Review Article

NANOSIZED CONTROLLED DRUG DELIVERY DEVICE: LIPID NANOPARTICLE

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ABSTRACT

Nanosized lipid particulate system review has been written till date to focus on various diseases. Lipid nanoparticles opened a new channel for effective delivery of various potential agents which are used to treat ailments like Cancer, Malaria, Leishmaniasis, Trypanosomiasis; problems associated with skin, lung, eye, cardiovascular system, central nervous system and emphasize was given on adjuvant property shown for proteins, peptides and vaccines delivery too. This delivery system is a new era technology which can be used to incorporate both lipophillic and hydrophilic molecules. This present article reveals special focus on preservation and applications of Nanostructure Lipid Carrier. Lipid Nanocarrier System is an innovative carrier system which improves physical stability, drug loading, protection of incorporated drugs from degradation, controlled and targeted release and excellent tolerability. The developed technology platform is proposed to be used for many other potential actives required to elicit prolonged action by various routes of delivery.

Keywords: Nanostructure lipid carrier (NLC), Pharmaceutical Applications, Nanotechnology, Targeted Drug Delivery.

INTRODUCTION

Lipid-based colloidal drug delivery is developing area and has contributed significantly to the advances in the field of targeted drug delivery[1]. Lipid Nanoparticles as a carrier system have a number of desirable features: (I) the ability to incorporate lipophilic and hydrophilic drugs[2], (II) low toxicity[3], (III) the ability of controlled release of incorporated drugs[4], (IV) their particulate matrix is easily biodegraded resulting in non-toxic degradation products[5] [6], (V) the ability to immobilize the drug in the solid particle matrix yielding in protection of the incorporated drug from degradation[5, 7], (VI) easy scale-up and manufacturing[2], (VII) low cost of excipients, i.e. lipids and stabilizers[8].

Nanostructure lipid carriers (NLCs) have been introduced in the last decade as an interesting alternative to the traditional colloidal carriers such as polymeric nanoparticles, liposomes, nanoemulsions; in virtue of the lower cost of raw materials, higher physical stability, ease of scale-up & manufacturing and great versatility[9-12]. NLCs are considered a smarter generation of Nanoparticles which possesses improved properties for drug loading, modulation of the delivery profile, and stable drug incorporation during storage[13, 14]. Nanometer scaled carrier systems like NLCs are efficient drug delivery and storage systems[15]. NLCs are acting as promising carriers for presenting several attractive features for various drug delivery systems[16], NLCs are nanoparticulate active substance vehicles and are attracting major attention as novel colloidal drug carriers for different uses. NLCs combine various advantages as controlled release and protection of active compounds[17]. NLCs are identical to an oil in water emulsion except that the liquid lipid (oil) portion of the emulsion is replaced by a solid lipid having a mean photon correlation diameter (PCS) ranging between 80-1000 nm[10, 16].

NLCs, the new generation of lipid nanoparticles, overcome the limitations associated with the solid lipid Nanoparticles (SLN) entitled as limited drug loading, risk of gelation, adjustment of drug release and drug expulsion during storage caused by lipid polymorphism[10, 17]. NLCs consist of a mixture of different lipid molecules i.e. solid lipid(s) is blended with liquid lipid(s)[17, 18]. It is further suggested that by changing the ratio of solid lipid and liquid lipid; release, permeation and hence pharmacodynamic activity can also be modulated. The resulting matrix of the lipid particles shows a melting point depression as compared to the original solid lipid, however, the matrix remains solid at body temperature[2]. Depending on the method of production and composition of lipid blend, different types of NLCs were obtained i.e. the imperfect, amorphous and multiple components[19]. When the content of liquid lipid was higher than the solubility of liquid lipid in the solid lipid, phase separation occurs. The structure of NLCs (showed in fig 1) should be such that it offers maximum space which could accommodate maximum drug. The different nanostructure model of NLCs are:

I) Type I : Highly imperfect matrix

II) Type II : Multiple oil/fat/water

III) Type III : Amorphous

- TYPE I (Imperfectly structured solid matrix): Spatially different lipids are mixed and the matrix formed contain imperfections to accommodate the drug in amorphous clusters [20, 21].
- TYPE II (Multiple oil in fat in water carrier): When lipids lack appropriate drug solubility, addition of a

higher amount of liquid lipid to the lipophilic phase displays the advantages of the solid matrix which prevented drug leakage while the liquid region show comparatively high solubility for lipophilic drugs. In type III, lipids are mixed in a way that prevents them from crystallizing. The lipid matrix is solid, but in an amorphous state. The absence of crystallization avoids drug expulsion by crystallization[20, 21].

- TYPE III (Structure less solid amorphous matrix): This kind of NLCs can be achieved by mixing solid lipids with special lipids. e.g. Hydroxy octacosanyl hydroxysterate, isopropylmyristate or medium chain triglycerides such as Miglyol 822. Drug expulsion caused by the process of the crystallization to beta-forms during storage is prevented by the special structure of the lipid matrix, since NLCs are solids in amorphous state, but not in crystalline state [10].

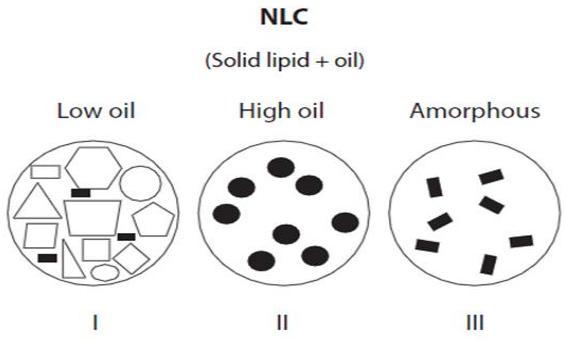

Figure 1: Structure of NLCs

The characteristics of lipids including the melting point, viscosity, crystallization form and hydrophilicity impact on the stability of NLCs solutions and for stability suitable emulsifiers reduce the interface tension and facilitate droplet dispersion during the homogenization process[22]. These are composed of biodegradable and physiological lipids exhibiting low cytotoxicity and low systemic toxicity[23]. These lipids or excipients used in commercial pharmaceutical preparation have approved status. The small size of lipid particles ensures the increased amount of drug penetrating into targeted organ or tissue and hence ensured targeted drug delivery.

NLCs proved to be an important tool when it is necessary to reduce systemic absorption, to supply the drug over prolonged period of time and when drug produces irritation in high concentrations[24,2,4]. Recently NLCs are used as delivery carriers for many hydrophilic & hydrophobic drugs and encapsulation of biologically active agents in NLCs can enhance their activity by acting as a drug reservoir[25].

NLCs are produced by one of the following techniques, namely

- High pressure homogenization[10, 26, 20]
- Micro-emulsion template technique[27]
- Solvent emulsification evaporation technique[28]
- Solvent displacement technique [29, 30]
- Solvent emulsification diffusion method [31]
- Phase inversion [32] and
- Membrane contractor technique [16, 33, 34]

FATE OF LIPIDS IN BODY

Lipids as carriers in various lipid based formulations have the potential of providing endless opportunities in the area of drug delivery due to their ability to enhance gastrointestinal solubilization and absorption via selective lymphatic uptake of poorly bioavailable drugs. The unique properties of lipids viz. their physiochemical diversity, biocompatibility and proved ability to enhance oral bioavailability of poorly water soluble, lipophilic drugs through selective lymphatic uptake have made them very attractive candidates as carriers for lipid based formulations[35-38]. The focus of this section is to simplify the understanding of the entire process by dividing it into three distinct phases:

a) **Digestive Phase**: The digestive phase initiates with the physical breakdown of lipid formulation into a coarse emulsion (lipid droplets ~0.5 µm) of high surface area due to shear produced by antral contraction, retropulsion and gastric emptying. This is accompanied with hydrolysis of the fatty acid glyceryl esters by gastric lipase secreted from chief cells in the stomach (capable of functioning in an acidic environment) which act at the oil/water interface. The dispersed lipid digestion products along with the undigested lipids then empty into the duodenum[39].

b) **Absorption Phase**: The journey of lipids from the GI lumen to the circulatory system in the presence of a powerful digestive system is of great significance for interpretation of the biopharmaceutical properties of lipid-based formulations and product development[40].

c) **Circulatory Phase**: The majority of administered drugs gain access to the systemic circulation by absorption into the portal blood and highly lipophilic drugs (log P > 5, solubility in Triglycerides > 50 mg/ml) gain access to the systemic circulation via lymphatic route which avoids hepatic first-pass metabolism. Therefore, highly metabolized lipophilic drugs may be potential candidates for lipid based drug delivery[41, 42]. The drug being transported in the circulatory system, in the form of either micelles or mixed micelles, may then be available in its free form, since upon dilution with a large volume of the lymph/blood, surfactant concentration may reduce below its Critical Micelle Concentration (CMC) value and micelle may dissociate into monomers[43]. The drug transported as lipid vesicles may remain intact for extended periods and thereby can result in

prolonged release of the encapsulated drug[44]. The various mechanisms of enhancement of drug bioavailability in the presence of lipids are shown in Fig 2.

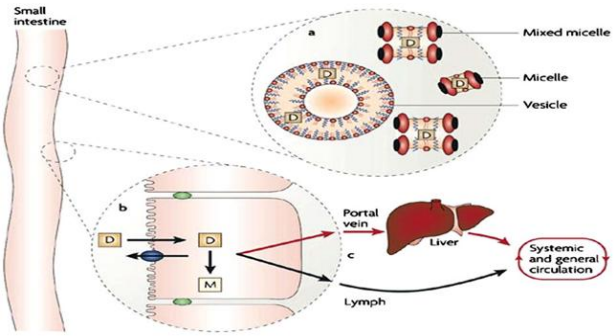

Figure 2: Various mechanisms of enhancement of drug bioavailability in the presence of lipids:

a) Solubilization of drug in the intestinal fluid by formation of colloidal species viz. vesicles mixed micelles and micelles.

b) Interference with enterocyte based transport and metabolic processes, thereby potentially changing drug uptake, efflux, disposition and the formation of metabolites (M) within the enterocyte

(c) By selective lymphatic uptake which reduces first-pass drug metabolism as intestinal lymph travels directly to the systemic circulation[45, 46].

Lipid based formulations are removed from the circulation by a mononuclear phagocyte system, largely in the liver and spleen, or by extravasation in areas where the vascular membrane is more permeable, such as sites of tumors, inflammation and infection[25].

PHARMACEUTICAL APPLICATION OF LIPID NANOPARTICLES

Lipid nanoparticles in treatment of cancer

Many anti-cancer agents have been formulated in lipid nanocarriers and their in-vitro and in-vivo efficacy have been evaluated. Lipid encapsulation enables increased antitumor efficacy and decreased toxicity due to higher accumulation of drug in tumors[47, 48]. NLCs have been used for delivery very poorly soluble drugs. NLCs are removed by extravasation at the sites of tumor, inflammation and infection where the vascular membrane is more permeable. They are also removed from the circulation by mononuclear phagocyte system mainly by liver and spleen so they reduce cadiotoxicity[49].

Topotecan is an anti-cancer drug used in the study by L.G Souza et al 2011, which showed NLCs made by micro emulsion technique were of satisfactory drug loading, entrapment and improved chemical stability and cytotoxicity[47].

In the research of Fang Li et al 2010; evaluated safety, efficacy, in-vitro cytotoxicity, pharmacokinetics, biodistribution, anti-tumour efficacy of bufadienolides loaded NLCs. Bufadienolides NLCs prepared by melt-emulsification and ultrasonic method showed high plasma concentration and lower clearance after IV administration as compared to bufadienolides solution. The biodistribution studies indicated that accumulation of bufadienolides in brain was higher and can be used for brain cancer[25].

Lipid nanoparticles for topical delivery

Lipid based colloidal drug delivery is the rapidly developing area and has contributed significantly to the advances in the field of targeted drug delivery due to their astonishing properties and numerous advantageous qualities for topical application. Particulate lipid mainly NLCs comprise of blend of solid and liquid lipids. These type of lipid nanoparticles enhance drug solubility and encapsulation efficiency in carrier lipid matrix using lipophilic solubilizers for topical delivery in terms of stability and skin localization. Lipid modification resulted in the formation of less perfect crystals offering space to accommodate the dissolved drug leading to high entrapment efficiency. It allows remarkable flexibility in drug loading, modulation of drug release, and improved performance in producing final dosage forms such as gels for topical application with excellent spreadability, appropriate viscosity, and skin sensitivity at the same time avoiding the undesirable qualities such as greasiness and stickiness of the currently marketed products. Prolonged and controlled drug release with main effects to improve skin penetration through the barrier i.e. SC and improved deposition at the target site i.e. skin; would be advantageous over commercially marketed products and would be highly beneficial and more appealing with better patient compliance[1, 9]. Nanosized particles are of growing interest for topical treatment of skin diseases to increase skin penetration of drugs and to reduce side effects[50]. Various recent works have been discussed in **table 1.**

Lipid nanoparticles in pulmonary treatment

Inhalation drug delivery represents a potential delivery route for the treatment of several pulmonary disorders. NLCs have greater stability against the shear forces generated during nebulization compared to polymeric nanoparticles, liposomes and emulsions[8, 53, 54]. NLCs are comprised of an inner oil core surrounded by an outer solid shell and hence allow the high payload of a lipophilic drug[8]. NLCs in pulmonary disorders seems to be promising strategy (discussed in table 2) since lung epithelium can be directly reached resulting in faster onset of action, desired dose and dosing frequency can be reduced as compared to other administered routes like oral and undesirable side effects of drugs can be avoided[8, 55-57]. Bioadhesive properties of NLCs are due to their small particle size as well lipophilic character lead to longer residence time in lungs[58, 59].

Table 1: Overview of drugs as topical agents incorporated into NLCs

Drug	Method	Lipid particle	Remarks	Reference
Ketoprofen	Simple blending and grinding using high energy micro mill	NLCs	Improved drug therapeutic efficacy and safety, allowing an improvement in the dissolution stability, high tolerability, percutaneous and the skin permeation properties of Ketoprofen.	9
Tacrolimus	High pressure homogenization	MNLCs	Lipid modification resulted in the formation of less perfect crystals offering space to accommodate the dissolved drug leading to high entrapment efficiency and topical delivery.	1
Psoralens	High shear homogenization	NLCs	Enhanced permeation and controlled release of psoralens were obtained with NLC due to improved skin absorption.	17
Nile red (model dye)	High pressure homogenization	NLCs	Increased delivery of dye into skin	50
Nitrooxide		NLCs	To increase penetration or storage of actives	51
Nitrosyl Ruthenium complex (NRC)	Micro emulsification	NRC-loaded SLNs, NLCs	NRC showed slow release from lipid particles and stability of drug in contact with skin homogenates was improved and the photochemical studies showed NRC-loaded SLNs increased the Nitric oxide release after light irradiation while nitric oxide degradation was prevented when entrapped in NLC.	52

Table 2: NLCs incorporated for drugs used in pulmonary treatment

Drug	Method	Lipid particle	Remarks	Reference
Itraconazole	High pressure homogenization	NLCs	NLCs possessed good storage stability and good carrier for poorly soluble drug. Nebulizing Itraconazole-loaded NLCs with a jet stream and an ultrasonic nebulizer had no influence on the particle size and the entrapment efficiency of 98.78% was achieved being a precondition for pulmonary application.	8
Celecoxib	High pressure homogenization	NLCs	NLCs formulation was able to release the drug in a controlled manner for a prolonged period of time and NLCs were able to deposit in the alveolar region of the lungs of mice as well as enhanced lung residence time	59

Table 3: Overview of drugs used in ocular treatment incorporated as NLCs

Drug	Method	Lipid Particle	Remarks	Reference
Triamcinoloe acetonide (TA)	High Pressure Homogenization	NLCs	NLCs increased ocular absorption and enhanced prolonged drug residence time in the ocular surface and conjunctival sac, by sustained drug release from the delivery system, it also reduced precorneal drug loss.	18
Lutein	Ultrasonication	NLCs	Lutein-loaded NLCs could protect the entrapped lutein in the presence of simulated gastric fluid and slowly released lutein in simulated intestinal fluid in an in-vitro study.	22
Cyclosporine	Melt emulsification	NLCs	The mucoadhesive properties of the thiolated non-ionic surfactant Cysteine polyethylene glycol stearate (Cys-PEG-SA) and NLC modified by this thiolated agent were evaluated. Cys-PEG-SA and its resultant NLC provided a promising system with prolonged residence time.	65

Table 4: Drugs incorporated in NLCs for cardiovascular agents

Drug	Method	Lipid particle	Remarks	Reference
Tashinone (TA)	Nanoprecipitation / solvent diffusion process	NLCs	Drug loading and stability were improved. The in-vitro incubation tests confirmed that TA-NLC could bind to apoA-I specifically. Macrophage studies demonstrated that TA-NLC incubated with native HDL could turn endogenous by association to apo-lipoproteins, which cannot trigger immunological responses and could escape from recognition by macrophages.	66
Nifedipine	Dry Roll milling followed by High pressure homogenization	NLCs	Nanoparticle suspensions were formulated with negatively charged phospholipid, dipalmitoyl phosphatidylglycerol in preventing coagulation to improve solubility and hence bioavailability of drug.	67
Lovastatin	Ultrasonication	NLCs	NLCs were developed to promote oral absorption of lovastatin. More than 70% lovastatin was entrapped in the NLCs. The in-vitro release kinetics demonstrated that lovastatin release could be reduced by up to 60% with lipid nanoparticles containing Myverol as the lipophilic emulsifier. NLCs showing the slowest delivery. The oral lovastatin bioavailability was enhanced from 4% to 24% and 13% when the drug was administered from NLCs containing Myverol and SPC as surfactants respectively.	13

Table 5: Overview of lipid particle for treatment of parasitic diseases

Drug	Method	Lipid Particle	Remarks	Reference
Artemether	Thin film hydration	Lipid Nanoparticles (LNPs)	High encapsulation and drug loading. LNPs resulted in sustain release which avoided fast drug metabolism and also avoided production of toxic compounds dihydroartimisinin.	[68]
Oryzalin	Emulsion solvent evaporation followed by high pressure homogenization	LNPs	Solubility of oryzalin increased which was confirmed by DSC studies. Cell viability studies determined that oryzalin in LNC decreased cell cytotoxicity.	[69]
Cytosine Guanine (CpG) containing oligo deoxynucleotides (ODN)	Encapsulation	LNP	Lipid mediated delivery has the capacity to increase the immunopotency of CpG ODN and wide variety of applications ranging from standalone agents to vaccine adjuvants for prophylaxis and treatment of infectious and malignant disease and as immunomodulatory agents for the amelioration of allergic and autoimmune disorders .	[70]

Table 6: Drugs incorporated in NLCs for treatment of central nervous system

Drug	Method	Lipid particle	Remarks	Reference
Baicalein	Ultrasonication	Tocol - NLCs	Tocol – NLCs proved to promising carriers for delivery as in-vivo results showed that the encapsulation of baicalein in IV administered NLCs significantly increased the plasma level and t1/2 compared to an equivalent aqueous solution. NLCs successfully targeted the main brain, especially the cortex and brain stem.	[80]
Bromocriptine	-	NLCs	In-vivo results showed bromocriptine NLCs have rapid onset of action and longer duration and higher brain levels as compared to that of solution, entrapment efficiency was also increased.	[79]

Lipid nanoparticles for ocular treatment

Many recent works have shown (**table 3**) that these lipid carriers have potential for delivering lipophilic drugs to the posterior segment of the eye and to avoid the complications associated with intraocular repeated injections. NLCs are most commonly used and have been reported to enhance drug absorption and bioavailability [18, 22]. These colloidal drug delivery systems were found to be taken up by the corneal epithelial cells, which serve as a reservoir to release the drug slowly to the surrounding tissue[60, 61]. Many drugs used in treatment of various eye diseases, drugs are almost insoluble in water and effectively excluded from the eye by uveal blood aqueous and blood retinal barriers, administration by traditional liquid eye drops is not possible and systemic administration fail to reach therapeutic drug levels [62, 63]. NLCs have been proved useful for ocular absorption enhance of drugs because of prolonged drug residence time in ocular surface and conjuctival sac[64].

Lipid nanoparticles in treatment of cardiovascular diseases

Lipid nanoparticles as a carrier system has superiorities mainly prolonged circulation time and increased area under the curve (AUC) with manageable burst effect. NLCs would provide highly desirable physic-chemical characteristics as a delivery vehicle for lipophilic drugs[66, 67]. Various cardiovascular agents have been formulated as NLCs are shown in table 4.

Lipid Nanoparticles for treatment of parasitic diseases

Novel colloidal delivery systems have gained considerable interest for anti-parasitic agents with focus on 3 major parasitic diseases viz. malaria, leishmaniasis and trypanosomiasis. Lipid Nanoparticles combine advantages of traditional colloidal drug carrier systems like liposomes, polymeric nanoparticles and emulsions but at the same time avoid or minimize the drawbacks associated with them. The delivery system should be designed in such a way that physic-chemical properties and pharmacokinetic properties are modulated of the anti-parasitic agents (formulated as NLCs shown in table 5) in order to improve biospecificity (targetablity) rather than bioavailability with minimization in the adverse effects associated with it. SLNs and NLCs have ability to deliver hydrophobic and hydrophilic drug with more physical and biocompatibility.

Lipid nanoparticles as adjuvants for proteins, peptides, antigens and vaccines

Lipid Nanoparticles have been used as adjuvants for proteins drugs (e.g bovine serum albumin, lysozyme), therapeutic relevant protein peptides (e.g. calcitonin[71,72], insulin[73], somatostatin, LNRH[74], Cyclosporin A[21], protein antigens (e.g hepatitis B[75], Malaria antigens) have been investigated for drug release, kinetics, protein stability and in-vivo activity[75, 76]. Lipid based system act as powerful immunological adjuvant inducing both cellular and humoral immunity for various bacterial and viral antigens relevant to human disease[76, 77]. The main factors influencing peptide and protein release from lipid

particles are the physico-chemical characteristics of the drug itself, particle size, lipid matrix composition, surfactants used, drug distribution throughout the matrix, method of preparation and production parameters.

Proteins antigen, vaccines intended for various therapeutic purpose are incorporated or adsorbed onto lipid particles (e.g. NLCs) and further administered by alternative route like nasal, pulmonary, mucosal etc. Lipid matrix improves release of protein in a controlled manner, avoids protein degradation after administration and improves protein stability[76, 77]. The incorporation efficiency of proteins was found to be more than 90% in many cases.

Lipid nanoparticles for central nervous system

Lipid nanoparticles like NLCs appear suitable as a delivery system due to prolonged release, targeted efficiency with lower side effects and less toxicity than other lipid system. NLCs are new generations of lipid nanoparticles produced from solid lipids[78]. Clinical failures of most potentially effective therapeutics for treating central nervous system (CNS) disorders are often not due to an insufficiency of drug potency but rather shortcomings in the method by which the drug is delivered and these problems are overcome by formulating NLCs and ensuring targeted delivery to brain are shown in table 6[79].

PRESERVATION OF LIPID NANOPARTICLES

Lipid nanoparticles mainly NLCs formulated used for various applications require preservation but these preservatives can impair the physical stability of disperse system. Therefore, preservative classification system is suggested and mechanistic model describing six key parameters affecting the physical stability of lipid. The Classification system of preservatives (CSP) in table 7 and corresponding preservatives identified in this new research study by Wasfy M. Obeidat *et al*, 2010[81, 82].

Table 7: Classification system of preservative

Class	Kind of impairment	Preservatives belonging to the group
I	No stability impairment	Propylene glycol Rokonsal PB5 Euxyl PE9010
II	Little stability impairment	Caprylyl glycol Phenonip
III	Strong impairment of Stability	Euxyl K700 Euxyl K702 MultiEx Naturotics Ethanol
IV	Stabilizing effect	Pentylene glycol Mixture of pentylene Propylene glycol

Product development involves ensuring the physical, chemical and also microbial stability of product during shelf life. The concentration of preservatives required in a formulation not only depends on the type of preservative, but also on the degree of exposure like microbial during the shelf-life. In the present investigations different mixtures of preservative was made to demonstrate the influence on size, physical stability, zeta potential[81].

The stability of colloidal system depends on various parameters:

1. Affinity of the preservative to the particle surface,

2. Surface hydrophobicity of the particles,

3. Anchoring of stabilizer onto/into surface,

4. Ability of preservative to reduce zeta potential,

5. Nature of the particle stabilizer,

6. Interaction of preservative with stabilizer layer.

The pH is also important for the physical stability as it strongly influence the change in pH can cause instability of a system (agglomeration) due to decrease in zeta potential. As basic rules for achieving stable preserved nanodispersions, the preservative should be as hydrophilic as possible with little affinity to the particle surface and ideally non-ionic to minimize zeta-potential reduction. The stabilizer should be bound as firm as possible to the surface, ideally being anchored into the particle matrix (as in NLC)[83].

QUALITY CONROL

Microscopic and Macroscopic techniques are used in development of colloidal system[84]. Various techniques like particle size analysis, zeta-potential, transmission electron microscopy, differential scanning calorimetry (DSC), X- Ray scattering, polarized light microscopy, laser diffraction (LD), field-flow fractionation (FFF) were performed to investigate the structure, mobility and molecular environment of the compounds. These techniques also reveal the physical and chemical stability of formulation, surface charge tend to determine the particles will flocculate or not[84, 85].

Particle Size: The particle size is important parameter in process control and quality assurance because physical stability of vesicle dispersion depends on particle size and as particle size decreases, surface area characteristics increases as a function of total volume, photon correlation spectroscopy (PCS) based on laser light diffraction provides an appropriate method for investigation and can be applied for particles ranging below 200 nm and up to 1µm [86]. For particles below 200 nm Rayleigh's theory holds that the scattering intensity to be proportional to the sixth potency of the particle diameter. Both, Fraunhofer's and Rayleigh's theories, are only approximations of Mie's theory which claims that the scattering intensity depends on the scattering angle, the absorption and the size of the particles as well as the refractive indices of both the particles and the dispersion medium.

Zeta-potential: Zeta-potential is electric potential in the interfacial double layer at the location of the slipping plane versus a point in the bulk fluid away from the interface. In other words, zeta-potential is the potential

difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle[87]. The zeta-potential of colloidal determines whether the particles within the liquid will tend to flocculate or not. The zeta-potential describes the nature of electrostatic potential near the surface of particle called zeta-potential. The zeta-potential indicates the degree of repulsion between adjacent charged particles. The colloids with high zeta-potential (negative or positive) are electrically stabilized while colloids with low zeta-potentials tend to coagulate or flocculate[88].

Transmission electron microscopy: It is a technique where colloidal samples could be visualized at high resolution. Sufficient contrast can be given to a thin film of the frozen sample by use of osmium tetra-oxide. This allows the sample to be viewed directly in the TEM (at a temperature of -196°C). The adjustment of the temperature to -196°C leads to a very low vapor pressure, so that the examination of the sample is possible by preservation of the microstructure despite the high vacuum. A disadvantage of cryo-TEM is the difficulty in establishing size classification of vesicles. Due to the fluid property of the vesicle dispersion prior to freezing, the sample film thickness increases from the center to the outside. Hence the smaller vesicles stay in the center where the film is thin while the larger ones linger at the outside margin in the thicker part of the film. In this outer part the vesicles are out of the field of view. Therefore the resulting distribution does not represent the true size distribution[88, 89].

X-ray scattering: With X-ray scattering experiments characteristic interferences are generated from an ordered microstructure. A typical interference pattern arises due to specific repeat distances of the associated interlayer spacings d. According to Bragg's equation d can be calculated:

$$d = n/\lambda\ 2\sin\Theta,$$

where λ is the wavelength of the X-ray being used, n is an integer and nominates the order of the interference, and Θ is the angle under which the interference occurs[90].

Differential scanning calorimetry (DSC): Thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. Both sample and reference are kept at same temperature, temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. The result of a DSC experiment is a curve of heat flux versus temperature or versus time. There are two different conventions: exothermic reactions in the sample shown with a positive or negative peak, depending on the kind of technology used in the experiment. This curve can be used to calculate enthalpies of transitions. This is done by integrating the peak corresponding to a given transition. It can be shown that the enthalpy of transition can be expressed using the following equation:

$$\Delta H = KA$$

Where ΔH is the enthalpy of transition, K is the calorimetric constant, and A is the area under the curve. The calorimetric constant will vary from instrument to instrument, and can be determined by analyzing a well-characterized sample with known enthalpies of transition[87-90].

CONCLUSION: TOWARDS FUTURE PROSPECTIVE

NLCs have shown edge over traditional colloidal carrier system and conventional methods. It promises effective treatment in a cost effective way where the diseases will be combated with maximum therapeutic effect and minimum intervention. The developed technology platform is proposed to be used for many therapeutic agents. The acceptance of lipids and surfactants used in the formulating lipid nanocarrier system is major hurdle in successful commercialization due to their toxicity issues. The other aspects of NLC such as sterilization, freeze drying, shelf life and large-scale industrial production have already been developed to a sufficient standard with the view of commercialization.

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