

The thermodynamic study of p-methoxycinnamic acid inclusion complex formation, using β -cyclodextrin and hydroxypropyl- β -cyclodextrin

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The thermodynamic study of *p*-methoxycinnamic acid inclusion complex formation, using β -cyclodextrin and hydroxypropyl- β -cyclodextrin

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Abstract

Objectives: Cyclodextrin's ability to form an inclusion complex with a guest molecule is a function of two factors. The first is steric and depends on the relative size of cyclodextrin cavity to the guest molecule, while the second is the thermodynamic interaction between the different system components. This study therefore aims to determine the effect of β -cyclodextrin and hydroxypropyl- β -cyclodextrin as complex formers, on thermodynamic parameter values (ΔH , ΔG , and ΔS) in the formation of inclusion complex with *p*-methoxycinnamic acid (*p*MCA).

Methods: The *p*MCA complex formation with β -cyclodextrin or hydroxypropyl- β -cyclodextrin was determined in 0.02 pH 4.0 M acetate buffer and 0.02 M pH 7.0 phosphate buffer, with a 0.2 μ value at 32, 37, and 42 \pm 0.5 $^{\circ}$ C. This experiment was carried out in a waterbath shaker until a saturated solution was obtained. Subsequently, *p*MCA concentration was determined using UV spectrophotometer at the maximum *p*MCA wavelength, at each pH.

Results: The result showed *p*MCA formed inclusion complex with β -cyclodextrin or hydroxypropyl- β -cyclodextrin and exhibited increased solubility with increase in β -cyclodextrin or hydroxypropyl- β -cyclodextrin concentration. This temperature rise led to a decrease in the complex's constant stability (*K*). Furthermore, the interaction showed a negative enthalpy ($\Delta H < 0$) and is a spontaneous processes ($\Delta G < 0$). At pH 4.0, an increase in the system's entropy

occurred ($\Delta S > 0$), however, at pH 7.0, the system's entropy decreased ($\Delta S < 0$).

Conclusions: The rise in *p*MCA solubility with increase in cyclodextrin solution concentration indicates an inclusion complex has been formed, and is supported by thermodynamic data.

Keywords: β -cyclodextrin; hydroxypropyl- β -cyclodextrin; inclusion complex; *p*-methoxycinnamic acid; solubility enhancement; thermodynamic.

Introduction

The compound *p*-methoxycinnamic acid (*p*MCA) is an ethyl *p*-methoxycinnamic derivative obtained from sand ginger plant (*Kaempferia galanga* Linn.), and known to inhibit the enzymes cyclooxygenase 1 and 2 [1], thus, functioning as an analgesic. According to Ekowati and Dyah [2], *p*MCA present in sand ginger exhibits an even greater painkiller activity, compared to acetosal [2]. However, *p*MCA has a low solubility of 0.712 mg/mL in water, at 25 $^{\circ}$ C [3]. This causes low *p*MCA dissolution rate, thus, leading to incomplete absorption and low bioavailability [4]. The formation of inclusion complexes is therefore a suitable effort to improve *p*MCA solubility.

An inclusion complex is a system between drug molecules (guest) trapped in the cavity of a complex forming material (host) [5, 6]. Factors influencing an inclusion complex's formation are the accordance between the host and guest cavities' sizes and polarities, as well as thermodynamic parameters [7, 8]. Cyclodextrin is a constituent of inclusions with the ability to the ingredients' solubility in water.

In addition, cyclodextrin (CD) is a cyclic oligosaccharide compound with at least 6 D-(+)-glucopyranose units bound to α -1,4 glycoside bonds, a toroidal shape, and a cavity with a hydrophobic interior and hydrophilic exterior. These compounds are able to increase an ingredient's solubility by forming an inclusion complex with the guest molecules and stabilizing with several interactions, including hydrogen bonds, Van der Waals bonds, hydrophobic interactions and

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electrostatic attraction. The cyclodextrin type most often used to form inclusion complexes is β -cyclodextrin, due to easy production, and relative inexpensiveness [8].

The cavity size of β -cyclodextrin and derivatives corresponds to the aromatic ring's size, thus the formation of inclusion complexes between these compounds and *p*MCA is highly likely [9].

Another commonly used cyclodextrin type for inclusion complex formation is hydroxypropyl- β -cyclodextrin (HP β CD). This is a derivative of β -cyclodextrin, with high solubility in water (above 50 g/100 mL, at 25 °C), due to the substitution of hydroxypropyl groups replacing the hydroxyl group in glucose. The compound is also less toxic and more environmentally friendly, compared to β -cyclodextrin [8, 10].

No previous studies have been conducted on the thermodynamics of inclusion complex formation between *p*MCA and cyclodextrin. This is a reversible reaction, and the complex compounds continuously formed undergo dissociation to produce free drugs and complex forming materials. However, the reaction is offset in a brief period of time, by the association of free drug molecules and complex forming materials. A molecule's ability to associate and dissociate to reach equilibrium is evident in the constant stability of complex formation (K) value [11]. This value is directly related to the complex formation free energy (ΔG) value [12], and is influenced by pH, because the drug compound's ionized or unionized form (guest) influences the inclusion complex formation [8].

Therefore, this study aims to determine the effect of β -cyclodextrin and hydroxypropyl- β -cyclodextrin as complex formers, on thermodynamic parameter values (ΔH , ΔG , and ΔS), in inclusion complex formation with *p*MCA, in a bid to better understand the interaction mechanism.

Materials and methods

The materials used in this study include *p*-methoxycinnamic acid (Sigma-Aldrich), β -cyclodextrin (Sigma-Aldrich), hydroxypropyl- β -cyclodextrin (Sigma-Aldrich), distilled water, disodium hydrogen phosphate (Sigma-Aldrich), sodium dihydrogen phosphate (Sigma-Aldrich), citric acid (Sigma-Aldrich), sodium citrate (Sigma-Aldrich), 96% analytical grade ethanol, and 96% technical grade ethanol.

Methods

Preparation buffer solutions

0.02 M pH 4.0 citrate buffer solution: This was prepared by mixing 0.02 M sodium citrate solution with 0.02 M citric acid solution and adding sodium chloride, to obtain a 0.20 ionic strength.

0.02 M pH 7.0 phosphate buffer solution: This was prepared by mixing 0.02 M disodium hydrogen phosphate solution with 0.02 M sodium dihydrogen phosphate solution, and adding sodium chloride, to obtain a 0.20 ionic strength.

Solubility testing

The *p*MCA's solubility in various β -cyclodextrin or hydroxypropyl- β -cyclodextrin concentrations (0.10×10^{-3} ; 2.5×10^{-3} ; 5.0×10^{-3} ; 7.5×10^{-3} ; and 10.0×10^{-3} M) was tested in pH 4.0 and 7.0 solutions, with 0.20 ionic strengths, at 32, 37, and 42 ± 0.5 °C (305, 310, and 315 K). About 5.0 mL of β -cyclodextrin or hydroxypropyl- β -cyclodextrin solutions with certain pHs and concentrations were placed into a 15-mL vial. The vials were then inserted in the waterbath shaker and the temperature was adjusted as required. After the trial temperature was obtained, about 15 mg of *p*MCA was added and the mixture was shaken at a frequency of 140/min, until a saturated solution was formed. An optimized determination of *p*MCA saturated solubility in distilled water was indicated by the absence of a rise in *p*MCA concentration, after shaking for 5 h, under the solubility test's conditions. Subsequently, the solution was collected with an injection syringe, filtered with a 0.22 μ m Millipore paper and the concentration was determined using UV spectrophotometer, at each pH's maximum *p*MCA λ . The maximum *p*MCA λ were discovered to be 307.0 and 286.0 nm, pH 4.0 and 7.0, respectively.

Results

The solubility of *p*-methoxycinnamic acid in cyclodextrin solutions

Figures 1 and 2 show the *p*MCA solubility study in various β -cyclodextrin (β CD) and hydroxypropyl- β -cyclodextrin (HP β CD) solution concentrations, carried out at different pHs and temperatures.

The complex stability constant and thermodynamic parameters

Table 1 shows the regression equation obtained from the curve of the relationship between the β -cyclodextrin (β CD) or hydroxypropyl- β -cyclodextrin (HP β CD) concentrations, and *p*MCA solubility.

The complex stability constant (K) is obtained using the formula below.

$$K_{(1,1)} = \frac{\text{slope}}{\text{intercept}(1 - \text{slope})} \quad (1)$$

Meanwhile, Table 2 shows the thermodynamic values of enthalpy (ΔH), free energy (ΔG), and entropy (ΔS) values, calculated using the formula below. The slope value obtained from each regression equation in Table 1 was then used to calculate the enthalpy value (ΔH cal/mol) using Eq. (2), where R represents the gas constant (1.987 calories/mol)

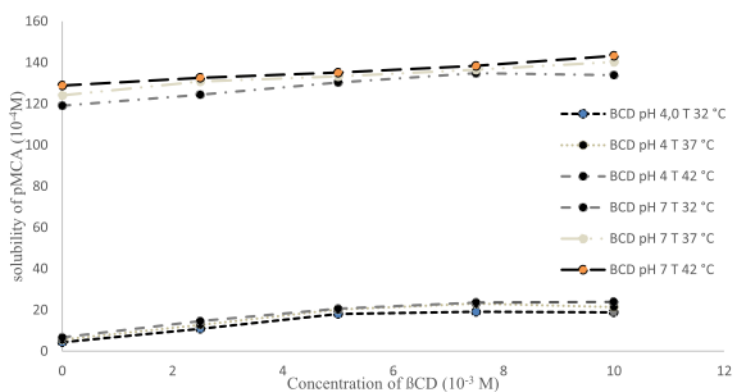


Figure 1: Solubility of *p*MCA in β -cyclodextrin (BCD) solutions at various pHs and temperatures.

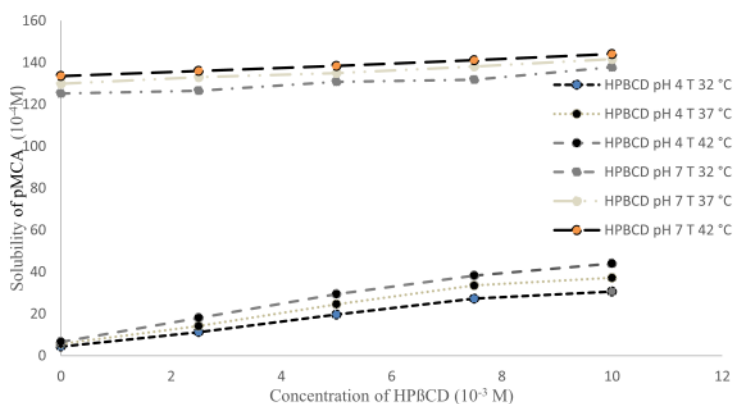


Figure 2: Solubility of *p*MCA in hydroxypropyl- β -cyclodextrin (HPBCD) solutions at various pHs and temperatures.

Table 1: Regression equation of the relationship between the *p*MCA solubility (M) and the concentration of cyclodextrin (M).

Cyclodextrin	pH	Temperature (°K)		
		305	310	315
β -cyclodextrin	4.0	$y = 0.14864x + 6.8120 \cdot 10^{-4}$ $r = 0.9045$	$y = 0.16724x + 8.2880 \cdot 10^{-4}$ $r = 0.9072$	$y = 0.17372x + 9.1680 \cdot 10^{-4}$ $r = 0.9418$
	7.0	$y = 0.16040x + 120.50 \cdot 10^{-4}$ $r = 0.9491$	$y = 0.15248x + 125.40 \cdot 10^{-4}$ $r = 0.9849$	$y = 0.13824x + 128.73 \cdot 10^{-4}$ $r = 0.9938$
Hydroxypropyl- β -cyclodextrin	4.0	$y = 0.27440x + 4.8700 \cdot 10^{-4}$ $r = 0.9923$	$y = 0.33000x + 6.4753 \cdot 10^{-4}$ $r = 0.9899$	$y = 0.38040x + 8.2140 \cdot 10^{-4}$ $r = 0.9918$
	7.0	$y = 0.12140x + 124.33 \cdot 10^{-4}$ $r = 0.9699$	$y = 0.11444x + 129.74 \cdot 10^{-4}$ $r = 0.9962$	$y = 0.10452x + 133.36 \cdot 10^{-4}$ $r = 0.9949$

$$\text{Slope} = -\Delta H / 2,303 R \quad (2)$$

Also, the free energy (ΔG cal/mol) was calculated using Eq. (3), where R represents the gas constant (1.987 calories/mol), T denotes absolute temperature (°K) and K indicates the complex stability constant value.

$$\Delta G = -2,303 RT \log K \quad (3)$$

The value of entropy (ΔS cal/mol) was calculated using Eq. (4), by entering ΔH value as obtained from Eq. (2) and ΔG

value as obtained from Eq. (3), where T denotes absolute temperature (°K).

$$\Delta G = \Delta H - T\Delta S \quad (4)$$

Discussion

The solubility of *p*MCA in various β -cyclodextrin or hydroxypropyl- β -cyclodextrin concentrations (0×10^{-3}

Table 2: The complex stability constant (*K*) and the thermodynamic parameters (ΔH , ΔG , and ΔS) at various pHs and temperatures.

1/T (°K)	pH	β -Cyclodextrin				Hydroxypropyl- β -cyclodextrin			
		<i>K</i> (M ⁻¹)	ΔH (cal/mol)	ΔG (cal/mol)	ΔS (cal/mol)	<i>K</i> (M ⁻¹)	ΔH (cal/mol)	ΔG (cal/mol)	ΔS (cal/mol)
305	4.0	256.30	-2,200.40	-3,361.89	3.81	776.53	-0.879.21	-4,033.57	10.34
310		242.31	-2,200.40	-3,382.42	3.81	760.64	-0.879.21	-4,085.51	10.34
315		229.32	-2,200.40	-3,402.48	3.82	747.44	-0.879.21	4,136.99	10.34
305	7.0	15.85	-4,391.34	-1,674.84	-8.91	11.11	-4,833.31	-1,465.48	-11.04
310		14.35	-4,391.34	-1,645.55	-8.86	9.96	-4,833.31	-1,418.58	-11.02
315		12.46	-4,391.34	-1,585.61	-8.91	8.75	-4,833.31	-1,354.97	-11.04

to 10.0×10^{-3} M) was tested in the medium of pH 4.0 and 7.0 buffer solutions, and the pH was selected based on the 4.04 *p*MCA pKa value. Based on the calculation using the Henderson–Hasselbalch equation, at pH 4.0 or pH equal to pKa, 50% of *p*MCA is in ionized form, while at pH 7.0 or 3 units above pKa, *p*MCA is about 99.9% ionized. The difference in the quantity of the guest molecule's unionized forms influences interaction with the hydrophobic cyclodextrin cavity.

The solubility study was conducted for a *p*MCA saturation time of 5 h. Figures 1 and 2 show the test results of *p*MCA solubility in β -cyclodextrin or hydroxypropyl- β -cyclodextrin. The *p*MCA solubility curve in β -cyclodextrin shows a BS type, meaning a rise in *p*MCA solubility occurred with increasing β -cyclodextrin concentration, but reached a plateau, and even tended to decrease at a certain point. This is because β -cyclodextrin's solubility in water is limited, thus, a complex deposition occurs at a certain concentration. Conversely, the *p*MCA solubility curve in hydroxypropyl- β -cyclodextrin shows an AL-type curve, meaning *p*MCA solubility rises with increasing hydroxypropyl- β -cyclodextrin concentration.

In the curves of *p*MCA solubility in β -cyclodextrin or hydroxypropyl- β -cyclodextrin, the calculated *r* value is greater, compared to the *r* table with a 95% confidence level of 0.754. This *p*MCA solubility curve's linearity shows the interactions occurring between guest molecules (*p*MCA) to form complexes with β -cyclodextrin or hydroxypropyl- β -cyclodextrin in a 1:1 M ratio. Table 1 shows the regression equation obtained from the *p*MCA solubility curve with β -cyclodextrin or hydroxypropyl- β -cyclodextrin concentration, while Table 2 shows the complex stability constant (*K*) value calculate using this equation.

According to Table 2, the increase in temperature causes a decrease in the complex stability constant (*K*). This shows the temperature rise causes the formed inclusion complex to become more unstable, meaning the interaction between

*p*MCA and β -cyclodextrin or hydroxypropyl- β -cyclodextrin is released more easily. The cyclodextrin inclusion complex formation is a reversible reaction and temperature rise causes the guest *p*MCA molecule in the host cavity to dissociate out of the cyclodextrin cavity, as indicated by the lower *K* value. In addition, a rise in pH reduces the complex stability constant (*K*), and the amount of ionized *p*MCA influences the compound's ability to enter the β -cyclodextrin or hydroxypropyl- β -cyclodextrin cavity. Ionized *p*MCA tends to come out of the hydrophobic cyclodextrin cavity, making the complex less stable.

At pH 4.0, hydroxypropyl- β -cyclodextrin's stability constant complex (*K*) is greater, compared to β -cyclodextrin, indicating the complex bonds formed in the hydroxypropyl- β -cyclodextrin are stronger. This is probably because hydroxypropyl- β -cyclodextrin has a hydroxypropyl group with the ability to provide steric barrier for *p*MCA to escape from the HP β CD cavity.

Based on Table 2, a curve of the relationship between $\log K$ and $1/T$ is plotted, with β -cyclodextrin and hydroxypropyl- β -cyclodextrin inclusion complex formation at pH 4 as well as 7. Each curve provided a regression equation with a correlation coefficient value (*r*)>0.9, indicating a relationship between $1/T$ and $\log K$. Subsequently, the slope obtained in each regression equation was used to calculate ΔH as in Eq. (2), as well as thermodynamic parameters (ΔG and ΔS), as in Eqs. (3) and (4).

The results of β -cyclodextrin or hydroxypropyl- β -cyclodextrin at various pH both produced a negative enthalpy value (ΔH), indicating an exothermic the dissolution process. Furthermore, the interaction between *p*MCA with β -cyclodextrin or hydroxypropyl- β -cyclodextrin is mainly hydrophobic. The free energy changes (ΔG) also had a negative sign, indicating a spontaneous the complex formation, while the entropy change (ΔS) in experiments with β -cyclodextrin or hydroxypropyl- β -cyclodextrin at pH 4.0 was positive, indicating an increase in system irregularities.

The regular water system surrounding the *p*MCA molecule becomes irregular because *p*MCA enters the cyclodextrin. This condition is not desirable because polar–nonpolar repulsion occurs between water molecules and the inner cyclodextrin cavity, thus, the condition is quickly replaced by the presence of guest molecules less polar than water molecules [13]. Meanwhile, in experiments with β -cyclodextrin or hydroxypropyl- β -cyclodextrin at pH 7.0, a negative value was obtained, indicating a lower system irregularity. This is possibly because the system irregularity caused by guest molecule entry and exit to and from the host molecule, no longer occurs. The cyclodextrin cavity is hydrophobic, thus, guest molecules in ionized form find it more difficult to enter.

Conclusions

Based on the results, *p*MCA was concluded to form an inclusion complex with both β -cyclodextrin and hydroxypropyl- β -cyclodextrin. The interaction between *p*MCA and hydroxypropyl- β -cyclodextrin is stronger, compared to β -cyclodextrin, as indicated by the higher complex stability constant (*K*) value. In addition, the temperature rise led to a constant decrease in stability, and the interaction showed a negative enthalpy ($\Delta H < 0$) as well as a spontaneous process ($\Delta G < 0$). In pH 4.0 a rise in the system's entropy occurred ($\Delta S > 0$), while in pH 7.0 a reduction in the system's entropy was observed ($\Delta S < 0$)

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