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Lipospheres: A Comprehensive Overview

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A B S T R A C T

The new drug molecules (more than 40%) are lipophilic in nature and showing poor water solubility and poor dissolution profile. Various techniques have been employed to formulate oral drug delivery system that would enhance the dissolution profile and in turn, the absorption efficiency of water insoluble drug. Solid dispersion, drug micronisation, lyophilisation, microencapsulation are some of the methods that have been used to enhance dissolution characteristics of water insoluble drugs. Among them, lipospheres are the promising particulate drug delivery systems for improving dissolution rate of water insoluble drugs. Lipospheres represents a novel drug delivery system in which water-insoluble lipid spheres forms a solid hydrophobic core, with a layer of phospholipids embedded on the surface of the core. Drugs or other biologically active agents may be contained in the hydrophobic core, in the phospholipid, attached to the phospholipids or a combination of the two. Lipid based carrier system (lipospheres) was adopted to eliminate the toxic effects associated with the use of polymers as carrier and entrapment of high amount of poorly bioavailable lipophilic compound. Due to its poor aqueous solubility of water insoluble or poorly soluble drug loaded lipospheres were found to be an effective natural carrier in terms of discrete particle size, encapsulation efficiency, and satisfactory in vitro release characteristics. The incorporated phospholipid could combine with drug by hydrophilic and hydrophobic interactions, consequently improved the drug entrapment efficiency and produced the sustained release rate.

Keywords: Lipospheres, Poor dissolution, Phospholipids, Sustained release

A R T I C L E I N F O

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1. Introduction

Various techniques have been employed to formulate oral drug delivery system that would enhance the dissolution profile and in turn, the absorption efficiency of water insoluble drug. Solid dispersion, drug micronisation, lyophilisation, microencapsulation, inclusion of the drug solution or liquid drug into soft gelatin capsules are some of the methods that have been used to enhance dissolution characteristics of water insoluble drugs. Among them, lipospheres are amongst the promising particulate drug delivery systems for improving dissolution rate of water insoluble drugs that were initially reported as a particulate dispersion of solid spherical particles between 0.2-100µm in diameter consisting of solid hydrophobic fat core such as triglycerides or fatty acids derivatives, stabilized by monolayer of phospholipids. Lipospheres represent a new type of fat based encapsulation system developed for parenteral and topical delivery of bioactive compounds and have been utilized in the delivery of anti-inflammatory compounds, local anaesthetics; antibiotics, anticancer agents, insect repellent, vaccines, proteins and peptides. The lipospheres are distinct from microspheres of uniformly dispersed material in homogenous polymer since they consist of two layers, the inner solid particle that contains the entrapped drug with phospholipids outer layer. The combination of solid inner core with phospholipid exterior confers several advantages on the lipospheres as compared with conventional microspheres and microparticles, including high dispersibility in aqueous medium, and a release rate for the entrapped substance that is controlled by the phospholipid coating and the carrier. Further, the substance to be delivered does not have to be soluble in the vehicle since it can be dispersed in the solid carrier. Lipospheres have a lower risk of drug –excipient interactions due to the solid nature of vehicle. Moreover, the drug release rate can be manipulated by altering either or both the inner solid vehicle or the outer phospholipid layer. The ease of preparation as compared to liposomes that have inherent problem of stability adds an advantage over other lipid systems [1]. Lipospheres represent a new type of fat based encapsulation system developed for parenteral and topical delivery of bioactive compounds [2]. 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 amonolayer of phospholipids (Fig 1).

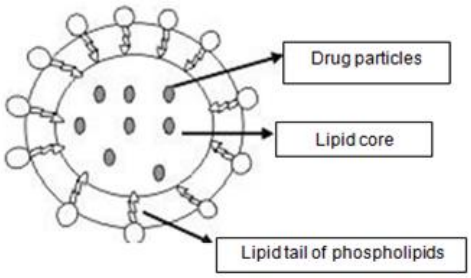

Figure 1: Structure of Liposphere

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, localanesthetics, antibiotics, anticancer agents, insect repellant and vaccines and adjuvants [3, 4, 5, 6].

Advantages

- Liposphere exhibit enhanced physical stability due to avoidance of coalescence.
- High dispersability in an aqueous medium.
- Low cost of ingredients.
- Ease of preparation and scale up.
- High entrapment of hydrophobic drugs.
- Controlled particle size.
- Extended release of entrapped drug after a single injection.
- Static interface facilitates surface modification of carrier particles after solidification of the lipid matrix [7].

Disadvantages

- Different lipid modifications and colloidal species coexist that may cause differences in solubility and melting point of active and auxillary species.
- Low drug loading capacity for hydrophilic proteins.
- Variable kinetics of distribution processes.
- High-pressure induced drug degradation.
- Insufficient stability data
- Toxic effects of organic residues after the production of polymers,
- Lack of large industrial scale production [7].

2. 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. Polymer lipospheres made up of PLA and lecithin showed a very broad particle size distribution from 2-100µm.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 [8].

Structure: Phospholipid content on the surface of lipospheres isdetermined by ^{31}P-NMR before and after manganese (Mn^{2+}) or proseodimium (Pr^{+3}) ion complexation and by trinitrobenzene sulfonic acid (TNBS) labelling using liposphere formulations containing phosphatidyl ethanolamine. Phospholipid content indicates the type of structureformed, as increase in the phospholipid content above certain limit has been reported to form other phospholipid structures like liposomes [9].

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. Preparation technique exhibits marked effect on the loading of protein in the carrier. Melt method produces lipospheres with highest loading. 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. The presence of surfactants also leads to reduced crystallinity responsible for higher incorporation efficiencies into lipid carriers. Phospholipids content has marked effect on encapsulation efficiency of proteins as it content increase up to 6% but further increase above 6% results in formation of other phospholipid structures like liposomes, micelles etc.[10]

Stability: Preservation of the integrity of protein can be maintained by avoiding exposure to higher temperatures using low melt lipid carriers. 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 [11].

Release Kinetics

Release of ahydrophilic 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 [12].

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.

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 (∈-PCL) and Poly lactide-co-glycolide (PLGA).

The phospholipids generally used to form the surrounding layer of lipospheres are pure-egg phosphatidyglycerol and phosphatidyl ethanolamine. Food-grade lecithin (96 % acetone insoluble) is used in the preparation of lipospheres for topical and veterinary applications [13].

3. Preparation of Lipospheres [7]

1. Melt dispersion technique

The lipid physical mixture containing lipid, phospholipids, cholesterol, etc., is prepared with and without a lipophilic model drug. The physical mixture is melted at 70°C and then emulsifiedinto a hot external aqueous phase maintained at 70°C containing suitable surfactant. The emulsion is mechanically stirred by using stirrer equipped with alternate impellers and maintained at 70°C. Then, the emulsion formulation is rapidly cooled to about 20°C by immersing the formulation into a ice bath and continuing the agitation to yield uniform dispersion of lipospheres. The obtained lipospheres is then washed with water and isolated by filtration through a paper filter.

2. Solvent evaporation technique

This technique is an alternative to the melt dispersion technique and it is considered with the objective of possibly minimizing theexposure to high temperatures of thermolabile compounds, such asproteins and nucleic acids. This technique is based on theevaporation of organic solvent in which lipids are dissolved andallowing the formation of solid microparticles. In particular, the lipid matrix is dissolved in an organic solvent such as ethyl acetate and maintaining the temperature about 50°C and then emulsifiedwith an external aqueous phase containing the surfactant agent. Theresulting oil-in-water emulsion is stirred for 6 to 8 h till completeevaporation of the solvent. The LS are recovered by filtrationthrough a filter paper.

3. Co-solvent solvent evaporation method

In this co-solvent - solvent evaporation method employing chloroform and N-methyl pyrollidone to create a clear solution,although low yield and large particle size is obtained, which isaltered by variation in the solvent used. Lipospheres made up ofpolar and non-polar lipids using synthetic stabilizers instead ofphospholipids which are the deviation from the definition of liposphere reported by Domb in his patent. Although their work is not related to protein delivery but they tried it with hydrophilic drug and reported around 50% entrapment by double emulsification method.

4. Solvent extraction method

The solvent extraction method is based on the dissolution of the triglyceride (i.e., tripalmitin) and the cationic lipid in the organic solvent (i.e., dichloromethane), and on the addition of an aqueous

polyvinyl alcohol (PVA) solution (0.5% w/w) used as extraction fluid. The solution and the extraction fluid are pumped into a static micro channel mixer, leading to the production of an O/W emulsion. The mixing leads to the production of fine lamellae, which subsequently disintegrate into droplets, allowing the formation of lipid microspheres dispersed in the extraction aqueous medium.

5. Multiple microemulsion

In this method a solution of peptide is dispensed in stearic acid melt at 70ºC followed by dispersion of this primary emulsioninto aqueous solution of egg lecithin, butyric acid andtaurodeoxycholate sodium salt at 70ºC. Rapid cooling of multiple emulsion forms solid lipospheres with 90%

entrapment of peptide. Sustained release is reported by multiple emulsification technique with inclusion of lipophilic counter ion to form lipophilic salt of peptide. Polymeric lipospheres have also been reported by double emulsification for encapsulation of antigen.

6. Sonication method

In this technique, the drug is mixed with lipid in a scintillation vial which is pre-coated with phospholipids. The vial is heated until the lipid melts, and then vortexed for 2min to ensure proper mixing ofthe ingredients. A 10 ml of hot buffer solution is added into the above mixture and sonicated for 10min with intermittent cooling until it reaches to the room temperature.

7. Rotoevaporation method.

In this technique, lipid solution with drug is prepared in a round bottom flask containing 100 grams of glass beads (3 mm in diameter) mixed thoroughly till a clear solution is obtained. Then, the solvent is evaporated by using rotoevaporizer under reduced pressure at room temperature and a thin film is formed around the round bottom flask and the glass beads. Raise the temperature upto 40°C until complete evaporation of the organic solvent. Known amount of 0.9% saline is added to the round bottom flask and the contents are mixed for 30min at room temperature and then the temperature is lowered to 10°C by placing in ice bath and mixing iscontinued for another 30min until lipospheres are formed.

8. Microfluidizer method

Lipospheres can also be prepared by using a microfluidizer which is equipped with two separate entry ports. From one entry port, a homogenous melted solution or suspension of drug and carrier is pumped and from second entry port, an aqueous buffer is pumped. The liquids are mixed in the instrument at elevated temperatures where the carrier is melted and rapidly cooled to form the lipospheres. The temperature of the microfluidizer can also be changed at any stage of the lipospheres processing to manipulate the particle size and distribution.

9. Polymeric lipospheres

Polymeric biodegradable lipospheres can also be prepared by solvent or melt processes. The difference between polymeric lipospheres and the standard liposphere formulations is the composition of the internal core of the particles. Standard lipospheres, as those previously described, consist of a solid hydrophobic fat core that is composed of neutral fats like tristearin,while in the polymeric lipospheres, biodegradable polymers such as polylactide (PLD) or PCL substitute the triglycerides. Both types of lipospheres are thought to be stabilized by one layer of phospholipid molecules embedded in their surface.

Preparation of Nanosize Lipospheres

Nanosize lipospheres are prepared by homogenization through serial filters of reduced pore size. In this system, the drug, triglyceride, phospholipid, andother additives are dissolved in a mixture of common surfactantssuch as Tweenand Spanand an organic solvent that is miscible withall components. This clear anhydrous solution spontaneously forms nanoparticles when gently mixed in aqueous solution. The particle size is controlled mainly by the formulation compositions. Cationic or anionic nanolipospheres can be obtained when adding a cationic or anionic lipid, such as stearyl amine, phosphatidilethanol amine, stearic acid, or phosphadilic acid, to the solution [14].

Table 1: Different types of lipospheres containing proteins and peptides[13]

S.No	Protein	Lipid composition	Method of preparation	Importance
1	Somatostatin	Glyceryl tripalmitate	a) Melt dispersion b)double emulsion (w/o/w)	In case of melt dispersion, yield and entrapment efficiency found to be higher than double emulsion
2	Thymocartin	Glyceryl tripalmitate	a) Melt dispersion b) Solvent evaporation	Melt technique produced particles with smooth surfaceand loading of 20%w/v as compared to other with 2%w/vonly.
3	Antigen (BSA)	Waxes, fatty alcohol,paraffin, poly lactides	a) Melt dispersion b) Solvent method	Polylactide proved to be the preferred core material for delayed release
4	R32NS1 Malaria	Tristearin, PLA, ∈-PCL	Melt dispersion	∈- PCL produced sustained release system as compared to other matrix material
5	D-Trp-6-LHRH	Stearic acid	Double emulsion (w/o/w)	Pseudo-zero order release with 10% drug released in 8hr.
6	Thymopentin	Stearic acid	multiple microemulsion	Pseudo-zero order release with 10% drug released in 6hr.
7	Triptorelin leuprolide	L-PLA, PLGA	Cosolvent-solvent evaporation	PVA was added alongwith phospholipids to stabilize the polymer emulsion with phospholipids polymer ratio as1.6
8	Hydrophilic model drug	Triglycerides, PLA, Eudragit RS100	Melt dispersion Double emulsion	Improved mechanistic properties were obtained by

			(w/o/w)Solvent evaporation	combination of biodegradable and non-biodegradable polymers
9	Papain	Triglycerides	w/o/w emulsion	Masked bitterness effectively and good chemical stability for 60 days

Evaluation of Lipospheres [1]

% Yield (%Y)

Dried lipospheres were accurately weighed, and considering the total amount of drug and excipient used for preparing the feed solution, the % yield of lipospheres was calculated using the following formula:

$$\% \text{ yield} = \frac{\text{Total weight of lipospheres}}{\text{Total material taken for emulsification}} \times 100$$

Angle of Repose

The angle of repose for the lipospheres of each batch was determined by the funnel method. Accurately weighed10gm of lipospheres were allowed to flow out of the funnel orifice fixed at a height of 2cm from the surface ona plane paper kept on the horizontal platform. The gradual addition of the lipospheres from the funnel mouth formed apile of granules on the surface this was continued until the pile touches the stem tip of the funnel. A rough circle was drawn around the pile base and the radius of the powder cone was measured. Angle of repose was calculated by using the following formula:

$\phi = \tan^{-1} h/r$

Where,

ϕ = angle of repose

h = height of the pile

r = Average radius of the powder cone

Bulk Density

Accurately weighed 10gm of lipospheres were placed into a 50ml cylinder of the bulk densitometer. Thevolume occupied by the sample was recorded. The bulk density was calculated as following formula:

$$\text{Bulk density} = \frac{\text{Weight of sample in grams}}{\text{Volume occupied by sample in ml}}$$

Tapped Density

Accurately weighed 10gm of lipospheres were poured gently through a glass funnel into a cylinder of bulk densitometer. The cylinder was tapped from a height of 2 inches until a constant volume was obtained. Volume occupied by the sample after tapping wererecorded and tapped density was calculated as follows by formula.

$$\text{Tapped density} = \frac{\text{Weight of sample in grams}}{\text{Volume occupied by sample after tapping in ml}}$$

Carr's Index (CI)

One of the important measures that can be obtained from bulk and tapped density determinations is the percent compressibility or the Carr's index, CI, which is determinedby the following formula:

$$\text{Carr}'\text{s index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}}$$

Characterization of Lipospheres [15]

Particle Size Analysis

The size of the lipospheres was determined by light scattering based on laser diffraction using the laser diffraction particle size analyzer, and the distribution modal size was estimated. Measurements were performed using a 45 mm focus objective, a beam length of 2.4 mm and obscuration levels from 5 to 10%.

Differential Scanning Calorimetry (DSC)

Samples of plain and drug loaded lipospheres of the selected formulation were submitted to DSC analysis using differential scanning calorimeter calibrated with indium. The analysis was carried out on 1 mg samples sealed in standard aluminum pans. Thermo grams were obtained at a scanning rate of 10°C/min using dry nitrogen flow of (25 ml/min). Each sample was scanned between zero and 400°C.

***In-Vitro* Release of drug from Lipospheres**

The release of drug from the prepared lipospheres was determined applying the membrane diffusion technique using molecular porous membrane. An accurately measured amount of lipospheres, equivalent to 2 mg drug, was suspended in 1 ml phosphate buffered saline of pH 7.4 and transferred to a glass cylinder having a length of 7 cm and diameter of 2.5 cm. This cylinder was fitted, before addition of lipospheres suspension, with the pre-soaked dialysis membrane and was suspended in the dissolution flask of the USP dissolution tester containing 100 ml phosphate buffered saline of pH 7.4. The apparatus was adjusted to a constant speed of 50 rpm and a temperature of 37°C. Samples were collected after 15 min, 30 min, 1 h and every hour over a period of 8 h and assayed spectrophotometrically for drug content at predetermined wavelength. The release data was subjected to kinetic treatment to determine the order of release.

Determination of Entrapment Efficiency

The entrapped drug concentration was determined by lysis of the lipospheres with absolute alcohol and sonication. Accurately weighed amount of loaded lipospheres (50 mg) was dissolved in 10 ml absolute alcohol and covered well with parafilm to prevent evaporation.The solution was sonicated for 15 min to obtain a clear solution. An aliquot of 1 ml of this solution was added to 9 ml of absolute alcohol. The solution was sonicated for another 15 min. The concentration of drug in absolute alcohol was determined spectrophotometrically after appropriate dilution. Unloaded lipospheres produced insignificant absorbance values at the same wavelength. The entrapment efficiency was calculated using the following formula

$$\text{Entrapment efficiency} = \frac{\text{Obtained drug concentration}}{\text{Theoretical drug concentration}} \times 100$$

***In Vivo* Anti-inflammatory Study of Lipospheres**

The anti-inflammatory activity of the selected lipospheres formulation applied both in solid and in dispersion form was estimated and compared to the marketed product using the rat paw edema test. Adult male albino rats of 6–8 weeks of age, each weighing 130–150 g were divided into five groups, of five animals each. Group I received topical saline application, group II received plain lipospheres, group III received lipospheres (F1) in solid pellet form as a paste, group IV received the same formula of group III but in dispersion form as a lotion, group V received the marketed Bristaflam cream. The volume of paw edema (ml) was measured in each animal using a plethysmometer to a precision of two decimal places. The rats were marked on the left hind paw just beyond the tibiotarsal junction, so that every time the paw was dipped in the electrolyte fluid column up to a fixed mark to ensure constant paw volume. The tested preparations were applied to the left hind paws of rats using an amount equivalent to 1 mg of drug. The area of application was occluded with a parafilm and was left in place for 2 h. The parafilm was then removed and the residual formulation on the surface was wiped off with cotton wool. After 2 h of topical application, initial paw volume of the rats was measured by dipping the rat paw into the electrolyte column just before carrageenan injection and the increase in volume due to fluid displacement was noted from a digital display, followed by the injection of 0.1 ml of 1% (*w*/*v*) carrageenan solution in saline in the sub plantar region of left hind paw of the rats. Measurement of paw volume was done after 1, 2, 3, 4, 5, 6, 7 and 8 h. The edema rate and inhibition rate of each group was calculated as follows:

$$\text{Edema rate (E\%)} = \frac{\text{Vt} - \text{Vo}}{\text{Vo}} \times 100$$

$$\text{Inhibition rate (I\%)} = \frac{\text{Ec} - \text{Et}}{\text{Ec}} \times 100$$

Where: Vo is the mean paw volume before carrageenan injection (ml),

Vt is the mean paw volume after carrageenan injection (ml),

Ec is the edema rate of control group, and

Et is the edema rate of the treated group

.

4. Conclusion

Lipid carriers like lipospheres have bright future due their inherent property to enhance the bioavailability of lipophilic drugs with poor water solubility. Lipospheres are solid, water insolublenano and microparticles composed of a solid hydrophobic corehaving a layer of a phospholipids embedded on the surface of the core. The hydrophobic core is made of solid triglycerides, fatty acid esters, or bioerodible polymers containing the active agent. Liposphere formulations were effective in delivering various drugs and biological agents including: local anaesthetics, antibiotics, vaccines, and anticanceragents with a prolonged activity of up to four to five days. 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. Lipospheres can be considered as a promising delivery system for oral delivery of peptide drugs like enzymes. Lipospheres were able to entrap the peptide at high levels and sustain its release over a prolonged time

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