

MANUFACTURING TECHNIQUES OF LIPOSPHERES: OVERVIEW

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ABSTRACT

Lipid microspheres, often called lipospheres (LS), have been proposed as new type of lipid-based encapsulation system for drug delivery of bioactive compounds especially lipophilic compounds. LS consist of solid microparticles with a mean diameter usually the size range between 0.2 to 500µm, composed of a solid hydrophobic fat matrix, where the bioactive compound(s) is dissolved or dispersed. The lipospheres have several advantages over other colloidal delivery systems (including nano & micro emulsions, nanaoparticles, hydrogels and liposomes). LS composed of triglycerides and monoglycerides are produced by melt dispersion technique, solvent evaporation or multiple emulsion method, sonication method, rotoevaporation method, microfluidiser method and co solvent method. The various bio active compounds are incorporated in to LS that can be administered in to various routes.

Keywords: Lipospheres, Controlled delivery, Lipids, melt dispersion.

INTRODUCTION

Many existing drug candidates have poor solubility in biological fluids, which results in low and highly variable bioavailability and a high food dependency after oral administration. Intravenous injection of these drugs is not possible because of their low solubility. Colloidal delivery systems (CDS) have gained the most attention to deliver the drugs in the body with enhancement of oral bioavailability, decrease in variability and food dependency, development of intravenous injectable formulations, drug targeting to specific tissues (with reduction of general toxicity), and life cycle management (protection by propriety formulation techniques).

It is expected that the modification/development of CDC will increase as a result of requirements for drug safety and of the increasing number of poorly soluble compounds in the pipeline and it is found to be crucial by many companies.

The main efforts to improve recent CDC are related to:

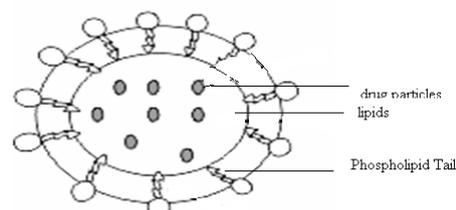
- Possibility of controlled release
- Possibility of drug targeting
- Increasing drug load
- Increasing feasibility of large-scale production
- Increasing physical and chemical storage stability
- Minimizing overall costs.

Particulate carriers (e.g., polymeric nano- and microparticles, fat emulsion, and liposomes) possess specific advantages and disadvantages. For instance, in the case of polymeric microparticles, the degradation of the polymer might possibly cause systemic toxic effects through the impairment of the reticuloendothelial system¹ or by accumulation at the injection site²; cytotoxic effects have indeed been observed *in vitro* after phagocytosis of particles by human macrophages and granulocytes³. In addition, organic solvent residues deriving from the preparation procedures, such as the

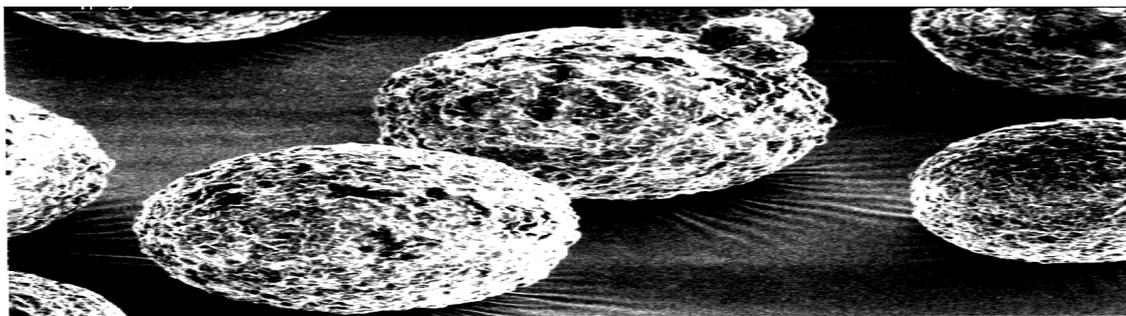
solvent evaporation technique often used for liposome⁴ and polyester microparticles⁵, can be present in the delivery system and could result in severe acceptability and toxicity problems⁶.

To overcome these adverse effects of polymeric colloidal delivery system, lipid microspheres, often called lipospheres (LS), are lipid (triglycerides) -based encapsulation system, developed for parenteral, oral and topical drug delivery of bioactives.⁷⁻¹⁸ Lipospheres consist of water dispersible solid microparticles, which have their diameter between 0.1 to 100 µm. These are comprises of a solid hydrophobic fat core (triglycerides) stabilized by a layer of phospholipids embedded in their surface. The internal core contains the bioactive compound, dissolved or dispersed in the solid fat matrix. The liposphere system has been used for the controlled delivery of various types of drugs including anti-inflammatory compounds, local anesthetics, antibiotics, and anticancer agents. They have also been successfully used as carriers for vaccines and adjuvants⁸⁻¹¹. Recently, lipospheres have been used for the delivery of peptides (Serratiopeptidase) and for oral drug-delivery.^{16, 17} Similar systems based on solid fats and phospholipids have been described^{19, 20}

Structure of Lipospheres



Optical Microscopy Photograph showing the morphology of lipospheres mmMorphology of Lipospheres



Scanning Electron Microscopy Photograph Showing the Morphology of Lipospheres

The use of lipid dispersion systems, such as liposomes and lipid emulsions, as lipophilic drug carriers has attracted great interest. The viability of the number of encapsulated chemotherapy agents in these lipid carriers have been demonstrated *in vivo*²¹⁻²³ Particularly, a LM, also called lipid emulsion formulation, is considered superior to others due to the fact that it can be produced in industrial scale, is stable during storage and highly biocompatible.²²⁻²⁴

The unique properties of lipids viz., their physicochemical diversity, biocompatibility and proven ability to enhance oral bioavailability of poorly water soluble, lipophilic drugs through selective lymphatic uptake have made them very attractive candidates as carriers for oral formulations.²⁵

With the above promises, the emerging field of lipid-based oral drug delivery system (LBODDS) has attracted considerable academic attention. Perhaps, some of the reasons for this include the complexity of their physicochemical properties, challenges in stability and manufacturing at the commercial scale, limited solubility of some poorly water-soluble drugs in lipids, their pre-absorptive gastrointestinal (GI) processing, a lack of knowledge about the *in vivo* behavior, influence of co-administered drugs/lipids and finally, the lack of predictive *in vitro* and *in vivo* testing methodologies. In spite of these limitations, lipids definitely offer the potential for enhancing drug bioavailability, through solubilization of drug, although other mechanisms of absorption enhancement have been implicated which includes, reduction of P-glycoprotein-mediated efflux, mitigation of hepatic first pass metabolism through enhanced lymphatic transport^{26,27,28}, and prolongation of gastrointestinal (GI)

transit time, or protection from degradation in the GI tract. Though, the formulation opportunities are yet to be fully explored. Formulation excipients capable of being digested in the GI tract play a major role in determining the rate and extent of absorption of drugs from the GI tract. Formulators need to have an in-depth knowledge of the GI digestive process for interpretation of the biopharmaceutical properties of lipid-based oral formulations and design relevant *in vitro* tests to mimic the physiological environment for the formulation. Continuous efforts are being made towards the design of a biorelevant dissolution media as well as to understand the *in vivo* colloidal behavior of the lipid-based formulations in the presence of endogenous solubilizing species viz., bile salts (BS), phosphatidylcholine (PL) and cholesterol (CL) and enzymes (lipase). The present review is a consolidated approach towards understanding the role of lipids (both exogenous and endogenous) in the process of bioavailability enhancement of lipophilic drugs, mechanisms involved in the digestive process and transcellular transport, challenges involved in formulation development with particular emphasis on solid dosage forms and advances made till date in the development of morphological evaluation of lipid digestion products, *in vitro* lipid digestion models, *in vivo* studies and *in vitro*-*in vivo* correlation.

LS can be administered by different routes such as orally, subcutaneously, intramuscularly, or topically or they can be used in cell encapsulation, thus allowing them to be proposed for treatment of a number of diseases.²⁹⁻³¹ For instance, the *in vivo* distribution of LS demonstrated a high affinity to vascular wells (including capillaries), inflamed tissues, and granulocytes.^{32,33}

Table 1: List of Drugs Incorporated in to Lipospheres by various Techniques

Drug	Lipid composition	Method of Preparation	References
Somatostatin	Glyceryl tripalmitate	Melt dispersion	Reithmeier et al., 2001a
Cyclosporin	Triglyceride	Multiple emulsion	Bekerman et al., 2004
Glipizide	paraffin wax and stearic acid	w/o/w emulsion	H. N. Shivakumar et al 2007
Butylmethoxydibenzoylmethane	Tristearin	Modified hydrophobic congealing method	
Carbamazepine	Precifac	Melt dispersion	V. Iannuccelli et al., 2008
Dexamethasone-21-palmitate	Soybean oil and lecithin	Melt dispersion	N. S. Barakat , 2006
Triptorelin leuprolide	L-PLA, PLGA	Cosolvent-solvent evaporation	K. Yokovama, et al., 1996
Nimodipine	Monostearin, caprylic/capric triglycerides	Solvent evaporation	Raisel et al., 2002
Serratiopeptidase	Paraffin wax & cetyl alcohol	water-in-oil-in-water double emulsion (w/o/w) method	F.-Q. Hu et al.,2008
Vinorelbine	-----	High pressure homogenization	Manju Rawat Singh et al.,2009
			Hong Yao Zhang, Xing Tang et al.,2008

Advantages

Lipospheres have versatile advantages over other delivery systems, such as:

- 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.
- 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

Disadvantages

- Different lipid modifications and colloidal species coexist that may cause differences in solubility and melting point of active and auxiliary species.
- Low drug loading capacity for hydrophilic compounds.
- Variable kinetics of distribution processes.
- High-pressure induced drug degradation
- Insufficient stability data.

Comparison of Lipospheres and Liposomes

To transport a large amount of drugs, suitable carriers are required. Liposomes are better drug carrier vehicles for drug delivery systems³⁴⁻³⁶ but these are relatively unstable and are not easily scale-up.

Lipid microspheres are widely used in clinical medicine for parenteral nutrition and these are very stable and can be stored for up to two years at room temperature. They have no particular adverse effects, even at high dose levels (500mg/ml) and accumulate in inflamed tissues and other lesions^{37, 38} due to these high lipophilic properties.

Table 2: Comparison of Lipospheres and Liposomes

	Lipospheres	Liposomes
Components	Soybean oil, Lipid. Water	Phospholipid, Water
Lipid membrane	Monolayer single membrane	Mono- or multi-layer double membrane
Emulsion form	O/W	W/O/W
Incorporable drugs	Compounds soluble in soybean oil and retainable in lipids.	Water soluble compounds and compounds retainable in lipids.
Particle diameter	200 – 300 nm	Various sizes
Safety in vivo	Safe Clinically used as intravenous nutrition in 100 ml doses	Liposomes made of some lipids are toxic.
Stability	Stable for 24 months at room temperature. Lipo preparation are stored at 4°C	Rather unstable
Large-scale production	Suitable for mass production but a high pressure apparatus is necessary.	A special apparatus is not necessary at the research level. An apparatus for mass production has recently been developed.

Formulation of Lipospheres

The formulation of lipospheres approach utilizes naturally occurring biodegradable lipid constituents. The internal hydrophobic core of lipospheres is composed of lipids, especially triglycerides, while the surface activity of liposphere is provided by the surrounding phospholipid layer. The neutral fats, stabilizers are additionally used in the preparation of the hydrophobic core of the lipospheres. The lipids and stabilizers used in liposphere are shown in Table 3.

Some biodegradable polymers also used in the preparation of polymeric lipospheres to enhance the stability of lipospheres, which includes:

- Low molecular weight poly(lactic acid)
- Poly (caprolactone).

The phospholipids are used to form the surrounding layer of lipospheres includes:

- Pure egg phosphatidylcholine (PCE)
- Soybean phosphatidylcholine (PCS)
- Dimyristoyl phosphatidylglycerol (DMPG) and
- Phosphatidylethanolamine (PE).
- Food grade lecithin (96% acetone insoluble)
- Lipospheres for topical and veterinary applications.

Table 3: Various lipids and stabilizers used in the formulation of lipospheres

Lipids	Stabilizers
Glyceryl monostearate	Gelatin 200 Bloom
Glyceryl monooleate	Pectin
Ethyl stearate	Carrageenan κ
Trilaurin	Carrageenan ι
Tristearin	Carrageenan λ
Tribehenin	Polyvinyl alcohol
Tripalmitin	Polyoxyethylene sorbitan trioleate
Trimiristine	Pluronic PE 8100
Cetyl alcohol	Lauryl sarcosine
Cholesterol	
stearic acid	
Hydrogenated vegetable oil	

Lipospheres are prepared by various techniques includes:-

1. Melt dispersion technique³⁹

The lipidic 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 emulsified into 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 LS. The obtained LS is then washed with water and isolated by filtration through a paper filter.

2. Solvent evaporation technique³⁹

This technique is an alternative to the melt dispersion technique and it is considered with the objective of possibly minimizing the exposure to high temperatures of thermolabile compounds, such as proteins and nucleic acids. This technique is based on the evaporation of organic solvent in which lipids are dissolved and allowing the formation of solid microparticles. In particular, the lipidic matrix is dissolved in an organic solvent such as ethyl acetate and maintaining the temperature about 50°C and then emulsified with an external aqueous phase containing the surfactant agent. The resulting oil-in-water emulsion is stirred for 6 to 8 h till complete evaporation of the solvent. The LS are recovered by filtration through a filter paper.

3. Co-solvent solvent evaporation method³⁹

In this co-solvent - solvent evaporation method employing chloroform and N-methyl pyrrolidone to create a clear solution, although low yield and large particle size is obtained, which is altered by variation in the solvent used. Lipospheres made up of polar and non-polar lipids using synthetic stabilizers instead of phospholipids which are the deviation from the definition of liposphere reported by Domb in his patent (Cortesi et al., 2003). 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. Multiple microemulsion⁴⁰

This method in which a solution of peptide is dispensed in stearic acid melt at 70°C followed by dispersion of this primary emulsion into aqueous solution of egg lecithin, butyric acid and taurodeoxycholate sodium salt at 70°C (Morel et al., 1994). Rapid cooling of multiple emulsion formed 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 (Morel et al., 1996). Polymeric lipospheres have also been reported by double emulsification for encapsulation of antigen (Amselem et al., 1996).

5. Sonication method⁴⁰

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 of the 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.

6. Rotoevaporation method.⁴⁰

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 is continued for another 30min until lipospheres are formed.

7. Microfluidizer method⁴⁰

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.

8. Solvent extraction method³⁹

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 microchannel 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.

9. Polymeric lipospheres⁴⁰

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.

Sterilization of lipospheres⁴⁰

Sterile liposphere formulations are prepared by sterile filtration of the dispersion in the hot stage during preparation using a 0.2- μ m filter, at a temperature that is 5°C above the melting point of the liposphere core composition. Heat sterilization using a standard

autoclave cycle decomposed the formulation. δ -Sterilization of liposphere formulations did not affect their physical properties.

Storage of lipospheres⁴¹

The liposphere formulations are stored in aqueous buffer, freeze dried, or in an ointment or cream base, in the freezer, refrigerator or room temperature. It is preferred to store the formulations suspended in an aqueous solution in the refrigerator for immediate use.

Preparation of Nanosize lipospheres¹⁸

Nanosize lipospheres is prepared by homogenization through serial filters of reduced pore size. An alternative method for in situ preparation of nanosize lipospheres, which have particle size below 100nm, is recently developed using dispersible concentrate oil system (18). In this system, the drug, triglyceride, phospholipid, and other additives are dissolved in a mixture of common surfactants such as Tween and Span and an organic solvent that is miscible with all components. Such organic solvents include ethanol, propanol, propylene glycol, low molecular weight-polyethylene glycol (PEG), N-methyl pyrrolidone (NMP), ethoxylated castor oil (Cremophor), and propylene-ethylene glycol copolymers (Poloxamer), and PEG conjugated a-tocopherol. 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, phosphatidylethanol amine, stearic acid, or phosphadilic acid, to the solution. This concept has been applied for improving the bioavailability of cyclosporin.

CONCLUSION

Lipid carriers have bright future due their inherent property to enhance the bioavailability of lipophilic drugs with poor water solubility. However, the limitations of these carriers like poor physiochemical properties of lipids, lack of drug solubility database in lipids and unavailability of standard methodologies for in vitro analysis, need to be addressed. Lipospheres are solid, water insoluble nano- and microparticles composed of a solid hydrophobic core having 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 anesthetics, antibiotics, vaccines, and anticancer agents 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. But on the basis of literature review only a few researches have been reported pertaining to protein, analgesics, pilocarpine, carbamazepine, bupivacaine, diazepam, local anesthetics etc.

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