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Photostability Enhancement of Miconazole Nitrate by Microsponge Formulation

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Abstract

The present study deals with the photostability enhancement of Miconazole nitrate by Ethyl Cellulose microsponge formulation. Miconazole nitrate is topical antifungal drug and poor aqueous solubility high lipophilic. Microsponge has well defined structure, porous, polymeric, crosslinked formulation. It has decrease toxicity and side effects of active ingredient. It also protect drug from biodegradation. The solubility studies for drug were performed. The microsponges were prepared using quasi emulsion solvent diffusion method. The prepared microsponges were subjected to in-vitro dissolution studies, fourier transform infra-red spectroscopy and differential scanning calorimetry. Stability also evaluate for formulation of microsponge. The in-vitro dissolution of microsponge was found to be slow compared to pure drug. The FTIR spectroscopy and DSC of drug and excipient confirm compatibility testing. Photostability study of pure drug and microsponge compare. Microsponge showed higher photostability as compared to plain drug and other physical mixture. Miconazole nitrate microsponge loaded hydrogel for topical delivery.

Key words: Photostability, Miconazole nitrate, Microsponge, Topical delivery

Contents

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1. Introduction

Topical preparations are used for the localized effects at the site of their application. Fungal infections are caused by a fungus, a type of microorganism. Common types of fungal infections are caused by the fungus tinea. Fungal infections are broadly classified either superficial or systemic. Systemic fungal infections need extensive treatment by oral or intravenous administration of antifungal drugs. Whereas, superficial fungal infections of the mucous membrane respond readily to topical application of antifungal agents. Common types of superficial fungal infections are ringworm (Tinea), Candida, Dermatitis etc[1]. The antifungal agents that have been or are currently being evaluated for use in treating fungal cells. These classes include the polyenes, nucleoside analogues, azoles, pneumocandins-echinocandins, pradimicins-benanomycins, nikkomycins, allylamines, thiocarbamates, sordarins and other targets[1].

Microsponge technology offers entrapment of ingredients and is believed to contribute towards reduced side effects, improved stability, increased elegance and enhanced formulation flexibility. Microsponges are microscopic spheres capable of absorbing skin secretions, therefore reducing oiliness and shine from the skin. Spherical particles composed of clusters of even tinier spheres are capable of holding four times their weight in skin secretions. Microsponge particles are extremely small, inert, indestructible spheres that do not pass through the skin. An ideal drug for superficial fungal infections would have broad spectrum activity. Antifungal drug is fungisidal rather than fungistatic effective with topical application. Antifungal drug have high effinity for the stratnum corneum, non sensitizing and avoid development of fungal resistance[2-12].

The major types of Fungal Infection are:

a) **Athlete's foot (*Tinea pedis*)**
   It's estimated that one in six people in the UK currently have athlete's foot. It's caused by a fungus that makes your skin itchy, flaky and red, and causes white cracks to appear, especially between your toes and on the side of your foot. sAthlete's foot is often picked up from walking bare foot on damp, contaminated floors in communal shower facilities, swimming pools or saunas.

b) **Nail infections (*Tinea unguium*)**
   These can occur on any part of your nail and take a long time to develop. They cause your nail to discolour and become crumbly. The surrounding tissue may also thicken. Toenails are usually affected more than fingernails.

c) **Ringworm of the groin (*Tinea cruris*)**
   This is also called 'jock itch' because it's more common in people that play sports and young males. It's contagious and can be passed from person to person by direct contact or contact with unwashed clothes. It can cause an itchy, red rash in your groin and the surrounding area.

d) **Ringworm of the body (*Tinea corporis*)**
   This often affects exposed parts of your body, such as your arms, legs or face, and causes a red, ring-shaped rash. Ringworm is contagious and can be caught by coming into contact with somebody who already has ringworm or touching contaminated items.

Like other azole antifungals, miconazole exerts its effect by altering the fungal cell membrane. Miconazole inhibits ergosterol synthesis by interacting with 14a demethylase, a cytochrome P-450 enzyme that is necessary for the conversion of lanosterol to ergosterol, an essential component of the membrane. Inhibition of ergosterol synthesis results in increased cellular permeability, causing leakage of cellular contents. Miconazole nitrate has also been shown in inhibit DNA synthesis and suppress intracellular concentrations of ATP. Like other imidazole antifungals, Miconazole can increase membrane permeability to zinc, augmenting its cytotoxicity. Miconazole does not appear to have the same effect on human cholesterol synthesis.

Miconazole Nitrate ($C_{18}H_{14}Cl_4N_2O \cdot HNO_3$).

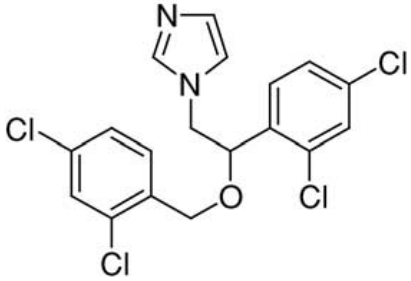


Figure 1. Structure of Miconazole Nitrate

A White Powder, Antifungal Agent, Class II drug(low solubility and high permeability), very slightly soluble in water, sparingly soluble in methanol, slightly soluble in ethanol(96%). Meltng poin range of drug 178ºC to 184ºC. Miconazole demonstrates broad *in vitro* and *in vivo* antifungal activity. Treatment of tinea pedis with miconazole nitrate has shown an excellent clinical response to once-daily therapy for 4 weeks with a mycologic cure rate of 80% at 2-weeks post treatment. Miconazole Nitrate is a broad-spectrum imidazole derivative whose antifungal activity is derived primarily from the inhibition of ergosterol biosynthesis, which is critical for cellular membrane integrity. It has fungicidal or fungistatic activity in vitro against a number of pathogenic fungi including the following dermatophytes and yeasts: *T.rubrum*, *T.violaceum*, *T.tonsurans*, *T.mentagrophytes*, *E.floccosum*, *M.canis*, *M.audouini*, *M. gypseum*, *C. albicans* and *M. furfur, Cryptococcus*. These effects are typical of topically applied imidazoles and miconazole has shown similar safety profiles as such agents.

Once-daily application makes it convienient for patients and could result in improved compliance. Miconazole is a broadspectrum therapy that is safe for pediatric use. Comparative trials have shown Miconazole to have comparable efficacy and safety as other topical antifungal imidazoles. Overall, it is a safe and effective alternative in the treatment of tinea infections caused by dermatophytic fungi. Side effects include Cardiac arrhythmias, Phlebitis, Pruritis, Arachnoiditis, Cardiac Arrest, Anaphylaxis, Drowsiness, Anorexia, Rashes, GIupset, Flushing, Nausea and vomiting, Local irritation. A Microsponge Delivery System (MDS) is patented, highly cross-linked, porous, polymeric microspheres that can entrap wide range of actives and then release them onto the skin. This system was employed for the improvement of performance of topically applied drugs. It is a unique technology for the controlled release of topical agents. Microsponge consists of microporous beads, typically 10-25 microns in diameter, loaded with active agent. When microsponge delivery system applied to the skin, the release of drug can be controlled through diffusion or other variety of triggers, including rubbing, moisture, pH, friction, or ambient skin temperature[8].

1.1 Advantages of Microsponge Delivery System[13,14]:

- It can also improve efficacy in treatment.
- They have better thermal, physical and chemical stability.
- These are non-irritating, non-mutagenic, non-allergenic and non-toxic.
- MDS allows the incorporation of immiscible products.
- They have superior formulation flexibility.
- In contrast to other technologies like microencapsulation and liposomes, MDS has wide
- range of chemical stability, higher payload and are easy to formulate.
- Liquids can be converted in to powders improving material processing.
- It has flexibility to develop novel product forms.
- It can absorb oil up to 6 times its weight without drying.
- It provides continuous action up to 12 hours i.e. extended release.
- Improved product elegancy.
- Lessen the irritation and better tolerance leads to improved patient compliance.
- MDS can improve bioavailability of the drugs.

1.2 Characteristics of Microsponges[11]:

- Microsponges are stable of pH upto 11
- Microsponges stable at the temperature up to 130ºC
- Microsponges compatible with most vehicles and ingredients
- Microsponges are self sterilizing as their average pore size is 0.25µm where bacteria cannot penetrate
- Microsponges have higher payload (50 to 60%), still free flowing
- Microsponges can be cost effective.

1.3 Characteristics of materials that are entrapped in Microsponges[12]:

Most liquid or soluble ingredients can be entrapped in the particles . Actives that can be entrapped in microsponges must meet following requirements,

- It should be either fully miscible in monomer or capable of being made miscible by addition of small amount of a water immiscible solvent.
- It should be water immiscible or at most only slightly soluble.
- It should be inert to monomers.
- It should be stable in contact with polymerization catalyst and conditions of polymerization.

2. Materials and Methods

3. Preparation of microsponges[4]

Drug loading in microsponges can take place in two ways, one-step process or by two step process as discussed in liquid-liquid suspension polymerization and quasi emulsion solvent diffusion techniques which are based on physico-chemical properties of drug to be loaded. If the drug is typically an inert non- polar material, will create the porous structure it is called porogen. Porogen drug, which neither hinders the polymerization nor become activated by it and stable to free radicals is entrapped with one-step process.

3.1 Quasi-emulsion solvent diffusion

As explained in Figure 2 the microsponges can also be prepared by *quasi-emulsion solvent diffusion* method using Ethyl Cellulose as polymer. The processing flow chart is presented in Figure 2. To prepare the inner phase, Ethyl Cellulose was dissolved in ethyl alcohol. Drug can be added to methyl alcohol. Polymer solution and drug solution dissolved under ultrasonication at 35ºC.This solution made iner phase. The inner phase was poured into the PVA solution in water (outer phase). Following 60 min of stirring, the mixture is filtered to separate the microsponges. The microsponges are dried in an air-heated oven at 40 ºC for 12 h and weighed to determine production yield (PY).

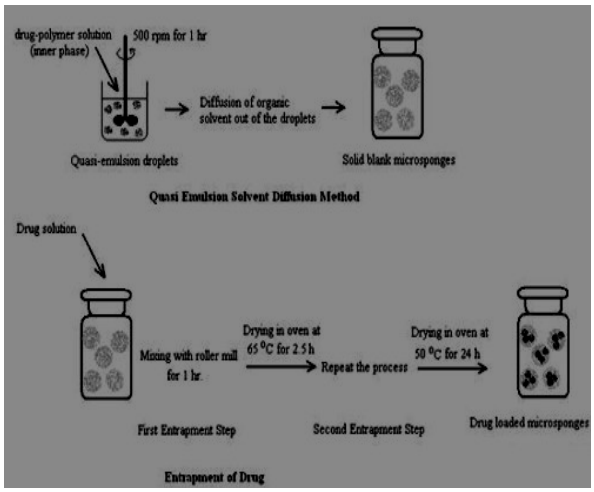


Figure 2. Preparation of Microsponges by Quasi Emulsion Solvent Diffusion Method

Trial batches study on different polymer for microsponge .The besy result was obtained in Ethyl cellulose microsponge. Ethyl cellulose give spherical and required micrometer size microsponge

Table 1. Preliminary trial batches of selection of polymer in internal phase

Ingredient	N1	N2	N3	N4	N5	N6
Internal phase						
Drug(mg)	100	100	100	100	100	100
HPMC K 100M (mg)	1000	-	-	-	-	-
Carbopol 940(mg)	-	1000	-	-	-	-
Eud.S-100(mg)	-	-	1000	-	-	-
Eud. RL 100(mg)	-	-	-	1000	-	-
Eud. RS 100(mg)	-	-	-	-	1000	-
Ethyl Celulose(mg)	-	-	-	-	-	1000
Ethanol(ml)	5	5	5	5	5	5
Methanol(ml)	5	5	5	5	5	5
External phase						
PVA(% w/v of water)	0.50	0.50	0.50	0.50	0.50	0.50
Water(ml)	100	100	100	100	100	100

Microsponge formation based on selection of polymer, volume of sovent, selection of internal and external phase. Best microsponge is that which is spherical and in proper size range and also provide control drug release.

Table 2. Formulations of microsponges batch F1 to F9

Formula	Drug: Ethyl Cellulose	Ethanol (ml)	Methanol (ml)	PVA (% w/v of water)	Water (ml)
F1	1:1	5	5	0.25	100
F2	1:2	5	5	0.25	100
F3	1:3	5	5	0.25	100
F4	1:1	5	5	0.50	100
F5	1:2	5	5	0.50	100
F6	1:3	5	5	0.50	100
F7	1:1	5	5	0.75	100
F8	1:2	5	5	0.75	100
F9	1:3	5	5	0.75	100

Optimization of formulation is selected based on the trial batches. Type of polymer , surfactant concentration and weight of polymer selected by trial batches.

Table 3. Preparation of microsponge loaded hydrogel Formulation

Components	MG1	MG2	MG3
Miconazole nitrate Microsponges	Eq. to 100 mg of drug	Eq. to 100 mg of drug	Eq. to 100 mg of drug
Carbopol 940 (g)	1	1.5	2
Triethanolamine (ml)	1	1	1
NaOH (2 % w/v) to Adjust pH 6.8	q.s.	q.s.	q.s.
Distilled Water (ml)	100	100	100

4. Evaluation parameter:

Preformulation study

Preformulation testing is defined as investigation of physical and chemical properties of a drug substance alone and when combined with excipients. It is the first step in rational development of dosage forms of drug substance. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms that can be mass-produced. Preformulation investigations are designed to identify those physicochemical properties and excipients that may influence the formulation design, method of manufacture, pharmacokinetic and biopharmaceutical properties of the resulting product[15-40].

Organoleptic property

This includes recording of state, Taste,odour and colour of the drug using descriptive terminology. Record of colour of early batches is very useful in establishing appropriate specifications for later production. Drugs generally have a characteristic odour and taste. Unpleasant ones are masked later during formulation. The results are shown in Table 4.

Melting range

The melting point of Miconazole nitrate was determined using melting point apparatus. The sample was placed in capillary which was placed in apparatus and the temperature at which the sample is start to melt was note down. The results are shown in Table 5.

Compatibility study

Drug excipient compatibility study for sucessfuly form of final formulation.

(1) *Differential scanning calorimetry (DSC) study*

DSC (Perkin Elmer Pyrisl DSC,Pyrisl TGA) was employed to determine glass transition temperatures(Tg) and crystalligation event of the formulation in their liquid state. Thermal analysis using DSC was carried out for sample (Thermal analyzer). Approximately 10mg of drug was weighed into aluminium samples were transferred to aluminium pan and sealed hermetically and the thermal behavior was determined in range of 50-220ºC at a heating rate of 5. Baselines were determined using an empty pan and all the thermograms were baseline-corrected. Transition temperatures were determined from the endothermic peak minima while transition enthalpies were obtained by integration of endothermic transitions using linear baseline. DSC of pure drug, polymer, physical mixture and formulation were obtained as shown in Figure3, Figure 4, Figure 5.

(2) *Fourier transform infrared spectroscopy (FTIR) study*

Drug-Excipients compatibility study was carried out using FTIR spectrophotometer (Perkin elmer, spectrum GX FTIR). The IR spectrum of miconazole nitrate was recorded using FTIR spectrophotometer with diffuse reflectanse principle sample preparation involved mixing the sample with Potassium bromide (KBr), triturating in glass mortar and finally placing in the sample holder. The spectrum was scanned over a frequency range 4000-400 cm^{-1}. The infrared absorption spectra of pure drug and physical mixture of polymer and drug were obtained as shown in Figure 6, Figure 7, Figure 8, Figure 9, Figure 10 and Table 6.

Analytical Method

1. **Determination of wavelength of Miconazole nitrate in Methanol and phosphate buffer solution (PBS) pH 7.4:**

   A solution of Miconazole nitrate was prepared in Methanol and phosphate buffer pH 7.4 . UV spectrum was taken using UV–visible spectrophotometer with 1cm smatched quartz cells was used. (UV-1800 Shimadzu). The wavelength of Miconazole nitrate was found to be 272 nm and 208 nm . Shown in Figure 11 and Figure 12.

2. **Preparation of standard Curve of Miconazole nitrate in Methanol:**

   Accurately weighed about 100 mg of Miconazole nitrate was transferred in to the 100 ml volumetric flask. Few ml of methanol was added to dissolve the drug properly with vigorous shaking and make the volume upto 100 ml with methanol. The concentration of resulting solution is of 1000 µg/ml. Then after prepare 100 µg/ml. Then take aliquots of 0.5, 1, 1.5, 2, 2.5 and 3 ml of above solution in five different 10 ml volumetric flask and make volume up to 10 ml with methanol. Take absorbance of series of solution and plot the calibration curve with the linear regression equation. A graph of absorbance Vs concentration was plotted and was found to be linear over a range of 5 to 30 µg/ml indicating its compliance with Beer's law . It was shown in Figure 13 and Table 8 .

3. **Preparation of Standard Curve of Miconazole nitrate in phosphate buffer pH 7.4**

   100 mg Miconazole nitrate was dissolved in phosphate buffer pH 7.4 and then the volume was made upto 100 ml with phosphate buffer pH 7.4 (stock solution- 1mg/ml). The samples of 5, 10, 15, 20 upto 50 mcg/ml were prepared from the stock solution an analyzed by UV spectrophotometer at 208 nm. It was shown in Figure 14 and Table 9 .

Particle size determination

The particle size was determined using an optical microscope.
The microscope was fitted with a stage micrometer to calibrate the eyepiece micrometer.
Calibration of the eyepiece micrometer
One division of the stage micrometer = 0.01mm = 10 µm
C = (SM X 10) / EM
Where;

C = correction factor
SM = Reading of stage micrometer which coincides with reading of eye-piece micrometer (EM).
The results are shown in Table 9 .

Surface morphology

The particle size, shape and surface morphology of microsponges were examined by SEM (JSM-5510, Jeol Ltd) operating at 15 kV. The samples were mounted on a metal stub with double adhesive tape and coated with platinum/palladium alloy under vacuum. The microsponges were then analysed by SEM. Pictures of the prepared microsponges were taken at different accelerating voltages at 100 and 500 µm working distance. The results are shown in Figure 15.

Determination of loading efficiency and production yield

A sample of dried microsponges equivalent to 10 mg was taken in to mortar and pestle and add little amount of phosphate buffer of pH 7.4 and allowed to stand for 24 hours. Then transfer content in to 100 ml volumetric flask and make up volume to 100 ml with phosphate buffer of pH 7.4. The solution was filtered through whatman filter paper (No. 41). From the resulting solution take 1 ml in to 10 ml volumetric flask and then make up volume to 10 ml with phosphate buffer of pH 7.4. Drug content was determined by UV spectrophotometer at 208 nm. The entrapment was calculated by using following formula. Result shown in Table 10. The loading efficiency (%) of the microsponges was calculated according to the following equation:
The loading efficiency (%) of the microsponges was calculated according to the following equation:

$$\text{Loading efficiency} = \frac{\text{Actual Drug Content in Microsponges X 100}}{\text{Theoretical Drug Content}} \quad \ldots\ldots\ldots (1)$$

The production yield of the microsponges were determined by calculating accurately the initial weight of the raw materials and the last weight of the microsponge obtained. It was shown in Table 5.7.

Production Yield= Practical mass of microsponges X 100…….. (2)
Theoritical mass (Polymer+drug)

In-Vitro Drug release study

Dissolution profile of microsponges was studied by microsponge (equivalent to 100mg of miconazole nitrate) dissolution apparatus USP XXIII with a modified basket consisting of 5µm stainless steel mesh. The speed of the rotation was maintained at 50 rpm using phosphate buffer pH 7.4 as dissolution medium(900ml). Temperature was maintained at 37±0.5°C. Sample aliquots were withdrawn from dissolution medium at predetermined time intervals and were analyzed by UVspectrophotometer (UV-1800 Shimadzu) at 208 nm**.** To determine the drug release mechanism and to compare the release profile differences among microsponges, the drug released amount versus time will be used. The results are shown in Figure 16 and Table 11.

Viscosity measurement

The viscosity of the prepared formulations was measured using Brookfield viscometer LVDV-III Ultra programbable model (Brookfield engineering laboratories inc. Ma) at room temperature. The selected formulations were poured into the sample in beaker and spindle did not touch the bottom of beaker. Viscosity was measured at 25ºC. The results are shown in Table 12 .

Spreadability study

An apparatus suggested by Mutimer et al modified suitably in the laboratory and was used for spreadability study. It was shown in Figure 17. The apparatus was made of wooden block with scale and two glass slides having a pan mounted on a pulley. Excess formulation was placed between two glass slides and 10 g weight was placed on the upper glass slide for 5 min to compress the formulation to uniform thickness weight (60g) was added to the pan. The time in seconds require to separate the two slides was taken as a measure of spreadability. The results are shown in Table 13.

The spreadability was calculated by using the following formula:

$S = (m \times l) / t$

S - Spreadability,
m - Weight tied to the upper slides,
l - length of glass slide, and
t - time taken in seconds.

Determination of pH

The pH of optimized microsponge loaded hydrogel was determined using digital pH meter (OAKTON, Eutech instruments). Microsponge loaded hydrogel (1 g) was weighed accurately and dispersed in 100 ml of purified water. Standardized using pH 7 buffers before use. The measurement of pH of each formulation was done in triplicate and mean values were calculated. Results are shown in Table 14.

Drug content studies

10 g of each gel (equivalent 10mg of drug) formulation were taken in 100 ml volumetric flask containing 50ml methanol and stirred for 30 minutes and allowed to stand for 24 hours in case of microsponge loaded hydrogel formulations. The volume was made up to 100 ml and 1ml of the above solution was further diluted to 10ml with methanol. The drug content was determined by measuring the absorbance at 272 nm using UV Visible spectrophotometer (UV-1800, Shimadzu). The results are shown in Figure 18 and Table 15.

Invitro diffusion study

Release of Miconazole nitrate from hydrogel was measured across dialysis membrane (12,000-14,000 molecular weight cut off, Himedia dialysis membrane) using Franz diffusion cell, with a diffusional area of 2 cm^2 and receptor volume of 11 ml. The membrane was soaked in receptor compartment for 15 min. One gram Carbopol gel was placed on membrane surface in the donor compartment. The receptor compartment of cell was filled with 11 ml of phosphate buffer pH 7.4 (physiological pH). During the experiment solution of receptor site was kept at 35±0.50 C stirred at 100 rpm with magnetic stirring bars. One ml aliquots was collected from receptor side at intervals of 0 to 12 hr and replaced by same volume of receptor to maintain the sink condition and constant volume. The sample was analyzed by UV Spectrophotometer (UV-1800 Shimadzu) for Phosphate buffer solution (PBS) pH 7.4. The withdrawn samples were filtered with whatman filter paper (90 mm Ø). The percentage cumulative drug release was determined from calibration curve.[14] The results are shown in Table 16.

In vitro antifungal activity using cup plate method

In vitro antifungal activity was carried out by cup plate (or cylinder plate) method. The cylinder plate method depends upon diffusion of antifungal from a vertical cylinder through a solidified agar layer in a petridish or plate to an extent such that growth of added micro-organism is prevented entirely in a zone around the cylinder containing antifungal agent. Evaluation of *in vitro* antifungal activity of microsponge loaded hydrogel was carried out by cup

plate method. The overnight grown culture of fungus was inoculated into the sterilized agar media plates. After solidification, wells were cut into the media and fixed with 100 mg of the specimens to be tested using plane gel and microsponge loaded hydrogel of Miconazole. The plates were incubated at room temperature and the widths of zone of inhibitions resulting after drug diffusion into media were measured. The results are shown in Figure 19 and Table 17.

Extrudability study

It is a usual empirical test to measure the force require to extrude the material from tube. Consist of a wooden block inclined at an angle of 45º fitted with a thin metal strip at one end. While the other end was free .The aluminium collapsible tube containing 10gm of gel was positioned on inclined surface of wooden block; 30gm weight was placed on free end of the aluminium strip and was just touched for 10 sec. The quantity of gel extruded from each tube was noted. More quantity extruded better was extrudability. The measurement of extrudability of each formulation was measured. The results are shown in Table 18.

Stability test

In any rational design and evaluation of dosage form for drug, the stability of the active component must be a major criterion in determining their acceptance or rejection. Stability of a drug can be defined as the ability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutic and toxicological specifications. Optimized formulation was selected and kept for stability studies. Formulations were packed in an aluminium foil and sealed tightly and studies were carried out for 30 days. The International Conference on Harmonization (ICH) Guidelines titled "stability testing of new drug substances and product" describes the stability test requirements for drug registration application in the European Union, Japan and the United States of America.

Stability testing of new drug substances and products [Q1A (R2)]

Formulations were packed in an aluminium foil and sealed tightly and studies were carried out for 30 days. Perticular interval of time drug content checked. Stability of the formulation measured. The results are shown in Table 19 .

Photo Stability testing of of drug product [Q1B]

Pure drug which is directly used in marketed preparation and drug in microsponge formulation are tested for photostability testing. The results are shown in Figure 20, Figure 21, Figure 22, Figure 23 and Table 20, Table 21, Table 22, Table 23.

3. Results and Discussion

Preformulation study

Organoleptic property:

This includes recording of state, Taste, odour and colour of the drug using descriptive terminology.

Table 4. Organoleptic properties of Miconazole nitrate

Properties	Results
State	Solid
Description	White powder
Taste	Tasteless
Odour	Odourless
Colour	White

Melting point:

The melting point of Miconazole nitrate was determined using melting point apparatus

Table 5. Melting point range of Miconazole nitrate

Experiment	Standard	Observation
Melting Point Range	178-184ºC	178-185ºC

Compatibility study

***Differential scanning calorimetry* (DSC) study**

Thermal analysis using differential scanning calorimetry (DSC) was carried out for sample (Thermal analyzer). The thermograms showed a sharp endothermic peak at 186.82ºC The DSC curve of physical mixture of drug and polymer physical mixture exhibited the characteristic peaks of the drug.

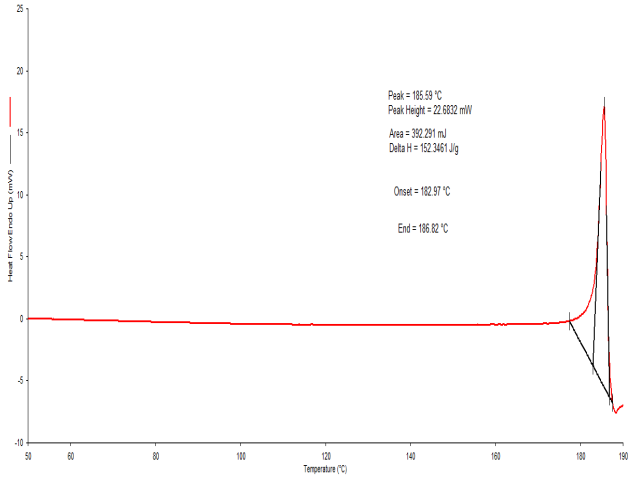


Figure 3. DSC spectra of Pure drug

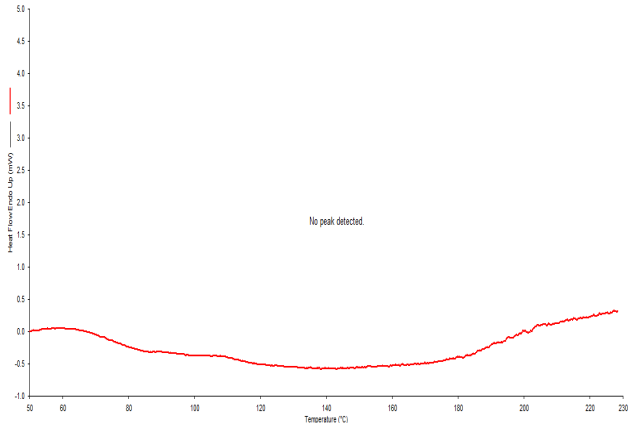


Figure 4. DSC spectra of Ethyl cellulose

Figure 5. DSC spectra of Physical mixture

Fourier transform infrared spectroscopy (FTIR) study

Drug-Excipients compatibility study was carried out using FTIR spectrophotometer. The FTIR studyof Miconazole nitrate revealed characteristic peaks which are shown in below table FTIR spectrum of miconazole nitrate (figure 7)was compared with refrence standard (figure 6). The infrared spectra of Miconazole nitrate pure powder, polymer, physical mixture and formulation are shown in the figure 8, 9 and 10. Thus, FTIR spectra of the physical mixtures and formulation when compared to pure drug thereby indicating the absence of any interaction at physical mixture level.

Table 6. Interaction studies of Miconazole nitrate with polymers through IR range spectroscopy

Group	Drug peak	Ethyl cellulose peak	Physical mixture	Formulation peaks	Observation
C-cl 785-540 cm^{-1}	637	-	637	637	No Interaction
C=C 1600-1475 cm^{-1}	1547	1623	1547,1623	1547,1623	No Interaction
C-0 1300-1000cm^{-1}	1217	1412	1217,1412	1217,1412	No Interaction
Aromatics out of plane blend 900-690 cm^{-1}	827	-	827	827	No Interaction
C-N 1350-1000cm^{-1}	1330	-	1330	1330	No interaction
C-H 2900-2800cm^{-1}	-	2977	2977	2977	No nteraction
C=0 1670-1820cm^{-1}	-	1735	1735	1735	No Interaction
O-H 3500-3000cm^{-1}		3459	3459	3459	No Interaction

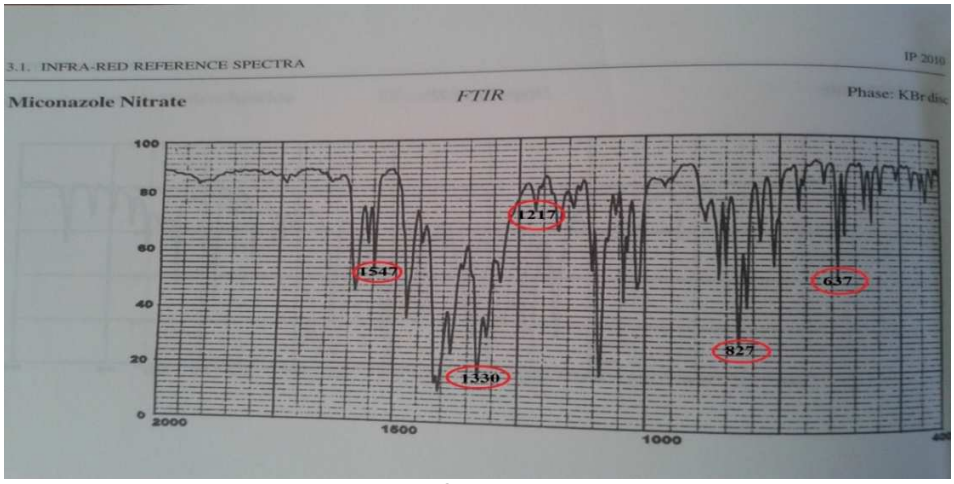


Figure 6. FTIR spectra of Miconazole nitrate in IP 2010

Figure 7. FTIR spectra of Miconazole nitrate

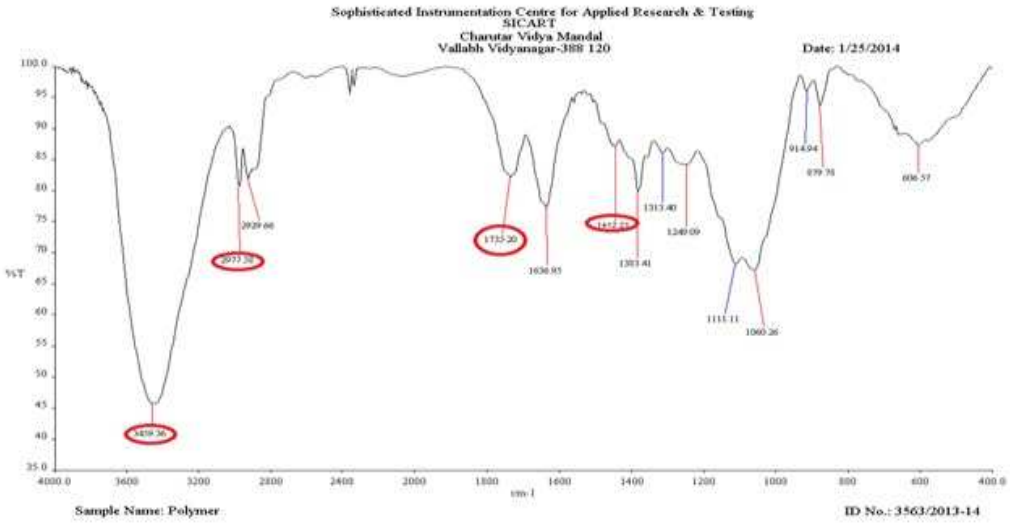


Figure 8. FTIR spectra of Ethyl Celluloses

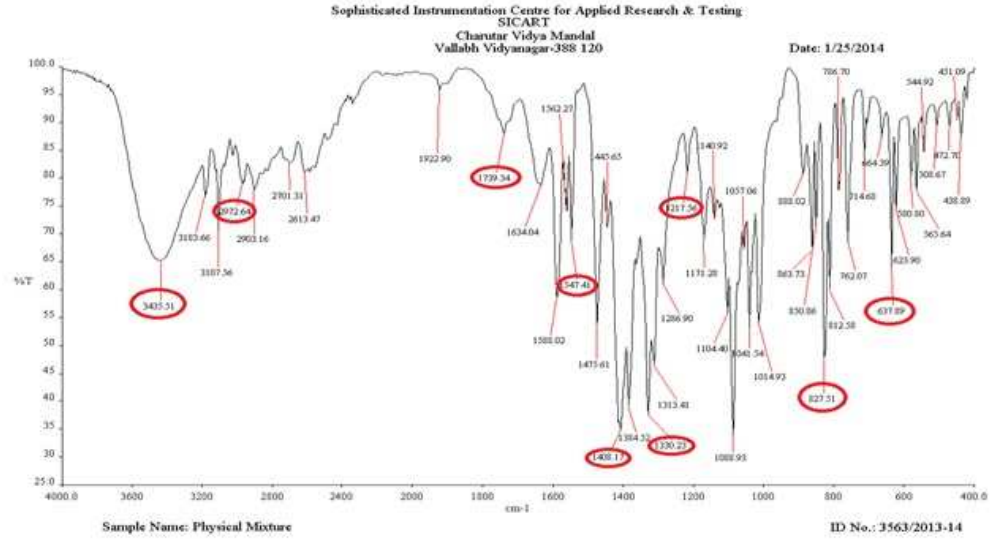


Figure 9. FTIR spectra of Physical mixture

Figure 10. FTIR spectra of Formulation

Analytical Method

a) Preparation of Calibration Curve of Miconazole nitrate in Methanol and phosphate buffer pH 7.4

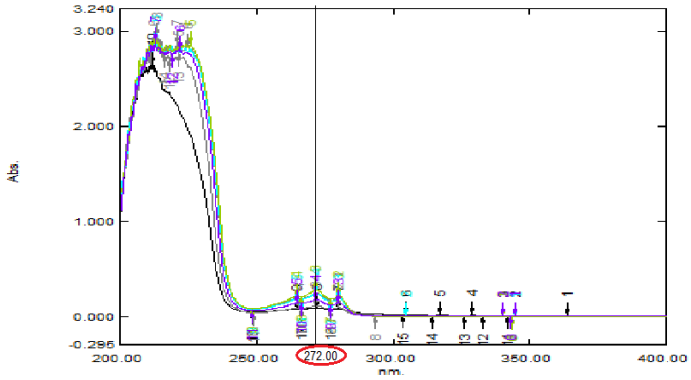


Figure 11. λmax of Miconazole nitrate in methanol 272 nm

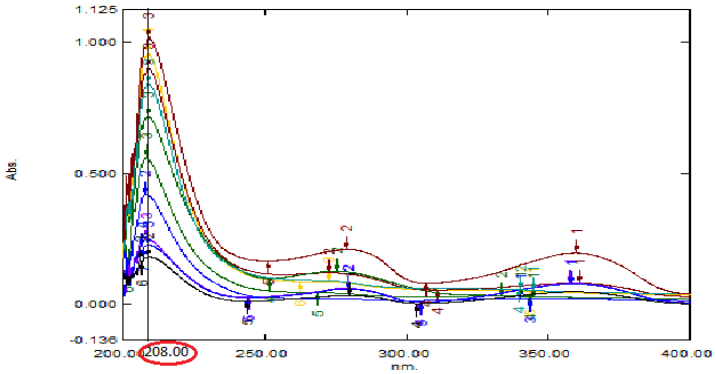


Figure 12. λmax of Miconazole nitrate in Phosphate buffer pH 7.4 208 nm

b) Preparation of standardCurve of Miconazole nitrate in Methanol (** Mean ± SD; n=3)

Table 7. Preparation of standardCurve of Miconazole nitrate in Methanol

Sr. no	Concentration (µg/ml)	Absorbance*
1	5	0.05 ± 0.002
2	10	0.11 ± 0.005
3	15	0.16 ± 0.01
4	20	0.21 ± 0.005
5	25	0.25 ± 0.005
6	30	0.30 ± 0.005

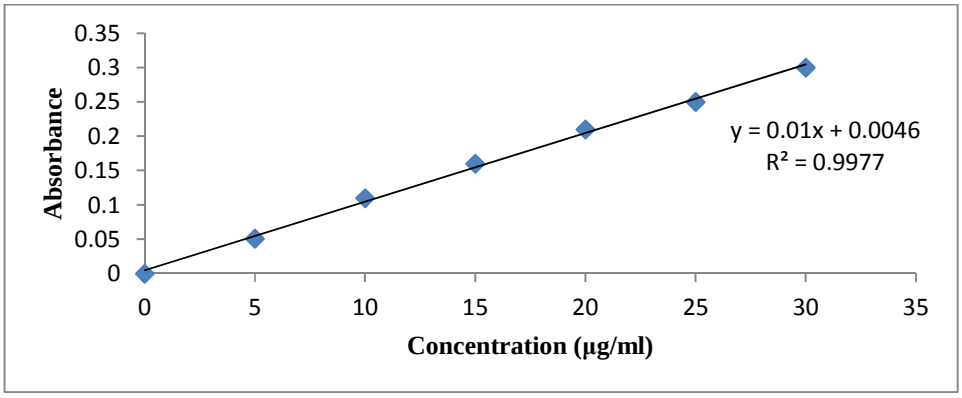


Figure 13. Standard curve of miconazole nitrate in methanol

c) Preparation of Standard Curve of Miconazole nitrate in Phosphate buffer pH 7.4

(* Mean ± SD; n=3)

Table 8. Preparation of standard Curve of Miconazole nitrate in Phosphate buffer pH 7.4

Sr. No.	Concentration (µg/ml)	Absorbance*
1	5	0.100 ± 0.005
2	10	0.225 ± 0.001
3	15	0.322 ±0.014
4	20	0.429 ± 0.001
5	25	0.541 ± 0.002
6	30	0.645 ± 0.001
7	35	0.743 ± 0.002
8	40	0.836 ± 0.003
9	45	0.952 ± 0.001
10	50	1.050 ± 0.011

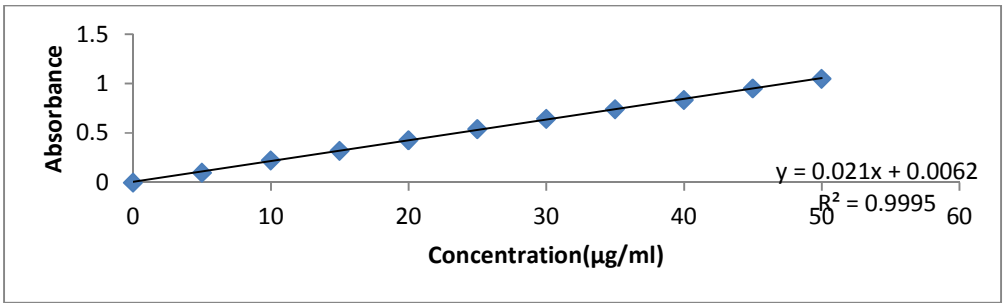


Figure 14. Standard curve of miconazole nitrate in phosphate buffer pH 7.4

Particle size determination of Ethyl Cellulose microsponge

(* Mean ± SD; n=3)

Table 9. Effect of formulation variables on particle size

Formula	Polymer type	Polymer wieght (mg)	PVA concentration (%w/v of water)	Particle size* µm
F1	Ethyl Cellose	500	0.25	29.69 ± 0.18
F2		1000	0.25	27.81 ± 0.20
F3		1500	0.25	27.08 ± 0.12
F4		500	0.50	23.44 ± 0.25
F5		1000	0.50	21.44 ± 0.09
F6		1500	0.50	20.07 ± 0.27
F7		500	0.75	19.36 ± 0.23
F8		1000	0.75	17.04 ± 0.12
F9		1500	0.75	14.82 ± 0.20

From above batches F5 batch has optimal partical size

Surface morphology

The particle size, shape and surface morphology of microsponges were examined by SEM.

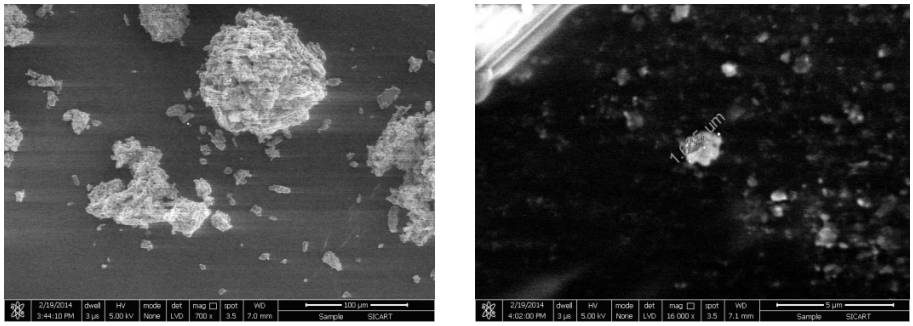


Figure 15. SEM of microsponge formulation

SEM shown that microsponge are pororous , spherical and in micrometer size

Determination of loading efficiency and production yield

The loading efficiency (%) and production yield of the microsponges were calculated according to the following equations

Loading efficiency = Actual Drug Content in Microsponges X 100…… (1)
Theoretical Drug Content

Production Yield= Practical mass of microsponges X 100…….. (2)
Theoritical mass (Polymer+drug)

Table 10. Loading efficiency and production yield of various microsponge Formulations

Formula	(%) Loading efficiency *	(%) Production yield *
F1	76.42 ± 1.15	51.7 ± 0.7
F2	80.64 ± 2.00	74.8 ± 1.0
F3	81.20 ± 1.05	76 ± 0.5
F4	70.52 ± 0.50	61.4 ± 0.7
F5	72.66 ± 1.00	65.3 ± 1.0
F6	74.35 ± 1.32	70 ± 0.4
F7	63.90 ± 0.78	43.87 ± 0.5
F8	65.82 ± 0.95	48.3 ± 0.5
F9	68.23 ± 1.12	52.2 ± 1.0

(* Mean ± SD; n=3)

Batch F5 has optimal (%) loading efficiency and highest (%) production yield

Drug release kinetics

Table 11. Drug release data of microsponge preparation

Time(hr)	Drug	F1	F2	F3	F4	F5	F6	F7	F8	F9
0.5	20.20	9.85	8.57	8.57	6.00	5.14	4.72	4.28	3.43	2.99
1	36.98	34.09	29.24	23.24	28.09	15.99	12.09	25.33	27.81	15.76
2	66.03	59.62	52.09	44.14	45.72	32.28	23.24	39.67	50.14	32.04
4	87.53	78.14	75.00	64.95	68.81	44.19	38.05	56.57	66.90	49.52
6	99.74	86.47	84.85	75.57	78.53	52.33	54.66	67.19	69.53	55.28
8	-	96.19	94.14	86.24	83.00	65.62	70.14	78.81	78.57	78.28

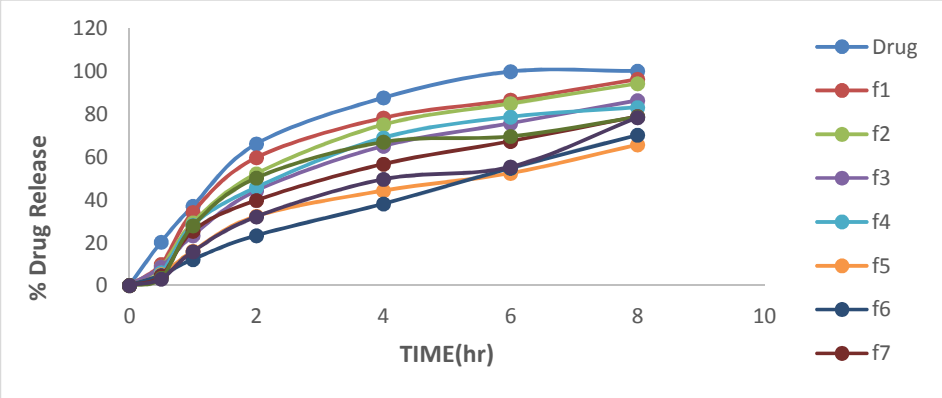


Figure 16. Drug release profile of microsponge loaded hydrogel

From above study F5 has require drug release

Viscosity measurement

Viscosity is an important parameter for characterizing the gels as it affects the mechanical and physical properties such as spreadability, consistency and hardness of the preparation which in turn are related to ease of product removal from container, ease of application on the skin surface and product feel on the application site as well as

release of drug. The viscosity of the prepared formulations was determined using Brookfield viscometer DV-III Ultra programbable model.

Table 12. Viscosity of various microsponge Formulations

Formulation	Viscosity(ps)*
MG1	1000 ± 4.5
MG2	1010 ± 5.5
MG3	1021 ± 2.3

(*Mean ± SD; n=3)

From above batches MG2 batch has require viscosity

Spreadability study

An apparatus suggested by Mutimer et al modified suitably in the laboratory and was used for spreadability study.

Figure 17. Spreadability apparatus

Table 13. Spreadability of various microsponge loaded hydrogel

Formulation	Spreadability(g.cm/sec)*
MG1	11.96 ± 0.02
MG2	11.05 ± 0 .05
MG3	10.62 ± 0.06

(*Mean ± SD; n=3)

From above batches MG2 has require spreadability

Determination of pH

The pH of optimized microsponge loaded hydrogel was determined using digital pH meter.

Table 14. pH of various microsponge loaded hydrogel Formulations

Formulation	pH*
MG1	6.7 ± 0.06
MG2	6.8 ± 0.06
MG3	6.8 ± 0.06

(*Mean ± SD; n=3)

From above batches MG2 and MG3 has require pH

Drug content studies

Microsponge loaded hydrogel 10 gm equivalent to 10 mg Miconazole nitrate was taken in 100 ml methanol = 100 µg/ml

1 ml from above solution was further diluted with 10 ml methanol = 10 µg/ml

(Test solution)

Absorbance of test solution (y) =0.904

Now, y = 0.01x+0.004

x = (y - 0.004) / 0.01

= (0.0904- 0.004) / 0.01

= 90

= x × Dilution factor

=90×100

=9000µg/ml

= 9 mg/100ml

Now 9mg = 100 %
10 mg = 90.00 %
So, the Miconazole nitrate content in the optimized microsponge loaded hydrogel formulation was found to be 90%.

Table 15. Drug content (%)data of Ethyl cellulose microsponge preparation

Formulation	Polymer type	Drug content (%)*
F1	Ethyl Cellulose	90.00 ± 0.57
F2		94.35 ± 1.0
F3		78.42 ± 1.3
F4		92.53 ± 0.7
F5		97.08 ± 0.5
F6		75.23 ± 2.0
F7		91.44 ± 1.23
F8		95.52 ± 0.5
F9		69.87 ± 1.0

(*Mean ± SD; n=3)

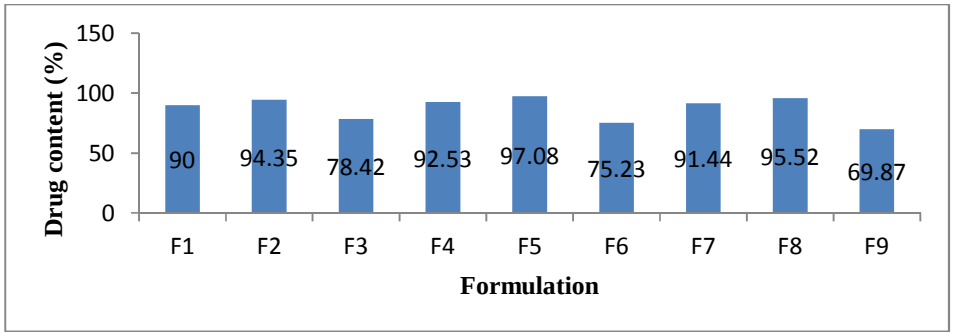


Figure 18. Drug release profile of microsponge loaded hydrogel

From above study F5 has higher drug content

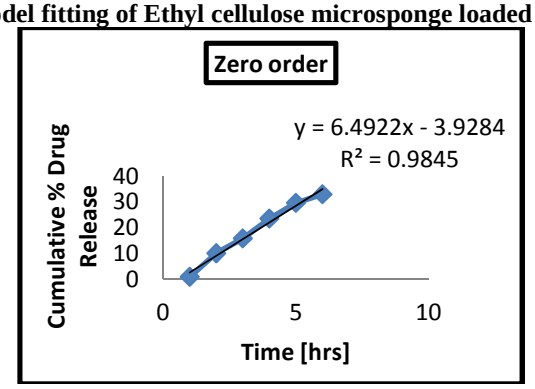
Invitro diffusion studies for drug release

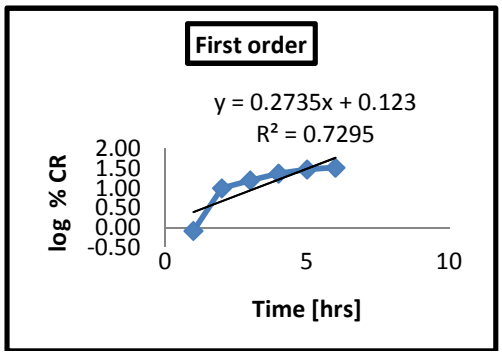
The drug release study from topical gel clearly indicated that the release of the drug was influenced by the concentration of the Carbopol 940. Very highly viscous solution and formed H-bond with drug molecule which reduced diffusion capacity. It was observed that maximum drug release from microsponge was achieved within 12 h. The release profile of Miconazole nitrate formulation is shown in Table 16.

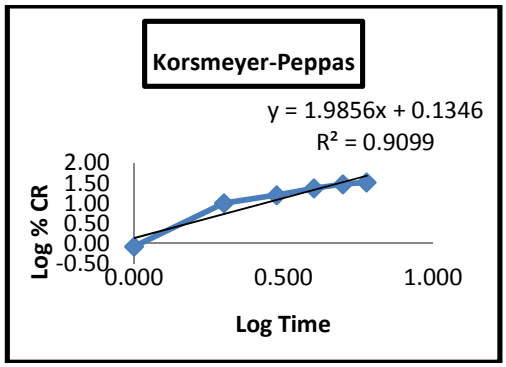
Table 16. Invitro diffusion studies for drug release

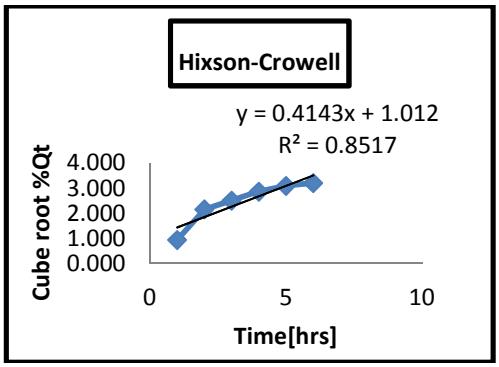
Time (hrs)	Maketed Preparation	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0	0
1	2.98	1.90	1.071	0.83	1.19	0.83	0.95	1.07	0.71	0
2	12.68	11.11	6.69	5.73	10.86	10.02	5.73	6.57	4.18	3.09
3	27.87	20.82	16.20	11.66	19.38	15.79	11.42	12.99	11.63	10.18
4	47.22	31.72	25.68	21.18	29.55	23.53	21.06	21.92	21.06	19.61
5	71.17	42.29	36.82	30.06	38.56	29.63	30.42	31.38	30.89	28.96
6	99.98	55.12	50.49	40.95	50.88	32.96	39.65	50.02	48.82	47.59
7	-	69.54	64.68	55.33	64.69	49.81	48.99	68.49	67.75	65.34
8	-	83.75	79.01	69.54	78.89	62.53	67.99	78.97	78.00	77.95
9	-	98.67	93.46	83.87	92.38	74.21	82.53	84.05	81.65	79.27
10	-	-	-	98.19	-	81.34	93.53	99.63	96.24	92.27
11	-	-	-	-	-	89.57	-	-	-	-
12	-	-	-	-	-	96.16	-	-	-	-

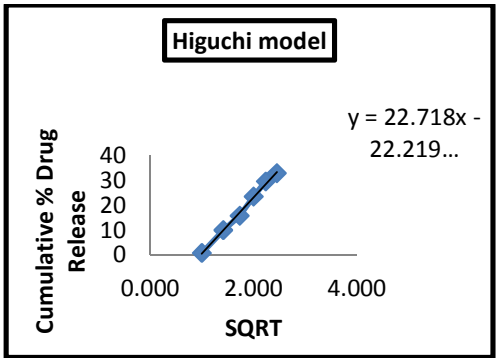
Model fitting of Ethyl cellulose microsponge loaded hydrogel











The best fitted model is higuchi model than other model

In vitro antifungal activity using cup plate method

On the basis of *in vitro* characterization studies, Miconazole nitrate microsponge loaded hydrogel formulation was compared with Marketed gel for *in vitro* antifungal activity by using cup plate method. Results of *in vitro* antifungal activity are shown in Figure 19 and Table 17 . Microsponge loaded hydrogel exhibited maximum antifungal activity after 24 hr comparable to marketed Miconazole nitrate gel.

Figure 19. Measurement of *in vitro* antifungal activity of Ethyl cellulose microsponge loaded hydrogel by cup plate method

Table 17. Comparisons of antifungal activity of Ethyl cellulose microsponge loaded hydrogel with marketed gel formulation

Organism	**Formulation**	**Zone of inhibition diameter in mm**
Cryptococcus	Control (Plane gel)	-
Cryptococcus	Pure drug	11.3 ± 0. 2
Cryptococcus	Microsponge loaded hydrogel	9.6 ± 0. 1
Cryptococcus	Marketed Miconazole nitrate gel	6.3 ± 0. 1

Ethyl cellulose Microsponge loaded hydrogel has more antifungal activity than marketed gel after 24 hr

Extrudability study

It is a usual empirical test to measure the force require to extrude the material from tube.

Table 18. Extrudability data for microsponge loaded hydrogel formulation

Formulation code	**Extrudability gm/cm^2**
MG1	5
MG2	3
MG3	1

From above batches MG2 has require extrudability

Stability test

The optimized microsponge gel was stable for at least 1 months. Stability studies were performed as per ICH guidelines. At fixed time interval drug content determination of this formulation shows that there were no significant changes in the values when compared to the initial formulations. Thus we may conclude that the drug does not undergo degradation on storage. The results of stability of microsponge gel formulation are indicated in Table which revealed that the formulation exhibits sufficient stability.

(a)Stability testing of new drug substances and products [Q1A (R2)]

Table 19. Stability data for microsponge loaded hydrogel formulation

Stability Condition	**Physical Stability**			**Drug Content**		
	No. of Days			No. of Days		
	10	20	30	10	20	30
250 C/60% RH	No change in appearance			90.35	89.72	89.65
400 C/75%RH	No change in appearance			92.04	91.83	91.71

Microsponge loaded hydrogel was stable after 1 month

(b) Photo Stability testing of of drug product

Pure drug as contol covered with aluminium foil, Microsponge as test sample (A_{T1}) taken Pure drug($\mathbf{A_{T2}}$).All sample are put in Uv chamber for particular peridor 48 hr .All sample absorbance taken after 12 hr, 24 hr, 36 and 48 hr and degaradation of drug in control, sample A_{T1} and $\mathbf{A_{T2}}$ determine. The absorbance of drug taken in methanol. The degradation of drug shown in figure 20, 21, 22 and 23. The result shown in table 20, 21, 22 and 23.

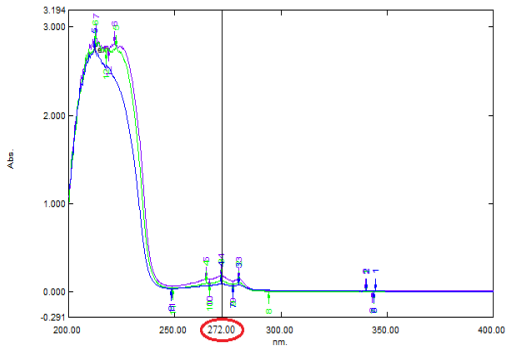


Figure 20. Stability data for microsponge loaded hydrogel formulation after 12 hr formulation

$\Delta A = A_T - A_O$
Where,
ΔA = Difference in absorbance
A_T = Absorbance of test
A_O = Absorbance of control

Table 20. Stability data for microsponge loaded hydrogel after 12 hr formulation

Sample	Absorbance
Control (A_O)	0.160
Microsponge formulation(A_{T1})	0.123
Pure drug(A_{T2})	0.086

$\Delta A = A_O - A_{T1}$
$= 0.160 - 0.123$
$= \boxed{0.037}$

$\Delta A = A_O - A_{T2}$
$= 0.160 - 0.086$
$\boxed{0.074}$

Absorbance difference between contol and test shown degradation. More the absorbance difference more the degradation of drug. The degradation of drug in microsponge was less compare to marketed product.

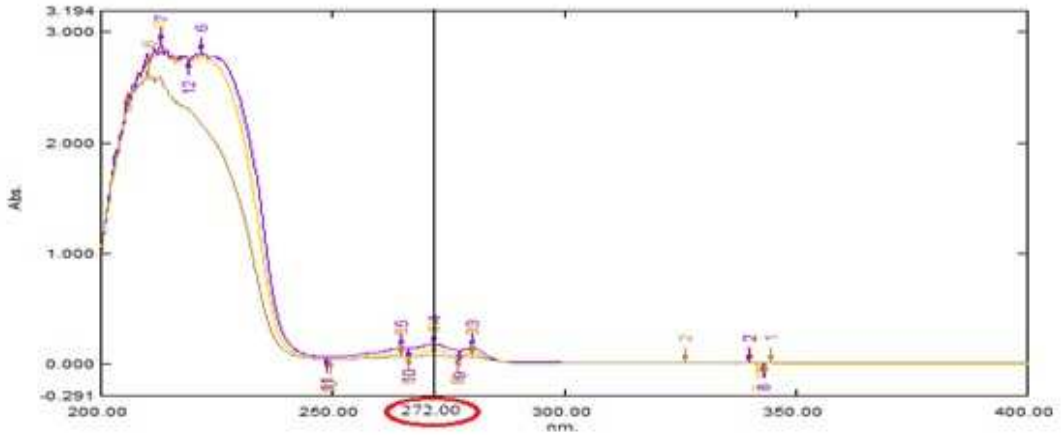


Figure 21. Stability data for microsponge loaded hydrogel formulation after 24 hr formulation

Table 21. Stability data for microsponge loaded hydrogel after 24 hr formulation

Sample	Absorbance
Control (A_O)	0.160
Microsponge formulation(A_{T1})	0.119
Pure drug(A_{T2})	0.079

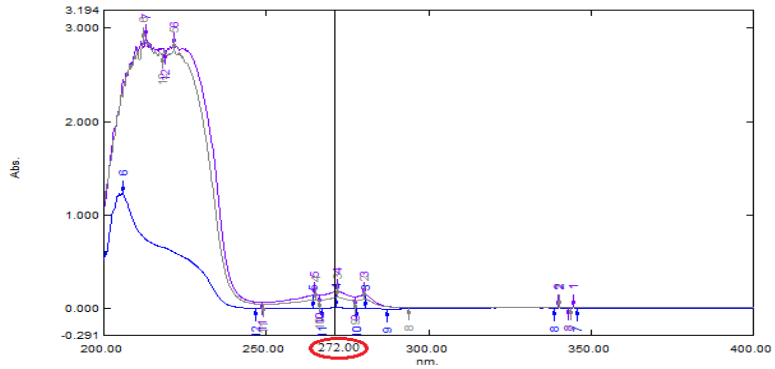


Figure 22. Stability data for microsponge loaded hydrogel formulation after 36 hr formulation

Table 22. Stability data for microsponge loaded hydrogel after 36 hr formulation

Sample	Absorbance
Control ($\mathbf{A_O}$)	0.160
Microsponge formulation($\mathbf{A_{T1}}$)	0.115
Pure drug($\mathbf{A_{T2}}$)	0.005

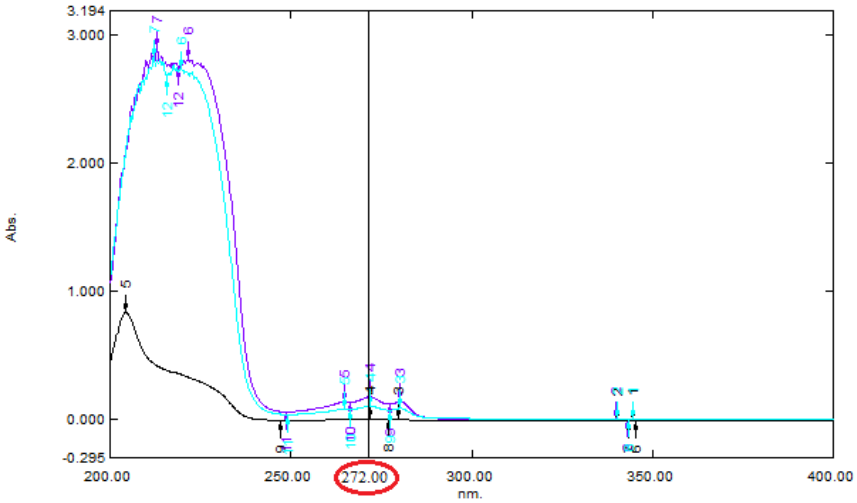


Figure 23. Stability data for microsponge loaded hydrogel formulation after 48 hr formulation

Table 23. Stability data for microsponge loaded hydrogel after 48 hr formulation

Sample	Absorbance
Control ($\mathbf{A_O}$)	0.160
Microsponge formulation($\mathbf{A_{T1}}$)	0.115
Pure drug($\mathbf{A_{T2}}$)	0.002

Discusion

Microsponge containing oxiconazole nitrate was prepared by quasi emulsion diffusion method using Ethyl cellulose. The FTIR spectral analysis suggested that there was no interaction between the drug and formulation additive. The drug exists in original form and available for the biological action. Weight of ethyl cellulose and concentration of PVA significantly affected on the dependent variables like particle size, loading efficiency and drug content, loading efficiency and drug content of the batch F5 containing 1000mg ethyl cellulose and 0.5% PVA produced microsponge with particle size 21.44 ± 0.09, loading efficiency 72.66 ± 1.00 and drug content was 97.08 ± 0.5. The optimized formulation F5 was selected on the basis of its good loading efficiency and particle size. The optimized microsponge formulations conveniently incorporated into carbopol gel with uniform drug content. It was evaluated for viscosity, spreadability, drug release.The gel containing 1.5 % carbopol 940 give viscosity 1010 ± 5.5 ps, spreadability 11.05 ± 0 .05 g.cm/sec and drug release at 12 h 96.16 %CPR and pH was 6.8 ± 0.06. Hence it was selected as optimized batch and it could meet the desired specifications. Stability studies indicated no significant changes over the period of one month were observed with respect to viscosity and drug content. In all the cases the best fit model was found to be Higuchi matrix. In conclusion, microsponge loaded hydrogel was successfully formulated to control the action of the drug.

4. Conclusion

Microsponge containing Miconazole nitrate was prepared by quasi emulsion diffusion method using Ethyl cellulose. The result shown that the concentration of ethyl cellulose and concentration of PVA significantly affected on the particle size, loading efficiency and drug content . Ethyl cellulose and PVA produced microsponge F5 containing batch with particle size 21.44 ± 0.09, loading efficiency 72.66 ± 1.00 and drug content 97.08 ± 0.5. The optimized microsponge formulations conveniently incorporated into carbopol 940 gel with uniform drug content. It was evaluated for viscosity, spreadability, drug release. The gel containing carbopol 940 give viscosity 1010 ± 5.5 ps, spreadability 11.05 ± 0 .05 g.cm/sec and drug release at 12 h 96.16 % CPR and pH was 6.8 ± 0.06. In conclusion, microsponge loaded hydrogel was successfully formulated to control the action of the drug.microsponge systems have been found to have good potential for prolonged drug release and therefore can be beneficial for use in

the treatment of various chronic fungal infections. Additional benefits such as dose reduction and reduced frequency of administration and avoiding related systemic side effects can be produced.

5. Acknowledgement

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