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Thymoquinone loaded dermal lipid nano particles: Box Behnken design optimization to preclinical psoriasis assessment

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ABSTRACT

The present study was to formulate and optimize Thymoquinone (TQ) lipid nanoparticles (NPs) formulations for the treatment of psoriasis. The formulation optimization was performed using the independent variables [(gelucire (A), capmul (B), sonication time(C)] at three levels (low, medium and high) and their individual and combined effects were assessed on size (Z_1), poly dispersity index (Z_2) and drug encapsulation (Z_3). The results of the present study revealed that the point prediction based TQNPopt showed low particle size (84.22 ± 3.31 nm), PDI (0.26 ± 0.02) and high entrapment efficiency (81.3 ± 4.11%). The drug release and dermal permeation result showed slow drug release (57.55 ± 5.38%) and enhanced dermal flux (5.77 μg/cm^2/h). The TEM image and thermal analysis revealed sealed, spherical shape particle and the disappearance of the characteristic endothermic peak of TQ. The skin irritation study score revealed the primary irritation index score (1.4) and further PASI score confirmed the reduction in all the parameters (erythema, edema, and thickening) in psoriatic model in compare to the toxic control group. The present study data revealed that the developed lipid nano particles formulation was found to be a potentially useful dermal carrier for TQ.

1. Introduction

Psoriasis is a chronic, immune-mediated skin disease that can lead to hyper proliferation, poor differentiation of epidermal keratinocytes substantial morbidity and mortality [1]. It ranges in severity from a few scattered red patches to scaly plaques and may involve the entire body surface. About 2–5% population affected by psoriasis in the western countries [2,3], and the treatment regimen depends on spread and disease severity. The successful treatment for psoriasis may bring back to skin with a clinically normal condition i.e., reduction in inflammatory cells and resolution of epidermal thickness [4]. The topical application is the main approach for the treatment of psoriasis and the application of topical corticosteroids are most commonly used. There are serious cutaneous and systemic side effects are one of the major concerns with the use corticosteroids [5]. The treatment of psoriasis varies depending on disease severity and spread. However, the topical application remains the approach of psoriasis treatment for majority patients. From the last decades the management of psoriasis was done by the use of topical corticosteroids. However, serious cutaneous and systemic side effects are one of the major concerns with the use corticosteroids [2].

Thymoquinone (TQ), is a lipid soluble benzoquinone and the main active constituent of *Nigella sativa* [6,7]. It is very popular and widely used herb in traditional system of medicine and impart therapeutic benefits in several diseases and ailments [8,9]. Additionally, the clinical trial report of psoriatic patients suggests the safety and therapeutic efficacy of *Nigella sativa.* The results showed good response to 65% of patients while 31% of patients suffered from relapse after 4 weeks of treatment cessations [10]. The oral formulations of TQ have not been successful due to the poor solubility and low bioavailability. The majority of oral administered drugs frequently encounter bioavailability problems due to poor solubility, poor dissolution and lack of dose proportionality [11]. The patients generally become noncompliant when they required to follow frequent dosage regimen for the long period of treatment.

For dermal treatment, lipid nanoparticles systems are mostly preferred because it helps the drug molecule to effective retention/penetration to the skin. The lipid nano particles (NPs) have been widely used due to their better encapsulation efficiency and drug release. The differences in the structures of the solid and liquid lipids are the main

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reason for higher drug entrapment. The more drug particles can be entrapped between lipid layers and/or fatty acid chains [12,13]. Thus, the particles can accommodates the active in molecular form or in amorphous clusters [14,15]. The variation in lipid matrix lead to lesser drug expulsion during storage and greater drug release profile can be achieved. For dermal application it can easily adheres to the skin by forming thin film and subsequently lead to an occlusive effect with reduction in the transepidermal water loss [14].

The use of design of experiment approach of optimization gives the solution to develop an optimized formulation [15]. It is considered a cost-effective approach than other usual processes of formulation optimization because it requires fewer runs than traditional approach [16]. The present study was designed to develop Box Behnken Design (BBD) based dermal thymoquinone lipid nano particles (TQNP) to assess the therapeutic potential in psoriasis treatment. The optimized formulation TQNPopt was converted into gel (TQNPopt gel) and evaluated for gel characteristic, skin permeation, anti-psoriatic activity and histopathological assessment.

2. Material and methods

2.1. Material

TQ was obtained from Sigma Aldrich (Germany), gelucire 44/14, capmul MCM were received as gratis sample from “Gattefosse India Pvt. Ltd., (Mumbai, India)”. Poloxamer 188, Tween 80, Carbopol 934®, Polyethylene glycol-400, and triethanolamine were purchased from S.D. Fine Chemicals, and Loba Chemie, India, respectively. All other chemicals and solvent used were of analytical grade.

2.2. Formulation of thymoquinone lipid nanoparticles

TQNPs were prepared by the combined method of melt-emulsification and ultrasonication process as per the reported procedure [17], and the formulation composition as per BBD design shown in Table 2. The formulations were prepared with TQ (50 mg), lipid blend i.e. gelucire 44/14 and capmul MCM (3.6–4.6%) were mixed and put on a hot plate to melt at 55 ± 2 °C to obtain a homogeneous and transparent oil phase. In a separate beaker 10 mL of the aqueous surfactant solution (tween 80 and poloxamer 188; 1:1 ratio; 4–6%) was taken and heated to same temperature. The aqueous surfactant phase was added drop wise into the lipid blend with continuous stirring. A translucent coarse dispersion was prepared with stirring and agitation using the magnetic stirrer. Finally, the prepared coarse dispersion was treated by probe sonicator (UP100H; Hielscher Ultrasonics GmbH, Berlin) at 50W to get smaller size particle in ice condition [18]. The temperature of the formulation was maintained at 4 °C, and sonication (3 cycles) was performed with a pause of 5 min during each cycle. The prepared TQNPs were freeze dried at a low pressure (0.5 milibar) to get solid form. The sample was frozen in a freezer for 4 h and then subjected to the freeze drying process for 8–12 h using mannitol (3%) to avoid the lysis of the NPs.

2.3. Box–Behnken design optimization

The application of optimization technique in the formulation using the design of experiments (DOE) approach has become a regular practice [19]. There are different DOE approach has been reported for the optimization but among them, Box–Behnken design (BBD) was selected because it requires lesser runs than other designs [20,21]. There are many experimental studies reported on diverse nanosized formulations that showed the utility of this approach in development of optimized formulations. [22,23]. A three factor, three-level BBD approach was used to optimize the TQNPs, the different independent and dependent variables used for the formulation were depicted in Table 1. The fifteen experimental compositions with the dependent variables (low, medium and high) were shown in Table 2. The selection of the optimized thymoquinone lipid nano particles (TQNPopt) was selected on the basis of maximum entrapment efficiency, minimum particle size, and PDI value by applying the point prediction method.

Table 1
Box Behnken design based thymoquinone lipid nanoparticle formulations.

Independent Variables	Low level (−)	Mid level (0)	High level (+)
A = Mixed lipid concentration (%)	3.6	4.6	5.6
B = Surfactant mixture (%)	4.0	5.0	6.0
C = Sonication time (second)	60	120	180
Dependent variables			
Z_1 = particle size (nm)	low		
Z_2 = PDI	low		
Z_3 = entrapment efficiency (%EE)	high		

2.4. Particle size

The particle size and PDI of developed TQNPs was assessed by diluting the sample (100 times) using double distilled water and mixed thoroughly with vigorous shaking (Zetasizer HAS 3000, Malvern instruments, UK). The PDI value < 0.1 indicate homogenous population of particles, while PDI values (> 0.3) indicate high heterogenecity among the particles.

2.5. Entrapment efficiency

The entrapment efficiency of TQNPs formulation was assessed by ultracentrifugation method. The sample was centrifuged at 30,000 rpm for 60 min using “ultracentrifuge (Remi Instruments, Mumbai, India)” to separate free TQ from NPs. The free TQ in the supernatant was diluted, and drug content in each formulation was determined by measuring the absorbance at 254 nm using UV spectrophotometer (Shimadzu, 1601, Kyoto, Japan). The drug entrapment percentage was calculated using the below formula [24].

$$Entrapment\ \ efficiency\ \ (\%) = \frac{Winintial - W\ \ free\ \ drug}{Winitial\ \ drag} \times 100$$

2.6. Transmission electron microscopy

The morphology of the TQNPopt was measured using an electron microscopy (Morgani 268D, Eindhoven, Netherlands). A drop of the formulation dispersion was taken on a carbon-coated copper grid and allowed to keep for 10 min for air drying. The sample was stained with phosphotungstic acid (1% w/v), excess was removed and the sample was visualized using soft imaging viewer software.

2.7. Thermal analysis

DSC analysis of TQ and lyophilized TQNPopt were carried out using DSC instrument (Pyris 6 DSC, Perkin Elmer, Massachusetts, USA). Each sample was sealed in a small aluminium pan and scanned in the range of 20–350 °C at 10 °C min^{-1} heating rate. The empty pan was used as a reference standard and inert nitrogen gas was inserted at a flow rate of 20 mL/min in the whole study.

2.8. Evaluation of TQNP loaded gel

The optimized formulation TQNPopt was converted to carbopol 934 (1%w/v) based gel for easy application. The gel was prepared by dispersing carbopol934 in distilled water with stirring for complete swelling of gelling agent. The carbopol sample was sonicated for 15 min to remove air bubbles, then triethanolamine (neutralizing agent) and

Table 2
Box Behnken design based Thymoquinone lipid nano particles with independent and dependent variables.

Code	Independent variables			Dependent variables		
	Total lipid concentration (%w/v)	Surfactant concentration (%w/v)	Sonication time (min)	Particle size (nm)	PDI	Encapsulation efficiency (%)
F1	–	+	0	108.34 ± 0.22	0.21 ± 0.2	68.5 ± 1.8
F2	0	0	0	92.3 ± 0.78	0.24 ± 0.8	71.9 ± 2.3
F3	–	0	–	88.5 ± 0.34	0.26 ± 0.4	70.5 ± 2.2
F4	0	–	–	94.5 ± 0.23	0.35 ± 0.1	69.5 ± 1.7
F5	+	–	0	101.5 ± 0.21	0.34 ± 0.4	62.5 ± 1.5
F6	0	0	0	91.3 ± 0.34	0.23 ± 0.8	72.3 ± 1.8
F7	0	–	+	88.9 ± 0.36	0.29 ± 0.1	69.8 ± 1.4
F8	+	0	+	71.1 ± 0.87	0.31 ± 0.3	63.5 ± 1.7
F9	+	+	0	82.8 ± 0.87	0.32 ± 0.4	66.5 ± 1.9
F10	0	0	0	92.9 ± 0.12	0.24 ± 0.7	72.9 ± 2.2
F11	0	+	+	89.6 ± 0.23	0.19 ± 0.5	81.9 ± 2.5
F12	–	0	+	92.1 ± 0.98	0.23 ± 0.8	71.3 ± 2.7
F13	0	+	–	74.1 ± 0.55	0.27 ± 0.5	77.1 ± 2.8
F14	–	–	–	94.2 ± 0.87	0.25 ± 0.2	79.7 ± 2.5
F15	+	0	–	80.2 ± 0.45	0.25 ± 0.7	82.3 ± 2.6

sodium benzoate (preservative) was added to obtain homogenous gel dispersion. Finally, the optimized TMQ lipid nano particle (TQNPopt) was added to the above gel base with gentle stirring to get the TQ lipid nano particles gel (TQNPopt gel). The same procedure is followed to prepare the TQ control gel (without NPopt).

2.9. Homogeneity and pH

The prepared optimized TQ lipid nano particles loaded gel (TQNPopt gel) was evaluated for homogeneity by visual inspection after the gel has been set in the container. The apparent pH was measured by pH meter in triplicate at 25 ± 1°C using digital pH meter (Mettler Tolledo, Japan) fitted with a glass microelectrode by allowing it to equilibrate for 1 min.

2.10. Spreadibility

The spreadibility was evaluated on the basis of the slip and drag characteristic of the gel. A modified apparatus consists of two glass slide with the lower side fixed to a wooden plate and upper one attached to a balance by a hook. The sample gel (1 gm) kept between the two glass slides and weight is applied to the upper plate. The change in the spread diameter due to spreading of the gel by application of weight was noted (n = 3) [25]. The spreadibility was calculated using the following formula

$$S = W*L/t$$

Where, S represents the spreadibility (g/sec), W is the weight in pan (gm), 'L' is the fixed length moved by the glass slide and 't' is the time (sec) required to separate the slides completely.

2.11. Texture analysis

The texture analysis of TQNPopt gel was characterized for the various textural parameters like firmness, cohesiveness, consistency, and index of viscosity. The formulation TQNPopt gel was placed into the identical glass jar and the testing surface was kept as flat as possible to avoid early triggering of the test [26]. The sample was introduced with care to avoid the introduction of air bubbles into the sample. The texture profile analysis was performed using a texture analyzer (Stable Micro System, UK) in the compression mode. The parameters are the resultant force-time plots, several mechanical parameters of the gels were determined.

2.12. Drug release study

The comparative TQ release study was carried out by placing TQNPopt gel and TQNPopt (containing equivalent to 5 mg of TQ) formulation in the pre-activated dialysis membrane (molecular weight 12000 D). This study was performed with phosphate buffer (250 mL) at 600 rpm and the temperature was maintained at 37 °C ± 0.5 °C. At different time intervals aliquots (2 mL) were withdrawn and replaced with equal volume of release media to maintain sink conditions. The sample was further filtered, diluted and analyzed by using UV-spectrophotometer at 254 nm. The drug release data of TQNPopt gel were fitted to various kinetic models like zero-order, first-order, Higuchi's model and Korsmeyer-Peppas models to assess the release mechanism.

2.13. Skin permeation study

The excised abdominal rat skin was taken to assess the permeation study using diffusion cells with an effective diffusion area of 1 cm^2 for 24 h. The hair was removed from skin and mounted between the donor (epidermis side facing the donor compartment) and receptor compartment of diffusion cell filled with phosphate buffer saline (pH 7.4) as diffusion media (10 mL) [27]. The both sample TQNPopt gel and TQGel (control) containing 5 mg of TQ was filled in the donor compartment. The receptor compartment was continuously stirred with the magnetic stirrer and temperature was maintained at 37 °C ± 0.5 °C during the whole study. At predetermined time intervals, aliquots (0.5 mL) were withdrawn and replaced with an equal volume of fresh PBS. The sample was filtered, diluted and analyzed for the drug content using HPLC [28].

2.14. In-vivo study

The albino rats (200–250 gm) were procured from the CAHF of Jamia Hamdard (Deemed University), New Delhi, India. The approval to carry out the in vivo studies was obtained from the IEAC, Jamia Hamdard, New Delhi and their guidelines were followed for studies. The animals were supplied free access to standard laboratory diet and water ad libitum and kept under standard laboratory conditions.

2.15. Skin irritation study

The comparative skin irritation study was assessed to check the irritation potential developed TQNPopt gel and standard irritant (formalin solution, 0.8%v/v) on albino rats and score were given using Draize score test [29]. The application sites were graded according to a visual scoring scale shown in Table 5. The rats were divided into 2

groups, Group I was treated with TQNPopt gel, and Group II received formalin as a standard irritant. Both the samples were applied to the back side of hair free rat skin, the animals were examined for irritation by giving visual scoring for erythema and edema. An adjacent area of untreated skin was served as a control. The score of primary irritation (SPI) for the control sites were calculated in the same manner as of test formulation. The difference between the summation of SPI scores of each group from the treated site and control site were calculated and were used for Primary Irritation Index (PII) determination.

2.16. Imiquimod (IMQ) induced psoriatic plaque like model

This psoriatic plaque like model was developed as per reported procedure [30–32]. IMQ was used to induce psoriatic plaque like model and the rats received a daily topical dose of 12.5 mg of commercially available IMQ cream 5% (Imiquad Cream, Glenmark) on the nude back for 6 consecutive days. For psoriasis assessment, the symptoms like the affected skin were treated twice daily with dermal TQNPopt gel (50 mg/cm^2) and standard marketed clobetasol propionate cream (Tenovate M cream; 40 mg/cm^2). The severity score of inflammation on the rat back skin was used as an objective scoring system basis of the clinical Psoriasis Area and Severity Index (PASI). Erythema, scaling, and thickening were scored independently on a scale from 0 to 4 as shown in Table 5. The animals were sacrificed and skin sections (5 μm thickness) were collected. The samples were fixed on the slide and stained with hematoxylin/eosin dye for clear visibility. These samples were observed in the light microscope (Motic digital microscope, DMB series) and compared with control skin sample [16].

3. Results and discussion

Box Behnken Design (BBD) with three-factors, three levels used for the optimization process of TQNPs. The design showed total fifteen formulations with three replicated points (Table 2). The quadratic model was found to be the most fitted model for all the responses. The three dimensional plots representing comparative effects of independent variables on each three responses are shown in Fig. 1(A–C). These three dimensional plots were known to evaluate the interaction effects of the individual factor on the every response and also useful in evaluating the effects of two factors on each response. The ranges for the observed responses (particle size, PDI and entrapment efficiency) were found between 71.1 and 108.34 nm, 0.19–0.35 and 62.5–82.3%, respectively.

3.1. Effect of independent variables on particle size

The three-dimensional plot (Fig. 1A) showed the individual and combined effects of independent variables on the particle size (Z_1). The variation in lipid blend concentration (A) affects the particle size of NPs formulation. As the ratio of solid lipid to liquid lipid decreases the particle size increased. At higher concentration of liquid lipid the particle size reduces due to the decrease in the viscosity of internal phase. The increase in surfactant concentration decreases the particle size due to the reduction in interfacial tension between the aqueous and lipid phases, leading to the formation of emulsion droplets of smaller size [33]. The higher surfactant concentration stabilized the particles by forming a stearic barrier on the particle surface and protects the particles to form coalescence into bigger ones [34]. The increase in sonication time leads to reduction in the particle size. At higher sonication time the breakdown of particles takes place. So the intermediate sonication time will be ideal to get the desired particle size range. The particle size range of all developed 15 formulations showed size range of 71.1–108.34 nm, which is primarily suitable for topical delivery [35,36].

3.2. Effect of independent variables on PDI

The PDI used as a measure of a unimodal size distribution and all formulations showed PDI value within the acceptable limits. The lower value of PDI indicates a homogenous population, while a higher PDI indicates high heterogeneity. The result of the study indicates that the PDI of all the fifteen formulations were in the range of 0.19–0.29 (Z_2). The wide variation in the PDI value achieved due to the effect of different independent variables used for the formulations. The 3D response surface plots (Fig. 1B) showed the interaction effect of independent variables on PDI. The positive variation in surfactant concentration and sonication time leads to an increase in PDI, while sonication time showed lesser effect than total lipid concentration. As the sonication time increase there was also an increase in PDI due to non-uniformed reduction in the particle size or may be due to some particle growth. The higher concentration of surfactant produces high PDI that may be due to production and stabilization of smaller particles.

3.3. Effect of independent variables on entrapment efficiency

The all independent variables have shown their effect on the encapsulation efficiency (62.5–82.3%) (Table 2). The interaction effects of independent variables on EE (Z_3) were shown on 3D response surface plots (Fig. 1C). It has been observed that the entrapment efficiency of TQ had been increased with an increase in the percentage of total lipid concentration. The entrapment efficiency increases as the ratio of liquid lipids into solid lipids increases which may change the crystal order of lipid blend. The higher imperfections in the crystal order give enough space to entrap the drug molecules, which ultimately improved drug entrapment efficiency. The entrapment in the formulation containing higher liquid lipid content indicates higher solubility of the drug which helps in improving EE. The total surfactant concentration also significantly affected the EE, as the surfactant concentration increases there is a gradual decrease in the entrapment efficiency was observed. The decrease in entrapment efficiency could be explained by partition phenomenon. At high surfactant concentration in the external phase lead to an increase in the partition of the drug from an internal to external phase of the medium [34].

3.4. Point prediction

The responses of all 15 formulations were fitted to different kinetic orders and the best-fitted model for the developed formulations was found to be quadratic. The quadratic model is highly desirable for the formulation as all the individual and combined independent variables significantly affects the dependent variables. The results of actual experimental value and the predicted experimental value (shown by software) were found to be very close and linear to each other. The optimized formulation TQNPopt was selected on the basis of the criteria of attaining the minimum particle size, and PDI with maximum entrapment efficiency by applying point prediction method of the software. The formulation composition with total lipid concentration (4.8%), surfactant mixture concentration (5.2%) and sonication time (110 s) was found to fulfill requisites of an optimum formulation. The optimized formulation has the entrapment efficiency of 81.3 ± 4.11% with particle size and PDI of 84.22 ± 3.31 nm and 0.26 ± 0.07 (Fig. 2), respectively. On the basis of the BBD design approach, the optimized formulation TQNPopt was selected and further converted to gel analyzed for skin permeation study, in vivo activity and skin irritation study.

3.5. Transmission electron microscopy

The transmission electron micrograph of the TQNPopt formulation is depicted in Fig. 3. The micrograph revealed more or less spherical shape and the formation of well identified discrete particles with sharp

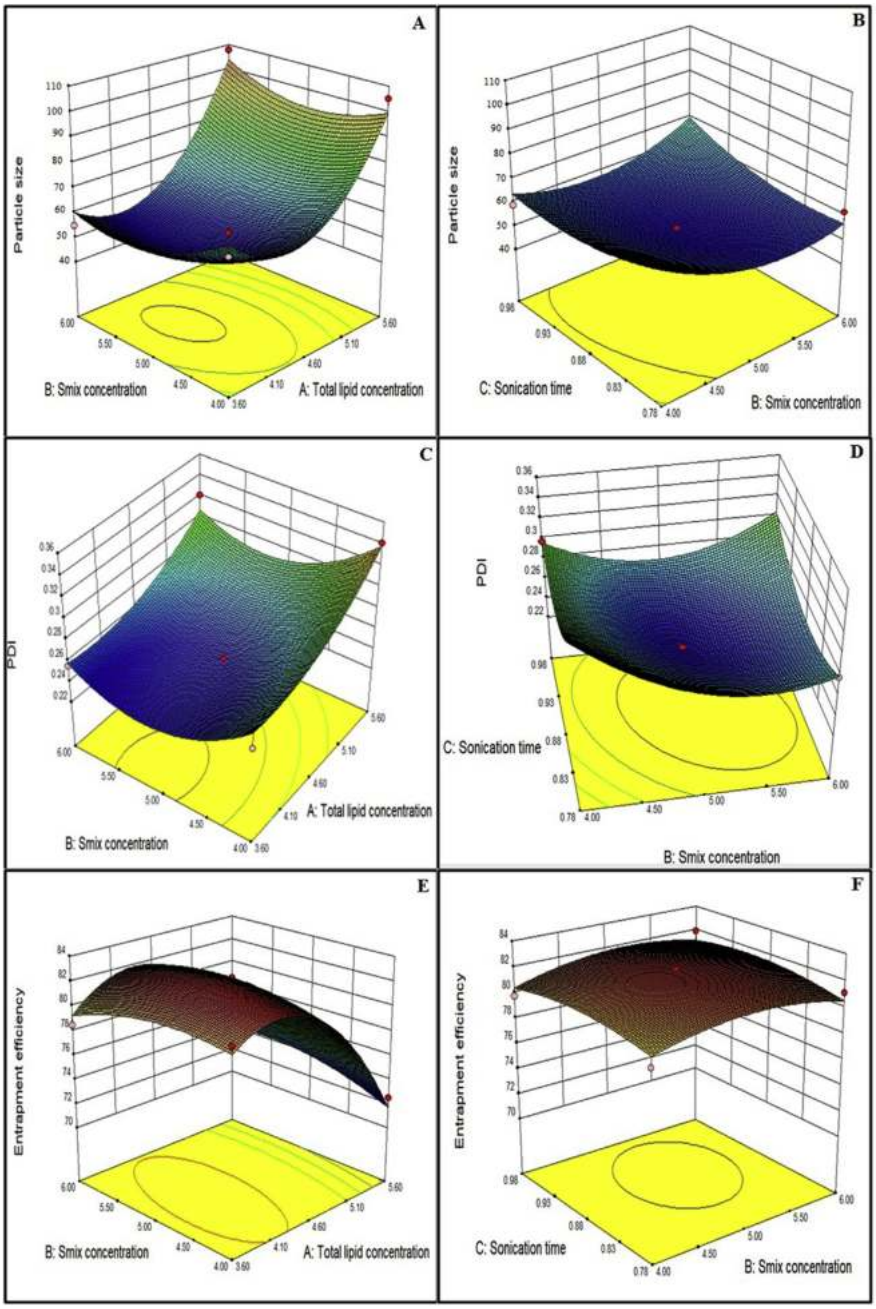

Fig. 1. Effect of independent variables (total lipid concentration; surfactant mixture and sonication time) on the particle size (Fig. 1A–B); PDI (Fig. 1C–D); encapsulation efficiency (Fig. 1E–F).

boundaries. The obtained results point out that the particles were evenly distributed, alienated from each other and shows the outline and core of the well identified particles.

3.6. Thermal analytical analysis

The changes in thermal behavior for TQ and TQNPopt were performed with DSC study and their image shown in Fig. 4(A–B). The thermal image of TQ showed a sharp characteristic endothermic peak at 48.29 °C, indicating its crystalline state of the drug and there was shifting of peak to 165.15 °C was observed in TQNPopt. The disappearance of the endothermic peak of TQ in the formulation might indicate the entrapment of the drug inside the lipid nano particles formulation [37].

3.7. Evaluation of TQNPopt gel

The developed TQNPopt dermal gel was assessed for various physical parameters and results were depicted in Table 3. The developed TQNPopt gel demonstrated a pleasant, smooth homogeneous appearance and was free from presence of any gritty particles. The pH of prepared dermal gel was found to be 6.85 ± 0.18, which are regarded as safe to avoid the risk of irritation upon application to the skin. The spreadability plays an important role in patient compliance and helps in uniform application at the site. The prepared gel formulation demonstrated the spreadability and extrudability of 16.87 ± 3.21 g cm/sec and 6.71 ± 0.9 g respectively.

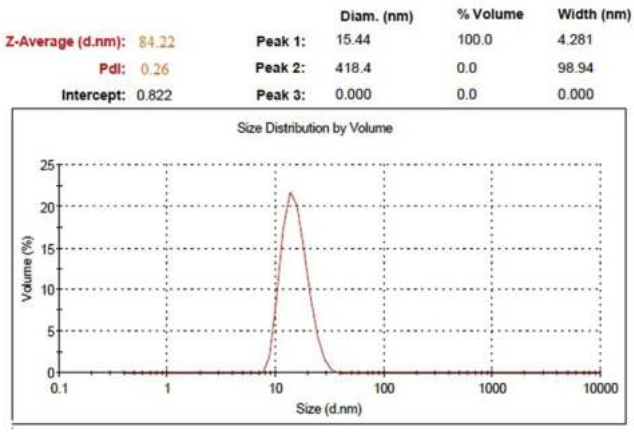

Fig. 2. Particle size and polydispersity index of optimized thymoquinone lipid nanoparticles.

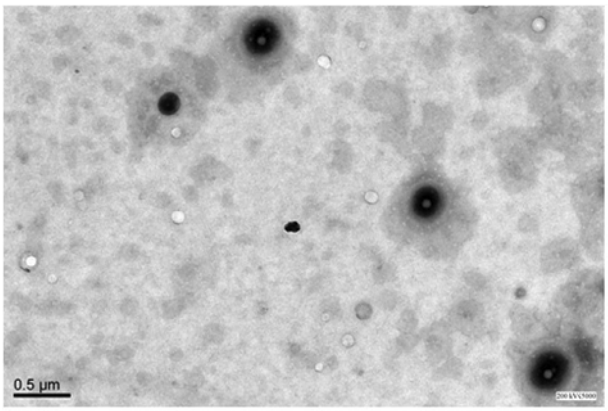

Fig. 3. Surface morphology of optimized thymoquinone lipid nanoparticles using TEM at scale bar (0.5 µm).

3.8. Texture analysis

The formulation TQNPopt gel was evaluated for texture analysis and the parameters such as cohesiveness, consistency firmness, and index of viscosity were evaluated. It was noted that the developed formulation showed cohesiveness (−360.58), consistency (2965.63), firmness (387.34), and index of viscosity (−1455.31) respectively for the developed topical gel formulation. The report of texture analysis obtained from software was presented in Fig. 5.

3.9. Drug release study

The comparative cumulative TQ release from TQNPopt gel and TQNPopt were calculated and found to be 57.55 ± 5.38 and 77.93 ± 4.66 after 24 h (Fig. 6). The formulation TQNPopt gel exhibited biphasic release behavior with the initial rapid release with slow release after 4 h. The presence of drug on the surface which may release quickly in the initial time and the slow release pattern due to the increased viscosity of gel or might be due to the entrapment of TQ in gel matrix, which is expected to hinder the process of drug diffusion from the lipid blend. The different rate controlling membrane like diffusion, swelling, and erosion are the most important mechanisms of formulations [38]. The drug release data were kinetically analyzed by using different mathematical models and the TQNPopt gel followed higuchi diffusion mechanism ($R^2 = 0.9991$) representing goodness of fit in terms of R^2 values followed by followed by the korsmeyer–peppas ($R^2 = 0.9663$), zero order ($R^2 = 0.9590$) and first-order ($R^2 = 0.9589$). The value of release exponent (n) for the proposed model was found to be less than 0.5 ($n < 0.5$), it implies that the NP loaded gel formulation follows fickian diffusion release mechanism.

3.10. Skin permeation study

The permeation profile across rat skin of TQNPopt gel yielded significantly higher ($p < 0.0001$) flux value i.e. 5.77 µg/cm^2/h, whereas TMQG (control) treated skin showed flux of 1.87 µg/cm^2/h. The formulation TQNPopt gel showed the enhancement ratio of 3.08 times higher than the control gel. The significant variation in the profile due to developed lipid nano particles comprised of lipids and surfactants and having nano size range. The presence of lipid and surfactant in the NPs not only offers stability to the preparation but also enhances the permeation of TQ by increasing the solubility. The nano size range particle able to penetrate into the deeper layer of skin very easily and produces greater absorption. It indicates that drug remains in the skin layers for longer period of time which is beneficial for dermal treatment of psoriasis.

3.11. Dermal irritation study

The dermal irritation score was recorded according to visual scoring scale presented in Table 4. The irritation study results indicated that TQNPopt gel treated animals showed slight skin irritation and there were not any significant changes in skin condition observed. The formulation TQNPopt gel treated group showed no erythema and edema with score (0.6 ± 0.02) and (0.8 ± 0.04). The standard irritant formalin solution treated skin showed higher irritation score for erythma (2.2 ± 0.38) and edema (3.4 ± 0.66). No obvious erythma, edema or inflammation was observed on skin after application of the dermal gel formulation whereas formalin solution showed significant skin irritation. The score of erythma and edema were added to calculate primary irritation index (PII) and the score [PII :- 1.4 and 5.6] was found for TQNPopt gel and standard irritant formalin solution. The compounds producing scores of 2 or less are considered negative [29]. The results of dermal irritation confirm their non-irritant nature as the score was found less than two. Hence, the developed dermal TQ lipid nano particles gel formulations are free of skin irritation.

3.12. Imiquimod (IMQ) induced psoriatic plaque like model

The psoriatic skin lesion shows scaling on the back of rat skin which is a typical phenomenon after topical application of IMQ. On day 2–4 after initiation of IMQ treatment, the treated rats began to show signs of thickening, erythema and scaling (very mild) on the back skin of the rat. The inflammation was visible and continuously increased up to day 5–7 in severity. After the application of TQNPopt gel treated group, there was observation of decrease in all the visible parameters. The control treated group also did not show any visible changes in all the parameters. The score in Table 5 showed the reduction in score of erythma, scaling and thickening from second day and on 4th day the score reaches to zero which matches with the control group. The standard treated group (Clobetasol) also shows reduction in all the parameters from the same day as the formulation treated group (Table 5). The cumulative score for both the standard (Clobetasol) treated and TQNPopt gel treated group was found to be closer. So from the scoring system of all groups, we can conclude that the formulation TQNPopt gel matches with the standard (Clobetasol) treated group. The toxic control group showed marked high scoring till all seven days. Further, the treated skin with TQNPopt gel and toxic control were assessed for internal changes by doing histopathology study. Both the treated skin were compared with untreated control skin and shown in Fig. 7(A–C). The image of control skin clearly shows the intact epidermis and viable dermis (Fig. 7A). The formulation TQNPopt gel treated skin did not reveal any change in the state of the skin (Fig. 7C). The thickness and appearance of the horny layer were found to be

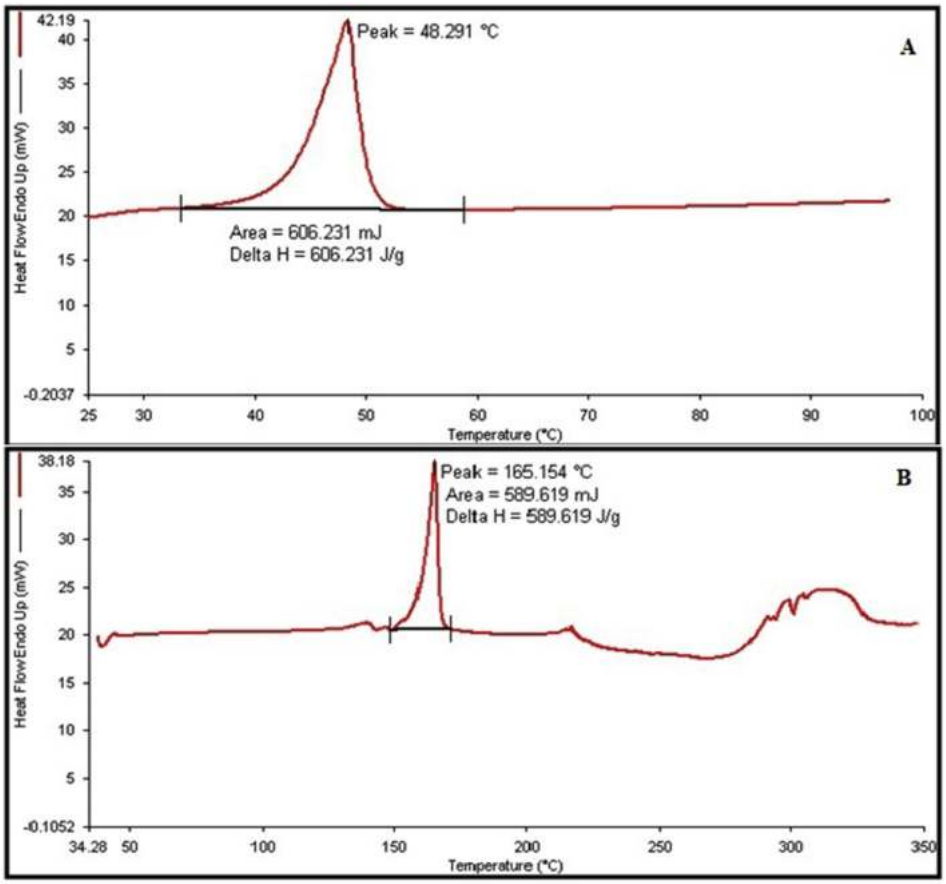

Fig. 4. Thermal analytical study profile of thymoquinone and optimized thymoquinone lipid nanoparticles.

Table 3
Physicochemical profile of optimized thymoquinone lipid nano particles loaded gel.

TQNPopt gel	Homogeneity	Drug Content (%)	pH	Spreadability (g.cm/sec)	Extrudability (gm)
	Good	93.35 ± 1.69	6.85 ± 0.18	16.87 ± 3.21	6.71 ± 0.9
	Cohesiveness (gm)	Consistency (gm.sec)	Firmness (gm)	Index of viscosity (gm.sec)	
	−360.58	2965.63	387.34	−1455.31	

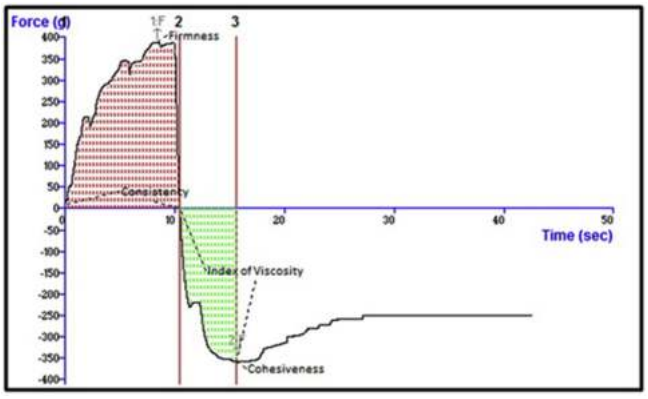

Fig. 5. Texture analysis (firmness, cohesiveness, consistency and index of viscosity) of optimized thymoquinone lipid nanoparticles loaded gel.

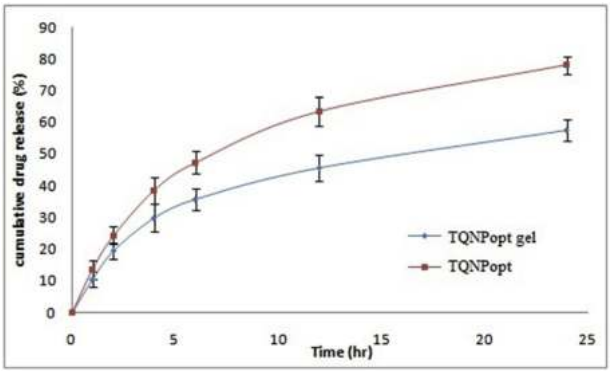

Fig. 6. Comparative drug release profile data of optimized thymoquinone lipid nanoparticles and optimized thymoquinone lipid nanoparticles loaded gel for 24 h.

Table 4
Average primary irritation index score of treated groups.

Animal	Control		TQNPopt gel		Formalin (0.8%)	
	Erythema	Edema	Erythema	Edema	Erythema	Edema
1	0	0	0	1	2	3
2	0	0	1	1	1	3
3	0	0	1	1	4	4
4	0	0	0	0	3	4
Mean score ± SD	0	0	0.6 ± 0.02	0.8 ± 0.04	2.2 ± 0.38	3.4 ± 0.66
PII	0		1.4		5.6	

Table 5
Erythema (ER), Scaling (SC) and Thickening (TH) score of treated groups as per clinical Psoriasis Area and Severity Index score.

Day of treatment	Control			IMQ + No treatment			IMQ + TQNPopt gel			IMQ + Clobetasol		
	ER	SC	TH	ER	SC	TH	ER	SC	TH	ER	SC	TH
0	0	0	0	4	4	4	4	4	4	4	4	4
1	0	0	0	4	4	4	3	2	3	2	2	3
2	0	0	0	3	4	4	1	1	0	1	1	2
3	0	0	0	3	3	4	1	0	1	0	1	1
4	0	0	0	3	2	3	0	0	0	0	1	0
5	0	0	0	2	2	2	0	0	0	0	0	0
6	0	0	0	1	1	2	0	0	0	0	0	0
7	0	0	0	1	1	2	0	0	0	0	0	0
Average PASI	0	0	0	3	3	3.57	1.28	1.0	1.14	1	1.28	1.4

0 – None; 1 – Slight; 2 – Moderate; 3 – Marked; 4 – Very marked

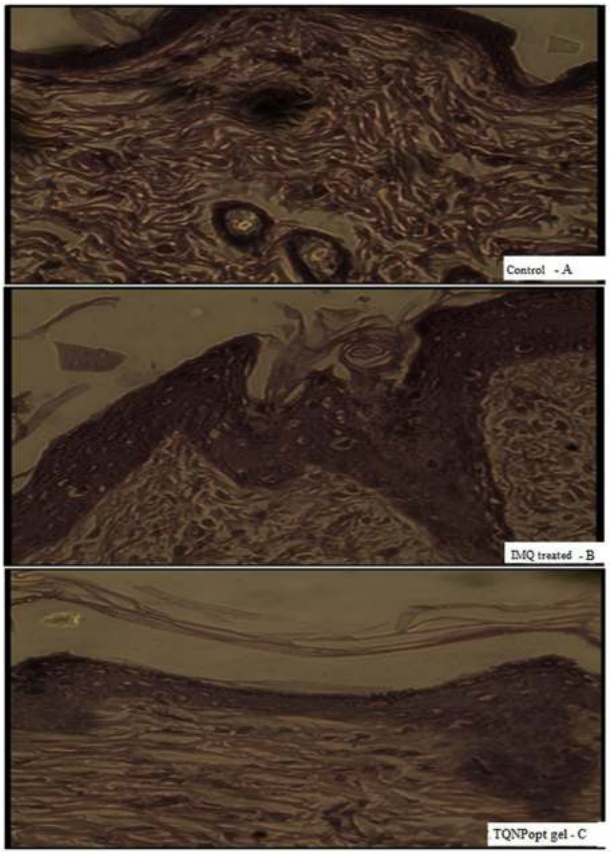

Fig. 7. Haemotoxylin and eosin stained skin section (A). Control skin (B). IMQ treated and (C). optimized thymoquinone lipid nanoparticles loaded gel.

unchanged in comparison to the normal untreated rat skin. The toxic control treated skin showed marked changes in the epidermis and dermis and also showing an irregular internal structure of the skin (Fig. 7B). There was a clear sign of visible inflammatory cell and redness to the skin confirms the score shown by visual scoring.

4. Conclusion

TQ lipid nano particles were formulated and statistically optimized using BBD design approach for the psoriasis treatment. The prepared formulation showed nano particle size (71.1–108.34 nm) with high encapsulation efficiency (62.5–82.3%), respectively. The optimized TMQ lipid nano particles gel (TQNPopt gel) showed high drug content (93.35 % ± 1.69), sustained drug release (57.55 % ± 5.38) and high flux (5.77 μg/cm^2/h). The skin irritation score of erythema (0.6 ± 0.02) and edema (0.8 ± 0.04) for TQNPopt gel treated group is significant lower than standard irritant. The psoriatic score showed reduction in all the visible parameters like erythma (1.28), scaling (1.0) and thickening (1.14). So, the present results demonstrate that NPs formulation is a potentially useful carrier for dermal delivery of thymoquinone.

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