Lipid Polymeric Hybrid Nanoparticles: Formulation Techniques and Effects on Glioblastoma

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ABSTRACT

Introduction:

In the pharmaceutical industry, liposomes and polymeric nanoparticles are the two most commonly studied delivery vehicles. A new technique uses lipid-polymeric hybrid nanoparticles (LPHNPs) with a polymeric core, and a shell made up of lipid-lipid-PEG lipids. They have properties which complement polymer nanoparticles and liposomes, and they have the potential to improve the physical stability and biocompatibility of the active pharmaceutical ingredient encapsulated in them. Evaporating the solvent from a dual-phase solution containing lipid and polymer is one of the most effective methods for producing the lipid polymeric hybrid nanoparticles. The LPHNPs applications has also been significantly expanded to include combinational and active targeted drug delivery, as well as delivery of genetic materials, vaccines, and diagnostic imaging agents, in addition to single drug delivery for anticancer therapy, like Glioblastoma.

Main goal:

The main agenda of this compilation was to address the effects of LPHNPs on Glioblastoma treatment. This compilation also highlights some of the formulation techniques and issues that arise during the preparation of LPHNPs. This review also discusses recent developments in core-shell lipid-polymer hybrid nanoparticles, which were conferred in considerable detail later in this article.

Conclusion:

The main issue which arises while using nanoparticles with polymer is entrapment efficiency. Because of their hybrid components, LPHNPs have proven to solve this problem to a large extent. The recent research trends suggest that lipid polymeric hybrid nanoparticles will prove to be highly effective or productive in treating diseases such as Glioblastoma.

Keywords:

Lipid Polymer hybrid nanoparticles; Lipid-based nanoparticles; Glioblastoma (GBM); TPGS; Core shell-type lipid polymer hybrid nanoparticles (CSLPHNPs); Drug delivery

INTRODUCTION

Lipid Polymeric Hybrid Nanoparticles (LPHNPs) are designed and synthesized to assist studies in lipid-membrane biotechnology and nano biotechnologies.[1-3] The LPHNPs technology gives fresh insight into the nanostructures and biological systems by providing various choices for the regulated release of the integrated components. By increasing the formula's solubility, dispersion, permeability, and overall bioavailability, such formulations improve the pharmacokinetics and pharmacodynamics characteristics of NPs. A novel method known as LPHNPs may be used to create extremely selective and efficient nanoparticles that are ultimately stable and successful in the drug delivery platform. LPHNPs are important in the treatment of cardiovascular disease, asthma, and infectious illness.[4] This medication delivery mechanism is important in the treatment of infection because it transports hydrophobic as well as hydrophilic medicines and regulates their release rate. The membranes define a cellular border and offer a framework for strict regulation of a variety of biological activities, including resource movement, signal transduction, trade, pathogenic pathways, intercellular structure, and extracellular matrix response. Because they are biocompatible and biodegradable, polymer and lipid-based nanocarriers, such as liposomes and polymeric nanoparticles, are effective product nanocarriers. Polymer nanoparticles made from natural or synthetic polymers have gotten a lot of interest since they can be loaded with water and water medications both to guarantee drug formulation stability and control. High stability and biocompatibility, extended circulation duration, high drug loading capacity, controlled drug release characteristics, and in vivo effectiveness are only a few of the advantages of polymeric nanoparticles. Liposomes are thought to have a high level of biocompatibility, a well-defined pharmacokinetics profile, and the ability to alter the surface, especially polyethylene glycol (PEG), resulting in a longer circulation duration. In the medication and gene delivery area, the benefits of both liposomes and polymeric nanoparticles are selected.[5] LPHNPs are core-shell nanoparticles that encapsulate a large number of hydrophobic medicines in a polymeric hydrophobic core that keeps them physically and chemically stable. These are made up of three distinct functional components: (i) a hydrophobic polymer core through which a significant amount of hydrophilic drugs is encapsulated; (ii) a hydrophilic polymer shell, which is an interfacial lipid monolayer that serves as a versatile and biocompatible shell with properties to increase nanoparticle stability and systemic circulation half-life. (iii) a lipid coating on the outside The middle lipid monolayer acts like a molecular barrier, reducing the loss of entrapped drugs over the course of the LPHNP formulation and preventing core degradation by preventing

water diffusion into the inner core at the core-shell interface, facilitating drug retention inside the polymeric core, as shown in (**Fig.1**). Such chemicals are typically assessed encapsulated within nanoparticle carriers or conjugated on the surface of nanoparticle carriers, with the carriers being controlled by the carrier matrix formulation. Many polymer lipid nanoparticles have a high drug encapsulation yield, good serum stability, a consistent drug release profile, or the capacity to target cells or tissues differently. It is also no surprise that nanoscale structures (less than 1000 nm) have been widely utilized as delivery vehicles for various medicinal substances, ranging from tiny molecular medicines, genes, and biopharmaceuticals to proteins and peptides in diagnostic imaging agents. Polymeric nanoparticles, liposomes, stable lipid nanoparticles, micelles, niosomes, gold nanoparticles, and carbon nanotubes have been utilized to deliver disease-targeting medicines for many years. LPHNPs development offers specific anticancer treatment strategies that can overcome the limitations of combination cancer therapy by performing a comprehensive function in essential therapy, protecting drugs in the bloodstream, increasing their biodistribution, and reducing their adverse drug effects by reaching individual targets.[6]

GEMZAR ®, a parenteral formulation with an intravenous (i.v.) infusion, is a generic gemcitabine medication that offers good therapeutic benefits in cancer treatment.[7] In another study, LPHNPs with cyclic arginine-glycine-aspartic acid modifications were created to deliver 10-hydroxycamptothecin to human breast cancer cells (MCF-7).[8] Core-shell type LPHNPs were developed in recent research (Zhang et al.) for children's shared genetic and treatment for head and neck malignancies. Successful LPHNPs that respond to external or internal stimuli to enhance therapeutic efficacy and site-specificity of treatments have recently been discovered. This new method of cancer treatment is speculative or appealing since it has been shown to improve therapeutic effectiveness while reducing medication side effects. Due to their considerable molecular weight, enzyme-mediated breakdown, negative charge, and providentially complex distribution, many advances in genetic nanomedicine have played a significant role in the delivery of nucleic acid medicines for the treatment of uncommon illnesses. Researchers have been able to circumvent this problem by using nanocarriers to deliver drugs to their exact target areas. The use of LPHNPs for biomedical imaging as a contemporary imaging approach has been attempted by the high encapsulation of therapeutic agents in the core and targeted by the customized surface, thanks to recent advancements in molecular imaging and nanotechnology.[9] When compared to PLGA and PLGA-PEG NPs, these hollow core-shell type LPHNPs had a greater siRNA encapsulation efficiency.[10] Several LPHNPs were created to improve multimodal contrast while also delivering medicines at the same time. LPHNPs with adjustable bioimaging

characteristics have been created by combining metallic gold nanocrystals with quantum dots for computed tomography (CT) and magnetic resonance imaging (MRI). These new systems have excellent compatibility with other materials, allowing for higher and simultaneous encapsulation of hydrophilic and lyophilic therapeutic molecules and improved pharmacokinetic profiles due to the presence of lipids, which may help encapsulated product respond more effectively. They also go through how to make LPHNPs with particular desired properties, among other things.

GLIOBLASTOMA

Glioblastoma, commonly known as glioblastoma multiforme (GBM), is a highly aggressive kind of primary brain tumor. Glioblastoma multiforme (GBM) is the most frequent and deadly kind of complicated treatment-resistant brain cancer, accounting for 45 %.[11, 12] GBM is the most common solid malignant tumor in the central nervous system. It is highly aggressive, invasive, and undifferentiated. This malignancy accounts for 12–15% of all brain tumors. It is the most severe and malignant primary brain tumor in adults. It originates from glial lineage cells and is the most severe and malignant primary brain tumor. Based on the degree of malignancy established by histopathological criteria, gliomas are classified from I through IV. Gliomas of classes II through IV, on the other hand, are very malignant and invasive. The WHO(World health organization) classifies GBM as a Grade IV tumor.Grade IV GBM patients have 12- to 18-month overall survival rate. Only around 5% of GBM patients survive longer than a year, and only about 25% of patients live longer than five years in severe cases. Nearly 60% of all adult brain cancers are astrocytic tumors (astrocytoma, anaplastic astrocytoma, and Glioblastoma), oligodendroglia, ependymoma, and mixed gliomas. This is not only due the late diagnosis of the disease but also due to insufficient care methods resulting from the lack of understanding of the triggers of glioblastoma development. Glioblastoma is the cancer that may be treated with a variety of methods, including surgery, radiation, and chemotherapy. While the number of FDA-approved cancer treatment medicines has increased dramatically in recent times, and considerable progress has been made in the molecular and cellular characterization of GBM, effective therapeutics against GBM still remains limited. Chemotherapy is not advised for the diagnosis of GBM since it may kill healthy cells alongside the malignant tissues. Open surgery, temozolomide chemotherapy, and radiation are the mainstays of current treatment. Nowadays, Raman and infrared spectroscopy (FTIR) are used to look for new diagnostic methods to detect the tiniest cancer features. The use of lipid polymer hybrid nanoparticles for the treatment of Glioblastoma multiform has been described with the help of number of

studies. Curcumin loaded LPHNPs for glioblastoma tissue in the rat glioma-2 (RG2) tumor model, for example, have anticancer efficacy when loaded on poly(lactic-co-glycolic acid)-1,2-distearoyl-glycerol-3-phospho-ethanolamine-N-[methoxy (polyethylene glycol)-2000] ammonium salt (PLGA-DSPE-PEG) hybrid nanop For the treatment of Glioblastoma, LPHNPs containing both pemetrexed and miR-21 antisense oligonucleotide (anti-miR-21) have been created.[13]

PATHOPHYSIOLOGY OF GLIOBLASTOMA (GBM)

GBM is the most frequent and malignant tumor found in the cerebral hemispheres, which develops from the du nord of low-grade astrocytoma, i.e., secondary Glioblastoma, or from the formation of original Glioblastoma .[14] Primary Glioblastoma affects mostly the older individuals and is characterized by the lack of heterozygozygosity on 10q (LOH 10q) (70%), TP53 mutation (less than 30%), and EGFR amplification (36 %). Secondary Glioblastoma, on the other hand, is way more common in younger individuals. Mutations in the TP53 gene are the first identifiable changes (65 %). The most common location for a genetic change in the route of primary or secondary glioblastomas is LOH 10q, although primary glioblastomas usually include the loss of the whole chromosome 10. In both primary and secondary glioblastomas, the P16INK4a/RB1 pathway plays a critical role. The EGFR/PTEN/Akt/mTOR signaling pathway is critical for primary glioblastoma progression. GBM has chronic cerebral edema, localized necrosis, hypervascularity, and fast cellular growth as histological characteristics.[15] These characteristics are due to the activation of EGFr by EGF activation, which is expressed on glioma cells via increasing vascular endothelial growth factor (VEGF) production. The pathogenomic, histopathologic, and clinical characteristics of these tumors in patients are explained by the production of VGEF by glioma cells in situ. Glioblastoma multiforme is one of the most aggressive form of brain cancers with the aggressive phenotype of intratumoral hypoxia, which is crucial in GBM. Growth, development, and angiogenesis of multiform tumors. Gliomas are divided into four categories based on their prognosis and histology, with three distinct types of tissue: ependymomas (which account for less than 10% of all cases), oligodendrogliomas (10-30 % of cases), and astrocytomas (which account for more than 30% of all cases) (more than 30 %). Within CpG dinucleotides, methylation of cytosine residues at the carbon 5 (mC) position by the DNA methyltransferases (DNMTs) enzyme family results in the transfer of a methyl group from S-adenosyl methionine to DNA, which is essential for human development and reproduction. It is possible to classify DNMTs into three categories, which are as follows: DNMT1, DNMT2, DNMT3a, and DNMT3b. It is the most abundant DNMT in the cell, and it is also the

most crucial.[16] It mostly transcribes during the cell cycle's S phase. Its action maintains DNA methylation patterns by acting mainly on hemimethylated DNA produced during semiconservative DNA replication. DNMT3a and DNMT3b create a methylation habit throughout embryo development, with strong expression in the foetus' stem cells and down-regulation in differentiated cells. **Figure1** explained the pathogenesis of glioblastoma multiforme.

Figure 1: Pathogenesis of glioblastoma multiforme

Role of LPHNPs in cancer therapy and Glioblastoma treatment:

The capacity to integrate therapeutic compounds with various physicochemical characteristics into LPHNPs, owing to their hybrid structure defined by polymer and lipid components with different properties, is the primary benefit of LPHNPs usage in delivering anticancer drugs.[17] Other emerging therapeutics, such as nucleic acids, proteins, and peptides, can be combined with hydrophobic drugs. Encapsulating hydrophobic drugs in the lipophilic polymeric core while charged biomolecules are conjugated or adsorbed to the lipid shell may be a feasible option in this context. salidroside (Sal) is a water-soluble anticancer drug, according to Wong et al. (2014), and the authors encapsulate it in this study using its affinity for the hydrophobic portion of the hybrid vector's outer lipid membrane.[18] The authors have used this technique to create tiny

nanoparticles with a high loading capacity that could effectively target the tumor after delivery. Anticancer drugs have a number of drawbacks, including unintended biodistribution, rapid drug clearance, and non-targeted activity, all of which can result in a variety of side effects in non-diseased tissues.[19] Therapeutic drugs can be administered in a controlled and selective manner to significantly reduce unfavorable systemic effects. LPHNPs are particularly well-suited for conjugating ligands aimed at over-expressed receptors on tumor cells, enhancing tumor targeting, and increasing the therapeutic effect of given medicines on tumor cells, thanks to their structure. Inactive targeting methods, ligands such as small molecules, peptides, antibodies, and aptamers have been used to functionalize the outer surface of hybrid nanoparticles. Electrostatic interactions or strong covalent attachment are used to connect targeting ligands to the outermost surface of LPHNPs.[19] Tumor cells internalize hybrid nanoformulations decorated with targeting ligands through a receptor-mediated endocytosis mechanism, with a higher affinity for diseased tissues and a lower affinity for non-diseased tissues. To increase the stability of hybrid nanosystems with a core-shell structure, a natural polymeric composite of chondroitin sulfate and chitosan was coated with a PEGylated lipid layer and used. The lipid layer was also functionalized with folic acid (FA) for targeted delivery to cancer cells. FA functionalization improved nanoparticle internalization by cancer cells in uptake studies using fluorescently labeled nanoparticles. Furthermore, active targeting caused the therapeutic agent (sorafenib) to be released for longer, resulting in a stronger antitumor effect. In another study, the group Gu et al. created a hybrid nanoformulation to overcome the drawbacks associated with the use of cisplatin and Indocyanine green (ICG), a photosensitizer used for clinical imaging and photothermal. The authors proposed a single-step sonication method for the production of folate-modified, cisplatin/ICG-loaded LPHNPs. The LPHNPs had excellent NIR penetration, as well as optimal monodispersity and stability.[19] In comparison to FRnegative A549 tumor cells, folate-functionalized LPHNPs showed improved targeting efficacy in MCF-7 tumor cells overexpressing folate receptors (FR). Furthermore, the functionalized formulation was more effective at mediating apoptosis and necrosis in MCF-7 cells than free PTX or ICG treatment alone, demonstrating that LPHNPs may be a suitable kind vector for multimodal tumor-targeted therapy. In line with the foregoing, Yugui et al.,(2019) used core-shell LPHNPs functionalized with FA and loaded with gefitinib, an EGFR inhibitor, and a radioisotope (yttrium 90) to develop an effective combination treatment for nasopharyngeal cancer in a recent study.[20] These FR-targeted nanosystems showed a synergistic chemoradiotherapy effect in vivo, with improved antitumor efficacy and 90% drug accumulation at the target site. Guo et al.,(2014) used hybrid nanoparticles with a PLGA polymeric core and a lipid shell functionalized

with transferrin in a similar study (Tf). A post-insertion process was used to conjugate Tf ligands to the lipid shell. TfR-mediated endocytosis was used to internalise the hybrid nanoformulations by A549 cells, with an efficiency of 2.8–4.1 times that of PLGA polymeric nanoparticles. Furthermore, the release rate of hybrid nanoparticles was lower than that of non-hybrid nanoparticles, indicating that hybrid nanovectors can promote long-term drug release.[21] GBM treatment has always been challenging, not only because of the blood-brain barrier (BBB), but also because of the disease's propensity to medication resistance.[22] CRISPR/Cas9, or clustered regularly interspaced short palindromic repeats (CRISPR/Cas9), is a gene-editing technique that has been revolutionized and may cure a variety of hereditary disorders, including human tumors. However, safe and efficient targeted delivery methods are currently few, especially in the central nervous system (CNS). As per Qiang Yang et al.,(2020), lipid-polymer hybrid nanoparticles (LPHNs-cRGD) were used to deliver CRISPR/Cas9 plasmids to temozolomide that target O6-methylguanine-DNA methyltransferase (MGMT), a drug resistance gene (TMZ). Focused ultrasound (FUS)-microbubbles (MBs) were utilised to open the BBB non-invasively and locally to further enable in vivo gene transfer into Glioblastoma. Gene editing efficiency and drug sensitivity modifications were tested both in vitro and in vivo.Therefore FUS-MBs can be used to open the BBB, while LPHNs-cRGD is used to target the delivery of the CRISPR/Cas9 system. The MBs-LPHNs-cRGD delivery system could be a viable option for effective targeted gene delivery in the treatment of glioblastoma.[22] As per Abbas Kaffashi et al.,(2017) research, an antineoplastic drug called farnesylthiosalicylic acid (FTA) loaded lipid–polyethylene glycol (PEG)–polymer hybrid nanoparticles would be effective against Glioblastoma. In vitro (in comparison to a non-malignant cell line, L929) and in vivo, the cytotoxicity of FTA-loaded nanoparticles and their efficacy on rat glioma-2 (RG2) cells were investigated. In vitro and in vivo studies have shown that farnesylthiosalicylic acid-loaded PLGA-DSPE-PEG-DOTAP hybrid nanoparticles are effective against Glioblastoma.Plumbagin, a naphthoquinone produced from medicinal leadwort with anti-cancer effects, has limited therapeutic promise owing to its difficulty to reach tumours in a targeted way at a therapeutic dosage after systemic injection.[23] The purpose of Intouch Sakpakdeejaroen et al.,(2020) study is to determine whether a novel tumor-targeted, lipid–polymer hybrid nanoparticle formulation of plumbagin can inhibit the growth of B16-F10 melanoma in vitro and in vivo. The transferrin-bearing lipid–polymer hybrid nanoparticles loaded with plumbagin resulted in the elimination of 40% of B16-F10 tumors and the regression of 10% of tumors after intravenous treatment.[24] Consequently, transferrin-bearing lipid–polymer hybrid nanoparticles that entrap plumbagin show promise as an anti-cancer nanomedicine. Overall, LPHNPs

has a promising role in the treatment of GBM. To enhance the accumulation of chemotherapeutic drugs, Andang Miatmoko. (2020)[25] prepared Polyethylene Oxide-b-Polymethacrylic Acid surface-modified Hybrid nanoparticles of Cisplatin. Surface modification helps to increase the positive ζ-potential, which improved the encapsulation efficiencies up to 5-18%. Furthermore, sodium cholate-modified hybrid nanoparticles had higher *in vivo* biodistribution in mice than compared to cisplatin solution. This experiment suggests that the surface modifications of cisplatin hybrid nanoparticles using Polyethylene Oxide-b-Polymethacrylic Acid could improve tumor accumulation. Carlotta Pucci et al., (2021)[26] focused on the encapsulation of nutlin-3a, a non-genotoxic drug, in a piezoelectric hybrid lipid-polymeric nanoparticle with ApoE scaffolding, which helps to reduce Glioma cell migration through the BBB. The modified nanoparticles also aid in the promotion of apoptotic and necrotic processes. Qiang Yang et al., (2021)[22] developed lipid-polymer hybrid nanoparticles for effective gene delivery of the clustered regularly interspaced short palindromic repeats (CRISPR) and its associated protein called 9 (CRISPR/Cas9) (LPHNs-cRGD). The LPHNs-cRGD was able to increase permeability through the BBB in the mice model. As a result, the prepared Lipid-polymer hybrid nanoparticles could be a promising treatment option for glioblastoma. Co-administration of chemotherapeutic drugs and nucleic acid is a new research dimension to overcome multidrug resistance while treating Glioblastoma. Pemetrexed and miR-21 antisense oligonucleotide (anti-miR-21) lipid-polymer hybrid nanoparticles (LPNs) have a significant therapeutic effect on glioblastoma treatment, according to Berrin Küçüktürkmen et al., (2017).[27] Anti-miR-21 significantly improves the accumulation of LPNs in U87MG glioblastoma cells, as per confocal microscopy analysis. Docetaxel-loaded poly (D, L-lactide-co-glycolide) Hybrid nanoparticles are developed by Kairong Shi et al. (2015).[28] These nanoparticles outperform brain endothelial C6 cell lines in terms of antitumor activity.

According to current studies, coadministration of chemotherapeutic medication and genes with Lipid Polymeric Hybrid Nanoparticles may increase drug payload in Glioblastoma cells. This method has the ability to enhance *in vitro* anticancer effects while minimising toxicity. The chronology of Lipid Polymeric Hybrid Nanoparticles research also suggests that scientists may use Lipid Polymeric Hybrid Nanoparticles as a theranostic agent in the future for the treatment of Glioblastoma.

LIPID BASED DRUG DELIVERY SYSTEM

Lipid-based drug delivery systems include a broad range of formulations, such as a lipidic excipient dissolved or suspended medicine.[29, 30] Lipophilic hydrocarbon chains are linked to a hydrophilic group such as glycerol, polyglycerol, or polyalcohol to form lipids. [31] Many authorized and commercialized lipid-related drug solutions aim for lipid formulation to solve drug delivery problems for medicines with significant formulation barriers. Solubilization of weakly water-soluble drugs, an increase in intestinal permeability, protection against enzymatic/chemical degradation, and a decrease in the first-pass metabolism are all issues that lipid-based formulations address. The pace and degree of oral absorption of a drug may be influenced by a variety of physicochemical and physiological factors. The lipid-based formulation can readily fix it.

POLYMERIC LIPID HYBRID NANOPARTICLES (LPHNPs)

Liposomes are recognized for their excellent biocompatibility, acceptable pharmacokinetic profile, and capacity to alter on the surface, particularly with polyethylene glycol (PEG), resulting in a longer circulation duration.[32] Physically and chemically unstable, fast drug release rates, and poor entrapment effectiveness are other drawbacks. LPHNPs may be loaded with both water-insoluble and water-soluble drugs to provide agreed stability and regulated drug release rates. They can be improved with either natural or synthetic polymers. LPHNPs is mainly made up of some biodegradable polymeric material core encased with medication and other materials. The phospholipid layer, or lipid PEG base, covers the core even further. By encapsulating the polymeric core system, non-toxic vehicles with a unilamellar or multilamellar structure are formed on lipid structures such as liposomes and niosomes that carry hydrophilic or lipophilic drug molecules. Polymeric nanoparticles, which are made of biodegradable polymers,[33] and are utilized as colloidal and micelles nanocarriers. Liposomal carriers provide a number of advantages, including improved compatibility, a better pharmacokinetic profile, a longer retention period, and effective surface modification. All of these characteristics, found in both liposomes and polymer carrier systems, contribute to the system's regulated and prolonged drug release as well as its stability profiles. LPHNPs have a shell-core structure that consists of a polymer core and a lipid shell. LPHNPs systems will out-perform their non-hybrid counterparts in enhancing the physicochemical characteristics of hybrid nanoparticles such as drug encapsulation, drug release control, physical stability, and cellular uptake with the necessary collection of lipids and polymers. Natural and manufactured polymers such as gelatin, sodium alginate, chitosan, and albumin are used to make polymer nanoparticles, which are biocompatible or biodegradable and non-toxic.

These are polymers that are found in nature. Other synthetic polymers include polyglycolide (PGA), polylactides (PLA), PLGA (polylactide co-glycolides), polyorthoesters, polyanhydrides, polycyanoacrylates, polymaleic acid, poly glutamic acid, poly (methyl methacrylate), poly (vinyl alcohol), poly (ethylene glycol), polyacrylamide, poly(acrylic acid), polyacrylamide, polyacrylic acid), polyacrylamide, polyacrylamide (methacrylic acid).[34] PLGA stands for poly lactide co-glycolides and is primarily used as a delivery vehicle for a variety of medicines, proteins, and other macromolecules. PLGA is biocompatible and biodegradable material that has previously been authorized by the US Food and Drug Administration to protect medications from degradation while also allowing for prolonged-release and tailored drug delivery. **Table 1** discussing different drugs and lipids encapsulated with PLGA to form different nanoformulations to target multiple cancers

Table 1: The Drugs and Lipids used while preparing PLGA based nanoformulations

S No.	Drugs (Single /combinational)	Lipid	Polymer	Methods of preparation	Treatment target	References
1.	Doxorubicin HCl	Lecithin/PEG2000/DSPE–PEG2000- FA	PLGA	Single-step assembly method	Carcinoma	[35]
2.	Docetaxel	Lecithin/DSPE–PEG2000-OMe/ DSPE–PEG2000-RGD	PLGA	Solvent extraction evaporation method	Glioblastoma	[36]
3.	Cisplatin	DSPE–PEG2000/lecithin/DSPE–PEG2000-FA	PLGA	Single-step sonication method	Breast cancer	[37]
4.	Paclitaxel and Triptolide	DSPE-mPEG5000, Injectable soybean lecithin	PLGA	Nanoprecipitation method	Lung cancer	[38]
5.	Curcumin	Lecithin/cholesterol/Chol-PEGRGD	mPEG-PLGA	emulsification solvent evaporation method	Melanoma	[39]
6.	Isoliquiritigenin	Lecithin/DSPE–PEG2000-Mal	PLGA-COOH	Modified single-step nanoprecipitation method	Breast cancer	[40]
7.	Norfloxacin	Soya lecithin, PVA	Poly Lactic Acid (PLA)	Emulsification Solvent Evaporation	Antimicrobial Activity	[41]
8.	10-Hydroxycamptothecin	EPC/DSPE–PEG/DSPE/H2N-PEG2K-OH	PLGA	Modified single emulsification solvent evaporation method	Breast cancer	[42]
9.	Mitomycin C	SPC/DPPE/DSPE–PEG-COOH/ DSPE–PEG2000-FA	PLA	Solvent evaporation method	Stomach and pancreatic cancer	[43]

| No.	Drug	Lipid	Polymer	Method	Application	Ref
10.	Paclitaxel	PEG/DSPE–PEG2000/DSPE–PEG2000-FA	PCL-PEG-PCL	Double step conventional method	Breast cancer	44
11.	Doxarubicin+combretastin	Cholestral,DSPE-PEG	PLA	Two step conventional method	Lewis lung carcinoma	45
12.	Doxorubicin	Lecithin/DSPE–PEG-COOH/ DSPE–PEG2000-FA	PLGA	Emulsification solvent diffusion method	Breast cancer	46
13.	Bupivacaine	Lecithin and DSPE-PEG2000	PLGA	Nanoprecipitation or self-assembly method	Local anesthetic Effects	47
14.	Psoralen	Soy lecithin	PLGA	Nanoprecipitation method	Breast cancer	48
16.	Rapamycin	DSPE-PEG2000, Soybean lecithin,	PLGA	Nanoprecipitation and Vortexing method	Infantile Hemangiomas	49
17.	Camptothecin, Fe3O4	Lecithin, DSPE-PEG	PLGA	Nanoprecipitation method	Breast cancer	50
18	GemicitabineHCL+Paclitaxel	Licithine,DSPE-PEG	PLGA	Nanoprecipitation method	Pancreatic cancer	50
19.	Docetaxel	DLPC/DSPE–PEG2000/DSPE–PEG2000-FA	PLGA	Single emulsification solvent evaporation method	Breast cancer	35, 51

Compared to other nanocarriers, LPHNPs has several distinct advantages, including the polymer lipid combination containing biocompatible polymers, the variety of lipid and polymer lipid combinations in which they are prepared, and their superior ability to encapsulate a variety of therapeutic agents. The development of a concept of core-shell nanostructures was inspired by liposomes and polymeric nanoparticles, in which the lipid membrane encases a polymer core. It has superior biocompatibility and physical strength. The encapsulation of many medicines increases drug loading, nanoparticle stability, drug extended-release in the blood, and systemic circulation half-life. LPHNPs provide various benefits, including high drug loading, fixed particle size, drug release control, and surface functioning with various ligands, such as antibodies, monoclonal antibodies, peptides, folate compounds, aptamers, and so on. A recent shift from a two-step to one-step approach has shown a considerable improvement in the manufacture of LPHNPs. In oncology, these are some potential medication delivery routes. LPHNPs is a relatively new discovery in nanotechnology, a modern technique for dispersing particulate matter also known as the supramolecular bio vector created by Bio Vector Therapy in the 1990s (SMVBV).[52] LPHNPs have retained their dominance in the delivery of medicines, vaccines, genomes, and proteins for many years. Lipid-based core is made up of inorganic components like silica and magnetic iron oxide, as well as organic materials including polysaccharides, polystyrene, and polyethylene. The primary polymeric core building element for

acting as a carrier for poorly water-soluble medicines is a hydrophobic or biodegradable polymer (Chitosan, PLGA). The regulated release of medicines from the body is part of the core type system. To support and vaccination adjuvants in intercellular targeting, particle size is less than 100nm.

Type of lipid polymer hybrid nanoparticles

Lipid-based nanoparticles are active carriers that increase product bioavailability within or across disease targets. **Figure 2** depicts the various kinds of Lipid Polymeric Hybrid Nanoparticles (LPHNPs) in more details.
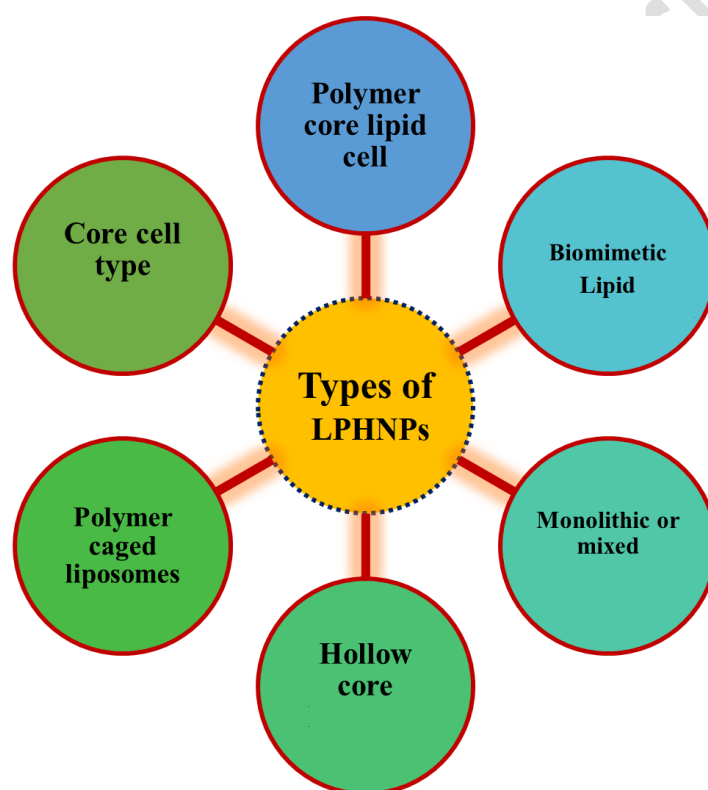
Figure 2: Different types of Lipid Polymeric Hybrid Nanoparticles (LPHNPs)

Based on particular Lipid and polymer combinations, the hybrid polymeric lipid nanoparticles were divided into many groups. The following sections go through each of those kinds in detail.

Polymer core lipid shells

Lipid polymer hybrid nanoparticles(LPHNs) in this form are the simplest to produce. It is made up of a polymer core that is coated with one or more lipids (lipid PEG and lipoidal shell).[53] The biomimetic consistency of the lipids, along with the structural advantage of the biodegradable polymer core, results in a

novel and effective delivery method that can be used to treat a variety of systemic and tropical diseases. Polymer and lipid coating make up the hybrid structure. The use of Psoralen-loaded lipid-polymer hybrid nanoparticles to improve doxorubicin effectiveness in multidrug-resistant patients has been described.[54] For targeted cancer therapy, a drug carrier comprised of a lipid-monolayer shell and a biodegradable polymer core with a continuous distribution and improved efficacy of the medicine offers great promise. The LPHNPs offer a number of benefits, including the capacity to load several drugs, the ability to control particle size and drug release, enhanced drug loading efficiency, and serum stability.

Monolithic lipid-polymer hybrid nanoparticles

Mixed lipid-polymer hybrid nanoparticles are also known as monolithic LPHNPs. PEG molecules are spread in a polymeric core matrix comprising drug molecules in this type of lipid or lipid nanoparticles.[55] Such a system is a development that can be comparable to a colloidal drug delivery vehicle. Phospholipids are an essential part of their architecture, but they are not versatile enough to enable structural alteration by PEGylation of high density.

Erythrocyte membrane-camouflaged polymeric nanoparticles

Biomimetic nanoparticles are erythrocyte vesicle nanoparticles. These nanoparticles, which are sub-100 nm in size and coated with an erythrocyte cell membrane (RBC) polymer, are used to make vesicles that imitate or duplicate the complex surface chemistry of the erythrocyte membrane. Such nanoparticles may cross the membrane barrier and efficiently deliver medications by staying in circulation for extended periods of time. This kind of nanoparticle, such as cell membrane-camouflaged nanoparticles, has recently been described as a medication carrier for cancer treatment by several studies. Polymer nanocarriers that are biomimetic-cell-camouflaged and have advantages similar to the functional versatility of natural cell membranes and the physicochemical adaptability of synthetic polymers are being studied as potential candidates for use in a therapeutic platform called "Erythrocyte-Membrane-Camouflaged Nanocarriers" that has tunable Paclitaxel release kinetics via macromolecules.[56]

6.4 *Polymer caged liposomes*

To preserve cell stability, this method includes the self-possession of polymers coated on the liposome's surface. For the processing of nucleic acids, proteins, and chemotherapeutic drugs, nanoscale polymer caged liposomes (20-200nm) have demonstrated excellent pharmacokinetic characteristics. The pH-responsive stable polymer-caged liposome may alter these pH-sensitive nanoparticles.[19] pH-sensitive polymer-caged

liposomes distribution system with excellent stability is the subject of a patent. The polymer caged liposomes were used in another study to describe photo actionable drug release techniques of liposomes. The tailored delivery of different kinds of peptides, peptones, and proteins for particular intracellular trafficking through PEG coating.

1.1 *Core-shell type lipid polymer-lipid hybrid nanoparticles (CS-LPHNPs)*

This kind of nanoparticle has an open inner core surrounded by a thick lipid coating, a polymeric coating on the inner core, and a lipid PEG coating on the outer core. Core-shell lipid polymer hybrid nanoparticles, which combine the mechanical benefits of biodegradable polymeric nanoparticles with the biomimetic advantages of liposomes, have emerged as a strong and efficient distribution vehicle.[19] A membrane made up of phospholipid membranes surrounds CS-LPHNPs, a biodegradable polymeric nucleus. The polymer and lipid complexes are typically constructed by mixing both liposomes and polymeric nanoparticles. The polymeric core surface is surrounded by a lipid or lipid multilayer bilayer. **Figure 3** depeicts the basic stracture of core-shell type of polymeric lipid hybrid nanoparticles.
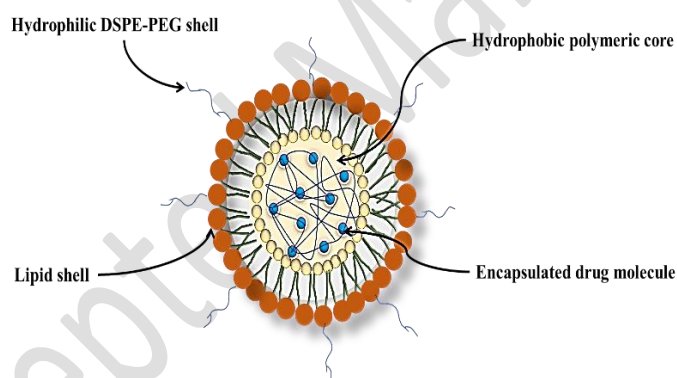
Figure 3: Core shell-type Polymeric lipid hybrid nanoparticles

ROLE of TPGS (D-tocopherol polyethylene glycol 1000 succinate) in LPHNPs

The FDA has authorized the Vitamin E TPGS or TPGS (D-tocopherol polyethylene glycol 1000 succinate) as a non-toxic adjuvant for effective medication distribution.[57] A water-derivative form of natural vitamin E with a lipophilic alkyl tail and a hydrophilic polar head part has been given the functions of TPGS. TPGS has a variety of physicochemical and biological characteristics that may be used in drug delivery, including improved drug solubility, improved drug penetration, high biocompatibility, and selective anticancer action. With high emulsification efficiency and cellular adhesion, TPGS is more than just a great emulsifier or absorption enhancer. It also has a high degree of hydrophilicity and is capable of penetrating cellular

membranes. The good compatibility of TPGS with a biodegradable polymer of poly(lactic-co-glycolic acid) improved transcellular or transcorneal product permeability, increased drug absorption, and reduced P-glycoprotein multidrug resistance. Multiple drug resistance (MDR) is a serious clinical issue that has a negative impact on the effectiveness and efficacy of cancer chemotherapy. MDR is shown in cancer cells via a variety of mechanisms. TPGS works as a strong excipient to overcome multidrug resistance in cancers by inhibiting the activity of ATP-dependent P-glycoprotein (MDR).[58] Other antioxidants and therapeutic effects have been seen in certain TPGS formulations.TPGS is a lipophilic antioxidant produced by enzymatic cleavage of a synthetic amphiphile. Vitamin E, often known as TPGS, is a water-soluble form of natural vitamin E produced using polyethylene glycol (PEG) 1000. A part of the hydrophilic polar head and the lipophilic alkyl tail form an amphiphilic structure.[59] TPGS may be used to improve the solubility, emulsification, permeation, and bioavailability of hydrophobic compounds. TPGS may be used as an anticancer treatment since it has been proven to trigger apoptosis in cancer cells. TPGS has a number of unique properties that make it a good candidate for use as a drug delivery nanocarrier, including the ability to solubilize poorly soluble medicines, improve drug cell penetration, and extend the product's blood circulation time. An efficient biological reaction agent capable of blocking P-gp in cell drug efflux and drug transport through cell barriers such as the endothelium of the brain. Normal immortalised breast cells and cancer cells respond to TPGS treatment in very different ways. As two human MDR cell lines (H460/taxR and KB-8-5) were treated with TS, the MDR effect was significantly reversed when compared to paclitaxel alone; however, the inhibitory impact was smaller than that observed with TGPS.

Method of preparation of Lipid polymeric hybrid nanoparticles

Lipid-polymeric hybrid nanoparticles are core-shell nanoparticle structures with polymer cores and lipid/lipid-PEG shells that have properties similar to both polymeric nanoparticles and liposomes, especially in terms of physical stability and biocompatibility. Nanoprecipitation, emulsification–solvent evaporation (ESE), spray drying and high-pressure homogenization are often used to make polymeric NPs. As seen in **Figure 4.**
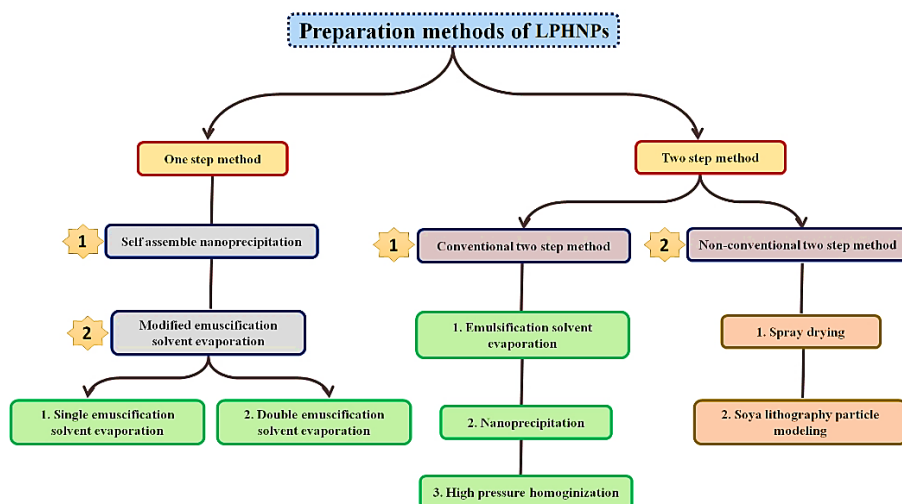
Figure 4: Methods of preparation of LPHNPs

Two broad methods can generally be used to synthesize LPHNPs; single-step processes and double-step methods; both ways of planning were mentioned below.

1.2 Single-step methods

The preparation of LPHNPs in a single step offers many advantages over two step technique, including great scalability and cost-effectiveness, as well as a traditional preparation method.. In the two-step approach, separating polymeric nanoparticles and lipid vesicles takes all of your time and energy. Maximum faults arise during the single preparation of LPHNPs in the two-step procedure. To address these issues, a single-step LPHNPs preparation was devised. For example, polymeric nanoparticles and lipid vesicles are required in the two-step process but not in the one-step phase. As a result, the one-step method only requires mixing polymer and lipid solutions; after that, they can be easily self-assembled to form a structure of LPHNPs using either nanoprecipitation or emulsification–solvent–evaporation, the same techniques that have been systematically used to prepare non-hybrid polymeric nanoparticles.[60]

1.3 Double-step methods

The dual-step technique is a commonly utilized methodology in the first stages of LPHNPs formation, and it is often employed to create monolayer, bilayer, or multilayer shells for LPHNPs.[61] Electrostatic interactions combine cationic lipid vesicles with anionic polymeric nanoparticles in this process. Nanoprecipitation and emulsification by solvent evaporation or homogenization via high-pressure, melt emulsification, and solvent injection is often used to produce LPHNPs. The polymeric core and lipid shell are typically produced in two steps; the two components are then combined using hydration and sonication to form the lipid-core polymer's structure. To begin with, lipid nanoparticles were made utilizing a variety

of techniques, including micro emulsification, ultrasonication, high-pressure homogenization (hot and cold), melt emulsification, solvent emulsification, and solvent injection. Using direct vortexing needle extrusion or high-pressure ultrasonic homogenization, the polymer solution was mixed with the lipid nanoparticles to create lipid-polymer hybrid nanoparticles. Two-step techniques are divided into two categories: standard and non-traditional two-step procedures, which are mentioned below.

1.3.1 Conventional two-step method

Small-scale preparations of lipid polymer hybrid nanoparticles are mostly done using conventional techniques. For hybrid nanoparticles, such methods are mostly utilized in small-scale preparations. Polymeric nanoparticles were made via emulsification solvent evaporation, nanoprecipitation, or high-pressure homogenization. The traditional two-step technique may be divided into two categories. The LPHNPs may be made by adding previously developed polymeric nanoparticles to (A) a dried thin lipid film made by dissolving the lipid in an organic solvent (e.g., chloroform) and evaporating it in a rotary evaporator, or (B) performed lipid vesicles made by hydration of the thin lipid film. During the purification step, differential centrifugation is used to separate free lipids and LPHNPs. For example, utilizing PLGA to assemble cationic lipid vesicles under continuous stirring or bath sonication at 30°C, a hybrid NP production technique was developed. The prepared particle size ranges from 200 to 400 microns in diameter, with a potential surface charge of 20-30 microvolts.

1.3.2 Non-conventional two-step method

The unconventional method is mostly utilised in the manufacture of large quantities of LPHNPs. Combining polymeric nanoparticles with lipid vesicles is the traditional two-step method. Spray drying and soft lithography particle moulding were utilised to make LPHNPs in an unconventional manner. The spray drying method was utilised to create nanoparticles with a size range of 400–500nm, which were then dispersed in an organic solvent (dichloromethane) containing different lipids. To make LPHNPs, the lipoid polymeric suspension was spray-dried using spray drying (SD) and spray freeze drying (SFD) to form dry powdered LPHNPs of levofloxacin through an inhalation route, a method similar to this was published on centred on the same research. A soft lithography moulding method, also known as particle replication in non-wetting templates (PRINT), was utilised to produce LPHNPs for the delivery of genetic material.[62] The organic solvent polymer PLGA, or genetic material such as siRNA, is dissolved and shed onto a sheet of

polyethylene terephthalate (PET), which is then heated in conformal contact with a PRINT mould, causing the polymer to spill into the mould and solidify when returned to ambient temperature. Following that, nanoparticles were removed from the mould and separated from PVA-coated PET sheets using an aqueous lipid solution, resulting in the formation of LPHNPs. These particles are required to be needle shapes with a length of 200nm and a (+) 5 mV zeta potential after freeze-drying. A similar technique was recently described for antipsychotic medication administration utilizing nanotechnology for nose-to-brain transfer. According to other researchers, nonsteroidal anti-inflammatory medications (NSAIDs) were incorporated into liposomes for topical therapy of inflammatory and degenerative diseases.

### 1.3.3   *Self-assembled nanoprecipitation method:*

Large yields of lipid-polymer particles below 100nm may be obtained using this self-assembled nanoprecipitation method. In two stages, the lipid shell and polymer core are produced separately and then joined to create a bilayer of LPHNPs. By using the self-assembly nanoprecipitation technique, lipid, polymer, and drug are united in one phase to create a monolayer of nanoparticles. A self-assembled nanoprecipitation technique dissolves the polymers and medicines to be encapsulated in water or another organic solvent before preparing the LPHNPs. It then heats Lipid or Lipid PEG water to 65-70°C to dissolve it and produce a consistent distribution. After that, the prepared solution was continuously stirred or sonicated. To precipitate the polymer, the polymeric drug solvent was supplied dropwise, and the Lipid or Lipid PEG molecule self-assembled with hydrophobic contacts across the polymer's core. The polymeric material was given a water-insoluble lipid tail. The surrounding layer was connected to a water-miscible head that created LPHNPs spherically secured by Lipid or Lipid PEG, after which the LPHNPs were centrifuged to remove excess Lipid and polymer, and the solvent evaporated. The particle size or PDI of hybrid nanoparticles is occasionally influenced by the polymeric material content, volume ratio, and solution mixing speed. The lipid-polymer ratio has an indirect impact on encapsulation uniformity, drug loading, and drug kinetics. To improve the efficacy of vincristine sulphate encapsulation and oral bioavailability, researchers used a self-assembled nanoprecipitation technique to create hybrid dextran sulphate nanoparticles. The self-assembled or nanoprecipitation technique is shown graphically in **Figure 5.**
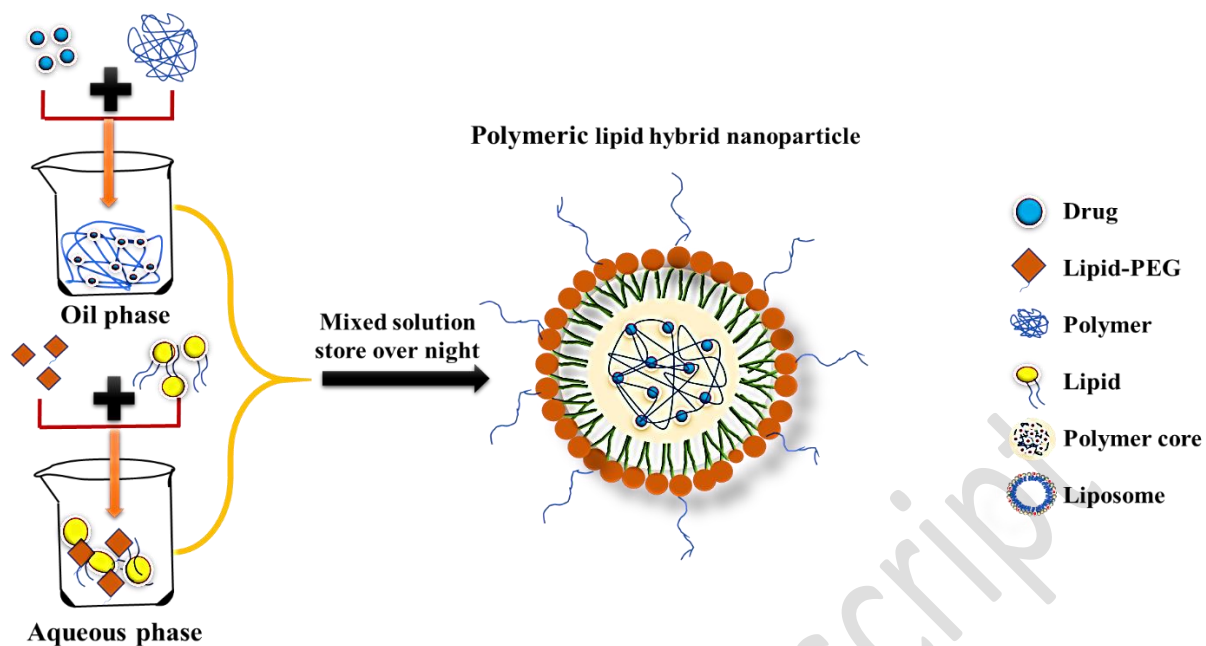
Figure 5: LPHNPs preparation by self-assembled or nanoprecipitation method

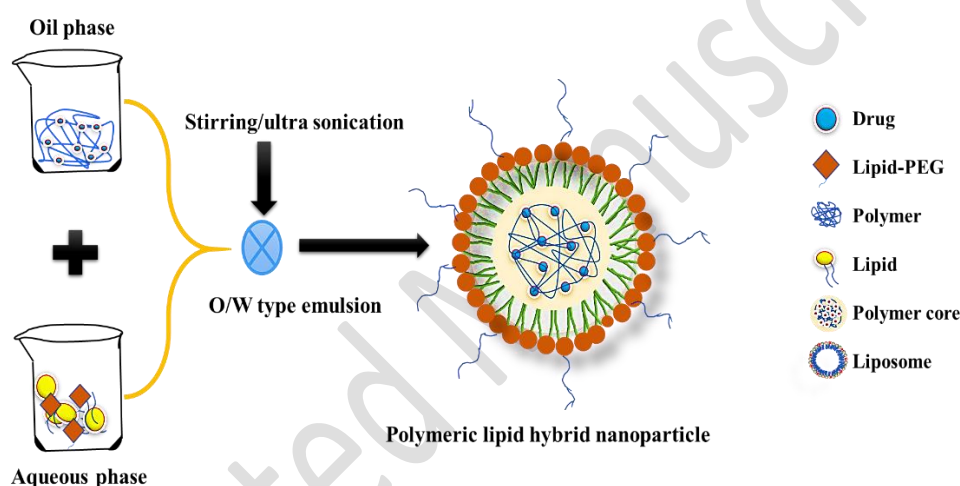### *1.3.4*   **Emulsification** *and solvent evaporation method*

The emulsification solvent evaporation (ESE) method may be classified into two subtypes: single and double

emulsification. Gurney et al. published the first paper on this technique in 1981. Various procedures such as

stirring, bath sonication, and homogeneous dispersion of Lipid into the water are used in this technique to

disperse the Lipid in water. It is then heated to a certain temperature in order to dissolve the organic solvent

polymer and medication. After the organic solution was injected dropwise in the aqueous process, the tiny

particles lipid coating was applied and mixed for 5 minutes to convert polymeric particles into small

particles. The dual step emulsification solvent evaporation method is shown graphically in **Figure 6.**
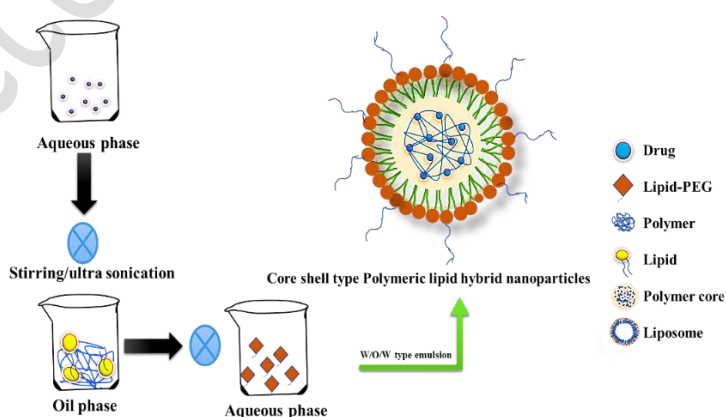
Figure 6: LPHNPs preparation by Dual step emulsification solvent evaporation method

8.2.2.1 Single emulsification solvent evaporation method

In this technique, the encapsulated medicinal substance or polymer is extracted into the organic phase (oil phase). The solution was then placed into a lipid water dispersion medium to create an oil in water emulsion (o/w) using ultrasonic content stirring. The organic solvent is evaporated under reduced pressure using a rotary evaporator, forming the polymer core while the lipid or lipid PEG self-assemblies in the surrounding region of the polymer core.[49] Due to the stable emulsion formation, this method is more frequently employed in the formulation of a large number of LPHNPs than nanoprecipitation. This technique was previously declared for the delivery of genes and medicines. Researchers recently announced nucleic acid-loaded lipid-polymer nanohybrid nanoparticles for anticancer treatment using this technique. The single emulsification methods are shown graphically in **Figure 7**.


Figure 7: LPHNPs preparation by single emulsification method

8.2.2.2 Dual emulsification solvent evaporation method

The best technique for creating polymeric nanoparticles is the double emulsification solvent evaporation process. This technique is important in the development of core-shell type LPHNPs, in addition to polymeric nanoparticle production. Its capacity to encapsulate both hydrophobic and hydrophilic drugs is maximised. This technique is mainly used to determine which drugs are insoluble in the organic phase but readily soluble in water. They can't dissolve a hydrophobic polymer core together. This technique is often used to make water-in-oil-in-water (w/o/w) emulsions. To begin, a water-in-oil emulsion (w/o) is created by mixing a medication with an aqueous polymer solution and an organic solvent containing a lipid. The emulsion is then transferred to another aqueous media to form a fresh water-in-oil-in-water (w/o/w) emulsion. A rotary

evaporator was used to evaporate the oil phase of the emulsion, resulting in the formation of LPHNPs.[63]
Drug encapsulation in nanoparticles reduces a number of drug-related drawbacks, such as leakage, enhances disease target, and improves the drug's long-term release. This technique loaded lipid polymer hybrid nanoparticles (LPHNPs) for cancer therapies, according to several researchers' gemcitabine hydrochloride findings. Hybrid nanoparticles of carboxymethylcellulose for excellent anticancer activities. The same technique is used to make nucleic acid-loaded lipid-polymer nanohybrids, which are used as new nanotherapeutics in anticancer treatment. This technique effectively produces core-shell type lipid polymer hybrid nanoparticles, which have the potential to provide single or combination medication delivery as well as optimal drug load capacity. The method dual step method shown graphically in **Figure 8.**
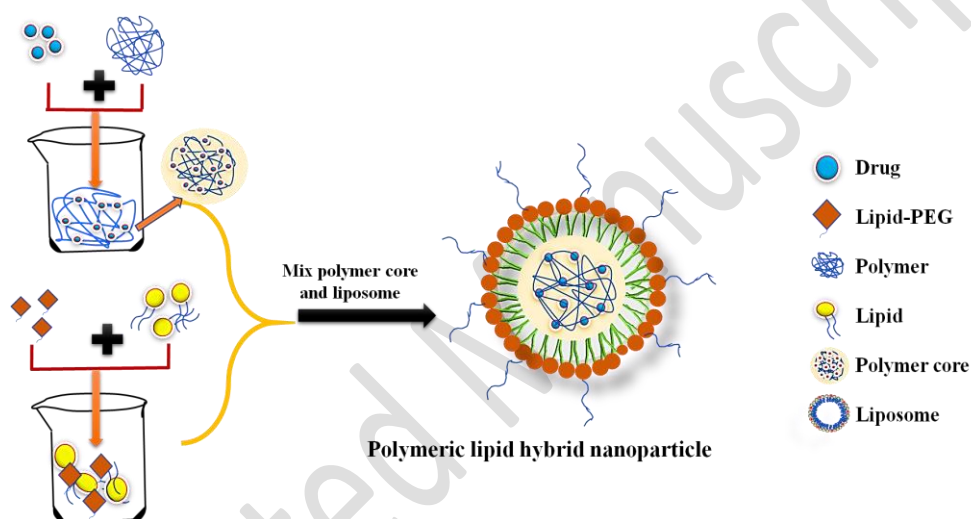
Figure 8: LPHNPs preparation by method dual step method

Characterization of Polymeric Lipid Hybrid Nanoparticles

1.4 Physiochemical properties of LPHNPs:

The in vivo profile of polymeric hybrid nanoparticles is determined by several physicochemical characteristics of nanoparticles, including particle size, shape, and zeta potential. Transmission electron microscopy (TEM), atomic force microscopy (AFM), scanning electron microscope (SEM), fluorescence microscopy, and confocal laser scanning microscopy was used to determine the structure and surface morphology of nanoparticles (CSLM). The real physical size, size distribution, structure, and surface appearance of polymer lipid hybrid nanoparticles were determined using transmission electron microscopy (TEM) and electron scanning. The physical size and size distribution may be determined by the dried nanoparticles attached to the silicon wafer substructure for electron microscopy (SEM) imaging. The interior

core-shell structure of the LPHNPs was examined using transmission electron microscopy (TEM). Cryo-electron microscopy (CEM) is one of the most efficient methods for studying polymer hybrid nanoparticles at shallow temperatures (using liquid nitrogen temperature).[64, 65]

1.5 Zeta potential, Particle size, and polydispersity index:

The hybrid nanoparticles' in vivo profiles were assessed by particle size, surface zeta potential, and shape, among other physicochemical characteristics. The average vesicle size has a high zeta potential. For the stability and efficiency of LPHNPs, particle size and polydispersity index are essential factors.[65, 66] One of the most critical variables in determining the lifetime of nanoparticles in systemic circulation and their ability to aggregate in tumor tissues passively is particle size. The particle size range of 10 to 150nm is widely recognized as helpful and desired for systemic medication delivery. Zeta sizer devices detect particle or molecule sizes from one nanometer to many microns using dynamic light dispersion. The dispersion of electrophoretic light and the molecular weight of static light diffusion determine the zeta potential and electrophoretic stability. The hydrodynamic distribution of nanoparticles and their size in the blank and drug-loaded polymer hybrid nanoparticles polydispersity index were calculated using the Dynamic Light Scattering (DLS) method (PDI). The PDI scale between 0-1 is ideal for particle distribution if the low PDI implies particle uniformity in particle preparation. When the zeta potential of the particle is between -30 and +30 mV, it remains stable. More than 30 mV in prepared particles, or -30 mV in prepared particles, implies high repulsive force and long-term stability. The Malvern Zeta sizer (Nano ZS instrument) was used to determine the real mean value of the zeta potential (mV), particle size (nm), polydispersity index (PDI), and particle size distribution under optimum temperature and experimental conditions. The Zeta Potential is a tool that may be used to determine the surface charge of nanoparticles in solution (colloids). For 10 to 15 cycles, particle size and potential zeta investigations were carried out. LPHNPs' relative particle size and a limited PDI were used to represent the average particle size. Different factors that influence particle sizes and hybrid polymer nanoparticles polydispersity index, such as polymer and lipid concentrations, either raise or reduce such concentrations of lipids and polymers. The formulation's particle size may have a significant impact.

Entrapment efficiency (%EE) and loading capacity (LC)

The most important parameters for defining polymer hybrid nanoparticles proposed to the drug loading range for any polymer or any excipient are entrapment efficiency (EE) and drug loading capacity (LC). It has to do with the prepared LPHNPs real drug entrapment ability. The medication was encased in a central polymeric or lipid shell, which increased drug loading capacity. In the hydrophobic polymer core has the greatest amount of hydrophobic medicines.[67] The drug loading method was often used to enhance one or both drug loads. Ion interaction between drugs is used by tiny molecules, and polymer may be a major method of boosting drug charges. Drug solubility, polymer and lipid phase miscibility, affinity, drug lipid load interactions, lipid density, aqueous phase pH, and preparation techniques are all variables that may influence entrapment effectiveness (EE) and drug loading ability (DL). The significance of drug loading and trapping is also affected by the preparation procedure (EE). The polymer lipid hybrid nanoparticles found many low molecular weight anticancer medicines, proteins, and nucleotides. The volume of medication encapsulated in the LPHNPs was measured using high-performance liquid chromatographic (HPLC), dialysis, centrifugation, and membrane filtering methods. The volume of LPHNPs encapsulating pharmaceuticals was measured using high-performance chromatographic liquid (HPLC), dialysis, centrifugation, and membrane filtering methods.

The drug loading capacity (DL) and entrapment efficiency (EE) were indicated below

$$\%EE = \frac{Amount\ of\ drug\ loaded\ in\ PLHNPs}{Total\ amount\ of\ drug\ added\ in\ formulation} \times 100$$

$$\%DL = \frac{Amount\ of\ drug\ loaded\ in\ PLHNPs}{total\ weight\ of\ PLNPS} \times 100$$

As for hydrophilic drugs, the hydrophobic product quickly partitioned between the lipid surfaces resulting in higher encapsulation of drugs close to hydrophilic drugs.

Drug release

Drug solubility, drug permeability, polymer degradation speed, and particle size interaction between polymer and drug potential particle charge may all influence drug release profiles.[3] The diffusion and release of chemically conjugated hydrolyzed pharmaceuticals is used to determine drug release for a mechanically encapsulated material using LPHNPs. Dialysis, high-performance liquid chromatography (HPLC), a mass spectrometer, and a semi-permeable membrane were used in the majority of in vitro drug release investigations. The drug release check of nanoparticles dialysis procedure may be carried out at 37°C in a

significant quantity of medium. At a temperature of 37°C, nanoparticles are poured into a huge volume of release media. The drug molecules are released from the nanoparticles and leach into the release environment through the diffusion process. The released drug and any leftover drug are collected in nanoparticles, and the solution is centrifuged for 30 minutes at 1200 RPM. A mass spectrometer analytical technique, such as high-performance liquid chromatography, was used to quantify the supernatant (HPLC).[68] The study of drug release is beneficial in the production of nanoparticles; drug release was studied in vitro using the USP type II dissolving technique at 50 rpm. Nanoparticles were prepared and submerged in PBS 900ml using a semi-permeable membrane. The experiment was carried out over many minutes at a time, with the pH 0.1HCL solution being maintained after the sample was collected. A UV spectrophotometer was used to analyse the samples.

1.6 Stability of LPHNPs

Physical and chemical stability are required to develop LPHNPs stability studies for both lipid polymer hybrid suspension and free dry nanoparticles to evaluate drug loss and changes in nanoparticle composition during storage. Microscopic inspection, entrapment efficiency, zeta potential, particle size and polydispersity index, physical and chemical assessment, optimization, and research of a produced nanocarrier system are all important factors to consider when evaluating formulated LPHNPs. As a consequence, the self-modified characteristics of nanoparticles on the lipid monolayer surface were utilized to improve the solubility and stability of DSC and TGA nanoparticles, allowing researchers to investigate the effects[69] of time and temperature on nanoparticle thermal properties. To investigate drug losses from nanoparticles and changes in the structure of nanoparticles under storage settings, stability experiments were conducted on suspended nanoparticles and lyophilized nanoparticles. The optimized formulation of HN-08 nanoparticles has been subject to accelerated stability studies according to ICH guidelines. Samples of lyophilized and suspension nanoparticles are kept at 4˚C/60 ± 5%, 25˚C/60 ± 5% RH for three months in glass vials lyophilized nanoparticles are distributed, sonicated, and characterized by duration, zeta potential

1.7 Cellular uptake and cytotoxicity

In vitro characteristics of drug-loaded LPHNPs for in vivo assessment of target cells include cellular uptake and cytotoxicity. Targeting drug-loaded nanoparticles with fluorescent samples such as isothiocyanate fluorescence (FITC) with cells using fluorescence microscopy, such as a confocal laser scanning

microscope, may be used to evaluate cell absorption of LPHNPs.[70] We can see the cell once the extra particles have been removed. The endocytosis mechanism is usually responsible for the in vitro cell absorption of formed nanoparticles. The surface of polymer lipid hybrid nanoparticles targeting ligands is conjugated to the nanoparticles to allow receptor-controlled endocytosis. The shape, size, type of drug load, and structure of nanoparticles interact with the biological system, influencing biological interaction, cell absorption and helping to decide cytotoxicity. To evaluate in vitro or in vivo cytotoxicity, specific approaches can be used, such as the ability to determine the immediate effects of NPs on target cells in the absence of secondary inflammatory effects; (ii) the ability to determine primary toxicity mechanisms in the absence of physiological and compensatory factors that can complicate interpretation in whole animals; and (iii) efficiency rate. The incubation cycle must be completed for a certain amount of time in order to assess cell cytotoxicity. After 72 hours of various cultures, appropriate assays such as the ATP and MTT assays are performed to evaluate the cell potential to free medications. Nanoparticles work directly to reduce drug-related adverse effects. Cell uptake and cytotoxicity have been studied using paclitaxel hybrid nanoparticles and MCF-7 breast cancer cells.

Immunocompatibilty

In vitro studies have shown that LPHNPs have the ability to carry poorly water-soluble drugs with high encapsulation and loading yields, as well as a tunable and sustained drug release profile, excellent serum stability, and differential cell targeting.[71] The LPHNPs may have the potential to be used as new adjuvants in vaccination procedures, in addition to being used in medication administration.[72] Although the immunological properties of these LPHNPs have been extensively studied; they have yet to be thoroughly investigated. If these nanoparticles are to be used for systemic drug administration or as adjuvants for immunization, their immunocompatibility features, such as complement system activation, plasma and serum protein binding capabilities, and activation of the coagulation cascade, must be better understood.[73] It's a biochemical cascade in the blood that's in charge of detecting and clearing foreign substances. The complement system is found in the bloodstream and is part of the innate immune system. This complex is made up of approximately 20 tiny proteins and protein fragments. These proteins are usually found in the body's circulation as inactive zymogens. The complement system is activated when they are triggered by a trigger or activator, which causes a reaction and the activation of an anti-cell-killing membrane assault complex. The complement system can be activated through three different pathways: the classical,

alternative, and lectin pathways. The most well-known of these channels is the classical pathway. Classical activation begins when the protein C1q recognizes activators and attaches to their surface, primarily through charges or hydrophobic contacts.[74] The alternative route to complement activation is activated as a result of C3b's covalent attachment to the hydroxyl or amino groups on the pathogen's surface, and the events that follow are similar to those that occur in the classical pathway. Determination of the immunocompatibility of material requires a thorough investigation of protein binding to its surface. Bound proteins can cause thrombosis on the surface of a substance by interacting with platelets and activating their own intracellular clotting cascade. Core-shell forming LPHNPs made of biocompatible, biodegradable polymers showed excellent immune system interaction characteristics. Core-shell forming LPHNPs made of biocompatible, biodegradable polymers revealed optimal immune system interaction characteristics. Polymer nanoparticles with immunogenic properties are made up of synthetic polyester and polyanhydride and are immunogenic.[75, 76] The immune system's primary functions are tissue homeostasis, or protecting the host against external agents such as poisons and pathogens, and tissue preservation. They are often known as a variety of environmental pollutants and xenobiotics in addition to other medicines, and they may have an impact on normal immune system function. LPHNPs' physicochemical characteristics improve charge, aggregation, lipid coating, lipid charge, lipid amount, liposome size, endotoxin contaminations, PEGylation, and drug delivery. Gene delivery, vaccine distribution, and improving the quality of anticancer, anti-inflammatory, and antiviral treatments may all benefit from focusing on the immune system. Because the vesicle mimics the form and size of harmful microorganisms, nanobacteria, viruses, endotoxin, and other pathogens, the liposomes are vulnerable to immune detection. Gene delivery via cationic polymer (e.g., PEI) and cationic lipid has minimal immunogenicity and toxicity for large-scale manufacturing (e.g., lipoplex, DOTAP). As a result, a non-viral formulation with a longer circulation time after systemic injection and lower absorption in non-targeted tissues may be used. Various studies have shown that altering the ratio of polymer to lipid on the surface of LPHNPs altered the adsorption patterns of human plasma and serum proteins. The results of their study's complement activation and coagulation test revealed that the lipid hybrid polymeric nanoparticles had good biocompatibility. The immunotoxicity of nanoparticles in carriers is influenced by their therapeutic loading. The inflammatory response, plasma coagulation time, cytotox ogenesis, and dose-limiting toxicity of therapeutic nucleic acids are all examples. The immunocompatibility of polymeric core nanoparticles and liposomes is a concern. Immunocompatibility and immunotoxicity characteristics of hybrid polymeric lipid nanoparticles must be assessed. Plasma coagulation, complement activation assay,

protein binding research, and platelet counter fusion test are among the in-vivo and in-vitro investigations available.

Application of the LPHNPs

Lipid polymer hybrid nanoparticles may be developed to contain and disperse a broad range of medicinal substances readily. These medicines may be put into nanoparticles in single doses or in combinations with two or more drug kinds. Medicines that are hydrophobic may be directly entrapped, whereas lipophilic drugs can be incorporated into the lipid shell. The LPHNPs has shown tremendous promise in delivering single or multiple drugs. They can help reduce medication resistance in a variety of ways. Wang et al., for example, have described an LPHNPs system for the targeted delivery of chemotherapy and radiation drugs for prostate cancer treatment.[3] Other than lung inflammation, vaccine treatment, and other therapeutic uses such as bioimaging, the usage of LPHNPs has just recently emerged. The discovery of nanoparticles and their uses are updating and improving the pharmacokinetics and pharmacodynamic characteristics of medicinal substances has been documented in clinical progress. To put it another way, the usage of LPHNPs in cancer therapy has expanded the number of chemotherapeutic medicines available. The dual nature of polymer lipid hybrid nanoparticles makes them a good platform for distributing single or many drugs. LPHNPs have recently been used to demonstrate the potential function of combination drug delivery for single drug administration of Paclitaxel to damaged vasculature. During the nanoprecipitation procedure, the anticancer medication (Docetaxel) was first encased in the polymeric core.[77] Rather of delivering medicinal drugs, LPHNPs have been used to encapsulate and distribute different imaging agents such as fluorescent dye, iron oxide, and quantum dots via a polymeric core system. Despite this, anticancer medication delivery has dominated LPHNP drug delivery applications. This is mainly due to the predominance of multidrug-resistant cancer cells as the most significant problem in chemotherapy, which necessitates the continuous development of better and more intriguing treatments. LPHNP drug delivery applications are classified into three types: single drug delivery, combinational drug delivery, and active targeted drug delivery. Targeted medication delivery, whether unique, combinational, or functional, is more effective for anticancer treatments. LPHNPs have been studied against a variety of cancers, including lung cancer, breast cancer, liver cancer, cervical cancer, prostate cancer, and others, have been reported to deliver a single chemotherapeutic medication. Successful anticancer treatment generally necessitates the use of several

chemotherapeutic medicines or the use of chemotherapy drugs in combination with other therapeutic agents such as genes and magnetic nanoparticles.

Recent advancement of the LPHNPS

LPHNPs, also known as liposomes, are the most recent advancement in nano-drug delivery systems, consisting of a polymer core and a lipid shell that gives nanoparticles their durability and biocompatibility.[78] There is an increasing number of new technologies in the field of nanomedicine to support the newest nanoformulations. The number of researchers studying LPHNPs takes into account desired characteristics in nanoparticles, including high drug loading efficiency, stability, controlled drug release, biocompatibility, and prolonged circulation durations, among other things. They combine the benefits of liposomes with polymeric nanoparticles, giving them a viable option in the area of medication and gene delivery. The use of LPHNPs has been broadened to include combinatorial and targeted treatments and the delivery of genes, vaccines, and diagnostic agents, rather than just single drugs. The use of LPHNPs in drug delivery and other kinds of hybrid nanoparticles and their applications in individual and combinatorial drug delivery and gene therapy.[22] LPHNPs are more stable and have a longer blood circulation duration when the polymer and lipids used in their production are chosen carefully. Modifications to drug-loaded LPHNPs are needed to enhance their physicochemical characteristics and site-specific drug distribution. This novel formulation takes a new approach to cancer treatment, overcoming the limitations of single- or multi-drug administration. LPHNPs have been shown to have appealing characteristics such as high stability, extended circulation duration, high biocompatibility, and the capacity to evade the immune system, according to several studies. These are used to transport anticancer drugs (doxorubicin indocyanine green paclitaxel), genes, and antimicrobials (Vancomycin), among other things. This new formulation takes a novel approach to anticancer treatment, allowing it to overcome the limitations of single or combinational anticancer delivery.

Future direction

The LPHNPS core cell type has a distinct shape and size. It has retained a prominent position in nanocarriers and is an excellent drug delivery platform. These nanoparticles contain a variety of properties that may be used to treat and target illnesses such as cancer, cardiovascular disease, diabetes, and bacterial infections, among others. These particles are often utilized to target a variety of malignancies.[79] LPHNPs provide a variety of benefits in drug delivery platforms, including high loading capacity for hydrophobic or poorly

water-soluble medicines, modified surface and surface charge regulated and sustained drug release, and in-vitro solid or highly effective in-vivo drug properties. Some nanocarrier technologies for medication delivery have been developed and authorized for clinical usage in past years. Simultaneously, a significant number of DDS are being tested in clinical studies for medicinal and diagnostic purposes. Nanocarriers, micro, and multiple emulsions are very helpful, but they have a number of drawbacks, including active ingredient degradation and formulation instability. Polymeric nanoparticles, solid lipid nanoparticles (lipids), gold nanoparticles, silica nanoparticles, silver nanoparticles, liposomes, polymeric micelles, and dendrimers are among the nanocarriers utilized (polymers). It helps to achieve the goal of regulated and site-specific medication release in a proactive and effective manner. The majority of current LPHNP research has focused on its in vitro effectiveness. They have a higher drug encapsulation efficiency, higher stability, more precise tissue or receptor targeting, biocompatibility, longer circulation duration, and a substantial biological reaction. A single-step technique is superior than a single-step or second-step method owing to its simplicity. The particle size, shape, homogeneity, entrapment efficiency, drug loading, drug release kinetics, and stability of LPHNPs may all be affected by the technique of production. The exterior layer of the nanoparticles was coated with lipid PEG and DSPE-PEG to enhance colloidal stability. Nanoparticles are a technology that delivers precise quantities of medicines to the cancer location while causing no harm to healthy cells. By drilling a stereotaxis and administering a precise therapeutic medication quantity without any side effects, the LPHNPs are directly delivered to the brain tumor. The correct amount of therapeutic drug delivery in the cancer cell (Glioblastoma) has been documented, particularly when two medicines are encapsulated into the polymer core and lipid layer, which may provide life-threatening illness treatment. It may be utilized to treat breast cancer, leukemia, and, in particular, glioblastoma brain tumors as a medication carrier (GBM). LPHNPS provides low molecular weight medicines, diagnostic agents, protein, peptide, and gene macromolecules. We think that lipid-based and non-lipid-based nanoparticles will be effective in identifying and treating glioblastoma brain cancers, as well as minimizing the negative effects of presently existing nanoparticles. We expect that the LPHNPS are more powerful, better for the illness, and better at reducing medication side effects and accurately administering single or multiple drug combinations. LPHNPS is primarily involved in the BBB delivery of breast cancer, leukemia, and glioblastoma brain tumors. LPHNPS is evolving in such a way that it will alter in the near future. We anticipate different advances and changes in this area in the next year, with researchers obtaining excellent results in diverse drug encapsulations. We expect many clinical investigations on these polymeric hybrid nanocarrier systems

as therapeutic drug delivery methods in the future years. We also show how LPHNPS may be used in medication delivery applications in the future. The researchers used lyophilization (freeze-drying) to convert the liquid LPHNPS formulation to a dry powder form, which is essential for long-term preservation. The two-stage technique for preparing LPHNPs is one of the most significant ways to minimize disadvantages and is especially useful for medication combination treatments.

Conclusion

Lipid Polymeric Hybrid Nanoparticles (LPHNPs) have demonstrated a remarkable range of successes in translating new clinical and drug delivery applications from the bench to the bedside, making a significant and long-lasting impact in the field of cancer treatment particularly in the treatment of Glioblastoma. The large-scale production of these LPHNPs has grown a lot of attention, and it's a big part of how translational research into these hybrid drug carriers is governed. On the other hand, the translation of LPHNPs into therapeutic applications is still in its early stages. Stability, toxicity, safety, and pharmacokinetics characteristics are the main elements that should be focused on. Before fabricating LPHNPs, the right solvent, material, and process must be chosen. The solvent used during synthesis, in particular, may improve the stability of the LPHNPs. During scaling-up, they may pose a threat to the environment. Furthermore, as LPHNPs scale up, their stability, binding ability, and circulation properties may be compromised. To address these issues, researchers should focus on developing solvent-free, easy-to-scale-up LPHNPs preparation techniques. The development of these hybrid NPs for cancer therapy may simplify clinical investigations in the future because they include clinically recognised components with better stability and biocompatibility than conventional carriers. PLHNPs exhibit highly desired targeted delivery, tumor accumulation, and deep penetration due to their small size and surface functionalization with various targeting ligands. They also include a variety of medicines, genes, vaccines, and imaging agents that can help with cancer treatment. They may help reduce degradation and protect medication molecules due to the outer PEGylated lipid layer. The polymeric nanocarrier system has developed a significant cancer-targeting alteration or therapeutic impact. Passive targeting of cationic NPs may be achieved while treating Glioblastoma with LPHNPs by electrostatically binding tumor endothelial cells to a negatively charged phospholipid. Cationic nanoparticles are also required for the delivery of small-molecule RNA (siRNA) to gene targets in Glioblastoma cells. Binding ligands include enzymes, nucleic acids (aptamer), peptides, nutrients, and carbohydrates. In fact, when compared to liposomes and polymeric nanoparticles, LPHNPs have already shown superiority in

several studies. Because of their unique properties, these LPHNPs have a wide range of biological applications, including anticancer therapy, immunotherapy, inflammatory treatment, and bioimaging. As a result, LPHNPs are innovative and appealing nanoplatforms for cancer therapy, and future clinical trials may focus on them.

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Sankha Bhattacharya, the second and corresponding author, contributed to the study's conception and design, material preparation, data collection, and analysis. Yudhishtir Singh Baghel, the first author, wrote the first draft of the manuscript. The final manuscript was read and approved by Sankha Bhattacharya. The authors declare no conflict of interest. The authors are responsible for the content and writing of the paper.

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