IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Formulation And Characterization Of Imiquimod Loaded Mocrisponge Gel

Ravi Shukla, Dr. Arun Patel*

Shri Ram Group of Institutions, Faculty of Pharmacy, Jabalpur- 482002, (M.P.)

Abstract

Actinic keratosis (AK), sometimes called solar keratosis or senile keratosis is a pre-cancerous area of thick, scaly, or crusty skin. The aim of proposed work is better patient compliance with effective targeting at specific site of action. Topical formulations reduce gastrointestinal tract irritation, stop the medicine from being metabolized in the liver in its first pass, and increase the drug's bioavailability. An objective of the study was to prepare microsponge of imiquimod by quasi- emulsion solvent diffusion method. Methods used were characterization of imiquimod microsponges loaded topical gel some methods like the visual examination, pH determination, spreadability, viscosity,drug content, *In vitro* diffusion studies using K C cell and Zeta Potential. The microsponge were prepared by quasi emulsion method and was evaluated for its different parameters which revealed many interesting results for efficient preparation of the microsponges. The formulation F3 has better results than other 9 formulations. F3 have its particle size 31.3 µm, percentage yield 79.27, Entrapment efficiency 93.44%, Drug content 83.04%, spreadability 14.4, pH 7.4, Viscosity 2564 cps, Cumulative Release 49.89 % in 24 hour, all these parameters are in optimized range for preparing a controlled release dosage form so showing itself as an optimised formulation in this project work.Microsponge delivery systems can precisely control the release rates or target drugs to a specific body site have a vast impact on the health care system. A microsponge delivery system can release its active ingredient on a timer mode and also in response to other stimuli. Therefore, microsponge has got a lot of potential and is a very emerging field which is needed to be explored. Microsponges constitute a significant part by virtue of their small size and efficient carrier characteristics.

Introduction: Actinic keratosis (AK), sometimes called solar keratosis or senile keratosis, is a pre-cancerous area of thick, scaly, or crusty skin. Actinic keratosis is a disorder of epidermalkeratinocytes that is induced by ultraviolet (UV) light exposure These growths are more common in fair-skinned people and those who are frequently in the sun.They are believed to form when skin gets damaged by UV radiation from the sun or indoor tanning beds, usually over the course of decades. Given their pre-cancerous nature, if left untreated, they may turn into a type of skin cancer called squamous cell carcinoma. Notably, AKs are frequently felt before they are seen, and it is occasionally advised to see a dermatologist because of the feel. from 2 to 6 millimeters, yet they have the potential to develop to several centimeters document Treatment by an is necessary since untreated lesions carry a 20% chance of developing into squamous cell carcinoma.Actinic keratoses are characterized by thick, crusty, scaly patches that frequently have a harsh, dry feel to them. They can grow to be several centimeters in diameter, although their typical size is between two and six millimeters. Notably, AKs are frequently felt rather than seen, and their texture has occasionally been likened to sandpaper. They could be tan, pink, red, dark, light, or a considering that exposure to the sun causes AK development.

The possibility of in situ or invasive squamous cell carcinoma (SCC) cannot be ruled out based only on clinical examination, hence a biopsy or excision can be considered for a conclusive diagnosis by histologic study of the lesional tissue if the clinical examination results are not typical of AK. There are several AK therapy methods available. One therapeutic option for several AK lesions in a skin region known as "field cancerization" is photodynamic therapy (PDT). A photosensitizer is applied to the skin, and then a bright light source is used to illuminate the area. Applying topical creams like imiquimod or 5-fluorouracil Seniors with fair skin and sensitivity to the sun are the usual patients with actinic keratoses. [4] Dorsal forearms, hands, and the cheeks, ears, and, in men, the bald scalp are among the regions where the lesions first appear after prolonged sun exposure. A single anatomic region may see a patient acquire several lesions, to the point where the lesions collide and result in confluent actinic keratosis over a sizable area.

This theory is supported by the challenge of developing clear standards for identifying the point at which an actinic keratosis transforms into an SCC. Ackerman asserts that there is no distinct boundary between actinic keratoses and thin SCCs, with actinic keratoses being referred to as "embryonic" SCCs and belonging to the evolutionary spectrum of SCCs.Thus, keratinocyticintraepidermalneoplasia and intraepidermal solar keratotic SCC are two proposed nomenclatures that would replace the term actinic keratosis. In clinical terms, the lesion manifests as an erythematous plaque on a sun-exposed area that is rough, scaly, poorly defined, and asymptomatic to slightly painful. Thick, sticky, hard to get rid of scales that feel better than look. The diameter of the lesion ranges from 1 mm to several centimeters. The dorsa of the hands, forearms, and face are sites of preference. Lentigines and wrinkles are frequently related.

Material and Methods

List of materials: Ethylcellulose, Polyvinyl alcohol, Glycerol, Dichloromethane, Magnesium stearate, Potassium dihydrogen phosphate, Sodium hydroxide above mentioned material choice will depend on availability of polymers and chemicals

List of Equipment: Electronic balance, High speed homogenisor, Hot air oven, Optical Microscope, pH meter, Dissolution apparatus, UV spectrophotometer, FT-IR spectrophotometer, SEM Analyser.

Methods

1. **Preformulation Studies:** Preformulation testing is the first step in the rational development of dosage forms of a drug substance. It can be defined as an investigation of physical and chemical properties of a drug substance alone and combination with excipients.

a. **Organoleptic evaluation of drug:** Organoleptic properties of the drug substance are very important for designing the dosage form. The colour, odour and tests of the drug are characterized.

b. **Solubility studies of drug in various organic and inorganic solvent:** A qualitative determination of the solubility was made by adding solvent in small incremental amount to a test tube containing fixed quantity of drug. After each addition, the system is vigorously shaken and observed visually.

c. **Determination of partition coefficient:** The partition coefficient determined by hand shaking method. The partition coefficient is defined as the ratio of unionized drug distributed between the organic and aqueous phase at equilibrium. For a drug delivery system, Lipophilic/Hydrophilic balance has been shown to be a contributing factor for rate and extent of drug absorption. Partition coefficient provides a

mean of characterizing Lipophilic/Hydrophilic nature of drug.

d. **Melting Point determination:**Melting point of drug determined by melting point apparatus.

It is performed by filling of drug in capillary tube and capillary tube and the thermometer were putin the apparatus. Now the point was noted at which the compounds starts melting.

e. **Determination of ƛ max and preparation of standard curve:**100 mg of drug sample was weighed accurately and dissolved in 100 ml of methanol in 100 ml of volumetric flask and stock solution was prepared. Dilution was prepared and scanned from 200 – 400nm by UV spectrophotometer.

f. **Drug Excipient Compatibility study:** Drug excipient interaction studies by FT-IR. It is Spectroscopy used to investigate and predict any physicochemical interactions between different components, in a formulation and therefore it applied to selection of suitable chemically compatible excipient. While selecting the ingredients, we would choose those which are stable, compatible and therapeutically acceptable. The aim of compatibility study was to test, whether there is any interaction between the excipients and the drug and compatibility between the drug and excipients.

g. **Formulation and Development of Microsponges:** Microsponges of imiquimod and Ethyl Cellulose was prepared by quasi-emulsion solvent diffusion method. The process involved formation of quasi-emulsion of two different phases i.e. internal phase and external phase similar to emulsions. The internal phase of drug-polymer solution (1: different ratio) made in a volatile solvent dichloromethane (10ml). And then it was added to external phase comprising the aqueous 5% (5mg/100mlwater) polyvinyl alcohol (PVA) solution with vigorous stirring. Glycerol (1-2ml), which was added at an adequate amount in order to facilitate plasticity. Stirring lead to the emulsion globules. The stirring was continued upto 6 hrs till the insoluble, rigid microparticles i.e. microsponges is formed. Then it was filtered to separate the microsponges. The microsponges were then dried in an air heated oven.

B. Characterization of drug loaded microsponges

(i). **Production yield:**The production yield of the microsponge was determined by calculating accurately the initial weight of the raw materials and the last weight of the microsponges obtained:

$$\text{Production yield} = \frac{\text{Practical Mass of microsponges}}{\text{Theoretical Mass (Drug + Polymer)}} \times 100$$

(ii). Entrapment efficiency: A sample of imiquimod microsponges (10 mg) was dissolved in 100 ml of phosphate buffer, freshly prepared (pH 5.5). The solutions were subsequently diluted suitably with the phosphate buffer pH 5.5and spectrophotometric absorbance was taken at the maximum wave length of imiquimod. The drug content was calculated from the calibration curve and expressed as the loading efficiency:

(iii). Particle size determination: Particle size analysis of loaded and unloaded microsponges can be performed by laser light diffractometry or any other suitable method. The values can be expressed for all formulations as mean particle size range. Cumulative percentage drug release from microsponges of different particle size will be plotted against time to study effect of particle size on drug release. Particles larger than 35 µm can impart gritty feeling and hence particles of sizes between 10 and 35µm are preferred to use in final topical formulation.

(iv). Surface topography/ Particle morphology by SEM: For morphology and surface topography, the prepared microsponges can be coated with gold– palladium under an argon atmosphere at room temperature and then the surface morphology of the microsponges can be studied by scanning electron microscope (JEOL Instrument, JSM-6360, Japan).

C. Formulation development of Fusidic acid microsponges loaded topical gel:

Microsponge loaded topical gel of imiquimod (100mg) was prepared step by step a clear dispersion of Carbopol was prepared in water using moderate agitation. A clear dispersion of carbopol (35 mg) is prepared in water (q.s) using moderate agitation. Triethanolamine (1-2 drops) is used to neutralise the formulation and subsequently preservatives Methyl paraben (3 mg) and Propyl paraben (1 mg) was added to resist the microbial growth. And then volume was maintained with water. Gel prepared were degassed with ultrasonication.

D. Characterization ofimiquimodmicrospongesloaded topical gel

- **The visual examination:** The examination considered a series of visual characteristics (consistency, colour, and homogeneity).
- **pH determination:** The pH of the prepared gel was measured using pH meter by putting the tip of the electrode into the gel and after 2 minutes the result was recorded.

- **Spreadability:** A sample of 0.1g of gel was pressed between 2 slides with 500g weights and left for about 5 min where no more spreading was expected. Diameters of spread circles were measured in cm and were taken as comparative values for spreadability (diameter of the spread circle –initial diameter.
- **Viscosity:** The viscosity of imiquimod loaded microsponge carbopol gel was measured in Brookfield viscometer, model-VL2 (Lemis Baltic) with spindle No 4.
- **Drug Content:** Imiquimodcontent in the gel was determined by taking required quantity of the gel which is equivalent to 10 mg of imiquimod transferred to 100 ml volumetric flask containing phosphate buffer (pH 5.5) and it allowed to sonicate and filtered. Then, suitably diluted and analyzed at λ max 205.
- **In vitro diffusion studies using KC cell:** In vitro release studies was performed using KC cell diffusion apparatus at 37 °C. The release medium is selected, while considering solubility of active ingredients to ensure sink conditions. Sample aliquots were withdrawn from the medium and analyzed by the suitable analytical method at regular intervals of time. Egg membrane was fitted at the donor side of the cell and predetermined amount of formulation was mounted on the membrane. The receptor medium is continuously stirred at and thermostated with a circulating jacket. Samples are withdrawn at different time intervals and analyzed using suitable method of assay.
- **Zeta Potential:** The zeta potential of imiquimod microsponge formulations were measured using Zetasizer (Malvern instruments, Worcestershire, UK). The measurement was performed at 250C. A sample of 1 ml was diluted using double distilled water).

E. Stability Studies: Stability of a drug has been defined as the ability of a particular formulation in a specific condition, to remain within its physical, chemical, therapeutical and toxicological specifications. The reason of stability testing is to provide evidence on how the quality of drug formulation varies with time under the influence of various environmental conditions such as temperature, humidity, light. From this study we know about recommended storage condition, re-test periods and shelf-life of the drug can be established.

1. **Stability studies are important for the following reasons:**This is an assurance given by the manufacturer that the patient would receive a uniform dose throughout the shelf life.The drug control administration insists on manufacturers on conducting the stability studies, identity, strength, purity and

quality of the drug for an extended period of time in the conditions of normal storage. Stability testing prevents the possibility of marketing an unstable product. Both physical and chemical degradation of drug can result in unstable product.

2. **Purpose of stability studies:** Stability studies are done to understand how to design a product and its packaging, such that product has appropriate physical, chemical and microbiological properties during a defined shelf life when stored and used.

3. **Storage conditions:** The selected formulations were subjected for three month stability study as per ICH guidelines. The selected formulations were placed in a wide mouth glass bottles, mouth of the bottle was tightly closed and packed in aluminum foils. In the present study, stability studies were carried out at 25ºC/60% and 40ºC/75% RH for a specific period of 3 months for the selected formulations.

RESULT AND DISCUSSION

Preformulation Study

Physical Characteristics: Organoleptic and physical properties of drug

Test	**Observations**
Colour	White
Taste	Bitter
Odour	Odourless
Form	Crystalline

Solubility: Solubility profile of drug

S. No.	Solvents	Solubility
1.	Water	Insoluble
2.	Methanol	Freely Soluble
3.	Ethanol (95%)	Freely Soluble
4.	Dimethyl formamide	Freely soluble
5.	Dimethyl sulfoxide	Freely soluble

Partition Coefficient:.

Material	Observation
Imiquimod Sovent system: Octanol: water	2.7

Melting point determination: Melting point of drug

Material	Observation
Imiquimod	292°C-294^{O}C

Determination of ƛ max and preparation of standard curve: The calibration curve of imiquimod was prepared in phosphate buffer pH 1.2 in table 5 shows the absorbance at λmax 226 nm for different concentration of imiquimod and figure 2 shows calibration curve.

Table 9: Absorbance of different aliquots of Imiquimod at 226 nm

Concentration(µg/ml)	Absorbance
5	0.0802
10	0.1727
15	0.2908
20	0.4030
25	0.5077

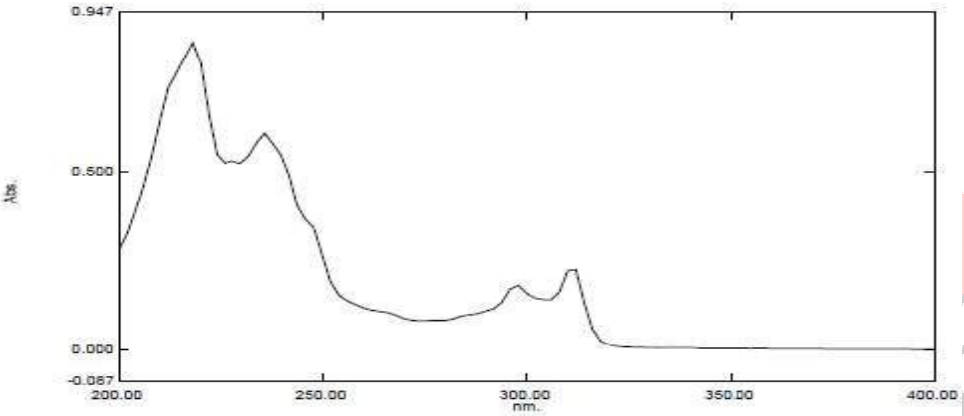


Fig. 1: UV Spectrum of Imiquimod in 0.1N HCl

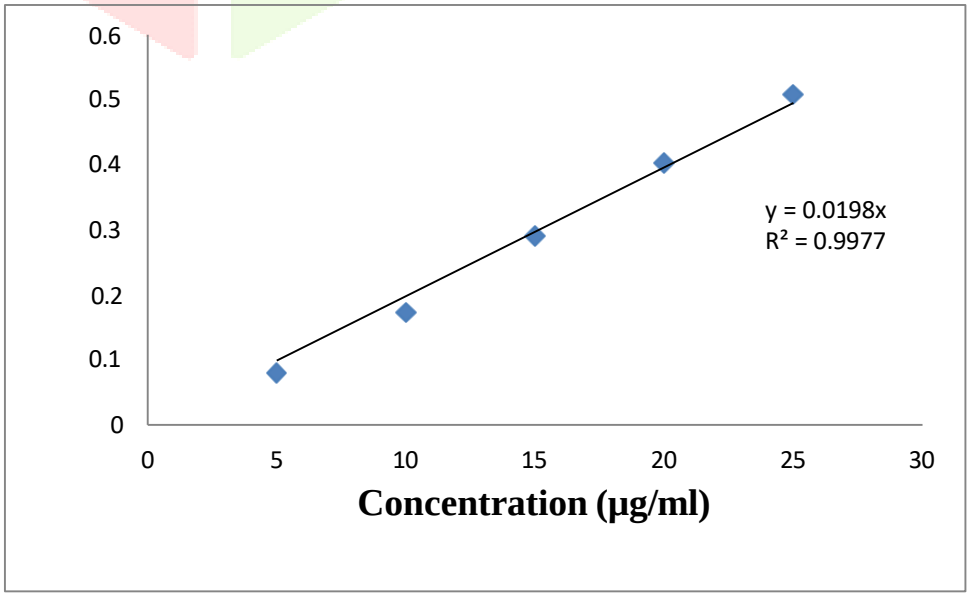


Fig. 2: Calibration curve of Imiquimod in 0.1N HCl buffer Drug –

Excipient Compatibility study:

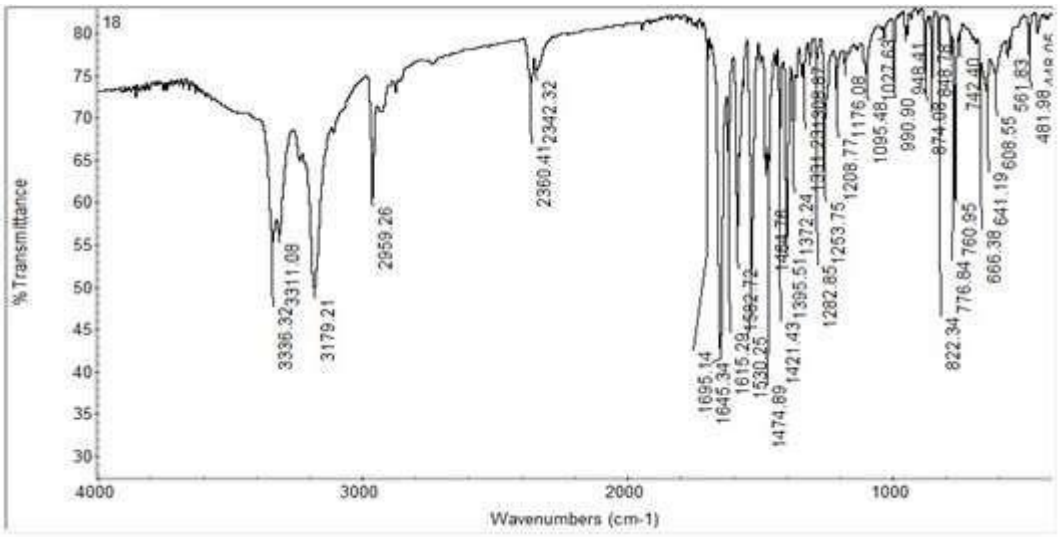
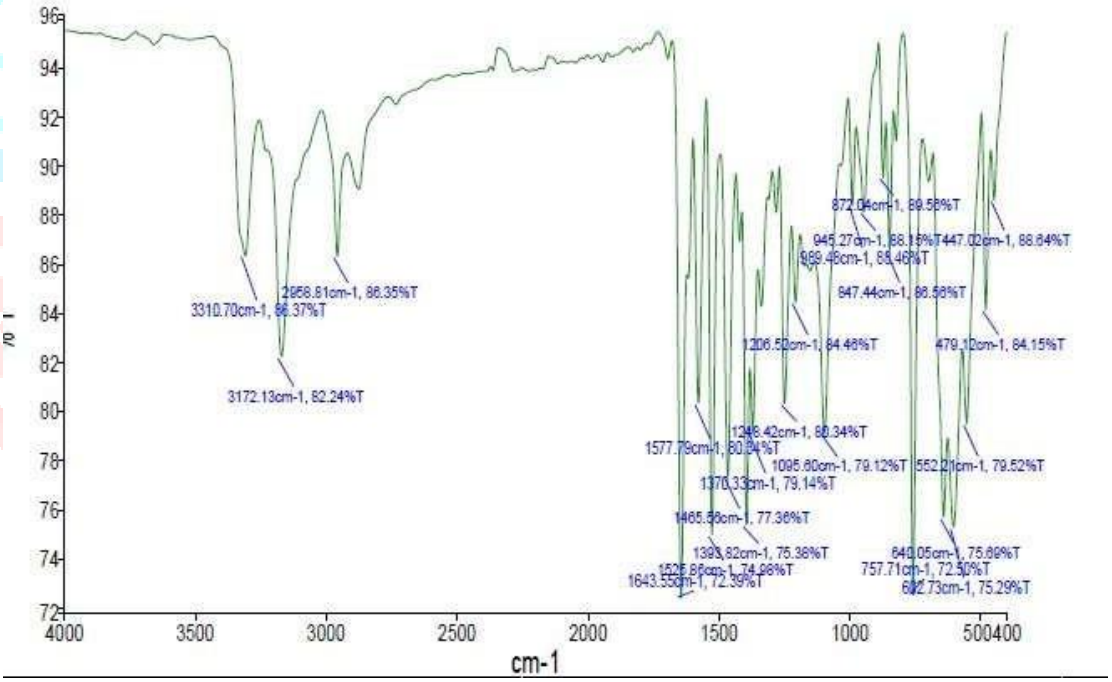


Figure 3: FTIR Spectrum of imiquimod



FTIR spectrum of imiquimod+ Excipients: Formulation and Development of Microsponges:

Table 10: formula for microsponge formulation

S. No.	Ingredient(mg /ml)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
1	Imiquimod	100	100	100	100	100	100	100	100	100	100
2	Ethylcellulose (polymer)	1	2	3	4	5	6	7	8	9	10
3	Polyvinylalcohol	500	500	500	500	500	500	500	500	500	500
4	Dichloromethane	10	10	10	10	10	10	10	10	10	10
5	Glycerol	1	1	1	1	1	1	1	1	1	1
6	Water	100	100	100	100	100	100	100	100	100	100

Characterization of drug loaded microsponges Production

yield and Entrapment efficiency:

Table 11: Results of Production yield and Entrapment efficiency

S. No	Formulation code	Production yield (%)	Entrapment Efficiency (%)
1	F1	20.60	85.12
2	F2	37.41	87.08
3	F3	79.27	93.44
4	F4	63.00	81.28
5	F5	70.13	76.11
6	F6	50.40	70.13
7	F7	25.17	60.24
8	F8	37.12	68.41
9	F9	20.13	72.21
10	F10	19.11	76.11

Particle size analysis of microsponge:

Table 12: Results of Particle size analysis of microsponge

S. No.	Formulation code	Particlesize (µm)
1	F1	28.7
2	F2	29.8
3	F3	31.3
4	F4	33.7
5	F5	37.2
6	F6	31.8
7	F7	31.9
8	F8	29.4
9	F9	27.5
10	F10	23.9

Surface topography/ Particle morphology by SEM: Scanning electron microscopy (SEM) was used to determine the Morphology of the prepared microsponges. 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 in formation about the specimens.

It was observed that the microsponges were spherical, and uniform with no drug crystals on the surface. The shape of the microsponges affects the surface area and surface area per unit weight of spherical

microsponges. The irregular shape of the particles may affect dissolution rate present in dissolution environment.

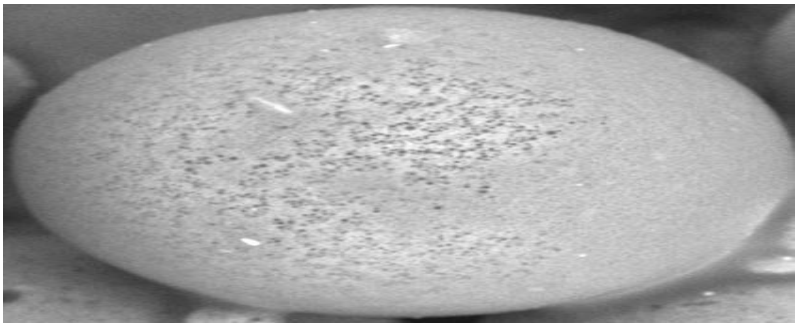


Figure 6: Microsponge

Formulation development of imiquimod microsponges loaded topical gel:

Gel of imiquimod was prepared by using following formula given in table. A clear dispersion of Carbopol was prepared in water using moderate agitation. A clear dispersion of carbopol (50 mg) is prepared in water (q.s) using moderate agitation. Triethanolamine (1-2drops) is used to neutralize the formulation and subsequently preservatives Methyl paraben (5 mg) and Propyl paraben (2 mg) was added to resist the microbial growth. And then volume was maintained with water. Gel prepared were degassed with ultrasonication.

Table 13: Formula for gel formulation

S. No.	Ingredient	Quantity(mg/ml)
1	Carbopol934P	50
2	Triethanolamine	2
3	Methylparaben	5
4	Propylparaben	2
5	Distilledwater	q.s

Characterization of imiquimod microsponges loaded topical gel

a. pH determination

Table 14: Results of pH of formulation

S. No.	Formulation code	pH
1	F1	6.9
2	F2	6.5
3	F3	7.4
4	F4	7.0
5	F5	7.4
6	F6	7.1
7	F7	7.2
8	F8	6.9
9	F9	7.4
10	F10	7.3

b. Viscosity measurement:

Table 15: Results of viscosity measurement

S. No.	Formulation code	Viscosity(cps)
1	F1	2874
2	F2	2745
3	F3	2564
4	F4	2731
5	F5	2345
6	F6	2814
7	F7	2781

8	F8	2498
9	F9	2791
10	F10	2747

c. Spreadability test:

Table 16: Results of Spreadability of microsponges

S. No.	Formulation code	Spreadability
1	F1	13.6
2	F2	13.8
3	F3	14.24
4	F4	14.3
5	F5	13.9
6	F6	14.2
7	F7	14.1
8	F8	13.5
9	F9	13.8
10	F10	13.6

d. Drug content determination: Table

17: Results of drug content studies

S. No.	Formulation code	Drug content (%)
1	F1	19.07
2	F2	27.82
3	F3	84.03
4	F4	74.03
5	F5	69.85
6	F6	63.02
7	F7	58.01
8	F8	51.06
9	F9	3705
10	F10	33.09

In-vitro diffusion studies using K Ccell:

Table 18: Results of diffusion study of different formulation

S. N.	T. (hr.)	Cumulative Release (%)									
		F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**
1	**1**	2.89	3.39	3.74	2.41	2.29	1.93	1.71	1.36	1.04	0.81
2	**2**	6.08	7.78	8.03	4.83	3.91	3.63	3.03	2.56	2.11	1.38
3	**3**	8.27	9.17	10.22	6.21	5.33	4.29	4.03	3.8	3.37	2.61
4	**4**	12.01	13.56	15.27	10.59	9.07	7.86	7.35	6.04	5.09	3.77
5	**5**	19.81	20.02	22.01	17.25	15.32	14.03	11.97	10.05	8.12	6.85
6	**24**	67.27	76.09	89.83	61.24	53.01	49.89	33.53	26.91	19.25	17.23

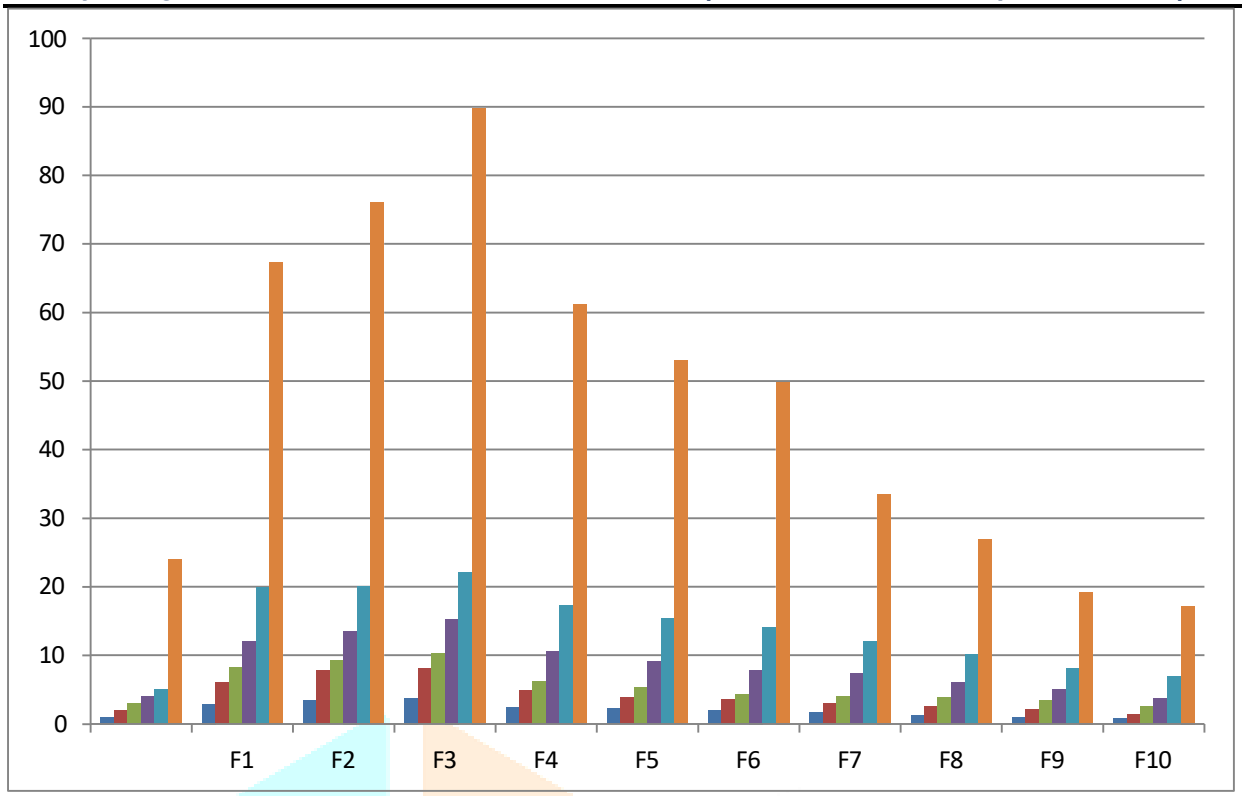


Fig. 7: % drug release of different formulation

Zeta Potential: The zeta potential value of imiquimod microsponge gel was found and which lies in ideal limit of ±10 to ± 30 mV. Imiquimod microspongic gel showed the zeta potential of–24.6 mV Figure 47. It indicates that systems may remain stable for longer period.

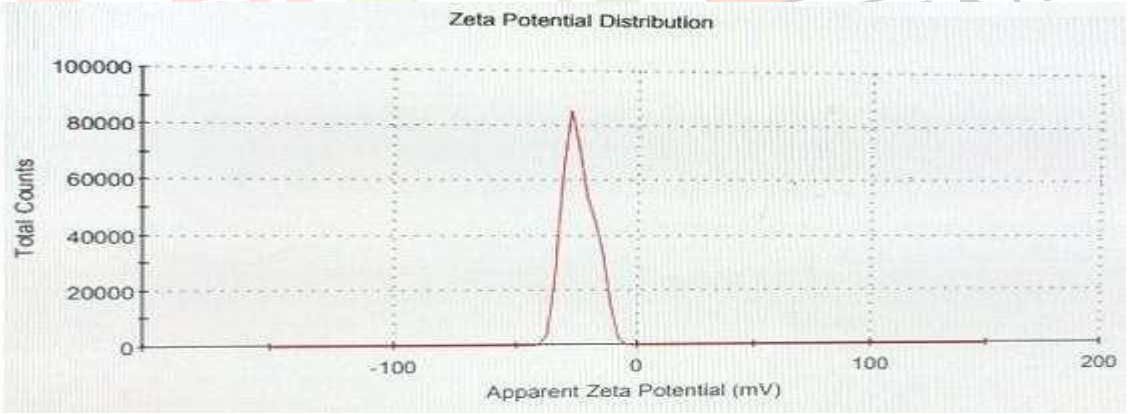


Fig. 8 : Zeta potential of microsponge gel

Stability Studies on developed delivery system was performed to study the effect of storage temperature on surface morphology and residual drug content:

Table 19: Results stability study of best {F3} formulation

PARAMETER S	Loaded Microsponges			
	Room temperature			
	10Day	30 Day	45 Day	90 Day
Colour	White	White	White	White
Odour	Odourless	Odourless	Odourless	Odourless
pH	7.4	7.3	7.2	7.2
Spreadability	14.4	14.3	14.2	14.2
Viscosity (CPS)	2564	2563	2562	2562
% Drug content	84.03	84.02	84.01	84.01

SUMMARY AND CONCLUSION

The microsponges was prepared by quasi emulsion method and was evaluated for its different parameters which revealed many interesting results for efficient preparation of the microsponges. The formulation F3 have better results than other 9 formulations. F3 have its particle size 31.3 µm, percentage yield 79.27, Entrapment efficiency 93.44%, Drug content 83.04%, Spreadability 14.4, pH 7.4, Viscosity 2564 cps, Cumulative Release 49.89 % in 24 hour, all these parameters are in optimized range for preparing a controlled release dosage form so showing itself as an optimised formulation in this project work. FTIR spectroscopy analyses indicated the chemically stable, amorphous nature of the drug in these microsponges. SEM photographs revealed the spherical nature of the microsponges in all variations.

However, at higher ratios, drug crystals were observed on the microsponge surface. With the revealed results by different evaluation parameters, it is concluded that microsponges dug delivery system has become highly competitive and rapidly evolving technology and more and more research are carrying out to optimize cost-effectiveness and efficacy of the therapy. It is a unique technology for the controlled release of topical agents and consists of microporous beads loaded with active agent and also use for oral as well as biopharmaceutical drug delivery. Microsponge delivery systems can precisely control the release rates or target drugs to a specific body site have a vast impact on the health care system. A microsponge delivery system can release its active ingredient on a timer mode and also in response to other stimuli. Therefore, microsponge has got a lot of potential and is a very emerging field which is needed to be explored. Microsponges constitute a significant part by virtue of their small size and efficient carrier characteristics.

References

1. Cooper J, Gunn C. Powder flow and compaction. In: Carter SJ, editor. Tutorial pharmacy, New Delhi: CBS Publishers and Distributors; 1986. p.211-33.
2. Loftus EV Jr, Sandborn WJ. Epidemiology of inflammatory bowel disease.
3. Gastroenterol Clin North Am 2002; 31:1-20.
4. Comoğlu T, Gönül N, Baykara T. Preparation and in vitro evaluation of modified release ketoprofen microsponges.Farmaco2003;58:101-6.
5. SinhaVR, Kumria R. Coating polymers for colon specific drug delivery: a comparative invitro evaluation. Acta Pharm 2003;53:41-7.
6. Kruis W, Bar-Meir S, Feher J, Mickisch O, Mlitz H, Faszczyk M. The optimal dose of 5-aminosalicylic acid in active ulcerative colitis: a dose-finding study with newly developed mesalamine. Clin Gastroenterol Hepatol 2003;31;1:36-4.
7. Cetinkaya A, Bulbuloglu E, Kurutas EB, Ciralik H, Kantarceken B, Buyukbese MA. Beneficial effects of N-acetylcysteine on acetic acid-induced colitis in rats.The Tohoku Journal of Experimental Medicine. 2005;206 (2): 131–139. [PubMed]
8. Devrim B, Canefe K. Preparation and evaluation of modified release ibuprofen

microspheres with acrylic polymers (Eudragit) by quasi emulsion Solvent diffusion method: effect of variables. Acta Pol Pharm 2006;63:521-34.

9. Park BS, Lee HK, Lee SE, Piao XL, Takeoka GR, Wong RY, Ahn YJ, Kim JH,2006. Antibacterial activity of Tabebuiaimpetiginosa Martius ex DC (Taheebo) against Helicobacterpylori. Journal of Ethnopharmacology; 105,255– 262
10. Jelvehgari M, Siahi-Shadbad MR, Azarmi S, Martin GP, Nokhodchi A. The microsponge delivery system of benzoyl peroxide: Preparation, characterization and release studies. Int J Pharm 2006; 308:124-32.
11. Asghar LF, Chandran S. Multiparticulate formulations approach to colon specific drug delivery: current perspectives. J Pharm Pharm Sci 2006;9:327-38.
12. Jelvehgari M, Siahi-Shadbad MR, Azarmi S, Martin GP, Nokhodchi A. The microsponge delivery system of benzoyl peroxide: preparation, characterization and release studies. Int J Pharm 2006;308:124-32.
13. Orlu M, Cevher E, Araman A. Design and evaluation of colon specific drug delivery system containing flurbiprofen microsponges. Int J Pharm 2006; 318:103-17.
14. Nokhodchi A, Jelvehgari M, Siahi MR, Mozafari MR. Factors affecting themorphology of benzoylperoxide microsponges. Micron 2007; 38:834-40.
15. Wang CC, Chiang YM, Sung SC, Hsu YL, Chang JK, Kuo PL, **2008**.
16. Plumbagininduces cell cycle arrest and apoptosis through reactive oxygen species/c-Jun N-terminal kinase pathways in human melanoma A375.S2 cells. Cancer Letter; 259,82-98.
17. Barollo M, Medici V, D'Incà R et al. Antioxidative potential of a combined therapy of anti TNFα and Zn acetate in experimental colitis. World Journal of Gastroenterology.2011;17(36):4099–4103.[PMCfree article].
18. Jain V, Singh R. Design and characterization of colon-specific drug delivery system containing paracetamol microsponges. Arch Pharm Res 2011; 34:733- 40.
19. Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, et al.

20. Host-microbe interactions have shaped the genetic architecture of inflammatory boweldisease.Nature 2012;491:119-24.

21. Redondo-Sendino A. An uncommon case of proximal Crohn's disease.

22. Semergen 2012; 38:539-42.

23. Kandhare AD, Raygude KS, Ghosh P, et al. Effect of hydroalcoholic extract ofHibiscus rosasinensis Linn. leaves in experimental colitis in rats. Asian Pacific Journal of Tropical Biomedicine. 2012; 2 (5) :337–344.

24. Rubin DC, Shaker A, Levin MS. Chronic intestinal inflammation: inflammatory bowel disease and colitis-associated colon cancer. Front Immunol 2012; 3: 107.

25. Gandhy SU, Kim K, Larsen L, Rosengren RJ, Safe S. Curcumin and synthetic analogs induce reactive oxygen species and decreases specificity protein transcription factors by targeting micro RNAs. BMC Cancer. 2012;12, article 564 [PMC free article] [Pub Med].

26. Goyal N, Rana A, Ahlawat A, Bijjem KR, Kumar P. Animal models of inflammatory bowel disease: a review.Inflammo pharmacology 2014; 22:219- 33.

27. Louis P, Hold GL, Flint HJ, **2014**. The gut microbiota, bacterial metabolites and colorectal cancer.Nature Reviews Microbiology;1-12.

28. Orridoni D, Arseneau KO, Cominelli F. Inflammatory bowel disease. Immunol Lett 2014; 161:231-5.

29. Yu DG, Xu Y, Li Z, Du LP, Zhao BG, Wang X. Coaxial electrospinning withmixed solvents: from flat to round eudragit L100 nanofibers for better colon-targeted sustained drug release profiles. Journal of Nanomaterials. 2014;2014:8pages.967.

30. Sareen R, Nath K, Jain N, Dhar KL. Curcumin loaded microsponges for colon targeting in inflammatory bowel disease: Fabrication, optimization, and in vitro and pharmacodynamic evaluation. Biomed Res Int 2014; 340701.

31. Shah N, Sharma O P, Mehta T, Amin A, **2015**. Design of experiment approach for formulating multi-unit colon-targeted drug delivery system: in vitro and in vivo studies, Drug Development and Industrial Pharmacy,1-11.

32. Prasad YG, Lei WX, Zhanju L. Psychological Stress Exacerbates Development of Inflammatory Bowel. Biomed Lett 2016; 2(1): 53-9.

33. Duan H, Lu S, Gao C, Bai X, Qin H, Wei Y, Wu X, Liu M, **2016**.

34. Mucoadhesive microparticulates based on polysaccharide for targetdual drug delivery of 5-aminosalicylic acid and curcumin to inflamed colon. Colloids and Surfaces B:Biointerfaces;145,510-519.

35. Gerola AP, Silva DC, Matsushita AFY, Borges O, Valente AJM, **2016**. The effect of methacrylation on the behavior of Gum Arabic as pH-responsive matrix for colon-specific drug delivery.European Polymer Journal;78,326-339.

36. Gowda R , Kardos G, Sharma A, Singh S, Robertson GP, **2016.** Nanoparticle- based celecoxib and plumbagin for the synergistic treatment of melanoma. Molecular Cancer Therapeutics;16 (3), 440–52.

37. Kumar B, Kulanthaivel S, Mondal A, Mishra S, Giri S, **2016**. Mesoporous silicananoparticle based enzyme responsive system for colon specific drug delivery through guar gum capping.Colloids and Surfaces B:Biointerfaces; 150, 352-361.

38. Nour SA, Abdelmalak NS, Naguib MJ, **2016**. Novel chewable colon targeted tablets of bumadizone calcium for treatment of ulcerative colitis: Formulation and optimization. Journal of Drug Delivery Science and Technology; 35, 172- 183.

39. Wang QS, Wang GF, Zho JU, Gao L, Cui Y,**2016**. Colon targeted oral drugdelivery system based on alginate-chitosan microspheres loaded with icariin in thetreatment of ulcerative colitis. International Journal of Pharmaceutics; 515 (1–2),176-185.

40. Reshma RS, Sreelatha KH, SomasundaramV, Satheesh KS, Revathy N, Nair RS,Srinivas P, **2016**. Plumbagin, a naphthaquinone derivative induces apoptosis inBRCA 1/2 defective castrate resistant prostate cancer cells as well as prostate cancer stem-like cells. Pharmacological Research; 105,134-145

41. Boshi T, Shaohua L, Shuyi W, Wei L, Wang D, Lin J, Bin H, Ke L, Zhenling W,Zewei Q, **2017**. pH-responsive poly (acrylic acid)-gated mesoporous silica and itsapplication in oral colon targeted drug delivery for doxorubicin. Colloids and Surfaces B:Biointerfaces;154,287-296.

42. Vijaykumar VA, Ravindra PB, Raghavendra VK, **2017.**Functionally modifiedpolyacrylamide-graft-gum karaya pH-sensitive spray dried microspheres for
43. colontargetingofananticancerdrug.InternationalJournalofBiologicalMacromolec ules;102,829-839.

44. Kumari A, JainA, Hurkat P, Tiwari A, Jain SK. Eudragit S100 coated microsponges for Colon targeting of prednisolone. Drug Dev Ind Pharm. 2018 Jun;44 (6): 902-913.

45. Othman MH, Zayed GM, Ali UF, Abdellatif AAH. Colon-specific tablets containing 5-fluorouracil microsponges for colon cancer targeting. Drug Dev Ind Pharm. 2020 Dec; 46 (12): 2081-2088.

46. Gandhi H, Rathore C, Dua K, Vihal S, Tambuwala MM, Negi P. Efficacy of resveratrol encapsulated microsponges delivered by pectin based matrix tablets in rats with acetic acid-induced ulcerative colitis. Drug Dev Ind Pharm. **2020** Mar; 46 (3): 365-375.

47. Rahman M, Almalki WH, Panda SK, Das AK, Alghamdi S, Soni K, Hafeez A, Handa M, Beg S, Rahman Z. Therapeutic Application of Microsponges-based Drug Delivery Systems. Curr Pharm Des. **2022**; 28 (8): 595-608.

48. Sher M, Sarfaraz RM, Iqbal S, Hussain MA, Naeem-Ul Hassan M, Hassan F, Bukhari SNA. Formulation and Evaluation of Hydroxypropyl methylcellulose-dicyclomine Microsponges for Colon Targeted Drug Delivery: In Vitro and In Vivo Evaluation. Curr Drug Deliv. **2022**; 19 (6) : 686-696.