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FORMULATION, CHARACTERIZATION AND EVALUATION OF TOPICAL GEL CONTAINING MICONAZOLE NITRATE FOR FUNGAL INFECTION

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ABSTRACT

Miconazole is an imidazole derivative and used for the treatment of local and synthetic fungal infection. The oral use of miconazole is not recommended as it has many side effects. The miconazole topical gel formulation is made for better patient compliance and to reduce the dose of drug and to avoid side effects like liver damage and kidney damage. Gel was formulated by using different polymers. FT-IR study confirmed the purity of drug and revealed no interaction between the drug and excipient.Agelconsistsofacross-linkedpolymernetworkthatis swollen in a liquid medium, with its properties being significantly influenced by the intersection between the solid-state polymer and the liquid component. The gel was formulated by using different gelling agent like Carbopol 934, hydroxypropyl methyl cellulose (HPMC) K4M and HPMC E15, guar gum the gel formulation was evaluated for their drug content, PH determination, in vitro diffusion properties, and anti-fungal efficiency.

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INTRODUCTION

GEL

Gel is a semisolid system consisting of dispersion made up of either small inorganic particles or large organic molecules enclosing an interpenetrated by liquid. The inorganic particles form a three-dimensional structure. Gels consist of two-phase system in which inorganic particles are not dissolved but merely dispersed throughout the continuous phase and large organic particles are dissolved into the continuous phase.

Classification of gels: Gels can be classified as depending upon colloidal phases and nature of solvent used, physical nature and rheological properties.

A.Based on colloidal: Two-phase system (Inorganic) – If the partial size of the dispersed phase is relatively large and form the three-dimensional structure throughout gel such a system consists of floccules of small particles rather than layer molecules and gel structure in this system is not always stable. They must be thixotropic-forming semisolids on standing and become liquid on agitation.

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Single phase system (organic) – These consists of large organic molecules existing on the twisted stands dissolved in a continuous phase. These organic molecules either natural or synthetic polymer are referred as gel forms.

B. Based on nature of solvent used Hydro gel (water based): In hydro gels water acts as a continuous liquid phase. E.g. gelatin, cellulose derivatives, poloxamer gel. Organic gels (with a non-aqueous solvent) – They contain a non-aqueous solvent on their continuous phase. E.g. Plastibase olag gel and dispersion of metallic state in oils. Xerogels – these are solid gels with low solvent concentration. They are formed by the evaporation of solvent leaving the gel framework behind. On contact with fresh fluid, they swell and can be reformed. E.g. tragacanth ribbons, dry cellulose and polystyrene.

C. Based on rheological properties they are classified into three types:

Plastic gel – Flocculated suspensions of aluminium hydroxide exhibit a plastic flow and the plot of rheogram gives the yield value of gels above which the elastic gel distorts and begins to flow.

Pseudo plastic gel - For example liquid dispersion of tragacanth, sodium alginate etc exhibit pseudo plastic flow. There is a decrease in the viscosity of this type of the gel with the increasing rate of shear, the rheogram results from the shearing action on the long chain molecules of the linear polymer. As the shearing stress increased the disarranged

molecules begin to align their long axis in the direction of flow with release of solvent from gel matrix.

Thixotropic gel- In this type of gel the bonds between the particles are very weak and can be broken down by shaking. The resultant solution will revert back to gel due to the particles colliding and linking together again, e.g. bentonite and agar.

D. Based on physical nature Elastic gel: Due to elastic behaviour of agar, pectin, guar gum the fibrous molecules being linked at the point of junction by relatively weak bond such as hydrogen bonds and dipole attraction. E.g. alginate and Carbopol.

Rigid gels – In this type of gel macromolecules in which the framework linked by primary valance bond e.g. Silica gel. Bases or gel forming polymers Polymer is simply a compound made up of repeating units. Polymers are used to give the structural network which is essential for the preparation of gels.

MATERIALS AND METHODS

Miconazole nitrate- Dhamtec pharma and consultants, Navi Mumbai, Carbopol- Loba Chemie Pvt limited, Mumbai, Hydroxy methyl cellulose- Yarrow Chem Products, Mumbai, Guar Gum- Loba Chemie Pvt limited, Mumbai, Benzyl alcohol- SDS fine- chem limited, Mumbai, Tween 80- Loba Chemie Pvt limited, Mumbai, Glycerin- SDS fine- chem limited, Mumbai, Triethanolamine- Loba Chemie Pvt limited, Mumbai.

INSTRUMENTS

Electronic weighing balance- Asha scientific company, Mumbai, pH meter- MC Dalal, Chennai, UV Spectroscopy- Shimadzu, Japan, Brookfield viscometer- Asha scientific company, Mumbai, Sonicator- SV scientific Pvt limited, Bangalore, Magnetic stirrer- Asha scientific company, Mumbai, Franz diffusion cell apparatus- T.C. Scientific glass works, Haryana.

METHODOLOGY

CALIBRATION CURVE OF MICONOZOLE NITRATE

Preparation of standard curve of miconazole nitrate

The content of miconazole nitrate was analysed by UV spectrophotometric analysis. Scanning of the stock solution was carried out in the UV range of 200 to 400 nm. The absorbance maximum was obtained at wavelength of 220 nm. Stock solution (1000 µg of miconazole nitrate was prepared by dissolving 100 mg of the drug in methanol to made up the volume 100 ml) and aliquot the portion of4 µg to 20 µg of miconazole nitrate were prepared. The absorbance values of the dilutions were noted at 220 nm against methanol taken as blank, and calibration curve was plotted.

Table 1. Calibration curve data of miconazole nitrate

S.No	Concentration	Absorbance at 220 nm
1.	0	0
2.	4	0.109
3.	8	0.220
4.	12	0.350
5.	16	0.478
6.	20	0.597

PREFORMULATION STUDY

Preformulation is branch of Pharmaceutics science that utilizes biopharmaceutical principles to determine physiochemical properties of the drug substances. Prior to the developments of any dosage form for new drug, it is essential that certain fundamental physical and chemical properties of drug are determine.

1. Identification of drug
2. Organoleptic properties
   a. Color
   b. pH
3. Melting point
4. Solubility profile
5. Development of standard curve
6. FT-IR

IDENTIFICATION OF DRUG (LAMBDA MAX)

The absorption maximum of the standard solution was scanned between 200-400 nm regions on Shimadzu double beam UV-Visible spectrophotomer. The absorption maximum obtained with the substance being examined corresponds in position and relative intensity to those in the reference spectrum.

ORGANOLEPTIC PROPERTIES

The color, odor and taste of the drug were recorded using descriptive terminology.

MELTING POINT

This method is involved in placing the sample in a capillary tube and running an experiment that will heat the sample until reaches is melting point. (IP 2014)

SOLUBILITY PROFILE

It is important to know about solubility characteristic of a drug in aqueous system, since they must possess some limited aqueous solubility to elicit a therapeutic response. The solubility of drug was recorded by using various descriptive terminology specified in Indian Pharmacopoeia, 2018.

Fourier Transforms Infra – Red (FTIR) Spectroscopy

FTIR study was carried out to check the identity of drug (Miconazole nitrate, Carbopol, Guar gum, HPMEC E15, HPMC K4M). Infrared spectrum of drug was determined on fourier transform infrared spectrometer using KBr dispersion method. The base line correction was done using dried potassium bromide. Then the spectrum of dried mixture of drug and potassium bromide was run followed by using FT-IR spectrophotometer. All samples were scanned from 400 to 4000cm-1.

Preparation of Miconazole Nitrate Topical Gel

Miconazole nitrate topical gel was prepared by the Cold method and Fusion method. Different weight ratios of the polymers Carbopol, Guar gum, HPMC E15, HPMC K4M with Drug Miconazole nitrate were incorporated in the formulations. For F1, F2 formulation, polymer such as Carbopol, Guar gum mixed with water respectively and allowed for 24hrs. After 24

hrs, Gelling agents were mixed with drug and stirred well to form a homogenous gel. For F3, F4 formulation HPMC E15, HPMC K4M mixed with water respectively and allowed to heat in a water bath. After dissolving of gelling agent, Gelling agents were mixed with drug and stirred well to form a homogenous gel.

Formulation of miconazole nitrate gel

Table 2. Formulation Code of Miconazole Nitrate Gel

Ingredients	F1	F2	F3	F4
Drug (gm)	1	1	1	1
Carbopol 934(gm)	1	-	-	-
Guar gum (gm)	-	1	-	-
HPMC E15 (gm)	-	-	1	-
HPMC K4M (gm)	-	-	-	1
Benzyl alcohol (ml)	2	2	2	2
Tween 80 (ml)	1	1	1	1
Glycerin (ml)	10	10	10	10
Triethanolamine (ml)	3	3	3	3
Water (ml)	qs	qs	qs	Qs

EVALUATION OF GEL

Physical appearance

All the prepared formulations were visually inspected for colour, consistency, phase separation and smoothness. All developed formulations were evaluated for clarity in gel form by visual observation against a black and white background.

Determination of pH

The gel pH was determined by the digital pH-meter, which was calibrated before each use with standard buffer solution at pH 4,7,9. 1 gram of gel was dissolved in 100ml of phosphate buffer solution and stored for two hours. The measurement of pH of each formulation is done in triplicate and average values are calculated and reported.

Homogeneity

After the generated gels were placed in the container, they were all visually inspected for homogeneity. They had examinations to check for aggregates and to see how they looked.

Viscosity

Using a Brookfield viscometer, consistency of the pre-assembled gel was estimated. The gel was rotated using shaft number 64 at speed of 20 and 30, and the corresponding of the average of three readings taken in one minute was noted as the viscosity of gels.

Drug content

Weighed 5gm of each gel formulation were transferred in 250ml of volumetric flask containing 10ml of alcohol and stirred for 15 min. the volume was made up to 50ml and filtered. 1ml of above solution was further diluted to 10ml with alcohol and again 1ml of the above solution was further diluted to 10ml with alcohol. The absorbance of the solution was measured spectrophotometrically at 220 nm

Grittiness and Stickiness

Grittiness and Stickiness of each formulated gels were determined by crushing it between two finger. The degree of grittiness and stickiness were determined comparing different formulations.

Spreadability

It displays the extent of the area to which the gel quickly spreads upon application to the skin or affected area. The usefulness of a detail also depends on how well-know it is. Spreadability is expressed in terms of the number of seconds it takes two slides to separate from gel that is sandwiched between them while being subjected to particular load. Better spreadability is achieved by dividing two slides in less time.

$S = M. L / T$

Where, M = wt. tied to upper slide

L = length of glass slides

T = time taken to separate the slides.

In- vitro diffusion studies through egg membrane

Preparation of egg membrane

From local department store egg was purchased. The skin was removed carefully from the outer region of the eff and separated from the underlying membrane. The outer skin of egg was removed with the help of 0.1 N HCl with constant stirring. After separating the full membrane, the membrane was washed with using phosphate buffer pH 7.4. the membrane was now used for further experimental work.

Drug Diffusion through egg membrane

The in-vitro release was carried out with the egg membrane using franz diffusion cell. The cell consists of two chambers, the donor and the receptor compartment. The donor compartment was open at the top and was exposed to atmosphere.

The temperature was maintained at 37 ± 0.5ºC and receptor compartment was provided with sampling port. The diffusion medium used PBS (pH 7.4). The drug containing film was kept in the donor compartment and was separated receptor compartment by the egg membrane.

The egg membrane was previously soaked for 24 hours in PBS. The donor and receptor compartments were held together using a clamp. The receptor compartment with 25 ml of PBS was maintained at 37 ± 0.5ºC and stirred with magnetic stirrer, to prevent the formation of concentrated drug solution layer below the dialysis membrane.

Samples of 1 ml were collected at predetermined time intervals and replaced with fresh buffer. The concentration of drug was determined by UV spectrophotometer at 220nm.

Antifungal studies

Antifungal activity was determined by agar diffusion test employing cup plate technique. The drug was allowed to diffuse through a solid agar medium. The standard minimum inhibitory concentration (MIC2 µg/ml) of control and developed formulations containing miconazole nitrate were prepared. A total of 60 ml of nutrient agar media was prepared and sterilized at 121ºC for 20 minutes in an autoclave; 0.5

ml of microorganism suspension was poured into the above medium which is maintained at temperature of 52ºC to 58ºC.

This will be done in an aseptic condition. Immediately 20 ml of the microbial agar suspension was poured into each petri plate. After solidification of the media, sterile solutions of the developed formulations were poured into the cup of sterile nutrient agar petri plates. This was previously seeded with test organisms (*Rhizopus*).

After allowing diffusion of the solutions for 2 hours, the agar plates were incubated at 37ºC for 24 hours. The zone of inhibition (ZOI) was measured around each cup and compared with that of control. Entire operation was carried out in a laminar airflow unit.

RESULTS AND DISCUSSION

PREFORMULATION STUDIES

SOLUBILITY STUDIES

The solubility profile of miconazole nitrate [API] was given below in the table.

Table 3. Solubility profile of miconazole nitrate

S.No	Solvents	Solubility
1.	Water	Partially soluble
2.	Ethanol	Freely soluble
3.	Methanol	Freely soluble

MELTING POINT

Melting point is determined by capillary tube method. The melting point of miconazole nitrate was found to be

Table 4. Melting point of miconazole nitrate

S.No	Observations
1.	183ºC
2.	181ºC
3.	182ºC
Average	182ºC

The melting point of Miconazole nitrate was found to be 182ºC

FTIR SPECTRAL STUDIES

The FTIR spectral studies for the drug miconazole nitrate, polymers (Carbopol 934, guar gum, HPMC K4M, HPMC E15) physical mixtures of drug and polymer were carried out to find any interaction between drug and polymers used in the formulation.Compatibility studies of the drug and polymers were carried out by IR spectroscopy [SHIMADZU]. The results are given below,

Table 5. FT-IR Spectral data of miconazole nitrate

S.No	Wave Number(cm^{-1})	Functional Group
2.	2900.82	C-H Aliphatic
3.	1411.89	C-H Bending
4.	3464.15	N-H Stretch
5.	1087.85	Ether C-O-C
6.	1327.03	NO2 Bending
7.	709.80	C-Cl Stretch

Table 6. FT-IR spectral data of Carbopol

S.No	Wave Number(cm^{-1})	Functional Group
1.	1172.72	Ether C-O-C
2.	1411.89	C-O Stretch
3.	2962.66	O-H Stretch
4.	2900.82	C-H Stretch
5.	1411.89	C-H Bending

Table 7. FT-IR spectral data of guar gum

S.No	Wave Number(cm^{-1})	Functional Group
1.	1411.89	C-H Bending
2.	2962.82	C-H Stretch
3.	3479.58	N-H Stretch
4.	3479.58	O-H Stretch

Table 8. FT-IR spectral data of HPMC E15

S.No	Wave Number(cm^{-1})	Functional Group
1.	2976.16	C-H Stretch
2.	1444.68	C-H Bending
3.	3510.42	N-H Bending
4.	1327.81	NO2 Bending
5.	1097.50	Ether C-O-C

Table 9. FT-IR spectral data of HPMC K4M

S.No	Wave Number (cm^{-1})	Functional Group
1.	1473.62	C-H Bending
2.	3464.15	O-H Stretch
3.	1327.03	O-H Bending
4.	1288.45	C-N Bending

The FT-IR spectral studies show that the drug is compatible with all the excipients. The FT-IR spectrum of physical mixture shows all the characteristic peaks of miconazole nitrate, thus confirming that no interaction of drug occurred with components of the formulation.

EVALUATION OF MICONAZOLE NITRATE

PHYSICAL APPEARANCE

Table 10. Physical Appearance of topical gels

S.No	Colour	Smoothness	Clarity	Phase Separation
F1	White	Yes	Clear	No
F2	White	Yes	Clear	No
F3	White	Yes	Clear	No
F4	White	Yes	Clear	No

The appearance of all the developed formulation is light white and are clear in visuals.The clarity of the formulation before

and after gelling was determined by visual examination of the formulation under light alternatively against white and black backgrounds.

pH

Table11. pH of miconazole nitrate topical gels	
Formulation code	**pH**
F1	6.7
F2	6.4
F3	6.8
F4	6.5

The pH of the formulation was near to 6.8. It shows that the gel was compatible with skin condition and does not cause any irritation on the skin.

DRUG CONTENT

Table12. Drug content for topical gels	
Formulation code	**% Drug Content**
F1	98.32%
F2	94.76%
F3	67.99%
F4	63.38%

The drug content of miconazole nitrate in the topical formulation of gel was found in satisfactory (ranging between 98.78 – 99.88%), indicating uniform distribution of the drug. The drug content of gels was found to increase as the polymer ratio was increased.

VISCOSITY AND RHEOLOGICAL MEASUREMENTS

Table13. viscosity studies		
Formulation code	**Rpm**	**Viscosity in cps (after gelling) (spindle no 62)**
F1	10	19250
F2	10	4500
F3	10	1500
F4	10	3800

The results obtained from the rheological study of the prepared gels (F1-F4) revealed that the viscosity decreased as the angular velocity or shear rate increased.

GRITTINESS AND STICKINESS

Table14. Grittiness and Stickiness of various formulations		
Formulation	**Stickiness**	**Grittiness**
F1	Non gritty	Non sticky
F2	Non gritty	Non sticky
F3	Non gritty	Non sticky
F4	Non gritty	Non sticky

SPREADABILITY

Table15. Spreadability of variousformulations		
Formulation Code	**Quantity (mg)**	**Diameter (cm)**
F1	500	4.8
F2	500	4.7
F3	500	4.6
F4	500	4.5

***IN VITRO* DIFFUSION STUDY**

Table16. *In-vitro* Drug Release of Miconazole Nitrate topical Gels				
Time Interval (hrs)	**% Drug Release**			
	F1	**F2**	**F3**	**F4**
15 min	10.65	10.77	9.27	8.26
30 min	17.47	12.86	11.21	10.17
1 hr	24.82	17.34	23.48	18.75
2 hr	55.64	28.46	30.56	27.39
3 hr	66.79	39.57	42.35	29.56
4 hr	79.56	45.64	55.11	33.47

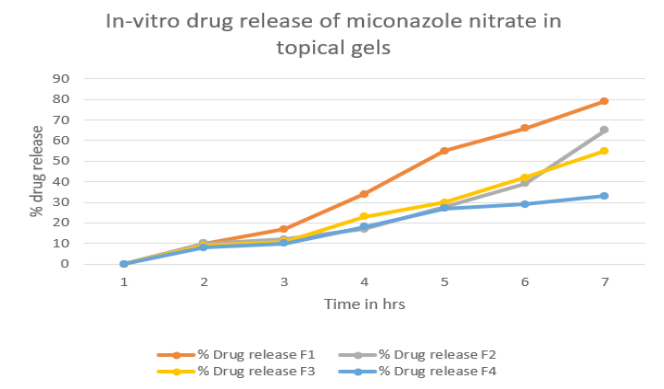

Figure 2. *In-vitro* **drug release of miconazole nitrate in topical gels**

The release of Miconazole nitrate was studied up to 6 hours and the Percentage Drug release for the formulations F1, F2, F3, F4 was found to be 79.56, 65.64, 55.11 and 33.47.The drug release from different formulations was increased in the following order F1>F2>F3>F4.All the formulations are passed the *in-vitro* Dissolution studies.The process of Drug release in most controlled release Devices is governed by Diffusion and the Polymer matrix has a strong influence on the Diffusion. The release rate was increased in the formulation F1 (Carbopol 934p) which is considered as best formulation.

ANTIFUNGAL ACTIVITY

Table17. Antifungal activity	
Formulation Code	Zone of Inhibition (mm)
F1	26
F2	28
F3	20
F4	22

Naturally, Guar gum having antifungal activity. So F2 formulation having greater antifungal activity when compared to other formulation.

CONCLUSION

An attempt to develop topical gel for miconazole nitrate using different polymers like Carbopol 934P, guar gum, HPMC E15, HPMC K4M was carried out for the purpose of attaining maximum antifungal effect and permeation through the human skin.The viscosity was found to be high for F1 formulation when compared with other formulated gels (F2, F3, F4). The reason behind this was F1 topical gel formulate by using Carbopol polymer. The concentration of the polymer, Carbopol can be developed in room temperature are the major reasons for having higher viscosity.The antifungal activity of the formulated topical gel was determined by using cup plate method. Naturally, guar gum having antifungal activity. So, F2 gel showed higher zone of inhibition against fungus when compared to other formulation.The formulation F1 and F2 shows better drug content when compare to other formulation. The formulation F1 and F2 shows a better *invitro* drug release profile across egg membrane, when compare to other formulation. This might be attributed to the nature of the polymer. From the study, F1 can easily permeate through the human skin.

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