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ORIGINAL ARTICLE

Inclusion complexation of diclofenac with natural and modified cyclodextrins explored through phase solubility, ^{1}H-NMR and molecular modeling studies

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Abstract Guest–host interactions were examined for neutral diclofenac (Diclo) and Diclofenac sodium (Diclo sodium) with each of the cyclodextrin (CD) derivatives: α-CD, β-CD, γ-CD and 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), all in 0.05 M aqueous phosphate buffer solution adjusted to 0.2 M ionic strength with NaCl at 20 °C, and with β-CD at different pHs and temperatures. The pH solubility profiles were measured to obtain the acid–base ionization constants (pK_as) for Diclo in the presence and absence of β-CD. Phase solubility diagrams (PSDs) were also measured and analyzed through rigorous procedures to obtain estimates of the complex formation constants for Diclo/CD and Diclo sodium/CD complexation in aqueous solutions. The results indicate that both Diclo and Diclo sodium form soluble 1:1 complexes with α-, β-, and HP-β-CD. In contrast, Diclo forms soluble 1:1 Diclo/γ-CD complexes, while Diclo sodium forms 1:1 and 2:1 Diclo/γ-CD, but the 1:1 complex saturates at 5.8 mM γ-CD with a solubility product constant (pK_{sp} = 5.5). Therefore, though overall complex stabilities were found to follow the decreasing order: γ-CD > HP-β-CD > β-CD > α-CD, some complex precipitation problems may be faced with aqueous formulations of Diclo sodium with γ-CD, where the overall concentration of the latter exceeds 5.8 mM γ-CD. Both ^{1}H-NMR spectroscopic and molecular mechanical modeling (MM^+) studies of Diclo/β-CD indicate the possible formation of soluble isomeric 1:1 complexes in water.

Keywords Complexation · Cyclodextrin · Diclofenac · ^{1}H-NMR · Molecular modeling · Phase solubility diagrams · pH-Solubility profiles · pK_a

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Introduction

Cyclodextrins (CDs) are torus shaped cyclic oligosaccharides consisting of (α-1,4)-linked α-D-glucopyranose units having a hydrophobic cavities and hydrophilic exterior surfaces. Natural CDs having 6, 7, and 8 glucopyranose units are called α-CD, β-CD and γ-CD, respectively, while HP-β-CD is the 2-hydroxypropyl derivative of β-CD. Inclusion complex formation of water-insoluble drugs with CDs have been used to enhance drug solubility and bioavailability [1, 2]. Diclofenac, 2-(2′,6′-dichloroanilino)phenylacetic acid, (Diclo) is an acidic non-steroidal anti-inflammatory drug whose neutral form is practically insoluble in water but is freely soluble in ethanol, methanol and in basic media. Anionic Diclo (Diclo sodium) is soluble in water at pHs exceeding 7. Both Diclo and Diclo sodium were reported to form soluble complexes with α-CD, β-CD, γ-CD and HP-β-CD in aqueous solution [3–13].

The aqueous 1:1 Diclo/CD complex formation constants (K_{11}) reported in the literature for neutral Diclo are: 1200 M^{-1} at pH 3.5 for α-CD [3]; 100.6 M^{-1} in

simulated gastric juice at pH 1.2 [4], ~1000 M^{-1} [5] at pH 1.2, 1500 M^{-1} at pH 2.0 [6], 331 M^{-1} at pH 3.0 [7] for β-CD; and 115.8 M^{-1} in simulated gastric juice for HP-β-CD) [4].

The K_{11} values reported for anionic 1:1 Diclo sodium/CD are: 1100 M^{-1} in water for α-CD [3]; 20 M^{-1} at pH 6.5 [6], 159 M^{-1} at pH 7.0 [7], 88.8 M^{-1} and 161 M^{-1} (isomeric 1:1 complexes) in water [8], 295 M^{-1} in water [9], ~1000 M^{-1} in water [5], 3100 M^{-1} in water [10], and 76 M^{-1} at pH 8.4 [11] for β-CD. It has been suggested that the soluble Diclo/HP-β-CD and Diclo sodium/HP-β-CD complexes are both of 1:1 and 1:2 stoichiometry [4]. In contrast, Diclo sodium was reported to form soluble 1:1 and 2:1 Diclo sodium/CD complexes with β-CD (K_{11} = 170 M^{-1}, K_{21}, = 22 M^{-1}), and also with HP-β-CD (K_{11} = 310 M^{-1}, K_{21} = 50 M^{-1}) [12]. In the presence of (0.1% mass/v) hydroxypropylmethyl cellulose (K4M) in simulated gastric juice, K_{11} values for neutral Diclo/β-CD were reported to increase from 100.6 in the absence of K4M to 330.46 M^{-1} in its presence, while that of Diclo/HP-β-CD increases from 115.6 to 392.03 M^{-1}, respectively [13]. Two pharmaceutical formulations involving Diclo sodium/γ-CD inclusion complexes have already been patented: one was for an oral dosage form assuming 1:2 Diclo sodium/γ-CD stoichiometry [14], while the other was for eye drops formulation [15].

The variation in K_{11} values reported so far for the Diclo/CD and Diclo sodium/CD complexes (Table 1) is obviously due to problems associated with the use of different media, in addition to methods of data analysis associated with the different experimental techniques used.

This work reports the results of an investigation of the complexation of Diclo and Diclo sodium, with each of α-CD, β-CD, γ-CD and HP-β-CD, under controlled conditions of buffer concentration, ionic strength, pH, and using the same experimental technique. This, coupled with rigorous nonlinear regression analysis of phase solubility diagrams (PSDs), allows obtaining more accurate complex formation constants and thus a more meaningful comparison between the complexation trends of different CDs used under same conditions. Estimates of the standard thermodynamics functions of Diclo sodium/β-CD are also reported, in addition to the results of ^{1}H-NMR spectroscopic and molecular modeling studies to explore possible sites of guest–host interactions.

Table 1 Complex stoichiometry and complex formation constants (K_{11} and K_{21}) that have been reported in the literature for Diclo/CD and Diclo sodium/CD complexation at different pHs (pK_a of acidic Diclo is 4.31)

Drug species	CD	pH (T°C)[a]	Stoichiometry	K_{11}	Technique	Ref.[b]
Diclo	α-CD	3.5	1:1	1200	Spectrofluorimetry	3
Diclo	β-CD	1.2	1:1, 1:2[c]	100.6	^{1}H-NMR, PSD	4
Diclo	β-CD	2.0	1:1	1500	PSD[d]	6
Diclo	β-CD	3.0 (25 °C)	1:1	331	PSD[d]	7
Diclo	β-CD	4.0	1:1	670	PSD[d]	6
Diclo	β-CD	Acidic	1:1	1000	Spectrofluorimetry	5
Diclo	HP-β-CD	1.2	1:1, 1:2[c]	115.8	^{1}H-NMR, PSD[d]	3
Diclo.Na	α-CD	Water	1:1	1100	Spectrofluorimetry	3
Diclo.Na	β-CD	5.5 (25 °C)	1:1	214	PSD 2005	7
Diclo.Na	β-CD	6.5	1:1	20	PSD[d]	6
Diclo.Na	β-CD	7.0 (25 °C)	1:1	159	PSD[d]	7
Diclo.Na	β-CD	Water (25 °C)	1:1	295	PSD[d]	9
Diclo.Na	β-CD	Water	1:1[e]	250[e]	^{1}H-NMR	8
Diclo.Na	β-CD	Water	1:1	1000	Spectrofluorimetry	5
Diclo.Na	β-CD	Water	1:1	3100	Ion-Sel.El.	10
Diclo.Na	β-CD	Water	1:1, 2:1[g]	170, 22[g]	^{1}H- & ^{13}C-NMR	12
Diclo.Na	β-CD	8.4 (25 °C)	1:1	76	AFC[f]	11
Diclo.Na	HP-β-CD	Water	1:1, 2:1[h]	310, 50[h]	^{1}H- & ^{13}C-NMR	12

[a] The temperature is only indicated wherever it was reported; otherwise it may be assumed to be room temperature

[b] Ref. stands for the reference number

[c] 1:2 Diclo/β-CD stoichiometry was suggested but only K_{11} value was reported

[d] PSD stands for phase solubility diagrams

[e] Isomeric 1:1 Diclo sodium/β-CD complexes with K_{11} = 88.8 M^{-1} and K'_{11} = 161 M^{-1}

[f] AFC stands for Affinity Capillary Electophoresis

[g] 2:1 Diclo sodium/β-CD complex were suggested (K_{11} = 170 M^{-1}, K_{21} = 22 M^{-1})

[h] 2:1 Diclo sodium/HP-β-CD complex were suggested (K_{11} = 310 M^{-1}, K_{21} = 50 M^{-1})

Materials and methods

Materials

Diclo was of pharmaceutical grade. β-CD and HP-β-CD were obtained from AVEBE while α-CD and γ-CD were obtained form Cerestar. All other chemicals were of analytical grade, and water was double distilled and deionized.

Methods

Instrumentation

Thermostatic water bath shaker (GFL 1083), UV/Visible spectrophotometer (Beckman Du 650i), pH meter (Jenway 3030), 400 MHz NMR spectrometer (GSK 400 JEOL).

pH-solubility profiles

Equal amounts of Diclo in excess of its inherent solubility were added to 50 mL of 0.05 M phosphate buffer solutions adjusted to 0.2 M ionic strength with NaCl at different pHs and 20 °C. The solutions were thermostatically shaken, let to settle, filtered and the absorbance measured. The concentration of each solution was determined spectrophotometrically using appropriate calibration curves at 275 nm. Estimates of the pK_a values were obtained using rigorous procedures discussed earlier [16].

Phase solubility diagrams

Phase solubility studies (PSDs) were performed as described by Higuchi and Connors [17]. PSDs were obtained at 20 °C where excess amounts of Diclo were added to flasks containing 50.0 mL 0.05 M phosphate buffer solutions adjusted to 0.2 M ionic strength with NaCl having different CD concentrations of each of α-CD, β-CD, γ-CD and HP-β-CD. The solutions were thermostatically shaken, let to settle, filtered and the absorbance measured. The concentration of each solution was determined spectrophotometrically against appropriate calibration curves at 275 nm. PSDs were also measured for Diclo/β-CD at pH 2.4 and different temperatures. Analysis of the PSDs to obtain estimates of the complex formation constants, corresponding to the measured A- and B_s-type PSDs [17], was carried out utilizing rigorous nonlinear regression procedures discussed earlier [18–20].

^{1}H-Nuclear magnetic resonance

An appropriate weight of Diclo calculated from the PSD at pH 6.5 was added to a solution of β-CD in D_2O. Chemical shifts are quoted relative to sodium 3-trimethylsilyl [2H4] propionate at 0.0 ppm but spectra were calibrated via the known position of the residual HOD resonance. ^{1}H-NMR spectra were obtained at 400 MHz and 25 °C on a JOEL GSX400 spectrometer fitted with a 5-mm probe.

Molecular modeling

Guest–host interactions were computationally simulated in vacuo for Diclo/β-CD complexation using the MM+ force field of the Hyperchem® molecular modeling software (release 6.03 professional, Hypercube Inc., Waterloo, Canada). Energy minimization was performed using the conjugate gradient algorithm (0.01 kcal/mol Å gradient). The initial molecular geometry of β-CD was obtained using X-ray diffraction data [21–24], and this was followed by energy optimization while imposing a restraint on the dihedral angles to the average values [23]. Diclo was built up from standard bond lengths and bond angles, and was further optimized with the MM+ force field.

The previously optimized structures of Diclo and β-CD molecules were allowed to approach each other along the symmetric x-axis passing through the center of the β-CD cavity. The phenylacetic acid and the dichlorophenyl sides of Diclo were allowed each to approach through both the wide and narrow rims of β-CD cavity. The energy of Diclo was initially computed (while β-CD was fixed) at 1 Å intervals, starting at x = –20 Å all through x = +20 Å from the origin of the Cartesian coordinate, which was designated by the center of the ether glucoside oxygen atoms of β-CD and an atom closest to the center of mass of the Diclo molecule. The binding energy ($E_{binding} = E_{complex} - \sum E_{components}$) was plotted against x for each longitudinal approach to indicate the energy minima. The whole Diclo/β-CD system of minimum energy thus obtained was allowed to interact, free of restrictions to either molecule, in order to arrive at the optimized 1:1 complex configuration. Attempts to obtain optimized Diclo/β-CD geometries in a water box of periodic boundary conditions proved to be computationally rather prohibitive, and were not pursued any further.

Results and discussion

pH solubility profiles and pK_a values

Figure 1 depicts of the pH-solubility profiles of Diclo in the absence and presence of 7.6 mM β-CD at 20 °C. Rigorous nonlinear regression analysis of the pH-solubility profiles [17] yielded the pK_a values listed in Table 2. The pK_a for acidic Diclo was estimated at 4.31, while that of the corresponding Diclo/β-CD complex is 4.50. The positive shift in the pK_a value on complexation which is given by $\Delta pK_a = pK_{a\ Complex} - pK_{a\ Diclo}$ is +0.19. This clearly indicates a lowering in acidity of Diclo on complexation, thus suggesting partial or complete inclusion of Diclo into the β-CD cavity.

The pK_a values of Diclo that have been reported in the literature are also listed in Table 2, which are 4.84 [5] and 4.18 [7]. We believe that the value of pK_a = 4.5 obtained in this work is more accurate, especially since it was determined for buffered aqueous solutions of Diclo and not mixed solvents.

Phase solubility diagrams (PSDs)

The PSDs of Diclo and Diclo sodium obtained for each of α-, β-, γ- and HP-β-CD in aqueous 0.05 M buffer solutions, adjusted to 0.2 M ionic strength with NaCl at 20 °C, are depicted in Fig. 2 (pH 2.4 for Diclo and pH 6.5 for Diclo sodium). The PSDs are all of the linear (A_L-type) indicating the formation of soluble complexes except for Diclo sodium/γ-CD, which belongs to the B_s-type (PSD with a descending portion), according to the classification of PSDs reported by Higuchi and Connors [17].

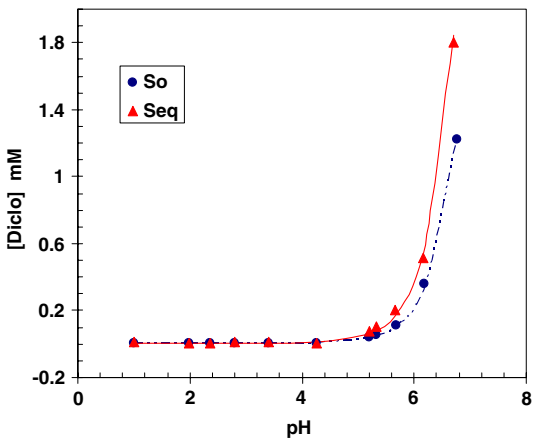


Fig. 1 pH-solubility profiles of Diclo in the absence and presence of β-CD (7.6 Mm) obtained in 0.05 M phosphate buffer adjusted to 0.2 M ionic strength with NaCl at 20 °C

Table 2 Estimates of pK_a values for Diclo and Diclo/β-CD complex obtained from pH-solubility profiles obtained for 0.05 M phosphate buffer adjusted to 0.2 M ionic strength with NaCl at 20 °C and shown in Fig. 1. ($\Delta pK_a = pK_{a\ Complex} - pK_{a\ Diclo}$)

$pK_{a\ Diclo}$	$pK_{a\ complex}$	ΔpK_a	Reference
4.0*	–	–	*
4.18	–	–	7
4.84	4.90	0.06	5
4.31	4.50	0.19	This work

* Merck Index, Twelfth edition

Estimates of the complex formation constants, which were obtained using rigorous procedures [18–20], are listed in Table 3. The results indicate that both Diclo and Diclo sodium form soluble complexes of 1:1 stoichiometry with each of α-, β- and HP-β-CD, with complex stabilities following the decreasing order: HP-β-CD > β-CD > α-CD. The presence of the peripheral and more flexible hydroxypropy groups in HP-β-CD seem to facilitate a better accommodation of the phenyl acetic acid and the more bulky dichlorophenyl moieties of Diclo than the more rigid β-CD, which can only accommodate the less bulky phenyl acetic acid moiety. On the other hand, the small α-CD cavity can only affect partial and less effective inclusion of either moieties, and hence exhibits the lowest complexation

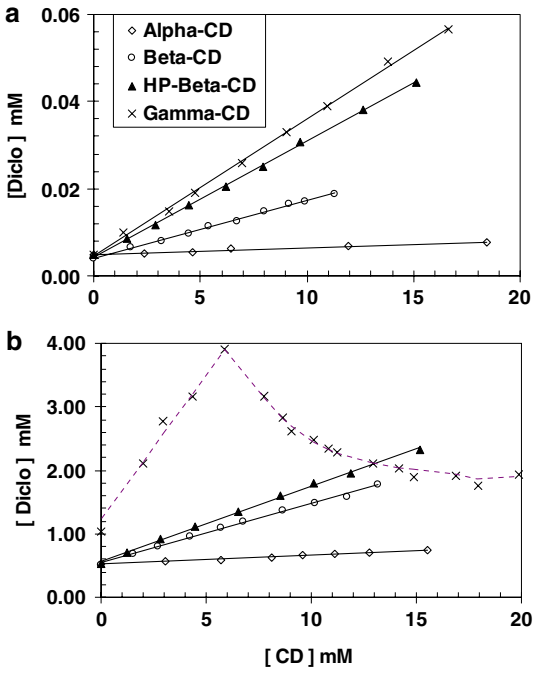


Fig. 2 PSDs of (a) Diclo (pH 2.4) and (b) Diclo sodium (pH 6.5) obtained for α-, β-, γ- and HP-β-CD in aqueous 0.05 M phosphate buffer solutions adjusted to 0.2 M ionic strength with NaCl at 20 °C

Table 3 Diclo/CD and Diclo sodium/CD complex formation constants for α-, β-, γ- and HP-β-CD estimated from PSDs (Fig. 2) for aqueous 0.05 M phosphate buffer solutions adjusted to 0.2 M ionic strength with NaCl, at pHs 2.4 and 6.5, respectively, all at 20 °C

CD	S_o (mM)	K_{11} (M^{-1})	K_{21} (M^{-1})	PSD type
Complex formation constants at pH 2.4				
α-CD	0.00486	32	–	A_L
β-CD	0.00410	336	–	A_L
HP-β-CD	0.00486	620	–	A_L
γ-CD	0.00486	515	3	A_L
Complex formation constants at pH 6.5				
α-CD	0.517	28	–	A_L
β-CD	0.509	190	–	A_L
HP-β-CD	0.517	237	–	A_L
γ-CD	1.042	715	110	B_S

A_L stands for a linear PSD while B_S stands for PSDs with a descending portion according to Higuch and Connors [16]

tendency towards Diclo and Diclo sodium among all CDs examined in this work (Fig. 2, Table 3).

It is also apparent from Table 3 that the K_{11} values are higher for neutral Diclo/CDs than for Diclo sodium/CDs under the same conditions. This trend reflects the fact that Diclo, which exhibits a much lower inherent solubility in water (S_o = 0.005 mM), is more hydrophobic than Diclo sodium (S_o = 0.51 mM at pH 6.5); therefore, neutral Diclo tends to escape from water bulk to include into the hydrophobic CD cavity more than the less hydrophobic Diclo sodium.

We believe that the K_{11} values estimated in this work (Table 3) provide an accurate basis for comparing the complexation tendencies of Diclo and Diclo sodium with CDs because the PSDs were measured under controlled conditions of same buffer species, buffer concentration, ionic strength, pH and temperature. The variability of K_{11} values reported in the literature (Table 1) clearly stem from the variability of aqueous media used, pH, ionic strength, in addition to possible problems with the methods of data analysis used to estimate K_{11} values for the different experimental techniques. This is more succinctly evident, for example, in the reported K_{11} values for Diclo sodium/β-CD in water and at the neutral pHs 6.5 and 7.0 (Table 1), which are: 20, 159, 295, 250, 1000, and 3100 M^{-1} [5–10]. Some workers have suggested the formation of soluble isomeric 1:1 Diclo sodium/β-CD complexes (e.g., bimodal complexes with K_{11} = 250 mM) [8] while, in addition to that, others suggested a probable 1:2 Diclo sodium/β-CD [25]. We have found that the probability for 1:2 complex formation is essentially zero, to within experimental error. Yet formation of isomeric 1:1 Diclo sodium/β-CD complexes cannot be excluded and has been successfully accounted for earlier [8].

Based on ^{1}H- and ^{13}C-NMR studies, it was also suggested that Diclo sodium forms 1:1 (possibly isomeric) and 2:1 Diclo sodium/CD complexes with both β-CD and HP-β-CD, and that the complex stabilities were higher for HP-β-CD (K_{11} = 310 M^{-1}, K_{21} = 50 M^{-1}) than for β-CD (K_{11} = 170 M^{-1}, K_{21} = 22 M^{-1}) at pH 6.5 [13, 26]. The results of this work do confirm that γ-CD forms 1:1 and 2:1 Diclo sodium/γ-CD complexes, but the 1:1 Diclo sodium/γ-CD complex reaches saturation and begins to precipitate above 5.8 mM γ-CD, with a solubility product constant pK_{sp} = 5.5 (the solid Diclo sodium/γ-CD complex was isolated and analyzed confirming 1:1 complex stoichiometry). This clearly indicates a problem with aqueous pharmaceutical formulations involving Diclo sodium/γ-CD where the overall concentration of γ-CD exceeds 5.8 mM. All other complexes indicated in Table 3 are soluble (A_L-type PSDs), where formation of isomeric 1:1 Diclo/CD and Diclo sodium/CD complexes are predominant over higher order complexes.

Thermodynamics

The thermodynamic functions corresponding to Diclo inherent solubility (S_o) and Diclo/β-CD complex formation were obtained from van't Hoff plots of ln S_o and ln K_{11} against $1/T$. The molar S_o and K_{11} values were estimated from PSDs measured at 10, 15, 20, 25, and 30 °C, and were converted to the mole fraction standard state. The results are listed in Table 4, which shows that complex formation is driven by favorable enthalpy (ΔH^o_{11} = –11 kJ/mol) and favorable entropy (ΔH^o = 44 J/mol.K) changes. In contrast, Diclo inherent solubility is hampered by unfavorable enthalpy (ΔH^o_{So} = 28 kJ/mol) and unfavorable entropy (ΔH^o = –21 J/mol.K) changes.

^{1}H-Nuclear magnetic resonance (^{1}H-NMR)

The 400 MHz ^{1}H-NMR chemical shifts (ppm) of Diclo sodium (δ_{Diclo}), β-CD ($\delta\beta_{-CD}$), and those of the corresponding Diclo sodium/β-CD and Diclo sodium/γ-CD complexes obtained in D_2O at 25 °C are listed in

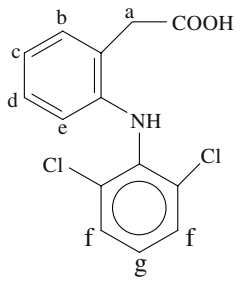
Table 4 Standard thermodynamic quantities corresponding to the inherent solubility of Diclo (S_o) and 1:1 Diclo/β-CD complex formation constant (K_{11}) at pH = 2.4

Parameter	For S_o	For K_{11}
ΔH^o (kJ/mol)	28	–11
ΔG^o (kJ/mol)	34	–24
ΔS^o (J/mol.K)	–21	44
S^o_o (mM)	0.0523	
K^o_{11} (M^{-1})	287	

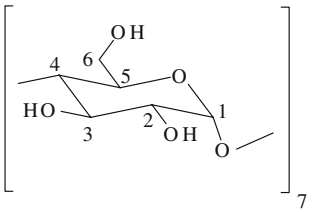
Table 5. The chemical shift displacements on complexation ($\Delta\delta_{ppm}$) are also listed in Table 5. The more significant upfield $\Delta\delta_{ppm}$ values exhibited by the inner CD cavity protons (H_3 and H_5) of β-CD and γ-CD clearly indicate inclusion complex formation in both cases. However, the outer cavity protons ($H_{6,6'}$), which protrude out of the narrow rim of the CD cavity, exhibit a significantly higher upfield $\Delta\delta_{ppm}$ value for γ-CD ($\Delta\delta_{ppm}$ = –0.058 ppm) than for β-CD ($\Delta\delta_{ppm}$ = –0.010 ppm). This suggests more probable inclusion of Diclo sodium into moth narrow and wide rims of the γ-CD cavity. Moreover, the chemical shift of the two equivalent Diclo sodium protons (a) at 3.679 ppm exhibit downfield $\Delta\delta_{ppm}$ values and split into two different resonances at 3.706 and 3.719 on complextion with γ-CD. This suggests that the two equivalent protons (a) experience different microenvironments within γ-CD, which may be attributed to a more significant restriction of rotational motion than for Diclo sodium/β-CD. The fact that the structure of γ-CD is larger and more flexible than that of the more symmetric and rigid β-CD allows this possible restriction of rotational motion due to more effective inclusion of bulky molecules into γ-CD.

In addition, the phenylacetic acid protons (a, b, c, and d) of Diclo sodium exhibit significant downfield $\Delta\delta_{ppm}$ values on complexation with β-CD, thus indicating effective inclusion of the phenylacetic acid moiety into the β-CD cavity. Moreover, proton (f) of the dichlorophenyl group also undergoes downfield $\Delta\delta_{ppm}$ suggesting a similar yet partial inclusion of the bulky dichlorophenyl, and hence possible formation of

Table 5 400 MHz ^{1}H-NMR chemical shift displacements ($\Delta\delta_{ppm}$) of Diclo/β-CD and Diclo/γ-CD protons on complexation for a mixture of 1.5 mM Diclo and 15 mM CD dissolved in 0.05 M deuterated phosphate buffer in D_2O at pH 6.5 and 25 °C. ($\Delta\delta_{ppm} = \delta_{Complex} - \delta_{pure\ component}$)



Assignment	n_H	δ_{Diclo}	Diclo/β-CD		Diclo/γ-CD	
			$\delta_{Complex}$	$\Delta\delta_{ppm}$	$\delta_{Complex}$	$\Delta\delta_{ppm}$
a	2	3.679	3.752	+0.073	3.706	+0.027
a′					3.719	+0.040
b	1	7.282	7.311	+0.029	7.292	+0.010
c	1	6.987	7.009	+0.022	6.997	+0.010
d	1	7.140	7.186	+0.046	7.101	–0.035
e	1	6.508	6.461	–0.047	6.335	–0.173
f	2	7.507	7.567	+0.060	7.398	–0.109
g	1	7.175	7.166	–0.009	7.121	–0.054



Assignment	Diclo/β-CD			Diclo/γ-CD		
	$\delta_{\beta\text{-CD}}$	$\delta_{Complex}$	$\Delta\delta_{ppm}$	$\delta_{\gamma\text{-CD}}$	$\delta_{Complex}$	$\Delta\delta_{ppm}$
H_2	3.667	3.646	–0.021	3.644	3.633	–0.011
H_3	3.972	3.945	–0.027	3.943	3.906	–0.037
H_4	3.591	3.578	–0.013	3.598	3.574	–0.024
H_5	3.872	3.837	–0.035	3.868	3.774	–0.094
$H_{6,6'}$	3.884	3.874	–0.010	3.880	3.822	–0.058

isomeric 1:1 complexes that has been reported earlier [8, 27]. In contrast, protons (f, and g) of the dichlorophenyl group exhibit very significant upfield $\Delta\delta_{ppm}$ values (–0.109 and –0.054 ppm) on complexation with γ-CD suggesting a different mode of interaction from that of β-CD. These observations added to the splitting of protons (a) indicated in Table 5 may suggest the formation of isomeric 1:1 Diclo sodium/γ-CD complexes which were suggested earlier [26, 27], or a more rigid Diclo sodium/γ-CD configuration of 2:1 stochiometry [28].

Molecular modeling

Molecular mechanical (MM+) modeling simulations of Diclo-β-CD interactions in vacuo were carried out, according to the procedure indicated in the ''Methods'' section above, in order to gain some insight as to the most likely groups of Diclo that may include into the β-CD cavity. Among the three likely groups examined were the carboxymethylene, the phenylacetic acid, the phenyl ring of phenylacetic acid, and the dichlorophenyl groups. From the binding energies of Diclo/β-CD complexes that have been optimized free of restrictions to either molecule, it was found that the most probable complex involves almost complete inclusion of the phenylacetic acid moiety ($E_{binding}$ = –24.5 kcal/mol) that is shown in Fig. 3. The binding energies of other less probable optimized structures were: –22.8 kcal/mol for partial inclusion of the dichlorophenyl moiety, –21.9 kcal/mol for the phenyl ring of phenyl acetic acid, and –19.7 kcal/mol for the carboxymethylene group. The small differences in the binding energies of the four optimized structures indicates that the possible formation of isomeric 1:1 Diclo/β-CD complexes in vacuo. Attempts to simulate 1:1 Diclo/CD interactions for β-CD or γ-CD in a water box of periodic boundary conditions proved computationally prohibitive and were not pursued any further.

Conclusion

The results of this investigation of the complexation of Diclo and Diclo sodium with each of α-, β-, γ- and HP-β-CD in aqueous solution under controlled conditions of buffer concentration, pH, ionic strength and temperature reveal the following: (a) The overall tendency of Diclo and Diclo sodium to form soluble complexes with the different CDs follows the decreasing order: γ-CD > HP-β-CD > β-CD > α-CD;

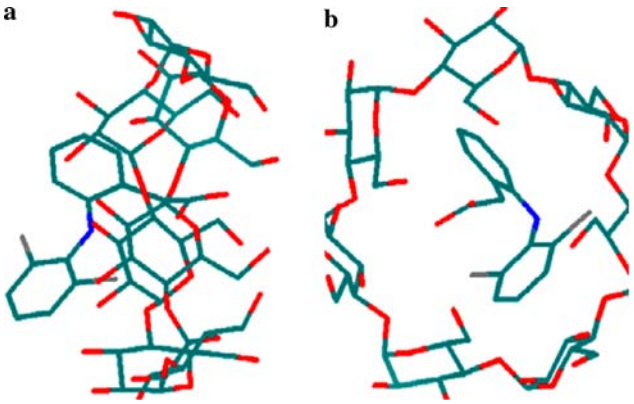


Fig. 3 Optimized geometry of the 1:1 Diclo/β-CD complex showing inclusion of the phenyl acetic acid side of the molecule through the wide rim of β-CD, (**a**) side view of β-CD (**b**) front view across the wide rim of β-CD

(b) However, Diclo sodium was found to form 1:1 and 2:1 Diclo/γ-CD complexes, with the 1:1 complex saturating at 5.8 mM γ-CD and having a solubility product constant (pK_{sp} = 5.5). Therefore, some complex precipitation problems may occur with aqueous formulations of Diclo sodium with γ-CD, where the overall concentration of the latter exceeds 5.8 mM γ-CD; (c) Thermodynamic studies indicate that Diclo/b-CD complex formation is favored by enthalpic and entropic changes; and (d) Both ^{1}H-NMR spectroscopic and molecular mechanical modeling (MM^+) studies of Diclo/β-CD indicate the possible formation of soluble isomeric 1:1 complexes.

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