Research Article

Designing a Topical Nanoliposomal Formulation of Ruxolitinib Phosphate

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Abstract

Background: The JAK-STAT pathway has been revealed to play a crucial role in the dysregulation of immune responses in autoimmune skin disorders. Ruxolitinib, a selective inhibitor of JAK1 and JAK2, potently suppresses cytokine signaling.

Methods: A topical emulgel containing ruxolitinib nanoliposome (RuxoLip) was prepared by the thin-film hydration method. Subsequently, its physicochemical characteristics were evaluated at 25±2°C/60±5% RH for 12 months. RuxoLip was assessed based on particle characteristics, Scanning Electron Microscopy (SEM), entrapment efficiency (EE), drug loading (DL), and Differential Scanning Calorimetry. The pH, density, viscosity, microbial assessment, *in vitro* drug release, and *in vivo* tape stripping test were evaluated on the emulgel of RuxoLip. Validating the analysis method was performed by UV spectroscopy.

Results: Nanoliposomal preparation was successfully formulated with a good particle size (218±2 nm), an EE of 67%, and a DL of 8%. The formulation was stable in a long-term condition. SEM showed that liposomes had a regular spherical surface. Moreover, *in vitro* drug release and the *in vivo* tape stripping test revealed good absorption and permeation, respectively.

Conclusion: The liposome dosage form is anticipated to be a perfect carrier for the topical drug delivery system of ruxolitinib in autoimmune skin disorders.

Introduction

Autoimmune skin diseases, which affect around 5% of the population,[1] are a group of inflammatory disorders, including lupus erythematosus, lichen planus, psoriasis, dermatomyositis, hidradenitis suppurativa, atopic dermatitis, alopecia areata, and vitiligo.[2] The JAK-STAT pathway is an intracellular signaling route that transmits cytokine signals from the cell membrane to the nucleus.[3] The binding of extracellular message ligands, including ILs and IFNs, to their special transmembrane receptors, leads to the activation of the cytoplasmic soluble JAK and initiates a cascade of molecular actions in the other components of the system, including STAT activity. This role enables STAT to translocate into the cell nucleus to activate the downstream transcription of pro-inflammatory signaling pathways.[4,5]

JAKs inhibition can simultaneously block the function of several cytokines. Consequently, JAK inhibitors are evolving as a novel class of drugs in dermatology.[3,6] Ruxolitinib phosphate is a potent selective inhibitor of JAK1 and JAK2 which control the signaling of growth factors and several cytokines that are vital for immune function. Ruxolitinib tablets (Jakafi°, Incyte Corporation) received FDA approval in 2011 for myelofibrosis and 2014 for polycythemia vera.[7,8] JAK inhibitors can also be used systemically in oral dosage forms or locally in topical preparations in dermatology. Previous studies revealed that treatment with oral ruxolitinib as an off-label indication improved autoimmune skin disorders.[5,9] When ruxolitinib is applied topically, it provides the chance to directly target various pathogenic pathways in the skin, and it is expected to indicate fewer adverse effects, compared to oral drug delivery.[5,10]

In recent pilot studies, topical ruxolitinib 1.5% cream was applied to manage skin disorders.[11-15] However, the use of conventional pharmaceutical dosage forms might be less effective due to lower permeability through the skin. Novel dermal drug delivery methods enhance the skin permeation of lipophilic and hydrophilic drugs; therefore, the in vivo activity of the active substance is stronger than that of conventional formulations.[16] Vesicular systems, such as liposomes, have been established for the

improvement of the skin penetration of drugs, especially for skin targeting. These lipid carriers have the benefits of controlling the release rate of the active ingredient and ensuring the localization of drugs in dermal layers.[17]

This study aimed to fabricate and assess the stability properties of liposomes containing ruxolitinib (RuxoLip) in emulgel form to be used topically in cutaneous autoimmune diseases. This is the first time that a novel skin drug delivery system is developed as a liposome for ruxolitinib instead of conventional formulations.

Materials and Methods
Materials
Ruxolitinib was obtained from Parsian Pharmaceutical Co. (Tehran, Iran). Lecithin and cholesterol were purchased from Duksan Pure Chemicals (Gyeonggi-do, South Korea) and Sigma-Aldrich (Missouri, USA), respectively. Chloroform, methanol, NaCl, KCl, and disodium hydrogen phosphate dodecahydrate were obtained from Merck (Darmstadt, Germany). Methylparaben and propylparaben were obtained from Alborz Bulk (Saveh, Iran). Potassium dihydrogen phosphate and polysorbate 80 were purchased from Sisco Research Laboratories Pvt. Ltd. (Maharashtra, India) and Oleon (Evergem, Belgium), respectively. Furthermore, ViscOptima™ SE (Sodium Polyacrylate [and] Ethylhexyl Cocoate [and] PPG-3 Benzyl Ether Myristate [and] Polysorbate 20) was obtained from Croda, (Snaith, United Kingdom). Soybean-Casein Digest Broth (Trypticase Soy Broth), Sabouroud Dextrose Agar, Mannitol Salt, and Cetrimide Agar were obtained from Liofilchem (Roseto, Italy). The dialysis membrane (MEMBRA-CEL MD44) with a molecular weight cut-off value of 12000-14000 Dalton was from Sigma-Aldrich (Department, Germany). Deionized water was prepared when required (Millipore, Bedford, MA).

Preparation of the liposomes
The thin-film hydration method was followed in the preparation of the liposomes. Several formulations of liposomes using various proportions of lecithin and cholesterol were fabricated. However, quantities of lecithin and cholesterol (4:1 w/w) were the best mixture because of good stability and other physicochemical characteristics.

Exact quantities of lecithin and cholesterol (4:1 w/w) were put in a dry, clean, round-bottom flask to dissolve p in 8 ml chloroform. Ruxolitinib powder was dissolved in 5 ml of methanol and added to the flask with glass beads. The solvents were removed using a rotary evaporator (Heidolph™, Germany) at 100 rpm under vacuum conditions and temperature (40±1°C) for 1 h until a thin film formed on the internal wall of the flask. Finally, the remained lipid film was hydrated with deionized water containing preservatives (methyl and propylparaben). To prepare the preserved water, 0.18% and 0.02% of methylparaben and propylparaben were accurately weighed, respectively, and the distilled water was then added and heated up to 90°C to dissolve completely.

The obtained vesicles were allowed to swell at 150 rpm for 2 h at the same temperature to get MLVs. The liposome suspensions were fabricated in triplicate. To reduce the size of MLVs, liposomal suspensions were sonicated using ultra probe sonication (Hielscher ultrasound technology UP400s, Germany) at 4°C, 80% amplitude, and 0.6 cycles for 9 min. To improve the rheological characteristics and increase the residence time of the drug on the skin, in this method, 1.2 % of a thickening agent (Sodium Polyacrylate, Ethylhexyl Cocoate, PPG-3 Benzyl Ether Myristate and Polysorbate 20) was completely dispersed in the liposomal suspension and mixed at 25±1°C and 1200 rpm for 20 min. following that, the pH was adjusted to 6.6 by NaOH 1 N. The final active concentration in emulgel was found to be 1.5% w/w. It means that 100 g of ruxolitinib emulgel contained a 1.5 g active ingredient of ruxolitinib phosphate.

Physicochemical characterization of the liposomes
Stability dtudy
Long-term stability studies (including appearance, particle aggregation, particle size, PDI, and ZP) were performed according to ICH guidelines at 25±2°C/60±5% RH for 12 months.

DLS measurements
Particle size, PDI, and ZP were assessed by DLS using Zetasizer (Malvern Instrument Ltd., Worcestershire, United Kingdom). The liposome formulations were diluted with deionized water (1:20) to obtain a suitable scattering intensity, and they were measured at a 90° scattering angle at 25±1°C.

Determination of pH, density, and viscosity of the RuxoLip emulgel
The preparation was tested for its pH, and the digital pH meter (Metrohm 827- Switzerland) was used to measure the pH of the nanoliposomal emulgel. For density measurement, the exact volume of the pycnometer was measured by filling it with water. The vessel was weighted full of the emulgel, and the density was simply measured using the following equation:

$$\rho = mV$$

ρ = density, m = mass, V = volume

The viscosity of RuxoLip in the emulgel was calculated by a Polyvisc Viscometer (Brookfield-USA). All assessments were performed in triplicate.

Morphological analysis of the vesicles
Initially, RuxoLip was diluted with deionized water at the ratio of 1:20. For the morphology study under SEM (MIRA3 TESCAN XMU, RMRC), the samples were coated with gold under vacuum using a gold coater, and they were visualized. The coating was performed in V=15 mA in 2 min with about 5-10 nm of thickness. Moreover, a SEM micrograph of RuxoLip in emulgel was captured to assess the stability of nanoliposomes in the gel base.

Entrapment efficiency (EE) and drug loading (DL)

RuxoLip was filtered by a cellulose acetate 0.22 μm syringe filter to separate the drug-loaded liposome from the free drug. Afterward, the separated nanoliposomes were dissolved entirely in a chloroform/methanol solvent. The absorbance of the dispersion was assessed using a UV-VIS spectrophotometer at 310 nm. Finally, the mass of the drug was calculated based on the standard calibration curve. The drug entrapment efficiency and the drug loading of the preparation were calculated using the following equations:

$$\%EE = \frac{M\ initial_{ruxolitinib} - M\ free_{ruxolitinib}}{M\ initial_{ruxolitinib}} \times 100$$

$$\%DL = \frac{W_{DL(weight\ of\ drug-loaded\ in\ nanoparticles)}}{W_{NL(weight\ of\ nanoliposomes)}} \times 100$$

Differential scanning calorimetry

Differential Scanning Calorimetry (DSC) investigation of the samples was conducted using Mettler Toledo DSC823 (Mettler Toledo, Greifensee, Switzerland) under the nitrogen flow of 80 ml/min at a heating ratio of 5 K/min between 25°C and 200°C. Samples including 1 mg of ruxolitinib powder, lecithin, cholesterol, ruxolitinib nanoliposomal, and nanoliposomes without the active ingredient (ruxolitinib free nanoliposomes) were put into a 40 μl aluminum sample pan and sealed with a lid to preserve the sample. An empty aluminum pan was used as the control. After all the examinations, the samples were cooled to zero.

In vitro and kinetic drug release study

The *in vitro* drug release assessments were performed using static Franz diffusion cells. Initially, a cellulose nitrate membrane was soaked in the receptor phase (phosphate buffer pH 7.4 containing 2% w/w Tween 80) overnight to fix the pore size throughout the release study. Approximately, 0.3 g of RuxoLip in emulgel was put on each membrane and devoted to each donor compartment. The heat was kept at 37°C, and 2 ml of the receptor phase was taken at time intervals of 0, 15, 30, 60, 90, 120, 150, 180, and 210 min and substituted in the donor section with the receptor phase to preserve the sink condition. The absorbance of the sample was measured at 310 nm by UV spectroscopy (CECIL CE2505, United Kingdom). For the evaluation of UV absorbance, the receptor solution was used as a blank to cover the tween 80 absorbance.

This method was performed on isolated abdominal skin of rats and excised abdominal human skin. The kinetic release models for the preparation were evaluated for zero-order, first-order, Hixson-Crowell, Korsmeyer-Peppas, and Higuchi models using KinetDS 3.0 rev.2010. In addition, the correlation coefficient (R^2) for each model was calculated based on the data obtained during the *in vitro* release study.

Tape stripping technique

In total, three young healthy female volunteers (mean age: 30±5.13) with no known history of dermatitis on the body were included in the test. Written informed consent was obtained from them, and the study protocol was approved by the Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.TIPS.REC.1397.142). In the next stage, 0-30 consecutive tapes were put on the skin surface area (2 cm^2) using a new tape for every three applications. Each trial participant had seven tape-stripped skin areas, and one skin area was chosen as a control site. At each site, 0.1 g of RuxoLip in emulgel was applied. After 10, 15, 20, 25, 35, 60, and 180 min, the tapes were applied using constant pressure on each site. The pressure was applied for around 10 sec, and the tape was detached with a uniform and rapid movement. From each tape-stripped area, the first tape and 29 consecutive tapes were separated, put into a microtube, dispersed in 1.5 ml of methanol, centrifuged with 13500 rpm for 30 min to extract the ruxolitinib, and analyzed using a UV spectrophotometer at 310 nm. This method was repeated for control tapes as the blank solution. The initial active ingredient applied on the skin was known and reduced from the amount absorbed into the tape to evaluate the amount of active ingredient passed the stratum corneum.

This method was repeated in 7-time intervals on the forearm. In this method, the percent of active ingredients that pass from the stratum corneum to viable epiderm was just evaluated; however, the percent of drugs in the derm cannot be detected in this method. The first tape showed the amount of unabsorbed drug, while the other tapes revealed the quantity of the drug that penetrated the SC to the viable epidermis and absorption quantity. Finally, the plots of drug penetration and unabsorbed percentage versus time were drawn. The experiments were performed in triplicate to ensure accuracy.[18]

Microbial assessment

In this section, 10 grams of RuxoLip in emulgel were mixed with 3 g of tween 80. To detect the bacteria, 2.4 g of tween 60 in water was added to the TSB medium for the homogeneity of emulgel in the TBS medium. Plates containing 25-250 colonies were counted, and the mean number of CFU/g was calculated. Two dilutions (10^{-1}, 10^{-2}) with the TSB culture medium were prepared, and 2 mL of pre-prepared dilution was added to two plates containing 20 mL culture medium of TSA, followed by incubation for 24-48 h at 30-35°C (pure plate). Moreover, 1 mL of 0.1 dilutions for fungal calculation permitted the agar to solidify at 25°C, followed by incubation for 5-7 days at 20-25°C. Colonies were calculated after the incubation, and it should be noted that all the examinations were performed in duplicate. Counting will pass in plates comprising fewer than 100 colonies for TAMC and in those containing less than 10 colonies for TYMC.[19]

Analytical validation

The analysis method was validated for accuracy, linearity,

precision, and solution stability. The validation method was conducted according to the ICH guidelines.

Linearity
The linearity of the analytical technique was assessed for five diverse concentrations (2, 4, 6, 8, and 10 µg/mL) of ruxolitinib in a 7.4 pH phosphate buffer with 2% tween 80. The experimentation was performed for three days in triplicate. The obtained data were used to plot the linearity curve, and the correlation coefficient equation and the regression equation were measured. Moreover, the LOD and LOQ of the proposed UV technique were determined. In addition, LOD and LOQ were determined based on the standard deviation of the slope and the response of the corresponding curve using the following equations:
LOD=3.3 (δ/S) and LOQ=10 (δ/S)
where δ signifies the standard deviation of the absorbance of the sample, and S represents the slope of the calibration curve.[20]

Precision
The precision of the UV technique was assessed based on the intraday and inter-day variations. The precision levels were measured for three diverse identified concentrations of ruxolitinib (2, 6, and 10 µg/mL), which were obtained from the stock solutions. For measuring intra-day precision, the absorbance of three concentrations was determined three times a day in triplicate. For inter-day precision, the absorbance was calculated daily for three days in triplicate. The RSD percentage was determined for three different concentrations.

Accuracy
Accuracy refers to the extent of agreement between the real value and the analytical value. The accuracy stages were determined for three diverse known concentrations of ruxolitinib (2, 6, and 10 µg/mL) using the following equation:
Accuracy (%) = calculated concentration/nominal concentration× 100
The solutions were evaluated by the same technique, and the sample concentration was measured in triplicate using the linearity curve.[21]

Solution stability
The absorbance of the same sample solution (6 µg/ml) was measured at the initial stage at intervals of 4, 8, and 24 h, followed by the determination of the cumulative %RSD.

Assay assessment
Ruxolitinib has UV absorbance, and the spectrophotometer was used due to the fast and inexpensive evaluation method. To extract the drug from the gel formulation, 10 mg of RuxoLip in emulgel was dissolved in 10 ml of chloroform and methanol at a 50:50 ratio. The gel was dissolved in the extracting solvent composition, further diluted as required, and centrifuged, followed by filtering through a 0.22 mm membrane filter before analysis. The experiment was evaluated in triplicates for nanoliposomal loaded gel-based preparations. Moreover, analysis by UV spectrophotometer and placebo were used as control.

Statistical analysis
Values in all the experiments are represented as mean±SD of three independent experiments. The statistical analysis of the variables was performed by the repeated measures ANOVA, and the significance level was established as $P<0.05$.

Results and Discussion
There is still a crucial therapeutic need for various skin disorders, including vitiligo, alopecia areata, atopic dermatitis, and others. There is increasing evidence that the JAK/STAT signaling pathway is the main pathway in different skin autoimmune disorders. The introduction of JAK inhibitors in dermatology will revolutionize the therapeutic result of numerous inflammatory skin disorders. JAK inhibitors, as topical or systemic drugs, could result in a great improvement.[6]

The first generation of JAK inhibitors includes ruxolitinib, which is a potent and selective inhibitor of both JAK1 and JAK2 proteins. As the topical cream which has approval from FDA was ruxolitinib phosphate (Opzerula 1.5%), phosphate salt of the drug was chosen. The safety profile of topical JAK inhibitors is higher than that of systemic drugs owing to their negligible systemic absorption.[10] Liposomes have been extensively researched for transdermal delivery of active ingredients.[22] The application of liposomes on the skin surface has been demonstrated to be effective in dermal targeted drug delivery. Liposomes increase the permeability of the skin for numerous entrapped drugs while reducing the adverse reactions of these drugs.[23]

The liposomes formulated by the thin-film hydration method exhibited suitable organoleptic characteristics (white and odorless) with acceptable stability. The stability of RuxoLip was evaluated for 12 months at 25±2°C/60±5% RH (Tables 1 and 2). As Table 1 shows, the mean particle size of RuxoLip was in the range of 218.33±2.08 to 243.33±5.77 nm. The particle size of liposomes is the main factor affecting their dermal penetration. Typically, it is suggested that liposomes with a particle size of ≥ 600

Table 1. Results of particle size, ZP and PDI of RuxoLip in long-term (25±2°C/60±5% RH).

Time (Month)	Initial	3rd	6th	12th	*P*-value
Z-average (nm)±SD	224±5.29	224.66±4.50	218.33±2.08	218.66±2.30	0.51
Zeta potential (mV)±SD	-40.33±0.57	-41.66±1.52	-39.66±0.57	-41.65±4.45	0.28
PDI±SD	0.213±0.00	0.20±0.01	0.197±0.01	0.235±0.00	0.12

Table 2. Results of physichochemical and microbial assessments of RuxoLip in emulgel during long-term stability (25±2°C/60±5% RH) conditions.

Test	Period of storage				
	Initial	3rd Month	6th Month	12th Month	*P*-Value
Description	White and homogenous emulgel				
Odor	Odorless				
pH (mean±SD)	6.51±0.01	6.7±0.08	6.58±0.01	6.87±0.107	0.51
Viscosity(cp) (mean±SD)	20443±51.31	21166±208.16	20541±74.87	20950±65.76	0.62
Density (g/cm3) (mean±SD)	0.98±0.00	0.970±0.00	0.975±0.00	0.98±0.003	0.18
Assay (g/100 g emulgel) (mean±SD)	103.03±0.89	99.7±0.36	103±1.73	99.69±0.60	0.23

nm cannot effectively deliver the active ingredients to the deep layer of the skin, mostly remaining on or inside the stratum corneum. However, liposomes with a particle size smaller than 300 nm can successfully deliver the active ingredients to the deeper layer of the skin.[23] The ZP of the formulation varied from -39.63±0.57 to -41.66±1.52 mV. The results of the ZP assessment indicated that the nanoliposome formulations located in the suitable mV range had good stability because the high surface charge prohibited the aggregation of particles.[24] ZP was shown to be on the negative side of -40 mV. This supports the fact that the lipids used in RuxoLip possess a negative charge.[25]

The nanoparticles that have ZP of less than -30 mV or more than +30 mV are considered a stable colloidal suspension that inhibits nanoparticles aggregation. Nanoparticles with ZP that drop between -30 and +30 mV specify poor colloidal stability and are expected to have aggregation, agglomeration, or flocculation. The suspension with a low ZP stimulates the accumulation of nanoparticles via van der Waals attractions. Moreover, the ZP offers an idea about whether charged drug molecules are trapped in the nanoparticle structure or just adsorbed on the surface of nanoparticles. Therefore, ZP is a suggestive tool for the long-term stability of nanoparticles.[26] The PDI of the prepared RuxoLip was between 0.197±0.01 and

0.266±0.01, indicating that these liposomal preparations are monodisperse. The PDI demonstrates the homogeneity of the droplet size and the width of size distribution, which is usually about 0.5 or less.[27,28]

Two main parameters related to different drug delivery systems are DL and EE. They demonstrate how much active ingredient is loaded in nanoliposomes. EE is defined as the loaded portion of the drug, which has a direct effect on drug release.[29] Furthermore, DL is determined as the fraction of the weight of the loaded drug to the weight of the liposomes. EE and DL depend on the active ingredient, nanoliposome properties, and the liposome synthesis method.[30] RuxoLip showed an EE of 67.71±2.35% and a DL of 8.58±0.648%. The relatively high EE might be due to their lipophilic nature, as well as better compatibility between ruxolitinib and the lipid material of liposomes.

The shape and surface morphology of RuxoLip and RuxoLip in emulgel were evaluated by SEM, as presented in Figure 1, respectively. This analysis indicated spherical-like particles in the nanoscale range with smooth and well-defined boundaries. The SEM micrograph of RuxoLip in emulgel base which was stored in 25±2°C/60±5% RH for 12 months was captured to assess the stability of nanoliposomes in the gel base.

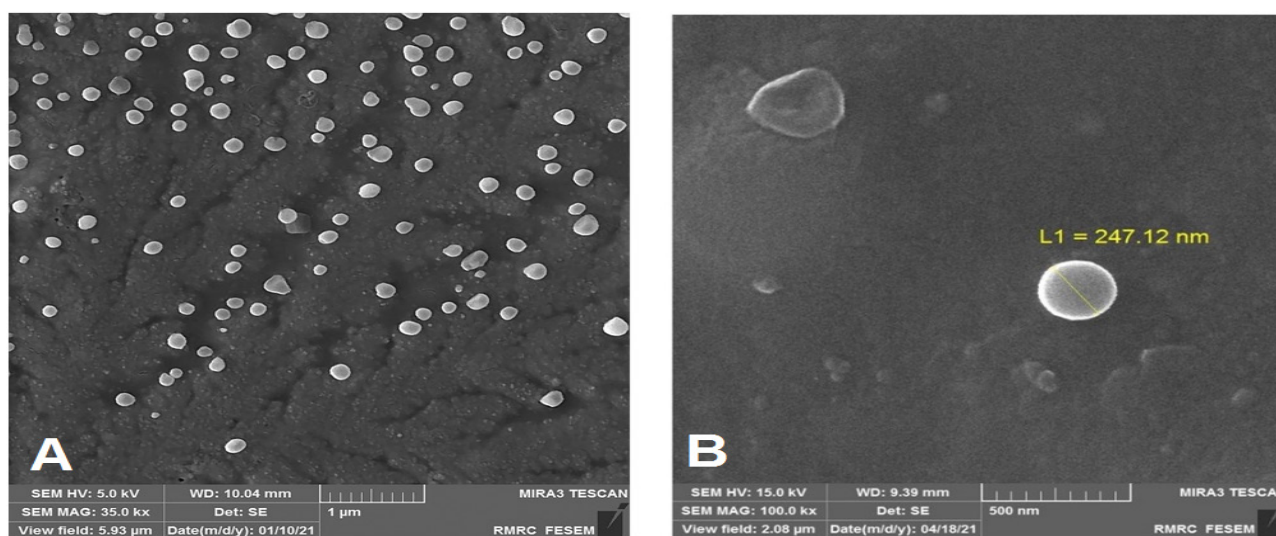The thermal behavior of RuxoLip was evaluated using

Figure 1. A) SEM micrograph of RuxoLip (Magnification: x2500), B) SEM micrograph of RuxoLip in emulgel (Magnification x100000).

DSC. A main application of DSC is to assess the thermodynamic variations of lipid bilayers after incorporating an active ingredient. These variations could be associated with the interactions of the lipid carrier with the active ingredient affecting the drug bioavailability. Thermodynamic factors are vital since they affect not only the pharmacokinetics of the drug but also the stability of RuxoLip under certain storage conditions. The results revealed DSC of cholesterol, lecithin, ruxolitinib powder, nanoliposome without ruxolitinib, and RuxoLip. The DSC curves showed a single endothermic peak in the regions of 150°C for cholesterol, 190°C for lecithin, 195°C for ruxolitinib powder, 112-126°C for nanoliposome without ruxolitinib, and 102-125°C for RuxoLip. The results of the DSC curve of lecithin, cholesterol, and ruxolitinib were very different from those of nanoliposome without ruxolitinib and RuxoLip. On the other hand, the DSC curves of lecithin and cholesterol can be dependent on the previous association of these molecules to form the liposome bilayer. Moreover, the results indicate that ruxolitinib can interact with lecithin and cholesterol during the preparation of the liposomes. When ruxolitinib was added to the lipid phase during the liposome preparation, the DSC curve shifted. The absence of sharp peaks in DSC indicates the homogeneity in the lipids forming the liposome and complete solubilization of ruxolitinib in the lipid bilayer or conversion of ruxolitinib crystal to amorphous form suggesting the reduction of lipid crystallinity in the liposomes.[31,32] (Figure S1 in Supplementary data).

The prepared RuxoLip in emulgel formulation showed no change in odor, color, pH, and phase separation during the storage period. As shown in Table 2, the mean pH at the initial, 1st, 2nd, 3rd, 6th, and 12th months was in the range of 6.51±0.01 to 6.87±0.10. The viscosity results were between 20443±51.31 and 21166±208.16 cp. The density of RuxoLip in emulgel was between 0.970±0.00 and 0.984±0.00 g/cm³. Finally, the microbial assessment during the entire period of stability confirmed that the formulation had no contaminations. In the preparation method of nanoliposome by rotary evaporator at 45°C for around 3.5 h, a total of chloroform was completely evaporated. Therefore, the assessment of the residual solvent amount in the final product was not performed.

The statistical analysis of the particle's morphological data and physicochemical properties showed that all the parameters were relatively constant during the study period. On the other hand, there was no significant alteration in particle size, PDI, ZP, density, assay, pH, and viscosity during assessments (P>0.05).

Adding nanoliposomes to the emulgel base increased the stability of the finished product. A study in 2019 exhibited that monocrotaline liposome dispersed into the carbomer, and glycerol matrix system could increase the stability of liposomes in terms of EE and *in vitro* drug release parameters.[33]

The *in vitro* release study of RuxoLip in emulgel within 210 min was performed by Franz diffusion cell, and the result is shown in Figure 2. Cumulative drug release from emulgel of RuxoLip in all the samples (synthetic membrane, rat, and human abdominal skins) during the entire period depicted a gradual increase of drug release reaching the highest percentage of around 70% at 210 min.

Based on our results, the release data were kinetically greatest fitted with the Korsmeyer-Peppas model in all points for all the samples with an R^2 value of 0.934. The Korsmeyer-Peppas model explains several release mechanisms instantaneously, including the swelling of the matrix, the diffusion of water into the matrix, and the dissolution of the matrix. The n values of the Korsmeyer-Peppas model are under the threshold for the Korsmeyer-Peppas (n=0.43). These values indicated a diffusion-controlled drug release mechanism. The value of n is less than 0.43 in all-time points and all membranes; therefore, a Fickian diffusion release mechanism was implied.

Cumulative permeation profiles obtained over 210 min were not significantly different for the cellulose nitrate membrane, as well as the rat and human skins. The rat skin illustrated lower drug permeation at primary time points in comparison with the other membranes because the thickness of hairless rat skin was lower than that in others. Furthermore, the pore size of the synthetic membrane is larger and more uniform than the rat skin.[34] A study revealed that the release of the drug of the synthetic membrane after 24 h of the experiment was higher than its permeation through the porcine ear epidermis for dapsone using Franz cells.[35]

However, the follicle density of the hairless rat skin is higher than human skin. As mentioned above, the mechanism of release was diffusion; therefore, the higher number of hair follicles reduced the permeation of ruxolitinib through the rat skin. The synthetic membrane revealed a ruxolitinib permeation profile that was similar to the one obtained for the human skin. The presence of lipid content in the stratum corneum of the human skin helps the permeation of drugs through lipid carriers, such as liposomes.[36,37] This synthetic membrane can be observed as an alternative to animal or human skin when assessing drug skin diffusion to reduce cost and time.[38] In another
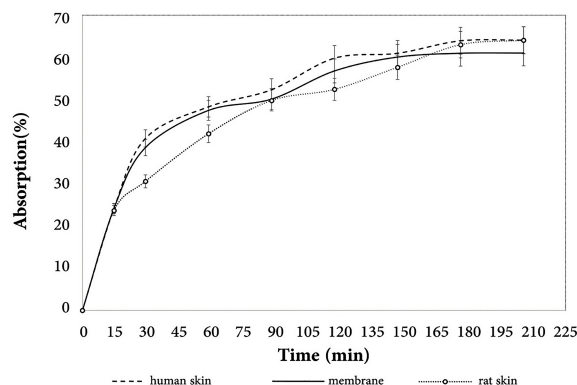

Figure 2. The cumulative release pattern of ruxolitinib from RuxoLip in emulgel during 210 minutes through synthetic membrane, rat skin, and human skin, n=3.

similar study, Glavas-Dodov et al.[39] showed that lidocaine HCl liposomal gel formulations provided a prolonged drug release rate because they acted as reservoir systems for the continuous delivery of a drug.

Tape stripping is a well-established *in vivo* method to examine skin penetration. The quantification of active ingredients within the skin is vital for topical and transdermal delivery investigations. It is a simple and minimally invasive way for testing the penetration of topically applied formulations through the SC.[40] In this method, the formulation to be examined is applied topically, and the cell layers of the stratum corneum are sequentially detached from the respective skin area using adhesive films.[41] Moreover, drug levels in the skin can be measured in the removed tape strips.[42] The penetration percentage of ruxolitinib in the different strips was indicated at 97% after 20 min of application. On the other hand, the percentage of the unabsorbed active ingredient was around 3%, which confirmed the penetration results (Figure 3).

In this study, the validation of the method was performed. LOQ, LOD, intra and inter-day precision, accuracy, and solution stability data were obtained. These parameters were evaluated at 2, 6, and 10 µg/mL of the ruxolitinib solution. Ruxolitinib obeys linearity within this concentration range, and the correlation coefficient was revealed to be 0.99. The RSD percentages of the LOD and LOQ were estimated at 1.30 and 2.43, respectively. Moreover, the percentage of RSD for intraday and inter-day precision was in the range of 1.58 to 3.58 and 1.64 to 1.98 at all levels, respectively, showing significant reproducibility. It is worth mentioning that the RSD percentage was not more than 5.0%.

The accuracy percentage at each level was found to be well within the range of 97.0%-103.0%, indicating an insignificant interference from the ingredients. (Table S1 in Supplementary data).

Therefore, it is shown that the method is accurate and precise in the range of 2 to 10 µg/mL of ruxolitinib. The solution stability examination showed that the solutions remained almost unchanged (RSD<5.0%), indicating that no significant degradation occurred within this period. The developed method was indicated to be simple, accurate, sensitive, and precise for the analysis of ruxolitinib.

It is worth mentioning that these findings for the first time show that RuxoLip in emulgel may represent a novel and effective topical treatment for patients with autoimmune skin disorders in a liposomal base. Moreover, the rationale for evaluating a topical formulation of ruxolitinib came from the recognition that Janus kinases modulate inflammatory cytokines.

Regarding the drawback of this study, one can refer to the lack of stability and efficacy comparison of the RuxoLip with a conventional formulation.

Conclusion

Topical JAK inhibitors may be a safer alternative, compared to oral ones, especially in cases where systemic therapy is contraindicated or not desired. In this study, topical ruxolitinib nanoliposomes were fabricated by the film-hydration method. Our stability evaluations confirm that ruxolitinib in the form of nanoliposomes enhances physicochemical stability with an appropriate permeation and release profile with an EE of 67%. In the formulation of nanoemulgel, 67% of active ingredients were entrapped into liposomes and 33% remained in the form of free drugs. In a permeation study, at first, the free drug was released, and then the drug entrapped in liposomes gradually released. However, about 70% of active ingredients were released across the synthetic membrane, abdominal rat skin, and abdominal human skin. Moreover, the analysis methods were indicated to be valid. The results of the current study show that RuxoLip in emulgel is a novel carrier to be used as a therapeutic agent in autoimmune skin disorders.

Ethical Issue

Written informed consent was obtained from volunteers, and the study protocol was approved by the Ethics Committee of Tehran University of Medical Sciences (IR. TUMS.TIPS.REC.1397.142).

Author Contributions

AN collected the data; MR and HAJ designed and supervised the study. SAN conducted the experiments. AF and MNK analyzed the experiments. AN wrote the initial draft. All authors revised the initial draft and approved the final version of the article.

Conflict of Interest
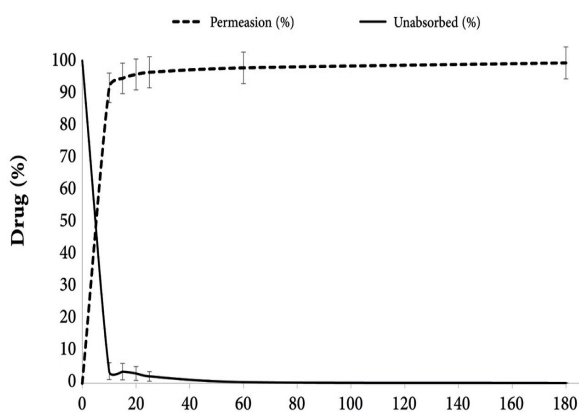
Authors declare no conflict of interest.

Figure 3. The permeation percentage of ruxolitinib through the SC against time and percentage of unabsorbed ruxolitinib in tape stripping technique, n=3.

Supplementary Data

Supplementary Data, Table S1 and Figure S1, are available at https://doi.org/10.34172/PS.2022.17.

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