Chapter 1: Preamble

1.1. Introduction

1.1.1. Overview on drug delivery system (DDS) development

The delivery system of drug is one of the conceptual approach to deliver the agents with therapeutic activity towards the body. A same concept is under use since ancient times by people when they used to utilise the medicines by simply chewing the plant part for healing. As the time lapse the various uncomfortable events especially during basic techniques for healing made community for finding another alternative techniques for treatment with more utilization capacity. Through efforts from ancient researchers in field of Ayurveda transform the concept to modify the form with new development in DDSs from solid as well as liquid form. By time lapse all the approaches were gradually modified in manufacturing techniques to extract the active constituents by approach of decoction, extraction and percolation. Modern modifications under medicines started by vaccine discovery (1885) and the purification methods of natural drugs. Number of commercial corporates were involved for genetic modifications of microorganism for the purpose of scale up.

Such kind of inventions’ segregation was very difficult to put under single strategy due to different properties and applications. This situation creates the need to manage reproducibility and reliability for application of medicines which gave a birth to new DDS. Principle aim of system for drug delivery was covered by various aspects with the administration routes for excretion. The approach for specific delivery should cover all the principle aim delivery system under defined manner as illustrated in figure 1.1.

Principal aim for developing the new delivery system for drug candidates is to reach the effective and safe concentration of drug in body. Traditional delivery systems of drugs are mostly characterized by quick and unrestrained release kinetics which generally leads to abrupt increase for drug concentration inside the body followed by similar level of decrease. Efficacy of number of therapeutic agent mostly depend on exploit on targeted molecules situated either in or to surface cell types. Many drugs usually interacts to enzyme or other molecules which are common by numbers of cells, but most frequently drug applies the action to particular cell type in anticipated therapeutic action. For obtaining therapeutic reaction, suitable
concentration of active ingredient must transported and absorbed towards site for action at a correct time and rate which can later adjusted for producing the concentration required for maintaining level of effect as required duration.

New delivery system for drug are recently developing for overcoming limitation in conventional delivery system to meet a need especially in healthcare profession. Such systems can characterised as drug release in controlled manner systems and targeted (specific site) delivery systems.

No any established definition of the definitions for DDSs are mainly assumed based on different basic parameters for entry route and dosage form.

![Diagram](Figure 1.1: Covering principles of DDS development)

### 1.1.2. Controlled DDS

All the aspects are necessity for producing a delivery of drug by controlled manner. During the beginning period of controlled delivery systems of drug, the controlled release utilize the polymeric matrix for rate control to deliver the drug as fixed dose. In recent days, the products with controlled release, the development become easier than earlier due to availability for advanced technology of fabrication
of matrix. The advancements from various efforts have made the most feasible in designing of delivery for wide categories of drugs (having different characteristics) as required. Various types of controlled DDS is explained in figure 1.2.

**Figure 1.2:** Types of controlled DDS

Controlled release mechanism over prolonged duration is greatly beneficial for active drugs which is rapidly metabolized and having a quick elimination from body post administration. A pharmaceutical technology is now grown as well as diversified quickly now a days. For the detailed understanding in derivation methods of controlled release along with a range of polymers can have barrier for involvement from a non-specialist. Almost all controlled release deliveries mainly aims to improve an effectiveness for drug therapy. With this improvement may take a form of rising the therapeutic activity in comparison to intensities of adverse effects, lowering the number in administrations of drug required at the time of treatment, eliminating a need for specialized type of drug administration.
For controlled delivery systems some advantages over other system (1) possibly formaintaining the drug level in plasma within therapeutically desirability, (2) possibilities for eliminating or reducing the adverse effects from regular systemic type administration in administration through local route from controlled delivery system,(3) administration of drug may improve & facilitated in needy areas even at medical command is unavailable, (4) drug administration with short half-life mayimportantly enabled, (5) constant small extents of drug might be low painful in compare to numerous huge doses, (6) progress of patient amenability, & (7) the utilisation of delivery systems may be resulting insomewhat lowcostly product and low wastage of drug. A 1st gen. in controlled delivery offered some drawbacks that is likely toxicity, essential for surgery for implanting the system, probable pain, and trouble for shutting off the drug release if required.

1.1.2.1. Nano sized drug carriers for controlled delivery mechanism

Nanotechnology science mainly devoted to modification, creation and material utilization, devices & related systems at nano sized range. It usually exploits for physical, chemical and biological material property which improves and diverse from the bulk of materials, for just due to they are at nano scale. (Duncan R 2011)(Moghimi S M 2005) Nanotechnologies are exist already in number of commercial products & industrial various applications i.e.; food, electronics, fuel, solar cells, chemical sector, batteries etc. Moreover, several claims of nanotechnology in the pharma and cosmetic field have transformed the principal administration of various drugs & cosmetics. Definitely, the usage of nano scaled carriers led to a meaning of a nanomedicine, a multidisciplinary matter area comprising many scientific corrections that has been definite as an application presentation for nanotechnology intreatment, prevention, monitor, identification & control for various biologicals.(Jain 2005)

This research area of nanomedicine have been categorised as:

- Nanotechnology diagnostics with imaging (molecular diagnostics, nanoparticle imaging, biosensor etc.)
- Nano-pharmaceuticals (targeted delivery of drug, nano-pumps and other devices etc.)
- Regenerative nano surgery and medicine (nanoscaffolds, nano surgery)
- Nanorobotics
Nano scaled carriers intended to medical applications comprise lots of engineered constructs, architectures, assemblies and particles systems along with numerous physicochemical characteristics, whose featured size from 1 to 1000 in pharma technology. Different kind of agent for diagnostic purpose or/and therapeutic purpose can be matrixed or encapsulated, in such these carriers attached or adsorbed on its surface. Various kind of nano scaled carriers i.e. nanocrystals, liposomes, polymeric nanoparticle, lipid nano particles, polymeric micelles, metal nanoparticles, dendrimers etc. are used for improving the delivery of therapeutic agents.

Previous studies shown that the nano scaled carriers are mostly beneficial in several applications of pharma and cosmetics. The Microemulsions and lipid composed vesicles were presented first time concurrently in 60s of 19th century. In 1959 Schulman et al. have shown the presence of small emulsion identical assemblies through electron microscope and later coined a word “microemulsion”, while liposomes were exposed by Sir Alec Bangham in 1961. (Johan 1996) (Bangham A D 1964)

Consecutively, colloidal categories carriers were projected in topical delivery and moreover, lipid nanoparticle was initially developed from the concept of microemulsions. In present study, the principal aim is to describe the design of lipid nanoparticles intended to dermal drug delivery. For understanding the most applicable potential of such carriers for first, routine issues for cutaneous permeation must be understand. Skin as an organ can provide several merits as platform for drug delivery while its barrier nature usually create difficult situation for majority of the drug to enter into biological system of human body through it.

1.1.3. Atopic dermatitis (AD)

AD is most mutual origin of occupational skin related disease in children and adults. It results from routine contact amongst susceptibility gene, skin barrier defect, pharmacological abnormality, host's environment, and immunology related factors. AD is skin related chronic inflammatory disease that can characterized through lesions of pruritic skin, skin dryness & infection of staphylococcus aureus. (Denby K S 2012)
However, exact pathogenesis for AD is partially understood till date. Recalcitrant type of pruritus is type of utmost upsetting indication that patient suffer. That is normally tracked by scratching type response, that intensifies inflammation of skin & primes to arrangement of circumstances called “itch and scratch cycle”. (Mihara K 2004) Onset for AD is usually yearly in life prominent to occurrence in adults (2–5%). (Darsow U 2010)

![Figure 1.3: Immunological routes in AD (Donald Y Leung 2003)](image)

Because of relapsing & prolonged progression of this disease, AD needs prolonged management approaches & immunologic investigations for eczematous skin (acute). AD lesions shows amplified occurrence for T type cells with production of Th22 & Th2 Interleukin cytokines IL-4/5/13/22. Raised incidences of IFN-g generating Th1 cells usual characteristic of (lesions) chronic lichenified (Leung D Y M 2004), whereas IL-17 generating Th17 cells initiate at lower level for chronic & acute type of lesions. (Gittler J K 2012) Immunological pathway understanding is given in figure 1.3. Latest investigation also covers part of insufficient response of host to cutaneous microbes & barrier of skin related irregularities which influence individual for developing AD. (Denby K S 2012) Sign formal functioning skin barrier can be providing through hereditary investigations presenting linking concerning AD & chromosome 1q21 (gene for epidermal differentiation). (Bowcock A M 2004).
Patient suffering AD necessitate subsequent topical based treatment through different emollient & anti-inflammatories that are basically topical type of glucocorticosteroids or inhibitors of calcineurin. (Knut S 2014) Recent investigations shows findings with tacrolimus (TCRLM) 0.1% and other formulations with other concentration & drug. TCRLM 0.1% found better in compare to lower strength corticosteroids. TCRLM formulations appeared with safe performance without sign for supporting related risk for malignancies/ skin atrophy. (Cury M J 2015) Figure 1.4 shows the reasonable factors for AD.

**Figure 1.4:** Reasonable factors for AD

### 1.1.4. Morphological overview of skin

Human skin as leading organ in body & it usually acts for leading target and foremost dermal & transdermal delivery of drug. Definitely, due to its easy access of wide surface, it got great potential of research interest as substitute route for conventional drug administration. Drug delivery via/into skin have a number of advantages including better bioavailability specially for those who have effects of
hepatic first pass. Still the percutaneous delivery is quiet puzzling. Definitely, now a
days a need for solving an individual unevenness amongst the changed locations on
skin & for operative barrier which this organ generate in between organism and
environment.

Definitely, primary role of skin is to perform as barrier for protecting human
body, inhibiting attack of pathogens and defending from chemical as well as physical
beatings, and from unregulated water loss and solutes loss. Such vital role is because
of design of a skin that composed of three efficient layers, namely dermis, epidermis
and hypodermis. \(\text{Simanski M 2014}\)

Epidermis layer’s main role includes to provide protection, which is outer
layer, even after its low thickness (0.002 to 5 mm) according to location of skin. This
epidermis is layer which is stratified squamous epithelial and it contains keratinocytes
prearranged in four key strata of stratum corneum, spinous, granular and basal.
However their arrangement is very specific. Physical barrier mainly confined in
topmost layer, which is horny layer (10-20 mm thick), containing in 15-25
compressed, type hexagonal, cornified & stackened cell set in lipoid intercellular areas.
\(\text{Walters K A 2002}\)

The most principal permeation barrier protects the humans from more water
loss as well as from harmful effect of highly toxic agents available in external
environment that consist of horny layer made up of corneocytes because of apoptosis
process of keratinocytes at a time of passage of such kind of cells from basal of
epidermis to external surface. This process also makes the cells to degrade the
phospholipids as well asceramides synthesise in granula portion which secrete into the
intercellular region where theceramides with long fatty acids, cholesterol as well as
cholesteryl sulfate from extremely disciplined lipid layers attending as cover. Water
level inskin at normal surface condition is low (10–15%). At similar time, nucleus of
keratinocytes usually degrades. Horny layer formed by 15 layers of corneocytes. Its
thickness is about 220–400 (nm) however the thickness of this layer is from 5 to 8
µm. \(\text{Cevc. 2004}\) Viable epidermis is situated below horny layer and dermis layer.
The hair follicles which reach from dermis to skin outer surface & pass through
epidermis is an important supplements of skin. In comparison to furred animal, the
follicles usually less in quantity in human. \(\text{Bouwstra J A 2003}\) The thickness of
stratum corneum (SC) differs significantly being mostly dense and thick at palms area. This layer’s barrier characteristics are generally connected to heavy density of 1.4 g/cm³, little level of hydration of 15 to 20% in assessment with other tissues of 70%, as well as its less surface for solute passage.

The properties of this barrier generally supported by nonstop desquamation of horny layer along with whole turnover happening each 2 to 3 weeks. (Walters K A 2002) The cells of stratum corneum layer generate from most deepest area of basal layer, epidermal stratum that experience some kind of physiological, morphologic & biochemical variations as they transfer from lamina of basal to superficial part of skin with density of newly formed keratinocytes. Melanocytes and merkel cells also existing together along with keratinocytes at basal.

Corneocytes mostly formed of insoluble keratins as well as lipids which are surrounded in a cornified envelope, though intercellular part of desmosomes and lipids that allow the cohesion of corneocyte.

**Figure 1.5:** Brick & mortar model - SC (J Hadgraft 2011)

Intercellular domain of lipid usually composed of a sheets of lamellar of approximately equal cholesterol, fatty acids and ceramides of long chain structure.
Under stratum corneum, the epidermis of 50 to 100 mm is present that has most important activity participation in regeneration of this layer. (Sinico C 2009) The functions of stratum corneum is not dependent only on one component but on the total structure, presented by Elias model of bricks of corneocytes that mortar is a lipid matrix as given in figure 1.5. (Elias 1983)

Epidermis donates to barrier over dense adherent junctions via cytoskeletal and desmosomes elements. At the time of differentiation of epidermal the diploidic substance are generated in keratinocytes that extruded in domains of extracellular part. The ceramides A & B bounded covalently to outer proteins & makes backbone for following accumulation of ceramides, fatty acids & cholesterol in lipid region of subcutaneous. Lipids are prearranged as several lipid bilayers that forms a part with semi crystalline like structure of gel & liquid crystals. (Sinico C 2009) Dermis part directly together with viable epidermis promotes the mechanical characteristics to skin. It is composed of elastins, collagen and glycosaminoglycans, all to gather they known as extracellular matrix and fibroblasts which prolong the extracellular matrix. Heavily vascularized dermis contains sweat glands, pilosebaceous glands, mast cells, dermal adipose & infiltrating leucocytes.

1.1.4.1. Absorption from percutaneous

Transdermal as well as dermal delivery of drug needs an effective permeation of most active substances via skin mostly through passive diffusion. Molecule exposed on skin may possibly utilise the two different diffusional pathway for penetration: transappendageal pathway or transepidermal pathway as shown in figure 1.5. Transappendageal pathway comprise passage through skin shunts such as hair follicles and sweat gland with related sebaceous glands. These pathway were usually reflected of less importance due to their comparatively less area (0.1 to 1%), current research indicates pilosebaceous may have contribution knowingly topical delivery by performing as less resistance route for nano scaled particles for entering stratum corneum. (Papakostas D 2011) (Manca M L 2014). This pathway also reflected as prospective passage route for polar molecules as well as ions. Furthermore, relative area for shunt may high impact on body areas like scalp, which has size and density for follicles of hair are more large in compare to other area of skin like back. (Bartosova L 2012) Moreover, follicles of hair as well as sebaceous linked to
different disorders related to dermatology like alopecia, acne and tumors. Thus, a countless attention in units of pilosebaceous as goal for localized delivery of drug, and shunts intended to delivery by transdermal route, even after particular part of follicular route in drug absorption is challenging to clarify because of absence of suitable animal study model to differentiate transport from follicular part to non-follicular part. (Knorr F 2009) Still, leading pathway for penetrating skin is reflected transepidermal passageway, ensuing the molecules able to pass intact, complete horny layer by utilising altered routes: transcellular, through corneocytes and matrix of lipid as mentioned in 2A of figure 1.5 and intercellular as mentioned in 2B of figure 1.5 through domains of lipid in between corneocytes. (Hadgraft J 2011) Structure and meaning of skin is broadly termed in lots of reported literature till date and it is recognised that tortuous but constant intercellular pathway promotes principal way for penetration of majority of drugs. (Wartewing S 2007) Still, hydrophilic components would first follow transcellular way due to aqueous surroundings because of higher volume of hydrated keratin in corneocytes. The absorption by percutaneous is being under study by in vitro & in vivo methods with a number of references concerning these practices have been together by regulation bodies for producing a proper guideline.

Now a days in academics and professionals used widely in vitro methods for assessment of penetration & permeation through skin due to appropriate model for predicting the dermal penetration in human, it can produce results rapidly, are not costly or time consuming and presents the more reproducible results. (Walters K A 2002) Furthermore, such kind of trials can be executed by means of on human or mammalian skin. But, trials should be executed following the guidelines of OECD.

Best usual technique for assessing in vitro penetration through skin involves the diffusion cell and previous reported studies confirms the appropriate presentation of these tests. Main drawback of in vitro tests is absence of evidence concerning effects of blood flow on permeation of drug, meanwhile the in vivo sink situations cannot be totally. (Bartosova L 2012) Design of diffusion cell may differ from normal 2 compartment “static” or further complexed “flow-through” scheme. They finished of inert solid material, normally glass while steel and Teflon material may use too. Static cell comprise the 2 compartments, where one is donor and another is receiver and
mostly of vertical type (Franz cells). They may differ in size, of receiver compartment of approximately 2 to 10ml. Skin specimens are placed between two compartments, where the outer layer of skin kept facing towards the donor part. The formulation is spread on surface. Receiver part has suitable fluid that mimics blood flow by continuously mixing with bar. The receiver buffer should nicely mimic the real condition of permeation and sink environments. Most ordinarily used liquids are phosphate buffer saline (PBS) in case of water soluble drug, although the solubilisers like albumin or alcohol or cyclodextrin are added for the water insoluble drugs. Receiver buffer are kept in thermostat condition for ensuring skin temperature (37±1°C). Drug permeating direction from donor to receiver part is derived as role of time by receptor buffer withdrawal from sampling port at specified interval. To confirm the sink condition, the withdrawal solution must substituted with equal volume of fluid of same type. Flow-through cells are suitable for less solubility in receptor medium.

1.1.4.2. The reservoir and barrier functioning

Although follicles of hair mainly permit straight entry for active components getting the outmost region of skin to blood or plasma vessels, such components largely penetrate & permeate skin by crossing a horny layer, exactly by spending a tortuous way in-between corneocytes. (Barry 2002) For that reason absorption of skin differs with physicochemical environment of component and functional area of application: active pharmaceutical component and other xenobiotics are nicely absorbed from region of forehead, low from postauricular, also low from stomach & minimum from region of palms or soles. (Tsai J C 2003) By learning the absorption of opioid, sudentanil and fentanyl uptake through foot region, thigh region, chest region and abdomen region was abnormally almost same, moreover age & gender special effects were not detected. (Roy S D 1990) To better absorbed, a component should have molecular weight <0.6 kDa, satisfactory solubility in water & oil and high level of partition coefficient. Additionally, it should functionally applied as saturated or supersaturated component system. (Moser K 2001)(Moser K K 2001) Though a moderately troubled barrier in some skin related diseases like psoriasis or atopic eczema may support API penetration via horny layer (Anigbogu A N 1996), an satisfactory penetration is quiet essential challenge for topical dosage form discovery. Traditional drug delivery, like ointment and cream, gives in API
acceptance of less percent, which additionally as rule is related to somewhat high individual deviation of uptake. Therefore, levels of API in unhealthy skin may sub therapeutic in few patients but prompting undesirable adverse effects. Additionally, high lipoid components can enter through the follicles of hair. (Münster U 2005) The uptake through follicular pathway may be significant with crystalline API particles or carrier with particulate nature with approx. size from 3 to 10 µm. Except horny layer, very small size not ignore penetration in follicular orifice. (Lampen P 2003) Also performing as permeation-barrier, horny layer can be consider also as reservoir for the substances which are topically applied. In recent times, saturable acceptance of UV filters was designated. (Teichmann A 2005). Examinations on primaquine confirmed the both binding to keratin of corneocyte and solubility of drug along with lipid fields subsidize to horny layer. (Heard C M 2003)

1.1.5. Topical treatment concept

Treatment by topical concept for any of skin diseases is one of attractive, since the systemic load by pharmaceutical ingredients moreover thus systemic adverse effects are compact in compare to oral or parenteral drug administration. Therefore the application of drug formulations to skin external surface was and still not only applicable for disease related to skin but likewise for the local antirheumatic rehabilitation for controlling the gastrointestinal adverse effects of NSAID drugs. Additionally, topical drug application generally avoids the main variabilities in plasma levels points uncharacteristically for frequent administration of quickly eliminated drugs candidates while it will also allow to bypass the first pass mechanism of drug molecules via liver post intestinal absorption. Thus transdermal drug formulation application increased still rising potential for the systemic treatment, for example the drugs subjected to widespread first-pass elimination like estrogens or glyceryl trinitrate and for extended suppression of severe pain. (Monika S K 2007)

This mode of application has been utilised for long through dermatologist who, still, have prolonged been unaware of quantity of drug reaching the viable skin layers or systemic circulation permitting the drug distribution within the organism. It was around 1960 where scientists inspecting the side-effect lying to highly effective glucocorticoids started to measure the effects toward the skin. It grow into possible by vasoconstriction assay (Barry B W 1974)(McKenzie A 1962) which is one improved
form and it was acknowledged as standard technique for development of glucocorticoids topical form by authorities.

The efficient barrier role done by horny layer disturbs the API absorption and also for toxic agents. The investigations performed on healthy volunteers presented the hydrocortisone absorption level was varying less than 1% of a total dose. After application, absorption in other areas i.e. forearm, back, forehead was found in range. (Feldmann R J 1969) Horny layer usually prevents a penetration for hydrophilic moieties much efficiently in comparison to lipophilic.

1.1.5.1. Improved penetration

To solve the low rates of uptake, recent findings in field of Pharma. Technology inspects penetration enhancers, with specific synergistic mixtures which can very much effective and nicely accepted. In detail, the peptide leuprolide with other lower molecular mass heparin permeate through pig skin via Franz cells, and heavy volumes were detected in plasma by applying the patches to skin of rats which were hairless. (Karande P 2004) Furthermore, micro-particles and nanoparticle are developing which does not rise the percutaneous absorption but also allow targeting of drug to skin. In this way they may have much potential for most improved ratio of benefit and risk for topical therapy. Prodrugs formation enabling packing to carrier system can be improved for topical therapy in diseased skin. These components are mainly activated at location of disease. Solid lipid nanoparticles (SLN) composed of lipids that are solidified and its surface is shielded by surfactant or emulsifier that stable the dispersion. Nanostructured lipid carriers (NLC) are the combinations of liquid lipid and solid lipids, liquid lipid is studied to be entrapped in solid matrix of solid lipid. (Müller R H 2002) or localized on surface of platelets (solid) and surfactant layer. (Jores K 2005) Such kind of carriers act as alternatives of polymeric nanoparticles (Wang Z 2003), liposomes (Yarosh 2001) and nanoemulsions (Müller R H 2000) microemulsions. (Subramanian N 2005)

1.1.6. Solid lipid for bioactive delivery

The -COOH group in fatty acid promotes a suitable site for conjugation to alcohol, through ester linkage. If fatty acid attached to alcohol by carbon chain, than obtained component is wax. When fatty acid and glycerolare conjugated, fatty acid part of obtained component called acyl group, & glycerol port referred as glyceride. A
tri-acyl-glyceride has 3 fatty acid conjugated with single molecule of glycerol. Triglyceride are normally mentioned as oils or fats, depending on nature either solid or liquid at regular temperature. (Glatz 2005) Lipid is waxy compound which is freely soluble to solvents which are non-polar.

Lipids is further grouped in categories depending on chemical configuration like homolipids, heterolipids and complex lipids. Homolipids are chemically esters of a fatty acid and elements present in its nature is hydrogen, carbon and oxygen thus they are simple lipid. Primary material of attention for delivery by oral route vehicle are usually long and medium chain structured fatty acids conjugated to glycerol molecule, which is referred as triacylglycerols. The elements present in heterolipids are principally nitrogen & phosphorus along with C, H and O for example glycolipids, sulfolipids and phospholipids. The importance will on phospholipids. The 2 major class of phospholipids found logically in qualities that are enough for pharma application. Complex lipids happen conjugate to proteins present in membranes of cell & subcellular particles. Mostlively tissues normally have greater content of complex lipid. Complex lipids includes chylomicrons, lipoproteins, etc. The lipoproteins are generally a complex of protein and lipid which is in control for passage of cholesterol & lipids in body. Basically, the lipoprotein comprises of polar core of esters, bounded by single layer for phospholipid that cholesterol & more specified apoproteins are fixed.

1.1.6.1. Characteristics of lipid in framing a DDS

1.1.6.1.1. Polymorphism and crystallinity of lipids

Number of pharmaceutical solids components occur in various forms. It is accepted that drug and other excipients may crystalline, amorphous or anhydrous, at different degree of either hydration or may be solvated along with trapped molecules of solvent, and variable in hardness, size and shape of crystals. Solid with amorphous nature comprise of disorderly engagements of molecules & don’t retain a divergent crystal lattice. In the crystalline state, principal molecules are organised in a static repeating range built of cells that is identified as lattice. Possession of suitable crystallinity is criterion for well lipoid particulate delivery system. Triglycerides that are mostly utilised in lipid matrices are crystallize with various forms of polymorphic. Most significant types are α & β type. Meanwhile formulation of lipid particle DDS
might include melting at specific temp, recrystallization occurs from melting obtained in stable $\alpha$ form of polymorph, that afterward undertakes a polymorphic conversion in stable $\beta$ through stable intermediary $\beta'$. (Anthony A A 2008) The $\beta$ form of polymorph specifically contains of extremely well-ordered, inflexible assembly with less loading volume of drugs. Formation of all kind of polymorphics is being shown between solid triglyceride lipid nano sized particles.

1.1.6.1.2. **Melting properties of lipid**

Triacylglycerol comprise the one melting point which happens at particular temperature. However, certain kind of lipids comprise extensive selection of various triacylglycerols, with various melting range & they will melt under large series of temperatures, which produce large endothermic transition under differential scanning colorimetry (DSC). Lipids with higher level of purity and sharp melting range omit drugs for recrystallization. More to solidification and melting point for individual lipid (triglyceride), in DDS, researcher is much interested & alarmed with mixture of triglycerides all the way through fat mixture. This usually influences on plasticity or range of melting. For developing the lipid particles (nano sized), lipids which have melting beyond to body temperature usually preferred. That will generally allow in-between others, the sustained mannered release of entrapped drug.

1.1.6.1.3. **Polymorphism & crystallinity vs drug release & loading**

Polymorphism & crystallinity finds a huge impact on characteristics of lipid used for lipid DDS. Factors such as capacity of loading of drug and release of drug generally be subject to high on crystallinity and on polymorphic type of lipid. Order of crystalline and density rise $\alpha$ to $\beta$ and most high for $\beta$ type of lipids (lipid). (Anthony A A 2008) Increased crystallinity has wide effect on loading of drug, meanwhile increased order normally decreases capability for incorporation of various molecules. Therefore loading capacity for poorly ordered forms of polymorphic is higher. (Jenning V 2000) Still, the benefit drives with particle in stable which are capable for transforming in metastable form of $\beta$ on storage. As significance of conversion, frequently drug discharge arises. The increased order of conditions (matrix) also decreases rate of diffusion for drug in particle & later decreases release rate.
1.1.7. Lipid selection in particulate DDS

In recent days the lipid DDS interest is increasing because of features like superior characterization for lipoid components and adaptability of formulations and different DDS selection. Besides profile of fatty acid of earlier indefinite lipids, many types of lipoid analysis techniques for characterization of lipids. Such recent techniques deliver the various types of analysis & it might use with mixture for selecting a proper lipid matrix in formulation.

Figure 1.6: Types of lipid DDS (particulate)

Widely used technique is DSC thermogram for analysing the oils, fats and mixtures. A details related to associated energy and temperature along with phase behaviours, polymorphic transformation, crystallization & data for solid fat estimation. DSC thermogram reports for damage in structures by related recordings found in far
form equilibrium state. One another technique is X-ray technique (diffraction / XRD) which also vital for properties elucidation of fats & related mixtures. Recordings of XRD promotes long and short spacing at particular temperature that sample should be under equilibrium. Meanwhile lipid related systems are somewhat sensitive with formulation processing history. Microscopy of polarized light (PLM) is one of technique for analysis for fat characterization in observation of micron sized structures of different polymorphic forms related to fat. Along with coupled in hot stage that is utilized to see the micron sized structural alterations in fats at time of melting, as lipid transform from crystalline to isotropic type of phase, for visualizing the crystallization from isotropic melts & for visibly notice undissolved drug into matrix of a lipid. One other potential tool is isothermal micro-calorimetry (IMC) for analysing a time mannered crystallization process in lipids and its matrices. It can apply for pure or mixed systems. On application basis, various lipid based DDS is given in figure 1.6.

Kinetic studies for isothermal crystallization for mixtures of related lipids by IMC too address a query on how this crystallinity for one component shows the effect to behaviour of crystallization of other. (Attama A A 2006)(Attama A A 2007) Atomic force type microscopy (AFM) is also can promote the priceless evidence almost for physicochemical properties of carriers which have an important role in determination of performance for particular DDS. This fact can’t gained from other technique because of unique capability of AFM to probe the nano range feature. In following table 1.1 examples of different lipids are presented which can be used with specific lipid in SLN preparation.

**Table 1.1:** Various ingredient for SLN preparation

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
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<tbody>
<tr>
<td>Lipids</td>
<td>Hydrogenated glyceride (Softisan142)</td>
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<tr>
<td></td>
<td>Triglyceride</td>
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<td></td>
<td>Tricaprine</td>
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<td></td>
<td>Trilaurine</td>
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<td></td>
<td>Trimyristine (Dynasan114)</td>
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<tr>
<td></td>
<td>Tripalmitine (Dynasan116)</td>
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<td></td>
<td>Tristearine (Dynasan118)</td>
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<tr>
<td>Hard fat</td>
<td>Emulsifiers</td>
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<td>----------------------------------------------</td>
<td>---------------------------------------</td>
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<tr>
<td>WitepsolW35</td>
<td>Soybean based lecithin</td>
</tr>
<tr>
<td>WitepsolH35</td>
<td>Phosphatidyglycerol</td>
</tr>
<tr>
<td>WitepsolE85</td>
<td>Phosphatidylcholine</td>
</tr>
<tr>
<td>WitepsolE85</td>
<td>Egg based lecithin</td>
</tr>
<tr>
<td>WitepsolS51</td>
<td>Poloxamer 188/182/237/338/407/908/238</td>
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<tr>
<td>WitepsolS55</td>
<td>Polysorbate 20/21/80</td>
</tr>
<tr>
<td>Glyceryl monosterat (Imwitor900)</td>
<td>Tyloxapol</td>
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<tr>
<td>Glyceryl palmitosterat (PrecirolATO5)</td>
<td>Sodium glycocholate</td>
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<tr>
<td>Glyceryl behenat (Compritol888®ATO) (COM)</td>
<td>Sodium cholate</td>
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<tr>
<td>Glyceryl caprat (CampulMCMC10)</td>
<td>Sodium taurodeoxy-cholate</td>
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<td>Cetyl palmitate</td>
<td>Sodium taurocholate</td>
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<td>Palmitic acid</td>
<td>Butanol</td>
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<td>Behenic acid</td>
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<td>Decanoic acid</td>
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<td>Bees-wax</td>
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<tr>
<td>Cocoa butter</td>
<td></td>
</tr>
<tr>
<td>Carnauba wax</td>
<td></td>
</tr>
</tbody>
</table>
1.1.7.1. Preparation of lipid nanoparticulate DDS

Number of methods exists for SLN DDS preparation. Method utilised is said by drug category especially for solubility & stability, administration route, lipid matrix, etc. Present section, importance laid on preparation of SLN and related nanoparticles, with various methods which can be implement to liquid crystal formulation.

Method related to homogenisation at high pressure (HPH) is appropriate for SLN and other related nano-particles preparation & that can done at raised temperature (hot HPH) or lower than room temperature (cold HPH). Particle size usually reduced due to shear, impact, turbulence and cavitation. In detailed, hot HPH technique comprise drug & lipid are first melted above 10°C of melting & mixed with surfactant (aqueous part) on same temperature and pre-emulsion (hot) generated by homogenisation at high speed (HSH).

This pre-emulsion further processed at temperature controlled HPH nearly 500-550 bar in single or multiple cycle. Resultant nanoemulsion is recrystallize by lowering down upto room temperature and further it will form SLN or other related nanoparticles. Cold HPH can be consider as compatible method for carrying the heat sensitive/ hydrophilic drug preparations. Lipid & drug made to melt in combination and further immediately crushed at liquid formed nitrogen temperature which will form microparticles of lipid (solid). Pre-suspension formed using homogenisation of particles at cold aqueous part of surfactant. That pre-suspension is further carried for homogenisation using HPH or lower than room temperature on previously defined homogenisation condition for preparing the SLN. Possibility for specific rise of temperature at the time of cold homogenisation must be in mind. All HPH methods are suitable to process a concentration of lipid about 40% & it normally results narrow size distribution of particle (PDI –polydispersityindex). (Lippacher A 2002)

Schematic presentation is given in figure 1.7 for HPH technique.

Gasco (Gasco M R 1993) had optimised compatible technique for SLN preparation by microemulsion. In this process, warm emulsion (micro) is arranged &
afterward, dispersed under continuous stirring of excessive water (below 10 °C) normally at 1:50 ratio with thermostated syringe. Excess water (below 10 °C) removed using filtration (ultra)/ lyophilisation to rise concentration of particle. Technical parameters like emulsion composition, device for dispersing, temperature effect & lyophilisation condition on structure and size of resultant SLN must be optimised. Excessive water removal from SLN dispersion is critical task for particle size. Even, higher concentration of cosurfactants and surfactants are required for formulation, but less/not desired for regulatory aspects and related application.
For preparation of SLN dispersion using solvent evaporation/emulsification, first lipoid material is dispersed in suitable solvent that is an emulsified into water phase. Further after vaporisation of solvent, particle dispersion will generated due to lipid precipitation in aqueous (water) medium.\textit{(Sjostrom B 1992)}
coworker (Siekmann B 1996) had formed cholesterol acetate based nanoparticles which have mean size with 29-30 nm by this same technique. Another method for SLN manufacturing involves the supercritical fluid/phase inversion technology. In the following table 1.2 describes past efforts for SLN preparation with various drug candidates.

**Table 1.2:** Examples of SLN with various entrapped agents

<table>
<thead>
<tr>
<th>Encapsulated component</th>
<th>Polymer</th>
<th>Technique used</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olanzapine</td>
<td>Hydrogenated soya-phosphatidylcholine</td>
<td>Modified HPH</td>
<td>(Vivek K 2007)</td>
</tr>
<tr>
<td>Gatifloxacin</td>
<td>Chitosan and Na-alginate</td>
<td>Coacervation (modified)</td>
<td>(Motwani S K 2008)</td>
</tr>
<tr>
<td>Insulin</td>
<td>Hydrophobised type cholesterol with pullulangyceryl-behenate, Campritol 888ATO, lecithin</td>
<td>Ultra-sonication</td>
<td>(Lu B 2006)</td>
</tr>
<tr>
<td>Clozapine</td>
<td>Dynasan114/116/112, Tristearin</td>
<td>Homogenization (hot)</td>
<td>(Venkateswarlu V 2004)</td>
</tr>
<tr>
<td>Rizatriptan</td>
<td>Tristearin &amp; Phospholipon80</td>
<td>Solvent injection (modified)</td>
<td>(Nair R 2011)</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>Compritol888ATO &amp;Miglyol 812</td>
<td>Homogenization (hot)</td>
<td>(Jenning G A 2000)</td>
</tr>
<tr>
<td>Vinpocetine</td>
<td>Glycerol-monostearate, lecithin</td>
<td>Solvent emulsification (ultrasonic)</td>
<td>(Luo Y 2006)</td>
</tr>
<tr>
<td>Gatifloxacin</td>
<td>Chitosan, Sodium alginate</td>
<td>Coacervation (modified)</td>
<td>(Motwani S K, 2008)</td>
</tr>
<tr>
<td>Drug</td>
<td>Formulations</td>
<td>References</td>
<td></td>
</tr>
<tr>
<td>--------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>--------------------------</td>
<td></td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>Paclitaxel-Tripalmitin&amp; phosphatidylcholine Micro-emulsion</td>
<td>(Cavalli R 2000)</td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>Insulin-Cetyl-palmitate Solvent evaporation</td>
<td>(Sarmento B 2007)</td>
<td></td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Methotrexate-Campritol888ATO, tween80, Cetyl alcohol Microemulsion (congealing)</td>
<td>(Misra A 20002)</td>
<td></td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>5-Fluorouracil-Dynasan114/118, lecithin &amp; triglyceride Solvent evaporation(emulsion (double))</td>
<td>(Yassin A E B 2010)</td>
<td></td>
</tr>
</tbody>
</table>

1.1.8. **Topically applicable SLN**

Area for huge potential especially for SLN & withinless duration-to the market are topical based products which are basically with SLN based technology, which means pharmaceutical along with cosmetic based formulations. SLN are usually considered as further generation delivery system of drug candidates after liposomes. (Silpa R 2012) Comparable to liposomes they comprise of good borne ingredients and because of small size they retain related adhesive characteristics on skin. Separatemerits of SLN isthe state(solid) of matrix (in particle), capability for shielding chemical basedexcipients against decomposition of chemical & related possibility for modulating release of drug. Distant from technological based merits, solidified form of SLN hasbenefit regards with product listing and registration for corporate pharmaceuticals and also for cosmetics. In Japan for products related to cosmetics need to prove that liposome is not qualitatively but then also quantitatively. In case of liposomeproof for qualitative presence is quite easy through microscopy (electron), butstillverydifficult for quantification and for showing that they still present with sufficient quantityat time of storage period forproduct. That big obstacle for introducing liposomal based cosmetics to principally Japan market. Indifference to quantitative analysis for SLN for cream is much simple & direct. A number of bases of cream don’t show melting lower to 100 °C, which mean SLN content in cream may quantified by melting behaviour obtained through DSC.
Storage stability can effortlessly supervised by simple visual observation of melting enthalpy alterations. Examination is also probable where cream comprises portion that melt lower to 100 °C. If overlapping found than determination for melting energy by time function is possible. Such kind of characteristic for SLN widen new kind of market in topical range products that contains colloidal type of carriers for drug.

Tackiness is one kind of common characteristics of nano sized particles. An example from routine is for iced sugar that adheres better with bakery products in compare with crystalline sugar. Similarly, SLN hypothetically forms the sticky film layer on skin. (Pinaki D 2010) Earlier presumed that SLN may form films with spheres with dense packing, however current studies recommend under force by application, spheres usually makes coherent film. (Wissing S A 2001) Such kind of lipoid film forming will re-establish injured defensive lipoid film on skin. Additionally, film may have some kind of effect of occlusion. Such occlusion was demonstrated through in vitro depths. (López G R 2015) SLN effect on barrier (properties) of skin is established. SLN used to create invisible hypothetical film (figure 1.8) which have attraction for SC that guarantees for release of drug in prolonged manner by time, decreases loss of water for transepidermal and expand hydration of skin. (Müller R 2007)

SLN were integrated in cosmetic cream (O/W) & verified in diffusion cell for its effect for penetration of drug and for occlusive. Base of cream & SLN loaded
cream was applied on skin & further analyse up to next 24 h (incubation). Occlusive properties was measured through staining a upright slices of skin by dye.

![Figure 1.9: Hypothetical targeting sites for skin (topical dosages)](image)

Natural skin demonstrated compressed SC along with layers of cornecytementically connected. Conventional cream (SLN-free) has shown considerably changed in structure. Altered outcome attained in cream with SLN, SC seemed little inflamed (swollen) & completewideness had risen. Quantitative calculation is tough through slices of skin by microscopic assessment. Additionally, loss of water through transepidermal was determined. Most marked and insignificantly varied impact was gained through SLN.

Discussion for effect under in vivo occlusive for SLN is slightly contentious which partly recognised to differences in formulation verified. From results found, it may resolved that SLN added in respective formulation have nosupplementary occlusive when formulation itself at present highly occlusive (petrolatum O/W). Occlusiveness improvement can attained through addition of SLN with proper configuration of light cream (O/W), hence growing moistening impact without having smoothness (glossy) of cream. Parameter for assessment of capability for delivery is impact on drug penetration to skin & accordingly beneficial effect and for cosmetic application impact on appearance of skin. Cosmetic excipients like tocopherol, coenzyme Q10 & retinol already been encapsulated in SLN. (Laouini A
However, there may possibilities of drug movement through formulation in case of topical dosages; it may permeate into skin (absorption), it may localize in skin layer (reservoir) or it may remain on skin (surface) as shown in figure 1.9.

![Figure 1.10: Interaction scheme for SLN & skin](image)

Hypothetical interaction scheme with skin is shown in figure 1.10 where follicles of hair accumulate the SLN further the lipid & drug will penetrate in skin followed by release of drug from SLN and possible targeting will follow as discussed in figure 1.10 (Jin Z 2006)

Skin caring characteristics for marketed retinol cream linked to similar cream having SLN (of retinol). (Jenning V 2001) In similar study, assessed parameter were covers elasticity of skin, moisture & roughness of skin for read out. Moisture in SLN formulation raised approximately to 33% in post 1 week observation related to normalised skin. Moreover, SLN cream (retinol) enhanced skin softness (10.3%), while the regular cream reached 4.1%. Encapsulation (EE) of ingredients in SLN defendsto chemical mediated degradation. Enhancement of stability was studied in case of coenzymeQ10. (Gokce E H 2012)&even for retinol. Products (cosmetic) with retinol, that currently launched, have to fashionedspecific cost related severe safety terms (like yellow light colour at time of production, defensive gassing with noble gas) and
less artistic packing (like aluminium is preferred as alternative to polyethylene). Retinol entrapped SLN stabilization gives various economical tricks for production/use of much attractive, consumer slanting packaging.

Major step onward expansion of ‘smart’ SLN that may exploited topical and routes of delivery. Smart SLN means release from in controlled manner of entrapped drug post that obtains initiating impulse. Such initiating impulses are usually rise of temperature or water loss from dispersion of SLN. As discussed above, that is known based on literature which forms alterations in stable lipid which leads drug removal that indirectly means release of drug through SLN. Such natural impact can exploited in organised way through settling an effect of triggering when drug release is main objective. It is known that assured section of adaptation and limitations within crystal lattice support drug inclusion. That directly means, during storage period of formulation of SLN a lipoid carrier must well-maintained in energy modification, when that smeared to skin or given to body conversion from much stable type modification will initiate. Such conversion clues to more methodical structure & less defectiveness, thus principal subsequently to discharge/release of drug through carrier. This kind of impact was exploited controlled manner release for retinol (SLN) that formulated in hydrogel or cream or gel. (Dasgupta S 2013)

Totally novel, currently exposed area for application is utilisation of SLN for sunscreen creams. (Bahman K 2015) Because of protective ozone reduction, a sudden rise in cancer of skin cancer, melanoma cancer shows toughest growth world-wide, specifically in Australia. (David A 2014) Adverse impacts of UV blocker is skin penetration and subsequently irritation. Sunscreen particulate system such as titanium bases were set penetrate possibly in skin. It can minimised or side-stepped by encapsulating particulate based sunscreen in SLN. (Threes G S 2011) Because of particulate character SLN are protective in terms to scattering a UV-light (like titanium dioxide). Additionally, a findings of molecular kind sunscreen and SLN with mixture demonstrate synergistic impact. Molecular sunscreen usually much effective post entrapment in SLN & at same moment adverse effects reduced. That widens perspective to novel category of formulations. (Müller R H 2000)
1.1.9. SLN characterization

Acceptable and correct characterization of SLN is mandatory for quality viewpoint. Still, SLN characterization is a serious task because of colloidal nature of particle & complex nature of DDS.

The main constraints that required to solve for SLN; poly dispersity index (PDI), size, distribution kinetics for size (zeta potential), crystallinity degree and polymorphism, existence for extra colloidal candidates, scale (time) for process of distribution, drug loading (DL), release behavior of drug & morphology. Size related characterization can carried through correlation (proton) spectroscopy (PCS), electron (transmission) microscopy (TEM), electron (scanning) microscopy (SEM), atomic force microscopy (AFM), tunneling (scanning) microscopy (STM), or freeze fracture EM. (Mukherjee S 2009)
1.1.9.1. **Size & zeta potential measurement**

PCS & laser diffraction (LD) generally in practical & powerful method for regular measurement of size of particle. Coulter method does not used in routine for measurement of SLN size due to complications in small size particle assessment & requirement of electrolyte that may weaken dispersion of colloids.

PCS (light scattering) will measure intensity variation of scatter light that caused due to movement of particle. That technique includes range of size (nanometer up to micron). It mean PCS as better tool for characterization of SLN, however that cant capture a large sized particle (micro). They visualized using measurement LD. That technique is usually based on necessity of angle for diffraction on radius of particle. Small sized particle may create much intense type of scattering at angle in comparison to large one. Most clear benefit for LD covers analysis of wider range of size from nanometer up to millimeter. Development for intensity of polarization differential scattering (PIDS) technique widely improved capacity of LD with means to size of particle. Although such progress, that is suggested for using PCS & LD consecutively. It should keep in attention that these methods don’t ‘analyze’ size of particle. Reasonably, they used to detect scattering of light that used for calculating size of size. For reference, some uncertainties might be produce in case of particles which are not spherical. Structures of platelet generally happen at time of crystallization of lipid and that also recommended for SLN. Additionally, complications may rise in LD and PCS capacities for test which have large number of population with various size. So, supplementary methods may useful like light based microscopy, although that is no more dedicate to sensitivity for nanometer size. That can give quick suggestion for presence & microparticle characters.

Electron microscopy (EM) delivers, with contrast to LD & PCS, straight details on shape of particle. Though, finder must give courtesy for possibility of artifacts that might arise from sample during preparation. For example, removal of solvent results the modification that effect the shape of particle. Zeta is one of principal characteristic of product containing SLN meanwhile higher the value estimated to particle deaggregation in absence to another confusing impactful factor like stearic stabilizers. That is generally measured through zeta sizer.
1.1.9.2. DLS technique

DLS known as quasielastic - light scattering (QELS) basically records deviation in scattered light intensity on time scale at microsecond level. This result of variation from interfering of scattered light through particular particles individually under guidance with brownion motion & quantified using compiling of autocorelation feature. Such feature is fit with exponential, or modification/ combination thereof, along withparallelconstant decay related to coefficient diffusion.

By using standard level expectation for sphere size, lower concentration & viscosity of medium for suspending, size of particle calculated through this same coefficient. Advantage of present technique areanalysis speed, calibration lack & sensitivity towards micrometer sized particle.

1.1.9.3. Static light scattering (SLS)

SLS is collaborativetechnique which have light scattering pattern from particle solutionis gathered & fit towardsequation of electromagnetic where size is initial variable. SLS technique is quick & rugged, however it needsmore clear preparation in compare to DLS & knowledge of optical quality.

1.1.9.4. Acoustic method

One other collaborativemethodology,dealings the dilution of waves of sound with mean to size determination through applying equation which is physically relevant. Additionally, electric field with oscillating produced from movement in particles with charge under impact of energy (sound) can detect for providing details on charge of surface.

1.1.9.5. Electron microscopy (EM)

TEM &SEM promotes way for observing nanoparticle directly, characterization on physical level for nanoparticle along with prior technique being much better for examination (morphological).

TEM has size limit (smaller) for certain detection. That is quite better validation for different techniques and it must knowing for statistically sample size (small) & impact which vacuum may have on particle.
1.1.9.6. AFM technique

This method comprise probe tip at sharpness at atomic scale is raster through sample for producing topological direction based map on forces which play in-between surface & tip. Probe can pulled through sample (in mode of contact), or it allows to mode of non-contact, along with perfect nature of specific force engaged attending to differentiate amongst sub-techniques.

Higher range resolution can obtain by such method, with capability for mapping sample as per characteristic in adding to size, e.g., attraction of colloidal or deformation resistance make this method more important tool. Basically AFM based single type force-spectroscopy (SMFS) has proven to strong technique for measuring forces of microcosmic that arise because of interactions like bonding (covalent), reorganization of host-guest, \( \pi-\pi \) interactions, forces of intercalating & hydrogen bonding.

1.1.9.7. Xray diffraction (XRD) and DSC

Geometric scattering radiation originating from plane of crystal in solid usually allow absence or presence of past to determine thus allowing crystallinity degree assessed. Other technique which is sight altered from application for materials in bulk, DSC can utilise for determining nature & speciation for crystallinity of SLN by measuring melting & glass transition range/point and related enthalpies.

DSC shows prominent role due to capability for providing physical structure based details for particle systems. Furthermore, utilisation of this method most often leads for harmonizing details about system. Physical nature based transformation in component are commonly attended by interactions in heat like its uptake at melt, emission especially while crystallization. DSC is definitely made for gaging such kind of heat related interchange in temperature program which permit for illustration a conclusion for structure based characteristics for samples. It is capable for observing & computing the tiny thermal based changes within sample & for identification of temperature that such events happens. It is one type of method that not straight disclose the source for thermal happening. The most precise nature of thermal changes has to resolute with corresponding techniques.
1.1.10. Sterilization of SLN

SLN sterilization is subject to discuss especially for pulmonary/parenteral route administration. Reported literatures has shown an autoclaving method for sterilisation of stabilised SLN and related study is also presented by Viveksarthi & co-worker. (Viveksarathi K 2015) SLN usually melt during heat application in autoclave & recrystallize during cooling. But, this method is impossible while certain specific structure has assumed by SLN in controlled manner through setting fabrication parameters. Such specific structure lead to preferred modulated type release profile & that would absent when melting of particles all through autoclav & recrystallize process by un-organised way. Specific temperature cant perform during sterilisation by autoclave if sterically stabilizing types of polymer is used in system like poloxamers. (Viveksarathi K 2015)(Ketan H 2010).

Past suggestion by Groves et al, that by sterilisation through heat application, phospholipid immediately displac from aqueous to oil phase. Such relocation happens in mixture with development of liquid cubic phase of crystalline at boundary during application of heat for sterilization that transforms to lamellar upon cold process. (Groves M J 1993) Autoclave temperature appears to very near to critical occultation temperature (CFT) of polymer, layer of polymer adsorption appears somewhat to failure prominent to insufficient aggregation of particle & stabilization. That phenomenon can sidestepped by decreasing temperature of autoclave & consecutively extending time. Physical stability in autoclave condition can’t be specified in broad means that mainly depend on SLN composition. Thus, above testimonial can studied as rough guideline.

Dispersion of SLN also sterilized through filtration same as emulsion intended to parenteral route. That is most significant to filter at liquid form, which allow particle with large size in compare to pore of filter. Filtration is much known for emulsions for parenteral use & quite easy to implement for SLN. (Constantinides PP 2000) Otherwise, SLN can formed high aseptically, and matching with parenteral emulsions. For sum up, dispersion of SLN can sterilized & formed aseptically by previously conventional techniques in industry.
1.1.11. Principle of solid lipid nanoparticles formulation

Generally interfacial tension have lesser value in compare with surface tension, mainly due to adhesive type forcein-between two different liquid at boundary usually more than liquid& gas. At interface, molecules behaves the grater potential than bulk type of phase molecule, such molecule create surface energy (free)to interfacial arrangement. At phase of agitation suchschemesreachesphere-shaped system, for lowers surface energy. Thus work must done for increasingdispersed particle surface.

For constant type temperature & pressure the recorded surface tension is denotes to Gibbs energy which is in unit area.

\[ W = \gamma \times \Delta A \]

Here, \( W \) relates to work in unit of ergs, \( \gamma \) relates to surface tension with unit of dyne/cm & \( \Delta A \) relates to increase (change) with surface area with unit of \( \text{cm}^2 \).

Molecules of surfactant of amphiphilic nature are usually adsorbed at interface due to adhesive type of forcein-between molecules of water & polar head of surfactant group usually are low with cohesive type of force amongst particularmolecules of water. Inmolecule (amphiphilic) to adsorb onmolecules which are at interface of molecules of surfactant which must balance to water & oil soluble group. The scale of HLB by Griffin gives hydrophile-lipophile measurement of balance in surfactant, thuscompetence of range of HLB range for surfactant may resolute. Higher HLB tends to hydrophile nature & lower HLB tends lipophile molecule.

SLN usually same with emulsion that formulatethroughlipids which are solid but not an oil, moreover that are solid at routinetemperature (room temp.) & body temp. withmelting with range between 40 to 60 °C. When melted lipid&water phase mixat pressurized agitation, gives very fine droplet structured of another phase (dispersed)in medium. However, because of interfacial type offorce for phasedistinct out & formsseparatelayer, thus 3rd component active agent (surface) is utilized for stabilizing entire system for reducing energy in system (disperse). Non-ionic& anionic type surfactant generally utilised for SLN formulation; and occasionally surfactant with cationic type may also utilised. Alcohol of traight chain,alkyl phenol, glycols (propylene) & fatty acid may also used in surfactant.
These understandings on basic principals mostly help to design the SLN and mainly for its stability formation. Such principal understanding also helps to select the right surfactants and lipid for SLN formation.

Solvent technique is proposed here especially for encapsulating molecules with higher level of stability with bioavailability issues, notwithstanding toxicological related issue due to solvent are mainly limiting part. The one main merit for such solvent based technique is the minor operating process temperature, that can be valuable for the thermosensitive drug. Finally, drug entrapment can be a most important: recently more complex molecules are encapsulated in SLNs. Such molecule profiles has various physico-chemical characteristics & stability related issue. The preferred groundwork technique must be the much appropriate to improve loading & EE in particulate system, deprived of obstructing the chemical related stability of molecule itself: process temperature & operating conditions mainly use in preparing particles may affect stability of the drug.

1.1.12. Work Plan

The entire work was designed based on the basic understandings. The strategical overview for work plan in given below. However, the detailed methodology will introduced in another section of methodology.

1) Review of literatures: It covers the survey from various articles from recent years in area with similar kind of work, especially for SLN production, development, SLN performance in topical DDS, TCRLM safety, excipient profile & performance, understandings the skin related absorption of SLN, understanding of advantages and disadvantages of each task of planned work.

2) Selection of compatible excipients for solid lipid nano formulation as well as topical formulation development.

3) Identification & confirmation of drug by Fourier transformed infrared spectroscopy (FTIR), DSC & NMR.

4) Excipient compatibility study by IR & DSC.

5) SLN preparation with entrapment drug and without drug by homogenization and solvent evaporation.

6) Implement SLN formulation in gel base for topical formulation development.

7) Characterization of SLN & gel formulation.
a. Morphology study of particles by SEM.
b. Characterization of particles by DSC.
c. Size distribution analysis by size analyser.
d. %EE by UV.

8) Evaluation of impact of different parameters and excipients in formulation using design experiment software on different characteristics of SLN.

9) Implement a SLN formulation in topical dosage (gel) form with suitable base.

10) Drug release study & kinetics understandings of gel formulation using diffusion cell.

11) Drug localisation & permeation evaluation by ex vivo technique.

12) Stability study.

13) Animal study.
1.2. Challenges

The principal problem with recent conventional delivery for drug specifically in topical DDS is related to targeted effect which is not possible through the simple dispersion of drug in vehicle. The ultimate solution for such episode is formation of delivery system that have more resident time i.e. prolonged drug localization. SLN are one of the approach against this problem. However, here are some problems that associated with SLN formation or post administration.

Large sized production of SLN is only feasible through specified facilities present in pharma unit. After exhaustive study on colloidal carrier, some terms during several decades, unusually only few found and launched in market representing that it seems todifficulties with fundamental ideas or with preparation of satisfactory colloidal carriers. Majority product are in category of cosmetic. In present context registration & further development for novel kind of system for administration might considered long for novel formulation; which means may be 10 or more years of duration. Thus, product semin market after significant time duration when that particular idea has effectively understood.

SLN or other categories of particles systems are very accurate at small scale designing. On large scale the phase transformation in lipid will follow the different behaviour, especially in case of melt technique the applicable temperature will follow the different mechanism for heat uptake by lipids. In that case the drug which are more temperature sensitive will face more problems for chances of degradation. In that case the lipid must be selected carefully. The process selection must carried from precise temperature control. Moreover, in such cases the process which usually avoids the heating phase can selected in that case. Presented research is best hands on example for such kind of risks. In that case, the method was selected which avoids the heat transformation. However, during the entire process at some of the stages the heat transformation cannot avoided. For example during HSH and HPH processing.
The one more topic is burst release, which frequently catches in SLN. Such event is effect of unentrapped drug fraction during processing of encapsulation. The effort must be directed under investigation related to such event specifically in production of prolonged release formulation. In case of IV administration by SLN, such system have risks to interact with reticulo-endothelial (RES) system. A biological system have possibility to take as foreign material and against it the body will answer back with defence response. Such kind of effects mostly due to diverse interplay within factors, and not restricted to fundamental properties of nanoparticle. Majority of nano material postassociating with biological matter, it will coated through proteins which called as “corona”. Definite workings of corona, may rise recognition of coated component by cell through RES. Actuality of corona on surface (of particle) creates “molecular signature” that used to in immune cell & normalize route for internalization. This route disturbs definitive fate of SLN in body.

Dosage selection is communal critical judgement in case of SLN. Other than injectable, a sturdy requisite to package SLN in proper units of dosage specifically in oral administration. Meanwhile a lower pH exposure within stomach with ionic strength can maintain the accumulation of particles. Thus SLN dispersion (aqueous) is not more compatible in oral administration.

In recent research the one more problem is related to risk analysis assessment. The risk analysis for process development cannot go through in similar way to solid dosages. The risk associated with some critical steps is purely depends on skilled work in preparation. The task like HSH processing, HPH processing, sonication processing etc. requires precise control on handling and working on it. In another words it just need skill for handling at each stages. Some of the processing techniques requires a critical dependency on selection of relative humidity (RH) level. For example in solvent evaporation technique for SLN preparation the RH plays a vital role in manufacturing stage. It requires a high level of RH in initial stages which will maintain the dissolved lipid in its own state. The final step requires a lower level of RH or there is no more impact of RH maintenance because ultimately it requires to evaporate the solvent. But if the RH level varies during initial phase, than it will show critical impact on solidification process. Solidification of lipid will indirectly effects on size or PDI of particle.
1.3. **Research objectives**

Recent study describes the current progress of novel nanotechnology which pays a potential consideration to characterisation for drug and particle interactions which are highly significance for skin uptake. Additionally presented research have demonstrated the studies on localization and following by a topical administration of drug loaded SLN for diseased skin (predicated) condition. The outcomes are compared with obtained alternative conventional carrier of same drug.

Following points covers the principal objectives that covers the intension for conducting this developmental work and related experiments.

- To promote drug localization in dermatological conditions like AD and eczema. In current research, development of SLN of TCRLM aims severe dermatological disease condition like AD and eczema, in which SLN based formulation mostly targets to promote drug localization in such dermatological conditions.
- To architecturally develop the highly lipophilic TCRLM loaded lipid based nano sized particles in which nano structure will form by solidification of lipid from biocompatible categories that free from producing unwanted toxic effect. Material screening will carried based on the compatibility study with drug
- To implement modified & applied processing technique for SLN development stage which covers the distinct consideration of thermo-labile& sensitive nature of TCRLM for preventing possible degradation in formulation stage.
- To characterize SLN based on basic characterization for its size, zeta charge and %EE. Optimization of SLN based on its optimum characteristic features like %EE, PDI and size which are main key parameters of it.
- To optimize SLN batch will further transform in topical gel based dosage form with suitable gel base for make it applicable in topical usage. This approach mainly designed to increase bioavailability of TCRLM in topical dosage form and to increase localization time of TCRLM which ultimately decrease dosing frequency.
• To evaluate localization and permeation of TCRLM from SLN, an ex vivo method will perform using animal skin in diffusion cell to present the better performance than in conventional topical dosage form of TCRLM. This study covers the principle aim of the research for drug localization approach.

• To perform In vitro experiment will for presentation of drug release from SLN via gel based formulation. This experiment is designed to identify the release performance in comparison to conventional marketed product.

• To perform In vivo study (animal study) of formulated topical formulation to evaluate skin irritation and sensitization effect produced by formulation if any. This study is designed to understand effect of biocompatible nature of formulation.
1.4. **Scope of research work**

The presented research comprise the development of novel product comprising TCRLM-SLN dispersed in gel base. Presented product have novel effect of localization which cannot find in conventional marketed product. The more focus is given on drug localization and precise SLN formation through optimisation experiments. Presented experiment design and other work revealed the practicality of developed SLN as active portion for topical gel, which may useful in topical formulation development for topical delivery of TCRLM. Thus developed practice unlocks the prospect for scaling up & widens occasion for its presentation to deliver largemultiplicity of drug in topical applications.

Novel SLN deals very resourceful platform that must reflected while functioning with drugs with solubility/ bioavailability related challenges. An increased range of drug under modification/ development of very less solubility in water & thus with bioavailability with poor class. Such nominated class II/ IV drugs. Novelty in formulation methods mandatory for producing complete drug product (for drug) which has adequate pharmacokinetics. The mutual approach of formulation along with this kind of compound emphasis on generating and stabilizing small sized particles of drug in effort to rise surface area accessible in dissolution in vivo & henceforth dissolution rate & accordingly tissue or plasma levels for drug. Stability of shelf-life & degradation (enzymatic) are principal areas of worry & design of formulation attentions on stabilizing drug during storage and for protection from enzyme (endogenous) degradation till it reach up to target (therapeutic). Such novel DDS well matched for bioactive formulation.

Lipid DDS can engaged for phytomedicines delivery in case of oral/ topical administration that considered as leading ways of phytomedicinals administration. Such kind of applications grasp countless potential in use & progress of phytomedicines which consider complications of its carriage owed to particular properties of physicochemical. Meanwhile many kind of phytomedicines typically have various pharmacological actions/ schedules, its delivery is capable for specific
part targeting in body where specific effect mandatory/ desired with means to SLN. Therefore, unwanted effects & excess unusual material would avoided. The allegation is along with effectiveness in preparation, only essential amount is used in formulation & passable dose absorbed & effectively conveyed to target for expected activity. SLN of natural constituents would useful in application related to nanomedicine where target specific delivery is essential like cancer treatment.

SLN development experiment designs have usefulness in active part for topical formulations like gel, cream and ointment which can further used for the development of sustained and site specific topical delivery of potential drugs. Such kind of development work widen the opportunity for further scale up and for its application to deliver wide variety of drug in topical applications.