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Preparation and Characterization of Two Solid Supramolecular Inclusion Complexes of Guaiacol with β- and γ-Cyclodextrin

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Solid inclusion complexes of β- and γ-cyclodextrin (CD) with guaiacol (Gua) were prepared and characterized using elemental analysis, matrix-assisted laser desorption ionization-time of flight mass spectrometry, powder X-ray diffraction, infrared spectroscopy, thermogravimetric analysis and nuclear magnetic resonance spectroscopy. Based on the results of elemental analysis and ^{1}H NMR, the host–guest stoichiometry of the two solid complexes was determined to be one-to-one. X-ray diffraction patterns of the inclusion complexes gave peak locations consistent with intermolecular complexations between CDs and Gua as well as shape and diffraction intensity of these peaks. The solubility of γ-CD was much less than that of α-CD in water through the complex formation of CD with Gua. Upon inclusion the thermal decomposition point of β-CD or γ-CD as host only slightly increased. However, the thermal stability of the complexed Gua was obviously higher in Gua–β-CD than in Gua–γ-CD. The inclusion phenomenon was attributed to the different strength of intermolecular interactions between host and guest. According to ^{1}H NMR titration measurements, binding constants between Gua and CDs were determined. Furthermore, ^{1}H NMR results indicated that the complexed Gua was likely to have stayed in the wider rim of the cavity of β- or γ-CD. PM3 calculations clearly showed that Gua–γ-CD had the most negative interaction energy, whereas Gua was near the secondary hydroxy rim of the cavity of γ-CD.

Cyclodextrins (CDs, Fig. 1) are cyclic oligosaccharides consisting of glucose subunits connected through glycosidic α-1,4 bond, forming a structure as a hollow truncate with one ring wider than the other.[1,2] The most common CDs are α-, β-, and γ-CD, which are composed of six, seven, and eight glucose units, respectively. CDs and their derivatives, such as β-cyclohepta(2,6-di-*O*-methyl)-dextrin (DMβ-CD), have been known to form inclusion complexes with a wide variety of guest molecules via noncovalent bonds.[3–6] Many papers have been published concerning the formation and spectral properties of guest–CD complexes in aqueous solution using various analytical methods.[7–11]

In aqueous solution, phenol and its analogs can be included into the CD cavity.[12,13] There have been a few structural reports on the inclusion complexes of CD with substituted phenols based on experimental measurements in aqueous solution or theoretical calculations in vacuo.[14–17] So far, the inclusion complexes of CDs with many organic guests have been successfully utilized in perfume, pharmaceutical, food supplement industries and other fields.[8,18] How to improve water solubility and thermal stability of aromatic organic guests and to control release of complexed guest molecules are two of the most interesting problems concerning the application of solid CD inclusion complexes in industry.[19–21] It should be noted, however, that less attention is paid to preparation and characterization of solid inclusion complexes of CDs with organic guests, relative to extensive research efforts on CD–guest complexation interactions in solution.[22–24]

Guaiacol (Gua), which has disinfectant properties, is an oil at room temperature due to low melting point. It is not only used as an expectorant but also used as a less expensive synthetic flavor chemical in flavor and fragrance industries. Moreover, as one of the most simple phenol derivatives, Gua is also an important structural unit for use in forming many specific polysubstituted phenols, such as 4-allyl-2-methoxyphenol and 4-hydroxy-3,5-dimethoxybenzoic acid.

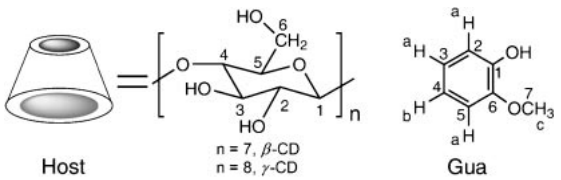

Fig. 1. Structural features of host and guest molecules.

Careful investigations on the formation, structure, character and stability of solid CD supramolecular complexes will not only help us to further realize the nature of inclusion phenomena, but also allow us to evaluate the potential application of CDs in many industries.[8,20] For these reasons, Gua was chosen as a guest, and β- and γ-CD were selected as hosts in this study. Their molecular structures are shown in Fig. 1.

In the present work, we examined the preparations of solid inclusion complexes of β-CD and γ-CD as well as α-CD and DMβ-CD with Gua and its derivative 4-hydroxy-3,5-dimethoxy-benzoic acid in aqueous solution. However, only two pure complexes, Gua–β-CD and Gua–γ-CD, were obtained. Under the same experimental conditions, no measurable precipitation was observed for the other six host–guest inclusion systems. The two solid inclusion complexes were characterized using elemental analysis (EA), nuclear magnetic

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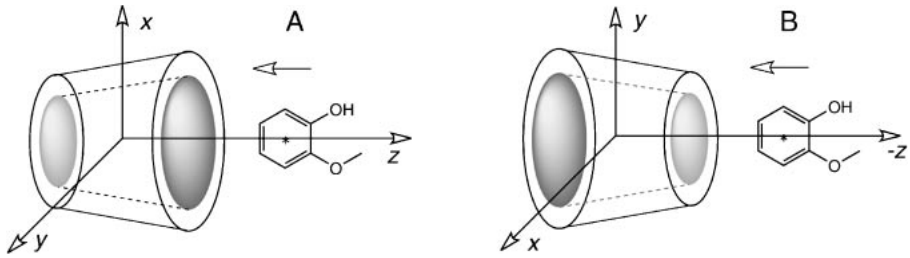

Fig. 2. Schematic sketches showing the relative positions and the centers of host and guest in two different staring geometries (L-form, Left; R-form, Right).

resonance (NMR), Fourier transform infrared spectroscopy (FT-IR), matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), thermogravimetric (TG) and X-ray powder diffraction (XRD) analyses. Binding constants between Gua and CDs were measured by using ^{1}H NMR titration method in a DMSO-d_6 solution. Moreover, PM3 method was also used to investigate the intermolecular complexations between Gua and γ-CD both in vacuo and in water.

Experimental and Method

Materials. α-CD was purchased from Nihon Toshin Chemical Company. β-CD was purchased from Shanghai Chemical Reagent Company and recrystallized twice from deionized distilled water. DMβ-CD and γ-CD were kindly donated by Harata. Gua and 4-hydroxy-3,5-dimethoxy-benzoic acid were obtained from Shanghai Chemical Reagent Company and used without further purification. All chemicals were of general purpose reagent grade unless otherwise stated.

Preparation of Solid Inclusion Complexes. Solid inclusion complexes were prepared by mixing a guest with α-, β-, γ-, or DMβ-CD and stirring for 48 h at 298.2 K. The initial molar ratio of guests to CDs was 10:1 in deionized water. The separated crystalline inclusion complexes were washed using small amounts of deionized water and alcohol (95%) three times, respectively, and dried for 24 h at 383.2 K in vacuo. Two pure solid inclusion complexes, Gua–β-CD and Gua–γ-CD, were obtained in this manner. However, the solid inclusion complexes of 4-hydroxy-3,5-dimethoxy-benzoic acid with CDs as well as those of Gua with α-CD and DMβ-CD were not obtained under the same experimental conditions.

Instrumentation and Measurement. XRD of the solid complexes was performed on a Philips X'Pert Pro X-ray diffractometer. The samples were irradiated with monochromatized Cu Kα and analyzed with $5^\circ \leq 2\theta \leq 40^\circ$. The voltage and current were 40 kV and 40 mA, respectively. EA were carried out on an Elementar Vario EL III elemental analyzer. FT-IR spectra were recorded on Bruker EQUINOX55 spectrometer and obtained in KBr pellets of the injected samples in a frequency range between 4000 and 450 cm^{-1}. TG curves were recorded on a Shimadzu TGA-50 thermogravimetric analyzer at the heating rate of 10.0 K min^{-1} under a nitrogen atmosphere. High-resolution MALDI-TOF mass spectra were recorded on a BIFLEX III TOF-MS from Bruker (Bremen, Germany) in positive mode. The instrument was equipped with a nitrogen laser ($\lambda = 337$ nm) to desorb and ionize samples. The sample solutions were prepared by dissolving the inclusion complexes in DMSO. 2,5-dihydroxybenzoic acid as matrix was also dissolved in DMSO. Then, the sample solution of the analyte was pipetted onto the layer of matrix and left in air at room temperature for evaporation of the solvent and for further analysis by MALDI-TOF MS.

^{1}H NMR and ^{13}C NMR spectra of the solid complexes were obtained on a Bruker NMR spectrometer at 300 and 75 MHz, respectively, at 298.2 K using DMSO-d_6 as solvent and TMS as internal reference. ^{1}H NMR titrations were performed by addition of stock solutions of CD to a solution of Gua at 298.2 K using DMSO-d_6 as solvent. The chemical shifts of protons in CD and Gua were monitored with Gua concentration kept constant (2.00×10^{-3} mol dm^{-3}), while the concentration of CD was gradually increased from 0 to 1.00×10^{-1} mol dm^{-3}.

Theoretical Studies of Inclusion Complexation between Host and Guest. PM3 method[25] was chosen to investigate the inclusion complexation between γ-CD and Gua both in vacuo and in water. All calculations reported in this paper were performed with the MOPAC software package.[26] The initial geometry of γ-CD was constructed with the help of the available crystallographic data[27] determined by X-ray crystal structure method and then fully optimized by PM3 without any symmetrical restrictions. The guest molecule, Gua, was also fully optimized. PM3 harmonic frequency calculations were performed for the equilibrium structures, characterizing them as true minima (all eigenvalues of the Hessian matrix were positive) on a potential energy surface. The optimum position of a complexation system was determined by trying several starting points rather than by a global search.

The glycosidic oxygen atoms of γ-CD were placed onto xy plane, and the center of its cavity was designated as the origin of the Cartesian coordinate system (see Fig. 2). The secondary OH rim of γ-CD was placed pointing toward the positive z-axis. The benzene ring of the Gua molecule was initially placed along z-axis as described in Fig. 2.[28] As the calculated ΔE_c values of γ-CD complexes of Gua depend on the starting geometries, two different starting geometries were considered in the present work.[15,28] Schematic sketches showing the relative positions of host and guest are depicted in Fig. 2.

The host–guest complexation process was simulated by making Gua penetrate into the cavity of γ-CD from either the wider (Fig. 2A, L-form) or the narrower (Fig. 2B, R-form) rim side and letting it pass through the cavity by steps. In every step, the geometry of the inclusion complex, Gua–γ-CD, was completely optimized by PM3 without any restrictions.

The formation of the supramolecular inclusion complex (HG) of Gua (guest, G) and γ-CD (host, H) can be represented by Eq. 1 as follows:

$$H + G = HG. \quad (1)$$

The complexation energy (ΔE_c) between G and H is the difference between the energy of the inclusion complex (ΔE_{HG}) and the sum of the energies of Gua ($\Delta E_{e,G}$) and γ-CD ($\Delta E_{e,H}$) in their

Table 1. Preparation of the Solid Inclusion Complexes of β-CD and γ-CD with Gua

Compound	Yield /%	BP[a] /K	DP[a] /K	Formulae	Found/% C	Found/% H	Calcd/% C	Calcd/% H
β-CD	—	—	596.0	$C_{42}H_{70}O_{35}\cdot 8H_2O$	—	—	—	—
γ-CD	—	—	598.7	$C_{48}H_{80}\ O_{40}\cdot 4H_2O$	—	—	—	—
Gua	—	478.2	—	$C_7H_8O_2$	—	—	—	—
Gua–β-CD	37.5	—	601.4	$C_{49}H_{78}O_{37}\cdot 6H_2O$	43.10	6.60	43.05	6.63
Gua–γ-CD	51.2	—	603.8	$C_{55}H_{88}O_{42}\cdot 8H_2O$	42.05	6.80	42.20	6.70

a) BP and DP represent the boiling point and melting-decomposition point of these compounds, respectively.

respective optimized equilibrium geometry. Thus, it can be calculated[29] according to Eq. 2:

$$\Delta E_c = \Delta E_{HG} - \Delta E_{e,H} - \Delta E_{e,G}. \quad (2)$$

In this way, a negative value of ΔE_c denotes that the complex formation is energetically favorable.

The deformation energy (ΔE_f^X) of a component (X) in an inclusion complex at its optimum position can be calculated from Eqs. 3 and 4 as follows:[30]

$$\Delta E_f^H = \Delta E_{e,H} - \Delta E_{c,H}, \quad (3)$$

$$\Delta E_f^G = \Delta E_{e,G} - \Delta E_{c,G}, \quad (4)$$

where ΔE_f^X corresponds to the energy difference between the energy of partners of the complex in their respective equilibrium geometry ($\Delta E_{e,X}$) and their energy at complex geometry ($\Delta E_{c,X}$).

Interaction energy (ΔE_i) is the difference between the energy of the complex and the sum of the energies of both partners at their complex geometry. Hence, ΔE_i can be calculated as follows:

$$\Delta E_i = \Delta E_{HG} - \Delta E_{c,H} - \Delta E_{c,G}. \quad (5)$$

Rearranging Eqs. 2, 3, and 4, gives the expresses of ΔE_{HG}, $\Delta E_{c,H}$, and $\Delta E_{c,G}$, then substituting them into Eq. 5 affords Eq. 6:

$$\Delta E_i = \Delta E_c + \Delta E_{e,H} + \Delta E_{e,G} - (\Delta E_{e,H} - \Delta E_f^H) - (\Delta E_{e,G} - \Delta E_f^G). \quad (6)$$

Accordingly, this interaction energy (ΔE_c) between Gua and γ-CD can also be calculated as the sum of the deformation energy of both molecules (Gua and γ-CD) and the complexation energy (see Eq. 7).

$$\Delta E_i = \Delta E_c + \Delta E_f^H + \Delta E_f^G. \quad (7)$$

Solvent effects were also taken into consideration during theoretical calculations. The calculations of solvation energy, i.e., hydration energy, in the present work were carried out using the PM3 method. The solvation model was the Conductor-like Screening Model (COSMO).[31] The COSMO algorithm is invoked using the keyword NSPA = 60 1SCF EPS = 78.4 (water has a dielectric constant of 78.4 at 298.2 K) and PM3 CHARGE = 0.

Results and Discussion

Preparation Analysis. Two pure solid CD inclusion complexes, Gua–β-CD and Gua–γ-CD, were prepared by a direct precipitation reaction described above. However, under the same experimental conditions, no measurable precipitation was observed for the other complexation systems investigated, possibly because of the very good solubility of these inclusion complexes in aqueous solution. Since the solubility of γ-CD in water is obviously higher than that of α-CD at room temperature, the results concerning preparation clearly reflected that the water solubility of supramolecular complexes of different CDs with the same guest did not depend entirely upon the solubility of CDs in water. In other words, the water solubility of γ-CD became much less than that of α-CD through complex formation with Gua. The yields of the obtained complexes were calculated on the basis of the initial concentration of host, and listed in Table 1. Moreover, the thermal decomposition temperatures, compositions and results of elemental analyses of these complexes are also summarized in Table 1.

The host–guest stoichiometries of the two solid supramolecular complexes were determined to be 1:1, based on the results of EA and further confirmed by comparing the relative integral areas of the proton signals between host and guest.

As shown in Table 1, the yield of Gua–γ-CD (51.2%) is 13.7% higher than that of Gua–β-CD (37.5%) under the same conditions, though the solubility of γ-CD in water is significantly higher than that of β-CD. This suggests that there could exist a strong intermolecular interaction between γ-CD and Gua. Since Gua is only slightly soluble in water, therefore, it should be reasonable that in Gua–γ-CD, Gua has no alternative but to penetrate only part way into the cavity of γ-CD. This inclusion phenomenon is proposed to explain the observed decrease in the water solubility of γ-CD.

Furthermore, as can be seen in Table 1, the thermal decomposition points (DP) of Gua–β-CD (601.4 K) and Gua–γ-CD (603.8 K) are somewhat higher than those of free β-CD (596.0 K) and γ-CD (598.7 K), respectively. The increase in thermal stability of CDs demonstrates that there is an intermolecular complexation between host and guest.

In order to further confirm the formation of inclusion complexes of Gua with β- and γ-CD, MALDI-TOF MS was employed. In the case of Gua–β-CD, two peaks with $m/z = 1281.7$ and 1297.7 were clearly observed, which could be assigned to the Na^+ adduct ion of Gua–β-CD [Gua–β-CD + Na]$^+$ and the K^+ adduct ion of Gua–β-CD [Gua–β-CD + K]$^+$, respectively. Similarly, in the mass spectrum of Gua–γ-CD, two peaks with $m/z = 1443.7$ and 1459.5 could be assigned to the Na^+ adduct ion of Gua–γ-CD ([Gua–γ-CD + Na]$^+$ and K^+ adduct ion of Gua–γ-CD [Gua–γ-CD + K]$^+$), respectively.

XRD Analysis of Solid Inclusion Complexes. The crystalline diffraction patterns of CDs should change when a guest molecule is partly or wholly included into CD cavity.[22,23,32] The differences in diffraction peak intensities and diffraction angles (2θ) can show whether there existed an interaction between CD and guest molecules.[7]

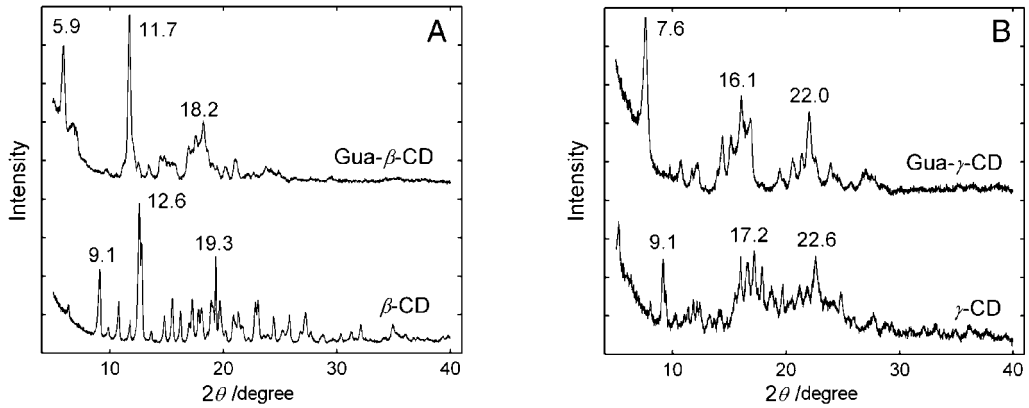

Fig. 3. XRD spectra of (A) β-CD and Gua–β-CD, and (B) γ-CD and Gua–γ-CD.

Table 2. Experimental IR and XRD Data of β-, γ-CD, and Their Complexes of Gua

Compounds	IR (Wavenumbers/cm^{-1})						XRD[a] (2θ/degree)		
	ν_{C-O}			$\nu_{C=C}$			I_1	I_2	I_3
β-CD	1028.7	1079.8	1157.4	—	—	—	12.6	19.3	9.1
γ-CD	1026.8	1080.2	1158.3	—	—	—	17.2	22.6	9.1
Gua	1024.5	1099.6	—	1260.8	1502.6	1598.8	—	—	—
Gua–β-CD	1031.1	1086.9	1160.4	1265.1	1506.9	1603.6	11.7	5.9	18.2
Gua–γ-CD	1031.7	1086.6	1161.8	1265.2	1506.9	1603.0	7.6	16.1	22.0

a) I_1, I_2, and I_3 represent the first, second, and third strongest peaks in X-ray diffractograms, respectively.

To confirm the existence of the supramolecular inclusion complexes of CDs with Gua, X-ray powder diffraction of the two solid samples was performed. The powder X-ray diffraction patterns of β-, γ-CD, and their inclusion complexes with Gua are shown in Fig. 3. Because Gua is an oleaginous liquid at room temperature, there is no powder X-ray diffraction data available.

As shown in Fig. 3A and Table 2, the 2θ values of the top three peaks in free β-CD at 9.1, 12.6, and 19.3° become 5.9, 11.7, and 18.2° in Gua–β-CD, respectively. The main peaks of Gua–β-CD shifted towards lower 2θ angles compared to those of free β-CD. On the whole, there are considerable differences in the XRD diffraction spectra of free β-CD and Gua–β-CD, including shapes, numbers and locations of diffraction peaks. Obviously, inclusion complexation decreases the numbers of diffraction peaks and increases the width in main peaks, clearly reflecting that Gua–β-CD is more amorphous than free β-CD.[33]

Figure 3B displays the XRD spectra of γ-CD and its inclusion complex of Gua. The free γ-CD has a characteristic pattern of multiple diffraction peaks at 9.1, 17.2, and 22.6°. The peak at 9.1° is characteristic of the two free hosts. However, the other two main peaks of γ-CD are quite different from those of free β-CD, reflecting a structural difference in the conformation of macrocyclic ring skeleton as well as in the molecular stacking in solid state between free β-CD and γ-CD.[7,34]

In the Gua–γ-CD spectrum, its top three peaks were observed at 2θ values of 7.6 (I_1), 16.1 (I_2), and 22.0° (I_3), all of which are rather different from those of free γ-CD. Upon inclusion with Gua, the three strongest peaks of γ-CD obviously shifted towards lower 2θ angles, and their intensities and widths also increased.

Furthermore, the morphological shape difference in the XRD diffraction diagrams, as can be seen in Fig. 3, is smaller between γ-CD and its complex of Gua than that between β-CD and its complex of Gua, suggesting relative small effects of host–guest complexation on the molecular arrangement of γ-CD in solid state before and after inclusion.[7]

FT-IR Spectroscopic Analysis of Solid Inclusion Complexes. FT-IR spectra are usually used to probe intermolecular interaction between host and guest. Exiguous spectra changes in the characteristic absorption bands of CD and guest, upon complexation, can be used to confirm the formation of a supermolecule as a new compound, though in general, the changes in IR spectra is quite small, because an inclusion interaction does not lead to the formation or rupture of chemical bonds.[23] FT-IR spectra of β-CD, γ-CD, Gua, and their inclusion complexes are shown in Fig. 4. As can be seen from Fig. 4, the positions and intensities of many spectroscopic bands of the Gua inclusion complexes of CDs show subtle changes in comparison with those of the same specific bands of free Gua and CDs.

In Fig. 4A, free β-CD shows prominent peaks at 3417.6 and 1028.7 cm^{-1}, contributing to ν_{OH} and ν_{C-O} of β-CD. There were a series of peaks at 720–1250 cm^{-1}, which is due to the ν_{C-C} stretching vibrations of β-CD in the fingerprint region. In the FT-IR spectra of Gua, the $\nu_{C=C}$ stretching vibrations of the aromatic moiety of Gua give two sharp peaks at 1502.6 and 1598.8 cm^{-1}.

As for Gua–β-CD, there were two peaks at 1506.9 (sharp) and 1603.6 (moderate) cm^{-1}, belonging to the $\nu_{C=C}$ stretching vibrations of Gua, and one small sharp peak at 1265.1 cm^{-1}, belonging to the ν_{C-O} stretching vibrations of Gua. The posi-

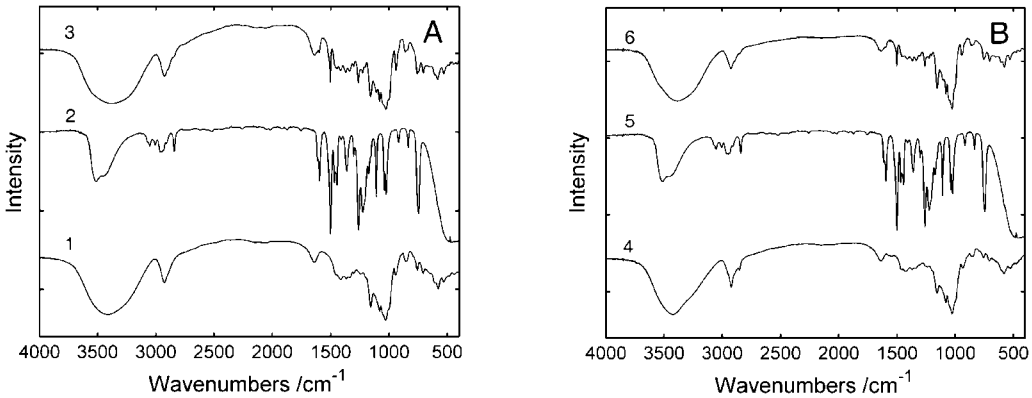

Fig. 4. FT-IR spectra of (A) 1. β-CD; 2. Gua; 3. Gua–β-CD and (B) 4. γ-CD; 5. Gua; 6. Gua–γ-CD.

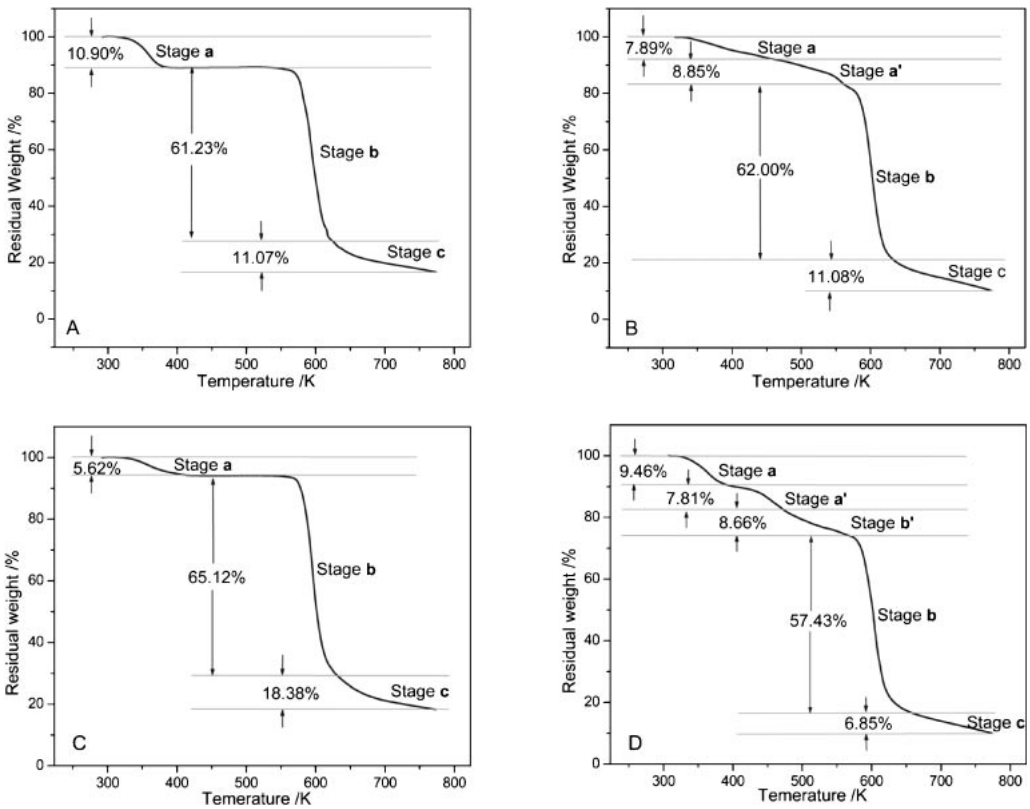

Fig. 5. TG curves of hosts and their complexes of Gua: (A) β-CD, (B) Gua–β-CD, (C) γ-CD, and (D) Gua–γ-CD.

tions and intensities of these peaks exhibited large changes relative to those of pure Gua, despite that there were no new covalent bonds formed between host and guest. The characteristic peaks of host and guest, as shown in Table 2, slightly shifted to higher wavenumbers. Furthermore, the intensities of these peaks obviously decrease upon inclusion, indicating that Gua has been introduced into the cavity of β-CD after complexation.[22]

In Fig. 4B, the stretching vibrations of ν_{C-O} in IR spectra of γ-CD were observed at 1026.8, 1080.2, and 1158.3 cm^{-1} respectively. The ν_{C-C} stretching vibrations of γ-CD lie within a given range of 709–1250 cm^{-1} in fingerprint region.

However, upon inclusion the characteristic peaks, attributed to the ν_{C-O} stretching vibrations of γ-CD, shifted to 1031.7, 1086.6, and 1161.8 cm^{-1}. The C=C stretching vibrations of Gua–γ-CD, gave weak absorptions at 1506.9 and 1603.0 cm^{-1}. These results show that there exist distinct intermolecular interactions between Gua and γ-CD.[23]

Consequently, FT-IR spectroscopic analyses of these host–guest systems suggest the formation of the two solid inclusion complexes Gua–β-CD and Gua–γ-CD. It should be noted that IR spectra of Gua–β-CD and Gua–γ-CD are very similar, whereas their XRD spectra were quite different, because many characteristic peaks of Gua in the IR spectra are overlapped by those of CDs.

TG Analysis of Solid Inclusion Complexes. Thermal analysis methods are widely used in host–guest chemistry for the thermodynamic investigation of inclusion complexations between CDs and organic guests.[23,35] Figure 5 displays the TG curves of β-CD, γ-CD, Gua–β-CD, and Gua–γ-CD.

Table 3. Thermal Decomposition Details of CDs and Their Inclusion Complexes

Compound	Stage **a**		Stage **a′**		Stage **b′**	Stage **b**		Stage **c**
	TP/K	TR/K	TP/K	TR/K	TR/K	TR/%	WL/%	RW/%
β-CD	356.6	323.5–383.2	—	—	—	573.0–623.3	61.23	16.80
Gua–β-CD	373.5	340.1–452.4	551.1	467.4–560.5	—	560.6–632.0	62.00	10.18
γ-CD	357.8	329.6–393.7	—	—	—	565.2–631.3	65.12	10.88
Gua–γ-CD	357.8	329.6–385.8	453.2	393.8–470.0	474.6–562.2	571.3–655.5	57.43	9.79

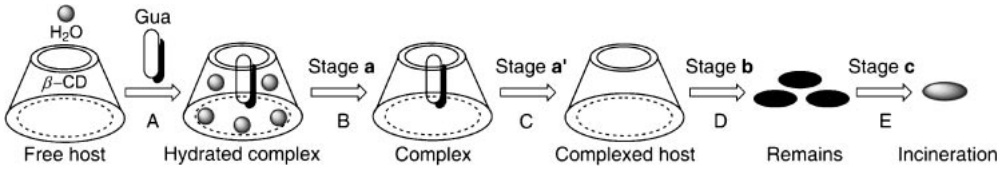

Fig. 6. A consecutive mechanism depicting the formation (A) and decomposition (B–E) of the inclusion complex of β-CD with Gua.

In Fig. 5A, the first weight loss of β-CD in the range from 323.5 to 383.2 K was about 10.90% (Stage **a**), corresponding to the release of about eight water molecules from β-CD cavity (calc. 7.71), with a maximum rate of mass loss at 356.6 K. No further change is observed until around 573.0 K after which β-CD begins to melt and decompose. The maximum rate of melting-decomposition was recorded at 596.0 K, corresponding to decomposition point (DP) of β-CD, as shown in Table 1. The melting-decomposition of β-CD appeared in the narrow range of 573.0–623.3 K with a weight loss of 61.23% in this stage (Stage **b**). Even though the free solid β-CD was subsequently heated up to 773.2 K, a residual weight of 16.80% (Stage **c**) could still be observed in the TG curve.

The TG curve of Gua–β-CD is significantly different from that of pure β-CD. When solid Gua–β-CD was heated from room temperature to 452.4 K, a weight loss of 7.89% (Stage **a**) occurred, corresponding to the release of six water molecules (calc. 6.00). In this stage, a continuous weight loss measurement, as in Fig. 5B, clearly showed slow and gradual releases of water from the β-CD complex in the broad temperature range from 340.1 to 452.4 K, which is rather different from the release of water molecules in free β-CD, indicating the difference in numbers of complexed and uncomplexed water molecules between the host–guest supermolecule (mainly containing complexed water) and the free host (mainly containing uncomplexed water).[35] In addition, one guest molecule (calc. 0.98) released from the complex in the temperature range of 467.4–560.5 K with a weight loss of 8.85% (Stage **a′**).

It is worth stressing that the molecular numbers of both water and guest based on the TG curve are exactly in accord with the measurement results of elemental analyses of the solid sample. A relatively rapid weight loss of complexed Gua occurred at around 551.1 K, due to its rapid boiloff. When the temperature was further increased to 560.0 K, the complexed Gua disappears entirely from its inclusion complex of β-CD. It should also be noted that until about 467.4 K, Gua is not released, and the two temperatures, 551.1 and 560.6 K, are markedly higher than the boiling point (478.2 K) of free Gua.

These observations strongly suggest that the intermolecular interactions between Gua and β-CD significantly improve the thermal stability of Gua. Above 560.0 K, a very large weight loss of 62.00% in the TG curve of Gua–β-CD was recorded in the temperature range of 560.6 to 632.0 K (Stage **b**, DP = 601.4 K, see Table 1), attributing to the melting-decomposition of the complexed β-CD. The residual weight of the inclusion complex of β-CD at 773.2 K was about 10.18% (Stage **c**). The temperature ranges (TR, K) of the thermal release and decomposition of the supramolecular inclusion complexes as well as the decomposition temperature points (TP, K), corresponding to a rapid weight loss (WL, %) process, and the residual weight (RW, %) at 773.2 K are all summarized in Table 3.

According to the findings in Fig. 5B and Table 3, a consecutive mechanism (A–E) concerning the formation and thermal decomposition of solid supramolecular inclusion complex of β-CD with Gua is suggested in Fig. 6. The formation of a hydrated inclusion complex, Gua–β-CD$\cdot 6H_2O$, is regarded as process A. Next the release of six water molecules and Gua in this complex early or late refer to processes B (Stage **a**) and C (Stage **a′**), respectively, and the melting-decomposition or carbonization of the complexed β-CD is regarded as process D (Stage **b**). Process E represents further incineration of the carbonized remains (Stage **c**).

It should be noted that there are very small differences in temperature range of decomposition and degradation of free β-CD (573.0–623.3 K) and complexed β-CD (560.6–632.0 K) as shown in Table 3. The melting-decomposition point (DP, 601.4 K) of the inclusion complex also is only slightly higher than that of free β-CD (DP, 596.0 K). However, at temperatures $>$ 632.0 K, more residual weight is always recorded in the TG curve of β-CD compared to that of Gua–β-CD.

As shown in Fig. 5C, the TG curve of γ-CD indicated the loss of hydrated water (Stage **a**, 5.62%, 4.10 water molecules) up to 393.7 K. In the temperature range from 565.2 to 631.3 K, γ-CD melted and decomposed (Stage **b**, 65.12%). The maximum rate of the thermal decomposition was recorded at 598.7 K (DP). The residual weight of γ-CD at 773.2 K was about 10.88% (Stage **c**). However, as can be seen in Fig. 5, the TG curve of Gua–γ-CD is significantly different from that of γ-CD or Gua–β-CD. Because the weight loss processes of Gua–β-CD and γ-CD can be divided easily into three and four stages respectively; however, a five-stage thermal decomposition process for Gua–γ-CD can be seen very clearly in Fig. 5D.

During the first stage, a weight loss of 9.46% should be ascribed to the release of about eight water molecules (Stage **a**, calc. 8.22) from the hydrated supermolecule of γ-CD with Gua.

The second weight loss process is a two-step Gua–γ-CD decomposition process (Stage **a′** and Stage **b′**): the first step showed a weight loss of 7.81% from 393.8 to 470.0 K, corresponding to the gradual evaporation of the complexed Gua (Stage **a′**, calc. 0.99), and the second step showed a weight loss of 8.66% from 474.6 to 562.2 K (Stage **b′**), corresponding to the slow melting and evaporation of the remaining complex, which was mainly γ-CD. It is worth stressing that the phase change temperature (448.4 K) of Gua after inclusion is significantly lower than the boiling point (478.2 K) of free Gua.

Moreover, even if the experimental temperature is below 470.0 K, complexed Gua can still be released entirely from the complex. In addition, due to the latent heat effects resulting from the phase changes of the first step, i.e., Stage **a′**, the remaining sample, mainly containing γ-CD, had a lower melting point than free γ-CD and lost 8.66% of its total weight in the broad temperature range of 474.6–562.2 K, due to a slow evaporation rate. The results described above are completely different from those found in free γ-CD and Gua–β-CD, because the initial onset temperature of the thermal decomposition of both free γ-CD and residual of Gua–β-CD are greater than 565.2 K. In addition, further heating would result in an abrupt weight loss of 57.43% in the temperature range from 571.3 to 655.5 K (Stage **b**) with the maximum rate of weight loss occurring near 603.8 K. The residual weight of complexed γ-CD at 773.2 K was about 9.79% (Stage **c**).

The above results indicate that, upon inclusion, the thermal decomposition point of β-CD or γ-CD as host only slightly increases. Gua after inclusion exhibits entirely different thermal behavior in its two CD complexes, Gua–β-CD and Gua–γ-CD, i.e., the thermal stability of the complexed Gua is obviously higher in the former than in the latter. Clearly, this inclusion phenomenon can be attributed to the effects of the different strength of interactions between Gua and CDs as well as between Gua itself. It is reasonable that the intermolecular interactions in the solid state decreases in the order: Gua–β-CD > Gua–Gua > Gua–γ-CD.

NMR Spectral Analysis of Solid Inclusion Complexes. NMR has been widely used to gain important information about the location of a guest in its complex of CD by chemical shift changes from protons of CD and guest in solution,[23,36] because the insertion of a guest molecule into the hydrophobic cavity of CD from two different rims will result in different chemical shift changes of the interior protons (H-3 close to the wider rim and H-5 close to the narrower rim) of the CD cavity.[22,37]

^{1}H NMR spectra of free Gua and its complexes of β- and γ-CD in DMSO-d_6 are displayed in Fig. 7. The ^{1}H and ^{13}C NMR chemical shifts (δ) of Gua, β-CD and γ-CD as well as the chemical shift changes ($\Delta\delta$) of their solid inclusion complexes in DMSO-d_6 are also listed in Table 4.

In the inclusion complex Gua–β-CD, the H-a proton signals of Gua were shifted downfield by 0.029 ppm. However, no obvious chemical shift changes of these protons of phenolic hydroxy and methoxy groups of Gua were observed. Moreover, among the CH or CH_2 protons of β-CD, the signals of H-3 and H-5 protons located inside β-CD cavity, were shifted slightly more downfield (0.019 and 0.010 ppm, respectively) than those of the other protons outside the cavity. In addition,

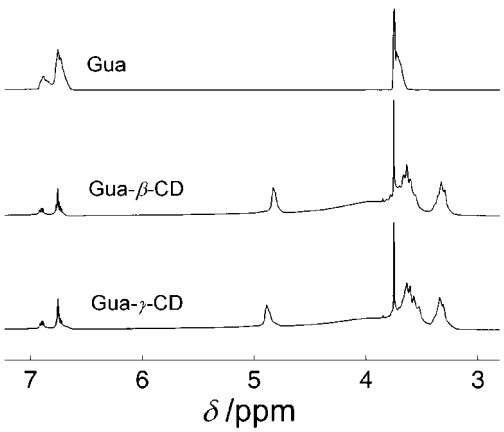

Fig. 7. ^{1}H NMR spectra (300 MHz, DMSO-d_6, 298.2 K) of Gua, Gua–β-CD and Gua–γ-CD.

Table 4. ^{1}H and ^{13}C NMR Chemical Shifts (δ, ppm) of Gua, β-CD, and γ-CD, and the Chemical Shift Changes ($\Delta\delta$, ppm) of Their Solid Inclusion Complexes in DMSO-d_6

Position	β-CD		γ-CD		Position	Gua		
	Free/ppm	$\Delta\delta$/ppm	Free/ppm	$\Delta\delta$/ppm		Free/ppm	$\Delta\delta_{\beta\text{-CD}}$/ppm	$\Delta\delta_{\gamma\text{-CD}}$/ppm
H-1	4.810	0.005	4.859	0.029	H-a	6.756	0.029	0.011
H-2	3.322	0.008	3.306	0.005	H-b	6.891	0.004	0.027
H-3	3.657	0.019	3.755	−0.004	H-c	3.748	−0.008	0.003
H-4	3.294	0.004	3.327	0.015				
H-5	3.598	0.010	3.567	0.006				
H-6	3.631	0.007	3.627	0.010				
C-1	102.300	−0.262	102.220	−0.374	C-1	146.619	0.020	0.011
C-2	72.410	−0.292	73.010	−0.300	C-2	147.701	0.066	0.084
C-3	73.470	−0.337	73.430	−0.399	C-3	115.616	0.030	0.076
C-4	81.900	−0.264	81.410	−0.343	C-4	119.229	0.140	0.066
C-5	72.730	−0.297	72.680	−0.389	C-5	120.953	0.103	0.103
C-6	60.320	−0.233	60.540	−0.425	C-6	112.600	0	0
					C-7	55.574	0.075	0.121

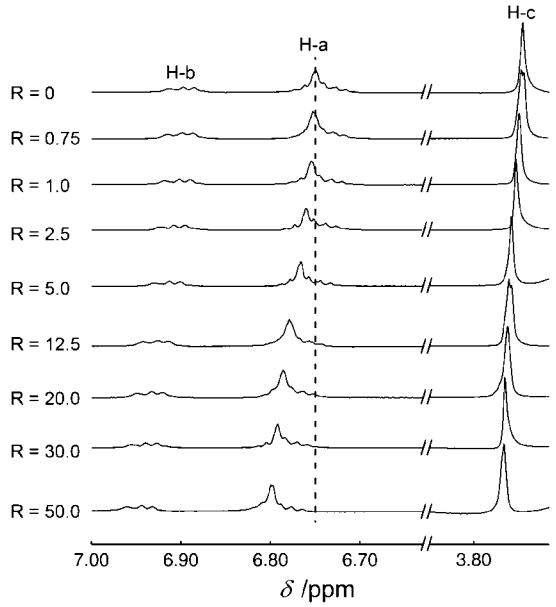

Fig. 8. Chemical shifts (δ) of the protons of benzene ring and methoxy groups in Gua for the ^{1}H NMR titration of the inclusion system of Gua and γ-CD. The values of R represent the molar concentration ratios of γ-CD to Gua.

the chemical shift change of H-3 proton is larger than that of H-5 proton. However, in the case of Gua–γ-CD, the signals of the H-3 and H-5 protons of γ-CD as well as the proton signals of methoxy groups of Gua showed only very small lateral shifts.

As ^{13}C NMR chemical shifts extend over a much larger scale than ^{1}H NMR does, it is particularly suitable for identifying the formation of inclusion complexes. The ^{13}C NMR data of β-CD, γ-CD, Gua, and their inclusion complexes are given in Table 4. Upon complexation, all of the signals of carbon atoms of β-CD and γ-CD shifted upfield, and the chemical shift changes of C-3 ($\Delta\delta$, -0.337 ppm for β-CD and -0.399 ppm for γ-CD) in the two complexes of Gua are bigger than of those of C-5 ($\Delta\delta$, -0.297 ppm for β-CD and -0.289 ppm for γ-CD). Moreover, the signals of the carbon atoms, especially C-4 atom ($\Delta\delta$, 0.140 ppm for β-CD) and C-5 atom ($\Delta\delta$, 0.103 ppm for γ-CD), in benzene ring of Gua aside from C-6 atom were shifted downfield.

It is noted that the observed $\Delta\delta$ values of the complexes dissolved in DMSO solution were very small. Their maxima were 0.029 ppm for proton and -0.425 ppm for carbon. This means that the solid inclusion complexes decompose in DMSO solution. In order to further investigate the complex geometries and the intermolecular complexation between Gua and CDs in solution, ^{1}H NMR titration measurements were carried out.

NMR Titration Measurements. ^{1}H NMR titrations were performed by adding stock solutions of CDs to a solution of Gua at 298.2 K using DMSO-d_6 as solvent. The continuous chemical shift changes in the protons of CD and Gua were observed with an increase in the concentration ratio of CD to Gua. Typical ^{1}H NMR spectra at each titration point of the inclusion system of Gua and γ-CD are shown in Fig. 8.

Quantitative analysis of the NMR titration data allows us to determinate the binding constants (K) between Gua and CDs.

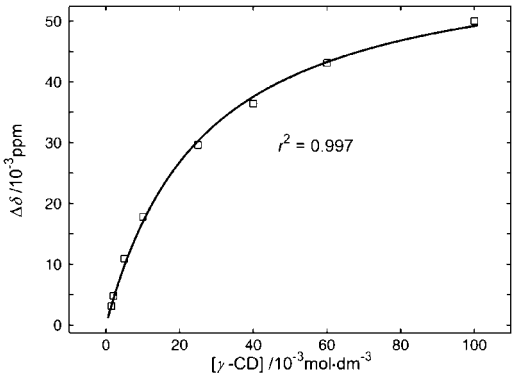

Fig. 9. Chemical shift changes ($\Delta\delta$) in the H-a protons of Gua (2.00×10^{-3} mol dm^{-3}) upon addition of γ-CD (0–1.00×10^{-1} mol dm^{-3}).

As shown in Fig. 9, the chemical shift changes of H-a proton of Gua upon addition of γ-CD in the concentration range from 0 to 1.00×10^{-1} mol dm^{-3} exhibited highly nonlinear characteristics. Therefore, the binding constant between Gua and γ-CD can be calculated. If the formation of γ-CD inclusion complex of Gua in solution is shown in Eq. 1, the relationship among the observed chemical shift changes of H-a proton signal in Gua, the initial molar concentration of Gua and γ-CD, and the value of K can be described by Eq. 8,[38,39]

$$\delta - \delta_G = \frac{(\delta_{HG} - \delta_G)}{2}\left\{[H]_0 + [G]_0 + (1/K) - \sqrt{([H]_0 + [G]_0 + (1/K))^2 - 4[H]_0[G]_0}\right\}, \quad (8)$$

where δ_G, δ_{HG}, δ represent chemical shift of Gua, inclusion complex of Gua, and observed chemical shift, respectively. Hence, the difference between δ and δ_G is the observed chemical shift changes ($\Delta\delta$) in the H-a proton signal in Gua with and without γ-CD. $[H]_0$ and $[G]_0$ represent the initial molar concentration of γ-CD and Gua, respectively. As can be seen in Fig. 9, the nonlinear relationship between the experimental data points and the fitted curve appears to be rather good. A correlation coefficient (r^2) of 0.997 was obtained, confirming that γ-CD interacts with Gua to form an inclusion complex of 1:1 stoichiometry. The calculated binding constant between Gua and γ-CD was 6.22×10^3 mol^{-1} dm^3. The inclusion system of Gua and β-CD was similarly treated, and the binding constant between Gua and β-CD was determined to be 5.74×10^3 mol^{-1} dm^3, which is smaller than that between Gua and γ-CD, indicating that there is a stronger intermolecular interaction between Gua and γ-CD than between Gua and β-CD in DMSO solution.

As can be seen in Fig. 8, a progressively increasing downfield shift of the benzene ring protons (H-a and H-b) in Gua with increasing molar fractions of γ-CD in sample solutions was clearly observed. This result is attributed to the formation of γ-CD inclusion complex of Gua in DMSO solution. The maximum $\Delta\delta$ values of the H-a, H-b, and H-c protons were 0.051, 0.047, and 0.031 ppm, respectively. Therefore, upon complexation, the benzene ring protons are more affected than the protons of the methoxy group in Gua. For the inclusion system of Gua and β-CD, a similar phenomenon was also

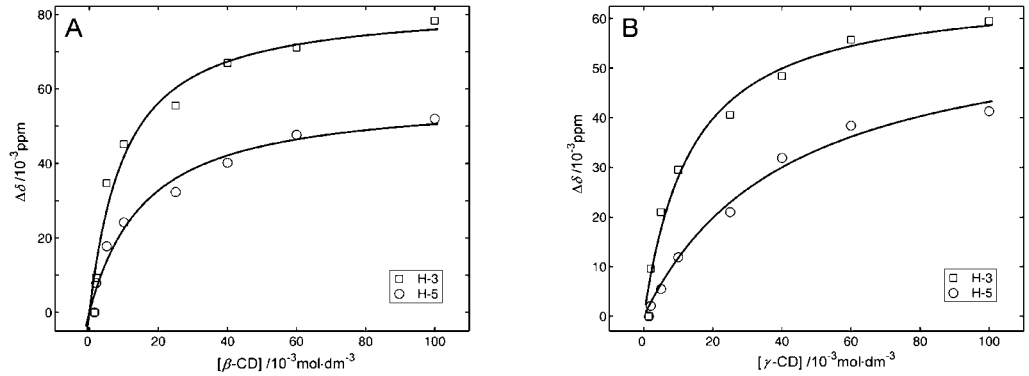

Fig. 10. Relationships between the chemical shift changes ($\Delta\delta$) of protons H-3 (□) and H-5 (○) of CDs and the concentrations of CDs. The concentration of Gua is kept constant at 2.00×10^{-3} mol dm^{-3}. (A) inclusion system of Gua with β-CD and (B) inclusion system of Gua with γ-CD.

observed. These findings suggest that the benzene ring of Gua is deeply embedded into the CD cavity and the methoxy group of Gua might project outside the cavity.

Intermolecular complexation between Gua and CD should lead to the chemical shift changes ($\Delta\delta$) in the protons (H-3 and H-5) of the inner cavity of CD. As shown in Figs. 10A and 10B, a markedly nonlinear increase of the $\Delta\delta$ values in the H-3 and H-5 protons of CDs with an increase in the relative concentration of CDs was observed. The maximum $\Delta\delta$ values of the H-3 and H-5 protons of Gua–β-CD were -0.075 and -0.052 ppm, respectively. For the Gua–γ-CD inclusion system, these values were -0.059 and -0.041 ppm, respectively. In other words, after complexation with Gua, due to the ring current effect, both H-3 and H-5 protons of CDs showed considerably large upfield shifts. These observations are in good agreement with those already published by other groups for the inclusion systems of β-CD and phenolic compounds.[11,37] However, the $\Delta\delta$ values are significantly larger than those listed in Table 4 for the solid inclusion complexes in DMSO solution. This is because complexation induced shifts corresponding to 100% complexation can be obtained by using NMR titration.

In both case, the $\Delta\delta$ value of H-3 protons located in the large end side is always bigger than that of H-5 protons located in the small end side of CD cavity. It is known that, if H-3 undergoes a shift in the presence of substrate, then the cavity penetration is shallow, whereas, when the H-5 resonance of CD also shifts, the penetration will be deep.[40] Therefore, according to the results from NMR titration experimental, it is reasonable to presume that the benzene ring of Gua in solution is likely to have stayed near the wider rim of the cavity of β- or γ-CD with its methoxy group projecting outside. In order to further investigate the details of complexation process and evaluate the stability of the geometry of CD inclusion complexes of Gua, PM3 calculations were carried out.

Calculated Results of Inclusion Complexation between γ-CD and Gua. ΔE_i is a reflection of the stability of a supramolecular complex. A negative value of ΔE_i means that the complex formation is energetically favorable. Theoretically speaking, if the value of ΔE_i of an inclusion complex is more negative, the complex should be more thermodynamically stable. ΔE_c, ΔE_f, and ΔE_i values of the inclusion complex Gua–γ-CD with two different starting geometries are listed in Table 5.

Table 5. Complexation Energies (ΔE_c), Deformation Energies (ΔE_f) and Interaction Energies (ΔE_i) in kJ mol^{-1} of the Inclusion Complex of γ-CD with Gua at Two Different Starting Geometries (L- and R-forms) in Vacuo and in Water

	Gua–γ-CD-L		Gua–γ-CD-R	
	in vacuo	in water	in vacuo	in water
ΔE_c	−60.5	−51.2	−33.5	−25.9
ΔE_f^H	6.59	6.72	6.34	7.03
ΔE_f^G	2.79	2.81	2.98	2.54
ΔE_f	9.38	9.53	9.32	9.57
ΔE_i	−51.1	−41.7	−24.2	−16.3

The calculated energies of CD–guest complexes depend on the starting geometries. The optimum position for guest into CD at different starting geometries can be determined according to complexation energy. The most stable structure can be obtained by comparing the characteristic energies of the complexes between different starting geometries at the optimum position. The detailed results of the inclusion processes of Gua with γ-CD and the most stable structure of Gua–γ-CD are shown in Fig. 11.

As can be seen in Fig. 11A, Gua–γ-CD with the starting geometry shown in Fig. 2A, Gua–γ-CD-L (L represents left), had the most negative ΔE_c near the secondary hydroxy rim ($Z = 200$ pm, $\Delta E_c = -60.5$ kJ mol^{-1}). Figure 11B shows the other structural form (Gua–γ-CD-R, R represents right) of Gua–γ-CD with the starting geometry in Fig. 2B. Its most negative complexation energy was near the center region of γ-CD cavity ($Z = -100$ pm, $\Delta E_c = -33.5$ kJ mol^{-1}). Clearly, the supramolecular structure of Gua with γ-CD described in Fig. 11A has a much lower complexation energy ($\Delta\Delta E_c = -27.0$ kJ mol^{-1}).

This phenomenon is attributed to the formation of intermolecular hydrogen bonds between the polar substituents (–OH or –OCH$_3$) in Gua and the hydroxys of γ-CD. The PM3 optimized structures of the two structural forms of Gua–γ-CD are illustrated with color photographs in Fig. 12. The hydrogen bond between –OH in Gua and a secondary hydroxys group of γ-CD (hydroxy group at C-3) is clearly shown in Fig. 12A. However, as shown in Fig. 12C, no intermolecular hydrogen

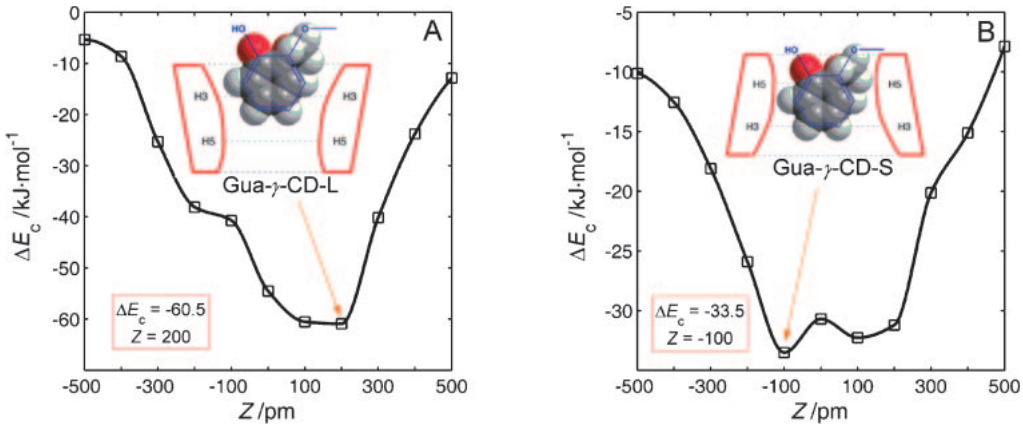

Fig. 11. PM3 complexation energy [ΔE_c/kJ mol^{-1}] curves of the host–guest inclusion complexations, (A) migration of Gua into γ-CD cavity from the secondary OHs rim and (B) migration of Gua into γ-CD cavity from the primary OHs rim.

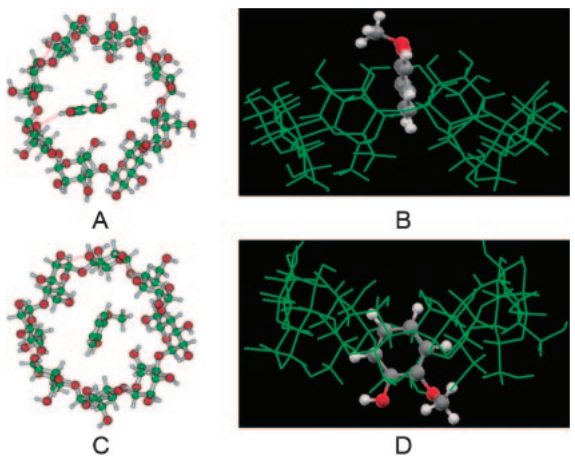

Fig. 12. PM3 optimized structures of Gua–γ-CD-L and Gua–γ-CD-R, (A) top view and (B) side view of the optimized structure of Gua–γ-CD-L; (C) top view and (D) side view of the optimized structure of Gua–γ-CD-R.

bonds form between Gua and γ-CD in the optimized structure of Gua–γ-CD-R. The side view of the optimized structure of Gua–γ-CD-L or Gua–γ-CD-R (see Figs. 12B and 12D) shows that the benzene ring of Gua is accommodated into the cavity of γ-CD. Furthermore, for either the two structural forms of the complex, when Gua is far from the center region of cavity of γ-CD, the host–guest inclusion system would become unstable (see Fig. 11).

The molecular inclusion of a guest molecule into CD cavity is sure to be accompanied by the deformation of both the guest and host molecules. Unlike α- and β-CD, γ-CD has a more flexible structure;[1] therefore, deformation of γ-CD during the complexation process cannot be neglected. Between the structural forms of Gua–γ-CD-L and Gua–γ-CD-R in vacuo, the ΔE_f^H values of γ-CD were 6.59 and 6.34 kJ mol^{-1}, respectively.

As can be seen in Table 5, there is no significant difference between the two calculated ΔE_f^H values of γ-CD under two different conditions, i.e., Gua enters γ-CD cavity from the secondary OHs rim or from the primary OHs rim. From Table 5, in any case the ΔE_f^G values of Gua are always obviously smaller than the ΔE_f^H values of γ-CD, because of the existence of the rigid benzene ring on the guest backbone.

In general, interaction of water with host or guest is important for making an inclusion complex in aqueous solution.[3] As can be found in Table 5, when solvent effects were taken into consideration during theoretical calculations, the values of ΔE_c of Gua–γ-CD-L or Gua–γ-CD-R were obviously different between in vacuo and in water ($\Delta\Delta E_c > 7.5$ kJ mol^{-1}), but the ΔE_f values of Gua–γ-CD were scarcely affected ($\Delta\Delta E_f < 0.3$ kJ mol^{-1}).

The structural form, Gua–γ-CD-L, of the inclusion complex has a relatively lower complexation energy ($\Delta\Delta E_c = -9.3$ kJ mol^{-1}) in vacuo than in water. For the other structural form, Gua–γ-CD-R, a similar energy difference of -7.6 kJ mol^{-1} is also found. However, there is only slight difference between the calculated ΔE_f values of Gua as well as γ-CD in vacuo and in water. In other words, in the present work the deformation of both host and guest molecule is not affected by the water environment around them.

The sufficiently large negative values of ΔE_i especially the extremely large negative ΔE_i value (-51.1 kJ mol^{-1} in vacuo, -41.7 kJ mol^{-1} in water) of the structural form Gua–γ-CD-L clearly demonstrate that Gua forms a stable complex with γ-CD near the secondary hydroxy rim. The results also suggest a significant intermolecular interaction between γ-CD and Gua.

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