ABSTRACT

Background:

Psoriasis is a psychosocially, and on occasion therapeutically, weakening issue that influences 1 to 3% of the populace all around. It is an immune-mediated disorder with hyperkeratosis and other inflammatory reactions. It essentially includes deviant differentiation and exorbitant growth of keratinocytes. Infections including T helper 1 (Th1) and T helper 17 cells (Th17) are closely linked with the pathogenesis of psoriasis. Around 80% of patients who are suffering from psoriasis vulgaris are topically treated. Psoriasis can be categorized as mild, moderate and severe conditions. Mild psoriasis leads to the formation of rashes, and when it becomes moderate, the skin turns into scaly. In severe conditions, red patches may be present on the skin surface and become itchy. With the discovery of novel carriers, limitation that arises in the traditional topical pharmaceuticals for the treatment of psoriasis is bypassed with protected and long-term utilize. Novel carriers, for example, liposome, niosome, nanoemulsion, nanostructured lipid carriers (NLCs), microemulsion, emulsomes, dendrimers, nanoparticles, hydrogel and ethosomes have for sure conveyed us nearer to the objective of safe and effective treatment of the disease. Stratum conium (SC) is the major challenge for the drug to get into the target tissues, via skin layers. Penetration enhancers added in the drug carriers help to increase the penetration capacity of drug through the outermost layer of the skin. The most favorable drug delivery should provide high penetration through SC and should not cause any irreversible changes to the skin barrier. There are many challenges in the transdermal delivery of drugs. There will be variability in percutaneous absorption due to the site, disease, age, etc. The first pass metabolic effect of skin is also one of the challenges for topical delivery. Novel drug delivery systems have a lot of advantages. They increase safety and efficacy levels. Drug targeting specificity and lowering systemic drug toxicity are the important merits of NDDS. Moreover, they have the ability to improve absorption rates and will prevent the biochemical degradation of pharmaceuticals.

Methods:

In the present study Homogenisation method is used for the formulation of nanoemulsion and NLCs, Thin film hydration method is used for the preparation of Deformable liposomes. In the preparation of nanoformulations three steps are involved

which are Preparation of nanoemulsion, NLCs and Deformable liposomes second is preparation of hydrogel and finally these formulations will be incorporated into gel with continuous stirring.

Method of preparation of nanoemulsion (NE) and nanostructured lipid carrier (NLCs): The aqueous and lipid phases of nanoemulsions were fabricated separately. The aqueous phase consisted of double-distilled water and PF68 (NE- 3.2%, *w/v*, *NLCs*- 3.5%, w/v), The lipid phase consisted of different ratio of squalene, SPC and Glyceryl monostearate only in case of NLCs. was added. Both phases were separately heated to 85°C for 15 min. The aqueous phase was then added into lipid phase and mixed under homogenization at 12,000 rpm for 20 min. Subsequently, a probe-type sonicator set at a power of 25 W was employed to treat the mixture for 15 min. A 10-ml volume was prepared for each batch.

Method of preparation of deformable liposomes: Deformable liposomes were prepared considering the thin film hydration method. Briefly, egg-yolk phosphatidylcholine (EPC) and sodium cholate mixed in a proportion of 86:14% w/w (5% w/v of final lipid concentration) were dissolved in ethanol and mixed with an ethanol solution of the antioxidant α-tocoferol (16 μM of final concentration). The lipid film was obtained through the evaporation of the organic solvent in a rotary evaporator at room temperature and at a pressure of 40 mbar. To assure the elimination of residual traces of ethanol, the lipid film was left under high vacuum (Vaccuu brand GMBH+CO, VSP 3000- Germany) at least 3 h. The hydration of the dried lipid film was performed with 7% v/v ethanol in ultra-pure water and then the mixture was vortexed at 2400 rpm and above the phospholipid phase transition for 15 min, at room temperature. Afterward, the vesicles were incubated for 2h at room temperature to swell and then extruded, at room temperature and polycarbonate filters of successive 0.2 and 0.1 μm pore size (Whatman Int. Ltd) to produce a homogeneous suspension. The increase of the extrusion number did not lead to better properties of the lipidic suspension in terms of size distribution.

Preparation of gel: Gel was chosen as a vehicle for incorporation of nanosystems for skin delivery. Carbopol 940 (0.25 g) was dispersed in distilled water (100 ml) by stirring at 800 rpm for 60 minutes. The mixture was neutralized by drop wise addition of triethanolamine. Mixing was continued until a transparent gel appeared, while the amount of the base was adjusted to achieve a gel with pH 5.5.

Incorporation of nanoemulsion or NLCs or deformable liposomes into carbopol 940 solution: Carbopol 940 (0.4% w/v) was dispersed in distilled water by stirring at 800

rpm for 60 minutes. The mixture was neutralized by drop wise addition of triethanolamine. Colloidal suspension was added to the mixture with continuous mixing till a transparent gel appeared; the pH 5.5 of the gel was adjusted with the help of base (triethanolamine) and final gel was kept overnight for swelling.

In the present work Preformulation studies were performed such as Melting Point, FT-IR Spectroscopy, Solubility, Partition coefficient etc. then blank nanoemulsion were optimized for surfactant concentration, lipid ratio, Homogenization speed, Homogenization time, Sonication time, size, shape, PDI then the optimization of drug loaded naoemulsion were done Centrifugation Test, Zeta Potential Determination, Globule Size and Polydispersity Index, motic microscopic image after that Optimization and characterization of Gel were performed on the basis of gelling concentration, pH, stability and percentage drug content. Percentage release of clobetasol propionate loaded nanoemulsion in 10% methanolic PBS (5.5 pH) was also studied. Skin retention studies were also performed comparison of drug release of marketed formulation and test sample by *in – vitro* release is also done. Pharmacokinetic studies and histopathological studies of animal (rats) have done.

Results:

Prepared nanoemulgel, NLCs loaded gel and deformable liposomes acting as carrier for low as well as high molecular weight drugs. Nano formulations 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%. 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 (72.56 ± 1.94%) > Nanoemulgel (66.83±2.05%) > deformable liposomes (61.67± 2.05%) > Topinate gel (57.67±1.63%). NLCs loaded gel showed 25.82% and nanoemulgel

showed 15.88% and deformable liposomes showed 6.93% more release than marketed formulation but 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 and the %age skin retention (in 24 hrs) was found to be in following sequence Nanoemulgel (63±1.28%) > NLCs (62.71 ± 3.09%) > deformable liposomes (34.12% ±2.18%) > Topinate gel (23.12 %) which shows that nanoemulgel showed 2.7 fold, NLCs loaded gel showed 2.7 fold and Deformable liposomes showed 1.4 fold retention in skin as compared to marketed gel. Because of this depot action in sebaceous gland and very small part of it will reach in the blood the cmax of the formulations was found to be Nanoemulgel (1.932 mcg/mL) > NLCs (2.063 mcg/mL)> Topinate gel (9.925 mcg/mL). This shows that because of presence of squalene in nanosystems they show depot action in sebaceous glands and there is 80.5% and 79.21% decrease in drug content in blood of nanoemulgel and NLCs loaded gel respectively than marketed formulation. 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.

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.