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LIPOSPHERES: RECENT ADVANCES IN VARIOUS DRUG DELIVERY SYSTEM

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Abstract

Use of lipids as commonly used excipients like emulsifiers; base for semisolid preparations such as ointments, creams; as flow modifiers for granule preparations in tablet making are known since ages in formulation technology. But researchers are looking at the application of lipids in drug delivery from a different facet. Much of research is now focused on using lipids as novel carriers for drug moieties. Lipid based drug delivery systems like solid lipid nanoparticles, lipospheres (LS) are being developed as substitutes for "polymer based delivery systems" due to the increasing toxicity related concerns of monomers after intracellular processing of polymers and attractive benefits offered by lipids as carriers. This article reviews lipospheres in particular as delivery system. Formulation of lipospheres, factors influencing the quality attributes of lipospheres, mechanisms behind drug loading, evaluation of lipospheres and challenges in the development of lipospheres are discussed in detail.LS composed of triglycerides and monoglycerides are produced by melt dispersion technique, solvent evaporation or multiple emulsion method. The various bio active compounds are incorporated in to LS that can be administered in to various routes.

Key Words: lipospheres, lipids, solid lipid nanoparticles, polymers, delivery system.

1. Introduction

Liposphere formulation is an aqueous micro dispersion of solid water insoluble spherical micro particles of particle size between 0.01 and 100 μm in diameter. The lipospheres are made of solid hydrophobic triglycerides with a monolayer of phospholipids embedded on the surface of the particle. Liposphere formulation is appropriate for oral, parenteral and topical drug delivery system. The solid core containing a drug dissolved or dispersed in a solid fat matrix and used as

carrier for hydrophobic drugs. Several techniques, such as solvent emulsification evaporation, hot and cold homogenization and high pressure homogenization have been used for the production of lipospheres[1]. Benefits of liposphere drug delivery system are;

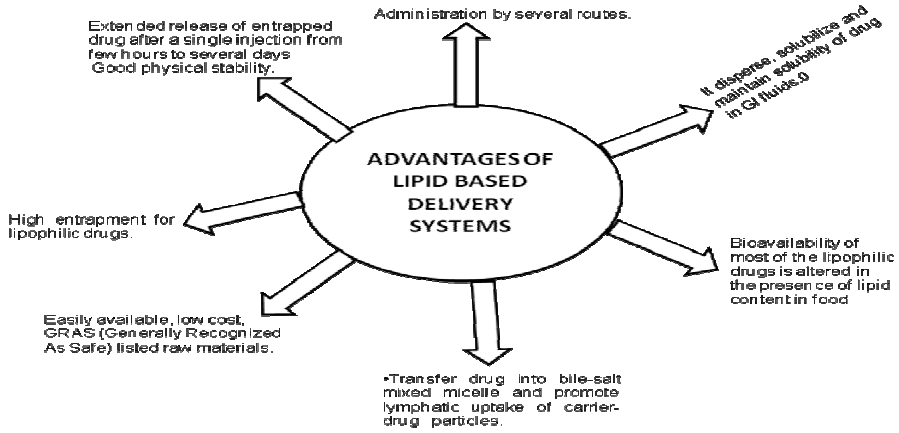
a) Improving drug stability; b) possibility for controlled drug release; c) controlled particle size; d) high drug loading. In addition, use of lipospheres for oral administration, it can protect the drug from hydrolysis, as well as improve drug bioavailability[2]. Therefore, the present review article is focused on achievements of lipospheres formulation to deliver the drugs in the targeted sites.

Due to several limitations with polymeric delivery systems, extensive attempts are being made to develop alternate carriers. Lipids [17] especially, are now being studied widely due to their attractive properties namely physicochemical diversity, biocompatibility, biodegradability, ability to increase the oral bioavailability of poorly water soluble drug moieties, thus making them ideal candidates as carriers for problematic drugs.

1.1 Advantages of lipid based delivery systems

- Physical stability of lipid dosage forms like polymorphic phase transitions of drug and Lipid based drug delivery systems like solid lipid nanoparticles (a technology owned by Skye Pharma) [18] and lipospheres are now being studied widely. Solid lipid nanoparticles [19-20] are nanosized lipid carriers in which lipidic core contain the drug in dissolved or dispersed state. These systems were designed to substitute polymeric carriers due to the inherent toxicity. Lipospheres are lipid based dispersion systems in which drug is dissolved or dispersed in lipidic core, the surface of which is embedded with emulsifier layer. Particle size of such lipid particles ranges from 0.2-100 micrometer (μm).

Figure-1: Advantages of lipid based delivery systems.



1.2 Application of lipospheres

1.2.1 Parenteral Route

Lipospheres have been exploited for the delivery of anesthetics like lidocaine [21] bupivacaine [22], for the parenteral delivery of antibiotics like ofloxacin, norfloxacin, chloramphenicol palmitate and oxytetracycline [23], and antifungal agents, such as nystatin and amphotericin B [24]; for the parenteral delivery of vaccines and adjuvant.

1.2.2 Transdermal route [25]

Properties of lipospheres like film forming ability, occlusive properties; controlled release from solid lipid matrix resulting in prolonged release of drug and retarded systemic absorption of drugs; increasing the stability of drugs which are susceptible to extensive hepatic metabolism, make them attractive candidates for topical delivery.

1.2.3 Oral delivery [26-27]

Several categories of drugs like antibiotics, anti-inflammatory compounds, vasodilators, anticancer agents, proteins and peptides are being formulated as oral lipospheres.

The formulation of Lipospheres are generally composed.

Table No.1: 3. Lipid core which is a combination of different lipids (fats, oils):

Triglycerides	Witepsol W35, Witepsol H35; Compritol 888 ATO (Glyceryl behenate); Dynasan 112; Precirol (Glyceryl palmito stearate); tricaprin, trilaurin, tripalmitin, tristearin, trimyristin.
Monounsaturated fatty acid	Cis forms of monounsaturated fatty acids have lower melting point than triglycerides hence used as a mixture with higher saturated fatty esters
Partially hydrogenate vegetable oils	Soybean oil, coconut oil, cotton seed oil.
Oils	olive oil, wheat germ oil, evenin primrose oil, arachis oil, safflower oil, corn oil, rice bran oil.

Waxes	Bees wax, spermaceti, cetyl palmitate, arachidyl oleate, carnauba wax, cetyl alcohol, cholesteryl butyrate

3.1 Active Pharmaceutical ingredient

3.2 Emulsifiers:

Phospholipids	pure-egg phosphatidyglycerol, phosphatidylethanolamine, dimyristoyl phosphatidylglycerol, soybean phosphatidylcholine
Surfactants	Tween-80, butyl alcohol

3.3 Stabilizers:

Gelatin, pectin, carrageenan, polyvinyl alcohol, polyoxyethylene sorbitan trioleate, Pluronic PE 8100, lauryl sarcosine.

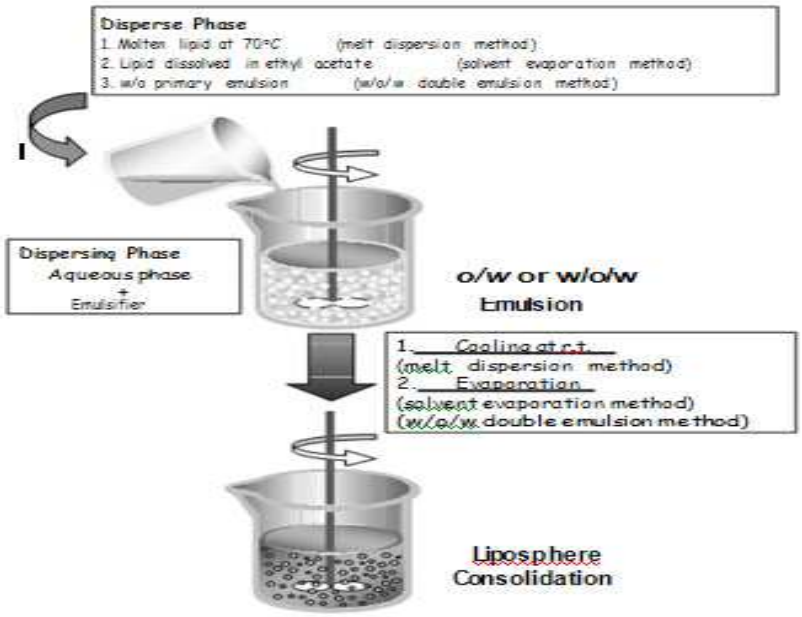
4. Methods of Preparation

4.1 Melt dispersion technique [28]

In this method, drug is dissolved or dispersed in the molten lipidic phase (figure2). Aqueous phase is composed of water or suitable buffer which is heated to the same temperature as lipid phase. The aqueous phase is kept under stirring during which emulsifier is added To the aqueous phase containing emulsifier, lipid phase containing drug is added drop by drop while maintaining the temperature and stirring speed. After this "hot emulsification phase", the temperature of the mixture is rapidly brought down to room temperature or below room temperature by adding ice cold water or ice under continuous stirring. This cold resolidification results in the formation of discrete lipospheres which can be filtered. Several drugs like bupivacaine, glipizide [29], aceclofenac [30], retinyl acetate, progesterone, sodium cromglycate, diclofenac [31], carbamazepine [32], C14-diazepam, proteins like somatostatin [33], thymocartin [34] , casein [35], bovine serum albumin [36], R32NS1 malaria antigen [37], tripalmitin based lipospheres [38] for labon-chip applications have been prepared by melt dispersion methods. Lipids carrying antigens exert their adjuvant effect to immunogenicity of antigens and the effect was found to decrease in the following order for the lipids studied:ethyl stearate>olive

oil>tristearin>tricaprin>corn oil>stearic acid. Also inclusion of negatively charged lipids like dimyristoyl phosphotidyglycerol in the lipid core was found to improve the antibody response to encapsulated malaria antigen.

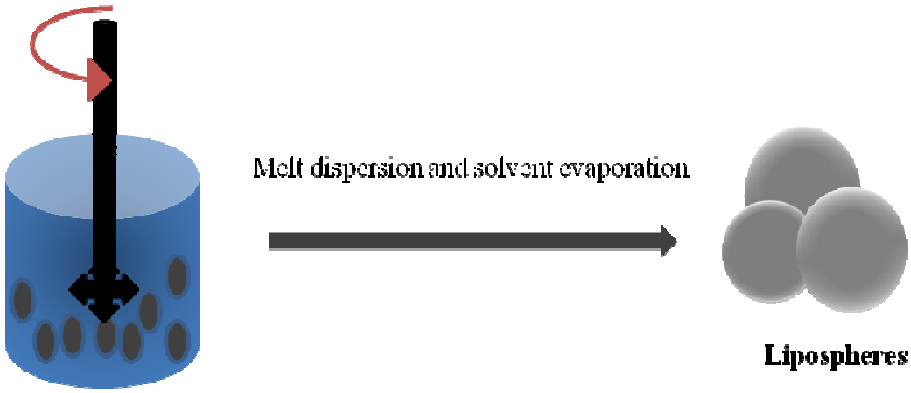
Figure-2: **Schematic representation of the methods of production of LS: melt dispersion and solvent evaporation.**



4.2 Solvent evaporation method [28]

In this method, lipid is dissolved in an organic solvent. Commonly used organic solvents include ethyl acetate, ethanol, acetone or dichloromethane. This lipid phase is emulsified into aqueous phase containing emulsifier. Organic solvent is evaporated by stirring the oil in water emulsion for 6-8 hrs under ambient conditions. Discrete lipospheres can be collected by filtration through paper filter after the water rises to the surface. Examples of the drugs formulated as lipospheres by this method include paclitaxel [39], thymocartin, bovine serum albumin, triptorelin leuprolide [40].

Figure-3: **Schematic representation of the methods of production of LS: melt dispersion and solvent evaporation.**



4.3 Microemulsion [41]

In this method, drug is added to the melted lipid. Aqueous phase is prepared by adding surfactant like Tween 80 into water maintained at same temperature as lipid phase. This is followed by the addition of co-surfactant like butyl alcohol to the aqueous phase. The aqueous phase containing surfactant and co-surfactant is added to lipid phase kept under stirring**.** Rapid cooling of the above mixture results on formation of discrete lipid particles. Flurbiprofen lipospheres[42] are prepared by this method. Presence of Tween80 at 2%, butyl alcohol at 2ml and water at 50ml found to give discrete lipospheres of superior quality.

5. **Recent Advances**

5.1 Production of Lipospheres for Bioactive Compound Delivery[43]

5.2 Lipospheres as Delivery Systems for Peptides and Proteins[44]

5.3 Lipospheres for Vaccine Delivery [45,]

5.4 Cationic Lipospheres as Delivery Systems for Nucleic Acid Molecules[46]

5.1 Production of Lipospheres for bioactive compound delivery

The solvent evaporation technique is often used for liposome and polyester. Microparticles can be present in the delivery system and could result in severe acceptability and toxicity problems.The drug delivery system concept is not new. Great progress has recently been made in the treatment of a variety of diseases. 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 system[47] or by accumulation at the injection site [48]. To solve these adverse effects, lipid microspheres, often called lipospheres (LS), have been proposed as a new type of fat-based encapsulation system for drug delivery of bioactive compounds.LS have been used for the controlled delivery of various types of drugs, including vasodilator and antiplatelet drugs, anti-inflammatory compounds, local anesthetics, antibiotics, and anticancer agents; they have also been used successfully as carriers of vaccines and adjuvant. Lopospheres (LS), under appropriate experimental conditions, can entrap both hydrophobic and hydrophilic drugs and can control the release of

the encapsulated drug. The encouraging results obtained in this study could propose LS for future *in vivo* studies, especially in the delivery of anti-infective and hormone.

5.2 Lipospheres as delivery systems for peptides and proteins

Delivery systems are designed to protect an incorporated drug from the environment during delivery and to provide a controlled release. The goal may be either to deliver a drug locally to specific sites in the body or to prepare a drug carrier system that acts as a reservoir at the site of injection over a certain time period[49] In recent years, a growing number of potential peptide and protein drugs has been discovered as a result of progress in biotechnology and genetic engineering. Unfortunately, protein drugs are subject to numerous chemical and physical instability mechanisms and rapid enzymatic degradation; therefore, they often show low bioavailability and have short *in vivo* half-lives, thus necessitating parenteral delivery[50]. To sustain therapeutic effects, these drugs have to be administered by infusion or via frequent injections.Therefore, alternative carrier substances have been investigated in recent years. Among them, lipidic materials have gained growing attention. Successful peptide or protein incorporation and delivery has been reported for liposomes[51]. Multivesicular liposome preparations [52], cubic phase gels [53], hollow lipid Microparticles [54], hollow lipid microcylinders[55], Microparticles[56-57], and solid lipid nanoparticles (SLN) for intravenous applications[58,59]

Table-2: **Examples of peptides and proteins incorporated into lipospheres [49].**

Peptide/Protein Drug	**Matrix Material**	**Preparation Method**
Antigen	Waxes, fatty alcohols, paraffins, hard fat	Melt method, solvent technique
[D-Trp-6]-LHRH	Stearic acid	w/o/w multiple micro emulsion
Thymopentin	Stearic acid	w/o/w multiple micro emulsion, o/w multiple micro emulsion
R32NS1Malaria antigen	Tristearin, Polylactide, Polycaprolactoe	Melt dispersion
Somatostatin	Triglycerides	Co solvent–solvent evaporation
Triptorelin, Leuprolide	L-PLA, PLGA 50:50, PLGA 75:25	Co solvent–solvent evaporation

Hydrophilic model drug	Triglycerides, PLA,Eudragit RS 100	Melt dispersion, solvent evaporation, w/o/w double emulsion

Note: LHRH = luteinizing hormone-releasing hormone; L-PLA = L-poly(lactic acid); PLGA = poly(lacticco-glycolic acid); PLA = poly(lactic acid); w/o/w = water/oil/water; o/w = oil/water.

5.3. Lipospheres for vaccine delivery

The tremendous advances of genetic engineering and the ability to obtain many synthetic recombinant protein antigens derived from parasites, viruses, and bacteria have revolutionized the development of new generation vaccines.

One approach to enhancing the bioavailability and effectiveness of peptide-based vaccines is the use of Microparticles as vaccine carriers. Several reports describing the improvement of immune response achieved by the association of antigens with lipid carriers such as liposomes [60-61] or microparticles like polymeric biodegradable microcapsules[62, 63] have been published. The ability of these delivery systems to enhance immunogenicity was related to the physicochemical characteristics of the particles. Lipospheres are fat-based encapsulation particulate systems developed for parenteral drug delivery [64-66] that also have been used successfully as carriers of vaccines and adjuvant [67]. Lipospheres have been used for topical applications, including with insect repellents and moisturizers with extended action. Lipospheres consist of water-dispersible solid microparticles composed of a solid hydrophobic fat core stabilized by one layer of phospholipid molecules embedded in their surface[68]. Manufacture of liposphere–vaccine formulations is accomplished by gently melting neutral fat in the presence of phospholipid and dispersing the mixture in an aqueous solution containing the antigen by vigorous shaking. Upon cooling of this mixture, a phospholipid-stabilized solid hydrophobic fat core containing the antigen forms spontaneously. Although the lipospheres seem to fit very well in vaccine formulations provided by injection or by oral intake. This chapter is an update of the, emphasis on the possible use of lipospheres for oral immunization. Particle size is a key factor, and it appears that particles of certain compositions in the size range of 50 to 3000 nm are capable of uptake and translocation[69-70]. Uptake increases with decreasing particle size. Surface hydrophobicity has a direct correlation with the immune response. Although the lipospheres seem to fit very well in vaccine formulations provided by injection or by oral intake.

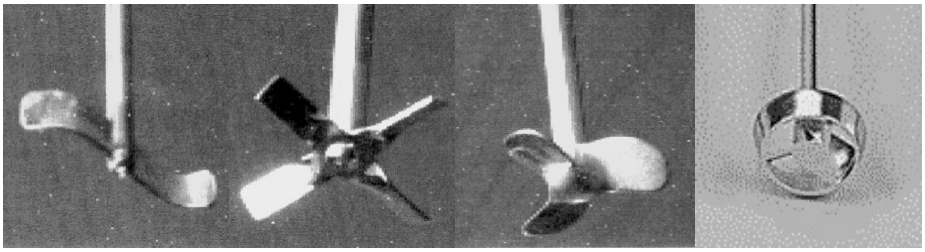
5.4 Cationic lipospheres (cls) as delivery systems for nucleic acid molecules

Gene delivery or the release of exogenous genetic material into cells or tissues at a pathological state, has recently received much attention as a therapeutic methodology for a number of acquired and inherited diseases, including cancer. Thus, the key to success for any gene therapy strategy is to design a vector able to provide safe and efficient gene transcription of the transgene in a variety of cells and tissues. In this view, the development of protocols aimed at obtaining optimal and efficient genetic transfer has been studied [71-73] and has led to the production of many delivery vehicles that are able to bind to DNA. The optimal carrier has to accumulate at sites of diseases such as infections, inflammations, and tumors and has to be a small, neutral, and highly serum-stable particle. Moreover, it has to be not readily recognized by the fixed and free macrophages of the reticuloendothelial system recent years; solid lipid nanoparticles have attracted increasing attention. However, only a few studies that have been aimed to obtain innovative nonviral transfection systems for gene therapy have been performed on CLS. In the last decade, the efficiency of nonviral transfection systems has improved several orders of magnitude. Although as yet none has proven to be effective enough *in vivo*, new developments are still ongoing. Among nonviral transfection systems, colloidal carriers such as CLS represent an alternative drug delivery system to emulsions, liposomes, and polymeric particles.

6. **Factors Influencing Quality Attributes of Lipospheres:**

6.1 Factors influencing morphology of lipospheres-see table no.03.

S.No.	Factors	Influence
01.	Drug loading	Proportion of larger particles formed was high on increasing the drug amount. At maximum drug: lipid (1:1)[74] insufficient coating of drug by lipid leads to the formation of aggregates during cooling phase resulting in irregular, fluffy and fragile particles.
02.	Type of lipid	Combination of apolar (tristearin, tripalmitin or tribehenin)[75] with polar lipids (glycery monostearate, glyceryl monooleate) gave lipospheres satisfactory in terms of size, shape and recovery.

03.	Type of impeller	Lipospheres were produced using different impeller types [76] and particle characteristics of formed lipospheres were studied. Impellers used were of rotor (2-blade, 3-blade) type, helicoidal rotor (4-blade) type, double truncated cone rotor. Lipospheres could not be produced using 2-blade rotor and resulted in the formation of elliptical particles. 

6.2 Factors influencing entrapment efficiency-see table no.04.

S.N0.	Factors	Influence
01	Type of lipid	Hydrophobicity of lipids promotes entrapment of drugs. Long chain triglycerides (tristearin and triarachidin) are generally more hydrophobic than short chain triglycerides like tricaprin and trilaurin. Accordingly the free drug contents of formulations containing the long chain triglycerides were found to be lower than short chain triglycerides[77]. Also long chain triglycerides were found to increase the bioavailability of drug as they increase in gastrointestinal residence time of drug compared to medium chain and short chain fatty acids [78] Lipid excipients reduce the activity of P-glycoprotein and MDR (multi drug resistant) associated protein 2 by down regulating the protein expression and increase in cell membrane permeability in addition to lymphatic uptake.

02	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 [79] revealed that 70-90% of phospholipid polar heads were accessible on liposphere surface thus enhancing the loadability of drug.
03	Effect of method of preparation:	Melt dispersion method was found [40] to be superior over solvent evaporation method in terms of entrapment efficiency as melt method promotes drug incorporation core where as solvent evaporation promotes drug incorporation in coat.

6.3 Factors influencing drug release: see table no.05.

S.No.	Factor	Influences
01	Release pattern	The release mechanism of drugs namely tetracaine, etomidate an prednisolone[80] 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.
02	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.
		Highest T8hr value was obtained with stearyl alcohol lipospheres [81]

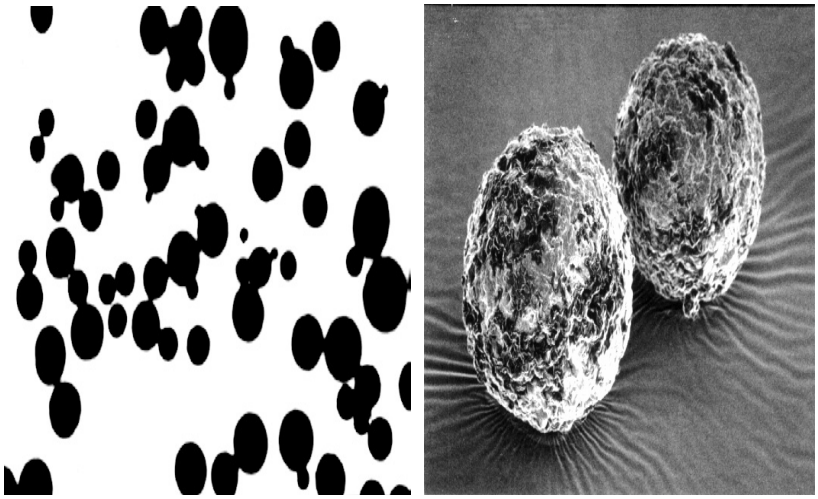
03	Type of lipid	compared to fatty acids like stearic acid. Stearyl alcohol possesses hydroxyl groups promoting matrix hydration by providing a hydrophilic pathway for water molecules to solubilize the drug and increase in dissolution rate. Lowest T8hr value was obtained from stearic acid lipospheres because of interaction of stearic acid with metal ions in medium forming sodium soaps which are crystals that contain fatty acid and metal carboxylate ion pairs retarding the release.
04	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 [40] resulted in a biphasic pattern (burst release followed by slow release)

7. **Evaluation of Lipospheres**

7.1 Morphology:

The size and surface characteristics of lipospheres can be determined by methods like electron microscopy, atomic force microscopy (AFM), nuclear magnetic resonance (NMR) and acoustic methods.

Figure-4: Morphology of liposphere by SEM (Scanning Electron Microscopy).



7.2 Entrapment efficiency:

Amount of drug loaded into lipospheres can be determined by first extracting the free drug (unencapsulated) by centrifugation into a suitable buffer. The encapsulated drug is then determined by dissolution-extraction of drug loaded Microparticles in Triton solution or in a solvent which can dissolve the Microparticles. Percentage(%) of the drug entrapped can be calculated using the formula:

Entrapment Efficiency Percentage = Entrapped drug/Total drug *100

7.3 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. 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 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. Filtered liposphere preparation can be filled into hard gelatin capsules and drug release from such capsules can be determined by using standard USP II (paddle) apparatus. Alternately, drug release from liposphere dispersions can be determined through dialysis membrane. Large pore dialysis membrane of 300,000 molecular weight (regular membrane pore size of 12,000 was found to affect the drug release) has been used to determine release from several drugs. For example, bupivacine from formulations containing 4% free drug was released immediately through dialysis membrane. A 2% loaded lipospheres of etoposide [91] released 90% of drug constantly over a period of 80 hrs.

Conclusion

Lipid based delivery systems like lipospheres offer new type of carrier system for lipophilic drugs. Easy availability of formulation ingredients and feasible, simple production techniques offer attractive option for formulation of lipospheres at industrial scale. Owing to the finer particle size of lipospheres and presence of a surface stabilized by emulsifier particles, bioavailability of several problematic drugs was found to increase. Recent works demonstrate sustained release of drugs entrapped in lipospheres. Hence lipospheres can be considered as new formulation approach for drug moieties. Lipid carriers have bright future due to 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. 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 the potential to be a major contributor to the search for better oral, parentral and topical drug delivery systems due to their improved adsorption and penetration. In addition, lipospheres could be suitable for low cost production, clinical and large-scale production. Therefore, Lipospheres could be considered as a promising delivery system for oral, parental and topical delivery of lipophilic drugs.

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