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## STANDARDIZATION OF PROCESS PARAMETERS INVOLVED ERYTHROMYCIN MICROSPONGES BY QUASSI EMULSION SOLVENT DIFFUSION METHOD

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### ABSTRACT

The aim of present work is to prepare microsponges of Erythromycin using Ethyl cellulose as polymer. Erythromycin has bacteriostatic activity which inhibits growth of bacteria. They mainly act by binding to the 50s subunits of bacteria, 70s r-RNA complex, and protein synthesis. Erythromycin is also used topically to treat acne. They are used to treat moderate to severe inflammatory acnes or acne that isn't getting better with other treatments. Erythromycin works to treat acne by reducing the amount of acne causing bacteria called "propionibacteria" acnes on the skin, it also lessens inflammation and redness. Erythromycin is easily inactivated by the gastric environment and produce gastric disturbances such as diarrhoea, nausea, abdominal pain and vomiting. Erythromycin microsponges were prepared using quassi emulsion solvent diffusion method. In order to standardize the microsp sponge formulation, factors affecting the physical properties of microsponges were determined. The SEM and DSC studies were carried out to study shape, morphology of microsponges and thermal analysis respectively Thus it was concluded that erythromycin can be formulated as microsp sponge gel that can release the drug up to 8hrs with reduced side effects.

**Keywords:** Acne, Erthromycin, Microsponges, Gel.

### INTRODUCTION

#### Microsp sponge Drug Delivery System

In recent years, there has been a considerable emphasis given to the development of novel microsp sponge based on the drug delivery systems, in order to modify and control the release behaviour of the drugs. By incorporation into a carrier system, it is possible to alter the therapeutic index and duration of the activity of drugs [1].

Microsponges are microscopic spheres capable of absorbing skin secretions, therefore reducing oiliness from the skin. Spherical particles composed of clusters of even tinier spheres are capable of holding four times their weight in skin secretions. Microsp sponge particles are extremely small, inert, indestructible spheres that do not pass through the skin. Rather, they collect in the tiny nooks and crannies of the skin and slowly release the entrapped drug, as the skin needs it. The microsp sponge system can prevent excessive accumulation of ingredients within the epidermis and the dermis. Potentially, the microsp sponge system can significantly reduce the irritation of effective drugs without reducing their efficacy. The empty spheres are then washed away with the next cleansing. The size of the microsponges can be varied, usually from 5 - 300  $\mu\text{m}$  in diameter,

depending upon the degree of smoothness or after-feel required for the end formula. Although the microsp sponge size may vary, a typical 25  $\mu\text{m}$  sphere can have up to 250000 pores and an internal pore structure equivalent to 10 ft in length, providing a total pore volume of about 1 ml/g. This resulted in a large reservoir within each microsp sponge, which can be loaded with up to its own weight of active agent. The microsp sponge particles themselves are too large to be absorbed into the skin and this adds a measure of safety to these microsp sponge materials [2].

The drugs which have been explored in microsp sponge delivery system are namely, Ibuprofen, Benzoylperoxide, Mupirocin, Ketoconazole, Ketoprofen [3,4,6-9].

In this study an attempt is made to combine the beneficial effects of microsponges along with the gel formulation. The study focuses on development of microsp sponge system of erythromycin, which is further incorporated into a gel for topical treatment of acne.

### MATERIALS AND METHODS

#### Materials

Erythromycin was received as gift sample from Micro Labs pharmaceutical Pvt. Ltd. Bangalore (India), Ethyl cellulose, ethanol, methanol, dichloromethane, Carbopol 450, was obtained from SD fine chemicals Ltd., Mumbai (India). All other chemical and reagents were of analytical grade.

### Preparation of Microsponges

#### Formulation of Microsponges of Erythromycin

Microsponges were prepared by Quasi emulsion solvent diffusion technique which requires two immiscible phases, internal and external phase with a surfactant which aids formation of an emulsion by reducing the interfacial tension.

#### Method of preparation of Microsponges

The required amount of erythromycin and ethyl cellulose were weighed accurately and dissolved in 20ml of DCM: Methanol (1:1) under sonication, this constitutes the internal phase. The surfactant PVA was weighed accurately and dissolved in distilled water at 60<sup>0</sup> C, which is the external phase. The external phase was allowed to cool to attain room temperature. The internal phase was taken in a burette and added drop wise to the external phase. During addition the emulsion was stirred using a Remi mixer at 800 rpm. Mixing was continued for about 2hrs to achieve complete diffusion of the external phase. The microsponges formed were filtered and dried in hot air oven at 40<sup>0</sup> C for a period of 12 hrs [5-9].

#### 1. Standardization of formulation and process parameters

In order to obtain the most satisfactory microsp sponge formulation, different formulation parameters such as concentration of retardant material, surfactant, and volume of external phase were standardized. The effect of stirring speed was also studied.

#### 2. Standardization of concentration of retardant material

Microsponges were prepared with varying concentration of ethyl cellulose (1%, 2%, 3%, 4%, 5% and 6%) in the internal phase, while other formulation parameters were kept constant. The resulted microsponges were washed with 25ml of distilled water. The minimum concentration of retardant material required for the formation of microsponges was determined by observing the microsponges for their physical characteristics (Table 1).

#### 3. Standardization of surfactant concentration

In order to know the optimum concentration of surfactant required for the formation of microsponges different concentration of polyvinyl alcohol i.e., 0.25%, 0.5 %, 0.75%, 1.0%, 1.5% and 2% w/v of the external phase were used. Polymer concentration was 3% and stirring speed was 800 rpm. The microsponges formed were observed for their physical characteristics (Table 2).

#### 4. Standardization of volume of external phase

Different volumes of external phase viz., 30 ml, 60 ml, 90 ml and 120 ml were used in order to standardize the volume of external phase. During this study, the polymer concentration was 3%, volume of internal phase was 20 ml with ratio of Dichloromethane: Methanol being 1:1 and stirring speed was 800 rpm. The surfactant concentration was 0.75% w/v (Table 3). The product was evaluated for physical characteristics, particle size and free drug content.

#### 5. Effect of stirring speed on the formation of microsponges

In order to evaluate the effect of stirring speed on the formation of microsponges, micro particles were prepared with different RPM of 500, 800, 1200 and 1500 keeping all the formulation parameters constant as shown in table 4.

#### 6. Evaluation of Drug Loaded Microsponges

The prepared microsponges were evaluated for the following parameters

##### a) Drug content

Microsponges equivalent to 100 mg of erythromycin were dissolved and made up to the mark in 100 ml volumetric flask with methanol, further 10 ml was diluted to 100 ml with methanol and the final dilution were made using distilled water to get a concentration within Beer's range. The absorbance was measured spectrophotometrically at 264 nm using blank microsponges treated in the same manner as sample.

##### b) Drug loading efficiency

The loading efficiency (%) was calculated according to the following equation [7].

$$\text{Loading efficiency} = \frac{\text{Actual ERY content in microsponges}}{\text{Theoretical ERY content}} \times 100$$

##### c) Percentage yield

The percentage yield of the microsp sponge was determined by calculating accurately the initial weight of the raw materials and the last weight of the microsp sponge obtained [7].

$$\text{Percentage yield} = \frac{\text{Practical weight of microsponges}}{\text{Theoretical wei}} \times 100$$

##### d) Particle size and size distribution

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 = 10µm

$$C = (SM \times 100) / EM$$

Where C = correction factor

SM = Reading of stage micrometer which coincides with reading of eye-piece micrometer (EM).

The particle diameters of around 100 microspheres were measured at random with optical microscope.

The average particle size was determined using the equation  $D(\text{mean}) = \sum nd / \sum n$

Where  $n$  = No of microspheres observed,  $d$  = mean size range.

## 7. Compatibility Studies

### a) FTIR studies

Instrument used was Shimadzu FTIR-8700 spectrophotometer. In this study, potassium bromide disc method was employed. IR study of pure drug, physical mixtures and polymer were done. The powdered sample was intimately mixed with dry powdered potassium bromide. The mixture was then compressed into transparent disc under high pressure using special dies. The disc was placed in IR spectrophotometer using sample holder and spectrum was recorded from 4000 to 500  $\text{cm}^{-1}$  [7].

### b) DSC studies

Differential scanning calorimetry studies were conducted using DSC Q2000. Sample was weighed (8.00-

10.00  $\pm$  0.5 mg) and placed in sealed aluminium pans. The coolant was liquid nitrogen. The samples were scanned at 100  $^{\circ}\text{C}/\text{min}$  from 200 to 250 $^{\circ}\text{C}$ . DSC thermo grams of pure erythromycin, physical mixtures and polymer were taken [11-13].

### c) (SEM) Scanning electron microscopy

Scanning electron microscopy (SEM) is an electron optical imaging technique that provides photographic images and elemental information. SEM is useful for characterizing the morphology and size of microscopic specimens with particle size as low as  $10^{-10}$  to  $10^{-12}$  grams. The sample was placed in an evacuated chamber and scanned in a controlled pattern by an electron beam. Interaction of the electron beam with the specimen produces a variety of physical phenomena that, when detected, are used to form images and provide elemental information about the specimens. Scanning electron microscopy (JSM 840 A) was used to study the surface morphology of the microsphere of formulation I. The samples were analyzed after they were gold sputtered using 25 nm gold film thickness [7-10].

**Table 1. Concentration of retardant material in the internal phase**

Formulation	F1	F2	F3	F4	F5	F6
Erythromycin(gm)	0.3	0.3	0.3	0.3	0.3	0.3
Ethyl cellulose (%)	1	2	3	4	5	6
Internal phase(ml)	20	20	20	20	20	20
External phase(ml)	90	90	90	90	90	90
PVA (%)	0.75	0.75	0.75	0.75	0.75	0.75

**Table 2. Standardization of surfactant concentration**

Formulation	F7	F8	F9	F10	F11
Erythromycin (gm)	0.3	0.3	0.3	0.3	0.3
Ethyl cellulose (%)	3	3	3	3	3
Internal Phase (ml)	20	20	20	20	20
External phase (ml)	90	90	90	90	90
PVA (%)	0.25	0.5	0.75	1.0	1.5

**Table 3. Standardization of volume external phase**

Formulation	F12	F13	F14	F15
Erythromycin (gm)	0.3	0.3	0.3	0.3
Ethyl cellulose (%)	3	3	3	3
Internal Phase (ml)	20	20	20	20
External phase (ml)	60	90	120	150
PVA (%)	0.75	0.75	0.75	0.75

**Table 4. Standardization of stirring speed**

Formulation	F16	F17	F18	F19
Erythromycin (gm)	0.3	0.3	0.3	0.3
Ethyl cellulose (%)	3	3	3	3
Internal phase (ml)	20	20	20	20
External phase (ml)	90	90	90	90
PVA (%)	0.75	0.75	0.75	0.75
RPM	500	800	1200	1500

**Table 5. Effect of concentration of ethyl cellulose on the internal phase**

Sl. No	Formulations	Formation of microsponges	Physical appearance	Particle size in $\mu\text{m}$	Production yield (%)
1	F1	+	Irregular	150-210 $\pm$ 7.35	64.4
2	F2	+	Spherical but collapses after some time	210-260 $\pm$ 6.95	72.4
3	F3	+	Spherical	260-375 $\pm$ 4.82	80.3
4	F4	+	Spherical	360-435 $\pm$ 3.78	84.7
5	F5	+	Spherical rigid	420-470 $\pm$ 3.76	88.5
6	F6	+	Spherical rigid	470-570 $\pm$ 2.67	90.5

+ indicates formation of microsponges.

#### 4) Effect of surfactant concentration

**Table 6. Effect of surfactant concentration**

Sl No	Formulations	Physical appearance	Mean particle size ( $\mu\text{m}$ )	Characteristics of emulsion
1	F7	Irregular	170- 220 $\pm$ 7.35	Irregular
2	F8	Uniform	210- 280 $\pm$ 6.91	Uniform
3	F9	Uniform	290- 350 $\pm$ 3.76	Uniform
4	F10	Irregular	350- 440 $\pm$ 2.48	Foaming
5	F11	Irregular	425- 480 $\pm$ 3.61	Foaming

#### 5) Effect of volume of external phase on microsponges

**Table 7. Effect of volume of external phase**

Sl. no	Formulations	Physical appearance	Particle size in $\mu\text{m}$	Drug content (%)	% entrapment
1	F12	Irregular, non-uniform	170-260 $\pm$ 4.56 $\mu\text{m}$	76.8	67.33
2	F13	Spherical, uniform	280-360 $\pm$ 3.82 $\mu\text{m}$	89.3	84.77
3	F14	Spherical, uniform	380-460 $\pm$ 4.39 $\mu\text{m}$	89.2	82.55
4	F15	Spherical, uniform	490-580 $\pm$ 5.67 $\mu\text{m}$	78.2	72.63

#### 6) Effect of process parameters

##### a) Effect of stirring speed on formation of microsponges

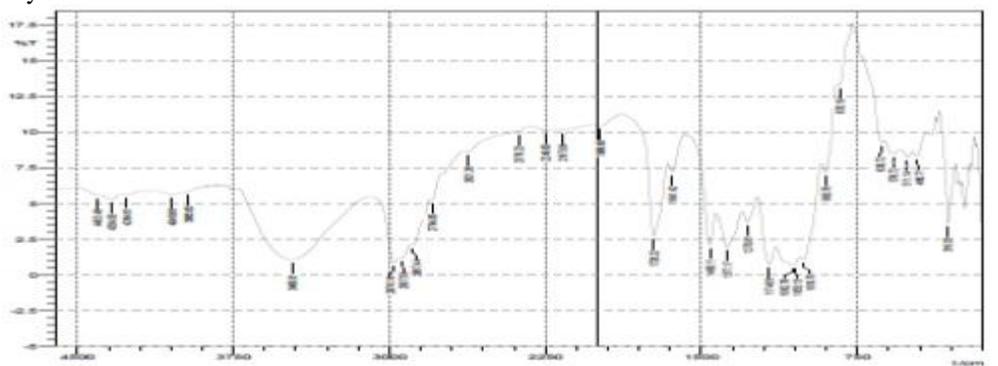
**Table 8. Effect of stirring speed**

Sl. No	Formulations	Physical appearance of microsponges	Mean particle size ( $\mu\text{m}$ )	Drug content	% Entrapment
1	F16	No microsponges were formed	-	-	-
2	F17	Uniform	290-430 $\pm$ 3.68	75.49	67.33
3	F18	Uniform	170-250 $\pm$ 4.34	91.33	88.77
4	F19	Uniform	100-180 $\pm$ 2.75	72.41	65.34

## RESULTS

**Fig 1. FTIR of physical mixture of pure erythromycin, ethyl Cellulose and PVA**

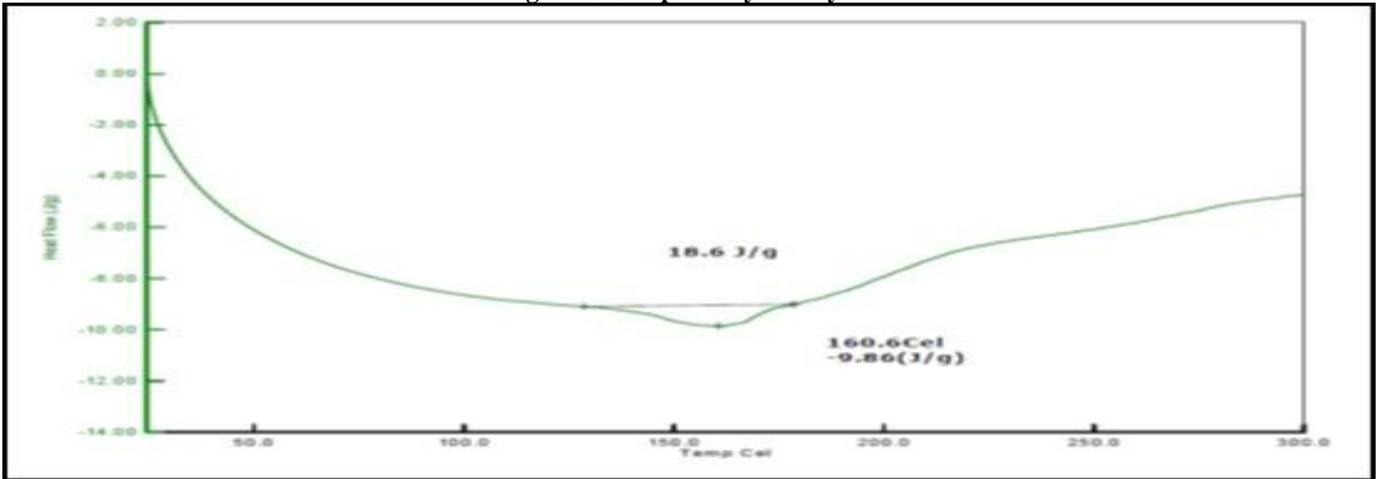
1) **FTIR studies** conducted on pure drug, polymer and physical mixtures showed that there is no marked interaction between drug and selected polymer



## 2) DSC studies

DSC studies were carried out for pure erythromycin and physical mixture of drug with Ethyl Cellulose, PVA.

**Fig 2. DSC of pure erythromycin**

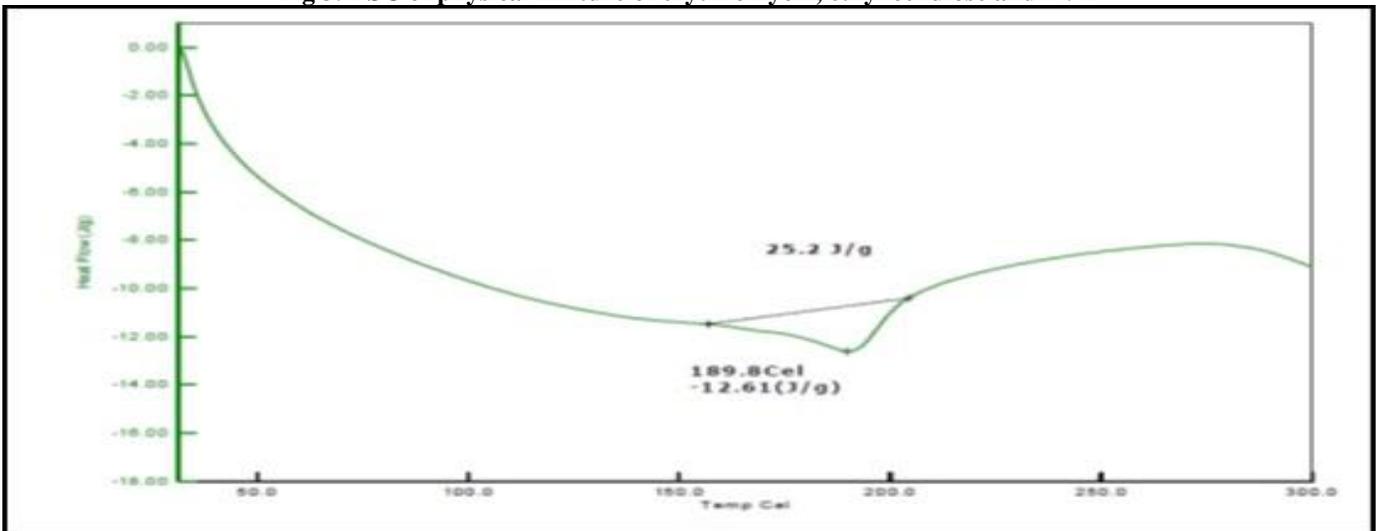


## 3) FORMULATION OF ERYTHROMYCIN MICROSPONGES

a) Effect of formulation parameters

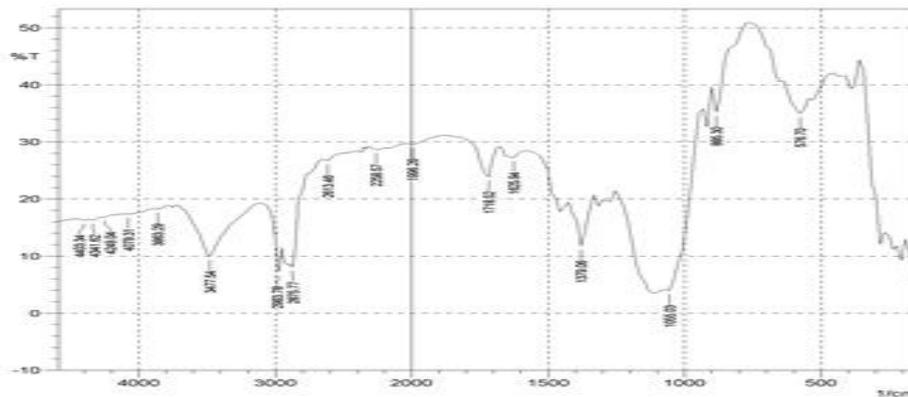
i) Concentration of retardant material in the internal phase

**Fig 3. DSC of physical mixture of erythromycin, ethyl cellulose and PVA**



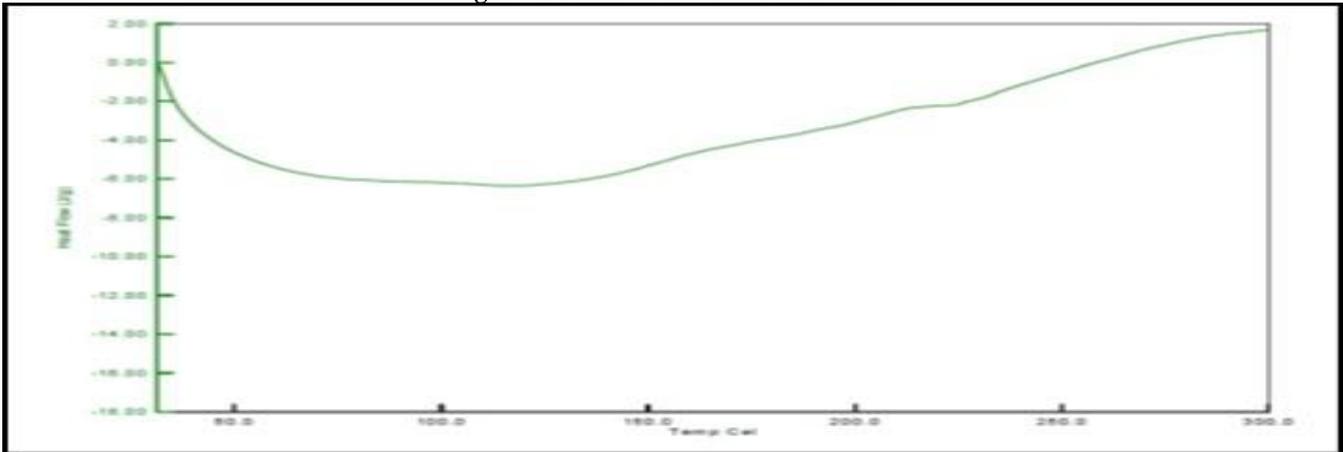
## 7) FTIR STUDIES

**Fig 4. FTIR studies for Formulation F-18**



8) DSC STUDIES

Fig 5. DSC studies of Formulation F-18



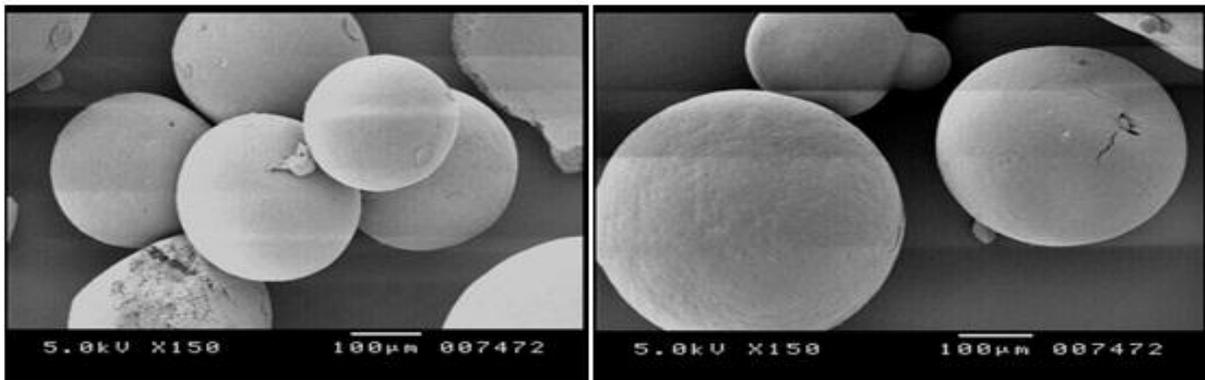
9) Morphological studies of microsponges

a) SEM studies

(a) and (b) representing microsponges are spherical in shape

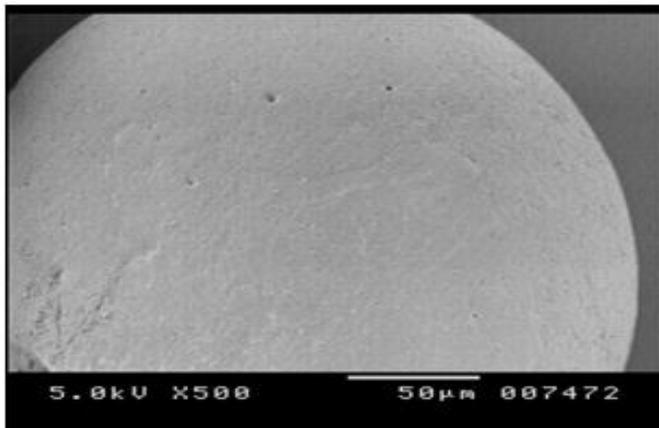
(c) Representing microsponges are porous and spongy in nature.

Fig 6. SEM of Formulation F-18



(a)

(b)



(c)

## DISCUSSION AND CONCLUSION

The present study was to formulate and evaluate the microsp sponge for topical sustained drug delivery of erythromycin for extended release. The study design focused on standardization of process parameters like standardization of concentration of retardant material table no (1), concentration of surfactant table no (2), volume of external phase table no (3) and the effect of stirring speed table no (4) involved in the preparation of erythromycin microsponges by quassi-emulsion solvent diffusion method. Ethyl cellulose was used as a polymer, poly vinyl alcohol as a surfactant, methanol and dichloromethane as solvents in the internal phase, water as external phase. By compatibility studies it was found there was no interaction between the drug and excipients. The best standardized F<sub>18</sub>

formulation showed good loading efficiency, production yield, particle size, drug content. The SEM results showed that the microsponges were spherical and porous in nature. Thus it was concluded that the selected anti-acne drug can be developed into microsponges and further can be incorporated into gel for topical application.

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