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Formulation and Evaluation of Topical Econazole Nitrate Microsponge Loaded Hydrogel

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ABSTRACT

Development of novel drug delivery systems for optimization of drugs efficacy and cost-effectiveness has become highly competitive and rapidly evolving area of interest. Several predictable and reliable systems were developed for systemic drugs under the heading of transdermal delivery system (TDS) using the skin as a portal of entry. Transdermal delivery can provide a number of advantages over conventional methods of drug administration, including enhanced efficacy, increased safety, and greater patient compliance. Microsponges are uniform, spherical and tiny sponge-like polymer particles having a myriad of interconnected voids of particle size range of 5 - 300µm. In this work, a microsponge based delivery system has been developed using different polymers such as Ethyl cellulose and Eudragit RS 100 by quasi-emulsion solvent diffusion method to provide a sustained release medication for topical delivery of Econazole nitrate and it is observed that; the drug entrapment efficiency and the size of the prepared microsponges were affected by the drug: polymer ratio, polymer type, solvent type, and the PVA concentration.

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INTRODUCTION

For thousands of years, medicinal substances have been applied to the skin in the belief that they could treat diseases, and in the past 60 years, there have been considerable advances in the understanding of the mechanisms that govern the absorption of chemicals through the skin. It is known that the skin is a formidable barrier to the passage of substances into and out of the body, but it is also an interactive interface that can be manipulated to allow it to become a viable pathway for drug transportation.

Transdermal delivery can provide a number of advantages over conventional methods of drug administration, including enhanced efficacy, increased safety, and greater patient compliance. By delivering a steady flow of drugs into the bloodstream over an extended period of time, transdermal systems can avoid the "peak and valley "effect of oral or injectable therapy and can enable more controlled, effective treatment.1,8 Some conventional dosage e.g., gels and ointments which are often aesthetically unappealing, greasiness and stickiness etc. that often result in lack of patient compliance. Other drawbacks of topical formulations are uncontrolled evaporation of active ingredient, unpleasant odor and potential incompatibility of drugs with the vehicles. Conventional topical formulations are designed to work on the outer layers of the skin. When the active ingredients of these formulations are released upon application, a highly concentrated layer of active ingredient is produced that is rapidly absorbed. Thus, there is a genuine need for delivery systems to maximize the amount of time to release of an active ingredient is present either on the skin surface or within the epidermis, while minimizing its transdermal penetration into the body. Moreover, as a result of the high concentration of active agents employed in the conventional topical dosage forms, several side effects are recorded insignificant users such as irritation and allergic reactions.

The microsponge drug delivery system fulfills these requirements. The Microsponge technology was developed by Won in 1987. In recent years, there has been considerable emphasis given to the development of microsponge base novel drug delivery systems, in order to modify and control the release behavior of the drugs. By incorporation into a carrier system, it is possible to alter the therapeutic index and duration of the activity of drugs. Microsponges are uniform, spherical and tiny sponge-like polymer particles having the myriad of interconnected voids of particle size range of 5 - 300µm. They are biologically inert particles that are made of synthetic polymers and the particles serve to protect the entrapped drug compound from physical and environmental degradation. Their high degree

of cross-linking results in particles that are an insoluble, inert and sufficient strength to stand up to the high shear commonly used in the manufacturing of creams, lotions, and powders. These microsponges have the capacity to entrap wide range of active ingredients such as emollients, fragrances, essential oils, sunscreens and anti-infective and are used as a topical carrier system. Its large capacity for entrapment of actives up to 3 times its weight differentiates microsponges from another type of dermatological delivery systems. The fundamental appeal of the microsponge technology from the difficulty experienced with conventional topical formulations in releasing active ingredients over an extended period of time. 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. This sustained release of actives to skin over time is an extremely valuable tool to extend the efficacy and lessen the irritation commonly associated with powerful therapeutic agents such as Retinoid or Benzoyl Peroxide. Microsponge polymers possess the versatility to load a wide range of actives providing the benefits of enhanced product efficacy, mildness, tolerability, and extended wear to a wide range of skin therapies. Although the microsponge size may vary, a typical 25µm sphere can have up to 250000 pores and an internal pore structure equivalent to 10 ft in length. The microsponge system can prevent excessive accumulation of ingredients within the epidermis and the dermis. Potentially, the microsponge system can significantly reduce the irritation of effective drugs without reducing their efficacy[2,3].

ADVANTAGES OF MDDS[1-6]

Microsponge technology offers:

• Enhanced product performance.

• Extended-release.

• Reduced irritation and better tolerance hence improved patient compliance Improved product elegance.

• Improved oil control: Microsponge can absorb oil up to 6 times its weight without drying.

• Improved formulation flexibility.

• Improved thermal, physical, and chemical stability.

• Flexibility to develop novel product forms.

• Microsponge systems are non-irritating, non-mutagenic, non-allergenic and non-toxic.

• Incorporation of immiscible products.

• Improves material processing e.g. liquid can be converted into powders.

• Increased bioavailability.

• Improves efficacy in treatment.

• Superior skin feels and exceptional product esthetics.

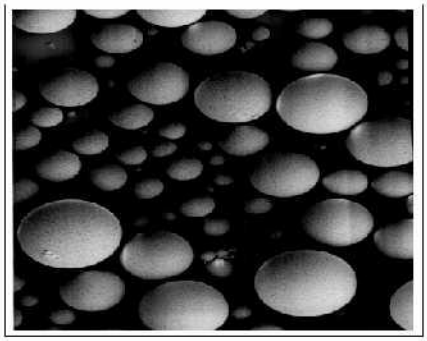


Figure: 1 Microsponge System

CHARACTERISTICS OF MICROSPONGES[1-15]

➢ Microsponges are stable over a pH range of 1 to 11.

➢ Stable up to 1300c temperature.

➢ Compactable with the many of the vehicles and active ingredients.

➢ Microsponge formulations are self-sterilizing as their average pore size is 0.25µm where bacteria cannot penetrate.

➢ It has higher payload (50 to 60%) and still free-flowing.

CHARACTERISTICS OF ACTIVE INGREDIENTS ENTRAPPED IN MICROSPONGE[1-17]

- Most liquid or soluble ingredients can be entrapped in the particles. Active ingredients that can be entrapped in microsponges must meet following requirements:

- It should be either fully miscible in a 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.

- The solubility of actives in the vehicle must be limited to avoid cosmetic problems; not more than 10 to 12% w/w microsponges must be incorporated into the vehicle. Otherwise, the vehicle will deplete the microsponges before the application.

- The spherical structure of microsponges should not collapse.

- Polymer design and payload of the microsponges for the active must be optimized for required release rate for given time period.

- It should be stable in contact with the polymerization catalyst and conditions of polymerization.

APPLICATION OF MICROSPONGE SYSTEMS[1-6]

Products under development or in the marketplace utilize the topical microsponge system in three primary ways,

1. As reservoirs releasing active ingredients over an extended period of time.

2. As receptacles for absorbing undesirable substances such as excess skin oils.

3. As closed containers holding ingredients away from the skin for superficial action.

Some other applications of MDS are described in **Table: 1**

Table 1: Application of microsponge systems

Active agent	Application
Anti-acne Eg. Benzoil peroxide	Maintained efficacy with decreased skin irritation and sensitization.
Anti dandruff Eg. Zinc pyrithone, Selenium sulfide	Reduced unpleasant odour with lowered irritation with extended safety and efficacy.
Anti fungal	Sustained release of active ingredients.
Anti inflammatory Eg. Hydrocortisone	Long lasting activity with reduction of skin allergic response and dermatoses.
Anti pruritics	Extended and improved activity.
Rubifacients	Prolonged activity with reduced irritancy greasiness and odour.
Skin depigmenting agents Eg. Hydroquinone	Improved stabilization against oxidation with improved efficacy and aesthetic appeal.
Sunscreens	Long lasting product efficacy, with improved protection against sunburns and sun related injuries even at elevated concentration and with reduced irritancy and sensitization.

Recent applications of microsponges are

- Microsponges from seaweed used for diagnosing diseases. Microsponges derived from seaweed help to detect the diseases such as heart disease, cancer, HIV quickly and at far lower cost than current clinical methods.

- Self-Assembling RNAi microsponges.

- Microsponge acts as both carrier and cargo for the delivery of gene-silencing RNA into cells.

- Cardiovascular engineering using microsponge technology.

- Microsponge technology is also used for Reconstruction of the vascular wall.

MATERIALS AND METHODS:

MATERIALS:

Econazole nitrate, Ethylcellulose, Eudragit RS 100, Dichloromethane and Carbopol was bought from Yarrow Chem Products; Ethanol, PVA, and Methanol was purchased from Chemco; Propylene glycol, Methyl parabens, Propyl parabens, Sodium Metabisulfite, Triethanolamine, Potassium dihydrogen orthophosphate and Sodium hydroxide was obtained from Spectrum Chemicals & Reagents and Rectified Spirit was purchased from Pamba Sugars and Chemicals.

METHODS:

1. PREFORMULATION STUDIES

1.1. Determination of melting point

A melting point of Econazole nitrate, Ethylcellulose, Eudragit RS100, Polyvinylalcohol, and Carbopol was determined by a capillary method using Thiele's apparatus.

1.2. Solubility

The solubility of Econazole nitrate, Ethylcellulose, Eudragit RS100, Polyvinylalcohol, and Carbopol were tested in various solvents such as distilled water, methanol, and ethanol.

1.3. FT-IR Spectroscopy

The FT-IR spectrum of the obtained sample of drug and polymers were compared with the standard functional group frequencies of Econazole nitrate, Ethylcellulose, EudragitRS100 and Carbopol respectively.

1.4. Compatibility between the drug and polymers

FT-IR spectroscopy was carried out to check the compatibility between drug and polymer. The compatibility between Econazole nitrate with ethyl cellulose and Eudragit RS100 was carried out in the ratio of 1:2 and that of Econazole nitrate and Carbopol in the ratio of 1:1.

2. PREPARATION OF STANDARD CALIBRATION CURVE

2.1. Preparation of phosphate buffer of pH 7.4.

Placed 250 ml of 0.2M potassium dihydrogen orthophosphate in 1000 ml volumetric flask, added 195.5ml 0.2M Sodium hydroxide and made up to the volume 1000 ml using distilled water.

2.2. Determination of λ max.

The absorption maximum of the standard solutions of Econazole nitrate was scanned between 200-400 nm regions on Shimadzu UV-visible spectrophotometer. The absorption maximum obtained with the substance being examined corresponds in position and relative intensity to those in the reference spectrum.

2.3. Preparation of standard curve of Econazole nitrate.

Accurately weighed 50 mg Econazole nitrate was dissolved in 25 ml of methanol and made up to 50 ml with phosphate buffer pH 7.4 in 50 ml volumetric flask to get the stock solution of 1000 µg/ml. From stock solution 5 ml was pipetted out and further diluted up to 50ml with buffer to get 100 µg/ml solutions. From 100µg/ml solution take 5ml and diluted to 50ml to get 10µg/ml solution from this aliquots of 2, 4, 6, 8, 10 ml were withdrawn and diluted to 10 ml with buffer to obtain a concentration range of 2-10µg/ml. The absorbances of the solutions were measured at 272 nm by using UV-Vis spectrophotometer. A graph of Concentration vs. Absorbance was plotted.

3. PREPARATION OF MICROSPONGES

Microsponges were prepared by Quasi-emulsion solvent diffusion method. The organic internal phase consisted of Ethylcellulose or Eudragit RS100 dissolved in 10ml dichloromethane or ethanol. The calculated amount of Econazole nitrate was added gradually with stirring. The resulting solution was then poured into 0.5% or 0.75% (w/v) of PVA solution in water (external phase of 100ml volume). The mixture was stirred at 1000 rpm for 6 hours at room temperature (RT) to remove dichloromethane or ethanol from the reaction flask. The formed microsponges were filtered, washed with distilled water, and dried overnight at RT.

4. EVALUATION OF DRUG LOADED MICROSPONGES.

The prepared microsponges were evaluated for the following parameters:

4.1. Particle Size Analysis

Particle size was determined using an optical microscope. The microscope was fitted with a stage micrometer to calibrate the eyepiece micrometer.

Calibration of eyepiece micrometer

1 eyepiece division = Number of division on stage micrometer(y) X Least count Number of eyepiece micrometer division(x)

4.2. Surface morphology of microsponge by Scanning Electron Microscopy (SEM).

The morphology of microsponge formulation was observed by scanning electron microscopy. Prepared microsponges were coated with platinum and studied by SEM (JEOL-JSM 6390, Japan) under vacuum at room temperature.

4.3. Production Yield

The production yield of the microsponges can be obtained by calculating accurately the initial weight of the solid raw materials and the last weight of the microsponge obtained after drying.

$$\textbf{Production yield} = \frac{\text{Practical mass of microsponge}}{\text{Theoretical mass (polymer+drug)}} \times 100$$

4.4. Drug content and Drug entrapment efficiency

About 50 mg of microsponge from all batches were accurately weighed and dissolved in 5ml methanol in 50 ml standard flask then made up to the volume with phosphate buffer pH 7.4. After appropriate dilution, the amount of drug was detected by a UV spectrophotometric method at 272 nm using blank microsponges treated in the same manner. The entrapment efficiency (%) was calculated according to the following equation:

Entrapment efficiency (%) = [Actual drug content in microparticles/Theoretical drug content] X100

4.5. *In vitro* drug release studies

The drug release rate of Econazole nitrate microsponges was studied using dissolution test apparatus by employing paddle method. Accurately weighed samples of microsponges equivalent to 100 mg Econazole nitrate were placed in 900 ml of phosphate buffer pH 7.4 with a paddle speed of 150 rpm and temperature of 37ºC ±0.5ºC was employed. Aliquots (1ml) were withdrawn at hourly intervals up to 6 hrs and assayed spectrophotometrically at 272 nm. The percentage of drug released at various time intervals was calculated.

5. EFFECT OF PHYSICAL PARAMETERS ON FORMULATION OF MICROSPONGE

5.1. Effect of Internal phase on the production of microsponges.

Effects of internal phase viscosity on EE% and particle size were evaluated.

5.2. Effect of polymer type on the production of microsponges.

Effects of different polymers including Ethyl cellulose and Eudragit RS100 on EE% and particle size were evaluated.

5.3 Effect of PVA concentration

In order to know the optimum concentration of emulsifier required for the formation of microsponges, different concentration of PVA 0.75% w/v of the external phase was used which has the formulation code F1-F7 and 50%w/v of external phase having formulation code F8-F12. In each formulation drug to polymer ratio, internal phase volume (10 ml), external phase volume (100 ml) was kept constant. The formed microsponges were observed for their particle size, drug content and entrapment efficiency and production yield.

5.4. Effect of solvent type on the formation of Microsponges

Effects of solvents such as ethanol and methylene chloride on a production of microsponge formulations F1-F12 were evaluated for particle size and EE%.

5.5. Effect of Drug: Polymer Ratio

The drug to polymer ratios 1:1 and 1:2 were taken to prepare different microsponge formulations which having the formulation code F1- F12. In each formulation, the amount of drug (0.5g), solvent (10 ml), PVA (0.75 %) were kept constant for F1-F7 and 0.50% for F8-F12. The formed particles were evaluated for their particle size, drug content and entrapment efficiency.

6. FORMULATION OF TOPICAL CARBOPOL HYDROGEL OF ECONAZOLE NITRATE AND ECONAZOLE NITRATE MICROSPONGES

A clear dispersion of Carbopol was prepared in water using moderate agitation. Econazole nitrate loaded microsponges were dispersed in propylene glycol and ethanol and added to Carbopol dispersion. Various ingredients viz. parabens, sodium Meta bisulfate and disodium edetate were dissolved in water and added to the drug solvent system. Triethanolamine was used to neutralize and volume was made with water. Various ingredients used in a formulation of a gel are mentioned in a table: 4

Table 4: Composition of Econazole nitrate microsponge gel

INGREDIENTS	FORMULATION (%W/W)
Econazole nitrate (equivalent to)	1
Carbopol 934	1
Propylene glycol	20
Ethanol	8
Methanol	0.04
Methyl parabens	0.18
Propyl parabens	0.02
Sodium metabisulfate	0.10
Disodium edentate	0.10
Triethanolamine	Q.S
Purified water q.s	100ml

7. EVALUATION OF TOPICAL GEL FORMULATIONS

Gels were evaluated for their Clarity, pH, Viscosity, Drug content, Skin irritation test and *in-vitro* diffusion studies by using a standard procedure.

7.1. Clarity

The clarity of various formulations was determined by visual inspection under black and white background and it was graded as follows: turbid, clear, very clear.

7.2. Homogeneity

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container for their appearance and presence of any aggregate.

7.3.pH

1gram of a gel was accurately weighed and dispersed in 100 ml of distilled water. The pH of the dispersion was measured by using digital pH meter.

7.4. Viscosity measurement

Brookfield digital viscometer (model DV-I+, Brookfield Engineering Laboratory, USA) was used to measure the viscosity (in cps) of the prepared gel formulations. The spindle number 4 (spindle code S 64) was rotated at 1.5 rpm for the viscosity measurement.

7.5. Drug content

About 1g gel was weighed and transferred to the 100ml volumetric flask. 5 ml methanol was added and shook well. Then made up to the volume using phosphate buffer pH 7.4. Serial dilutions were made using phosphate buffer pH 7.4 to get a concentration within Beer's range. The absorbance was measured spectrophotometrically at 274 nm against blank.

7.6. *In vitro* evaluation of topical gel formulations

The *in vitro* release of Econazole nitrate and Econazole nitrate microsponge from the formulations was studied through cellophane membrane using an open end cylinder. The medium used was freshly prepared phosphate buffer pH 7.4.Cellophane membrane, previously soaked overnight in the dissolution medium, was tied to one end of a specifically

designed glass cylinder (open at both ends and of 5 cm diameter). The formulation was accurately weighed and placed in a membrane and attached to this assembly. The cylinder was attached to the metallic driveshaft and suspended in 50 ml of dissolution medium maintained at 37± 1°C so that the membrane just touched the receptor medium surface. The medium was stirred at 50 rpm using magnetic stirrer. Aliquots, each of 1ml volume, were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium. The aliquots were diluted with the receptor medium and analyzed by UV-Vis spectrophotometer at 272 nm.

7.7. *Ex-vivo* Diffusion Study

Through Rat Abdominal Skin

A phosphate buffer of pH 7.4 was used for *ex -vivo* release as a receptor medium. The pretreated skin of albino rat was used in Franz diffusion cell. The gel sample was applied to the skin and then fixed in between donor and receptor compartment of the diffusion cell. The receptor compartment contained phosphate buffer of pH 7.4. The temperature of diffusion medium was thermostatically controlled at 37±1ºC by surrounding water in a jacket and the medium was stirred by the magnetic stirrer at 100 rpm. The sample at predetermined intervals was withdrawn and replaced by equal volume of fresh fluid. The samples withdrawn were spectrophotometrically estimated at 302nm using phosphate buffer pH 7.4 as a blank.

7.8. Skin irritation test

Irritancy test was carried out to determine the possible localized reaction of the selected formula on the skin since skin safety is of prior consideration for transdermal delivery systems. A single dose of 200mg of the selected medicated formulations (10 mg TX) was applied to the left side of the shaved back of male albino rabbits (1.5±0.5kg) and the right side was considered as control. The control area was further divided into two sub-areas, one receiving the selected formulation unloaded with the drug (positive control) and the other receiving no treatment(negative control).The development of erythema was monitored daily for 6 days. The test was approved by the IAEC (**CPCSEA NO: 1702/EO/C/13/CPCSEA).**

IEAC Reg. No: 62/2013/UCP, RIMSR.

8. STABILITY STUDY OF OPTIMIZED FORMULATION

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light and enables recommended storage conditions, re-test periods and shelf lives to be established In any rational drug design or evaluation of dosage forms for drugs the stability of the active component must be major criterion in determining their acceptance or rejection. Stability is defined as the extent to which throughout its period of storage and use (i.e. its shelf life), the same properties and characteristics that it possessed at the time of its manufacture. Stability testing is performed to ensure that drug products retain their fitness for use until the end of their expiration dates.

Procedure.

Optimized microsponge loaded Carbopol gel formulation was subjected to stability studies at 40ºC and 75% Relative humidity for a period of 45 days. The samples were withdrawn after 45 days and were evaluated for drug content and *in vitro* drug release.

RESULTS AND DISCUSSION:

6.1. PREFORMULATION STUDIES

1. Determination of melting point

The melting point of various samples was determined and shown in Table: 6. Experimental values are identical with the reference values. This indicates the purity of the sample. If any impurity is present, that will cause variation in the melting point of given substances.

Table: 6 Melting points of different samples

Sl.No.	SAMPLES	MELTING POINT
1	Econazole nitrate	about 165 °C, with decomposition
2	Ethyl cellulose	245°C
3	Eudragit RS 100	179°C
4	PVA	188°C
5	Carbopol	Decomposition occurs within 30 minutes at 260°C

2. Solubility

Solubility in different solvents was determined and tabulated in Table: 7

Table 7: Solubility data

Samples	Water	Ethanol	Methanol	Propylene glycol	Acetone
Econazole nitrate	Very slightly soluble	Slightly soluble	Soluble	Slightly soluble	Slightly soluble
Ethyl cellulose	Practically insoluble	Slightly soluble	Soluble	Practically insoluble	Soluble
Eudragit RS 100	Practically insoluble	Slightly soluble	Soluble	Slightly soluble	Soluble
Poly vinyl alcohol	Soluble in hot water	Insoluble	Insoluble	Slightly soluble	Practically insoluble
Carbopol	Soluble	Slightly soluble	Slightly soluble	Slightly soluble	Soluble

3. IR spectroscopy

The IR spectrum of Econazole nitrate is recorded by FTIR spectrometer which was then compared with standard functional group frequencies. The spectrum is shown in figure: 2.

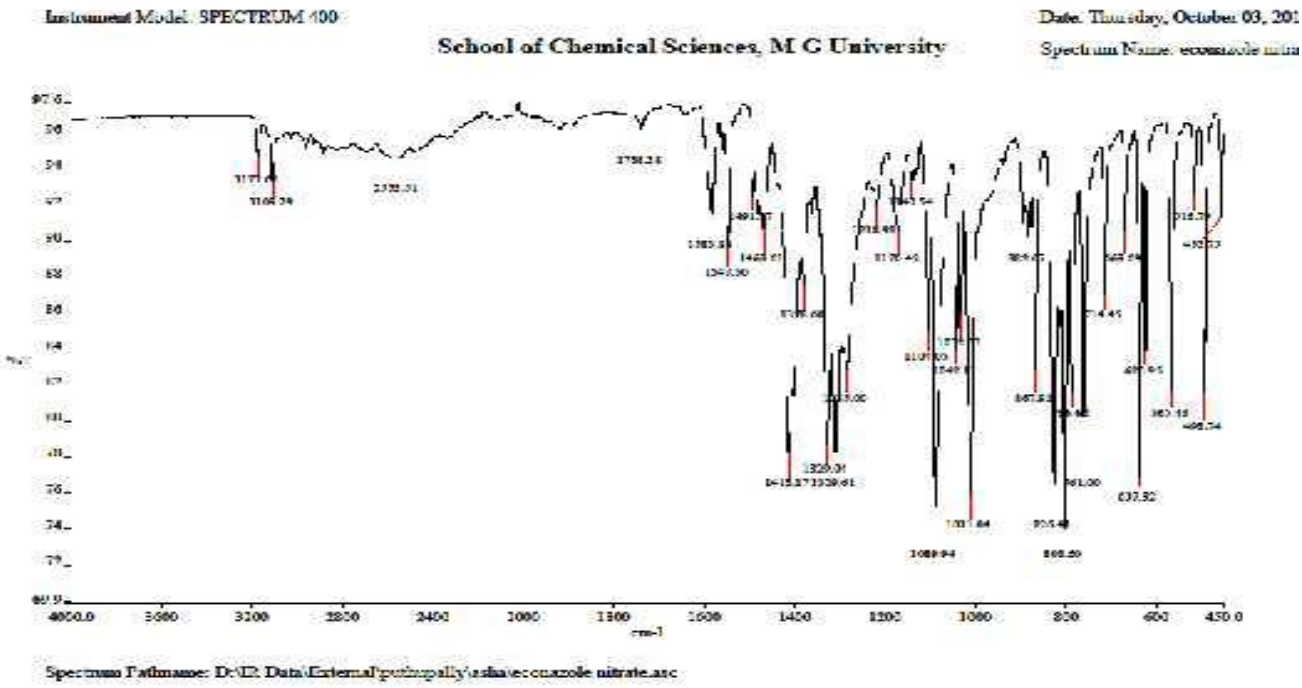


Figure: 2 IR spectrum of Econazole nitrate

Table: 8 Interpretation of IR spectrum of Econazole nitrate

Functional group	Normal range (cm^{-1})	Observed peak (cm^{-1})
Nitrate symmetrical vibrations	1300-1255	1285
C-O-C band(Aromatic ether)	1170-1114	1170.45
C-cl band	1300-1150	1218.98
Hetero aromatic ring stretching vibrations	1600-1300	1309.61
C-C bending vibration	Below 500	493.74

4. Compatibility studies

The FTIR spectrums of Econazole nitrate with different polymers used in the formulation are shown in figures. The major peaks observed in drug spectrum were also observed in spectrums of a drug with the polymer; therefore it could indicate that there was no incompatibility between drug and polymers.

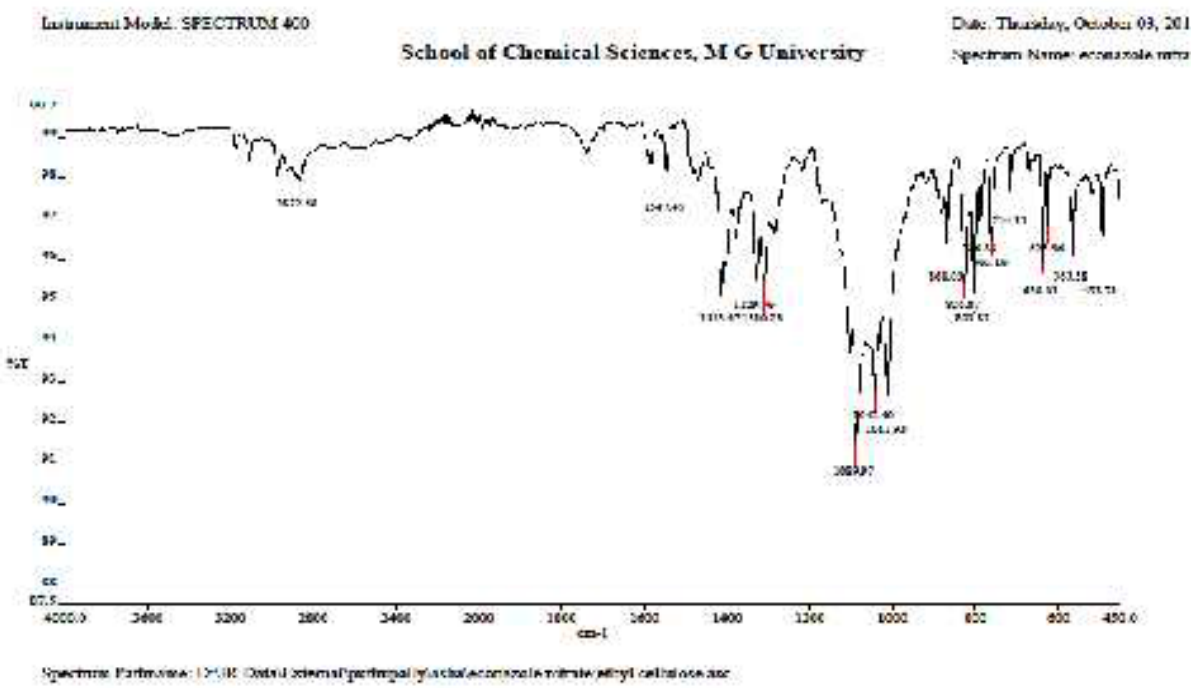


Figure: 3 IR spectrum of Econazole nitrate - Ethylcellulose combination

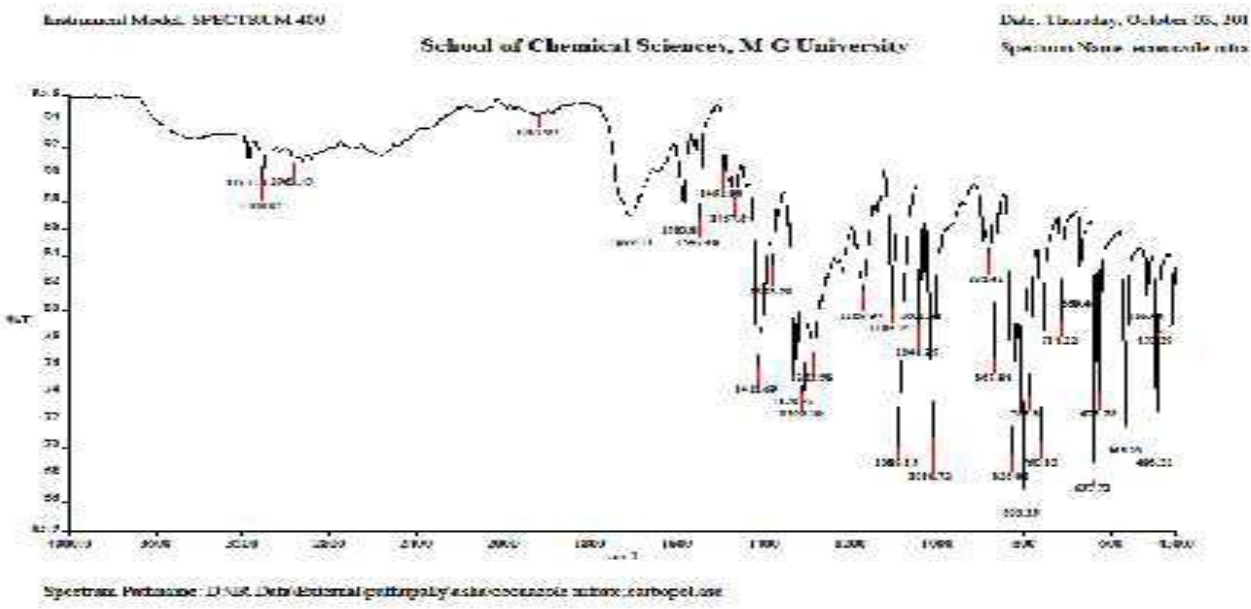


Figure: 5 IR spectrum of Econazole nitrate-Carbopol combination

6.2. PREPARATION OF STANDARD CALIBRATION CURVE

1. Preparation of phosphate buffer pH 7.4

Phosphate buffer was prepared and pH measured using digital pH meter and was found to be 7.4.

2. Determination of λmax of Econazole nitrate

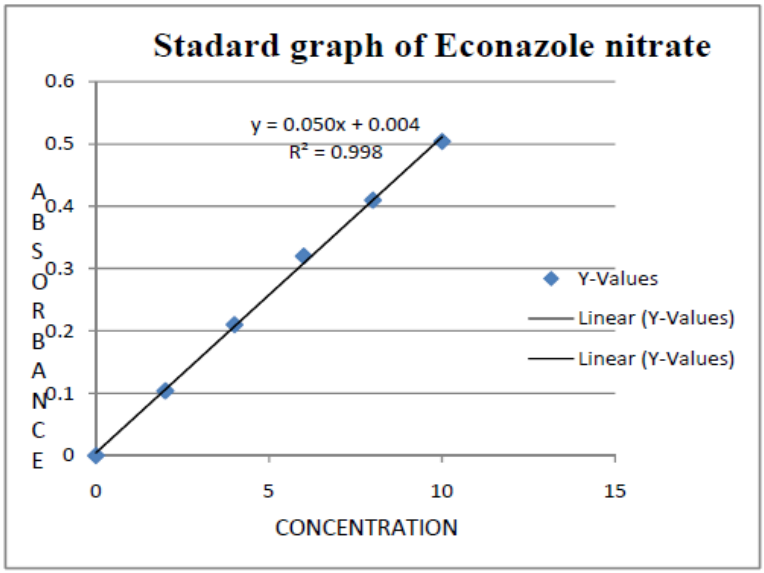
The absorption maximum for Econazole nitrate was found to be 272 nm.

3. Standard calibration curve of Econazole nitrate

The absorbance of Econazole nitrate standard solutions having a concentration range 2-10 µg/ml in phosphate buffer pH 7.4 were shown in table 8. The curve was found to be linear in the range of 2-10 µg/ml at λmax 272 nm. The calculation of the drug content, *in-vitro* release, and stability studies are based on this calibration curve.

Table 9: Standard calibration curve for Econazole nitrate in phosphate buffer pH 7.4 at 272nm

Concentration(µg/ml)	Absorbance
0	0
2	0.104
4	0.210
6	0.320
8	0.410
10	0.504



Graph 1: standard calibration curve for Econazole nitrate in phosphate buffer pH 7.4 at 272nm

6.3. FORMULATION OF ECONAZOLE NITRATE LOADED MICROSPONGES

Econazole nitrate microsponges were prepared by Quasi-emulsion solvent diffusion method by using different polymers like ethyl cellulose and Eudragit RS 100. Prepared formulations were subjected to evaluation.

Table 10: Composition of different microsponge formulation.

Formulation code	Components					
	Econazole nitrate (g)	Ethyl Cellulose (g)	Eudragit RS 100 (g)	Ethanol (ml)	Methylene Chloride (ml)	PVA (%)
F1	0.5	0.5	-	10	-	0.75
F2	0.5	0.5	-	-	10	0.75
F3	0.5	-	0.5	-	10	0.75
F4	0.5	1	-	10	-	0.75
F5	0.5	-	1	10	-	0.75
F6	0.5	1	-	-	10	0.75
F7	0.5	-	1	-	10	0.75
F8	0.5	0.5	-	10	-	0.5
F9	0.5	0.5	-	-	10	0.5
F10	0.5	-	0.5	-	10	0.5
F11	0.5	1	-	10	-	0.5
F12	0.5	1	-	-	10	0.5

6.4. EVALUATION OF ECONAZOLE NITRATE LOADED MICROSPONGES

1. Particle Size Analysis

Particle size was determined using an optical microscope.

Calibration of eyepiece micrometer

1 eyepiece division = (Number of divisions in stage micrometer(y) / Number of divisions in eyepiece micrometer(x)) × 100

Table: 11 Calibration of eye piece micrometer

Number of divisions in stage micrometer	0	6	9
Number of divisions in eyepiece micrometer	10	14	16

One division of eyepiece micrometer (A) = 6/14×10 = 4.28µm

(B) = 9/16×10 = 5.62µm

Average value = (A+B)/2 = 4.95µm

Each division of eyepiece micrometer = **4.95µm.**

Table: 12 Particle size of microsponge preparations

Formulation code	Particle size (µm)
F1	40.786±2.70
F2	44.5±1.13
F3	37.094±3.70
F4	59.004±1.40
F5	38.744±1.41
F6	49.89±2.11
F7	39.80±3.43
F8	92.64±1.41
F9	82.96±1.52
F10	47.124±2.17
F11	103.75±3.85
F12	89.49±2.12

2. Surface morphology

From SEM studies it was found that the samples had porous and almost spherical nature. The pores were induced by the diffusion of the solvent from the surface of the microparticles.

SEM image of a best formulation (F2) in which ethyl cellulose is used as polymer was given in figure –6 and 7

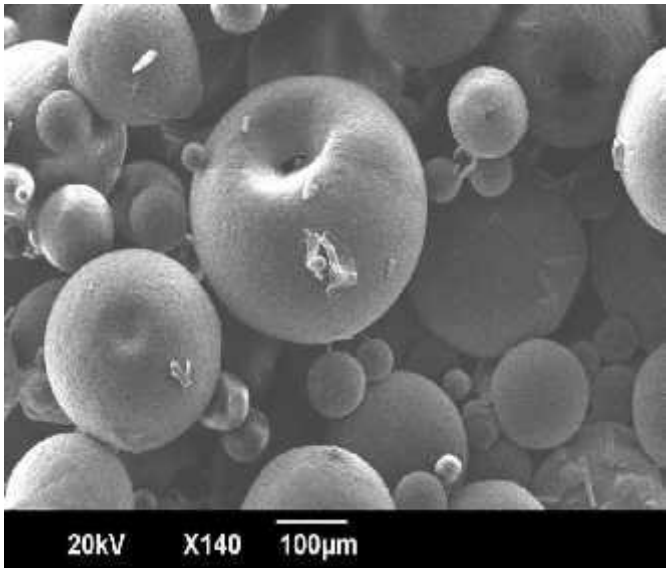


Figure: 6 SEM photograph of microsponge formulation F2 (whole image)

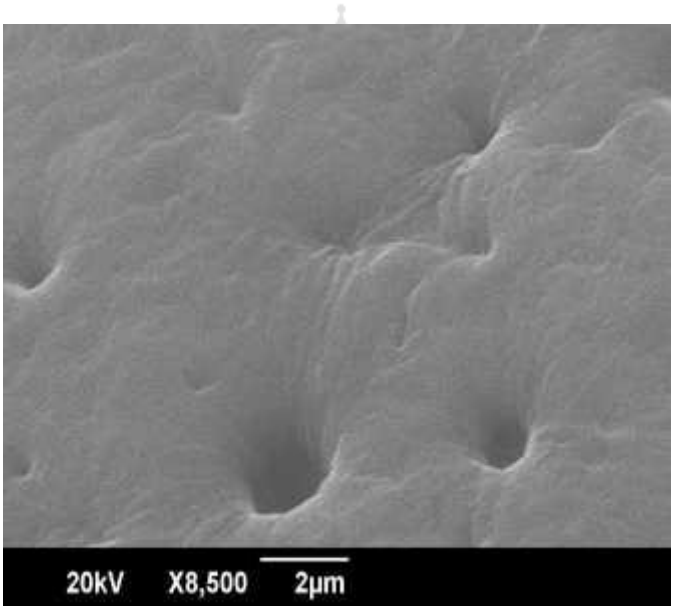


Figure: 7 SEM photograph of microsponge formulation F2 (surface image)

SEM image of formulation F3 in which Eudragit RS 100 used as polymer are shown in

Figure-8 and 9

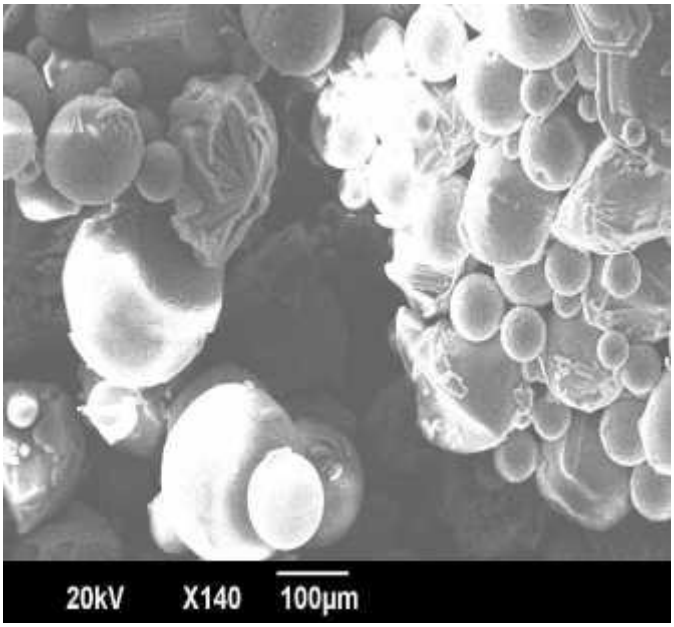


Figure: 8 SEM Photograph of microsponge formulation F3 (whole image)

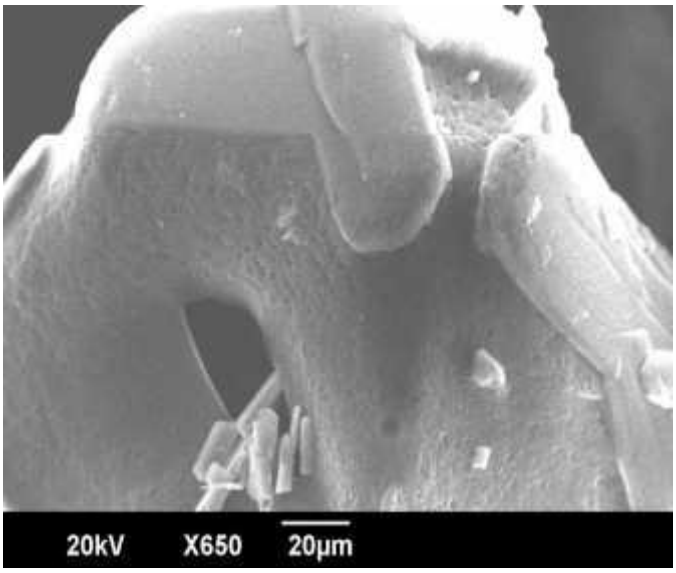


Figure: 9 SEM Photograph of microsponge formulation F3 (surface image)

3. Production Yield

The production yield of all prepared microsponge formulations was determined and the results were given in Table: 13

Table: 13 Production yield of prepare microsponges

Formulation Code	Production yield (%)
F1	51.8
F2	75.6
F3	63.8
F4	77.33
F5	76.67
F6	52
F7	78
F8	61.8
F9	76.2
F10	82.1
F11	88
F12	69.2

4. Drug content and Drug entrapment efficiency

Drug content and Drug entrapment efficiency were determined and shown in table no.14:

Table 14: Drug content and % EE estimation of microsponge formulations.

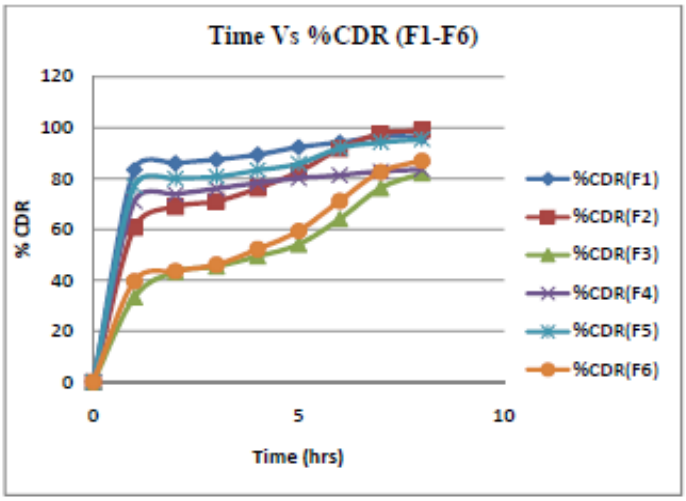
Formulation code	Absorbance	Concentration	Concn*dilution factor /1000 (mg/ml)	Drug content (%)	Theoretical Drug content in 50 mg	%E.E
F1	0.226	4.358	21.79	43.58	25	87.16
F2	0.238	4.605	23.03	46.05	25	92.11
F3	0.224	4.329	21.64	43.29	25	86.58
F4	0.166	3.185	15.92	31.85	16.66	95.59
F5	0.162	3.106	15.53	31.06	16.66	93.21
F6	0.156	2.988	14.94	29.88	16.66	89.66
F7	0.158	3.027	15.135	30.27	16.66	90.85
F8	0.221	4.27	21.35	42.70	25	85.40
F9	0.228	4.408	22.04	44.08	25	88.16
F10	0.198	3.816	19.08	38.16	25	76.32
F11	0.159	3.047	15.233	30.47	16.66	91.44
F12	0.138	2.632	13.16	26.32	16.66	79

5. *In vitro* drug release studies

The release profiles from the Econazole nitrate microsponge formulations were studied. The profiles showed a bi-phasic release with an initial burst effect. In the first hour, about 16.87-83.34 % of the drug was released. Cumulative release for the microsponges after 8 hrs ranged from 59.51-98.89 %. Drug release from the formulations decreased with increase in the amount of polymer in the microsponges. *In vitro*, drug release data of formulations were tabulated on Table15 and 16. The graphical representations are shown below:

Table: 15 %CDR of microsponge formulations F1-F6

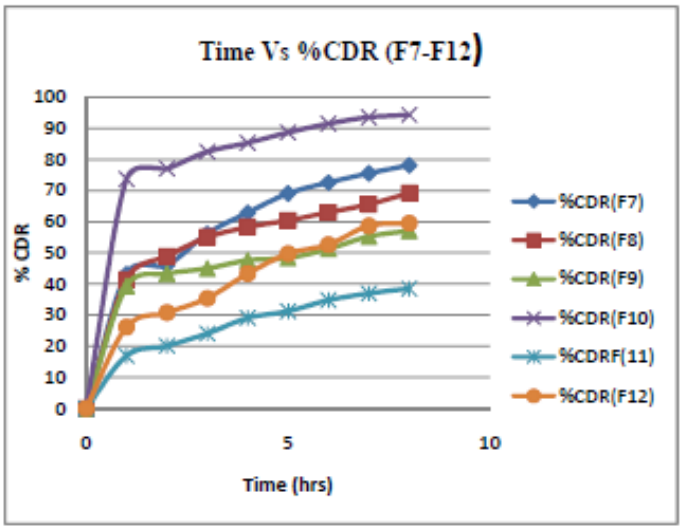
Time (hrs)	% cumulative drug release of microsponge formulations F1-F6					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	83.34	60.61	33.21	70.91	76.95	39.61
2	85.92	68.83	43.21	73.78	79.88	43.56
3	87.40	71.05	45.39	76.03	80.67	46.12
4	89.30	76.10	49.35	78.25	83.25	52.20
5	92.26	82.59	54.03	80.12	85.84	59.38
6	94.13	91.56	64.04	81.28	91.96	71.18
7	96.38	97.72	76.20	82.79	94.20	82.63
8	96.49	98.89	81.96	83.58	95.37	86.97



Graph: 2 Time Vs %CDR for formulations F1-F6

Table: 16 %CDR of microsponge formulations F7-F12

Time (hrs)	% cumulative drug release of microsponge formulations F7-F12					
	F7	F8	F9	F10	F11	F12
0	0	0	0	0	0	0
1	43.18	41.39	39.27	73.70	16.87	26.11
2	45.99	48.88	43.22	77.02	20.07	30.75
3	56.14	55	44.96	82.45	24	35.38
4	62.87	58.26	47.53	85.31	29.03	43.28
5	69.07	60.41	48.31	88.65	31.18	49.71
6	72.57	63	51.21	91.45	34.73	52.63
7	75.53	65.58	55.21	93.54	36.93	58.65
8	78.14	69.20	57.03	94.31	38.42	59.51



Graph: 3 Time Vs %CDR for formulations F7-F12

6.5. EFFECT OF PHYSICAL PARAMETERS ON FORMULATION OF MICROSPONGE

1. Effect of Internal phase on the production of microsponges.

The particle sizes of microsponges were directly proportional to the viscosity of dispersed phase. Hence larger particle size was obtained due to increasing the amount of polymer available per microsponge. With an increase in polymer concentration, viscosity also increased and can impede drug mobility in the droplets which were observed as an increase in the EE%. And also the EE% may be improved simply by a greater proportion of polymer with respect to the amount of drug available.

6.5.2. Effect of polymer type on the production of microsponges.

Concerning the polymer type Ethylcellulose significantly increased the mean particle size when compared to Eudragit RS100. This could probably be due to increasing the viscosity of the dispersed phase containing EC, as the formed globules can be hardly divided into smaller particles, hence larger droplets were formed and the mean particle size was increased.

When compared with Eudragit RS100, Ethylcellulose significantly shows improved drug entrapment efficiency. This may be due to increased viscosity of the internal phase containing the ethyl cellulose polymer, reducing the drug mobility outside the formed droplets, and hence entrapping larger amount of Econazole nitrate.

Table: 17 Effect of polymer type on particle size and EE%

POLYMER TYPE	Ethyl cellulose	Eudragit RS 100
EE (%)	79 to 95.59	76.32 to 92.11
Particle size(µm)	40.786 to 103.75	37.094 to 47.124

3. Effect of PVA concentration on the production of microsponges.

PVA concentration has a key role to play in the preparation of microsponges. Entrapment efficiency of Econazole nitrate increases with increase in PVA concentration due to the nonionic nature of the emulsifier. And also there was a reverse proportionality between the PVA concentration and the mean particle size. This may due to decreasing the surface tension of the continuous phase upon increasing the surfactant concentration which results in the reduction of the particle size.0.75% PVA solution provided good microsponges.

Table: 18 Effect of PVA concentration on particle size and EE%

%PVA Concentration	0.50	0.75
EE (%)	76.32 to 91.44	86.58 to 95.59
Particle size(µm)	47.124 to 103.75	37.094 to 59.0004

5. Effect of a drug-polymer ratio in the production of microsponges.

Entrapment efficiency may be improved simply by the greater proportion of polymer with respect to the amount of drug available. And also increasing the polymer fraction

significantly increased the particle size. This may be due to increase in viscosity hindered the breaking of an emulsion into smaller droplets resulting in microsponges with larger particle size.

Table: 20 Effect of drug polymer ratio on particle size and EE%

Drug polymer ratio	1:1	1:2
EE (%)	76.32 to92.11	79 to 95.59
Particle size(µm)	37.094 to 92.64	38.744 to103.75

6.6. FORMULATION OF TOPICAL CARBOPOL HYDROGEL OF ECONAZOLE NITRATE AND ECONAZOLE NITRATE MICROSPONGES

Formulations of Econazole nitrate and Econazole nitrate microsponge gel was developed using carbopol 934, alcohol, methylparaben, propylparaben, propylene glycol, Triethanolamine, and water. Carbopol 934 was used as polymer; alcohol as a penetration enhancer; methylparaben and propylparaben as preservatives, Triethanolamine as Ph balancer and water as a vehicle.

6.7. EVALUATION OF TOPICAL GEL FORMULATIONS

Gels were evaluated for their Clarity, pH, Viscosity, Drug content, Skin irritation test and *in-vitro* diffusion studies, *ex-vivo* diffusion studies by using a standard procedure.

1. Clarity

The clarity of various formulations was determined by visual inspection under black and white background and it was found to be clear.

2. pH

The pH value of developed formulation of Carbopol gel (F2) was found to be 6.4

3. Homogeneity

Developed formulation (F2) showed good homogeneity with an absence of lumps. The developed preparation is much clear and transparent.

4. Viscosity measurement

The viscosity of various formulated Econazole nitrate gels was measured using Digital Brookfield viscometer. The rheological behavior of all formulated gel was studied. In-gel system, consistency depends on the ratio of solid fraction, which produces the structure to a liquid fraction.

Table: 21 Evaluation parameter of gel formulation

Formulation	Clarity	pH	Homogeneity	Viscosity
Pure Econazole Nitrate gel	Clear	6.8	Good	58770
EN microspongic gel(F2 gel)	Clear	6.4	Good	59749

5. Drug content

After formulating Econazole nitrate gel and F2 microspongic gel, the drug content of the formulated gels was estimated by UV spectrophotometer at λmax 272nm. The percentage drug content of all prepared gel formulations was found to be 97.93% and99.85%.

Table: 22 Drug content of formulated topical gel

Formulation	Abs.	Conc (µg/ml)	Conc*dilution factor / 1000 (mg/ml)	Weight taken (g)	Theoretical drug content (mg)	%drug Content
Pure Econazole nitrate gel	0.533	10.42	5.21	0.532	5.32	97.93
Econazole nitrate MS gel	0.538	10.52	5.26	0.527	5.27	99.85

6. *In vitro* evaluation of topical gel formulations

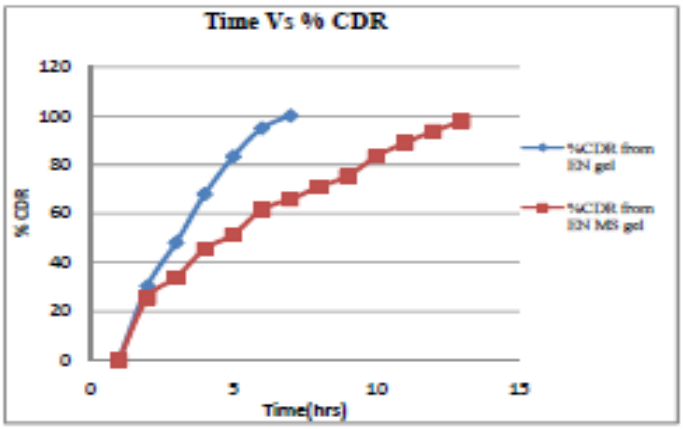
The release profiles obtained for the Econazole nitrate microsponge formulation & Econazole nitrate are represented in Table 23 and Table24. The profiles showed a bi-phasic release with an initial burst effect. In the first hour, about 25.53% of the drug was released. Cumulative release for the microsponges after 12 hrs about 97.90 %.

Table: 23 *In vitro* drug release from Econazole nitrate gel

Time (hrs)	Absorbance	Conc. (µg/ml)	Amount in 1ml(mg)	Amount in 20 ml (mg)	Correction factor	Cumulative release	% Release
0	0	0	0	0	0	0	0
1	0.043	0.76	0.076	1.52	0.076	1.596	30.34
2	0.064	1.17	0.117	2.34	0.193	2.533	48.15
3	0.086	1.61	0.161	3.22	0.354	3.574	67.94
4	0.102	1.92	0.192	3.84	0.546	4.386	83.38
5	0.113	2.14	0.214	4.28	0.76	5.04	95
6	0.114	2.16	0.216	4.32	0.976	5.296	100.06

Table: 24 *In vitro* drug release from EN microsponge gel

Time (hrs)	Absorbance	Conc (µg/ml)	Amount in 1ml (mg)	Amount in 20ml (mg)	Correction factor	Cumulative release	% Release
0	0	0	0	0	0	0	0
1	0.037	0.639	0.0639	1.279	0.0639	1.3429	25.53
2	0.046	0.817	0.0817	1.634	0.1456	1.7796	33.83
3	0.059	1.07	0.107	2.14	0.2526	2.3926	45.49
4	0.064	1.17	0.117	2.34	0.3696	2.7096	51.51
5	0.074	1.369	0.1369	2.738	0.5065	3.2445	61.68
6	0.076	1.41	0.141	2.82	0.6475	3.4675	65.92
7	0.079	1.47	0.147	2.94	0.7945	3.7345	71
8	0.081	1.51	0.151	3.02	0.9455	3.9655	75.39
9	0.088	1.64	0.164	3.28	1.1095	4.3895	83.45
10	0.091	1.7	0.17	3.4	1.2795	4.6795	88.96
11	0.093	1.74	0.174	3.48	1.4535	4.9335	93.79
12	0.094	1.76	0.176	3.52	1.6295	5.1495	97.90



Graph: 4 *In vitro* drug release profile from pure EN gel and F2 microsponge gel

7. *Ex-vivo* diffusion study

Through Rat Abdominal Skin

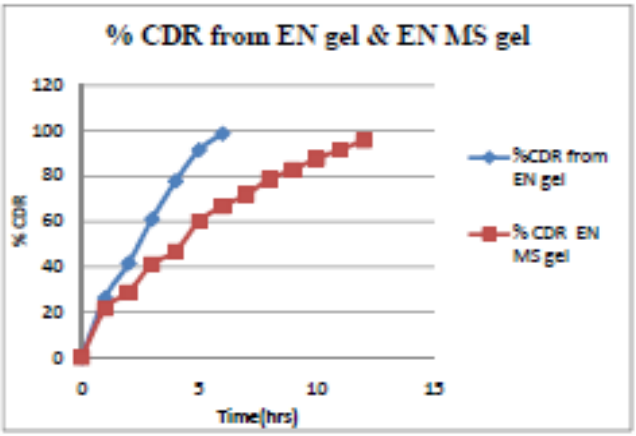
The release profile obtained for Econazole nitrate microsponge gel and pure EN gel formulations through rat abdominal skin were shown in Table:25 and Table26 .In the first hour, about 21.55% of the drug was released. Cumulative release of a gel after 12 hrs about 95.71 %.

Table: 25 *Ex vivo* drug release from pure EN gel

Time (hrs)	Absorbance	Conc. (µg/ml)	Amount in 1ml (mg)	Amount in 20 ml(mg)	Correction factor	Cumulative release	% Release
0	0	0	0	0	0	0	0
1	0.038	0.659	0.0659	1.318	0.0659	1.3839	26.30
2	0.056	1.01	0.101	2.02	0.1669	2.1869	41.57
3	0.078	1.45	0.145	2.9	0.3119	3.2119	61.06
4	0.096	1.8	0.18	3.6	0.4919	4.0919	77.79
5	0.109	2.06	0.206	4.12	0.6979	4.8179	91.59
6	0.113	2.14	0.214	4.28	0.9119	5.1919	98.71

Table: 26 *Ex vivo* drug release from EN microsponge gel

Time (hrs)	Absorbance	Conc. (µg/ml)	Amount in 1ml (mg)	Amount in 20 ml(mg)	Correction factor	Cumulative release	% Release
0	0	0	0	0	0	0	0
1	0.032	0.54	0.054	1.08	0.054	1.134	21.55
2	0.04	0.698	0.0698	1.396	0.1238	1.5198	28.89
3	0.054	0.975	0.0975	1.95	0.2213	2.1713	41.28
4	0.059	1.07	0.107	2.14	0.3283	2.4683	46.93
5	0.073	1.35	0.135	2.7	0.4633	3.1633	60.13
6	0.078	1.45	0.145	2.9	0.6083	3.5083	66.69
7	0.081	1.51	0.151	3.02	0.7593	3.7793	71.84
8	0.086	1.61	0.161	3.22	0.9203	4.1403	78.71
9	0.088	1.64	0.164	3.28	1.0843	4.3643	82.97
10	0.09	1.68	0.168	3.36	1.2523	4.6123	87.68
11	0.091	1.7	0.17	3.4	1.4223	4.8223	91.67
12	0.092	1.72	0.172	3.44	1.5943	5.0343	95.71



Graph: 5 *Ex vivo* drug release profile from EN gel and F2 microsponge gel

8. Skin irritation test

The absence of skin irritation in gel formulation is acceptable to a patient. Skin irritation test was performed on 3 healthy Albino rabbits, but there was no erythema, edema or reddening of the skin. F2 microsponge gel formulation was found to be free from irritation. This observation indicates acceptability of these gels for topical use.

6.8. KINETIC MODELING

The results obtained of *in-vitro* release studies were attempted to fit into various mathematical models as follows:

- **Zero order rate kinetics** Cumulative percent drug released Vs. Time
- **First order kinetics** Log Cumulative percent drug retained Vs. Time
- **Higuchi matrix** Cumulative percent released Vs. a square root of Time
- **Korsmeyer Peppas** Log cumulative percent drug released Vs. Log Time

1. Release kinetics of Econazole nitrate microsponge gel

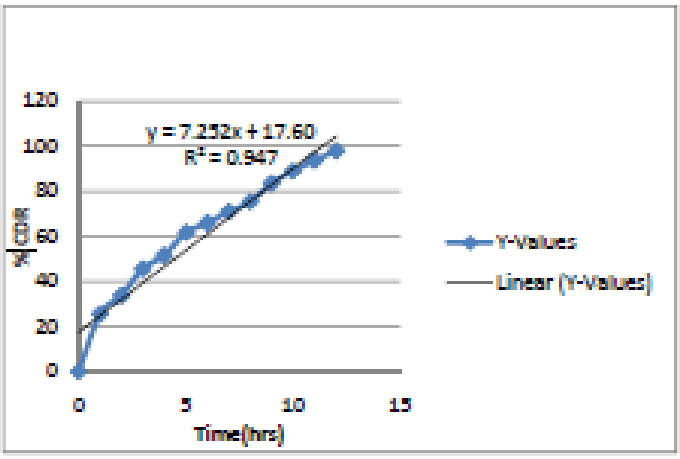
Data obtained from *in-vitro* study was fitted into various kinetic models such as Zero order, First order, Higuchi matrix, and Peppas**.** The regression coefficient (r) of zero order, first order, Higuchi matrix, and Peppas are tabulated in Table: 27.

Table: 27 Kinetic release profile of Econazole nitrate loaded microsponge (F2) containing Carbopol gel

Time (hr)	Root time	Log time	% CDR	Log %CDR	% drug retained	Log % drug Retained
0	0		0		100	2
1	1	0	25.53	1.41	74.47	1.87
2	1.41	0.301	33.83	1.53	66.17	1.82
3	1.73	0.4771	45.57	1.66	54.43	1.74
4	2	0.602	51.51	1.71	48.49	1.69
5	2.24	0.699	61.68	1.79	38.32	1.58
6	2.45	0.7782	65.92	1.82	34.08	1.53
7	2.65	0.8451	71	1.85	29	1.46
8	2.83	0.9031	75.39	1.88	24.61	1.39
9	3	0.9542	83.45	1.92	16.55	1.22
10	3.16	1	88.96	1.95	11.04	1.04
11	3.32	1.0414	93.79	1.97	6.21	0.79
12	3.46	1.0792	97.9	1.99	2.1	0.32

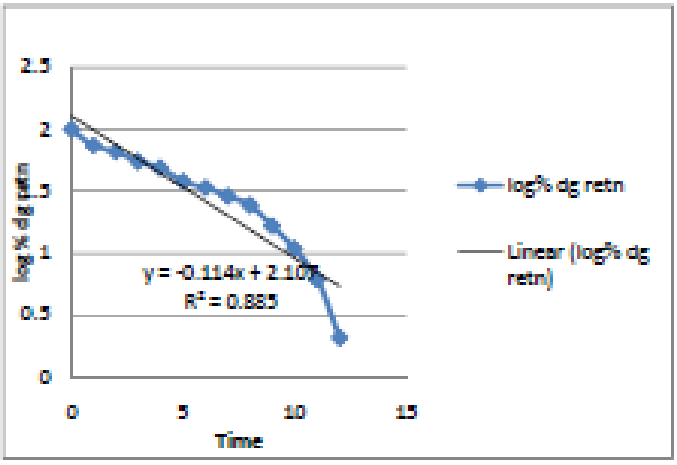
Plots of Zero order, First order, Higuchi matrix, and Peppas are depicted in Graph 7-10**.** The regression coefficient (r) of zero order, first order, Higuchi matrix, and Peppas are tabulated in Table: 28.

% Cumulative drug released Vs time



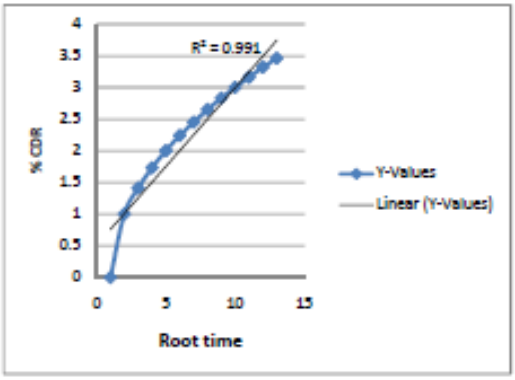
Graph 6: Kinetic release profile of Econazole nitrate loaded microsponge (F2) containing Carbopol gel - Zero order.

Log % drug retained Vs time



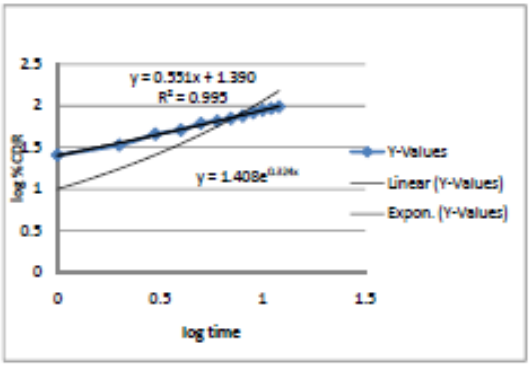
Graph 7: Kinetic release profile of Econazole nitrate loaded microsponge (F2) containing Carbopol gel- First order.

% Cumulative drug release Vs Root time



Graph 8: Kinetic release profile of Econazole nitrate loaded microsponge (F2) containing Carbopol gel - Higuchi matrix.

Log time Vs log % cumulative drug release



Graph 9: Kinetic release profile of Econazole nitrate loaded microsponge (F2) containing Carbopol gel - Peppas model.

Table: 28 Model fitting for the release profile of Econazole nitrate microsponge gel from formulation F2 by using 4 different models.

Formulation	Zero order R	First order R	Higuchi matrix R	Korsmeyer-Peppas		Best fit model
				R	N	
Microspongic gel of F2	0.947	0.885	0.991	0.995	0.324	Higuchi matrix

Based on highest regression value, the best fit model for F2 microsponge gel was observed as Higuchi matrix model. The n value obtained from Peppas model was found to be 0.324; this reveals that F2 microsponge gel formulation follows Fickian diffusion.

6.9. STABILITY STUDY OF OPTIMIZED FORMULATION

Prepared F2microsponge containing topical gel formulation was subjected to the stability study at elevated temperature (40ºC) and relative humidity 75% for a period of 45 days. A sample was withdrawn after 45 days and was evaluated for parameters such as drug content and *in-vitro* drug release. The observations are shown in Table 29. Formulation showed the slight decrease in drug content of Econazole nitrate at 40ºC after 45 days of storage.The *in-vitro* drug release from the F2 microsponge formulation was also decreased after stability study period. This may be due to the decrease in the relative drug content. From the stability studies, it was confirmed that Econazole nitrate microsponge containing topical gel formulation remained stable at 40ºC and 75% relative humidity.

Table: 29 Stability study of optimized formulation

Formulation	Storage condition	Before stability study		After stability Study	
		Drug content	% Drug release	Drug content	% Drug release
F2 microsponge gel	40·C and 75% relative humidity	99.85	97.90	95.24	94.12

CONCLUSION:

Microsponge based delivery system has been developed using different polymers such as Ethyl cellulose and Eudragit RS 100 by quasi-emulsion solvent diffusion method to provide a sustained release medication for topical delivery of Econazole nitrate. From the study, the following conclusions can be drawn.

The drug entrapment efficiency and the size of the prepared microsponges were affected by the drug: polymer ratio, polymer type, solvent type, and the PVA concentration.

1. Physical characterization showed that entrapment efficiency (EE %) and mean particle size were increased with increasing polymer fraction.

2. EC significantly increased the EE% and mean particle size when compared to Eu RS100.

3. With an increase in PVA concentration EE% increased and mean particle size decreased. The minimum concentration of PVA required to produce microsponges was found to be 0.50% w/v.

4. Regarding solvent type ethanol shows increased EE% and particle size as compared to methylene chloride.

- From SEM studies it was found that the samples had porous and almost spherical nature.

- The study results showed that out of 12 formulations, optimum formulation (F2) consisted of Ethylcellulose and Econazole nitrate in 1:1 ratio, methylene chloride as solvent and 0.75% PVA as emulsifier, shows better release profile(98.89% release at 8th hour), Entrapment efficiency(92.11%) and optimum particle size(44.5±1.13µm).

- The release profile of the Econazole nitrate microsponges loaded Carbopol topical gel was compared with that of the pure Econazole nitrate gel. From the results, it can be concluded that the microsponge gel could sustain the drug release over a period of 12 hours when compared to the 99-100% release at the end of 6 hours from the pure Econazole nitrate gel.

- By model fitting of the data obtained by drug release profile we can conclude that the mechanism of drug release from F2 microsponge gel was Fickian and it followed Higuchi matrix model.

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