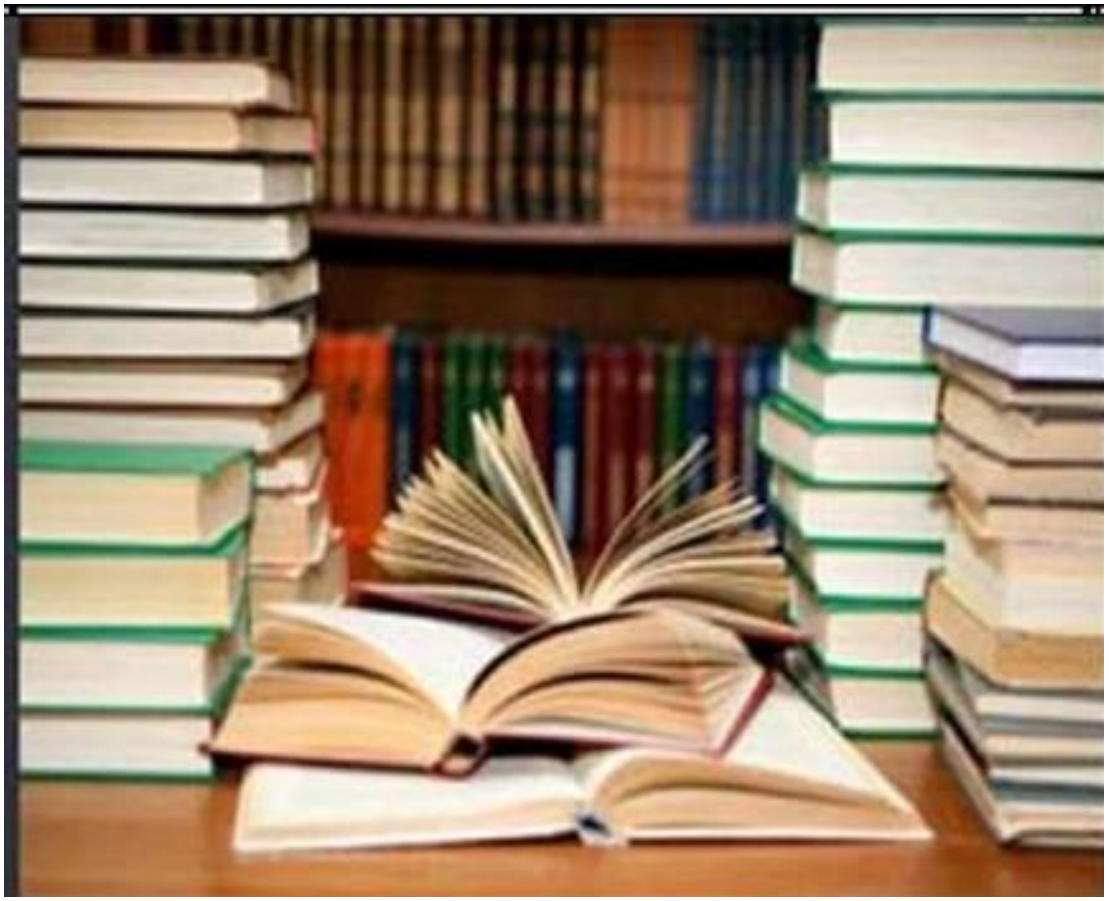
CHAPTER 6: SUMMARY AND CONCLUSION



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SUMMARY

Psoriasis vulgaris is an autoimmune disease caused by inappropriate activation of the cellular immune system. Psoriasis is a psychosocially debilitating disorder that affects 1 to 3% of the population worldwide. It involves in excessive growth and deviant differentiation of keratinocytes. There is an increase in proliferation of epidermis with dilation of dermal capillaries, infiltration of inflammatory cells in skin layers (dermis, epidermis), and localized infiltration into skin layers. It leads to localized skin deregulation that plays a major role in the development of scaly erythematous plaques. Other symptoms are swelling of the skin, pain, itching and skin flaking.

Sebaceous glands are small oil (sebum) producing glands that are attached to hair follicles. These glands are not evenly distributed all over the skin but are more prevalent on the face, scalp, chest and neck. As our present study is on Plaque psoriasis which is most often found on the scalp, the lower back, the face, the palms and soles of feet these areas are sebaceous gland rich areas(www.psoriasis.org). About 10 percent or more of body-surface area is covered in chronic plaque psoriasis.

Clobetasol propionate is a very potent topical corticosteroid class compound. Topical steroids are used in addition to moisturizers for treating inflammatory skin conditions such as eczema and dermatitis. Owing to its anti-inflammatory, antipruritic and immune-modulating properties, clobetasol propionate is used to treat psoriasis. Clobetasol propionate induces phospholipase A2 inhibitory proteins, thereby controlling the release of the inflammatory precursor arachidonic acid from membrane phospholipids by phospholipase A2. It was also found that if clobetasol propionate (CP) was incorporated in the nanosystems it shows significant increase in their anti-inflammatory activity. It was reported that CP-loaded nanoemulsion significantly increased NTPDase (Nucleoside triphosphate diphosphohydrolases) activity in lymphocytes. This membrane protein is responsible for the hydrolysis of extracellular ATP (Adenosine triphosphate) which is responsible for cell proliferation,

differentiation and inflammatory processes.

For topical formulations use of conventional excipients could serve the purpose only to a 0limited extent of absorption, penetration, and retention through psoriatic barrier cells. With the discovery of newer chemicals like squalene biocompatible and biodegradable materials like phospholipids and novel drug delivery technologies like deformable liposomes, SNLs, liposomes, nanostructured lipid carriers (NLCs), microemulsions and nanoemulsions *etc*. have the possibility to improve the efficiency and safety of the topical products to a great extent and also improve the absorption, penetration and retention in skin. It was found that the addition of corticosteroid and a keratolytic agent such as salicylic acid in nanocarrier would result in enhancement and sustaining of corticosteroid delivery rate leading to better anti-psoriatic activity. Clinical use of corticosteroid is restricted to some extent due to its poor permeability across the skin. So to increase its permeation across the skin, nanocarriers were prepared and characterised.

The aim of this study is to develop a "squarticles," (Nanoemulsions and NLCs) and deformablr liposomes composed of clobetasol propionate loaded nanosystems gel and evaluate its potential in the psoriatic animal model. The nanosystems loaded gel was prepared by dispersing the clobetasol propionate loaded nanosystems in carbopol gel base and its efficacy was evaluated in rat. The Imiquimod model used to induce psoriasis in Wistar mice based on scoring to check severity and histopathology. We also had used squalene in our formulation which is a sebum derived lipid and shows its affinity towards sebaceous glands and because of which drug shows depot effect in it and retention of drug in the skin was increased.

The aim of this study was to develop a 'squarticles,' (NLCs, and Nanoemulsion) and deformable liposomes composed of clobetasol propionate loaded nanosystems and to evaluate its potential in the psoriatic animal model. In this research work we have shown that these systems will improve drug deposition in the skin layers as sebum derived lipid were used which shows affinity towards sebaceous gland, these

nanosystems enhance drug penetration through the keratinized psoriatic skin cells because of their flexible nature. After drug deposition in sebaceous gland minimum absorption in the systemic circulation will occur, which in results reduce systemic side effects and hence, may provide a better therapeutic effect as compared to the conventional delivery system.

Formulations prepared in this research revealed some advantages for drug therapy over conventional carriers, including - increased solubility, the ability to enhance storage stability, improved permeability, reduced adverse effects, prolonged half-life, and tissue-targeted delivery; hence these nanosystems hold great promise for reaching the goal of controlled and targeted delivery. Objective of this research work was to increase the permeation rate through psoriatic barrier cells and retention time in pilosebaceous gland by preparing nanostructured lipid carrier loaded gel and nanoemulgel of clobetasol propionate which results in increased permeation through psoriatic barrier skin cells, and improved local availability at the target site and less toxicity. The existence of squalene and fatty esters in the nanosystems tends to increase the drug uptake into the hair follicles. Better partitioning of drug in pilosebaceous glands results in improved therapeutic efficacy. The drug would be available at targeted site over an extended period of time which will lead to increased duration of action as well as minimize dosing frequency.

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 Clobetasol propionate. λmax of Clobetasol propionate was found to be at 239 nm in methanolic PBS at 5.5 pH. The reported λmax is also 239 nm. The results ensured the identification and purity of Clobetasol propionate. All other studies such as organoleptic properties, IR spectroscopy, and melting point confirmed the identity and purity of Clobetasol propionate.

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- A standard analytical method for quantifying Clobetasol propionate 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 Clobetasol propionate.
- Solubility studies also revealed that it has very low aqueous solubility.
- Prepared nanoemulgel, NLCs loaded gel and deformable liposomes acting as carrier for low as well as high molecular weight drugs. It also increases the effectiveness of the clobetasol propionate by enhancing permeation and penetration of the drug through scaly keratinized barriers of stratum corneum.
- Homogenisation method was used for the formulation of Squarticles. There are three steps involved in the formulation of Squarticles (nanoemulsion and NLCs) which were (i) preparation of nanoemulsion, NLCs and Deformable Liposomes (ii) preparation of hydrogel and (iii) finally, nanosystems loaded gels will be produced by the incorporation of nanoemulsion, NLCs and Deformable Liposomes into a gel with continuous stirring.
- Squarticles were optimised by using different surfactant concentration, lipid ratio, homogenisation speed and sonication time entrapment efficiency, drug loading.
- The clobetasol propionate is a potent drug (dose 0.05% through transdermal route) and we have selected its concentration on the bases of studies reported (Gordon 1998; Feldman 2005). The loaded nanoemulsion formulation has a particle size of 240.5 ± 9.2, PDI 0.282 ± 0.03 nm, entrapment efficiency (E.E.) 89.8 ± 7.11% w/v and drug loading 45.12% w/v.
- Drug loaded NLCs was optimized and having a particle size of 254 ± 10.11 nm, zeta potential -56.11 ± 6.21 mV, PDI 0.140, entrapment efficiency 89.8 ± 6.13%, and drug loading 45.14% w/v.
- Drug loaded deformable liposomes has a particle size 232.1±6.35, PDI 0.169±0.02, entrapment efficiency (E.E.) 72.9% and drug loading 52.2%.

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- Nanosystems loaded gel were prepared in different batches by using different concentration of carbopol 940 w/v with formulation codes and optimized for the various parameters such as pH, spreadibility, % of carbopol and % drug content. Deformable liposomes loaded gel prepared by using carbopol 940 was 0.25%w/v found to be optimized.
- Because of easy penetration and retention nanosystems showed 85% decrease in average PASSI score, marketed formulation showed 50% decrease in average PASSI score and there is no change in disease group.
- Histopathological study fluorescence microscopic images (stained with haematoxylin and eosin dye and images of the slides were visualised at 10X magnification) of mouse skin with in vivo topical administration of marketed and noamoemulgel formulation. (a) Control Group (Keratinised stratified squamous epithelium Normal subepidermal tissue are present). (b) Diseased Group (Mild acanthosis Moderate inflammation And Parakeratosi is present). (c) Topinate gel (marketed formulation) Standard Group (Keratinised thin stratified squamous epithelium and Moderate inflammation was found). (d) Clobetasol propionate loaded nanoemulgel Treated Group (Keratinised stratified squamous epithelium and Mild inflammatory was seen). This histopathological study showed the increased therapeutic effectiveness of antipsoriatic drug.
- Nanosystems are smaller in size, Particle size of nanoemulsion, NLCs loaded gel, deformable liposome was found to be 240.5nm, 254.6nm and 232.1nm respectably. Large amount of surfactant was used in their preparation and because of their flexible nature they easily penetrate the thick scales of dead keratinized layer of psoriatic skin.
- *In-vitro* release study of marketed gel, and nanosystems (Nanoemulgel, NLCs and Deformable loaded gel) was carried out using dialysis bag method. The dissolution medium used 100 ml of 10% methanolic phosphate phosphate buffer saline pH 5.5 in a conical flask. This flask was taken in incubator shaker and

speed of shaker was maintained at 60 rpm at 37^0 c.

- The release profiles for optimized gel formulations (drug-loaded Nanoemulsions, NLCs, and deformable liposomes) and marketed gel (Topinate gel) were determined in triplicate. Here, the formulations are arranged according to their release rate NLCs > Nanoemulgel > deformable liposomes > Topinate gel.
- Due to presence of squelene the system showed affinity towards sebaceous gland and it showed depot effect. Amount of drug retained in the skin was calculated by subtracting the unabsorbed drug over epidermal surface of skin from the initial drug content of the formulation applied.
- Franz diffusion cell with an effective diffusion area of 1.00 cm^2 was used for the experiment. The rat abdomen skin was cleaned, shaved and placed between the donor and receptor compartments of Franz diffusion cell with the stratum corneum facing donor compartment. Drug permeation profiles through rat skin, optimised Nanosystems loaded gel formulation, and marketed gel were taken.
- Nanosystems owning better membrane deformability and due to smaller size could penetrate the scaly keratinised psoriatic skin through the hydrophilic pathways and pore between the skin cells. But due to the presence of squalene the system will show an affinity towards sebaceous gland and very small part of it will reach in the blood and skin will shows depot effect.
- The skin depot effect for optimized gel formulations (drug-loaded Nanoemulsions, NLCs) determined. In vitro penetration and skin retention are graphically shown. This study indicates that by using squalene in the formulation we can use sebaceous glands as a depot for sustained release of the drug.
- Comparative study is also shows nanoemulgels gives better retention as compared to NLCs loaded gel.
- Pharmacokinetic studies were performed HPLC method was developed in rat plasma for estimation of clobetasol propionate. A linear response was obtained in the range of 1–10mg/mL with correlation coefficient r^2 = 0.9992 which proves

that there is the least penetration of the drug in the blood circulation and our system has shown deposition in pilosebecious glands.

- Biopharmacokinetic parameters of optimised clobetasol propionate loaded Nanoemulgel and NLCs loaded gel and marketed formulation by using HPLC method (n=6) shows that there is around 80% decrease in drug content of nanosystems as compared to marketed formulation.

CONCLUSION

Clobetasol propionate was successfully formulated in nanoemulgel, NLCs and deformable liposomes. These nanosystems reveals some advantages for drug therapy over conventional carriers, including - increased solubility, the ability to enhance storage stability, improved permeability, reduced adverse effects, prolonged half-life, and tissue-targeted delivery; hence these nanosystems hold great promise for reaching the goal of controlled and targeted delivery. Objective of this project work is to increase the permeation rate and retention time in pilosebaceous gland by preparing nanostructured lipid carrier loaded gel and nanoemulgel of clobetasol propionate which results in increased permeation through psoriatic barrier skin cells, and improved local availability at the target site and less toxicity. The existence of squalene and fatty esters in the nanostructured lipid carrier tend to increase the drug uptake into the hair follicles. Better entrapment efficiency of drug results in improved therapeutic efficacy. The drug would be available at targeted site over an extended period of time which will lead to increased duration of action as well as minimize dosing frequency.