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# Complexation of nevirapine with β-cyclodextrins in the presence and absence of Tween 80: characterization, thermodynamic parameters, and permeability flux

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**Abstract** The work is undertaken to evaluate the effect of Tween 80 on the complexing ability of β-cyclodextrins to encapsulate the poorly soluble antiretroviral agent, nevirapine. The phase solubility diagram indicates 1:1 stoichiometry and is supported by electronspray ionization mass spectrometry. The complexes were characterized by DSC, FT-IR, and XRD in the solid state. The ternary systems were autoclaved before being lyophilized for the best results. Proton NMR suggests that the methyl pyridine ring of the drug is involved in inclusion and enters from the wider side of the cavity which was confirmed by COESY NMR. Solution calorimetry, a direct method to determine the thermodynamic parameters, was used to determine the complexation constant ($K$) and other thermodynamic properties. The process is associated with negative $\Delta H$ and positive $\Delta S$ indicating a stable inclusion complex. The value of $K$ follows the order β-CD < HP-β-CD < M-β-CD. The molar enthalpy of solution in autoclaved solid formulation is less endothermic as compared to additive molar enthalpy of solution obtained by summation of enthalpy of solution of individual components suggesting synergistic interaction between the drug and its constituents. A threefold increase of the in vitro permeability flux was observed for binary systems which was elevated to fourfold for autoclaved ternary complexes.

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**Keywords** Nevirapine · Inclusion complexes · Tween 80 · Permeability studies · Thermodynamics

## Introduction

Nevirapine, 11-cyclopropyl-5,11-dihydro-4-methyl-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one (NEV), a well-known antiretroviral agent belonging to Class II of the Biopharmaceutics Classification System (BCS) has low solubility. Consequently, dissolution of this drug has been considered to be the rate-limiting step for absorption [1, 2]. Encaging the drug in hydrophilic cyclodextrins to form inclusion complexes can overcome its undesirable physicochemical properties resulting in increased aqueous solubility and stability [3–5]. The inclusion yield and efficiency of cyclodextrins has been reported to improve dramatically when used in the presence of several additives such as water soluble polymers [6–8], organic acids [9, 10], surfactants [11, 12], and alcohols [13]. Even the quaternary complexes have also been studied and characterized [14]. This solubilization enhancement is synergistic than being additive. The use of third component has emerged as an effective strategy to decrease the bulk of cyclodextrins needed to incorporate a given amount of drug in the dosage forms [15]. The ability of different cyclodextrins to solubilize the given drug is frequently evaluated by comparing the complexation constants ($K$) of the inclusion complexes which is a key factor for deciding the solubilization efficacy of different cyclodextrins [16]. Therefore, the determination of complexation constant in solution is the subject of active research [17, 18]. Solution calorimetry is the most important of the techniques described in the literature for directly measuring the complexation constant and other thermodynamic properties associated with encapsulation

[19]. The present work investigates the complexation of nevirapine with β-cyclodextrin (β-CD) along with its methyl-β-CD (M-β-CD) and hydroxypropyl-β-CD (HP-β-CD) derivatives including the studies in the presence of non-ionic surfactant (Tween 80). The focus is tilted toward numerical values of *K* and enthalpy of formation associated ($\Delta H$) with the binary as well as ternary complexes in order to establish the potential of Tween 80 on complexing efficacy of these cyclodextrins. The success of the study has been supported by enhancement in the flux through cellulose membrane.

## Experimental

### Materials

Nevirapine was obtained as a gift sample from Ranbaxy Laboratories Pvt. Ltd. (Poanta Sahib, India). β-CD and HP-β-CD were purchased from Hi-media Lab. Pvt Ltd. (Mumbai, India), and M-β-CD was purchased from Sigma-Aldrich, Co. (St. Louis, USA). All other chemicals were of analytical grade.

#### *Preparation of complexes*

Binary complexes were prepared using nevirapine with cyclodextrins in 1:1 molar ratio by (a) *physical mixing (PM)* in a pestle motar for 25 min and keeping it in a desiccator at room temperature for 5 days; (b) *kneading (KN)* the drug and cyclodextrin using methanol (about 15% of the total weight of the drug and cyclodextrin) for 90 min to obtain a homogenous paste and drying at room temperature overnight before screening through a 150 mesh sieve; (c) *lyophilizing* (*LY*) the drug and cyclodextrin solution in water (the solutions were stirred for 12 h and prefrozen at −80 °C) before being lyophilized; (d) *co-evaporating (Co-evp)* the drug and cyclodextrins in desired molar ratio were dissolved in ethanol in separate flasks. The solutions were added together, and the clear mixtures were then vacuum evaporated on a rota vapor at 70 °C at 200 rpm to get a solid residue complex which took 2 h.

Ternary complexes were prepared by adding Tween 80 (0.1% v/v) to a solution of drug and selected cyclodextrin in water and autoclaving at 120 °C for half an hour and freeze drying at −80 °C.

#### *Effect of cyclodextrins on the solubility of nevirapine in presence and absence of Tween 80*

Excess amount of nevirapine was added to 10 mL of phosphate buffer (pH 7.4) of selected cyclodextrins with or without 0.1% v/v Tween 80. The mixture was shaken at 37 °C for 24 h (MSW-275 Macro scientific works, Delhi), and the sample withdrawn after 24 h was filtered through 0.45 μm membrane filter and analyzed spectrophotometrically (Perkin Elmer precisely, Lambda 25, UV/VIS spectrophotometer) at 237 nm.

### Analysis in solid state

#### *Electronspray ionization mass spectroscopy (ESI-MS)*

ESI-MS study was performed using a Q-ToF quadrupole time of flight mass spectrometer (Waters) equipped with an electronspray source. The sample was introduced via a syringe pump at a flow rate of 5 μL/min. The spray voltage was set to 2.5 kV in the positive mode, and the heated metal capillary temperature was set at 80 °C.

#### *Differential Scanning Calorimetry (DSC)*

DSC curves were obtained on DSC (Q20, TA Instruments-Waters LLC, USA) which was calibrated using pure indium. The temperature range was from 50 to 350 °C with a heating rate of 10 °C per min.

#### *X-Ray powder diffraction (XRPD)*

X-ray diffractometer (XPERT-PRO, PANalytical, Netherlands) with Cu as tube anode, voltage 40 kV, 35 mA, angular range 5, and fixed divergence slit was used.

### Analysis in solution phase

#### *Nuclear Magnetic Resonance (NMR) Spectroscopy*

NMR spectra were obtained in deuterated dimethylsulfoxide on Bruker Advance II 400 NMR spectrometer operating at 400 Hz.

#### *Microcalorimetric study*

Isoperibol solution calorimetry (ISC) model 4300 (Calorimetry Science Corporation, UTAH, USA) was used to determine the enthalpy of solution. It is a semi-adiabatic calorimeter consisting of 25 mL silvered Dewar flask in a constant temperature bath held at 37 °C (±0.0001 °C). The sample is filled into ampoule which is shattered automatically by plunger and temperature change noted. Complexation thermodynamics of nevirapine with β-CD (and its derivatives) was determined by measuring the enthalpy of solution of drug in pure buffer (pH 7.4) and in buffered solution (pH 7.4) of cyclodextrins with and without 0.1% Tween 80. The solutions of cyclodextrins were prepared

over a concentration range of 0.001 to 0.01 M for binary and ternary system.

### Dissolution study

The dissolution profile of the nevirapine, its binary, and ternary complexes were obtained using USP XII apparatus equipped with paddle type tribune. Dissolution media consisted of 900 mL of phosphate buffer (pH 7.4), previously filtered, degassed, and maintained at 37 ± 0.5 °C. The stirring speed was set at 70 rpm. The amount of inclusion complex added was equivalent to 50 mg of nevirapine. The aliquots were withdrawn and analyzed after 15, 30, 60, 90, 120 min and then every 120 min till the absorbance of the solution attains a constant value. Each dissolution study was performed on duplicate batches.

### Permeability study

The permeation study was performed through a semi-permeable cellulose membrane (dialysis tubing, high retention seamless cellulose tubing, 12000 Dalton, Himedia, Mumbai, India). The cellulose membrane was placed in Franz-type diffusion cells; the surface area of membrane in the diffusion cells was 1.77 $cm^2$. The receptor phase (20 mL) consisted of phosphate buffer (pH 7.4). The membrane of the diffusion cell was kept at 37 °C by circulating water through an external jacket. The donor phase consisted of 5 mL of aqueous suspension or solution of 1 mg nevirapine or its equivalent amount of NEV–cyclodextrin complexes with or without Tween 80. Samples of receptor fluid (0.5 mL) were withdrawn at various intervals up to 6 h and replaced with fresh buffer solution and analyzed spectrophotometrically at 237 nm.

## Results and Discussion

### Effect of cyclodextrins on the solubility of nevirapine

The phase solubility diagram (Fig. 1a) reveals that the solubility of nevirapine increases linearly as a function of cyclodextrin concentration indicating it to be $A_L$ type for all the CDs. The slope of these $A_L$ type systems was less than one indicating 1:1 stoichiometry. Solubility increase was the highest for complex with M-β-CD, being ~40 times higher than that of the drug at 100 mmoles $L^{-1}$ concentration of M-β-CD. The solubilization strength of cyclodextrins follows the order: β-CD < HP-β-CD < M-β-CD. It may be noted that the intercepts of all the straight lines obtained from regression analysis correspond to the solubility of pure drug (0.01 ± 0.006 mM).

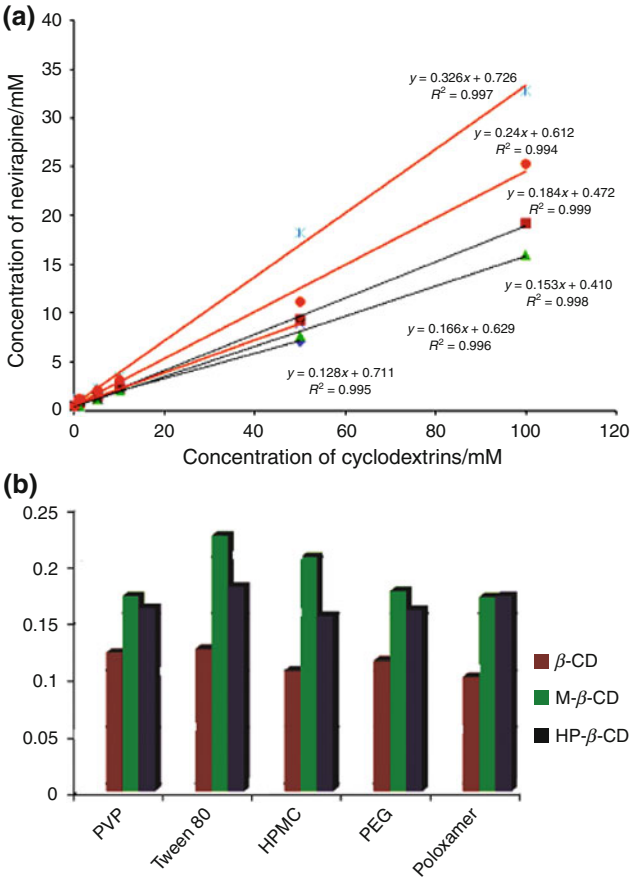


**Fig. 1** **a** Phase solubility diagrams of nevirapine with (*filled diamond*) β-CD, (*filled square*) M-β-CD, and (*filled triangle*) HP-β-CD in phosphate buffer (pH 7.4) and with (*x*) β-CD (*) M-β-CD and (*filled circle*) HP-β-CD in the presence of 0.1% Tween80 at 37 °C. Each point represents the mean of three determinations. **b** Solubility increase of nevirapine in buffer solution at 37 °C containing 30 mM of β-CD, M-β-CD, and HP-β-CD (saturation solubility) in the presence of 0.1% w/v of different polymers

### Selection of third component

The effect of various additives (polyvinylpyrrolidine (PVP), Tween 80, hydroxylpropyl methyl cellulose (HPMC), polyethylene glycol (PEG), and poloxmer on the solubilizing efficiency is shown in Fig. 1b. This experiment is very important for the proper choice of third component as some additives can produce the opposite effects [12]. The best performance was shown by Tween 80 (0.1% v/v) which resulted in 1.7 fold increase in solubility as compared to the same concentration of binary complex (Fig. 1b). The addition of surfactant does not change the stoichiometry of the complex (1:1) as the slope still continues to be less than 1. The ratio between the slopes of the phase solubility curves of ternary and binary systems, an index of the relative solublizing efficiency, is found to be 1.77 for M-β-CD, 1.58 for HP-β-CD, and 1.25 for β-CD, confirming the greater effectiveness of ternary system.

Analysis in solid state

*Electronspray ionization mass spectroscopy (EI-MS)*

In EI-MS, the species existing in solution can be transferred into the gas phase without breaking the non-covalent interaction present between the host and guest [20]. Figure 2 suggests that peak observed at *m/z* 267, corresponds to $[NEV + H]^+$. The peaks at m/z 1311 and at m/z 1577 correspond to $[M\text{-}\beta\text{-CD} + H]^+$ and $[NEV + M\text{-}\beta\text{-CD} + H]^+$, respectively, suggesting a 1:1 complexation of nevirapine with methyl-$\beta$-CD. Similarly, peaks at m/z 1647 and 1402 indicate the formation of $[NEV + HP\text{-}\beta\text{-CD} + H]^+$ and $[NEV + \beta\text{-CD} + H]^+$ complexes.

*Differential Scanning Calorimetry (DSC)*

The DSC trace of nevirapine exhibited a sharp melting endotherm at 247.7 °C. The thermal profile of nevirapine remained well recognizable in kneaded as well as co-evaporated products but with a reduction and broadening along with a shift in melting peak (Fig. 3). However, Patyi et al. [4] have shown the complete absence of melting endotherm in spironolactone-CD (1:2) complexes, whereas melting peak is visible in 1:1 complexes. It is well documented that in the cyclodextrin complexes the degree of interaction varies depending upon the nature of drug and methods of preparation [21, 22]. Thus, appearance of drug melting in co-evaporated complexes is ascribed to incomplete inclusion with some interaction. Accordingly, the complete disappearance of drug endothermal peak in the lyophilized complexes (NEV-CD LY) indicated drug amorphization and/or true inclusion. In all the ternary systems (NEV-CD-Tween 80), except with $\beta$-CD, the fusion endotherm of nevirapine was totally absent indicating the absence of any crystalline phase (Fig. 3).

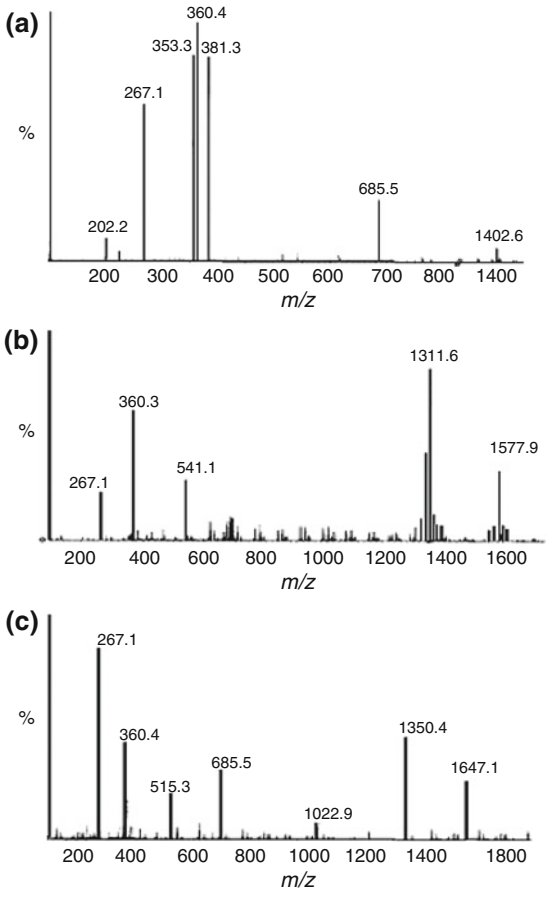


**Fig. 2** Mass spectra of nevirapine with **a** $\beta$-CD, **b** M-$\beta$-CD, **c** HP-$\beta$-CD

*X-ray powder diffraction (XRPD)*

The characteristic diffraction peaks relevant to crystalline nevirapine appear at $2\theta = 13.15°$ and 25.61° (Fig. 4). The diffraction patterns of the investigated PM and Co-evap system of nevirapine and cyclodextrins at 1:1 molar ratios are apparently superposition of diffraction pattern of individual components, indicating that drug maintained its crystallinity in the respective physical mixture. The KN system presented a diffraction pattern with fewer peaks of lower intensity. It was no longer possible to distinguish the characteristic peaks of the drug in the lyophilized system (NEV-CD LY) which showed a modified and halo pattern suggesting the formation of amorphous inclusion complex. The diffraction pattern for ternary system (NEV-CD LY) is also completely diffused showing its amorphous nature (Fig. 4).

## Analysis in solution phase

Nuclear Magnetic Resonance spectroscopy (NMR)

NMR plays a vital role in the analysis of cyclodextrin complexes because it can provide detailed spatial information on the interaction between guest molecule and cyclodextrin in the solution [23]. NMR studies reveal changes in the chemical shifts of protons attached to methyl pyridine rings of nevirapine indicating its entry in the cyclodextrin cavity (Table 1). The H-3 of methylpyridine ring shows an upfield shifts indicating its interaction with hydrogen atoms inside the cavity (Fig. 5a). A downfield shift was observed for the protons H-2 and methyl protons of the methyl pyridine ring which is due to interaction with the oxygen atoms inside the cyclodextrin cavity. The inclusion mode can be further explained on the basis of the size of methyl pyridine ring. The distance between the H-2 proton and protons of the methyl group of

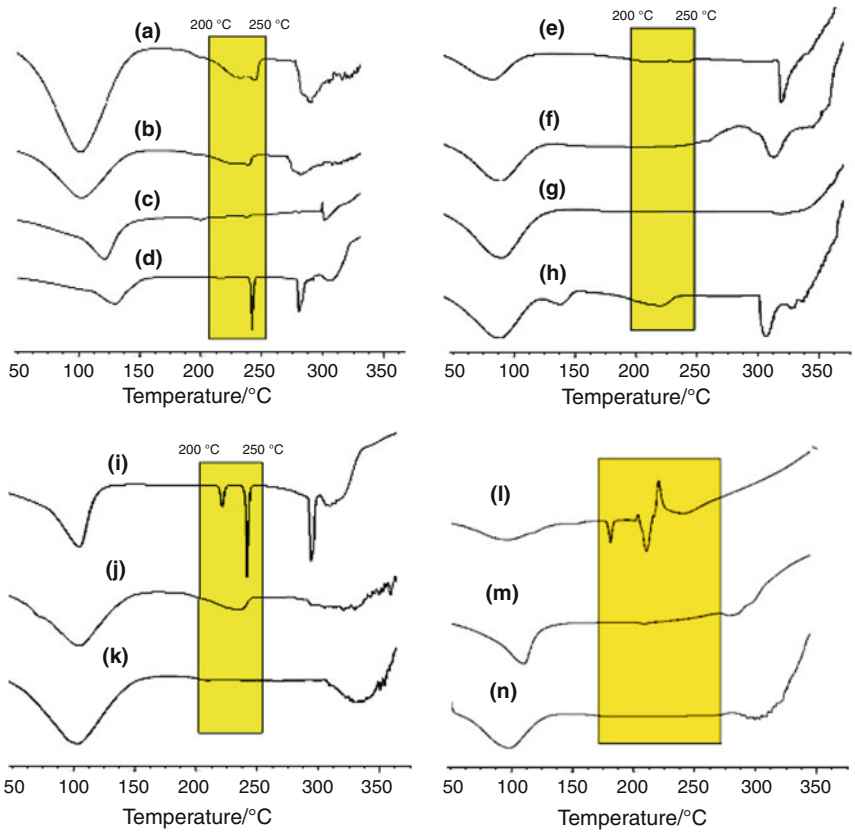


**Fig. 3** DSC curves of different nevirapine cyclodextrin systems (*a*) NEV-β-CD PM complex (*b*) NEV-β-CD KN complex (*c*) NEV-β-CD LY complex (*d*) NEV-β-CD co-evap complex (*e*) NEV–M-β-CD PM complex (*f*) NEV–M-β-CD KN complex (*g*) NEV–M-β-CD LY complex (*h*) NEV–M-β-CD co-evap complex (*i*) NEV-HP-β-CD PM complex (*j*) NEV-HP-β-CD KN complex (*k*) NEV-HP-β-CD LY complex (*l*) NEV-β-CD complex containing 0.1% Tween 80 (*m*) NEV–M-β-CD complex containing 0.1% Tween 80 and (*n*) NEV-HP-β-CD complex containing 0.1% Tween 80

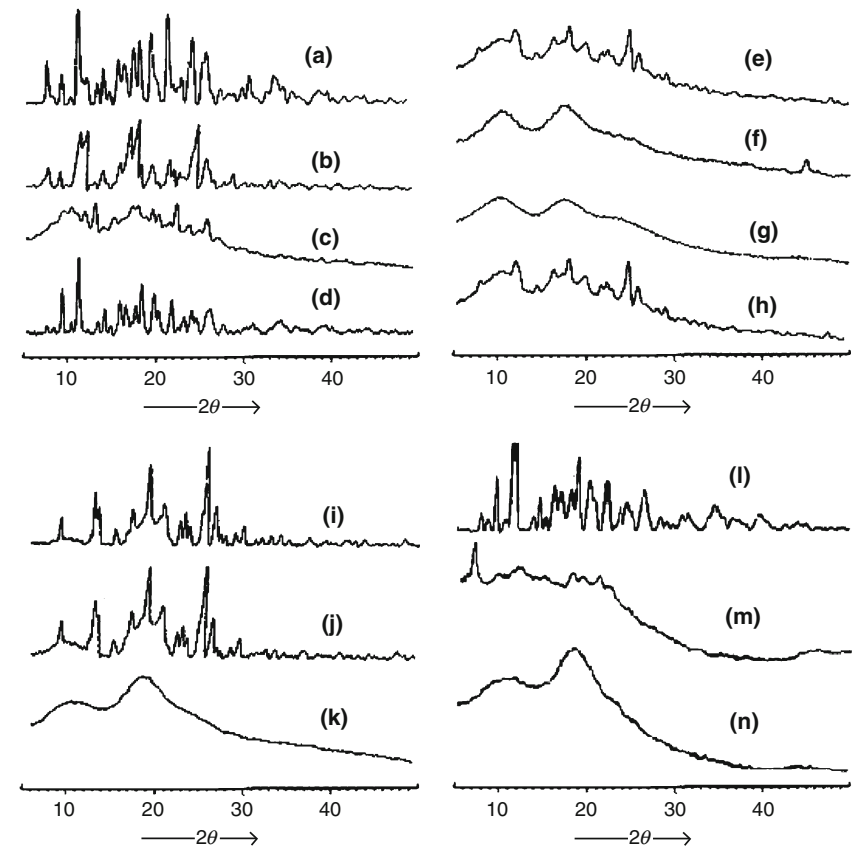


**Fig. 4** X-ray diffraction pattern of different nevirapine cyclodextrin systems (*a*) NEV-β-CD PM complex (*b*) NEV-β-CD KN complex (*c*) NEV-β-CD LY complex (*d*) NEV-β-CD co-evap complex (*e*) NEV–M-β-CD PM complex (*f*) NEV–M-β-CD KN complex (*g*) NEV–M-β-CD LY complex (*h*) NEV–M-β-CD co-evap complex (*i*) NEV-HP-β-CD PM complex (*j*) NEV-HP-β-CD KN complex (*k*) NEV-HP-β-CD LY complex (*l*) NEV-β-CD complex containing 0.1% Tween 80 (*m*) NEV–M-β-CD complex containing 0.1% Tween 80 and (*n*) NEV-HP-β-CD complex containing 0.1% Tween 80

**Table 1** Variation in chemical shifts of some nevirapine protons in the presence of $\beta$-CD, M-$\beta$-CD, and HP-$\beta$-CD

| | $\beta$-CD $\Delta\delta$/ppm | M-$\beta$-CD $\Delta\delta$/ppm | HP-$\beta$-CD $\Delta\delta$/ppm |
|---|---|---|---|
| H-2 | 0.0049 | 0.0013 | 0.0036 |
| H-3 | −0.0086 | −0.0115 | −0.0069 |
| Methyl-protons | 0.0057 | 0.0027 | 0.0052 |
| H-11a | 0.0008 | −0.0002 | −0.0152 |
| H-11b,c | −0.0018 | 0.0015 | 0.0013 |
| H-11d,e | −0.0012 | 0.0011 | −0.0013 |

$\Delta\delta = \delta_{complex} - \delta_{free}$

methylpyridine ring of nevirapine is ~5.271 Å suggesting the ability of the whole part of the molecule to be inside the cavity (Fig. 5b, c). The entrance of drug cyclopropane ring can be ruled out because of its relatively smaller size (cis distance: 2.641 Å, trans distance: 3.185 Å) as compared to the cavity size of $\beta$-CD and its derivatives allowing the drug to move in and out of the cavity. The small change in cyclopropyl protons chemical shift can be attributed to the interaction with the hydrogen atoms outside the cavity or structural rearrangement.

The 2D $H^1$-$H^1$ COESY spectra (Fig. 6) were further used to support the 1:1 stoichiometry and geometry of the inclusion. The figure shows the 2D $H^1$-$H^1$ COESY spectra of the drug with cyclodextrins. The off-diagonal peaks are observed between the H-3 and H-5 protons of $\beta$-cyclodextrins (present on the wider side) and methyl protons of methyl pyridine ring suggesting its entrance from the wider side of the cavity. Similar cross peaks were found in M-$\beta$-CD and HP-$\beta$-CD but due to random substitution the peaks could not be clearly identified.

*Microcalorimetric study*

The solution calorimetry has been employed to confirm the stoichiometry as well as the stability constants and thermodynamic parameters associated with the binding process of the complex formed [24]. The enthalpy of solution of nevirapine ($\Delta_{sol}H$) was determined in buffered aqueous solution showing exothermicity. The molar enthalpy of solution ($\Delta_{sol}H_{(M)}$) is −2.01 kJ/mol. The enthalpy of solution of nevirapine ($\Delta_{sol}H_{(CD)}$) in the presence of cyclodextrins ($\Delta_{sol}H_{(M)(CD)}$) was found to be more exothermic which is attributed to interaction between drug and cyclodextrins. Enthalpy of interaction between drug and cyclodextrin per liter of solution ($\Delta_{sol}H_{int(exp)}$) was determined by Eq. 1 where *v (l),* is the volume of sample cell (0.025 L).

$$\Delta_{sol}H_{int(exp)} = \left(\Delta_{sol}H_{(CD)} - \Delta_{sol}H\right)/v(l) \quad (1)$$

Table 2 shows the calorimetric data over the whole concentration range for nevirapine and M-$\beta$-CD. Similar

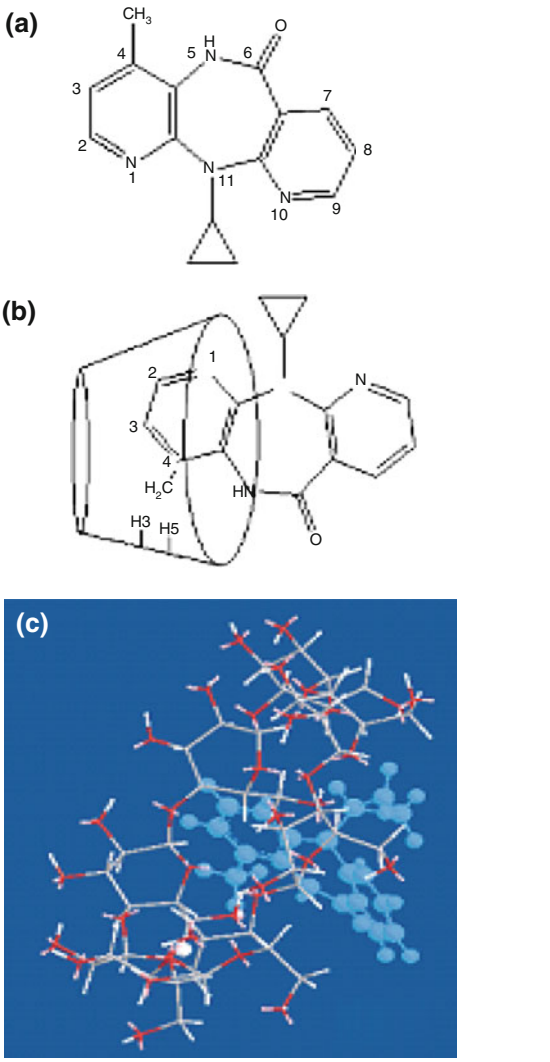


**Fig. 5** **a** The chemical structure of nevirapine, **b** proposed inclusion mode of drug into cyclodextrin cavity, **c** molecular representation of inclusion complex of nevirapine with $\beta$-CD drawn using ligandFit module of Accerlys (Discovery studio 2.0 version)

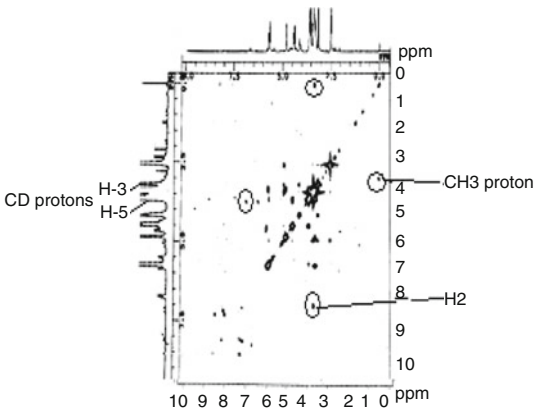


**Fig. 6** Proton COESY spectrum showing off diagonal peaks of methyl pyridine ring of nevirapine with $\beta$-CD

**Table 2** Enthalpy of solution of nevirapine ($\Delta_{sol}H_{(CD)}$) in buffered solution of M-β-CD over a range of initial concentrations, interaction enthalpy per liter ($\Delta_{sol}H_{int(exp)}$) and molar enthalpy of interaction ($\Delta_{sol}H_{int(M)}$) between nevirapine and M-β-CD

| $X_2$ | a/ mM | b/ mM | $\Delta_{sol}H_{(CD)}$/ J × $10^{-2}$ | $\Delta_{sol}H_{int(exp)}$/ J/l | $\Delta_{sol}H_{int(M)}$/kJ/ mol |
|---|---|---|---|---|---|
| 0.095 | 2.36 | 0.25 | −18.44 | −2.62 | −1.01 |
| 0.113 | 2.60 | 0.33 | −20.72 | −3.32 | −1.19 |
| 0.162 | 2.19 | 0.43 | −1.89 | −4.35 | −1.66 |
| 0.186 | 2.03 | 0.46 | −21.77 | −4.62 | −1.86 |
| 0.207 | 1.99 | 0.52 | −22.86 | −5.14 | −2.05 |
| 0.255 | 1.94 | 0.66 | −25.88 | −6.44 | −2.47 |
| 0.318 | 1.77 | 0.83 | −27.93 | −7.61 | −2.94 |
| 0.359 | 1.63 | 0.92 | −28.38 | −8.06 | −3.16 |
| 0.415 | 1.47 | 1.04 | −28.69 | −8.52 | −3.39 |
| 0.483 | 1.41 | 1.32 | −29.78 | −9.22 | −3.57 |
| 0.446 | 1.12 | 1.14 | −28.05 | −8.92 | −3.92 |
| 0.584 | 0.84 | 1.19 | −25.64 | −8.21 | −3.37 |
| 0.603 | 1.07 | 1.56 | −24.48 | −7.85 | −3.32 |
| 0.720 | 0.75 | 1.93 | −21.73 | −7.18 | −2.67 |
| 0.801 | 0.65 | 2.67 | −20.16 | −6.77 | −2.04 |
| 0.893 | 0.43 | 3.58 | −13.95 | −4.73 | −1.18 |

results are obtained for β-CD and HP-β-CD. The magnitude of interaction depends upon the concentration of the drug as well as cyclodextrins. The enthalpy of interaction per mole of both the drug and cyclodextrin ($\Delta_{sol}Hint_{(M)}$) was calculated using Eq. 2

$$\Delta_{sol}H_{int(M)} = \left(\Delta_{sol}H_{int(exp)}\right)/(a+b) = \left(\Delta_{sol}H_{(M)(CD)} - \Delta_{sol}H_{(M)}\right)/(1+(x_2/x_1)) \quad (2)$$

$\Delta_{sol}H_{(M)(CD)}$ is the molar enthalpy of solution of drug in buffered aqueous solution of cyclodextrins; $x_1$ and $x_2$, apparent mole fractions of the drug and cyclodextrin ignoring the concentration of buffers; *a, b* are the molar concentrations of drug and cyclodextrin, respectively.

The stoichiometry was ascertained utilizing continuous variation method (Job's plot) by plotting $\Delta_{sol}H_{int(M)}$ against $x_2$ for all the complexes (Fig. 7). The plot is a symmetric bell-shaped curve showing minima at $x_2 = 0.5$ indicating 1:1 stoichiometry for all the NEV: CD complexes.

The equilibrium constants have been calculated assuming the following reaction:

$$NEV + CD \leftrightarrow NEV : CD \quad (3)$$

The experimentally determined interaction enthalpy per liter ($\Delta_{sol}H_{int(exp)}$) is proportional to the product of molar concentration of drug-cyclodextrin complex (c) in solution at equilibrium and enthalpy of complexation *(ΔH)* i.e.

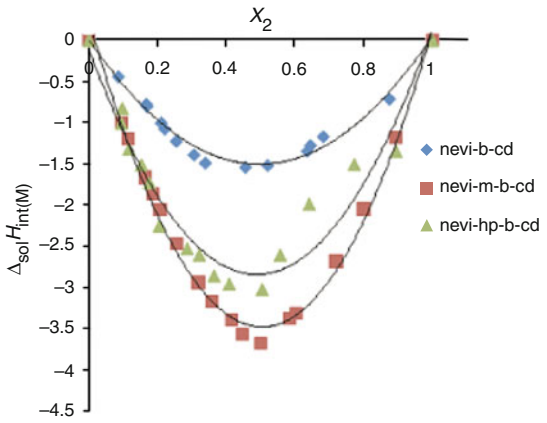


**Fig. 7** Plot of molar enthalpy of interaction ($\Delta_{sol}H_{int(M)}$) of nevirapine with different cyclodextrins versus mole fraction ($x_2$) of β-CD, M-β-CD and HP-β-CD at pH 7.4

$$\Delta_{sol}H_{int(exp)} = \Delta H \times c \quad (4)$$

The equilibrium constant for the above equation is given by Eq. 5 which can be rearranged to Eq. 6:

$$K = c/\{(a-c)(b-c)\} \quad (5)$$

$$c^2 - (a+b+1/K)c + ab = 0. \quad (6)$$

The physically acceptable solution for the above quadratic equation is given in Eq. 7:

$$c = \left[(a+b+1/K) - \sqrt{\{(a+b+1/K)^2 - (4ab)\}}\right]/2 \quad (7)$$

Putting Eq. 7 in Eq. 4,

$$\Delta_{sol}H_{int(exp)} = \Delta H \times \left[\left(A - \sqrt{\{(A)^2 - 4ab\}}\right)/2\right] \quad (8)$$

The best fit values of the thermodynamic parameters *K* and *ΔH* were determined by our computer program utilizing an iterative non-linear least square regression method to minimize the value of $\Sigma\Delta_{sol}H_{int(exp)} - \Delta_{sol}H_{int(calc)})^2$. The values of free energy of inclusion (Δ*G*) and entropy of inclusion (Δ*S*) are given in Table 3.

The same method was also used to determine the stability constant and other thermodynamic parameters in the presence of Tween 80. Nevirapine behaved endothermically in buffered aqueous solution of Tween 80 (0.1%) and molar enthalpy of solution of nevirapine ($\Delta_{sol}H_{(M)}$) is 11.9 kJ/mol. The enthalpy of solution of nevirapine in the buffered aqueous solution of Tween 80 (0.1%) and cyclodextrins ($\Delta_{sol}H_{(M)(CD)}$) was found to be less endothermic than molar enthalpy of solution in buffer containing 0.1% Tween 80. This is attributed to interaction between all the three components. The interaction enthalpy per liter of solution $\Delta_{sol}H_{int(exp)}$ was calculated using Eq. 2. Subsequently, the values

**Table 3** Thermodynamic parameters associated with the inclusion of nevirapine with $\beta$-CD, M-$\beta$-CD, and HP-$\beta$-CD at pH 7

| System | $K$/M$^{-1}$ | $\Delta H$/kJ mol$^{-1}$ | $\Delta G$/kJ mol$^{-1}$ | $\Delta S$/J mol$^{-1}$K$^{-1}$ |
|---|---|---|---|---|
| Nevirapine + $\beta$-CD | 890 ± 2 | −8.20 ± 0.02 | −17.50 ± 0.01 | 28.94 ± 1.8 |
| Nevirapine + M-$\beta$-CD | 2340 ± 6 | −12.60 ± 0.02 | −19.99 ± 0.01 | 22.08 ± 2.5 |
| Nevirapine + HP-$\beta$-CD | 2140 ± 4 | −10.60 ± 0.02 | −19.76 ± 0.01 | 29.56 ± 1.7 |
| Nevirapine + $\beta$-CD in 0.1% Tween80 | 955 ± 1 | −9.88 ± 0.02 | −17.68 ± 0.01 | 25.18 ± 2.2 |
| Nevirapine + M-$\beta$-CD in 0.1% Tween80 | 2737 ± 7 | −13.40 ± 0.04 | −20.40 ± 0.01 | 22.58 ± 3.5 |
| Nevirapine + HP-$\beta$-CD in 0.1% Tween80 | 2200 ± 3 | −11.80 ± 0.03 | −19.84 ± 0.01 | 25.92 ± 2.8 |

**Table 4** Observed molar enthalpy of solution ($\Delta H_{formu}$), additive molar enthalpy of solution ($\Sigma \Delta H_i x_i$) and enthalpy of interaction ($\Delta_{sol} H_{int}$) of autoclaved lyophilized ternary complexes of nevirapine

| Formulation No. | Type of CD | $\Sigma \Delta H_i x_i$/ kJ mol$^{-1}$ | $\Delta H_{formu}$/ kJ mol$^{-1}$ | $\Delta_{sol} H_{int}$/ kJ mol$^{-1}$ |
|---|---|---|---|---|
| I | $\beta$-CD | 22.67 | 16.66 | −6.02 |
| II | M-$\beta$-CD | 28.49 | 16.29 | −12.20 |
| III | HP-$\beta$-CD | 24.14 | 15.45 | −8.68 |

of $K$, $\Delta H$, $\Delta G$, and $\Delta S$ were calculated utilizing Eqs. 3 and 9 and are given in Table 4.

The magnitude of $K$ reflects the efficiency of guest molecule to be included inside the cavity of the cyclodextrin molecule both for binary and ternary systems (Table 3). The absolute value of $K$ increases in the order $\beta$-CD < HP-$\beta$-CD < M-$\beta$-CD. This shows that substituent groups assist in binding by lengthening the cavity and also by increasing the hydrophobicity. However, the effect may be counterbalanced by the presence of hydroxyl group in case of HP-$\beta$-CD which is reflected by somewhat smaller value of $K$ in binary (2140 M$^{-1}$) as well as ternary complexes (2200 M$^{-1}$). The complexation ability of M-$\beta$-CD significantly increases for both binary ($K$ = 2340 M$^{-1}$) as well as for ternary system ($K$ = 2737.5 M$^{-1}$) as methylation enlarges the cavity of substituted cyclodextrin toward nevirapine making the environment around it more hydrophobic and allows for increased adaptability of cyclodextrin toward the guest through enhanced flexibility.

As mentioned in the literature multiple driving forces including hydrophobic as well as van der Waals interactions exerting simultaneously lead to true inclusion. Table 3 shows that enthalpy of binding ($\Delta H$) is negative in all the cases reflecting an exothermic interaction. Negative enthalpy changes are accounted for by pronounced van der Waals interactions. The inclusion phenomenon in the present study is also accompanied by a favorable entropic term ($\Delta S > 0$) which may be due to release/restructuring of water molecules inside and around the cyclodextrin cavity. The value of entropy of binding follows the order: M-$\beta$-CD < HP-$\beta$-CD < $\beta$-CD. The highest magnitude of negative $\Delta H$ and smaller value of positive $\Delta S$ for M-$\beta$-CD is due to appreciable enthalpy entropy compensation taking place in the cyclodextrin complexation [25].

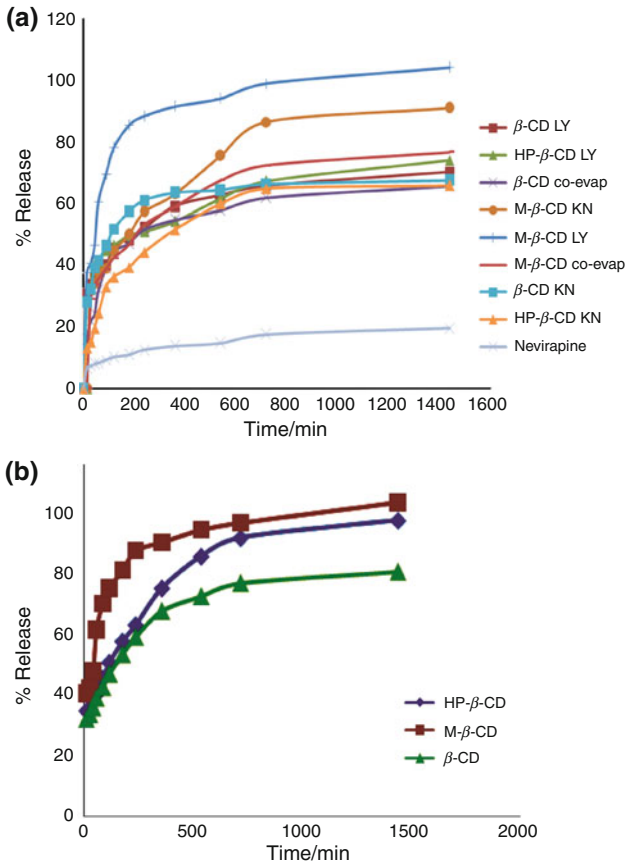


**Fig. 8** **a** Nevirapine release rate profile in phosphate buffer (pH 7.4) from binary inclusion complexes; (*gray cross*) nevirapine, (*blue square*) NEV-$\beta$-CD KN complex, (*brown square*) NEV-$\beta$-CD LY complex, (*violet cross*) NEV-$\beta$-CD co-evap complex, (*brown circle*) NEV-M-$\beta$-CD KN complex, (*blue bar*) NEV-M-$\beta$-CD LY complex, (*brown dash*) NEV-M-$\beta$-CD co-evap complex, (*orange triangle*) NEV-HP-$\beta$-CD KN complex and (*green triangle*) NEV-HP-$\beta$-CD LY complex. **b** Dissolution profile of lyophilized ternary complexes of NEV-cyclodextrin-0.1% w/v Tween 80 (*dark green triangle*) NEV-$\beta$-CD complex, (*dark red square*) NEV-M-$\beta$-CD complex and (*violet rhombus*) NEV-HP-$\beta$-CD complex. (Color figure online)

The NEV-M-β-CD-Tween 80 ternary complex is associated with higher value of equilibrium constant accompanied by favorable enthalpic and entropic terms. The higher binding constant value illustrates that the presence of Tween 80 facilitates the inclusion. The less positive $\Delta S$ value can be ascribed to greater structural restraints as a consequence of ternary complexation.

## Enthalpy of interaction in autoclaved ternary complexes

The enhancement of solubility as well as the complexation constant in the ternary complexes led us to the autoclaving of the ternary components to get a solid formulation. The improvement of solubility after autoclaving is attributed to the enhancement of interaction of the components in the ternary system. The enthalpy of interaction between various constituents after autoclaving was estimated by determining the enthalpy of solution of formulation ($\Delta H_{\text{formu}}$) as well that of pure components. The molar enthalpy of interaction ($\Delta H_{\text{int}}$) in autoclaved solid formulation containing drug, cyclodextrins, and Tween 80 was calculated from:

$$\Delta_{\text{sol}} H_{\text{int}} = \Delta H_{\text{formu}} - (\Sigma \Delta H_i x_i) \quad (9)$$

$$\Delta_{\text{sol}} H_{\text{int}} = \Delta H_{\text{formu}} - (\Delta H_1 x_1 + \Delta H_2 x_2 + \Delta H_3 x_3) \ldots\ldots\ldots \quad (10)$$

$\Delta H_1$, $\Delta H_2$, and $\Delta H_3$ are the molar enthalpy of solution of nevirapine, cyclodextrin, and Tween 80, respectively; $x_1$, $x_2$, and $x_3$ are mole fraction of nevirapine, cyclodextrin, and Tween 80, respectively, in the formulation. $\Sigma \Delta H_i x_i$ is the sum of contribution of various constituents toward total enthalpy of solution.

Numerical values of $\Delta H_{\text{formu}}$ along with the additive molar enthalpy of solution obtained by summation of solution enthalpies of individual components are given in Table 4 for comparison.

A lower value of endothermic enthalpy of solution of our autoclaved ternary complexes as compared to additive molar enthalpy of solution is suggestive of favorable interactions between the drug and its constituents (Table 4). The results show that interaction is strongest for the formulation containing M-β-CD. This is in agreement with the DSC and PXRD results confirming its highly amorphous nature.

## Dissolution studies

The release profiles of binary and ternary complexes prepared by different methods are given in Fig. 8a and b. In all binary systems, the dissolution rate was observed to be maximum for the complexes prepared by lyophillization than by other methods. The increase in dissolution rate for the complexes of nevirapine prepared with β-CD, M-β-CD, and HP-β-CD followed the order PM < Co-evap < KN < LY. However, M-β-CD had a greater effect on enhancing the dissolution rate of the drug. The heightened effectiveness of M-β-CD can be attributed to its greater ability to amorphize and consequently to solublize the complexed drug. The dissolution profile of nevirapine cyclodextrin ternary systems containing 0.1% Tween 80 showed further increase in the dissolution rate (Fig. 8b). It is clear from the plot that the addition of non-ionic surfactant markedly enhances the dissolution rate of nevirapine as compared to binary systems.

**Table 5** In vitro permeation characteristics of nevirapine in lyophilized binary (NEV-CD) and ternary (NEV-CD-Tween 80) complexes through cellulose membrane (value ± SD)

| Time/min | % release of Nevirapine | % release of Nevirapine in binary complexes | | | % release of Nevirapine ternary complexes | | |
|---|---|---|---|---|---|---|---|
| | | β-CD | M-β-CD | HP-β-CD | β-CD | M-β-CD | HP-β-CD |
| 0 | 2.13 ± 0.02 | 5.39 ± 0.02 | 8.71 ± 0.04 | 8.08 ± 0.03 | 8.31 ± 0.05 | 11.70 ± 0.04 | 10.33 ± 0.03 |
| 30 | 2.77 ± 0.03 | 7.54 ± 0.03 | 10.21 ± 0.02 | 8.84 ± 0.03 | 9.30 ± 0.04 | 15.49 ± 0.02 | 11.86 ± 0.01‘ |
| 60 | 3.19 ± 0.02 | 9.08 ± 0.03 | 14.51 ± 0.02 | 12.52 ± 0.03 | 13.93 ± 0.04 | 17.03 ± 0.02 | 13.90 ± 0.05 |
| 90 | 3.95 ± 0.02 | 10.33 ± 0.02 | 16.49 ± 0.05 | 14.48 ± 0.01 | 15.70 ± 0.03 | 18.91 ± 0.05 | 16.89 ± 0.05 |
| 120 | 4.71 ± 0.03 | 13.30 ± 0.03 | 18.53 ± 0.05 | 18.47 ± 0.02 | 17.89 ± 0.07 | 21.32 ± 0.02 | 20.30 ± 0.04 |
| 180 | 5.67 ± 0.03 | 15.85 ± 0.02 | 21.90 ± 0.06 | 20.74 ± 0.03 | 20.87 ± 0.02 | 24.54 ± 0.01 | 23.69 ± 0.00 |
| 240 | 7.12 ± 0.03 | 17.97 ± 0.01 | 25.92 ± 0.03 | 22.51 ± 0.03 | 24.10 ± 0.03 | 28.76 ± 0.03 | 27.34 ± 0.03 |
| 300 | 8.55 ± 0.02 | 19.89 ± 0.03 | 27.67 ± 0.04 | 24.71 ± 0.04 | 26.69 ± 0.06 | 32.34 ± 0.01 | 29.28 ± 0.04 |
| 360 | 11.39 ± 0.01 | 22.89 ± 0.05 | 29.67 ± 0.01 | 26.90 ± 0.06 | 28.51 ± 0.02 | 37.52 ± 0.04 | 31.28 ± 0.04 |
| R*/mg $cm^2 min^{-1}$ | 0.011 | 0.023 | 0.029 | 0.026 | 0.028 | 0.033 | 0.030 |

R* (Permeability flux)

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference/$P \leq 0.05$

Permeability Studies

The cyclodextrins induced bioavailability of drug was assessed by in vitro measurement of drug permeability through cellulose membrane as the barrier model. In this study, permeation was performed on lyophilized binary complexes of nevirapine with $\beta$-CD, M-$\beta$-CD, and HP-$\beta$-CD (Table 5). The in vitro study was also performed on autoclaved ternary systems containing 0.1% Tween 80. Table 5 shows that there is a significant increase ($P < 0.05$) in the permeability flux (R) of nevirapine in binary complex as compared to drug alone that permeated through the cellulose membrane. The extent of enhancement of drug permeation flux is found to be dependent on the type of cyclodextrin and permeation follows the order of M-$\beta$-CD > HP-$\beta$-CD > $\beta$-CD. A further increase in permeation is obtained when a surfactant is present as the ternary component. Thus, it can be concluded that the permeation improve significantly when nevirapine is complexed with cyclodextrins, and this improvement is more prominent in the presence of Tween 80.

## Conclusions

The data suggest higher efficiency of M-$\beta$-CD in the solubility and, consequently, the permeability of nevirapine as compared to native and HP-$\beta$-CD. The effect is further enhanced in the presence of Tween 80. The DSC and XRPD studies reveal that the lyophilized product to be most amorphous. The highest value of $K$ for NEV–M-$\beta$-CD complex supports its higher potential for complexation. It is heartening to note that the autoclaved NEV–M-$\beta$-CD–Tween 80 complex represents an effective formulation to enhance the physicochemical parameters of this poorly soluble anti-HIV drug.

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