—Reviews—

Lipid Carriers: A Versatile Delivery Vehicle for Proteins and Peptides

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Lipid based carriers have attracted increasing scientific and commercial attention during the last few years as an alternative material for the delivery of peptides and proteins concerned with stability issues. This article presents an overview of different types of biocompatible and versatile lipid-based carriers employed for the delivery of therapeutic proteins and peptides. Such delivery systems are discussed and exemplified regarding both more traditional lipid based delivery systems such as liposomes and lipid emulsions as well as more novel structures, *e.g.*, lipid microtubules, microbubbles, and solid lipid nanoparticles.

Key words——lipid; protein; peptide; delivery system

INTRODUCTION

Proteins and polypeptides are important classes of bioactive agents that play key roles in controlling various bodily functions required for good health when administered in the correct quantities at the appropriate body site and at the correct time. These materials are synthesized in nature in miniscule quantities, usually at only one site and diffuse or are actively transported to their site of action. There they may react with one specific molecule or a group of molecules at a receptor site in order to produce the appropriate physiological response.[1] Advances in biotechnology have resulted in a significant increase in the number of therapeutic peptides and proteins that are reaching the market. This trend is expected to continue and escalate in the future. The total global market for protein drugs was $47.4 billion in 2006 and by the end of 2011 is expected to reach $55.7 billion with an average annual growth rate (AAGR) of 3.3%.[2]

Proteins and peptides have many attractive properties but they also have disadvantages that limit their widespread acceptance by patients and physicians such as numerous chemical and physical instability mechanisms, rapid enzymatic degradation which is responsible for low oral and transdermal bioavailability and short *in vivo* half lives, which often necessitate their frequent delivery.[3] The development of delivery systems for this rapidly expanding class of therapeutic agents is the major challenge. Unfortunately, modern drug delivery methods involve delivering relatively massive amounts of drug or biological response modifier throughout the body once or twice a day. Sites all over the body may be affected in addition to the one that is being targeted. So, a pharmaceutical delivery system is needed that mimics the natural process as closely as possible and delivers the necessary small quantity of active material close to its target tissue. These goals can be achieved by delivery of drugs specifically to diseased sites for the effective pharmacodynamic profile or by preparation of a drug carrier system that acts as a reservoir at the site of application for the appropriate time period. Moreover, poor bioavailability of these labile molecules far too often results in not only higher patient costs and inefficient treatment, but also, more importantly, increased risks of toxicity or even death.[4]

Several controlled release formulations based on matrix materials such as PLGA, poly (lactic acid) (PLA), for protein and peptide drugs are currently available on the market but the synthetic polymer matrix has its own compatibility drawbacks.

Need of Lipid Carriers Lipid based carrier systems represent drug vehicles composed of physiological lipids such as phospholipids, cholesterol, cholesterolesters and triglycerides. Lipid carriers owing to the biological origin of the carrier material offer a number of advantages making it an ideal drug delivery vehicle (Table 1).

- Controlled release devices based on lipids as natural materials and which are derived from

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Table 1. Overview of Different Lipid Carriers

S. No	Carriers	Size range	Composition	Features	Common preparation techniques
1.	Liposomes	25 nm-few microns	Natural or synthetic phospholipids	Bilayered vesicles containing an aqueous volume entirely enclosed by a membranous lipid bilayer	Passive loading • Mechanical dispersion • Solvent dispersion • Detergent removal Active loading
2.	Solid lipid nanoparticles	50–1000 nm	High melting point fats of natural or synthetic origin	Submicron colloidal carriers containing solid hydrophobic core having a monolayer of phospholipid coating	• High-pressure homogenisation • Microemulsion formation • Precipitation • As lipid nanopellets
3.	Oily suspensions	Globule size 10 nm-few microns	Natural or synthetic oils	Dispersions of peptides and proteins in oils of high viscosity for sustained-release of proteins	Dispersion technique
4.	Submicron lipid emulsions	lipid globules 1–100 nm	Lipids, hydrophilic liquid, surfactants	Multicomponent fluid made of water, a hydrophobic liquid, and one or several surfactants resulting in a stable system	Emulsification technique • o/w • w/o • w/o/w • w/o/o
5.	Lipid implants	Variable size according to application site	Natural or synthetic lipid compacts	Drug dispersions in lipid or fat compressed modules	Lipid compression
6.	Lipid microtubules/microcylinders	$<1\ \mu m$	Natural or synthetic lipids with suitable surfactant	Self-organizing system in which surfactants crystallize into tightly packed bilayers that spontaneously form cylinders	Self emulsification
7.	Lipid microbubbles	few microns	Lipids, polymers, proteins, phospholipids	Gas-filled microspheres stabilised by phospholipids, polymers or proteins with low density and high elasticity of these bubbles	Sonication
8.	Lipid microspheres (Lipospheres)	0.2–100 μm	High melting lipid, phospholipids	Water dispersible solid microparticles composed of a solid hydrophobic fat core stabilised by a monolayer of phospholipid molecules embedded in a microparticle surface	• Melt method • Multiple microemulsion • Cosolvent method • Preincorporation into lipophilic carriers

body tissue constituents exhibit a high biocompatibility after administration.[5–7]

- Lipophilic matrices are not as susceptible to erosion phenomena as polymeric systems.[8]
- Simple manufacture by compressing or moulding.
- Slower water uptake after administration results in a less detrimental environment for incorporated proteins.[9]

Limitations

- High-pressure homogenization is the commonly used preparation technique that induces prominent drug degradation in high molecular weight compounds and long chain molecules, for example DNA and albumin.[10]
- Lipid crystallization results in several polymorphic forms with different melting behaviors, different capacities to incorporate drugs and different particle shapes responsible for coexistence of different lipid modifications and colloidal species.
- Variable kinetics of distribution processes.

- Low drug loading capacity as a result of the crystalline nature of the lipid, with even minor difference in chain length of triglycerides forming separate lipid crystals.[10]

Types of Lipid Based Carriers Potential lipid-based carrier systems for controlled delivery of peptides and proteins are as follows (Fig. 1, Table 2):

- Liposomes
- Solid lipid nanoparticles
- Oily suspensions
- Submicron lipid emulsions
- Lipid implants
- Lipid microtubules and microcylinders
- Lipid microbubbles
- Lipid microspheres (Lipospheres)

Liposomes Liposomes are concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of natural or synthetic phospholipids. Liposomes are formed when thin lipid films or lipid cakes are hydrated and stacks of liquid crystalline bilayers become fluid and swell. The hydrated lipid sheets detach during agitation and self associate to form large multilamellar vesicles (MLVs), which prevent interaction of water with the hydrocarbon core of the bilayer at the edges. These large vesicles can be changed in terms of vesicle shape and morphology by energy input in the form of sonic energy and mechanical energy. Liposomes are characterised in terms of size, surface charge and number of bilayers. Small unilamellar vesicles (SUVs) are surrounded by a single lipid layer (25–50 nm) whereas several lipid layers separated by an intermittent aqueous layer surround large unilamellar vesicles (LUV).

These alter the pharmacokinetic profile of the loaded drug to a great extent, especially in the case of proteins and peptides,[11] and can be easily modified by surface attachment of hydrophilic polymers (polyethylene glycol-units) making it a stealth liposome (a registered trade name of Liposome Technology, Inc.) and thus increase its circulation half-life due to their invisibility to the body's immune system.[12] The use of liposomes as protein carriers improve functioning as a circulating microreservoir for sustained release after intravenous administration. The application of liposomes containing human recombinant IL-2 inhibited the growth of intradermal tumours for one week.[13]

Generally a protein is expected to reside in the aqueous compartment of the liposome; however, the hydrophobic part of the protein may interact with the lipid membrane. Such protein-lipid interactions may or may not affect the bioactivity of the protein. A protein that can transform into a 'molten globule' state

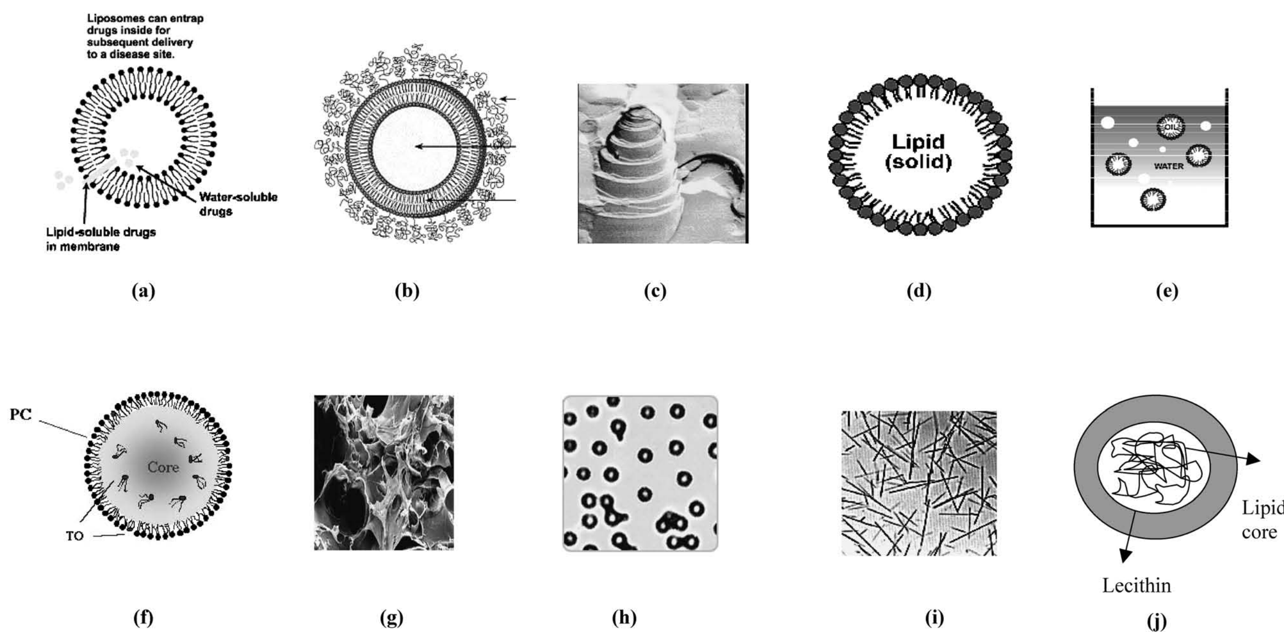

Fig. 1. Different Types of Lipid Based Carriers
(**a**) Liposomes (**b**) Stealth Liposomes (**c**) Cochlear liposomes (**d**) Solid lipid nanoparticles (SLN) (**e**) Oily suspensions (**f**) Lipid emulsions (**g**) Lipid implants (**h**) Microbubbles (**i**) Microtubules (**j**) Lipospheres

Table 2. Lipid Based Carriers for Proteins and Peptides

S. No	Lipid carriers	Protein used	Advantages	Disadvantages	Remarks	Ref
1.	Liposomes	β-Glucuronidase (GUS)	Biocompatible Amphiphilic Ease of modification	Limited stability	The feasibility of using liposomal dry powder aerosols for protein delivery was demonstrated	96
2.	Liposomes	Interleukin-2	Biocompatible Amphiphilic Ease of modification	Limited stability	Local delivery of liposomal IL-2 to the lungs facilitated bioactivity and reduced toxicity	13
3.	Depo-Foam (MVLs)	Interferon α-2a	Sustained release Increased entrapment	Toxicity studies required	Exhibited sustained release of IFN for one week with retention of activity	97
4.	Solid lipid nanoparticles (SLN)	Salmon calcitonin	Better stability	Decreased entrapment	Developed chitosan coated lipid nanopaticles and showed slow release due to the affinity of the peptide for the lipids and the absence of degradation of the lipid matrix	98
5.	Oil suspensions	GHRH	Sustained release Non-Toxic	Stability studies required	Sustained delivery of the growth hormone for over 2 weeks. Peptide stability was substantially increased within the oil solution.	99
6.	Lipid implants	Insulin	Biocompatible Sustained release Stable	Decreased entrapment	Demonstrated suitability of lipids as excipient in sustained release insulin implants	6
7.	Submicron lipid emulsions	Calcein	Improved bioavailability Better entrapment	Stability studies required	The bioavailability of calcein was increased using microemulsion concentrate and preformulated w/o microemulsions	100
8.	Lipid microtubules	Myoglobin, albumin, and thyroglobulin	Safe Non-inflammatory Ease of modification Increased entrapment	Marked burst effect	Sustained release from microtubule-hydrogel system without contact of protein with organic solvent	77
9.	Microcylinders	Albumin	Safe Non-inflammatory Ease of modification	Rapid diffusion	Released 50% of protein within 8 days	76
10.	Microbubbles	IgG	Controlled delivery Stable	Limited loading	Enhanced local and systemic immune responses associated with receptor-mediated loading of alveolar macrophages	101
11.	Lipid coated microbubbles	Luciferase protein	Controlled delivery Stable	Limited loading	Six-fold higher cardiac luciferase uptake compared with control groups that did not include bubbles	102
12.	Lipid Microspheres	Insulin	Biocompatible Stable	Limited entrapment	The biological effect was extended in proportion to the amount of lipid present.	103
13.	Lipospheres	Cyclosporine	Enhanced entrapment Stable	Non predictable release	All formulations were reproducible and stable at room temperature for at least 6 months, with full activity of cyclosporine retained.	104

[**Abbreviations**: IgG: Immunoglobulin G; MVLs: Multivesicular liposomes; GHRH: Growth Hormone Releasing Hormone; DMPC: Dimyristoyl phosphatylcholine; Ch: cholesterol; PG: Phosphatidyl Glycerol]

appears to exist in either water soluble or membrane bound form. In the molten globule state, the protein exists in an unfolded intermediate conformation. This conformation makes it easier for the hydrophobic part of the protein to partition into the lipid bilayers. This mechanism has been proposed for the interaction of rec-human granulocyte colony stimulating factor (rhG-CSF) and tumour necrosis factor (TNF-α) with lipid vesicles. However, charge interactions can be equally important and must be considered.[14,15]

The incorporation of IL-2 in liposomes was strongly dependent on the charge of liposomes and pH and ionic strength of hydration medium.[16] The highest incorporation efficiency (81%) was achieved with negatively charged liposomes composed of phosphatidylcholine/phosphatidyl glycerol (9:1). Co-injection of IL-2 containing liposomes resulted in enhancement of an immune response.[16]

(i) A new approach is the **DepoFoam™ system-** These multivesicular liposomes (MVLs) (1–100 μm) contain multiple non-concentric internal aqueous compartments and lead to an increase in the encapsulation efficiency. MVLs could be a versatile vehicle for controlled and sustained delivery of proteins and their release could be controlled up to several weeks. After subcutaneous injection, the release of encapsulated peptide and protein was shown to be prolonged up to 7 days, *e.g.* DepoInsulin,[17] and up to 3 weeks, *e.g.* DepoLeuprolide® formulation.[18] Furthermore, MVLs as depofoam particles were stable for 1 year at 4°C in a long-term storage stability study.[19] In a recent work by Langston, *et al*,[20] Leridistim (a protein from the MPO family) was encapsulated in multivesicular liposomes (Depo Foam™) for sustained delivery, and a single injection of Leridistim MVLs was demonstrated to result in elevated neutrophil counts for 10 days, in contrast to only 2 days for un-encapsulated Leridistim.

(ii) Another lipid-based structure is the cochlear phospholipid structure. The cochlea is a cylindrical structure with spirally curved bilayers.[21] These structures are formed by negatively charged phospholipids like phosphatidylserines (PS). The addition of cationic calcium ions causes them to bind to the oppositely charged head groups and the spherical structures collapse to roll or stack into essentially solid lipid structures. There is no trapped water in these cochlear structures so that any drug or guest molecule becomes trapped in between the adjacent lipid sheets protected from the surrounding aqueous medium.[22,23] They are non-toxic as phosphatidylserine is mainly obtained from soybeans. Cochleates are readily lyophilized to free flowing powders that can be incorporated into capsules for oral administration or resuspended in aqueous vehicles for injection.

Thus, liposomes can serve as a versatile carrier for fragile molecules like proteins and peptides by offering certain advantages:

- Suitable systems for drugs that are rapidly excreted or metabolized.
- An undesirable peaks and troughs in the pattern of circulating drug levels can be avoided as observed in conventional dosage forms.
- Liposomes can be injected into the circulation and thus can serve as intravascular drug depot.
- Passive macrophage targeting and RES accumulation can be achieved for the treatment of ailments caused by intracellular pathogens.
- Amphiphilic character, biocompatibility, and ease of surface modification.

Liposomal dosage forms can be used in drug targeting by positioning of marker proteins such as antibodies or cytokines upon the surface of liposomes or in the preparation of vaccine formulations.

Solid Lipid Nanoparticles (SLN) SLN particles made of solid lipids are submicron colloidal carriers (50–1000 nm). These consist of a solid hydrophobic core having a monolayer of phospholipid coating. The solid core contains drug dissolved or dispersed in the solid high melting fat matrix. The hydrophobic chains of phospholipids are embedded in the fat matrix. Depending on the type and concentration of the lipid, emulsifier (0.5-5%) is added for the physical stabilization of the system, *e.g.* poloxamer 188, polysorbate 80, lecithin, polyglycerol methylglucose distearate, sodium cocoamphoacetate or saccharose fatty acid esters. SLN are prepared by various techniques[24] such as high-pressure homogenization,[25] microemulsion formation,[26] precipitation,[27] and as lipid nanopellets.[28] Initial work on SLN on the oral delivery of lipid nanopellets was reported by Speiser

in 1990. Being in the solid state, the lipid components of SLN degrade more slowly as drug mobility in a solid lipid is low compared with the liquid lipid providing controlled and long-lasting drug release.[28)]

Different delivery routes have been exploited such as parenteral,[29)] pulmonary[30)] and topical.[31,32)] SLN are non-toxic in comparison with polymeric nanoparticles.[33)] Recently, chitosan coated lipid nanoparticles for oral salmon calcitonin delivery have been used for oral administration of peptidal drugs.[34)] SLN are more appropriate for incorporation of lipophilic proteins due to their hydrophobic nature and small size, which can be easily dissolved in the melted mixture.[35)] Lysozyme was entrapped successfully into various lipids by the use of a solubilisation technique.[36)]

A problem in using SLN as drug carrier, however; is the burst release observed for various active agents after intravenous administration.[37)] SLN offers a futuristic approach for the encapsulation of proteins with a low solubility (*e.g.*, cyclosporine), in drug targeting, and as an effective adjuvant for vaccines to give a maximum immune response by optimizing surface properties. These can be applied for oral drug delivery in the form of aqueous dispersions or they can be used as additives in traditional dosage forms such as tablets, capsules or pellets.

This offers biomedical as well as industrial benefits over existing systems in terms of:

- Biodegradability and good tolerability.
- Ease of industrial scale-up due to availability of cost-effective techniques such as high-pressure homogenisation, and microemulsion technology paving its way for use by the pharmaceutical industry.
- Increased drug stability and high drug payload.
- Lack of biotoxicity of the carrier.
- Avoidance of organic solvents.
- Feasible incorporation of lipophilic and hydrophilic drugs.

Oil Suspensions Oil suspensions are dispersions of peptides and proteins in oils for sustained-release of proteins with short half-life. These exhibit higher viscosity than the viscosity of an aqueous phase. Viscosity of the system can be further increased by the addition of gelling agents such as aluminium monostearate affecting the process parameters like drug solubility and drug transfer rate or the distribution coefficient of compounds in an oily medium and the surrounding tissue. Generally, a lipophilic drug with a high distribution coefficient accumulates in the oily medium decelerating effective drug actions.

Long-acting injectable depot formulations for super-agonist analogues of luteinizing hormone-releasing hormone (LH-RH), with oils (peanut oil or sesame oil) and a gelling agent (aluminium stearate) was successfully developed.[38)] FDA approved Posilac ® containing a complex of bovine somatotropin and zinc with the use of sesame oil and aluminium monostearate as suspension medium.[39)] Protein/polyol/oil parenteral suspensions for the prolonged release of granulocyte colony stimulating factor have been developed.[40)] The first approved *i.v.* lipid emulsion for parenteral administration was Intralipid which consisted of 10–20% soybean oil droplets stabilized by a monolayer of egg yolk mixed phospholipids (1.2%) and glycerol (2.25%) as an osmotic agent. Since then, a number of commercially available oil suspensions consisting of soyabean oil, cottonseed oil as the oil phases have appeared.[41)]

It may be anticipated that other drugs may be presented in similar vehicles, especially oil soluble proteins. Oil suspensions under investigation have apparently revealed potential as drug carriers for peptides and proteins.

Microemulsions Microemulsions are also termed "transparent emulsion," "micellar emulsion," or "swollen micellar emulsion." Rosano and Clausse (1987) described "microemulsion" as any multicomponent fluid made of water (or a saline solution), a hydrophobic liquid (oil), and one or several surfactants resulting in systems that are stable, isotropic, and transparent with low viscosity.[42)] Depending upon the type of continuous phase-water or oil, microemulsions can be compounded to be hydrophilic or hydrophobic, respectively. The inclusion of proteins in microemulsions is the basis for a number of research topics.

Proteins hosted in microemulsions may also find application in research into enzyme activity and protein separation; they can alter surfactant self-association and phase behaviour and can promote the formation of novel solvents and materials.[43)] Microemulsions have been used as a model system to understand membrane transport behaviour.[44,45)] They can also be used to separate and concentrate proteins, as the solubilisation of individual proteins depends on the protein properties.[46)] Goklen and Hatten separated cytochrome *c* from lysozyme using an Aerosol-OT-

based microemulsion system.[47] The separation of xylose reductase[48] and lysozyme from egg white[49] have been reported using microemulsion technology, and the ability of reversed micellar systems to act as a bioseparation technique for isolation and purification of proteins has been reviewed by Pires, *et al*.[50] Promising application of the ability of microemulsions to separate and concentrate proteins may be in the separation and purification of heterogeneous proteins, such as caseins and whey proteins, resulting in large-scale production of individual proteins with increased value. Recently, the incorporation of immunoglobulin G[43] and α-lactalbumin[51] into microemulsion systems formulated with AOT, a non-food-grade anionic surfactant and isooctane has been reported.

The shearing force required for mixing in the formulation of a peptide-lipid emulsion should be minimised so that it does not denature the protein. Surfactants or a mixture of surfactants with HLB matching the lipid phase will reduce the need for high shear mixing. A multiple emulsion (w/o/w) containing bovine growth hormone for sustained release has also been described in patent literature.[52] Recently Cilek, *et al*. formulated stable lecithin based microemulsions containing rh-insulin.[53]

(i) **Sub-micron lipid emulsions** are potential drug carriers for lipophilic and amphiphilic drugs with many favourable properties:

- Site specific delivery can be designed by attaching ligands specific for cellular receptors to the surface of the emulsion globules.[54,55]
- Provide possibilities for preparing better tolerated intravenous formulations of poorly soluble drugs.

(ii) **Self emulsifying drug delivery systems** (SEDDS) consist of anhydrous solutions of the drug in oil containing surfactant and cosurfactant, which spontaneously emulsify when added to an excess of water.[56] A SEDDS can be made by simple, direct mixing under low shear conditions and packed into soft gelatin capsules without undergoing exposure to extreme conditions. The final product is generally a finely divided submicron emulsion that forms very rapidly when added to water. Thus, stability to hydrolysis is increased during storage and transportation of the anhydrous oil phase. In addition, it can be designed to dissolve hydrophobic drugs as the small droplets are formed so quickly that mass-transfer from oil to water phase is usually very rapid.

Spontaneous emulsification makes SEDDS good candidates for the oral delivery of hydrophobic drugs since the drug is presented as a fine (submicron) emulsion that has a large surface area across which diffusion can take place rapidly, facilitating absorption into the body. They are particularly useful for the formulation of proteins and peptides which would otherwise become degraded by the extreme temperature and shearing conditions encountered during homogenisation.

These formulations offer a number of advantages:

- No requirement of transporting water from the production facility to the application site.
- No exposure of drug to an aqueous environment until immediately before application.
- A major difference between SEDDS and microemulsions is that the order of mixing for the SEDDS concentrate is unimportant since there are no structures formed at this stage.

Recently SEDDS have been delivered as liquids absorbed by powders such as colloidal silicon dioxide or microcrystalline cellulose.[57] Thus SEDDS could specifically be utilised for the delivery of oil soluble proteins and peptides requiring minimal preparation conditions.

Lipid Implants Fats and waxes are ideal candidates for implant preparation as they exhibit high compressibility as compared to polymeric systems. Lipid implants can easily be produced by compacting with a tablet press or a hydraulic press. Lipid implant preparation protects the entrapped drug from harsh conditions of heat and exposure to organic solvents, making it particularly suitable for peptides and proteins.

- For example, Kent, *et al*. studied implant matrices made up of cholesterol for sustained macromolecule release for the first time[58]
- Wang, *et al*. proposed insulin dispersion in a pellet disk made by compressing a cholesterol mixture and the implantation of these pellets within rats reduced blood glucose levels for up to 24 days.[59]
- Later on, palmitinic acid was presented as a promising excipient in sustained insulin release.
- Pure cholesterol and cholesterol/lecithin implants were used for prophylaxis in livestock.[60]
- Cady, *et al*. prepared a series of C10 - C20 fatty acid salts of a synthetic growth hormone releas-

ing hexapeptide (tri-fatty acid hexapeptide salt) and compressed into implants. The implants were partially coated with either biodegradable polymers or non-biodegradable polymers.[61,62]

- Steber, *et al*. prepared implants based on triglycerides for the delivery of polypeptides and proteins such as somatotropin[63,64] Recent works have shown gelatin as a release modifier to adjust release from triglyceride matrices.[65,66] In hyaluronidase loaded implants neither the lipid nor the manufacturing process affected protein stability.[67]
- Lipid implants as a controlled release system for interleukin-18 were recently investigated by Koennings, *et al*. The manufacturing procedure of solid-in-oil (s/o) dispersion technique had no detrimental effects on protein stability in which protein particles in the micrometer range were first prepared by co-lyophilisation with polyethylene glycol (PEG) followed by compression of the powder mixture in a specially designed powder compacting tool.[68]

Thus lipid implants can be used for sustained release of proteins and peptides with less detrimental effects on their activity and integrity.

Lipid Microtubules/Microcylinders Lipid microtubules are a self-organising system in which surfactants crystallise into tightly packed bilayers that spontaneously form cylinders of less than 1 μm in diameter during a controlled cooling process. For the first time, Yanger and Schoen described lipid microtubules made from the diacetylenic lipid 1,2- bis (tricosa-10,12-diynoyl) -sn-glycero-3-phosphocholine.[69,70] These microtubules are hollow and open-ended, with walls formed by one or more lipid bilayers. The chiral interactions between lipid molecules cause the bilayer to twist and form a tubular structure.[71,72] Tubules either dissolve or enzymatically degrade from the tubule ends resulting in a constant rate of tubule degradation because of the tight packing of the lipids and highly ordered walls.[73] Thus, these tubules form continuous release systems independent of any macroscopic encapsulation or delivery devices. Microtubules have a lumen diameter of approximately 0.5 μm and an average length of 20–45 μm, that provides large storage volume relative to the diameter.[71,74]

- They are stable in physiological solutions at 37oC for prolonged periods of time.[75,76]
- Lipid microtubules are safe, non-inflammatory and provide ease of site specific delivery for proteins and peptides.[77,78]

In spite of advantages offered by their small size it also allows them to easily diffuse away from the injection site. To circumvent this effect these are embedded in carriers like agarose hydrogel which allows small molecules such as proteins and nucleic acids to diffuse through while the microtubules remain in the hydrogel.[79] A number of proteins like transforming growth factor-β, nerve growth factor and high molecular weight kininogen have shown sustained release properties in the microtubules.[76–78] The usefulness of the microtubule delivery system extends to therapeutic drug delivery, cancer therapy, tissue regeneration, gene and protein delivery. Its role in sustained release of proteins and nucleic acids could be exploited for biomedical applications.

Lipid Microbubbles Lipid-coated microbubbles represent a new class of agents with both diagnostic and therapeutic applications. These consist of gas-filled microspheres stabilised by phospholipids, polymers or proteins and used as contrast enhancers in ultrasonic diagnostics due to the low density and high elasticity of these bubbles. Stabilisation of microbubbles by lipid coatings creates low-density particles with unusual properties for diagnostic imaging and drug delivery. Such microbubbles are prepared by simple sonication of a protein (typically albumin) or polymer solution, which generates mm-sized bubbles. Generally, microbubbles made up of Perfluorocarbon (PFC) gases entrapped within lipid coatings are sufficiently stable for circulation in the vasculature as blood pool agents. Bioactive compounds can be incorporated into these carriers for site-specific delivery.[80]

Ultrasound targeted microbubble destruction (UTMD) could allow delivery of protein to ultrasound-accessible organs while keeping systemic concentrations and side-effects at a low level. It is based upon attaching or incorporating bioactive substances onto or within microbubble shells and then destroying microbubbles in the target organ, by ultrasound, after systemic administration.[81] This process releases the transported substance into the surrounding tissue.

- The ultrasound-targeted microbubble destruction can substantially and non-invasively augment organ specific delivery of proteins.

- Microbubbles are stable for many months as ultrasonic contrast agents, carrying labile and potent drugs like proteins.[82]

Lipospheres Lipospheres represent a new type of fat based encapsulation system developed for parenteral and topical delivery of bioactive compounds.[83–86] Lipospheres were first reported by Domb, as water dispersible solid microparticles with a particle size between 0.2–100 μm in diameter composed of a solid hydrophobic fat core stabilised by a monolayer of phospholipid molecules embedded in a microparticle surface.[85] Lipospheres can contain a biologically active agent in the core, in the phospholipids, adhered to the phospholipids, or a combination of the two.[85] Lipospheres are prepared by solvent and melt technique. In the melt method, lipophilic agent is melted together with the lipid core material or dissolved in melted core material whereas in the solvent method, organic solvents are employed to dissolve the active agent, solid carrier and the phospholipid component.[85] Alternative techniques have also been proposed for hydrophilic drugs such as the multiple microemulsion[87] cosolvent method[88] and preincorporation into lipophilic carriers.[85]

Lipospheres have successfully been used to incorporate and deliver a variety of substances, including anti-inflammatory compounds,[89] local anesthetics,[90] antibiotics,[85] insect repellents[91] and vaccines and adjuvants.[92] But only a countable number of research projects are being done in terms of protein and peptide delivery by lipospheres. Amselem, *et al.* reported around 80% encapsulation of R32NS1 in tristearin lipospheres.[86] Saraf, *et al.* (2006) also demonstrated the successful delivery of hydrophilic bioactive- HBsAg by double emulsion-solvent evaporation (w/o/w).[93] A number of research teams have conducted extensive investigation on the effects of various parameters like; different lipid composition, ratio of ingredients, preparation procedures on encapsulation efficiency, size distribution and release characteristics.[87,88,94,95]

Lipid particles degrade faster in the presence of surfactants which enable the contact with lipases leading to burst release.[8] Polymer lipospheres with matrices made up of biodegradable polymers have been investigated to obtain longer release periods.[95] Extensive investigation to determine the protein stability in lipospheres is needed.

CONCLUSION

There has been tremendous growth in the development of lipid based delivery systems in recent years due to the ever increasing demands of biocompatible carriers for drugs. The likely increased importance of lipid based drug delivery systems also stems from an increasing fraction of biopharmaceutical drugs, notably recombinant proteins and antibodies in particular, emerging on the market and in the drug discovery work. These systems offer real opportunities for providing "biological stability" through preservation of protein secondary and tertiary structure, avoidance of aggregation, and elimination of chemical and enzymatic degradation. The most successful system from among the different lipid based delivery systems is difficult to choose, but clearly stability and ease of preparation will be important concerns, as will the biological response to the different carrier systems. However, given the array of new and old lipid based drug delivery systems, rapid progress in the understanding of the physicochemical properties of these systems, as well as knowledge of their preparation and analysis, provides an interesting future for these systems in drug development, particularly for fragile molecules like proteins. Consequently, this somewhat shadowy existence should not persist any longer and in the near future a growing number of attributes of lipid based delivery are expected to be recognized.

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