DEVELOPMENT AND EVALUATION OF MICONAZOLE NITRATE ENTRAPPED POLYMERIC NANOPARTICLES

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***Abstract*: The aim of the study was to Development and Evaluation of Miconazole Nitrate entrapped polymeric nanoparticles by nanoprecipitation method using different drug and polymer ratio. Miconazole Nitrate is an antifungal drug with a poor aqueous solubility, which requires the development of new delivery systems to improve its therapeutic effects. Fungal infections are caused by microscopic organisms that invade the epithelial tissue. The drug nanoparticles were prepared using chitosan and Eudragit RS 100 as drug loading polymer. These nanoparticles can effectively direct drug delivery to specific targets and improve drug stability and controlled drug release. Nanoparticles of different ratios were formulated and analyzed for drug content, entrapment efficiency, particle size, zeta potential and in vitro drug release studies. Acetone: Water (1:1) co-solvent system is selected for preparing Miconazole Nitrate loaded nanoparticles using different polymers. Different formulations of nanoparticles were evaluated by using different parameters such as pre & post- formulation studies. F1 & F10 were showed the best drug release within 12 hrs.**

***Keywords*: Nanoparticles, nanoprecipitation method, Miconazole Nitrate and Topical drug delivery system.**

INTRODUCTION [1-6]

Novel drug delivery system aims to deliver drug at a rate directed by the needs of the body during the period of treatment and channel the active entity to the site of action. Nanoparticles represent a very promising drug delivery system of sustained and targeted drug release. Nanoparticles are generally defined as particulate matter particulate dispersions or solid particles with at least one dimension that is less than 100 nm. Depending upon the method of preparation of nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed. **Nanoparticles have been prepared by different techniques such as** solvent evaporation, Nanoprecipitation, Emulsification/solvent diffusion, Salting out, Emulsion, Interfacial polymerization & Ionic gelation or coacervation of hydrophilic polymers.1-6

Nanoparticles are generally defined as particulate matter particulate dispersions or solid particles with at least one dimension that is less than 100 nm. This definition puts them similar size domain as that of ultrafine particles (air borne particulates) and places them as a sub-set of colloidal particles.

The drug molecule is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation of nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are complex in which the drug is cramped to a cavity surrounded by an individual polymer membrane, while nanospheres are matrix complex in which the drug is physically and invariably dispersed. In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as poly (ethylene glycol) (PEG) known as long-circulating particles, have been used as prospective drug delivery devices because of their capability to pass on for a prolonged period time target a particular organ, as transporter of DNA in gene therapy, and their capability to supply proteins, peptides and genes.

The major objective in designing nanoparticles as a delivery system are to manage particle size, surface properties and to free of pharmacologically active ingredients in order to attain the target -specific action of the drug at the therapeutically optimal rate and dose regimen. Though liposomes have been used as potential carriers with unique advantages including protecting drugs from degradation, targeting to site of action and reduction toxicity or side effects, their applications are limited due to inherent problems such as low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components and poor storage strength. On the other hand, polymeric nanoparticles offer some specific advantages over liposomes. For instance, they help to increase the stability of drugs/proteins and possess useful controlled release properties.

ADVANTAGES OF NANOPARTICLES [7-9]

Nanoparticles offers various advantage in drug delivery system. These advantages include:

- Nanoparticles have many remarkable advantages over conventional and traditional drug delivery system.
- Nanoparticles are sustained release form at the site of localization, they change organ distribution of drug substances. They increase drug flow in blood, bioavailability, therapeutic effectiveness and lower easily penetrates to cell walls, blood vessels, stomach epithelium and blood–brain barrier.
- Nanoparticle increases the aqueous solubility of poorly soluble drug, which upgrade bioavailability of drug.
- As a targeted drug transporter nanoparticles decrease drug toxicity and enhance effective drug distribution.
- By using polymers drug release from nanoparticles can be modified which construct polymeric nanoparticle an ideal drug delivery system for cancer therapy, vaccines, contraceptives and antibiotics.

- Useful to determine various diseases.
- Increased stability of ingredients.
- Extend shelf life.
- Used in dental surgery also as filling the cavities in teeth.
- Change the method of drug delivery to improve customer acceptance or decrease manufacturing costs.
- Nanoparticles can be deliver by various routes including oral, nasal, parenteral, intra-ocular etc.
- In the minute areas of body nanoparticles shows better drug delivery as compare to other dosage form and target to a specific cell type or receptor.
- Due to small particle size nanoparticles overcome resistance by physiological barriers in the body.

MATERIALS AND METHODOLOGY

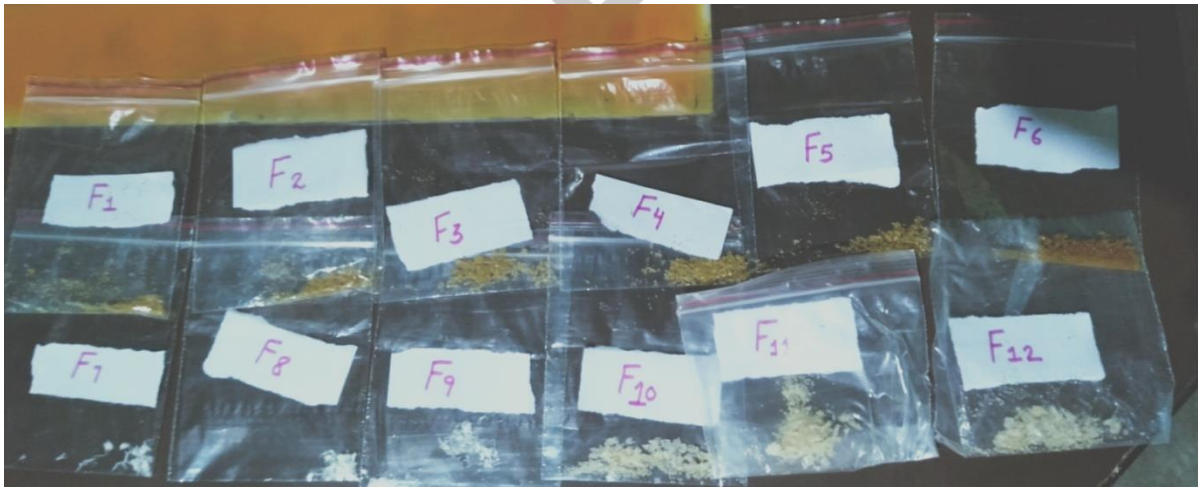
MATERIAL: Miconazole Nitrate was a gift sample from Akums Pvt Ltd, Haridwar, Uttarakhand, India. Eudragit RS-100, Carbopol 940 , Chitosan , Dimethyl sulfoxide(DMSO), Triethanolamine were taken by the college i.e. GRD (PG) IMT 214 Rajpur Road Dehradun, Uttarahand.

METHODS

PREPARATION OF NANOPARTICLES [10-14]

There are different kind of method of preparation but we prepared the nanoparticles by using nanoprecipitation method. Drug was dissolved in water, and then cosolvent (acetone) was added into this solution. A cosolvent was needed in order to make the inner phase more homogeneous. Then polymer and 150 mg of propylene glycol were dissolved in chloroform, and this solution was added to the drug solution to form dispersion. The dispersion was added to 10 ml of aqueous ethanol solution (70%). After 5 minutes of mixing, the organic solvents were removed by evaporation at 35° under normal pressure, nanoparticles were separated by using cooling centrifuge (10000 rpm for 20 min), supernatant were removed and nanoparticles washed with water and dried at room temperature in a desicator. By following the above mentioned procedure the other batches of nanoparticles with different ratio of drug and polymer 2:0.5, 2:1, 2:1.5, 2:2, 2:2.5 and so on were prepared and named FMN^1, FMN^2 , FMN^3 , FMN^4, FMN^5 and so on respectively.

FORMULATION CODE	DRUG (NANOPARTICLES) (%)	CHITOSAN (%)	EUDRAGIT RS 100 (%)	ACETONE (ml)	PEG 400 (ml)	CHLOROFORM (ml)	WATER (ml)
FMN_1	2	3.0	-	10	5	10	10
FMN_2	2	4.0	-	10	5	10	10
FMN_3	2	4.5	-	10	5	10	10
FMN_4	2	5.0	-	10	5	10	10
FMN_5	2	5.5	-	10	5	10	10
FMN_6	2	6.0	-	10	5	10	10
FMN_7	2	-	4.0	10	5	10	10
FMN_8	2	-	5.0	10	5	10	10
FMN_9	2	-	6.0	10	5	10	10
FMN_{10}	2	3.0	3.0	10	5	10	10
FMN_{11}	2	4.0	4.0	10	5	10	10
FMN_{12}	2	5.0	5.0	10	5	10	10



PRE-FORMULATION STUDIES OF DRUG [15-18]

Preformulation studies are defined as the investigation of physical & chemical properties of the drug molecule. Objective of the preformulation study is to establish and compose the stable, efficacious and safe dosage form by obtaining kinetic rate profile, similarity or closeness with drug and the other ingredients.

A) **Organoleptic properties:** It includes colour, taste, odour and texture.

B) **Bulk characterisation studies**: It includes bulk density &tapped density.

C) **Powder flow properties**: It includes Hausner's ratio and angle of repose.
   i) **Angle of repose**
   ii) **Hausner's ratio**

D) **Solubility analysis:** It includes Intrinsic solubility determination, Partition coefficient & Dissolution studies .
Intrinsic solubility determination: Amount of drug is dissolved in a medium and mix it at constant temperature, withdraw the samples at interval time, clarify with filtration process and assayed it using UV, HPLC & GLC.
Partition coefficient: It may be defined as the ratio of concentrations of a compound in a mixture of two immiscible phases at equilibrium**.**
Dissolution studies: The speed or rate at which drug substance dissolves in a medium is called dissolution rate.

E) **Maximum wavelength of drug**
It refers to the wavelength in the absorption spectrum where the absorbance is maximum. It is generally also called as lambda max. Symbol denoted as λ.

F) **Standard curve or calibration curve of drug**
It is a type of graph used as a quantitative research technique. Many samples with well known properties are measured and graphed, which then permits the same properties to be determined for unknown samples by interpolation on graph.
We were prepared standard curve of drug in ethanol,6.8 phosphate buffer and 7.4 phosphate buffer.

G) **Stability studies**
Solution stability: These studies include the effect of pH, ionic strength, co-solvent, light, temperature and oxygen.

H) **FTIR Studies**
The drug excipient compatibility studies were performed by using FT-IR spectrophotometer . The FTIR spectra of drug and polymers were analyzed separately and then correlated for incompatibility.
The scanning range was 400 - 4000 cm -1. The peaks obtained in the spectra's of each drug with polymer correlates with the peaks of drug spectrum. This indicates that the drug was compatible with the formulation components.

CHARACTERISATION OF PREPARED MICONAZOLE LOADED NANOPARTICLES [18-20]

- **Loading Efficiency**
Drug content in the formulation was determined by extracting the drug from the nanoparticles with 0.1 M hydrochloric acid. In this method, the nanoparticles (50 mg) were stirred in 50 ml of 0.1 M hydrochloric acid until dissolved; it was filtered through a whatman filter paper and the drug content was determined, after suitable dilution, at 246 nm by UV spectrophotometry. The loading efficiency (L) of the nanoparticles was calculated by given below formula:

$$L\ (\%) = (Qn / Wn) \times 100$$

Where,
Wn is the weight of the nanoparticles and
Qn is the amount of drug present in the nanoparticles.

- **Entrapment Efficiency**
For determination of drug entrapment, the amount of drug conc. Present in the formulations was determined by UV spectrophotometer at 246 nm. A standard calibration curve of drug was plotted for this purpose. Then percentage entrapment of a drug was calculated by given below formula:
% Drug Entrapment = Experimental drug content/total drug content× 100
- **Drug-Excipient Compatibility Studies**
The drug excipient compatibility studies were performed by using FT-IR spectrophotometer. The FT-IR spectra of drug, polymers, and formulations were analyzed separately and then correlated for incompatibility.
- **In vitro release studies**
The in-vitro drug release studies were carried out using Franz diffusion cell. The formulation was applied on dialysis membrane which was placed between the donor and receptor compartment of the franz diffusion cell. The temperature of the cell was maintained at 37˚C by circulation jacket. This whole assembly was kept on a magnetic stirrer and the solution was stirred continuously using a magnetic bead. A similar blank set was run simultaneously as a control. Sample 5 ml was withdrawn at suitable time intervals and replaced with equal amounts of fresh dissolution media. Samples were analysed spectrophotometrically at 246 nm.
- **Stability study**
The stability study was carried using the batch F1 & F10. The stability of drug loaded nanoparticles was evaluated in terms of its drug content, pH and physical parameters. The stability of nanoparticles was evaluated in PBS (pH 6.8). Nanoparticles formulation was incubated at 5-8° and 37 ± 1° for a period of 60 d. After specified time intervals, the suspension was centrifuged at 15,000 rpm for 1 h, supernatant was removed and nanoparticles were dissolved in

dichloromethane. After adding of water and separation, the amount of drug was detected by UV-Vis spectrophotometrically method at 246 nm.

- **Particle Size & Particle Size Distribution**
  The particle size and particle size distribution of the formulation was determined by photo correlation spectroscopy with a zeta master (Malvern Instruments, UK) equipped with the Malvern PCS software. Every sample was diluted with distilled water. The surface charge (Zeta potential) was determined by measuring the zeta potential the nanoparticles using a Malvern zeta sizer (Malvern Instruments, UK). Samples were prepared by diluting with distilled water.

RESULT & DISCUSSION

❖ PRE-FORMULATION STUDIES

- **Organoleptic properties:**

Parameters	Interference
Colour	White
Odor	Odorless
State/Form	Smooth
Melting Point	130˚C

- **Powder flow properties**

S.NO.	Parameters	Interference	Type of Flow
1.	Hausner's ratio	1.189	Good Flow
2.	Carr's index	27.8	Excellent
3.	Angle of Repose	0.66	Excellent

- **Bulk characterisation studies**

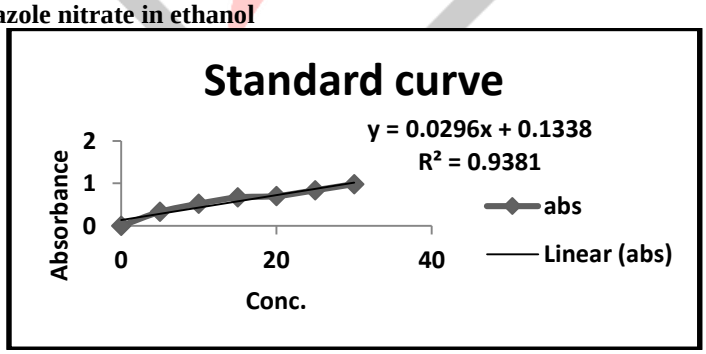
S.No.	Parameters	Interference
1.	Tapped Density	0.615
2.	Bulk Density	0.444
3.	Hygroscopicity	0.48

- **Solubility analysis:**

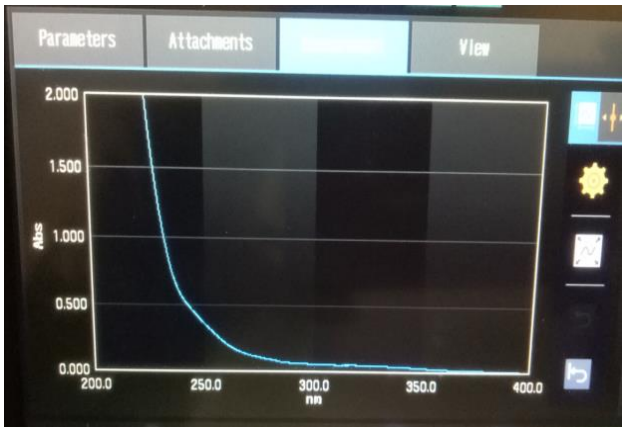
S.No.	Solvent used	Absorbance	Interference
1.	Methanol	0.394	++
2.	Ethanol	0.896	++++
3.	Water	0.012	+
4.	Acetone	0.765	+++
5.	Chloroform	0.459	+++

Partition coefficient of pure drug (Miconazole nitrate) was found **1.42.**

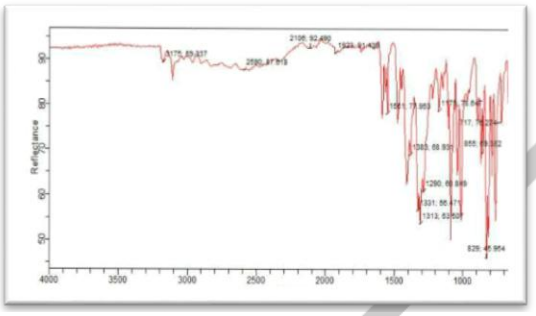
- **Standard graph of miconazole nitrate in ethanol**



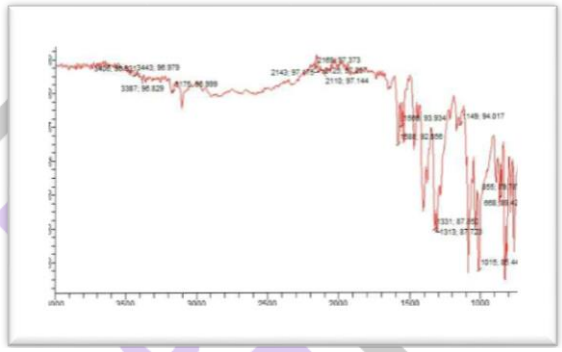
- **Maximum wavelength of drug**
  Maximum wavelength of Miconazole Nitrate was measured by using UV-Visible spectrophotometer was 246 nm.



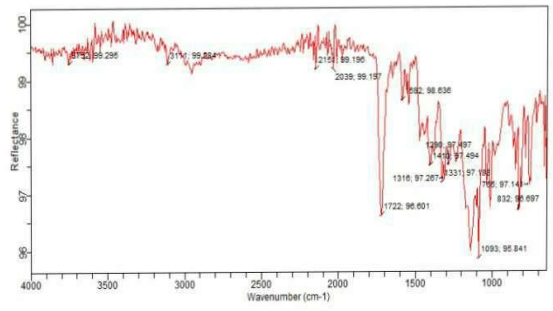
- **FTIR Studies**



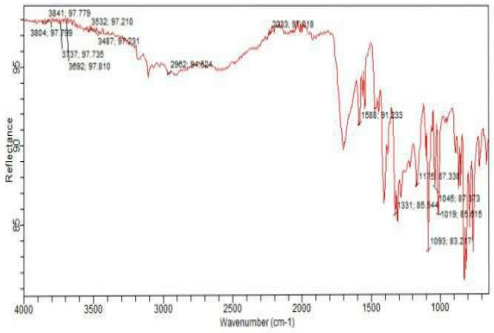
IR Spectra of Miconazole Nitrate



IR- Spectra of Miconazole Nitrate + Chitosan



IR Spectra of
Miconazole Nitrate + Eudragit RS 100



IR Spectra of
Miconazole Nitrate + Carbopol

- **CHARACTERISATION OF MICONAZOLE NITRATE LOADED NANOPARTICLES**

- **Entrapment efficiency of Nanoparticles**

FORMUALTION CODE	ENTAPPED EFFICIENCY (%)
FMN[1]	49.25
FMN[2]	48.65
FMN[3]	49.05
FMN[4]	49.15
FMN[5]	48.1
FMN[6]	48.9
FMN[7]	48.95
FMN[8]	49.0
FMN[9]	48.45
FMN[10]	49.8
FMN[11]	48.4
FMN[12]	49.10

- **In-Vitro drug release from Nanoparticles**

TIME (Hrs)	FMN 1	FMN 2	FMN 3	FM N4	FM N5	FM N6	FM N7	FM N8	FM N9	FMN 10	FMN 11	FMN 12
0	0	0	0	0	0	0	0	0	0	0	0	0
1	20.3	26.5	28.1	29.2	29.9	30.1	25.8	29.4	32.4	30.3	32.5	36.5
2	26.3	30.0	33.6	33.2	31.8	32.7	29.3	32.7	36.5	37.0	38.4	40.3
3	30.2	34.6	39.8	38.1	33.6	36.4	32.4	35.8	39.6	40.8	45.6	46.5
4	36.1	39.3	43.6	40.3	36.8	38.3	36.8	39.4	42.3	49.8	49.0	59.6
5	43.5	43.1	46.8	42.3	39.2	42.6	40.4	42.4	47.3	56.3	53.3	61.3
6	48.4	48.5	53.1	47.1	40.2	46.4	48.5	48.1	50.0	59.5	56.4	65.4
7	52.5	53.4	55.6	48.7	49.2	48.9	55.7	50.9	53.1	63.5	65.6	68.6
8	58.1	59.4	59.9	50.3	43.3	50.4	65.3	53.9	54.7	72.3	72.8	70.8
9	62.5	63.3	67.1	53.6	49.8	52.6	73.4	65.9	58.9	80.7	78.3	74.6
10	77.3	66.5	69.2	58.8	52.4	53.0	85.3	70.8	60.0	87.6	85.2	79.7
11	85.9	69.4	69.8	61.3	55.6	54.9	90.9	75.7	65.8	91.5	89.0	82.9
12	**96.8**	71.5	70.2	65.9	59.4	55.2	95.8	78.9	70.8	**97.9**	90.2	83.3

CONCLUSION

In the present research work, it was concluded that Developed and evaluated Miconazole nitrate entrapped polymeric nanoparticles loaded gels by reducing first pass metabolism and increasing the bioavailability. Miconazole nitrate is an imidazole derivative, used for the topical as well as systemic fungal infections and used to treat ringworm and other skin infections.The nanoparticles were prepared by nanoprecipitation method using 2 different polymers in different ratios such as Chitosan 3%, 4%, 4.5%, 5%, 5.5% and 6%, Eudragit RS 100 4%, 5% and 6% along with other excipients.

It can be concluded that the formulation that incorporated the use of drug loading polymers in different ratios i,e smaller the concentration of polymers used, higher the drug release rate of the nanoparticles that is one of the factors to get better bioavilability and bypass the first pass effect.

Based on the above research work following conclusion can be drawn from the methodology:

❖ **Pre-formulation studies:**

Various preformulation studies were conducted on the sample of the drug powder to check the purity and compatibility study, it can be concluded that:

- Miconazole nitrate were recognised by using Organoleptic properties including colour, odour and it was found to be solid white crystalline powder, odourless.
- Melting point was estimated by capillary method.
- From the solubility behaviour, it was estimated by using various solvent like methanol, ethanol, chloroform & water, the drug sample was found in it.
- In UV spectrometry, calibration curve were prepared by using different medium such as ethanol, 6.8 PBS & 7.4 PBS and observed R^2values.
- Compatibility studies means that the drug was compatible with other excipients by using FTIR.

❖ **Post-formulation studies of Nanoparticles**

- F1-F6 contains chitosan used as drug loading polymer with different concentrations, F6-F9 contains Eudragit RS 100 used as drug loading polymer with different concentrations and F10-F12 contains both chitosan as well as Eudragit RS100 used as drug loading polymer with different concentrations.
- The drug entrapment efficiency was higher in F1 & F10 formulation i.e 49.5 & 49.8 % respectively.
- In the dissolution study, F1 & F10 were the best formulations possessed drug content i.e 98.5 & 99.6% respectively by using UV spectrophotometry.
- Similarly F1 & F10 showed the in-vitro drug release by using franz diffusion cell i.e 96.8 & 97.9 % respectively, i.e particle size decreases with decreasing concentration of drug loading polymers.

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