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LIPID-BASED NANOPARTICLES

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Abstract

Lipid-based nanoparticles (LNPs) are nano-sized particles composed of lipids, which are natural or synthetic molecules. It comprises of phospholipid bilayer in its structure. The unique properties of lipids such as biocompatibility and versatility, have spurred the development of various lipid based nano formulations. It has different types of lipid-based nanoparticles including liposomes, lipid nanoemulsions, solid lipid nanoparticles, nanostructured lipid carriers, and lipid-polymer hybrid nanoparticles. This review focused on the preparation methods and applications of LNPs. The various production techniques, such as injection, sonication, microfluidization, homogenization, microemulsion, nanoprecipitation, and evaporation methods were discussed. Among the various nanomaterials, lipid-based nanoparticles (LNPs) have shown remarkable pharmacological performance and therapeutic outcomes. Additionally, these carriers can enhance the drug distribution, bioavailability, encapsulation efficiency, drug loading capacity, pharmacokinetic properties and thus, results in minimizing the adverse side effects. The LNPs as promising carriers for targeted drug delivery in gene therapy and cancer therapy.

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1. Introduction

Nanoparticles are defined as particles with at least one spatial dimension, not exceeding 100 nm. Reducing the particle size of materials to the nanometer scale makes possible to increase their total surface area. Such objects are called nanoparticles, and the methods for their production are the subject of nanotechnology. The origin of term ''Nano'' is derived from the Greek word ''Nanos'', which means ''dwarf'' [1]. According to their origin, they can be divided in-to two types. These are produced by the erosion of geological materials and the degradation of biological materials, mainly vegetable residues, and can be produced by combustion processes. The second type is engineered nanoparticles (designed), include fullerenes, carbon nanotubes, quantum dots and nanofibers.

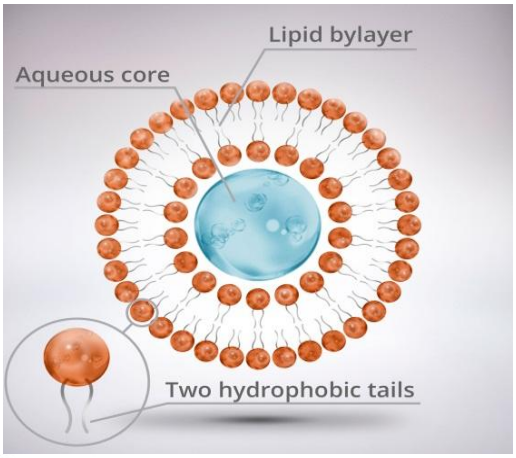
Lipid-based nanoparticles (LNPs) are highly adaptable class of nanocarriers that have gained widespread usage in medical research and pharmacology.It has several benefits, such as safeguarding drugs from *invivo* degradation, boosting their solubility and efficacy, targeted drug delivery to the disease site, regulating drug release and altering drug bio distribution.

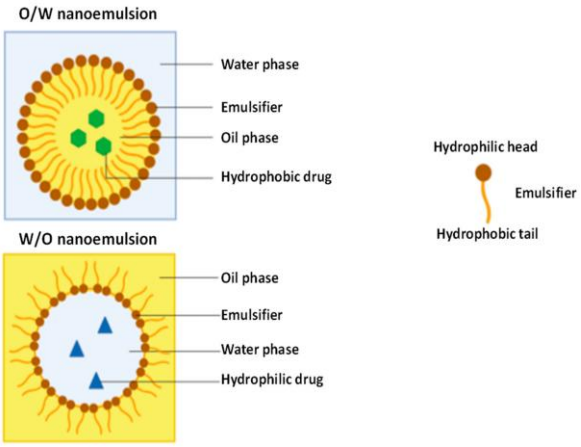
Over the past two decades, several nanoparticle-based therapeutic agents have been developed for the treatment of diabetes, asthma, allergies, infections, pain and others [2]. Lipid nanoparticles have garnered significant research interest in recent years due to their potential in drug delivery systems, as their lipophilic nature enables them to effectively address challenging the obstacles, physiological barriers, such as blood-brainbarrier. Other important properties are high skin compatibility, higher penetration through the stratum corneum and show low toxicity.

The current market value of liposomal therapeutics used for cancer treatment (e.g., Doxil, Myocet, DepoCyt, Marqibo). In addition to cancer treatment LNP- based therapies have gained FDA approval for treating other diseases, including COVID-19 vaccine (Spikevax,Comirnaty) and Amyloidosis (Onpattro), by delivering mRNA and siRNA respectively [3].

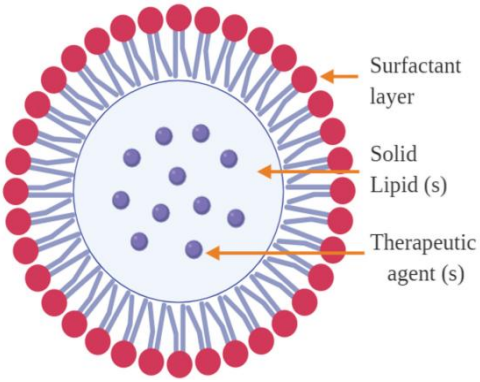
2 Types of Lipid Based Nanoparticles

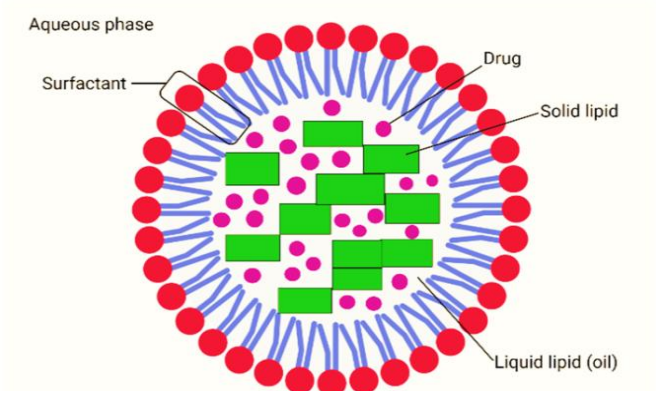
LNPs can be categorized into five sub-groups like, liposomes, lipid nanoemulsions, solid lipid nanoparticles, nanostructured lipid carriers,and lipid –polymer hybrid nanoparticles.



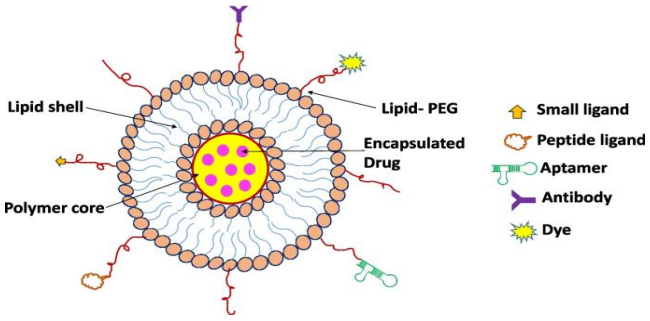


(A)Liposomes **(B)Lipidnano emulsions**





(C)Solid lipid nanoparticles (D)Nanostructured lipid carriers



(E) Lipid-polymer hybrid nanoparticle

Fig.1: Schematic illustrations depicts the structure of various lipid-based nanoparticles

2.1 Liposomes

Liposomes are first discovered in 1965 by Bangham and his colleagues, do self-assembling nanosized lipid vesicles comprise one or more concentric phospholipid bilayers that enclose discrete aqueous spaces. Liposomes are spherical structures composed of phospholipid bilayers, containing hydrophilic drugs in their inner aqueous core and incorporating hydrophobic drugs within the lipid layers. Itssize ranges from 0.01-1µm.The vesicle's size is a critical factor in determining the half-life of liposomes within the bloodstream.Both the number of bilayers in it and size of liposomes have a great influence on drug encapsulation [4].

Liposomes occur naturally in living organisms, for example, in blood, but can also be produced for the pharmaceutical and cosmetic industries.

Liposomes can be synthesized into unilamellar or multilamellar vesicles, with sizes varying from 20-1000nm, depending on the formulations and synthesis procedures [5]. Particle size is an important parameter for pharmaceutical applications of liposomes. Small unilamellar liposomes (≤100nm) exhibit higher encapsulation efficiency (EE %) of the drug,improved drug half-life, and the ability to evade the immune system upon administration[6].

Preparation Methods Of Liposomes

A)Ethanol injection

In this method, a lipid ethanol solution is rapidly injected into an excess of 0.16M KCl, upon continuous stirring dissolution of ethanol occurs and thus resulting in the formation of multilamellar vesicles (MLV)-type liposomes. A significant disadvantage of this method is the heterogeneous size of the particles obtained (30-110nm) [7].

B) Sonication method

Sonication stands out as the most recognized and widely used technique for synthesizing liposomes, particularly small unilamellar vesicles (SUVs). This method is based on the size transformation and involves the subsequent sonication of MLVs prepared by thin-film hydration method, using sonic energy usually under an inert atmosphere including nitrogen or argon. The sonication method enables homogenous dispersion of small vesicles using probe type or bath type sonicator with a potential for greater tissue penetration.

The probe tip sonicator (generally used for small volumes) needed high energy to the lipid suspension. Accumulation of energy at the tip of the sonicator probe can cause a local rise in temperature, this overheating of lipid suspension causes degradation. Therefore, the reaction vessel must be immersed

in water or ice bath to control the temperature. During sonication (up to 1h), more than 5% of lipids can de-esterify. In addition, the use of a titanium-coated probe causes contamination of the solution, which must be removed by centrifugation.

The bath sonicator is most widely used instrumentation for preparation of small unilamellar vesicles (SUV) [8].They find application in situations requiring a high volume of thinly dispersed lipids. Liposome dispersion is placed in a bath type sonicator. Controlling the temperature of the dispersion is much easier than the probe type. The sonicated liposomes can be secured in a sterile vessel other than probe or in an inert atmosphere [9].

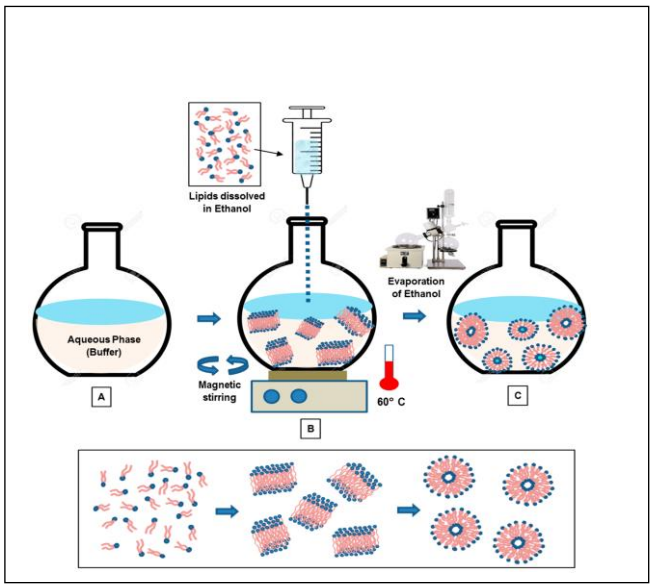


Fig.2: Diagrammatic representation of (a) ethanol injection method and (b)sonication method forpreparation of liposomes.

Applications of Liposomes

1) Liposomes are mainly used for drug delivery. Liposomal drug carriers can provide great benefits comparative to traditional drug substances. They protect the encapsulated drug from degradation and premature metabolization during release in to the human body [10].

2) In foods, liposomes have greater usage due to their ability to increase bioactive dissolution rate and bioavailability. Liposomes can be used to encapsulate an aqueous phase in order to decrease the vapour pressure of a matrix, allowing for the lowering of water activity without decreasing the moisture content, and this preventing the growth of microorganisms in foods that contain nutrients such as proteins or sugars [11].

3) Nanotechnology-based approaches in cosmetics are growing exponentially with the aim of developing novel formulations that can confer aesthetic and therapeutic benefits to the people. In particular, such cosmetic formulations referred as “cosmeceuticals”, they have both cosmetic and medicinal properties [12]. Liposome based nanoformulations are gaining interest to prepare antiperspirants, creams, lipsticks, deodorants, moisturizers, hair care produces etc., and can also be successfully used to deliver vitamins, anti-oxidants and other bioactive molecules.

4) Liposomes are used to deliver drugs approved by the Food and Drugs Administration (FDA), like the anthracyclines Doxil (doxorubinicin) and DaunoXome (daunorubincin), for the treatment of ovarian cancer, breast cancer, multiple myeloma, and sarcomas [13].

2.2 Lipid Nanoemulsions

Lipid nano emulsions are another type of LNP, that consist of biphasic dispersion of two immiscible liquids, in which one liquid is dispersed within the other in the form of Nano droplets. The spherical biphasic liquid droplets ranging the size from 50-500nm [14].They are composed of an internally dispersed oil phase covered by an external continuous phase. Nanoemulsions can be formulated either as oil-in-water (o/w) or water-in-oil (w/o) droplets, serving to transport hydrophobic or hydrophilic active compounds, respectively.To stabilize these small droplets, different types of emulsifiers, such as surfactants, phospholipids, proteins, polysaccharides or polymers such as polyvinyl alcohols, can be added during the synthesis procedure. These surfactants whether ionic or nonionic, prevent droplet aggregation thought electrostatic repulsion or steric hindrance, hydration and thermal fluctuation interaction[15].

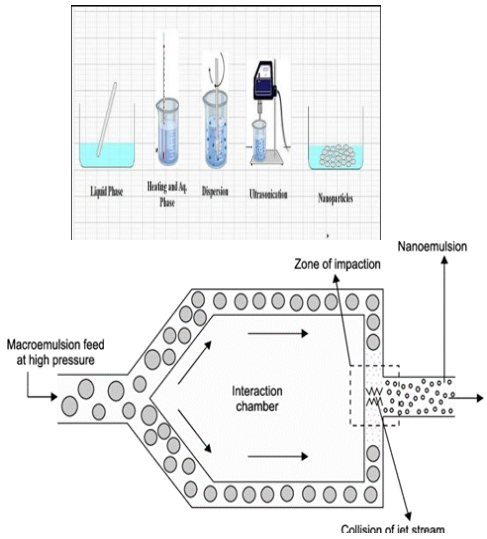
Preparation Methods of Lipid Nanoemulsions

A) High shear homogenization or high-speed stirring

Shear stress primarily facilitates the reduction of particle size in this method. This high shear mixers use a rotor or stator system to produce the shear stress [16]. In this setup, fluid flows between a stationary platform and an internally rotating one. The rotation of the inner device functions as an impeller, generating turbulent flow that intensifies shear forces.

B) Microfluidization

A microfluidizer is a patented mixing device that uses a high-pressure positive displacement pump (5 to 135MPa) which repeatedly forces a coarse emulsion through an interaction chamber consisting through small channels known as microchannels, the particle size is reduced until reaching the desired dimensions. Turbulent flow along with cavitation causes droplet disruption and nanoemulsion formation. Following this step, the bulk emulsion undergoes filtration to eliminate larger droplets, resulting in the formation of a homogeneous nanoemulsion. This technique is suitable for its use at industrial scale [17].



(a) (b)

Fig.3: Diagrammatic representation of (a) high shear homogenization and (b) microfluidization to obtain lipid nanoemulsions.

Applications of Lipid Nanoemulsions

Lipid nano emulsion can be directly employed for active delivery and targeting as drug delivery systems or can act as template for preparation of polymeric nanoparticles and lipid nano capsules. Nano emulsion have a colloidal structure that enables solubilization and encapsulation of hydrophobic drugs, reducing adverse effects. They are mainly used for topical drug delivery, but have potential for other modes of administration like intravenous, ocular, intranasal, and oral delivery [18]. Nanoemulsions find applications in the food industry as flavoring, coloring, nutraceuticals, or preservative agents.

Resveratrol, a natural polyphenol found in grape skins, blueberries, raspberrieshave many functional properties like antioxidant, anticancer, and antiobesity. Nanoemulsion based delivery systems have been used to encapsulate resveratrol.

2.3 Solid Lipid Nanoparticles (SLNs)

Solid lipid nanoparticles (SLNs) are colloidal carriers that were first synthesized in 1991 as an alternative to liposome, emulsion, and polymeric micro and nanoparticle-based systems [19]. Muller and co-workers were the first to name those colloidal systems as SLNs. They described a production method using high pressure homogenization of melted lipid in water at high temperature.

SLNs consists of solid lipid matrices entrapping hydrophobic drugs, forming nanoscale particles with lipid a core. They are stable at room temperature and at human body temperature [20]. SLNs usually are in spherical shape and a diameter in the range of 50-1000 nm. It consistsof a core of solid lipids in which a bioactive component is embedded, and this structure is stabilized by a surfactant coating.

The incorporation of cationic lipids into SLN shell formulations can enhance blood-brain-barrier penetration, and gene transfection efficiency.

2.4 Nanostructured Lipid Carriers (NLCs)

NLCs are the second generation of SLNs, but it contains a combination of solid and liquid lipids, resulting in a more stable matrix and improved drug loading capacity, which was first shown for retinol. Muller et al. [21] have shown that it is possible to overcome this limitation by adding liquid lipids that alter the crystal structure of lipid matrix.

Nanostructured lipid carriers are characterized by a partially crystallized lipid matrix with a poorly ordered structure, which significantly reduces the risk of drug leakage (uncontrolled release) during storage.

Preparation Methods for SLNs & NLCs

A) Membrane contractor method

In this method, pressure is applied to promote the passing of the melted lipids through a membrane (**Fig 4**). The lipids form droplets, whose size depends on the pore size of the membrane. The aqueous phase, which contains the surfactants, flows tangentiallyto the membrane and removes the formed lipiddroplets. The emulsion is then cooled down to allow lipids to solidify and form SLNs or NLCs [22].

B) Microemulsion method

This method was firstly developed and patented in the 90's by Gascoet al. to produce solid lipid microparticles (**Fig 4**). Briefly, the lipids and the cargo are heated above the melting point of the lipid solution is mixed with an aqueous phase, which is at a temperature equal to lipids melting point and contains the surfactant and co-surfactant. This mixture is kept at a high temperature to obtain the microemulsion.Finally, the hot ME solution is added, under stirring, over a cold-water solution (2-10 °C), resulting in lipid solidification and the formation of the microspheres. Another variant of this technique consists of cooling of the hot ME without water addition. The cooling process of the solution under stirring drives the solidification of the lipids and the reduction of the particle size, yielding SLNs and NLCs with sizes below 300nm [23].

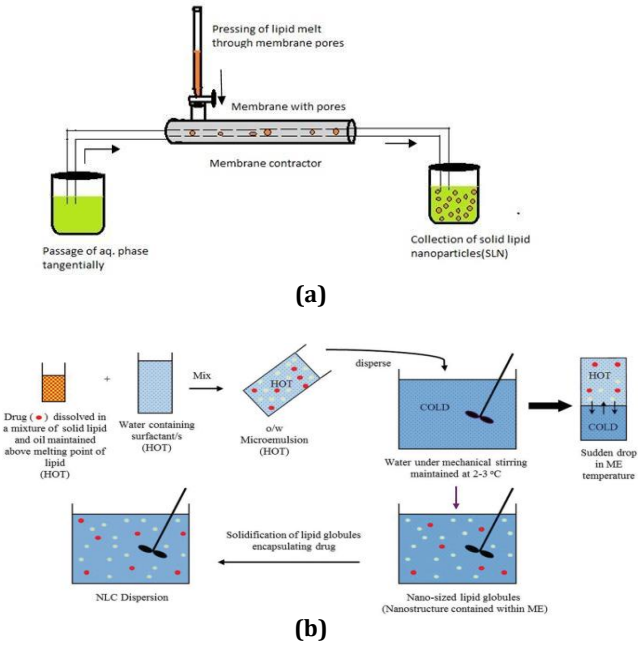


Fig .4: (a) Membrane contractor method and (b) Microemulsion method for preparation of SLNs and NLCs.

Applications of SLNs & NLCs

LNP subgroup	Active substance	Disease/ applications	Products	Reference
Solid lipid nanoparticles	doxorubicin	hepatocarcinoma	Doxorubicin Transdrug (DT)(phase III clinical trial)	24
Solid lipid nanoparticles	oxiconazole	Tinea fungal infection	Oxiconazole nitrate solid lipid nanoparticles loaded gel (phase I clinical trial)	25
Solid lipid nanoparticles	Halobetasol propionate	Inflammation	Pluronic gel Halobetasol propionate-loaded lipid nanoparticles	26
Solid lipid nanoparticles	siRNA targeting transthyretin gene	Amyloidosis	Onpattro (patisiran)	27
Nanostructured lipid carriers	Acitretin	Psoriasis	Acitretin precirol ATO 5/ oleic acid / tween 80(Randomized controlled trial)	28
Nanostructured lipid carriers	All-trans retinoic acids	Keratinization disorders	Oleic acid /cetyl palmitate/ cineole/limonene/transcutol/butylated hydroxytoluene/tween 20/tween 80	29
Nanostructured lipid carriers	Self –amplifying RNA	COVID-19	THEMBA II T-CELL Vaccine (phase I/II clinical trial)	30
Nanostructured lipid carriers	mRNA-1273	COVID-19	Spikevax	31

2.5 Lipid Polimer Hybrid Nanoparticles (LPHNPs)

Lipid polymer hybrid nanoparticles combines both lipid-based and polymer-based components, offering the benefits of both systems. It has polymer cores that contain therapeutic substances lipid/lipid-PEG (polyethylene glycol)shells as a "stealth" coating for improved *in vivo*circulation [32]. This unique structural composition offers optimal biocompatibility and physical stability, making them an ideal vehicle for drug delivery. These nanoparticles can effectively encapsulate various types of drugs(pharmaceuticals), including nucleic acidsand exhibit enhanced stability and controlled release properties.

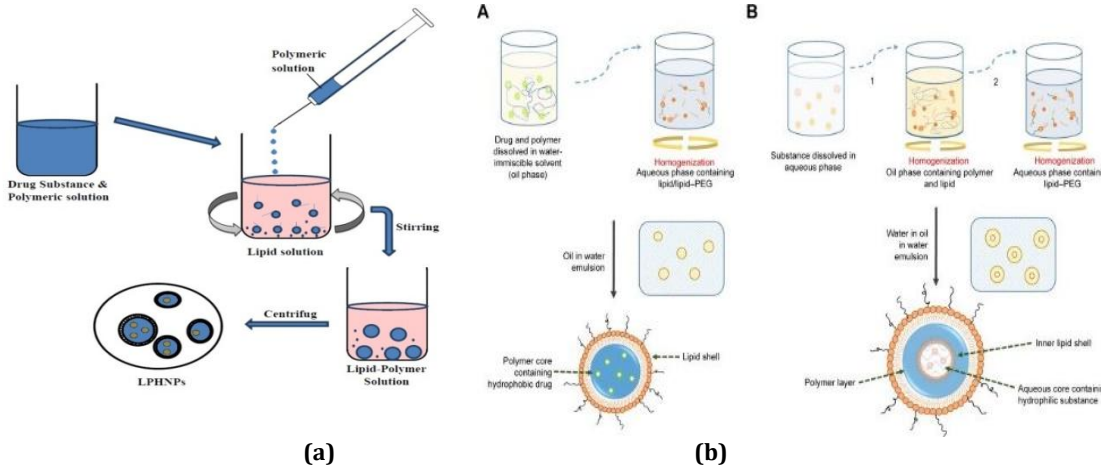
Preparation Methods of LPHNPs

A) Nanoprecipitation

Nanoprecipitation method requires that the drug and polymer are together dissolved in water miscible organic solvent and the lipid/lipid-PEG dissolved in water. It is mandatory to heat the lipid/lipid-PEG solution to achieve a homogeneously dispersed liquid crystalline phase. Then addition of polymer drop wise to the aqueous dispersion of lipids under continuous stirring. This enhances the polymer to coil into NPs with concurrent self-assemblage of hydrophobic interactions. Hydrophobic tails of lipids are directed towards inner NP and the hydrophilic head face out toward the external aqueous solution. The hydrophobic tails of lipid-PEG merge into the inner lipid shell while PEG chains pop out to aqueous environment, sterically stabilizing the hybrid [33]. The organic media is evaporated and the LPHNPs, thus formed, and are centrifuged.

B) Emulsification-solvent evaporation

This method is subcalssified into single and double emulsification methods. A single Emulsification solvent evaporation method is used for drugs soluble in hydrophobic solvents. In this method an oil-in-water (o/w) emulsion is formed, where in the water immiscible oil phase containing the polymer and the drug is mixed with an aqueous phase containing dissolved lipid under ultrasonication or under constant stirring. The polymer core is formed by evaporation of organic media and lipids assemble around the polymer core concomitantly. As are placement, the lipid can concurrently be dissolved in oil phase with the polymer. A double Emulsification-solvent evaporation method (w/o/w) is applied for water soluble drugs. The aqueous solution of drug is emulsified in organic solvent containing polymer and lipid to form 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 [34].



(a) **(b)**

Fig.5: (a) Nanoprecipitation and (b) Emulsification-solvent evaporation method for preparation of LPHNPs.

Applications of LPHNPs

LPHNPs have wide range of applications in cancer therapy and delivery of protein based therapeutic agents that is small interfering RNA, nucleic acid and genes delivery and can be used for oral drug delivery of many drugs. Doxorubicin loaded polymer-lipid hybrid nanoparticles (Dox-PLN) were designed administered intratumorally in mice. At a dose of 0.1mg and 0.2 mg, 70 % and 100% tumor growth delay was observed, respectively. These results indicated that Dox-PLN expressed as cytotoxic activity against solid tumors and improved therapeutic efficacy [35].

LPHNPs not only used for delivering of therapeutic agents but also used for diagnostic purpose. Different type of imaging agents such as fluorescent dyes, quantum dots and iron oxide or incorporated in polymer core. Lipid-polymer and lipid-quantum dot nanoparticles were prepared in single step with narrow size distribution [36].Physico-chemical properties can be controlled by varying the experimental condition. Such multicomponent nanoparticles can be used in therapy and in diagnosis simultaneously.

LPHNPs used for nasal delivery of an antiviral drug, tenofovir. The enhanced fluidityimproves the drug permeation through membrane phospholipids, which increase the bioavailability of drug [37].

3 Characterization of Lipid- Based Nanoparticles

1) Cryo-Transmission Electron Microscopy

Electron microscopy allows the direct visualization of nanomaterials;therefore, it is an important means of investigating nanosized pharmaceutical forms. It is the useful method for analyzing liquid dispersions in a very close way to their native state, revealing morphology and inner details. Milky nano particle sized liquid dispersions can be accurately identified by cryo-TEM. The images taken from cryo-TEM observation are useful to measure the particle dimensions by a scale bar.

2) X-rays Diffraction Studies

X-ray diffraction studies were frequently conducted on lipid dispersions in the past decades. It is well known that the morphology of nanosized drug carriers directly influences the release and encapsulation efficiency of the active molecule. Therefore, an investigation of internal structural organization of nanosystem becomes very important.

X-ray diffraction analysis is the most explored tool to achieve the task. Generally, in lipid-based formulations, lipids can aggregate into too many structures via interplay parameters including water concentration, temperature, or the presence of different components. The phase stability is described by these lipid arrangements. Lipid phases are most often detected in drug delivery nanosystems such as lamellar, hexagonal, and cubic phases [38].

3) SdFFF

The Sedimentation Field Flow Fractionation (SdFFF) technique is meant for the separation and characterization of nanoparticles based on gravitational or centrifugal force as an external force the factors most influencing the FFF analysis can be summarized as sample preparation, properties of liquid carrier, channel size and influence of applied external force.SdFFF is not only limited to analyze size distribution but also useful in varies tasks such as fractionation of nucleic acids (<70nm), proteins (40-300nm), polysaccharides assemblies (0.1-1μm), cells (10-20μm), and virus like particles (10-80nm) [39].

4) Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR is advantageous analytical technique for structural architecture and quantitative analysis of nanosized materials. This phenomenon is shown by nuclei having non-zero spin under influence of an external strong magnetic field, which create variation in the energy between spin up and down states. The transition between these two phases can be sensed by electromagnetic radiations. In the physical characterization of nanosystems, NMR can be applied to investigate the coordination or interactions between the ligand and the surface of diamagnetic or antiferromagnetic nanoparticles [40]. The high-resolution technique becomes a useful tool for gathering information regarding dissolved ormobile constituents, especially in solid-state.It can be employed for structural characterization of nanoparticles of lipids. Particularly, interaction of entrapped drug molecules with lipid core can be examined with the help of this tool. It can be employed to characterize functionalized lipid nanoparticles such as stabilization of nanoparticles via PEGylation. The blend of lipids namely tripalmitin, lecithin, and polyethylene glycol (PEG)-stearate has been used to produce lipid nanoparticles by anemulsification-solvent evaporation technique.

5) Zeta potential and surface charge

The zeta potential is the measurement technique, which is based on electrophoresis. The particles in their respective suspending media are exposed to electrical field. The charged moieties will exhibit drift under the influence of electrical field (positive ones travel towards the negative electrode and vice-versa). The thin layers of ion and solvent around the particle will drift along with particles. The zeta values will not fluctuate during analysis and more reliable results are obtained.

4 Discussion and Conclusion

Lipid-based nanoparticles (NPs) typically highlight their versatility, effectiveness, and wide- ranging applications in various fields such as pharmaceuticals, cosmetics, and in food industries. It often emphasizes the diverse types of LNPs, including liposomes, lipid nanoemulsion, solid lipid nanoparticles, nanostructured lipid carriers, and lipid-polymer hybrid nanoparticles, each offering unique advantages for drug delivery, and gene therapy. The discussion is about the preparation methods, which is important of optimizing formulation parameters to achieve desired NP characteristics such as size, stability, drug encapsulation efficiency and controlled release kinetics. The techniques like sonication, solvent evaporation, solvent injection, emulsification, nanoprecipitation and microfluidization are commonly discussed. The applications of LNPs, including targeted drug delivery to specific tissues/ells, improved bioavailability of poor soluble drugs, sustained release formulations, and minimization of side effects. Additionally, their potential for therapeutic applications, combining therapy and diagnostics. The significant impact of LNPs in advancing drug delivery and therapeutic strategies. Here, also discussed about the characterization methods of LNPs like, cryo-TEM, x-ray diffraction studies, SDFFF, NMR spectroscopy, zeta-potential and surface charge.

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