REVIEW ARTICLE

Accepted 23 June 2001
Revised 14 June 2001
Received 7 September 2000
Indian J. Pharm. Sci., 2001, 63(6) 450-458

Lipid Microspheres as Drug Delivery Systems

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Recent advances in the isolation techniques, medicinal chemistry, biotechnology and the capacity for screening new drug compounds have been able to produce several lead molecules. More than likely many potential drugs have been lost because of inadequate formulation strategies during different stages of evaluation. In many cases, the loss could have been due to solubility problems. To maximize the drug potential of all molecules, there has been a renewed effort to find formulation strategies for compounds inadequately soluble in water. Many different drug delivery systems for lipid-soluble compounds have been suggested, but lipid microsphere (LM) drug delivery system has great potential. The system is time tested, safe and stable at room temperature, easily mass-produced yet cheaper. The drug distribution and targeting can be achieved by manipulating the size, surface charge and surface legands of LM. At present, commercially available lipid microsphere formulations are few but in future this system will find considerable share in the market.

One approach to increasing the beneficial action of drug and decreasing systemic adverse effects is to deliver the necessary amount of drugs to the diseased sites, where they are most needed, for the appropriate time period. Several carrier systems such as liposomes, lipid microspheres, nanoparticles and albumin microspheres have been used to achieve the above objective. The design of appropriate delivery system must take into account the nature of the target and physiological barriers to targeting as well as factors such as drug loading and drug release, stability of the carrier system and its biocompatibility and biodegradation. Targeting with microspheres can be divided into passive methods that rely upon physiological and physicochemical determinants such as entrapment in capillary beds or uptake by phagocytic cells, and active method whereby the particle is directed to a specific site through the use of surface coatings (surfactants, glycolipids, monoclonal antibodies) or a material sensitive to an external influence (applied magnetic field). Lipid microspheres may find wide clinical applications because their physical characters are comparable with that of liposomes that are proved to be excellent drug delivery systems and are relatively stable at room temperature and easily mass produced.

The lipid microspheres (LM) are prepared by emulsifying biocompatible oil or triglyceride containing lipid soluble drugs using homogenizer and/or ultrasonicator, lecithin or phospholipids being added as emulsifying agents. This basic LM preparation has been marketed as a nutritional supplement (Intralipid, Celepid) and is given in doses of 300-500 ml. The structures of lipid microsphere and liposome are presented in fig. 1. The average size of LM ranges between 200-600 nm. Liposomes are excel-

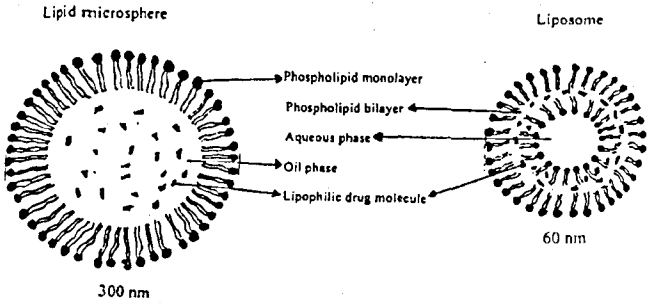

Fig. 1: Structure of Lipid Microsphere and Liposome

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TABLE 1: COMPARISON OF THE PROPERTIES OF LIPID MICROSPHERES AND LIPOSOMES

LIPID MICROSPHERES	LIPOSOMES
A parenteral lipid microsphere systems, such as Intralipid, consist of a water phase with droplets composed of a triglyceride core (diam 200-400 nm) stabilized with a phospholipid monolayer (2-3 nm). The phospholipid monolayer stabilizes the system by long-range repulsive electrostatic forces and short-range hydration forces.	Excess phospholipids exist as dispersed liposomes, unilamellar closed aggregates with water core (diam 60-90 nm).
The surface characteristics are mainly determined by phospholipid(s) used, other homing devices and to some extent by the lipids used as oil phase.	The surface characters are determined by the phospholipids used and the homing devices.
The lipophilic drug is in the lipid phase and the drug entrapment capacity of the system is higher than that of liposomes.	The lipophilic drug is located in the hydrophobic portion of the liposome and the entrapment capacity of the liposomes is less compared to LM
Production cost is low, easily massproduced and the approved shelf-life is normally 18 or 24 months at room temperature, but could remain stable for much longer. Sterilization of the system is easy.	Production cost is high and stability is less. Aseptic production procedures also contribute to the production cost of the formulation.

lent drug carrier vehicles for Drug Delivery Systems[1-3]. However, liposomes are relatively unstable and are not easily mass-produced. Lipid microspheres, themselves, are very stable and can be stored for upto two years at room temperature. They have no particular adverse effects, even at dose levels of 500 ml[4].

Liposomes Vs lipid microspheres:

Because of some similarities in the processing and excipients used, a comparison with liposomes should highlight the capabilities of lipid microsphere drug delivery system. Because of similarity in the tissue distribution of lipid microspheres and liposomes[5], many studies performed with liposomes can be applied to those with lipid microspheres[1-3]. Table 1 shows the similarities and differences between the liposomes and lipid microspheres. It can be seen from the Table 1 that lipid microspheres offer several advantages over liposomes. Parenteral lipid emulsions are normally ready to use formulations stored at room temperature, unless the stability of the formulated drug itself limits this type of formulation. Liposome formulations normally are lyophilized because of stability issues and require reconstitution before use. The lack of a lyophilization step during manufacture is another advantage of the lipid microspheres. There is less pain on injection than with solvent based or solubilized formulations. Therefore, the knowledge accumulated from studies involving liposomes can be utilized for proper design of LM drug delivery systems.

General method of preparation of lipid microspheres:

Various ingredients required to prepare a stable lipid microsphere drug delivery systems are presented in Table 2. The drug, emulsifiers, co-emulsifiers and antioxidants are dissolved in oil phase, the system is warmed to 70-80° if required[4]. Oxygen has to be removed with the help of vacuum application and bubbling the nitrogen gas through the system. Water-soluble components of the formulation are dissolved in some quantity of water and this aqueous system is made oxygen free. The aqueous phase is added to the oil phase at the same temperature. A crude emulsion is prepared with vigorous shaking. The crude emulsion is homogenized in a high-pressure homogenizer (e.g. Manton-Gaulin homogenizer) at pressure between 100 and 500 bars. The homogenization is repeated for 3-4 times to obtain a particle size below 10 μm or several times to obtain a particle size below

TABLE 2: VARIOUS INGREDIENTS USED IN THE FORMULATIONS OF LIPID MICROSPHERE

Category	Examples
Oil phase	Soybean oil, safflower oil, sunflower oil, sesame oil, cotton seed oil, coconut oil, corn oil, olive oil, peanut oil, fish oil, wheat germ oil, Walnut oil, diacetyl monoglecerides, medium and long chain triglycerides.
Emulsifiers	Soy lecithin, egg lecithin, phosphatidyl choline, phosphatidyl, ethanolamine, phosphatidyl inositol, dimyristoylphosphatidyl choline, dimyristoylphosphatidyl ethyl-N-demethyl propyl ammonium hydroxide.
Co-emulsifiers	Poly(oxyethylene)-6-glycerol trioleate, poly (oxyethylene)-6-glycerol linolase, nonionic surfactants (Tween 80, Span 80, Span 65, Myrj 52, Pluronic F-68), acetylated-mono-glycerides.
Stabilizers	Phosphatides, polyethylene glycols, polypropylene glycols, polyglycerol mono oleate.
pH adjusting agents	Free fatty acids (oleic acid, linoleic acid, stearic acid, palmitic acid) and their sodium and potassium salts, sodium hydroxide.
Agents for adjusting isotonicity	Glycerine, sorbitol, xylitol, dextrose.
Preservative	Benzoic acid, sorbic acid, para amino bezoic acid, methyl paraben, propyl paraben.
Antioxidants	Ascorbic acid, ∝-tocopherol, butylated hydroxy anisole.

1 μm and preferably in the range of 200 to 600 nm[6]. A microemulsion (with globule size <10 μm) can be subjected for ultrasonication (e.g. Branson ultrasonicator) to obtain globules size in the range of 200-600 nm[7]. In a study it is reported that as the number of shear applications increased, the mean diameter of the emulsion droplets decreased sharply and reached a minimum constant value at ten cycles[8,9] (fig. 2). If necessary water is added so as to dilute the emulsion to the desired strength. The pH and isotonicity adjustments are done with the help of suitable agents, filled into ampoules and sealed. The pH of LM as a pharmaceutical is normally adjusted between 5-7, even though the stability of emulsion is excellent between pH 7-9 for LM with egg yolk lecithin because the deterioration of egg yolk lecithin and soybean oil by oxidation can occur at an alkaline pH[10]. The pH may be set from considering the stability of

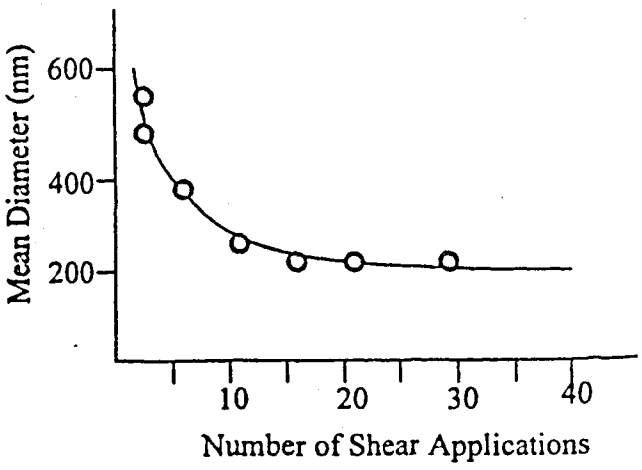

Fig. 2: Effect of number of shear applications on mean diameter of Lipid Microsphere formulation

the active drug contained in the formulation[11]. Antioxidants such as ∝-tocopherol are used to prevent oxidative degradation of both the oil phase and phospholipids used as emulsifier.

The product can be sterilized by autoclaving. Heat sterilization is preferred because of its advantages with respect to manufacturing ease and product safety, but alternative methods are feasible. Groves and Herman[12] reported that the phospholipids rapidly relocate during autoclaving from aqueous phase to oil phase, forming a cubic liquid crystalline phase, the bulk of oil which is converted to a lamellar phase on cooling, and that this organization of interfacial material accounts for the enhanced stability of phospholipid emulsions after heat sterilization[12].

Stability of lipid microsphere drug delivery systems:

Higuchi and Misru used the surface potential of lipid particles for the evaluation of stability of o/w emulsions[13]. Lawrence and Mills evaluated the flocculation of LM by measuring the absorbency using ultraviolet spectrophotometer[14]. Washington and Davis measured the zetapotential as an indicator of the surface potential to evaluate the stability of LM[15]. Surface potential of lipid particles as an indicator of stability of LM systems is used[16-18]. Handa *et al.* evaluated the stability of LM from the strength of membrane measured by the surface pressure on the interfacial membrane in the lipid particles[19]. Takamura *et al.* reported by measuring the zeta potential in the solutions of different pH's that the absolute value of zeta potential increases as the pH increases, resulting in a stable system[9]. The relationship of pH and terminal heat sterilization by autoclaving on the stability of phospholipid stabilized LM evaluated using droplet size and zeta potential measurements[10]. Nasirideen *et al.* evaluated the stability of LM at different temperatures (4, 25 and 40°) for a period of 6 mo by measuring changes in pH, droplet size, viscosity and percentage oil separation[20]. Accelerated stability studies under stress conditions (i.e., autoclaving and shaking) and long-term storage tests demonstrated that the tirilazad lipid emulsions had excellent physical stability over the study period of 16 mo[21]. The effect of lysine, a water-structure breaker, on the stability of phospholipid-stabilized lipid emulsions has been reported[22].

A quantitative theory of the interaction between lyophobic disperse particles was worked out independently by Derjaguin and Landan in the USSR and by Verway and Overbeek in the Netherland in the early 1940s[23,24]. The DLVO theory may be used accurately to evaluate the stability of a lyophobic dispersion.

Retention rate of drug in LM:

Solubility of drug in the oil phase and its partition coefficient between the oil phase and aqueous phase determine the entrapment and retention of the drug in LM. The retention rate of drug in lipid particles is the most important factor among the physicochemical properties of LM. If the drug in the lipid particles moves into water phase freely, LM is meaningless as drug delivery system. A pH dependent phase distribution of prostaglandin E_1 in LM is determined by ultrafiltration[25]. Retention rate of prostaglandin E_1 in lipid particles was determined by the equilibrium dialysis method[26]. The drug concentration in aqueous phase and oil phase is used to calculate the partition coefficient of drug between these phases. First diffusion equation of Fick can be used to formulate an equation for drug release from LM[1].

$$dm/dt=Dm.S.K/h(C_{wmax}-C_w) \quad (1)$$

Where, dm/dt is the change of the total amount of drug in the water phase, Dm is the diffusion coefficient in the interfacial membrane, S is the surface area of the interfacial membrane, K is the partition coefficient of the drug between interfacial membrane and water phase, h is the thickness of the interfacial membrane, C_w is the drug concentration in the water phase and C_{wmax} is the saturation solubility of the drug in water phase.

The potential usefulness of lipid emulsions as parenteral drug delivery system for lipophilic drugs was examined in tumor-bearings rats. The simulation studies were used to predict the drug release pattern from the system[27]. The kinetics and thermodynamics of delivery of lipophilic antioxidants from LM to cells in culture are determined. The rate of transfer is directly proportional to the concentration of particles in contact with the cells[28]. Influence of temperature and cosurfactants on drug release from lipid emulsions is reported[29].

Biodistribution of lipid microspheres:

In rats, a high proportion of intravenous lipid microspheres is distributed to the liver as well as spleen[30]. Extensive incorporation of radiolabeled LM in to the liver tissue and Reticulo Endothelial System (RES) has been observed in animals[31] and humans[32].

Antiinflammatory drugs:

Mizushima *et al.* reported that corticosteroids incorporated in lipid emulsions are taken up by RES and some inflammatory cells much more than free corticosteroids, resulting in a stronger antiinflammatory activity[31,33]. Removal of lipid emulsion particles by the RES and lipid accumulation in the RES have been reported in human beings receiving lipid emulsion[34,35]. It was found that at 30% edema inhibitory dose, LM-indomethacin was about 1.5 times more potent than free indomethacin, indicating possible localization of LM at the inflammatory site[36]. Using LM, average diameter 0.2 micron and containing methyl and ethyl esters of biphenylacetic acid, in the carrageenan paw edema tests in rats, it was found that their antiinflammatory activities were enhanced 3 to 8 times over that of free biphenylacetic acid esters[37].

Prostaglandins:

Prostaglandin E_1 (PGE_1) and prostacycline (PGI_2) lipid microsphere preparations were developed for passive targeting to the sites of inflammation and vascular lesions. Favorable pre-clinical and clinical results have been achieved with these preparations[38,39]. After intravenous injection into spontaneously hypertensive rats (SHR) with vascular lesion or rabbits with catheter induced arteriosclerotic lesions, LM accumulated in the lesions and especially in the subendothelial spaces[40]. Marked enhancement in antithrombic activity of isocarbocyclin, a new stable prostacyclic analogue, following its incorporation into lipid microspheres has been reported[41,42]. Treatment of infantile primary pulmonary hypertension with PGI_2 analog in lipid microspheres produced marked reduction in the pulmonary arterial pressure and resistance[43].

Antitumor agents:

Two lipophilic anticancer drugs were formulated as LM and found to be useful for site specific targeting[44]. An antitumor drug (9-oxo,-15-hydroxy-delta-7,10,13-prostatrienoic acid methyl ester) was incorporated into lipid microspheres of 0.2 micron diameter and found to have a significantly greater antitumor activity than the free drug[45]. Takenaga *et al.* reported that lipid microspheres (200 nm) and lipid nonospheres (50 nm) of a lipophilic antitumor drug (1,3-bis(2-chloroethyl)-1-nitrosourea) significantly enhanced antitumor activity with reduced toxicity, when compared with the corresponding doses of free drug[46]. Prolonged survival time of sarcoma 180-bearing mice treated with lipid microsphere-entrapped antitumor marine coral prostanoids is reported[47].

Ophthalmic drugs:

A 3H-labeled hydrocortisone 17-butyrate 21-propionate (HBP) ophthalmic suspension and 3H-labeled HBP lipid microspheres were applied to rabbit eyes, which were then enucleated at fixed intervals to determine the level of 3H-labeled HBP in ocular tissues. The lipid microspheres were shown to deliver the drug to the anterior ocular tissues more efficiently than the ophthalmic suspension[48,49].

Other drugs:

Concentrations of cyclosporin in lymph-ducts were about 46 times higher after 2 h and 10 times higher after 3 h following oral administration of lipid microsphere formulation, compared with those of conventional formulation[50]. Blood-brain-barrier permeability to LM containing the prostaglandin I_2 analogue has been evaluated for an *in vitro* system of primary cultured monolayers of bovine brain capillary endothelial cells, by a capillary depletion study in rats and by an *in-situ* brain perfusion study in normal and 4-vessel-occluded fore brain ischaemic rats. The results indicated that the drug in LM was transported through the BBB by endocytosis of LM and by simple diffusion of the drug released from LM[51].

Fluoresence-labeled lipid microspheres were used to study their transport across the human endothelial cells cultivated on microporous membranes mimicking the luminal and spaces of blood vessels. The results suggested that endothelial cells transport LM by transcytosis. The interleukin-1 beta induced increase of transcytosis in endothelial cells would explain why LM preferentially accumulates in inflammatory tissues[52].

Transport of ketoprofen across rat skin was enhanced by formulating it in lipid microspheres[53]. In a study it is suggested that immunomodulatory agents incorporated into LM might selectively regulates the function of CD4+or CD8+T cells when these are activated[54].

Factors influencing the biodistribution of lipid microspheres and liposomes:

The components, size and surface charge of the microspheres influence their biodistribution. In addition, specific legands anchored on the surface of these microspheres leads to the targeting to specific sites.

Lipids used in the formulation:

The lipids and phospholipids used in the formulation of lipid microspheres and liposomes influence their distribution in the body. The zeta potential of the mean particle size of oil droplets in 10% (w/w) o/w type emulsion decreased with increasing the emulsifier concentration and then leveled off at more than 1.2% (w/w)[8]. Kurihara *et al.* reported that LM prepared with polyoxyethylene-60-hydrogenated castor oil are more stable to lipoprotein lipase and showed low uptake by RES organs, long circulations in the plasma and high distributions in tumors compared with conventional lecithin stabilized LM[55]. Liposomes containing spingomyelin were taken up by the liver and spleen to a greater extent than those prepared from phosphatidyl choline-cholesterol[56].

Particle size:

Small unilamellar vesicles were cleared less rapidly than were large multilamellar ones[57]. The small-globule emulsions resulted in a long retention and the large-globule emulsions led to a relatively short retention[58]. The uptake clearance of large-globule size emulsions in the liver and spleen are much larger than those of small-globule size emulsions[59,60]. A reduction in size and coating with egg sphingomylin on the surface of the droplets resulted in avoidance of the reticuloendothelial system[61]. LM prepared with phospholipids modified with polyethylene glycol or the addition of surfactants containing polyethylene glycol showed long retention time in plasma[62].

Surface charge of microspheres:

Washington and Davis reported that the absolute value of the zeta potential increase according to the amount of oleic acid added[15]. Takamura *et al.* reported that absolute value of zeta potential increases as the pH increases, resulting in a stable system[9]. The zeta potential of the LM decreased with increasing emulsifier concentration and then leveled off at more than 1.2% (w/w)[8]. Piemi *et al.* prepared positively and negatively charged lipid microspheres using stearylamine or deoxycholic acid, respectively[63]. Negatively charged liposomes are not taken up by the phagocytes. The lipids, phosphatidyl serine, phosphatidyl inositol and dicetyl phosphate are used to give negative charge around the lipid microspheres[64]. Positively charged liposomes were found to have greater apparent volume of distribution in the body than that of neutral and negatively charged liposomes[65]. Gershanik *et al.* reported that cyclosporin A incorporated in positively charged oil globules was preferentially taken up by the rat mucosal cells due to enhanced electrostatic interaction than that of cyclosporin A incorporated in negatively charged oil globules[66]. Schwendener *et al.* reported that the degree of liposomal cell interaction with macrophages can be improved by increasing the degree of positive charge using stearylamine[67]. A stable positively charged lipid microsphere system was prepared with phospholipids, poloxamer 188 and stearylamine and found to have good stability[68].

Homing devices or surface legands:

The uptake of lipid microspheres by ploymorphonucleocytes in areas of inflammation was enhanced 2-3 fold when lipid microspheres were coated with homogenous IgG[69]. The mannosegrafted pentamidine liposomes were best in lowering spleen Leishmania parasite load in comparison to those bearing glucose or galactose[70]. Inhibition of phagocytosis by erythrocyte membrane sialoglycoprotein containing liposomes has been reported by Utsumi *et al*[71]. An antitumor drug entrapped in the membrane of small sonicated liposomes bearing antitumor monoclonal antibodies can be delivered to antigenic tumor cells and exert more efficient anti-tumor activity than does the free drug[72].

Future trends:

One of the major problems of LM using as drug delivery system is its rapid uptake by liver, spleen and other RES. For drug delivery purposes, it may be necessary to control and modify the uptake of the droplets. Adding block copolymers of ethylene oxide and propylene oxide to stabilize an emulsion prolongs circulation time. Preparation of small particles with negative charge also improves the circulation time of the microspheres. Another strategy for avoiding rapid clearance uses phospholipids modified with polyethylene glycol or the addition of surfactants. Positively charged LM may be used for drug targeting to RES. A continuing challenge is to tailor the surface properties of LM to achieve the desired bioditribution. Optimizing certain characters could endow LM with targeting properties, a line of research that has been prompted with liposomes.

Results of various studies carried out with LM reveal the fact that it can be used for drug delivery as well as

TABLE 3: DRUGS STUDIED FOR LM DRUG DELIVERY

Drug	Reference
Pregnanolone	73
Barbituric acid	74
Cyclandelate and nitroglycerin	75
Indomethacin	36, 76
Biphenylacetic acid	37
Prostaglandin E_1 and prostaglandin I_2	77
Hydrocortisone 17-butyrate 21-propionate	48,49
Isocarbocycline	41
Dexamethasone	31
Cyclosporin A and cyclosporins	50,78
Naproxen	20
Ketoprofen	53
Antitumor drugs	45-47
Ubidecarnone, progestarone, nifedipine and grisofulvin	07
Phenyramidol, mecamylamine base Hexobarbitol, phenylbutazone, lidocaine	79
Ibudilast	80
Propofol	81
Tirilazad	21

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TABLE 4: COMMERCIALLY AVAILABLE LM DRUG DELIVERY SYSTEMS

Drug	Company	Brand name
Dexamethasone Palmitate	Green Cross	Limethasone
Flurbiprofen axetil	Green Cross	Lipfen
Flurbiprofen axetil	Kaken	Ropion
Prostaglandin E_1	Green Cross	Liple
Prostaglandin E_1	Taisho	Palux
Parenteral Nutrition	Clintec, Pharmacia, Kabipharmacia	Intralipid
Diazepam	Pharmacia, Dumex	Diazemuls
Propofol	Zeneca, ICI	Diprivan

for drug targeting (Table 3). The success of developing a desired lipid microsphere drug delivery system of a particular drug depends on how best one tailors lipid microsphere size and surface properties. At present commercially available lipid microsphere formulations are few (Table 4) but in future this system will find considerable share in the market.

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