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FORMULATION, DEVELOPMENT AND OPTIMIZATION OF DAPSONE DEPOT INJECTION IN ACNE: *IN-VITRO*/*IN-VIVO* STUDY

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ABSTRACT: The aim of this work is to design and develop a controlled release drug delivery system of the anti-acne agent (dapsone), also used as a leprostatic agent. Dapsone is a strong anti-inflammatory agent that makes it a more powerful treatment in dermatological disorders; its treatment in acne requires long term steady-state concentration in plasma. Poor patient compliance and long term treatment by an oral route leading to dapsone resistance, which stimulates the development of depot preparation. Depot injection consists of a PLGA polymer containing matrix, which gives control release up to 1 month. Microspheres were formed by solvent extraction/evaporation technique. Various parameters, like a selection of solvents, selection of drug: polymer ratio, glycolide: lactide ratio, and evaporation temperature, were important and optimized based on the results. The entrapment efficiency of microspheres was found between 40% to 70%. The initial burst of the drug was controlled and found to be 9.8% within 24 h of release. The inactive ingredients used for formulation development were found to be compatible based on FTIR comparison. The glass transition temperature of the microsphere was found to be 53.45 °C. The residual solvents like methanol and ethyl acetate were found within ICH limits in the finished product. The *in-vitro* release profile found between 80% to 95% after 30 days. The C_{max} of microsphere was found to be 2.04 mcg/mL which is lower as compared to immediate-release (4.82 mcg/mL). The controlled release of drugs from the microsphere provides constant plasma drug content for a long period of time and improves patient compliance by reducing dosage frequency.

INTRODUCTION: Acne is the most common disease amongst adolescents with a pervasiveness rate of ~85% [1]. The lesions are most prone to area which has the most sebaceous gland.

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The acne lesions are divided into two categories, non-inflammatory and inflammatory. Inflammatory acne is more critical and not easy to control because of the involvement of the host immune system. It includes papule, pustule, nodular, and cystic acne. In this lesion, the follicular epithelium is damaged and causes intra-dermal inflammation.

If the dermal cell rupture occurs, elicits the severe inflammatory response in the dermis layer. An interesting thing in acne is a spontaneous resolution by follicular cycling process [2].

Dapsone is an anti-microbial and anti-inflammatory agent known to treat moderate to severe acne. The oral route is known to cause dose-dependent hematological reactions. The treatment requires long term exposure, *i.e.*, up to 3 months by oral route and 6 months by topical route. The first proven case for dapsone resistance was found during the treatment of leprosy may be due to low dose-dependent therapy [3]. Depot injection therapy also can be preferred for leprosy to reduce the dose-dependent side effects. The world health organization (WHO) stated that "a formulation of dapsone or a derivative of dapsone, that, on monthly administration, would provide bactericidal concentrations of dapsone in the tissues, without risk of toxicity, is desirable [4]. The microsphere is the well-known technology for depot preparation, which helps to control the release from days to months. Microsphere technology encapsulates the drug inside the polymer to maintain the plasma concentration and gives sustained release. WHO suggested that one-month administration of dapsone depot injection will impart bactericidal concentration of drugs in tissues without any toxicity risk after intramuscular administration of dapsone injection gives variation between men and women patients [5]. Development of depot injection by solvent extraction and evaporation technique is well known, and dapsone is a model drug used for the development of depot injection with different evaluation parameters like initial burst, residual solvent, drug loading, *in-vitro* dissolution, and particle size. The research article explores the formulation and its critical parameters for the development of dapsone depot injection.

MATERIALS AND METHODS:

Materials: Dapsone (Suzhou Bichal Biological Technology Co., Ltd), PLGA polymer (Evonik Degussa, Mumbai), Ethyl acetate (Spectrum Pvt. Ltd.), Methanol (Spectrum Pvt. Ltd.), Polyvinyl alcohol (Merck Pvt. Ltd.), Methylene Chloride (Spectrum Pvt. Ltd.), Na-CMC(Ashland), Mannitol (Roquette Freres), Poloxamer (BASF). 33 Central composite designs were conducted using Design-Expert® 9 software from State-ease, Inc.

Method of Microspheres Preparation: The method of microsphere preparation is solvent extraction evaporation technique [6] and required quantity of PLGA polymer dissolved in ethyl acetate, and the drug phase solubilized separately in ethyl acetate: methanol solution. Both polymer and drug phases were mixed and called a dispersed phase (DP). 1% w/w PVA solution prepared, which was used as a continuous phase (CP).

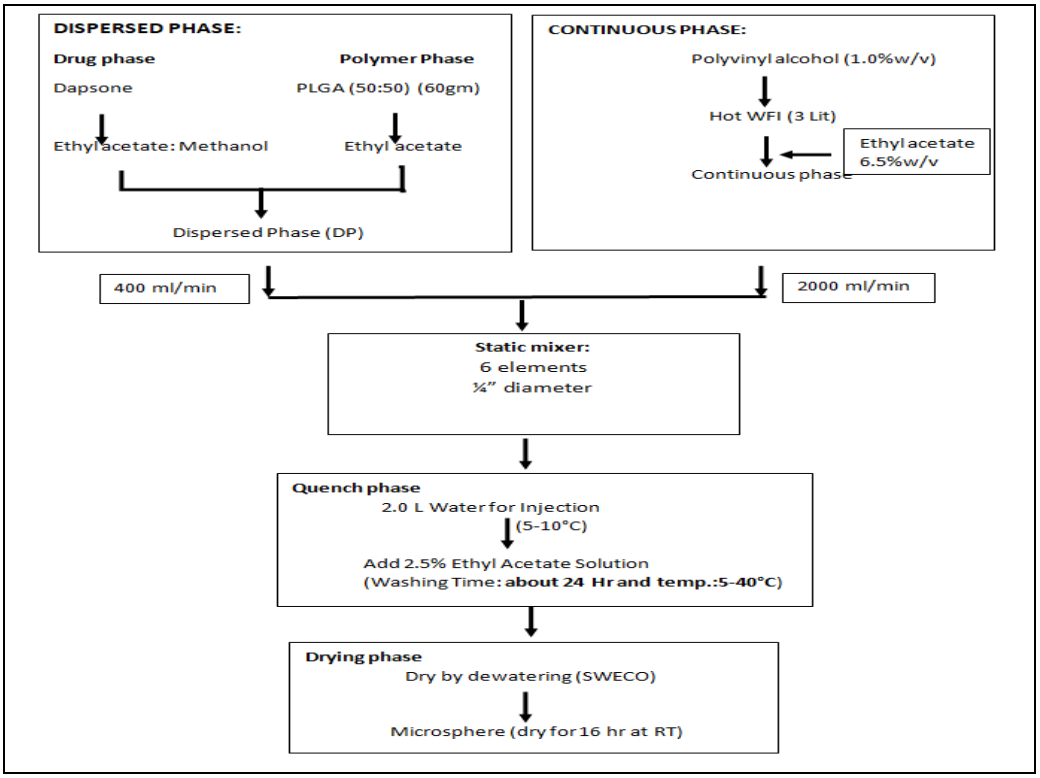

FIG. 1: METHOD OF MICROSPHERES PREPARATION

The DP and CP phase passed through static mixer [7] (Cole-Parmer Pvt. Ltd.) containing a diameter of ¼" at ratio of 1:5, respectively. The solution coming at the outlet of static mixer was poured in to quench phase, which contains 2.5% w/w ethyl acetate: water solution. The microsphere treated at different temperatures to enhance the evaporation of the organic solvent and reduce the residual solvent as shown in **Table 1**. The microspheres passed through 25# sieve and dried in sweco. The microsphere process flow is shown in **Fig. 1**.

Design of Experiment (DOE): An efficient output can be provided by using the DOE with a minimum number of trials to optimize the solvent extraction / evaporation method. DOE is an efficient method for exploring variability between process variables and responses. The number of experiments depends on process variables, and pre-design experiments gives better knowledge for understanding important process variables that affect responses. Here, 3^3 central composite designs were adopted to analyze the interaction of each level of independent factors on desired responses. The design was generated within the domain of levels using the design-expert® 9 software. Based on preliminary trials following three factors were found to be having a significant impact on microsphere characteristics:

- Temperature of quench phase(A)
- Flow rate (B)
- Drug: polymer ratio (C)

The first independent variable temperature of quench phase (A) range was evaluated from 25 °C to 50 °C. The ratio of DP: CP (1:5) was fixed based on the preliminary evaluation. Different flow rates of DP from 150 mL/min to 400 mL/min were evaluated as a second independent variable (B). drug: polymer ratio (C) in the range of 0.5 to 1.5 was evaluated as a third independent variable. The set of experiments are shown in **Table 1.**

TABLE 1: DESIGN MATRIX AND MEASURED RESPONSES

Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
	A: temperature	B: flow rate	C: drug: polymer ratio	Residual EA (Y1)	Particle size (D90) (Y2)	% Drug release at T24 (Y3)
	°C	mL/min		PPM	micron	%
1	25.00	400.00	0.50	20000	89	10
2	37.50	275.00	1.00	2100	154	4
3	50.00	400.00	1.50	1200	75	5
4	37.50	275.00	1.00	2400	148	6
5	37.50	275.00	1.00	2409	159	2
6	37.50	275.00	1.84	14596	140	2
7	37.50	275.00	1.00	2985	139	9.5
8	50.00	275.00	1.00	142	137	25
9	25.00	400.00	1.50	25874	85	0.5
10	25.00	150.00	0.50	21475	180	4
11	37.50	68.00	1.00	1986	368	0.5
12	37.50	275.00	0.50	2598	174	25
13	50.00	400.00	0.50	1486	90	11
14	37.50	485.22	1.00	1459	59	22
15	50.00	150.00	0.50	1474	189	14
16	50.00	150.00	1.50	8695	198	2
17	37.50	275.00	1.00	1452	159	7
18	16.48	275.00	1.00	28796	157	0.5
19	25.00	150.00	1.50	15896	181	0.2
20	37.50	275.00	1.00	2145	130	8

Optimization and Validation of Model: Optimization of the model was done by using analysis of variance (ANOVA) is linked with the experimental design shown in **Table 1** [8], which displays b-coefficients, F-values, and p-values of model terms.

After the selection of the levels of the independent variables, various confirmation batches were taken, and the response of confirmation batches was compared with the predicted response value to validate the model equation.

Microsphere Characterization:

Particle Size Distribution: The particle size distribution was analyzed by a laser diffraction method (Beckman Coulter Inc.) The microspheres were suspended in diluents solution (Diluents contains Na-CMC, mannitol, and poloxamer), sonicated for 30 sec, and analyzed with a pre-defined set of parameters.

Viscosity of the Dispersed Phase: The dispersed phase viscosity measured by Brookfield viscometer (DV2T model) with LV spindle assembly with a torque range of 40% to 70% with different RPM at 25 °C, which directly correlates to the particle size and drug entrapment efficiency of the finished microsphere.

External and Internal Morphology: The internal as well as external morphology of microspheres we reanalyzed by scanning electron microscopy (SEM). The samples were mounted onto aluminum specimen stubs using double-sided adhesive tape and fractured with a razor blade. The samples were then sputter-coated with gold/palladium for analysis by SEM.

Thermal Analysis and Compatibility Study: The glass transition temperature (TG) was analyzed by differential scanning calorimetry (DSC) mettle toledo using the modulated DSC mode. The temperature was regulated at ± 1 ℃ with a ramping rate of 10 °C/min with a range of 0 °C to 70 ℃. The drug-excipient compatibility study was carried out by FTIR (Fourier-transform infrared spectroscopy).

Residual Solvent: Residual solvents were analyzed using gas chromatography and characterized for ethyl acetate and methanol, which was used during microsphere formation. Both the solvents were analyzed and controlled as per ICH (International Conference on Harmonisation) Q3C guidelines.

Drug Loading Inside PLGA Microspheres: A sample of 50 mg microsphere was dissolved in 60 ml of acetonitrile then sonicated for 1 min and microsphere once dissolved to make up with water up to 100 ml.

The sample was filtered through a 0.22 micron PVDF (polyvinyl dine difluoride) filter and injected in the RP-HPLC system. The separation was obtained with the Luna C18 column with mobile phase methanol: water (40:60) ratio, injection volume 5 µl, with run time 20 min and flow rate of 1 ml /min [9].

% Entrapment efficiency = Actual loading / Theoretical loading × 100

% Drug loading = Weight of drug in microsphere / Weight of microsphere × 100

% Yield = Weight of microsphere / Total expected weight of Drug and polymer × 100

Initial Burst Release of Dapsone from PLGA Microsphere: About 95 mg of microspheres were dissolved in 25 ml of dilute hydrochloric acid (2 mL in 100 mL of water) solution and was shaken for 15 min then filtered through 0.22 µm PVDF filter and the filtrate was analyzed by high-performance liquid chromatography (HPLC). Conditions of the HPLC assays described previously [9].

Molecular Weight Determination: Microspheres were dissolved in tetrahydrofuran and sonicated until dissolve completely. 5600, 33000, 120000, and 333000 standard molecular weights were determined based on retention time in HPLC.

Release Kinetics: *In-vitro* release data were evaluated with various kinetic models (zero-order, first-order, highchair, and Korsmeyer-Peppas) to understand the mechanism of drug release from the microsphere [10].

In-vitro **Dissolution by Bottle Rotating Apparatus:** Saline phosphate buffer of pH 7.4 was used as dissolution media, 4.2 gm of Na_2HPO_4, and 0.74 gm of KH_2PO_4 were dissolved in 80% water and sonicated for 5 min. then 24 gm NaCl and 0.6 gm KCl were added in it and sonicated for another 5 min. Finally, the volume was made up to 3 Lit.

The osmolality of the solution was checked and maintained within 270 ± 20 mOsm. Microspheres were added in 90 mL of dissolution media based on saturation solubility and later analyzed in HPLC [11]. The study has been conducted in triplicates to understand the variability.

In-vivo **Studies:** The protocol for the *in-vivo* drug permeation study was approved (protocol no. IP/PCOG/PHD/23/2018/021) by the Institutional

Animal Ethics Committee. The study was conducted on adult Sprague Dawley rats.

A total of 12 animals were divided into two groups after acclimatization and administered a single intramuscular dose of 30 mg (0.1 mL), irrespective of body weight. Acclimatization of animals was done for one week prior to the experiments (temperature: 25 ± 2 °C and relative humidity: 50-60% under natural light/dark conditions).

Group I: IR (immediate-release) dosage form

Group II: CR (controlled release) depot injection

The blood samples were collected at specified time intervals- 0 (pre-dose), 1, 3, 7, 14, 21, and 28 days post-dose. The drug content was analyzed by HPLC method [12-15].

In-vitro **Antimicrobial Study:** The lowest concentration of test microsphere required to inhibit the growth of *p. acne* (ATCC 6919) bacteria known as minimal inhibitory concentration (MIC), which was measured by optical density (OD). Samples were prepared as 100 µg/ml stock in peptone broth; working stocks (8 µg/ml) were prepared from the mother stock (100 µg/ml). 100 mg of microspheres were dissolved in DMSO (240 µl).

It is diluted up to 1% of DMSO with different concentration of 8, 4, 2, 1, 0.5, 0.25, and 0.125 µg and then used for the study. Ciprofloxacin was used as positive control and peptone broth inoculated without a test compound used as a negative control. Treated cultures were incubated at 37 °C. The test plates were observed after 24-48 h, and OD @ 590 nm is measured in plate reader [16].

RESULTS:

Experimental Design: The current study focuses on the formulation and optimization of the manufacturing process and parameters based on 3^3 central-composite design experiments. Microsphere formulation was developed using a solvent extraction-evaporation technique. The solvent extraction process was done using water as an extraction medium and evaporation by means of temperature. The main objective of this research work is to develop a depot formulation of dapsone as a microsphere, which is supposed to deliver the drug for a period of up to 1 month.

As per ICH guidelines, residual solvent (s) need to be controlled at or below the threshold level to have a safe product for human use [17]. Ethyl acetate (EA) is used to dissolve the polymer, and at the end of microsphere preparation, residual amounts of EA can be found in the finished product.

Here, residual EA is selected as the first response variable (Y_1). It is well known that the particle size of the microsphere is an important *in-vitro* parameter governing *in-vivo* drug release [18]. Hence, D90 was selected as the second response variable (Y_2) in the study. Dapsone has various systemic side-effects; hence there will be higher chances of adverse effects with higher burst release [19].

Therefore, the T24 burst release (Y_3), was selected as the critical attribute. A randomized, central composite design (CCD) was selected as an experimental design in order to evaluate the effect of three input factors stated above (A, B, and C) on three critical product characteristics (Y_1, Y_2, and Y_3). In order to evaluate response Y_1, multiple regression analysis methods were used to generate a quadratic statistical model.

$$Y_1 = b_0 + b_1 A + b_2 B + b_3 C + b_{12} AB + b_{13} AC + b_{23} BC + b_{11} A^2 + b_{22} B^2 + b_{33} C^2$$

Where b_0 is the arithmetic means the response of 20 runs, and b_1, b_2, and b_3 are the estimated coefficients for the factors A, B, and C, respectively. The following equation was derived by the best-fit method to describe the relationship between the residual EA (Y_1), the quenching phase temperature (A), DP flow rate (B), and drug: polymer ratio (C).

$$\text{Residual EA} = + 2131.47 - 8682.83 * A + 0.93 * B + 2006.92 * C - 1998.25 * AB + 830.00 * AC + 493.25 * BC + 5034.10 * A^2 + 544.63 * B^2 + 2958.04 * C^2$$

In order to evaluate response Y_2, multiple regression analysis methods were used to generate a linear statistical model.

$$Y_2 = b_0 + b_1 A + b_2 B + b_3 C$$

Where b_0 is the arithmetic means the response of 20 runs, and b_1, b_2, and b_3 are the estimated coefficients for the factors A, B, and C, respectively.

The following equation was derived by the best-fit method to describe the relationship between the D 90 (Y_2), the quenching phase temperature (A), DP flow rate (B), and drug: polymer ratio (C).

Particle size (D 90) = + 150.64 - 1.22 * A - 68.02 * B-4.85 * C

In order to evaluate response Y_3, multiple regression analysis methods were used to generate a linear statistical model.

$$Y_3 = b_0 + b_1A + b_2B + b_3C$$

Where b_0 is the arithmetic means response of 20 runs, and b_1, b_2, and b_3 is the estimated coefficients for the factors A, B, and C, respectively. The following equation was derived by the best-fit method to describe the relationship between the T24 (Y_3), the quenching phase temperature (A), DP flow rate (B) and drug: polymer ratio (C).

% Drug release at T24 = + 7.91 + 4.28 * A + 3.11 * B - 5.12 * C

Optimization and Validation of Model: Based on the experimental design and design space, the optimized factors were found to be (A) temperature 37.3 °C, (B) flow rate 314 ml /min, (c) drug: polymer ratio 0.8.

The comparison of predicted results and observed results was made for optimized independent variables such as flow rate, temperature, and drug: polymer ratio of microsphere for validation.

The predicted and the observed results were found within ± 5%, as shown in **Table 2**. Percent residual calculation formula shown below:

Percent residual = Predicted results – Observed results / Predicted results × 100

TABLE 2: OPTIMIZED FACTORS VALIDATION

S. no.	A	B	C	Y1-pre.	Y1-obs.	% Res.	Y2-pre.	Y2-obs.	% Res.	Y3-pre.	Y3-obs.	% Res.
1	37.09	314	0.8	2000	1984	0.80	152.068	150	1.36	10.214	9.8	4.05
2	37.09	314	0.8	2999.98	2890	3.67	151.176	150	0.78	10.256	10.1	1.52
3	37.08	314	0.8	2000	1940	3.00	154.015	155	-0.64	10.1224	10.2	-0.77
4	37.09	314	0.8	2999.99	2870	4.33	150.123	149	0.75	10.3057	10.2	1.03
5	37.06	314	0.8	2999.99	2930	2.33	155.59	156	-0.26	10.0209	9.7	3.20
6	37.06	314	0.8	2999.99	2990	0.33	148.074	146	1.40	10.2697	10.1	1.65
7	37.04	314	0.8	2999.99	2989	0.37	134.372	135	-0.47	10.5529	10.4	1.45

Microsphere Characterization:

Particle Size Distribution: Decreasing flow rate shows an increase in the particle size, but the optimum particle size considering injectability was set to D (90) 134 µm as shown in **Fig. 2**.

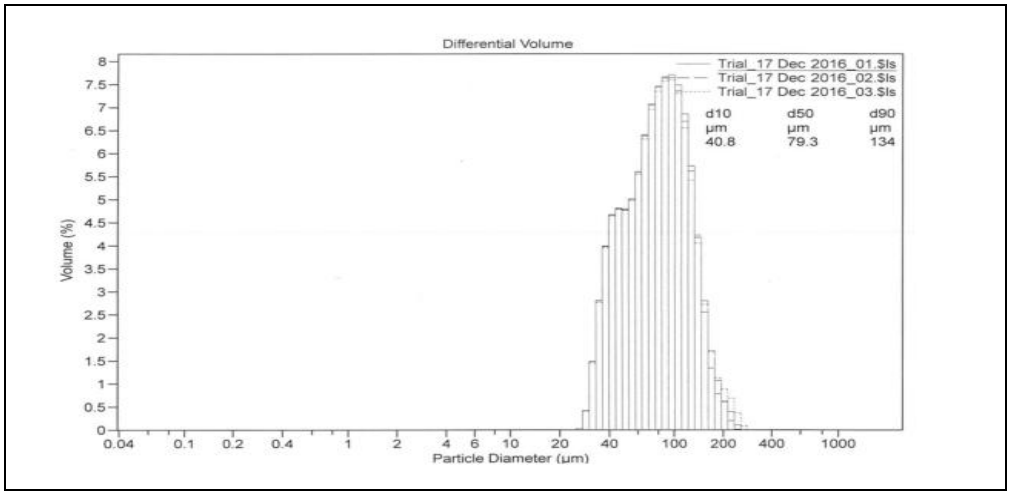

FIG. 2: VOLUME STATISTICS OF MICROSPHERE

Viscosity of the Dispersed Phase: Dispersed phase viscosity was directly affecting drug entrapment and particle size. **Table 3** shows viscosity data of the dispersed phase at different RPM (from 2 to 8) to achieve optimum torque. From the above results, it was a clear indication that the viscosity of the dispersed phase is directly proportional to particle size and entrapment efficiency.

TABLE 3: VISCOSITY OF DISPERSED PHASE AT 25 °C

S. no.	RPM	Torque (%)	Viscosity (cp)	Particle size (D90-µm)	Entrapment efficiency (%)
1	8	53.0	70.5	94	35
2	4	55.0	105.6	135	65
3	2	54.4	140	320	70

External and Internal Morphology: Microsphere morphology was found to be a smooth and spherical surface, but during the process, when the temperature was increased to accelerate the solvent removal process leads to pore formation at the microsphere surfaces **Fig. 3C** and **D** and also leads to the rough surface of the microsphere.

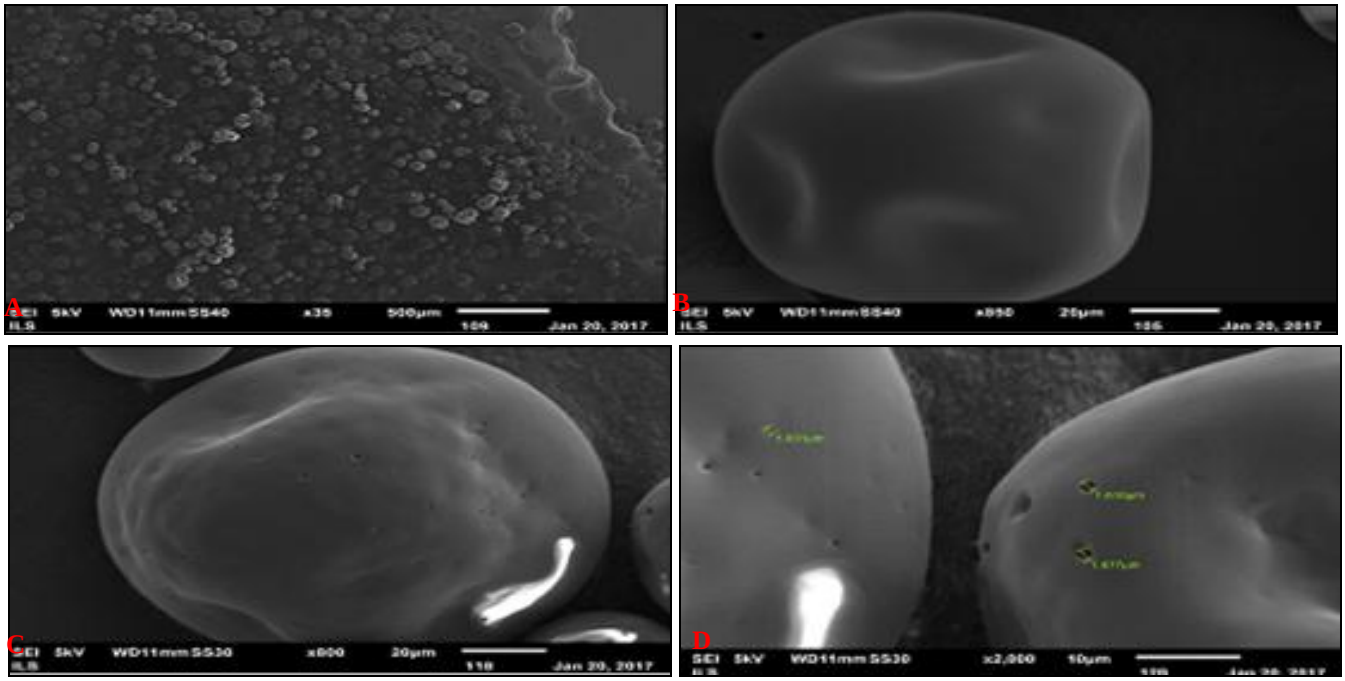

FIG. 3: SEM ANALYSIS OF MICROSPHERES: (A) MICROSPHERE FORMED AT 37 °C WITH LOW MAGNIFICATION, (B) MICROSPHERE FORMED AT 37 °C WITH HIGH MAGNIFICATION, (C) MICROSPHERE FORMED AT 50 °C WITH HIGH MAGNIFICATION, (D) MICROSPHERE FORMED AT 50 °C WITH PORE SIZE

Thermal Analysis and Compatibility Study: The process of solvent evaporation should be done below the TG of the polymer. The TG of the polymer was found to be 44.32 °C and for microsphere 53.45 °C as shown in **Fig. 4**. The drug and polymer were found to be compatible and non-reactive during the manufacturing process, and it is confirmed by FTIR analysis, as shown in **Fig. 5**.

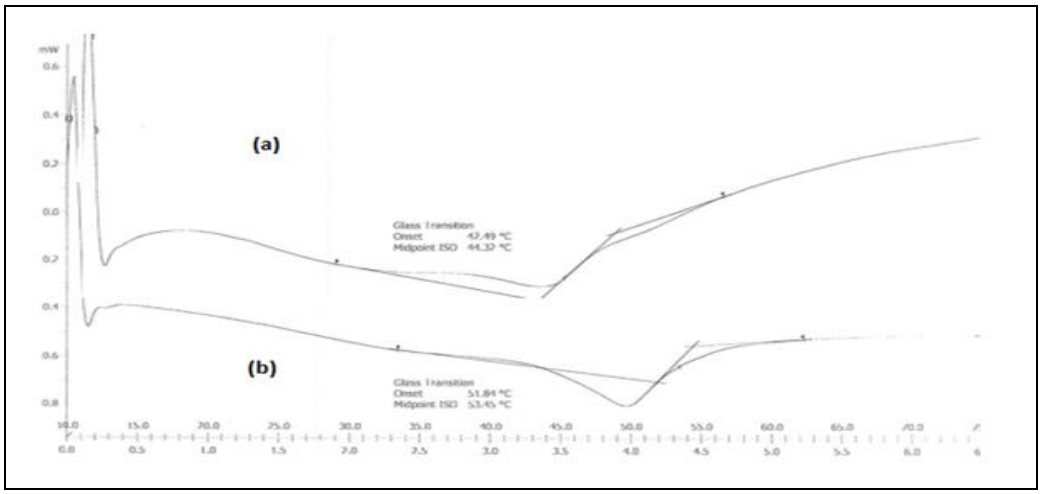

FIG. 4: DSC THERMAL ANALYSIS OF POLYMER AND MICROSPHERES: (A) DSC GRAPH OF POLYMER, (B) DSC GRAPH OF MICROSPHERES

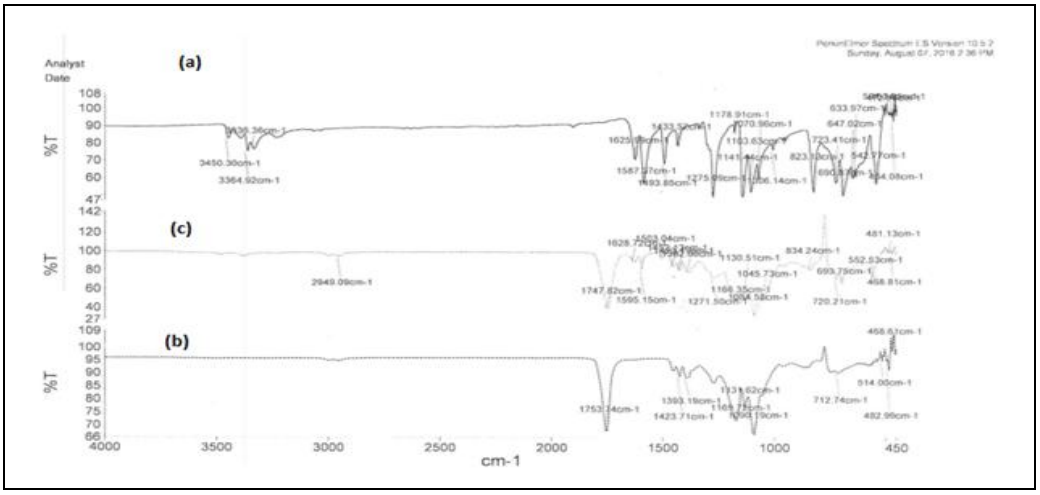

FIG. 5: FTIR ANALYSIS OF DRUG (A), POLYMER (B) AND MICROSPHERE (C)

Residual Solvents: The organic solvents may be harmful to human exposure, so the residual solvent levels need to be controlled in microspheres as per ICH guideline Q3C.

Methanol (class 2 solvent) and ethyl acetate (class 3 solvent) were used during the formulation of the microsphere, where methanol was not detected while ethyl acetate was found to be 2000 ppm in finished microspheres. All the residual solvents were found to be well within the ICH limit [20].

Determination of Drug Loading, Yield, and Initial Burst: Drug entrapments of microspheres were found from 40% to 70%based on changing in-process parameters. Drug entrapment in the optimized formulation was found to be 65%, and an initial burst of the microsphere was found 9.8% (24 h release) in the optimized formulation. The percentage yield varied from 70.9% to 85.5%.

Molecular Weight Determination: Molecular weights of microspheres were found to be Mw 54921 and Mn 32003. The polydispersity index of the microsphere was found to be 1.74, which suggested moderate polydispersed particles [21].

Release Kinetics: The microsphere release mechanism was studied using respective correlation coefficients for different release models in **Table 4**. The drug released from the microsphere was found to be non-fickian diffusion-controlled (n = 0.823).

TABLE 4: RELEASE KINETIC STUDIES OF DAPSONE MICROSPHERES

Formulation	Zero-order model	First-order model	Higuchi model	Korsmeyer-peppas model
	R^2	R^2	R^2	R^2
Optimized formulation	0.9676	0.9388	0.9099	0.9835

***In-vitro* Dissolution (IVR):** IVR profiles of different batches were shown in **Fig. 6**.

The release of optimized formulation with (A) temperature 37.3 °C, (B) flow rate 314 mL/min, (c) drug: polymer ratio 0.8 shown in **Fig. 6**.

There were selected batches (including minimum and maximum level) release profiles at different levels shown for better visualization.

***In-vivo* Studies:** Immediate release (IR) formulation and controlled release (CR) formulation was injected intramuscularly in rats to compare *in-vivo* drug release over a period of 28 days. The concentration of dapsone was measured in plasma using HPLC method [22].

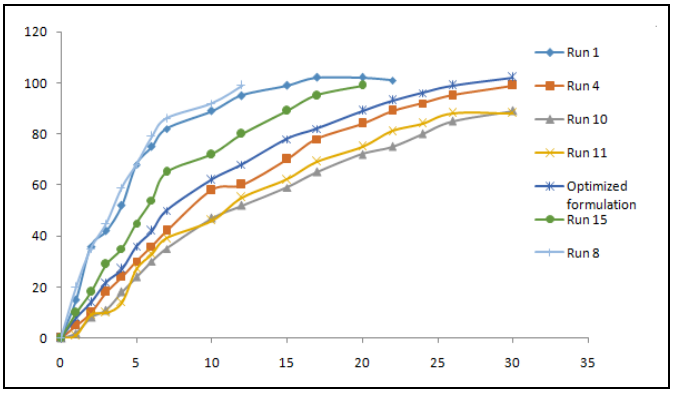

FIG. 6: *IN-VITRO* RELEASE PROFILE

The mean plasma profiles were observed, as in **Fig. 7**. The Pharmacokinetic parameters are given in **Table 5.**

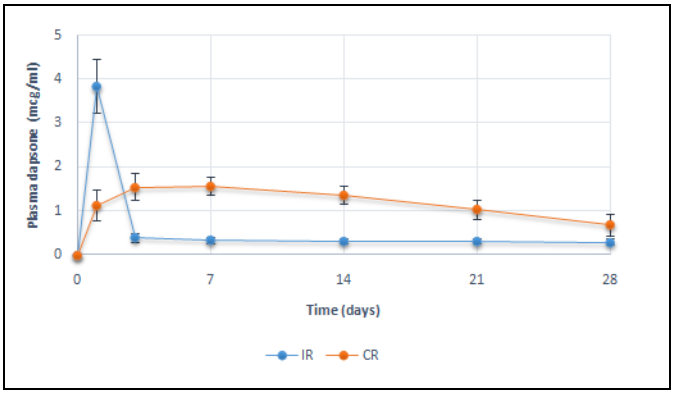

FIG. 7: MEAN PHARMACOKINETIC PROFILES

TABLE 5: MEAN PHARMACOKINETIC PARAMETERS

Parameter	IR	CR
Cmax (mcg/mL)	4.82 (0.61)	2.04 (0.27)
T_{max} (d)	1	3 (1-7)
AUC0-28 (mcg *d/mL)	17.5 (2.17)	34.08 (5.88)

All values are presented as arithmetic mean (SD) T_{max} is presented as median (range).

***In-vitro* Antimicrobial Study:** The microspheres were showed strong anti-microbial effects against *p. acne* bacteria with MIC value of 1 µg/mL. The ciprofloxacin as positive control showed a MIC of 0.5 µg/mL. The placebo doesn't show any activity against *p. acne* bacteria.

DISCUSSION: The microspheres were formulated with solvent extraction and evaporation technique. Rate of extraction and evaporation is very important to control the rate of release by controlling the porosity and channel formation inside the microspheres.

As the temperature increased, the rate of evaporation of residual solvents was also increased, and it has resulted in an acceleration of the release and initial burst of the drug. Various polymer parameters are also important to provide a controlled release profile like molecular weight, lactide: glycolide ratio, and inherent viscosity.

The polymer with higher molecular weight and inherent viscosity can hold drugs for a longer period of time and can retard the drug release. In PLGA, the lactase monomer is hydrophobic, and glycolide part is hydrophilic in nature. The increase in the lactase ratio will retard the drug release by repelling water to penetrate inside the microsphere.

The viscosity of the dispersed phase is a very critical and important factor that impacts entrapment efficiency and particle size of microspheres. The optimized formulation with temperature 37.3 °C, Flow rate 314 ml/min, drug: polymer ratio of 0.8 microspheres have entrapment efficiency of 65% and initial burst of 1.2%. The morphology of the microsphere was smooth, and no pores were found at the surface. The extraction and evaporation time and temperature were designed based on criteria to meet residual solvent limit and to reduce initial burst. The release mechanism of the microsphere was found to be non-fickian diffusion controlled. From the mean pharmacokinetic profile, it is evident that IR injection is associated with higher C_{max} as opposed to CR formulation, where microspheres were able to control release. Geometric mean (C_{max}) for IR is observed as 4.82 mcg/mL; whereas 2.04 mcg/mL is for CR formulation. This virtue of CR is helpful in preventing high drug levels in the blood, which may prevent several adverse events as compared to IR treatment.

At the same time, CR was able to sustain drug concentration >1 mcg/mL for a duration of 20 days (*i.e.,* study duration). In contrast, IR could hold a drug level at >1.0 mcg/mL for 3 days only. CR formulation is able to provide therapeutic drug levels above the MIC for a prolonged time. Such a strategy may be useful in preventing several adverse effects associated with higher blood levels, and at the same time, it will be efficacious for a prolonged duration. These properties of CR formulations can be utilized to minimize dosing frequency and improve patient compliance with lesser adverse events.

CONCLUSION: Depot injection of dapsone microspheres was prepared using DOE. The requirement of a dapsone depot is to improve patient compliance with minimum dose frequency. The optimized formulation was evaluated in rats for *in-vivo* drug absorption. The prepared formulation was able to maintain the concentration for a longer period of time in the systemic circulation. The statistical approach gives freedom to the scientist to examine more than one factor at a time and to evaluate the impact of one factor on another. The developed formulation strategy can also be implemented in leprosy to reduce the dose-

dependent side effects and tolerance to improve patient compliance.

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