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DESIGN AND EVALUATION OF AN ANTIFUNGAL DRUG WITH CORTICOSTEROID LOADED MICROSPONGE HYDROGEL.

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ABSTRACT: This study focuses on the development of a topical gel formulation using microsponges entrapping clotrimazole and Beclomethasone dipropionate to enhance treatment effectiveness. Clotrimazole is known for its antifungal, local anti-infective and growth-inhibiting properties while Ethyl cellulose serves as a glucocorticoid, corticosteroid and anti-inflammatory agent. The microsponges were prepared using the Quasi emulsion solvent diffusion method with ethyl cellulose as the polymer and PEG-200 as the plasticizer. The characterization of the microsponges involved assessing production yield, entrapment efficiency, viscosity, FTIR, particle size analysis and drug diffusion from the gel base. The microsponges appeared as free-flowing white powders with production yields ranging from 41% to 80%. A higher amount of polymer resulted in decreased drug content in the microsponges and the encapsulation efficiency varied from 41.3% to 73.8%. Scanning Electron Microscopy (SEM) revealed that the microsponges were porous but not uniformly spherical. The mean particle size ranged from 25.42µm to 53.43µm. FTIR and DSC studies demonstrated the compatibility of the drug and polymer during microsponge preparation. The drug release profiles of the microsponge gel formulations indicated a sustained drug release pattern when evaluated using pH 5.4 phthalate buffer. Among the formulations (F1 to F12) F6 showed promising sustained drug release of 91.92% for Clotrimazole and 90.35% for Beclomethasone dipropionate respectively. These findings suggest the potential of the developed gel formulation for improved topical drug delivery.

Keywords – Clotrimazole; Beclomethasone dipropionate; Ethyl cellulose; Microsponge; Quasi emulsion solvent diffusion method.

1. Introduction

Microsponges are polymeric delivery system composed of porous microspheres that are mostly used for prolonged topical administration.[1] Microsponge drug delivery is a patented polymeric system consists of porous microspheres. These are tiny sponge like a spherical particle that consists of inter-connecting voids within a non-collapsible structure with a large porous surface through which active ingredient is released in controlled manner.[2] Microsponges are designed to deliver a pharmaceutically active ingredient efficiently at minimum dose and also to enhance stability, reduce side effects and modify drug release profile due to microspheres that can entrap a wide variety of substances and formulate different products like creams, liquids, gels and tablets. MDS can gives a increased efficacy for topical with enhanced safety and stability property. [3, 4, 5]

In challenging area of research microsponges having controlled release of drugs on epidermis with promising that drug remains primarily localized and does not enter into the systemic circulation in significant amounts. Drugs which are applied on skin to get controlled release and do not enter in to the systemic circulation in significant amount hence it is a challenging area of research.[6] Microsponge particles are extremely small, inert spheres that do not pass through the skin and prevent the accumulation of ingredients on skin due to this reason reduce the irritation and efficacy of drugs. And these microsponges which trigger the drug release by response to change in temperature, pressure and solubility. In 1987 the microsponge technology was developed by Won and original patents were assigned to advanced polymer systems.[7] The size of the microsponge may have 10-25µ in diameter and having 250000 pores. Due to large reservoir within in each microsponge, the bacterial contamination or entrapment in the microsponge is less because the size of pore diameter is smaller but the bacteria having the size range from 0.007 to 0.2µm hence it cannot penetrate in to tunnel structure of microsponge.[8]

Conventional delivery system formulations of topical drugs are intended to work on outer layers of the skin. Hence such products require high concentration of active agents to be incorporated for effective therapy because of their low efficacy as delivery system. Due to this reason in recent years develop novel drug delivery systems called microsponges there has been considerable emphasis given in order to modify and control the release behavior of drugs by incorporating in to a carrier system and alter the duration of activity and therapeutic index of drug. Due to this reason archived targeted and controlled release of drugs.[9]

2. Materials and method

2.1 Materials

Clotrimazole was gifted by Wex ford pvt. Ltd. Beclomethasone dipropionate was acquired by Micro Labs. Ethyl cellulose, Methyl paraben & Propyl paraben was bought from Ozone international. Ethyl alcohol was liberal blessing from fisher scientific. Poly vinyl alcohol was bought Loba Chemicals. Carbopol 934 was bought from Yarrow Chemicals Pvt. Ltd. Triethanolamine, PEG 200, Sodium hydroxide and Potassium hydrogen phthalate was procured by Sd fine Chemicals.

2.2 Methods

2.2.1 *Preformulation studies*

Preformulation is defined as a phase and development process where physical, chemical and mechanical properties of a new drug substance are characterized alone and when combined with excipients in order to develop stable, safe and effective dosage form. A thorough understanding of physical properties may ultimately provide a rationale for formulation design, or support the need for molecular modification or merely confirm that there is no significant barrier to the formulation development. Hence preformulation studies on the obtained sample of drug include colour, taste, solubility analysis, melting point determination and compatibility studies.

2.2.2 *Spectrophotometric determination of clotrimazole*

In this experiment, 100 mg of clotrimazole was dissolved in ethanol and phthalate buffer pH 5.4 to make stock I. From stock I, 10 ml was pipetted into another flask to form stock II. Working solutions of clotrimazole (2-10 µg/ml) were prepared from stock II. Absorbance of these concentrations was measured using a Shimadzu UV-visible double beam spectrophotometer. A calibration curve was obtained by plotting concentration versus absorbance. The method was validated for linearity, accuracy and precision and followed Beer-Lambert's law in the 2-10 µg/ml concentration range.

2.2.3 *Spectrophotometric determination of Beclomethasone dipropionate*:

In this experiment, 100 mg of Beclomethasone dipropionate was dissolved in ethanol and phthalate buffer pH 5.4 to create stock I. From stock I, 10 ml was transferred to another flask to form stock II. Working solutions of Beclomethasone dipropionate (2-10 µg/ml) were prepared from stock II. Absorbance of these concentrations was measured using a Shimadzu UV-visible double beam spectrophotometer. A calibration curve was obtained by plotting concentration versus absorbance without using a drug sample as a blank. The method was validated for linearity, accuracy and precision and followed Beer-Lambert's law in the concentration range of 2-10 µg/ml. The absorbance of the drug sample was measured at 239 nm.[10]

2.2.4 *Preparation of Microsponges*

Table 1: Different concentrations of polymer and same concentration of drug

	Internal phase			**Internal phase**		
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
Clotrimazole	100mg	100mg	100mg	200mg	300mg	400mg
Beclomethasone dipropionate	20mg	20mg	20mg	40mg	60mg	80mg
Eudragit RS 100	120mg	240mg	360mg	120mg	120mg	120mg
Ethanol	10ml	10ml	10ml	0.2ml	0.2ml	0.2ml
PEG 200	0.2ml	0.2ml	0.2ml	200mg	300mg	400mg
	External phase			**External phase**		
PVA	50mg	50mg	50mg	50mg	50mg	50mg
Water	200ml	200ml	200ml	200ml	200ml	200ml

	Internal phase			**Internal phase**		
	F7	**F8**	**F9**	**F10**	**F11**	**F12**
Clotrimazole	100mg	100mg	100mg	100mg	100mg	100mg
Beclomethasone dipropionate	20mg	20mg	20mg	20mg	20mg	20mg
Eudragit RS 100	120mg	240mg	360mg	120mg	240mg	360mg
Ethanol	10ml	10ml	10ml	10ml	10ml	10ml
PEG 200	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml
	External phase			**External phase**		
PVA	50mg	50mg	50mg	50mg	50mg	50mg
Water	200ml	200ml	200ml	200ml	200ml	200ml

3. Characterization of microsponges

3.1 Percentage Yield

The prepared microsponges of all batches were accurately weighed. The measured weight of prepared microsponges was divided by the total amount of all the excipients and drug used in the preparation of the microsponges, which gives the total percentage yield of microsponges. It was calculated by using following equation.[11]

$$\% \text{ yield} = \frac{\text{Actual weight of product}}{\text{Total weight of excipients and drug}} \times 100$$

3.2 *Morphological study using SEM*

The morphological studies were carried out by scanning electron microscope (SEM). Microspheres were scanned and examined under electron microscope HITACHI SU 1500, Japan connected with Fine coat, JEOL JFC-1100E Ion sputter. The sample was loaded on copper sample holder and sputter coated with carbon followed by Gold.

3.3 Morphological study using DSC

Differential scanning calorimetry (DSC) is a technique in which the difference in the amount of required to increase the sample and reference is measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. Generally, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. The reference sample should have a well-defined over the range of temperatures to be scanned. DSC studies were carried out for pure drug and polymers. DSC scans were performed by using an automatic thermal analyzer system. (DSC60 Shimadzu Corporation, Japan). Sealed and perforated aluminium pans were used in the experiments for all the samples. Temperature calibrations were performed using indium as standard. An empty pan sealed in the same way as for the sample was used as a reference. The entire samples were run at a scanning rate of 10°C/min from 50-200°C.

3.3Particle size determination

Determination of average particle size of clotrimazole and Beclomethasone dipropionate loaded microsponges was determined by using an optical microscope using calibrated ocular and stage micrometer under regular polarized light. A minute quantity of microsponges was spread on a clean glass slide and the average particle size was calculated by measuring 15 particles of each batch.

3.4Drug entrapment studies

Microsponges equivalent to 100mg of the drug were taken for evaluation. The amount of drug entrapped was estimated dissolving with 100ml 5.4 pH phthalate buffer solution with the aid of sonication. The solution was filtered and the absorbance was measured Spectro-photometrically at 260nm and 239nm after suitable dilution. The amount of drug loaded and entrapped in the microspheres was calculated by the following formulas.

$$\% \text{ Drug entrapment} = \frac{\text{Amount of drug actually present (DC)}}{\text{Theoretical drug load expected}} \times 100$$

4 Formulation of clotrimazole and Beclomethasone dipropionate microsponge gel

Clotrimazole and Beclomethasone dipropionate microsponge gel is prepared by using following formula.

TABLE 2: Composition of clotrimazole and Beclomethasone dipropionate microsponge gel

Ingredients	Quantities
Clotrimazole	400mg
Beclomethasone dipropionate	80mg
Ethyl cellulose	120mg
Ethanol	10ml
Carbopol 934	100mg
Glycerine	3ml
Methyl paraben	20mg
Propyl paraben	10mg
SLS	5mg
Triethanolamine	Qs to adjust pH
Lavender oil	qs
Purified water qs	200 ml

Carbopol 940 of 1 and 2% w/v is taken in a beaker containing water and allowed to soak for 24hrs. The prepared gel is neutralised by sufficient quantity of triethanolamine. Disperse the prepared microsponge in ethanol and pour in to the prepared gel. Glycerine acts as humectants, methyl paraben and propyl paraben acts as preservatives, SLS acts as surfactant and lavender oil acts as a flavouring agent. All were added slowly with continuous stirring to form homogeneous gel.

5. Evaluation of microsponge gel

5.1 Percentage Yield: [12]

The empty container was weighed in which the gel formulation was stored then again, the container was weighed with gel formulation. Then subtracted the empty container weighed with the container with gel formulation, it gives the practical yield. The Percentage yield was calculated by the formula.

$$\text{Percentage Yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

5.2 Determination of pH

Weighed 50gm of each gel formulations were transferred in 10ml of beaker and measured it by using the digital pH meter. pH of the topical gel formulation should be between 3 – 9 to treat the skin infections.

5.3 Viscosity & Consistency

All formulated gels were subjected to viscosity measurement by using a Brookfield digital viscometer (Analytical Technologies) by using spindle # 2 at room temperature. The measurement of consistency of the gel formulations was done by dropping a cone attached to a holding rod from distance of 10cm in such a way that it should fall on the centre of the glass cup filled with gel. The penetration by cone was measured from the surface of the gel to the tip of cone inside the gel. The distance travelled by cone was noted after 60sec.[13]

5.4 *In vitro* diffusion study:

Cellophane membrane was used for this study in Franz diffusion cell. 5gm of gel is placed in donor compartment the gel was applied uniformly to the membrane. The membrane was mounted between the compartments of the Frantz diffusion cell. Reservoir compartment was filled with 100ml phthalate buffer of pH 5.4. The study was carried out at 37 ± 1°C and speed was adjusted until the vortex touches the membrane and it carried out for 10h. 5ml of sample was withdrawn from reservoir compartment at 60min interval and absorbance was measured spectrophotometrically at 260nm and 239nm. Each time the reservoir compartment was replenished with the 5ml volume of phthalate buffer pH 5.4 solutions to maintain constant volume.[14]

Fig 1: Franz diffusion cell with skin mounted between compartments

6. Release Kinetics

Data obtained from the (NP-1) *In vitro* release studies were fitted to various kinetic models such as zero order, first order, Higuchi model and Kosmeyer peppas model. The four models of data treatment are as follows.[15, 16, 17]

6.1 Zero-order release equation

$$Q = Q_0 - K_0 t$$

"Q" is the amount of drug released at time "t"

K_0 is zero-order release rate constant

A plot of fraction of drug release against time will be linear, if the release obeys zero order release kinetics

6.2 First-order release equation

$$\ln Q = \ln Q_0 - K_1 t$$

"Q" is the amount of drug released at time "t"

Q_0 is the amount of drug remaining in the formulation

Thus, a plot of the logarithm of the fraction of drug remained against time will be linear, if the release obeys first order release kinetics.

6.3 Higuchi's square root of time equation

It defines a linear dependence of the active fraction released per unit of surface(Q) on the surface root time.

K_2 is Higuchi square root of time-release rate constant. A plot of the fraction of drug released against root of time will the linear if the release obeys Higuchi equation.

This equation describes drug release as a diffusion process based on the Fick's Law square root time dependent.

$$Q = K_2 t_{1/2}$$

6.4 Korsmeyer-Peppas equation

Q/Q_0 is fraction of drug release at time "t". "K" is a constant and 'n'is diffusion exponent indicating the mechanism of drug release. The 'n' value could be used to characterize different release mechanisms as follows:

$$Q/Q_0 = K t^n$$

'n' Mechanism

0.5 Fickian Diffusion (Higuchi Matrix)

0.5 < n < 1 Anomalous Transport (First order)

1 Case-II Transport (Zero order release)

n > 1 Super case-II Transport

7. Results

7.1 Identification of pure drug

The FTIR spectrum of the Clotrimazole pure drug was found to be similar to the standard spectrum of clotrimazole as in I.P. The spectrum of clotrimazole showed the following functional groups at their frequencies.

Characteristic group	**cm $^{-1}$**
C-H stretch	3166, 3063
C-C stretch	1584
C-N stretch aromatic (ring)	1566
C-C stretch aromatic (ring)	1492
Aromatic C-H out of the plane bending	765, 741 (strong)

The FTIR spectrum of the Beclomethasone dipropionate pure drug was found to be similar to the standard Spectrum of Beclomethasone dipropionate as in I.P. The spectrum of Beclomethasone dipropionate showed the following Functional groups at their frequencies.

Characteristic group	**cm $^{-1}$**
C-H stretch	1411
C-C stretch	1543

The FTIR spectrum of the Ethyl cellulose polymer was found to be similar to the standard spectrum of Ethyl cellulose as in I.P. The spectrum of Ethyl cellulose showed the following functional groups at their frequencies.

Characteristic group	**cm $^{-1}$**
C-H stretch	2919
C-C stretch	1543

7.2 Solubility analysis

Practically insoluble in water.
Freely soluble in ethanol, methanol, chloroform.

7.3 Melting point

7.3.1 Melting point of clotrimazole

The melting point of the clotrimazole pure drug was found to be 148^{o}C and the range is 147^{0}C to 150^{o}C.

7.3.2 Melting point of Beclomethasone dipropionate

The melting point of the Beclomethasone dipropionate pure drug was found to be 118^{o}C and the range is 117^{0}C to 120^{o}C.

7.3.3 Melting point of Ethyl cellulose

The melting point of the ethyl cellulose polymer was found to be 133^{0}C and the range is 129^{0}C to 133^{0}C.

7.4 Compatibility studies

7.4.1 FTIR Study

From the FTIR spectra of the pure drug and the combination spectra of drug with the polymers it was observed that all the characteristic peaks of clotrimazole and Beclomethasone dipropionate were present in the combined spectra as well thus indicating the compatibility of the drug with the polymers. The individual FTIR spectra of the pure drug Clotrimazole, Beclomethasone dipropionate, polymers Ethyl cellulose as well as the combination spectra of the drug and polymers physical mixture are shown in the Figure 26 to 28. It was found that the drug was compatible with polymer in physical mixture.[18]

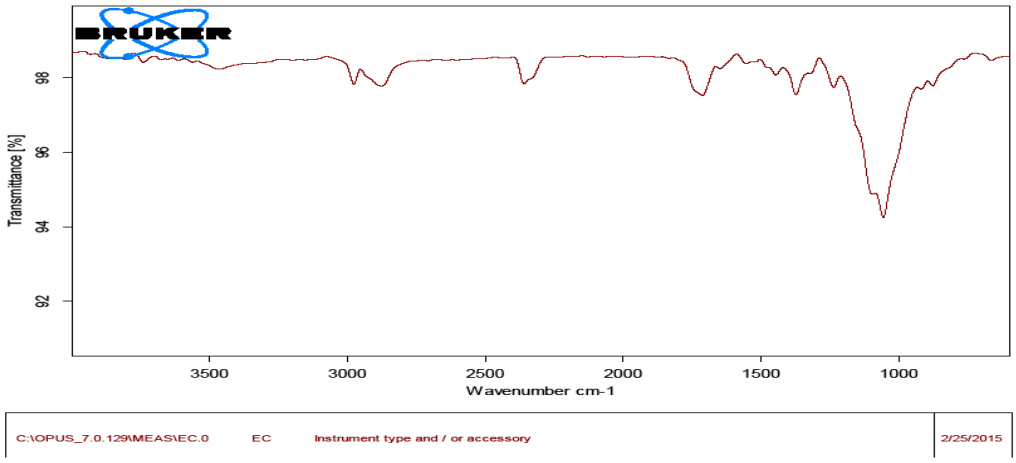

Fig 2: IR spectra for ethyl cellulose

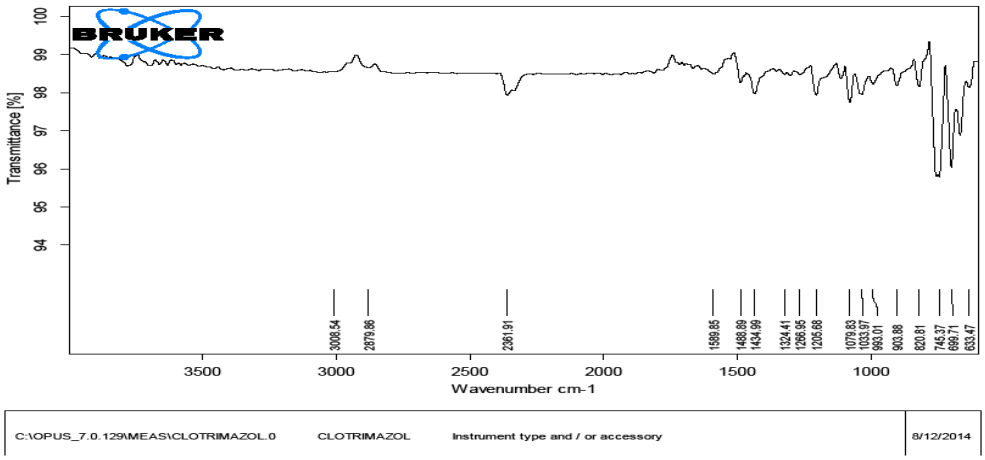

Fig 3: IR spectra for Clotrimazole

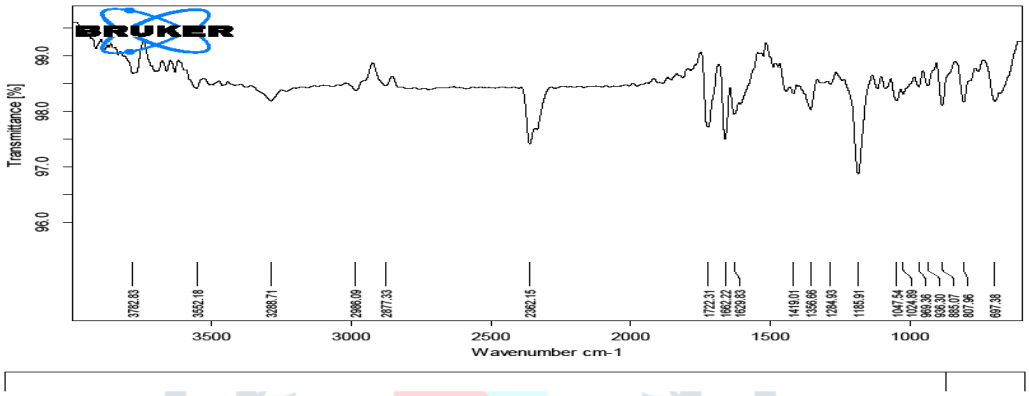

Fig 4: IR spectra for Beclomethasone dipropionate

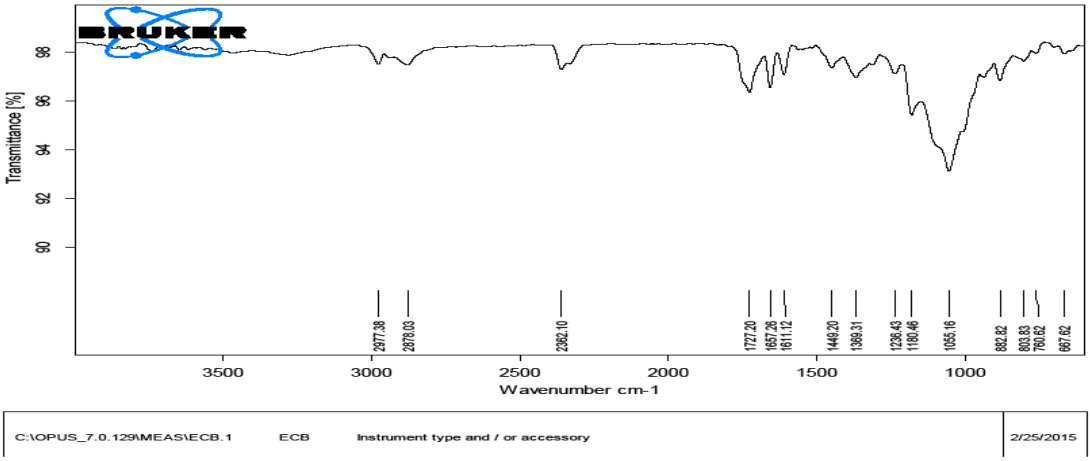

Fig 5: IR spectra for combination of Ethyl cellulose and Beclomethasone dipropionate

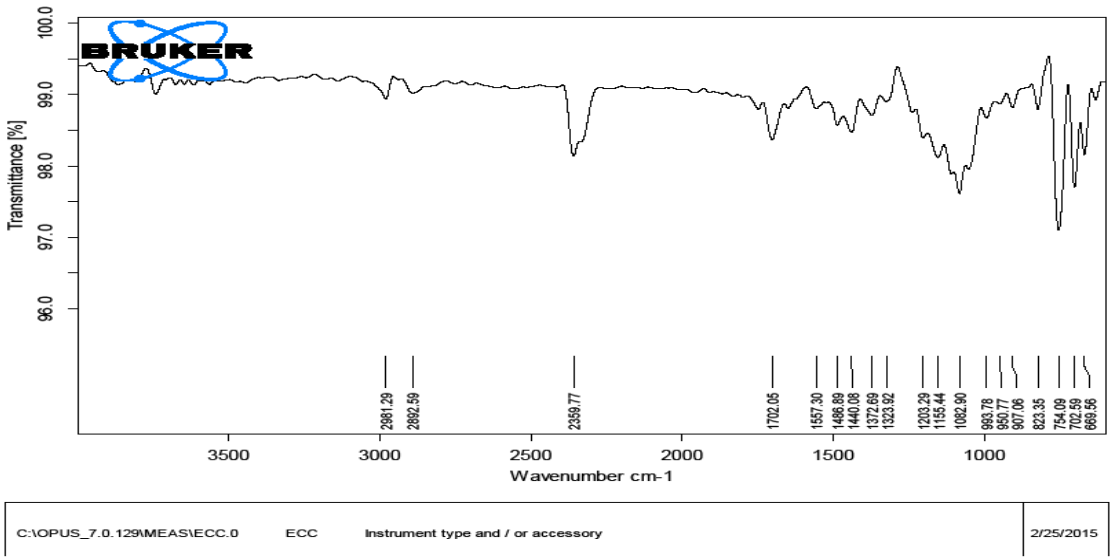

Fig 6: IR spectra for Ethyl cellulose and Clotrimazole

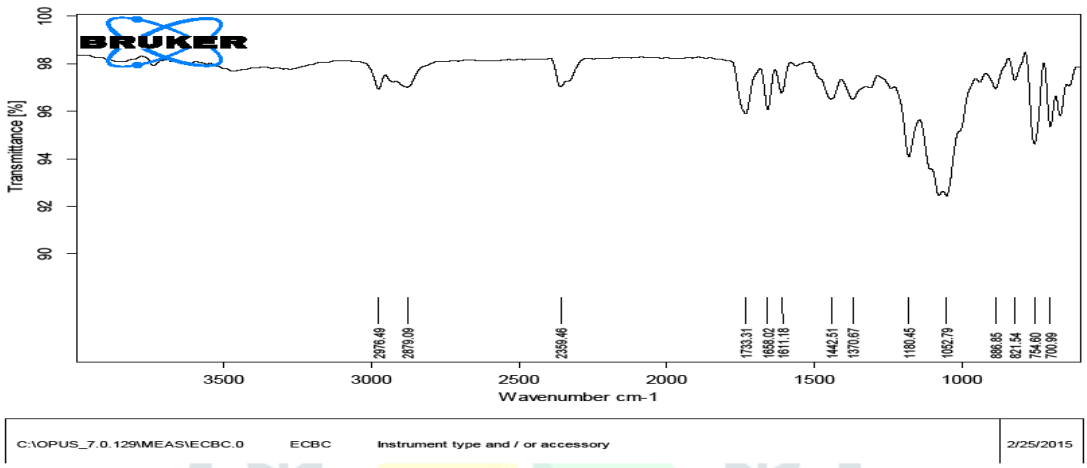

Fig 7: IR spectra for the combination of Ethyl cellulose, Beclomethasone dipropionate and Clotrimazole

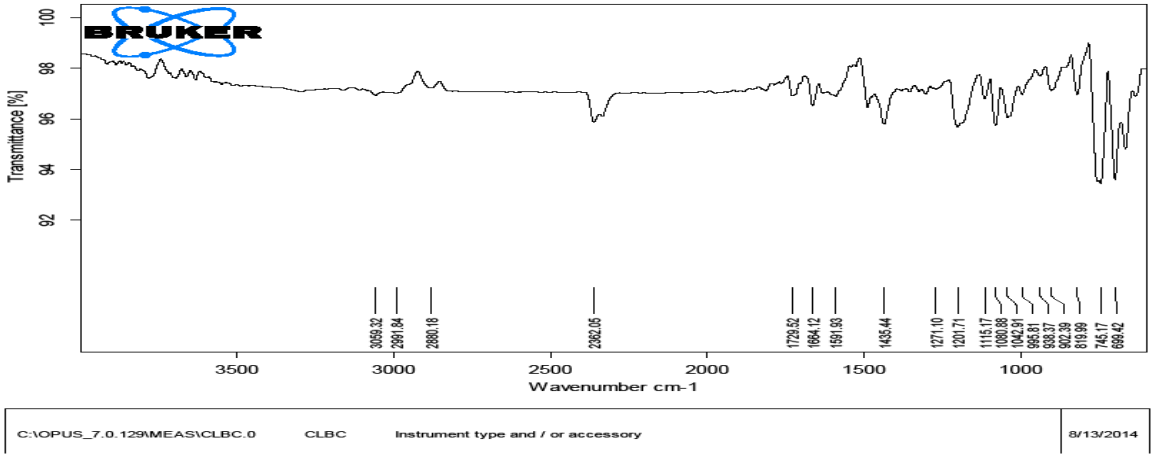

Fig 8: IR spectra for combination of Clotrimazole and Beclomethasone dipropionate

7.4.2 UV Spectrophotometric study

- **Standard calibration curve of clotrimazole in phthalate buffer (pH-5.4)**

The absorbance of standard solutions of Clotrimazole ranging from 2-10µg/ml in phthalate buffer (pH 5.8). The curve was found to be linear in the range of 2-10µg/ml at λ max 260nm. The regression value was found to be 0.999 as shown in Figure 9.

TABLE 3: Standard calibration curve of Clotrimazole in Phthalate buffer (pH 5.4) at 260nm

Sl no	Concentration (µg/ml)	Absorbance
1	0	0.0
2	2	0.15
3	4	0.32
4	6	0.49
5	8	0.667
6	10	0.82

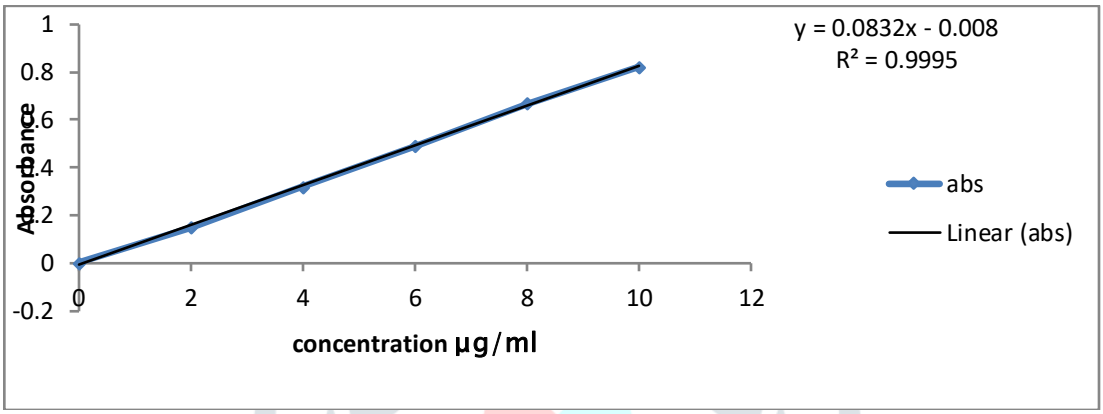

Fig 9: Calibration curve of Clotrimazole

- **Standard calibration curve of Beclomethasone dipropionate phthalate buffer (pH 5.4)**

Table 4 shows the absorbance of standard solution of Beclomethasone dipropionate ranging from 2-10µg/ml in phthalate buffer (pH 5.8). The curve was found to be linear in the range of 2-10µg/ml at λ max 239nm. The regression value was found to be 0.999 as shown in the Figure 10.

TABLE 4: Standard calibration curve of Beclomethasone dipropionate in Phthalate buffer (pH 5.4) at 239nm

Sl no	Concentration (µg/ml)	Absorbance
1	0	0.0
2	2	0.05
3	4	0.106
4	6	0.156
5	8	0.205
6	10	0.270

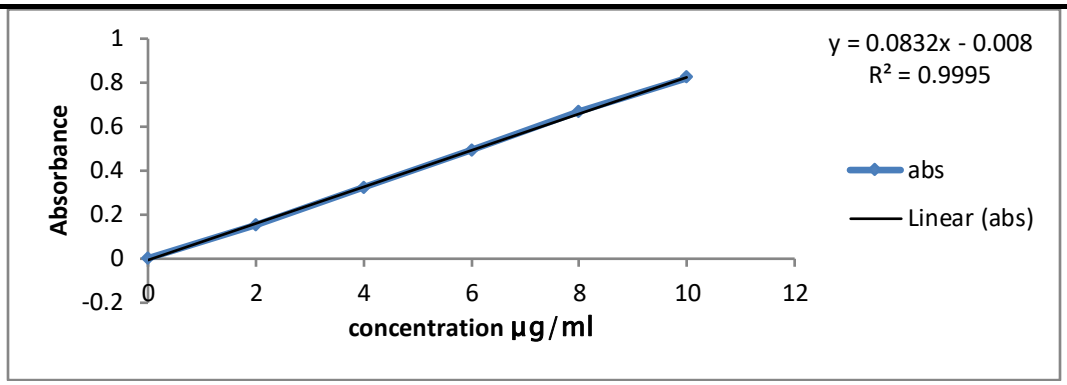

Fig 10: Calibration curve of Beclomethasone dipropionate

7.4 Formulation of microsponges loaded with Clotrimazole and Beclomethasone dipropionate

Clotrimazole is an antifungal with short half-life, poor oral bioavailability, and minimal vaginal and topical absorption. It was selected as a model drug to formulate a microsponge delivery system for topical delivery. Beclomethasone dipropionate is a corticosteroid with short half-life. It was selected as a model drug to formulate a microsponge delivery system for topical delivery. Ethyl cellulose is a (a synthetic polymer) biocompatible, hydrophobic polymer, which prolong the release of water-soluble and water insoluble drugs from microsponges. The organic solvent chosen is ethyl alcohol (10ml) and has a capacity to solubilize ethyl cellulose. This has been incorporated to form internal phase for emulsification with external Phase. Microsponges were prepared by employing Quasi emulsion solvent diffusion technique. Ethyl alcohol was evaporated from an internal phase which was mixed with an aqueous dispersion medium containing polyvinyl alcohol (PVA). Incorporation of Polyvinyl alcohol to the aqueous phase was necessary to impart emulsification. The microsponges prepared by the above technique were spherical in shape.

7.5 Evaluation

7.5.1 Percentage yield

Percentage yield of different formulation F1 to F12 were calculated the yield was found to be 62.2%, 70.1%, 79.3%, 86.6%, 83.9%, 85.3%, 44.7%, 53.2%, 57.4%, 74%, 70.11%, and 78.78%. The percentage practical yield slightly increases with increase in the concentration of polymer respectively.

TABLE 5: Percentage yield of Clotrimazole & Beclomethasone dipropionate loaded microsponges

Sl no	Theoretical yield (mg)	Practical yield (mg)	Percentage yield (%)
F1	290	120	41.3
F2	410	190	46.3
F3	530	300	56.6
F4	410	290	70.7
F5	530	390	73.5
F6	650	520	80.0
F7	290	120	41.3
F8	410	155	37.8
F9	530	170	32.01
F10	410	210	51.2
F11	530	240	45.2
F12	650	340	52.3

7.5.2 Particle size analysis

Average particle sizes of microsponges were determined by optical microscopy as shown in Table. The mean particle size for the formulation F1 to F12 containing eudragit RS-100 and ethyl cellulose was found to be in range from 25.42 □m to 53.43 □m. There is a decrease in eudragit RS-100 & ethyl cellulose polymer concentration in the microsponges from F1 to F12.

60^{th} division of eye piece micrometer is coinciding with 80^{th} division of stage micrometer. 60^{th} division of eye piece micrometer = 800µm [one smallest division of stage micrometer = 10µm]
Therefore, one division of eye piece micrometer = 800/60 = **13.33µm**

34^{th} division of eye piece micrometer is coinciding with 40^{th} division of stage micrometer.
34^{th} division of eye piece micrometer = 400µm
Therefore, one division of eye piece micrometer = 400/34 = **11.74µm**

Average = 13.33+11.74/2
= 25.094/2
= **12.54µm**

Therefore, one division of eye piece micrometer = **12.54µm**

TABLE 6: Individual particle size (µm) for different formulations

F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
25.08	25.08	25.08	25.08	37.62	112.8	37.62	50.16	37.62	37.62	37.62	50.16
37.62	25.08	25.08	37.62	50.16	50.16	50.16	37.62	37.62	50.16	50.16	62.17
25.08	37.62	25.08	25.08	62.7	25.04	37.62	75.24	50.16	37.62	62.17	50.16
37.62	37.62	25.08	25.08	25.08	50.16	50.16	25.04	50.16	25.08	25.08	37.62
25.08	12.54	37.62	37.62	37.62	37.62	37.62	25.04	25.08	50.16	62.7	50.16
25.08	12.54	25.08	37.62	50.16	25.04	50.16	37.62	25.08	37.62	50.16	37.62
25.08	25.08	50.16	25.08	62.7	37.62	50.16	50.16	37.62	25.08	37.62	50.16
25.08	25.08	25.08	25.08	25.08	37.62	50.16	25.04	37.62	50.16	100.32	87.78
25.08	25.08	12.54	37.62	37.62	25.04	50.16	37.62	37.62	37.62	50.16	37.62
12.54	50.16	25.08	25.08	25.08	37.62	25.08	50.16	50.16	25.08	25.08	37.62
25.08	37.62	37.62	37.62	50.16	37.62	50.16	25.04	37.62	50.16	37.62	50.16
25.08	25.08	25.08	25.08	37.62	25.04	25.08	25.04	37.62	50.16	50.16	62.17
25.08	25.08	37.62	25.08	25.08	37.62	25.08	37.62	25.08	37.62	62.7	75.24
25.08	37.62	25.08	25.08	12.54	50.16	25.08	75.24	12.54	50.16	50.16	62.7
25.08	25.08	25.08	25.08	50.16	37.62	12.54	62.7	25.08	62.7	37.62	50.16

TABLE 7: Average particle size (µm) of Clotrimazole & Beclomethasone dipropionate microsponges

Sl No	Formulation code	Particle size
1.	F1	25.91
2.	F2	28.62
3.	F3	25.42
4.	F4	29.26
5.	F5	39.29
6.	F6	43.44
7.	F7	38.44
8.	F8	42.62
9.	F9	35.11
10.	F10	41.8
11.	F11	49.32
12.	F12	53.43

7.5.3 DSC Studies

Differential scanning calorimetry (DSC) is a technique in which the difference in the amount of required to increase the sample and reference is measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. Generally, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. The reference sample should have a well-defined over the range of temperatures to be scanned.
DSC studies were carried out for pure drug and polymers. DSC scans were performed by using an automatic thermal analyzer system. (DSC60 Shimadzu Corporation, Japan). Sealed and perforated aluminium pans were used in the experiments for all the samples. Temperature calibrations were performed using indium as standard. An empty pan sealed in the same way as for the sample was used as a reference. The entire samples were run at a scanning rate of 10°C/min from 50-250°C. The figures are showed from fig 11 to 14.

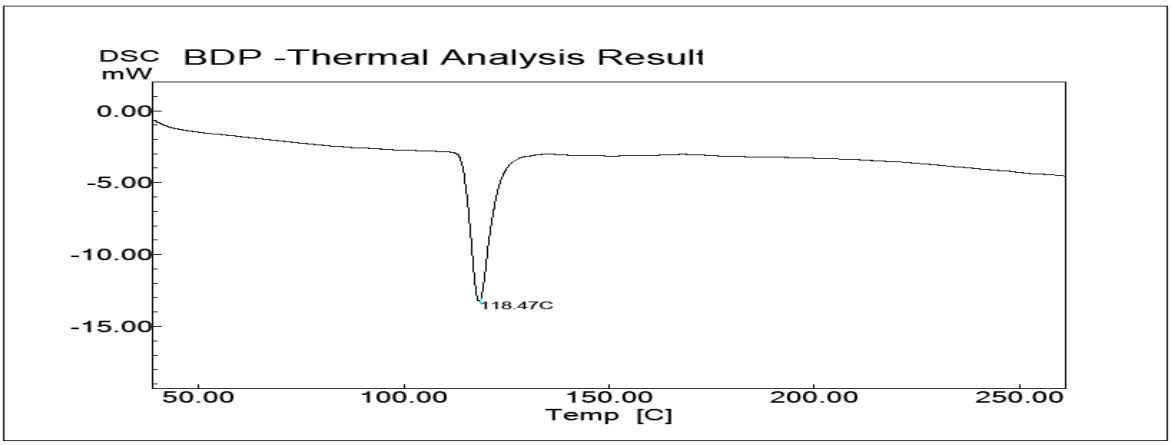

Fig 11: DSC spectra of pure Beclomethasone dipropionate

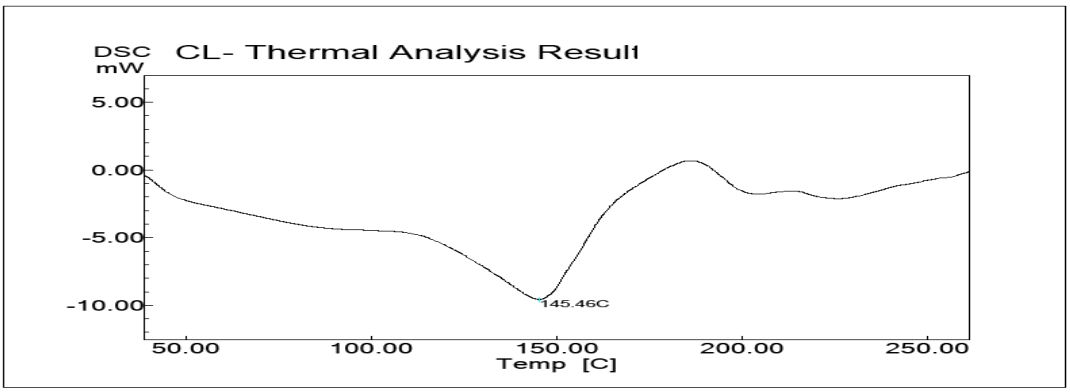

Fig 12: DSC spectra of pure Clotrimazole

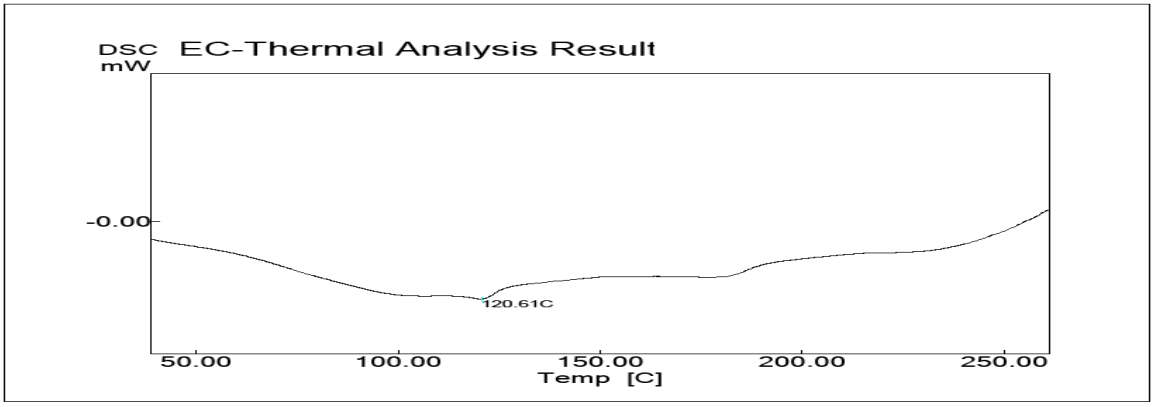

Fig 13: DSC spectra of pure Ethyl cellulose

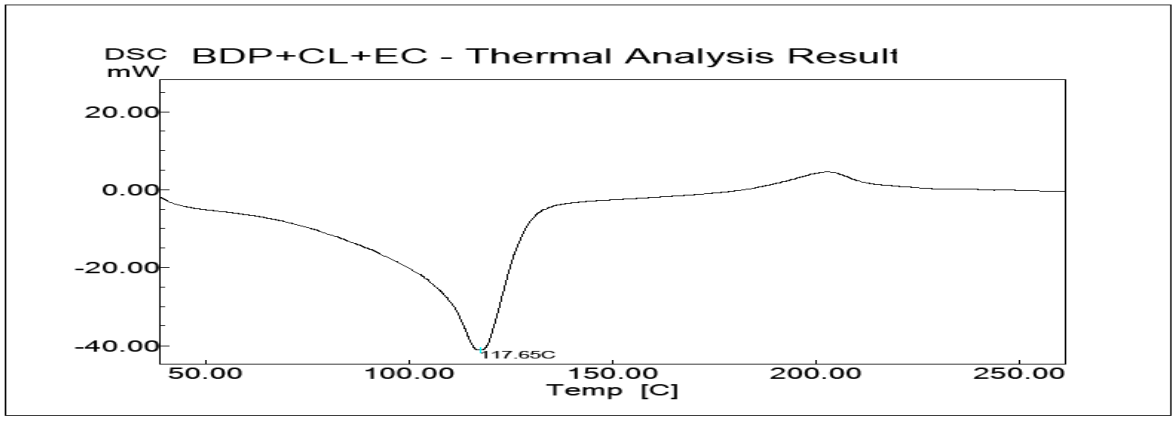

Fig 14: DSC spectra of physical mixture of Beclomethasone dipropionate + Clotrimazole + Ethyl cellulose

7.5.4 Drug entrapment studies:

The percentage entrapment efficiency is shown in Table 8. As the polymer concentration was increased the percentage encapsulation efficiency was decreased. The results of encapsulation efficiency were showed at higher Drug: Polymer ratios the available polymer can encapsulate more amount of drug. The % entrapment efficiency is shown in Table 8. Microsponge formulation F6 showed higher encapsulation efficiency.

TABLE 8: Drug entrapment of Clotrimazole & Beclomethasone dipropionate loaded microsponges

Formulation code	% Drug Entrapment
F1	41.3
F2	29.26
F3	22.64
F4	58.53
F5	67.92
F6	73.8
F7	41.3
F8	29.26
F9	22.64
F10	58.53
F11	67.92
F12	73.8

7.5.5 Physical parameters of gels:

Physical parameters of gel formulations incorporated with Clotrimazole & Beclomethasone dipropionate loaded microsponges which are shown in Table 16. The consistency reflects the capacity of the gel to get ejected in uniform and desired quantity when the tube is squeezed. Consistency is inversely proportional to the distance travelled by falling cone. All developed gel formulations showed viscosities in the range of 2750-2990 cps.

TABLE 9: Viscosity of Clotrimazole & Beclomethasone dipropionate loaded microsponge hydro gel

FORMULATION CODE	VISCOSITY (cps)	
	1%	**2%**
F1	2989 cps	5672 cps
F2	2845 cps	5876 cps
F3	2865 cps	5962 cps
F4	2972 cps	5823 cps
F5	2845 cps	5829 cps
F6	**2751 cps**	5510 cps
F7	2799 cps	5729 cps
F8	2834 cps	5789 cps
F9	2876 cps	5811 cps
F10	2865 cps	5889 cps
F11	2976 cps	5764 cps
F12	2832 cps	5792 cps
Marketed gel	2534 cps	

7.5.6 *In Vitro* drug release studies:

The drug release from the microsponge hydrogel were studied by Franz diffusion cell method. The *In-vitro* release profiles of Clotrimazole and Beclomethasone dipropionate from Ethyl cellulose microsponges are shown in Table 10 to 13 and Fig 15 to 18 . The cumulate percentage release of clotrimazole and Beclomethasone dipropionate varied from14.80 to 91.92 and 50.6 to 90.3 respectively depends on the drug polymer ratio for 10hrs

TABLE 10: *In vitro* drug release for clotrimazole F1 to F6

Time (min)	Batch code					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
60	18.212	17.845	18.312	23.412	25.675	20.515
120	22.623	20.676	21.345	29.645	37.769	33.212
180	29.315	25.849	24.824	36.487	40.342	39.924
240	35.169	29.628	26.709	45.672	47.701	47.413
300	41.204	32.151	30.236	53.128	49.802	53.621
360	45.673	36.182	34.190	63.750	61.507	59.457
420	51.324	41.024	39.925	65.418	68.915	66.012
480	62.597	55.683	54.628	67.746	71.212	75.129
540	71.645	59.355	56.159	73.687	75.854	83.910
600	74.896	67.802	60.924	79.504	83.158	91.927

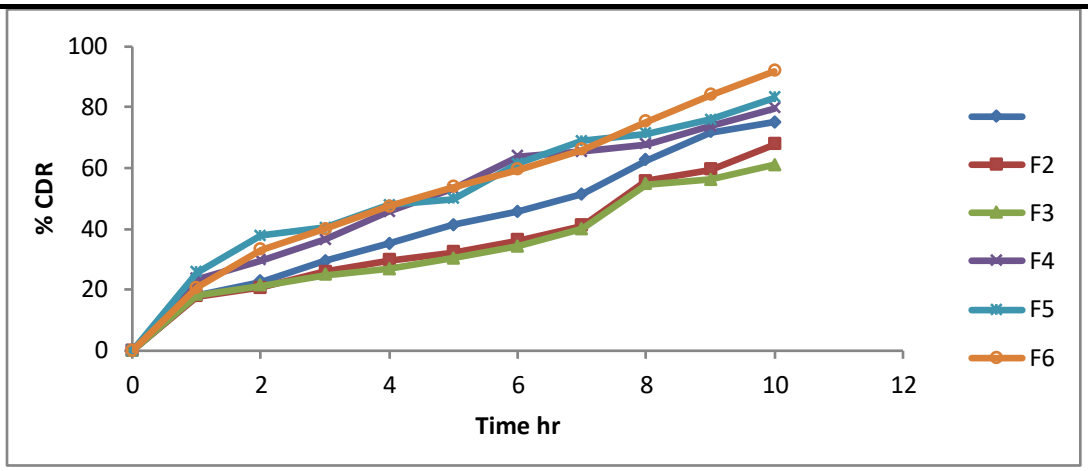

Fig 15: *Invitro* drug release for clotrimazole from F1 to F6

TABLE 11: *In Vitro* drug release for Clotrimazole from F7 to F12

Time	Batch code					
	F7	F8	F9	F10	F11	F12
0	0	0	0	0	0	0
60	16.761	14.812	15.619	22.464	24.190	23.912
120	21.019	19.910	18.320	27.324	38.670	29.760
180	25.230	22.601	23.102	32.670	45.100	37.415
240	30.623	25.405	26.091	37.810	49.674	43.610
300	35.092	29.672	28.912	41.672	52.620	47.910
360	41.325	32.015	33.120	47.916	58.430	52.314
420	47.719	39.612	38.621	54.032	64.723	59.617
480	56.012	43.213	41.910	61.314	69.917	66.712
540	57.321	51.021	48.240	65.012	74.790	72.816
600	69.910	61.990	58.872	70.167	79.991	80.912

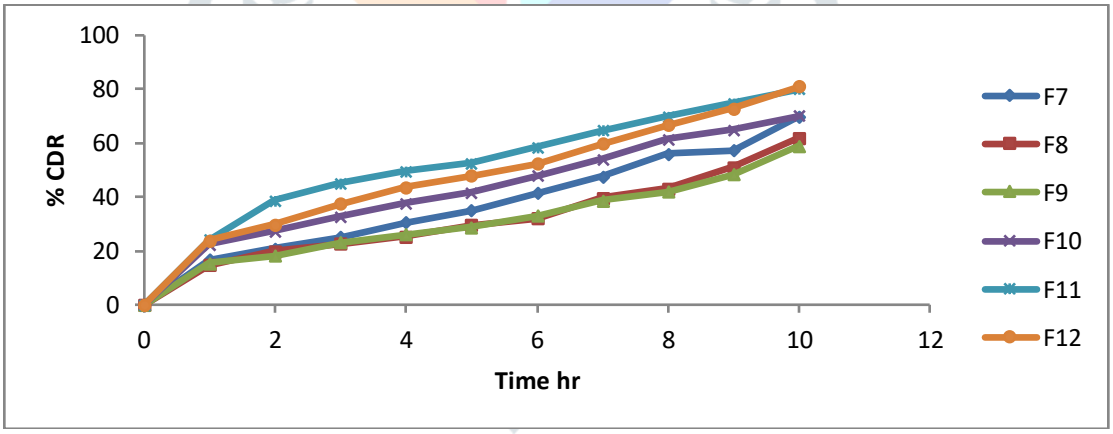

Fig 16: *In vitro* drug release for Clotrimazole from F7 to F12

TABLE 12: *In Vitro* drug release for Beclomethasone dipropionate from F1 to F6

Time	Batch code					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
60	17.615	17.92	19.216	20.212	22.156	21.216
120	23.452	19.645	20.314	26.156	34.561	31.346
180	31.615	26.132	23.110	31.347	39.990	38.124
240	33.458	31.025	27.620	39.487	43.109	45.365
300	39.346	33.156	31.210	45.513	48.650	49.907
360	41.955	35.624	35.012	49.716	57.656	55.754
420	48.912	40.152	38.915	55.612	62.714	64.168
480	55.826	54.214	50.014	62.742	69.816	76.912
540	67.758	56.432	54.915	69.814	72.250	82.364
600	71.050	64.762	56.724	72.616	76.891	90.354

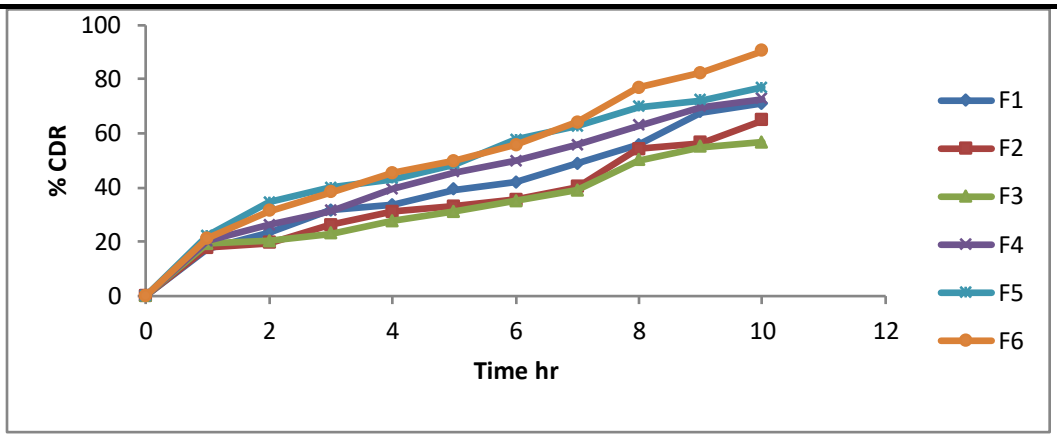

Fig 17: *In vitro* drug release for Beclomethasone dipropionate from F1 to F7

TABLE 13: *In vitro* drug release for Beclomethasone dipropionate from F7 to F12

Time	Batch code					
	F7	F8	F9	F10	F11	F12
0	0	0	0	0	0	0
60	17.925	15.612	16.901	20.123	22.140	23.176
120	22.395	18.013	18.453	25.032	29.346	30.250
180	29.995	21.190	24.609	30.113	34.710	36.725
240	34.594	24.032	28.101	35.345	39.462	42.179
300	40.618	30.543	29.010	39.912	45.670	49.601
360	43.019	34.623	31.612	43.031	49.789	55.612
420	46.192	39.105	35.932	49.604	52.617	61.702
480	56.820	42.603	39.902	56.901	59.457	68.543
540	62.447	49.910	45.312	63.181	65.710	71.414
600	66.992	58.033	56.703	67.015	72.617	78.614

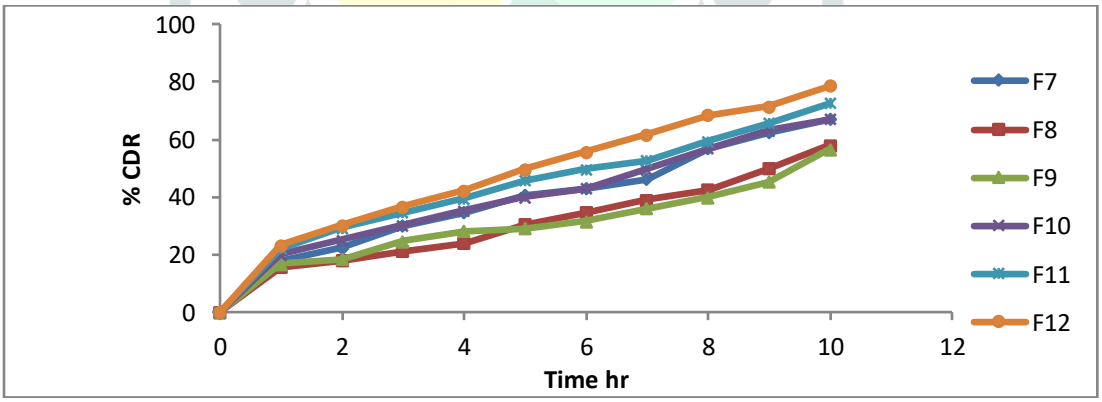

Fig 18: *In vitro* drug release for Beclomethasone dipropionate from F7 to F12

7.5.7 Scanning Electron Microscopy:

The determination of shape and surface morphology was done by scanning electron microscope HITACHI SU 1500, Japan. The microsponges of clotrimazole and Beclomethasone dipropionate with ethyl cellulose were smooth, porous and discrete spherical. Scanning electron photomicrographs of the formulation F6 are shown in Figure 19 and 20 respectively. The surface topography reveals that the microsponges were porous due to the rapid escape of the volatile solvents during formulation. Inward dents were seen on the surface probably due to collapse of the walls of the microsponges during the *In situ* drying process.

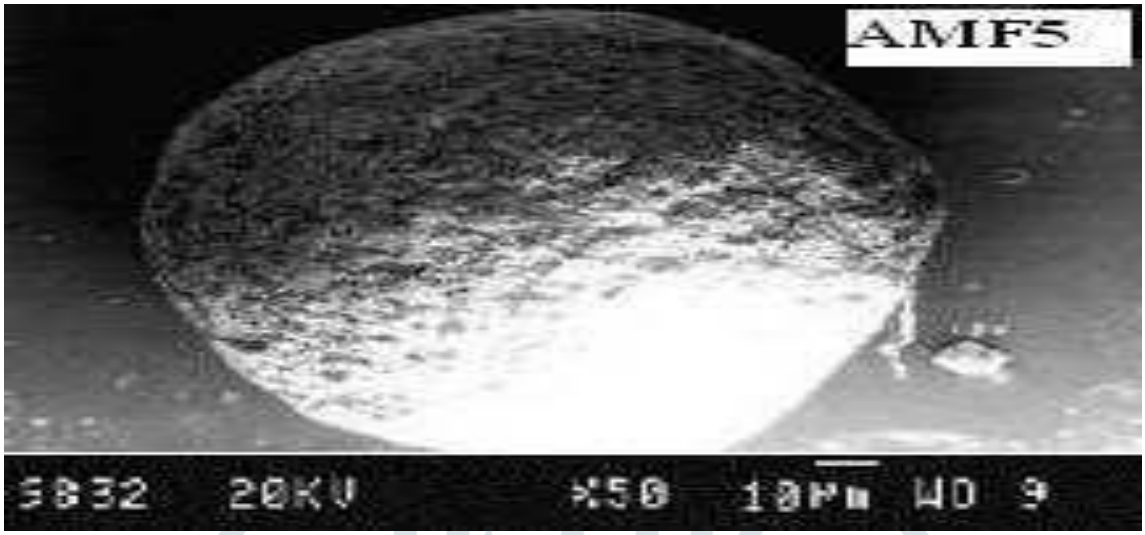

Fig 19: SEM for F6 formula

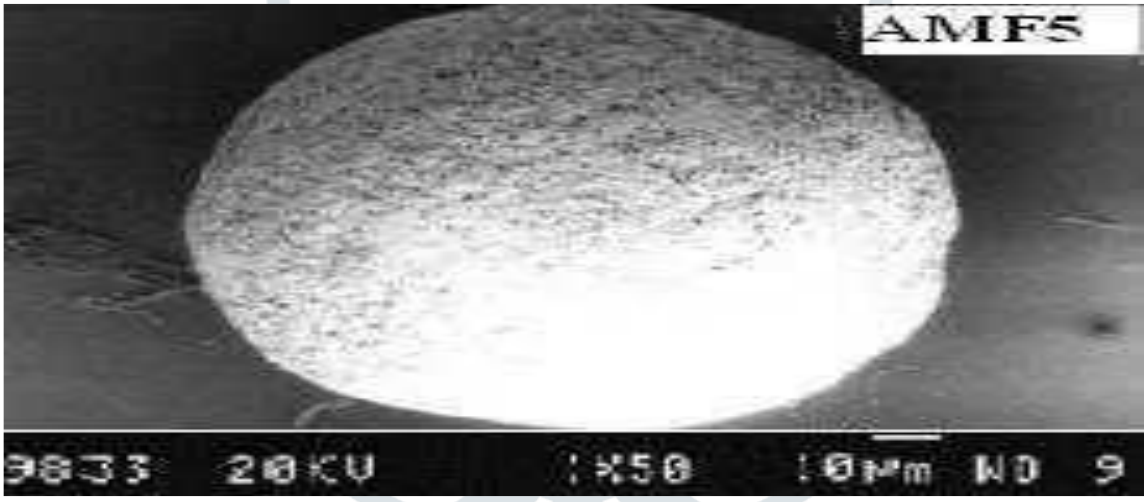

Fig 20: SEM for F6 formula

7.5.7 *In-vitro* drug release kinetics

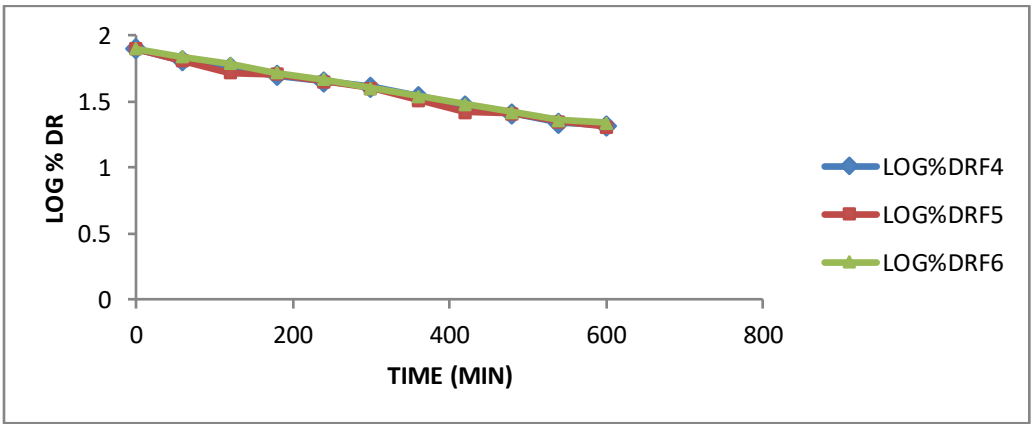

Fig 21: Plot of log % drug remaining vs time of Beclomethasone dipropionate with Ethyl cellulose

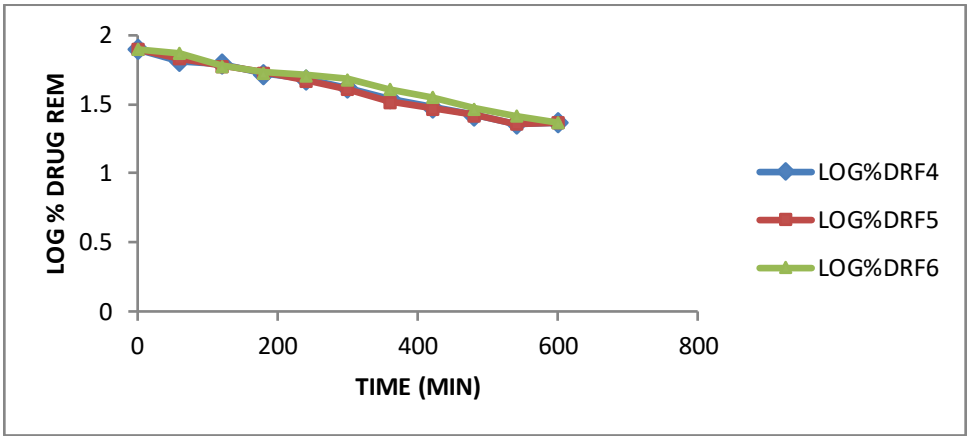

Fig 22: Plot of log % drug remaining vs Time of Clotrimazole with Ethyl cellulose

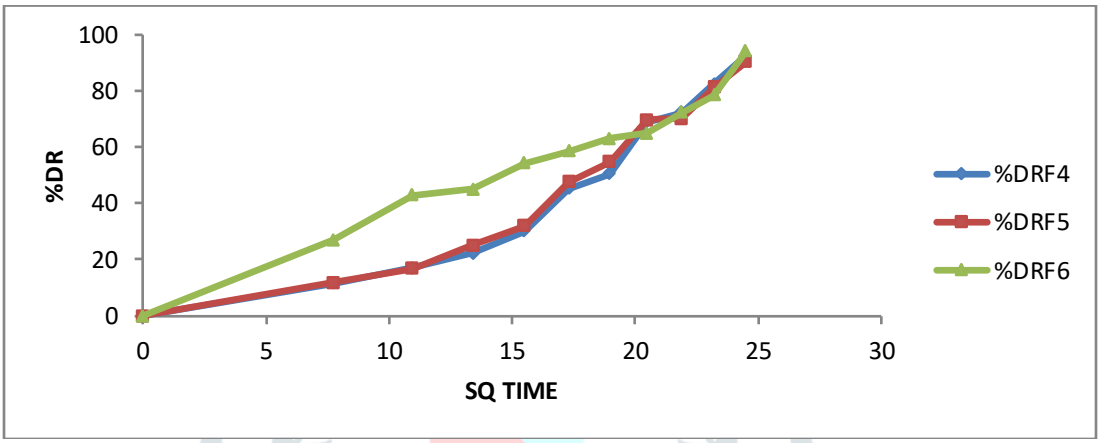

Fig 23: Plot of % drug release vs square root of Time for Beclomethasone dipropionate with Ethyl cellulose

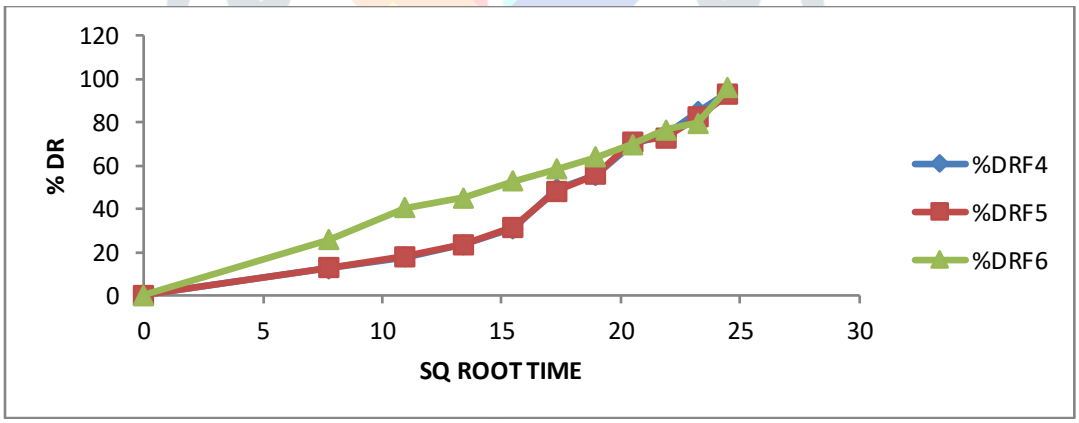

Fig 24: Plot of %drug release vs square root of Time for Clotrimazole with Ethyl cellulose

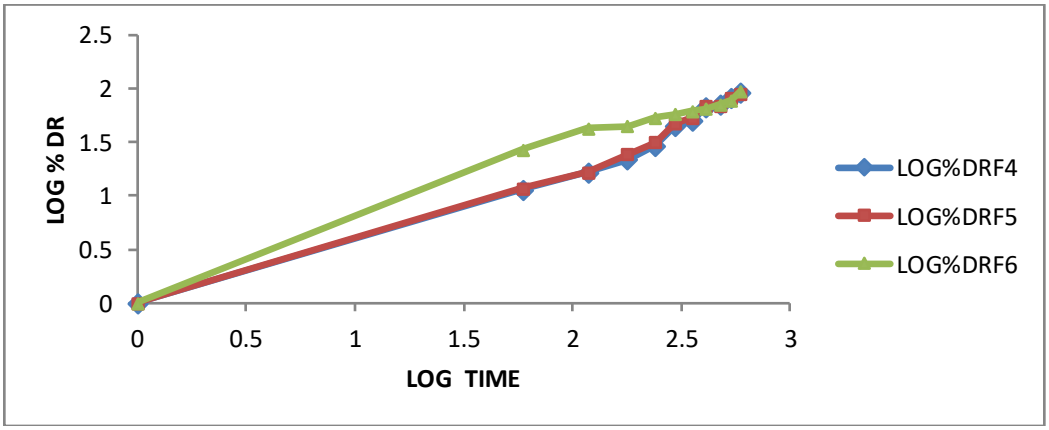

Fig 25: Plot of log of % drug release vs log Time for Beclomethasone dipropionate with Ethyl cellulose

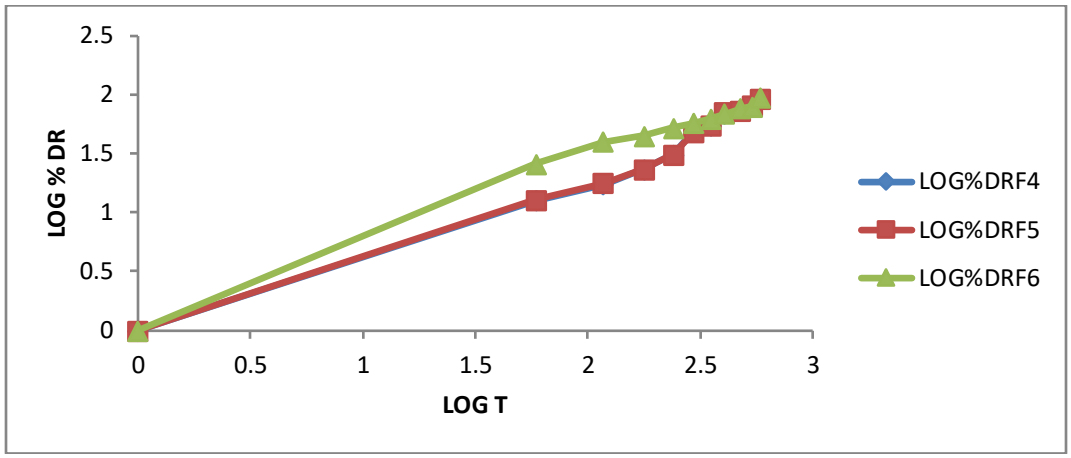

Fig 26: Plot of log of % drug release vs log Time for Clotrimazole with Ethyl cellulose

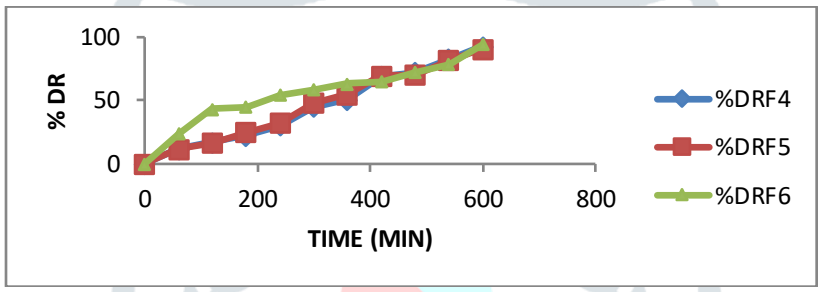

Fig 27: Plot of % drug release vs Time for Beclomethasone dipropionate with Ethyl cellulose

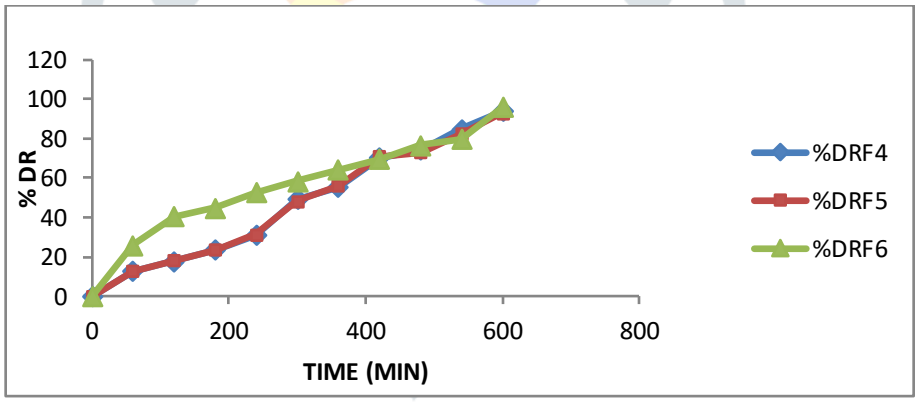

Fig 28: Plot of % drug release vs time for Clotrimazole with Ethyl cellulose

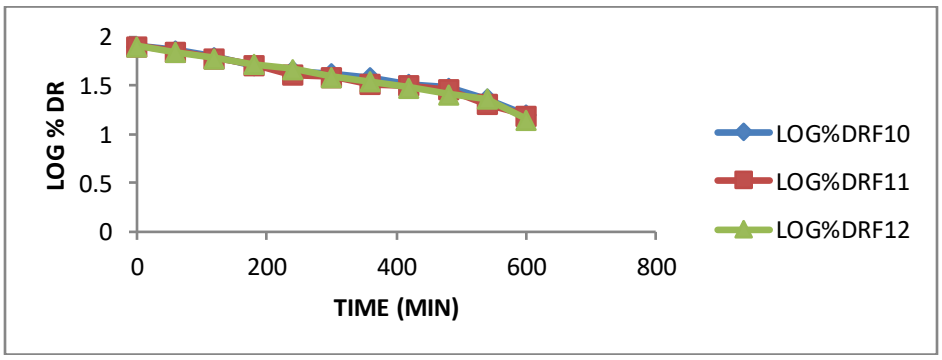

Fig 29: Plot of log % drug remaining vs Time for Beclomethasone dipropionate with eudragit RS

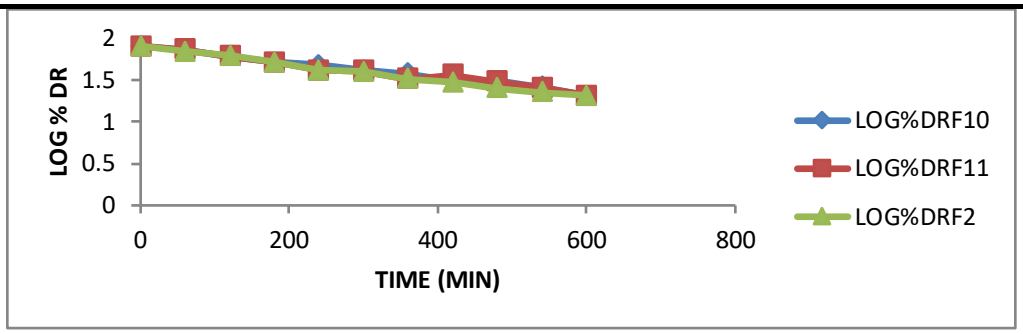

Fig 30: Plot of log % drug remaining vs Time for Clotrimazole with eudragit RS

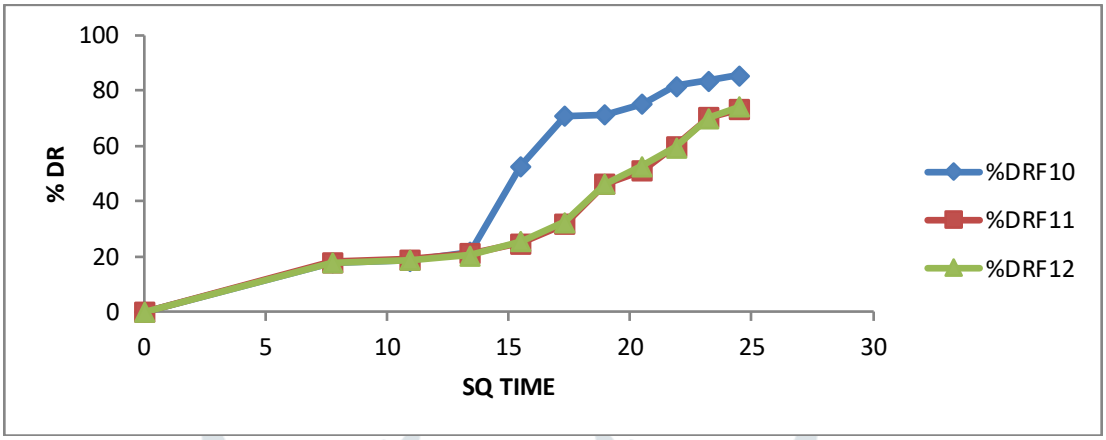

Fig 31: % Drug release vs square root of time for Beclomethasone dipropionate with eudragit RS

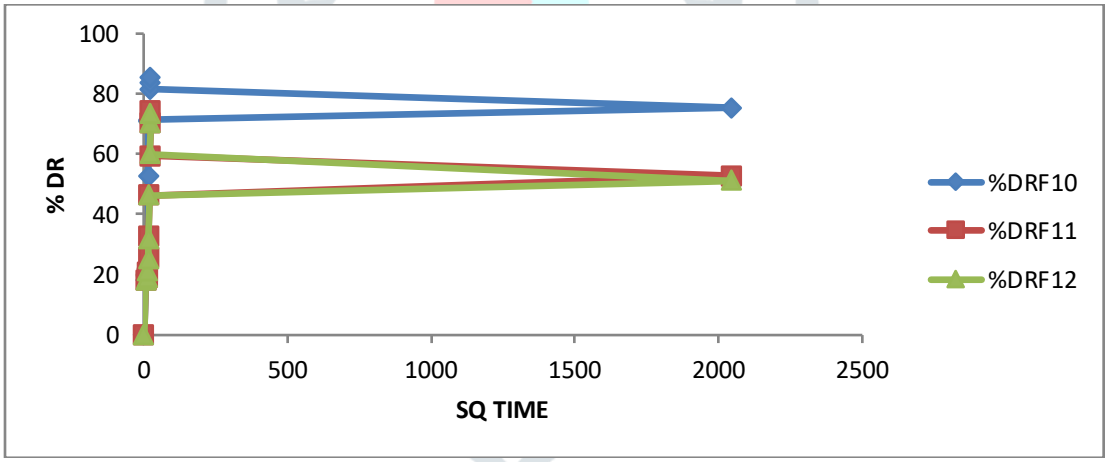

Fig 32: % Drug release vs square root of time for Clotrimazole with eudragit RS

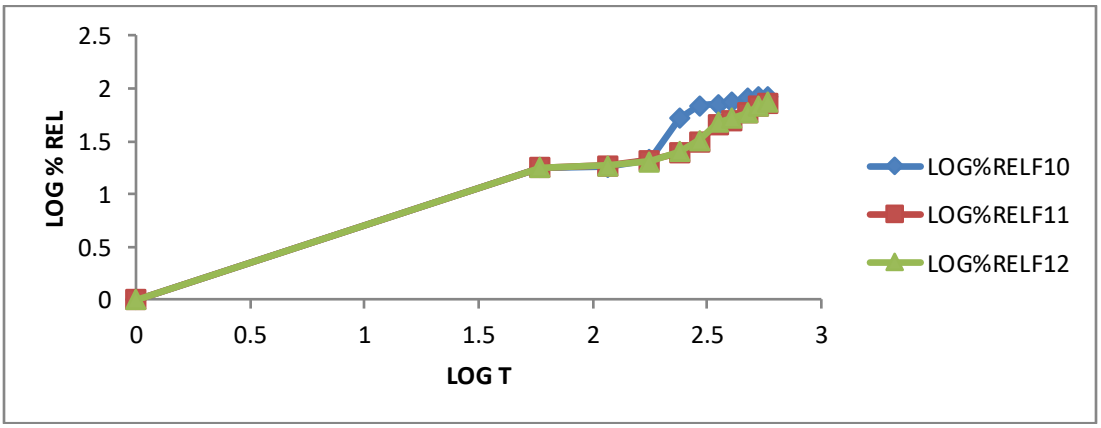

Fig 33: Plot of log % releasing vs log T for Beclomethasone dipropionate with eudragit RS

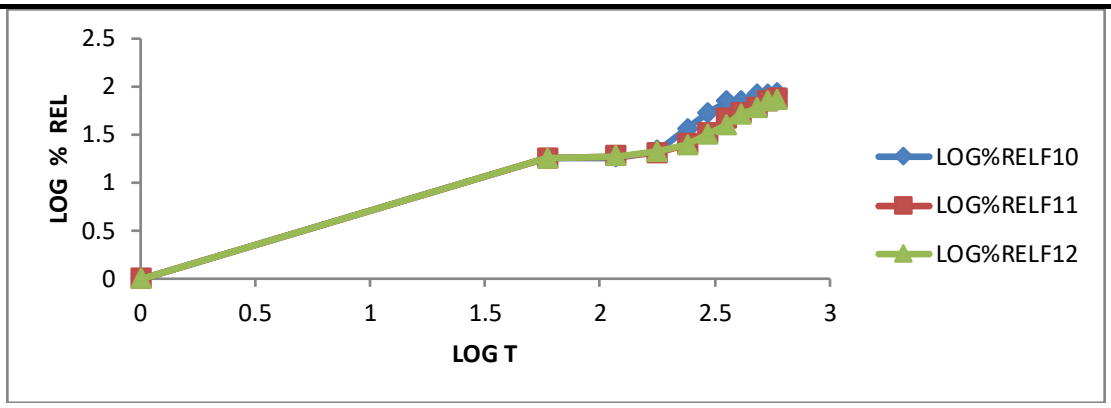

Fig 34: Plot of log % releasing vs log t for Clotrimazole with eudragit RS

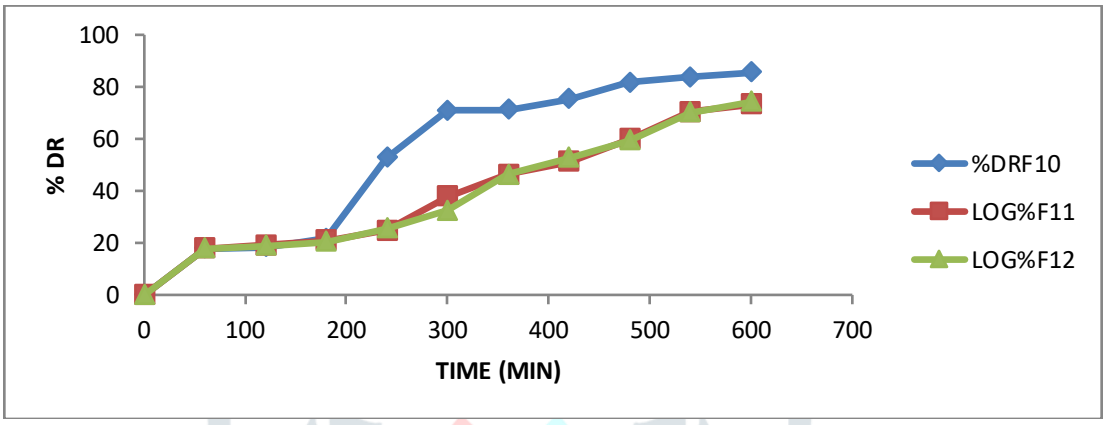

Fig 35: Plot of % drug releasing vs time for Beclomethasone dipropionate with eudragit RS

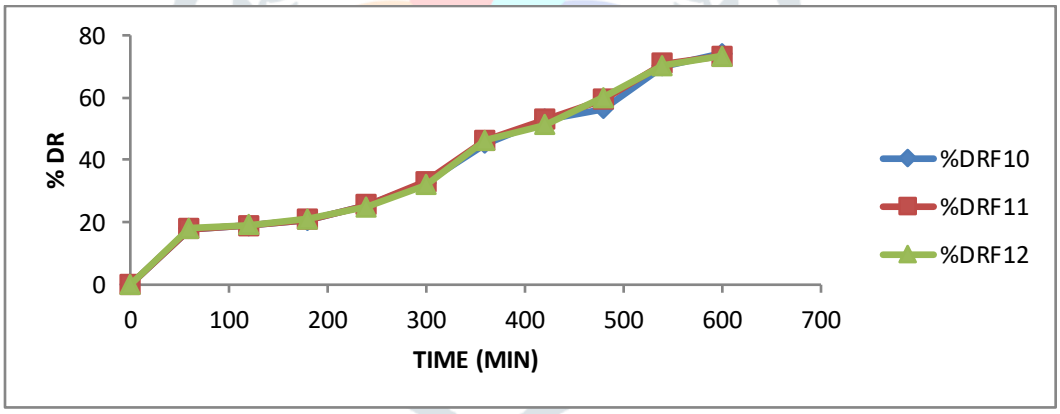

Fig 36: Plot of % drug releasing vs time for Clotrimazole with eudragit RS

CONCLUSION

A topical polymeric microsponge formulation of clotrimazole and Beclomethasone dipropionate was formulated by using ethyl cellulose and eudragit RS 100 polymer. From this study following conclusions were obtained. By considering the solubility studies of the drug and polymer and the rate of diffusion of the solvent used, the internal phase suitable for the preparation of microsponges was found to be ethanol and the external phase was found to be water. The concentration of the polymer required to produce microsponges with good physical and morphological characteristics was found to be 9-13% w/w of the drug. The minimum concentration of an emulsifier PVA required to produce microsponges was found to be 50mg per 200ml. The particle size range increases as increase in amount of polymer in the formulation. The SEM photograph reveals that not all particles are spherical as such, but they contain enormous pores. So microparticles were found spongy in nature. The DSC thermal studies showed that there are constituents that have not undergone to give any reaction product. The ratio of drug polymer ratio required to produce microsponges with good encapsulation capacity was found for the formulations F5, F6 & F11and F12. Below this ratio, the microsponges formed showed low capacity to encapsulate the drug and above this range there may be no further increase in the encapsulation efficiency. Hence it was concluded that F5, F6 & F11 and F12 were optimum ratios of drug: polymer to produce good microsponges. By the drug release studies from the gel formulations AMF1 to AMF5, it can be concluded that AMF5 formulation shows controlled drug release.

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