Formulation,Optimization,and Evaluation of Cyclodextrin-Based Nanosponges as a Novel Topical Gel Delivery System for Antifungal Activity

Nihal Ashish Tamboli, Vishal Umesh Kulkarni, Rohan Londhe

Dbatu University College name Shri Ganpati Institute of Pharmaceutical Sciences and Research Centre Tembhurni

Introduction:-

1.1. Fungal Infection :

Superficial fungal infections of the skin can be caused by dermatophytes, yeasts and nondermatophytes. The various fungal organisms that cause skin infection are constantly competing with each other for their particular environmental niche, which can result in the emergence of a dominant species. Knowledge of current epidemiological trends in the incidence of cutaneous fungal infections is important in diagnosis and treatment of the disease. Singapore is a country in Southeast Asia located at the southern tip of Peninsular Malaysia, 136.8 kilometres north of the Equator. It has a cosmopolitan population of 4.45 million people, with Chinese (76.7%), Malays (14%) and Indians (7.9%) forming the majority.

This is an epidemiological study into recent trends of fungal skin infections seen at the National Skin Centre, which is a tertiary referral centre for skin diseases in the country.
Superficial fungal infections are caused by many fungi, which can invade various aspects of the human body.(1–3) These infections include dermatophytes, which infect keratinized epithelium, hair follicles, and nail apparatus; Candida sp, which needs a warm, humid environment; Malassezia sp, which requires a humid microenvironment and lipids to grow; and Trichosporon sp and Hortae sp. Dermatophytes infect nonviable, keratinized cutaneous structures such as stratum corneum, nails, and hair.(1–3) An epidermal dermatophytosis infection is called epidermomycosis, dermatophytosis of hair and hair follicles is called trichomycosis, and dermatophytosis of the nail apparatus is called onchomycosis. Three genera of dermatophytes exist: Trichophyton sp, Microsporum sp, and Epidermophyton sp. Trichophyton rubrum is the most common cause of epidermal dermatophytosis and onchomycosis.(4)

1.1.1. Transmission

Dermatophyte infections can be transmitted from person to person through fomites, transmitted from animals to humans, and, least commonly, acquired from soil. Predisposing factors for complicated dermatophyte infections include atopic diathesis, such as cell-mediated immune deficiency for T rubrum, prolonged immunosuppression with use of topical glucocorticoids, and systemic immunocompromised states.(4)

1.1.2. Diagnosis

It is important to recognize characteristic patterns of inflammation to diagnose fungal infections. The highest numbers of hyphae are located in the active border of infection, the best area to obtain a sample for potassium hydroxide examination. The active border is scaly, red, and slightly elevated. One can find vesicles in the active border when inflammation is intense. Many ways of obtaining a diagnosis exist through laboratory examinations.5,6 The most important test for the diagnosis of dermatophyte infection is direct visualization under microscopy of the branching hyphae in keratinized material. One should collect scale with a scalpel blade, place on the center of a microscopy slide, and cover with a coverslip. Potassium hydroxide, 5% to 20% solution, is applied at the edge of the coverslip. The preparation should be gently heated under low flame until bubbles begin to expand, clarifying the preparation.
Potassium hydroxide dissolves the material that binds cells together but does not distort the epithelial cells or fungi. Under microscopy, dermatophytes will be recognized as septated, tubelike structures called hyphae and mycelia. A slight back-and-forth rotation of the focusing knob can aid in visualization of the entire segment of the hyphae, which may be at different depths.5,6 A Wood's lamp can be used to visualize hairs infected with Microsporum species, which fluoresce in a greenish color in a dark room. Fungal cultures are grown on Sabouraud's glucose medium, but it is usually not necessary to know the species of dermatophyte infecting the skin, as the same oral and topical agents are effective against all of them. Distal and lateral subungual onchomycosis are best visualized using periodic acidSchiff or methenamine silver stains, which are more sensitive than potassium hydroxide preparation or fungal culture.(5)

Deep Inflammatory Lesions

Zoophilic fungi such as Trichophyton verrucosum from cattle may produce a very inflammatory skin infection. This infection is more common in northern regions where cattle are confined in close quarters in the winter season. The round, intensely inflamed lesion has a uniformly elevated, red, boggy, pustular surface. The pustules are follicular and represent deep penetration of the fungus into the hair follicle. A fungal culture helps identify the animal source of the infection. A distinctive form of inflammatory tinea called Majocchi's granuloma is caused by T rubrum and was originally described as occurring on the lower leg of women who shave, but it is also seen at other sites on men and children. The primary lesion is a follicular papulopustule or inflammatory nodule. Intracutaneous and subcutaneous granulomatous nodules arise from these initial inflammatory tinea infections. Lesions have necrotic areas containing fungal elements which are surrounded by epitheliod cells, giant cells, lymphocytes, and polymorphonuclear leukocytes, and are believed to be a result from rupturing of infected follicles into the dermis and subcutis.(4)

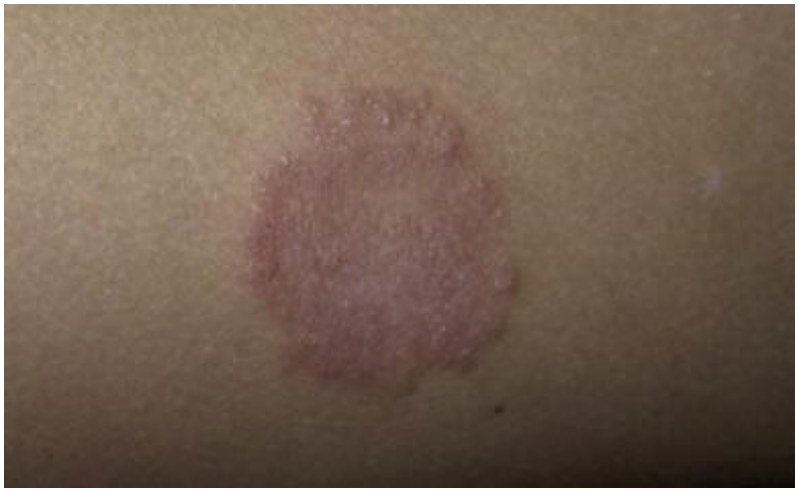


Fig. 1. Tinea corporis. *(From Elewski BE, Hughey LC, Sobera JO, et al. Fungal diseases. In: Bolognia JL, Jorizzo JL, Schaffer JV, editors. Dermatology. 3rd edition. Philadelphia: Saunders; 2012; with permission.)*

TINEA OF THE HAND

Tinea of the dorsal aspect of the hand, or tinea manuum, has all the features of tinea corporis. Tinea of the palm has the same appearance as the dry, diffuse, keratotic form of tinea on the soles. The dry keratotic form can be asymptomatic; hence,patients may be unaware of the infection, attributing the dry, thick, scaly surface to hard physical labor. Tinea of the palms is frequently seen in association with tinea pedis. The usual pattern of infection involves 1 foot and 2 hands or 2 feet and 1 hand. Treatment is the same as for tinea pedis.(4)

Fungal infections that are treated with topical steroids often lose some of their characteristic features. Topical steroids decrease inflammation and give the false impression that the rash is improving while the fungus continues to thrive secondary to cortisone-induced immunologic changes. When treatment is stopped, the rash returns but in a different manner. Scaling at the margins can be absent. Diffuse erythema, diffuse scale, scattered pustules or papules, and brown hyperpigmentation may all result. A once localized process may have expanded greatly and the intensity of itching is variable. Tinea of hand is most often seen on the groin, the face, and the dorsal aspect of the hand. Tinea infections of the hand are frequently misdiagnosed as eczema and treated with topical steroids. Hyphae are easily seen when scaling reappears.(4)

1.2 Characteristics of Nanosponges :-

1. Nanosponges are porous particles, used mainly to encapsulate the poorly soluble drugs.

2. The nanosponges have high aqueous solubility and are capable of carrying both lipophilic and hydrophilic drugs.

3. Nanosponges compositions are stable throughout a pH range of 1 to 11 and at temperature up to 300 °C.

4. Nanosponges are non-irritating and non-mutagenic, non-allergic and non-toxic and protect the drug from physiological degradation.

5. Nanosponges may encapsulate several types of pharmacological molecules by generating(12)

Advantages

1. Nanosponge provides the site-specific drug delivery and predetermined release.
2. A Smaller quantity of the drug comes in contact with the normal tissue hence produces
3. These formulations are soluble in water and are capable of encapsulating hydrophobic
4. Nanosponge formulation is used to hide undesirable taste of drug substance and to convert liquid substances to solids.
5. Particles can be prepared smaller or larger by varying the proportion of cross-linker to
6. Due to their average pore size, 0.25 um, bacteria cannot penetrate in nanosponges.
7. Nanosponges improve stability.
8. Nanosponges are self-sterilizing.
9. Nanosponges improve formulation flexibility.
10. Nanosponges are used to improve dissolution (12)

Disadvantages

1. Nanosponge may face problem of loading capacities.
2. Formulation of nanosponge includes only small molecules.(12)

1.2.2 Factors that influence nanosponge formation: (13)

I. Type of polymer- The creation and functionality of nanosponges can be influenced by the kind of polymer utilised. The nanosponge's cavity should be big enough to fit a specific-sized drug molecule for complexation.

II. Type of drugs- The following features of the drug molecules should be present when they are complexed with nanosponges. Molecular weight of drug should be between 100 to 400 daltons. Drug molecule should consist of less than five condensed rings.Drug solubility in water should be less than 10mg/ml.Melting point of the substance should be less than 250℃

III. Temperature- Drug/nano sponge complexation can be impacted by temperature fluctuations. In general, as the temperature rises, the strength of the drug/nanosponge complex's apparent stability constant falls. This may be because the contact forces between the two substances-van der Waal and hydrophobic interactions, for example decrease as the temperature rises

IV. Method of preparation- The method of loading the drug into the nanosponge can affect drug/nanosponge complexation, however, the effectiveness of a method depends on the nature of the drug and polymer. In many cases freeze drying is found to be most effective for drug complexation.

V. Degree of substitution- The kind, amount, and location of the substituents on the parent molecule may have a significant impact on the nanosponge's capacity to complex

1.2.3. Preparation of nanosponges :-

1. Solvent Diffusion Method :- (14)

Different ratios of polyvinyl alcohol (PVA) and ethyl cellulose (EC) can be used to create nanosponges (PVA). The dispersed phase containing ethyl cellulose and drug are
dissolved in 20 millilitre dichloromethane and slowly added to a definite amount of polyvinyl alcohol in 150 millilitre of aqueous continuous phase. The reaction mixture is stirred at 1000 rpm for 2h. The Nanosponges formed are collected by filtration and dried in oven at 40℃ for 24h. The dried nanosponges are stored in vacuum desiccator to ensure the removal of residual solvents.

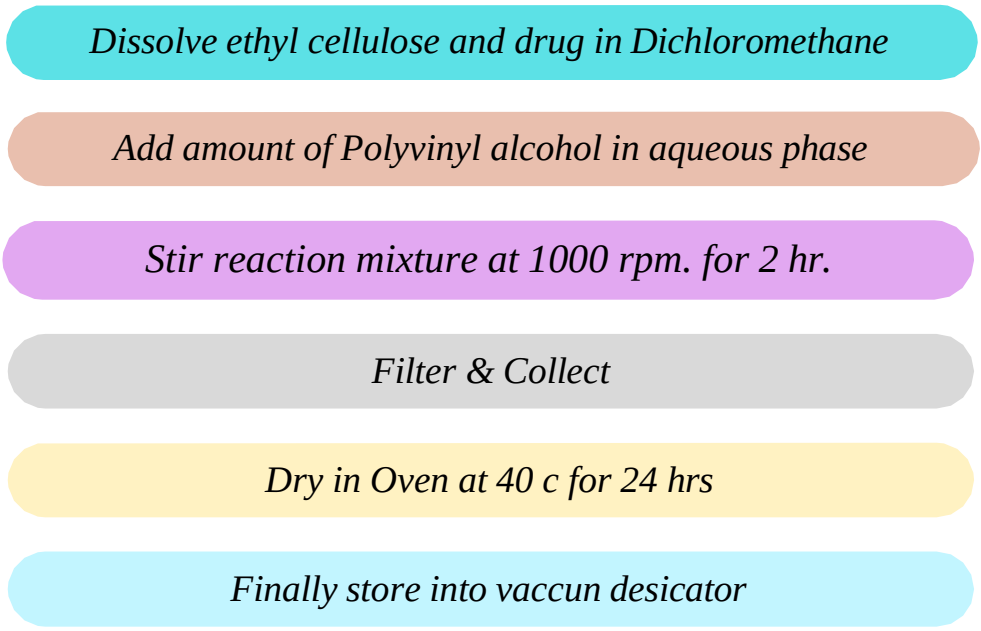


Fig5-f low diagram for preparation of nanosponge

2. Nanosponges prepared from β-Cyclodextrins :- (15)

Nanosponges are recently formulated by hyper cross linked cyclodextrins nano structure to form the 3- dimensional networks; a generally spherical structure, about the size of protein. with channels and pores inside. Nanosponges are obtained by reacting cyclodextrin with cross linker like isocynates, dimethyl carbonate, diphenyl carbonate and carboxylic acid,

carbonyl diimidazoles and dianhydrides. The surface charge, porosity, density and pore sizes of nanosponges can be controlled to attach with different molecules. Nanosponges with low cross linking give a quick drug release.

3. Solvent Method

In this method, polymer is mixed with a suitable solvent, mainly in a polar aprotic solvent such as dimethylformamide (DMF) or dimethylsulfoxide (DMSO). This mixture is added to cross linker in an excess quantity, the ratio for cross linker/ molar ratio is preferred as 1:4. The reaction is carried out at temperature ranging from 100℃ to the reflux temperature of the solvent, for time ranging from 1 to 48 hr. The cross linkers which may be preferred are dimethyl carbonate and carbonyl diimidazole. After their action is finished, the solution is left to cool to room temperature.

4. Ultrasound- Assisted Synthesis :- (16)

In this method, nanosponges can be obtained by reacting polymers with cross- linkers inthe absence of solvent and under sonication. The resulting nanosponges will have a sphere-like shape, be homogeneous in size, and be less than 5 microns. Use of pyromelitic anhydride or di-phenyl carbonate as a cross-linker is part of this procedure. Here, the polymer and cross- linker are mixed in a flask. The flask is placed in an ultrasound bath filled with water and heated to 90℃ and sonicated for 5 hours. Then, the solid is ground in a mortar and soxhlet extraction is done with ethanol to remove either impurities (or) unreacted polymer. After Purification nanosponges are stored at 25℃.

1.2.4. Characterization of nanosponges

The following approaches can be used to characterize inclusion complexes generated between the drug and nanosponges.

1. Solubility Studies

The widely used approach to study inclusion complexation is phase solubility method described by Higuchi and Connors, which examines the effect of nanosponge, on the solubility of drug. The phase solubility diagrams indicate the degree of pomplexation.

2. Microscopy studies-

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) can be used to study the microscopic aspects of the drug, nanosponges and the product (drug/nanosponge complex).
Even if there is a noticeable difference between the raw material's crystallisation state and the product made via co-precipitation, the difference between the raw material's crystallisation state and the product as viewed under an electron microscope points to the creation of inclusion complexes.

3. Particle size and polydispersity-

The particle size can be determined by dynamic light scattering using 90 Plus particle sizer equipped with MAS OPTION particle sizing software. From this the mean diameter and polydispersity index can be determined.

4. Zeta potential- (19)

The zeta potential is known as the measure of a surface charge, which is measured using additional electrode in the particle size equipment and for this type of zeta potential determination, samples of the NSs should be diluted with KCL. 0.1 mole/L and should be placed in the electrophoretic cell, where the electric field of 15 V/cm is applied. Thus, the mean hydrodynamic diameter and the polydispersity index of particles can be calculated using the cumulated analysis after averaging of all the total measurements.

5. Entrapment efficiency. (7)

The weighed amount of drug loaded nanosponges are kept in suitable solvent and soaked for 3hr. Samples are then filtered and analysed by using UV spectrophotometer. The actual drug content can be determined by the amount of drug which is entrapped in nanosponges. The weighed amount of drug loaded nanosponges (50mg) is kept in 10 ml solvent and soaked for 3 h. The samples are filtered and analyzed at . max of drug against blank using UV spectrophotometer (Shimadzu, PharmaSpecUV-1700, Japan).

Encapsulation efficiency can be calculated by following formula:

$$\textit{Entrapment efficiency (\%)} = \frac{\textit{Total amount of drug-Free unentrapped drug} \times 100}{\textit{Total amount of drug}}$$

6.Fourier Transform Infra-Red spectroscopy- (17)

Infra-Red spectroscopy is used to estimate the interaction between nanosponge excipients and the drug molecules in the solid state. When a complex is formed, nanosponge bands frequently vary very little, and if fewer than 25% of the guest molecules are encapsulated in the complex, bands that may be attributed to the included portion of the guest molecules are quickly hidden by the bands of the nanosponge spectrum. The method is less illuminating than other approaches and is often unsuitable for the detection of inclusion complexes.

7.X-ray diffractiometry and single crystal X-ray structure analysis- (18)

Powder X-ray diffractiometry can be used to detect inclusion complexation in the solid state. When the drug molecule is liquid since liquids have no diffraction pattern of their own, then the diffraction pattern of a newly formed substance clearly differs from that of uncomplexed nanosponge. This difference of diffraction pattern indicates the complex formation. When the drug compound is a solid substance, a comparison has to be made between the diffractogram of the assumed complex and that of the mechanical mixture of the drug and polymer molecules. A diffraction pattern of a physical mixture is often the sum of those of each component, while the diffraction pattern of complexes are apparently different from each constituent and lead to a "new" solid phase with different diffractograms. Diffraction peaks for a mixture of compounds are useful in determining the chemical decomposition and complex formation.

The complex formation of drug with nanosponges alters the diffraction patterns and also changes the crystalline nature of the drug. The intricate creation causes some peaks to change, some to look new, and some to become sharper than before.

8.Single crystal X-ray structure analysis- (18)

This method is used to determine the detailed inclusion structure and mode of interaction. The interaction between the host and guest molecules can be identified and the precise geometrical relationship can be established. This information obtained during the analysis leads to know about the formation of inclusion complexes.

1.2.5. **Applications: (7)**

1) Nanosponges for Drug Delivery

2) For protein delivery

3) In anti-cancer therapy

4) In anti- viral therapy

5) In anti-miotic therapy

6) Other applications

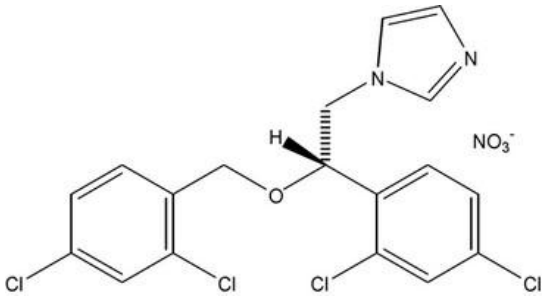
Biomedicine application

- Analytical application
- In agriculture
- In food industry

6.Drug Profile

1.Miconazole Nitrate (35)

Structural Formula



IUPAC NAME - 1-[2-(2,4-Dichlorophenyl)-2-[(2,4-dichlorophenyl)-methoxy]-ethyl]-1H-imidazole.

Physico Chemical Properties -

Molecular Formula - $C_{18}H_{15}Cl_4N_3O_4$

Molecular Weight- 479.14 g/mol

- **Colour:** White to almost white crystalline powder
- **Odour**: Odourless or faint characteristic odour
- **Taste**: Bitter
- **Boiling Point**: Not applicable / decomposes on heating
- **Melting Point:** 178–184°C
- **Octanol–water partition coefficient** (Log P): 6.63
- **Refractive index**: 1.6200

Solubility - Soluble 1 in 6250 of water, 1 in 312 of alcohol, 1 in 75 in methanol, 1 in 525 of chloroform, 1 in 1408 of isopropanol, 1 in 119 of propylene glycol, freely soluble in dimethylsulfoxide, protect from light

Major Sources -Synthetic (chemically synthesized antifungal drug)It is a synthetic imidazole derivative, produced by chemical synthesis in pharmaceutical manufacturing.

Pharmacological Actions

- Antifungal agent – primarily used to treat fungal infections.
- Broad-spectrum antifungal activity – effective against dermatophytes, yeasts (e.g., Candida), and molds.
- Mechanism of action: Inhibits ergosterol synthesis in the fungal cell membrane, increasing membrane permeability and causing fungal cell death.
- Antibacterial activity (mild) – also shows some activity against certain Gram-positive bacteria.

Pharmacokinetics

Foster and Stefanyszyn [102] reported that miconazole nitrate may penetrate the ocular compartments better than either natamycin or amphotericin B after intravenous subconjunctival, or topical administration in rabbits. The concentration of the drugs in cornea and in aqueous humor after either topical or subconjunctival administration were high, and a further threefold increase in the level was seen if the corneal epithelium had been removed prior to drug therapy. Miconazole nitrate was found

Major Uses

Miconazole nitrate is widely used to manage fungal infections affecting the skin, nails, and mucous membranes. It is commonly prescribed for conditions such as nail fungal infections, candidiasis, ringworm, athlete's foot, and other dermatophytic infections.

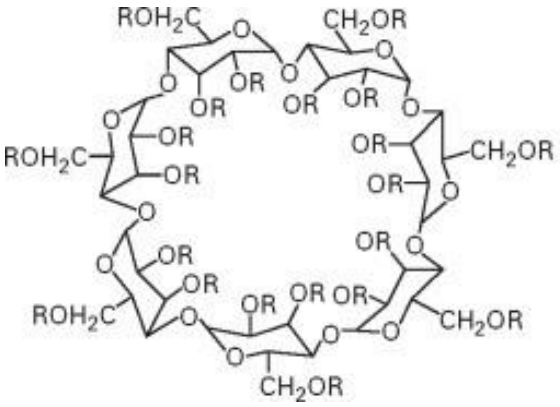
Toxicity

Miconazole nitrate is generally considered safe when used as prescribed; however, it may cause mild side effects such as skin irritation, redness, burning sensation, or itching at the site of application. In rare cases, allergic reactions or hypersensitivity may occur. Systemic toxicity is minimal due to its low absorption through the skin.

6.Drug Profile

2 .β-Cyclodextrin (40)

Structural Formula



IUPAC NAME -Cyclomaltoheptaose (or) β-Cyclodextrin

Physico Chemical Properties -

Molecular Formula - $C_{42}H_{70}O_{35}$

Molecular Weight-1134.98 g/mol

- **Colour –** White crystalline powder
- **Odour –** Odourless
- **Taste –** Slightly sweet
- **Boiling Point –** Not applicable
- **Melting Point –** Above 260°C
- **Octanol–water partition coefficient** (Log P) – −14.3
- **Refractive Index –** Not commonly reported / not applicable for solid powder

Solubility - β-Cyclodextrin is freely soluble in water and dimethyl sulfoxide, slightly soluble in methanol, and practically insoluble in most organic solvents such as chloroform and ether.

Major Sources -β-Cyclodextrin is a naturally derived cyclic oligosaccharide obtained through enzymatic conversion of starch. It is commonly produced from natural polysaccharides using cyclodextrin glycosyltransferase.

Pharmacological Action

β-Cyclodextrin acts mainly as a pharmaceutical excipient and drug carrier. It improves the solubility, stability, and bioavailability of poorly water-soluble drugs by forming inclusion complexes.

Major Uses

β-Cyclodextrin is widely used in pharmaceutical formulations to enhance drug solubility and controlled release. It is also utilized in nanosponges, topical gels, oral formulations, and other advanced drug delivery systems.

Toxicity

β-Cyclodextrin is generally regarded as safe when used within recommended limits. However, excessive use may cause mild gastrointestinal discomfort or irritation depending on the route of administration.

8. MATERIALS AND EQUIPMENTS :

The raw materials such as drug, polymer, excipients, solvents and chemicals requiredforthepresent work were procured from different sources. For the formulation and evaluationofthenanosponges and gel, the following materials were used.

Sr. No	***Drug/Polymer/Excipients/Solvents***	***Manufacturer/Supplier***
1.	β Cyclodextrin	Aromaaz International pvt. ltd
2.	Micanazole Nitrate	Yarrow Pharma, Mumbai
3.	Polyvinyl alcohol (PVA)	S.D Lab Chemical Centre, Mumbai
4.	Dichloromethane	S.D Lab Chemical Centre, Mumbai
5.	Carbopol 934	S.D Lab Chemical Centre, Mumbai
6.	Triethanolamine	S.D Lab Chemical Centre, Mumbai

Table1:List of Drug, Polymers, Excipients and Solvents used in Formulation

Sr. No.	*Ingredients*	*Manufacturer/Supplier*
1.	Ethyl Cellulose	S.D Lab Chemical Centre, Mumbai
2.	Disodium Hydrogen Phosphate	S.D Lab Chemical Centre, Mumbai

Table 2: List of Chemicals and Reagents used in Evaluation study

All the other ingredients used were of analytical grade and were used as procured.

Sr. No.	*Instruments*	*Manufacturer/Supplier*
1.	Weighing balance	Adventure, Ohaus, AR-0640 USA
2.	Ultra Sonicator	Enertech electronics Pvt Ltd, Mumbai, India
3.	Magnetic Stirrer (5000 rpm)	4EMS, Remi Equipment's Pvt. Ltd, Mumbai
4.	Hot Air Oven	Sriji chemicals, Mumbai, India
5.	Optical or Binocular Microscope	Longmeditech
6.	Digital pH meter	Systronics µ pH system 362
7.	Laboratory Centrifuges	Remi Equipment's Pvt. Ltd, Mumbai
8.	UV Visible Spectrophotometer	Systronics (INDIA) Limited
9.	Particle Size Analyzer	HORIBA SZ-100 for windows [Z type] Ver.1.90
10.	Zeta sizer	HORIBA SZ-100 for windows [Z type] Ver.1.90

Table 3: List of Instruments used in the Formulation and Evaluation

10. Result & Disscusion

Characterization of Miconazole Nitrate

Organoleptic properties:

Miconazole nitrate was observed for the following properties and the observations are recorded below:

Color: White to off-white crystalline powder

Odor: Odorless or faint characteristic odor

Appearance: Crystalline powder

Solubility: Slightly soluble in water and freely soluble in methanol and ethanol

Melting point:

The melting point of Miconazole nitrate was determined using a melting point apparatus and was found to be within the range of 178–185°C, which was in agreement with the reported literature values. The observed value indicated the purity and identity of the procured drug sample.

Partition coefficient:

The partition coefficient study was carried out to determine the lipophilic nature of Miconazole nitrate. The drug exhibited a higher partition coefficient value, indicating its hydrophobic nature and suitability for nanosponge formulation.

Identification of Miconazole Nitrate

1) Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy was carried out to identify the characteristic functional groups present in Miconazole nitrate. The obtained spectrum showed characteristic peaks corresponding to different functional groups, confirming the identity and purity of the drug sample.

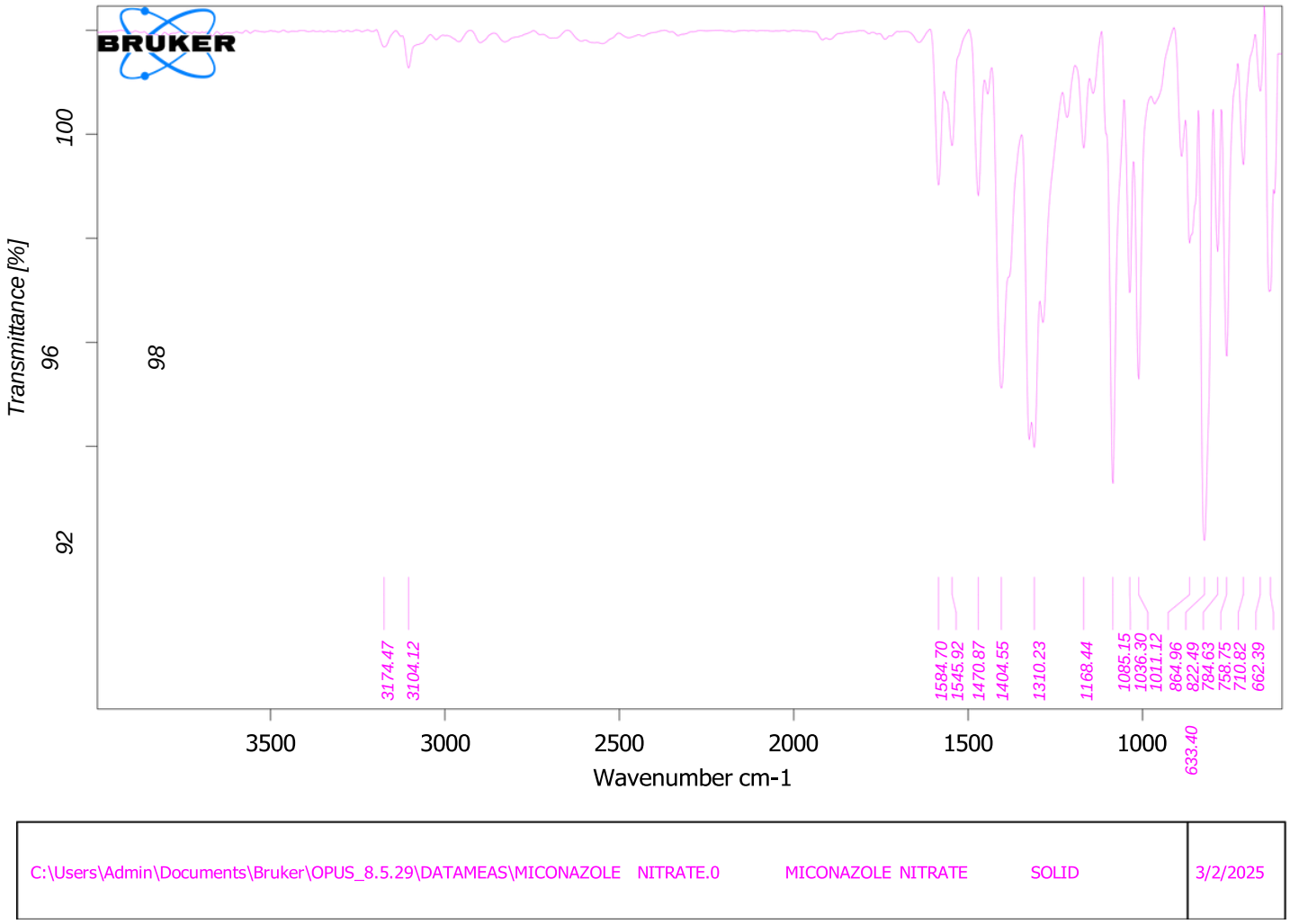


Figure No. 6: FTIR spectrum of Miconazole nitrate

Sr. No	Wave number (cm^{-1})	Functional group
1	~3150–3000	Aromatic C–H stretching
2	~1580–1500	C=C aromatic stretching
3	~1300–1100	C–N stretching
4	~1000–800	C–Cl stretching
5	~3400–3200	O–H stretching (if present)

Table No. 8: Wave number and functional groups of Miconazole nitrate

10.1. Drug Exipients Studies

Drug–excipient compatibility study was carried out to evaluate the possible interaction between Miconazole nitrate and excipients used in the formulation development of nanosponges. Compatibility study is an important parameter because interaction between drug and excipients may affect stability, efficacy, and drug release characteristics of the final formulation.

In the present work, Miconazole nitrate was formulated using β-Cyclodextrin and Polyvinyl Alcohol (PVA). The compatibility study was performed using FTIR spectroscopy by comparing characteristic peaks of pure drug and physical mixtures of drug with excipients.

Ingredients	Category	Function in formulation
Miconazole nitrate	Drug	Antifungal agent
β-Cyclodextrin	Polymer	Used for nanosponge
Polyvinyl Alcohol (PVA)	Stabilizer/Surfactant	Maintains stability and prevents
Dichloromethane	Organic solvent	Used for preparation of
Distilled Water	Vehicle	Used for preparation of

Table 9: Excipients used in formulation of Miconazole nitrate nanosponges

The FTIR spectra of pure Miconazole nitrate and physical mixtures of drug with excipients were analyzed. Characteristic peaks of Miconazole nitrate were observed and compared with those of physical mixtures.

Functional Group	Pure Drug (cm^{-1})	Drug–Excipient Mixture (cm^{-1})	Observation
Aromatic C–H stretching	3174.47	3170–3180	Retained
Aromatic C=C stretching	1584.7	1580–1590	Retained
C–N stretching	1310.23	1305–1315	Retained
C–O stretching	1168.44	1160–1170	Retained
C–Cl stretching	822.49	820–825	Retained

Table 10: Characteristic FTIR peaks of Miconazole nitrate and drug–excipient mixture

10.4. Evaluation of drug loaded Nanosponges:

10.4.1. Visual inspection:

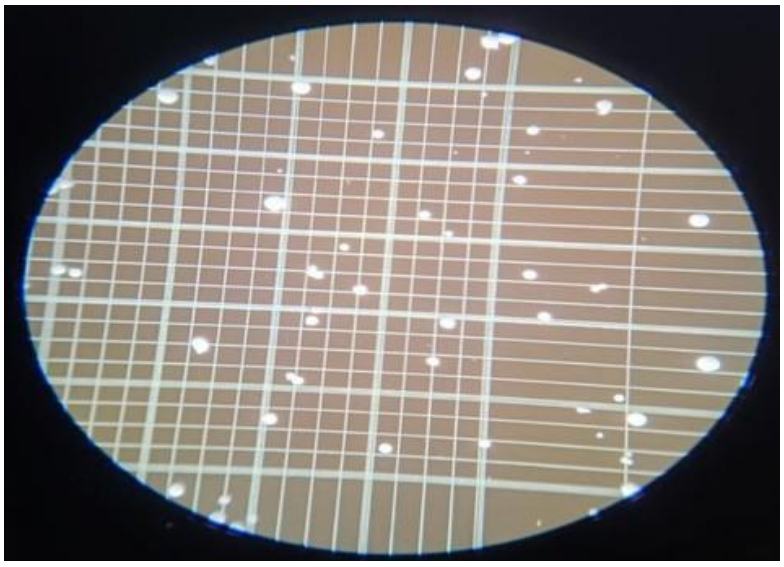


Fig.7: Microscopy of optimized nanosponge formulation (F4)

In this Figure, the nanosponges were observed using the optical microscope. The microsponges were spread on neubauer's chamber; small cube of the size 1 µm contained more than four nanosponges. From the Figure it could be primarily concluded that the obtained product was in micro range.

10.4.2. Determination of production yield and Entrapment efficiency

Table 11: Production Yield and Entrapment Efficiency

Batch	Production yield (%)	Entrapment Efficiency
F1	70.35 ± 0.2 %	80.32 ± 1.98
F2	74.26 ± 0.1 %	75.63 ± 2.43
F3	80.30 ± 0.3 %	78.98 ± 2.12
F4	84.41 ± 0.2 %	86.26 ± 2.34
F5	82.37 ± 0.4 %	83.48 ± 1.79
F6	84.21 ± 0.1 %	72.93 ± 2.21
F7	72.44 ± 0,3 %	82.17 ± 1.92
F8	69.42 ± 0.2 %	73.67 ± 2.54
F9	71.10 ± 0.1 %	66.23 ± 1.82

The % production yield of all batches was found to be ranged from 69.42 to 84.41 %. It was found that the production yield was greatly affected by polymer concentration as well as by concentration of polyvinyl alcohol. The % entrapment efficiency of all batches was ranged from 66.23 to 86.26%, It was found that the entrapment efficiency was greatly affected by polymer concentration as well as by concentration of polyvinyl alcohol

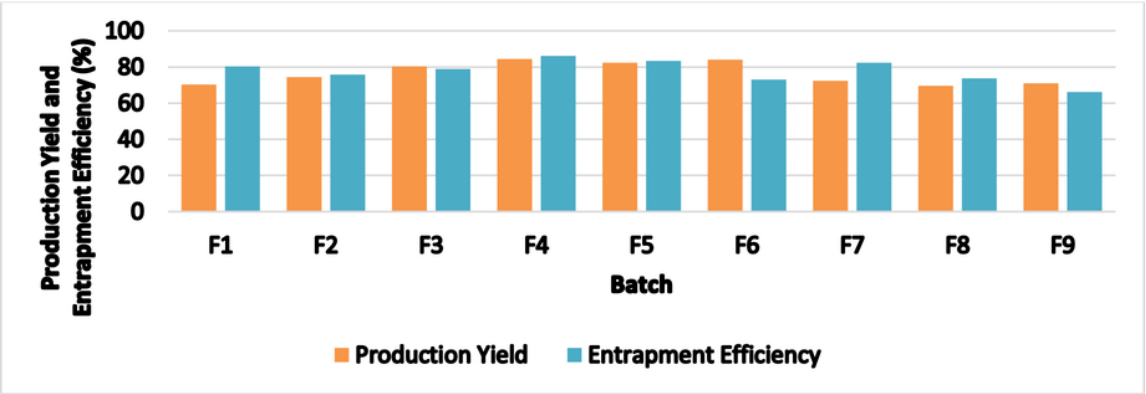


Fig.8: production yield and Entrapment efficiency graph

10.4.3. Determination of Particle size and PDI:

The visual inspection of all batches for particle size using optical and binocular microscope revealed that the particle size was increased with the increase in the Eudragit RS100 amount. This might be due to increasing polymer wall thickness which led to the larger size of microsponges. The F4 batch possessed more percent of intact, uniform, spherical particles in optical microscopy; so the batch F4 was chosen for further analysis. A mean particle size of formulation F4 was found to be 317.7nm and PDI of 0.464.

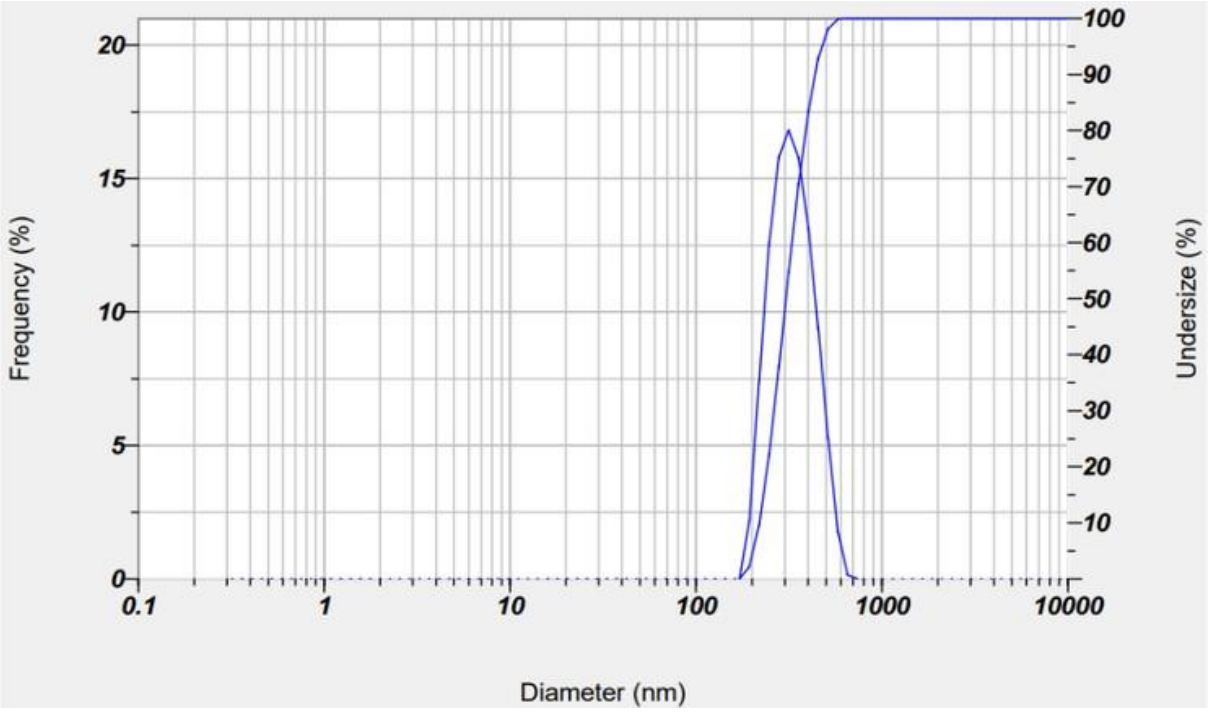


Fig.9: Particle size of optimized formulation Nanosponges (F4)

10.4.4. Determnation of Zeta potential :

Highly negative or highly positive zeta potential value indicates good physical stability of the formulation. The optimized microsponge formulation of escitalopram oxalate was measured by using zeta sizer at 25°C, it was found to be -3.6 mV and it indicated the formulation to be stable.

Fit Summary | Model | ANOVA | Diagnostics | Model Graphs

mary

2: Zeta potential

	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
ar	< 0.0001		0.9766	0.9623	
2FI	0.4145		0.9758	0.9645	
tic	**0.0120**		**0.9979**	**0.9890**	**Suggested**
bic	0.3953		0.9990	0.9682	**Aliased**

Sequential Model Sum of Squares [Type I] | Model Summary Statistics

Sequential Model Sum of Squares [Type I]

Response 2: Zeta potential

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean vs Total	3660.25	1	3660.25			
Linear vs Mean	94.57	2	47.29	168.19	< 0.0001	
2FI vs Linear	0.2305	1	0.2305	0.7912	0.4145	
Quadratic vs 2FI	**1.38**	**2**	**0.6901**	**27.17**	**0.0120**	**Suggested**
Cubic vs Quadratic	0.0643	2	0.0321	2.70	0.3953	Aliased
Residual	0.0119	1	0.0119			
Total	3756.51	9	417.39			

Select the highest order polynomial where the additional terms are significant and the model is not aliased.

Fig.10: Zeta potential of optimized formulation of Nanosponges (F4)

10.4.5. Scanning electron microscopy (SEM) :

From SEM photographs, it was clear that the Nanosponges so obtained were having the spherical and the spongy nature of the microsponges was clearly evident, thus it could be concluded that the adopted method for the preparation of Nanosponges was useful.

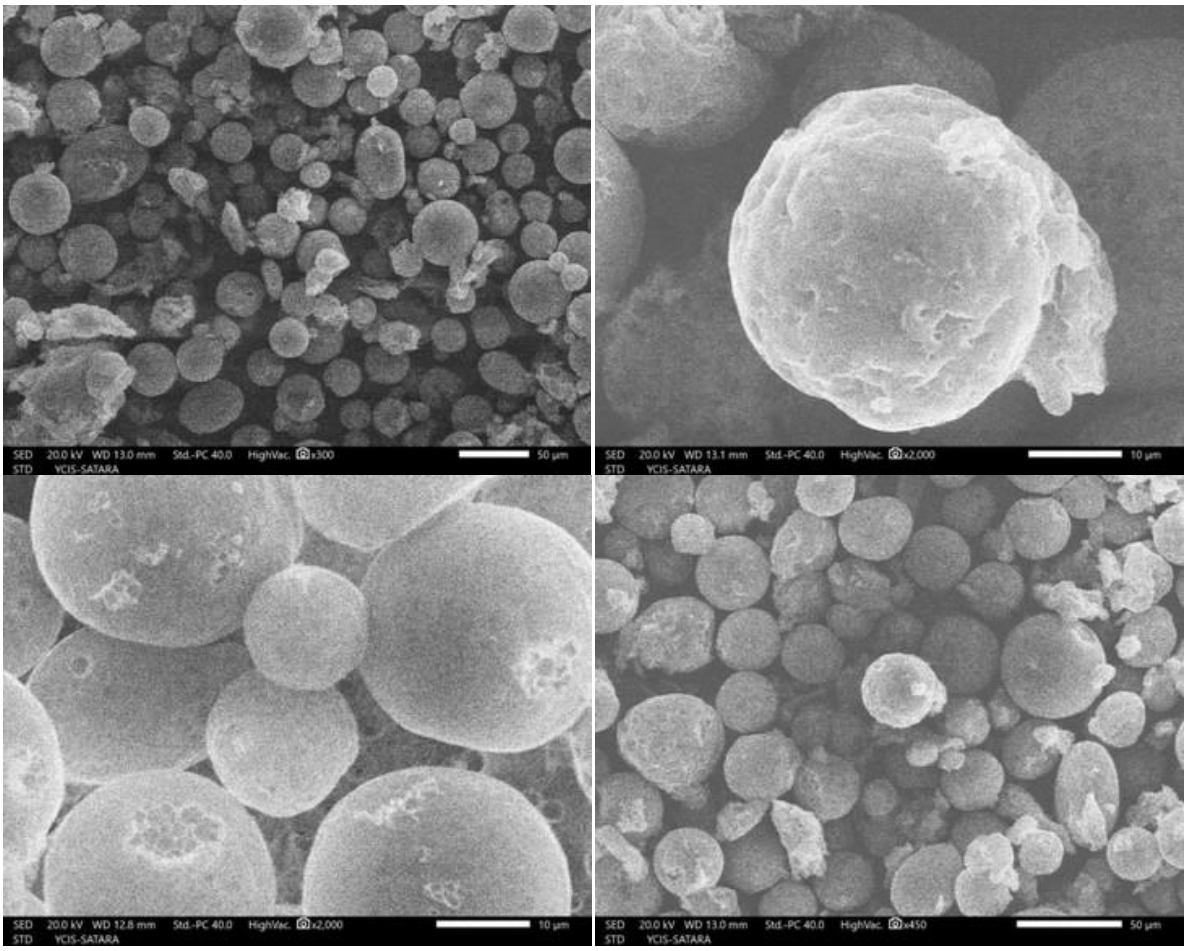


Fig.11: SEM of optimized formulationNanosponges (F4)

10.4.6. FTIR of optimized Nanosponge formulation (F4)

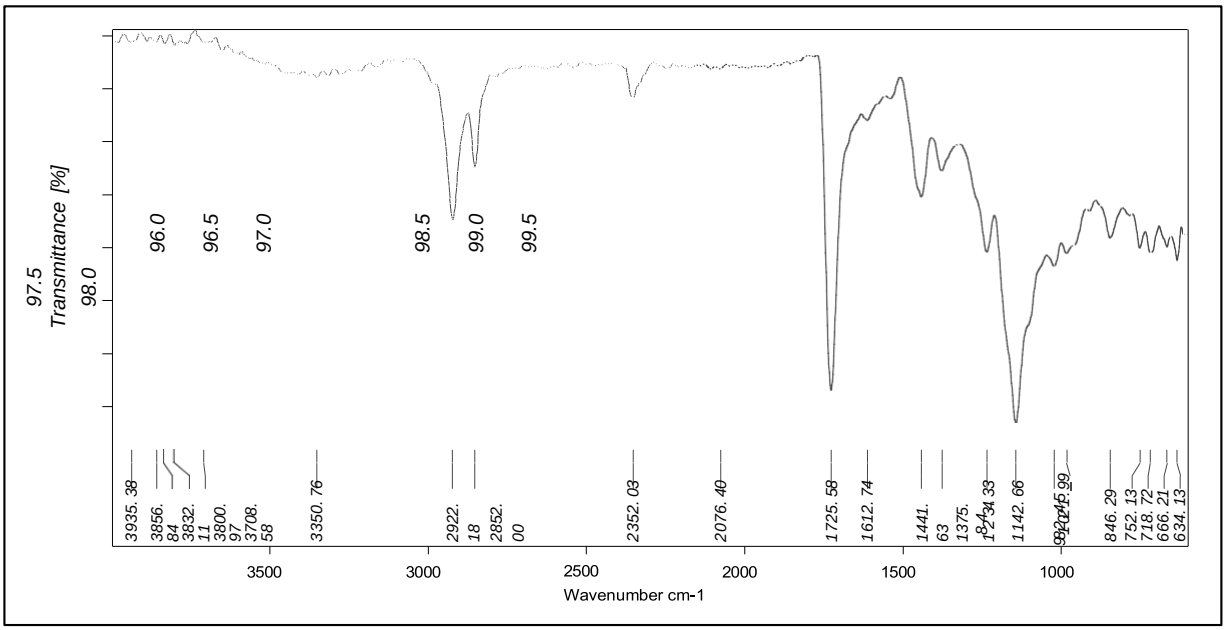


Fig.12: FTIR of optimized Nanosponges formulation (F4)

Table 12: FTIR interpretation of Optimized Nanosponge formulation (F4)

Standard Range	Wavelength (cm^{-1})	Functional group
3000-3500	3350	O-H
1650-1750	1725	C=O
2850-2950	2922	C-H
1300-1230	1234	C-O
1300-800	1142	C-C

The FTIR spectrum of optimized Nanosponge formulation of Chaulmoogra oil and excipients was compared to the spectra of each component separately. Significant peaks of the corresponding and excipients were seen in the FTIR spectrum.

10.5. Evaluation of Micanazole nitrate Nanosponges gel

10.5.1. Physical appearance

Table 13: Physical appearance of optimized MNZ NS formulation

Sr. No	***Physical appearance***	***Results***
1	Appearance	Smooth
2	Odour	Characteristic
3	Physical state	Semi solid

10.5.2. Determination of pH:

The pH ofMicanazole nitrate Nanosponges gel was found to be 6.9±0.36, the pH value was found to be within the range of skin pH (6.5 ± 0.12).

Table 14: pH of optimized MNZ NS gel formulation

Sr. No	***Test***	***Observation***			***Average ±Std.***
1.	pH	6.5	6.5	6.5	6.5 ± 0.12

10.5.3. Determination of spreadability:

The spreadability of prepared Micanazole nitrate Nanosponges gel was found to be 67.58±6.39 g.cm/sec. The spreadability values were found to be satisfactory.

Table 15: Spreadability of optimized MNZ NS gel formulation

Sr. No	***Test***	***Observation***			***Average ±Std.***
1.	Spreadability (gm.cm/sec)	62.22	65.88	74.66	***18.24 ± 0.56***

10.5.4. Determination of viscosity:

The viscosity of prepared Micanazole nitrate Nanosponges gel was found to be 9450 ± 124cps.

Table 16: Viscosity of optimized Micanazole nitrate Nanosponges gel formulation

Analysis	***Run 1***	***Run 2***	***Run 3***	***Mean***	***SD***
Viscosity	9421	9430	*9440*	***9450***	1.24

10.5.5. In-Vitro drug diffusion study:

In vitro drug diffusion study was carried out using phosphate buffer solution pH 7.4 a magnetic stirrer and a Franz diffusion assembly. From the results it could be revealed that formulation gave better drug release and at the end of 8 hours cumulative amount of drug release was found to be 87.51%. The percent cumulative drug diffusion calculated is shown in table given below.

Table 17: Drug diffusion in % in hours

Time in hours	***% Drug Diffusion***
0	0
1	33.63
2	38.53
3	50.29
4	63.35
5	68.25
6	76.41
7	85.55
8	87.51

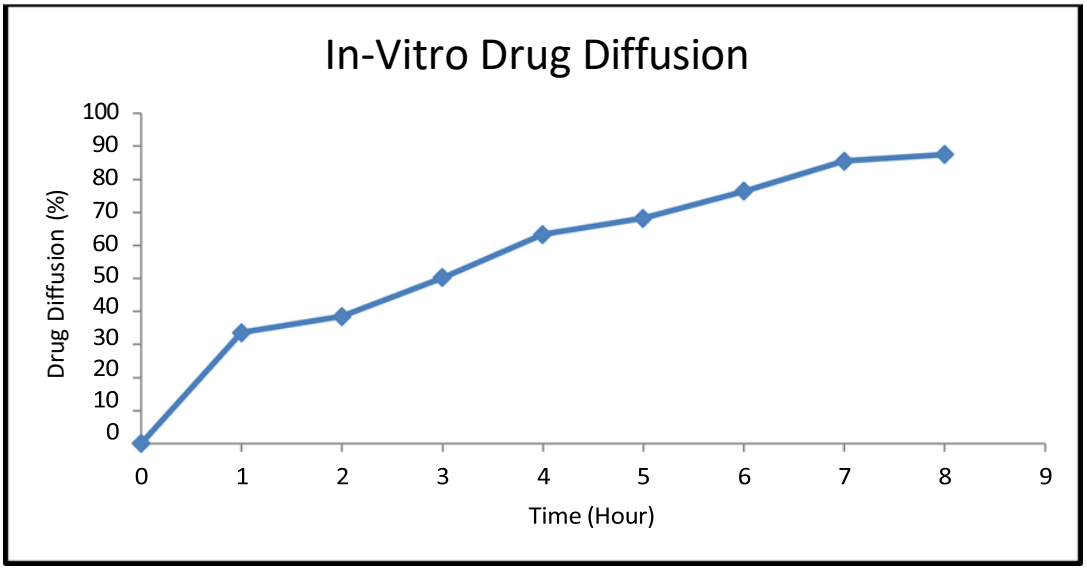


Fig.13: In vitro drug diffusion of Micanazole nitrate Nanosponges gel(F4)

10.6. Optimization of MNZ NS by response variables effects (3^2 factorial designs)

Traditional design of the pharmaceutical formulations are based on time consuming approach of changing one variable at a time which does not take into consideration the joint effect of independent variables. Thus, factorial design can serve as an essential tool to understand the complexity of the pharmaceutical formulations.

Table 18: Response Design summary

Response	*Name*	*Units*	*Observations I*	*nimum M*	*ximum M*	*an*	*Std. Dev.*	*Ratio*
R1	Particle size	nm	9.00	262.98	317.7	296.41	15.77	1.21
R2	PDI		9.00	0.376	0.507	0.4649	0.0440	1.35
R3	Drug entrappement	%	9.00	66.23	86.26	77.74	6.22	1.30

A 3^2 full factorialdesignwas selected and the 2 factors were evaluated at 3 levels. The amount of EudragitRS100(A)and polyvinyl alcohol (B) were selected as independent variables and the dependentvariableswereparticle size, PDI and % entrapment efficiency. The data obtained was treatedusingStat-EaseDesign Expert software**.**

11. Conclusion

• In conclusion, Miconazole nitrate nanosponges were successfully formulated and characterized. An optimal custom design approach was applied for optimization of the formulation.
• Among all prepared formulations, the optimized batch was selected because it exhibited a desirable particle size of 244.481 nm, zeta potential of 22.301 mV, and entrapment efficiency of 81.934%, indicating satisfactory drug loading and good formulation stability.
• The statistical analysis and optimization studies confirmed that β-Cyclodextrin concentration and stirring speed significantly affected the characteristics of the nanosponge formulation.
• The contour plots and 3D surface plots demonstrated the influence of formulation variables on particle size, zeta potential, and entrapment efficiency of the developed nanosponges.

13.Referances

1. Tiwary AK, Sapra B, Jain S. Innovation in transdermal drug delivery: Formulation and techniques. Recent Pat Drug Deliv Formul 2007;1:23-36.
2. Chong S, Fung HL, In: Hadgraft J, Guy RH, editors. Transdermal drug delivery. development issues and research initiatives. New York: Marcel Dekker; 1989. p. 135-54.
3. Singh A, Singh MP, Alam G, Patel R, Vishvakarma D, Datt N. Expanding opportunities for transdermal delivery systems: An overview. J Pharm Res 2011;4:1417-20.
4. Ansel HC, Allen LV and Popovich NG. Pharmaceutical dosage forms and drug delivery system. 7th ed. New York: Lipponcott Williams and Wilkins; 2002.
5. Patel RP, Baria AH. Formulation and evaluation consideration of transdermal drug delivery system. Int J Pharm Res 2011;3:1-9.
6. Kumar JA, Pullakandam N, Prabu SL, Gopal V. Transdermal drug delivery system: An overview. Int J Pharm Sci Rev Res 2010;3:49-54.
7. Jain NK. Advances in controlled and novel drug delivery. 1st ed. New Delhi: CBS Publishers and Distributors; 2001. p. 108-10.
8. Soni M, Kumar S, Gupta GD. Transdermal drug delivery: A novel approach to skin permeation. J Pharm Res 2009;2:1184-90.
9. Naik A, Kalia YN, Guy RH. Transdermal drug delivery: Overcoming the skin's barrier function. Pharm Sci Technol Today 2009;3:318-26.

10. Flynn GL. Percutaneous Absorption. 3rd ed. New York: Marcel Dekker; 1985.
11. Hadgraft J. Skin Deep. Eur J Pharm Biopharm 2004;58:291-9.
12. Jain NK, Controlled and novel drug delivery. 1st Ed., CBS Publisher and Distributors, New Delhi. 2001:100-129.
13. Robinson JR, Lee VH. Controlled drug delivery fundamentals and applications. 2nd Ed. New York. 2005:523-536.
14. Jain NK. Pharmaceutical product development. 1st Ed. CBS Publisher and Distributors. New Delhi. 2002:221- 228.
15. Kumar D, Sharma N, Rana AC, Agarwal G, Bhat ZA. A review: transdermal drug delivery system: a tools for novel drug delivery sestem. Int. J Drug Dev. Res. 2011;3(3):70- 84.
16. Wilson R, Waugh A, Grant A. Anatomy and physiology in health and illness. 9th Ed. 2001 pg. 363-366.
17. Mehta R. Topical and transdermal drug delivery: what a pharmacist needs to know. InetCE. 1st Ed., Arizona;2004:1-10.
18. Ramteke KH, Dhole SN, Patil SV. Transdermal drug delivery system: a review. J Advanced Sci. Res. 2012;3(1):22-35.
19. Singh MC, Naik AS, Sawant SD. Transdermal drug delivery system with major emphasis on transdermal patches: a review. J Pharm Res. 2010;3(10):2537-2543.
20. Jalwal P, Jangra A, Dhaiya L, Sangwan Y, Saroha R. A review on transdermal patches. Pharm Res. J. 2010; 3:139- 149.
21. Dhawan S, Aggarwal G. Development, fabrication and evaluation of transdermal drug delivery system- a review. Pharm info.net. 2009:1-25.
22. Yadav V. Transdermal drug delivery system: review. Int. J Pharm Sci. Res. 2012;3(2):376-382.
23. Patel D, Chaudhary SA, Parmar B, Bhura N. Transdermal drug delivery system: a review. The Pharm Innovation. 2012;1(4):66-75.
24. Dhiman S, Thakur GS, Rehni AK. Transdermal patches: a recent approach to new drug delivery system. Int. J Pharmacy Pharm Sci. 2011;3(5):26-34.

25. Sharma RK, Keleb E, Mosa EB, Aljahwi AAZ. Transdermal drug delivery system-design and evaluation. Int. J Advances Pharm Sci. 2010;1:201-211.

26. Sandhu P, Bilandi A, Kataria S, Middha A. Transdermal drug delivery system (patches), applications in present scenario. Int. J Res. Pharm Chem. 2011;1(4):1139-1151.

27. Zaki Rizkalla CM, Latif Aziz R, Soliman II. In vitro and in vivo evaluation of hydroxyzine hydrochloride microsponges for topical delivery. AAPS PharmSciTech 2011;12:989–1001 doi: 10.1208/s12249- 011-9663-5.

28. Chowdary KPR and Rao YS. Mucoadhesive Microspheres for Controlled Drug Delivery. Biol. Pharm. Bull. 2004; 27(11):1717-1724. doi: 10.1248/bpb.27.1717.

29. Nacht S, Katz M. The microsponge: a novel topical programmable delivery system, in: D. W. Osborne, A. H. Amman (Eds.), Topical Drug Delivery Formulations, Marcel Dekker: New York, Basel 1990; 299- /325.

30. Roy A. Microsponge as a novel drug carrier system: a review. World Journal of Pharmaceutical Research, 2015; 4(12): 680- 701. https://wjpr.s3.ap south1.amazonaws.com/article_issue/1448877486.pdf

31. Hans et al, A Review on Microsponge Delivery System, Journal of Drug Delivery & Therapeutics. 2019; 9(3-s):1032-1040. DOI https://doi.org/10.22270/jddt.v9i3-s.2938

32. Dutta, D., Goyal, N., Sharma, D.K. Formulation, and development of herbal microsponge sunscreen gel. Journal of Cosmetic Dermatology. 2021;21(4):1675-1687. doi: 10.1111/jocd.14274.

33. Teeranachaideekul V, Müller RH, Junyaprasert VB. Encapsulation of ascorbyl palmitate in nanostructured lipid carriers (NLC) effects of formulation parameters on physicochemical stability. Int J Pharm. 2007;340(1–2):198–206. doi:10.1016/j.ijpharm.2007.03.022 doi: 10.1016/j.ijpharm.2007.03.022.

34. Siddiqui IA, Sanna V. Impact of nanotechnology on the delivery of natural products for cancer prevention and therapy. Mol Nutr Food Res. 2016;60(6):1330–1341. doi:10.1002/mnfr.201600035

35. Arnab et al. Herbal microsponge incorporated sunscreen gel: A novel strategy; Biomedicine: 2022; 42(5): 844-850. DOI:10.51248/.v42i5.2016

36. Sena M. Beauty industry analysis 2016-cost & trends, 2016. Available from https://www.franchisehelp.com/ industry-reports/beauty-industryreport/.

37. Jadoul A, Bouwstra J, Preat V. Effects of iontophoresis and electroporation on the stratum corneum: review of the biophysical studies. Adv Drug Deliv Rev 1999;35(1):89 105. DOI: 10.1016/s0169-409x(98)00065-9

38. Osmani RA, Aloorkar NH, Ingale DJ, Kulkarni PK, Hani U, Bhosale RR, et al. Microsponges based novel drug delivery system for augmented arthritis therapy. Saudi Pharm J 2015;23:562–72. DOI: 10.1016/j.jsps.2015.02.020

39. Osmani RA, Aloorkar NH, Thaware BU, Kulkarni PK, Moin A, Hani U, et al. Microsponge based drug delivery system for augmented gastroparesis therapy: formulation development and evaluation. Asian J Pharm Sci 2015;10:442–51. DOI:10.1016/j.ajps.2015.06.003

40. Li SS, Li GF, Liu L, Jiang X, Zhang B, Liu ZG, et al. Evaluation of paeonol skin-target delivery from its microsponge formulation: in vitro skin permeation and in vivo microdialysis. PLoS ONE 2013;8(11):e79881. DOI: 10.1371/journal.pone.0079881

41. Jain V, Singh R. Design and characterization of colon-specific drug delivery system containing paracetamol microsponges. Arch Pharm Res 2011;34:733–40. doi: 10.1007/s12272-011-0506-4.

42. Pawar AP, Gholap AP, Kuchekar AB, Bothiraja C, Mali AJ. Formulation and evaluation of optimized oxybenzone microsponge gel for topical delivery. J Drug Deliv 2015;2015:1–9. https://doi.org/10.1155/2015/261068

43. Kumar PM, Ghosh A. Development and evaluation of silver sulfadiazine loaded microsponge based gel for partial thickness (second degree) burn wounds. Eur J Pharm Sci 2017;96:243–54. DOI: 10.1016/j.ejps.2016.09.038

44. Rajeshwari S, Swapna V (2019) MDS as a neoteric cornucopia for drug delivery systems. Int J Curr Pharm Res 11(3):4–12

45. Ravi V, Ravi G, Bose PSC, Saritha D (2019) MDS- a comprehensive review: success and challenges. Indo Am J Pharm 9(7):3056–3067

46. Tiwari A, Mishra MK, Shukla A, Yadav SK (2016) Microsponge: an augmented drug delivery system. Am J Pharm Tech Res 6(6):79–95

47. Kawashima Yoshiaki , Niwa Toshiyuki , Takeuchi Hirofumi , Hino Tomoaki , Ito Yoji, Control of Prolonged Drug Release and Compression Properties of Ibuprofen Microsponges with Acrylic Polymer, Eudragit RS by Changing their Intraparticle porosity, Chemical & Pharmaceutical bulletin 1992;40(1):196-201.

48. Kaity Santanu, Maiti Sabyasachi, Ghosh Ashoke Kumar , Pal Dilipkumar, Ghosh Animesh , Banerjee Subham, Microsponge: A Novel Strategy For Drug Delivery System, Journal of Advanced Pharmaceutical Technology and Research, 2010; 1:283-290.

49. Nanda Shivani, Microsponge drug delivery syatem: an overview, World Journal of Pharmacy and Pharmaceutical sciences, 2013; 2(3):1032-1043. 50. Aritomi H, Yamasaki Y, Yamada K, Honda H, Koshi M. Development of sustained release formulation of chlorpheniramine maleate using powder coated microsponges prepared by dry impact blending method. Journal of Pharmceutical Science and Technology, 1996; 56(1):49-56.

51. Namrata Jadhav, Vruti Patel, Siddesh Mungekar, Gaurav Bhamare, Manisha Karpe, Vilasrao Kadams. Microsponges delivery System: An updated review, current status and future prospects, J of scienti. And innov Res. 2013; 2(6):1097-1110.

52. John I D' Souza and Harinath N. Topical anti-inflammatory gels of fluocinolone acetonide entrapped in eudragit based microsponge delivery system. Research J Pharm and Tech, 1(4), 2008, 502. https://rjptonline.org/AbstractView.aspx?PID=2008-1-4-101

53. Mahant S, Kumar S, Nanda S, Rao R; Microsponges for dermatological applications: Perspectives and challenges, Asian Journal of Pharmaceutical Sciences 15 (2020) 273 291 doi: 10.1016/j.ajps.2019.05.004

54. Deore Mayuri B, Salunkhe K.S., Pawbake G., Chaudhari S.R. and Gaikwad P.R.Microsponges As A Modified Drug Delivery System World Journal of Pharmaceutical Research, 4(3):657-667.

55. Patel SB, Patel HJ, Seth AK. Microsponge drug delivery system: an overview. J Global Pharma Technol 2010;2(8):1–9. https://www.researchgate.net/publication/287938322_Microsponge_drug_delivery_system_An_overview

56. Orlu M, Cevher E, Araman A. Design and evaluation of colon specific drug delivery system containing flurbiprofen microsponges. Int J Pharm 2006;318(1):103–17. DOI: 10.1016/j.ijpharm.2006.03.025

57. Patravale VB, Mandawgade SD. Novel cosmetic delivery systems: an application update. Int J Cosmet Sci 2008;30(1):19–33. doi: 10.1111/j.1468-2494.2008.00416.x.

58. Osmani RA, Aloorkar NH, Kulkarni AS, Harkare BR, Bhosale RR. A new cornucopia in topical drug delivery: microsponge technology. Asian J Pharm Sci Technol 2014;4:48

60. https://www.researchgate.net/publication/261873915_A_new_cornucopia_in_topical_drug_delivery_Microsponge_technology

59. Chanchal D, Swarnlata S. Novel approaches in herbal cosmetics. J Cosmet Dermatol 2008;7(2):89–95. DOI: 10.1111/j.1473- 2165.2008.00369.x

60. Chadawar V, Shaji J. Microsponge delivery system. Curr Drug Deliv 2007;4(2):123–9. DOI: 10.2174/156720107780362320

61. Embil K, Nacht S. The microsponge® delivery system (MDS): a topical delivery system with reduced irritancy incorporating multiple triggering mechanisms for the release of actives. J Microencapsul 1996;13(5):575– 88. doi: 10.3109/02652049609026042.

62. Orthaber, K., Pristovnik, M., Skok, K., Peric, B., Maver, U. Skin cancer and its treatment: novel treatment approaches with emphasis on nanotechnology. Journal of Nanomaterials. 2017; 2:1-20. https://doi.org/10.1155/2017/2606271

63. Pandit, A.P., Patel, S.A., Bhanushali, V.P., Kulkarni, V.S., Kakad, V.D. Nebivolol loaded microsponge gel for healing of diabetic wound. AAPS Pharm Sci Tech. 2017;18(3):846- 854. DOI: 10.1208/s12249-016-0574- 3

64. Srivastava R et al, Micosponges: a futuristic approach for oral drug delivery, Expert Opin Drug Deliv, 2012; 9(7): 863-8. doi: 10.1517/17425247.2012.693072.

65. Kawashima, Y., Niwa, T., Handa, T., Takeuchi, H., Iwamoto, T., Itoh, K. Preparation of controlled-release microspheres of ibuprofen with acrylic polymers by a novel quasi emulsion solvent diffusion method. 2308104_522683_47_57Journal of Pharmaceutical Sciences. 1989;78(1):68-72. https://doi.org/10.1002/jps.2600780118

66. Rizkalla, C.M., Aziz, L. R., Soliman, II. In vitro and in vivo evaluation of hydroxyzine hydrochloride microsponges for topical delivery. Aaps Pharmscitech. 2011;12(3):989 1001. DOI: 10.1208/s12249-011-9663-5

67. Orthaber, K., Pristovnik, M., Skok, K., Peric, B., Maver, U. Skin cancer and its treatment: novel treatment approaches with emphasis on nanotechnology. Journal of Nanomaterials. 2017; 2:1-20. https://doi.org/10.1155/2017/2606271

68. Amrutiya, N., Bajaj, A., Madan, M. Development of microsponges for topical delivery of mupirocin. AAPS PharmSciTech. 2009; 10(2):402- 409. DOI: 10.1208/s12249-009 9220-7

69. Nokhodchi, A., Jelvehgari, M., Siahi, M.R., Mozafari, M.R. Factors affecting the morphology of benzoyl peroxide microsponges. Micron. 2007;38(8):834-840. DOI: 10.1016/j.micron.2007.06.012

70. Kane, J.G. (1965). Some minor fatty oils of India. Fette Seifen Anstrichmittel, 67: 396 400.

71. Vimal, O.P. (1983). Potential of nonedible seed oils. Soaps, Detergents and Toiletries Rev.13: 24–26.