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Preparation, Characterization And Evaluation Of Statistically Optimized Dapsone Loaded Nano Crystals

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ABSTRACT

Dapsone is a widely used antileprotic drug which is prevalent in many countries. It is highly lipophilic and possess a very low aqueous solubility makes it as a suitable candidate for preparation of nanoparticles. Nanosuspension of Dapsone is prepared by nanoprecipitation method. The DOE approach is used to determine the effect of process parameters like rate of injection and stirring time, material parameters like solvent and stabilizer ratios. The optimized formulation is subjected to various evaluation tests and freeze dried for further evaluation. The nanoparticles were found to be intact in SEM/TEM analysis and there were no drug interactions between the formulation ingredients. The predicted formula from the DOE confirms the laboratory evaluation. The pharmacokinetic parameters like bioavailability of the formed nanoparticles have improved significantly when compared with that of pure Naproxen.

KEY WORDS: Dapsone, Nanosuspension, Nanoprecipitation, DOE, SEM/TEM, bioavailability

INTRODUCTION

Leprosy is one of the major health concerns globally. It is a chronic disease caused by Mycobacterium leprae which is more prevalent in tropical counties like India. The standard treatment for leprosy includes the multi drug therapy contains rifampicin, clofazimine and dapsone, whereas as rifampicin is administered under supervision, dapsone is administered as non-supervised which leads to irregular administration which leads to severe conditions like microbial resistance. [1-3]

Dapsone being an important drug in the regimen its non-supervision type and poor aqueous solubility may lead to non-compliance. To overcome the disadvantages and to increase bioavailability various techniques have been reported. [4-6]

Nano technology offers many advantages and solution to the problems encountered during the preparation of a formulation. Nanosuspension is the most widely and accepted formulation technique to increase the bioavailability of poorly water-soluble drugs. The bottom-up technique is most preferred method in preparation of nano suspension as it is easy to formulate and few manufacturing steps are involved. Previous reports have been published increase of bioavailability of dapsone using nano technology. [7-10]

The usage of design of experiments and quality by design have been mandatory during the formulation development as they offer many advantages over the conventional methods like minimal utilization

of resources, reduced number of trials and broader understanding of the parameters involved. [11-12]

The present aim of the research work is to prepare nanoparticles of dapsone and evaluate them using various evaluation parameters and to compare the bioavailability enhancement with that of pure drug using design expert.

MATERIALS AND METHODS

Materials

Dapsone was obtained as a gift sample from Dr Reddy's Laboratories, Hyderabad. All other ingredients like Acetone, Poloxamer 188 were procured from Sigma Aldrich. All other ingredients were used of analytical grade or higher.

Methods [13]

Preparation of Nanosuspension

Solvent antisolvent precipitation method previously reported was employed as the preparation technique. Briefly dapsone was dissolved in Acetone which acts as a solvent and is added slowly at a predetermined rate into an antisolvent mixture (water + poloxamer 188(stabilizer)) under stirring at determined time and speeds.

Lyophilization

Dapsone nanosuspensions were lyophilized with mannitol. Nanosuspensions were distributed into vials and frozen at -80^{0}C for 4 h. Then, it was transferred to a freeze-dryer and dried at a pressure of 0.098 Mbar for 24h at -80±0.5^{0}C. The lyophilized naproxen nanosuspensions were found to be dispersible.

Experimental design

A full factorial design (2^4) is used to design the experiment. Based on previous studies and literature review the parameters were selected. The experimental design is designed in such a way to assess various parameters like critical process parameters, material attributes and process parameters. Each factor is used at two levels based on the preliminary experiments. The parameters along with the identified levels are given in the table 1.

The responses selected are particle size and PDI which plays a key role in determining the stability of the product.

Statistical analysis

The experimental data obtained was subjected to statistical analysis using various methods like regression and ANOVA. The polynomial expression obtained through the software is noted.

Table 1: Parameters along with levels

Sl no	Type	Name of the parameter	Low level	High level
1		Rate of Injection	0.2 ml/min	0.6 ml/min

	Critical process parameter	Stirring time	15 min	30 min
2	Formulation parameter	Solvent: Anti Solvent Ratio	1:20	1:10
		Stabilizer: Drug	1:10	1:5

EVALUATION OF THE NANOPARTICLES [14]

SEM/TEM

The optimized, stabilized nanosuspension was used further for the evaluation parameters. The SEM/TEM analysis was generally used to identify the shape, surface morphology of the particles. Freshly prepared nanosuspension is suitably diluted and it was poured on carbon coated paper, dried and observed under microscope with suitable magnification.

Particle size, zeta potential and PDI

Zeta potential indicates the potential present at the double layer and it plays a major role in determining the stability of the nanosuspension. The measurement of zetapotential is done by sing Malvern zetasizer by loading the nanosuspension into capillary cells.

ATR

The ATR (Attenuated Total Reflectance) studies are performed to identify any interaction between the drug and other formulation ingredients. The pure drug and the final formulation ATR were recorded using Bruker instrument and analysed for any interactions.

DSC

DSC is a measurement of the thermal behaviour of the suspension. The freeze-dried suspension is loaded on to a standard aluminium pan and the temperature is increased at intervals of 10^{o}C/min and the values are recorded. Q200 (TA instruments) is used to determine the sample thermal behaviour.

Dissolution studies

Dapsone nanosuspension dissolution study was performed eight station dissolution test apparatus (Electro lab TDT08L) employing a USP II (Paddle) type apparatus at speed of 50 RPM and temperature of 37 ± 1^{o}C. The dissolution media (900 ml) used was phosphate buffer (pH 7.4). The drug release from the nanosuspension is measured by using the validated method, the dissolution data is analysed for various release kinetics.

In vivo pharmacokinetic studies

The optimized formulation was selected for ethe determination of various pharmacokinetic parameters and bioavailability studies. The institutional animal ethical committee approved all the protocols and the procedures were followed according to the established guidelines. Male wistar albino mice were selected for the study and they are acclimatized for 7 days prior to the experiment. All the animals had free access to food and water. The mice were fasted overnight before the administration of the nanosuspension (p.o 8 mg/kg BW). The mice divided into two groups and control

group had access to pure water. The blood was collected from retroorbital plexus, centrifuged ad 2500 RPM and analysed for the drug content according to the developed and validated method.

RESULTS AND DISCUSSION

Evaluation of Nanosuspension

SEM/TEM

The SEM and TEM images are shown in the figure 1,2, the images showed the spherical nature of the particles and showed no sign of aggregation and the surface morphology is also clear.

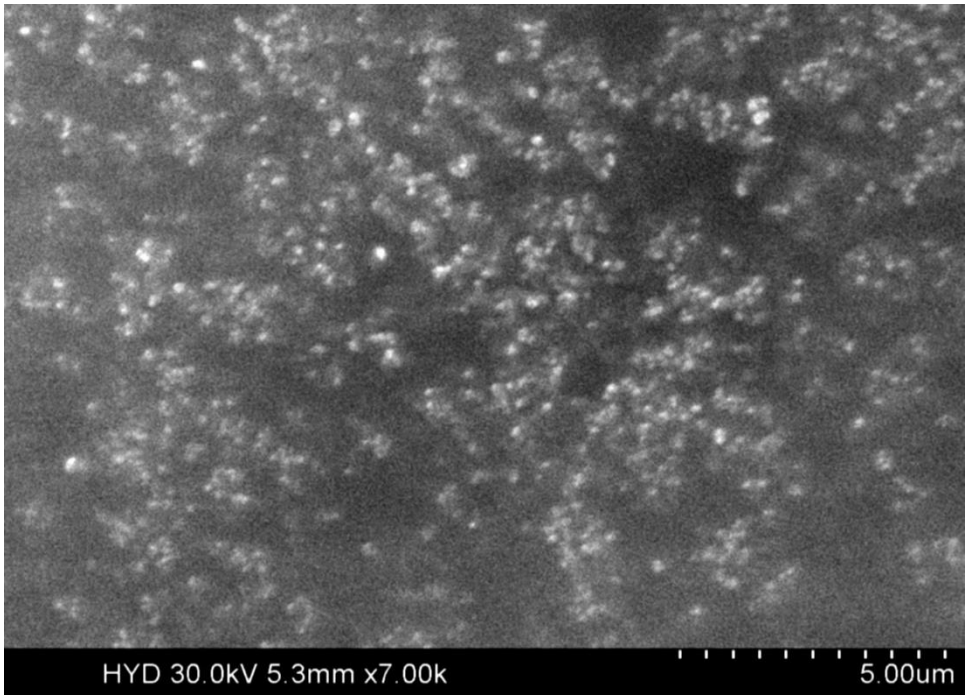


Figure 1: SEM image of Dapsone NanoSuspension

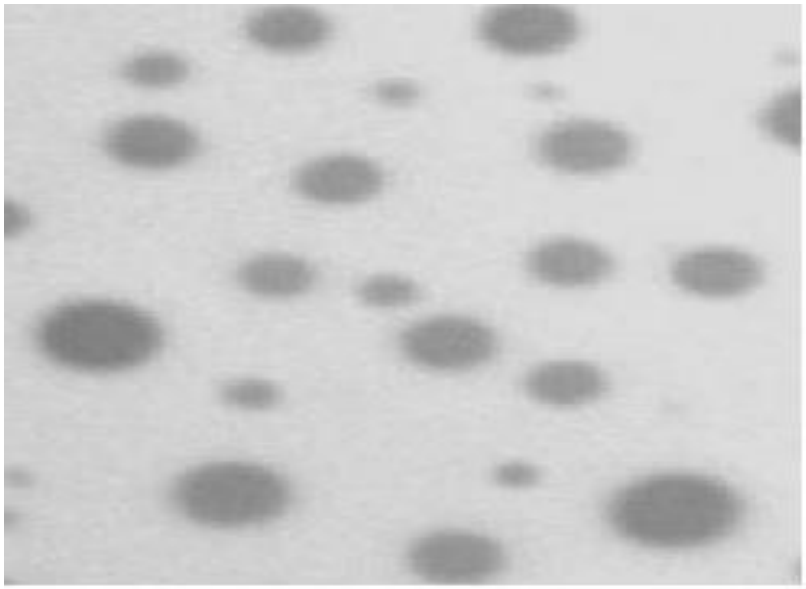


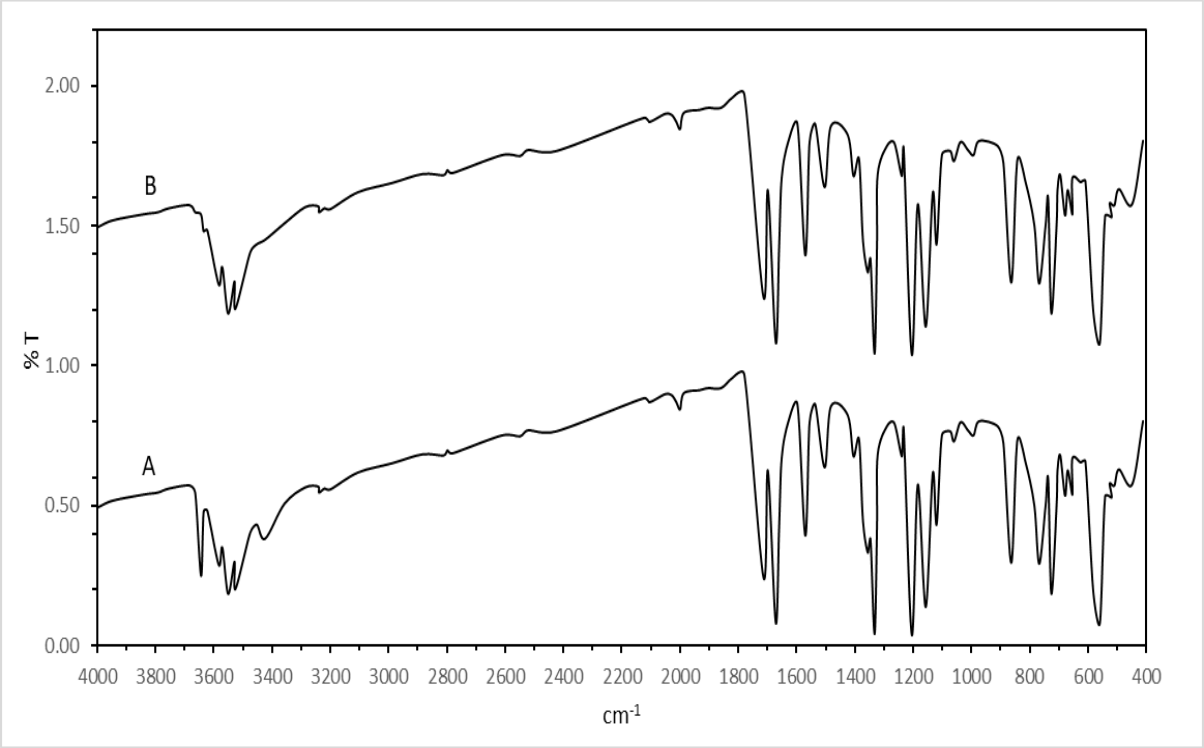
Figure 2: TEM image of Dapsone Nanosuspension

ATR

The ATR spectrums of the pure Naproxen along with the final formulation is shown in the figure 3, which shows there is no sign of major interactions between the drug and the other ingredients. The characteristic peaks of Naproxen showed are given in table 2

Table 2: Characteristic peaks observed in ATR spectrum

Type of peak	Characteristic value	Pure	Formulation
-NH stretching	3400	Yes	Yes
-NH_2 bending	1590	Yes	Yes
-SO_2 symmetric	1143	Yes	yes
- SO_2 asymmetric	1180	Yes	Yes

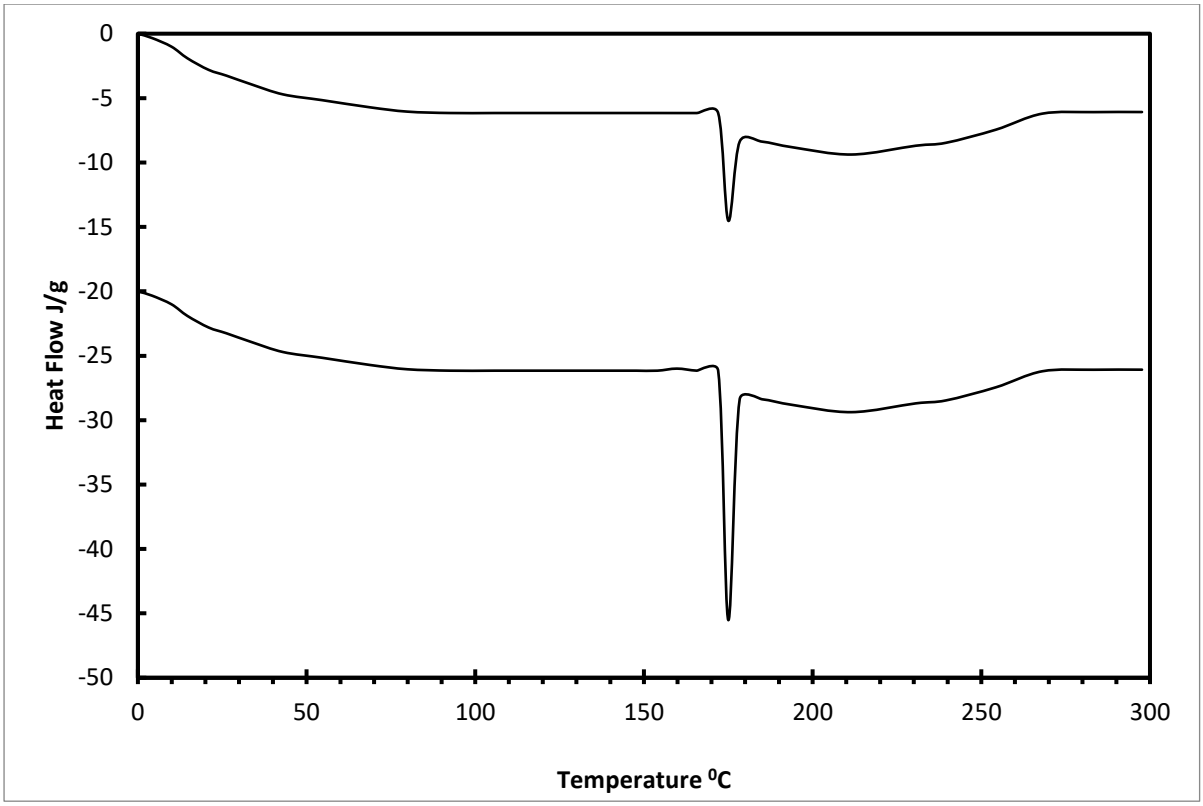


* A indicates pure Dapsone and B indicated final formulation

Figure 3: ATR Spectrum

DSC

The DSC of the pure drug showed and peak at 175^oC while the peak of the combined formulation showed slightest change in peak at 176^oC which shows no interaction between the drug and the ingredients. The retention of the peak showed the crystalline nature of the drug.

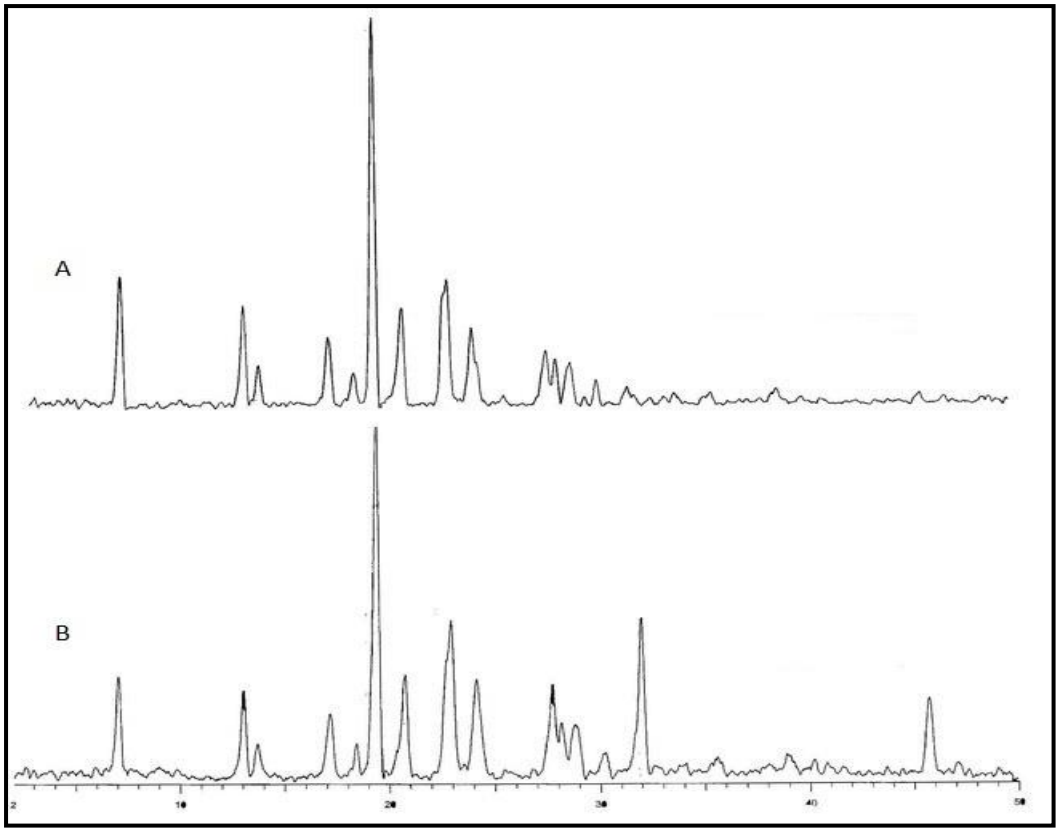


* A indicates pure Dapsone and B indicated final formulation

Figure 4: DSC thermogram

PXRD

The PXRD studies of the pure drug and the final formulation are shown in figure 5. The spectra indicate the crystalline nature of the drug the peaks are present at 19.10^0, 24.26^0 and 26.10^0 shows the same and there is no major interactions between the drug and crystallinity of the drug is retained.



* A indicates pure Dapsone and B indicated final formulation

Figure 5: PXRD

Particle size

The particle size of the nanosuspension is determined by the Malvern zetasizer and the values are noted in the table. All the values obtained were in acceptable size and it has been showed that the process parameters can be a potential influencer in determining the particle size and PDI.

Zeta potential

The zetapotential of the final selected formulation was found to be 50.2 which is generally considered as stable formulation as the zeta potential is sufficient enough to make stable nanosuspension. The zeta potential graph is shown in figure 6.

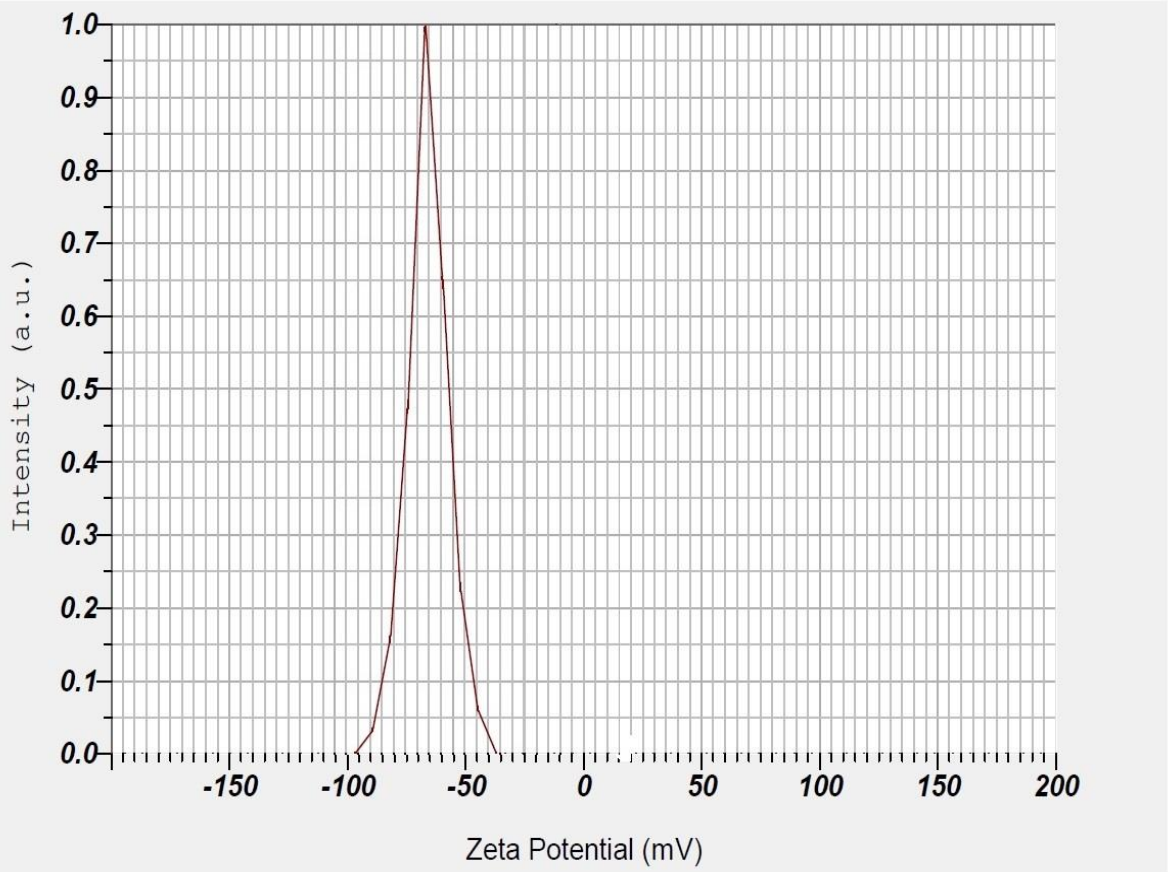


Figure 6: Zetapotential graph of Dapsone nanosuspension

Effect of process and parameters

The effects of the various parameters and the responses are shown the table3, the parameters along with the responses are shown in table 3

Table 4: ANOVA table for response particle size

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	93263.75	5	18652.75	15.29	0.0002	significant
A-Stirring Time	1980.25	1	1980.25	1.62	0.2315	
B-Rate of Injection	10302.25	1	10302.25	8.44	0.0157	
C-Solvent: Anti Solvent	24964.00	1	24964.00	20.46	0.0011	
D-Stabilizer: Drug	23256.25	1	23256.25	19.06	0.0014	
CD	32761.00	1	32761.00	26.85	0.0004	
Residual	12200.00	10	1220.00			
Cor Total	1.055E+05	15				

Table 3: Responses and critical process attributes of Dapsone Nanosuspension

Run	A:Stirring Time (min)	B: Rate of Injection (ml/min)	C:Solvent:Anti Solvent	D:Stabilizer: Drug	Particle Size (nm)	PDI
1	30	0.6	1:10	1:10	250.1±18.2	0.35±0.02
2	15	0.6	1:20	1:10	260.4±22.6	0.36±0.01
3	15	0.6	1:10	1:10	222.1±17.2	0.32±0.02
4	15	0.2	1:20	1:5	196.3±18.5	0.28±0.04
5	30	0.6	1:10	1:5	283.6±21.6	0.19±0.02
6	30	0.2	1:20	1:10	310.2±13.9	0.2±0.01
7	15	0.6	1:20	1:5	239.2±8.5	0.34±0.02
8	15	0.2	1:20	1:10	208.9±17.5	0.3±0.06
9	30	0.6	1:20	1:5	233.7±11.9	0.33±0.04
10	15	0.6	1:10	1:5	19.6±11.3	0.15±0.01
11	30	0.2	1:10	1:10	136.2±11.3	0.22±0.02
12	15	0.2	1:10	1:10	222.2±14.4	0.31±0.06
13	15	0.2	1:10	1:5	32.8±2.8	0.17±0.02
14	30	0.6	1:20	1:10	206.8±11.8	0.29±0.02
15	30	0.2	1:20	1:5	173.4±11.2	0.26±0.06
16	30	0.2	1:10	1:5	29.1±6.8	0.16±0.01

The Anova table for the particle size was showed in the table showing the p value less than 0.05 indicating the model is significant and the responses can be predicted.

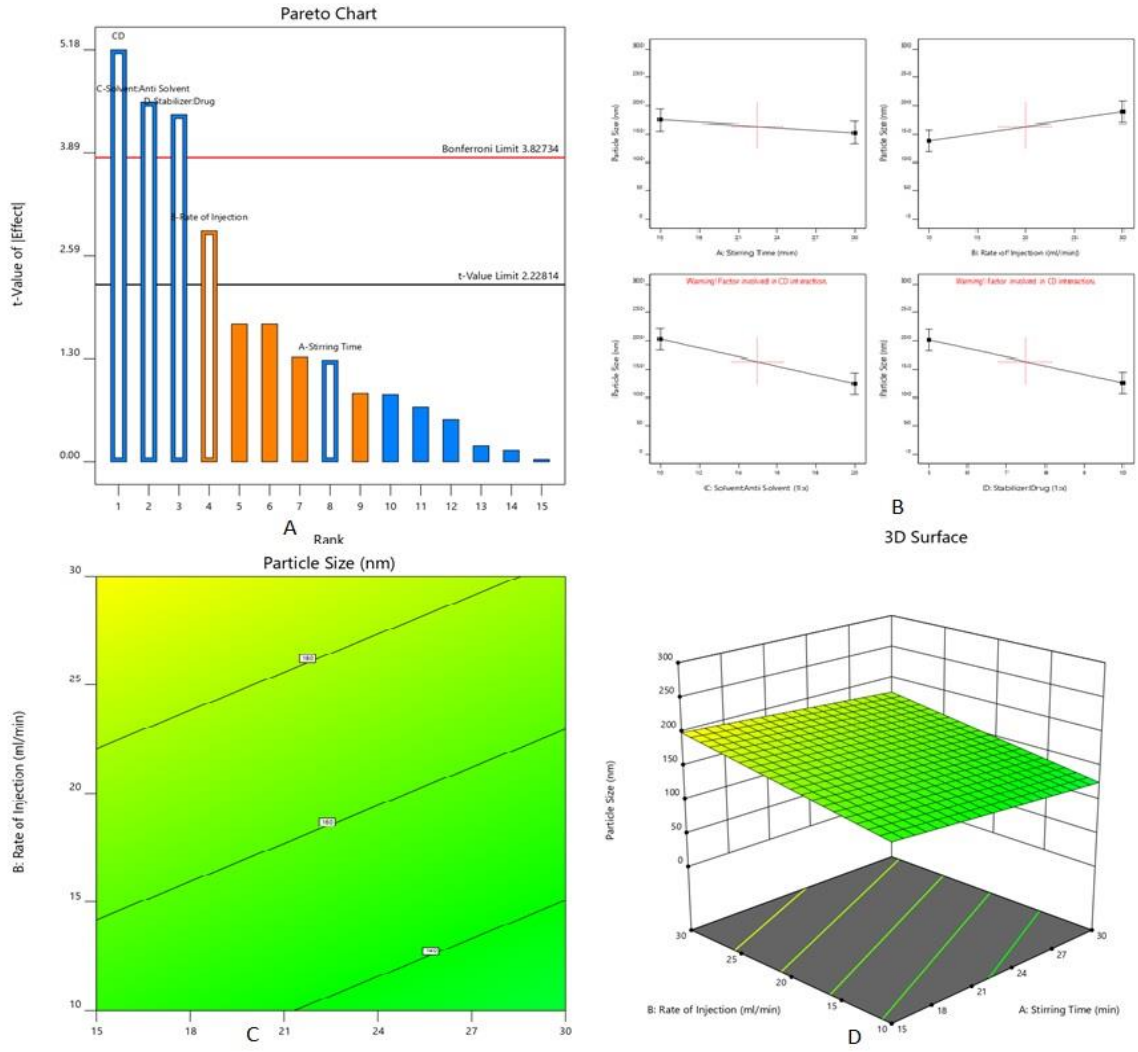


Figure 7: A. Pareto chart, B. Main effects, C. Contour Plot, D. 3D surface response plot of response Particle Size

The pareto chart shown in figure showed that the selected process variables and material variables showed a response over the mean particle size of the Dapsone nanosuspension. The main effects of all the parameters are shown in figure which showed that the material variables have more effect than the process variables. Among all the parameters the antisolvent: solvent ratio has most influence on the particle size. An interaction also reported that as the antisolvent ration increases the particle size decreases and the opposite effect was observed with stabilizer concentration. Among all the parameters the stabilizer: drug concentration has most influence on the particle size. An interaction also reported that as the antisolvent ration increases the PDI decreases and the opposite effect was observed with stabilizer concentration. Among all the combinations the point predicted formulation obtained was selected for further evaluation. The final selected formula was shown in the table.

Table 5: ANOVA table for response PDI

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0654	5	0.0131	10.10	0.0012	significant
A-Stirring Time	0.0033	1	0.0033	2.55	0.1413	

B-Rate of Injection	0.0116	1	0.0116	8.92	0.0137	
C-Solvent: Anti Solvent	0.0150	1	0.0150	11.58	0.0067	
D-Stabilizer: Drug	0.0138	1	0.0138	10.65	0.0085	
CD	0.0218	1	0.0218	16.78	0.0022	
Residual	0.0130	10	0.0013			
Cor Total	0.0784	15				

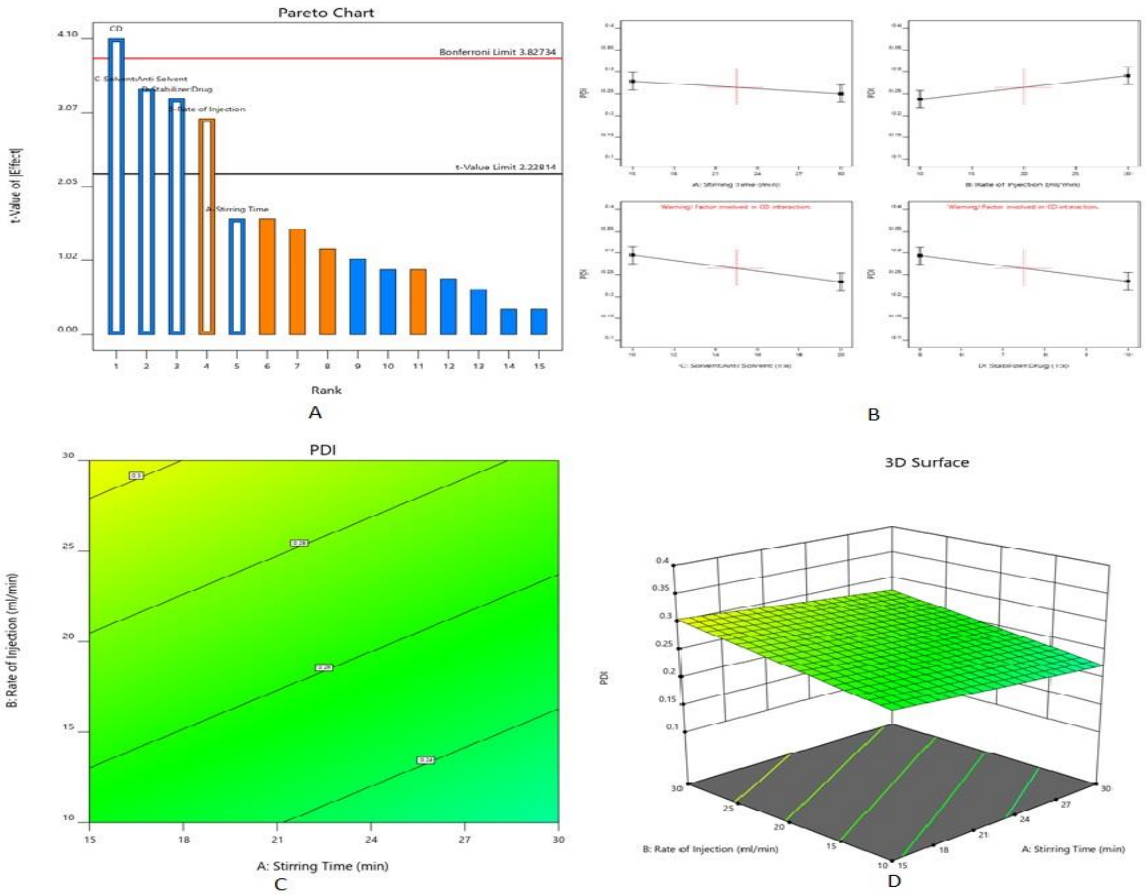


Figure 8: A. Pareto chart, B. Main effects, C. Contour Plot, D. 3D surface response plot of response PDI

Table 6: Point prediction for optimum formulation

Stirring Time	Rate of Injection	Solvent: Anti Solvent	Stabilizer: Drug
22.5 min	0.4 ml/min	1:15	1:7.5

Invitro dissolution studies

The best selected Dapsone nanosuspension was subjected to dissolution and the data was shown in tables and graphs, the pure dapsone does not show any significant dissolution whereas the Dapsone nanosuspension showed a maximum release within 30 min. The release kinetics showed that it followed first order mechanism and the release kinetics are shown in table 7. The dapsone nanosuspension follows first order release kinetics.

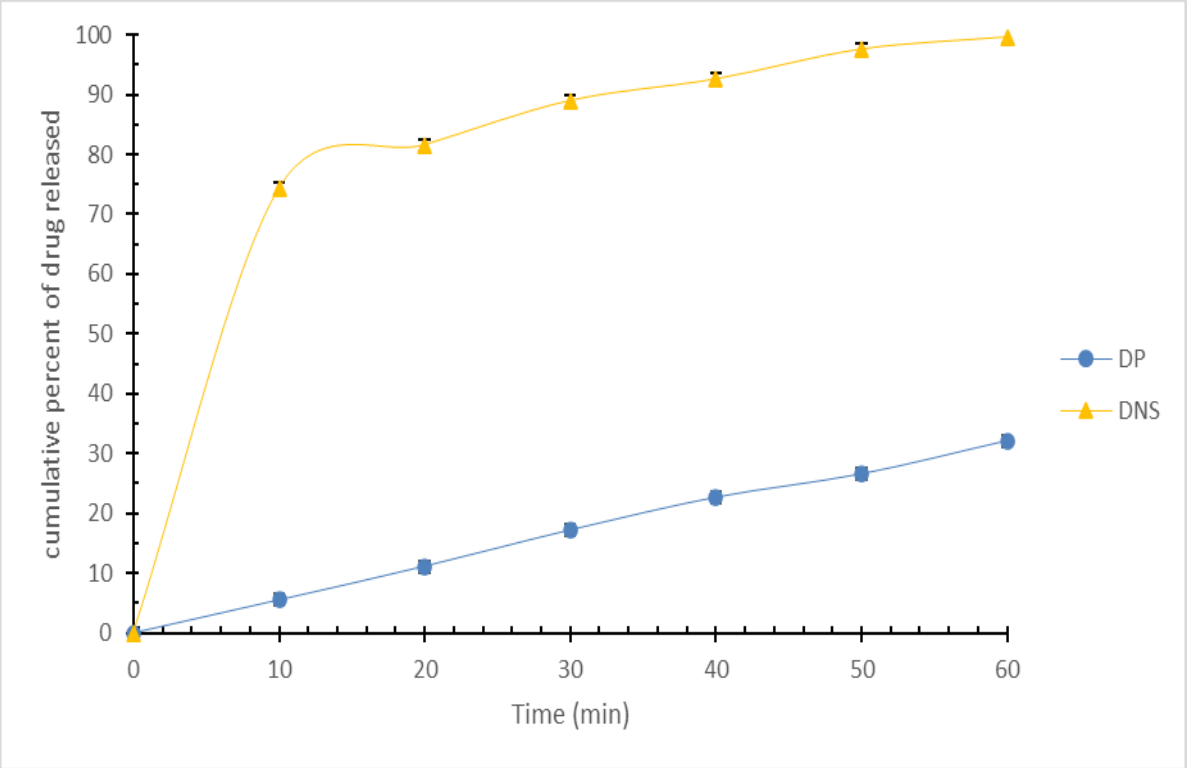


Figure 9: Drug release plot of Dapsone Nanosuspension

Table 7: Drug release kinetics of Dapsone Nanosuspension

Formulation	Zero Order		First Order	
	Equation	R^2	Equation	R^2
DP (Dapsone Pure)	y = 0.5346x + 0.3886	0.9975	y = 0.0145x + 0.7099	0.918
DNS (Dapsone Nano Suspension)	y = 1.2724x + 38.184	0.6234	y = 0.0025x + 1.8589	0.9492

Pharmacokinetic data

The pharmacokinetic data of the pure drug and the Dapsone nanosuspension formulation was shown in table 8 and figure 10, it was evident that the Dapsone nanosuspension formulation showed better pharmacokinetic parameters when compared to pure drug.

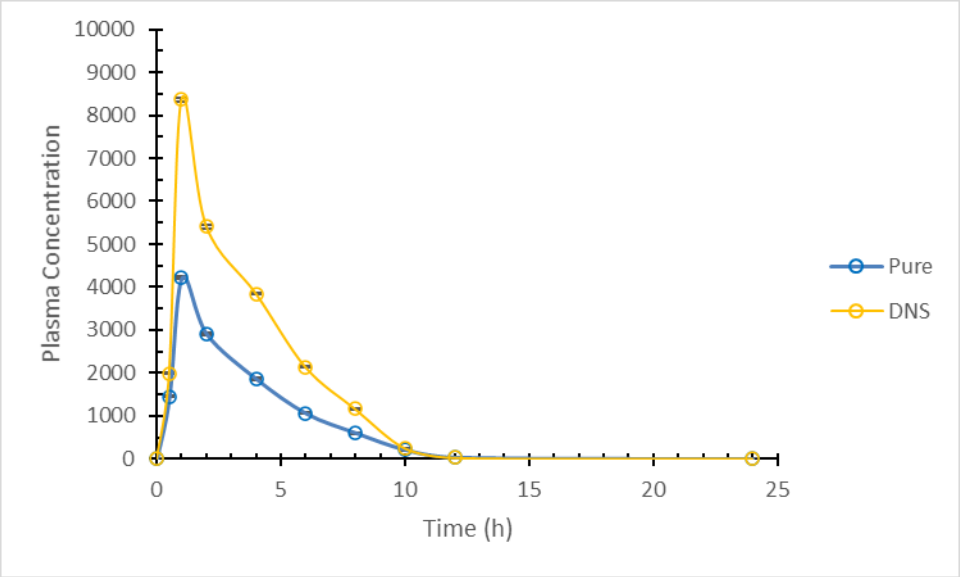


Figure 10: Pharmacokinetic drug profile of Dapsone Nanosuspension

Table 8: Pharmacokinetic profile of Dapsone Nanosuspension

Parameter	Unit	Pure	DNS
t1/2	H	1	0.722
Tmax	H	1	1
Cmax	ng/ml	4216	8364
AUC 0-t	ng/ml*h	15800	30184.5
AUC 0-∞	ng/ml*h	15854.82241	30210.5483
MRT	H	3.480	3.4270

CONCLUSION

Dapsone owing its low solubility and irritability nanoparticulate approach is preferable as it overcomes the major disadvantages of the drug. The nanosuspension can be prepared conveniently and the various process and material attributes plays a major role in determining the efficiency of the delivery system, the material variable interactions are also taken into consideration while designing a suitable dosage form. The dapsone nanoparticles have been successfully evaluated for various pharmacokinetic parameters.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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