IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
December 2020 Vol.:20, Issue:1

Development and Evaluation of Ursolic Acid Loaded Solid Lipid Nanoparticle Based Gel for the Treatment of Acne

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Shreeja Sawant[1], Sheela Yadav[2], Geeta Bhagwat[3], Shifa Haju[4], Rutuja Sawant*[5]

1. Research Scholar, Department of Pharmaceutics, H.K College of Pharmacy, Oshiwara, Jogeshwari (w), Mumbai- 400102, India. 2. Associate Professor (HOD), Department of Pharmaceutics, H.K College of Pharmacy, Oshiwara, Jogeshwari (w), Mumbai- 400102, India. 3. Assistant Professor, Department of Pharmaceutics, H.K College of Pharmacy, Oshiwara, Jogeshwari (w), Mumbai- 400102, India. 4. Research Scholar, Department of Pharmaceutics, H.K College of Pharmacy, Oshiwara, Jogeshwari (w), Mumbai- 400102, India.5. Research Scholar, Department of Pharmaceutics, H.K College of Pharmacy, Oshiwara, Jogeshwari (w), Mumbai- 400102, India.

Submitted: 12 November 2020
Revised: 02 December 2020
Accepted: 22 December 2020

Keywords: Ursolic Acid, Solid Lipid Nanoparticles, Topical delivery

ABSTRACT

Ursolic acid is a pentacyclic terpenoid exhibiting a wide range of pharmaceutical properties. The purpose of this study was to develop Solid lipid nanoparticles containing Ursolic Acid (UA) by high-pressure homogenization technique and evaluate for the topical treatment of acne. The prepared solid lipid nanoparticles were evaluated for their particle size, zeta potential, entrapment efficiency, surface morphology, *in-vitro* drug release, and *ex-vivo* skin permeation study. The gel was additionally characterized for its pH, drug content, and spreadability. The prepared nanoparticles were spherical and of size below 250 nm with negative zeta potential. Incorporation of prudently chosen excipients made possible a relatively high entrapment efficiency of almost 71%. The drug release pattern was found to be biphasic, with an initial burst release followed by sustained release up to 8 hours and simultaneously minimizing permeation through the skin, i.e., systemic exposure. A stability study showed that room temperature was the best condition for nanoparticle storage. The results of the study suggest that SLNs of UA have the potential to be used in the therapy of acne.

INTRODUCTION:

Acne vulgaris may be a chronic inflammatory disorder of the pilosebaceous unit (1). Acne is a common human skin disorder affecting people of all races and ages. It has been reported in 2010, that acne affects approximately 9.4% of the population. It affects about 90% of people during their teenage years and sometimes in adulthood. Moderate and severe cases of acne affect 20% of people. Acne vulgaris is largely a disease of adolescence, occurring in approximately 85% of this population, it can appear at any age(2). Acne is a multifactorial disease affecting pilosebaceous follicles and arises from the interplay of four pathogenic factors:

1. Increased sebum production.

2. Follicular hyperkeratinization may lead to follicular obstruction.

3. It leads to Colonization by the causative agent, Propionibacterium acnes.

4. Host inflammatory responses triggered as a result of bacterial infection(3).

Although acne isn't life-threatening, it causes significant psychological disability(4). Treatment options include antibiotics, antiseborrheic, topical retinoids, salicylic acid, antiandrogen treatment. These are given either orally or topically, but many of the above treatments cause undesirable side effects such as erythema, scaling, burning, bacterial resistance. Hence topical therapy is the first-line treatment of acne as it has the advantage of reducing undesirable side effects of API as fewer drugs enter the systemic circulation as compared to oral or parenteral administration(5). The drug delivery onto the skin is recognized as an effective means of therapy for local dermatologic diseases like acne.

Hence, the present study has been undertaken to develop Ursolic acid loaded solid lipid nanoparticles for the treatment of acne topically with sustained-release potential.

MATERIALS AND METHODS:

Ursolic acid (UA) was purchased from Maysar Herbals, Haryana., India. Tween 80 was a kind gift from Mohini Organics, Mumbai India. Compritol ATO 888 was obtained as a gift sample from Gattefosse, India. Kolliphor EL was a gift received from BASF, India. All the excipients were of analytical grade.

PREPARATION OF SLN

Screening of components (solubility studies)

Because of the lipophilicity of UA, a solubility study was initiated in different solid lipids and surfactants. Drug with gradual increment was added in a vial containing different solid lipids held in a hot water bath until the drug became completely solubilized in the particular solid lipid. Known amounts of surfactants were mixed with an excess of the drug and shaken for about 72 hours in an orbital shaker at 37 ± 2°C. The calculated amount of drug dissolved in each excipient was determined spectrophotometrically at 206 nm.

Fabrication of SLNs

Several formulation techniques have been employed in SLN preparation with high-pressure homogenization and microemulsion being the most commonly used(6,7). O/W microemulsion was prepared by dispersing the hot aqueous phase into the melted lipidic phase at a temperature 10°C above the melting point of lipid in a water bath. The aqueous phase was gently dropped on the organic phase with constant agitation, at 1500 rpm using a magnetic stirrer for 15 minutes. This emulsion was immediately subjected to high-pressure homogenization at 1000 bar pressure for further particle size reduction. The influence of various surfactants on the characteristics of the SLN (EE, particle size, and zeta potential) was investigated.

For the preparation of SLN based topical gel, the gelling agent Carbopol 934 (2% w/w) was dispersed in the prepared SLN dispersion. It was then neutralized to pH 7 using triethanolamine.

Particle size and Polydispersity Index

The particle size and polydispersity index of UA-SLN were determined using Horiba Nanoparticle SZ 100 and Malvern Zetasizernano ZS. A suitably diluted suspension formulation (lyophilized SLNs) was used for the analysis(8). The sample was diluted with filtered deionized water in polystyrene cuvettes and was observed at a fixed angle of 90º at 25±0.1ºC.

Zeta Potential

The zeta potential of the SLNs-A was determined in folded capillary cells by Laser Doppler Anemometry using Malvern zeta sizer. The zeta potential was measured on samples well-dispersed in deionized water at temperature, 25±0.1°C.

Entrapment efficiency (EE)

Determination of the amount of drug entrapped within the lipid matrix of the system is essential because it may affect the drug release and skin deposition. Entrapment efficiency (EE) of SLNs was determined by measuring the concentration of unentrapped drug in an aqueous medium by centrifugation method(9). A known amount of SLN suspension was centrifuged at 12,000 rpm (Remi microcentrifuge machine BL-135D) for 30 min. at 25ºC. After completion of centrifugation, the supernatant liquid was collected and filtered through a syringe filter (0.22 µm). Free drug available in this supernatant liquid was further analyzed for UV by suitable dilution of solution (see calibration curve for dilution method) for drug content. The encapsulated amount of the drug was calculated by subtracting the initial amount of the drug from the free amount of the drug in nanodispersion. A percentage of entrapment efficiency was calculated by using the following formula. Where the initial amount of drug is the drug that was taken initially during nanoformulation preparation.

Percentage EE was then calculated as follows:

$$\% EE = (Wa-Wb) /WA\times100$$

Where Wa is the amount of initial drug used for the assay

Wb is the amount of supernatant of the free drug after centrifugation of the aqueous dispersion.

% EE is the % entrapment efficiency.

Transmission electron microscopy (TEM)

The particles in the SLN dispersion were visualized by TEM studies. A few drops of the diluted dispersion was placed on a carbon-coated copper grid and left for 1 minute to adhere to it. One drop of phosphotungstic acid (1% w/v) was placed on this and was allowed to dry

under an IR lamp for 5 minutes. The sample was then viewed under TEM and photographs were taken.

Differential scanning calorimetry (DSC)

The thermal behavior of pure drug and drug-loaded SLN dispersion was studied by DSC. Samples (about 3 mg) were placed in aluminium pans and sealed. The samples were heated from 25°C to 300°C at a heating rate of 5°C/min using nitrogen as purge gas (50 ml/min) and endotherms were recorded.

***In-vitro* release study**

In vitro drug release could be achieved using dialysis tubing. The solid lipid nanoparticle dispersion is placed in pre-washed dialysis tubing which can be hermetically sealed(10). A quantity of nanoparticles equivalent to 1mg UA was placed into a dialysis bag that was immersed into 20 ml phosphate buffer solution and the system was maintained at 37°C under mild agitation of 300 RPM/min on a magnetic stirrer. At predetermined time intervals, aliquots of the release medium (1 ml) were withdrawn intermittently (0, 5, 10, 15, 30, 45 min, 1, 2, 4, 5, 6, and 8 h) and assayed for drug release and replaced by 1 ml of fresh buffer in receptor compartment. UA in the release medium was quantified by UV spectrophotometry at 206 nm and the cumulative release of UA was calculated based on a pre-generated calibration curve.

***Ex-vivo* skin permeation studies**

Permeation study was carried out using goat abdominal skin (obtained from local abattoir) as a model of human skin. The skin was installed in between the donor and receptor chamber of vertical, jacketed Franz cells (receptor volume of 20 ml, pH buffer 5.5) with an effective permeation area 3.14 cm^2, constantly stirred with a magnetic bead and thermostatted at 37 ± 1°C. Later, the test formulations (UA-SLN dispersion and UA-SLN gel equivalent to 400 mcg of UA) were gently placed in the donor chamber. At predetermined time intervals over 8 h (0.5, 1, 2, 4, 6, and 8 h), samples (1 ml) were withdrawn from the receptor chamber (immediately replaced with an equal volume of fresh medium) and % UA permeated in receptor chamber was analyzed by UV spectrophotometer at 206 nm with suitable dilutions.

Stability Studies

The prepared formulation was subjected to stability studies at 25 ± 2°C/60 ± 5% RH and 40 ± 2°C/75% ± 5% RH in a stability chamber for 2 months. After measuring initial drug content, the formulations were filled in glass vials & sealed, and stored at prescribed conditions in the stability chambers. After each periodic interval of one month, the content was periodically determined.

RESULT AND DISCUSSION:

Screening of components (Solubility studies)

The lipid itself is the key ingredient of lipid nanoparticles that influence their drug loading capacity, their stability, and also the sustained release behavior of the formulations(11). The selection of appropriate lipids is crucial before their use in the preparation of lipid nanoparticle dispersions hence solubility study was carried out. Among solid lipids, Compritol 888 ATO demonstrated the highest solubilizing capacity for UA and was selected as the solid lipid for the SLN system. Results are shown in Figure No. 1. Although there are no specific guidelines for the selection of appropriate lipids. Crystallization in lipids with longer chains of fatty acids is lower than that with shorter fatty acid chains(11). This is in agreement with the reported literature. Solubility data of UA in surfactants are shown in Figure No. 2. Pluronic® and Tween® are the most commonly used non-ionic surfactants(12). As discussed, most surfactants contain a hydrophilic moiety (ethylene oxide) and a hydrophobic moiety (hydrocarbon chain). It was observed that the combination of Cremophor EL & Tween 80 had the highest solubility for UA (2.5 mg/2ml). Tween 80 has multiple polar functional groups contributing to the high hydrophilicity i.e. ether, hydroxyl, ester groups, and thus, high HLB (HLB 15) of the molecule. Hence it can solubilize UA. Cremophor EL is Polyoxyl 35 castor oil i.e., a propylene glycol ester of fatty acid, and a medium-chain length triglyceride though there are some polar functional groups in the molecule like hydroxyl and carbonyl group with high HLB (HLB-12-1).

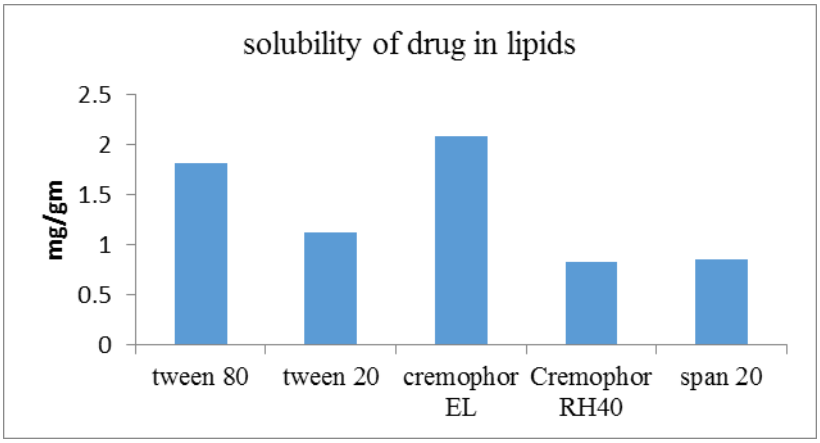

Figure No. 1: Solubility of the drug in lipids

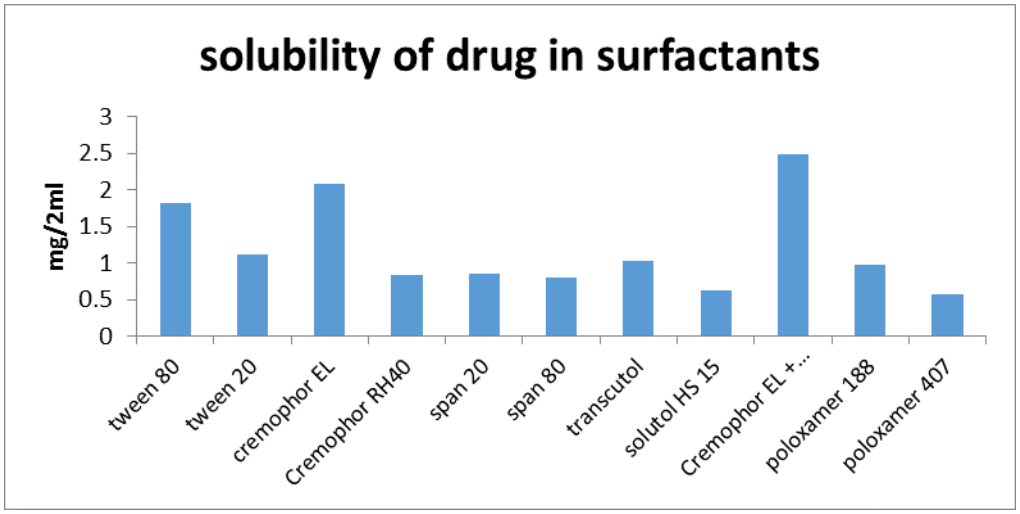

Figure No. 2: Solubility of the drug in surfactants

Fabrication of solid lipid nanoparticle:

Gasco et al. (1997) developed SLNs based on dilution of microemulsion(13). The SLNs were fabricated by microemulsion technique followed by high-pressure homogenization technique resulting in small-sized lipid nanoparticles. Preparation of solid lipid nanoparticles via microemulsion method was performed at a temperature above the melting point of the lipid. O/W microemulsion was prepared by dispersing the hot aqueous phase into the melted lipidic phase. Formulations were prepared using lipid compritol ATO 888 and a combination of surfactants Tween 80 and cremophor EL and deionized water as the dispersion medium. Appropriate quantities of lipid, surfactant, and water were weighed and mixed at a temperature of 10°C above the melting point of the lipid in a water bath. The aqueous phase is consisting of water-soluble surfactant and the oily phase consists of lipid-soluble

surfactant. The water phase was heated to the same temperature as the lipid phase and added dropwise under mild stirring to the lipid melt. These two phases were mixed by using a magnetic stirrer at 1500 RPM for 15 minutes and visualized for clarity. A transparent, thermodynamically stable system was formed when the compounds were mixed in the correct ratio for the microemulsion formation. The prepared microemulsions were immediately subjected to high-pressure homogenization at 1000 bar pressure for further particle size reduction.

Particle size and zeta potential

A novel topical treatment for acne vulgaris using Nanotechnology and UA was produced and evaluated. Particle size is crucial for skin permeation it is described that the optimum nanoparticle size for follicular permeation lies between 200 -600 nm 8 and thus a transcutaneous permeation via the pores is promoted.

The particle size of the SLN dispersion was found to be below 600 nm, with a polydispersity index (PI) below 0.7 which is indicative of the uniform distribution of particles. It was observed that the particle size of the drug-loaded dispersion 246.6 nm. Results are shown in Figures No. 3 and 4.

Zeta potential is an important criterion for examining the storage stability of lipid particles. The zeta potential of optimized formulations of UA-SLN was -26.1 mV. The higher negative value of zeta potential indicates repulsive interaction between SLNs, and thus prevents aggregation of nanoparticles also the negative zeta potential of the particles may facilitate interaction with pilosebaceous units that is crucial because the zeta potential of the skin has been described as being positive. Besides, tween 80 used in this study also provided stearic stability to achieve stable formulation(14).

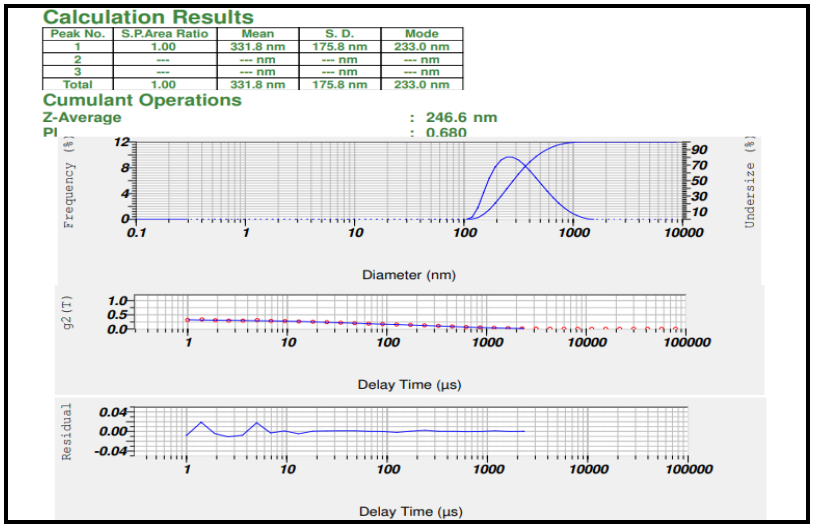

Figure No. 3: Particle size of an optimized batch made by high-pressure homogenization

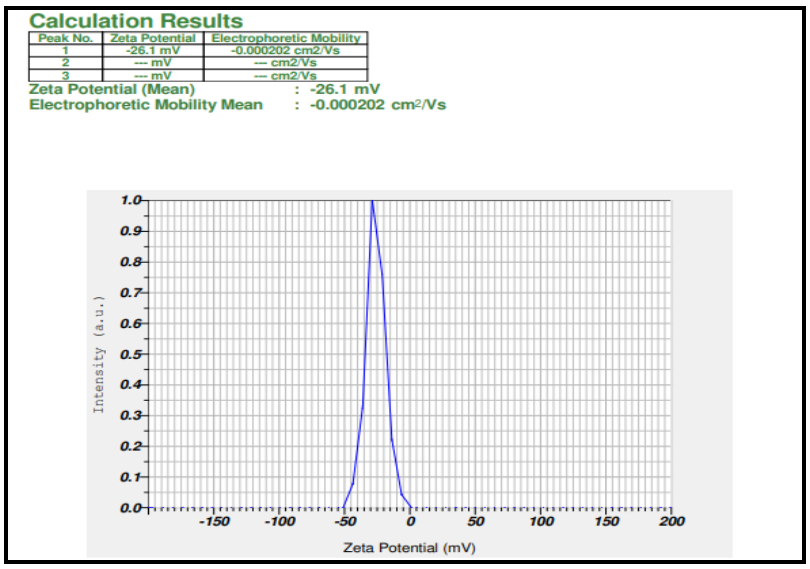

Figure No. 4: Zeta potential of UA-loaded SLN prepared by high-pressure homogenization

Entrapment efficiency

Entrapment efficiency indicates the percentage of the drug that has been loaded within the lipid SLNs concerning the feed drug concentration. The greater entrapment efficiency (71 %) of UA-SLN was found at 3.5% surfactant concentration. We believe the loss in entrapment efficiency is probably due to surface leaching of the entrapped drug. The high lipophilicity and better compatibility between drug and lipid may result in the high EE of SLNs

formulations and might prove beneficial in reducing skin irritation due to minimal or no contact of the drug to the skin surface(15). The results are shown in Table No. 1.

Table No. 1: % Entrapment efficiency of UA-SLN

Sr. No.	Formulation code	% Entrapment efficiency
1.	G-7	71

Transmission electron microscopy

TEM results confirmed the spherical shape of prepared UA-SLN, and photomicrographs of the prepared formulation are suggestive of their nanometric size range and narrow size distributions. The TEM image also showed a uniform particle size distribution. The results are shown in Figure No. 5.

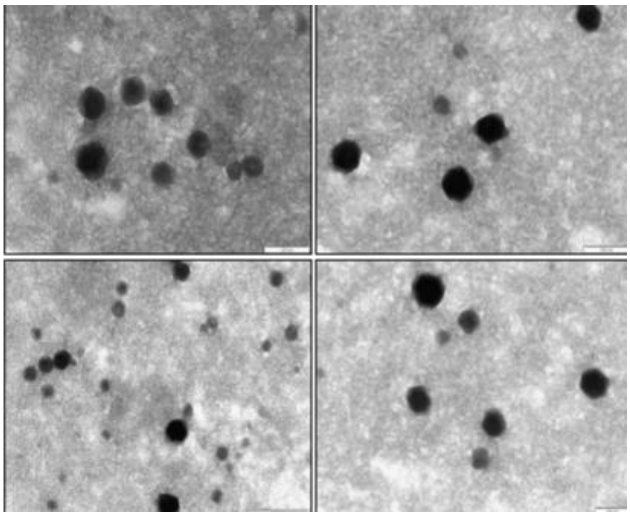

Figure No. 5: TEM images of UA-SLN dispersion

Differential scanning calorimetry

DSC allows the determination of thermotropic phase transitions quantitatively and is especially useful for the investigation of the complex behavior of polymorphic triacylglycerols(16). DSC studies were carried out to investigate the thermal behavior of the UA-SLN and the pure drug. DSC curve of pure UA (Fig.6) showed a first exothermic event at 201 °C, related to crystalline transition, and an endothermic peak corresponding to melting point in 284 °C, indicating the crystalline nature of the drug. DSC curve of UA- nanoparticles is shown in Figure No. 7. The peak corresponding to the UA melting point was not observed,

indicating the encapsulation of the drug. UA was dissolved in the compritol ATO888, which makes them an ideal excipient in the production of SLN. We observed the exothermic decomposition peak of Ursolic acid disappeared in the thermogram of Optimal UA-SLN, suggesting that Ursolic acid is integrated into the lipid(17).

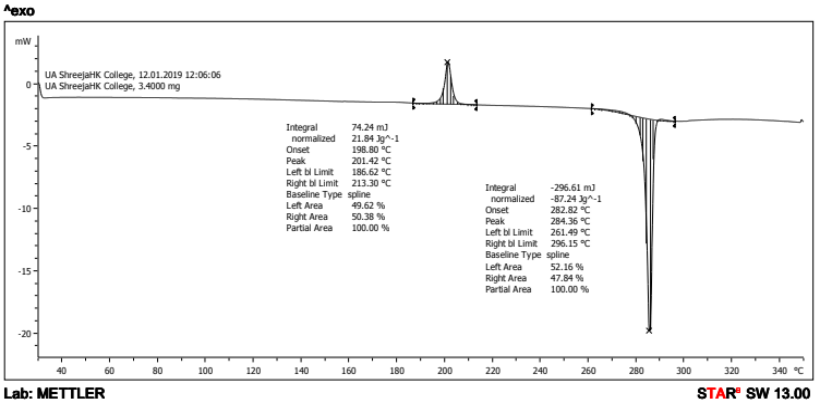

Figure No. 6: DSC of pure drug

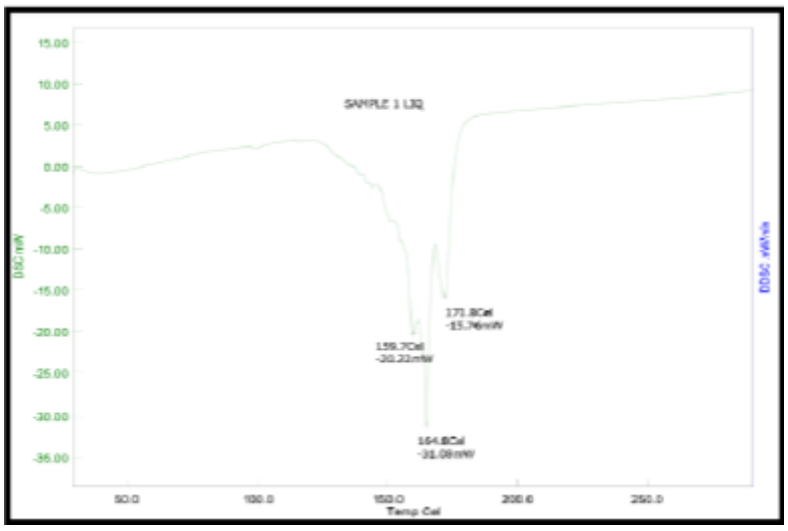

Figure No. 7: DSC of UA-Loaded SLN

In-vitro release study

The drug release was studied intermittently starting from 0 hrs to 8 hrs. The initial burst release of 22.42% and 19.57% from UA-SLN and UA-SLN gel was an interesting observation respectively and followed the slow and sustained release. The initial burst was probably due to the rapid release of drug adsorbed on the surface or presence of drug just underneath the stratum of the SLNs. The slower and sustained release, however, attributed to

the diffusion of drug molecules through the lipid matrix of SLNs. Comparing the drug release from nanoparticulate dispersion and nanoparticles in gels (Figure No. 8), the release of UA was slower from the gel formulation: 46.71% at the end of 8 hours. Drug release was decreased after the incorporation of nanoparticulate dispersion into gels. This result was probably due to the release-retarding effect of the polymeric matrix of gelling agents(18).

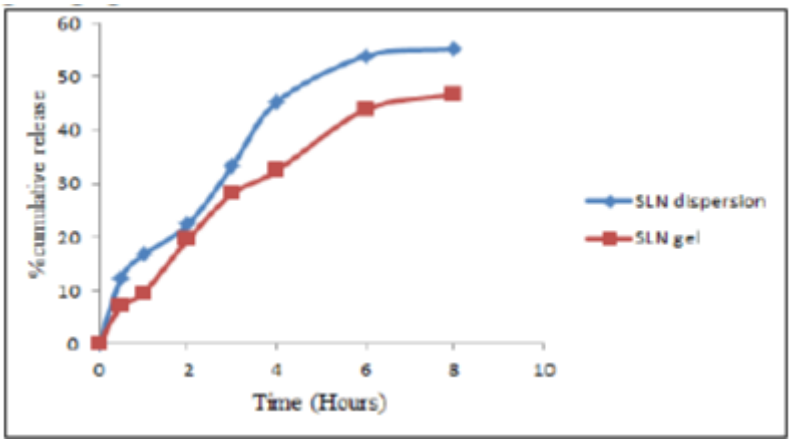

Figure No. 8: *In-vitro* release profile of UA loaded SLN dispersion and SLN-gel

***Ex-vivo* skin permeation studies**

Permeation study was carried out using goat abdominal skin (obtained from local abattoir) as a model of human skin. Franz diffusion cells were used to evaluate the skin uptake and skin targeting potential of SLNs. An ideal topical formulation for acne should give minimum permeation through the skin and maximum skin deposition. The drug should be restricted into the stratum corneum layer and should not pass into the systemic circulation. The penetration should give a superficial cosmetic effect but not a systemic pharmaceutical effect. It is reported that less drug permeation through the skin in the case of SLN gel indicates its skin targeting ability, which is desirable for effective topical therapy.

The permeation ability of UA from SLNs formulation into goatskin was determined by sampling goat from 0 to 8 hrs. From Figure No. 9, it can be seen that the plain drug gel allowed much greater quantities of the drug to permeate through the skin, compared to the SLN dispersion and gel. The SLN gel and dispersion allowed less drug to permeate through the skin than the plain drug gel respectively. They thus had lower flux rates than the plain drug gel. The plain drug gel had a flux of 42.61 μg/cm^2 hr, respectively, whereas the SLN gel

and dispersion had much lower fluxes of 19.55 and 29.67 µg/cm^2 hr, respectively. These lower fluxes indicate skin deposition.

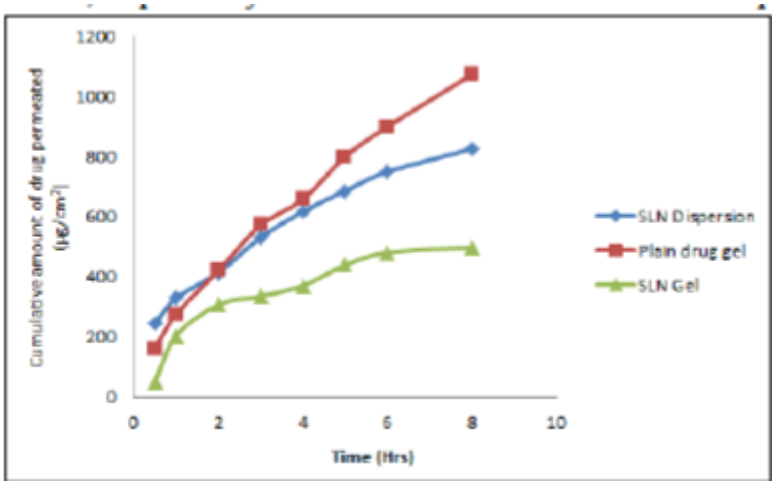

Figure No. 9: *Ex-vivo* skin permeation study of SLN Dispersion, SLN gel, and plain drug gel

pH, Drug content, and Spreadability

The pH of the SLN gel was determined using a digital pH meter (Lab India) after appropriate calibration. One gram of each formulated gel was dispersed in 30 ml of distilled water, then the pH was measured which was noted by bringing the electrode near the surface of the formulations and allowing it to equilibrate for 1 min(19,20). It was found to be in the range of 6.0 to 7.0, which is suitable for use on the skin. The drug content of the SLN gel was determined and found to be 97 %. Spreadability was measured based on slip and drag characteristics of the gels and was found to be 12.69 gms. cm./sec.This is indicative of good spreadability which is an important requirement for any topical gel formulation to ensure good patient compliance.

Stability studies

According to International Conference on Harmonization (ICH) guidelines, samples of optimal UA-SLN dispersion and UA-SLN gel were stored in airtight glass vials at 25 ± 2°C/60 ± 5% RH and 40 ± 2°C/75% ± 5% RH in a stability chamber for 2 months. No considerable variations in clarity and phase separation were observed demonstrating good physical stability of SLNs.

CONCLUSION

Several studies have been reported for the effective topical delivery of different kinds of anti-acne molecules. The various colloidal carriers like liposomes, lipid-nanoparticles, and microemulsion were employed to either improve the physicochemical properties of drugs or to improve their pre-clinical efficacy. However, there are no studies have been reported on UA loaded SLNs. A novel topical formulation with UA-SLN was prepared and evaluated. UA-SLNs were prepared using Compritol ATO 888 as lipid matrix, Tween 80 as a surfactant, and distilled water as a dispersion medium using a high-pressure homogenization method. The UA-SLN demonstrated efficient encapsulation of UA, showed desirable quality control parameters like particle size & zeta potential. TEM studies revealed spherical particles and the formation of a 'drug-enriched shell' type of SLN. *Ex-vivo* permeation studies demonstrated a lower permeation rate, which led to increased skin drug deposition and eventually, reduced risk of skin irritation. Results of stability testing showed that optimal UA-SLN dispersion was stable for 2 months at varying temperatures. Considering the results of the current study, directions for future work include the design and development of combination SLNs (i.e., UA + Pentacyclic triterpenoids) through to our current strategy to target two or more pathogenic factors of acne i.e., P acne & S.epidermidis and long-term stability tests and efficacy test with human volunteers.

Conflict of interest

The authors report no conflict of interest.

Acknowledgments

The authors would like to thank Gattefosse, Mumbai, India, for the supply of Compritol 888 ATO and Mohini Organics, Mumbai for providing Tween-80 as a gift sample.

REFERENCES:

1. Bhambri S, Del Rosso JQ, Bhambri A. Pathogenesis of acne vulgaris: recent advances. J Drugs Dermatol JDD. 2009 Jul;8(7):615–8.
2. Picardo M, Eichenfield LF, Tan J. Acne, and Rosacea. Dermatol Ther. 2017 Jan;7(Suppl 1):43–52.
3. M T, M M. Pathogenesis of acne. Med Electron Microsc Off J Clin Electron Microsc Soc Jpn. 2001 Mar 1;34(1):29–40.
4. Beauty Restoration Institute, Wong A. Oxidative Stress in Acne Vulgaris. Clin Dermatol Ther. 2016 Sep 2;3(1):1–6.

5. Schäfer-Korting M, Mehnert W, Korting H-C. Lipid nanoparticles for improved topical application of drugs for skin diseases. Adv Drug Deliv Rev. 2007 Jul 10;59(6):427–43.
6. Wissing SA, Kayser O, Müller RH. Solid lipid nanoparticles for parenteral drug delivery. Adv Drug Deliv Rev. 2004 May 7;56(9):1257–72.
7. Torchilin VP. Nano particulates as drug carriers [Internet]. London: Imperial College Press; 2006 [cited 2020 Nov 29]. Available from: http://site.ebrary.com/id/10201228
8. Esposito E. Production, Physico-Chemical Characterization and Biodistribution Studies of Lipid Nanoparticles. J Nanomedicine Nanotechnol. 2015;06(01).
9. Kumar VV, Chandrasekar D, Ramakrishna S, Kishan V, Rao YM, Diwan PV. Development and evaluation of nitrendipine loaded solid lipid nanoparticles: influence of wax and glyceride lipids on plasma pharmacokinetics. Int J Pharm. 2007 Apr 20;335(1–2):167–75.
10. Abdelbary G, Fahmy RH. Diazepam-loaded solid lipid nanoparticles: design and characterization. AAPS PharmSciTech. 2009;10(1):211–9.
11. Pardeshi C, Rajput P, Belgamwar V, Tekade A, Patil G, Chaudhary K, et al. Solid lipid based nanocarriers: an overview. Acta Pharm Zagreb Croat. 2012 Dec;62(4):433–72.
12. Manjunath K, Reddy JS, Venkateswarlu V. Solid lipid nanoparticles as drug delivery systems. Methods Find Exp Clin Pharmacol. 2005 Mar;27(2):127–44.
13. Garud A, Singh D, Garud N. Solid Lipid Nanoparticles (SLN): Method, Characterization and Applications. Int Curr Pharm J. 2012 Oct 3;1(11):384–93.
14. Patzelt A, Richter H, Knorr F, Schäfer U, Lehr C-M, Dähne L, et al. Selective follicular targeting by modification of the particle sizes. J Control Release Off J Control Release Soc. 2011 Feb 28;150(1):45–8.
15. Jain AK, Jain A, Garg NK, Agarwal A, Jain A, Jain SA, et al. Adapalene loaded solid lipid nanoparticles gel: an effective approach for acne treatment. Colloids Surf B Biointerfaces. 2014 Sep 1;121:222–9.
16. Westesen K, Siekmann B. Investigation of the gel formation of phospholipid-stabilized solid lipid nanoparticles. Int J Pharm. 1997 May 12;151(1):35–45.
17. Pokharkar VB, Mendiratta C, Kyadarkunte AY, Bhosale SH, Barhate GA. Skin delivery aspects of benzoyl peroxide-loaded solid lipid nanoparticles for acne treatment. Ther Deliv. 2014 Jun;5(6):635–52.
18. Shinde UA, Parmar SJ, Easwaran S. Metronidazole-loaded nanostructured lipid carriers to improve skin deposition and retention in the treatment of rosacea. Drug Dev Ind Pharm. 2019 Jul;45(7):1039–51.
19. Kumar S, Himmelstein KJ. Modification of in situ gelling behavior of carbopol solutions by hydroxypropyl methylcellulose. J Pharm Sci. 1995 Mar;84(3):344–8.
20. Srividya B, Cardoza RM, Amin PD. Sustained ophthalmic delivery of ofloxacin from a pH triggered in situ gelling system. J Control Release Off J Control Release Soc. 2001 Jun 15;73(2–3):205–11.