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Fexofenadine/Cyclodextrin Inclusion Complexation: Phase Solubility, Thermodynamic, Physicochemical, and Computational Analysis

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Interactions of fexofenadine (Fexo) with cyclodextrins (CDs: α- β-, γ-, and HP-β-CD) were investigated by several techniques including phase solubility, differential scanning calorimetry (DSC), X-ray powder diffractometry (XRPD), ^{1}H-nuclear magnetic resonance (^{1}H-NMR) and molecular mechanical modeling (MM^+). The effects of CD type, pH, ionic strength, and temperature on complex stability were also explored. Fexo/CD complex formation follows the decreasing order: β-CD > HP-β-CD > γ-CD > α-CD (i.e., at pH 7.0 and 30°C, K_{11} = 1139, 406, 130, and 104 M^{-1}, respectively). The linear correlation of the free energy of Fexo/β-CD complex formation (ΔG_{11}) with the free energy of inherent Fexo solubility (ΔG_{So}), obtained from the variation of K_{11} with inherent Fexo solubility (S_o) at different pHs and ionic strengths, was used to measure the contribution of the hydrophobic character of Fexo to escape from water by including into the hydrophobic CD cavity. The hydrophobic effect (desolvation) contributes about 76% of the total driving force towards inclusion complex formation, while specific interactions contribute –7.7 kJ/mol. Moreover, Zwitterionic Fexo/β-CD complex formation appears to be driven both by favorable enthalpy (ΔH° = –23.2 kJ/mol) and entropy (ΔS° = 15.2 J/mol.K) changes at pH 7.0. ^{1}H-NMR and MM^+ studies indicate multimodal inclusion of the piperidine, carboxypropylphenyl, and phenyl moieties into the β-CD cavity. MM^+ computations indicate that the dominant driving force for complexation is Van der Waals force with very little electrostatic contribution. ^{1}H-NMR, DSC, and XRPD studies indicate the formation of inclusion complex in aqueous solution and the solid state.

Keywords fexofenadine; cyclodextrin inclusion complexation; complex formation constants; thermodynamics; hydrophobic effect; ^{1}H-NMR; molecular modeling

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INTRODUCTION

There has been a great interest in using CDs in different pharmaceutical applications. The parent CDs (α-, β-, and γ-CD) and their derivatives are widely used to improve the solubility of water insoluble compounds through inclusion complexation. There is a wealth of literature describing the effect of various factors on inclusion complex formation (e.g., pH and buffer type). For example, ionized species of ibuprofen and hydrochlorothiazide do not contribute significantly to complex formation with CD, and protonated diazepam showed a lower tendency to complex than neutral diazepam (Menard et al., 1990; Perlovich et al., 2003). Protonated basic drugs were also found to have a lower complexation tendency than the neutral species (Backensfeld et al., 1991). A combination of pH adjustment and CD was also reported to improve the solubility of thiazolobenzimidazole (Tinwalla et al., 1993), nimesulide (Piel et al., 1997), flavopiridol (Li et al., 1998) and sulfisoxazole (Gladys et al., 2003). The solubility of some dihydropyridine derivatives in aqueous HP-β-CD was found to be lower in citrate than phosphate buffers, an effect related to a lower solubility product of the citrate salt (Müller & Albers, 1992). Spray-dried ketoconazole/β-CD complexes prepared from aqueous citric and hydrochloric acid solutions showed some pH dependence of their dissolution behavior (Esclusa-Diaz et al., 1996). A study of the complexation of ziprasidone with β-CD-sulfobutyl ether showed that different salts of ziprasidone including tartrate, esylate, and mesylate exhibit different phase solubility diagrams with different intercepts and slopes (Kim et al., 1998). This indicates that the type of counter anion may affect both the solubility of the drug and its complex thus yielding different values of the complex formation constant even at the same pH.

Fexofenadine (Fexo), α,α-dimethyl-4-[1-hydroxy-4-[4-(hydroxydiphenyl-methyl)-1-piperidinyl] butyl] benzeneacetic acid, is used to treat symptoms of allergic conditions. It is a selective, nonsedating H_1-receptor antagonist. It is available under the trademark TELFAST and ALLEGRA in tablet and capsule dosage forms containing 60, 120, and 180 mg of Fexo.HCl. It is rapidly absorbed after oral administration, and has a relatively low absolute bioavailability of about 30% (Dresser et al., 2005; Russell et al., 1998) that is not limited by solubility, but rather limited by the first pass effect (Tannergren et al., 2003). The octanol/water Log P values are reported at 5.18 ± 0.40 for Fexo and 0.49 ± 0.01 for Fexo.HCl (Lin et al., 2005).

Different patents describe novel formulations of various active ingredients including Fexo. In these formulations, cyclodextrin (CD) is used as a solubilizer to improve drug delivery in different dosage forms (Chen & Patel, 2000; Illum et al., 2000), as one of the pharmaceutical excipients that may be used to produce nano- and micro-particles (Williams et al., 2002), and as a carbohydrate-based dispersing agent to form liquid and semi-solid pharmaceutical compositions (Meyer et al., 2002).

Recently, highly soluble binary inclusion complexes of various amphoteric compounds, such as Fexo, with CDs were prepared by dehydration (evaporation or freeze-drying) of a saturated aqueous solution of both components (Badwan et al., 2005). However, guest-host interactions of Fexo with CDs involving inclusion complex characterization have not yet been reported.

In this work, the effects of CD type, pH of solution, ionic strength, and temperature on the interaction of Fexo with CDs were studied. A quantitative estimate of the contribution of the hydrophobic character of Fexo and specific interactions to complex stability with β-CD was determined from the linear dependence of the free energy of complex formation (ΔG_{11}) on that of the inherent Fexo solubility (ΔG_{So}), obtained at different pHs and ionic strengths. The thermodynamic parameters were also obtained to evaluate the driving forces for complex formation. In addition, DSC, XRPD, ^{1}H-NMR, and MM^+ studies were carried out to verify inclusion complex formation, and to explore guest-host interaction sites.

EXPERIMENTAL

Chemicals

Fexo.HCl and CDs (α-, β-, HP-β-, and γ-CDs) were provided by The Jordanian Pharmaceutical Manufacturing Company (JPM, Jordan). The neutral form (Fexo) was prepared by neutralization of Fexo.HCl (10 mmoles), dissolved in sufficient amount of water, with 0.1 M NaOH solution; the precipitate was collected and dried at 40°C. All other chemicals were of analytical grade obtained from Merck/Germany and Surechem/UK.

Preparation of Fexo.HCl/β-CD Solid Complex

By Freeze-Drying: The Fexo.HCl/β-CD complex was prepared by dissolving a mixture of Fexo.HCl (3.8 mmoles) and β-CD (7.6 mmoles) in sufficient amount of water. The sample was freeze-dried (Heto FD3, Heto-Holten A/S, Denmark) and the solid was collected.

By Evaporation: The Fexo.HCl/β-CD complex was prepared by dissolving a mixture of Fexo.HCl (3.8 mmoles) and β-CD (7.6 mmoles) in sufficient amount of water. The sample was evaporated under vacuum at 80°C (RV06-ML, IKA-WERKE, Germany) and kept at 40°C overnight. The solid was collected and dried at 110°C for 1 h.

To ensure that this procedure does not affect the chemical stability of Fexo.HCl and its complex after drying at 110°C, samples equivalent to 50 mg Fexo.HCl from Fexo.HCl and its complex with β-CD were dissolved each in 0.01 M hydrochloric acid solution and tested by high performance liquid chromatographic method for assay and related compounds determinations [Fexofenadine hydrochloride USP monograph, 2006]. The results indicated that both Fexo.HCl and its complex with β-CD are highly stable, where the assay values proved higher than 99% and the level of impurities less than 0.3%.

Preparation of Physical Mixtures of Fexo.HCl/β-CD

Saturated solutions of Fexo.HCl and β-CD were individually prepared; portions of each were evaporated under vacuum while other portions were freeze-dried. The collected solids of Fexo.HCl and β-CD were physically mixed in a molar ratio of 1:2, respectively, using mortar and pestle for those obtained by evaporation, for those obtained from freeze-drying, and also for untreated samples. In the case of evaporation, β-CD was dried at 110°C for 1 h prior to mixing with Fexo.HCl to prevent overlapping of the broad endothermic peak corresponding to water with that of Fexo.HCl at 145°C as indicated by DSC.

Determination of Acid-Base Ionization Constants (pK_as)

By UV/Visible Spectrophotometry: A stock solution of Fexo (15 mM) was prepared by dissolving 1.5 mmoles in 100 mL of methanol, which was diluted further with 0.05 M citrate buffers of different pHs ranging from 1 to 12.7 to obtain final solutions having fixed concentration of 0.60 mM. The pH of solution was adjusted by either diluted HCl or NaOH solution to obtain the desired pH. The absorbencies of these solutions were measured using first derivative UV/visible spectrophotometry at 273 nm (Du-650i, Beckman, U.S.A.). It should be noted that first derivative UV spectrophotometry was consistently used throughout this work following careful examination of absorption spectra against β-CD concentration at different pHs, which offered the maximum differences in absorbance and the absence of interferences.

The two pK_as of Fexo were determined following the procedure discussed earlier (Al-Omari et al., 2006a). Using the following acid-base equilibria relevant to Fexo

$$HA \rightleftharpoons H^+ + A^-; K_a = \frac{(H^+)y[A^-]}{[HA]} \quad (1)$$

$$H_2A^+ \rightleftharpoons H^+ + HA; K_{a1} = \frac{(H^+)[HA]}{y(H_2A^+]} \quad (2)$$

The measured absorbance at different pHs (A) was nonlinearly regressed against the predicted absorbance (A^P) by minimizing the function $SSE = \sum (A^P - A)^2$ where

$$\begin{aligned} A^P &= \{\varepsilon_{A^-}[A^-] + \varepsilon_{HA}[HA] + \varepsilon_{H_2A^+}[H_2A^+]\} \\ &= C\{\varepsilon_{A^-} f_{A^-} + \varepsilon_{HA} f_{HA} + \varepsilon_{H_2A^+} f_{H_2A^+}\} \end{aligned} \quad (3)$$

while ε_{A^-}, ε_{HA}, and $\varepsilon_{H_2A^+}$ are the molar absorptivities of A^-, HA, and H_2A^+, respectively, C is the molar concentration of Fexo which was kept constant at 0.60 mM, and f_{A^-}, f_{HA}, and $f_{H_2A^+}$ are the fractions of A^-, HA, and H_2A^+ present in solution at each pH.

By pH Solubility Profile: Excess amounts of Fexo were added to 0.05 M citrate buffers (50 mL) of pHs ranging from 2.5 to 10.5. The samples were mechanically shaken in a thermostatic bath shaker at 30°C to attain equilibrium (2 days), an aliquot was filtered using a 0.45 μm filter (cellulose acetate or cellulose nitrate, Advantec MFS Inc., Duplin). The final pH of each solution was measured by pH-meter (Jenway, 3030, England). The Fexo content was determined using first derivative UV/visible spectrophotometry at 270 nm (Isosbestic point).

Referring to the acid/base equilibria of Fexo indicated in Eq. (1–2) above, estimates of the ionization constants of Fexo (pK_a and pK_{a1}) and the solubility product (pK_{sp}) of the $(FexoH^+)_2$ citrate^{2-} salt were obtained by nonlinear regression of the measured inherent solubility (S_o) of Fexo against pH (Al-Omari et al., 2006a) according to

$$S_o{}^P = [HA] + [A^-] + [H_2A^+] = [HA]\{1 + \frac{K_a}{y(H^+)} + \frac{(H^+)}{yK_{a1}}\} \quad (4)$$

or

$$S_o{}^P = [HA]\{1 + \frac{K_a}{y(H^+)} + (\frac{K_{sp}}{y^6[X^{2-}]})^{1/2}\} \quad (5)$$

where X^{2-} denotes the citrate^{2-} ion species, and K_{sp} is the solubility product of the salt given by

$$(H_2A^+)_2X^{2-}{}_{(s)} \rightleftharpoons 2H_2A^+ + X^{2-}; K_{sp} = y^6[H_2A^+]^2[X^{2-}] \quad (6)$$

Eq. (4) was automatically switched into Eq. (5) in the pH region where S_o was limited by saturation of the $(FexoH^+)_2$ citrate^{2-} salt (pH < 7). The best estimates for pK_a, pK_{a1}, and pK_{sp} were obtained by minimizing $SSE = \sum (S_o^P - S_o)^2$ where S_o^P is the predicted value of S_o.

Phase Solubility Studies

Solubility studies were performed as described earlier (Higuchi & Connors, 1965). Excess amounts of Fexo were added to 50 mL of the desired aqueous CD solutions ranging in concentration from 0 to 100 mM. The solutions include: 0.05 M citrate buffers of pHs ranging from 2.3 to 7.0, in 0.01 M HCl (pH = 2.0) having different ionic strengths (μ = 0.01 to 0.80) by adding different amounts of KCl. The samples were mechanically shaken in a thermostatic bath shaker (GFL, 1086, Germany) to attain equilibrium (2 days); an aliquot was filtered using a 0.45 μm filter (cellulose acetate, Advantec MFS Inc., Duplin). The pH of the filtrate was measured by pH-meter. The Fexo content was determined using the same methods described above in the pH solubility profile section.

The measured phase solubility diagrams (PSD)s were rigorously analyzed to obtain estimates of complex formation constants (K_{ij}) through linear and nonlinear regression analysis, which were discussed earlier (Al Omari et al., 2006a; Zughul & Badwan, 1998). Correction for citrate buffer species/β-CD complexation was carried out according to procedures discussed earlier (Al-Omari et al., 2006b; Al-Omari et al., 2007). The fitting indicated the formation of 1:1 and 1:2 Fexo:CD complexes. As the K_{12} values obtained by rigorous analysis varied with no general trends and represent only not more than 10% of the K_{11} values, so K_{11} was used as an index to study the effect of various factors on complex stability as discussed below.

Estimation of Thermodynamic Parameters

Gibbs and van't Hoff equations were used to estimate the thermodynamic parameters ΔH^o, ΔS^o, and ΔG^o by regressing ln $K_{11}{}^x$ and ln $S_o{}^x$ against 1/T, where x stands for the mole fraction standard state. K_{11} (M^{-1}) and S_o (M) were transformed to mole fraction units by multiplying K_{11} with 55.5 and dividing S_o by 55.5, where 55.5 represents the number of moles of water in 1 L water (Al Omari et al., 2006a).

Differential Scanning Calorimetry (DSC)

The thermal behaviors of all samples of Fexo.HCl, β-CD, a physical mixture of Fexo.HCl and β-CD, and the isolated

Fexo.HCl/β-CD solid complex (untreated, evaporated, and freeze-dried samples of each) were studied by DSC (910S, TA instrument). Accurately weighed sample of each equivalent to 5 mg Fexo.HCl was heated in a sealed aluminum pan, using an empty pan sealed as reference, over the temperature range of 30 to 300°C, at a rate of 10°C/min. Indium standard was used for calibrating the temperature.

X-Ray Powder Diffractiometry (XRPD)

The XRPD patterns of all samples of Fexo.HCl, β-CD, a physical mixture of Fexo.HCl and β-CD, and Fexo.HCl/β-CD complex (untreated, evaporated, and freeze-dried samples of each) were measured using an X-ray diffractometer (Philips PW 1729 X-Ray Generator). The Radiation generated from a Co K_α source and filtered through Ni filters (λ = 1.79025 Å) at 40 mA and 35 kV was used. The instrument was operated over the 2θ range of 10–80°.

Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR)

Samples were dissolved in 99.98% D_2O and filtered before use. ^{1}H-NMR spectra were obtained at 400 MHz and 25°C (GSX400, JEOL, Japan). Chemical shifts are quoted relative to sodium 3-trimethylsilyl [D_4] propionate at 0.0 ppm, but spectra were calibrated via the known position of the residual HOD resonance, which was used as a reference.

Molecular Modeling

Molecular mechanical modeling was performed by MM^+ force field using HyperChem Version 6 software (Hypercube, Canada) as described earlier (Al Omari et al. 2006a; Taraszewska et al., 2003). Interaction energies were computed for the drug approaching from its phenyl and carboxyl groups through the wide and narrow rims of β-CD cavity. For the 1:2 complex, the second β-CD molecule was allowed to approach the most stable 1:1 complexes obtained from the phenyl and carboxyl approaches to assess the most probable 1:2 complex configuration. The binding energies ($E_{binding} = E_{complex} - \Sigma E_{components}$) corresponding to energy minima were computed together with their electrostatic (E_{es}) and Van der Waals (E_{vdw}) contributions.

RESULTS AND DISCUSSION

Acid-Base Ionization Constants (pK_as)

Analysis of the variation of the first derivative UV absorptivity of a fixed concentration of Fexo (0.60 mM) at 273 nm with pH (Figure 1a) according to Eq. (3) yielded the values: pK_a = 10.3 and pK_{a1} = 4.2 corresponding to ionization of the piperidine and the carboxylic moieties, respectively. The corresponding analysis of the variation of inherent Fexo solubility (S_o) with pH (Figure 1b) according to Eqs. (4–5) yielded: pK_a = 10.2 and pK_{a1} = 4.2, which are in close agreement with those

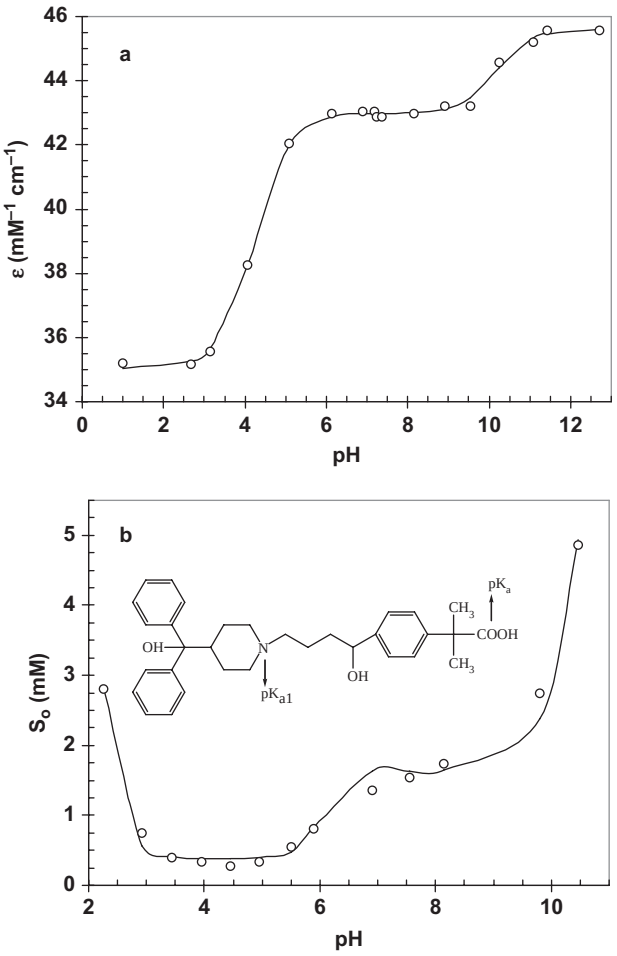


FIGURE 1. Plots of the variation of (a) the absorbance of a fixed concentration of Fexo (0.60 mM) at 273 nm, and (b) the inherent solubility of Fexo (S_o) against pH, both measured in 0.05 M Citrate buffer at 30°C.

obtained from the UV spectrophotometric method above. The solubility product of $(FexoH^+)_2$ $citrate^{2-}$ salt was estimated at pK_{sp} = 9.9.

Effect of CD Type

Figure 2 depicts PSDs obtained for Fexo against each of α-, β-, HP-β-, and γ-CD concentration in 0.05 M citrate buffers (pH = 7.0) and 30°C. At this pH, Fexo exists as a Zwitterion at an inherent solubility S_o = 0.87 mM. The K_{11} values were 1139, 406, 130, and 104 M^{-1} for β-CD, HP-β-CD, γ-CD, and α-CD, respectively (Table 1). The relatively lower K_{11} values for α-CD and γ-CD complexes are most likely due to α-CD having a small cavity size that reduces the probability of including the rather bulky groups of Fexo, while γ-CD has a large cavity size which lowers effective interactions with Fexo (Nalluri et al., 2003; Taraszewska et al., 2003). The binding of Fexo with HP-β-CD (K_{11} = 406 M^{-1}) is relatively less than that with β-CD

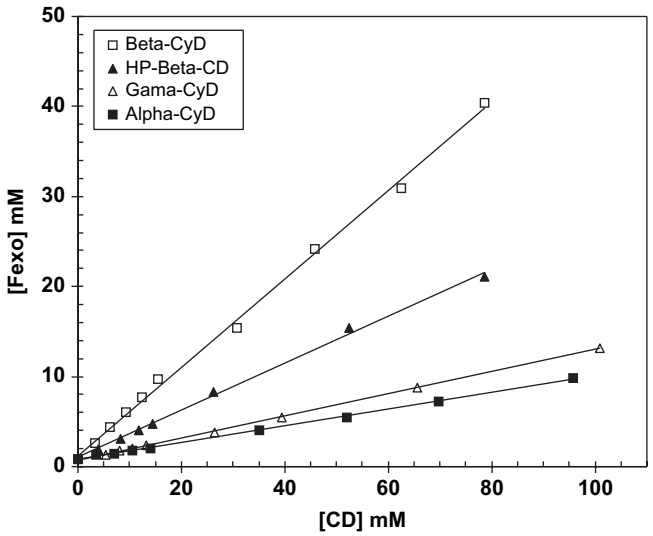


FIGURE 2. PSDs of the Fexo/CD systems for α-, β-, γ- and HP-β-CD obtained in 0.05 M Citrate buffer at pH 7.0 and 30°C.

TABLE 1
Estimates of Complex Formation Constant (K_{11}) for Fexo/CDs Systems Obtained in 0.05 M Citrate Buffer at pH 7.0 and 30°C. The Enhancement Factor (EF) = S_{eq}/S_o, Where S_{eq} and S_o Are the Solubilities of Fexo in the Presence (15 mM) and Absence of CD, Respectively

CD	Phase Solubility Diagram Type	K_{11} (M^{-1})	EF
α-CD	A_L	104	2.4
β-CD	A_L	1139	9.7
γ-CD	A_L	130	2.9
HP-β-CD	A_L	406	5.6

(K_{11} = 1139 M^{-1}), probably due to the presence of substituent hydroxypropyl groups in HP-β-CD, which may hamper inclusion of some relatively large and flexible guest molecules into the CD cavity via steric hindrance (Ribeiro & Veiga, 2002; Yoshida et al., 1989). In the case of β-CD, the PSD exhibits solubility synergism, where the solubility of β-CD increased in the presence of Fexo.citrate Zwitterions from 20 to 80 mM at pH 7.0 and 30°C (Al Omari et al., 2006c; Badwan et al., 2005).

Effect of pH and Ionic Strength

Figures 3a and 3b depict the Fexo/β-CD PSDs obtained in 0.05 M citrate buffer at different pHs (ranging from 2.3 to 7.0) and in 0.01 M HCl (pH 2.0) adjusted to different ionic strengths (μ = 0.01 to 0.80) by adding different amounts of KCl, respectively. The corresponding K_{11} values were listed in

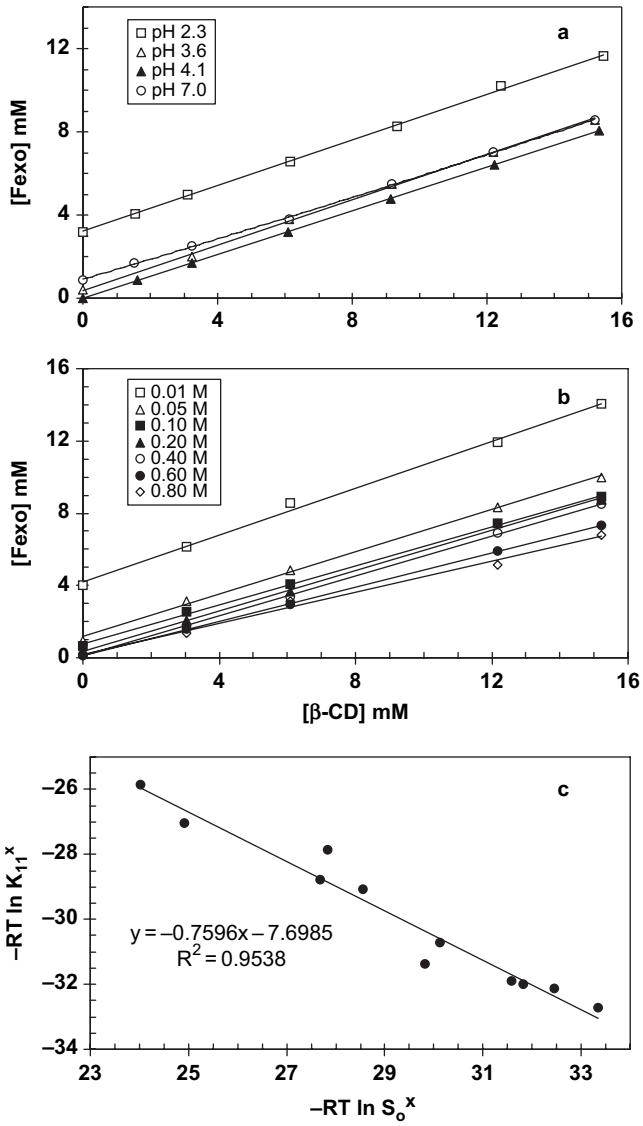


FIGURE 3. PSDs of the Fexo/β-CD system obtained in 0.05 M Citrate buffer at different pHs (a), and in 0.01 M HCl (pH 2.0) at different ionic strengths and 30°C (b), while (c) is a plot of $-RT \ln K_{11}^x$ against $-RT \ln S_o^x$ for the data in Table 2 (*x* Denotes the mole fraction standard state).

Table 2. The K_{11} value appears to increase for protonated Fexo with pH and μ. The increase in K_{11} as pH increases is mainly a reflection of the corresponding decrease in the inherent solubility (S_o) of Fexo (i.e., as S_o decreases, Fexo becomes more hydrophobic thus increasing its tendency to escape from water bulk into the CD hydrophobic cavity). This indicates a significant contribution of the hydrophobic character of Fexo as a driving force for complex formation, though other factors must be involved (Liu & Guo, 2002).

A quantitative measure of the contribution of the hydrophobic effect (desolvation) to complex formation was obtained by two different procedures. One was to obtain estimates of K_{11}

TABLE 2
Estimates of Complex Formation Constant (K_{11}) of the Fexo/β-CD Complexation Obtained in 0.05 M Citrate Buffer at Different pHs and in 0.01 M HCl Solution (pH 2.0) Adjusted to Different Ionic Strengths (μ) by the Addition of KCl, Both at 30°C (S_o Represents the Inherent Solubility of Fexo)

pH	μ	S_o (mM)	K_{11} (M^{-1})
Fexo in 0.05 M Citrate Buffer			
2.3	0.01	2.80	825
3.6	0.05	0.40	4596
4.1	0.08	0.20	5705
7.0	0.30	0.88	1139
Fexo in 0.01 M HCl Solution			
2.0	0.01	3.99	513
	0.05	0.94	1649
	0.10	0.66	1845
	0.20	0.36	3561
	0.40	0.18	5946
	0.60	0.14	6248
	0.80	0.099	7855

from PSDs measured at different pHs where S_o varies with pH (Al Omari et al., 2006a). The second route followed was to measure the PSDs at same pH but different ionic strengths where S_o decreases with an increase in ionic strength (El-Barghouthi et al., 2005). The negative slope of the linear plot of the free energy of complex formation ($\Delta G^o = -RT \ln K_{11}^x$) against the free energy of inherent Fexo solubility ($\Delta G^o = -RT \ln S_o^x$) would indicate how much of the driving force for complex stability is contributed by the hydrophobic character of Fexo, while the intercept indicates the contribution of other factors including specific interactions. Figure 3c shows this plot of all data listed in Table 2. The linear variation of $-RT \ln K_{11}^x$ against $-RT \ln S_o^x$ indicates that almost 76% (slope) of the tendency for complex formation is driven by the hydrophobic character of Fexo, while other factors including specific interactions contribute about –7.7 kJ/mol (intercept) to complex stability.

Thermodynamics

The PSDs of Fexo/β-CD obtained in 0.05 M citrate buffers at pH 7.0 and different temperatures are shown in Figure 4. The corresponding thermodynamic parameters obtained from the van't Hoff plots of $\ln K_{11}^x$ and $\ln S_o^x$ against 1/T are listed in Table 3. At pH 7.0, where Fexo exists as a Zwitterion, its solubility is impeded both by enthalpy ($\Delta H° = 8.3$ kJ/mol) and entropy ($\Delta S° = -64.5$ J/mol.K) changes.

The 1:1 complex formation appears to be driven both by enthalpy ($\Delta H^o = -23.2$ kJ/mol) and entropy ($\Delta S° = 15.2$ J/mol.K) changes for Zwitterion Fexo/β-CD complextaion at pH 7.0, which is mainly attributed to Van der Waals interactions and solvent disordering (Ventura et al., 2005).

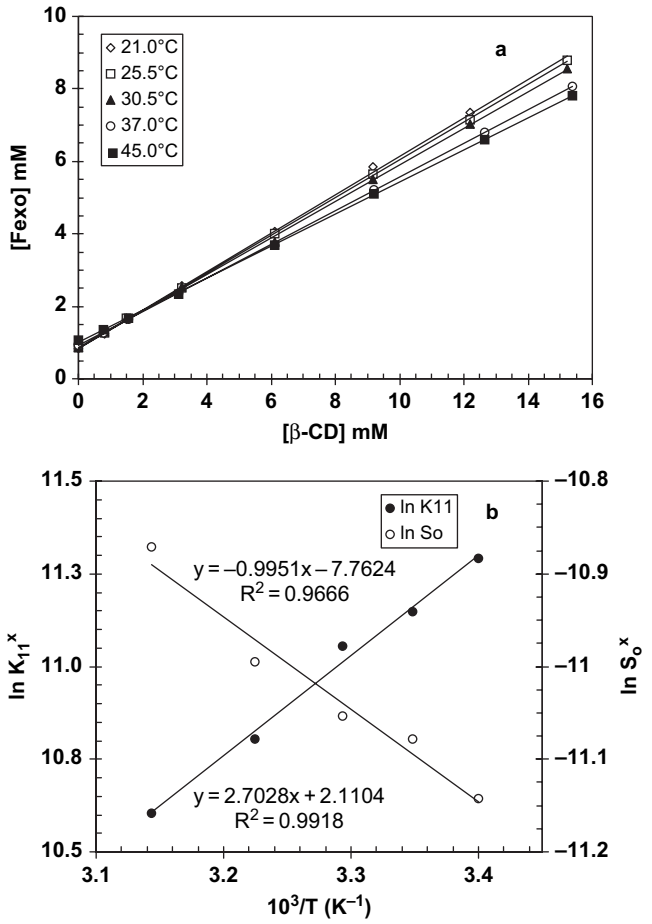


FIGURE 4. (a) PSDs of the Fexo/β-CD system in 0.05 M Citrate buffer at pH 7.0 and different temperatures and (b) van't Hoff plots of $\ln K_{11}^x$ and $\ln S_o^x$ against 1/T (*x* Denotes the mole fraction standard state).

Differential Scanning Calorimetry (DSC)

Fexo.HCl has an endothermic peak at about 210°C, which disappeared in freeze-dried sample (DSC thermogram is not shown) indicating its transformation to an amorphos state (Kumar et. al., 2000). Since the disappearance of Fexo.HCl peak was also observed in both the physical mixture and the complex, this indicates that the DSC technique cannot prove inclusion complex formation in freeze-dried samples. However, by the evaporation method (Figure 5), the Fexo.HCl endothermic peak at 210°C disappeared while new peaks appeared at about 90 and 145°C, indicating the formation of a pseudomorph (Henton & Mccarty, 1995). These two peaks were retained in the physical mixture of Fexo.HCl and β-CD, but disappeared in the thermogram of the complex prepared by evaporation indicating that Fexo.HCl forms an inclusion complex with β-CD.

TABLE 3
Estimates of Complex Formation Constants (K_{11}) of the Fexo/β-CD System Obtained in 0.05 M Citrate Buffer at pH 7.0 and Different Temperatures, and the Thermodynamic Parameters Corresponding to Inherent Fexo Solubility (S_o Obtained in the Absence of β-CD) and Complex Formation Constant (K_{11}) Obtained from van't Hoff Plots

T (°C)	S_o (mM)	K_{11} (M^{-1})	
21.0	0.80	1444	
25.5	0.85	1249	
30.5	0.88	1139	
37.0	0.93	888	
45.0	1.00	726	
	ΔG^o (kJ/mol)	**ΔH^o (kJ/mol)**	**ΔS^o (J/K.mol)**
Solubility of Fexo (S_o)	27.5	8.3	–64.5
Fexo/β-CD complex	–27.7	–23.2	15.2

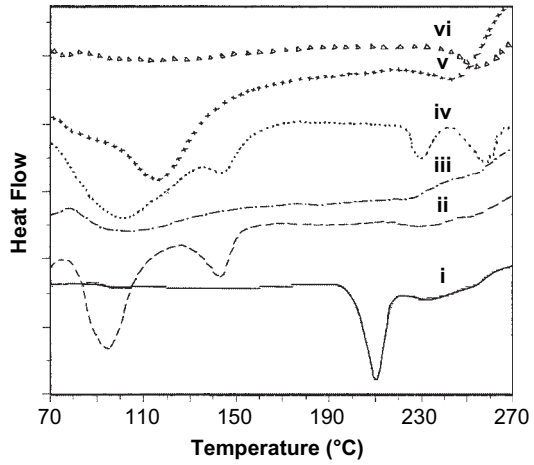


FIGURE 5. DSC thermograms of Fexo.HCl, β-CD, a physical mixture of Fexo.HCl and β-CD, and Fexo/β-CD complex obtained through evaporation under vacuum. In the thermograms, the roman numerals refer to (i) untreated Fexo.HCl, (ii) evaporated Fexo.HCl, (iii) evaporated β-CD, (iv) A physical mixture of evaporated Fexo.HCl and evaporated β-CD, (v) Fexo.HCl/β-CD complex and (vi) Fexo.HCl/β-CD complex dried at 110°C.

The appearance of a new peak at about 230°C in the physical mixture indicates partial transformation of the pseudomorph to another polymorph; this peak did not appear in the thermogram of the evaporated Fexo.HCl, nor did it appear in that of the complex even after it was kept drying for 1 h at 110°C.

X-Ray Powder Diffractometry (XRPD)

The XRPD patterns of Fexo.HCl, β-CD, physical mixtures, and complexes, which were prepared by evaporation method, are presented in Figure 6. The diffraction pattern of Fexo.HCl/β-CD complexes prepared by evaporation method were devoid of the diffraction peaks apparent in their corresponding physical mixtures, thus indicating inclusion complex formation (Figure 6a). It was also observed that the physical mixture prepared from freeze-dried Fexo.HCl and freeze-dried β-CD

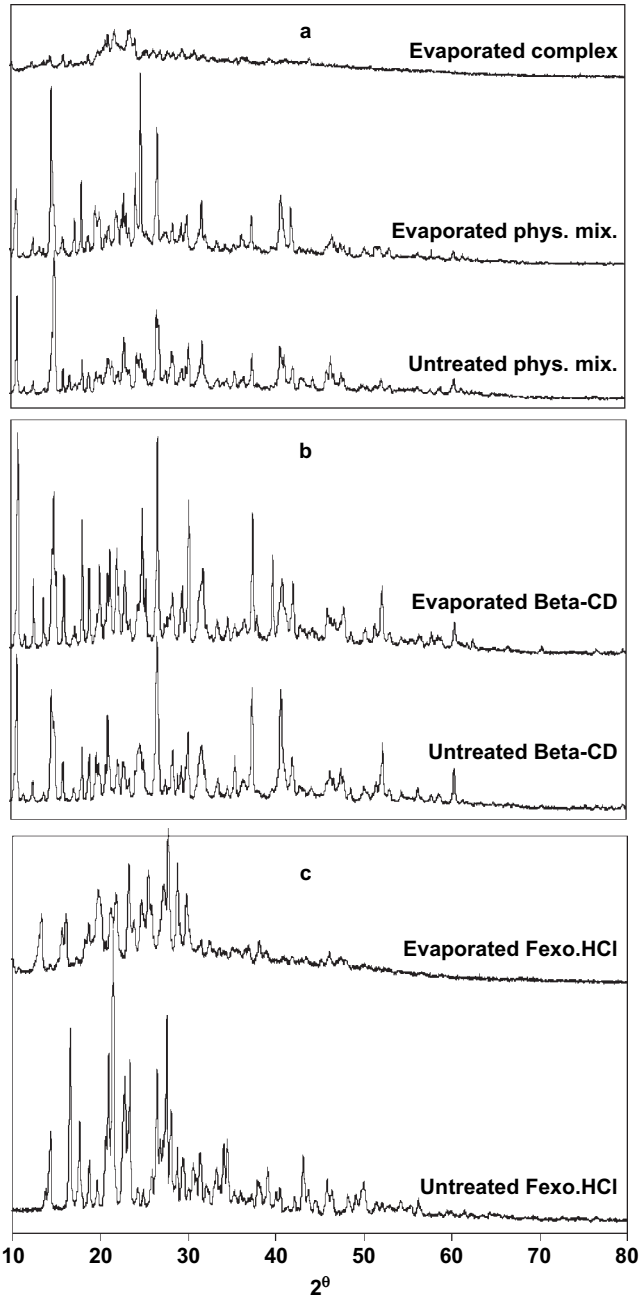


FIGURE 6. XRPD patterns of: (a) Evaporated Fexo.HCl/β-CD complexes and physical mixtures of untreated and evaporated Fexo.HCl and β-CD components, in addition to those of untreated and evaporated β-CD (b) and Fexo.HCl (c).

exhibit low intensity peaks compared with those prepared by evaporation, and with those of untreated components, which clearly suggest that Fexo.HCl was transformed into its amorphos state while β-CD became less crystalline by freeze-drying method (DSC thermograms are not shown). On the other hand, both Fexo.HCl and β-CD remained crystalline following evaporation; this crystallinty was also retained in their physical mixture following evaporation (Figures 6b and 6c).

Proton Nuclear Magnetic Resonance (^{1}H-NMR)

The ^{1}H-NMR spectra of Fexo.HCl, β-CD, and Fexo.HCl/β-CD complex are shown in Figure 7, while their corresponding proton assignments are listed in Table 4. Inspection of the table shows that the upfield chemical shift displacement (Δδ) on complexation is highest for the inner-cavity β-CD protons H_3 (–0.174 ppm) and H_5 (–0.111 ppm). This indicates deep penetration of Fexo into the β-CD cavity (Connors, 1997), whereas protons H_1, H_4, and $H_{6,6}$ exhibit relatively lower though significant upfield Δδ (–0.055 to –0.064 ppm) upon complexation, and proton H_2 demonstrates the least Δδ (–0.011 ppm).

As to Fexo, all protons of the carboxypropyl phenyl group, the hydrocarbon chain (C_IH through $C_{IV}H_2$) and the piperidine group V, and VI, show sizeable downfield Δδ values on complexation ranging from 0.046 to 0.139 ppm. Only proton VII showed a lower upfield Δδ of 0.015 ppm indicating low interaction with β-CD. On the other hand, aromatic protons of the carboxypropylphenyl group are shielded demonstrating upfield Δδ of –0.034 and –0.050 ppm for protons a and b, respectively. In contrast, those of the diphenyl carbinol moiety exhibit both upfield and downfield Δδ. For example, the four protons labeled c whose resonance occurs at 7.550 ppm in Fexo.HCl split into two resonances of equal intensity at 7.654 and 7.671 ppm in the Fexo.HCl/β-CD complex, which indicates that two sets of protons c are subjected to different microenvironments on complexation with β-CD. On the other hand, protons d experiences a relatively slight Δδ (–0.019 ppm), while protons e is not significantly affected upon complexation, aside from a slight splitting. Whether protons d split on complexation could not be recognized due to strong overlap with protons b in the complex. The fact that protons c undergo a more significant downfield Δδ suggests a more pronounced proximity to a deshielding environment than protons d and e, which may result from proximity to the extensive hydroxyl group network present on either side of the β-CD cavity.

Molecular Modeling and Optimal Complex Configurations

The most probable 1:1 Fexo/β-CD complex configurations obtained from MM^+ energy minimization are depicted in Figures 8a and 8b. The binding energies indicate that inclusion of the piperidine moiety (Figure 8a: $E_{binding}$ = –36.9 kcal/mol) offers the most stable complex structure obtained via the

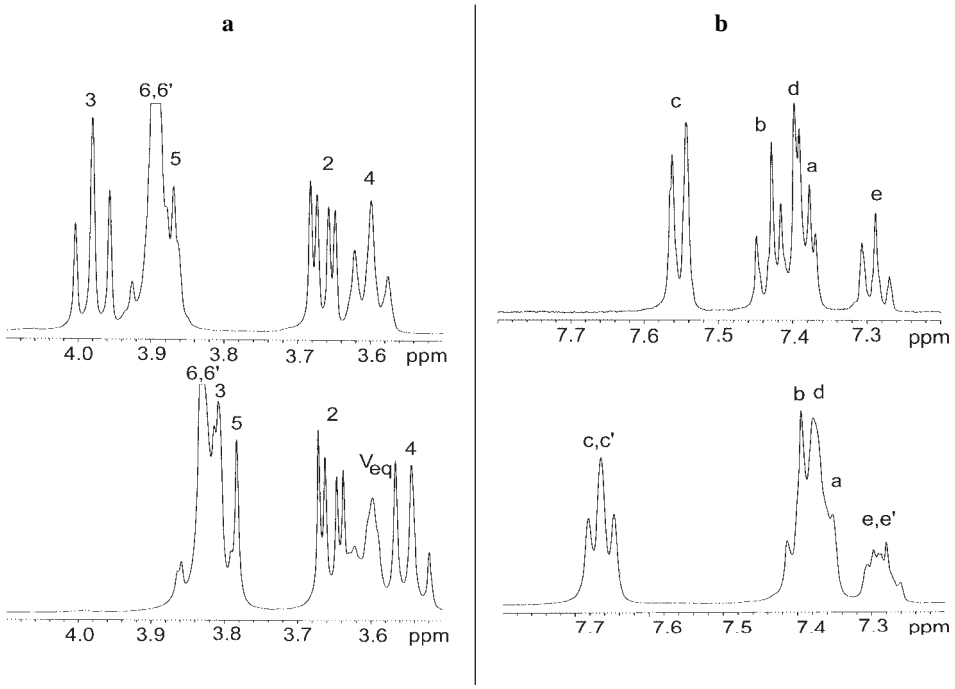
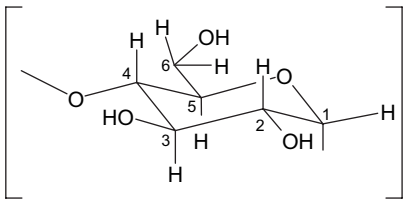
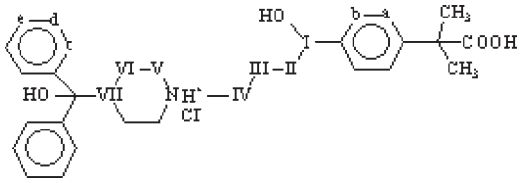


FIGURE 7. 400 MHz ^{1}HNMR spectra of the Fexo.HCl/β-CD system in D_2O at 25°C (a) for protons of β-CD and (b) for aromatic protons of Fexo.HCl. The upper and lower traces correspond to the compound before and after complexation, respectively.

TABLE 4
The 400 MHz HNMR Chemical Shifts in ppm (δ) of β-CD, Fexo.HCl and the Fexo.HCl/β-CD Complex and the Corresponding Chemical Shift Displacements (Δδ) Obtained in D_2O at 25°C (n_H Denotes the Number of Protons)



β-**CD** ($n = 7$)



Fexo.HCl

Assignment	n_H	Multiplicity	δ_{ppm}	$\delta_{Complex}$	$\Delta\delta_{ppm}$
β-CD					
H_4	7	triplet	3.598	3.543	–0.055
H_2	7	quartet	3.665	3.654	–0.011
H_5	7	triplet	3.893	3.782	–0.111
$H_{6,6}$'	14	multiplet	3.893	3.829	–0.064
H_3	7	triplet	3.980	3.806	–0.174
H_1	7	doublet	5.086	5.029	–0.057
Fexo.HCl					
$(CH_3)_2C$-	6	singlet	1.527	1.627	0.100
III	2	multiplet	1.659	1.770	0.111
II, VI	6	multiplet	1.797	1.843	0.046
IV	2	triplet	2.970	3.109	0.139
VII	1	multiplet	2.956	2.971	0.015
V_{ax}	2	multiplet	3.072	3.189	0.117
V_{eq}	2	multiplet	3.498	3.597	0.099
I	1	masked by HOD resonance at 4.7 ppm			
e	2	triplet	7.287	7.278	–0.009
e'		triplet	7.287	7.296	0.009
a	2	doublet	7.393	7.359	–0.034
d	4	triplet	7.394	7.375	–0.019
b	2	doublet	7.452	7.402	–0.050
c	4	doublet	7.550	7.654	0.104
c'		doublet	7.550	7.671	0.121

carboxypropylphenyl approach through the wide rim of the β-CD cavity. Inclusion of a phenyl group from the diphenylcarbinol approach may occur but with a lower probability (Figure 8b: $E_{binding} = -30.5$ kcal/mol). The most stable 1:2 complex configuration appears to occur via complete inclusion of the piperidine moiety into β-CD cavity and the carboxypropylphenyl moiety into the second both occurring via the carboxypropylphenyl group approach (Figure 8c: $E_{binding} = -63.2$ kcal/mol). Other isomeric 1:2 complex configurations are likely occur but somewhat at a lower probability (Figure 8d: $E_{binding} = -61.6$ kcal/mol). Individual contributions of Van der Waals and electrostatic forces to the binding energies indicate that Van der Waals provides the dominant driving force for 1:1 and 1:2 Fexo/β-CD complex formation.

CONCLUSION

The results of this study on Fexo/CD complexation under different conditions reveal the following: The relatively high K_{11} value for the complexation of Fexo with β-CD indicates the existence of a better geometric fit than with α- or γ-CD cavities. In contrast, the lower K_{11} value for complexation with HP-β-CD

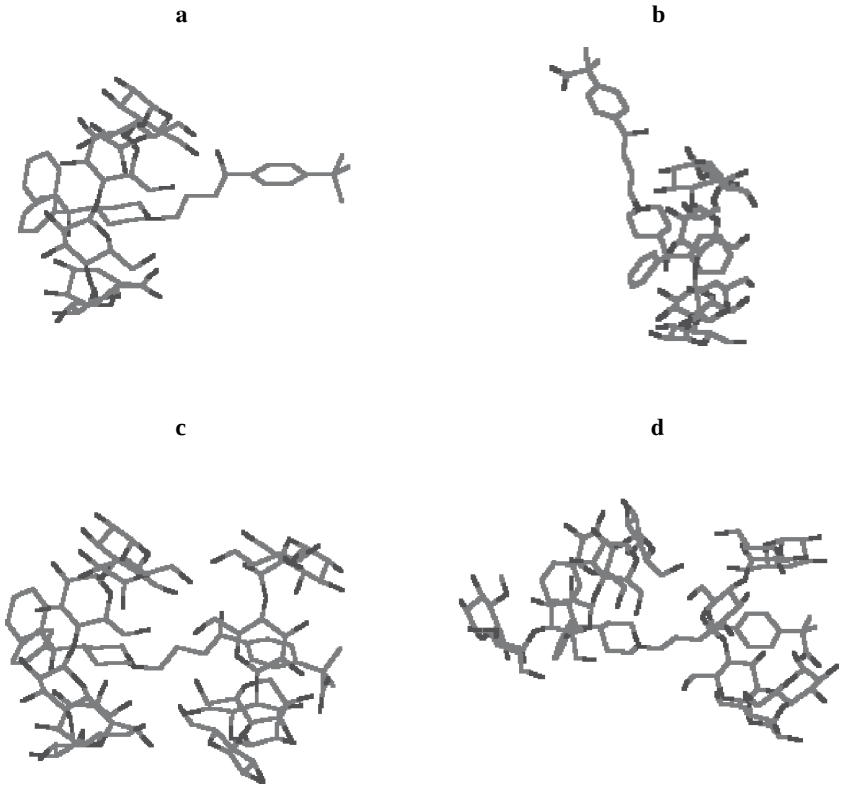


FIGURE 8. Side views of the most probable Fexo/β-CD configurations obtained for the 1:1 (a and b) and 1:2 complexes (c and d).

is likely due to the presence of hydroxypropyl groups in HP-β-CD, which may hamper inclusion of the relatively large and flexible guest molecules (Fexo) due to steric hindrance. Both protonated and Zwitterionic Fexo have a tendency to complex with β-CD. The hydrophobic character of Fexo contributes 76% of the total driving force for Fexo/β-CD complex formation, while other factors including specific interactions contribute about –7.7 kJ/mol. Complex formation of Zwitterionic Fexo with β-CD ($\Delta G° = –27.7$ kJ/mol) is driven both by favorable enthalpy ($\Delta H° = –23.2$ kJ/mol) and entropy ($\Delta S° = 15.2$ J/mol.K) changes.

All the data obtained from the PSDs, DSC, XRPD, ^{1}HNMR, and MM^+ indicate the formation of inclusion complex between Fexo and β-CD in solution and the solid state.

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