

## Lipid-Polymer Hybrid Nanoparticles for Topical Drug Delivery System

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### Abstract

Human skin not only functions as a permeation barrier (mainly due to the stratum corneum layer), but also provides a unique delivery pathway for therapeutic and other active agents. These compounds penetrate via intercellular, intracellular and transappendageal routes, resulting in topical delivery (into skin strata) and transdermal delivery (to subcutaneous tissues and into the systemic circulation). Lipid-polymer hybrid nanoparticles (LPHNPs) are next-generation core-shell nanostructures, conceptually derived from both liposome and polymeric nanoparticles (NPs), where a polymer core remains enveloped by a lipid layer. Although they have garnered significant interest, they remain not yet widely exploited or ubiquitous. Recently, a fundamental transformation has occurred in the preparation of LPHNPs, characterized by a transition from a two-step to a one-step strategy, involving synchronous self-assembly of polymers and lipids. Owing to its two-in-one structure, this approach is of particular interest as a combinatorial drug delivery platform in oncology. In particular, the outer surface can be decorated in multifarious ways for active targeting of anticancer therapy, delivery of DNA or RNA materials, and use as a diagnostic imaging agent.

**Keywords:** Lipid-polymer hybrid nanoparticle, Topical delivery, Drug delivery, Gene delivery.

## Introduction

Nanotechnology is a compelling medicinal platform with the potential to greatly impact the delivery of a plethora of therapeutics, encompassing small molecule therapeutics, genes, RNAs, peptides, and diagnostic imaging agents, as well as holding great promise for improving the therapeutic index and pharmacokinetics of several drugs under systemic settings<sup>1-4</sup>. In general, these payloads are encapsulated within or covalently grafted on the surface of the nanocarriers, and after being systemically incorporated, their release is monitored by factors such as formulation of the matrix, pH of the microenvironment, and temperature of the surroundings<sup>5-7</sup>. The inherent potential of nanoparticles (NPs) for therapeutic cargo delivery is primarily attributable to a few key parameters, including average nanometric size, homogeneity, surface potential, and drug loading, among others<sup>8,9</sup>. Surface-coated immuno-inert NPs can also skillfully bypass the reticuloendothelial system yielding increased bioavailability of encapsulated drugs<sup>10</sup>. The plausible advantages of nanocarriers are summarized as follows: 1) improvement to a drug's overall pharmacokinetic and pharmacodynamic properties without alteration of its molecular structure; 2) enhanced effective tissue targeting, cellular targeting, and molecular targeting; 3) the ability to circumvent many inherent biological impediments; 4) targeted and nontargeted drug delivery to their respective site of action (cytosol, nucleus, etc) and enhanced therapeutic index of the drug; 5) delivery of multiple drugs with differing chemical properties<sup>11,12</sup>. The barrier function of skin can be attributed

mostly to the stratum corneum layer of the epidermis, and this skin barrier also regulates the transport of compounds into the skin. Approaches that deliver drugs/active compounds through the skin barrier are referred to the topical route of administration (as opposed to the enteral and parenteral routes of administration). Passive and active skin penetration enhancement methods have been successfully used to improve the efficiency of either the topical delivery (the drugs/active compounds are delivered into skin strata), or transdermal delivery (drugs/active compounds are delivered into subcutaneous tissues and are taken up systemically into the body). Topically applied therapies are promising for the treatment of skin diseases such as psoriasis, contact dermatitis, and skin cancers, since the drugs are delivered directly into skin stratum<sup>10</sup>. Nano-sized drug carriers have attracted much attention in the past decade as options in formulations for topical therapy. Nanoparticles (including nanospheres, solid lipid nanoparticles, micelles, and micellar-like nanoparticles), liposomes, and nanoemulsions are among the most studied systems. Inorganic and metal nanoparticles such as quantum dots and gold nanoparticles are widely used for diagnosis, and are not in the scope of this review. Rather, this paper focuses on topically applied, polymeric nanoparticle-based drug delivery systems. Nanoparticles made of natural polymers (e.g. chitosan) and synthetic biodegradable polymers (e.g. poly(lactide-co-glycolide) and poly( $\epsilon$ -caprolactone)) as well as non-degradable polymers (e.g. polyacrylates) are discussed. Special emphasis is given to the tyrosine-derived nanospheres since they consist of natural occurring metabolites and have been utilized in several

products already marketed for patients. The most recent advances in the nanoparticle-based topical delivery systems elicited a few interesting questions: do the ultra-small nanoparticles penetrate into skin, can we manipulate hair follicle stem cells via drug-loaded nanoparticles that localize in the hair follicles, and will the less invasive vaccination using the combination of microneedle techniques and vaccine-loaded nanoparticles become routine clinical practice<sup>8,9</sup>. Lipid-polymer hybrid nanoparticles (LPHNs) are emerging nanoparticles drug delivery systems that have advantage of both state i.e. liquid and solid state. LPHNs remains in solid state at body temperature<sup>13</sup>, hence incorporation into the carbopol gel make them easy and consistent delivery of the drug to the targeted site. Due to their existent in both state, they showed a control release of drug. LPHNs are polymeric nanoparticle basically composed of three subsequent layers as 1) an inner hydrophobic core layer where the encapsulation of large amount of hydrophobic drug is possible; 2) an interfacial lipid layer that act as a flexible and biocompatible shell; and 3b) an outer hydrophilic polymer stealth layer to enhance the circulation time and stability of the LPHNs<sup>14</sup>. The coexistence of lipid and polymer possessing different physicochemical properties, such as their lipophilic and hydrophilic behavior lead to design a large variety of delivery system, and also have versatile capability of loading varying types of drugs, (Y.W. Xiao suggested that LPHNs are the best drug delivery system for the highly hydrophilic drug, because these drug possess the problem of rapid clearance from the body and low therapeutic recovery)<sup>13</sup> as well as they can be easily conjugated with the targeting moiety to deliver a drug to its target site. Topical infections cover, localized surface infection due to accidental injury, surgery, abrasion and major complication of burns and topical disease covers bacterial infection, plant warts attack, and fungal growth etc.<sup>15</sup>. Topical antibiotics of lipid-polymer hybrid nanoparticles play an important role to deliver drug in such kind of topical infection and disease, due to its controlled and prolonged drug delivery to the surface of the infection, and avoid frequent application of the medicament to the infected and painful area, hence, lead to an increase in patient compliances.

On the other hand making use of biodegradable, biocompatible and non-toxic polymer such as Poly lactic acid has shown great therapeutic potential as a drug delivery system. PLA is a hydrophobic polymer and exhibits a good mechanical strength widely used in the manufacturing of containers, surgical equipment, and other delivery appliances.

### Advantages of lipid-polymer hybrid nanoparticle

- The solid polymeric core act as a cytoskeleton that provides mechanical stability, controlled morphology, and high available specific surface area.
- The lipid shell enveloping the core is biocompatible and exhibits behavior similar to that of cell membrane.
- Improved encapsulation of hydrophobic drug, high drug entrapment efficiency and drug loading capacity for a number of the drug compared to liposomes or Polymeric Nanoparticles.
- The LPHNs entrap and deliver multiple hydrophilic and hydrophobic therapeutic agents simultaneously.
- Particles smaller than 100 nm are promising for intracellular drug targeting<sup>13</sup>.

### Topical and transdermal delivery across skin barrier

The permeation barrier properties of human skin elicit challenging but exciting delivery avenues for drugs and other compounds into the skin strata (topical delivery) and/or to the systemic exposure (transdermal delivery). Currently, there are more than 30 transdermal products in the US market, and it is expected that the topical and transdermal drug delivery market will reach \$32 billion in 2015; formulations of a number of low molecular weight drugs and macromolecules have been developed and some are currently under clinical trial. Compared to the other delivery routes, topical and transdermal delivery approaches have the unique advantages: a) for skin diseases, topical delivery approaches directly deliver drugs to the site of the diseases cells/tissues; b) smaller amounts of drugs are needed to produce a therapeutic effect; c) plasma level peaking of drugs will be avoided; d) increase bioavailability due to elimination of hepatic first-pass metabolism; and e) greatly enhanced patient compliance by eliminating frequent dosing.

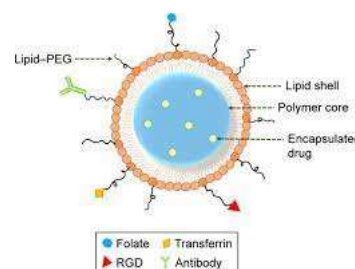
The cutaneous penetration pathways are a) through stratum corneum via intercellular/intracellular routes, followed by the viable epidermis and dermis via partitioning/diffusion; and b) through the appendageal pathway. These routes result in topical and transdermal delivery<sup>14</sup>.

### Passive and active skin penetration enhancement methods

It was not until 1960s-1970s that the scientists reached the consensus that a) the stratum corneum is the rate-limiting barrier against percutaneous drug penetration, and b) the specific content, composition and structure of the stratum corneum lipids selectively and effectively inhibit the penetration of chemicals. In addition, not all the compounds can penetrate through the stratum corneum barrier; the ones with moderate lipophilicity (octanol-water partition coefficient between 10 and 1000) and molecular weight less than 500 Daltons are able to permeate the stratum corneum and penetrate into deeper layers of skin<sup>8</sup>.

### Structure elucidation and mechanism of hybrid formation

As can be inferred from their name, LPHNs merge the features of both polymeric NPs and liposomes. They consist of three building blocks as illustrated in Figure 1. These are 1) a polymer core encapsulating the drug, 2) a lipid monolayer surrounding the polymer core, and 3) an outer lipid-PEG layer, a steric stabilizer prolonging systemic circulation of the LPHNs by evading immune destruction. The middle lipid monolayer behaves like a molecular barricade that mitigates the loss of entrapped drugs over the course of the LPHNP formulation and protects the core from degradation by preventing the diffusion of water into the inner core<sup>16,17</sup>.



**Figure 1: Structure of a lipid-polymer hybrid nanoparticle (LPHNP) comprises of a polymer core encapsulating a payload, a lipid shell, and an outer lipid-PEG layer<sup>20</sup>.**

The molecular mechanics of fusion between lipid and polymer is still under investigation. It is apparent that distinguished methods of LPHNP manufacturing have different mechanisms of formation. For instance, in single-step methods, the polymer precipitates from the organic solvent when added to aqueous media containing lipids, which subsequently spontaneously self-assemble into a monolayer surrounding the core. PEGylated lipids also self-assemble during this step, wherein a lipid moiety clings onto the surface of the polymer core and the PEG chain extends externally toward the aqueous environment. During the two-step method, a plausible mechanism of LPHNP formation may involve an initial bilayer structure formation and adherence to the core, with subsequent disintegration of the bilayer owing to the hydrophobic interaction between polymer and lipid chains. The hybrid formation is thermodynamically favorable, with respect to hydrophobic, van der Waal, and electrostatic interactions<sup>18,19</sup>.

## Methods for preparation of LPHNPs

### Two-step method

#### Conventional method

During initial days of study, a typical two-step method was frequently employed to form LPHNPs, wherein preformed polymeric NPs were mixed with preformed lipid vesicles and the latter was surface-assimilated onto the former, propelled by electrostatic forces. The polymeric NPs are generally prepared by nanoprecipitation<sup>21</sup>, emulsification-solvent evaporation (ESE)<sup>22</sup>, or high-pressure homogenization<sup>23</sup>, the two-step method can be subcategorized into two types: A) direct addition of the previously formed polymeric NPs to dried lipid film, or B) preformed NPs added to preformed lipid vesicles, made by initial hydration of the thin lipid film. In either case, the hybrids are assembled by the input of external energy via vortexing and/or ultrasonication of the suspension and heating at a temperature beyond the phase transition temperature of the lipid constituent. In purification step, free lipid and LPHNPs are separated by differential centrifugation. For instance, a method was developed for hybrid NP preparation using PLGA combined with cationic lipid vesicles (FA-OQLCS/ PEG-OQLCS/Chol) under continuous stirring or bath sonication at 30°C, yielding stable LPHNPs with average sizes between 200 and 400 nm and a surface potential of (+) 20–30 mV. Using different precursors, Thevenot et al and Troutier et al utilized a similar method to make LPHNPs<sup>24,25</sup>.

#### Nonconventional method

Aside from the abovementioned methodologies, few other methods going beyond convention have also been implemented to manufacture LPHNPs. For example, polymeric NPs (ie, polyglutamic acid, polylysine) of average size 400–500 nm were produced by spray drying, dispersed in DCM containing the lipids (tripalmitin, tristearin, cetyl alcohol). This suspension was later spray-dried again to prepare LPHNPs of size range 0.9–1.2  $\mu\text{m}$  with aspray dryer that was inappropriate for the production of NPs<sup>26</sup>.

The recently marketed nanometric spray dryer can be used to produce smaller hybrid NPs as well. Additionally, in recent years, a particle molding method by soft lithography called particle replication in nonwetting templates (PRINT) was explored to prepare LPHNPs for the delivery of genetic materials<sup>27</sup>.

### One-step method

The limitation of the two-step method is that preparing polymeric NPs and lipid vesicles separately makes the process inefficient in terms of energy and time spent. The commonly available and more efficient alternative is a onestep method.

Preformed lipid vesicles and polymeric NPs are not prerequisites for the one-step method. The method solely requires mixing of lipid and polymer solutions that subsequently tend to self-assemble to form LPHNPs. The most common processes are nanoprecipitation and/or ESE, both of which are often implemented for the production of nonhybrid polymeric NPs. Here, the lipids/PEG-lipids used function as stabilizing agents for the hybrid produced, while ionic or nonionic surfactants (PVA, DMAB, poloxamer) are generally used as stabilizers in the preparation of regular, nonhybrid polymeric NP<sup>28</sup>.

### Nanoprecipitation

Traditional nanoprecipitation method requires that the drug and polymer are dissolved together in a water-miscible organic solvent (viz, acetone, EtOH) and the lipid/lipid-PEG dissolved in water. It is mandatory to heat the lipid/lipid-PEG solution beyond its gel-to-liquid transition temperature in order to achieve a homogeneously dispersed liquid crystalline phase. This is followed by drop wise addition of the polymer to the aqueous dispersion of lipid under continuous stirring. This triggers the polymer to coil into NPs with concurrent self-assembly of the lipids surrounding the polymer owing to hydrophobic interactions, where hydrophobic tails of the lipids are directed toward the inner NP and the hydrophilic head groups face out toward the external aqueous solution. The hydrophobic lipid tails of the lipid-PEG merge into the inner lipid shell, while its PEG chains pop out to the aqueous environment, sterically stabilizing the hybrid. The organic media is evaporated and the LPHNPs, thus formed, are centrifuged. A promising noninvasive delivery of mRNA-based vaccines, developed recently, involves postinsertion of the PEGylated lipid vesicles following nanoprecipitation.<sup>44</sup> A few novel approaches are being adopted by a number of research groups to improve upon one-step methods<sup>28</sup>.

### Emulsification-solvent evaporation

This method can further be subclassified into single and double emulsification methods as depicted in, respectively. A single ESE method is used for drugs soluble in hydrophobic solvents (oil phase). In this method, an oil-in-water (o/w) emulsion is formed when the water-immiscible oil phase containing the polymer and the drug is mixed with an aqueous phase containing dissolved lipid under ultrasonication or constant stirring. Next, the polymer core is formed by evaporation of the organic media and the lipids assemble around the polymer core concomitantly. As an ostensible replacement, the lipid can concurrently be dissolved in the oil phase with the polymer. A double ESE method (w/o/w) is applied for water-soluble drugs. First, the aqueous solution of the drug is emulsified in an organic solvent (oil phase) containing polymer and lipid to form a w/o mixture. A w/o/w emulsion is generated when the mixture is emulsified again in an aqueous phase containing the lipid-PEG, followed by subsequent oil phase evaporation, to yield the LPHNPs. As evident, the hybrids produced by the double ESE method contain certain structural anomalies. It is composed of 1) an inner aqueous core surrounded by lipid layer, 2) a polymer layer in between, and 3) an outer lipid-PEG shell. Generally, the ESE method produces LPHNPs that are larger than those produced by conventional nanoprecipitation<sup>29,30</sup>.

## Characterization of LPHNPs

### • Particle size, polydispersity and zeta potential

The LPHNPs are characterized for their particle size (nm), polydispersity index (PDI) and zeta potential (mV) by dynamic light scattering (DLS) method using Malvern Zetasizer Nano ZS (Malvern Instruments, UK) at standard temperature and experimental condition<sup>31</sup>. The average particle size defined as



the relative size of the particle in the LPNPs, and a narrow PDI reveals about particle homogeneity in the prepared formulation. The zeta potential value having larger than +30 mV or -30 mV reveals about its maximum repulsion force and long term stability of the prepared LPNPs. All the measures of particle size, PDI, and zeta potential was run trice for 15 cycle by using software.

#### • **Entrapment efficiency (%EE)**

The entrapment efficiency (%EE) of the lipid-polymer hybrid nanoparticles is determined by measuring the concentration of untrapped drug in the supernatant when it exposed to a high speed centrifugation. Briefly, the LPNPs are subjected to ultracentrifugation (REMI equipped with TLA-45 rotor) at 14000 rpm at 4 C for 15 min, the procedure is repeated- till a clear supernatant was obtained. Then the amount of untrapped drug present in the supernatant is determined by UV/Vis spectrophotometry at 272 nm<sup>32</sup>. The each supernatant were analyzed thrice and the %EE was calculated by following formula:

$$\%EE = \frac{[(\text{Drug added} - \text{Free "untrapped drug"}) / \text{Drug added}] \times 100}{(1)}$$

#### • **Particle morphology of LPNPs by scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM)**

In the present study, the surface morphology of the lipid-polymer hybrid nanoparticles is studied by using SEM, TEM, and AFM. The Scanning electron microscope (SEM) is performed by using lyophilized sample of LPNPs. Briefly, the LPNPs are lyophilized by Freeze-dryer (Labconco lyophilizer, ¼ hp refrigeration system) using trehalose (50 mg/ml) as a cryoprotectant in deionized water by maintaining the freezing temperature for LPNPs at -60°C and vacuum pressure of 0.018 mbar, till complete drying of the LPNPs and stored in closed tight container. Then lyophilized LPNPs sample is mounted on double sided carbon tape and uniformly coated with gold by the help of ion-sputter for 10 min and examine under SEM (CSIR-CEERI, Pilani, Rajasthan) operated at 20 kV at different magnification of 2,700x, and 19000x. TEM (AIIMS, Delhi) operated at 200 kV at a magnification of 9900x is used to assess the size and morphology of the LPNPs.

TEM visualizes transparent LPNPs as bright spherical areas against dark background. The TEM study is performed accordingly reported elsewhere. Briefly, 1 ml of the LPNPs is taken in an Eppendorf tube and then diluted 10 times with deionized water, then 200 µl of the sample is pipetted and stained with 2% w/v phosphotungstic acid for 30 s on a coated copper grid and the excess of the material is removed by filter paper, and then allowed to completely dried. Two grids are prepared for each sample; the microscope magnification is calibrated and then viewed randomly.

Atomic Force Microscope (AFM) is carried out to determine the surface properties as height and diameter of the LPNPs. An AIST FP tip no. 01 and AC mod is used for imaging the LPNPs. Controlled software, and an Advance Integrated Scanning tool for Nano Technology (AIST-NT) Model: Smart SPM 1000, (NIIFF, Russia) in tapping mode is used to analyze the images. In this imaging technique, around 10 µl of LPNPs is safely secured on a freshly cleaved Mica slips and incubated for 5 min. The surplus matter was removed from the surface of slips gently by deionized water. Then sample is dried on spin coater to produce a thin film. Each sample is scanned by placing under the lens and analyzed at different magnification and three dimensional structures are observed by displaying amplitude, height and phase signal of the cantilever in the traced direction simultaneously<sup>33</sup>.

#### • **Attenuated total reflection- Fourier transform infrared spectroscopy (ATR-FTIR)**

The Infrared spectra of LPNPs loaded norfloxacin, composed of soya lecithin (lipid), PLA (polymer), stearylamine (charge inducer), and PVA (poly vinyl alcohol, stabilizer) are analyzed by using a ATR-FTIR spectra (Bruker EQUINOX 55 FTIR spectrophotometer equipped with a liquid nitrogen cooled mercury cadmium telluride (MCT)) at room temperature (25 ± 1 C) and a set detector of nominal resolution of 2 cm<sup>-1</sup> for each spectra. A diamond is used as an internal reflection element, placed at an incidence angle of 45°, scans 32 times and gives one reflection that is equivalent to 21 resolutions. An advanced ATR correction is applied to all spectra, and the region from 4000 to 400 cm<sup>-1</sup> is selected to run the spectra and peak fitting is done by using Opus software.

#### • **Differential scanning calorimetry (DSC)**

In this study, DSC is performed by using DSC (Model: DSC 204 F1 PHOENIX). Equipped with calorimeter (DSC 60), flow meter (flow controller FCL 60), thermal analyzer (TA 60) and operating software (NETZSCH Proteus Thermal software). In this study, the optimized sample LPNPs-8 is placed in aluminium pans, crimped with hydraulic press and then heated under a nitrogen flow (30 mL/ min) at a scanned temperature rate of 5 C/min from 25 C to 300 C<sup>34</sup>, set on isothermal programming. The same procedure is repeated for the physical mixture of the drug and excipient and a concordance result was predicted.

#### • **Thermogravimetric analysis (TGA)**

TGA studies of norfloxacin, soya lecithin, PLA, polyvinyl alcohol (PVA) and optimized formulation LPNPs-8 is done in order to study physical and chemical properties with the help of PROTEUS Thermal analysis (TGA 400). TGA is also used to determine weight loss, vaporization, sublimation, absorption, adsorption, etc. TGA is generally used to conclude selected characteristics of samples that show either weight loss or gain due to decomposition. The small amount of samples is taken in a crucible and after tarring the weight of crucible was kept in assembly and software was made to run. The amount of weight loss graphs are obtained and reported<sup>35</sup>.

#### • **In-vitro drug release**

In-vitro drug release of the optimized formulation is studied on cellulose acetate membrane which was soaked for 24 h prior work so that it can easily tie to diffusion tube. Diffusion tube was clamped and dipped in phosphate buffer pH 6.8 in beaker maintained at temperature 37° C. About 1 gm of LPNPs was added in diffusion which is a donor compartment and covered with parafilm to avoid evaporation of formulation. The phosphate buffer 6.8 is kept in receiver compartment and stirred continuously at 500 rpm. From receptor compartment 3 ml solution is withdrawn at 0,1,2 ... ..8, .....and 24 h respectively at particular time interval and replaced by buffer solution so that volume of receptor solution kept constant during drug release. The drug concentrations in the aliquot are determined by UV/VIS spectroscopy against appropriate blank<sup>36-38</sup>.

#### • **Antimicrobial activity studies**

The microbiological assay of standard drug solution and optimized formulation is performed in nutrient agar plate employing cup plate method. The nutrient agar media (2.8% w/v) is prepared and sterilized at 121°C for 21 min at 15 lbs pressure and poured into sterile petri plates. Media is allowed to solidify and then loop full bacteria is inoculated (Staphylococcus aureus MTCC 3160 and Pseudomonas aeruginosa MTCC 1688 from grown bacterial suspension) with the help of swapping method followed by boring in the plate

(9 cm in diameter and 5 cm in thickness) with the help of cork-borer to obtain definite size of hole. Drug solution and optimized LPNPs formulation equivalent to 2, 4, 6, and 8 mg/ml of drug is poured in hole with crystal violet dye and incubated for 24 h at 37°C. Further the zone of inhibition is measured by using Vernier caliper in mm and antimicrobial effect is compared with the standard drug. The entire operation except the incubation is carried out in a laminar airflow unit<sup>39</sup>.

#### • Skin irritation studies

Solution and optimized formulation is assessed by Draize patch tested on albino Wistar rat. The animals are housed at 25°C and hair of the back is trimmed short, 24 h prior to beginning of the assay. The animals are divided into three groups of six each. Two milliliters of solution and LPNPs are applied on the hair-free skin of rats by spreading uniformly. The animals are protected by using nylon mesh, supported by the plastic squares having small pores. Any visible changes such as erythema at 24, 48 and 72 h after the application of formulations is observed. The mean erythema scores were recorded (ranging from 0 to 4), depending on the degree of erythema<sup>40</sup>.

#### • Stability studies

Stability studies are performed for both lipid-polymer hybrid nanoparticles suspension and lyophilized nanoparticles to investigate the loss of drug from nanoparticle and change in nanoparticle structure during storage condition. Optimized formulation LPNPs nanoparticles are subjected to accelerated stability studies as per ICH<sup>40</sup>, for a period of 9 month. Further the sample evaluation is made by microscopic observation, particle size, PDI, zeta potential, and entrapment efficiency.

## Application of lipid-polymer hybrid nanoparticle system

### Drug delivery

Today drug delivery is the most challenging phenomenon for the researcher as well as Pharma companies. In recent the most carriers are discovering for drug delivery but they have some little bit limitation. Here LPHNPs develop as a carrier that overcomes these limitations and shows high bioavailability. The drug delivery through the hybrid Nanoparticle has been dominated by the delivery of various drugs; here we are focus on the delivery of the anticancer drug. The multidrug-resistant are present in the cancer cells that are challenging to the delivery of the chemotherapeutic agent to the cells<sup>41-43</sup>.

### Single-drug delivery

In the single drug delivery, various types of chemotherapeutic agents or drugs are used for the different types of cancer cells like Breast cancer, Prostate cancer, Lung cancer, Liver cancer, cervical cancer<sup>42, 44</sup>. In previous research, very less *in vivo* data were available for delivery of single drugs from LPHNPs. These are mostly designed for combinational and active targeted drug delivery. The single drug delivery from the LPHNPs has low *in vitro* cytotoxicity, biocompatible with drug, good cellular uptake and high drug release kinetic. According to Chin-Hang *et al.*, and Zhang *et al.* the LPHNPs exhibit high uptake in prostate and cervical cancer cells than in another delivery system<sup>44</sup>. Liv *et al.* reported that LPHNPs exhibit sustains release kinetic like 33% in 12 hr and 100% in 7 d<sup>45</sup>. The LPHNPs formulation is widely used for the delivery of fluoroquinolone antibiotics for lung infection therapy because particle easily penetrates the thick mucus layer nearby the bacteria and prevents lung infection. The antibiotic-loaded

LPHNPs as inhaler product has been establishing for lung infection disease<sup>46</sup>.

### Combinational drug delivery

The combination of a chemotherapeutic agent or drug is highly effective for cancer therapy. In combinational drug therapy, chemotherapeutic or drug is used with another therapeutic agent like Gene, Magnetic Nanoparticle<sup>42, 47</sup>, etc. Develop a LPHNPs carrier that carries multiple drugs at a precise ratio that helps in drug release in a controlled manner and overcomes the limitation of a single drug delivery system. The multiple drug delivery through LPHNPs generally employs two-drug-incorporate into one system. The multidrug loading in the LPHNPs system follows the one-step method in which drug is covalently conjugated with polymer and another drug is conjugate within lipid and mix properly by sonication technique to form multiple drugs loaded LPHNPs. In the two-step method, the drug is entrapped or encapsulated into the polymeric core and another drug conjugate with lipid. The lipid is adsorbed on the polymeric core and forms multiple drug-loaded LPHNPs. In contrast the second method, the drugs are conjugate to the LPHNP system with the help of hydrolyzable linker and this drug-conjugate linker gets hydrolyzed upon reaching the cancer cell resulting in the drug are released separately. According to Sengupta *et al.*, the LPHNPs for multi-drug delivery containing an anti-angiogenesis and another chemotherapeutic drug, resulting one inhibit the growth of tumor cells by cutting the blood supply to the cell and another kill the existing tumor cell<sup>48</sup>.

### Actively targeted drug delivery

In the anticancer therapy, the delivery of chemotherapeutic agents should be targeted because the chemotherapeutic agents kill the cancerous cell as well as healthy cells. In the targeted drug delivery system, the maximum amount of drug reaches the cancer cell, not to other cells. In which the targeting moiety is attached to the LPHNP system that easily recognizes the cancerous cell and kills them. The targeted vs untargeted drug delivery system. For example, folic acid is the one such targeting moiety has high affinity to bind the cancer cells because folate receptor is present on most of the cancer cells. Hence the target moiety (folic acid) is attached to the LPHNP system before the preparation<sup>42</sup>.

### Gene delivery

Today the delivery of nucleic acid is a very challenging process for the pharmaceutical company. The delivery of nucleic acid is very useful in the treatment of chronic disease, genetic disorder, cancers and another diagnostic purpose<sup>49</sup>. The cationic lipid and cationic biodegradable polymer-based Nanoparticle are widely used in the gene delivery system<sup>50</sup>. The lipid and polymer-based non-viral carrier systems have various advantages like low immunogenicity, low toxicity absence of viral recombination low production cost<sup>51</sup>, but have some limitations like cytotoxicity, stability into the serum, high duration of gene expression and large particle size. The lipid-polymer hybrid Nanoparticle is a more reliable carrier for gene delivery than other carriers because these are biodegradable, stable and long-lived Nanoparticle vector delivery systems. The plasmid Deoxyribonucleic acid encoding luciferase receptor gene encapsulated into the polymer core and lipid is adsorbed on the core<sup>52</sup>. The LPHNPs (100-400 nm) is able to transfer the luciferase gene in the prostate cancer cell.

### Deoxyribonucleic acid (DNA) delivery

According to Zhong *et al.*, here they are reporting that 3 Deoxyribonucleic acid (DNA) incorporation methods for the transfection efficiency in the preparation of LPHNPs-loaded

Deoxyribonucleic acid (DNA) luciferase gene. The LPHNPs consist of a polymer and lipid and prepared by a double emulsification solvent evaporation method by using Polylactic Glycolic acid (PLGA) as a polymer and N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl-sulfate or 3 $\beta$ -[N-(N',N'-dimethylaminoethane)-carbamoyl]cholesterol (DC-Chol) as a lipid<sup>52</sup>. There are 3 methods are used for evaluation –

1. Deoxyribonucleic acid adsorbs on the cationic lipid shell of LPHNPs by electrostatic adsorption technique before the preparation of LPHNPs. This method is called out method.
2. Deoxyribonucleic acid is encapsulated into the aqueous hollow core of the LPHNPs this system is called in method.
3. In the last method combine the both in and out method.

### Si-RNA delivery

Small interfering Ribonucleic acid (SiRNA) is an important tool for gene therapy and help in suppressing the expression of the specific gene by the RNA interference process. Ex. The delivery of Small interfering Ribonucleic acid to the cancer cells that initiate the RNA interference pathway to block the protein expression into the tumor initiation and progression<sup>48</sup>. The formulation method for developing a Small interfering Ribonucleic acid delivery system is some as a Deoxyribonucleic acid (DNA) delivery system (Polyplexes and Lipoplexes)<sup>42</sup> but Deoxyribonucleic acid (DNA) delivery has some limitations like poor stability during oral or systemic administration thus to overcome this problem develop a new system called Small interfering Ribonucleic acid (SiRNA) development.

### Diagnostic imaging agent delivery

The LPHNPs are also used for the delivery of diagnostic imaging agents for the medical diagnostic approach. The common diagnostic agents like quantum dots (QD), inorganic nanocrystals, Barium sulfate, Gastrograffin, etc are used in computed tomography (CT), magnetic resonance imaging (MRI), X-Ray/Mammography, Ultrasound, Fluoroscopy, Nuclear Medicine/Molecular Imaging, and Angiography/Interventional, etc. According to Mieszawska *et al.*, they prepare gold particle and quantum dots loaded two LPHNP systems by nanoprecipitation technique<sup>53, 56</sup>. In which the gold particle and quantum dots are incorporated separately into the Polylactic Glycolic acid (PLGA) polymer by esterification reaction, and the lipid adsorbs on the polymer core. The *in vitro* bioimaging application of gold particle-loaded LPHNPs and quantum dots loaded LPHNPs are done on the macrophage cells of the mouse<sup>54, 55</sup>.

### Conclusion

The LPHNP system are the most promising class of nanocarriers, they have various versatile drug delivery applications in the pharmaceutical field. The main goal of the researcher to develop an LPHNPs based drug delivery system for effective and safe therapy of clinic use and increases the efficacy and reduce the toxic side effects. In recent the most of carriers are discover for the drug delivery but they have some little bit limitations, LPHNPs carrier overcome these limitation and various problems associated with lipid-based Nanoparticle and polymeric nanoparticle like drug leakage, polymer toxicity, unstable during storage, less permeable, etc, because the polymer core is coated with lipid monolayer that prevents the drug leakage, and the lipid layer easily permeate into the phospholipid bilayer of human skin resulting maximum drug rich to the targeted site and show the high bioavailability. LPHNPs have displayed a remarkable range of successes in translating new clinical and drug delivery applications from bench to bedside, with a significant and

lasting impact in the field of topical drug delivery system. Indeed, in some cases, LPHNPs have already demonstrated superiority compared with liposomes and polymeric NPs. From the perspective of industrial production and scalability, efficient and simple large-scale production of NPs has already been developed.

### References

1. Farokhzad O, Langer R. Nanomedicine: developing smarter therapeutic and diagnostic modalities. *Adv Drug Deliv Rev.* 2006; 58(14):1456-1459. <https://doi.org/10.1016/j.addr.2006.09.011>
2. Shi J, Kantoff PW, Wooster R, Farokhzad OC. Cancer nanomedicine: progress, challenges and opportunities. *Nat Rev Cancer.* 2017; 17(1):20-37. <https://doi.org/10.1038/nrc.2016.108>
3. Ferrari M. Cancer nanotechnology: opportunities and challenges. *Nat Rev Cancer.* 2005; 5(3):161-171. <https://doi.org/10.1038/nrc1566>
4. Kim BY, Rutka JT, Chan WC. Nanomedicine. *N Engl J Med.* 2010; 363(25):2434-2443. <https://doi.org/10.1056/NEJMra0912273>
5. Kamaly N, Xiao Z, Valencia PM, Radovic-Moreno AF, Farokhzad OC. Targeted polymeric therapeutic nanoparticles: design, development and clinical translation. *Chem Soc Rev.* 2012; 41(7):2971-3010. <https://doi.org/10.1039/c2cs15344k>
6. Kamaly N, Yameen B, Wu J, Farokhzad OC. Degradable controlled-release polymers and polymeric nanoparticles: mechanisms of controlling drug release. *Chem Rev.* 2016; 116(4):2602-2663. <https://doi.org/10.1021/acs.chemrev.5b00346>
7. Nair SS, Chitosan-based transdermal drug delivery systems to overcome skin barrier functions, *Journal of Drug Delivery and Therapeutics.* 2019; 9(1):266-270 <https://doi.org/10.22270/jddt.v9i1.2180>
8. Albanese A, Tang PS, Chan WC. The effect of nanoparticle size, shape, and surface chemistry on biological systems. *Annu Rev Biomed Eng.* 2012; 14(1):1-16. <https://doi.org/10.1146/annurev-bioeng-071811-150124>
9. Cho EJ, Holback H, Liu KC, Abouelmagd SA, Park J, Yeo Y. Nanoparticle characterization: state of the art, challenges, and emerging technologies. *Mol Pharm.* 2013; 10(6):2093-2110. <https://doi.org/10.1021/mp300697h>
10. Alexis F, Pridgen E, Molnar LK, Farokhzad OC. Factors affecting the clearance and biodistribution of polymeric nanoparticles. *Mol Pharm.* 2008; 5(4):505-515. <https://doi.org/10.1021/mp800051m>
11. Burgess P, Hutt PB, Farokhzad OC, Langer R, Minick S, Zale S. On firm ground: IP protection of therapeutic nanoparticles. *Nat Biotechnol.* 2010; 28(12):1267-1270. <https://doi.org/10.1038/nbt.1725>
12. Farokhzad OC, Langer R. Impact of nanotechnology on drug delivery. *ACS Nano.* 2009; 3(1):16-20. <https://doi.org/10.1021/nn900002m>
13. Jain S, Kirar M, Bindeliya M, Sen L, Soni M, Shan M, Purohit A, Jain PK. Novel drug delivery systems: an overview. *Asian J Dent Health Sci.* 2022; 2(1):33-39 <https://doi.org/10.22270/ajdhs.v2i1.14>
14. Shi J, Kantoff PW, Wooster R, Farokhzad OC. Cancer nanomedicine: progress, challenges and opportunities. *Nat Rev Cancer.* 2017; 17(1): 20-37. <https://doi.org/10.1038/nrc.2016.108>
15. Kim BY, Rutka JT, Chan WC. Nanomedicine. *N Engl J Med.* 2010; 363(25):2434-2443. <https://doi.org/10.1056/NEJMra0912273>
16. Ferrari M. Cancer nanotechnology: opportunities and challenges. *Nat Rev Cancer.* 2005; 5(3):161-171. <https://doi.org/10.1038/nrc1566>
17. Zhang L, Chan JM, Gu FX, et al. Self-assembled lipid-polymer hybrid nanoparticles: a robust drug delivery platform. *ACS Nano.* 2008; 2(8): 1696-1702. <https://doi.org/10.1021/nn800275r>
18. Wakaskar RR. General overview of lipid-polymer hybrid nanoparticles, dendrimers, micelles, liposomes, spongosomes and



- cubosomes. *J Drug Target*. 2018; 26(4):311-318. <https://doi.org/10.1080/1061186X.2017.1367006>
19. Fang RH, Aryal S, Hu CM, Zhang L. Quick synthesis of lipid-polymer hybrid nanoparticles with low polydispersity using a single-step sonication method. *Langmuir*. 2010; 26(22):16958-16962. <https://doi.org/10.1021/la103576a>
  20. Barenholz Y. Doxil(R)-the first FDA-approved nano-drug: lessons learned. *J Control Release*. 2012; 160(2):117-134. <https://doi.org/10.1016/j.jconrel.2012.03.020>
  21. Mandal B, Bhattacharjee H, Mittal N, et al. Core-shell-type lipid-polymer hybrid nanoparticles as a drug delivery platform. *Nanomedicine*. 2013; 9(4):474-491. <https://doi.org/10.1016/j.nano.2012.11.010>
  22. Thevenot J, Troutier AL, David L, Delair T, Ladavière C. Steric stabilization of lipid/polymer particle assemblies by poly(ethylene glycol)-lipids. *Biomacromolecules*. 2007; 8(11):3651-3660. <https://doi.org/10.1021/bm700753q>
  23. Mieszawska AJ, Gianella A, Cormode DP, et al. Engineering of lipid-coated PLGA nanoparticles with a tunable payload of diagnostically active nanocrystals for medical imaging. *Chem Commun (Camb)*. 2012; 48(47):5835-5837. <https://doi.org/10.1039/c2cc32149a>
  24. Fenart L, Casanova A, Dehouck B, et al. Evaluation of effect of charge and lipid coating on ability of 60-nm nanoparticles to cross an in vitro model of the blood-brain barrier. *J Pharmacol Exp Ther*. 1999; 291(3):1017-1022.
  25. Cserr HF, Ostrach LH. Bulk flow of interstitial fluid after intracranial injection of blue dextran 2000. *Exp Neurol*. 1974; 45(1):50-60. [https://doi.org/10.1016/0014-4886\(74\)90099-5](https://doi.org/10.1016/0014-4886(74)90099-5)
  26. Wang H, Zhao P, Su W, et al. PLGA/polymeric liposome for targeted drug and gene co-delivery. *Biomaterials*. 2010; 31(33):8741-8748. <https://doi.org/10.1016/j.biomaterials.2010.07.082>
  27. Troutier AL, Delair T, Pichot C, Ladavière C. Physicochemical and interfacial investigation of lipid/polymer particle assemblies. *Langmuir*. 2005; 21(4):1305-1313. <https://doi.org/10.1021/la047659t>
  28. Hitzman CJ, Elmquist WF, Wattenberg LW, Wiedmann TS. Development of a respirable, sustained release microcarrier for 5-fluorouracil I: In vitro assessment of liposomes, microspheres, and lipid coated nanoparticles. *J Pharm Sci*. 2006; 95(5):1114-1126. <https://doi.org/10.1002/jps.20591>
  29. Li X, Anton N, Arpagaus C, Belleiteix F, Vandamme TF. Nanoparticles by spray drying using innovative new technology: the Büchi nano spray dryer B-90. *J Control Release*. 2010; 147(2):304-310. <https://doi.org/10.1016/j.jconrel.2010.07.113>
  30. Bershteyn A, Chaparro J, Yau R, et al. Polymer-supported lipid shells, onions, and flowers. *Soft Matter*. 2008; 4(9):1787-1791. <https://doi.org/10.1039/b804933e>
  31. Cheow WS, Hadinoto K. Factors affecting drug encapsulation and stability of lipid-polymer hybrid nanoparticles. *Colloids Surf B Biointerfaces*. 2011; 85(2):214-220. <https://doi.org/10.1016/j.colsurfb.2011.02.033>
  32. Li Y, Wong HL, Shuhendler AJ, Rauth AM, et al. Molecular interactions, internal structure and drug release kinetics of rationally developed polymer-lipid hybrid nanoparticles. *J Contr Rel*. 2008; 1: 60-70. <https://doi.org/10.1016/j.jconrel.2008.02.014>
  33. Shah P, Desai P, Singh M. Effect of oleic acid modified polymeric bilayered nanoparticles on percutaneous delivery of spantide II and ketoprofen. *J Contr Rel*. 2012; 158: 336-345. <https://doi.org/10.1016/j.jconrel.2011.11.016>
  34. Agrawal U, Gupta M, Vyas SP. Capsaicin delivery into the skin with lipidic nanoparticles for the treatment of psoriasis. *Artif Cells Nanomed Biotechnol*. 2013; 23:456-460.
  35. Ranjan OP, Shavi GV, Nayak UY, Arunqam K, Averineni RK, et al. Controlled release chitosan microspheres of mirtazapine: in vitro and in vivo evaluation. *Arch Pharm Res*. 2011; 11:1919-1929. <https://doi.org/10.1007/s12272-011-1112-1>
  36. Babu RJ, Kanikkannan N, Kikwai L, Ortega C, Andega S, et al. The influence of various methods of cold storage of skin on the permeation of melatonin and nimesulide. *J Control Rel*. 2003; 86: 49-57. [https://doi.org/10.1016/S0168-3659\(02\)00368-1](https://doi.org/10.1016/S0168-3659(02)00368-1)
  37. P. Zhao, H. Wang, M. Yu, Z. Liao, X. Wang, et al., Paclitaxel loaded folic acid targeted nanoparticles of mixed lipid-shell and polymer-core: In vitro and in vivo evaluation, *Eur. J. Pharm. Biopharm.* 2012; 81:248e256. <https://doi.org/10.1016/j.ejpb.2012.03.004>
  38. Ling G, Zhang P, ZhangW, Sun J, Meng X, et al., Development of novel self assembled DS-PLGA hybrid nanoparticles for improving oral bioavailability of vincristine sulfate by P-gp inhibition. *J Contr Rel*. 2010; 148:241-248. <https://doi.org/10.1016/j.jconrel.2010.08.010>
  39. Rajak H, Jain DK, Dewangan PK, Patel V, Agrawal N. Application of microwaves in organic synthesis: speeding up the process of drug discovery. *RGUHS J. Pharm. Sci*. 2013; 3(1):14-20.
  40. Jubie S, Rajeshkumar R, Yellareddy B, Siddhartha G, Sandeep M, et al., Microwave assisted synthesis of some novel benzimidazole substituted Fluoroquinolones and their antimicrobial evaluation. *J Pharmaceu Sci Res*. 2010; 2:69-76.
  41. ICH Q1A (R2), Stability Testing of New Drug Substances and Products, International Conference on Harmonization, U.S. Department of Health and Human Service Food and Drug Administration, 2003, pp. 4e20. CPMP/ICH/2736/99.
  42. Bose RJC, Ravikumar R, Karuppagounder V, Bennet D, Rangasamy S, Thandavarayan RA. Lipid-polymer hybrid nanoparticle-mediated therapeutics delivery: advances and challenges. *Drug Discov Today*. 2017; 22(8):1258-65. <https://doi.org/10.1016/j.drudis.2017.05.015>
  43. Chaudhary K, Parihar S, Sharma D, A critical review on nanoscience advancement: in treatment of viral infection, *Journal of Drug Delivery and Therapeutics*. 2011; 11(6):225-237 <https://doi.org/10.22270/jddt.v11i6.5030>
  44. Mandal B, Bhattacharjee H, Mittal N, Sah H, Balabathula P, Thoma LA, Wood GC. Core-shell-type lipid-polymer hybrid nanoparticles as a drug delivery platform. *Nanomedicine*. 2013; 9(4):474-91. <https://doi.org/10.1016/j.nano.2012.11.010>
  45. Zhang L, Chan JM, Gu FX, Rhee JW, Wang AZ, Radovic Moreno AF, Alexis F, Langer R, Farokhzad OC. Self-assembled lipid-polymer hybrid nanoparticles: a robust drug delivery platform. *ACS Nano*. 2008; 2(8):1696-702. <https://doi.org/10.1021/nn800275r>
  46. Chu CH, Wang YC, Huang HY, Wu LC, Yang CS. Ultrafine PEG-coated poly(lactic-co-glycolic acid) nanoparticles formulated by hydrophobic surfactant-assisted one-pot synthesis for biomedical applications. *Nanotechnology*. 2011; 22(18):185601. <https://doi.org/10.1088/0957-4484/22/18/185601>
  47. Wang Y, Kho K, Cheow WS, Hadinoto K. A comparison between spray drying and spray freeze drying for dry powder inhaler formulation of drug-loaded lipid-polymer hybrid nanoparticles. *Int J Pharm*. 2012; 424(1-2):98-106. <https://doi.org/10.1016/j.ijpharm.2011.12.045>
  48. Shah J, Patel S, Bhairy S, Hirlekar R. Formulation optimization, characterization and in vitro anti-cancer activity of curcumin loaded nanostructured lipid carriers. *Int J Curr Pharm Sci*. 2022; 14(1):31-43. <https://doi.org/10.22159/ijcpr.2022v14i1.44110>
  49. Ebos JML, Lee CR, Kerbel RS. Tumor and host-mediated pathways of resistance and disease progression in response to antiangiogenic therapy. *Clin Cancer Res*. 2009; 15(16):5020-5. <https://doi.org/10.1158/1078-0432.CCR-09-0095>
  50. El-Anead A. An overview of current delivery systems in cancer gene therapy. *J Contr Rel*. 2004; 94(1):1-14. <https://doi.org/10.1016/j.jconrel.2003.09.013>
  51. Jain S, Purohit A, Nema P, Vishwakarma H, Qureshi A, Jain PK. Pathways of targeted therapy for colorectal cancer. *J Drug Deliv Ther*. 2022; 12(5):217-221. <https://doi.org/10.22270/jddt.v12i5.5602>
  52. Bivas Benita M, Romeijn S, Junginger HE, Borchard G. PLGA-PEI nanoparticles for gene delivery to pulmonary epithelium.

- European J Pharm Biopharm. 2004; 58(1):1-6.  
<https://doi.org/10.1016/j.ejpb.2004.03.008>
53. Lee M, Kim SW. Polyethylene glycol-conjugated copolymers for plasmid DNA delivery. Pharm Res. 2005; 22(1):1-10.  
<https://doi.org/10.1007/s11095-004-9003-5>
54. Zhong Q, Chinta DM, Pamujula S, Wang H, Yao X, Mandal TK, Luftig RB. Optimization of DNA delivery by three classes of hybrid nanoparticle/DNA complexes. J Nanobiotechnol. 2010; 8(1):6.  
<https://doi.org/10.1186/1477-3155-8-6>
55. Mieszawska AJ, Gianella A, Cormode DP, Zhao Y, Meijerink A, Langer R, Farokhzad OC, Fayad ZA, Mulder WJ. Engineering of lipid-coated PLGA nanoparticles with a tunable payload of diagnostically active nanocrystals for medical imaging. Chem Commun (Camb). 2012; 48(47):5835-7.  
<https://doi.org/10.1039/c2cc32149a>
56. Purohit A, Jain S, Nema P, Vishwakarma H, Jain PK. Intelligent or smart polymers: advance in novel drug delivery. J Drug Deliv Ther. 2022; 12(5):208-216. <https://doi.org/10.22270/jddt.v12i5.5578>