Indian Journal of Natural Products and Resources
Vol. 12(1), March 2021, pp 158-163

Tea tree and jojoba oils enriched bigel loaded with isotretinoin for effective management of acne

Jovita Kanoujia[1,2], Nikita[1], Poonam Parashar[1], Neelu Singh[1] and Shubhini A. Saraf[1]

[1]Department of Pharmaceutical Sciences, Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Raebareli Road, Lucknow 226025, Uttar Pradesh, India

[2]Amity Institute of Pharmacy, Amity University of Madhya Pradesh, Maharajapura, Gwalior 474005, India

Received 03 May 2020; Revised 31 December 2020

The study is aimed at the preparation of isotretinoin (ITR) loaded bigel for acne therapy. Bigels were enriched with tea tree and jojoba oils known to bestowed soothing properties. Bigels were evaluated for the physiochemical parameters viz. pH, viscosity, etc, *in vitro* release, and *ex vivo* permeation. The results displayed enhanced retention and antimicrobial potential of optimized B2 gel when compared with plain gel and other formulations. Thus the bigel of isotretinoin could be a promising delivery system for the treatment of acne.

Keywords: Acne, Bigel, Jojoba oil, Tea tree oil.

IPC code; Int. cl. (2015.01)- A61K 9/00, A61K 36/00, A61P 17/10

Introduction

Acne vulgaris of the face is reported to be most common in the Indian population comprising ~1% of all dermatology patients[1]. The pathogenesis of acne is related to many factors, such as excess sebum production, overgrowth of *Propionibacterium acnes* in obstruction of the pilosebaceous unit, and inflammation induced by bacteria[2]. Many approaches have been used as well as many topical formulations developed to treat skin problems such as eczema, psoriasis, acne, and wound healing[3]. Thus, cosmetic manufacturers struggle to find ways to produce an effective product that matches all the needs of consumers[4]. The product should meet the need of all age groups and skin type. as well as environmental factors such as pollution along with the efficacy of the formulations[5]. A substantial number of shortcomings regarding cosmetic products has been reported comprising of instability and high sensitivity towards pH, temperature, light, and oxidation process as well as irritation and allergy upon application[6]. All these considerations have been a driving force for the development of new technology for manufacturers[7]. Recently it has been suggested that isotretinoin (ITR) should be used for the management of moderate-severe acne[8]. ITR is a drug of choice in the treatment of all types of acne, including recalcitrant, severe and nodulocystic[9]. The most widely employed route of its administration is oral intake, but oral administration is reported to be associated with severe side-effects such as skin dryness and psychological disorders[10]. Consequently topical delivery, of ITR has been opined[11]. However, it is associated with several hiccups like irritation, erythema and peeling of the skin. Hence to overcome these side effects, the current study is embarked upon to develop of "optimized" Bigel of ITR. The developed system was characterized and evaluated for skin compliance, skin transport characteristics, and anti-acne potential[12].

Gels are semi-solid formulations and are broadly classified into hydrogel and organogel based on the nature of the continuous phase. Hydrogels have an aqueous phase system, while, organogel include a polar solvent as continuous phase[12,13]. Bigels are formulated by mixing of both aforementioned gels i.e., hydrogel and organogel in defined proportions. It has been found that the drug release rates from the organogels can be increased many folds by converting the organogels into a bigel[14]. Bigels are soft solids that belong to a class of interfacially jammed emulsion gels[15]. These classes of biphasic gels are quite unexplored and have been gaining attention in the food as well as pharmaceutical industries as they can simultaneously deliver both lipophilic and

*Correspondent author
Email: shubhini.saraf@gmail.com
Supplementary tables and figures are available online only.

hydrophilic drugs[16]. Due to the presence of hydrogels, these gels provide enhanced hydration of the stratum corneum thereby providing a cooling and moisturizing effect[17] and are now being studied extensively. Tea tree oil (TTO) is an essential oil, steam-distilled from the plant *Melaleuca alternifolia*. It has a minimum content of terpinen-4-ol and a maximum content of 1, 8-cineole. Terpinen-4-ol is a major TTO component that exhibits strong antioxidant, anti-inflammatory properties and broad-spectrum antimicrobial activity against bacterial, viral, fungal, and protozoal infections affecting skin and mucosa[18]. Several studies have suggested the uses of TTO for the treatment of acne vulgaris, seborrheic dermatitis, and chronic gingivitis[19]. Jojoba (*Simmondsia chinensis*) is a long-lived, drought-resistant, perennial plant oil that is the main biological source of wax esters and has a multitude of potential applications[20]. Jojoba oil has an anti-inflammatory effect and can be used on a variety of skin conditions including skin infections, skin ageing, as well as wound healing[21]. Moreover, jojoba has been shown to play a role in cosmetic formulations such as sunscreens and moisturizers and also enhances the absorption of topical drugs[22].

Materials and Methods

Materials

Isotretinoin was obtained from Navketan Pharma, Aurangabad, India. Tea tree and jojoba oils were purchased from Sugandhco (P) Limited, Lucknow. Span 60 (S-60) was purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. All chemicals were of analytical grade and were purchased from Himedia, Mumbai unless otherwise specified.

Preparation and optimization of bigel

Preparation of bigels involved a three-step process. The first process involved the development and optimization of the organogel and the second step comprised of the formulation and optimization of the hydrogel. The third step was the preparation of bigel by the dropwise addition of molten organogel in hydrogel.

Preparation and optimization of organogel

The preparation and optimization of organogel were based on critical gelling agent concentration. Organogel formulations were prepared by varying the concentrations (10, 15, 20, and 30 % w/w) of S-60, to decide critical gel concentration (the lowest concentration and viscosity at which organogel was first developed with good thermodynamic stability). Organogels were prepared by dissolving different concentration of S-60 in 80 g of jojoba oil and 5 g of tea tree oil. The mixing was carried out at 70 °C with a stirring speed of 500 RPM and subsequently cooled to 25 ^{o}C. ITR (0.05% w/w) loaded organogel was prepared in the same way and further mixed with hydrogel to form bigel. All the organogels were optimized based on thermodynamic stability and low viscosity (Suppl. Table 1).

Preparation and optimization of hydrogel

Hydrogel (1% w/w) was prepared by soaking the weighed quantity of carbopol (1 g) in the required quantity of distilled water as per the established method[23] the author's lab. The stable hydrogel was formed after occasional stirring.

Preparation of bigel

Bigels were prepared by dropwise addition of molten organogel in carbopol hydrogel (70 °C, 500 RPM) until a homogenous mixture was obtained. Based on the composition, the mixture either formed a bigel or remained as a phase-separated system when cooled to room temperature. Ten formulations were developed by varying the composition of organogel and hydrogel with (B-1 to B-5) and without isotretinoin (DB-1 to DB-5) (Suppl. Table 2). The bigel formation was confirmed by tube inversion test (Suppl. Fig. 1).

Characterization of bigel

Drug-excipient compatibility study

The chemical interactions between the drug and components of the bigels were analyzed using Fourier Transform Infrared Spectroscopy (FTIR) (FTIR-8400S Shimadzu, Japan). The FTIR Spectrum of ITR along with excipients such as carbopol 934 and span 60 were observed and analyzed.

Physical appearance

The physical appearance of the bigel formulations was visually observed for change in colour, homogeneity, consistency, and phase separation. Tube inversion test was also carried out to confirm the development of bigel.

Determination of pH and viscosity

The pH of the bigel formulations was determined by using a digital pH Meter (NIG333, New Delhi). For pH determination, bigel (0.1 g) was suitably diluted with distilled water and then the pH of the solution was measured.

The viscosity of bigels was studied using a digital Viscometer (Labsonic LT-730). Bigel (30 g) was kept in a beaker. Viscosity was determined using spindle no. 4 at a velocity of 60 rpm. The Experiments were done in triplicate at room temperature.

Leaching of oil

The leaching of the internal phase was qualitatively studied using the filter paper method. Square slabs of 1.9 cm diameter of all five formulations (B-1 to B-5) and hydrogel was kept on a filter paper and incubated at 37 °C for 24 hours. The quantification of the leaching of the internal phase was done as per the reported literature[24].

Fluorescent microscopy

The microarchitecture and the nature of the bigel were determined using a Fluorescent Microscope (Nikon Eclipse). The bigels for the fluorescence microscopy were prepared by dissolving fluoresce in isothiocyanate dye (FITC 0.1% w/w) in organogel. The organogels in the molten state were mixed with hydrogel to form dye loaded bigels. The prepared samples were analyzed by fluorescence microscopy.

Thermal properties

The melting points (Tm) and the thermal profiles of the developed bigels were analyzed using a differential scanning Calorimeter (Pyris Diamond TG/DTA, PerkinElmer (Singapore). A platinum crucible was used with alpha alumina powder as a reference. The analysis was performed under a nitrogen environment maintained at a flow-rate of 150 mL/min.

Drug content

The drug content of bigel was determined by dissolving the 0.1 g of bigel in the 5 mL of methanol. The solution was mixed followed by vortexing for 5 minutes and filtered. The absorbance was measured using a UV-Visible Spectrophotometer (Labtronics Model LT-2910) at 332 nm to calculate the content ITR in bigel.

Texture analysis

Bigels were analyzed for hardness, adhesiveness, spreadability, and extrudability using CT3 Texture Analyzer (Brookfield Engineering Laboratories, USA). For the study of hardness and adhesiveness, TA 10 probe and TA-BT-KI fixture were used by filling the bigel (30 g) in a beaker. The TA 10 probe was allowed to compress into the sample (speed of 1 mm/s to a depth of 30 mm) with a trigger load of 4 g to complete 1 cycle of the experiment. The hardness was noted in term of force required to deform the bigel. The spreadability of bigels was measured by using a male and female cone-type probe and extrudability was determined by the TA-DEC probe. The experiment was performed for a comparative study of bigel formulations vs marketed gel. Data were generated using Texture Pro CTV1.3 software.

In-vitro drug release studies

A modified Franz's diffusion cell was used to study the *in-vitro* release profiles of ITR from the bigels (3.14 cm^2 area and 20 ml volume). The release studies were carried out in phosphate buffer (pH 7.2). Weighed quantity of (0.5 g) of bigel was placed in a formerly activated dialysis membrane mounted between the receptor and donor compartment. The donor compartment was filled with 20 mL of the buffer as dissolution media, kept on stirring at 100 rpm and the temperature was maintained 32±1 °C. Samples were collected at regular time intervals over a period of 24 hours and analyzed at 332 nm using a UV-Visible spectrophotometer. Experiments were done in triplicate.

Release kinetics study

The drug release data of the formulations (bigel) was plotted using various kinetic models i.e. zero order, first order. Higuchi's kinetics and Korsmeyer's equation to evaluate the drug release mechanism and kinetics as mentioned in literature.

Ex-vivo permeation and ex-vivo retention studies

The experiment was carried out in Franz diffusion assembly using goatskin (obtained from the local slaughterhouse, Lucknow), with the optimized formulation (B-2) in triplicates. Hair was removed carefully with an electric applicator and the skin was rinsed with physiological phosphate buffer (pH 7.2). The adhering fatty tissues below the skin were removed using isopropyl alcohol. The skin section was clamped between the donor and the receptor chamber of the vertical diffusion cell with an effective diffusion area of 3.14 cm^2 and a cell volume of 20 mL. The receptor chamber was filled with a freshly prepared mixture of physiological PBS (pH 7.2). The diffusion cells were maintained at 37±0.5 °C using a recirculating water bath and the fluid in the receptor chamber was stirred continuously at 300 rpm on a magnetic stirrer. Accurately weighed

1.0 g of the gel was placed in the donor chamber. Samples (2 mL) were withdrawn at a regular time interval, and the same volume of PBS (pH 7.2) was added to maintain the sink condition and then analyzed at 332 nm using a UV-visible spectrophotometer. Experiments were performed in triplicate.

Ex-vivo antimicrobial Studies

The antimicrobial activity of optimized bigel (B-2) and conventional formulation (10 mg/mL) was carried out against *Staphylococcus aureus*, one of the bacteria responsible for causing acne, using the disc diffusion method. The Kirby-Bauer test was used to determine the zone of inhibition[25]. The concentrations of each formulation were incorporated into the filter paper discs and the disc of each formulation was placed into the agar medium for 24 hours at 37 °C. It gave a clear zone of inhibition around the disc, indicating the antimicrobial activity of the formulations. The zones of inhibition so obtained were measured, and the results were compared. This study was performed in triplicate for each formulation.

Stability studies

The accelerated stability of the bigels was carried out by the freeze-thaw method. In this method, bigels were alternatively kept at -20 ^{o}C (freezing) and 70 ^{o}C (thawing) for 15 min. The study was performed for 5 cycles, and the bigels were checked visually for any signs of destabilization (phase separation) after each cycle. The bigels were allowed to reach room temperature upon completion of five cycles and finally evaluated for their organoleptic characteristics along with drug content and pH. The long-term stability of bigels was estimated by incubating the weighed samples at 30±2.0 ^{o}C and 65% RH ± 5% RH for 3 months. The bigels were observed for any changes in the visual appearances (e.g. colour change, phase separation or syneresis) and drug content at regular intervals of time (0, 10, 30, 60, and 120 days respectively).

Result and Discussion

Based on the literature reported, different organogel formulations were prepared by varying the concentrations of SP-60 to decide critical gel concentration i.e., the lowest concentration at which organogel was first developed and which has the lowest viscosity and thermodynamic stability (Suppl. Table 1). The organogel containing 15% w/w SP-60 was found to be optimized as it displayed no signs of phase separation, low viscosity, and thermodynamic stability.

Characterization of bigel

Drug-excipient compatibility study

In Suppl. Fig. 2a, ITR was characterized by bands around 1700–1500 and 1300–1100 cm^{-1} that correspond to C=O and C–O stretching vibrations. The IR spectrum of ITR along with the excipients present in the bigel closely resembles the IR spectrum of ITR, carbopol 934, SP-60. The characteristic bands of ITR present with reduced intensity as shown in the FTIR spectrum of bigel probably due to the restriction inside the formulation matrix. The absorption band at 3400 cm^{-1} due to hydroxyl stretching was not affected in the formulation, which emphasized the absence of any possible interaction between the drug and the formulation components used.

Physical appearance

The prepared bigel formulations (Suppl. Table 2) were visually tested for colour, physical appearance, homogeneity, and texture. The physicochemical properties of the gel formulations are given in Suppl. Table 3. The results displayed B-1 and B-2 bigel formulation possessed good homogeneity, smoothness and texture with no phase separation as compared to other prepared bigels. Therefore, the two physiochemical stable bigels B-1 and B-2 were taken for further studies and formulations B-3, B-4, and B-5 were dropped out. Further fluorescence microscopy of these samples was performed. The droplet size and population of bigels are shown in Suppl. Fig. 3. The fluorescent micrographs showed non-uniformly distributed spherical droplets. The droplets were smaller and lesser in number in the bigels containing lower proportions of the organogel. The dispersed droplets showed green fluorescence, suggesting the presence of organogel as the dispersed droplets indicating the formation of oil/water emulsion gel.

pH and viscosity measurement

The pH of all the gel formulations was in the range of 6.5 to 7.0, which lies in the normal pH range of the skin and would not produce any skin irritation[26]. There was no significant change in pH values as a function of time for all formulations.

The viscosity of the bigels increases with an increase in the concentration of organogel as shown in Supplementary Table 3. The increase in viscosity could be attributed to a higher concentration span-60 giving physical strength and stability of organogel[27].

Leaching of oil from bigel

The leaching of the internal phase was qualitatively studied using the filter paper method. Hydrogel B1 and B2 displayed no signs of leaching, however, hydrogels B3, B4, and B5 displayed leaching in a range of 1.4±0.5, 2.2±0.3, and 2.7±0.4 cm respectively (Suppl. Table 3). This could be attributed to the formation of a rigid gel network of the system towards which oil displayed higher affinity and remains entrapped into it preventing leaching from the gel[28].

Thermal analysis

An increase in the melting points of the bigels was observed with an increase in the proportion of the organogel of B-1 and B-2. An increase in the solid component is associated with the increase in the orderliness at the molecular level[29]. Hence, a higher amount of energy is required. From the results, it may be assumed that the bigels prepared with higher proportions of organogel lead to the formation of formulations with higher thermodynamic stability (Suppl. Fig. 4).

Drug content and texture analysis

The drug content of all the formulation was found to be in a range of 92.1±2.33 to 98.85±0.13 (Suppl. Table 3). Formulation B-1 and B-2 displayed extrudability of 318.8±2.44 and 315.0±3.11 mJ respectively. The adhesive force was found to be 215.66±2.13 and 242.33±1.99 g and spreadability of 5.0±0.3 and 7.6±0.5 respectively. The texture analysis profile is shown in Suppl. Table 4. The adhesive force of B-2 was found high when compared to B-1, while B-2 showed good sticky behaviour with high spreadability, less hardness, and a little difference in extrudability (easy to extrude the formulation from the packaging of the formulation) led to an optimized formulation B-2 (Suppl. Table 4).

***In-vitro* drug release profile and release kinetics**

A significantly (P >0.05) higher release of 80±3.22% was displayed from B-2 when compared with 60±2.31% of conventional gel. The release was diffusion mediated release which could prove to be a better topical delivery (Suppl. Fig. 5). The prepared bigel follow Korsmeyer-peppas model indicating the release to be diffusion mediated[30] (Suppl. Table 4).

***Ex-vivo* permeation and retention studies**

Ex-vivo per cent permeation was found to be 54.05±2.31% of the optimized formulation B-2 and 41.48±3.22% of conventional gel which indicated that a less percentage of the drug was permeated in comparison to conventional gel over a period of 24 hours and was diffusion mediated with a permeation flux of 0.903 J. However, an *ex-vivo* retention study of the optimized bigel formulation after 24 hours displayed retention of 23±2.0% drug on the dermal layer. This gives auxiliary evidence that the formulation is suitable for topical delivery as the retention aids in enhancing local effect[31](Suppl. Fig. 6).

***Ex-vivo* antimicrobial studies**

The formulation displayed a significantly higher (P >0.05) zone of inhibition when compared to conventional gel. The zone of inhibition was found to be 32±0.127 and 17±0.513 mm for bigel and conventional gel respectively after an incubation period of 48 hours.

Stability studies

The formulations were subjected to short term stability through a freeze-thaw cycle (5 cycles) and no significant difference in drug content, physical appearance, and pH of the bigel was observed (Suppl. Table 5). This indicates that the formulation possesses good stability at different temperatures. Further, the optimized batch B-2 was subjected to long term stability and stored in vials at 30°C±2ºC over a period of 120 days. The formulation revealed no significant change in the aforementioned physicochemical characteristics. However, Drug content and the pH value insignificantly decreased with time at temperature 30°C±2ºC. The result of the stability study suggests the ITR bigel B-2 can be used for a long time and can be kept at room temperature. The method of preparation was facile, reproducible, and suitable for scale-up.

Conclusion

The current study is based on an easy and economical method for novel bigels preparation. The bigels with higher proportions of organogel were smooth, stable and biocompatible in nature and showed higher viscosity. The bigels with lower proportions of organogel showed higher drug release following diffusion mediated Korsemeyer Peppas model which is ideal for a diffusion mediated release system. Hydrogel present in it reduces the dryness (as a side effect) of the drug. The developed bigels may be tried as matrices for topical delivery of an antiacne drug. It is expected that the two gel systems

in bigels may synergize enhancement of hydration of stratum corneum, moisturizing effect, spreadability, and drug penetration as desired for topical application.

Conflict of interest

The authors declare no conflict of interest

References

1 Bhavsar B, Choksi B, Sanmukhani J, Dogra A and Haq R, Clindamycin 1% nano-emulsion gel formulation for the treatment of acne vulgaris: results of a randomized, active controlled, multicentre, phase IV clinical trial, *J Clin Diagn Res*, 2014, **8**, YC05.

2 Nishijima S, Kurokawa I, Katoh N and Watanabe K., The bacteriology of acne vulgaris and antimicrobial susceptibility of Propionibacterium acnes and Staphylococcus epidermidis isolated from acne lesions, *J Dermatol*, 2000, **27**, 318-23.

3 Prasad S, Mukhopadhyay A and Kubavat A, Efficacy and safety of a nano-emulsion gel formulation of adapalene 0.1% and clindamycin 1% combination in acne vulgaris: A randomized, open label, active-controlled, multicentric, phase IV clinical trial, *Indian J Dermatol Venereol Leprol*, 2012, **78**, 459.

4 Wang P, Tang H and Zhang P, Highly efficient and biocompatible nanoparticle-based photosensitizer for treatment of acne vulgaris, *Nanomedicine*, 2018, **13**, 2629-36.

5 Khalid N, Che Wel C, Alam SS and Mokhtaruddin S, Cosmetic for modern consumer: The impact of selfcongruity on purchase intention, *Int J Asian Soc Sci*, 2018, **8**, 34-41.

6 Kale S N and Deore S L, Emulsion micro emulsion and nano emulsion: A review, *Sys Rev Pharm,* 2017, **8**, 39.

7 Nast A, Dreno B and Bettoli V, European evidence-based (S3) guidelines for the treatment of acne, *J Eur Acad Dermatol Venereol*, 2012, **26**, 1-29.

8 Ramli S, Chyi K, Zainuddin N, Mokhtar W and Rahman A, The Influence of Surfactant/Co-Surfactant Hydrophilic-Lipophilic Balance on the Formation of Limonene-Based Microemulsion as Vitamin C Carrier. *Sains Malays,* 2019, **48**, 1035-42.

9 Shah SK and Alexis AF, Acne in skin of color: practical approaches to treatment *J Dermatol Treat*, 2010, **21**, 206-11.

10 Raza K, Singh B, Singal P, Wadhwa S and Katare O P, Systematically optimized biocompatible isotretinoin-loaded solid lipid nanoparticles (SLNs) for topical treatment of acne, *Colloids Surf B***,** 2013, **105**, 67-74.

11 Liu J, Hu W, Chen H, Ni Q, Xu H and Yang X, Isotretinoin-loaded solid lipid nanoparticles with skin targeting for topical delivery, *Int J Pharm*, 2007, **328**, 191-5.

12 Layegh P, Mosallaei N, Bagheri D, Jaafari M R and Golmohammadzadeh S, The efficacy of isotretinoin-loaded solid lipid nanoparticles in comparison to Isotrex® on acne treatment, *Nanomed J*, 2013, **1**, 38-47.

13 Di Michele L, Fiocco D, Varrato F, Sastry S, Eiser E and Foffi G, Aggregation dynamics, structure, and mechanical properties of bigels, *Soft matter*, 2014, **10**, 3633-48.

14 Sagiri S, Behera B and Rafanan R, Organogels as matrices for controlled drug delivery: A review on the current state, *Soft Materials*, 2014, **12**, 47-72.

15 Behera B, Singh V and Kulanthaivel S, Physical and mechanical properties of sunflower oil and synthetic polymers based bigels for the delivery of nitroimidazole antibiotic–A therapeutic approach for controlled drug delivery, *Eur Polym J*, 2015, **64**, 253-64.

16 Wakhet S, Singh V K and Sahoo S, Characterization of gelatin–agar based phase separated hydrogel, emulgel and bigel: A comparative study, *J Mater Sci Mater*, 2015, **26**, 118.

17 Rehman K, Amin M C I M and Zulfakar M H, Development and physical characterization of polymer-fish oil bigel (hydrogel/oleogel) system as a transdermal drug delivery vehicle, *J Oleo Sci*, 2014, ess14101.

18 Halcón L and Milkus K, Staphylococcus aureus and wounds: A review of tea tree oil as a promising antimicrobial, *Am J Infect Control*, 2004, **32**, 402-8.

19 Pazyar N, Yaghoobi R, Bagherani N and Kazerouni A, A review of applications of tea tree oil in dermatology, *Int J Dermatol*, 2013, **52**, 784-90.

20 Lin T-K, Zhong L and Santiago J L, Anti-inflammatory and skin barrier repair effects of topical application of some plant oils, *Int J Mol* Sci, 2018, **19**, 70.

21 El Mogy N S, Medical effect of Jojoba Oil in the treatment of skin diseases, Google Patents, 2004.

22 Rawlings A and Lombard K, A review on the extensive skin benefits of mineral oil, *Int J Cosmet Sci,* 2012, **34**, 511-8.

23 Parashar P, Pal S, Dwivedi M and Saraf S A, Augmented Therapeutic Efficacy of Naringenin Through Microemulsion-Loaded Sericin Gel Against UVB-Induced Photoaging, *AAPS Pharm Sci Tech*, 2020, **21**(6), 1-13

24 Bordenave N S, Janaswamy and Yao Y, Influence of glucan structure on the swelling and leaching properties of starch microparticles, *Carbohydrate polymers*, 2013, **103**, 234-243.

25 Ruparelia J P, Chatterjee A K, Duttagupta S P and Mukherji S, Strain specificity in antimicrobial activity of silver and copper nanoparticles, *Acta Biomater*, 2008, **4**(3), 707-716.

26 Pal R R, Parashar P, Singh I and Saraf S A, Tamanu oil potentiated novel sericin emulgel of levocetirizine: repurposing for topical delivery against DNCB-induced atopic dermatitis, QbD based development and in vivo evaluation, *J Microencapsul*, 2019, **36**, 432-46.

27 Cok M, Viola M and Vecchies F, N-isopropyl chitosan, A pH-and thermo-responsive polysaccharide for gel formation, *Carbohydr Polym,* 2020, **230**, 115641.

28 Singh B and Kumar R, Designing biocompatible sterile organogel–bigel formulations for drug delivery applications using green protocol, *New J Chem*, 2019, **43**, 3059-70.

29 Javed H, Shah SNH and Iqbal FM, Formulation development and evaluation of diphenhydramine nasal nano-emulgel, *AAPS Pharm Sci Tech*, 2018, **19**, 1730-43.

30 Singh N, Parashar P, Tripathi CB, Kanoujia J, Kaithwas G and Saraf SA, Oral delivery of allopurinol niosomes in treatment of gout in animal model, *J Liposome Res*, 2017, **27**, 130-8.

31 Gómez-Segura L, Parra A, Calpena-Campmany AC, Gimeno Á, Gómez de Aranda I and Boix-Montañes A, Ex vivo permeation of carprofen vehiculated by PLGA nanoparticles through porcine mucous membranes and ophthalmic tissues, *Nanomaterials*, 2020, **10**, 355.