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SOLUBILITY ENHANCEMENT OF NEVIRAPINE BY HYDROTROPIC SOLUBILIZATION

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Abstract:

Solubility enhancement of Nevirapine (NVP) was carried out by hydrotropic solubilization technique by using hydrotropic agent like citric acid. The Fourier transform-IR (FTIR) studies indicated the possibility of bonding of Nevirapine with the citric acid. DSC was used to characterize the purity of nevirapine by showing melting point. Analytical method validation was carried out with 6.8 buffer. Syrup was formulated as a trial batch (50 ml). Stability testing of syrup was carried by freeze-thaw cycling testing. In present investigation solubility of Nevirapine was increased by 105.97 fold.

Keyword*: Nevirapine, hydrotropic solubilization, solubility enhancement, stability.*

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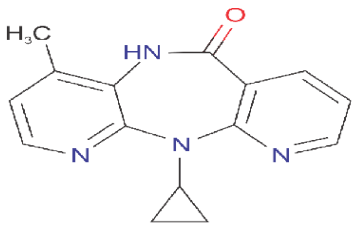
INTRODUCTION

Formulation of a solution is not less than a challenge due to a few technical problems as instability and low aqueous solubility of the drugs. Special solubilization techniques are sometimes required and great care has to be taken to maintain pharmaceutical elegance with regard to taste, appearance and viscosity. Liquid preparations have some distinct advantages over more widely used solid dosage forms viz. faster absorption and quicker therapeutic response and ease to swallow to make them more suitable and acceptable for the pediatric and geriatric use.[1]

In the pharmaceutical analysis and formulation development fields is most often required to increase the aqueous solubility of poorly water-soluble drugs.

Nevirapine is a drug belonging to a class of pharmacological agents known as the non-nucleoside reverse transcriptase inhibitor (NNRTI) of HIV-1. Chemically it is 11- cyclopropyl-4-methyl-5,11-dihydro-6H-dipyrido{3,2-b:2',3'-e}{1,4}diazepine-6-one2.



Nevirapine binds directly to reverse transcriptase (RT) and blocks the RNA-dependent and DNA-dependent DNA polymerase activities by causing a disruption of the enzyme's catalytic site. Nevirapine is used for the treatment of HIV-1 infection. Nevirapine was having extensive first pass metabolism, and relative Elimination half-life of about 45 hrs. [2], [3]

As nevirapine have poor aqueous solubility, it needs to enhance solubility by solubilization method. Various solubilization methods, such as miceller solubilization, pH adjustment, cocrystallization, hydrotropic, solid dispersion etc. are used for solubility enhancement.

Hydrotropes are a class of chemical compounds that affect a several fold increase in the solubility for sparingly soluble solute under normal conditions. This phenomenon, termed hydrotropy can be considered to be a unique and unprecedented solubilization technique [4] Hydrotropy is a solubilization process whereby addition of a large amount of second solute results in an increase in the aqueous solubility of another solute. Additives salts that increase solubility in given solvent are said to "salt in" the solute and those salts that decrease solubility "salt out" the solute. Several salts with large anions or cations that are themselves very soluble in water result in "salting in" of non-electrolytes called "hydrotropic salts" a phenomenon known as "hydrotropism"[5], [6], [7], [8] Hydrotropic method is economic, safe and user friendly method, so we selected hydrotropic solubilization.

The objective of the present study was to enhance the solubility of Nevirapine by using various hydrotropic agent, and also formulation of the Nevirapine dosage form sing hydrotropes.

MATERIAL AND METHODS

Materials

Nevirapine was kindly received as gift sample from Mylan laborites, Aurangabad. Sodium acetate, sodium citrate, sodium chloride, citric acid, urea, sodium carbonate etc. were purchased from research fine lab (AR grade).

Methods

Preparation of hydrotrope

Solubilization method was used for the preparation of the hydrotropes. The different solutions of hydrotropic agent was prepared in 10%, 20%, 30%, 40% and then calibration curve of Nevirapine in that solutions was taken. And the solubility of NVP was calculated in different hydrotropic solutions like citric acid, sodium acetate, urea, sodium citrate, sodium chloride, sodium carbonate. The solution which shows highest solubility of NVP was taken as final hydrotropic agent. [9]

Saturation solubility of Nevirapine in 0.1N HCL, 6.8 phosphate buffer and water

The saturation solubility of drug was determined in 0.1N HCL, 6.8 phosphate buffer and water. The saturation solubility studies were conducted according to method given by Higuchi and Connors in triplicate. In order to determine saturation solubility, an excess amount of Nevirapine was added to vials having 10 ml of distilled water. The vials are subjected to rotary shaking and sonication and allowed to stand for equilibration for 24 hrs, after that samples were filtered through whattman filter paper and filtrate was analyzed by UV Spectrophotometer at 282 nm after appropriate dilutions. [10]

Analytical method validation

The analytical method validation of Nevirapine was carried out in 6.8 phosphate buffer. Ultra violet absorption spectrum of Nevirapine was obtained in 6.8 phosphate buffer in the scanning range of 200-400 nm. All the system suitability & validation parameters (Accuracy, Precision, Limit of detection, Limit of quantitation, Linearity, Range) were studied as per ICH Q_2R_1 guideline on Analytical Method Validation. [11], [12]

Determination of solubility of Nevirapine in Different Concentrations of Hydrotropic Agents

The solubility of Nevirapine was determined in different solutions of hydrotropic agents like citric acid, sodium acetate, urea, sodium citrate, sodium chloride, sodium carbonate. The construction of calibration curve of different hydrotropic agents was done and from that determination of solubility in different concentrations of hydrotropic agents like 10%, 20%, 30% and 40% was done. The saturation solubility studies were conducted according to method given by Higuchi and et al. In order to determine saturation solubility, an excess amount of Nevirapine was added to vials having 10 ml of distilled water. The vials are subjected to rotary shaking and sonication for 12 hrs and allowed to stand for equilibration for 24 hrs, after that samples were filtered through whattman filter paper and filtrate was analyzed by UV Spectrophotometer at 282 nm after appropriate dilutions. [13]

Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy was employed to characterize further the possible interaction between the drug and hydrotropic agent. Shimadzu FTIR spectrometer Prestige 21 with DRS assembly was used in Attenuated total reflectance (ATR) mode for collecting FT-IR spectra of samples. The spectra's were collected over the range of 4000-400 cm^{-1} in 45 scans, with a resolution of 5 cm^{-1} for each sample. [14]

Differential scanning calorimetry

The possibility of any interaction of between the drug and hydrotropic agent was employed by differential scanning calorimetry. Thermal analysis of the Nevirapine was performed using a differential scanning calorimeter DSC-60A Shimadzu calorimeter. The sample powders (9mg) were placed in aluminum pans, sealed hermetically and then these hermetically sealed aluminum pans were heated at a scanning rate of 20°C/min from 50° to 300°C under constant purging dry nitrogen flow (100 ml/min). Empty aluminum pan was used as a reference. [15]

Preparation of syrup: For formulation of syrup, 40% solution of citric acid in water was prepared. Sucrose dissolves in 20 ml hydrotropic solution, then add sodium benzoate in it as preservative. Nevirapine dissolves in 20 ml hydrotropic solution, sonicate it till dissolve the drug. Make up volume with hydrotropic solution. [16], [17]

Stability Study of Syrup

Formulated syrup was subjected to physical and chemical stability testing at three different temperatures (ambient, moderate and relatively higher temperature). The syrup was filled in 10 ml colorless glass vials and vials were plugged and sealed. The vials were kept at room temperature. [18]

Freeze-Thaw Cycling Testing of Syrups

Two vials of each type of syrups were subjected to stress testing. For 24 hr, the vials were stored at 4^0 C in refrigerator and then vials were kept at 40^0 C in oven for 24 hr. After this again vials were observed to check turbidity and precipitation, if any. There was no precipitation and turbidity in syrup formulation at the end of this testing [19]

RESULT AND DISCUSSION

Saturation solubility of Nevirapine

In the present study saturation solubility of Nevirapine 0.1N HCL, 6.8 phosphate buffer and water results of saturation solubility of nevirapine in µg/ml showed in table 1.

Table 1: Saturation solubility of nevirapine

Sr. No.	Solubility in	Solubility (µg/ml)
1.	0.1N HCL	4529
2.	6.8 Phosphate buffer	3300
3.	Water	10.935

Analytical Method Validation

Analytical method validation was carried out which shown in table 2. From the data observed it was concluded that the developed method is fast, best and accurate.

Table 2: Analytical method validation

Sr no	Parameter	Result	ICH standard
1	Accuracy	98.34	98±102%
2	Linearity	0.999	>0.997
3	Range	05-25	-
4	Intraday precision	0.4836	NMT 2
5	Interday precision	0.4988	
6	Limit of Detection(LOD)	0.174275	< 2
7	Limit of Quantitation(LOQ)	0.528105	< 2

Determination of solubility of Nevirapine in different concentrations of hydrotropic agents

Solubility of nevirapine in different concentrations of hydrotropic agent (mg/ml) was reported in table 3 with slope of line and R^2. From this data we were concluded that maximum solubility of nevirapine was shown with citric acid that's why citric acid was selected for further study. The solubility enhancement graphically showed in figure 1.

Table 3: Solubility of Nevirapine in Different Concentrations of Hydrotropic Agent (mg/ml)

Sr. No.	Hydrotropic agent	Solubility (mg/ml)	Slope	R^2
1	Sodium citrate		Y=0.01107x-0.00188	0.99883
	10% Solution	0.9376		
	20% Solution	1.7521		
	30% Solution	2.5100		
	40% Solution	3.1485		
2	Sodium acetate		Y=0.01513x+0.00026	0.99925
	10% Solution	0.1590		
	20% Solution	0.5981		
	30% Solution	1.5765		
	40% Solution	2.2341		
3	Sodium carbonate		Y=0.00283x+0.00585	0.87222
	10% Solution	0.9688		
	20% Solution	2.1670		
	30% Solution	3.2064		
	40% Solution	4.2712		
4	NaCl		Y=0.00675x+0.05212	0.91926
	10% Solution	0.0447		
	20% Solution	0.0890		
	30% Solution	0.1342		
	40% Solution	0.1790		
5	Citric acid		Y=0.02779x-0.02899	0.99926
	10% Solution	2.9380		
	20% Solution	5.8775		
	30% Solution	8.713		
	40% Solution	11.52		
6	Urea		Y=0.0370x+0.4086	0.99933
	10% Solution	2.1247		
	20% Solution	3.2094		
	30% Solution	4.2754		
	40% Solution	4.9145		

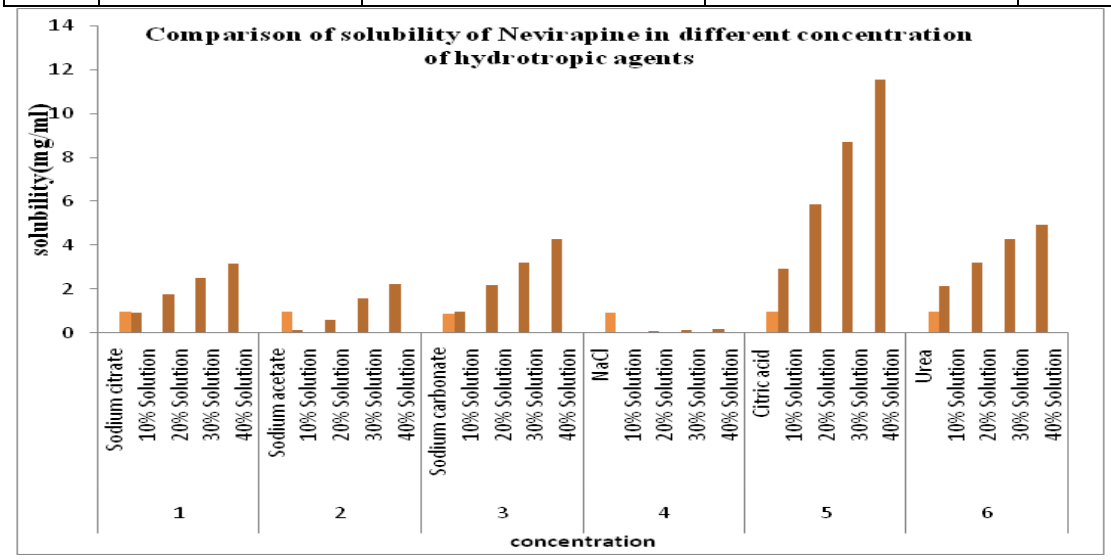


Fig 1: Solubility of Nevirapine in Different Concentrations of Hydrotropic Agent (mg/ml)

Nevirapine shows solubility increasing in linear manner in Citric acids different concentration and also shows higher solubility than other hydrotropic agents. So the citric acid selected as hydrotropic agent for further study for preparation of hydrotrope. Citric acids 40% concentration shows highest solubility so it was used for preparation of hydrotrope. According to solubility enhancement ratio, solubility increases by 105.97 fold.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of nevirapine were showed in figure 2.The data obtained by FT-IR of Nevirapine was matches with reported values which were reported in literatures for Nevirapine of good quality. FTIR of nevirapine, citric acid, and nevirapine+ citric acid showed in figure.2,3,4 and table 4,5,6.

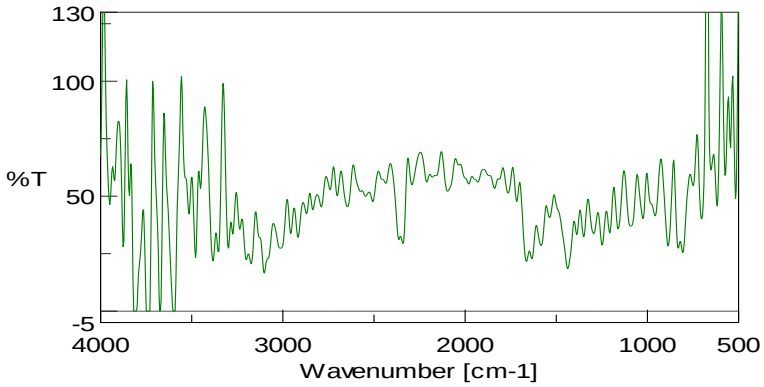


Fig 2: FT-IR spectrum of Nevirapine

Table 4: FT-IR Frequency Data Correlated with Reported Frequency

Functional group	Reported value	Observed value
-NH stretching	3193-3255 cm^{-1}	3224 cm^{-1}
-CH stretching	2873-2953 cm^{-1}	2931 cm^{-1}
-C=N stretching	1612-1739 cm^{-1}	1654 cm^{-1}
-C=O aromatic stretching	3359-3517 cm^{-1}	3440 cm^{-1}
C-C stretching	980-1160 cm^{-1}	1045 cm^{-1}

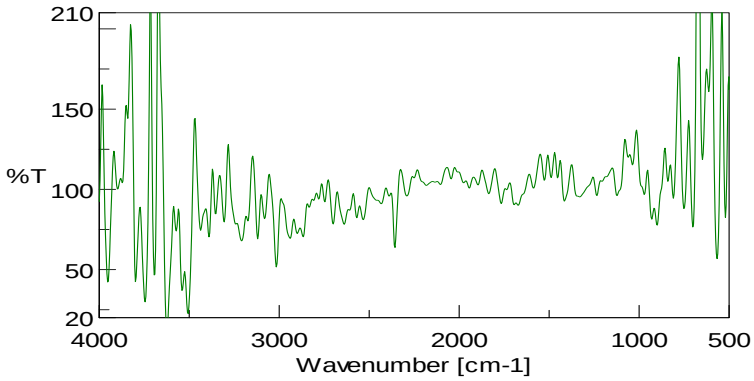


Fig 3: FT-IR spectrum of Citric acid

Table 5:FT-IR Frequency Data Correlated with Reported Frequency

Functional group	Reported value	Observed value
O-H stretching (bonded)	**3120-3180 cm^{-1}.**	**3174 cm^{-1}.**
O-H deformation	1122-1330 cm^{-1}.	1234 cm^{-1}.
C-O stretching	1330-1450cm^{-1}.	1415 cm^{-1}.

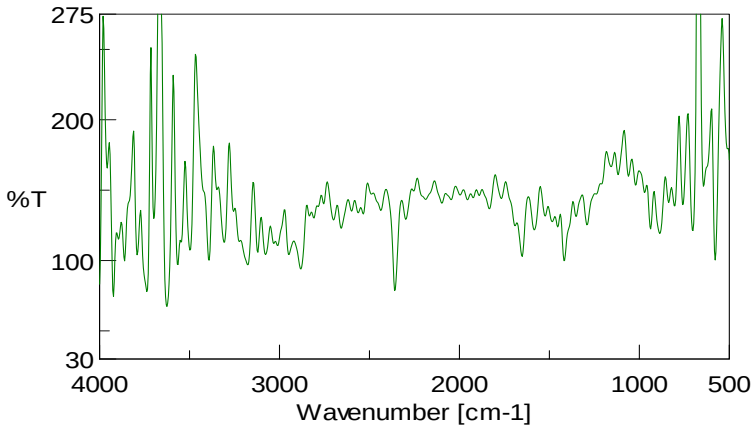


Fig 4: FT-IR of NVP+ CA

Table 6: FT-IR Frequency Data Correlated With Reported Frequency

Functional group	Reported value	Observed value
N-H stretching	3390–3563 cm^{-1}	3498 cm^{-1}
O-H stretching bonded	2699-2807 cm^{-1}.	2753 cm^{-1}
C=C=C stretching disubstituted	1924-1997 cm^{-1}	1955 cm^{-1}
-C-H in plane deformation	1118-1292 cm^{-1}	1157 cm^{-1}
≡C-H deformation	578-701 cm^{-1}	644 cm^{-1}

Differential Scanning Calorimetry

DSC results of nevirapine shown in figure 5, which indicates the thermogram of the Nevirapine indicates the reported M.P.(246-248° C) of the Nevirapine and their actual M.P. obtained by the DSC(246.60). The data obtained by DSC of Nevirapine was matches with values which were reported in literatures and nevirapine are of good quality.DSC result of citric acid and NVP + citric acid was shown in fig.6, 7. Fig. 7 indicates that citric acid affect thermal property of nevirapine.

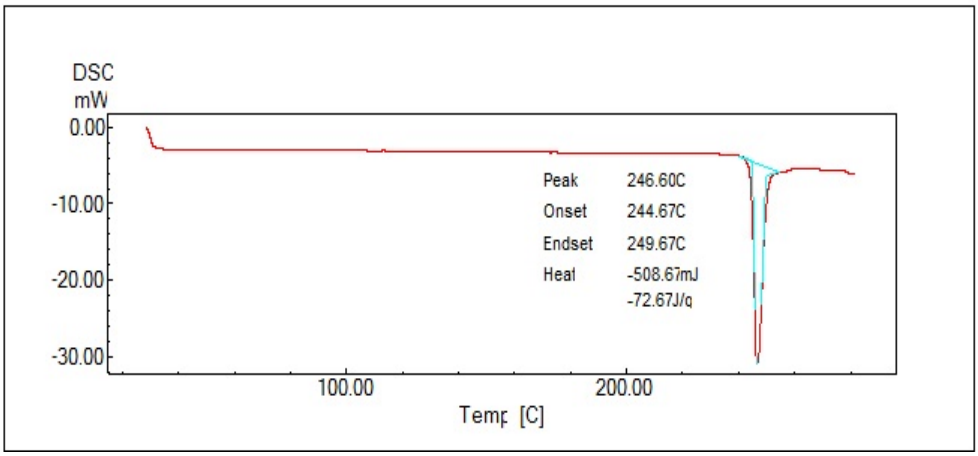


Fig 5: DSC Thermogram of Nevirapine

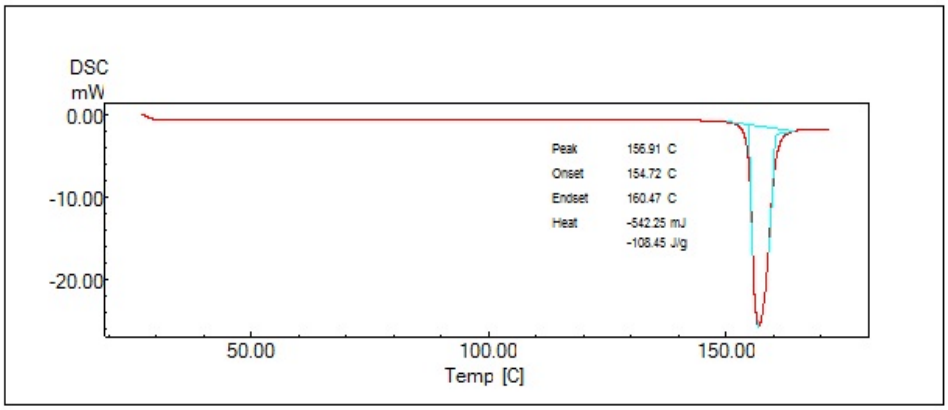


Fig 6: DSC of citric acid

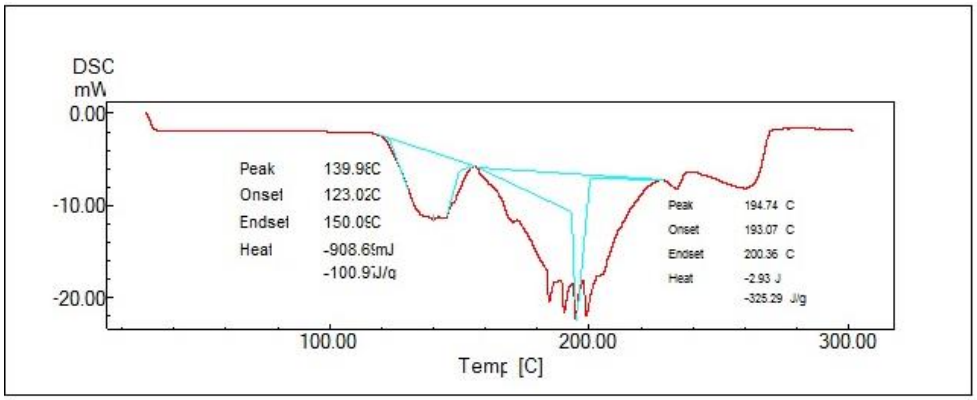


Fig 7: DSC Thermogram of Nevirapine-Citric acid

Table 7: Formula for Syrup

Sr. No.	Ingredients	Quantity taken(gm)(50 ml)
1.	Nevirapine	0.5
2.	Citric acid	38
3.	Sucrose	10
4.	Sodium Benzoate	0.5
5.	Distill water	q. s.

Preparation of Syrup:

According to method given by R.K Maheshwari and et al. formulation of syrup was carried out. A batch (50ml) on as trial basis was formulated and stability study was carried out [16].

Stability Study

Stability of syrup was checked by Freeze Thaw - cycling Testing. No change in color was observed. Clear solution is observed. No precipitation observed during stability study.

Table 8: Stability Study Data

Condition	**Cycle**	**Physical parameter**		
		Color	**Clarity**	**Precipitation**
4^0C in refrigerator,	7 cycle Alternatively	Colourless	Clear solution	No precipitation
40^0C in oven				

CONCLUSION

In the present study as Nevirapine poorly soluble in water by adding hydrotropic agent like citric acid we enhance solubility of Nevirapine by solubilization method by 105.97 fold. As our main motto is to enhance solubility we just made trial batch of Nevirapine syrup and stability study shown that syrup is stable. We can formulate nevirapine as syrup dosage form.

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