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Original Article

Title: Effect of γ-cyclodextrin derivative complexation on the physicochemical properties and antimicrobial activity of hinokitiol

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ABSTRACT

The aim of this study was to evaluate the physicochemical properties of solid dispersion on mixtures of hinokitiol (HT) and γ-cyclodextrin (γ-CD) and of HT and (2-hydroxypropyl)-γ-cyclodextrin (HP-γ-CD). Differential scanning calorimetry revealed that coground HT/γ-CD at a molar ratio of 1:1 and HT and HP-γ-CD at molar ratios of 1:1 and 1:2 lacked an endothermic peak due to melting of HT crystals. Powder x-ray diffraction revealed that HT crystal showed a halo pattern respectively, by mixing and grinding of the CDs and HT. Thus, coground HT/γ-CD and HT/HP-γ-CD at a molar ratio of 1:1 had molecular interaction. Assessment of dissolution revealed that ground mixtures had improved dissolution of HT compared to HT crystals, ground HT alone, and physical mixtures containing HT. ^{1}H-^{1}H NOESY NMR suggested that the 7-membered ring and isopropyl group of HT were located within the cavity of γ-CD and HP-γ-CD. The antimicrobial tests indicated that ground mixtures exhibited a minimum inhibitory concentration (MIC) of 20 μg/mL against *Bacillus subtilis*, 40 μg/mL against *Staphylococcus aureus*, and 20 μg/mL against *Escherichia coli*. GMs were found to have 4 times more antimicrobial activity than HT crystals. Ground mixtures also exhibited MIC of 160 μg/mL against *Pseudomonas aeruginosa* and they were found to 2 times more antimicrobial activity than HT crystals. Improvement in antimicrobial activity with the formation of inclusion complexes is presumably due to increase the solubility of HT as a result of the formation of HT/CD inclusion complexes.

Keywords: hinokitiol, cyclodextrin, ground mixture, molecular interaction, antimicrobial activity

Introduction

Hinokitiol (HT) is a crystalline substance with a 7-membered ring structure that is extracted from the essential oil of certain trees. Since HT is a tropolone derivative, it has similar anti-bacterial action [1], antifungal action [2, 3], anti-inflammatory action [4], antioxidant activity [5], melanin inhibition [6] and anti-tumor action [7]. HT has exceptional biochemical and pharmacological activity. These properties have led to the use of HT in oral care products in the pharmaceutical field [8, 9]. HT is naturally occurring and it is known to exhibit exceptional cytotoxic and antimicrobial activity against oral pathogens and oral squamous epithelial cancer cell lines, so it can be used safely [10]. Medical use of HT is anticipated to increase in the future. HT is also used as a preservative in food since it inhibits the enzymatic browning of foods [11]. HT acts by degrading certain proteins, scavenging free radicals, and chelating metals, so it has been approved as a food additive and preservative. HT has been used in various fields as mentioned earlier, and its further use is anticipated in the future. However, HT has poor solubility since it comes from essential oil. HT is also unstable to light, potentially resulting in photolysis, polymerization, and condensation and therefore limiting its effective use. Improved stability to heat, solubility in water, and stability to light are needed to capitalize on the properties of HT to develop various products.

Cyclodextrin (CD) is, based on the number of glucopyranose units it contains, classified into α-, β-, and γ-CD. CD is widely used as a host molecule because of the inclusion complexes formed by each CD. The area near the inlet of the CD cavity is hydrophilic while the inside is hydrophobic. CD is known, as a result of hydrophobic interaction in an aqueous solution, to accept various hydrophobic guest molecules into its cavity to form inclusion complexes [12]. The physical and chemical properties of the guest molecule are affected by inclusion, i.e. its solubility is improved [13], its stability

is improved [14], is antibacterial activity is improved [15], its bioavailability is better controlled [16, 17] and its taste is improved [18]. CDs have been utilized in various fields, including pharmaceuticals and foods. As an example, α-lipoic acid is a coenzyme involved in energy production that is present in mitochondria. However, α-lipoic acid is sensitive to physical changes, light, and heat. The inclusion complex was prepared using α- lipoic acid and α-CD, β-CD and γ-CD inclusion style different results in type of CD is obtained. When α-lipoic acid forms inclusion complexes with γ-CD, though, it is reported to have improved stability against heat, light, and pH changes [19]. Since the type of guest molecule is determined by it having a size and shape that fits in the CD cavity, CDs allow the stereoselective formation of inclusion complexes. Therefore, the form of inclusion is known to differ depending on the type of CD, even when using the same guest molecule [20]. When candesartan cilexetil is used as a guest molecule, complex formation is affected by steric hindrance since the size of the CD cavity depends on the type of CD [21].

Antimicrobial agents must be effective in small amounts, long-lasting, safe, and easy to handle. Therefore, natural antimicrobial agents reduce the impact on the environment and on the human body. This study coground HT to allow its use as a natural antimicrobial component to form HT/CD inclusion complexes in order to evaluate the physicochemical properties of HT and study its dissolution. Furthermore, this study examined the effect that inclusion complex formation had on the antimicrobial activity of HT.

Materials and methods

Materials

HT was a bulk powder manufactured by Wako Pure Chemical Industries Co. Ltd.

γ-CD was donated by Cyclo Chem Co. Ltd (Tokyo, Japan). γ-CD was used after storage at 40°C, 82% RH for 7 days. (2-hydroxypropyl)-γ-cyclodextrin (HP-γ-CD) was a bulk powder manufactured by Sigma-ALDRICH Co. Ltd. (Tokyo, Japan). HP-γ-CD was used 0.6 substitutions. All other chemicals and solvents were of analytical grade.

Preparation of physical mixtures and ground mixtures

Physical mixtures (PMs) were prepared by mixing γ-CD or HP-γ-CD with HT at different molar ratios (HT:γ-CD=2:1, 1:1, and 1:2) for 1 minute using a vortex mixer. Ground mixtures (GMs) were prepared by grinding PMs (1.0 g) for 60 min using a vibrating rod mill (TI-500ET, CMT Co.) with an aluminum pan.

Physicochemical characterization

Differential scanning calorimetry (DSC)

Thermal behavior of samples was recorded using a differential scanning calorimeter (Thermo plus Evo, Rigaku). All samples were weighed (2 mg) and heated at a scanning rate of 5.0°C/min with a nitrogen flow rate of 60 mL/min between 30 and 100°C. Aluminum pans and lids were used for all samples.

Powder x-ray diffraction

Powder x-ray diffraction (PXRD) was performed with a powder x-ray diffractometer (MiniFlex II, Rigaku) using Cu $K\alpha_1$ radiation, a voltage of 30 KV, and a current of 15 mA. Sample powders were placed in glass sample holders. Samples were scanned from 3° to 35° (2θ) at a rate 4°/min.

Fourier transform infrared spectroscopy

Fourier transform infrared (FT-IR) absorption spectra of samples were recorded with a spectrometer (FT/IR-410, JASCO) using the potassium bromide (KBr) disk method. Scanning was performed 16 times over a range of 650-4000 cm^{-1} with a resolution of 4 cm^{-1}. Tablets were prepared by adding KBr to the sample (ratio of sample:KBr of 1:10 by weight) and manually compressing the mixture.

Nuclear magnetic resonance (NMR) measurement using ^{1}H-^{1}H nuclear Overhauser effect difference spectroscopy (NOESY)

Nuclear magnetic resonance (NMR) spectroscopy was performed using a Varian NMR System 700 MHz spectrometer (manufactured by Agilent Technologies, Inc.). Spectroscopy conditions were a solvent system (D_2O:H_2O=9:1), a resonant frequency of 699.6 MHz, a pulse width of 90°, 256 increments, a scan time of 0.500 s, a relaxation delay of 1.5 s, and a temperature of 25°C.

Dissolution study

Dissolution testing of samples was performed using a dissolution apparatus (NTR-593, Toyama Sangyo) at 37±0.5°C with 900 mL of distilled water that was stirred at 50 rpm using the paddle method. The samples were weighed accurately to be equivalent to 50 mg of HT. Ten mL of the dissolved sample was collected at 0, 5, 10, 15, 30, and 60 min through 0.45-μm membrane filters. An equal volume of fresh dissolution medium maintained at the same temperature was added after removing the sample to keep the volume of dissolution medium constant. Five mL of filtered sample was diluted to 50 mL with distilled water:methanol (2:8). The concentration of HT in samples of the diluted solutions was analyzed using UV spectroscopy at 240 nm.

Antimicrobial study

The minimum inhibitory concentration (MIC) of HT was measured using agar dilution procedure. This procedure essentially followed the M7-A7 standard of the Clinical and Laboratory Standards Institute (CLSI) [22]. Test bacteria used were two strains of Gram-positive bacteria (*Bacillus subtilis* NBRC3134 and *Staphylococcus aureus* JCM2413) and two strains of Gram-negative bacteria (*Escherichia coli* JCM5491 and *Pseudomonas aeruginosa* JCM6119). Mueller-Hinton II agar (MHA, Becton, Dickinson and Company, NJ) was used as a test medium. HT was added to have an concentration of 320 μg/mL in MHA. The mixture was stirred, then sequentially diluted with MHA to make a serial two-fold dilution. The test plates were prepared and an inoculum (5-μL) of the bacterium adjusted to 2.0×10^6 CFU/mL, was spotted onto a test plate. After test plates were incubated for 24 hours at 37 °C, the growth of each strain was observed to determine the MIC [23].

Results and Discussion

DSC

Changes in thermal behavior are reported to appear as a result of the formation of inclusion complexes [24]. Thus, DSC was performed to examine the thermal behavior of GMs (Fig. 2, 3). HT crystals and ground HT alone were found to produce an endothermic peak at around 53°C due to the melting point of HT (Fig. 2a, b). An HT/γ-CD PM (HT:γ-CD=1:1), an HT/HP-γ-CD PM (HT:HP-γ-CD=1:1), an HT/γ-CD GM (ground for 60 min, HT:γ-CD=2:1), and an HT/HP-γ-CD GM (ground for 60 min, HT:HP-γ-CD=2:1) all produced an endothermic peak at around 53°C due to the melting of HT crystals (Fig. 2e, f. Fig.3e, f). However, HT/γ-CD GMs (ground for 60 min, HT:γ-CD=1:1 and 1:2) and HT/HP-γ-CD GMs (ground for 60 min, HT:HP-γ-CD=1:1

and 1:2) did not produce an endothermic peak due to the melting of HT crystals (Fig. 2g, h. Fig.3g, h).

The aforementioned changes in thermal behavior presumably indicate molecular interaction in HT/γ-CD, HT/HP-γ-CD, and the GMs. In addition, cogrinding presumably resulted in molecular interaction of HT and γ-CD at a molar ratio of 1:1.

PXRD

PXRD was performed to examine the crystalline state of HT/γ-CD, HT/HP-γ-CD, and coground mixtures of the two. HT crystals and ground HT alone produced a peak characteristic of HT at 2θ=10.3° and 23.7° (Fig. 4a, b). An HT/γ-CD PM (HT:γ-CD=1:1) and an HT/HP-γ-CD PM (HT:HP-γ-CD=1:1) produced a diffraction peak due to HT crystals at 2θ=10.1° and 23.6° and a diffraction peak due to γ-CD at 2θ=12.2° and 16.2° (Fig. 4g, h). In contrast, an HT/γ-CD GM (ground for 60 min, HT:γ-CD=1:1) and an HT/HP-γ-CD GM (ground for 60 min, HT:HP-γ-CD=1:1) did not produce a diffraction peak due to HT crystals and or a diffraction peak due to γ-CD or HP-γ-CD, but they did produce a halo pattern (Fig. 4i, j). Amorphous state, has been reported that there is a possibility of some interaction is occurring [25].

FT-IR analysis

PXRD and DSC presumably indicated molecular interaction of HT and γ-CD or HT and HP-γ-CD. Thus, FT-IR spectroscopy was performed to examine molecules in a solid state (Fig. 5). FT-IR spectroscopy revealed that HT crystals produced peaks presumably due to the carbonyl group (producing a peak at around 1608 cm^{-1}) and the hydroxyl group (producing a peak at around 3200 cm^{-1}) within the HT molecule (Fig. 5a). An HT/γ-CD PM (HT:γ-CD=1:1) and an HT/HP-γ-CD PM (HT:HP-γ-CD=1:1)

both produced absorption peaks due to the carbonyl group and hydroxyl group within the HT molecule as were similarly noted with HT crystals (Fig. 5c, e). However, an HT/γ-CD GM (ground for 60 min, HT:γ-CD=1:1) caused a lower absorption peak (at around 1608 cm^{-1}) due to the carbonyl group of HT. An HT/γ-CD GM (ground for 60 min, HT:γ-CD=1:1) and an HT/HP-γ-CD GM (ground for 60 min, HT:HP-γ-CD=1:1) caused the peak due to the hydroxyl group (at around 3200cm^{-1}) to disappear or they caused a higher absorption peak (Fig. 5d, f). CD is known to take up a water molecule when it does not include a guest molecule, and it replaces the water molecule with the guest molecule as the latter approaches [26]. This probably results in a stable energy state. A peak due to the water of crystallization present in the 8-membered ring of γ-CD was noted at around 1653 cm^{-1}. This peak disappeared with an HT/γ-CD GM (ground for 60 min, HT:γ-CD=1:1) and an HT/HP-γ-CD GM (ground for 60 min, HT:HP-γ-CD=1:1). Thus, dehydration of the water in CD crystals [27] presumably produced hydrophobic molecular interaction.

Dissolution study

Assessment of the physical properties of compounds in a solid state suggested that HT/γ-CD and HT/HP-γ-CD had molecular interaction as a result of the cogrinding of HT and CD. A dissolution test was performed with HT crystals, HT ground for 60 min, an HT/γ-CD PM (HT:γ-CD=1:1), an HT/HP-γ-CD PM (HT:HP-γ-CD=1:1), an HT/γ-CD GM (ground for 60 min, HT:γ-CD=1:1), and an HT/HP-γ-CD GM (ground for 60 min, HT:HP-γ-CD=1:1) to determine the solubility of HT as a result of molecular interaction. Results indicated that HT was present as crystals in the HT-HP-γ-CD PM (HT:HP-γ-CD=1:1) and in the HT/γ-CD PM (HT:γ-CD=1:1), resulting in limited contact and slow dissolution. In contrast, an HT/HP-γ-CD GM (ground for 60 min,

HT:HP-γ-CD=1:1) had a dissolution rate of around 90% and an HT/γ-CD GM (ground for 60 min, HT:γ-CD=1:1) had a dissolution rate of around 80% at 5 min. In the initial stages, HT was almost completely dissolved. In the initial stages, HT crystals dissolve little, and a dissolution rate of around 30% was noted at 60 min. This indicates that an HT/γ-CD GM (ground for 60 min, HT:γ-CD=1:1) and an HT/HP-γ-CD GM (ground for 60 min, HT:HP-γ-CD=1:1) had improved dissolution of HT compared to HT crystals (Fig. 6).

In GMs, the formation of inclusion complexes is affected by HT and CD in a solid state. GMs presumably had improved dissolution compared to HT crystals and PMs.

^{1}H-^{1}H NOESY NMR

Results of a dissolution test revealed that GMs had improved dissolution compared to HT crystals alone. Molecular interaction between HT and γ-CD or between HT and HP-γ-CD in solution may affect solubility. Thus, ^{1}H-^{1}H NOESY NMR spectroscopy was performed to assess the state of molecules in an aqueous solution.

An HT/γ-CD GM (ground for 60 min, HT:γ-CD=1:1) produced cross-peaks between the peaks at H-A, B, C, and D due to the 7-membered ring of HT and the peaks at H-3 and H-5 due to γ-CD, and an HT/γ-CD GM (ground for 60 min, HT:γ-CD=1:1) also produced cross-peaks between the peak at H-F due to the isopropyl group of HT and the peaks at H-3 and H-5 due to γ-CD (Fig. 7a, b). Similarly, an HT/HP-γ-CD GM (ground for 60 min, HT:HP-γ-CD=1:1) produced cross-peaks between the peaks at H-A, B, C, and D due to the 7-membered ring structure of HT and the peaks at H-3, 5 and 6 due to γ-CD. An HT-HP-γ-CD GM (ground for 60 min, HT:HP-γ-CD=1:1) also produced cross-peaks between the peak at H-F due to the isopropyl group of HT and the peak at H-3 due to HP-γ-CD (Fig. 8c, d).

These findings suggest that HT in a GM of HT/γ-CD enters the γ-CD cavity and that the 7-membered ring of HT is located near the wider edge of γ-CD. Protons of the isopropyl group of HT enter the CD cavity and are located near the narrower edge of CD. In a HT/HP-γ-CD GM, the 7-membered ring of HT enters the CD cavity and is located near the narrower edge of γ-CD. Protons of the isopropyl group of HT enter the CD cavity and are located near the wider edge of CD. In HT/γ-CD inclusion complexes, the isopropyl group of HT enters the CD cavity from its wider edge and the 7-membered ring of HT is located at the narrowed edge. In HT/HP-γ-CD inclusion complexes, the 7-membered ring of HT enters the CD cavity and the isopropyl group of HT is located at its wider edge. These findings suggest that HT/γ-CD and HT/HP-γ-CD have different forms of inclusion.

Antimicrobial study

Inclusion complexes were found to have improved dissolution of HT. Thus, an antimicrobial test was performed with 4 strains of bacteria to determine whether inclusion complex formation affects the antimicrobial activity of HT. MICs were also calculated (Table 1).

HT crystals exhibited a MIC of 80 μg/mL against *B. subtilis*, 160 μg/mL against *S. aureus*, 80 μg/mL against *E. coli*, and 320 μg/mL against *P. aeruginosa*. Ground HT alone exhibited almost the same MIC as HT crystals exhibited. An HT/γ-CD PM (HT:γ-CD=1:1) and an HT/HP-γ-CD PM (HT:HP-γ-CD=1:1) were not found to have improved antimicrobial activity compared to HT crystals. However, an HT/γ-CD GM (ground for 60 min, HT:γ-CD=1:1) and an HT/HP-γ-CD GM (ground for 60 min, HT:HP-γ-CD=1:1) exhibited a MIC of 20 μg/mL against *B. subtilis*, 40 μg/mL against *S. aureus*, and 20 μg/mL against *E. coli*. These GMs were found to have 4 times more

antimicrobial activity than HT crystals. In addition, an HT/γ-CD GM (ground for 60 min, HT:γ-CD=1:1) and an HT/HP-γ-CD GM (ground for 60 min, HT:HP-γ-CD=1:1) exhibited a MIC of 160 μg/mL against *P. aeruginosa*. These GMs were found to have 2 times more antimicrobial activity than HT crystals. The mechanism for the antimicrobial and microbicidal action of HT is known to be via metabolic inhibition of the cell membrane in conjunction with reduced permeability of the cell membrane and suppressed respiration [28]. A study involving the formation of inclusion complexes by cefdinir, an antibiotic, and HP-β-CD, an analog of β-CD, reported that those complexes improved the antimicrobial activity of cefdinir against 2 species of bacteria, *S. aureus* and *E. coli* [27]. Formation of inclusion complexes with CD improved the antimicrobial activity of cefdinir by enhancing its solubility (normally, it is poorly soluble) and improving its stability. In the current study, the antimicrobial activity of HT improved with the formation of inclusion complexes. This is presumably evidence of the improved solubility of HT. A study reported that inclusion of chlorogenic acid in β-CD improved the stability of chlorogenic acid and helped to improve its antimicrobial activity without diminishing its antimicrobial activity [29]. Similarly, inclusion of HT in CD in the current study improved the stability of HT and may have helped to improve its antimicrobial activity. The stability of HT and inclusion complexes must be assessed in the future.

Conclusion

Use of cogrinding was found to produce HT/γ-CD and HT/HP-γ-CD inclusion complexes in a solid state. Assessment of the physical properties of the compounds in a solid state and in solution revealed that the molar ratios for the inclusion complexes were HT:γ-CD=1:1 and HT:HP-γ-CD=1:1.

Improved solubility of HT was noted with GMs. The mechanism for this action was molecular interaction in a solid state and in solution.

Moreover, improved solubility of HT resulted in improved antimicrobial activity of HT for GMs. CD is reported to form inclusion complexes with other drugs, resulting in improved stability to light [30]. Similarly, HT formed inclusion complexes with CD in the current study, inhibiting photolysis. If a high level of antimicrobial activity can be maintained, then HT can be used to develop products such as food additives and pharmaceuticals. In addition, this approach may allow effective use of HT in humans and in the environment.

In the future, sites of molecular interaction must be elucidated in further detail. Complexes formed HT by different CDs must also be examined and compared using various CDs and CD analogs. Moreover, methods of preparation besides cogrinding must also be studied.

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FIGURE LEGEND

Table 1 Antimicrobial activity of hinokitiol, PMs and GMs

Fig. 1 Chemical Structures of (a) HT and (b) γ-CD and HP-γ-CD

Fig. 2 DSC curves of HT/γ-CD systems

(a) HT crystal, (b) HT ground 60min, (c) γ-CD, (d) γ-CD ground 60min, (e) PM(HT:γ-CD=1:1) (f) GM60min (HT:γ-CD=2:1), (g) GM60min (HT:γ-CD=1:1), (h) GM60min (HT:γ-CD=1:2)

Fig. 3 DSC curves of HT/HP-γ-CD systems

(a) HT crystal, (b) HT ground 60min, (c) HP-γ-CD, (d) HP-γ-CD ground 60min, (e) PM(HT:HP-γ-CD=1:1) (f) GM60min (HT:HP-γ-CD=2:1), (g) GM60min (HT:HP-γ-CD=1:1), (h) GM60min (HT:HP-γ-CD=1:2)

Fig. 4 PXRD patterns of HT/CDs systems

(a) HT crystal, (b) HT ground 60min, (c) γ-CD, (d) γ-CD ground 60min, (e) HP-γ-CD,

(f) HP-γ-CD ground 60min, (g) PM (HT:γ-CD=1:1), (h) PM (HT:HP-γ-CD=1:1), (i) GM60min (HT:γ-CD=1:1), (j) GM60min (HT:HP-γ-CD=1:1)

▲:HT, ●:γ-CD

Fig. 5 FT-IR spectra of HT/CDs systems

(a) HT crystal, (b) γ-CD, (c) HP-γ-CD, (d) PM (HT:γ-CD=1:1), (e) PM (HT:HP-γ-CD=1:1), (f) GM 60 min (HT:γ-CD=1:1), (g) GM60min (HT:HP-γ-CD=1:1)

Fig. 6 Dissolution profiles of HT/CDs systems in 900 mL of water (37±0.5ºC)

◇ :HT crystal, ◆ :HT ground 60min, ○ :PM (HT:γ-CD=1:1), □ :PM (HT:HP-γ-CD=1:1), ●:GM60min (HT:γ-CD=1:1), ■:GM60min (HT:HP-γ-CD=1:1)

Results were expressed as mean±S.D. (n=3)

Fig. 7 ^{1}H-^{1}H NOESY NMR spectrum of HT/CDs in D_2O

(a) GM60min (molar ratio of HT:γ-CD = 1:1) X is 7.0-7.5 and the Y axis is 3.2-4.0,

(b) GM60min (molar ratio of HT:γ-CD = 1:1) X is 0.8-1.5 and the Y axis is 3.2-4.0

Fig. 8 ^{1}H-^{1}H NOESY NMR spectrum of HT/CDs in D_2O

(c) GM60min (molar ratio of HT:HP-γ-CD = 1:1) X is 6.9-7.5 and the Y axis is 3.3-4.0,

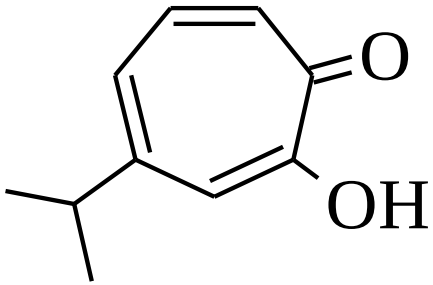
(d) GM60min (molar ratio of HT:HP-γ-CD = 1:1) X is 3.3-4.0 and the Y axis is 3.3-4.0

Table 1 Antimicrobial activity of hinokitiol, PMs and GMs

	MIC (μg/mL)			
	B.sub	*S.a*	*E.coli*	*P.aer*
γ-CD	-	-	-	-
HP-γ-CD	-	-	-	-
HT crystal	80	160	80	320
HT ground	40	40	80	320
PM (HT:γ-CD=1:1)	40	80	80	320
PM (HT:HP-γ-CD=1:1)	40	80	80	320
GM (HT:γ-CD=1:1)	20	40	20	160
GM (HT:HP-γ-CD=1:1)	20	40	20	160

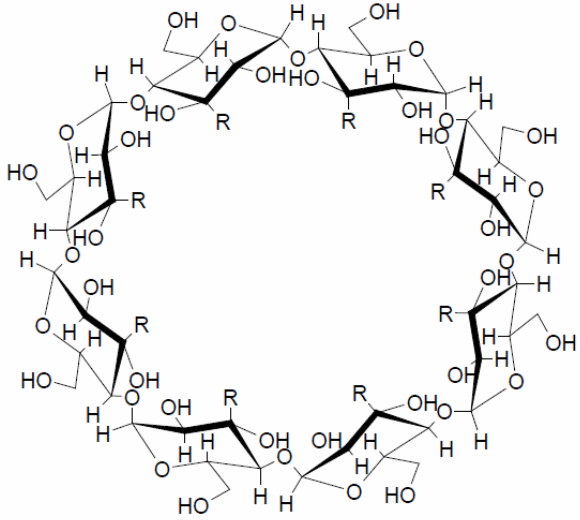
B. sub : Bacillus subtilis
S. a : Staphylococcus aureus
E. coli : Escherichia coli
P. aer : Pseudomonas aeruginosa

(a)



Hinokitiol (HT)
Mf: $C_{10}H_{12}O_2$
Mw: 164.2

(b)



R = H
γ-Cyclodextrin

R = H or $CH_2CH(OH)CH_3$
Hydroxypropyl-γ-Cyclodextrin

γ-Cyclodextrin (γ-CD)
Mf: $C_{48}H_{80}O_{40}$
Mw: 1297.1

Hydroxypropyl-γ-Cyclodextrin (HP-γ-CD)
Mw: 1580.0

Fig. 1 Chemical Structures of (a) HT and (b) γ-CD and HP-γ-CD

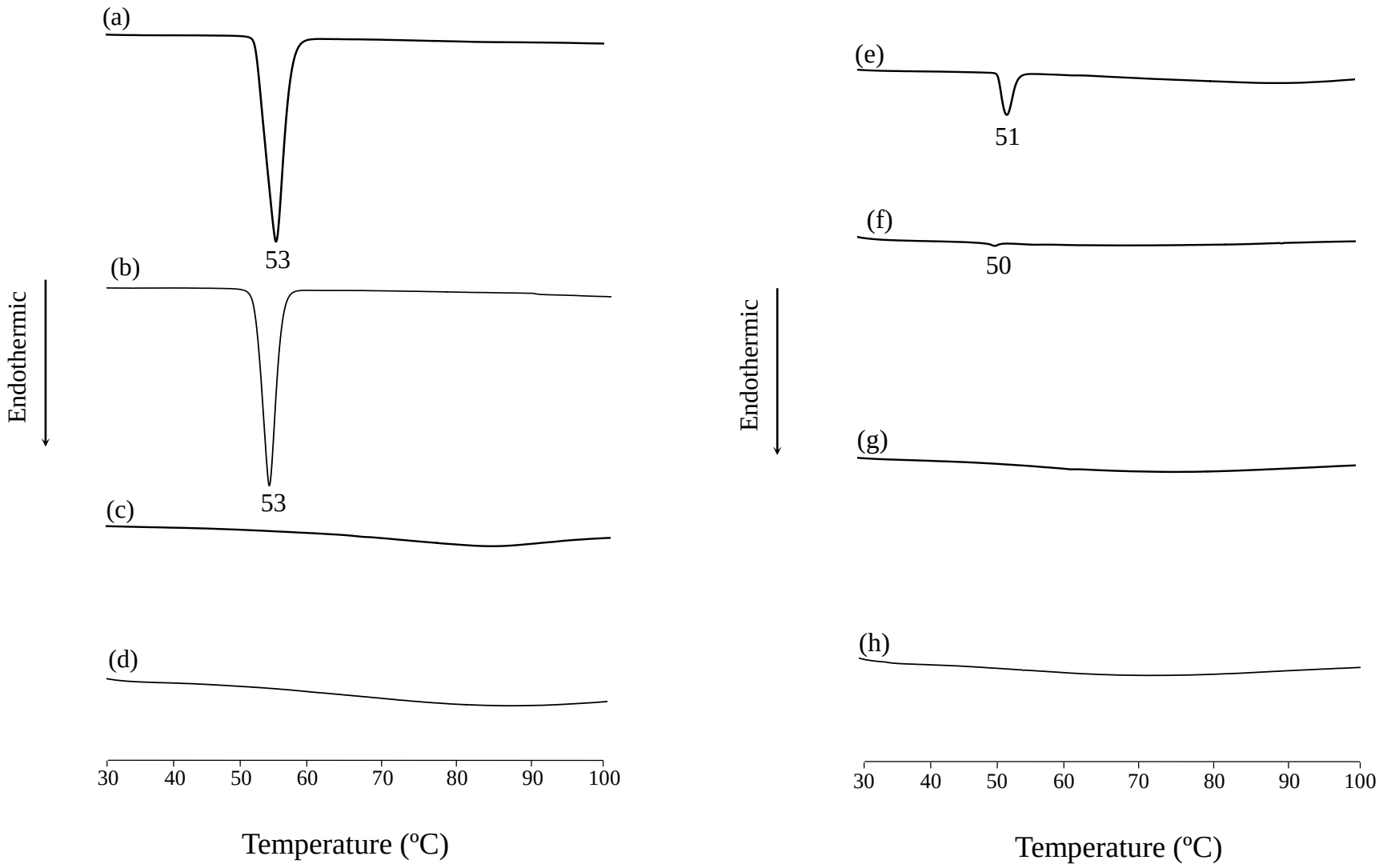


Fig. 2 DSC curves of HT/γ-CD systems

(a) HT crystal, (b) HT ground 60min, (c) γ-CD, (d) γ-CD ground 60min, (e) PM(HT:γ-CD=1:1) (f) GM60min (HT:γ-CD=2:1), (g) GM60min (HT:γ-CD=1:1), (h) GM60min (HT:γ-CD=1:2)

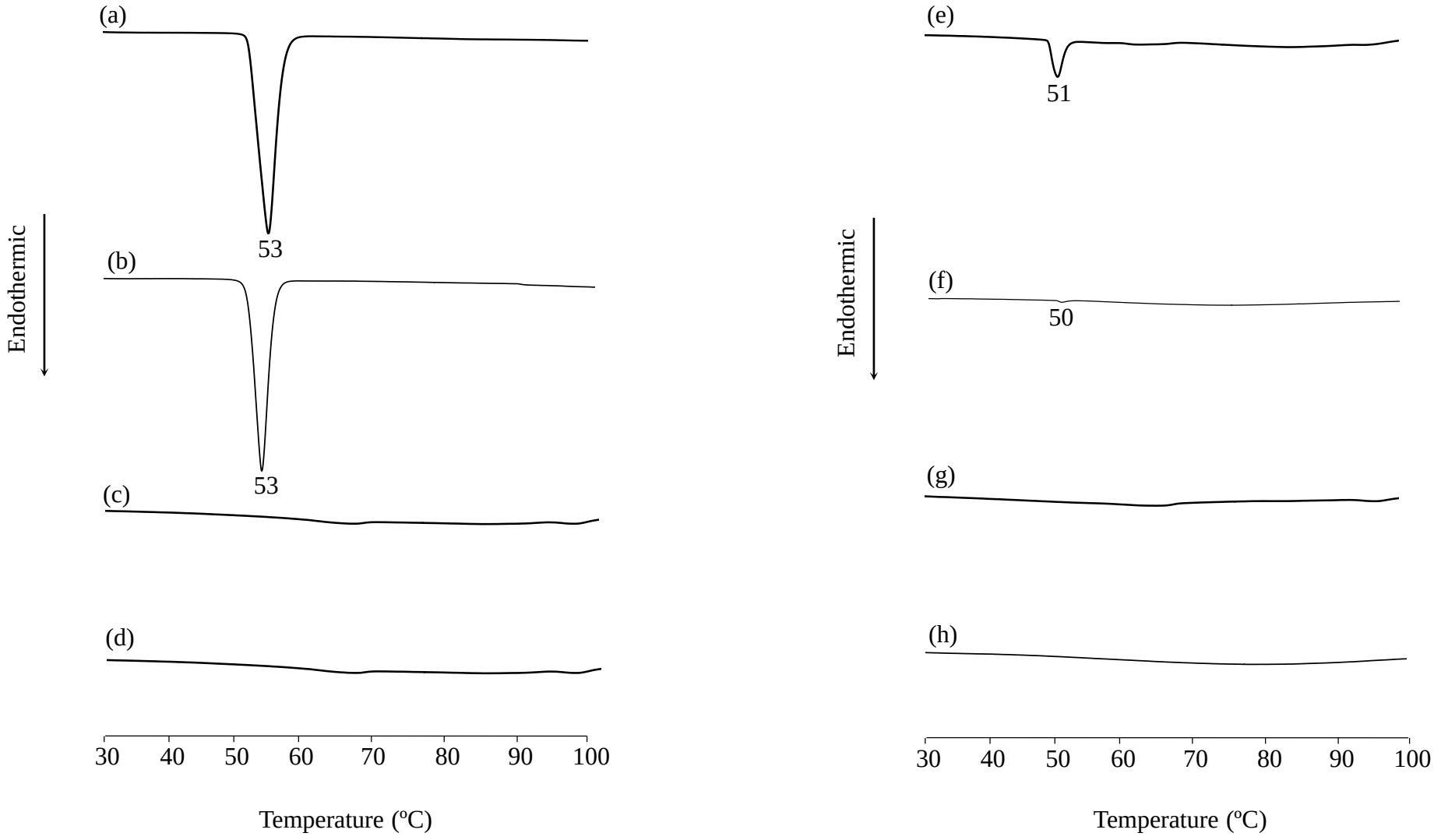


Fig. 3 DSC curves of HT/HP-γ-CD systems

(a) HT crystal, (b) HT ground 60min, (c) HP-γ-CD, (d) HP-γ-CD ground 60min, (e) PM(HT:HP-γ-CD=1:1) (f) GM60min (HT:HP-γ-CD=2:1), (g) GM60min (HT:HP-γ-CD=1:1), (h) GM60min (HT:HP-γ-CD=1:2)

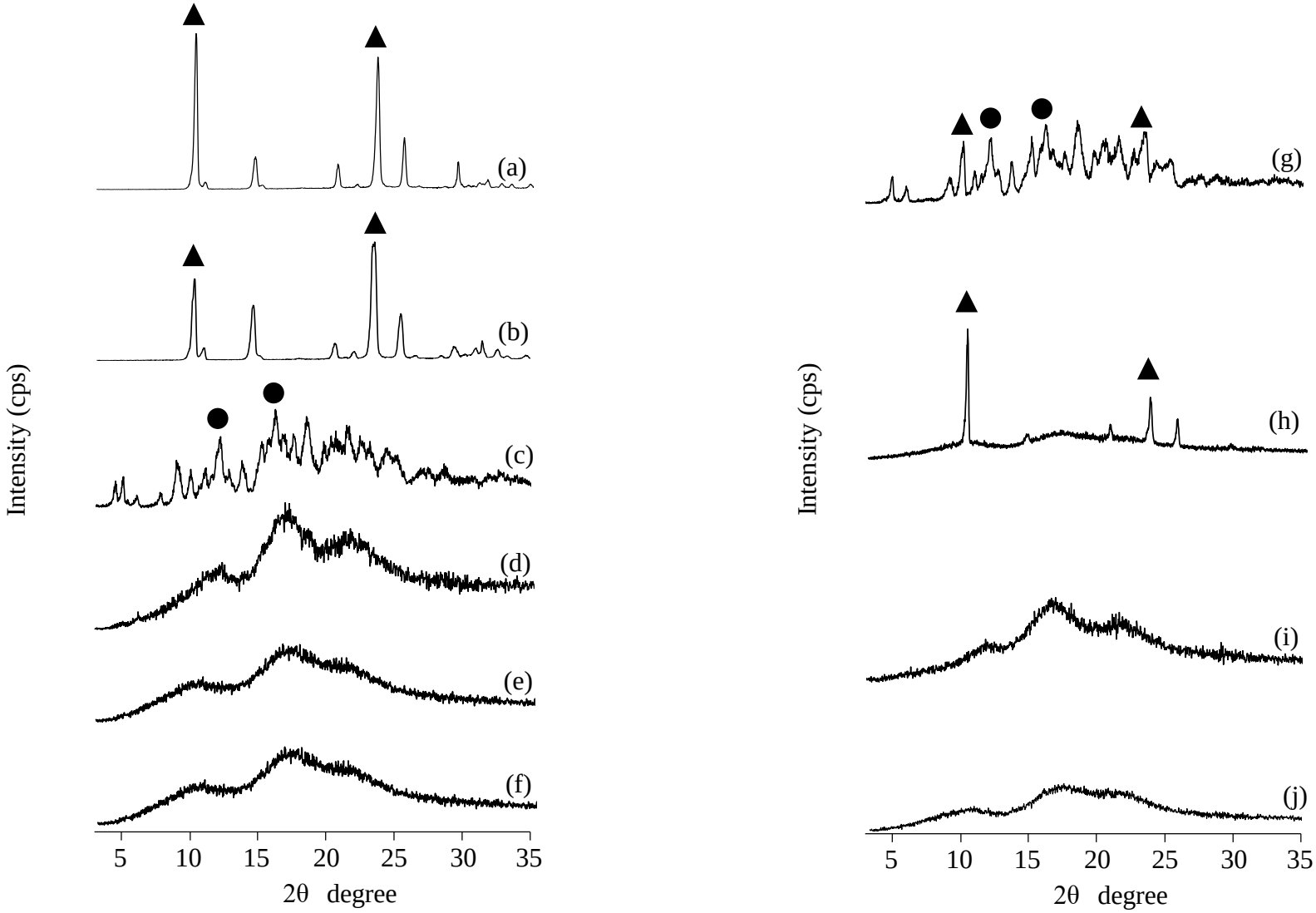


Fig. 4 PXRD patterns of HT/CDs systems

(a) HT crystal, (b) HT ground 60min, (c) γ-CD, (d) γ-CD ground 60min, (e) HP-γ-CD, (f) HP-γ-CD ground 60min, (g) PM (HT:γ-CD=1:1), (h) PM (HT:HP-γ-CD=1:1), (i) GM60min (HT:γ-CD=1:1), (j) GM60min (HT:HP-γ-CD=1:1)

▲:HT, ●:γ-CD

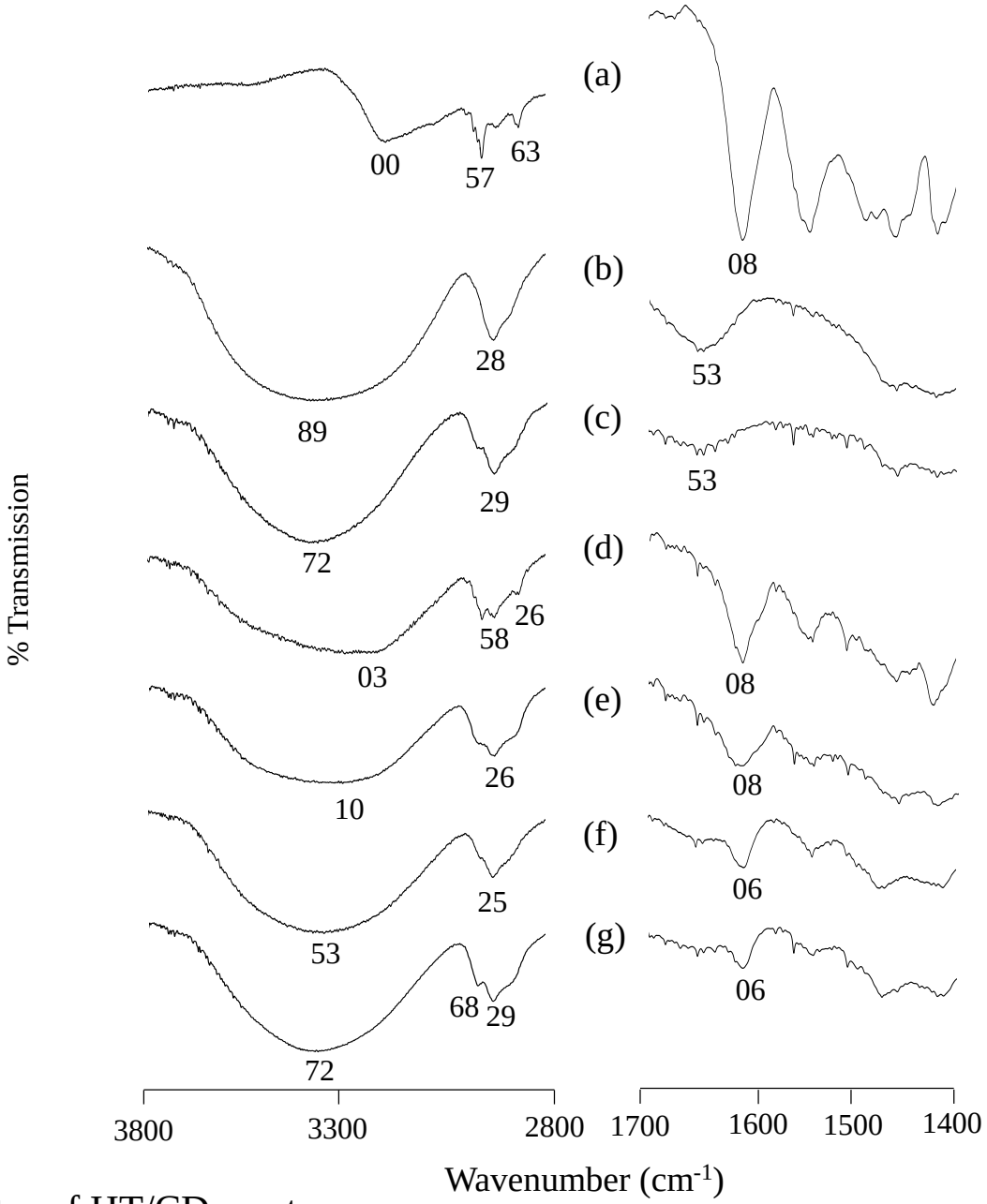
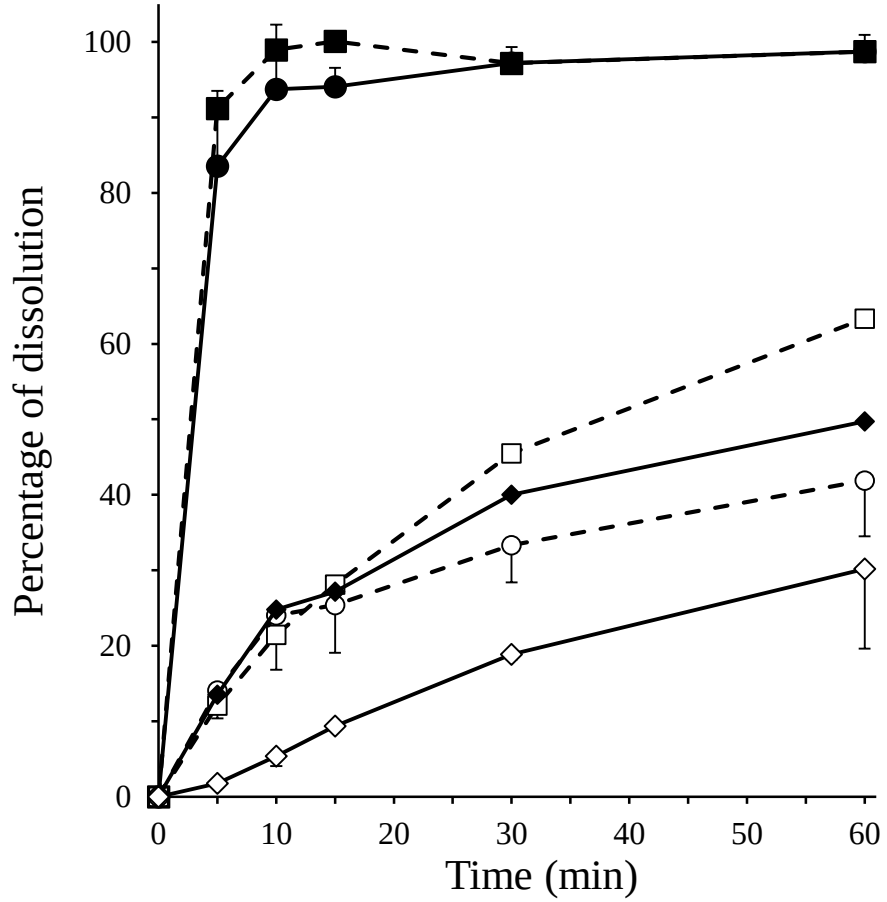


Fig. 5 FT-IR spectra of HT/CDs systems

(a) HT crystal, (b) γ-CD, (c) HP-γ-CD(d) PM (HT:γ-CD=1:1), (e) PM (HT:HP-γ-CD=1:1), (f) GM 60 min (HT:γ-CD=1:1), (g) GM60min (HT:HP-γ-CD=1:1)



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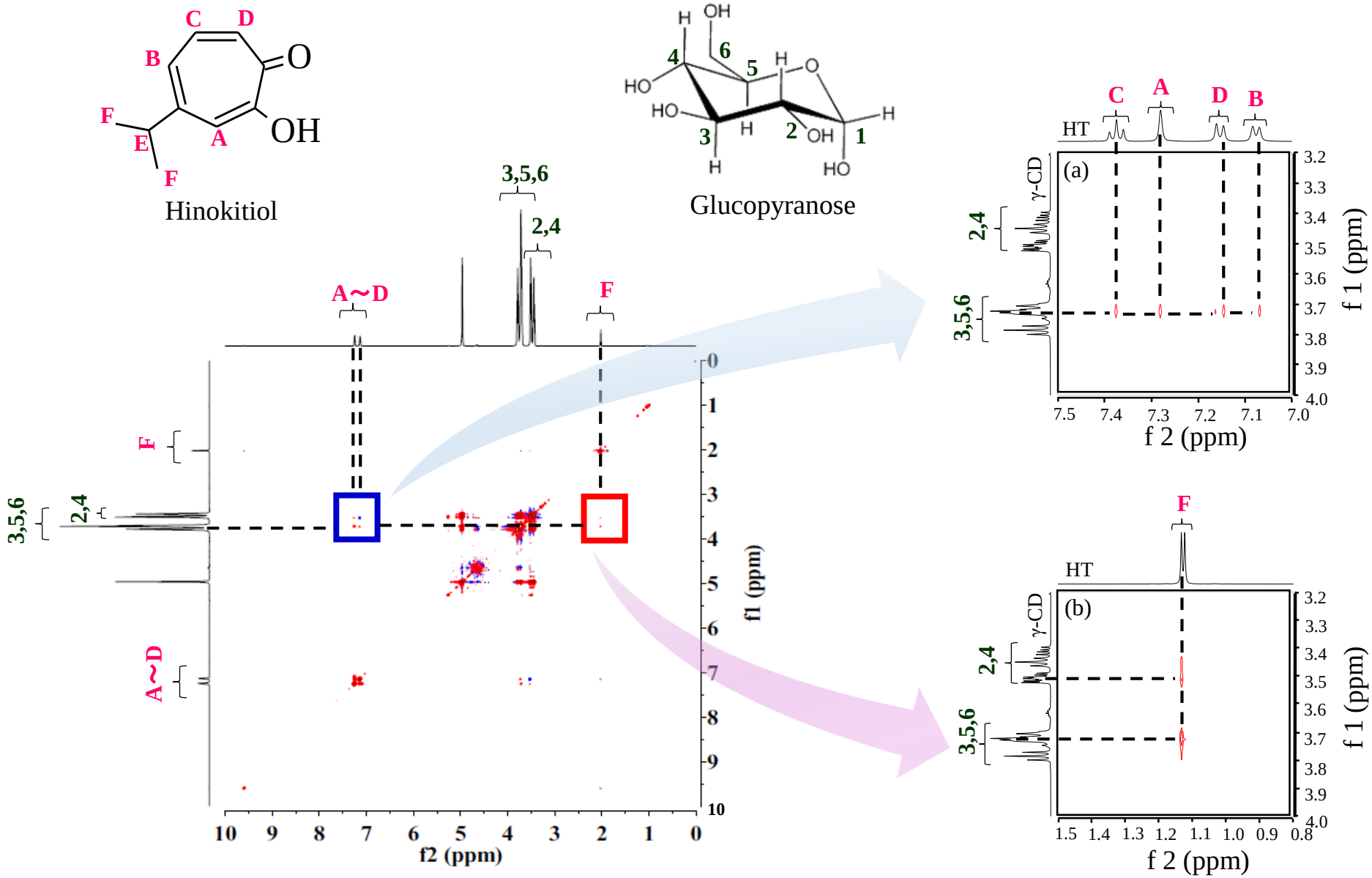
◇:HT crystal, ◆:HT ground 60min, ○:PM (HT:γ-CD=1:1), □:PM (HT:HP-γ-CD=1:1), ●:GM60min (HT:γ-CD=1:1), ■:GM60min (HT:HP-γ-CD=1:1).

Results were expressed as mean±S.D. (n=3)



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(a) GM60min (molar ratio of HT:γ-CD = 1:1) X is 7.0-7.5 and the Y axis is 3.2-4.0,
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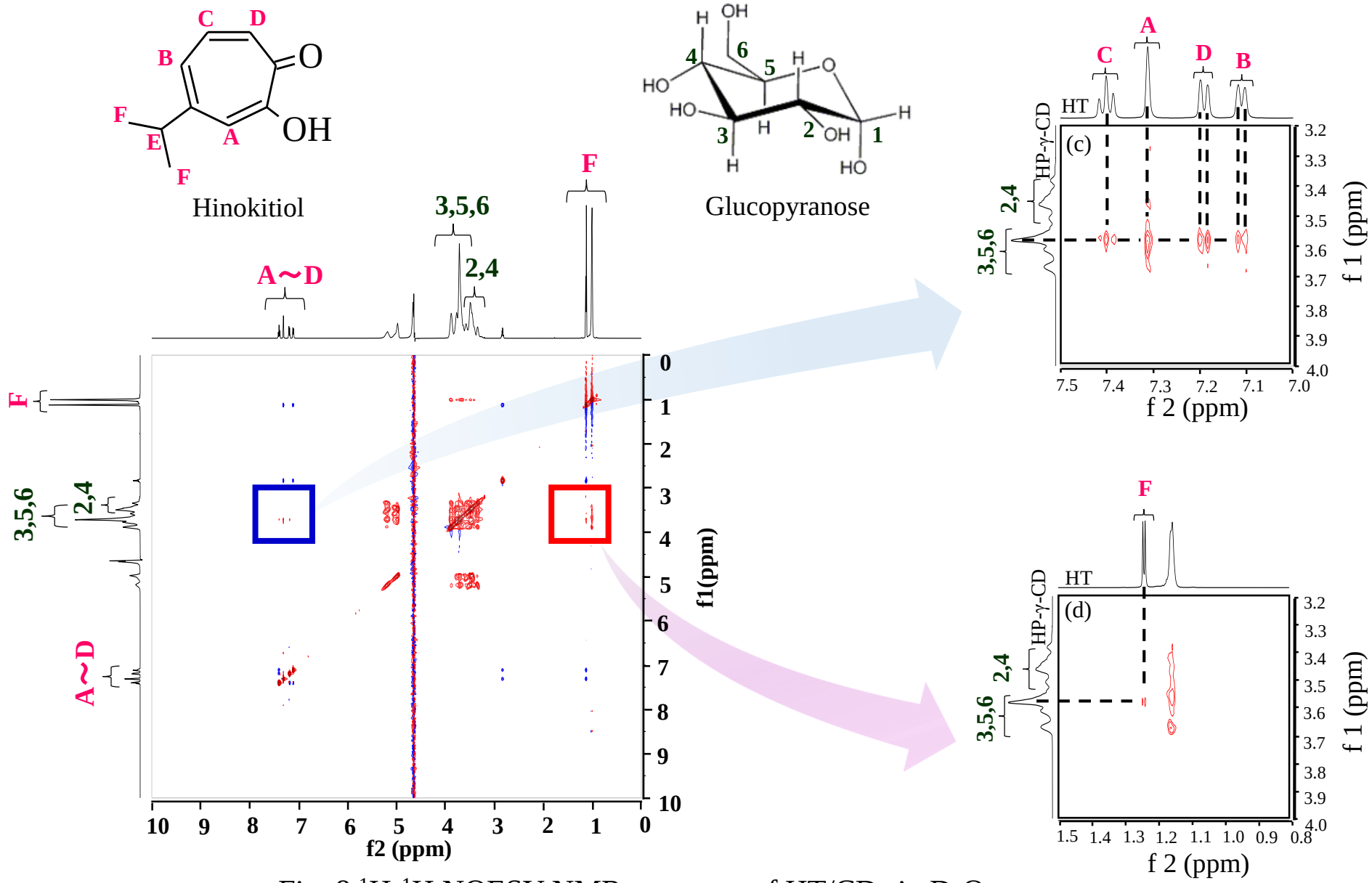

Fig. 8 ^{1}H-^{1}H NOESY NMR spectrum of HT/CDs in D_2O

(c) GM60min (molar ratio of HT:HP-γ-CD = 1:1) X is 6.9-7.5 and the Y axis is 3.3-4.0,

(d) GM60min (molar ratio of HT:HP-γ-CD = 1:1) X is 3.3-4.0 and the Y axis is 3.3-4.0