International Journal of Life Science Research Archive

ISSN: 0799-6640 (Online)

Journal homepage: https://sciresjournals.com/ijlsra/

(RESEARCH ARTICLE)

Check for updates

Formulation and evaluation of proniosome loaded sertaconazole nitrate for topical application

Shailaja D [1], Latha K [1,*], Manasa D [1], Shirisha A [1], Padmavathi R [1], Naseeb Basha Shaik [1] and Shailaja T [2]

[1] *G Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad (Telangana), India.*
[2] *Govt. Polytechnic for Women, Nizamabad.*

International Journal of Life Science Research Archive, 2021, 01(02), 023–037

Publication history: Received on 08 August 2021; revised on 09 September 2021; accepted on 11 September 2021

Article DOI: https://doi.org/10.53771/ijlsra.2021.1.2.0060

Abstract

Proniosomal technology is a novel solution for poorly soluble drugs. Proniosomes are water-soluble carrier particles which are coated with non-ionic surfactants. Proniosomal gels were prepared by coacervation phase separation method using non-ionic surfactants, lipid carriers and cholesterol as a membrane stabilizer. FTIR compatibility studies revealed that the drug and excipients were compatible. All formulations were evaluated for pH, drug content, extrudability, spreadability, viscosity, *in-vitro*, *ex-vivo*, skin irritation and stability studies. Among formulations prepared, F80H1 has shown higher % EE (83.02) and least diffusion through dialysis membrane i.e., 17.68%. With *ex-vivo* studies, F80H1 formulation has shown highest skin deposition and lower flux of sertaconazole nitrate through the rat skin. F80H1 was selected as final optimized formulation. F80H1 exhibited good stability and SEM studies revealed that the vesicles were spherical in shape. The optimized formulation was found to follow zero order release kinetics and korsmeyer-peppas release mechanism. F80H1 found to be non-irritant and stable from skin irritation and stability studies.

Keywords: Anti-fungal drugs; Proniosomal gel; Sertaconazole nitrate; Topical delivery

1. Introduction

Topical drug delivery systems mainly provide localized effects at their site of application by use of penetration of drug into the underlying layers of skin or into the layers of mucous membranes. The topical drug delivery system is usually used when other drug administration systems are fails or it's mainly utilized in pain management, contraception and enuresis. These drug delivery systems include a wide variety of pharmaceutical dosage form like liquids, semisolid preparation, solid powders and sprays. Gels, creams and ointments are the most widely used semisolid preparations for the topical drug delivery [1].

Topical drug delivery involves the application of formulation containing the drug to the skin to treat the cutaneous disorders like acne, psoriasis. Gel is known as a two-component, cross linked three-dimensional network which consists of structural materials interposed by an adequate but proportionally large amount of liquid to form an infinite rigid network like structure that immobilizes the liquid continuous phase within the structure. Polymers such as inorganic particles or organic macromolecules are the most structural materials that forms the network of the gel. Cross links can be formed via physical or chemical interactions [2].

Proniosomal technology is said to be a novel solution for poorly soluble drugs. Proniosomes are either liquid crystals or anhydrous free flowing formulations. Proniosomes are water-soluble carrier particles which are coated with non-ionic surfactants [3]. The hydration of proniosomes on brief agitation in hot aqueous media immediately before use leads to

* Corresponding author: Latha K
G Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad (Telangana), India.

the formation of niosomal dispersion [4]. Proniosomal gel is an intermediate state of formation of niosome. Less quantity of continuous phase (aqueous phase) tends to the formation of liquid crystalline compact mass of proniosomes. Further, these proniosomes can be hydrated in two ways: i) By undergoing hydration in skin itself by using water present in the skin after application topically on skin; ii) By hydrating using aqueous systems i.e. purified water, 7.4 Phosphate buffer saline (PBS) and saline solution with or without agitation and sonication.

Sertaconazole nitrate is a highly lipophilic antifungal agent that belongs to the imidazole class which exhibits broad spectrum antifungal activity, having white or almost white powder, practically insoluble in water, soluble in ethanol, sparingly soluble in alcohol and in methylene chloride. It does not appear to be appreciably absorbed systemically, hence given by topical application. Sertaconazole nitrate is a poorly soluble drug that belongs to BCS class II. Proniosomes loaded SN topical gel was prepared using different non-ionic surfactants such as Span 80, Span 60 and Span 20 and lipids like Phospholipon 90H, Phospholipon 80H and Soya lecithin and using cholesterol as a membrane stabilizer. Proniosomal delivery of sertaconazole nitrate can provide controlled release over a prolonged period of time by which dose frequency and adverse effects can be reduced.

2. Material and methods

2.1. Materials

Sertaconazole Nitrate was obtained from Varahi Pvt. Ltd., Soya Lecithin and Cholesterol were purchased from Yarrow chem products Pvt. Ltd., Phospholipon 90H, 80H was obtained from Lipoid, Span 80 was obtained from Sisco research lab Pvt. Ltd., Span 20 was obtained from NR Chemicals, Span 60 and Ethanol were obtained from SD fine Chem Pvt. Ltd., Methanol was obtained from Merck life science Pvt. Ltd.,

2.2. Preparation of proniosomes by coacervation phase separation method

In this present study proniosomes are prepared by accurately weighing quantity of carrier, surfactant, cholesterol along with the drug are taken into a clean and dry wide mouthed glass beaker and small quantity of solvent is added to it. These mixtures have to heated on water bath at 60 to 70^0C until surfactant mixture dissolved completely. To prevent the loss of solvent, the open end of the glass beaker can be covered with a lid. Very little quantity of aqueous phase (0.5 ml) has to be added and again heated for 5 min to form liquid crystalline compact mass. The mixture brought to be allowed to cool down at room temperature till the dispersion gets converted to a proniosomal gel [5].

2.3. Experimental design

Preliminary trials were conducted to optimize the final formulations based on the observation of vesicles under optical microscope by choosing the right combination of carriers.

Table 1 Gel formulation trials with Span 60 and phospholipon 80H

F	80H (mg)	Span 60 (mg)	CHO (mg)	Ethanol (ml)	7.4 PBS	Gel formation	Vesicle formation
F8S1	400	1600	200	0.5ml	0.5ml	+	-
F8S2	600	1400	200	0.5ml	0.5ml	+	-
F8S3	800	1200	200	0.5ml	0.5ml	+	-
F8S4	1000	1000	200	0.5ml	0.5ml	+	-
F8S5	1200	800	200	0.5ml	0.5ml	+	-
F8S6	1400	600	200	0.5ml	0.5ml	+	-
F8S7	1600	400	200	0.5ml	0.5ml	+	-
F8S8	1800	200	200	0.5ml	0.5ml	+	-

Note: S-60=Span 60; CHO-Cholesterol; 80H=Phospholipon 80H; PBS= Phosphate buffer saline; + Observed; - Not observed;

Different non-ionic surfactants like Span 80, Span 60 and Span 20 and carriers like soya lecithin, Phospholipon 90H and Phospholipon 80H were used for the study. The total amount of the surfactant and carrier in all the formulations (Table: 1-7) were fixed to 2 grams and their ratios were changed in each formulation in order to know the effect of each on the vesicle formation, entrapment efficiency and drug release.

Table 2 Gel formulation trials with Span 60 and phospholipon 90H

F	90H (mg)	Span 60 (mg)	CHO (mg)	Ethanol (ml)	7.4 PBS (ml)	Gel Formation	Vesicle formation
F9S1	400	1600	200	0.5ml	0.5ml	+	-
F9S2	600	1400	200	0.5ml	0.5ml	+	-
F9S3	800	1200	200	0.5ml	0.5ml	+	-
F9S4	1000	1000	200	0.5ml	0.5ml	+	-
F9S5	1200	800	200	0.5ml	0.5ml	+	-
F9S6	1400	600	200	0.5ml	0.5ml	+	-
F9S7	1600	400	200	0.5ml	0.5ml	+	-
F9S8	1800	200	200	0.5ml	0.5ml	+	-

Note: S-60=Span 60; CHO-Cholesterol; 90H=Phospholipon 90H; PBS= Phosphate buffer saline; + Observed; - Not observed;

Table 3 Gel formulation trials with Span 80 and phospholipon 90H

F	90H (mg)	Span 80 (mg)	CHO (mg)	Ethanol (ml)	7.4 PBS (ml)	Gel formation	Vesicle formation
F90H1	400	1600	200	0.5ml	0.5ml	+	+
F90H2	600	1400	200	0.5ml	0.5ml	+	+
F90H3	800	1200	200	0.5ml	0.5ml	+	+
F90H4	1000	1000	200	0.5ml	0.5ml	+	-
F90H5	1200	800	200	0.5ml	0.5ml	+	-
F90H6	1400	600	200	0.5ml	0.5ml	+	-
F90H7	1600	400	200	0.5ml	0.5ml	+	-
F90H8	1800	200	200	0.5ml	0.5ml	+	-

Note: S-80=Span 80; CHO-Cholesterol; 90H=Phospholipon 90H; PBS= Phosphate buffer saline; + Observed; - Not observed;

Table 4 Gel formulation trials with Span 80 and phospholipon 80H

F	80H (mg)	Span 80 (mg)	CHO (mg)	Ethanol (ml)	7.4 PBS (ml)	Gel formation	Vesicle formation
F80H1	400	1600	200	0.5ml	0.5ml	+	+
F80H2	600	1400	200	0.5ml	0.5ml	+	+
F80H3	800	1200	200	0.5ml	0.5ml	+	+
F80H4	1000	1000	200	0.5ml	0.5ml	+	-
F80H5	1200	800	200	0.5ml	0.5ml	+	-
F80H6	1400	600	200	0.5ml	0.5ml	+	-
F80H7	1600	400	200	0.5ml	0.5ml	+	-
F80H8	1800	200	200	0.5ml	0.5ml	+	-

Note: S-80=Span 80; CHO-Cholesterol; 80H=Phospholipon 80H; PBS= Phosphate buffer saline; + Observed; - Not observed

Table 5 Gel formulation trials with combination of phospholipon 80 H and phospholipon 90H with Span 80

F	80H (mg)	90H (mg)	S-80 (mg)	CHO (mg)	Ethanol (ml)	7.4 PBS (ml)	Gel formation	Vesicle formation
FC1	600	400	1800	200	0.5ml	0.5ml	+	+
FC2	800	200	1800	200	0.5ml	0.5ml	+	+
FC3	200	800	1800	200	0.5ml	0.5ml	+	+
FC4	500	500	1800	200	0.5ml	0.5ml	+	+
FC5	400	600	1800	200	0.5ml	0.5ml	+	-

Note: S-80=Span 80; CHO-Cholesterol; 80H=Phospholipon 80H; PBS= Phosphate buffer saline; + Observed; - Not observed;

Table 6 Gel formulation trials with combination of phospholipon 80H, phospholipon 90H and soya lecithin as lipid carriers using Span 80

F	Lecithin (mg)	80H (mg)	90H (mg)	S-80 (mg)	CHO (mg)	E (ml)	7.4 PBS (ml)	Gel formation	Vesicle formation
FHS1	900	900	-	1800	200	0.5ml	0.5	+	-
FHS2	900	-	900	1800	200	0.5ml	0.5	+	-

Note: S-80=Span 80; CHO-Cholesterol; 80H=Phospholipon 80H; 90H=Phospholipon 90H; PBS= Phosphate buffer saline; + Observed; - Not observed;

Table 7 Gel formulation trials using Soya lecithin and different Non-ionic surfactants

F	Lecithin (mg)	S-80 (mg)	S-20 (mg)	S-60 (mg)	CHO (mg)	E (ml)	7.4 PBS (ml)	Gel formation	Vesicle formation
FL1	900	1800			200	0.5ml	0.5	+	+
FL2	900		1800		200	0.5ml	0.5	+	+
FL3	900			1800	200	0.5ml	0.5	+	+

Note: S-80=Span 80; S-60=Span 60; S-20=Span 20; CHO-Cholesterol; E- Ethanol; PBS= Phosphate buffer saline; + Observed; - Not observed;

In the preliminary trial studies, previously Span 60 was used as a surfactant in the place of Span 80 and observed for the vesicle formation and in neither of formulation vesicles were seen. So, Span 60 was not used further. Trials were conducted using Span 80 with the same formula. Vesicles were observed under the optical microscope when Span 80 was used. So, Span 80 was chosen as an optimized surfactant for F80H.

In the case of F90H, previously, Span 60 was used as a surfactant and trials were conducted and no vesicle formation was seen in all the formulations. Then Span 80 was used as a surfactant in place of Span 60 with the same formula. Vesicle formation is seen when Span 80 was used. So, Span 80 was chosen as an optimized surfactant for F90H also.

In both categories, out of nine preliminary trials in each category (F90H and F80H), four formulations were optimized based on vesicle formation and these formulations were chosen for further studies.

2.4. Characterization and evaluation of proniosomal gels

2.4.1. Drug excipients compatibility studies of dosage form by FTIR

It is done to check the compatibility between the drug and excipients because the drug will be subjected many physical and chemical changes [6].

2.4.2. Physicochemical evaluation of formulated proniosomal gels

Physical appearance

The prepared gel was examined for clarity, color, homogeneity and the presence of foreign particles [7].

Determination of pH

The pH of gel is determined in order to check the gel compatibility with the skin. By using digital pH meter. 1 g of gel was dispersed in 100 ml of distilled water and stored for 2 hrs at room temperature. Process repeated for 3 times and average values obtained were calculated [8].

Drug content

Sertaconazole nitrate content in gel was determined by dissolving 100 mg of gel in 10 ml methanol (solvent) by sonication. The solution was filtered through the Whatman filter paper no.42. Absorbance was measured after suitable dilutions at 261nm in UV-spectrophotometer. The experiment was done in triplicate and average values were calculated [8].

Homogeneity

It was determined by visual inspection for the appearance of gel and presence of any aggregates [9].

Extrudability

A 15 g of gel was filled in aluminum tube. Plunger was adjusted to hold the tube. The pressure of 1kg/cm^2 was applied for 30 sec. The quantity of gel extruded was weighed. The % of gel extruded was calculated and grades were allotted (+ + + Excellent, + + Good, + fair) [10].

Spreadability

The spreadability of the formulated gel was determined by measuring the spreading diameter of 1g of gel between 20x20 cm glass plates after1 min. The spreadability was calculated by using the formula [11].

$$S = \frac{ml}{t}$$

Where,

S = Spreadability

m = weight (g) tied to the upper glass slide

l = length of the glass slide

t = time taken in seconds

Determination of viscosity

The sample 1-2 ml was applied in the measurement champers and the results were displayed on the screen of VISCO lab 3000. The determination of viscosity for each formulation was done in triplicate and average values were calculated [12].

Vesicle morphology under optical microscope

Vesicle morphology study involves the measurement of shape and size of proniosomal vesicles. Optical microscopy is used to study morphology of vesicles.

Entrapment efficiency (EE %)

To determine the encapsulation capability of proniosomal derived niosomal vesicles, EE% was done using centrifugation method. Weighed amount of gel was taken and it is suspended in 10 ml of 7.4 PBS and centrifuged at 7000 rpm at 40°C for 40 min. The supernatant containing unentrapped drug was separated and required dilutions were made with 7.4 PBS and analyzed for concentration of unentrapped drug by UV-Vis spectrophotometer at 261 nm [5].

Vesicle morphology, particle size and size distribution

Scanning electron microscopy (SEM) is used for the in-depth observation of shape of the vesicles. Proniosomal vesicle size can be measured by using Zetasizer (Zetasizer Ver. 7.12 Malvern instruments) in two conditions: with agitation and without agitation. Hydration without agitation results in larger sized vesicles. Hydration with agitation by vortex mixer results in smaller vesicles. Polydispersity index can be measured using zeta sizer [13].

Zeta potential

Zeta potential can be analyzed by Zetasizer (Zetasizer Ver. 7.12 Malvern instruments). The optimized formulation was hydrated with distilled water and was converted to Niosomes, so formed niosomes were used to determine the zeta potential [13].

2.5. Drug release studies

2.5.1. In-vitro diffusion studies

Diffusion studies were performed using Franz diffusion cell. The cell was locally fabricated and the volume capacity of receptor compartment was 25ml with an area of 4.9 cm^2. In between donor and receptor compartment, the dialysis membrane used for diffusion studies was placed. The temperature was maintained at 37°C. Gel formulation was uniformly applied on membrane and clamped together. The receptor compartment was filled with 25 ml of pH 7.4PBS and the hydrodynamics within the receptor compartment were maintained by continuous stirring on magnetic stirrer at 300 rpm. At predetermined time intervals, 5ml of sample was withdrawn for every hour and replaced with an equal volume of pH 7.4 PBS buffer for a period of 8 hours. The samples were analyzed using UV-Visible double beam spectrophotometer at 261nm [14].

2.5.2. Ex-vivo skin permeation studies

Male Wister rats (150-180 g) were sacrificed by over ether anesthesia and the hair was removed from abdomen using an animal hair clipper. Abdominal pores and skin section was excised and observed for existence of cuts and wounds. With the use of scalpel, the fat adhering on dermis was removed and finally it was washed with running water. The skin was hold on at −20°C and used for study within a week. The thawed rat skin was mounted onto diffusion cell such that the dermis side was in contact with receptor solution instead of the dialysis membrane. Proniosomal gel was applied to the stratum corneum facing the donor compartment. The amount of the drug permeated across rat skin was analyzed by regular withdrawal of samples and analyzing using UV-Visible double beam spectrophotometer at 261nm. For calculating the flux, graph was plotted considering the cumulative amount of drug permeated time. The flux ($\mu g/cm^2/hr2$) was obtained from the slope of the linear line [15].

2.6. Skin deposition studies

At the end of the permeation study, skin was removed and the surface was washed 2-3 times with7.4 PBS then cut into pieces. Each skin sample was soaked in flask with 20 ml of methanol for 24 hrs. Then the methanolic skin samples were sonicated in an ultrasound bath sonicator four times for 30 min each, in order to extract all the amount of drug accumulated in the skin pieces. The methanolic solution was then filtered and analyzed by UV spectrophotometer at 261 nm against a blank [11].

2.7. Skin irritation studies

The test was carried out on healthy rabbit weighing between 1.5-2 kg. Before setting the formulations, the unbraided pores and skin was wiped cleaned with rectified spirit. The control was kept on the right dorsal surface of rabbit and optimized formulation was placed on the left dorsal surface of the same rabbit. The experiment was conducted for 3 days and the application sites were graded according to a visual scoring scale [16].

2.8. Stability studies

Stability studies were carried out by keeping optimized formulations in glass containers with polypropylene closure for one month at room temperature and refrigerator conditions. Known amount of gel was taken out at different time intervals like 0, 1^{st}, 2^{nd}, 4^{th} week and was analyzed for phase separation, vesicle formation, drug content, entrapment efficiency and percentage drug release [17].

3. Results and discussion

3.1. Drug excipient compatibility studies of optimized formulation

Drug excipient compatibility studies revealed that pure drug characteristic peaks were retained and no additional peaks were added throughout the formulation process where the drug was affected by physical and chemical changes like heating, mixing at different rpms, addition of solvents and storage conditions.

It can be concluded that the drug and excipients were found be stable. The results were shown in Figure 1.

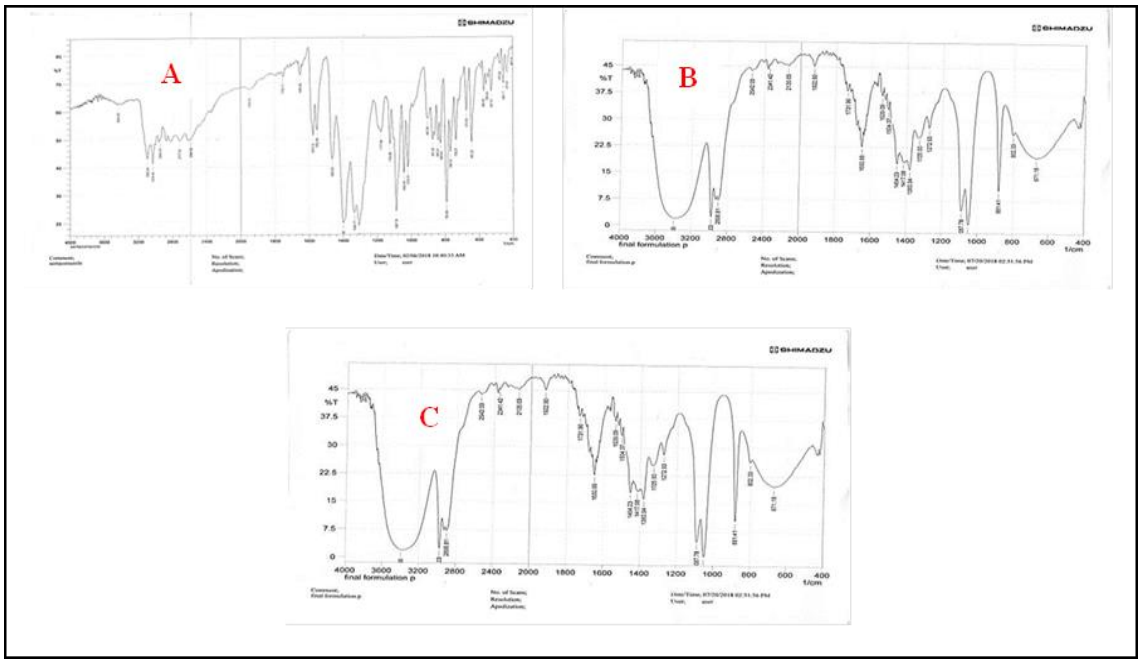

Figure 1 FTIR spectra of A) Sertaconazole nitrate B) Phospholipon 80H C) F80H1 formulation

3.2. Physicochemical evaluation of proniosomal gels

Table 8 Physico-chemical evaluation of prepared proniosomal gel formulations

Formulation	PH	Drug content (%)	Homogeneity	Spreadability (g.cm/sec)	Extrudability
F80H1	7.03±0.56	98.14±0.58	+++	11.07±0.12	+++
F80H2	6.94±0.75	97.41±0.22	+++	12.12±0.24	+++
F80H3	6.91±0.22	96.28±0.19	+++	11.63±0.16	++
F90H1	6.89±0.86	98.24±0.28	+++	12.03±0.32	++
F90H2	6.87±0.42	97.07±0.43	+++	11.2±0.14	+++
F90H3	6.68±0.36	98.01±0.38	+++	11.09±0.24	+++
FC1	6.56±0.96	98.15±1.01	+++	11.6±0.04	+++
FC2	5.93±0.12	96.92 ±0.54	++	10.02±0.62	++
FC3	6.82±0.08	97.49±0.03	+++	12.19±0.42	+++
FC4	5.97±0.06	98.45±0.43	+++	11.7±0.28	+++
FL1	7.06±0.12	96.17±0.26	+++	12.08±0.16	+++
FL2	6.98±0.08	97.22±0.49	++	10.12±0.45	++
FL3	6.73±0.12	95.07±0.11	++	10.19±0.61	++

Note: All values are expressed as mean ±SD, n=3; +++ -Excellent; ++ -good; + -Satisfactory;

pH of all the formulations were found in the range of 5.93 to 7.06 that suits the skin pH, indicating skin compatibility. Skin compatibility is the primary requirement for a good topical formulation [18]. The content of drug per 100 mg of gel ranged from 95.07% to 98.45% which indicates that efficient loading and uniform distribution of drug in the formulations.

Homogeneity was evaluated by visual observation. The physical appearances of all gel formulations were whitish semisolid. Good homogeneity was seen in all formulations without lumps. The value of spreadability varies from 10.02-12.19 g.cm/s indicating that the gels are easily spreadable by small amount of shear. All gel preparations indicated a good spreadability. The results were summarized in Table 8.

The extrudability of all formulations was found to be good and compatible. The extrusion of the gel from the tube is important during its application and in patient acceptance. Viscosity of all the prepared formulation in which vesicle formation was seen have shown optimum gel viscosity (>25000 cps).

3.2.1. Vesicle morphology under optical microscope

Proniosomal vesicle morphology was studied using optical microscopy. The optimized formulations have shown vesicles with spherical shape and uniform size (See Figure 2)**.**

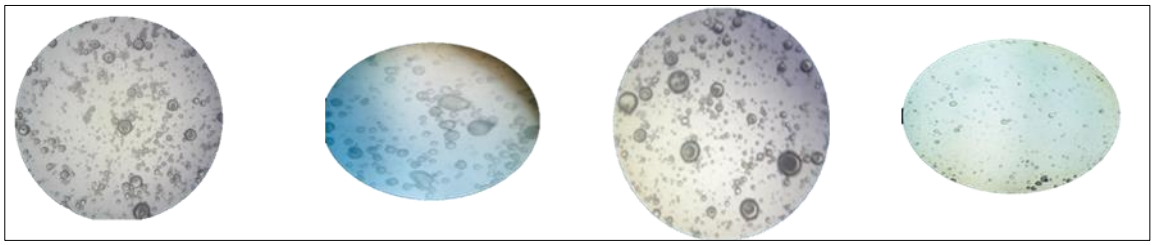

Figure 2 Vesicles observed under optical microscope for the optimized formulations

3.2.2. Entrapment efficiency of proniosomal gel

The entrapment efficiency denotes the ability of niosomal vesicles in entrapping the drug. EE% is determined using centrifugation method.

Table 9 Entrapment efficiency of the prepared proniosomal gel formulations

Formulations	Entrapment efficiency (%)
F80H1	83.02±0.12
F80H2	74.64±0.16
F80H3	72.41±26
F90H1	78.09±0.21
F90H2	73.25±0.17
F90H3	62.10±0.25
FC1	58.64±0.21
FC2	49.26±0.32
FC3	71.72±0.23
FC4	60.43±0.15
FL1	76.12±0.36
FL2	61.40±0.12
FL3	53.91±0.29

Note: All values are expressed as mean ±SD, n=3;

Among all the 13 formulations, F80H1 has shown highest EE [19].

3.2.3. Vesicle morphology under Scanning electron microscope

The hydrated niosomal suspension was used to know the vesicle morphology. SEM revealed that the vesicles were spherical in shape and no aggregation between the vesicles is seen (See Figure 3).

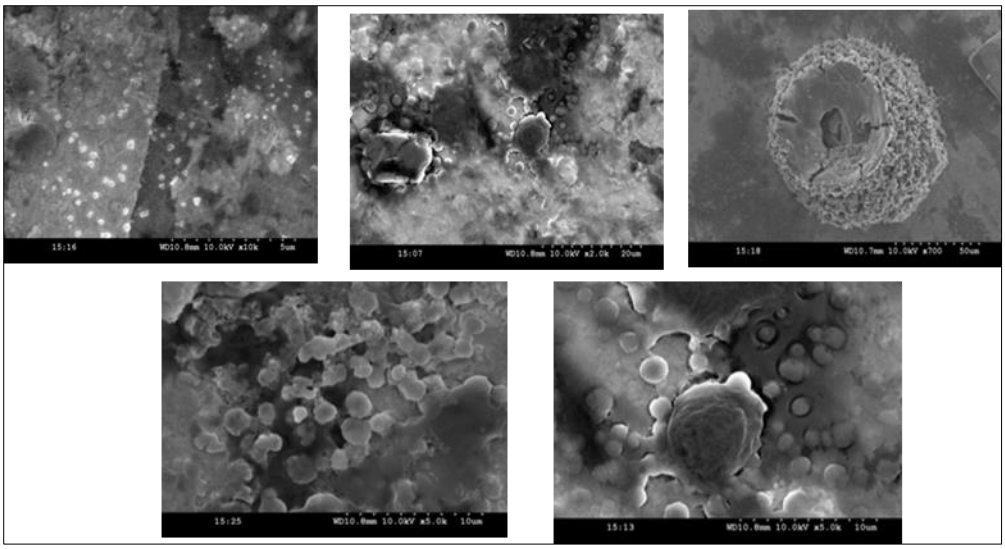

Figure 3 Scanning electron microscopy images of optimized formulation

3.2.4. Particle size and size distribution

The particle size of F80H1 was found to be 532.6±2.8 nm and PDI was 0.375 which indicates uniform distribution and homogeneity of the preparation 25. The vesicles found were in Nano range which helps in passage across the anatomical constraints in the skin. This might be due to the Hydrophobicity. Cholesterol seems to increase the Hydrophobicity due to which the surface free energy available will decreases leading to reduction of vesicle size [20].

3.2.5. Zeta potential

Zeta potential analysis is a measure of net charge of niosomes. The zeta potential of F80H1 was found to be -33.1 mV which indicates that the formulation is physically stable and strongly influenced by the non-ionic surfactant used in the formulation [21].

3.3. Drug release studies

3.3.1. In-vitro diffusion studies

The *in-vitro* diffusion studies are done to know the amount of the drug that has diffused across the dialysis membrane over a period of time. Studies were carried out for the F90H, F80H with span 80 formulations.

The results of %drug release of all the formulations are shown in Figure 4-7. F90H1 and F80H1 were selected as optimized formulations among all, as they shown highest %EE least amount of drug release compared to other F90H and F80H formulations. So, both F80H1 and F90H1 are selected for further studies.

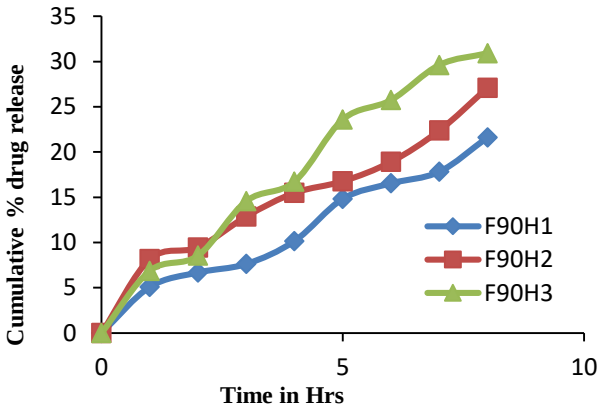

Figure 4 *In-vitro* diffusion profile of F90H formulations

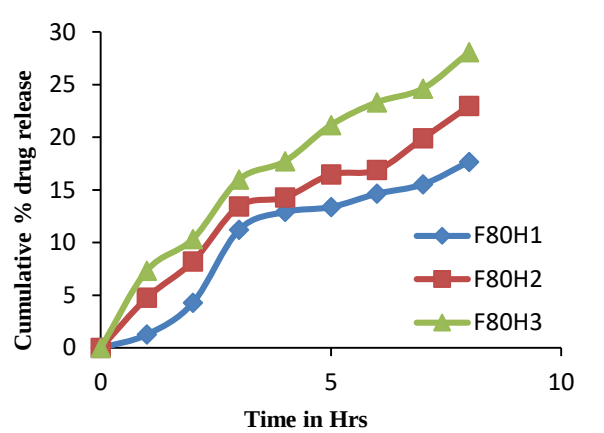

Figure 5 *In-vitro* diffusion profile of F80H formulations

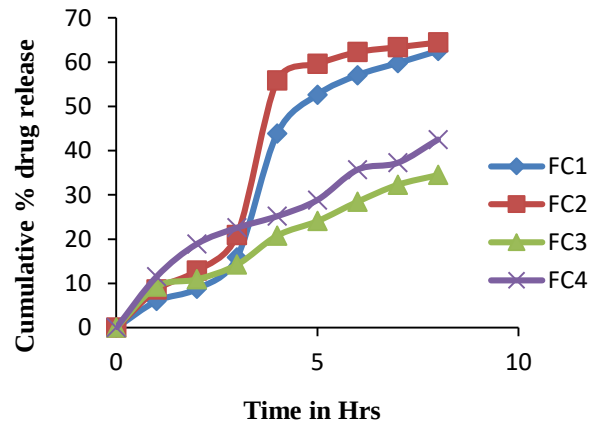

Figure 6 *In-vitro* diffusion profile of FC formulations

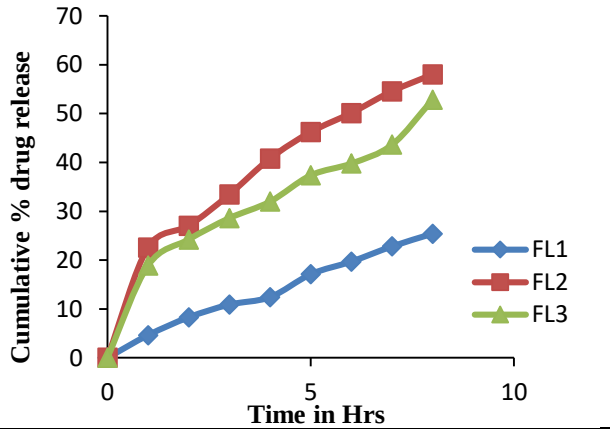

Figure 7 *In-vitro* diffusion profile of FL formulations

3.3.2. Ex-vivo skin permeation

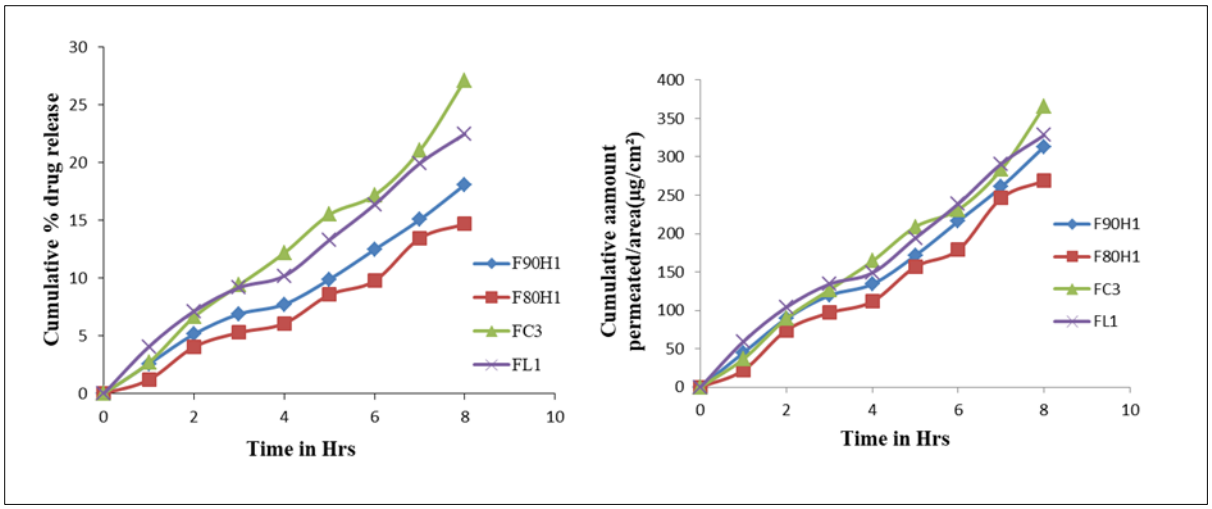

Figure 8 *Ex-vivo* drug permeation profile of optimized formulations

Among all the thirteen formulations, four formulations i.e., F90H1, F80H1, FC3 and FL1 are selected as optimized formulations as they have shown highest % EE, least *in-vitro* drug release in a controlled release pattern over a period

of eight hours compared to other formulations in their respective categories. So, here *Ex-vivo* studies are done for F90H1, F80H1, FC3 and FL1.The drug release profile was given in Figure 8.

From the results obtained, it was observed that the cumulative amount permeated from F90H1, F80H1, FC3, FL1 increased in the order of FC3>FL1>F90H1>F80H1.

3.4. Skin deposition studies

Skin deposition studies were performed for all the four formulations in order to know the actual amount of SN accumulated into the skin [22].

Table 10 Skin deposition of optimized formulations

Formulation Code	Ex-vivo % release	% Skin deposition
F90H1	18.09±0.061	81.02±1.8
F80H1	14.68±0.075	84.31±0.92
FC3	27.13±0.035	72.80±0.76
FL1	22.48±0.047	77.46±1.3

Note: Value were expressed as mean ±SD; n=3;

The percentage skin deposition was higher for F80H1 i.e. 84.31% and lower for FC3 i.e. 72.80%. F90H1 and FL1 also retarded the drug permeation well and provided 81.02% and 77.46% of skin deposition respectively (See Figure 9)

F80H1 was selected as final optimized formulation as its %EE was higher and *in-vitro* drug release, *ex-vivo* skin permeation were less and skin deposition was higher when compared to all other formulations.

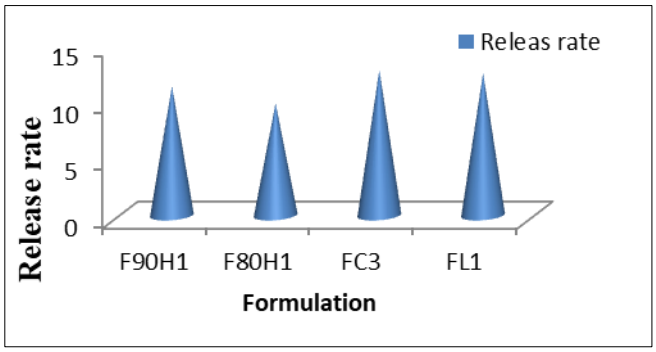

Figure 9 *Ex-vivo* release rate for the optimized formulations

3.5. Skin irritation studies

Skin irritation studies were conducted on the depilated rabbit. Skin reaction at the site of application was assessed and scored according to Draize method. Result is given in Table 11

Table 11 Skin irritation studies

Formulation	Grade formation of erythema and edema		
	24 hours	48 hours	72 hours
F80H1	0	0	0
Control gel	0	0	0

Note: Grade formation of erythema and edema; 0-none; 1-slight; 2-well defined; 3-moderate; 4-severe erythema and edema;

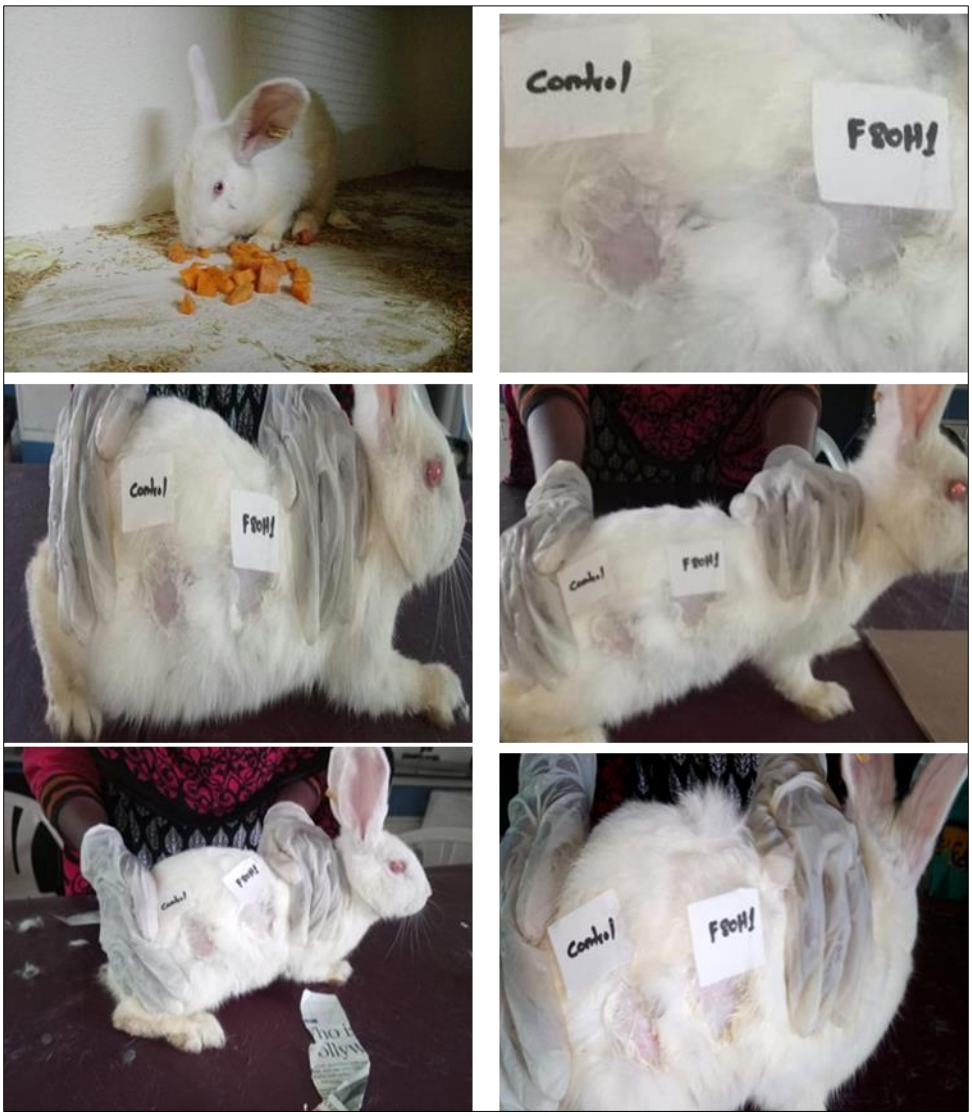

Figure 10 Skin irritation studies for F80H1 formulation on rabbit skin

The formulation F80H1 and control gel were tested for skin irritancy by applying on rabbit skin as shown in Figure 10

3.6. Stability studies

The stability studies were performed for F80H1 formulation at room temperature and refrigerator conditions and the results were showed in Figure 11.

The stability studies for F80H1 were conducted for one-month period of time at room temperature. The formulation was analyzed for EE%, drug content, ability of vesicle formation and % drug release. The formulation was found to be stable, with insignificant change in above mentioned parameters. The graphical representation of % release studies was shown in Figure 12.

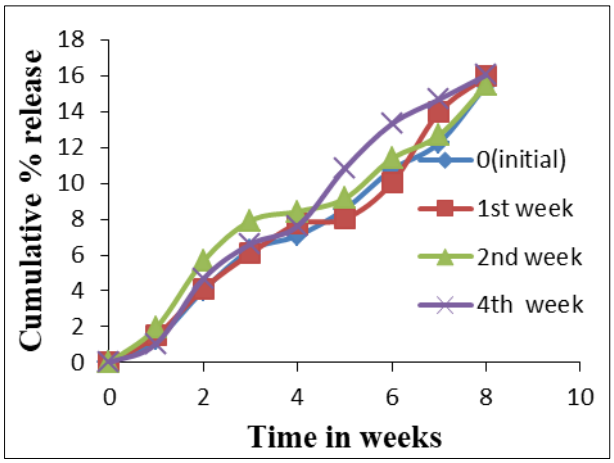

Figure 11 Stability study of F80H1 at room temperature

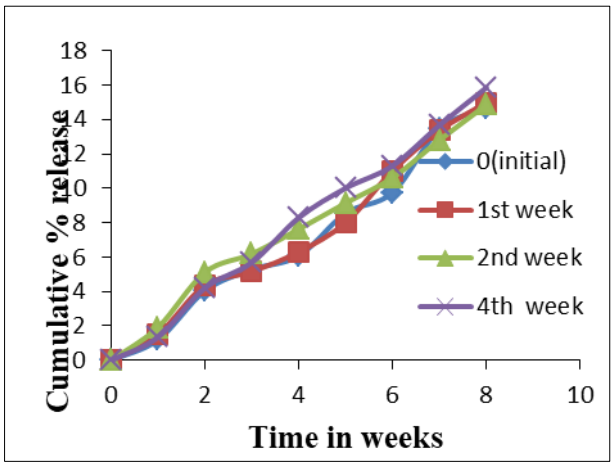

Figure 12 Stability studies of F80H1 at refrigerator conditions

4. Conclusion

The present study mainly focuses to incorporate sertaconazole nitrate into proniosomal vesicles for increasing the permeation of the drug and accumulation in the skin was achieved successfully. Encapsulation can improve the solubility and reduce the skin irritancy problems. In this present work, Proniosomes loaded SN topical gel was successfully prepared by using different non-ionic surfactants such as Span 80, Span 60 and Span 20 and lipids like Phospholipon 90H, Phospholipon80H and Soya lecithin and using cholesterol as a membrane stabilizer. Drug-excipient compatibility studies concluded that the drug and excipients are compatible with each other. EE%, *In-vitro* release studies were conducted and based on the results, four formulations were optimized i.e., F80H1, F90H1, FC3 and FL1 as they have shown higher EE% and least cumulative amount permeation. *Ex-vivo* studies and skin retention studies were performed for 8 hours. F80H1has shown least cumulative amount permeation across rat skin i.e., 268.77µg/cm^2 and high skin deposition of SN i.e., 84.31% and selected as optimized formulation. SEM studies revealed that the vesicles were spherical in shape and particles size was found to be 532.6±2.8 nm and zeta potential was found to be -33.1 mV indicating that the formulation is physically stable. PDI was found be 0.375 indicates the uniform distribution and homogeneity of the preparation. Skin irritation studies were performed and it is indicated that the control, optimized and did not cause any skin irritation. The optimized formulations were found to be stable for one month at room temperature and refrigerator conditions. Hence, finally it was concluded that the prepared floating microspheres of sertaconazole nitrate may prove to be potential candidate for safe and effective controlled drug delivery over a prolonged period of time which can reduce dosing frequency.

Compliance with ethical standards

Acknowledgments

We are grateful to G. Pulla Reddy College of Pharmacy, Hyderabad, India, for their constant encouragement and support in publishing papers.

Disclosure of conflict of interest

The authors have no conflicts of interest, financial or otherwise.

Statement of ethical approval

Institutional Animal Ethical Committee, IAEC meeting NO-1, dated 10/2/2018, ID NO.GPRCP/IAEC/10/18/02/PCE/AE-3-Rats-M-4/Rabbits-M-2.

References

[1] Verma A, Singh S, Kaur R, Jain UK. Topical gels as drug delivery systems: A review. Int. J. Pharm. Sci. Rev. Res. Nov 2013; 23(2): 374-82

[2] Goyal S, Sharma P, Ramchandani U, et al. Novel Anti-Inflammatory Topical Herbal Gels Containing Withania somnifera and Boswellia serrata. Int J Pharm Biol Arch. 2011; 2: 1087–1094.

[3] Khatoon M, Shah KU, Din FU, et al. Proniosomes derived niosomes: recent advancements in drug delivery and targeting. Drug Deliv. 2017; 24: 56–69.

[4] Bachhav AA. Proniosome: A novel non-ionic provesicules as potential drug carrier. Asian Journal of Pharmaceutics (AJP): Free full text articles from Asian J Pharm. Sep 2016; 10: 10(03).

[5] Benipal G. Design, development and evaluation of proniosomal gel of an antifungal drug–ketoconazole. Int J Pharm Sci RevRes. 2015; 31(2): 265-72.

[6] Kumari K, Sara UVS, Sachdeva M. Formulation and Evalution of Topical Hydrogel of Mometasone Furoate using Different Polymers. Int J Pharm Chem Res. 2013; 2: 89–100.

[7] Sagar S, Lavanya S, Sagar V, Nanjwade BK. Formulation and evaluation of proniosomal gel of capecitabine. Indo American Journal of Pharmaceutical Sciences. 1 Aug 2017; 4(8): 2513-20.

[8] Rathod HJ, Mehta DP. A review on pharmaceutical gel. International Journal of Pharmaceutical Sciences. 2015; 1(1): 33-47.

[9] Gupta A, Mishra AK, Singh AK, Gupta V, Bansal P. Formulation and evaluation of topical gel of diclofenac sodium using different polymers. Drug invention today. 1 May 2010; 2(5): 250-3.

[10] Chandira RM, Pasupathi A, Bhowmik D, et al. Design, Development and Formulation of Antiacne Dermatological Gel. J Chem Pharm Res. 2010; 2: 401–414.

[11] Sawant AA, Mohite SK. Formulation and Evaluation of Itraconazole Emulgel for Topical Drug Delivery. Asian J Pharm Technol. 2015; 5: 91.

[12] Kakar R, Rao R, Goswami A, Nanda S, Saroha K. Proniosomes: An emerging vesicular system in drug delivery and cosmetics. Der Pharmacia Lettre. 2010; 2(4): 227-39.

[13] Sowmya J, Gowda DV, Srivastava A. Topical Gels: A Recent Approach for Novel Drug Delivery. Asian journal of biomedical and pharmaceutical. 2014; 4: 62-70.

[14] Rita B, Lakshmi PK. Preparation and evaluation of modified proniosomal gel for localised urticaria and optimisation by statistical method. J Appl Pharm Sci. 2012; 2: 85–91.

[15] Fang JY, Yu SY, Wu PC, et al. *In vitro* skin permeation of estradiol from various proniosome formulations. Int J Pharm. 2001; 215: 91–99.

[16] Mohamed MI, Zaky HT, Kandile NG. Microwave-assisted synthesis of antimicrobial agents based on pyridazine moiety. J Enzyme Inhib Med Chem. 2013; 28: 1307–1315.

[17] Ammar HO, Ghorab M, El-Nahhas SA, et al. Proniosomes as a carrier system for transdermal delivery of tenoxicam. Int J Pharm. 2011; 405: 142–152.

[18] Alam MI, Baboota S, Kohli R, et al. Pharmacodynamic evaluation of proniosomal transdermal therapeutic gel containing celecoxib. ScienceAsia. 2010; 36: 305–311.

[19] Sambhakar S, Paliwal SK, Sharma S, et al. Formulation and development of risperidone loaded niosomes for improved bioavailability: In vitro and in vivo study. Acta Pol Pharm - Drug Res. 2017; 74: 1859–1873.

[20] Ibrahim MMA, Sammour OA, Hammad MA, et al. *In vitro* evaluation of proniosomes as a drug carrier for flurbiprofen. AAPS PharmSciTech. 2008; 9: 782–790.

[21] Singh S, Trivedi S, Jain S. Design and development of proniosome based transdermal delivery of ondansetron hydrochloride. Int J Pharm Biol Res. 2012; 3(5): 191-201.

[22] Abdou EM, Ahmed NM. Terconazole proniosomal gels: effect of different formulation factors, physicochemical and microbiological evaluation. J Pharm Drug Deliv Res. 2016; 5(1): 1-6.