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FORMULATION AND EVALUATION OF MICROSPONGES GEL FOR TOPICAL USE

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ABSTRACT

The purpose behind undertaking this project was to formulate and evaluate gel containing microsponges of Clobetasol Propionate (CP) to deliver CP in a perennial manner for far-reaching time period to reduce application frequency, hypersensitive reactions and to improve safety than conventional formulation. Quasi-emulsion solvent diffusion method was employed using Ethyl Cellulose as a polymer and microsponges with varied drug–polymer ratios were prepared. For optimization purposes, diverse factors affecting microsponges physical properties were investigated. Microsponges were characterized by SEM, FT-IR, and for particle size analysis and evaluated for drug content and drug loading, morphology and *in-vitro* release study as well. No chemical interactions were found between drug and polymer used which can be seen in results of compatibility studies. Drug-polymer ratio manifested luminary effect on production yield, drug content and drug loading efficiency, drug release and particle size. SEM results revealed spherical microsponges with porous surface and had 22.09 µm mean particle size. The best microsponges formulations were then incorporated into carbopol gel and were evaluated for their viscosity, pH and *in-vitro* drug release study. The microsponges gel results depicted that gel formulation G-F4 with 1:1 drug–polymer ratio was more efficient to give extended drug release upto 75.75 % at the end of 12 h while conventional formulation exhausted extremely only after 2.5 h. Thus, the formulated microsponge-based gel of clobetasol propionate would be a likely substitute to conventional therapy for safer and efficient treatment of inflammation, psoriasis and chronic hyperkeratotic eczema.

KEYWORDS: Clobetasol Propionate, Microsponges, Polymer (Ethyl cellulose), Extended release.

1. INTRODUCTION

Psoriasis is a relatively common skin disease that affects approximately 2% of the world's population. It is a chronic and T-cell-mediated autoimmune disorder with hyper proliferation of the epidermis and inflammatory reactions of the dermis and epidermis.[1] The treatment of psoriasis varies depending on disease severity and spread. However, topical medications remain the mainstay of psoriasis treatment for most patients. As seen in the literature, topical corticosteroids and particularly super potent ones are the most widely prescribed medications for the topical treatment of psoriasis for decades in the world. They are available in numerous vehicles including powders, sprays, lotions, solutions, creams, emollient creams, ointments, gels and medicated tapes.[2] Although serious cutaneous and systemic side effects of the corticosteroids have limited their use, they are still among the most effective treatments.[1]

Clobetasol Propionate (CP) is a dihalogenated highly potent glucocorticoid[4] topical steroid. CP is a white or almost crystalline[3,5] tasteless powder. A derivative of prednisolone with high glucocorticoid activity and low mineralocorticoid activity.[3,4]

It has been used in clinical practice because of its anti-inflammatory, anti-pruriginous, and vasoconstrictor activities,[5] used topically in treatment of psoriasis.[4] CP has been shown to suppress the hypothalamic-pituitary-adrenal (HPA) axis at the lowest doses tested. Cushing's syndrome, hyperglycemia and unmasking of latent diabetes mellitus can also result from systemic absorption of topical corticosteroids. Children may be more susceptible to systemic toxicity from use of topical corticosteroids.[29]

Local adverse reactions with topical corticosteroids may occur more frequently with the use of occlusive dressings and higher potency corticosteroids, including Clobetasol propionate. These reactions include: folliculitis, acneiform eruptions, hypopigmentation, perioral dermatitis, allergic contact dermatitis, secondary infection, striae and miliaria.[6]

Topical corticosteroids can be absorbed from normal intact skin. Inflammation and other disease processes in the skin may increase percutaneous absorption.[29]

Being a highly potent topical corticosteroid side effects and adverse effects of drug could occur due to burst release of drug on application. So, there is a need to modify the release

pattern in a way to control the delivery of actives to a predetermined site in the human body. By limiting the release and delivery rate of actives from the topical formulation on to the epidermal layer would lead to increase the drug residence time as well as reduces the absorption of drug in the systemic circulation resulting in reduced side as well as adverse effects. The Microsponge delivery system (MDS) is a polymeric microsphere system uniquely fulfilling these above requirements by providing topical controlled drug delivery systems.

Microsponges are polymeric delivery systems consisting of porous microspheres[7,8,9] these are tiny sponge like spherical particles that consists of myriad of interconnecting voids within a non-collapsible structure with large porous surface.[7,10] The size of these microsponges can be varied, usually from 5-300 µm in diameter.[7] Microsponges are used as topical drug carriers that allow an even and sustained rate of release and therefore reduce irritation while maintaining efficacy. In addition, the microsponge particles themselves are too large to be absorbed into the skin and this adds a measure of safety to the microsponge materials.[11]

Microsponges drug delivery systems have been extensively used for oral administration and bone & tissue engineering and could also be useful to deliver several drugs into the skin.[8,12] However, there are few researches about dermal application of microsponges. Several drugs such as: Benzoyl peroxide, hydrocortiscone, zinc pyrithione, selenium sulphide, hydoquinones, suncscreens[9,15] were successfully encapsulated into microsponges for several purposes.

The aim of this study was to develop a topical drug delivery system in order to provide the prolonged and controlled release of clobetasol propionate, minimize the systemic drug absorption and reduce the possible side effects of the drug. For this purpose, microsponges loaded with Clobetasol propionate were prepared and characterized. The effects of different drug-polymer ratios and polyvinyl alcohol (PVA) concentrations on the characteristics of the microspheres were evaluated. Selected microsponges formulation was formulated into the carbopol gel base. Carbopol gel is more easily spreadable base for the wide surfaces like psoriatic lesions compared to cream or ointment formulations. Release profiles of Clobetasol propionate from carbopol gel formulations were compared with commercial product of the drug.

2. MATERIAL AND METHODS

Table 1: Showing material used in preparation of microsponges

S.no	Chemicals	Source
1.	Clobetasol Propionate	Gift sample by Glenmark Pharmaceutical Ltd., Baddi, H.P.
2.	Ethyl Cellulose	SD Fine Chem Ltd.
3.	Tri ethyl citrate	Merk Specialities pvt. Ltd
3.	Poly Vinyl Alcohol	Bombay Drug House Pvt. Ltd
4.	Di Chloro Methane	Merk Specialities pvt. Ltd
5.	Carbopol- 934 LR	SD Fine Chem Ltd.
6.	Distilled water	Made in laboratory through distilled water assembly by Perfit India.

2.1. PRE-FORMULATION STUDIES OF DRUG

2.1.1. Physical Characterization and Identification of Clobetasol Propionate:

The drug identification is based on the following parameters:

2.1.1.1. Organoleptic property of drug

Drug (*Clobetasol Propionate*) was physically characterized on the basis of color, nature, odor and taste. All these parameters were recorded and compared with standard.

2.1.1.2. Determination of Melting Point

The reported Melting point (M.P) of CP is in range 195.5-197C [14], approximately 196 a/c *USP 2*4).

Melting point of the Clobetasol propionate was determined by capillary tube method. A capillary tube was filled with drug crystals about 3 mm high. Then capillary tube was put (open end down) into the crystals and tapped on the bottom of the crystallization dish to got the crystals into the tube. The crystals were forced to slide to the bottom of the tube by tapped the tube (open end up) on the lab bench. Then the capillary tube was inserted in the melting point apparatus. The temperature of apparatus was then increased to make a rapid determination of melting point. Melting process was observed through the magnifying lens. Procedure was repeated 3 times to obtained readings in triplicate.[13]

2.1.1.3. Solubility of Clobetasol propionate.[5]

Table 2: Solubility profile of Clobetasol propionate.

S.NO	SOLVENT	SOLUBILITY
1.	Distilled water	Insoluble
2.	Di chloro methane	Soluble
3.	Alcohol	Sparingly soluble
4.	Chloroform	Soluble
5.	Acetone	Slightly soluble
6.	Dimethyl sulphoxide	Soluble

2.1.1.4. Partition Coefficient

For determination of partition coefficient of drug, equal ratio of chloroform and water (H_2O) *i.e.*10 ml each was taken in a separating funnel. In this mixture excess amount of drug was added and shaked properly for mixing of drug in both the phases. The solution mixture was left for 24 h for proper separation of drug into two phases i.e. chloroform and water. After 24 h chloroform and water phases were individually took out in the separate beakers. For better clearance the obtained filtrate was sonicated for 15 min at 80 Hertz (Hz) and then diluted. The absorbance was checked at 242.4 nm. The same procedure was repeated in triplicate for better accuracy.[16,17]

2.1.1.5. Infrared Spectroscopy

Fourier Transform Infrared (FTIR) spectra of CP were recorded over the wavelength range of 4500 to500 cm^{-1}. It was done using Fourier Transform Infrared Spectrophotometer using KBr pellet method. The characteristics Infrared (IR) absorption peaks of CP were studied and spectral interpretation was done. *(Shown in appendix A).*

2.1.1.6. UV spectroscopy[18,19]

Clobetasol propionate has characteristics UV absorption at λ max (MeOH) 237 nm (ε=15000).[19] Calibration curve of CP was plotted using methanolic water (40:60) by keeping concentration range of 5-30 µg/ml. The drug was analyzed spectrophotometrically by Double Beam Spectrophotometer (shimadzu 1800 UV-VIS) in range 200- 400 nm. (Regression coefficient r^2 = 0.999).

2.1.1.7. Drug–excipient interaction study

Drug–excipient interactions were investigated by FTIR study. IR spectra were recorded to check compatibility of drug with excipients, using FTIR spectrophotometer (FTIR, A-410, Jasco, Japan) over wavelength range of 4500 to500 cm^{-1} at resolution of 4 cm^{-1}. KBr dispersed samples were compressed in pellets by applying 5 tons pressure for 5 min using hydraulic press. Formed pellets were kept in light path and spectra were recorded. The characteristics IR absorption peaks of CP were studied and spectral interpretation was done.

3.0. FORMULATION DEVELOPMENT OF MICROSPONGES[20,21]

The microsponges were prepared by ***Quasi-emulsion solvent diffusion method***. The internal phase consists of ethyl cellulose dissolved in 20 ml of dichloromethane. This was followed by addition of CP dissolved under ultra sonication. The surfactant PVA (50 mg) was weighed

accurately and dissolved in 90 ml of distilled water at 60˚C. The surfactant mixture was allowed to cool to room temperature. The internal phase containing Clobetasol propionate and ethyl cellulose polymer was added drop wise with stirring at 400 rpm. After 3h of stirring, microsponges were formed due to the removal of solvent, dichloromethane (DCM) from the system by evaporation. The microsponges were washed 3 times with distilled water, filtered and dried overnight at room temperature. For the evaluation of the effect of drug: polymer ratio on the physical characteristics of microsponges, six different ratios of the drug to ethyl cellulose (1:1, 1:2, 1:3) with varying DCM amount were employed.

The obtained microsponges were then stored in a glass container for further use i.e. for characterization and incorporation into gel base to make microspongic gel.

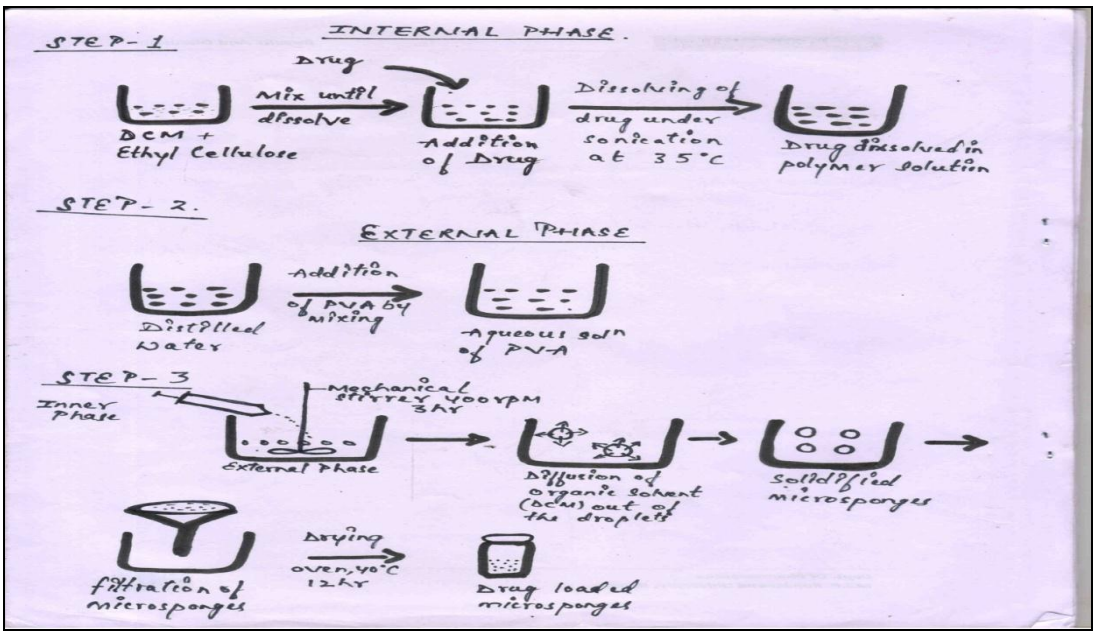

Figure 1: Showing steps involved in preparation of microsponges by quasi-emulsion solvent diffusion method.

3.1. FORMULATION CHART FOR MICROSPONGE PREPARATION

Table 3: Showing all formulations composition.

S. no	Formu-lation Code	Drug: Polymer Ratio	Drug (Clobetasol propionate)	Ethyl- Cellulose (polymer) (mg)	Di Chloro Methane (ml)	Poly Vinyl Alcohol (mg)
1.	**F 1**	1:1	750	750	20	50
2.	**F 2**	1:2	500	1000	20	50
3.	**F 3**	1:3	350	1125	20	50
4.	**F 4**	1:1	750	1000	20	75
5.	**F 5**	1:2	500	500	20	75
6.	**F 6**	1:3	350	1125	20	75

4.0. EVALUATION OF MICROSPONGES

4.1. Physical appearance

The microsponges were physically evaluated visually for their appearance, color and flow property.

4.2. Production yield[22]

Microsponges production yield was determined by the formula mentioned below:

$$Production\ Yield = \frac{Practical\ mass\ of\ microsponges}{Theoretical\ mass *} \times 100$$

4.3. Loading efficiency[22]

Loading efficiency of microsponges was determined by the below written formula:

$$Loading\ Efficiency = \frac{Actual\ drug\ content\ in\ microsponge}{Theoretical\ Drug\ Content} \times 100$$

4.4. Actual drug content[22]

The weighed amount of drug loaded microsponges (50 mg) was dissolved in 100 ml solvent, methanol: water (40: 60) with continuous stirring. Filtered samples (using whatmann filter paper) were analyzed at 242.4 nm against blank using UV spectrophotometer (Shimadzu 1800, Japan). Estimation of drug content for all batches was done using the following expressions:

$$Actual\ drug\ content\ (\%) = \frac{M_{act}}{M_{mms}} \times 100$$

Where, M_{act} *= actual CP content in weighed quantity of microsponges,*

$M_{mms} = weighed\ quantity\ of\ microsponges$

4.5. Morphology and Surface topography[21]

The morphology and surface characteristics of the microsponges were examined using a scanning electron microscope (SEM analyzer, GEOL 5400, USA) operating at 15Kv at Babasaheb Bhimrao Ambedkar University (BBAU), Lucknow, U.P., India.

Dried microsponges were coated with gold–palladium alloy for 45 s under an argon atmosphere before observation. SEM photographs were recorded at magnification of × 500, 1000, and 10,000.

4.6. Infrared spectroscopy

It was done using a Fourier Transform Infrared Spectrophotometer (FTIR A-410, shimadzu) using KBr pellet method at SRMS College of Engg. & Tech., Bareilly, U.P., India.

FTIR spectra of CP and microsponge formulation were recorded in the wavelength range of 4500 to 500 cm−1. *(Shown in appendix A& B).*

4.7. In-vitro release study[23,24]

The pH of normal healthy human skin is between 4.5 and 6. However, the pH value rises beyond 6, when a person actually suffers from a skin problem or skin disease. So, the drug release studies of Clobetasol propionate microsponge were done in methanolic water.[23]

The in-vitro release of microsponge formulations were studied using cellophane membrane using modified apparatus. The dissolution medium used was methanolic water (40: 60). Cellophane membrane previously boiled for 1 h in the dissolution medium (methanolic water; 40:60), was tied to one end of a specifically designed glass cylinder (open at both ends). Formulation (equivalent to 10 mg of Clobetasol propionate) was accurately placed into this assembly. The cylinder was attached to stand and suspended in 100 ml of dissolution medium maintained at 37 ± 1°C and the membrane was just touching the receptor medium surface. The dissolution medium was stirred at 100 RPM speed using teflon coated magnetic bead. Aliquots each of 1ml (diffusion medium) was withdrawn at a specific interval and replaced by an equal volume of the receptor medium to maintain the sink condition. The samples were taken periodically for 12 h. The samples were measured spectrophotometrically at 242.4 nm by UV spectrophotometer (Shimadzu UV-VIS 1800 Pharma spec.).

The cumulative percentage of drug released was plotted against time to find the drug release behavior of all microsponge formulations.

4.8. Particle size analysis

Particle size analysis was executed by optical microscopy using optical microscope as well as by SEM.

5.0. INCORPORATION OF CLOBETASOL PROPIONATE MICROSPONGES INTO GEL[22,23]

To obtain a suitable topical formulation for application, microsponges were incorporated into a gel base.

For preparing CP microsponge gel, 1 g of Carbopol 941 LR was uniformly dispersed in beakers containing sufficient quantity of water and was allowed to hydrate overnight. Then it was mixed with 5 g of glycerin to form paste. Later 95 ml of water was added slowly to paste under constant stirring, followed by drop wise triethanolamine addition to adjust pH to 6.5–7.5. Clobetasol propionate microsponges equivalent to 0.05% w/w of drug were dispersed into gel base and stirred until a viscous smooth translucent gel was obtained.[25]

5.1. CHARACTERIZATION OF CLOBETASOL PROPIONATE LOADED MICROSPONGE TOPICAL GEL

From the in-vitro release studies, the best microsponge formulations F1 and F4 was selected and incorporated into gel base and evaluated for various parameters *viz*. homogeneity, pH of the gels, viscosity, drug content and in-vitro permeation study.

5.2. Homogeneity[26]

Both developed gels were tested for homogeneity by visual inspection after the gel has been set in the container. They were tested for their appearance and presence of any aggregates.

5.3. Viscosity[25,26]

The intrinsic viscosity of gel formulations were measured using a Model Brookfield Viscometer. The viscometer was operated at 100 rpm using a T-F (code 96) spindle. In order to obtain stable display readings, all measurements were recorded 60 sec after the commencement of spindle rotation and a maximum of three (3) readings were taken to obtain an average viscosity value.

5.4. Determination of pH[27]

The pH of extemporaneously manufactured CP gel formulations were measured using the pH meter.

Diverse gel formulations pH was recorded using digital pH meter. 5g gel was dispersed in 45 ml distilled water at 27 ºC and solution pH was measured. The measurements were taken within twenty four (24) h of manufacture of the extemporaneous formulations. The pH

measurements were recorded in triplicate to generate an average pH value for each formulation.[27]

5.5. Drug content[25]

The CP loaded microsponges gel (1g) was accurately weighed and dissolved in methanolic water (40:60) solvent system, sonicated for a period of 10-15 min and volume was made to 100 ml in volumetric flask with methanolic water (40: 60). From this 10 ml was pipetted out and diluted to 100 ml with methanolic water and the final dilution was made using methanolic water to got the concentration within Beer's range. The absorbance of filtered samples (using whatmann filter paper) was analyzed spectrophotometrically at 242.4 nm against blank gel, treated in the same manner as sample. Estimation of drug content for F1 and F4 formulations were done using the following expressions:

$$\textit{Actual drug content (\%)} = \frac{M_{act}}{M_{mms}} \times 100$$

Where, M_{act} = actual CP content in weighed quantity of microsponges,

M_{mms} = weighed quantity of microsponges

5.6. In-vitro release study[24,27]

The *in-vitro* release of microsponges gel formulations were carried out using cellophane membrane using modified apparatus. The dissolution medium used was methanolic water (40: 60). Cellophane membrane previously boiled for 1h in methanolic water and was tied to one end of a specifically designed glass cylinder (open at both ends). 16.766 mg gel formulation (equivalent to 10 mg w/w of Clobetasol propionate) was accurately placed on the cellophane membrane. The cylinder was attached to stand and suspended in 100 ml of dissolution medium (methanolic water) maintained at 37 ± 1°C and the membrane was just touching the receptor medium surface. The dissolution medium was stirred at 100 rpm speed using teflon coated magnetic bead. Aliquots each of 1ml (diffusion medium) was withdrawn at a specific interval and replaced by an equal volume of the receptor medium to maintain the sink condition. The samples were taken periodically for 12 h. The samples were measured spectrophotometrically at 242.4 nm by UV spectrophotometer (Shimadzu UV-VIS 1800 Pharma spec.).

The cumulative percentage of drug released from gel was plotted against time to find the drug release behavior of all microsponges formulations.

6.0. RESULTS AND DISCUSSION

6.1. PRE-FORMULATION STUDY

6.1.1. Characterization of Clobetasol Propionate pure drug

6.1.1.1. Description: Clobetasol Propionate (CP) was found white to creamy white crystalline powder.

6.1.1.2. Melting point: Melting point of Clobetasol propionate was found to be in the range of 195 - 196.5 ºC (literature standard 195.5 -197 ºC).

6.1.1.3. Solubility: Soluble in methanol, chloroform and Di chloro methane while very slightly soluble in acetone and insoluble in water.

6.1.2. SPECTROSCOPIC STUDIES

6.1.2.1. IR Spectroscopy: IR Spectra of Clobetasol propionate in its pure form was recorded. Results are depicted in *appendix* A and *Table 4.*

Table 4: IR spectrum interpretation of clobetasol propionate.

Functional group	Wave number observed (cm^{-1})
1661	C=C stretching of the aliphatic non- conjugated alkene.
1608	C=O stretching of the ketone.
1734	C-Cl stretching of chlorine.
1066	COO stretching of the ether.
1009.78	O-H bending of the alcohol.

6.1.2.2. UV Spectroscopy: (Determination of λ max)

For UV spectroscopy Clobetasol propionate in methanolic water (40: 60) was scanned from 200-400 nm. The λ max was found to be 242.4 nm.

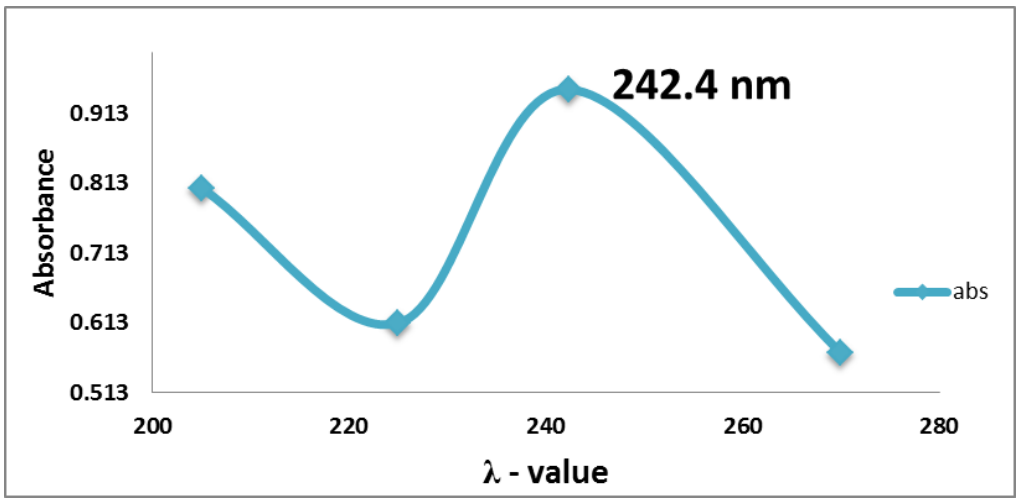

Figure 2: Showing maximum lambda max of clobetasol propionate.

6.1.2.3. DRUG -EXCIPIENT COMPATIBILITY STUDIES

6.1.2.3.1. Physical Change

No physical changes such as: discoloration, change in texture etc. were observed during compatibility study.

6.1.2.3.2. FTIR Study

FTIR spectra of 'pure drug' and 'drug entrapped microsponges' were compared to study incompatibility of drugs with excipients. Principal peaks of microsponge entrapped drugs were compared with peaks of pure drugs.

Vibrational peaks of Clobetasol propionate detected in drug loaded microsponges formulation associated with C=C stretching of the aliphatic non-conjugated alkene, C-Cl stretching of chlorine, COO stretching of the ether and O-H bending of the alcohol were identified at 1665.60, 1738.90, 1112.01 and 1009.78.

Principle peaks of drugs were retained; broadening of peaks may be due to overlapping of peaks of polymer system and drug in microsponge formulation. Thus, IR spectroscopy results depicted that CP was compatible with selected polymer, excipients and possess good stability in all microsponge formulations.

As experimental values were in good agreement with standard, procured drug was supposed to be pure.

6.2. EVALUATION OF CLOBETASOL PROPIONATE MICROSPONGES

6.2.1. Physical appearance

White color microsponges particles were obtained by quasi- emulsion solvent diffusion method. Flow properties of drug were noted to be poor while it has been observed that microsponges of Clobetasol propionate had good flow properties.

6.2.2. Production Yield

Production yield (PY) of all formulations of Clobetasol propionate microsponges was observed in the range 62.667 to 83.50 for formulations F1 to F3 and 66.90% to 86.650 % for formulations F4 to F6 *(Table 5).*

Table 5: Production yield of Clobetasol propionate microsponges formulations.

Formulation code	Production yield
F 1	62.667±0.907
F 2	70.670±0.855
F 3	83.50±0.681
F 4	66.90±0.815
F 5	73.437±0.637
F 6	86.650±0.655

***each value is average of three separate determinations ± SD**

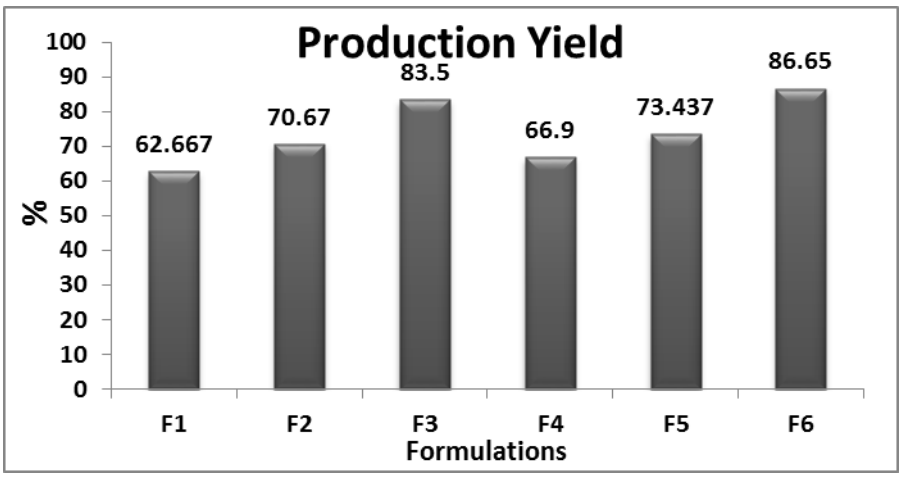

Figure 3: Graphical presentation of Production yield of Clobetasol propionate microsponges formulations.

It was found that the production yield was greatly affected by drug: polymer ratio as well as by concentration of poly vinyl alcohol (PVA). Moreover, increase in the drug: polymer ratio resulted into increased production yield from formulation F1 to F3 and F4 to F6.

With the high PVA conc. (750 mg) in formulations F4 - F6, the production yield was high as compare to formulations F1 –F3 which contained low PVA conc. (50 mg) in them. This was for the reason that the abridged dichloromethane diffusion rate from concentrated solutions to aqueous phase at higher drug: polymer concentrations provides additional time for formation of droplet, thereby improved yield.

6.2.3. Drug loading efficiency and drug content

At all ratios of drug: polymer employed, the mean amount of drug loaded in the prepared microsponges was lower than the theoretical value, since the drug loading efficiency did not reach 100%. This could be attributed to dissolution of some drug in the solvent or aqueous phase employed. Loading efficiency (LE) outcomes reflected that higher drug: polymer ratios

led to superior drug loadings. Use of the higher amount of polyvinyl alcohol for formulations F4, F5 and F6 while preparing microsponges caused slightly increased viscosity of the dispersed phase. When solvent was diffused out, nearly all of the dispersed phase was converted to solid microsponges and estranged particles emerged. The reason behind utmost drug loading efficiencies for these formulations was availability of maximum polymer amount to each drug unit in contrast to the rest of formulations.

Table 6: Drug loading efficiency of Clobetasol propionate microsponges formulations.

Formulation code	(%) Drug loading efficiency	% Actual drug content
F1	81.130±1.310	64.620±0.803
F2	78.662±0.709	34.02±0.816
F3	74.510±0.734	21.45±1.032
F4	88.377±0.741	66.33±0.613
F5	80.533±0.701	36.36±0.775
F6	76.273±1.030	22.017±0.076

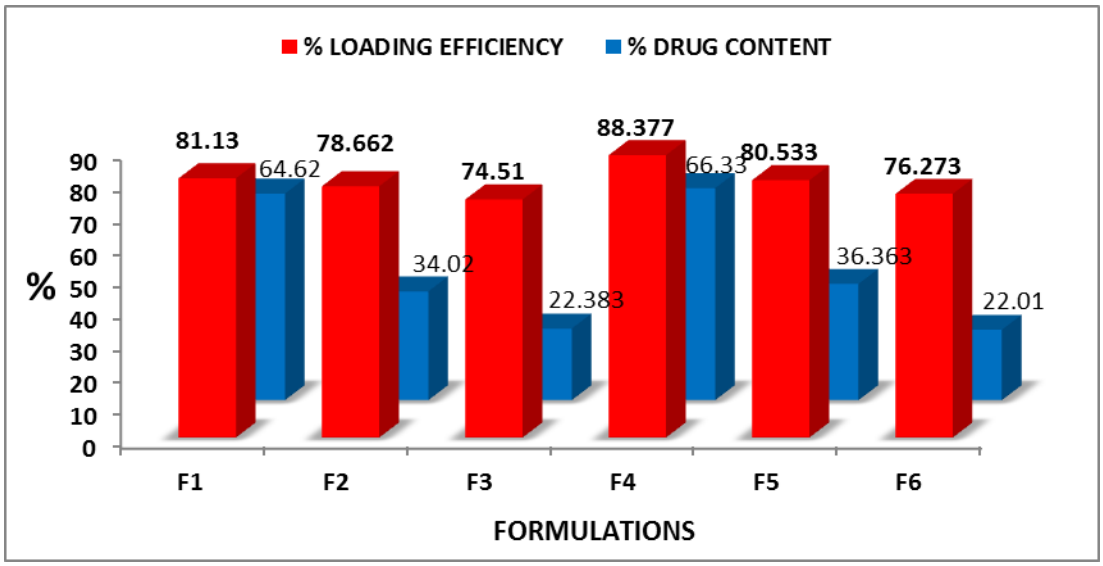

Figure 4: Graphical presentation of loading efficiency of Clobetasol propionate microsponges formulations.

The loading efficiency was noted in the range 81.130 – 74.510% for formulations F1- F3 while loading efficiency was noted in the range 88.377– 76.273 % for formulations F4- F6 as shown in *Table 6.*

Actual drug content of formulations decreased with increase in drug: polymer ratio in formulations F1-F3 and F4-F6. But formulations with high conc. of polyvinyl alcohol have more drug content as compare to formulations that contains less amount of polyvinyl alcohol. Drug content was found to be in range 64.62±0.80 to 21.45±1.03 for formulations F1- F3

while it was found in range 66.33±0.613 to 22.017 for formulations F4- F6 as shown in *Table 6.*

6.2.4. Scanning electron microscopy

Morphology and surface topography of prepared microsponges by quasi-emulsion solvent diffusion method were investigated by SEM. The representative SEM photographs of the microsponges are shown in *Figures 5-10.*

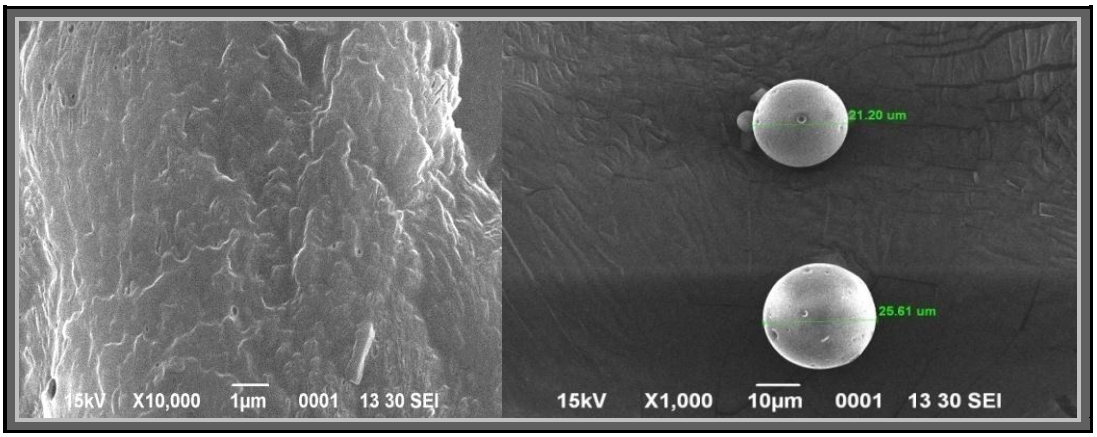

Figure 5: SEM images of microsponges F1 formulation.

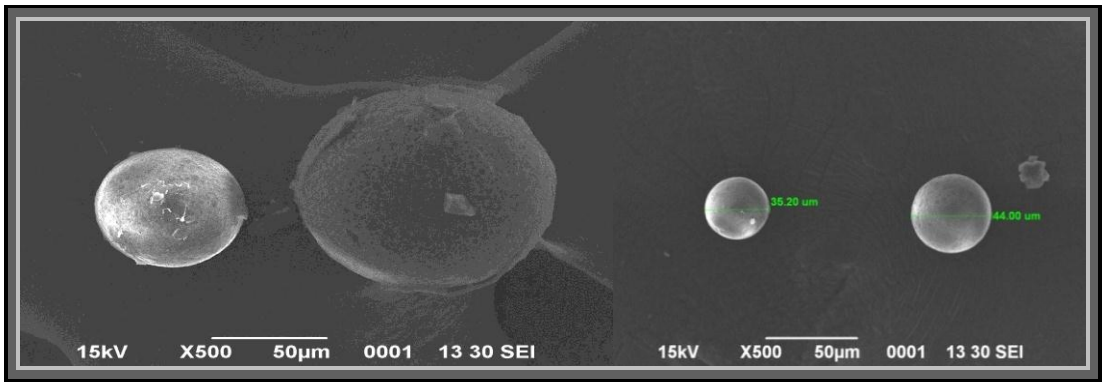

Figure 6: SEM images of microsponges F2 formulation.

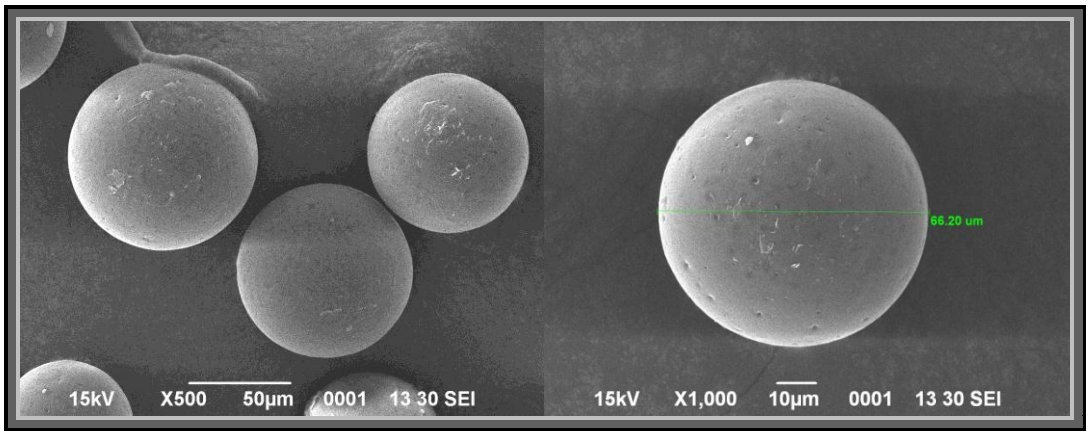

Figure 7: SEM images of microsponges of F3 formulation.

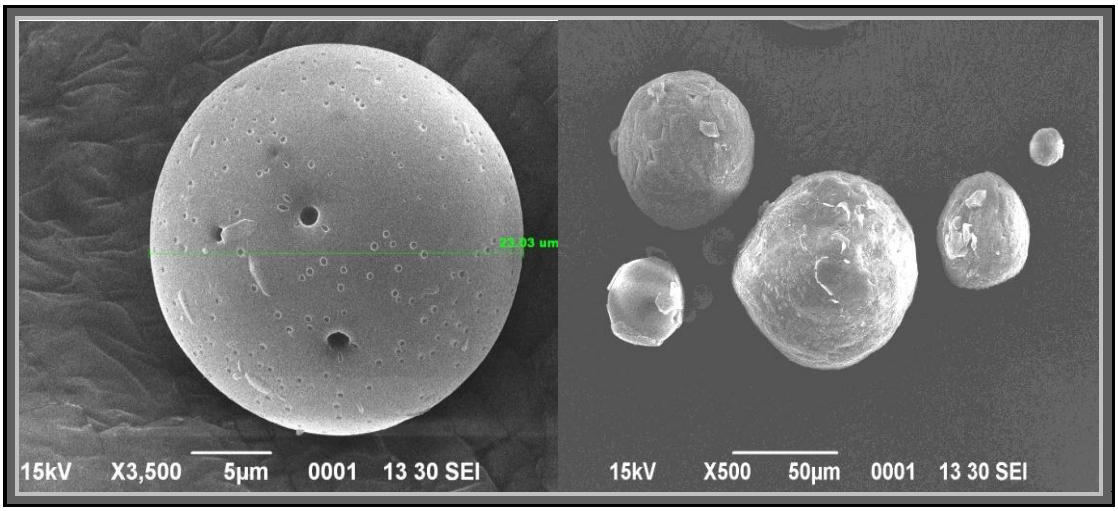

Figure 8: SEM images of microsponges of F4 formulation.

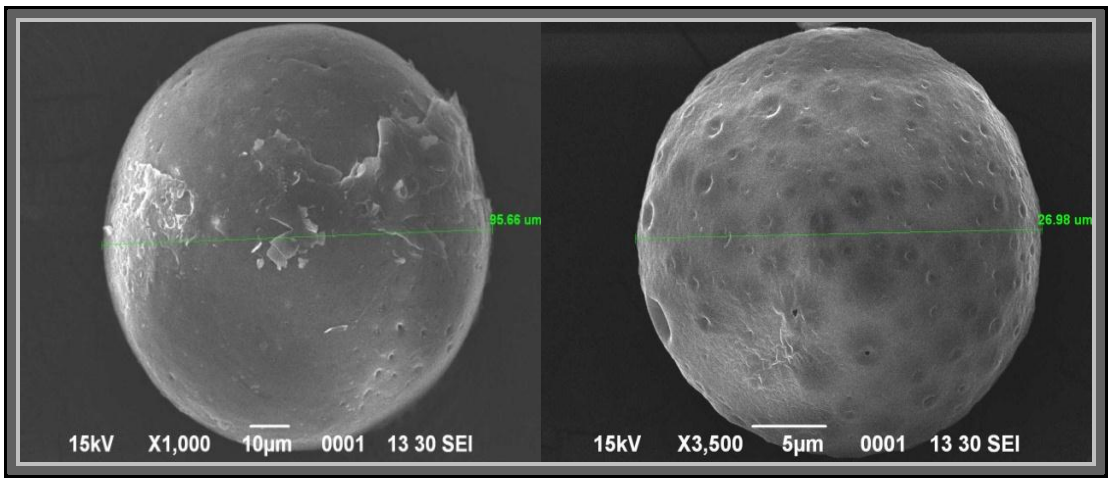

Figure 9: SEM images of microsponges F5 formulation.

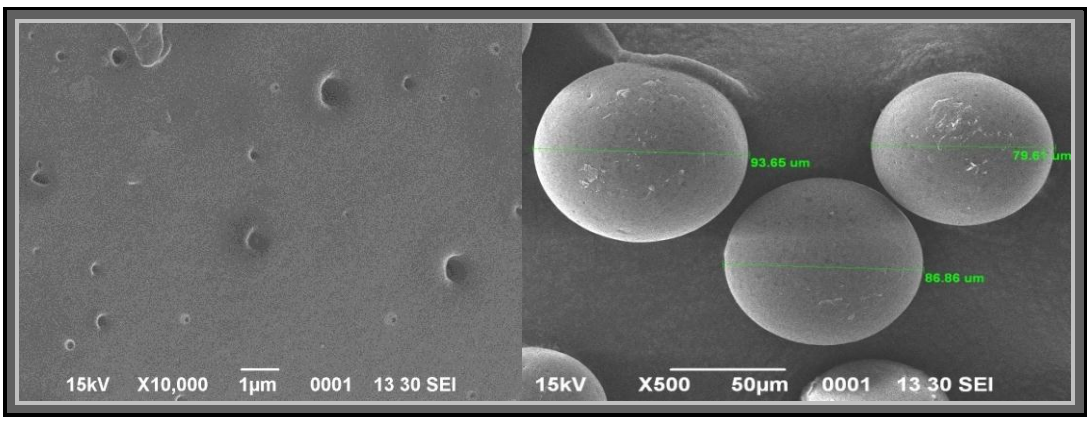

Figure 10: SEM images of microsponges F6 formulation.

The representative SEM images of microsponges as shown in *Fig.5- Fig.10.* SEM results indicate that microsponges formed were highly porous, predominantly spherical and no Clobetasol propionate crystals were observed visually on the surface. Closer view of a

microsponge revealed the characteristic internal pores on the surfaces. By diffusion of dichloromethane from surface of microsponges pores were induced. Moreover, it was supposed that the distinctive internal structure comprised spherical cavity enclosing a stiff shell assembly of drug and polymer. The internal structure consisted of numerous annulled spaces and appearance of particles was such that they were perfect to be called microsponges. The microsponges were also observed under optical light microscope *(Fig. 11)*, which showed that formed microsponges were spherical in each single entity or in form of bunches and had porous nature.

Figure 11: Optical light microscopy images of microsponges.

6.2.5. Particle Size analysis

The mean particle size of microsponge formulations should be in the range of 5-300 µm. The observed particle size by optical optical light microscopy was found in range from 22.09 µm to 90.57 µm as shown in *table 7.*

Visual inspection of all batches for particle size using optical microscope revealed that the particle size was increased with increase in ethyl cellulose amount, i.e. with an increase of drug: polymer ratio. This might be due to the fact that polymer available at higher drug: polymer ratio was in greater amount thereby increasing polymer wall thickness, which consequently led to larger microsponges. In addition with increasing amount of polyvinyl alcohol, particle size was found to be increased, credited to the rise in apparent viscosity at increased concentrations. It results in larger emulsion droplet formation and finally in greater microsponge size. Optimized batches possessed greater percentage of intact, uniform and spherical particles during optical microscopy.

Table 7: Showing particle size of microsponges by optical microscopy.

Formulations	Particle Size (µm)
F 1	22.09±0.91
F 2	30.94±4.11
F 3	59.30±24.48
F 4	26.66±1.036
F 5	71.22±25.94
F 6	90.57±3.43

It was found that the observed particle size by optical microscopy matches to the results of particle size that has obtained from scanning electron microscopy. So, we can say that the optical microscopy results are more relying.

6.2.6. In- vitro drug release from microsponges

The *in-vitro* drug release profiles of CP fabricated in all formulations of microsponges were conducted taking methanolic water (40: 60) as diffusion medium. The drug release was observed to decline within range of 88.28% to 77.40% with respect to rise in drug: polymer ratio from 1:1 to 1:3 (in F1- F3 which contained PVA 50 mg) and 79.16% to 71.29% for formulations F4- F6 (that contained PVA 75 mg). The reason behind is that as the drug: polymer ratio has increased for each microsponge to encapsulate drug, polymer amount available was greater. It led to thickening of the polymer matrix wall, thus extending

diffusion path and ultimately lessening drug release rate. The highest drug release i.e. 88.28 % was found for F1 while the lowest 74.06% for F6 was observed *(see table 8).*

Table 8: Showing cumulative % drug release of all formulations.

Cumulative % Drug Release									
Time (h)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**G -F1**	**G - F4**	**Marketed Formulation**
0.5	10.74 ± 0.47	9.88 ± .20	7.93 ± 0.49	8.29 ± 0.56	6.93 ± 1.10	5.25 ± 0.55	8.84 ±0.57	6.18 ± 0.50	17.66 ± 0.72
1.0	15.97 ± 0.60	14.39 ± 0.48	12.25 ± 0.49	13.56 ± 0.57	11.70 ±1.32	10.26 ± 0.52	13.21 ± 0.82	11.43 ± 0.80	30.33 ± 0.89
1.5	21.58 ± 0.70	18.93 ± 0.48	16.61 ± 0.65	18.40 ± 0.13	17.31± 1.33	14.91 ± 0.68	19.18 ± 0.80	15.49 ± 0.91	46.32 ± 0.61
2.0	27.63 ± 0.58	23.12 ± 0.82	21.44 ± 0.66	22.17 ± 0.13	22.57 ± 1.34	19.20 ± 1.04	25.21 ± 1.28	19.22 ± 0.62	68.56 ± 0.72
2.5	32.19 ± 0.60	27.35 ± 1.04	25.04 ± 0.51	26.33 ±.01	25.93 ±1.18	23.25 ± 0.92	29.36 ± 0.75	24.43 ± 1.06	98.60 ± 0.82
3.0	37.57 ± 0.65	32.82 ± 0.65	30.38 ± 0.51	31.96 ± 1.07	29.71 ± 1.54	27.89 ± 0.67	35.09 ± 0.72	28.26 ± 0.72	
3.5	41.71 ± 1.08	37.15 ±1.06	34.49 ± 0.52	36.50 ± 0.69	32.35 ± 1.39	29.81 ± 0.74	39.72 ± 1.23	33.55 ± 0.87	
4.0	48.07 ± 1.81	42.31 ± 0.52	40.34 ±0.58	42.29 ± 1.02	38.98 ± 1.37	34.65 ± 0.67	44.39 ± 0.83	38.53 ± 0.87	
5.0	54.38 ± 0.37	48.72 ± 2.94	45.81 ± 0.52	48.36 ±1.00	42.48 ± 1.22	40.77 ± 0.89	49.49 ± 0.92	44.28 ± 0.76	
6.0	60.74 ± 0.96	53.59 ± 0.96	50.92 ± 2.49	55.40 ± 0.97	47.95 ± 0.89	46.96 ± 1.06	53.47 ± 0.56	48.29 ± 0.65	
7.0	67.16± 1.00	59.71 ±61.5	56.07 ± 0.90	61.24 ±1.20	52.36 ± 1.50	50.71 ± 0.80	58.65 ±0.94	54.13 ± 0.62	
8.0	72.86 ± 1.05	64.68 ± 0.49	62.12 ± 1.41	66.40 ±0.98	58.70 ± 0.79	54.92 ± 0.22	63.88 ± 0.99	58.59 ± 0.72	
9.0	76.66 ± 0.92	69.29 ± 0.71	66.10 ± 0.92	71.39 ± 0.95	63.56 ± 1.57	59.99 ± 0.78	73.44 ± 0.68	63.45 ± 0.60	
10.0	81.28 ± 1.16	72.75 ±0.80	70.96 ± 0.72	76.99 ± 1.45	72.18 ± 1.5	63.86 ± 0.80	78.03 ± 1.17	67.63 ± 0.83	
11.0	85.93 ± 1.03	75.00 ± 0.57	73.75 ± 0.96	80.76 ±1.08	70.67 ± 1.59	67.77 ± 0.81	81.48 ± 0.99	72.56 ± 1.19	
12.0	88.28 ± 1.26	79.73 ±0.72	77.40 ± 0.52	84.17 ± 0.96	74.46 ± 1.61	71.29 ± 0.70	85.36 ± 0.57	75.75 ± 0.74	

It has been reported that with increasing amount of PVA from F4 to F6, the drug release went on decreasing. It might be due to fact that the polymer matrix releases drug after complete swelling and the time required for swelling of polymer is more as compare to formulations with less amount of PVA. The declined release rate with increased amount of PVA for formulations F4–F6 was from 79.16% to 71.29%.

6.2.7. Comparative graph of all formulations showing Cumulative% drug release

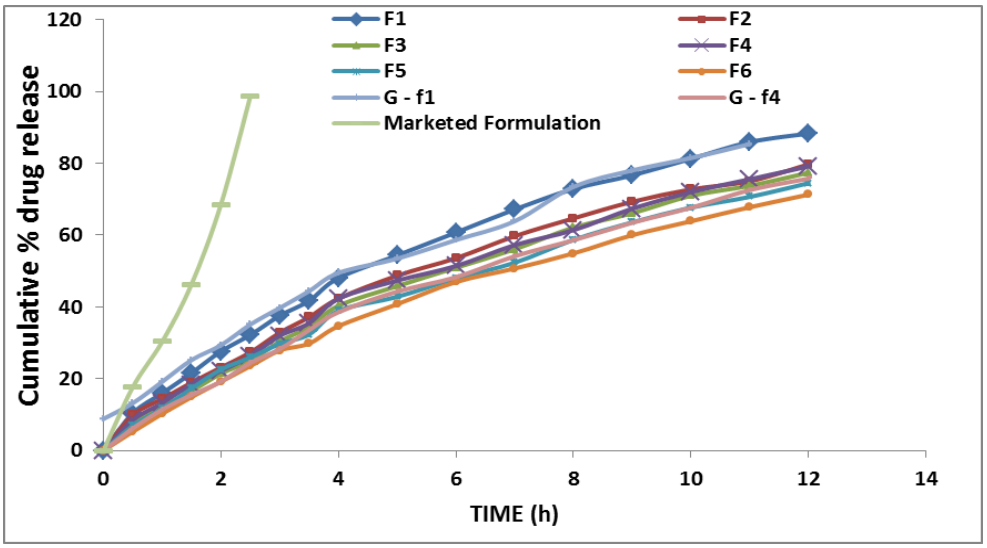

Figure 12: In-vitro drug release profiles of Clobetasol propionate microsponges formulations, microsponge gel and marketed formulation (n = 3, mean ± SD).

6.3. EVALUATIONS OF MICROSPONGES GEL

As formulation F1 and F4 exhibited drug release 83.10% and 81.55% after completion of 12 h, and was also found superior in terms of physiochemical characterization, production yield, actual drug content, entrapment efficiency, morphology, surface topography, particle size, percentage of intact porous microsponges and other physical parameters. In addition to drug release, they are assumed to be the best and most efficient formulations to give an extended drug release among all the formulations, so incorporated into carbopol gel and led to the various parameters of the gel as well.

6.3.1. Determination of Viscosity

Results of viscosity determination of gel showed that gel of microsponge formulation F4 loaded with CP and PVA conc. (75 mg) was found more viscous and gritty than gel of microsponge formulation F1 loaded with CP and PVA conc. (50 mg). The results of viscosity are shown in the table below:

Table 9: Showing results of different parameters of microsponges loaded gel.

Gel Formulations	G – F1	G - F4
Appearance	Translucent gel	Translucent gel
Homogeneity	Good	Good
pH	5.50	5.55
Viscosity	6500±500*	7566.66±404.1*
% Drug content	88.57±1.13	88.40±1.054

***Each value is average of three separate determinations ±SD**

6.3.2. In-vitro drug release from microsponges gel G - F1 and G - F4 formulation

Drug release from F1 and F4 formulation of microsponges gel was found to be 80.67% and 76.37%. It was found that gel containing PVA (75 mg) gave slightly less release as compare to gel with PVA (50 mg) conc.

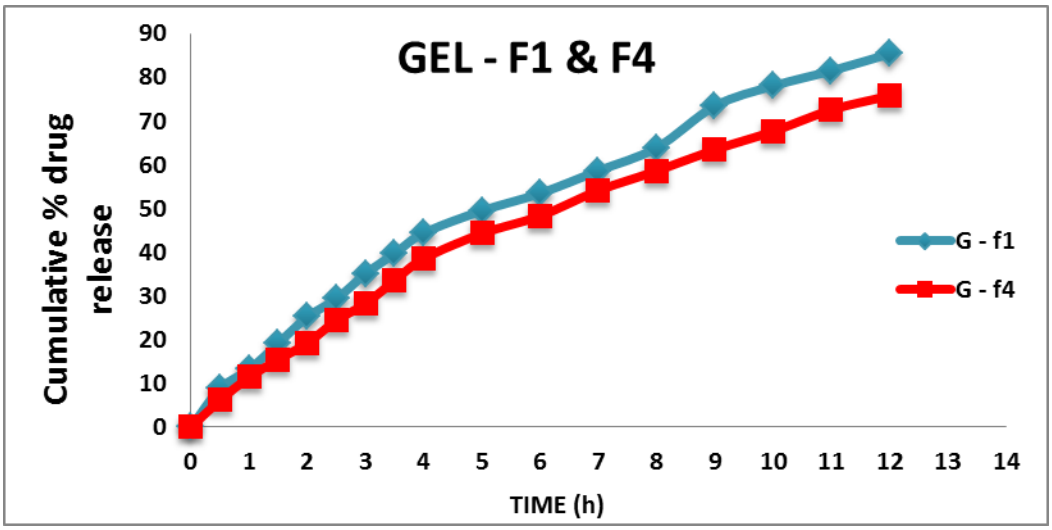

Figure 13: Showing cumulative % drug release of G - F1 and G –F4.

6.3.3. Release profile of marketed formulation

The drug release study for conventional marketed formulation containing pure unentrapped CP was carried out; release profile obtained was depicted in *Fig. 14*. The conventional formulation released 98.60% drug at the end of 2.5 h only and got exhausted. In contrary microsponges based formulation gave drug released gradually up to 12 h, and thereby would be effective in minimizing eczema, ulcers and other side effects of Clobetasol Propionate.

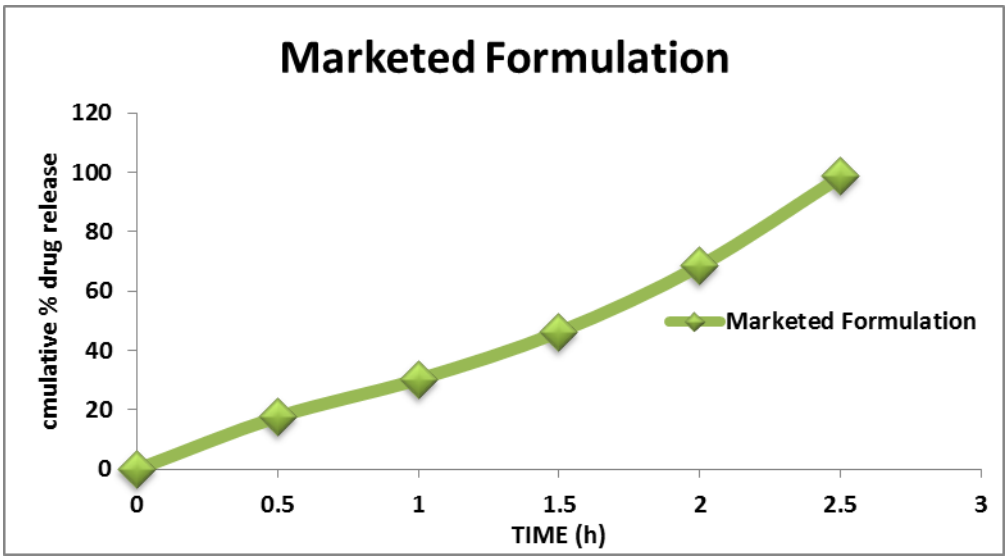

Figure 14: Showing Cumulative % Drug release of marketed formulation.

6.4. EFFECT OF VARIABLES ON FORMULATION

6.4.1. Effect of drug-polymer ratio

Increase in drug: polymer (D:P) ratio (F1–F3 & F4–F6) has been found to increase the production yield while drug content, loading efficiency and % drug release were found to be decreased *(table 10).* The reason behind this is as D:P ratio went on increasing, the polymer amount available for each microsponges to encapsulate the drug was greater, thus resulting to rising polymer matrix wall thickness which led to extended diffusion path and ultimately to slower drug release. Consequently the amounts of drug diffused from the formulations were decreased at higher D: P ratio.

Table 10: Actual drug content, encapsulation efficiency, production yield and % CDR (*n* =3).

Formu-lations	Drug: polymer ratio	PVA Conc. (mg)	Production Yield (%)	Loading Efficiency (%)	Actual drug content (%)	Particle size (µm)	% CDR
F1	1:1	50	62.667 ±0.907	81.130 ±1.310	64.620 ±0.803	22.09 ±0.91	88.28 ± 1.26
F2	1:2	50	70.670 ±0.855	78.662 ±0.709	34.02 ±0.816	30.94 ±4.11	79.73 ± 0.72
F3	1:3	50	83.50 ±0.681	74.510 ±0.734	21.45` ±1.032	59.30 ±24.48	77.40 ± 0.52
F4	1:4	75	66.90 ±0.815	88.377 ±0.741	66.33 ±0.613	26.66 ±1.036	84.17 ± 0.96
F5	1:5	75	73.437 ±0.637	80.533 ±0.701	36.36 ±0.775	71.22 ±25.94	74.46 ± 1.61
F6	1:6	75	86.650 ±0.655	76.273 ±1.030	22.017 ±0.076	90.57 ±3.43	71.29 ± 0.70
G – F1	1:1	50	-	-	-	-	85.36 ± 0.57
G- F4	1:1	75	-	-	-	-	75.75 ± 0.74

6.4.2. Effect of composition of external phase

Composition of external phase was altered for formulations F4–F6 by changing the concentration of PVA from 50 mg to 75 mg. It has been observed that on increasing the amount of PVA, production yield, and particle size were increased while slight decrease in drug release, drug content and loading efficiency was noticed *(table 10).*

6.5. CONCLUSION

Controlled drug delivery via the polymer based systems has been proposed to be prevailing both in present and in future; as having numerous potential advantages for scientific as well

as economic reasons. The thought behind developing polymeric microsponge delivery system was to deliver clobetasol propionate in a continual manner for extensive time period to reduce application frequency, hypersensitive reactions and to improve safety than marketed conventional formulation. The method implemented was quasi-emulsion solvent diffusion; found to be simple, reproducible and rapid. Formed microsponges were spherical shape, have high porosity and good flow. SEM photographs revealed the spherical nature of the microsponges in all variations. No drug crystal was observed at the surfaces of microsponges. Furthermore, the drug was compatible with the polymer of the microsponges. Varied drug–polymer ratio reflected remarkable effect on particle size, drug content and encapsulation efficiency.

Gel formulation

G-F4 with 1:1 drug–polymer ratio was found more efficient to give extended drug release (75.75% at 12 h); while conventional formulation exhausted extremely earlier (98.60% at 2.5 h). Thus, gel containing microsponges prepared in this study was found to be promising as new-fangled delivery system offering prolonged release of Clobetasol propionate in treating skin disorders viz., psoriasis, eczema etc.

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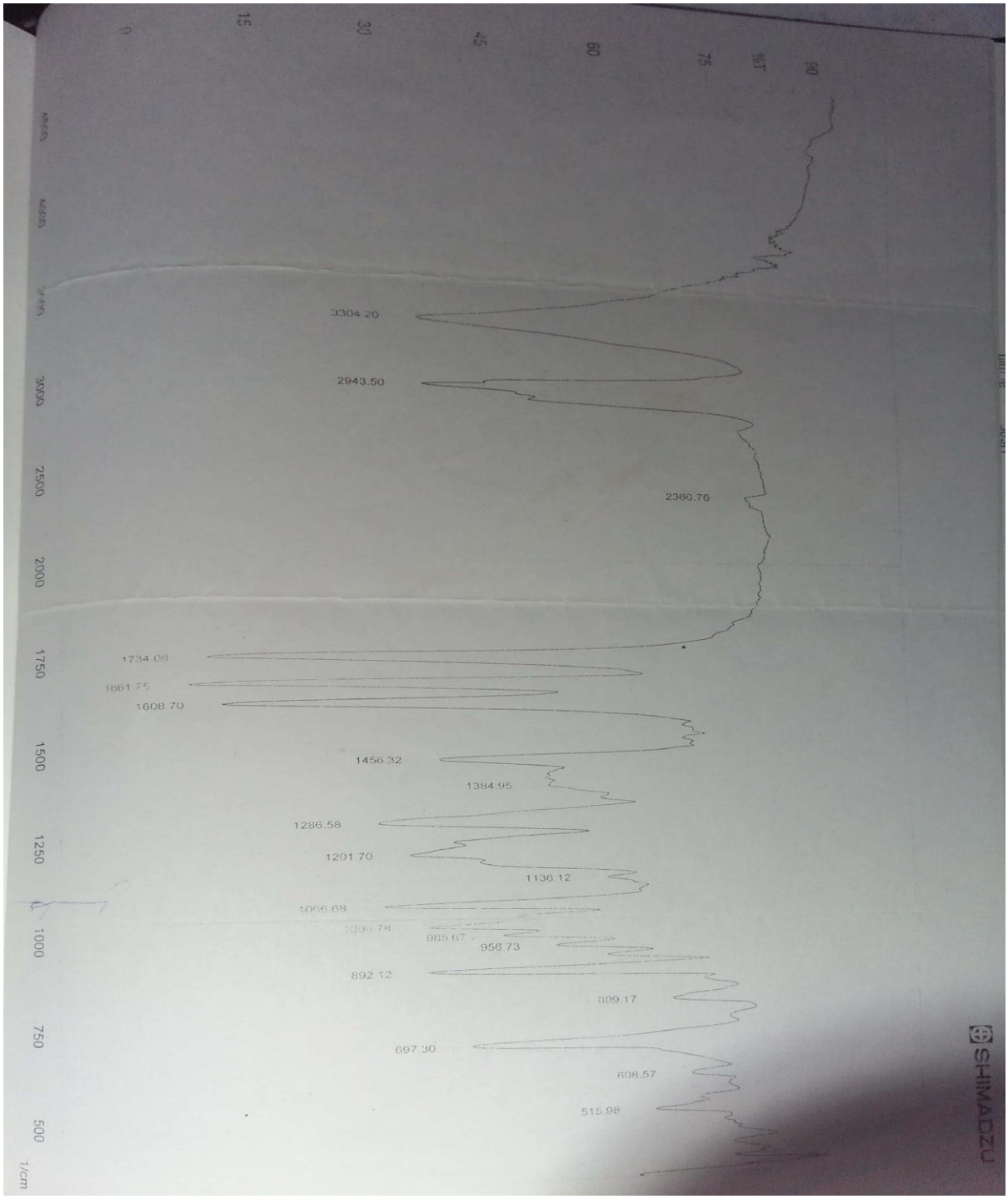
Appendix – A

FTIR SPECTRA OF PURE DRUG CLOBETASOL PROPIONATE



Appendix – B

FTIR SPECTRA OF MICROSPONGES FORMULATION INCORPORATED WITH CLOBETASOL PROPIONATE