Abstract

Psoriasis is an immune-mediated chronic skin disorder characterized by erythematous, sharply demarcated papules, hyperproliferative keratinocytes, and massive infiltration of leukocytes with dermal inflammation. It affects approximately 2-3% of the total world's population.

Apremilast was the selective phosphodiesterase 4 (PDE4) inhibitor approved by the United States Food and Drug Administration (USFDA) in 2014 for treatment of psoriasis. Apremilast prevents the degradation of cyclic adenosine monophosphate (cAMP), which thereafter reduces the production of pro-inflammatory cytokines tumor necrosis factor (TNF)-α, interleukin (IL)-23, and interferon (IFN)-γ. It increases the production of anti-inflammatory mediator IL-10. The oral administration of Apremilast exhibits adverse events such as headache, nausea, diarrhoea, abdominal pain, depression, upper respiratory tract infection, nasopharyngitis, decreased appetite, fatigue and insomnia. The topical delivery of Apremilast is expected to reduce the systemic adverse effects and increase patient compliance. The low solubility of Apremilast is one of the limitations to deliver through the topical route. The skin condition is inflamed and thickened in psoriasis condition, which further hinders skin permeation and efficacy of the drug. Lipid-based nanocarriers can be a suitable alternative for delivery of the drugs through topical route in psoriasis condition.

In this dissertation, lipid nanocarriers (solid lipid nanocarriers, nanostructured lipid carriers, lyotropic liquid crystal nanoparticles) were explored for the topical delivery of Apremilast. These lipid nanocarriers exhibit advantages for skin delivery such as high drug loading, stability, sustained release, and skin retention. The overall objective was to design and characterize three lipid nanocarriers i.e. solid lipid nanocarriers, nanostructured lipid carriers, and lyotropic liquid crystal nanoparticles of Apremilast to improve skin permeation, prolong skin retention and sustained release at the targeted site.

A simple, accurate, robust isocratic HPLC method was developed to estimate Apremilast in pre-formulation samples, nanoformulation, in-vitro release, ex-vivo release and skin biological samples. The developed HPLC method was validated according to regulatory guidelines. The method was linear in the range of 100 to 10000 ng/mL concentration ($R^2 \geq 0.999$) at 229 nm. The limit of detection and limit of quantification for Apremilast was found to be 30 ng/mL and 100 ng/mL, respectively. The intraday and inter-day precision % relative standard deviation was less than 2%. The developed method was successfully evaluated for specificity and its application for in-vitro and ex-vivo drug release studies of nanoformulations.

Apremilast-loaded solid lipid nanocarriers (SLNs) were developed by the quality by design approach. SLNs were prepared using hot emulsification, followed by size reduction using probe sonication. The optimized batch showed size and entrapment were found to be 167.70 ± 1.5 nm (0.238 ± 0.022 PDI) and 63.84 ± 0.93%, respectively. The in-vitro drug release of SLNs dispersion showed extended-release up to 18 h and followed the Korsmeyyar-Peppas model. In-vitro cell line study, the MTT assay depicted the formulation excipients had no effect, and high internalization was observed with SLNs dispersion (1.4-fold) in comparison to free Coumarin-6. The C_t value reduction in the relative expression of TNF-α mRNA was 3-fold higher with SLNs dispersion compared to the positive control. The ex-vivo skin retention and dermal distribution study performed by Coumarin-6 dye-loaded SLNs depicted an increase in permeation and retention compared to free Coumarin-6. The dermato-pharmacokinetic study of SLNs formulation exhibited AUC_{0-24} 1082.812 ± 80.610 μg/cm^2.h in the epidermis and 1074.629 ± 137.553 μg/cm^2.h in the dermis. The C_{max} in the epidermis and dermis were 98.311 ± 2.121 μg/cm^2 and 68.685 ± 5.657, respectively. The T_{max} of the formulation was found to be 6.00 h and 8.00 h in the epidermis and dermis, respectively.

Apremilast-loaded nanostructured lipid carriers were prepared by hot emulsification followed by size reduction. The characterization results of the selected formulation showed its spherical

shape with a particle size of 157.91 ± 1.267 nm (0.165 ± 0.017 PDI). The entrapment efficiency and zeta potential were found to be 69.144 ± 0.278% and -16.75 ± 1.40 mV, respectively. The in-vitro release study revealed a controlled release of Apremilast from NLCs up to 24h and followed the first-order release model. The ex-vivo study showed 3-fold enhanced skin retention compared to conventional gel preparation. The C_t value reduction in the relative expression of TNF-α mRNA was 4.02-fold higher with NLCs dispersion compared to the positive control. The formulation depicted improved psoriasis efficacy indicating reduced TNF-α mRNA expression. The cytotoxicity and skin irritation study revealed that the prepared formulation has no toxicity and irritation. The dermato-pharmacokinetic study of NLCs formulation exhibited AUC_{0-24} 2112.538 ± 140.428 µg/cm^2.h in epidermis and 847.120 ± 83.051 µg/cm^2.h in dermis. The C_{max} in the epidermis and dermis were found to be 109.123 ± 4.838 µg/cm^2 and 62.444 ± 3.563 µg/cm^2, respectively.

The Lyotropic liquid crystalline nanoparticles (LCNPs) formulation was prepared using a high shear homogenizer for size reduction. The particle size, zeta potential and entrapment efficiency of the optimized LCNPs formulation were found to be 173.25 ± 2.192 nm (PDI 0.273 ± 0.008), –21.46 ± 1.30 mV, and 75.028 ± 0.235%, respectively. The in-vitro drug release showed the controlled release for 18 h and followed the first-order release mechanism. The C_t value reduction in the relative expression of TNF-α mRNA was 3.73-fold higher with LCNPs dispersion compared to the positive control. The ex-vivo studies revealed that drug retention was 2.61 and 14.59-fold higher in stratum corneum and viable part of the skin in LCNPs formulation comparison to free drug-loaded gel. The dermato-pharmacokinetic study of LCNPs formulation exhibited AUC_{0-24} 2872.747 ± 93.70 µg/cm^2.h in epidermis and 550.750 ± 155.88 µg/cm^2.h in dermis. The C_{max} in the epidermis and dermis were found to be 187.744 ± 2.22 µg/cm^2 and 66.824 ± 8.53 µg/cm^2, respectively. The T_{max} of the formulation was found to be 6.00 h in the epidermis and dermis, respectively.

The in-vivo studies of the three lipid formulations depicted are non-irritant on swiss albino mice with no signs of erythema after application. The amount of Apremilast retained in skin layers was estimated after 12 h and 24 h. The amount of Apremilast in stratum corneum after 12 h in SLNs, NLCs, and LCNPs were 10.91 ± 1.75 µg/cm^2, 11.15 ± 1.13 µg/ cm^2, and 10.77 ± 1.42 µg/ cm^2, respectively. The amount of Apremilast in viable part of skin after 12 h in SLNs, NLCs, and LCNPs were 4.00 ± 1.29 µg/cm^2, 4.81 ± 1.78 µg/cm^2, and 8.00 ± 0.94 µg/cm^2, respectively. In the free drug-loaded gel amount of Apremilast in stratum corneum after 12 h and 24 h was 2.16 ± 0.60 µg/cm^2 and 1.62 ± 0.85 µg/cm^2. The amount of Apremilast in viable part of skin after 12 h and 24 h was 2.06 ± 0.56 µg/cm^2 and 1.85 ± 0.82 µg/cm^2. The amount of drug retained in the skin was higher in SLNs (3.53 fold in 12 h, 2.32 fold higher in 24 h), NLCs (3.78 fold in 12 h, 3.22 fold higher in 24 h), and LCNPs loaded gel (4.44 fold in 12 h, 2.59 fold higher in 24 h) compared to free drug-loaded gel. The overall study concluded that the designed Apremilast-loaded lipid nanocarriers (solid lipid nanocarriers, nanostructured lipid carriers, lyotropic liquid crystal nanoparticles) provided improved in-vitro efficacy, enhanced skin retention, and prolonged-release compared to conventional topical preparation.

The outcome of the present study emphasizes an industrial feasible process for the development of Apremilast-loaded lipid carriers for topical delivery. The developed formulations are free from permeation enhancers (solvents), and all ingredients used are within the inactive ingredient guidelines (IIG) limits.