Journal of Pharmaceutical and Biomedical Analysis 47 (2008) 535–540

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Review

Effect of β-cyclodextrin and hydroxypropyl β-cyclodextrin complexation on physicochemical properties and antimicrobial activity of cefdinir

Omair Aleem [a], Bhanudas Kuchekar [a], Yogesh Pore [a,*], Sameer Late [b]

[a] Department of Pharmaceutical Chemistry, Government College of Pharmacy, Karad, Maharashtra 415124, India
[b] College of Pharmacy and Health Sciences, Mercer University, 3001 Mercer University Drive, Atlanta, GA 30341, USA

ARTICLE INFO

Article history:
Received 14 September 2007
Received in revised form 19 December 2007
Accepted 11 February 2008
Available online 15 February 2008

Keywords:
Cefdinir
Cyclodextrins
Dissolution
Inclusion complex
Antimicrobial activity

ABSTRACT

The solid-state properties, dissolution profile and antimicrobial activity of inclusion complexes of cefdinir (CEF) with β-cyclodextrin (βCD) and hydroxypropyl β-cyclodextrin (HPβCD) were investigated. The phase solubility profiles of cefdinir with βCD and HPβCD were classified as A_L-type, which indicates the formation of 1:1 stoichiometry inclusion complexes. Stability constants with 1:1 molar ratio obtained from the phase solubility diagrams were 120.38 ± 1.07 and $58.60 \pm 1.20\ M^{-1}$ for βCD and HPβCD, respectively. Binary systems of CEF with βCD and HPβCD prepared by kneading method were characterized by Fourier transformation-infrared spectroscopy (FTIR) and X-ray powder diffractometry (XRD). The aqueous solubility of CEF was enhanced by 101% for βCD and 23.4% for HPβCD, respectively. The dissolution profiles of inclusion complexes were determined and compared with those of CEF alone and their physical mixtures. The dissolution rate of CEF was increased by βCD and HPβCD inclusion complexation moderately. However, the antimicrobial activity of CEF was increased significantly ($p < 0.001$) by βCD and HPβCD inclusion complexation against *S. aureus* and *E. coli*. In all these studies, HPβCD had superior antimicrobial activity than that of βCD while βCD had greater effect on solubility enhancement of CEF.

Contents

* Corresponding author. Tel.: +91 9890237303/2164 274133; fax: +91 2164 271196.
E-mail addresses: yogeshvpore@rediffmail.com, poreyogesh@yahoo.co.in (Y. Pore).

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.02.006

1. Introduction

Cefdinir (CEF; [6*R*-[6α,7β(*Z*)]]-7-[[(2-amino-4-thiazolyl) (hydroxyimino) acetyl] amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo [4.2.0]-oct-2-ene-2-carboxylic acid; Fig. 1) is a semi-synthetic third generation broad-spectrum oral cephalosporin active against Gram-positive and Gram-negative bacteria [1]. It is used in the treatment of acute chronic bronchitis, rhinosinusitis and pharyngitis [2,3]. However, the oral bioavailability of CEF is only 21–25% [2], which is mainly due to its poor aqueous solubility. Poor bioavailability may in turn provide lower antimicrobial activity and this may give rise to the development of resistance by the microorganisms. Therefore, attempts were made in order to improve the solubility and dissolution rate of CEF using cyclodextrins. Further, the effect of cyclodextrin inclusion complexation on antibacterial activity of CEF was also investigated.

Cyclodextrins (CDs) are popular for their ability to form inclusion complex that increase the aqueous solubility and driving force for diffusion across the biological membrane for lipophilic drugs [4–7]. However, while forming inclusion complex with hydrophobic drugs, they do not alter their molecular structure and permeability characteristics [8]. Complexation with cyclodextrins has been extensively used to enhance the aqueous solubility and dissolution rate of poorly water-soluble drugs [9–11]. CDs have become popular due to their hydrophilic nature and ability to improve the solubility of poorly water-soluble drugs, enhancement in physicochemical properties and chemical stability of drugs [12]. Due to hydrophobic central cavities, CDs are capable of forming stable complexes with properly sized guest molecules [13]. Some chemically modified CDs such as hydroxypropyl β-cyclodextrin (HPβCD) have gained importance, because of their suitable cavity sizes and greater hydrophilicity. CDs act as excellent carriers for the hydrophobic drug molecules in solution phase and deliver them to the surface of the biological membrane [14]. It has been also reported that, CDs can improve antimicrobial properties of chemotherapeutic agents by increasing their release rate from inclusion complex [15].

The objective of the present work was to study the effect of cyclodextrin inclusion complexation on aqueous solubility, dissolution rate and antimicrobial activity of CEF. The inclusion complexes of CEF with βCD and HPβCD were prepared by kneading method. The solubility type and the stability constants of the complexes were established according to phase solubility studies. The dissolution properties of inclusion complexes were studied and compared with CEF alone and physical mixtures. Antimicrobial activity of inclusion complexes was checked by cup-plate method against Gram-positive species *S. aureus* and Gram-negative species *E. coli* and compared with CEF alone and physical mixtures. Fourier transformation-infrared spectroscopy (FTIR) and X-ray powder diffractometry (XRD) were used to characterize the solid-state properties of CEF, physical mixtures and inclusion complexes.

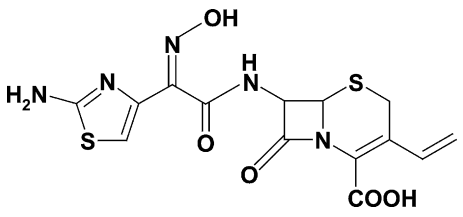

Fig. 1. Chemical structure of cefdinir.

2. Materials and methods

2.1. Materials

Cefdinir was kindly supplied by Lupin Ltd., Mumbai, India as a gift sample. β-Cyclodextrin (βCD) and 2-hydroxypropyl β-cyclodextrin (HPβCD) were kindly supplied by Panacea Biotech, Chandigad, India. The degree of substitution for 2-hydroxypropyl β-cyclodextrin (HPβCD) was 0.65 [16]. All the reagents used were of analytical grade. Double distilled water was used throughout the experiments.

2.2. Phase solubility studies

Phase solubility studies were carried out in water in triplicate according to the method described by Higuchi and Connors [17]. Excess amount of CEF (50 mg) was added to 20 ml of aqueous solutions containing various concentrations of βCD or HPβCD (0–0.01 M). Then, the suspensions were shaken on rotary shaker at 25 ± 2 °C for 4 days. After equilibrium was achieved, the samples were filtered through 0.45 μm membrane filter and appropriately diluted. The concentration of CEF was determined spectrophotometrically (Shimadzu UV–vis Spectrophotometer, 1700, Japan) at 286 nm. The apparent stability constants K_s were calculated from phase solubility diagrams with the assumption of 1:1 stoichiometry according to the following equation:

$$K_s = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (1)$$

S_0 is the solubility of CEF in absence of CDs.

2.3. Preparation of solid binary systems

The following binary systems of CEF with βCD and HPβCD were prepared in 1:1 molar ratio.

2.3.1. Preparation of physical mixtures of CEF with βCD and HPβCD

The physical mixtures (PM) of CEF–βCD and CEF–HPβCD in 1:1 molar ratio were prepared by mixing individual components that had previously been sieved through sieve no. 60.

2.3.2. Preparation of inclusion complex by kneading method

CEF and CDs with 1:1 molar ratio were accurately weighed and transferred to mortar. The mixtures were then triturated in a mortar with a small volume of water–ethanol (1:1 v/v) solution till a homogenous paste was formed. The paste formed was kneaded for 45 min and then dried at 45 °C in an oven. The dried masses were pulverized and sieved through sieve no. 60.

2.4. X-ray powder diffractometry

The XRD patterns of CEF, CEF–βCD, CEF–HPβCD inclusion complexes and physical mixtures were recorded by using Philips Analytic X-Ray—PW 3710 (Holland) diffractometer with tube anode Cu over the interval 5–70°/2θ. The operation data were as follows: generator tension (voltage) 40 kV, generator current 30 mA and scanning speed 2°/min.

2.5. Fourier transformation-infrared spectroscopy

Infrared spectra were obtained using a PerkinElmer spectrum-one FTIR spectrometer using KBr disks. The samples were previously ground and mixed thoroughly with KBr. The KBr disks

were prepared by compressing the powder. The scanning range was kept from 4000 to 450 cm^{-1}.

2.6. Saturation solubility studies

Saturation solubility studies were performed in distilled water in triplicate according to the method reported by Higuchi and Connors [17]. Excess of pure drug, physical mixtures and inclusion complexes were added to 20 ml of distilled water taken in screw cap tube and shaken for 24 h in rotary flask shaker at a room temperature to achieve the equilibrium. In preliminary studies, it was found that equilibrium solubility was achieved in 24 h and therefore samples were shaken for 24 h. Appropriate aliquots were then withdrawn and filtered through Whatman filter paper no. 41. The filtrate so obtained was analysed spectrophotometrically at 286 nm. The results obtained from saturation solubility studies were statistically validated using ANOVA (Tukey–Kramer multiple comparisons test).

2.7. Dissolution studies

The dissolution rate studies of CEF alone, physical mixtures and inclusion complexes were performed in triplicate in a dissolution apparatus (model: Veego DA-6-D tablet dissolution test apparatus, Mumbai, India) using the paddle method (USP Type II). Dissolution studies were carried out using 900 ml of 0.1 M phosphate buffer (pH 7.0) at 37 ± 0.5 °C at 100 rpm. 300 mg of CEF or its equivalent amount of CEF–CDs complexes was added to 900 ml of 0.1 M phosphate buffer (pH 7.0). Samples of 5 ml were withdrawn at time intervals of 5, 10, 20, 30, 50 and 60 min. The volume of dissolution medium was adjusted to 900 ml by replacing each 5 ml aliquot withdrawn with 5 ml of fresh 0.1 M phosphate buffer (pH 7.0). The solutions were immediately filtered through 0.45 μm membrane filter, suitably diluted and the concentrations of CEF in samples were determined spectrophotometrically at 286 nm. The results of dissolution studies were statistically validated using ANOVA (Tukey–Kramer multiple comparisons test).

2.8. Antimicrobial studies

In vitro antimicrobial studies of inclusion complexes were performed by cup-plate method against Gram-positive species *S. aureus* and Gram-negative species *E. coli* in the concentration of 0.2 μg/ml (MIC 0.1–0.5 μg/ml). The activities were compared with pure CEF and physical mixtures. CEF was dissolved in small amount of dimethyl formamide and suitably diluted with distilled water to obtain the final concentration. Similarly inclusion complexes and physical mixtures were dissolved separately in distilled water and suitably diluted with the same to obtain the concentration of 0.2 μg/ml. Aqueous solutions of pure βCD and HPβCD were used as a placebo control. The plates were incubated at 37 °C for 24 h and the zone of inhibition was measured in mm. The results of antimicrobial studies were statistically validated using ANOVA (Tukey–Kramer multiple comparisons test).

3. Results and discussion

3.1. Phase solubility studies

The phase solubility profiles of CEF–βCD and CEF–HPβCD are presented in Fig. 2A and B, respectively. These plots showed that aqueous solubility of the drug increases linearly as a function of βCD and HPβCD. The phase solubility profile of CEF with βCD and HPβCD can be classified as A_L-type. The linear host–guest correlation coefficient $r = 0.9877$ ($r^2 = 0.9756$) with a slope (m) of 0.08765 and $r = 0.9966$ ($r^2 = 0.9932$) with a slope (m) of 0.0688 suggested the

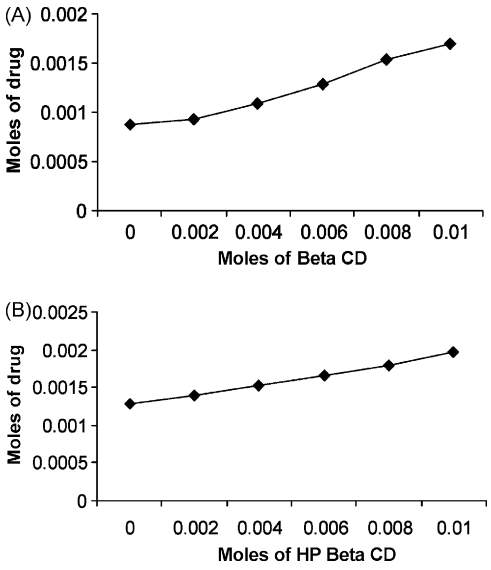

Fig. 2. (A) Phase solubility diagram of CEF–βCD system in water. (B) Phase solubility diagram of CEF–HPβCD system in water.

formation of a 1:1 complex with respect to βCD and HPβCD concentrations. The apparent stability constants, $K_{1:1}$ obtained from the slope of the linear phase solubility diagrams were 120.38 ± 1.07 and 58.60 ± 1.20 M^{-1} for βCD and HPβCD, respectively (Eq. (1)). The $K_{1:1}$ values suggested that CEF formed more stable complex with βCD than with HPβCD, which may be because of a steric hindrance of hydroxypropyl group of HPβCD which has restricted the entry of guest molecule into the CD cavity [13].

3.2. Fourier transformation-infrared spectroscopy

Fig. 3A illustrates the FTIR spectra of CEF, βCD, physical mixture and CEF–βCD inclusion complex. IR spectrum of CEF (a) is characterized by principal absorption peaks at 3300 cm^{-1} (O—H stretch COOH), 2978 cm^{-1} (C—H stretch cyclic), 2898 cm^{-1} (C—H stretch), 1781 cm^{-1} (C=O), 1667 cm^{-1} (C=C alkene), 1610 cm^{-1} (C=C aromatic), 1544 cm^{-1} (N—H bending), 1428 cm^{-1} (C—N stretch) and 656 cm^{-1} (C—S). The IR spectrum of βCD (b) shows prominent peaks at 3398 cm^{-1} (O—H), 2925 cm^{-1} (C—H), 1643 cm^{-1} (H—O—H bending), 1157 cm^{-1} (C—O), 1028 cm^{-1} (C—O—C).

The IR spectra of PM (c), shows peaks of both CEF and βCD with decrease in the peak intensity. However some peaks of CEF at 3300, 2978, 2898 and at 656 cm^{-1} were disappeared indicating strong physical interaction of CEF with βCD. In the IR spectra of inclusion complex (d) the peaks of CEF at 3300, 2978, 2898 and at 656 cm^{-1} completely disappeared indicating that cephem ring with carboxylic functional group of guest had been entrapped in the hydrophobic cavity of host molecule. The peak of OH group of βCD at 3398 cm^{-1} was shifted towards lower frequency 3303 cm^{-1} due to intermolecular hydrogen bonding with CEF. The peak at 1649 cm^{-1} in IR spectra of βCD due to water of crystallization, also disappeared in both PM and inclusion complex [13]. These changes occurred in IR spectra of samples indicated formation of inclusion complex in solid state.

Fig. 3B shows the FTIR spectra of CEF, HPβCD, physical mixture and CEF–HPβCD inclusion complex. The IR spectrum of HPβCD (b) shows prominent peaks at 3390 cm^{-1} (O—H), 2928 cm^{-1} (C—H), 1647 cm^{-1} (H—O—H bending). In physical mixture the broad peak of HPβCD (c) at 3390 cm^{-1} was shifted to 3365 cm^{-1}. All the peaks of CEF completely disappeared with a shift of 1781–1767 cm^{-1}. The FTIR spectra of HPβCD complex (d), shows complete disappearance

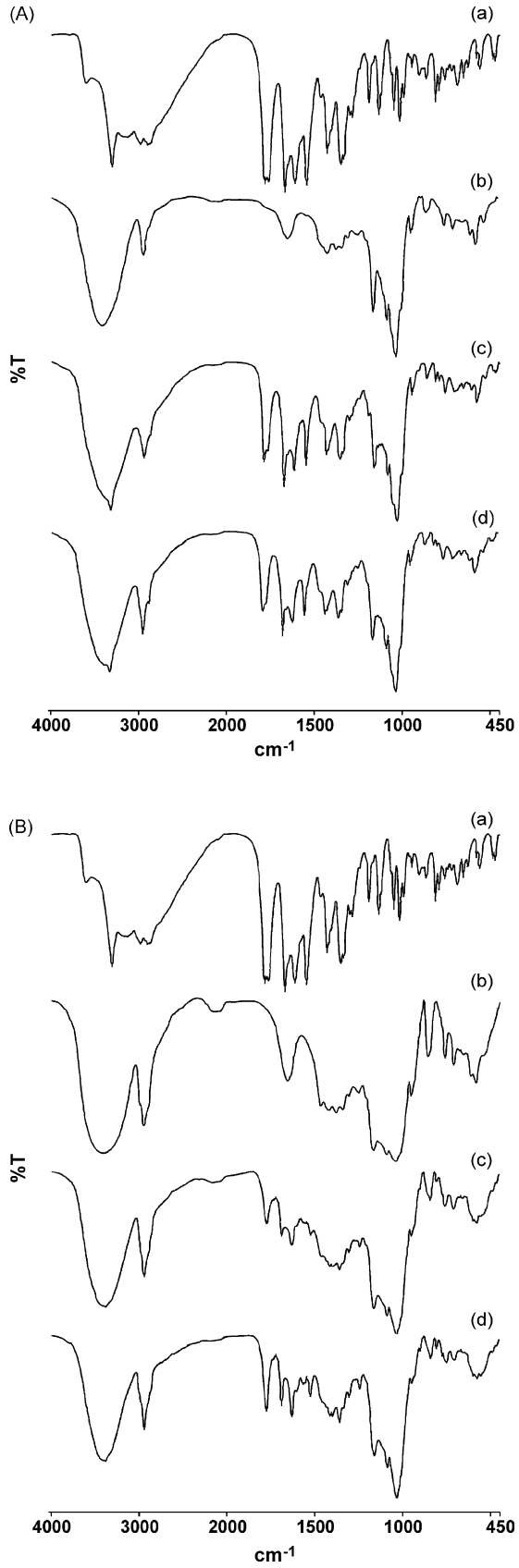

Fig. 3. (A) FTIR spectra of CEF–βCD systems: (a) CEF; (b) βCD; (c) physical mixture and (d) inclusion complex. (B) FTIR spectra of CEF–HPβCD systems: (a) CEF; (b) HPβCD; (c) physical mixture and (d) inclusion complex.

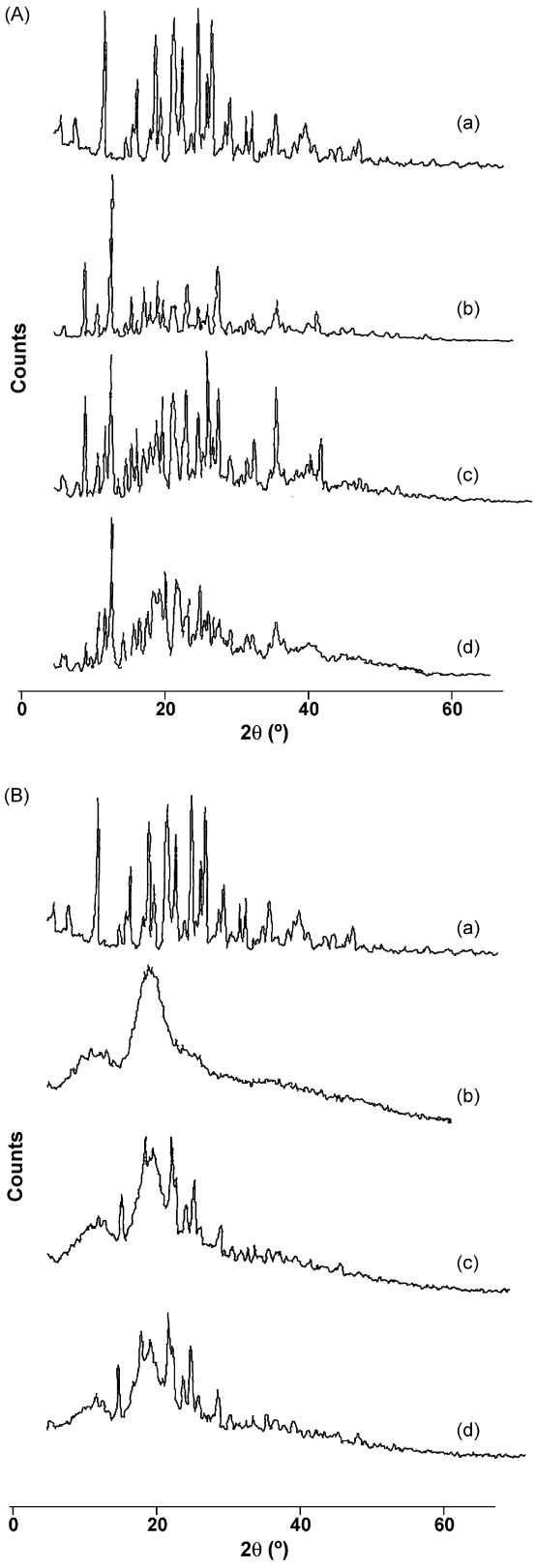

Fig. 4. (A) XRD patterns of CEF–βCD systems: (a) CEF; (b) βCD; (c) physical mixture and (d) inclusion complex. (B) XRD patterns of CEF–HPβCD systems: (a) CEF; (b) HPβCD; (c) physical mixture and (d) inclusion complex.

Table 1
Solubility data of pure cefdinir, physical mixtures and inclusion complexes

System	Solubility in water at 25 °C (μg/ml)* (mean ± S.D.)	S.E.M.
Cefdinir (CEF)	431.97 ± 1.56	0.89
βCD physical mixture	720.13 ± 1.49[†]	0.86
βCD complex	869.27 ± 1.76 [†,a,c]	1.02
HPβCD physical mixture	493.00 ± 1.61[†]	0.93
HPβCD complex	531.93 ± 1.66[†,b]	0.96

* Indicates mean of three experiments; S.D.: standard deviation; S.E.M.: standard error of mean; † p value compared to pure CEF ($p < 0.001$); [a]p value compared to βCD physical mixture ($p < 0.001$); [b]p value compared to HPβCD physical mixture ($p < 0.001$); [c]p value compared to HPβCD complex ($p < 0.001$).

of the CEF peaks at 3300, 2978, 2898 and at 656 cm^{-1} with strong decrease in peak intensity. This suggested that, CEF could form inclusion complex with HPβCD in solid state. All the binary systems of CEF–CD did not show any new peaks, indicating no chemical bond formation in the complexes.

3.3. X-ray powder diffractometry

The XRD pattern of CEF showed (Fig. 4A and B) peaks that were intense and sharp, indicating its crystalline nature. Crystallinity was determined by comparing some representative peak heights in the diffraction patterns of the binary systems with those of a reference. CEF showed sharp peak at 26° (2θ) with highest peak intensity 231. The relative degree of crystallinity (RDC) was calculated according to the equation:

$$\mathrm{RDC} = \frac{I_{\mathrm{sam}}}{I_{\mathrm{ref}}} \quad (2)$$

where I_{sam} is the peak height of the sample and I_{ref} is the peak height at the same angle for the reference with the highest intensity [18]. The peak height at 26° (2θ) was used for calculating the RDC of kneaded and physical mixture binary system. The RDC values of corresponding binary systems of CEF–βCD (Fig. 4A) were 0.6233 and 0.7532, respectively. However, the RDC values of corresponding binary systems of CEF–HPβCD (Fig. 4B) cannot be calculated as the peak of CEF at the same angle completely disappeared in these binary systems. The diffraction pattern of all binary systems of CEF also showed other peaks of CEF with decrease in the peak intensity indicating reduction in crystallinity. However in the binary systems of CEF–HPβCD, the crystallinity of CEF was found to be reduced to a greater extent, evidenced by complete disappearance of intense of peaks of CEF. Further, XRD studies of CEF–HPβCD inclusion complex (4B-d) shows typical and characteristic hollow pattern of HPβCD suggesting formation of inclusion complex in solid state. In case of CEF–βCD inclusion complex (4A-d), the intense and sharp peaks of CEF were highly diminished indicating formation of inclusion complex. From the XRD studies it could be concluded that there might be presence of some amorphous entities of pure CEF in inclusion complexes.

3.4. Saturation solubility studies

All the binary systems of CEF showed enhancement in the aqueous solubility as compared to pure drug alone (Table 1). The 1:1 inclusion complex of CEF with βCD showed higher solubility than all other binary systems of CEF. The enhancement in the solubility of complex is mainly attributed to the formation of stable inclusion complex of CEF with βCD. The stability constant, 120.38 ± 1.07 M^{-1} suggests that CEF and βCD are having sufficient affinity towards each other to form stable inclusion complex, as the solubility of complex was found to be increased by 101%. The physical mixture of CEF–βCD has also shown higher solubility (66.68%) than the pure drug. The enhancement in aqueous solubility of CEF can be explained in terms of wetting property and hydrophilicity of βCD with simultaneous reduction in the crystallinity of the drug caused by the kneading process and inclusion into the hydrophobic cavity of the βCD [19]. However, the binary systems of CEF–HPβCD have shown little increment in the solubility as compared to CEF–βCD binary systems. The solubility of pure CEF has been increased only by 23% when complexed with HPβCD. This may be due to lower $K_{1:1}$ value (58.60 ± 1.20 M^{-1}) of CEF–HPβCD complex. The low value of stability constant indicates less affinity of HPβCD towards CEF. However, overall effect of cyclodextrin complexation is the significant improvement in aqueous solubility of CEF as shown by the p values in Table 1.

3.5. Dissolution rate studies

The dissolution curves of CEF–βCD and CEF–HPβCD binary systems in 0.1 M pH (7.0) phosphate buffer at 37 ± 0.5 °C are shown in Fig. 5A and B, respectively. The release rate profiles were expressed as the percentage of drug released (vs.) time. The dissolution time of CEF from inclusion complexes and physical mixtures was determined and further evaluated. Table 2 shows % drug dissolved at 5 min (DP_5), at 10 min (DP_{10}), at 30 min (DP_{30}) and at 60 min (DP_{60}).

According to these results, all the DP values obtained for physical mixtures of both the cyclodextrins were not significantly different than that of CEF alone ($p > 0.05$). The kneaded products have shown higher dissolution rate as compared to the physical mixtures and pure drug. At DP_5 and DP_{10} ($p < 0.05$), and at DP_{30} and DP_{60} ($p < 0.01$), inclusion complex prepared with HPβCD had a significant difference with respect to drug CEF alone whereas, inclusion complex prepared with βCD showed significant effect only at DP_{30} ($p < 0.05$) and DP_{60} ($p < 0.01$) with respect to CEF alone. However, there were no significant differences for all the DP values when inclusion complexes were compared amongst the two CDs.

The moderate enhancement in dissolution rate has been attributed to the formation of inclusion complexes in the solid state with reduction in the crystallinity of CEF, as confirmed by XRD studies. The dissolution rate increase for inclusion complexes was due to greater hydrophilicity, higher wetting effect, mechanical treatment, which increased the contact between the drug and the carrier

Table 2
The dissolution parameters of pure cefdinir, physical mixtures and inclusion complexes in phosphate buffer pH 7 at 37 ± 0.5 °C

System	DP_5* ± S.D.	DP_{10}* ± S.D.	DP_{30}* ± S.D.	DP_{60}* ± S.D.
Pure CEF	62.48 ± 3.9	71.76 ± 4.1	76.47 ± 3.5	82.12 ± 4.3
βCD PM[§]	67.81 ± 4.2	72.35 ± 3.7	77.12 ± 4.3	85.22 ± 4.1
βCD KN	72.42 ± 3.8	80.28 ± 4.5	88.89 ± 3.9 ($p < 0.05$)[‡] ($p < 0.05$)[!]	99.65 ± 3.4 ($p < 0.01$)[‡] ($p < 0.01$)[!]
HPβCD PM[§]	71.03 ± 3.7	73.04 ± 3.8	77.22 ± 3.9	90.32 ± 4.1
HPβCD KN[#]	74.63 ± 4.5 ($p < 0.05$)[†]	83.08 ± 3.5 ($p < 0.05$)[†]	90.95 ± 4.2 ($p < 0.01$)[†] ($p < 0.05$)[ψ]	99.89 ± 4.0 ($p < 0.01$)[†]

*Indicates mean of three experiments; S.D.: standard deviation; PM: physical mixture; KN: kneaded product (complex); DP_5: % dissolved at 5 min; DP_{10}: % dissolved at 10 min; DP_{30}: % dissolved at 30 min; DP_{60}: % dissolved at 60 min; † indicates p value compared to pure CEF: significant at all DP values; ψ indicates p value compared to HPβCD PM: significant at only DP_{30}; ‡ indicates p value compared to pure CEF: significant at only DP_{30} and DP_{60}; ! indicates p value compared to βCD PM: significant at only DP_{30} and DP_{60}; § indicates p value compared to pure CEF: not significant at all DP values ($p > 0.05$); # indicates p value compared to βCD KN: not significant at all DP values ($p > 0.05$).

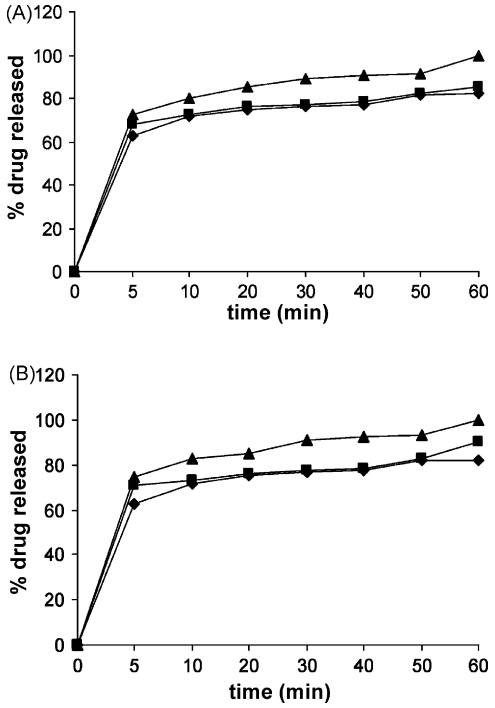

Fig. 5. (A) The dissolution diagram of CEF–βCD systems at 37 ± 0.5 °C: (♦) CEF; (■) physical mixture and (▲) inclusion complex. (B) The dissolution diagram of CEF–HPβCD systems at 37 ± 0.5 °C: (♦) CEF; (■) physical mixture and (▲) inclusion complex.

Table 3
Antimicrobial activity of pure cefdinir, physical mixtures and inclusion complexes

System	Zone size (mm)* mean ± S.D.			
	S. aureus	S.E.M.	*E. coli*	S.E.M.
CEF	3.1 ± 0.36	0.21	2.9 ± 0.31	0.18
βCD (placebo control)	–	–	–	–
HPβCD (placebo control)	–	–	–	–
βCD physical mixture	6 ± 0.35[†]	0.20	6 ± 0.25	0.15[‡]
βCD complex	7 ± 0.25[†,a]	0.15	8 ± 0.35	0.20[‡,d]
HPβCD physical mixture	5 ± 0.32[†]	0.19	5.9 ± 0.32	0.19[‡]
HPβCD complex	11 ± 0.35[†,b,c]	0.20	12.1 ± 0.26	0.15[‡,e,f]

* Indicates mean of three experiments; S.D.: standard deviation; S.E.M.: standard error of mean; [†] *p* value compared to pure CEF (*S. aureus*: $p < 0.001$); [a] *p* value compared to βCD physical mixture ($p < 0.05$); [b] *p* value compared to HPβCD physical mixture ($p < 0.001$); [c] *p* value compared to βCD complex ($p < 0.001$). [‡] *p* value compared to pure CEF (*E. coli*: $p < 0.001$); [d] *p* value compared to βCD physical mixture ($p < 0.001$); [e] *p* value compared to HPβCD physical mixture ($p < 0.001$); [f] *p* value compared to βCD complex ($p < 0.001$).

and ability to form stable inclusion complex of the βCD and HPβCD [20].

3.6. Antimicrobial studies

The antimicrobial activity of all binary systems of CEF with βCD and HPβCD against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) species was checked by cup-plate method and compared with the pure CEF. The results are summarized in Table 3. These studies revealed that all binary systems of CEF have shown greater antimicrobial activity than CEF alone. However, CEF–HPβCD inclusion complex has shown significant and highest zone of inhibition against both the microorganisms as compared to pure CEF alone and its all other binary systems. The greater antimicrobial activity of CEF–HPβCD inclusion complex may be due to the ability of HPβCD to release the drug readily from the inclusion complex [15]. Further, the low stability constant of CEF–HPβCD complex favours the rapid release of drug from the inclusion complex. On the contrary, the high value of stability constant of CEF–βCD complex perhaps hinders the release of pure drug from the complex. Thus HPβCD was found to be superior to βCD in enhancing antimicrobial activity of CEF as shown by the *p* values in Table 3.

4. Conclusion

The present investigation revealed that CEF can form inclusion complex with both βCD and HPβCD in solid state. The stoichiometry of complex formation is in 1:1 molar ratio with better stability constant. From these results, it can be assumed that the formation of the inclusion complex of CEF with either βCD or HPβCD can increase the aqueous solubility of CEF. The improved dissolution rate may be due to increase in solubility, brought about by complexation, amorphizing power of CDs and mechanical treatment. From these evidences it can be concluded that the aqueous solubility and dissolution rate of CEF can be significantly increased by forming an inclusion complex with CDs. Further, it is found that CDs can also improve the antimicrobial activity of CEF in vitro by increasing its release rate. In all these studies, HPβCD was found to be superior to βCD with respect to antimicrobial activity while βCD was found to be superior with respect to enhancement in aqueous solubility of CEF.

Acknowledgements

The authors are thankful to Shivaji University, Kolhapur, Maharashtra, India for providing IR and XRD facilities. Authors are very much thankful to Principal, Govt. College of Pharmacy, Karad, Maharashtra, India for providing laboratory facilities and constant encouragement.

References

[1] S. Budavari (Ed.), The Merck Index, Whitehouse Station, Merck and Co. Inc., New Jersey, NY, 1996, p. 316.
[2] C.M. Perry, L.J. Scott, Drugs 64 (2004) 1433–1464.
[3] A. Marchese, D. Saverino, E.A. Debbia, A. Pesce, G.C. Schito, J. Antimicrob. Chemother. 35 (1995) 53–66.
[4] J.A. Bruce, J. Pharm. Sci. 89 (2000) 429–441.
[5] M. Mar, L. Thorsteinn, M. Gisli, J. Control. Release 59 (1999) 107–118.
[6] C. Gian, C. Paolo, L. Simone, J. Pharm. Sci. 91 (2002) 2399–2405.
[7] T. Loftsson, N. Bodor, in: E.W. Smith, H.I. Maibach (Eds.), Percutaneous Penetration Enhancers, CRC Press, Boca Raton, 1995, pp. 335–342.
[8] H. Brun, M. Paul, N. Razzouq, M. Binhas, S. Gibaud, A. Astier, Drug Dev. Ind. Pharm. 32 (2006) 1123–1134.
[9] M.A. Vandelli, G. Salvioli, A. Mucci, R. Panini, L. Malmusi, Int. J. Pharm. 118 (1995) 77–83.
[10] C.M. Fernandes, M.T. Vieira, F.J. Viega, Eur. J. Pharm. Sci. 15 (2002) 79–88.
[11] H.A. Archontaki, M.V. Vertzoni, M.H. Athanassiou-Malaki, J. Pharm. Biomed. Anal. 28 (2002) 761–769.
[12] W. Xianhong, T. Fei, J. Zhijun, L. Ziuyang, J. Pharm. Biomed. Anal. 34 (2004) 517–523.
[13] L. Longxiao, Z. Suyan, J. Pharm. Biomed. Anal. 40 (2006) 122–127.
[14] M. Masson, T. Loftsson, G. Masson, E. Stefanson, J. Control. Release 59 (1999) 107–118.
[15] H.V. Doorne, E.H. Bosch, C.F. Lerk, Pharm. World Sci. 10 (1988) 80–85.
[16] T. Loftsson, M.E. Brewster, M. Masson, Am. J. Drug Deliv. 2 (2004) 262–275.
[17] T. Higuchi, K.A. Connors, Adv. Anal. Chem. Instrum. 4 (1965) 117–212.
[18] J.A. Ryan, J. Pharm. Sci. 75 (1986) 805–807.
[19] D. Derle, S.H.S. Boddu, M. Magar, Indian J. Pharm. Educ. Res. 40 (2006) 232–236.
[20] C.M. Fernandes, M.T. Vieira, F.J.B. Veiga, Eur. J. Pharm. Sci. 15 (2002) 79–88.