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Formulation and Evaluation of Miconazole Nitrate Loaded Novel Nanoparticle Gel for Topical Delivery

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The aim of the present research work is to design miconazole-loaded chitosan nanoparticles that could potentially assemble in wrinkle and hair follicles to provide prolong release to the skin tissue. The amount of drugs required for the preparation of nanoparticles was determined by studying the entrapment efficiency of preliminary batches. The emulsification/Solvent evaporation method was used for the preparation of nanoparticles. Different proportions of Miconazole Nitrate and Chitosan were dissolved in DCM. The size of the globules in the emulsion was reduced by a high energy shearing using a probe Sonicator at 50 % amplitude for 10 Minutes, followed by the addition of 10 ml 2% PVA. After overnight evaporation of DCM, for isolation of dried NPs, the NPs dispersion was centrifuged at 15,000 RCF for 30 minutes. The obtained particles were dispersed in de-ionized water and freeze-dried. 3^2 full factorial design was selected for optimization purposes. Prepared batches were evaluated for various parameters such as entrapment efficiency, production yield, particle size, zeta potential, and SEM. F5 batch was found to be optimized which was then used for the preparation of gel. Three levels of Carbopol934 and propylene glycol were used for the

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optimization of gel. The prepared gel was also evaluated for pH, drug content, viscosity, and spreadability. From the study, it was concluded that nanoparticle gel can be used for the treatment of various skin infections over the conventional gel.

Keywords: Gel; nanoparticle; carbopol; solvent evaporation; antifungal.

1. INTRODUCTION

The incidence of fungal infections is increasing at an alarming rate, presenting an enormous challenge to healthcare professionals. This increase is directly related to the growing population of immunocompromised individuals, resulting from changes in medical practice such as the use of intensive chemotherapy and immunosuppressive drugs [1].

Nanotechnology is a rapidly growing science of producing and utilizing nano-sized particles, that measure in nanometers [2]. Using nanotechnology in the pharmaceutical field, quicker and much cheaper treatments can be developed. Nanoparticles (NPs) were prepared by the solvent evaporation method initially. Formulation of emulsion by dissolving polymer solution in a volatile solvent is used in this method [3,4,5,6].

Miconazole is an imidazole antifungal agent used as Miconazole nitrate for the treatment of superficial candidiasis and of skin infections dermatophytosis and pityriasis versicolor.

Present research work aims to design miconazole-loaded chitosan nanoparticles that could potentially assemble in wrinkle and hair follicles to provide prolong release to the skin tissue. They will provide sustained release of the drug at the site of infection.

2. MATERIALS AND METHODS

Miconazole nitrate was used as a gift sample from GlaxoSmithKline Pharmaceuticals Ltd. Nashik. Chemicals like Chitosan, Dichloromethane, Polyvinyl alcohol, Carbopol934, Propylene glycol, Triethanolamine, Methyl Paraben, and Propyl Paraben were obtained from Modern Science, Nashik. The obtained chemicals were used without further purification.

2.1 Preformulation Studies

Preformulation studies are designed to guarantee the development of a stable, safe, and therapeutically effective dosage form. Preformulation testing is designed to assess the influence of physicochemical properties of drugs and excipients on formulation properties of dosage form, method of manufacturer, pharmacokinetic and biopharmaceutical properties of the resulting product. A full understanding of physicochemical properties may eventually confirm that no significant barriers are present for the formulation development. Organoleptic properties such as colour, odour and appearance, solubility and melting point, UV analysis, FTIR, DSC, and compatibility study were carried out.

2.1.1 Organoleptic characterization of drug

Organoleptic characterization of Miconazole Nitrate was done by observing color, odor, and appearance.

2.1.2 Melting point

The melting point is the temperature at which solid converts into Liquid. The melting point of Miconazole nitrate was studied by Thiele's tube method [7].

2.1.3 Solubility

The solubility of a drug in water, methanol, ethanol, and 6.8 buffer solutions was determined by the shake flask method. The drug was dissolved in solid excess in 10 ml of respective solvent. The solutions were stirred for 48 hours on the magnetic stirrer under thermostated circumstances until the solubility equilibrium is achieved. The solutions were left to sediment under thermostat circumstances. The solution was filtered. Aliquots were taken from the clear part of the solution. The aliquotes were suitably diluted and the absorption was measured with UV-Spectrophotometer Shimadzu, UV-2450 [8].

2.1.4 Ultraviolet (U. V.) spectrum

The Miconazole Nitrate was subjected to UV spectroscopic analysis to find out the wavelength (λ_{max}) at which it shows maximum absorbance. The drug was accurately weighed and dissolved

in methanol to obtain a stock solution of 1000 µg/ml. This solution was then suitably diluted with the same solvent to get a solution of concentration 100 µg/ml. Then the UV spectrum of this concentration was recorded over the wavelength range 200-400 nm. The same procedure was repeated for the phosphate buffer pH 6.8 [9,10].

2.1.5 Fourier Transform Infrared (FTIR) spectrum

The dry sample of the drug was mixed with KBr in the ratio of 1:9. The sample was triturated and finally placed in a sample holder. The spectrum was scanned over the frequency range 4000-400 cm^{-1} in FTIR instrument Shimadzu IRAffinity-1S. The spectral analysis was done, by standard absorbance of the functional groups [11].

2.1.6 Differential Scanning Calorimetry (DSC)

The DSC analysis of the drug sample of Miconazole Nitrate was performed using the DSC instrument (Shimadzu, DSC 60). A small amount of Miconazole Nitrate (2 to 3 mg) was accurately balanced in an aluminum pan, hermetically sealed with the help of a crimper, and the sample pan and reference pan are kept in a DSC analyzer. The sample was heated from ambient temperature 40 ℃ to 400 ℃, with a heating rate of 10℃/minute. Inert atmospheres were provided by purging nitrogen gas flowing at 100 ml/min [11].

2.1.7 Dose strength determination

The amount of drug to be added is a very crucial parameter to be determined. For the determination of the strength of the drug to be incorporated, there was only a way, to prepare trial batches of nanoparticles with different strengths of drug and chitosan, dichloromethane, PolyVinyl alcohol (PVA) as polymer, solvent, and stabilizer respectively. Then trial batches are evaluated for percent entrapment efficiency. And based on entrapment efficiency, the strength of the drug was selected. For such purpose 4 formulation batches of was been prepared with different strengths (15, 25, 50, 75 mg) of the drug and evaluated its percent entrapment efficiency.

2.2 Formulation of Miconazole Nitrate Loaded Nanoparticle

The emulsification/ Solvent evaporation method was used for the preparation of nanoparticles. Different proportions of Miconazole Nitrate and Chitosan were dissolved in Dichloromethane (DCM). Globule size in the emulsion was reduced by a high energy shearing using a probe sonicator (Athena Technology) at 50 % amplitude for 10 minutes, followed by the addition of 10 ml 2% PVA. After overnight evaporation of DCM, for isolation of dried NPs, the NPs dispersion was centrifuged at 15,000 Relative Centrifugal Force (RCF) for 30 minutes (REMI equipment). The obtained pellet was dispersed in de-ionized water and freeze-dried using a freeze dryer (VirTis benchtop K).

2.3 Optimization of Formulation

For optimization of Miconazole nitrate loaded NPs, 3^2 randomized full factorial design was selected. The design was applied to study the effect of the concentration of chitosan and DCM on the formulation. The amount of chitosan (x_1) and the amount of DCM (x_2) were selected as independent variables. These two factors were evaluated at 3 levels as higher, middle, and lower levels with coding +1, 0, and -1 respectively. Levels of X_1 were selected as 5, 75, and 100 mg and for X_2 levels selected were 2, 4, and 6 ml. The dependent or response variables included entrapment efficiency (Y_1) and particle size (Y_2).

Table 1. Trial batches for dose strength determination

Formulation code	Drug Dose Strength (mg)	Chitosan (mg)	Dichloromethane (ml)	Polyvinylalcohol (2 %) (ml)
T1	15	65	2	10
T2	25	75	3	10
T3	50	85	4	10
T4	75	25	5	10

Table 2. Composition of different batches of Miconazole loaded NPs for optimization

Formulation Code	Miconazole (mg)	Chitosan (mg) (X_1)	DCM (ml) (X_2)	PVA (2%) (ml)
F1	25	50	2	10
F2	25	50	4	10
F3	25	50	6	10
F4	25	75	2	10
F5	25	75	4	10
F6	25	75	6	10
F7	25	100	2	10
F8	25	100	4	10
F9	25	100	6	10

2.4 Characterization of Prepared Nanoparticles

2.4.1 Physical appearance

The physical appearance of prepared NPs was observed visually. The white powder was observed.

2.4.2 Drug entrapment efficiency

The percentage of incorporated Miconazole nitrate was determined spectrophotometrically at 272 nm. After centrifugation of the aqueous suspension, the amount of the free drug was detected in the supernatant and the amount of incorporated drug was determined as the result of the initial drug minus the free drug [12].

The entrapment efficiency is calculated using the following formula:

$$EE = [W_1 \text{ (initial drug)} - W_2 \text{ (free drug)}] * 100 / W1 \text{ (initial drug)} \quad (1)$$

2.4.3 Production yield

The production yield was determined by the following formula [13].

$$\text{Production yield} = (\text{Practical yield}) * 100 / (\text{Theoretical yield}) \quad (2)$$

2.4.4 Particle size determination

The mean particle size and size distribution of nanoparticles were determined by Malvern Zetasizer at room temperature. Before measurement, batches were diluted with filtered double distilled water until the appropriate concentration of particles was achieved to avoid multi-scattering events. The colloidal mixture of NPs was added to the sample dispersion unit with a slight shaking to minimize the particulate aggregation by inter-particle interaction [14].

2.4.5 Bulk density

An accurately weighed quantity of NPs was carefully poured into the graduated cylinder. Volume was measured by the use of a measuring cylinder directly from its graduation marks which are labeled as ml. The measured volume was called as the bulk volume and bulk density was calculated by the following formula.

$$\text{Bulk Density} = \text{Mass of sample in gram} / \text{The volume occupied by the sample in ml} \quad (3)$$

2.4.6 Tapped density

The measuring cylinder containing a known weight of NPs was tapped 100 times by using tap density apparatus. The minimum volume occupied by NPs in the cylinder is noted. The tapped density was calculated using the formula.

$$\text{Tapped Density} = \text{Mass of sample in gm} / \text{The volume occupied by the sample in ml after tapping} \quad (4)$$

2.4.7 Hausner's ratio

It is a number that is correlated to the flowability of powder material. Hausner's ratio is calculated by the following formula.

$$\text{Hausner's ratio} = \text{Tapped density} / \text{Bulk density} \quad (5)$$

2.4.8 Carr's index

An important measure that can be obtained from bulk density and tapped density is as follows.

$$\text{Carr's Index} = (\text{Tapped density} - \text{Bulk density}) * 100 / \text{Tapped density} \quad (6)$$

2.4.9 Angle of repose

The angle of repose of the powder blend was determined by using the funnel method. The correctly weighed powder was taken in a funnel. NPs powder was poured up to a certain height through the funnel. The diameter of the powder cone was measured and the angle of repose was calculated by using the following equation.

$$\tan \theta = \frac{h}{r} \quad (7)$$

Where,

r and h are the radius and height of the powder cone respectively.

2.5 Selection of Optimized Formulated Batch

The optimized batch was selected as per the finding obtained during optimization. The batch that shows the least particle size with maximum entrapment efficiency will be considered. Also, results obtained during the micromeritic study were considered for optimization of the formulation.

2.6 Lyophilization

Developed formulations were converted into dry powder using the lyophilization technique. The effect of the cryoprotecting agent such as sucrose, mannitol, lactose, ß-cyclodextrin, fructose, trehalose, dextrose, and PERLITOL on the stability of nanoparticles was studied. In 5 ml of formulation, cryoprotectant was added in increasing concentration 3%, 5%, 7%, and lyophilized for 24 hours at – 50 °C temperature and 0.011 mbar pressure. The lyophilized nanoparticles were reconstituted in double-distilled water and analyzed for particle size [15].

2.7 Evaluation of Optimized Nanoparticle Batch

2.7.1 Fourier Transfer Infrared (FTIR) spectroscopy

The FTIR spectrum of Miconazole nitrate-loaded nanoparticles was recorded in the wavelength range of 4000 to 400 cm^{-1}. The characteristics absorption peaks of Miconazole Nitrate were studied.

2.7.2 Determination of drug loading

Ten milligrams of NPs were weighed and were dissolved in 10 ml of methanol followed by centrifugation at 5000 rpm for 15 minutes. Filtration is carried out and then the filtrate was analyzed at 272 nm using UV-Visible spectrophotometry. The experiment was carried out in triplicate and drug loading was calculated from the following equation [16,17].

$$\text{Drug loading} = \text{Weight of drug-loaded in NP} * 100 / \text{Weight of nanoparticle} \quad (8)$$

2.7.3 Particle size and Poly Dispersibility Index (PDI) value

The mean particle size analysis and PDI value were done with the help of Malvern zetasizer to evaluate the effect of the concentration of polymer on size. PDI is an index of width or spread or variation within the particle size distribution. Monodisperse samples have a lower PDI value, whereas PDI of the higher value indicates a wider particle size distribution and polydisperse nature of the sample [18]. PDI can be calculated by the following equation. Where Δd is the width of distribution denoted by SD and d_{avg} is the average particle size denoted by MV (nm) in the particle size datasheet.

$$PDI = \Delta d / d_{avg} \quad (9)$$

2.7.4 Zeta potential

Zeta potential determines the stability of the formulation by measuring the charge of the drug-loaded droplet surface. Zeta potential for the optimized batch was measured using Malvern Zetasizer. For the determination of zeta potential, nanoparticles were diluted with 0.1 mM KCl and placed in the electrophoretic cell with15 V/cm electric field [18].

2.7.5 Scanning Electron Microscope (SEM)

The shape and surface morphology of Miconazole-loaded NPs was observed by scanning electron microscope. SEM study was carried out using ZEISS, Japan, and the sample was coated by gold ion and the coating was performed for 5-6 min and the sample was analyzed at 250X Scanning.

2.7.6 Differential Scanning Calorimetry (DSC)

DSC was performed to characterize the physical state of prepared Miconazole Nitrate loaded NPs.

About 3-4 mg of prepared NPs was weighed, crimped into aluminium pan, and analyzed at the scanning temperature range from 40 ℃ to 400 ℃ at a heating rate of 100 ℃/ minute.

2.8 Formulation of Miconazole Nitrate Loaded Nanoparticle Gel

Preparation of 2% Miconazole Nitrate gel containing nanoparticles was carried out by the following method. Nanoparticles equivalent to 2 % of the drug were weighed and dispersed in 20 ml of water in one beaker. In another beaker, Carbopol934 was soaked overnight in a sufficient quantity of water. After 24 hours to this remaining ingredient i.e., methyl and propylparaben as a preservative were added. Triethanolamine is added drop by drop to neutralize the pH of a formulation. Different concentrations of Carbopol934 and Propylene Glycol were used for the formulation of gel as shown in Table 3.

2.9 Evaluation of Nanoparticle Gel

2.9.1 pH

The pH of the prepared gel is measured using a digital pH meter (Lab India, 6E404). The pH of the topical gel formulation should be between 3 to 9.

2.9.2 Drug content

1 gm of gel which was quantity equivalent to the dose of the drug was dissolved in 100 ml of phosphate buffer pH 6.8. A sample (5 ml) was taken from this solution and diluted to 25 ml, then Miconazole Nitrate concentration was determined by measuring the absorbance at 272 nm using UV-visible Spectrophotometer [19].

% of drug content = Amount of drug obtained after centrifugation * 100 / Amount of drug taken

2.9.3 Spreadbility test

The Spreadability of the gel formulation was determined by using sliding plate apparatus and by measuring the diameter of 1 gm of gel between horizontal plates after 1 minute. The standardized weight tied on the upper plate was 125 gm. An excess of gel is placed between two glass slides and a 1000 gm weight is placed on them for 5 minutes, to compress the sample to a uniform thickness. The bottom slide is anchored to the apparatus and weights are placed in the pan. The time in seconds needed to separate the two slides is taken as a measure of spreadability. A shorter time interval indicates better spreadability. Spreadability was determined by using the following formula.

$S = M \times L / T$

S = spreadability.
M = weight tied to the upper slide.
L = length of the glass slide.
T = Time taken to separate two slides (sec).

2.9.4 Viscosity

The viscosity of prepared gel was measured using Brookfield viscometer (Brookfield Engineering, spindle LV6) at different RPM viscosity was measured and noted. The measurement was made over the whole range of speed settings from 5-100 rpm with 10 seconds between two successive speeds.

2.9.5 *In-vitro* drug release studies

The *in- vitro* release studies were performed using modified Franz diffusion cell to evaluate the amount of Miconazole Nitrate released from each formulation. These cells consist of donor compartment, acceptor compartment, Dialysis membrane 70, magnetic stirring, thermostatic water bath, and sampling device [20]. Dialysis membrane 70 (Hi-Media, Mumbai, India) of pore size 2.4 nm, molecular weight cut-off between 12,000-14,000 was used for drug release study [21]. The surface area of the diffusion membrane was 3.14 cm^2. The receptor compartment (50 ml) consists of a phosphate buffer of pH 6.8, and is stirred by the magnetic bead at 500 to 600 rpm for proper sample withdrawal and maintained at 37°C ± 0.5°C. Nanoparticle gels equivalent to 2% of Miconazole Nitrate were placed in the donor compartment. After a certain time interval, 2 ml of the sample was withdrawn from the receiver compartment through the side tube and the same volumes of freshly prepared 6.8 pH phosphate buffer were added. The samples were analyzed by UV spectrophotometer at 272 nm. For each formulation, the release studies were performed in triplicate.

3. RESULTS AND DISCUSSION

3.1 Preformulation Studies

3.1.1 Organoleptic properties

The sample of drug received was studied for its organoleptic characters such as colour, odour,

and appearance. The appearance of powder is a crystalline powder. The colour is white and found to be odourless.

3.1.2 Melting point

The melting point of Miconazole nitrate was determined by using Thiel's tube and found to be 181 ℃. The melting point of the drug is found to be near mentioned in official standards.

3.1.3 Solubility

The drug Miconazole nitrate was found practically insoluble in distilled water. The solubility of pure Miconazole nitrate in ethanol, methanol, and phosphate buffer pH 6.8 was found to be 2.9 mg/ml, 11.9 mg/ml, and 11.15 mg/ml respectively. It indicates that the drug is poorly soluble in ethanol, sparingly soluble in methanol as well as in phosphate buffer ph 6.8.

3.1.4 Ultraviolet spectrum

The UV spectrum of Miconazole nitrate solution in methanol and phosphate buffer pH 6.8 exhibited wavelength of absorbance maximum at 271.73 nm, 272.90 respectively. This is near the reported value. However, keeping in mind the probable concentrations likely to be encountered while carrying out *In-vitro* release studies and considering the predicted theoretical λ_{max} involved, the working λ_{max} was decided as 272 nm [22].

3.1.5 Fourier transform Infrared (FTIR) spectrum

The powdered mixture of Miconazole nitrate and KBr was taken in a sample and the spectrum was recorded by scanning in the wavelength region of 4000-400 cm^{-1} using an FTIR spectrophotometer. The FTIR spectrum of Miconazole Nitrate was shown in Fig. 2. The absorption bands shown by Miconazole nitrate are characteristics of the groups present in its molecular structure. The presence of absorption bands corresponding to the functional groups present in the structure of Miconazole nitrate confirms the identification and purity of the gifted Miconazole nitrate sample [23].

Table 3. Composition of Miconazole Nanoparticle gels Containing Various Polymers

Ingredients	Formulation Code								
	G1	G2	G3	G4	G5	G6	G7	G8	G9
Nanoparticle equivalent to (gm)	2 %	2 %	2 %	2 %	2 %	2 %	2 %	2 %	2 %
Carbopol 934 (gm)	0.2	0.4	0.6	0.2	0.4	0.6	0.2	0.4	0.6
Propylene glycol (ml)	5	5	5	10	10	10	15	15	15
Methyl paraben (gm)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Propyl paraben (gm)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Triethanolamine (ml)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Distilled water upto (ml)	20	20	20	20	20	20	20	20	20

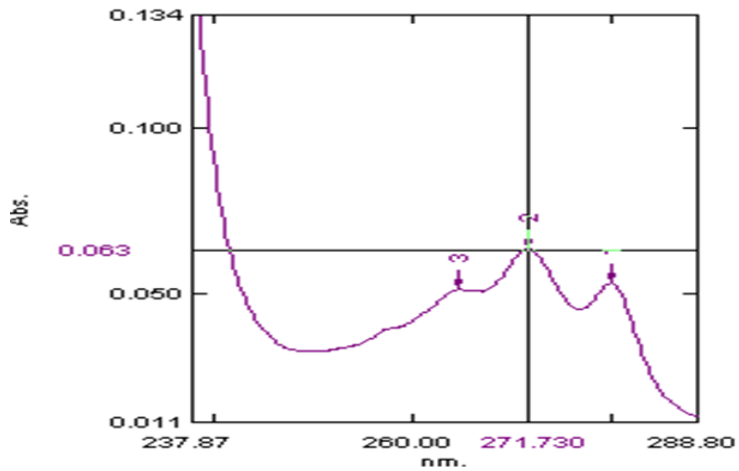

Fig. 1. UV spectrum of Miconazole nitrate in methanol

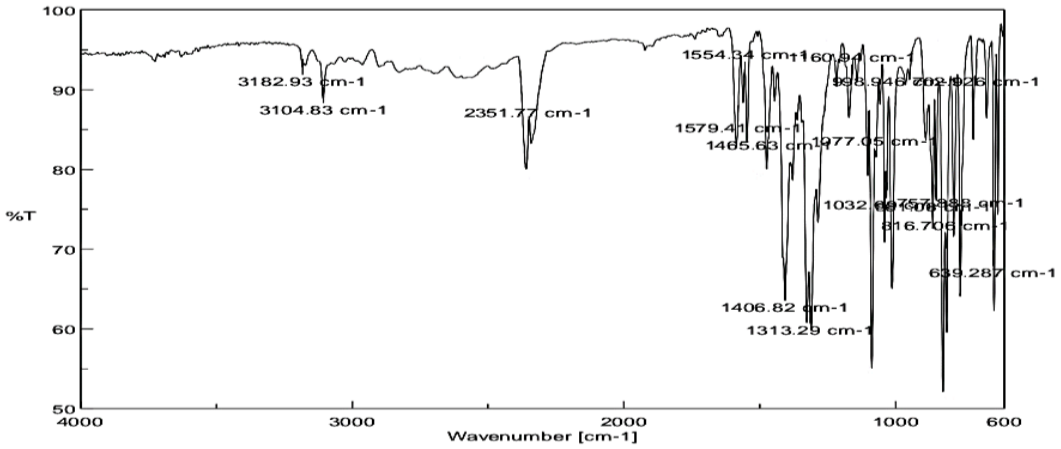

Fig. 2. FTIR spectrum of miconazole nitrate

3.1.6 Differential Scanning Calorimetry (DSC)

DSC thermogram for Miconazole nitrate is shown in Fig. 3. DSC spectra of miconazole nitrate show a peak at 182.90 ℃, which coincides with the melting point of miconazole nitrate [24].

3.1.7 Dose strength determination

For the determination of dose strength, the different trial formulations are formulated according to Table 1 and evaluated for % entrapment efficiency. The results of this study are indicated in Table 4.

Above result shown that the T2 batch shows a higher % entrapment efficiency (92.08 %), which contains 25 mg of the drug. Hence 25 mg concentration of drug was used for the optimization of further batches.

3.2 Optimization of Formulation

To study the optimization of Miconazole Nitrate loaded NPs, 3^2 randomized full factorial design was selected. % Entrapment efficiency and particle size of nanoparticles have been used as dependent variables for the optimization process. Results of optimization obtained are summarized in Table 5.

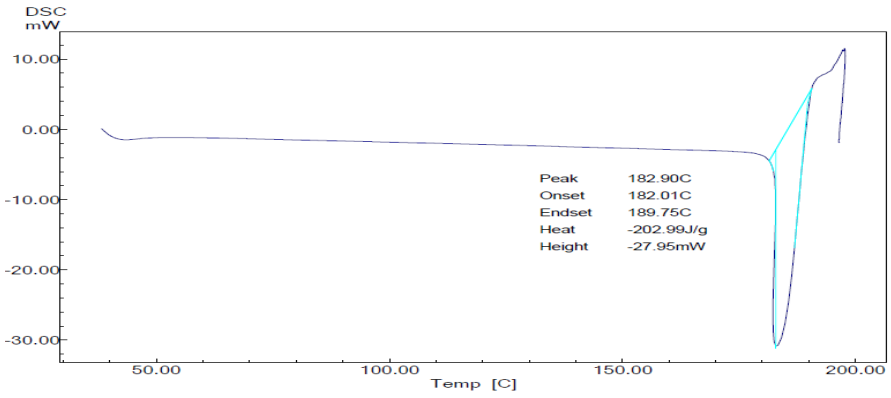

Fig. 3. DSC spectra of miconazole nitrate

Table 4. Trial batches for dose strength determination

Formulation code	% Entrapment efficiency
T1	60%
T2	92.08%
T3	74.02%
T4	85.53%

Table 5. Drug entrapment, particle size, and production yield of formulated batches

Formulation code	Drug entrapment (%)	% Production Yield	Particle size (nm)
F1	83.40 %	84.41 ± 0.13	259.1
F2	77.20 %	77.74 ± 0.03	271.3
F3	77.60 %	78.54 ± 0.13	268.9
F4	89.60 %	85.69 ± 0.02	248.2
F5	93.28 %	92.56 ± 0.13	234.8
F6	90.36 %	88.53 ± 0.01	245.5
F7	84.44 %	84.78 ± 0.04	256.8
F8	72.40 %	74.46 ± 0.47	277.4
F9	80.00 %	81.73 ± 0.12	260.6

Depending upon the results obtained, batch F5 showed maximum drug entrapment (93.28 %) and particle size of 234.8 nm.

3.3 Characterization of Prepared Nanoparticles

3.3.1 Physical appearance

The physical appearances of prepared nanoparticles are observed visually. It is found to be a white crystalline particle having good flowability.

3.3.2 Production yield

The percentage production yield of F1 to F9 batches was found to be in the wide-area from 74.46% to 92.56 %. F5 batch has a good % production yield of 92.56 %. Hence from the study, it can be concluded that concentration of chitosan and time of stabilizer affects the production. The production yield may vary due to change in polymer concentration. Table 5 indicates the percantegThe percentage production yield of nine batches of the nanoparticle is listed in Table 5.

3.3.3 Particle size determination

The particle size of different prepared batches is indicated in Table 5.

3.3.4 Micromeritic properties

Prepared batches were evaluated for various micrometric properties like bulk density, tapped density, Hausner's ratio, Carr's index, and angle of repose. The results are indicated in Table 6. Among the various formulations batch, F5 showed excellent flow properties.

From the above evaluation data batch, F% showed excellent results overall, and hence batch F5 is considered optimized and was used for further studies.

Table 6. Results of micrometric evaluation of formulated batches

Formulation Code	Bulk Density (gm/ml)	Tapped Density (gm/ml)	Hausner's ratio	Carr's Index (%)	Angle of Repose (°)
F1	0.63 ± 0.08	0.78 ± 0.08	1.20	16.01 ± 0.03	26.73 ± 0.05
F2	0.76 ± 0.04	0.91 ± 0.01	1.23	15.00 ± 0.01	28.05 ± 0.03
F3	0.48 ± 0.03	0.55 ± 0.04	1.14	12.72 ± 0.04	23.31 ± 0.07
F4	0.55 ± 0.01	0.61 ± 0.03	1.18	15.38 ± 0.01	29.24 ± 0.01
F5	0.52 ± 0.03	0.58 ± 0.03	1.13	11.87 ± 0.04	22.68 ± 0.02
F6	0.56 ± 0.06	0.65 ± 0.03	1.17	15.19 ± 0.06	27.15 ± 0.05
F7	0.50 ± 0.09	0.58 ± 0.01	1.16	12.19 ± 0.05	26.61 ± 0.04
F8	0.58 ± 0.04	0.70 ± 0.03	1.20	17.14 ± 0.06	28.50 ± 0.08
F9	0.52 ± 0.06	0.60 ± 0.02	1.15	13.33 ± 0.07	30.79 ± 0.03

**Result expressed in mean (n=3) ± SD (Standard Deviation)*

3.4 Lyophilization of Nanoparticles

Usually, NPs formulation shows an increase in particle size in a short time during the storage. It limits the shelf life. Therefore, required to be lyophilization which offers chemical and physical stability by preventing Ostwald ripening and hydrolysis reactions. The suggested mechanism of stabilization of nanoparticles is the water replacement hypothesis. During lyophilization, hydrogen bonds are formed between cryoprotectants and polar groups at the surface of nanoparticles at the end of the drying process. These cryoprotectants preserve the native structures of NPs by serving as water substitutes. The amorphous nature of NPs and cryoprotectants allows maximal H-banding between NPs and stabilizer molecules. Various cryoprotects used are sucrose, mannitol, lactose, fructose, and dextrose. The results of the use of various cryoprotectants are shown in Table 7.

It was observed that after the addition of cryoprotectants there is a slight increase in particle size due to the formation of cryoprotectant layer around NPs. From the above data, 5 % w/v of mannitol was selected among other cryoprotectants for lyophilization.

3.5 Characterization of the Optimized Batch

The optimized batch F5 was further characterized for the following parameters.

3.5.1 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectrum of the optimized nanoparticle is shown in Fig. 4. The FTIR spectrum clearly indicates no interaction between excipients & drugs within the nanoparticle. The FTIR spectrum of the optimized batch was like a pure drug. FTIR spectra of optimized formulation showed substantial variations in the fingerprint region i.e., 600 to 1500 cm^{-1}. This established the development of a bond among excipients and Miconazole Nitrate. Due to cross-linking, there is a noteworthy alteration in the downshift and upshift in the formulation, observed by as S-O, and C-N stretching. Hence it is evident that no chemical reaction is occurring in between drug and excipient.

3.5.2 Drug loading

Drug loading was determined by the procedure described in material and methods. The Miconazole nitrate content of optimized NPs was found to be 86.64%.

3.5.3 Particle size

Malvern zeta sizer was used for particle size determination of optimized batch. The graph shows the particle size increasing order due to an increase in the concentration of polymer. The average particle size of optimized batch F5 is found to be 234.8 nm. The size distribution graph for particle size analysis is shown in Fig. 5.

3.5.4 Poly dispersibility index (PDI)

PDI value of batch F5 formulation is found to be 0.355. The mid-Range monodisperse of the optimized batch was revealed by the PDI study. Therefore, it can be stated that the polymer-based nanoparticle prepared by the solvent evaporation method has exhibited a homogeneous size distribution.

3.5.5 Zeta potential

Zeta potential gives the type of charge present on the surface of the nanoparticle and the stability of the prepared formulation. It is the electric potential in the interfacial double layer i.e., the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. It is used for the quantification of the magnitude of the charge.

Table 7. Influence of various cryoprotectants and their concentration on particle size

Concentration (% W/V)	Particle size (nm)				
	Sucrose	Mannitol	Lactose	Fructose	Dextrose
3%	N	N	N	N	N
5%	191.32	161.31	170.02	182.81	174.44
7%	248.29	210.42	215.84	257.19	220.67

**N indicates that batches failed to produce NPs*

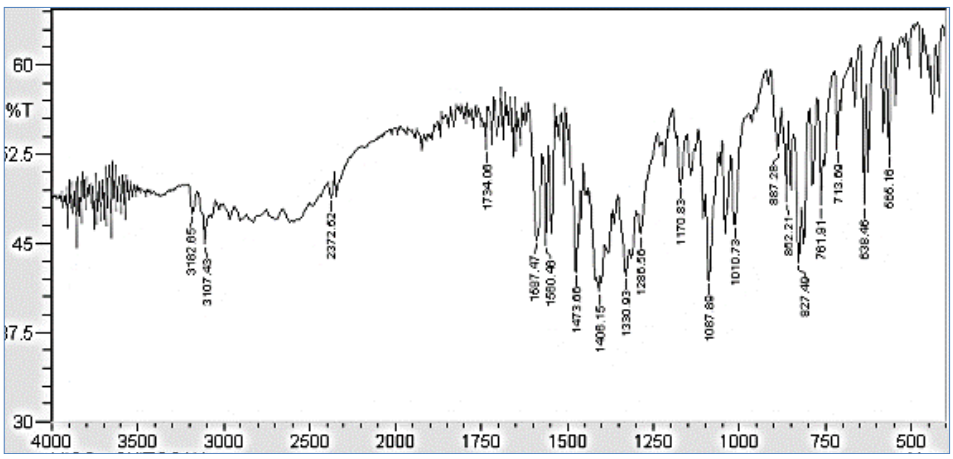

Fig. 4. FTIR spectra of Miconazole nitrate nanoparticles, Batch F5

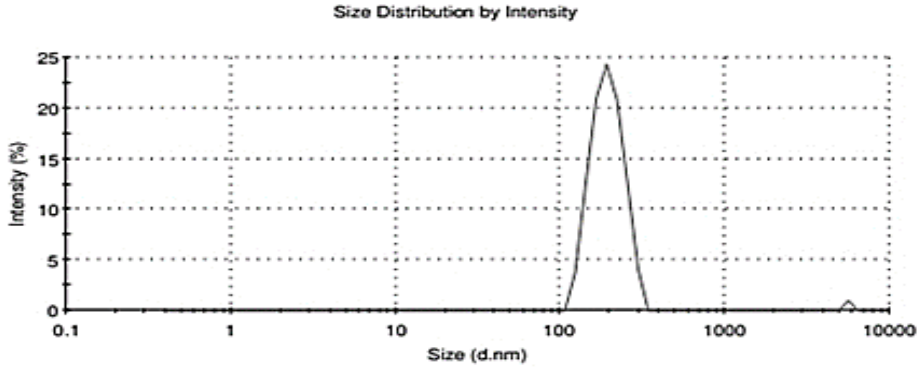

Fig. 5. Particle size analysis of optimized formulation, batch F5

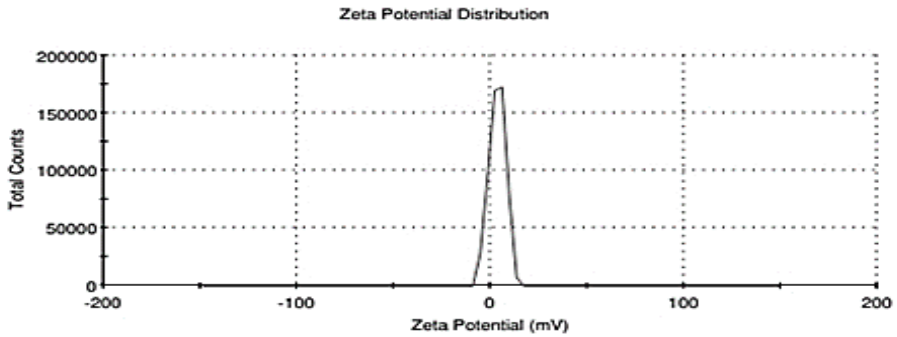

Fig. 6. Zeta potential of optimized F5 formulation

Fig. 5 indicated a graph of Zeta potential. The zeta potential of the optimized batch is found to be 3.48 which is considered good for the stability of nanoparticles.

3.5.6 Scanning electron microscopy (SEM)

The surface morphology of drug particles can be studied by Scanning electron microscopy (Fig. 7). SEM photographs of the optimized batch confirm that nanoparticles were of spherical shape. The surface structure of the nanoparticle was porous with no drug crystal on the surface.

The size is affected by the encapsulation.

3.5.7 Differential Scanning Calorimetry (DSC)

DSC of the optimized batch was executed to find any potential interaction between drug and

polymer and shown in Fig. 8. The sharp endothermic peak observed in the DSC thermogram matches the melting point of the drug at 182.90 ℃**.** The DSC spectra of optimized nanoparticles display a minor discrepancy in endothermic peak as compared to the pure drug while the intensity of peak is somewhat decreased.

The potential reason behind this observation is maybe a decrease in the crystalline size of the drug. The DSC thermogram of the F5 batch shown a broad endothermic peak at 161.69 ℃. It can be concluded that peak broadening may be due to the encapsulation of the drug in the nanoparticle.

3.6 Evaluation of nanoparticle-based Gel

3.6.1 Physical appearance

Physical appearance i.e., clarity and homogeneity were observed visually. From the results, it is clearly evident that all the gel formulations were clear without any aggregates.

3.6.2 Actual drug content

The drug content of the prepared formulation was analyzed. From the results it was evident that drug distribution was uniform and drug content was also satisfactory. The percentage drug content was found to be between 59.89 ± 0.12 to 90.57 ± 0.03. The actual drug content formulated gel formulations are shown in Table 8.

3.6.3 Spreadability

Correct dosage transfer to the target site is based on the spreadability property of the semi-solid dosage form. The spreadability of prepared nanoparticle gel formulation was in the range between 5.81 to 8.34, results are shown in Table 8.

3.6.4 PH

The pH mete was used to determine the pH of the gel formulation. The results were reported in Table 8. Preparations are considered non-irritant as the pH of the prepared formulations was in the range compatible with the normal pH range of the skin.

3.6.5 Viscosity

Brookfield viscometer with spindle LV6 was used to measure the viscosity of the gel. An increase in share rate causes a decrease in viscosity which shows the pseudoplastic behavior of prepared gel. The result indicated the viscosity of gel formulation was consistent.

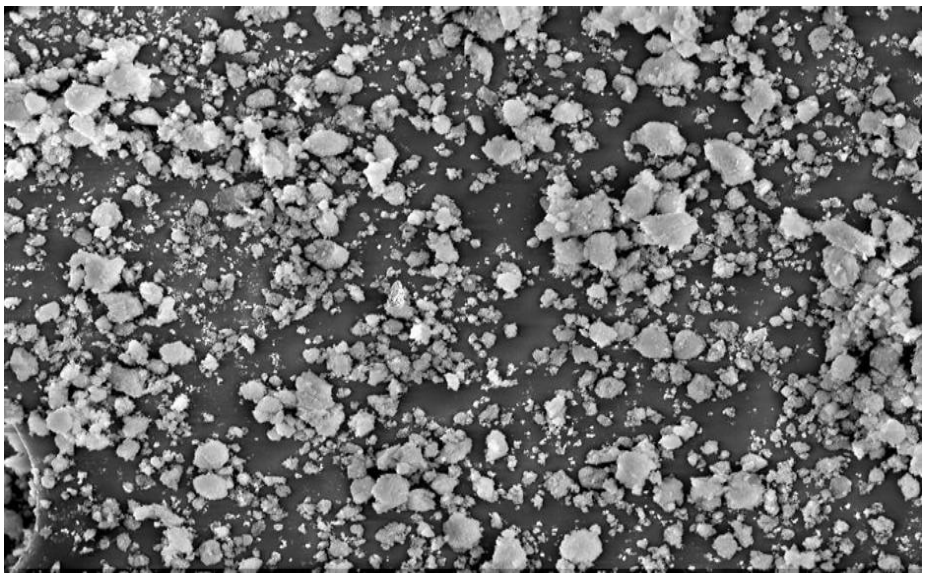

Fig. 7. SEM of optimized F5 formulation

3.6.6 *In-vitro* drug release study

A drug diffusion study was performed on all gel formulations using Franz diffusion cell. The cumulative % release of gel from formulations G1 to G9 has good penetration power, % drug release, and prolonged effect. The release of the gel was found to be sustained over 6 hours. The results of this study signify that administration of gel in nanoparticle form has sustained drug release. The obtained results are shown in Fig. 8.

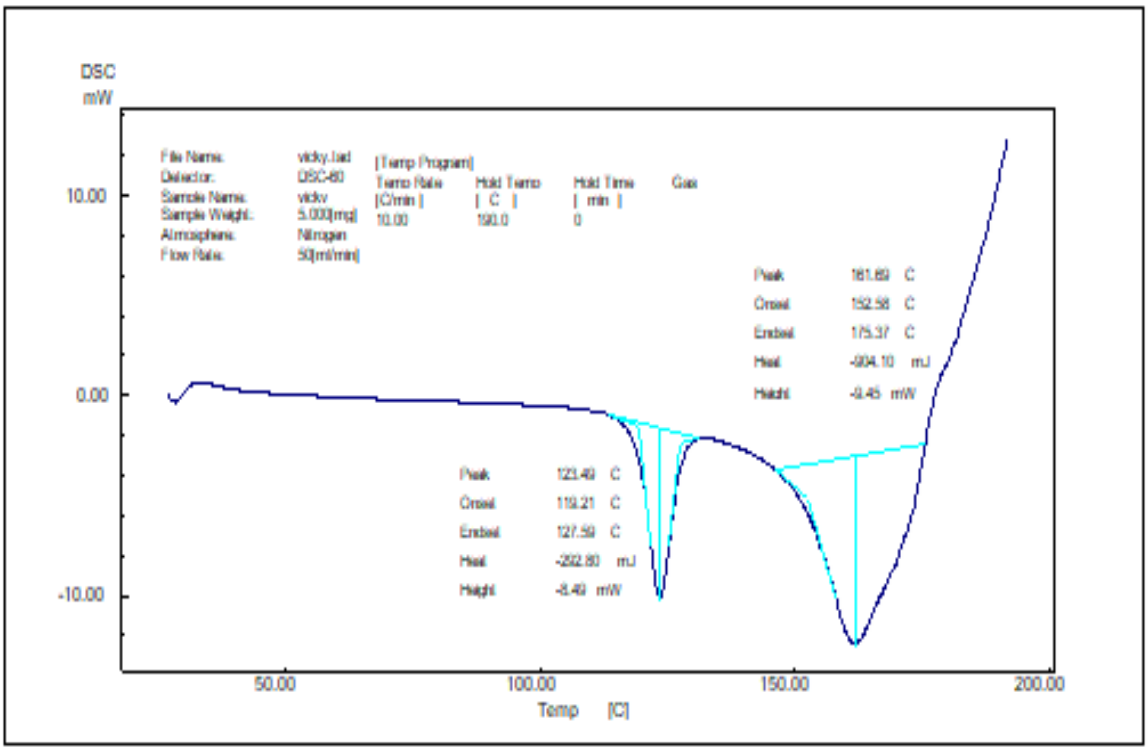

Fig. 8. DSC of optimized F5 formulation

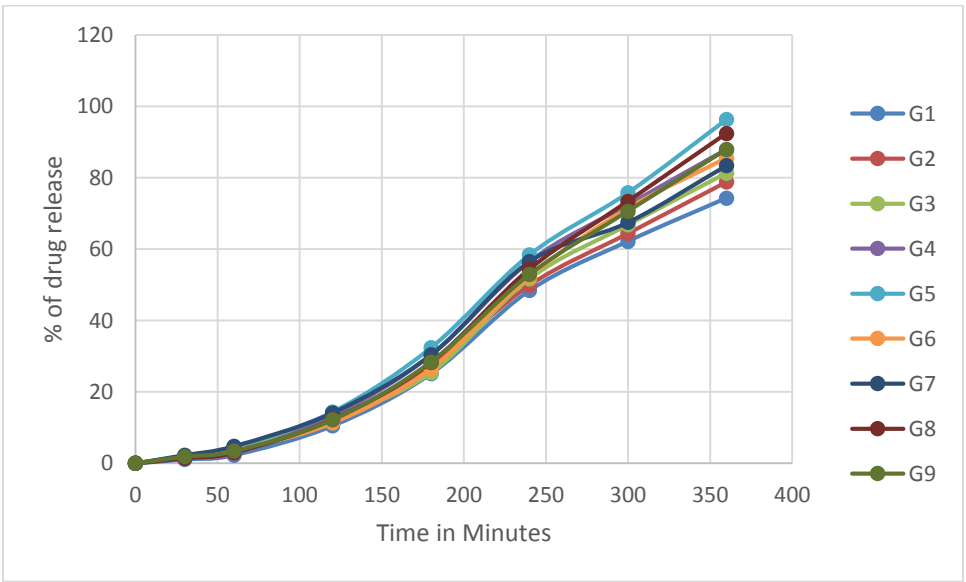

Fig. 9. *In-vitro* drug release pattern of miconazole Nitrate Nanoparticle gel formulations

Table 8. Actual drug content, Spreadability, and pH of the Formulation

Formulation code	Actual Drug Content	Spreadability (gm.cm/sec)	pH
G1	59.89 ± 0.12	5.813	6.9
G2	62.55 ± 0.36	6.2721	7.1
G3	71.53 ± 0.43	6.5321	6.8
G4	74.52 ± 0.80	6.6335	7.1
G5	60.47 ± 0.66	6.1326	7.0
G6	80.73 ± 0.34	6.8291	7.1
G7	69.34 ± 0.61	6.2489	6.9
G8	90.57 ± 0.03	8.3411	6.7
G9	84.66 ± 0.23	7.0611	7.0

4. CONCLUSION

The present study aimed to develop a Miconazole nitrate-loaded NPs with the parameters of its physio-chemical properties. NPs of Miconazole has been developed using the Emulsification/solvent evaporation method. All the prepared formulation shows satisfied organoleptic properties. As no uncountable peaks were observed in FT-IR analysis, so it confirmed the purity of the developed formulation and no interaction of excipients with the drug. It was observed that the Emulsification and Solvent evaporation method was a useful method for the successful incorporation of the drug Miconazole with high entrapment efficiency. Furthermore, it could be presumed that if the nanometer range particles were obtained, the bioavailability might be increased. The result obtained from the response of experiment it was confirmed that the formulation F5 in NPs was optimized as they meet all specifications for bulk density, tapped density, angle of repose, Carr's index, Hausner's ratio, entrapment efficiency, drug content, SEM, Zeta potential and particle size determination. SEM photographs showed a spherical structure having a porous surface. Particle size was observed as 234.8 nm. of optimized batch F5. The entrapment efficiency of the optimized batch was found to be 93.28 %. The drug excipient compatibility study for one month at 50ºC /75% RH did not show any changes in the physical properties. The stability study of the drug shows no changes in the physical appearance, entrapment efficiency. Nanoparticle gel prepared and evaluated for viscosity, Spreadability of nanoparticle gel and results are obtained, which shows nanoparticle formulation shows superior to the marketed conventional gel in the treatment of skin infection such as athletes' foot, jock itch, ringworm, and fungal skin infection (candidiasis). The optimized gel formulation was examined for visual appearance and it was found to be transparent. The pH of the formulation was found to be in between the skin pH range which is in tolerable range for the transdermal route. Hence it is concluded that the above formulation can be more effective than conventional gel used in athletes' foot, jock itch, ringworm, and fungal skin infection (candidiasis).

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable

ETHICAL APPROVAL

It is not applicable

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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