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Design, Development and Characterization of Nanoparticulate Drug Delivery System

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ABSTRACT

Background: Recently, nanoparticles are becoming popular in drug delivery.The present research work was designed to formulate and evaluate polymeric nanoparticle loaded gel of dapsone (DS). An attempt was made to develop topical gel with better skin permeation rate and antimicrobial activity.

Methods: The polymeric nanoparticles formulation of dapsone were prepared by nanoprecipitation method and evaluated forits *in vitro* characteristics. The effect of DS concentration of poloxamer 407, stirring speed and volume of acetoneon particle size and entrapment efficiency was studied using factorial design. The optimized nanoparticle formulation was incorporated into the gel using carbopol-974P as a gelling agent. The nanoparticle loaded gel was characterized for pH, viscosity, drug content, *in vitro* drug release.

Results: The optimized dapsone nanoparticle formulation showed particle size of 156.3nm with polydispersity index 0.541 and 91.48±1.82% drug entrapment. The nanoparticle loaded gel was clear, having good viscocity and spreadability and antimicrobial activity against *propionibacterium acne.*

Conclusion: In the present study, poly (lactic-co-glycolic acid) loaded dapsone nanoparticles were successfully prepared by using nanoprecipitation method. It was demonstrated that the poloxamer-407 as a surfactant produce nanoparticles with maximal particle size reduction and stable zeta potential value. The developed nanoparticles were successfully loaded into gel by using carbopol 974P as gelling agent. The results of in-vitro diffusion study showed that the developed nanoparticle loaded gel had the ability to release the drug for the duration of about 7 hours. This nanoparticle loaded gel was clear, transparent, the pH was neutral and its viscosity was good and spreadability is excellent and easily spread, gave antimicrobial activity high effectiveness inhibiting the growth of *propionibacterium acne* bacteria.

Keyword: Polymeric nanoparticle, Dapsone (DS), Nanoprecipitation method, poly (lactic-co-glycolic acid) (PLGA)

1. INTRODUCTION [1, 2, 3, 4]

Acne is a multifactorial inflammatory skin disease that primarily affects adolescents and young adults, causing a significant physical, psychological, and psychosocial burden on those who suffer from it. Acne is characterised by increased sebum production as a result of the androgenic effect on the pilosebaceous unit, which leads to a chronic inflammatory disorder, microbial colonisation by *propionibacterium acne,* altered keratinization, and inflammation of hair follicles in various areas of the body such as the forehead, cheek, chest, neck, and back. Normal skin follicles were blocked, and an overgrowth of bacteria, *propionibacterium acne,* causes destruction of the follicle lining, allowing follicular material to enter the dermis and cause an inflammatory response.

1.1 CLASSIFICATION OF ACNE

Acne is broadly classified into four classes such as papular, pustular, comedonal, and pustulocytic acne.

The presence of papules usually on the face, back, and posterior region of the body can be recognised as a papular type of acne. Generally presence of bacteria, heat, oil, and dirt is responsible factors for causing this type of acne. Inflammatory lesions of size less than 5 mm can also be seen in the affected area. Pustular acne can be characterized by small bumps on the skin surface filled up with pus or fluid; these pustules are generally present in the areas like face, neck, and chest.

The acne with non-inflammatory comedones usually present in the area of facial skin is recognised as a comedonal acne. They are also called microcomedones if their size is 1mm or more than 1 mm.

Pustulocystic type of acne can be recognised by the presence of cyst with scarring in the groin, buttocks, and armpit region. They affect the deeper skin layers of the body.

Current treatment like topical therapies with benzoyl peroxide, tretinoin and clindamycine causes skin irritation, dryness, burning, erythema and peeling. Extensive use of topical and oral clindamycin has resulted in increasing clindamycin resistant microorganism which has increased inappropriate therapeutic responses systemic therapies including oral antibiotic like doxycycline, erythromycin, and minocycline resulted in gastrointestinal upset, emergence of resistance of *propionibacterium acne,* vertigo, hyperpigmentation of skin and oral mucosa. Also, other treatment like surgical and photodynamic are very costly technique and not affordable by all the patients. [5, 6, 7]

Hence, dapsone was introduced to treat acne. Dapsone has a longer half-life, fewer side effects and drug interactions, and safety in pregnancy. Dapsone (4-[4-aminobenzene) sulfonyl]aniline) is an antibacterial agent with a bacteriostatic effect and is used in a wide range of indications due to its unique properties, namely long-term treatment safety, immediate reduction and control of skin lesions. steroid-sparing and CNS-protective effect. The mechanism of topical application of dapsone in the treatment of acne vulgaris may involve a combination of anti-inflammatory and antibacterial effects. Dapsone is a Class II BCS drug with low water solubility. Oral administration of dapsone has several side effects, including hemolyticanemia, nausea, and headache. Dapsone side effects are related to the production of metabolites. In the liver, dapsone is acetylated by N-acetyltransferase to form monoacetyldapsone, and after enzymatic hydroxylation, dapsone hydroxylamine is formed, which is mainly responsible for the generation of side effects. These side effects reduce the practicality of its oral use in treating skin conditions.Because of unsuitability of dapsone for the oral route; topical route is another alternative. [1,2,8]

Hence in this study, nanoparticulate based topical gel formulation of dapsone was prepared for increasing solubility and penetration, reducing side effects, improve patient compliance and site-specific delivery.

2. MATERIALS AND METHODS:

Dapsone was gifted by Atul Ltd. Mumbai. PLGA was gifted by corbion biomaterials, Netherlands. PVP K30 and PVA were purchased from alpha chemical lab, India. Poloxamer 407 was purchased from BASF Chemicals India Private Limited, Inidia. All the solvents were purchased from Molychem, Mumbai. All the solvents and chemicals were of analytical grade.

3. FORMULATION OF NANOPARTICLES

Dapsone nanoparticles were prepared by the nanoprecipitation method. Dapsone, PLGA polymer, and poloxamer 407 were dissolved in organic solvent (acetone) at room temperature, and cyclo mixed to get clear solution, then the resultant organic solution was added dropwise into distilled water with subsequent agitation by a magnetic stirrer, stirring was continued until complete evaporation of organic solvent. [9, 10]

4. PREFORMULATION STUDY [11, 12, 13]

It is the study of physical and chemical properties of drug prior to its formulation process which is intended to provide a scientific data to support a dosage form design and to evaluate product efficacy and stability.

4.1 Solubility of dapsone

Solubility of drug dapsone was determined in distilled water, methanol, ethanol, acetone, ethyl acetate, dichloromethane (DCM).

4.2 Melting point determination

Melting point of the drug dapsone was determined by capillary method. The melting point of the drug was determined for the determination of any impurity. The obtained result was compared with the reported data.

4.3 Fourier Transform Infra-Red (FTIR) Spectroscopy

A Fourier-transform infrared spectroscopy (Shimadzu IR Spirit, Lab solution, Japan) was used to study the drug excipient compatibility studies. FTIR of the pure drug dapsone and a mixture of drug with excipients were taken. Infrared spectra were recorded. The sample was placed in the IR sample holder. A background scan was taken followed by the IR scan of the sample in the range of 4000-400 cm^2. The peaks of the pure drug were compared with the physical mixture of drug and excipients.

4.4 Differential Scanning Calorimetry (DSC)

A thermal property of pure drug powder was investigated using DSC TA 25. About 20mg of sample and alumina was filled in aluminum pan in sample and control compartment of furnace. The heat runs were set from 30°C to 300°C with increment at a heating rate of 10 °C/min under an inert environment using nitrogen gas. Ultrahigh purity nitrogen was used as the purge gas at a flow rate of 50 ml/min. Alumina was used as a reference standard.

4.5 Ultraviolet-Visible (UV-Visible) spectroscopy

Determination of λ max and construction of calibration curve

The UV system consisted of a Shimadzu UV-visible spectrophotometer with 10mm quartz cuvettes were used for determination of λ max and construction of calibration curve.

Determination of maximum wavelength

10 mg of dapsone was weighed accurately and dissolved in 10 ml Methanol to get 1000 µg/ml stock solution and further diluted with methanol to get 10µg/ml. The UV spectrum was recorded in the range 200-400 nm using methanol as blank. The absorbance maxima for the drug were found to be 291 nm.

Construction of Calibration curve

1) In methanol

10mg of dapsone was weighed accurately and dissolved in methanol (1000 µg/ml). 1 ml of this solution was diluted to 10 ml with methanol (100 µg/ml). 1 ml of this solution was diluted again to 10 ml with methanol (10 µg/ml) from this solution different dilutions were prepared with methanol in concentration range of 2,3,4,6 and 8 µg/ml. The absorbance of these solutions was measured at λ max 291nm using methanol as a blank solution by UV-visible Spectrophotometer. Absorbance value was plotted against the concentration to obtain standard graph.

2) In phosphate buffer pH 7.4

10 mg of dapsone was weighed accurately and dissolved in methanol (1000 µg/ml). 1ml of this solution was diluted to 10ml with phosphate buffer pH 7.4 (100 µg/ml). 1ml of this solution was diluted again to 10ml of phosphate buffer pH 7.4 (10 µg/ml) from this solution different dilution were prepared with phosphate buffer 7.4 in concentration range of 2,3,4,6 and 8 µg/ml. The absorbance of these solutions was measured at λ max 291nm using phosphate buffer 7.4 as a blank solution by UV-visible spectrophotometer. Absorbance value was plotted against the concentration to obtain standard graph.

4.6 SELECTION OF METHOD

In this study, four different methods which are 1) Emulsion Solvent Evaporation, 2) Nanoprecipitation, 3) Solvent diffusion, 4) Evaporation assisted solvent antisolvent interaction (EASAI) were evaluated. The most suitable method was identified by optimum particle size and entrapment efficiency.

4.7 SELECTION OF POLYMER

Two polymers namely, Polyvinyl alcohol (PVA) and Poly (lactic-co-glycolic acid) (PLGA) were studied. The most suitable polymer was selected based on particle size and entrapment efficiency.

4.8 SELECTION OF SURFACTANT/ STABILIZER

In this study, four different surfactant/ stabilizer polyvinyl alcohol (PVA), polyvinylpyrrolidone K30 (PVP K-30), poloxamer 407, and poloxamer 188 were studied and evaluated based on particle size and entrapment efficiency.

5. FORMULATION, OPTIMIZATION AND CHARACTERIZATION OFNANOPARTICLES OF DAPSONE

Trial batches were taken for the screening of independent factors. The independent factors which are considered for screening are mentioned below,

Sr. No.	Independent factors	Lower limit (-)	Higher limit (+)
1)	Concentration of dapsone	20mg	40mg
2)	Concentration of PLGA	30mg	60mg
3)	Concentration of poloxamer 407	60mg	80mg
4)	Volume of organic solvent	3ml	8ml
5)	Stirring speed	800rpm	1200rpm
6)	Flow rate	0.5ml/min	1ml/min
7)	Stirring time	3hr	6hr

Table 1 : Independent factors for screening

The factors were evaluated for particle size and entrapment efficiency. Based on results obtained the factors which influence the particle size and entrapment efficiency were selected further for the optimization.

5.1 OPTIMIZATION OF NANOPARTICLES BY USING FACTORIAL DESIGN

Factorial design of 2 level, 3 factor was designed to optimize the formulation. The design expert software was used for the optimization.

Run	Factor 1: Concentration of poloxamer 407 (mg)	Factor 2: Stirring speed (rpm)	Factor3: Volume of acetone (ml)
1	60	800	3
2	80	800	3
3	60	1200	3
4	80	1200	3
5	60	800	8
6	80	800	8
7	60	1200	8
8	80	1200	8

Table 2: Factorial design for optimization of nanoparticles

5.2 CHARACTERIZATION OF OPTIMIZED BATCH [14]

5.2.1 Particle size, polydispersity index and zeta potential

The mean particle size, polydispersity index (P.I.) and zeta potential of the resulting nanoparticles were determined by Horiba Nanopartica SZ100 particle size analyzer by placing the formed nanosuspension in four-sided transparent polystyrene cuvette and electrophoretic cell respectively for analysis.

5.2.2 Drug entrapment efficiency

The resultant nanosuspension was centrifuged at 12,000rpm, 4°C for 30min. The nanoparticle pellet was settled down and washed twice with double distilled water to remove unentrapped drug completely, and supernatant was collected. The amount of unentrapped drug in the supernatant was determined by using UV spectroscopy at 291nm. The percentage drug entrapment was calculated by using formula:

Entrapment Efficiency (%) = $\frac{Total\ amount of drug - Amount\ of\ free\ drug}{Total\ amount\ of\ drug} \times 100$

5.2.3 Morphology of Dapsone and dried powder of nanoparticles by Field Emission Gun-Scanning Electron Microscopy (FEG-SEM)

The outer macroscopic structure of plain dapsone powder and dried powder of dapsone nanoparticles were investigated by FEG-SEM (Field emission gun-scanning electron microscope). The powder samples were stuck on stubs using a conducting carbon tap. The samples were then coated with Pt (~10nm thickness). These were then analyzed under FEG-SEM. The obtained images were examined for the surface morphology of dapsone crystals.

5.2.4 Differential scanning calorimetry of optimized nanoparticle batch

A thermal property of optimized nanoparticle batch was investigated using DSC TA 25. About 20 mg of sample and alumina was filled in aluminum pan in sample and control compartment of furnace. The heat runs were set from 30°C to 300°C with increment at a heating rate of 10 °C/min under an inert environment using nitrogen gas. Ultra high purity nitrogen was used as the purge gas at a flow rate of 50 ml/min. Alumina was used as a reference standard.

6. FORMULATION OF NANOPARTICLE LOADED GEL [15]

A topical nanoparticulate gel containing dapsone was formulated using carbopol 974P, propylene glycol and methyl paraben.

6.1 EVALUATION OF NANOPARTICLE LOADED GEL [16, 17, 18]

Evaluation of gel was done based on following parameters:

6.1.1 Appearance

All developed gels were tested for appearance by visual inspection. They were tested for presence of any aggregates.

6.1.2 Spreadability

Spreadability was determined to obtain uniform and optimum spreading. 1 gm of gel placed over one of the slide, over which a second glass plate was placed. A weight of 1000gm was allowed to rest on the upper glass plate for 5min. The distance travelled by upper slide after removing of weight was calculated and spreadability was calculated using formula,

$$S = ML/T$$

Where,

S, is the spreadability of gel

M, is the weight (g) tied on the upper plate

L, is the length of glass plates

T, is the time taken for plates to slide the entire length.

6.1.3 Determination of pH

For determining pH of a gel, 1 gm of gel was dispersed uniformly in 20-30ml of water and volume was making up till 100ml and it was kept aside for 2 hours. Then the pH of that solution was measured using digital pH meter.

6.1.4 Drug Content

Gel equivalent to 10 mg drug was mixed vigorously in 10ml acetone- methanol mixture till maximum gel gets dissolved. The Solution was then filtered using whatman filter paper and 1ml of the filtered solution was again diluted using 10ml methanol. The absorbance of the final solution was taken in UV spectrophotometer at 291nm.

6.1.5 In-vitro Diffusion study [19]

The *In-vitro* diffusion study was carried by modified franz-diffusion method using dialysis membrane. The compartment was filled with 30 ml of phosphate buffer pH 7.4. The aliquot of 2ml was taken in suitable interval of time for 24 h and analyzed using UV spectrophotometer at 291 nm.

6.1.6 Antimicrobial study

The developed formulations of nanoparticle loaded gel and marketed gel were checked for their antibacterial activity against *propronibacterium acne*, using the cup plate method. The zone of inhibition for each sample was observed, measured and expressed in mm.

6.1.7 Histopathology study[20]

Histopathological study by using pork skin was carried out to assess any skin irritation. The treated skin (optimized nanoparticle loaded gel was applied to skin) and untreated skin were collected was stored in 10% formalin solution. The samples were dehydrated using ethanol, embedded in paraffin and stained with hematoxylin and eosin. The skin samples were then observed under light microscope and compared with the untreated (control) skin sample and evaluated for any skin irritation effect.

6.1.8 Stability Study

Chemical and physical stability of nanoparticle loaded gel was assessed for a period of 3 months under storage conditions; 25±2 °C/ 60±5%RH as per ICH conditions.

7. RESULT AND DISCUSSION:

7.1 PREFORMULATION STUDY

7.1.1 Solubility of Dapsone

Dapsone was found freely soluble in ethanol, methanol, acetone, ethyl acetate, dichloromethane (DCM) and was practically insoluble in water.

7.1.2 Melting point determination

Melting point of the drug dapsone was found to be 176 °C. At temperature 170°C the slight change in the consistency of dapsone powder filled in capillary tube was observed. Melting was started at 172°C and at 176°C it was observed that the drug dapsone was completely melted.

7.1.3 Fourier Transform Infra-Red (FTIR) Spectroscopy[21]

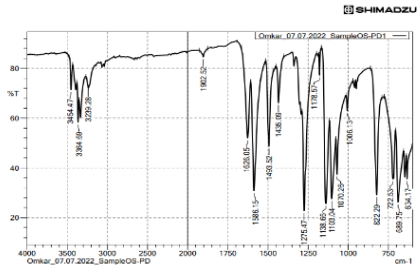


Figure 1: FTIR spectra of Dapsone

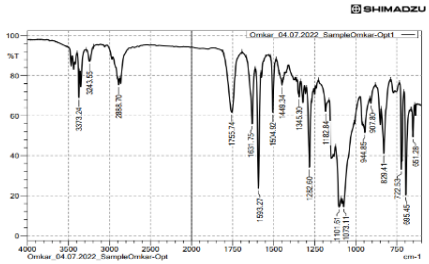


Figure 2: FTIR of physical mixture

The pure drug spectrum and physical mixture showed the characteristic stretching's. The spectrum of drug and physical mixture indicate that there is no chemical interaction between drug and excipients. These observations were similar to the data reported in literature.

Stretching	**Standard range**	**Observed range for drug dapsone**	**Observed range for physical mixture**
C-C aromatic	1600-1680	1626.05	1631.75
N-H stretch	3100-3500	3364.69	3373.24
N-H bend	1640-1550	1586.15	1593.27
S=O stretch	1140-1445	1178.57	1182.84

Table 3: FTIR observations of dapsone and physical mixture

7.1.4 Differential Scanning Calorimetry (DSC) [21]

The thermogram of pure dapsone drug powder exhibited sharp endothermic peak at 178 °C which is near to its melting point and thus indicating its crystalline nature which is described in figure 3. The obtained result was in accordance with the reported data in the literature.

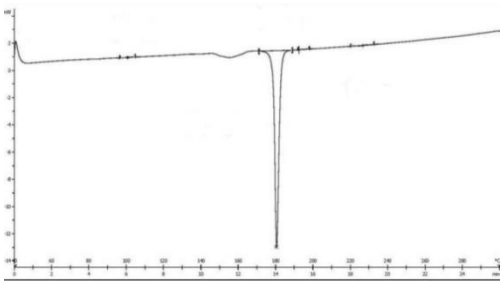


Figure 3: DSC of dapsone

Determination of λ max and construction of calibration curve

Figure 4 reflects the UV-Visible spectrum of 10µg/ml standard solution of dapsone in methanol. Wavelength of maximum absorption (λ max) for dapsone was found to be at 291 nm for the maximum absorptivity. Many researchers who have worked on dapsone reported the same observations as dapsone λ max.

Based on the preformulation studies performed to identify the drug; the obtained results of FTIR spectra, DSC thermogram and UV-Visible spectrum confirms the drug is dapsone. The obtained calibration curve of dapsone in methanol suggested that, dapsone obeying Beer's law was in the linearity range of 2,3,4,6 and 8µg/ml and the obtained experimental data (y = 0.0813x + 0.044 with correlation coefficient of 0.998) were used to estimate the dapsone in drug content uniformity study. Also, the obtained calibration curve of dapsone in phosphate buffer pH 7.4. The curve suggested that, dapsone obeying Beer's law was in the linearity range of 2,3,4,6 and 8µg/ml for buffer solution and the obtained experimental data (y = 0.089x + 0.012 with correlation coefficient of 0.997) were used to estimate the dapsone*in-vitro* diffusion study.

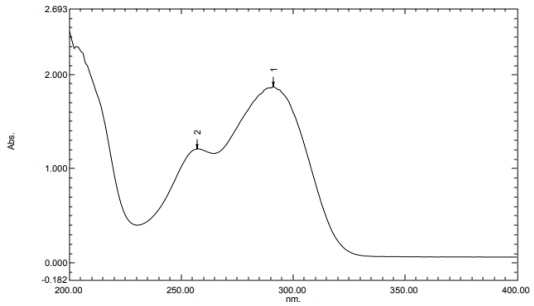


Figure 4: UV-Visible spectrum of dapsone

7.2 SELECTION OF METHOD

In this study, four different methods were evaluated which are 1) Emulsion Solvent Evaporation, 2) Nanoprecipitation, 3) Evaporation Assisted Solvent Antisolvent Interaction (EASAI), 4) Solvent diffusion. The most suitable method was identified by optimum particle size and Entrapment efficiency.

Sr. No.	Method	Instrument used	Solvent used	Results and Conclusion
1)	Emulsion Solvent Evaporation	A) Probe sonicator Sonication Time: 20min,30min,40min Pulse Time: 5sec	Ethyl Acetate	Particle size was found in the range of 3470.2nm to 7226.5nm and entrapment efficiency was found between 30 % to 89.06%.
			Dichloromethane (DCM)	Small aggregates were observed after sonication when DCM was used.
		B) High Speed homogenizer Speed: 8000rpm, 10000rpm Time: 10min, 20min, 30min	Ethyl Acetate	Particle size was found in the range of 3262.7nm to 3845.2nm and entrapment efficiency was found between 47.54 % to 86.03%.
			Dichloromethane (DCM)	Particle size was found in the range of 3196.0 nm to 6069.4 nm with entrapment efficiency 37.5 % to 76.26%.
2)	Nanoprecipitation A) When surfactant is added to aqueous phase	Magnetic stirrer (800rpm-1400rpm)	Acetone	Particle size was found in the range of 423.4nm to 5758.4nm with entrapment efficiency 32.2 % to 90.2%.
	B) When surfactant is added to organic phase			Particle size was found in the range of 90.3 nm to 1191.1 nm with entrapment efficiency 78.3 % to 95.54%.
3)	Evaporation Assisted Solvent Antisolvent Interaction (EASAI)	Magnetic Stirrer (1200rpm, 1400rpm)	Acetone, Methanol, Ethanol	Clear solution observed with particle size more than 10,000nm.
4)	Solvent Diffusion Method	Magnetic Stirrer (800rpm-1400rpm)	Acetone, Acetonitrile	Particle size was found in the range 2851nm to 5819.6nm with entrapment efficiency 67.5% to 87.8%.

Table 4: Results observed for different methods

According to results obtained it was observed that nanoprecipitation method (surfactant in organic phase) found to give optimum particle size and entrapment efficiency, so it was further selected for the study.

7.3 SELECTION OF POLYMER

In this study, two polymers which are Polyvinyl Alcohol (PVA) and Poly (lactic-co-glycolic acid) (PLGA) were studied. They were evaluated based on particle size and entrapment efficiency. The most suitable polymer was identified by optimum particle size and entrapment efficiency.

Sr. No.	**Polymer**	**Concentration**	**Results and discussion**
1)	Polyvinyl Alcohol (PVA)	10mg to 120mg (0.1%w/v-1% w/v)	Particle size was found in the range of 140.2 nm to 5059.9 nm and the maximum entrapment efficiency observed was 88.7%.
2)	Poly (lactic-co-glycolic acid) (PLGA)	10mg to 200mg	Particle size was found in the range of 179.0 nm to 4559.8 nm and the maximum entrapment efficiency observed was 91.45%.

Table 5: Results observed for polymer

According to results obtained it was observed that Poly (lactic-co-glycolic acid) (PLGA) polymer found to give optimum particle size and entrapment efficiency, so it was further selected for the study.

7.4 SELECTION OF SURFACTANT

In this study, PVP K30, PVA, HPMC K5, poloxamer 407, poloxamer 188 as the surfactant were evaluated. The most suitable surfactant was identified by optimum particle size and Entrapment efficiency. The higher molecular weight of poloxamer 407 and its relatively longer polymer chains lead to a higher degree of steric stabilization for the drug particles, which can enhance particle dispersion and result in a greater extent of size reduction. Hence according to results of particle size and entrapment efficiency the poloxamer 407 surfactant was used for further study.

Sr. No.	**Surfactant/ Stabilizer (0.1%-1%)**	**Results and discussion**
1)	PVA (Poly vinyl alcohol)	Smallest particle size found was 134 nm, particle aggregation was seen in some batches, and entrapment efficiency was between 32.2 % to 95.54%.
2)	PVP K-30	Clear solution was observed, particle size found was more than 10000 nm
3)	Poloxamer 407	Colloidal suspension was observed. Smallest particle size found was 90.3 nm with entrapment efficiency 80.8%.
4)	Poloxamer 188	Colloidal suspension was observed. Particle size was in the range of 3525.4 nm to 7236.1 nm with entrapment 67.2% to 85.42%.

Table 6: Results observed for different surfactants

Nanoprecipitation method by using magnetic stirrer instrument, PLGA as a polymer and poloxamer 407 as a stabilizer found to give optimum particle size and entrapment efficiency. Trial batches were taken for selection of independent factors for optimization.

Sr. No.	**Independent factors**	**Lower Level (-)**	**Higher Level (+)**
1)	Concentration of drug	20mg	40mg
2)	Concentration of PLGA	20mg	40mg
3)	Concentration of poloxamer 407	60mg	80mg
4)	Volume of organic solvent	3ml	8ml
5)	Stirring speed	800rpm	1200rpm
6)	Flow rate	0.5ml/min	1ml/min
7)	Stirring time	2hr	6hr

Table 7: List of independent factors for optimization

From trial batches it was found that concentration of poloxamer 407, stirring speed, and volume of organic solvent influences the particle size and entrapment efficiency.

7.5 FORMULATION, OPTIMIZATION AND CHARACTERIZATION OFNANOPARTICLES OF DAPSONE

7.5.1 Formulation and optimization

Factorial design of 2 level, 3 factor was designed to optimize the formulation. The concentration of poloxamer 407, stirring speed and volume of organic solvent were selected as independent variables. The high and low levels for independent variables were selected based on preliminary trials and extensive literature survey. Based on particle size and entrapment efficiency due to above factors, optimized batch was selected for further studies.

Sr. No.	Independent factors	Lower level (-)	Higher level (+)
1)	Concentration of poloxamer 407 (mg)	60	80
2)	Stirring speed (rpm)	800	1200
3)	Volume of organic solvent (ml)	3	8

Table 8: Independent factors for factorial design

Run	Concentration of poloxamer 407(mg)	Stirring speed (rpm)	Volume of acetone (ml)	Particle size (nm)	Entrapment efficiency (%)
1	60	800	3	855.5	90.2
2	80	800	3	122.1	91.65
3	60	1200	3	817.0	92.65
4	80	1200	3	422.0	92.64
5	60	800	8	253.2	76.84
6	80	800	8	1014.1	80.8
7	60	1200	8	309.8	78.3
8	80	1200	8	1191.1	84.3

Table 9: Results obtained for factorial design

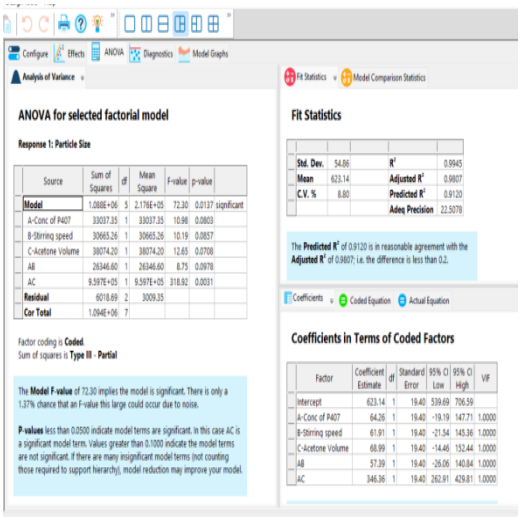


Figure 4: ANOVA for particle size

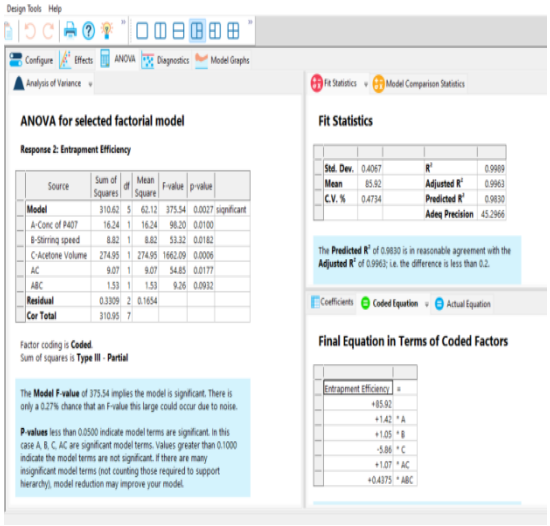


Figure 5: ANOVA for entrapment efficiency

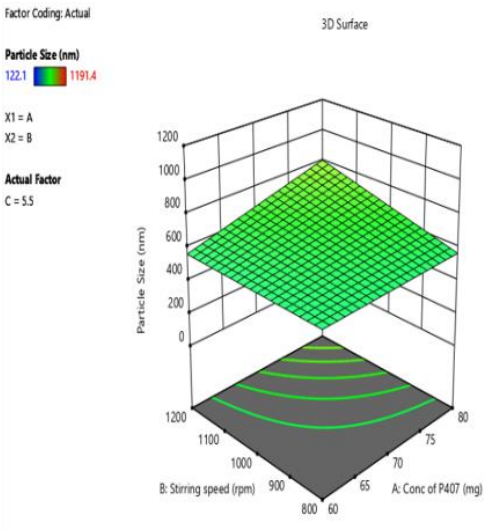
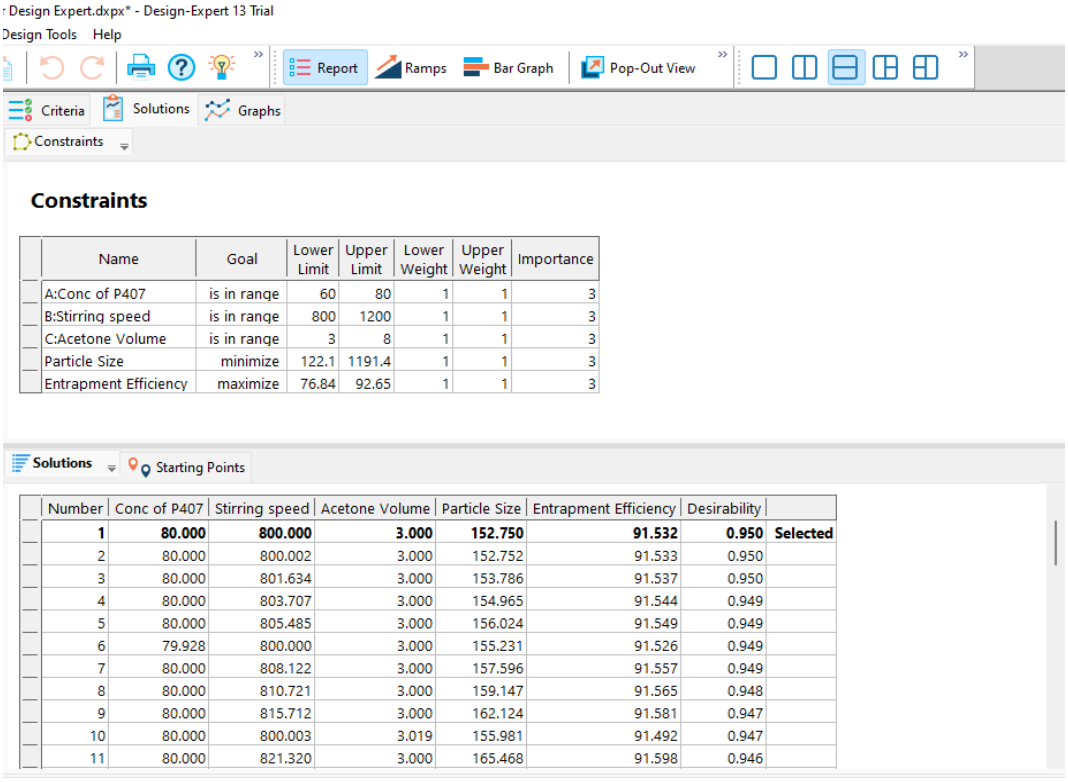


Figure 6: 3D surface graph of particle size and entrapment efficiency



Constraints

	Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
	A:Conc of P407	is in range	60	80	1	1	3
	B:Stirring speed	is in range	800	1200	1	1	3
	C:Acetone Volume	is in range	3	8	1	1	3
	Particle Size	minimize	122.1	1191.4	1	1	3
	Entrapment Efficiency	maximize	76.84	92.65	1	1	3

Solutions | Starting Points

	Number	Conc of P407	Stirring speed	Acetone Volume	Particle Size	Entrapment Efficiency	Desirability	
	1	**80.000**	**800.000**	**3.000**	**152.750**	**91.532**	**0.950**	**Selected**
	2	80.000	800.002	3.000	152.752	91.533	0.950	
	3	80.000	801.634	3.000	153.786	91.537	0.950	
	4	80.000	803.707	3.000	154.965	91.544	0.949	
	5	80.000	805.485	3.000	156.024	91.549	0.949	
	6	79.928	800.000	3.000	155.231	91.526	0.949	
	7	80.000	808.122	3.000	157.596	91.557	0.949	
	8	80.000	810.721	3.000	159.147	91.565	0.948	
	9	80.000	815.712	3.000	162.124	91.581	0.947	
	10	80.000	800.003	3.019	155.981	91.492	0.947	
	11	80.000	821.320	3.000	165.468	91.598	0.946	

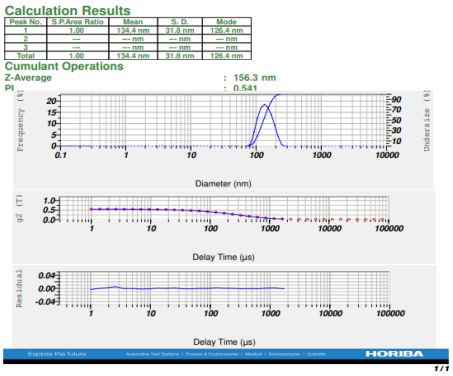
Figure 7: Solutions given by design expert software

7.5.2 Characterization of optimized dapsone nanoparticles

7.5.2.1 Particle size, polydispersity index and zeta potential

Optimized batch was found to be efficient with average particle size in the range of 100 nm to 200 nm, zeta potential in the range of -20mV to -40 mv, PI in the range of 0.4 to 0.8. The smaller particle size of formulation ultimately provides good solubility and permeability to the formulation. Also, the higher zeta potential value of formulation indicates good stability (i.e., the dispersion will resist aggregation).

- **Particle size: 156.3 nm**
- **Polydispersity index: 0.541**
- **Zeta potential: -37.8 mV**



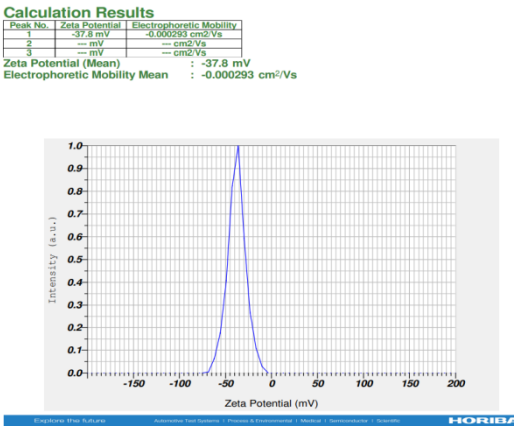


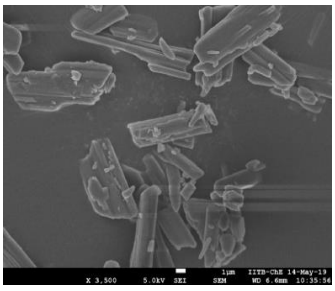
Figure 8: Particle size of optimized batch **Figure 9: Zeta potential of optimized batch**

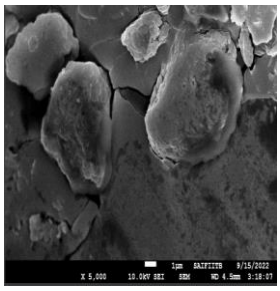
7.5.2.2 Entrapment efficiency of optimized batch

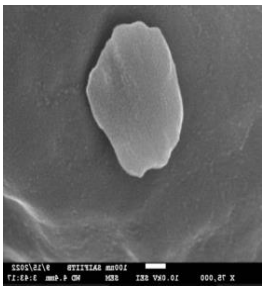
Entrapment efficiency of optimized batch was determined using UV–visible spectroscopy and it was found to be 91.48 ±1.82%**.**

7.5.2.3 Morphological analysis and comparison of drug and nanoparticles by Field Emission Scanning Electron Microscopy (FEG SEM)

Photo image obtained by SEM analysis is presented in Figure 10 and Figure 11. Dapsone appeared to be made up of rod shape crystalline structures of nearly 1 µm size. Dried nanoparticulate powder appears to be in spherical aggregate.







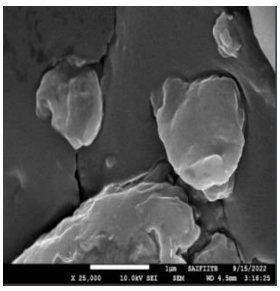


Figure 10: SEM image for drug **Figure 11: SEM image of dried nanoparticles**

7.6 FORMULATION OF NANOPARTICLE LOADED GEL

A topical nanoparticle loaded gel was formulated using carbopol 974 P, propylene glycol and methyl paraben.

7.7 EVALUATION OF NANOPARTICLE LOADED GEL

The Nanoparticle loaded gel of dapsone was evaluated for appearance, spreadability, viscosity, pH, drug content and *In-vitro* diffusion study.

Experiment	**Viscosity (cps)**	**Spreadability (gm.cm/sec)**	**pH**	**Drug Content**	**Appearance**
Optimized nanoparticle loaded gel	34000	3.1	7.1	89.7 %	Clear

Table 10: Evaluation of Gel

7.7.1 *In-vitro* release study [22]

In vitro diffusion studies of optimized nanoparticle loaded gel and marketed gel formulations were performed and compared by modified franz-diffusion using dialysis membrane. Phosphate buffer of pH 7.4 was used as diffusion media. The results showed that the developed gels had the ability to release the drug for the duration of about 7 hours. The amount of drug release from nanoparticle loaded gel was found to be maximum 73.48% at the end of 7 hours and it is slightly better than marketed gel. Results are shown in figure 12.

Time (Hrs.)	**%CR of nanoparticle loaded gel**	**%CR of marketed gel**
1	6.20	5.92
2	16.14	15.76
3	33.58	33.53
4	44.35	42.28

5	52.23	50.86
6	64.27	58.84
7	73.48	65.36
24	79.50	71.93

Table 11: *In-vitro* release of nanoparticle loaded gel and marketed gel

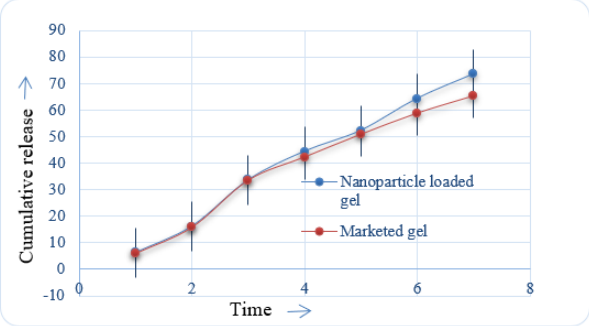


Figure 12: *In-vitro* release of nanoparticle loaded gel and marketed gel

7.7.2 Antimicrobial Study

The results of the study showed that the nanoparticle loaded gel and marketed gel indicates the presence of potent antibacterial activity; antimicrobial activity was based on measurement of inhibition zones formed around the disc. From the observation of the agar plate containing optimized formulation of nanoparticle loaded gel and marketed gel against *propionibacterium acne* gave a sufficient zone of inhibition similar to that of the drug dapsone. So, it can be concluded that nanoparticle loaded gel showed a sufficient anti-acne activity in the antibacterial test performed.

Sr. No.	Name of Sample	Zone of Inhibition (mm)
1	Marketed gel	17.58
2	Nanoparticle loaded gel	19.45

Table 12: Zone of inhibition of marketed gel and nanoparticle loaded gel

Figure 13: Nanoparticle loaded gel

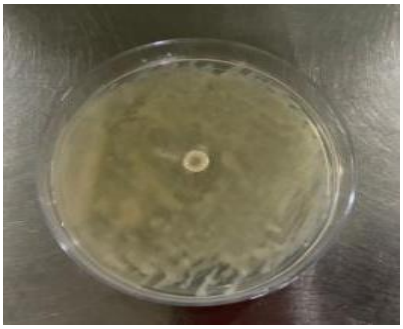


Figure 14: Marketed gel

7.7.3 DSC compatibility study

Melting point of dapsone was measured by differential scanning calorimetry at scanning rate of 10^0 /min. It exhibits melting endothermic peak at temperature of 179.86 ^{0}C as shown in Figure 15. This gives an indication that the drug has crystalline nature with high purity. For nanoparticle, the melting point of dapsone disappeared as in figure 16 giving a strong indication that the drug is entrapped by the polymer.

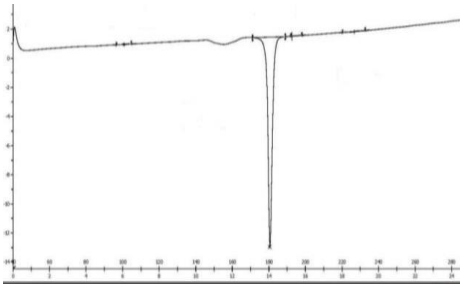


Figure 15: DSC study of dapsone

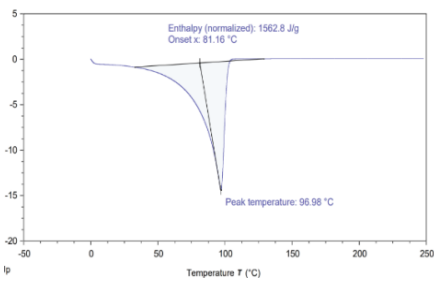


Figure 16: DSC of nanoparticle batch

7.7.4 Histopathology study

The result suggests that nanoparticulate formulation does not shows any sign of irritation or damage to epidermis which indicates the safety and efficacy of developed formulation.

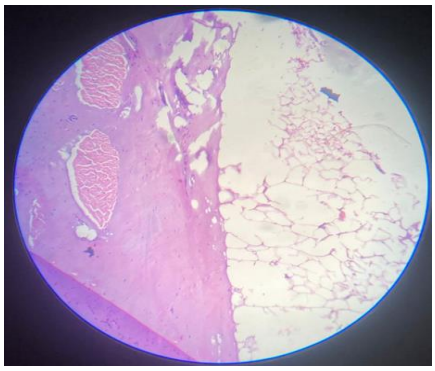


Figure 17: Untreated skin

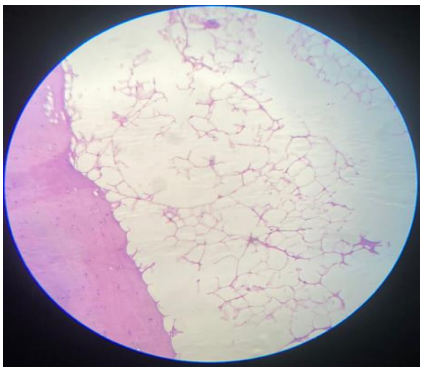


Figure 18: Formulation treated skin

7.7.5 Stability assessment of dapsone nanoparticle loaded gel formulations at 25°C ± 2°C / 65 % ± 5 % RH For 2 Months

The optimized nanoparticle loaded gel formulation was evaluated at the time interval of 30 and 60 days for all the parameters like appearance, spreadability, viscosity, pH and % drug content. The observations of stability studies of optimized formulation are shown in table 13 and it did not show any significant change in these parameters after stability studies. This confirms the stored gel formulation were stable for the storage period.

Sr. No.	Parameters	0 day	30 days	60 days
1	Appearance	Clear	Clear	Clear
2	Spreadability	1.73 gm.cm/sec	1.65 gm.cm/sec	1.63 gm.cm/sec
3	Viscosity	36000 cps	35700 cps	35500cps
4	PH	7.1	7.2	7.2
5	Drug content	89.3%	87.5%	86.%

Table 13: Stability assessment of nanoparticle loaded gel

8. SUMMARY AND CONCLUSION

In the present study, poly (lactic-co-glycolic acid) loaded dapsone nanoparticles were successfully prepared by using nanoprecipitation method. It was demonstrated that the poloxamer-407 as a surfactant produce nanoparticles with maximal particle size reduction and stable zeta potential value. The developed nanoparticles were successfully loaded into gel by using carbopol 974P as gelling agent. The results of this study lead to the conclusion that the formulated nanoparticle loaded gel as a topical drug delivery system promising the approach which is utilized for improving efficacy of dapsone in the treatment of acne. DSC study indicated that there is no interaction between the drug and excipients. The results of in-vitro diffusion study showed that the developed nanoparticle loaded gel had the ability to release the drug for the duration of about 7 hours. This nanoparticle loaded gel was clear, transparent, the pH was neutral and its viscosity was good and spreadability is excellent and easily spread, gave antimicrobial activity high effectiveness inhibiting the growth of *propionibacterium acne* bacteria.

Conflict of Interest

The authors report no conflicts of interest.

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