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Formulation and characterization of Dapsone topical gel for the treatment of Leprosy

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ABSTRACT:
Leprosy, commonly known as Hansen's disease, is a global health problem that can range from tuberculoid to lepromatous (i.e., paucibacillary to multibacillary illness) depending on the host immunological response. Leprosy, commonly known as Hansen's disease, is a global health problem that can range from tuberculoid to lepromatous (i.e., paucibacillary to multibacillary illness) depending on the host immunological response. Orally administered dapsone has been linked to hematologic responses such as methemoglobinemia, hemolysis, and agranulocytosis, particularly in patients with glucose-6-phosphate dehydrogenase (G6PD) impairment. Topical therapy reduces the likelihood of these responses. The study's major goal is to achieve an effective drug concentration at the intended site of action for a long enough time to elicit a reaction.

Key Words: dapsone, topical gel, pharmaceutical, leprosy, cutaneous disorder.

I. INTRODUCTION

Topical delivery is defined as the application of pharmaceutical dosage form to the skin for direct treatment of cutaneous disorder, with the intent of confining the pharmacological or other effect of drug to the surface of the skin. Topical drug delivery system includes dosage form like semisolids, liquid preparation, sprays and solid powder. Most widely used semisolid preparation for topical drug delivery includes gels, creams and ointments.

Gels are defined as semi rigid system in which the movement of dispersing medium is restricted by an interlacing three-dimensional network of particles or solvated macromolecules of the dispersed phase[1].

Leprosy, also known as Hansen’s disease, is a worldwide health problem, varying from tuberculoid to Lepromatous Leprosy (i.e., paucibacillary to multibacillary disease) according to the host immune response. It is caused by Mycobacterium leprae, and affects the skin, eyes, and nerves, leading to skin lesions, eye pain, loss of vision, weakness, and numbness. The final diagnosis is based on a combination of findings on skin biopsy, smear, and physical examination. The treatment options differ according to the clinical manifestations. Leprosy can be associated with type 1 (reversal) and type 2 (erythema nodosumleprosum) immunologic reactions that may occur at any time before, during, or after the start of treatment. Oral administration of DAP is associated with several adverse effects, including hemolytic anemia, peripheral neuropathy, nausea, and headache. These side effects diminish its feasibility for treating skin diseases by the oral route. Many of the adverse effects of DAP are related to the production of metabolites. In the liver, DAP is acetylated by N-acetyltransferase which produces mono-acetyl DAP, and upon enzymatic hydroxylation, DAP hydroxylamine is produced, which is primarily responsible for the development of adverse effects. Because of the therapeutic relevance of DAP, it is desirable to reduce its adverse effects using nanotechnology. Most infections due to Gram-positive organisms can be treated with quite a small number of antibiotics.

II. MATERIALS AND METHOD

MATERIAL

Dapsone drug was received as a gift sample from Glenmark pharmaceutical, Ltd. Mumbai, India carbopol 971P and methanol was obtained from grey scientific Ambala and other chemicals utilized (Loba chemicals Pvt. Ltd., Mumbai) was purchased from local supplier. All the other reagents were used of analytical grade were used in the development of the topical gels.

PREPARATION OF ANTI-MICROBIAL GEL

Carbopol 924 was dispersed in 05 ml of distilled water with continuous stirring and left for overnight for swelling. Further of DAPSONE and PEG 400 was mixed to above mixture and volume was made up to 10 ml by adding remaining part of distilled water. The entire ingredient was mixed properly with Carbopol 924 to form a smooth anti-microbial gel. Finally different formulation is made for the adjustment of required pH of about 4.5-5.5, to form a gel of required consistency. The prepared anti-microbial gel was subjected to various evaluation parameters.Gels can be prepared using following ratio of ingredients[2].

S.No.	Ingredients	F1	F2	F3
1.	Dapsone	100mg	100mg	100mg
2.	Carbopol 924	0.15 gm	0.15 gm	0.15 gm
3.	Ethanol	0.4 ml	0.4 ml	0.4 ml
4.	PEG 400	0.5 gm	0.5 gm	0.5 gm
5.	Distilled water	10 ml	10 ml	10 ml

Table 1. Depicting formulation of anti-microbial gel

EVALUATION OF ANTI-MICROBIAL GEL

MEASUREMENT OF PH

The pH values of different formulations were measured using a calibrated digital pH meter at room temperature in triplicate[3].

MEASUREMENT OF VISCOSITY

The measurement of viscosity of the prepared gel was done with a Brookfield Viscometer. The gels were rotated at 0.3, 0.6 and 1.5 rotations per minute. At each speed, the corresponding dial reading was noted. The viscosity of the gel was obtained by multiplication of the dial reading with factor given in the Brookefield Viscometer catalogues[4].

HOMOGENEITY

After the gels have been set in the container, all developed gels were tested for homogeneity by visual inspection. They were tested for their appearance and presence of any aggregates[5].

GRITTINESS

All the formulations were evaluated microscopically for the presence of any appreciable particulate matter which was seen under light microscope. Hence obviously the gel preparation fulfils the requirement of freedom from particular matter and from grittiness[6].

SPREADABILITY

Two sets of glass slides of standard dimensions were taken. The anti-microbial gel formulation was placed over one of the slides. The other slide was placed on the top of the gel, such that the gel was sandwiched between the two slides in an area occupied by a distance of 7.5cm along the slides. Hundred g weight of gel was placed on the upper slides so that the gel was between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of gel adhering to the slides was scrapped off. The two slides in position were fixed to a stand without slightest disturbance and in such a way that only upper slides to slip off freely by the force of weight tied on it. A 20 g weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 7.5 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated for three times and the mean time was taken for calculation. Spreadability was calculated by using the following formula:

$$S = m \times l/t$$

where, S= spreadability
m-weight tied to upper slides (20 g)
l- length of the glass slide (7.5 cm)
t- time taken in sec.

SWELLING STUDIES

To determine the swelling index of prepared topical gel, 1gm of gel was taken on petri dish and then placed separately in a 50 ml beaker containing 10 ml distilled water. Then the samples were removed from beakers at different time intervals and put it on dry place for some time after it re weighed. Swelling index was calculated as follows:

$$\text{Swelling Index (SW) \%} = [(W_t - W_o) / W_o] \times 100$$

Where, (SW) % = Equilibrium percent swelling,
W_t = Weight of swollen gel after time t,
W_o = Original weight of gel at zero time[7]

ANTIMICROBIAL STUDY[8-10]

Test organisms and Inoculums:

Gram positive: Staphylococcus aureus

Gram negative: E. coli

Standard: Povidone iodine Media: -Dehydrated nutrient agar media was used and was prepared in distilled water.

The composition of the media was as given below;

- Agar 15.0%
- Peptic digests of animal tissue 5.0%
- Sodium chloride 5.0%
- Beef extract 1.5%
- Yeast extract 1.5%
- pH 7.4 ± 0.2 at 25°c
- Distilled water 1000 ml

The medium was autoclaved at 15 lbs per square inch pressure at 121°C

PREPARATION OF MEDIA

Dehydrated nutrient agar media (28gm) was accurately weighed and suspended in 1000 ml of distilled water in a conical flask. It was heated on a water bath to dissolve the medium completely.

STERILIZATION OF MEDIA

The conical flask containing the nutrient agar medium was plugged with the help of non-absorbent cotton bung. The mouth of the conical flask and the cotton bung were properly covered with aluminium foil. The medium was then sterilization by autoclaving at 15 lbs per square inch pressure for 20 minutes.

METHOD: CUP AND PLATE METHOD

The sterile nutrient agar medium at a temperature between 40°C to 50°C was immediately poured into the sterile Petri plates to give a depth of 3 to 4 mm, by placing the plates on a level surface. The plates were then allowed to solidify. Each plate was then inoculated with 0.1ml of the solution of test organisms prepared in water for injection. The wells in each plate were bored in the centre that was filled with gel. The plates were then incubated at 37° C for 24 hours. After incubation, zonal inhibition (inhibition around each well) was measured and this value was taken as an indicator for the antimicrobial activity.

STABILITY STUDY

Stability studies of developed formulations were carried out using ICH guideline for accelerated testing with required modifications. All the developed formulations were selected and filled in to test tubes and stored at temperature of 40±2°C/75±5%RH for a period of 3 months. After the period of 3 months, samples were tested for visual appearance, pH and drug content[11,12].

TEM STUDY

Samples were prepared by placing a drop of gel on a carbon-coated 300-mesh copper grid. The suspension was left to adhere on the carbon substrate for about 2 min., to allow its absorption in carbon film and the excess liquid was drawn off with the filter paper. Subsequently a drop of 2% (w/v) aqueous solution of uranyl acetate was applied for 35 seconds for contrast enhancement and again the solution in excess was removed by the tip of filter paper. The sample was air-dried and observed under the Transmission electron microscope (PU Chandigarh) at 90kV[13].

PERMEABILITY STUDIES (DIFFUSION CELL)

Phosphate buffer of pH 5.5 was used for in vitro release study as a receptor medium. The pretreated skin cellophane membrane was used in diffusion cell. The gel sample was applied on the cellophane membrane and then fixed in between donar and receptor. The receptor compartment contained phosphate buffer of pH.The temperature of diffusion medium was thermostatically controlled at 37º±2º by surrounding water in jacket and the medium was agitated by magnetic stirrer. The sample at predetermined intervals were withdrawn and replaced by equal volume of fresh fluid. The samples withdrawn were uv-spectrophotometrically estimated at 293nm against their respective blank.

III. RESULT AND DISCUSSION

Aforesaid mentioned methods were described in the methodology for the development and evaluation of gel containing dapsone as a drug. These formulations were intended to produce immediate release of drugs. The result and discussion are described under different heading as follows. Antiacne gel of dapsone was evaluated for various parameters. In the present study, eight formulations were prepared by varying the polymer concentration, and by using differentpolymers.

As described in the methods, FT-IR studies were carried out on pure drugs and along with the polymer. There were no any kind of interaction was observed between drug and

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excipients used in the development of gel. IR spectra of dapsone and overlay of carbopol 971P, physical mixture combinations are shown Figure 1 & 2 respectively and their observed peaks are given in table2.

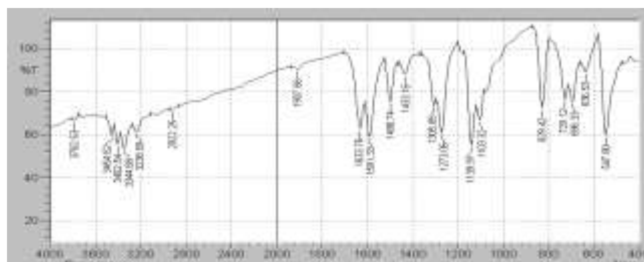

Figure 1. Infrared spectra of Dapsone

Interpretation of chemical group	Observed peaks	Standard ranges
C-C Aromatic	1633.76	1600-1680
N-H stretch	3344.68	3100-3500
N-H bending	1633.76	1640-1550
S=Ostretch	1433.16	1140-1445

Table 2. Interpretation of Pure drug by FT-IR

DSC THERMOGRAPH OF DAPSONE

Melting point of Dapsone was measured by Differential scanning Calorimetry at scanning rate of 10^0C/min. It exhibits melting endothermic peak at temperature of 179.86^0 C as shown in Figure8.

Parameters	Observed results
Melting point determination	182^0 C
DSC method	179.86^0 C
Reference range	178-83 ^{0} C

Table 3. Interpretation of DSC Thermogram of Dapsone

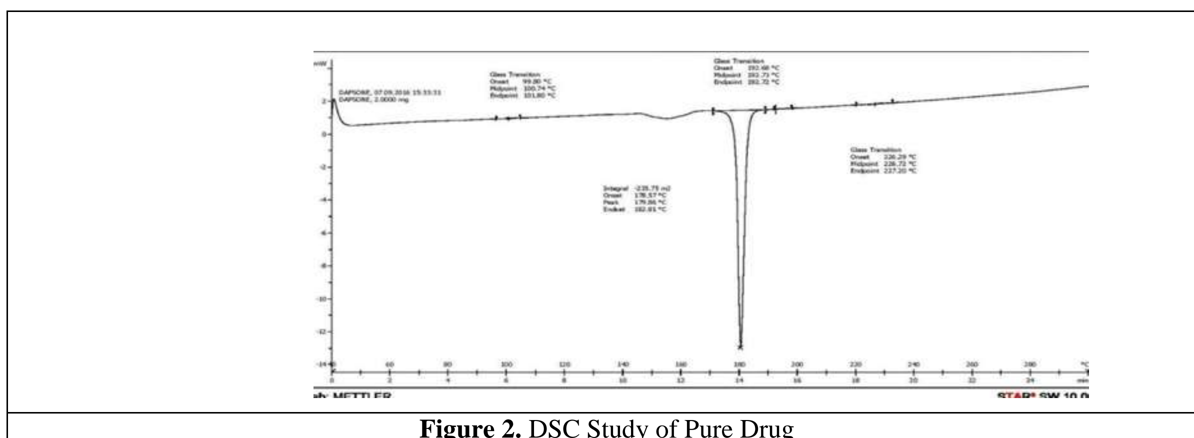

Figure 2. DSC Study of Pure Drug

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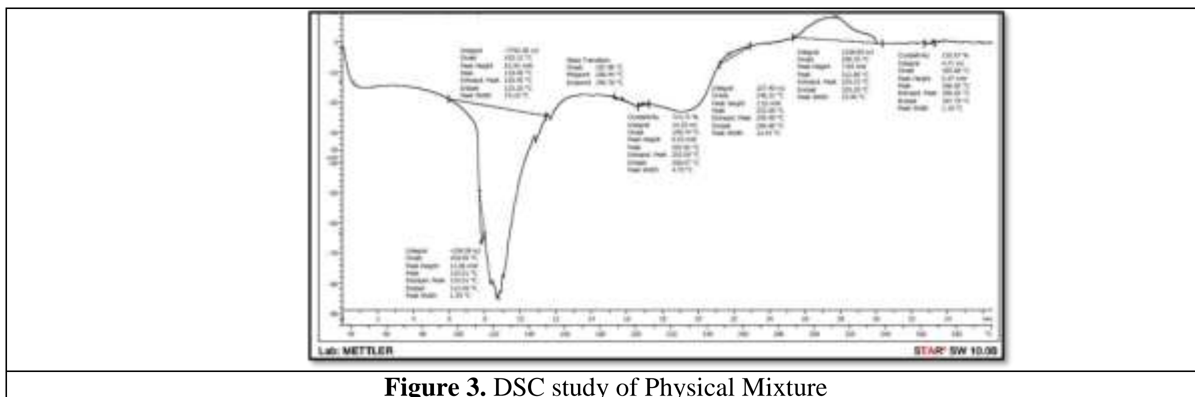

Figure 3. DSC study of Physical Mixture

After the gels have been set in the container, all developed gels were tested for homogeneity by visual inspection and appearance.

PREPARATION OF ANTIMICROBIAL GEL

Figure No. 4: Antimicrobial Gel

EVALUATION
PH

The pH of all the four formulations was in the range of the pH of the skin i.e. 4 to 5.5 which are in range shown in below table

S.No.	F1	F2	F3
pH	4.11	4.09	4.10

Table 4. pH of formulation F1,F2,F3

VISCOSITY
Viscosities of the gels were measured by the Brookfield viscometer in centipoises. The viscosity of different formulations at different rpm are given below;

S.No.	F1	F2	F3
RPM	2.5	5	10
Viscosity (CPS)	216	401	862

Table 5. Viscosity of formulation F1,F2,F3

ANTIMICROBIAL STUDY
Test organism
Gram positive bacteria: Staphylococcus aureus
Gram negative bacteria:E.coli
Standard: Povidone iodine

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Test organism	Standard	F1	F2	F3
S.aureus	2.7cm	2.5cm	2.6cm	3cm
E.coli	2.5cm	3.4cm	2.5cm	3.4cm

Table 6. Zone of inhibition of different gel formulations

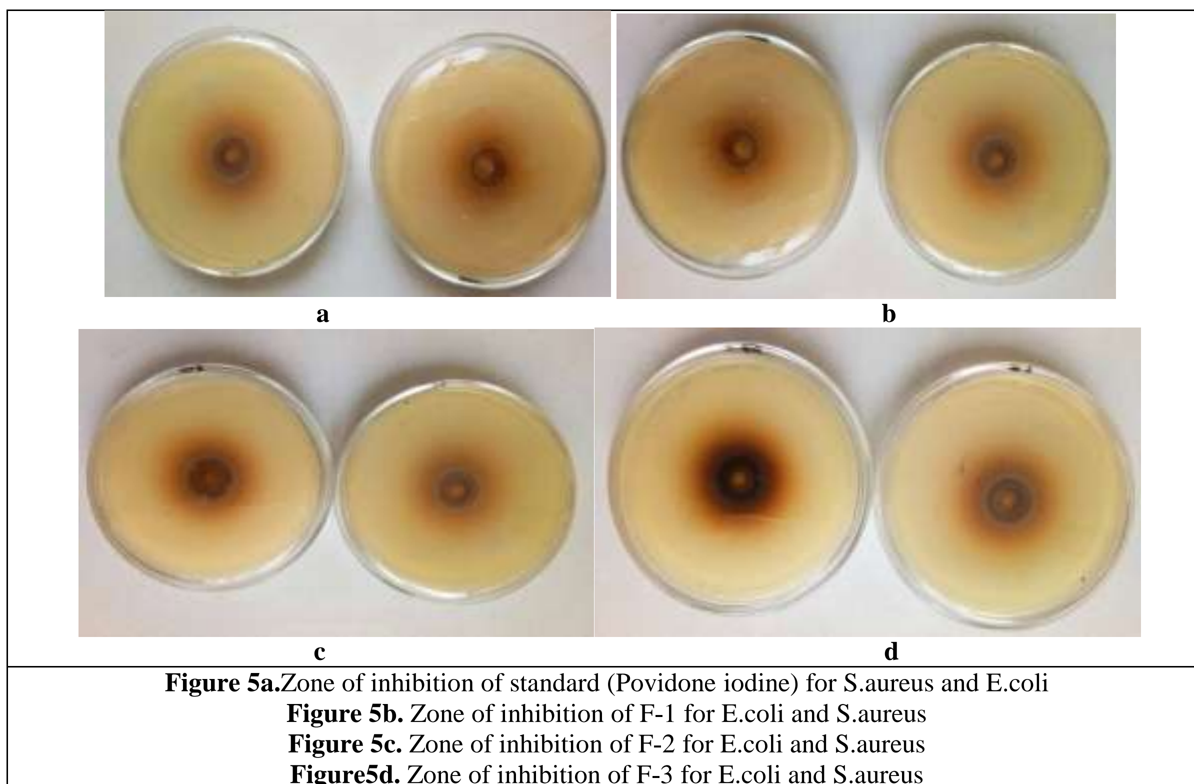

Figure 5a. Zone of inhibition of standard (Povidone iodine) for S.aureus and E.coli
Figure 5b. Zone of inhibition of F-1 for E.coli and S.aureus
Figure 5c. Zone of inhibition of F-2 for E.coli and S.aureus
Figure5d. Zone of inhibition of F-3 for E.coli and S.aureus

The antimicrobial activity of herbal gel was found to be better than standard Povidone iodine as the zone of inhibition of dapsone gel was found to be better than the zone of inhibition of against both the bacteria S. aureus and E. coli than the zone of inhibition of the herbal gel and the values are given in the Table. Hence dapsone gel was found to be more effective in treating the wound healing. It was found that F-3 gel formulation was having best antimicrobial activity when compared with marketed formulation.

STABILITY STUDY

Stability testing of the different gel formulations were shown in table. The prepared gel formulations stored at room temperature, accelerated condition or at refrigerated temperature over a period of 3 month to study the effect of environmental conditions on the four prepared gel formulations.

The stability study was performed to investigate the effect of storage condition in order to check that the formulation withstand the environmental challenges. In stability studies, the gel exhibited changes in pH and on visual observation and was found to be stable at refrigerated condition. At refrigerated condition also the formulation F-3 was stable. The results of stability studies showed that there are no changes in the parameters of F-3 for 3 months at refrigerated condition and are shown in the given below table:
Convert these pictures in real table formats

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Table7: Effect of Temperature on Stability (physical appearance) of dapsone gel after One Month

S. No.	Storage conditions	C-1	F-1	F-2	F-3
1	Room temperature (25-28^{0}C)	No change	change	No change	No change
2	Humidity chamber (40^{0}C)	No change	change	No change	No change
3	Refrigeration temperature (4^{0}C)	No change	No change	No change	No change

Table 8: Effect of Temperature on Stability (physical appearance) of dapsone gel after second month

S.No.	Storage conditions	C-1	F-1	F-2	F-3
1	Room temperature (25-28^{0}C)	No change	Change	Change	Change
2	Humidity chamber (40^{0}C)	No change	Change	change	No change
3	Refrigeration temperature (4^{0}C)	No change	No change	No change	No change

Table 9: Effect of Temperature on Stability (physical appearance) of dapsone gel after third month

S.NO.	Storage conditions	C-1	F-1	F-2	F-3
1	Room temperature (25-28^{0}C)	Change	Change	fades	Change
2	Humidity chamber (40^{0}C)	Change	Change	Change	Change
3	Refrigeration temperature (4^{0}C)	No change	Change	Change	No change

Table 10: Effect of Temperature on Stability (pH) of dapsone gel after one month

S.NO.	Storage conditions	C-1	F-1	F-2	F-3
1	Room temperature (25-28^{0}C)	4.14	4.15	4.24	5.27
2	Humidity chamber (40^{0}C)	3.99	4.02	4.16	4.25
3	Refrigeration temperature (4^{0}C)	4.16	4.18	4.34	4.54

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Table 11: Effect of Temperature on Stability (pH) of Anti-microbial gel after two months

S.NO.	Storage conditions	C-1	F-1	F-2	F-3
1	Room temperature (25-28^{0}C)	4.10	4.09	4.20	5.20
2	Humidity chamber (40^{0}C)	3.91	4.03	4.12	4.21
3	Refrigeration temperature (4^{0}C)	4.14	4.13	4.14	4.38

Table 12: Effect of Temperature on Stability (pH) of dapsone gel after three months

S.NO.	Storage conditions	C-1	F-1	F-2	F-3
1	Room temperature (25-28^{0}C)	4.02	4.06	4.15	5.17
2	Humidity chamber (40^{0}C)	3.83	4.00	4.07	4.15
3	Refrigeration temperature (4^{0}C)	4.03	4.10	4.12	4.22

After one month of Storage at Room Temperature, in Humidity Chamber and refrigerated temperature there is no change in appearance of the prepared gel formulations. After 30-90 days in different storage conditions the colour of F-3 gel does not fades in refrigerated condition where other formulation were not stable in their state and decreases the pH. Formulation F-3 was more stable as compared to other formulation and colour was not change. The stability study of formulation is without preservatives, if we used preservatives than stability will be increased as usual.

SPREADABILITY

All the prepared gels using different polymers in different concentrations were spreadable. The formulation first showed the maximum spreadability followed by second and third and the results are tabulated in table no.13.Spreadability decreased with the increase in the concentration of the polymer.

S No.	Formulation Code	Spread ability (g.cm/sec)
1.	F – 1	94.49 ± 1.04
2.	F – 2	78.99 ± 1.96
3.	F – 3	54.04 ± 0.608

Table 13. Results of spreadability of gel (mean ± standard deviation)

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Fig 6. Gel subjected to spreadability determination

SWELLING STUDIES

Hydrogel can swell thousands of time then its dry weight of the hydrogel. The release of drug from hydrogel particles depends on the swelling behaviour. As the hydrogel swells, the network pores open and drug release occurs. Swelling studies of the anti-dapsone gel were done as dynamic equilibrium study. The results are tabulated in table no. 14 and graphically represented in the figure no. 10. It may be concluded that as the quantity of the polymer increased, the swelling ability of the formulations also increased.

Time (hours)	F – 1(ml)	F – 2 (ml)	F – 3(ml)
0	0	0	0
1	13.9 ± 5.45	30 ± 8.16	40 ± 8.16
2	33.3 ± 4.71	43.3 ± 4.64	53.33 ± 4.64
3	36.6 ± 4.71	56.6 ± 4.71	63.33 ± 4.64
4	43.3 ± 4.69	66.6 ± 4.71	76.67 ± 4.64
5	70 ± 8.16	83.3 ± 4.69	86.67 ± 4.71

Table 14. Results of percentage swelling index of dapsone gel (mean ±)

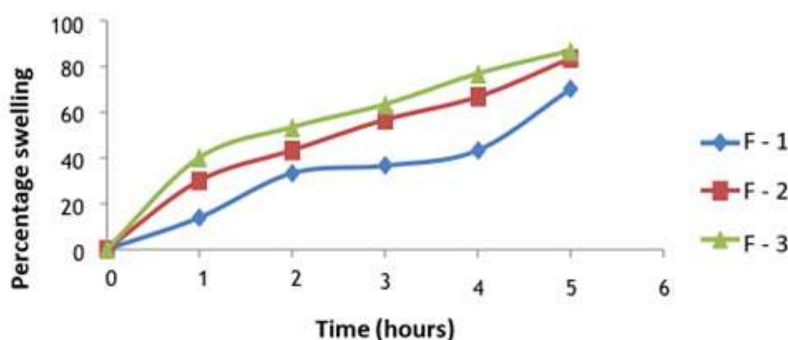

Figure 7. Percentage swelling index v/s time profile of formulations F - 1, F - 2 and F – 3

TRANSMISSION ELECTRON MICROSCOPY

The morphology of hydrogel was analysed by the help of transmission electron microscope [FEI Technai G^2 F20 Netherland]. The results are shown in figure given below. Most of the particles were spherical with only few irregular shaped particles.

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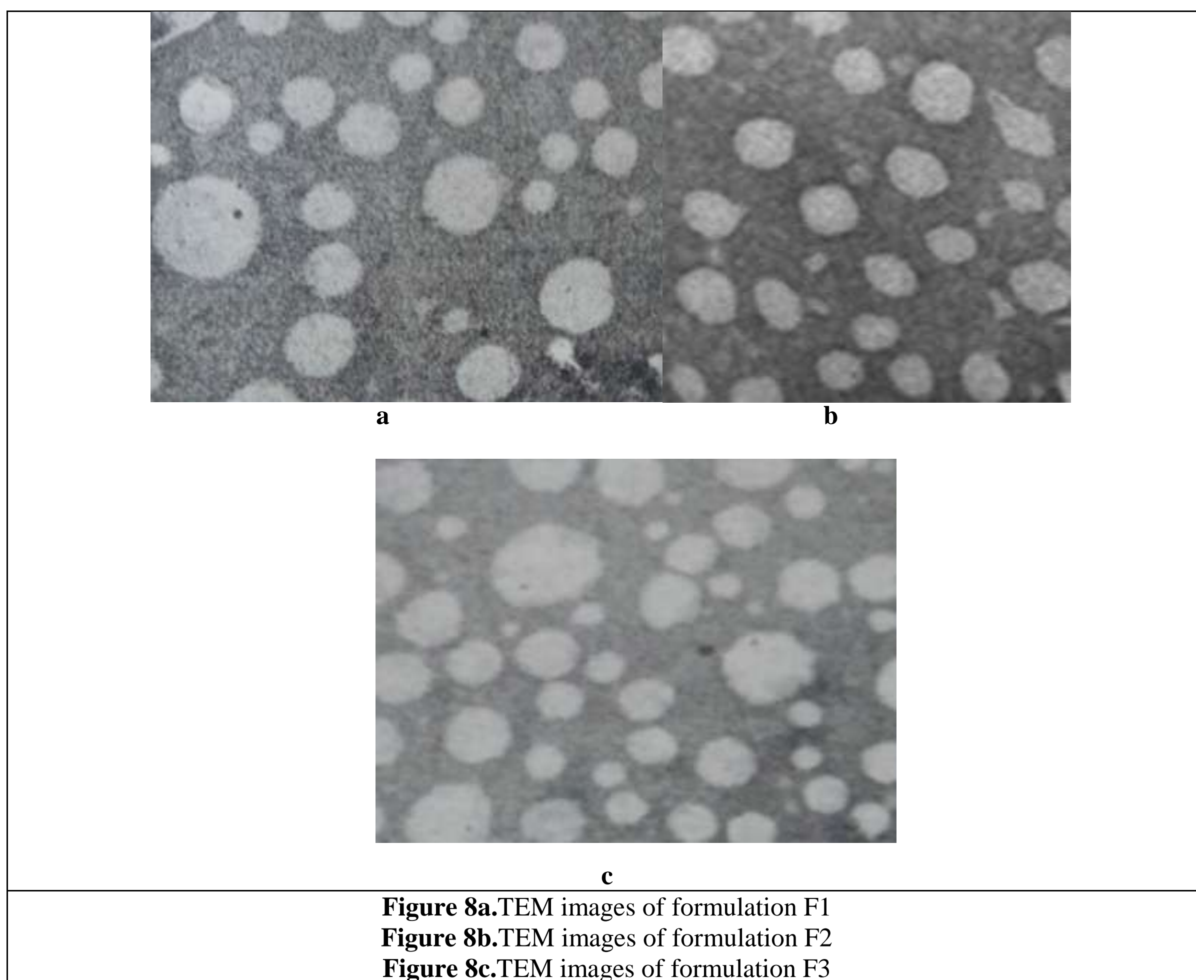

Figure 8a.TEM images of formulation F1
Figure 8b.TEM images of formulation F2
Figure 8c.TEM images of formulation F3

IN-VITRO RELEASE STUDIES

Formulations were performed using the diffusion cell apparatus with dialysis membrane. PBS pH 5.5 was used as diffusion media. The initial rate of drug release was found to be rapid due to incomplete gel formation, but as the time progresses the release rate decreases due to the complete formation of gel batch result was found to be for F1 86.78%, F2 97.9% F3-90.42%\at the end of 90 min. The results showed that the developed Gels had the ability to release the drug for the duration of about 90 minutes. In vitro release study indicated that the release of drug varied according to the type and concentration of polymer utilized in development of gel formulations. The amount of drug release from F2 Batch was found to be maximum 97.92% at the end of 90 minute.

Time (min)	F1	F2	F3
0	0	0	0
15	21.96	18.36	24.48
30	35.4	31.42	36.5
45	46.99	43.37	50.6
60	57.86	60.5	61.48
75	72.32	78.7	75.94
90	86.78	97.92	90.42

Table 15. drug release of Formulated Gel

IV. CONCLUSION

Leprosy is a big task to treat because of its chronicity and continuation which builds adolescent and psychologically distressed. A range of systemic and topical drugs are available which targets the different phase of leprosy. Inflammation is also measured to be key factor in pathogenesis of leprosy. Systemic antibiotic and other anti-inflammatory drugs are being utilized in management of leprosy. In conclusion, the formulated gel as a topical drug delivery system promising the approach which are utilized for improving efficacy of dapsone in the treatment of leprosy. FTIR and DSC study indicated that there is no interaction between the drug and excipients. On the basis ofin-vitro drug diffusion study optimize batch F2 was selected showing 98.9 % drug release at the end of 90 min. This gel was clear, transparent. the pH was neutral and its viscosity was easily removed from container and spreadability is excellent and easily spread, gave antimicrobial activity high effectiveness in inhibiting the growth of microorganisms. The gel was stable at room temperature. The quality control tests results were within the acceptable limits. Hence in future such type of topical drug delivery may utilize for the leprosy treatment.

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