International Journal of Current Trends in Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijctpr

Research Article **Open Access**

Formulation and Evaluation of Topical Proniosomal Gel of an Anti Fungal Drug-Itraconazole

Srividhya Vardhani CH*, Nirosha M

Department of Pharmaceutics, Oil Technological and Pharmaceutical Research Institute, JNTUA Ananthapuramu, India

A B S T R A C T

Proniosomal gels are the liquid crystalline compact Niosomal hybrids that can be converted into niosomes immediately upon hydration or used as such in the topical/ transdermal applications. Itraconazole is an Anti fungal drug which on Oral administration has Bioavailability of 55% only because of first pass hepatic metabolism. In order to bypass the First pass effect and also to decrease other Oral route side effects like Dry mouth, Nausea, Vomiting etc they can be formulated into Proniosomal gels for an effective Transdermal drug delivery. The Present study was aimed at preparing twelve formulations of Topical Proniosomal gels with different concentrations of surfactants and cholesterol. All the prepared formulations were evaluated for Physical appearance, pH, Vesicle size analysis, Vesicle shape and surface characteristics, % Entrapment efficiency, *In-vitro* drug release studies and Stability studies. Among all the formulations F4 was found to exhibit optimum results for all the evaluation tests and In vitro drug release was found to be 91.9% at the end of 9th hour. The drug release of all formulations followed zero order kinetics on fitting the drug release data to various kinetic models. Thus Proniosomal gels are the promising approach for Itraconazole to overcome the gastrointestinal side effects and to increase the drug bioavailability.

Keywords: Itraconazole, Proniosomal gels, Coacervation phase separation method, Non ionic surfactants, Cholesterol.

A R T I C L E I N F O

CONTENTS

Article History: Received 28 September 2016, **Accepted** 29 October 2016, **Available Online** 15 November 2016

***Corresponding Author**
Srividhya Vardhani CH
Department of Pharmaceutics,
Oil Technological and
Pharmaceutical Research Institute,
JNTUA Ananthapuramu, India
Manuscript ID: IJCTPR3140

PAPER-QR CODE

Citation: Srividhya Vardhani CH. Formulation and Evaluation of Topical Proniosomal Gel of an Anti Fungal Drug-Itraconazole. *Int. J. Curnt. Tren. Pharm, Res.*, 2016, 4(6): 307-313.

1. Introduction

Proniosomes are defined as the dry formulations which are coated with carriers such as non-ionic surfactants[1] and these Proniosomes can be converted into Niosomes upon hydrating with hot water right before the use. As Niosomes are associated with various drawbacks such as physical instabilities like fusion, aggregation of particles and leakage of the drug to overcome these disadvantages Niosomes are formulated into Proniosomes. Proniosomal gels are a mixture of non ionic surfactant, lecithin and cholesterol. On addition of a small quantity of water or gelling agent they appear as translucent gels and liquid lamellar crystals of vesicular bilayers, the lamellas stacked together are termed as compact Niosomes which can be used for Topical/Transdermal drug delivery. Proniosomal gels are becoming more popular because of a wide range of applications and better percutaneous absorption compared to other semi solid preparations. These Proniosomal gels can resist the physiological stress caused by skin flexion, mucociliary movement adapting to the shape of the applied area for a controlled drug delivery. A suitable carrier must be used to deliver the drug to the site of action and to achieve the optimum drug release. Thus the main objective of the present study is to avoid the first pass hepatic metabolism, reduce the Gastro intestinal side effects and to improve the drug bioavailability.

Itraconazole [2] is an Antifungal drug with an oral bioavailability of 55%. It is most widely used to treat fungal infections like Systemic mycosis, Histoplasmosis, Onchomycois, Aspergillosis, Chromo mycosis, Candidiasis, Blastomycosis and Cryotococcal Meningitis. The available dosage forms of Itraconazole are tablets, solutions etc. Due to the most common GI side effects of Itraconazole like convulsions, dry mouth, decreased urine, irregular heartbeat, loss of appetite, nausea and vomiting etc. they are formulated into proniosomal gels.

2. Materials and Methods

Materials: Itraconazole (Hetero drugs, Hyderabad), Cholesterol (Sisco Laboratories, Mumbai), HPMC k 30 (G.M Chemicals, Maharastra), Span20 (SBFCL Chemicals Ltd. Mumbai), Span 80(SBFCL Chemicals Ltd. Mumbai), Lecithin (Sd fine chem. Ltd. Mumbai), Ethanol (Alkem Laboratories, Mumbai).

Methods:

Preformulation Studies

Description: The received sample of API was subjected to determination of its nature and viewed visually for the determination of its colour and then the results were compared with the official books.

Determination of melting point: Melting point of a compound helps in the identification of sample and to establish its purity. It can be checked by placing the sample in glass capillary tube and heated using melting point apparatus.

Drug - Polymer Compatibility Studies:

Drug polymer compatibility studies were performed by using FTIR (Fourier Transform Infrared Spectroscopy). An infrared (IR) spectrum was obtained using the KBr disk method (2 mg sample in 200 mg KBr). The scanning range was 400 to 4000 $cm^{-1}$and the resolution was 1 cm^{-1}.

Formulation Development:

Itraconazole Proniosomal gels were formulated by using Coacervation phase separation method. This is the most widely choosen method of preparation with varying concentrations of Itraconazole, cholesterol, span 20, span 80, lecithin and HPMC K 30. Where, cholesterol acts as a membrane stabilizer[5], non ionic surfactants[6] for uniform vesicle formation, lecithin[8] acts as permeation enhancer and HPMC K30[7] acts as a gelling agent. The compositions were shown in the Table No.2.

Preparation of Proniosomal gel of Itraconazole [9]

First all the ingredients i.e., drug, surfactants, cholesterol and lecithin were weighed accordingly and transferred into a wide mouthed glass vial. Then add required quantities of ethanol and stir well. All the ingredients were heated by mixing with a glass rod. To prevent the loss of solvent the open end of the glass tube must be covered with a lid. Heating must be continued on a water bath at 60-70^{0}C until the surfactant has been dissolved properly. After the surfactant has been dissolved add measured quantities of HPMC K 30 and then the mixture has to be cooled down to room temperature till the dispersion forms Proniosomal gel.

Figure 1: Steps involved in the Formulation of Proniosomal gels

Evaluation Studies

Evaluation of Proniosomal Gels

Morphological Evaluation [10]

Physical Appearance: The physical appearance of the Proniosomal gels has to be viewed by naked eye for characterizing the colour and physical state and then viewed under optical microscope at 40x magnification to determine the crystal characteristics of the drug.

Determination of pH:

The pH of the twelve Proniosomal gel formulations was determined by using digital pH meter.

Shape and Surface Characteristics of the Vesicles:

The Proniosomal gels was hydrated with a hydrating medium and place the Niosomal suspension thus obtained on a slide then observe under the Scanning Electron Microscope.

Vesicular Size Distribution: The size of the vesicles of Niosomal suspension formed after the hydration of Proniosomal gel was determined by observing the dispersion under the optical microscope at 100 x magnifications or by using SEM analysis.

Drug Entrapment Efficiency by Centrifugation [11]:

The Niosomal suspension prepared by dispersing the Proniosomal gel in phosphate buffer was centrifuged at 18000 rpm in a cooling centrifuge at a temperature of 20^{0}C

for 30 min to separate the drug entrapped in Niosomes. The sediment vesicles were collected, diluted with phosphate buffer saline and analyzed**.**

% Entrapment efficiency =

$$\frac{\textbf{Amount of entrapped drug}}{\textbf{Amount of total drug}} \textbf{ X 100}$$

***In-vitro* Drug Release Studies:**

The *In-vitro* drug release studies can be performed by using Franz diffusion cell. The dialysis cellophane membrane was mounted between the donor and receptor compartments and the capacity of the receptor compartment was 30ml. The area of the donor compartment was 2.54cm^2. The weighed quantity of Proniosomal gel was placed on one side of the dialysis membrane and phosphate saline of pH 7.4 is used as a receptor medium. The receptor compartment was surrounded by water jacket to maintain the temperature of 37±0.5^{0}C. The heat was maintained using thermostatic hot plate with a magnetic stirrer. The receptor fluid was stirred by using a Teflon coated magnetic bead fitted to the magnetic stirrer. At every sampling interval the withdrawn samples were replaced with equal amounts of fresh receptor liquid. The samples thus withdrawn were analyzed spectrophotometrically. The maintenance of sink conditions is essential [14].

Stability Studies:

Stability studies were performed to determine the ability of vesicles to retain the drug by placing the Proniosomal gel at three different temperature conditions like Room temperature (25±2^{0}C), Refrigeration temperature (4-8^{0}C) and in Oven temperature (45±2^{0}C). During the stability studies the samples of Proniosomal gels are to be placed in aluminium foil sealed glass vials. The samples were withdrawn at regular time intervals for a period of 3 months and observed microscopically for the change in consistency, pH and % entrapment efficiency [15].

3. Results and Discussions

All the twelve formulations were evaluated for various parameters.

Preformulation Studies: The Preformulation studies like description, melting point determination and assay were performed.

Description:

After visual observation and under compound microscope it was observed that API sample was white amorphous powder.

Melting point: Melting point of the pure API was found to be 166.3 ºC which is within the range of 166.2-166.5ºC as reported in literature, thus indicating purity of the drug sample.

Drug –Polymer Compatibility Studies by FTIR

The physicochemical compatibility of the drug and the polymer was established through FTIR studies. In the physical mixture of Itraconazole with Surfactants, the major peaks were obtained almost at the same wave numbers. However, additional peaks were obtained in physical mixtures which could be due to presence of polymers but there is no influence in the drug peaks, which indicates that there is lack of significant interaction between the drug and polymers and the entrapment of drug only by physical process. So, it was concluded that the drug and polymers used in the formulation were compatible with each other.

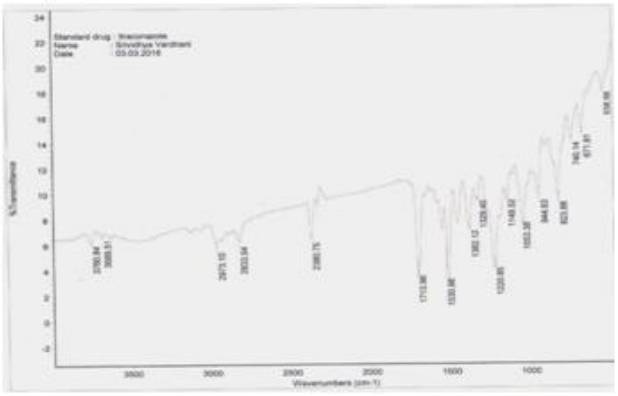

Figure 2: FTIR Spectra of Itraconazole

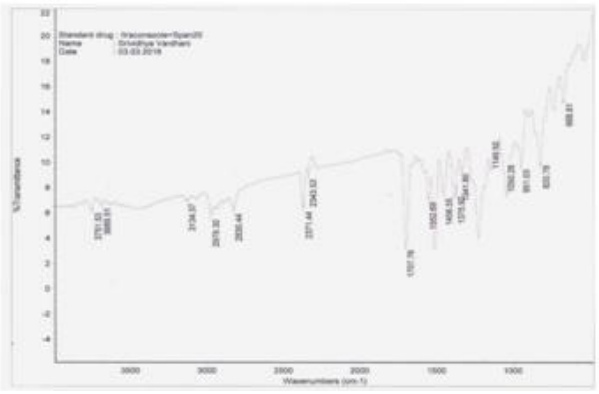

Figure 3: FTIR Spectra of Itraconazole + Span 20

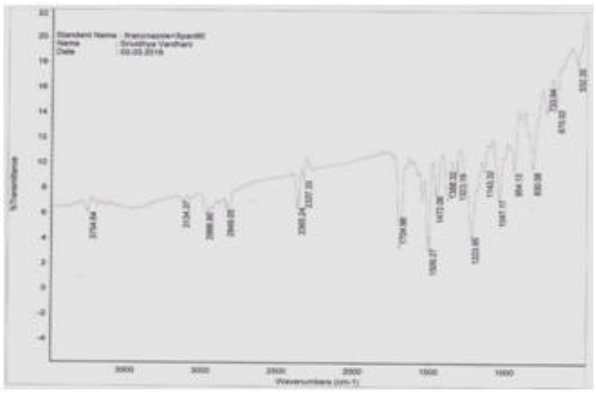

Figure 4: FTIR Spectra of Itraconazole + Span 80

Evaluation of Proniosomal Gels

Physical Appearance: The physical appearance of the Proniosomal gels has been viewed by naked eye and the results were mentioned in Table No.3. The results indicate that the physical state of all the formulations is jelly in nature with light yellow in colour.

Determination of pH:

pH of all the formulations were determined by using pH meter and the pH of the twelve formulations were within the range of 6.4-6.93 which avoids the skin irritation as it is

within the acceptable range for Topical gels. The results were tabulated in the following Table No. 3.

Shape and surface characteristics of the vesicles

The shape and surface characteristics of the formulations can be revealed by using SEM analysis. The SEM images revealed that the Niosomes formed were spherical with sharp boundaries. The surface characteristics of Proniosomes of all formulations were uniform with high wall rigidity. The SEM image of optimized formulation F1, F2 is shown in Fig. No.6

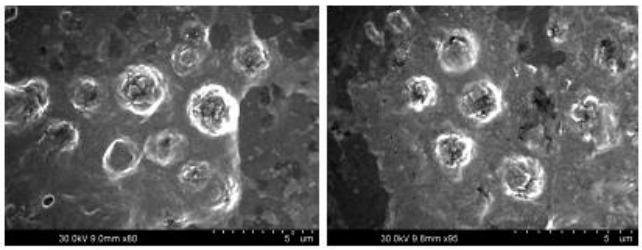

Figure 5: SEM image of Proniosomal gel formulation (F1), (F2)

Vesicular Size Distribution: At least 100 vesicles are to be measured using stage and optical microscope or through SEM analysis the results of the vesicle size distribution were tabulated in Table No. 4. For this purpose the Proniosomal gel was hydrated with phosphate buffer with agitation. The mean vesicle size was between 1 µm -5 µm.

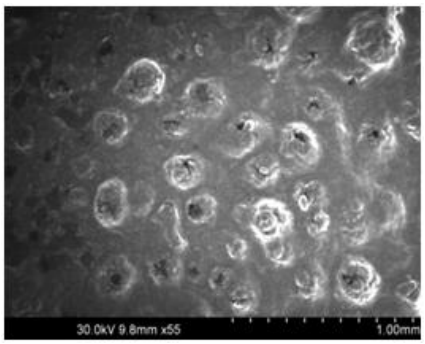

Figure 6: SEM image of Proniosomal gel formulation (F9)

Drug Entrapment Efficiency:

The entrapment efficiency was determined for all the twelve formulations. The Niosomes prepared from span 20 containing Proniosomal gel has shown better entrapment efficiency compared to those containing span 80. This is because the entrapment efficiency depends on the structure of the surfactant. Longer is the saturated alkyl chain of the surfactant higher is the entrapment efficiency. Since the alkyl chains of the surfactant span 80 bend and forms membrane, this leads to lowest entrapment efficiency. The percentage entrapment efficiency of all the twelve formulations was listed in Table No. 4. The results suggest that the formulation F3 has shown the highest entrapment efficiency and Formulation F2 has shown the least Entrapment efficiency.

***In-vitro* Drug Release Studies:**

The *in vitro* drug release studies were performed for all the twelve formulations. The permeation of drug from Proniosomal gels containing Span 80 is high compared to that of Span 20. This is due to the unsaturated alkyl chain structure in Span 80 that led to leakier Niosomal membrane. Hence, fast drug release was observed in case of Itraconazole Proniosomal gels prepared using Span 80. Increasing the cholesterol content resulted in the formation of more intact bilayers as a barrier for drug release through vesicles and thus decreases the leakage of drug from vesicles. With the increase in the cholesterol content beyond the limit disruption of linear structure of vesicle takes place. The percentage drug release of all the twelve formulations at the end of 9hrs is in the following order F2> F4> F7> F11> F9> F5> F6> F8> F12> F10> F1> F3. The graphs were plotted by taking time on x- axis and cumulative percentage drug release on y- axis. The graphs were mentioned in Fig. No. 5 and 6. From the results of the *in vitro* drug release studies it indicates that formulation F2 has shown the highest linear drug release and formulation F3 has shown the least drug release it may be due to more quantity of Span 80 in F2 which is more leakier in nature than Span20.

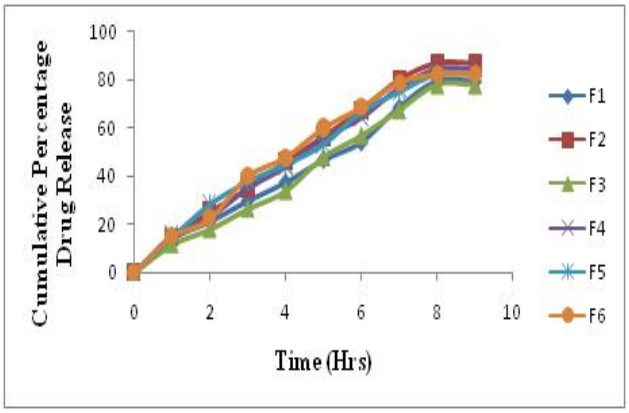

Figure 7: *In-vitro* drug release of formulations F1-F6

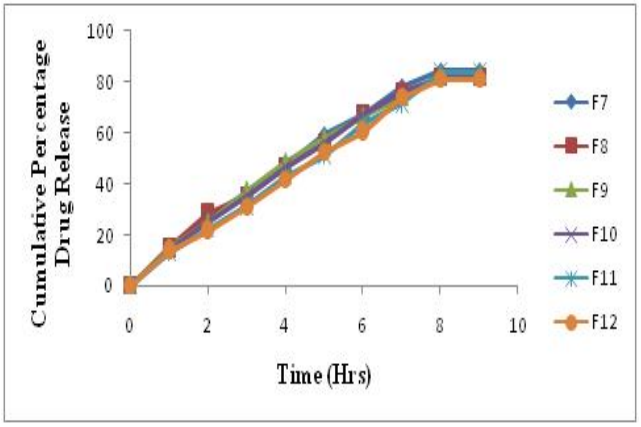

Figure 8: *In-vitro* drug release of formulations F7-F12

Analysis of Drug Release Kinetics [12]:

The *in vitro* drug release data so obtained was fitted to various kinetic models like Zero Order and First order. Results were shown in Table No.5. The release of drug from the Proniosomal gel was best fitted to zero order kinetics and all the formulations followed the zero order drug relelase [13].

Stability studies: Stability studies were performed to determine the ability of vesicles to retain the drug by placing the optimized formulation F9 at three different temperature conditions like Room temperature (25 ± 2^0C), Refrigeration temperature ($4\text{-}8^0$C) and in oven (45 ± 2^0C). The results were mentioned in Table No. 6.

Table 1: List of components used in the formulation of proniosomal gel [3]

S. No	Components	Examples
1	Membrane Stabilisers	Cholesterol, Lecithin
2	Nonionic surfactants	Span 20,40,60,80, Tween 20,60,80, Polyoxyethylene 4 lauryl ether
3	Coating materials	Sucrose stereate, Sorbitol, Lactose monohydrate, Maltodextrin

Table 2: Formulation table of Itraconazole Proniosomal gels

Formulation code	Itraconazole (mg)	Span 20 (mg)	Span 80 (mg)	Cholesterol (mg)	Lecithin (mg)	HPMC K 30 (mg)
F1	100	1000	-	100	100	300
F2	100	-	1000	100	100	300
F3	100	900	-	200	100	300
F4	100	-	900	200	100	300
F5	100	500	500	100	100	300
F6	100	450	450	200	100	300
F7	100	250	750	100	100	300
F8	100	750	250	100	100	300
F9	100	200	600	200	200	300
F10	100	600	200	200	200	300
F11	100	200	600	100	300	300
F12	100	600	200	100	300	300

Table 3: Physical Appearance and pH of Proniosomal gels

S. No.	Formulation Code	Colour	Physical state	pH
1	F1	Light yellow	Gel	6.93
2	F2	Light Yellow	Gel	6.8
3	F3	Light yellow	Gel	6.9
4	F4	Light yellow	Gel	6.8
5	F5	Light yellow	Gel	6.7
6	F6	Light yellow	Gel	6.8
7	F7	Light yellow	Gel	6.8
8	F8	Light Yellow	Gel	6.4
9	F9	Light yellow	Gel	6.8
10	F10	Light yellow	Gel	6.87
11	F11	Light yellow	Gel	6.9
12	F12	Light yellow	Gel	6.8

Table 4: Mean vesicle size & Percentage Entrapment Efficiency of Proniosomal gels

S. No.	Formulation Code	Mean vesicle size (µm) Mean±S.D, n=3	Percentage Entrapment Efficiency Mean±S.D, n=3
1	F1	5±1.1	84.1±1.1
2	F2	4.84±1.1	78±1
3	F3	1.73±1.8	84.8±1.2
4	F4	5±0.6	78.3±1.7
5	F5	2.2±1.2	79.8±2
6	F6	2.13±0.7	81.3±1.6
7	F7	2.39±1.1	80.2±1.8
8	F8	2.29±1.1	83±2.1
9	F9	1.0±0.9	81.4±1.3
10	F10	2.41±1.3	83.6±1.1

11	F11	2.54±1.5	79.2±1.6
12	F12	3.14±1.6	81±1.4

Table 5: Mechanism of Drug Release of all Proniosomal gel formulations

S. No.	Formulation Code	Regression coefficient values(R^2)	
		Zero order	First order
1	F1	0.992	0.834
2	F2	0.994	0.849
3	F3	0.991	0.851
4	F4	0.997	0.829
5	F5	0.992	0.833
6	F6	0.994	0.816
7	F7	0.993	0.837
8	F8	0.992	0.849
9	F9	0.997	0.845
10	F10	0.992	0.854
11	F11	0.995	0.858
12	F12	0.996	0.849

Table 6: Stability data of Itraconazole Proniosomal gels

Temperature	Physical Appearance	pH	% Entrapment Efficiency
Initial findings	Light yellow	6.8	81.4
Refrigeration temperature (4-8^{0}C)	Light yellow	6.78	80.3
Room temperature (25±2^{0}C)	Light yellow	6.75	80.1
Oven temperature (45±2^{0}C)	Faint yellow	6.6	79.2

4. Conclusion

In the present study we had prepared twelve formulations of proniosomal gels by coacervation phase seperation method and evaluated them for physical appearance, pH, vesicle shape and surface characteristics, vesicle size analysis and surface morphology, % entrapment efficiency, in vitro drug release studies and stability studies. All the formulations had shown optimum results for all the tests. Thus we conclude that the itraconazole is suitable for formulating into topical proniosomal gels. This formulation is advantageous for overcoming the gastro intestinal side effects, avoiding the hepatic first pass metabolism and high patient compliance. Hence further work is recommended to support its efficacy claims by in-vivo studies.

5. References

[1] Abbas pardakhty et al., Nano Niosomes in drug, vaccine and gene delivery: A rapid overview. Nano medicine journal, **2013**, 1(1): 1-12.

[2] Itraconazole- Drug bank, (http:// www.drugbank.ca/drugs/DB01167). Accessed 06 september 2016.

[3] Wlave J.R et al., Proniosomes: A surrogated carrier for improved trasdermal drug delivery system. International journal of research in ayurveda and pharmacy, **2011**, 2(3): 743-750.

[4] Shalin K. Parikh et al., Development and validation of UV spectrophotometric method for estimation of Itraconazole bulk drug and pharmaceutical formulation. International journal of drug development and research, **2011**, 3(2): 324-328.

[5] Raymond C Rowe. Handbook of Pharmaceutical Excipients, 6th edition, Pharmaceutical Press and the American Pharmacists Association, (2009): 86-88.

[6] Hypromellose- Wikipedia, (https:// en.wikipedia.org/wiki/Hypromellose). Accessed on 08 September 2016.

[7] Raymond C Rowe et al., Handbook of Pharmaceutical Excipients, 6th edition, Pharmaceutical Press and the American Pharmacists Association, (2009**)**: 326-329.

[8] D. Nagasamy venkatesh et al., Proniosomes: A superior drug delivery system. International journal of pharmaceutical sciences and drug research, **2014**, 6(3): 178-182.

[9] Yasam venkata ramesh et al., Proniosomes: A novel nano vesicular transdermal drug delivery. International journal of pharmaceutical sciences and drug research, **2014**, 6(3): 178-192.

[10] Neeraj bhandari et al., Proniosomes - A surrogate for transdermal drug delivery. International journal of pharmaceutical research and bioscience, **2012**, 1(6): 10-26.

[11] Narashimhan B., Mallapragada S.K., Peppas, Release kinetics, data interpretation, in:

Encyclopedia of controlled drug delivery, Mathiowitz E. Ed., John Wiley and Sons, Inc, New York (1999).

[12] Hadjiioannou T.P., Christian G.D., Koupparis MA. Eds., Quantitative calculations in pharmaceutical practice and research. VCH Publishers Inc., New York (1993).

[13] D. Akhilesh et al., Review on span-60 based non-ionic surfactant vesicles (Niosomes) as novel drug delivery. International journal of research in pharmaceutical and biomedical sciences, **2012**, 3(1).

[14] Samita single et al., Proniosomes for penetration enhancement in Transdermal system. International journal of drug development & research, **2012**, 4(2): 1-13.