1. Introduction

1.1. Topical delivery system

Drug application to skin can be classified under two categories: topical and transdermal delivery system. Transdermal delivery systems are discrete dosage forms that, when applied to intact skin, are designed to deliver the drug through the skin to the systemic circulation. TDS comprise an outer covering which is called a drug reservoir that release the drug and second is the controlling membrane. A contact adhesive may be applied to some or all parts of the system and the skin interface, and a protective liner that is removed before the patient applies the system. Topical delivery systems are intended for localized action on one or more layers of the skin while transdermal systems use the percutaneous route for systemic drug delivery, where the skin is not the primary target organ (Kaur et al 2016) (Lingan et al 2011) (Mezei et al 1980).

Topical dosage forms include solutions, colloidal suspensions, emulsions, semisolids (e.g., foams, ointments, pastes, creams, and gels), solids (e.g., powders and aerosols), and sprays. Topical drug delivery is commonly used for the treatment of local infections of the nose, eye and skin. Conventional topical drug delivery systems suffer from drawbacks such as poor retention and low bioavailability. Topical administration has been gaining much attention over the last few years. The successful formulation of topical delivery products requires the careful manipulation of defensive barriers and selection of a soluble drug carrier. Extensive research is required to develop newer topical drug delivery systems aiming either to improve the efficacy or to reduce side effects compared to current patented systems (Tiwari et al 2012) (Muller et al 2004) (Lee et al 1986).

Advantage of Topical Drug Delivery System

- Provide sustained drug release action
- Lower fluctuations in plasma drug levels
- Avoidance of first-pass metabolism
- Improve patient compliance as it is pain free and ease of administration
- Provide local or systemic (in transdermal) effects
- Low cost
1.1.1. The skin as a barrier for penetration of anticancer drug

The skin is the largest organ of the body and is comprised of three layers: the epidermis, dermis and hypodermis. The epidermis plays a role in the penetration of drug substances into the skin. It is the outer avascular layer of the skin, primarily composed of keratinocytes. Because of cellular differentiation, the epidermis is divided into different layers, which are formed by the division of basal cells from the inner part of the body toward the surface. The stratum corneum is the major barrier for the penetration of substances into the skin because of its heterogeneous composition and packed organization of corneocytes and the intracellular lipid matrix. The corneocytes are flat anucleated squamous cells packed primarily with keratin filaments and surrounded by a lipid matrix composed primarily of ceramides, cholesterol, and free fatty acids. Two other cell types that are important in the context of skin tumor composition, melanocytes and Langerhans, are embedded between the basal keratinocytes. Melanocytes are dendritic cells capable of melanin production, and Langerhans cells are antigen presenting cells that are responsible for the immune response to the skin. Because the skin is a heterogeneous organ, this wide variety of cell types can generate several types of benign and malignant tumors. The development of these tumors is associated with many factors, but most of these cancers are related to excess ultraviolet radiation (UV) exposure. Following sun exposure-induced damage, the stratum corneum of tumor lesions usually presents with hyperkeratinisation, a factor known to hamper drug penetration. Topical anticancer administration therefore requires a well-designed formulation to increase drug penetration into the thicker stratum corneum and to favor drug penetration into the deep skin layers, where tumors are usually located (Muller et al 2004) (Barua et al 2014) (Das et al 2016).

**Barriers in TDS**

Barrier function of the skin by the horny layer of the stratum corneum impairs the penetration and absorption of drugs. This layer prevents the penetration of hydrophilic compounds much more efficiently as compared to lipophilic compounds. Therefore, there has been wide interest in exploring new techniques to increase drug absorption through the skin. Novel topical drug delivery systems, with the use of nanotechnology in dosage form design, have been used to facilitate overcoming the skin barrier.
The main barrier of the skin is located in the outermost layer, the stratum corneum. The stratum corneum consists of corneocytes surrounded by lipid regions. As most drugs applied onto the skin permeate along the lipid domains, the organization and composition of the lipid component is considered to be very important for the skin barrier function. This layer generally consists of long chain ceramides, free fatty acids and cholesterol. Additional defensive features of the stratum corneum that have a negative influence on skin penetration, include the low pH, the presence of enzymes on the skin, and the transcutaneous concentration gradient (Paudel et al 2010) (Weiss 2011).

**Factors affecting skin absorption of topically applied drugs are as follow:**

- Size and shape
- Superficial charges
- Lipophilicity
- Presence of penetration enhancers
- Type of formulation
- Physical state of the stratum corneum

### 1.1.2. Drug penetration in the skin

Now days, significant attention has been given to understand the mechanisms by which drugs penetrate through skin. It was known that substances that penetrate the skin by three different routes: through the stratum corneum between the corneocytes (intercellular route); through these cells and the intervening lipids (intracellular route); or through the skin appendages, such as hair follicles and sweat glands. Molecules with adequate solubility in water and oil, with a log of oil/water partition coefficients between 1 and 3 and a molecular weight lower than 0.6 kDa, may penetrate the skin. Therefore, topical administration is limited to hydrophobic and low-molecular weight drugs. Most anticancer drugs are hydrophilic, have low oil/water partition coefficients, high molecular weights and ionic characters, they do not easily penetrate the stratum corneum. Drug permeation through the stratum corneum can be described with Fick’s second law

\[ J = D_m C_v P/ L \]
where \( J \) is the flux, \( D_m \) is the diffusion coefficient of the drug in the membrane, \( C_v \) is the drug concentration in the vehicle, \( P \) is the drug partition coefficient and \( L \) is the stratum corneum thickness.

It can be seen that the flux of a drug through the skin is governed by the diffusion coefficient of the drug in the stratum corneum, the concentration of the drug in the vehicle, the partition coefficient between the formulation and the stratum corneum and the membrane thickness. Using this equation, it is a simple matter to determine which parameters can be manipulated to increase drug flux through the stratum corneum. Formulations containing chemical penetration enhancers or the use of physical penetration methods, such as iontophoresis and electroporation, may alter one or more of these parameters to increase drug penetration in the skin. For instance, chemical enhancers can disrupt the stratum corneum barrier and increase the diffusion coefficient of the drug through the altered membranes. Alternatively, enhancers can alter the solvent nature of the skin and improve partitioning between the formulation and the stratum corneum. Nano carriers can increase drug concentration in the vehicle and so increase drug flux. Physical penetration methods can modify drug penetration routes through the stratum corneum, making it less tortuous and facilitating drug penetration (Pathan et al 2009).

1.1.3. Penetration enhancers for topical delivery system

Penetration enhancers have been widely used in topical formulations. These are chemicals that, when added to the topical formulation, generally favour drug diffusion reversibly disturbing the structure of the stratum corneum, increasing drug diffusivity and increasing the solubility in the skin. There are many well-known substances that may act as penetration enhancers. Fatty acids, oleic acid, azone, dimethyl sulfoxide (DMSO) and terpenes all increase the drug diffusion coefficient by disordering the stratum corneum lipid matrix. Propylene glycol, ethanol, transcutol and N-methyl pyrrolidone act by increasing a drug’s solubility in the skin. Monoolein, a frequently studied substance is used to enhance the skin penetration of anticancer drugs, causing a temporary and reversible disruption of the stratum corneum and increasing drug penetration (Mathur et al 2010).

Different approaches have been developed to increase skin permeability, such as the use of chemical enhancers, the application of an electric field (e.g., iontophoresis and electroporation) and the use of nanocarriers, such as liposomes and polymeric solid lipid
nanoparticles (Mitragotri 2000) (Marren 2011). These methods permeate through the stratum corneum targeting cancerous cells (Figure 1).

Figure 1: Different Methods to improve drug penetration through the skin

1.2. Nanocarriers for topical delivery of anticancer drug

Semi-solid conventional formulations, such as creams, ointments and gels, have been used for topical administration of drugs for many years. Simple application of the formulation on the skin’s surface, however, is not sufficient to allow the drug to reach the site of action. This means that it is important for the formulation to aid in drug penetration through the different skin layers to reach the tumor site. Nanocarriers could improve skin targeting, improving the drug’s ability to reach and penetrate into tumor cells. Moreover, nanocarriers can improve
drug stability and reduce skin irritation by avoiding direct contact of the drug with the skin’s surface. Different nanocarriers have been used for topical application. This section will discuss the most frequently studied, topically applied carriers for the treatment of skin tumors (Gupta et al 2012) (Taveira and Lopez 2011).

1.2.1. Liposomes: Liposomes are one of the most researched nanocarriers for the treatment of cancer. They are colloidal particles and are biocompatible and biodegradable, consisting primarily of phospholipid vesicles. These vesicles are in turn composed of one or several lipid bilayers. Phospholipids are able to self-assemble into vesicular structures when dispersed in an aqueous medium because of their amphiphilic characteristic. The non-polar tails orient toward non-polar tails of other phospholipid molecules present in the medium in an attempt to avoid the water. This process forms lipid bilayers that are separated by the polar heads of the phospholipids. Because of this special arrangement, liposomes are able to entrap both hydrophilic and hydrophobic compounds in the aqueous compartments or within the lipid bilayer, respectively. Moreover, lipid bilayers are biocompatible with the stratum corneum, increasing the liposome’s affinity for the skin and making them able to release drugs directly to this membrane. Traditional liposomes are composed primarily of phospholipids. Liposomes formed with different components have been developed in an attempt to increase the stability of the vesicles and their ability to penetrate through different membranes, especially the stratum corneum. In this way, elastic liposomes, also called ultra-deformable or ultra-flexible liposomes, are the new generation of liposomes. These contain surfactants, other amphiphiles or ethanol in their composition, which improve the flexibility of the lipid bilayer. Transfersomes, niosomes and ethosomes were the names given to the first, second and third flexible liposome generations; respectively Transfersomes are composed of phosphatidylcholine and sodium cholate. Ethosomes consist of a mixture of phosphatidilcoline and ethanol, and niosomes are non-ionic surfactant vesicles. The ability of these vesicles to deform gives them the ability to pass through narrow pores, such as the pores present on the skin surface, possibly improving the penetration of drugs carried by these vesicles into the deep skin layers. New generations of liposomes have been well studied in the context of topical administration and have also been introduced into the field of topical skin cancer treatments (Sercombe et al 2015) (Allen et al 2013) (Deshpande et al 2013) (Park et al 2002).
Niosomes: They are non-ionic surfactant vesicles made up of single chain surfactant molecules in combination with cholesterol. These nanoparticles generally resemble the same characteristics as that of liposomes, however are considered more stable. Niosomes were thought to improve the horny layer properties, both by reducing trans epidermal water loss and by increasing smoothness through replenishment of lost skin lipids following fusion to coenocytes. Niosomes have the ability to modify the structure of the stratum corneum through their surfactant properties, in order to make the layer looser and more permeable (Okore et al 2011) (Mujoriya et al 2011).

Transfersomes: They are a novel type of liquid-state vesicles that consist of phospholipids and an edge activator, which is often a single chain surfactant (e.g., Sodium cholate, Span 60, 65, 80, and Tween 20, 60, 80) that destabilizes the lipid bilayers of the vesicles and increases their deformability by lowering the interfacial tension. They are considered the first generation of highly elastic or deformable vesicles. This feature is thought to enable transfersomes to squeeze themselves through intercellular regions of the stratum corneum under the influence of the transdermal water gradient. They have been reported to penetrate intact skin in vivo with efficiency similar to subcutaneous administration, provided that the elastic vesicles are topically applied in non-occlusive conditions (Gupta et al 2012) (Benson 2006).

Ethosomes: They are another novel lipid carrier that has shown enhanced skin delivery of encapsulated compounds. The ethosome system is mainly composed of phospholipids, a relatively high concentration of ethanol (20–50%) and water. The high alcohol concentration allows the formation of soft, malleable and highly fluid vesicles. Ethanol is a well-known permeation enhancer that is suggested to provide a synergistic mechanism with the vesicles and skin lipids. The inclusion of ethanol may provide the vesicles with soft flexible characteristics, which allow them to more easily penetrate into deeper layers of the skin. Phospholipid vesicles containing ethanol may also influence the bilayer structure of the stratum corneum to enhance drug penetration (Verma and Pathak 2010) (Bhalaria et al 2009).

1.2.2. Polymeric and lipid nanoparticles

Polymeric nanoparticles: The polymeric nanoparticles are prepared using biodegradable and biocompatible polymers having size in the nanometer range. Depending on the preparation
techniques, templates as well as conditions used such as stirring speed, pH, ionic strength, type and concentration of surfactant/stabilizer and temperature etc., nanoparticles with desired properties can be prepared. Polymeric nanoparticles can be classified as nanocapsules and nanospheres. Nanospheres have a solid matrix while nanocapsules have a shell that surrounds a core which is usually oily. Anticancer drugs can be encapsulated inside or be associated with the nanoparticle surface. Nanocapsules have a polymeric shell with an interior phase that is often oily (Rao et al 2011) (Chan et al 2010).

The advantages of nanoparticles for topical delivery are enlisted below

- Physically stable as compared to liposomes
- Encapsulated active compound can be stabilized
- Site-specific targeting, thus low dose of the active compound is required
- Provide controlled release
- Their small size (<50 nm) enables penetration through skin.

**Solid lipid nanoparticles:** Solid lipid nanoparticles (SLNs) have been studied since the 1990s and are considered new relative to liposomes and polymeric nanoparticles. Solid lipid nanoparticles (SLN) are formed by a matrix of lipids which are biodegradable raw materials that are physiologically well tolerated. SLNs are primarily composed of lipids, which are solid at room temperature, dispersed in water. They are similar to nanoemulsions, but the inner liquid lipid is replaced with a solid lipid. This structure can improve sustained drug release because drug mobility is lower in SLNs. When compared to liposomes, SLNs exhibit greater stability, prolonged drug release and greater ease in sterilization and in scaling the manufacturing process to an industrial level. The absence of organic solvents in the preparation of SLNs is a huge advantage compared to polymeric nanoparticles. However, low drug loading and drug expulsion during storage can be a limiting factor for some therapeutic treatments. Both SLNs and polymeric nanoparticles have been shown to promote sustained drug release and protection against drug degradation when topically applied. In addition, they allow for modifications to matrix softness and superficial charges, adjustments that may improve skin targeting (Figure 2). The exact mechanism by which these particles increase drug penetration through the skin is not completely understood, but efforts to understand this property have been made by developing and characterizing different nanoparticles. It appears that nanoparticles can closely contact the superficial junctions of corneocytes clusters and furrows, possibly favouring drug accumulation for several hours. This would allow for the
sustained release of anticancer drugs. However, there are controversies regarding the ideal mean diameter, flexibility and superficial charge of nanoparticles to contribute to skin penetration. Studies of nanoparticles have not been limited to the examination of cytotoxic cancer drugs; numerous studies have also been performed that use these systems to deliver antiproliferative drugs. SLNs have been shown to increase drug stability and to decrease drug irritation. In summary, most studies have described the advantages of drug encapsulation in nanoparticles by demonstrating increased drug stability, sustained release and improved skin penetration and cytotoxicity. Despite such promising results, more studies should be performed to elucidate the mechanisms by which nanoparticles increase the ability of anticancer drugs to penetrate the skin (Mukherjee et al 2009) (Almeida et al 2007).

Additional features are the avoidance of organic solvents during the preparation and amenability to large scale production and sterilization. Furthermore, the great ability of SLNs to facilitate the contact time of active substances with the stratum corneum, because of the small size of the particles and consequently the high surface area, leads to the high permeation of the carried substances into the viable skin (Ekambaram et al 2012).

Figure 2: Drug incorporation models of SLN (a) solid solution model, (b) core-shell model (drug-enriched Shell) and (c) core-shell model (drug-enriched core)
Nanostructured lipid carriers (NLC): NLC are colloidal carriers characterized by a solid lipid core consisting of a mixture of solid and liquid lipids, and having a mean particle size in the nanometer range. This nanostructure improves drug loading and firmly retains the drug during storage. NLC system minimizes some problems associated with SLN such as low payload for some drugs; drug expulsion on storage and high water content of SLN dispersions. The basic idea is that by giving the lipid matrix a certain nanostructure, the payload for active compounds is increased and expulsion of the compound during storage is avoided. Ability to trigger and even control drug release should be considered while mixing lipids to produce NLC. Newer methods of generating NLC have been developed. Nanoparticle drug carrier systems are potential formulations to improve the therapeutic effectiveness and safety profile of conventional cancer chemotherapies. Different types of nanoparticles have been investigated for topical delivery (Muller et al 2007) (Fang et al 2013).

1.3. Nanostructure lipoidal carrier

Nanostructure lipid carriers (NLC) are the new generation of lipid nanoparticles, as novel colloidal drug carriers. NLC were developed to overcome the limitations associated with the SLN. SLN consist of solid lipids, while NLC consist of a mixture of specially blended solid lipid (long chain) with liquid lipid (short chain), preferably in a ratio of 70:30 up to a ratio of 99.9:0.1. Commonly observed disadvantages of SLN include limited drug-loading capacity, drug expulsion during storage, and relatively high water content in the dispersions. It has been well documented that NLCs were developed to overcome the limitations of SLN. The addition of a liquid lipid to a solid lipid creates a less-ordered crystal lattice with an increased number of imperfections, attaining high drug encapsulation and stable drug incorporation during storage.

Drug release from nano lipid particles occurs by diffusion and by lipid particle degradation in the body. NLCs accommodate the drug because of their highly unordered lipid structures. Lipid particles are preferentially suited to incorporate lipophilic drugs; hydrophilic drugs can only be incorporated at a low percentage. They also have applications in cosmetics, food and
agricultural products. These have been utilized in the delivery of anti-inflammatory compounds, cosmetic preparation, topical corticosteroid therapy and also increases bioavailability and drug loading capacity (Loo et al 2013) (Souto et al 2007) (Naseri et al 2015) (Souto and Muller 2005).

1.3.1. Type of NLC

- **Highly imperfect matrix:** In this solid lipids and liquid lipids are blended. The difference in the structures of the lipids and special requirements in the crystallization process lead to a highly disordered, imperfect lipid matrix structure which offer more space for drug and amorphous clusters of drugs as shown in Figure 3.

- **Multiple O/F/W type:** Usually the drug solubility is more in liquid lipids than in solid lipids. Based on this, particles were produced with a high content of liquid lipids (oils) (Figure 3). During the production process, the liquid lipid particles are cooled from the molten state to room temperature leads for crystallization and form solid particles. At high oil concentrations a miscibility gap of the two lipids occurs during the cooling phase, leading to phase separation that means precipitation of tiny oily nano compartments.

- **Non-crystalline amorphous NLC:** In this method lipids are mixed in such a way that crystallization is avoided. The lipid matrix is solid, but in an amorphous state (Figure 3). The absence of crystallization avoids drug expulsion (Kumar et al 2012).
Figure 3: Structures of NLC. Class I (Imperfect type), Class II (Non crystalline, amorphous), Class III (Multiple type)

1.3.2. Advantage of NLC

- NLCs have a higher drug-loading capacity
- Avoid or lessen expulsion of active compounds during storage
- Enhanced drug loading due to enhanced solubility of liquid lipid than that of solid lipid
- NLC carriers are composed of physiological and biodegradable lipids, exhibiting low systemic toxicity and low cytotoxicity (Uner 2006) (Saupe et al 2005)

1.3.3. Preparation technique

Commonly used methods for preparation of NLC are high pressure homogenization at elevated or low temperatures (including hot homogenization and cold homogenization), solvent emulsification, evaporation or diffusion, supercritical fluid (supercritical fluid extraction of emulsions (SFE)), ultrasonication or high speed homogenization and spray drying. Their processes and comparison have been summarised in Table 1.

**Homogenization Process:** Homogenization can be of two types either hot homogenization or cold processes (Figure 4). The pharmaceutical compound is dissolved or dispersed in the melted lipid before HPH, in both the processes. Melted dispersion passed through the narrow gap in homogenizer. Average particle size is in sub-micron region. Homogenization has several advantages including large-scale production, no use of organic solvent, improved product stability and improved loading of drugs, but specific high pressure and temperature conditions pose challenges to its application for dosage form development.

**Hot homogenization technique:** The drug is dissolved in melted lipid. The drug-containing lipid melt is dispersed in the molten state in a hot aqueous surfactant solution. Dispersion is performed using a high-speed stirrer. The pre emulsion obtained is then homogenized using a high pressure homogenizer at a pressure ranging from 100 to 1500 bar. Typically one to three homogenization cycles are sufficient. After that cooling the oil-in-water nanoemulsions to room temperature or below, leads to lipid crystallization and formation of lipid nanoparticles. This technique can be successfully applied to lipophilic and insoluble drugs, but it is not
entirely suitable for hydrophilic drugs. During homogenization, the hydrophilic drug partitions to the aqueous phase resulting in low entrapment efficiency (Joshi et al 2006) (Thatipamula et al 2011).

**Cold homogenization technique:** This technique is much more suitable for hydrophilic drugs. The drug is incorporated in the melted lipid. If the solubility of the hydrophilic drug in the lipid is too low, surfactants can be used for solubilization of the drug. The drug-containing lipid melt is solidified in dry ice or liquid nitrogen to increase the brittleness of the lipid and to ease the milling process. After milling the microparticles obtained are dispersed in a cold aqueous surfactant solution. This lipid suspension is homogenized at room temperature. The solid state of the matrix minimizes partitioning of the drug to the water phase. Many heat-sensitive drugs can be incorporated into lipid nanoparticles by this technique since the thermal exposure of the drug is relatively short (Figure 5). Most lipid nanoparticles produced by hot homogenization are characterized by an average particle size of below 500 nm (Patel et al 2013) (Sanap et al 2014).

![Diagram](image)

**Figure 4:** Diagrammatic representation of hot high pressure homogenization and cold high pressure homogenization
In general, the advantages of the high pressure homogenization technique over others are narrow particle size distribution of the product with a low content of microparticles, higher lipid particle content in the dispersions, avoidance of organic solvents, acceptability of the homogenization equipment by the regulatory authorities (even for parenteral products), scale-up feasibility and the availability of homogenization production lines in industry. Depending on the size of production-scale homogenizers, a wide production range (500–60000 l/h) is possible.

**Solvent emulsification /evaporation:** In the method of solvent evaporation by precipitation in o/w emulsions, lipid dissolved in an organic solvent is emulsified in a water bath under mechanical stirring in an aqueous phase containing surfactant. Stirring of the resulting oil-in-water emulsion is maintained under ambient conditions to allow evaporation of the solvent. The solvent evaporation process can be performed under reduced pressure. Upon evaporation
of the solvent, a lipid nanoparticle dispersion is formed by precipitation of the lipid material in the aqueous medium. Depending on the solid lipid and surfactant used, particles with average diameters of 30–100 nm can be obtained. A particle size of around 30 nm can be obtained for cholesteryl acetate nanoparticles stabilized with a blend of phosphatidylcholine and sodium glycocholate. Avoidance of heat during the preparation is the most important advantage of this method. Solvent emulsification-diffusion in an aqueous system is based on emulsion solvent diffusion in water similar to evaporation by precipitation in o/w emulsions. Lipid is dissolved in the organic phase in a water bath since it cannot be completely dissolved in the organic phase at room temperature. Addition of water to the resultant organic solution under mechanical agitation results in coacervation and formation of lipid nanoparticles. The lipid nanoparticle dispersion obtained is then separated by centrifugation, by evaporation of the solvent under reduced pressure. In the solvent emulsification-evaporation or -diffusion methods, the suspensions obtained are fairly dilute due to the limited solubility of the lipids in the organic solvents used (Figure 6) (Emami et al 2012) (Nabi et al 2013).

Figure 6: Emulsification solvent evaporation technique in the preparation of lipid carrier system

**Supercritical fluid extraction of emulsions (SFEE):** This method uses a supercritical fluid such as carbon dioxide for solvent extraction from o/w emulsions. Carbon dioxide is a good option, but it cannot dissolve many drugs. Therefore, supercritical anti solvent precipitation (SAS) can be an alternative method to SFEE (Chen et al 2009).
Ultra sonication or High Speed Homogenization (US or HSH): US or HSH do not involve organic solvents, large amount of surfactants or additives. These techniques are easy and require familiar tools which are available in almost every laboratory. Melted lipid is added and dispersed in an aqueous surfactant solution under high shear homogenization or ultrasonication. Then the emulsion is cooled down to room temperature. While a homogenizer such as a rotor-stator homogenizer is required for high shear, ultrasonication can be performed using a probe. Low dispersion quality is a disadvantage of high shear homogenization and ultrasonication. Dispersion quality of the lipid nanoparticles produced by these techniques is often affected by the presence of microparticles leading to physical instability upon storage (Figure 7) (Puglia et al 2013) (Chen et al 2009).

Figure 7: High shear homogenization and ultrasonication techniques in the preparation of NLC
Spray drying: This system is a substitute to lyophilization method that leads to the production of pharmaceutical products from aqueous dispersion. Spray drying is a cost effective method rather than lyophilization. The high temperatures and shear forces used in this method, leads to particle aggregation. According to previous studies only those lipids with melting point greater than 70 °C are suitable for spray drying (Puglia et al 2013) (Nandiyanto et al 2011).

Table 1: Comparison of different production techniques along with their advantages and limitations

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
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| Hot High Pressure Homogenization | • Avoidance of organic solvents  
• Easy to scale up  
• Ready availability of instruments easily  
• Regulatory approved process | • High temperature cause degradation  
• Burst release at starting  
• Coalescence of particles  
• Conformation changes in protein |
| Cold High Pressure Homogenization | • Reduced exposure of drug to heat  
• Can also be used for hydrophilic and temperature sensitive drug | • Provides a high Polydispersity Index to the formulation |
| Melt Emulsification / Ultrasound | • No organic solvent left in final formulation  
• No burst release  
• High lipid concentration  
• Avoidance of organic solvent  
• Higher Drug loading | • Contamination  
• Broader particle size range  
• Metallic particle |
| Emulsification Solvent evaporation | • Avoidance of heat during production process so suitable for thermo labile drugs  
• Simple procedure | • Solvent Residues in final dosage form |
| Emulsification Solvent diffusion | • Simple process  
• Faster drug release | • Low lipid content  
• Organic solvent residue  
• Low drug loading and... |
1.4. Topical Nano lipoidal carrier system

Several problems have been listed with conventional topical preparations such as low uptake due to the barrier function of the stratum corneum and absorption to the systemic circulation. Much attention has been given in recent years to the lipoidal carrier system for topical application. Many features, which these carrier systems exhibit for dermal application of cosmetics and pharmaceutics, have been elucidated. SLN and NLC are composed of physiological and biodegradable lipids that show low toxicity. The small size ensures a close contact to the stratum corneum and can increase the amount of drug penetrated into the skin. Due to the occlusive properties of lipid nanoparticles, an increased skin hydration effect is observed. Furthermore, lipid nanoparticles are able to enhance the chemical stability of compounds sensitive to light, oxidation and hydrolysis.

Advantage of Topical NLC

1. Increase of skin occlusion: The lipid film formation on the top of the skin and the subsequent occlusion effect was reported for lipid nanoparticles. By using very small lipid particles, which are produced from highly crystalline and low melting point lipids, the highest occlusion can be reached. Comparing NLC with varying oil content showed that an increase in oil content leads to a decrease of the occlusive factor.

2. Increase of skin hydration and elasticity: The reduction of trans epidermal water loss caused by occlusion leads to an increase in skin hydration after dermal application of SLN, NLC or formulations containing them. An in vivo study showed that the SLN-containing o/w cream increased the skin hydration significantly more than the conventional o/w cream.
3. Enhancement of skin permeation and drug targeting: The stratum corneum in healthy skin has typically a water content of 20% and provides relatively an effective barrier against percutaneous absorption of exogenous substances. Skin hydration after applying SLN or NLC leads to a reduction of corneocytes packing and an increase in the size of the corneocytes gaps. This facilitates the percutaneous absorption and drug penetration to the deeper skin layers.

4. Improve benefit/risk ratio: Skin atrophy and systemic side effect occurred after applying traditional dosage form could be avoided when drug was formulated as NLC. NLC can enhance skin penetration of incorporated actives, promote the epidermal targeting and minimize the systemic side effects and therefore, the benefit/risk ratio is improved.

5. Enhancement of UV blocking activity: Some side effects of organic UV blockers have been reported due to the penetration of these compounds into the skin thereby causing skin irritation and allergic reactions. This penetration can be reduced by incorporating these compounds in lipid nanoparticles. Improving the UV blocking activity allows the reduction of the concentration of the UV blocker while maintaining the protective level of the conventional formulation. Encapsulation of inorganic sunscreens into NLC is therefore a promising approach to obtain well tolerable sunscreens with high SPF.

6. Enhancement of chemical stability of chemically labile compounds: Enhancement of chemical stability after incorporation into lipid nanocarriers was proven for many cosmetic actives e.g. coenzyme Q 10, ascorbyl palmitate, tocopherol (vitamin E) and retinol (vitamin A) (Aliasgharlou et al 2016) (Kong et al 2012) (Han et al 2012) (Paolino et al 2011)

Limitations of Topical Nano delivery system

The specific challenge of designing a therapeutic system is to achieve an optimal concentration of a certain drug at its site of action for an appropriate duration. The greatest challenge for dermal penetration is the tough horny layer, that is, stratum corneum (SC), the uppermost layer of the skin, which is the rate limiting step for epidermal drug transport. The physicochemical factors of drug like log pKₐ, solubility, and molecular mass also play an important role in the selection of components for the topical delivery vehicle. For acidic and unstable drugs special consideration has to be made on the excipient selection for topical
vehicle which will not only mask the irritation potential of drug due to acidic group but also maintenance of chemical integrity (Hajare et al 2014) (Dev et al 2015) (Fang et al 2011) (Lau et al 2008)

- Not all drugs are suitable for topical delivery
- Drugs that require high blood levels cannot be administered
- Drugs or drug formulation may cause sensitization or irritation which must be evaluated fairly early in the development process

1.5. Skin cancer

Skin cancer is the most common form of malignancy in the United States and many other countries. In India the trend is on a rise melanoma represents only a very small proportion of skin cancer incidence, but it accounts for the vast majority of skin cancer deaths. Indeed, at the early stage, melanoma can be surgically removed, with a survival rate of 99%, while metastasized melanoma causes the death of 80% of patients within 5 years from the diagnosis. Other types of skin cancers, basal cell carcinoma and squamous cell carcinoma, are the most common diseases (Miller et al 1994) (Brash et al 1991).

1.5.1. Type of skin cancer

There are three major types of skin cancers: basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanoma. The first two skin cancers are grouped together as non-melanoma skin cancers. Other unusual types of skin cancer include Merkel cell tumors and dermatosarcoma protruberans. The vast majority of skin cancers are basal cell carcinomas and squamous cells carcinomas. While malignant, these are unlikely to spread to other parts of the body. They may be locally disfiguring if not treated early. A small but significant number of skin cancers are malignant melanomas. Malignant melanoma is a highly aggressive cancer that tends to spread to other parts of the body. These cancers may be fatal if not treated early (Armstrong et al 2001) (Harwood et al 2006).
1.5.2. Cause of skin cancer

Important cause of skin cancer is as follows

- Ultraviolet (UV) light exposure, most commonly from sunlight is frequent cause of skin cancer. Exposure to ultraviolet (UV) radiation, namely UVA and UVB wavelengths, is the major environmental risk factor for development of NMSC. More specifically, intensive intermittent UV exposure is a risk factor for BCC whereas chronic, cumulative exposure is a risk factor for SCC.
- Immunosuppression, or impairment of the immune system, which protects the body from germs or substances that cause an allergic reaction
- Exposure to unusually high levels of radiations, such as X-rays
- Skin type and family history
- Contact with certain chemicals, such as arsenic and hydrocarbons in tar, oils, and soot (Leiter and Garbe et al 2008) (Naryanan et al 2010) (Boffetta et al 1997)

1.5.3. Symptoms of skin cancer

A basal cell carcinoma (BCC) usually looks like a raised, smooth, pearly bump on the sun-exposed skin of the head, neck, or shoulders. Others signs include:

- Small vessels may be visible within the tumor
- A central depression with crusting and bleeding (ulceration) frequently develops
- A BCC often appears as a sore that does not heal
- A squamous cell carcinoma (SCC) is commonly a well-defined, red, scaling, thickened bump on sun-exposed skin. It may ulcerate and bleed, and left untreated, may develop into a large mass (Kris et al 2003) (Nooijer et al 2001)

1.5.4. Nanotechnology in skin cancer

Nanotechnology is a generalization for techniques, materials, and equipment that operate at the nanoscale. It is a revolutionary approach that consists of the design, characterization, preparation, and application of structures, devices, and systems by controlling shape and size at the nanoscale. These biomimetic features, together with their high surface-to-volume ratio and the possibility of modulating their properties, raised the interest of the use in biomedical
application with potential applications in imaging, diagnosis, and therapy. Over the past two decades, the rapid developments in nanotechnology have allowed the incorporation of multiple therapeutic, sensing, and targeting agents into nanoparticles, for detection, prevention, and treatment of oncologic diseases.

Nano medicine has an enormous potential to improve the selectivity in targeting neoplastic cells by allowing the preferential delivery of drugs to tumours owing to the enhanced permeability and retention effect (EPR). Furthermore, specific binding of drugs to targets in cancer cells or the tumour microenvironment increases the effectiveness of the specific treatment of cancer cells, while leaving healthy cells intact. Nanoparticles (NP) can also improve the solubility of poorly water-soluble drugs, modify pharmacokinetics, increase drug half-life by reducing immunogenicity, improve bioavailability, and diminish drug metabolism. They can also enable a controlled release of therapeutic compounds and the simultaneous delivery of two or more drugs for combination therapy. In addition, by reducing the drug doses, it is also possible to reduce side effects and ameliorate the patients compliance. These engineered nanocarriers also offer the opportunity to use the combination of imaging and drug therapy to monitor effects in real time, as well as the possibility to join the delivery of drug with energy (heat, light, and sound) for synergistic anticancer therapeutic effects (Nohynek et al 2008) (Ferrari et al 2005) (Alvia et al 2011).

1.5.5. Drug approved for Skin Cancer

Drugs approved by the Food and Drug Administration (FDA) in India for skin cancer, including for basal cell carcinoma and melanoma.

Drugs Approved for Basal Cell Carcinoma: Aldara (Imiquimod), Efudex (Fluorouracil--Topical), Erivedge (Vismodegib), 5-FU (Fluorouracil--Topical), Imiquimod, Odomzo (Sonidegib), Sonidegib, Vismodegib

Drugs Approved for Melanoma: Aldesleukin, Cobimetinib, Cotelic (Cobimetinib), Dabrafenib, Dacarbazine, DTIC-Dome (Dacarbazine), IL-2 (Aldesleukin), Imlygic (TalimogeneLaherparepvec), Interleukin-2 (Aldesleukin), Intron A (Recombinant Interferon Alfa-2b), Ipilimumab, Keytruda (Pembrolizumab), Mekinist (Trametinib), Nivolumab, Opdivo (Nivolumab), Peginterferon Alfa-2b, Pembrolizumab, Proleukin (Aldesleukin), Recombinant Interferon Alfa-2b, Sylatron (Peginterferon Alfa-2b), Tafinlar (Dabrafenib),
1.6. Photoinduced tumour

The sun emits UV radiation, which covers a small part of the electromagnetic spectrum from 400 nm to 100 nm. Within the UV portion of the spectrum, the biological effects of the radiation vary significantly with wavelength range. Sun light emits UV radiation in the UVA (320–400 nm), UVB (280–320 nm), and UVC (200–280 nm) bands, but because of absorption in the atmosphere's ozone layer, 99% of the UV radiation reaches the earth's surface is UVA. UV radiation is a very prominent environmental toxic agent. UVB radiation (wavelengths between 280 and 320 nm) is partially absorbed by the ozone layer and the remaining portion that reaches the earth can cause severe damage on biological organisms, while UVA (wavelengths >320 nm) is not absorbed by ozone layer and comparatively is not damaging to biological organisms.

UVB radiation, comprising approximately 5–10% of the entire spectrum of UV radiation reaching the surface of the earth is characterized by a relatively high energy and responsible for the most important biological effects including sunburn, erythema, pigmentation, Vitamin D3 synthesis, immunosuppression, inflammation, photo aging, and carcinogenesis. UVB is absorbed in the stratum corneum of the skin by cellular chromophores. Effected factors in the skin are melanin, cellular DNA, proteins, lipids, and amino acids. UVB directly damages the DNA strand, resulting in the formation of pyrimidine dimers and distortion of repair mechanisms, which lead to mutations. The reactions induced by UVB radiation are immediate, resulting in the release of inflammatory mediators (e.g., histamine, serotonin, and prostaglandins) which lead to dilation of capillaries and the development of erythema and edema. UVB easily penetrates through water and quartz glass. However, these rays are filtered through clouds and windowpanes. Greatest ray intensity is reached during the summer, between the hours of 10 am and 5 pm (Reddi et al 1990) (Guardiano et al 1989) (Bugaj et al 2011)
1.7. Phytochemicals for skin cancer

Phytoconstituents are gaining popularity as ingredients in cosmetic formulations because they can protect the skin against exogenous and endogenous harmful agents and can help remedy many skin conditions. Exposure of skin to sunlight and other atmospheric conditions causes the production of reactive oxygen species (ROS), which can react with DNA, proteins, and fatty acids, causing oxidative damage and impairment of antioxidant system. Such injuries damage the regulation pathways of skin and lead to photo aging and development of skin cancer. The effects of aging include wrinkles, roughness, appearance of fine lines, lack of elasticity, and de- or hyperpigmentation marks. Herbal extracts act on these areas and produce healing, softening, rejuvenating, and sunscreen effects (Kowalczyk et al 2010) (Liu 2004).

Various synthetic agents are used as photoprotectives, but they have limited use because of their potential toxicity in humans and their ability to interfere in certain selected pathways of multistage process of carcinogenesis. Several botanical compounds have been shown to be antimutagenic, anticarcinogenic, and nontoxic and have the ability to exert striking inhibitory effects on a plethora of cellular events at various stages of carcinogenesis. Because multiple pathways are involved in photocarcinogenesis, a mixture of several botanical antioxidants working through various mechanisms, in conjunction with the use of sunscreens, could also be an effective approach for reducing UV-generated ROS-mediated photo damage, immunosuppression, and skin cancer in humans. Few examples include tea polyphenols, curcumin, silymarin, garlic compounds, apigenin, resveratrol, ginkgo biloba, beta-carotenoids, and ascorbic acid. There is a need to develop herbal formulations that could combat the harmful effects of both UV-A and UV-B radiations. The present researches are aimed to develop novel strategies to reduce the occurrence of skin cancer and delay the process of photo aging (Lee et al 2011) (Surh et al 2003).

Phytochemicals uses are increased in humans now days, although an increasing number of well conducted studies were done with a lower risk off side effects, as well as lower relapse after initial treatment completion. There is a wide range of phytochemicals, but one of the largest and well-known groups being the polyphenols. The health benefits of phytochemical are being consistently highlighted in the medical use and hence there is an increasing interest among medical practitioners and their patients, especially those with cancer who have a particular interest in over the counter drugs (Table 2). There are three major groups of
phytochemicals: the polyphenols which can be subcategorized as the flavonoids, phenolic acids and other non-flavonoid polyphenols; the terpenoids, which can be subcategorized as the carotenoids and non-carotenoid terpenoids; and the thiols, which includes the glucosinolates, allylicsulfides and non-sulphur containing indoles. There are other phytochemical group, which although have some properties within these groups, have been classified within a miscellaneous category and examples of these include the chlorophylls and capsaicin.

**Photo protective:** The use of active photoprotectives is very beneficial in facing the deleterious effects of UV rays. Important categories of beneficial phytoconstituent include phenolic acids, flavonoids, and high molecular weight polyphenols. Naturally occurring phenolic acids include hydroxycinnamic acid and hydroxybenzoic acid. High molecular weight polyphenols, also known as tannins, include condensed polymers of catechins or epicatechins and hydrolyzable polymers of gallic or ellagic acids. Many flavonoids, such as quercetin, luteolin, and catechins, are found to be better antioxidants than ascorbic acid, alpha-tocopherol, and beta-carotene. Other photoprotective phytoconstituents include curcumin, garlic compounds caffeic and ferulic acid, apigenin, genistein, resveratrol, nordihydroguaiaretic acid, carnosic acid, silymarin, tea polyphenols, *Capparisspinosa* extract, *Culcitiumreflexum* leaf extract, *French maritime* pine bark extract, *Ginkgo biloba* extract, Grape seed extract, *K. triandra* root extract, *Prunuspersica* flower extract, *S. officinalis* root extract, and *Sedum telephium* leaf extract (Proteggant et al 2003) (Pathak et al 1982).

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Mechanism of action on skin</th>
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<tbody>
<tr>
<td>Quercetin</td>
<td>Antioxidant</td>
</tr>
<tr>
<td></td>
<td>Protects from activities of glutathione peroxidase, reductase, catalase</td>
</tr>
<tr>
<td>Green Tea Polyphenols</td>
<td>Reduce production of cyclobutane pyrimidine dimers</td>
</tr>
<tr>
<td></td>
<td>Protects from UV induced erythema and edema</td>
</tr>
<tr>
<td></td>
<td>Reduce H₂O₂ and NO production and lipid peroxidation</td>
</tr>
<tr>
<td>Compound</td>
<td>Effects</td>
</tr>
<tr>
<td>-------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Silymarin</td>
<td>Inhibits skin sunburn, skin edema and cellular apoptosis</td>
</tr>
<tr>
<td></td>
<td>Inhibits induction of ODC and COX-2 activity</td>
</tr>
<tr>
<td></td>
<td>Inhibits catalase activity</td>
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<tr>
<td>Curcumin</td>
<td>Inhibits lipid peroxidation and arachidonic activity</td>
</tr>
<tr>
<td></td>
<td>Inhibits Ornithine decarboxylase activity</td>
</tr>
<tr>
<td></td>
<td>Enhances glutathione activity</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>Inhibits ODC and COX-2 activity</td>
</tr>
<tr>
<td></td>
<td>Controls increased level of lipid peroxidation</td>
</tr>
<tr>
<td>Genistein</td>
<td>Reduces edema</td>
</tr>
<tr>
<td></td>
<td>Inhibits ultra violet induced $H_2O_2$ production</td>
</tr>
</tbody>
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1.8. Literature Survey

The present literature survey was undertaken to gain a better insight in to the Nanostructured Lipoidal Carriers.

1. Cao et al (2012) prepared a formulation of silybin using a combination of solid dispersion, gel matrix and porous silica nanoparticles (PSNs) and established the in vitro/in vivo correlations (IVIVCs). It was concluded that Silybin could be released up to 72 hr with first-order release kinetics and from PSNs with Higuchi kinetics.

2. Han et al (2012) developed Nanostructured lipid carriers (NLC)-based gel as potential topical system for flurbiprofen (FP) topical delivery. It was observed that NLC remained within the colloidal range and it was uniformly dispersed after suitably gelation by carbopol. It was indicated through in vitro permeation studies through rat skin that FP-NLC-gel had a more pronounced permeation profile compared with that of FP loaded conventional gel.

3. Kumbhar et al (2012) developed an optimized nanostructured lipid carrier (NLC) for bicalutamide (BCT), a poorly water-soluble drug, and to investigate its phase transition behavior during the NLC processing. Finally, NLCs made of Precirol® ATO 5 (solid lipid) and triacetin (oil) was found to possess the potential to entrap the poorly water-soluble drug, bicalutamid and the system could be tailor-made to meet the desired drug release.

4. Kamble1 et al (2012) reviewed types of NLC, preparation methods and characterisation of SLN and NLC. The review covered in brief the comparative study of SLN and NLC of some drugs by researchers. It was concluded that NLC are attractive alternatives to micro and nanoemulsions, liposomes and nanoparticles, but a detailed study of possibility of meeting industrial needs such as process scale up, equipment qualification and validation is required.

5. Fangueiroa et al (2012) reported a $3^3$ full factorial design study to optimize SLNs formulations for hydrophilic biomolecules. Coating SLNs with alginate improves their stability and could also provide better mucoadhesive properties. The results suggested the use of SLNs based multiple emulsions for the incorporation of several hydrophilic drugs, such as proteins and peptides.
6. Phiriyawirut et al (2012) proposed silymarin-loaded electrospun cellulose acetate (CA) fibers in which silymarin was added in various amounts (i.e., 2.5-20 wt. % based on the weight of CA powder). The results found that the CA fiber and silymarin-loaded electrospun CA fibers were not degraded by these test solutions.

7. Spada et al (2012) developed silymarin emulsions designed for the skin delivery and determined influence of hydroxypropyl-β-cyclodextrin on the extract delivery and permeation. This research demonstrated that the penetration capability of formulations is strictly influenced by the phytocomplex, moreover phytocomplex increases flavonoids stability and in vitro release behaviour.

8. Severino et al (2012) reviewed the optimization of the production process of SLN and NLC by High Shear Homogenization (HSH) and High Pressure Homogenization (HPH). To build up the surface response charts, a $2^2$ full factorial design based on 2 independent variables was used to obtain an optimized formulation. This factorial design study has proven to be a useful tool in optimizing SLN (~100 nm) and NLC (~300 nm) formulations.

9. Martinho et al (2011) reviewed new drug delivery systems like lipid, proteic and polymeric technologies to provide new sustained drug delivery with better body distribution, drug protection from the harsh external environment and avoidance of drug clearance. This review covered the generalities of these new carriers and their new advances in drug delivery.

10. Javed et al (2011) reviewed diverse pharmacological activities of silymarin including hepatoprotective, antioxidant, anti-inflammatorv, anticancer, and cardio protective activity. Pharmacokinetics related to absorption, distribution, metabolism, and excretion of silymarin revealed poor absorption, rapid metabolism, and ultimately poor oral bioavailability. This article critically reviews the recent published literature on various techniques for increasing the bioavailability of silymarin.

11. Rabea et al (2011) formulated nanoemulsions with increased silymarin solubility (and so its oral bioavailability) as well as therapeutic activity. The results indicated an excellent potential of the nanoemulsions formulation for the reversal of CCl4-induced liver toxicity in rats as compared to standard silymarin.
12. Choi et al (2011) developed formulation to enhance the solubility of poorly water soluble drug silymarin, Shirasu-porous glass membrane system was used. Results suggested that dry membrane nanoemulsion could be a promising solid dosage form for poorly water soluble drug like silymarin.

13. Kovacevic et al (2011) studied the crystallization effect of polyhydroxy surfactants caused by surfactant structure but also the interaction of the surfactant and lipid matrix molecules. It was concluded that polyhydroxy surfactants proved suitable for the stabilization of SLN and NLC dispersions; they are therefore an interesting newly applied class of stabilizers for future dermal products.

14. Wun et al (2011) studied that incorporation of liquid lipid can improve the loading capacity of drugs in the NLCs. It was found that NCLs were less crystalline than the bulk lipid, and polysorbate 80 was a better dispersing agent for NLC than polysorbate 20.

15. Sherbiny et al (2011) studied the development and characterization of a series of sodium alginate-based pH responsive hydrogel microspheres encapsulating poly(d,l-lactic-co-glycolic acid) (PLGA) nanoparticles (NPs). The effect of the drying technique (air- or freeze-drying) on the size of the developed particles was determined.

16. Jia et al (2010) developed nanostructured lipid carriers for parenteral delivery of silybin. Compared with silybin solution, silybin-NLC showed higher AUC values and a prolonged residence time of drug in the blood circulation. Therefore, nanostructured lipid carriers may hold some promise to deliver silybin for therapy of liver disease.

17. Doktorovova et al (2009) developed nanostructured lipid carriers (NLC) for topical delivery of fluticasone propionate (FP) to improve the safety profile and decrease the adverse-side effects commonly reported in topical cortico therapy. Results revealed a low-crystalline structure and confirmed the incorporation of FP into the particles.

19. Junyaprasert et al (2009) developed the Q10-loaded NLC dispersions composed of varying solid lipid/oil ratios. It was concluded that, the chemical stability of Q10 entrapped
in NLC was higher than that in NE and relied on the amount of oil content in NLC, i.e. the higher oil content, the lower percent Q10 remaining.

20. Pardeike et al (2009) stated that SLN and NLC exhibit many features for dermal application of cosmetics and pharmaceutics, i.e. controlled release of actives, drug targeting, occlusion and associated with it penetration enhancement and increase of skin hydration. Furthermore, an overview of the cosmetic products currently on the market was given and the improvement of the benefit/risk ratio of the topical therapy was highlighted.

21. Teeranachaideekul et al (2007) studied enhancement of the chemical stability of ascorbyl palmitate (AP) after incorporation into nanostructured lipid carriers (NLC). It was concluded that the chemical stability of AP-loaded NLC can be improved by selecting suitable types of lipid, surfactant, and proper storage conditions, i.e. cold temperature and flushing with nitrogen gas or inert gas.

22. Souto et al (2007) developed nanostructured lipid carriers (NLC) composed of cetyl palmitate with various amounts of caprylic/capric triacylglycerols (as liquid lipid) and Coenzyme Q10. It was found that NLC showed a biphasic release pattern, i.e. in comparison to nanoemulsions; NLC provided a fast release initially for skin saturation followed by a slow and prolonged release profile to maintain the skin concentration of Q10.

23. Muller et al (2007) gave an overview of the cosmetic benefits of lipid nanoparticles, enhancement of chemical stability of actives, film formation, controlled occlusion, skin hydration, enhanced skin bioavailability and physical stability of the lipid nanoparticles as topical formulations. Special formulation challenges for these products were discussed.

24. Agarwal et al (2006) reviewed the pharmacological activities of natural derived products such as silymarin, curcumin, resveratrol, or silymarin with the potential to treat cancer. The advantage of plant-derived products was that they have better anticancer potential, due to less chance of drug resistance.

25. Fraschini et al (2002) reviewed pharmacological property of Silymarin. It was concluded that Flavonoid silymarin may act in four different ways: (i) as antioxidants, scavengers (ii) as cell membrane stabilisers and permeability (iii) as promoters of ribosomal RNA synthesis,
stimulating liver regeneration; and (iv) as inhibitors of the transformation of stellate hepatocytes into myofibroblasts.

26. Naik et al (2004) determined whether how encapsulation of lipophilic compounds in polymeric nanoparticles is able to improve topical delivery to the skin. The penetration of octyl methoxycinnamate, highly lipophilic sunscreen, into and across porcine ear skin in vitro was investigated. Confocal laser scanning microscopy (CLSM) was used to visualize the distribution of nanoparticles, charged with Nile red (NR), a lipophilic and fluorescent dye.

27. Calderon et al (2013) prepared micro-particles and nano-particles by using cross-linked chitosan with tripolyphosphate (TPP) due to the biocompatibility, bio-adhesion ability and the potential power as penetration enhancer of this polymer. Particles were characterized by Fourier-transformed infrared (FTIR) spectroscopy, X-ray diffraction, SEM, Zeta potential and particle size. Encapsulation efficiency and release profiles in flow through diffusion cells were also determined. Slug Mucosal Irritation (SMI) assays was applied as an alternative to the Draize test to predict the mucosal irritation of the selected formulation.

28. Gaba et al (2015) studied the development and evaluation of Terbinafine HCl loaded nanostructured lipid carrier (NLC) for the treatment of fungal infection via topical administration. TH-NLC was prepared using high pressure homogenization technique using Glycerol Monostearate (GMS) as solid lipid, Labrasol as liquid lipid and Pluronic F-127 as surfactant, binary lipid phase was selected in the ratio 6:4 w/w (solid:liquid lipid ratio). The pharmacodynamics studies indicated that TH-NLC (771 ± 41.797 CFUs) gel efficiently reduced the fungal burden in shorter duration of time as compared to marketed formulation.

29. Jeengar et al (2016) evaluated the anti-inflammatory potential of curcumin in combination with emu oil from a nanoemulgel formulation in experimental inflammation and arthritic in vivo models. Nanoemulsions were prepared using emu oil, Cremophor RH 40 and Labrafil M2125CS as oil phase, surfactant and co-surfactant. The optimized curcumin loaded nanoemulsions with emu oil was incorporated into carbopol gel for convenient application by topical route. The anti-inflammatory efficacy was evaluated in carrageenan induced paw edema and FCA induced arthritic rat model in terms of paw swelling, weight indices of the liver and spleen, pathological changes in nuclear factor kappa B, iNOS, COX-2 expression and inflammatory cytokines.
30. Ghate et al (2016) formulate NLCs of tretinoin for reducing the skin irritation potential, increasing the drug loading capacity and prolonging the duration of action. The NLCs were optimized using the response surface methodology based on the particle size. NLCs of tretinoin were prepared by hot melt microemulsion and hot melt probe sonication methods. The properties of the optimized NLCs such as morphology, size, Zeta potential, stability and in vitro drug release were investigated. The results showed that the irritation potential of tretinoin was reduced, the drug loading was increased and the drug release was prolonged by the incorporation into the NLCs.

31. Patel et al (2013) reviewed advantages and potential limitations of SLN for the use in topical pharmaceutical formulations. Features discussed, included stabilisation of incorporated compounds, controlled release, occlusive, film formation on skin including in vivo effects on the skin. As a novel type of lipid nanoparticles with solid matrix, the nanostructured lipid carriers (NLC) are presented, the structural specialities described and improvements discussed, for example, increase in loading capacity, physical and chemical long-term stability, triggered release and potentially supersaturated topical formulations. For NLC, the technologies to produce the final topical formulation are described, especially the production of highly concentrated lipid nanoparticle dispersions.

32. Benjamin et al (2015) developed Farnesol-loaded nanoparticles which effectively attenuated biofilm virulence in vivo using a clinically relevant topical treatment regimen in a rodent dental caries disease model. Strikingly, treatment with farnesol-loaded nanoparticles reduced both the number and severity of carious lesions, while free farnesol had no effect. Nanoparticle carriers have great potential to enhance the efficacy of antibiofilm agents through multi targeted binding and pH-responsive drug release due to micro environmental triggers.

33. Farid et al (2014) reviewed structure and physical-chemical features, the beneficial effects (e.g., enhanced antioxidant, anti-inflammatory, anti tumor, anti-aging properties) exerted by polyphenols. Interestingly, an increasing number of studies suggest that controlled-topical delivery of nano-encapsulated polyphenols (e.g., (−)-epigallocatechin-3-gallate (EGCG), resveratrol) might overcome some limitations frequently observed with topical or systemic bulk polyphenols (e.g., bioavailability, pharmacokinetics, targeting efficacy, toxicity and
safety). Therefore, topical application of nano-polyphenols would represent valuable preventive and therapeutic options to provide clinical benefits for individuals with certain skin conditions (e.g., skin inflammation, skin cancers, premature skin aging, skin wound healing).

34. Salim et al (2016) reviewed growing interest in using nanoemulsions in topical applications, due to their high stability and their optical transparency or translucency, which make them good and very dermatologically attractive. A good selection of oils and surfactants would enhance the transdermal treatment efficacy. This review highlights the potential of drug-loaded nanoemulsions for the treatment of psoriasis towards achieving better efficacy and eliminating side effects.

35. Pandita et al (2016) developed azelaic acid loaded nanostructured lipid carriers (NLCs). They were prepared by solvent diffusion-solvent evaporation method for enhancing their dermal retention and moreover, slow release of drug from NLCs can avoid side effects associated with its usage. The optimized formulation was characterized for size, morphology and zeta potential. Mean particle size of azelaic acid loaded NLCs was 81.57±9.6 nm with low poly dispersity index (PI) i.e. 0.208±0.021 and high zeta potential of -29.3±1.21 mV was obtained. Results of transmission electron microscopy (TEM) imaging demonstrated spherical shape of NLCs with uniform surface. Ex vivo permeation studies of azelaic acid loaded NLCs gel showed biphasic drug release pattern with initial burst release followed by sustained release and also led to slower release profiles compared to plain drug loaded gel and drug solution i.e. 21.06±0.99, 38.71±1.47 and 78.79±2.52% of the drug was permeated from NLCs gel, plain gel and drug solution, respectively with 51.6±0.74, 95.25±1.23 and 184.59 μg/cm2/hr flux values respectively. The results concluded that the developed NLCs have enormous potential to improve the penetration of the azelaic acid through stratum corneum with utmost retention in the skin which is the pre-requisite for the topically applied formulations for the management of skin diseases and the avoidance of systemic adverse effects associated with its usage.

36. Souto et al (2005) developed ketoconazole stability in aqueous SLN and NLC dispersions, as well as the physicochemical stability of these lipid nanoparticles, which might be useful for targeting this drug into topical route, minimizing the adverse side effects and providing a controlled release. Lipid particles were prepared using Compritol 888 ATO as solid lipid. Ketoconazole loading capacity was identical for both SLN and NLC systems (5%
of particle mass). SLN were physically stable as suspensions during 3 months of storage, but the SLN matrix was not able to protect the chemically labile ketoconazole against degradation under light exposure. In contrast, the NLC were able to stabilize the drug, but the aqueous NLC dispersion showed size increase during storage. Potential topical formulations are light-protected packaged SLN or NLC physically stabilized in a gel formulation.

37. Paolino et al (2016) found that a 20% suspension of lutein in safflower oil (FloraGLO® Lutein) represents a good raw material for the production of creams and other semisolid formulations. However, the high viscosity of FloraGLO® and poor chemical stability of lutein in the suspension represents a practical limitation to its use. An efficient method was proposed in this study for taking benefit of the liquid oily composition of FloraGLO®, by realizing a nanostructured carrier system (NLC) able to ensure a controlled release of lutein and improve its permeability across the skin. NLC were prepared with different percentages of FloraGLO® as the liquid phase of NLC. The physical stability of NLC was assessed by storage at room conditions and by accelerated analysis. All the produced nanocarriers were perfectly tolerated on the skin. In an in vivo model of UV-induced skin erythema, the lutein-loaded NLC were able to improve the photo-protective effects of the antioxidant compared to the commercial suspension, when the NLC formulations were applied before inducing the erythema. This study also proved for the first time the possibility of converting a liquid formulation into a solid, modified release nanocarrier with more manageable formulates features.

38. Bhaskar et al (2009) prepared aqueous dispersions of lipid nanoparticles – flurbiprofen solid lipid nanoparticles (FLUSLN) and flurbiprofen nanostructured lipid carriers (FLUNLC) by hot homogenization followed by sonication technique and then incorporated into the freshly prepared hydrogels for transdermal delivery. They are characterized for particle size, for all the formulations, more than 50% of the particles were below 300 nm after 90 days of storage at RT. DSC analyses were performed to characterize the state of drug and lipid modification. Shape and surface morphology were determined by TEM which revealed fairly spherical shape of the formulations. Further they were evaluated for in vitro drug release characteristics, rheological behaviour, pharmacokinetic and pharmaco dynamic studies. The bioavailability of flurbiprofen with reference to oral administration was found to increase by 4.4 times when gel formulations were applied. Anti-inflammatory effect in the Carrageenan-induced paw edema in rat was significantly higher for B1 and A1 formulation than the orally administered flurbiprofen. Both the SLN and NLC dispersions and gels enriched with SLN
and NLC possessed a sustained drug release over period of 24 h but the sustained effect was more pronounced with the SLN and NLC gel.

39. Choi et al (2010) developed topical nanostructured lipid carrier (NLC) systems which has a high occlusive property. Various NLC dispersions were successfully formulated with Compritol 888 ATO as a solid lipid, Labrafil M 1944 CS as an oil, and Tween 80 as a surfactant. The increase of oil content (5 to 50%) led to the decrease in the occlusion factor in the order of SLN > NLC-5 > NLC-15 = NLC-30 > NLC-50. Particle size of lipid particulates was in the range of 100 to 160 nm. NLC-based carbogels were prepared by the employment of humectants such as urea, glycerin, and Tinocare GL to carbomer gel. NLC-30 gel formulations containing 4 or 8% of lipid particles showed improved occlusive effect in vitro, compared to NLC-free gel base. Even though NLC-free gel base revealed comparable occlusion effect by itself, the occlusion factor of 4% NLC-30 gel was about 2-fold higher than that of NLC-free gel base.

40. Rawia et al (2014) prepared nystatin loaded solid lipid nanoparticles (NystSLNs) using the hot homogenization and ultrasonication method. The prepared NystSLNs were characterized in terms of entrapment efficiency, particle size, zeta potential, transmission electron microscopy, differential scanning calorimetry, rheological behavior and in vitro drug release. A stability study for 6 months was performed. A microbiological study was conducted in male rats infected with Candida albicans, by counting the colonies and examining the histopathological changes induced on the skin of infected rats. The results showed that SLNs dispersions are spherical in shape with particle size ranging from 83.26±11.33 to 955.04±1.09 nm. The entrapment efficiencies ranged from 19.73±1.21 to 72.46±0.66% with zeta potential ranging from -18.9 to -38.8 mV and shear-thinning rheological behaviour. A least number of colony forming unit/ml (cfu/ml) was recorded for the selected Nyst SLN compared to the drug solution and the commercial Nystatin® cream present in the market.

41. Moghddam et al (2016) developed and optimized topically applied nimesulide-loaded nanostructured lipid carriers. Box-Behnken experimental design was applied for optimization of nanostructured lipid carriers. The independent variables were ratio of stearic acid: oleic acid (X1), poloxamer 188 concentration (X2) and lecithin concentration (X3) while particle size (Y1) and entrapment efficiency (Y2) were the chosen responses.
42. Hyon song et al (2014) developed topical preparations of voriconazole (VRC) for the treatment of mycotic infections of the skin, a nanostructured lipid carrier-based hydrogel (NLC-gel) formulation and its physical characteristics, *in vitro* skin permeation, and retention profiles were examined. A VRC-loaded NLC dispersion, consisting of Precirol ATO 5, Labrafil 1944 CS, and Tween 80, was prepared by high-pressure homogenization and embedded into Carbopol 940 hydrogel. The lipid nanoparticles in the hydrogel were approximately 210 nm in size, with a spherical shape and zeta potential of −30 mV. In a skin permeation study using a Franz diffusion cell mounted with depilated mouse skin, the NLC-gel was superior to conventional cream and microemulsion-based gel formulations, showing 2.8- and 1.7-fold greater flux values, respectively. In addition, the NLC-gel led to markedly greater accumulation of VRC in deeper skin layers as compared with the reference formulations.

43. Woo et al (2014) formulated cost effective stearic acid-oleic acid nanoparticles (SONs) with high loading of salicylic acid, by melt emulsification method combined with ultrasonication technique. The physicochemical properties, thermal analysis and encapsulation efficiency of SONs were studied. TEM micrographs revealed that incorporation of oleic acid induces the formation of elongated spherical particles. The optimized SON was further incorporated in cream and *in vitro* release study showed a gradual release for 24 hours, denoting the incorporation of salicylic acid in solid matrix of SON and prolonging the *in vitro* release.

44. Bahari et al (2015) reviewed SLN and NLC for particle size and size distribution. They reported numerous merits in drug delivery. Size is the most important index in nanocarriers affecting its drug delivery efficiency. The influence of preparation conditions and type of lipid components on the size of SLN and NLC was investigated in this study.
1.9. Objective of the study

Along with the growth and development of pharmaceutical technology, uses of plant derived actives have attracted more attention due to their lower cost and fewer side-effects. Among various plant derived medicines being used through the ages, antioxidants hold much popularity, they have been used since thousands of years for different beneficial effects. One of the under explored properties of flavonoids is that, they have protective potentials against DNA damage and non-melanoma skin cancers. Besides, they also possess anti-inflammatory and immuno modulating characteristics. Keeping these properties in mind, a nano formulation could be designed with one or more of these antioxidants for their anti-cancer potential.

The primary objective of the present study was to design, development and optimization of process and product parameters of silymarin-NLC, through design of experiments. Physicochemical characterization would be evaluated by parameters such as compatibility, mean particle size, zeta potential, entrapment efficiency, in vitro release and stability studies as per ICH guidelines. Aim of this research work was to formulate a stable topical carrier system of silymarin by amalgamating the positive attributes of lipids and surfactants for NLC formulation, designed for its protective as well as associated inhibitory activity of silymarin on the proliferation of cell line.

NLC would be designed to assess targets at the protein and gene level, and evaluated by in vivo studies by western blot and Reverse transcription polymerase chain reaction (RT-PCR) analysis. As a topical gel, ease of application and feasible synergistic effect of silymarin and nano lipoidal carrier formulation may provide a possible protectant action against Photoinduced tumorigenicity. The novelty of this work is the possible synergistic effect of drug and excipients, formulation optimization, and it’s in vivo studies in 7, 12-dimethylbenz[a]anthracene (DMBA) mice model, at enzyme level.
1.10. Plan of work

Based on the extensive literature survey, it was thought worthwhile to o research with the following plan of work

1.10.1. Preformulation study
- Organoleptic Evaluation
- Identification of Drug sample by Infrared spectrum
- Identification of Drug sample by absorption spectrum
- Solubility study
- Compatibility study
- Differential scanning calorimetry (DSC) analysis

1.10.2. Preparation of standard plot of drug
- Scanning and preparation of standard curve of drug using UV-Spectrophotometry in methanol
- Scanning and preparation of standard curve of drug using UV-Spectrophotometry in PBS of pH 6.8 for \textit{in-vitro} release study

1.10.3. Formulation Development
- Selection of lipid and surfactant
- Preparation of NLC by various methods:
  - Preparation of silymarin NLC gel
    - Ultrasonication or High speed homogenizer
    - Melt emulsification and low temperature solidification
    - Hot high pressure homogenization
  - Preparation of silymarin NLC gel

1.10.4. Optimization of NLC
- Optimization of process parameters
- Optimization of formulation parameters

1.10.5. Characterization and Evaluation of NLC
- Physicochemical properties
  - Particle size and polydispersity index
  - Analysis of surface morphology
- Zeta Potential Measurement
- Encapsulation and loading capacity of NLC
- \textit{In-vitro} release study of NLC
- \textit{In-vitro} permeation study
1.10.6. Photostability study

1.10.7. *In vitro* anti-cancer activity by sulfo rhodamine-B (SRB) assay

1.10.8. In vivo studies
  - Western blotting
  - Reverse transcription polymerase chain reaction (RT-PCR)

1.10.9. UV induced skin edema study

1.10.10. Skin Irritation study

1.10.11. Stability studies
  - Effect of process parameters on stability
  - Effect of product parameters on stability

1.10.12. Statistical analysis of data

1.10.13. Computation and compilation
References


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