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DESIGN, DEVELOPMENT AND EVALUATION OF MICONAZOLE NITRATE TOPICAL GEL FOR FUNGAL INFECTIONS

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Keywords:

Miconazole Nitrate gels, Carbopol 940, Hydroxy propyl methyl cellulose, Sodium carboxy methyl cellulose, Sodium alginate, topical

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ABSTRACT: In this investigation, Miconazole Nitrate topical gels were designed by employing various bioadhesive polymers like polyacrylates (carbopol 940), cellulose derivatives (hydroxyl propyl methyl cellulose and sodium carboxy methyl cellulose) and natural polymers (sodium alginate) alone and in combination. The prepared gels were also characterized for different parameters like visual appearance, pH, consistency, rheological studies, drug content, in vitro release studies, stability studies and antifungal activity. From the results it was found that all the formulations were free of gritty particles and were found to be uniform in consistency. The values of pH were within the range of the skin pH that is in between 5.9 and 6.8, which indicates these gels cannot cause any local irritation to the skin surface. The consistencies of the gels were found to be excellent. The viscosity values of the gel increased in the following manner: carbopol and HPMC >carbopol>carbopol and SCMC >carbopol and sodium alginate > Sodium alginate >SCMC: indicating that carbopol in combination with HPMC was found to be the most viscous gel when compared to the others. The drug content was found to be uniform among various batches prepared and were in the range from 98.78±0.14 to 99.88±0.1%. The release of drug from all the formulations at the end of 8 hours study ranged from 68.78±0.42 to 99.88±0.05%. Gels containing carbopol 940 and HPMC and carbopol 940 alone released the drug at a sustained manner when compared to the gels containing SCMC and sodium alginate alone which released at a faster rate within 8 hours. The antifungal assay revealed that the gels containing carbopol 940 and HPMC in combination and carbopol 940 alone showed the maximum zone of inhibition values of about 15mm and 14mm respectively upto 72 hours. They possessed the maximum antifungal activity. The stability studies revealed good physical stability for all formulated preparations.

INTRODUCTION: Drug administration through topical is a localized system of delivery of drug anywhere in the body through ophthalmic, rectal, vaginal and the skin as topical routes. Skin is one of the most readily accessible organs on the human body for topical administration and is the main route of topical drug delivery.[1]

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Gels which are made with water are termed as hydrogels while that with an organic liquid are termed as organogels. Hydrogels include the composition of water- soluble materials such as cellulose derivatives and natural gums.

These pseudohydrogels swell to a great extent and the consitutuent molecules dissolve from the surface of the matrix. Drug molecules tend to release through the spaces in the network and also by degradation as well as dissolution of the matrix. Miconazole Nitrate, an antifungal agent is often prescribed for the treatment of various topical fungal infections such as Candidiasis, Coccidio

idomycosis, Crypto coccosis, Para coccidio idomycosis, and infections due to Pseudeli escheria. The drug undergoes substantial first pass metabolism and only half the amount of it is bioavailable systemically. To avoid this, delivery of Miconazole Nitrate through skin delivers the potential advantage of bypassing the hepato-gastro first pass metabolism associated with oral administration.[2]

Although the gel formulation of Miconazole Nitrate seems to be highly useful, there is a lack of literature on the present work. Therefore in this present work it was planned to formulate and evaluate Miconazole Nitrate gels Using various bioadhesive polymers such as cellulose derivatives, sodium alginate and polyacrylates.

MATERIALS AND METHODS:

Materials:

Miconazole Nitrate was obtained as a gift sample from Fourrts India Limited, Chennai, Carbopol 940 (Himedia Laboratories, Lobachemie), Sodium alginate (Loba Chemie), Hydroxyl propyl methyl cellulose (Loba Chemie), Sodium carboxy methyl cellulose (Merck), Peppermint oil, Methyl paraben (Loba Chemie), Triethanolamine (Rankem), Propylene glycol (Sisco Research Laboratories Pvt. Ltd). All chemicals either of analytical or pharmaceutical grade, were used without further purification.

Method:

Gels were formulated by cold mechanical method described by Schmolka *et al.* (1972) [7, 8]. Stated quantity of polymers (carbopol 940, HPMC, SCMC and sodium alginate) were weighed and was sprinkled on the surface of purified water for 2 hrs. Then continuous stirring was done with the help of a mechanical stirrer so that the polymer gets completely soaked in water. With incessant stirring, triethanolamine was added which acts as a neutralizer to maintain the pH of the gel.

Then the required amount of propylene glycol was added to the gel, which amplifies the penetration, followed by the addition of required quantities of methyl paraben that acts as a preservative. Finally the drug, Miconazole Nitrate was incorporated to the gel with continuous stirring until complete dispersion takes place. Six formulations of the topical gel were prepared by incorporation of various polymers alone and in combination. The prepared gels were packed in wide mouthed containers and were kept in dark and cool place.[3] (Refer **Table 1**).

TABLE 1: PREPARATION OF VARIOUS TOPICAL GELS

Ingredients	F1(gm)	F2(gm)	F3(gm)	F4(gm)	F5(gm)	F6(gm)
Miconazole Nitrate	0.6	0.6	0.6	0.6	0.6	0.6
Carbopol 9	0.5	0.5	-	1	0.5	-
HPMC	0.5	-	-	-	-	-
SCMC	-	-	1	-	0.5	-
Sodium alginate	-	0.5	-	-	-	1
Methyl paraben	0.5	0.5	0.5	0.5	0.5	0.5
Propylene glycol	2.5	2.5	2.5	2.5	2.5	2.5
Triethanolamine	q.s	q.s	q.s	q.s	q.s	q.s
Distilled water	upto to 30g	upto to 30g	upto to 30g	upto to 30g	upto to 30g	upto to 30g

EVALUATION OF THE GELS – caps lock:

Visual Appearance:

The prepared gels were visually inspected for clarity, colour and transparency. Smears of gels were prepared on glass slide and observed under the microscope for the presence of any gritty particles.

Surface pH:

An alkaline or acidic formulation can lead to irritation of the skin and hence checking of the surface pH is an important parameter. This test was performed by dipping a digital glass electrode inside the prepared gel.[4, 5]

Consistency:

To measure the consistency, a cone was attached to a rod at distance of 10cm and it had to be dropped in such a way that it had to fall on the centre of the glass cup with the gel. The penetration of the conefrom the surface of the gel to the tip of the cone inside the gel was measured. The distance travelled by the cone was noted down after 10 seconds.[6, 7]

Rheological Study:
The viscosities of the various prepared gels were determined by using a Brook Field viscometer (model-DVE) with spindle LV3 at a temperature of about 35±2 ºC.

Drug Content Estimation:
A gram of the gel was weighed accurately and placed inside a tightly closed volumetric flask with 10ml of methanol. Shake the flasks for 10 minutes and then 100ml of the prepared phosphate buffer of pH 7.4 was added and was further subjected to sonication. The absorbance of the above solution was determined using a spectrophotometer at λ_{max} 220nm for its drug content against an appropriate blank solution.[2, 8, 9]

***In-vitro* Release Studies:**
The *in - vitro* release studies were done by using open ended cylinders using egg membrane. A glass cylinder was taken which had both its ends open of dimensions 12cm height, 2.1cm outer diameter and 1.5cm inner diameter. The egg membrane was then tied to one end of the open ended glass cylinder compartment. Calculated quantity of gel (1gm) was weighed and was placed in the donor compartment and this apparatus was immersed into a beaker containing 100ml of phosphate buffer of pH 7.4.

The cell was submerged into a depth of 1cm below the surface of the buffer solution in the receptor compartment and was agitated using magnetic stirrers and the temperature needed was maintained at 37±1ºC throughout the study. Aliquots of 5ml sample were withdrawn and were replaced periodically with the fresh buffer pH7.4 solution at intervals of fifteen minutes for the first hour and every half an hour for the next seven hours. The amount of drug released was estimated by using UV spectrophotometer at 220nm.[10]

The *in vitro* release of the best two formulations was compared with the marketed product (Daktarin gel- Miconazole Nitrate 20mg/g topical gel) by using the same methods as stated above.

Anti-Fungal Assay:
Preparation of potato Dextrose Broth (PDB):
Antifungal activity of sample was determined by antifungal susceptibility test. Prepare PDB Broth and inoculate the culture. Then it was kept in shaker for a day. The potato dextrose agar was weighed as 3.9g and dissolved in 100ml using distilled water and then1gm of agar was added. Then, the medium was kept for sterilization. After sterilization, the media was poured in to sterile petriplates and were allowed to solidify for twenty minutes. After solidification, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension.

The disc were placed in PDA plate and 20µl of (20 µl of Different concentrations: 1000µg, 750µg, 500µg, 250µg) each sample were added. each samples were placed in the disc. The plates were kept at room temperature. Then the microbial growth was determined by measuring the diameter of zone of inhibition. After incubation for 18 hours to 72 hours at 25ºC, the fungal growth was observed and the percentage inhibition was measured.

Stability Studies:
All the selected formulations that showed a sustained release were subjected to stability testing as per ICH norms and WHO guidelines at a temperature of about 40±2ºC as well as at room temperature for the same period of time. Samples were withdrawn at the end of every month and analysed for drug content, appearance and pH.[5,7]

RESULTS AND DISCUSSIONS:
Visual Appearance:
Formulation F1was found to be white in colour and F2 and F6 were light brown in colour due to sodium alginate and F3, F4 and F5 were found to be transparent. All the formulations were free of gritty particles and were found to be uniform in consistency.

Surface pH:
The values of pH were within the range of the skin pH that is in between 5.9 and 6.8, this indicates these gels cannot cause any local irritation to the skin surface. **Table 2** shows the surface pH of all the formulations.

Consistency:
The consistency reflects the capacity of the gel to get ejected in uniform and desired quantity when

the tube is squeezed. Relation between the consistency in terms of distance travelled by the cone was almost 6 mm in all the developed formulations but was found to be 10 mm in marketed preparation. Consistency is inversely proportional to the distance travelled by falling cone. Refer **Table 2.**

TABLE 2: VALUES OF pH, CONSISTENCY AND DRUG CONTENT OF THE DEVELOPED GELS

Formulation	pH	Consistency (60sec)	Drug content (%)
F1	6.2	6mm	99.98
F2	6.4	6mm	99.94
F3	6.2	5mm	99.95
F4	5.9	6mm	98.97
F5	6.8	6mm	98.87
F6	6.7	5mm	99.78

Rheological study:

The viscosity values of the gel increased in the following manner: carbopol and HPMC >carbopol>carbopol and SCMC >carbopol and sodium alginate > Sodium alginate >SCMC: which indicates that carbopol in combination with HPMC was found to be the most viscous gel when compared to the others. Refer **Table 3**.

TABLE 3: RHEOLOGICAL STUDIES

S. No	Sample	RPM	Centipoise or mPa.s	%torque
1	F1	0.3	28880	92.4
		0.5	16710	89.1
		0.6	14450	92.5
2	F2	1.0	7941	84.7
		1.5	5613	89.8
		2.0	4613	98.4
3	F3	4.0	1303	55.6
		5.0	1158	61.8
		6.0	1052	67.3
4	F4	0.3	28910	92.5
		0.5	18900	89.9
		0.6	13410	85.8
5	F5	0.3	24310	77.8
		0.5	15230	81.2
		0.6	12920	82.6
6	F6	4.0	1205	49.8
		5.0	1099	52.7
		6.0	1072	64.9

Drug Content Estimation:

The drug content was found to be uniform among various batches prepared and was found to be in the range from 98.78±0.14 to 99.88±0.1%. The drug content determination also showed that the drug was uniformly distributed throughout the gel. Refer **Table 2**.

In Vitro Release Studies:

The results of in vitro permeation studies of gel formulations across egg membrane are depicted in **Table 4**, **Figure 1** and **Table 5**, **Figure 2**. The drug release from all the formulations at the end of 8 hours ranged from 68.78±0.42 to 99.88±0.05%. It can be observed that gels containing carbopol 940 and HPMC and carbopol 940 alone released the drug at a sustained manner when compared to those gels containing SCMC and sodium alginate alone which released at a faster rate within 8 hours. The release from the two best formulations that is F1 and F4 were compared with the release from the marketed formulation. The release profile was found to be sustained for the formulated formulations when compared with the marketed gel.

TABLE 4: *IN - VITRO* RELEASE STUDIES

Time (mins)	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)
0	0	0	0	0	0	0
15	1.52	5.25	8.99	3.46	6.42	6.23
30	5.72	6.67	10.67	6.001	8.67	8.99
45	7.67	9.89	13.68	8.12	12.29	10.758
60	9.99	12.457	15.76	10.75	15.83	11.59
90	11.45	17.83	17.843	12.008	17.93	16.76
120	12.14	20.112	21.96	13.96	19.64	19.78
150	13.21	27.74	27.83	15.53	22.18	23.42
180	16.75	30.22	30.66	17.83	24.56	26.43
210	20.05	39.97	39.145	19.85	30.23	30.74
240	24.56	45.39	43.674	24.28	39.64	39.53
270	30.97	50.75	51.67	31.09	43.54	49.96
300	35.61	56.11	59.99	38.667	50.77	56.43
330	39.88	60.12	64.66	42.21	59.9	60.58
360	42.57	65.53	70.909	49.96	67.34	69.44
390	49.51	71.05	77.64	58.765	74.2	75.7
420	52.01	79.99	84.623	63.98	79.96	80.394
450	59.96	84.21	90.008	70.006	87.54	89.32
480	68.78	90.86	99.88	77.24	94.19	96.32

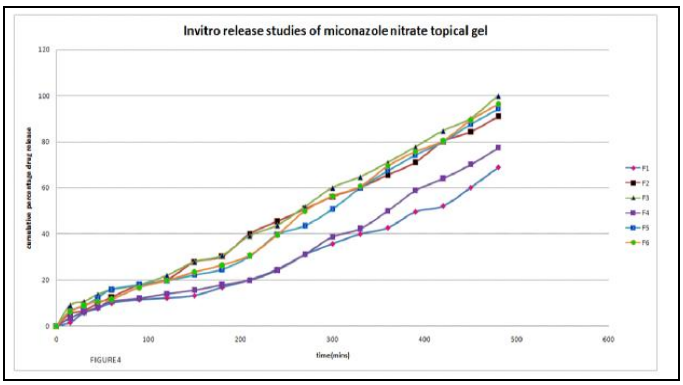


FIGURE: 1

TABLE 5: *IN - VITRO* RELEASE OF F1 AND F4 COMPARED WITH MARKETED GEL

Time (mins)	F1 (%)	F4 (%)	Marketed gel (%)
0	0	0	0
15	1.45	4.97	2.32
30	4.72	6.051	8.53
45	7.657	9.12	11.65
60	9.45	11.45	15.38
90	11.40	12.907	18.17
120	12.14	13.916	24.89
150	13.89	15.03	30.14
180	16.25	17.953	41.09
210	21.05	19.85	45.36
240	23.96	24.78	49.07
270	30.97	31.09	52.66
300	35.61	39.126	58.96
330	38.21	43.18	62.98
360	43.95	53.31	69.34
390	49.91	59.809	71.97
420	52.58	67.96	77.89
450	60.02	72.88	85.86
480	68.06	79.567	90.12

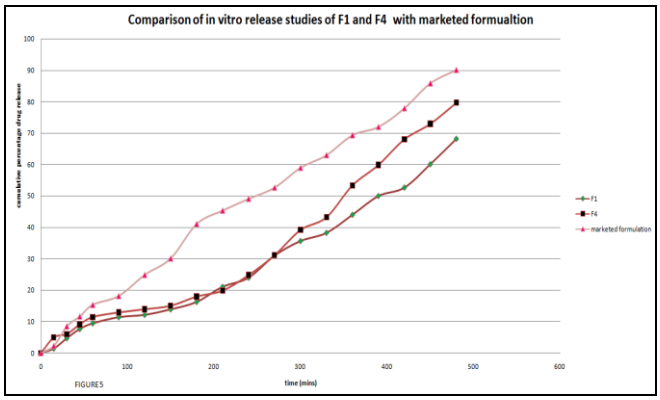


FIGURE: 2

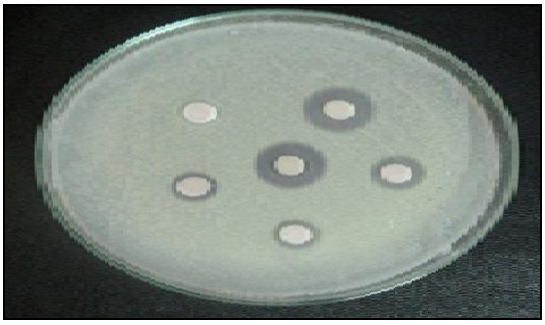
Anti-Fungal Assay:

Table 6 shows the anti-fungal assay values. The studies revealed that the gels containing carbopol 940 and HPMC in combination and carbopol 940 alone showed the maximum zone of inhibition values of about 15mm and 14mm respectively upto 72 hours. They possessed the maximum antifungal activity. The zone of inhibition was found to be 17mm for the marketed formulation.

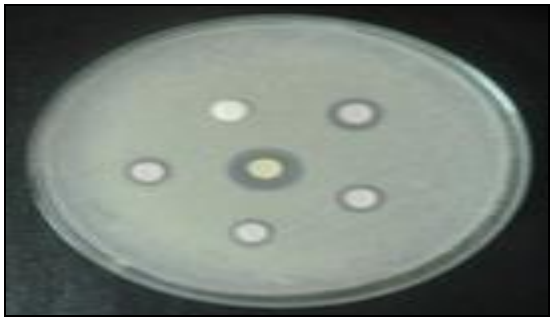
TABLE 6: ANTI- FUNGAL ASSAY

S. No	Sample	Zone of inhibition(mm)					Negative Control
		1000 µg	750 µg	500 µg	250 µg	Std	
1.	F1	15	14	11	10	14	-
2.	F2	11	10	9	8	14	-
3.	F3	13	12	11	8	14	-
4.	F4	14	13	10	9	14	-
5.	F5	12	10	9	8	14	-
6.	F6	12	12	11	10	14	-
7.	Marketed gel	17	16	14	13	14	-

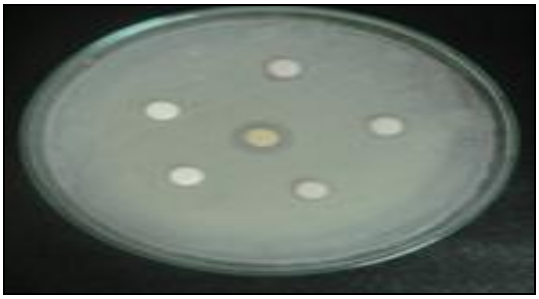
Microorganisms: (*Candida albicans*),
Standard: Amphotericin B (100 mg/ml) 20 µl per disk
Negative control: DMSO



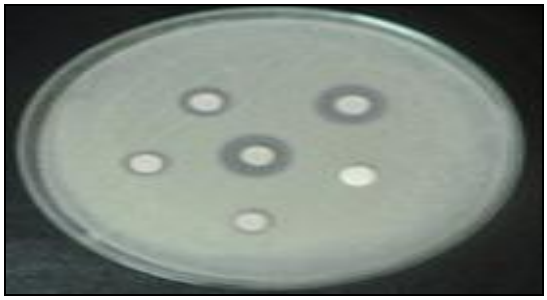
F1 FORMULATION



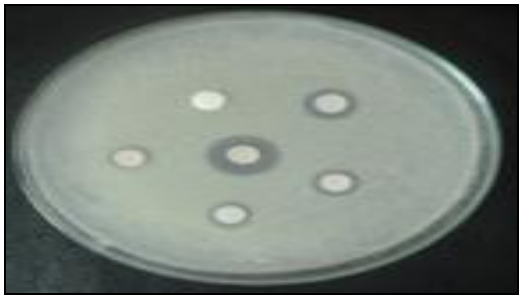
F3 FORMULATION



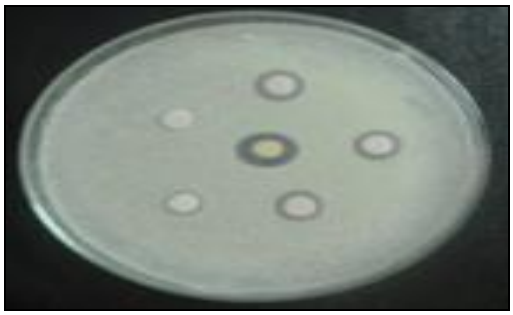
F2 FORMULATION



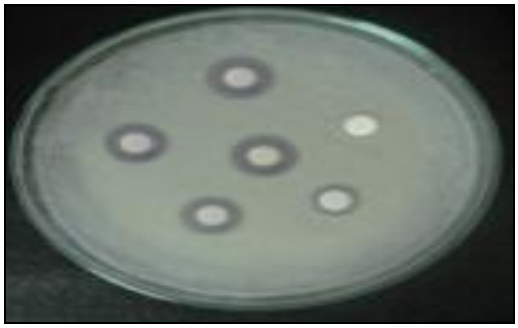
F4 FORMULATION



F5 FORMULATION



F6 FORMULATION



MARKETED FORMULATION

The above figures represent the zone of inhibition for the prepared gels and the marketed gel.

Stability Studies:

There were no significant variations in the drug content, consistency, pH and physical appearance of the gels after storing at the prescribed temperatures as mentioned earlier. All the gels showed promising results. This indicates that the drug was stable even after 3 months of short term storage. Refer **Table 7**.

TABLE 7: STABILITY STUDY OF VARIOUS DEVELOPED GELS

Sample no.	Months	Appearance	pH	Drug content
F1	0	Clear	6.2	99.98
	1	Clear	6.2	99.95
	2	Clear	6.1	99.90
	3	Clear	6.1	99.86
F4	0	Clear	5.9	98.80
	1	Clear	5.9	98.73
	2	Clear	5.8	98.23
	3	Clear	5.7	97.99

CONCLUSION: The present work aimed towards developing the topical gel of Miconazole Nitrate for the treatment of Candidiasis using various gelling agents like carbopol 940, HPMC, SCMC, sodium alginate in combination as well as alone. Miconazole Nitrate is an antifungal agent belonging to the imidazole group of drugs. Since oral route causes GI side effects and first pass metabolism there is a decrease in the bioavailability, in order to prevent this disadvantage, gels containing Miconazole Nitrate were prepared. Six formulations of the topical gels (F1, F2, F3, F4, F5 and F6) were prepared using different polymers as mentioned earlier.

Thus it was concluded that Miconazole Nitrate gels can be successfully prepared by using some of the polymers which made the gel bioadhesive, making it stay on the skin for a prolonged period of time and releasing the drug over an sustained manner providing a slow diffusional path length which will be helpful to avoid more fluctuations and also reduces the cost of therapy.

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