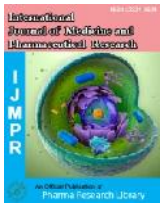
International Journal of Medicine and Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijmpr



Research Article **Open Access**

Development and Characterization: Proniosomes of Ketoconazole for the Management of Candidiasis

Akanksha Jethmal*[1], Amit K Agrawal[2], Archana Tiwari[2]

[1]*Department of Pharmaceutics, Vedica College of B.Pharmacy, Bhopal, M.P, India*
[2]*Department of Pharmaceutics, Patel College of Pharmacy, Bhopal, M.P, India*

A B S T R A C T

The aim of present study was to prepare proniosomal gel for the topical drug delivery. Ketoconazole was used as proniosomal gel with sorbitol by using surfactant (span 60), Cholesterol, Lecithin. Proniosomal gel loaded with ketoconazole was prepared by slurry method. The properties of the proniosome such entrapment efficiency (%EE), Zeta potential, Microscopy and vesicle size, physical parameter of proniosomal gel were investigated. The morphology of Proniosomal gel was observed by SEM (Scanning Electron Microscopy). The drug release behaviour was studied by dialysis membrane. The results shows % entrapment efficiency of formulation $L_3S_1D_1$ was found to be 96.5±2.3. Vesicle size of formulation $L_3S_1D_1$ was large 8.6±0.4 with zeta potential -38.5and zeta deviation was -5.16. Stability studies results show that there were no significant changes observed in the vesicle size, drug retained of formulation at 5°C ± 3°C. It confirms that formulation $L_3S_1D_1$ was stable at the end of 45 days. Ketconazole proniosomal gel *in-vitro* drug release was conducted for $L_3S_1D_1$ formulation. The formulation was found to provide 86.25% release with a period of 4 hours.

Keywords: Proniosomes, Proniosomal gel, Ketoconazole, *In-vitro* cumulative % drug release, cholesterol, Antifungal

A R T I C L E I N F O

CONTENTS

Article History: Received 09 June 2015, **Accepted** 29 July 2015, **Available Online** 10 August 2015

***Corresponding Author**
Akanksha Jethmal
Department of Pharmaceutics,
Vedica College of B.Pharmacy,
Bhopal, M.P, India
Manuscript ID: IJMPR2606

PAPER-QR CODE

Citation: Akanksha Jethmal, et al. Development and Characterization: Proniosomes of Ketoconazole for the Management of Candidiasis. *Int. J. Med. Pharm, Res*., 2015, 3(4): 1080-1085.

1. Introduction

Over the last two decades, there has been a dramatic increase in the rate of superficial and invasive fungal infections. Treatment of severe life threatening skin fungal infections with ketoconazole has shown to be emerge as an efficient therapy and occupies a prominent position among the alternatives of treatment [1]. However, topical delivery of ketoconazole resulted in systemic absorption, skin irritation and therefore failed to achieve mycological eradication [2]. Therefore, these problems create the poor patient compliance and compromising the efficacy of the therapy. Moreover, the topical administration of bioactives is however a challenging field in drug delivery with the intricacy in controlling and not determining the exact amount of drug that reach the different skin layers. Ketoconazole has emerged as the primary treatment option for virtually all forms of susceptible *Candida* infections in both immunocompetent and immunocompromised hosts. The lipophilic nature of ketoconazole poses problems in a suitable topical dosage form for topical delivery [3].

Candidiasis is a fungal infection caused by fungi that belongs to genus candidia. Candidia fungi normally lives on the skin and mucous membranes without causing infectionovergrowth of this disease can cause symptoms to develop. Candidiasis vary depending on the area of the body that is infected [4]. Ketoconazole an imidazole antifungal agent, is used both in treatment of systemic or topical fungal infection. Ketoconazole is lipophilic and is practically insoluble in water [5].

Proniosomes are dry formulations of surfactant coated carrier, which can be measured out as needed and rehydrated by brief agitation in hot water. These "proniosomes" minimize problems of niosomes physical stability such as aggregation, fusion and leaking and provided additional convenience in transportation, distribution, storage and dosing. Proniosome derived niosomes are superior to conventional niosomes in convenience of storage, transport and dosing.

Proniosomes of ketoconazole for the attainment of better therapeutics in candidiasis. The drug is 99% protein bound moreover, it is hepatotoxic hence a cutaneous/topical delivery is more beneficial for the attainment of better therapeutic results. To overcome the side effects associated with conventional delivery and formulation is envisaged for the drug. Prepared proniosome shall penetrate deeper in the skin and led to better and faster therapeutics effect as compared to conventional formulation.

The objective of this study was to prepare proniosomal gel of ketoconazole by using Slurry method. Cholesterol and lecithin are used as lipids and Span 60 as surfactants. Ketoconazole is a poorly water-soluble drug. The various formulations were prepared with different ratios of lipids and surfactants. The Vesicle Particle size, zeta potential, entrapment efficiency, Scanning electron microscopy (SEM) and *In-vitro* release of Ketoconazole loaded Proniosome were investigated.

2. Materials and Methods

Materials:

Lecithin was purchased from Himedia Laboratories Pvt. Ltd. Mumbai, India. Ketoconazole was obtained as gift sample from Modern Labs, Indore. Cholesterol was purchased from Ranbaxy Laboratory, New Delhi. India. Span 60 (Sorbitan monostearate) was purchased from Himedia, India. Other chemicals used were all analytical grade.

Preparation of Proniosomes by Slurry method:

The proniosomes were prepared by slurry method with slight modification in concentration of span 60 and cholesterol.[6] 500mg of Mannitol was placed in a 100ml of round bottom flask attached to a rotary evaporator.180mg Lecithin, 160mg Span 60, 40mg Cholesterol and 20mg Ketoconazole mixture in 12ml Chloroform and 3ml Isopropylalcohol mixture (4:1) was added slowly onto Mannitol powder bed. Care was taken not to overweight the powder paste. The rotary evaporator was maintain at a temperature of 65°C using water bath and the flask was rotated at 60 rpm under vacuum.The dried material was finally removed and kept under vacuum overnight.

Preparation of Ketoconazole Proniosomes Gel:

Gel was prepared with the formula optimized. Proniosomes powder was weighed into screw cap vials to which was added water at 80°C. The vials were vortexed mixed for completed and uniform hydration. The Proniosomal preparation were then converted into gel by approximately diluting the proniosomes and adding Carbopol 934 (1%w/v) for ease of handling. The final Ketoconazole concentration achieved was 2%w/w.

Characterization of Proniosomal gel:

I. Percentage Entrapment Efficiency [7**]:**

Percent entrapment efficiency was determined by centrifugation method. In this the proniosomes were hydrated centrifuged at 18000 rpm for 40 minutes at 5°C in order to separate unentrapped drug. The supernatant was discarded. The pellet was digested with triton × 100 (1% w/v) and the entrapped drug was extend with PBS (pH 7.4). The solution was filtered and absorbance noted after suitable dilution. The drug concentration in the resulting solutions was assayed spectrophotometrically at 221.6 nm. Entrapment Efficiency is expressed as the % of drug entrapped.

$$\text{\% entrapment efficiency} = \frac{\text{Drug in proniosomes}}{\text{Total drug}} \times 100$$

II. Microscopy and Vesicle Size [8**]:**

A drop of proniosomal gel was spread on a glass slide and examined for the vesicle structure and presence of insoluble drug crystals under the optical microscope with varied magnification power. Photomicrographs were taken for proniosome using a digital camera with 100X optical 200m.

III. Vesicle Morphology [9**]:**

Shape and surface morphology of proniosomes was studied using scanning electron microscopy (SEM). The proniosome gel was mounted on metal stubs and the stud was then coated conductive with sputter coater attached to

the instrument. The photographs were taken using a JEOL-6390A scanning electron microscope.

IV. Zeta Potential and Zeta Sizer [10**]:**

Zeta Potential is a measure of the charge residing on the surface or near surface of a suspended particle. Zetatrac determines zeta potential by measuring the response of charged particles to an electric field. In a constant electric field particles drift at a constant velocity. Through the velocity, the charge and zeta potential are determined. Zetatrac utilizes a high frequency AC electric field to ascillates the charged particles. The Brownian motion power spectrum is analyzed with the nanotrac controlled reference technique of particle sizing to determine the Modulated Power Spectrum, a component of the power spectrum resulting from the oscillating particles. Zeta potential is calculated from the MPS signal.

V. Physical Parameter of Proniosomal Gel:

Proniosomes gel formulations were characterization for spreadability and homogeneity.

V.a. Spreadability:

It was determined by wooden block and glass slide apparatus. Weights of about 10g were added to the pan and the time was noted for upper slide (movable) to separate completely from the fixed slide. Spreadability was then calculated by using the formulation.

S = M . L / T

Where,

S = Spreadability

M = Weight tied to upper slide

L = Length of glass slide

T = Time taken to separate the slide completely from each other

V.b. Homogeneity:

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. Preparations were tested for their appearance and presence of any aggregates.

VI. *In-vitro* diffusion study [11**]:**

Invitro diffusion studies of proniosomal gel was carried out using a dialysis bag as a donar compartment. Proniosomal gel equivalent 200 mg of drug was taken in a dialysis membrane and placed in a beaker containing PBS (pH 7.4) of 100ml, which acted as the receptor compartment. Previously, the dialysis membrane was soaked in warm water for 10min and both ends were sealed with closure clips after adding the proniosomal preparation.

The beaker was placed over a magnetic stirrer (100rpm) and maintained at 37±1˚C. At predetermined time intervals during 24 hrs, aliquots (1ml) were withdrawn and replace with PBS (pH 7.4). The sink condition was maintained throughout the experiment. Samples withdrawn were suitably diluted and analyzed spectrophotometrically at 221.6 nm for Ketoconazole.

VI.1 Release Kinetics:

To analyse the mechanism for the release and release rate kinetics of the dosage form, the data obtained was fitted into zero order, first order, Higuchi matrix. By comparing the r values obtained, the best –fit model was selected.

(i) Zero Order Kinetics:

Drug dissolution from dosage forms that do not disaggregates and release the drug slowly, assuming that the area does not change and no equilibrium condition are obtained can be represented by the following equation.

Qt = Qo + Kot

Where,

Qt = Amount of drug dissolved in time t

Qo= Intial amount of drug in the solution

Kt= Zero order release constant

(ii) First Order Kinetics:

To study the first order rate kinetics the release rate data were fitted to the following equation.

Qt = Qo + K1 t/2.303

Where,

Qt= Amount of drug release in the time t.

Qo= Intial amount of drug in the solution.

K1 = First order release constant.

(iii) Higuchi Model:

Higuchi developed several theoretical models to study the release of water soluble and low soluble drug incorporated in semisolids and or solid matrics. Mathematical expression was obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media.

The Higuchi is

Qt = KH× t1/2

Where**,**

Qt = Amount of drug release in time t.

KH = Higuchi dissolution constant.

The results obtained from in-vitro drug release studies were shown in table adopting three different mathematical models of data treatment as follows:

• % Cum. Drug release vs Time (Zero order rate kinetics)

• % Cum. Drug retained vs. Time (First order rate kinetics)

• % Cum. Drug release was plotted against √T (root time) (Higuchi model)

VII. Stability Study [12**]:**

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under influence of variety of environmental factors such as temperature, light and to establish a retest period for drug substance or a shelf life for the drug product and recommended storage condition. The optimized formulation was tested for stability studies. Formulation was divided into 3 sample sets and stored at:

• 5°C ± 2°C in refrigerator.

• 25°C ± 2°C

• 40°C ± 2°C

The samples were withdrawn after 15, 30, 45 days tested for drug retained.

3. Results and Discussion

Preparation of Proniosomes by Slurry method:

Proniosomes of ketoconazole was successfully prepared by Slurry method. Proniosomes was converted into ketoconazole proniosomes gel. The different formulations were prepared using varying ratios of lipids Lecithin: Cholesterol, Surfactant:Lipid and Drug:Excipients (table 1,2,3). The best formulation was selected after the optimized characterization by % Entrapment Efficiency and Vesicle size formulations

Table 1: Optimization of Lecithin:Cholesterol Ratio for The Preparation of Proniosomes

Formulation Code	Lecithin : Cholesterol	% Entrappment Efficiency	Vesicle Size (µm)
L_1SD	8:1	80.4 ±2.3	7.8 ± 0.35
L_2SD	7:2	76.3 ±2.5	6.2 ±0.24
L_3SD	4:1	81.6 ±1.8	7.9 ±0.05
L_4SD	2:7	65.9 ±1.5	6.1 ±0.28
L_5SD	6:3	77.4 ±2.0	5.9 ±0.25
L_6SD	5:4	70.9 ±1.9	5.8 ±0.24

Values are expressed as Mean ± S.D.; n = 3

Table 2: Optimization of Surfactant:Lipid

Formulation Code	Surfactant : Lipid	% entrapment efficiency	Vesicle Size (µm)
$L_3S_1D_1$	4:1	95.4 ±2.0	8.5 ±0.40
$L_3S_1D_2$	7:3	85.6 ±2.8	7.3 ±0.32
$L_3S_1D_3$	3:2	83.5 ±2.2	7.3 ±0.31
$L_3S_1D_4$	2:3	88.6 ±2.1	7.3 ±0.30
$L_3S_1D_5$	3:7	75.3 ±1.8	6.6 ±0.25
$L_3S_1D_6$	1:4	60.5 ±1.5	6.1 ±0.27

Values are expressed as Mean ± S.D.; n = 3

Table 3: Optimization of Drug: Excipient (SPAN 60 and Cholesterol)

Formulation Code	Drug:Span60: Cholesterol:Lecithin	% entrapment efficiency	vesicle Size (µm)
$L_3S_1D_1$	1:50	96.5±2.3	8.6 ±0.40
$L_3S_1D_2$	2:100	85.4 ±1.8	8.4 ±0.38
$L_3S_1D_3$	3:150	81.2 ±1.6	8.2 ±0.35
$L_3S_1D_4$	4:200	79.5 ±1.2	7.4 ±0.31
$L_3S_1D_5$	5:250	75.2 ± 1.2	7.0 ±0.30
$L_3S_1D_6$	6:300	70.3 ±1.5	6.2 ±0.28

Values are expressed as Mean ± S.D.; n = 3

Table 4: *In-Vitro* Drug Release of Proniosomal Gel Formulation ($L_3S_1D_1$)

S. No	Time (min)	Cumulative drug release	% Cumulative drug release	Drug remaining	Sq. Root Time
0	0	0	0	0	0
1	30	8.396±0.6	8.396±0.6	91.61±2.5	5.477
2	60	25.387±1.2	25.387±1.2	74.62±2.8	7.745
3	90	35.217±1.7	35.217±1.7	64.79±1.2	9.486
4	120	55.325±2.7	55.325±2.7	44.68±2.2	10.954
5	150	65.231±1.2	65.231±1.2	34.77±1.7	12.247
6	180	75.321±2.7	75.321±2.7	24.68±1.2	13.416
7	210	80.231±2.0	80.231±2.0	19.77±0.9	14.491
8	240	86.298±2.5	86.298±2.5	13.71±0.6	15.491

Percentage Entrapment Efficiency and Vesicle Size:

The entrapment efficiency is maximum in Lecithin: Cholestrol ratio of optimized preparation L3SD i.e. 81.6±1.8 (table 1). The entrapment efficiency is maximum in Surfactant:Lipid ratio of optimized preparation L3S1D i.e. 95.4±2.0 (table 2). The entrapment efficiency is maximum in Drug:Excipient ratio optimized formulation L3S1D1 i.e. 96.5±2.3(table 3).

Vesicle size is large in Lecithin:Cholestrol ratio of optimized preparation L3SD i.e. 7.9±0.05.

Vesicle size in Surfactant: Lipid ratio of optimized preparation L3S1D i.e. 8.5±0.4.

Vesicle size in Drug:Excipient ratio optimized formulation L3S1D1 i.e. 8.6±0.4.

Microscopy:

In optical microscopy the photograph shows that proniosomes are unilamellar vesicles having spherical shape and no aggregation or agglomeration was observed optimized formulation L3S1D1 shown figure 1. In this formulation the vesicle size is large. In optical microscopy the photograph shows that proniosomes are unilamellar vesicles having spherical shape and no aggregation or agglomeration is observed shown in figure 2. But in this (L3S1D6) formulation the vesicle size is small.

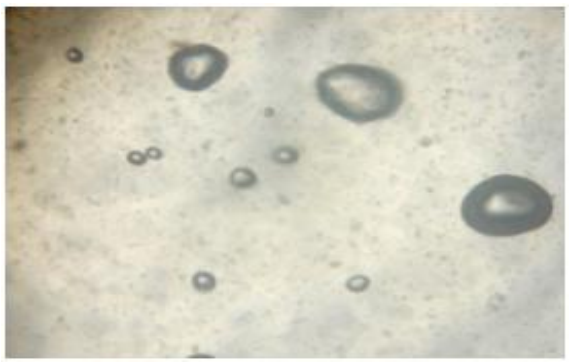


Figure 1: Optical Microscopy of Proniosome Gel (Ketoconazole) (L3S1D1)

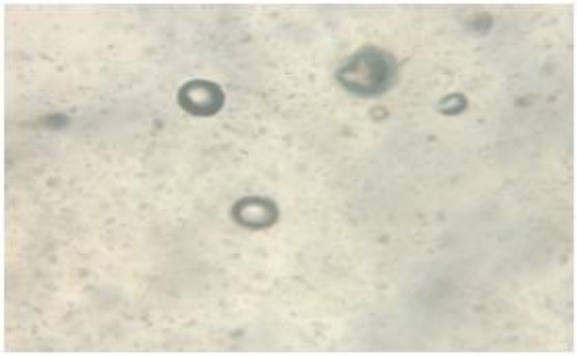


Figure 2: Optical Microscopy of Proniosome Gel (Ketoconazole) (L3S1D6)

Vesicle Morphology:

SEM image of proniosomes showed that most of vesicles are well identified, spherical and discrete with sharp boundaries having large internal aqueous space.

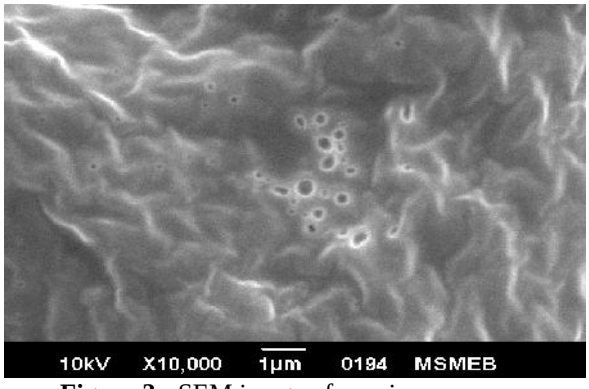


Figure 3: SEM image of proniosomes

Zeta potential and zeta size:

Zeta potential and zeta sizer are used for determining the colloidal properties of the prepared.The L3S1D1 formulation of zeta potential is -38.5 and zeta deviation is -5.16. Then the zeta potential result is good. The L3S1D1 formulation of zeta Sizer is z-averge is 3111 and diameter of peak1 is 2819. Zeta Potential and Zeta Sizer of L3S1D1 formulation which was found to have a better physical stability.

Spreadability:

The value of spreadibility of all proniosomal gel formulations ranged from 13.4 to 14.3 (g.cm/sec). The values of spreadability indicate that the gel is easily spreadable with minimal of shear. Spreadibility of proniosomal gel formula is good it is easily spreadable.

Homogeneity:

All developed gel showed good homogeneity with absence of lumps. All developed preparations were clear and transparent.

***In-vitro* release:**

The release study was conducted for L3S1D1 formulation. The formulation was found to provide 86.25% release with a period of 4 hours. The Higuchi describes the release from the system where solid drug is dispersed in insoluble matrix. Drug release is good in L3S1D1 formulations.

Stability Study:

Stability Studies were carried out after storing the selected formulation at 3 different temperature 5˚C ± 3˚C, 25˚C ± 2˚C and 40˚C ± 2˚C for 45 days. The vesicle size and % residual drug content was monitored every 15 days. Results showed that there were no significant changes observed in the vesicles size, drug retained of formulation at 5˚C ± 3˚C (table 5). It confirms that formulation $L_3S_1D_1$ was stable at the end of 45 days. On the other hand nominal change in formulation was observed in the vesicle size, % residual drug content diffusion after 45 days at 25˚C ± 2˚Cand 40˚C ± 2˚C temperature (table 6 and 7). Acceleration in drug leakage at higher temperature as observed in storage stability suggested keeping the proniosomal gel in the refrigeration condition.

Table 5: Data Showing Stability Studies of Proniosomal Gel ($L_3S_1D_1$) AT 5˚C ± 3˚C

Time (days)	Vesicle Size(µm)	(%) Residual Drug Content
0	5.6 ±0.28	100±1.5
15	5.5±0.27	100 ±0.02
30	5.5±0.27	99.99 ±1.54
45	5.4±0.21	99.99 ±2.0

Values expressed are Mean S.D., n = 2

Table 6: Data Showing Stability Studies of Proniosomal Gel ($L_3S_1D_1$) AT 25˚C ± 2˚C, 60 ± 5% RH.

Time (days)	Vesicle Size(µm)	(%) Residual Drug Content
0	5.6 ±0.28	100 ±1.5
15	5.6±0.28	99.9±1.0
30	5.7±0.29	99.9±1.5
45	5.7±0.29	99.8±2.0

Values expressed are Mean S.D., n=2

Table 7: Data Showing Stability Studies of Proniosomal Gel ($L_3S_1D_1$) AT 40˚C ± 2˚C, 75 ± 5% RH.

Time (days)	Vesicle Size(µm)	(%) Residual Drug Content
0	5.6 ±0.28	100.00±1.4
15	5.7±0.29	99.8±1.8
30	5.7±0.29	99.7±1.1
45	5.8±0.30	99.3±2.0

Values expressed are Mean±S.D., n=2

4. Conclusion

A successful attempt was made to develop proniosomal gel for topical delivery of ketoconazole using different ratio of span and cholesterol concentration and evaluated for different in-vitro and skin irritancy test. From the results obtained it can be concluded that, In stability studies L3S1D1 is observed in storage stability studies suggested keeping the proniosmal gel in the refrigeration condition. Proniosomes are dry formulations of surfactant coated carrier, which can be measured out as needed and rehydrated by brief agitation in hot water. These "proniosomes" minimize problems of niosomes physical stability such as aggregation, fusion and leaking while providing additional convenience in transportation, distribution, storage and dosing. Proniosome derived niosomes are superior to conventional niosomes in convenience of storage, transport and dosing. Stability of dry proniosomes is expected to be more stable than enhanced as compared to pre-manufactured niosomal formulation. Proniosomes gel of ketoconazole for the attainment of better therapeutics in candidiasis. The drug is 99% protein bound moreover, it is hepatotoxic hence a cutaneous/topical delivery is more beneficial for the attainment of better therapeutic results.

5. References

1. Hatakka K, Ahola AJ, Yliknuuttila, Korpel R, Probiotics reduces of prevalences of oral candida in the elderly-a randomized controlled trail, J Dent Res., **2007**, 86(2): 30-125.
2. Santos VR, Pimenta FJ, Ageiar MC, Oral candidiasis treatment with ethanol propolis extract, J Dent. Res., **2005**, 19(7): 4-654.
3. Viviane F, Proniosomes as a stable carrier for oral Acyclovir: Formulation and Physiochemical Characterization, Journal of America Science, **2012**, 8(9): 417-425.
4. Banker GBS, Rodes CT, Marcel Dekker, Modern Pharmacist, New York, 2nd: vol 40, **1979**; 263-311.
5. Goud B.Agaiah, Formulation and Evaluation of Megesterol Proniosomal Systems, International Journal of Pharmacy and Biological Sciences, **2012**, 2(2): 67-76.
6. Chandra A, Sharma PK, Proniosome based drug delivery system of Piroxicam, African J of Pharma. And Pharmaco, **2008**, 2(9): 184-190.
7. Almira I. Blazek-Welsh1 and Rhodes DavidG, Maltodextrin-Based Proniosomes, AAPS Pharma Sci. Tech., **2001**, 18(5): 656-661.
8. Alsarra I.A, Bosela A.A.S., Mahrous, Proniosomes as a drug carrier for transdermal delivery of Ketorolac, Eur. J. Pharma. Biopharma, **2005**, 59: 485-490.
9. Hu C and Rhodes D.G, Proniosomes: a novel drug carrier Preparation, Int. J.Pharma., **2000**. 206(1): 22-110.
10. Gupta A, Prajapati SK, Balamurugan M, Singh M and Bhatia D, Transdermal drug delivery system for Captopril, Trop. J Pharma Res., **2007**, 6(2): 93-687.
11. Escarbibiano .E, Calpeno AC, Queralt J, Obach R, Domenech J, Assessment of Diclofenac Permeation with different formulations : Anti-inflammantory study of a selected formula, Eur. J. Pharma Sci., **2003**, 19(3): 10-203.
12. Ammar H.O., M. Ghorab, S.A. El-Nahhas, I.M. Higazy, Proniosomes as a carrier system for transdermal delivery of tenoxicam, International Journal of Pharmaceutics, **2011**, 1(2): 142-152.