WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 5, 225-262. Review Article ISSN 2277–7105

COMPREHENSIVE INTERPRETATION OF NANOSTRUCTURED LIPID CARRIERS (NLC): A PROMISING NEW GENERATION LIPID CARRIER FOR THE DELIVERY OF CANCER CHEMOTHERAPEUTICS

Parikshit Banerjee[1], Arpan Mahanty[1], Rakotomalala Manda Heriniaina[3,4], Tiantian Li[1], Geng Tengjiao[1], Biplab Mallick[2] and Bo Wang[1]*

[1]Department of Pharmaceutics, School of Pharmacy, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, China.

[2]BCDA College of Pharmacy and Technology, 78, Jessore Road (S), Hridaypur, Kolkata-700127, India.

[3] State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, China.

[4]Jiangsu Key Laboratory of TCM Evaluation and Translational Research, China Pharmaceutical University, Nanjing 211198, China, Tongjiaxiang 24, Nanjing 210000, China.

Article Received on
15 March 2017,

Revised on 05 April 2017,
Accepted on 26 April 2017

DOI: 10.20959/wjpr20175-8459

***Corresponding Author**
Bo Wang
Department of Pharmaceutics, School of Pharmacy, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, China.

ABSTRACT

In the last couple of years, Nano-carries have attracted large amount of consideration in drug delivery specifically for cancer chemotherapy. There are shortcomings which greatly hindered anti-cancer drug delivery such as poor-aqueous solubility, toxicity towards normal tissues, limited targeting ability, inefficient large-scale production output, rapid degradation and quick release from the formulations. The amount of studies analyzing formulations based on nanostructured lipid carriers (NLCs) has been significantly increased. This increase in NLC investigation is possibly due to its ability to overcome the physiological obstacles and enhanced understanding of mechanism of action NLCs in vitro and in vivo via different route of administration in recent years. Toxicity related concerns like- presence of organic residues and polymers are tactically bypassed by using nanostructured lipid carriers. In this review we have discussed the types, preparation procedure and physicochemical characterization technique of NLCs. In addition, the roles NLCs can play in cancer

chemotherapy have also been discussed. It is expected that in foreseeable future NLCs will /stand out as a carrier with the ability to target cancers more efficiently and will present better clinical outputs.

KEYWORDS: Nanostructured lipid carriers, cancer chemotherapy, physiological obstacles, stability, clinical outputs.

INTRODUCTION

Cancer is regarded as one of the most life threatening disease and responsible for large number of deaths all over the world.[1] It is a kind of disease that can eventualize in any ethnic group at any age and even most of the times precautions cannot even ensure to defend its occurrence and with current state of knowledge available to the scientific community it is essential to detect it as early as possible to enhance survival rate of the patients suffering from this notorious disease. Currently, available treatments include combination therapy using surgery, radiation and chemotherapy. Usually, after removing the tumor surgically, radiation and chemotherapy have given to the patients to ensure complete annihilation of any traces or tumor cells and tissues to restrict any chance of relapse of this disease.[2] There are several groups of anti-cancer agents available which execute their actions by interdicting cell division in tumor cells which suppress the metastasis and hence exterminate cancer.[3] Disastrously, these cytotoxic agents have wide range of limitations like poor-aqueous solubility, high dosing often reaching maximum tolerated dose, low targeting ability, toxicity towards normal cells and narrow therapeutic window.[4] Such hurdles greatly limits their application in clinical settings which have led to the endless search for therapeutic molecules with better therapeutic and safety profile or to find new delivery carriers which can deliver both old and new therapeutics with better efficiency and can help to improve the outputs of anti-cancer therapy.

In the breakthrough of pharmaceutical research today, the novel technologies have led to the discovery of several potent compounds. To confirm the progress in therapeutic drug therapy, only the introduction of new drug molecules is not satisfactory enough due to the possible poor-aqueous solubility and low bioavailability of newly discovered drug molecules. Poor water solubility and low bioavailability are one of the most widespread obstacles which limit the use of many promising therapeutics drug molecules in clinical settings. So, to overcome this barriers constant need to develop drug delivery carriers which can conquer these obstacles effectively and can provide better loading capacity, free of toxicity, longer chemical

stability and adequate targeting capability is always evident in pharmaceutical industry. Expediency of manufacturing process and cost-effectiveness is also essential for successful industrialization of these delivery carriers.[5-7]

Nanocarriers have shown to be very effective in delivery of therapeutic cargo in wide range of diseases including cancer. It is widely acknowledged that nanoparticlulate delivery systems are far more effective than other conventional chemotherapy in terms of safety, drug resistance, therapeutic effectiveness and patient compliance. Interesting in vitro data achieved from the experiments are recurrently failed to match with the data obtained form in vivo experiments or in clinical situations. The rationale behind this failures are high drug toxicity due to inefficient targeting ability and general distribution, inadequate drug concentration in vivo due to fast metabolism, poor solubility of drugs in formulations, and inconsistent plasma drug levels.[6] Available colloidal systems such as liposomes, polymeric nanoparticles, micro and nanoemulsions also have their own shortcomings like rapid degradation in stomach or intestinal pH or in bile salt in case of oral delivery, limited physical and chemical stability during storage,[8-10] lack of large-scale production method, rapid uptake by reticule-endothelial system (RES) during intravenous delivery, presence of the residue of organic solvent and toxicity due to the use of polymers[11,12] and many more to say. As an anti-cancer drug delivery system, nanocarriers need to have some definite characteristics such as particle size below 200nm, ideal zeta potential, higher loading capacity, surface hydrophilicity to dodge phagocytosis, biodegradability and biocompatibility and lowest possible antigenicity.[13]

In the early 90s, professor R.H. Müller from Germany and Professor M. Gasco from Italy had started to investigate the possible effectiveness of a novel nanoformulation called solid lipid nanoparticles (SLNs).[14,15] These nanoparticulate formulations are solely based on lipids even though other ingredients like surfactants are also needed. In last few decades voluminous work has been done on lipid nanocarriers to deliver cytotoxic drugs. Investigation on Lipid carriers like solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLC) are also largely increased due to their cost-effective and safe composition and feasibility to scale up.[16] This large number of investigations has found several difficulties associated with SLNs such as phase transition of lipids, low encapsulation, crystal growth during storage etc.[17] To overcome these drawbacks related to SLNs, unstructured matrix containing both solid and liquid lipid was introduced and later named as NLCs,[18-20] which

claimed to have the ability to clear up issues coupled with SLN because of the presence of solid lipid and liquid lipid in an ideal ratio.[21] NLCs are also well-considered as second generation of lipid nanocarriers. Contrary to the SLNs, NLCs are capable of higher drug loading by realizing imperfect organization of solid lipid matrix by adding liquid lipid with solid lipid. This expands drug stacking ability of NLCs which significantly reduce the expulsion of drugs and also maintain physical stability during storage.[6,21-23] NLCs also have less water content in it and less susceptible to abnormal gelation.[21,24,25] This characteristics of NLCs made them a promising alternative not only to polymeric nanoparticles but also to other lipid based delivery systems such as liposomes, SLNs, nanoemulsions etc. Compared to other colloidal nanocarriers NLCs are found to be more beneficial specifically in terms of in vivo stability and drug loading and further considering their easy and cost-effective manufacturing process it can be said confidently that this carriers are sensible choice for the delivery of therapeutic cargo whenever and wherever it needed with or without modifications.[6,14,21,26,27]

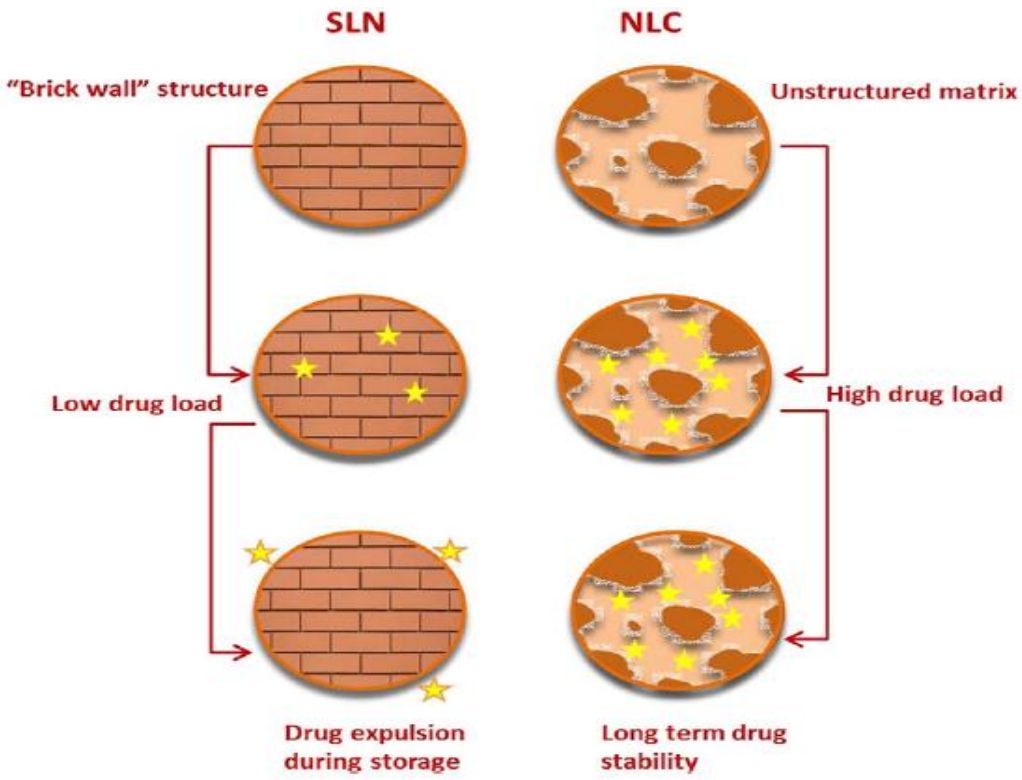


Figure 1: Schematic illustration of SLN structure versus NLC structure, where NLC advantages over SLNs are also highlighted[141]

The current review is focused on nanostructured lipid carriers (NLCs) and the aim is to- (i) interpret the major difficulties commonly encountered while using lipid nanoparticles (LPs), (ii) to unfold the fundamentals steps have been taken to overcome those obstacles and (iii) to

give comprehensive synopsis on relevance of NLC formulations as an anti-cancer drug delivery system.

Components of NLC Formulation

The essential parts in making of NLC embrace solid lipids, liquid lipid, water and emulsifiers. NLCs are prepared with lipids as a core excipient by mixing solid and liquid lipids in an appropriate ratio.[20] Solid lipids constructs the main fraction of the NLC matrix that keeps them in solid state at normal temperature and choice is completed on the premise of their physiochemical structure, miscibility, solubility, polymorphic nature and crystallinity.[28]

Generally, NLCs are composed of imperfect matrix of solid lipid consist of a mixture of both solid lipid and liquid lipid in an optimal ratio typically ranges from 70:30 up to 99.9:0.1 and surfactant concentration ranges from 1.5%-5% (w/v) but this amounts varies time to time depending on the output of preparation method.[29] There are numerous combinations of lipids and surfactants have been used in literature and all those components are available commercially as marketed product and/or have approval from regulatory agencies (GRAS= Generally Recognized As Safe, US FDA IIG).[30,31]

Collectively, possible production scale-up, use of GRAS ingredients and enhance safety make NLCs an excellent drug delivery system for future pharmaceutical market.[32]

Standards for selecting Excipients

The excipients like liquid lipid, solid lipoid and surfactants are picked considering their restrictive status (GRAS standing as per USFDA), purity, chemical stability, solubility of drug within the lipoid parts, miscibility of solid and liquid lipids, concentration of solid and liquid lipids, concentration of surfactants, edibleness, fate of digestible products (generally biodegradable lipids are endorsed), processing temperature and value of excipients. Generally, excipients with low level of peroxides or aldehydes are chosen as these contaminants might alter the chemical stability of the dissolved drug.[33]

Lipids

Compounds that exhibit solubility in organic solvents such as chloroform, acetone and benzol are much insoluble in water. These are often fatty acids like saturated fatty acid, linoleic acid or are often oils like olive oil, vegetable oil, corn oil, peanut oil and herbaceous plant oil. Solid lipids are the the esterified derivatives of glycerin and fatty acids and have specific

characteristics in terms of temperature and polarity. These solid lipids are well tolerated GRAS substances. Some examples embrace beeswax, wax, Dynasan® 118 (glycerin organic compound of designated saturated, even numbered and branchless fatty acids of plant origin), Precifac (palmityl palmitate), saturated fatty acid, Apifil (PEG-8 beeswax), Cutina CP (cetyl palmitate) and Compritol® 888 ATO (glyceryl behenate). Liquid lipids (oils) are entity like carboxylic acid esters or alcohols (2- octyldodecanol). Attributable to wonderful polar character, they are considered nearly as good solvent for hydrophobic medication as compared to hydrocarbons. Some examples embrace Cetiol V (decyl oleate), Miglyol 840 (propylene glycol dicaprylate), oleic acid, vegetable oil and olive oil. These lipids are amphiphilic (except castor oil and davana oil) attributable to presence of each lipotropic portion (hydroxyl group) and also the deliquescent portion (the esterified chain). The melting temperature or fusion temperature for a supermolecule will increase and decrease with changes in their mass and unsaturation of the fatty acid chain severally.[24,33-34]

Surfactants

Hydrophilic, lipophilic and amphiphilic surfactants units are used in NLC. Water-insoluble surfactants like Span 20, Span 80, Myverol 18-04K penetrate and fluidize biological membranes. The soluble surfactants like Poloxamer 188 and Tween 80 solubilize the membrane elements. These surfactants tend to point out toxicity thanks to their interaction with skin elements.[35] Cationic surfactants like linear alkyl-amines and alkyl-ammoniums are more toxic than anionic surfactants like soaps and alternative carboxylates. The non-ionic surfactants (such as ethoxylated linear alcohols) are thought to be the safest of all the surfactants. hydrophilic surfactants like polysorbates or polyethoxylated vegetable oil derivatives are advised as non-irritant and safer. Irrespective of the positioning of application, non-ionic surfactants are wide utilized in the formulations thanks to their nontoxic nature inside an explicit limit.[36] it's been seen that Pluronic F68, polysorbates, polythene glycol (PEG), polyvinyl alcohol are substances normally used as stabilizer within the formation of NLC.[37-39] A mix of surfactants in specific ratio is thought to produce higher stability by preventing the particle aggregation for particulate distributed system like NLC.[6] In a number of the recent studies, PEG has been used in NLC to enhance its circulation half-life that helps in accumulating higher concentration of drug to the tumour tissues.[40, 41]

Additives

Other excipients like preservatives, cryoprotectants and antioxidants are additional to reinforce the steadiness of NLC.

Preservatives

Since the NLC formulations are developed by employing comparatively high water content, so preservatives are incorporated in it.[42-44] Usually, ethanol, propylene glycol and pentylene glycol are used as preservatives in NLC. it's been ascertained that NLC preserved with 5-10% w/w propylene glycol didn't show any significant amendment in particle size or in zeta potential in comparison with the non-preserved formulation.[45] Equivalently, the use of 5% pentylene glycol was conjointly tested to produce stable NLC over a period of 4 month.[45, 46]

Antioxidants

Oxidation of unsaturated carboxylic acid and sometimes the drug molecules could cause major chemical instability in liquid formulation. Antioxidants incorporated to defend the chemical degradation of liquid lipids utilized in NLC. lipophilic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), β-carotene, α-tocopherol, and propyl gallate is used for this purpose.[47]

Cryoprotectants

Cryoprotectants are best-known to supply stable unimodal size distribution of particles on reconstitution or re-dispersion of lyophilized formulations.[48,49] NLC as binary compound dispersion may result in the difficulty of instability attributable to reaction and oxidation of lipid phase due to the presence of water and other element therein, thus need freeze-drying (lyophilization). Significantly, in freeze-drying of nano-sized particles, cryoprotectants are used for the convenience of re-dispersion. Vital work has been done to date during this explicit space addressing the improvement of variables like type and concentration of cryoprotectants and freeze temperature on the overall quality of NLC.[50,51] PEG4000, Avicel, dextrose, sucrose, sorbitol and aerosil are some additive ordinarily used as cryoprotectants in R&D and usually utilized by researchers while carrying out freeze-drying of NLC.[48,49,52,53] The concentration of cryoprotectants ought to be inside the limit of 1-5% and increase in concentration of cryoprotectants result in rise in particle size of NLC.[54]

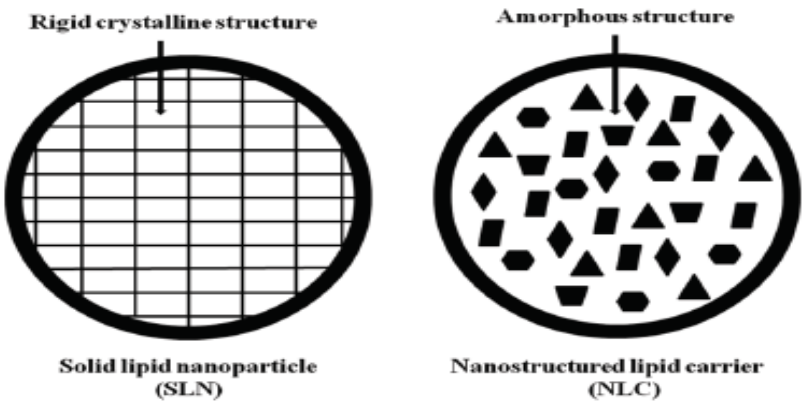


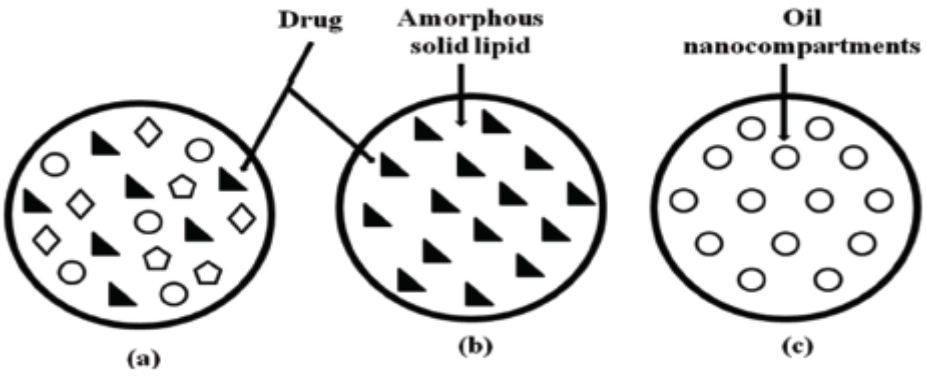
Figure2: Structure of solid lipid nanoparticle (SLN) and nanostructured lipid carrier (NLC).[142]

TYPES OF NLC

Type I or Imperfect crystals: This contains a imperfect matrix with several void in it where the drug molecules can accommodate in sufficient quantity. This NLCs can be prepared by mixing solid lipids with partialy different liquid lipids such as: glycerides containing different fatty acid to achieve long distance between fatty acid chains.

Type II or Amorphous Structure: It defends expulsion of drug during storage by preventing by blocking re-crystallization procedure. It can be produces by adding liquid lipid such as isopropylmyristate or Medium Chain Triglycerides which potentially inhibit recrystallization in solid state.

Type III or Multiple Structure: This type prohibit the crystallization of liquid lipid and hence minimize drug expulsion from the NLCs. It can be produced by mixing higher amount of liquid lipid with solid lipid which produce ultra-small nano-oil compartments where significant amount of drug molecules can stored.



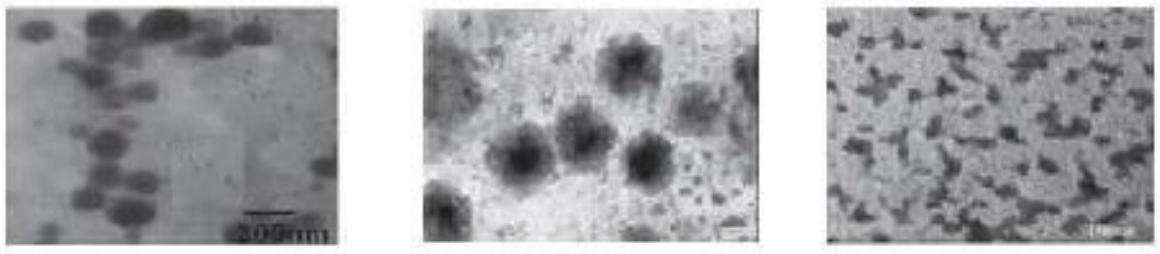


Figure3: Types of NLC (a) imperfect crystal, (b) amorphous, and (c) multiple type and their respective TEM image.[142]

METHODS OF PREPARATION OF NANOSTRUCTURED LIPID CARRIERS:[55,56,57]

High Pressure Homogenization (HPH) technique

This technique is extremely effective. Principle involved is high homogenization by pushing the liquid through high pressure (100 – 2000 bar) within a gap of couple of microns. This method evolved from its use within the preparation of o/w emulsion for parenteral medications.

Hot homogenisation technique (HHT)

In this technique the drug is added to the lipid at 5-10^{0}C above melting temperature of the lipid, stirring is done with addition of aqueous wetting agent solution along with lipid dispersion at a similar temperature. Emulsion obtained is then homogenized, and ensuing hot o/w microemulsion is cooled to a lower temperature to realize NLCs. It's applied for lipophilic and insoluble medication. Hence the exposure time is brief with higher temperature as most of the heat sensitive medication processed safely. This method doesn't suit incorporating the water-soluble medications since; when homogenization is finished low encapsulation efficiency is also resulted in higher proportion of drug left in water. During this technique some drawbacks elicited by the temperature are drug degradation and drug distribution into the aqueous section.

Cold homogenisation technique (CHT)

In order to beat the issues of hot homogenization technique such as: (i) drug degradation as a result of heat (ii) Partitioning of hydrophilic compounds into aqueous section at elevated temperature (iii) Particle growth and crystallization, cold homogenization technique has been utilized. The principle concerned is dispersion of drug in molten lipid. Cool the melted portion and solid lipid is formed the lipid microparticles. The lipid microparticles then spread in an exceedingly cold surfactant solution. Homogenization is conducted at room temperature or below, generating NLCs. Compared to hot homogenization, there's increase in size of the

particle and polydispersity index is seen in cold homogenization. The cold homogenization solely reduces the thermal exposure of drug, however it will not avoid heat totally as a result of melting of the lipid/drug mixture in the first step .This technique is a lot appropriate for thermo-sensitive products. It‟s an energy intensive method and leads to formation of big particle size & broader size distribution.

Ultrasonication/High Speed Homogenization

The principle concerned in NLCs preparation is ultrasonication or high speed homogenization. Both ultrasonication and high speed homogenization is critical for smaller particles. During this technique drug is added to the hot melted lipid solution followed by the addition of hot aqueous section to the lipid phase. Then emulsification is done by probe sonicator or by high speed stirrer. Emulsion produced is sonicated by probe sonicator, then the obtained nanoemulsion is filtered to obtain NLCs. Advantage during this technique is not any temperature elicited degradation, nominal shear stress and equipment's are simple to use. Disadvantage during this technique is chance of metal contamination, particle growth upon storage, broader particle size distribution.

Solvent emulsification - diffusion methodology Technique

Solvent emulsification - diffusion method is dispersion of lipid matrix in water. Under reduced pressure emulsification is finished in an aqueous compound section and within the aqueous compound medium lipid gets precipitated therefore the nanoparticle dispersion is created. The range of average particle diameter is found to be within the range of 30-100 nm. Advantage of this methodology is absence of thermal stress. It's an especially energy intensive method and involves use of organic solvents.

Solvent evaporation/emulsification technique

Principle concerned during this methodology is that in an exceedingly water incompatible organic solvents (like- cyclohexane, chloride, toluene, chloroform), drugs and lipids are dissolved. By applying high speed homogenizer emulsification is done in a binary compound section and at reduced pressure (40-60 mbar). The prepared emulsion is evaporated to get rid of organic solvent by stirring at room temperature.

Microemulsion based technique

Microemulsions comprises of two-phase systems comprising of oil and binary compound phases. Principle of this methodology is concerning microemulsion dilution. These is done by

stirring an optically transparent mixture of surfactant, co-emulsifier, lipid and water at 65-70ºC and in cold water (2- 3ºC) the obtained microemulsion is distributed under stirring. The quantity ratios of the produced microemulsion to cold water were found to be in the range of 1:25 to 1:50. As a result, suspension of lipid particles created. It is filtered and after that washed with dispersion medium and NLCs are obtained. Advantage of this methodology is low energy is needed and is on technically stable. Disadvantage of this is Low nanoparticle concentrations.

Spray drying methodology

Principle of this methodology is that a binary compound NLC dispersion is modified into a solid product by spray drying. Merits of this methodology are cheaper cost as compared to drying up. Some of the demerits are: lipid having over 70°C of melting temperature is used and frequent particle aggregation.

Supercritical fluid (SCF) methodology

The good alternative of solvent for this methodology was found to be CO2 (99.99%). It is considered being safer as oxidization of drug material is impossible. This method usually uses miscible SCF-CO2 organic solvents (DMSO, DMFA). this method produces nanoparticles by varied methodology that are: i) Rapid expansion of supercritical solution (RESS) ii) Particles from gas saturated solution (PGSS) iii) Gas/supercritical antisolvent (GAS/SAS) iv) Supercritical fluid extraction of emulsions (SFEE).

Double Emulsion methodology

This methodology is appropriate for principally water-soluble drugs and therefore the principle is predicated on solvent emulsification-evaporation methodology. Double emulsification is administered to make w/o/w double emulsion. Stabilizers are added to encapsulate the drug and to stop drug from dividing into external water section throughout solvent evaporation within the external water section of w/o/w double emulsion. In solution drug is dissolved and it's blended in liquefied lipid, stabilizer is added to stabilize it. Then this stabilized emulsion is distributed in binary compound phase containing hydrophilic surfactant. Hence results in production of double emulsion that is then stirred and isolated by filtration. Advantage during this methodology is higher proportion of microparticles are produced.

Precipitation methodology

The technique concerned during this methodology is; Glycerides dissolved (e.g. chloroform) in an organic solvent and in a binary compound section the solution undergoes emulsification. Later the organic solvent gets removed by evaporation and the nanoparticles are obtained through the precipitation of lipid.

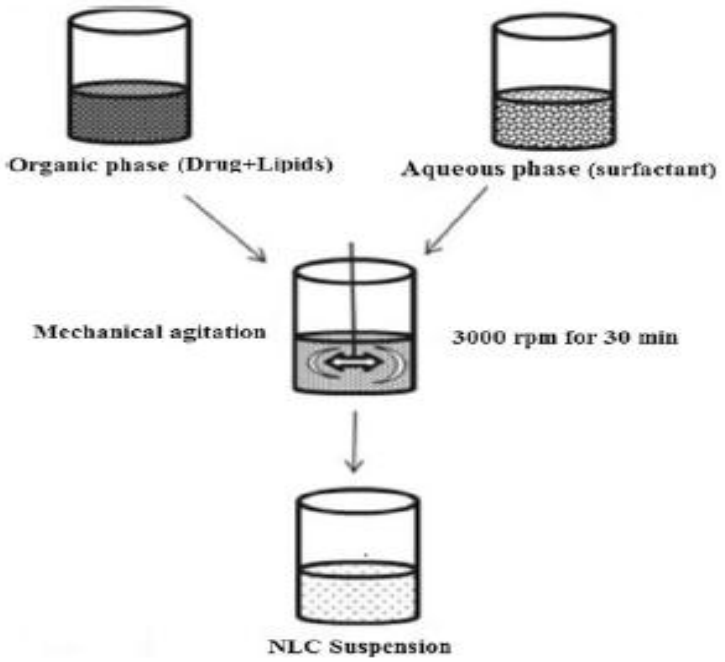


Figure 4: Output of NLC nanosuspension.[137]

FACTORS INFLUENCING FORMULATION OF NLC

The major factors influencing formulation of NLC are as Follows:

Effect of Lipids

Lipids (solid and liquid lipids) enhance the solubility of hydrophobic drug molecules leading to improvement of their oral bioavailability. The choice of lipids relies on the solubility of drug within the lipid section. Lipids having most drug solubility chosen for lipoid section of NLC. In general, lipids represent 5-10% of total composition of NLC. Enhancing the overall lipid content of the formulation ends up in increase drug loading but it conjointly ends up in multiplied particle size and reduced stability.[58]

Effect of Surfactants

Surfactants are utilized to retain the steadiness of distributed system like emulsion and/or nanoparticulate dispersions by minimizing the aggregation of dispersed phase.[59] Like different lipidic nanoparticles, the type and concentration of the surfactants powerfully affects

the particle size of NLC. Sometimes 1-5% stabilizing agents are employed for the preparation of NLC. However, higher concentration of stabilizers relative to the lipid ends up in smaller nanoparticles.[58] The decrease in stabilizing agent concentration ends up in increase in particle size throughout storage.[60] Typically non-ionic surfactants (Tween 80, poloxamer 188) are used to prepare NLC. Non-ionic surfactants result in tiny particle size of NLC and supply steric stability to NLC formulations. Such associate observation has been reported by Han et al. wherever ionic and non-ionic surfactants used compositely resulted in a very stable disperse system, with slender particle size distribution and smallest mean particle size.[61]

Effect of Hydrophile-lipophile Balance (HLB)

Values of Surfactants Generally to stabilize the NLC system ought to be over 10. It greatly affects the stability, particle size distribution, polydispersity index, and entrapment potency of the NLC system. HLB values of lipid section used for the preparation of NLC plays a serious role for the choice of surfactants. The HLB values of surfactants should be up to or larger than the desired HLB value of lipoid section.[62,63] As the concentration of water-soluble surfactant will increase, the general HLB value of the NLC system also increases and this reduces the surface free energy of the dispersed phase, which ends up in decrease in particle size distribution of NLC system. Reduction in particle size distribution, more increase the general expanse of the mixture system and ultimately improves the entrapment efficiency.[62]

Effect of Process Parameters

Effect of Temperature

It is suggested that the temperature of the solid lipid must be 5-10 °C on top of its melting point then solely it's shows solvent capacity for the drug. Sometimes high release is determined with increase in temperature.[56] In a study, it was found that the lower temperature of dispersing medium led to the lower diffusion rate of organic dispersion part and consequently formed the comparatively big particles with wide size distribution.[64] On the opposite hand, if the temperature is too high the lipids can degrade.[59] Hot temperature could also cut back the emulsifying capability of surfactants as these have cloud point less than 85°C.[64]

Effect of Stirring Time

Stirring at a continuing speed is needed to boost the drug emulsification because it affects the particle size of the composition. Usually, stirring for 3-5 minutes at higher rotation results in formation of a coarse emulsion.[59]

Effects of Formulation Techniques

Formulation techniques greatly have an effect on the particle size and polydispersity index (PDI).[59] Until now, high pressure homogenization (HPH) has been found to be the foremost successful technique to scale back the particle size compared other strategies mentioned.[65]

CHARACTERIZATION OF NANOSTRUCTURED LIPID CARRIERS

Characterization of the NLC is incredibly necessary attributable to complexity of the system and mixture size of NLC. Moreover, appropriate characterization of the formulations is critical to control the standard, stability, and release dynamics of NLC. Thus, correct and sensitive characterization strategies should be used. Following parameters will be evaluated that have direct impact on stability and release dynamics of NLC.[66]

Particle Size

Particle size plays a crucial role within the uptake of NLC from GIT upon oral intake and their clearance by the reticuloendothelial system. Definite determination of the particle size is very necessary for in vivo performance. Photon correlation spectroscopy (PCS)[67-69] and optical laser diffraction[70,71] are the foremost techniques used for the determination of particle size of NLC. The variation of the intensity of the scattered light-weight, caused by movement of nanoparticles, is measured by these techniques. PCS is comparatively correct and sensitive methodology. However, this system will detect the nanoparticles of size ranges from few nanometers to concerning about 3μm.[72,73] This size range is efficient to characterize NLC. On the other hand, laser diffraction able to detect larger particle sizes (>3 μm).[72,73]

Polydispersity Index (PDI)

Since NLC are typically polydisperse in nature, mensuration of polydispersity index (PDI) is crucial to understand the size distribution of the NLC.[66] The lower the PDI value, the more of monodispersed the nanoparticle dispersion is. Most of the researchers settle for PDI worth of 0.3 as optimum worth.[74,75] PDI will also be measured by PCS.[67,76]

Zeta potential

The zeta potential (ZP) indicates the general charge a particle attains during a explicit medium. Stability of the NLC during storage will be foreseen from the ZP worth. High ZP indicates extremely charged particles. Generally, high ZP (negative or positive) inhibits aggregation of the particles attributable to electrical repulsion and stabilizes the NLC

dispersion. Conversely, in case of low ZP, attraction exceeds repulsion and also the dispersion coagulates or flocculates.[66] However, this assumption isn't appropriate for all mixture dispersions, significantly the dispersion which contains steric stabilizers. The ZP of the nanodispersions will be determined by PCS.[67-69]

Morphology

Microscopic techniques like scanning microscopy (SEM), transmission microscopy (TEM), and atomic force research (AFM) are typically used to determine the form and morphology of NLC. These techniques can also be done to confirm the particle size and size distribution.[66] SEM utilizes lepton transmission from the sample surface, whereas TEM utilizes lepton transmission through the sample. Though traditional SEM is not adequately sensitive to the nanosize, whereas, field emission SEM (FESEM) can detect nanoparticles effectively.[77] Sample preparation greatly influences the results of SEM analysis. Cryogenic FESEM can even be useful wherever liquid dispersion is frozen by N2 and images are taken at the frozen condition. AFM provides a three-dimensional surface profile not like microscopy which provides solely two-dimensional pictures of a sample. AFM directly provides structural, mechanical, functional, and topographical details concerning surfaces with metric linear unit to A scale resolution.[78]

Crystallinity and Polymorphism

Determination of the crystallinity of the parts of NLC formulations is vital because the lipid matrix and also the drug might bear a polymorphic transition resulting in a undesirable drug expulsion throughout storage.[79] Differential scanning measure (DSC)[68,69] and X-Ray diffractometry (XRD)[71] are most typically used techniques to work out the polymorphic behavior and crystallinity of the parts of the NLC. DSC provides info on the melting and crystallization behavior of all solid and liquid constituents of NLC, whereas XRD will acknowledge specific crystalline compounds supported their crystal structure.[80] DSC utilizes the very fact that lipid macromolecule modifications possess different melting points and melting enthalpies. In XRD, the monochromatic beam of X-ray is diffracted at angles determined by the spacing of the planes within the crystals and the kind and arrangement of the atoms that is recorded by a detector as a pattern. The intensity and position of the diffractions are distinctive to every kind of crystalline material. XRD pattern will predict the style of arrangement of lipid molecules, part behavior, and characterize the structure of macromolecule and drug molecules.[81,82]

Drug release from NLCs

Since the NLC carries with it solid lipid matrix, they supply controlled release for the drug.[14] The prolonged release can be dominated by the number of liquid macromolecule in the formulation.[18] In most of the cases, a biphasic drug release is discovered with an initial burst discharge followed by release at a relentless rate in all probability from the solid macromolecule core. The drug dissolved in liquid lipid exist at the outer boundary shows initial burst release.[83] Attributable to high drug stacking in the unorganized lipid matrix, impulses like temperature, agitation and warmth can even trigger burst release.[62,84-88]

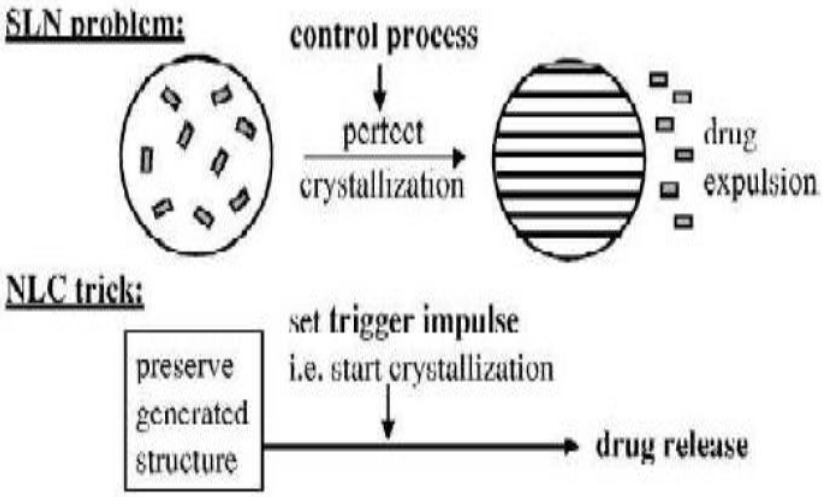


Figure 5: Triggered release of drug from NLC the transform form of SLN.

Stability of nanostructured lipid dispersions

NLCs might contain other colloidal structures, such as micelles, mixed micelles, liposomes and nanoemulsions which contribute to their stability. There also are some major stability problems throughout storage, like increase in particle size, gelation of the dispersion and drug expulsion from the lipid matrix. Possible gelation is attributable to formation of the network and lipid bridges between the particles. The physical stability of those dispersions is mostly investigated by measurement of particle size, surface charge by using Photon correlation spectrum analysis (PCS), Laser diffraction (LD), zeta potential (ZP) and thermal analysis through Differential scanning measuring (DSC).

ADVANTAGES OF NANOSTRUCTURED LIPID CARRIERS OVER OTHER LIPID BASED CARRIERS

The following sections explicate varied mechanisms and theories that allow NLC system overcome general limitations with conventional lipoid formulations and prove them to be an

improved formulation style. it's renowned that NLC are new kind of lipid nanoparticles which provide the benefits of improved drug loading capability alongside stable drug incorporation throughout storage.[18,89] The potential explanations for these fascinating attributes of NLCs mentioned intimately in the subsections below.

Higher drug loading and entrapment potential

In SLN, it's discovered that the drug quantity soluble within the melted lipid before particle production is higher than within the final SLN and such higher drug concentration within the soften lipid would possibly result in immediate drug expulsion throughout the cooling method. In distinction, in NLC the solid matrix of the supermolecule nanoparticle contains a liquid-oil section in which drug solubility is higher, thus increasing the overall drug loading capability.[18,90] Thus, in NLC, liquid lipids content affects the defense potency to an excellent extent as a result of they cause many crystal defects in solid matrix and cause imperfections in extremely ordered crystal matrix providing sufficient area for significant quantity of drug to lodge with success.[91]

Modulation of drug release pattern

NLC exhibit a biphasic drug release pattern that's, initial burst release of drug followed by a sustained release at a continuing rate. The liquid lipoid placed within the outer layers of the nanoparticles forms drug-enriched casing that ends up in burst release of the drug at the initial stage. Unlike SLN, these oil-enriched outer layers possess well higher solubility for lipotropic drugs;[92] thus, the next quantity of drug may well be simply loaded, additionally as free by the drug diffusion or the matrix erosion.[93] The initial quicker drug release part is followed by slow release from the solid lipoid core. Apparently, in NLC it's feasible to improvise the discharge profiles as a operate of the lipoid matrix composition like by varied the quantity of liquid lipoid content with relation to solid lipoid.[94]

Long-term stability of incorporated drug during Storage

The conception of NLC that's, macromolecule matrices that are solid, but not crystalline comes from the very fact that crystallization process itself causes the expulsion of the drug. By victimization special mixtures of solid lipids and liquid lipids, the particles become solid once cooling however don't crystallize.[14] This affecting the particle size, defense potency and in vitro drug release characteristics, the liquid lipids constitutional the solid macromolecules in NLC[95] additionally resolve the hitch of changes primarily crystallinity and polymorphism that will occur upon semi-permanent storage. As crystallization happens

attributable to supersaturation, the presence of liquid macromolecule is probably going to carry on to sub saturation condition of the solid lipid, thus mitigating crystallization.[94]

Minimum level of surfactant with maximum drug loading potential

NLC are simply stable with a minimum possible concentration of surfactants beside best results of stability, entrapment, and release. Sometimes, even 0.5-1% of the surface-active agent is enough for developing stable NLC of oleophilic medication. Additionally, full array of excipients is accessible being of accepted state in distinction to LE wherever there is an awfully very little scope to play with excipients. This attribute makes NLC way more well-liked formulation approach than LE where high and restricted use of surfactants is a difficulty of concern.

Drug Encapsulation Efficiency

Determination of drug-loading potency is incredibly necessary for NLCs since it affects the discharge characteristics.[99] The lipotropic drug molecules could homogeneously distribute in the supermolecule matrix or enrich the core or particulate shell. Aqueous and surface phases provide locations for loading hydrophilic medicine. The requirement to achieving high loading capability is related to solubility of the drug within the lipids. The solubility thought to be above needed as a result of it decreases once cooling down the melt and should even be lower within the solid lipids.[14] The encapsulation proportion of the medicine in NLCs relies on the separation of the interior and external phases. To separate the dispersions, different techniques like ultrafiltration, centrifugation, gel filtration by Sephadex, and chemical analysis techniques are used.[100] As compared to SLNs, the incorporation of liquid oil to solid lipid in NLCs results in huge crystal order disturbance. The resulting matrix indicates nice imperfectness within the lattice and leaves extra space to accommodate the medicine. The encapsulation efficiency and loading capability of the medicine thus improved.

DRUG CANDIDATES FOR NANOSTRUCTURED LIPID CARRIERS

In general, the criterion used to classify the medicine includes BCS, which means that binary compound solubility and membrane permeability are 2 major factors limiting drug absorption.[101] Considering the biopharmaceutical obstacles in oral absorption of lipotropic medicine like UWL, P-gp flow, intra-enterocyte, and internal organ metabolism, BCS alone isn't a satisfactory tool for selecting drug candidates for advanced lipid-based formulations. There is a necessity of a modified organization, which also takes into consideration attributes

like drug metabolism, disposition, and also the role of transporters as they have an effect on the absorption method to an surprisingly massive extent.

A review of these drugs, done by Wu and Benet,[96] classified the drugs in categories I-IV of BCS such medicine in categories I and II were metabolized and eliminated, in distinction, categories III and IV medicine were eliminated unchanged as shown in Figure 6, This is a core criterion for the tailored organization, namely biopharmaceutics drug disposition classification system (BDDCS). In accordance with this method, the extent of metabolism (or major route of drug elimination) substitutes membrane permeability as classification condition.

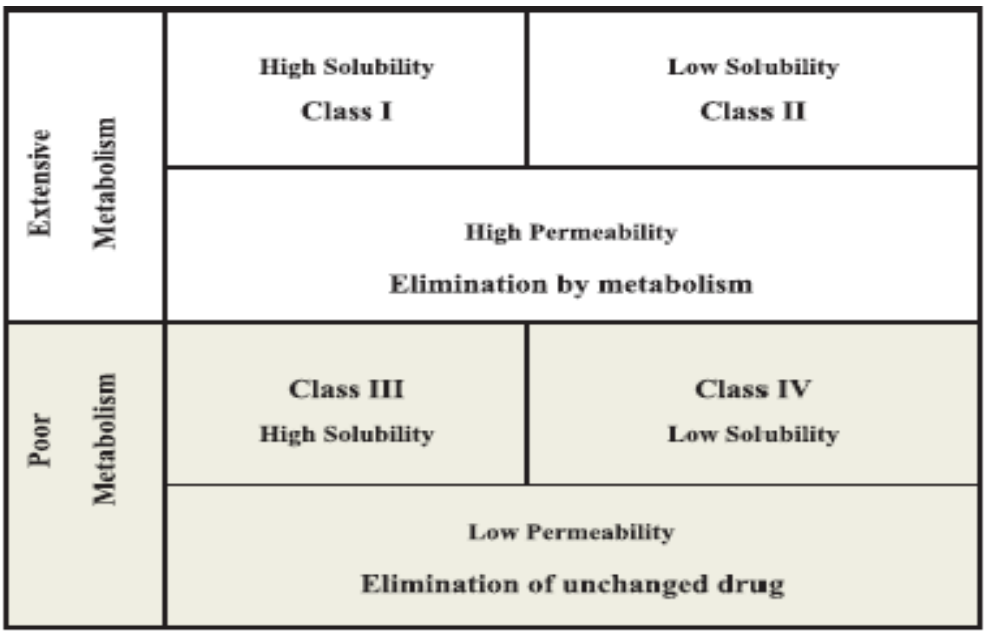


Figure6: Depiction of a biopharmaceutics drug disposition classification system based on metabolism and elimination of drug.[144]

Significantly, this method takes into consideration the information of flow transporters and pre-systemic metabolism. Lipotropic and poorly water soluble drugs, that are classified as category II or IV are identified to be potential substrates for enteral flow transporters such as P-gp[96,102,103] and are identified to be metabolized by intestinal CYP enzymes. Consequently, BDDCS might play a vital role in distinct appropriate drug candidates that are expected to benefit from NLC formulations. As per this classification, absorption of class II drugs may be greatly increased probably by selection of these lipids within the formulations that influence metabolism and/or flow. The understanding of a specific transporter(s) within the disposition of a selected drug can guide appropriate lipidic excipient choice, with intent of modulating

this result and rising bioavailability. Hence, BDDCS helps in selecting the suitable drug candidate for appropriate lipid carrier and maximizes the advantages from co-administration of appropriate lipids in NLC.[104]

DRAWBACKS ENCOUNTERED DURING CANCER THERAPY AND HOW NLC OVERCOME IT

Numerous sorts of malignancy, particularly solid tumors, possess challenges to accepted chemotherapy. Notwithstanding the advancement in therapeutic cocktails, the conclusion of therapy remains unsatisfactory. For demonstration, the response rate of pancreatic cancerous ailment, esophageal cancerous disease and gonad cancerous disease to therapy are well beneath 2%.[105] Even in patients with malignancies that are a lot of perceptive to chemotherapeutical agencies, e.g. For breast cancers, the clinical conclusions are usually below expectation.[106] In evaluation to alternative pharmaceutical categories, cytotoxic anticancer prescribed drugs carry distinctive difficulties such as poor specificity, high toxicity and status to induce pharmaceutical resistance. Conventionally administered cytotoxic agencies usually extensively and indiscriminately are a part of body tissues and serum protein in an extremely unpredictable manner. solely a little a part of the drugs reach the tumor growth location.[107] This might each reduce the therapeutic effectuality and boost general pharmaceutical toxicity. Moreover, even if cytotoxic medicines are able to completely inhibit cancerous cells, they're conjointly venomous to noncancerous cells, notably due to rapid splitting up. The conventional tissue toxicities frequently occur even once maximized therapeutic doses of anticancer medicines are administered. The poor specificity of cytotoxic medicine in terms of each pharmaceutical bio distribution as well as pharmacology of those medicines at the cellular level impersonates a major challenge to productive antitumor remedy. This poor aspect impact profile of cytotoxic pharmaceuticals has significantly diminished the therapeutic worth of the prescribed drugs. Wishing on the alternative of medication, totally different organs or tissues may be affected by the non-specific activity of the cytotoxic agencies.[108,109] Whereas edge effects like nausea, vomiting, fatigue, and hair loss are ordinarily caused by the majority cytotoxic prescribed drugs, some side-effects are drug-specific. For demonstration, anthracyclines will origin cardiotoxicity.[110,111] Some of these side-effects are additive and life-threatening. Cytotoxic medicine usually show steep dose-response bend and high dose intensity is required to control therapeutic success.[112] This ends up in a troublesome quandary for clinicians to pick out between high drug doses with a high risk of usual tissue toxicities or reduced pharmaceutical doses with reduced

chances of therapeutic answer. Additionally, there are alternative obstacles to the optimal presentation of standard therapy. Cancerous diseases work through mechanisms at cellular grade that weaken the toxicity of chemotherapeutic agencies they're exposed to. These protective behaviors against mechanisms are typically categorized as "cellular" pharmaceutical resistance. The foremost outstanding one is that the multidrug resistance (MDR), which engages untiring effluence of a broad kind of cytotoxic pharmaceutical substances out of the protoplasm by membrane based transporters.[113-115] In supplement to cellular mechanisms, cancerous units in solid tumors are inclined to be a lot of resistant to therapy than non-aggregating cancerous cells as a result of numerous pharmaceutical permeation obstacles, that makes it difficult to realize high intra-tumoral drug concentration in solid tumors.[113] This sort of drug resistance, or sometimes mentioned as "non-cellular" drug resistance, could farther lead to compromised clinical outcomes even if associate degree of the anticancer drug has powerful in vitro effectuality. We believe NLC supply a pledge to beat a minimum of a number of these obstacles.

APPROACHES TO IMPROVE ANTICANCER POTENTIAL OF CHEMOTHERAPEUTIC DRUGS THROUGH NLC

So far variety of antitumor moieties are inducted and developed as NLC. Diagnosis testing done on cell lines or victimization animal models have shown promising effects involving the development within the antitumor activity of cytotoxic drugs with adverse effects.

Approaches to overcome obstacles with anti-cancer drug delivery are described below:

Tumor-specific Targeting

Through NLC as mentioned earlier most cytotoxic medicine exhibit narrow therapeutic window since they have very poor targeting ability. Many times, the chosen dose is near most tolerated dose so providing a challenge to an efficient drug delivery.[116] Recently, NLC are shown to exhibit nice potential to target tumor cells. Tumor targeting is possible through passive or active drug targeting.[116]

Passive Tumor Targeting Through NLC

Abnormalities in neoplasm vessels result in increased vascular permeability since the administered nanocarriers extravagate and get localized within the interstitial space. This retention by passive development is termed as „Enhanced permeability and Retention‟ (EPR) effect.[117,118] The EPR impact in solid tumors was introduced by Matsumura and Maeda in 1986.[119-121] Their findings advised that the neovasculature in most solid tumors sometimes

have an abnormal architecture compared to traditional tissues and organs, containing defective epithelium cells with massive aperture, rough vascular alignment, absence of a smooth-muscle layer, wide lumen and broken receptors for angiotensin (AT-II).[119] Also, the pore size of neoplasm vessels typically ranges from a 100 to 780 nm. Of these anatomical imperfectness, along with functional abnormalities, result in in depth leakage of plasma parts into the neoplasm tissue, such as macromolecules, supermolecule particles, nanoparticles.[122] Further, the dysfunctional body fluid clearance, and the slow blood vessel recovery in neoplasm tissue indicate that macromolecules are preserved in neoplasm sites, whereas extravasations into neoplasm interstitium continues.[121] Passive targeting is accomplished by sterilization the shape, size and surface characteristics of the NLC. However, there stay important barriers to move that usually result in low drug concentrations at the neoplasm site and, consequently, low therapeutic effectivity.[123] What is more, passive targeting suffers from a number of constant limitations of traditional therapy like an inability to actively distinguish healthy tissue from neoplasm tissue.[124] Wang et al. engineered nanostructured lipid carriers for co-encapsulating paclitaxel (PTX) and antibiotic drug (DOX) for combinable lung cancer medical care.[125] After blood vessel administration, NLCs quickly get attached with opsonins in blood and cleared by the reticuloendothelial system (RES) at intervals couple of minutes. Therefore, NLCs continuously targeted in tissues with an affluent RES, like liver, spleen and body fluid nodes. This passive targeting/EPR effect is favorable if the neoplasm exists in RES. Moreover, the toxicity may be reduced as a result of fewer drug molecules distributed to alternative organs.[126]

Active neoplasm targeting Through NLC

The incorporation of active targeting ligands is meant to improve and enhance nanoparticle accumulation at the tumor site. The ligands unremarkably used folic acid, hyaluronic acid, protein (Fr), antibody (transzumab), aptamers and peptides.[126] Actively targeted medicine to the neoplasm sites offer many distinct benefits over non-targeted medicine. The most advantage includes high drug retention within the neoplasm tissue. Dose connected adverse effects are also expected to be less.[127] Most actively targeted NLC can be designed to exhibit passive endocytosis or endocytosis involving specific interactions with the receptors.[128] A study done by Khajavinia et al. showed that beta globulin conjugated stearylamine NLC of etoposide to focus on K562 acute myelogenous malignant neoplastic disease cells exhibited 15-fold reduction in IC50 than the free etoposide.[129] Though ligand joined and receptor mediate targeting through NLC can expeditiously target tumors however it must be noted that

the targeted nanostructured lipid carriers (NLC) is removed by the RES at intervals minutes before they're ready to bind to tumor cells.

Fabrication of Long Circulating NLC to avoid clearance by reticuloendothelial system

Generally, the nanostructured macromolecule carriers are cleared by blood and RES,[130] therefore it's necessary to increase the current circulation time of NLC, and this has been made possible to some extent. The NLC with surface modification by explicit hydrophilic polymers don't seem to be simply recognized by the RES.[131] PEGylation may be used to surface modification technique to reduce immunogenicity and thus convey long current property. High molecular weight of PEG (PEG 20,000 to 50,000) offers increased recirculation of smaller NLC whereas low mass of PEG (PEG 3400 to 10,000) will increase the hydrodynamic radius of huge nanocarriers[132,133] Some deliquescent materials, like polyethylene glycol (PEG), poloxamers or poloxamines and numerous amphipathic polymers, the lipophilic a part of which can be attached into the inner core of the NLC and therefore the hydrophilic part of which may cover the surface and widely used for this purpose.[126]

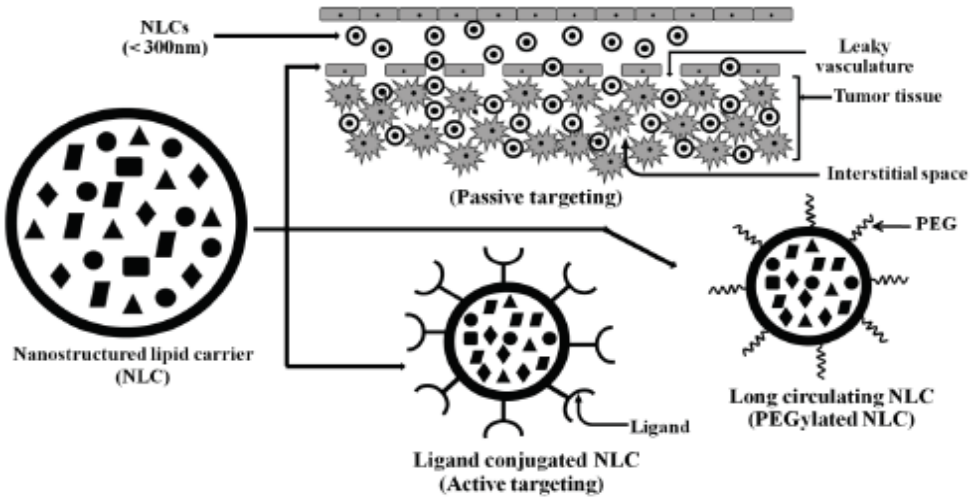


Figure 7: Targeting strategy of NLC. (1) The passively targeted NLC that exhibit a short t1/2 (2) The ligand conjugated NLC that exhibit site specific tumor targeting by receptor mediated endocytosis (3) The PEGylated NLC which elope RES.[142]

Although for different cancers, RES clearance is anticipated a foremost barricade to general cytotoxic pharmaceutical consignment by NLC. Once a particulate pharmaceutical carrier is cased with amphiphilic polymers the carrier becomes a lot of immune to RES clearance. This is often partially because these polymers favor exterior surface assimilation of proteins that suppress body process in vivo.[130] This type of polymer-coated drug delivery strategy is

usually mentioned to as "stealth", for his or her proficiency to ability to evade the barricade of immune system, or more justly "long – circulating" drug carriers. This kind of systems is ready to remain within the circulation for prolonged times, with a half-life of some of hours in animal models to as high as 55h in human subjects.[130,134,135] Long-circulating NLC formulations were ready by coating the nanoparticles with a polymer compound. To facilitate more protected attachment of the hydrophilic compound up on the surfaces of the lipid cores, compound molecules were pre-conjugated to lipophilic moieties to create amphiphilic concealment outer layer agents. By increasing the engrossment of the outer layer it absolutely was shown that the zeta potentials of nanoparticles were shrunken and their mean diameters were inflated.[136] The use of higher concentration of lipid and compound which moderately enlarged the polydispersity catalogue. Hence, one ought to settle for in mind that outer layer NLC with a stealth modification might have an effect on the clearance rates by the RES, which considerably control NLC presentation, security or formulation stability.

Approaches to Combat Multidrug Resistance (MDR) Through NLC

Despite tremendous advancement in cancer treatment, risk of resistance to therapy (multidrug resistance- MDR) may be a challenge. The improved effluence rate of drug caused by overexpressed ABC transporters, P-gp and multidrug resistance-associated macromolecule one (MRP1), reduce cell uptake, resistance to Topoisomerase II are few number of the reasons for MDR. Therefore, modern analysis into reversing MDR is primarily targeted on block specific drug efflux. With the introduction of multifunctional NLC which proved to be an alternative carrier for chemotherapeutics to overcome MDR. Such NLC have confirmed fascinating drug delivery characteristics like delivery of hydrophobic agents, decrease drug clearance, less non-specific cellular uptake, delivery of multiple therapeutic payloads, targeted drug delivery, manageable drug release and lesser pharmacokinetic interactions.[121,137] Recently NLC are developed to encapsulate or attach multifunctional agents like malignant tumor medication, antibodies or ligands targeting MDR cancer cells, nucleic acid, and inhibitors of P-gp to inhibit different contributors to MDR completely. Paclitaxel – doxorubicin drug loaded NLC showed high cytotoxicity in adriamycin resistant MCF-7 cell line and paclitaxel resistant human gonad neoplastic cell line. The reversal power of paclitaxel NLC for these 2 types of cells was 34.3 and 31.3-fold, whereas for doxorubicin NLC was 6.4 and 2.2-fold.[138]

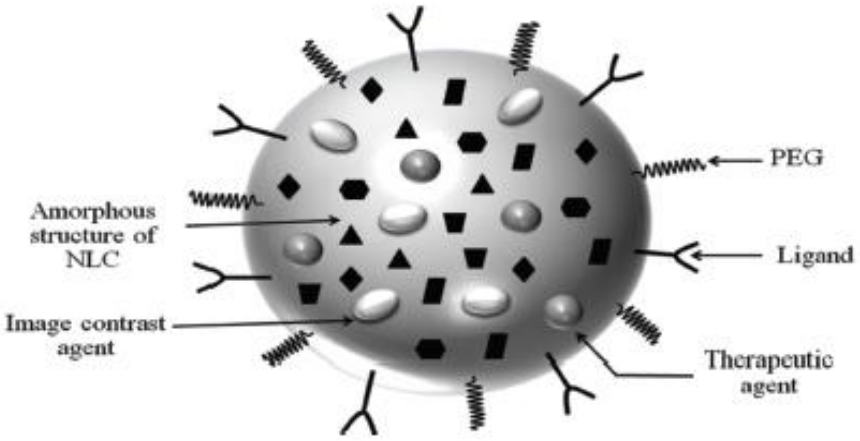


Figure 8: Illustration defining the different characteristics of NLC such as imaging property, drug carrying and targeting capacity with long circulating behavior. [142]

GENE DELIVERY FOR CANCER THERAPY BASED ON NANOSTRUCTURED LIPID CARRIERS

Following the discovery of the many therapeutic genes, increasing attention has been paid to the analysis and development of those genes for clinical medical aid. Several medical aid techniques involve the development of well-organized vehicles that may deliver foreign genes like siRNA, deoxyribonucleic acid to focus on cancerous cells. Recently, the potential of NLC used as a vehicle for gene delivery has additionally attracted nice interest.[131,132] For example, Han et al. developed transferrin-modified antibiotic (DOX) and enhanced inexperienced visible radiation supermolecule inclusion body (pEGFP) (DNA) co-encapsulated NLC (T-NLC) and solid lipoid nanoparticles (T-SLN) for the treatment of carcinoma.[132] Theses results confirmed high transfection efficiency of NLCs and established NLCs as a promising gene delivery system.[133,134]

CURRENT AND FUTURE DEVELOPMENTS

The selection of vehicles is very important for drug delivery to exert most activity and cause least adverse effects. Some novel nanocarriers studied to load medicine for medical care. Among them, NLCs have gained abundant interest in recent years owing to the wide drug carrier efficiency and safety. It‟s expected that the utility of NLCs in basic analysis and therefore the clinical setting will be a lot of in depth within the future owing to imperative desires to discover new therapies like treatments for cancer, neurodegenerative disease, and inflammation. The interest to develop different routes and to treat different diseases with NLCs ought to be continued to increase their applications. Permeation via the epithelial duct and BBB is also a future trend. The mixture of two therapeutically active agents to be

enclosed in an exceedingly single nano-system is another thought for future development. Though some benefits of NLCs for drug delivery incontestable, the mechanisms for increased effectiveness aren't totally understood. Hence, these mechanisms need to be explored additionally with the goal of elucidation and effectiveness improvement of NLC for future drug delivery.

REFERENCE

1. WHO: Cancer. http://www.who.int/mediacentre/factsheets/fs297/en/ (Accessed 29 January, 2017).
2. Bhosle J, Hall G. Principles of cancer treatment by chemotherapy. Surgery - Oxford International Edition, 27(4): 173-7.
3. Boyle P, Levin B. World cancer report 2008: IARC Press, International Agency for Research on Cancer, 2008.
4. Feng S-S, Chien S. Chemotherapeutic engineering: Application and further development of chemical engineering principles for chemotherapy of cancer and other diseases. Chemical Engineering Science, 2003; 58(18): 4087-114.
5. Barratt GM. Therapeutic applications of colloidal drug carriers. Pharmaceutical Science & Technology Today, 2000; 3(5): 163-71.
6. Mehnert W, Mäder K. Solid lipid nanoparticles: Production, characterization and applications. Advanced Drug Delivery Reviews, 2001; 47(2–3): 165-96.
7. Mainardes RM, Silva LP. Drug delivery systems: past, present, and future. Current drug targets, 2004; 5(5): 449-55.
8. Gregoriadis G, Florence AT. Liposomes in Drug Delivery. Drugs, 1993; 45(1): 15-28.
9. Choi MJ, Maibach HI. Liposomes and Niosomes as Topical Drug Delivery Systems. Skin Pharmacology and Physiology, 2005; 18(5): 209-19.
10. Selvamuthukumar S, Anandam S, Krishnamoorthy K, Rajappan M. Nanosponges: A novel class of drug delivery system-review. Journal of Pharmacy & Pharmaceutical Sciences, 2012; 15(1): 103-11.
11. Smith A, Hunneyball lM. Evaluation of poly(lactic acid) as a biodegradable drug delivery system for parenteral administration. International Journal of Pharmaceutics, 1986; 30(2): 215-20.
12. Lherm C, Müller RH, Puisieux F, Couvreur P. Alkylcyanoacrylate drug carriers: II. Cytotoxicity of cyanoacrylate nanoparticles with different alkyl chain length. International Journal of Pharmaceutics, 1992; 84(1): 13-22.

13. 13. Arias JL, Gallardo V, Gómez-Lopera SA, Plaza RC, Delgado AV. Synthesis and characterization of poly(ethyl-2-cyanoacrylate) nanoparticles with a magnetic core. Journal of Controlled Release, 2001; 77(3): 309-21.
14. MuÈller RH, MaÈder K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery–a review of the state of the art. European journal of pharmaceutics and biopharmaceutics, 2000; 50(1): 161-77.
15. H. Muller R, Shegokar R, M. Keck C. 20 Years of Lipid Nanoparticles (SLN & NLC): Present State of Development & Industrial Applications. Current Drug Discovery Technologies, 2011; 8(3): 207-27.
16. Joshi MD, Müller RH. Lipid nanoparticles for parenteral delivery of actives. European Journal of Pharmaceutics and Biopharmaceutics, 2009; 71(2): 161-72.
17. Jores K, Mehnert W, Mader K. Physicochemical investigations on solid lipid nanoparticles and on oil-loaded solid lipid nanoparticles: a nuclear magnetic resonance and electron spin resonance study. Pharmaceutical research, 2003; 20(8): 1274-83.
18. Muller RH, Radtke M, Wissing SA. Nanostructured lipid matrices for improved microencapsulation of drugs. Int J Pharm, 2002; 242(1-2): 121-8.
19. Muchow M, Maincent P, Muller RH. Lipid nanoparticles with a solid matrix (SLN, NLC, LDC) for oral drug delivery. Drug Dev Ind Pharm, 2008; 34(12): 1394-405.
20. MuÈller RH, MaÈder K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery–a review of the state of the art. European journal of pharmaceutics and biopharmaceutics, 2000; 50(1): 161-77.
21. H. Muller R, Shegokar R, M. Keck C. 20 Years of Lipid Nanoparticles (SLN & NLC): Present State of Development & Industrial Applications. Current Drug Discovery Technologies, 2011; 8(3): 207-27.
22. Joshi MD, Müller RH. Lipid nanoparticles for parenteral delivery of actives. European Journal of Pharmaceutics and Biopharmaceutics, 2009; 71(2): 161-72.
23. Jores K, Mehnert W, Mader K. Physicochemical investigations on solid lipid nanoparticles and on oil-loaded solid lipid nanoparticles: a nuclear magnetic resonance and electron spin resonance study. Pharmaceutical research, 2003; 20(8): 1274-83.
24. Muller RH, Radtke M, Wissing SA. Nanostructured lipid matrices for improved microencapsulation of drugs. Int J Pharm, 2002; 242(1-2): 121-8.
25. Muchow M, Maincent P, Muller RH. Lipid nanoparticles with a solid matrix (SLN, NLC, LDC) for oral drug delivery. Drug Dev Ind Pharm, 2008; 34(12): 1394-405.

26. Shidhaye SS, Vaidya R, Sutar S, Patwardhan A, Kadam VJ. Solid lipid nanoparticles and nanostructured lipid carriers--innovative generations of solid lipid carriers. Current drug delivery, 2008; 5(4): 324-31.
27. Muller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv Drug Deliv Rev, 2002; 54(1): S131-55.
28. Saupe A, Wissing SA, Lenk A, Schmidt C, Muller RH. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) -- structural investigations on two different carrier systems. Bio-medical materials and engineering, 2005; 15(5): 393-402.
29. Jenning V, Thunemann AF, Gohla SH. Characterisation of a novel solid lipid nanoparticle carrier system based on binary mixtures of liquid and solid lipids. Int J Pharm, 2000; 199(2): 167-77.
30. Jenning V, Mader K, Gohla SH. Solid lipid nanoparticles (SLN) based on binary mixtures of liquid and solid lipids: a (1)H-NMR study. Int J Pharm, 2000; 205(1-2): 15-21.
31. Müller RH, Dingler A, Schneppe T, Gohla S. Large scale production of solid lipid nanoparticles (SLN™) and nanosuspensions (DissoCubes™). Handbook of pharmaceutical controlled release technology, 2000; 359-76.
32. Uner M. Preparation, characterization and physico-chemical properties of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC): their benefits as colloidal drug carrier systems. Die Pharmazie, 2006; 61(5): 375-86.
33. Souto EB, Müller RH. Lipid nanoparticles: effect on bioavailability and pharmacokinetic changes. Drug delivery: Springer, 2010; 115-41.
34. Pardeike J, Hommoss A, Muller RH. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. Int J Pharm, 2009; 366(1-2): 170-84.
35. Strickley RG. Solubilizing excipients in oral and injectable formulations. Pharmaceutical research, 2004; 21(2): 201-30.
36. Nanjwade BK, Patel DJ, Udhani RA, Manvi FV. Functions of lipids for enhancement of oral bioavailability of poorly water-soluble drugs. Scientia pharmaceutica, 2011; 79(4): 705-27.
37. Lim SB, Banerjee A, Onyuksel H. Improvement of drug safety by the use of lipid-based nanocarriers. Journal of controlled release: official journal of the Controlled Release Society, 2012; 163(1): 34-45.

38. Jannin V, Musakhanian J, Marchaud D. Approaches for the development of solid and semi-solid lipid-based formulations. Adv Drug Deliv Rev, 2008; 60(6): 734-46.
39. Christie WW. High-performance liquid chromatography and lipids: Pergamon Press, 1987.
40. Attwood D, Florence AT. Surfactant systems: Their chemistry, pharmacy and biology. 1983. London: Chapman and Hall, 794.
41. Hauss DJ. Oral lipid-based formulations: enhancing the bioavailability of poorly water-soluble drugs: CRC Press, 2007.
42. Schafer-Korting M, Mehnert W, Korting HC. Lipid nanoparticles for improved topical application of drugs for skin diseases. Adv Drug Deliv Rev, 2007; 59(6): 427-43.
43. Rosenblatt KM, Bunjes H. Poly (vinyl alcohol) as emulsifier stabilizes solid triglyceride drug carrier nanoparticles in the alpha-modification. Molecular pharmaceutics, 2009; 6(1): 105-20.
44. Gu X, Zhang W, Liu J, Shaw JP, Shen Y, Xu Y, et al. Preparation and characterization of a lovastatin-loaded protein-free nanostructured lipid carrier resembling high-density lipoprotein and evaluation of its targeting to foam cells. AAPS Pharm Sci Tech, 2011; 12(4): 1200-8.
45. Zhang X, Pan W, Gan L, Zhu C, Gan Y, Nie S. Preparation of a dispersible PEGylate nanostructured lipid carriers (NLC) loaded with 10-hydroxycamptothecin by spray-drying. Chemical & pharmaceutical bulletin, 2008; 56(12): 1645-50.
46. Zhang X, Gan Y, Gan L, Nie S, Pan W. PEGylated nanostructured lipid carriers loaded with 10-hydroxycamptothecin: an efficient carrier with enhanced anti-tumour effects against lung cancer. The Journal of pharmacy and pharmacology, 2008; 60(8): 1077-87.
47. Pereda J, Ferragut V, Quevedo JM, Guamis B, Trujillo AJ. Effects of ultra-high pressure homogenization on microbial and physicochemical shelf life of milk. Journal of dairy science, 2007; 90(3): 1081-93.
48. Lucks JS, Müller RH. Medication vehicles made of solid lipid particles (solid lipid nanospheres SLN). EP0000605497. 1991.
49. Diels AM, Michiels CW. High-pressure homogenization as a non-thermal technique for the inactivation of microorganisms. Critical reviews in microbiology. 2006; 32(4): 201-16.
50. Hommoss A. Preservative system development for argan oil-loaded nanostructured lipid carriers. Die Pharmazie, 2011; 66(3): 187-91.

51. Obeidat WM, Schwabe K, Muller RH, Keck CM. Preservation of nanostructured lipid carriers (NLC). European journal of pharmaceutics and biopharmaceutics: official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik eV, 2010; 76(1): 56-67.
52. Fang M, Jin Y, Bao W, Gao H, Xu M, Wang D, et al. In vitro characterization and in vivo evaluation of nanostructured lipid curcumin carriers for intragastric administration. International journal of nanomedicine, 2012; 7: 5395-404.
53. Hu FQ, Jiang SP, Du YZ, Yuan H, Ye YQ, Zeng S. Preparation and characteristics of monostearin nanostructured lipid carriers. Int J Pharm, 2006; 314(1): 83-9.
54. Varshosaz J, Eskandari S, Tabbakhian M. Freeze-drying of nanostructure lipid carriers by different carbohydrate polymers used as cryoprotectants. Carbohydrate Polymers, 2012; 88(4): 1157-63.
55. Emami J, Rezazadeh M, Varshosaz J. Formulation of LDL targeted nanostructured lipid carriers loaded with paclitaxel: a detailed study of preparation, freeze drying condition, and in vitro cytotoxicity. Journal of Nanomaterials, 2012; 2012: 3.
56. 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 and surfaces B, Biointerfaces, 2010; 80(2): 213-8.
57. Li X, Wang D, Zhang J, Pan W. Preparation and pharmacokinetics of docetaxel based on nanostructured lipid carriers. The Journal of pharmacy and pharmacology, 2009; 61(11): 1485-92.
58. Xia Q, Kong R, editors. Freeze-drying and characterization of vitamin a palmitate-loaded nanostructured lipid carriers (NLC) 2011: Trans Tech Publ.
59. Tips GRE, Tips T, Pharma MBA. Formulation development and characterization of solid lipid nanoparticles containing nimesulide, 2009.
60. Hentschel A, Gramdorf S, Muller RH, Kurz T. Beta-carotene-loaded nanostructured lipid carriers. Journal of food science, 2008; 73(2): N1-6.
61. Dubey A, Prabhu P, Kamath JV. Nano structured lipid carriers: a novel topical drug delivery system. International Journal of PharmTech Research, 2012; 4(2): 705-14.
62. Bunjes H, Koch MH, Westesen K. Influence of emulsifiers on the crystallization of solid lipid nanoparticles. Journal of pharmaceutical sciences, 2003; 92(7): 1509-20.
63. Gaba B, Fazil M, Ali A, Baboota S, Sahni JK, Ali J. Nanostructured lipid (NLCs) carriers as a bioavailability enhancement tool for oral administration. Drug delivery, 2015; 22(6): 691-700.

64. Lippacher A, Muller RH, Mader K. Semisolid SLN dispersions for topical application: influence of formulation and production parameters on viscoelastic properties. European journal of pharmaceutics and biopharmaceutics: official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik eV, 2002; 53(2): 155-60.
65. Han F, Li S, Yin R, Liu H, Xu L. Effect of surfactants on the formation and characterization of a new type of colloidal drug delivery system: nanostructured lipid carriers. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2008; 315(1): 210-6.
66. Seyfoddin A, Al-Kassas R. Development of solid lipid nanoparticles and nanostructured lipid carriers for improving ocular delivery of acyclovir. Drug development and industrial pharmacy, 2013; 39(4): 508-19.
67. Kovacevic A, Savic S, Vuleta G, Muller RH, Keck CM. Polyhydroxy surfactants for the formulation of lipid nanoparticles (SLN and NLC): effects on size, physical stability and particle matrix structure. Int J Pharm, 2011; 406(1-2): 163-72.
68. Yuan H, Miao J, Du YZ, You J, Hu FQ, Zeng S. Cellular uptake of solid lipid nanoparticles and cytotoxicity of encapsulated paclitaxel in A549 cancer cells. Int J Pharm, 2008; 348(1-2): 137-45.
69. Iqbal MA, Md S, Sahni JK, Baboota S, Dang S, Ali J. Nanostructured lipid carriers system: recent advances in drug delivery. Journal of drug targeting, 2012; 20(10): 813-30.
70. Das S, Chaudhury A. Recent advances in lipid nanoparticle formulations with solid matrix for oral drug delivery. AAPS Pharm Sci Tech, 2011; 12(1): 62-76.
71. Jores K, Mehnert W, Drechsler M, Bunjes H, Johann C, Mader K. Investigations on the structure of solid lipid nanoparticles (SLN) and oil-loaded solid lipid nanoparticles by photon correlation spectroscopy, field-flow fractionation and transmission electron microscopy. Journal of controlled release: official journal of the Controlled Release Society, 2004; 95(2): 217-27.
72. Fang JY, Fang CL, Liu CH, Su YH. Lipid nanoparticles as vehicles for topical psoralen delivery: solid lipid nanoparticles (SLN) versus nanostructured lipid carriers (NLC). European journal of pharmaceutics and biopharmaceutics: official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik eV, 2008; 70(2): 633-40.
73. Steichen SD, Caldorera-Moore M, Peppas NA. A review of current nanoparticle and targeting moieties for the delivery of cancer therapeutics. European journal of

pharmaceutical sciences: official journal of the European Federation for Pharmaceutical Sciences, 2013; 48(3): 416-27.

74. Pardeike J, Weber S, Haber T, Wagner J, Zarfl HP, Plank H, et al. Development of an itraconazole-loaded nanostructured lipid carrier (NLC) formulation for pulmonary application. Int J Pharm, 2011; 419(1-2): 329-38.
75. Muller RH, Runge SA, Ravelli V, Thunemann AF, Mehnert W, Souto EB. Cyclosporine-loaded solid lipid nanoparticles (SLN): drug-lipid physicochemical interactions and characterization of drug incorporation. European journal of pharmaceutics and biopharmaceutics: official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik eV, 2008; 68(3): 535-44.
76. Muller RH, Runge S, Ravelli V, Mehnert W, Thunemann AF, Souto EB. Oral bioavailability of cyclosporine: solid lipid nanoparticles (SLN) versus drug nanocrystals. Int J Pharm, 2006; 317(1): 82-9.
77. Zhang J, Fan Y, Smith E. Experimental design for the optimization of lipid nanoparticles. Journal of pharmaceutical sciences, 2009; 98(5): 1813-9.
78. Anton N, Benoit JP, Saulnier P. Design and production of nanoparticles formulated from nano-emulsion templates-a review. Journal of controlled release: official journal of the Controlled Release Society, 2008; 128(3): 185-99.
79. Souto EB, Wissing SA, Barbosa CM, Muller RH. Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery. Int J Pharm, 2004; 278(1): 71-7.
80. Sahana B, Santra K, Basu S, Mukherjee B. Development of biodegradable polymer based tamoxifen citrate loaded nanoparticles and effect of some manufacturing process parameters on them: a physicochemical and in-vitro evaluation. International journal of nanomedicine, 2010; 5: 621-30.
81. Shahgaldian P, Da Silva E, Coleman AW, Rather B, Zaworotko MJ. Para-acyl-calix-arene based solid lipid nanoparticles (SLNs): a detailed study of preparation and stability parameters. Int J Pharm, 2003; 253(1-2): 23-38.
82. Souto EB, Mehnert W, Muller RH. Polymorphic behaviour of Compritol888 ATO as bulk lipid and as SLN and NLC. Journal of microencapsulation, 2006; 23(4): 417-33.
83. Estella-Hermoso de Mendoza A, Campanero MA, Mollinedo F, Blanco-Prieto MJ. Lipid nanomedicines for anticancer drug therapy. Journal of biomedical nanotechnology, 2009; 5(4): 323-43.

84. Uprit S, Kumar Sahu R, Roy A, Pare A. Preparation and characterization of minoxidil loaded nanostructured lipid carrier gel for effective treatment of alopecia. Saudi pharmaceutical journal: SPJ: the official publication of the Saudi Pharmaceutical Society, 2013; 21(4): 379-85.
85. Huang ZR, Hua SC, Yang YL, Fang JY. Development and evaluation of lipid nanoparticles for camptothecin delivery: a comparison of solid lipid nanoparticles, nanostructured lipid carriers, and lipid emulsion. Acta pharmacologica Sinica, 2008; 29(9): 1094-102.
86. Zhao C, Liu Y, Fan T, Zhou D, Yang Y, Jin Y, et al. A novel strategy for encapsulating poorly soluble drug into nanostructured lipid carriers for intravenous administration. Pharmaceutical development and technology, 2012; 17(4): 443-56.
87. Kumbhar DD, Pokharkar VB. Engineering of a nanostructured lipid carrier for the poorly water-soluble drug, bicalutamide: physicochemical investigations. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2013; 416: 32-42.
88. How CW, Rasedee A, Manickam S, Rosli R. Tamoxifen-loaded nanostructured lipid carrier as a drug delivery system: characterization, stability assessment and cytotoxicity. Colloids and Surfaces B: Biointerfaces, 2013; 112: 393-9.
89. Zhang W, Li X, Ye T, Chen F, Sun X, Kong J, et al. Design, characterization, and in vitro cellular inhibition and uptake of optimized genistein-loaded NLC for the prevention of posterior capsular opacification using response surface methodology. Int J Pharm, 2013; 454(1): 354-66.
90. Muller RH, Petersen RD, Hommoss A, Pardeike J. Nanostructured lipid carriers (NLC) in cosmetic dermal products. Adv Drug Deliv Rev, 2007; 59(6): 522-30.
91. Zhuang CY, Li N, Wang M, Zhang XN, Pan WS, Peng JJ, et al. Preparation and characterization of vinpocetine loaded nanostructured lipid carriers (NLC) for improved oral bioavailability. Int J Pharm, 2010; 394(1-2): 179-85.
92. Zur Muhlen A, zur Muhlen E, Niehus H, Mehnert W. Atomic force microscopy studies of solid lipid nanoparticles. Pharmaceutical research, 1996; 13(9): 1411-6.
93. Hu F-Q, Jiang S-P, Du Y-Z, Yuan H, Ye Y-Q, Zeng S. Preparation and characterization of stearic acid nanostructured lipid carriers by solvent diffusion method in an aqueous system. Colloids and Surfaces B: Biointerfaces, 2005; 45(3): 167-73.
94. Tiwari R, Pathak K. Nanostructured lipid carrier versus solid lipid nanoparticles of simvastatin: comparative analysis of characteristics, pharmacokinetics and tissue uptake. Int J Pharm, 2011; 415(1-2): 232-43.

95. Muchow M, Maincent P, Müller RH. Lipid Nanoparticles with a Solid Matrix (SLN®, NLC®, LDC®) for Oral Drug Delivery. Drug Development and Industrial Pharmacy, 2008; 34(12): 1394-405.
96. Wu CY, Benet LZ. Predicting drug disposition via application of BCS: transport/absorption/ elimination interplay and development of a biopharmaceutics drug disposition classification system. Pharmaceutical research, 2005; 22(1): 11-23.
97. Wandel C, Kim RB, Stein CM. "Inactive" excipients such as Cremophor can affect in vivo drug disposition. Clinical pharmacology and therapeutics, 2003; 73(5): 394-6.
98. Cornaire G, Woodley J, Hermann P, Cloarec A, Arellano C, Houin G. Impact of excipients on the absorption of P-glycoprotein substrates in vitro and in vivo. Int J Pharm, 2004; 278(1): 119-31.
99. Joshi M, Patravale V. Nanostructured lipid carrier (NLC) based gel of celecoxib. Int J Pharm, 2008; 346(1-2): 124-32.
100. Sawant KK, Dodiya SS. Recent advances and patents on solid lipid nanoparticles. Recent patents on drug delivery & formulation, 2008; 2(2): 120-35.
101. Amidon GL, Lennernas H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharmaceutical research, 1995; 12(3): 413-20.
102. Lennernas H. Intestinal drug absorption and bioavailability: beyond involvement of single transport function. The Journal of pharmacy and pharmacology, 2003; 55(4): 429-33.
103. Constantinides PP, Wasan KM. Lipid formulation strategies for enhancing intestinal transport and absorption of P-glycoprotein (P-gp) substrate drugs: in vitro/in vivo case studies. Journal of pharmaceutical sciences, 2007; 96(2): 235-48.
104. O'Driscoll CM, Griffin BT. Biopharmaceutical challenges associated with drugs with low aqueous solubility--the potential impact of lipid-based formulations. Adv Drug Deliv Rev, 2008; 60(6): 617-24.
105. Ewesuedo RB, Ratain MJ. Principles of cancer chemotherapy. Oncologic Therapies: Springer, 2003; 19-66.
106. Early Breast Cancer Trialists' Collaborative G. Polychemotherapy for early breast cancer: an overview of the randomised trials. The Lancet, 1998; 352(9132): 930-42.
107. Schilsky RL, Milano GA, Ratain MJ. Principles of antineoplastic drug development and pharmacology: Marcel Dekker, 1996.

108. Skeel RT, Khleif SN. Handbook of cancer chemotherapy: Lippincott Williams & Wilkins, 2011.
109. Torchilin V. Tumor delivery of macromolecular drugs based on the EPR effect. Adv Drug Deliv Rev, 2011; 63(3): 131-5.
110. Powis G. A unique opportunity to study human toxicology. The Toxicity of Anticancer Drugs, 1991; 1-9.
111. Lu P, editor Monitoring cardiac function in patients receiving doxorubicin, 2005: Elsevier.
112. Kalyanaraman B, Joseph J, Kalivendi S, Wang S, Konorev E, Kotamraju S. Doxorubicin-induced apoptosis: implications in cardiotoxicity. Oxygen/Nitrogen Radicals: Cell Injury and Disease: Springer, 2002; 119-24.
113. Hryniuk WM, Figueredo A, Goodyear M. Applications of dose intensity to problems in chemotherapy of breast and colorectal cancer. Seminars in oncology, 1987; 14(S4): 3-11.
114. Gieseler F, Rudolph P, Kloeppel G, Foelsch UR. Resistance mechanisms of gastrointestinal cancers: why does conventional chemotherapy fail? International journal of colorectal disease, 2003; 18(6): 470-80.
115. Baird RD, Kaye SB. Drug resistance reversal--are we getting closer? European journal of cancer (Oxford, England: 1990), 2003; 39(17): 2450-61.
116. Wong HL, Bendayan R, Rauth AM, Li Y, Wu XY. Chemotherapy with anticancer drugs encapsulated in solid lipid nanoparticles. Advanced drug delivery reviews, 2007; 59(6): 491-504.
117. Prabhakar U, Maeda H, Jain RK, Sevick-Muraca EM, Zamboni W, Farokhzad OC, et al. Challenges and key considerations of the enhanced permeability and retention effect for nanomedicine drug delivery in oncology. Cancer research, 2013; 73(8): 2412-7.
118. Mohanty C, Das M, Kanwar JR, Sahoo SK. Receptor mediated tumor targeting: an emerging approach for cancer therapy. Current drug delivery, 2011; 8(1): 45-58.
119. Maeda H. The enhanced permeability and retention (EPR) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting. Advances in enzyme regulation, 2001; 41: 189-207.
120. Fang J, Nakamura H, Maeda H. The EPR effect: Unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect. Adv Drug Deliv Rev, 2011; 63(3): 136-51.

121. Hu CM, Zhang L. Therapeutic nanoparticles to combat cancer drug resistance. Current drug metabolism, 2009; 10(8): 836-41.
122. Brigger I, Dubernet C, Couvreur P. Nanoparticles in cancer therapy and diagnosis. Advanced drug delivery reviews, 2002; 54(5): 631-51.
123. Gu FX, Karnik R, Wang AZ, Alexis F, Levy-Nissenbaum E, Hong S, et al. Targeted nanoparticles for cancer therapy. Nano today, 2007; 2(3): 14-21.
124. Wang Y, Zhang H, Hao J, Li B, Li M, Xiuwen W. Lung cancer combination therapy: co-delivery of paclitaxel and doxorubicin by nanostructured lipid carriers for synergistic effect. Drug delivery, 2016; 23(4): 1398-403.
125. Lin X, Gao R, Zhang Y, Qi N, Zhang Y, Zhang K, et al. Lipid nanoparticles for chemotherapeutic applications: strategies to improve anticancer efficacy. Expert opinion on drug delivery, 2012; 9(7): 767-81.
126. Danhier F, Feron O, Preat V. To exploit the tumor microenvironment: Passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. Journal of controlled release: official journal of the Controlled Release Society, 2010; 148(2): 135-46.
127. Minko T, Rodriguez-Rodriguez L, Pozharov V. Nanotechnology approaches for personalized treatment of multidrug resistant cancers. Adv Drug Deliv Rev, 2013; 65(13-14): 1880-95.
128. Khajavinia A, Varshosaz J, Dehkordi AJ. Targeting etoposide to acute myelogenous leukaemia cells using nanostructured lipid carriers coated with transferrin. Nanotechnology, 2012; 23(40): 405101.
129. Moghimi SM, Szebeni J. Stealth liposomes and long circulating nanoparticles: critical issues in pharmacokinetics, opsonization and protein-binding properties. Progress in lipid research, 2003; 42(6): 463-78.
130. Rudmann DG, Alston JT, Hanson JC, Heidel S. High molecular weight polyethylene glycol cellular distribution and PEG-associated cytoplasmic vacuolation is molecular weight dependent and does not require conjugation to proteins. Toxicologic pathology, 2013; 41(7): 970-83.
131. Gao W, Xiang B, Meng TT, Liu F, Qi XR. Chemotherapeutic drug delivery to cancer cells using a combination of folate targeting and tumor microenvironment-sensitive polypeptides. Biomaterials, 2013; 34(16): 4137-49.
132. Woodle MC, Storm G. Long circulating liposomes: old drugs, new therapeutics: Springer Science & Business Media, 2013.

133. Park JW. Liposome-based drug delivery in breast cancer treatment. Breast cancer research: BCR, 2002; 4(3): 95-9.
134. Zara GP, Cavalli R, Bargoni A, Fundaro A, Vighetto D, Gasco MR. Intravenous administration to rabbits of non-stealth and stealth doxorubicin-loaded solid lipid nanoparticles at increasing concentrations of stealth agent: pharmacokinetics and distribution of doxorubicin in brain and other tissues. Journal of drug targeting, 2002; 10(4): 327-35.
135. Yang X, Yi C, Luo N, Gong C. Nanomedicine to overcome cancer multidrug resistance. Current drug metabolism, 2014; 15(6): 632-49.
136. Zhang XG, Miao J, Dai YQ, Du YZ, Yuan H, Hu FQ. Reversal activity of nanostructured lipid carriers loading cytotoxic drug in multi-drug resistant cancer cells. Int J Pharm, 2008; 361(1-2): 239-44.
137. Xue HY, Wong HL. Tailoring nanostructured solid-lipid carriers for time-controlled intracellular siRNA kinetics to sustain RNAi-mediated chemosensitization. Biomaterials, 2011; 32(10): 2662-72.
138. Han Y, Zhang Y, Li D, Chen Y, Sun J, Kong F. Transferrin-modified nanostructured lipid carriers as multifunctional nanomedicine for codelivery of DNA and doxorubicin. International journal of nanomedicine, 2014; 9: 4107-16.
139. Shao Z, Shao J, Tan B, Guan S, Liu Z, Zhao Z, et al. Targeted lung cancer therapy: preparation and optimization of transferrin-decorated nanostructured lipid carriers as novel nanomedicine for co-delivery of anticancer drugs and DNA. International journal of nanomedicine, 2015; 10: 1223-33.
140. Han Y, Li Y, Zhang P, Sun J, Li X, Sun X, et al. Nanostructured lipid carriers as novel drug delivery system for lung cancer gene therapy. Pharmaceutical development and technology, 2016; 21(3): 277-81.
141. Beloqui A, Solinis MA, Rodriguez-Gascon A, Almeida AJ, Preat V. Nanostructured lipid carriers: Promising drug delivery systems for future clinics. Nanomedicine : nanotechnology, biology, and medicine, 2016; 12(1): 143-61.
142. Rizwanullah M, Ahmad J, Amin S. Nanostructured Lipid Carriers: A Novel Platform for Chemotherapeutics. Current drug delivery, 2016; 13(1): 4-26.
143. Selvamuthukumar S, Velmurugan R. Nanostructured lipid carriers: a potential drug carrier for cancer chemotherapy. Lipids in health and disease, 2012; 11: 159.

144. Khan S, Baboota S, Ali J, Khan S, Narang RS, Narang JK. Nanostructured lipid carriers: An emerging platform for improving oral bioavailability of lipophilic drugs. International journal of pharmaceutical investigation, 2015; 5(4): 182-91.