RESEARCH ARTICLE

Topical delivery of Bruton’s tyrosine kinase inhibitor and curcumin-loaded nanostructured lipid carrier gel: Repurposing strategy for the psoriasis management

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ABSTRACT

This work investigates the synergistic potential of the nanostructured lipid carrier (NLC) gel of Ibrutinib with Curcumin as a repurposing strategy to treat psoriasis. In the present work, various components such as liquid lipid, solid lipid, and surfactant were selected and optimized based on the solubility of each drug, size, and polydispersity index. The optimized NLC consists of Capryol PGMC as liquid lipid, Glyceryl Mono Stearate as solid lipid, and Pluronics-F-127 as a surfactant. The prepared NLCs have a particle size of 95.12±3.39 nm with PDI of 0.285±0.009, exhibiting high entrapment efficiency (86.04±2.86%) for IBR and 87.25±2.14% for CUR) with spherical geometry. CI value of 0.283 suggests synergism. Carbopol 940 was used as a gelling agent and has shown improved flux compared to plain drug gel. Anti-psoriatic studies in BALB/c mice indicated negligible skin irritation and improved histopathological features of psoriasis. Moreover, a reduced amount of inflammatory markers (TNF-alpha, IL-6, IL-22, and IL-23), and psoriasis severity score was observed with prepared gel than the IMQ group. The study suggested integrated benefits of repurposing Ibrutinib with Curcumin as NLC topical gel and it could possibly reduce remission of Psoriasis like inflammation and merit additional investigation.

1. Introduction

Psoriasis is a dermal disorder frequently painful, affecting roughly approximately 1–3% of the world’s population, among these approximately 17% of patients have mild to moderate conditions. Psoriasis is associated with high morbidity (Parisi et al. 2013; Woo et al. 2017), and distress level, and decreased quality of life (Rahol et al. 2012). It is characterized as an extreme inflammatory disorder of the skin eventuated because of the immoderate proliferation of epidermal cells which leads to the appearance of scales, thick red patches, and abrasion on the skin along with hypergranulosis, hyperkeratosis (Harden et al. 2015), and parakeratosis which births enormous infiltration of inflammatory markers (Deng et al. 2016). Adaptive and innate immune cells, which act as shields of our body against bacteria, trigger an autoimmune response that forms the fundamental outlining cause of Psoriasis. In a nut-shell, the healthy skin cells are attacked by our own T-cells which yields sky-rocketing levels of pro-inflammatory cytokines in the blood (Cai et al. 2012; Schön 2019). The balance between cell birth and death is lost leading to hyper-proliferation of dermal cells afflicting more than 10% of the skin surface in regions like knee, scalp, folded skin, elbows, and legs (Goliniska et al. 2019). Years of research has led to understanding of few of the causative factors for Psoriasis which include sunburn, genetics, infection, and medicament-derived symptoms, deficiency of vitamin D, etc. (Roberson and Bowcock 2010).

Psoriasis is a chronic and progressively degrading skin disorder and its management varies with its severity. Topical agents like antibiotics, methotrexate, biologics, and anti-inflammatory agents form the first line of defense against Psoriasis (Rendon and Schäkel 2019). Phototherapy and systemic therapy are other promising therapy options to curtail the menace of Psoriasis. Despite all the available therapeutic advances psoriasis still bears some unmet clinical needs for its management and demands for long-lasting, safe and effective treatment options (Zhang and Wu 2018). Repurposing of drugs can be a viable option for its management and long-term remission of disease.

Tyrosine kinase enzymes play a significant part in the progression of psoriasis, as they are key components that drives cellular response array and induce inflammation (Nadeem et al. 2020). One of the kinase enzyme is Bruton’s tyrosine kinase (BTK) and activation of this signaling induction leads to increase in the concentration of inflammatory markers (such as tumor necrosis factor-a (TNF-a), and interleukins (IL-23), and oxidative signaling in dendritic cells (DCs) and gamma delta T cells that are present in dermis and epidermis of the skin, so impeding this signaling pathway will reduce the burden of dermal inflammation in Imiquimod (IMQ)-induced psoriasis (Al-Harbi et al. 2020). Ibrutinib (IBR) is a BTK inhibitor initially approved as an anticancer molecule that can be repurposed as a therapeutic option as it decreases the expression of an inflammatory signaling pathway in the pathogenesis of Psoriasis-like inflammation. Moreover, Curcumin (CUR) is a natural

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compound, employed to manage a variety of maladies, particularly cancer, and chronic inflammatory disorder. CUR found to decrease the various inflammatory cascade involved in the psoriasis progression (Noorafshan and Askhani-Esfahani 2013). It has recently been recommended as a viable therapeutic choice to manage psoriasis as it lowers the concentration of various pro-inflammatory mediators (Kang et al. 2016). A combination of these two drugs results emerges as an effective therapeutic option to manage chronic conditions like psoriasis. Also, it leads to achieving synergism and reducing the dose of drug, and improving efficacy and patient compliance. It eliminates the compensatory mechanism as well as dose-related off-target side effects (Lebwohl et al. 2004).

Repurposing of IBR in the management of psoriasis is of greatest advantage as IBR inhibits the BTK pathway and the role of CUR is to inhibit the MAPK and NF-kB pathway thus these two molecules can offer multiple targets for psoriasis management. Topical lipid-based systems are preferred because they penetrate deeper into the skin layers. Nanostructured lipid carriers (NLCs) are widely employed as lipid-based nano-formulations offering benefits of both lipid emulsion systems and nanotechnology as well (Souto et al. 2020). In the present studies, Ibrutinib with Curcumin (IBC) loaded NLCs are prepared and characterized, and encased in gel. Repurposing of IBR with CUR for anti-psoriatic potential as synergistic potential was assessed using the IMQ-induced psoriatic mouse model.

2. Material and method

2.1. Material

Ibrutinib was obtained as gift sample from the MSN Labs Pvt. Ltd, Hyderabad, India. Curcumin, cellulose dialysis tubing (12 000 mwco), poloxamer-407 (Pluronic® F-127) were acquired from Sigma Aldrich®. Carbopol 934/940, Cholesterol was purchased from AVRA chemicals. High-performance liquid chromatography (HPLC) grade solvents (Orthophosphoric acid (OPA), and acetonitrile) were obtained from ADVENT Chembio Pvt. Ltd, India. Hydroxyl propyl methylcellulose (E-15 grade) (HPMC E-15), tri-fluorooracetic acid, acetone were procured from SRL sciences, India. Polysorbate (Tween®80) was from SD fine chemicals, India. Palmitic acid, gyceryl monostearate (GMS), stearic acid, trimyristin, trilaurin, compritol, Labrafil® (LBF), Capryol™ PGMc, Labrasol® (LBSL), Lauraglycol® (LGL), and Capmul MCM8® were obtained as gift sample from Gattefosse, France. RPMI media, FBS, Trypsin were procured from Hyclone. Mouse enzyme-linked immunosorbent assay (ELISA) kits IL-17 Kit (DY421-05; R&D Systems), Mouse IL-22 Kit (DY582-05; R&D Systems), Mouse IL-23 Kit (DY1887-05; R&D Systems), and ancillary kits were purchased from R&D systems, Minneapolis, USA.

2.2. Fourier-transform infrared (FTIR) spectroscopy

ATR FT-IR was used to study any feasible interaction between the drug and excipients used in the formulation (PerkinElmer, Inc., USA). Individual plain drug (IBR and CUR) along with the mixture of excipients were taken in the same ratio as per the NLC formulation and screened using universal ATR from 400 to 4000 cm⁻¹ (Movasaghi et al. 2008).

2.3. In vitro cell line studies

2.3.1. Cytotoxicity assay and determination of combination index (CI)

The CI technique put forwarded by Chou and Talalay (Chou 2010) was exploited to find the ratio of IBR and CUR plain drug and in NLC formulation that inhibit human keratinocytes (HaCat) cell proliferation synergistically in in vitro condition. Initially, the cytotoxicity (IC₅₀) of free drug (IBR/CUR) and in combination (IBC) as well as NLC formulation (individual drug formulation, and in combination) against HaCat cell were calculated by employing (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (MTT) cell viability assay. To determine the IC₅₀ values, HaCat cells were seeded and allowed to adhere for 24 h using RPMI (rotations per minute) as culture media followed by the treatment of individual drug and formulation and cytotoxicity was assessed. For CI estimation, HaCat cells were treated at various multiples of their IC₅₀ values of IBR and CUR. Further MTT assay was performed to determine the combination effect for plain drug and formulation as well.

Combination index (CI) was determined to check the interaction of two molecules in the form of synergism, additive, or antagonistic effect.

The CI can be enumerated based on Chou and Talalay equation:

\[
(\text{CI}) = \left( \frac{\text{D}1}{\text{D}x} \right) + \left( \frac{\text{D}2}{\text{D}x} \right) - \left( \frac{\text{D}1}{\text{D}x} \right) \left( \frac{\text{D}2}{\text{D}x} \right),
\]

where (Dx1 and (Dx2 are the concentration of individual drug (1 and 2), required to inhibit 50% cells (IC₅₀), and (D1) and (D2) are the concentration of drug 1 and drug 2 required at IC₅₀ inhibition when taken together. Value of CI less than 1 suggests synergism, a CI of >1 stands for antagonistic effect, and the value equals to 1 indicates additive effect (mChou 2018).

2.4. Screening studies

2.4.1. Screening of liquid lipid

Selection of liquid lipid was carried out depending upon the capacity of the liquid lipid to solubilize both the drugs i.e. IBR and CUR. Concisely, in a 1.5 ml microcentrifuge tube 1 ml of individual liquid lipid was taken. Excess quantity of IBR and CUR were added, vortexed, and kept at 37°C for 24 h. Samples were centrifuged at 10 000 rpm for 10 min; further, 0.45 µm syringe filter was used to filter the supernatant. Some amount of methanol was added to solubilize residual drug adsorbed to the membrane. Finally, the samples were diluted appropriately and analyzed using RP-HPLC.

2.4.2. Screening of solid lipid

Different solid lipids (500 mg of each) were taken into a glass vial (3 ml), which was further heated at a temperature 5°C more than their melting point to guarantee absolute melting. IBR and CUR was added in increments to these molten lipids, and any undisolved drug was visually verified.

2.4.3. Partitioning or affinity study

Saturated solution of IBR and CUR was prepared by putting surplus quantity of both drug in Milli-Q water. Remaining undisolved drug was removed by using membrane filter (0.22 µm). Each solid lipids (500 mg) were put in 5 ml above solution and heated up to 75°C. Further, samples were kept in a shaking water bath (Julabo, SW 22) for 1 h, maintained at 75°C. Afterwards samples were allowed to attain room temperature (RT), further samples were centrifuged at 10 000 rpm for 10 min to separate lipid
and aqueous phase. Methylene chloride was used to dissolve solid lipid, which was then appropriately diluted. The content of IBR and CUR were estimated using RP-HPLC in both the aqueous and lipid phases.

### 2.4.4. Screening of solid lipid and liquid lipid ratio
NLC are made up of liquid and solid lipids, the compatibility of which is critical for the optimized and stable formulation. The compatibility of the chosen lipids was checked at different ratios of solid and liquid lipids (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, and 30:70). The lipids in above-mentioned ratio were blended well and kept in capillary tube to determine the melting point (Stuart SMP 30). Additionally, solid-liquid lipid matrices were kept on watch glass at RT for solidification. Gentle pressure was applied using Whatmann filter paper to check oozing of liquid lipid from matrices.

### 2.4.5. Screening of surfactant
Different surfactants based on their HLB were selected for screening. Batches were formulated using these surfactants (Pluronic F-127, Tween-80, Span-20, and Egg lecithin) at 0.5% and 1% and Particle size, and polydispersity index (PDI) was observed for 15 days as indicator of stability. Most stable and surfactant posing desired size and PDI would be selected for the final formulation.

### 2.4.6. Optimization of process parameters
Batches were primed with varying stirring speeds (800 rpm, 1000 rpm, 1200 rpm) and stirring times (60 min, 120 min), and they were analyzed for particle size and PDI.

### 2.5. Preparation and optimization of NLC
NLC containing IBC were formulated as per the reported method with slight modification (Rangaraj et al. 2020). Mixture of GMS (solid lipid) and Caproyl-90 (liquid lipid) in the ratio of 75:25 were used to prepare the IBC-loaded NLC using Pluronic® F-127 as the surfactant at 1% concentration. Briefly, GMS, Caproyl PGMC 90, IBR and CUR were added in a 10 ml beaker containing mixture of organic solvent (1:1 ratio of acetone and ethanol) as organic phase. To another 25 ml beaker, 1% Pluronic F-127 surfactant was added in 10 ml of distilled water and kept for stirring to ensure proper mixing. Both the organic as well as aqueous phases were heated at 60°C on a magnetic hot plate (Remi 1 MLH). Hot organic phase was poured to preheated aqueous phase at same temperature under stirring for 1 min at 500 rpm. Further, the mixture was allowed to sonicate using probe sonicator for 15 min at 40% amplitude, with a pulse cycle of 15 s on and 5 s off (Sonics and Materials, Inc., Vibra cell VCX 750) followed by continuous stirring at 1000 rpm for complete evaporation of the organic phase to obtain IBC-NLCs. FITC-loaded NLC were prepared with the same procedure using FITC (0.25% w/v). The prepared NLCs were then characterized for the size, PDI, and entrapment efficiency (EE) (%).

### 3. Characterization of NLC containing IBC
#### 3.1. Determination of particle size, polydispersity index, and zeta potential
Dynamic light scattering was used to check size and PDI, and the Stokes-Einstein relation was used to convert Brownian motion measurements into size and distribution of particles. For the measurement of size and zeta potential, prepared formulations were diluted 10 times with the help of Milli-Q water and kept in size analyzer and zeta seizer cuvette. The size, PDI and zeta potential was computed with help of Zetasizer Nano ZS (Malvern Instrument Ltd., Worcestershire, UK) at 25°C in triplicates (Xia et al. 2016).

#### 3.2. Determination of entrapment efficiency
The entrapment efficiency was calculated using ultracentrifugation technique. Concisely, 1 ml of the prepared NLC was allowed to centrifuged (for 40 min at 4°C at 60 000 rpm) using an ultracentrifuge (Thermo Scientific Sorvall MX 150 Plus) (Elmowafy et al. 2017). Further, HPLC was used to measure the quantity of free drug (Wfree) using supernatant at 258 nm and 427 nm. The % of entrapped drug can be calculated by subtracting unentrapped drug amount from the total drug added in the formulation and % EE was calculated as follows:

\[
EE(\%) = \frac{WT-W_{free}}{WT} \times 100, \quad (2)
\]

where \(W_T\) is total drug added and \(W_{free}\) is free drug.

#### 3.3. Transmission electron microscopy
Transmission electron microscopy (TEM) analysis was done to investigate surface morphology of prepared NLC. The samples were diluted with Milli-Q water and then put on a copper grid, followed by staining (1% w/v uranyl acetate). The grid was washed and set aside for drying. The sample was then analyzed using electron microscope JEM 1400 (HR) (JOEL, Japan) with 120.0 kV as accelerating voltage and data was processed using Radius software. The analysis was carried out at 25°C (Elmowafy et al. 2017).

#### 3.4. Preparation of NLC-loaded gel
Carbopol gel (1% w/v) was prepared by soaking Carbopol-940 and stirred continuously to prepare a uniform gel. Triethanolamine as cross-linking agent was added to carbopex solution to make proper consistency and gel was further evaluated for the gel characteristics like pH, spreadability factor, and rheology.

#### 3.5. pH, gel spreadability factor and rheological behavior
pH of the prepared formulation loaded gel was calculated using digital pH meter. The spreadability factor of NLC-loaded gel was evaluated at room temperature using modified parallel plate technique. A glass plate was placed over another plate having 100 mg of prepared gel in parallel direction. The initial diameter was calculated with two different directions and average was taken, and weights varying from 5 to 100 g were driven into the NLC gel at intervals of 30 s with each inclusion, the spreading area was measured by calculating diameter. Spreadability factor can be calculated as follows:

\[
S_f = \frac{A}{W} \quad (3)
\]

where \(S_f\) (cm² g⁻¹) is the spreadability factor, calculated as a ratio of the final area (A) in cm² to the final weight (W in g) stress applied on the gel.

Rheology of prepared formulation was done using modular compact rheometer (MCR102 instrument, Anton Paar, Germany). Briefly, a small portion of gel was kept on the plate, and a cone was attached at the top of the rheometer at 25°C. The
formulation was assessed for viscosity by varying the shear stress (80–250 Pa). The rheological functioning of gel was assessed by indicating shear rate versus shear stress and viscosity in graph.

### 3.6. In vitro drug release studies

In vitro drug release study for IBC loaded NLC and free drug were conducted with modified dialysis bag method (Nagaich and Gulati 2016). Concisely, formulation equivalent to 1 mg/ml of individual drug concentration were taken in pre-activated membrane sealed at either ends and kept in a beaker containing of 50 ml of phosphate buffer pH 6.8 with 0.25% Tween 80 as release media at 37 °C, and 150 rpm. At definite time points, 1 ml of sample was taken and replaced with fresh buffer. Experiments were conducted in triplicates. Drug content from the samples were calculated by using HPLC. The release pattern of formulation was compared with the free drug suspension kept for release under the similar conditions (Rapalli et al. 2020; Tawfeek et al. 2020).

The Franz diffusion apparatus was used to evaluate drug diffusion from the gel. Dialysis membrane (MWCO 12 000 daltons) after activation was kept on the receptor compartment of Franz diffusion cell with diffusion area of 3.14 cm² filled with 30 ml of release media at 37 °C at 150 rpm. The formulation was evenly spread on the membrane, 1 ml of sample was taken from sample compartment at the different time points and replenished with same media followed by analysis of drug content using HPLC. To evaluate the release kinetics, data was fitted with various release kinetic models.

### 3.7. Ex vivo permeation study

The Animal Ethics Committee of the Institute of Pharmaceutical Education and Research NIPER Hyderabad approved the animal experiment protocol (NIP/02/2020/PE/417) to carry all animal studies. All the animal experiments were done using male Bagg Albino research mouse strain (BALB/c) mice (6–8 weeks with 25–28 g) in accordance with ARRIVE 2.0 guideline. For the permeation study mice were anaesthetized and dorsal hairs were shaved. After 24 h, the skin of animal was excised and the extra attached fat on the skin was removed. The skin was cleaned thoroughly with buffer and kept at −20 °C prior use. The permeation study was carried out using Franz diffusion apparatus with effective permeation area of 3.14 cm² with 30 ml of receptor cell capacity. The release media was put in the diffusion cell and equilibrated for 1 h at 37 ± 0.5 °C before ex vivo permeation study. Before use, the skin was hydrated for 1 h in phosphate buffer solution and then mounted on a diffusion cell with stratum corneum facing toward the donor compartment. Optimal gel formulation equivalent to 1 mg of drug was spread evenly on the skin. At regular intervals, 1 ml of material was taken, filtered, and evaluated using a validated HPLC technique. The skin penetration from NLC gel was compared with plain drug gel. The amount of drug penetrated (g/cm²) from the prepared formulations over time was shown (h). The straight-line slope of the presented curve was used to calculate the permeation flux (J) (g/cm²/h⁻¹). All the experiments were conducted in triplicates (Doppalapudi et al. 2017).

### 3.8. Skin distribution studies using confocal microscopy

The confocal laser scanning microscopy (CLSM) was used to check the skin permeation of prepared formulations. For distribution of prepared NLC loaded animals were kept in central animal house facility at NIPER-Hyderabad under monitored conditions (12 h light/12 h of darkness at 22 °C with 50% relative humidity (RH)) for a week to acclimatize. The distribution of prepared formulation into skin layers was examined using shaved mice. The Fluorescein isothiocyanate (FITC) (0.25% w/v) loaded NLC formulations were prepared as per the procedure described in Section 2.4. FITC loaded NLC and plain drug were applied uniformly to the skin and kept for 6 h in dark. The animals were sacrificed after the completion of study, and the skin sections were collected and kept at 4 °C in 30% sucrose solution. The experiment was executed in psoriatic and healthy animal. Sections were cut with the help of cryotome (Leica Biosystems, Germany). The sections were mounted on slides and examined using a microscope (Nikon Corporation, Japan) (Jain et al. 2016).

### 3.9. In vivo anti-psoriatic efficacy study

IMQ generated psoriatic plaque model was used to assess anti-psoriatic activity of NLC as topical gel. All experimental methods performed as per relevant ethical guidelines for laboratory animal use and care (U.K. animal Act, 1986 and associated guidelines, EU directive 2010/63/EU for animal experiments, NIH guidelines-Publications No. 8023, revised 1978). Pilot study was conducted at two varying doses (HD and LD) to estimate the dose of IBR and CUR (see supplementary data) before efficacy study. The dorsal hair of the mice was shaved 24 h prior to the induction. Psoriasis was developed in mice by applying 62.5 mg of marketed Imiquamod cream (Imiquimod cream, 5% w/w) on the skin (5 cm²), and left ear regularly for six days. For efficacy study animals were categorized into 10 groups: Normal Control (Healthy animals); IMQ control (diseased animals); Betamethasone control (animals with induction and receiving marketed Betnovate-N ointment); CUR PD (plain drug) gel; IBR PD gel; IBC PD gel; Formulation Gel (IBR NLC gel); Formation Gel (CUR NLC gel); Formation Gel (IBC NLC gel); Plain carbopol gel.

In final study, mice in formulation group received treatment with NLC gel with a 10 mg/kg of IBR and 20 mg/kg of CUR dose from the third day from induction and continued for 6 regular days. Whereas, animals in Betamethasone control group, were given marketed Betnovate-N (100 mg of 0.01% of betamethasone propionate ointment) regularly for 6 days. On the 2nd, 4th, and 6th day of induction, psoriasis area severity index (PASI) scores and changes in body weights were recorded. Animals were sacrificed on the 7th day from induction, and scoring severity and weight of spleen were noted. Vernier caliper was employed to calculate the ear thickness and compared. Skin of the animals were taken and stored at −80 °C for further interleukin determination with ELISA. Similarly, spleen was removed, weighed and compared with other groups from sacrificed animals (Wu et al. 2004; Panonnummal and Sabitha 2018).

#### 3.9.1. Scoring severity of skin inflammation

The extent of thickness of left ear and skin, scaling and redness over the skin was calculated using psoriasis area severity index (PASI) scoring. Animal were evaluated for inflammatory scoring to check the hallmark of psoriasis, and how well it was responding to treatment. The intensity of the PASI parameters is represented by a rating from 0 to 4 (from no evidence to extreme condition) (Naldi 2010).

#### 3.9.2. Histopathological evaluation

Skin sections were examined to measure the histo-pathological changes with psoriasis induction and treatment as well.
Activation of psoriasis can be measured by changes in the pathological condition that may include infiltration of inflammatory markers, hypergranulosis, parakeratosis, and acanthosis. For histo-pathology study, collected skin sections were attached in 4% formaldehyde, fixed using paraffin and sectioned (5 μm of thickness). The mounted sections were further stained with hematoxylin and eosin dye (H & E staining). Pictures were captured using microscope (Leica ICC 50W, Leica Biosystems, Germany) (Hu et al. 2020).

3.9.3. Enzyme-linked immunosorbent assay for cytokine determination

Cytokines have greater impact in the progression of psoriasis, as their amplification increases psoriatic inflammation that ultimately leads to keratinocyte hyper proliferation. Thus, quantification of these individual cytokines becomes more crucial to investigate the impact of treatment. Sandwich ELISA was used to measure cytokine concentration. For this, skin samples were taken and weighed, followed by the homogenization for 5 min at 3000 rpm with the help of tissue hand homogenizer (Remi Electrokinetic, Ltd., India). The hominization carried out in 7.4 pH Tris buffer (containing 150 mM NaCl and 1% TritonTM X-100). The extraction buffer was taken in the double quantity that of the skin weight. Further, the homogenates were centrifuged (at 10 000 rpm for 5 min at 4 °C). The supernatant was then removed and stored at −80 °C. The measurement of cytokine levels was done by using mouse ELISA kits (R&D Systems, Inc. Minneapolis, USA) as per the provided protocol by the manufacturer. Briefly, 96 pre-coated well plate was coated using 50 μl of capture antibody prior to the experiment for overnight at RT. The plate were then washed using washing buffer followed by the addition of 100 μl of standards with varying concentration and skin supernatant samples. 50 μl of detection antibody was further added after washing following 50 μl of mixture of color reagent A & B. After 10 min of incubation, stop solution (50 μl) was added in each well in dark. Finally, absorbance was taken at 450 nm using multimode reader and concentration of interleukin for different group was calculated and compared (Jain et al. 2016).

3.10. Statistical analysis

The statistical difference was determined using Graph Pad Prism 5.0 (Graph Pad Software Inc., La Jolla, CA). Data was presented as mean ± SD (n = 3) for optimization and characterization of formulation. Animal ranking, and ELISA data are presented as mean ± SEM (n = 6). Statistical significance was predicted using one way analysis of variance (ANOVA), followed by the Bonferroni multiple comparison and P value was stated for each note.

4. Results and discussion

4.1. Fourier-transform infrared (FTIR) spectroscopy

Possible interactions between the free drugs (IBR and CUR), and excipients such as GMS, Pluronic® F-127, physical mixture was examined in the range of 4000–400 cm−1 using FT-IR (Perkin Elmer). As shown in Figure 1(C), free drug CUR had peaks at 3371, 2959, 1589, 1377 cm−1, IBR at 1652, 1613, 1520, 1241, 1147, 1100, 986 and 953 cm−1 (Shi et al. 2019). Pluronic® F-127 shows peaks at 2882, 1632.45, 1582.24, 1374, 1360, 1281, 1149, 1060, 1012, 963 and 947 cm−1 (Su et al. 2003). GMS showed characteristic peaks at 3067.99, 2156.6, 1632.81, 1611.04, 1582.22, 1491.08, 1374, 1360, 1281, 1149, 1060, 1012, 963 and 947 cm−1. Absence of major shift in the observed spectra indicated there is no solid-state interactions amongst free drugs and the polymers (Figure 1(A)).

4.2. In vitro cell line studies

4.2.1. Cytotoxicity assay and determination of combination index (CI)

The in vitro efficacy of free drug, free drug combination, and NLC formulation was carried out in HaCat cell lines. The combination of drug killed more cells than individual drug alone at each concentration. Data suggested higher encapsulation for the both the drugs in formulation than plain drug. The increased cytotoxicity with NLC formulation resulted due to improved cellular internalization (Zhang et al. 2008). Also, combining these two drugs as plain drug suspension and as NLC formulation produced (

![Figure 1](image-url)
synergism as the value of CI value was calculated as 0.476 and 0.283 for plain drug combination and NLC formulation respectively (Figure 1(B)) as per Chou Talalay equation. In vitro cell culture study suggested 1:2 was the suitable drug combination ratio to produce synergism. The inhibitory effectiveness is correlated with drug concentration for both individual drugs and drug combinations. These findings suggested that, in comparison to simple mixture of two drugs, drug delivery systems such as NLC containing both drugs can inhibit the growth of HaCat cells more effectively as indicated by the CI values which are lower than one showing synergism. Further, more studies are needed to justify this ratio as synergistic ratio for IBR and CUR for the management of psoriasis.

4.3. Screening studies

4.3.1. Screening of liquid lipid
The primary requirement of any formulation is to have higher drug loading and high entrapment which can be achieved by incorporating lipid with high drug solubility. Therefore, screening of lipid is done based on the solubility drug in it. As demonstrated in Figure 2(A) IBR demonstrated highest solubility in Capryol™ PGMC (46.09 ± 1.611 mg/ml), and CUR exhibited highest solubility in Capryol™ PGMC (27.43 ± 1.14 mg/ml) thus chosen for the further formulation as it shows better compatibility.

4.3.2. Screening of solid lipid
The solubility of IBR and CUR were also measured in glyceryl mono stearate, palmitic acid, stearic acid, compritol, trilaurin, and trimyristin. Both the drug has shown maximum solubility and compatibility in GMS (67.06 ± 1.62 mg/500 mg for IBR and 23.13 ± 0.50 mg/500 mg for CUR) as visually observed (Figure 2(B)). Therefore, GMS was chosen as solid lipid.

4.3.3. Screening of solid lipid and liquid lipid ratio
GMS exhibited melting point at 68.2°C without addition of any liquid lipid. Afterwards addition of liquid lipid results into the decrease in the melting point that indicates the miscibility of liquid and solid lipids (Figure 2(C)). Furthermore, mixtures of lipids when observed using Whatman® filter papers, no oozing out of liquid lipid was noticed up to 70%. All the possible ratios beyond 70:30 were tried and based on the stability of formulation for the period of 15 days 75:25 (solid: liquid lipid) was selected for further studies (shown in Table 1). Appropriate ratio of solid and liquid lipid is crucial not only to carry the drug inside the lipid but also to preserve the consistency at RT.

4.3.4. Screening of surfactant
Selection of surfactant was done by formulating different batches with GMS as solid lipid and Capryol™ PGMC as liquid lipids, with each of individual surfactant such as Tween-80, Pluronic F-127, Span-20, and Egg lecithin. Amongst all the solubilizers, formulation with Pluronic® F-127 at 1% w/v concentration have shown compatibility in GMS (67.06 ± 1.62 mg/500 mg for IBR and 23.13 ± 0.50 mg/500 mg for CUR) as visually observed (Figure 2(B)). Therefore, GMS was chosen as solid lipid.

Table 1. Screening of solid lipid and liquid lipid ratio.

| Ratio (solid:liquid lipid) | Size ± SD | PDI ± SD |
|---------------------------|-----------|----------|
| 90:10                     | 274.03 ± 16.85 | 0.4 ± 0.13 |
| 80:20                     | 170.13 ± 15.31 | 0.272 ± 0.035 |
| 70:30                     | 108.4 ± 7.32   | 0.285 ± 0.009 |
| 75:25                     | 95.12 ± 3.39   | 0.308 ± 0.001 |

Figure 2. (A) Screening of liquid lipid, (B) Screening of solid lipid, (C) Graphical representation of change in melting point with addition of liquid lipid i.e. Capryol™ PGMC (n = 3, data presented as mean ± SD).
the lowest particle size and PDI (shown in Tables 2 and 3). Hence, Pluronic® F-127 was chosen as stabilizer for final formulation development.

### 4.3.5. Optimization of process parameters

Particle size and PDI of the prepared formulation can be affected by various process parameters such as stirring time, stirring intensity. Thus, the process parameters were optimized to get desired particle size and PDI. It was observed that there is decrease in particle size on increasing the stirring rate from 800 rpm to 1000 and 1200 rpm, and sonication time from 5 to 10 min and 15 min. Basic role of stirring time for this preparation technique is to ensure complete evaporation of the organic solvent from the formulation. Moreover, it also affects the particle size, PDI and somewhat entrapment efficiency of the drug hence hyper tuning stirring rate plays an important role. 1000 rpm was found to have desired results (shown in Table 4).

### 4.4. Formulation and characterization of IBC loaded NLC

Melt emulsification-ultra sonication technique was utilized to prepare IBC loaded NLCs. For the preparation of NLC, GMS and caproyl PGMC at 75:25 ratio used as solid lipid and liquid lipid, and 1% Pluronic F-127 was used as surfactant. The prepared NLC exhibited size of 95.12 ± 3.39 nm with PDI of 0.285 ± 0.009. The zeta potential of prepared NLC was observed as -33.2 ± 0.84 mV (Figure 3(A,B)). The prepared NLC exhibited higher entrapment efficiency for both the drugs (86.04 ± 2.86% for IBR and 87.25 ± 2.14% for CUR). TEM analysis of prepared NLC exhibited the spherical and mono-disperse particles (Figure 3(C)). The particle size of FITC loaded NLC was observed as 98.22 ± 4.35 nm with PDI of 0.292 ± 0.012.

### 4.5. Selection and optimization of polymer for gel preparation

The gel-forming potential and spreadability of different polymers were evaluated. Among various polymer, Carbopol-934 at 1% concentration provided optimum viscosity. The prepared gel was further characterized for gel properties.

### 4.6. pH, Rheological behavior and gel spreadability factor

The pH of the prepared gel was found to be in the range of 5.94–6.08. Rheological behavior was determined using compact rheometer and graph between shear rate vs viscosity and shear stress was plotted (Figure 4(A,B)). The viscosity of prepared gel decreases on increasing the shear rate that suggested the shear-thinning behavior of gel as shown in the graph. This pseudo-plastic behavior seen in formulation confirms high spreadability due to viscosity decreasing with application of a certain force, while also having the property of remaining at the application site without draining (Rathod and Mehta 2015).

The prepared gel’s spreadability factor was calculated to be 0.274 ± 0.05, indicating better spreadability under low stress. Additionally, the gel expands to the plate’s maximum dimensions when 100 g of force is applied, indicating a strong spreadability. The prepared gels must have good spreadability and meet the ideal requirements for topical application because spreading the gel aids in its uniform application to the skin. The patient’s compliance with the treatment is also thought to be influenced by this. The skin has a dehydrated keratinous coating in conditions like psoriasis, which calls for the proper hydration and prepared formulation is found to be have desired characteristics (Figure 4(C)).

### 4.7. In vitro drug release and ex vivo permeation study

In vitro release testing is a crucial analytical tool used in life cycle management to examine and determine product behavior at various stages of product development. Also, the facile penetration of topically administered gel through the skin is needed to have therapeutic efficacy in the skin. To determine the amount of drug penetrated through the dialysis membrane and skin, release and permeation study was conducted. Release study concluded that at the end of 8 h both plain drugs (IBR and CUR) were released more than 95% drug. However, in case of NLC formulation, nearly 55% release of drug was observed in a controlled fashion at the end of 12 h. Moreover, diffusion study revealed that within 12 h near 60% drug was slowly diffused through the membrane. The
prepared NLC follows zero-order kinetics for IBR with $R^2$ of 0.995 and CUR with 0.992 values. These results demonstrate that constant drug delivery throughout time which is required for chronic cutaneous illnesses such as psoriasis in order to improve patient compliance (Figure 5(A)).

Likewise, in ex vivo permeation was performed in the skin of mouse. NLC loaded gel formulation have demonstrated increased permeation shown in term of permeation flux and compared with plain drug gel. Data obtained from permeation study showed 2.44 times and 4.05 times increased permeation of IBR and CUR from formulation than gel containing plain drug. The 24 h permeation flux ($J$) was calculated to be $6.08 \pm 0.25$ g/h.cm² and $7.5 \pm 0.86$ g/h.cm² for IBR and CUR with NLC loaded gel shows enhanced drug flux and penetration through skin (Figure 5(B)).

4.8. Skin distribution studies using confocal microscopy

The dermal distribution of FITC plain solution and FITC-loaded NLC gel was executed to measure the penetration or distribution of the NLC loaded gel into the different layers of skin. Many aspects such as solubility, hydrophobic nature and log P values also attributes to the dermal penetration of the drug. FITC has log P value of 4.3 and having structural resemblance to the CUR thus chosen for the dermal distribution studies. Fluorescent images of plain dye and dye loaded NLC gel have demonstrated in Figure 6. The study certified that the plain FITC solution could not penetrate into the deeper tissues as the fluorescence appears on the superficial layer only whereas, in case of FITC loaded NLC gel they have shown improved penetration and depicted high intensity of dye even after 6 h owing to its nano size and high penetration ability of lipids in the fabrication of NLC. Moreover, prepared gel improved the skin hydration and permeation of the formulation into the skin (Jain et al. 2016).

4.9. In vivo efficacy study

Here, using imiquimod-induced mouse model for psoriasis, we evaluated the preclinical safety and repurposing therapeutic efficacy of a novel NLC loaded with a BTK inhibitor and CUR as a topical gel. In this model, imiquimod (IMQ), a toll-like receptors (TLR)7/8 ligand and vigorous immune modulator, instigate and exacerbates a phenotype similar to psoriasis. IMQ, induces psoriasis by causing hyper-proliferation of DCs and keratinocytes, and recapitulates same features in mouse as human psoriasis (Bandyopadhyay and Larregina 2020). These pre-clinical studies started with an initial pilot study for dose determination. The final study was performed at the dose of IBR and CUR (10 mg/kg and 20 mg/kg). Therapeutic efficacy studies suggested that treatment with NLC loaded gel combined with both the drug ameliorated the degree of psoriasis at varying levels. Moreover, in comparison with the other formulation groups, IMQ control animals showed significantly high PASI score. However, scores for cornified layer pathology features (hyperkeratosis, parakeratosis) and skin scaling and thickening were lower than those seen in the IMQ group (Gudjonsson et al. 2007). Animals in the Betamethasone control and formulation gel groups demonstrated better remarkable phenotypic recovery. Although formulation with individual drug reduces the severity of disease to some extent but IBC loaded NLC gel demonstrated high performance compared to single drug NLC gel and gel containing plain drug, suggesting strong rationale for synergism, yet more extensive studies are required to explore the mechanism of synergism using IBC loaded NLC gel. Furthermore, the skin state revealed through histology study...
suggested that both betamethasone control and NLC gel animals resembled that of the normal group, suggesting that the treatment was more effective.

4.9.1. Scoring severity of skin inflammation
Psoriasis area severity index is a qualitative assessment for the progression of disease and treatment success. On the 3rd day of induction mice began to exhibit scaling, erythematous plaques, skin thickness and ear thickness to varying degree indicating the psoriatic lesions (Langley and Ellis 2004). By the end of 5th day, these psoriatic lesions and other PASI score got intensified progressively. A numeric score range from 0 to 4 was allotted based on the severity of the psoriasis (Gottlieb et al. 2003). The score for normal control group animals was recorded as zero, as the animal did not receive any vehicle or formulation. The severity of psoriasis including scaling over the skin, thickness was noticed in IMQ control animals, and thus had highest severity score after six days. In other groups, the severity of the condition improved as therapy progressed. Overall, the PASI score was recorded in the order of IBC NLC gel > IBR NLC gel > Betamethasone control > CUR NLC gel > plain drug gel > carbopol gel > IMQ control. Similarly, the body weight also varies with the severity of

Figure 4. Rheological parameters of IBC loaded NLC gel: (A) Graph between Shear rate vs Shear stress, (B) Graph between Shear rate and viscosity, and (C) Spreadability factor of IBC loaded NLC gel and plain gel.

Figure 5. (A) In-vitro drug release of IBC NLC; (B) Ex-vivo permeation study of IBC loaded NLC gel.
Figure 6. Skin distribution study using confocal microscopy. (A) Plain FITC in healthy skin, (A') FITC loaded NLC formulation in healthy skin, (B) Plain FITC in Psoriatic skin, (B') FITC loaded NLC formulation in psoriatic skin.

Figure 7. Assessment of anti-psoriatic potential of IBC loaded NLC gel on IMQ induced mouse model. Erythema, scaling, and skin thickening was recorded on 0, 2nd, 4th and 6th day on a scale from 0 to 4. (A) spleen weight to body weight ratio; (B) ear thickness; (C) Change in body weight; (D) PASI scoring for erythema; (E) PASI scoring for scaling; (F) PASI scoring for skin thickening.
psoriasis. IMQ control group animals have shown significant loss in the body weight. However, in case of betamethasone control and formulation group, initially loss of body weight was observed but improvement was observed after the therapy started (Figure 7).

Application of imiquimod also results into the increased thickness and inflammation in left ear than right ear. After completion of therapy on 7th day, reduction in the thickness of ear was seen in treatment group when compared with IMQ control. The back skin thickness was more in IMQ control animals than in normal control and formulation control group. On the other hand higher ratio of spleen/body weight indicates higher inflammatory burden and stronger immune response (Bronte and Pittet 2013). In this study, spleen was isolated weighed after the sacrifice of the animal from each group and compared. When compared to IMQ animals, the spleen/body weight ratio, and size was found to be lower in formulation group. Furthermore, histopathological psoriasis hallmarks of skin inflammation in the epidermal and dermal layers of the skin were also reduced in NLC gel, confirming the therapeutic efficacy of two drugs in treatment of psoriasis pathology. This shows that the NLC gel attributed to high efficacy in psoriasis.

4.9.2. Histopathological evaluation

Histopathological was carried out to measure the efficacy of the formulation in normalizing the skin in IMQ-induced psoriasis model. H&E staining was used to dye skin sections and images were taken. Skin H&E staining indicated significant stratum corneum thickening (hyperkeratosis), parakeratosis (i.e. retention of nuclei in st. corneum), and acanthosis (thickening of malphighian layer) and infiltration of inflammatory cell in the dermis in the IMQ control group. IMQ control animal shown increased infiltration of leukocytes and acanthosis and irregularity in epidermal layer compared to other groups. These features in treatment group caused by IMQ were improved to varying degrees after administration of NLC gel formulations. Normal control animals represent the healthy skin with proper and intact epidermis and dermis. Our formulation showed normalized histopathological features and skin thickness as that of normal control group. The histopathological features of skin for all the groups are shown in Figure 8(B).

4.9.3. Enzyme-linked immunosorbent assay for cytokine determination

Infiltration of pro-inflammatory cytokines are some key features in the progression of psoriasis like inflammation. Moreover, it has been suggested that the IL-23/IL-17 axis is an important index of psoriasis progression. The hypersecretion of pro-inflammatory cytokines promotes proliferation and inflammation of keratinocyte in psoriasis. TNF- \(\alpha\) regulates immune response, and inflammation, and its aberrant production is linked to the emergence of psoriasis and other immunological diseases (Schottelius et al. 2004). Also, IL-23 is well recognized for its involvement in cell survival and proliferation (Girolomoni et al. 2017). IL-22 is responsible for hyper-proliferative keratinocyte; demonstrating its importance in the IMQ produced psoriasis model (Wolk et al. 2009). In mouse skin, IMQ induces psoriasis-like inflammation by either inducing mRNA or protein expression of TNF-\(\alpha\), IL-23, IL-17, IL-2, and IL-6,
and effective anti-psoriasis therapy inhibited the expression of the aforementioned cytokines. In our study we employed the ELISA to determine the levels of IL-2, IL-23, IL-17, IL-22, and TNF-α in skin homogenates as per the protocol provided. The levels of cytokines were calculated and compared with all other groups as demonstrated in Figure 9.

ELISA of skin samples showed higher inflammatory markers in IMQ control group apart from the treatment groups (p < 0.05). However, the cytokines level in PD gel and carbopol gel, did not reduced significantly. In comparison with normal control and betamethasone control group, animals of IMQ control group showed an increased level of TNF-α by the folds of 2.18 and 1.88 respectively. Treatment with NLC gel showed an improved efficacy than individual drug formulation. When compared with IMQ control group, animals who received NLC gel have shown 3.03, 4.15, 4.1, 2.81, 2.79, and 1.82 times decreased level of TNF-α, IL-23, IL-6, IL-2, IL-17, and IL-22. The psoriasis induction with IMQ in the skin tissues of the animals caused an increase in the levels of these inflammatory cytokines in the animals from the IMQ control group. Individual drug formulation have lesser impact compared to combined drug formulation suggesting strong evidence for the synergism, however, further studies are required to generate more evidence to establish and validate the mechanism of synergism.

In addition to offering adequate hydration for dry skin, NLC gel could achieve high therapeutic efficacy. In conclusion, our research indicates that an IBC-loaded NLC gel may be an effective topical anti-psoriasis treatment because it inhibits abnormal keratinocyte proliferation, inhibits the inflammatory response by downrating the IL-23/IL-17 axis, and sufficiently hydrates dry skin.

5. Conclusion

Psoriasis is an inflammatory dermal condition involving relapsing of hyperkeratosis and inflammation. It is a need of hour to develop novel chemical moieties whilst maintaining perfect balance of therapeutic efficacy and potential combination drug regimen to this dreadful dermal disorder. In the present work, for the first time we have developed NLC system co-loaded with IBR and CUR transformed into carbopol gel to improve the treatment efficacy for psoriasis management. The combination of IBR and CUR provides a synergistic effect in treating psoriasis as demonstrated
through the in vitro cell lines studies by exploiting the Chou–Talalay equation. The topical delivery of IBC-loaded NLC gel significantly improved the efficacy on IMQ-induced psoriasis plaques in BALB/c mice, by alleviating the symptoms of IMQ-induced incrasassion, and decreasing the concentration of pro-inflammatory cytokines. The experimental data indicate that repurposing IBR with CUR can be a potential drug combination as NLC carrier gel for psoriasis therapy. In vitro, in vivo studies, results suggested that IBC-loaded NLC gel not only improved the phenotypic and histological symptoms of psoriasis but also decreased the pro-inflammatory markers in collected biological samples. These findings suggest that further research with larger treatment groups is necessary to determine the significance of synergistic effects on specific pathology features.

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HJ: Conceptualization, writing-original draft, methodology, and writing – review and editing. GD: Methodology and writing – review and editing. AB: Writing – review and editing. HD: Writing – review and editing. NR: Writing – review and editing. CG: Supervision. SS: Conceptualization and resources.

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References
Al-Harbi NO, Nadeem A, Ahmad SF, Bakheet SA, El-Sherbeeny AM, Ibrahim KE, Alzahrani KS, Al-Harbi MM, Mahmood HM, Alqahtani F, et al. 2020. Therapeutic treatment with lbrutinib attenuates imiquimod-induced psoriasis-like inflammation in mice through downregulation of oxidative and inflammatory mediators in neutrophils and dendritic cells. Eur J Pharmacol. 877:173088.

Bandyopadhyay M, Larregina AT. 2020. Keratinocyte-polyamines and dendritic cells: a bad duet for psoriasis. Immunity. 53(1): 16–18.

Bronte V, Pittet MJ. 2013. The spleen in local and systemic regulation of immunity. Immunol. 39(5):806–818.

Cai Y, Fleming C, Yan J. 2012. New insights of T cells in the pathogenesis of psoriasis. Cell Mol Immunol. 9(4):302–309.

Chou T-C. 2010. Drug combination studies and their synergy quantification using the Chou-Talalay method. Cancer Res. 70(2):440–446.

Deng Y, Chang C, Lu Q. 2016. The inflammatory response in psoriasis: a comprehensive review. Clin Rev Allergy Immunol. 50(3):377–389.

Doppalapudi S, Jain A, Chopra DK, Khan W. 2017. Psoralen loaded liposomal nanocarriers for improved skin penetration and efficacy of topical PUVA in psoriasis. Eur J Pharm Sci. 96:515–529.

Elmowafy M, Ibrahim HM, Ahmed MA, Shalaby K, Salama A, Hefesha H. 2017. Atorvastatin-loaded nanostructured lipid carriers (NLCs): strategy to overcome oral delivery drawbacks. Drug Deliv. 24(1):932–941.

Giorolomoni G, Strohal R, Puig L, Bachelez H, Barker J, Boehncke W-H, Prinz J. 2017. The role of IL-23 and the IL-23/TH 17 immune axis in the pathogenesis and treatment of psoriasis. J Eur Acad Dermatol Venereol. 31(10):1616–1626.

Golińska J, Sar-Pomian M, Rudnicka L. 2019. Dermoscopic features of psoriasis of the skin, scalp and nails–a systematic review. J Eur Acad Dermatol Venereol. 33(4):648–660.

Gottlieb AB, Chaudhari U, Baker DG, Perate M, Dooley LT. 2003. The National Psoriasis Foundation Psoriasis Score (NPF-PS) system versus the Psoriasis Area Severity Index (PASI) and Physician’s Global Assessment (PGA): a comparison. Journal of Drugs in Dermatology: JDD. 2(3):260–266.

Gudjonsson JE, Johnston A, Dyson M, Valdimarsson H, Elder JT. 2007. Mouse models of psoriasis. J Invest Dermatol. 127(6):1292–1308.

Han L, Wang T. 2016. Preparation of glycerol monostearate from glycerol carbonate and stearic acid. RSC Adv. 6(41):34137–34145.

Harden JL, Krueger JG, Bowcock AM. 2015. The immunogenetics of psoriasis: a comprehensive review. J Autoimmun. 64:66–73.

Hu JZ, Billings SD, Yan D, Fernandez AP. 2020. Histologic comparison of tumor necrosis factor-α inhibitor–induced psoriasis and psoriasis vulgaris. J Am Acad Dermatol. 83(1):71–77.

Jain A, Doppalapudi S, Domb AJ, Khan W. 2016. Tacrolimus and curcumin co-loaded liposphere gel: synergistic combination towards management of psoriasis. J Control Release. 243:132–145.

Kang D, Li B, Luo L, Jiang W, Lu Q, Rong M, Lai R. 2016. Curcumin shows excellent therapeutic effect on psoriasis in mouse model. Biochimie. 123:73–80.

Langley RG, Ellis CN. 2004. Evaluating psoriasis with psoriasis area and severity index, psoriasis global assessment, and lattice system physician’s global assessment. J Am Acad Dermatol. 51(4):563–569.

Lebwohl M, Menter A, Koo J, Feldman SR. 2004. Combination therapy to treat moderate to severe psoriasis. J Am Acad Dermatol. 50(3):416–430.

mChou T-C. 2018. The combination index (CI< 1) as the definition of synergism and of synergy claims. Amsterdam: Elsevier.

Movasaghi Z, Rehman S, Ur Rehman DI. 2008. Fourier transform infrared (FTIR) spectroscopy of biological tissues. Appl Spectrosc Rev. 43(2):134–179.

Nadeem A, Ahmad SF, Al-Harbi NO, El-Sherbeeny AM, Alasmari AF, Alanzai WA, Alasmari F, Ibrahim KE, Al-Harbi MM, Bakheet SA, et al. 2020. Bruton’s tyrosine kinase inhibitor suppresses imiquimod-induced psoriasis-like inflammation in mice through regulation of IL-23/IL-17A in innate immune cells. Int Immunopharmacol. 80:106215.

Nagaich U, Gulati N. 2016. Nanostructured lipid carriers (NLC) based controlled release topical gel of clobetasol propionate: design and in vivo characterization. Drug Deliv Transl Res. 6(3):289–298.

Naldi L. 2010. Scoring and monitoring the severity of psoriasis. What is the preferred method? What is the ideal method? Is PASI passé? facts and controversies. Clin Dermatol. 28(1):67–72.
Noorafshan A, Ashkani-Esfahani S. 2013. A review of therapeutic effects of curcumin. Curr Pharm Des. 19(11):2032–2046.
Panonnurnral R, Sabitha M. 2018. Anti-psoriatic and toxicity evaluation of methotrexate loaded chitin nanogel in imiquimod induced mice model. Int J Biol Macromol. 110:245–258.
Parisi R, Symmons DP, Griffiths CE, Ashcroft DM, Identification and Management of Psoriasis and Associated Comorbidty (IMPACT) project team 2013. Global epidemiology of psoriasis: a systematic review of incidence and prevalence. J Invest Dermatol. 133(2):377–385.
Raho G, Koleva DM, Garattini L, Naldi L. 2012. The burden of moderate to severe psoriasis. Pharmacoeconomics. 30(11):1005–1013.
Rangaraj N, Pailla SR, Shah S, Prajapati S, Sampathi S. 2020. QbD aided development of ibrutinib-loaded nanostructured lipid carriers aimed for lymphatic targeting: evaluation using chylomicron flow blocking approach. Drug Deliv Transl Res. 10(5):1476–1494.
Rapalli VK, Kaul V, Gorantla S, Waghule T, Dubey SK, Pandey MM, Singhvi G. 2020. UV Spectrophotometric method for characterization of curcumin loaded nanostructured lipid nanocarriers in simulated conditions: method development, in-vitro and ex-vivo applications in topical delivery. Spectrochim Acta, Part A. 224:117392.
Rathod HJ, Mehta DP. 2015. A review on pharmaceutical gel. International Journal of Pharmaceutical Sciences. 1(1):33–47.
Rendon A, Schäkel K. 2019. Psoriasis pathogenesis and treatment. IJMS. 20(6):1475.
Roberson ED, Bowcock AM. 2010. Psoriasis genetics: breaking the barrier. Trends Genet. 26(9):415–423.
Schön MP. 2019. Adaptive and innate immunity in psoriasis and other inflammatory disorders. Front Immunol. 10:1764.
Schottelius AJ, Moldawer LL, Dinarello CA, Asadullah K, Sterry W, Edwards IC. 2004. Biology of tumor necrosis factor-α–implications for psoriasis. Exp Dermatol. 13(4):193–222.
Shi X, Song S, Ding Z, Fan B, Huang W, Xu T. 2019. Improving the solubility, dissolution, and bioavailability of ibrutinib by preparing it in a coamorphous state with saccharin. J Pharm Sci. 108(9):3020–3028.
Souto EB, Baldim I, Oliveira WP, Rao R, Yadav N, Gama FM, Mahant S. 2020. SLN and NLC for topical, dermal, and transdermal drug delivery. Expert Opin Drug Deliv. 17(3):357–377.
Su Y-L, Liu H-Z, Guo C, Wang J. 2003. Association behavior of PEO–PPO–PEO block copolymers in water or organic solvent observed by FTIR spectroscopy. Mol Simul. 29(12):803–808.
TawfEEK HM, Abdellatif AA, Abdel-Aleem JA, Hassan YA, Fathalla D. 2020. Transfersomal gel nanocarriers for enhancement the permeation of Iromoxicam. J Drug Delivery Sci Technol. 56:101150.
Wolk K, Haugen HS, Xu W, Witte E, Waggie K, Anderson M, Vom Baur E, Witte K, Warszawska K, Philipp S, et al. 2009. IL-22 and IL-20 are key mediators of the epidermal alterations in psoriasis while IL-17 and IFN-γ are not. J Mol Med (Berl). 87(5):523–536.
Woo YR, Cho DH, Park HJ. 2017. Molecular mechanisms and management of a cutaneous inflammatory disorder: psoriasis. IJMS. 18(12):2684.
Wu JK, Siller G, Strutton G. 2004. Psoriasis induced by topical imiquimod. Australas J Dermatol. 45(1):47–50.
Xia D, Shrestha N, van de Streek J, Mu H, Yang M. 2016. Spray drying of fenofibrate loaded nanostructured lipid carriers. Asian J Pharm Sci. 11(4):507–515.
Zhang X-G, Miao J, Dai Y-Q, Du Y-Z, Yuan H, Hu F-Q. 2008. Reversal activity of nanostructured lipid carriers loading cytotoxic drug in multi-drug resistant cancer cells. Int J Pharm. 361(1-2):239–244.