RESEARCH ARTICLE

To Study the Significance of Processing Variables Using Quality by Design for Optimization of Nanoparticulate System of Cilnidipine

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ABSTRACT

This investigation aimed to prepare Cilnidipine Nanoparticles by nanoprecipitation ultrasonication method and to study the significance of processing variables by applying quality by design. Cilnidipine is fourth-generation dual L/N-type Ca^{2+} channel blocker used for the management of hypertension. It is BCS class-II drug exhibiting lower aqueous solubility, which tends to lower bioavailability. The combination of Poloxamer 188 and Tween 80 was used as a stabilizer. The design of the experiment is one of the tools of Quality by design. Plackett-Burman design was applied for the screening of processing variables, which are significant for the method. The processing variables screened were stirring speed, antisolvent ratio, drug concentration, polymer concentration, stabilizer concentration. The effect of each parameter evaluated by particle size, entrapment efficiency, and drug release at 10 minutes of prepared Nanoparticles of Cilnidipine. Analysis of variance and Pareto-plot of Plackett-Burman design were utilized to find the significance of the factor and extent of the effect. The surface morphology of Cilnidipine Nanoparticles was studied by SEM. The Pareto plot, as well as statistical analysis of design, had shown that the Concentration of drug, solvent: antisolvent ratio and concentration of poloxamer 188 were the significant parameters for the method. The stabilizer concentration, the stirring speed, and the antisolvent ratio had a negative effect of while the concentration of drug has a positive effect on the particle size of Nanoparticles and drug release at 10 minutes and positive effect of entrapment efficiency of Cilnidipine Nanoparticles. The Cilnidipine Nanoparticles were characterized by FTIR and DSC analysis.

Keywords: Processing variables, Nanoparticulate System, Cilnidipine, Plackett-Burman Design.

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INTRODUCTION

Cilnidipine (CLN) is a new dihydropyridine Ca-channel blocker that had given excellent effect clinically on cardiovascular diseases. [1] Cilnidipine is diester 1,4-dihydropyridine-3,5-dicarboxylic acid and it blocks both L and N-type ca- channels. [2] It only possesses reno, neuro and cardioprotective action out of all antihypertensive drugs. It is a yellow, odorless crystalline powder having molecular weight 492 g/mol. CLN is included in BCS class 2 drug which has

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low aqueous solubility and high permeability. The drug with poor aqueous solubility shows the poor dissolution rate which resulted in low oral bioavailability from an effective dose of 10 mg. [3] Development of poorly water-soluble drugs formulations is always a challenging task for formulators and In the future also 40% of newly developed drugs expected to be poorly soluble. [4]

The solubility can be enhanced by different traditional strategies like micronization, co-solvency technique, utilizing permeation enhancers, utilizing surfactant but they may have limited use. even though considerable results have been obtained by formulating hydrophobic drugs utilizing techniques like emulsions, liposomes, microemulsions, solid dispersion technology and inclusion complexes with cyclodextrins, but for all drugs, anyone approaches not applicable. At present, the need is for a unique technology that may overcome the problems related to the formulation of hydrophobic drugs which improve their clinical efficacy and optimize their therapy related to pharmacoeconomics. [4]

The drug powder is converted to drug nanoparticles by basic two techniques like bottom-up technology and top-down technology. [5] A pharmaceutical nanosuspension is characterized as all-around finely scattered strong medication particles in a fluid vehicle for either oral furthermore, topical and Parenterals use. The molecule estimate circulation of the strong particles in nanosuspension is typically not exactly less than one micron. Nanosuspension has demonstrated its capability to beat the issues related to the less water-soluble drugs and lipid dissolvable medications with the effortlessness and the points of interest over different strategies. [6]

The present study aimed to increase the dissolution rate of less water-soluble drugs CLN by the nanoparticulate system. Nanoparticles were prepared by the nanoprecipitation method. This technique was developed and patented by Fessi and co-workers. [7-8] In these techniques the Nano size is achieved by one single step. This method requires one solvent in which drug is highly soluble and one solvent in which drug is insoluble. Important thing is that these two solvents must be miscible with each other. By the means of rapid desolvation of the polymer particles nanoprecipitation observed. At the point when the polymer-containing dissolvable polymer diffuses into the dispersed medium, it precipitates out following entrapment of drug. The moment arrangement of the Nanoparticles is because of the Marangoni impact, which is a result of interfacial turbulences occur at the interface of the dissolvable and the non-dissolvable solvent and result from complex and cumulated marvels, for example, stream, dispersion and surface strain varieties. [9] Nano size range of 100–300 nm with narrow unimodal distribution can be achieved by this technique. Entrapment efficiency value up to 100% can

be obtained due to zero drug leakage towards the outer medium. [7, 10]

In this method, many processing variables were required to be optimized. To select the critical processing variables which have a significant effect on Cilnidipine Nanoparticles the Plackett Burman design (PB Design) was utilized. The objective of this design is the screening of important parameters among the various parameters. The PB Design is a two-level factorial design and the impact of every factor was given by the equation

$$Eai = 2(\Sigma Hai - \Sigma Lai)/N ----- (I)$$

Where Eai is the concentration effect of a particular variable, Hai is the response at the higher level, Lai is the response at the lower level and N is the total number of trials.

A positive sign in the model for a reaction demonstrates an impact that favors and the negative sign shows a reverse connection between reactions. In the Plackett-Burman experimental design, the linear equation is expressed as

$$R = B_0 + B_1A_1 + B_2A_2 + B_3A_3 + B_4A_4 + \ldots + B_NA_N ---- (II)$$

Where R is the response, B0 is the constant and B1, B2….BN are the coefficients of the variables A1, A2,... AN. [11-15]

MATERIALS AND METHODS
Materials
Cilnidipine was obtained as a gift sample from Laksh Finchem, Anand. The poloxamer 188 was obtained as a gift sample from BASF, Germany. The tween 80 was purchased from Loba Chemical Pvt. Ltd., India.

Preparation of Cilnidipine Nanoparticles
The formulation of nanosuspension was performed by the nanoprecipitation ultrasonication method. Precipitation with sonication was used to prepare nanosuspension. The specific amount of stabilizers Poloxamer 188 and tween 80 were dissolved into distilled water. The CLN drug was dissolved in dimethyl sulphoxide (DMSO) to prepare an organic solution of the drug. This solution was injected into the stabilizer solution maintained at 5°C under stirred conditions. Comminution was carried out on mechanical stirrer for a specific period at optimum speed. Continuous sonication for a specific period was applied via the probe sonicator. The temperature was maintained at 5°C to get less particle size. [16] The solution was sonicated by probe sonicator for 15 minutes. The obtained nanosuspension was centrifuged using ultracentrifuge at 14000 RPM for 5 minutes at 5°C. The supernatant solution was replaced by a stabilizer solution with appropriate concentration. To store it for a longer period it was lyophilized using lyophilizer. [17]

Plackett-Burman design
Plackett-Burman design was applied to screen out the significant parameter out of various processing parameters. The processing parameters screened were Drug Conc. in mg/ml, Solvent/Antisolvent Ratio,

Stirring Speed, Conc. of Poloxamer 188 and Conc. of Tween 80 indicated in table 1. The effect of each parameter evaluated using particle size, entrapment efficiency, and *in vitro* drug release. A total of 12 experimental trials were carried out with 5 independent factors and 3 dependant factors generated by Design-Expert®. The levels of each variable were selected based on preliminary studies.

Table 1: Experimental factors and values

Independent factors	Coded value	LEVELS	
		-1	+1
Drug Conc.	X_1	20	40
Solvent/ Antisolvent Ratio	X_2	1:10	1:30
Stirring Speed	X_3	800	1200
Conc. of Poloxamer 188	X_4	0.1	0.3
Conc. of Tween 80	X_5	0.05	0.15

Characterization of Cilnidipine Nanoparticles
Particle size
Mean particle size and polydispersity index (PI) of the prepared nanosuspension and raw drug were checked by the Zetatrac instrument (microtrac, Mumbai). Whenever required suitably diluted Nanoparticles were added to the instrument test unit and estimation was done with the assistance of microtrac 10.6.1 programming.

Entrapment efficiency
For the determination of entrapment efficiency, prepared nanosuspension formulation was centrifuged at 10000 rpm for 10 min. The supernatant solution was analyzed by a UV-visible spectrophotometer at 240 nm using 1.2 pH simulated gastric fluid as a blank. Entrapment Efficiency was calculated by the following equation. [18]

$$\% \text{ Entrapment Efficiency} = \frac{\text{Total Drug Content in Nanoparticles} \times 100}{\text{The total amount of drug}}$$

In-vitro Drug release
In vitro release of drugs was performed utilizing the USP dissolution Apparatus II setup (Electrolab Dissolution Tester TDT-06P, USP). Simulated gastric fluid (1.2 pH) was taken as a dissolution medium. The temperature was maintained at 37 ± 0.5℃, and the paddle speed used was 100 rpm. Cilnidipine Nanoparticles containing the equivalent of 10 mg drug was added into the 250 ml dissolution medium. Samples containing each of 5 ml were withdrawn at a different time interval (2, 5, 10, 15, 30, 45, 60, 90 and 120 min) and passed through a 0.45µm syringe filter. The determination of the samples was done using a UV spectrophotometer at 240 nm. [19]

Solid-state characterization
Fourier Transmission Infra-Red Spectroscopy (FTIR)
FTIR analysis of pure drug CLN and CLN Nanoparticles were performed using FTIR CARY 630 (Agilent Technologies) to check the interaction between drug and excipients. The samples were added into the sample port for recording the IR spectra. The spectrum was recorded in the region 400–4000 cm⁻¹.

Differential scanning calorimetry (DSC)
DSC study was performed to analyze the drug and excipients compatibility study of pure drug, and final formulation. DSC analysis was carried out using aluminum sample pans in Differential Scanning Calorimetry (DSC-60, Shimadzu Japan) at a heating rate of 10°C per minute in the range 30 to 300°C using differential scanning calorimetry (DSC).

Scanning electron microscopy (SEM)
The surface morphology of CLN Nanoparticles was studied with a field emission scanning electron microscopy. Before observation, the samples was fixed onto metal stubs using double-sided sticky tape previously secured onto aluminum stubs and then coated with gold under a vacuum and photographs were taken at various magnifications.

RESULTS AND DISCUSSION
Preparation of CLN Nanoparticles
Nanoparticles of CLN were prepared by the nanoprecipitation ultrasonication method. The drug was dissolved in Dimethyl sulphoxide (DMSO) solvent that was selected based on the highest solubility. The organic solution of CLN was added into an aqueous solution containing stabilizers Poloxamer 188 and Tween 80. The resultant solution was stirred by mechanical stirrer at various speeds. The spontaneous precipitates were observed due to the instant desolvation of the drug. By using the stabilizer in the concentration range of 0.1 to 0.3%, 200 to 500 nm particle size of Nanoparticles was formed. The stirring speed was kept in the range of 800 to 1200 rpm. The larger particle was formed when stirring speed was kept greater than 1200 and less than 800. If the antisolvent amount increases yield of Nanoparticles was decreased and the removal of the solvent becomes difficult so the ratio of antisolvent was selected from 1: 10 to 1: 30. Plackett Burman design was applied for the screening of factors in which levels of parameters selected based on preliminary trials.

Particle size determines the performance of drugs like drug solubility and dissolution behavior in media. Entrapment efficiency determines the performance of drugs like how much amount of drug entrapped in particles and dissolution at 10 minutes determines how much amount of drug released at 10 minutes.

Plackett Burman design
In the design total, 12 batches have been prepared. The Particle size, entrapment efficiency, and drug releases at 10 minutes of all 12 batches were in the range of 236-431 nm (table 2), 90.94% to 95.87% and 90% to 98 %. Here 11th batch was considered optimized with 236 nm particle size (figure 1), 95.57 entrapment efficiency, and 98.36% drug release at 10 minutes. The statistical analysis results (table 3) showed that R^2 value greater than 0.9. The *p*-value is less than 0.05 proves the significance of design. The *F*-Ratio was observed greater than theoretical F value 0.008. The correlation of R^2, *p*-value and *F*-Ratio had shown that linear relationship between predicted and observed value. [20]

The equation (III to V) according to coded value for particle size, entrapment efficiency (%EE) and drug releases at 10 minutes (D_{10}) are the following (table 5).

Particle size = $352.91 + 33.08X_1 - 30.25X_2 - 19.91X_3 - 24.41X_4 - 19.58X_5$------------ (III)

% EE = $91.83 - 1.1X_1 + 1.21X_2 + 0.69X_3 + 0.95X_4 + 0.593X_5$--------------------------- (IV)

$D_{10} = 95.01 - 0.9X_1 + 1.192X_2 + 0.75X_3 + 0.97X_4 + 0.73X_5$---------------------------(V)

Table 2: Plackett Burman design with responses

RUN	X_1	X_2	X_3	X_4	X_5	R^1	R^2	R^3
1	-1	-1	-1	1	-1	362 ± 8.88	92.46 ± 0.077	95.45 ± 0.088
2	-1	-1	1	-1	1	351 ± 3.785	92.78 ± 0.035	95.26 ± 0.036
3	1	-1	1	1	1	359 ± 4.04	92.04 ± 0.03	95.01 ± 0.036
4	-1	1	1	-1	1	268 ± 4.03	94.09 ± 0.037	97.53 ± 0.041
5	-1	-1	-1	-1	-1	441 ± 3.60	87.76 ± 0.2	90.48 ± 0.032
6	1	-1	-1	-1	1	397 ± 3.21	88.81 ± 0.050	93.15 ± 0.025
7	1	1	-1	-1	-1	412 ± 2.08	90.94 ± 0.025	92.94 ± 0.02
8	1	-1	1	1	-1	389 ± 3.05	89.83 ± 0.04	93.56 ± 0.04
9	-1	1	-1	1	1	261 ± 3.511	94.96 ± 0.032	98.42 ± 0.05
10	1	1	1	-1	-1	395 ± 2.08	90.86 ± 0.026	94.85 ± 0.026
11	-1	1	1	1	-1	236 ± 3.21	95.57 ± 0.037	98.36 ± 0.025
12	1	1	-1	1	1	364 ± 2.58	91.86 ± 0.025	95.12 ± 0.08

R^1 = Particle Size; R^2 = Entrapment Efficiency (%EE); R^3 = Drug Releases At 10 Minutes (D_{10})

Table 3: ANOVA of particle size, entrapment efficiency, and dissolution rate at 10 minute

Response	F-Ratio	R^2 value	P-value
Particle Size	9.358706	0.941461	0.00843
% EE	7.893815	0.931688	0.012901
D10	7.52558	0.928694	0.014503

Table 4: Statistical analysis of particle size, entrapment efficiency, and dissolution rate at 10 minute

Response/ Factors	*p*-value		
	Particle size	% EE	D10
Drug Concentraion	0.008084	0.016569	0.036594
Solvent/ Antisolvent Ratio	0.011983	0.011105	0.012419
Speed	0.057735	0.083029	0.068157
Concentration of Poloxamer 188	0.028413	0.029337	0.027845
Concentration of Tween 80	0.060917	0.127993	0.071962

Table 5: Coefficient of variable for of particle size, entrapment efficiency, and dissolution rate at 10 minute

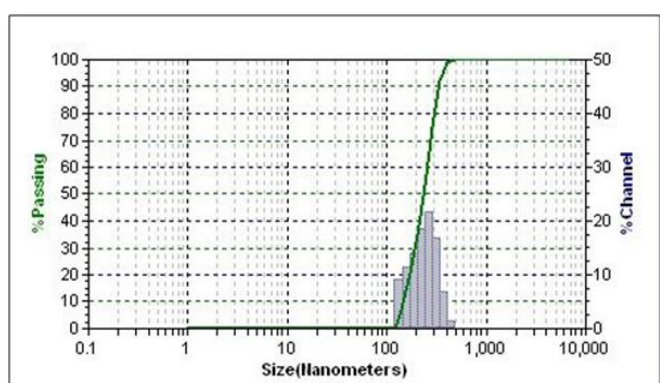| Response	Intercept	X_1	X_2	X_3	X_4	X_5
Particle Size	352.91	33.08	-30.25	-19.91	-24.41	-19.58
% EE	91.83	-1.10	1.21	0.69	0.95	0.593
D10	95.01	-0.90	1.192	0.75	0.97	0.73

Fig. 1: Particle size distribution of Nanoparticles of Cilnidipine using zetasizer
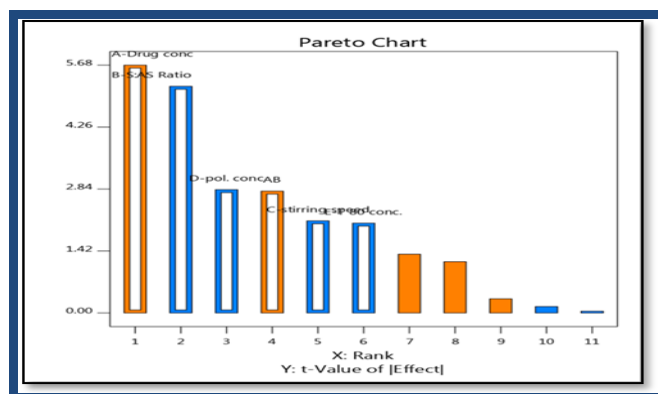

Fig. 2: Pareto chart of the effect of Independent factors on the particle size.

Effect of processing variables on particle size

The concentration of drug, solvent: antisolvent ratio and concentration of poloxamer 188 had shown *a p-value* less than 0.05 (table 4) which shows that they were the significant parameters for the method. Pareto chart (Figure 2) showed that these three variables *t value* is also greater than 2.5 which show a significant effect on the particle size of Nanoparticles of Cilnidipine. The stirring speed and tween concentration have not significant effect on particle size. The amount of drugs has an inverse relationship with the span of the precipitated particles. The extent of the span of drug particles diminishes with an enhancement of the number of drugs. This reason is the reliance on the nucleation rate on the number of drugs in their liquid medium from which the drug is precipitate out. [21] A solvent to antisolvent proportion is a method variable, which had a negative impact on the size of the Nanoparticles. As this proportion enhances the size of Nanoparticles diminishes radically. At the point when the solution of the drug is added to the antisolvent, a fast decrease for focus happens with an expansion in the measure of antisolvent prompting quick precipitation of the drug into Nano-sized particles. At the point when the blending velocity goes higher up, the high exceptional speed delivers a lot of heat energy, which improves the temperature prompting increment in the size of Nanoparticles. [22-26] The stabilizer concentration had a negative effect on particle size as the amount of stabilizer increase than the size of particles was decreased. The scanning electron microscopy of pure drug CLN (figure 6) and Nanoparticles of CLN (figure 7) shows that there was a reduction in the size of particles of the drug.

In the Pareto plot the blue color bar shows negative effect on particle size. The orange color bar shows positive effect on particle size. White color space between bar shows that significant effect. The stirring speed and tween 80 concentration shows less significant effect on particle size. [27]

Effect of processing variables on % Entrapment Efficiency

The concentration of drug, solvent: antisolvent ratio and concentration of poloxamer 188 had shown a *p-*

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value less than 0.05 (table 4) which shows that they were the significant parameters for the entrapment efficiency. Pareto chart (figure 4) showed that these three variables t value is also greater than 2.5, which shows a significant effect on entrapment efficiency of Nanoparticles of Cilnidipine. The stirring speed and tween concentration have not significant effect on particle size. The drug concentration had a negative effect on entrapment efficiency as the amount of drug increases entrapment efficiency decreases. Solvent: antisolvent ratio, the concentration of Poloxamer 188, concentration of Tween 80 and stirring speed had shown a positive effect on entrapment efficiency.

The orange color was observed for solvent antisolvent ratio, stirring speed and concentration of stabilizers Poloxamer 188 and Tween 80, which indicates positive effect on entrapment efficiency. As amount of antisolvent increases, the more amount of drug can be entrapped. Drug concentration shows negative effect by the blue color bar.

Effect of processing variables on the dissolution rate at 10 minutes

The concentration of drug, solvent: antisolvent ratio and concentration of poloxamer 188 had shown a p-value less than 0.05 (table 4) which shows that they were the significant parameters for the entrapment efficiency. Pareto chart (figure 4) showed that these three variables t value is also greater than 2.5, which shows a significant effect on particle size and entrapment efficiency of Nanoparticles of Cilnidipine. Drug concentration had shown a negative effect on drug release .the all other variables solvent: antisolvent ratio, the concentration of poloxamer 188, concentration of tween 80 and stirring speed had shown a positive effect on drug release as per table 5.

The orange color was observed for solvent antisolvent ratio, stirring speed and concentration of stabilizers Poloxamer 188 and Tween 80, which indicates positive effect on the dissolution rate at 10 minute. Drug concentration shows negative effect by the blue color bar. The higher stirring speed lowers the particle size and increases the drug release rate. The stabilizer amount up to certain concentration range lowers the particle size and increases the drug release rate. The higher drug concentration particle size increases and the drug release rate decreases.

Solid-state characterization

Fourier Transmission Infra-Red Spectroscopy (FTIR) Analysis

FT-IR spectra of CLN, Poloxamer 188, physical mixture and Nanoparticles of CLN are given in Figure 5. The study of IR spectra of CLN demonstrated the presence of N-H stretching and of C=O stretching is clearly shown by bands at 3292 cm^{-1} and 1697 cm^{-1}.Strong and prominent peak at 1524 cm^{-1} and 1348 cm^{-1} confirms the existence of NO$_2$ in the structure. Presence of secondary amine is confirmed by weak band at 3289 cm^{-1}. Aromatic structure in the drug is confirmed by the characteristic absorption bands at 3086 cm^{-1} and at

1523-1491 cm^{-1} due to C–H stretch and C-C stretching respectively. The spectrum illustrates CH3 aliphatic stretch at 2944 cm^{-1} along with extensive NO$_2$ vibrations at 1577 and 1348 cm^{-1}. The IR spectrum had showed that not any major interaction between CLN drug and its Nanoparticles.
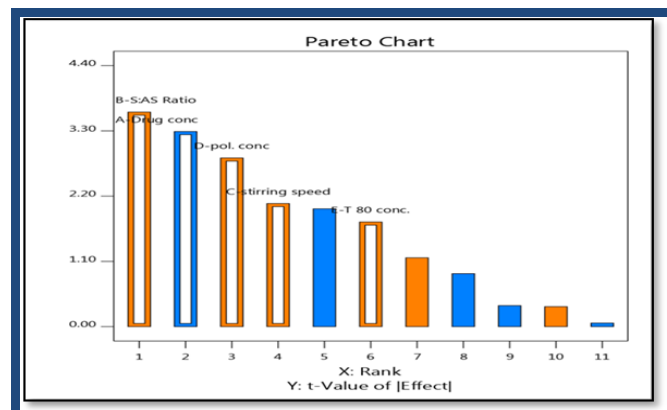

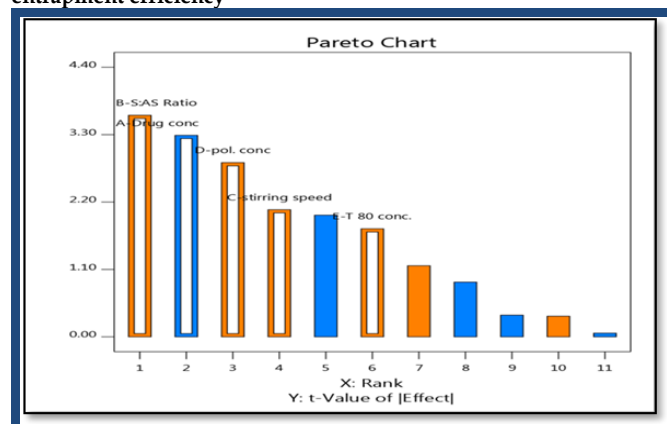**Fig. 3: Pareto chart of the effect of independent factors on the entrapment efficiency**

Fig. 4: Pareto chart of the effect of independent factors on the dissolution rate at 10 minute
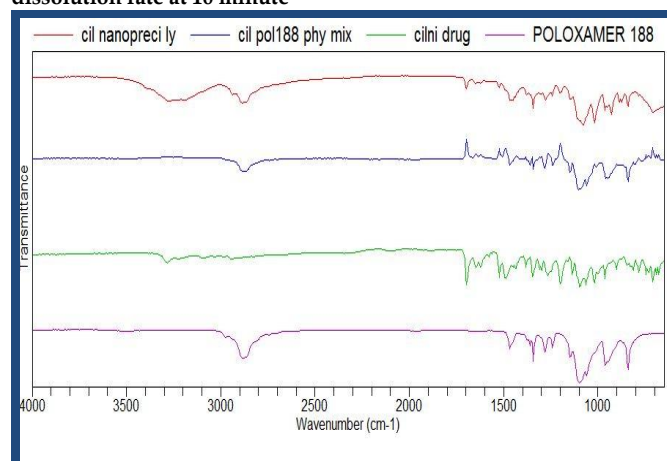

Fig. 5: FTIR spectrum of pure drug and Nanoparticles of Cilnidipine by NP-US method

Differential Scanning Calorimetry (DSC) study

The graph of the DSC pure drug CLN and CLN Nanoparticles (Figure 8 and 9) exhibited sharp endothermic melting peaks at 111.99°C and 112.14°C respectively. The graph showed no major interaction between CLN drug and CLN Nanoparticles.
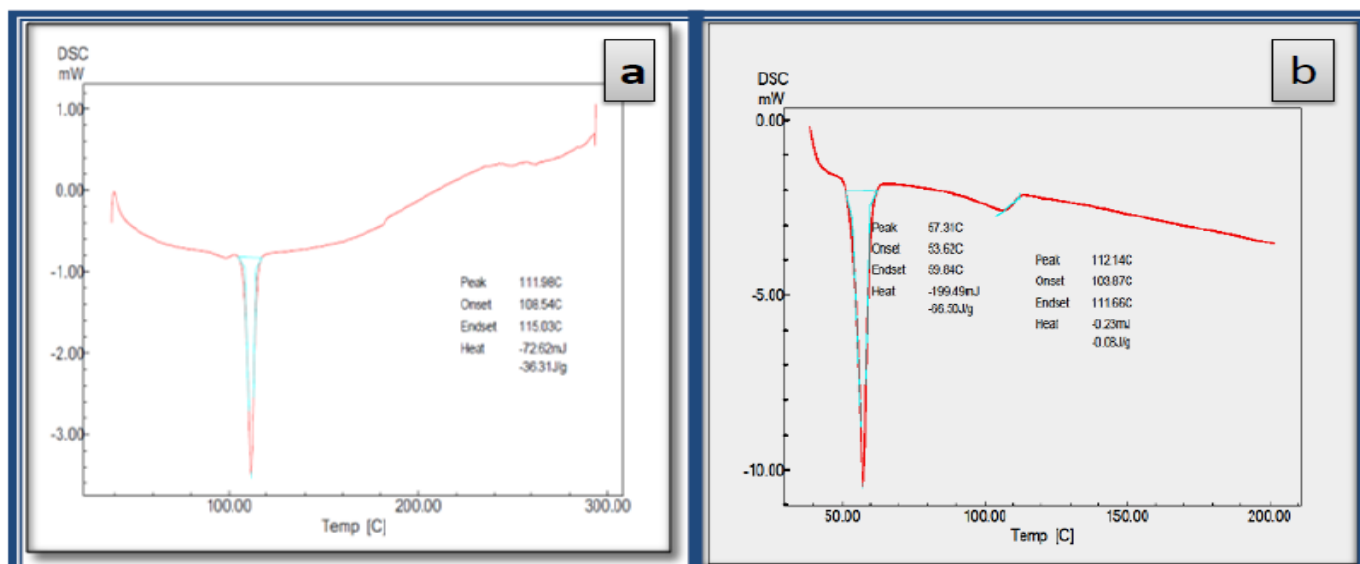
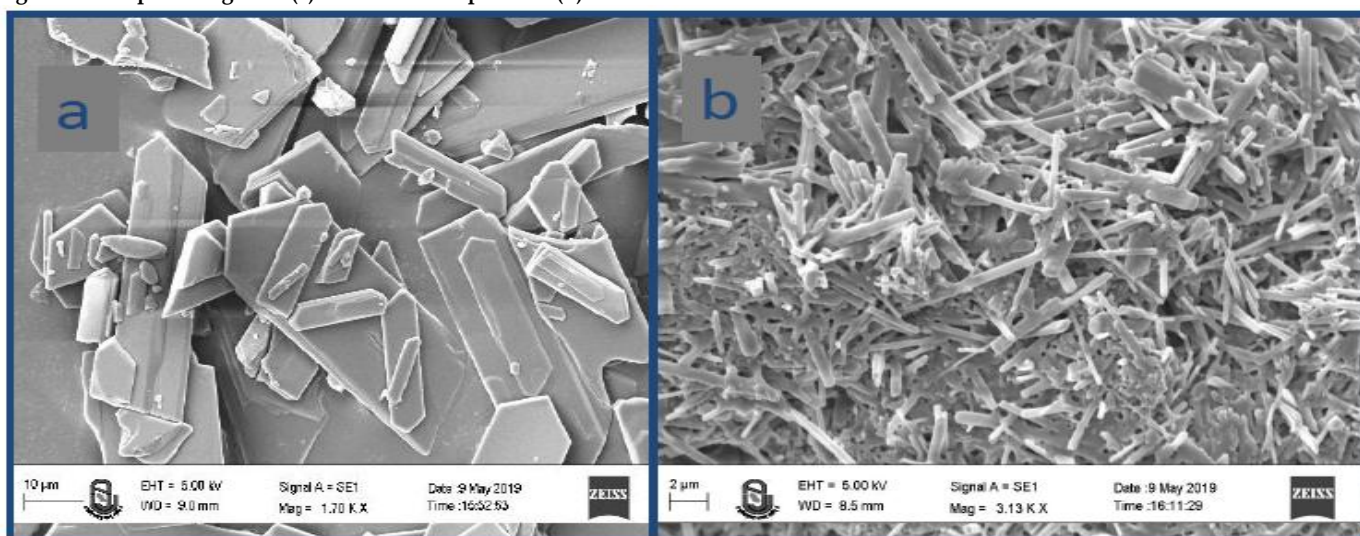**Fig. 6: DSC of pure drug CLN (a) and CLN Nanoparticles (b).**

Fig. 7: scanning electron microscopic image of CLN(a) and Nanoparticles of CLN

Scanning Electron Microscopy (SEM)

Scanning electron microscopy was performed to identify the morphology of the pure drug sample and optimized CLN Nanoparticles. Scanning electron microscopy image of CLN Nanoparticles (figure 7) had shown that size of particles decreased as compare to pure drug CLN after formulating the Nanoparticles (figure 6).

In the current research, the effect of some process variables on the particle size, entrapment efficiency, and *in vitro* drug release of the Nanoparticles of Cilnidipine was studied by Plackett- Burman design. The lower Particle size, higher drug release, and higher entrapment of drugs can be obtained by enhancing the concentration of stabilizers, enhancing the stirring speed, enhancing the solvent: antisolvent ratio and decreasing the amount of drug.

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