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ORIGINAL ARTICLE **OPEN ACCESS**

Formulation and Evaluation of Antifungal Topical Gel by Applying Full Factorial Design

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ABSTRACT
The aim of the present study was to develop an antifungal gel formulation for topical delivery of Miconazole Nitrate. Miconazole Nitrate is broad-spectrum azole antifungal drug. The Miconazole Nitrate antifungal topical gel were designed by employing carbopol grades (carbopol 934 and carbopol 940) in combination. In present research 3^2Full factorial design has been applied to study effect of carbopol grades on quality attributes of Miconazole Nitrate topical gel formulation. Miconazole Nitrate topical gel formulation batches (F1-F9)have been formulated as per runs obtained in factorial design and further evaluated for pH, viscosity, spredability, extrudability, drug content, in vitro drug diffusion study etc. In drug diffusion study at the end of 8 hours F1 batch showed faster drug release with lowest viscosity among all the batches and vice versa result have been observed with batch F9. Both dependent variables showed carbopol 940 quite more responsible for increasing viscosity and extending drug release.
Keywords: *Miconazole Nitrate, Carbopol 940, Carbopol 934, Factorial design, topical gel*

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INTRODUCTION

There are two types of medication preparations that can be applied topically to the skin. Products that are used for local action fall under this category. The active ingredients either remain on the skin's surface or penetrate into the epidermal layers in this case. Although they may reach the dermis, they are not absorbed into the blood stream. This is a category defines as topical drug delivery system. By passing through the various layers of the skin, the active ingredients are delivered into the general blood circulation, where they produce a therapeutic response. [1,2,3] They treat local dermatological conditions by delivering drugs locally rather than systemically. Corticosteroids, antifungals, antivirals, antibiotics, antiseptics, local anesthetics, and antineoplastics are examples of drugs delivered topically.[2,4,5]

Topical drug delivery has various benefits over other modes of administration. The fundamental advantage of topical distribution is that when the topical medicine is administered to the afflicted tissues, it has low systemic adverse effects. Second, by transporting the active via the skin, topical drug delivery devices avoid first pass metabolism. This prevents the active's metabolism before it reaches the place of action. Topical products are simple to use and appropriate for self-medication, which can improve patient compliance.[6]Miconazole Nitrate is an azole antifungal that is used to treat a range of diseases, including Candida overgrowth. Miconazole Nitrate is unique among azoles in that it is expected to work via three distinct pathways. The principal mechanism of action is suppression of the CYP450 14- lanosterol demethylase enzyme, which results in altered ergosterol synthesis and reduced cell membrane composition and permeability, resulting in cation, phosphate, and low molecular weight protein leakage. Furthermore, miconazole inhibits fungal peroxidase and catalase while having no effect on NADH oxidase activity, resulting in an increase in the formation of reactive oxygen species (ROS) [7,8]. Increased intracellular ROS causes pleiotropic effects and eventually, apoptosis. Finally, miconazole increases intracellular farnesol levels, most likely due to suppression of lanosterol demethylation. This molecule contributes in quorum sensing in Candida, inhibiting the shift from yeast to mycelial forms and, as a result, the creation of antibiotic-resistant biofilms. Farnesol is also an inhibitor of drug efflux ABC transporters, namely Candida CaCdr1p and CaCdr2p, which may contribute to the enhanced efficacy of azole treatments. [9,10]

The oral use of Miconazole Nitrate is not recommended as it has many side effects. Miconazole Nitrate topical gel formulation is made for better patient compliance and to reduce the dose of drug and to avoid the side effects like liver damage and kidney damage.[10]In this research work we implemented factorial design in which effect of multiple factors are investigated simultaneously. A 3^2 factorial design was

applied to study the effect of carbopol grades on quality attributes of Miconazole Nitrate antifungal gel formulations.

MATERIAL AND METHODS

Carbopol 934, carbopol 940 and triethanolamine (Research–Lab Fine Chem. Industries, Mumbai), Miconazole Nitrate (Yarrow chem. Product Dombivali, Mumbai), ethanol(SD. Lab. Chemical, Mumbai), Distilled water were used throughout the experiment (Satara college of pharmacy, Satara).

METHODS

PREFORMULATION STUDY

FTIR studies were performed to investigate the compatibility between drug and polymer.

FACTORIAL DESIGN

Factorial design is the most efficient way to study two or more factors in an experiment. In this study, there are 2 factors and 3 levels, is called as 3^2 factorial designs. Carbopol 934 and Carbopol 940 were selected as independent variables and viscosity and drug release were selected as responses. The levels of the two components were chosen based on the preliminary research conducted before to performing the experimental design. Throughout the study, all other formulations and processing factors were remained constant. The table summarizes the translation of coded levels to experimental units and runs, as well as the factor combinations used in the experiment.[11]

PREPARATION OF GEL

From the 3^2 factorial design 9 gel formulation batches were prepared by following method. Stated quantity of polymer (Carbopol 934 and Carbopol 940) were weighed (Table No. 1) and was sprinkled on surface of water and allowed to sock for 24 hours. With incessant stirring, triethanolamine was added as a neutralizer to adjust the pH of the gel. Miconazole is dissolved in suitable quantity of ethanol which act as a stabilizer and mixed with above prepared gel.

EVALUATION

a) pH

pH of prepared gels (F1-F9) was determined using a digital pH meter(pH system 362) by placing the glass electrode completely into the gel formulations.

b)VISCOSITY

The viscosity of gel formulations (F1-F9) was determined by using a Brookfield viscometer(DVE model) with spindle LV4 at temperature 25±3 ^oC.

c) SPREADABILITY

To evaluate the spreadability of the prepared gel formulations (F1-F9), 1 g of gel was put in the centre of the glass plate. Another glass plate with the same size was placed on top of this one. After that, a weight of 1000 g was gently put to the upper side of the plate, causing the gel to spread out in between the plates. The weight was removed after one minute, and the diameter of the spread area (cm) was measured.[12]

d)EXTRUDABILITY

A closed collapsible tube carrying about 20g of prepared gel formulations (F1-F9) was firmly pressed at the crimped end. The cap was removed, and the gel was extruded. The extruded gel was collected and weighed. The percent extruded gel was determined.[13]

$$\% \text{ Extrudability} = \frac{\text{Amount of sample extruded}}{\text{Initial weight of sample}} \times 100$$

E) Drug Content

Drug content of prepared gel formulations (F1-F9) was determined. A gram of gel was precisely weighed and put in a perfectly closed volumetric flask with 10ml of methanol. After shaking the flasks for 10 minutes, 100ml of the prepared phosphate buffer with a pH of 7.4 was added and sonicated. The absorbance of the above solution was measured at 272nm against a suitable blank solution using a spectrometric analysis(Spec UV–1700).[13]

f) IN-VITRO DRUG DIFFUSION STUDY

A Franz diffusion cell was used for the in-vitro drug release study of prepared formulations (F1-F9). The formulation was applied to the surface of a cellophane membrane that was sandwiched between the donor and receptor compartments of a Franz diffusion cell. As a diffusion medium, phosphate buffer pH 7.4 was utilised. A flowing water jacket kept the temperature of the cell at 37^0C. The entire assembly was placed on a magnetic stirrer, and the solution was continually stirred with a magnetic bead. At appropriate time intervals, the sample was withdrawn and diluted up to 10ml with the same solvent. The cumulative percent drug release was estimated after spectrophotometric analysis(Spec UV-1700) of the samples at 272 nm.[13,14]

G) ANTIFUNGAL ACTIVITY

Antifungal activity of optimized gel formulation (F1) was done by cup and plate method against the standard test microorganism (*Candida albicans*).

Table No. 1 Composition of topical gel

Sr. No.	Ingredients	Formulations								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Carbopol934 (gm)	0.2	0.2	0.2	0.5	0.5	0.5	0.8	0.8	0.8
2	Carbopol940 (gm)	0.2	0.5	0.8	0.2	0.5	0.8	0.2	0.5	0.8
3	Ethanol (ml)	3	3	3	3	3	3	3	3	3
4	Miconazole (gm)	2	2	2	2	2	2	2	2	2
5	Triethanolamine (ml)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
6	Distilled Water (ml)	100	100	100	100	100	100	100	100	100

Table No. 2 Physical characteristics of topical gel

Formula	pH	Viscosity (cps)	Spredability (cm)	Extrudability (%)	Drug content(%)
F1	5.64±0.2	3542±3	12.7±0.2	97.6±0.31	99.33±0.31
F2	5.8±0.3	3740±2	12.4±0.3	96.6±0.37	98.72±0.22
F3	5.9±0.03	4286±2.5	12.2±0.1	92.92±0.34	98.93±0.29
F4	5.25±0.2	3742.6±4	11.8±0.1	92.44±0.25	97.85±0.24
F5	5.88±0.2	3998±2	11.7±0.2	88.93±0.33	98.57±0.37
F6	5.26±0.2	4389.3±4	11.6±0.2	86.16±0.15	97.3±0.22
F7	5.80±0.03	4223.6±2	10.8±0.1	87.35±0.32	97.72±0.24
F8	5.70±0.2	4547.3±2	10.4±0.1	85.01±0.2	98.21±0.1
F9	5.70±0.2	5179.3±2.5	10.4±0.3	85±0.27	97.93±0.13

*n=3

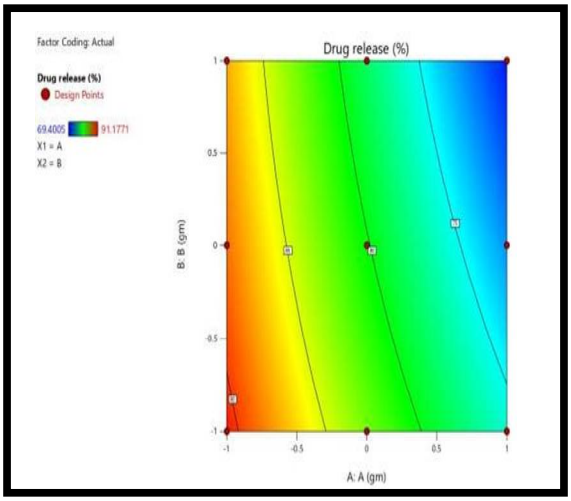

Fig 1 Contour plot of Drug release of carbopol 934 and carbopol 940

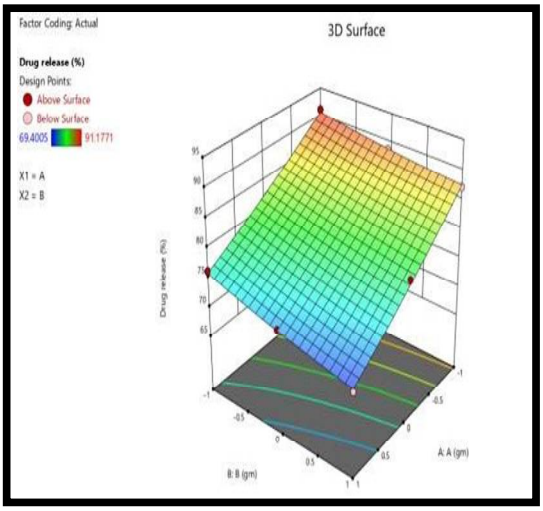

Fig 2 3D surface plot of Drug release of carbopol 934 and carbopol 940

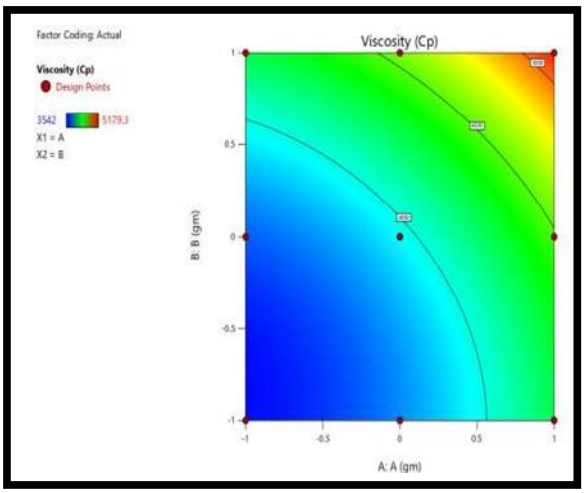

Fig 3 Contour plot of viscosity of carbopol 934 and carbopol 940

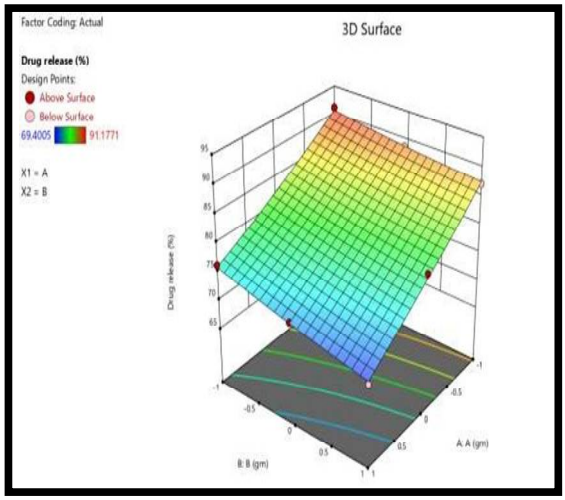

Fig 4 3D surface plot of viscosity of carbopol 934 and carbopol 940

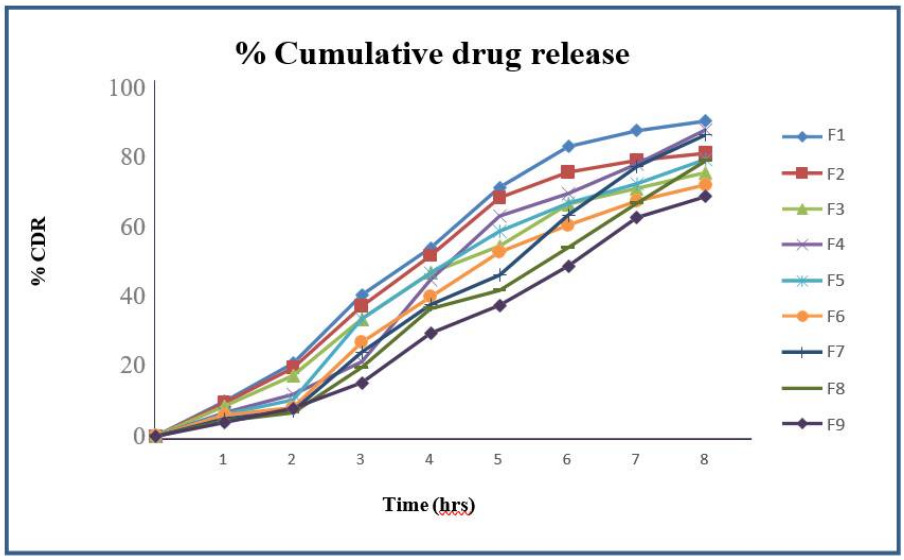

Fig 5 % Cumulative drug release of all formulations F1-F9

Fig 6 Antifungal activity of formulation, control and standard

RESULT AND DISCUSSION

In FTIR study Miconazole Nitrate showed characteristic peaks C-Cl stretching at 783.28 cm^{-1}, C=C stretching at 1041.77 cm^{-1}, C-O stretching at 1088.9 cm^{-1}, C-N stretching at 1641.13 cm^{-1}. Similar peaks were observed in spectra of drug and polymer in combination which indicated no interaction between drug & polymer and they compatible with each other. Miconazole Nitrate antifungal topical gel was developed successfully using a combination of carbopol 940 and carbopol 934. The various evaluation tests of all formulations like pH, viscosity, extrudability, spredability, drug content, invitro drug diffusion and antifungal test were evaluated successfully. Selection of carbopol grade is the measure key factor in deciding viscosity and drug release from gel[1-4]. It was discovered that carbopol 934 had a significant effect on extending Miconazole Nitrate release from gel formulations when combined with carbopol 940. (F1, F4, and F7; F2, F5, and F8; F3, F6, and F9). It was discovered that carbopol 940 showed quite more positive effect than carbopol 934 that means carbopol 940 is quite more responsible for increasing viscosity and extending drug release with increase inconcentration [5-9]. (Fig 1-4).

Physical characteristics like pH, viscosity, extrudability, spredability, drug content, and invitro drug diffusion of the optimized gel formulation (F1) were found to be pH is 5.64 ± 0.2, viscosity is 3542 ± 3 Cp, spredability is 12.7 ± 0.2 cm, extrudability is 97.6± 0.31 % and drug content is 99.33± 0.31 %. In-vitro drug diffusion of optimized F1 formulation was found to be 91.1771± 0.12 % at the end of 8 hours. The viscosity was found to be increased with increment in the amount of carbopol 940 and extended in drug release. The viscosity of batches F1, F4 and F7 was found to be 3542± 3, 3742.6± 4 and 4223.6± 2 cp respectively. The percentage drug release of batches F1, F4 and F7 was found to be 91.1771± 0.12, 88.6644 0.13 and 87.1568±0.12 % respectively. The viscosity of batches F2, F5 and F8 was found to be 3740 ± 2, 3998 ± 2 and 4547.3 ± 2. The percentage drug release of batches F2, F5 and F8 was found to be 81.9641 ± 0.15, 80.1365 ± 0.13 and 79.1162± 0.13. The viscosity of batches F3, F6 and F9 was found to be 4286 ± 2.5, 4389.3 ± 4 and 5179.3 ± 2.5 The percentage drug release of batches F3, F6 and F9 was found to be 76.2685 ± 0.07, 72.7507 ± 0.13 and 69.4005 ± 0.06 [10-19]. (Fig. 5) Antibacterial studies of optimized gel formulation (F1) exhibited the highest zone of inhibition against *Candida albicans* (F1) (15 mm), standard marketed formulation (S) (14 mm). (Fig 6) This formulation (F1) showed its effectiveness against *Candida albicans*; hence it can be used as an antifungal agent in topical gel.

CONCLUSION

From present investigation it can be concluded that selection of carbopol type and concentration is major key factor in deciding viscosity and Miconazole Nitrate release from gel. Therefore both the grades are preferred in extending drug release.It has been concluded that carbopol 934 has a significant influence on extending miconazole release from gel formulations when combined with carbopol 940.

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