

## FORMULATION DEVELOPMENT AND EVALUATION OF PRNIOSOMAL GEL OF ETHINYLESTRADIOL AND LEVONORGESTREL FOR ANTIFERTILITY TREATMENT

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### ABSTRACT

**Objective:** The purpose of this research was to develop and formulate proniosomal gel drug delivery system of ethinylestradiol and levonorgestrel for antifertility treatment that is capable of efficiently delivering entrapped drug over an extended period of time.

**Methods:** Ethinylestradiol and levonorgestrel are encapsulated in various formulations of proniosomal gel composed of various ratios of span surfactant, cholesterol, soya lecithin, and alcohol as aqueous phase prepared by coacervation-phase separation method. The prepared formulations characterized for drug encapsulation efficiency, size distribution, *in vitro* release studies, and vesicular stability at different storage conditions were carried. Stability studies for proniosomal gel were carried out for a few weeks. Morphological size and shape of the vesicles are characterized using optical microscopy and scanning electron microscopy (SEM). Stability studies for proniosomal gel were carried out for 3 months.

**Results:** Morphological size and shape of the vesicles are characterized using optical microscopy and SEM, particles are found to be spherical, size of the particles is in the range of 46.4–80.6 nm, and permeation studies showed good control release for prolonged period of time. The encapsulation efficiency of proniosomal gel formulations is in the range of 74–80% and *in vitro* permeation studies proved that good amount of drug is permeated and has reasonably good stability characteristics as well.

**Conclusions:** The results suggest that proniosomal gel formulations of ethinylestradiol and levonorgestrel may be used for transdermal delivery for antifertility treatment. The dried proniosomal formulation could act as a promising alternative to niosomes.

**Keywords:** Estradiol, Formulation development, Levonorgestrel, Transdermal matrix patches.

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### INTRODUCTION

Contraception was used by women in the form of pills, intrauterine device, Norplant, foaming tablets, nasal spray, and condoms, and coitus interruptus was used by men. Limitation of contraceptive is mainly their side effect and failure rate related to the consumer compliance. Vesicular structures such as niosomes, ethosomes, transfersomes, and liposomes are favorable systems to go this permeation barrier. But their main disadvantage is their instability, which can be overcome by the usage of provesicular tactics like proniosomes, proliposomes and provesicular, protransfersomes procedures which can further enhance the efficacy of vesicles. These approaches may triumph over pores and skin barrier properties and beautify percutaneous absorption. In these systems, hydrophilic and lipophilic pill drugs may be integrated. The proniosomes are dry formulation which is water-soluble carrier, covered with surfactant, it is dehydrated to make niosomal dispersion and can be rehydrated by agitating in hot aqueous media within few minutes. The ensuing niosomes are uniform in size. Proniosomes are the latest development in novel drug delivery device. These are most advanced drug provider in a vesicular system which overcomes demerits of liposomes and niosomes [1,2].

Ethinylestradiol is regularly utilized in female hypogonadism, and treatment of prostate cancer, as the estrogenic component of combined oral contraceptive and it is frequently used in menopausal symptoms, as the estrogen for menstrual disorders and estrogenic component for combined oral contraceptive and with the daily dose of ethinylestradiol is 20–50 µg [3].

Levonorgestrel is a powerful progestogen and has been broadly prescribed as a contraceptive steroid for female fertility regulation.

It has been found that, if progestin alone is used as a contraceptive, it produced episodes of irregular and unpredictable spotting and numerous untoward effects. Their side effects were the main reasons why women discontinued its use. The daily dose of levonorgestrel is 30–37 µg in step with day. Alone use of progestin is less efficacious than combination oral contraceptives.

Therefore, the study indicates that the combined mixture of ethinylestradiol and levonorgestrel turned into a powerful contraceptive formulation. Therefore, the objective of the study is to formulate the proniosomes of levonorgestrel and ethinyl estradiol for antifertility treatment [4].

### MATERIALS AND METHODS

#### Materials

Levonorgestrel and ethinylestradiol were received as a gift sample from Cochin and Ontop Pharmaceuticals Ltd., Bengaluru. Cholesterol, soya lecithin, Span 20, and alcohol were procured from S. D. Fine Chemical Ltd., Mumbai, and Central Drug Store. All chemicals were used as received without any further purification.

#### Method of preparation

Prniosomal gel was prepared by a method known as coacervation-phase separation method. Precise amounts of non-ionic surfactant, cholesterol, lecithin, and drug (10 ml) were weighed and taken in a clean and dry wide-mouthed glass flask of 5.0 ml capacity, and alcohol was further added to it. All the ingredients such as cholesterol and lecithin were mixed well with a glass rod; the open end of the glass bottle was covered with a lid to prevent the loss of solvent from it and warmed over a water bath at 60–70°C for about 5 min until the surfactant mixture was dissolved completely. Then, the aqueous phase

(0.1% glycerol solution) was added and warmed on a water bath until a clear solution was formed which was converted into proniosomal gel on cooling. The gel so obtained was preserved in the same glass bottle in dark conditions for characterization [5]. Compositions of proniosomal gel formulations are given in Table 1.

### Characterization of proniosomal gel

#### Physical appearance

The prepared gel was viewed by the naked eye to characterize color and physical state of gel. Proniosomal gel was also viewed by optical microscope, to observe the characteristics of gel by spreading proniosomal gel on a slide which was hydrated with phosphate saline (pH 7.4) to produce niosome. A drop of niosomal suspension was placed on a slide and after placing coverslip, is further observed under microscope [6].

#### Vesicle size analysis and size distribution

Hydrated proniosomal gel as niosomal suspension was evaluated for niosomal size. The dispersion was observed under optical microscope at  $\times 100$  magnification. The size of 200 vesicles was measured using a calibrated ocular and stage micrometer fitted in the optical microscope [6].

#### Viscosity study

About 1 g of proniosomal gel was taken in a tube and 10 ml of distilled water was added on it and then stored for 7 h. Results showed that the proniosomal gel mixture obtains a homogenous mixture. Proniosomal gel viscosity was determined by Brookfield Viscometer at 0.5, 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, and 100 with increasing and decreasing rpm [6].

#### Rate of spontaneity

Rate of spontaneity is calculated by counting the number of niosomes formed after hydration of proniosomes for 15–20 min.. Approximately 10 or 20 mg of proniosomal gel is transferred to a glass bottle and spread uniformly around walls [7]. 2 ml of saline solution (0.154M NaCl) was added along the walls and left aside for 20 min. Then, a drop was withdrawn and placed on Neubauer chamber to count the number of vesicles. The number of niosomes eluted from proniosomes was counted [7].

#### Scanning electron microscopy (SEM)

The surface morphology and size distribution of proniosomes were perceived by SEM. A double-sided tape was affixed on the aluminum stubs, and the proniosomal powder was evenly spread over it. The aluminum stub was further kept in a vacuum chamber of SEM [8].

#### Thermal analysis

Differential scanning calorimetry (DSC) analysis was studied to determine the physical state of the drug within the gel and also, to determine the possible interactions between drug and the vesicle ingredients. For this, 6 mg of each sample (drug, Span 20, drug-free, and drug-loaded proniosomes) was sealed in a standard aluminum pan and subjected to analysis using DSC-4000 (PerkinElmer, USA). The samples were scanned at a heating rate of  $10^{\circ}\text{C}/\text{min}$ . An empty pan was used as a reference. The thermograms were obtained in the temperature range of  $40\text{--}350^{\circ}\text{C}$  [9,10].

#### Drug entrapment efficiency

Proniosomal gel (100 mg) was distributed in brine solution and warmed slightly for the formation of niosomes. Then, the solution was centrifuged at 18,000 rpm for 40 min at  $37^{\circ}\text{C}$ . The subsequent solution was filtered and examined spectrophotometrically at 281 nm and 247 nm for ethinylestradiol and levonorgestrel against 30% v/v PEG 200 [11,12].

The percentage encapsulation efficiency was calculated from Equation.

$$\% \text{Efficiency} = \left( 1 - \left( \frac{\text{Unencapsulated drug}}{\text{Total drug}} \right) \right) \times 100$$

#### In vitro release

*In vitro* release studies of proniosomal gel were performed using Keshary-Chien cell permeation system. Cellophane membrane was settled in between the half-cell of Keshary permeation cell [13,14]. Donor half-cell containing proniosomal gel formulation with 10 mg drug was adhered to the outer side of the membrane, and the receptor half-cell had a solution containing 30% v/v PEG in saline solution to maintain the sink condition. The receptor compartment was surrounded by a water bath for maintaining the temperature  $37 \pm 2^{\circ}$  for 24 h. The top of the donor compartment was 10 ml, and the area of donor compartment was 1.31 cm. At the 45 min of the time interval, 5 ml sample was taken from the receptor compartment and the same volume of 30% v/v PEG saline solution was added in it. Samples collected were analyzed ultraviolet (UV) spectrophotometrically for ethinylestradiol at 281 nm and for levonorgestrel against the respective blank solution.

#### Stability studies

Stability studies were determined by means of keeping the proniosomal gel at three different temperatures, that is, refrigerator temperature at  $4\text{--}6^{\circ}\text{C}$ , room temperature at  $37 \pm 20^{\circ}\text{C}$ , and in the oven at  $45 \pm 20^{\circ}\text{C}$ . Throughout the study, proniosomal formulations have been stored in aluminum foil-sealed glass vials. The temperature glass vials containing formulation are fully protected with aluminum foil [15-17].

## RESULTS AND DISCUSSION

The formulation variables greatly influence the characteristics as well as the performance of the obtained formulation. The proniosomal formulations were optimized by "factor at one time." First, a different grade of the commonly used surfactants, that is, spans or tweens was screened to choose the suitable surfactant for the formulations. The amount of the selected surfactant was varied thus at high and low levels. Similarly, the amount of the lecithin and the cholesterol was also varied to high levels to study their influence on the formulation characteristics. Finally, the drug amount was optimized. Tweens and spans are the popular non-ionic surfactants used as vesicle-forming agent in the preparation of niosomes and proniosomes. On evaluating different grades of spans, for example, 20, 40, 60, or 80 and tweens, for example, 20, 60, or 80, it was found that their nature considerably affected the drug entrapment and the final vesicle size. Percentage entrapment efficiency of the proniosomes was found to be higher with spans than with tweens which might be attributed to the high hydrophobicity of spans than tweens. Spans also have low hydrophile-lipophile balance

Table 1: Composition of proniosomal gel formulations

S. No	Drug (mg)	Surfactant (span 20) (mg)	Soya lecithin (mg)	Cholesterol (mg)	Alcohol (ml)
F1	10	50	100	100	0.5
F2	10	50	150	100	0.5
F3	10	100	100	-	0.5
F4	10	100	-	100	0.5
F5	10	50	100	150	0.5

value and higher phase transition temperature (Tc); thus, greater amount of the drug was expected to be encapsulated in the lipid layers of the vesicles. Higher phase transition temperature (Tc) further aids in the formation of ordered gel with less leaky lipid bilayers.

The amount of the selected surfactant “span 20” was further varied at low level (50 mg) and high level (100 mg). The surfactant concentration was found to be an important variable in the formation of niosomal vesicles and encapsulation of drug therein. The variation in the concentration greatly affected the entrapment efficiency with a predominant effect on vesicle size also. Lecithin acts as cosurfactant in the formation of smaller size proniosomal vesicles and also improves the drug permeation into vesicles by increasing hydrophobicity. The non-hydrogenated lecithin used in this study allowed the chains to bend, loosening the adjacent molecule that assembled to form the niosomal membrane. The loosely packed membrane was thus formed which showed high permeability. The concentration of cholesterol also plays an important role in the formation and drug entrapment in the vesicles, due to these, molecules house themselves as “vesicular cement” into the bilayers of the surfactant membranes. This mechanism is responsible for the permeability, rigidity, and stability of the niosomal vesicles.

**Investigation of physicochemical compatibility of drug and polymer**

*DSC analysis*

No major peak of drug is observed in drug loaded formulation and no major difference was observed in the drug-free and drug-loaded proniosomes. The endothermic peak of the niosomal bilayer was changed from 84.88°C to a broad wide peak at 90.64°C after drug loading due to some cholesterol molecules (Fig. 1). DSC thermogram of proniosome (D) with drug (C), blank proniosomes (B), span 20 (A). It indicates that there is integration between the span 20 molecules, cholesterol and soya lecithin and also indicates the amorphous nature of the drug since no peak is observed in the drug-loaded proniosome formulation.

*Physicochemical characterization of patches*

The study of organoleptic properties of the proniosomal gel formulations F1–F5 is presented in Table 2. Soya lecithin gave a yellowish colour to the formulation F1, F2, F4 and F5. F2 shows the best spreadability and homogeneity among the five formulations. Color of formulations F1, F2, F4, and F5 was found out to be yellowish-white, and F3 showed white color due to the absence of soya lecithin (Table 2). Homogeneity of F1

is best in all of the formulation; spreadability of F1, F2, and F5 showed excellent spreadability in all formulations (Table 2). There was no oily feel in any of the formulations. Proniosomal gel was also analyzed by an optical microscope at ×40 to represent coloration and physical phase of gel. Optical microscopy revealed that niosomes have been round and homogenous. The proniosomes were found to have a sweet fragrance and were tasteless.

*Vesicle size analysis*

Niosomes formed from the formulation of proniosomes have vesicle size very similar to each other. Gel containing formulation F2 produces the smallest vesicular sized niosomes (Table 3).

*Viscosity study*

Viscosity was determined at different rpm. Proniosomal gel formulations were studied for physical characteristics viscosity using Brookfield Viscometer (Table 3).

*Rate of spontaneity*

Rate of spontaneity was determined from the prepared formulation of proniosomal gel; the prepared formulation result is shown in Table 3, that is, prepared with span 20. The rate of hydration of all formulations was not significantly distinctive (Table 3).

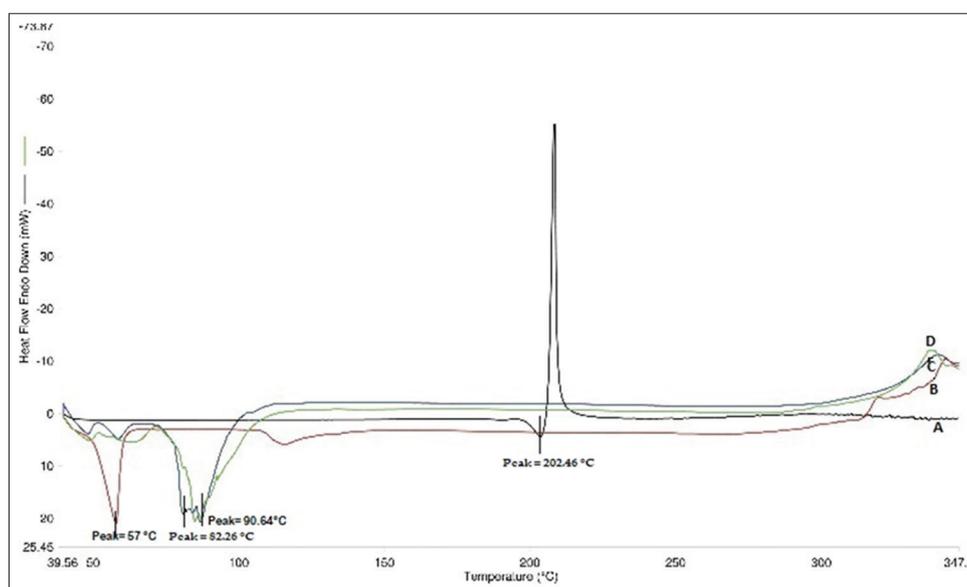
*Drug entrapment efficiency*

Among all formulations, Span 20 has shown the highest encapsulation efficiency and mixed formulation with Span 20 showed least encapsulation efficiency (Table 3).

**Table 2: Organoleptic controls\* at the end of 24 h**

Control	F1	F2	F3	F4	F5
Ease of absorption	++	++	++	++	++
Spreadability	+++	+++	++	++	+++
Oily feel	-	-	-	-	-
Homogeneity	+++	++	+	++	++
Stickiness	+++	++	++	+++	++
Water dilution	++	++	++	++	++
Odor	+	+	+	+	+
Color	YW	YW	W	YW	YW

–: Unavailable, +: Moderate, ++: Good, +++: Best, W: White, YW: Yellowish-white, \*mean±SD, n=3. SD: Standard deviation



**Fig. 1: Differential scanning calorimetry thermogram report**

### SEM

SEM images of optimized proniosomal gel, Span 20 formulations, size distribution, and morphology of proniosomes were determined by SEM as shown in Fig. 2, proniosomal gel formulations were examined, and images purely indicate spherical-shaped particles with a size range of 46.4 nm, 62.4 nm, 72.8 nm, and 80.2 nm. The morphology of the niosomes formed on hydration of proniosomal formulations and the effect of the amount of cholesterol in vesicle formation were also evaluated using SEM.

### In vitro drug release

*In vitro* drug release was carried out by using domestically fabricated Keshary Chien diffusion cell through cellophane membrane. It was apparent that the incorporation of a drug into vesicular systems resulted in sustaining the drug release profile for a longer period of time. Ethinylestradiol and levonorgestrel were released from the proniosomes with an initial fast release phase followed by a sustained release pattern. The initial fast release was due to desorption of the drug molecules that were present on the surface of formed niosomes. In the later phase, the ethinylestradiol and levonorgestrel release was regulated by diffusion through the inflated bilayers and exhibited sustained release pattern. The percentage cumulative release in 24 h was found ranging in 74–86%, and F2 system has shown the maximum percentage of release in 24 h as shown in Table 4. The optimized proniosomes (F2) showed a significant sustained drug release which was 86.42% after 24 h. The formulation prepared with high cholesterol content (familial combined hyperlipidemia) showed a more sustained release of ethinylestradiol and levonorgestrel with reduced initial burst release effect. Because the high cholesterol content facilitated the formation of compact niosomal bilayers which further reduced permeability and thus, hindered the release of drug entrapped. When the release data were fitted into various kinetic models, it was found that ethinylestradiol and levonorgestrel release from the proniosomal formulations followed Higuchi kinetics. This model suggested the diffusion controlled release of the drug.

### Stability study

Stability was scrutinized to determine the fraction of drug entrapped inside the vesicles of proniosomal formulation at temperature 4–6°C, 37°C, and 45±2°C for 48 days. Based on the results of vesicular size, Percentage drug content and Percentage entrapment efficiency (Table 5). From the result of the stability study test, it showed that the proniosomal formulations were highly stable at room temperature as well as refrigeration temperature.

As evident from the results, ethinylestradiol and levonorgestrel leakage from the vesicles may be due to the phase transition of the surfactant and lipid at 37°C (Table 5). However, no significant change was observed in the vesicular size or percentage entrapment of the formulations when stored at 4–8°C and 45±2°C for the period of 90 days.

### CONCLUSIONS

The results of the study indicated that ethinylestradiol and levonorgestrel proniosomal gel containing lecithin, cholesterol, and in combination of surfactant Span 20 formulations was prepared successfully using coacervation-phase separation method. F2 formulation showed highest

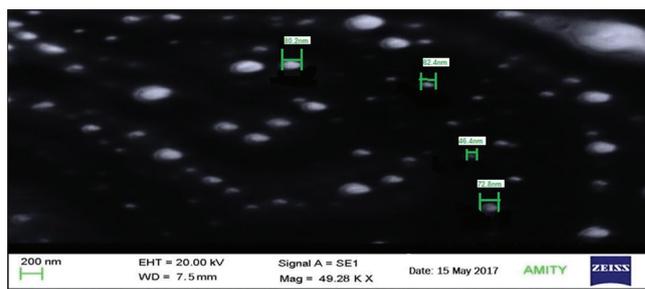


Fig. 2: Scanning electron micrograph of proniosomes

Table 3: Physicochemical evaluation of proniosomal gel formulation

Formulation code	Viscosity study*	Vesicle size (nm)*	Spontaneity* (niosome/mm <sup>3</sup> )	Percentage of drug entrapment*
F1	16	395.2	(9.687±0.883)×10 <sup>3</sup>	80.42±0.61
F2	15	385.4	(9.563±0.578)×10 <sup>3</sup>	86.85±0.85
F3	13	315.6	(5.477±0.315)×10 <sup>3</sup>	78.71±0.42
F4	15	365.6	(6.687±0.695)×10 <sup>3</sup>	79.25±0.66
F5	16	340.6	(6.681±0.752)×10 <sup>3</sup>	81.12±0.96

\*Mean, n=3

Table 4: Cumulative percentage drug release\*

Time (h)	F1	F2	F3	F4	F5
0.5	4.75±0.34	5.29± 1.08	3.65±0.867	0.4.04±0.35	4.51±1.3
1	7.94±0.34	8.76±1.76	6.3±0.34	5.08±1.36	5.23±0.42
2	10.23±1.23	11.89±0.54	9.21±0.28	0.10.16±1.75	9.78.15±0.08
3	15.86±0.91	17.21±1.37	14.29±1.73	16.24±0.45	16.15±0.98
4	21.23±0.14	22.78±1.41	21.76±1.87	20.69±0.78	21.87±1.98
6	34.67±0.56	36.98±1.43	33.72±1.54	34.03±1.3	33.89±0.65
12	56.23±1.47	58.91±1.79	56.98±1.46	55.27±1.62	55.38±1.48
24	80.42±0.67	86.42±0.61	76.08±9.98	74.85±0.56	80.51±0.67

\*Mean±SD, n=3. SD: Standard deviation

Table 5: Stability study analysis\*\*

Characteristics	Before storage	After 3 months' storage		
		4–8°C	37±2°C	45±2°C
Vesicle size (nm)	288.2	375.2	385.2	185.2
Percentage entrapment	77.95±0.12	80.42±0.08	85.05±0.85	63.85±0.69
Percentage drug content	79.27±0.04	83.76±0.23	86.03±0.61	75.12±0.35

\*\*Mean±SD, n=3. SD: Standard deviation

entrapment efficiency. Proniosomal gel formulations were evaluated for the entrapment efficiency, UV spectroscopy, viscosity, vesicle size and shape with SEM, and optical microscopy, and the results were found in the acceptable range. The absence of cholesterol content in formulations F4 was found to cause low percentage entrapment efficiency (78.1%) which might be due to leakage of the vesicles. Further, cholesterol absence reduces vesicles size of the formulation. F4 showed the lowest drug release (74.85%) because soya lecithin is absent in the formulation. F3 showed the lowest drug release (74.85%) because soya lecithin is absent in the formulation. Increased level of soya lecithin in F1 and F2 showed high drug release (80.42% and 86.01%, respectively). F4 showed the higher drug entrapment efficiency due to higher level of cholesterol. *In vitro* dissolution studies showed satisfactory results, and the permeation studies showed good control release for prolonged period of time. Permeation studies showed the highest permeation of proniosomal gel formulation F2, and *in vitro* rat skin permeation studies proved that good amount of drug is permeated and has good stability characteristics. The study showed that the proniosomal gel formulation was quite stable at refrigeration and room temperature as well. All the results suggest that ethinylestradiol and levonorgestrel drug proniosomal gel formulations may be used as an antifertility treatment and can be further subjected to clinical trials to find out the adverse effects [18]. The high stability of the system further favors the potential of proniosomes for systemic delivery of therapeutics.

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#### AUTHORS' CONTRIBUTIONS

All the authors have contributed equally.

#### CONFLICTS OF INTEREST

The authors have declared no conflicts of interest.

#### REFERENCES

- Kakar R, Rao R, Goswami A, Nanda S, Saroha K. Proniosomes: An emerging vesicular system in drug delivery and cosmetics. *Der Pharm Lett* 2010;2:227-39.
- Vyas SP, Khar RK. Novel carrier systems. In: Jain NK, editor. Targeted and Controlled Drug Delivery. New Delhi, India: CBS Publishers and Distributors Pvt Ltd; 2010.
- Singh S. Niosomes: A role in targeted drug delivery system. *Int J Pharm Sci Res* 2013;4:550-7.
- Balasubramaniam A, Kumar VA, Pillai KS. Formulation and *in vivo* evaluation of niosome-encapsulated daunorubicin hydrochloride. *Drug Dev Ind Pharm* 2002;28:1181-93.
- Yoshioka T, Sternberg B, Florence AT. Preparation and properties of vesicles (niosomes) of sobitan monoesters (Span 20, 40, 60, and 80) and a sorbitantristearate (Span 85). *Int J Pharm* 1994;105:1-6.
- Karki R, Mamatha GC, Subramanya G, Udupa N. Preparation, characterization and tissue disposition of niosomes containing isoniazid. *Rasayan J Chem* 2008;1:224-7.
- Tavano L, Infante MR, Riya AM, et al. Role of aggregate size in the hemolytic and antimicrobial activity of colloidal solutions based on single and gemini surfactants from arginine. *Soft Matter* 2013;9:306-19.
- Walker RB, Smith EW. The role of percutaneous penetration enhancers. *Adv Drug Deliv Rev* 1996;18:295-301.
- Paolino D, Muzzalupo R, Ricciardi A, Celia C, Picci N, Fresta M, et al. *In vitro* and *in vivo* evaluation of bola-surfactant containing niosomes for transdermal delivery. *Biomed Microdevices* 2007;9:421-33.
- Barry BW. Lipid protein partitioning theory of skin penetration enhancement. *J Control Release* 1991;15:237-48.
- Schreier H, Bouwstra J. Liposomes and niosomes as topical drug carriers: Dermal and transdermal drug delivery. *J Control Release* 1994;30:1-15.
- Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes-novel vesicular carriers for enhanced delivery: Characterization and skin penetration properties. *J Control Release* 2000;65:403-18.
- Patel KK, Kumar P, Thakkar HP. Formulation of niosomal gel for enhanced transdermal lopinavir delivery and its comparative evaluation with ethosomal gel. *AAPS Pharm Sci Tech* 2012;13:1502-10.
- Ammar HO, Ghorab M, El-Nahhas SA, Higazy IM. Proniosomes as a carrier system for transdermal delivery of tenoxicam. *Int J Pharm* 2011;405:142-52.
- Gupta A, Prajapati SK, Balamurugan M, Singh M, Bhatia D. Design and development of a proniosomal transdermal drug delivery system for captopril. *Trop J Pharm Res* 2007;6:687-93.
- Mishra A, Kapoor AN, Bhargava SH. Proniosomal gel as a carrier for transdermal drug delivery of clotrimazole. *Int J Pharm Pharm Sci* 2012;4:610-14.
- Patil HN, Hardikar SR, Bhosale AV. Formulation development and evaluation of proniosomal gel of carvedilol. *Int J Pharm Pharm Sci* 2012;4:191-7.
- Chauhan BS, Naved T. Herbal contraceptives: Evaluation of antifertility potential of *Hibiscus rosa-sinensis* (Linn). *Asian J Pharm Clin Res* 2018;11:36-41.