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## Design and Characterization of Micro-Crystals of A Model Antihypertensive Drug for Enhanced Dissolution Rate

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### ABSTRACT

Elevated bioavailability is an advantage for most of the poorly soluble drugs. The present scenario of research investigation is concentrated on different techniques to alter the solubility characteristics of weakly soluble drugs and crystallization phenomenon is one amongst them. The low solubility problem can be solved by changing the crystal habit of drug. So, in the present research an attempt has been made to modify the solubility characteristics of Nifedipine, an anti-hypertensive drug, using solvent change method, Solvent evaporation technique and solvent change precipitation technique. Among them solvent change method gave a better formulation (NIF-MC-6) showing better dissolution (91.36% at the end of 240mins) as compared to pure drug and micro-crystals formulated using other methods. The formulated crystals of Nifedipine were subjected to various physico-chemical parameters like size distribution, solubility studies, in-vitro dissolution studies, drug content, FT- IR, DSC, crystallographic studies by PXRD and crystal morphology by SEM studies. The micro-crystals produced with PVPK30 and chloroform. FT-IR Results showed that there was no chemical interaction between the drug, solvent and the stabilizer. PXRD of micro-crystals showed higher peak height than pure drug indicating that crystal habit modification occurred in the micro-crystals without any polymeric changes and were found to be smaller in size than pure drug and free from any interactions. SEM studies indicated that the crystals are present in rectangular and square shape. The DSC curve showed that Nifedipine appeared an endothermic peak at about 174°C corresponding to its melting. However, the crystals prepared with PVP K30 shows shift of endothermic peak towards lower temperature at 170.82°C respectively, dictating decreased melting point of the drug in the formed crystals, which accounted for increased solubility of the drugs.

**Keywords:** Microcrystals, Nifedipine, PVPK30, Solvent evaporation technique, solvent change method, solvent change precipitation technique.

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## INTRODUCTION

During formulation development by the various pharmaceutical industries more than 40% of active ingredients are found to be poorly water soluble. This problem is directly associated with poor dissolution characteristics. Dissolution rate in the gastro-intestinal tract is the rate limiting factor for the absorption of BCS class II-drugs of drugs and so they suffer from poor oral bioavailability. An increase in the dissolution rate of those drugs can enhance the blood-levels to a safe, clinically suitable level. Various methods are commonly used to improve dissolution characteristics and bioavailability of poorly water-soluble drugs<sup>1,2</sup> such as size reduction<sup>3</sup>, the use of surfactants<sup>4</sup>, the formulation of solid dispersions<sup>5</sup>, complexation with cyclodextrins, and the transformation of crystalline drug to amorphous state<sup>6</sup>.

In addition to the general solubility enhancement techniques described above, drug particle size reduction has often been used, in regards to the Noyes–Whitney and Ostwald–Freundlich equations, to raise dissolution of poorly water soluble active substances<sup>7</sup>. Crystallization is a phenomenon in which solid particles formed by solidification under favorable conditions of a chemical element or a compound, whose boundary surfaces are planes symmetrically arranged at definite angles to one another in a definite geometric form. In the matter, particles are present randomly due to thermal agitation. Crystallization varies from precipitation in that the compound is deposited from a supersaturated solution. The precipitation mechanism takes place when solutions of the compound react chemically to form a product, which is sparingly soluble in the liquid and therefore deposits out. The polymorphic variations will have a definite influence on the solubility and there by changes in bioavailability of a particular compound can obtain due to structural variations resulting from different arrangements of molecules in the solid state<sup>8</sup>. Nifedipine is a second-generation di hydro pyridine calcium channel blocker (CCB), appears to decrease blood pressure without causing these unfavorable adverse events. Is a long-acting di hydro pyridine CCB with high vascular selectivity, thus it has many of the properties that are desirable in an **antihypertensive** agent. As its microcrystalline forms exhibit poor micromeritic properties and also has poor aqueous solubility which is ideal for the present. And so this drug was found to be ideal for the present development studies.

## MATERIALS AND METHOD

### Materials

Nifedipine (NIF) was procured as a gift sample from Anglo French Drug and Industries ltd (Bangalore, India), Povidone (PVP, K-30) was procured as a gift sample from Ce-chem

Pharmaceuticals, Bangalore, India. Acetone, Isopropyl Alcohol (IPA), Chloroform, Dichloromethane (DCM), Methanol, Potassium dihydrogen phosphate (PDP) and Sodium Hydroxide (NaOH) pellets were of AR grade.

### **Methods:**

#### **Preparation of NIF-MC-1 and 2 By Solvent Evaporation Technique<sup>9</sup>**

Prepared 4gm of drug solution in 20 ml of methanol respectively. The above prepared drug solution was added drop-wise to beaker containing water with or without adding PVPK30 (0.5 gm). By continuous stirring (slow and turbulent) the biphasic layer formed due to water immiscible solvent is evaporated by maintaining the temperature corresponding to their boiling point. Micro-precipitation takes place due to the evaporation of water immiscible solvents and re-precipitation of drug in water. The precipitated crystals were filtered using Whatmann filter paper no. 1 and dried at 60°C for 1 hour.

#### **Preparation of NIF-MC-3, 4, 5, 6, 7 and 8 by Solvent Change Method**

Drug was dissolved in suitable solvents i.e. DCM, Chloroform and Acetone, heated to boiling point of that particular solvent. The drug solution was poured quickly into water maintained at 20°C under continuous stirring with paddle device, 400 (±5) rpm. After 25 mins of stirring, micro crystals formed and were separated from the solution by filtration. Micro crystals were dried at 45°C for 12 hours.

#### **Preparation of NIF-MC-9 and 10 by Solvent Change Precipitation Technique<sup>10</sup>**

The solvent change precipitation was conducted by instantaneously mixing two liquids in the presence of a stabilizing agent. The organic phase (solvent phase) was a methanolic solution of drug at gm/ml, a stabilizing agent in the aqueous phase. The aqueous phase was poured rapidly from a beaker into the methanolic drug solution under stirring using a magnetic stirrer. The process was carried out at room temperature. Crystals were collected by filtration

### **Evaluation and characterization of the prepared Micro-crystals**

#### **Percentage yield**

The practical percentage yield was calculated from the weight of dried spherical agglomerates (Practical mass) to the initial weight (Theoretical mass) the percentage yield was calculated by using the following formula:

$$\% \text{ Yield} = \frac{\text{Practical Mass}}{\text{Theoretical Mass}} \times 100$$

#### **Solubility studies**

The solubility of Nifedipine in water was determined by taking excess quantity of micro-crystals and adding to 100 ml volumetric flask filled with water and sonicated. The solution was filtered through whatmann filter paper no. 1 and the drug concentration was determined spectrophotometrically at 238 nm.

### **Particle size analysis**

Particle size of the prepared micro-crystals was determined by optical microscopy. The optical microscope was fitted with an ocular micrometer and a stage micrometer. The eyepiece micrometer was calibrated. The particle diameters of more than 200 micro-crystals were measured randomly by optical microscope.

### **Fourier-transform infrared spectroscopy (FT-IR)**

1-2mg of Nifedipine alone and the prepared Nifedipine microcrystal formulations were weighed and mixed properly with potassium bromide uniformly. FTIR was performed by KBr Pellet method. Drug and KBr were taken in 1:100 ratios and ground in mortar for even distribution of sample and KBr. Then it was prepared in the form of disk by applying pressure of 5 tons for 5 min using a hydraulic press and subjected to IR. The IR- spectrum of the pellet from 450-4000cm<sup>-1</sup> was recorded.

### **Scanning Electron Microscopy (SEM)**

The samples for scanning electron microscopy were prepared by gently sprinkling the micro-crystals on a double adhesive tape, which is stuck to an aluminum stub. The stubs were then coated with gold using a sputter coater under high vacuum and high voltage to achieve a film thickness of 30nm. The samples were then imaged using a 3 kV electron beam.

### **Powder X-Ray Diffraction (PXRD)**

X-ray powder diffraction patterns were used to detect possible polymorphic transition during the crystallization process. X-ray powder diffraction were obtained at room temperature (25<sup>0</sup>C) using Broker <sub>a</sub>XS D8 Advance diffractometer (Cu K<sub>α</sub> source  $\lambda = 1.5418 \text{ \AA}$ ).

### **Diffractional Scanning Calorimetry (DSC)**

Samples of about 1-3 mg were weighed and placed in aluminium pans and the lids were crimped using a shimadzu crimper. An empty pan sealed in the same way as for the sample was used as a reference. Thermal behaviour of the samples was investigated under nitrogen gas at scanning rate of 10<sup>0</sup>C/min, covering a temperature range of 30-300<sup>0</sup>C.

### **Accelerated Stability study**

Stability studies were performed as per ICH guidelines. physical appearance was observed for formulations at the end of the 1 month storage period at 5<sup>0</sup>C / ambient, 25<sup>0</sup>C / 60% RH & 40<sup>0</sup>C / 75% RH conditions. The micro-crystals from the selected and optimized batch (NIH-MC-6) were packed

in the light protective containers and are tightly closed with caps, they were studied for physical appearance and kept under the accelerated conditions like raised temperature and moisture up to period of 1 month and studied for the %CDR weekly.

## RESULTS & DISCUSSION

### Drug Content, Solubility and In-Vitro Drug Release Study

For untreated drug the drug content was considered to be 100%; however drug content of the micro-crystals was found to be in the range of 95.09 to 99.19 %. The percentage yield shows the result for the prepared micro-crystals in the range 75.70 to 96.66% respectively. Micro-crystals showed nearly two fold higher enhanced solubility than the pure sample in water at the temperature 28<sup>0</sup>C. Micro-crystals NIF-PVPK30-chloroform (NIF-MC-6) showed highest solubility as compared to the untreated drug and other micro-crystal formulations prepared. (**Table-4**)

**Fig No-1** dictates the results of dissolution studies. NIF-MC-6 showed fastest dissolution rate, with approximately 91.35 % of the drug being released within 4hrs min compared to the micro-crystals prepared with other solvents, stabilizing agents and just 40.91 % for the control (untreated drug). More than 50 % of the drug from NIF-MC-6 crystals was dissolved in 150 mins. NIF-MC-6 containing pvpk30 as stabilizing agent and chloroform as solvent presented a dramatic increase in the solubility and dissolution rate of the drug. PVPK30 of low viscosity grades were used for the micro-crystals stabilization as these water soluble polymers offers adequate surface active properties when compared to other commonly used Stabilizing agents. Reduced particle size of the crystals, increases their specific surface area as well as the adsorption. Moreover, the solubility results advocate to the output of dissolution studies. Based on Noyes-Whitney equation an increase in saturation solubility leads to an increase in dissolution velocity<sup>11</sup>.

### FT-IR

The characteristics absorption peaks of Nifedipine were obtained at 3332.29cm<sup>-1</sup>, 3061.33cm<sup>-1</sup>, 2996.36 cm<sup>-1</sup>, 2952.81 cm<sup>-1</sup>, 2841.80 cm<sup>-1</sup>. The principle peaks obtained for the combinations were almost similar to that of the drug (**Fig-2 & 3**).

### SEM, XRD and DSC Analysis

The SEM photomicrographs of the untreated drug are shown in **Fig-4**. Pure Nifedipine was characterized with larger particle size. In contrast, micro-crystals were with rough surfaces indicating adsorption on their surfaces. NIF-MC-6 and NIF-MC-4 (Figure 5 and 6) indicates smaller particle size with rough surface area. According to the Rasneck, if a hydrophobic substance is inserted to the stabilizer molecule, the drug particle size decreases<sup>12</sup>. Rough surface area of the

crystals is due to the presence of stabilizing agents which result in higher dissolution rate. **Powder X-ray** diffraction pattern confirmed physical nature of the raw material and micro-crystals (**Fig-7**). The X-ray diffraction pattern of the micro-crystal formulation NIF-MC-6 and 4 showed that Nifedipine peak intensity was much higher than the pure drug. This could be due to the increase in crystalline nature of prepared micro-crystals. These results from **Fig:-8 and 9** could explain the observed enhancement of solubility and dissolution of Nifedipine micro-crystals.

The **DSC** curve showed (**Fig-10**) that Nifedipine appeared an endothermic peak at about **174<sup>0</sup>C** corresponding to its melting. However, the crystals prepared with PVP K30 shows shift of endothermic peak towards lower temperature at **170.82<sup>0</sup>C** respectively (**Fig-11**). Shift of the endothermic peak towards lower temperature dictates decreased melting point of the drug in the formed crystals. This decreased melting point accounts for increased solubility of the drugs. Usually this may be caused by a variation of the different crystal habit or to a reduction in particle size. From the DSC analysis of the crystals, positive influence of the polymer on the solid state of the drug was attested.

#### Accelerated Stability study

The in-vitro drug release determination of optimized formulation NIF-MC-6 at a fixed time interval of 7 days showed (**Fig-12**) that there was no significant change in the values when compared to the initial drug release of the formulation. Thus we may conclude that the drug does not undergo degradation on storage. These results are the indication of good stability of the product.

**Table No.1:Formulation design of NIF-MC-1 and 2 by Solvent Evaporation Method:**

Formulation code	Amt of Drug (gm)	Stabilizing agent with Amount (gm)	Organic solvent used	Qty. of Organic solvent (ml)	Stirring speed (rpm)	Temp (°C)
NIF-MC-1	4	PVP K30(0.5)	Methanol	20	400	25
NIF-MC-2	4	Without PVPk30	Methanol	20	400	25

**Table No.2:Formulation design of NIF-MC-3, 4, 5, 6, 7 and 8 by Solvent Change Method:**

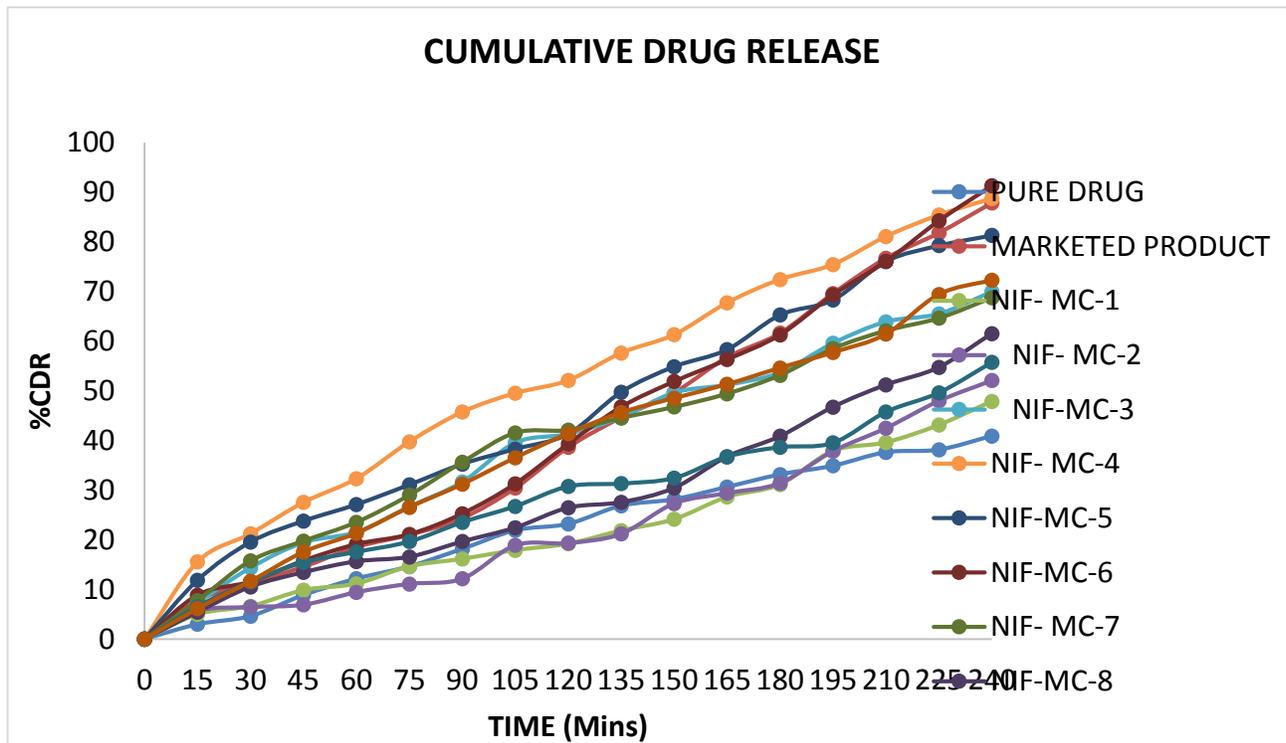
Formulation Code	Amt Of Drug (Gm)	Stabilizing Agent With Amount (gm)	Organic Solvent Used	Qty. Of Organic Solvent (ml)	Stirring Speed (RPM)	Temp (°C)
NIF-MC-3	4	Without PVP K30	DCM	20	400	39
NIF-MC-4	4	PVP K30(0.5)	DCM	20	400	39
NIF-MC-5	4	WithoutPVPk30	Chloroform	20	400	61
NIF-MC-6	4	PVPK30(0.5)	Chloroform	20	400	61
NIF-MC-7	4	Without PVPK30	Acetone	20	400	56
NIF-MC-8	4	PVP K30(0.5)	Acetone	20	400	56

**Table No.3: Formulation design of NIF-MC-9 and 10 by Solvent Change Precipitation Technique:**

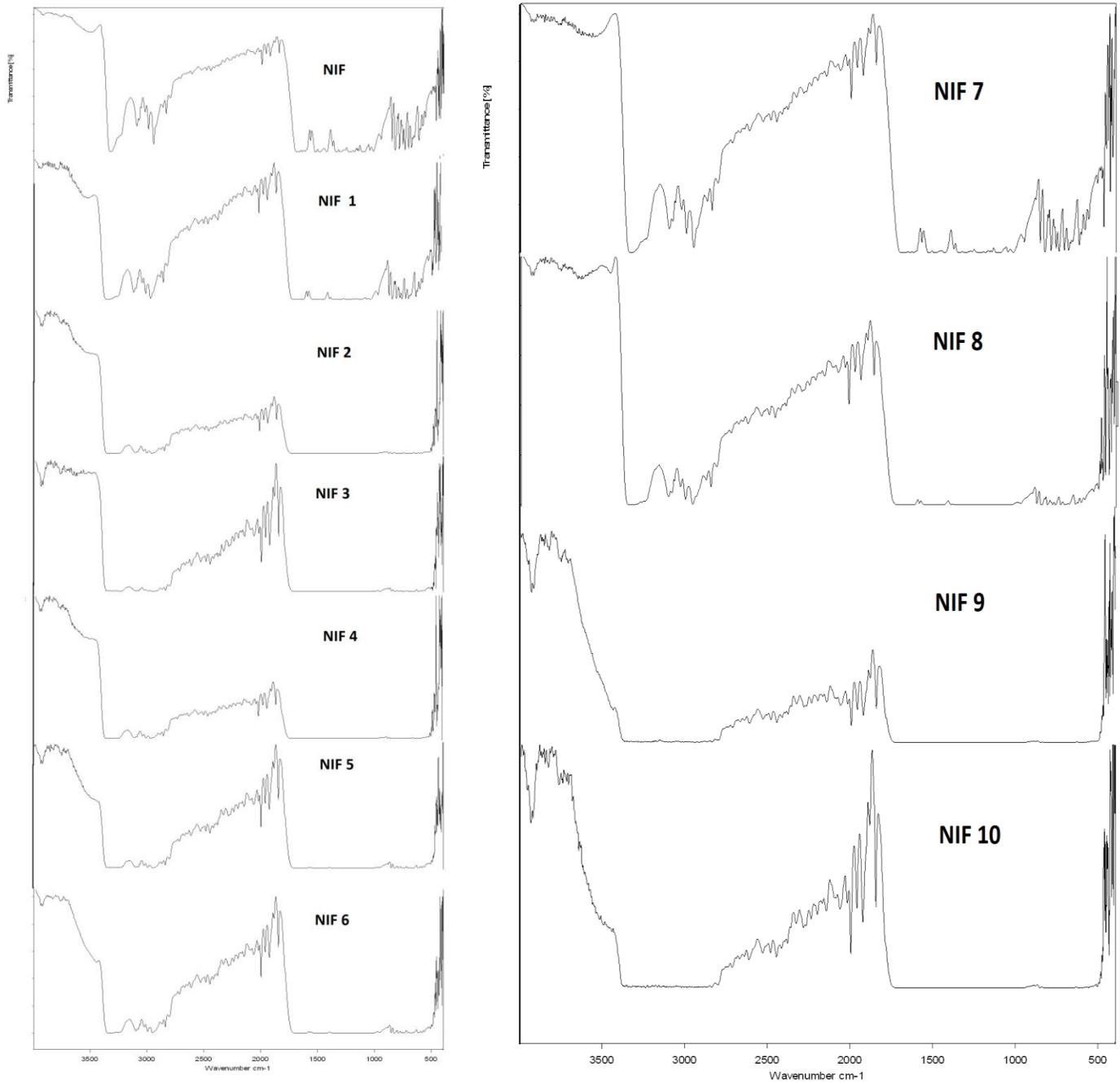
Formulation Code	Amt of Drug (gm)	Stabilizing Agent	Organic Solvent used	Qty of Organic solvent (ml)	Stirring Speed (RPM)	Temp (°C)
NIF-MC-9	4	PVP K30	IPA	20	400	25
NIF-MC-10	4	Without PVPk30	IPA	20	400	25

**Table No.4 : Observed data for Melting point (°C), Percentage Yield (%), Drug Content (%), Solubility in water (µg/ml) ±SD, Avg. Particle size Distribution (µm)**

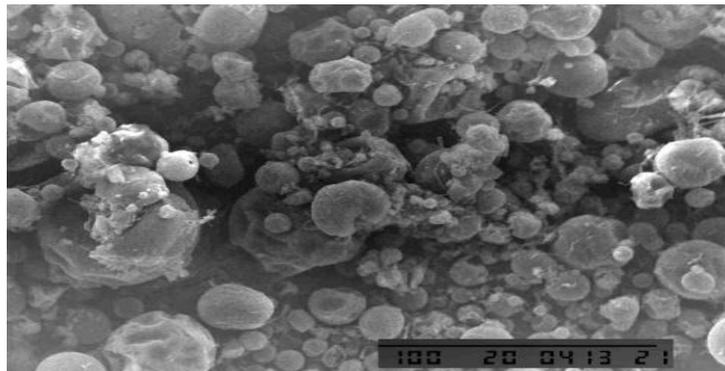
Sl No.	Formulations	Observed Melting point (°C)	Percentage Yield (%)	Drug Content (%)	Water Solubility (µg/ml) ±SD	Avg. Particle size Distribution (µm)
01	Nifedipine Pure Drug	173	--	--	61.89 ± 0.002	--
02	NIF-MC-1	170	75.70	95.09	130.22 ± 0.001	34.50
03	NIF-MC-2	179	83.86	97.77	118.01 ± 0.002	31.55
04	NIF-MC-3	181	91.49	99.09	130.22 ± 0.001	33.31
05	NIF-MC-4	172	64.84	97.75	118.01 ± 0.002	30.11
06	NIF-MC-5	171	93.81	98.09	130.22 ± 0.001	32.90
07	NIF-MC-6	170	96.66	97.17	118.01 ± 0.002	35.02
08	NIF-MC-7	171	92.31	99.19	130.22 ± 0.001	36.01
09	NIF-MC-8	178	91.42	97.97	118.01 ± 0.002	38.75
10	NIF-MC-9	173	95.61	99.09	130.22 ± 0.001	33.67
11	NIF-MC-10	180	95.55	96.77	118.01 ± 0.002	35.00



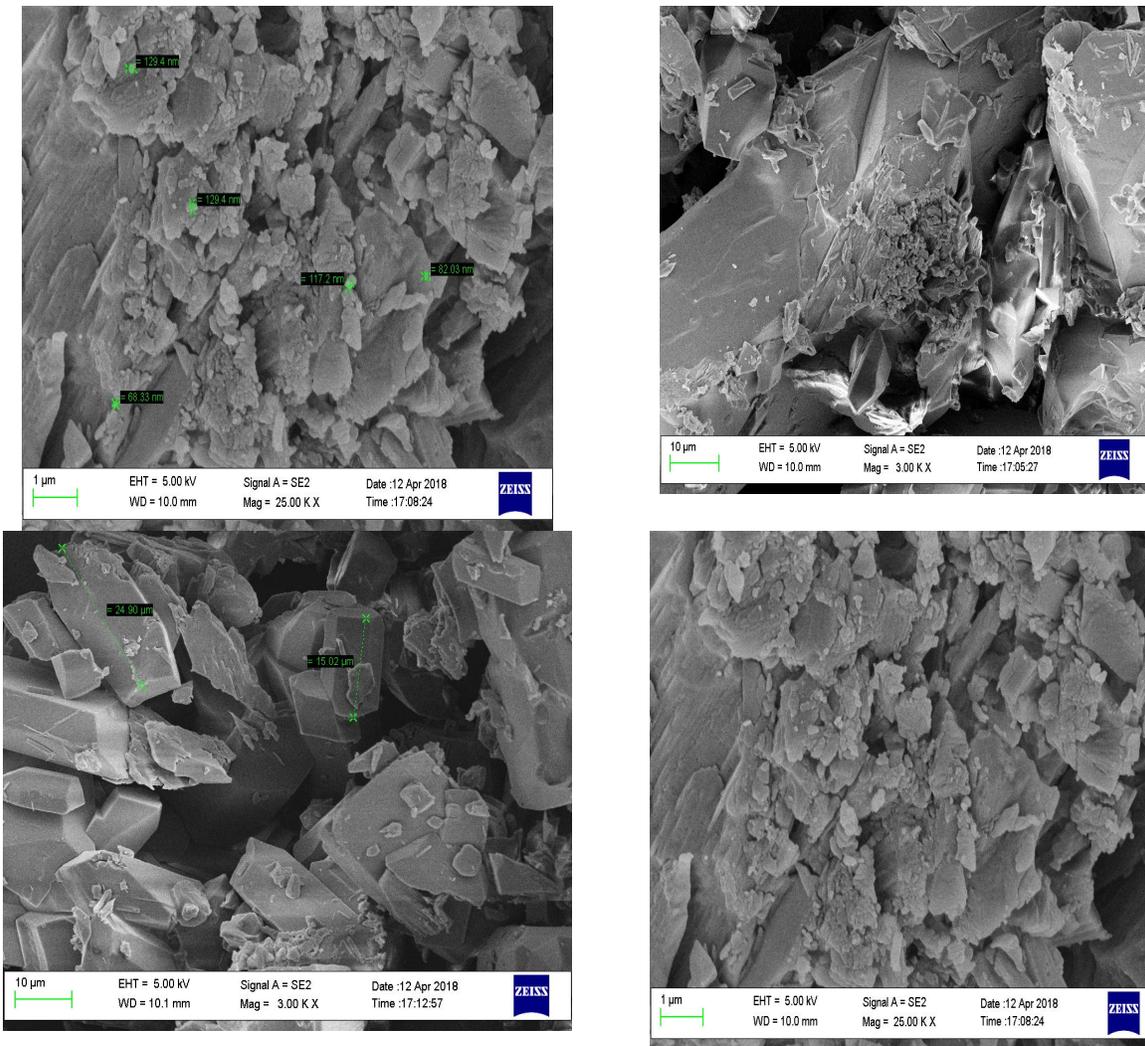
**Figure:1.**In-vitro drug release studies of pure drug, Marketed Product (Depin 10), and prepared Micro-crystal formulations.



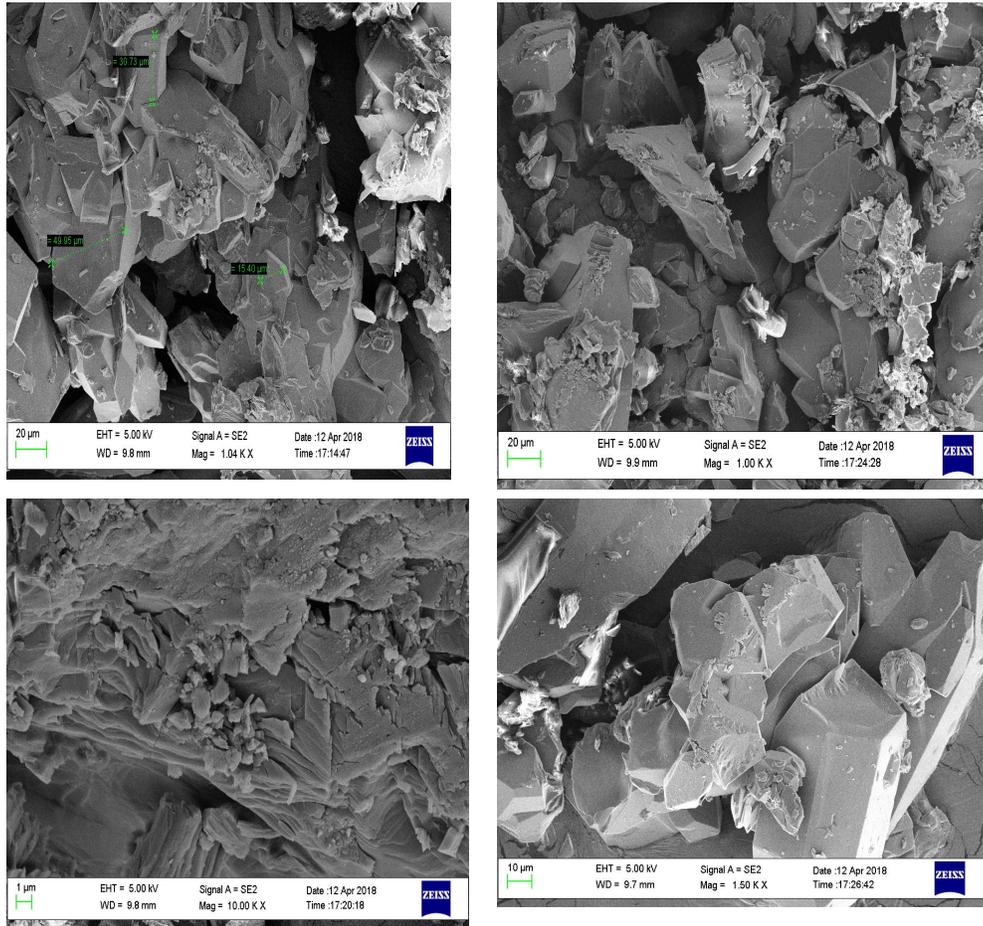
**Figure: 2 and 3, FT-IR spectra of Pure Nifedipine(NIF) and Nifedipine Microcrystal Formulations NIF-1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.**



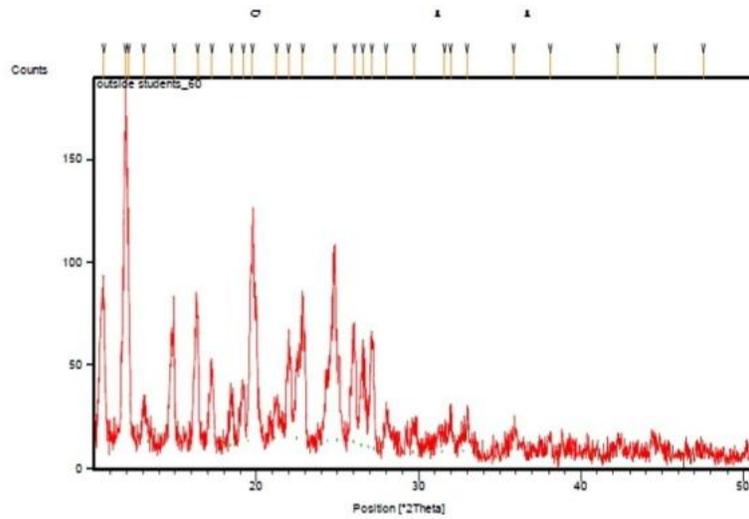
**Figure 4: SEM photograph for Pure Nifedipine**



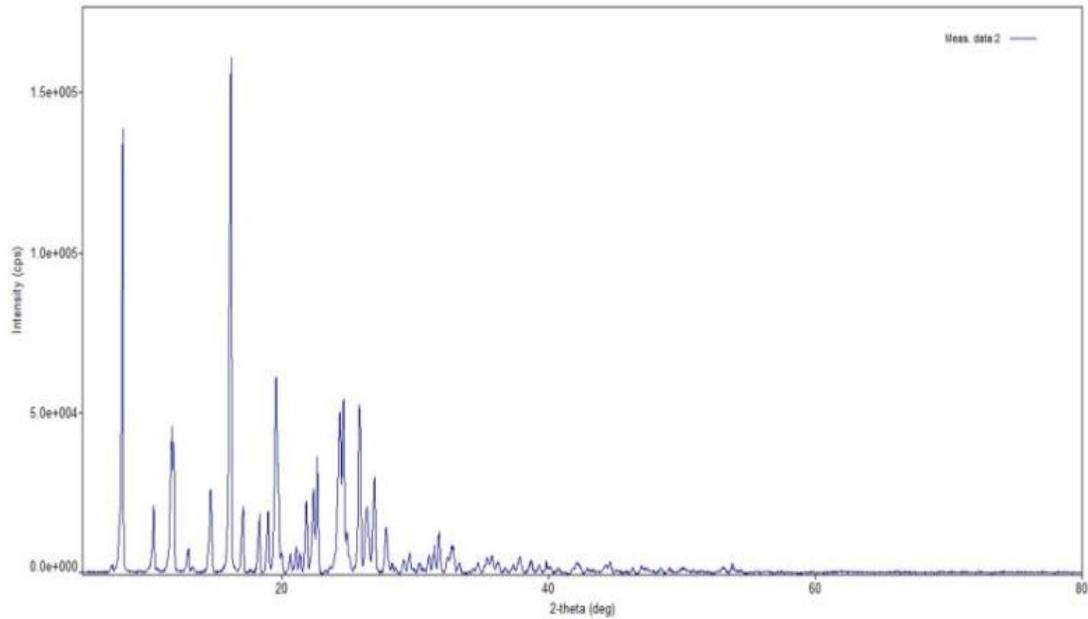
**Figure 5: SEM photograph for NIF-MC-6**



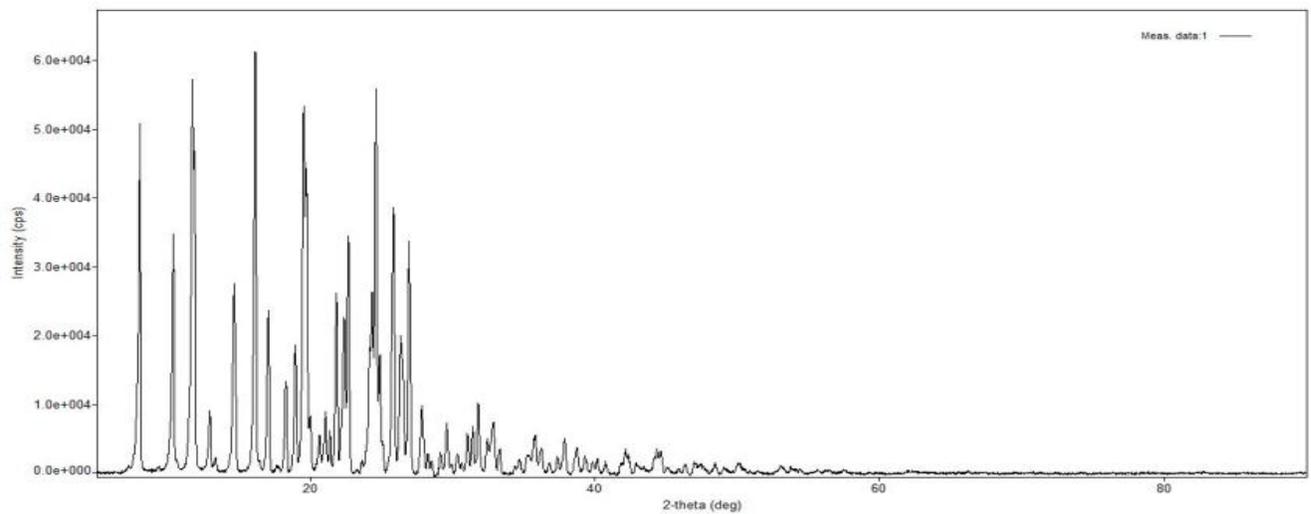
**Figure 6: SEM photograph for NIF-MC-4**



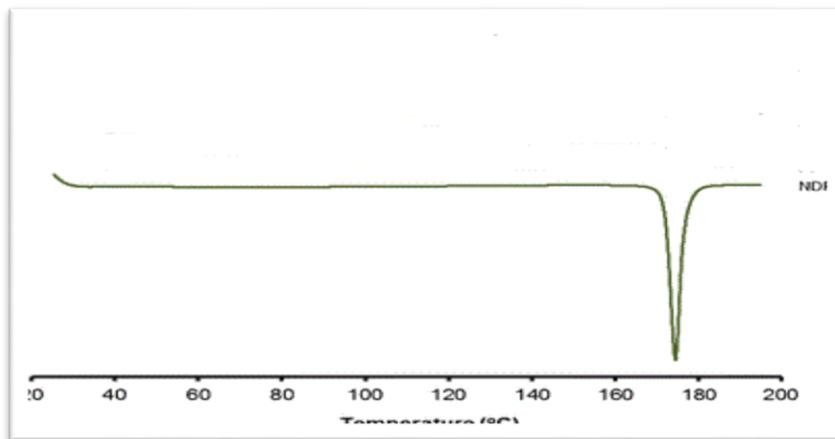
**Figure7: PXRD of pure Nifedipine**



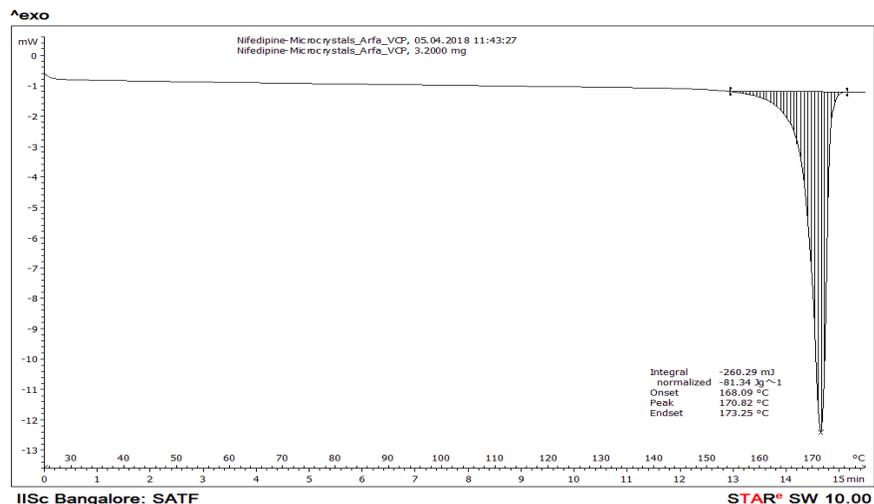
**Figure 8: PXRD of NIF-MC-6**



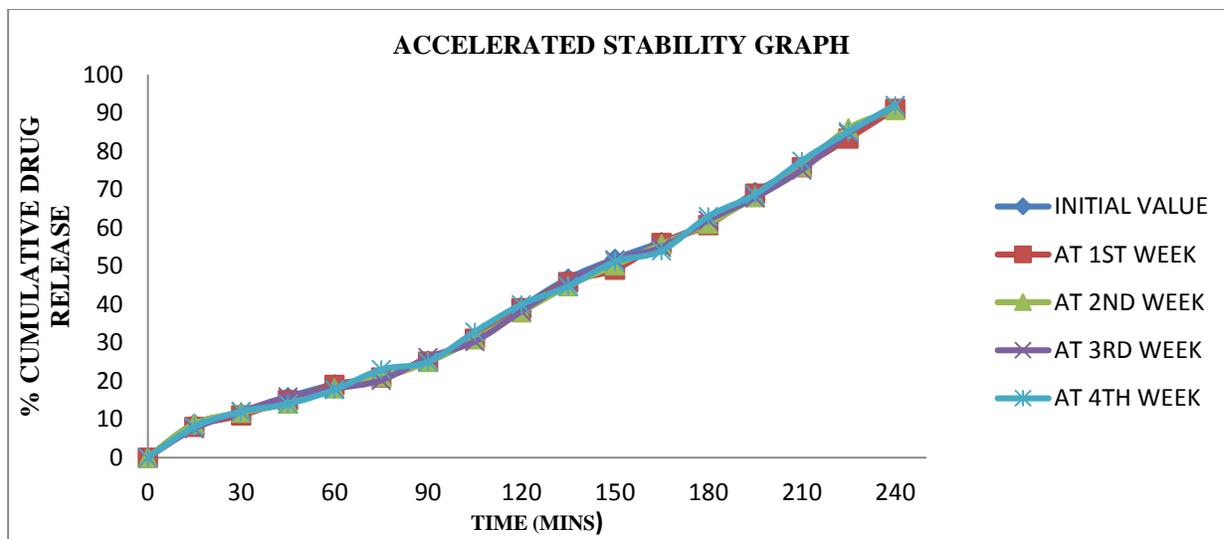
**Figure 9: PXRD of NIF-MC-4**



**Figure 10: DSC of pure Nifedipine**



**Figure11: DSC of NIF-MC-6**



**Figure 12: Graphical representation of Accelerated stability study of optimized formulation NIF-MC-6, for the period of 1 month with weekly cumulative drug release observations.**

## CONCLUSION

In this study the prepared Micro crystals of NIF-MC-6, prepared by Solvent Change Method exhibited good solubility, dissolution characteristic and modified physicochemical properties in comparison to that of the pure drug. Thus the results proved that this method can be used to formulate micro-crystals of poorly soluble CCB Nifedipine for the treatment of hypertension.

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