Summary and conclusion

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Psoriasis is a chronic autoimmune skin disorder that affects 2-3% of the world population. It is characterized by increased keratinocyte proliferation and the production of inflammatory mediators. Different treatment strategies include topical, systemic, phototherapy, and biologics are evolved for controlling and management of psoriatic conditions. Topical therapies are preferred for treatment of mild to moderate psoriasis conditions, whereas systemic therapies are preferred in severe disease conditions. The immunosuppressants, biological agents and recently approved PDE4 inhibitors are the systemic therapies used in psoriasis.. Due to the limitations of existing therapies and new discoveries in the pathogenesis of psoriasis, it has paved a path for newer molecules for targeting at the molecular level. Small molecules PDE4 inhibitors had proved efficacious. There was a decrease in pro-inflammatory mediators IL's, TNF-α, with reduced flaking, scaling, and joint tenderness and swelling.

The oral administration of Apremilast exhibits adverse events such as headache, nausea, diarrhoea, upper respiratory tract infection, and nasopharyngitis. To overcome these limitations, 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 nanoparticulate systems are well explored for delivery of the drugs in skin disorders targeting skin layers by increasing skin permeation and retention. The ease of preparation technique, low manufacturing cost, biocompatibility, protection of the drug from degradation, controlled release, and increased skin retention make the lipid nanoparticles a potential and ideal topical drug delivery. This can increase the efficacy of the drugs and reduce the systemic absorption of the drug.

To meet the objective in the present study, we have investigated topical delivery of Apremilast using lipid nanocarrier systems (SLNs, NLCs, and LCNPs) for the treatment of psoriasis. The SLNs, NLCs, and LCNPs were developed and characterized for improved skin permeation and retention in in-vitro and in-vivo. The developed formulation was evaluated for size, entrapment efficiency, in-vitro drug release, ex-vivo drug release, skin retention, skin permeation, in-vitro cytotoxicity study, psoriasis efficacy studies, dermatokinetic studies and in-vivo skin retention, irritation study.

Analytical development is the foremost step in drug product development. To meet the requirement a sensitive, rapid, selective, accurate and precise method was developed for routine analysis of Apremilast loaded lipid-based nanoformulation for topical application. The method was developed on the eclipse XDB-C18 analytical column with a mobile phase consisting of acetonitrile and 10 mM potassium phosphate buffer 50:50 ratio with a flow rate of 0.8 mL/min. The samples were analyzed at 229 nm wavelength using a PDA detector. The method was found linear in the range of 100 to 10000 ng/mL with 30 ng/mL as LOD and 100 ng/mL as LOQ, respectively. The developed method was tested for stability-indicating, and there was no interference of impurities and formulation excipients with the retention time of Apremilast. The developed method was found to be specific, and it was applicable for the determination of entrapment efficiency, assay, in-vitro release, and permeation study without any matrix effect of formulation and skin samples. The developed method was utilized for estimation of the Apremilast in biological skin samples which can be applicable for dermatokinetics studies.

For topical delivery of Apremilast, three types of lipid nanocarriers i.e. SLNs, NLCs, and LCNPs were designed. Lipids were selected with the maximum solubility criteria. The Precirol ATO 5 was selected as a solid lipid for the development of SLNs, and Labrafil M 2125 was selected as a liquid lipid for the development of NLCs. Glyceryl monooleate was utilized for the development of LCNPs. To study the effect of formulation and process variables on desired

characteristics of finished formulation, QbD (design of experiments) was implemented. Box-Behnken experimental design was employed with 17 trials (3 factors and 3 levels) using design-expert software (Design-Expert® 8.0, State-Ease Inc., Minneapolis, USA). The influence of independen varioable including the amount of lipid, percent of surfactant and probe sonication time (for SLNs, NLCs) and homogenization time (for LCNPs) were investigated on response variables i.e. particle size and entrapment efficiency. For formulation optimization, the independent variables were studied at low, medium, and high levels. Formulation was selected for furher optimization based on desirability for particle size lower than 200 nm with PDI less than 0.300 and entrapment efficiency greater than 60%. The selected optimized formulation exhibited the entrapment efficiency as LCNPs > NLCs > SLNs. The complex structure of LCNPs is expected for high entrapment efficiency compared to NLCs and SLNs. The presence of liquid oil in NLCs enhanced entrapment efficiency compared to SLNs. The in-vitro drug release studies demonstrated the prolonged-release up to 18 h for all the lipid nanocarriers.

The cytotoxicity studies of optimized lipid nanocarriers were performed on HaCaT cell lines demonstrated the formulation excipients were non-toxic. The cell uptake studies performed using Coumarin-6 dye demonstrated the higher internalization in lipid nanocarriers (NLCs > LCNPs > SLNs) compared to free Coumarin-6. The in-vitro psoriasis efficacy studies conducted in the HaCaT cell lines demonstrated the high efficacy in reducing the TNF-α mRNA with lipid nanocarriers compared to the free drug. The suppression of TNF-α mRNA was found to be highest in NLCs followed by LCNPs and SLNs formulation. As demonstrated in cell uptake studies, higher internalization of Apremilast may reduce the TNF-α mRNA expression.

The lipid nanocarriers dispersion was loaded into Carbopol 974P gel. The amplitude test and frequency sweep test performed on the prepared gel exhibited a high degree of crosslinking with significantly greater storage modulus (G') compared to loss modulus (G"). The lipid

nanocarriers loaded gel exhibited high occlusive nature. The difference in the occlusive nature of the three formulations was minimal (SLNs > NLCs > LCNPs). The skin permeation studies demonstrated the higher skin retention of developed lipid nanocarriers compared to free drug-loaded gel. Among the three developed formulations LCNPs loaded gel followed by NLCs loaded gel and SLNs loaded gel. The percent permeated was higher in NLCs loaded gel followed by SLNs loaded gel and LCNPs loaded gel, respectively. The structural similarities of LCNPs with skin membrane favours the high retention in skin layers. The ex-vivo dermal distribution studies (Coumarin-loaded SLNs, NLCs, and LCNPs) demonstrated the higher retention in skin layers. The dermatokinetic study results showed higher $AUC_{0\text{-}24}$ in LCNPs loaded gel followed by NLCs loaded gel and SLNs loaded gel, respectively. The higher elimination rate constant (K_e) was observed in SLNs loaded gel followed by NLCs loaded gel and LCNPs loaded gel, respectively. The results indicated prolonged skin retention of drug with LCNPs loaded gel followed by NLCs loaded gel and SLNs loaded gel, respectively.

The in-vivo studies of desgined nanocarriers performed on swiss albino mice demonstrated the formulation had no signs of irritation or erythema. The skin retention studies were performed for 12 h and 24 h. The concentration of the drug in the stratum corneum and viable part of the skin was estimated. The improved skin retention was observed in optimized lipid nanocarriers compared to free drug-loaded gel. The studies revealed a mostly similar concentration of drug in stratum corneum after 12 h. A high amount of drug in viable part was observed in LCNPs loaded gel followed by NLCs loaded gel and SLNs loaded gel. In the case of 24 h, the high amount of drug was retained in the NLCs loaded gel followed by LCNPs and SLNs loaded gel in stratum corneum and viable part of the skin. A high amount of drug retention was observed in 12 h compared to 24 h. The same results were observed in the dermatokinetic study. It was expected due to the clearance of the drug from the skin layers.

The results collectively conclude that the Apremilast can be delivered by lipid nanocarriers by topical route to improve skin permeation and retention. This can minimize the systemic adverse effects and increase the efficacy with a reduced dose. Among designed lipid nanocarriers preparations, LCNPs formulation required low energy which favors large scale-production. The dermatokinetic study showed the higher AUC_{0-24} in LCNPs loaded gel. In-vivo studies illustrate improved skin permeation and retention of Apremilast when delivered via LCNPs.

Overall, the work concluded that the designed lipid nanocarriers improved the skin permeation, prolong retention, and sustained release compared to conventional topical preparation of Apremilast. The formulations designed with QbD approach and minimum processing steps provided the opportunity for ease of scale-up and industrial translation.

Outcomes

Since the decade, lipid nanocarriers have attained great attention towards the delivery of topical therapeutics to improve permeation and skin retention. The direct outcome of the present study emphasizes an industrial feasible process for the development of Apremilast-loaded lipid carriers for topical delivery. The developed formulation can improve skin permeation and skin retention with improved dermatokinetic properties in the skin. The improved topical availability of the Apremilast supports the enhanced efficiency of the developed formulation. The developed formulations are free from permeation enhancers (solvents), and all ingredients used are within the inactive ingredient guidelines (IIG) limits.

Future scope and directions

Further studies based on the present outcomes could include

- The surface modification of the formulation can be explored to estimate the skin permeation, and skin retention.
- The developed formulation can be explored for a clinical study.
- Further scale-up to larger batch size can be explored for industrial approach.