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Hybrid Nano-carriers for Potential Drug Delivery

Asadullah Madni, Nayab Tahir, Mubashar Rehman,
Ahmed Raza, Muhammad Ahmad Mahmood,
Muhammad Imran Khan and
Prince Muhammad Kashif

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Abstract

Nanocarriers have provided the versatile platform for the delivery of various therapeutic and diagnostic agents. Liposome, niosomes, polymeric and solid lipid nanoparticles are the most promising nanocarriers that have been entered in the clinical trials and become commercially available. However, each system has been associated with some problems that can be minimized by using the combinatorial approach of hybrid nanocarriers. These hybrid systems combine the benefits of different structural components to synergize the outcome of the therapy. In this chapter, the different types of hybrid nanocarriers have been described with particular emphasis on the brief rationale for the development of these hybrid nanocarriers along with different fabrication approaches with greater emphasize on the lipid polymer hybrid nanoparticles. A brief description factors governing the optimized response characteristics and their potential application of these hybrid nanoparticles are also presented.

Keywords: core shell hybrid nanoparticles, drug delivery, hybrid nanoparticles, nanoflowers

1. Introduction

In the recent decades, pharmaceutical nanotechnology has opened a new era for the research in the design and characterization of drug delivery systems (DDS) and biotechnological products. A variety of novel drug delivery systems and strategies emerged for diagnostic and therapeutic applications that explored the different structural components, fabrication methods and mechanisms of drug delivery and targeting [1]. These DDS emphasized on

the use of multiple nanomaterials and therapeutic moieties that renovate the current pharmaceutical industry and biomedical sciences toward the better drug therapy [2]. These nanosized particles were utilized for the delivery of various molecules including different drugs, proteins, nucleic acid and other diagnostic agents. Some of these compounds may be encapsulated inside while others were adsorbed on the surface of these nanoparticles. These nanocarriers can amend the pharmacokinetics and pharmacodynamics of drug by enhancing the solubility, permeability and bioavailability in multiple ways. The availability of the encapsulated compound depends upon the nature of formulation components and the other external stimuli which enable the controlled as well as targeted delivery of these encapsulated compounds within the cellular microenvironment [3]. All these parameters ultimately achieve the higher concentration of the encapsulated drug that efficiently reaches the potential target site without affecting the normal tissues. These nanocarriers also aid to implement the concept of rational therapeutics by providing the tunable drug delivery systems based on the patient therapeutic demands.

Despite of excellent in-vitro performance, some drugs demonstrate poor in-vivo results because of low aqueous solubility, poor membrane penetrability, rapid clearance by the reticuloendothelial system, complex pathophysiological states of the disease and uncertain plasma levels leading to drug toxicity, thus, requiring such drug delivery systems that overcome these problems [4]. Latest developments in the material sciences, polymer engineering and nanotechnology have enabled multidisciplinary research to formulate and evaluate different novel drug delivery systems that claimed increased drug solubility, penetration and retention at the targeted site in the body [5].

Among the different nanoparticulate systems, nanoparticles of different composition and lipid based vesicular carriers (liposome, lipid nanocarriers, solid lipid nanoparticles and drug lipid conjugates) have been frequently employed for the medical applications. The nanoparticles may provide versatility in terms of composition. As, these include the polymeric nanocarriers, mesoporous nanoparticles, metal coated (gold, iron and silver), inorganic nanoparticles, quantum dots, carbon nanotubes, dendrimers and magnetic nanoparticles [6, 7]. Furthermore, all these systems were modified to mimic the desired therapeutic properties through different modification method and ligands such as (i) increase in the retention time and stability of the system, (ii) stimuli triggered release, (iii) targeted delivery of various agents and (iv) administration of dual modalities simultaneously [8, 9].

Liposomes and niosomes have been considered as most promising domains among the lipid vesicular carriers. Liposomes are defined as the lipid vesicles having the single or multiple layers of the lipid providing the encapsulation of different therapeutic moieties while niosomes have the same morphology but contain nonionic surfactants instead of phospholipids as major structural components. They provide the better biocompatibility profile, easy surface modification of the vesicles, versatility in the loading of hydrophobic and hydrophilic drugs and improved pharmacokinetic properties [10, 11]. However, drug leakage or fast release from the system, reproducibility, poor physical and chemical stability on storage, higher cost and scale up issues are the major drawbacks associated with the vesicular systems [12, 13].

Nanoparticles (polymeric, organic/inorganic, mesoporous silica, calcium carbonate and different metals, i.e., iron, silver and gold) established the second domain of the nanocarriers. These systems prove superiority in terms of smaller particle size, structural integrity, versatility in the polymeric materials, improved drug loading and release profile. They also provide the targeting capabilities in the case of magnetic iron oxide nanoparticles and better cellular interactions in case of organic and inorganic nanoparticles [14]. Similar to that of vesicular systems, these polymeric nanoparticles have some limitations in term of polymer toxicity, presence of toxic organic solvents, poor entrapment of hydrophilic drugs, polymer degradation and drug leakage before reaching the site of action [15].

The problems associated with the liposomes, polymeric nanoparticles and other carrier systems can be reduced by using a novel combinatorial approach of “hybrid nanoparticles” (HNPs) that utilizes the positive attributes of two different components. These hybrid nanoparticles (HNPs) exploit the benefits of both systems (lipid and polymer/organic and inorganic materials) and the release profile of drug is based on the erosion and degradation of the core material by hydrolysis with in turn determined by water permeation into the outer shell layer and composition of the polymer. The core materials may be protected by the application of multiple layers of the shell materials and the interface of these layer acts as a site for the functionalization of the carrier system for the dual modalities of treatment and diagnosis [16].

Similarly, core shell hybrid nanoparticles using different oils, metal oxides, organic and inorganic components also provide newer system that has multilayered structure having the inner core outer shell with a suitable lipid or oil at the interface to develop a core shell hybrid structure. Recently, use of green approach offer more facile and potentially successful system with the added advantage of solvent-free nanohybrids with greater efficiency.

Such novel system consists of three different structural components as follows:

- (i) The inner most core made up of different polymers (poly-lactic-co-glycolic acid [PLGA], polycaprolactone [PCL] and chitosan), lipids (cationic, anionic, zwitterion and neutral phospholipids and nonionic surfactants), inorganic materials (silica, iron oxide) and organic materials (polysaccharides) that encapsulate the therapeutically active moiety.
- (ii) The intermediate lipid layer that covers the polymeric/inorganic core and enhance the biocompatibility of that system. It also acts as barrier to minimize the drug leakage and control the rate of polymer/inorganic core degradation by controlling the water permeation into the core.
- (iii) The outer most lipid or polymer-conjugate which act as a layer for functionalization of the system by making it target specific through the use of different ligands or increased its circulation and retention time by coating with the PEG. This layer may be modified with a suitably charged moiety to attach the antibodies, aptamer and other such molecules by electrostatic forces [17]. Different types of the hybrid nanocarriers having different morphology and different structural components **Figure 1**.

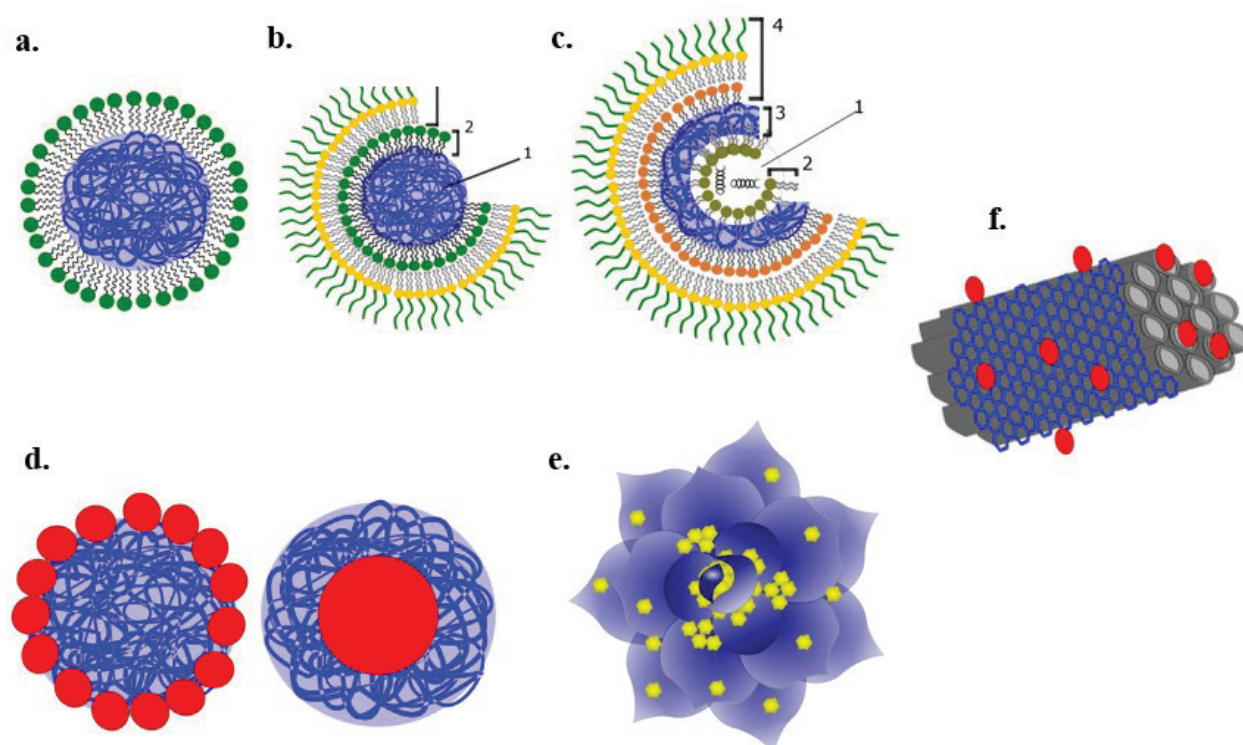


Figure 1. Structure of lipid-polymer hybrid nanoparticles; (a) polymer core-lipid shell hybrid, (b) 3 layers polymer-lipid hybrid nanoparticles consisting of polymeric core (1) and two lipid layers (2,3) shell, (c) 4 layers hollow core lipid-polymer hybrid, consisting of hollow core (1) covered by reverse surfactant layer (2), polymeric shell (3), and outer shells of two lipids (4). (d) organic core-inorganic shell and inorganic core-organic shell hybrid, (e) inorganic (metallic)-protein hybrid nanoflowers, and (f) graphene oxide coated mesoporous silica-inorganic hybrid nanoparticles.

In this chapter, the different types of hybrid nanocarriers have been described with particular emphasis on the brief rationale for the development of these hybrid nanocarriers along with different fabrication approaches with greater emphasize on the lipid polymer hybrid nanoparticles. A brief description factors governing the optimized response characteristics and their potential application of these hybrid nanoparticles are also presented.

2. Method of preparation

Different methods have been employed for the fabrication of hybrid nanocarriers depending upon their chemical composition and applications. The lipid-polymer hybrid, polymer-inorganic hybrid, metal (gold, silver or iron) polymer, silica (SiO_2) based hybrid nanosystems and hybrid polymeric nanocarriers have been most widely investigated [18]. Most of these hybrid carriers utilized two distinctive fabrication approaches. First, a two-step conventional approach process, in which the inner core and outer shell are prepared separately and then are coincubated for the formation of hybrid nanoparticle. The second approach is the single step, in which various state-of-the art techniques of the self-assembling are being incorporated. These processes are further modified with different chemical moieties to obtain versatile hybrid nanoparticles meeting specific need of therapy [19]. In the present chapter, we will focus on the two step conventional as well as single step formulation approaches along

with recent innovations have been presented in order to prepare the hybrid nanocarriers with versatile characteristics.

2.1. Conventional two-step method

It was the first technique employed for the fabrication of hybrid nanocarriers. The inner core and outer shell components are prepared in two separate steps employing suitable polymers and chemicals and are then combined to form the hybrid nanoparticle [17]. The foremost type of core shell hybrid nanoparticles contained a core of the polymeric nanoparticles and an outer shell of preformed lipid component such as liposome or lipoparticles in appropriate ratios [20]. Further, the single or multilayered shell is prepared with other techniques such as sonication [21], extrusion or high pressure homogenization and vortexing [22]. The polymeric core is prepared by emulsification-solvent evaporation or solvent diffusion [23], desolvation [24], nanoprecipitation [25, 26], sonication [27] and high pressure homogenization [28] depending upon the hydrophobicity of the loading drugs, their applications [29] and the size of the core.

The single step method is applied when the core materials such as polymers, silica and organic substances are miscible with the drug payload and also are solubilized in the organic solvent [30, 31]. The double emulsification step is employed when the compound is immiscible with the organic solvents and does not form covalent linkage with the core material. As this method requires multiple steps for mixing of different components, relatively larger hybrid nanoparticles are produced [32]. Further, any of the suitable technique such as ultrasonication or extrusion by high pressure homogenization also reduces the particle size as the polymer solution is passed through the nozzle under high pressure. Furthermore, the freeze drying or cooling at normal temperature produced free flowing characteristic particles [33, 34]. Another recent innovation is the application of nanoprecipitation method for the preparation of polymeric core. The polymer is dissolved in the suitable solvent and then precipitated by using the nonsolvent component [26].

The formed polymeric core and lipid vesicles are mixed by vortexing, extrusion, film hydration and ultrasonication techniques in order to formulate the hybrid nanoparticles. The mixing processes provide the energy for the fusion or adsorption of the shell on the inner core material. Additionally, the electrostatic forces among these components also play their role for fabrication of hybrid nanoparticles [35]. It is worth mentioning here that mixing process must be carried out above the phase transition temperature of the lipid component. The formed hybrid nanoparticles are separated by the ultracentrifugation process [36, 37]. Different investigators such as Liang et al. [38] and Zhao et al. [39] prepared the hybrid nanoparticles and nanocells by the emulsification solvent evaporation technique employing the paclitaxel loaded polymeric nanoparticles as core and the PEG or folic acid conjugated octadecyl-quaternary lysine-modified chitosan and cholesterol as lipid shell [38, 39].

2.2. Modified two-step methods

The modifications to the conventional two step method such as spray drying and lithographic molding processes have also been employed for fabrication of hybrid nanoparticles [29]. The inner core is prepared by the spray drying which is dispersed in an appropriate

solvent containing the lipid, polymer or any inorganic material. The spray dried lipid coated core shell hybrid nanoparticles were collected after the completion [17].

Freeze or spray dried inhalation hybrid nanoparticles of levofloxacin, ciprofloxacin and isoniazid coated with multiple layers of the lipids were prepared using double emulsion solvent evaporation technique. These hybrid nanoparticles showed better inhalation efficiency, emitted particle size and diameter compared to the conventional two step methods [37, 40]. Another investigations employed nanospray drying for fabrication of hybrid nanoparticles using polyglutamic acid, poly lysine nanoparticles coated with the lipid materials [41]. Recently, Keloglu et al. [42] employed jet spray drying technique for the fabrication of hybrid microfibers-nanoparticles having low density and greater strength using PLGA and poly lactic acid (PLA) [42].

A soft lithography particle molding technique was also utilized for the preparation of hybrid nanocarriers for the delivery of genes to various diseases. De Simon and his coworkers prepared the nanosized particles using the particle replication approach on the silicon wafers. The technique was referred to as Particle Replication in Nonwetting Templates (PRINT) [43]. The process involve the dissolution of the polymer (e.g., PLGA, PLA) in an organic solvents such as dimethyl formamide, methyl acetate and/or dimethyl sulfoxide along with the material to be encapsulated. The PRINT molding device was employed to fabricate the nanoparticles which later were harvested with the help of polyethylene terephthalate sheet [43]. It produces the particles of different shapes and a wide size range depending upon the size of the molding cavities [44].

2.3. Single-step preparation methods

The low encapsulation efficiency due to the leakage of the drugs from the inner core during second step, batch variability and large time consumption are the common problems associated with the conventional two step methods [45]. These constraints can be overcome by designing the simple method that utilized the single step approach and also provide better control on the content uniformity, reproducibility and other characteristics of the system. The method involves the mixing of two different solutions containing the polymer and lipid that self-assembled to form the particles with the core shell hybrid structure [46]. The polymer is dissolved in an appropriate organic solvent while the lipid solution is prepared in the water that may utilize the small fraction of organic solvent as solubilizing agent. The solution containing polymer is added to the lipid phase where the polymer precipitate to formed the nanoparticles and the lipid is self-assembled at the surface to form the hybrid nanoparticles. Single-step preparation is usually achieved by nanoprecipitation, emulsification-solvent evaporation and solvent diffusion methods. These methods and their appropriate modifications are discussed here.

2.3.1. Emulsification solvent evaporation method

Emulsification solvent evaporation method is the most commonly employed single step approach for the fabrication of hybrid nanocarriers. The single emulsification solvent evaporation [47] and double emulsification solvent evaporation (DESE) techniques are employed depending upon the nature and solubility of encapsulating drug. In the ESE method, the oil phase is formed by dissolving the polymer and the drug in the water immiscible organic solvent.

The aqueous solution containing the lipid portion which act as a stabilizer itself during the self-assembling process [48, 49]. The organic phase is then added dropwise into the aqueous phase under the sonication or stirring at the constant speed that results in the formation oil in water emulsion. During the emulsification process, the hydrophobic part of the lipid is adsorbed on the inner core material while the hydrophilic parts arrange themselves toward the aqueous medium forming the lipid coated hybrid nanoparticles [45, 50].

The single ESE method is employed for the encapsulation of hydrophobic drugs with low aqueous solubility [51]. Recently, the folate conjugated lipid polymer hybrid nanoparticles have been prepared by the emulsification solvent diffusion method for the targeted delivery of the doxorubicin using phosphatidylcholine (lecithin 99%) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DPSE)-PEG-COOH as lipid portion and PLGA as a polymeric portion [52]. The ESE method was also employed to formulate dual ligand hybrid nanocarriers for the targeted delivery of docetaxel. The hybrid nanoparticles possessed a uniform monolayer of the lipid over the polymeric core. The cell interaction studies revealed better endocytosis profile with sustained release of the drug by preventing the diffusion of the aqueous medium in the polymeric core. However, the particle were relative larger compared to that prepared by the nanoprecipitation method. This might be attributed to higher drug loading that maintained the therapeutic concentration for the longer period of time [53].

The double emulsification solvent evaporation (DESE) has been employed for the hydrophilic drugs and nucleic acid such as siRNA (small interfering ribonucleic acid) which are not dissolved in different organic solvents along with the other suitable polymers or the core/shell materials [54]. The aqueous solution of desired substance is prepared and is then emulsified in the organic/oil phase containing the lipid and polymer. The resultant primary emulsion is again added to another aqueous solution containing the lipid (lecithin, phosphatidylcholine or DSPE) or surface ligand (PEG, half antibodies, aptamer) and a water-in-oil-in-water (w/o/w) multiple emulsion is prepared. The evaporation of the organic phase results in the formation of hybrid nanoparticles [55]. The particles with hollow core covered with an appropriate shell provide the space for the internalization of hydrophilic and small molecules. The evaporation of the organic solvent provides the multilayered shell which has larger size as compared to the other methods [17].

Su et al. [56] prepared the reduction sensitive hybrid nanoparticles of doxorubicin using chitosan with the sodium dodecyl sulfate employing the double emulsification solvent evaporation method. The amphiphilic chitosan and lipid base micelles core provided a unique nanoconfiguration that is enveloped by the triglycerides which enhanced the loading efficiency and provided the drug release profile up to eight folds [56].

2.3.2. Nanoprecipitation

This method is also known as salting out method. It is a well known method for fabrication of hybrid nanoparticles of size less than 100 nm. This method employs two miscible solvents with different solubilizing capacity for the polymer. First, the polymer core is formed by solubilizing in solvent of greater solubility designated as good solvent which is then added to less soluble solvent designated as poor solvent. The two solutions are mixed by dropwise addition,

stirring or sonication. Good solvent being miscible with poor solvent diffuses into later, leaving behind the core nanoparticles due to the precipitation of the polymer [19].

The core forming polymer and lipophilic drug are solubilized in a water-miscible organic solvent like acetone, acetonitrile or ethanol [57]. The lipids, inorganic salts or silica are dispersed in water with moderate heating (~60–75°C) and/or addition of hydroalcoholic mixtures for proper dispersion of the lipids.

The hydrophilic drugs are added to the aqueous phase containing dispersed lipids [58]. The polymer containing organic phase is then added dropwise to lipid dispersion with continuous stirring to precipitate the polymer into nanoparticles. The monodispersed hybrid nanoparticles are collected after suitable application of vortexing, homogenization or ultrasonication [55, 59]. Concurrent to the precipitation process, the self-assembly of lipid molecules around the polymer molecules occurs due to the hydrophobic interactions. The polymer core captures the hydrophobic tails of lipid while the heads are facing toward the aqueous phase [17, 60]. Continuous stirring of dispersion for several hours is helpful in uniform lipid coating of hybrid nanoparticles and to ensure the complete removal of organic solvent [55]. Rotary evaporator may also be helpful for the removal of organic solvents [58].

The literature suggests 10% ethanolic solution is employed for solubilization of lipids and PEG may enhance the stability of hybrid nanoparticles [61]. According to the study of Ling et al. [58], dextran sulfate and lecithin/PEG-PLGA hybrid nanoparticles can entrap higher amounts of hydrophilic moiety, the vincristine.

Wang et al. [62] developed PLGA/TPGS-lecithin hybrid nanoparticles using a modified nanoprecipitation method. The PLGA was dissolved in acetone while lipids were dispersed in either aqueous or 4% ethanolic aqueous solution. An inverse-phase nanoprecipitation method (i.e. aqueous phase was added dropwise into organic phase consisting of acetone, the PLGA and the paclitaxel). Initially, the formation of hybrid nanoparticles was slow due to the higher proportion of organic phase in the mixture. Continuous stirring and addition of water boosted the diffusion which leads to solidification of the hybrid nanoparticles. A stable hybrid nanoparticle formulation with low value of PDI (~0.1) was observed at 5:1 aqueous to organic phase ratio [62].

2.3.3. Sonication

Sonication is a fast technique for the fabrication of hybrid nanoparticles which utilizes ultrasonic waves rather than vortexing, solvent evaporation or heating. In this method, the two solutions designated as organic and aqueous phases lead to formation of inner core (polymer) and outer shell or coating materials (lipids), respectively. The sonication has been employed by Fang et al. [63] for the fabrication of hybrid nanoparticles of lecithin-PEG and PLGA by using this approach. The PLGA was dissolved in acetonitrile while the lecithin and the PEG were added in 4% ethanol solution. The former solution was carefully pipetted into the hydroalcoholic solution (aqueous to organic ratio was kept as 10:1). The hybrid nanoparticles were produced as this 'cocktail' mixture was placed in sonicator bath for five minutes at a frequency of 42 kHz and a power of 100 W. The main advantage of this technique is the formation of stable hybrid nanoparticles with short processing time and production rate is 20 times than other processes [63].

The sonication technique has been employed for PLGA and docetaxel hybrid nanoparticles by Liu et al. [64]. In another study, Mandal et al. [65] developed erlotinib loaded hybrid nanoparticles of PCL in which erlotinib and PCL were dissolved in acetone and added to the aqueous phase containing lipids. Hybrid nanoparticles were produced after sonication for 10 minutes at a frequency of 67 kHz and a power of 200 W [65].

A unique method using the combination of modified nanoprecipitation and sonication methods is presented for the fabrication of hybrid nanoparticles. In this method, the lipids melt was mixed with ethanolic solution of Elacridar, a chemosensitizer, and placed in vacuum oven until complete removal of solvents. The doxorubicin being hydrophilic drug was added in water with surfactant (Pluronic-F68) and heated (72–74°C). The drug and surfactant dispersion was mixed with Elacridar-lipid mixture. The whole mixture was stirred for 10 min and then ultrasonicated for two cycles of three minutes. It produced submicron sized lipid emulsion which was dispersed in 4–9 times higher volume of cold water (maintained 4°C) which leads to the formation of hybrid nanoparticles [66, 67].

2.3.4. Green technology for the preparation of hybrid nanocarriers

The use of green technology has revolutionized the synthesis of hybrid nanocarriers due to the ecofriendly procedures that mitigate the threats of toxic impurities and use of the organic solvents. These ecofriendly approaches also provided low operating cost, better stability, compatibility and minimum health hazards [68]. The literature has suggested the successful implementation of solvent free approaches to formulate nanosized systems for the targeted delivery of different therapeutic and diagnostic moieties.

The heat chill method has been employed to prepare micelles using the amphiphilic diblock and triblock copolymers of polycaprolactone (PCL) for the encapsulation of insulin without using any organic solvent and has provide better stability of the entrapped proteins which are liable to denaturation in the presence of different organic solvents [69].

Kumar et al. prepared the green PLGA-oil hybrid nanoparticles of resveratrol employing the acrysol oil (a derivative of castor oil) as nontoxic solvent. The nanoparticles have a smooth outer morphology with improved drug release and stability profile [70].

2.3.5. Preparation of organic/inorganic hybrid nanoparticles

The concept of combining the characteristics of organic and inorganic components is quite old since the time of Egyptian inks. However, the modern organic-inorganic hybrid systems are not prepared by simple mixing these materials but may involve the weak electrostatic linkages (H-bonding or van der Waals forces) or strong chemical bonds, i.e., covalent bonds [71]. Multiple strategies are employed for the preparation of these hybrid particles. These include (i) polymerization of the different monomers, organosilanes and the metal oxides, (ii) self-assembly of different structural components at nanoblock level with different organic and metal components, (iii) the functionalization of preformed nanocarriers with different organic compounds and (iv) making the core with organic materials and coating with the silica and different metallic components [72, 73].

Structural components	Physicochemical properties			Application	References
	Size (nm)	Zeta potential (mV)	Entrapment efficiency (%)		
PLA DPPC PEG-PE	278 ± 16	(+) 20 to 50	N/A	Steric stabilization of hybrid nanoparticles was enhanced at least up to 150 mM NaCl (for more than 1 year at 4°C).	[27]
Paclitaxel PLGA PEGylated octadecyl-quaternized lysine modified chitosan	194 ± 7	22 ± 4	87 ± 2	Folic acid modified polymer core lipid shell hybrid carrier for targeted anti-cancer therapy. Higher internalization up to 14.8 folds was observed in flow cytometry. It also showed higher cytotoxicity than commercial preparation (Taxol®). After 2 hours administration, it showed 3.70 fold higher bio distribution than Paclitaxel injection.	[39]
Levofloxacin Ciprofloxacin Ofloxacin PLGA Phosphatidylcholine (PC), Stearic Acid (SA)	420 260 360	-26 +26 -22	19 4 5	The amount of the polymer and lipid were optimized for highly efficient hybrid system. Hybrid nanoparticles showed higher size and drug encapsulation in comparison to polymeric carriers. Different antibiotics like levofloxacin, ciprofloxacin and ofloxacin were encapsulated. Ciprofloxacin showed less EE due to less lipophilicity. Oppositely charged drug and lipid prevented nanoparticle formation which was remedied by the addition of counter ionic surfactant.	[45]
Doxorubicin PLGA DEPE-PEG Lecithin	118.7 ± 0.75	15.19 ± 3.85	45.76 ± 6.58	Folate receptor mediated drug delivery of anti-cancer agent, doxorubicin, resulted in higher cell internalization and enhanced cell-killing effect toward MCF-7 cells with a significantly lower IC50.	[52]
PLGA DSPE-PEG Poly (β-aminoester) poly-1	280 ± 70	(+) 40 ± 7	N/A	mRNA loaded pH sensitive particles reached cytosol offering low cytotoxicity followed by translation at a frequency of ~30%. Intranasal administration of abovementioned system led to <i>in vivo</i> expression of protein as soon as 6 hours after administration.	[57]
Vincristine PLGA Poly ethylene glycol (PEG) Dextran sulfate	121.8–133	-8.5 to -14.6	64.7 to 93.6	Vincristine loaded hybrid nanocarriers resulted in 3.3-fold increase in apparent bioavailability, while its uptake was 12.4-fold higher than plain drug solution.	[58]

Structural components	Physicochemical properties			Application	References
	Size (nm)	Zeta potential (mV)	Entrapment efficiency (%)		
Docetaxel PLGA Lecithin PEG	70–80	-30 to -35	59 ± 4	Docetaxel loaded Hybrid nanoparticle exhibited 20 hours as T ₅₀ . These carriers also exhibited good stability in 10% bovine serum albumin and in 10% plasma solution.	[59]
Paclitaxel PLGA Soybean lecithin D- α -tocopheryl polyethylene glycol 1000 succinate (TPGS)	120–150	-15 to -20	>80	Developed carriers provided sustained release up to 8 days with a high tumor targeting potential through EPR effect. It also showed superior antitumor efficacy by inhibiting 58.8% volume of tumor at day 28.	[62]
PLGA DSPE-PEG	65	-47.7	N/A	A new quick single step preparation method is reported which needs 5 min to get accomplished. This method increased the production rate 20-fold without compromising determinant features of hybrid particles. Particles developed such exhibited good colloidal stability in PBS and serum over 5 days.	[63]
Docetaxel PLGA DEPE-PEG ₂₀₀₀	263.6	-20.74	66.88	Folic acid conjugation increased 38.2% for 0.5 hour incubation and 54% increase for 2 hours incubation during cell uptake study. Cell viability studies showed that formulation was 93.65% more effective than commercial preparation Taxotere®.	[64]
Erlotinib DEPE-PEG ₂₀₀₀ Dipalmitoylphosphatidylcholine (DPPC) N-[1-(2,3-Dioleoyloxy) propyl]-N,N,N-trimethylammonium methyl-sulfate (DOTAP)	161–271	-47	77.18	Erlotinib loaded Core Shell Lipid Polymer Hybrid Nanoparticles demonstrated 170 nm size with 66% Entrapment efficiency and greater uptake and efficiency in A549 cells.	[65]
Doxorubicin Elacridar Pluronic F-68	187–272	-19.7 to -22.9	71.2–89.3	Formulation shows up to 89% encapsulation efficiencies of Dox and GG918 in PLN with more uptake and cytotoxicity of Dox to MDR cells	[67]

Structural components	Physicochemical properties			Application	References
	Size (nm)	Zeta potential (mV)	Entrapment efficiency (%)		
Resveratrol PLGA	375 ± 13	-22 ± 1.6	76 ± 4.2	G-PONHs have higher biocompatibility and stability, but moderate cytotoxicity compared to standard NPs. It also involves the application green synthesis approach for the hybrid nanocarriers	[70]
Paclitaxel Poly-lactic-co-glycolic acid (PLGA) Soybean lecithin 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG)	186.9 ± 8.52	-29.5 ± 2.0	81.34 ± 3.41	More drug reaches target site crossing Blood brain barrier and survival time for mice was PtxR-FPLNs (42 days), Ptx-FPLNs (38 days) compared to PtxR (18 days) and Paclitaxal (14 days)	[76]
Melatonin Poly lactic acid (PLA) Didodecyldimethylammonium bromide (DDAB) Cetyltrimethylammonium bromide (CTAB)	180–218	+15.4 to -36.1	90.35	Coating with cationic lipids provides sustained and prolonged drug release, a pronounced benefit in ophthalmic application	[77]
Docetaxel PLGA DEPE-PEG ₂₀₀₀ Soybean lecithin	60–70	-40 to -60	~62	The system provides 62% entrapment efficiency and almost 50% drug release in 20 hours. The incorporation of PEG provides stability over 120 hours. TC 50 value ranged between 4.58 and 5.55 mg.	[78]
PLGA DSPE-PEG	50–150	N/A	N/A	Different lipid ratios were evaluated for the entrapment, particle size, stability of the system. The current system provides that pH sensitive release and targeting which aggregate the drug in the acidic tumor microenvironment.	[79]
Poly caprolacton (PCL) Glyceryl tripalmitate	58–2009	-5.82 to -46.31	5.81–60.32	The system indicates biphasic release of the drug in which the burst release is presented in the initial hour. The cellular uptake was 83.3% in L929 cells. It also provides better colloidal stability over 120 hours.	[81]
Human IgG Poloxamer-188	135–799	+16.7 to +17.9	30.3–60	The system was loaded with the SiRNA. Which show the loading capacity of up to 2.04%, entrapment efficiency 60% in the optimized formulation. It provides the targetibility with the antibody and the sustain release was demonstrated by 20% release over the study time.	[82]

Structural components	Physicochemical properties			Application	References
	Size (nm)	Zeta potential (mV)	Entrapment efficiency (%)		
PCL Grape leaf extract Curcumin	~291	-24.3		The resulting drug delivery system improves the antimicrobial efficacy against two bacterial strains in addition to antifungal activity and can be an alternative approach to antibacterial agents.	[83]
Doxorubicin Epoxidized soybean oil Pluronic F68	200–350	-23.1	70 to 80	The system promotes cytotoxicity of DOX against MDR to about eightfolds. Uptake and retention of drug by MDR was also significantly increased.	[84]
Carboxymethyl chitosan Calcium phosphate PEG	102 ± 1.7	-8.25 ± 0.76	78	It gives excellent delivery of siRNA to cancer proximity through EPR effect. Particularly hTERT containing nanoparticles promotes silencing of hTERT expression and induction of cell apoptosis pathways.	[85]
Doxorubicin Sorafenib	126.3 ± 16.4	-21.4 ± 4.6	90.5 ± 3.4 and 70.8 ± 2.8	DOX-SOR combination in iRGD conjugated HNPs produces more apoptotic rates up to 44.7% than 33.4% with plain drug combinations or 37% with HNPs without iRGD.	[86]
Doxorubicin Mitomycin C	~150	~ -25	>90	Co delivery of anticancer agents using HNPs is more effective than simple combination therapy with advantages of high local delivery. It provides the 2–4 fold increase in the cellular uptake of the drug in the cancer cells.	[87]
Lipid modified PEG	24 ± 5	-38 ± 1	N/A	The system describes engineering of functionalized lipid conjugated polymeric nanoparticles with more specific targeting approach and imaging and bio sensing based on fluorescence.	[88]
Doxorubicin Combretastatin A4 PLGA PC DSPE-PEG Cholesterol	180–200	N/A	N/A	The system contains two different therapeutic agents which provide the site targeted release of the anti-angiogenesis agent and the anti-cancer drugs.	[89]
Curcumin PLGA DPPC DSPE-PEG	171.6 ± 8.2	N/A	N/A	By treating the metastatic breast cancer cells with the lipid-polymer hybrid nanoparticles of Curcumin decreased the adhesion onto tumor necrosis factor by 70% in capillary flow.	[90]

Structural components	Physicochemical properties			Application	References
	Size (nm)	Zeta potential (mV)	Entrapment efficiency (%)		
Docetaxel PLA Chitosan	208–255.7	-21.3 to + 52.4	75.9	PLA/chitosan nanoparticles provide rapid initial release of 40% drug in 5 hours and 70% cumulative release in 24 hours.	[91]
Docetaxel Curcumin PLGA	169.6 ± 4.6	-35.7 ± 1.9	89.8 ± 3.1 and 81.9 ± 5.6	The drugs loaded hybrid nanoparticles showed enhanced cytotoxicity and tumor growth inhibition.	[92]
Docetaxel DSPE-PEG PLGA	110 ± 13.5	-25.67 ± 1.45	77.65 ± 0.57	The system increases the cellular uptake of docetaxel 2.5 folds and anti-proliferative activity 2.69–4.23 folds.	[93]
Doxorubicin Chitosan Hyaluronic acid	264 ± 2.2	-12.3 ± 2.0	97.8 ± 1.3	It is used to deliver anticancer drugs which results in enhanced circulation half-life and reduce the elimination of drug	[94]

Table 1. Hybrid nanoparticles with different structural components and their applications.

In conventional sol-gel approach, the hydrolysis process is used to obtain the hybrid system. The reaction involves the organically modified metal oxides which crosslinks with the polymers of multiple functionalities. These components may or may not be present in the organic solvents and possibly trapped within the inorganic material. However, use of self-assembling procedures in last few decades provided new methods for the fabrication. During the process, the inorganic materials (triblocks) were arranged by the use of organic surfactants. The preparation of the mesoporous hybrid with multiple functionalities provide highly porous surface which further modified based on the applications [74].

Shen and Shi [75] reported a method for preparation of the organic/inorganic hybrid based dendrimers. The metal or inorganic nanoparticles were entrapped in the dendrimers template to provide a modified surface morphology which can be tuned by different functional components to provide the biocompatibility and better colloidal stability [75] (**Table 1**).

3. Factors affecting hybrid nanocarriers

Hybrid nanoparticles are trimmed to an acceptable level of particle size, drug carrying capacity and site specificity through incorporation and adjustment of ratios of different chemical components. The variations of structural components of HNPs have an obvious influence on HNPs' characteristics [17, 96]. The principal factors of HNPs' formulation are (i) lipid/polymer ratio, (ii) PEGylation and (iii) polymer nature.

3.1. Lipid/polymer ratio

The lipid covering the polymeric core provides substantial benefits to HNPs and their distinction over nonhybrid nanoparticles. The ratio of two building blocks (lipid-polymer) of hybrid particles have significant role in stabilizing the formulation, monodispersibility and encapsulation efficiency [45, 97].

At a lower L/P ratio, the nanoparticle surfaces are not entirely covered with lipids, which can form bridges with lipid part of other particles causing aggregation and formation of larger particles. At a relative higher lipid concentration, it tends to decrease the production yield as whole amount is not incorporated in particles and free lipids will arrange themselves to form liposomes can affect the homogeneity of formulation. Therefore, the concentration of lipids should be optimized that cover to polymeric core on the basis of particle size and production yield [59, 98]. Chew et al. prepared HNPs with PC and PLGA carrying antibiotics with W_{PC}/W_{PLGA} value <15- up to 90%. At lipid amount below 15% larger particles were formed (800–1000 nm) and a sharp decrease in particle size was observed at an optimum concentration i.e. 30% lipids, an optimum particle size (260–400 nm) and 80% production yield was achieved. The lipid ratio above the optimum concentration i.e. 30% did not reduce particle size but it decreased the yield as the entire lipid was not utilized [45].

An optimum lipid to polymer ratio also provides the colloidal stability of HNPs by providing an optimum surface charge density which is responsible for electrostatic repulsive forces that prevent

particle coalescence and stabilizes the formulation. In case where the lipid part is insufficient and the resulting electrostatic repulsive forces are weak, some agents like PEG can be incorporated in the formulations to provide steric repulsion and stabilization of the HNPs [52, 80–98].

The charge on lipid part which is responsible for electrostatic repulsion between particles is shielded when mixture of cationic and zwitterionic lipids is employed. Anionic heads of zwitterionic lipids face outwards which reduces of cationic lipids charge and promotes aggregation of particles. However, the higher cationic lipid concentration may overcome this charge screening and aggregation can be minimized [59, 99]. The zwitterionic lipid such as 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) produces less aggregation than a cationic lipid, 1,2-dipalmitoyl-3-trimethylammonium propane (DPTAP). Therefore, it zwitter ionic lipid provides more stability than ionic lipids [55, 59].

The two potential benefits that lipid augments to the HNPs are the encapsulation efficiency and retardant release of the incorporated drugs. The former is achieved by preventing drug leakage during self-assembling process, whereas the latter is due to reduced interaction of lipids with dissolution medium [17, 100]. The charge on the surface of lipids and drugs also affects the entrapment efficiency due to interaction of surface charge of HNPs and the charge of the drug. The loading of ciprofloxacin in the PLGA-PC hybrid system is not successful due to the interaction of cationic drug with the anionic lipids [19, 78].

A significant higher percent encapsulation of docetaxel (59 ± 4) was achieved in HNPs assembled from lecithin, DSPE-PEG and PLGA i.e. compared to PLGA-PEG nanoparticles with 19 ± 3 (mean \pm SD). This effect is attributed to the fencing action of lipids which keeps hydrophobic drugs within the core and retards water penetration. The lipid-polymer hybrid formulations also provide a sustained release of drug when compared with nonhybrid formulation due to less water penetration and reduced escape of drug molecules from polymeric core. A consistent 50 % release of docetaxel from lecithin-PLGA hybrid system was observed compared to the PLGA-PEG NPs and PLGA NPs released same amount of drug in 10 hours and 7 hours, respectively. The pH of the dissolution media also affected the encapsulation of drugs, for example, the erlotinib EE % was 77.1%, 28.83% and 18.45% at pH values of 7.4, 5.4 and 3.4, respectively [55, 59, 78, 100].

3.2. PEGylation

The steric stabilization of HNPs systems to withstand salt solutions, buffer actions and uptake by macrophages is provided by the appropriate surface modification by employing PEG. The term is called as PEGylation. PEGs can escalate circulation times of HNPs by preventing particle aggregation, opsonization and adsorption of plasma proteins [27, 78].

Incorporation of PEG-lipid affects the colloidal stability of HNPs by two ways (i) chain length of PEG-lipid and (ii) molar Ratio of PEG-lipid. HNPs coated with PEG-lipid longer chains exhibited more stability than the shorter chain PEG-lipid coated particles. Similarly, at the fixed chain length more PEG-lipid incorporated onto polymer core and thickness of lipid shell increased which lowered the zeta potential and hence stability is enhanced [78, 80].

Yang et al. studied the effect of lipid/polymer ratio and PEGylation on HNPs prepared from PLA/mPEG-PLA polymer and BHEM-Chol cationic lipid. HNPs prepared from mPEG-PLA were smaller and more stable in PBS at the given lipid/polymer ratio than PLA alone [61].

Fang et al. formulated HNPs using 0.10–0.35 lipid-PEG/PLGA ratios without incorporating lecithin. Initially particle reduced with increase in lipid amount and optimized at 0.30 lipid-PEG/PLGA ratio after which further increase in lipids did not affect particle size and PDI. At an optimized (0.30 lipid-PEG/PLGA) ratio, lipid-PEG was replaced with mole equivalents of lecithin. The stable particles of 60 nm were obtained at 50% lipid-PEG replacement. Upon 70% lipid-PEG replacement, the size was increased to 100 nm and at 80% lipid-PEG replacement with lecithin, the unstable particles were obtained. This instability of particles is due to the replacement of higher lipid-PEG content, a major stability component of HNPs [63].

3.3. Nature of polymer

The characteristics such as density and surface charge play an important role in the fabrication of HNPs [35]. The density of polymer also has substantial effect on stability and particle size [59, 78]. HNPs fabricated from high density polymer are less stable toward increasing ionic strength of medium due to the higher sedimentation rate when electrostatic charges are shielded. PLA is 1.18 times denser than poly(styrene); hence, HNPs prepared with PLA core have less colloidal stability toward increasing ionic strength of medium [35]. Zhang et al. evaluated that change in viscosity of PLGA polymer from 0.19 to 0.82 resulted a decrease in particle diameter from 92.7 nm to 66.7 nm [59].

Adsorption of lipid over polymeric particle surface to form lipid shell depends upon curvature and surface charge of particle. Cationic lipids exhibit more adsorption than zwitter ions toward the anionic polymeric core due to the electrostatic attractions polymeric core from anionic polymer PLA has greater affinity for DPTAP cationic lipid than the zwitterionic DPPC. Lipid rearrangement around polymeric core can be quick and complete if the affinity between polymer and lipid is high. Larger size distribution and free lipid structures are observed when lipids cannot rearrange around polymeric core due to weaker affinity. Modification in pH of medium can improve the affinity of polymer for lipid by surface charge variation at different pH levels [35, 101, 102].

4. Applications of hybrid nanocarriers

Hybrid systems combine properties of two or more materials, thus, appear superior to individual material system. Usually, one component of hybrid system is active, whereas other is used to improve biocompatibility, circulation life and targeting. Many new hybrid systems use second material to improve efficiency of first materials. By suitable selection of materials, hybrid systems find wider applications in medical field. Hydrophilic polymers have been widely used to impart stealth property to nanoparticles. However, stealth coating does

not improve or impart new functional aspect of nanoparticles. Thus, many researchers do not regard PEG coated as hybrid systems. Similarly, nanoparticles conjugated with targeting ligands cannot be regarded as hybrid system.

4.1. Lipid polymer hybrid nanoparticles (LPHN)

LPHN consists of a drug containing polymeric core which is coated by a lipid shell. In these systems, inner polymer core contains drug and lipid shell is used to enhance penetration through biological membranes and to control drug release. Polymeric core can be made from hydrophilic or hydrophobic polymer. Term lipid-polymer hybrid is also used for systems that contain polymer core with lipid coating. Lipid is preferred carrier material for hydrophobic drugs due to higher encapsulation efficiency and extended release pattern. A polymeric coating is applied over lipid core to impart certain characteristics required for novel biomedical applications.

In addition of polymeric and lipid layers, surface of LPHN may be modified with different materials. In one study, a hydrophobic drug was loaded in a hydrophobic biodegradable polymer to enhance encapsulation efficiency of a hydrophobic drug. Then, a lipid layer is applied to stabilize core and shell, and to prolong drug release. Finally, hydrophilic polymeric layer, consisting of DSPE-PEG (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-carboxy (polyethylene glycol)2000) was applied to improve pharmacokinetics of LPHN. The three-layer LPHN showed high encapsulation and sustained release of hydrophilic drug [59]. A hydrophilic polymer monolayer may be applied to LPHN to escape phagocytosis and early removal from body. Generally, polyethylene glycol (PEG) is used to provide this stealth property and enhance circulation time of nanoparticles. PEG will attract water to make an aqueous layer which protect LPHN from attachment of opsonin proteins and let it escape the uptake by reticuloendothelial (RES) system. Hydrophilic polymer layer can also enhance colloidal stability of LPHN due to steric hindrance effect [5]. As stealth layer can also hinder interaction with target cells, PEG can be conjugated with other monomers or polymers to form block copolymers that are specific to certain stimuli. This approach enables long circulating LPHN that can shed stealth layer when come in contact with target cells. The stimuli could be intracellular and extracellular protease enzymes, low pH or reducing agents [103].

Selection of polymeric matrix plays a major role in drug delivery properties of LPHN. LPHN are commonly used for poor water soluble or hydrophobic drugs. A hydrophobic polymer core can encapsulate higher amount of hydrophobic drug and vice versa. Two or more drugs could also be loaded into the core of LPHN. On the other hand, LPHN with hydrophilic and hydrophobic drug could be made to contain one drug in core and the other in shell. Wong et al. [67] prepared LPHN containing lipid core to encapsulate hydrophobic drug Elacridar (GG918) and hydrophilic shell of hydrolyzed polymer of epoxidized soybean oil (HPESO) to encapsulated doxorubicin. They found that both drugs were released in sustained manner for more than 72 hours (**Figure 2**). Simultaneous delivery of chemosensitizer GG918 was able to revert multidrug resistance to anticancer drug doxorubicin. These simultaneously loaded LPHN showed better efficacy than free drug solution or LPHN of any of the two drugs.

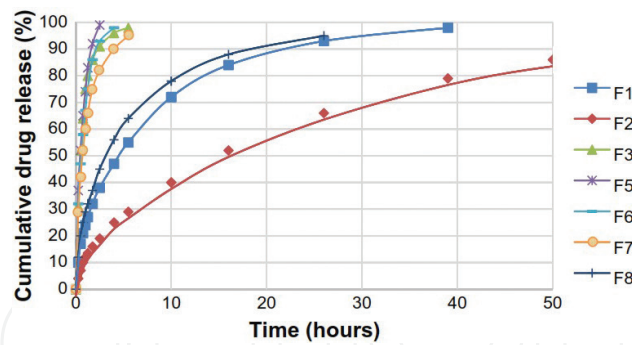


Figure 2. Drug release from gold nanoparticles containing solid lipid nanoparticles. F1 (5mg diacerein) and F2 (10 mg diacerein) show sustained release of drug for 39 and 48 hours. However, F3 had same composition as F2 but drug release was conducted at 40°C. F5 and F6 contained increasing amount of hydrophilic gold nanoparticles with 5 mg diacerein. F7 contained gold nanoparticles same as F6 but 10 mg diacerein. Finally, F8 contained lipophilic gold nanoparticles with 10 mg diacerein. Drug release was faster at higher temperature (F3) as compared to 37°C. Furthermore, hydrophilic gold nanoparticles containing formulations F5, F6 and F7 released drug in less than 5 hours, whereas hydrophobic gold nanoparticles showed prolonged release up to 25 hours.

As a wide variety of polymers and lipids are available, LPHN can be prepared to theoretically load any therapeutic moiety. Nucleic acid based therapeutics i.e. plasmid DNA, antisense oligonucleotide, small interfering RNA and small hairpin RNA, have shown promise to cure many diseases. LPHN have emerged as nonviral carriers for nucleic acid products with low toxicity, immunogenicity and cost of production. Cationic polymers and lipids have been widely investigated for this purpose. Cationic groups can bind negatively charged nucleic acid molecules and deliver to target cells. Zhong et al. [104] prepared LPHN with biodegradable PLGA and two cationic lipids i.e. 1, 2-dioleoyl-3-trimethylammonium-propane (DOTAP) or 3 β -[N-(N',N'-dimethylaminoethane)-carbonyl]cholesterol (DC-Chol). LPHN were prepared by two either with cationic lipid core so that DNA is loaded inside core or with cationic lipid shell so that DNA is loaded on surface. The *in vitro* evaluation was done in human embryonic kidney cells. They found that LPHN with DNA on surface showed higher transfection efficiency than those with DNA inside core. Next, they prepared LPHN with polymer both inside core and on the surface which showed efficiency similar to that of LPHN with DNA on surface. This study concluded that LPHN can show transfection efficiency about 600 times higher than unbound DNA. However, cationic lipids and polymers may have some problems on their own. They may interact with biological components, be nonbiodegradable or toxic after systemic administration. These factors are controlled by hydrophobic chain length, nature of cation group and linkage. To solve these problems, Shi et al. [54] prepared novel LPHN with four distinct layers. First is a hollow core i.e. aqueous droplet containing nucleic acid which is coated by an inner lipid layer of cationic lipid ethylphosphocholine.

The cationic lipid orients itself in such a way that cationic group faces inward and its hydrophobic chain faces outward. Third layer is formed by ester terminated PLGA. It is a hydrophobic polymer that intermingles with protruding chains of cationic lipid. Finally, self-assembled lecithin and DSPE-PEG form outer coating to facilitate transfection and to impart stealth property to LPHN. This LPHN system release loaded siRNA in sustained manner up to 6 days and enhanced gene expression in mice.

A recent trend in drug delivery research has focused on the development of human-like vesicular drug delivery system. This concept emerged when exosome were found to be responsible for cell to cell communication in tumors and regulate tumor microenvironment. It was believed that exosomes isolated from patients may be filled with antitumor drugs and injected back to the patients for personalized treatment. As isolation of exosomes from patients is complicated and very costly, this dream was realized by synthesizing surface antigens of exosomes by genetic engineering and grafting on the surface of drug containing liposomes or other vesicular systems [5]. In addition to this, many bacterial and viral antigens have been used. These antigens are used for the delivery of vaccine and act as immune adjuvant i.e. enhance immune response to vaccine. Moreover, polymeric core produce better adjuvant effects than lipid core. Bershteyn et al. [105] prepared PLGA core and phospholipid bilayer coated LPHN that were stabilized by PEG for simultaneous loading of antigen and adjuvant. The protein adjuvant was covalently bonded on surface and lipophilic adjuvants, such as monophosphoryl lipid A and α -galactosylceramide, which were loaded in lipid bilayer. Immune response was shown at dose as low as 2.5 ng which was detectable after 100 days. It was also found that α -galactosylceramide shows rapid rise in antibody titer whereas monophosphoryl lipid A produced response in sustained manner. Interestingly, coloaded of both adjuvants with antigen further increased antigen titer by 12 fold. These results show that LPHN can reduce dose of antigen to reduce cost and side effects.

Term LPHN may also be extended to nanoparticle systems consisting of two or more polymer at least one of which is lipophilic. A hydrophilic shell may be applied to drug containing hydrophobic (or lipophilic) polymeric core to impart mucoadhesion or to make them stealth. For example, PEG or chitosan coating has been widely used to improve circulation life of sustained release solid lipid nanoparticles [106]. On the other hand, a hydrophobic polymer shell may be formed over hydrophilic polymer core to enhance LPHN absorption through biological membranes. This approach is especially useful for oral administration of therapeutic macromolecules [107]. Recently, Liu et al. has synthesized supramolecular vectors for gene delivery. First, adamantyl-terminated polyethyleneimine was admixed with β -cyclodextrin to encapsulate nucleic acid, i.e., DNA or siRNA which was further coated with adamantyl-PEG. The supramolecular vector was stabilized by host-guest interaction. This LPHN system showed low toxicity and high transfection efficiency during in vitro experiments. Graphene is another two-dimensional framework of carbon atoms that is investigated for hybrid applications. When treated with suitable reagents, it can be oxidized, hydroxylated, carboxylated or halogenated. These functional groups can be conjugated with different materials desired for biomedical applications [108].

4.2. Inorganic/organic hybrid nanoparticles (IOHN)

IOHN are synthesized from organic and inorganic materials. Most commonly, core is made of inorganic materials and the shell of an organic material is applied to improve its pharmacokinetic parameters. On the other hand, inorganic shell may be applied to the core of organic materials to impart different properties. IOHN are interesting because they offer properties of both materials. Like organic polymer, they can be functionalized with different groups. Like metallic nanoparticles, inorganic shell provides physical and chemical stability

to polymeric core. Generally, the inorganic portion is developed by reduction of metal ions to zerovalent state. Inorganic core is synthesized by mixing metal ions solution with a reducing agent with or without heating. However, inorganic shell may be synthesized either by reduction of metal ions on polymeric core or by deposition of preformed metal colloids on organic core.

Methods for synthesis of organic core and shell have already been discussed in detail in a chapter. We have prepared IOHN consisting of gold core with fatty acid shell. First, gold nanoparticles were synthesized with lecithin bilayer (hydrophilic surface) and lecithin monolayer by acid treatment (hydrophobic surface). The gold nanoparticles were added to molten fatty acids and emulsified with aqueous surfactant solution. Upon cooling, we found that gold nanoparticles with hydrophobic surface are more stable as compared to gold nanoparticles with hydrophilic surface [109]. The presence of gold nanoparticles in core enhanced drug release rate from lipid nanoparticles. This can be attributed to the presence of gold nanoparticles that push drug toward periphery and reduce diffusion path length (**Figure 1**). In another study, we prepared an organic core of lecithin and inorganic shell of gold nanoparticles. First, lecithin nanoparticles were prepared and loaded with drug. Next, preformed gold nanoparticles were adsorbed on its surface. We found that drug release was controlled by both gold nanoparticles. Gold nanoparticles retard release of drug due to physical barrier. Lecithin controlled release of anti-inflammatory drug from core in pH-dependent manner [110]. Gold is also known to possess anti-inflammatory effect. In this study, gold shell was found to synergize anti-inflammatory effect of encapsulated drug diacerein by many folds (**Figure 3**).

Various organic materials have been used to prepare IOHN to improve their performance. The materials that are used to synthesize or stabilize nanoparticles may impart specific function. The most pronounced function is enhanced penetration inside target cells which in turn controls toxicity of IOHN. Freese et al. [47] studied toxicity of gold nanoparticles with different organic coatings with neutral, positive and negative charge. The results showed that IOHN with positive charge coating shows more internalization in cells, and thus, higher toxicity. The cell membrane has a negative charge, whereas the IOHN are positively charged particles. This charge difference triggers the rapid binding to the cell surface and internalization of these IOHNs. As gold can cause toxicity at higher dose, higher internalization in cell will lead to high toxicity [111].

Metallic nanoparticles smaller than 100 nm are usually responsive to different stimuli, a technique that has been widely employed in diagnosis and therapy. IOHN with metallic core can be used for thermotherapy of cancer whereby IOHN produces heat when exposed to external magnetic field. Similarly, metallic moieties, i.e., nanoparticles or tagged polymers, can be bound to core of organic materials. These nanoparticles will be targeted to cancerous tissues and magnetic moieties will produce hyperthermia under external stimuli. When core of organic material is loaded with drug, inorganic part can release the drug by hyperthermia-mediated degradation of core after reaching the target site [5]. In addition to magnetic field, inorganic nanoparticles are also responsive to infrared and ultrasound waves. This makes IOHN interesting candidates for biomedical imaging of targeted tis-

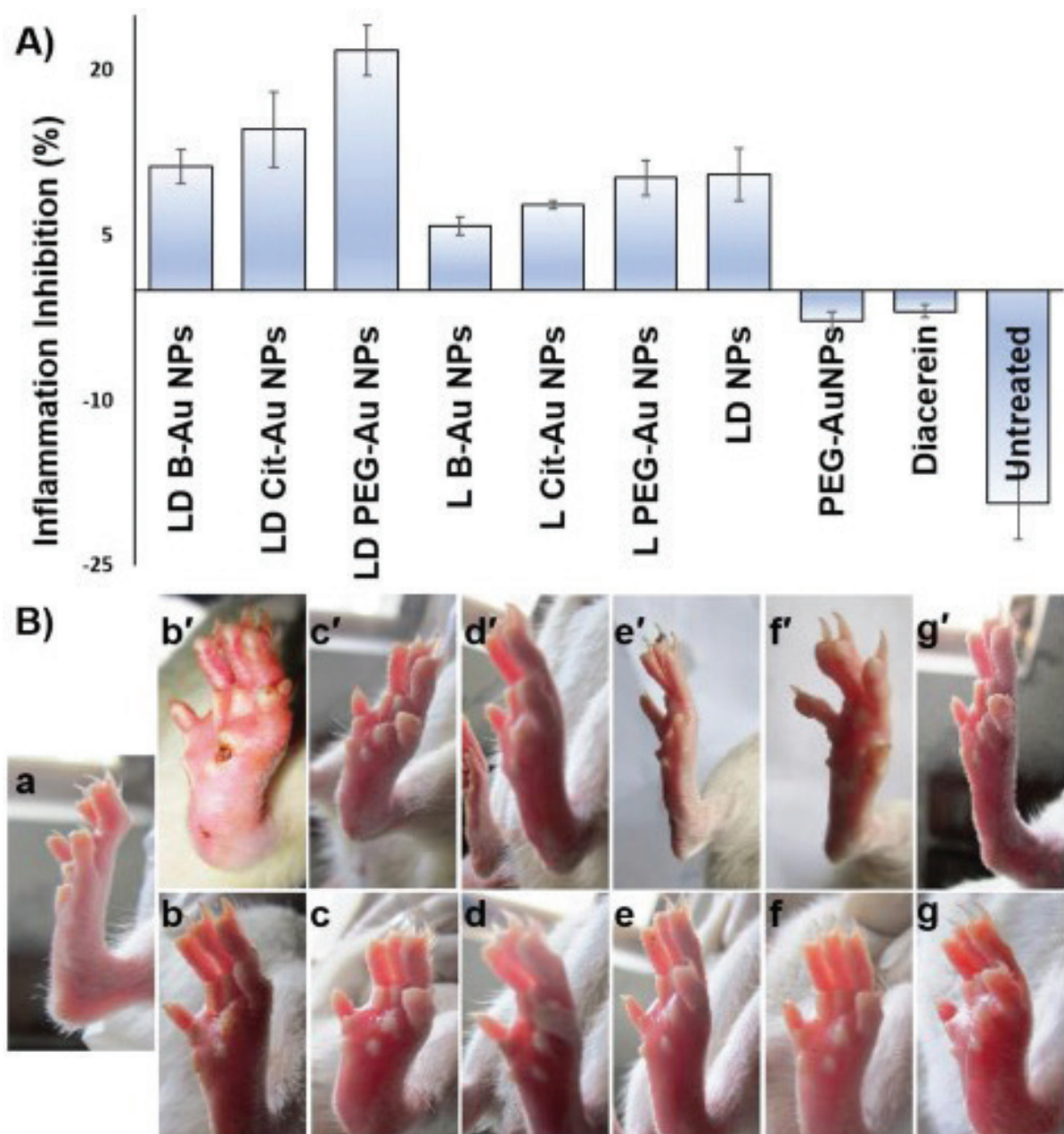


Figure 3. Efficacy of anti-inflammatory drug encapsulated in lecithin core-gold shell hybrid nanoparticles; (A) Anti-inflammatory effect of diacerein is synergized in the presence of gold as compared to pure drug, diacerein. PEG-AuNP = PEG coated gold nanoparticles, LD-NP = diacerein loaded lecithin nano[articles, L PEG-AuNP = Lecithin nanoparticles surface coated with PEG coated gold nanoparticles, L Cit-AuNP = Lecithin nanoparticles surface coated with citrate coated gold nanoparticles, L B-AuNP = Lecithin nanoparticles surface coated with sodium borohydrate coated gold nanoparticles, LD PEG-AuNP = L PEG-AuNP loaded with diacerein, LD Cit-AuNP = L Cit-AuNP loaded with diacerein, LD B-AuNP = L B-AuNP loaded with diacerein. (B) represents decrease in swelling as measured by Vernier caliper before (a, b, c, d, e, f, g) and 3 h after (b', c', d', e', f', g') from untreated (b), diacerein (c), PEG-Au NPs (d), LD PEG-Au NPs (e), LD Cit-Au NPs (f), and LD B-Au NPs (g) treatments groups, while a is normal rat paw.

sues. More recently, multimodal IOHN have ensured imaging and drug release from the same system after systemic administration. This target can be achieved in two ways. First, magnetic field of low frequency or intensity is applied for imaging of IOHN. Once in cancer

tissue, intensity or frequency is increased to produce hyperthermia-based cell killing or drug release [112]. Secondly, inorganic materials responsive to more than one stimulus can be used. One stimulus aids in imaging, whereas second stimulus will lead to drug release or thermotherapy [113].

IOHN have also been prepared with hollow core enclosed inside a hybrid shell. Hollow core IOHN can be prepared by many ways. First strategy is to make layer of inorganic or organic material which is then stabilized by other component of IOHN system. Similarly, it can consist of a mixed shell of inorganic and organic materials enclosing hollow core. Metal-tagged polymers with amphiphilic nature self-assemble to form micelles in aqueous solution or after reaching the target microenvironment [114]. Whole virus or virus capsid has been investigated as drug delivery systems by many researchers due to its inherent high penetration in cells.

Portney et al. [115] hybridized virus capsid with quantum dots and single-wall carbon nanotubes to yield hybrid structures that can find various applications. These hybrid structures are very stable to chemical and mechanical stress. IOHN with metallic core and organic shells have been widely investigated for diagnostic application. Although, organic shell usually employed to improve the pharmacokinetics and targeting properties of the metallic nanoparticles but may be beneficial by enhancing the diagnostic efficiency of the system. The most prominent example is nucleic acid-based biosensors with metallic core. When metallic nanoparticles aggregate, they show blue shift due to increase in size. Metallic core is coated with single-stranded DNA (ssDNA) that can identify specific sequence on target DNA and bind it. In bioassay, when metallic nanoparticle conjugated ssDNA start bind target DNA, they come close to each other and test solution color changes from red to blue. This indicates the presence of target DNA as visualized by naked eye or through UV-visible spectrophotometer [116].

4.3. Metalloprotein hybrid nanoflowers (MPHNs)

Although MPHN can be categorized as inorganic-organic hybrid NP, they are discussed here separately due to difference in structure and many fold increased surface area. The flower-like structure of MPHN is due to the presence of proteins that stabilize metallic crystals in the structure. Proteins act as glue and hold metallic crystals in a pattern which mimics flower petals. Unlike inorganic-organic hybrids, synthesis of MPHN occurs in three stages. First stage is the growth stage in which metal ions bond with proteins through amide bond. This acts as nucleation site leading to growth of primary crystals. In the second stage, metalloprotein crystals aggregate to form larger structures bearing primary petals like structures. Finally, anisotropic growth on metalloprotein aggregates leads to formation of complete petals. Generally, their size lies in the range of 2–30 μm which is another reason to differentiate MPHN from IOHN. MPHN is mostly used for bioassay whereby desired enzyme is conjugated with metallic part. Encapsulation efficiency of enzymes in MPHN has been achieved up to 66%. Enzyme loading above or below this limit decreases encapsulation efficiency. Nevertheless, enzyme efficiency of MPHN varies between 85% and 100%. Enzyme efficiency higher than free enzyme is due to many reasons. MPHN shows high surface area due to petal-like projections. The petals also have hole-like spaces between them that may be up to 100 nm in diameter. It is

also observed that immobilized enzyme shows cooperative interaction to enhance enzyme efficiency. Similarly, metal ions, such as copper, calcium and manganese, may also help enzyme in catalysis. Copper (Cu^{2+}) is the most widely used metal with different enzymes. Cu^{2+} and laccase enzyme MPHN have been developed for detection of phenols. The prepared MPHN was adsorbed on filter, and a mixture of phenol and 4-aminoantipyrine was added to it. Laccase-assisted reaction of both compounds produced red antipyrine dyes in 5 minutes. The changes in color will be visible with the naked eye, and UV-visible spectrophotometer can be used for quantitative detection. The MPHN-coated filters are reusable and are much faster than chromatography and mass spectrometry based methods. Likewise, MPHN of Cu^{2+} and horseradish peroxidase was prepared for detection of phenol and hydrogen peroxide. This MPHN was able to detect very low amounts of phenol ($1 \mu\text{M}$) and hydrogen peroxide ($0.5 \mu\text{M}$) as change in color was observed with the naked eye. It has been found that hydrogen peroxide induces cell death at concentration higher than $50 \mu\text{M}$ and the limit of detection of free enzyme is around $20 \mu\text{M}$. Thus, these MPHNs will be very efficient to detect slight changes in hydrogen peroxide efficiently even below its threshold level. Cu^{2+} and trypsin MPHN have been used to carry out proteolysis which is an important step in protein identification. The enzyme efficiency of proteolytic MPHN is similar or superior to free enzyme but are fast and reusable.

Another form of nanoflowers is synthesized using deoxyribonucleic acid (DNA) which, like proteins, possesses high number of nitrogen molecules and serves as a template for nanoflowers. In one study, a drug and a dye molecule was bonded to DNA that was used to synthesize nanoflowers. These nanoflowers showed multimodal property of drug delivery and imaging by using FRET technology. More recently, capsular MPHNs have been prepared with improved characteristics. This technique involved coating of MPHN with protamine and silica. Then, metallic core is removed from capsular MPHN system. Capsular nanoflowers show higher enzyme efficiency and improved stability in harsh environmental conditions.

4.4. Mesoporous silica hybrid nanoparticles

Silica has been widely used in drug delivery due to its nontoxic and biocompatible nature. Silica shell has been applied to metallic nanoparticles to reduce their toxicity in various biomedical applications. Mesoporous silica nanoparticles (MSNPs) are silica materials with mesopores of up to 50 nm. They are also termed as hollow mesoporous silica nanoparticles due to the fact that mesopores are hollow. The advantages of MSNP are enhanced surface area and that hollow mesopores can be loaded with therapeutic molecules. First, MSNPs were loaded with drugs. Later, MSNPs were used for the delivery of different dyes and macromolecules such as enzymes. MSNP hybrids have been prepared with both organic and inorganic materials. One problem with the use of MSNP is the leakage of drugs from pores. Sreejith et al. [117] used graphene oxide (GO) coating on MSNP to prevent leakage of drugs. After drug loading, GO coating is applied which acts as blanket to physically block the pores. GO coating also prevents encapsulated drug from environmental degradation. In addition to applications in drug delivery, MSNPs are also used for diagnosis and imaging.

Maji et al. [118] prepared MSNP-GNP (gold nanoparticle) hybrids for detection of hydrogen peroxide. They coated MSNP with graphene oxide, and GNPs were coated on this surface.

The hybrids were first used for electrochemical detection of hydrogen peroxide in the presence of other biological molecules. Later, MSNP-GNPs were successfully used for *in vivo* imaging in mice. MSNP surface can be modified with different functional groups that provide opportunities to form hybrid with different materials [118].

5. Conclusion and future prospects

Hybrid nanocarriers provide a novel platform that synergizes the effects of therapeutic and diagnostic agents through tunable properties such as particle size, structure, composition, preparatory method and easy surface and charge modifications. Here, we describe the different parameters related to development, optimization as well as characterization to obtain a robust platform for the drug delivery and other biomedical applications. We can still try to focus some unmet challenges of this novel drug delivery system. These challenges include development and optimization of the application of target ligands in appropriate ligand density that will improve the pharmacokinetics as well as pharmacodynamics profiles of all the drugs loaded in these hybrid nanoparticles either single or in combination with other therapeutic and diagnostic agents. Similarly, development of these hybrid nanocarriers at large scale has received less attention. So it is a key parameter to translate the system for large-scale applications by using the different methods mentioned in the section of method of preparation especially the one-step self-assembly method that is likely to improve the production in a facile and economic manner.

Author details

Asadullah Madni*, Nayab Tahir, Mubashar Rehman, Ahmed Raza, Muhammad Ahmad Mahmood, Muhammad Imran Khan and Prince Muhammad Kashif

*Address all correspondence to: asadpharmacist@hotmail.com

Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

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