Journal of Pharmaceutical and Scientific Innovation
www.jpsionline.com
Research Article

PREPARATION AND EVALUATION OF LIPOSOME ENTRAPPED HYDROGEL COMPLEX SYSTEMS OF ITRACONAZOLE FOR ENHANCED TRANSDERMAL PERMEATION

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DOI: 10.7897/2277-4572.031100
Published by Moksha Publishing House. Website www.mokshaph.com

Received on: 09/12/13 Revised on: 19/01/14 Accepted on: 06/02/14

ABSTRACT
In this study, liposomal hydrogel complex drug delivery system to enhance transdermal permeation of itraconazole as a model drug was developed. The complex systems were prepared by incorporating drug liposome consisting of biocompatible lipid, into carbopol to form hydrogel. The systems were evaluated for encapsulation efficiency, particle size, zeta potential and *ex vivo* release behavior for skin permeability. FT-IR studies were done to find for any drug excipient interactions. The particle size was ranging from 94.2 nm to 104.8 nm with low PdI indicating the formation of monodisperse system. The % of drug released from the formulation was ranging from 48.04 % to 99.92 % in 24 h. In terms of skin permeability, complex liposomal hydrogel has proved to have greater skin permeation compared to simple liposomal system, simple hydrogel system and the plain drug suspension (485.49, 362.06, 226.03 and 172.25 $\mu g/cm^2/h^1$). From the release kinetics we can conclude the drug is releasing by diffusion mechanism and also due to erosion of the gelling agent. It was found that liposome in hydrogel complex systems improved skin permeability of the drug when compared to control with high flux and high permeability coefficient. These results indicate that liposome in hydrogel systems can function as probable drug delivery systems to enhance transdermal permeation of the water-insoluble itraconazole for treating the topical infections.
Keywords: Cholesterol, hydrogel, liposomes, itraconazole and lecithin.

INTRODUCTION

Stratum corneum is the outermost layer of the skin, functioning as a primary barrier to protect the skin from potentially harmful environmental agents. In addition, it prevents the loss of moisture to the outside environment, the intercellular lipids in the stratum corneum helps in maintaining homeostasis of the skin. The trasdermal delivery of drugs is rapidly increasing in the formulation development in enhancing the bioavailability of many drugs. When drugs are administered via transdermal route skin barrier is harmfully affected. Therefore, recently research has been focused in investigating a variety of drug delivery systems aimed at promoting better transport of active drugs by using colloidal drug delivery systems such as micro emulsions, solid lipid nano-particles and liposome's for topical delivery[1-4]. Liposomes are enclosed vesicles containing a lipid bi layer composed of unimers that usually have a hydrophilic head and a hydrophobic tail and are oriented so that the hydrophobic head groups are inside the bi layer. Liposomes are highly biocompatible with low toxicity that helps in conniving drug delivery system with improved bioavailabilty[5]. Hydrogels are 3-dimensional networks consisting of hydrophilic polymers that swell in aqueous solution retaining large amount of water without dissolving. Hydrogels formulated with cellulose have biodegradable properties, high permeation of active materials with high degree of swelling and no associated toxicity or irritation makes them as ideal polymers for delivery of drugs through transdermal route as delivery vehicles[6–8]. Fungal diseases are increasing each year due to the ease of transmission from person to person[9]. Effective treatment options are necessary to avoid the spreading of the disease to peripheral organs leading to potential death[10-11]. Superficial infections are caused by many species like *Aspergillus*, *Candida*, *Tinea*, *Pneumocystis* and *Histoplasma*. These species causes fungal infection conditions like athlete's foot, finger and toe nail infections, yeast infections, oral thrush and ringworm. Some systemic and opportunistic fungal infections can enter the bloodstream and result in more serious disease in those with compromised immune system[12-14]. Itraconazole (ITZ) is commonly used in the treatment of fungal infections. Though oral and parenteral route are commonly used for the treatment of fungal infections because of wide bio-distribution of drugs to other tissues the actual amount reaching the site of action is less[15-17]. Hence, high drug dosing is required for proper treatment which increases the toxicity and cost of the treatment. More efficient way of delivering the drug to combat fungal infections and reducing the cost of the treatment is by using transdermal route of delivery. The efficacy of topical administration of antifungal drugs depends on the penetration through the skin. Use of hydrogels for treatment of infection limits the penetration of drug through intact skin (especially class IV drug) thereby creating an urge to develop formulations for transdermal route with a combination approach. One such approach is preparing niosomes or liposomal formulation and incorporating the same in the hydrogels which enhances increase permeability of the drug for treating chronic conditions. The concept of liposomal hydrogels may potentially increase the permeability of drug through the stratum corneum. Hence, the aim of our present study is to develop liposomal hydrogels of itraconazole and evaluate for their enhanced Transdermal permeation.

MATERIALS AND METHODS

Materials

Itraconazole was obtained as gift sample from Dr. Reddys Labs., Pvt. Ltd. (Hyderabad, India). Lecithin (phosphatidyl choline) and cholesterol were purchased from Sigma Aldrich; carbopol was purchased from S.D fine and all other reagents and solvents used were of analytical grades.

Analytical Method Development for Itraconazole

A spectrophotometric method was developed for analysis of itraconazole. Briefly, the stock solution was prepared by dissolving 100 mg of itraconazole in 100 mL of saline phosphate buffer with 2 % SLS and subsequent dilutions were made to get concentrations of 5 - 25 µg/mL. The absorbance was measured using double beam Jasco UV spectrophotometer at 263 nm.

Preparation of Liposomes

Multilamellar liposomes were prepared by thin film hydration technique. In brief, accurately weighed quantities of itraconazole, lecithin and cholesterol were dissolved in chloroform-methanol in 3:1(% v/v; 10 ml) ratios in a round-bottomed flask. The chloroform methanol mixture was evaporated by using rota evoparator (lobarota 4000, Heidolph, Germany) at 60°C for a period 30 minutes at 125 rpm. After complete evaporation of organic solvent mixture thin film was hydrated using 10 ml phosphate buffer pH 7.4. The formulation variables have been presented in (Table 1)

Preparation of Liposomal Hydrogels

Itraconazole liposomal hydrogels were prepared by incorporating formulated liposome into 2 % carbopol 934. Weighed quantity of the carbopol 934 was dissolved in 15 mL of distilled water and stirred thoroughly to get homogenous slurry. The Itraconazole liposomal formulations were incorporated into the prepared carbopol gels and mixed thoroughly and the pH was adjusted to neutral with triethanolamine.

Characterization of Liposomes and Liposomes Entrapped Gels

Physico-chemical Characteristics

The formulated liposomal hydrogels were checked for color, odour and pH immediately after preparation and on 15th day.

Fourier Transforms Infrared Spectroscopy (FT-IR)

FT-IR spectra were taken for the dried samples using FT-IR 8400S (Shimadzu, Japan) to determine the possible interactions between the drug and polymers. The plain drug, lecithin and cholesterol, formulation and 1:1 physical mixture (5 mg) were taken and mixed with 100 mg of KBr. About 50 mg of this mixture was compressed to form a pellet using a hydraulic press at 15 tonnes pressure. The prepared pellets were scanned from 4,000 to 400 cm^{-1} using FT-IR spectrophotometer.

Entrapment Efficiency (EE)

Entrapped drug in the liposomes was estimated after removing the unentrapped drug. The unentrapped drug was separated by subjecting the dispersion to centrifugation in a cooling centrifuge at10000 rpm at a temperature of 4°C for 10 minutes. The supernatant was removed and the pellet of liposomes was washed with 5 ml buffer to remove any unentrapped drug. The washing was combined with supernatant and was analyzed for drug content at 252 nm to calculate the entrapment efficiency.

$$entrapment\ efficiency = \frac{amount\ of\ drug\ entrapped}{total\ amount\ of\ drug} \times 100$$

Particle Size

The mean particle size and size distribution were determined by photon correlation spectroscopy with a Zeta-sizer Nano ZS (Malvern, UK) at 25°C. The prepared liposomal formulations were diluted to the appropriate concentration with distilled water before measurement of droplet size. The average diameter and poly dispersity index of samples were measured by photon correlation spectroscopy (Nano ZS90, Malvern Instruments, and U.K) at 633 nm. The measurement was performed at 25°C using a He-Ne laser.

Microscopy

The formulated liposomes were observed under binocular microscope (PJRM-700 Qosmo, India) at suitable magnification to identify the unilamellar and multilamellar liposome formation.

Ex vivo Studies

Ex vivo release studies by using albino rat skin as the membrane was performed to study the delivery of the drug through the skin. *Ex vivo* skin permeation study was performed by using Franz diffusion cells. Adult albino rats of 180 - 200 g were depilated with trimmer and skin samples were excised and were clamped between the donor and the receptor chamber of Franz diffusion cells with the stratum corneum facing the donor chamber. The formulations containing itraconazole were placed in the donor compartment. The receptor compartment was filled with saline phosphate buffer with 2 % SLS as the dissolution medium and maintained at 37 ± 0.5 ºC and stirred at 600 rpm. Samples were withdrawn at regular intervals for 24 h and analyzed by UV-Spectrophotometer. The experiment was conducted in triplicates. Steady state flux (Jss. µg/cm^2/h^1) and lag time (T-lag/h) were calculated from the slope and intercept of the straight line obtained by plotting the cumulative amount of itraconazole permeated versus time in steady condition. Permeability Coefficient (Kp.Cm.h^{-1}) was calculated by dividing the flux obtained by the initial concentration of drug in the donor compartment[18].

RESULTS AND DISCUSSION

Physico-chemical Characteristics

The liposomal formulations were evaluated for their physico-chemical properties and found to be colorless and odorless. The liposomal hydrogels were also found to be odorless, translucent and with neutral pH. The properties of formulations immediately and on 15 day did not show any differences in their properties indicating the physical stability of the formulations. The physico-chemical properties of itraconazole liposomal hydrogel complex suggest that it has a good potential for topical drug delivery.

Table 1: Composition of the itraconazole formulations

Formulation	ITZ (mg)	Lecithin (mg)	Cholesterol (mg)	Carbopol 934
F I	25	100	20	20.55
FII	50	100	20	21.08
FIII	75	100	20	21.53
SL	25	100	20	20.44

Table 2: Particle size, PdI and Entrapment efficiency (EE) of the complex liposomal hydrogel systems (Mean ± S.D)

Form. code	Particle size	PdI	EE
F I	94.2 ± 2.26	0.196 ± 0.002	65.92 ± 3.10
F II	102.63 ± 3.48	0.267 ± 0.001	63.28 ± 2.46
F III	104.8 ± 2.21	0.239 ± 0.004	68.46 ± 1.24

Table 3: Data of Flux and permeability coefficient of the formulations

Form. code	FLUX JSS. µg/cm^2/h^1	Kp. Cm.h^{-1}
F I	485.4962	97.09924
FII	460.3053	92.06107
FIII	276.1832	55.23664
SL	362.0611	72.41221
SG	226.0305	45.20611
PD	172.5573	34.51145

Table 4: Curve fitting data for all formulations of itraconazole formulations (R^2)

Form. code	0°	1°	Higuchi's	Peppa's
F I	0.909	0.944	0.935	0.975
FII	0.682	0.863	0.958	0.921
FIII	0.788	0.925	0.978	0.974
SL	0.570	0.906	0.643	0.920
SG	0.557	0.923	0.935	0.892
PD	0.9997	0.952	0.762	0.993

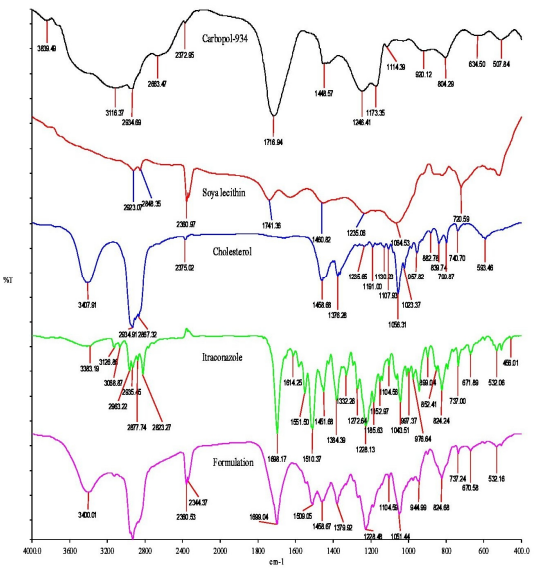

Figure 1: FT-IR overlay of the drug and excipients

		Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	104.4	Peak 1:	130.2	98.8	66.96
PdI:	0.239	Peak 2:	4980	1.2	617.6
Intercept:	0.960	Peak 3:	0.000	0.0	0.000
Result quality	Good				

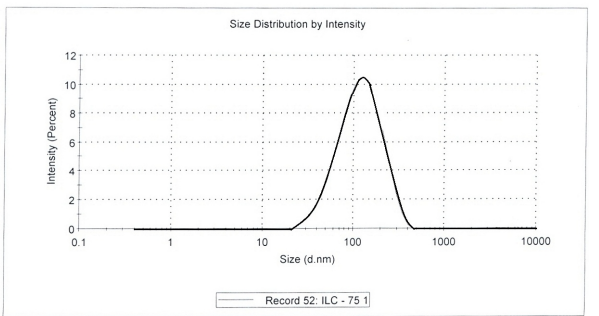

Figure 2: Globule size distribution curve of ITZ liposomal formulation

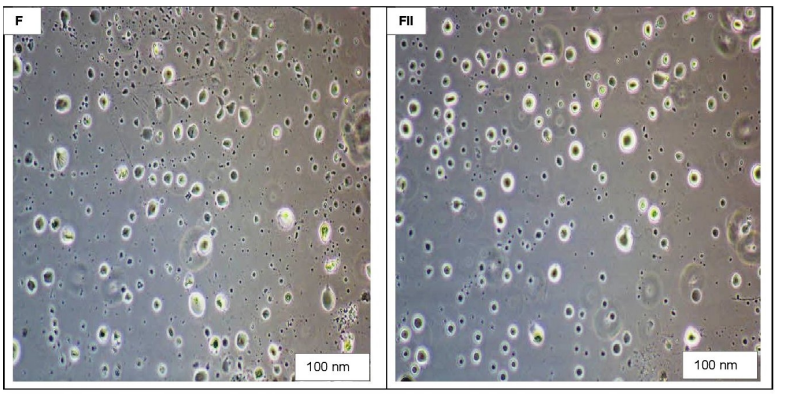

Figure 3: Microscopic view of itraconazole multilamellar vesicles (magnification: 100X)

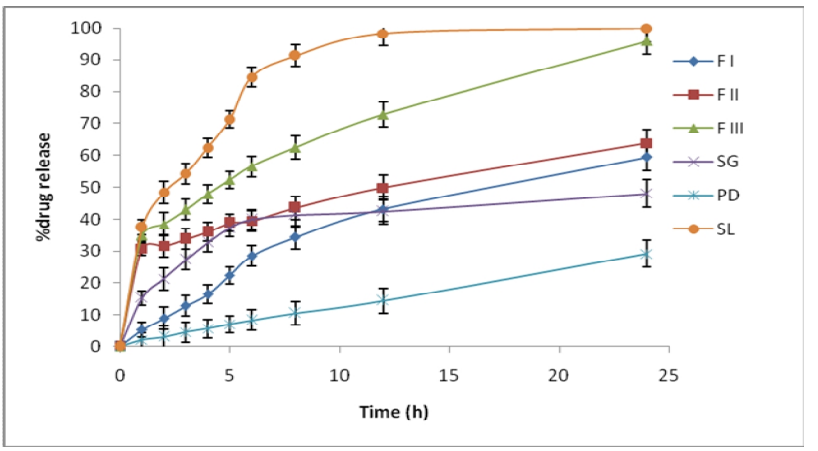

Figure 4: *Ex vivo* release profiles of complex hydrogels, simple liposomes (SL), simple hydrogel (SG) and plain drug (PD)

The other important criteria for selection of material in the complex development is, the selected components are pharmaceuctically acceptable, non-irritating and non-sensitive to the skin and all the excipients fall under the GRAS category.

Fourier Transforms Infrared Spectroscopy (FT-IR)

FT-IR studies of the complex systems results revealed the drug excipient compatibility. In IR spectra of itraconazole pure drug, many peaks were found prominently at various wave numbers indicating the presence of functional groups and substitutions peaks. Peak at 1044.10 cm^{-1}, wave number due to C-O stretching (primary alcohol), prominent peak at 1273.90 cm^{-1}due to C=O stretching. Peak at 1698.1 indicates -C=O of aromatic ring. Broad peaks appeared between 3383.19 cm^{-1}, 3126.47 cm^{-1} wave number was due to N-H stretching group and N-H group structure. Peaks appeared at 2963.22 cm^{-1} and 2877.74 cm^{-1} were because of CH-Ar stretching and C-H stretching aromatic respectively. All these peaks were appeared unchanged in IR spectra of formulations without any additional peaks (Figure 1).

Entrapment Efficiency (EE)

The amount of drug entrapped into the liposome and in liposomal formulations was determined. The entrapment efficiency was in the range of 63.28 to 68.46 %. A good amount of drug was entrapped in the liposome formulations prepared.

Particle Size and Zeta Potential

The mean particle size of the liposomal formulation of ITZ was found to be 94.74 ± 4.8 to 104.6 ± 3.2 nm. The formulation FI has low particle size and it was observed that as the concentration of the cholesterol increased the particle size also increased. Polydispersity basically relates the ratio of standard deviation to mean droplet size. This signifies the uniformity of droplet size within the formulations. The higher the value of the poly dispersity the lower is the uniformity of the droplet size within the formulations[18]. The average mean particle size with PdI values below 0.2 (Table 2), indicated a narrow droplet size distribution and signifies a better stability of the formulation. (Figure 2)

Microscopy

The microscopic images of the formulated liposomes were viewed under binocular compound microscopy. The pictures shows the formation of both unilamellar and multilamellar vesicles with drug entrapped inside them (Figure 3).

***Ex vivo* Diffusion Study**

In this study we developed a complex liposomal hydrogel system of itraconazole an insoluble drug in order to enhance transdermal permeation of the drug. The release of liposomal hydrogel was compared with the single liposomal formulation and single gel system. Franz diffusion cell was used to compare the permeability's of the formulations *ex vivo*. From the results (Figure 4) it is clear that in terms of skin permeability, complex liposomal hydrogel has high flux (485.4962 $\mu g/cm^2/h^1$) with high permeation coefficient (97.09

$Cm.h^{-1}$) compared to the single liposomal system, single hydrogel system and the plain drug suspension (Table 3). The data was also fitted to various kinetic models to study the transport mechanism of drug through the membrane by a diffusion exponent. It was found that the drug was released by diffusion behavior. The drug also released by erosion of hydrogel. From the results we can confirm that use of complex liposomal hydrogel system can be a potential to increase transport of drug.

CONCLUSION

Design of complex liposomal loaded hydrogel system was developed. We studied for encapsulation efficiency, particle size, PdI, drug interactions and for drug release. The use of hydrogels may temporarily destroy the skin barrier by hydration of the skin and it also protects the epidermis from water evaporation, and the liposomal formulation will help to support the intercellular lipid which was destroyed when skin barrier was disrupted. In this way liposomes support the defense system of the skin. Results of our study strongly recommend the complex liposomal hydrogel system as an effective drug delivery in enhancing transdermal permeation of itraconazole.

REFERENCES

1. Rosen H and Abribat T. The rise and rise of drug delivery, Nat Rev Drug Discov 2005; 4: 381-385. http://dx.doi.org/10.1038/nrd1721
2. Garg AK, Negi LM and Chauhan M. Gel containing ethosomal vesicles for transdermal delivery of aceclofenac, Int J Pharm Pharm Sci 2010; 2: 102-108.
3. P Garidel, B Folting, I Schaller, A Kerth. The microstructure of the stratum corneum lipid barrier: mid-infrared spectroscopic studies of hydrated ceramide:palmitic acid: cholesterol model systems, Biophysical Chemistry 2010; 150: 144–156. http://dx.doi.org/10.1016/j.bpc.2010.03.008
4. GSK Pilgram, AME Pelt, JA Bouwstra, HK Koerten. Electron diffraction provides new information on human stratum corneum lipid organization studied in relation to depth and temperature, J. Invest. Dermatol 1994; 113: 03–409.
5. GE El Maghraby, BW Carry, AC Williams. Liposome and skin: from drug delivery to model membrane, Eur. J. Parm. Sci 2008; 34(4–5): 203–222. http://dx.doi.org/10.1016/j.ejps.2008.05.002
6. A Sannino, C Demitri, M Madaghiele. Biodegradable cellulose-based hydrogels: design and applications, Materials 2009; 2(2): 353–373. http://dx.doi.org/10.3390/ma2020353
7. NA Peppas, P Bures, W Leobandung, H Ichikawa. Hydrogels in pharmaceutical formulations, Eur. J. Pram. Biopharm 2000; 50: 27–46. http://dx.doi.org/10.1016/S0939-6411(00)00090-4
8. C Chang, L Zhang. Cellulose-based hydrogels: present status and application prospects, Carbohydrate Polymers 2011; 84: 40–53. http://dx.doi.org/10.1016/j.carbpol.2010.12.023
9. Pinner RW, Teutsch SM, Simonsen L, Klug LA, Graber JM, Clarke MJ and Berkelman RL. Trends in infectious diseases mortality in the United States, JAMA 1996; 275: 189-193. http://dx.doi.org/10.1001/jama.1996.03530270029027
10. Armstrong GL, Conn LA and Pinner RW. Trends in infectious disease mortality in the United States during the 20th century, JAMA 1999; 281: 61-66. http://dx.doi.org/10.1001/jama.281.1.61
11. Morens DM, Folkers GK and Fauci AS. The challenge of emerging and re-emerging infectious diseases, Nature 2004; 430: 242-249. http://dx.doi.org/10.1038/nature02759
12. Ronaldo N, Harvey F, Brahm J, Philpott Howard J, Alexander G, Csewell M, Fagan E and Williams R. Fungal infection: a common, unrecognized complication of acute liver failure, J Hepatol 1991; 12: 1-9. http://dx.doi.org/10.1016/0168-8278(91)90900-V
13. Richardson MD. Changing patterns and trends in systemic fungal infections, J Antimicrob Chemother 2005; 56: 5-11. http://dx.doi.org/10.1093/jac/dki218
14. De Gregorio MW, Lee WM, Linker CA, Jacobs RA and Ries CA. Fungal infections in patients with acute leukaemia, Am J Med 1982; 73: 543-548. http://dx.doi.org/10.1016/0002-9343(82)90334-5
15. Davis A. Medicines by design, National Institutes of Health 2006; 474: 4-14.
16. Kopacek KB. Administration and kinetics of drugs, The Merck Manuals; 2007.
17. Wei C, Bing G, Hao W, Jun P, Weiyue L and Huimin H. Development and evaluation of novel itraconazole-loaded intravenous nanoparticles, Into J Pharmaceutics 2008; 362(1-2): 133-140.
18. Klang V, Matsko NB, Valenta C and Hofer F. Electron microscopy of nanoemulsions: an essential tool for characterization and stability assessment, Micron 2012; 43: 85-103. http://dx.doi.org/10.1016/j.micron.2011.07.014

Source of support: Nil, Conflict of interest: None Declared

QUICK RESPONSE CODE	ISSN (Online) : 2277 –4572
	Website http://www.jpsionline.com

How to cite this article:

Sunitha Sampathi, Thirupathi Ajimera, Vijaya Kuchana. Preparation and evaluation of liposome entrapped hydrogel complex systems of Itraconazole for enhanced transdermal permeation. *J Pharm Sci Innov*. 2014;3(1):25-29 http://dx.doi.org/10.7897/2277-4572.031100