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Effect of PVP K30 and/or L-Arginine on Stability Constant of Etoricoxib–HPβCD Inclusion Complex: Preparation and Characterization of Etoricoxib–HPβCD Binary System

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The effect of polyvinyl pyrrolidone (PVP) K30 and/or L-arginine on etoricoxib–HPβCD complex was investigated. The phase solubility profiles were classified as A_L-type, both in absence or presence of auxiliary substances used. The apparent stability constant (K_c) of binary complex obtained at room temperature, 371.80 ± 2.61 M^{-1}, was decreased with the addition of PVP and arginine indicating no benefit of addition of auxiliary substances to promote higher complexation efficiency. Therefore, solid etoricoxib–HPβCD binary systems were prepared and characterized by proton nuclear magnetic resonance spectroscopy (^{1}HNMR), X-ray powder diffractometry, Fourier transformation-infrared spectroscopy, and dissolution studies. Among all binary systems, a lyophilized product showed superior performance in enhancing dissolution of etoricoxib.

Keywords etoricoxib; HPβCD; stability constant; phase solubility; ^{1}HNMR; dissolution rate

INTRODUCTION

The enhancement of oral bioavailability of poorly water-soluble drugs remains one of the most challenging aspects of drug development. An oral bioavailability of such drugs is influenced by a variety of factors that affect their absorption from the gastrointestinal tract. One determinant factor for absorption is drug dissolution that is influenced by the solubility of the drug in the gastrointestinal fluids. A variety of drug delivery systems have been developed over the years to improve the release and the dissolution of drugs. Cyclodextrin (CD) inclusion complexation is one effective approach to achieve ideal therapy for drugs with poor aqueous solubility (Loftsson, Hreinsdottir, & Masson, 2005).

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CDs served as a versatile carrier for the poorly soluble drugs by increasing its solubility and dissolution rate through formation of inclusion complex. However, high molecular weight, high cost, relatively low water solubility, and potential toxicity limit the use of CDs (Loftsson, Fridriksdóttir, & Gudmundsdóttir, 1996; Szente & Szejtli, 1999; Thompson, 1997; Uekama, Hirayama, & Irie, 1998). Further, due to lower complexation efficiency (CE) of CDs, a large amount of CDs is frequently needed to solubilize small amounts of a poorly water-soluble drug. Strengthening the complexation and solubilization efficiency of CDs is one of the possible ways to reduce their workable amount in drug delivery systems. Hydroxyacids or certain low-molecular-weight acids can strongly increase the solubilizing capacity of CD toward poorly water soluble drugs due to simultaneous effect of salt formation and inclusion complexation (Esclusa-Diaz et al., 1996; Fenyvesi, Vikmon, SzemanSzejtli, Ventura, & Pasini, 1994; Mura, Faucci, & Bettinettib, 2001a; Redenti, Szente, & Szejtli, 2000; Vikmon et al., 1994). The effect of polyvinyl pyrrolidone (PVP) K25, arginine, and other hydrophilic polymers at different pH on naproxen solubilization with CDs has been also reported (Cirri, Maestrelli, Corti, Furlanetto, & Mura, 2006; Mura et al., 2005; Valero, Perez-Revuelta, & Rodriguez, 2003). The positive effect of addition of small amounts of PVP K30 or hydroxypropylmethyl cellulose (HPMC) to a vinpocetine–CD system in improving both the complexing and solubilizing efficiencies of the CDs has been also studied (Ribeiro, Loftsson, Ferreira, & Veiga, 2003; Veiga, Ribeiro, & Ferreira, 2003). Thus the enhanced complexation can be achieved with the formation of ternary systems between a drug, CD, and a suitable auxiliary substance.

2-Hydroxypropyl-β-cyclodextrin (HPβCD) (Figure 1) is a hydroxyalkyl β-CD derivative which is widely used in pharmaceutical formulations owing to its inclusion ability, high water solubility, and low toxicity (Chen et al., 1996). Further, toxicological studies revealed that HPβCD is well tolerated by the

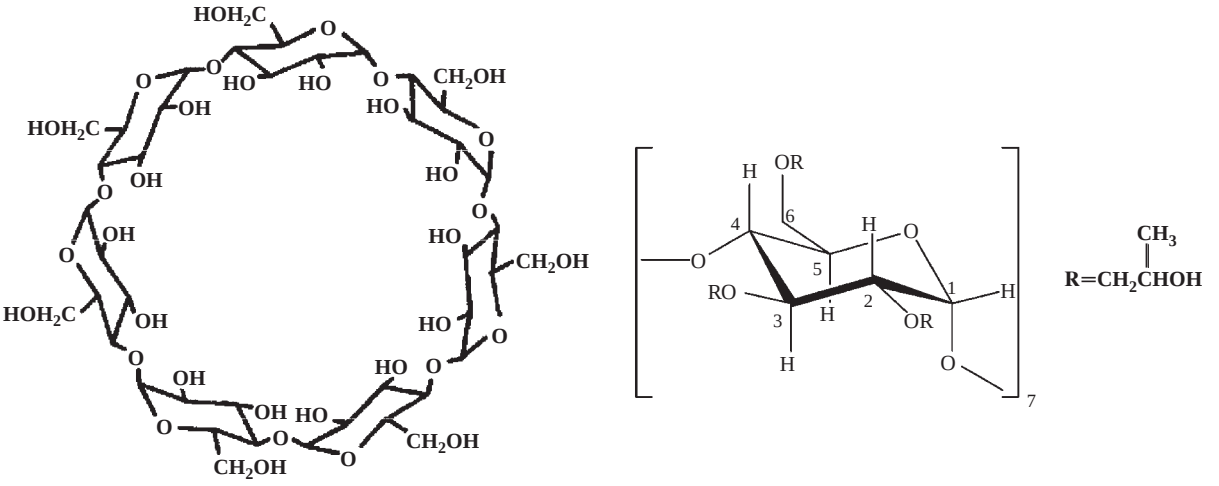

FIGURE 1. HP-β-cyclodextrin.

human body both by intravenous and oral administrations (Lazaro, Ferreira, & Gimeneza, 2007). Several papers have reported the NMR studies on structural features of CD inclusion complexes in solution. (Bettinetti, Melani, Mura, Monnanni, & Giordano, 1991; Mulinacci, Melani, Mazzi, & Vincieri, 1993; Nishijo, Ushiroda, Ohbori, Sugiura, & Fujii, 1997). In this article a combined effect of HPβCD and PVP K30 and/or L-arginine on complexation of etoricoxib has been addressed. The structure of inclusion complex has been further characterized and confirmed by ^{1}HNMR studies.

Etoricoxib (5-chloro-2-[6-methyl pyridin-3-yl]-3-[4-methyl sulfonylphenyl] pyridine) is a novel, selective second-generation cyclooxygenase-2 inhibitor administered orally as an analgesic and anti-inflammatory drug (Agrawal & Porras, 2001; Rodrigues, Halpin, & Geer, 2003). However, its very low aqueous solubility and poor dissolution can cause formulation problems and limit its therapeutic application by delaying the rate of absorption and the onset of action (Cochrane, Jarvis, & Keating, 2002). Various anti-inflammatory drugs have been complexed with CDs, thus obtaining further advantages such as dose lowering, reduction of side effects (gastric irritation) and taste masking (Bettinetti, Gazzania, Mura, Giordano, & Setti, 1992; Fromming & Szejtli, 1994). The method of complexation may play a role in drug solubilization (Uekama et al., 1998).

This work was aimed to investigate the solid-state properties of etoricoxib in its binary systems with HPβCD and to improve its dissolution profile. The effect of water-soluble polymers, namely, PVP K30 and amino acid, L-arginine in the concentrations of 0.5% (wt/vol), 1% (wt/vol), and 1.5% (wt/vol) separately on phase solubility curve of etoricoxib–HPβCD was also investigated. The results obtained from the phase solubility studies served as a basis for proper choice of none of the carrier for ternary systems. Therefore, only binary systems of etoricoxib–HPβCD were prepared and characterized. The solubility type and the stability constants of the complexes were established according to the methods described by Higuchi and Connors. The inclusion complexes of etoricoxib with HPβCD were prepared by kneading and lyophilization method whereas the physical mixture was prepared by mixing individual components. A proton magnetic resonance spectroscopy (^{1}HNMR) was used to study the structure and geometry of the inclusion complex whereas X-ray powder diffractometry (XRD) was employed to investigate solid-state properties of binary systems of etoricoxib. A drug: CD interaction was studied by using Fourier transformation-infrared spectroscopy (FTIR). The dissolution behavior of etoricoxib and its binary systems were further evaluated.

MATERIALS AND METHODS

Materials

Etoricoxib was supplied by Unichem Ltd. (Mumbai, India) as a gift sample. HPβCD was kindly provided by Panacea Biotech (Chandigarh, India). L-Arginine and PVP K30 were purchased from S.D. Fine Chemicals (Mumbai, India). All the reagents were of analytical grade. Double distilled water was used throughout the experiment.

Methods

Phase Solubility Studies

Phase solubility studies were carried out in distilled water in triplicate according to the method described by Higuchi and Connors (1965). Excess amount of etoricoxib (50 mg) was added to 20 mL of aqueous solution containing various concentrations of HPβCD (0–0.01 M) with or without fixed concentrations of PVP or L-arginine (0.5, 1, and 1.5%, wt/vol). Then, the suspensions were shaken on rotary shaker at 25 ± 2°C for 48 h. After equilibrium was achieved, the samples were filtered through 0.45-μm membrane filter and appropriately diluted. The concentration of etoricoxib was determined spectrophotometrically (Shimadzu 1700, Kyoto, Japan) at 283 nm.

The apparent 1:1 stability constants were calculated from the phase solubility diagrams, according to the following equation:

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

where S_0 is the solubility of etoricoxib in absence of CDs.

Preparation of Solid Binary Systems

The following binary systems of etoricoxib were prepared at 1:1 molar ratio.

Preparation of Physical Mixture of Etoricoxib–HPβCD. The physical mixture (PM) of etoricoxib-HPβCD binary system in 1:1 molar ratio was prepared by mixing individual components that had previously been sieved through sieve no. 60.

Preparation of Inclusion Complexes by Kneading Method. Etoricoxib and HPβCD with 1:1 molar ratio were accurately weighed and transferred to a mortar. The mixture was then triturated in a mortar with a small volume of water–ethanol (1:1, vol/vol) solution till a homogenous paste was formed. The paste that formed was kneaded for 45 min and then dried at 45°C for 24 h in an oven. The dried mass was pulverized and sieved through sieve no. 60.

Lyophilized Binary Products. Equimolar amounts of etoricoxib and HPβCD were dissolved in methanol and in water, respectively. The two solutions were sonicated for 20 min and mixed for 2 h at 50°C. Furthermore, the resultant clear solution was frozen in a deep freezer at –20°C and then the frozen solution was lyophilized in a freeze-dryer (Khera Instruments, Delhi, India) at –40°C until sample was completely dried.

Proton Nuclear Magnetic Resonance Spectroscopy

Proton nuclear magnetic resonance spectroscopy (^{1}HNMR) spectra of etoricoxib, HPβCD, inclusion complexes, and physical mixture were recorded in $CDCI_3$, D_2O, or DMSO (d6) on Varian Mercury YH-300 (Palo Alto, CA, USA) NMR spectrophotometer at an operating frequency of 300 MHz.

Fourier Transformation-Infrared Spectroscopy

Infrared spectra were obtained using a JASCO FTIR-5300 (Tokyo, Japan) 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 4,000 to 400 cm^{-1}.

X-Ray Powder Diffractometry

The XRD patterns of etoricoxib, HPβCD, inclusion complexes, and physical mixture were recorded by using Philips Analytic X-Ray—PW 3710 (Almelo, 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.

In Vitro Dissolution Rate Studies

The dissolution rate studies of etoricoxib alone, physical mixture, and inclusion complexes were performed in triplicate in a dissolution apparatus (Lab India, Model Disso 2000 Tablet dissolution test apparatus, Mumbai, India) using the paddle method (USP Type II). Dissolution studies were carried out using 900 mL of phosphate buffer (pH 7.4) at 37 ± 0.5°C at 50 rpm. About 120 mg of etoricoxib or its equivalent amount of etoricoxib–HPβCD complexes was added to 900 mL of phosphate buffer (pH 7.4). Samples of 5 mL were withdrawn at time intervals of 2, 5, 10, 15, 20, 25, 30, 45, 60, 90, and 120 min. The volume of dissolution medium was adjusted to 900 mL by replacing each 5-mL aliquot withdrawn with 5 mL of fresh phosphate buffer (pH 7.4). The solutions were immediately filtered through 0.45-μm membrane filter, suitably diluted, and the concentrations of etoricoxib in samples were determined spectrophotometrically at 283 nm. The results of dissolution studies were statistically validated using ANOVA (Tukey–Kramer Multiple Comparisons Test).

RESULTS AND DISCUSSION

Phase Solubility Studies of Binary and Ternary Systems

The phase solubility profiles of etoricoxib in aqueous HPβCD solutions with and without addition of hydrophilic carriers, PVP or arginine (0.5, 1, and 1.5%, wt/vol) are shown in Figure 2A and B. The solubility of etoricoxib increased linearly as a function of HPβCD concentration, suggesting AL-type phase solubility diagrams in all cases and indicating the formation of a complex. As the slopes of these solubility diagrams were all less than 1, the possible stoichiometry assessed was 1:1 and apparent stability constants (K_c) of the binary and ternary complexes were calculated using Equation 1. The estimated values of S_0, slopes of phase solubility diagrams, K_c, and CE of HPβCD are presented in Table 1. It was observed from the phase solubility diagrams that the addition of auxiliary substances used to the complexation media at room temperature resulted in decrease in the slopes, which was reflected in stability constant of etoricoxib. Thus stability constant of etoricoxib in HPβCD aqueous solution was 371.80 ± 2.61 M^{-1} when no hydrophilic carrier were present, but decreased to 314.60 ± 1.89, 164.15 ± 2.26, and 123.11 ± 2.12 M^{-1}, respectively, with the addition of 0.5% (wt/vol) PVP, 1% (wt/vol) PVP, and 1.5% (wt/vol) PVP to the complexation media. Similarly, the stability constant of etoricoxib in HPβCD aqueous solution was decreased to 369.90 ± 1.96**,** 365.09 ± 2.57, and 362.35 ± 2.09 M^{-1}, respectively, with the addition of 0.5% (wt/vol) arginine, 1% (wt/vol) arginine, and 1.5% (wt/vol) arginine to the complexation media. For the binary system, the linear host–guest correlation coefficient r = .9989 (r^2 = .9977) with a slope of 0.1659 suggested the formation of a 1:1 complex with respect to HPβCD concentrations. For ternary systems with 0.5% (wt/vol) PVP, r = .9974 (r^2 = .9948) with a slope 0.1652,

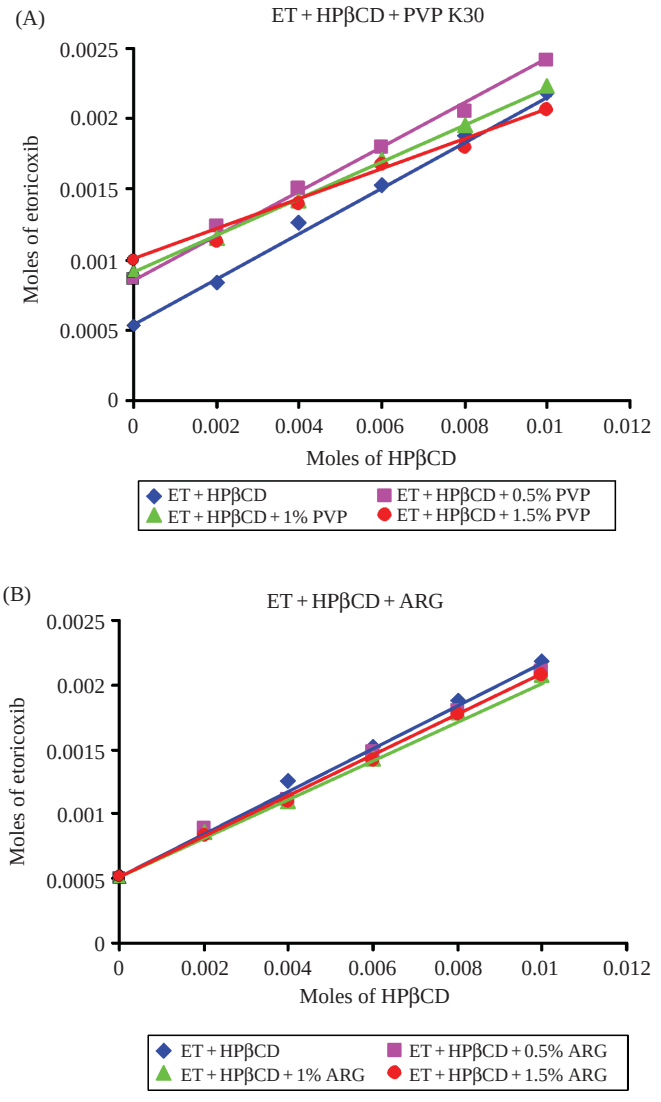

FIGURE 2. (A) Phase solubility diagram of etoricoxib–HPβCD system in water in presence of PVP K30, (B) Phase solubility diagram of etoricoxib–HPβCD system in water in presence of L-arginine.

1% (wt/vol) PVP, r = .9995 (r^2 = .9991) with a slope 0.1317, and for 1.5% (wt/vol) PVP, r = .9951 (r^2 = .9903) with a slope 0.1090 suggested the formation of 1:1 complex. For ternary systems with 0.5% (wt/vol) arginine, r = .9984 (r^2 = .9968) with a slope 0.1579, 1% (wt/vol) arginine, r = .9988 (r^2 = .9977) with a slope 0.1567, and for 1.5% (wt/vol) arginine, r = .9992 (r^2 = .9983) with a slope 0.1565 suggested the formation of 1:1 complex. The line equations from the linear regression analysis for these systems were

$$y = 0.1659x + 0.0005401 \text{(binary system)}, \quad (2)$$

$$y = 0.1652x + 0.0006252 \text{ (ternary system with 0.5\% [wt/vol] PVP)}, \quad (3)$$

$$y = 0.1317x + 0.0009072 \text{ (ternary system with 1\% [wt/vol] PVP)}, \quad (4)$$

$$y = 0.1090x + 0.0009653 \text{ (ternary system with 1.5\% [wt/vol] PVP)}, \quad (5)$$

$$y = 0.1579x + 0.0005254 \text{ (ternary system with 0.5 [wt/vol] arginine)}, \quad (6)$$

$$y = 0.1567x + 0.0005110 \text{ (ternary system with 1\% [wt/vol] arginine)}, \quad (7)$$

$$y = 0.1565x + 0.00050019 \text{ (ternary system with 1.5\% [wt/vol] arginine)}. \quad (8)$$

Thus in all ternary systems containing either PVP or arginine the stability constant of etoricoxib was decreased notably with increase in the concentration of auxiliary substances used. The effect was remarkable in presence of PVP than arginine. In addition to the stability constants, the complexation efficiency of HPβCD in various ternary systems was also calculated from the following equation (Brewster & Loftsson, 2007):

$$\text{complexation efficiency (CE)} = D_0 K_{1:1} = \frac{\text{slope}}{1 - \text{slope}}, \quad (9)$$

where D_0 is the intrinsic solubility of drug.

It is more convenient to compare the CE than $K_{1:1}$ values as CE is less sensitive to errors related to estimation of intrinsic drug solubility (Brewster & Loftsson, 2007). The values of complexation efficiency of HPβCD in various ternary systems are presented in Table 1. From these values, it is evident that neither PVP nor arginine has improved the complexation efficiency of HPβCD. On the contrary, the CE values were decreased with increase in the concentration of auxiliary substances.

From these results, it could be concluded that the addition of water-soluble carriers could not offer any advantage toward complexing efficiency of HPβCD.

Many papers have reported that certain hydrophilic carriers like PVP K30 or HPMC are known to interact with drug molecules, CD, and drug–CD complexes in aqueous solution to form ternary complexes resulting in increased solubility of drug (Acartürk, Ki¸slal, & Çelebi, 1992; Loftsson et al., 1996; Ribeiro et al., 2003; Usui, Maeda, Kusai, Nishimura, & Yamamoto, 1997; Valero et al., 2003). Several mechanisms may contribute to the improved drug solubility in the presence of water-soluble polymers. These include the formation of micelles or aggregates which show higher K_c values than the simple binary drug–CD complexes, accounting for their higher solubility (Cochrane et al., 2002). Further, hydrogen bond formation, Van der Walls

TABLE 1
Effect of Water-Soluble Polymer, PVP K30 (0.5 [wt/vol], 1% [wt/vol], and 1.5% [wt/vol]) and Amino Acid, L-arginine (0.5 [wt/vol], 1% [wt/vol], and 1.5% [wt/vol]) on the Intrinsic Solubility (S_0), Slope of Phase Solubility Diagrams and Stability Constant (K_c) for Binary and Ternary Systems of Etoricoxib with HPβCD

System	S_0 (μg/mL)	Slope	r^2	K_c (M^{-1})[a] Mean ± *SD*	K_{TS}/K_{BS}	CE
Drug–HPβCD	191.71	0.1659	.9977	371.80 ± 2.61	—	0.1989
0.5% (wt/vol) PVP K30	225.71	0.1652	.9948	314.60 ± 1.89[b]	0.84	0.1978
1% (wt/vol) PVP K30	331.10	0.1317	.9991	164.15 ± 2.26[b]	0.45	0.1516
1.5% (wt/vol) PVP K30	356.72	0.1090	.9903	123.11 ± 2.12[b]	0.33	0.1224
0.5% (wt/vol) arginine	181.93	0.1579	.9968	369.90 ± 1.96[c]	0.99	0.1875
1% (wt/vol) arginine	182.50	0.1567	.9977	365.09 ± 2.57[d]	0.98	0.1858
1.5% (wt/vol) arginine	183.73	0.1565	.9983	362.35 ± 2.09[e]	0.97	0.1855

K_{TS}/K_{BS} is the ratio of K_c for ternary and binary complexes.
[a]Indicates mean of three readings; *SD*, standard deviation; CE, complexation efficiency.
[b]*p* value compared to pure etoricoxib ($p < .001$), that is, significant.
[c]*p* value compared to pure etoricoxib ($p > .05$), that is, not significant.
[d]*p* value compared to pure etoricoxib ($p < .05$), that is, significant.
[e]*p* value compared to pure etoricoxib ($p < .01$), that is, significant.

interactions, or hydrophobic interactions between drug, CD, and the water-soluble polymer and polymer viscosity also contribute to enhanced solubility (Ribeiro et al., 2003).

However, in this study, the phase solubility curve of etoricoxib–HPβCD in presence of PVP K30 in all concentrations showed increase in the intrinsic solubility of drug with decrease in the slope, resulting in significant decrease in the stability constant of etoricoxib ($p < .001$). Earlier papers have reported the enhancement in complexation efficiency of CDs in presence of hydrophilic polymers where *K* values were only modified after certain types of treatment such as autoclaving (Cappello, Carmignani, Iervolino, Immacolata la Rotonda, & Saettone, 2001; Loftsson & Fridriksdóttir, 1998). Further, it has been also reported that PVP, does not increase the ability of drug–CD complexation but act as a driving force for the formation of complex depending on its percentage used (Valero et al., 2003). In contrast, positive effect of addition of PVP K30 on naproxen complexation with HPβCD was evaluated at different temperature conditions where higher temperature was supposed to decrease stability constant of naproxen (Mura, Faucci, Manderioli, & Bramanti, 2001b).

In this study where all the phase solubility studies were carried out at similar conditions, that is, at room temperature (25°C), PVP K30 has significantly decreased the stability constant of etoricoxib which might be because of the lower viscosity conferred by the polymer in complexing media (Ribeiro et al., 2003). Further, at higher polymer concentrations, the solubility decreases possibly due to formation of water-insoluble inclusion complexes between the polymer and CD molecules (Loftsson & Fridriksdottir, 1998). Also at room temperature PVP might have not formed aggregates with drug or HPβCD responsible for enhancement of solubilizing power of HPβCD.

In this article, we also studied the effect of L-arginine on etoricoxib–HPβCD inclusion complex. The phase solubility curves of L-arginine in all concentrations almost superimpose the curve of etoricoxib–HPβCD in absence of arginine. It was observed that there were no significant changes in the slope of the curve, intrinsic solubility and stability constant of etoricoxib when arginine was used in 0.5% concentration level ($p > .05$). However, at higher concentrations of arginine; 1% (wt/vol) ($p < .05$) and 1.5% (wt/vol) ($p < .01$), the stability constant of etoricoxib was found to be decreased.

It has been reported that, the basic amino acid L-arginine simultaneously interact with both the CD (via hydrogen bonding) and the drug (via electrostatic interactions and salt formation) (Berge, Bighley, & Monkhouse, 1977; Laveneziana, Speranza, Raulli, & Paredi, 1996). In the presence of the basic amino acid that acts as a counter-ion, the drug gives rise to an amphiphilic structure characterized by a strongly hydrophobic portion and a hydrophilic polar head. In the presence of HPβCD, the hydrophobic portion of this amphiphilic structure (i.e., the drug molecule) can interact with the hydrophobic CD cavity, whereas, at the same time, the hydrophilic portion can act as a surfactant toward the CD complex, lowering the aqueous surface tension, and thus favoring its solubility (Mura et al., 2005). However, arginine has moderately decreased the stability constant of etoricoxib–HPβCD complex. The reason lies in the fact that etoricoxib does not contain any acidic functional group able to form salt with arginine which could have formed an amphiphilic structure. The obtained results are in full agreement with the mechanism already suggested.

Proton Nuclear Magnetic Resonance Spectroscopy

Figures 3 and 4 shows ^{1}HNMR spectra of etoricoxib (A), HPβCD (B), PM (C), kneaded (D), and lyophilized products (E). ^{1}HNMR spectra of etoricoxib, in the absence of HPβCD, exhibited a pair of doublets, each integrating for two protons

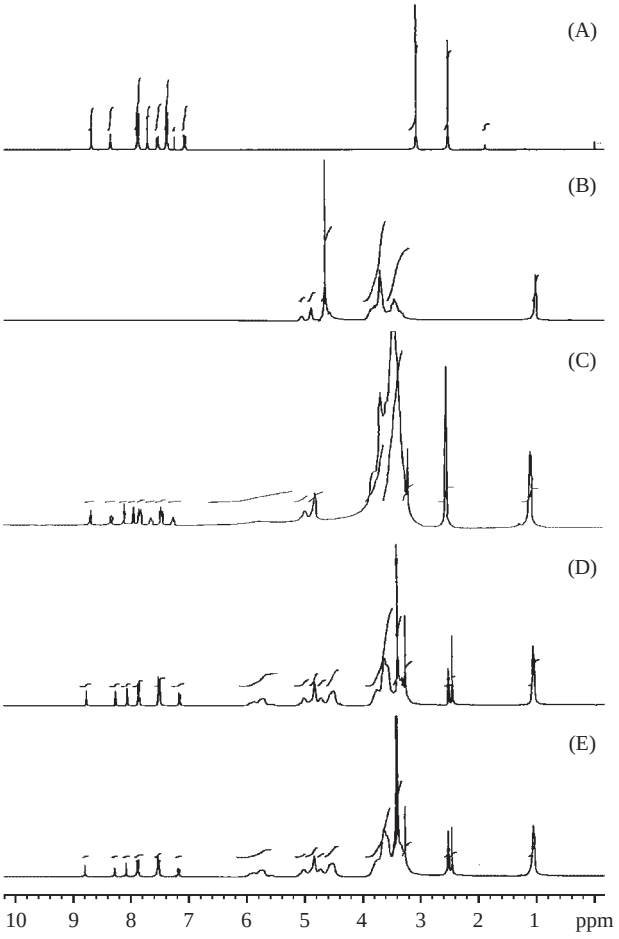

FIGURE 3. [1]HNMR spectra of etoricoxib–HPβCD binary systems: (A) etoricoxib, (B) HPβCD, (C) physical mixture, (D) kneaded product, and (E) lyophilized product.

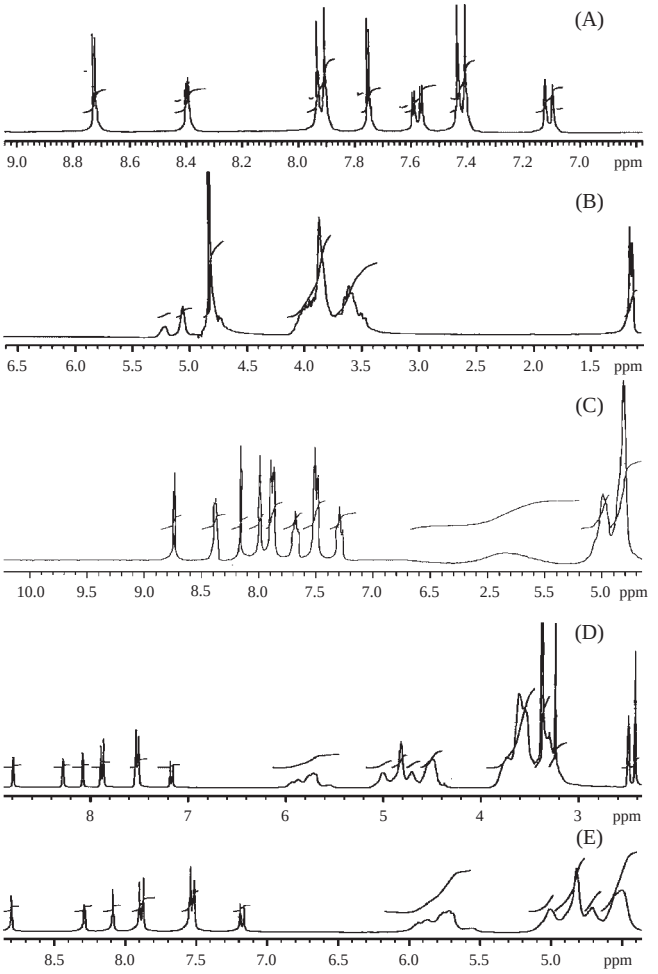

FIGURE 4. Partial (resolved) [1]HNMR spectra of etoricoxib–HPβCD binary systems: (A) etoricoxib, (B) HPβCD, (C) physical mixture, (D) kneaded product, and (E) lyophilized product.

and showing A2B2 pattern, at 7.563 and 7.403 which could be assigned to H-1,4 and H-2,3, respectively, of the methyl sulfonyl-substituted aromatic ring. The signal for H-5 of methyl group of methyl sulfonyl-substituted phenyl ring appeared as a singlet at 3.087. A singlet at 2.537 for three protons was due to CH_3 group of pyridine ring (H-6). Three doublets appeared at 7.092, 7.902, and 8.697 were assignable to H-7, H-8, and H-9 protons, respectively, of methyl-substituted pyridine ring. Another two doublets appeared at 7.724 and 8.366, each integrating for one proton, was assigned to chloro-substituted pyridine ring protons (H-10 and H-11).

Significant changes in the nature and position of signals for the protons of etoricoxib were observed in the presence of HPβCD in PM, kneaded, and lyophilized products. The signals for H-1,4 of methyl sulfonyl-substituted aromatic ring exhibited upfield shift in both the binary systems of etoricoxib with HPβCD whereas, in PM these protons exhibited a significant downfield shift. A very significant upfield shift was observed in the H-2,3 signal in kneaded and lyophilized products. However, the signals for H-2,3 were shifted to downfield in PM. The peaks for H-5 proton of the methyl of sulfonyl group moved to downfield. Downfield shift changes were also observed in the signals for H-7, H-8, H-9, and H-10 protons of the two pyridine rings in the presence of HPβCD whereas upfield shifts were observed for H-6 and H-11 protons of methyl group of pyridine- and chloro-substituted pyridine ring, respectively, in kneaded and lyophilized products. The signals for all protons except H-6, of etoricoxib exhibited a significant downfield shift in PM. The chemical shift change values for various protons of the guest are given in Table 2. In all the spectra of kneaded and lyophilized systems of etoricoxib with HPβCD, the signals for H-5′ and H-3′ of HPβCD, situated inside the HPβCD cavity, exhibited high field shifts compared to pure HPβCD whereas, only H-3′ protons of HPβCD exhibited high field shift in PM. The chemical shift change ($\Delta\delta$) values for HPβCD protons are given in Table 3.

TABLE 2
^{1}HNMR (300 MHz) Chemical Shift Change (Δδ) Values for Various Protons of Etoricoxib in the Presence of HPβCD in $CDCl_3$ at 25°C

System	H-1,4	H-2,3	H-5	H-6	H-7	H-8	H-9	H-10	H-11
PM	0.327	0.278	0.083	–0.037	0.199	0.252	0.042	0.264	0.007
KN	–0.030	–0.221	0.290	–0.111	0.063	0.172	0.097	0.170	–0.083
LP	–0.024	–0.241	0.295	–0.038	0.071	0.187	0.108	0.184	–0.076

Negative values indicate upfield shift.
PM, physical mixture; KN, kneaded product; LP, lyophilized product.

TABLE 3
^{1}HNMR (300 MHz) Chemical Shift Change (Δδ) Data for HPβCD Protons in the Presence of Etoricoxib in D_2O at 25°C

System	H-3′	H-5′	ΔδH-5′/ΔδH-3′
KN	–0.138	–0.368	2.67
LP	–0.0719	–0.363	5.04
PM	–1.169	–0.196	0.167

Negative values indicate upfield shift.
KN, kneaded product; LP, lyophilized product; PM, physical mixture.

The complexation pattern of guest into host cavity of CDs reported in earlier papers suggest the insertion of the less polar (nonpolar) portion of the guest into the CD cavity. NMR spectroscopy is a powerful tool for the study of CD inclusion complexation because of its sensitivity (Schneider, Hacket, Rudiger, & Ikeda, 1998). The penetration of guest generally takes place from the wider rim side of the cavity. The changes occurred in NMR signals clearly indicate the host–guest interactions. The changes in chemical shifts of H-3′ and H-5′ protons of CDs in the presence of guest molecule indicate that inclusion in cavity has taken place in kneaded and lyophilized systems as these protons are situated inside the cavity (Ali, Asmat, Maheshwari, & Koketsu, 2005). A deep penetration of guest into the HPβCD cavity results in the chemical shift of both the protons H-3′ and H-5′ of HPβCD whereas the shift in only H-3′ protons occurs when the cavity penetration is shallow (Bergaron & Rowan, 1976). The ratio for the chemical shift changes for these protons, ΔδH-5′/ΔδH-3′, gives the idea regarding the depth of inclusion of the guest into the HPβCD cavity. This ratio was found to be very small in PM as compared to kneaded and lyophilized products (Table 3). From these results, it could be possible to interpret that there might exists a strong interaction between etoricoxib and HPβCD in PM and that the drug might be approaching toward HPβCD for the formation of complex. The ΔδH-5′/ΔδH-3′ ratio was observed to be highest in lyophilized products indicating higher stability of inclusion complex. The stability of inclusion complex is related to the magnitude of the chemical shift changes for H-3′ and H-5′ protons of HPβCD; higher the value of ΔδH-3′ and ΔδH-5′, greater is the stability of the complex (Rekharsky et al., 1995). The protons of the guest molecule situated inside the HPβCD cavity in complex, experience upfield shift changes due to the shielding effect by the cavity while, all remaining protons of the guest, which are outside the cavity, generally, show downfield shift changes on complexation (Ali, Asmat, Maheshwari, & Koketsu, 2005). Significant upfield shift changes observed in the NMR signals of H-3′and H-5′ of HPβCD in kneaded and lyophilized systems of etoricoxib clearly indicate the inclusion of aromatic part of the guest into the HPβCD cavity due to hydrophobic interaction (Ali, Maheshwari, & Asmat, 2004). The higher values of ΔδH-5′ and ΔδH-3′ protons might be attributed to the deep penetration of the etoricoxib from wider rim of HPβCD (Nakajima, Sunagawa, Hirohashi, & Fujioka, 1984) as from earlier studies; most of the guest protons have experienced downfield shifts. Further, both the protons of the methyl sulfonyl-substituted aromatic ring; H-1,4 and H-2,3, exhibited significant upfield shifts indicating its penetration inside the HPβCD cavity. However, H-10 and H-11 protons of chloro-substituted pyridine ring have also exhibited remarkable upfield and downfield shifts, respectively, indicating the possibility of penetration of chloro-substituted pyridine ring inside the HPβCD cavity. Therefore, existence of two different topologies of complex formation could be possible for each aromatic ring as entry may occur through either the wider or smaller rim of HPβCD, which results in either shallow or deep penetration of guest molecule. Figure 5 illustrates possible arrangements of inclusion equilibria of etoricoxib and HPβCD inclusion complex. The chemical shift values obtained in NMR signals of kneaded and lyophilized products suggests Arrangement 1 for methyl sulfonyl-substituted aromatic ring with HPβCD where, the etoricoxib enters from wider rim and penetrates deep so that the methyl sulfonyl group protrudes outside the cavity and interacts with 2′-OH of HPβCD at 6 position. This was evidenced by upfield shift of H-1,4 of etoricoxib in kneaded and lyophilized products, which might be attributed to close proximity of these protons with H-5′ whereas H-2,3 of etoricoxib were in the proximity of both H-3′ and H-5′ of HPβCD as these protons have experienced higher upfield shifts than H-1,4 in

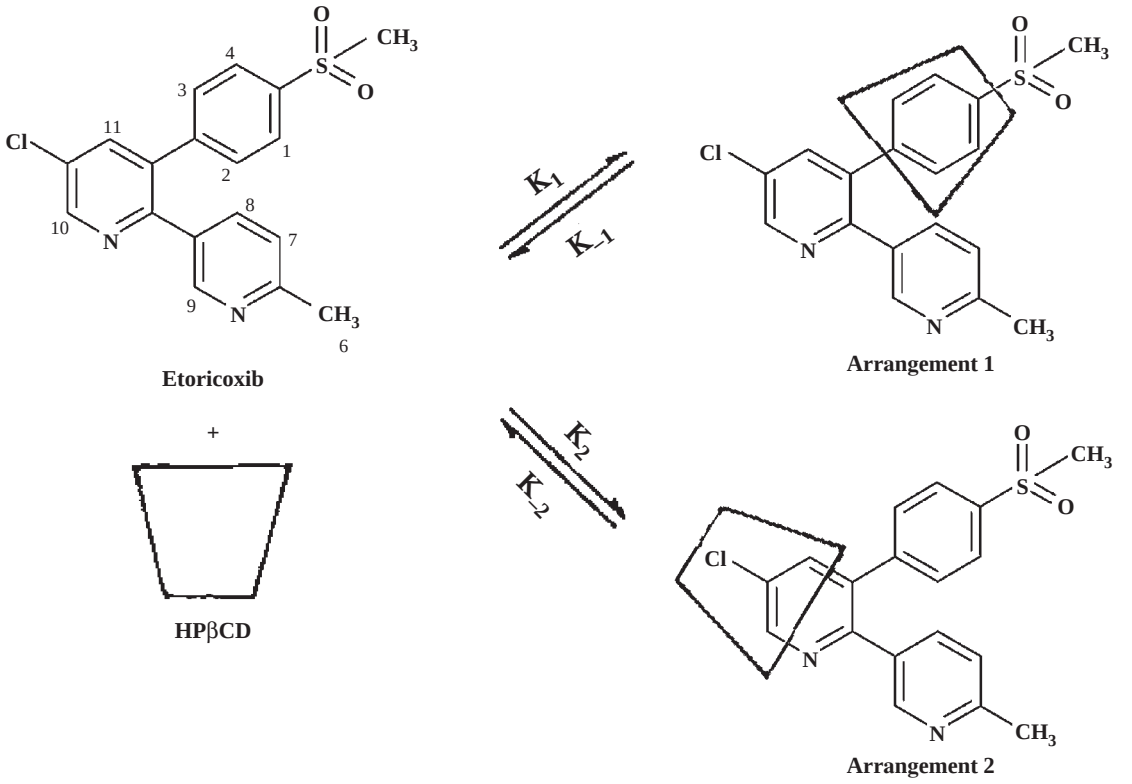

FIGURE 5. A schematic representations of the inclusion equilibria of etoricoxib with HPβCD.

binary systems. The high upfield shift of H-10 and downfield shift of H-11 of chloro-substituted pyridine ring suggests Arrangement 2 indicating the possibility of penetration of the chloro-substituted pyridine ring from wider side but only a part of the ring enters the cavity and the cavity penetration would be shallow. The deep penetration would result in upfield shift of H-11 also. The obtained results were in full agreement with the NMR studies already reported on CD complexation.

Fourier Transformation-Infrared Spectroscopy

Figure 6 illustrates the FTIR spectra of etoricoxib, HPβCD, physical mixture, and etoricoxib–HPβCD inclusion complex. IR spectrum of etoricoxib (Figure 6A) is characterized by principal absorption peaks at 3,057 cm^{-1} (C−H stretch aromatic benzene), 3,026 cm^{-1} (C−H stretch pyridine), 2,962 cm^{-1} (C−H stretch aliphatic CH_3 *asym*), 2,916 cm^{-1} (C−H stretch aliphatic CH_3 *sym*), 1,680 cm^{-1} (C=C aromatic), 1,562 cm^{-1} (C=N), 1,053 cm^{-1} (S=O sulfonyl), 1,084 cm^{-1} (C−Cl), 839 cm^{-1} (*p*-substituted benzene) and 748 cm^{-1} (pyridine).

Figure 6B shows the FTIR spectra of HPβCD. The prominent peaks were characterized at 3,395 cm^{-1} (O−H), 2,930 cm^{-1} (C−H), 1,647 cm^{-1} (H−O−H bending) and 1,035 cm^{-1} (C−O−C). In physical mixture, the broad peaks of HPβCD (Figure 6C) at 3,395 and 2,930 cm^{-1} were shifted to 3,408 and 2,928 cm^{-1}. All prominent peaks of etoricoxib were completely disappeared. The FTIR spectra of KN (Figure 6D) and LP (Figure 6E) shows complete disappearance of the etoricoxib peaks at 3,057, 3,026 2,962, 2,916, 1,053, and 1,084 cm^{-1}. However, significant decrease in the peak intensities was observed for 1,680 and 1,562 cm^{-1} in the physical mixture and the kneaded product. The peaks of etoricoxib at 839 cm^{-1} (shifted to 844 in LP) and 748 cm^{-1} appeared with strong decrease in peak intensity. The peak at 1,647 cm^{-1} in IR spectra of HPβCD due to water of crystallization, was also disappeared in both PM and LP but appeared in KN (Longxiao & Suyan, 2006). The peak of OH group of HPβCD at 3,395 cm^{-1} was shifted toward lower frequency 3,393 cm^{-1} in KN due to intermolecular hydrogen bonding with etoricoxib indicating strong physical interaction of etoricoxib with HPβCD. This suggested that, etoricoxib could form inclusion complex with HPβCD in solid state. All the binary systems of etoricoxib–HPβCD did not show any new peaks, indicating no chemical bond formation in the complexes (Ford, 1986).

X-Ray Powder Diffractometry

The XRD pattern of etoricoxib showed (Figure 7) intense and sharp peaks, indicating its crystalline nature. The peak intensities of pure etoricoxib and its corresponding binary systems are presented in Table 4. Crystallinity was determined by comparing some representative peak heights in the diffraction patterns of the binary systems with those of a reference. The relation used for the calculation of the crystallinity was the

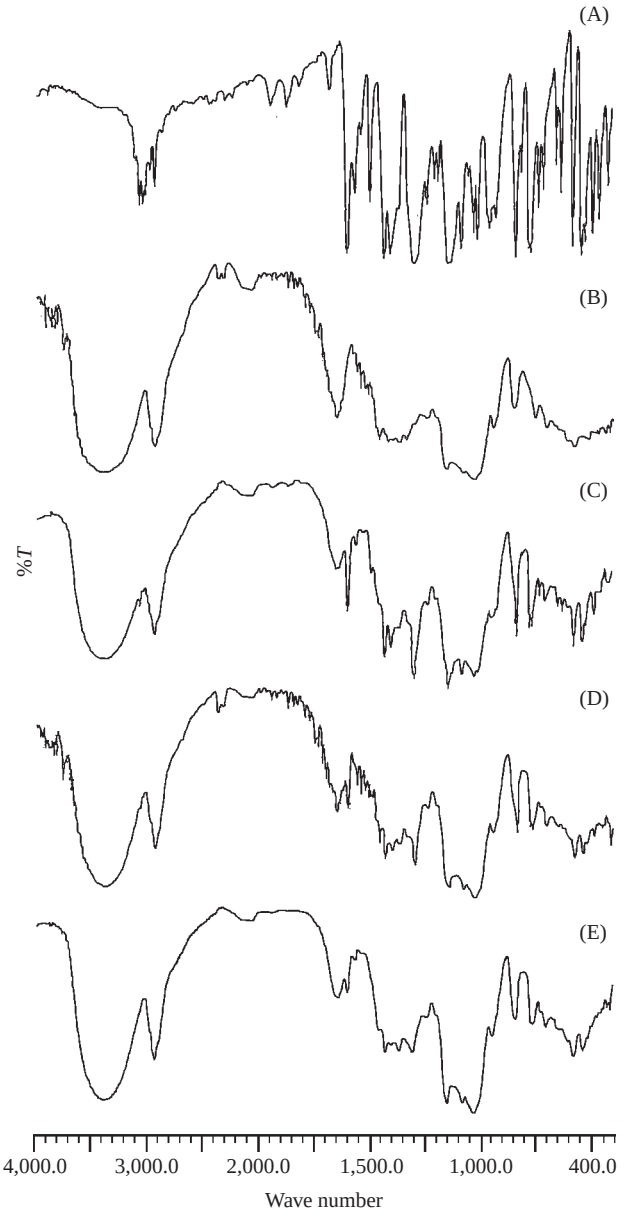

FIGURE 6. FTIR spectra of etoricoxib–HPβCD binary systems: (A) etoricoxib, (B) HPβCD, (C) physical mixture, (D) kneaded product, and (E) lyophilized product.

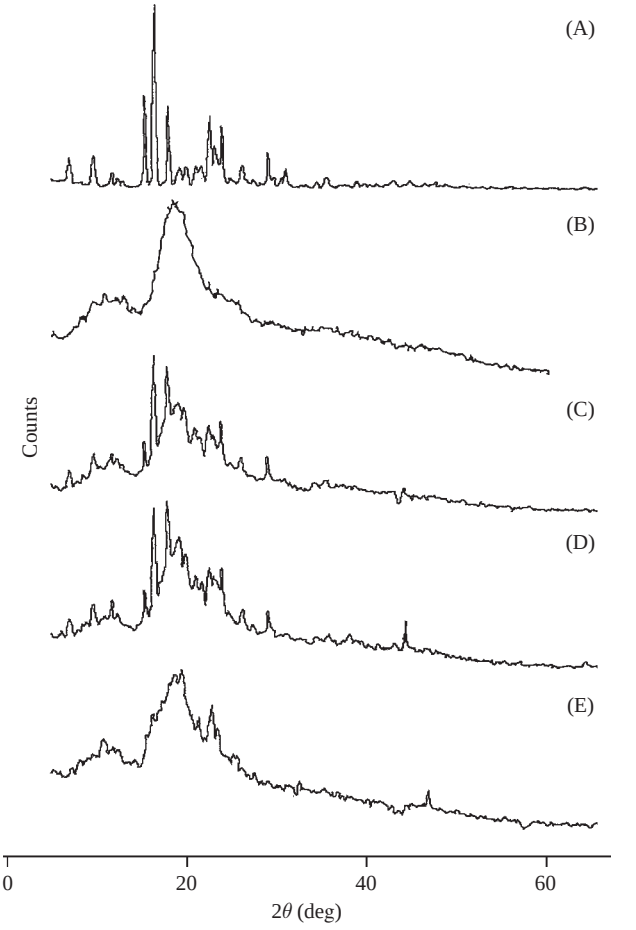

FIGURE 7. XRD patterns of etoricoxib–HPβCD binary systems: (A) etoricoxib, (B) HPβCD, (C) physical mixture, (D) kneaded product, and (E) lyophilized product.

TABLE 4
Peak Intensities of Etoricoxib in the XRD Patterns of Etoricoxib–HPβCD Binary Systems

		Drug–HPβCD Binary System		
2θ	Drug	PM	KN	LP
16.44	790	—	—	—
16.66	511	243	—	—
15.34	428	—	—	—
22.72	357	139	—	53
15.57	331	79	—	—
24.10	286	130	108	—

PM, physical mixture; KN, kneaded product; LP, lyophilized product.

relative degree of crystallinity (RDC) = I_{SA}/I_{REF}, where I_{SA} is the peak height of the sample under investigation and I_{REF} is the peak height of the same angle for the reference with the highest intensity (Ryan, 1986). The peak intensities of pure etoricoxib were used as a reference for the calculation of RDC values in binary systems.

Etoricoxib (Figure 7A) showed sharp peaks at 16.44°, 16.66°, 15.34°, 22.72°, 15.57°, and 24.10° (2θ) with peak intensities of 790, 511, 428, 357, 331, and 286, respectively, whereas a halo-pattern was recorded for HPβCD (Figure 7B) demonstrating its amorphous state. The relative decrease in the crystallinity in binary systems of etoricoxib–HPβCD were calculated by considering the peak heights at the angle 16.66° (2θ), 24.10° (2θ), and 22.72° (2θ) for the physical mixture, kneaded, and lyophilized products, respectively.

The diffraction pattern of physical mixture (Figure 7C) showed peaks of etoricoxib and HPβCD with little decrease in the peak intensity of etoricoxib indicating reduction in crystallinity (RDC = 0.4755). The kneaded system presented a diffraction pattern (Figure 7D) quite similar to that of PM. However the crystallinity of etoricoxib was reduced to a greater extent as compared to physical mixture as almost all peaks of etoricoxib except peak at 24.10 were disappeared in diffraction pattern of kneaded system (RDC = 0.3776).

Figure 7E shows the diffraction pattern of lyophilized product. The disappearance of almost all peaks except at 22.72° in XRD pattern of lyophilized product clearly indicated a marked reduction the crystallinity of etoricoxib (RDC = 0.1484). Furthermore, the obtained patterns were diffused suggesting the amorphous state reached by the lyophilization technique.

In conclusion, the diffraction pattern of etoricoxib–HPβCD prepared by lyophilization technique was completely diffuse, similar to that of HPβCD indicating that the drug was no longer present in crystalline state but was transformed to amorphous form. However, the peaks of etoricoxib in the kneaded system were less intense indicating a strong interaction of etoricoxib with HPβCD resulting in a significant loss of the crystallinity of etoricoxib (Mura et al., 1998; Veiga, Teixeira-Dias, Kedzierewicz, Sousa, & Maincent, 1996).

In Vitro Dissolution Rate Studies

The dissolution curves of etoricoxib–HPβCD binary systems in phosphate buffer pH (7.4) at 37 ± 0.5°C are shown in Figure 8. The release rate profiles were expressed as the percentage of drug released (vs.) time. The dissolution time of etoricoxib from inclusion complexes and physical mixtures was determined and further evaluated. Table 5 shows % drug dissolved at 2 min (DP_2), at 15 min (DP_{15}), at 30 min (DP_{30}) and dissolution efficiency at 45 min (DE_{45}).

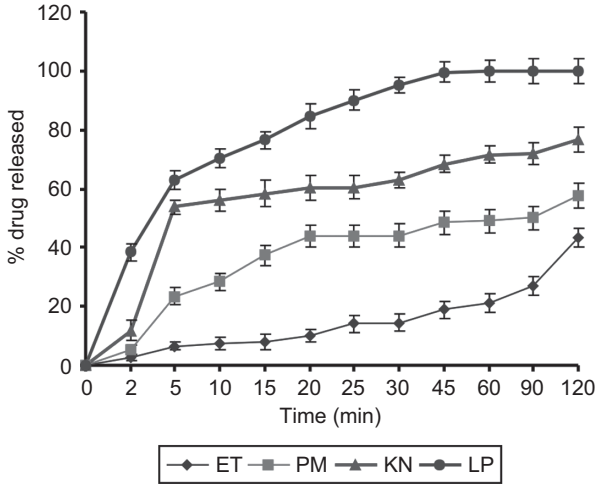

FIGURE 8. The dissolution curves of etoricoxib–HPβCD system at 37 ± 0.5°C. ET, etoricoxib; PM, physical mixture; KN, kneaded product; LP, lyophilized product.

The DE is defined as the area under dissolution curve *upto* the time *t* expressed as percentage of the area of rectangle described by 100% dissolution in the same time (Khan, 1975). The dissolution efficiency at 45 min was calculated as follows:

$$DE_{45} = \frac{\text{AUC of dissolution curve at 45 min}}{\text{AUC of rectangle at time 45 min}}, \qquad (10)$$

where, AUC is area under the curve.

The DE_{45} values were compared statistically using ANOVA (Tukey–Kramer Multiple Comparisons Test).

According to these results, it is evident that all binary systems of etoricoxib with HPβCD have shown faster dissolution than etoricoxib alone. It can be seen that the increase in dissolution rate of etoricoxib was 4.5-fold greater from physical mixture within 15 min whereas, it was sevenfold greater from the kneaded system at the same time. However, lyophilized product has shown higher dissolution rate than physical mixture and kneaded product. The increase in dissolution rate of etoricoxib was 9.4-fold greater from lyophilized product within 15 min.

When DE_{45} values of physical mixture were statistically compared with pure etoricoxib, a significant difference was found between the dissolution profile of etoricoxib and physical mixture ($p < .001$). Further kneaded product has shown significant improvement in the dissolution profile of etoricoxib than pure drug and physical mixture ($p < .001$). The lyophilized product has shown excellent dissolution among all other binary systems of etoricoxib studied, when compared statistically ($p < .001$).

In addition, the extent of the dissolution enhancing effect was found to be dependent on the method used for the preparation of inclusion complexes. The enhancement in dissolution rate from physical mixture was possibly due to a local solubilization action operating in the microenvironment or the hydrodynamic layer surrounding the drug particles in the early stages of the dissolution process. Further, HPβCD dissolves in a short time thus improving the wettability and hence dissolution of the drug particles (Goldberg, Gribaldi, Kanig, & Myersohn, 1966; Ismail, 1991).

The kneaded product displays a dissolution rate greater than the physical mixtures but smaller than lyophilized product. The higher dissolution rate of kneaded product might be attributed to reduction in crystallinity of the drug due to the formation of inclusion complex (kneaded product) in the solid state, evidenced by XRD studies.

The significant enhancement of the dissolution efficiency that occurred with lyophilized product has been attributed to (1) surfactant-like properties of the carriers, which can reduce the interfacial tension between water-insoluble drugs and the dissolution medium; (2) an increase of etoricoxib solubility upon complexation in the solid state; (3) to the high energetic amorphous state/reduction of the crystallinity following

TABLE 5
The Dissolution Data of Pure Etoricoxib and its Various Binary Systems with HPβCD in Phosphate Buffer (pH 7.4) at 37 ± 0.5°C

System	DP_2[a] ± *SD*	DP_{15}[a] ± *SD*	DP_{30}[a] ± *SD*	DE_{45}[a] ± *SD*
Etoricoxib	2.60 ± 0.9	8.13 ± 2.6	14.42 ± 2.8	11.57 ± 2.35
PM	5.30 ± 1.4	37.39 ± 3.3	44.02 ± 3.9	37.60 ± 3.90[b]
KN	11.89 ± 3.5	58.18 ± 4.5	62.87 ± 2.6	56.80 ± 3.03[b,c]
LP	38.44 ± 3.0	76.39 ± 2.9	95.26 ± 2.8	81.23 ± 3.14[b,d]

[a]Indicates mean of three readings (n = 3); *SD*, standard deviation; PM, physical mixture; KN, kneaded product; LP, lyophilized product; DP, % drug dissolved; DE, dissolution efficiency.
[b]Indicates p value compared to pure etoricoxib ($p < .001$), that is, all significant.
[c]Indicates p value compared to PM ($p < .001$), that is, significant.
[d]Indicates p value compared to PM and KN ($p < .001$), that is significant.

complexation (as confirmed by XRD studies) (Dollo et al., 1999; Lin & Kao, 1989; Moyano, Arias-Blanco, Ginés, & Giordano, 1997). From the experimental results it could be concluded that the extent of the dissolution rate-enhancing effect was found to be dependent on the method used for the preparation of inclusion complex. The lyophilized product showed highest dissolution rate among all binary systems studied. These results demonstrate that it could be possible to achieve the high energetic amorphous state of drug with lyophilization technique suggesting its advantage over kneading method (Erden & Celebi, 1988). Furthermore, NMR studies revealed that the stability of inclusion complex prepared by lyophilization technique (lyophilized product) was greater than the kneaded one which might have further contributed for enhanced dissolution rate of etoricoxib. The obtained results are in full accordance with physical characterizations of different formulations studied.

In conclusion, the dissolution rate increase for inclusion complexes was due to greater hydrophilicity, higher wetting effect, and mechanical treatment, which increased the contact between the drug and the carrier and the ability to form stable inclusion complex of HPβCD (Fernandes, Vieira, & Veiga, 2002).

CONCLUSION

This study shows that the addition of PVP K30 or L-arginine either decreases or fails to modify the drug: HPβCD stability constant. Therefore only binary systems of etoricoxib and HPβCD were prepared and characterized by ^{1}HNMR, FTIR, and XRD studies. ^{1}HNMR spectroscopy studies revealed that inclusion complexes of etoricoxib and HPβCD were formed by the penetration of aromatic rings of guest into the HPβCD cavity from wider rim side and that methyl sulfonyl-substituted ring penetrates deep whereas inclusion of chloro-substituted pyridine ring is shallow. Moreover, the values of Δδ H-5′(Δδ H-3′ demonstrate higher stability of inclusion complex prepared by lyophilization technique (lyophilized product) than the kneaded one. FTIR spectra suggested a strong physical interaction between a drug and HPβCD but little or no chemical interaction between them. Further, XRD data confirmed a significant decrease in the crystallinity of etoricoxib in kneaded system and a presence of some amorphous entities in lyophilized product. In comparison of dissolution data, all binary systems have improved the dissolution rate of etoricoxib to a great extent. However, lyophilized product was found to be superior to all other binary systems in enhancing dissolution profile of etoricoxib. Based on the results, it could be concluded that, inclusion complex of etoricoxib could be formulated with HPβCD with like high dissolution rate suggesting a possible enhancement of its oral bioavailability.

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