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Formulation and evaluation of Miconazole nitrate nanoparticle for topical delivery

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ABSTRACT

Miconazole nitrate (MN) is a broad-spectrum antifungal agent of the imidazole group. The drug is primarily used as a topical treatment for cutaneous mycoses11; poor dissolution and lack of absorption make it a poor candidate for oral administration. However, MN can be used as a systemic antifungal agent when amphotericin B or ketoconazole is either ineffective or contraindicated. MN's poor skinpenetration capability presents a problem in the treatment of cutaneous diseases by topical application. Nanoparticle ointment has been prepared as the nanoparticle for the topical delivery. The present study intends to carry out Formulation and Evaluation of Miconazole Nitrate loaded Chitosan Nanoparticle formulation and to compare with the marketed formulation. In this work, the nanoparticles were prepared by Ionic gelation method. The optimized nanoparticles were characterized by using different parameter such as FTIR, SEM, entrapment efficiency, drug release study. The antifungal activity of the MN NPs was evaluated for 3 different formulations which all showed good results. Further by using the formulationF3 -of MN NPs, the nanoparticle Ointment was formulated and evaluated for appearance, viscosity, pH, Spreadability, in vitro release, and its antifungal activity was carried out. All showed good results. The formulated Ointment showed good zone of inhibition. And finally the formulated ointmentwas compared for its antifungal activity with a marketed formulation, which showed better antifungal activity than the marketed formulation.

Keywords: Miconazole Nitrate,Chitosan, Nanoparticles, Ionic gelation method, Antifungal ointment, Candida Albicans, Zone of Inhibition.

INTRODUCTION

The biological effects of a drug in a patient depend upon the pharmacological properties of the drug. These effects arise due to the interaction between the drug and receptors at the site of action of the drug. However, the efficacy of this drug-target interaction stands undermined unless the drug is delivered to its site of action at such a concentration and rate that causes the minimum sideeffects and maximum therapeutic effects [1]. Targeted drug delivery aims to achieve the same. Targeted drug delivery, also known as smart drug delivery, is a method of treatment that involves the increase in medicament in one or few body parts in comparison to others. Therefore, it delivers the medication only to areas of interest within the

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body. This offers an improved efficacy of treatment and also reduces side effects [2]. It differs from the conventional drug delivery system in that, it gets release in a dosage form while the former functions by the absorption of drug across biological membrane [3].

Nanoparticles are generally characterized by their size, morphology and surface charge, using such advanced microscopic techniques as scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM). The average particle diameter, their size distribution and charge affect the physical stability and the in vivo distribution of the nanoparticles. Electron microscopy techniques are very useful in ascertaining the overall shape of polymeric nanoparticles, which may determine their toxicity.

The main drawback of conventional method, in which,40%of new drug are hydrophobic and hydrophilic in nature. Because of that they possess low aqueous solubility and poor distribution profiles.And they also show unfavourable pharmacokinetics and toxicity. These problems can be overcome by using nanoparticles drug delivery system. Nanoparticle is small in size and that increases in surface area. There by fast dissolution of active agent in human body. Nanoparticles provide greater bioavailability,less toxicity and improve therapeutic effect of drug [28].

Topical and transdermal products are important classes of drug delivery systems and their use in therapy is more useful. Topical products to treat superficial infections from long time, for transdermal products skin is used as alternative route for systemic and in regional therapies. Topical drug delivery which avoids first-pass metabolism and effectively reduces the applied dose of drug. Topical administration of drugs to the skin by applying ointments, creams, gels directly to an external body surface by spreading and rubbing.The stratum corneum of the skin acts as a principle barrier to the permeation of topically applied drugs [5]. Now-a-days fungal infection of skin is one of the most common dermatological problems in worldwide. Dermatophytes are one of the frequent causes of tinea and onchomycosis.Candida species is one of the fungi for the most widespread superficial cutaneous fungal infection. When the immune system is weakened the candida can spread ing deeper tissues and also in blood which cause systemic candidiasis [6]. Many of antifungal agents are there like Amphotericin. Nystatin.,Azoles, such as miconazole, fluconazole, ketoconazole and posaconazole, have been administered by novel methods. And flucytosine,have also been employed. Many of them have low bioavailability when orally given therefore topical treatment of the skin infection has been mainly used due to its eminence over oral treatment to avoid systemic adverse effects, target the site of infection for application of drug formulation and to increase the patient compliance. So now a days The vesicular, colloidal and nanoparticulate carriers systems are used for the topical antifungal treatment [7].The present work was aimed to formulate Miconazole nitate nanoparticle ointment by ionic gelation method and evaluate its antifungal activity.

MATERIALS AND METHODS

Preformulation study

Delivery of any drugs needs a suitable dosage form to achieve optimum therapeutic efficacy. In this study, the preformulation studies conducted are solubility analysis, development of analytical range (λmax), physical characterization like color, odor etc. and compatibility studies

Ftir spectra of mn and chitosan

The FTIR analysis of Miconazole Nitrate was carried to evaluate the functional groups that might be involved in its formulation.

DEVELOPMENT OF ANALYTICAL METHODS

Standard graph preparation

Materials

Miconazole Nitrate Pure Drug, Methanol, Standard flask, UV-Visible Spectrophotometer.

DETERMINATION OF ΛMAX

A calibration curve of miconazole nitrate was constructed in methanol. Dilutions of miconazole in methanol at concentrations ranging from 200 µg/ ml to 12.5 µg/ ml (05 different concentrations) were made. The absorbance of each sample was determined at 272 nm with spectrophotometer. Methanol was taken as blank. The experiments were performed in triplicate and mean absorbance value was taken. A calibration curve was plotted with concentration as x-coordinate and absorbance as y-coordinate. Linear regression analysis was performed [8].

Preparation of miconazole nitrate loaded chitosan nanoparticle

Chitosan –Tripolyphosphate nanoparticles were prepared by applying a modified inotropic gelation method using chitosan and tripolyphosphate as positive and negative charges,respectively

Preparation of drug solution

Miconazole Nitrate solution was prepared by dissolving 1g pf MN in to 100ml of Dimethyl sulfoxide solution under the magnetic stirrer for 48 hours.Then filter the solution by using membrane

Preparation of polymer solution

Different concentrations of chitosan (200mg,400mg,600mg, 800mg and1mg)dissolved in 1%v/vAcetic acid solution (1ml in 100ml water) to a concentration of 20ml. Mix 10 ml drug solution with 10 ml of chitosan solution - Keep for 15 minutes at room temperature - Stirring at 700rpm in magnetic stirrer - Add 8ml of STPP solution (1mg/ml) drop wise on to the drug +chitosan solution - Sonicate the prepared solution at frequency 10000MHZ for 45min - Centrifuge at 28,000rpm (twice for 30 min at 100C) Layophilize at 400c for 24 hours. Each of the different concentration such as 1mg/ml-5mg/ml solution are prepared by this method (9,10). The size of nanoparticle are analysed by SEM analysis. (Table: 1)

Table 1: Composition of MN nanoparticle

FORMULATIONS	MICONAZOLE NITRATE	CHITOSAN (w/v)	STPP (m)
F1	1mg	1mg	8ml
F2	1mg	1.5mg	8ml
F3	1mg	2mg	8ml

Solubility

Solubility of Miconazole Nitrate was determined by dissolving in different solutions.

EVALUATION OF MICONAZOLE NITRATE NANOPARTICLE

Determination of particle size,shape and surface morphologyScanning electron microscope (sem)

SEM images were taken using a Hitachi TM 3000 Tabletop Scanning electron Microscope in the Pharmacognosy Division, Centre for Medicinal Plants Research, AryaVaidyaSala, Kottakkal. Samples were placed on two-sided carbon conducive-adhesive tape pasted on the specific aluminum stub. Voltage and magnifications were set depending on the sample

Fourier transform infrared spectroscopy (FTIR)

About 1–2 mg of MN were mixed with dry potassium bromide and the samples were examined at transmission mode over wavenumber range of 4,000 to 400 cm−1.

Drug entrapment efficiency

Take 1ml of drug solution in tp cuvette,add 8ml of ethanol in order to dissolve free drug, Measure the absorbance at 282nm,let the absorbance be M (total drug), Prepared nanoparticle suspension were centrifuged at 2000 RPM for 30 min, The supernatant was collected

and the particle were washed with water and then subjected to another cycle of centrifugation, Measure the absorbance of the supernatant solution obtained after centrifugation of chitosan nanoparticle at 282nm.Let the absorbance be M(free drug), The entrapment efficacy calculated by using following formula (11)

% Entrapment efficiency = M(Total drug) − M(Free drug)/ M (Total drug) * 100, M-Mass of drug

Determination of invitro drug release kinetics

The in-vitro release of MN NPs dispersion was determined using dialysis bag diffusion technique. Dialysis bag consists of 12,000-14,000 MW cut-off. Prior to the experiment, the membrane was washed with warm Milli-pore distilled water (70° C) for 1 hr and then rinsed thrice with Milli- pore water to remove the glycerine [1-5].

Preparation of phosphate buffer ph

Phosphate Buffer of pH 7.4 at 0.2 M: Accurately weighed 8 g of sodium hydroxide and 27.2 g of potassium di-hydrogen phosphate for the preparation of 1000 mL 0.2 M potassium di-hydrogen phosphate and 1000 mL 0.2 M sodium hydroxide. From that 50 mL of of 0.2 M potassium di-hydrogen phosphate and 39.1 mL sodium hydroxide is measured and mixed together and made up the volume to 200 mL with distilled water to produce pH 7.4 [IP., 2014].

Protocol

2 mL of suspension were placed inside the dialysis bag, tied at both ends and dipped in the dissolution media of pH 7.4 Phosphate Buffer solution at a temperature of 37±0.5°C.. Two millilitres of aliquot were withdrawn at particular time intervals and replaced by an equal volume of a fresh dissolution medium. After suitable dilution, the samples were determined spectro-photometrically by measuring the absorbance at 282 nm. The concentration of test samples was calculated by using the regression equation of the calibration curve. The percentage in-vitro drug release was calculated [12].

Where first order rate constant expressed in units of time-1.The data obtained are plotted as log cumulative percentage of drug remaining Vs time which would yield a straight line with a slope of -K/2.303.

MATHEMATICAL MODELLING FOR DRUG RELEASE KINETICS

Drug release kinetics was determined by the following kinetic equations such as zero order, first order, Higuchi model, Korsmeyer-Peppas model etc.

Zero-order model

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation:

$Q0 – Qt = K\ ot$

Where,

Qt amount of drug dissolved in time t

Q0 initial amount of drug in the solution,

(most times Q0 = 0) and K0 is the zero order release constant expressed in units of concentration/time. To study the release kinetics, data obtained from in vitro drug release studies were plotted as cumulative amount of drug released versus time.

First order model

This model has also been used to describe absorption and/or elimination of some drugs, although it is difficult to conceptualize this mechanism on a theoretical basis. The release of the drug which followed first order kinetics can be expressed by the equation:

$dc/dt = -Kc$

Higuchi model

Model expression is given by the equation

$Ft= Q = AD (2 C – CS)$

Where,Q- amount of drug released in time t per unit area. A, C - drug initial concentration, Cs-drug solubility in the matrix media. D - Diffusivity of

the drug molecules (diffusion coefficient) in the matrix substance.

In general way it is possible to simplify the Higuchi model as (generally known as the simplified Higuchi model):

Ft = Q =KH×t1/2

KH Higuchi dissolution constant. The data obtained were plotted as cumulative percentage drug release versus square root of time.

Korsmeyer-Peppas model

To find out the mechanism of drug release, first 60% drug release data were fitted in Korsmeyer- Peppas model

Mt/M□ = Ktn

Mt /M∞ - fraction of drug released at time t.

K - Release rate constant,

n - Release exponent

In this model, the value of n characterizes the release mechanism of drug. To study the release kinetics, data obtained from in vitro drug release studies were plotted as log cumulative percentage drug release versus log time. Diffusional release mechanism of polymeric films is given below. (13) (Table:2).

Table 2: Interpretation of diffusional release mechanism from polymeric film

Release exponent (n)	Drug transport mechanism
0.5	Fickian diffusion
0.5<n<1.0	Anomalous transport (Non-Fickian diffusion)
1.0	Case- 2 transport
Higher than 1.0	Super Case 2 transport

Evaluation of antifungal activity of mn against candida albicans

Sterilization

All the glass wares for the present study were washed thoroughly with detergent and water and rinsed with distilledwater. The glasswares and the media were autoclaved at 1210C and 15 lbs for 20 minutes. The inoculation was done in Laminar Air Flow (LAF) Chamber. The in vitro antimicrobial analysis is carried out against Candida albicans by microtitre plate assay at different concentrations

Potato dextrose broth media and preparation

The potato dextrose agar was weighed 3.9g and dissolved in 100ml using distilled water and then 1gm of agar was added,and then the medium kept for sterilization. After sterilization,the media was poured in to sterile petriplate and were allowed to solidify for 20min. (14) (Table:3)

Table:3-formulation of culture media

Ingredients	Working formula (50ml)
Potato Infusion	10g
Dextrose	1g

WELL DIFFUSION METHOD

After solidification, an L shaped glass rod is placed into a beaker containing sufficient amount of 95% ethyl alcohol to cover the lower bent position. With a micropipette,place a loop full of organism culture randomly on the petri plate.Sterlise the rod and cool it for 10-15 seconds.Remove the petridish cover and rotate the plate manually. While rotating lightly touch the sterile

bent to the surface of potato dextrose agar and

move it to and fro to spread the culture over the surface. Replace the cover, resterlise the rod. The antifungal study was done by well diffusion method. Then by using borer make wells on the petri plate (15). Then add nanoparticle suspension to the wells. Incubate all the petridish for 24 hrs. Zone of inhibition then observed, which is suggested by the clear area around the well. (16). (Table: 4).

Table 4:Antifungal analysis

	C1	C2	C3	CONTROL
Nanoparticles	F1	F2	F3	
Culture	100ml	100ml	100ml	100ml
PDB	100ml	100ml	100ml	100ml

PREPARATION OF NANOPARTICLE OINTMENT

Preparation of ointment containing drug loaded chitosan nanoparticle

By using Miconazole Nitrate loaded chitosan nanoparticle an ointment is prepared (Table:5)

Table 5: Composition of ointments

INGREDIENTS	%W/W FORMULATION
Nanoparticle	4gm
Boric acid	1gm
Zinc oxide	1gm
Hard paraffin	1gm
Microcrystalline wax	2.5gm
Liquid Paraffin	20gm
Cetostearyal alcohol	13gm
Sodium Lauryl sulphate	1gm
Methyl paraben	3.5gm
Propyl paraben	0.2gm
Propylene glycol	15gm
Purified water	q.s

The ointment was prepared by heating Zinc oxide,Boric acid,Microcrystalline wax, Sodium lauryl sulphate, Methyl paraben in a vessel attains 750C to 800C.Oil phase was added by heating soft paraffin and Cetosteryl alcohol in stainless steel till the temperature attains 750C to 800C.Both water phase and oil phase mixed in to mass.The mass was stirred and cooled for 1.5 hours.Active ingredients like nanoparticle and ethylene glycol were made in to homogenous slurry for 30 min.The slurry was transferred in to vessel and homogenization was continued for 1.5 hours.Then it was cooled and again stirred till ointment is obtained. Temperature was maintained to 350C to 370C [17].

EVALUATION OF OINTMENTS

Physical appearance (or) visual inspection

The prepared formulations were evaluated for their clarity, colour, and smell [18].

Determination of ph

The pH values of ointments were measured with a digital pH meter at room temperature. 1g of formulation were dissolved in 100ml of distilled water and stored for 2 hrs. The measurement of pH

of each formulation is measured triplicate and average values were depicted.

Rheological evaluation

The viscosity of the preparation should be such that the product can be easily removed from the container and easily applied to the skin. By using Brookfield viscometer the viscosity of the preparation is determined.Spindle used is spindle no:3 at 30 RPM 44

Loss on drying

Loss on drying was determined by placing ointment in Petridis on water bath and dried for 1050C.

Spreadability testing

Spreadability was developed to access the spreading property of the liquids, cosmetics etc. It was developed to study the amount of spread of a sample [19].

IN VITRO DRUG RELEASE STUDY

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

COMPARISON OF ANTIFUNGAL ACTIVITY OF PREPARED FORMULATION WITH MARKETED FORMULATION

Potato dextrose broth media and preparation

The potato dextrose agar was weighed 3.9g and dissolved in 100ml using distilled water and then 1gm of agar was added,and then the medium kept for sterilization. After sterilization,the media was poured in to sterile petriplate and were allowed to solidify for 20min.

Well diffusion method

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RESULT & DISCUSSION

Preformulation studies

Nanoparticles prepared are subjected to preformulation studies.F1,F2, F3 are the three formulation prepared by varying concentration of drug and the polymer.

Preformulation studies

Nanoparticles prepared are subjected to preformulation studies.F1,F2, F3 are the three formulation prepared by varying concentration of drug and the polymer Formulation after visual inspection were found that (Fig: 1).

FTIR SPECTROSCOPY

FTIR spectra of MN

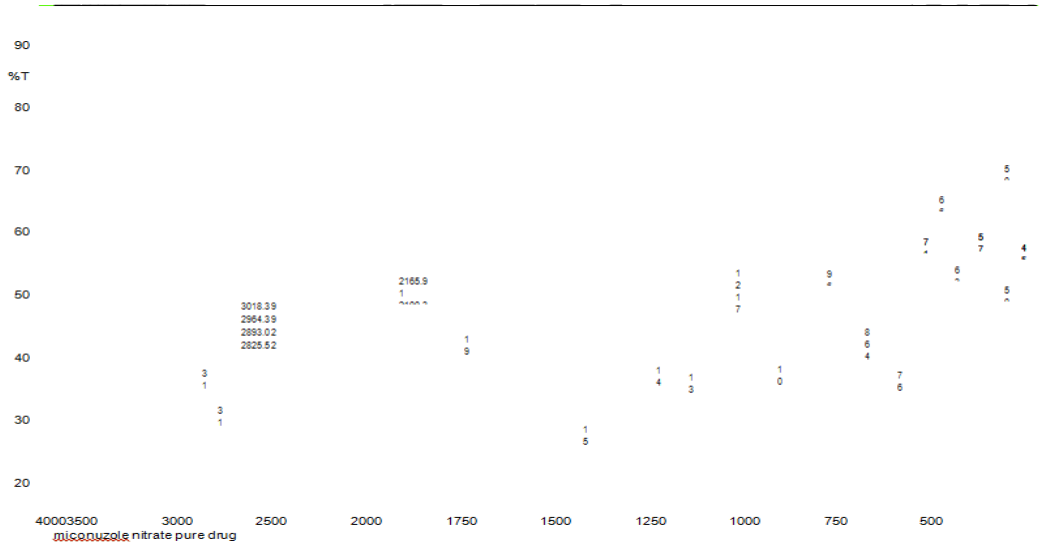

Fig 1: FTIR spectra of MN

The FTIR spectrum shows characteristic peaks of MN such as aromatic C=N stretching (1,637.45 cm-1), aliphatic C–H stretching (2,964 cm-1), aromatic C–H stretching (3,018 cm-1).

FTIR SPECTRA OF CHITOSAN

FTIR Studies of chitosan were performed and has been showed in the fig;9 The spectra of Chitosan shows vibration of amine at 1373cm-1.The OH stretching 2869cm-1.The peaks 1568cm-1and 1548 cm-1 are attributed to the CoNH2 and NH2 groups respectively. (Fig:2).

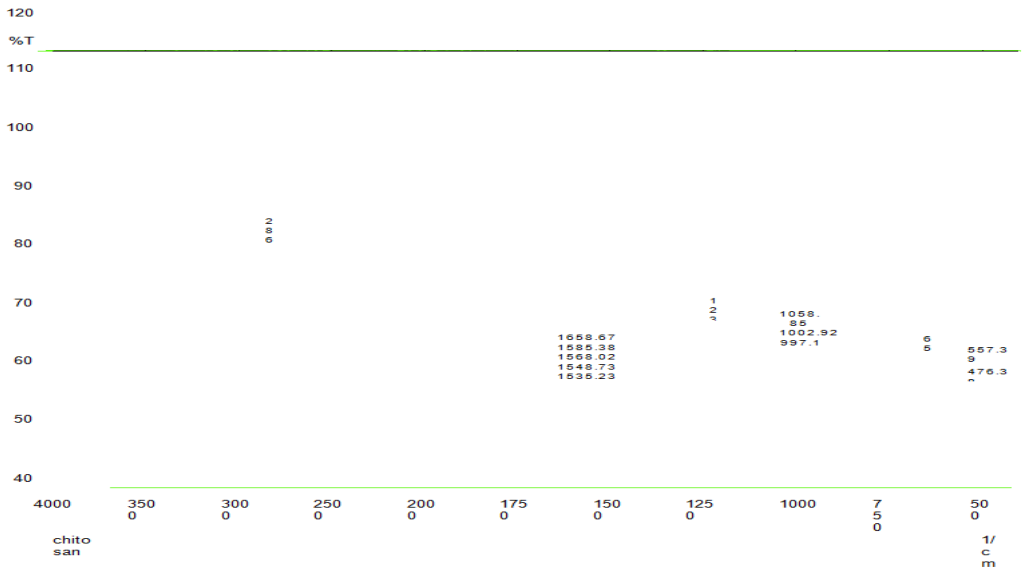

Fig 2: FTIR spectra of Chitosan

DEVELOPMENT OF ANALYTICAL METHODS

Determination of □ max

Accurately weighed 10mg of MN was dissolved in methanol and made upto 100ml.Out of this solution different concentration were pipette and diluted upto 10ml by methanol.The drug solution were scanned (200-400)against reagent blank (methanol) and the absorption spectra was recorded. The absorption maximum (□ max) was observed at 272nm.

Standard graph preparation

The standard solution of MN was prepared by dissolving accurately about 10mg of MN in 100ml of ethanol (100mg/ml).Then 0.5,1,1.5,2,2.5,was pipette and diluted upto 10ml by methanol to obtain concentration of 5,10,15,20,25 mg/ml of solution. The UV Visible spectroscopy of the drug MN was performed at wavelength of 272 nm.The standard graph of the drug was plotted by taking the concentration of drug solution on the X axis and the corresponding absorbance values on the Y axis. (Table:6) (Fig:3).

Concentration (mg/ml)	Absorbance (nm)
5	0.124
10	0.245
15	0.364
20	0.461
25	0.609

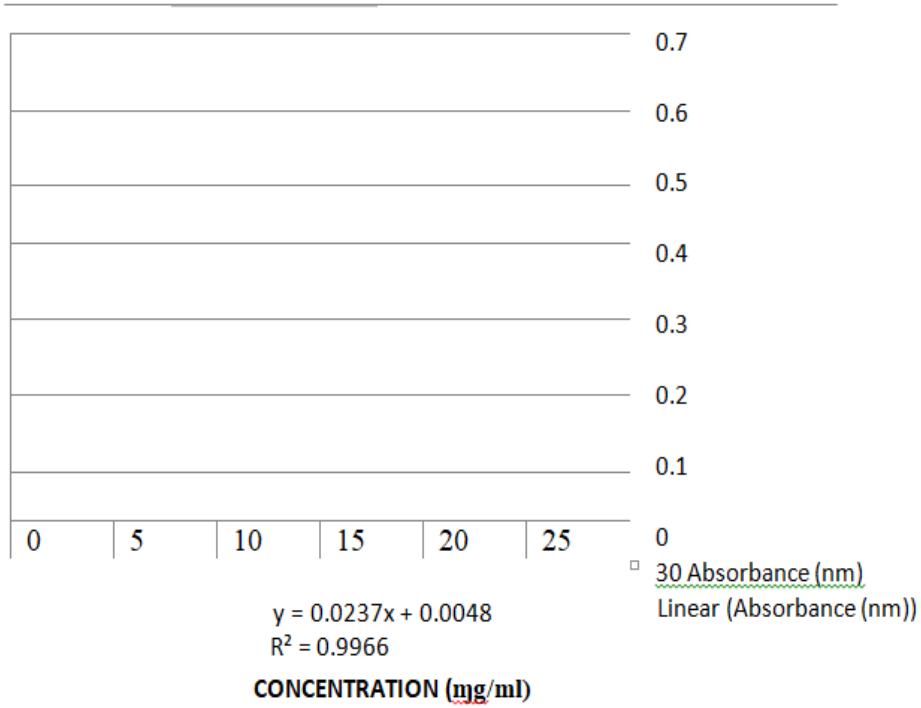

Fig: 3-std graph of MN

Preparation of miconazole nitrate nanoparticle

Nanoparticle suspension was prepared by varying concentration of the polymer (Chitosan) in the ratio 1:1,1:1.5,1:2 using inotropic gelation method. The ratio 1:2 of nanoparticle showed more effective action compared to other ratios,which was further confirmed by the evaluation method.

SOLUBILITY

Table 7:Solubility of MN

SOLVENT	SOLUBILITY
Water	Insoluble
DMSO	Soluble
Methylene chloride	Freely soluble
Tetra hydro furan	Sparingly soluble
Alcohol	Very slightly soluble

VALUATION OF MICONAZOLE NITRATE

Determination of nanoparticle size,shape and surface morphology, scanning electron microscopy

The SEM image of MN nanoparticles by inotropic gelation method is shown in the figure. It gave a clear image of highly dense chitosan nanoparticles. The MN nanoparticles were spherical in shape with varying particle size ranges. The larger nanoparticles may be due to the aggregation of the smaller ones. The SEM image showing MN nanoparticles confirmed the development of Nano structures. (Fig:4).

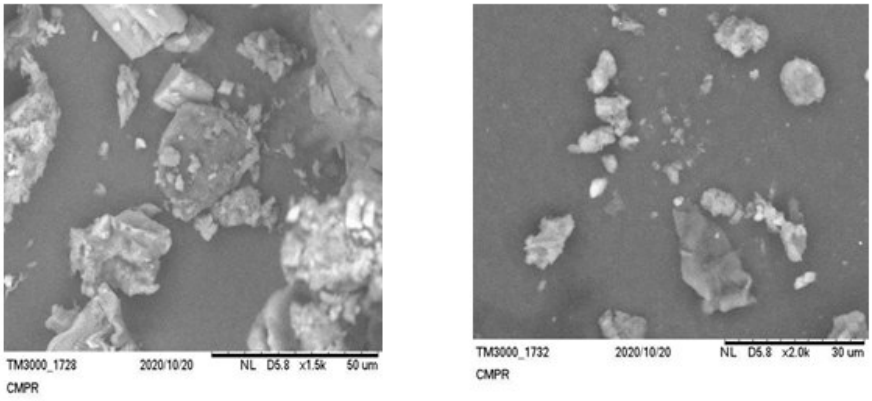

Fig 4 SEM images of MN nanoparticle

In the IR spectrum of Nanoparticle, peaks corresponding to MN disappear or are buried in the peaks of Chitosan indicating drug entrapment in the Matrix.So this indicates the Chitosan-TPP,it may have formed the coating around the drug core as suggested from the above data. The shift in 1566cm-1 and 1530cm-1 in the FTIR spectra of nanoparticle caused due to the interaction between NH3+ groups of chitosan and phosphate groups of TPP.The peak at 1012cm-1 which appears in the FTIR spectra of chitosan nanoparticle shows characteristics P=O Stretching vibration from phosphate group.

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

MN nanoparticle (Formulation f3) (Fig:5)

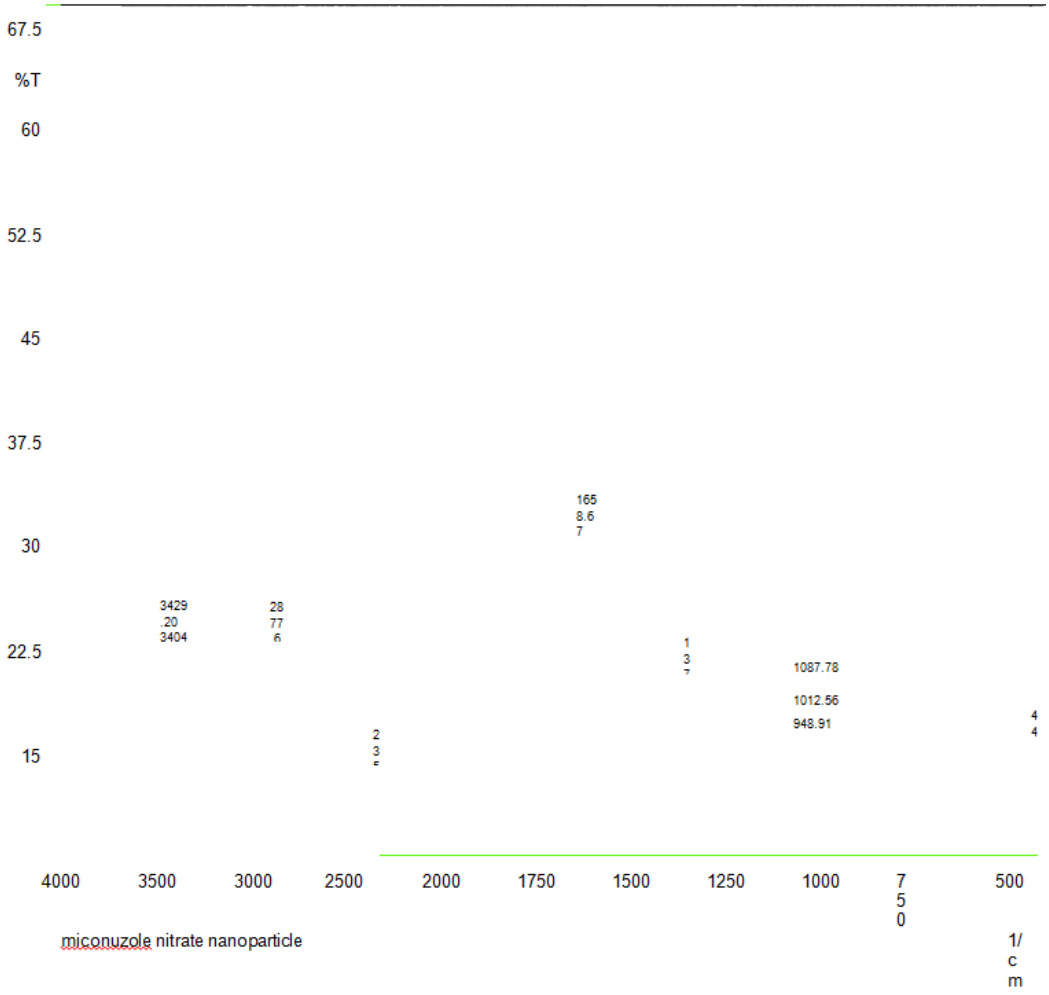

Fig 5: FTIR spectra of MN NPs

Entrapment efficiency

The principle of drug loading of nanoparticles is surface adsorption. 0.5 mg of dried Nanosuspension in 10 mL of acetone was allowed to centrifuge at 10,000 rpm for 90 min. The nanoparticles free from entrapped drug were detected in the filtrate, which was separated and its absorbance were examined by UV-Visible spectrophotometer at 282 nm. The entrapment efficiency was calculated. (Table:8).

Table:8 Entrapment efficiency of MN NPs

Batches	Absorbance 282 nm	%EE
F1	0.058	76.3%
F2	0.051	77.2%
F3	0.042	81.81%

Four batches of samples were evaluated and the entrapment efficiency of samples was found to be76.3%,77.2%,81.81% Out of which, F3 batch shows optimum %EE compared to other three batches. Hence from these results the optimized %EE of the nanoparticles was found to be 81.81%.

Invitro drug release

In-vitro drug release study of optimized formulation was done by means of Dialysis bag diffusion technique. Accurately weighed 6 mL of suspension were placed inside the dialysis bag, tied at both ends and dipped in the dissolution media of pH 7.4 Phosphate Buffer solution. 2mL of sample were withdrawn at suitable time intervals (1,2,3,4,5,6,7,8 hours) and the absorbance was measured at 282 nm and later percentage drug release was calculated. (Table:9).

Table 9:Data of % drug release

S no	Time	Absorbance (nm)	Concentration	Actual concentration	Amount of drug released	Cumulative amount of drug release	% Cumulative Drug Release
1	0	-	-	-	-	-	-
2	1	0.031	0.568	32.9	0.0329	0.329	18.2%
3	2	0.043	0.972	48.6	0.0486	0.498	27.4%
4	3	0.052	1.74	87	0.087	0.880	48.92%
5	4	0.063	1.88	94.1	0.0941	0.959	53.3%
6	5	0.079	2.28	114.1	0.114	1.161	64.5%
7	6	0.094	2.62	131	0.131	1.3356	74.2%
8	7	0.096	2.71	135.1	0.135	1.378	78.5%
9	8	0.098	2.81	140.5	0.1405	1.443	79.21%

From the in-vitro drug release study of MN nanoparticles,% drug release was found to be 79.21%at 8 hours.

MATHAMATICAL MODELLING FOR DRUG RELEASE KINETICS INTERPRETATION OF DATA

Determination of the order of release of drug from MN nanoparticles, using graphical method.The results obtained from invitro release studies were attempted to fit into various models as follows: Cumulative percentage drug released V/S Time (Zero order rate kinetics). Log cumulative percentage drug released V/S Time (First order kinetics). Cumulated percentage released V/S square root of Time (Higuchi's classical diffusion equation). Log of cumulative percentage drug released V/S Log Time (Korsmeyer-Peppas, exponential equation).

Zero order kinetics

The graph was plotted with % drug release on y axis and time on x axis.The zero order rate constant (K0) for MN nanoparticles was determined from the slope (K0 = Slope × 2.303), which was found to be 5.53. The Regression coefficient were also determined to find the correlation, between X and Y values. The regression coefficient of the MN nanoparticles was found to be 0.949

First order kinetics

The graph were plotted with log % drug remaining on X axis and time on Y axis.The first order rate constant (K1) for MN nanoparticles was determined from the slope (K0 = Slope × 2.303), which was found to be 0.084. The Regression coefficient were also determined to find the correlation between X and Y values.

The regression coefficient of the NPs nanoparticles was found to be 0.625. These values were compared with the R2 values of the zero order kinetics plots. It was very difficult to predict exact order of kinetics of the formulations but the R2 values showed a fair linearity, with the regression value of 0.949, that the MN nanoparticles was found to be close to be zero order kinetics, drug release was independent of concentration.

MECHANISM OF DRUG RELEASE: HIGUCHI MODEL

In this model, the data obtained were plotted as cumulative percentage drug release versus square root of time. The R2 value for MN nanoparticles was found to be 0.973 which indicated goodlinearity with n value. The n value for MN nanoparticles was found to be 6.41

Korsemeyer peppas model

In this model, the graph was plotted between log % drug release and log time. The R2 value for MN nanoparticles was found to be 0.666 which indicated fair linearity with n value. The n value for MN nanoparticles was found to be 1.440. (Table: 10).

Table:10 R2 values of kinetic models

RELEASE KINETICMODELS	R2 VALUES
Zero order kinetics	0
First order Kinetics	0
Higuchiplot	0
Kosmeyer-Peppasplot	0

In vitro drug release study data of the prepared formulation MN NPs was subjected to goodness of fit test by linear regression analysis according to Zero order, first order and Korsemeyer-Peppas model to ascertain the mechanism of drug release. The model that best fitted the release data was evaluated by correlation coefficient (R2).The R2 values of nanoparticles in various models are given above in the table. With the R2 value of 0.949, that the MN nanoparticles was found to be close to be zero order kinetics, indicating that drug release was independent of concentration. The release profile of the MN nanoparticles could be best explained by Higuchi model, as the plot showed high linearity with R2 value 0.973. The diffusion mechanism of the drug release was further confirmed by KorsemeyerPeppas plot, that showed fair linearity (R^2 =0.666), with slope values more than 0.5, indicating that drug release mechanism from the prepared MN nanoparticles follows Non-Fickian diffusion. This findings was in accordance with other reported works

EVALUATION OF ANTIFUNGAL ACTIVITY

Well diffusion method

The Potato dextrose media was autoclaved, cooled and poured in to the petri plates and kept for 15 minutes to solidify. With a micropipette ,place a loop full of organism culture randomly on the petri plate.Sterlise the L rod and cool it for 10-15 seconds remove the petridish cover and rotate the plate manually. While rotating lightly touch the sterile bent to the surface of potato dextrose agar and move it to and fro to spread the culture over the surface. Then with the help of sterile well puncture (5 mm diameter), wells were made in the inoculated plate and labelled. 100µL of each prepared formulation of NP suspension F1, F2, F3 were transferred in to the well with the help of micropipette. Then the plates were incubated at 370C for 48 hours and observed for the zone of inhibition, which is suggested by the clear area around the well The antifungal activity of MN was performed by well diffusion method. Formulation 3 (F3) showed more zone of inhibition compared to other formulations (F1&F2). (Table:11).

Table 11:Antifungal activity of MN nanoparticle

Samples	Zone of inhibition
F1	14mm
F2	18mm
F3	21mm

PREPRATION OF NANOPARTICLE OINTMENT

Evaluation of ointment

Physical appearance/ visual inspection

The appearance of the NP Ointment were visually observed and found to be white in colour and clear

DETERMINATION OF PH

The pH of prepared NP ointment in 10% distilled water was determined at room temperature using digital pH meter immersing in a depth of 0.5cm in a beaker contains ointment .The determination of pH was carried out in triplicate and the average pH of ointment was recorded. (Table:12).

Table:12-determination pH of ointment

Trials	A	B	C	Avg
pH	6.1	6.5	6.8	6.4

Rheological Evaluation

The viscosity of formulated ointment was performed by using Brookfield viscometer using spindle number 3 and determination was carried out in triplicate and the average reading was determined. (Table:13).

Table 13:-determination of viscosity

Sl.No	RPM	Cp TRIALS	Cp trials			Average
			A	B	C	
3	30	2min	1163	1161	1160	1162cps

Loss on drying

% Loss on drying of ointment was obtained by drying the ointment in water bath at 1050C. %LOSS ON DRYING =39%W/W

DETERMINATION OF SPREADABILITY

Two glass slide of 20*20 cm were selected. The Spreadability test was done by placing 2g of prepared ointment on a glass slide holding horizontally upward. The ointment formulation was sandwiched between this slide in an area occupied by a distance of 60cm along 100gm weight was placed on the upper slide. So that the ointment between the two slides was pressed uniformly to form thin layer. The weight was removed and fixed on the stand without slightest disturbances and in such a way only the upper slide to side off freely, to force of weight tied to it. A 20 g of weight is tied on to the upper side, the time taken to travel the distance of 6cm and seprate away from the lower slide under certain of weight was noted.The determination was carried out in triplicate band average values are recorded. (Table: 14).

Table:14-Determination of Spreadability

TRIALS	A	B	C	AVERAGE
Spreadability	7.7	7.2	7.0	7.3

IN VITRO DRUG RELEASE

The in - vitro release studies were done by using open ended cylinders using egg membrane.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. (Table:15).

Table: 15 In vitro drug release of prepared formulation

SL NO.	Time (hour)	%Cumulative release of formulation
1	**0**	**0**
2	**1**	**12.8%**
3	**2**	**23.4%**
4	**3**	**33.6%**
5	**4**	**45.2%**
6	**5**	**50.6%**
7	**6**	**62.3%**
8	**7**	**75.4%**
9	**8**	**78.1%**

COMPARISON OF ANTIFUNGAL ACTIVITY OF PREPARED FORMULATIONWITH MARKETED FORMULATION

The comparison was done by Well Diffusion method. Fungus used was Candida albicans. Microbial suspension of C .albicans was spreader onto the solidified potato dextrose media by using sterile L rod. Then with the help of sterile well puncture (5 mm diameter), wells were made in the inoculated plate and labelled. Formulated ointment containing drug loaded nano particles were aseptically transferred onto the inoculated plates and left to be incubated for 2 days and same as for marketed formulation. The zone of inhibition obtained was calculated. From the study the test formulation prepared formulation (F3) was found to more zone of inhibition when compared with the marketed formulation (standard).(zole ointment). (Table: 16).

Table 16:Antifungal analysis of ointment

SAMPLES	ZONE OF INHIBITION
Standard	20 mm
Test	23mm

SUMMARY AND CONCLUSION

The present study deals with preparation of Miconazole Nitrate loaded chitosan nanoparticle ointment by inotropic gelation method and from the best formulation has been incorporated into ointment andcompared with the marketed formulation. The major goal in designing the nanoparticle ointment as a targeted drug delivery is to achieve the site specific and prolonged action Nanoparticle were prepared and its preformulation study was conducted and its organoleptic properties and analytical ranges were determined. In this study Three formulation of MN nanoparticle were successfully synthesized using Inotropic gelation method by varing concentration of the polymer Chitosan (1:1,1:1.5 and 1:2). The formulation of MN NPs was confirmed by the colour, which was further confirmed by FTIR.The SEM result also revealed the presence of NPs in the sample by its particle size. The entrapment efficiency of the MN NPs reveals the 80.02% of the

drug was successfully absorbed into the NPs. The in-vitro drug release study was performed by using dialysis bag diffusion technique, and the cumulative drug release was found to be 80.21% at 8 hours and its release kinetics were also performed. The release kinetics study of the Nanoparticles was also performed and found that the drug release kinetics follow zero order kinetics and the graph fitted into Higuchi plot and Korsemeyer-peppas plot. Next step to be performed was the determination of antifungal activity of Nanoparticles. It was performed in well diffusion method using Candida albicans as fungus and the zone of inhibition of the four formulations were observed among which formulation 3 showed more zone of inhibition of 21mm

By analysing all these evaluation parameter of MN Nanoparticle, it was observed that the formulation F3 obtained best results compared to other formulations, and hence Nanoparticle Ointment was prepared by using F3 formulation. The appearance of NP ointment was visually observed and was found to be white in colour and clear in nature. The pH of the formulation was determined using digital pH meter and was found to be 6.9. The viscosity of the prepared gel was determined by using Brookfield viscometer and was found to be 1162cps. Loss on drying of ointment was found to be 39%w/w.The Spreadability of the prepared gel was found to be 7.3 g.cm/sec. The in-vitro drug release study of the prepared gel was performed by using diffusion bag technique. 2 mL of the sample were withdrawn at the time intervals of 1, 2, 6, 10, 12, 20 and 24 hours and the absorbance were measured at 282nm and the percentage drug release was calculated. From the data, the maximum % drug release of F3 was found to be 79.21%. The antifungal activity of prepared nanoparticle ointment was performed by well diffusion method and compared with marketed formulation. From this study the prepared NP formulation showed more zone of inhibition compared to marketed gel with 23mm.

Hence, the formulation F3 containing Miconazole Nitrate nanoparticles has met the objectives of the present study by designing the nanoparticle ointment as a targeted drug delivery which has achieved the site specific and prolonged action. The developed formulation overcome and alleviates the drawback and limitation of Miconazole nitrate. This project suggests that Miconazole Nitrate loaded nanoparticle formulation is more efficient than the marketed formulation for fungal infections for targeted drug delivery. Thus this study may hold promise forfurther studies.

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