formulation assessment of microsponges loaded gel for reinforced acne treatment

Dr. Shubhangi Aher¹, Prachi Giri²

IPA MSB’s Bombay College of Pharmacy (Department of Pharmaceutics), Kalina, Santacruz, Mumbai-400098

Abstract: Background: Dapsone (DAP) is an antibacterial and anti-inflammatory agent used in the treatment of acne vulgaris. But it has some side effects like mild irritation and dryness and also cause orange-brown discoloration of skin which limits the topical application of the DAP.

Objective: The formulation was prepared to overcome the drawbacks such as irritation, dryness and discoloration related to the skin. This was achieved by developing a gel formulation using microsponges for effective skin delivery of DAP.

Methods: Quasi emulsion solvent diffusion technique was employed in preparation of DAP loaded microsponges. The formed microsponges were embedded in Hydroxypropyl methylcellulose gel for topical application. The microsponges were assessed for entrapment efficiency, particle size analysis, production yield and in-vitro drug release. The prepared DAP loaded microspnge gel was assessed for its pH, viscosity, spreadability, in-vitro drug release, ex-vivo permeation, anti-microbial activity and HET-CAM for irritation study.

Results: The DAP loaded microsponges were successfully fabricated and possessed good entrapment efficiency and smaller particle size. The scanning electron microscopy illustrated spherical and spongy particles. DAP loaded microsponges gel showed no irritation and promising antibacterial activity against Staphylococcus aureus.

Conclusion: The results obtained confirm that DAP loaded microsponges gel can be an effective strategy for management of acne.

Keywords: Microsponges, Acne, Dapsone, Gel

Introduction

Acne is persistent chronic inflammatory disease of the pilosebaceous follicles, which affects the seborrheic areas like face, back and chest. It is characterized by comedones (open/white and closed/black comedones), papules, pustules, nodules, cysts and scars. (Dawson & Dellavalle, 2013) Acne vulgaris is prevalent condition impacting individuals worldwide. Acne emerges during puberty between age of 10 to 13 and it is most noticeable in people with oily skin. (Bickers et al., 2006) Oral and topical treatments are involved in the treatment of acne. Topical treatments comprise of antibiotics, retinoids and its analogs are used in treatment of mild to moderate acne. For severe acne oral treatments are employed. (Feldman & Hancox, 2004)

The pathogenic factors responsible for development of acne are: abnormal sebum excretion, abnormal keratinization, ductal colonization with Propionibacterium acnes (P. acnes) and release of inflammatory mediators onto skin. (Vasam et al., 2023) Topical antibiotics are used in the treatment of the acne as they have antibacterial and anti-inflammatory activity. DAP is a synthetic sulfone with dual anti-inflammatory and antibacterial properties.(Zhu and Stiller, 2001) DAP functions similar to sulphonamides by inhibiting synthesis of dihydrofolic acid. The inhibition occurs by binding competitively with para-amino-benzoate for active site of dihydropteroate synthetase. (Coleman, 1993)

Microsponges are characterized by their porous and polymeric structure, have primary application in extended topical administration. The purpose of microsponges is to optimize the effective delivery of active pharmaceutical ingredients at low doses while improving the stability, mitigating side effects and modifying drug release profiles. (Orlu et al., 2006) The sustain release of the drug from the formulation into the epidermis ensures that the drug is predominantly confined to the targeted area, with only a limited amount entering the systemic circulation. This approach serves as a mechanism to manage and minimize potential side effects.(Embil and Nacht, 1996)

This research aims to combine the advantageous properties of microsponges within a gel formulation. The microsponges were prepared by Quasi emulsion solvent diffusion technique subsequently, these microsponges were added into the gelling agent. The microsponges formulated were further evaluated for particle size, production yield and entrapment efficiency. The resulting gel formation ensures a sustained release of the drug.
Materials and Methods

Materials

The materials required for the present investigation were procured from different sources. Dapsone (DAP) was provided as gift sample from Aadhar Lifesciences Pvt. Ltd., Solapur, India. Ethocel, Methocel K100 M were procured from Colorcon. Polyvinyl alcohol (PVA) was obtained from S. D. Fine Chemical, while Dichloromethane (DCM) were sourced from Loba Chemie Pvt. Ltd.

Methods

Formulation of microsponges

Quasi emulsion solvent diffusion method was employed in the present investigation. In this technique organic phase (internal) is added dropwise to the aqueous phase (external) under continuous magnetic stirring. The organic phase is composed of ethylcellulose and DAP in dichloromethane. The obtained mixture is filtered using Whatmann filter paper and dried in oven at 40°C (Jadhav et al., 2013).

Optimization of microsponges

Box-Behnken experimental design was adopted for statistical optimization process. To achieve high precision of design, $3^3$ i.e. 3-factor, 3-level box-behnken design with 15 experimental runs having 3 replicate of central points was used. The effect of three independent variables that is ethylcellulose (X1), polyvinyl alcohol (X2) and stirring speed (X3) on the responses such as entrapment efficiency (Y1), particle size (Y2) and % production yield (Y3) was evaluated.

Characterization of microsponges

Particle size analysis

The particle size of microsponges was analysed using optical microscope. The optical microscope was fitted with a stage micrometer to calibrate the eye piece micrometer. Approximately 5 mg of powdered microsponges was spread on glass slide and particle size was determined using stage micrometer (Ravi and Senthil 2013).

Calibration of eyepiece micrometer,

1 division of stage micrometer = 0.01mm = 10µm

$$C = SM \times 100$$

Where, $C$= Correction factor, $SM$= Stage micrometer reading, $EM$= Eye piece micrometer reading

The average particle size of microsponges was determined by the formula

$$D = \frac{\sum nd}{\Sigma n}$$

Where, $D$ = Average particle size, $n$ = No. of microsponges, $d$ = Average size range

Production yield

The formulated microsponges were weighed using a digital weighing scale. The % production yield of microsponges was determined by final weight of microsponges to the theoretical weight of the raw materials. The theoretical weight of microsponges was calculated by adding the mass of polymer and drug. (Yadav V et al., 2017).

$$\%\text{ production yield} = \frac{\text{Practical mass of microsponges}}{\text{Theoretical mass}} \times 100$$

Entrapment efficiency

The entrapment efficiency of DAP loaded microsponges was determined by indirect method. Accurately weighed (10mg) of DAP loaded microsponges were added to dialysis bag 12-14 kDa MWCO (Previously hydrated in Phosphate buffer saline pH 7.4) and placed in 10mL of the Phosphate buffer saline pH 7.4 in water bath shaker at 37°C ± 2°C, 60±05 rpm for 15 mins. The aliquots were taken at an interval of 5 minutes (Khattab and Nattouf 2021).
Scanning electron microscopy

The prepared microsponges were analysed for the surface morphology and topography using scanning electron microscopy FEI, Quanta 200 operating at 20kV equipped with a digital image processor (Yehia et al., 2022).

Thermal analysis

Differential scanning calorimetry (DSC) is thermal technique where the difference in amount of heat required to increase the temperature of sample is measured. The thermogram of DAP, ethylcellulose, DAP loaded microsponges gel were obtained using DSC 1 STARe system (Mettler, Toledo, Switzerland). The sample was placed on an aluminium pan which was sealed and exposed to heat at constant rate of 10 °C over temperature range of 30-300 °C. The nitrogen gas is purged at the rate of 10 mL/min during an experiment to maintain an inert environment and endotherm was recorded (Bhatia and Saini 2018).

Fourier transform infrared spectroscopy

Using fourier transform infrared spectroscopy (FTIR-ATR Shimadzu IR spirit) the spectra of DAP, ethylcellulose, polyvinyl alcohol, physical mixture and DAP loaded microsponges were recorded in the range of 4000-400 cm⁻¹ at resolution of 4 cm⁻¹ (Bansode et al., 2019).

Powder X-Ray diffraction

X-Ray diffraction patterns were recorded using X-Ray diffraction instrument (Rigaku, MiniFlex600) between 5 to 60° at 20 angle at speed of 4°/ min. The XRD instrument was equipped with a detector D/teX Ultra with a generator voltage and current of 40 kV and 15 mA, respectively. About 500 mg of powder sample was placed on the sample holder, the excess powder was scrapped off from the slide using the microscope slide. The prepared slide was placed in the X-ray diffraction slot for scanning of sample (Rajkumar and Bhise 2010).

In-vitro release studies

The in-vitro release of microsponges was performed using dialysis bag method. Approximately 10 mg of DAP loaded microsponges were weighed and added to the dialysis membrane which was previously soaked in phosphate buffer saline pH 7.4. The temperature was maintained at 37 ± 0.5°C under stirring (50 rpm). Aliquots of 1 mL were withdrawn at specified time while maintaining the sink conditions, these aliquots were further analysed using UV spectroscopy method (V -1900, Shimadzu, Japan) at λ max of 291 nm (Bhatia and Saini 2018).

Formulation of Dapsone (DAP) loaded microsponges gel

Gelling agents namely HPMC K4M and HPMC K100M were evaluated for their gel forming ability with DAP loaded microsponges. The gelling agents were hydrated for 24 hours. Suitable gelling agent was selected on basis of compatibility with microsponges formulation and ability to spread easily.

Characterization of microsponges loaded gel

Visual morphology

The organoleptic properties, such as colour, texture, consistency, homogeneity, and physical appearance of gel containing microsponges were checked by visual observation.

pH measurement

The pH of the microsponges loaded gel was measured using digital pH meter (Universal Enterprises, Mumbai, India). 10% dispersion of the gel was prepared in the Milli-Q water and it was further analysed (Thavva and Baratam 2019).

Spreadability

Spreadability of gel was determined by Parallel-plate method by placing 0.1 g of microsponges loaded gel within a circle of 1 cm diameter premarked on a glass plate over which a second glass plate was placed. For 5 minutes, a weight of 100 g was allowed to rest on the upper glass plate. The spreading of the gel caused an increase in diameter and change in the diameter was noted. (Dantas et al, 2016).

Viscosity

The viscosity of the placebo gel was determined using a Brookfield viscometer LVT model. At room temperature, dial readings were taken with spindle numbers LV 3 at 0.6,1.5,3,6,12,30 and 60 rpm, respectively. The viscosity was
calculated in centipoise and a rheogram of rpm versus viscosity was plotted. Also, the viscosity of microsponges loaded gel was determined using helipath spindle f at 0.6,1.5,3,6,12,30 and 60 rpm (Nurman et al., Nov 2019).

In-vitro release studies

The in-vitro release study of the DAP loaded microsponges gel was performed using cellophane membrane. The cellophane membrane was previously soaked in phosphate buffer saline pH 7.4 overnight. 100mg of DAP loaded microsponges gel was added to the cellophane membrane and was placed in water bath shaker having temperature 37± 0.5°C under constant stirring of 50 rpm. 1 mL aliquots were withdrawn and sink conditions were maintained. The aliquots were further diluted and analysed using UV spectroscopy method (V -1900, Shimadzu, Japan) at λmax of 291 nm.

Ex-vivo permeation

The ex-vivo permeation study for DAP loaded microsponges gel was performed using Franz diffusion cell apparatus. The receptor compartment of the Franz diffusion cells has volume of 20mL, the tissue of appropriate size was cut and placed between the donor and the receptor compartment. The epidermal side of tissue is in direct contact with the donor medium. The dermal side of the tissue was in contact with the receptor medium (phosphate buffer saline pH 7.4). Aliquots of 1mL were withdrawn from the receptor compartment while eliminating air bubbles (Vernekar et al, 2019).

Microbiological test by Cup-plate method (IP 2007)

It is a qualitative assessment to compare the antibacterial efficacy of developed formulation with the marketed formulation. The microorganism involved in the analysis was Staphylococcus aureus ATCC 6538. The test organism (0.2mL) was inoculated in sterile nutrient agar (autoclaved) and poured into petri plate for solidification. On solidification of nutrient agar, the cavity was formed using sterile cork borer of 4mm diameter. Further, the formulations were poured into the cavity of different petri plates. The formulations were allowed to diffuse through the sterile agar medium and then the plates were inverted and incubated at 37°C for 24 hours. The zone of inhibition was determined using an antibiotic zone reader (Naveen NR 2023).

Hen’s egg chorioallantoic membrane (HET-CAM) test for skin irritation.

The HET-CAM test is performed to check any skin irritancy of the developed formulation by observing the changes that occur in the chorioallantoic membrane of the egg after exposure to the test substances. Irritation is caused by a variety of circumstances, one of which is irritation induced by different formulation components. Skin Irritation studies (Hen’s egg test-Chorioallantoic i.e. HET-CAM) for DAP loaded microsponges gel formulation was evaluated. The purpose of the study is to evaluate the tendency of a test substance to elicit toxicity in the chorioallantoic membrane to assess its potential skin irritation. The beginning of (i) haemorrhage (ii) coagulation and (iii) capillary lysis is used to assess the effects.

Procedure:

1. White leghorn fertile chicken eggs were procured from Central poultry development organization and were cleaned before incubation.
2. The eggs were rotated regularly until day 8 in an incubator at 37± 0.3 °C and 40± 2%. The eggs were observed under the candle light and eggs with broken shells were discarded.
3. On day 9, the eggs were taken out of the incubator for the test. The eggs were placed with wider part upwards and labelled, cut and pared off by using scalpel and tweezers.
4. Around 0.2 mL of 1% NaOH (positive control) and 0.9% NaCl (Negative control) were added to the chorioallantoic membrane.
5. The internal membrane was exposed, around 0.2 g of the test sample was applied to the chorioallantoic membrane and was observed for 300 seconds for sign of lysis, haemorrhage or coagulation (Naveen NR et., 2023).

The observation of the CAM was done by monitoring the endpoints for 5 min and a numerical score depending on the extent of lysis, haemorrhage or coagulation was assigned to each chorioallantoic membrane. These endpoints were evaluated using two different methods of analysis as given in Table No.1.

Stability study ICH Q1A(R2)

The stability study demonstrates how the quality of a drug substance or drug product changes over time in response to changes in environmental conditions like temperature, humidity and light. The stability study of formulation gives us idea about potential excipients reaction, long term drug stability and possible drug expulsion from formulation. It also
assesses the stability of formulation at different environment and storage condition. The DAP loaded microsponges gel was stored at 25°C ± 2°C at 60%RH ± 5%RH and 40°C ± 2°C at 75% RH ± 5%RH according to ICH guidelines Q1A(R2). The formulation was tested at 0, 30, 60 and 90 days. The formulation was tested for appearance, total drug content, spreadability and pH (Abraham J 2010).

Results

DAP loaded microsponges were successfully formulated by quasi-emulsion solvent diffusion technique. The formulated microsponges were embedded in Hydroxypropyl methylcellulose gel for topical application in treatment of Acne.

The effect of polymer concentration, emulsifier concentration and stirring speed on entrapment efficiency, production yield and particle size were observed. Responses of various batches using Box-Behnken design are given in Table No. 2

Analysis of variance was applied to determine significance of effects of independent variables and their interactions on Y1, Y2 and Y3.

\[ Y_1 = +93.46 + 5.10 X_1 + 0.5450X_2 + 0.7563X_3 - 3.33X_1X_2 - 2.76X_1X_3 + 1.13X_2X_3 - 5.47X_1^2 + 1.92X_2^2 + 0.2400X_3^2 \]
\[ Y_2 = +12.00 - 1.20X_1 - 2.39X_2 - 1.26X_3 \]
\[ Y_3 = +78.97 + 3.84X_1 + 2.68X_2 - 0.7688X_3 \]

Characterization of Microsponges

The particle size of microsponges was analysed using optical microscope. The mean diameter ranged from 8.15 to 20.5 μm. Particle size of microsponges is given in Table No. 2.

The production yield of the microsponges batches varied from 70% to 86.3%. The MS8 batch of microsponges has highest production yield i.e. 86.3%. Highest entrapment was observed in batch MS8 i.e., 97.3% of the formulation batches. MS8 batch was further utilized for the preparation of microsponges loaded gel as it has desired properties like minimum particle size, high entrapment efficiency and production yield.

The scanning electron microscopy images of optimized microsponges have spherical shape and were porous in nature. Differential scanning calorimetry graph showed a sharp endothermic peak at 178.34°C for DAP, this corresponds to the melting point range of DAP. The P-XRD of DAP shows sharp peaks which indicates the crystalline nature of the drug while the blank microsponges shows no sharp peaks indicating the amorphous nature of ethylcellulose.

The FTIR spectra of DAP and DAP loaded microsponges is shown in Figure No. 5 DAP showed principle peaks at 1135.26 (Symmetric SO2 stretch), 1276.46 (Asymmetric SO2 stretch), 1434.77 (Aromatic C=C stretch), 1497.52 (Aromatic C=C stretch), 1587.37 (Aromatic C=C stretch), 3465.69 (N-H stretch).

In-vitro release of microsponges showed a sustant release from the porous particles. The optimized batch (MS8) showed % cumulative release of 76.43% in 8 hours.

Characterization of Microsponges loaded gel

The formulated microsponges gel was milky white in color and had no presence of lumps. DAP loaded microsponges gel (MS8G) had a pH of 5.8 ± 1.3 which was within the range of skin pH. The viscosity of MS8G was 156000 cP and the spreadability was found to be 6.8 ± 2.5 cm.

DAP loaded microsponges gel (MS8G) showed % cumulative release of 52.19% within 8 hours, whereas the marketed formulation depicted 97.48% within 4 hours. The ex-vivo permeation through porcine ear skin was found to be 33.16% for MS8G in 8 hours while the marketed formulation depicted 97.48% of permeation.

The developed formulation was compared with marketed formulation for antibacterial efficacy that showed positive activity against Staphylococcus aureus bacteria. HET-CAM assay performed depicts no irritation on applying the MS8G formulation to chorioallantoic membrane as there was no lysis, hemorrhage or coagulation observed.

Discussion

Quasi emulsion solvent diffusion technique was employed in manufacturing of DAP loaded microsponges. As this method avoids solvent toxicity and is easy, reproducible and rapid technique. This method of preparation is widely reported in literature for fabrication of microsponges.
Dichloromethane was used as internal phase as it has capacity to dissolve both DAP and Ethylcellulose. The internal phase (DCM + Ethylcellulose + DAP) is dispersed into external phase (PVA+ MilliQ water), the droplets obtained are due to interfacial tension between external phase and internal phase.

Increased entrapment of drug was observed by increasing the drug:polymer ratio. The high solubility of drug and polymer in Dichloromethane formed homogenous dispersion creating porous microsponges increasing the entrapment of drug. High concentration of polymer had positive influence on the production yield. Increase in emulsifier concentration leads to more % production yield and formed smaller particles of microsponges.

Ethylcellulose exhibits delayed action of release. Hence, increased ethylcellulose concentration extended the drug release from microsponges particle, achieving sustained release of DAP through microsponges.

Prominent peaks observed in FT-IR spectra of DAP shifted in DAP loaded microsponges spectra from the wavelength observed in DAP spectra, that depicted no similarity with pure DAP drug.

The thermal curves of developed formulation indicates single broad peak that represents all excipients are compatible. The melting temperature of formulation decreased compared to DAP drug indicating drug is entrapped in formulation.

SEM images provide the morphology and surface topography of microsponges. Visual information obtained was regarding its spherical nature and porosity. Porous nature of microsponges is due the diffusion of organic solvent from the surface of the microsponges. The absence of drug crystals in SEM confirmed the entrapment of drug in microsponges.

The X-Ray diffraction (XRD) of DAP indicates sharp peaks at 10.62°, 15.69°, 20.99°, 21.27° and 22.69° depicting the crystalline nature of DAP. The XRD of blank microsponges indicate absence of sharp peaks owing to the amorphous nature of ethylcellulose. Developed formulation (MS8G) XRD pattern indicated decrease in the intensity of the peaks leading to decrease in the crystallinity of microsponges concluding the presence of DAP in the pores of microsponges.

The in-vitro release of DAP from microsponges was found to be 76.43% for MS8 in phosphate buffer saline pH 7.4 in period of 8 hours. Ethylcellulose showed sustained release of the DAP from the microsponges particles.

**Dapsone loaded microsponges gel**

The DAP loaded microsponges gel was formulated using HPMC K100M as its viscosity was good compared to HPMC K4M. The placebo gel of HPMC K100M has a neutral pH, which is suitable for the skin. The pH of the formulated gel was pH 5.8 ± 1.3 and is in the range of pH of the skin.

The application of gel was easy due to good viscosity and this helped in good retention of gel on the acne prone skin. Spreadability indicates the extent of area to which gel spreads readily on its application. Incorporation of DAP loaded microsponges into gel did not significantly affect spreadability. High spreadability value indicates their ability to spread easily on the skin.

The in-vitro release from microsponges gel showed a release of 52.19% within 8 hours and marketed formulations showed release of 97.48% in 4 hours of time period. The marketed gel showed 97.48% of permeation from the porcine ear skin in 6 hr while the optimized formulation showed permeation of 33.16% in 8 hr.

Antibacterial assay for DAP loaded microsponges gel and marketed gel against Staphylococcus aureus is given in Figure No 10. The results reveal that DAP has antibacterial activity against Staphylococcus aureus. The zone of inhibition was determined using antibiotic zone reader. The zone of inhibition for Staphylococcus aureus with marketed formulation was 27 mm and optimized formulation (MS8G) was 26.6 mm. This, concludes that the developed formulation exhibits same antibacterial activity as marketed formulation.

Skin irritation studies were mimicked using Chorioallantoic Membrane (CAM) of egg. The HET-CAM assay is performed to determine the potential irritancy of chemicals. CAM is filled with arteries, veins and capillaries that show haemorrhage, lysis or coagulation on injury and these changes are examined for 300 seconds. Irritation score was assessed by two methods that is Method A and Method B. Negative control (0.9% NaCl) showed lysis after 5 min which accounts to non-severe irritation. Lysis and haemorrhage was observed after 0.5 min and coagulation after 5 min in positive control (0.1N NaOH) indicating severe irritation. On application of DAP loaded microsponges gel to CAM, lysis was observed after 5 min and this indicates non-severe irritation indicating that formulation is safe for topical application.

The DAP loaded microsponges gel was observed for physical changes and it depicted no change in the appearance at 25°C ± 2°C at 60%RH ± 5%RH and 40°C ± 2°C at 75 % RH ± 5%RH. There was no significant change observed in the pH of the formulation. Also the formulation showed good spreadability and drug content did not reveal any significant change. The formulation was found stable for period of 3 months.
Conclusion

This study focuses on the investigation of potential of ethylcellulose microsponges formulation for topical delivery of DAP, a BCS class-II drug used in treatment of acne. The formulated microsponges were prepared by quasi emulsion solvent diffusion technique using mechanical stirrer and yielded porous particles. On evaluation of various formulations MS8 was most promising composition as it exhibited sustain release of DAP over period of 8 hours. The optimized microsponges were successfully incorporated into HPMC K100M gel and characterized. Encapsulation of drug into porous microsponges reduces DAP induced irritation on topical application. This delivery system presents new and potentially superior alternative to conventional gel for treatment of acne.

Abbreviations

DAP – Dapsone
BCS – Biopharmaceutical classification system
HPMC – Hydroxy propyl methylcellulose
HET-CAM – Hen’s egg chorioallantoic membrane
XRD – X-Ray diffraction
FTIR – Fourier transform infrared spectroscopy
DSC – Differential scanning calorimetry
DCM – Dichloromethane
PVA – Polyvinyl alcohol

Acknowledgement

The authors are grateful to Aadhar Lifesciences Pvt. Ltd. for providing the gift sample of drug for research work. The authors would like to express their gratitude to Dr. Pravin Tirmali, IR technology services Pvt. ltd., for providing assistance for carrying out X-Ray diffraction patterns. The authors would also like to thank Bombay College of Pharmacy for providing essential resources and laboratory facilities.

References


Table No. 1 Protocol for calculation of Irritation score (IS)

Table No.2 Optimization of polymer concentration, emulsifier concentration and stirring rate by Box Behnken design
### Irritation score (IS) analysis method A

<table>
<thead>
<tr>
<th>Irritation response</th>
<th>IS score at each time point</th>
<th>0.5min</th>
<th>2min</th>
<th>5min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysis</td>
<td></td>
<td>5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td></td>
<td>7</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Coagulation</td>
<td></td>
<td>9</td>
<td>7</td>
<td>5</td>
</tr>
</tbody>
</table>

### Irritation score (IS) analysis method B

0 to 0.9: Non-irritant  
1 to 4.9: Slight irritant  
5 to 8.9: Moderate irritant  
9 to 21: Severe irritant

### IS calculation

According to the following scheme, the sum of the scores assigned at each time point to the occurrence of the corresponding effect:

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>0.5min</th>
<th>2min</th>
<th>5min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysis</td>
<td>5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>7</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Coagulation</td>
<td>9</td>
<td>7</td>
<td>5</td>
</tr>
</tbody>
</table>

### Calculation of Irritation

IS = \[(301 - HT/300) \times 5\] + \[(301 - LT/300) \times 7\] + \[(301 - CT/300) \times 9\]

<table>
<thead>
<tr>
<th>MS9</th>
<th>1:2</th>
<th>90</th>
<th>2000</th>
<th>92.73</th>
<th>8.3</th>
<th>86</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS10</td>
<td>1:3</td>
<td>30</td>
<td>1750</td>
<td>95.83</td>
<td>13.4</td>
<td>84</td>
</tr>
<tr>
<td>MS11</td>
<td>1:3</td>
<td>60</td>
<td>1500</td>
<td>94.24</td>
<td>9.2</td>
<td>86.7</td>
</tr>
<tr>
<td>MS12</td>
<td>1:3</td>
<td>60</td>
<td>2000</td>
<td>94.05</td>
<td>9.8</td>
<td>74.75</td>
</tr>
<tr>
<td>MS13</td>
<td>1:3</td>
<td>90</td>
<td>1750</td>
<td>92.56</td>
<td>12.6</td>
<td>84.75</td>
</tr>
</tbody>
</table>

Figure No.1 3D Response surface plots showing effect of polymer concentration and emulsifier concentration on  
(a) Entrapment efficiency  
(b) Production yield  
(c) Particle size
Figure No. 2 Scanning electron microscopy of DAP loaded microsponges

Figure No. 3 Thermal analysis spectra for (a) Pure drug (b) DAP loaded microsponges gel

Figure No. 4 FT-IR spectra of (a) Pure drug (b) Ethylcellulose (c) DAP loaded microsponges gel
Figure No. 5 X-Ray diffraction of (a) Pure drug (b) Ethylcellulose (c) DAP loaded microsponges

Figure No. 6 In-vitro drug release profile of DAP loaded microsponges
Figure No. 7 In-vitro drug release profile of DAP loaded microsponges gel and marketed formulation

Figure No. 8 Ex-vivo permeation study of Dapsone loaded microsponges gel and marketed formulate.
Figure No. 9 Antimicrobial assay of (a) DAP loaded microsponges gel (b) Marketed formulation

<table>
<thead>
<tr>
<th>Time of Exposure</th>
<th>0.5min</th>
<th>2min</th>
<th>5min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td><img src="negative_control.png" alt="Image" /></td>
<td><img src="negative_control_2.png" alt="Image" /></td>
<td><img src="negative_control_5.png" alt="Image" /></td>
</tr>
<tr>
<td>NaCl 0.9% w/v</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive control</td>
<td><img src="positive_control.png" alt="Image" /></td>
<td><img src="positive_control_2.png" alt="Image" /></td>
<td><img src="positive_control_5.png" alt="Image" /></td>
</tr>
<tr>
<td>NaOH 0.1N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test Sample</td>
<td><img src="test_sample.png" alt="Image" /></td>
<td><img src="test_sample_2.png" alt="Image" /></td>
<td><img src="test_sample_5.png" alt="Image" /></td>
</tr>
<tr>
<td>(MS8G)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure No. 10 HET-CAM assay images of Negative control, Positive control and Test sample (MS8G)

Table No. 3 Stability study of DAP loaded microsponges gel at 25°C ± 2°C at 60%RH ± 5%RH and 40°C ± 2°C at 75 % RH ± 5%RH
<table>
<thead>
<tr>
<th>Temperature</th>
<th>25°C ± 2°C at 60%RH ± 5%RH</th>
<th>40°C ± 2°C at 75 % RH ± 5%RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluation</td>
<td>Time in months</td>
<td></td>
</tr>
<tr>
<td>Appearance</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>No change</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>Total drug content</td>
<td>98.24 ± 0.923</td>
<td>96.37 ± 0.73</td>
</tr>
<tr>
<td>Spreadability</td>
<td>6.7±1.2</td>
<td>6.89±2.2</td>
</tr>
<tr>
<td>pH</td>
<td>5.89±2.3</td>
<td>6.1±1.1</td>
</tr>
</tbody>
</table>