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Host–Guest Complexation of Oxicam NSAIDs with β-Cyclodextrin

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Abstract: *Spectroscopic and molecular modeling techniques have been employed to study the interaction of the oxicam group of nonsteroidal antiinflammatory drugs (NSAIDs) with a polysaccharide such as β-cyclodextrin (β-cd). β-cd is a good drug delivery system and is known to reduce harmful side effects of these drugs in the gastrointestinal tract and to increase their clinical efficacy. A detailed understanding of such host–guest interaction helps in designing a better drug delivery system coupled with increased therapeutic potential. However, there exists a controversy as to which prototropic form of piroxicam, a drug belonging to the oxicam group, becomes encapsulated in the host and also the stoichiometry of binding. In this study, we have revisited that controversy using steady state fluorescence, absorption, fluorescence anisotropy measurements, and molecular modeling techniques. In addition, we have for the first time studied the interactions of two other oxicam drugs, viz. tenoxicam and meloxicam, with β-cd in aqueous solution. In all cases the neutral forms of these drugs were incorporated in the β-cd cavity with a binding stoichiometry of 1:1 host : guest. The values of the binding constants for piroxicam, meloxicam, and tenoxicam with β-cyclodextrin are 134 ± 21, 114 ± 15, and 115 ± 13* M^{-1}*, respectively. Molecular modeling studies show that the minimum energy configuration gives favorable interaction energy between the host and the guest in the complex with 1:1 stoichiometry when the conjugated rings of the drugs are inside the hydrophobic bucket-like cavity of β-cd and the third ring is exposed to the solvent.* © 2004 Wiley Periodicals, Inc. Biopolymers 75: 355–365, 2004

INTRODUCTION

Encapsulation of drugs in different host systems not only serves as an effective tool for delivering drugs to appropriate targets, but in some cases is also known to increase their therapeutic efficacy. A detailed understanding of the basic principles that govern the interaction of such complexes is important for better design of delivery systems coupled with efficient modulation of clinical efficacy. β-Cyclodextrin (β-cd), a cyclic oligosaccharide, serves as an effective drug delivery system for several nonsteroidal antiinflammatory drugs (NSAIDs). It is composed of D(+)-glucopyranose units linked by an α-(1,4)-glycosidic linkage. The molecule is shaped like a truncated cone, with a smaller and a larger diameter opening at the primary hydroxyl and secondary hydroxyl faces of the cyclic sugar network, respectively. The exterior of the molecule is hydrophilic and the cyclodextrins are soluble in water. However, the interior of the cavity

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consists of a ring of C–H groups, a ring of glucosidic oxygens, and another ring of C–H groups; thus the inner cavity is relatively hydrophobic. The internal diameter of the cavity is 6.5 Å and the depth is 8 Å.[1] Because of its ability to form inclusion complexes with various molecules (guests) of suitable dimension, leading to the modification of some physicochemical properties of the guest, it has found extensive application in the pharmaceutical industry. For piroxicam, a drug belonging to the oxicam group of NSAID, it has been reported earlier that, not only does the encapsulation of this drug in β-cyclodextrin impart better tolerability in the gastrointestinal (GI) tract,[2–6] but it also increases the therapeutic efficacy.[7–9] Studies have therefore been devoted to understand the host–guest interaction of piroxicam with β-cd.

However, there exists a controversy regarding both the nature of the prototropic species of piroxicam (the guest molecule) that are incorporated into the cyclodextrin cavity (the host) and the stoichiometry of binding of piroxicam with the host (β-cyclodextrin).[10–13] Some work has indicated that the neutral form of piroxicam gets encapsulated in β-cd,[10,13] while evidence that the zwitterionic form acts as the guest molecule also exists.[11] Different binding stoichiometries of the host : guest have been indicated, which include 1 : 1[10,12] and 2 : 1[13] host–guest complexes. In this work we have attempted to revisit the problem of the host–guest complexation of piroxicam and settle the existing controversy. In addition, we have also extended our studies to two other oxicam drugs, viz. tenoxicam and meloxicam. This is the first report of the interaction of tenoxicam and meloxicam with β-cd in aqueous solution. Considering piroxicam as the parent compound, tenoxicam and meloxicam can be prepared by isosteric substitution of the aryl and the pyridyl ring of piroxicam, respectively (Figure 1). It is important to understand the nature of the guest in the cyclodextrin cavity for the three oxicam drugs, which is essential to elucidate the reason behind their improved efficacy on encapsulation. This study also allows us to compare the interactions of these three drugs with β-cd under identical conditions.

The association process of oxicam drugs with β-cd has been studied using UV-visible absorption, fluorescence spectroscopy, and fluorescence anisotropy measurements. The intrinsic fluorescence of the three drugs has been exploited to identify the principal prototropic forms these are incorporated into the β-cd cavity, the stoichiometry of the complexes, and the binding constants. In all cases the host–guest stoichiometry was found to be 1 : 1. Molecular modeling studies were employed to judge the feasibility of such complex formation and also to present some realistic structure of the 1 : 1 complexes of these three drugs with β-cd.

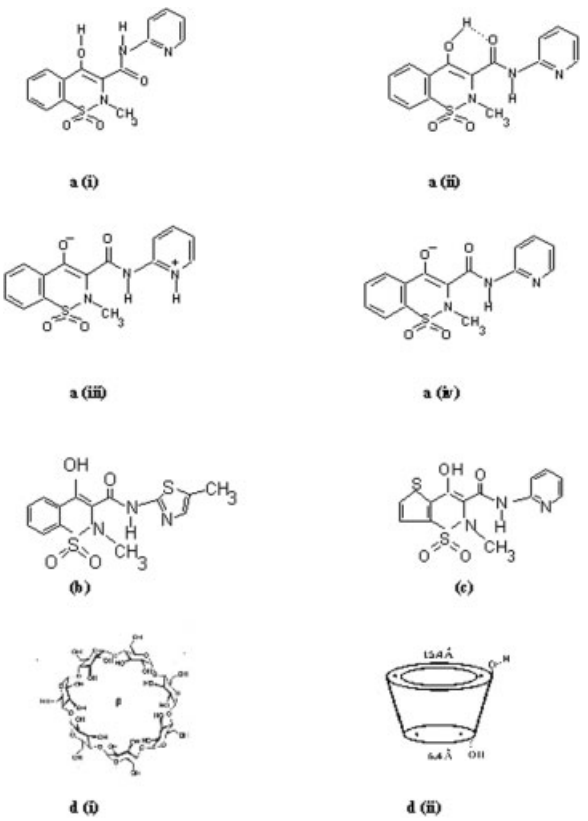

FIGURE 1 Structure of (a) different prototropic forms of Piroxicam (i) open conformer (neutral form), (ii) closed conformer (neutral form), (iii) zwitterionic form, (iv) anionic form; (b) meloxicam, (c) tenoxicam, (d) (i) β-cyclodextrin, and (ii) dimension of the bucket like cavity of β-cyclodextrin.

EXPERIMENTAL

Materials. Piroxicam, tenoxicam, and β-cyclodextrin were purchased from Sigma Chemicals, meloxicam was from LKT laboratories (MN, USA), and all were used without further purification. Since the drugs piroxicam, meloxicam, and tenoxicam are sparingly soluble in water, the 0.5 mM stock solutions were prepared in spectroscopic-grade ethanol (Merck, Germany) and were diluted by triple distilled water according to the desired concentration. Concentrations of the drugs in each sample were kept constant at 30 μM, hence the percentage volume of ethanol present in the samples did not exceed 6% (vol/vol). A maximum of 6% vol/vol ethanol in the sample solutions was equivalent to $\sim$ 100 mM of ethanol. Van Stam et al.[14] have adequately demonstrated that this amount of ethanol in solution does

not significantly disrupt the β-cd complexes. Stock solutions of β-cd (20 mM) were prepared in triple distilled water. The pH of the samples were adjusted by adding either dilute HCl or dilute NaOH solution.

Absorption and Fluorescence Measurements. Absorption spectra were recorded by Shimadzu UV2101PC spectrophotometer and fluorescence excitation, emission, and anisotropy data were recorded by Jobin Yvon Fluoromax-3 (Horiba, Japan) spectrofluorimeter. Before taking the absorption data, the baseline correction was done using the corresponding solvent (i.e., bulk water with 6% ethanol vol/vol). A pair of 10 × 10 mm path length quartz cuvettes were used for absorption experiments. The OD of the samples were always kept below 1 OD unit. For fluorescence emission and excitation, a 2 × 10 mm path length quartz cuvette was used to avoid any blue edge distortion due to the inner filter effect.[15] All measurements were done at room temperature (298 K).

Anisotropy measurement. Fluorescence anisotropy (r) was determined by an inbuilt matched set of polarizer and analyzer using the following equation:[15]

$$r = (I_{VV} - GI_{VH})/(I_{VV} + 2GI_{VH})$$

The emitted light intensities with vertical (V) and horizontal (H) polarizer/analyzer arrangements were measured as I_{VV}, I_{VH}, I_{HV}, and I_{HH}, where the first and second subscripts refer to the orientation of the polarizer and analyzer, respectively. G is the grating efficiency factor that gives a measure for the sensitivities of the detection systems for vertically and horizontally polarized light and is given by the ratio I_{HV}/I_{HH}.

Molecular Modeling Protocol. Piroxicam, meloxicam, and tenoxicam are not very common molecules and hence the topology and parameters for these molecules are not directly available in standard molecular mechanics and molecular dynamics (MD) packages like CHARMM etc.[16] We therefore have prepared the topologies of these molecules for use in CHARMM. In doing so, we have assigned the atom types of the atoms involved in these drugs following CHARMM[16] atom type definition. This makes most of the bonds, angles, dihedrals, and all the nonbonded parameters directly available from the combined CHARMM all-parameters set for nucleic acid and proteins.[17] Those are not available were obtained either by comparison with similar groups in the CHARMM parameter set or from literature data.[18] The Cartesian coordinates of these molecules were generated by the freeware ArgusLab 2.0.0.[19] This program uses semiempirical quantum mechanical calculations with the AM1 parameter set and restricted Hatree Fock self-consistent field approach to optimize the geometry of the desired structure. The partial atomic charges of the atoms in each molecule were estimated by fitting the partial atomic charges to reproduce the electrostatic potential at 1200 space points around the molecules using the ElectroStatic Potential (ESP) facility available in the MOPAC 7.0 package.[20] The atomic coordinates and the topology file of β-cyclodextrin were downloaded from the Internet (Hanyang University, Seoul, Korea, August, 1995). The potential energy of the individual free drug molecules and β-cyclodextrin were minimized in vacuum by 5000 steepest descent steps and a distance-dependent dielectric constant was used to partially mimic the effect of solvents.[21] A spherical cutoff method was used to tackle the nonbonded interactions with a cutoff value of 12.0 Å and was smoothly switched to zero in the range (10–11) Å.[22] Each drug molecule was then manually docked into the β-cd cavity using the freeware WebLab ViewerLite.[23] While docking, care was taken to incorporate our experimental information, such as 1:1 stoichiometry was maintained and the conjugated rings were placed into the cavity in such a way that the enolic –OH remained partially accessible to the solvent. A total number of 10 starting structures, which differ in relative position and orientation between the host and the guest, were generated in each case. Each of these initial model complexes were energy minimized following the same protocol as mentioned above. The lowest energy model of the complex was chosen as the representative structure of the corresponding host–guest complex in each case.

RESULTS

Identification of the Nature of Guest in β-cd Host

To revisit the controversy of piroxicam-β-cd inclusion complex, our first aim was to identify the prototropic form of the drug that gets encapsulated in the host, β-cd. To do that, we have studied the change in the excitation spectra of the drug with increasing concentrations of β-cd at pH 5.5, i.e., when no acid or alkali has been added to the aqueous solution. Since the pK_a values for the enolic OH and the pyridyl ring of piroxicam are 1.86 and 5.46, respectively,[24] the isoelectric pH of the drug is around 3.66. Hence at pH 3, the maximum population of piroxicam is either in the neutral or zwitterionic forms, which cannot be spectroscopically separated and are therefore together termed the "global neutral" form. At pH values above 3.66, some of the molecules also exist in the anionic form. Therefore, at pH 5.5, there exists a mixed population of anionic and global neutral forms in solution, though the anionic form predominates in aqueous solution at this pH. It should be mentioned that, for tenoxicam, the pK_a values of the enolic OH and the pyridyl ring are 1.07 and 5.34, respectively, and the isoelectric pH is at 3.2, whereas, for meloxicam, only one pK_a that of the enolic OH is available,[24] which is 4.08. For meloxicam, there is no evidence for the existence of the zwitterionic form. So, the term global neutral can be used only for piroxicam and

tenoxicam, but not for meloxicam. In our earlier studies[25,26] we have seen that the anionic form of piroxicam absorbs near 360 nm and the neutral form absorbs near 330 nm in ethanol. However, in polar solvents like water, the absorption maximum for anion is around 353 nm. We have determined the extinction coefficients of the global neutral and the anionic forms of piroxicam to be 3.52×10^4 and $2.43 \times 10^4\ M^{-1}\ cm^{-1}$ in water and the quantum yields are 11.1×10^{-4} and 3.52×10^{-4}, respectively. Therefore, at pH 5.5, when there is predominantly anion in the solution, it is difficult to detect the relatively small quantity of neutral forms by absorption spectra as their extinction coefficients do not differ much. The presence of mixed species in solution is attributed to a broad peak in absorption spectrum, from which a little shift in the position of absorption maximum is difficult to detect. On the other hand, the quantum yield for the neutral form is 3.15 times greater than that of the anion. So, a change in the mole fraction of the two species in the ground state can be better identified by the corresponding changes in the excitation spectra. Figure 2(a) shows that, with increasing concentration of β-cd, the excitation maximum of piroxicam shifts from 353 to 324 nm. The excitation spectra were recorded keeping the $\lambda_{emission}$ at the corresponding emission maximum of the sample. Similar studies have been conducted for the other two oxicam drugs viz., tenoxicam and meloxicam. The extinction coefficients of the neutral and anionic forms of tenoxicam are 2.94×10^4 and $2.26 \times 10^4\ M^{-1}\ cm^{-1}$ and the quantum yields are 2.12×10^{-4} and 1.17×10^{-4}, respectively. For meloxicam also, the extinction coefficients of the neutral and the anionic forms are very close (1.41×10^4 and $1.22 \times 10^4\ M^{-1}\ cm^{-1}$, respectively) whereas their quantum yields have a greater difference (for neutral form, it is 4.78×10^{-4} and for the anion it is 2.23×10^{-4}). Hence, to identify the principal structural form in solution and also how it changes from one form to another, excitation spectra could give much more precise information than the absorption spectra. For meloxicam and tenoxicam with increasing concentration of the host, the blue shifts in the excitation maxima occurred from 363 to 346 nm and 370 to 350 nm, respectively [Figures 2(b) and (c)]. Such a large shift indicates that the principal structural form of these drugs changes from the anion in aqueous solution to the global neutral and/or neutral form in nonpolar solvent. The dielectric constant inside the β-cd cavity is known to be close to that of absolute alcohol.[27] Hence it is evident that the neutral/global neutral forms of the drugs are encapsulated by their host, which offers a relatively nonpolar microenvironment

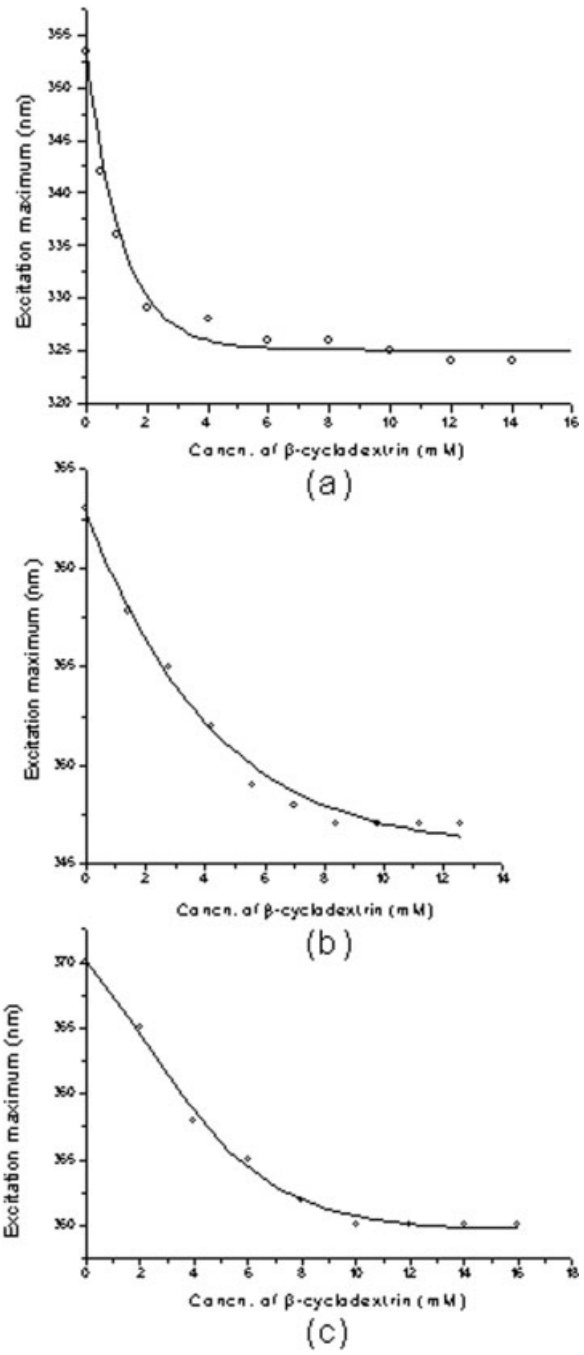

FIGURE 2 Plot of excitation maximum (nm) versus concentration of β-cyclodextrin (mM) for (a) piroxicam, (b) meloxicam, and (c) tenoxicam at pH 5.5.

to the guest molecules. As these oxicam NSAIDs are extremely sensitive to their microenvironment, such that the environment dictates the principal prototropic form to predominate in solution,[25] it is quite likely that, as more drug molecules are incorporated in the β-cd cavity, they are transformed from the anionic to the neutral form. Figures 2(a–c) show that, after

reaching a certain concentration of the host, there is no further blue shift in the excitation spectra, indicating that most of the guest molecules have already been encapsulated within the host.

To identify that the encapsulation occurs for the neutral form(s) of the drugs only and not for the anionic forms, fluorescence anisotropy of the inclusion complexes was recorded with increasing host concentration. In bulk aqueous solution, a fluorophore is free to rotate in the solvent, whereas the rotational and tumbling motions are restricted when it faces a more rigid environment, which results in an increase in the anisotropy of the fluorophore. Therefore, an increase in anisotropy is indicative of the complexation of a fluorophore with another larger molecule. The greater the number of complexed fluorophores, the higher the value of fluorescence anisotropy. Hence, to study the formation of inclusion complexes between oxicam NSAIDs and β-cd, fluorescence anisotropy of the samples was studied. The anisotropy values have been recorded for both the prototropic species, i.e., the neutral and the anionic forms by monitoring at the corresponding emission and the excitation maximum for the species. The emission and excitation maxima for the neutral/global neutral and the anionic forms have already been determined in our earlier studies.[25,26] Figure 3(a) shows the change in anisotropy value of piroxicam with increasing β-cd concentration at pH 5.5 monitored at the excitation maximum of the global neutral form (330 nm). The increase in the anisotropy value indicates that the global neutral forms are being incorporated into the β-cd cavity, where they face a more rigid environment. Interestingly, when the anisotropy was monitored at the excitation maximum of the anionic form of piroxicam (360 nm), no systematic change in the value of anisotropy was obtained with increasing host concentration (data not shown). This shows that, at pH 5.5, where a mixed population exists, it is only the global neutral form that is incorporated into the β-cd cavity while the anionic form is left behind in the bulk aqueous phase. To maximize the population of the global neutral and anionic forms, similar studies were carried out at pH 2.5 and at pH 10, respectively [Figures 3(b) and (c)]. As expected, the anisotropy of the neutral form at pH 2.5 increased with increasing host concentration [Figure 3(b)], whereas no such changes were seen in the case of the anionic form at pH 10 [Figure 3(c)]. Figures 4(a) and (b) show the increase in anisotropy values of the neutral/global neutral forms of meloxicam and tenoxicam, respectively, at pH 5.5 with increasing host concentration. In these cases too, the anionic forms of these drugs were left behind in the bulk aqueous phase.

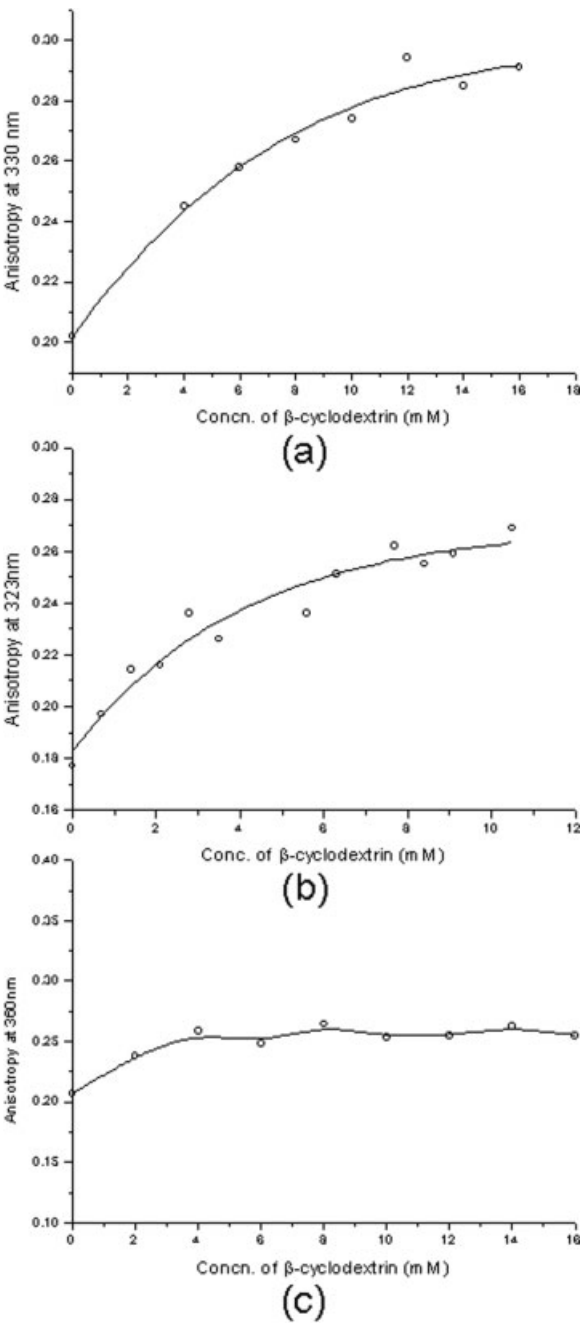

FIGURE 3 Change in anisotropy with increasing concentration of β-cyclodextrin (mM) for piroxicam at (a) pH 5.5, (b) pH 2.5, and (c) pH 10, monitored at 330, 323, and 360 nm, respectively.

Determination of Host : Guest Binding Stoichiometry and Binding Constant

Another part of the controversy regarding piroxicam-β-cd host–guest complexation includes the host : guest binding stoichiometry. The major conflict regarding the host : guest binding ratio is whether it is

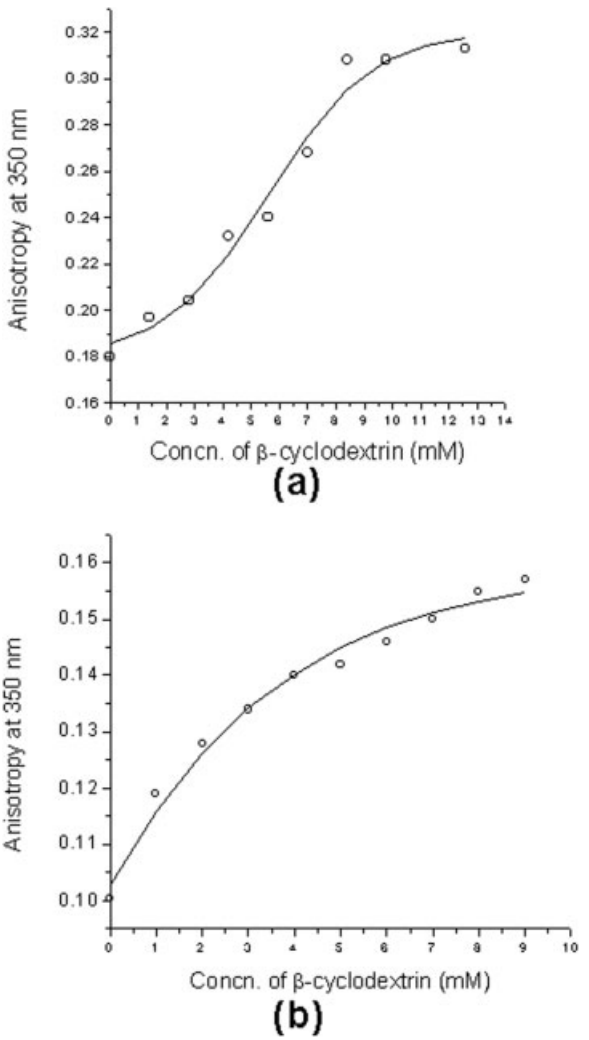

FIGURE 4 Change in anisotropy at 350 nm for (a) meloxicam and (b) tenoxicam at pH 5.5.

2:1 or 1:1. The stoichiometry of binding and the binding constants of the drugs with the host have been determined using a modified Benesi–Hildebrand equation. The binding constant K of the association reaction of oxicam drugs with β-cd

$$\text{O} + n\text{CD} \rightarrow \text{O-CD}n$$

is given by

$$K = [\text{O-CD}n]/[\text{O}][\text{CD}]^n \quad (1)$$

where n is the stoichiometry of the complex. Eq. (1) can be re written as

$$K = [\text{O-CD}n]/(C\text{o} - [\text{O-CD}n])(C_{\text{CD}} - [\text{O-CD}n])^n \quad (2)$$

where Co and C_{CD} represent the analytical concentration of oxicam drugs and β-cd respectively. In all of our experiments we have always used large excess of β-cd (1–12 mM) relative to oxicam drugs (concentration kept constant at 30 μM). Hence we can assume $C_{\text{CD}} \gg [\text{O-CD}n]$. Eq. (2) then reduces to

$$K = [\text{O-CD}n]/C_{\text{CD}}{}^n(C\text{o} - [O\text{-CD}n]) \quad (3)$$

For the low concentrations (30 μM) of oxicam drugs used in our experiments, the fluorescence intensity is proportional to the concentration of the fluorophore. Hence the fluorescence intensity of the complex is given by

$$F_{\text{O-CD}n} = Q[\text{O-CD}n]k_1 \quad (4)$$

where Q represents the quantum yield of the complex and k_1 is an instrumental constant. Substituting Eq. (4) in (3) and rearranging Eq. (3) gives

$$C\text{o}/(F_{\text{O-CD}n}) = \{(1/C_{\text{CD}}{}^n) \times (1/Kk_1Q)\} + (1/k_1Q) \quad (5)$$

Keeping the concentration of the guest molecule constant, with increasing concentration of the host β-cd, an increase in fluorescence emission intensities of the oxicam drugs was observed. If F is the fluorescence intensity at a particular concentration of β-cd and F_0 is the intensity in the absence of β-cd, then

$F_{\text{O-CDn}} = F - F_0$ and equation (5) reduces to

$$C\text{o}/(F - F_0) = \{(1/C_{\text{CD}}{}^n) \times (1/Kk_1Q)\} + (1/k_1Q) \quad (6)$$

A plot of $C\text{o}/(F - F_0)$ versus $1/C_{\text{CD}}{}^n$ was created for $n = 1, 2, 3$ etc. The binding stoichiometry n corresponds to a linear plot and the binding constant K was calculated as the ratio of the intercept to the slope.

The experiments were carried out at pH 3 to ensure the predominant presence of the neutral form in solution, which is involved in complexation with β-cd. The fluorescence intensities were monitored by exciting the samples at the absorption maxima for the corresponding neutral forms of the drugs. The peak intensities for piroxicam, meloxicam, and tenoxicam were monitored at 470, 510, and 500 nm, respectively. After reaching a certain concentration of the host, the fluorescence emission intensities did not increase further. The $C\text{o}/(F - F_0)$ versus $1/C_{\text{CD}}{}^n$ plots (Eq. (6) of piroxicam [Figure 5(a)], meloxicam [Figure 6(a)], and tenoxicam [Figure 6(b)] at 25°C assuming a 1 : 1 association between the host and the guest gives a linear plot. Linear regression was done with correla-

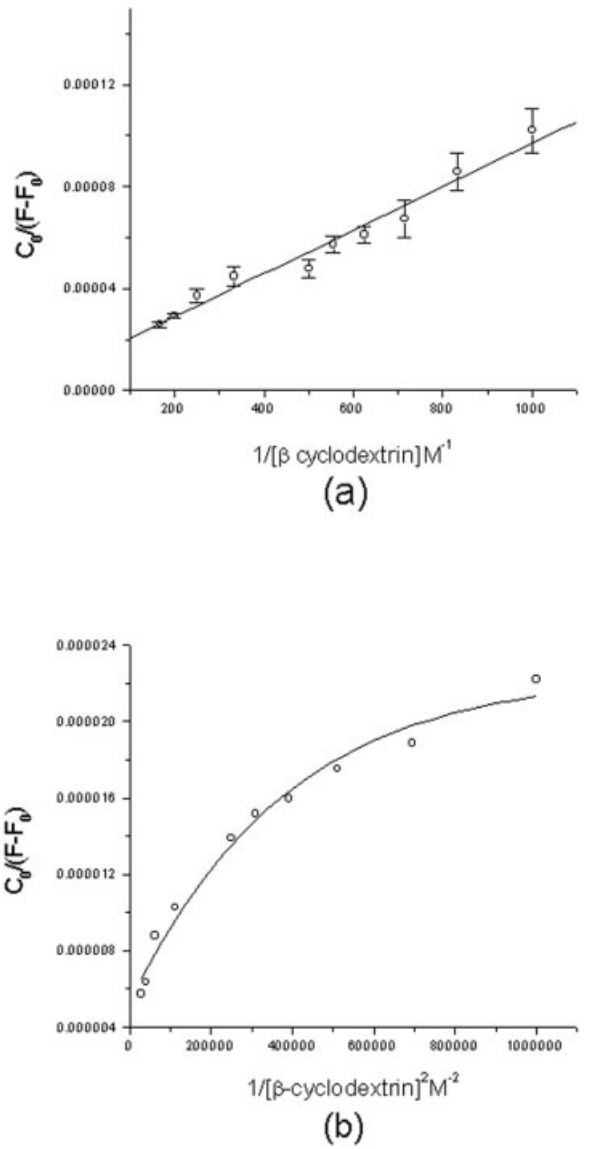

FIGURE 5 Benesi–Hildebrand plot for (a) 1 : 1 guest : host complex (linear plot) and (b) 1 : 2 guest : host complex (nonlinear plot) of piroxicam and β-cyclodextrin. Error bars indicate the standard deviations of data points taken from at least three different sets.

tions of 0.97, 0.98, and 0.99 for piroxicam, meloxicam, and tenoxicam, respectively. However, assuming 2 : 1 host : guest complex, i.e., putting $n = 2$ in Eq. (6) yields a nonlinear plot as shown in Figure 5(b), which indicates that the stoichiometry of binding of β-cd with piroxicam is 1 : 1 and not 2 : 1. This is also true for meloxicam and tenoxicam. The binding constants (K) for piroxicam, meloxicam, and tenoxicam have been calculated from the ratio of intercept to the slope of the linear Benesi–Hildebrand plots. Each experiment was repeated at least three times and the standard deviations in the data points have been indicated by the error bars. The values of the binding constants are 134 $\pm$ 21 M^{-1} for piroxicam, 114 $\pm$ 15 M^{-1} for meloxicam, and 115 $\pm$ 13 M^{-1} for tenoxicam.

Effect of Changing pH on the Host : Guest Complex

From the previous data, it is evident that the neutral forms of the drugs are incorporated in the host. We made an attempt to understand in which form the drug piroxicam might be delivered to blood. With this aim,

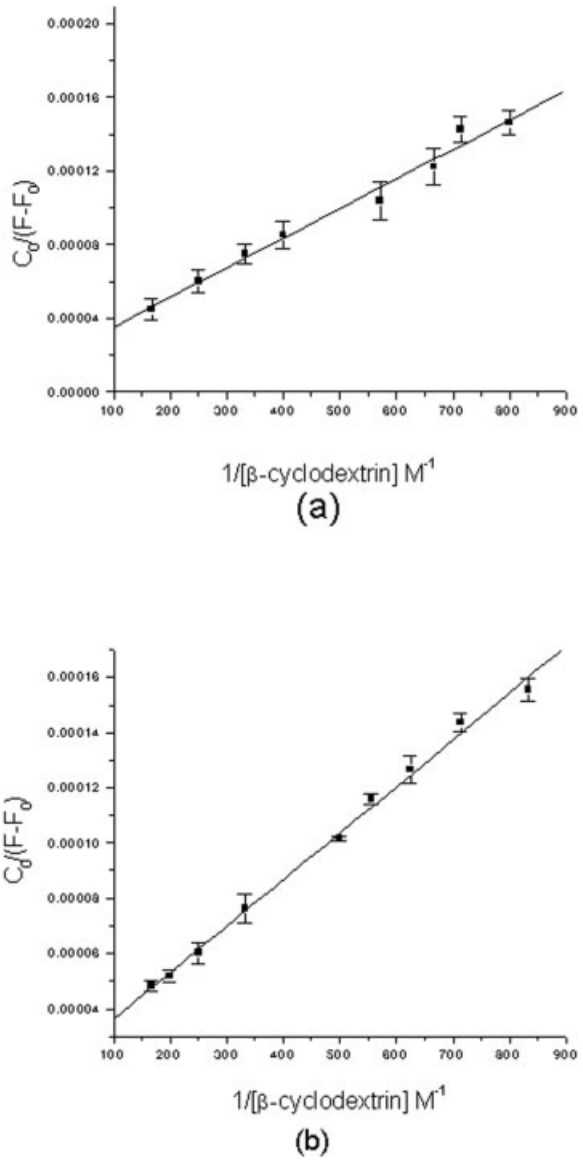

FIGURE 6 Benesi–Hildebrand plot of 1 : 1 complex of (a) meloxicam and (b) tenoxicam with β-cyclodextrin. Error bars indicate the standard deviations of data points taken from at least three different sets.

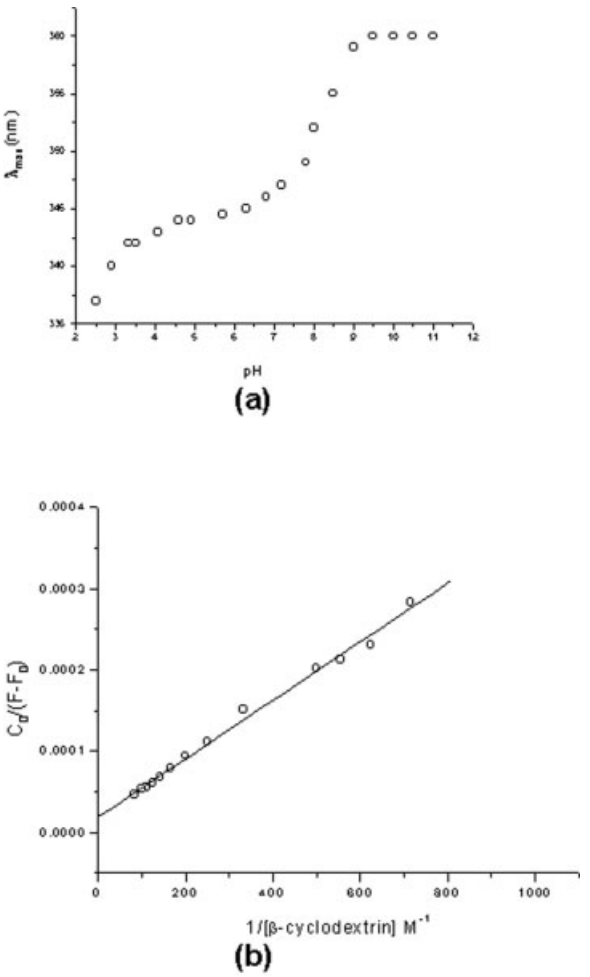

FIGURE 7 (a) Change in absorption maximum of piroxicam–β-cyclodextrin complex with pH: [piroxicam] = 30 μM and [β-cyclodextrin] = 12 mM. (b) Benesi–Hildebrand plot of 1 : 1 complex of anionic form of piroxicam with β-cyclodextrin at pH 9.7.

we have conducted a pH-dependent absorption study in which at first the pH of the solution was made to be 2.5 to make the predominant structural form of piroxicam global neutral. A significantly high concentration of the host (12 mM) then was added so as to assure the formation of the inclusion complex. The pH of the solution was then increased up to pH 11 in very small steps by adding alkali. The absorption maximum shifted to a higher wavelength (from 337 to 360 nm) [Figure 7(a)] in going from pH 2.5 to 9 and remained at 360 nm at pH $>$ 9. At 350 nm, an isosbestic point was observed. The pH was not increased beyond pH 11 in order to avoid the deprotonation of β-cd. It should be noted that in the bulk aqueous solution the absorption maximum of the anionic form of piroxicam is at 353 nm, whereas, in ethanol, it is at 361 nm.[26] The shift in the absorption maximum from 337 to 360 nm indicates that the principal prototropic species changes from neutral to anionic form with increasing pH though the drug still resides in the host cavity. Figure 7(a) also shows that there is almost an abrupt red shift in the absorption spectra near pH 7.4, i.e., at physiological pH, the drug predominates in its anionic form, but still it remains in the host. An effort was made to find the binding constant of anionic piroxicam with β-cd. Figure 7(b) shows the modified Benesi–Hilderbrand plot using fluorescence data of 1 : 1 guest : host complex of anionic form of piroxicam and β-cd at pH 9.7. It should be mentioned that the anionic form of piroxicam does not get encapsulated in β-cd, so to do this we proceeded as follows. First, we adjusted the pH of the solution to 2.5 to get the neutral form of piroxicam, then a certain concentration of β-cd was added, which varied from sample to sample. This resulted in the neutral form being incorporated in β-cd as was evident from the absorption maximum (337 nm). Alkali (dilute solution of NaOH) was then added to each sample to attain pH 9.7. It was ensured that each sample had an absorption maximum at 360 nm, which indicated that the anionic form of piroxicam was still encapsulated in the β-cd cavity. The binding constant obtained was $K = 52\ M^{-1}$, which is significantly lower than that of the neutral form of piroxicam (134 $\pm$ 21 M^{-1}). This is an expected result since the charge of the anionic form of piroxicam disfavors its binding in the hydrophobic cavity of β-cd compared to the neutral form.

Molecular Modeling Studies

Figures 8(a)—(c) show the top view and side view of the lowest energy model of each host–guest complex. In each case, a part of the guest molecule is accommodated in the β-cd cavity. The long axis of the drugs is oriented along the axis of the β-cd in each case, with the conjugated rings lying within the cavity and the third ring exposed to the solvent through the larger opening of the bucket-like cavity of β-cd [Figures 8(a)—(c)]. In all the cases the enolic –OH group was found to remain near the surface of the β-cd cavity and hence was exposed to the solvent. No bad contact between the host and guest were observed. In all of the host–drug systems, H-bonding between the sulfur oxygen of the benzothiazene ring and H of CH_2OH of the β-cd molecule were observed with a geometry having an average acceptor donor distance ($d_{D\text{-}A}$) of 2.675 Å and an average angle ($\theta_{D\text{-}H\ldots A}$) of 175°. It is interesting to point out that, in piroxicam and tenoxicam, only one sulfur oxygen of the benzothiazene ring was involved in H-bonding with β-cd, whereas, for

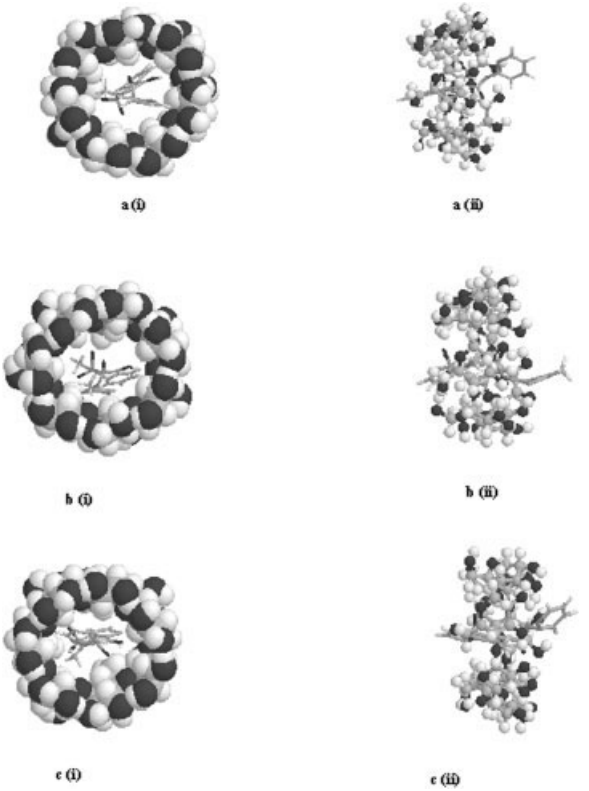

FIGURE 8 (i) Top view and (ii) side view of energy-minimized structures of the complexes of β-cyclodextrin with (a) piroxicam, (b) meloxicam, and (c) tenoxicam.

meloxicam, both the sulfur oxygens are involved in H-bonds. Table I represents the total energy of the molecules both in the free and in the complexed form as well as the interaction energy between the drug and the β-cd in the complex. In all cases of the three drugs and β-cd, the self-energies of the free molecules are not very different from their counterparts in the complexes. This implies that no significant distortions are introduced in the drugs or β-cd when they form the complexes. It should be mentioned that the absence of explicit solvent does not allow us to consider the hydrophobic effect in a proper way. Despite this drawback, we still get a favorable interaction energy between the two components of the host–guest complex. The solvophobic effect will further improve the stabilization of the complex. The conjugated ring being predominantly hydrophobic in nature, its incorporation into the β-cd cavity should be partially stabilized by the solvophobic effect, while the interaction of the third ring with β-cd is expected to be guided by the electrostatic effect. This is reflected in similar interaction energy of host–guest complexes of piroxicam and tenoxicam with β-cd where the third ring is the same, viz. the pyridyl ring. Detailed MD simulations of these complexes in aqueous solutions are in progress and will be communicated in a separate paper.

DISCUSSION

The oxicam NSAIDs studied here show that they are capable of forming inclusion complexes with the host, β-cyclodextrin. As mentioned earlier, there are several controversies regarding the prototropic species of piroxicam that is incorporated into β-cd. In previous works by Escandar[13] and Kim et al.,[10] spectrofluorimetric studies were done on a piroxicam β-cd complex in aqueous solution. In both the works it has been depicted that the neutral form is encapsulated in host cavity. However, in a solid-state NMR and Raman spectroscopic study by Redenti et al.,[11] it was shown that in a 1:1 host–guest inclusion compound, piroxicam mainly assumes the zwitterionic structure. In our work, we have identified the structural form to be the global neutral form that is involved in formation of the host–guest complex in aqueous solution. This allowed us to settle the controversy as to which structural form of piroxicam prevails in the inclusion complex. Though there exists no previous data on meloxicam and tenoxicam inclusion complex with β-cd in aqueous solution, our study on these two oxicam drugs also conclusively shows that the neutral form of meloxicam and the global neutral form of tenoxicam take part in forming the host–guest complex.

Table I Self-energy of Host and Guest and Their Interaction Energy in the Complexes

	Piroxicam	Meloxicam	Tenoxicam	β-Cyclodextrin
Energy of individual free molecule (kcal/mol)	25.93	54.99	78.36	608.59
Energy of β-cyclodextrin in complex (kcal/mol)	619.49	629.36	625.15	—
Energy of drug molecules in β-cyclodextrin complex (kcal/mol)	29.28	64.19	82.54	—
Total energy of complex (kcal/mol)	601.38	637.06	661.16	—
Interaction or stabilization energy (kcal/mol)	−47.39	−56.51	−46.53	—

The second part of the controversy on piroxicam–β-cd complex involves the binding stoichiometry with the host. Some of the earlier studies using different methods like spectrofluorimetry,[10] microcalorimetry,[12] Raman, and solid state NMR,[11] have shown the stoichiometry of binding to be 1:1 for the piroxicam–β-cd inclusion complex. However, in a study by Escander,[13] a 2:1 host–guest complex is proposed. In our work we have obtained 1:1 binding stoichiometry. We have shown that, in the modified Benesi–Hildebrand plot, a linear fit was obtained only for a 1:1 complex and not for a 2:1 guest– host complex of β-cd–piroxicam.

The high value of the equilibrium constant (134 ± 21 M^{-1}) of the β-cd–piroxicam inclusion complex at pH 3 indicates that the encapsulation of the guest is dependent on the hydrophobic interactions between the drug in the apolar cavity of the host. In previous work by Dalmora and Oliveira[9] complexation of piroxicam with β-cd was studied within the pH range 4.5 to 6.0 and it was indicated that the complex formation was facilitated at a lower pH. Since the isoelectric pH of piroxicam is 3.66,[24] the global neutral species, i.e., the mixture of neutral and the zwitterionic form, predominates in solution around this pH. A better complexation at low pH supports our result that the global neutral form is incorporated in the host. Our studies on meloxicam and tenoxicam show for the first time that, in aqueous solution, both the oxicam drugs are capable of forming a host–guest complex with β-cd. It is the neutral/global neutral forms of the drugs that are incorporated in the β-cd cavity in a 1:1 binding stoichiometry. Molecular modeling studies of the host–guest complexes also indicate that, for all three drugs, the lowest energy model for the complexes formed by encapsulation of the neutral forms of the drugs in 1:1 stoichiometry have favorable interaction between the drugs and β-cd. Also, in each case, the enolic –OH group of the drugs is found to be near to the surface of the β-cd cavity where it is more accessible to the solvent. All of these findings together provide additional support in favor of our present model.

Since the oxicam NSAIDs studied here show better complex formation and stability at low pH, it can be concluded that, when the drugs are encapsulated in the host and administered orally, they encounter the GI mucosal lining in the complexed form. The drug might be delivered to body fluid even in the encapsulated form as revealed from our pH-dependent study [Figure 7(a)]. It shows that once the complex is formed, increasing pH converts the drug (piroxicam) to its anionic form that still faces an apolar environment, indicating that the anionic form is not ejected from the β-cd cavity into the bulk aqueous phase. If the anionic form of the drug (piroxicam) has to come out in bulk solvent we should have gotten the absorption maximum at 353 nm. However, the absorption maximum remained at 360 nm even at pH 9, which is the characteristic peak position for the piroxicam anion in a relatively nonpolar solvent such as ethanol.[26] Clinical studies on piroxicam indicate that β-cd encapsulation shows better GI tolerability and therapeutic efficacy. Our study demonstrates the stability of the complexes at low pH and the possible delivery of the drugs at a higher pH in the encapsulated form. Our results also indicate that the increased GI tolerability and therapeutic efficacy could be either due to the fact that the drugs should remain in the encapsulated form in the stomach and/or the fact that only a particular prototropic form might be transported to the target. However, to isolate which factor improves the pharmacological efficacies, further studies are required.

CONCLUSION

Our study elucidates the interaction of oxicam NSAIDs with the drug delivery system β-cd at the molecular level. For the three drugs, piroxicam, meloxicam, and tenoxicam, only the neutral forms are encapsulated as guest in the host β-cd system with 1:1 binding stoichiometry and binding constants of 134 ± 21, 114 ± 15, and 115 ± 13 M^{-1}, respectively. This also settles the controversy regarding the piroxicam–β-cd complex. Once encapsulated, the neutral form of piroxicam responds to the change in bulk pH by converting to the anionic form without being ejected out of the host. This is important if one considers the diverse pH conditions the drugs face *in vivo*. Molecular modeling studies show the minimum energy configuration gives favorable interaction between the drugs and β-cd when the conjugated rings of the drugs are inside the hydrophobic bucket-like cavity of β-cd and the third ring is exposed to the solvent.

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