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ORIGINAL ARTICLE

Evaluation of the Antimycotic activity of Terconazole proniosomal Gel

[1]Rehab Bahy[*], [2]Doaa A. Helal

[1] Department of Microbiology and Immunology, Faculty of Pharmacy, Fayoum University, Fayoum, Egypt.
ORCID ID: 0000-0002-2868-928X

[2] Department of pharmaceutics, Faculty of Pharmacy, Fayoum University, Fayoum, Egypt
ORCID ID. 0000-0002-1222-8112

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Corresponding Author:
Rehab Bahy
Department of Microbiology and Immunology, Faculty of Pharmacy, Fayoum University, Fayoum, Egypt.
ORCID ID:
0000-0002-2868-928X
Tel.: 01144738122
rhb11@fayoum.edu.eg

ABSTRACT

Background: *Terconazole (TCZ) counted is an antifungal drug with a broad spectrum of activity against Candida species. TCZ has been shown to be more active than imidazoles.* ***Objectives:*** *The aim of this research was to assure the potential of proniosomalgelfor the topical delivery of terconazole, and to detect its MIC against Candida albicans comparing it with the reported MIC of terconazole cream 0.8%.* ***Methodology:*** *The coacervation phase separation process was used to develop Terconazole-loaded proniosomal gels. The particle size (PS) and entrapment efficiency percentage of the produced proniosomal gels were assessed and in-vitro drug release. The particle size varies from (17.06–to 48.1 nm), range of EE% is (83.41%–95.66%). F4 (span 60, tween 80 (1:1), and cholesterol) have the highest EE% (95.66%), was tested by Transmission electron microscopy (TEM) and a zeta potential (ZP) test were performed.* ***Results:*** *The outcomes revealed a nanosized spherical vesicleofterconazole. Proniosomal gel MIC was (32µg/ml) lower than the reported MIC of market terconazole cream 0.8% (128µg/ml).* ***Conclusion:*** *Proniosomal gel achieved the desired sustained topical effect.There was no significant change in EE percent, PS, or ZP in physical stability investigation.*

INTRODUCTION

Candidiasis is a mycotic infection induced by *Candida albicans*, the most serious public health threat of all mycoses now. The vaginal mucosa, skin, oropharynx, bronchi, lungs, and gastrointestinal tract are the most typically affected areas. Candidiasis comes in acute and chronic forms, and it can be superficial or severe. Some antifungal agents, particularly those used against Candida albicans, are ineffective against some fungus species. Inability to respond to antimycotic therapy could be induced by the strains intrinsic resistance or acquired during treatment. Developing an antimycoticdrug with lowest possible Candida resistance, or perhaps designing a controlled release approach capable of boosting the therapeutic efficiency of antifungal drugs, is one of current challenges[1-3].

Terconazole (TCZ) counted as a triazole antifungal drug with a broad spectrum of activity[4]. TCZ has been discovered to be one of the most effective azoles against Candida species[5], and its principal mechanism of action was similar to that of imidazoles, in that it inhibits the fungus cytochrome P-450. TCZ, on the other hand, has been shown to be more active than imidazoles[6].

Hence TCZ is weakly soluble in water and to improve the efficacy of poorly soluble medicines, various ways were used to integrate them into drug delivery systems. Poorly soluble medicines may be embedded into lipid carriers with nanometric dimensions to improve their solubility and bioavailability[7]. TCZ has been encapsulated in a several drug delivery systems, including proniosomal gel bilosomes, and self-nanoemulsifying systems, overcoming its low solubility[8].

Vesicleofterconazoles such asniosomes, liposomes, and proniosomes have diverse compared to conventional dosage forms, there are a number of advantages. Vesicles can entrap both hydrophilic as well as lipophilic medicines in the aqueous layer or in the or inner lipid membrane[1-3]. Proniosomalare structurally analog to the bilayer liposome and noisomebut more stable[9]. Niosomes' physical stability difficulties are reduced, and storage, transportation, distribution, and handling are easily accomplished.[10] The aim of this work is to assure the potential of proniosomal gel for the topical delivery of terconazole, and to detect its MIC against *Candida albicans* comparing it with the reported MIC of terconazole cream 0.8%.

METHODOLOGY

Materials:

Terconazole was supplied as a free sample from Pharco, Egypt. Sorbitan monoesters (Span 20, 60) and Polysorbates (Tween 80), and Cholesterol were

kindly supplied by the Egyptian (EPICO), Egypt. All additional chemicals were purchased from El-Nasr Company Co. Egypt, and were of analytical quality.

Preparationof terconazoleproniosomal gel

The method of coacervation phase separation was used for preparation of proniosomal formula using different nonionic surfactants with different ratios of the surfactant: cholesterol[7]. Different formulations with different compositions were shown in (Table 1). The surfactant and cholesterol were combined with 0.5 mL absolute ethanol in a beaker, and then **0.8%** of Terconazole was added. The beaker was then covered to prevent solvent loss and heated in a water bath (55°C–60°C) about 5 minutes while shaking until the solvent was completely dissolved.0.15 ml of distilled water (55°C–60°C) was applied and allowed to warm in the water bath for 5 minutes, yielding a translucent solution [8]. The dispersion was turned to gel by allowing the fluid to cool.

Table1: Composition of different terconazole proniosomal formulations using different nonionic surfactants with different ratios of surfactant: cholesterol.

Code	Span20 (%)	Span60 (%)	Tween80 (%)	Cholesterol (%)
F1	90	-	-	10
F2	-	90	-	10
F3	45	-	45	10
F4	-	45	45	10
F5	70	-	-	30
F6	-	70	-	30
F7	35	-	35	30
F8	-	35	35	30
F9	50	-	-	50
F10	-	50	-	50
F11	25	-	25	50
F12	-	25	25	50

Each formula contains 0.8% terconazole.

Evaluation of the prepared Proniosomal gels formulations:

pH measurement:

A digital pH meter was used to determine gel pH of (3310, Jenway, UK). After dissolving 0.1g of gel in 10ml of distilled water, the electrode was dipped into the gel and reading was taken.[11]. Triplicate determinations for each formulation were done, mean ±SD was deduced [8, 12].

Particlesize (PS), Polydispersity Index (PDI), and Zeta potential analysis (ζ) of terconazole proniosomes:

Theparticlesize (PS), Polydispersity Index (PDI), andsurface charge (ZP)of proniosomes were determined using dynamic light scattering with Malvern Zetasizer (Malvern Instrument Ltd, UK). Distilled water was used to dilute the proniosomal gel (1:2500) (v/v) before measurements[9]. Polydispersity index (PDI) was identified for assessing size of particle size distribution and the homogeneity of the proniosomal vesicles. Zeta potential was also determined to confirm the stability of proniosomes[13]. Each investigation was resolved in triplicate.

***Determination of entrapment efficiency (EE%)*:**

In glass tube, 0.5 g of gel was placed, along with 10 ml phosphate buffer (pH 5.5). In a sonicator, this aqueous solution was sonicated, followed by centrifugation at 15.000 rpm at 20°C for 30 min (Ultracentrifuge, Eppendorf, Germany). Supernatant was collected and tested for unentrapped terconazole content particles using an ultraviolet (UV) spectrophotometer (Shimadzu UV spectrophotometer, Japan) at λ_{max} 227 nm[2, 13].

$$\mathbf{EE\%} = \frac{\mathbf{Totalamountofdrug - Unentrappeddrug}}{\mathbf{Totalamount\ of\ drug}} \boldsymbol{x}\ \mathbf{100}$$

In-Vitro drug release study:

Membrane diffusion technique was used to measure the release of terconazole from various proniosomal gels. The 20 mg of terconazole proniosomal gel was kept in a glass tube with a diameter of 2.5 cm and length of 10 cm that had previously been covered with a presoaked cellulose membrane has a molecular weight cutoff of 12,000Daltons.The lower end of the gel tube has just brushed the surface of the diffusion medium, which is 100 ml phosphate buffer (pH 5.5) at 37°C with a magnetic stirrer agitated at 100 rpm. Aliquots of three-milliliter sample were taken at a different time interval (0, 0.5, 1, 2, 4, 6, 8 and 12 h) and replaced with equal volume to keep the constant volume of the receptor's phase. Using a UV spectrophotometer, the drug concentration in the collected samples was determined at max 227 nm. The formulas with the highest EE percent and the maximum in vitro drug release were candidates for further investigation.

Transmission electron microscopy (TEM)

The optimized formula will be visualized using a transmission electron microscope (TEM, Jeol – JXA-840A – Electron Microscope – Japan). Prior to analysis, the sample was diluted with dist. water 10 times. A drop of resulting suspension was dropped on a copper grid that had been film-coated, generating a thin liquid film. Which were then stained negatively using a phosphotungstic acid solution containing 2% (w/v) phosphotungstic acid. After air drying, stained films were photographed by transmission electron microscopy.

Stability studies

Physical stability for the optimised formula was tested by storing it in well-closed bottles in the refrigerator three months at 4°C1°C. Physical appearance, percent EE, PS, and ZP were all considered in the stability study [6].

Percentages of EE, PS, and ZP were tracked versus storage time.

In vitro evaluating antifungal activity

The antimycotic activity was investigated for the selected formulation, which demonstrated the highest EE percent and extended drug release, was evaluated using *Candida albicans* strain ATCC 10231.

Preparation of standard inoculums

The yeast from a 24-hour culture on Sabouraud dextrose agar was used to make the inoculum, which was suspended in a sterile saline solution (0.85 percent). Using a spectrophotometer set to 530nm, the turbidity of the culture was adjusted to match the final concentration of a 0.5 McFarland standard ($0.5\text{-}2.510^{3}$). At 37°C, the bacteria were grown in nutritional agar broth.

Preparation of drug dilutions

The chosen formula was diluted with the blank solution of saline solution (0.85%) to meet the final concentration of 256, 128, 64, 32, 16, 8, and 4 µg/ml. Normal saline was used as a blank.

Determination of Minimum Inhibitory Concentration (MIC) using Agar Well diffusion method

The MIC of Terconazole proniosomal gel against *C. albicans* strain were determined by the agar well diffusion method where20 ml of Sabouraud dextrose agar were melted, cooled to 55°C and than inoculated with 1ml of the organism suspension. The inoculated agar was poured into a 15 cm diameter assay plate and set aside to cool on a flat surface. Eight wells, each 10 mm diameter, were cut out of the agar once the media had solidified. and 200 µl of each seven drug concentrations and blank solution were placed into each well, three plates were used for triplicate repetition and incubated at 35°C for 24 hours[10], results were analyzed using the same criteria for fluconazole[14] NCCLS disc diffusion breakpoints were used for the interpretative breakpoints[15].

Statistical analysis:

One-way analysis of variances was used to establish significance of difference between groups in statistical analysis; P 0.05 was considered as statistically significant.

RESULTS

As indicated in Table 2, pH of proniosome formulations was within the physiological range of topical administration, ranging from 5.5 to 6.9.

Table2: Physical appearance, pH, particle size (PS), polydispersity index (PDI), Zeta potential analysis (ζ) and the entrapment efficiency percentage of terconazolein different proniosomal gels.

Code	Physical appearance	pH	Particle Size±SD (nm)	PDI±SD	Zeta potential analysis (ζ)	EE%±SD
F1	Viscousgel	5.5	33.47±1.07	0.23±0.023	-22.1	89.64±0.98
F2	Whitecreamy gels	5.6	19.80±1.10	0.10±0.003	-24	91.37±1.09
F3	Paleyellowgels	6.2	30.10±1.63	0.27±0.150	-19.6	85.43±1.99
F4	Whitecreamy gels	6.0	23.41±0.51	0.16±0.050	-30	95.66±1.24
F5	Pale yellow viscous gel	5.6	31.20±0.67	0.22±0.055	-21.6	87.56±1.94
F6	Whitecreamy gels	6.2	20.40±0.94	0.20±0.013	-23.9	88.24±1.02
F7	Pale yellow viscous gel	6.1	30.52±0.57	0.13±0.021	-22.9	90.21±1.32
F8	Whitecreamy gels	5.5	28.45±0.74	0.26±0.028	-27.3	83.14±1.07
F9	Pale yellow viscous gel	5.9	33.54±0.41	0.22±0.019	-20.6	87.74±1.54
F10	Whitecreamy gels	5.9	29.20±0.67	0.28±0.150	-23.6	86.24±1.02
F11	Paleyellowviscousgel	6.9	30.40±0.94	0.17±0.060	-23.9	88.21±1.32
F12	Whitecreamy gels	6.4	32.52±0.57	0.23±0.053	-21.9	81.14±1.07

*SD: Standard deviation

The Particle size of the prepared pronisomal gel was found to be in the nanosize range (19.80–33.54nm). The vesicles were distinct and separated without aggregation [Figure2]. It was observed that vesicles prepared of span 60 show the smallest size than vesicle prepared of span 20.

The entrapment efficiency percent of terconazole formulations are clarified in Table 2. F4 (span 60: tween 80: cholesterol) and F2 (span 60: cholesterol) resulted in the highest entrapment efficiency(95.66% and 91.37%, respectively).

Figure 1 showed the rate of terconazole release from certain proniosomal gel having EE% greater than 88% (F4, F2, F7, F1, F6, and F11: 95.66, 91.37, 90.21, 89.64, 88.24, and 88.21% respectively). At P< 0.05, the proniosomal gel formulation (F4) had the highest EE percent and the longest drug release, making it the best formula. It's possible that the slower release of terconazole from proniosomal prepared formulations

compared to a manufactured control gel (1.5 percent HPMC containing 0.8 percent w/w terconazole) is due to drug encapsulation in vesicles, which allows for a longer drug release rate. The proniosomal gel (F4) that was selected revealed. At P 0.001, the selected proniosomal gel (F4) demonstrated a significantly longer drug release of 50.2 percent 5.25 percent after 6 hours, compared to 95.11 percent 2.11 percent for the control.

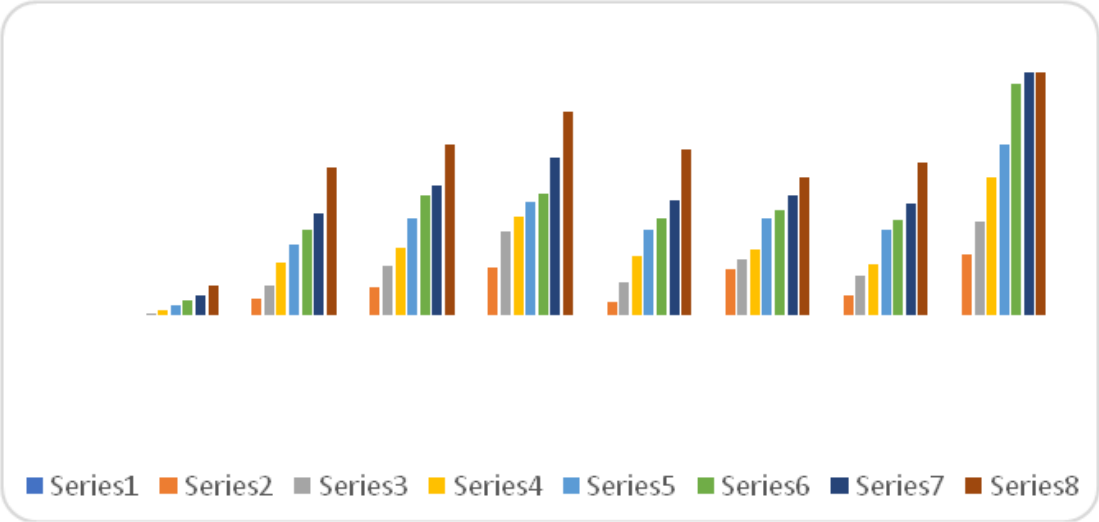

Fig. 1: *In vitro* terconazole release from proniosomal gel

Figure 2 showed that the optimized proniosomal formula (F4) was nanosized, smooth, nanovesicles with sharp edges and homogeneous nanovesicles

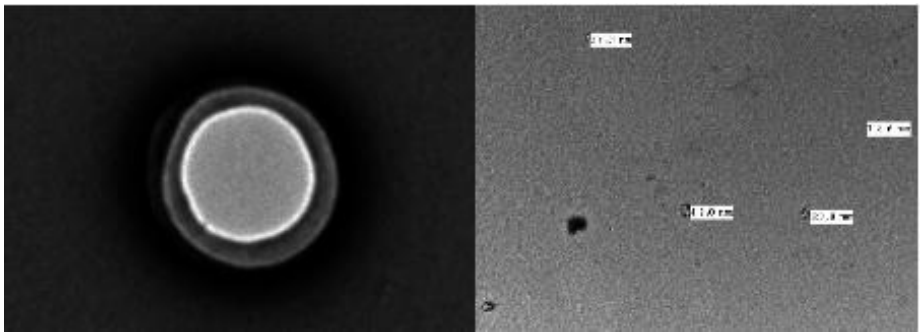

Fig. 2: Transmission electron micrograph of TERCONAZOLE-loaded proniosome (F4)

The selected optimum formulation was subjected to a stability test. It was kept at 4°C 1°C for 3 months and the PS of the vesicles, ZP, and percent EE were measured.

The physical look remained unchanged. Furthermore, the EE percent, PS, and ZP of the terconazole proniosomal gel did not alter significantly at P > 0.05 [Table 3].

Table 3: Particle size, zeta potential, and percentage entrapment efficiency determined for the selected terconazole proniosome formulation when fresh and after storage in the refrigerator for 3 months at 4°C

Form	PS (nm)±SD			ZP (ζ)			EE% ± SD		
	Initial	At1 Month	At3 Months	Initial	At1 Month	At3 Months	Initial	At1 Month	At3 Months
F4	23.41 ±0.51	24.51 ±1.51	26.14 ±1.64	−30.9	−30.9	−29.45	95.66 ±1.24	94.4 ±0.95	93.05 ±1.12

The susceptibility results were analyzed for the proniosomal formula to detect its MIC using the same criteria for fluconazole [16], the diameters of the clear zone of inhibition of growth were illustrated in table (4) and interpretation of results according to NCCLS criteria (document M60), where zone of inhibition ≥19 mm is susceptible, 18—13 mm susceptible dose dependent and ≤12 is resistance.

Table 4: Correlation between drug concentration and diameter of inhibition zone

Drug concentration µg/ml	**256**	**128**	**64**	**32**	**16**	**8**	**4**
Diameter of clear zone – diameter of blank mm	28	26	25	20	15	13	10
Interpretation	S	S	S	S	SDD	SDD	R

DISCUSSION

Spans are lipophilic surfactant, it forms smaller vesicles to minimize the surface free energy [17]. By increasing ZP the vesicle repulsion has improved, preventing reaggregation and ensuring the system's electrical stability.

Span60 has the greatest phase transition temp (50°C) and entrapment effectiveness percent[2,18]. Hydration temperature which used to form niosomes should normally be higher than the system's gel–to–liquid phase transition temperature, resulting in less leaky niosomes with high entrapment efficiency. The lower the surfactant's HLB, meaning better drug entrapment efficiency also stability, as seen in proniosomes made using span 60.[19].

The vesicular structure, which is made up of span 60, tween 80, and cholesterol and is thought to represent a significant barrier to drug diffusion and release. The period required for the hydration of proniosomes into niosome vesicles before initiating the release of the medication over the cellophane membrane is referred to as the delayed release of proniosomal gel formulations[20].

Because of its high phase transition temperature and low permeability, the non-significant change in size and EE percent in the span 60 surfactant-based formulation can be explained[21]. This research demonstrated proniosomal gel's exceptional stability and appropriateness for topical terconazole administration.

The MIC of proniosomal Terconazole against *C. albicans* was found to be 32 µg/ml which considered to be equivalent to predetermined and published data of Terconazole Cream 0.8% which had MIC of 128 µg/ml against *C. albicans*[9].

CONCLUSION

The coacervation phase separation approach would be used to successfully prepare a terconazole-loaded proniosomal gel. The entrapment effectiveness of terconazole in the produced proniosomal gel ranged from 95.6 to 83.1 and PSs were in the nanosize range (19.80–33.54nm). TEM reveals the formation of smooth and spherical vesicles after hydration.

The most acceptable surfactant mixtures for the manufacture of proniosomes were discovered to be F4, which contains a 1:1 mixture of span 60 and tween 80. In vitro release experiments revealed that the manufactured proniosomal gel contains terconazole, which is an effective topical drug delivery technology that delivers encapsulated medication over time. Furthermore, following storage for for 3 months, there was no significant change in the EE percent, PS, or ZP of terconazole proniosomal gel. The MIC of proniosomal gel preparation (32µg/ml) was lower than the reported MIC of market terconazole cream 0.8% (128 µg/ml).

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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REFERENCES

1. Teaima MH, Abdelhalim SA, El-Nabarawi MA., et al, Non-ionic surfactant based vesicular drug delivery system for topical delivery of caffeine for

treatment of cellulite: design, formulation, characterization, histological anti-cellulite activity, and pharmacokinetic evaluation. Drug Dev Ind Pharm, 2018. 44(1): p. 158-171.

2. Helal DA, Teaima MH, Abd El-Rhman D, et al, Preparation and Evaluation of Niosomes Containing an AntiCellulite Drug. Inventi Rapid: Pharm Tech 2015(2).
3. Mainardes RM, Urban MC, Cinto PO, et al, Colloidal carriers for ophthalmic drug delivery. Curr Drug Targets, 2005. 6(3): p. 363-71.
4. Blazek-Welsh AI and Rhodes DG, Maltodextrin-based proniosomes. AAPS PharmSci, 2001. 3(1): p. E1.
5. Gupta A, Prajapati SK, Balamurugan M, et al, Design and development of a proniosomal transdermal drug delivery system for captopril. Trop J Pharm Res 2007. 6: p. 687-693.
6. Moustafa MA, El-Refaie WM, Elnaggar YSR, et al, Gel in core carbosomes as novel ophthalmic vehicles with enhanced corneal permeation and residence. Int J Pharm, 2018. 546(1-2): p. 166-175.
7. Mokhtar M, Sammour OA, Hammad MA, et al, Effect of some formulation parameters on flurbiprofen encapsulation and release rates of niosomes prepared from proniosomes. Int J Pharm, 2008. 361(1-2): p. 104-11.
8. El-Nabarawy NA, Teaima MH, and Helal DA, Assessment of Spanlastic Vesicles Of Zolmitriptan For Treating Migraine In Rats. Drug Des Devel Ther, 2019. 13: p. 3929-3937.
9. Teaima MH, Yasser M, El-Nabarawi MA, et al, Proniosomal Telmisartan Tablets: Formulation, in vitro Evaluation and in vivo Comparative Pharmacokinetic Study in Rabbits. Drug Des Devel Ther, 2020. 14: p. 1319-1331.
10. Magaldi S, Mata-Essayag S, Hartung de Capriles C, et al, Well diffusion for antifungal susceptibility testing. Int J Infect Dis, 2004. 8(1): p. 39-45.
11. Rao M, Kadam M, Rao S., Formulation and evaluation of topical formulation for cutaneous tuberculosis. J Drug Deliv Ther, 2018. 8: p. 102-116.
12. Abdel-Rashid RS, Helal DA, Omar MM, et al, Nanogel loaded with surfactant based nanovesicles for enhanced ocular delivery of acetazolamide. Int J Nanomedicine, 2019. 14: p. 2973-2983.
13. Abdel-Rashid RS, Helal DA, Alaa-Eldin AA, et al, Polymeric versus lipid nanocapsules for miconazole nitrate enhanced topical delivery: in vitro and ex vivo evaluation. Drug Deliv, 2022. 29(1): p. 294-304.
14. Tulin EE, Ecleo ZT. Cytokine-mimetic properties of some Philippine food and medicinal plants. J Med Food, 2007. 10(2): p. 290-9.
15. Clinical and Laboratory Standards Institute, C, CLSI supplement M60, in Performance standards for antifungal susceptibility testing of yeasts2017, Wayne PA.
16. Li T, Zhu Y, Fan S, et al, A randomized clinical trial of the efficacy and safety of terconazole vaginal suppository versus oral fluconazole for treating severe vulvovaginal candidiasis. Med Mycol, 2015. 53(5): p. 455-61.
17. Alsarra IA, Bosela AA, Ahmed SM, et al, Proniosomes as a drug carrier for transdermal delivery of ketorolac. Eur J Pharm Biopharm, 2005. 59(3): p. 485-90.
18. Kibbe AH, Handbook of Pharmaceutical Excepients, ed. 32000, Washington,DC: AmericanPharmaceutical Association.
19. Guinedi AS, Mortada ND, Mansour S, et al, Preparation and evaluation of reverse-phase evaporation and multilamellar niosomes as ophthalmic carriers of acetazolamide. Int J Pharm, 2005. 306(1-2): p. 71-82.
20. Thakur R, Anwer MK, Shams MS, et al, Proniosomal transdermal therapeutic system of losartan potassium: development and pharmacokinetic evaluation. J Drug Target, 2009. 17(6): p. 442-9.
21. Sandeep G, VasaviReddy D, Devireddy SR, Formulation and evaluation of terconazolepro-niosomal gel for topical administration. J Appl Pharm Sci, 2014. 4: p. 98-104.