CHAPTER 6: SUMMARY AND CONCLUSION

6.1 Summary

Plaque Psoriasis is a chronic inflammatory disorder that is particularly characterized by abnormal proliferation and disrupted differentiation of keratinocytes along with reverse occurrence of inflammatory lesions and neovascularization. Being an autoimmune disease, it is manifested by immunological and biochemical alteration and thus activates immune cells. Thus, it is associated with increased levels of pro-inflammatory markers, chemokines, growth factors, and cytokines such as TNF-α, IL-17, and IL-23. Cytokines play an essential role in the pathophysiology of psoriatic like a skin condition.

The treatment options include topical therapy, phototherapy, and systemic therapy. For severe cases, systemic therapy along with topical therapy is recommended. Systemic therapy includes corticosteroids, immunosuppressant drugs such as cyclosporine or retinoids. The use of these drugs systemically or topically results in much severe toxicity like renal and hepatic toxicity, etc as well as patient noncompliance. Among all the available treatments, none approach can cure completely, safely, and effectively.

Resveratrol (3,5,4′-trihydroxy *trans*-stilbene) is a naturally occurring polyphenolic compound, mainly originates from berries, grapes, and red wine. It has been reported to show potent antioxidant, anti-inflammatory, anti-proliferative and anti-cancer effects.

Resveratrol is a known activator of sirtuin genes especially *SIRT1* present in the skin which leads to modulation in immune cells and decreases proliferation and promotes differentiation. Along with it, it has inherent antioxidant property, thus it possesses strong chemopreventive as well as antipsoriatic effects. However, Resveratrol is a natural originated molecule but is therapeutic efficacy could not be utilized due to its poor biopharmaceutical properties such as low solubility and poor systemic bioavailability.

Treatment now, thus, aims at higher efficacy through the delivery of drugs in a sustained (temporal) and site-specific (spatial) manner. Topical skin delivery is the most ideal and route of choice for several dermatological disorders including psoriasis.

But due to the psoriatic skin conditions and allied changes in epidermal anatomy such as hard, inflamed, and thicker keratodermas, and scaly horny barrier, the

formulation development for its management is a challenging task. The conventional formulations available in the market are not fulfilling the requirements as they lack the aesthetic and cosmetic properties as well as they show certain toxicity and poor patient compliance in long-term use. Developing novel drug delivery systems with improved dermatological benefits can outweigh the existing challenges. Along with it, they are associated with some other advantages such as ease of administration, reduced dosing frequency, sustained-release drug, and drug targeting at the required dermal site for improved therapeutic benefit to psoriatic patients.

The stratum corneum acts as the main barrier and challenge to be overcome for the permeation of the bioactive molecule. Overcoming this horny barrier by enhancing the permeation and at the same time, retaining the maximum amount of drug in deeper layers, and thus, avoiding systemic absorption to enhance the dermatological benefits via topical route is the need of the hour.

In the past few decades, several lipids and polymeric novel topical drug delivery systems have been exploited to overcome the barriers of topical skin delivery of drugs and bioactive.

Among many nanosystems attempted for dermatological benefits, micellar drug delivery systems and nanoemulsions are a few of the successful approaches having better skin permeation properties, skin compatibility, and high stability.

The present work was focused on the formulation and evaluation of Resveratrol loaded polymeric micelles (PM) and lipidic based nanoemulsion (NE) delivery systems and their corresponding hydrogels (PMG and NEG). The delivery systems were formulated using QbD and systematic optimization (DoE) based approach.

The proposed systems are novel approaches for formulating Resveratrol containing delivery systems for the treatment of Plaque Psoriasis. The developed system was evaluated for *in vitro* skin permeation abilities as well as *in vivo* antipsoriatic efficacy. It was envisaged that these novel delivery systems may follow maximum pathways of skin absorption and hence, may provide a better therapeutic effect as compared to the conventional delivery system.

The summary of the whole study is given below:

- Preformulation studies were carried out to confirm the identity and purity of the drug, establishment of analytical technique, and assessment of some important features of Resveratrol. λ_{max} of Resveratrol in methanol was found to be 306 nm (USP 2016). The reported λ_{max} is also 306 nm. The results ensured the identification and purity of Resveratrol. All other studies such as organoleptic properties, IR spectroscopy, and melting point confirmed the identity and purity of Resveratrol.
- A standard analytical method for quantifying Resveratrol was developed using both the UV spectroscopic method and the HPLC method. Both methods were found to be reliable in terms of linearity, accuracy, and precision.
- Partition coefficient results confirmed the highly lipophilic nature of Resveratrol.
- Solubility studies also revealed that it has very low aqueous solubility.
- Resveratrol loaded Polymeric Micellar formulation (PM) was formulated constituted of a mixture of two block copolymers i.e. Pluronic P123 and Pluronic F127.
- QbD supported optimization was done by employing the Design of Experiment (DoE) with a holistic perception of product and processes optimization was employed to formulate Resveratrol loaded polymeric micelles.
- As per the ICH guidelines, defined QTPP and related characteristics for the development of Resveratrol loaded polymeric micelles with better skin retention, permeation, and pharmacodynamic properties were selected. Similarly, CQAs were identified as accountable to fulfill defined QTPP along with its pertinent explanation. Particle size, micellar incorporation efficiency, and skin retention potential of micelles were chosen as CQAs.
- Ishikawa fishbone diagram was created for the risk assessment during formulation. It was done to layout the influential formulation and process variables/ parameters affecting selected CQAs. Failure mode and effect analysis (FMEA) was executed to screen out the crucial parameters affecting the quality, safety, and efficacy of the drug product.

- Mixed pluronic based polymeric micellar dispersion was successfully developed using a thin-film hydration method.
- Response surface methodology using Face Centered Central Composite Design (FCCCD) was employed for the optimization of Resveratrol loaded micelles.
- Two variables i.e. the percentage of Pluronic P123 in the mixture of two Pluronics (P123 and F127) (X1) and the amount of Resveratrol (X2) were varied at three different levels i.e. low, medium, and high represented by coded levels (-1, 0 and +1).
- The identified CQAs (dependant attribute) were micellar incorporation efficiency (Y1), particle size (Y2), and extent of skin deposition (Y3).
- Thirteen batches in triplicate were developed and analyzed for selected CQAs.
- Design-Expert software was employed to navigate and validate the design.
- The generated model, equation, perturbation curve, 2D contour plot, and 3D plots ratified that the concentration of Pluronic P123 and the amount of resveratrol has a significant effect on MIE ($p < 0.0001$). The higher the proportion of P123 as compared to F127, the higher is the encapsulation of Resveratrol.
- While lower levels of the amount of Resveratrol added during the formulation of micelles, higher MIE was achieved.
- The analysis of particle size confirmed that the positive relationship with both the selected CMAs.
- The skin deposition of Resveratrol, at the lowest levels of both CMAs, was found to be less, however, it increased at intermediate levels, and then either the increment has declined or it reduced at higher levels of CMAs.
- Multiple response optimizations through the checkpoint analysis and desirability approach were used.
- Constraints were set for desired responses i.e. MIE (Y1) to be maximum, particle size (Y2) to be in range ($100 \text{ nm} < Y2 < 200\text{nm}$), and skin deposition (Y3) also to be maximum.
- The optimized level of P123 concentration was found to be 0.584 i.e. 73.5 %. The optimum value lies in between intermediate level (0 i.e. 50 %) and high level (+1 i.e. 90%) tested. While the optimum level of the Resveratrol amount to be used

was found to be -0.940 i.e. 51.5 mg. This value is very near to the minimum level tested (i.e. -1) with a Desirability value of 0.916.

- The predicted and observed responses were compared and the percentage prediction error was within specified limits.
- The observed MIE was 93.45 ± 2.34 %, particle size was 142.67 ± 6.98 nm with skin deposition of 51.63 ± 1.97 %. The predicted and observed responses were compared and the percentage prediction error was within specified limits.
- PDI and zeta potential of prepared micelles were found to be 0.165 ± 0.096 and -33.65 ± 2.45 mV respectively. All the parameters were also found to be optimum for topical skin delivery and indicate stable colloidal carriers.
- Morphological evaluation by TEM revealed that polymeric micelles obtained were spherical particles with a well-identified structure, uniform size, and distribution in the aqueous medium.
- To improve the skin applicability properties of the formulated colloidal formulation, it was converted into the carbomer-based hydrogel i.e. micellar hydrogel (PMG).
- The size and PDI of colloidal carriers incorporated in the gel were observed to be statistically similar to the size and PDI of colloid in the form of a dispersion, indicating the structural stability of micelles in the gel.
- Conventional hydrogel (CG) was formulated to be used as a control for comparative purposes. Each gel was formulated to have 1% w/w Resveratrol. All the developed gels were colorless, translucent, and free from any gritty particles.
- An alternative to this, Resveratrol loaded vitamin E-based nanoemulsion (NE) was also optimized, developed, and evaluated.
- Preliminarily, various excipients such as oil (castor oil, ethyl oleate, olive oil, coconut oil, vitamin E and their combinations); surfactants (tween 20 and tween 80); and co-surfactants (propylene glycol, PEG 400, and transcutol P) were screened based on the solubility of resveratrol in them.

- A mixture of ethyl oleate and vitamin E (1:1) as oil, tween 80 as a surfactant, and transcutol P as co-surfactant were selected as major excipients to be used in the formulation of resveratrol loaded nanoemulsion.
- Optimization of the ratio of surfactant:co-surfactant i.e. (Smix) was carried out by plotting pseudo ternary phase diagrams. The phase diagram at Smix ratio 3:1 has shown the highest area of emulsification and thus it was selected for further optimization.
- High energy emulsification is a widely used and accepted method for the preparation of nanoemulsion (NE).
- The effect of different formulation variables on selected CQAs was studied by employing a 3^2 full factorial design consisting of two factors which were varied at three levels i.e. high, intermediate, and low.
- The different levels of independent variables (CMAs) i.e. X1 = concentration of oil (% v/v) and X2 = concentration of Smix (% v/v) were selected as low, medium, and high. Dependant variables or response variables (CQAs) were selected as Y1 [globule size (nm)], Y2 [% cumulative drug permeation], Y3 [permeation flux (J) (µg hr^{-1} cm^{-2})] and Y4 [skin deposition (µg cm^{-2})].
- The ranges of responses for all batches were 143 to 340 nm (globule size), 3.25 to 4.92 % (% cumulative resveratrol permeation), 2.04 to 10.16 µg hr^{-1} cm^{-2} (permeation flux) and 157 to 976 µg cm^{-2} (skin deposition).
- Each response was fitted to a second-order quadratic model and the best-fitted model was determined. Model significance was identified and confirmed by ANOVA, lack-of-fit test, and multiple correlation coefficients (R^2) tests.
- The lowest level of oil, as well as Smix, resulted in minimum globule size. However, the impact of the concentration of Smix on globule size was more as compared to the concentration of oil.
- On increasing oil concentration, percent permeation was found to be increased while it was decreased upon increasing the concentration of Smix.
- Permeation flux is also affected positively by increasing oil concentration, while a negative effect was observed by increasing the Smix ratio.

- Similar to it, skin deposition is also highly affected by the concentration of Smix in a negative manner. However, increasing oil concentration resulted in higher skin deposition. Both variables have a significant quadratic effect on the skin deposition of bioactive.
- Optimization was done using the desirability approach and checkpoint analysis.
- Constraints for responses were set as minimal globule size, maximal percent permeation, and skin deposition and flux in the required range.
- Predicted values of the responses suggested by the software were found to be as Y1= 160.289 nm, Y2= 4.692 %, Y3 = 7.000 µg hr^{-1} cm^{-2} and Y4 = 657.047 µg cm^{-2}. The optimized combination of formulation variables was obtained at oil concentration (X1) = 9.8% and at a concentration of Smix (X2) = 2.5 % with the corresponding desirability (D) value of 0.784.
- Experimental values at this combination checkpoint was found to be as Y1 = 168.3 ± 4.98 nm, Y2 = 4.81 ± 0.65 %, Y3 = 7.62 ± 0.39 µg $hr^{-1}cm^{-2}$ and Y4 = 668.65 ± 11.98 µg cm^{-2}.
- An acceptable range of percentage prediction error (less than 10 %) and desirability value was observed, which indicated a lofty degree of predictive ability and validated the generated design for the evaluation and optimization of nanoemulsion.
- PDI and zeta potential of optimized nanoemulsion formulation were found to be 0.181 ± 0.079 and -29.57 ± 3.43 mV respectively.
- Due to poor skin applicability of formulated nanoemulsion formulation, it was incorporated into carbomer based hydrogel (NEG).
- The globules were found to be dark spherical droplets with bright surroundings and the absence of any aggregation was confirmed by TEM.
- Both the developed hydrogels i.e. PMG and NEG were also evaluated for some other important evaluations such as pH, spreadability, viscosity, all of which were found to be optimum.
- The dialysis bag method was employed to determine the *in vitro* release profile of Resveratrol from formulated hydrogels. The dissolution medium

used was 500 mL of 3:7 (v/v) ethanol-pH 7.4 phosphate buffer which was maintained at 32 ± 0.5 °C and 50 rpm.

- Results indicated that conventional gel showed a burst release of Resveratrol of around 80% in the first two hours only. No such burst release was observed in either of the colloidal dispersion or hydrogel. PM and PMG showed a sustained release of up to 12 h, while NE and NEG showed approximately 80 % release in just six hours.
- CG and Resveratrol solution both were found to show first-order release kinetics While, both emulsion and micellar colloids as well as corresponding hydrogels showed to follow the Higuchi model which revealed that the release of Resveratrol is occurring in a sustained pattern following the diffusion mechanism.
- The *ex vivo* skin permeation study was performed using the freshly excised skin from pig ear (obtained from the local slaughterhouse).
- PM and PMG successfully enhanced Resveratrol permeation compared to plain carbopol gel, showing the enhancement ratio of 2.934 and 2.741 reflected by a high permeation flux i.e. 7.32 ± 0.54 µg cm^{-2} h^{-1} and 6.83 ± 0.86 µg cm^{-2} h^{-1} respectively as compared to plain carbopol Gel i.e. 2.50 ± 0.65 µg cm^{-2} h^{-1} and the increase was found to be statistically significant ($p < 0.01$).
- Results also clearly indicated that nanoemulsion and nanoemulgel formulations showed a higher rate of skin permeation as compared to conventional gel. The permeation flux of resveratrol from nanoemulsion and nanoemulgel was 7.62 ± 0.39 and 7.18 ± 0.57 µg cm^{-2} h^{-1} respectively which were found to be significantly higher ($p<0.001$) than permeation flux obtained from conventional gel i.e. 2.50 ± 0.65 µg cm^{-2} h^{-1}. The permeability coefficient of both nanoemulsion and nanoemulgel was observed to be three times higher than conventional gel.
- The value of Resveratrol retained in the dermal layer by PM, PMG and CG was quantified as 246.64 ± 9.4 µg cm^{-2} (51.63 ± 1.97 %), 253.90 ± 5.47 µg cm^{-2} (53.15 ± 3.24), and 49.40 ± 9.62 µg cm^{-2} (10.34 ± 2.01 %) respectively. The skin deposition by PM and PMG is approximately five times higher than CG. The

high deposition can be ascribed because micelles are supposed to form a depot in a deeper layer of skin.

- The present studies confirmed the superior permeation and skin deposition potential of both PMG and NEG as compared to conventional gel and thus, could be used successfully for topical/dermal delivery of Resveratrol. Thus, found to be highly efficient and appropriate for skin targeting and providing enhanced dermatological benefits.
- C6 loaded NEG and PMG were compared with CG for skin targeting properties using CLSM.
- Results confirm the efficiency of nanoemulgel for targeting required bioactive into the skin and can act as a potential topical drug delivery system in the treatment of various skin related disorders like psoriasis.
- Free radical scavenging and antioxidant property of the developed formulation were evaluated by conducting the DPPH assay method.
- Among all tested formulations, the sequence of antioxidant potential was found to be NEG (IC_{50} = 6.03 µg/mL) Ascorbic Acid (IC_{50} = 8.72 µg/mL) > Res Solution (IC_{50} = 10.62 µg/mL) > PMG (IC_{50} = 11.99 µg/mL). Ascorbic Acid was used as a control for comparison purposes, exhibited comparable DPPH scavenging activity with plain Resveratrol solution.
- Results revealed that Resveratrol retained its potential antioxidant effect in its formulations also and it is indicated that the formulation process and materials attributes have not hampered the activity of Resveratrol, thus considered to be optimum to be used as a topical delivery vehicle.
- *In vivo* efficacy studies (antipsoriatic activity) of developed formulations were assessed using IMQ-induced psoriatic-like plaque model in Swiss Albino Mice.
- Animal Protocol was duly permitted by the Institutional Animal Ethics Committee, ISFCP, Moga [ISFCP/IAEC/CPCSEA/Meeting No. 23/2018/ Protocol No. 391]. Studies were carried out according to the CPCSEA guidelines. 2-3 months old Swiss Albino mice weighing 20-25 g were utilized.

- *In vivo* studies ratified the significant effectiveness of PMG and NEG as compared to conventional gel (CG). A significant reduction in PASI score, splenomegaly, serum cytokines levels, and hyperkeratosis by both micellar based and nanoemulsion based hydrogel formulations showed their higher effectiveness and better therapeutic and dermatological benefits.
- Histopathological alterations in skin samples were analyzed by histological evaluation of skin samples.
- The application of Resveratrol loaded formulations to the disease-induced animals have shown considerable improvement in skin thickness, as well as alleviation of other signs such as lessened parakeratosis and healing of keratinocytes, was observed.
- As compared to disease control and CG group, PMG and NEG formulation has shown significant improvement in skin histology as histological images of animals of PMG and NEG treated group was quite similar to the normal control group. Improved signs revealed the enhanced efficacy of the Resveratrol loaded PMG and NEG for the treatment of various dermatological benefits such as psoriasis.
- Formulated polymeric micellar hydrogel and nanoemulgel were subjected to stability studies to evaluate the effect of different storage conditions. The study was conducted by keeping the formulations at "refrigerated conditions (5 ± 3 ºC)" and "room temperature (25 ± 2 ºC/ 60 ± 5 % RH)" for 6 months. Results concluded that both hydrogels should be stored at lower temperatures for maximum stability and minimum loss of its therapeutic efficacy.

6.2 Conclusion

The target site of application for several skin disorders such as psoriasis is the dermal layer of skin. Efficient dosage forms controlling the delivery of bioactive/drug at the target site of disease is the prerequisite for maximal therapeutic benefits. The Resveratrol is a known antioxidant and anti-inflammatory natural molecule and also inhibits the proliferation of keratinocytes. Poor biopharmaceutical properties inhibit its utilization for the treatment of skin diseases. Several studies investigated the use of novel carrier systems for its improved permeation into the skin. In the present study, QbD and DoE driven development and optimization of Resveratrol loaded polymeric micelles (PM) and Resveratrol loaded nanoemulsion (NE) and its corresponding carbomer based hydrogel i.e. PMG and NEG were carried out. Results ratified that both polymeric and lipidic nano delivery systems resulted in better skin permeation and enhanced deposition of Resveratrol in deeper skin layers. *In vitro* DPPH assay proved that formulation procedure has not hampered the antioxidant potential of Resveratrol. *In vivo* studies concluded that both colloidal delivery systems enhanced the desired therapeutic benefits for topical treatment of plaque psoriatic like skin condition. Thus, the present study revealed substantial evidence for utilizing Resveratrol loaded polymeric micelles as well as vitamin E based nanoemulsion as an alternative therapy for the treatment of Plaque psoriasis with better dermatological benefits. Though, the obtained results are seminal to conclude their effectiveness, further clinical trials along with long term stability studies are also required to validate the obtained results.