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FORMULATION AND EVALUATION OF MUCOADHESIVE THERMOSENSITIVE PLURONIC LECITHIN ORGANOGEL OF MICONAZOLE NITRATE FOR VAGINAL CANDIDASIS

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ABSTRACT

The present investigation deals with development of mucoadhesive thermosensitive pluronic lecithin organogel of miconazole nitrate for vaginal candidasis. To develop more effective treatment for vaginal candidasis, Miconazole 1% (MZN) was formulated as pluronic lecithin organogel (PLOs). Total of 8 formulation have been formulated F1-F8 by keeping the soya lecithin as constant upto 4% for all the formulation and then changing the concentration of carbopol 934 and pluronic f 127(by keeping carbopol 934 0.2% and pluronic f 127 15%-50% from F1-F4 and carbopol 934 0.8% and pluronic f 27 15%-50% from F5 to F8). For all the PLOs formulations composed of miconazole nitrate (1%), Spreadability, rheological behaviour, drug content (%), mucoadhesive force, gelling capacity and *in-vitro* release profiles of the different formulations were determined. In vivo antifungal activity of MZN, tested against Candida albicans vaginitis in agar plate, was significantly prolonged after vaginal delivery using PLOs. These results indicate that MZN-containing F4 formulation of vaginal PLOs are safe, convenient and provide effective treatment of vaginal candidasis with reduced dosing interval.

Key words: Vaginal candidasis, Miconazole nitrate, Mucoadhesive thermosensitive pluronic lecithin organogel.

INTRODUCTION

Vaginal candidasis is caused by the overgrowth of a fungal species, *Candida albicans*, in the vaginal flora. The symptoms of vulvovaginal candidasis include pruritus (itching), soreness, change in vaginal discharge, and dyspareunia and can disrupt sexual and social functioning [1,2].

PATHOGENESIS

The infective organism is a fungus that reproduces by budding:

- 90% are due to *Candida albicans*.
- 5% are due to *Candida glabrata*.

Other fungal infections of the vagina are caused by sacchromyces cereviciae (brewer's yeast) and rarely, Trichosporon spp.Candida is a normal commensal organism in the vagina. Pathological infection usually follows a change in the local environment or a decrease in the host's susceptibility to infection.However, recent research suggests that symptomatic candidasis is due to an exaggerated immunological response to the presence of candida rather than a failure of immune mechanisms [3].

Miconazole, a synthetic imidazole derivative, exhibits a broad spectrum of antimicrobial activity for the use in the systemic treatment and local treatment of vaginal and topical fungal infections. It is particularly active against Candida species, Trichophyton species, Epidermophyton species and Microsporum species as well as possessing some activity against gram-positive bacteria.

The exact mechanism of miconazole's antifungal activity has not been fully established. The primary site of

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action appears to be the cell membrane. Studies with C. albicans indicate that at low concentrations, miconazole acts primarily on the yeast cell membrane resulting in selective inhibition of the uptake of precursors of RNA and DNA (purines) and mucopolysaccharides (glutamine). Depending on dose and duration of exposure, yeast cells show progressive degradation of cytoplasmic organelles and the cell wall. Growth, cell permeability and respiration of C. albicans are inhibited at low miconazole concentrations. These observations indicate that miconazole may have multiple actions and/or pathways to inhibit and/or kill microbial cells.

ORGANOGELS

An organogel is a non-crystalline, non glassy thermo reversible (thermoplastic) solid material composed of a liquid organic phase entrapped in a three-dimensionally cross-linked network. The liquid can be, for example, an organic solvent, mineral oil, or vegetable oil. The solubility and particle dimensions of the structurant are important characteristics for the elastic properties and firmness of the organogel. Often, these systems are based on self-assembly of the structurant molecules [4].

MATERIALS

Miconazole nitrate from strides lab, Soya lecithin from rolex chemicals ltd, Pluronic f 127 from s d fine chemicals, Sorbic acid, Isopropyl myristate, Carbopol 934 from rolex chemical ltd.

COMPATIBILITY STUDIES

FTIR STUDIES (FOURIER TRANSFORMED INFRARED)

Instrument used was Shimadzu FTIR-8700 spectrophotometer. In this study, potassium bromide disc method was employed. IR study of pure drug, physical mixtures and polymer were done. The powdered sample was intimately mixed with dry powdered potassium bromide. The mixture was then compressed into transparent disc under high pressure using special dies. The disc was placed in IR spectrophotometer using sample holder and spectrum was recorded from 4000 to 500cm^{-1}.

METHODS

FORMULATION OF PLURONIC LECITHIN ORGANOGEL

Pluronic lecithin organogel were prepared by cold method technique which requires aqueous phase and oil phase both are mixed by mechanical stirrer with slowly.

Method of preparation of Pluronic lecithin organogel

PLOs were prepared by using the cold method. Aqueous phase were prepared by dispersing Carbopol 934 in citrate-phosphate buffer (0.1 M, pH 4.0) at 4^{O}C with gentle mixing. Pluronic f 127was then added to carbopol 934 solutions and allowed to dissolve over night at 4^{O}C. Oil phase was prepared by dissolving soya lecithin and Sorbic acid in appropriate quantity of isopropyl myristate. The mixture was kept overnight at 4ºC in refrigerator for complete dissolution of its constituents. Miconazole nitrate was initially dissolved in the mixture of methanol and polyethylene glycol (PEG) 400 (3:5) and mixed with lecithin isopropyl myristate solution., Finally aqueous phase (70%) was slowly added in oil phase (30%) in with stirring at 400 rpm using mechanical stirrer [5].

EVALUATION OF GEL FORMULATIONS

DETERMINATION OF MUCOADHESIVE FORCE

The mucoadhesive force of organogel on vaginal mucosal tissues was determined by means of mucoadhesive force measuring apparatus, fabricated in our laboratory.Vaginal mucosal tissues were removed from Sprague-Dawley rats and tissue were stored frozen in phosphate buffer at pH 5.5, and thawed to the room temperature before use. At the time of testing, a section of tissue was secured (keeping the mucosal side out) to the upper side of a glass vial using a cyanoacrylate adhesive. The diameter of each exposed mucosal membrane was1.5 cm. The vials were equilibrated and maintained at 37°c for 10 min. One vial with a section of tissue was connected to the balance and the other vial was fixed on a height adjustable pan. To the exposed surface of the tissue attached on the vial, a constant amount of 0.1 g organogel was applied. Before applying the organogel, 150µl of simulated vaginal fluid was evenly spread on the surface of the test membrane. The height of the vial was adjusted such that the organogel could adhere to the mucosal surface of both vials. Immediately, a constant force of 0.5 N (Newton) for 2 min was applied to ensure intimate contact between the tissue and the sample. The upper vial was then moved upwards at a constant force, while it was connected to the balance. Weights were added at a constant rate to the pan on the other side of the modified balance until the two vials were separated. The mucoadhesive force, expressed as the detachment stress in dynes/cm^2, was determined from the minimal weights needed to detach the tissues from the surface of each formulation, using the following Eqn.

Detachment stress (dynes/cm^2) = (m×g)/a,

Where ‘m’ is the weight added to the balance in grams; ‘g’ is the acceleration due to gravity taken as 980 cm/s^2; and ‘a’ is the area of tissue exposed.

Effect of varying contact time (1, 2, 3, 5 and 10 min) was investigated for some of the organogel preparations to optimize initial contact time. In brief, formulations were allowed to be in contact with mucosa for carrying contact time (1, 2, 3, 5, and 10 min.), and the mucoadhesive force was determined as discussed above. Contact time that resulted in maximum mucoadhesive strength was selected as optimum contact time required for adequate adhesion. All the above mentioned experiments were carried out in triplicates.

PH OF THE GELS

The pH of gel was determined after diluting and dispersing it in distilled water using digital pH meter [6].

SPREADABILITY

For the determination of Spreadability, excess of sample was applied between the two glass slides and was compressed to uniform thickness by placing 1000gm weight for 5 min. Weight (50gm) was added to the pan. The time required to separate the two slides, i.e., and the time in which the upper glass slide moves over the lower plate was taken as measure of Spreadability (S).

$$S=M\times L/T$$

Where M=weight tide to upper slide, L=length moved on the glass slide, T=time taken

GELLING CAPACITY

The gelling capacity was determined by placing a drop of the system in a vial containing 2ml of simulated vaginal fluid (pH 5.5) freshly prepared and equilibrated at 37°C and visually assessing the organogel formation and noting the time for gelation and the time taken for the organogel formed to dissolve. Different grades were allotted as per the organogel integrity, weight and rate of formation of organogel with respect to time.

VISCOSITY

The viscosity of the different gel formulations were determined by using a Brookfield viscometer with spindle no 7 at 50 rpm [7].

DRUG CONTENT

1gm of gel was accurately weighed dissolved using 10ml of 0.1m HCL and 50 ml of isopropyl alcohol, sonicated for a period of 10-15mins and made up to the mark in 100 ml volumetric flask with isopropyl alcohol. From this 10 ml was pipette out and diluted to 100 ml with isopropyl alcohol and the final dilution was made using isopropyl alcohol to get a concentration within Beer's range. The absorbance was measured spectrophotometrically at 272nm against blank gel treated in the same manner as sample.

IN-VITRO RELEASE STUDIES

In-vitro studies of the gel were carried out across the egg membrane extracted by using the concentrated HCL. The receptor compartments were filled with 0.1M citrate- phosphate buffered saline (PBS) pH 5.5, Study was carried out using excised egg membrane. The entire setup was placed on a thermostatic magnetic stirrer and the temperature was maintained at 37^0C throughout the study. Release studies were carried out over a period of 24 hrs at regular intervals. Samples were withdrawn and analyzed spectrophotometrically at 272 nm.

ANTIFUNGAL EFFICACY STUDIES

The antifungal efficacy study against Candida albicans was determined by agar diffusion method employing 'cup plate technique'. Sterile solutions of miconazole nitrate in DMSO (standard solution) and the developed organogel having the pH adjusted to 7.0 were poured into cups (0.1 ml of 0.1% w/v) bored into sterile malt yeast agar previously seeded with test organism. After allowing diffusion of the solutions for 2 h, the agar plates were incubated at 37°Cfor 48 h. The zone of inhibition (ZOI) was measured and compared with that of pure drug. The entire operation was carried out in aseptic condition throughout the study. Each solution was tested in triplicate. Both positive and negative controls were maintained throughout the study.

RESULTS

COMPATILITY STUDIES

By comparing the FTIR spectra of pure miconazole nitrate, with its physical mixture it was observed that there was no considerable difference in their spectral values. This was justified by the presence of two peaks of N-H stretch and aliphatic C-H stretch at around 2829 cm^{-1} and 2891 cm^{-1} respectively in all the mixtures. Similarly, C-CL stretch and C-N stretch were seen at around 823 cm^{-1} and 1101 cm^{-1} respectively in all the mixtures. Since there is no change in the nature and position of the bands it was concluded that the drug maintains its identity without going any chemical interaction with the polymers used.

The prepared pluronic lecithin organogel formulations were characterized on the basis of Spreadability, rheological behaviour, drug content (%), Mucoadhesive force, gelling capacity and in vitro release profiles. The prepared pluronic lecithin organogel formulations were white to pale yellow viscous creamy preparation with a smooth and homogenous appearance. The pH values of the prepared formulation ranged from 5.9 to 6.6, which are considered acceptable to avoid the risk of irritation on application to the skin as pH of skin 5.5.

The values of the Spreadability indicate that the organogel is easily spreadable by small of shear. The Spreadability of all formulations was found to be from 10.10 to 12.90. Viscosity determinations of the prepared organogel were carried out by Brookfield viscometer. The high viscosity was found in organogel contain high level of the carbopol 934 concentration.

The drug content in organogel was found in range of 93.5 % to 99.4 %. The higher drug content found in F4 i.e. 99.4% due to the optimum concentration of pluronic F127 and soya lecithin. The *in vitro* release profiles of miconazole from its various organogel formulations are represented in Table 4. Higher drug release was observed with formulations F4. This finding may be due to presence of optimum level of carbopol 934 (0.8%) and soya lecithin (4%).

ANTIFUNGAL EFFICACY STUDIES

MIC ranges (mg/ml) was 0.5-16. No intrazonal growth was observed in the antifungal method. Inhibition zones diameters ranged from 5 to 35mm. Interclass correlation coefficients(ICCs) and 95% confidence intervals for comparing the methods were calculated using log2 transformed data.

Figure 1: FTIR of pure miconazole nitrate

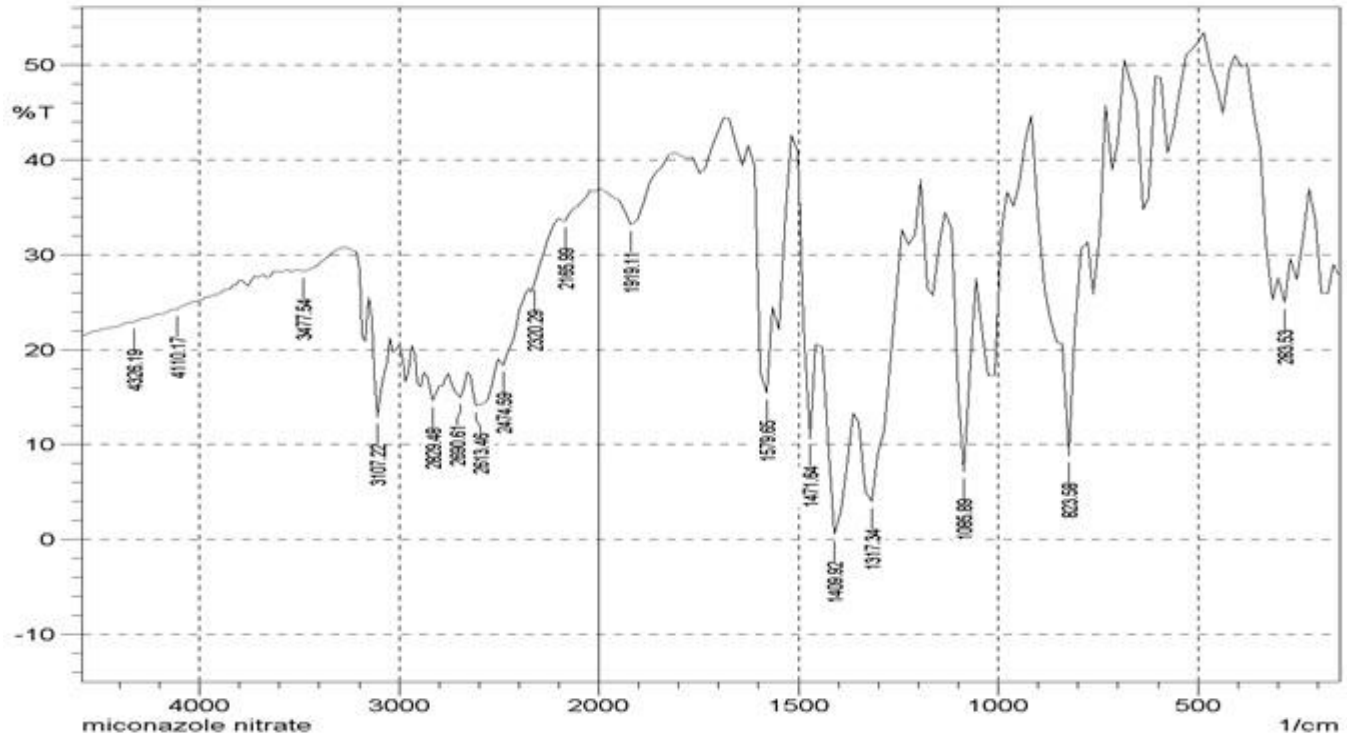

Figure 2: FTIR of Carbopol 934

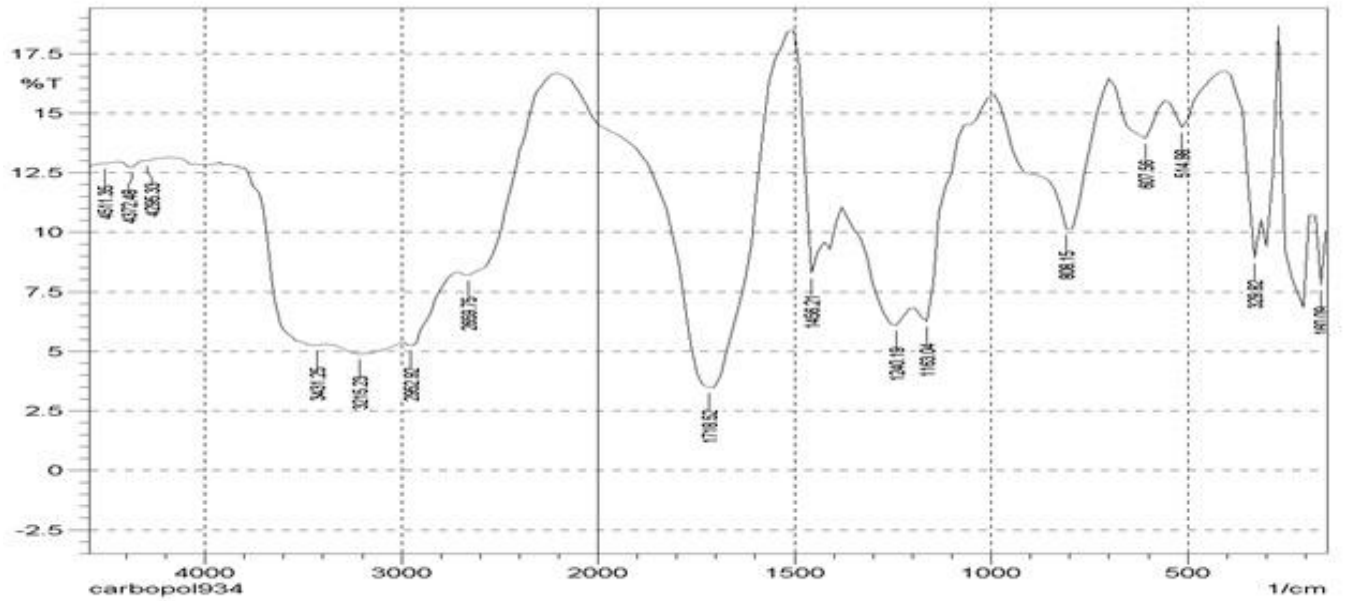

Figure 3: FTIR of Pluronic f 127

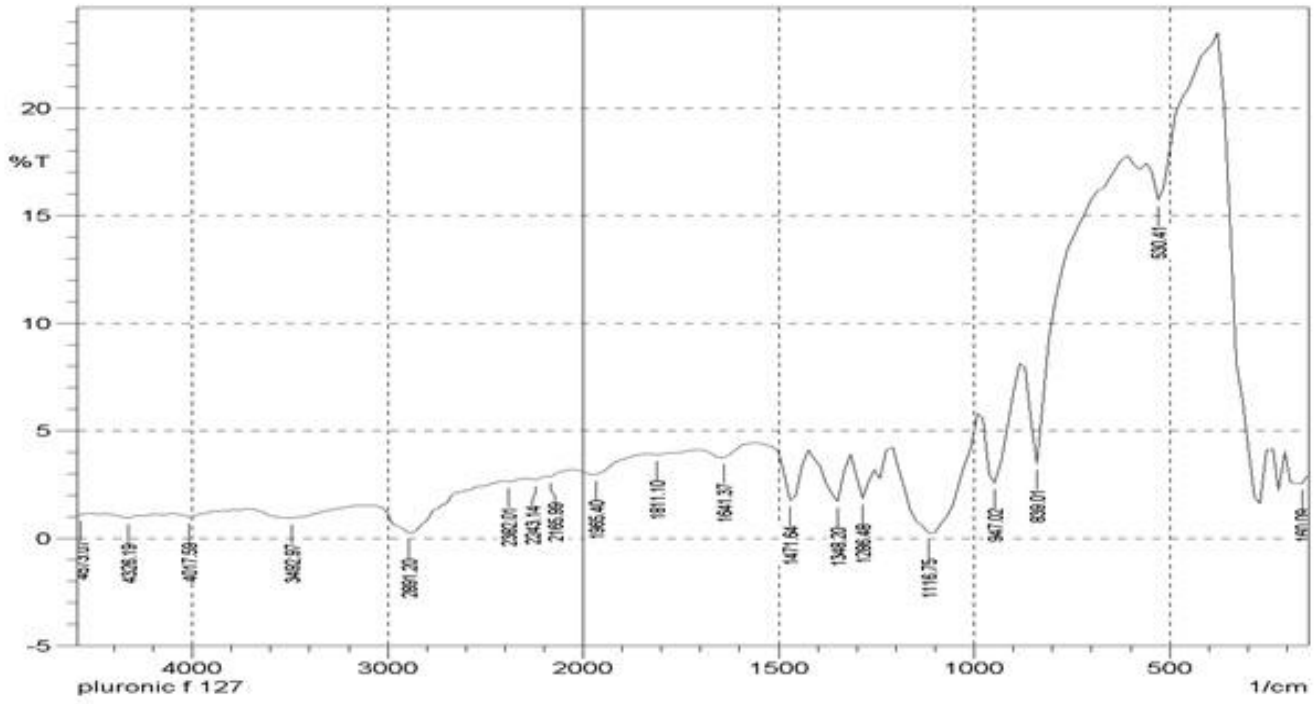

Figure 4: FTIR of physical mixture of Miconazole nitrate, Carbopol 934 and Pluronic f 127.

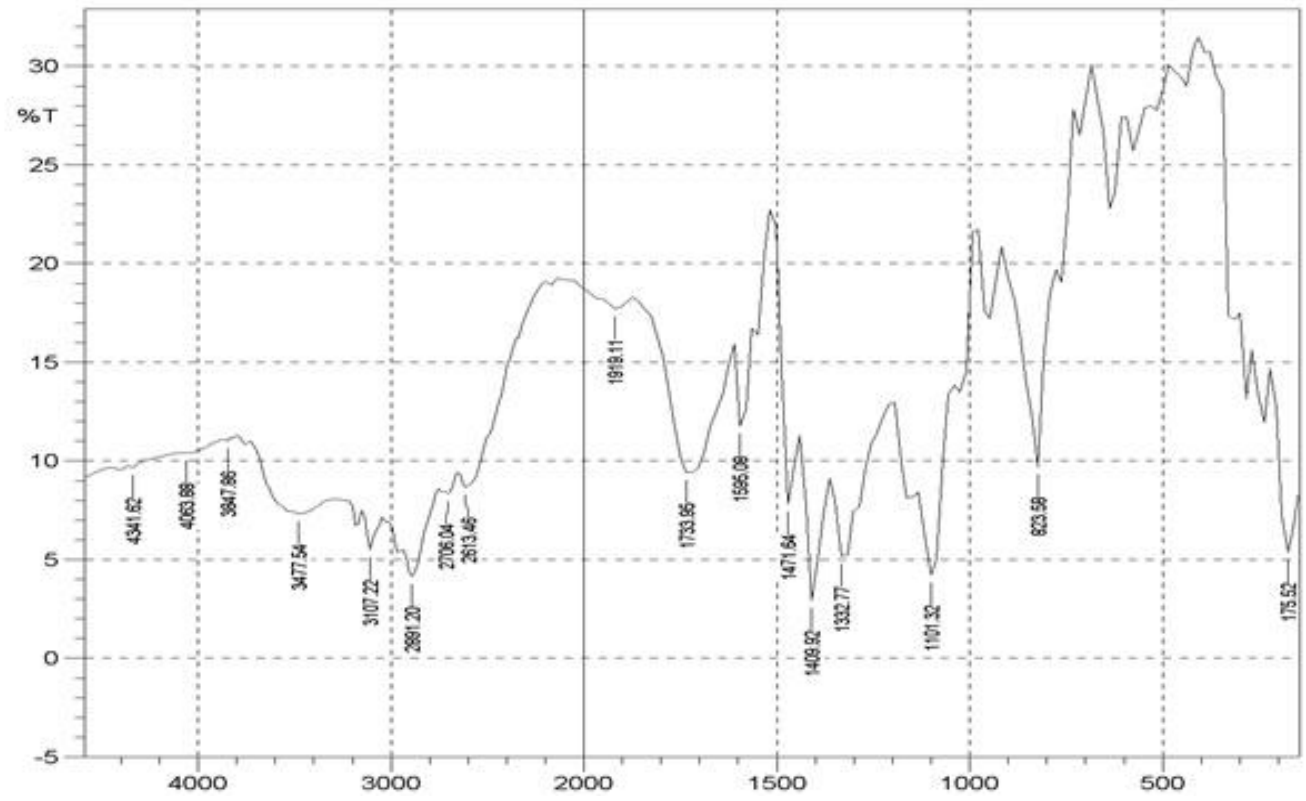

Figure 5. Viscosity of organogel formulations

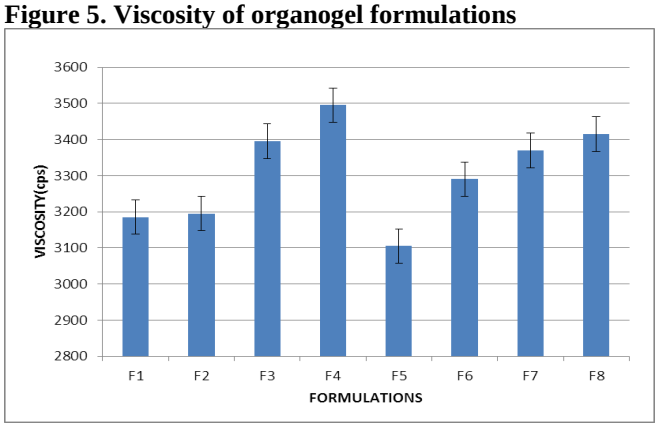

Figure 6. Spreadability of organogel formulations

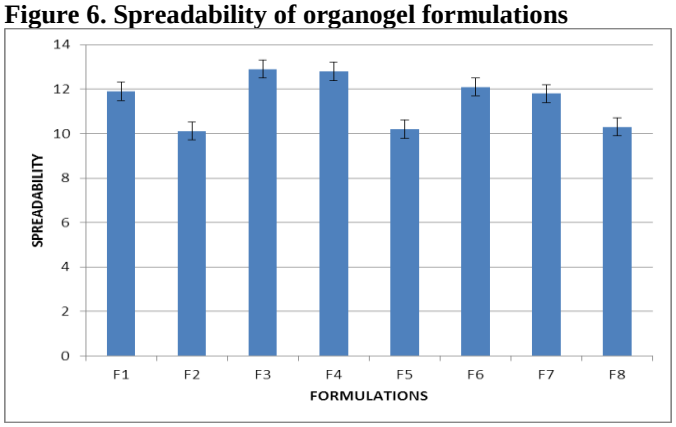

Figure 7. Mucoadhesive force of organogel formulations

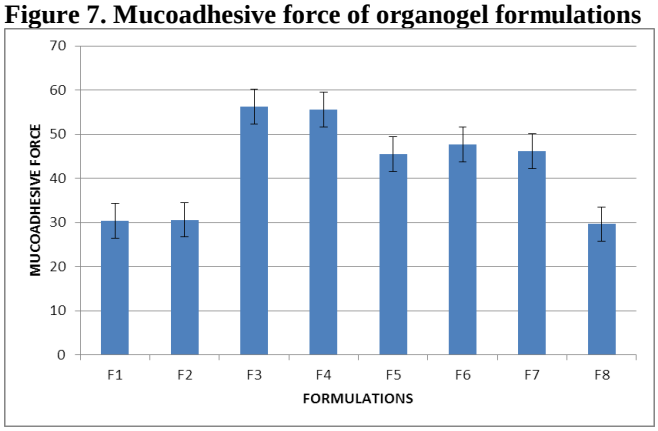

Figure 8. Drug content of organogel formulations

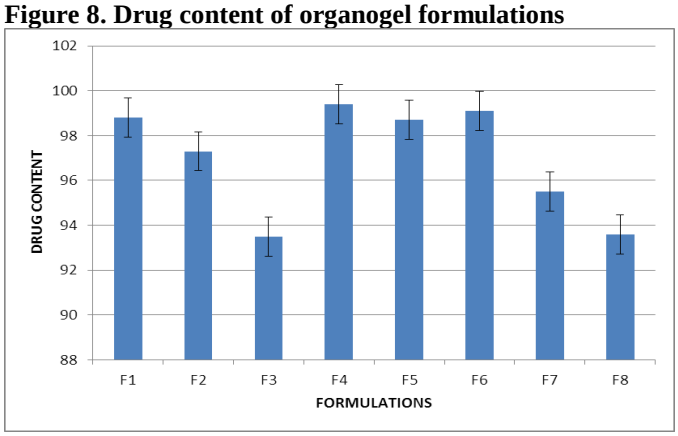

Figure 9. % Cummulative drug release of organogel formulations (F1-F8)

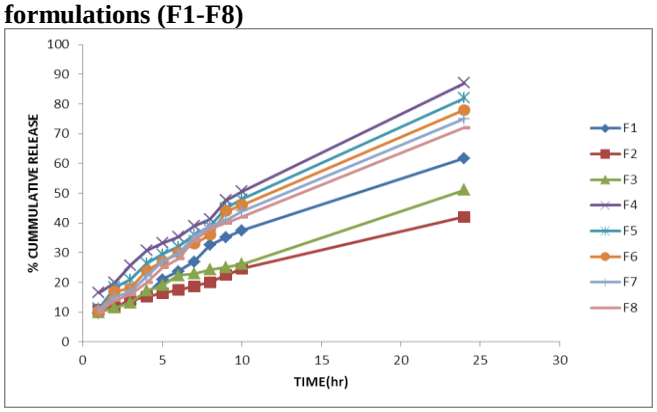

Figure 10. Antifungal efficacy study against candida albicans by agar diffusion method employing cup plate technique A: Front view

Table 1. Composition of various formulations

FORMULATION	Miconazole (%)	Soya Lecithin (%)	Carbopol 934 (%)	Pluronic F127 (%)	Isopropyl Myristate Upto (ml)	Sorbic Acid (%)	Water upto (ml)
F1	1	4	0.2	15	100	0.2	100
F2	1	4	0.2	25	100	0.2	100
F3	1	4	0.2	35	100	0.2	100
F4	1	4	0.2	50	100	0.2	100
F5	1	4	0.8	15	100	0.2	100
F6	1	4	0.8	25	100	0.2	100
F7	1	4	0.8	35	100	0.2	100
F8	1	4	0.8	50	100	0.2	100

EVALUATION OF PLURONIC LECITHIN ORGANOGEL

Table 2. Characteristics of various miconazole nitrate organogel formulations

Formulations	pH*	Viscosity* (cps)	Spreadability (g.cm/s)
F1	6.3	3185±15	11.90±0.5
F2	5.9	3195±60	10.12±0.3
F3	6.4	3395±45	12.90±0.4
F4	6.6	3495±35	12.80±0.4
F5	6.5	3105±25	10.20±0.4
F6	6.2	3290±20	12.10±0.1
F7	6.0	3370±25	11.80±0.6
F8	6.2	3415±45	10.30±.07

*Average of three determination.

TABLE 3. Characteristics of various miconazole nitrate organogel formulations

Formulations	Drug Content (%W/W)	*Mucoadhesive Force (dynes/ cm^2)	Gelling Capacity
F1	98.8±0.49	30.9±2.8	+
F2	97.3±0.68	30.6±0.7	+
F3	93.5±.044	56.3±5.3	+++
F4	99.4±0.75	55.6±4.2	+++
F5	98.7±0.55	45.5±1.2	++
F6	99.1±0.47	47.7±1.5	++
F7	95.5±0.33	46.2±2.4	++
F8	93.6±1.44	29.6±1.0	+

*Average of three reading; + gel after few minutes, dissolved rapidly; ++gelation immediately, remains for few hours; +++ gelation immediately, remains for extended period.

Table 4. Percentage Drug Release from Organogel Formulations (F1-F8)

Time [h]	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	9.76±0.37	10.83±0.29	10±0.57	16.5±0.73	11.3±0.31	10.5±0.3	11±0.66	10±0.54
2	11.88±0.3	11.71±0.45	11.5±0.45	20±0.38	18.3±0.39	17±0.51	15±0.76	14±0.35
3	13.21±0.4	14.1±0.31	13.2±0.58	25.8±0.34	21±0.18	18±0.59	17±0.44	16±0.54
4	16.09±0.3	15.1±0.40	17.2±0.69	30.7±0.25	26.4±0.89	24±0.73	22±0.62	20±0.84
5	21±0.35	16.4±0.46	19±0.54	33.2±0.12	29.4±0.82	27±0.83	27±0.41	25±0.94
6	23.74±0.5	17.4±0.29	22.3±0.48	35.3±0.67	32±0.64	30±0.6	29.4±0.64	28±0.15
7	26.9±0.9	18.72±0.29	22.9±0.55	38.9±0.64	36±0.53	33±0.9	35.5±0.58	34±0.19
8	32.5±0.5	20.03±0.38	24.3±0.72	41.3±0.59	39±0.42	36±0.41	39±0.64	38±0.51
9	35.22±0.3	22.52±0.47	25.12±0.7	47.6±0.49	45±0.17	44±0.71	41±0.76	40±0.23
10	37.48±0.3	24.65±0.39	26.13±0.8	50.6±0.43	48±0.83	46±0.42	44±0.85	42±0.11
24	61.7±0.41	42.06±0.36	51.06±0.3	87±0.11	82±0.19	78±0.49	75±0.69	72±0.78

CONCLUSION

The present study was to design, develop and evaluate the pluronic lecithin organogel incorporated gel for topical controlled drug delivery of miconazole nitrate for extended release. Miconazole nitrate is easily inactivated by the gastric environment and have less absorption from the GIT. The study design focused on standardization of process parameters and formulation parameters involved in the preparation of miconazole pluronic lecithin organogel by cold method. Carbopol 934 was used as a polymer to exhibit mucoadhesive property and Pluronic f 127 used as a thermosensitive polymer, soya lecithin as a surfactant, methanol and polyethylene glycol-400 as solvents in the oil phase, water as aqueous phase. By compatibility studies it was found there was no interaction between the drug and excipients. The best standardized F4 formulation showed good mucoadhesive force, gelling capacity, pH, drug content and viscosity**.** The best formulation F4 was incorporated into gels and gels were evaluated for physical parameters and showed controlled release upto 24 hrs.

Thus it was concluded that the selected antifungal drug can be developed into organogel. The data in this study support the potential effectiveness of a vaginal gel with mucoadhesive properties to ensure longer residence time in the application site because of prolonged release properties controlled release of the incorporated drug is achieved, suggesting better patient compliance and higher therapeutic efficacy.

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