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Article

Development of amphotericin b Based organogels against mucocutaneous fungal infections

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Amphotericin B is a broad spectrum antifungal agent used to treat fungal infections. Organogel is a semisolid preparation in which the apolar phase gets immobilized within spaces of the three-dimensional structure. The current study aimed at the formulation and comparative evaluation of sorbitan monostearate organogels and pluronic lecithin organogels (PLO). Different compositions of span 60 based organogels were prepared by varying the concentrations of the span 60 and PLO gels were prepared by varying the concentration of Pluronic F 127. The developed organogels were subjected to various characteristics such as pH, viscosity, spreadability, extrudability, and drug release studies. The optimized formulations were evaluated against *Candida albicans and* carried out *ex vivo* release study. The optimized formulation was selected from span 60 based organogels, and pluronic lecithin organogels were S1 and P1, respectively. The optimized formulation (S1) showed effective inhibition against *Candida albicans*. The skin irritation test was carried out on albino mice for optimized formulations and results showed that no irritation to the skin. Based on the results, organogels prepared by sorbitan monostearate showed better antifungal activity, and also all the formulations were found to be stable and safe throughout the study period.

Keywords: Amphotericin B. Organogel. Span 60. Pluronic F127. Lecithin.

INTRODUCTION

For many decades, topical drug delivery system is considered as the best and easiest way of administration of therapeutic agents for local effect; however, it also shows some systemic effects. Absorption of the drug through topical application is easy because of the presence of innumerable blood vessels in the skin. Amongst various topical formulations, the semisolid system holds keen importance due to its ease of absorption through the skin layers. Topical dosage forms are more effective and less toxic than conventional dosage forms. There are different factors which can affect the topical absorption of drugs such as particle size of the drug, chemical nature of the drug, physiological factors such as moisture of the skin, skin texture, skin temperature, age, sex, and disease conditions etc. (Quindós, 2014). Numerous novel topical formulations have evolved in the last decades (Nakhat *et al*., 2005). Gels are the convenient form of topical delivery system which has a lot of application in the pharmaceutical industry. Compared to other topical semisolid preparations like ointment and cream, the gel has got better application property and stability. It is also providing controlled drug delivery. Nowadays, several novel gels are available in the market. Among which organogel, which is a new type of gel system became popular in a short time because of its various advantages. The popularity of organogel increases because of its penetration power through the skin layers without the addition of chemical enhancer, ease of preparation, and it can accommodate both hydrophilic and lipophilic therapeutic agents (Voltan, Fusco-Almeida, Mendes-Giannini, 2014).

Cutaneous fungal infection is one of the most common diseases in all over the world. Many fungi

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which are occurring in our environment can cause various types of infections in different parts of the body in both humans and animals. There are several drugs available to treat these types of fungal infections such as ketoconazole, clotrimazole, and miconazole etc. Amphotericin B is one of the broad spectrum antifungal agent which is usually used to treat systemic fungal infection by intravenous administration. Topical application of amphotericin B is limited because of its low absorption through mucosa or skin. Due to its lipophilic nature, it does not dissolve in an aqueous medium.

Various topical formulations are available such as cream, gel, lotion, but these are undesirable for many drugs due to its poor absorption and other side effects. To avoid all these drawbacks, it is a new idea to prepare a formulation of organogel incorporating with amphotericin B as the therapeutic agent. Organogel has better penetration power through the skin layers without any chemical catalyst, and it is a good carrier for lipophilic drugs. These novel drug delivery systems can also produce controlled drug delivery; it can also significantly improve its performance in terms of efficacy, safety and stability (Xie *et al*., 2014).

MATERIAL AND METHODS

Amphotericin B was received as a gift sample from Cipla Pvt. Ltd. Mumbai, India. Pluronic F127 was procured from Sigma Aldrich, USA. Soya lecithin, isopropyl myristate, isopropyl palmitate, and sorbic acid were obtained from Hi-Media laboratories, Mumbai, India. All the materials used in this study are of analytical grades.

METHOD OF PREPARATION OF ORGANOGEL

For the preparation of organogels two different organogelators were selected, like sorbitan monostearate based organogel and lecithin based organogel.

Preparation of sorbitan monostearate organogel

Sorbitan monostearate (span 60) based organogels were prepared by a simple method, in which required the amount of span 60 was dissolved into isopropyl myristate (10% w/v) in a beaker and 2% w/v of polysorbate was added to the above solution. This mixture was heated to 60°C in a water bath, and a homogenous solution was obtained. In another beaker, 0.1% w/w of amphotericin B was dissolved in 5 mL of dimethyl sulfoxide (DMSO), and this was added to the homogenous solution with continuous stirring at room temperature (Chen, Playford, Sorrell, 2010). The solution was further cooled, and a semisolid gel was obtained. Six different formulations were prepared by changing the concentration of span 60 (Chandrasekar, 2011). The compositions of different formulations were summarized in Table I.

TABLE I - Sorbitan monostearate organogel formulations from S1 to S6

Formulation code	Drug (%w/v)	Span 60 (% w/v)	Tween 20 (% w/v)	DMSO (%w/v)	Isopropyl myristate (w/v)
S1	0.1	18	2	2	100
S2	0.1	20	2	2	100
S3	0.1	22	2	2	100
S4	0.1	24	2	2	100
S5	0.1	26	2	2	100
S6	0.1	28	2	2	100

Preparation of pluronic-lecithin organogel (lecithin based)

Pluronic lecithin orange consists of aqueous phase as well as the oil phase. The aqueous phase was prepared by dissolving the required amount of Pluronic F127 in cold water with continuous stirring, and this dispersion was stored in a freezer for 24 h to obtain a clear polymer solution. Potassium sorbate was added as a preservative (Jose, Charyulu, 2016). Similarly, the oil phase was prepared by dissolving the specified quantity of soya lecithin in isopropyl palmitate with continuous stirring. Sorbic acid was added as a preservative. This solution was kept at room temperature for 24 h. Finally, the organogel was prepared by mixing of oil phase with the aqueous phase. Amphotericin B (0.1%w/w) was dissolved in a minimum quantity of DMSO and added to the oil phase. The oil phase containing the drug was added in drop wise manner to the aqueous phase with continuous stirring (Petrikkos, Skiada, 2007). The formulae for preparation of pluronic lecithin organogel are given in Table II.

Physicochemical Characterization of Organogel

Compatibility studies

The FTIR spectra of the pure drug (amphotericin B), pluronic F127, span 60 and the formulations were recorded on an FTIR spectrometer (Shimadzu FTIR 8300 Spectrophotometer). The signals arising out of various intramolecular stretching and bending vibrations have been expressed in wave numbers. The spectra obtained for drug, physical mixture and the final formulation was compared.

Physical appearance

The prepared organogel was visually inspected for its colour, homogeneity, and consistency.

Measurement of pH

The pH of all the formulations was determined by placing the electrode of electronic pH meter in contact with the surface of the prepared gel and allowing equilibrating for 1 min (Singh, Pramanik, Pal, 2015).

Gel-sol transition temperature

The gel-sol transition temperature of all gels was determined by incubating the organogels in a temperature bath at a temperature ranging from 25 ºC – 60 ºC. The temperature of the water bath increased by 5°C every 5 min. The temperature was noted at which the gel started to flow when the beaker was inverted (Mohamed, 2004).

TABLE II - Formulation of pluronic lecithin organogel from P1 to P6

Components	Content	Formulation code					
		P1	P2	P3	P4	P5	P6
Drug(% w/v)	Amphotericin B	0.1	0.1	0.1	0.1	0.1	0.1
Oil phase (% w/v)	Soya lecithin	10	10	10	10	10	10
	Potassium sorbate	0.4	0.4	0.4	0.4	0.4	0.4
	Isopropyl palmitate up to	100	100	100	100	100	100
Aqueous phase (%w/v)	Pluronic F 127	30	34	38	42	46	50
	Sorbic acid	0.4	0.4	0.4	0.4	0.4	0.4
	Distilled water up to	100	100	100	100	100	100

Viscosity

The viscosity of all the organogel was determined by using Brookfield viscometer (Brookfield DV-II+ Pro). The samples were taken in a beaker and viscosity was measured using spindle no: 96 at room temperature and increase in angular velocity 5 rpm to 100 rpm and constructed rheogram(Gendy, Jun, Kassem, 2002).

Spreadability

All the prepared formulations were evaluated for the spreadability by using two glass plates. A circle of 1 cm diameter was marked in one of the glass plates, and 0.5 g of the formulation was placed on it. The second glass plate placed over the first glass plate, and 1kg of weight was kept over it for 5 min. Spreadability of the gel was measured by increased diameter after 5 min (Shivhar, Jain, Mathur, 2009).

Extrudability test

Extrudability test of all the prepared formulations was done by using Monsanto hardness tester. About 15 g of the prepared formulation was placed in the aluminium tube, and the plunger of the tester was adjusted to hold the tube properly. About 1 kg/cm^2 pressure was applied to it for 30 sec. The amount of formulation extruded out was weighed, and the procedure was repeated at three equidistance places of the tube (Hosadurga *et al*., 2014).

Drug content determination

The amphotericin B showed maximum absorbance in the UV region at its characteristic wavelength (409 nm). A calibration curve was constructed at different amphotericin B concentrations. Since other ingredients in the formulation give no absorbance at 409 nm, the absorbance obtained from the formulation would be exclusively from amphotericin B. The limits of detection (LOD), and the limits of quantification (LOQ) were found to be 0.24 and 0.72 µg/mL respectively. From each formulation, weigh accurately 1 g of formulation and dissolved in 2 mL DMSO in a 100 mL volumetric flask. The volume was made up to the mark with phosphate buffer of pH 7.4. Again 1 mL from this stock solution was taken and diluted to 100 mL with the same buffer, and the absorbance was measured at 409 nm by using UV/Visible spectrometer. This absorbance was correlated with the calibration curve, and drug content was determined (Shaikh *et al*., 2009).

In vitro drug release

In vitro, drug release studies were carried out in modified *in vitro* permeation apparatus using dialysis membrane (MWCO 1000 Daltons). The phosphate buffer of pH 7.4 was used as the dissolution medium. The dialysis membrane was soaked in the dissolution medium for overnight. Accurately weighed 1 g of organogel and placed in the centre of the dialysis membrane and tied to one of the opening ends of the specially designed glass cylinder (glass cylinder with an opening at both ends and a diameter of 3.4 cm called as donor chamber). The glass cylinder was attached to the metallic shaft and dipped in a beaker containing 50 mL of phosphate buffer of pH 7.4, and it is placed in such a way that the dialysis membrane just touched to the receptor dissolution medium surface. The dissolution medium was kept at a temperature of 37+ 0.5 ºC and stirred throughout the studies at 50 rpm. In a specified time interval, aliquots of 3 mL sample from the receptor medium were withdrawn, and the same volume was replaced with phosphate buffer of pH 7.4 to maintain perfect sink conditions. The absorbance of the samples was measured by UV spectrometer at 409 nm after suitable dilution (Khan *et al*., 2013).

Screening of antifungal activity of the formulation against *Candida albicans*

The antifungal activity studies of the optimized formulations were carried out by the cup plate method (Hammer, Carson, Riley,1999). The organism used for the study was *Candida albicans* ATCC-14053. The optimized formulations (S1 and P1) were taken for the antifungal activity study. The DMSO was used as the control. Pure Amphotericin B was used as a reference standard, and it was dissolved in a minimum volume of DMSO. The culture medium was prepared by dissolving 6.5 g of sabouraud dextrose agar in 100 mL distilled water by heating and sterilized by autoclaving at 15 lbs/sq inch pressure for 15 min. After that, the medium was poured into three Petri plates and kept in an incubator at 37±2 ºC. A loopful of the organism was transferred from the mother culture to the sterilized sabouraud dextrose agar medium by spread

plate method. The Petri plates were marked into four equal parts. The first part was marked as S (standard), the second part was marked as T (test), the third part was marked as S6 (formulation), and the fourth part was marked as P1 (formulation). The cup or holes about 8mm in diameter were cut into the centre of each part of the medium with a sterile cork borer. The hole S (standard) was filled with the solution of 0.01mg amphotericin B in DMSO and T (test) was filled with placebo gel. The optimized formulations S6 and P1 were weighed equivalent to 0.01mg of drug and placed in the holes S1 and P1 respectively. The plates were transferred into biological oxygen demand incubator and maintained at 37±2 ºC for 24-48 h. After incubation, the Petri plates were observed for the zone of inhibition. The diameter of the zone of inhibition was reported in mm (EI Maghraby, Barry, William,2008).

Ex-vivo permeation studies

The modified validated ex-vivo permeation apparatus was used for the ex-vivo drug release studies through pig skin. The medium used in this study was a phosphate buffer of pH 7.4. The pig ear skin was collected from the local slaughter house and cleaned it properly. The collected skin with suitable size was stored at -20 ºC. Before the study, the pig ear skin was taken out of the freezer and allowed to come into room temperature. Weighed accurately 1g of the organogel and placed in the centre of the pig ear skin and tied to the specially designed glass cylinder, which was donor compartment (Schwartz *et al*., 2014). The glass cylinder was then attached to the metallic shaft and suspended in 50 mL dissolution medium so that the membrane just touched to the receptor surface. The temperature of the dissolution medium was maintained at 37+ 0.5 ºC throughout the experiment, and the medium was stirred at 50 rpm using a magnetic stirrer. In specified intervals, 3 mL sample was taken from the receptor medium, and the same volume was replaced with phosphate buffer of pH 7.4. The filtered samples were analyzed by UV spectrometer at 409 nm after suitable dilution (Tazrart *et al*., 2017).

Skin irritation test

After the approval from the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), skin irritation test was carried out for optimized formulations on Albino mice. For the studies, eight mice of either sex were selected. The selected mice were caged together and provided with normal food. The mice were anaesthetized using anaesthetic ether. Hairs were depleted from the back of mice by depilatories, and an area of 2 cm^2 was marked. After 24 h of depilation, the mice were subjected to irritation studies. The animals were categorized into four sets; each set contains two animals in which, and one was used as control, and the other one was used as a test. On the control, 100 mg of gel without drug was applied, and on the test, 100 mg of the optimized formulation was applied. The skin surface was observed for any visible change such as erythema (redness) after 24, and 48 h of the formulation application. The mean erythemal scores were recorded depending on the degree of erythema: no erythema = 0, slight erythema (barely perceptible-light pink) = 0.5, moderate erythema (dark pink) = 1 (Nava *et al*., 2011).

Stability studies

The accelerated stability studies for the optimized formulation were carried out as per the ICH guidelines (Nakhat *et al*., 2005). The optimized gels were filled in a collapsible tube and stored away from light at 25± two ºC and 40±2 ºC and 75% relative humidity (RH) for three months. After storage, the samples were tested for their visual appearance, pH, % drug content and % drug release (Bhalodia, Shukla, 2011).

RESULTS AND DISCUSSION

Compatibility studies

FTIR spectrum of amphotericin B showed a characteristic sharp peak at 3413.15 cm^{-1} due to the amine group, peak at 2923.22 cm^{-1} representing hydroxyl group and C-O stretching peak at 1181 cm^{-1}. When compared with the pure drug, no considerable change in the IR peaks of the drug was observed in spectra of optimized formulations; it indicates the absence of any incompatibility between drug and excipients.

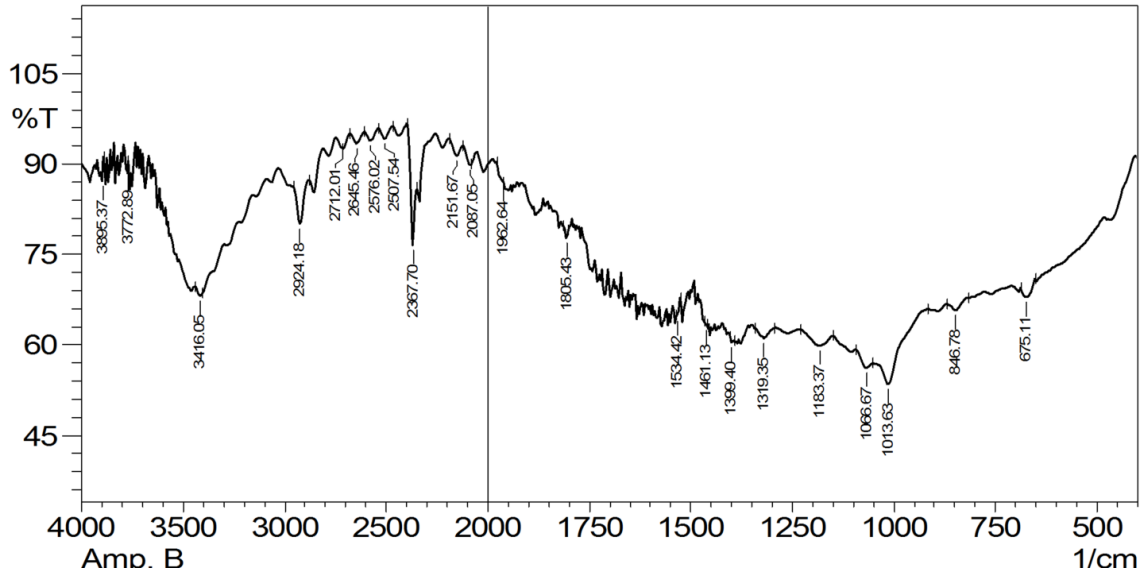

FIGURE 1 - FTIR spectrum of amphotericin B.

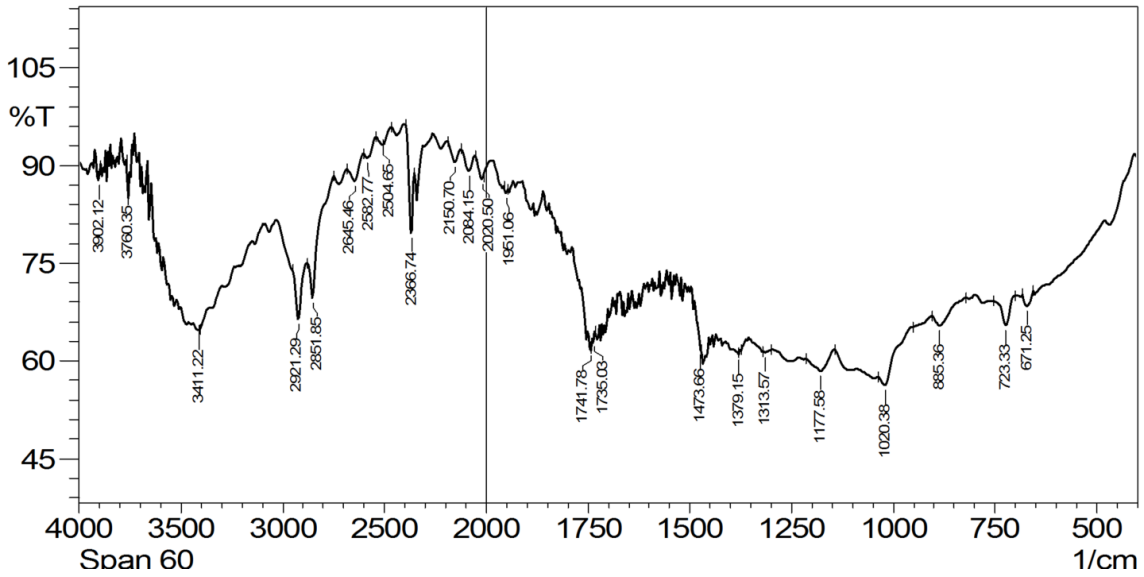

FIGURE 2 - FTIR spectrum of span 60.

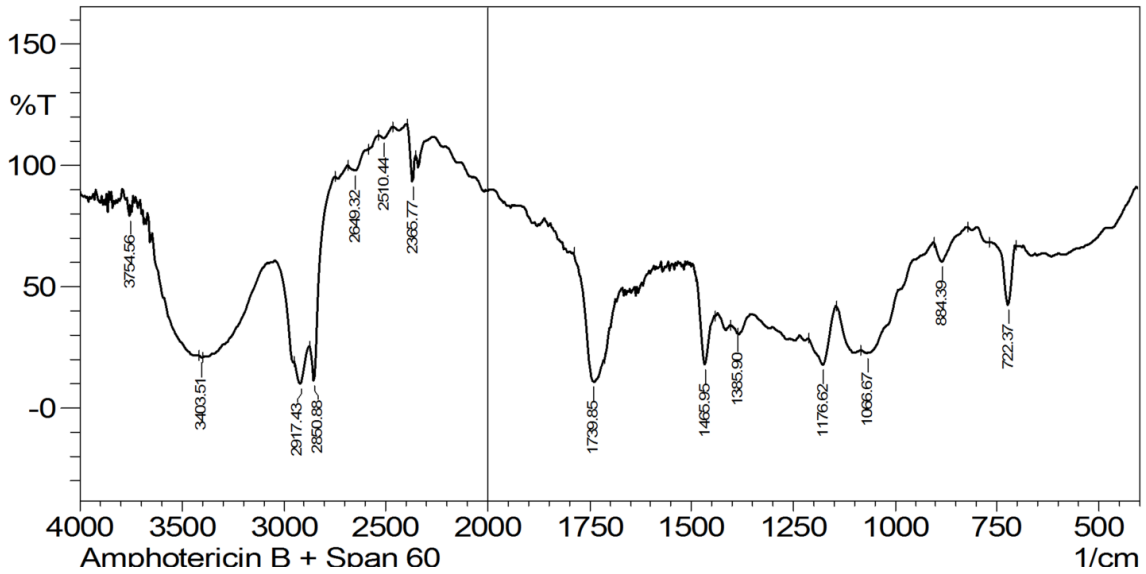

FIGURE 3 - FTIR spectrum of optimized formulation S1.

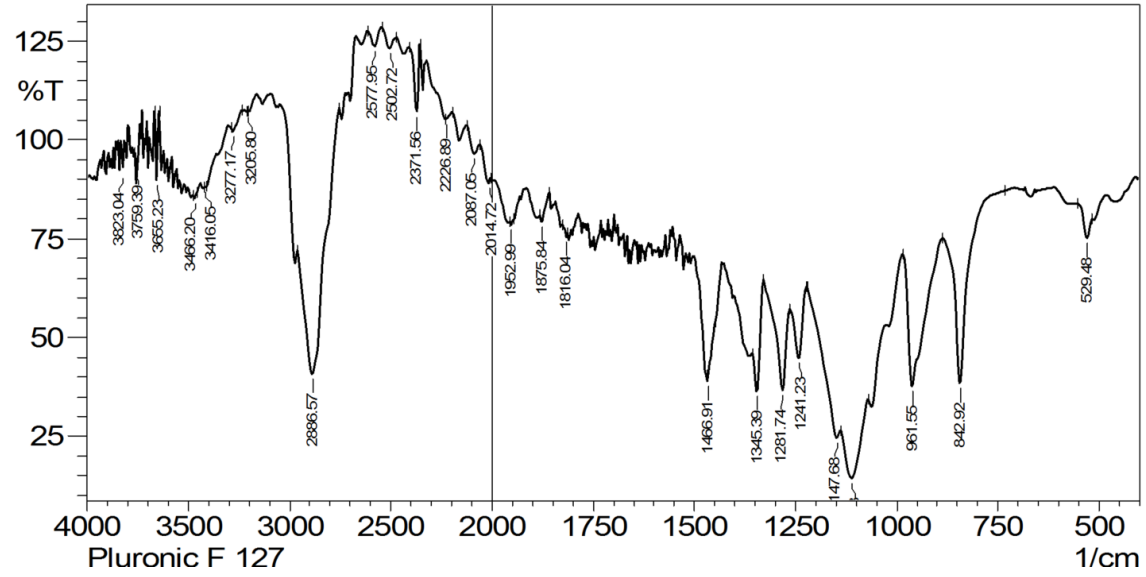

FIGURE 4 - FTIR spectrum of pluronic F127.

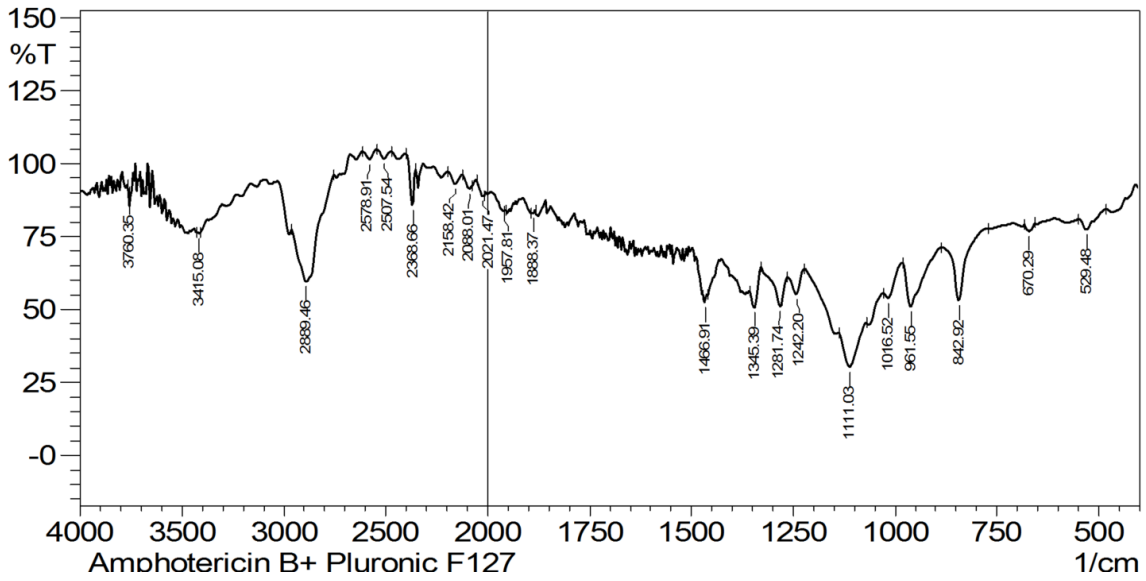

FIGURE 5 - FTIR spectrum of optimized formulation P1.

Physical appearance

All the developed organogel was creamy light yellowish, non-transparent and showed good homogeneity. The span 60 based organogels was appeared to be little more yellowish than the pluronic lecithin organogel. The results were depicted in Table III.

The pH of all the formulations was measured and enlisted in Table III. The pH of all the formulations was remained within the normal skin pH range, indicated that it would not cause any irritation upon administration (Essa, 2010).

Gel-sol transition temperature

The gel-sol transition temperature of all the formulations was found to be in the range of 42 °C-50 °C as enlisted in Table III. In the case, both types of organogels the gel-sol transition directly depends on the viscosity of the system. The gel-sol transition temperature of all the pluronic lecithin organogels was found to be more than that of the span 60 based organogel.

Viscosity, Spreadability and Extrudability

The viscosity of all the prepared formulation was determined by Brookfield viscometer, and the data were shown in Figure 6 and 7. The viscosity of the span 60 based organogel was increased gradually from the formulation S1 to S6 due to the increased concentration of span 60. In the case of pluronic lecithin organogel, formulation P6 showed the highest viscosity and P1 showed the least viscosity indicated that the direct effect of polymer concentration on viscosity. However, viscosities of all the pluronic lecithin organogel were found to be more than that of the span 60 based organogel. The rheogram was constructed for all the organogel formulations and it was found that all the formulation exhibits shear thinning property (Essa, 2010). The values of the spreadability (Table IV) of the formulated organogel showed that only a small amount of force is required to spread the gel easily. The entire span 60 based organogels showed better spreadability when compared to pluronic lecithin organogel (Gupta, Nappinnai, Gupta, 2010). Extrudability of all the formulations were determined and listed in Table IV. The results implied that more than 90% of the contents were extrudable indicating they have excellent extrudability. (>90% extrudability: excellent, >80% extrudability: good, >70% extrudability: fair). The extrudability studies indicated that suitable consistency of the gel was required to extrude the gel from the tube uniformly.

TABLE III - Physical appearance, pH and gel-sol transition temperature

Formulation code	Appearance	pH± SD*	Gel-sol transition temperature(°C)
S1	**Non transparent, light yellow**	6.9±0.1	42
S2		6.4±0.3	43
S3		6.3±0.1	44
S4		6.7±0.2	45
S5		6.1±0.1	46
S6		6.5±0.2	47
P1		7.1±0.1	45
P2		6.8±0.2	46
P3		7.4±0.2	47
P4		7.3±0.1	48
P5		7.0±0.3	49
P6		7.1±0.2	50

(*SD=Standard Deviation)

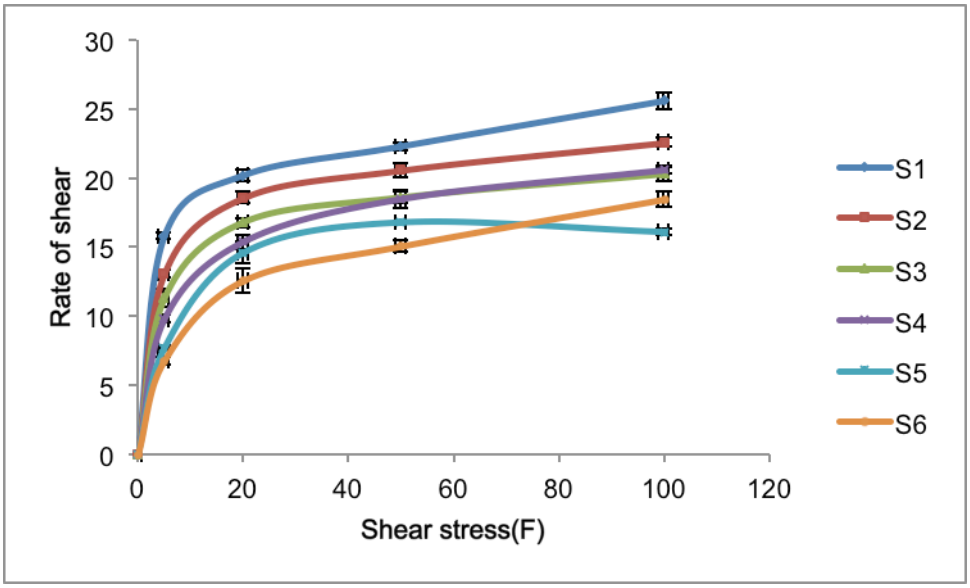

FIGURE 6 - Rheogram of span 60 based organogel.

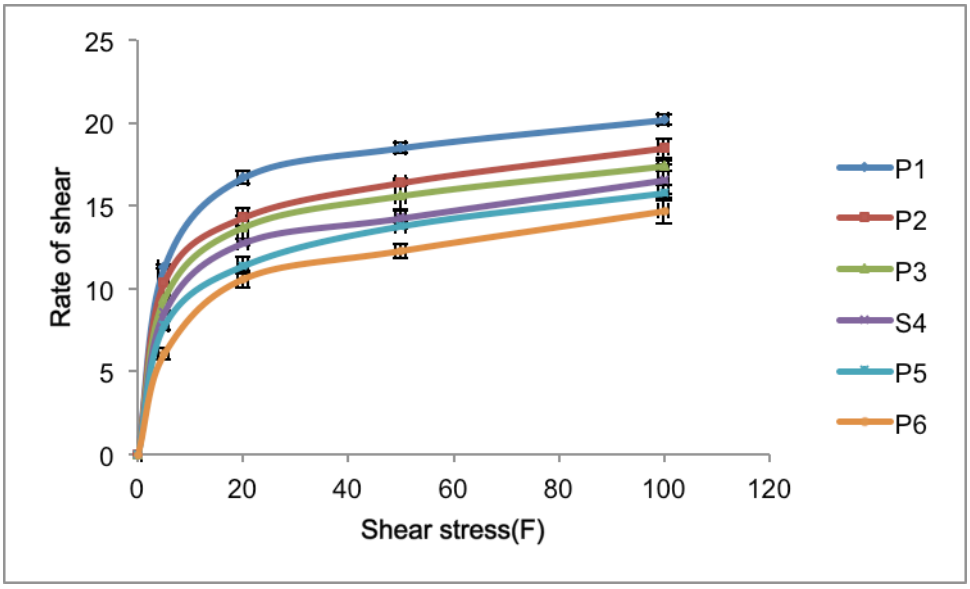

FIGURE 7 - Rheogram of pluronic lecithin organogel.

Drug content

The drug content of developed gels was found to be in the range of 96-99% (Table IV). There was no significant difference in the drug content among the organogels, indicated the drug was uniformity distributed in the gel base.

TABLE IV - Spreadability, extrudability, drug content

Formulation code	Spreadabilty (mm)	Extrudability	Drug content (%)
S1	25.12±0.01	**Excellent**	99.46±0.002
S2	24.11±0.01		98.24±0.005
S3	23.02±0.05		98.55±0.011
S4	22.26±0.01		97.46±0.010
S5	20.10±0.20		97.10±0.003
S6	19.25±0.04		97.46±0.002
P1	17.24±0.01		99.08±0.001
P2	16.56±010		96.26±0.010
P3	15.21±0.24		98.20±0.001
P4	13.56±0.03		97.72±0.004
P5	12.25±0.04		96.93±0.002
P6	9.10±0.02		97.45±0.001

In vitro drug release studies

In vitro drug release profile of all the formulated span 60 based organogel was given in Figure 8. The study was carried out for eight h using modified *in vitro* permeation apparatus through the dialysis membrane. All the formulations showed extended drug release for eight h. However, S1 showed the highest drug release of 99.4% at the end of 8 h. The order of drug release from the formulation S1 to S6 varied as follows, S1>S2>S3>S4>S5>S6. Among the formulations from S1 to S6, as the concentration of span 60 increased, the drug release was decreased. The decrease in drug release was due to an increase in alkyl chain length of surfactant. The formulation S1 was selected as the best due to its highest drug release within eight h (Tavano *et al*., 2010).

In vitro drug release profile of pluronic-lecithin organogel was also carried, and results were shown in Figure 9. The drug release from all the developed gel was extended to 12 h. The order of drug release from the formulation P1 to P6 varied as follows, P1>P2>P3>P4>P5>P6. The drug release values from P1 to P6 indicated that, as the polymer concentration increased, the drug release was decreased, which might be due to extensive formation of network-like structures with very high viscosity. The formulation P1 was selected as the best due to its effective prolonged release of the drug throughout 12 h (Srinivas *et al*., 2010).

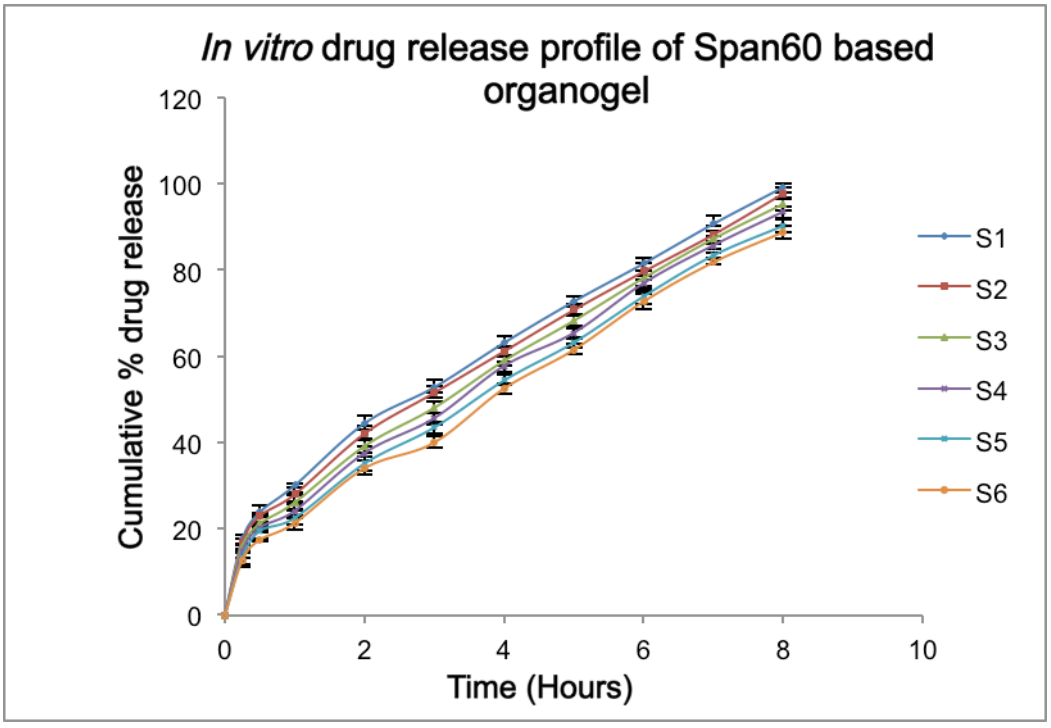

FIGURE 8 - Drug release profile of span 60 based organogel in phosphate buffer of pH 7.4 as a function of hours.

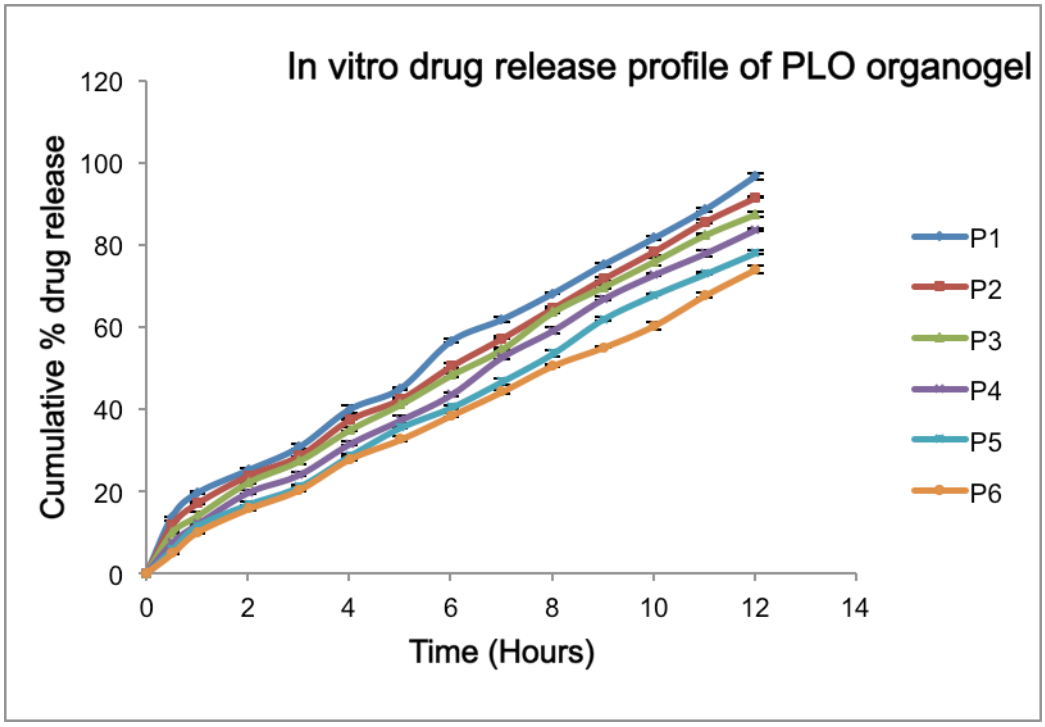

FIGURE 9 - Drug release profile of pluronic lecithin organogel in phosphate buffer of pH 7.4 as a function of hours.

Kinetic studies

In the case of span 60 based organogel, the release kinetics best-fit model indicated that formulations S1 to S4 followed the Higuchi model while S5 and S6 followed first order release. The optimized formulation S1 followed the Higuchi model with the R2 value of 0.993 (as shown in Table V).

In the case of pluronic lecithin organogel, the release kinetics best-fit model indicated that formulations P1 to P4 followed Korsmeyer-Peppas model and P5 and P6 followed zero order release. The results were shown in Table VI. The optimized formulation P1 best fit to the Korsmeyer Peppas model with R2 value of 0.990. The values of release exponent "n" obtained by applying the Peppas equation was found to be less than 0.45, which revealed that the drug release follows Fickian diffusion (Ning *et al*., 2005).

In vitro antifungal activity against *Candida albicans*

The in the vitro antifungal study was carried out by cup plate method to compare the effectiveness of the optimized formulations to controls against *Candida albicans* and results were given in Figure 10. The zone of inhibition produced by the optimized formulations S1 and P1 were compared with the zone of inhibition produced by the standard pure drug. The formulation of S1 and P1 showed a zone of inhibition of 14.21±0.002 mm and 10.11±0.005 mm, respectively. So the formulation S1 had comparatively higher antifungal activity than formulations P1.

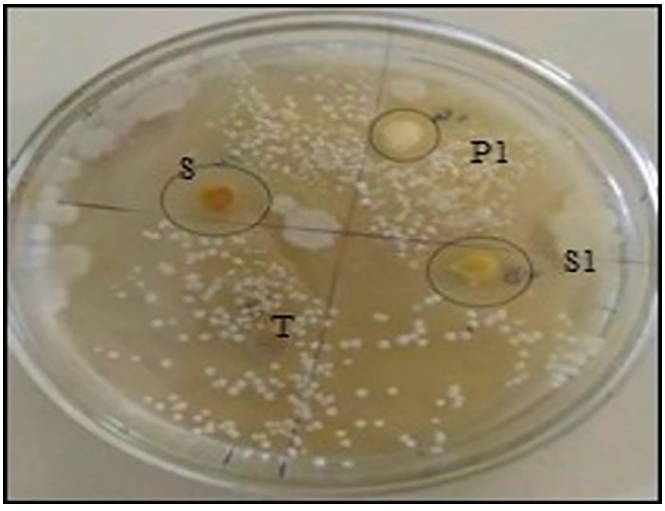

FIGURE 10 - Comparison of antifungal activity of formulation S1 and P1 against *Candida albicans* by cup plate method.

TABLE V - Kinetic models of formulations S1 to S6

Formulation code	Kinetic models								
	Zero order		First order		Higuchi		Korsmeyer-peppas		
	R^2	k	R^2	k	R^2	k	R^2	k	n
S1	0.960	-0.191	0.985	-0.0017	0.993	4.289	0.955	0.691	0.343
S2	0.961	-0.188	0.986	-0.0016	0.99	4.19	0.958	0.689	0.191
S3	0.971	-0186	0.985	-0.0015	0.986	4.13	0.963	0.687	0.174
S4	0.975	-0.184	0.986	-0.0014	0.982	4.06	0.968	0.686	0.157
S5	0.980	-0.178	0.983	-0.0013	0.978	3.94	0.973	0.684	0.142
S6	0.983	-0.177	0.980	-0.0013	0.972	3.88	0.977	0.683	0.125

TABLE VI - Kinetic models of formulations P1 to P6

Formulation code	Kinetic models								
	Zero order		First order		Higuchi		Korsmeyer-peppas		
	R^2	K	R^2	k	R^2	k	R^2	k	n
P1	0.978	-0.130	0.969	-0.0009	0.967	3.06	0.990	0.6615	0.051
P2	0.982	-0.122	0.984	-0.0008	0.969	2.88	0.995	0.6551	0.030
P3	0.989	-0.121	0.992	-0.0007	0.962	2.84	0.997	0.6576	-0.005
P4	0.993	-0.115	0.994	-0.0006	0.950	2.68	0.994	0.6544	-0.046
P5	0.993	-0.105	0.992	-0.0006	0.951	2.46	0.987	0.6453	-0.070
P6	0.996	-0.101	0.995	-0.0006	0.951	2.34	0.977	0.6448	-0.102

Ex vivo drug release studies

The ex vivo drug release studies of the optimized formulations (S1 and P1) were carried out using a modified ex vivo permeation apparatus through the pig membrane, and the results were given in figure 11. The release of the drug from the formulation S1 through pig membrane was found to be 99.3%, but the formulation P1 showed only 88.2%. The ex vivo drug release profile showed a better release of formulation S1 when compared to P1. The formulation S1 showed maximum drug release within ten h. The probable reason could be the stronger interaction between S1 formulation and lipids in the pig membrane, causing their rearrangement and thus facilitated drug penetration. This result was in agreement with the previous data reported by Staub (Staub, Schapoval, Bergold, 2005).

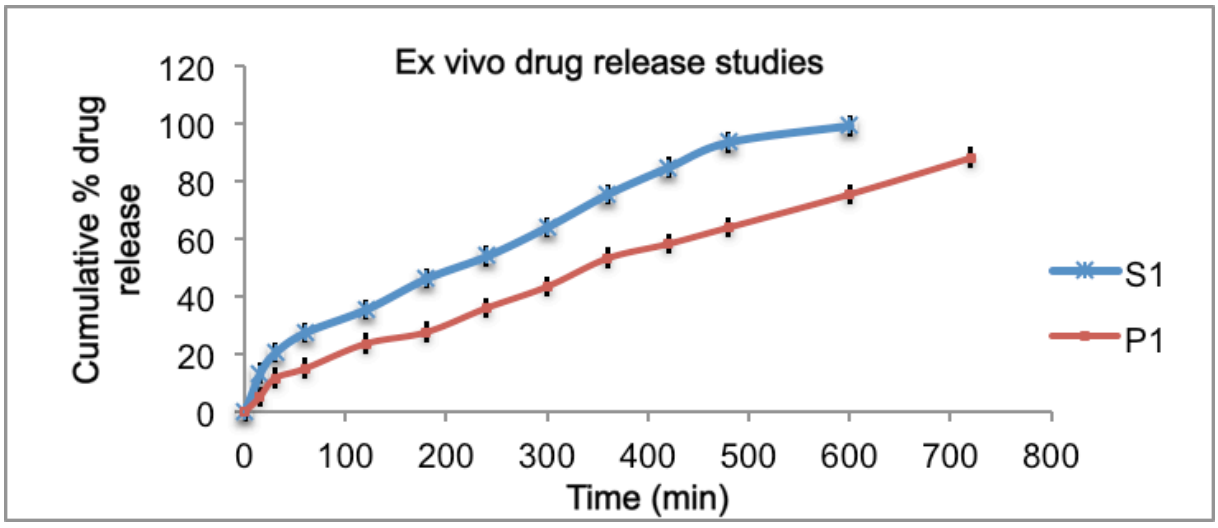

FIGURE 11 - *Ex vivo* drug release profile of formulation S1 and P1 through pig membrane.

Skin irritation studies

The result obtained from the skin irritation study was given in Table VII. The optimized formulations were applied to the skin of mice as per the procedure is given in methodology (Figure 12(a)) and observed for sensitivity reaction up to 48 h. There was no sign of development of erythema at the end of 24 h after application of formulations (Figure 12(b)). From the set 2 and set 5 animals, the test a animal showed slight, patchy erythema and graded as 0.5 at the end of 48 h (Figure 12(c)). The results obtained were graded according to the standard grading system, and the average primary irritation was calculated. The primary irritation index on each mouse was calculated. The average primary irritation index was found to be 0.027. The results indicated that optimized formulations S1 and P1 were compatible with skin (Staub, Schapoval, Bergold, 2005).

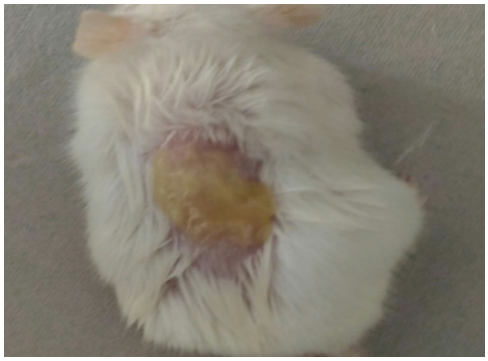

FIGURE 12(a) - Mice skin for observation of erythema at 0 h.

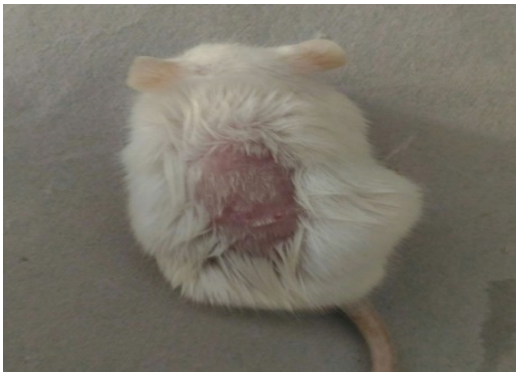

FIGURE 12(b) - Mice skin for observation of erythema at 24 h.

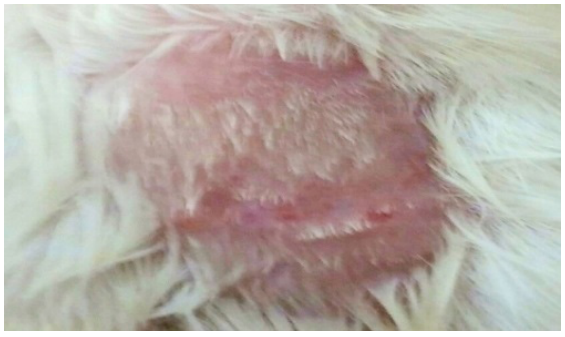

FIGURE 12(c) - Mice skin for observation of erythema after 48 h.

Stability studies

Stability studies were conducted for the optimized organogels for three months, and results were given in the Table VIII. The optimized formulations were evaluated for visual appearance, drug content, drug

TABLE VII - Results of skin irritation study

Formulation code	Study sample	Type	Parameter			Primary irritation index
			Erythema*			
			0 h	24 h	48 h	
S1	Set 1	Control	0	0	0	P11=0/3=0
		Test	0	0	0	P11=0/3=0
S1	Set 2	Control	0	0	0	P11=0/3=0
		Test	0	0	0.5	P11=0.5/3=0.16
S1	Set 3	Control	0	0	0	P11=0/3=0
		Test	0	0	0	P11=0/3=0
P1	Set 4	Control	0	0	0	P11=0/3=0
		Test	0	0	0	P11=0/3=0
P1	Set 5	Control	0	0	0	P11=0/3=0
		Test	0	0	0.5	PII=0.5/3=0.16
P1	Set 6	Control	0	0	0	P11=0/3=0
		Test	0	0	0	P11=0/3=0

* Average primary irritation index = 0 +0 + 0 + 0,16 +0 + 0 + 0 + 0 + 0 + 0,16 + 0 +0,12 = 0.027

release and pH. There was no significant variation in visual appearance, drug release, drug content and pH of both span 60 based and pluronic lecithin organogels. The results indicated that the optimized formulations were found to be physically and chemically stable throughout the study period. This result was in agreement with the previous data reported by Nesseem (Nesseem, 2001).

TABLE VIII - Stability studies of optimized formulations S1 and P1

Formulation code	Parameters evaluated	Storage temperatures	
		25+2 ºC±SD	40+2 ºC±SD
S1	Visual appearance	Yellowish creamy and no phase separation	
	Drug content*	99.46±0.002	99.98±0.012
	pH*	6.90.1±0.1	7.10±0.2
	% Drug release	99.4±0.001	100.01±0.002
P1	Visual appearance	Yellowish creamy and no phase separation	
	Drug content	99.25±0.101	99.97±0.002
	pH	7.1±0.1	7.1±0.1
	% Drug release	69.7±0.001	71.01±0.002

(*SD=Standard Deviation)

CONCLUSION

The development of safer and more effective drug delivery systems of antifungal agents has radically changed the treatment options of fungal infections. In this study, different gel-based systems were developed for amphotericin B and evaluated for its efficacy to prevent fungal infections. Based on the results, organogels prepared by sorbitan monostearate showed better antifungal activity, and also all the formulations were found to be stable and safe throughout the study period. Certainly, these findings can be applied for the treatment of topical fungal infections.

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CONFLICT OF INTEREST

The authors disclose no conflict of interest.

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