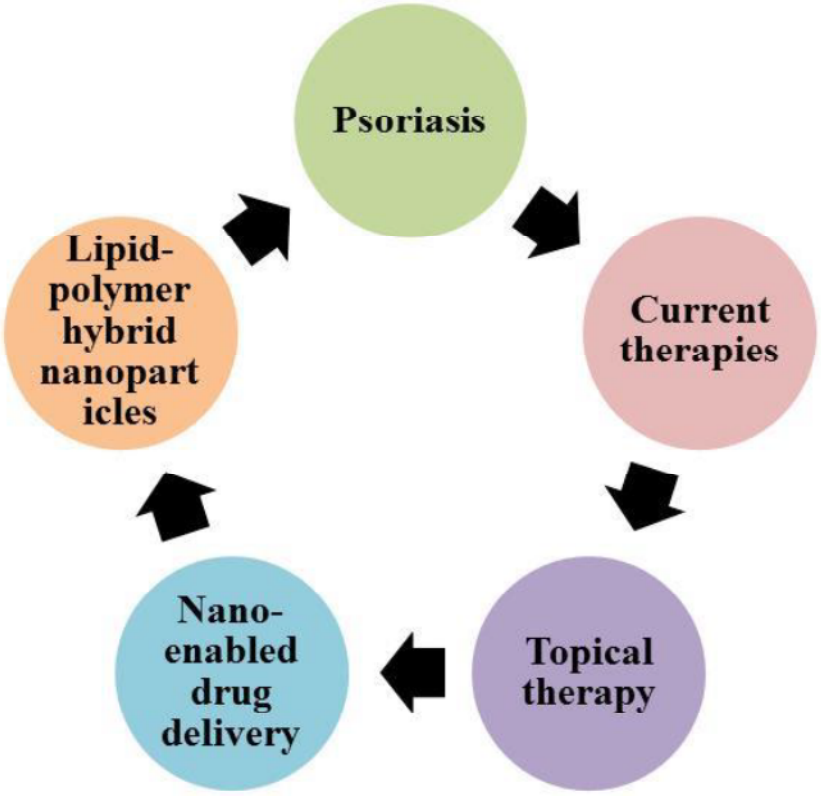
CHAPTER 1

INTRODUCTION



- Psoriasis
- Currently available therapies (Systemic, topical and photodynamic therapy)
- Conventional drug delivery versus nanotherapeutics
- Nano-carrier based approach
- Lipid-polymer hybrid nanoparticles
- Outline of current research work
- Objectives of the research work

1.1. Psoriasis

Psoriasis is a non-contagious, chronic skin disease involving erythema, scaling and thickening additionally accompanied by insistent itching, swelling, pain, inflammation, bleeding and skin lesions revealing a significant degree of hyperkeratosis and hyperplasia. The therapeutic strategies are mainly focused on symptomatic relief rather than the complete cure. It has a massive negative impact on patients' lives, affecting individuals (both men and women) of all ages (most commonly at the age of 50–69). The incidence of psoriasis ranges from 0.09% to 11.43%, imposing a severe global problem with a minimum of 100 million people suffering worldwide. It affects skin and nails majorly and is accompanied by several comorbidities, including cardiovascular diseases, arthritis, metabolic syndrome, depression and inflammatory bowel disease. Countless psoriasis patients suffer needlessly as a result of an inappropriate or delayed diagnosis and scarce treatment opportunities. Amongst 1.3% to 34.7% of psoriasis sufferers develop psoriatic arthritis, a chronic, inflammatory condition affecting joints leading to disability, and around 4.2% to 69% of patients develop nail changes. Its occurrence is more common in financially rich countries and generally affects the population from the older category. Psoriasis greatly affects at emotional, physical and social level hindering quality of life leading to noticeable loss of productivity, mental well-being, resulting into social exclusion and frustration [1, 2].

Psoriasis is now regarded as an autoimmune skin disease with marked presence of erythema, scaling and thickening additionally accompanied by insistent itching, swelling, pain, inflammation, bleeding and skin lesions revealing significant degree of hyperkeratosis and hyperplasia. It is intervened by the activation of T cells leading to hyperproliferation of keratinocytes with drastic reduction in the epidermal keratinocytes turnover. Although genetic predisposition is the causative factor still the etiology remains vague [3-7]. It is

reported that this disease can be triggered by both internal and external triggering factors, including stress, sunburn, mild trauma, systemic drugs and infections**.** Recent reports claim that it is caused mainly due to domination of pro-oxidants such as reactive oxygen species (ROS) over skins' self-antioxidant defense system resulting into severe dermal inflammation [8, 9].

Currently assessable treatment strategies include oral, systemic, topical and photodynamic therapy. Clinically, corticosteroids (topical) are employed as first-line medication for treating psoriasis. Second-line therapy (including calcipotriene, anthralins and corticosteroids (intralesional injection)) are given when first-line therapy does not yield optimum results. Further, these medications are complemented with keratolytics, moisturizers, and climatotherapy to deliver improved therapeutic effects. Other therapies include cyclosporine, retinoids, fumaric acid esters, methotrexate, mycophenolate mofetil, hydroxyurea, vitamin D analogues, sulfasalazine, leflunomide etc [10]. It was also found that the topical application of antioxidants has resulted in significant amelioration of psoriatic inflammation that might be due to the quenching of free radicals and other ROS. Several antioxidant molecules are reported that showed amelioration of psoriasis that includes berberine [11], epigallocatechin-3-gallate [12], resveratrol [13], mangeferin [14], glabridin [8], propylthiouracil [15], quercetin [16], proanthocynidins [9] etc. All the above-mentioned therapeutic agents directly or indirectly hinder the cellular proliferation by inhibiting inflammatory immunological response resulting into a symptomatic relief.

1.2. Clinical types of psoriasis

It is classified into numerous categories depending upon the incidence site, the form of lesions, and the manner in which the individual types react to the given medications. Psoriasis vulgaris or plaque psoriasis being the typical form affecting

approximately 90% of cases. Initially, the small papules (lesions) develop, which subsequently fuse into plaques casing huge skin areas that are pruritic, silvery-white, scaly, erythematous, and abruptly usually existing on the scalp, the outer sides of the limbs and trunk [17-19]. Guttate psoriasis is caused due to immune system stimulation when Group A Streptococcus infects tonsils and ascends very fast even though treated easily with UV therapy in comparison to plaque psoriasis. The pink color papule turns scaly in the later stages and this type is typically witnessed in teens, and nearly 33% of the sufferers develop plaque psoriasis during their adult lifetime [20, 21]. One more severe and modified form of this disease is erythrodermic psoriasis that may self-ascend or due to augmentation of any other earlier existing variant of the disease and involves extreme loss of body proteins leading to failure of retaining body temperature and water content and needs hospitalization. The treatment can be further exaggerated by retardation of growth, staphylococcus infections, arthropathy and pustulosis [17]. The skin is usually enclosed with multiple merged pustules in case of pustular psoriasis and based on the phenotypic observations, it is classified into Acrodermatitis continua of Hallopeau (ACS) and psoriasis pustulosa palmoplantaris (PPP). The prime position of both types includes extreme body parts such as feet and hands. Nevertheless, while ACS is typically witnessed at remote site such as tips of toes and fingers, disturbing the nails structure whereas, PPP is restricted to soles and palms. The lesions are difficult to cure as they can relapse in localized pustular psoriasis; but, they have negligible systemic effects. Even there is a high rate of relapse in generalized pustular psoriasis and is complemented by electrolyte imbalance and fever [22].

1.3. Pathogenesis of psoriasis

Numerous steps are involved in the progress of this disease which can be broadly divided into the following stages that involve T-cells stimulation followed by its relocation to distressed skin lesions, whereby they release cytokines (Figure 1.1) [4]. The Langerhans'

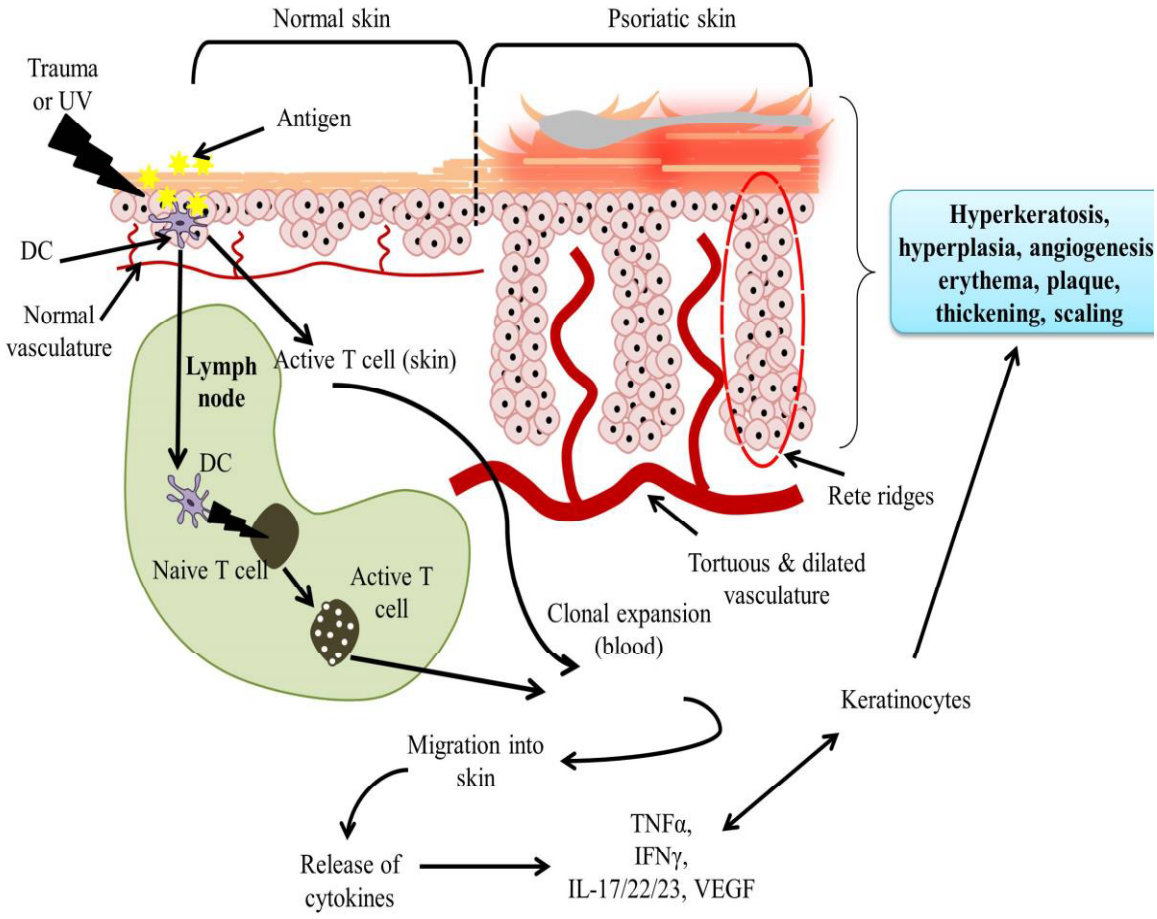


Figure 1.1. Pathophysiology of psoriasis [10].

cells (epidermis) and dendritic cells (dermis) perform as antigen-presenting cells (APC) upon activation by any internal or external antigen in the APC activation process. Once stimulated, these cells drift from the skin to the lymph nodes and trigger naive CDRA45+ T cells with assistance from the leukocyte function antigen-1 (LFA-1) and the immune cell adhesion molecule (ICAM-1). Both the LFA-1 and ICAM-1 lead to stable binding between the T cell and the APC via an improved interface between the CD4/CD8 co-receptors and T cell

receptors with Major Histocompatibility Complex of APC, resulting in signal generation. Additional interactions crucial for proper stimulation resulting in co-stimulatory signals happen between the APCs and T cells, which include CD2 with LFA3, CD40L with CD40, and CD28 on the T cell with B7 (CD86 and CD80) on the APC. Further, the apoptosis of T cells or anergetic effect can result due to a lack of these interactions. After T cells activation, they multiply and differentiate into CD45RO+ type 1 effector and central memory cells as a result of the release of cytokines including tumor necrosis factor-alpha (TNF-α), interleukin (IL)-2, IL-12 and interferon-gamma (IFNγ) [23, 24]. Subsequently, the T cells enter into the skin as a result of the interaction of cutaneous lymphocyte-associated (CLA) antigen (surface protein of T cells) with P selectin and E selectin endothelial cells (skin vasculature). These migrated T cells start affecting the keratinocytes by expressing proteins like CD40, ICAM-1, and major histocompatibility complex II as a result of greater levels of TNF-α and IFN-γ secreted by the type 1 cytotoxic T cells and TH1 cells. Further, T cells trigger hyperproliferation of the keratinocyte resulting in rapid growth of the epidermis. The cytokines such as TNF-α control the levels of type 1 vasoactive intestinal peptide receptor mRNA produced by keratinocytes, which stimulates its hyperproliferation and secretion of proinflammatory cytokines (IL-8, IL-6 and RANTES). Further, cytokines IL-8 and VEGF are responsible for the development of a characteristic appearance of a psoriatic skin lesion i.e., neutrophil infiltration and vascular proliferation [25, 27].

There are numerous proposed theories for the initiation of psoriasis, of which one theory (Koebner's phenomenon) states that any chemical, physical or ultraviolet damage to the epidermal tissue results in keratinocyte stimulation with subsequently higher levels of cytokines which later on trigger T cells leading to more inflammation on account of greater levels of cytokines released ending into a surge of keratinocytes and T-cells. According to the second theory, in trauma-induced psoriasis, there is an interaction of the helper T cells with

the epidermal Langerhans' leading to cytokines release which activates keratinocytes. The third theory proposes that the effects associated with Koebner's phenomenon are the result of an inappropriate attack by the killer T cells on keratinocytes [5]. It has been clearly observed that the biochemistry of psoriatic cells is altered (over expressed markers (epidermal proliferation- keratins like K10, K6, K17, and K16, ornithine decarboxylase and epidermal growth factor (EGF) receptor) and lower expression (keratins for differentiation K2, K1 and K10 and Filaggrin required for the development of Stratum Corneum). As the disease is autoimmune, the levels of immunomodulatory cytokines (interleukin-1 (IL-1), IL-8, IL-6, and TNF) expressed within the keratinocytes are not the same compared to normal cells wherein the IL-10 is under expressed whereas TNF and IL-8 are over expressed suggesting lack of balance between the anti-inflammatory and pro-inflammatory cytokines [6, 28, 29]. In addition to the above-mentioned theories, recently, it was proposed that reactive oxygen species (ROS) play a key role in the pathogenesis of this disease. It was stated that environmental, genetic and/or immunological factors increase the ROS level that further induces oxidative stress (OS) reaction leading to increased levels of malodialdehyde, nitric oxide synthase (iNOS), superoxide anion (O_2^-), nitric oxide (NO) and other ROS with a simultaneous reduced levels of superoxide dismutase, catalase (CAT), glutathione peroxidase (GSH-Px) resulting into higher production of several cytokines (IL-17, IL-12, IL-22, IL-19, IL-23, IFN-γ, transforming growth factor beta (TGF-β) and TNF-α) via mitogen activated protein kinase (MAPK), nuclear factor kappa B (NF-κB) and Janus kinase-signal transducers and activators of transcription (JAK-STAT) signaling pathways. These higher levels of pro-inflammatory cytokines further stimulates VEGF and T cells culminating into psoriatic symptoms (neutrophils and lymphocytes infiltration, parakeratosis, hyperkeratosis, angiogenesis, etc.) [9].

1.4. Currently available therapies

The currently available therapies for psoriasis management include systemic, phototherapy, and topical. Of these, phototherapy (PUVA and UVB irradiation) involves high costs and is not suitable for long-term application [30, 31]. The systemic and topical therapies have been a vital part of the psoriasis treatment regimen. Even if topical therapeutic strategies are supportive in relieving the psoriatic symptoms, still systemic therapies are needed in 20% of cases. The concern with practicing systemic therapies (calcineurin inhibitors and immunosuppressive agents) are their extreme levels of adverse effects including nephrotoxicity and hepatotoxicity due to cyclosporine and methotrexate; cancer due to cyclosporine and PUVA and teratogenicity due to retinoids. Additionally, these formulations demonstrate poor targeting to the desired site (deeper dermal layers) with massive distribution to undesired organs, causing toxicity. Further, as a result of high plasma protein binding, the overall levels of the drug reaching the desired site of action is very less. Because of these concerns, the long-term application of this treatment strategy is challenging to continue.

In this aspect, topical therapy based approaches are comparatively safer where drugs are directly delivered to the skin tissue (target site involved in the origination of disease), thus avoiding exposure of the drug to rest body organs [32-38]. Several topical preparations available as over the counter (OTC) products for the management of psoriasis are composed of retinoids, corticosteroids, salicylic acid, anthralin, coal tar and vitamin D analogues but are associated with side effects such as skin irritation, thinning of the skin, and dilated blood vessels and are not suitable from long term point of view [39]. Most of the drug molecules that have been discovered and are being used in pharmaceuticals are hydrophobic in nature, thereby posing challenges in their delivery. However, its conventional preparations (gels, creams, ointments, etc.) exhibit lower efficacy and cause local toxicity (*e.g* ., skin atrophy,

skin infections, stretch marks, and redness) due to rapid loco-regional drug release and systemic toxicity (*e.g* ., suppression of hypothalamic-pituitary-adrenal (HPA axis)) as a result of systemic leaching of drug [40-44]. To avoid these toxicities and improve the efficacy of drugs, new carriers are required that should penetrate deeper into viable epidermis without systemic absorption and release the drug at the local site at a controlled rate over a prolonged period of time in addition for being affordable.

1.5. Nano-carrier based topical drug delivery

Innovative advancement in the formulation field intends to lessen the adverse effects of the conventional therapies, along with enhancing *in vivo* efficacy leading to improvement in the quality of life of patients. The same could be accomplished by restricting the delivered drug to the desired site (only deeper dermal layers) along with negligible exposure to other normal organs. In order to address these challenges, the focus has been switched to nanotherapeutics. There are several reports stating that nano-carriers are preferred for their promising delivery of therapeutic agents to localized skin surface due to their smaller size, greater permeation through biological barriers, better surface properties, high skin deposition, and sustained drug release properties and were able to prove advantageous in treating psoriasis (Figure 1.2) [10, 32, 37, 35]. These nanosystems include both lipidic (vesicular systems, microemulsions or nanoemulsion, solid lipid nanoparticles (SLNs), nanostructure lipid carriers (NLCs) etc.) and polymeric nano-carriers (nanoparticles, micelles, nano-conjugate, dendrimers etc.). These nanosystems offer many benefits over conventional delivery systems, including prolonged drug release profile, protection of the active principle from the destructive bio-environment, lower dose leading to lesser side effects, targeting drug to the active site and altering the dermatokinetic parameters. Both polymeric and lipidic nanoparticles have been extensively reported to offer several advantages; however certain disadvantages are also associated with these systems [45, 32].

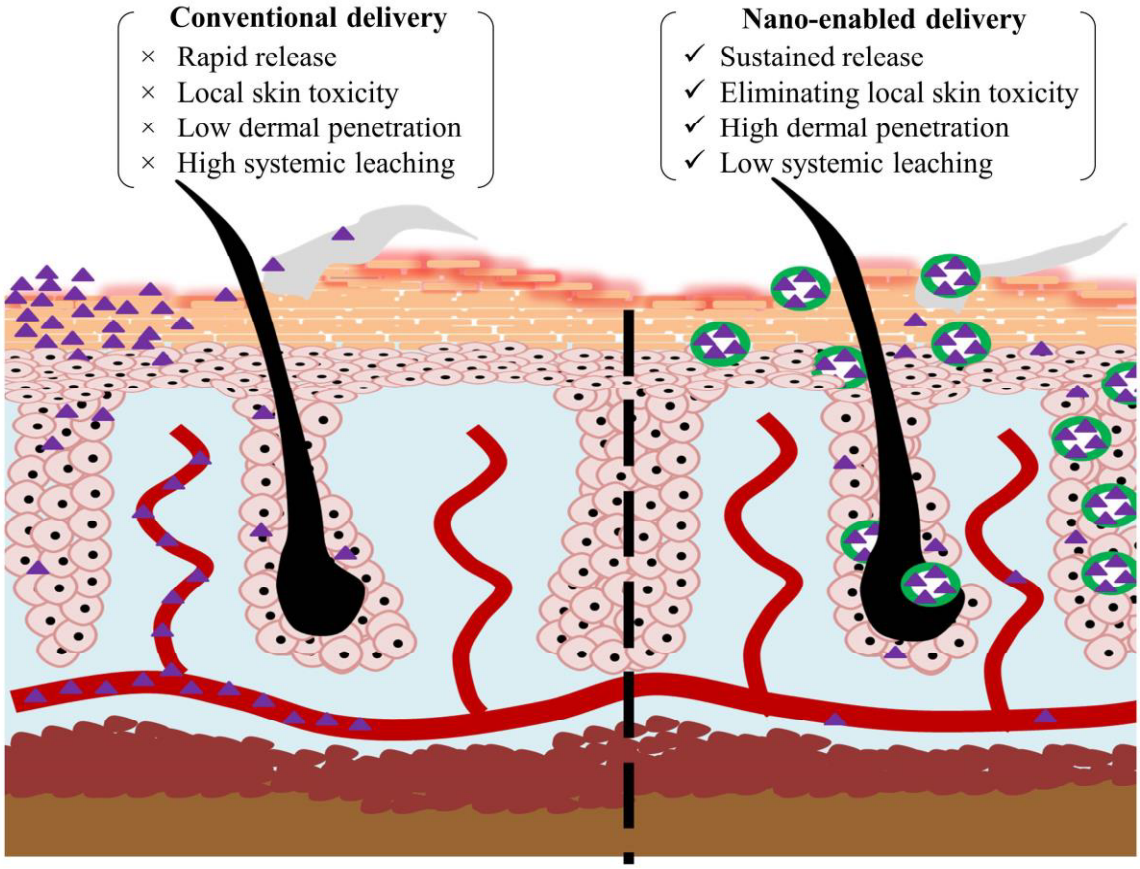


Figure 1.2. Advantages of nano-carrier based drug delivery systems over conventional formulations [10]

1.5.1. Lipidic nano-systems

1.5.1.1. Vesicular systems

Liposomes are key members of this family which are defined as bi-layered vesicular systems composed of cholesterol, phospholipids, and long-chain fatty acids. They can be classified based on their size: (i) large unilamellar vesicles (0.10 mm), ii) multilamellar vesicles (0.05 mm) and (iii) small unilamellar vesicles (0.025–0.05 mm). They offer the ability to deliver both lipophilic and hydrophilic drugs *via* cutaneous route because of the resemblance of their lipid composition with that of skin structure, enabling them to pierce the epidermal barrier without difficulty in comparison to other conventional formulations [46-

48]. For instance, the drug disposition (dermal distribution and systemic absorption) of triamcinolone acetonide was improved significantly when delivered as multilamellar liposomes constructed from cholesterol and dipalmitoyl phosphatidylcholine. The results demonstrated that liposomal formulation delivered a higher drug level at the dermis (~4.8-fold) with lower systemic leaching (~2.1-fold) compared to conventional formulation [49]. In another study, liposomes containing betamethasone dipropionate reduced scaling and erythema accompanying atopic eczema prominently as compared to commercial formulation [50]. It was observed by Knudsen et. al. that calcipotriol loaded liposomes were able to penetrate the skin layers significantly when delivered as a liquid state rather than gel state with the obtained values of 62.5±8.2% and 49.8±9.9%, respectively, concluding that fluidity of vesicular vehicle plays a key role in determining the degree of topical delivery [51]. When lipopolymer named poly(ethylene glycol)-distearoylphosphoethanolamine was incorporated in the liposomal formulation of calcipotriol, had resulted into higher colloidal stability without any interference with skin penetration and drug delivery properties. It was also concluded that the size of this vesicular system is an important factor for determining skin penetration, with small unilamellar vesicles giving better results compared to large multilamellar vesicles [52].

Another member of this class is called as transferosomes that are highly deformable and elastic owing to the presence of an edge activator that destabilizes the bilayer structure of the vesicle. These vesicles are considerably smaller than any other nanosystem and possess a water-filled core enclosed by bilayers formed from surfactants and lipids [46, 53-57]. Deformable liposomes containing MTX was developed using oleic acid (OA) and phosphatidylcholine at 1:2.5 molar ratio respectively, and its comparison was done with two conventional liposomal systems constructed from cholesterol (CH) and PC prepared at various ratios, 1:2 and 1:9 respectively. All the formulations exhibited size 80–140 nm and

narrow PDI desirable for successful topical delivery. It was found that conventional liposomes (PC9:CH1 and PC2:CH1) exhibited higher entrapment efficiency, drug loading and colloidal stability compared to deformable liposomes (PC2.5:OA1) whereas, deformable liposomes resulted into deeper skin penetration of MTX compared to conventional liposomes, which was due to elastic and deformable properties of OA and in addition to this, OA is reported to have good penetration enhancing action [58]. In another report Gizaway et. al. developed transferosomes loaded with betamethasone di-propionate by thin film hydration technique that showed good physical (drug release and colloidal stability) and chemical stability (drug content) [59].

Ethosomes are another reformation of the liposomal system (sizes 30 nm- few µm) that contains higher quantities of alcohol that results in enhancement of drug delivery to deeper skin layers by increasing its permeation by opening up new channels through skin and are similar to liposomes in delivering both hydrophilic and lipophilic drug molecules. Zhang et al. developed both psoralen loaded conventional liposomes (using Lipoid S 100) and ethosomes (using Lipoid S 100 and varying amounts of ethanol) and found that the skin penetration of active was enhanced in the case of ethosomes (most optimal formulation was found to be the one with 40% v/v of ethanol and 5% w/v of Lipid S 100) that could be supportive in increasing the efficiency of PUVA therapy and decreasing the toxicity [60].

Further, the features of both ethosomes and transferosomes were combined to develop transethosomes for enhancing the drug delivery to the deeper dermal layer and are prepared from ethanol, phospholipid, edge activator, and water [46, 61, 62]. Niosomes are biodegradable and biocompatible vesicular systems formulated using non-ionic surfactants along with cholesterol and/or lipids and offer numerous benefits over liposomes as they are stable and osmotically active. Aggarwal et al. formulated niosomes loaded with tazarotene by

thin-film hydration method that was subsequently incorporated in carbopol 940 based topical gel. This niosomal gel (NMG5) displayed better skin retention of the drug with greater values of local accumulation efficiency and reduced cumulative permeated amount in comparison to marketed product and plain gel [63]. In another study, niosomal formulation containing MTX was developed by thin-film hydration method using Design-Expert® software. These niosomes exhibited improved $AUC_{0–10}$ (1.15 mg. h/cm^2) and dermal distribution (22.45%) in comparison to drug solution (0.49 mg.h/cm^2 and 13.87%, respectively) [64].

Further, the advancement of these vesicular systems by doping ceramide onto conventional vesicular system resulted in cerosomes used for achieving localized delivery of tazarotene with good skin deposition leading to clinical effectivity in treating psoriatic patients in comparison to Acnitaz® gel (marketed formulation). The inclusion of ceramide into the vesicular nanosystem imparted several better properties in comparison to vesicles without ceramides such as slower drug release, higher drug loading, and greater skin deposition, which was attributed to the interaction of keratin in the skin with ceramide leading to fusion and localized drug retention [65].

1.5.1.2. Nanoemulsion

These are dispersed systems wherein the oil phase is dispersed in the aqueous phase (o/w type) and globules are within the nano-range (20-500 nm), additionally stabilized by surfactants [66, 67]. Commonly used corticosteroid i.e. clobetasol propionate was formulated into nanoemulsion by aqueous phase titration method using tween 20, eucalyptus oil, distilled water and ethanol as the surfactant, oil phase, aqueous phase and co-surfactant, respectively. Further, *in vivo* results in Wistar rats suggested that the nanoemulsion significantly inhibited edema up to 84.15% at 12 h compared to the Glevate cream (marketed product) that suppressed edema up to 40.99% at 12 h [68]. In another study, nanoemulsion of calcipotriol

was developed using oil phase (sunflower oil) and emulsifier (exopolysaccharides) derived from *Bacillus amyloliquefaciens* that efficiently ameliorated psoriatic inflammation as a result of greater deposition of the drug at target site supplemented with reduced side effects suggesting that exopolysaccharides served as medical material for dermatological application [69]. Furthermore, clobetasol propionate was co-delivered with calcipotriol using nanoemulsion that exhibited controlled drug release with substantial improvement at *in vitro, ex vivo* and *in vivo* levels as compared to the Betagel® (marketed product) [70]. Moreover, the fashion of merging drug with excipients having functional activity for getting synergistic anti-psoriatic efficacy endeavored wherein nanoemulsion loaded with tacrolimus was prepared using Kalonji oil (functional excipient) led to a synergistic anti-psoriatic effect. The obtained nanoemulsion displayed a sustained and biphasic drug release profile with better local accumulation (4.33-folds), negligible systemic delivery with significantly enhanced *in vivo* efficacy in comparison to conventional marketed product (Tacroz). Such sort of strategy has opened a new area in exploring functional excipient for dermatological management using nanotechnology based approach [71]. Further, TPGS based microemulsion (ME) containing tacrolimus showed substantial enhancement in topical drug delivery followed by improved anti-psoriatic efficacy in comparison to Protopic® (commercial ointment). In addition to this, TPGS ME vehicle employed modest anti-psoriatic activity apparent from the *in vitro* and *in vivo* data signifying its key role as a potential adjuvant that, together with the drug, leads to synergistic effect for psoriasis management [72].

1.5.1.3. Solid lipid nanoparticles and nanostructured lipid carriers

These lipidic nano-carriers had aroused as a smart substitute to the earlier conferred vesicular systems and emulsions as a result of enhanced control over the drug release profile and delivery portion. When the dispersed oil globules of nanoemulsion are replaced completely by solid lipids, then the resulting dispersion is called solid lipid nanoparticles

(SLNs), and similarly, these too are stabilized by surfactants exhibiting size (10 to 1000 nm) with spherical geometry. The lipid from the central hydrophobic core is capable of solubilizing added hydrophobic drug molecules. There are several established mechanisms explaining enhanced skin permeability of these nanosystems such as skin hydration, increased drug residence time and the interaction between lipidic components of these systems with stratum corneum [73-77]. When the dispersed solid lipid portion of SLNs is replaced partly by liquid lipid (oil) then the resulting nano-carriers are called nanostructured lipid carriers (NLCs) [78]. NLCs offer several advantages over SLNs, such as better drug loading and stability due to the presence of oil that reduces the crystallinity of the core matrix and imparts fluidity. Even these upgraded lipid nanosystems improve residence time similar to that of SLNs and have an inverse relation with the particle size [79-83].

Zhang et al. improved the topical delivery of betamethasone 17-valerate (BMV) by formulating it into SLNs and concluded that the composition of lipid matrix was very important for delivering the steroidal molecule specifically to the epidermal layers along with minimal systemic leaching. For this, they had compared two SLNs: monostearin SLNs and beeswax SLNs with no significant difference between the size, PDI and charge and found that percutaneous flux was higher [J = 0.3970 ± 0.0372 μg/ (cm^2 h)] for beeswax SLNs in comparison to monostearin SLNs [J = 0.1547± 0.009 μg/(cm^2 h)] whereas the portion of drug delivered to epidermal layers was comparatively higher for monostearin SLNs keeping BMV lotion and suspension as control [42]. Similarly, systemic delivery and skin deposition of mometasone furoate was improved when the drug was delivered as SLNs (prepared from Tefose-63 and glyceryl monostearate) that further exhibited sustained drug release profile compared to its conventional forms [84]. It is clear from the previous two research attempts that the lipid core composed of glyceryl monostearate demonstrated improved skin permeation of hydrophobic drug molecules.

Dermal drug distribution (deeper skin layers) and systemic leaching of fluocinolone acetonide and triamcinolone acetonide were improved by delivering it as NLCs [85, 86]. Further, the local toxicity associated with tazarotene was reduced when delivered as both SLNs and NLCs as a result of sustained drug release along with enhanced skin retention [87]. A clinical study demonstrated that NLCs loaded with acitretin exhibited sustained drug release profile and higher skin deposition (1.72-folds) compared to plain Act gel leading to significantly reduced skin irritation potential and scores for clinical psoriasis area and severity index (PASI) [88]. In another study, poor skin permeability associated with all-trans retinoic acid improved when delivered as SLNs and NLCs that displayed controlled drug release [89]. Ridolfi et al. compared two types of SLNs: i) with chitosan (SLN-chitosan-TRE) and ii) without chitosan (SLN-TRE), which were loaded with tretinoin (TRE) in order to address challenges of local side effects improve the stability of the active. They observed that the inclusion of chitosan was beneficial along with imparting additional antibacterial activity and was non-cytotoxic towards HaCaT cells [90].

Comparative evaluation amongst various nanosystems, including liposomes (LPs), nanoemulsions (NEs) and solid lipid nanoparticles (SLNs) loaded with retinyl palmitate (RP) were carried out. It was concluded that NEs exhibited highest flux (0.37 ± 0.12 µg/h) in comparison to SLNs (0.10 ± 0.05 µg/h) and LPs (0.15 ± 0.09 µg/h) apparent from *ex vivo* skin permeation studies whereas, SLNs imparted greatest photoprotection (~8%) compared to NEs (~21%), LPs (~15%) and free RP solution (~45%) [91]. Tripathi et al. demonstrated that methotrexate-loaded NLCs were better compared to SLNs in terms of entrapment efficiency and skin deposition [92]. In another study, it was found that SLNs were promising nano-carriers compared to microemulsions in enhancing the stability of anthralin [93]. Further, the washability (reducing staining potential) and efficacy (lowered PASI score, lower expression

of IL-22, IL-17, and IL-23) of dithranol were improved after loading it in NLCs in comparison to dithranol ointment [94].

Thermosensitive SLNs loaded with tacrolimus were fabricated that demonstrated improved dermal distribution (deeper dermal layers) and reduced skin irritation or erythema in comparison to reference product however suffered from the major drawback of drug leakage [95]. Further, several tacrolimus loaded nano-carriers such as solid lipid nanoparticles (Tac-SLN), liquid crystalline nanoparticles (Tac-LCNP), liposomes (Tac-liposomes) and nanostructured lipid carriers (Tac-NLC) were compared with Tacroz™ Forte (marketed product) and free Tac loaded gel. The obtained values of dermal bioavailability for the above-mentioned formulations i.e., Tac-NLC, Tac-LCNP, Tac-SLN, and Tac-liposomes were 12.5, 14, 11.5, and 3.7-folds higher, compared to free Tac loaded gel and ~2, 2.5, and 2-folds enhancement in dermal bioavailability, respectively compared to Tacroz™ Forte. Tac-liposomes demonstrated lower dermal bioavailability compared to marketed product. But, *in-vivo* efficacy data proved substantial enhancement in treatment in groups treated with Tac-SLN and Tac-NLC, which was attributed to the deposition of high Tac level in the deeper skin layers, which is the target region for psoriasis treatment compared to other formulations [96].

Furthermore, the squalene, one of the constituents of sebum, was used to formulated another type of lipidic nano-carrier named squarticles for their excellent properties to target the deeper dermal layers with insignificant systemic leaching [97]. Dadwal et al. showed improved *in vivo* anti-psoriatic efficacy (skin permeation) of clobetasol propionate after delivering it as squarticles in comparison to marketed product (Topinate gel) [98]. In another study, methotrexate and calcipotriol were successfully co-delivered by the topical route *via* NLCs consisting of squalene and Precirol ATO 5 [99].

Lipidic nano-systems, including vesicular systems, microemulsions or nanoemulsion, solid lipid nanoparticles (SLNs) and nanostructure lipid carriers (NLCs) offer advantages such as lower cost of manufacturing, higher encapsulation efficiencies, less number of steps that are involved, and no toxicity issues. These are too associated with limitations including burst release, limited opportunities for chemical modifications, instability and high polydispersity index, drug partitioning, drug expulsion, etc. [45, 100, 101]. Table 1.1 demonstrated various lipidic nanosystems explored for the treatment of psoriasis.

Table 1.1. List of lipidic nanosystems explored topically for psoriasis management [10]

Carrier	**Therapeutic agent**	**Benefits**
Liposomes	Betamethasone di-propionate, Triamcinolone acetonide, Calcipotriol, Psoralen, Retinyl palmitate, Bexarotene, Methotrexate, Tacrolimus, Cyclosporine A	-Higher skin permeation -Lower systemic leaching -Lower skin toxicity -Enhanced anti-psoriatic efficacy
Ethosomes	Psoralen	- Enhanced efficacy - Higher skin deposition and permeation - Lower toxicity
Transferosomes	Betamethasone di-propionate	-Improved stability -Enhanced efficacy and safety -Better tolerance
Cerosomes	Tazarotene	-Improved anti-psoriatic efficacy at clinical level
Niosomes	Methotrexate, Tazarotene	-Better skin permeation -Scalable process with greater drug loading -Stability
Nanosomes	Acitretin	-Improved drug deposition into viable skin layers -Superior skin permeation -Improved skin tolerability -Greater anti-psoriatic activity
Microemulsion and Nanoemuslion	Calcipotriol, Clobetasol propionate, Cyclosporine A, Retinyl palmitate, Tacrolimus	-Sustained drug release with better percutaneous absorption -Lower skin irritation -Greater anti-psoriatic efficacy
Solid lipid nanoparticles (SLNs)	Mometasone furoate, Betamethasone 17-valerate, 8-methoxsalen, Tretinoin,	-Reduced degradation of drug -Sustained release profile -Reduced systemic toxicityand

	Cyclosporine A, Methotrexate, Tacrolimus, Anthralin, calcipotriol, Betamethasone dipropionate	better dermal drug delivery -Lower skin irritation -Enhanced in vivo anti-psoriatic efficacy
Squarticles	Clobetasol propionate	-Enhanced in vivo anti-psoriatic efficacy due to higher skin permeation
Nanostructured lipid carriers (NLCs)	Triamcinolone acetonide, Fluocinolone acetonide, All-trans retinoic acid, Acitretin, Methotrexate, Tazarotene, Calcipotriol, Anthralin, cyclosporine	-Sustained-release kinetics -Higher dermal retention and permeation -Higher stability -Lower toxicity and skin irritation -Enhanced anti-psoriatic activity
Liposphere	Curcumin, Tacrolimus	-Enhanced efficacy -Controlled release profile -Better skin penetration
Liquid crystalline nanoparticles	Tacrolimus	-Better skin penetration and sustained drug release profile

1.5.2. Polymeric nano-systems

1.5.2.1. Polymeric nanoparticles

These are formulated using several biocompatible and biodegradable polymers of which poly(ε-caprolactone) (PCL) and poly(lactic-co-glycolic acid) (PLGA) are extensively studied because of their numerous benefits and are regarded as GRAS. Tang et al. fabricated sustained-release cyclosporine A (CsA)-loaded poly (ethylene glycol)-*b*-poly(d,l-lactide-co-glycolide) (PEG-PLGA) nanoparticles that demonstrated enhanced safety and efficacy profile with IC_{50} values of 35 ng/mL (compared to free drug, 30 ng/mL) showing marked suppression of both inflammatory cytokine and T cell proliferation [102]. Further, CsA deposition in the dermal layer and stratum corneum were 4.85 and 6.6 folds higher when delivered as PLGA nanoparticles in comparison to free drug along with eliminating systemic leaching [103]. Additionally, skin permeation and drug release of CsA were studied and compared amongst PLGA nanoparticles and nanoparticles fabricated from PLGA-PEG-

PLGA (triblock copolymer) wherein both nanosystems possessed approximately similar size and PDI of ~30 nm and ~0.2 respectively. It was found that nanoparticles developed from triblock copolymer showed faster drug release (>80%, 1 day) and deeper skin permeation compared to PLGA nanoparticles that showed sustained drug release profile (39.6% at 1 day and >90% at 4 day) which was attributed to a greater degree of hydration in case of triblock copolymer than di-block copolymer [104].

In order to address the problems of rapid degradation and toxicity linked with psoralens, these nanosystems can function as an promising strategy [105]. PLGA nanoparticles encapsulated with benzopsoralen (BP) were fabricated for targeting the powerhouse of the target cells, including neutrophils, eosinophils, and macrophages, and induced apoptosis strongly resulting in enhanced therapeutic efficacy when coupled with UV treatment [106]. In another study, BP PLGA nanoparticles specifically targeting vascular endothelial cells (ECs) were developed which, when combined with UV treatment, can be used for treating psoriasis [105].

Even the nanoparticles designed from poly-Ɛ-caprolactone (PCL) demonstrated enhanced skin permeation followed by improved efficacy and simultaneous reduction of toxicity profile [107]. Poor solubility associated with anthralin was addressed by delivering it in the form of PEG-b-PCL nanoparticles that has resulted in its 120 times rise in water solubility of drug with additional sustained drug release profile [108]. In another study, side effects and systemic leaching of hydrocortisone acetate were minimized by delivering it as PCL nanoparticles possessing size (190 to 230 nm), zeta potential (−3 to −5 mV), and EE (~62%) [109].

The benefits of using cationic nanoparticles for topical drug delivery have been previously reported, which was due to the presence of positive charge on nanoparticles that

interacts with skin tissue bearing negative charge. Retinol was formulated into cationic nanoparticles composed of Eudragit RS 100 that eliminated the degradation of the drug and improved the stability for up to 2 months along with substantially enhanced drug residence time [110]. In another attempt, skin retention of CsA was enhanced by incorporating protamine in nanomaterial matrix and could be efficaciously used for delivering the drug by transdermal route [111]. Further, a substantially higher level of clobetasol propionate in the epidermis was achieved by delivering it as lecithin/chitosan cationic nanoparticles that possessed higher entrapment (92.2%) in comparison to commercial cream and plain chitosan gel, which is highly desirable for topical therapy using steroids [35].

Lately, CsA was loaded in PLGA (50:50 ratio of each monomer) nanocapsules embedded in a silicone-based polymer that serves as a platform for topical delivery for effective management of dermal inflammation [112]. Aggarwal et al. designed nanosponges loaded with tazarotene by emulsion solvent evaporation method that showed lower systemic delivery and improved localized skin retention efficiency in comparison to the marketed formulation and plain gel [63]. The dermal bioavailability of cholecalciferol was significantly improved by Ramezanli et al. by formulating it into TyroSpheres (nanospheres) that possessed both hydrophobic and hydrophilic segments from desaminotyrosyl alkyl ester and PEG that imparted self-assembling features and possessed size and PDI of 60–70 nm and 0.19, respectively. These nanosystems were able to prevent drug degradation (both photo-degradation and hydrolysis) and were able to distribute the drug to deeper dermal layers (desired site for psoriasis treatment) with no cytotoxicity demonstrated in HaCaT cells [113].

1.5.2.2. Micelles

These are self-assembled nanostructures (ranging from 10-100 nm in size) with excellent colloidal stability, formed by the aggregation of amphiphilic copolymers above

CMC and exhibit higher cutaneous deposition (deeper dermal layers), resulting in their tremendous application in topical drug delivery [46]. Poor skin permeability of tacrolimus leading to reduced anti-psoriatic efficacy was addressed successfully by delivering it via polymeric micelles fabricated using methoxy poly(ethylene glycol)-dihexyl substituted polylactide di-block copolymer that showed specific delivery of the drug to deeper skin layers [114]. Jin et al. developed core-shell type self-assembled micelles wherein zinc phthalocyanine was conjugated with PEG chain of Brij 58 exhibiting smaller size and zeta potential of 25 nm and −15 mV, respectively. These micelles demonstrated an almost complete cure of psoriatic inflammation in the guinea pig psoriasis model when supplemented with UV treatment thoroughly backed by histopathological data and can be used as a promising approach for psoriasis management [115].

1.5.2.3. Nano-conjugates

These are nano-ranged conjugates of drugs with polymer or peptides involving pH or enzyme-sensitive cleavable functional groups demonstrating reduced clinical side effects and improved *in vivo* efficacy. The systemic toxicity and *in vivo* efficacy of fluocinolone acetonide was improved by delivering it as polypeptide (poly-L-glutamic acid (PGA)) self-assembled nano-conjugate exhibiting a size of 50-100 nm. PGA was selected for preparing conjugate as it possessed several carboxylic acid groups on its structural backbone, offering sites for conjugation reaction in addition to its ability to permeate and pierce biological barriers. The resulting conjugate did not hinder the anti-inflammatory action of active rather substantially improved it. This was apparent after a 1.4-fold and 8.5-fold reduction in tissue levels of IL-23 and INF-γ compared to a pure drug resulting in significantly enhanced *in vivo* anti-psoriatic efficacy [116]. Similarly, cutaneous irritation associated with retinal was eliminated by administering it as retinal grafted self-assembled chitosan nanoparticles, which were called as proretinal nanoparticles (PRN) [117]. The dermal irritation or local dermal

toxicity associated with methotrexate was eliminated by delivering it as nano-conjugate using water-soluble star-shaped polymethacrylic copolymer that was attributed to the slower drug release profile preventing sudden loco-regional exposure of skin to higher drug levels [118]. Another attempt was made wherein 5-aminolaevulinic acid was conjugated by Fmoc chemistry (solid-phase peptide synthesis strategy) to cell-penetrating peptide (Penetratin). It was demonstrated that the peptide conjugation has not hampered the pharmacological conversion of the drug to protoporphyrin IX (photosensitizer) [119]. The disadvantage of these systems is that they are applicable to only those drug molecules or polymers that have reactive functional groups such as carboxylic, hydroxyl, amine, or sulfhydryl that can undergo coupling reactions.

1.5.2.4. Dendrimers

Dendrimers are nano-sized, 3D globular, macromolecular hyperbranched structures composed of a central core enclosed by numerous concentric shells called generations which consisted of branching units with several free functional groups at terminal endings. Therapeutic drug molecules can be loaded within the hydrophobic void spaces enclosed by branching units within the concentric shells or can be covalently bound to the peripheral functionalities. They catch several applications in enhancing the permeation of numerous hydrophobic drug molecules across the skin and exhibit numerous benefits compared to other polymeric nano-carriers for topical delivery [120-122, 46]. Skin penetration of anthralin was significantly improved from 2.72±0.31 to 11.61±1.80 mg/cm^2/h after delivering it *via* fifth-generation polypropylene imine (PPI) dendrimers compared to its free solution besides reducing 2.3 folds of primary irritation index [123]. In another attempt, the skin permeation of 8-methoxypsoralene was accelerated by delivering as G3.5 and G2.5 poly(amido amine) dendrimers complex that can aid as a promising approach for several dermatological treatments [124].

However, these systems exhibit lower encapsulation efficiencies and higher burst release compared to other polymeric systems. Attempts to improve entrapment efficiency and burst release from these systems were made by structural alterations of these hyperbranched structures. For this, the model drug used was methotrexate (MTX) and dendrimer used was polyester-co-polyether (PEPE). It was demonstrated that switching dendritic systems on to higher generation side with higher aromatic rings was able to improve the encapsulation efficiency and reduce the burst effect as a result of larger void spaces [125]. Despite these improvements, the encapsulation efficiency was still on the lower side.

Even though the above-mentioned polymeric nano-carriers offer several advantages such as smaller particle size with a narrow size distribution, possible chemical modifications over the surface, prolonged drug release, and stability, but at the same time, these are associated with disadvantages such as lower drug entrapment, multiple steps that are involved in the preparation method, use of large quantities of organic solvents, scalability and cost of manufacturing [45, 100, 101]. Table 1.2 demonstrated various polymeric nanosystems explored for the treatment of psoriasis.

Table 1.2. List of polymeric nanosystems explored topically for psoriasis management [10]

Carrier	**Therapeutic agents**	**Benefits**
Nanoparticle (Matrix type)	Hydrocortisone acetate, Betamethasone-21-acetate, Cyclosporine A, Benzopsoralen	-Controlled release with better stability -Lower systemic toxicity with enhanced skin permeability
Nanosponge	Tazarotene	-Better skin permeation -Scalable process with higher drug loading -Stability
Nanocapsule	Cyclosporine A	-Enhanced efficacy
Micelle	Tacrolimus	-Lack of transdermal penetration -Improved TAC deposition in deeper dermal layers -Lower systemic leaching and associated side effects

Nanospheres (TyroSpheres)	Cholecalciferol	-Sustained drug release -Higher epidermal penetration with better dermal bioavailability -Protection against photodegradation and hydrolysis resulting into higher stability
Nanogel	Acitretin	-Enhanced anti-psoriatic action -Better drug permeation and dermal retention -Reduced skin irritation and/or systemic adverse effects

1.5.3. Lipid-polymer hybrid nanoparticles

In order to get the benefits of both systems, lipid-polymer hybrid nanosystems have been developed. This newer class of nano-carriers combines advantages of both lipidic and polymeric nano-carriers such as good drug loading capacities, a more controlled drug release, improved cellular uptake and biocompatibility, avoiding the disadvantages associated with them. Based upon the structure of these nanosystems, they are categorized into a. monolithic, b. core-shell, c. biomimetic lipid-polymer nanoparticles and d. polymer-caged liposomes [45, 100, 101]. Monolithic systems are the ones where the polymeric matrix contains uniformly distributed lipids that together form a core where hydrophobic drug molecules could be loaded [126]. Core- shell type hybrid systems are made of polymeric core upon which lipids are arranged in layers forming lipid shells around the central polymeric core. These types of systems are also modified such that there is a central hollow core formed by using lipids with surrounding polymeric shell followed by a lipid shell [127-129]. Biomimetic lipid-polymer nanoparticles are made by coating the RBC's membrane onto the polymeric core and are sometimes also referred to as erythrocyte membrane-camouflaged polymeric nanoparticles [130, 131]. Polymer-caged liposomes are formed by anchoring polymers on the surface of liposomes [132]. Their pharmaceutical applications apart from drug delivery include vaccine adjuvants (for boosting immune response), gene delivery (DNA (pLuc, pEGFP-N2), SiRNA

delivery (anti-GFP, anti-Luc and KIF11)), and diagnostic agent delivery. These characteristics of lipid-polymer hybrid [117] nanoparticles have encouraged their applications in the delivery of chemotherapeutics, proteins, peptides and vaccines [100, 133, 101].

Cai et al. formulated amoxicillin-loaded monolithic lipid-polymer hybrid nanoparticles around 200 nm comprising of pectin sulfate, phospholipids and rhamnolipid that demonstrated complete and sustained drug release and proved effective in eradicating H. pylori in the biofilm form [134]. Further, Zhao et al. co-delivered Hypoxia-inducible factor 1α (HIF1 α) siRNA and gemcitabine using a biocompatible polymer-core lipid shell type of hybrid system for effective pancreatic cancer treatment. These hybrid systems were fabricated using ε-Poly-lysine, mPEG-PLGA and PEGylated lipid bilayer using double emulsion method where cationic ε-Poly-lysine effectively complexes negatively charged si-HIF1 α on the surface and encapsulate gemcitabine in the hydrophilic core and PEGylated lipid bilayer coated on the outer surface prevents aggregation of nanoparticles and improve the serum stability by preventing the degradation of si-RNA. These hybrid system demonstrated excellent capability to suppress tumor metastasis in orthotopic tumor model [135]. In another research work authors had designed a hollow core lipid–polymer–lipid systems by modified double emulsion solvent evaporation technique that encapsulated siRNA within the innermost hollow core lined with layer of cationic lipid. The layer present immediately above this cationic layer is composed of PLGA that loaded water insoluble drugs imparting sustained drug release profile. The outermost neutral PEG layer (from PEGylated lipid) present above the PLGA layer, provided stealth effect [136]. Biomimetic lipid-polymer hybrid nanoparticles has also grabbed enormous attention in designing nanosystems with significantly improved residence time in vivo. Hu et al. designed RBC membrane-camouflaged PLGA nanoparticles with the particle size 130nm and zeta potential -12.7 mV. These biomimetic lipid-polymer hybrid

nanoparticles proved to be more efficacious ($t_{1/2}$: 39.6 h) than PEG nanoparticles ($t_{1/2}$:15.8 h) in improving residence time in vivo [137]. Aoki et al. developed a multimodal thermoactivatable polymer-grafted liposome (polymer-caged liposomes) with 123± 11 nm diameter, which offered increased antitumor therapeutic effect by remaining stable for 8 hours after administration and MRI imaging capability for localized tumor [138].

1.5.4. Scale-up of lipid-polymer hybrid nanoparticles

Within the class of lipid-polymer hybrid nanoparticles, monolithic system is simple and scalable nevertheless could accomplish maximum advantages of a hybrid system. Most of the researchers had used nanoprecipitation and emulsion solvent evaporation techniques for preparing these hybrid systems, which finds difficulty during scale-up owing to the numerous critical steps involved with the complex formulation process imposing challenges for industrial applications. Further, the reported methods for preparing these hybrid nanosystems are restricted to lab-scale due to the equipment's used i.e. probe sonicator and high shear homogenizer that are lacking scalability. There is still an unmet requirement to prepare novel lipid-polymer hybrid nanoparticles exhibiting higher entrapment of hydrophobic drugs formulated using biocompatible excipients having minimal toxicity with prolonged drug release profile, improved stability with negligible burst release, deterrence of drug partitioning, leakage and expulsion effect.

High pressure homogenization is profitable for preparing nano-formulations from the industry standpoint as the process is easily scalable from lab level to commercial level [139]. There is sound evidence that the high-pressure homogenizer is more efficient compared to a high shear homogenizer and ultrasonicator, which could be credited to the mechanism of size reduction i.e. impact forces apart from cavitation and shear stress, respectively [140]. In high pressure homogenization, extensional force (shear stress) is the major leading force

responsible for size reduction with some additional input from cavitation and impact forces. When the coarse dispersion is forced under pressure through a narrow gap at very high velocity, droplets are first elongated in the inlet area followed by disintegration in the turbulent and cavitating flow in the discharge area and are finally impacted on the inner chamber wall. When the dispersion enters the discharge area, there is a large pressure drop resulting into formation of vapor bubbles (cavities) in liquid dispersion that further collapse generating shock waves which produces energy required for breaking droplets [141, 142].

1.6. Gaps in existing research

Even though it is well established that topical therapy is a more promising strategy compared to phototherapy and systemic approaches that harness the advantages of directly delivering therapeutic agents to the site of action, eliminating undesired distribution of therapeutic agents to undesired body organs that ultimately lead to toxicity issues. The challenge that come across topical drug delivery is poor permeation across biological skin layers of which stratum corneum imparts major barrier functions. Nanoparticles-based strategies offer several advantages over conventional formulations (ointments, creams and gels) in improving skin permeation, lowering of dose, better efficacy, lower side effects and reduce systemic leaching of topically applied drug molecules. Nano-carriers reported in the literature are majorly divided into two types; lipidic and polymeric. Even though both of these types offer several advantages over conventional systems however they are associated with certain disadvantages as well. Lipidic nanosystems are associated with limitations, including burst release, limited opportunities for chemical modifications, instability and high polydispersity index, drug partitioning, drug expulsion, etc whereas the above-mentioned polymeric nano-carriers exhibits disadvantages such as lower drug entrapment, multiple steps that are involved in the preparation method, scalability and cost of manufacturing. So there is a requirement of a carrier that combines the

advantages of these both types of systems with simultaneously eliminating their disadvantages. In order to get the benefits of both systems, lipid-polymer hybrid nanosystems have been developed. This newer class of nano-carriers combines advantages of both lipidic and polymeric nano-carriers such as good drug loading capacities, a more controlled drug release, improved cellular uptake and biocompatibility, avoiding the disadvantages associated with them. Within the class of lipid-polymer hybrid nanoparticles, a monolithic system is simple and scalable nevertheless could accomplish maximum advantages of a hybrid system. Most of the researchers had used nanoprecipitation and emulsion solvent evaporation techniques for preparing these hybrid systems, which finds difficulty during scale-up owing to the numerous critical steps involved with the complex formulation process imposing challenges for industrial applications. Further, the reported methods for preparing these hybrid nanosystems are restricted to lab-scale due to the equipment's used i.e., probe sonicator and high shear homogenizer that are lacking scalability. There is still an unmet requirement to prepare lipid-polymer hybrid nanoparticles exhibiting higher entrapment of hydrophobic drugs formulated using biocompatible excipients having minimal toxicity with prolonged drug release profile, improved stability with negligible burst release, deterrence of drug partitioning, leakage, and expulsion effect. High-pressure homogenization is profitable for preparing nano-formulations from the industry standpoint as the process is easily scalable from lab level to a commercial level.

1.7. Outline of current research work

The current thesis work is directed to fabricate and assess platform technology comprising of monolithic lipid-polymer hybrid nanoparticles for topical delivery of hydrophobic drug molecules for effective treatment of psoriasis. These are prepared using a

combination of an amphiphilic di-block copolymer, solid lipid, liquid lipid, and surfactant. The central core of these nanoparticles is made up of the hydrophobic block of polymer and lipids (both solid and liquid), whereas the hydrophilic block of the polymer forms a hydrophilic shell around the core of nanoparticles responsible for stabilization. In order to assess the applicability of the lipid-polymer hybrid system, drugs including clobetasol propionate, cholecalciferol and coenzyme Q10 were loaded individually within this core. Further, the process of preparing these hybrid nanosystems is less complex involving fewer critical steps making it industrially viable. These novel monolithic lipid-polymer hybrid nanoparticles offer several advantages such as higher entrapment efficiencies, sustained drug release profile, higher storage stability without any drug expulsion, better cellular uptake, deeper skin penetration with higher retention, no systemic leaching with and improved *in vivo* anti-psoriatic efficacy in imiquimod-induced psoriasis-like skin inflammation in Swiss albino mice.

1.8. Objectives of the research work

To acheive the aim of the thesis, the following objectives have been designed that were divided into different chapters focusing on each objective.

Objective 1. Bioanalytical method development and validation for the analysis of clobetasol propionate, cholecalciferol and coenzyme Q10

Objective 2. Development and evaluation of clobetasol propionate loaded lipid-polymer hybrid nanoparticles

Objective 3. Development and evaluation of cholecalciferol loaded lipid-polymer hybrid nanoparticles

Objective 4. Development and evaluation of coenzyme Q10 loaded lipid-polymer hybrid nanoparticles

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