CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
http://doi.org/10.5281/zenodo.3732268

Available online at: http://www.iajps.com Review Article

NANO CARRIERS IN DETAIL: A REVIEW

Apoorva More [1*], Akkshata Parab [2]

[1]Department of Quality Assurance, St. John Institute of Pharmacy and Research, Palghar, Maharashtra, India – 401404. Email: apoorvamore8@gmail.com, Phone: +91 9665544609

[2]Department of Pharmaceutics, VIVA Institute of Pharmacy, Virar, Maharashtra, India – 401305. E-mail: akkshataparab@gmail.com, Phone: +91 9890618685

Article Received: January 2020 **Accepted:** February 2020 **Published:** March 2020

Abstract:

To date, numerous nano-drug systems for different routes of administration have been evolved, including dendrimers, nanocrystals, emulsions, liposomes, solid lipid nanoparticles, micelles, and polymeric nanoparticles. In particular, functionalized nano drug systems can offer modification of delivery time, provide targeted delivery to specific tissues and enhance in vivo solubility and thus the bioavailability of poorly soluble drugs. Thus, nano-drug systems could reduce the administration frequency while offering maximized pharmacological effects and minimizing systemic side effects, leading to better therapeutic efficiency and clinical results. More attention has been placed over past few years on lipid-based drug delivery system to overcome some limitations of conventional formulations. Promising delivery systems include solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs). The purpose of this paper is to review the overall nano-drug systems information which includes the reasons to choose nano carriers, along with composition / ingredients, preparation method, characterization method, advantages and limitations of SLNs and NLCs as nano carriers.

Keywords: *Nano carriers, solid lipid nanoparticles, nanostructured lipid carriers, composition, preparation methods, characterization methods*

Corresponding author:

Apoorva More,

Department of Quality Assurance,
St. John Institute of Pharmacy and Research,
Palghar, Maharashtra, India – 401404.
Email: apoorvamore8@gmail.com
Phone:+91 9665544609

QR code

Please cite this article in press Apoorva More and Akkshata Parab., ***Nano Carriers In Detail: A Review,*** *Indo Am. J. P. Sci, 2020; 07(03).*

1. INTRODUCTION:

1.1. History

The roots of Nanotechnology can be traced back from a lecture delivered by Richard Feymann (Nobel Laureate) in 1960, when he speculated this future scientists and engineers would build structures from atoms and molecules. He quoted, "There's plenty of Room at the Bottom" at an American Physical Society meeting at Caltech. [1] In 1974, Norio Taniguchi coined the term "nanotechnology". [2] In 1980, K. Eric Drexler developed and popularized the concept of nanotechnology, he is also the founder of the field molecular nanotechnology. [2] According to Kreuter, a nanoparticle is a solid colloidal particle ranging in size from 1 to 1000 nm. [3]

Nanotechnology is an interdisciplinary field where technological developments are made on nanometer scale which offers various comprehensive applications. [3] The prefix "Nano" comes from Latin '*nanus'* which means *dwarf* or *very small*. It is used from last decade for an ever-increasing application to different fields of the knowledge. [4] National Nanotechnology Initiative defined '*Nanotechnology*' as the study and use of structures roughly in size range of 1 to 100 nm. [5] The overall goal of Nanotechnology is to diagnose as accurately, early as possible and to treat effectively without any possible side effects.

Nano medicine have greater opportunities where conventional techniques cannot be used. This field developed exponentially with major aim for targeted drug delivery. Nanotechnology is utilized for design, production, and application of material at atomic, molecular and macromolecular scales to intentionally alter and manipulate the materials or surfaces at nanometre scale resulting in nano sized material with new desired properties. Nanoparticle mediated delivery of drugs, vaccines, and diagnostics is rapidly developing area of nanomedicine that brings together the scientific disciplines of nanotechnology, pharmacology, immunology, and chemistry. This huge potential of nanoparticles and nanomaterials in modern medicine is reflected by intensive basic and oriented research. Well focused scientific programmes supporting the research in nanotechnology and medicine are proposed in EU, USA as well as in technically developed Asian countries like Japan, Korea and China. [6-8]

1.2. General information [9,10]

Nanoparticles are submicron sized colloidal structures made of synthetic or semi synthetic polymers. The first reported nanoparticles were based on non-biodegradable polymeric systems. Even though polymeric nanoparticles have proven to be excellent drug carriers there are few associated limitations such as inclusion of residues from organic solvents used during formulation, toxicity of the polymer, and production scale up. The continual search and maneuring towards physical stability improvisation of liposomes resulted in development of solid core nano particles in eighties as an alternative drug carrier. A particular advantage offered by lipid colloidal carriers is the increase in bioavailability of poorly water-soluble drugs. Melt-emulsified nanoparticles based on lipids that are solid at room temperature offers several advantages when compared to nano-emulsions, nano-suspensions, mixed micelles, liposomes and polymeric nanoparticles. Nano carriers have high ratio of surface area to volume, show improved pharmacokinetics and bio distribution of therapeutic agents. They minimize toxicity by their preferential accumulation at the target site.

The various generations of Nano carriers:

- **First generation:** Nanospheres and nanocapsules (best known; most accessible).
- **Second generation**: Nanoparticles coated with hydrophilic polymers such as polyethylene glycol (PEG); pegylated nanoparticles.
- **Third generation**: Combining a biodegradable core and polymer envelope (PEG) with a membrane recognition ligand (still under development)

2.DIFFERENT TYPES OF NANCARRIER SYSTEMS:

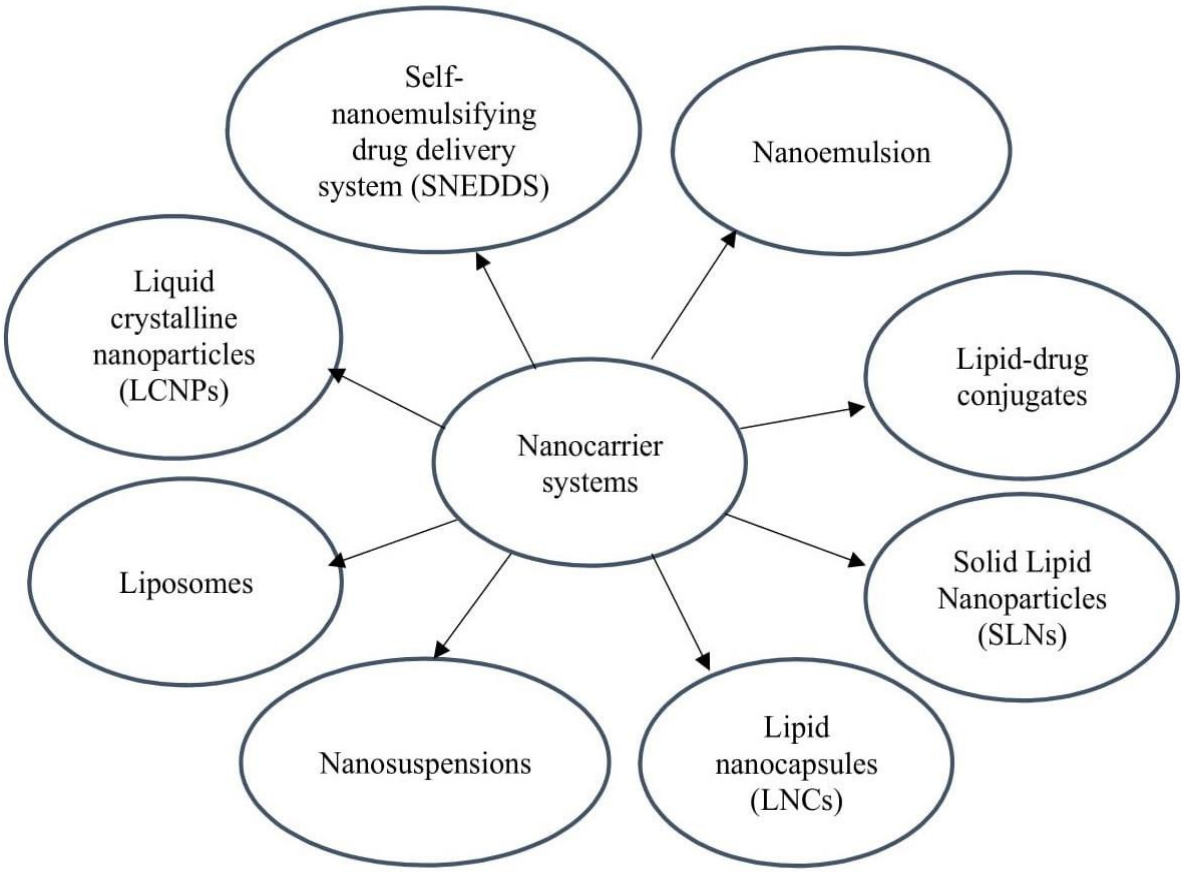

Fig 1. Different types of nanocarriers

Finding solutions to deliver drugs at specific sites is not a contemporary issue but remains as a crucial biomedical aspect. The constant development and design of optimized strategies involving polymers, nanoparticles, liposomes, and others active or passive delivery systems has been a revolution on the way that the drugs exert their actions and several novel strategies are developed and tested all years.[11]

2.1 Nanoemulsions: [12]

Nanoemulsions are heterogeneous, non-equilibrium systems consisting of oil droplets dispersed in an aqueous medium and stabilized by surfactant molecules. The oil droplets act as a reservoir for hydrophobic drugs in a nanoemulsion. Moreover, nanoemulsions are regarded as kinetically stable, isotropic and transparent without any apparent coalescence during the long time storage. The nanoemulsions are usually stabilized by large amount of surfactants, which can improve drug solubilisation, protect active compound against physicochemical and enzymatic degradation and modify the permeability of the GIT membrane. Non-ionic surfactants are commonly preferred due to their less toxicity, less affected by pH and ions than ionic and amphiphilic surfactants, and better compatibility with biological systems. Combinations of different surfactants have also been employed to decrease the droplet size and improve the stability of nanoemulsions. Methods used for the production of nanoemulsions include high-pressure homogenization, microfluidization, ultrasonication, spontaneous emulsification and so on. The advantages of nanoemulsions are increased drug loading, tissue targeting and enhanced permeability.

2.2 Lipid-drug conjugates: [13]

To overcome the limitation of limited loading capacity for highly potent hydrophilic drugs and drug expulsion during storage, lipid-drug conjugates have been made. Lipid-drug conjugates nanoparticle are prepared either by formation of a salt with a fatty acid or alternatively by covalent linkage (e.g. to ester or ethers). Further process is perform an aqueous surfactant solution to a nanoparticle formulation using high pressure homogenisation. The lipids that can be used for formulation of lipid–

drug conjugates include phospholipids, fatty acids such as stearic acid, oleic acid, docosahexaenoic acid, etc. and lipoamino acids.

2.3 Solid lipid nanoparticles (SLNs):[14-17]
Solid lipid nanoparticles (SLNs) are composed of melt-emulsified solid lipids like highly purified triglycerides, monoglycerides, hard fats, complex glyceride mixtures as matrix materials. As they are derived from biodegradable and compatible lipids, SLN represents a comparatively stable system with protective effects against serious drug toxicity and harsh external environment in comparison to the conventional nanoparticles. In addition, they also offer the advantages of avoidance of organic solvents in their preparation, controlled release of drugs and excellent tolerability. Of the available methods for preparation, cold high-pressure homogenisation process, hot homogenization of melted lipids at elevated temperatures and microemulsion technology are considered as the most feasible methods for large scale production of SLNs. SLN with highly ordered crystalline is capable to encapsulate both hydrophobic and hydrophilic active ingredients. Although solid lipid nanoparticles (SLNs) have attracted increasing attention due to its advantages, SLNs have several limitations, for example, low loading efficiency for some drugs which owing to the densely packed lipid crystal network. Furthermore, SLNs also show considerable expulsion of the drug during storage.

2.4 Lipid nanocapsules (LNCs):[18]
Lipid nanocapsules (LNCs) provide a new nanotechnology which contributes to oral drug delivery development. LNCs are another kind of lipid nanoparticles, composed of an internal liquid or semi-liquid oil core and an external lipid layer solid as a core-shell structure. LNCs with the unique properties such as controlled release profiles and high bioavailability, represent a promising biocompatible drug delivery platform in nanometer range with narrow size distribution. The phase inversion temperature (PIT) method proposed by Shinoda and Saito led to lipid nanocapsules preparation with good mono-dispersion. LNCs prepared by PIT method is based on three main components: an oil phase, an aqueous phase and a non-ionic surfactant. Furthermore, the temperature cycling process crossing the phase-inversion zone (PIZ) plays another role on LNCs formulation. Increasing the number of cycles promotes LNC formation and improves the quality of LNC dispersion. Recently, many lipophilic drugs have been developed in LNCs form for instance, ibuprofen loaded LNCs for pain treatment; indinavir, an inhibitor of HIV1 protease; various hydrophobic anticancer agents. Consequently, LNCs provide an attractive drug delivery approach for highly lipophilicity drug substances that are usually unsuitable for oral use.

2.5 Nanosuspensions: [19,20]
Nanosuspensions are nanoscale colloidal dispersion of solid drug particles which are stabilized by surfactants, polymers or a combination of both. The key difference from conventional suspensions is that the particle size distribution of the solid particles in nanosuspensions is usually < 1 µm. Nanosuspensions engineering processes presently used are media milling, high pressure homogenization, microprecipitation-high pressure homogenization, emulsion diffusion method and melt emulsification method. Owing to the enhanced drug solubility, increased surface-volume ratio of the nanocrystals, and improved dissolution rate, oral nanosuspensions have been specifically used. Furthermore, nanosuspensions are available in various dosage formats such as tablets, pellets, and capsules following different manufacturing techniques. Nevertheless, the major challenges in nanosuspensions preparation are maintaining colloidal stability and particle size of the nanosuspensions during storage. The appropriate selection of the surfactants and/or steric stabilizers and the method of fabrication have been sought to prevent the nanocrystal aggregation to achieve the nanosuspensions with long-term storage and physiological stability.

2.6 Liposomes: [14,21,22]
Liposomes are a form of self-assembled lipid bilayer vesicles which composed of one or more aqueous compartments are completely enclosed by hydrophilic and/or hydrophobic molecules. Due to the core (aqueous)-shell (lipidic) structure, liposomes are available for encapsulating hydrophilic drugs in the aqueous core, hydrophobic agents in the lipidic shell, meanwhile, amphiphilic molecules distributed through the hydrophobic-hydrophilic layers. In addition, using biologically and natural lipids makes liposomes highly biocompatible and suitable for in vivo use. Recently, research on liposomes technology has been extensively investigated for the delivery of various therapeutic and bioactive agents, decreasing toxicity and increasing their accumulation at target sites. Nitesh Kumar et al developed lecithin-based silymarin liposomes. The results showed that incorporating phytosomal form of silymarin in liposomes had better in vitro and in vivo hepatoprotection and better anti-inflammatory effects in histopathological changes. Therefore,

liposomes can be used in the oral delivery of lipophilic drugs to increase its oral bioavailability.

2.7 Liquid crystalline nanoparticles (LCNPs): [23]

Liquid crystalline nanoparticles (LCNPs), which combine the properties of both liquid and solid states, are self-assembled from polar amphiphilic lipids in the presence of excess water. LCNPs are generally prepared by dispersing the liquid crystalline matrix formed into water phase using high-energy fragmentation, such as ultrasonication, microfluidization, or homogenization. Normally, LCNPs enhance the oral bioavailability of lipophilic drug by improvement of bioadhesiveness, membrane fusing properties, superior encapsulation, solubilization, etc.

2.8 Self-nanoemulsifying drug delivery system (SNEDDS): [14,20,24]

Self-nanoemulsifying drug delivery systems (SNEDDS) are isotropic mixtures of oil, surfactant, co-surfactant and drug that rapidly form fine oil-in-water (o/w) nanoemulsions when introduced into aqueous medium under mild agitation. In the human body, the agitation required for formation of nanoemulsions is provided by digestive motility of the gastrointestinal tract. In comparison with the ready to use nanoemulsions or nanosuspensions, SNEDDS have shown many advantages such as: physical or chemical stability profile improvement in long term storage; possibility of filling into soft/hard gelatin capsules, which results in attractive commercial viability and patient acceptability; no palatability-related issues. In recent years, SNEDDS have attracted more and more attention as the mean to enhance the oral bioavailability of poorly soluble and highly metabolized drugs. Nevertheless, conventional SNEDDS also require a relatively large amount of surfactants, which may induce GI irritation and side-effects. In order to achieve a safe and efficient delivery system for the poor oral bioavailability drugs, we have designed a novel self-nanoemulsifying drug delivery system with high proportion lemon essential oil as carrier for lipophilic drugs.

Examples of different types of nanocarriers: [26-36]

Table 1. Examples of different types of nanocarriers

Sr No	Active Ingredient	Biological activity	Type of nanosystem	Application
1	Artemisia arborescens	Antiviral activity	Herbal liposome	Increase in activity and stability
2	Silybin	Antihepatotoxic activity	Nanoparticulate formulation	Sustained release and targeted drug delivery
3	Berberine	Antineoplastic activity	SNEDDS	Sustained release
4	Curcumin	Antitumor, antioxidant and antioxidant activities	SLN	Increase in stability
5	Gliclazide	Antidiabetic activity	SEDDS	Improved systemic availability
6	Cilostazol	Antiplatelet activity	Nanosuspension	Increase in bioavailability
7	Ketorolac Tromethamine	NSAIDs activity	Lipid nanocapsules	Enhanced and prolonged the anti-inflammatory effects
8	Ketoprofen	NSAIDs, analgesic and antipyretic activities	Nanoemulsions	Good permeation when applied in skin
9	Cyclosporin A	Antiinflammatory activity	Inhalable dry emulsion	Enhanced activity in lungs
10	Lidocaine	Anesthetic activity	Solid lipid nanoparticle	Controlled dermal permeation and duration of action
11	Diclofenac sodium	NSAID	Solid-in-oil nanosuspension	Increased percutaneous absorption

3. REASONS TO PREFER NANO CARRIERS [8, 37]

Drug delivery with nanocarriers can be used to improve pharmacokinetics, obtain proper targeting and reduce toxicity of therapeutic agents. Solubility of hydrophobic materials and drugs can be increased by using nanocarriers which can also enhance the stability of drugs. NPs enable the therapeutic agent targeting and delivering to the brain. Drug carriers

which are created by nanotechnology offer the opportunity to penetrate or overcome some

biological barriers such as blood–brain barrier and tight epithelial junctions. These systems can be used to provide targeted (cellular or tissue) delivery of drugs, improve bioavailability, sustain release of drugs or solubilize drugs for systemic delivery.

The major reasons to go for the nanosystems include:

1. Protection of drugs.
2. Prolonged drug effect.
3. Increase in an amount of drug at target site.
4. Reduced toxicity.
5. Amenable to sterilization.

3.1 Protection of drugs:
Poorly soluble drugs can be encapsulated, and therapeutic molecules can be protected by using nanotechnology. Nanostructures can protect drugs from hydrolytic and enzymatic degradation (i.e., nucleases and proteases). They even prevent drugs from hepatic first-pass metabolism and increase the blood residence time.

3.2 Prolonged drug effect:
They can be tailor-made to achieve both controlled drug release and disease-specific localization by tuning the polymer characteristics and surface chemistry. It has been established that nanocarriers can become concentrated preferentially to tumours, inflammatory sites, and at antigen sampling sites by virtue of the elevated permeability and retention (EPR) effect of the vasculature. Once accumulated at the target site, hydrophobic biodegradable polymeric nanoparticles can act as a local drug depot depending on the dimensions and structure of the carrier, providing a source for a continuous supply of encapsulated therapeutic compound(s) at the disease site, e.g., solid tumours.

3.3 Increase in amount of drug at target site:
The nanosize of these particles allows for efficient uptake by a variety of cell types and selective drug accumulation at target sites.The use of biodegradable materials for nanoparticle formulation allows for sustained drug release within the target site over a period of days or even weeks. Biodegradable nanoparticles formulated from PLGA and PLA have been developed for sustained drug delivery and are especially effective for drugs with an intracellular target.

3.4 Reduced toxicity:
Nanosystems can be used for reducing toxicity. There are a few anticancer drugs which contain platinum, but there are some disadvantages of platinum such as nephrotoxicity and neurotoxicity. Also, they caused developing drug resistance limiting their uses. But nanocarrier-based delivery of platinum complexes gives the opportunity to reduce non-target toxicity. Also, in some cases nanocarriers prevent to develop drug resistance against platinum. Moreover, these drug delivery systems can be used for multidrug resistant cancer treatment. Paclitaxel loaded NPs were eight times more efficient than Taxol plus XR958.

3.5 Amenable to sterilization:
Several techniques are available for the removal of microbial contamination from nanoparticles developed for use in nanomedicine applications. These techniques include filtration, autoclaving and irradiation, as well as formaldehyde, ethylene oxide and gas plasma treatments. Of all these sterilization techniques, filtration may potentially remove microbial contamination without altering the physicochemical properties of the carrier nanoparticles, nor affecting their toxicity and functionality. However, no single process may be applied to all nanoparticle preparations and, therefore, it is recommended that each nanoparticle-drug system should be validated on a case-by-case basis. Sterile filtration is a commonly used technique for the physical removal of microorganisms from chemically and thermally sensitive liquids, through the use of 0.22 μm membrane filters. This technique has been shown to be widely applicable as it doesnot appear to have any adverse effects on the nanoparticles. Poly(ε-caprolactone) (PEC) nanospheres of mean diameter size below 200 nm were successfully sterilized by filtration with 0.2 μm cellulose acetate membrane filters without alteration of their size, morphology or concentration. Filter sterilization through 0.22 μm filters has also been successfully implemented for the sterilization of PEGylated poly (γ-benzyl-L-glutamate) (PBLG) NPs and polyester NPs.

4. APPROACHES FOR PREPARATION OF NANOCARRIERS: [38-41]
Top-Down approach:
Begins with a pattern generated on larger scale and reduced to nano scale i.e. breaking down the bulk material into smaller dimension. The various methods included are milling, attrition, lithography, etc. These methods are very slow and costly to manufacture. They are not appropriate for large scale production. The biggest problems are the imperfection of surface structure, significant crystallographic damage to processed patterns and contaminations.

Bottom-up approach:
It starts with atoms or molecules and build up to nanostructures by consolidation. After miniaturization there is further self-assembly

process leading to formation of nanostructures. Fabrication is much less expensive. It is more favourable method. The various methods included are sol-gel processing, chemical vapour deposition, and laser pyrolysis, atomic or molecular condensation. Advantages of this method are fewer defects, homogenous chemical composition, better short- and long-range ordering.

5. LIPOSOMES AS DRUG CARRIERS

Liposomal carriers can be applied by invasive (e.g. i.m., s.c., i.d.) as well as non-invasive (transdermal and mucosal) routes. Depending on the method used to prepare the liposomes, final liposomal product can vary in size, number of lamellae (unilamellar, oligo- or multilamellar vesicles) and have different physical and chemical characteristics that may greatly affect the amount of the encapsulated substance.

For the pharmaceutical field, liposomes as a carrier are very attractive for the following reasons:

1.Easy encapsulation of hydrophilic and hydrophobic drugs
2.Preparation of drugs from natural lipids, which are readily biodegradable and of low toxicity
3.Preparation of liposomes of varying size and morphology
4.Reduction in adverse effects of drugs
5.Specific drug delivery to the target organ or tissue
6.Targeting of drug and gradual drug release

5.1. Methods for preparation and characterisation of liposomes

Various techniques have been developed during the last fifty years for preparation of liposomes and other lipid based particles. Selection of appropriate method is dictated by factors such as administration route (e.g., i.v. injection, transdermal/mucosal administration), lipid composition (saturated versus unsaturated lipids), stability of substances which are to be encapsulated (e.g. proteins, DNA, viruses, low molecular drugs), conditions for preparation (reconstitution of membrane proteins), morphology and size of final liposomal preparation, postforming modification of liposomes (binding of various ligands – e.g. antibody, saccharide ligands, protective polymers – PEG) and scaling-up ability of the method (application of technology for industrial production) etc. The size, morphology, lipid composition and surface modification are the most important parameters affecting interaction of liposomes with cells, their biodistribution and stability in the body, stability during technological processes (e.g. sterilisation by microfiltration, γ-irradiation or thermal sterilisation; fluid drying or lyophilisation), stability during transportation and storage in dry or liquid formulation. [42-45]

5.2. The size and morphology of liposomes

The most commonly used parameter for the distribution of liposomes is classification according to their size and number of lamellae. Liposomes can have different sizes ranging from 20 nm to 100 nm with the lipid bilayer approximately 4 nm thick. They can be divided into small (SUVs; 10-100 nm) and large (LUVs; 100-1000 nm) unilamellar vesicles, which are of the order of only a few units of bilayers, and the large multilamellar vesicles MLV (100 nm - 20 nm) containing dozens concentrically arranged bilayers as multivesicular MVV vesicles (100 nm - 20 μm) which are arranged as nonconcentric lamellae.

5.3. Classification of liposomes according to the function and nature of lipid membranes

Liposomes can be classified according to the structure of the membrane and the surface modification into several basic types that have different uses in the design and preparation of systems for targeting various molecules to cells. [46]

1. Conventional liposomes - non-specific interactions with the environment, instability in serum
2. Sterically hindered liposomes (Stealth liposomes) - long-term circulation in the bloodstream
3. Targeted liposomes – targeting is mediated via specific interaction with surface bound ligand structure on cell membranes (ligand-receptor interaction, antibody - epitope)
4. Cationic liposomes - the ability to interact with negatively charged DNA/RNA and induction of its condensation; they electrostatically interact with the negatively charged DNA to form complexes (lipoplexes), protect the DNA from degradation by nucleases, promotes entering into the cells via endocytosis or phagocytosis.

5.4 Methods of liposome preparation

Liposome preparation methods are based on the natural properties of phospholipids to form a lipid bilayer in an aqueous medium spontaneously. During interaction of water with the phospholipid film, phospholipid bilayer fragments are formed first. These intermediates are then converted into various stable vesicular structures. Membrane fragments formed in the process of hydration are the consequence of minimization of the interaction between water molecules and the hydrophobic parts of the phospholipids. The distance between the individual lamellae formed in multilamellar vesicles is a result of steric repulsive and attractive van der

Waals forces. General procedures for preparing liposomes include the preparation of lipid and aqueous phases, followed by hydration of the lipids in an aqueous medium, which finally leads to the desired final formation of liposomes[47]

Classification of liposome preparation methods:

1. Mechanical dispersion method (vortexing, sonication, high pressure homogenization)
2. Detergent dispersion method (dispersion of phosphatidylcholine solubilized with detergent to form micelles)
3. Two-phase dispersion methods (ethanol injection and ether injection, reverse-phase evaporation, and the recently introduced method of nanofluidic mixing)

Basic stages of liposome preparation:

1. Biphasic physical dispersion of lipids in the aqueous phase to form an emulsion of proliposomes and solubilization of lipids in a detergent to form micelles
2. Removal of the organic solvent or detergent to form liposome vesicles
3. Secondary processes to alter the morphology, size and stability of the primarily formed liposomes (extrusion through a membrane filter, the process of freezing and thawing, lyophilisation with cryoprotectants)
4. Chemical modification of liposomes (ligand binding)

5.4.1. Detergent removal method

Another primary method for preparation of liposomes is based on transformation of mixed lipid-detergent micelles into liposomes during the process of detergent removal. This can be achieved for example by dialysis using various devices (flow dialyzer ultrafiltration), removal of detergent by sorbents, or simple dilution of the mixed micelle solution). Liposomes prepared by this method are unilamellar and extremely homogeneous in size distribution. The preparation is particularly suitable for reconstitution of membrane proteins. The principle of the method consists in converting small mixed micelles formed by phospholipids using a suitable detergent (e.g. octylglucoside, deoxycholate) into disc shaped micelles, their coalescence and finally disc micelles of critical size vesiculate to form liposomes. This process is driven by reducing the concentration of detergent removed by dialysis.

5.4.2 Proliposome-liposome method

The proliposome-liposome method is based on proliposomal preparation of hydrated stacked bilayer sheets in a water-ethanol solution. Generally, the organisation of a lipid/ethanol/water mixture can be described in terms of a three-phase diagram which can be divided into the following principal areas: lipid dissolved in aqueous ethanol, hydrated bilayers suspended in aqueous ethanol, and a liposomal area. Spontaneous formation of liposomal suspensions is accomplished by addition of excess aqueous phase to a lipid mixture. This technique is simple and practical and is characterized by an extremely high entrapment efficiency, when compared with other methods based on passive entrapment. The technique is suitable for encapsulation of a wide range of drugs with various water and alcohol solubilities.

6.SLN AS DRUG CARRIERS: [9,48,14]

Lipid nanoparticles (LNPs) are intensively studied drug delivery systems derived from o/w emulsions, they combine the advantages of polymeric nanoparticles, conventional emulsions and liposomes, while simultaneously avoiding their disadvantages. They are capable to improve the insufficient physicochemical properties of biopharmaceutical classification system (BCS) II (low water solubility, high permeability) class pharmaceutical actives, such as nonsteroidal anti-inflammatory drugs (NSAIDs), enhancing their bioavailability.

Nanostructured lipid carriers (NLCs) appeared as new drug delivery system in late 1990's. NLCs are second generation Solid Lipid Nanoparticles (SLNs). SLN are novel lipid-based formulations which constitutes of biodegradable lipids such as highly purified triglycerides, monoglycerides (MGs), hard fats, complex glyceride mixtures or even waxes, which are solid at physiological temperature. SLN consists of pure solid lipid while NLCs are made of solid lipids that entrap incompatible liquid lipid in a Nano compartment within nanoparticle.

6.1. NLCs are preffered over other lipid formulations because: [8,49]

- Higher drug loading capacity.
- Less drug leakage during storage.
- Improved bioavailability.
- Physical stability.
- Modulation of drug release profile (prolonged release and targeting).

Due to the lipophilic nature of their matrices, NLCs are considered predominantly useful for administering lipophilic compounds. NLCs are highly used in specialized areas like brain targeting, tumour targeting and gene delivery. The composition of nanoparticle shells significantly affects the in vitro release of medicine and its

performance in blood. The presence of liquid lipid of dissimilar fatty acid composition than solid lipid increases imperfections in the lipid matrix crystals and thereby increases ability to encapsulate drug molecules. Liquid oils usually used for NLCs comprises digestible oils from natural sources. Generally used solid lipids to produce NLCs include tripalmitin, glycerylbehenate (Compritol), glyceryldistearate (Precirol) and cetylpalmitate.

6.2. FEATURES OF NLCs:

They were developed to overcome the short comings of SLNs. The main purpose was to avoid lipid recrystallization which causes expulsion of enclosed active substances, as detected in SLNs. Lipid transforms to more perfect β modifications which leads to increase in perfection of crystals creating less space to accommodate drug molecules leading to drug expulsion. NLCs are solid but not crystalline so no drug expulsion due to crystallization. They acquired significance in drug delivery owing to their ability to accommodate a surplus quantity of drugs and their greater stability as compared to SLNs. They can load hydrophilic as well as hydrophobic drugs. The NLCs are biodegradable and can also be surface modified for better targeting. Their cost effectiveness and easy administration for drugs that cannot be formulated as aqueous solutions can be also counted as their advantage.

6.3. Structural models of NLCs: [8,49,50]

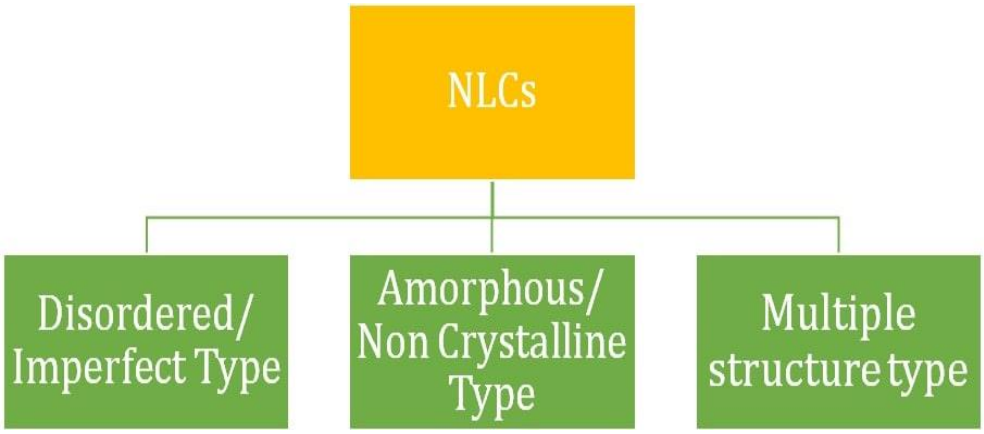

Fig 2: Types of NLCs

Table 2. Different structural models of NLC

Sr no	Type	Details
1	Disordered/ imperfect type	Involves mixed solid and liquid lipids that conform to disordered state to matrix. Appears between crystal and liquid lipids. Increases drug penetration through lipid bilayer.
2	Amorphous/ non crystalline type	Addition of mixture of varying lipid prevent crystal formation. Matrix is structure less and amorphous. Prevent leakage of loaded drugs.
3	Multiple structure type	High liquid lipid concentration Structurally similar to oil/fat/water micro emulsion. Solid matrix of lipid nanoparticle containing tiny liquid Nano compartments of oil in which drugs are more soluble so they have more drug loading capacity. These Nano compartments are surrounded by solid lipid matrix which helps to give prolonged drug release. More drug solubility, slow drug release, high drug loading. Avoid loss due to decomposition of solid lipids.

6.2 Composition of NLCs: [27, 51-53]
The general components include solid lipid(s), emulsifier(s) and water. Several emulsifiers with different charge and molecular weights (e.g. Soybean lecithin, polysorbates, PVA) have been used for the emulsification and stabilization of lipid dispersions. Occasionally, the surface properties of SLNs are altered by surface modification or coating with polymers in order to avoid phagocytic uptake by macrophages and/or to improve the pharmacokinetics of these colloidal carriers.

In the SLNs, there is a high interfacial tension at the surface of the lipid particles that are dispersed in the aqueous medium. Owing to the small particle size of the dispersed lipid and the resulting enormous surface area, there is increased free energy of the system which cause SLNs to be thermodynamically unstable. In order to decrease the surface free energy, these colloidal particles have a tendency of flocculation, aggregation and crystal growth. Stabilizers are, therefore, used to reduce the free surface energy of the particles by decreasing the interfacial tension and prevent particle aggregation by electronic repulsion or steric stabilization.[46] A variety of surface active agents such as bile salts, phospholipids, poloxamers, polysorbates etc. have been used for stabilization of SLNs. [47]

Table 3. Composition of NLC

Ingredients	Examples	Role in formulation
Solid-lipids	Tristearin, stearic acid, cetylpalmitate, cholesterol, Precirol® ATO 5, Compritol® 888 ATO, Dynasan®116, Dynasan® 118, Softisan® 154, Cutina® CP, Imwitor® 900 P, Geleol®, Gelot® 65, Emulcire® 62	Controlled release of drugs.
Liquid-lipids	Medium chain triglycerides, paraffin oil, 2-octyldodecanol, oleic acid, squalene, isopropyl myristate, tocopherol, Miglyol® 812, Transcutol®HP, LabrafilLipofile®WL1349, Labrafac® PG, Lauroglycol®FCC,Capryol® 90	Solubilizes for lipophilic drugs and penetration enhancers.
Hydrophilic emulsifying agents	Pluronic® F69 (poloxamer 188), Pluronic® F127 (poloxamer 407), Poly sorbates like Tween 20, Tween 40, Tween 80, polyvinyl alcohol, Solutol® HS15, trehalose, sodium deoxycholate, sodium glycocholate, polyglycerol methyl glucose Distearate	Stabilize lipid dispersions.
Lipophilic Emulsifiers	Myverol® 18-04K, Span 20, Span 40, Span 61	For fabrication of NLCs (optional).
Amphiphilic emulsifiers	Egg lecithin, soya lecithin, phosphatidylcholine, Gelucire® 50/13, phosphatidylethanolamines	For fabrication of NLCs(optional).

6.3. Methods of preparation of NLCs: [8, 27, 51-59]

Table 4. Different methods to prepare SLN and NLC

Sr No	Name of method	Details	Advantages	Disadvantages
1	High pressure homogenization (High temperature, high pressure homogenization / Low temperature, high pressure homogenization)	In this method, the drug is dissolved or dispersed in a lipid melt prepared by melting solid-lipids and mixing them with liquid-lipids. This lipid melt is added to aqueous phase containing surfactants and stirred at high pressure till emulsion begins to form. Then the pre-emulsion is homogenized at temperatures above melting point of lipids to yield nanoparticles.	Avoidance of organic solvents Can be used for large scale production This method is suitable for insoluble and lipophilic drugs but not suitable for hydrophilic drugs.	At high temperatures, there is reduction of viscosity; probability of degradation of drug and carrier The size of Nano particles depends on the pressure and time.
2	Ultrasonic emulsion evaporation method	In this method solid lipids are melted and mixed with liquid lipids along with drug to form organic phase. This organic phase is added to aqueous phase containing required amount of surfactants and dispersed by means of probe sonication. The emulsion is cooled down and solvent is evaporated to form NLCs. After formation of stable emulsion, excess oil phase is evaporated by two ways: By heating under reduced pressure or by evaporation while stirring continuously	Avoidance of heat during precipitation.	Toxicology due to presence of solvent residues
3	Solvent dispersion method	In this method, solid lipids, liquid lipids and drug are dissolved in solvents like ethanol, acetone, isopropanol which are water miscible to form organic phase which is slowly added to water containing emulsifiers. The NLCs are obtained by centrifugation.	Speed, Simplicity, Less requirement of sophisticated instruments	Not entirely for industrial production. Presence of residual solvent.
4	Film-ultrasonic method	The solid lipids, liquid lipids and drugs are dissolved in volatile organic solvent which can be easily removed by vacuum evaporation in later stages. To this organic solution, surfactants are added in aqueous solution to form film of mixed lipids. Ultrasound probe or ultrasonic dispersion method is used to obtain small and uniform NLCs.	Simplicity, Practicality, Yield of small and uniform particles	Toxicology due to presence of solvent residues

5	High temperature emulsion evaporation-low temperature curing	Organic phase and aqueous phase are heated till they attain same temperature, then organic phase is added to aqueous phases so that an emulsion is formed. Then the resulting liquid is heated till organic solvent is evaporated. The remaining liquid is cooled and dispersed in ice-cold water of temperature 0°C-4°C and desired NLCs are formed as dispersion solution.	Simplicity, Speed.	Not entirely suitable for industry. Presence of residual solvents.
6	Micro emulsion method	Lipid carrier is heated till it melts, then drug, emulsion and auxiliary emulsion along with deionized water are added to it resulting in transparent oil-in-water micro emulsion which is then quickly dispersed in ice-water to form NLCs in dispersion system. Key factors: Size of nano particles, particles from microemulsion, dilution are extremely close to temperature difference between cold water and micro emulsion. Rapid cooling + solidification→ Prevent aggregation	Low drug content. Simple method.	Abundance of emulsifiers
7	Melt emulsification method	Solid lipid and liquid lipid are melted and mixed with the drug. This is then added to aqueous phase containing surfactant which is stirred to form coarse emulsion which once homogenized under high pressure form NLCs.	No organic solvent residues. No initial time burst release. Dispersions with high lipid concentrations.	Not entirely suitable for industrial production. Residual organic solvent is sometimes present.

6.4. Method of drug release from sln and nlc: [50, 60]

The general principles of drug release from lipid nanoparticles are as follows:

1. There is an inverse relationship between drug release and the partition coefficient of the drug.
2. Higher surface area due to smaller particle size in nanometer range gives higher drug release.
3. Slow drug release can be achieved when the drug is homogenously dispersed in the lipid matrix. It depends on type and drug entrapment model of SLN.
4. Crystallinization behaviour of the lipid carrier and high mobility of the drug lead to fast drug release.

There is an inverse relationship between crystallization degree and mobility of drug. The drug incorporation model of SLN is crucial to the drug release pattern. It is related to the composition and production method of SLN. For example in the case of production by cold homogenization technique the drug-loaded lipid phase remains mainly in the solid state. The model for incorporating drugs with a solid solution appears here. Drug release is delayed over several weeks, as the drug molecularly distributed in colloidal particles is very limited in mobility. Quick initial drug release (burst effect) occurs in the first 5 minutes of the drug-enriched shell model (i.e. around 100 percent within < 5 min) as a result of the particle outer layer due to the large drug deposit surface area on the particle surface. With increasing particle size, burst release is reduced and sustained release could be obtained when the particles were large enough, i.e. lipid microparticles The type of surfactant and its concentration, which interacts with the outer shell and affects its structure, should be noted as the other important factor, since a low concentration of surfactants results in a minimal burst and prolonged release of the drug

The drug release is regulated by membrane in the drug-enriched core model and is governed by the Fick law diffusion, since the lipid surrounds the drug as a membrane.

The oil content of the particles solves the drug in the case of NLC – the new generation SLN – combining controlled release characteristics with high drug load capacity. The imperfect type and amorphous type of NLC, in particular, provide much more flexibility to achieve the desired prolonged release. The particle size that directly affects drug release levels depends on various parameters such as SLN formulation composition (such as surfactant / surfactant mixture, amount of drug incorporated, lipid and drug structural properties), production methods and conditions (such as time, production temperature, equipment, sterilization, and lyophilization).All of these parameters have been investigated extensively and data have been reported for years in the literature. Additionally, surface modifiers can change the particle size to reduce the phagocytic uptake such as polyethylene oxide and PEG.The effect of surface modifiers on particle size and on drug release rate is discussed in upcoming sections.

6. METHODS OF CHARACTERIZATION OF NANOCARRIERS: [59, 61-64]

7.

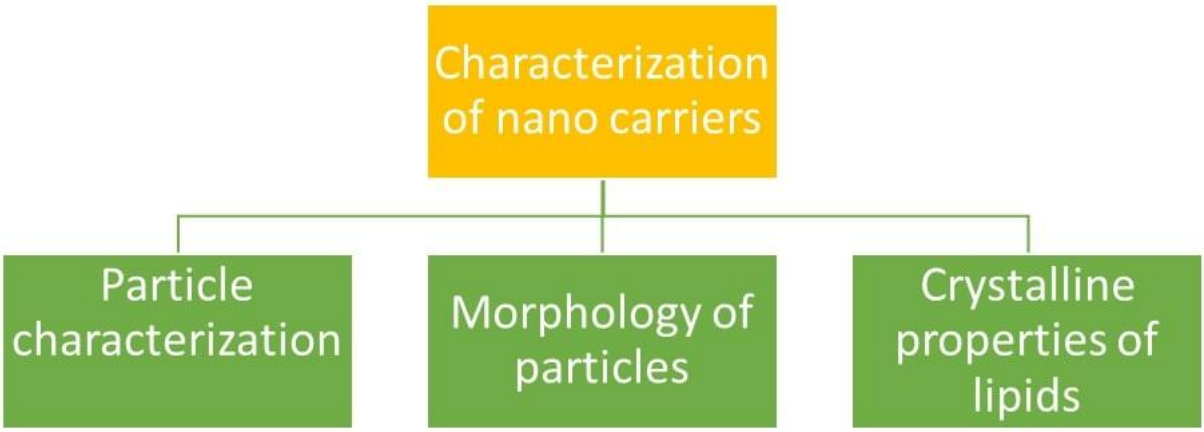

Fig 7.1: Methods for analysis of nanoparticles

7.1. Particle characterization:

7.1.1 Zeta potential: The zeta potential values were analysed by determining the particle electrophoretic velocity using a Zeta-sizer to study stability of colloidal particles based on repulsion forces. The zeta potential is an important parameter that allows predictions on the physical stability of colloidal dispersions. In theory, higher values of zeta potential, either positive or negative, tend to stabilize the suspension. Usually, aggregation phenomena are less likely to occur for charged particles with pronounced zeta potential (>|20|), due to the electrostatic repulsion between particles with the same electrical charge. In this study, the zeta potential mean value registered for all formulations was around ±30 mV, which predicts a good long-term stability.

7.1.2. Surface charge: The measurement of zeta potential allows prediction about the storage stability of colloidal dispersions. At higher zeta potential, particle aggregation is likely to occur, due to electrical repulsion.

7.1.3. Particle size: This can be determined by zetasizer. This property dictates the physical stability of NLCs. The particle size and particle size distribution (PSD) of these materials are of great importance to the end user because they influence key colloid properties such as rheology, film gloss, surface area and packing density. Moreover, to prevent the aggregation of fine particles into much larger, undesirable units, steps must be taken to prevent particles from sticking together (aggregating) due to interparticle collisions in the liquid medium. This can be accomplished by creating an interparticle electrical and/or steric energy barrier. For very fine particles, a combination of both electrical and steric barriers may be necessary to prevent aggregation.

7.1.4. Polydispersity index: It is used as an indicator of uniformity of size. The lipid carriers are homogenous in size when polydispersity index is below 0.30. For monomodal dispersion, we would theoretically expect a perfectly exponential decay of the autocorrelation function of the scattered light intensity. PDI is derived from an extra term in the fit

of an exponential, and describes how broad the range of sizes is. Mathematically, PDI could be any positive number, but generally is only reported up to a value of 1. Values around 0.1 may be considered good for a monomodal dispersion. Higher values mean that the distribution is broad, or that the particle size distribution is multimodal, in which case the average size value reported alongside PDI becomes less relevant, and a distribution analysis is more representative.

7.2. Morphology of particles:

7.2.1. AFM: Using the Atomic Force Microscope (AFM), individual particles and groups of particles can be resolved and unlike other microscopy techniques, the AFM offers visualization and analysis in three dimensions. Nanoparticles were applied on different kinds of substrates, in varied concentrations and were imaged by AFM in dynamic mode. The resultant images were found to be influenced by the kind of substrate used, the nature of the surface of the nanoparticle and also the dilution of the nanoparticle suspension. The nature of the substrate plays a very important role of influencing the number of the nanoparticles and the distribution of the particles that are deposited onto its surface.

7.2.2. TEM: Specifically, both the AC-TEM and AC-STEM techniques can be employed to image materials comprised of single atoms to small size NPs. Though a suitable electron beam conditions should be adapted for the imaging of materials having NPs of size 5 nm or close. Used to study the internal structure of nanostructures.

7.2.3. SEM: The SEM functions by bombarding a conductive particle surface with an electron beam from a Tungsten filament that under a KV potential range between 1530KV. As a result, an inelastic scatter of the secondary electron from the surface of the particle occurs and can be determined by a special SEM detector. It is used to study the surface topology of sample.

7.3. Crystalline properties of lipids:

7.3.1 DSC: Basically, DSC measures heat flow as a function of temperature applied to a sample going through freezing, melting, crystallization, and glass transition. The thermal properties that can be measured by using DSC include eutectic crystallization temperature (Tx), eutectic melting temperature (Te), glass transition temperature (**T0g**) and ice melting temperature (Tim). Among these critical temperatures, the glass transition temperature T0g, is one of the most important thermo physical properties of the formulation.

7.3.2 DTA and ERA: In addition to DSC, other instruments, such as differential thermal analysis (DTA) and electrical resistance analysis (ERA), are also commonly used in determining the thermo physical properties

7.4. Other methods for characterization:

7.4.1. Measurement of entrapment efficiency and drug content: Determines how much of the drug is encapsulated.

$$\text{Load content (\%)} = \frac{Ws \times 100\%}{W \text{ lipid}}$$

$$\text{Entrapment efficiency (\%)} = \frac{Ws \times 100\%}{W \text{ total}}$$

Where Ws represents the amount of drug in the NLC, Wlipid is the weight of the vehicle, and Wtotal represents the amount of drug used in the formulation.

7.4.2. Percentage yield: the percentage yield can be determined by dividing the weight of recovered nanoparticles with the weight of drug and lipids used for preparation of nanoparticles.

$$\text{Percentage Yield} = \frac{\text{Weight of recovered nanoparticles} \times 100}{\text{Theoretical weight (drug + lipids)}}$$

7.4.3. In vitro drug release studies: These are performed by dialysis method or bioassays. These methods help to study release profile and drug loading capacity of the carriers. Flow-through Franz diffusion cells, cell culture characterization are some of the methods used for in vitro studies.

8. ADVANTAGES OF NANO CARRIERS: [18, 49, 51, 53]

The advantages of using nanoparticles for drug delivery are a result of two main basic advantages: small (nano) size and use of biodegradable materials.

1. Better physical stability
2. Ease of preparation and scale-up
3. Increased dispersability in an aqueous medium
4. High entrapment of lipophilic drugs and hydrophilic drugs
5. Controlled particle size
6. An advanced and efficient carrier system,
7. Increase of skin occlusion,
8. Extended release of the drug,

One of the carriers of choice for topically applied drugs because their lipid components have an approved status or are excipients used in commercially available

topical cosmetic or pharmaceutical preparations,

9. Small size of the lipid particles ensures close contact to the stratum corneum thus increasing drug penetration into the mucosa or topically,
10. Improve benefit/risk ratio,
11. Increase of skin hydration and elasticity and
12. These carriers are highly efficient systems due to their solid lipid matrices, which are even generally recognized as safe or have a regulatory accepted status.

9. LIMITATION OF NANO CARRIERS: [64-67]

1. Cytotoxicity related to the nature of matrix and concentration,
2. Irritative and sensitising action of a few surfactants,
3. Application and efficiency in case of protein and peptide drugs and gene delivery systems still need to be better explored,
4. Lack of sufficient preclinical and clinical studies with these nanoparticle systems in case of bone repair.

10. MARKETED PREPARATIONS: [68-73]

Table 5: Marketed preparations containing nanocarriers

Sr no	Product name	Manufacturer	Indication
1	Duopafei®	Qilu, China	Tumour targeting
2	Xylocain® gel	AstraZeneca Pty Ltd	Local anaesthetic
3	Nano Repair Q10®, Nanovital Q10®, Cutanova®	Dr. Rimpler GmbH	Restructuring Anti-aging serum
4	Garnier Micellar Cleansing Water	Garnier	Skin care
5	nanoXIM• Care Paste	FLUIDINOVA	Dental care
6	Restasis®	Allergan	Ophthalmic Emulsion

11. PATENTS: [74-78]

Table 6. Patents on Nanocarriers

Sr no	Patent Number	Title	Information	Inventor
1	US20080206341A1	Nucleic acids incorporated in solid lipid nanoparticles (SLN)	Process of preparation of solid lipid nanoparticles (SLN) containing nucleic acids, particularly polynucleotides and oligonucleotides	Maria Rosa Gasco
2	WO2014115137A1	Skin anti-aging cosmetic composition	Anti-aging cosmetic skin composition consisting of niacinamide, coenzyme Q10 or its equivalent, caprylic / capric triglyceride and geranylgeranylpropanol, lepidium sativum sprout extract and gold.	Erez Zabari
3	US20080020089 A1	Lipid nanoparticle based	Relates to novel particle forming delivery agents	Chen T et al

		compositions and methods for delivery of biologically active drugs.	including cationic lipids, microparticles, and nanoparticles that are useful for delivering various molecules to cells also features compositions, and methods of use for the study, diagnosis, and treatment of traits, diseases and conditions that respond to the modulation of gene expression and/or activity in a subject or organism.	
4	EP2344134B1	Nanocarriers for drug delivery	novel nanocarriers, comprising of PEG and oligo-cholic acids, can self-assemble under aqueous conditions to form core-shell (cholane-PEG) structures that can carry PTX in the hydrophobic interior.	University of California
5	US8057823B2	Lipid nanocapsules, preparation process and use as medicine	Deals with a process for preparing them and to their use for manufacturing a medicament intended especially to be administered by injection, orally or nasally	Universited Angers Ethypharm

12. CONCLUSION:

The use of nanocarriers for the topical treatment of skin diseases is very attractive, since epidermal lipids are found in high amounts within the penetration barrier thus decreasing their systemic absorption by localization in skin layers. Besides liposomes, solid lipid nanoparticles and nanostructured lipid carriers showed potentials in dermal targeting as well as cosmetic products by overcoming the stability problems of liposomes. Moreover, transdermal drug application has gained increasing importance for systemic treatment, e.g. for medications subject to significant first-pass removal such as glyceryl trinitrate or estrogens, and for the continuous suppression of chronic pain. Extensive research has been conducted on the use of nanoemulsions, microemulsions, liposomes and most importantly lipid nanoparticles for the transdermal delivery of various drug groups. Specific needs, considerations, approaches, and limitations in nanocarrier drug delivery were also discussed on the basis of the method of preparation. Solubility of hydrophobic materials and drugs can be increased by using nanocarriers which can also enhance the stability of drugs. These systems can be used to provide targeted delivery of drugs, improve bioavailability, sustain release of drugs or solubilize drugs for systemic delivery. Lipid nanoparticles are intensively studied drug delivery systems derived from o/w emulsions, they combine the advantages of polymeric nanoparticles, conventional emulsions and liposomes, while simultaneously avoiding their disadvantages. SLN consists of pure solid lipid while NLCs are made of solid lipids that entrap incompatible liquid lipid in a Nano compartment within nanoparticle.

13.REFERENCES:

1. Feynman's Talk. Available at http://www.zyvex.com/nanotech/feynman.html
2. Hornigold, T. What Are the Origins of Nanotechnology? Available at

https://www.azonano.com/article.aspx?ArticleID=4859
3. Prijic, S.; Sersa, G; Magnetic nanoparticles as targeted delivery systems in oncology;*Radiology and Oncology* 2011, *45* (1), 1–16.
4. Sovan Lal Pal, Utpal Jana. Nanoparticle: An overview of preparation and Characterization.*Journal of Applied Pharmaceutical Science* 2011; 01(06):228-234.
5. Sutradhar, K. B.; Khatun, S.; Luna, I. P. Increasing Possibilities of Nanosuspension. *Journal of Nanotechnology* 2013, *2013*, 1–12.
6. Grimaldi N, Andrade F, Segovia N, Ferrer-Tasies L, Sala S, Veciana J, et al. Lipid-based nanovesicles for nanomedicine. Chem Soc Rev. 2016;45(23):6520–45.
7. Kapoor M, Lee SL, Tyner KM. Liposomal Drug Product Development and Quality: Current US Experience and Perspective. AAPS J. 2017 May;19(3):632–41.
8. Li Q, Cai T, Huang Y, Xia X, Cole SPC, Cai Y. A Review of the Structure, Preparation, and Application of NLCs, PNPs, and PLNs. Nanomaterials. 2017 Jun;7(6):122.
9. Chawla Vinay; Saraf, S. A. In *Nanocolloidal Carriers: Site Specific and Controlled Drug Delivery* ; CBS Publishers & Distributors Pvt Ltd; pp 172–190.
10. Caban S, Aytekin E, Sahin A, Capan Y. Nanosystems for drug delivery. *OA Drug Design & Delivery* 2014 Jan 18;2(1):2.
11. Mainardes, R.; Silva, L. Drug Delivery Systems: Past, Present, And Future. Current Drug Targets 2004, 5 (5), 449-455.
12. Kazi, M. Lipid-Based Nano-Delivery For Oral Administration Of Poorly Water Soluble Drugs (Pwsds): Design, Optimization And In Vitro Assessment. Advanced Technology for Delivering Therapeutics 2017, 1-21.
13. Irby, D.; Du, C.; Li, F. Lipid–Drug Conjugate For Enhancing Drug Delivery. Molecular Pharmaceutics 2017, 14 (5), 1325-1338.
14. Kalepu, S.; Manthina, M.; Padavala, V.: Oral Lipid-Based Drug Delivery Systems – An overview. Acta Pharmaceutica Sinica B, 2013, 3 (6):361-372.
15. Mukherjee, S.; Ray, S.; Thakur, R., Solid Lipid Nanoparticles: A Modern Formulation Approach in Drug Delivery System, *Indian Journal of Pharmaceutical Sciences*2009, *71* (4), 349.
16. Mehnert, W.; Mader, K. Solid Lipid Nanoparticles Production, Characterization And Applications. *Advanced Drug Delivery Reviews* 2001, *47* (2-3), 165-196.
17. Huynh, N.; Passirani, C.; Saulnier, P.; Benoit, J. Lipid Nanocapsules: A New Platform For Nanomedicine. International Journal of Pharmaceutics 2009, 379 (2), 201-209.
18. Mohammadi-Samani, S.; Ghasemiyeh, P., Solid lipid nanoparticles and nanostructured lipid carriers as novel drug delivery systems: applications, advantages and disadvantages, *Research in Pharmaceutical Sciences*2018, *13* (4), 288.
19. Agrawal, Y.; Patel, V. Nanosuspension: An Approach To Enhance Solubility Of Drugs. Journal of Advanced Pharmaceutical Technology & Research 2011, 2 (2), 81-87.
20. R. Ravichandran.Nanoparticles in Drug Delivery: Potential Green Nanobiomedicine Applications, *International Journal of Green Nanotechnology: Biomedicine*, 2009, 1:2, B108-B130.
21. Porter, C.; Pouton, C.; Cuine, J.; Charman, W. Enhancing Intestinal Drug Solubilisation Using Lipid-Based Delivery Systems. Advanced Drug Delivery Reviews 2008, 60 (6), 673-691.
22. Sharma, A.; Sharma, U. Liposomes In Drug Delivery: Progress And Limitations. International Journal of Pharmaceutics 1997, 154 (2), 123-140.
23. Nanjwade, B. Functions Of Lipids For Enhancement Of Oral Bioavailability Of Poorly Water-Soluble Drugs. Scientia Pharmaceutica 2011, 79 (4), 705-727.
24. Mo, J.; Milleret, G.; Nagaraj, M. Liquid Crystal Nanoparticles For Commercial Drug Delivery. Liquid Crystals Reviews 2017, 5 (2), 69-85.
25. Akhlaquer Rahman, M.; Harwansh, R.; Aamir Mirza, M.; Hussain, S.; Hussain, A. Oral Lipid Based Drug Delivery System (LBDDS): Formulation, Characterization And Application: A Review. Current Drug Delivery 2011, 8 (4), 330-345.
26. Fadda M.; Sinico C.; Lai F.; Logu D. Liposomal incorporation of artimisia arborescence L. Essential oil and *in vitro* antiviral activity, Eur J Pharma Biopharma 2005, 59, 161-168.
27. Jia L, Zhang D, Li Z, Duan C, Wang Y, Feng F, et al. Nanostructured lipid carriers for parenteral delivery of silybin: Biodistribution and pharmacokinetic studies. Colloids Surf B Biointerfaces 2010, 80, 213-8
28. Ruan J, Liu J, Zhu D, Gong T, Yang F, Hao X, et al. Preparation and evaluation of self-nanoemulsified drug delivery systems (SNEDDSs) of matrine based on drug-phospholipid complex technique. Int J Pharma 2010, 386, 282-90.
29. Kakkar V, Singh S, Singla D, Sahwney S, Chauhan AS, Singh G, et al. Pharmacokinetic

applicability of a validated liquid chromatography tandem mass spectroscopy method for orally administered curcumin loaded solid lipid nanoparticles to rats. J Chromatogr B Analyt Technol Biomed Life Sci 2010;878:3427-31

30. Nipun, T.; Ashraful Islam, S. SEDDS Of Gliclazide: Preparation And Characterization By In-Vitro, Ex-Vivo And In-Vivo Techniques. *Saudi Pharmaceutical Journal* 2014, *22* (4), 343-348.
31. Jinno, J.; Kamada, N.; Miyake, M.; Yamada, K.; Mukai, T.; Odomi, M.; Toguchi, H.; Liversidge, G.; Higaki, K.; Kimura, T. Effect Of Particle Size Reduction On Dissolution And Oral Absorption Of A Poorly Water-Soluble Drug, Cilostazol, In Beagle Dogs. Journal of Controlled Release 2006, 111 (1-2), 56-64.
32. Varshosaz, J.; Hajhashemi, V.; Soltanzadeh, S. Lipid Nanocapsule-Based Gels For Enhancement Of Transdermal Delivery Of Ketorolac Tromethamine. *Journal of Drug Delivery* 2011, *2011*, 1-7.
33. Sakeena, M.; F.A., M.; Z.A., G.; M.M., K.; S.M., E.; A.S., M.; M.N., A. Formulation And In Vitro Evaluation Of Ketoprofen In Palm Oil Esters Nanoemulsion For Topical Delivery. *Journal of Oleo Science* 2010, *59* (4), 223-228.
34. Onoue, S.; Sato, H.; Ogawa, K.; Kojo, Y.; Aoki, Y.; Kawabata, Y.; Wada, K.; Mizumoto, T.; Yamada, S. Inhalable Dry-Emulsion Formulation Of Cyclosporine A With Improved Anti-Inflammatory Effects In Experimental Asthma/COPD-Model Rats. *European Journal of Pharmaceutics and Biopharmaceutics* 2012, *80* (1), 54-60.
35. Pathak, P.; Nagarsenker, M. Formulation And Evaluation Of Lidocaine Lipid Nanosystems For Dermal Delivery. *AAPS PharmSciTech* 2009, *10* (3), 985-992.
36. Piao, H.; Kamiya, N.; Hirata, A.; Fujii, T.; Goto, M. A Novel Solid-In-Oil Nanosuspension For Transdermal Delivery Of Diclofenac Sodium. *Pharmaceutical Research* 2007, *25* (4), 896-901.
37. Müller, R.; Radtke, M.; Wissing, S., Nanostructured lipid matrices for improved microencapsulation of drugs, *International Journal of Pharmaceutics*, 2002, *242* (1- 2), 121–128.
38. Pattekari, P.; Zheng, Z.; Zhang, X.; Levchenko, T.; Torchilin, V.; Lvov, Y. Top-Down And Bottom-Up Approaches In Production Of Aqueous Nanocolloids Of Low Solubility Drug Paclitaxel. *Physical Chemistry Chemical Physics* 2011, *13* (19), 9014.
39. Geetha, G,; Poojitha, U.; Khan, Arshad. Various techniques for preparation of nanosuspension - a review. *Pharma Research*, 2014. 3. 30-37.
40. https://www.ttu.ee/public/m/Mehaanikateadus kond/Instituudid/Materjalitehnika_instituut/M TX9100/Lecture11_Synthesis.pdf (accessed Jan 26, 2020).
41. Thassu, D.; Deleers, M.; Pathak, Y. In *Nanoparticulate Drug Delivery Systems*; informahealthcre: New York, 2007; pp 1-32.
42. Chen C, Zhu S, Huang T, Wang S, Yan X. Analytical techniques for single-liposome characterization. Anal Methods. 2013;5(9):2150–7.
43. Eloy JO, de Souza MC, Petrilli R, Abriata Barcellos JP, Lee RJ, Marchetti JM. Liposomes as carriers of hydrophilic small molecule drugs: Strategies to enhance encapsulation and delivery. Colloid Surf B-Biointerfaces. 2014, 1;123:345–63.
44. Mallick S, Choi JS. Liposomes: Versatile and Biocompatible Nanovesicles for Efficient Biomolecules Delivery. J Nanosci Nanotechnol. 2014, 14(1):755–65.
45. Patil YP, Jadhav S. Novel methods for liposome preparation. Chem Phys Lipids. 2014; 177:8–18.
46. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, et al. Liposome: classification, preparation, and applications. Nanoscale Res Lett. 2013, 22;8:102.
47. Shmeeda H, Even-Chen S, Honen R, Cohen R, Weintraub C, Barenholz Y. Enzymatic assays for quality control and pharmacokinetics of liposome formulations: Comparison with nonenzymatic conventional methodologies. In: Duzgunes N, editor. Liposomes, Pt A. San Diego: Elsevier Academic Press Inc; 2003. p. 272–92.
48. Souto E.; Muller R. In *Nanoparticulate Drug Delivery Systems*; informahealthcre: New York, 2007; pp 213-234.
49. Ana Beloqui, MaríaÁngelesSolinís. Nanostructured lipid carriers: Promising drug delivery systems for future clinics *Nanomedicine.* 2016; 12(1):143-62.
50. zur Mühlen, A.; Schwarz, C.; Mehnert, W. Solid Lipid Nanoparticles (SLN) For Controlled Drug Delivery – Drug Release And Release Mechanism. *European Journal of Pharmaceutics and Biopharmaceutics* 1998, *45* (2), 149-155.
51. Mohammadi-Samani, S.; Ghasemiyeh, P., Solid lipid nanoparticles and nanostructured lipid carriers as novel drug delivery systems:

applications, advantages and disadvantages, *Research in Pharmaceutical Sciences*2018, *13* (4), 288.
52. Chia-Lang Fanga, Saleh A. Al-Suwayeh. Nanostructured Lipid Carriers (NLCs) for Drug Delivery and Targeting. *Recent Patents on Nanotechnology*, 2013; 7(1):41-56.
53. Iqbal, M. A.; Md, S.; Sahni, J. K.; Baboota, S.; Dang, S.; Ali, J., Nanostructured lipid carriers system: Recent advances in drug delivery, *Journal of Drug Targeting*2012, *20* (10), 813–830.
54. Verma S, Kumar S, Gokhale R *et al.*, Physical stability of nanosuspensions: Investigation of the role of stabilizers on Ostwald ripening. *Int. J. Pharm.* (2011) 406(12), 145-152.
55. Rosenblatt KM, Bunjes H., Poly(vinyl alcohol) as emulsifier stabilizes solid triglyceride drug carrier nanoparticles in the alpha-modification. *Mol. Pharm.* (2009) 6(1), 105-120.
56. Narang, J.; Khan, S.; Baboota, S.; Ali, J.; Khan, S.; Narang, R., Nanostructured lipid carriers: An emerging platform for improving oral bioavailability of lipophilic drugs, *International Journal of Pharmaceutical Investigation*2015, *5* (4), 182.
57. Sarabjotkaur, UjjwalNautyal. Nanostructure Lipid Carrier (NLC): the new generation of lipid nanoparticles. *Asian Pac. J. Health Sci.*, 2015; 2(2): 76-93.
58. Jaiswal, P.; Gidwani, B.; Vyas, A.,Nanostructured lipid carriers and their current application in targeted drug delivery, *Artificial Cells, Nanomedicine, and Biotechnology*2014, *44* (1), 27–40.
59. AbhishekGarg, SharadVisht. Formulation, Characterization and Application on Nanoparticle: A Review. *Der PharmaciaSinica*, 2011, 2 (2):17-26.
60. Üner, M.; Yener, G. Importance of Solid Lipid Nanoparticles (SLN) in various Administration Routes and Future Perspective. International Journal of Nanomedicine 2007, 2(3), 289-300.
61. Bhatia, S.,Nanoparticles Types, Classification, Characterization, Fabrication Methods and Drug Delivery Applications, *Natural Polymer Drug Delivery Systems*2016, 33–93.
62. Jenning, V.; Thünemann, A. F.; Gohla, S. H., Characterisation of a novel solid lipid nanoparticle carrier system based on binary mixtures of liquid and solid lipids, *International Journal of Pharmaceutics*2000, *199* (2), 168–177.
63. Rao, A.; Schoenenberger, M.; Gnecco, E.; Glatzel, T.; Meyer, E.; Brändlin, D.; Scandella, L. ;Characterization of nanoparticles using Atomic Force Microscopy.; *Journal of Physics: Conference Series*2007, *62*, 971–976.
64. Puglia, C.; Damiani, E.; Offerta, A.; Rizza, L.; Tirendi, G. G.; Tarico, M. S.; Curreri, S.; Bonina, F.; Perrotta, R. E.;Evaluation of nanostructured lipid carriers (NLC) and nanoemulsions as carriers for UV-filters: Characterization, in vitro penetration and photostability studies ; *European Journal of Pharmaceutical Sciences*2014, *51*, 221– 227.
65. Reddy RN and Shariff A: Solid Lipid Nanoparticles: An Advanced Drug Delivery System. *Int J Pharm Sci Res*. 2013; 4(1); 162-171.
66. Zhang, G.; Qiao, J.; Liu, X.; Liu, Y.; Wu, J.; Huang, L.; Ji, D.; Guan, Q. Interactions Of Self-Assembled Bletilla Striata Polysaccharide Nanoparticles With Bovine Serum Albumin And Biodistribution Of Its Docetaxel-Loaded Nanoparticles. *Pharmaceutics* 2019, *11* (1), 43.
67. Zhao, X.; Sun, Y.; Li, Z. Topical Anesthesia Therapy Using Lidocaine-Loaded Nanostructured Lipid Carriers: Tocopheryl Polyethylene Glycol 1000 Succinate-Modified Transdermal Delivery System. *Drug Design, Development and Therapy* 2018, *Volume 12* (1), 4231-4240.
68. Zhang, G.; Qiao, J.; Liu, X.; Liu, Y.; Wu, J.; Huang, L.; Ji, D.; Guan, Q. Interactions Of Self-Assembled Bletilla Striata Polysaccharide Nanoparticles With Bovine Serum Albumin And Biodistribution Of Its Docetaxel-Loaded Nanoparticles. *Pharmaceutics* 2019, *11* (1), 43.
69. Zhao, X.; Sun, Y.; Li, Z. Topical Anesthesia Therapy Using Lidocaine-Loaded Nanostructured Lipid Carriers: Tocopheryl Polyethylene Glycol 1000 Succinate-Modified Transdermal Delivery System. *Drug Design, Development and Therapy* 2018, *Volume 12* (1), 4231-4240.

70. Dr. Rimpler INTENSIVE Serum Nanorepair Q10 20ml. Available at https://www.m-beauty24.de/Dr-Rimpler-INTENSIVE-Serum-Nanorepair-Q10-20ml/en

71. Micellar Cleansing Water - Facial Cleanser & Makeup Remover - Garnier
https://www.garnierusa.com/about-our-brands/skinactive/micellar-cleansing-water
72. Product - Care Paste. Available at https://fluidinova.com/nano-hydroxyapatite-toothpaste-ingredient-for-teeth-remineralization-and-sensitivity (accessed Jan 26, 2020).
73. Prescription Eye Drops For a Type of Chronic Dry Eye Patients. Available at

https://www.restasis.com/ (accessed Jan 26, 2020).
74. US20080020058A1 - Lipid nanoparticle based compositions and methods for the delivery of biologically active molecules - Google Patents. https://patents.google.com/patent/US20080020058A1/en (accessed Jan 25, 2020).
75. WO2014115137A1 - Skin anti-aging cosmetic composition - Google Patents. https://patents.google.com/patent/WO2014115137A1/en
76. US8057823B2 - Lipid nanocapsules, preparation process and use as medicine - Google Patents. https://patents.google.com/patent/US8057823B2/en
77. EP2344134B1 - Nanocarriers for drug delivery - Google Patents. https://patents.google.com/patent/EP2344134B1 (accessed Jan 26, 2020).
78. US20080206341A1 - Lipid Nanoparticles as Vehicles for Nucleic Acids, Process for Their Preparation and Use - Google Patents. https://patents.google.com/patent/US20080206341A1/en