

## Original Research Article

# Inclusion Mechanism and Heat Stability of the Complex of 4'-Hydroxychalcone and Hydroxypropyl- $\beta$ -Cyclodextrin

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### Abstract

**Purpose:** To study the inclusion mechanism and heat stability of the complex formed between 4'-hydroxychalcone and hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD)

**Methods:** The inclusion mechanism of the complex of 4'-hydroxychalcone and HP- $\beta$ -CD was investigated by phase solubility method. The physicochemical properties of the complex were evaluated by Fourier transform infrared spectroscopy (FT-IR) and x-ray diffractometry (XRD) while the heat stability of the complex was measured by thermogravimetric/differential scanning calorimetry (TG/DSC).

**Results:** The stability constants of the complexes were higher than those of  $\beta$ -CD at all temperatures. For the formation of the complex, Gibbs energy (-16.87 KJ/mol at 20 °C) was negative, and positive entropy (31.15 KJ/mol) along with a slightly positive enthalpic change (163.89 J/mol) was also found. IR and XRD analysis showed that the physical phase of 4-hydroxychalcone was changed and completely dispersed in HP- $\beta$ -CD while TG/DSC data indicate that the starting decomposition temperature of 4'-hydroxychalcone (200 °C) was significantly enhanced to 397 °C due to the formation of inclusion complex with HP- $\beta$ -CD in the solid state.

**Conclusion:** The stability constant of HP- $\beta$ -CD for 4'-hydroxychalcone is higher than that of  $\beta$ -CD. The inclusion is a spontaneous process, driven by hydrophobic forces. The heat stability of 4'-hydroxychalcone is significantly enhanced by complexing with HP- $\beta$ -CD.

**Keywords:** 4'-Hydroxychalcone, Hydroxypropyl- $\beta$ -cyclodextrin, Inclusion complex, Phase solubility, Thermogravimetry, Differential scanning calorimetry

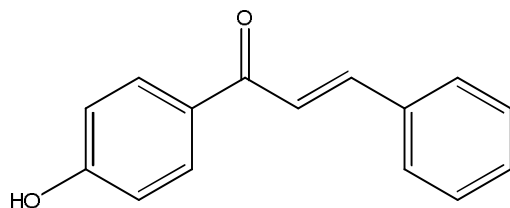
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## INTRODUCTION

Chalcones, a kind of flavonoid, are polyphenolic compounds that usually exist in plants as secondary metabolites. Several beneficial properties have been attributed to these dietary compounds, including antioxidant, anti-inflammatory, and anticarcinogenic, anticancer activities [1-3]. But chalcones are poorly water soluble, which severely restricts its application in medicinal and food formulations.

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of six ( $\alpha$ -cyclodextrin), seven ( $\beta$ -cyclodextrin), eight ( $\gamma$ -cyclodextrin) or more glucopyranose units linked by  $\alpha$ -(1, 4) bonds, which have been used extensively as additives to increase the solubility of poorly water-soluble organic compounds by the formation of an inclusion complex [4,5]. Hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD), a hydroxyalkyl derivative of cyclodextrin, is an alternative to  $\alpha$ -,  $\beta$ - and  $\gamma$ -cyclodextrin, with improved water solubility and may be slightly more toxicologically benign. FDA

has approved the application of HP- $\beta$ -CD in food, agriculture and the pharmaceutical field [6].



**Figure 1:** Chemical structure of 4'-hydroxychalcone

To the best of our knowledge, there are few reports exploring the complex of HP- $\beta$ -CD and chalcone. In this study, in order to investigate the supramolecular complexation between chalcone and HP- $\beta$ -CD, 4'-hydroxychalcone (Figure 1) and two CDs ( $\beta$ -CD and HP- $\beta$ -CD) were selected for phase-solubility study. The physicochemical properties of the obtained complexes were investigated by IR and XRD. And their heat stabilities were also studied by TG/DSC analysis.

## EXPERIMENTAL

### Materials and chemicals

4'-Hydroxychalcone (> 95 %) was obtained from Tokyo Chemical Industry Co Ltd (Tokyo, Japan) while (2-hydroxypropyl)- $\beta$ -cyclodextrin (average MW 1460) was purchased from Sigma (St Louis, MO). Other chemicals were of analytical grade unless stated otherwise.

### Phase solubility studies

Phase solubility studies were carried out according to the method described by Higuchi and Connors [7]. An excess amount of 4'-hydroxychalcone (about 50 mg) was added to a series of test tubes each containing 5 mL of  $\beta$ -CD and HP- $\beta$ -CD solutions at the concentrations ranging from 0 to 10 mM, and the mixture was vortexed for 2 min to disperse the sample. The test tubes were shaken in a rotary water bath (180 rpm) set at 20-40 °C for 72 h. After that, the mixture was filtered through a 0.45  $\mu$ m membrane filter and appropriately diluted. The concentrations of the dissolved 4'-hydroxychalcone in the solution were determined by measuring their absorbance with a TU-1810PC UV spectrophotometer (Purkinje, Beijing, China) at 320 nm comparing them with the corresponding standard curves, respectively. The apparent stability constants ( $K_s$ ) of the complexes were calculated from phase-solubility diagrams as in Eq 1.

$$K_s = \text{slope/intercept (1-slope)} \dots\dots\dots (1)$$

### Preparation of inclusion complex of 4'-hydroxychalcone and HP- $\beta$ -CD

4'-Hydroxychalcone (0.224 g, 1 mM) and HP- $\beta$ -CD (1.460 g, 1 mM) was mixed in 25 mL of distilled water, stirred for 72 h at 40 °C and filtered through a 0.45  $\mu$ m membrane filter to remove undissolved material. The filtrate was freeze-dried (Alpha 1-2, Christ, Germany) and the resultant powdery material was weighed and collected as the inclusion complex of 4'-hydroxychalcone and HP- $\beta$ -CD for the following analysis.

### Preparation of physical mixture of 4'-hydroxychalcone and HP- $\beta$ -CD

4'-Hydroxychalcone (0.224 g, 1 mM) and HP- $\beta$ -CD (1.460 g, 1 mM) was mixed thoroughly in a small beaker at room temperature, respectively. The obtained product was collected as the physical mixture of 4'-hydroxychalcone and HP- $\beta$ -CD.

### Fourier transform infrared spectroscopy (FT-IR)

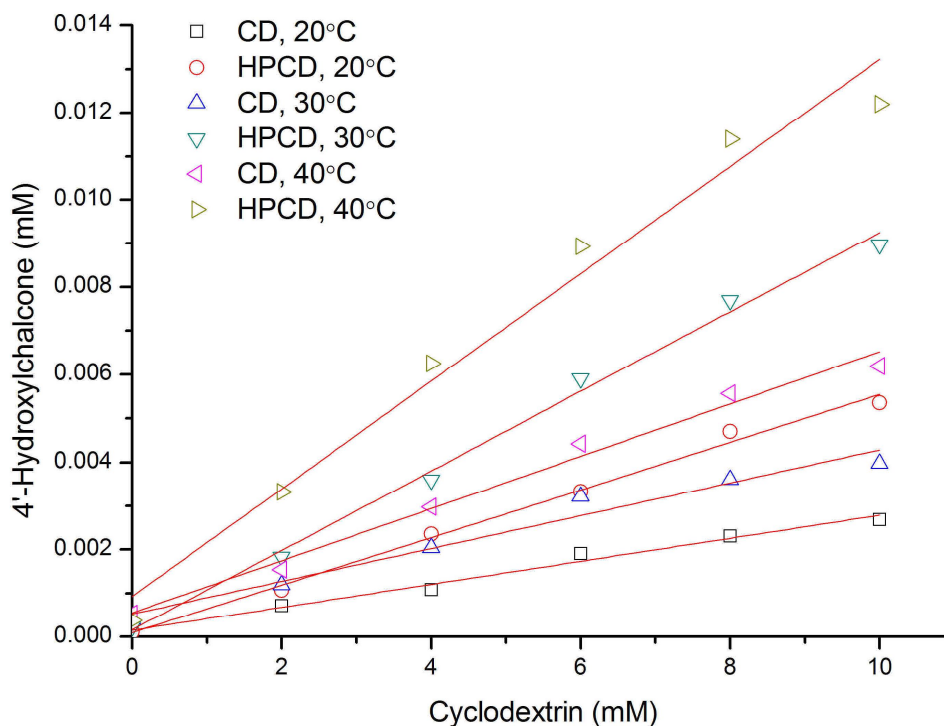
FT-IR was conducted using a Tensor 27 FT-IR spectrometer (Bruker, Germany). The spectrum in the mid-IR (400-4000  $\text{cm}^{-1}$ ) spectral region was collected. The procedure consisted of grinding the sample together with KBr into a fine powder, placing the powder into the sampling cup, smoothing the powder, and compressing the powder bed into the holder using a compression gauge. The sample was placed in the light path and the spectrum was obtained.

### X-ray diffractometry (XRD)

Monochromatic Cu Ka radiation (wavelength = 1.54056  $\text{\AA}$ ) was produced by a D8 Advance X-ray diffractometer (Bruker, Germany). The powdery samples were packed tightly in a rectangular aluminum cell prior to exposure to the X-ray beam. The scanning regions of the diffraction angle,  $2\theta$ , were 5 - 80° and radiation was detected with a proportional detector.

### Thermogravimetric/differential scanning calorimetry measurement (TG/DSC)

The Q600 TG/DSC system (TA, USA) was adjusted to operate at the following conditions: dynamic atmosphere of nitrogen (99.999 %) at 100 ml/min and heating rate of 20 °C /min from 20 to 500 °C, with sample mass of about 5 mg.



**Figure 2:** Phase-solubility diagrams of inclusion complexes formed by 4'-hydroxychalcone and cyclodextrins

## RESULTS

### Phase solubility result

The phase-solubility plots of different chalcone-CD complexes were showed in Figure 2. It was found the concentration of 4'-hydroxychalcone increased with the increasing CD concentrations. The stability constants ( $K_s$ ) of the complexes, calculated from the slopes of the linear phase-solubility plots, were summarized in Table 1. Of the three temperatures, the stability constants were highest for the complexes formed with HP- $\beta$ -CD, followed by  $\beta$ -CD.

For the formation of an inclusion complex, there must be a favorable net energetic force that drives the guest into the cyclodextrin cavity [4]. To understand the thermodynamic forces driving

the inclusion process between chalcones and CDs, The thermodynamic parameters were calculated from the phase solubility. Based on the Van't Hoff equation (Equation (2)), the changes of enthalpy and entropy were revealed. The Gibbs energy change was also computed from the stability constants using Equation (3) [8].

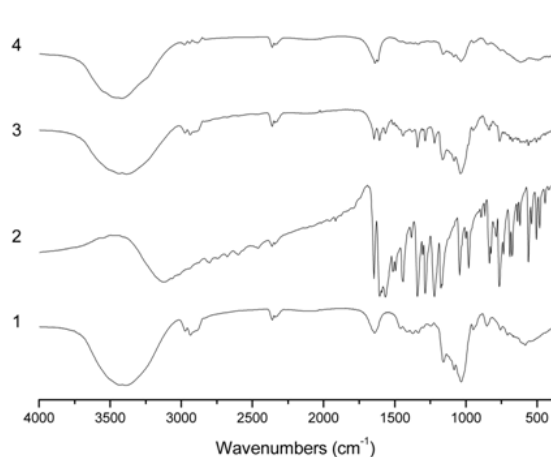
$$\ln K_c = -(\Delta H/RT) + (\Delta S/R) \dots\dots\dots (2)$$

$$\Delta G = \Delta H - T\Delta S \dots\dots\dots (3)$$

Where R and T are the gas constant and temperature (K), respectively. These thermodynamic parameters for the formation of inclusion complexes between chalcones and CDs were summarized in Table 1.

**Table 1:** Thermodynamic parameters for the formation of inclusion complexes between 4'-hydroxychalcone and HP- $\beta$ -CD

Compound	Temperature (°C)	$K_s$ ( $M^{-1}$ )	$\Delta G$ (KJ/mol)	$\Delta H$ (KJ/mol)	$\Delta S$ (J/mol)
4'-Hydroxychalcone	$\beta$ -CD	20	479.04	-14.98	
		30	683.10	-16.56	31.07
		40	1083.84	-18.13	
	HP- $\beta$ -CD	20	989.74	-16.87	
		30	1651.45	-18.51	31.15
		40	2235.93	-20.15	



**Figure 3:** IR spectra of HP-β-CD (1), 4-hydroxychalcone (2), their physical mixture (3) and complex (4)

### FT-IR spectra

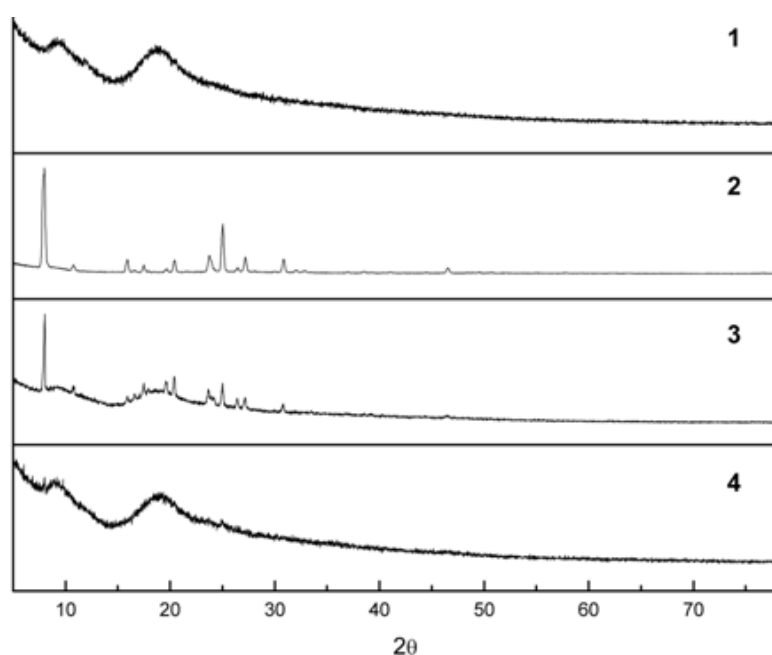
The infrared spectra of HP-β-CD, 4'-hydroxychalcone, their physical mixture and the complex were shown in Figure 3. The FT-IR spectrum of HP-β-CD showed prominent absorption bands at  $3387\text{ cm}^{-1}$  (for O-H stretching vibrations),  $2935\text{ cm}^{-1}$  (for C-H stretching vibrations) and  $1158$ ,  $1083$  and  $1033\text{ cm}^{-1}$  (for C-H, C-O stretching vibration). The FT-IR spectrum of 4'-hydroxychalcone and 4-hydroxychalcone consisted of the prominent absorption bands of the hydroxyl group (about  $3122\text{ cm}^{-1}$ ), the aromatic conjugated carbonyl

group (about  $1646\text{ cm}^{-1}$ ) and the aromatic nucleus (about  $1566$ ,  $1512$ ,  $1494$ ,  $1442\text{ cm}^{-1}$ ). The IR spectra of the physical mixture of HP-β-CD and chalcones displayed the spectral addition effect and were essentially a combination of the spectra of HP-β-CD and corresponding chalcone. However, in the spectra of their inclusion complex, several small but characteristic absorption peaks of chalcone between  $400$  and  $1600\text{ cm}^{-1}$  almost disappeared.

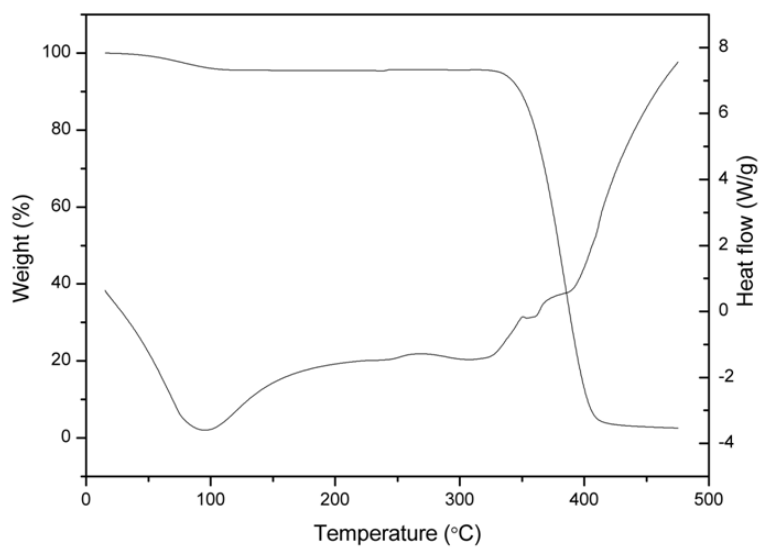
### XRD

The powder x-ray diffraction patterns of HP-β-CD, 4'-hydroxychalcone, their mixture and complex were shown in Figure 4. The powder diffraction pattern of 4'-hydroxychalcone displayed sharp crystalline peaks, which is the characteristic of an organ molecule with crystallinity. In contrast, HP-β-CD showed two broad peaks lacking crystalline peaks. The XRD pattern of the physical mixture of the powder of 4'-hydroxychalcone and HP-β-CD showed essentially a superposition of the patterns of the two compounds, confirming that no inclusion was formed between them and both retained their original physical characteristics.

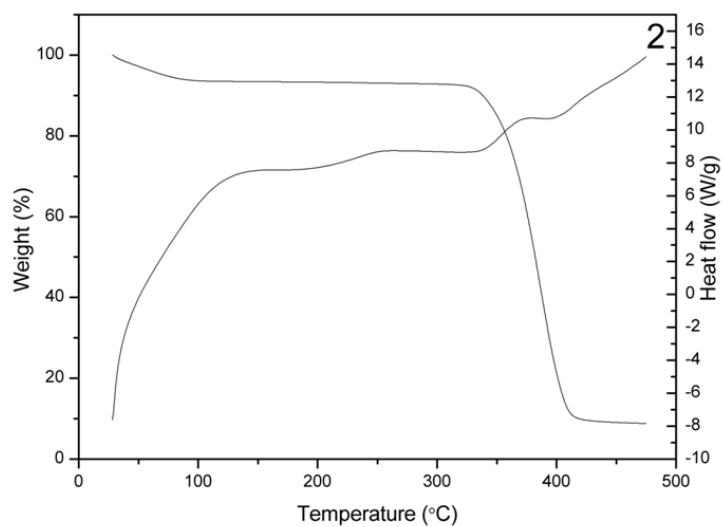
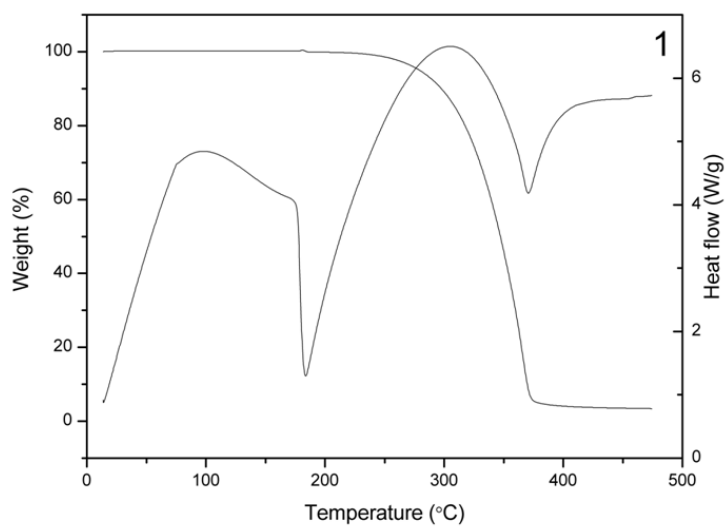
In contrast, the XRD pattern of the inclusion complex was virtually the same as that of the amorphous HP-β-CD and exhibited none of the characteristic peaks of 4'-hydroxychalcone.



**Figure 4:** XRD patterns of HP-β-CD (1), 4-hydroxychalcone (2), their physical mixture (3) and complex (4)



**Figure 5:** TG/DTA curves of HP- $\beta$ -CD



**Figure 6:** TG/DTA curves of 4'-hydroxylchalcone (1) and its complex (2)

## TG/DSC

The TG and DSC thermograms of HP- $\beta$ -CD, 4'-hydroxychalcone and their inclusion complex were illustrated in Figure 5-6, respectively. It could be clearly found that the TG and DSC curves of HP- $\beta$ -CD and 4'-hydroxychalcone mainly have one step or one peak, indicating that both the samples were pure compounds. Along with the increase of the temperature, HP- $\beta$ -CD began to decompose at about 320 °C and this ended at about 420 °C. The decomposing peak value (about 360 °C) was obtained from the DSC curve. And the fusion and degradation of HP- $\beta$ -CD were completed in one step.

Different from this, the DSC curves of 4'-hydroxychalcone had an obvious endothermic peak where the weights of 4'-hydroxychalcone had not decreased, suggesting that 4'-hydroxychalcone melted at about 177 °C. Then 4'-hydroxychalcone began degradation at about 200 °C, ending at about 400 °C. At the DSC curve of each complex, there were two endothermic peaks at 344 and 397 °C, respectively. The latter should be the decomposition peak of 4'-hydroxychalcone, with 344 °C being the peak for HP- $\beta$ -CD. Furthermore, the DSC curve showed the disappearance of the melting peak of 4'-hydroxychalcone.

## DISCUSSION

Phase solubility study is the quantitative determination of the solubility of a guest substance at the various concentrations of CD, which can yield the solubility diagram of the dissolved guest substance against different concentrations of CD. In this study, the phase-solubility plots of different chalcone-CD complexes all displayed a typical AL type diagram (i.e., linear increases in chalcone solubility with increasing CD concentrations) within the concentration range studied (Figure 2), indicating 1:1 molecular complexes between the chalcones and CDs [7]. And the stability constants were highest for the complexes formed with HP- $\beta$ -CD, followed by  $\beta$ -CD, which suggested that HP- $\beta$ -CD had the greater capacity to form inclusion complexes with chalcones than the parent  $\beta$ -CD.

For Table 1, it could be found that the Gibbs energy values were negative for all the complexes, indicating that the inclusion was a spontaneous process and thermodynamically favored. Furthermore, the positive enthalpic and entropic values indicated that the inclusion

process was endothermic and mainly entropically driven. The positive entropy together with a slightly positive enthalpic change means that the driving force for the process is essentially hydrophobic interaction [9]. Similar temperature effect on the stability constant was also observed in the complexation of morin with  $\beta$ -CD and HP- $\beta$ -CD [10]. It was inferred that when the chalcone was free in solution, it may have a strong interaction with its solvent shell. Upon forming an inclusion complex with CDs, this solvent shell was broken up, leading to a partly unfavorable enthalpic change.

According to IR and XRD result, it could be found that that the physical phase of 4'-hydroxychalcone had been changed, which were completely dispersed in HP- $\beta$ -CD. For DSC/TG, it could be concluded that the presence of 4'-hydroxychalcone in the cavity of HP- $\beta$ -CD made it degrade at a lower temperature. The starting decomposition temperature of 4'-hydroxychalcone increased, due to the protection of HP- $\beta$ -CD. The decomposition process should be that along with the increase of the temperature, HP- $\beta$ -CD decomposed first and exposed 4'-hydroxychalcone to the heat, 4'-hydroxychalcone melted and degraded almost at the same time under so high temperature. A similar result has been reported by [11]. DSC/TG analysis suggested the heat stability of 4'-hydroxychalcone could be significantly improved by forming inclusion complex with HP- $\beta$ -CD.

## CONCLUSION

Chalcone is a kind of flavonoid with many reported health-promoting properties. However, its low solubility and stability in water impedes its application in pharmaceutical and functional food products. HP- $\beta$ -CD has greater capacity to form inclusion complexes with 4'-hydroxychalcone than the parent  $\beta$ -CD. Thus, the driving force for the complexation process is hydrophobic interaction. The heat stability of 4'-hydroxychalcone is significantly improved by formation of inclusion complex with HP- $\beta$ -CD.

## ACKNOWLEDGEMENT

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