European Journal of Pharmaceutics and Biopharmaceutics 70 (2008) 819–827

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Inclusion complexes of tadalafil with natural and chemically modified β-cyclodextrins. I: Preparation and *in-vitro* evaluation

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ARTICLE INFO

Article history:
Received 10 April 2008
Accepted in revised form 13 June 2008
Available online 4 July 2008

Keywords:
Tadalafil
Cyclodextrin
Chemically modified cyclodextrin
Inclusion complexes
Physicochemical characterization
In-vitro dissolution

ABSTRACT

The aim of this work was to investigate the inclusion complexation between tadalafil, a practically insoluble selective phosphodiesterase-5 inhibitor (PDE5), and two chemically modified β-cyclodextrins: hydroxypropyl-β-cyclodextrin (HP-β-CD) and heptakis-[2,6-di-*O*-methyl]-β-cyclodextrin (DM-β-CD), in comparison with the natural β-cyclodextrin (β-CD) in order to improve the solubility and the dissolution rate of the drug in an attempt to enhance its bioavailability. Inclusion complexation was investigated in both the solution and the solid state. The UV spectral shift method indicated guest–host complex formation between tadalafil and the three cyclodextrins (CDs). The phase solubility profiles with all the used CDs were classified as A_p-type, indicating the formation of higher order complexes. The complexation efficiency values (CE), which reflect the solubilizing power of the CDs towards the drug, could be arranged in the following order: DM-β-CD > HP-β-CD > β-CD. Solid binary systems of tadalafil with CDs were prepared by kneading and freeze-drying techniques at molar ratios of 1:1, 1:3 and 1:5 (drug to CD). Physical mixtures were prepared in the same molar ratios for comparison. Physicochemical characterization of the prepared systems at molar ratio of 1:5 was studied using differential scanning calorimetry (DSC), X-ray diffractometry (XRD), and Fourier-transform infrared spectroscopy (FTIR). The results showed the formation of true inclusion complexes between the drug and both HP-β-CD and DM-β-CD using the freeze-drying method at molar ratio of 1:5. In contrast, crystalline drug was detectable in all other products. The dissolution of tadalafil from all the prepared binary systems was carried out to determine the most appropriate CD type, molar ratio, and preparation technique to prepare inclusion complexes to be used in the development of tablet formulation for oral delivery of tadalafil. The dissolution enhancement was increased on increasing the CD proportion in all the prepared systems. Both the CD type and the preparation technique played an important role in the performance of the system. Irrespective of the preparation technique, the systems prepared using HP-β-CD and DM-β-CD yielded better performance than the corresponding ones prepared using β-CD. In addition, the freeze-drying technique showed superior dissolution enhancement than other methods especially when combined with the β-CD derivatives.

1. Introduction

Tadalafil is a potent and selective phosphodiesterase-5 inhibitor used for the treatment of erectile dysfunction which was recently approved by the FDA in November 2003 [1]. Compared to sildenafil and vardenafil, tadalafil has the advantages of longer duration of action of approximately 36 h, and minimized potential for vision abnormalities due to its high selectivity for PDE5 versus PDE6 [2,3]. However, it has the disadvantage of poor aqueous solubility [4]. This may cause formulation problems and lead to highly variable blood levels, and irreproducible clinical response (therapeutic failure or exaggerated side effects). Therefore, it is important to introduce effective methods to enhance the solubility and dissolution rate of the drug aiming to improve its bioavailability, increase the predictability of the response and/or reduce the dose.

Complexation with CDs has been widely used to enhance the bioavailability of poorly soluble drugs by increasing the drug solubility, dissolution and/or permeability [5–8].

The most widely used natural cyclodextrin, β-CD, is limited in its pharmaceutical applications due to its limited aqueous solubility (1.85 g/100 ml) [9]. Therefore, chemically modified β-CDs have been synthesized to overcome this problem. Examples include heptakis-(2,6-*O*-dimethyl) β-CD (DM-β-CD) and hydroxypropyl-β-CD (HP-β-CD).

Literature lacks any data about the effect of cyclodextrins on enhancing solubility and dissolution rate of tadalafil. Thus, the aim of this work was to study the interaction of tadalafil with the parent β-CD and its water soluble derivatives, HP-β-CD and DM-β-CD, in both solution and solid state, aiming to develop a

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doi:10.1016/j.ejpb.2008.06.024

soluble form of the drug as a primary step in the development of tadalafil tablet formulation. The UV spectral shift method and phase solubility technique were used to investigate the interaction of tadalafil with cyclodextrins in the solution state. Solid tadalafil–cyclodextrin binary systems were prepared using different preparation methods. DSC, XRD and FTIR were used to evaluate the physicochemical properties of the prepared systems in order to clarify any interaction between the drug and the used carriers. *In-vitro* dissolution studies of all the prepared systems were carried out to investigate the effect of the molar ratio, CD type and preparation method on tadalafil dissolution.

2. Materials and methods

2.1. Materials

Tadalafil (Dr. Reddy's Laboratories, India) was a gift sample from Luna pharmaceuticals company (Egypt). β-CD (MW = 1135 Da) and HP-β-CD (average degree of substitution; 0.8 and MW = 1460 Da) were purchased from Sigma–Aldrich (GmbH, Germany). DM-β-CD (MW = 1331.4 Da) was obtained from Fluka (Buchs, Switzerland). All other chemicals and solvents were of analytical grade and used without further purification.

2.2. UV spectroscopic measurements

Complex formation between tadalafil and CDs was studied in distilled water using the spectral shift method [10]. The concentration of tadalafil was kept constant at 0.04 mmole/L, while the cyclodextrins concentrations were increased from 5 to 15 mmole/L. In the study, stock solution of the drug in ethanol and a stock solution of CD in water were prepared. Aliquots from each solution were transferred to a 25 ml volumetric flask and the volume was made up using distilled water so that the required concentrations are obtained. The prepared solutions were stirred for 2 h, filtered through 0.45 μm membrane filter (Ustar LB, USA), and the UV absorption spectra were recorded (UV spectrophotometer-1601 PC, Shimadzu, Japan) in the wavelength range from 200 to 400 nm against blank solutions containing the same concentrations of cyclodextrins. The recorded spectra were compared to the spectrum of free tadalafil.

2.3. Phase solubility studies

The effects of CDs on the solubility of tadalafil were investigated according to the phase solubility technique established by Higuchi and Connors [11]. Excess amounts of tadalafil (50 mg) were added to 25 ml of either distilled water or aqueous solutions containing increasing concentrations of the CDs (2–20 mM) in 50 ml stoppered glass bottles. The concentration range of CDs was set based on the maximum solubility of β-CD in water at 37 °C, being of the lowest solubility among the applied CDs (1.85 g/100 ml ≈ 16 mmole/L at 25 °C). The obtained suspensions were shaken at 37 ± 0.5 °C for 7 days in a thermostatically controlled shaking water bath (Model 1083, GLF Corp., Germany). After equilibrium attainment, aliquots were withdrawn, filtered through a 0.45 μm membrane filter (Ustar LB, USA), and assayed spectrophotometrically (UV-1601 PC, Shimadzu, Japan) for tadalafil content at 283.6 nm against blank solutions containing the same concentrations of cyclodextrins. Each experiment was carried out in triplicate. Phase solubility diagrams were obtained by plotting the solubility of tadalafil, in mM, versus the concentrations of the cyclodextrins used. The complexation efficiency (CE), which reflects the solubilizing power of the CDs towards the drug, was calculated from the straight line of the phase solubility diagrams according to the equation [12]

$$CE = S_0 K_{1:1} = \text{slope}/1 - \text{slope}$$

where S_0 represents the drug solubility in the absence of cyclodextrins, $K_{1:1}$ is the apparent stability constant, where $K_{1:1} = \text{slope}/S_0(1 - \text{slope})$.

2.4. Preparation of tadalafil–CD solid binary systems

Solid inclusion complexes of tadalafil with β-CD, HP-β-CD and DM-β-CD were prepared using kneading and freeze-drying techniques in molar ratios of 1:1, 1:3 and 1:5 (drug to CD). Physical mixtures were also prepared in the same molar ratios for comparison.

2.4.1. Physical mixtures

Physical mixtures of tadalafil and CDs were prepared by thoroughly mixing the two components in a mortar for 30 min.

2.4.2. Kneading method

The calculated amounts of tadalafil and cyclodextrin were accurately weighed, transferred to a mortar and triturated with small volume of ethanol–water (50:50, v/v) solution. The slurry obtained was kneaded for 30 min and then dried under vacuum at room temperature in the presence of calcium chloride as a dehydrating agent.

2.4.3. Freeze-drying method

Lyophilization monophase solution method was applied. Appropriate quantities of cyclodextrins and tadalafil were dissolved in distilled water and glacial acetic acid, respectively. The resulting solutions were mixed by stirring. The clear monophase solution was frozen at −20 °C, and subsequently freeze-dried for 24 h at −50 °C using a freeze-dryer (Novalyphe-NL 500; Savant Instruments Corp., USA).

2.5. Physicochemical characterization of tadalafil–CD solid binary systems

DSC thermograms, X-ray diffractograms and FTIR spectra were recorded for pure tadalafil, pure cyclodextrins, and their binary systems prepared by different techniques in molar ratio of 1:5 (drug to CD).

2.5.1. Differential scanning calorimetry

DSC analysis was performed using a Shimadzu differential scanning calorimeter (DSC-50, Shimadzu, Japan). The apparatus was calibrated with purified indium (99.9%). Samples (3–4 mg) were placed in flat-bottomed aluminium pan and heated at a constant rate of 10 °C/min, in an atmosphere of nitrogen in a temperature range of 20–400 °C.

2.5.2. X-ray diffractometry (XRD)

The X-ray diffraction patterns were recorded at room temperature using a Scintag diffractometer (XGEN-4000, Scintag Corp., USA). The samples were irradiated with Ni-filtered Cu K_α radiation, at 45 kV voltage and 40 mA current. The scanning rate employed was 2°/min over a diffraction angle of 2θ and range of 3°–70°.

2.5.3. Fourier-transform infrared spectroscopy (FTIR)

The FTIR spectra were recorded using a Bruker FTIR spectrophotometer (Model 22, Bruker, UK) according to the KBr disc technique. The smoothing of the spectra and the baseline correlation procedures were applied. The spectra were saved using a Lotus

123 computer program. The FTIR measurements were performed in the scanning range of 4000–400 cm^{-1} at ambient temperature.

2.6. In-vitro dissolution studies

The dissolution of pure tadalafil and the prepared binary systems was performed using a USP dissolution tester, apparatus II (VK 700, Vankel, USA). The dissolution medium consisted of 900 ml of 0.1 N HCl. A sample equivalent to 10 mg tadalafil of the prepared systems was spread on the surface of the dissolution medium. The stirring speed was 100 rpm, and the temperature was maintained at 37 ± 0.5 °C. At selected time intervals for a period of 120 min, aliquots each of 5 ml were withdrawn from the dissolution medium through a 0.45 μm membrane filter (Ustar LB, USA) and replaced with an equivalent amount of the fresh dissolution medium. Concentrations of tadalafil were determined spectrophotometrically at 283.6 nm using the regression equation of a standard curve developed in the same medium. Each experiment was carried out in triplicate. The initial dissolution rate (IDR, % dissolved/min) was computed over the first 5 min of dissolution [13]. Additionally, the dissolution profiles were evaluated on the basis of the dissolution efficiency parameter at 60 min (DE_{60}, %), calculated from the area under the dissolution curves and expressed as a percent of the area of the rectangle described by 100% dissolution in the same time [14].

2.7. Statistical analysis

The DE_{60} data of the binary systems were statistically analyzed using two-way ANOVA to test the significance of the effects of the preparation method, cyclodextrin type and molar ratio at $p \leqslant 0.05$. Multiple comparisons between different preparation methods, molar ratios and cyclodextrin types were then performed according to Scheffé test using SPSS® software, version 7.5 (SPSS Inc., Chicago, IL). Differences were considered significant at $p \leqslant 0.05$.

3. Results and discussion

3.1. UV spectroscopic measurements

Figs. 1–3 show the effect of CDs concentrations on the absorption spectra of tadalafil in aqueous solutions. Increasing the concentration of all CDs from 5 to 15 mmole/L resulted in an increase in the absorbance of tadalafil without any shifts of λ_{max}. The observed hyperchromic shift might be due to the perturbation of the chromophore electrons of the drug due to the inclusion into the cyclodextrin cavity [15]. It could be indicative of cyclodextrin guest–host complex formation [15,16].

3.2. Phase solubility studies

The phase solubility diagrams of tadalafil with β-CD, HP-β-CD and DM-β-CD in distilled water at 37 ± 0.5 °C are shown in Fig. 4. The solubility of tadalafil increased as a function of the CDs concentrations due to the formation of inclusion complexes [11]. However, other interactions may be involved, such as aggregation of cyclodextrins and their complexes into water soluble aggregates that are capable of solubilizing water insoluble drugs via non-inclusion complexation or micelle-like structure [17]. The coefficient of determination (r^2) values of the phase solubility diagrams with all CDs were <0.990 (0.988, 0.988 and 0.980 for β-CD, HP-β-CD and DM-β-CD, respectively); therefore, the diagrams were classified as A_p-type curves [18]. Such positive deviations from linearity could suggest the formation of higher order inclusion complexes with respect to cyclodextrin [19]. The complexation efficiency (CE) values revealed that the solubilizing power of CDs towards the drug follows the order DM-β-CD (0.00372) > HP-β-CD (0.00319) > β-CD (0.00271). The highest CE value exhibited by DM-β-CD could be ascribed to the presence of methyl groups that

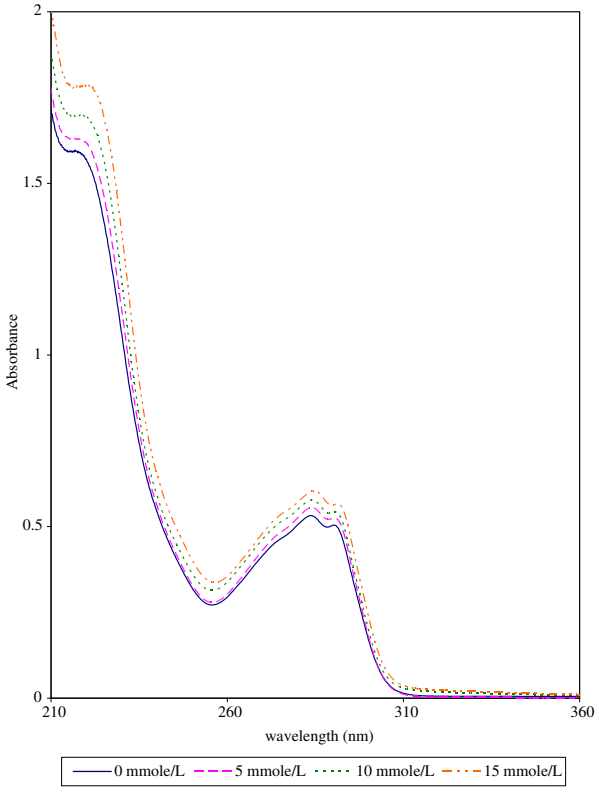

Fig. 1. Differential ultraviolet absorbance spectra of tadalafil in presence of β-CD.

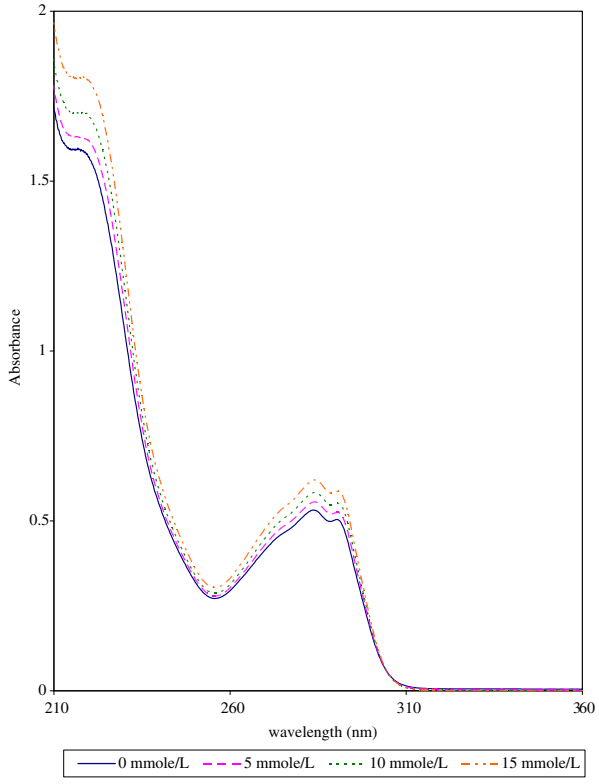

Fig. 2. Differential ultraviolet absorbance spectra of tadalafil in presence of HP-β-CD.

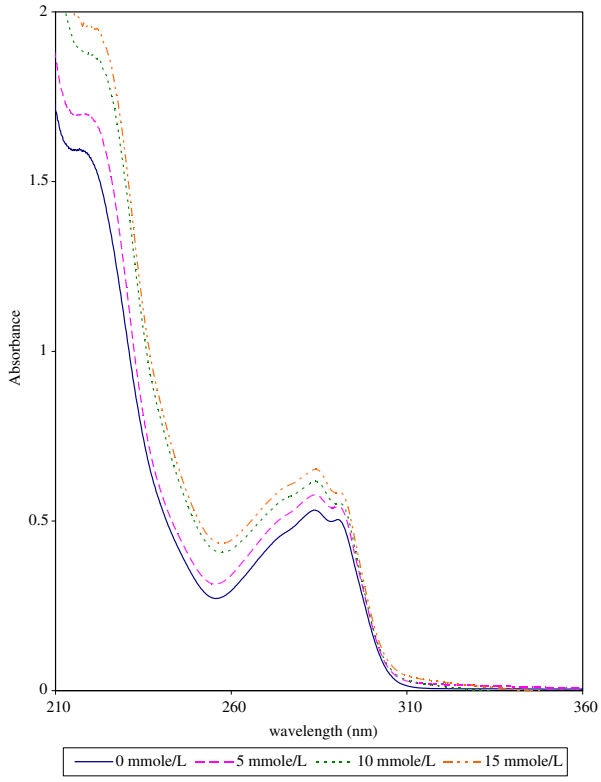

Fig. 3. Differential ultraviolet absorbance spectra of tadalafil in presence of DM-β-CD.

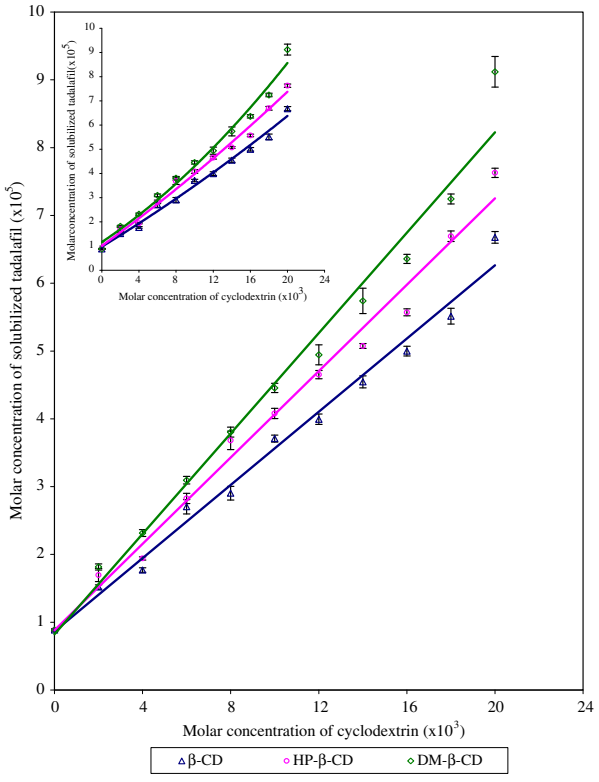

Fig. 4. Phase solubility diagrams of tadalafil with different cyclodextrins in distilled water at 37 ± 0.5 °C.

expand the hydrophobic region of the CD cavity and thus increase its affinity towards tadalafil [20].

3.3. Preparation of tadalafil–CD solid binary systems

Solid binary systems of tadalafil with β-CD, HP-β-CD and DM-β-CD were prepared using kneading and freeze-drying techniques. Based on the results obtained through the phase solubility studies, which proved the possibility of formation of higher order complexes between tadalafil and different cyclodextrins, 1:3 and 1:5 (drug to CD) molar ratios were chosen for the preparation of solid binary systems in addition to the conventional 1:1 molar ratio. Physical mixtures were also investigated in the same molar ratios for comparison.

3.4. Differential scanning calorimetry (DSC)

DSC was performed for the pure drug, pure cyclodextrins and their binary systems prepared by different techniques at a molar ratio of 1:5 (drug to CD) (Figures not shown). The DSC thermogram of tadalafil was typical of a crystalline substance, exhibiting a sharp endothermic peak at 299.95 °C, corresponding to the melting point of the drug. The drug endothermic melting peak completely disappeared in the DSC thermograms of the freeze-dried systems (FD) prepared using HP-β-CD and DM-β-CD. This could indicate amorphous solid dispersion or molecular encapsulation of the drug into the cyclodextrin cavity [21].

On the other hand, the drug melting endotherm was recorded in all the other investigated systems, but with marked broadening and reduction in intensity. The marked broadening of the observed effect, especially in case of HP-β-CD, might suggest the masking of the drug melting endotherm or the fusion between drug melting and CD decomposition due to the overlapping of the vicinity of the two effects, as described by Liu et al. [22]. Also, the marked reduction in intensity of the melting peak in the aforementioned systems could be attributed to the low drug to CD molar ratio (1:5) (the drug content of the prepared systems did not exceed 10% by weight of the system). Therefore, the DSC could not be considered as a discriminative analysis tool between these systems. However, the existence of the drug melting peak in these systems could suggest that no true inclusion is formed in either of these systems.

3.5. X-ray diffractometry (XRD)

Figs. 5–7 show the XRD patterns for pure components and their binary systems prepared by different techniques at molar ratio of 1:5 (drug to CD). The diffraction pattern of tadalafil powder revealed several sharp high intensity peaks at diffraction angles (2θ) of 7.8°, 10.2°, 12.2°, 14.5°, 18.2°, 22.2° and 24.5° suggesting that the drug existed as crystalline material. Both pure β-CD and DM-β-CD showed a crystalline diffractogram, while a diffuse halo-pattern was recorded for HP-β-CD demonstrating its amorphous nature. Similar observations have been reported by other authors [23,24]. The diffraction patterns of the investigated PMs correspond to the superposition of those of the pure components. However, lower intensities of the diffraction peaks were observed due to particle size reduction during mixing and dilution of the pure crystalline components [25]. Overlapping of some tadalafil diffraction peaks with those of β-CD was evident. The diffractograms of the KN systems showed almost similar diffraction behavior to the PMs. The crystallinity of tadalafil was higher in the kneaded tadalafil-β-CD than the corresponding PM. Similar increment in crystallinity was observed for Griseofulvin-2-HP-β-CD kneaded systems, and was attributed to the formation of mixed crystalline particles during the desiccation process [26].

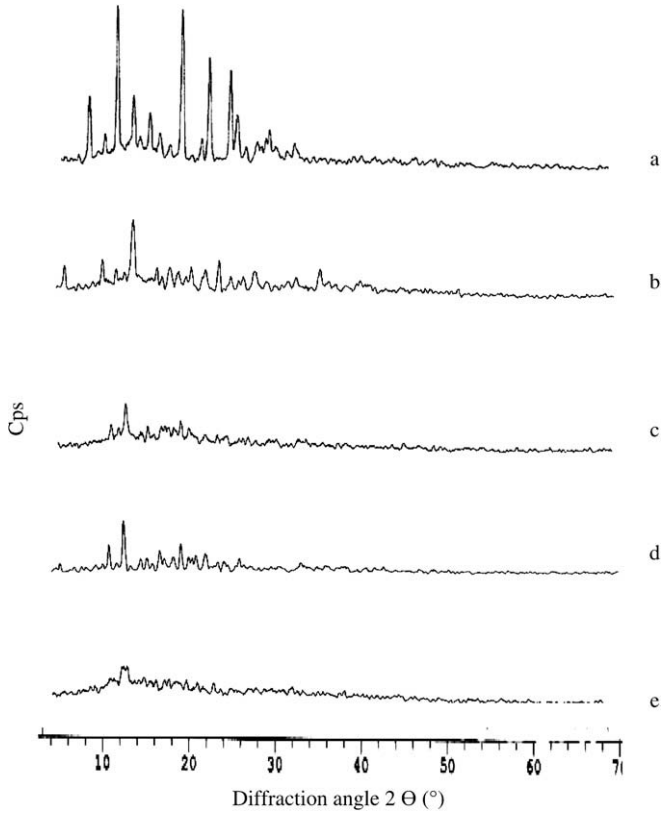

Fig. 5. X-ray diffraction patterns of tadalafil-β-CD systems: (a) pure tadalafil; (b) pure β-CD; (c) PM 1:5; (d) KN 1:5; (e) FD 1:5.

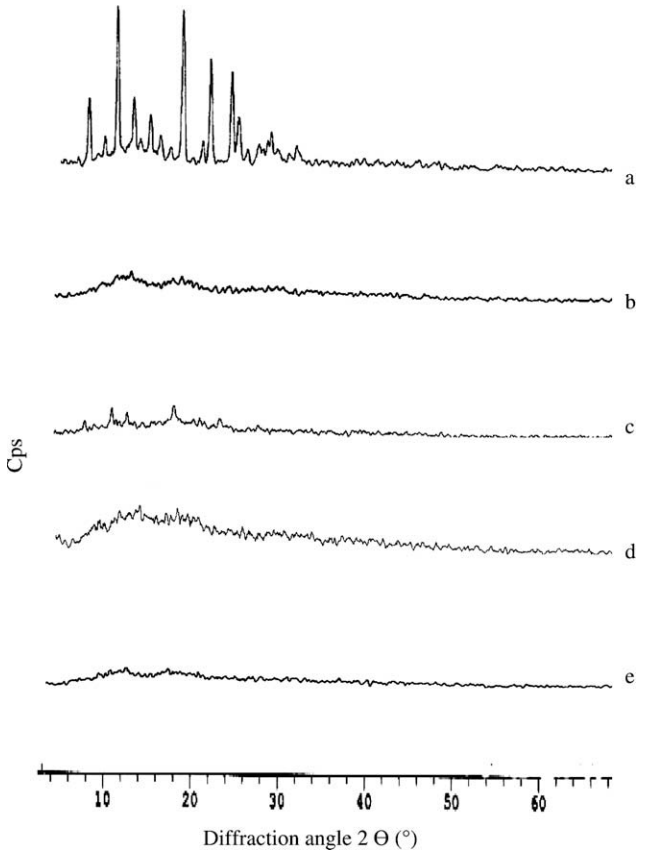

Fig. 6. X-ray diffraction patterns of tadalafil–HP-β-CD systems: (a) pure tadalafil; (b) pure HP-β-CD; (c) PM 1:5; (d) KN 1:5; (e) FD 1:5.

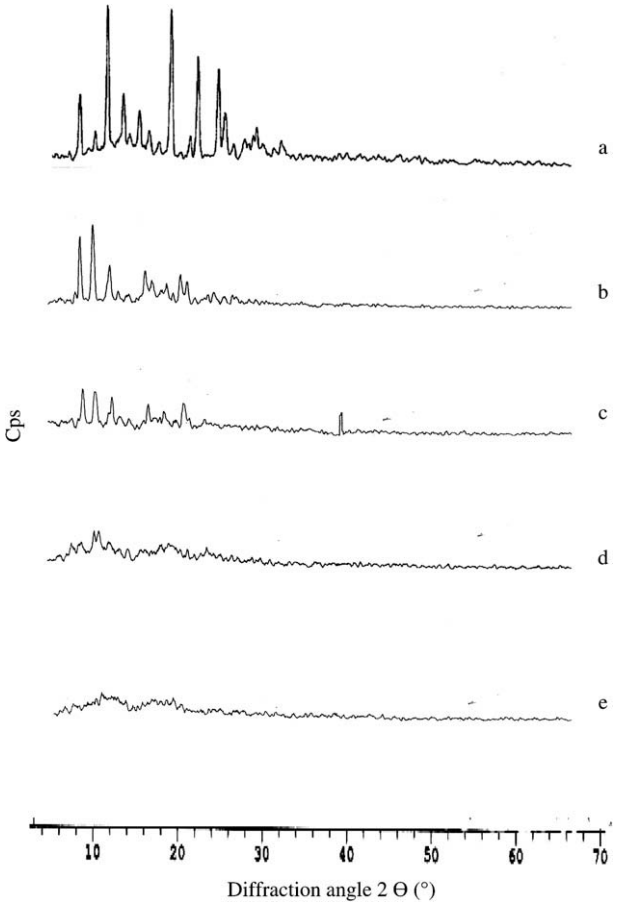

Fig. 7. X-ray diffraction patterns of tadalafil–DM-β-CD systems: (a) pure tadalafil; (b) pure DM-β-CD; (c) PM 1:5; (d) KN 1:5; (e) FD 1:5.

The presence of tadalafil peaks in the diffractogram of tadalafil-β-CD, freeze-dried system could suggest the presence of the free crystalline drug, although reduction in number and intensities were observed. On the other hand, the diffractograms of both FD systems prepared using HP-β-CD and DM-β-CD showed a typical diffuse pattern indicating the entirely amorphous nature of tadalafil in both systems. According to Williams et al. [27], lack of crystallinity is an added evidence for the formation of inclusion complex. However, since the amorphization of the drug can be a sequence of the lyophilization process, it is possible that the X-ray data cannot discriminate whether the drug–CD lyophilized systems obtained are true inclusion complexes or homogenous dispersed mixtures of the amorphous components [28]. Nevertheless, having in account the results of the DSC analysis, one can assume the formation of new solid phases that might be credit to the formation of inclusion complexes.

3.6. Fourier-transform infrared spectroscopy (FTIR)

The FTIR spectra of pure components and their binary systems prepared by different techniques at molar ratio of 1:5 (drug to CD) are shown in Figs. 8–10. The FTIR spectrum of tadalafil showed characteristic absorption band at 3326 cm^{-1} corresponding to the –NH stretching vibration. Two intense absorption bands attributed to the carbonyl stretching vibration were found at 1676 and 1649 cm^{-1}. Other sharp bands appeared at 3059 cm^{-1} (C–H aromatic stretching), 2904 cm^{-1} (C–H aliphatic stretching), 1500–1400 cm^{-1} (C=C aromatic stretching) and 1240 cm^{-1} (C–N stretching). The FTIR spectra of the pure CDs

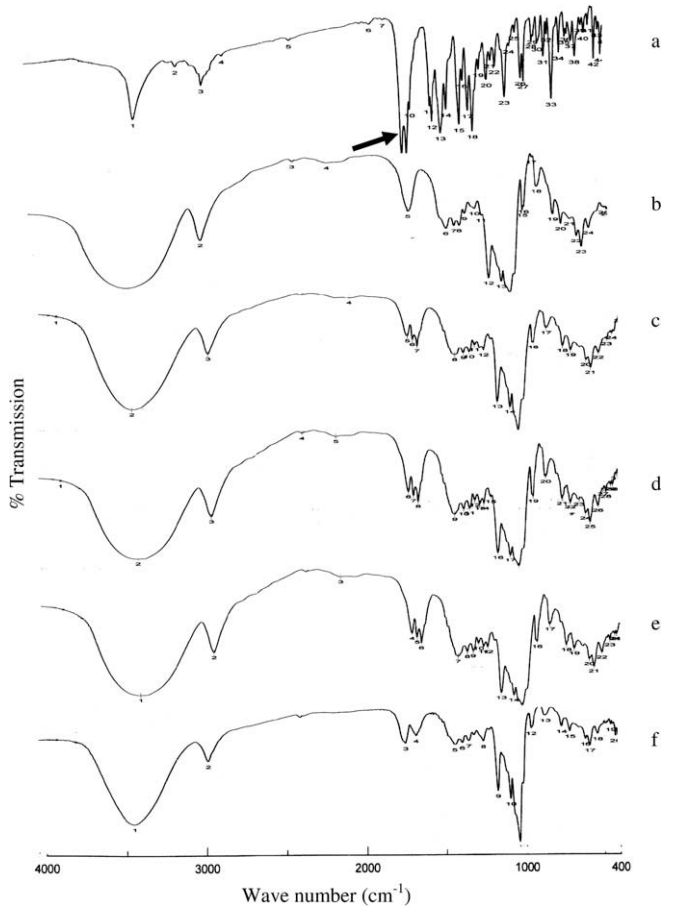

Fig. 8. FTIR spectra of tadalafil-β-CD systems: (a) pure tadalafil; (b) pure β-CD; (c) PM 1:5; (d) KN 1:5; (e) FD 1:5.

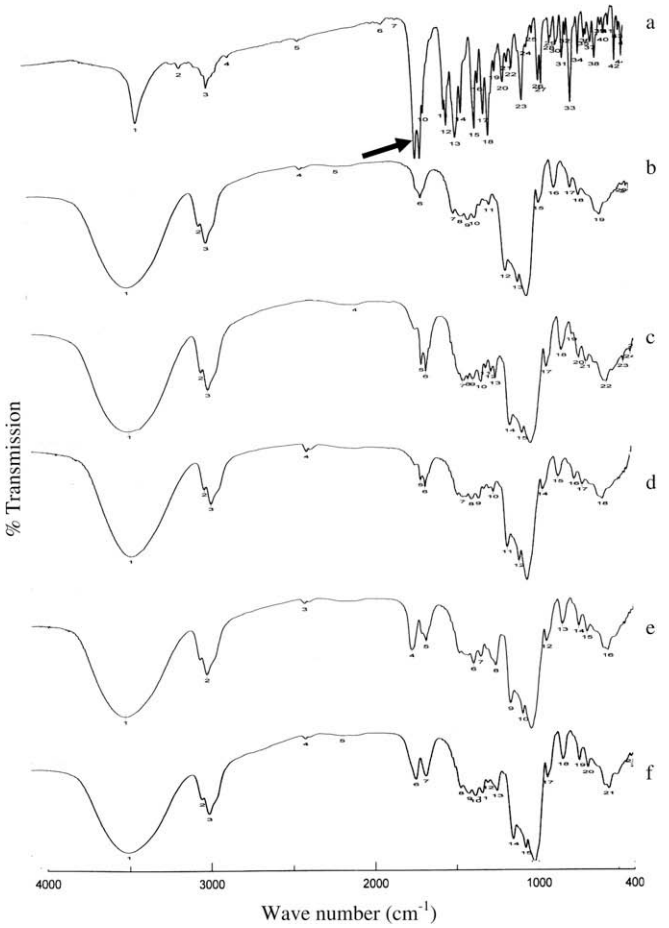

Fig. 9. FTIR spectra of tadalafil–HP-β-CD systems: (a) pure tadalafil; (b) pure HP-β-CD; (c) PM 1:5; (d) KN 1:5; (e) FD 1:5.

illustrated intense broad absorption bands at 3500–3300 cm^{-1} corresponding to the free –OH stretching vibration. The vibration of –CH and $-CH_2$ groups appeared in the region 2950–2600 cm^{-1}. A shorter band appeared in the region 1650–1640 cm^{-1} that could be ascribed to the hydrated bonds within cyclodextrin molecules. Another large band, assigned to the C–O–C stretching vibration, displaying distinct sharp peaks occurred between 1200 and 1030 cm^{-1} [8,29]. In this study the characteristic –NH stretching band of tadalafil was masked in all the prepared systems by the broad intense band corresponding to the free –OH vibration of CDs. This effect was expected due to the low drug to CD molar ratio (1:5), which resulted in systems containing less than 10% by weight of the drug. Also, there was an overlap between the carbonyl stretching of the drug at 1649 cm^{-1} and the band corresponding to the hydrated bonds within CD molecules at 1650–1640 cm^{-1}. Therefore the carbonyl stretching of tadalafil at 1676 cm^{-1} was the main characteristic band used to assess the drug–CD interactions (indicated by black arrow in the figures).

The FTIR spectra of all the PMs and the KN products did not show any significant changes with respect to the FTIR spectra of the pure components, and in particular the characteristic carbonyl stretching band of tadalafil. On the other hand, the FTIR spectra of the FD products exhibited a shift of the characteristic tadalafil carbonyl stretching band to higher frequencies up to 1739 cm^{-1}, with concomitant broadening and decrease in intensity. Similar shifts have been reported for other drugs, and were explained by the dissociation of the intermolecular hydrogen bonds associated with crystalline drug molecules [7,21]. This might be indicative of tadalafil monomeric dispersion as a consequence of the interaction with CDs, which could result in its inclusion in the hydrophobic cavity of the carrier. The broadening and decrease in the intensity of the drug carbonyl stretching band observable in these systems might be due to its restriction within the cyclodextrin cavity [30].

3.7. In-vitro dissolution studies

The dissolution profiles of tadalafil from its solid drug–CD binary systems in 0.1 N HCl are demonstrated in Figs. 11–13. The reported values are the arithmetic mean of three measurements ± standard deviations. The percent amount dissolved was calculated according to a predetermined drug content for each product. The initial dissolution rate during the first 5 min (IDR) and the extent of dissolution after 60 min (DP_{60}) of all the systems are compiled in Table 1. Additionally, the dissolution efficiency data calculated based on 60 min (DE_{60}) are presented in Table 2.

It was evident that no dissolution was achieved for pure tadalafil even after 120 min, under the specified dissolution conditions. The hydrophobic property of the drug prevented its contact with the dissolution medium causing it to float on the surface, and consequently hindering its dissolution. Tadalafil dissolution was enhanced when physically mixed with CDs due to local solubilization action of the carrier operating in the microenvironment of the drug, or the hydrodynamic layer surrounding drug particles in the early stages of the dissolution process, since CDs dissolve in a short time. This action resulted in an *in-situ* inclusion process causing a rapid increase in the amount of dissolved drug.

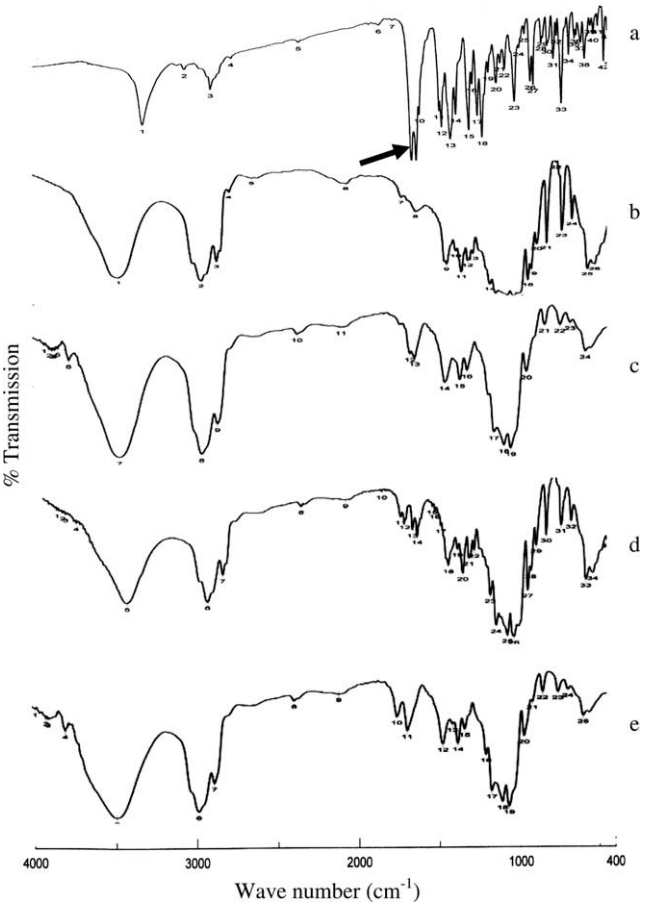

Fig. 10. FTIR spectra of tadalafil–DM-β-CD systems: (a) pure tadalafil; (b) pure DM-β-CD; (c) PM 1:5; (d) KN 1:5; (e) FD 1:5.

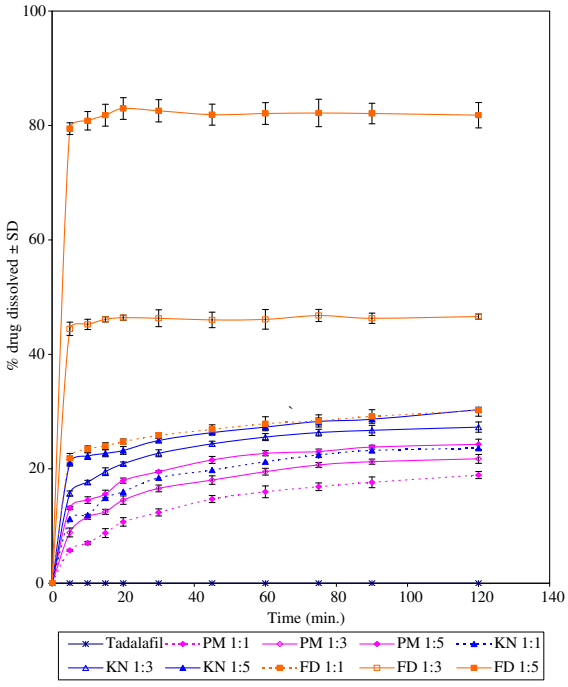

Fig. 12. Dissolution profiles of tadalafil from tadalafil–HP-β-CD binary systems in 0.1 N HCl at 37 ± 0.5 °C (mean ± SD, $n = 3$).

The increase in the drug dissolution rate from physical mixtures could be, to a lesser extent, due to the surfactant-like properties of cyclodextrins, which reduce the interfacial tension between the water insoluble drug particles and the dissolution medium, thus improving the wettability and dissolution of the drug [31]. The KN products showed approximately the same dissolution behavior of the physical mixtures with slightly more enhancement in tadalafil dissolution, which is in complete accordance with the

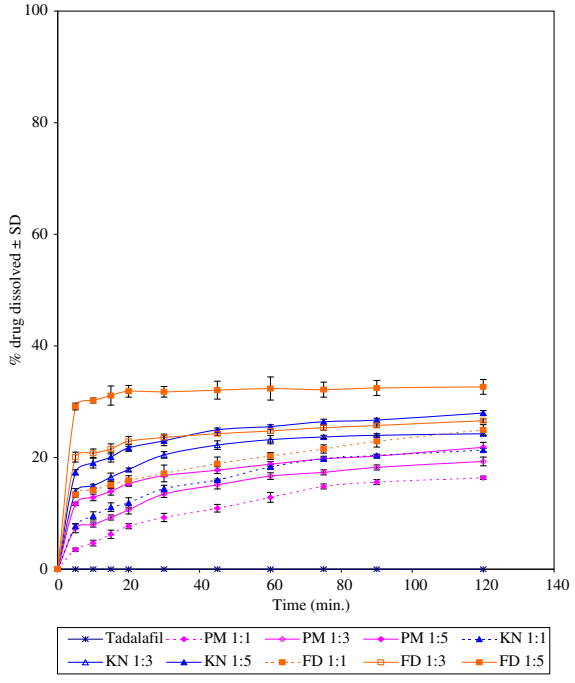

Fig. 11. Dissolution profiles of tadalafil from tadalafil-β-CD binary systems in 0.1 N HCl at 37 ± 0.5 °C (mean ± SD, $n = 3$).

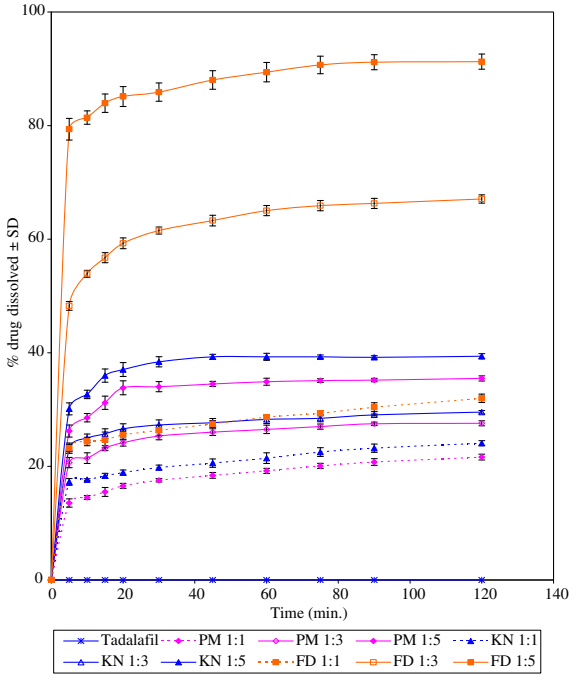

Fig. 13. Dissolution profiles of tadalafil from tadalafil–DM-β-CD binary systems in 0.1 N HCl at 37 ± 0.5 °C (mean ± SD, $n = 3$).

Table 1
Dissolution parameters of different tadalafil–CD binary systems (values are mean ± SD, $n = 3$)

Tadalafil–CD system	Molar ratio	Initial dissolution rate (IDR % dissolved/min)			Extent of dissolution after 60 min (DP_{60} %)		
		PM	KN	FD	PM	KN	FD
Tadalafil-β-CD	1:1	0.70 ± (0.06)	1.54 ± (0.08)	2.67 ± (0.09)	12.87 ± (0.88)	18.33 ± (0.33)	20.28 ± (0.61)
	1:3	1.46 ± (0.15)	2.77 ± (0.12)	4.02 ± (0.18)	16.67 ± (0.58)	23.21 ± (0.73)	24.77 ± (0.45)
	1:5	2.34 ± (0.06)	3.47 ± (0.09)	5.83 ± (0.12)	18.82 ± (0.45)	25.55 ± (0.33)	32.37 ± (2.08)
Tadalafil–HP-β-CD	1:1	1.15 ± (0.03)	2.24 ± (0.03)	4.37 ± (0.17)	15.99 ± (1.03)	21.26 ± (0.16)	27.89 ± (1.21)
	1:3	1.77 ± (0.09)	3.14 ± (0.08)	8.89 ± (0.23)	19.50 ± (0.44)	25.55 ± (0.61)	46.12 ± (1.71)
	1:5	2.63 ± (0.06)	4.21 ± (0.12)	15.89 ± (0.21)	22.72 ± (0.17)	27.3 ± (0.33)	82.09 ± (1.90)
Tadalafil–DM-β-CD	1:1	2.71 ± (0.15)	3.45 ± (0.11)	4.64 ± (0.12)	19.21 ± (0.46)	21.45 ± (0.94)	28.67 ± (0.29)
	1:3	4.13 ± (0.18)	4.64 ± (0.18)	9.65 ± (0.15)	26.52 ± (0.74)	28.28 ± (0.74)	65.03 ± (0.89)
	1:5	5.25 ± (0.33)	6.03 ± (0.21)	15.87 ± (0.38)	34.91 ± (0.45)	39.29 ± (0.61)	89.41 ± (1.71)

β-CD, beta cyclodextrin; HP-β-CD, hydroxypropyl beta cyclodextrin; DM-β-CD, dimethyl beta cyclodextrin; PM, physical mixture; KN, kneaded products; FD, freeze-dried products.

Table 2
DE_{60} of different tadalafil–CD binary systems (values are mean ± SD, $n = 3$)

Tadalafil–CD system	Molar ratio	Dissolution efficiency (DE_{60} %)		
		PM	KN	FD
Tadalafil-β-CD	1:1	8.43 ± (0.52)	13.14 ± (0.43)	16.38 ± (0.90)
	1:3	12.04 ± (0.45)	18.72 ± (0.53)	22.15 ± (0.45)
	1:5	15.41 ± (0.21)	21.65 ± (0.51)	30.21 ± (1.09)
Tadalafil–HP-β-CD	1:1	11.40 ± (0.58)	16.61 ± (0.28)	24.46 ± (0.62)
	1:3	14.96 ± (0.47)	21.03 ± (0.10)	44.04 ± (1.08)
	1:5	18.14 ± (0.08)	23.59 ± (0.28)	78.49 ± (1.71)
Tadalafil–DM-β-CD	1:1	16.39 ± (0.39)	18.75 ± (0.52)	25.16 ± (0.67)
	1:3	23.57 ± (0.48)	25.63 ± (0.78)	57.42 ± (0.49)
	1:5	31.48 ± (0.44)	35.60 ± (0.45)	82.155 ± (1.53)

physicochemical characterization. The limited improvement effect of the kneading method is the direct result of employing a semi-solid medium during the systems preparation, where the interactions between the drug and the cyclodextrin might be limited [5,31]. The observed slight increment in drug dissolution compared to the PMs is probably due to the increase in the drug-carrier contact surface as a consequence of the more drastic mechanical treatment [5].

It was obvious that the FD systems showed marked increase in tadalafil dissolution compared with the other methods, especially for the water soluble β-CD derivatives, showing a burst effect of more than 75% during the first 5 min that could be attributed mainly to the formation of soluble inclusion complexes of the drug with CDs and the high energetic amorphous state or reduction of the crystallinity following complexation as reported previously [32]. Additionally, Betageri and Makarla [33] stated that the marked increase in the dissolution rate might be due to the formation of solid solution of the drug in the FD products as a result of the complete inclusion of the drug into the CD cavities. The particle size was reduced to the molecular size when the carrier brought the drug into the dissolution medium, leading to fast dissolution. The observed lower increment in drug dissolution from β-CD freeze-dried systems compared to HP-β-CD and DM-β-CD products reflects the inability of the parent CD to promote true inclusion complexation with tadalafil even under reduced temperature and pressure, as confirmed by the physicochemical characterization. This could result from the highly hydrophobic property of the drug and the low water solubility of β-CD [34]. The use of glacial acetic acid in freeze-drying technique was found to improve tadalafil complexation with CDs. This finding is in full agreement with the work of Johnson et al. [35], who used glacial acetic acid as a solvent to improve cyclodextrin complexation with an anti-hepatitis drug. Furthermore, Loftsson et al. [36] used glacial acetic acid to enhance the complexation efficiency of cyclodextrins with water insoluble basic drugs through drug ionization in aqueous medium. Being volatile and miscible with water in all proportions, acetic acid is considered as a good candidate for freeze-drying medium [36].

The influence of tadalafil: CD molar ratio on the dissolution was clear, where the drug dissolution was enhanced on increasing the cyclodextrin proportion. PMs showed the least effect for the molar ratio since the enhancement in dissolution is mainly due to the wetting effect of the cyclodextrins, to which cyclodextrins contribute to an equal extent, with their different molar ratios [31,37]. However, the increase in dissolution might also be due to the availability of more CD molecules in the hydrodynamic layer surrounding the drug to undergo in-situ inclusion of drug molecules. Conversely, the most pronounced effect for the molar ratio was observed for the FD products due to better dispersion and/or inclusion of the drug with increasing the cyclodextrin molar ratio during preparation.

In addition to the preparation method and the drug to CD molar ratio, the effect of the CD type was also evident on the dissolution of tadalafil, where the solid binary systems prepared using HP-β-CD and DM-β-CD exhibited superior enhancement in tadalafil dissolution compared with that prepared using the parent β-CD, especially on using the freeze-dried technique. This could be explained on the basis of greater water solubility, better wetting ability and higher complexing power of β-CD derivatives towards the drug in the solid state. The increment in drug dissolution from tadalafil–DM-β-CD systems was also higher than the corresponding ones with HP-β-CD, probably due to stronger interaction between tadalafil and DM-β-CD and/or better inclusion of the drug molecules into the CD hydrophobic cavity that expanded by the two methyl groups [20]. The previous findings are in perfect agreement with the values of CE obtained for tadalafil with the used CDs.

The results of the two-way ANOVA performed on the DE_{60} data revealed the presence of significant differences among the different CD types, preparation methods and molar ratios at $p \leqslant 0.05$

($F_{2,54}$ = 3114.99, 7563.34 and 5147.61 for CD type, preparation method and molar ratio, respectively). The computed *F*-values could indicate that the dissolution of tadalafil from its binary systems was dependent mostly on the preparation method followed by the molar ratio and finally CD type.

Multiple comparisons between the different preparation methods for each CD type at each molar ratio according to Scheffé test revealed that the freeze-drying technique exhibited the most significant effect on the dissolution enhancement of tadalafil compared to the other methods at $p \leqslant 0.05$. In addition, multiple comparisons between the different molar ratios for the freeze-dried products of each CD according to Scheffé test revealed that the molar ratio of 1:5 (drug to CD) exhibited the most significant improvement on the dissolution efficiency compared to the other molar ratios for all cyclodextrins at $p \leqslant 0.05$. These results confirmed that the freeze-dried systems prepared at a molar ratio of 1:5 (drug to CD) showed the most superior and significant effect on the dissolution pattern of tadalafil. Therefore, the DE_{60} of these systems were statistically compared using Scheffé test to separate the effect of different cyclodextrins at $p \leqslant 0.05$. The results showed a significant difference between β-CD and both of HP-β-CD and DM-β-CD, while no significant difference was observed between HP-β-CD and DM-β-CD. Accordingly, the FD system, tadalafil–HP-β-CD, prepared at a molar ratio of 1:5 was chosen for further formulation into tablet due to the well-documented safety profile of HP-β-CD and lower cost compared to DM-β-CD [38].

4. Conclusion

Solid binary systems of tadalafil with β-CD, HP-β-CD and DM-β-CD were prepared using kneading and freeze-drying techniques in 1:1, 1:3 and 1:5 (drug: CD) molar ratios. From the above results, it is possible to conclude that both HP-β-CD and DM-β-CD were able to form true inclusion complexes with tadalafil at a molar ratio of 1:5 using the freeze-drying technique. The dissolution of tadalafil was markedly enhanced in both systems, showing an initial burst effect of more than 75% in the first 5 min. No significant difference was found between the two systems at $p \leqslant 0.05$. Therefore, the FD system of tadalafil with HP-β-CD prepared at a molar ratio of 1:5 could be chosen for the formulation of tadalafil tablets due to the well-documented safety profile of HP-β-CD and lower cost compared to DM-β-CD.

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