ABSTRACT

Psoriasis is a non-contagious, autoimmune (T cells mediated) skin disease with a marked presence of 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 incidence of psoriasis ranges from 0.09% to 11.43%, imposing a severe global problem with a minimum of 100 million people suffering worldwide. Although genetic predisposition is the causative factor still the etiology remains vague and the therapeutic strategies are mainly focused upon getting symptomatic relief rather than complete cure.

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. The systemic and topical therapies have been a vital part of the psoriasis treatment regimen. The concern with practicing systemic therapies are their extreme levels of adverse effects, associated with massive distribution to undesired organs with poor targeting to the desired site (deeper dermal layers). 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 other body organs. Several topical preparations available for the management of psoriasis are composed of retinoids, corticosteroids, salicylic acid, anthralin, coal tar, and vitamin D analogs 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 use. 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. 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 the drug. 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 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; they were able to prove advantageous in treating psoriasis. These nano-systems 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 nano-systems offer many benefits over conventional delivery systems including prolonged drug release profile, protection of the active principle from the destructive bio-environment, lower dose, 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. Lipidic nano-systems 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.

In order to get the benefits of both systems, lipid-polymer hybrid nano-systems 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.

The research work disclosed in the present thesis entitled **"Lipid-polymer Hybrid Nanoparticles for Topical Delivery of Hydrophobic Molecules in a Psoriasis Mouse Model"** systematically provides the development of a scalable monolithic lipid-polymer hybrid nano-carrier for delivering small hydrophobic molecules including clobetasol propionate, cholecalciferol (Vitamin D), and coenzyme Q10 for treating imiquimod (IMQ) induced plaque psoriasis in *Swiss albino* mice. This doctoral work has been divided into six chapters to achieve the objective of the thesis.

C**hapter 1** of the thesis provides an introduction to psoriasis, its clinical types, treatment strategies, limitation of conventional formulations, advantages, and disadvantages of nano-enabled systems (including both lipidic and polymeric) followed by focusing on the aspects of lipid-polymer hybrid (LPH) nanoparticles. Further, this chapter focuses on the different types of LPH nano-systems followed by objectives of current thesis work.

Chapter 2 of the thesis describes the reverse-phase high-performance liquid chromatography (RP-HPLC) methods for the analysis of clobetasol propionate, cholecalciferol and coenzyme Q10. A reverse-phase HPLC based analytical (for CP, vitamin D3 and CoQ10) and bioanalytical method (for CP) were developed and validated as per ICH Q2R1 and USFDA guidelines, respectively. For the analytical methods, calibration curve depicted the better correlation coefficient and linearity in the concentration range (0.25-100 µg/mL for CP, 0.05-100 µg/mL for vitamin D3 and 0.5-100 µg/mL for CoQ10). The developed method could be employed for the determination of drug loading, entrapment efficiency and drug release studies of nano-formulation. Similarly, the bioanalytical methods of CP was developed in both the skin and plasma samples of Swiss albino mice with a

dynamic calibration range of 0.025-1 µg/mL. These validated bioanalytical methods could be utilized for skin permeation studies and determining systemic leaching.

Chapter 3 of the thesis describes scalable and stable clobetasol propionate loaded monolithic lipid-polymer hybrid nanoparticles (CP/LPNs) consisting of a combination of lipids (solid and liquid) and an amphiphilic copolymer, mPEG-PLA. For this several lipids (solid and liquid) and surfactant were screened and a scalable platform was established for preparing nanoparticles. CP/LPNs gel showed a sustained *in vitro* clobetasol release for 7 days with no burst release and 6-month stability at 2-8 °C and room temperature. Further, CP/LPNs showed an improved cellular uptake with significant growth inhibition of HaCaT cells. These spherical nanoparticles demonstrated deeper skin penetration with undetectable quantities leaching systemically. Efficacy assessment showed significantly improved PASI score, reduced skin damage, and proliferation after treatment with CP/LPNs gel as compared to marketed product (Clobetamos™). Collectively, the enhanced cellular uptake, high skin penetration with increased skin retention, and improved efficacy demonstrate the potential of these LPNs for future clinical application.

Chapter 4 outlines the application of the established LPH system to load cholecalciferol and evaluate its efficacy in treating IMQ-induced psoriasis mice model. VD3/LPNs gel too demonstrated enhanced anti-psoriatic efficacy similar to that of CP/LPNs gel, proving the therapeutic advantage of this hybrid nano-carrier. These monolithic LPNs exhibited spherical morphology with a lower particle size (123.1 nm) with narrow PDI (0.234) and efficient encapsulation (76.80%) demonstrating a sustained release profile of VD3 for up to 3 days following a Korsemeyer-Peppas release model. *In vivo* efficacy assessment in imiquimod (IMQ)- induced psoriatic mouse model demonstrated enhanced anti-psoriatic activity of VD3 with improved PASI score when delivered as LPNs gel as

compared to the free VD3 gel that were further supported by histopathology and immunohistochemistry

Chapter 5 outlines the application of the established LPH system to load Coenzyme Q10 and evaluate its efficacy in treating IMQ-induced psoriasis mice model. CoQ10/LPNs gel too demonstrated enhanced anti-psoriatic efficacy similar to that of CP/LPNs gel and VD3/LPNs gel, proving the therapeutic advantage of this hybrid nano-carrier proposing it as an platform technology. These monolithic LPNs exhibited spherical morphology with particle size (121± 11.61 nm), PDI (0.252±0.073), and encapsulation (78.57±3.88 %) demonstrating a sustained release profile of CoQ10 for up to 3 days following a Hixson-Crowell model demonstrating non-fickian release diffusion. *In vivo* anti-psoriatic efficacy was carried out using Imiquimod (IMQ)-induced plaque psoriatic mouse model demonstrated significant enhancement in the efficacy of CoQ10 when administered as CoQ10/LPNs gel as compared to the free CoQ10 gel characterized by marked improvement in PASI scores that were additionally supported by thorough histopathology followed by immunohistochemical analysis (IHC) which was attributed to the improved ROS scavenging activity.

Chapter 6 provides conclusions of the research work and its future scope. These LPNs could serve as a platform for delivering various hydrophobic small molecules with improved dermal distribution leading to enhanced anti-psoriatic efficacy.