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# Liposphere: A Versatile Drug Delivery System - An Over View.

**Ganesan V*, and Allimalarkodi S.**

Department of Pharmaceutics, The Erode College of Pharmacy, Perundurai Main Road, Vallipurathanpalayam (p.o), Veppampalayam, Erode – 638112, Tamilnadu, India.

**ABSTRACT**

Drug carrier systems are being developed with the aim of changing the distribution of an active substance with in the body and thus increasing the pharmacological efficacy and are reducing its toxicity. In this respect colloidal particles (25nm to 1µm in diameter) have shown some very promising results. Researchers are looking at the application of lipids in drug delivery from the different facet; they focused on using lipids as novel carriers for drug moieties. Both hydrophobic and lipophilic therapeutics can be delivered successfully into deep and peripheral tissues by encapsulating them with crystalline lipids as liposphere. Lipospheres are meant to deliver both therapeutic moieties with enhanced efficacy and added stability to reach out intended tissue areas. In this articles we are compiling the key points for typical formulation of liposphere and discussed about the methods of preparation like melt methods, solvent evaporation and multiple emulsion to scale up the liposphere, initial screening factors, to find out a dimensions , storage issues , enhancing solubility and permeability characteristics of class II and IV drug candidate as liposphere.

**Keywords:** Colloidal carriers, Liposphere, formulation methods, morphology, entrapment efficiency, storage.

***Corresponding author***

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## INTRODUCTION

The particulate drug carriers that have been investigated for sustaining the therapeutic activity are based on the synthetic polymers or natural macromolecules. The drawback of this particulate system like, liposomes, microparticles, micro emulsions and nanoparticles are being the degradation of the polymer [1]. Organic solvents residue present in the delivery system that could result in the severe acceptability and toxicity problem [2]. In recent years much of research is now focused on using lipid as novel carriers for drug molecules which is the promising technique to increase the bioavailability of poorly soluble drug their by increasing their solubility in biological media of choice or increasing their dissolution kinetics. Liposphere represent a new type of fat based encapsulation system developed for oral, parenteral and topical delivery of bioactive compounds and have been utilized in the delivery of anti inflammatory compounds [3] , local anaesthetics [4], antibiotics [5] anticancer agents [6] insect repellants , vaccines [7] , proteins and peptides. Liposphere are composed of solid lipid core surrounded by a single unit phosphor lipid layer that may entrap the drug or enrich its coat with the drug. The emulsifier or stabilizer is used to form uniform coat around the core material and to facilitate partition of the drug between the lipid and aqueous phases [8], [9]. In liposphere the combination of solid inner core with phospholipid exterior confers several advantages on the liposphere as compared with conventional microsphere 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 [10]. Lipids may present an interesting alternative to polymeric materials due to their ability to form a variety of morphological states (e.g. emulsions, solid lipid nanoparticles, microparticles) and their biocompatibility. In our study, we investigated the potential physiological lipids such as triglycerides as a matrix material for microparticles suitable for subcutaneous or intramuscular injection.

Beneficial advantages of liposphere drug delivery system are;

1) Improving drug stability;
2) Possibility for controlled drug release;
3) Controlled particle size;
4) High drug loading efficiency.
5) Protect the drug from hydrolysis during oral administration hence improve bioavailability.

Due to several limitations with polymeric delivery systems, extensive attempts are being made to develop alternate carriers. Lipids especially, are now being studied widely due to their attractive properties namely physicochemical diversity, biocompatibility, biodegradability, ability to increase then oral bioavailability of poorly water soluble drug moieties, thus making them ideal candidates as carriers for problematic drugs[11]. The present review is aimed to focus on various fundamental and applied aspects of liposphere in the field of drug delivery system especially techniques involved in their production, characterization and different application.

### Techniques widely employed in preparation of Lipospheres

### Melt Dispersion technique

In this technique, Liposphere were prepared by using a lipid mixture, constituted of cetyl alcohol and cholesterol (2: 1 w/w) and gelatin as the stabilizer. Gelatin was selected from eight natural or synthetic emulsifiers, namely gelatin (200Bloom), pectin, carrageenan k, carrageenan l, carrageenan λ, poly vinyl alcohol, poly oxy ethylene 20 sorbitan trioleate (poly sorbate 85) and lauryl sarcosine. The lipidic mixture, both with or without a lipophilic model drug, was selected at 70˚C and then emulsified into an external aqueous phase containing a suitable surfactant. The emulsion was mechanically stirred by a stirrer equipped with alternative impellers. Then the emulsion was heated to the same temperature as the melted lipidic phase. The milky formation was then rapidly cooled to about 20˚C by immersing the formulation flask in a cool water bath without stopping the agitation to yield a uniform dispersion of lipospheres. The obtained lipospheres were then washed with water and isolated by filtration through a filter paper.

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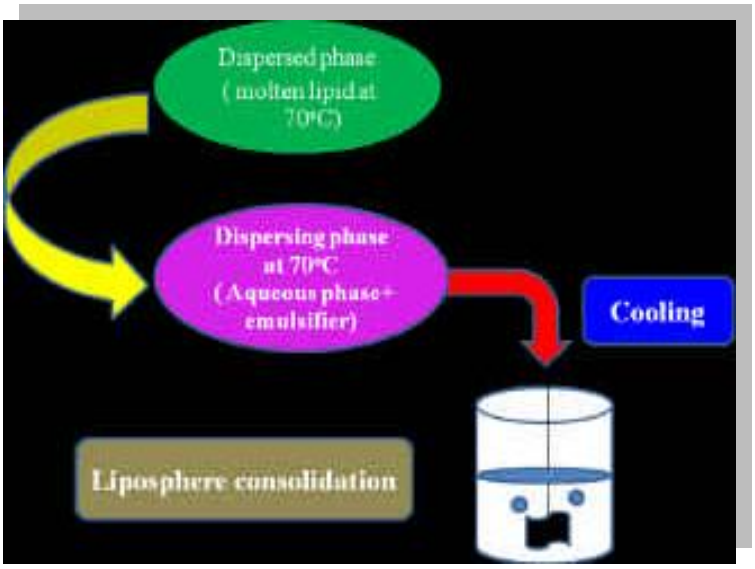


**Figure 1: Preparation of lipospheres by melt dispersion method**

**Solvent evaporations technique**

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 thermo labile compounds. This technique is based on the evaporation of organic solvent in which lipids are dissolved and allowing the formation of solid nanoparticles. In particular, the lipidic matrix is dissolved in an organic solvent such as ethyl acetate, ethanol, acetone or dichloro ethane and maintaining the temperature about 50 to 60°C and then emulsified with an external aqueous phase containing the surfactant agent. The resulting oil in water emulsion is stirred between 6 to 8 hr until evaporation of the solvent. The liposphere are recovered by filtration through a filter paper, dried and stored. [12, 13].

**Co-Solvent method**

Use of co-solvent in liposphere development facilitates the enhanced solubility of lipids and lipophilic drugs and to obtain clear homogeneous solutions. This was evident when chloroform was used as co-solvent to prepare peptide loaded lipospheres and to solubilize the polyacetic acid, hydrogenated soyabean, phosphotidyl choline and thereby clear solution of N-methyl pyrolidone containing peptide. Selection of co-solvent was the result of miscibility which affects the output [14].

**Multiple micro emulsion method**

The uniform size about 300 nm with 90% entrapment efficiency was reported with multiple micro emulsion method. In this method, the hydrophilic drugs were dissolved in aqueous phase, and this solution was added to the lipid phase to yield primary emulsion at high temperatures. Then, the solution was added to the oil phase containing hydrophobic emulsifier to yield uniform size lipospheres [15].

**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 is 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 10 min with intermittent cooling until it reaches to the room temperature.

**Ultrasonication or High Speed Homogenization**

This technique is a dispersing technique, which was initially used for the production of solid lipid micro or nano particle dispersion. Ultrasonication based on the mechanism of cavitation. Stepwise procedure

is followed for ultrasonication method. The drug was added to previously melt solid lipid then the heated aqueous phase was added to the melted lipid and emulsified by probe sonication or by using high speed stirrer or aqueous phase added to lipid phase drop by drop followed by magnetic stirring. The obtained pre emulsion was ultrasonicated using probe. sonicator with water bath (0˚C). Production temperature was kept at least 5˚C above the lipid melting point in order to prevent recrystallization during the process. The obtained nanoemulsion (O/W) is then filtered through a 0.45µmmembrane in order to remove impurities carried in during ultrasonication. The obtained solid lipid nanoparticles are stored at 4˚C. To increase the stability of the formulation it is necessary to lyophilize with the help of lyophilizer to obtain freeze –dried powder and sometime mannitol (5%) was added into solid lipid nanoparticles as cryoprotector[16].

**Rotoevaporation method**

In this technique, lipid solution with drug is prepared in a round bottom flask containing 100gms of glass beads (3mm 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.0.9% of saline was added to the vessel and the contents are mixed for 30min at room temperature. Then the temperature is lowered to 10˚C by placing in ice bath and mixing is continued for another 30min until liposphere are formed.

**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 any stage of the lipospheres processing to manipulate the particle size and distribution[17] .

**Solvent extraction method**

The solvent extraction method is based on the dissolution of the triglyceride and the cationic lipid in the organic solvent and on the addition of an aqueous polyvinyl alcohol 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 [18].

**Spray drying method**

Spray drying provides smaller size particles with homogenous distribution compared to other methods. The shape of particles was affected by drying rate, viscosity and surface tension of the drying liquid. The key parameters involved in this method were inlet and outlet temperature, feeding rate, drying gas medium, gas flow rate, gas humidity and residence time. The rate of particle formation was controlled by these key parameters. As complete removal of solvent was observed, the chance of toxicity was also minimized. This technique was highly applicable for food industry in producing peptide loaded lipospheres [19] .

**Spray congealing method**

Spray congealing was successfully employed for preparing lipid microparticles loaded with therapeutics such as clarithromycin, theophylline, verapamil and indomethacin. The molten lipid containing dispersed drugs at 70˚C was made to flow into the spray congealer specifically into cyclone which was maintained at -20 ˚C, which lead to separation of solid particle that were again made to atomize to remove adhered condensed water. The atomization pressure and spraying temperature affect the particle size distribution and also product yield [20,21,22,23].

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**Factors Influencing Quality Attributes Of Lipospheres**

**Factors influencing morphology of lipospheres**:

**Amount of lipid:**

With an increase in amount of lipid, there was a decrease in particle size of lipospheres but the dispersion formed was viscous with larger droplets and larger lipospheres.

**Stirring speed:**

A non-linear relationship was observed between stirring speed [14] Rasiel et al 2002] and particle size after a certain speed range, after which particle size decreased only gradually and slowly.

**Type of surfactant:**

Using cationic lipospheres in the formulation will improve the drug loading and physical stability due to the charge component. In view of this influence of different cationic lipids on morphology was studied. $DDAB_1$[24] (didodecyl dimethyl ammonium bromide) gave irregular particles; $DDAC_{18}$ (dimethyl-dioctadecyl-ammonium chloride) in formulations not only improved the yield and morphology of lipospheres but also produced larger proportion of smaller particles. Best spherical shape was obtained with $C_{18}VBr_2$ **(**1,1 diocatadecyl-4,4 bipyridinium bromide).

**Effect of stabilizer:**

Natural stabilizers [25] like gelatin, pectin, carragenans λ, t resulted in particles of 150 and 250 µm. Carragenan λ produced dispersions of viscosity compatible with filtration process. Synthetic stabilizer like 1% polyoxyethylene, polyoxypropylene block copolymer (Pluronic PE) 8 and 100 did not stabilize O/W emulsion resulting in large aggregates. 1% polyoxyethylene sorbitan trioleate or polyvinyl alcohol resulted in particles of 190µm and 120 µm and recoveries of 54%, 78%. Lauryl sarcosine resulted in the formation of very fine O/W emulsion of 10±4.1 µm size.

**Factors influencing entrapment efficiency:**

**Amount of phospholipid:**

As the phospholipid (coat) amount increases, formation of alternative systems like liposomes was observed which will compromise drug entrapment. Experiments with triglyceride: phospholipid at a 1:0.5 to 1: 0.25 w/w [26] revealed that 70-90% of phospholipid polar heads were accessible on liposphere surface thus enhancing the loadability of drug.

**Effect of drug: lipid:**

As the amount of drug increases [27], entrapment in lipid decreases due to limited room available in the lipid structure. Loadability of drug also depends on the solubility of drug in lipid and nature of drug. Naturally, entrapment is high for lipophilic drugs like retinyl acetate and progesterone and low for hydrophilic drugs like sodium cromoglycate.

**Viscosity of dispersion:**

Increase in viscosity of dispersion by adding hydrophilic polymer to dispersion medium reduces the crystallization tendency (which is discussed in detail in following sections) and increases entrapment efficiency of drug into lipid particles.

**Factors influencing drug release:**

Muhlen et.al, studied the release mechanism of drugs namely tetracaine, etomidate and prednisolone [28] entrapped in lipid particles. Dynasan 112 (glycerol trilaurate), Compritol 888 ATO (glycerol behenate) were used as lipid carriers and Pluronic F 68 (Poloxamer 188), Lipoid S 75 (soy lecithin), Lipoid KG were used as emulsifiers. Tetracaine and etomidate lipospheres have shown burst release and prednisolone lipospheres gave prolonged release.

**Effect of drug loading mechanism:**

The drug release pattern [29] depends upon the way the drug gets incorporated in lipospheres. Drug solution can either distribute uniformly throughout the lipid matrix, in which case due to increased viscosity of lipid matrix, prolonged release as evidenced in prednisolone lipospheres can result (OR) during solidification stage of liposphere preparation, lipids might solidify first forming large crystals of pure lipid cores around which drug enriched outer shell forms resulting in burst release.

**Effect of particle size:**

Smaller particles have larger surface area exposed to dissolution medium and higher diffusion coefficient. If the drug resides in the outer shell diffusion distance becomes shorter resulting in fast (burst) release.

**Nature of drug:**

Hydrophobic drugs like retinyl acetate and progesterone from lipospheres were released at a slower rate, 27% of total drug within 8hrs and 63% of total drug within 8hrs whereas release from hydrophilic drug sodium cromoglycate containing lipospheres gave 100% release within 5hrs. Presumably, drug-lipid interactions might control the release of drug.

**Effect of stabilizer:**

Lipospheres formulated with gelatin as stabilizer released 80% of total drug in 8hrs resulting in sigmoid mode of release whereas formulations with Poloxamer 407 [25] resulted in a biphasic pattern (burst release followed by slow release).

**Factors influencing stability of lipospheres:**

Major problem [29] with lipid particulate systems is that their tendency to undergo transition from disordered to highly ordered state during cooling phase of liposphere preparation. As a result the drug entrapped in disordered chains gets expelled during conversion of lipid to ordered state.

**Effect of Emulsifier and type [30]:**

Presence of emulsifier delays recrystallization tendency and thus prevents recrystallization at room temperature. Natural emulsifiers like egg phosphotidylcholine and soybean phosphotidylcholine resulted in shorter shelf life of lipospheres due to leaky nature of such membranes compared with synthetic emulsifiers.

**Effect of drug amount:**

Presence of drug prevents the formation of unstable crystalline forms of lipid and promotes conversion to stable form. For example incorporation of tetracaine at 5% had no influence on melting point and melting enthalpy compared to drug free lipid particles but a 10% incorporation of tetracaine reduced the crystallinity of compritol lipid particles by 40%.

**Evaluation of Lipospheres:**

**Measurement of Particle size and Zetapotential**

Photon correlation spectroscopy (PCS) and laser diffraction (LD) are the most powerful techniques for routine measurements of particle size. The morphology of lipospheres was characterized by transmission electron microscopy where interaction of the electrons with lipid surface produces the images and SEM where electronic transitions with particle surface produce the images. Field Emission SEM (FESEM) can be effective in case of particles that were not recognized by SEM where sample preparation may damage the particle morphology. Cryogenic FESEM, where liquid nitrogen was used to freeze the liquid dispersion, produced microscopic images in the frozen state. The above methods were of two dimensional analyses of the particles and three-dimensional profiles which include structural, mechanical, functional and topographical information of lipospheres were given by atomic field microscopy [31]. Zeta potential measurements allow predictions about the storage stability of colloidal dispersion. It is clearly the surface properties of colloidal systems are critical in determining their drug carrier potential, since they will control their interactions with plasma proteins. Zeta potential measurements will give information about overall surface affected by changes in this environment (e.g. pH, presence of counter- ions, adsorption of proteins). Charge shielding by PEG or other hydrophilic groups can be used to predict the effectiveness of the barrier function against opsonisation *in vivo*. Zeta potential can also be used to determine the type of interaction between the active substance and the carrier: i.e. whether the drug is encapsulated within the body of the particle or simple adsorbed on the surface. This is important because adsorbed drug may not be protected from enzymatic degradation, or may be released very rapidly after administration [32].

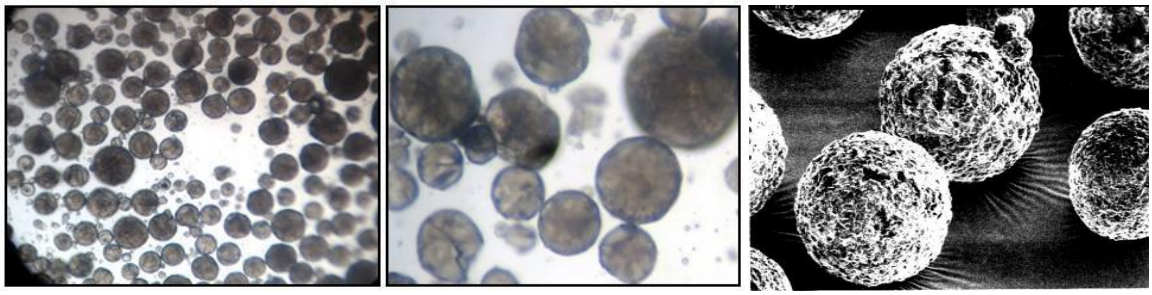

**Figure 2: Scanning Electron Microscopy Photograph Showing the Morphology of Lipospheres**

**Nuclear magnetic resonance (NMR):**

Nuclear magnetic resonance spectroscopy being widely used for analyzing the nature of the lipids and the lamillarity of the lipid particles formed, using paramagneticions like manganese (Mn2+) and preseodimium (Pr3+) that form complexes with the lipids based on the polar groups available. Liposphere formulation containing phosphotidyl ethanolamine were identified using the trinitrobenzene sulfonic acid labeling with evidence of 70-90% of the phospholipid polar heads in the particle surfaces.[33,34].

**X-ray diffraction (powder X-ray diffraction) and differential scanning calorimetry (DSC):** The geometric scattering of radiation from crystal planes within a solid allow the presence or absence of the former to be determined thus permitting the degree of crystallinity to be assessed. Another method that is a little different from its implementation with bulk materials, DSC can be used to determine the nature and speciation of crystallinity within nanoparticles through the measurement of glass and melting point temperatures and their associated enthalpies [35].

**Acoustic methods [36]:**

Acoustic spectroscopy measures the attenuation of sound waves as a means of determining size through the fitting of physically relevant equations. In addition, the oscillating electric field generated by the movement of charged particles under the influence of acoustic energy can be detected to provide information on surface charge.

**Entrapment Efficiency:**

The entrapment efficiency is defined as the drug entrapped in the lipid based particles, relative to the total amount of drug added, that is percent of drug included in the particles versus percent of drug remaining in the dispersion medium, which can be calculated from Equation 1. The EE increases with drug concentration. The EE depends on the polymer concentration as well. This was evident with that of EE of gentamycin, which was depended on PEG and EE and subsequent microencapsulation were increased gradually with PEG concentration [37]. The EE was also affected by the lipid composition/ratio used in formulating the lipospheres. The reason behind it may be due to the presence of small amounts of fat in the inner core of the lipospheres which lead to saturation of the fat core of the lipospheres by the drug incorporated in dispersion [38]. The EE also depends upon the drug solubility in the solvent system used for processing. Various co-solvents such as ethanol, dimethyl sulfoxide and dimethyl formamide been often used in the formulation of lipospheres since they aid in a higher drug entrapment[10]. Ultrafiltration and microdialysis were considered as the most reliable techniques for EE quantification, while result obtained by ultracentrifugation, the fastest and easiest technique, but not always accurate [39]. Loading capacity (LC)was the percentage of drug incorporated into the lipid particles, relative to the total weight of the lipid phase (drug + lipid)and it would be computed from the Equation 2. LC being an important parameter for characterization and optimization of lipid-based drug carrier , depends mainly on the solubility of the drug under investigation in the core lipid/lipids blend, miscibility of drug melt and lipid melt, chemical and physical stature of the SLM and the polymorphic state of the lipid. The reported LC values range between 1% for prednisolone, 20-25%for cyclosporine A (CsA) and up to 50% for extremely lipophilic compound Vitamin E [40].

$$\text{Entrapment efficiency (EE \%)} = \frac{\text{Mass of drug in lipospheres}}{\text{Initial weight of drug}} \text{X100 ---------- (1)}$$

$$\text{Drug Loading (\% w/w)} = \frac{\text{Amount of drug in lipospheres}}{\text{Mass of recovered lipospheres}} \text{X 100 ---------- (2)}$$

**Release Kinetics:**

Development of reliable in vitro dissolution testing methodology is important as it aids in selection of excipients, assesses the performance of formulation during different phases of drug development, for regulatory approvals, claiming biowaivers and substitute clinical studies (if the *in vitro* dissolution test can act as surrogate for in *vivo* studies). For newer formulations like lipospheres for which there are no established methods, a reliable and reproducible dissolution testing method becomes even more important. Methods like “dispersion test”, “digestion test” have been designed for laboratory testing in order to establish *invitro* - *invivo* correlation (IVIVC). pH stat test [60] or titra-stat test is another development in this direction. In this, a known or measured volume of sodium hydroxide solution is titrated against formulation containing lipid to which pancreatic lipase and calcium ions (to activate lipase) are added to mimic in *vivo* conditions. Neutralization reaction takes place between sodium hydroxide and fatty acids. As hydrogen ions are consumed or liberated during the course of reaction, amount of reagent added to maintain deviating pH to the set pH value is measured. Most of the works reported the use of USP II (Paddle) dissolution apparatus or large pore dialysis tubing to determine release from liposphere formulations[41].

**Suitable Formulations of Lipospheres**

Lipospheres being possible alternative to avoid the side effects resulting from the oral administration. The aceclofenac was formulated into lipospheres successfully to sustain the release topically [42]. The antigen or immunogen, alone or in combination with a phospholipid carrier were able to form lipospheres with aid of melt method and also with solvent preparation. One of the most promising approaches for the delivery of poor water-soluble drugs is the use of layer-by-layer assembly technology for the encapsulation of the lipid based drugs. This technique permits the step-wise adsorption of the various components as the layer growth is governed by their electrostatic attraction and allows the formation of multi-layer shells with nanometer-scale precision. The application of layer-by-layer assembly for emulsions, nanoparticles and capsule based delivery

systems for lipid based drugs were extensively developed [43]. The lipid microparticles as a parenteral controlled release device for peptides were also established [44].

**Suitable Storage Condition**

As the storage conditions are important for lipid dispersion, the formulated lipospheres can be stored at 4°C in order to prevent the degradation of the coat and core material and thereby maintaining the structural integrity. Lipospheres are very stable after 3 months storage at 2-8°C manifested by low leakage rate (<7%) and no major changes in particle size.[42] Oxytetracycline injectable lipospheres meant for veterinary use were analyzed for the injectability when stored at 4°C showed stability irrespective of the lipid used in liposphere formulations [44]. If proper storage conditions were not maintained the problems of stability could be aroused leading to failure and may cause toxicity due to degradation of lipids.

**Stability Studies**

Many studies have conducted on liposphere stability at various stress conditions. Among them, the photolysis based stability testing was proven to be a benchmark. The photolysis based stability studies were explained with an example of butyl methoxy dibenzoyl methane (BMDBM), a sunscreen agent complexed with hydroxyl propyl-β-cyclodextrin (HP-β-CD). The lipospheres of BMDBM were developed with tristearin and hydrogenated soybean phosphotidylcholine. The resulting cream was undertaken for photo degradation study about 3 months in which permeability of lipospheres and also the release of drug were evaluated. It was learnt that the lipospheres are able to provide further superior protection to the drug in formulation, apart from the protection provided by HP-β-CD inclusion complexation [45].

## CONCLUSION

Lipospheres as crystal lipid particles composed of lipids which are typically selected and basis of stability and physiochemical parameters. Lipid carriers have bright future due to their inherent property to enhance the bioavailability of lipophilic drugs with poor solubility. Lipospheres are solid water insoluble nano and microparticles composed of a solid hydrophobic core have a layer of phospholipids embedded on the surface of the core. Though the phospholipids are prone to degradation the formulation can be stable and firm with lipid and stabilizers. The sensitive and potent drug can be formulated as liposphere to enhance stability at low doses. Liposphere formulations are well suitable to administer by the most common routes. Liposphere formulation are effective in delivering various drug and biological agents including local anesthetics, antibiotics, vaccines and anticancer agents with prolonged activity upto four to five days. Melt dispersion, solvent evaporation and micro emulsion methods enabled us to produce liposphere whose morphology and size where influenced by the experimental parameters employed. Lipid based microspheres appear to be ideal candidates for administering antibacterial agents, because they are bio degradable , they do not have to be removed after the treatment period and they possess mucoadhesive properties. The lipospheres are expedient for commercial scale production of life saving drugs and cosmetics

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