

## Solubility Enhancement of Nicergoline Poorly Water Soluble Drug by Novel Melt Sonocrystallization Technique

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### Abstract

### Original Research Article

**Objective:** Nicergoline, in an oral drug delivery system suffers from low bioavailability, which mainly results from poor aqueous solubility. The present study shows that porous structures formed on the surface of the Nicergoline using melt sonocrystallization technique enhance the aqueous solubility. **Methods:** Melt sonocrystallization process was developed for Nicergoline in which Nicergoline melt was poured in deionized water and simultaneously subjected to ultrasonic energy for 20 min at amplitude 80%. The obtained MSC form of nicergoline was evaluated for Flow Properties, Particle size analysis, Scanning electron microscopy (SEM), X-ray diffractometry (XRD), Fourier transformed infrared spectroscopy (FTIR), solubility and dissolution rate. **Result:** Micromeritic and rheological properties of MSC form were found to be superior to the original form of Nicergoline. The irregular agglomerates with porous surface were obtained having different crystal habit which increases solubility and dissolution rate. FTIR shows thermal behaviour of pure Nicergoline and MSC Nicergoline has no significant difference. Low intensity peaks in XRD of treated Nicergoline were noticed crystals habit changes and lattice defects during processing have causes favourable changes in the physicochemical properties of Nicergoline. **Conclusion:** The use of melt sonocrystallization technique is promising technique that may afford powder with improved flow as well as improved solubility and dissolution.

**Keywords:** Nicergoline; Melt sonocrystallization technique, Solubility, Dissolution etc.

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## INTRODUCTION

The in vivo performance of drug administered depends on the solubility and characteristics of the tissue permeability. The poor bioavailability and variable solubility profile are due to poor solubility in water. Therapeutic medications are regularly administered systemically when first disseminated in the body once administered. By dispersing in the body, the drug is essentially diluted from the original concentration in the formulation / dosage form. The absorption of the drug and thus its bioavailability is determined by the degree of solubility and permeability of drugs.

In the solid dosage form, the dose to be delivered is not normally a physical problem. However, due to limitations in aqueous solubility and volume, the dose can become a significant formulation challenge for parenteral preparations. The choice of solubilization

method will depend upon how efficiently the drug can be solubilized, stability in the system, and biocompatibility of the vehicle for a given drug delivery route. For solid dosage forms, alteration in the solid phase may enhance dissolution. For parenteral, the four most commonly used techniques for solubilization are pH adjustment, co-solvent addition, micelle inclusion through surfactant addition and complexation which have been frequently practised.

Various powder flow problems have been encountered in industries during handling of powders. For instance, when flowing out of a storage bin or hopper, powders may form a rat-hole, which is a self-supporting vertical channel extending from outlet to the top surface of the powder. Sometimes, arches are formed at hopper outlet, leading to an intermittent flow. Arches can form in bulk solids because of two reasons -

- i. Particle interlocking
- ii. An increase in cohesive strength

The particle interlocking occurs when particles lock together mechanically at the outlet. These particles are irregular shapes have a greater chance of forming arches. Cohesive arches can form where particles bond together physically, chemically or electro-statically.

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- pH adjustment
- Co solvent addition
- Micelle inclusion through surfactant addition
- Complexation

### IMPORTANCE OF FLOW PROPERTIES

According to Prescott and Barnum [1], several processes used to manufacture pharmaceuticals involve powder handling. Quality of the final product depends upon complex powder flow or flow of a blend during manufacturing, compression or granulation. Problems such as arching, rat-hole formation, flooding and segregation may develop in equipment. Thalberg et al., [2] suggested that for successful product development for inhalation, it is important to adjust and control the flow properties of powders during processing and formulation. According to Fitzpatrick et al., [3], powder properties affect powder behaviour during storage, handling and processing and often connected to the flow pattern inside the vessel. Particle segregation and material flow interruptions are major problems during direct-compression tableting operations because of the wide particle size distribution encountered [4].

### SOLUBILITY

The majority of pharmaceutical compounds and the brand new bioactive compounds that consequences from high throughput screening programs showcase very low solubility in water. Such compounds may additionally be afflicted by insufficient dissolution in the course of the gastrointestinal tract and consequently acquire inferior systemic after oral management [5]. Therapeutic pills are frequently given systemically if after they administered they'll distribute in the course of the frame. By distributing in the frame, the drug is largely diluted out from its unique concentration within the system/dosage shape. Hence, the components are truly a drug concentrate.

The choice of solubilization method will depend upon how efficiently the drug can be solubilized, stability in the system, and upon the biocompatibility of the vehicle for a given drug delivery route. For solid dosage forms, it may be possible to alter the solid phase to enhance dissolution. For parenteral, the four most commonly used techniques for solubilization are pH adjustment, co-solvent addition, micelle inclusion through surfactant addition and complexation which have been frequently practised.

The most chemical entities are water-insoluble lipophilic compounds or BCS class II compounds, so it can be quite challenging for formulation scientist to develop usable pharmaceutical products for such compounds. According to the BCS system, drugs are classified according to their permeability and solubility to assess whether or not an IVIVC can be attained Class II drugs are poorly soluble in aqueous media, but are easily transported across the gut mucosa.

The poor solubility and low dissolution rate of poorly water-soluble drugs especially those belonging to class II of BCS can be enhanced by the addition or complexation with a hydrophilic carrier. Improvement of drug bioavailability of poorly-water soluble drugs remains one of the most challenging aspects of drug development. The rate and extent of absorption of class II compounds is highly dependent on the performance of the formulated product.

The solubility behaviour of a drug is a key determinant of its oral bioavailability. Consideration of the modified Noyes-Whitney equation provides some hints as to how the dissolution rate of very poorly soluble compounds is improved to minimize the limitation to oral availability:

$$\frac{dc}{dt} = \frac{AD(C_s - C)}{h}$$

Where  $dc/dt$  is the rate of dissolution, A is the surface area available for dissolution, D is the diffusion coefficient of the compound,  $C_s$  is the solubility of the compound in the dissolution medium, C is the concentration of drug in the medium at time t and h is the thickness of the diffusion boundary layer adjacent to the surface of the dissolving compound.

An increase in the rate of dissolution is desired in many industrial fields like Hydro-metallurgy or pharmaceutical where the people are almost dealing with sparingly soluble solids. Rates of dissolution are increased conventionally by changing physical states by size reduction, phase transformation or amorphization. Changes in chemical states by compounding with foreign substances could also accelerate the dissolution process.

The main possibility for improving dissolution according to the analysis is to increase the surface area available for dissolution by decreasing the particle size of the solid compound and by optimizing the wetting characteristics of the compound surface.

### FACTORS AFFECTING SOLUBILITY

The solubility depends on the physical form of the solid; the nature and composition of solvent medium as well as temperature and pressure of system are as:

#### A. Particle size

The particle size and solubility are inversely co-related but the surface area to volume ratio is

directly proportional to the solubility. The solubility is completely depends on particle texture like particle becomes minute, the surface area to volume ratio goes up. The larger surface area allows a greater interaction with the solvent.

### B. Temperature

Energy absorption is directly proportional to the solubility:

Energy absorbed in the process – increases temperature – solubility increases,

Energy released in the process – decreases temperature – solubility decreases.

- Generally, the temperature increment increases the solubility of a solid solute.

### C. Pressure

For solutes in the gaseous state, pressure is again directly proportional to the solubility:

- increased pressure increases the solubility,
- decreased pressure decreases the solubility.

For gaseous solutes, an increase in pressure increases solubility and a decrease in pressure decrease the solubility. For solids and liquid solutes, changes in pressure have practically no effect on solubility.

### D. Molecular size

Molecular size will affect the solubility. The larger the molecule or the higher its molecular weight the less soluble the substance. Larger molecules are more difficult to surround with solvent molecules in order to solvate the substance.

### E. Polarity

Generally non-polar solute molecules will dissolve in non-polar solvents and polar solute molecules will dissolve in polar solvents. The polar solute molecules have a positive and a negative end to the molecule. If the solvent molecule is also polar, then positive ends of solvent molecules will attract negative ends of solute molecules. This is a type of intermolecular force known as dipole-dipole interaction.

### F. Rate of solution

The rate of solution is a measure of how fast substances dissolve in solvents.

### Melt Sonocrystallization (MSC) Technique

Melt sonocrystallization is a novel particle engineering techniques, developed to modify the physicochemical, micromeritic and biopharmaceutical properties of the drug. Ultrasound (US) was introduced in the traditional process of pharmaceutical technology of few years ago. For instance, several workers reported US assisted compaction and US spray congealing of variety of systems where physical modification of structure of drug or excipients was done to improve drug release and compaction properties of drug. Besides these effects on solid, US may also act on a liquid or

melt mixtures causing cavitation and extreme molecular motion, which divides the drop of material into number of microdrops of narrow size range [6]. One of the mechanical effects cause by ultrasonication is disaggregation or de-agglomeration of the particle assembling.

Cavitation is an important phenomenon of ultrasonication. The energy produced due to collapsing of bubbles at very high temperature was responsible for breaking of particles. The so generated shock waves can cause the particle collide into one another with great force since these are similar charge particles. Problem of agglomeration is greatly reduced.

There are reports on application of ultrasonic (US) energy during crystallization, i.e. sonocrystallization. US energy has been used to achieve nucleation at moderate super saturation during the crystallization process or terminal treatment to achieve deagglomeration and to obtain desired crystal habit. Fini *et al.*, [7] had studied US assisted compaction of various drugs with excipients such as Di-sodium hydrogen phosphate, concentrated HCl and methanol. While low melting paraffin wax is also used. Significant changes in the crystal properties were observed due to US treatment.

### Advantages of melt sonocrystallization

- Faster nucleation which is fairly uniform throughout the sonicated volume.
- Relatively easy nucleation of materials for which nucleation is difficult otherwise.
- Generation of smaller, purer and more uniform crystals with smooth surfaces.
- Mechanical size reduction methods have some significant drawbacks. Thus, the use of power ultrasound in crystallization is a method of generating particles of controlled size.
- Great importance during manufacturing and is a key factor in product performance.

## MATERIALS AND METHODS

**Materials:** Nicergoline (Inga Laboratories P. Ltd., Mumbai, India); Methanol, Liq. Paraffin, Conc. Sulfuric Acid, Potassium Bromide (S.D. Fine Chemicals Ltd., Mumbai, India) were purchase from the sources indicated.

## METHODS

### DRUG IDENTIFICATION STUDIES

#### PHYSICAL APPEARANCE

The drug sample was received from Inga Pharmaceuticals Pvt. Ltd, Mumbai. The physical appearance of the drug was determined by visual inspection.

#### MELTING POINT DETERMINATION

The melting point of nicergoline was determined by capillary rise method. A capillary tube

was taken and blocked from one side and the drug was filled in it by tapping method up to 1/4<sup>th</sup> of its length. Then the tube was placed in the apparatus and noted the last temperature at which drug got melted. This was performed thrice and an average was taken.

#### **SULPHURIC ACID TEST**

Dissolve 2 mg of drug in 2 ml of sulphuric acid, blue colour developed. Blue colour indicates that the drug is in pure form.

#### **FOURIER TRANSFORMED INFRARED SPECTROSCOPY (FT-IR)**

The infrared spectroscopy of the drug was carried out for confirmation of the identity of the drug (Japanese Pharmacopoeia, 17<sup>th</sup> edition). A small amount of drug is mounted in Infrared compartment and scanned between 4000-450 cm<sup>-1</sup> wave numbers using a Bruker alpha 2.

#### **UV SPECTROSCOPIC ANALYSIS**

Accurately weight 50mg of drug and dissolve in alcohol and dilute to 100ml with same solvent known as "stock solution". Dilute 5ml of the stock solution to 50ml with alcohol. Examine between 220nm and 350nm, the solution shows an absorption maximum at 288nm.

#### **PREPARATION OF CALIBRATION CURVE OF NICERGOLINE**

#### **PREPARATION OF CALIBRATION CURVE IN DISTILLED WATER**

Accurately weighed 10 mg of the drug (NICERGOLINE) was transferred into the 50 ml of volumetric flask. The drug was dissolved in 10ml of methanol as co-solvent and volume make up to 50 ml with gradual addition of the distilled water by 10 ml, it is referred as "stock solution" to make a solution of 200µg/ml. From the stock solution, aliquots were made of following concentration 0-20 µg/ml and transferred into a series of 10 ml volumetric flasks and make up the volume up to mark with distilled water. The absorbance of standard solutions was measured at 287.6 nm. Mean absorbance of three reading were taken to check the reproducibility. The observed absorbance was then subjected to regression analysis for study of linearity and optical characteristics.

#### **PREPARATION OF CALIBRATION CURVE IN PHOSPHATE BUFFER (pH – 6.8)**

Accurately weighed 10 mg of the drug (NICERGOLINE) was transferred into the 50 ml of volumetric flask. The drug was dissolved in 10ml of methanol as co-solvent and volume make up to 50 ml with gradual addition of the phosphate buffer pH 6.8 by 10ml, it is referred as "stock solution" to make a solution of 200µg/ml. From the stock solution, aliquots were made of following concentration 0-20 µg/ml and transferred into a series of 10 ml volumetric flasks and make up the volume up to mark with distilled water.

The absorbance of standard solutions was measured at 287.6nm. Mean absorbance of three reading were taken to check the reproducibility. The observed absorbance was then subjected to regression analysis for study of linearity and optical characteristics.

#### **PREPARATION AND CHARACTERIZATION OF MELT SONOCRYSTALLIZED FORM OF NICERGOLINE (MSC NIC)**

The original form of nicergoline 10 g was melted in a test tube on a paraffin oil bath maintained at 200°C. The molten mass was poured into a beaker containing 100 ml of double distilled water maintained at 75 – 80°C and sonicated for 4 min using an ultrasonic bath at a frequency of 33 ± 3 kHz at 80 % amplitude with the power rating of 60 watts. The product was separated by filtration using Whatman filter paper #1. The product was dried at room temperature overnight and kept in a desiccator to get melt sonocrystallized form of nicergoline (MSC NIC).

#### **CHARACTERIZATION OF MSC NIC FORM OF DRUG**

##### **PERCENTAGE YIELD**

The product obtained was weighed and percentage (%) yield was calculated using formula for the MSC NIC form.

$$\% \text{ Yield} = (\text{Practical Yield/Theoretical Yield}) \times 100$$

##### **PHYSICAL APPEARANCE**

The physical appearance of prepared MSC NIC form of the Nicergoline was also determined by visual inspection and compared with the pure drug.

##### **MELTING POINT**

The melting point of MSC NIC was determined by capillary rise method. A capillary tube was taken and blocked from one side and the drug was filled in it by tapping method up to 1/4<sup>th</sup> of its length. Then the tube was placed in the apparatus and noted the last temperature at which drug got melted. This was performed thrice and an average was taken.

#### **SULPHURIC ACID TEST**

Dissolve 2 mg of drug in 2 ml of sulphuric acid, blue colour developed. Blue colour indicates that the drug is in pure form.

#### **FOURIER TRANSFORMED INFRARED SPECTROSCOPY (FT-IR)**

The infrared spectroscopy of the prepared MSC form was carried out for confirmation of the identification (Japanese Pharmacopoeia 17<sup>th</sup> edition). A small amount of drug is mounted in Infrared compartment and scanned between 4000-450 cm<sup>-1</sup> wave number sat 4 cm<sup>-1</sup> resolution using a Bruker alpha 2.

#### **UV SPECTROSCOPIC ANALYSIS**

Accurately weight 50mg of drug and dissolve in alcohol and dilute to 100ml with same solvent known as "stock solution". Dilute 5ml of the stock solution to 50ml with alcohol. Examine between 220nm and 350nm, the solution shows an absorption maximum at 288nm.

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#### PREPARATION OF CALIBRATION CURVE IN PHOSPHATE BUFFER (pH – 6.8)

Accurately weight 10 mg of the MSC form (NICERGOLINE) was transferred into the 50 ml of volumetric flask. The drug was dissolved in 10ml of methanol as co-solvent and volume make up to 50 ml with gradual addition of the phosphate buffer (pH 6.8) by 10ml, it is referred as "stock solution" to make a solution of 200µg/ml. From the stock solution, aliquots were made of following concentration 0-20 µg/ml and transferred into a series of 10 ml volumetric flasks and make up the volume up to mark with distilled water. The absorbance of standard solutions was measured at 287.8nm. Mean absorbance of three reading were taken to check the reproducibility. The observed absorbance was then subjected to regression analysis for study of linearity and optical characteristics.

#### MICROMERITIC STUDIES

##### PARTICLE SIZE AND ITS DISTRIBUTION

The particle size of NIC and MSC NIC were determined by Dynamic laser scattering by using (Mastersizer) particle size analyzer equipped with an argon laser. Suspension of nicergoline was prepared in distilled water. The suspension was added to the sample cell attached inside the coulter counter then started the analysis. The addition of sample drop wise in the sample cell was continued up to the obscuration rate of 10%. Particle size analysis was based on the refractive index (RI) of both the material. The size distribution was expressed as volume median diameter (VMD) and span. The data was obtained for quantification of powder in terms of skewness, kurtosis, interquartile

coefficient of skewness (IQCS), specific surface area, span and variance by using the following equation:

$$IQCS = [(c-a) - (a-b)] / [(c-a) + (a-b)]$$

Where,

a is the median diameter,

b and c are the lower and upper quartile points.

$$Span = D(90\%) - D(10\%) / D(50\%)$$

Where,

D (90%) is the median diameter at 90 % cumulative size,

D (50%) and D (10%) cumulative size [8].

#### RHEOLOGICAL PROPERTIES

NIC and MSC NIC powders were characterized for bulk density, tapped density, Carr's compressibility index, angle of repose and Hausner's ratio. Dynamic angle of repose of NIC and MSC NIC was determined by preparing a laboratory step up where we kept the height as constant and poring 1 g of drug powder in funnel and allow the drug to flow from it and form a hip. The angle made by the hip was recorded and dynamic angle of repose each sample was calculated.

The bulk density was obtained by dividing the weight of sample by the final volume in cm<sup>3</sup> of the sample contained in the cylinder [9]. Hausner's ratio was determined by dividing the tapped density by bulk density. The percent compressibility index was calculated by using the equation:

$$\% \text{ Compressibility Index} = (\text{Tapped density} - \text{poured density}) / \text{Tapped density} \times 100$$

#### EQUILIBRIUM SOLUBILITY DETERMINATION

The equilibrium study was determined by shake flask method. The excess amount of the drug was taken and placed in the 25 ml of conical flask. The solubility was determined in double distilled water. The mixture was shaken for 72 h in a thermostatic water bath shaker at temperature of 37 ± 2°C. The sample was filtered with the help of Whatman filter paper no. 1. This filtrate was centrifuged for 15 min at 3000 rpm (503 x g). The amount of drug dissolved was then analyzed spectrophotometrically.

#### DETERMINATION OF INTRINSIC DISSOLUTION RATE (IDR)

Intrinsic dissolution rate was determined by using rotating disc method [10]. Disc of NIC and MSC NIC was prepared by compressing 30 mg of each with a compression force of 9-ton using 13 mm flat faced IR disc punch and compaction pressure of 600 mm Hg with dwelling time of 5 min. The top and sides of compressed disc was coated using low melting point paraffin wax (58 – 60°C) and fixed to the holder of the rotating basket leaving one face to exposed (surface area = 1.131 cm<sup>2</sup>) that was cleared of residual wax. The dissolution of compressed disc of NIC and MSC NIC was conducted in double distilled water and stirred at

100 rpm and maintained at  $37 \pm 0.5^\circ\text{C}$ . 5 ml of aliquots were withdrawn from the media containing double distilled water and replaced with the fresh media. The aliquots were filtered by using Whatman filter paper # 1, diluted and analyzed spectrophotometrically. The linear portion of each dissolution profile was used to derive the intrinsic dissolution rate.

$$\text{IDR} = \frac{a}{t} \times \frac{1}{A}$$

Where,

a is amount of dissolved drug in media (mg),  
A is surface area ( $\text{cm}^2$ ),  
t is time (min).

### X-RAY DIFFRACTION (XRD)

Samples of NIC and MSC NIC were subjected to XRD analysis. X-ray diffraction (XRD) patterns were recorded using Advance X-ray diffractometer (Rigaku Ultima IV, Japan). The samples were irradiated with mono chromatized Cu  $K\alpha$  radiation, generated at 1.54239 Å wavelength, at 30 kV and 30 mA. Afterwards, the samples were step scanned between  $10-80^\circ$  at  $2\theta$  scale. The obtained data was used to calculate crystallinity index, using equation.

$$\% \text{ Crystallinity index} = (I_{020} - I_{\text{am}} / I_{020}) \times 100$$

Where,  $I_{020}$  is intensity at  $22.5^\circ$  and  $I_{\text{am}}$  is lowest 2  $\theta$  value near  $18^\circ$ .

### SCANNING ELECTRON MICROSCOPY

The photomicrographs of both NIC and MSC NIC were obtained using scanning electron microscope

(ZEISS EVO 18). Particles were coated with thin gold layer by sputter coater unit under an argon atmosphere in order to make them conductive. The coating time was 5-6 min. Surface morphology of both the powders was studied by observing the obtained photomicrographs at an acceleration voltage of 20 kV.

### DIFFERENTIAL SCANNING CALORIMETRY (DSC)

Thermal behavior of NIC and MSC NIC was estimated using a Differential scanning calorimeter (Beckman Coulter LS 13 320) equipped with an intercooler in order to assess the change in chemical properties of powders. Indium was used as a standard to calibrate the differential scanning calorimetry (DSC) temperature and enthalpy scale. The samples were hermetically sealed in aluminum pans and heated at a constant rate of  $10^\circ \text{C}/\text{min}$ , over a temperature range of  $0-350^\circ\text{C}$ . An inert atmosphere was maintained by purging with nitrogen at a flow rate of 100 ml/min.

## RESULT AND DISCUSSION

### DRUG IDENTIFICATION TEST

#### PHYSICAL APPEARANCE

Nicergoline (provided by Inga Laboratories, Mumbai) on visual inspection appeared as white to light yellow color crystal or crystalline powder.

#### DETERMINATION OF MELTING POINT

For the determination of melting point, capillary rise method was suggested in the Pharmacopoeias as the standard technique.

#### Melting point determination of Nicergoline

Drug	Experimental Valur ( $^\circ\text{C}$ )	Literature value ( $^\circ\text{C}$ )
NIC	$135.8 \pm 0.107$	136

From the result, the melting point confirmed that the drug was nicergoline.

#### SULPHURIC ACID TEST

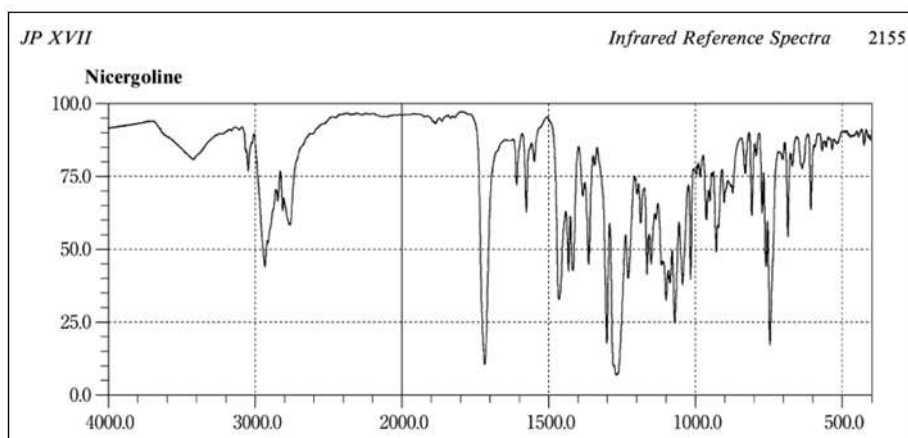
Dissolve 2 mg of drug in 2 ml of sulphuric acid, a blue color develops it indicates that the sample of drug is in the pure form.

### FOURIER TRANSFORMED INFRARED SPECTROSCOPY (FT-IR)

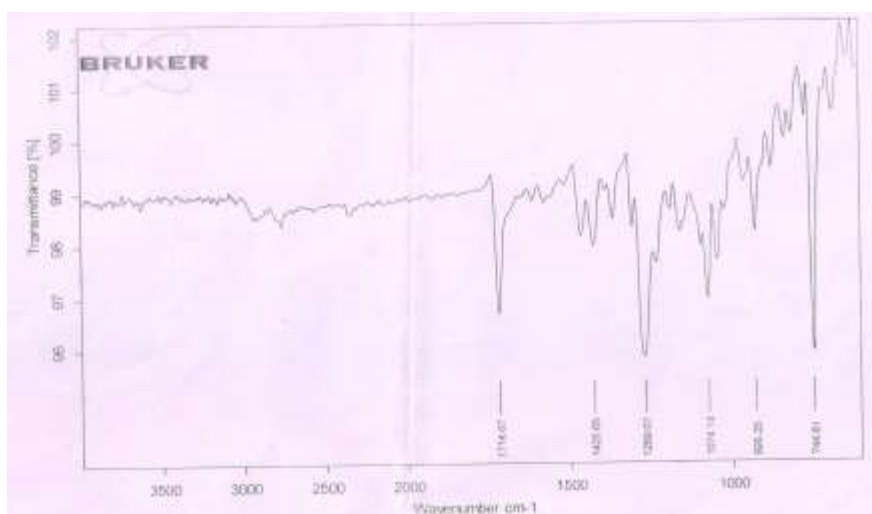
FT-IR spectra of the drug was scanned at  $4000-450 \text{ cm}^{-1}$ . The interpretation of FT-IR spectra of the nicergoline was given in Table and FT-IR spectra were shown in Figure (a and b)

#### Interpretation of FT-IR spectra of nicergoline

Reported Peaks ( $\text{cm}^{-1}$ )	Observed Peak ( $\text{cm}^{-1}$ )	Inference
3000-3700	3377.12	N-H
1600-2000	1714.67	C=O Stretch
1400-1470	1425.65	C-H
1180-1360	1269.07	C-N
800-1200	1074.14	C-C
735-770	744.61	o-distribution



(a): Reference FT-IR spectrum of Nicergoline



(b): FT-IR Spectrum of Nicergoline (Observed)

The FT-IR spectra showed characteristic N-H, C=O, C-H, C-N, C-C stretching bands at  $3377.12\text{ cm}^{-1}$ ,  $1714.67\text{ cm}^{-1}$ ,  $1425.65\text{ cm}^{-1}$ ,  $1269.07\text{ cm}^{-1}$ ,  $1074.14\text{ cm}^{-1}$  respectively shown in figure (b). The ortho-distribution at  $744.61\text{ cm}^{-1}$  was also observed. FT-IR spectra of the drug were matched with reference spectra. The presence of all characteristic peaks confirmed that the drug was nicergoline.

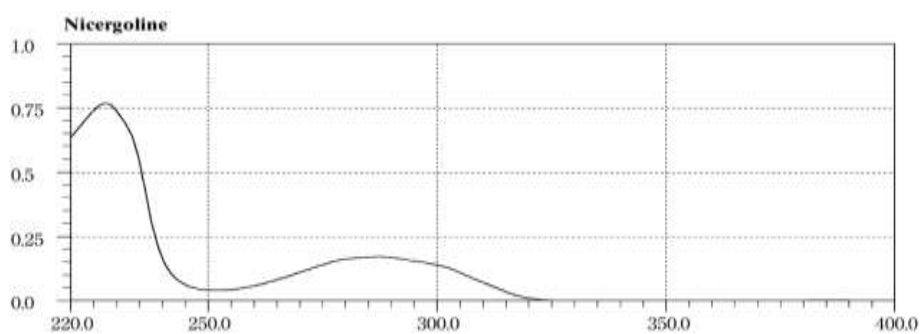
#### UV SPECTROSCOPIC ANALYSIS

Accurately weight 50 mg of drug and dissolve in alcohol and dilute to 100ml with same solvent. 5ml

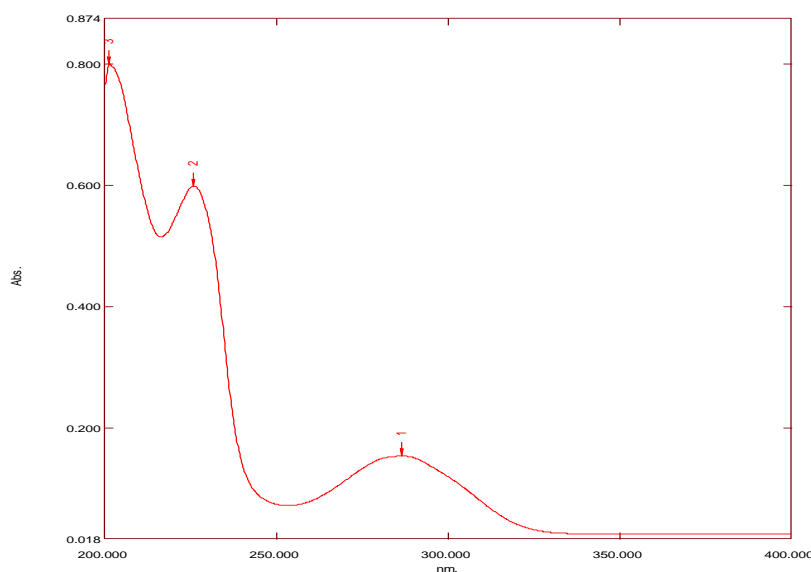
of sample was withdrawn from the stock solution and diluted up to 50ml with the same solvent. Observe the sample between 220nm to 350nm. The solution shows an absorption maximum at 288nm and an absorption minimum at 251nm [11].

#### UV – visible spectroscopic data of nicergoline

S. No.	Media	Experimental $\lambda_{\text{max}}$ (nm)	Literature $\lambda_{\text{max}}$ (nm)
1.	Ethanol	287.6	288



(a): Reference UV spectrum of Nicergoline



(b): UV spectrum of sample Nicergoline

The UV spectra showed characteristic maximum absorption at 288nm and minimum absorption at 251nm respectively shown in figure (b). The presence of both maximum absorption and minimum absorption at same wavelength confirmed that the drug was nicergoline.

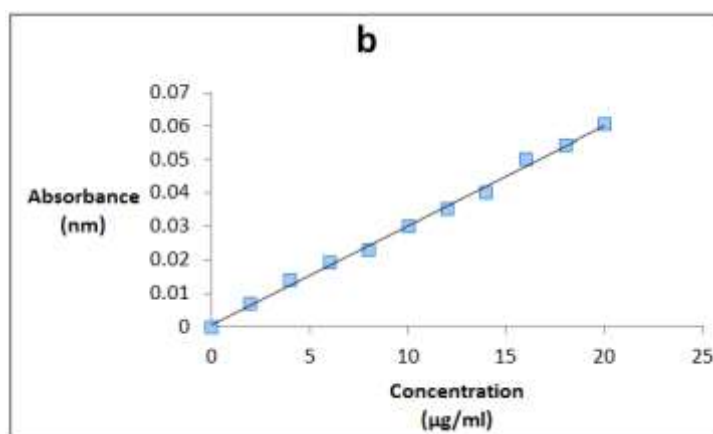
#### PREPARATION OF CALIBRATION CURVE OF NICERGOLINE

#### PREPARATION OF CALIBRATION CURVE IN DISTILLED WATER

The calibration curve of nicergoline was prepared in the concentration range 0– 20 $\mu$ g/ml in distilled water. The absorbance value of the respective concentration was present in the Table. The data was plotted without deviation and the calibration curve obtained following Beer's – Lambert's law with  $r^2$  value 0.993 and the regression equation was found that  $y = 0.01581x - 0.01182$ .

Calibration curve of nicergoline in distilled water

S. No.	Concentration ( $\mu$ g/ml)	Absorbance (nm)	Regression Analysis
1	0	0	<b>Equation of line:</b> $y = 0.01581x - 0.01182$ <b>Correlation coefficient:</b> $R^2 = 0.993$ <b>Slope (m):</b> 0.01581 <b>Intercept:</b> 0.01182
2	2	0.029	
3	4	0.053	
4	6	0.076	
5	8	0.107	
6	10	0.139	
7	12	0.173	
8	14	0.21	
9	16	0.244	
10	18	0.279	
11	20	0.306	



Calibration curve of nicergoline in distilled water



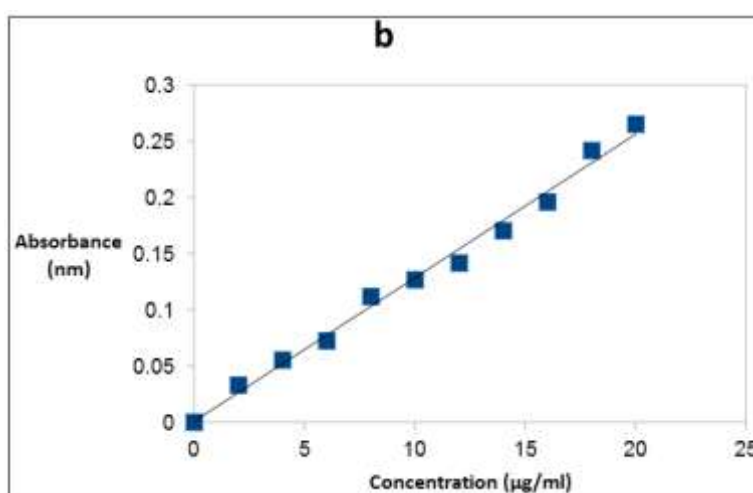
### PREPARATION OF CALIBRATION CURVE IN PHOSPHATE BUFFER (pH – 6.8)

The calibration curve of nicergoline was prepared in the concentration range 0– 20 µg/ml in phosphate buffer pH 6.8. The absorbance values of the

respective concentration was present in the Table. The data was plotted without deviation and the calibration curve obtained following Beer's – Lambert's law with  $R^2$  value 0.99 and the regression equation was found that  $y = 0.0127x$ .

**Calibration curve of Nicergoline in Phosphate buffer (pH 6.8)**

S. No.	Concentration (µg/ml)	Absorbance	Regression Analysis
1	0	0	<b>Equation of line:</b> $y = 0.0127x + 0.0012$ <b>Correlation coefficient:</b> $R^2 = 0.99$ <b>Slope (m):</b> 0.0127 <b>Intercept:</b> 0.0012
2	2	0.033	
3	4	0.056	
4	6	0.073	
5	8	0.112	
6	10	0.127	
7	12	0.142	
8	14	0.171	
9	16	0.196	
10	18	0.242	
11	20	0.265	



**Calibration curve of nicergoline in phosphate buffer pH 6.8**

### MELT SONOCRYSTALLIZED NICERGOLINE (MSC NIC)

The MSC form of the nicergoline was successfully prepared with the procedure and used for the below studies:

#### CHARACTERIZATION OF MSC NIC FORM PERCENTAGE YIELD

The process yield of various batches of MSC NIC was in the range of 84 –95 % w/w. Loss of drug in aqueous phase was found to be less than 1 % w/w for sonicated drug. Also it showed increase in loss of drug with the increase in the sonication time and amplitude. It might be due to the micronization of crystals which facilitates the solubilization of the drug in aqueous

phase. As the sonication time and amplitude increase number of micronized particle also increases so that the yield of the drug gets reduced.

#### PHYSICAL APPEARANCE

By visual inspection it was observed that MSC NIC appears as white to light yellow color crystal or crystalline powder as per (Japanese pharmacopoeia, 17<sup>th</sup> edition). It showed that the physical properties were not changed after the application of the sonication energy.

#### MELTING POINT

Melting point of MSC NIC was determined by capillary rise method and found to be 123.9° C.

**Table: Melting point determination of MSC NIC**

Drug	Experimental value (°C)	Literature value (°C)
MSC NIC	123.9 ± 0.374	---

**SULPHURIC ACID TEST**

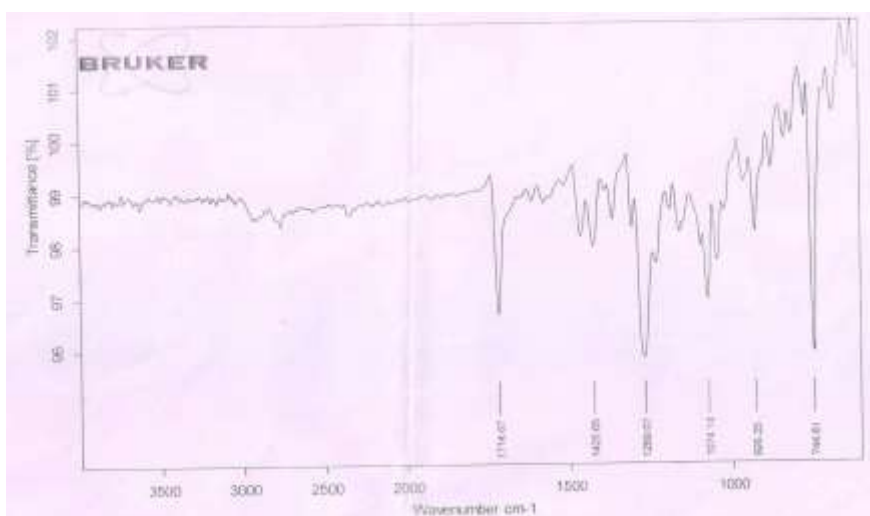
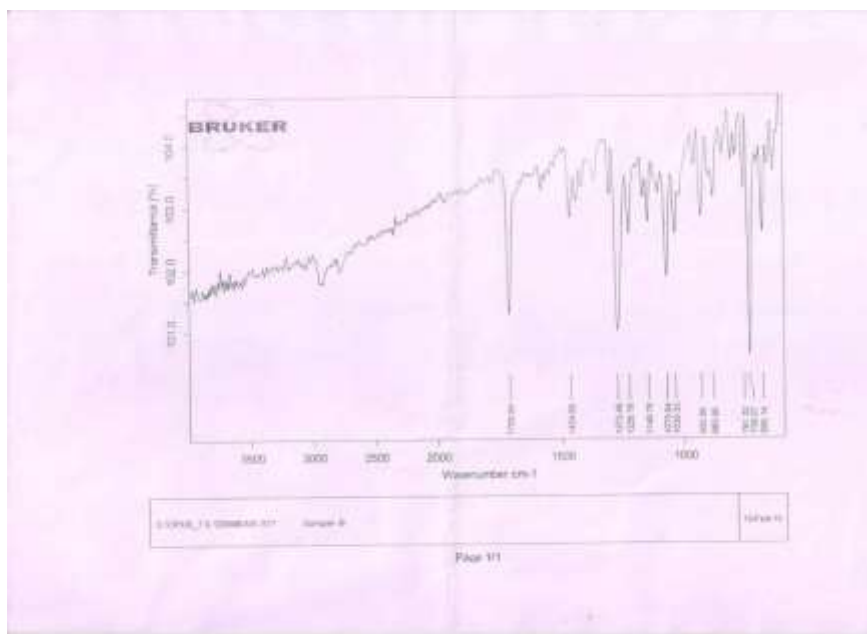
On dissolving 2 mg of MSC form of drug in 2 ml of sulphuric acid, a blue color develops it indicates that the MSC form of drug was confirmed as same as the pure form (European Pharmacopoeia, 2008) [11].

**FT-IR OF MSC NIC**

FT-IR spectra of the MSC form was scanned at 4000-450  $\text{cm}^{-1}$ . The interpretation of FT-IR spectra of the nicergoline was given in Table 6.7 and FT-IR spectra are shown in Figure (a) and (b).

**Table: Interpretation of FT-IR spectra of MSC NIC**

Band frequencies of drug ( $\text{cm}^{-1}$ )	Band frequency of MSC form ( $\text{cm}^{-1}$ )	Inference
3377.12	3379.18	N-H
1714.67	1709.94	C=O Stretch
1425.65	1464.66	C-H
1269.07	1273.48	C-N
1074.14	1073.94	C-C
744.61	738.07	o-distribution

**Figure (a): FT-IR Spectrum of standard Nicergoline (Observed)****Figure (b): FT-IR Spectrum of MSC Nicergoline (Observed)**

The FT-IR spectra showed characteristic N-H, C=O, C-H, C-N, C-C stretching bands at 3379.18  $\text{cm}^{-1}$ , 1709.94  $\text{cm}^{-1}$ , 1464.66  $\text{cm}^{-1}$ , 1273.48  $\text{cm}^{-1}$ , 1073.94  $\text{cm}^{-1}$  respectively shown in figure (b). The ortho-

distribution at 744.61  $\text{cm}^{-1}$  was also observed. FT-IR spectra of the drug and MSC form were matched. The presence of all characteristic peaks confirmed that their

was no change in the chemical structure MSC form drug (nicergoline).

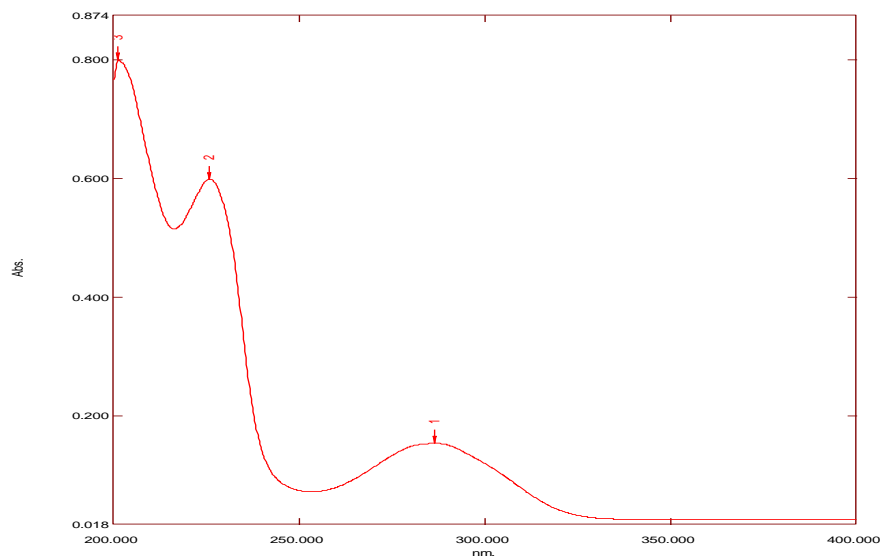
### UV-VISIBLE SPECTROSCOPY (UV) OF MSC NIC FORM

Accurately weight 50mg of drug and dissolve in alcohol and dilute to 100ml with same solvent. 5ml

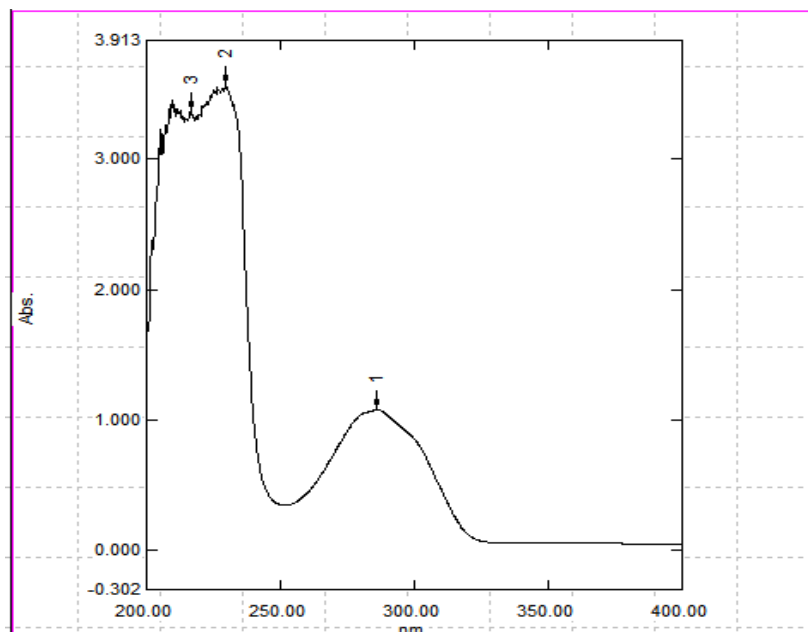
of sample was withdrawn from the stock solution and diluted up to 50ml with the same solvent. Observe the sample between 220nm to 350nm. The solution shows an absorption maximum at 288nm and an absorption minimum at 251nm [11].

**Table: UV – visible spectrometric data of MSC nicergoline**

S. No.	Media	Experimental $\lambda_{max}$ (nm)	Literature $\lambda_{max}$ (nm)
1.	Ethanol	287.8	_____



**Figure (a): UV spectrum of standard Nicergoline**



**Figure (b): UV spectrum of MSC Nicergoline**

The UV spectra showed characteristic maximum absorption at 288nm and minimum absorption at 251nm respectively shown in figure (b) by MSC NIC similar to standard NIC. The presence of

both maximum absorption and minimum absorption at same wavelength confirmed that the drug was nicergoline.

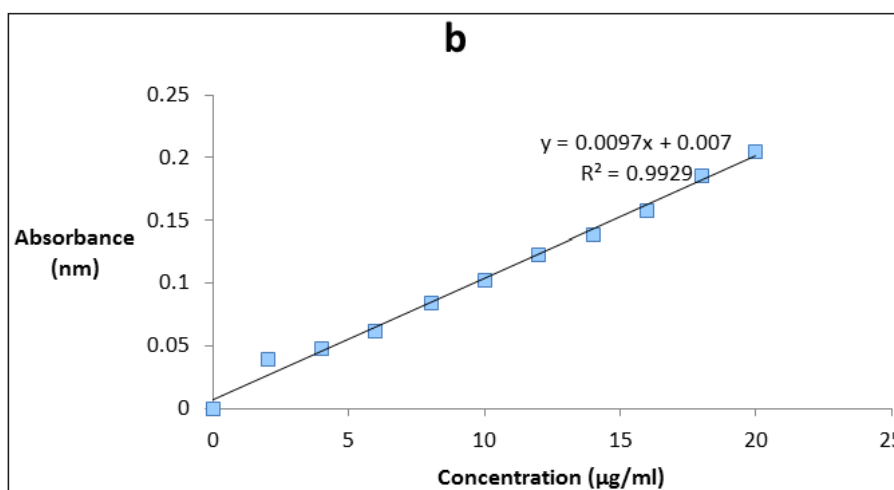
**PREPARATION OF CALIBRATION CURVE OF MSC FORM OF NICERGOLINE  
PREPARATION OF CALIBRATION CURVE IN  
DISTILLED WATER**

The calibration curve of MSC nicergoline was prepared in the concentration range 0– 20µg/ml in

distilled water. The absorbance values of the respective concentration were present in the Table. The data was plotted without deviation and the calibration curve obtained following Beer's – Lambert's law with  $r^2$  value 0.992 and the regression equation was found that  $y = 0.003x - 0.002$ .

**Table: Calibration curve of MSC nicergoline in distilled water**

S. No.	Concentration (µg/ml)	Absorbance (nm)	Regression Analysis
1	0	0	<b>Equation of line:</b> $y = 0.003x - 0.002$ <b>Correlation coefficient:</b> $R^2 = 0.992$ <b>Slope (m):</b> 0.003 <b>Intercept:</b> 0.002
2	2	0.006	
3	4	0.011	
4	6	0.014	
5	8	0.021	
6	10	0.029	
7	12	0.034	
8	14	0.041	
9	16	0.047	
10	18	0.057	
11	20	0.063	



**Figure b: Calibration curve of MSC nicergoline in distilled water**

**PREPARATION OF CALIBRATION CURVE IN  
PHOSPHATE BUFFER (pH – 6.8)**

The calibration curve of nicergoline was prepared in the concentration range 0– 20µg/ml in phosphate buffer pH 6.8. The absorbance values of the

respective concentration was present in the Table. The data was plotted without deviation and the calibration curve obtained following Beer's – Lambert's law with  $r^2$  value 0.99 and the regression equation was found that  $y = 0.01x + 0.01$ .

**Table: Calibration curve of MSC form in phosphate buffer pH 6.8**

S. No.	Concentration (µg/ml)	Absorbance (nm)	Regression Analysis
1	0	0	<b>Equation of line:</b> $y = 0.01x + 0.01$ <b>Correlation coefficient:</b> $R^2 = 0.99$ <b>Slope (m):</b> 0.01 <b>Intercept:</b> 0.01
2	2	0.030	
3	4	0.048	
4	6	0.062	
5	8	0.084	
6	10	0.102	
7	12	0.122	
8	14	0.139	
9	16	0.158	
10	18	0.186	
11	20	0.205	

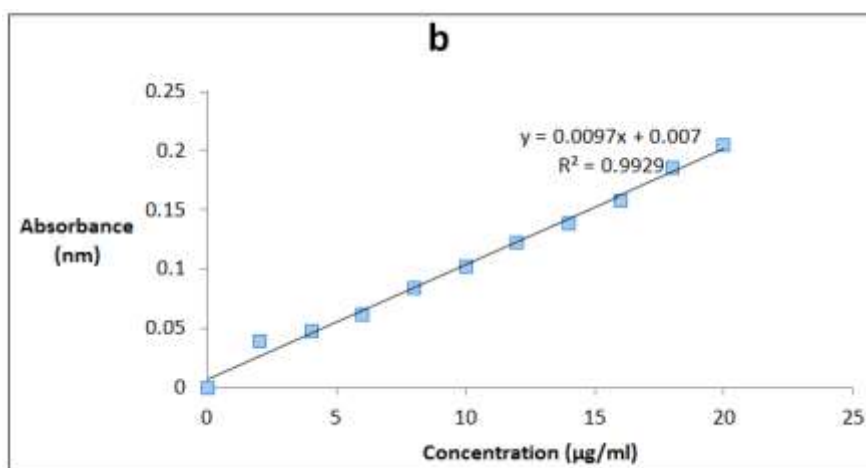


Figure: Calibration curve of nicergoline in phosphate buffer pH 6.8

## MICROMERITIC STUDIES

### PARTICLE SIZE AND ITS DISTRIBUTION

Mean particle size of the MSC form was reduced to 2.7621µm (MSC NIC) from 4.7779 µm (NIC) as shown in Table. The quantum reduction of mean particle size was 42.26 %. Reduction in particles is due to the fact that application of ultrasonic energy to the melted form results in enhanced kinetic energy of molecules and further collision causes drops to get reduced to micro-drops with narrow size range of particles [12].

Skewness and Kurtosis are the statistical parameters in particle size distribution and used for the analyses of micronized and controlled atomization as well as also for melt sonocrystallization [13]. Skewness of MSC NIC was reduced from 0.922 to 1.320 as compared to NIC (Figure a & b) indicating that the particles of MSC NIC tend to be more symmetrically distributed with respect to NIC.

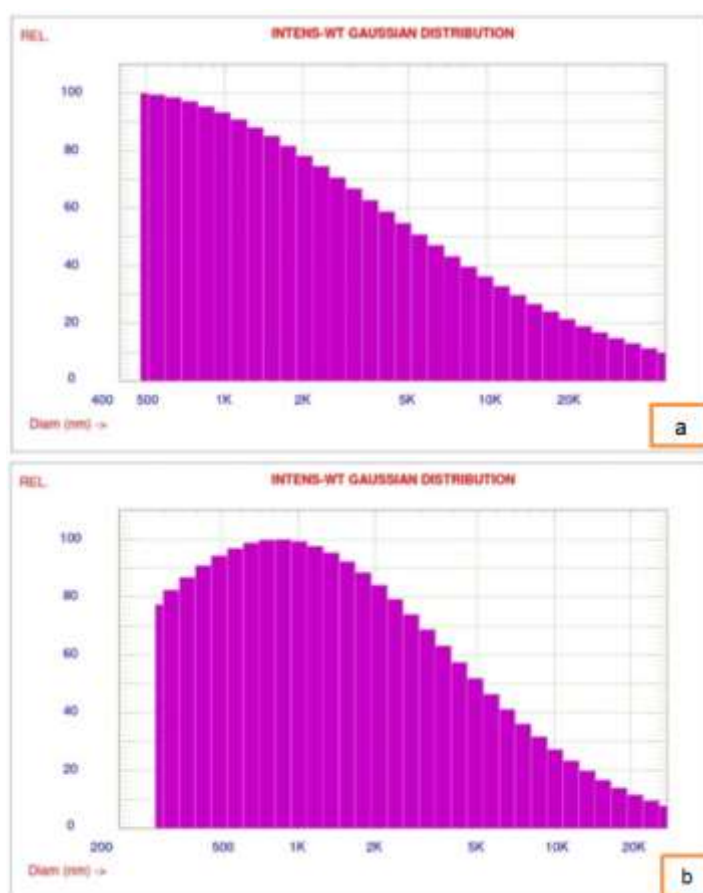


Figure a & b: Frequency distribution curves of a) NIC and b) MSC NIC

Similarly, the value of kurtosis in MSC NIC was found to increase from 0.290 to 2.154. As compared to NIC form kurtosis specifies the shape of distribution and normal distribution of particles. Thus

positive value of kurtosis in both the cases depicted peakness of the distribution. Modification in degree of peakness in case of MSC NIC form was due to sonocrystallization.

**Table: Comparative micromeritic data of MSC NIC with reference to NIC**

Parameter	NIC	MSC NIC
Mean diameter (nm)	4777.9	2762.1
Standard deviation	10478 nm	4272.9 nm
Specific Surface area (cm <sup>2</sup> /ml)	6937	10730
Skewness	0.922	1.320
Kurtosis	0.290	2.154
IQCS	-0.482	0.417
Types of skewness of curve	Positive	Positive
Types of Kurtosis	Leptokurtic	Leptokurtic
Span	3.97	3.5
Variance	4.809	2.393
Median	6100	4900
Mode	497	248
D <sub>50</sub> (nm)	4777.9	2762.1

A high span value indicates a wide size distribution with large particles and a high polydispersity index [14]. Conventional particle size reduction methodology may result in large span value but the melt sonocrystallization technique provides an opportunity for producing powder of low span value that indicates narrow particle size distribution and uniformity [15]. The values of variance in case of original and MSC form were observed to be 4.809 nm<sup>2</sup> and 2.393 nm<sup>2</sup> respectively. This indicates that the MSC NIC has lower variance value specifying least variability or lies near to the mean as compared to NIC.

#### RHEOLOGICAL STUDY

Improvement in flowability and packing properties of powder particles is essential and can be assessed by angle of repose [16]. Angle of repose in

case of NIC was found to be  $49 \pm 1.24^\circ$  which specifies that it possessed very poor flow property. In MSC NIC, it was found to be  $33 \pm 1.72^\circ$ . Thus, indicating that application of MSC technique resulted in improvement of flow properties [9].

Bulk density and tapped density of MSC NIC form were higher than the original form of NIC. Carr's compressibility index and Hausner's ratio of melt sonocrystallized form were lower than their original form. The important factors that affect bulk density of powder and its flow properties are inter-particulate interaction including friction and adhesion. Melt sonocrystallized form of drug showed reduction in inter-particulate interaction due to decrease in surface roughness, thus increase in flow properties [9].

**Table: Comparative flow property data of NIC and MSC NIC**

Parameter	NIC	MSC NIC
Dynamic angle of repose (°)	$49 \pm 1.24$	$33 \pm 1.72$
Density (g/cc)	Bulk density	$0.715 \pm 1.48$
	Tapped density	$0.5 \pm 1.26$
Carr's Index (%)	$33 \pm 1.62$	$15 \pm 1.79$
Hausner's ratio	$1.5 \pm 1.13$	$1.18 \pm 1.70$

#### EQUILIBRIUM SOLUBILITY

Equilibrium solubility study of MSC as well as pure form of drug revealed higher solubility of MSC NIC as compared to the original form of the drug in distilled water. In distilled water, the solubility enhanced from  $259.59 \pm 1.41$  mg/ml (NIC) to  $315.46 \pm$

$1.36$  µg/ml (MSC NIC). 1.22 fold solubility enhancement was observed from NIC to MSC NIC in distilled water. Solubility of NIC in phosphate buffer, pH 4.5 was found to be  $227.68 \pm 1.15$  mg/m which increased to  $297.46 \pm 1.23$  mg/ml (MSC NIC).

**Table: Equilibrium solubility data of NIC and MSC NIC of drug**

Media	Solubility (mg/ml)		% Enhancement
	NIC	MSC NIC	
Double distilled water	259.59	315.46	121.52%
Phosphate buffer (pH 4.5)	$227.68 \pm 1.15$	$297.46 \pm 1.23$	130.64%

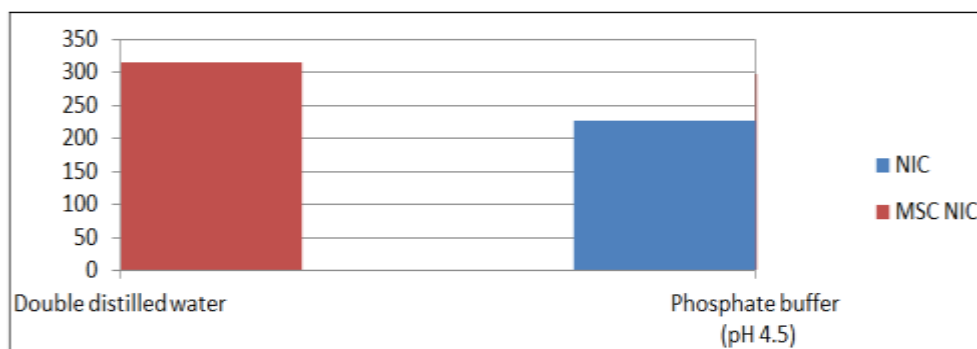


Figure: Bar diagram depicting solubility of NIC and MSC NIC in double distilled water and phosphate buffer, pH 6.8.

### DETERMINATION OF INTRINSIC DISSOLUTION RATE (IDR)

Measurement of intrinsic dissolution rate (IDR) is an important tool in pharmaceutical research allowing characterization of the different crystal forms of drugs by exposing a constant surface area to the dissolution medium. It is useful in predicting absorption problems due to dissolution rate [17]. The IDR of NIC was performed in different media at different pH. Intrinsic dissolution rate for NIC was  $0.135 \pm 1.39$  mg/cm<sup>2</sup>/min in double distilled water and  $0.098 \pm 1.50$  mg/cm<sup>2</sup>/min in phosphate buffer, pH 4.5 which get increased to  $0.242 \pm 1.42$  mg/cm<sup>2</sup>/min and  $0.195 \pm 1.26$  mg/cm<sup>2</sup>/min respectively in MSC NIC form.

Intrinsic dissolution rate (IDR) profiles for original and MSC form of drug are shown in Figure 3.9. The intrinsic dissolution rate of MSC NIC was significantly ( $p < 0.05$ ) enhanced as compared to NIC (Table 3.12).

Increment in intrinsic dissolution rate may be due to reduction in particle size resulting in higher specific surface area as compared to NIC. During dissolution the individual particles were exposed to the dissolution media causing higher intrinsic dissolution rate [18]. From this result it could be expected that MSC NIC will exhibit comparatively better dissolution rate and consequently, better absorption than NIC.

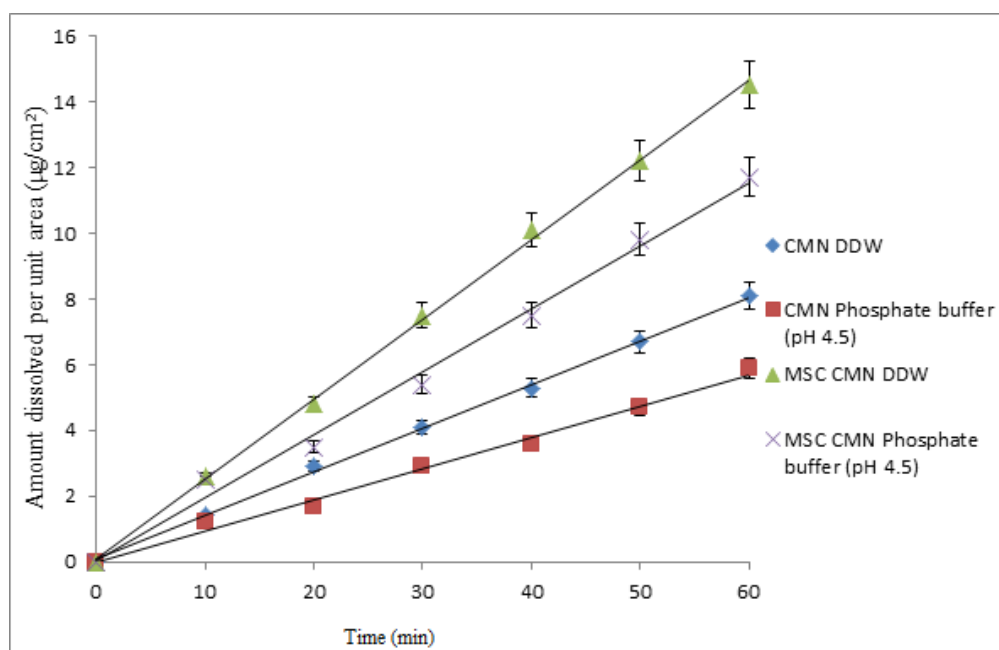


Figure: Intrinsic dissolution rate profile of NIC and MSC NIC in different dissolution media

Table: Intrinsic dissolution rate of NIC and MSC NIC of drug in double distilled water and phosphate buffer, pH 4.5

Media	IDR (mg/cm <sup>2</sup> /min)	
	NIC	MSC NIC
Double distilled water	$0.135 \pm 1.39$	$0.242 \pm 1.42$
Phosphate buffer, pH 4.5	$0.098 \pm 1.50$	$0.195 \pm 1.26$

### X-RAY DIFFRACTION (XRD)

XRD is the technique employed for the identification of crystalline solid phases. Every crystalline solid phase has unique XRD pattern by which it can be identified. From XRD study, it was observed that the diffraction peaks of NIC were also detectable in MSC NIC form (Figure 3.10), suggesting that the MSC NIC did not undergo any modification in crystal. Also, confirming the absence of new reflections and other crystal phases. However, difference in relative intensity of peaks of NIC and MSC NIC were detected. The peaks of MSC NIC have relatively smaller intensities in comparison to NIC. This may be due to markedly different crystal habit or differences in

the crystallinity or particle size of the samples. Therefore, the relative abundance of the planes exposed to X-ray source had altered, producing variations in relative intensities of the peak. Low-intensity peak in the X-ray pattern of the MSC NIC was observed as compared to its original form. This may be due to the presence of uniform crystals in the both the samples. Also, the defects present in the crystal structure may alter the crystal habit of drug [19]. The change in crystallinity was confirmed by calculating crystallinity index. The % crystallinity index of NIC was 326.2% that reduced to 196.7% for MSC NIC. The total percent reduction in the crystallinity index was 129.5 %, confirmed reduction of crystallinity in MSC NIC.

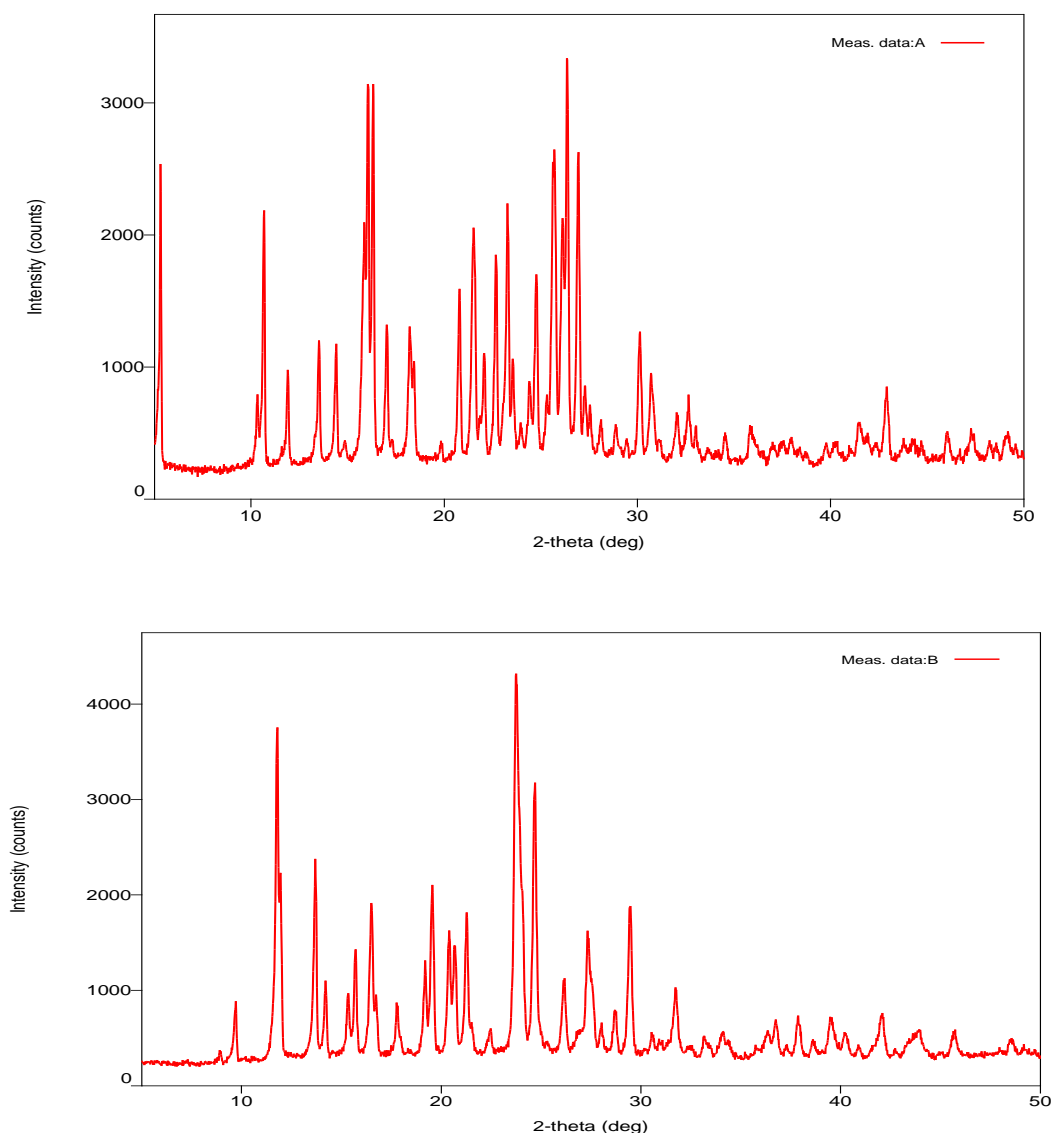


Figure: XRD spectra of a) NIC and b) MSC NIC

### SCANNING ELECTRON MICROSCOPY

Scanning electron microscopy was used to visualize the morphology of original as well as MSC forms of drug. SEM images of NIC (Figure 3.11 A)

showed the presence of large prismatic-cube crystalline particles with rough surface edges. Slight imperfections on planar surface were impressions/remnants of the conventional crystallization process [20]. The MSC



NIC (Figure 3.11 B & 3.11 C) particles were observed to be regular, uniform and almost spheroid in shape with pores in their crystalline structure. Some pitted and shrunken areas were also visualized on the surface of MSC NIC particles. As sonication of the molten mass may create cracks and increases the intra-particulate

porosity. Similar results were also reported by El Kamel et al., [18] for flurbiprofen and by Maheshwari et al., [21] for ibuprofen. Thus, sonocrystallization can be considered as an effective tool to affect surface morphology and structure of crystalline form of drug.

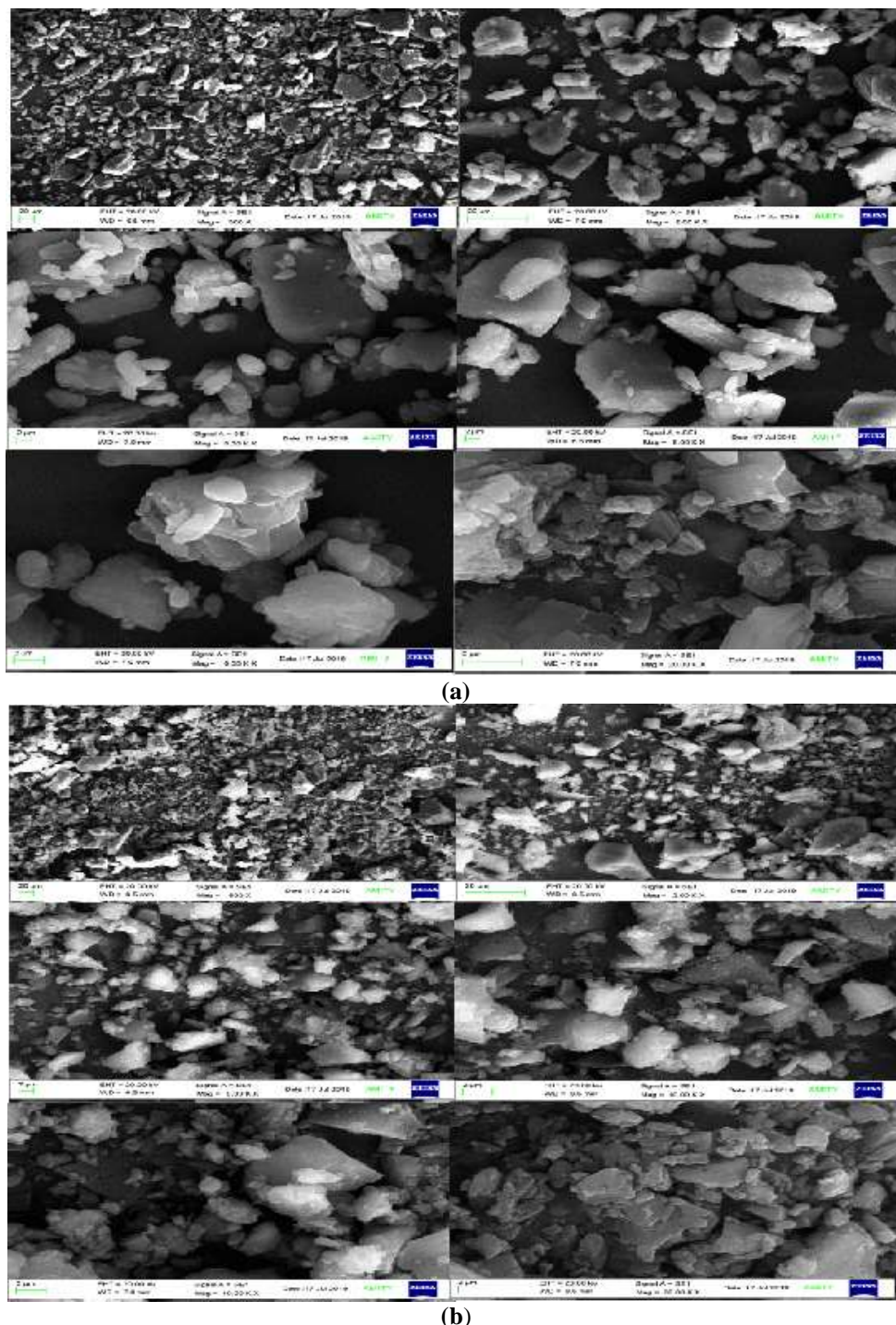


Figure: Scanning Electron Micrograph of A) NIC at 1000X, B) MSC NIC at 1000X, C) Single crystals of MSC NIC at 2000X

#### DIFFERENTIAL SCANNING CALORIMETRY (DSC)

The thermograms of NIC and MSC NIC had shown in Figure 3.12. The DSC scan of NIC exhibited a

single endotherm at 136.48 °C with a normalized energy of 103.416 J/g. The MSC NIC form showed a characteristic crystallization peak at 123.47°C, with a normalized energy of 266.745 J/g.

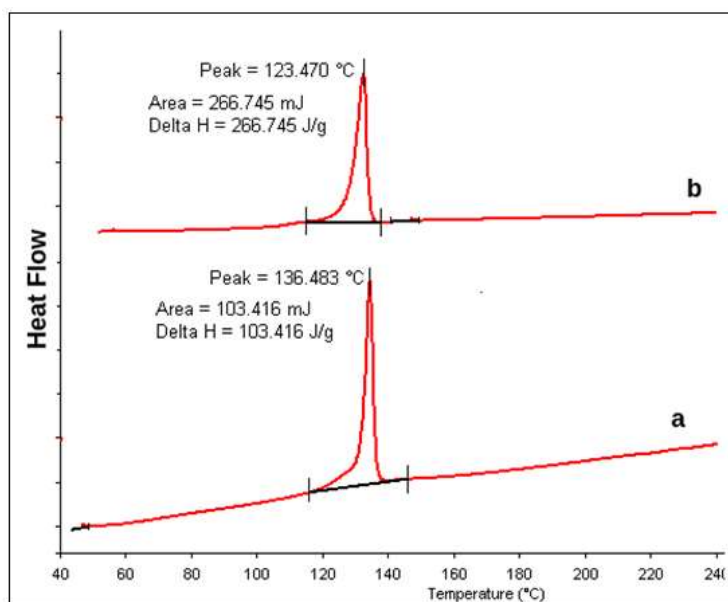


Figure: DSC thermogram profiles of a) NIC and b) MSC NIC.

The sharp endotherm with the minor shift in melting point was obtained, which may be caused due to different crystal structures or reduction in size of crystal. These observations are similar to the reports described by Maheshwari et al., [21], where changes in thermal properties of ibuprofen after MSC were observed. Broadening and asymmetry were observed due to the different crystal sizes and crystal habits of nicergoline.

## SUMMARY AND CONCLUSION

The present study revealed that the application of melt sonocrystallization can be used to improve the flow properties, enhance the solubility and dissolution of poorly water soluble drug nicergoline. This technique had the advantage of producing particles with desired biopharmaceutical properties without the use of organic solvents or the addition of other excipients. An attempt had been made to overcome these problems and melt sonocrystallization technique had been employed to produce MSC NIC. The product obtained after MSC showed reduced particle size, higher solubility and intrinsic dissolution rate, better flow as compared to original form of drug.

## REFERENCES

1. Prescott JK, Barnum RA. On powder flowability. *Pharmaceutical technology*. 2000 Oct;24(10):60-85.
2. Thalberg K, Lindholm D, Axelsson A. Comparison of different flowability tests for powders for inhalation. *Powder technology*. 2004 Sep 8;146(3):206-13.
3. Fitzpatrick B. Distributed caching with memcached. *Linux journal*. 2004;124.
4. Prescott JR, Hutton JT. Cosmic ray contributions to dose rates for luminescence and ESR dating: large depths and long-term time variations. *Radiation measurements*. 1994 Apr 1;23(2-3):497-500.
5. Mellaerts R, Mols R, Kayaert P, Annaert P, Van Humbeeck J, Van den Mooter G, Martens JA, Augustijns P. Ordered mesoporous silica induces pH-independent supersaturation of the basic low solubility compound itraconazole resulting in enhanced transepithelial transport. *International journal of pharmaceuticals*. 2008 Jun 5;357(1-2):169-79.
6. Rasenack N, Bernd W. Crystal habit and tableting behaviour, *International Journal of Pharmaceutics*, 2002; 244, 45–57.
7. Fini A, Holgado MA, Rodriguez L, Cavallari C. Ultrasound-compacted indomethacin/polyvinylpyrrolidone systems: Effect of compaction process on particle morphology and dissolution behavior. *Journal of pharmaceutical sciences*. 2002 Aug 1;91(8):1880-90.
8. Wirtz J, Chew P. The effects of incentives, deal proneness, satisfaction and tie strength on word-of-mouth behaviour. *International journal of service industry management*. 2002 May 1.
9. Martin A. *Physical pharmacy and pharmaceutical sciences*, New Delhi, India: Walters Kluwer Pvt. Ltd, 1999.
10. Rienecker MM, Suarez MJ, Gelaro R, Todling R, Bacmeister J, Liu E, Bosilovich MG, Schubert SD, Takacs L, Kim GK, Bloom S. MERRA: NASA's modern-era retrospective analysis for research and applications. *Journal of climate*. 2011 Jul;24(14):3624-48.
11. *European pharmacopoeia*, 2008.
12. Khan MA, Akhtar N, Sharma V, Pathak K. Product development studies on sonocrystallized curcumin for the treatment of gastric cancer. *Pharmaceutics*. 2015 Jun;7(2):43-63.

13. Chamoli A, Bhandari CM. Teleportation of unknown state by qutrits. *International Journal of Quantum Information*. 2008 Apr;6(02):369-78.
14. Padhi R, Ali SF. An account of chronological developments in control of distributed parameter systems. *Annual Reviews in Control*. 2009 Apr 1;33(1):59-68.
15. Dhumal RS, Biradar SV, Yamamura S, Paradkar AR, York P. Preparation of amorphous cefuroxime axetil nanoparticles by sonoprecipitation for enhancement of bioavailability. *European Journal of Pharmaceutics and Biopharmaceutics*. 2008 Sep 1;70(1):109-15.
16. Aulton ME. *The science of dosage form design*, Churchill Livingstone, Ed. 2002; 2:114-116.
17. Ansel LV, Popovich NG, Ansel HC. *Pharmaceutical dosage forms and drug delivery systems*, New York, USA, Lippincott Williams and Wilkins, 2006; 8, 105.
18. El-Kamel AH. Improvement of physicochemical and biopharmaceutical properties of flurbiprofen using melt sonocrystallization technique. *Drug Development Research*. 2008 Feb;69(1):34-41.
19. Stovall DM, Givens C, Keown S, Hoover KR, Rodriguez E, Acree WE, Abraham MH. Solubility of crystalline nonelectrolyte solutes in organic solvents: mathematical correlation of ibuprofen solubilities with the Abraham solvation parameter model. *Physics and Chemistry of Liquids*. 2005 Jun 1;43(3):261-8.
20. Kushwaha T, Shankar V. Are multichannel customers really more valuable? The moderating role of product category characteristics. *Journal of Marketing*. 2013 Jul;77(4):67-85.
21. Manish M, Harshal J, Anant P. Melt sonocrystallization of ibuprofen: Effect on crystal properties. *European journal of pharmaceutical sciences*. 2005 May 1;25(1):41-8.