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Review Article

NANOSTRUCTURE LIPID CARRIERS: A PROMISING TOOL FOR THE DRUG DELIVERY IN THE TREATMENT OF SKIN CANCER

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ABSTRACT

Skin cancer is the most common type of cancer having a very high rate of incidence, exceeding the sum of all other types of cancers. Current topical treatments for non-melanoma skin cancer and their precursor lesions, such as actinic keratosis includes conventional treatments using semisolid formulations of 5-fluorouracil, diclofenac, and imiquimod. Photodynamic therapy is another topical treatment which is used and approved by the US Food and Drug Administration. However, these conventional treatments present various side effects such as severe inflammation, pain, long duration of treatment, and unappealing scars leading to noncompliance of the patients. Hence, the main objective of this review is to highlight the advantages of nanostructured lipid carriers (NLCs) as promising carriers for cytotoxic drugs due to their potential to increase the solubility and bioavailability (BA) of poorly water-soluble and lipophilic drugs. The topical administration of anticancer drugs through NLCs has many advantages such as reduced side effects, reduce degradation, and enhanced penetration of the drug through the stratum corneum (SC) and thus increased drug targeting and therapeutics and also cost benefits. NLCs are composed of mixture of solid lipids and liquid lipids, because of that they have adequate capacity to accommodate large amount of drug as compared to SLN. Being lipid-based drug delivery systems, NLCs have been proved as better drug delivery carriers for cytotoxic drugs due to their potential to increase the solubility and BA of poorly water-soluble and lipophilic drugs. This review includes the applications and recent developments in topical drug delivery using NLCs. The structures, preparation techniques, modulation of drug release, long-term stability of NLCs and their physicochemical characterization are systematically described in this review. The potential of NLCs in the drug therapy of skin cancer has been highlighted.

Keywords: Skin cancer, Nanostructured lipid carriers, Topical drug delivery, Drug targeting.

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INTRODUCTION

Skin cancer is the most common type of cancer having a very high rate of incidence, exceeding the sum of all other cancers combined [1]. There are three types of skin tumors, namely cutaneous malignant melanoma, basal cell carcinoma (BCC), and squamous cell carcinoma (SCC). Out of which BCC and SCC are classified as non-melanoma skin cancers, with BCC being the most common and constituting 75% of cases [2]. The high incidence rate of non-melanoma skin cancers makes them a major challenge in terms of the management of public health. Moreover, these cancers can have a huge impact on health-care costs of the country. Non-melanoma skin cancers are not fatal but can destroy facial sensory organs such as the nose, ear, and lips [3]. Therefore, these lesions should preferably be treated using non-invasive techniques. Some of the well-established treatments for non-melanoma skin cancer include curettage, surgery, cryotherapy, and chemotherapy [4,5]. Current topical treatments for non-melanoma skin cancer and their precursor lesions, such as actinic keratosis include conventional methods using semisolid formulations of 5-fluorouracil [6], diclofenac [6], and imiquimod. Another topical treatment also used and approved by the US Food and Drug Administration is photodynamic therapy (PDT) [7]. However, these conventional treatments have various side effects such as severe inflammation, pain, long duration of treatment, and unappealing scars leading to noncompliance of the patients [8].

Melanoma skin cancers are the aggressive metastatic type of cancer and cause death. These cancers are associated with chronic exposure to sunlight and further stimulation of melanocytes, which are pigment-producing cells [9]. As melanoma has a much higher mortality rate than non-melanoma skin cancers, along with non-invasive techniques, invasive interventions are required [10].

Treatments for melanoma, in turn, are primarily surgical because these tumors can be resistant to traditional chemo- and radiotherapies [11]. Nonsurgical treatments for melanomas are limited to adjuvant therapies, such as immunotherapy, biochemotherapy, gene therapy, and PDT [10,11].

Therefore to increase patient compliance and to reduce surgical costs and undesirable scars, the topical administration of anticancer drugs has been investigated. The topical administration of anticancer drugs has many advantages, namely reduced side effects and increased drug targeting and therapeutic and also cost benefits. The major challenge for the topical treatment is to increase penetration of the anticancer drug in sufficient levels to kill tumor cells [12].

Therefore, to overcome skin barriers and to reach malignant skin cells and tumor several techniques and formulations have been developed for facilitating drug penetration into the deeper layers of the epidermis. The use of chemical penetration enhancers is the simplest strategy, causing temporary and reversible disruption of the SC bilayers and leading to increased anticancer drug penetration into the skin malignancies. Moreover, nanoparticle (NP) delivery systems have also attracted great interest as they can protect anticancer drugs against degradation and, combined with physical methods, significantly increase the tumor penetration of the drug.

NANOCARRIER DELIVERY SYSTEMS FOR CONTROLLED TOPICAL DRUG DELIVERY

Many nano delivery systems have been studied for the treatment of skin cancers, including liposomes, dendrimers, polymersomes, carbon-based NPs, inorganic NPs, protein-based NPs, and nanostructured lipid carriers (NLCs). Structural properties for a good skin penetration are moderate lipophilicity and low molecular weight – to overcome the

SC barrier – as well as sufficient water solubility – to cross the viable epidermis. In addition, the formulation can strongly influence skin absorption of the respective drug. Classical penetration enhancers such as alcohols, fatty acids, or propylene glycols can intercalate with the SC lipid and influence their conformational order or interact with the drug itself, manipulating drug solubility [13]. However, modification of the skin surface may come along with irritation or damage of the skin barrier functions. Thus, highly efficient and well-tolerated drug delivery systems are looked for. A broad spectrum of different nanocarriers such as liposomes, solid lipid NPs (SLN), NLCs, polymeric NPs, nanoemulsion, and quantum dots have been developed and studied for topical drug delivery [14]. NPs in drug delivery can be designed as various systems such as nanocrystals and nanosuspension, nanotubes and nanowires, ceramic NPs, liposomes, polymeric NP, hydrogel NPs, copolymerized NPs, polymeric micelles, dendrimers, functionalized nanocarriers, SLN, and NLCs [15].

They can reduce degradation and may enhance the penetration of the drug to the target site. Furthermore, NPs can control drug release from the formulation and therefore allow sustained drug delivery. Intact NPs may penetrate through the human skin or enhance drug delivery through influencing the lipid composition of the SC, or the drug solubility is still questionable. Suggested modes for the action of nano drug delivery systems on the skin include [16,17]:

- The interaction of nano drug delivery systems with SC lipids impairs the SCs barrier function. The drug released directly on the skin surface easily overcome the disturbed skin barrier.
- The nanoparticulate drug delivery systems exhibit stronger permeability and allow skin penetration. Loaded NPs enter the skin and release the drug to the site of disease.
- Penetration of intact nanoparticulate drug delivery system into hair follicles and sebaceous glands.

Special care needs to be addressed to particle toxicity. Exposure to NPs, especially in combination with environmental factors such as ultraviolet (UV) radiation or allergens, can trigger hypersensitivity, atopic dermatitis, and skin barrier defects. In particular, lesions similar to atopic dermatitis were detected by UV irradiation in combination with titanium dioxide nanoparticles in DS-Nh mice [18]. Furthermore, immunostimulation in mice was observed by carbon nanotubes [19].

Lipid-based drug delivery systems have been proved as promising carriers for cytotoxic drugs due to their potential to increase the solubility and bioavailability (BA) of poorly water-soluble and/or lipophilic drugs [20]. The combination of the nanoparticulate delivery system with lipids resulted in the development of a new class of NPs commonly known as SLNs [21]. The lipids which are used to prepare lipid NPs are usually biocompatible and biodegradable lipids having low acute and chronic toxicity [22]. SLN has many benefits such as lack of organic solvent usage during the production and easy scale-up [23]. As SLN are composed of solid lipids only. Therefore, during formulation, a part of the lipid crystallizes in a higher energy modification (α or β`). Further on storage, these modifications can transform to more organized lower energy, β modification. As this modification has a high degree of alignment, there is a small number of imperfections in the crystal lattice which further leads to drug expulsion [24]. Apart from polymorphic transition, SLNs also show some disadvantages as drug carriers including an unpredictable gelation tendency, and low incorporation due to the crystalline structure of solid lipids [25,26].

To overcome these limitations of the SLNs, second-generation encapsulation systems have been developed by incorporating liquid carrier oil into the solid lipid matrix to form NLCs; thus, NLCs were introduced. NLCs have shown to have improved active drug encapsulation and delivery properties compared to SLNs. The major advantage of NLC as drug delivery system is its ability to accommodate large quantities of drugs as a result of the formation of a less ordered lipid matrix with many imperfections [27-30]. These are prepared by mixing physically different kind of solid and liquid (oil) lipids [31].

TYPES OF NLCs

NLCs are classified into three different types depending on the basis of the type of lipid used in their production (Table 1). NLCs Type 1 (Fig. 1a) composed of solid lipids and liquid lipids in low concentration. These are mixed so that the matrix of the NLCs is unable to form highly ordered structures resulting in structural imperfections due to the different chain lengths of the various fatty acids and mixture of mono-, di-, and triacylglycerols used during their preparations [27,30]. NLCs Type II (Fig. 1b) composed of special lipids that do not recrystallize after homogenization and cooling of nanoemulsions such as hydroxyl

Table 1: Different types of NLCs

S. No.	Type of model	Name of the model	Characteristics	Method
1	I	Imperfect crystal type	Consists of a matrix with numerous voids and vacancies to accommodate the API	By mixing solid lipids with a sufficient amount of liquid lipids
2	II	Amorphous type	Minimize the API expulsion during storage by delaying the recrystallization phenomenon	By mixing special lipids that do not recrystallize After homogenization and cooling of a nanoemulsion
3	III	Multiple types	Avoids drug expulsion by avoiding crystallinity	Very small oily nanocompartments created inside the solid lipid matrix of NPs by the phase separation process

NPs: Nanoparticles, NLCs: Nanostructured lipid carriers

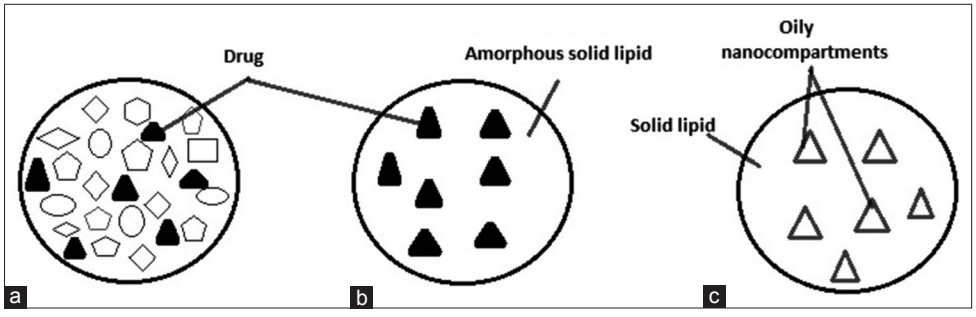

Fig. 1: (a-c) Types of nanostructured lipid carriers

Table 2: List of commonly used excipients for formulating NLCs [75]

S. No.	Ingredient	Material
1	Solid lipids	Monostearin, Tristearin, stearic acid, cetyl palmitate, cholesterol, Precirol® ATO 5, Compritol® 888 ATO, Dynasan® 116, Dynasan® 118, Dynasan® 114, Softisan® 154, Cutina® CP, Imwitor® 900 P, Geleol®, Gelot® 64, Emulcire® 61, Apifil, Glyceryl monostearate (GMS)
2	Liquid lipid	Medium chain triglycerides, paraffin oil, 2-octyl dodecanol, oleic acid, squalene, isopropyl myristate, Vitamin E, Miglyol® 812, Transcutol® HP, Labrafil Lipofile® WL 1349, Labrafac® PG, Lauroglycol® FCC, Capryol® 90
3	Hydrophilic emulsifiers	Pluronic® F68 (poloxamer 188), Pluronic® F127 (poloxamer 407), Tween 20, Tween 40, Tween 80, polyvinyl alcohol, Solutol® HS15, trehalose, sodium deoxycholate, sodium glycocholate, sodium oleate, polyglycerol methyl glucose distearate, Apifil
4	Lipophilic emulsifiers	Myverol® 18-04K, Span 20, Span 40, Span 60, Soybean Lecithin

NLCs: Nanostructured lipid carriers, PEG: Polyethylene glycol

octacosanol hydroxystearate, isopropyl myristate, and dibutyl adipate are blended in such a way which prevents crystallization. This results in the formation of NLCs with solid amorphous matrices. In NLCs, Type III (Fig. 1c) solid lipids are mixed with oils in high concentration, for example, medium [32] and long-chain triacylglycerols [33], and oleic acid [34] so that the solubility of the oil molecules in the solid lipid is exceeded. On cooling of the nanoemulsion, the lipid droplets reach the miscibility gap resulting in precipitation of oil, thereby resulting in the formation of tiny oil droplets. Subsequent solidification of the solid lipid surrounding these droplets leads to stabilization of the oily nanocompartments. Increase in loading capacity for a drug of higher solubility in liquid lipids than in solid lipids is the advantage of this model [35]. The exact structure of the NLCs depends on the formulation's procedure and composition [36].

MATERIALS USED FOR PREPARING NLCs

Lipids, water, and emulsifying agents constitute the essential ingredients for the formulation of NLCs (Table 2). Economic, non-irritating, and capable of being sterilized before application are essential properties for lipids which are involved in NLCs for fabricating the inner cores. Normally, all the ingredients should be approved by regulatory authorities for clinical applications and for "generally recognized as safe" status. The glyceryl behenate (Compritol® 888 ATO), glyceryl palmitostearate (Precirol® ATO 5) [37,38], fatty acids (e.g., stearic acid) [39], triglycerides (e.g., tristearin), glyceryl monostearate [40], steroids (e.g., cholesterol), and waxes (e.g., cetyl palmitate), Apifil [41] include commonly used lipids for NLCs [42-45], these are solid at room temperature and melt at higher temperatures (e.g., >60°C) during the preparation process. Liquid oils usually used for NLCs may consist of digestible oils from natural sources like castor oil [46], olive oil [47], Siberian pine seed oil and fish oil [48]. The medium chain triglycerides, such as Miglyol® 812 [42,43] are most commonly used liquid lipids (Jenning *et al*. 2000) [36]. Some other substances such as 2-octyl dodecanol, paraffin oil, propylene glycol dicaprylocaprate (Labrafac®) [49], isopropyl myristate or palmitate [50], Cetyl resinoleate [51] and squalene [52], Vitamin E (α tocopherol) and other tocols [53] can be used. Stability, ease of production on a large scale, and good solubility in lipophilic drugs are beneficial qualities of tocols [54]. The fatty acids, such as oleic acid, linoleic acid, and decanoic acid, are also included in NLCs as they also have penetration enhancing property for topical delivery. Various emulsifying agents have been used to stabilize the lipid dispersions. To prevent particle aggregation efficiently the combination of emulsifiers are found very effective [55]. Pluronic F68 (poloxamer 188), polysorbates (Tween), polyvinyl alcohol, and sodium deoxycholate are commonly employed hydrophilic emulsifiers. Lipophilic or amphiphilic emulsifiers such as Span 80 [50] and lecithin [39] are commonly used for production of NLCs if required. To prevent uptake by the reticuloendothelial system and to prolong the circulation time of drugs polyethylene glycol, sometimes supplementary in NLCs, resides on the nanoparticulate shell [56]. Table 2 summarizes the detailed information concerning to the materials used for NLCs. Ability for preservation is the another prerequisite for NLCs' stability. The preservatives have adverse effect on the physical stability of lipid dispersions. Hydrolite® 5 is evidenced suitable for the preservation of coenzyme Q10-loaded [57].

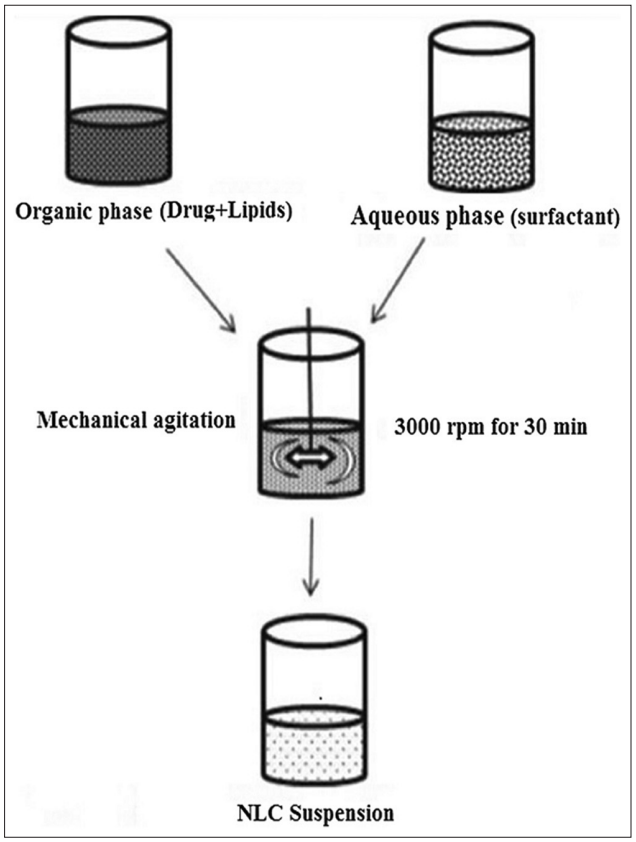

Fig. 2: General scheme for the preparation of nanostructured lipid carriers

METHODS OF PREPARATION OF NLCs

There are various formulation techniques that exist for the production of the NLCs which have been adopted from the previous techniques, which were used to formulate the polymeric NPs. The general scheme for the preparation of NLCs is shown in Fig. 2. The techniques are: Double emulsion [58], emulsification-sonication [59], high pressure homogenization [38,37,41] melt emulsification, microemulsion [60-62], probe sonicator [31], solvent diffusion [63], solvent emulsification-evaporation [64], solvent injection/solvent displacement [65], and phase inversion [66,67] technique based on membrane contractor [58]. Out of the above used formulation techniques, high-pressure homogenization and microemulsion techniques have demonstrated strong potential for scaling up to industrial production scale [68,69]. Table 3 gives the summary of the different methods of preparation of NLCs [70].

MODULATION OF RELEASE OF ENCAPSULATED DRUG

Release of encapsulated drug from lipid particles follows preferably the process of diffusion and also simultaneously by degradation of lipids in

Table 3: Various methods of preparation of NLCs

Method	Method	Advantages	Limitations	References
High-Pressure Homogenization (HPH) (a) Hot HPH (b) Cold HPH	Lipid and drug are melted and combined with an aqueous surfactant at the same temperature. By using high shear device, a hot pre-emulsion is formed which is then processed in a temperature controlled HPH. The obtained nanoemulsion recrystallizes on cooling down to room temperature forming NLCs. In Cold HPH, lipid and drug are melted together and rapidly ground under liquid nitrogen forming microparticles. A pre-suspension is formed by homogenization of the particles in a cold surfactant solution which is then subjected to HPH	Avoidance of organic solvent Short production time. Possibility of large scale Production thermolabile drug can be formulated by cold HPH	Complete avoidance of drug exposure to high temperature is not possible	[37,38,41]
Solvent diffusion	To ensure initial thermodynamic equilibrium, organic solvents are saturated with water. The transient oil-in- water emulsion is passed into water under continuous stirring, which leads to solidification of dispersed phase forming lipid NPs due to diffusion of the organic solvent.	Water-immiscible solvents are used to dissolve lipids	Ultrafiltration or lyophilization is required Organic solvents residue may remain in the formulation	[63]
Solvent emulsification-evaporation	Lipid is dissolved in water-immiscible organic solvent, and then emulsified in an aqueous phase containing surfactants under continuous stirring. The organic solvent will evaporate during emulsification, resulting in lipid precipitation	Suitable for thermolabile drugs	Organic solvent may remain in the formulation Ultrafiltration or evaporation is required Very dilute NPs are produced	64
Emulsification sonication	Formulating the nanoemulsion is same as HPH, after which it is ultrasonicated using probe sonicator	High shear mixing	During sonication, metallic contamination may occur in the product.	[59]
Microemulsion	Lipid melted above the melting point simultaneously the aqueous surfactant is heated at same temperature. A hot microemulsion is formed which is poured into cold water forming nanoemulsion, which then recrystallizes to form NLC	Industrial scale production is possible	Use of high concentration of surfactant which is not desired Strong dilution of particle suspension due to large volume of water used	[60-62]
Phase inversion	Lipid, drug, water, and surfactant are mixed together on magnetic stirring, three heating and cooling cycles are performed which is then diluted with cold water causing phase inversion of the emulsion and breaking resulting in NLC	Thermolabile drugs can be incorporated. Avoidance of organic solvent	Cumbersome technique	[66]
Solvent injection/solvent displacement	Basic principle is similar to the solvent diffusion. Lipids are dissolved in a water-miscible solvent and quickly injected into an aqueous solution of surfactants through an injection needle	Easy handling and fast production process without technically sophisticated equipment	Use of organic solvents	[65]
Membrane contractor	In a porous membrane, lipids are passed pressure, small lipid droplets are formed. Simultaneously, the aqueous phase is circulated inside the membrane module and sweeps away lipid droplets from the pore. After cooling at room temperature, lipid NPs are formed.	Simple methodology and equipment	–	[58]

HPH: Homogenization, NPs: Nanoparticles, NLCs: Nanostructured lipid carriers

the body. In cases, when it might be desirable to have a controlled quick release in spite of diffusion and degradation, then this quick released should be triggered by an impulse after the administration of particles. NLCs accommodate the high concentration of pharmaceutical agent due to their highly unordered lipidic arrangements. By applying the initiate impulse to the matrix to alter in a more ordered structure, such a desired blew pharmaceutical release can be started. NLCs of certain organizations can be triggered this way; for demonstration when applying the particles to the skin incorporated in a cream. Boost in warmth and water evaporation leads to a boost in the pharmaceutical issue rate (Fig. 3). Based on these observations, cyclosporine-lipid particles are under development to heal psoriasis [71]. After application to the skin, accelerated issue from the lipid particles should lead to a supersaturated scheme (similar to microemulsions, but without

high surfactant) resulting in an improved penetration of cyclosporine into the skin [72].

LONG-TERM STABILITY

It has been observed that during long-term storage of dispersions, element aggregation can happen as in the case of SLNs [73]. At lower lipid concentrations particles diffuse in the dispersion medium; the collision of particles can lead to perikinetic flocculation (Fig. 4a). In the highly concentrated NLC dispersions the particles pattern a "pearl-like network;" thus, the particles are in a restricted place and will not undergo collision and perikinetic flocculation. On administration, the particles are diluted with body fluids (gastrointestinal fluids, for example), the mesh is decimated issuing single, non-aggregated particles (Fig. 4b). Thus, the low concentrated dispersions aggregated during storage time, whereas the gel-like NLC dispersion remained stable throughout storage and, after dilution, and single particles were obtained displaying no size boost [74].

PHYSICOCHEMICAL CHARACTERIZATION OF NLCs

The physicochemical characterization for NLCs is important to confirm quality control and stability. Both physical and chemical properties can be determined for NLCs. The parameters that must be determined for NLCs are particle size and zeta potential, differential-scanning calorimetry (DSC), X-ray, nuclear magnetic resonance (NMR), and Raman spectroscopy, encapsulation efficiency, and drug-release studies [75].

Particle size

Photon correlation spectroscopy (PCS) also known as dynamic light scattering and laser diffraction is the most powerful methods for routine measurement of particle size. PCS measures the fluctuation of the scattered light intensity produced by particle movement and covers a determined size range from several nm to 3 μm [55]. The larger size can be detected by laser diffraction. This determination is based on the effect of diffraction angle on a particle radius. Main factors that influence particle size are types and ratios of lipid and emulsifier used in the preparation of NLC. The increase in the concentration of emulsifiers results in more complete emulsification and more rigid structure and thus reduction in the size [35].

Zeta potential

Zeta potential determination is done to check if the cationic surface is achieved. Sometimes a negative charge of the particulate surface is needed to stabilize the nanoparticulate systems during storage. The determination of surface charge is done to assess the dispersion of NLCs for aggregation properties affecting particle stability in the application. In general, charged particles tend to show particle aggregation or fusion is less likely than uncharged particles because of the electrostatic repulsion. A positively charged surface of NLCs is efficient for entering the blood–brain barrier (BBB) due to binding to the paracellular area of the BBB, an area rich in anionic sites [76].

Electron microscopy

The electron microscopy is used for observing the shape and morphology of the particles. The particulate radius and size distribution of NLCs can be measured by scanning electron microscopy (SEM). It employs electrons transmitted from the surface of the sample, while transmission electron microscope (TEM) uses electrons transmitted through the specimen. SEM possesses high resolution and easy preparation of the samples. TEM allows visualization of NLCs after freeze-drying or freeze-thawing.

Atomic force microscopy (AFM)

AFM is an important study for morphological and surface features that are particularly small. Real quantitative data in three dimensions with

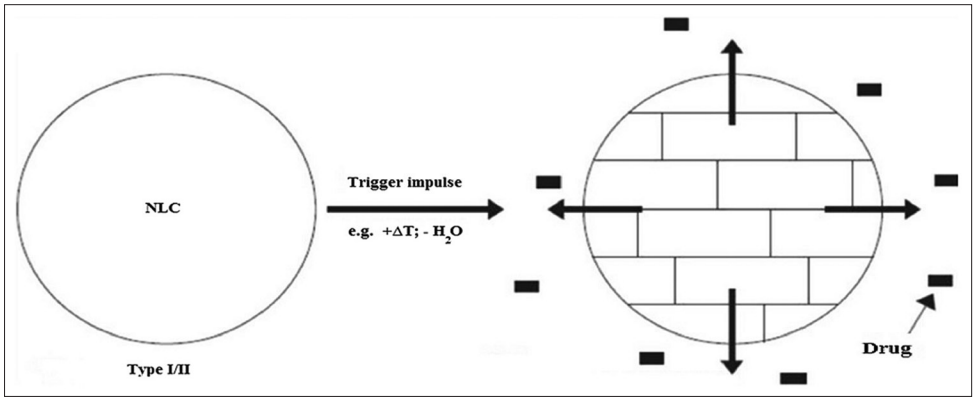

Fig. 3: Triggered pharmaceutical releases from nanostructured lipid carrier by starting the alteration from a highly disorganized lipid structure to more organized stable modifications

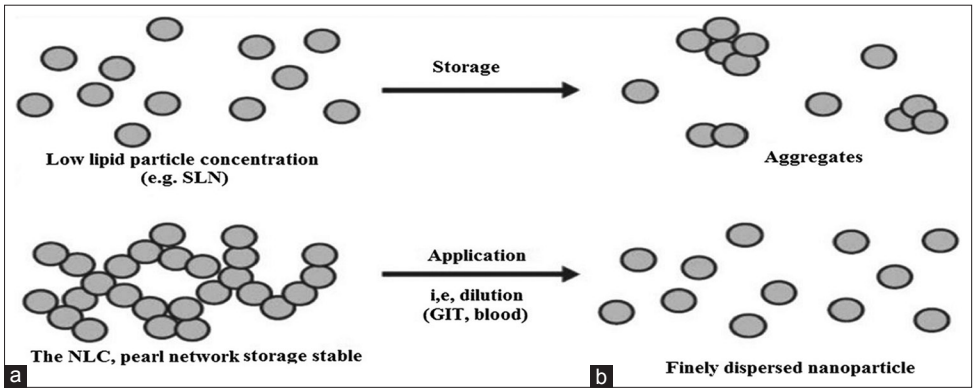

Fig. 4: (a and b) Aggregation method in reduced intensified dispersions (upper) and pearl-like network in nanostructured lipid carrier dispersions with stabilizing effect

minimal sample preparation times, flexibility in ambient functional conditions, and effective magnifications at the nano levels are some of the benefits of AFM over electron microscopy [77].

Surface tension

The surface tension of the lipid NPs is often measured by the Wilhelmy plate method. The surface tension of water at 20°C is 72.8 dynes/cm. Reduction in the surface tension is observed following the increase of emulsifier concentration due to the emulsification process of the whole system. The measurement of the contact angle is another method for detecting surface tension of the nanoparticulate systems [78].

DSC

DSC gives an insight into the melting and recrystallization behavior of the solid lipids from SLNs and NLCs. DSC determination uses the fact that various lipid modifications have various melting points and enthalpies. The degree of crystallinity of NLCs is calculated from the ratio of NLCs enthalpy to bulk lipid enthalpy, which is calculated on the basis of total weight taken [32]. As the ratio of liquid lipid increases, the degree of crystallinity of NPs decreases. This result proves that the liquid oil is the main factor in lowering the crystallinity and increasing the less-ordered structure of NLCs. The decline of enthalpy and reduction of the melting point of the lipids occur in the NLCs that have a smaller size, a higher surface area, and a greater number of emulsifiers. The loading of liquid oil leads to crystal order disturbance, resulting in more space to include drug molecules. DSC profiles are advantageous to suggest the preferential drug dissolution in solid or liquid lipids [79].

X-ray diffraction

Both DSC and X-ray diffraction are widely used to investigate the purity of lipids. The phenomenon of polymorphism is commonly observed in lipid molecules composed of along hydrocarbon chain [33]. The crystalline order of NLCs can be elucidated by wide-angle X-ray diffraction. The length of the long and short spacing of the lipid lattice can be assessed by means of X-ray scattering.

Parelectric spectroscopy

Parelectric spectroscopy is based on the frequency dependency of dipole density and mobility when exposed to a change of electromagnetic field. This approach is employed to recognize the structure and dynamics of SLNs and NLCs. Parelectric spectroscopy is proven to be a versatile tool as it offers insight into experimental details and function of open-ended coaxial probes to be used when performing measurements on liquid dispersions, and even when testing living material for medical diagnostic aims.

NMR

Proton NMR spectroscopy is performed to investigate the mobility of the solid and liquid lipids in the inner core of NLCs which is related to the width at half amplitude of the signals [80]. Broad signals and small amplitudes are features of molecules with restricted mobility and strong interactions [81]. The interaction of liquid oil with the solid lipid is indicated by the higher line width of NLCs compared to the physical mixture of the materials added in the preparation of NLCs. Immobilization of the NPs of NLCs is stronger in comparison to SLNs with completely crystallized cores.

Raman spectroscopy

Raman spectroscopy detects vibrations of molecules after excitation by a strong laser beam. Water causes only broad peaks at 3500 cm^{-1}. The symmetric stretching modes of the methylene groups at 2840 cm^{-1} and the sharp band of the asymmetric stretching mode at 2880 cm^{-1} are both indicators of a high degree of conformational order of hydrocarbon chains occurring in NLCs [82].

Molecular environment/polarity

The lipophilic fluorescent dye Nile red can be used as a marker determined by fluorometric spectroscopy. The molecular environment or polarity of NLCs is elucidated because of the solvatochromism of Nile red. Nile red is a lipophilic benzophenoxazone known to show strong fluorescence in organic solvents and lipid environments. Corresponding to high lipophilicity, the emission maximum of Nile red is near 600 nm. The emission spectra of Nile red can shift to shorter wavelengths with decreasing environmental polarity [82]. On the other hand, the emission maximum moves to a longer wavelength, and the reduction of the fluorescence intensity is observed when Nile red is relocated into a more polar environment such as an aqueous phase or nanoparticulate shell. In NLCs, Nile red is preferentially located in the fluid lipid phase [36].

Drug encapsulation efficiency

Determination of drug-loading efficiency is very important for NLCs since it affects the release characteristics [32]. The lipophilic drug molecules may homogeneously distribute in the lipid matrix or enrich the core or particulate shell. Aqueous and interfacial phases are the main locations for loading hydrophilic drugs. The prerequisite to achieving high loading capacity is sufficient solubility of the drug in the lipids. The solubility should be higher than required because it decreases when cooling down the melt and may even be lower in the solid lipids [21]. The encapsulation percentage of the drugs in NLCs is based on the separation of the internal and external phases. Different techniques such as ultrafiltration, ultracentrifugation, gel filtration by Sephadex, and dialysis are commonly used for separation the dispersions. As compared to SLNs, the incorporation of liquid oil to solid lipid in NLCs leads to massive crystal order disturbance. The resulting matrix indicates great imperfection in the lattice and leaves more space to accommodate the drugs. The entrapment efficiency and loading capacity of the drugs are thus improved.

Drug release

The prolonged half-life and retarded enzymatic attack in systematic circulation may be achieved by the controlled or sustained release of the drugs from NLCs. The drug release behavior from NLCs dependents on the number of factors, i.e. the production temperature, composition of emulsifier, and quantity of oil incorporated in the lipid matrix. The drug present in the outer shell of the NPs as well as on the surface is released in a burst manner, while the drug incorporated into the particulate core is released in a sustained manner [30]. Prolonged release of the drugs can be described considering both drug partitioning between the lipid and water, as well as the barrier of the interfacial membrane. The *in vitro* drug release from nanoparticles is measured using dialysis method or the Franz cell. Enzymatic degradation of lipid NPs may depend on a relevant amount by the composition of the particles.

PROBLEMS ENCOUNTERED IN CANCER CHEMOTHERAPY AND THE ADVANTAGES OF NLCs

Although there is great advancement in therapeutic drug delivery mechanisms in the treatment of cancer, the results are not satisfactory. For instance, the response rate of pancreatic malignancies, esophageal malignancies, and ovarian malignancies to chemotherapy is well underneath 20% [83]. Even in patients with malignancies that are more discerning to chemotherapeutic agents, for example, breast cancer, the clinical observations are generally not up to the expectations [84]. In comparison to other pharmaceutical drug categories, anticancer agents present some difficulties such as poor specificity, high toxicity, and pharmaceutical resistance. Conventionally administered cytotoxic agents show an unpredictable and often extensive body tissues and serum protein binding. Only a small fraction of the drugs come to the tumor location resulting in the reduction of therapeutic efficacy and increase in systemic pharmaceutical toxicity [85]. Furthermore, anticancer drugs should destroy cancerous units preferentially, but rather, in reality, they destroy healthy units, particularly to rapidly splitting up units, for example, skeletal part marrow units and units of the gastrointestinal tract. The poor specificity of cytotoxic drugs in terms of both pharmaceuticals biodistribution and pharmacology at the cellular level presents a significant challenge to effective anticancer treatment. Most common toxic effects are nausea, vomiting, fatigue, and hair loss. Some of the anticancer agents even result in cardiotoxicity.

Cytotoxic drugs normally display a narrow therapeutic index. This leads to difficulty for clinicians to select between high drug doses with a high risk of usual tissue toxicities (maximum tolerated concentration) or reduced pharmaceutical doses with reduced probabilities of therapeutic success (minimum effective concentration). In addition, the multidrug resistance, which engages hardworking efflux of a broad variety of cytotoxic pharmaceutical substances out of the cytoplasm by membrane-bound transporters [86,87] is another obstacle to the effective conventional chemotherapy. Along with the cellular mechanisms, cancerous cells in solid tumors are more resistant to chemotherapy than separate non-aggregated cancerous cells due to permeability issues, which make it tough to achieve a high intratumoral pharmaceutical concentration in solid tumors [88]. This type of drug resistance, or occasionally mentioned as "non-cellular" drug resistance, may farther lead to compromised clinical outcomes even though an anticancer drug has powerful *in vitro* efficacy.

As the clinical outcomes of conventional cancer therapy are not up to the expectations, NLCs can be used as an alternative drug carrier in the anticancer drug delivery. Due to erratically regulated tumor angiogenesis a tumor has a defective, leaky vasculature and a badly formed lymphatic system resulting in the insufficient drainage of interstitial fluid inside a tumor. This aids submicron sized particles to extravasate preferentially into the tumor and to be retained there. This is called the "enhanced permeability and keeping" (EPR) effect [89]. This EPR effect can be advantageous for correctly formulated nanoparticulate drug delivery system such as NLC to achieve passive tumor targeting. By modifying the exterior physicochemical properties of NLC, the distribution of NLC in the body can be additionally manipulated to target them to the tissue of interest [55]. This increases the concentration of pharmaceutical at the tumor sites and thus minimizes systemic pharmaceutical toxicity.

Among other nano delivery systems such as polymeric systems and liposomes, NLC have the advantages of improved stability, protection of sensitive pharmaceuticals from degradation, controlled drug delivery, and less tedious method of preparation. Large scale production NLC is economical and easier as required for large scale production of liposomal formulations. They have lesser storage and drug leakage difficulties compared to liposomes. NLC lacks the significant toxicity and acidity involved with a number of biodegradable polymeric materials. Due to the heterogenic nature of anticancer drug compatibility problems with polymers occurs. Thus, this versatility of NLC as a pharmaceutical carrier makes them a promising drug delivery system for diverse anticancer cytotoxic agents.

TOPICAL DELIVERY AND APPLICATION OF NLCs

NLCs are studied extensively for topical administration of various pharmaceuticals. Drug penetration through the skin is always limited by the relative impermeability of the SC. Another concern is that the formulations should deliver the drug to the action site in a sufficient amount. Nanoparticulate systems enhance skin absorption and also target the drug in the skin and/or its substructures [36]. The BA of drugs through topical route can be enhanced using nanocarriers due to their nanosize enabling close contact to SC [90]. NPs may have close contact with superficial junctions of SC and furrows between corneocyte islands, allowing superficial dispersion of the active agents. Particles from an adhesive layer occluding the skin after the evaporation of water from the nanosystems applied to the skin surface. Hydration of SC thus rises to reduce corneocyte packing and widens inter-corneocyte gaps and also influences partitioning of the drug into SC [91]. Intact NPs sized >100 nm are not likely to permeate the SC due to their dimensions and rigidity [92]. Although the particles do not penetrate across SC, uptake of the components is to be expected. Lipid NPs attaching to the skin surface would allow lipid exchange between SC and the nanocarriers due to epidermal lipids are rich in SC [93]. Lipid NPs have the potential to deliver drugs through the follicles [94]. Furthermore, each follicle is associated with sebaceous glands, which release sebum, creating an environment enriched in lipids [95]. This environment is helpful for trapping of lipid NPs. Sebum is a blend of triglycerides, squalene, and waxes. Some glyceride lipids present in NLCs may quicken the entrance into the follicles/sebaceous glands. The probable mechanisms involved in skin permeation enhancement by NLCs. Drug carriers intended for dermal application should be biocompatible and nonirritant. NLCs are basically composed of biodegradable and well-tolerated lipids, usually the same ones used in the pharmaceutical and cosmetic industries. It is recommended that cutaneous administration of NLCs may reduce the risk of acute and chronic toxicity. The encapsulation of drugs into the lipid matrix may also diminish the possible irritation induced by the drugs due to the avoidance of direct contact.

NLCs containing nitrosyl ruthenium complex (NRC) were developed and characterized to further explore their topical administration for applications such as skin cancer treatment. NRC-loaded NLC was formulated using the micro emulsification method. *In vitro,* drug release studies of NRC from NLCs showed sustained release. NRC-loaded NLC performed released and protected nitric oxide (NO) degradation *in vitro* and is a promising carrier for topical delivery of NO [96]. Tryptanthrin loaded was developed and characterized. It was found that NLC had zero-order kinetics and sustained release properties. The results demonstrated that the NLC formulation is a potential carrier with sustained release behavior and cytotoxicity effects and allowed tryptanthrin to be taken up by breast cancer cells. These results suggested that NLCs can potentially be exploited as a drug carrier for topical use in the future [75]. Coenzyme Q10 (Q10) is an endogenous cellular antioxidant. NLCs loaded with Coenzyme Q10 were formulated by high-pressure homogenization technique and characterized. Tape-stripping test was performed and it was found that penetration of coenzyme Q10 into the skin was enhanced using NLCs as compared with emulsion and liquid paraffin coenzyme Q10 [57]. Calcipotriol is a Vitamin D3 analog used for the treatment of psoriasis. Calcipotriol leads to cutaneous irritant reactions. It can be used in combination with topical corticosteroids, UV B, psoralen-UVA, acitretin, cyclosporine, tazarotene, and methotrexate. NLCs loaded with calcipotriol and methotrexate were developed and characterized and it was confirmed that NLC systems are a promising carrier for the topical delivery of antipsoriatic drugs. A good *in vitro* and *in vivo* correlation was obtained. The skin permeation of methotrexate can be potentiated using hyper proliferative skin as the penetration barrier. This proved that the drug in the aqueous phase also exhibits enhanced delivery with NLCs [97]. Topical cyproterone acetate (CPA) is used in the treatment of skin diseases but has poor skin penetration. CPA was incorporated into NLCs and formulation was characterized. 2–3 fold increase in drug absorption through excised human skin was observed [38]. BA of ketoprofen is limited by its very low water solubility [98]. Ketoprofen was complexed with cyclodextrin (Cd) to increase drug solubility and dissolution properties, and the complex was loaded into NLC by using ultrasonification techniques, to improve drug delivery through the skin. Drug Cd complex (drug-Cd)-loaded NLC system, formulated into a xanthan hydrogel, exhibited better drug permeation properties than those of the plain drug suspension or the plain drug-loaded NLC, due to the combined effect of the solubilizing effect of Cd and the penetration enhancer properties of NLC [44]. Celecoxib was loaded in NLC using the microemulsion template technique and size of NLCs was determined by PCS and SEM studies. Drug encapsulation efficiency was also determined. These celecoxib loaded NLCs were incorporated in the topical gel. The NLCs based gel is evaluated for *in vitro* release and *in vitro* skin permeation using rat skin. Pharmacodynamic study using Aerosil-induced rat paw edema model was performed. Both skin permeation and rat paw edema pharmacodynamic studies were performed in comparison with a micellar gel having the same composition as that of the NLC gel except for the solid lipid and oil. The NLC-based gel showed faster onset and prolonged activity until 24 h [99]. NLC containing clotrimazole was prepared using hot high-pressure homogenization technique and evaluated for the physical stability, the entrapment efficiency and *in vitro* release profile. The particle size was analyzed by PCS and linear discriminant showing no increase in size during 3 months of storage at 4, 20, and 40°C. For all batches, the entrapment efficiency was higher than 50%. The found

Table 4: Applications of NLCs

Drugs	Method	Excipients	Application
Methotrexate	Hot homogenization/Ultrasonication	Witepsol1 S51, oleic acid, polysorbates 60 (Tween 60) and 80 (Tween 80)	Delivery of methotrexate for topical therapy of psoriasis [110]
Artemether	Hot homogenization/Ultrasonication	Compritol® 888 pellets and Transcutol® P, Phospholipon® 85G, Polysorbates 80 and 20, Macrogol 4000, Pluronic F68, Sorbitan monostearate	Delivery of artemether for topical therapy for malaria [111]
Quercetin	Emulsion evaporation–solidification	TPGS, soy lecithin, glyceryl monostearate, stearic acid, Media chain triglyceride	Topical delivery of quercetin [112]
Fluconazole	Solvent diffusion method	Compritol 888 ATO, Oleic acid, Pluronic F-68 (PF68) and Sephadex G-50	Topical delivery of fluconazole for treatment of cutaneous candidiasis [113]
Tocopherol	Hot homogenization/Ultrasonication	Pluronic F68, tripalmitin, oleic acid, Tween 80, sodium dodecyl sulfate	Topical delivery of tocopherol [104]
Paclitaxel	Melt emulsification technique	Glyceryl Monostearate, Soybean oil, soy lecithin, hexadecyl trimethyl ammonium bromide, and hyaluronic acid	Subcutaneous and IV delivery of paclitaxel for tumor therapy [114]
Capsaicin	Solvent diffusion method	CAP, L- α Egg phosphatidylcholine (PC), Pluronic F-68 Sephadex G-50 Compritol 888 ATO, Oleic acid	Topical delivery of capsaicin for the treatment of psoriasis [115]
Tretinoin	Melt emulsification technique	Phosphatidylcholine, Compritol 888, Isopropyl myristate, butylated hydroxytoluene, cholesterol, and absolute alcohol	Topical delivery of tretinoin for the treatment of psoriasis [116]
Fluocinolone acetonide	Melt emulsification technique	Compritol_888 ATO, Methanol, acetone, Polysorbate 80, and Miglyol 812	Topical delivery of fluocinolone acetonide for the treatment of psoriasis [117]
Clotrimazole	Hot homogenization/Ultrasonication	Tween 80, Tween 20, Pluronic F68 Chromophore EL Compritol 888 ATO, Precirol_ ATO 5 Geleol™ Suppocire_NC, Labrafac™ CC, Dynasan_114, Dynasan_118, and Imwitor_900 K	Topical/oral/parenteral delivery of clotrimazole for treating antifungal infection [49]
Chitosan Oligosaccharides	Melt-emulsification and ultrasonication technique	Compritol 888 ATO, Gelucire 44/14, Solutol HS-15, Miglyol 812N,	Ocular delivery of chitosan oligosaccharides [43]
Meloxicam	Hot homogenization/Ultrasonication	Geleol, Compritol 888 ATO, Precirol ATO5, Miglyol_812, methanol, Chromasolvv	Topical delivery of meloxicam [118]
Triamcinolone acetonide	Homogenization/Ultrasonication	Precirol®ATO5, Palmitic acid, Stearic acid, Lutrol, Triamcinolone acetonide, Squalene	Ocular delivery of triamcinolone Acetonide for antiangiogenic activity [119]
Ofloxacin	Homogenization/Ultrasonication	Oleic acid, Tween 80, OFX, Compritol HD5 ATO	Ocular delivery of Ofloxacin for treatment of bacterial keratitis [120]
Cyclosporine A	Melt-emulsification and ultrasonication technique	4-Nitrophenyl chloroformate, PEG Monostearate, l-Cysteine, 5,5 dithiobis, Glyceryl palmitostearate, Propylene glycol dicaprylate, Tween 80	Ocular delivery of Cyclosporine A [121]
Clotrimazole, climbazole, ketoconazole	Melt-emulsification and ultrasonication technique	Tween R 80, triethanolamine, Compritol, Labrafac	Topical delivery of antifungal drug [122]
Dexamethasone acetate	Homogenization/Ultrasonication	Mygliol 812, Compritol ATO888, Carbomer 940, Tween 80	Topical delivery of Dexamethasone acetate [123]
Enoxaparin	Solvent diffusion method	Tristearin, Oleic acid, Tween-80	Topical delivery of Enoxaparin [124]
Tripterine	Solvent evaporation method	Glyceryl behenate, isopropyl myristate, Precirol ATO-5, Soybean lecithin Pluronic F68, acetone, and ethanol	Topical delivery of tripterine [125]
Calcipotriol and methotrexate	Homogenization/Ultrasonication	Squalene, Pluronic® F68, Nile red, sulforhodamine B, Precirol® ATO 5, Myverol™ 18-04K fluorescein isothiocyanate	Delivery of calcipotriol and methotrexate for topical therapy of psoriasis [97]
Aceclofenac	Melt-emulsification, low-temperature solidification, and high-speed homogenization methods	Cetyl Palmitate, stearic acid, Phospholipon 90G, oleic acid, and isopropyl myristate tristearin, Pluronic F68, carrageenan, Carbopol 940P, xanthan gum, HPMC, and chitosan	Topical delivery of aceclofenac [126]

NLCs: Nanostructured lipid carriers

results also confirmed the use of these lipid NPs as a prolonged release system for lipophilic drugs over a period of 10 h [100]. Silva *et al*. developed NLC loaded with minoxidil and incorporated these NLCs into freshly prepared Carbopol or perfluorocarbon-based hydrogels. Size of Minoxidil-loaded NLC remained <500 nm after mixing with both types of hydrogels [101]. Indomethacin-loaded SLN and NLC were developed and evaluated mean particle size and percentage of drug encapsulation efficiency. Increased indomethacin encapsulation efficiency was found in NLC in comparison with SLN due to the organization and distribution of the different types of lipid [102]. Various other pharmaceutical agents were loaded in NLCs and successfully characterized. Some heat-sensitive bioactive compounds such as β-carotene and tocols which are widely used in the pharmaceutical and cosmetic fields were initially difficult to produce in NLCs [103]. As the high-temperature high-pressure homogenization technique used in the preparation of NLCs can cause degradation of heat sensitive compounds. Therefore, a novel preparation process was developed to minimize the degradation of heat-sensitive active compounds during the preparation of NLCs [23]. Tocopherol was formulated as NLCs and nanoemulsion using homogenization technique to overcome the problems due to its stronger antioxidant nature than tocopherol acetate but due to its viscous form, poor water solubility, instability to light and skin irritation issues it is not used in the current marketed formulations. Tocopherol loaded was characterized for particle size and zeta potential *in vitro* release study was performed using the dialysis method, and skin permeation was carried out using human cadaver skin. It was concluded that tocopherol loaded NLCs proved to be a stable, non-irritant drug delivery system [104]. Oxybenzone loaded NLCs were developed using the solvent diffusion method using liquid lipid Miglyol 812 and oleic acid. After entrapment of oxybenzone in NLCs, it was incorporated into gel base without any crystallization. The topical application of gel formulation containing NLCs of oxybenzone enhance its sun screening efficacy and safety by about six-fold while overcoming solubility and skin irritancy problem probably due to the film formation over the skin, which itself acts as a physical barrier to UV radiations [105]. Alpha-lipoic acid (LA) is a potent antioxidant. Its low water solubility and strong sulfur smell are a major challenge which can be overcome using lipid nanocarrier system. It was an inference that the water-soluble NLC could be suitable colloidal carriers for the water-insoluble LA and their physicochemical and biological activities suggest their potential use for topical delivery [41]. Acitretin is loaded in NLCs which were prepared and characterized for determining its clinical significance in healing psoriasis [106]. Three psoralen derivatives were loaded in SLNs and NLCs to examine their ability to permeate the skin. Enhanced permeation and controlled release of psoralen delivery were both achieved using the NLCs. The *in vitro* nude mouse skin permeation showed that NLCs increased psoralen flux 2.8 times over that of a conventional emulsion [52]. Flurbiprofen loaded NLCs were prepared and evaluated. An increase in stability by 4.5-fold by NLCs is found when compared to phosphate buffered saline [107]. An improved flurbiprofen delivery is observed when the drug is loaded in Compritol NLCs; this is attributable to the particle size and crystallinity [108]. Fluticasone propionate is a glucocorticoid used in the management of skin disorders connected with inflammation such as atopic dermatitis and psoriasis. Fluticasone propionate loaded NLCs were prepared for topical fluticasone delivery with the aim to improve safety and reduce side effect commonly reported in topical corticotherapy [109]. Benzocaine and lidocaine are local anesthetics. NLCs formulation of BENZO and LIDO were developed to study the percutaneous absorption of these local anesthetics from NLCs employing *in vitro* and *in vivo* techniques, and to determine the possibility of achieving a drug-sustained release from NLCs into the skin [46] Pathak and Nagarsenker 2009 also developed LIDO loaded NLC and compared with marketed LIDO formulation for their *in vivo* guinea pig using pinprick test. It was concluded that the LIDO loaded NLC gel exhibited five- to six-fold increase in the duration of an esthesia as compared to marketed formulation [45]. Enoxaparin-loaded NLCs were prepared by the solvent diffusion technique. The effect of formulation and process variables on the physicochemical properties of prepared NLCs was studied and characterized. *In vitro* skin permeation studies revealed the better passage of enoxaparin by NLCs than of plain drug. The *in vivo* skin retention was monitored by fluorescence microscopy (Table 4).

CONCLUSION

As conventional skin cancer therapy leads to various side effects like severe inflammation, pain, long duration of treatment and unappealing scars leading to noncompliance of the patients. The above comprehensive review of literature suggests that NLCs have the potential to serve as multidrug nanocarriers due to enhanced drug loading, physicochemical stability, drug permeation, and limited skin irritation.

Lipid-based drug delivery systems have been proved as promising carriers for cytotoxic drugs due to their potential to increase the solubility and BA of poorly water-soluble and/or lipophilic drugs. The combination of the nanoparticulate delivery system with lipids resulted in the development of SLN. In general, physiological lipids (biocompatible and biodegradable) are employed to prepare lipid NPs with low acute and chronic toxicity. Besides SLN possess several advantages such as lack of organic solvent usage during the production processes and ease of scale of production, as they are formulated from solid lipids only. Therefore, during formulation, a part of the particles crystallizes in a higher energy modification (α or β`). Further on storage, these modifications can transform to the low power, more organized β modification. Due to this modification high degree of alignment, the number of imperfections in the crystal lattice is small; this leads to drug expulsion. Apart from polymorphic transition, SLNs also show some disadvantages as drug carriers including an unpredictable gelation tendency, and low incorporation due to the crystalline structure of solid lipids. To overcome these limitations of the SLNs, second-generation encapsulation systems have been developed by incorporating a liquid carrier oil into the solid lipid matrix to form so-called NLCs. NLCs have shown to have improved bioactive compound encapsulation and delivery properties compared to SLNs. The major advantage of NLC/ delivery system is its ability to incorporate large quantities of drugs as a result of the formation of a less ordered lipid matrix with many imperfections. These are prepared by mixing solid and liquid (oil) lipids. Thus, the development of NLCs has been focused largely on efforts made to improve formulation related difficulties associated with SLNs. The primary objective for formulating NLCs should be the design of stable formulation with potential marketability. Drug-loaded NLCs formulation have shown a great potential to be given by oral, topical, and parenteral route, as it improves the oral BA by bypassing the first pass effect and also overcomes the disadvantage of SLN formulation. The NLC formulation of various lipophilic/hydrophilic drugs mentioned above has shown great potential when compared with the marketed formulations of the same drug. NLCs are biocompatible, biodegradable, non-irritating, and non-sensitizing. No toxicity is reported till now, as the physiological lipids and excipients which are approved by regulatory authorities for clinical applications and for their "generally recognized as safe" status is used in the production of NLCs.

AUTHOR'S CONTRIBUTIONS

Mrs. Arti Majumdar has been compiled the data and summarized all the information, Dr. Nidhi Dubey has supervised the manuscript, and Dr. Neelesh Malviya has been involved in drafting the manuscript or revising it critically for important intellectual content.

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