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ORIGINAL ARTICLE

Effect of hydrophilic polymer on complexing efficiency of cyclodextrins towards efavirenz-characterization and thermodynamic parameters

Renu Chadha · Poonam Arora · Swati Bhandari · Dharam Vir Singh Jain

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Abstract The present work describes the effect of PVP on the complexation efficiency of cyclodextrins towards efavirenz, a poorly soluble antiretroviral agent imparting irritating sensation to buccal cavity. The phase solubility study indicates 1:1 stoichiometry for binary and ternary systems. DSC and XRPD revealed complete inclusion only in the lyophilized systems. The ternary systems were autoclaved before being lyophilized for the best results. Proton NMR suggests that the chlorobenzene part of benzoxazinone ring of the drug is involved in inclusion and was confirmed by 2D-COESY. The thermodynamic parameters, indicative of complexation efficiency were calculated calorimetrically by determining the interaction enthalpy of efavirenz with cyclodextrins in the presence and absence of PVP. The value of stability constants increased in the order β-CD < HP-β-CD < M-β-CD and is still higher in the presence of PVP illustrating the facilitation of the inclusion. Molar enthalpy of interaction of autoclaved solid formulation determined calorimetrically indicated stronger interaction for efavirenz:M-βCD-PVP system (−12.20 kJ/mol) which showed highest solubility and dissolution rate. The in vitro measurement of permeability showed a ten fold increase in the flux for the autoclaved formulation containing efavirenz-M-β-CD-PVP. In conclusion, encapsulation by cyclodextrins increases the solubility and suppresses the oral irritation of efavirenz. PVP further increases the complexation efficiency and decreases the bulk of cyclodextrins.

Keywords Cyclodextrins · Efavirenz · Polyvinylpyrrolidine · Calorimetry · Stoichiometry

R. Chadha (✉) · P. Arora · S. Bhandari
University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh 160014, India
e-mail: renukchadha@rediffmail.com

D. V. S. Jain
Department of Chemistry, Panjab University, Chandigarh 160014, India

Introduction

Efavirenz (Fig. 1), (4*S*)-6-chloro-4-(cyclopropylethynyl)-4-(trifluromethyl)-1,4-dihydro-2*H*-3,1-benzoxazin-2-one, is a HIV-1 specific, non-nucleoside reverse transcriptase inhibitor, used for the treatment of AIDS in combination with other retroviral agents [1]. Unfortunately, it exhibits low solubility [2–4] in aqueous gastric fluid and imparts a strong and prolonged burning sensation to the mouth when incorporated in water in the liquid formulations [4–6] and needs a potential drug carrier which can also mask its unpalatable taste. Engaging the drug in hydrophilic cyclodextrins to form inclusion complexes can provide an ideal solution under such conditions. The role of cyclodextrin and its derivatives in improving the physicochemical properties such as solubility [7, 8], palatability [9, 10] and decreasing intestinal irritation [11] by forming reversible inclusion complexes is well established, however, the amount of cyclodextrin that can be incorporated is limited because of the various issues such as cost, toxicology and increase in the bulk of the solid dosage form. The addition of third component is emerging as a successful corrective measure which minimizes the amount of cyclodextrin used and enhances the extent of complexation. Multiple technological alternatives e.g. addition of water soluble polymers [12–16], surfactant [17–19], co-solvents

[20, 21], volatile acids or bases, organic salts [22, 23] and amino acids [24, 25] have been used as third components. Among all these alternatives, the addition of water soluble polymers has been widely used. The complexation efficiency is affected by the type of cyclodextrin as well as the polymer and the interactions between them. The present work describes the inclusion complexes of efavirenz with β-CD and its methyl and hydroxyl propyl derivatives [26]. The role of PVP in improving the complexation efficacy of cyclodextrins towards drug is also a part of present investigation. The ability of different cyclodextrins to solubilize a given drug is frequently evaluated by comparing the stability constants [27]. Therefore, emphasis has been laid on determining the equilibrium constant along with other thermodynamic parameters in the absence and presence of third component. For this, solution calorimetry has been employed that has been found to be the most suitable for the binding studies [28]. As far as we know not much information is available except for one report by Sathigiri et al. [23] on efavirenz and cyclodextrin inclusion complexes but the thermodynamic parameters and the effect of PVP is lacking.

Experimental

Materials

Efavirenz 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 and M-β-CD was purchased from Sigma-Aldrich. All other chemicals were of analytical grade.

Preparation of binary complexes: Efavirenz with β-CD, M-β-CD and HP-β-CD were mixed in 1:1 molar ratio by different methods as given below.

Physical mixing (PM)

Efavirenz and cyclodextrins were mixed together in the 1:1 molar ratio in a pestle motar for 25 min and were kept in a desiccator.

Kneading (KN)

The kneaded complexes were prepared by mixing the drug with cyclodextrins using about 15% methanol of the total weight of the drug and cyclodextrin mixture. The solvent was gradually added and kneading was performed for 90 min to obtain a homogenous paste. The paste was then dried at room temperature overnight, then ground and finely screened through a 150 mesh sieve.

Lyophilization (LY)

Efavirenz and different cyclodextrins were dissolved in 100 mL of triply distilled water and magnetically stirred at room temperature for 12 h. The solutions were then frozen at −80 °C and freeze dried in a lyophillizer.

Co-precipitation (Co-ppt)

The required amount of efavirenz and cyclodextrins were dissolved separately in ethanol and water. Then the cyclodextrin solution was slowly added to alcoholic solution of efavirenz to give precipitates of the complex formed. The resulting complex was then filtered and dried at room temperature to give efavirenz cyclodextrin complex. The solid residue was passed through a 150 mesh sieve.

Preparation of ternary complex formulation

Polyvinylpyrrolidine (PVP) (0.20%) was added to 1:1 molar solution of drug and different cyclodextrins in water; the mixture was then autoclaved at 120 °C for half an hour and freeze dried at −80 °C to obtain the solid complex.

Characterization of binary (drug cyclodextrin) and ternary (drug cyclodextrin polymer) complexes

Effect of cyclodextrins on the solubility of efavirenz

Phase solubility studies for both binary and ternary systems were carried out according to Higuchi and Connors (30). Excess amount of efavirenz was added to 10 mL of aqueous solution of β-CD, M-β-CD, and HP-β-CD. The aqueous solution used for efavirenz was phosphate buffer (pH 7) with or without a fixed PVP concentration of 0.20% v/v. The concentration of cyclodextrins was varied over a range of 0.001–0.2 M for the binary and 0.01–0.08 M for the ternary mixtures. The mixtures were shaken at 37 °C for 72 h and the samples were withdrawn at 24 h. The aliquots were filtered through 0.45 μm membrane filter and analyzed spectrophotometrically at 250 nm. The shaker made by MSW-275 Macro scientific works, Delhi was used. The standard plot of efavirenz was prepared by dissolving a weighed amount of the drugs in phosphate buffer (pH 7), suitably diluted and absorbance measured at wavelength 250 nm on a spectrophotometer.

Analysis in solid state

Differential Scanning Calorimetry (DSC)

DSC thermograms were obtained on DSC, (Q20, TA Instruments-Waters LLC, USA). The calorimeter was

calibrated for temperature and heat flow accuracy using the melting of pure indium (mp 156.6 °C and ΔH of 25.45 J g^{-1}). The temperature range was 50–250 °C with a heating rate of 10° per minute.

X-Ray powder diffraction (PXRD)

Powder diffraction patterns were recorded on an X-ray diffractometer (XPERT-PRO, PANalytical, Netherlands) with Cu as tube anode. The diffractograms were recorded under the following conditions: voltage 40 kV, 35 mA, angular range five and fixed divergence slit.

Fourier Transform Infrared spectrometry (FT-IR)

The FT-IR spectra were obtained on FT-IR spectrometer, Mode spectrum RXI, Perkin Elmer, England over the range 400–4,000 cm^{-1}. Dry KBr (50 mg) was finely ground in an agate mortar and samples of drug and their complexes (1–2 mg) were subsequently added and mixed gently. A manual press was used to form the pellets.

Analysis in solution phase

Nuclear Magnetic Resonance (NMR) Spectroscopy

Bruker Advance II 400 NMR spectrometer operating at 400 Hz was used for studying proton NMR spectra of inclusion complexes and pure drug in deuterated dimethylsulfoxide.

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 with temperature resolution, close to 1 μK, which corresponds to a heat resolution of 1–4 mJ in a 25 mL reaction vessel. The apparatus consists of 25 mL silvered Dewar flask in a constant temperature bath held at 37 °C (± 0.0001 °C). The drug was filled into batch adaptor sealed on both sides with 'O' rings and cover glass which was then inserted into the Dewar flask containing the buffer. The ampoule was shattered automatically by means of a plunger and temperature change noted. The performance of the instrument was tested by measuring enthalpy of solution of potassium chloride (17.031 kJ/mol) in triple distilled water, which was in good agreement with known enthalpy of solution of 17.322 kJ/mol. The precision of any individual measurement was better than ±0.03 kJ/mol for three consecutive experiments.

Complexation thermodynamics of efavirenz with β-CD and its derivatives was determined by measuring the enthalpy of solution of drug in pure buffer (pH 7) and in buffered solution of cyclodextrins (pH 7) with and without 0.20% PVP. The solutions of cyclodextrins were prepared over a range of 0.001–0.2 M for binary and 0.01–0.08 M for ternary systems. The enthalpy of interaction was calculated by the equation:

$$\Delta H_{int(l)(calc)} = \frac{\Delta_{sol}H_{(CD)} - \Delta_{sol}H}{v(l)} \quad (1)$$

$\Delta H_{int(l)(calc)}$ enthalpy of interaction between drug and cyclodextrin per liter of solution, $\Delta_{sol}H$ and $\Delta_{sol}H_{(CD)}$ are enthalpy of solution of drug in buffer and in buffered aqueous solution of cyclodextrins, respectively, $v(l)$ volume of sample cell (0.025 L).

Dissolution study

The dissolution profile of the efavirenz and 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. The media was previously filtered, degassed and maintained at 37 ± 0.5 °C. The stirring speed was set at 50 rpm. Inclusion complexes equivalent to 50 mg of efavirenz was filled in hard gelatin capsules. 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 of efavirenz and its cyclodextrin complexes with or without PVP through a semi-permeable cellulose membrane (dialysis tubing, high retention seamless cellulose tubing, 12,000 Dalton) was investigated. 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 consisted of phosphate buffer (pH 7). The membrane of the diffusion cell was stirred with a magnetic bar and 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 efavirenz or its equivalent amount of efavirenz-cyclodextrin complexes with or without water-soluble polymer. Samples of receptor fluid (1 mL) were withdrawn at various intervals up to 6 h and replaced with fresh buffer solution. The samples were analyzed spectrophotometrically at 250 nm.

Statistical analysis

Data was expressed as mean ± S. D. and Permeability flux (R) of complexes were statistically assessed by one-way

ANOVA followed by Turkey test using Jandel sigma stat 2.0 version. The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = < 0.001$).

The value of equilibrium constant (K) and enthalpy of binding (ΔH^{o}) were determined by our computer program utilizing an iterative non-linear least square regression method to minimize the value of Σ $(\Delta H_{int(l)(exp)} - \Delta H_{int(calc)})^2$.

Where ($\Delta_l H_{int}(l)$ experimentally determined enthalpy of interaction per litre of solution) and $\Delta H_{int(calc)}$ is calculated enthalpy of interaction per litre of solution using Eq. 4.

Results and discussion

Effect of cyclodextrins on the solubility of efavirenz

Phase solubility study

The effect of β-CD, M-β-CD, and HP-β-CD concentration on the solubility of efavirenz in phosphate buffer (pH 7) at 37 °C was investigated (Fig. 2). The solubility of efavirenz was found to be 0.44 mmoles L^{-1} in the absence of cyclodextrins. The phase solubility diagram revealed that the solubility of efavirenz increased linearly as a function of increasing cyclodextrin concentration indicating it to be A_L type for all the CDs [29]. The slope of these A_L type systems was less than one indicating 1:1 stoichiometry. Solubility increase was highest for the complex with M-β-CD, being $\sim$12.4 times higher than that of the drug at 20 mmoles L^{-1} concentration of M-β-CD. The increment in the solubility of drug seems to depend upon inclusion ability of cyclodextrin molecules with the solubilization strength decreasing in the order: M-β-CD > HP-β-CD > β-CD.

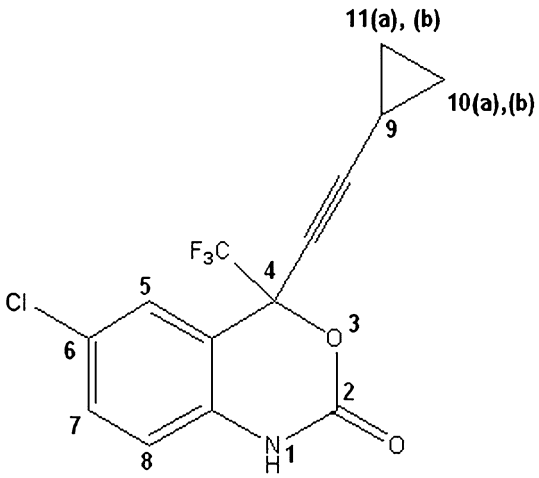

Fig. 1 The chemical structure of efavirenz

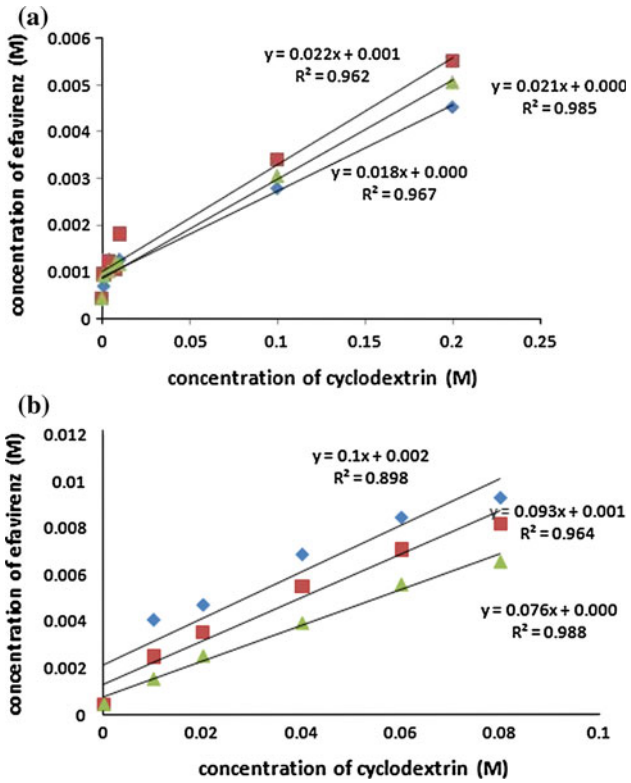

Fig. 2 Phase solubility diagram of efavirenz with (**a**) (*filled diamond*) β-CD, (*filled square*) M-β-CD and (*filled triangle*) HP-β-CD and (**b**) (*filled triangle*) β-CD, (*filled diamond*) M-β-CD and (*filled square*) HP-β-CD in the presence of 0.20% PVP at 37 °C

Selection of the third component

As mentioned earlier, addition of water soluble polymers increases the solubilizing efficiency of a drug, however, few reports are available showing the adverse effect of polymer on solubility. Therefore, selection of suitable polymer is very important. The effect of various additives (polyvinylpyrrolidine (PVP), Tween80, hydroxylpropyl methyl cellulose (HPMC), polyethylene glycol (PEG) and poloxamer) on the solublizing efficiency is shown in Fig. 3 and PVP is found to be the most effective in increasing the drug solubility. Further studies shows that the solubility of drug in presence of 0.2%w/v PVP is 1.02 mmoles L^{-1} exhibiting a 2.3 fold increase in comparison to solubility of drug in pure buffer (0.44 mmoles L^{-1}). The solubility of drug is 3.4 mmoles L^{-1} in binary system where only M-β-CD (0.08 mol L^{-1}) is present, suggesting 7.7 fold enhancement. Interestingly, the solubility of efavirenz jumped to 9.27 mmoles L^{-1} in the presence of 0.2%w/v PVP, whereas, the M-β-CD is still 0.08 mol L^{-1}. This

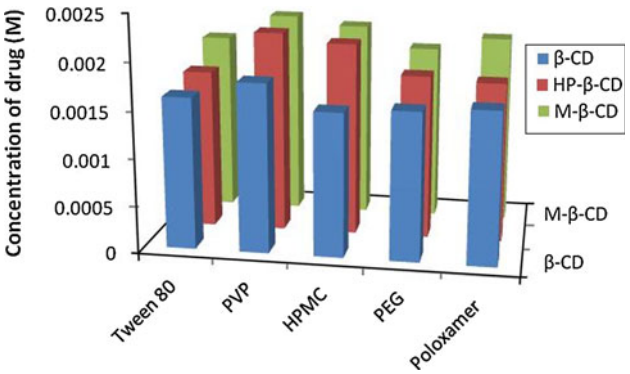

Fig. 3 Effect of different additives (0.20%) on the solubility of efavirenz in presence of 30 mM of β-CD, M-β-CD, and HP-β-CD

suggests a 2.7 fold increase in solubility of efavirenz on switching over from binary system to ternary system and overall there is a 21 times increase in solubility of efavirenz in drug- M-β-CD (0.08 mol L^{-1})—PVP (0.2%w/v) system in comparison to solubility in pure buffer. This shows that when M-β-CD and polymer are present together in solution, increase in solubility is greater (Fig. 2b) as compared to when they are used separately suggesting the increase in solubility upon addition of PVP to the binary system is synergistic than simply additive. The favourable effect of PVP on drug –CD complex is documented in literature; however, none of the studies have reported the exact nature of interaction. It can be attributed to the fact that the solubility of cyclodextrins is enhanced in the presence of PVP.

The linear phase solubility curve in the presence of 0.2%w/v PVP shows it to be A_L type. The addition of polymer does not seem to change the stoichiometry in all the complexes (1:1) as the slope still continues to be less than one. The ratio of the slopes of the phase solubility curves of ternary and binary systems, is an index of the relative solubilizing efficiency, and is found to be 4.54 for M-β-CD, 4.42 for HP-β-CD and 4.22 for β-CD, confirming the greater effectiveness of ternary system. This led to the preparation of ternary inclusion complexes containing the drug and cyclodextrins in the presence of 0.2% PVP. These ternary complexes were autoclaved before being lyophilized keeping in mind the results reported by Pose-Vilarnovo et al. [30] and Mura et al. [31]. The authors have reported that the addition of the polymer without further treatment of the system is ineffective on the capacity of cyclodextrins to complex the drugs [32]. However, this statement was not found to be true in the case of solubility enhancement of trocainamide with HP-β-CD in the presence of PVP [33]. The improved solubilization after autoclaving may be due to the increased electrostatic forces operating at higher temperatures leading to a facilitated fitting of the guest molecule into the cavity [34].

Characterization of binary (drug cyclodextrin) and ternary (drug cyclodextrin polymer) complexes

Analysis in solid state

Differential Scanning Calorimetry (DSC)

The DSC thermogram of the pure drug, PM, KN, LY and Co-ppt complexes of β-CD, M-β-CD and HP-β-CD with efavirenz are given in Fig. 4. The lyophilized as well as kneaded systems containing M-β-CD and HP-β-CD showed complete disappearance of the peak due to melting of efavirenz (138.4 °C). This suggests an inclusion complex with complete amorphous nature has been formed. The PM, Co-ppt complexes and the complexes involving β-CD showed smaller and broader endotherm in the region of melting of pure drug.

The presence of hydrophilic polymer in multicomponent complexes could show different physico-chemical properties from the individual or binary system. The melting peak completely disappeared in the ternary complexes (Fig. 5). The disappearance of endothermic peak of the drug is attributed to the amorphous state and the inclusion complexation of the drug inside the cavity.

Powder X-ray diffractometry (PXRD)

The PXRD pattern of efavirenz showed peaks that are intense and sharp, indicating its crystalline nature (Fig. 6). The characteristic diffractional peaks relevant to crystalline efavirenz appear at $2\theta = 6.07^{o}$. PXRD analysis depicted that lyophilized complexes involving M-β-CD and HP-β-CD were completely amorphous in nature as compared to the

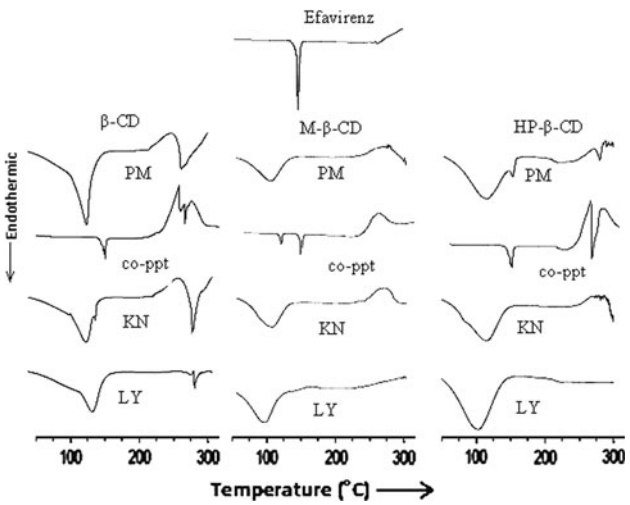

Fig. 4 DSC thermogram of efavirenz and its physical mixtures (PM), kneaded (KN), lyophilized (LY) and co-precipitated (co-ppt) complexes with β-CD, M-β-CD and HP-β-CD

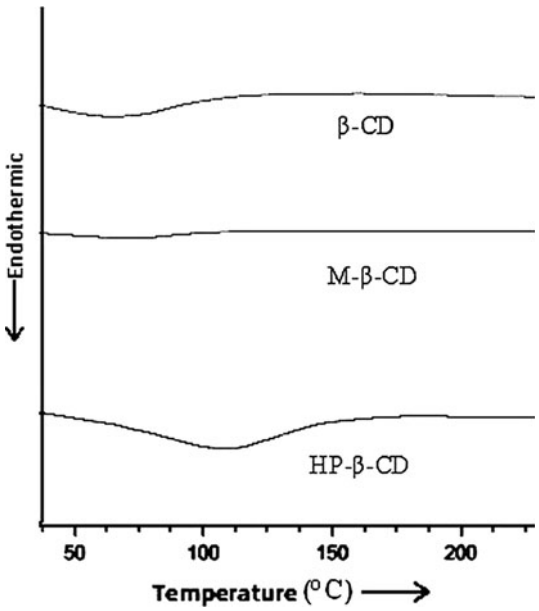

Fig. 5 DSC thermogram of efavirenz and its ternary complexes with β-CD, M-β-CD, and HP-β-CD

raw materials confirming the existence of complete inclusion. The inclusion complexes prepared by kneading method showed peaks of much diminished intensity. The diffraction patterns of the investigated physical mixtures were apparently the superposition of diffraction pattern of components in each mixture, indicating that drug maintained its crystallinity in the respective physical mixtures. The co-precipitated system presented a quite similar diffraction pattern.

The PXRD pattern of ternary complexes showed fewer and less intense peaks in complex with β-CD whereas complete amorphous product was obtained in complex with M-β-CD and HP-β-CD (Fig. 7). This indicates that all the lyophilized ternary complexes are markedly less crystalline than the pure components. The above results show that out of all the methods employed for preparation of complexes, the lyophilized method is the best method.

Fourier Transform Infra-red (FTIR) spectroscopy

FTIR of pure efavirenz showed absorption bands at 3319.6 cm^{-1} for N–H stretch and at 1749.7 and 1650.4 cm^{-1} for carbonyl stretching band. The absorption band at 1180.8 cm^{-1} was denoted for stretching vibration of C–O. Apart from these the $–CH_2$ stretching and bending vibrations were also observed (Fig. 8).

The –N–H stretch shifted to 3379.9, 3425.5 and 3399.2 cm^{-1} in lyophilized system of efavirenz with β-CD, M-β-CD and HP-β-CD and to 3421, 3426.3 and 3425.5 cm^{-1} in PM, KN and LY of M-β-CD, respectively. The peak at 1749.7 cm^{-1} for carbonyl group disappears for M-β-CD in the lyophilized system whereas the intensity of peaks is reduced to medium for β-CD, HP-β-CD and. Besides this, C=C bands near 1497 cm^{-1} of the aromatic ring were also shifted maximum in the lyophilized complexes than other systems. The overall intensity of IR absorption pattern of lyophilized complexes of all the CDs showed a decrease suggesting appreciable interaction between the drugs and CDs. Also a number of peaks disappeared in the complexes. Addition of polymer to drug

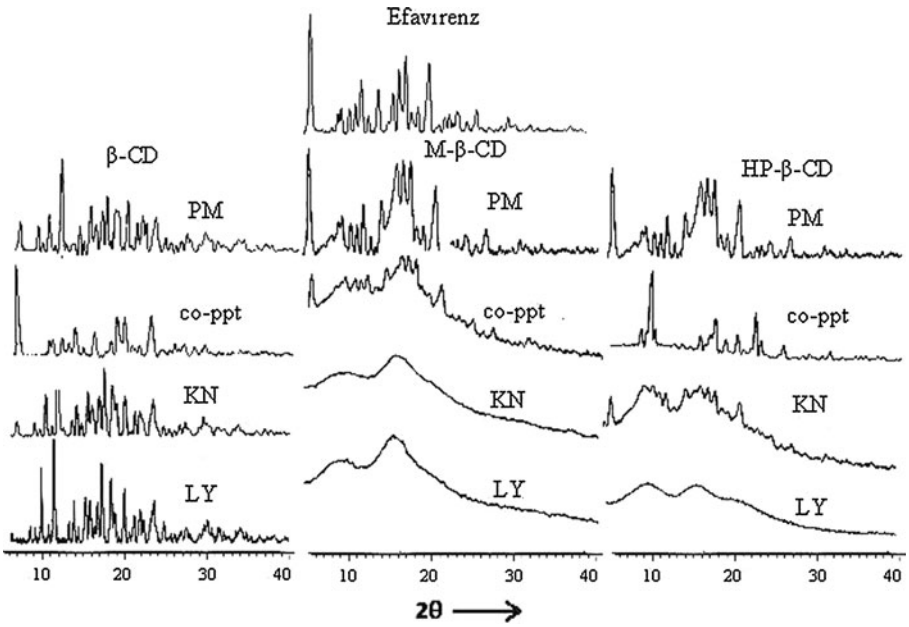

Fig. 6 XRPD pattern of efavirenz and its physical mixtures (PM), kneaded (KN), lyophilized (LY) and co-precipitated (co-ppt) complexes with β-CD, M-β-CD and HP-β-CD

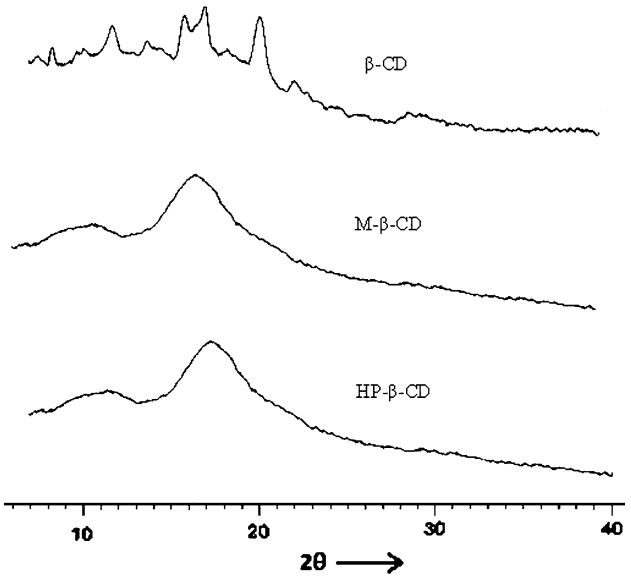

Fig. 7 XRPD pattern of efavirenz and its ternary complexes with *β*-CD, M-*β*-CD and HP-*β*-CD

CD complex resulted in appreciable changes in the FTIR spectra of efavirenz as can be seen in Fig. 9.

Analysis in solution phase

Proton NMR spectroscopic studies

Proton NMR studies have revealed changes in the chemical shifts of protons H-5,7 and 8 of aromatic ring of efavirenz suggesting its potential to enter the cyclodextrin cavity (Table 1). The H-5 and H-8 show upfield shifts indicating their interaction with hydrogen atoms inside the cavity [35, 36]. A downfield was observed for the proton H-7 may be due to interaction with the oxygen atoms of the cyclodextrin ring inside the cavity. The entrance of drug cyclopropane ring can be ruled out because of the relatively smaller size of cyclopropane ring (cis distance: 2.62 Å, trans distance: 3.17 Å) as compared to the cavity size of *β*-CD and its derivatives allowing the drug to move in and out of the cavity (Table 2). 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 proposed structure of the complex is further supported by 2D H^1–H^1 COESY analysis. The off-diagonal peaks are displayed between the cavity protons H-3′ and H-5′ protons of *β*-CD with H-7 and H-8 of efavirenz. This suggests deep penetration of chlorobenzene ring of the drug from the wider side of the cavity (Fig. 10). The most probable structure of the complex is drawn using ligandFit module of Accerlys (Discovery studio 2.0 version) (Fig. 11). Similar cross peaks were found in M-*β*-CD and HP-*β*-CD due to random substitution of M-*β*-CD and HP-*β*-CD, the peaks could not be clearly identified.

Microcalorimetric study

The stability constants and other thermodynamic parameters associated with the binary and ternary complexes are determined using solution calorimetric technique [37]. The molar enthalpy of solution of efavirenz ($\Delta_{sol}H_{(M)}$) was determined in buffered aqueous solution and was found to be exothermic (−8.22 kJ/mol). The molar enthalpy of

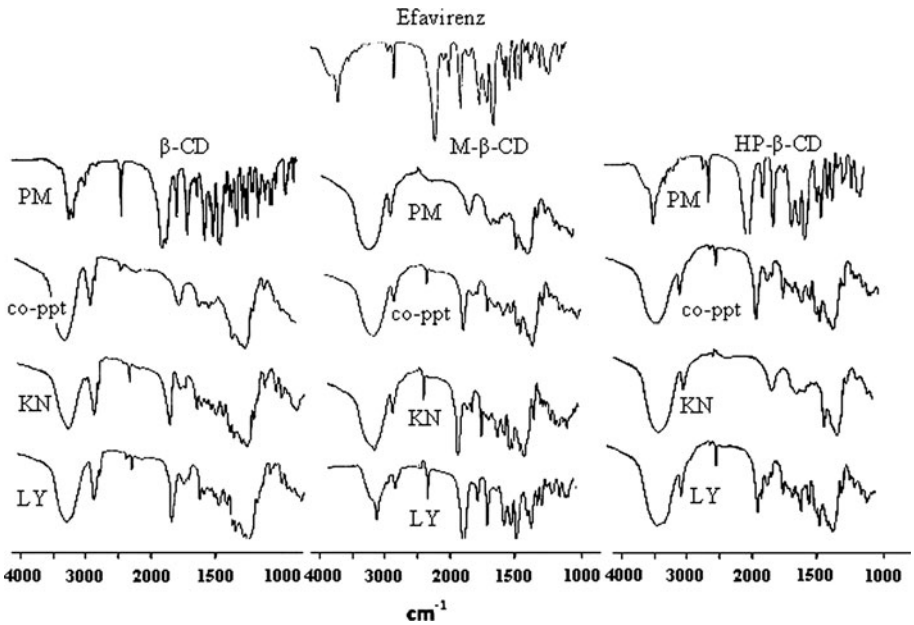

Fig. 8 FTIR spectra of efavirenz and its physical mixtures (PM), kneaded (KN), lyophilized (LY) and co-precipitated (co-ppt) complexes with *β*-CD, M-*β*-CD and HP-*β*-CD

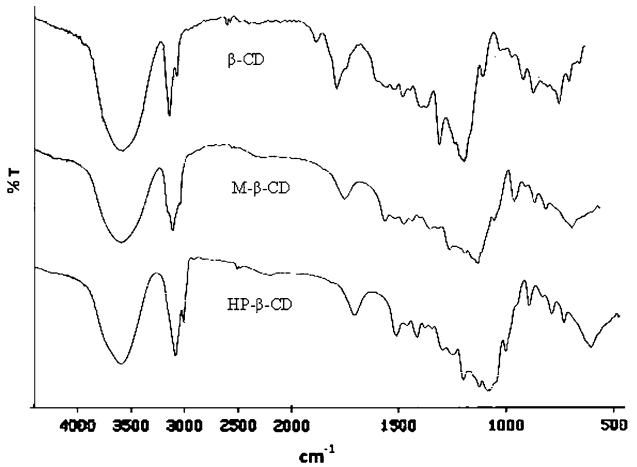

Fig. 9 FTIR spectra of efavirenz and its ternary complexes with β-CD, M-β-CD and HP-β-CD

Table 1 Variation of ^{1}H chemical shifts before and after inclusion

	β-CD ($\Delta\delta$)	HP-β-CD ($\Delta\delta$)	M-β-CD ($\Delta\delta$)
H-1	0.0065	–	–
H-5	−0.00115	−0.00245	−0.0001
H-7	0.0048	0.0028	0.00665
H-8	−0.00105	−0.0045	−0.0019
H-9	−0.00436	−0.00549	−0.00149
H-10 a,b	0.00066	0.00434	0.00465
H-11a,b	−0.001125	−0.0072	−0.0113

The significance of a, b in 10(a, b) indicates the two protons attached to carbon number10

The significance of a, b in 11(a, b) indicates the protons attached to carbon number11

($\Delta\delta$) = δ (complex) - δ (free)

Table 2 Distances between different atoms of efavirenz

Protons of efavirenz	Distance (Å)
H_5–H_8	4.92
H_7–H_9	10.85
H_8–H_9	9.66
H_{10a}–H_{11a} (cis)	2.62
H_{10a}–H_{11b} (trans)	3.17
Cl^-=O	8.11

solution of efavirenz in the presence of cyclodextrins ($\Delta_{sol}H_{(M)\ (CD)}$) as well as in the presence of both cyclodextrins and PVP (0.20%) was found to be more exothermic than $\Delta_{sol}H_{(M)}$ and the exothermic effect of the system further increased in the presence of both cyclodextrins and PVP. This increase is attributed to the interaction between drug, cyclodextrins and PVP in binary and ternary systems. The magnitude of difference depends upon the

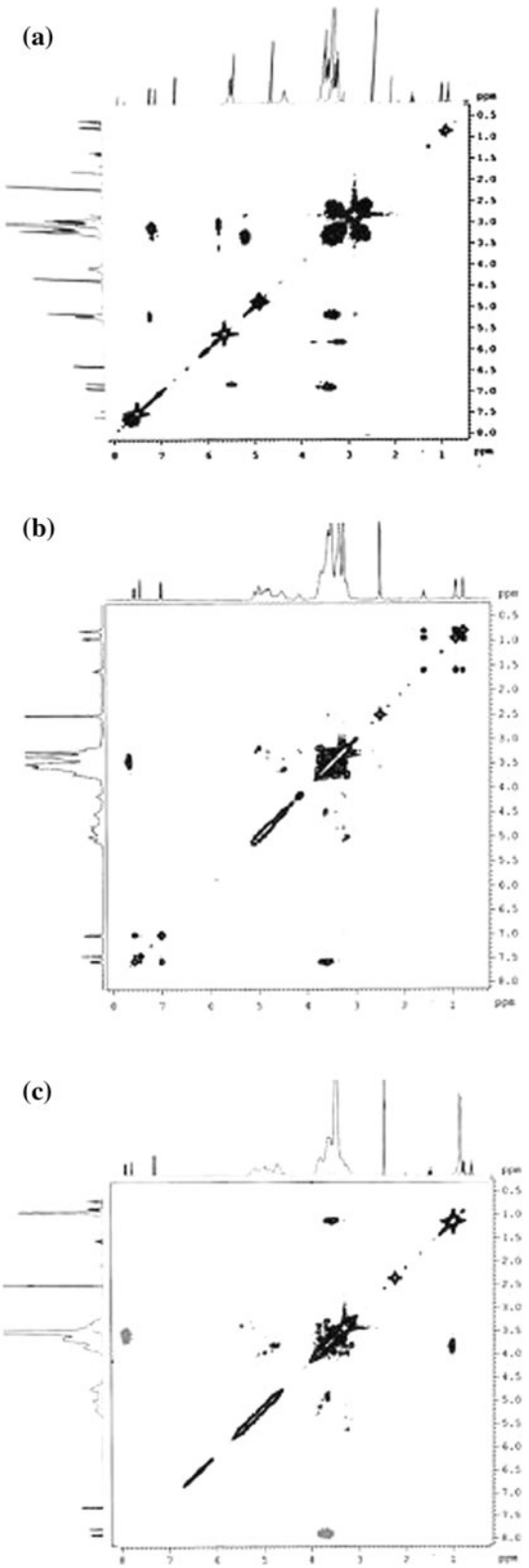

Fig. 10 ^{1}H COESY of efavirenz with (**a**) β-CD, (**b**) M-β-CD and (**c**) HP-β-CD

Table 3 Enthalpy of solution under different initial concentrations of drug and cyclodextrins and derived quantities at pH 7

X_2	*a* (M)	*b* (M)	$\Delta_{sol}H_{(CD)}$ ($J \times 10^{-1}$)	$\Delta H_{int(l)}$ (J/l)	$\Delta H_{int(M)}$ (kJ/mol)
0.084	8.06	0.74	−18.438	−7.53	−0.85
0.106	6.89	0.81	−16.209	−8.16	−1.06
0.146	4.99	0.85	−12.331	−8.28	−1.42
0.232	2.99	0.90	−8.157	−8.06	−2.07
0.327	1.82	0.88	−5.448	−6.82	−2.52
0.404	1.18	0.80	−3.733	−5.18	−2.60
0.505	0.78	0.79	−2.719	−4.44	−2.81
0.572	0.59	0.80	−3.011	−7.13	−2.87
0.564	1.08	1.40	−3.212	−3.93	−2.82
0.702	0.59	1.38	−2.285	−4.30	−2.18
0.728	0.95	2.54	−3.970	−8.09	−2.32
0.792	0.76	2.90	−3.277	−6.82	−1.86
0.854	0.54	3.18	−2.367	−5.00	−1.34
0.914	0.32	3.42	−1.409	−3.00	−0.80

concentration of the drug as well as cyclodextrins. The enthalpy of interaction per mole of drug and cyclodextrin ($\Delta H_{int\ (M)}$) was calculated using the Eq. 2. Table 3 shows the calorimetric data over the whole concentration range for efavirenz and M-β-CD. Similar results are obtained for β-CD and HP-β-CD.

$$\Delta H_{int(M)} = \frac{\Delta H_{int}(l)}{a+b} = \frac{\Delta_{sol}H_{(M)\,(CD)} - \Delta_{sol}H_{(M)}}{l + (x_2/x_1)} \quad (2)$$

$\Delta_{sol}H_{(M)\ (CD)}$ 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 $\Delta H_{int(M)}$ against x_2 for efavirenz with β-CD and its derivatives (Fig. 11). It can be seen that the plot shows a symmetric bell-shaped curve with efavirenz with all the cyclodextrins. The plot

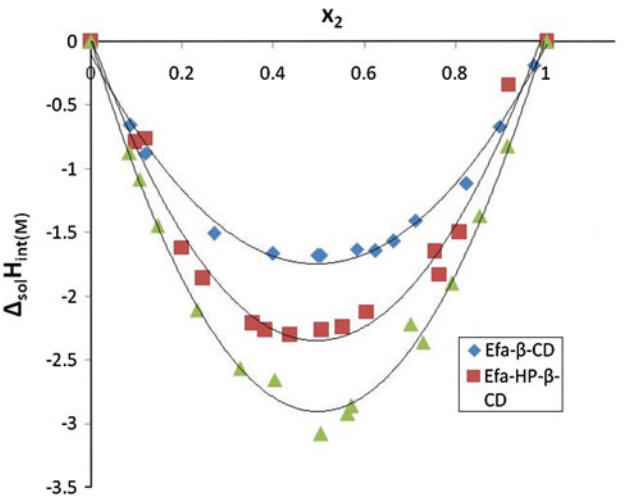

Fig. 12 Plot of $\Delta_{sol}H_{int(M)}$ versus mole fraction (x_2) of efavirenz with β-CD, M-β-CD and HP-β-CD at pH 7

showed maximum at $x_2 = 0.5$ indicating it to be a 1:1 drug: CD complex (Fig. 11).

The equilibrium constants for efavirenz with β-CD, M-β-CD, and HP-β-CD, have been calculated assuming the following reaction:

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

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

$$\Delta H_{int(l)(calc)} = \Delta H^\circ \times c \quad (4)$$

The equilibrium constant for reaction Eq. 3 is given by the equation:

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

Where K is the equilibrium constant and c is the molar concentration of drug-cyclodextrin complex at equilibrium. Rearranging Eq. 5 we obtain the quadratic Eq. 6,

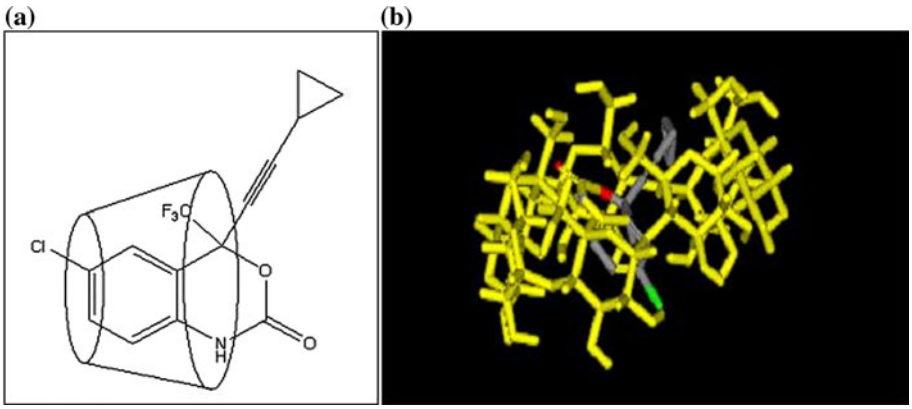

Fig. 11 (**a**) Inclusion mode of drug into cyclodextrin cavity. (**b**) Molecular representation of inclusion complex of efavirenz with β-CD

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

The physically acceptable solution for the above quadratic equation is:

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

Putting, A = a + b + 1/K, the concentration of complex [c] is given by the Eq. (8)

$$c = \left[A - \sqrt{\{(A)^2 - 4\ ab\}}\right]/2 \quad (8)$$

Putting Eq. 8 in Eq. 4,

$$\Delta H_{int(calc)} = \Delta H^o \times \left[\left(A - \sqrt{\{(A)^2 - 4ab\}}\right)/2\right] \quad (9)$$

The best fit values of the thermodynamic parameters K and ΔH^o were determined by our computer program utilizing an iterative non-linear least square regression method to minimize the value of $\Sigma(\Delta H_{int(l)(calc)} - \Delta_{sol}H_{int(calc)})^2$ and are given in Table 4. Similar calculations were performed to determine the binding parameters in the presence of PVP.

The association constants for 1:1 inclusion complexes of efavirenz with β-CD, M-β-CD and HP-β-CD (Table 4) have been found to be within the range of 1,000–2,500 M^{-1} which is optimum for the use of cyclodextrins as drug carrier [38]. The absolute value of K decreases in the order of M-βCD > HP-β-CD > β-CD. This shows that substituent groups assist in binding by lengthening the cavity and also by increasing the hydrophobicity which allows increased adaptability of cyclodextrin towards the guest through enhanced flexibility. However, the effect may be counterbalanced by presence of hydroxyl group in case of HP-β-CD which is reflected by somewhat smaller value of K in binary as well as ternary complexes. Table 4 shows that enthalpy of binding ($\Delta H°$) is negative in all the cases reflecting an exothermic interaction. Large negative enthalpy changes are probably due to 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 release/restructuring of water molecules inside and around the cyclodextrin cavity. The highest magnitude of negative $\Delta H°$ and smaller value of positive $\Delta S°$ for M-β-CD is due to appreciable enthalpy entropy compensation taking place in the cyclodextrin complexation. As mentioned above no data is available for direct comparison of binding parameters except the work reported by Sathigiri et al. [23]. These authors have mentioned the apparent stability constant determined by phase solubility studies. Though the numerical value differs from ours, the stability constant follows the same order as in present study.

The binding parameters for the ternary complexes are given in Table 4 and are found to be higher illustrating that presence of PVP facilitates the inclusion. Such a synergistic effect can be explained by assuming PVP induced higher complexation efficiency of cyclodextrins towards efavirenz which is reflected by almost 27.4, 18.5, 14.4% increase in the stability constant of efavirenz with β-CD, HP-β-CD and M-β-CD, respectively. These ternary complexes are accompanied by more negative ΔH values which are also ascribed to stronger binding between the complexing agent and the drug. However, the ΔS values are found to be less positive as compared to binary complexes. This is attributed to structural resistance as a consequence of ternary complexation.

The PVP interacts with drug-CD complex forming drug-CD-polymer aggregates as a co-complex i.e. complex between drug-CD complex and a polymer chain. This shows that PVP has shown more affinity for the complex than for free drug [39, 40]. This co-complex is sustained by hydrogen bonding between carbonyl oxygen of PVP and proton of N–H of benzoxazinone ring of the drug [41]. Besides this, PVP is also stated to be effective for stabilizing the amorphous state, the form in which drug exists in inclusion complexes [41]. The XRPD and DSC very well show the conversion of crystalline drug into amorphous form in binary as well as ternary complexes.

Table 4 Thermodynamic parameters associated with the inclusion of efavirenz with β-CD, M-β-CD and HP-β-CD at pH 7 (value ± S.D.)

System	K (M^{-1})	ΔH^o (kJ mol^{-1})	ΔG^o (kJ mol^{-1})	ΔS^o (J $mol^{-1}K^{-1}$)
Efavirenz + β-CD	1040 ± 4	−8.20 ± 0.03	−17.90 ± 0.05	31.31 ± 1.04
Efavirenz + HP-β-CD	1830 ± 5	−10.60 ± 0.03	−19.36 ± 0.05	28.26 ± 1.15
Efavirenz + M-β-CD	2150 ± 5	−10.70 ± 0.02	−19.78 ± 0.05	29.28 ± 1.86
Efavirenz + β-CD in 0.20% PVP	1325 ± 5	−8.80 ± 0.03	−18.53 ± 0.05	31.38 ± 1.94
Efavirenz + HP-βCD in 0.20% PVP	2170 ± 6	−11.20 ± 0.02	−19.80 ± 0.05	27.74 ± 1.84
Efavirenz + M-β-CD in 0.20% PVP	2470 ± 4	−13.90 ± 0.03	−20.13 ± 0.05	20.11 ± 1.38

Enthalpy of interaction in autoclaved ternary complexes

The ternary complexes showed an increase in the solubility as compared to the drug and binary complexes. Thus, it was decided to measure the extent of interaction in the autoclaved solid ternary formulation. The enthalpy of interaction between various constituents was estimated by determining the enthalpy of solution of formulation as well that of pure components. The molar enthalpy of interaction in autoclaved solid formulation containing drug, cyclodextrins and PVP was calculated from the equation:

$$\Delta H_{int} = \Delta H_{formu} - (\Sigma \Delta H_i x_i) \quad (12)$$

$$\Delta H_{int} = \Delta H_{formu} - (\Delta H_1 x_1 + \Delta H_2 x_2 + \Delta H_3 x_3) \quad (13)$$

ΔH_{int} molar enthalpy of interaction, ΔH_{formu} molar enthalpy of solution of formulation, $\Sigma \Delta H_i x_i$ sum of contribution of various constituents towards total enthalpy of solution, ΔH_1, ΔH_2, ΔH_3 molar enthalpy of solution of efavirenz, cyclodextrin and PVP, respectively, x_1, x_2, x_3 mole fraction of efavirenz, cyclodextrin and PVP, respectively, in the formulation. Numerical values of ΔH_{formu} along with the additive molar enthalpy of solution obtained by summation of solution enthalpies of individual components are given in table for comparison.

A lower value of endothermic enthalpy of solution of autoclaved ternary complexes as compared to additive molar enthalpy of solution is suggestive of synergistic interactions between the drug and its constituents (Table 5). The results show that interaction is strongest for the formulation containing M-β-CD.

Dissolution studies

The release profiles of binary and ternary complexes of efavirenz prepared by different methods are given in Fig. 13. Increase in dissolution rate was recorded for the solid complexes as compared to pure drugs. In all binary systems, the dissolution rate was observed to be maximum for the complexes prepared by lyophilization than by other methods in agreement with the earlier study. The increase in dissolution rate followed the order PM < Co-evap < KN < LY for the all complexes. Moreover, M-β-CD had

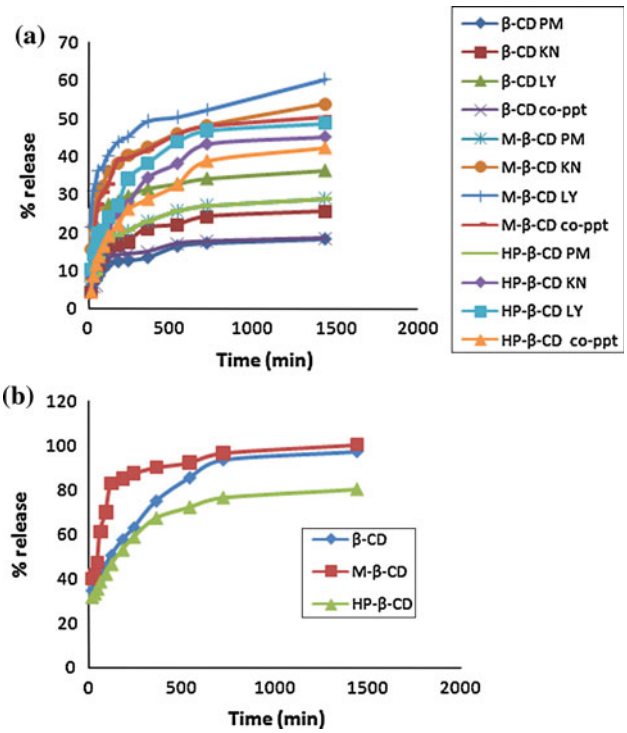

Fig. 13 Dissolution study of efavirenz (**a**) binary systems and (**b**) ternary systems

greatest effect on enhancing the dissolution rate of the drug. This can be attributed to true inclusion as well as greatest ability to amorphize and consequently to solubilize the complexed drug. The dissolution profile of efavirenz cyclodextrin ternary systems containing 0.20% PVP showed further increase in the dissolution rate. It is clear from the plot that the addition of polymer markedly enhances the dissolution rate of efavirenz as compared to binary systems. The % release was 83% in 120 min for efavirenz-M-β-CD-PVP ternary system as compared to 40% for efavirenz-M-β-CD binary system.

The dissolution of the complexes suggests that the release rate was higher for the ternary complexes as compared to the binary systems. So, it was decided to perform the in vitro measurement of the autoclaved ternary complexes of efavirenz. In these complexes, the molecules of efavirenz and drug-CD complexes the drug is present more or less in PVP matrix through interaction between the

Table 5 Enthalpy of interaction in autoclaved ternary complexes of efavirenz

Formulation no.	Type of CD	Additive molar enthalpy of solution (kJ mol^{-1}) ($\Sigma \Delta H_i x_i$)	Observed molar enthalpy of solution (kJ mol^{-1})	ΔH_{int} (kJ mol^{-1})
I	β-CD	22.67	16.66	−6.02
II	HP-β-CD	24.14	15.45	−8.68
III	M-β-CD	28.49	16.29	−12.20

Table 6 In vitro permeation characteristics of efavirenz and its binary and ternary complexes through cellulose membrane (value ± S.D.)

Time (min)	% Release of efavirenz	% Release of efavirenz in binary complexes			% Release of efavirenz ternary complexes		
		β-CD	HP-β-CD	M-β-CD	β-CD	HP-β-CD	M-β-CD
0	1.67	6.88	12.08	10.63	13.75	18.33	15.63
30	3.62	10.34	16.02	14.49	18.18	37.16	20.36
60	4.22	12.74	21.61	19.38	24.30	43.82	26.17
90	5.68	14.41	28.32	22.02	27.19	56.42	33.73
120	6.17	18.47	32.23	24.78	30.42	62.58	38.75
240	7.11	20.02	36.35	29.36	34.44	72.99	47.98
360	8.30	22.68	39.62	31.66	37.21	83.31	51.47
R*	0.009	0.022	0.031	0.041	0.033	0.056	0.089

* Permeability flux (mg cm^2 min^{-1})

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = < 0.001$)

exterior of the complex and PVP and this state is responsible for the higher dissolution rate.

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 efavirenz with β-CD, M-β-CD and HP-β-CD. The in vitro study was also performed on autoclaved ternary systems containing 0.20% PVP. The permeation was measured by the flux which improved significantly ($P < 0.001$) when efavirenz was complexed with cyclodextrins, and this improvement was more prominent in the presence of PVP as shown in Table 6. The extent of enhancement of drug permeation flux is found to be dependent on the type of cyclodextrin and permeation is in the order of M-β-CD > HP-β-CD > β-CD. The presence of polymer further increased the flux. Thus, it can be concluded that the permeation improve significantly when efavirenz is complexed with cyclodextrins, and this improvement is more prominent in the presence of PVP.

Conclusions

Our results suggest that the solubilizing efficiency and of efavirenz can be enhanced maximum by complexation with M-β-CD as compared to β-CD and HP-β-CD which is further enhanced in the presence of PVP. The DSC and XRPD analysis of the complexes in solid state revealed that true inclusion exists for the lyophilized system. The stability constant and thermodynamic parameters determined by calorimetry suggest the highest efficiency of the system. Dissolution and permeability studies support best complexation and solubility on complexation with M-β-CD in the presence of PVP.

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