Research Article

Formulation and characterization of drug loaded niosomes for antifungal activity

S. B. Shirsand[1], G. Ganesh Keshavshetti[2]

[1]Department of Pharmaceutical Technology, HKE's Matoshree Taradevi Rampure Institute of Pharmaceutical Sciences, Gulbarga, Karnataka, India,
[2]Department of Pharmaceutics, SVET's College of Pharmacy, Humnabad, Bidar, Karnataka, India

Correspondence: G. Ganesh Keshavshetti, Department of Pharmaceutics, SVET's College of Pharmacy, Humnabad - 585 330, Bidar, Karnataka, India.
Phone: +91-7588200501. E-mail: gkeshavshetti@yahoo.co.in

ABSTRACT

The aim of this work was to prepare and evaluate drug loaded niosomes for antifungal activity. In this study, niosomes containing ciclopirox olamine were prepared by ether injection technique using nonionic surfactant (Span 40 and Span 60) and cholesterol at different concentrations. The prepared formulations were evaluated for optical microscopy, drug entrapment efficiency, drug content, in-vitro drug release study and stability studies. Among the ten formulations, formulations CNS41 to CNS45 (containing span 40) and formulation CNS61 (containing span 60) showed good drug release. CNS61 have shown higher entrapment efficiency as compared to formulations prepared by span 40. So, after considering all these parameters, formulation CNS61 was found to be the best formulation having vesicle size 8.81 µm, entrapment efficiency of 71.45±0.44 and drug release of 55.345±1.009 at the end of 24 hrs. The present study indicated that all the formulations prepared by ether injection method using non-ionic surfactant are showed sustain drug release rate for 24 h. Slope values of peppas log–log plots are between 0.634 to 0.763 suggesting that the drug release by non-fickian release mechanism.

Keywords: Niosomes, antifungal, ciclopiroxolamine

Introduction

Fungal disease is ubiquitous in the world and antifungal medication accounts for sales of more than US$ 1 billion annually. The most fungal disorder is relatively benign but can become life threatening in immunocompromised or malnourished population. The mainstay of management of fungal infection and dermatophytes associated with skin and nail injuries has been oral and topical antifungals.[1] Most antifungal drug substances are lipophilic compounds, which are practically insoluble in water. A number of strategies to deliver antifungal using nanocarriers are developed to facilitate drug targeting infected cells that result in improved antifungal activity.[2]

Niosomes are vesicular, microscopic lamellar structures analogous to liposomes, made of non-ionic surfactants that self-assemble in aqueous media, yielding closed bilayers.[3] Due to the presence of can accommodate drug molecules with a wide range of solubility, thus moreover surfactants contribute to the overall penetration enhancement of compounds primarily by adsorption at interfaces, by interacting with biological membranes and by alteration of the barrier function of the stratum corneum, as a result of reversible lipid modification.[4]

Ciclopirox olamine (CPO), a broad-spectrum antifungal, is a hydroxypyridone derivative that has mechanism of action different from other marketed antifungal agents such as the azoles and the allylamines. It has a broad spectrum of action against dermatophytes, yeasts, filamentous fungi, and bacteria. It has a biological half-life of 1.7 h and bioavailability of <5% with prolonged use. A remarkable feature of the drug is that no single case of fungal resistance has been reported so far.[5]

Therefore, the present work is an attempt toward the development of niosomal gel delivery systems for CPO. The encapsulation of antifungal drug in niosomes increases the half-life, providing prolonged drug delivery and minimizes the side effects, as well as drug accumulation, will be high after incorporation.

Materials and Methods

CPO was received as a gift sample from Kumar Organic Products Ltd., Bengaluru, Karnataka, India. All the other reagents were

Access this article online

Website: sjandd.sperpublications.com | E-ISSN: ***

How to cite this article: Shirsand SB, Keshavshetti GG. Formulation and characterization of drug loaded niosomes for antifungal activity. SPER J Adv Nov Drug Deliv 2016;1(1):12-17.

Source of Support: Nil, **Conflict of Interest:** None declared.

of analytical grade and were purchased from Sigma. Dialysis membrane-70 (2229.31 mm Avg. flat width) was purchased from HiMedia Laboratories Pvt. Ltd., Mumbai. All other chemicals were of analytical grade and procured from the authentic sources.

Preliminary identification tests for CPO

CPO was identified (by preliminary tests such as melting point and solubility test) and was checked for compatibility of the drug with non-ionic surfactants (Span 40 and Span 60) by Fourier transform infrared (FTIR) study.

Identifications tests

a. Preliminary tests: In the preliminary tests the state, color, odor, ignition test and Bayer's test were performed to identify the drug. The appearance of drug was observed visually
b. Melting point: Melting point of CPO was determined using Thiele Tube's method[6]
c. *Solubility studies:* Solubility measurements were performed according to the method of Higuchi and Connors. In brief, solubility studies of CPO were carried out in water and methanol. Saturated solutions were prepared by adding an excess drug to the vehicles and shaking on the shaker for 48 h at 25 ± 0.5°C under constant vibration. After this period the solutions were filtered, diluted and analyzed by ultraviolet (UV)-spectrophotometer.[7]
d. FTIR spectroscopy study: IR spectrum of CPO was determined on FTIR spectrophotometer using potassium bromide dispersion method. The baseline correction was done using dried potassium bromide. Then, the spectrum of mixture of drug and potassium bromide was run followed by drug with excipients in the wavelength region between 4000 and 400/cm.

The primary objective of this investigation was to identify a stable storage condition for drug in solid state and identification of compatible excipients for formulation. To analyze the compatibility between drug and excipients, proposed to incorporate into the formulation. IR studies were carried out taking the physical mixture of drug and other excipients as a test sample.

Preparation of CPO loaded niosomes by ether injection method

Niosomes containing CPO were prepared by ether injection technique using nonionic surfactant (Span 40 and Span 60) and cholesterol at different concentrations given in Table 1. Cholesterol and surfactant were dissolved in 5 ml of chloroform. Accurately weighed (100 mg) of CPO was dissolved in the above lipid solution. The resulting solution was slowly injected at a rate of 1 ml/min into 20 ml of hydrating solution phosphate-buffered saline (PBS) pH 7.4. The solution was stirred continuously on magnetic stirrer, and temperature was maintained at 55-65°C. As the lipid solution was injected slowly into aqueous phase, the differences in temperature between phases cause rapid vaporization of chloroform, resulting in spontaneous vesiculation and formation of niosomes.[8,9]

Evaluation of CPO niosomes

Morphological characterization

Optical microscopy

The vesicle formation was confirmed by optical microscopy in ×45 resolution. The niosome suspension placed over a glass slide and fixed over by drying at room temperature, the dry thin film of niosome suspension observed for the formation of vesicles.[10]

Entrapment efficiency[11,12]

Entrapment efficiency of niosomes was determined by exhaustive dialysis method. The measured quantity of niosomal suspension was taken into a dialysis tube to which dialysis membrane was securely attached on one side. The dialysis tube was suspended in 100 ml PBS pH 7.4 containing 10% v/v methanol, which was stirred on a magnetic stirrer. The unentrapped drug was separated from the niosomal suspension into the medium through membrane. At every hour entire medium (100 ml) was replaced with fresh medium (for about 6-7 h) till the absorbance reached a constant reading indicating no drug is available in un-entrapped form. The withdrawn samples were checked for absorbance at 306.20 nm. Amount of entrapped drug was obtained by subtracting amount of unentrapped drug from the total drug incorporated.

Table 1: Formulations of CPO niosomes prepared by ether injection method

Ingredients	Formulation code									
	$CNS4_1$	$CNS4_2$	$CNS4_3$	$CNS4_4$	$CNS4_5$	$CNS6_1$	$CNS6_2$	$CNS6_3$	$CNS6_4$	$CNS6_5$
Ratios (Drug:surfactant:cholesterol)	1:1:0.2	1:1.5:0.3	1:2:0.4	1:1:0.8	1:1:1	1:1:0.2	1:1.5:0.3	1:2:0.4	1:1:0.8	1:1:1
CPO (mg)	100	100	100	100	100	100	100	100	100	100
Span 40 (mg)	100	150	200	100	100	-	-	-	-	-
Span 60 (mg)	-	-	-	-	-	100	150	200	100	100
Cholesterol (mg)	20	30	40	80	100	20	30	40	80	20
Chloroform (ml)	5	5	5	5	5	5	5	5	5	5
PBS (pH 7.4) (ml)	20	20	20	20	20	20	20	20	20	20

CNS4: Ciclopirox olamine niosomes containing span 40, CNS6: Indicates ciclopirox olamine niosomescontaining span 60, CPO: Ciclopirox olamine, PBS: Phosphate-buffered saline

$$\text{Percent entrapment} = \frac{\text{Total drug} - \text{Diffused drug}}{\text{Total drug}} \times 100$$

Drug content[13,14]

Niosomes preparation equivalent to 1 mg of CPO (drug) was taken into a standard volumetric flask. Then, they were lysed with 50% n-propanol by shaking. Then, 1 ml of this was subsequently diluted with PBS (pH 7.4). The absorbance was measured at 301.0 nm and calculated drug content from calibration curve.

In-vitro drug release study[15,16]

The release of CPO from niosomal formulations was determined using membrane diffusion technique. The niosomes left after removal of unentrapped drug were dialyzed into a beaker containing 100 ml of PBS pH 7.4 containing 10% v/v methanol (to maintain sink condition), which acted as receptor compartment. The temperature of receptor medium was maintained at 37 ± 0.5°C and agitated using magnetic stirrer. Aliquots of 5 ml sample were withdrawn periodically and after each withdrawal same volume of medium was replaced. The collected samples were analyzed using UV spectrophotometer at 306.20 nm. The tests were carried out in triplicate.

Stability studies

Optimized formulation was preserved at refrigerated temperature (4-8 ± 1°C) and room temperature (25 ± 2°C) for 30 days. At the 30th day shape, percentage entrapment efficiency and percentage drug release of vesicles were measured. The results were compared with the initial shape, percentage entrapment efficiency and percentage drug release of both samples.[17]

Results and Discussion

The preliminary study indicates that the drug was white crystalline powder with melting point 143.17°C and drug was slightly soluble in water and highly soluble in methanol given in Table 2.

IR study

The IR spectrum of the pure drug CPO displayed characteristic peaks at 3415.00/cm for the O-H stretch, 2927.47, 2852.97/cm peaks for C-H stretch, 1592.38/cm for C=C stretch, 1634.31/cm for C=O stretch, 1450.22/cm for C-C stretch, and 893.24/cm for N-H wag. All the above characteristic peaks of the pure drug were also found in the IR spectrum of the formulation CPO niosomes containing span 40 (CNS4) (peaks at 3423.00, 2924.19/2851.34, 1597.14, 1632.00, 1467.03 and 886.57/cm due to O-H stretch, C-H stretch, C=C stretch, C=O stretch, C-C stretch and N-H wag, respectively) and formulation CPO niosomescontaining span 60 (CNS6) (peaks at 3424.00, 2921.20/2852.13, 1598.02, 1633.12, 1467.91 and 893.35/cm due to O-H stretch, C-H stretch, C=C stretch, C=O stretch, C-C stretch and N-H wag, respectively). The presence of above peaks indicates undisturbed structure of drug in the above formulations. Hence, there are no drug-excipient interactions. IR spectra of CPO (pure drug), CNS4 and CNS6 along with other excipients are shown in Figure 1.

Table 2: Solubility studies of CPO in water and methanol

Liquid vehicle	Solubility (mg/ml)*
Water	<1
Methanol	48

*Values represented as mean of three determinations. CPO: Ciclopirox olamine

Vesicles of CPO

In the present work, CPO niosomes were prepared using various non-ionic surfactants (Span 40 and Span 60) along with cholesterol in different proportions (1:0.2, 1:03, 1:0.4, 1:0.8 and 1:1) by ether injection method given in Table 1. The prepared niosomes were evaluated for preliminary characteristics such as shape and size analysis.

The hydrophilic-hydrophobic segments of non-ionic surfactants and a balance between them are of paramount importance for noisome

Table 3: Vesicle size and shape of niosomes prepared by ether injection method

Formulation code	Particle size*±SD (μm)	Shape of the vesicles
$CNS4_1$	9.21±1.90	Spherical
$CNS4_2$	9.08±2.11	Spherical
$CNS4_3$	8.89±2.41	Spherical
$CNS4_4$	7.24±2.58	Spherical
$CNS4_5$	7.02±1.18	Spherical
$CNS6_1$	8.81±1.82	Spherical
$CNS6_2$	8.65±2.38	Spherical
$CNS6_3$	8.04±2.42	Spherical
$CNS6_4$	7.09±1.28	Spherical
$CNS6_5$	6.36±1.23	Spherical

*Values represented as mean±SD (n=25). SD: Standard deviation, CNS4: Ciclopirox olamine niosomes containing span 40, CNS6: Indicates ciclopirox olamine niosomescontaining span 60, CPO: Ciclopirox olamine

Table 4: Entrapment efficiency and drug content of CPO niosomes prepared by ether injection method

Formulation code	Percentage entrapment efficiency*±SD	Drug content*±SD
$CNS4_1$	55.23±1.34	98.35±0.87
$CNS4_2$	60.12±0.67	97.88±1.89
$CNS4_3$	65.98±1.09	99.01±0.76
$CNS4_4$	62.34±0.89	97.67±0.34
$CNS4_5$	64.19±0.56	98.04±0.67
$CNS6_1$	71.45±0.44	99.06±1.25
$CNS6_2$	74.21±1.54	98.67±1.01
$CNS6_3$	79.06±1.02	97.45±0.76
$CNS6_4$	73.23±0.87	97.88±0.72
$CNS6_5$	76.34±0.06	98.15±0.39

*Values represented as mean±SD (n=3). CNS4: Ciclopirox olamine niosomes containing span 40, CNS6: Ciclopirox olamine niosomescontaining span 60, CPO: Ciclopirox olamine, SD: Standard deviation

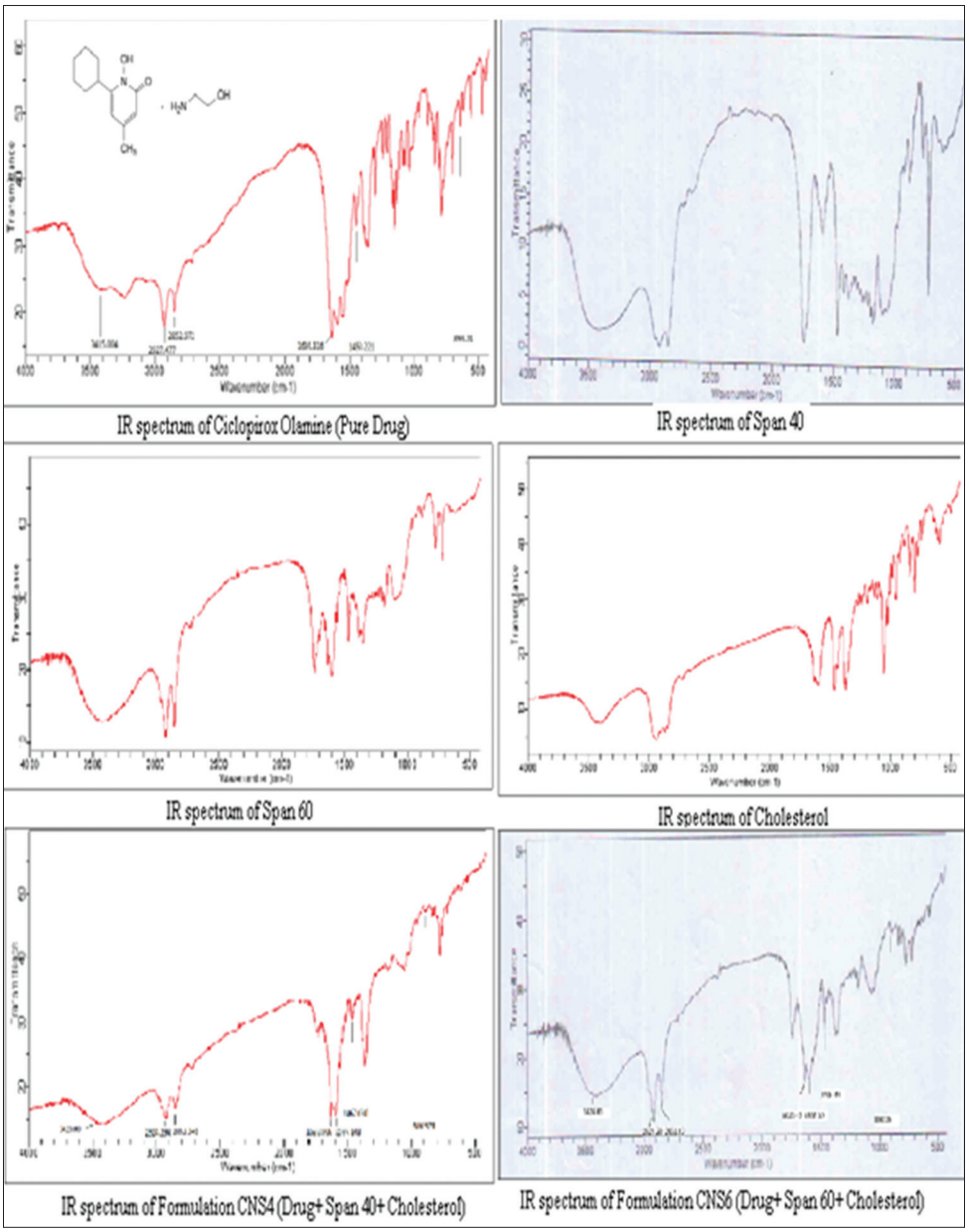

Figure 1: Infrared spectra

vesicle formation. The prepared vesicles were studied under ×45 magnification to observe the formation of vesicles. The niosomes were observed as spherical vesicles with smooth surface. The diameter of the formulated niosomes was found to be in the range of 6.36 ± 1.23 to 9.21 ± 1.90 µm (Table 3).

The entrapment efficiency of CPO niosomes was in the range of 55.23 ± 1.34 to 79.06 ± 1.02%. The drug content was found to be in the range of 97.45 ± 0.76 to 99.06 ± 1.25% (Table 4). The *in-vitro* drug release from the niosomal formulations was in the range of 44.278 ± 1.192 to 65.213 ± 0.253% at the end of 24 h given in Table 4.

Entrapment efficiency

The entrapment efficiency of the prepared CPO niosomes was measured by dialysis method. The entrapment efficiency was determined by subtracting the amount of drug dialyzed from the total amount of drug in the formulation. In all the prepared formulations, the impact of cholesterol and surfactant on entrapment efficiency was significant. The results of entrapment efficiency were shown in Table 4.

Among all the formulations, formulations containing span 60 showed maximum entrapment efficiency compared with other formulations prepared by span 40. The entrapment efficiency increased in the order of span 60 (C16) > span 40 (C16), this could be due to the surfactant chemical structure. All span types have the same head group and different alkyl chain. Increasing the alkyl chain length is leading to higher entrapment efficiency.[12,16] The span 40 and span 60 have same head groups but it has different transition temperature such as span 40 has 42°C and span 60 has 53°C. Increasing the

Table 5: *In-vitro* release data of CPO niosomes prepared using drug, span and cholesterol

Time in h	Formulation code									
	$CNS4_1$	$CNS4_2$	$CNS4_3$	$CNS4_4$	$CNS4_5$	$CNS6_1$	$CNS6_2$	$CNS6_3$	$CNS6_4$	$CNS6_5$
1	8.125	7.653	6.875	6.453	6.023	6.090	6.121	5.809	6.213	5.543
2	11.243	10.543	9.654	11.984	10.243	7.456	7.067	6.897	7.276	6.998
3	14.761	13.875	11.867	14.410	12.777	9.764	9.436	9.432	10.546	9.432
4	17.560	17.654	14.547	17.998	16.987	11.712	11.574	11.112	12.342	10.543
5	20.564	20.412	18.532	22.786	22.142	14.871	16.371	13.006	19.641	12.767
6	24.870	24.098	21.860	26.911	23.541	18.450	22.712	15.543	23.871	13.009
7	27.351	29.123	23.987	32.098	28.465	22.897	27.423	19.564	28.354	16.957
8	31.301	31.870	27.650	36.654	31.211	27.143	31.045	20.989	33.172	20.654
16	48.980	49.650	45.975	53.614	49.125	40.445	38.032	32.876	41.63	33.876
24	65.213	61.231	57.243	63.112	60.078	55.345	49.163	44.278	52.876	46.845

CNS4: Ciclopirox olamine niosomes containing span 40, CNS6: Ciclopirox olamine niosomescontaining span 60, CPO: Ciclopirox olamine

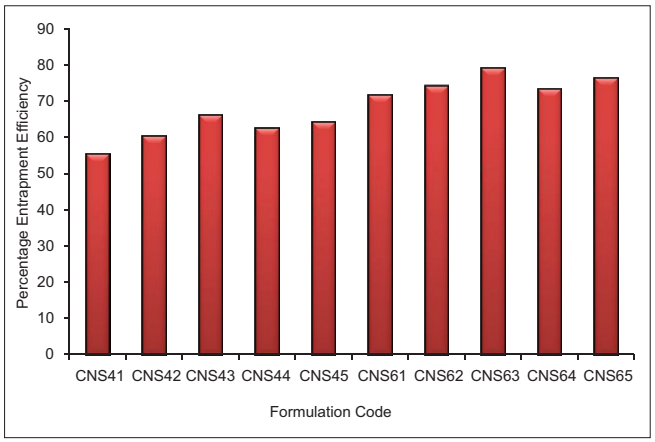

Figure 2: Entrapment efficiency of ciclopirox olamine niosomes prepared by ether injection method

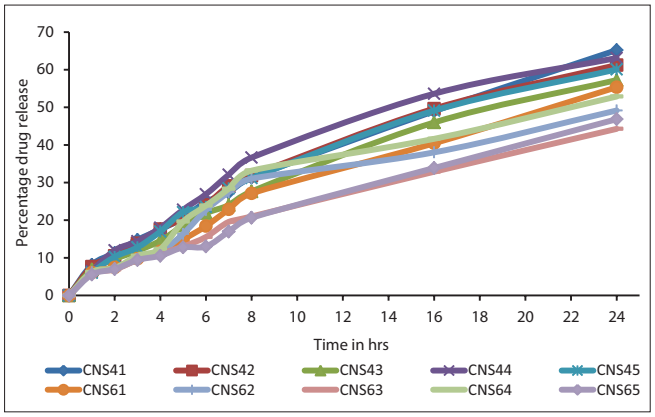

Figure 3: Comparative cumulative drug release versus time plots (zero-order) of niosomal formulations containing ciclopirox olamine

phase transition temperature is leading to higher entrapment efficiency.[12,16]

The order of entrapment efficiency increased as the lipophilicity of the surfactant increased (hydrophilic-lipophilic balance [HLB] value decreased).[12]The HLB value of the surfactant span 60 was 4.7 whereas span 40 was 6.7.

Incorporation of cholesterol is known to influence vesicle stability and permeability.[12] The effect of cholesterol on CPO entrapment was varied according to the nonionic surfactants used (Figure 2).

In-vitro release studies

The cumulative percentage of drug release in 24 h from various niosomal formulations were shown in Table 5 and Figure 3.

Among the five formulations from $CNS4_1$ to $CNS4_5$ prepared by ether injection method using span 40 as surfactant, $CNS4_1$ shows maximum drug release in 24 h. The order of decreasing percentage drug release in 24th h were $CNS4_1$ > $CNS4_2$> $CNS4_3$ > $CNS4_4$ > $CNS4_5$.

Among the five formulations from $CNS6_1$ to $CNS6_5$ prepared by ether injection method using span 60 as surfactant, $CNS6_1$ shows maximum drug release in 24 h. The order of decreasing percentage drug release in 24th h were $CNS6_1$ > $CNS6_2$> $CNS6_3$ > $CNS6_4$ > $CNS6_5$.

Among the 10 formulations, formulations $CNS4_1$ to $CNS4_5$ and formulation $CNS6_1$ (containing span 60) showed good drug release. $CNS6_1$ have shown higher entrapment efficiency as compared to formulations prepared by span 40. Hence, after considering all these parameters, formulation $CNS6_1$ was found to be the best formulation having vesicle size 8.81 µm, entrapment efficiency of 71.45 ± 0.44 and drug release of 55.345 ± 1.009 at the end of 24 h.

All the niosomal formulations release profiles were subjected to various kinetic equations such as first order plots, Higuchi diffusion plots, and Peppas log-log plots. The regression coefficient values of these kinetic equations are very nearer to one, suggesting that plots are fairly linear. Slope values of Peppas log–log plots are between 0.634 and 0.763 suggesting that the drug release by non-Fickian release mechanism, i.e., the drug were released by a combination of both diffusion and erosion controlled drug release.

Conclusion

This study indicated that all the formulations prepared by ether injection method using non-ionic surfactant are showed sustain drug release rate for 24 h. Slope values of Peppas log–log plots are between 0.634 and 0.763 suggesting that the drug release by non-Fickian release mechanism. Among all the formulations, formulation $CNS6_1$ was found to be the best formulation having vesicle size 8.81 μm, entrapment efficiency of 71.45 ± 0.44 and drug release of 55.345 ± 1.009 at the end of 24 h.

Acknowledgment

The authors are thankful to the Principals of SVET"s College of Pharmacy, Humnabad and HKES's MTRIPS, Gulbarga for providing the necessary facilities to carry out the research work and Kumar Organic Products Ltd., Bengaluru, Karnataka for providing the gift sample of CPO.

References

1. Pappas PG, Rex JH, Sobel JD, Filler SG, Dismukes WE, Walsh TJ, *et al*. Guidelines for treatment of candidiasis. Clin Infect Dis 2004;38:161-89.
2. Wagh VD, Deshmukh OJ. Itraconazole niosomes drug delivery system and its antimycotic activity against *Candida albicans*. ISRN Pharm 2012;2012:653465.
3. Agrati C, Marianecci C, Sennato S, Carafa M, Bordoni V, Cimini E, *et al*. Multicompartment vectors as novel drug delivery systems: Selective activation of Tγδ lymphocytes after zoledronic acid delivery. Int J Nanomed 2011;7:153-61.
4. 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.
5. Gupta AK, Bluhm R. Ciclopirox (Loprox) gel for superficial fungal infections. Skin Therapy Lett 2004;9:4-5, 9.
6. Blank W. Micromelting - Point determination with Thiele tube. Ind Eng Chem Anal 1993;5:74-5.
7. Higuchi T, Connors KA. Phase solubility techniques. Adv Anal Chem Instrum 1965;4:117-212.
8. Baillie AJ, Coombs GH, Dolan TF, Laurie J. Non-ionic surfactant vesicles, niosomes, as a delivery system for the anti-leishmanial drug, sodium stibogluconate. J Pharm Pharmacol 1986;38:502-5.
9. Rogerson A, Cummings J, Willmott N, Florence AT. The distribution of doxorubicin in mice following administration in niosomes. J Pharm Pharmacol 1988;40:337-42.
10. Gupta KS, Nappanee M, Gupta VR. Formulation and evaluation of topical meloxicam niosomal gel. Int J Biopharm 2010;1:7-13.
11. Tavano L, Muzzalupo R, Cassano R, Trombino S, Ferrarelli T, Picci N. New sucrose cocoate based vesicles: Preparation, characterization and skin permeation studies. Colloids Surf B Biointerfaces 2010;75:319-22.
12. Tamizharasi S, Dubey A, Rathi V, Rathi J. Development and characterization of niosomal drug delivery of gliclazide. J Young Pharm 2009;1:205-9.
13. Lingan MA, Sathali AA, Vijaykumar MR, Gokila A. Formulation and evaluation of topical drug delivery system containing clobetasol propionate niosomes. Sci Rev Chem Commun 2011;1:7-17.
14. Srinivas S, Kumar YA, Hemanth A, Anitha M. Preparation and evaluation of niosomes containing aceclofenac. Dig J Nanomater Biostruct 2010;5:249-54.
15. Hayes CA. Antifungal ketoconazole composition for topical use. European. Patent. (EP1309352B1); 2002.
16. Gupta V, Barupal AK, Ramteke S. Formulation development and *in vitro* characterization of proliposomes for topical delivery of aceclofenac. Indian J Pharm Sci 2008;70:768-75.
17. Sabarikumar K. Bioavailability enhancement of aceclofenac niosomes containing surfatants and cholesterol. Int J Biol Pharm Res 2012;3:354-9.