Chapter - 1
Introduction

1. Skin

Skin serves as an outermost barrier protecting the body from the external environment. It is the largest organ of the body, preventing the entry of external pathogens and chemicals. This goes in hand with its numerous functions, including vitamin D metabolism, perception as a sense organ, and the main site for topical drug delivery [1].

The skin is mainly composed of stratified squamous keratinized epithelium cells. The three main layers include the outermost epidermis, the middle dermis, and the innermost hypodermis. The epidermis is devoid of blood vessels, and therefore essential nutrients have to diffuse across the dermal-epidermal junction. The five sub-layers of the epidermis include the stratum corneum (SC), stratum lucidum, stratum granulosum, stratum spinosum, and stratum germinativum. The arrangement of these individual layers is from the outside to the inside. The viable epidermis is the epidermis without the SC.

The epidermis is in direct contact with the outside environment, responsible for its primary purpose as a barrier. Therefore, it restricts pathogens and drugs from entering and prevents the loss of water from the body. The SC has a thickness of 15-20 µm consisting of 10-20 layers of corneocytes lodged in a lamellar lipid bilayer structure composed of desmosome-linked epithelial cells. This distinctive disposition helps to prevent the entry of molecules larger than 500 Da. On the other hand, the viable epidermis is made up of Langerhans cells, Merkel cells, and keratinocytes at different phases of differentiation [2]. Melanin synthesis is also a significant function of the viable epidermis, apart from immunology and sensory perception. Percutaneous transport of drugs through lipophilic skin is possible due to phospholipids, cholesterol, and esters [3–5]. The anatomy of the skin is illustrated in **Figure 1.1.**

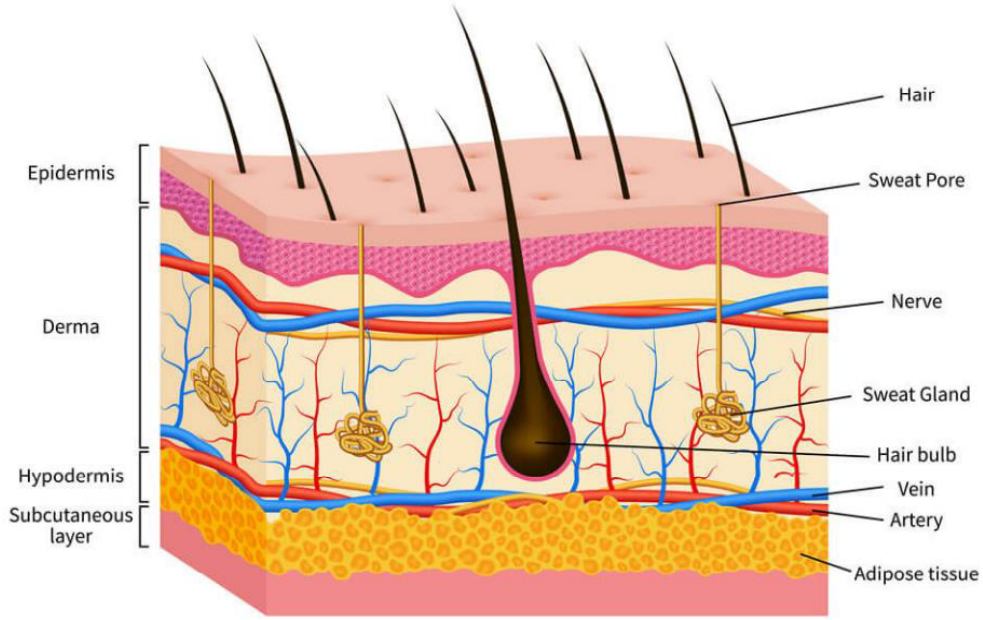


Figure 1.1. Anatomy of the skin.

Image source: https://www.thedermspecs.com/blog/skin-anatomy-101/

SC has the presence of mobile, continually renewable outer layers. They assist in removing cancerous cells, particulate matter of a solid nature, and any foreign bodies [6]. The dermis is composed of collagen fibrils which play a role in its flexible nature and mechanical support. The dermis serves as an important location for fluid and cellular exchange between the blood, lymph, and skin. It is primarily made up of fibroblasts that create connective tissue constituents, melanocytes, nerves, and mast cells. Below the dermis is the inner subcutaneous tissue referred to as the hypodermis, which acts as an energy deposit. Here the adipocytes held together by the collagen fibers provide a protective mechanical pillow for the human body. The dermis also serves as a connecting link for the skin to the blood vessels and nerves. There is also various appendages impaling the skin, such as hair follicles, sweat and sebaceous glands arising from the dermis [7]. The skin also helps in immunology through the antigen-presenting cells, i.e., Langerhans cells and dermal dendritic cells located in the skin. Their association with mast cells, specific T lymphocytes, and keratinocytes is a critical prerequisite for this activity [8].

1.1. Psoriasis

Psoriasis is a chronic autoimmune disorder of the skin characterized by erythematous and scaly plaques with a predisposition for the scalp, extensors of the limbs, lumbosacral area, and genitalia. The psoriatic condition affects approximately 125 million (2-3%) of the world population. Psoriasis can occur in any age group but mainly affects the age group of 15 to 25. It leads to psoriasis arthritis in the age group of 30 to 50. Owing to genetic and environmental factors, the prevalence of psoriasis includes age, gender, geography, and origin. It is highly pervasive in higher latitudes and Caucasians compared to other ethnic categories [8].

Even though it is considered an autoimmune disorder, there is no evidence of autoantigen that is responsible for psoriasis. However, there is evidence for genetic predisposition [9]. Psoriasis involves the skin, nails, and associated problems with several comorbidities. Psoriasis leads to localized and generalized skin lesions and demarcated red plaques usually covered with white or silver scales. Psoriatic plaques are characterized by; i) dysregulation of proliferation and maturation of keratinocytes leading to acanthosis, hypogranulosis, and parakeratosis, ii) proliferation of dermal blood vessels, and iii) infiltration of the skin by inflammatory cells (activated CD4+, CD8+ T lymphocytes, CD3 T cells, dendritic cells, macrophages, mast cells, and neutrophils). It is the condition in which the patient suffers from numerous comorbidities like arthritis, cardiovascular diseases, and diabetes. Psoriasis is not a fatal/life-threatening disease but, the psoriatic patient gets psychologically disturbed, which leads to declension in quality of life [10].

Psoriasis condition is classified based on the clinical manifestations of the skin with a few distinctions. Psoriasis Vulgaris or plaque-type psoriasis are cognate in scientific prose. Psoriasis Vulgaris most commonly observed in 90% of psoriasis cases, including raised demarcated, erythematous red patches with silvery-white scales on the skin. It occurs in places such as the trunk, the extensor surfaces of limbs, and the scalp of the body. Inverse psoriasis,

also termed flexural psoriasis, is observed in intertriginous locations like underarms, groin, and behind the knee. It includes erosive red patches, which may appear smooth or shiny. Guttate psoriasis is a type of psoriasis in which the lesion or plaques appear as tiny dots. It is commonly found in children as well as adolescents and triggered mainly by streptococcal infections. It is the second most occurring psoriasis, which affects up to 10% of patients after the psoriasis Vulgaris. One-third of patients suffering from guttae psoriasis may develop psoriasis Vulgaris. Pustular psoriasis is characterized by white multiple coalescing pustules (blisters of non-infectious pus) encircled by red skin. In erythrodermic psoriasis condition, 90% of the total body surface gets affected with the erythematous and inflamed regions. It is infrequent, and up to 3% of psoriasis patients may get affected. Symptoms include severe itching, pain and necessitate emergency treatment [11].

Based on the affected body surface area, plaque thickness, redness, and scaling severity of disease are usually considered as mild, moderate, or severe. If the affected body surface is less than 3% area, it is deemed to be mild, 3-10% affected area is considered moderate, and more than 10% is considered a severe psoriatic disease condition.

Disruption of skin leads to activation of immune response to protect itself from foreign bodies. The Langerhans cells (dendritic cells), termed antigen-representing cells, get activated by peripheral antigens produced by keratinocytes in response to damage. Keratinocytes act as sensors for foreign or microbial agents and tissue damage by pattern recognition receptors. Keratinocytes on stimulation to peripheral antigens lead to secondary mediators like cytokines, pro-inflammatory chemokines, and antimicrobial peptides. This immune dysregulation and keratinocyte differentiation with sustained inflammation lead to psoriasis [12]. Inflammatory cytokines involved in the disease are elevated in psoriasis skin lesions. The severity can be correlated with cytokines in serum and neovascularization in the skin (erythema).

1.2. Pathogenesis of psoriasis

Psoriasis is a multifactorial disorder associated with genetic, environmental, and immunological factors. Obesity, infection, trauma, and deficiency of active Vitamin D3 are the modifying factors. The genetic predisposition in psoriasis is high. If one of the parents has psoriasis, the child has about a 10% chance of getting psoriasis. If both the parents have psoriasis, the chance increases to 50% [13]. Individuals possessing HLA class I antigen, specifically HLA Cw6, are associated with positive family history and early and acute onset of psoriasis. Race and geographical location also affect its prevalence. Psoriasis prevalence is the highest (3%) in Scandinavian countries and Northern Europe in contrast to Japan, where its incidence is 0.2%. Both acquired, and immune changes are responsible for the development of psoriatic plaques [14].

Apart from the above factors, skin is the protective layer of the body with a over-all area of about 20 square feet and shields the body from microorganisms, temperature, cold, and water loss. Stress on the skin due to various factors such as Koebner phenomenon (trauma), infection by Streptococcus pyogenes, psychological stress, metabolic syndrome indirectly aggravates psoriasis by disturbing the innate and adaptive immune system of the skin [15].

1.2.1. T-cell and cytokine-mediated pathogenesis

Various cells like Langerhans cells, keratinocytes, endothelial cells, and monocytes are involved in psoriasis that causes skin lesions in the disease state. T-cells play a crucial role in inflammation. T-helper1 (Th1) cells are thought to be the main reason for psoriasis disorder. Based on the investigation related to pathology, inflammation is a combination effect of Th1 and Th17. The identification of Th17 and Th22 cells with their cytokines interleukin (IL)-22, IL-23 has explored a path for discovering new drugs. The T-cell mediated pathogenesis mechanism is illustrated in **Figure 1.2.** Th17 cells generate different cytokines like IL-17A, IL-17F, and IL-22, which induce keratinocyte proliferation, tumor necrosis factor-α (TNF-α),

chemokine ligand (CXCL) 1, and CXCL8 production [16]. TNF-α accelerates the infiltration of inflammatory cells (lymphocytes, monocytes, and neutrophils) into the skin layer.

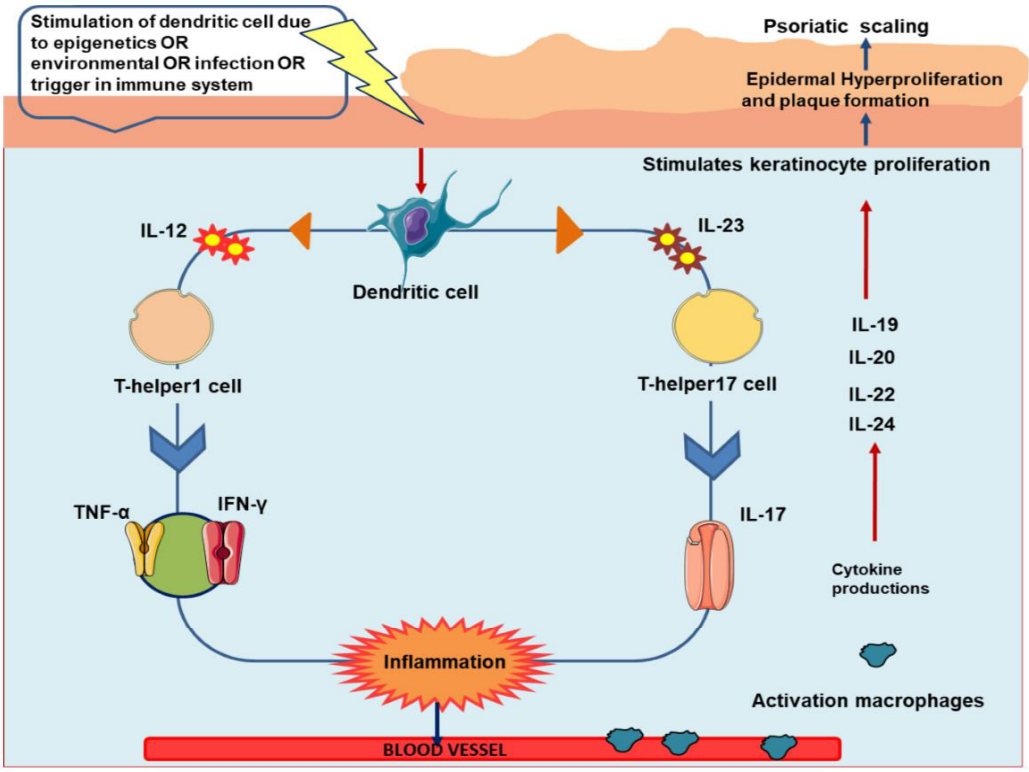


Figure 1.2. T-cell mediated pathogenesis mechanism in psoriasis.

Image source: Psoriasis: pathological mechanisms, current pharmacological therapies, and emerging drug delivery systems. Drug Discov. Today, Oct. 2020, DOI: 10.1016/j.drudis.2020.09.023.

The stimulation of the dendritic cell triggers the immune system. The dendritic cell further activates Th1 cell and Th17 by the production of IL-12 and IL-23, respectively. Th1 cell further produces inflammatory cytokines interferon (IFN)-γ and TNF- α, whereas Th17 cell produces IL-17. These inflammatory cytokines further activate macrophage accumulation and production of cytokines which cause inflammation, keratinocyte proliferation and neovascularization. The hyperproliferation of keratinocytes leads to scale formation and neovascularization, leading to erythema.

Psoriasis is a complex disorder in which various subsets of T-cells such as natural killer (NK) cells, NK-T cells, and gamma delta T cells (ϒδT) are involved. The activation of the T-cell is mediated by antigen-representing cells (Langerhans cell). The factor responsible for activating

the antigen representing cells is still under investigation [10,17]. Th1 and Th2 cytokines act through Janus kinase (JAK) signaling pathways, whereas Activator 1 (ACT1) adaptor protein and nuclear factor kappa B (NFκB) mediates Th17 responses [11]. Inflammation and angiogenesis are increased by TNF-α, vascular endothelial growth factor (VEGF), and proteases produced by macrophages continuously. Keratinocyte proliferation is increased by mediators produced by keratinocytes, Langerhans cells (dendritic cells), macrophages, and T-cells. Keratinocytes produce a large number of cytokines, growth factors, which play key role in proliferation and inflammation. TNF-α, VEGF, IFN-Ɣ, and IL-8 are produced by mast cells, which are responsible for augmentation of T-cells and neutrophils at the site of inflammation. TNF-α activates the nuclear factor signal pathway (NF-kB1), affecting keratinocytes and lymphocyte cell survival, proliferation, and anti-apoptotic effect [10,18].

Phosphodiesterase-4 (PDE-4) is an enzyme that exhibits a key role in psoriasis pathogenesis. It degrades the levels of its substrate cyclic adenosine monophosphate (cAMP) to adenosine monophosphate (AMP), which subsequently leads to the production of pro-inflammatory mediators [19].

Role of other factors in psoriasis

Psoriasis also found to be a genetic disposition, which is supported by family aggregation. Genes coding for HLA (human leukocyte antigen) protein are thought to be persuasive. The suspected genes in psoriasis include psoriasis susceptibility 1 (PSORS1) on 6p21.3, PSORS2 on 17q, CX3CL1 (fractalkine) and CX3CR1 (receptor) as their products act as chemotaxis for T-lymphocytes, monocytes, and natural killer cells [20].

Antimicrobial peptides exhibit a crucial role in the host defense protection from pathogens (bacteria, fungi, and few viruses). Antimicrobial peptides consist of 12-50 amino acids and act

as chemotactic agents, neo-vascularization agents, and controllers of cell proliferation. The psoriatic skin lesions mostly exhibit S100 proteins, β-defensins, and cathelicidins [21–24].

Micro Ribonucleic acid (miRNA) is found to exhibit a specific role in psoriasis. Few RNAs were found to be upregulated, whereas few RNAs were found to be downregulated. The research findings in miRNA became a leading target for the delivery of miRNA, which is downregulated and is anti-sense for upregulated miRNA [25,26]. The miRNA 146a, miRNA-203, miR-21 is found to be upregulated in psoriasis. Few miRNAs like miR-99a and miR-125b are downregulated in psoriasis [27,28]. Recent findings in psoriasis pathogenesis revealed the involvement of Lipocalin-2, Galectin-3, Vaspin, Gαq and Glycation end products in the pathogenesis of psoriasis.

1.3. Therapies to treat psoriasis

Based on the severity of psoriasis, the treatment approach is varied for the management of psoriasis. Psoriasis treatment includes topical therapy, systemic therapy, phototherapy, and biological agents. Even though multiple strategies are existing for the treatment of psoriasis, the topical route of therapy is the most widely preferred. Topical therapy is preferred as first-line therapy in case of mild to moderate psoriasis conditions. Large surface area and localized action of the drugs avoid unwanted systemic adverse effects and are mostly preferred by the patients. This includes corticosteroids, vitamin D3 analogs, calcineurin inhibitors, retinoids, and over-the-counter therapies (coal tar, dithranol, emollients). In moderate to severe psoriasis conditions, systemic therapies are preferred. The selection of systemic therapy also considers the presence of comorbidities for effective therapy and patient-related factors. The approved drugs for systemic therapy were methotrexate, cyclosporine, and acitretin until 2000. New therapies like PDE4 inhibitors, JAK inhibitors, monoclonal antibodies have been developed and utilized for effective therapy by systemic route. Various therapies and drugs used for psoriasis treatment are mentioned in **Table 1.1.** [29].

Table 1.1. Tabulated compilation of all therapy, drug examples, and their target [29,30].

Approved pharmacotherapy	**Drug examples**	**Mechanism of action**	**Marketed formulation**
Corticosteroids (Topical)	Hydrocortisone, Clobetasol propionate, Amcinonide, Betamethasone, Halcinonide, Fluocinonide, Desoximetasone , Mometasone furoate	These bind to glucocorticoid receptors and alter the protein synthesis that is responsible for inflammation. These also act by suppression of immune cells (dendritic cells and macrophages).	Creams, Gels, Aerosols, Lotions, Solutions.
Vitamin D analogs (Topical)	Calcipotriene, Maxacalcitol, Tacalcitol, Calcitriol	It acts through vitamin D receptors on keratinocytes and activates transcription of genes that influence growth, differentiation, and inflammation in keratinocytes. It also exhibits immunomodulatory effects on monocytes, macrophages, T cells, and dendritic cells.	Cream, Gel, Ointment
Retinoids	Topical: Tretinoin, Tazarotene, Bexarotene, Adapalene, Oral: Etretinate, Acitretin	Retinoids act by binding with retinol-binding protein. Retinol is then metabolized into Retinaldehyde in the presence of enzyme retinol dehydrogenase and converted to Retinoic acid by enzyme Retinaldehyde dehydrogenase. Retinoic acid gets bounded by the Cellular Retinoic Acid Binding Protein and makes it more polar, which enters into the nucleus and binds to Retinoic acid receptors and Retinoic X receptors. These receptors heterodimerize and bind to a sequence of DNA known as the Retinoic acid response element. It further activates the gene transcription of the target genes.	Topical: Cream, Gel Oral: Capsule
Immunosuppressants (Oral)	Methotrexate, Cyclosporine	Cyclosporine acts by reducing IL-4-producing $CD4^+$ T cells; Methotrexate acts by decreasing	Tablet, capsule

		the frequency of circulating skin-homing CLA^+ T cells.	
Calcineurin inhibitors (Topical)	Tacrolimus, Pimecrolimus, Sirolimus	Calcineurin inhibitors suppress the synthesis of pro-inflammatory cytokines. This further reduces cytokine Th1 and Th2 dependent IL 2, 3, 4 and 5, INF-γ and TNF-α.	Ointment
Phototherapy (Topical)	Psoralen, 5-Aminolevulinic acid	Cellular damage is primarily in the DNA of cell nuclei	Cream, ointment
Miscellaneous (Topical)	Anthralin, Fumaric acid esters, Coal tar and Emollient	Anti-inflammatory and anti-proliferative effects. Emollient maintain proper skin hydration, prevents irritation.	Gel, Cream
PDE4 enzyme inhibitors	Apremilast (Oral), Crisaborole (Topical)	Inhibition of PDE4 enzyme results in increased intracellular cAMP concentration, which further activates the cAMP-dependent protein kinase A. This step results in a cascade of signalling steps, including activation of specific transcription factors and inhibition of NF-κB. Consequently, this leads to increased anti-inflammatory cytokines like IL-10 and decreased levels of pro-inflammatory cytokines like TNF-α, IL-23, IFN-β, and γ.	Apremilast (Tablet), Crisaborole (Ointment)

Table source: Psoriasis: pathological mechanisms, current pharmacological therapies, and emerging drug delivery systems," Drug Discov. Today, Oct. 2020, doi: 10.1016/j.drudis.2020.09.023.

1.3.1. PDE4 inhibitors

Phosphodiesterase is an important class of enzymes involved in the hydrolysis of secondary intracellular messengers, which play a vital role in psoriasis and psoriatic arthritis. PDE has total of 11 families, the PDE4 family (consisting of 4 genes A-D) has demonstrated high substrate specificity to cyclic adenosine monophosphate (cAMP) and is majorly present in keratinocyte cells of the skin. PDE4 inhibitors are novel, low molecular weight compounds found to show anti-inflammatory effects. Inhibition of the PDE4 enzyme results in increased

intracellular cAMP concentration, which further activates the cAMP-dependent protein kinase A (PKA). This step results in a cascade of signaling steps like activation of specific transcription factors (cAMP response element-binding protein-CREB) and inhibition of NF-κB. Consequently, leading to increased levels of anti-inflammatory cytokines like IL-10 and decreased levels of pro-inflammatory cytokines like TNF-α, IL-23, IFN-β, and γ [31]. The mechanism of the PDE-4 inhibitor is depicted in **Figure 1.3**.

1.4. Apremilast: A PDE4 inhibitor for the treatment of psoriasis

Apremilast (a phthalimide derivative) is the first PDE4 inhibitor approved by the United States Food and Drug Administration (USFDA) to treat psoriasis and psoriatic arthritis in 2014. It is well absorbed when given orally as a tablet with doses of 10, 20, and 30 mg (Otezla®, Celgene), showing ~73% bioavailability [32]. Several preclinical studies have proved the potential of Apremilast to completely restore the normal histology of the skin [33].

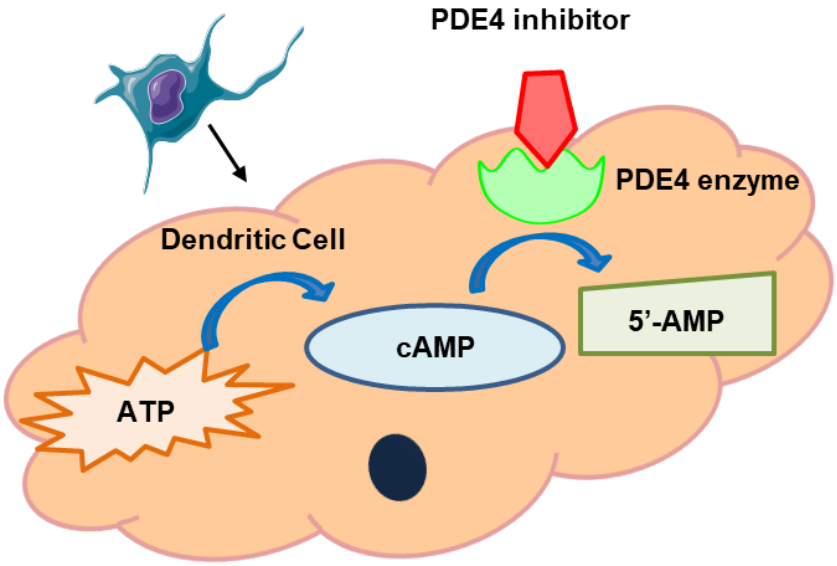


Figure 1.3. Mechanism of phosphodiesterase-4 (PDE-4) inhibitor.

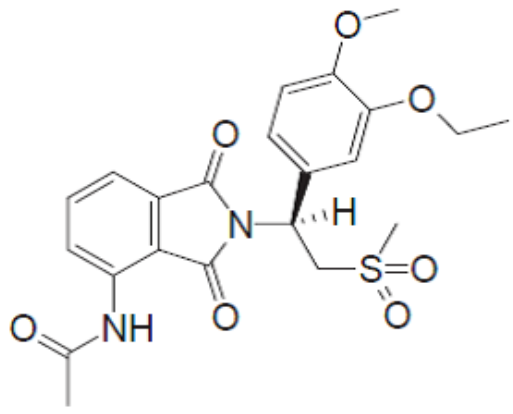
(Adenosine triphosphate (ATP) is converted to cyclic adenosine monophosphate (cAMP) in the presence of adenylate cyclase. cAMP inhibits inflammatory cells and cytokines and epidermal vascular infiltration. But cAMP was further converted into 5'- adenosine monophosphate (5'-AMP) in the presence of PDE 4. Apremilast inhibits PDE 4 and thereby

increases cAMP levels which leads to a decrease in inflammatory cytokines and epidermal infiltration.)

Image source: Psoriasis: pathological mechanisms, current pharmacological therapies, and emerging drug delivery systems. Drug Discov. Today, Oct. 2020, DOI: 10.1016/j.drudis.2020.09.023.

The efficacy and safety of apremilast were established through well-conducted clinical trials which showed superior efficacy over placebo. Unlike the clinical trials that followed strict protocols in patient follow-up and treatment plan, the real-world data have revealed even better efficacy with the achievement of Psoriasis Area and Severity Index (PASI)-75 in half of the studied population . In brief, the clinicians who participated in a study view apremilast as an attractive option for the individualized treatment of psoriasis and consider that objective indicators of effectiveness should be contemplated together with safety, convenience, and patient satisfaction. In particular, they appreciated the versatility of the drug as an option for patients who are not candidates for other treatments as well as its favorable safety profile and positive impact on symptoms, quality of life, and lesions in difficult-to-treat areas [34,35].

1.4.1. Drug properties

Name:	Apremilast
Chemical Name:	N-[2-[(1S)-1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl) ethyl]-2,3-dihydro-1,3-dioxo-1H-isoindol-4-yl] acetamide
Structural Formula:	
Molecular Formula	$C_{22}H_{24}N_2O_7S$

Molecular Weight:	460.5 g/mol
Appearance:	A white to pale-yellow crystalline powder
Solubility:	Insoluble in aqueous buffers irrespective of pH range, soluble in acetone, acetonitrile, methylethylketone, methylene chloride and tetrahydrofuran.
Water Solubility:	0.0503 mg/mL (Predicted)
Polymorphism:	Exhibit polymorphism in 7 polymorphic forms (designated A-G) of the active substance. The desired form B was found to be the most thermodynamically stable anhydrous form of Apremilast.
PKa:	4.83
Log P:	1.79
Melting Point:	156.1°C
Half-life:	6-9 h.
Absorption:	On oral administration, Apremilast is absorbed with an absolute bioavailability of 73%, with peak plasma concentrations (C_{max}) occurring at a median time (T_{max}) of 2.5 h.
Distribution:	Apremilast exhibit approximately 68% of human plasma protein binding. The apparent volume of distribution (V_d) of Apremilast is 87 L.
Metabolism:	Succeeding oral administration in humans, Apremilast is a major circulating component (45%) followed by inactive metabolite M12 (39%), a glucuronide conjugate of O-demethylated Apremilast. It is extensively metabolized in humans, with up to 23 metabolites identified in plasma, urine, and feces. Apremilast is prone to

	metabolism by both cytochrome (CYP) oxidative metabolism with subsequent glucuronidation and non-CYP mediated hydrolysis.
Elimination:	The Apremilast plasma clearance is about 10 L/h in healthy subjects, with a terminal elimination half-life of approximately 6-9 h. Next to oral administration of radio-labelled Apremilast, about 58% and 39% of the radioactivity is recovered in urine and feces, respectively, with about 3% and 7% of the radioactive dose recovered as Apremilast in urine and feces, respectively.
Indication:	Apremilast is indicated for the treatment of patients with moderate to severe plaque psoriasis and psoriatic arthritis.
Mechanism of action:	Apremilast is a small-molecule, inhibitor of PDE4 specific for cAMP. The PDE4 inhibition results in amplification of intracellular cAMP levels.

Table 1.2. Summary of Apremilast pharmacokinetic parameters after 30 mg twice daily in psoriasis and psoriatic arthritis

Indication and Dose	**Cmax (ng/mL)**	**t½ (h)**	**AUC0-T (ng•h/mL)**	**CL/F (L/h)**	**V/F (L)**
Psoriasis 30 mg BID (N=166)	382 (36.4)	9.67 (22.3)	3425 (42.0)	8.76 (41.2)	122 (24.4)
Psoriatic Arthritis 30 mg BID (N = 61)	423 (42.6)	8.88 (25.4)	3425 (42.0)	8.07 (36.5)	103 (26.1)

AUC= Area under curve; BID = Twice daily; C_{max} = Maximum concentration; CL/F = apparent clearance; $t_{1/2}$ = terminal half-life; V/F = apparent volume of distribution. Values are displayed as geometric mean (coefficient of variation [%]).

Table source: OTEZLA® (Apremilast tablets 10 mg, 20 mg, and 30 mg) Product monograph.

1.4.2. Adverse effects

The most common adverse effects were diarrhoea, nausea, upper respiratory tract infection, nasopharyngitis, tension headache, and headache. Few rare adverse effects (< 2%) include major cardiac events, malignancies, serious infections, depression, suicide attempts, migraine, weight loss [36].

1.5. Topical drug delivery systems for the treatment of psoriasis

In severe psoriasis conditions, oral and systemic therapy is preferred. Topical treatment is most preferred as the first-line treatment by 80% of the psoriasis population in mild to moderate conditions and is recommended for long-term maintenance therapy. Topical drug delivery is preferred for the delivery of drugs to localized chronic disease conditions as a large surface area of the skin acts as an attractive route for drug delivery. A topical drug delivery system is advantageous over oral therapy as it bypasses first-pass metabolism, enhanced bioavailability at the site of action and reduced unwanted systemic effects [37]. Topical therapy targets the keratinocytes proliferation, hyperkeratosis, immune cells and inflammation in the skin. Further, topical administration of therapeutics is considered for pro-active management to decrease psoriasis number of relapses. Conventional formulations such as gels, ointments, creams, lotions, and foams are widely used for topical delivery [38].

1.5.1. Limitations of conventional topical therapy

The conventional topical therapies (ointment, cream, and gel) exhibit poor permeation and low absorption due to the skin's barrier properties. A high concentration layer of the therapeutic moiety is created due to the formulation partitioning upon their application to the skin. Stickiness, greasiness, unpleasant odor of ointments have gone a long way in decreasing patient compliance. Since these delivery systems exhibit low efficiency, many drugs are loaded with vehicles resulting in allergies and irritations [39,40].

Skin penetration of drugs from these delivery systems is also comparatively less due to delivery being unpredictable and imprecise. Ideally, the drug must move to the site of action at notable amounts and then remain at this site in the required concentration for a while. Penetration enhancers like propylene glycol and dimethyl sulfoxide are used to provide a convenient access path to the required site. They enhance the rate of transport across the epidermis barrier but may exhibit certain limitations. It includes an increased amount of systemic circulation, a high risk of side-effects (cause skin irritation or other allergic conditions), and can alter the organized lipid structure, cell membrane and components. The increased amount of drug in the systemic circulation may cause unwanted side effects [41]. Hyperkeratosis and the formation of rigid scales in psoriasis skin further restrict the permeation of drugs. These lead to a low rate of drug absorption after the application of topical conventional dosage form. Therefore, a high dose and frequent application of conventional delivery systems are needed to achieve therapeutic efficacy. This reduces patient adherence and benefit to risk ratio. To overcome these limitations of conventional therapy, advanced drug delivery systems (nanocarriers and targeted-based treatment) are explored to deliver the therapeutics [39,42].

1.5.2. Role of nanocarriers in topical delivery for treatment of psoriasis

Reports of nanocarriers-based drug delivery (as mentioned in Table 1.3.) in psoriasis demonstrated enhanced efficacy and reduced toxicity compared to their conventional topical preparations (ointments, gels, creams). The topical delivery of drug-loaded nanocarriers has shown improved permeation and efficacy at a low dose with minimum systemic side effects. The particle's small size, shape, and surface charge increase the permeation by fluidizing the stratum corneum on application. The smaller the particle size, the better the interaction and penetration into the skin. The particles with < 20 nm can permeate through intact skin; however, in disease conditions, imperfect skin barrier may lead to the permeation of larger particles. The

literature revealed that the particle size in a range of 50-200 nm remains on the skin surface or stratum corneum [43].

1.5.3. Mechanism of nanocarriers permeation through the skin

After topical application of formulation (on to the skin), passive diffusion is mainly responsible for the transport of substances possible by three routes - intercellular, transcellular, and appendageal. Small, tiny molecules take up the intercellular pathway, also referred to as penetrants. SC may also physically hinder the entrance of small particles due to lipid channels of 19 nm [8]. The volume, molecular weight, hydrophilicity, and lipophilicity are some of the factors that control the diffusion process. Active transport of drugs may be facilitated via numerous protein transporters of the skin. The location and presence of tight junction proteins may also change in skin disorders like psoriasis [4,44].

The nanoparticles with elasticity can be translocated through skin appendages. They permeate into deeper layers through hair follicles or intercellular spaces. The solid particles form a thin film and get embedded in the stratum corneum. The smaller size favours the close contact of the nanocarriers with skin membrane and reduces transepidermal water loss (TEWL) (occlusive effect). The occlusive effect leads to hydration of the skin. Hydration of skin further enhances the gaps between corneocytes, increasing permeation into skin layers [45]. The encapsulated drug gets retained in the skin layers and prolongs the drug release. The permeation of nanocarriers on the topical application is depicted in **Figure 1.4.**

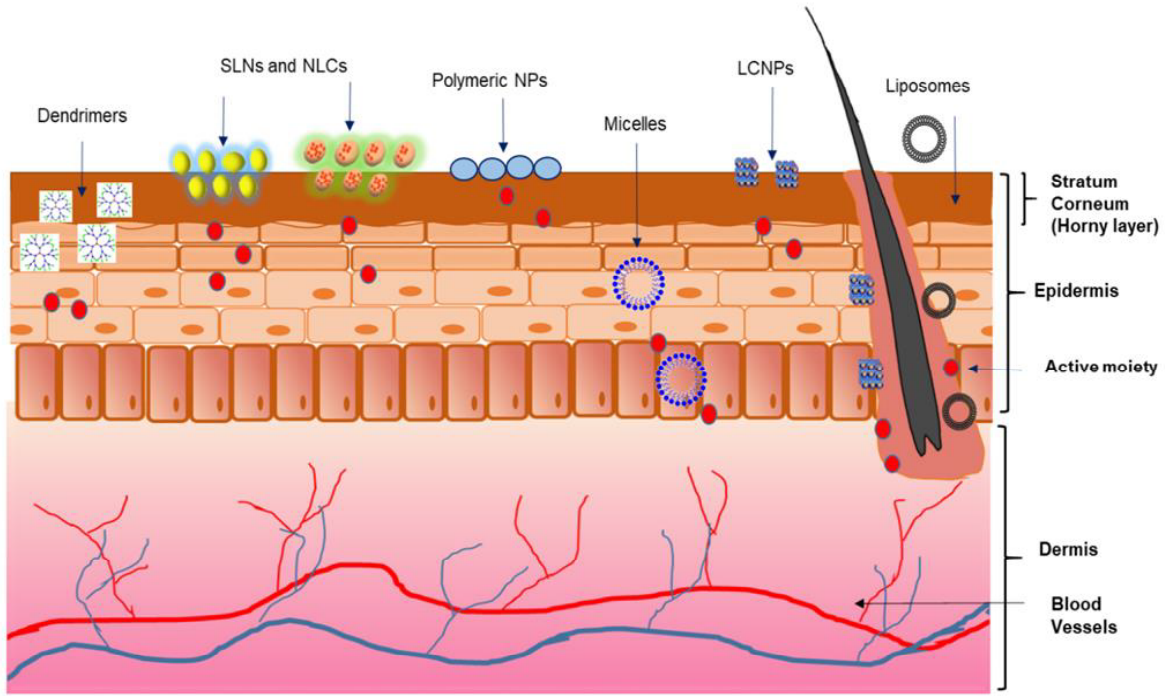


Figure 1.4. Nanocarriers permeation upon topical application

Image source: Psoriasis: pathological mechanisms, current pharmacological therapies, and emerging drug delivery systems," Drug Discov. Today, Oct. 2020, doi: 10.1016/j.drudis.2020.09.023.

To date, various nanocarrier-based formulations are explored for the delivery of therapeutics in psoriasis. Nanocarriers explored for psoriasis therapy include liposomes, solid lipid nanocarriers (SLNs), nanostructured lipid carriers (NLCs), polymeric micelles, polymeric nanoparticles, nanocapsules, ethosomes, lyotropic liquid crystalline nanoparticles (LCNPs) [40,42]. Various therapeutic agents have been attempted to be delivered using nanocarrier systems. **Table 1.3.** summarizes different techniques along with the molecules studied.

Table 1.3. Various nanocarriers used for topical delivery therapeutics used in psoriasis [39]

Nanocarrier	**Drug**
SLNs	Fluocinolone Acetonide, Halobetasol propionate, Capsaicin, Calcipotriol and betamethasone dipropionate
NLCs	Tacrolimus, Methotrexate, Fluocinolone Acetonide, Capsaicin
Liposome	Calcipotriol, Methotrexate, Tazaretone, Cyclosporine

Ethosome	Tacrolimus, Psoralen, Cyclosporine
Transferosome	Methotrexate, Tacrolimus
Niosome	Methotrexate, diacerein
Microemulsion	Tacrolimus, Dithranol, Methotrexate, Tazarotene, Babchi oil (Psoralea corylifolia)
Nanocapsule	Dithranol
Dendrimers	Dithranol, 8-methoxypsoralen
Micelle	Cyclosporine A, Tacrolimus
Gold nanoparticle	Methotrexate
Emulsome	Capcaisin, Dithranol
LCNPs	Tacrolimus
Niosome	Methotrexate and Trioxysalen

Table source: The nanocarriers for topical delivery in psoriasis, n: R. Shegokar (Ed.), Deliv. Drugs, Elsevier Inc., Germany, 2020: pp. 75–96.

1.6. Lipid nanocarriers

Lipid nanocarriers such as SLNs, NLCs, LCNPs, liposomes and nanoemulsion were found to be effective in stratum corneum permeation due to their nano-sized and lipophilic nature. Lipid nanocarriers have been found for better absorption due to the interaction of carrier lipid with biological lipid. Additionally, SLNs, NLCs, LCNPs are the lipid nanocarriers that can provide advantages like high drug loading, stability, sustained release and skin retention on topical administration.

1.6.1. Solid lipid nanocarriers

SLNs are lipid nanoparticles that are prepared by utilizing solid lipid (stearic acid, triglycerides like tristearin/ tripalmitin, waxes like carnauba wax, glyceryl monostearate, glyceryl dibehenate, cetyl palmitate, glyceryl palmitostearte) as a matrix media and stabilized by surfactants in an aqueous media. SLNs provide the advantages of increased stability and

extended-release of drug. Therefore, SLNs find their use in follicular delivery, epidermal targeting, and improve skin hydration because of the occlusive nature.

Pradhan et al. and his co-workers developed fluocinolone acetonide SLNs as a topical delivery system for the treatment of psoriasis. The developed SLNs preparation showed particle size of 107.4 ± 1.25 nm with a PDI of 0.193. The fluocinolone acetonide loaded SLNs formulation revealed the maximum amount of drug in the epidermis (46.06 μg/ml) compared to free drug (17.83 μg/mL) , indicating high skin retention. The results exhibited reduced side effects with fluocinolone acetonide SLNs preparation due to the particular accumulation in the epidermis [46]. Bikkad et.al. developed halobetasol propionate containing SLNs. The selected SLNs formulation exhibited 84-94% entrapment efficiency, with the average particle size being 200 nm. The SLNs formulation exhibited improved skin deposition (up to 90%) compared to plain gel (up to 56%) [47]. The studies showed that SLNs can be suitable carriers for topical delivery to disease site with enhanced therapeutic efficacy in skin disorders.

1.6.2. Nanostructured lipid carriers (NLCs)

NLCs were developed as a new generation of lipid nanocarriers to overcome challenges associated with SLNs like limited drug loading, lipid polymorphism, and stability. NLCs are prepared by the mixing of different lipid molecules, i.e., solid lipids with liquid oil. The blend of these two leads to the generation of unique nanostructures inside the matrix. A crystalline structure is formed, showcasing many imperfections, thus allowing space for the drug [48,49]. NLCs have been explored for topical delivery in the treatment of psoriasis. Acitretin-loaded NLCs with the particle size of 223 nm loaded gel showed 81.38 % deposition in human cadaver skin, whereas acitretin plain gel showed 47.28 % deposition. Clinical studies conducted on patients with psoriasis showed that the formulation was effective in treating psoriasis and reducing the local side effects [50]. Essaghraoui and his co-workers developed cyclosporine-loaded NLCs for topical delivery. The mean particle size of SLNs and NLCs formulation was

found to be less than 200 nm. The in-vitro cell viability studies were performed on fibroblast cells and keratinocyte cell lines. NLCs formulation showed more sensitivity with keratinocytes compared to fibroblasts. The skin permeation studies conducted through pig ear skin demonstrated higher permeation of cyclosporine when loaded in NLCs (2.55 ± 0.13 μg/cm^2) compared to SLNs (1.05 ± 0.07 μg/cm^2). The occlusive nature of SLNs and NLCs improves the permeation in comparison to conventional formulation [51].

1.6.3. Lyotropic liquid crystalline nanoparticles

Liquid crystalline nanoparticles (LCNPs) are self-assembled mesophases that exhibit properties of both ordered solid and isotropic liquids. It is also called mesophase, which indicates that it has a unique structure that is in between the ordered solid phase and true liquid phase. LCNPs are bi-continuous cubic phase formulations that have been utilized as transdermal delivery systems for transporting drugs via the topical route. They are being used as a vehicle in controlled drug delivery systems. They show the potential to incorporate a lipophilic drug in the hydrophobic core of the LCNPs and a hydrophilic drug in inner aqueous chambers. They are also safe, non-toxic, biocompatible, and bio-adhesive polar liquids [52].

Various types of amphiphiles like phytantriol, oleyl glycerate, phytanyl glycerate, glyceryl monooleate, lecithin, silicone oil and hydrogenated castor oil are used to prepare LCNPs. For preserving the nanoparticulate dispersion, surface active agents are also required in the generation of LCNPs. Poloxamers are widely used to disperse oily substances in water. LCNPs help in solubilizing hydrophobic, amphiphilic, and hydrophilic active agents to impart controlled release to the preparation [53]. In one study, tacrolimus-loaded LCNPs were prepared with the objective to treat psoriasis. The study of drug permeation and retention disclosed a profound increment in tacrolimus quantity, which permeated and retained due to LCNPs. The 65% of tacrolimus was retained with LCNPs formulation, whereas the tacrolimus solution prepared in propylene glycol exhibited 25% skin retention. The studies showed that

LCNPc can be a suitable choice to improve the drug permeation through stratum corneum and can retain the drug in the skin layers for prolong time. [54].

1.7. Quality by design approach for formulation development

The preparation of nanocarriers is a complex and sensitive process. The process involves various material and unit operations. Minor variation in the process affects the physicochemical properties (size, shape, composition, crystallinity, polydispersity, morphology, solubility, viscosity, zeta potential, drug loading, and drug release), biological parameters. (permeation, bioavailability, and stability) and pharmacological behavior. Commercialization of nanoformulations have shown promise in preclinical studies, is impeded due to lack of scale-up data and regulatory issues for the formulations. Achieving consistent results in scale-up and manufacturing ability of nanocarriers is difficult due to poor reproducibility of the existing process. The development of nanocarriers with suitable safety, efficacy, and stability is a challenging task which consists of multifarious hurdles [55]. Both the processes can be influenced by many factors, including surfactant concentration, the volume of the surfactant solution, mixing time, mixing speed, product exposure for size reduction time, and temperature conditions. There is a need to know about the interaction between each constituent and the effect of process parameters with minute changes in the concentration of constituents. Complete knowledge on the interaction between minor changes in each constituent along with process parameters will help in the large-scale production of nanocarrier formulations with desired predetermined quality [56].

Quality by Design (QbD) is the "systematic approach with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management". The FDA has given a clear description on a risk-based approach to current good manufacturing practices (cGMP)-based pharmaceutical industries in the year 2004. QbD helps in understanding the factors controlling the formulation parameters and

manufacturing variables. The QbD-based approach is useful for the development of robust formulations which can expedite the regulatory approvals of nanocarriers-based formulations. QbD can contribute to understanding the multiple factors affecting the product with the application of the design of experiment (DOE) technique. DOE is an approach where interaction between two material attributes, process parameters along with interaction between process and material attributes can be studied. It provides complete and thorough knowledge related to the formulation, helps in solving problems faced during manufacturing and scale-up. Continuous process consistency is required as end-product testing alone cannot confirm the product quality, QbD depicts the risk assessment and risk control for the product and process involved [57]. The objective of QbD in drug delivery is to derive meaningful product quality specifications based on safety, efficacy, and clinical performance. It helps in the investigation of variable factors in material and process and ultimately enhances the process capability with minimum product variability. QbD approach designs a scientific structure of understanding the process and products related to its root cause analysis, product development, and manufacturing efficiencies [58]. In this work, formulation optimization was performed by identifying quality target product profile, critical quality attributes, critical material attributes and critical process parameters.

1.8. Current status of research on topical delivery of Apremilast

Few studies were explored for topical delivery of Apremilast using nanocarriers. Kushwaha and his co-workers, a novel nail lacquer formulation, was designed to treat nail psoriasis to enhance the ungual and trans-ungual delivery of Apremilast. In-vitro studies revealed a cumulative amount of Apremilast delivered in nail plate was ~3 fold (0.52 ± 0.07 μg/cm^2) higher in comparison to control formulation without enhancers (0.19 ± 0.02 μg/cm^2). The studies performed in human subjects revealed the cumulative amount of Apremilast retained in the free distal edge of the nail plate was ~2 fold (0.93 ± 0.14 μg/mg) more associated to

control (0.41 ± 0.04 µg/mg) [59]. Madan et al. developed NLCs for topical delivery of Apremilast. The developed formulation exhibited a particle size of 758 nm, with 0.339 polydispersity index and the entrapment efficiency of the prepared formulation was 85.5%, with a zeta potential of −33.3 mV. Ex-vivo skin permeation results reprsented sustained drug release, and 60.1% of Apremilast skin deposition [60]. Parmar and Bansal developed nanocrystal-based formulations of Apremilast for improved topical delivery. The nanosuspension prepared by the wet milling technique exhibited a average mean particle size of 200 nm. In ex-vivo studies, nanosuspension showed 2.6 and 3.2 fold drug penetration in stratum corneum and viable layers, respectively, over micro-suspension. Nanogel showed 2.7 and 2.4 fold drug penetration in stratum corneum and viable layers, respectively, compared to microgel. Nanocream showed 1.2 and 2.8 fold drug penetration in stratum corneum and viable layers, respectively, over micro cream [61]. In a study, Apremilast microemulsion was prepared for topical application, the study depicted 479.35 ± 102.85 µg/g skin/cm^2 Apremilast skin retention. In-vivo anti-inflammatory efficacy studies showed the microemulsion had reduced the inflammation with reduced pro-inflammatory cytokines. The research indicates as topical delivery of Apremilast is a potential strategy to treat skin inflammation [62].

1.9. Gaps in the current research

To overcome the limitations of the oral therapy (adverse effects) of Apremilast topical delivery was investigated. Apremilast is the BCS II drug, and conventional topical preparation exhibits poor permeation through the skin due to low solubility. The literature showed the hope that lipid nanocarriers can be a potential delivery system to overcome the limitations of skin permeation and efficacy with conventional topical preparations. However, limited lipidic nanocarriers were explored for the topical delivery of Apremilast in a psoriasis disease condition. Further, the reported lipid-based nanocarrier systems were prepared with complex preparation techniques or showed higher particle size (> 200 nm). The particle size with less

than 200 nm exhibits a more significant occlusive effect and improves the permeation [8,63]. There is a need to develop the lipid nanocarrier with a particle size less than 200 nm. There is a need to understand the effect of the formulation and process parameters to get the desired characteristic nanocarriers. Further, there is a need to understand the scale-up ability of the formulation at the lab level to emphasize industrially feasible process. There is a need for the development of formulation with a simple process (minimal steps and minimal use of organic solvents) for commercial application. The rate and extent of drug permeation in the skin layers need to be investigated to understand the dermatokinetics of topical Apremilast loaded nanocarriers.

1.9.1. Objective and specific aims of the current work

Considering the gaps of the existing research, it was proposed to develop the Apremilast-loaded lipid nanocarriers for topical delivery with improved permeation,skin retention and prolonged release at disease site.

The specific aims laid to achieve the major objectives include

I. Preparation and optimization of lipid nanocarriers for topical delivery of the Apremilast using the QbD approach.
   - Design and optimization of lipid nanocarriers with QbD based approach to understand the impact of formulation and process parameters on desired characteristics of nanocarriers such as particle size (< 200 nm) and entrapment efficiency.
   - Physicochemical characterization of designed nanocarriers and topical preparation

II. In-vitro, ex-vivo characterization and dermatokinetic study of the developed nano-formulations.

III. To study the in-vivo skin irritation and skin retention study of the selected formulations.

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