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Design, characterization and skin permeating potential of Fluocinolone acetonide loaded nanostructured lipid carriers for topical treatment of psoriasis

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ABSTRACT

The aim of the current study was to develop and optimize Fluocinolone acetonide (FA) loaded nanostructured lipid carriers (NLC) and to evaluate its potential as topical delivery system for management of psoriasis. FA loaded NLCs were successfully developed by modified microemulsion method and optimized using 3-level Box–Behnken design. NLCs were evaluated for particle size, polydispersity index, zeta potential, drug entrapment efficiency and drug loading. Further X-ray diffraction (XRD) and Differential scanning calorimetry (DSC), *in vitro* release, *in vitro* skin distribution and stability study were also performed. Transmission electron microscopy confirmed spherical shape of prepared NLCs. Complete encapsulation of drug in the nanoparticles was confirmed by XRD and DSC. Release study showed prolonged drug release from the NLCs following Higuchi release kinetics and Zero order release kinetics, whereas pure FA suspension exhibited faster drug release following Zero order release kinetics with R^2 value of 0.995. Stability study confirmed that NLCs were stable for 3 months at 4 °C. Furthermore, *in vitro* skin distribution studies showed presence of significant amount of FA in the epidermal and dermal layer of skin when treated with FA loaded NLCs suspension while plain FA suspension showed significantly lesser amount of FA in the epidermis and dermis. Moreover, selective retention of FA in the epidermis might eliminate adverse side effects associated with systemic exposure. Thus FA loaded NLCs could be a potential system for psoriasis treatment but to create clinical value of the present system further studies are needed in clinically relevant models.

1. Introduction

Psoriasis is a T cell mediated autoimmune, inflammatory skin disorder differentiated by red, scaly and raised patches [1]. In psoriasis, erratic remissions and reversions occur for a lifelong period. Moreover it is coupled with pain, distress, physical disability and psychological discomfort [2,3].

The treatment of psoriasis varies depending on disease severity and spread. However, topical medications remain the mainstay of psoriasis treatment for most patients. Topical corticosteroids are frequently used for the management of psoriasis for decades. Numerous formulations including powders, sprays, lotions, solutions, creams, emollient creams, ointments, gels, medicated tapes etc are available for psoriasis treatment. However, serious cutaneous and systemic side effects are one of the major concerns with the use corticosteroids [4].

Recently, nano structured lipid carriers (NLC) which are lipid nanoparticles or colloidal carriers have been explored as potential topical delivery vehicle. NLCs has been reported to offer several advantages over conventional topical products owing to their ability to prolong the drug release, mitigate skin irritation, and protect of drug from potential degradable opportunities. Additionally, the high specific surface area of the particles ensures excellent contact with the affected site on the skin, facilitating the transfer of drug more efficiently [7]. NLC is constituted from a solid matrix enclosing liquid lipid inside which the drug is diffused [5,6]. Drawbacks coupled with solid lipid nanoparticles such as limited drug loading capacity, adjustment of drug release, and drug discharge during storage might be prevented by the application of this new type of lipid carrier. Generally, since NLCs are prepared using biodegradable lipids, they are safe and highly tolerable in patients.

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Fluocinolone acetonide (FA) is a synthetic hydrocortisone derivative which is primarily used in dermatology to reduce skin inflammation for the management of skin disorders like psoriasis. Their clinical success for the management of psoriasis is attributed to their vasoconstrictive, anti-inflammatory, immunosuppressive, and antiproliferative property [8].

However, in dermatological application, systemic and topical administration of topical glucocorticoids have been restricted owing to their adverse effects and dose dependent side effects such as dermal atrophy, steroidal acne, reduced pigmentation, allergic contact dermatitis etc.

Currently, researches are being focused toward the development of new approach in order to get enhanced benefit-risk ratio of glucocorticosteroids. NLCs could be a potential delivery carrier for improving solubility and enhancing therapeutic concentration of FA to the target tissue. NLCs permits prolonged release of the drug at the target and minimize dose dependant side effects. Therefore exploring the potential of NLCs loaded with FA appears valuable.

The aim of the present work was to develop and optimize 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, the extent of *in vitro* FA delivery into the epidermis and dermis and stability of the developed formulation were also evaluated.

2. Materials and methods

2.1. Materials

FA was generous gift from Encube Ethicals Pvt. Ltd. (Mumbai, India). Compritol® 888 ATO was kind gift from Gattefossé (France). Methanol, acetone, polysorbate 80, and miglyol 812 were purchased from Sigma Aldrich (India). Purified water from ultrapure water system (Synergy UV water purifier system, India) was used throughout the study.

2.2. Preparation of FA loaded NLC

NLCs were developed by modified microemulsion method [9]. Weighed quantity of Compritol® 888 ATO, Miglyol®812 and the drug were dissolved into 5 ml of mixed organic solvent of methanol and acetone (1:1, v/v) in a water bath at 70 °C. Aqueous phase containing varying concentration of polysorbate 80 was also heated up to 70 °C and added drop by drop into the molten lipid phase under high speed homogenizer (ultra turrax IKA 10) having 10,000 rpm for 5 min. Hot pre-emulsion thus obtained was sonicated (Frontline sonicator, India) and then cooled at room temperature. Nanoparticles were obtained by centrifugation of NLCs dispersion at 12,000 rpm for 30 min. The NLCs pellet thus obtained were redispersed in distilled water.

2.3. Experimental design

In the present work, response surface methodology using a 17-run, 3-factor, 3-level Box–Behnken design was employed to optimize the process. Design-Expert software (Trial Version 9, Stat-Ease Inc., MN) was used for experimental designing. The design consisted of replicated center points and a set of points lying at the midpoints of each edge of the multidimensional cube. This defines the region of interest used to evaluate the main effects, interaction effects, and quadratic effects of the formulation ingredients, and to optimize the formulation. The quadratic model generated by the design was:

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

In which Y is the measured response of the dependent variables associated with each factor-level combination; A_0 to A_9 are the regression coefficients of the respective variables and their interaction terms computed from the observed experimental values of measured response. X_1, X_2 and X_3 are the codes for independent variables. Independent variables were concentration of lipid (X_1), concentration of surfactant (X_2) and the concentration of drug (X_3) which were represented by level −1, 0 and +1, which corresponds to the low, middle, and high values respectively (described in Table 1). The measured responses Y_1 = particle size (PS) and Y_2 = entrapment efficiency (%EE) with constraints applied are described in Table 1.

Box–Behnken design employed for the study has been shown in Table 2. Further 3D response surface graph were plotted for depicting the effects of the predetermined factors on the measured responses. 3D response surface plots are helpful in explaining the relationship between independent variables and responses/dependent variables. When these plots are carefully observed, the qualitative effect of each variable on each response parameter could be visualized.

Table 1
Variables and their levels in the Box–Behnken design.

Variables	Levels		
	−1	0	+1
Independent variables			
X_1 = Liquid lipid: Total lipid	2:11	3:11	5:11
X_2 = Amount of surfactant (v/v)	1.5	2	2.5
X_3 = Amount of drug (w/v)	0.025	0.50	0.75
Dependent variables			Constraints
Y_1 = Particle size (PS)			Minimize
Y_1 = Entrapment efficiency (%EE)			Maximize

Table 2
Box–Behnken design for the study of three experimental factors in coded and actual levels with experimental results.

Formulation code	Actual value of variables			Response values	
	X_1	X_2	X_3	Particle size (nm)	Entrapment efficiency (%)
F1	5:11	2.5	0.50	218.32	82.21
F2	3:11	2.5	0.75	158.09	94.12
F3	5:11	2	0.75	232.44	78.31
F4	3:11	2	0.50	172.57	88.72
F5	2:11	2	0.25	164.16	74.11
F6	3:11	2	0.50	169.3	87.82
F7	5:11	2	0.25	222.28	80.21
F8	3:11	1.5	0.75	182.52	91.96
F9	3:11	1.5	0.25	179.81	89.31
F10	2:11	2.5	0.50	141.18	73.97
F11	3:11	2	0.50	182.92	87.72
F12	2:11	2	0.75	162.08	74.97
F13	3:11	2	0.50	172.81	88.12
F14	3.11	2	0.50	179.18	89.76
F15	5:11	1.5	0.50	249.61	76.18
F16	3:11	1.5	0.25	151.64	90.14
F17	2:11	1.5	0.50	170.41	70.98

Where, X_1 = Ratio of Liquid lipid: Total lipid, X_2 = Amount of surfactant (v/v) and X_3 = Amount of drug (w/v).