type of fat based encapsulation system developed for parenteral and topical delivery of bioactive compounds (Domb et al., 1990). Lipospheres were reported for the first time by Domb and Maniar as a particulate dispersion of solid spherical particles of a particle size between 0.2-100 μm in diameter consisting of solid hydrophobic fat core such as
triglycerides or fatty acid derivatives, stabilized by a monolayer of phospholipids (Fig 1) (Domb et al., 1990). Internal core contains the drug dissolved or dispersed in solid fat matrix. Inconsistent nomenclature is found in relation to lipospheres as nanoscale particles are termed as solid lipid nanoparticles (SLN). Lipospheres are restricted to the stabilizing material of a phospholipid layer as given in the definition by Domb. These have been utilized in the delivery of anti-inflammatory compounds, local anesthetics, antibiotics, antinecancer agents, insect repellant and vaccines and adjuvants (Masters et al., 1998; Khopade et al., 1997; Amselem et al., 1996; Domb et al., 1995).

![Fig. 1 Structural feature of Lipospheres.](Fat core stabilized by a monolayer of phospholipids)

**Table 1.** Different types of lipospheres containing proteins and peptides.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Protein</th>
<th>Lipid composition</th>
<th>Method of preparation</th>
<th>Remarks</th>
<th>References</th>
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<tbody>
<tr>
<td>1.</td>
<td>Somatostatin</td>
<td>Glyceryl tripalmitate</td>
<td>a) Melt dispersion b) double emulsion (w/o/w or s/o/w)</td>
<td>In case of melt dispersion, yield and entrapment efficiency found to be higher than double emulsion</td>
<td>Reithmeier et al., 2001a</td>
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<td>2.</td>
<td>Thymocartin</td>
<td>Glyceryl tripalmitate</td>
<td>a) Melt dispersion b) Solvent evaporation</td>
<td>Melt technique produced particles with smooth surface and loading of 20%w/v as compared to other with 2%w/v only.</td>
<td>Reithmeier et al., 2001b</td>
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<td>3.</td>
<td>Antigen (BSA)</td>
<td>Waxes, fatty alcohol, paraffin, hard fat and poly lactides</td>
<td>a) Melt dispersion b) Solvent method</td>
<td>Polylactide proved to be the preferred core material for delayed release</td>
<td>Domb et al., 1990</td>
</tr>
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<td>4.</td>
<td>R32NS1 Malaria antigen</td>
<td>Tristearin, PLA, ε-PCL</td>
<td>Melt dispersion</td>
<td>ε-PCL produced sustained release system as compared to other matrix material</td>
<td>Amselem et al., 1996</td>
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<td>5.</td>
<td>D-Trp-6-LHRH</td>
<td>Stearic acid</td>
<td>Double emulsion (w/o/w)</td>
<td>Pseudo-zero order release with 10% drug released in 8hr.</td>
<td>Morel et al., 1994</td>
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<td>6.</td>
<td>Thymopentin</td>
<td>Stearic acid</td>
<td>w/o/w, o/w multiple</td>
<td>Pseudo zero order release with 10% drug</td>
<td>Morel et al., 1996</td>
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<td>microemulsion</td>
<td>released in 6 hr.</td>
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<td>7.</td>
<td>Triptorelin</td>
<td>L-PLA, PLGA</td>
<td>Cosolvent-solvent</td>
<td>PVA was added along with phospholipids to</td>
<td>Raisel et al., 2002</td>
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<td>leuprolide</td>
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<td>evaporation</td>
<td>stabilize the polymer emulsion with</td>
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<td>phospholipids polymer ratio as 1:6</td>
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<td>8.</td>
<td>Hydrophilic</td>
<td>Triglycerides, PLA</td>
<td>Melt dispersion</td>
<td>Improved mechanistic properties were</td>
<td>Cortesi et al., 2003</td>
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<td></td>
<td>model</td>
<td>PLA, Eudragit RS100</td>
<td>(w/o/w/Solvent</td>
<td>obtained by combination of biodegradable</td>
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<td>drug</td>
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<td>evaporation</td>
<td>and non biodegradable polymers</td>
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<td>9.</td>
<td>Papain</td>
<td>Triglycerides</td>
<td>w/o/w emulsion</td>
<td>Masked bitterness effectively and good</td>
<td>Barbosa et al., 2004</td>
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<td>chemical stability for 60 days</td>
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<td>10.</td>
<td>Cyclosporine</td>
<td>Triglyceride</td>
<td>w/o/w emulsion</td>
<td>A Cmax of 1300 ng/mL was found after 2 h of</td>
<td>Bekerman et al., 2004</td>
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<td>oral intake of four capsules, each loaded</td>
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<td>with 50 mg cyclosporine.</td>
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PLA- Polylactic acid; PLGA- Poly lactic-co-glycolide; PCL- Polycaprolactone; BSA- Bovine serum albumin; D-Trp-6-LHRH- Luteinizing hormone releasing hormone.

**Advantages**
- Liposphere exhibit enhanced physical stability due to avoidance of coalescence (Domb et al., 1990).
- High dispersability in an aqueous medium.
- Low cost of ingredients.
- Ease of preparation and scale up.
- High entrapment of hydrophobic drugs.
- Controlled particle size.
- Reduced mobility of incorporated drug molecules responsible for reduction of drug leakage, circumvention of instabilities due to interaction between drug molecules and emulsifier film.
- Extended release of entrapped drug after a single injection.
- Static interface facilitates surface modification of carrier particles after solidification of the lipid matrix (Muller, 1991).

**Disadvantages**
- Different lipid modifications and colloidal species coexist that may cause differences in solubility and melting point of active and auxiliary species (Reithmeier et al., 2001).
- Low drug loading capacity for hydrophilic proteins.
- Variable kinetics of distribution processes.
- High-pressure induced drug degradation (Reithmeier et al., 2001).
- Insufficient stability data (Domb et al., 1996).
Materials Used

Lipospheres can entrap protein or peptide drugs in the internal hydrophobic core, in the phospholipid, attached to the phospholipids or a combination of the two (Domb et al., 1990).

Classical lipospheres consist of lipid based matrix. The neutral fats used in the preparation of the hydrophobic core are tricaprin, trilaurin, tristearin, stearic acid, ethyl stearate and hydrogenated vegetable oil.

Polymer lipospheres consist of matrices made from biodegradable polymers such as poly (lactic acid) (PLA), polycaprolactone (ε-PCL) and Poly lactide-co-glycolide (PLGA).

The phospholipids generally used to form the surrounding layer of lipospheres are pure-egg phosphatidyglycerol and phosphatidylethanolamine. Food-grade lecithin (96 % acetone insoluble) is used in the preparation of lipospheres for topical and veterinary applications.

Preparation of Lipospheres

Two preparation methods have been reported for drug loaded lipospheres (Domb, 1993):

Solvent technique

In this technique, all solid components such as drug, solid carrier and phospholipids are dissolved in an organic solvent. Commonly employed solvents are acetone, ethyl acetate, ethanol or dichloromethane. This is followed by solvent evaporation and the resulting solid is mixed with warm buffer solution until a homogeneous dispersion of lipospheres is obtained.

Melt technique

In this method, drug is dissolved or dispersed in the melted solid carrier followed by addition of warm buffer solution containing phospholipid with constant mixing and rapid cooling to obtain the uniform dispersion of lipospheres is obtained.

Polymeric lipospheres can also be prepared by a solvent or melt process. These differ from classical lipospheres in terms of the composition of the internal core of the particles composed of biodegradable polymers. Both types of lipospheres are stabilized by a layer of phospholipid molecules.

Miscellaneous technique

Protein drugs are principally hydrophilic in nature, thus, the entrapment of water soluble agents in the hydrophobic core of lipospheres has been achieved by modification in the existing methods of preparation.

Multiple microemulsion

Morel et al reported a method in which a solution of peptide was dispensed in stearic acid melt at 70ºC followed by dispersion of this primary emulsion into aqueous solution of egg lecithin, butyric acid and taurodeoxycholate sodium salt at 70ºC (Morel et al., 1994). Rapid cooling of multiple emulsion formed solid lipospheres with 90% entrapment of peptide. Sustained release was reported by multiple emulsification technique with inclusion of lipophilic counter ion to form lipophilic salt of peptide (Morel et al., 1996).

Polymeric lipospheres have also been reported by double emulsification for encapsulation of antigen (Amselem et al., 1996).

Cosolvent method

Raisel et al reported cosolvent-solvent evaporation method employing chloroform and N-methyl pyrollidone to create a clear solution (Raisel et al, 2002). Although low yield and large particle size was obtained, which was altered by variation in the solvent used. Cortesi et al also reported lipospheres made up of polar and non-polar lipids using synthetic stabilizers instead of phospholipids which were the deviation from the definition of liposphere reported by Domb in his patent (Cortesi et al., 2003). Although their work was not related to protein delivery but they tried it with hydrophilic drug and reported around 50% entrapment by double emulsification method.

Entrapment into lipid carriers

Reithmeier et al reported successful incorporation of model peptides like insulin, somatostatin and thyromucarin into lipid microparticles (Reithmeier et al., 2001a; 2001b). Domb described a method of antigen incorporation into
lipospheres lay pre-entrapment in multilamellar liposomes followed by dispersion into ethyl stearate melt containing L-alpha lecithin (Domb et al., 1990).

Properties

Morphology
Lipospheres are characterized in terms of morphology by various microscopic techniques such as optical and electron microscopy. Lipospheres prepared by melt method showed unimodal shape with average particle size between 5-15µm with less than 2% of particles greater than 100µm. Homogeneous formulation of lipospheres containing antigen were prepared by melt dispersion with 100% of particles having an average diameter of about 7±3µm (Amselem et al., 1996). Polymer lipospheres made up of PLA and lecithin showed a very broad particle size distribution from 2-100µm (Amselem et al., 1996). Inclusion of lipid A in the composition of the polymeric lipospheres reduced their mean particle size by a factor 0.25 regardless of the polymer type (Amselem et al., 1992).

Structure
Phospholipid content on the surface of lipospheres is determined by 31P-NMR before and after manganese (Mn ²⁺) or prosedimium (Pr ³⁺) ion complexation and by trinitrobenzene sulfonic acid (TNBS) labeling using liposphere formulations containing phosphatidylethanolamine (Barenholz et al., 1993). Phospholipid content indicates the type of structure formed, as increase in the phospholipid content above certain limit has been reported to form other phospholipid structures like liposomes (Domb et al., 1996).

Entrapment efficiency
Loading capacity of drug in lipid carriers depends on the type of lipid matrix, solubility of drug in melted lipid, miscibility of drug melt and lipid melt, chemical and physical structure of solid lipid matrix and the polymorphic state of the lipid material (Muller et al., 2000). Preparation technique exhibits marked effect on the loading of protein in the carrier. Melt method produces lipospheres with highest loading (Masters et al., 1998). But it is associated with the drawback of difference in crystallization behavior of lipids as determined by DSC. Reithmeier et al reported the single endothermic peak in case of lipid bulk material and microparticles prepared by solvent evaporation method resulting from the melting of the stable crystalline form (β- modification) and three peaks were detected for microparticles prepared by melt dispersion technique (Reithmeier et al., 2001a). High drug loading capacities have been reported for unstable modifications with lower crystalline order, as less perfect crystals with many imperfections offer more space to accommodate drugs (Mehnert et al., 2001). The presence of surfactants also leads to reduced crystallinity responsible for higher incorporation efficiencies into lipid carriers. Phospholipids content also exerted marked effect on encapsulation efficiency of proteins as increase in its content above 6% showed a large increase due to increased stability of the primary emulsion or electrostatic interactions between peptide and lecithin (Reithmeier et al., 2001b). But further increase above 6% have been reported to form other phospholipid structures like liposomes, micelles, mixed micelles etc (Mehnert et al., 2001).

Stability
Countable number of research work has been done dealing with protein delivery through lipospheres. Stability of proteins in terms of physical, chemical and conformational features is an important prerequisite to establish the utility of the procedures adopted for encapsulation. The extent to which they occur is dependent on the temperature and pH of the solution (Wang, 1999). Preservation of the integrity of protein can be maintained by avoiding exposure to higher temperatures using low melt lipid carriers (Domb et al., 1990). Morel studied the thermal stability of peptide thymopentin by exposure to higher temperatures for longer period of time. Results reported variation in stability results in water and microemulsions (Morel et al., 1996). Different methods have been reported by various researchers to identify the degradation products released from lipospheres, HPLC being commonly utilized method. But no such observation have been published for peptides or proteins released from lipospheres (Raisel et al., 2002; Morel et al., 1996; 1994).

Release Kinetics
Drug kinetics is a crucial parameter of evaluation for a drug delivery system (Domb et al., 1996). Release of a hydrophilic substance from a lipophilic matrix depends on matrix material composition, properties of the incorporated drug such as solubility in lipid and aqueous medium, drug carrier interaction, drug loading, presence of surfactants, particle size, and method of preparation (Vogelhuber et al., 2003; Zhu et al., 2000; Zur Mahlen et al., 1998; El Shanawamy, 1993). Domb claimed that the release of drug from lipospheres depends on phospholipids coating and the
carrier (Domb et al., 1990). Morel et al investigated the release behavior of protein from lipospheres by using multicavity microdialysis cell (Morel et al., 1994; 1996; Raisel et al., 2002). They observed pseudo zero order release with 10% of drug loading released in 8 hr in case of Luteinizing hormone releasing hormone (LHRH) (Morel et al., 1994) and 6 hr in case of thymopentin (Morel et al., 1996). Zur Muhlen observed that the initial burst release increased with the decreasing particle size due to increased surface area and short diffusion distance of the drug (Zur Muhlen et al., 1998). Reithmeier studied the effect of different triglyceride/phospholipid ratios on the release profile. Phospholipids content exerted accelerating effect on encapsulation efficiency and burst effect. Burst release was absent in absence of phospholipids followed by incomplete release due to decreased entrapment and interaction of drug with the carrier material (Reithmeier et al., 2001a; 2001b).

Polymer lipospheres are superior to lipid lipospheres in terms of long duration release. Different polymers such as PLA, PLGA, and PCL have been utilized as matrix material, where the extent of release was found to be dependent on degradation behavior, molecular weight of polymer and copolymer composition (Amselem et al., 1996; Gopferich, 1996). Polymer matrices loaded with triptorelin made up of L-PLA showed extended release for upto 30 days with initial burst (Raisel et al., 2002). Polymer lipospheres without the presence of phospholipids showed a faster release profile than classical lipospheres (Raisel et al., 2002). But, on degradation and erosion polymer matrices undergoes constant changes with detrimental effects on protein drugs whereas triglyceride matrices preserve the integrity and bioactivity of encapsulated model peptides serving as a promising alternative to polymer matrices (Maschke et al., 2000).

Conclusion

The liposphere approach employs a fat lipid environment to achieve desired goal for controlled and safe delivery of drugs. Lipospheres have been successfully utilized for the delivery of variety of substances with the potential of targeting while avoiding systemic side effects. But on the basis of literature survey only a countable number of researches have been reported pertaining to protein delivery. Although attempts have been made to investigate various processing parameters for effective delivery of proteins and peptides, but there is a need for further optimization.

Lipospheres offer the advantages of good physical stability and dispersability with the ease of freeze drying and reconstitution before use. These further provide both the surface interface necessary for solubilization and proper orientation as potential carriers for protein drugs like vaccines, hormones and growth factors etc. Polymeric lipospheres can serve as alternative for extended release of entrapped drug. Thus, there is a need of further investigations with a wider spectrum of proteins and peptides to establish the clinical utility and industrial scale up of these novel lipid formulations for delivery of proteins.

References


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