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Fabrication, optimization and characterization of Triamcinolone acetonide loaded nanostructured lipid carriers for topical treatment of psoriasis: Application of Box Behnken design, *in vitro* and *ex vivo* studies

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ABSTRACT

Psoriasis is a highly inflamed, chronic, autoimmune skin disorder affecting 2–5% of the world population. Complete cure for psoriasis is still lacking and there remains a substantial challenge for world health systems to explore a new drug moiety or delivery system which could safely and effectively manage psoriasis without compromising patient compliance. Present work was aimed to develop, optimize and investigate the potential of nanostructured lipid carriers (NLC) for secure and efficient delivery of Triamcinolone acetonide (TA). TA loaded NLCs were effectively fabricated by modified microemulsion method and examined for particle size, zeta potential, polydispersity index, drug entrapment efficiency, drug loading, transmission electron microscopy, X ray diffraction and Differential scanning calorimetry study. Release study demonstrated prolonged TA release from NLCs following Higuchi release kinetics with $r^2 = 0.995$, while pure TA suspension showed quicker drug release obeying Zero order kinetics with R^2 value of 0.993. *In vitro* skin distribution studies demonstrated the presence of significant quantity of TA on the epidermis when treated with TA loaded NLCs suspension. Adverse side effects linked with systemic exposure might be removed by selective drug accumulation in the epidermis. Conclusively TA loaded NLCs might be a efficient carrier for effective management of psoriasis.

1. Introduction

Psoriasis is a T cell mediated autoimmune disorder characterized by inflammatory, red, scaly patches that constantly shack its scale due to over growth of epithelial cells [1]. Unpredictable remissions and reversions take place in psoriasis for lifetime which makes the condition of psoriasis patient very pity. The condition gets pathetic day by day thereby influencing the manner a patient view himself and the manner he is observed by others. Furthermore pain, distress, physical discomfort and psychological distress are also key hurdles faced by patients suffering from psoriasis [2].

The treatment option for psoriasis varies depending on the extent and sternness of diseases. However, topical medications remain the bastion of psoriasis treatment. Among topical medications, corticosteroids are recurrently used worldwide for effectual management of psoriasis. Conventional formulations including sprays, powders, lotions, solutions, emollient creams, ointments, gels, creams, medicated tapes etc are accessible for psoriasis treatment. But serious cutaneous and systemic side effects associated with corticosteroids have limited their use though they still stay the most effective drug [3].

Lack of safe and efficient management of psoriasis using conventional approach has directed to develop and implement novel approach for psoriasis treatment resulting into more convenient, acceptable and suitable treatment. Nano structured lipid carriers (NLCs) are the new invention of lipid nanoparticle gaining gigantic attention as novel colloidal carriers for topical drug delivery. NLCs are comprised of solid matrix surrounding liquid lipid inside which the drug is diffused [4,5]. Negative aspect linked with solid lipid nanoparticles such as modification of drug release, inadequate drug loading capacity and drug liberation while storage condition might be avoided by using this new generation lipid carrier [6,7].

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NLCs present numerous merits such as extended drug release, shielding of active drug moiety and insignificant skin irritation. In addition, tiny sizes of NLC assure the seal contact of nanoparticles with the outermost layer of skin i.e. stratum corneum thereby ensuring enhanced penetration of encapsulated agent into the skin. Since biodegradable lipids are principal constituent of NLCs, they offer low toxicity and outstanding acceptability for topical application. Furthermore NLCs have proved their suitability for dermatological purpose especially for the management of skin ailments [8,9].

Triamcinolone acetonide (TA) is mainly used to treat skin diseases like psoriasis. Vasoconstrictive, immunosuppressive, anti-inflammatory and antiproliferative effects of TA suggest its clinical competence for the treatment of psoriasis [10].

Though, adverse effects and dose dependent side effects such as steroidal acne, dermal atrophy, skin irritation, itching, decreased pigmentation, allergic contact dermatitis etc restricts the use of systemic and topical glucocorticoids for dermatological disorders [11,12].

Currently, extensive exploration is being carried out to accomplish better benefit-risk ratio of glucocorticosteroids by employing novel drug delivery approach. Among various novel drug delivery carriers NLCs might be a probable carrier for accomplishing increased solubility as well as therapeutic concentration of drug to the aimed location. NLCs permits prolonged drug release at the target site thereby minimizing dose dependant side effects and dosing frequency. Thus exploring therapeutic potential of TA loaded NLCs for topical route seems valuable.

Present work deals with fabrication and optimization of nano structured lipid carriers using design expert software and to explore the *in vitro* characterization of the optimized formulation for particle size, zeta potential (ZP), drug entrapment efficiency (EE), transmission electron microscopy, powder X-ray diffraction (PXRD), Differential Scanning Colorimetry (DSC) and drug release. Furthermore, extent of ex vivo distribution of drug across skin and stability issue of the fabricated formulation was also investigated.

2. Materials and methods

2.1. Materials

TA was a kindly gifted by Ciron drugs Pvt Ltd (India). Compritol® 888 ATO was received from Gattefossé (France). The dialysis membrane (Molecular weight cut off 10,000–12,000 Da) was obtained from HiMedia Laboratories (India). Acetone, methanol and miglyol 812 were obtained from Sigma Aldrich (India). Purified water used during the study was supplied by ultra-pure water system (Synergy UV water purifier system, India) was.

2.2. Preparation of TA loaded NLC

Modified emulsification–ultrasonication method was used to fabricate TA loaded NLCs [13]. In brief, weighed amount of Compritol® 888 ATO, Miglyol®812 and the drug were dissolved into mixture of methanol and acetone (1:1 v/v) in a water bath placed at 70 °C. Aqueous phase was prepared using varying concentration of poloxamer 188 and heated up to 70 °C. The resultant mixture was poured drop by drop into the molten lipid phase under high speed homogenizer (Ultra turrax basic IKA 10) at 10000 rpm for 10 min.

Pre-emulsion thus prepared was sonicated (Frontline sonicator, India) for 10 min. The resultant pre emulsion obtained was then cooled at room temperature. NLCs were separated by centrifugating the NLCs dispersion at 12,000 rpm for 30 min. The nanocarriers obtained were further dispersed in distilled water and lyophilized for 24 h (Heto power dry LL 3000 Lyophilizer, Stuttgart, Germany).

2.3. Experimental design

Process optimization was done employing response surface methodology of Design-Expert software (Trial Version 10, Stat-Ease Inc., MN). Box-Behnken design having 17-run, 3-factor, 3-level specification was used for optimization. The experimental design comprised of replicated center points and a set of points present at the midpoint of every periphery of multidimensional cube. It represents the region of concern for investigating the key effects, interaction effects, quadratic effects of the formulation ingredients and for further optimization of the formulation. The quadratic model produced by the experimental design was:

$$Y = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_4X_1X_2 + A_5X_2X_3 + A_6X_1X_3 + A_7X_1^2 + A_8X_2^{\ 2} + A_9X_3^{\ 2} \quad (1)$$

Y signifies measured response of dependent variables linked with each factor-level combination; A_0 to A_9 are the regression coefficients of the respective variables and their interaction terms calculated from observed experimental results of measured response. X1, X2 and X3 represent the codes for independent variables. Independent variables were X_1 = Liquid lipid concentration: Total lipid concentration (LLC: TLC), X_2 = surfactant concentration (SC) and X_3 = drug concentration (DC). Independent variables were designated by level −1, 0 and + 1 which corresponds to the low, middle, and high values respectively (Table 1). The measured responses Y1 = particle size (PS) and Y2 = entrapment efficiency (% EE) with limit used for optimizing TA loaded NLCs has been shown in Table 1.

Box-Behnken design used for optimization has been shown in Table 2. Moreover 3D response surface graph were also plotted for presentic the influence of the predetermined factors over calculated responses. 3D plots are useful for exploring the relationship between independent variables and responses/dependent variables. Careful observation might allow observing the qualitative effect of every variable over every response parameter.

2.4. Mean particle size and polydispersity index (PDI)

The mean particle size and PDI of TA loaded NLCs were measured by Malvern Zetasizer ZS 90 (Malvern Instruments Inc., UK). Samples were prepared by dissolving drug loaded NLCs in adequate quantity of ultra purified water prior to the conduct of experiment.

2.5. Zeta potential measurement

Zeta potential (ZP) is nothing but the electric charge on the surface of particle. It is a prime restraint to envisage the physical

Table 1
Variables and their levels in the Box-Behnken design for optimization of TA loaded NLCs.

Variables	Levels		
	−1	0	+1
Independent variables			
X_1 = LLC:TLC	3:11	4:11	5:11
X_2 = Amount of surfactant (w/v)	1.5	2	2.5
X_3 = Amount of drug (w/v)	0.050	0.1	0.15
Dependent variables			Constraints
Y1 = Particle size (PS)			Minimize
Y1 = Entrapment efficiency (%EE)			Maximize

Where, w/v = weight/volume; LLC:TLC = Liquid lipid concentration: Total lipid concentration.