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A COMPREHENSIVE REVIEW ON LIPID POLYMER HYBRID NANOPARTICLES

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ABSTRACT
Solid lipid nanoparticles, polymeric nanoparticles, Liposomes, micelles, gold nanoparticles, dendrimers, niosomes and carbon nanotubes have been commonly used as nanocarriers for many years but they are suffering from their own inherent limitations which are enumerated in literature such as drug leakage and high water content of dispersions. Intentionally, drug loaded lipid polymer hybrid nanoparticles (LPHNPs) are developed to overcome all the limitations of traditional nanosystems. LPHNP gives advantages of both polymeric nanoparticles and lipidic nanoparticles along with liposomes. LPHNPs restricts the release of water soluble drugs because of its structure in which lipid coats polymeric core, that ultimately leads to increase entrapment efficiency. Therefore in ingenious one can encapsulated oil or water soluble drugs while developing LPHNPs. The present review is focused on the drug selection criteria, approaches, different formulation strategies, and characterization of LPHNPs for oral drug delivery.

KEYWORDS: Lipid polymer hybrid nanoparticles, Polymer lipid hybrid nanoparticles, Entrapment efficiency, Liposomes, Polymeric nanoparticles, Traditional nanosystem.

INTRODUCTION
Existing nanocarriers such as lipidic nanoparticles, polymeric nanoparticles, liposomes, polymeric micelles, gold coated nanoparticles, dendrimers, niosomes, and carbon nanotubes have been commonly used as promising drug delivery system for many years. Among these polymeric nanoparticles and lipidic nanoparticles along with liposomes are the important classes which are currently under investigation. These nanocarrier system has many advantages but also having some limitations in terms of physicochemical and biological properties.

When we are talking about lipidic nanoparticles along with liposomes, they are suitable for both hydrophilic and lipophilic drug candidates. Liposomes are vesicles which having spherical shape. This drug delivery system is attractive because of their advanced biocompatible and biodegradable properties. As we know every system having some limitations, liposomes also having some limitations that are burst release kinetics of encapsulated drugs, low drug loading capacity, limitation of physical along with chemical stability and short circulation half life of vesicles.[1]

Polymeric nanoparticles (PNPs) proved momentous therapeutic potential as nanoparticles. During storage, these polymeric nanoparticles having high structural integrity as well as high stability when compared with liposomes.[2] On the other hand, polymeric nanoparticles and liposomes facing difficulty in encapsulation of drugs having hydrophilic nature due to their escape to the external aqueous phase.

Intentionally, drug loaded lipid polymer hybrid nanoparticles (LPHNPs) are developed to overcome all the limitations of polymeric nanoparticles, lipidic nanoparticles and liposomes. LPHN gives dual benefits i.e. of both liposomes and polymeric nanoparticles[3] in which polymeric core coat by lipid layer (Fig. 1). LPHNs restricts the release of water soluble drugs because of its structure in which lipid coats polymeric core, that ultimately leads to increase entrapment efficiency. Therefore in ingenious one can encapsulated oil or water soluble drugs in polymeric core which gives robust structure.[4] External lipid core function as: a) act as biocompatible safeguard b) a model for surface modification c) provides control release of water soluble drug.

At the laboratory scale, there are mainly two methods to fabricate LPHNPs, one is single or one step (self assembled) and another is two-step method. In the two-step method, the lipid shell and the polymeric core are prepared separately and then allowed to fuse together. For this fusion, there are varieties of mixing techniques reported in literature. They include direct hydration,

sonication, or extrusion, to form LPHNPs.[5] To cause the electrostatic interactions to impel the fusion process, the anionic polymeric core is allowed to interact with the cationic lipid vesicle. However, another single-step approach is now preferred in comparison to earlier, because it is quite easier and more convenient to fabricate LPHNs. In this single-step method the lipid, polymer and incorporated drugs are fused together, and LPHNPs formation takes place by self-assemble way. A self-assembly physical method was recently devised for preparation of LPHNPs which having the attractive characteristics of polymeric carrier and lipid carrier.[6]

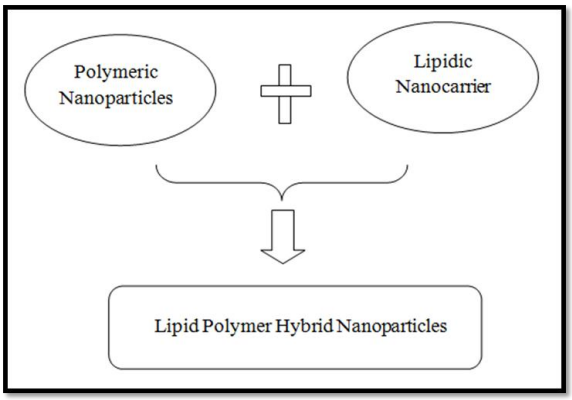

Fig. 1: Schematic representation of LPHNPs

Advantages

- LPHNPs show sustained drug release profile.
- Improve the encapsulation of hydrophobic drug, high drug entrapment efficiency and drug loading capacity for number of drugs compared to liposomes, lipidic nanoparticles, and PNPs.
- LPHNPs have a capability to incorporate hydrophilic as well as hydrophobic drug candidate.
- LPHNPs simultaneously deliver multiple hydrophilic and hydrophobic therapeutic agents.
- The structure provides the lipid monolayer at the interface of hydrophilic core and lipophilic shell reduces water penetration.

Model Drug Selection Criteria

When we are going to select the drug for any drug delivery system, there are some criteria. On the basis of this, the drug should withstand for all parameters.

- The drugs which are having poor aqueous solubility.
- Having poor bioavailability.
- Those who are undergoes into extensive presystemic metabolism.
- Drug which have half life more than of 2 h.

Such can be formulated in LPHNPs. One can incorporate hydrophilic or hydrophobic or ionic (cationic/ anionic) drug in such system, this is one of the important advantage.

Synthesis: LPHNs are prepared by two-step process or by one-step process. The polymeric core and lipoid shell are linked through hydrophobic interaction, Van der Waals forces, electrostatic interactions or other non-covalent forces are involved in it.[7] The hydrophilic polymer stealth layer is often conjugated to the lipid shell through covalent bond. According to nature of drug (hydrophilicity/lipophilicity or anionic/cationic), the formulator is going to choose polymer, lipid, surfactant, cosurfactant and along with method. The details are reported in table no. 1.

Table- 1: LPHNPs formulated previously and reported in literature.

Sr. No.	Drug	Activity/Category	Polymer	Lipid	Method	Reference
1.	Norfloxacin	Antibiotic	Poly lactic acid	Soya lecithin	Emulsion solvent evaporation (ESE)	[8]
2.	Itraconazole	Antifungal	Poly (ε-caprolactone)	Soya lecithin	ESE	[9]
3.	Methotrexate and Aceclofenac	Anti Cancer	Polycaprolactone	Phospholipid S100	Single step self-assembled nano-precipitation technique	[10]
4.	Methotrexate	Anti Cancer	Polycaprolactone	Phospholipon-S100	Single step self-assembled nano-precipitation technique	[11]
5.	Moxifloxacin hydrochloride	Antibacterial	Hyaluronic acid Chitosan	1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine. Egg phospholipid	Ionotropic gelation method	[12]
6.	Paclitaxel and Tetrandrine	Anti Cancer	PLGA	DSPE-PEG	Nanoprecipitation and self-assembly process.	[13]
7.	Methotrexate	Antineoplastic	PLGA	Phospholipid (Lipoid S100) leutrol® F68	Modified Single-level nanoprecipitation technique	[14]
8.	Docetaxel	Anti Cancer	Dextran	Compritol 888 ATO	ESE	[15]
9.	Doxorubicin	Anti Cancer	HPESO (hydrolyzed polymer of epoxidized soybean oil)	Stearic acid,	Ultrasonication method	[16]
10.	Doxorubincin	Anti Tumor	PLGA	DSPE-PEG2k	Single-step assembly method	[17]
11.	Gemcitabine hydrochloride	Anti Cancer	PLGA	DSPE-PEG soya phosphatidylcholine (SPC)	Double emulsion solvent evaporation (DESE)	[1]
12.	Levofloxcine Ciprofloxcine Tobramycin Ofloxcine	Antibiotic	PLGA (Purasorb 5004A)	Stearylamine	DESE, ESE	[18]
13.	Carbamazepine	Antiepileptic	Poly vinyl alcohol	Compritol® 888 ATO(glyceryl dibehenate), Transcutol® Glyceryl tripalmitate	Two step method – high speed homogenization followed by ultrasonication	[19]
14.	Capsaicin	Anti-nociception agent	PLGA	1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC)	DESE	[20]
15.	Enoxaparin	Anticoagulant	Chitosan	Glyceryl monooleate	Self-assembly process fixed with an ultrasonic treatment.	[6]
16.	Amphotericin B	Anti Fungal	Gelatin	Lecithin	Two-level desolvation Method	[21]
17.	Zidovudine	Anti HIV	Pemulen TR-2®NF	Acconon®CO-7 (PEG-7 glyceryl cocoate)	Melt emulsification-probe sonication	[22]
18.	Verapamil	Antiarrhythmic	Pluronic F68	Dodecanoic acid	Modified emulsion method followed by ultrasonication	[23]
19.	Tamoxifen	Anti Cancer	Chitosan	Lecithin (70% phosphatidylcholine)	Modified emulsion solvent evaporation MESE	[24]

20.	Doxorubicin	Anti Cancer	PLGA	DSPE-PEG	Single-step sonication technique	[25]
21.	Levofloxacin	Anti Bacterial	PLGA	Phosphatidylcholine	ESE	[26]
22.	Verapamil HCl	Antiarrhythmic	Dextran sulfate	Compritol ATO 888 Precirol ATO 5 dodecanoic acid	Co-precipitation method	[27]
23.	Folfirinox	Anti Cancer	PLGA PLA	DSPE-PEG	Double emulsion method	[2]
24.	Adriamycin	Anti Cancer	PLGA	DSPE-PEG	Single-level nanoprecipitation method.	[28]
25.	Paclitaxel	Anti Cancer	PLGA	DSPE	Nanoprecipitation method	[29]
26.	Doxorubicin and mitomycin	Anti Cancer	Pluronic F68 (PF68)	Myristic acid	Microemulsion technique	[30]
27.	Vancomycin	Anti Bacterial	Eudragit RS100	Glyceryl tripalmitate	Hot high pressure homogenization (HHPH)	[31]

A) Two step method

Two step process involves the preparation of polymeric core and lipid core individually. Then merge the both individually prepared core by needle extrusion, high pressure homogenizer or by simply vortexing. Polymer core can prepare through emulsion method, high pressure homogenizer or nanoprecipitation. The different method used to fabricate LPHNPs are depicted in **Fig. 2**.

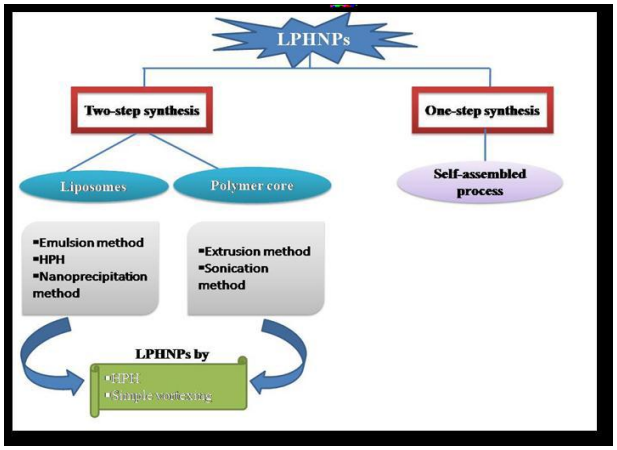

Fig. 2: Methods used for fabrication of LPHNPs.

Double emulsion solvent evaporation method (DESE)

Double emulsion solvent evaporation method is also one of two step method for preparing LPHNPs. If the drug is hydrophilic and cannot be dissolved into the organic solvent, a water-in-oil-in-water (w/o/w) double emulsion method is needed to prepare the polymer core. In this, water-in-oil (w/o) emulsion is prepared by adding a hydrophilic drug containing aqueous solution into a polymer-containing organic solvent. The resulting w/o emulsion is subsequently added into a second aqueous solution to form w/o/w double emulsion.[18] The double emulsion is then hardened in an aqueous solution by evaporating the organic solvent. Generally, this emulsion method involves multiple steps in preparation and also use of high energy homogenization which further results in large polymer particles yielding high polydispersity index. During the preparation heat is avoided in this method, which is the most important advantage for thermolabile drug candidates.

A) One-step method

One-step methods of LPHNPs preparation include the modified solvent extraction/evaporation and nanoprecipitation method. Compared with the two-step methods, one-step methods save time and are convenient, so have been studied to a greater extent and used in more applications. Examples of one-step methods are the emulsion evaporation method, the modified solvent extraction/evaporation method, the ultrasonic method, the high-pressure homogenization method, thin-film hydration, the ultrasonic dispersion method, and the nanoprecipitation method.

1. Emulsion solvent evaporation method (ESE)

This method is used for the drugs which are soluble in water immiscible solvents i.e. in oil phase.

In this type of process, very first step is to form the organic phase and then formation of aqueous phase. Organic phase is prepared by dissolving the polymer in organic solvent such as dichloromethane (DCM), ethyl acetate etc. Followed by, drug dissolution or dispersion in polymer solution. The aqueous phase is prepared by dissolving the lipid and finally aqueous phase is added to the organic phase lead to form O/W system. After formulating the stable solution, the removal of organic solvent should be done by evaporation under reduced pressure or by continuously homogenization.[26]

2. Modified solvent extraction/evaporation method (MSE)

In modified solvent extraction/evaporation method, the polymer and the drug were dissolved in a water-insoluble organic solvent such as dichloromethane, chloroform, or ethyl acetate. The lipid was then mixed with water to form an aqueous phase. The organic phase was added to the aqueous phase, the suspension was dispersed by ultrasound, and the organic phase was transformed into tiny nanoparticles. The nanoparticles were coated with a lipid layer, and the samples were separated and purified by organic solvent removal. Due to the limited solubility of lipid in organic solvent, the obtained suspensions are fairly diluted. There are limited applications of LPHNPs by this method.[24]

3. Ultrasonic method

In an aqueous surfactant solution, melted lipids, drug and polymer are added and dispersed under probe ultrasonication. The sample was then cooled down and solidified to form NLCs.[22] This method is effortless and requires usual tools that are available in a laboratory. The disadvantage of this method is the low dispersion quality. The dispersion quality of the LPHNPs produced by these methods is often affected by the presence of microparticles, leading to physical instability upon storage. The lipid concentration is low (<1%) and the surfactant concentration is comparatively high. Metal contamination is the other important problem with ultrasonication.

4. High-pressure homogenization method (HPH)

High pressure homogenization refers that the polymer and drug are heated and melted, then they are dispersed in aqueous phase which having the presence of lipid. This step leads to form a primary emulsion is then subjected to high pressure, and decompression expansion, after which the fluid droplets are gradually broken down by high shear forces to the desired nanoparticulate diameter range. This method can be successfully used to insoluble i.e. lipophilic drugs, is also suitable for hydrophilic drugs. The advantages are avoidance of organic solvents and suitable for large-scale production at industrial scale up.[19]

The method is basically of two types namely, hot HPH and cold HPH. In hot HPH, the temperature of system is increased and can cause the degradation of thermolabile drugs. This hot HPH technique is more convenient for thermostable drugs. In cold HPH, additionally chillers are used to reduce the increased temperature due to high pressure. This cold HPH method is more suitable for thermolabile drugs.

5. Thin-film hydration and ultrasonic dispersion

In this method, the polymers and drugs are dissolved in an appropriate organic solvent, subjected to vacuum evaporation to remove the organic solvent, and used to produce a layer of polymer film. A lipid aqueous solution is added, and the mixture is subjected to ultrasonic dispersion using an ultrasound probe to form LPHNPs. This method is most habitually used due to its ease and practicality, and its ability to yield small and uniform particles.[32]

6. Nanoprecipitation method

The main advantage of this nanoprecipitation method is its rapidity and reproducibility for fabricating LPHNPs. In this approach, polymers and drugs are first dispersed in a solvent miscible with water (e.g., acetone and acetonitrile). Then, the solution is dropped into a lipid-containing aqueous phase, and the nanoparticles are mixed by spinning and homogenization. This preparation method is based on the principal of emulsification. This method involves use of a lipid substitute for surface-active agents then lipids, polymers, and drugs are dissolved in the oil phase, and the oil phase is mixed with the water phase to form an O/W emulsion. The hydrophobic region of the lipid attaches to the polymer core, and the hydrophilic end of the lipid extends to the aqueous phase to efficiently form LPHNPs.[14] Toxicological problems may result from solvent residues since the product obtained by this method.

Use of Lipids in Nanoparticles

A lipid coat over the NPs may increase its dissolution and there by bioavailability. Furthermore it provides protection to the particulate system from drug retention as well as water permeation. PEG can also be linked as a targeted ligand.[33] There are different types of lipid reported in table- 2.

Table. 2: Different types of lipids.

Fatty acids	Cationic lipids	Glycerides	Hard fats
Saturated • Capric acid (C_{10}) • Lauric acid (C_{12}) • Myristic acid (C_{14}) • Stearic acid (C_{18}) and its sodium salt • Behenic acid (C_{22}) • Palmitic acid (C_{16}) • Palmitic acid (C_{16}) **Unsaturated** • Oleic acid (C_{18}) • Docosahexanoic acid (C_{22})	• N[1-(2,3dioleyloxy) propyl]-N,N,N trimethylammonium chloride (DOTMA) • Dioctadecylamidoglycyl spermine (DOGS) • 1,2-dipalmitoyl-3-trimethylammonium- propane (DPTAP) • [N-(N',N'-dimethylaminoethane)- carbamoyl] cholesterol (DC-Chol)	• Glyceryl behenate (*Compritol ®888 ATO*) • Glyceryl behenate (mono) • Glyceryl palmitostearate (*Precirolol ®ATO 5*) Glyceryl monostear ate (*Imvitor®9 00*)	Witepsol ® of the following series • W- with higher hydrox yl value* (*Witeps ol®W 35*) • H- with low hydroxyl value* (Witeps ol®H 35, H 42) • E- with melting points above body temperature (*Witepsol®E*

APROCHES of LPHNPs

Recently based on literature[34] the various types or approaches for LPHNP are as follows

I.Polymer core lipid shell nanoparticles (PCLSNPs)
II.Hollow core/shell lipid- polymer- lipid hybrid nanoparticles (HSLPLHNPs)
III.Lipid bilayer coated polymeric particles (LBCPNPs)
IV.Polymer caged nanoparticles (PCNPs)

I. Polymer core lipid shell nanoparticles (PCLSNPs)

As the name indicates for this type of nanoparticles, the polymeric core is surrounded by lipid. Here the lipid membrane may be one or more. The aqueous buffer or water is placed in between the polymeric core and lipid membrane. In this kind of system, the polymer deals with delay in drug release and enhanced the lipid shell stability as shown in Fig. 3. This system is much suitable for hydrophobic drugs but not for hydrophilic drugs. This can be overcome by using complex of polymer and lipid. For the same, well reported example in literature[35], Salidroside (Sal) is the potent antitumor drug with high aqueous solubility. PCLSNPs were developed by using PLGA-PEG-PLGA triblock polymer and lipids (lecithin and cholesterol), this kind of incorporation gives better entrapment efficiency, small particle size and increased tumor cell uptake.[35] PCLSNPs have limitations of drug properties such as, hydrophilicity and another is anionic charge. Furthermore such problems are solved in literature. Alginate coated chitosan core shell nanoparticles for oral delivery of enoxaparin were prepared by using cationic polymer chitosan (opposite charge that of drug) which act as a charge stabilizer.[36] Here we are highlighting the critical parameters in the production of PCLSNPs. They are as - 1) Ratio of drug to polymer concentration. An improper concentration leads to formation of lumps which ultimately fails the nanoparticulate formulation. 2) Presence of charge which leads to the interaction between drug and polymer that causes instability to the nanoparticulate formulation.[15]

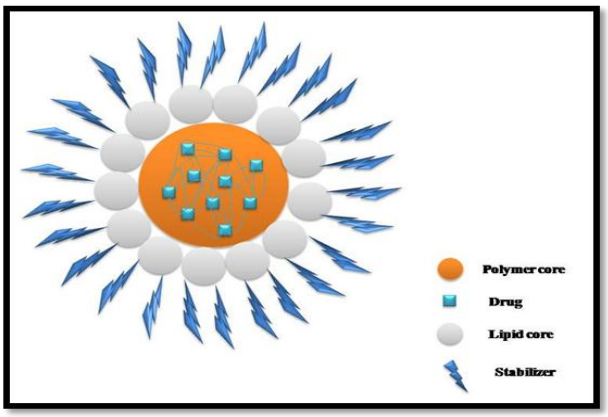

Fig. 3: Polymer core-lipid shell NPs.

II. Hollow core/shell lipid- polymer- lipid hybrid nanoparticles (HSLPLHNPs)

As compared to other LPHNPs, this type of system is different because they exhibit dual properties i.e. of PEGylated lipoplexes as well as of PLGA nanopaticles.[37] The double emulsion solid evaporation technique (DESE) can be used for the production of HSLPLHNPs. The main function of PEG lipid layer in this system is allow the particle to evade its recognition as a macrophage, enhanced stability during circulation, slow drug release pattern and protection from polymer degradation.[38,34] The polymer layer which is at middle provides sustained drug release.[34] The anionic drug is more efficiently encapsulated in positively charged hollow core (formed by cationic lipid) than polymer alone.[39] Sometimes combination of lipids may used to compose an outer layer which provides the stability of system. One lipid may self assemble itself at water-oil interphase with their hydrophilic head facing towards aqueous droplet and the tail towards polymeric phase and thus forming a complex with PEG. While formulation of such complexes, the both lipid must be below their respective critical micelle concentration, otherwise risk of liposome and micelle formation could be increased. Nonetheless, the important parameters to be considered for its development are inner cationic lipids, outer PEG chain length, middle polymer composition /molecular weight.[40] For the development of this HSLPLHNPs system the important parameters are: 1) inner cationic lipids, 2) outer PEG chain length, and 3) composition of middle polymer.

III. Lipid bilayer coated polymeric particles (LBCPNPs)

To enhance the residence time, bypass of macrophage uptake and systemic clearance are the most important parameters of nanoparticles. Hence in LBCPNPs there are two lipid layers which are blend of membrane vesicle and particles. Various approaches were used to enhance residence time, from which the PEGylation is mostly preferred one but its immunological responses have been reported for a limited number of cases. Then to enfold the nanoparticles RBCs were used because they have potential for long time circulation which may easily protect it from macrophage uptake. It has been reported that this combination could retain the functionality of membrane-associated protein.[41] RBC-membrane derived vesicles were formed from RBC and conjugated with PLGA NPs to obtain RBC-membrane-camouflaged PNPs.[42] The LBCPNPs provide the sustained release in a better way than the liposomes and traditional PNPs. Moreover, RBC may exhibit slower release because it possesses denser lipid barrier against drug release. Besides the advantages it has the drawback that different blood groups have different types of antigens on the surface of the erythrocytes that have to be cross matched during blood transfusion which is really a challenging aspect.[43]

IV. Polymer caged nanoparticles (PCNPs)

Basically these are liposomes, for better effect they made up with the surface modification of polymer. Polymer-caged liposomes are more stable and possess pH-sensitive behavior.[44] Due to this not only change in surface properties but drug release also flexible. Cross linking of polymer offers enhancement of stability and reduction in drug leakage of liposomal NPs which is represented in Fig. 4. This cross linked polymer cage gives shield of drug and often serves as a pH-responsive trigger to enhance drug release in the acidic environments generally seen in solid tumors and endosomes. Varying in degree of cross linking in polymer cage provides the surface potential for *in vivo* circulation lifetime of the nanocarriers to be tuned. During this, pH-sensitive lipid components[45], such as dioleoylphosphatidylethanolamine[46] and acid-labile PEG,[44,47] have been used to enhance drug release, which have a size of 100 nm and are called polymer-caged nanobin (PCN). Addition of targeting ligand or imaging agent is possible by chemical modification. The lipid shell is surrounded by steric stability and repulsion of drug is decreases due to polymer cage. At low pH targeted site the drug release is restricted by surface of polymer cage such as tumor interstitium and cellular endosomal vesicles.[48]

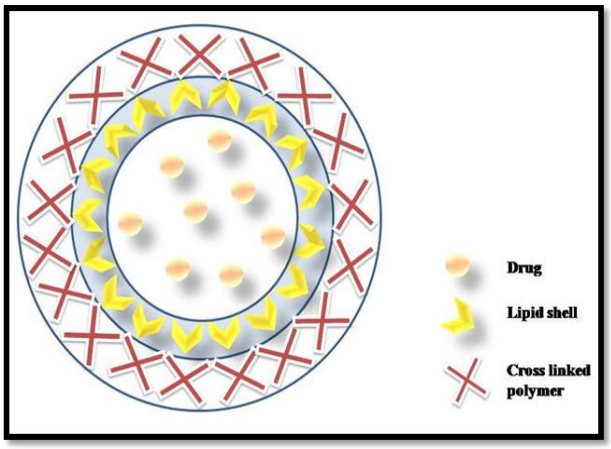

Fig. 4: Polymer caged NPs.

Surface Functionalization of Lipid Polymer HYBRID Nanoparticles

To maintain the stability of the LPHNPs the PEG layer is essential, both in *in vitro* by reducing nanoparticulate aggregation and in *in vivo* by allowing the particles to avoid recognition by the reticulo-endothelial system (RES) and also from other immune cells. Moreover, the PEG molecules also provide functional groups for further modification of the LPHNPs with targeting ligands for cell or tissue specific drug delivery. Many targeting ligands have been disclose by researchers including monoclonal antibodies, antibody fragments, aptamers, peptides and small molecules such as folic acid. In principle all these targeting ligands can be conjugated with the lipid polymer hybrid nanoparticles to enhance delivery efficiency.[7]

Characterization of Lipid Polymer Hybrid Nanoparticles (LPHNPs)

1. Particle size, zeta potential and polydispersity index (PDI)

Particle size is a surface morphology of formulated nanoparticles. Generally we say that particle size of nanoparticles should be in the range of 100-300 nm which prevent the presystemic metabolism by absorbing through the lymphatic transport system instead of portal vein or hepatic pathway. There are some techniques for measuring particle size of LPHNPs are DLS (Dynamic Light Scattering), SEM (Scanning Electron Microscopy), TEM (Transmission Electron Microscopy), AFM (Atomic Force Microscopy) also helps to measure particle size in addition to particle shape. Hydrodynamic size and distribution can be measured by DLS. This method is quick and not so complicated. It does not require any additional sample before measurement. Nevertheless when the particles are not identically spherical, then this technique does not provide definite physical size of particles and posses large polydispersity index (PDI). A narrow PDI reveals about particle homogeneity in the prepared NPHNPs. Most researchers accept the PDI value around or less than 0.3. Zeta potential (mV) is the potential at the hydrodynamic shear plane and can be determined from the particle mobility under an applied electric field. This is also measured by DLS method using Zetasizer at standard temperature and experimental conditions.[49] Nanoparticles with a zeta potential between -10 and +10 mV are considered approximately neutral, while nanoparticles with zeta potentials of greater than +30 mV or less than -30 mV are considered strongly cationic and strongly anionic, respectively. For better stability of LPHNPs the zeta potential should be in the range of +30 mV to -30 mV.

2. Entrapment efficiency (% EE) and Drug loading (% DL)

Entrapment efficiency percent (EE %) was determined by measuring the concentration of unentrapped free drug in aqueous medium.[8,50] The aqueous medium was separated by centrifugation. The EE (%) and drug-loading percentage (DL %) were calculated by using Equations 1 and 2 as

$$EE(\%) = \frac{\text{Total weight of drug used} - \text{Free unentrapped drug}}{\text{Total weight of drug used}} \times 100$$

----------Equation 1

$$DL(\%) = \frac{\text{Weight of drug used - Drug in suppernatent}}{\text{Weight of lipid used}} \times 100$$

----------Equation 2

3. Differential scanning calorimetry (DSC)

DSC is a thermal analysis apparatus measuring how physical properties of a sample change, along with temperature against time. In other words, the device is a thermal analysis instrument that determines the temperature and heat flow associated with material transitions as a function of time and temperature. DSC is executed by placing the sample (Sample size: from 0.5 to 2 mg) in pan (pans of Al, Cu, Au, Pt, and or graphite) to avoid reactions with samples and with regard to the temperature range of the measurement, then it is crimped with hydraulic press at specified atmosphere (atmospheres: nitrogen, air, oxygen, argon, vacuum, controlled or mixed gases), flow rate, and temperature. The thermogram of individual components (pure drug, lipid, polymer) and physical mixture are compare with drug loaded LPHNPs for final interpretations. Peak of pure drug missing in drug loaded LPHNP formulation that indicates the crystalline nature of drug is converted into amorphous form and the thermogram of physical mixture will help us to interpret the compatibility between drug and used excipients.[9]

4. Fourier transform infrared spectroscopy (ATR-FTIR): FTIR spectroscopic analysis is performed to detect any possible physicochemical interaction between the formulation components.[19] In this technique the solid lyophilized sample is used as in case of DSC study. The infrared spectra of LPHNPs loaded drug, which is composed by lipid, polymer, stabilizer should be perform (range: 4000-400 cm^{-1}). Individual infrared spectra of each component is compare with drug loaded LPHNPs. The infrared spectra of pure drug and drug loaded LPHNPs when compared it will shows the absence of drug peak in LPHNPs which clearly indicates the crystalline nature of drug is converted into amorphous in LPHNPs.

5. Powdered X-Ray diffraction (pXRD).

X-ray diffraction is a unique method to determine crystallinity of a compound. Pure drug, physical mixture and the optimized formulation are subjected for pXRD analysis. All the XRD diffractogram or patterns are recorded at ambient temperature at 2θ diffraction angle in a range of 0 to 40°.[14] Diffractogram of pure drug is always compared with drug loaded LPHNPs for interpretation. Specific 2θ peaks of drug are compared here. Nonexistence of 2θ peaks of drug in LPHNPs indicates the conversion of crystalline state of drug into amorphous.

6. *In vitro* drug dissolution study

The *in vitro* studies refer to study the biological characteristics of drug without using animal or human models as these *in vitro* tests predicts the exact *in vivo* behavior. For the nanoparticles, generally dialysis bag (according to the molecular weight or size) are used. These are also called as dialysis tubing and method is reported in literature as modified beaker method. Dialysis membrane act as a semi permeable membrane which allows the flow of minute drug molecules based on differential diffusion. For this experiment, 0.1 N HCl (pH 1.2) and phosphate buffer saline (PBS) pH 6.8 are used as a dissolution (200 – 250 mL) medium.[21] Membrane is allowed to soak overnight in double distilled water or dissolution media. Specific amount of drug equivalent optimized LPHNPs are transferred to dialysis bag. Then sealed firmly on both ends bags are dispersed in dissolution media. Initially drug release in 0.1 N HCl (pH 1.2) for 2 h followed by PBS pH 6.8 for remaining h. The drug release study period is depending upon the half life of drug along with used polymer and lipid system. At specific time interval, the amount (5 mL) of medium is replaced with fresh medium of same temperature to maintain the sink condition. Finally the amount of drug release in medium is determined by (at respective wavelength i.e. λmax of drug) using UV visible spectroscopy or suitable analytical technique such as HPLC/UPLC.[51]

7. Kinetic modelling

In vitro dissolution has gained prime importance in the drug delivery development. Under certain conditions it can be used as a surrogate for the assessment of Bioequivalence. Several kinetics models/ theories from literature describe the process of drug dissolution from particular LPHNPs. Literature suggests several models that represent the drug dissolution profile where f_t is a function of time (t) related to the amount of drug dissolved from the pharmaceutical formulation such as Zero order kinetics, First order kinetic, Higuchi model, Hixson crowell model, Weibull model, Korsmeyer–Peppas model etc as.[31]

a) Zero-order model:

$Q=k.t+Q_0$---------- Equation 3

b) First-order model

$Q=Q_0e^{kt}$ ---------- Equation 4

c) Higuchi model

$Q=k.t^{1/2}$---------- Equation 5

d) Hixson–Crowell model

$Q^{1/3}=kt+Q_0^{1/5}$-------- Equation 6

e) Weibull model

$Q=1\exp[-(t)^{b/a}]$------ Equation 7

f) Korsmeyer–Peppas model

$Q=k.t^n$---------- Equation 8

Where

Q = the amount of drug released in time t,

Q_0 = the start value of Q,

k = the rate constant,

a = the time constant, and b is the shape parameter,

n = the release or diffusional exponent, an indicative of drug release mechanism.

To understand the release kinetics (best fit model) and dissolution enhancement (model independent parameter), the drug release data are used to calculate the squared correlation coefficient (r^2) and mean dissolution time (MDT) using software. The optimized LPHNPs have follows one of the above release kinetic models based on closeness of squared correlation coefficient (r^2) to 0.999 or 1. Peppas[52] used this "n" (release exponent) value to interpret or characterize the different release mechanisms such as Fickian or non Fickian model. This kinetic modeling with *in vitro* dissolution data also involved in calculation of dissolution efficiency.

8. Pharmacokinetic study

Pharmacokinetic study gives the measurement of pharmacological response of drug (LPHNPs formulation) onto the body. Here the trials are taken on the animal models like rats, rabbits, guinea pig etc. This is also referred or called as preclinical studies. According to the study, the animal model is chosen and they are divided into groups. Generally two groups are referred as a test and standard group. They are placed in suitable cages present in the animal house with provided laboratory conditions like ambient temperature of 25–30°C and 45–55% relative humidity with a 12 h dark and light cycle. Animals are fed with a pellet diet along with water libitum. All the rats generally kept on fasting state of 12 h before the experiment are needed to start. But have free access to water. Depending on the route of administration of developed/formulated system, the dose and blood withdrawal site will be finalized. These sites may be oral, intravenous, nasal etc. In standard group the pure drug suspension is given and for test group the developed LPHNPs formulation is given. After specific interval of time, specific volume of blood (0.5 to 1 mL) is withdrawn from site. These blood samples are subjected for centrifugation to separate the plasma. The drug plasma concentration is estimated by using suitable bioanalytical technique such as HPLC or UPLC like analytical techniques. The conditions for analysis like type of column, mobile phase composition, flow rate, and internal standard etc. should be referred from literature. The results of bioanalysis are analyzed with the help of kinetica software[29] to estimate C_{max}, T_{max}, AUC. The bioavailability is calculated from AUC of two groups. There are two types of bioavailability: one is absolute bioavailability (F) and another is relative bioavailability (Fr). The measurement of bioavailability is done by using following equation:

$$F = \frac{(AUC)\,oral}{(AUC)\,iv} \times 100$$ ----------Equation 9

$$Fr = \frac{(AUC)test}{(AUC)std} \times 100$$ ----------Equation 10

In vivo studies need to be conducted as per the guidelines laid down by CPCSEA (committee for the purpose and control and supervision of experiments on animals) of country. Prior to the study the proposals should get sanctioned from IAEC (institute animal ethical committee).

9. *In vitro in vivo* correlations (IVIVC)

A simple in vitro dissolution test on the drug product will be insufficient to predict its therapeutic efficacy. Establishing correlations in between *in vitro* dissolution test and *in vivo* studies gives the guarantee of reproducibility of biological response from pharmaceutical formulation. The main objective of developing and evaluating an IVIVC is to enable the dissolution test to serve as a surrogate for *in vivo* bioavailabity studies in human beings.[53]

These correlations are of three types 1. Correlations based on the plasma level data, here few *in vitro* dissolution parameters such as % cumulative release, MDT etc are compared with AUC, Cmax, K_a (absorption rate constant), MRT (mean residence time) etc. 2. Correlations based on urinary excretion data, here the above cited *in vitro* dissolution parameters are compared with the amount of drug excreted unchanged in the urine, cumulative amount of drug excreted as a function of time (t), and 3. Correlations based on the pharmacological response, here the above cited *in vitro* dissolution parameters are compared with an acute pharmacological effect such as LD_{50} in animals.

Three IVIVC levels have been categorized as level A, B, and C. Level A is highest category of correlations. *In vitro* dissolution and *in vivo* absorption curves should be superimposible. In level B, both curves are not expected to become superimposible but few points should match such as MDT with MRT, whereas level C is based on single point correlation, one dissolution time point should match to AUC, C_{max}, or t_{max}.

10. Accelerated stability studies and estimation of shelf life (i.e. expiry date)

The accelerated stability studies are carried out according to international conference on harmonisation (ICH) Q1A (R2) guide lines (2003) (FDA, 2003), with the optimized LPHNPs formulation. Sealed vials of freshly prepared freeze-dried nanoparticles subjected in stability chamber maintained at 25 ± 2 ◦C/60 ± 5 % RH. Nanoparticles dispersions subjected to stability test were analyzed over 3 month period for particle size, PDI, zeta potential, % EE and drug content in nanoparticles with a sampling frequency of one month. Here the *in vitro* dissolution profile of optimized LPHNPs formulation before and after stability is compared to calculate similarity factor (f_2). The f_2 values can be calculated using equation 11 and should be in the range of 50 – 100. Values closer to 100 indicates excellent similarity in dissolution profile where as below 50 indicates poor results.[52] Drug content data can be analyzed using the Sigma Plot software to establish the expiry date or shelf life of optimized LPHNPs.[54]

$$f2 = 50 \times \log\left(1 + \left(\frac{1}{n}\right)\sum_{j=1}^{n}(Rj - Tj)^2\right) - 0.5 \times 100$$-----Equation 11

APPLICATIONS

LPHNPs are having the various versatile applications such as in drug delivery, gene delivery, delivery of diagnostic agents, and delivery of drug conjugates. The previously reported applications in literature are summarized in table - 3.

Table. 3: Various versatile applications of LPHNPs.

1.	Drug delivery	[55]	LPHNPs are desirable for: Intracellular drug release, Targeted drug delivery, Protection of normal tissues, Reduction of adverse effect to the system.
		[56,57]	LPHNPs act as a carrier for anti-angiogenesis, anticancer/antibiotic drugs. They inhibit the growth of tumor cells, where as anticancer agent interact with topoisomerase II which leads to termination of tumor cell inducing apoptosis.
2.	Delivery of diagnostic imaging agent		LPHNPs are used to deliver variety of imaging agents such as ion oxide, fluorescent dyes, and quantum dots by incapsulating them into polymer.
		[58]	Metallic gold nanocrystals (AuNPs) and quantum dot loaded LPHNPs can be formulated through the nanoprecipitation method. In the center of lipid and polymer, the quantum dots located and then linked by an esterification reaction, thus forming PLGA polymer conjugates.
		[3]	Quantum dots used to replace the hydrophobic polymer to fabricate lipid quantum dots LPHNPs by rapid mixing method within a microfluidic device.
3.	Gene delivery	[59]	LPHNPs exhibit high stability and good bioavailability compound with liposomes and PNPs. In one preparation of LPNs containing DNA, unmodified LPNs were composed of a PEI core and a polycarbonate three OA glycerin ester two stearic acylphosphatidylcholine lipid shell. Modified LPNs were delivered to HEK 293 cells and MB MDA 231 breast cancer cells. The transfection efficiency was higher than when using liposomes; the colloid was more stable, and the toxicity to the HEK 293 and MB MDA 231 breast cancer cells by the PLNs was low.

4.	Delivery of drug conjugate	[29]	The preparation of LPHNPs by pass the BBB (blood brain barrier) by targeting to rich glioma cells.
5.	Protein delivery	[5]	LPHNPs used to deliver proteins/peptides. They preserve their stability and release them in a controlled manner. The LPHNPs showed greater encapsulation efficiency.

Future Perspective

Lipid-polymeric hybrid nanoparticles may be produced with different polymers and solid lipids of synthetic or natural origin, but their combination must be carefully regarded to avoid undesirable interactions with each other and with the drug, negatively affecting the carrier features. Thus, the interactions between polymers, lipids, and drugs within the nanoparticulate formulation need to be clearly debriefed to assure the drug stability and the mechanisms of drug release and targeting. When the composition of hybrid nanoparticles is properly regarded, they can load a wide array of drugs to be administered through different routes and according to the therapeutic target.[60] The polymers of hybrid nanoparticles may play important roles in prolonging nanoparticles half-life in the bloodstream, protecting the drug stability, creating a diffusion barrier that can control the release kinetics of drugs and also act as targeting moiety for specific drug delivery. The characterization of LPHNPs should be conducted at pharmacodyanamic as well as pharmacokinetic level. Results of both the (*in vitro* and *in vivo*) studies should be compared to establish IVIVC. Stability studies should be investigated by researchers at long term level. The data of this stability studies should be used to estimate expiry date, Furthermore the efforts should be taken for industrial scale up. The hybrid nanoparticles have been mainly used in cancer therapy due to their ability for target drug delivery, increasing the therapy efficacy and reducing potential side effects and toxicity; or even used to deliver proteins such as insulin for oral administration due to the ability of carriers to protect proteins from enzymatic degradation and increase the intestinal uptake. These abilities of nanoparticles have the potential to drastically improve the quality of life of patients. Besides the positive results obtained so far, further work is needed to deeply understand the interactions of hybrid nanoparticles with cells, revealing potential toxicity issues and showing their safe application. The ongoing developments of lipid-polymeric hybrid nanoparticles have proposed these carriers as good candidates to deliver a wide array of drugs and have ability to capture the market in near future. Therefore, the quality of life of patients may be drastically improved and severe pathological conditions may be treated by these promising carriers.

CONCLUSION

A variety of both hydrophilic as hydrophobic drugs can be loaded into lipid polymer hybrid nanoparticles with high encapsulation efficiency due to the association between polymers and solid lipids that favor the drug partitioning in the lipid phase and also improve the release profile, resulting in a controlled drug release. There are different approaches to formulate desire LPHNPs. Furthermore, these nanoparticles could be administered by several routes to have access more specific to the site of action (targeted approach).

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