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Solubility enhancement of nevirapine by cocrystallisation technique

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ABSTRACT

The objective of present study was to enhance solubility of Nevirapine by cocrystallisation technique.Nevirapine is non-nucleoside reverse transcriptase inhibitor (NNRTI) class of antiretroviral and having poor aqueous solubility and dissolution profile. The method used for co-crystal formation was neat grinding method. The co-crystals were prepared by using conformer Maleic acid. The method used for co-crystal formation was neat grinding method. Further prepared co-crystals were characterized by Powder X-ray diffractometry (PXRD), Differential Scanning Calorimetry (DSC), Fourier Transformation Infra-red Spectroscopy (FTIR). Moreover they were studied for melting point determination, flow property studies and dissolution studies. All the performed study revealed formation of co-crystals, improvement in micromeritic properties and dissolution behavior of drug. The result shows that solubility of Nevirapine was improved by 106 folds via crystal engineering technique.

KEYWORDS: Co-crystallization, dissolution behavior, micromeritic properties, Nevirapine.

INTRODUCTION

Many of the drugs (API) are facing various problems like poor aqueous solubility, poor dissolution profile and poor stability thus affecting bioavailability and therapeutic effect. [1] To overcome these problems various methods have been used to improve aqueous solubility of poorly water soluble drugs. These strategies include micronization [2,3,4] use of salt forms, [5] co-solvent approach, [6] micellar solubilisation[7] and complexation with cyclodextrins. Crystal engineering approaches, which can potentially be applied to a wide range of crystalline materials, offer an alternative and potentially fruitful method for improving the solubility, dissolution rate and subsequent bioavailability of poorly soluble drugs. The challenges of low aqueous solubility provide an ideal situation for the application of crystal engineering techniques for improving bioavailability. [8]

Nevirapine (Fig. 1) (11-cyclopropyl-5, 11- dihydro-4-methly-6H-dipyrido [3, 2-b: 2,3-e] [1,4]diazepin- 6-one,a dipyridodiazepinone) is a non-nucleoside reverse transcriptase inhibitor of human immunodeficiency virus type 1 (HIV-1). It acts by binding directly to allosteric site on reverse transcriptase and inhibits activities of both the RNA and DNA-dependant DNA polymerase. It is widely used in treatment of HIV-1 infection and is non-competitive inhibitor of the viral replicative enzyme reverse transcriptase. However it has very poor aqueous solubility and dissolution properties. [9]

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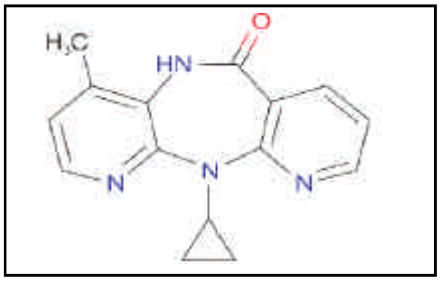

Figure 1: Chemical structure of Nevirapine.

The present work was oriented towards improving physicochemical properties of Nevirapine by preparing cocrystals using neat grinding method [10] with the help of different co-crystals forming agents. The micromeritic properties of Co-crystals were studied. The improvement in physicochemical properties is confirmed by characterizing prepared co-crystals by using several techniques such as Fourier transformation-infrared spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), X-ray powder diffraction analysis(XPRD), melting point determination and dissolution test.

MATERIALS AND METHODS

Materials:

Nevirapine was obtained as a gift sample from Mylan laboratory Ltd, Aurangabad, Maharashtra, India. Maleic acid and other conformers (Extra Pure Grade) were purchased from ResearchFineLab, Mumbai, India. All the reagents were of analytical grade. Distilled water was used throughout the experiment.

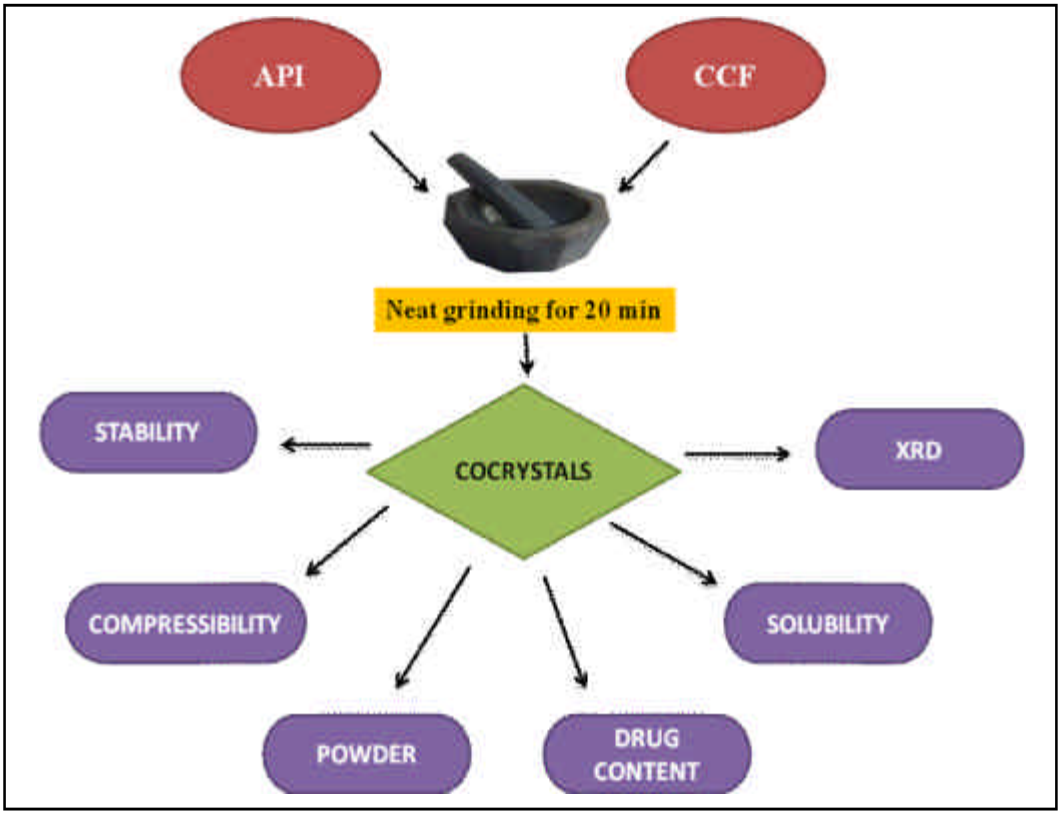

Figure 2 : Screening of Nevirapine co-crystals

Methods:

Preparation of Co-crystals:

Nevirapine was co-crystallized with different GRAS listed co-crystalformers in the 1:1 stoichiometric ratio. The neat grinding method was used for screening of co-crystals because this mechanical technique is one of the green chemistry technique thus eco-friendly. Total batch size for initial screening was of 1gm. An accurately weighed quantity of Nevirapine and CCF was transferred to clean mortar pestle. Then manual grinding was done for 20 minutes. After grinding these co-crystals were collected and stored. [11]

Determination of Saturation solubility of Nevirapine:

The saturation solubility of Nevirapine 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. [12]

Determination of physical constant (melting point):

The melting points of pure drug and co-crystals were determined using the open capillary tube method. [13]

Pre-formulation characteristics of Co-crystals:

After co-crystallization the pre-formulation characterization of co-crystals and their comparison with Nevirapine were carried out. The pharmaceutical processing properties i.e. angle of repose, bulk density, tapped density, Carr's index and Hausner's ratio were studied in comparison to pure Nevirapine.[14,15,16]

Solid state characterization:

Fourier transformation infrared spectroscopy (FTIR):

Shimadzu FTIR spectrometer Prestige 21 with DRS assembly was used in Attenuated total reflectance (ATR) mode for collecting FT-IR spectra of Nevirapine, Maleic acid and cocrystals. 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.[17]

Powder X-ray diffractometry (PXRD):

Generally PXRD technique is used for determining polymorphism, change in crystal habit modifications in drugcrystals and generation of new crystal form. The PXRD data of pure Nevirapine and co-crystals were recorded on a Philips Analytic X Ray – PW 3710 (Philips, Almelo, The Netherlands) diffractometer with tube anode Cu over the interval 10-70º/2θ under the following set ofconditions: The Generator tension (voltage): 45 kV and Generator current: 40 mA. [18]

Differential Scanning Calorimetry (DSC):

Thermal analysis of the Nevirapine, Maleic acid and cocrystals were performed using a differential scanning calorimeter DSC-60A Shimadzu calorimeter. The sample powders (7mg) were placed in aluminium pans,

sealed hermetically and then these hermetically sealed aluminium 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 aluminium pan was used as a reference.[17]

Dissolution studies:

The % drug release of pure drug that is Nevirapine and selected cocrystal (NVP-MA) was calculated from the data obtain by in vitro dissolution study of drug and cocrystal. The dissolution studies were carried out in 6.8 phosphate buffer and 0.1N HCl, so the % drug release of pure drug and cocrystal were calculated from that.[19]

RESULTS AND DISCUSSION

Determination of Saturation solubility of Nevirapine:

The saturation solubility of Nevirapine were determined in 0.1N HCl, 6.8 phosphate buffer and water and results were as shown 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

Determination of Melting point and saturation solubility of cocrystals:

The melting point and saturation solubility of prepared cocrystals were tabulated in table 2.On the basis of the solubility results of the co-crystals prepared from which three conformers (Maleic acid, cinnamic acid and malonic acid) were selected for the further study. The Solubility of all the cocrystal formed using these three CCF were studied in triplicates. And their melting point was checked to finalize the conformer best suited for the drug candidate. And finally Maleic acid was selected as conformer as it showing consistent results.

Table 2: Melting point and saturation solubility of cocrystals

Co-crystals of Nevirapine with	Co-crystals solubility(µg/ml)	M.P. of Co-crystals(°C)
PABA	954.7±1.23	161-163
Sodium saccharine	905.2±1.20	264-265
Benzoic acid	105.80±0.61	170-172
Oxalic acid	981.50±0.53	229-231
Tartaric acid	262.20±0.25	194-196
Ferrulic acid	3225.1±1.26	163-165
Gallic acid	875.3±0.89	204-206
Mandelic acid	87.60±0.10	188-190
Malonic acid	1523.3±1.13	205-207
Urea	102.90±0.36	238-240
Hippuric acid	311.18±0.54	171-173
Glutaric acid	134.83±0.24	90-92
Cinnamic acid	1217.6±1.06	240-242
Maleic acid	1162.2±1.14	193-195
Malonic acid	618.41±0.75	245-247
Citric acid	166.60±0.28	145-147
4-Hydroxy benzoic acid	939.10±0.74	207-209

Pre-formulation characteristics of Co-crystals:

After the co-crystallization the pre-formulation characterization of co-crystals was done and their comparison with Nevirapine as shown in table 3.

Table 3: Pre-formulation characteristics of drug and co-crystal

	Angle of repose (°C)	Bulk density (gm/cm³)	Tapped density (gm/cm³)	Carr's index (%)	Hausner's ratio
Pure drug	32.34	0.280	0.440	57.14	1.57
Co-crystal	28.61	0.400	0.5714	42.85	1.42

The results obtained shows that co-crystallization improves the Powder characteristics i.e. angle of repose, bulk density, tapped density, Carr's index and Hausner's ratio of co-crystals when compared to pure drug Nevirapine.

Solid state characterization:

FTIR Studies:

FT-IR data of Nevirapine and Maleic acid:

Figure 3, 4 and table 4, 5 indicates the interpretation of the IR spectra of the Nevirapine and Maleic acid respectively,which give evidence that the Nevirapine and Maleic acid are of good quality. The data obtained by FT-IR of both was matches with reported values.

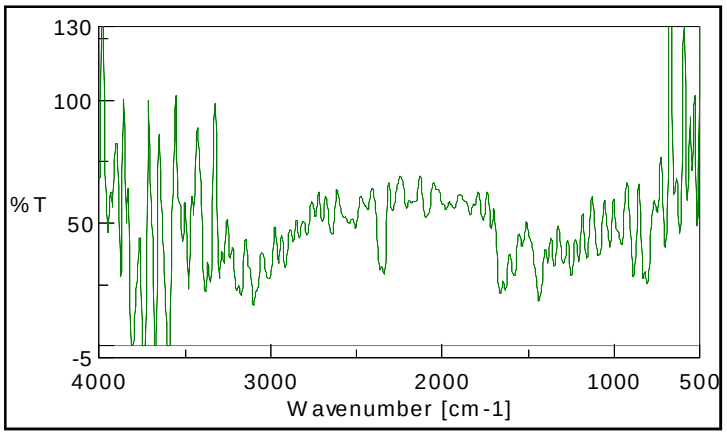

Figure 3: FT-IR spectrum of Nevirapine

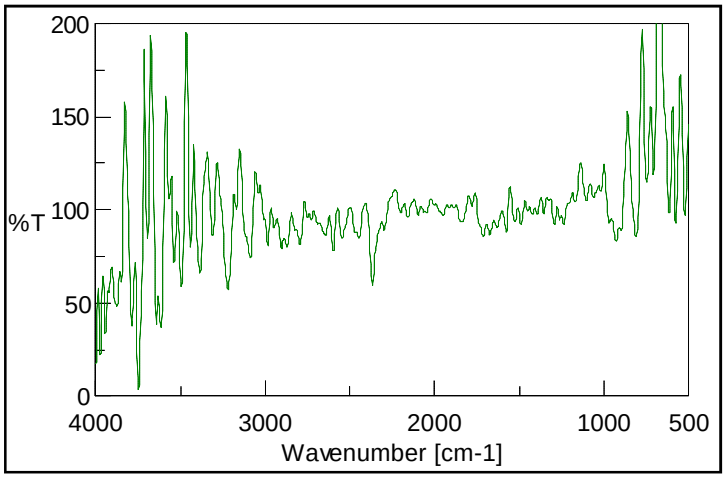

Figure 4: FT-IR spectrum of Maleic acid

Table 4: FT-IR frequency data of nevirapine correlated with reported frequency

Functional groups	Reported value	Observed value
-NH stretching	3310-3360 cm^{-1}	3355.53 cm^{-1}
-CH stretching	2862-2882 cm^{-1}	2869.56 cm^{-1}
-C=N stretching	1250-1340 cm^{-1}	1295 cm^{-1}
-C=O aromatic stretching	1705-1725 cm^{-1}	1716 cm^{-1}

Table 5: FT-IR frequency data of maleic acid correlated with reported frequency

Functional group	Reported value	Observed value
O-H stretching (bonded)	2500-3300 cm^{-1}	3043 cm^{-1}
C-H aromatic	1050-1400 cm^{-1}	1699 cm^{-1}
C=C stretching	1625-1680 cm^{-1}	1631 cm^{-1}

FT-IR spectra of co-crystal NVP-MA:

The spectra of the co-crystal shows nearly both the peaks present in the Nevirapine and the maleic acid and also show some new peaks which indicates that there may be new bonds formed due to crystallisation as shown in figure 5 and table 6.

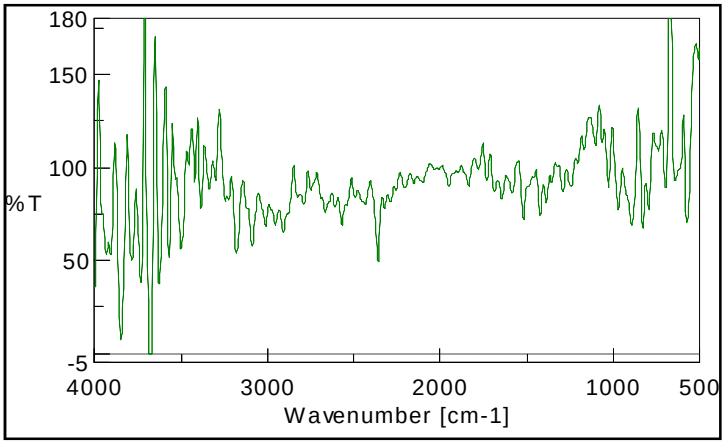

Figure 5: FT-IR spectrum of Nevirapine-Maleic acid (co-crystal)

Table 6: FT-IR frequency data of nvp cocrystals correlated with reported frequency

Functional group	Reported value	Observed value
N-H Stretching	3440-3480 cm^{-1}	3455 cm^{-1}
C=O stretching	1630-1680 cm^{-1}	1639 cm^{-1}
O-H stretching (bonded)	3450-3550 cm^{-1}	3455 cm^{-1}
C=C aromatic	1450-1600 cm^{-1}	1570 cm^{-1}
$-CH_3$ stretching	2953-2972 cm^{-1}	2954 cm^{-1}
N-H stretching	3382-3416 cm^{-1}	3386 cm^{-1}

PXRD Studies:

The Powder X-ray diffractometry (PXRD) patterns of pure Nevirapine and co-crystals are shown in figures 6 and 7.It was noticed in figures that unique PXRD patterns of cocrystals was different from the drug. Peak intensities of Nevirapine and co-crystals at various diffractionangles (2?) are shown in table 7 and 8. It is evident from the observation that the cocrystals exhibited different peak positions patterns from drug. This could be explained on the basis of change in internal crystalstructure having different crystalline habit than that of drug crystal habit. This shows that formation of different crystalline structure was takes place.

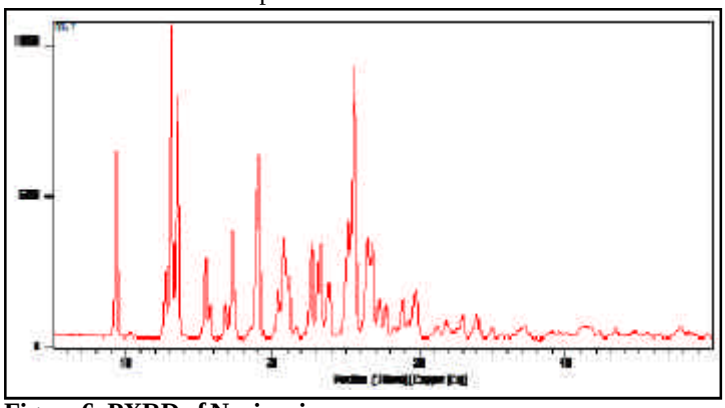

Figure 6: PXRD of Nevirapine

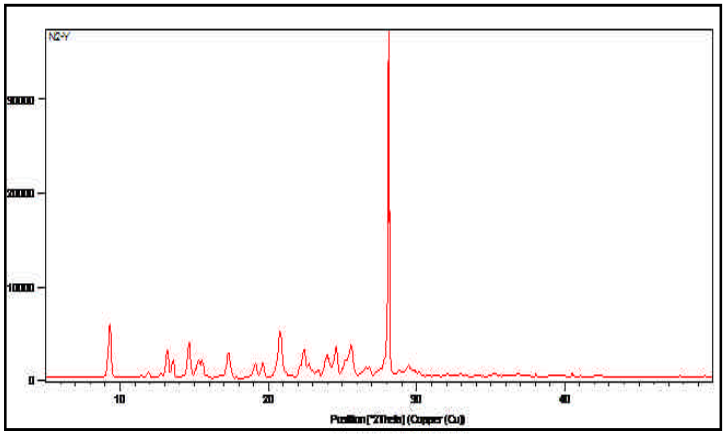

Figure 7: PXRD of Nevirapine - Maleic acid cocrystals

Table 7: PXRD interpretation of nevirapine

Nevirapine	
2q	Intensity
9.3446	60.21
13.1450	100.00
13.5531	73.30
19.1201	57.66
25.5844	88.70

Table 8: PXRD interpretation of nevirapine cocrystal

Cocrystal of Nevirapine and Maleic acid	
2q	Intensity
28.1123	100.00

Differential Scanning Calorimetry (DSC):

Figures 8, 9 and 10indicate the thermogram of the Nevirapine, Maleic acid and cocrystals of NVP and MA [Nevirapine and Maleic acid] respectively. The data obtained by DSC of Nevirapine and Maleic acid matches with values which were reported in literatures and of good quality. The NVP-M.A. co-crystal showed formation of co-crystal by DSC data, and has a melting point of 184.5°C. DSC data for drug and co-crystals is summarized in table 9.

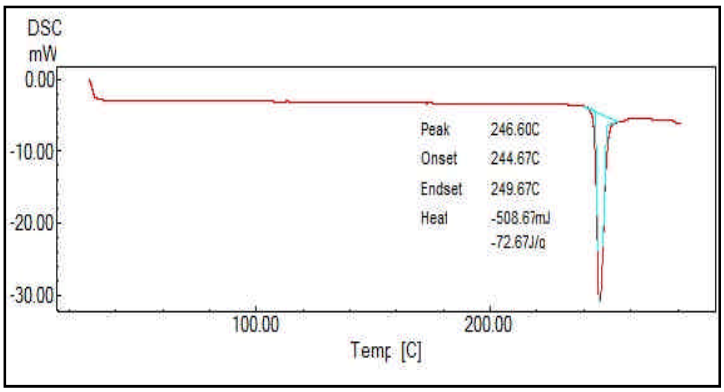

Figure 8: DSC Thermogram of Nevirapine

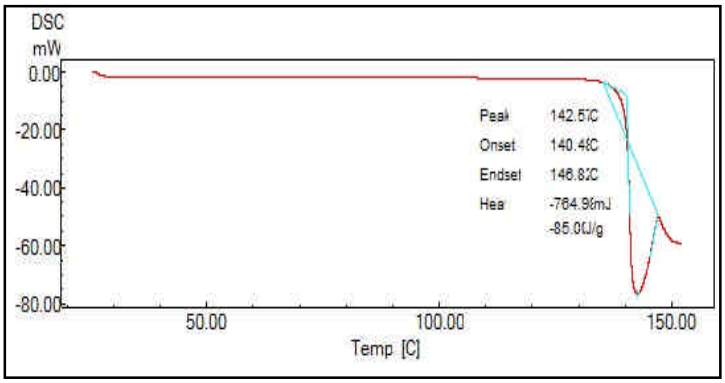

Figure 9: DSCThermogram of Maleic acid

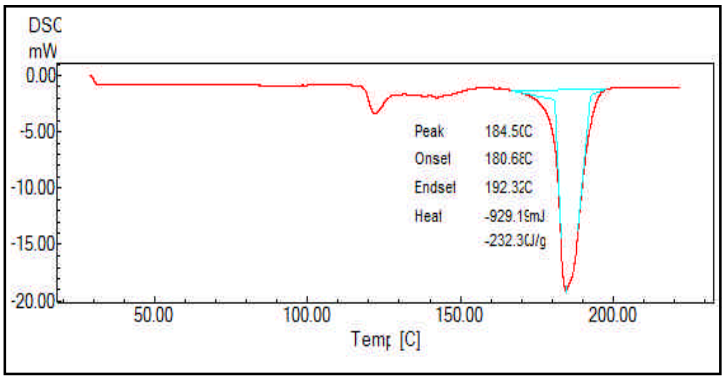

Figure 10: DSC Thermogram of Nevirapine-Maleic acid's cocrystals

Table 9: DSC data for NVP co-crystals

Sr no	Name	Theoretical melting point	Melting Point by DSC
1	Nevirapine	242-246°C	246.60°C
2	Maleic acid	134-136°C	142.50°C
3	NVP-MA	—	184.50°C

Dissolution studies:

Powder dissolution of pure drug and co-crystals in 6.8 phosphate buffer and 0.1 N HCl is summarized in table 10.

Table 10: % drug release (drug, co-crystal) in phosphate buffer 6.8 and 0.1N Hcl

Sr no	Time (min)	% drug release (Pure drug in 6.8 buffer)	%drug release (co-crystal in 6.8 buffer)	% drug release (Pure drug in 0.1N HCl)	%drug release (co-crystal in 0.1N HCl)
1	10	5.50	21.87	15.62	64.80
2	20	8.28	34.59	23.53	67.30
3	30	8.58	43.72	35.20	76.20
4	40	12.69	49.47	40.23	77.40
5	50	17.82	56.78	48.25	83.40
6	60	22.78	61.04	49.81	102.10
7	70	23.09	65.39	56.52	-
8	80	26.31	70.13	61.05	-
9	90	30.47	71.38	61.98	-
10	120	34.46	74.58	65.35	-

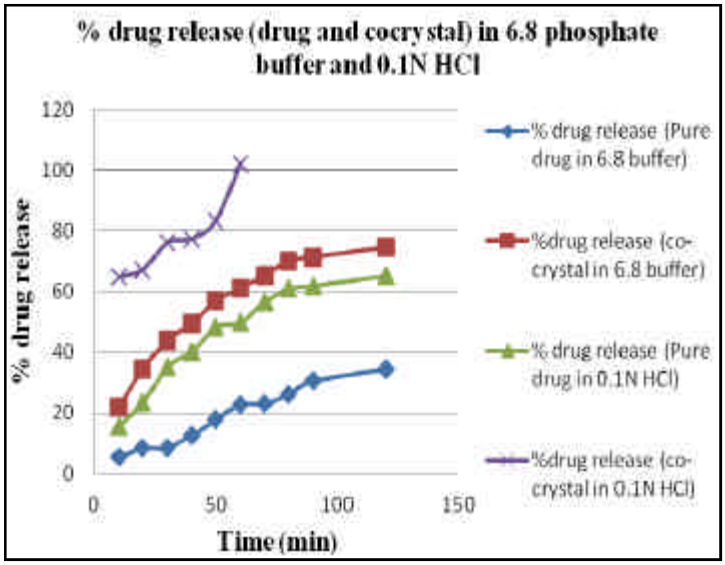

Figure 11: % drug release in 6.8 buffer and 0.1N HCl

From the figure 11 it can be concluded that co-crystal formed had shown more release as compared to pure drug in 0.1N HCl and 6.8 phosphate buffer.

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