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RESEARCH ARTICLE

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Study on inclusion complexes of hydroxypropyl-β-cyclodextrin with tanshinone IIA

Abstract The inclusion complex formation of tanshinone IIA (Tan IIA) with hydroxypropyl-β-cyclodextrin (HPCD) was studied by using phase solubility method, and the formation constant for tanshinone IIA-HPCD- dextrin was determined. The effect of temperature on the reaction was studied through thermodynamics, and the changes inentropy, enthalpy, and free energy of the reaction were calculated. The molecular modeling provided further evidence for the formation of the inclusion complexes. Stable inclusion complex in solid state was characterized by IR spectra.

Keywords hydroxypropyl-β-cyclodextrin, tanshinone IIA, phase solubility method, inclusion complex, thermodynamics, molecular modeling

1 Introduction

Salvia miltiorrhiza is the root of the salvia of the Lamiaceae plant. Its two major efficacies are promoting blood circulation by removing blood stasis and antibiosis and anti-inflammation. Based on these two effects, various preparations of Salvia miltiorrhiza have been applied to treat many clinical symptoms in recent years, including cardiovascular diseases, hepatitis, trauma, skin diseases, and baldness. Its curing effect on coronary heart disease is remarkable and has attracted a lot of attention from the medical field [1,2]. The fat-soluble active ingredient of Salvia miltiorrhiza tanshinone IIA (Tan IIA) is of ortho quinoid structure and its chemical structure is shown in Fig. 1 [3]. As it is insoluble in water, the use and the efficacy of this drug is quite limited. For a better application of this drug, the solubility and stability of Tan IIA must be increased. Usually organic solvents, latent solvents, and surfactants are used to solve this problem. However, these reagents are limited in use due to their toxicity and causes irritation. Hydroxypropyl-β-cyclodextrin (HPCD) is a hydrophilic derivative of β-cyclodextrin. It is stable under alkaline conditions, heat, and light. It is used as the solubilizer, stabilizer, and osmotic-promoting reagents in the drug. Since HPCD is low in toxicity, safe, and effective, it is considered to be a highly potential auxiliary material for injection [4]. Therefore, HPCD can be used to solubilize Tan IIA to get a relatively stable water solution. A report about this aspect has not been published in China. This experiment used the phase-solubility method to study the solubilization effect of HPCD on Tan IIA for a better application of Tan IIA [5,6].

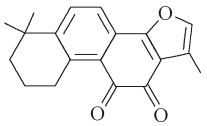

Fig. 1 The structure of tanshinone IIA

2 Instruments and reagents

HP-8453 ultraviolet spectrophotometer; Shimadzu 8300 FT-IR spectrometer; HZS-H water bath vibrator (Harbin Donglian Electronic&Technology Development Co. Ltd); KQ3200 ultrasonic instrument (Kunshan Ultrasonic Instrument Co. Ltd); Control of Tanshinone IIA (National Institute for the Control of Pharmaceutical and Biological Products); HPCD (Aldrich product); other reagents are all analytically pure. The water used in the experiment was distilled water after three times' distillation.

3 Methods and results

3.1 The solubilization experiment of HPCD on Tan IIA

3.1.1 The drawing of ultraviolet standard curve

The control of Tan IIA was accurately weighed and dissolved in a little methanol. A pH 6.8 phosphate buffer was added

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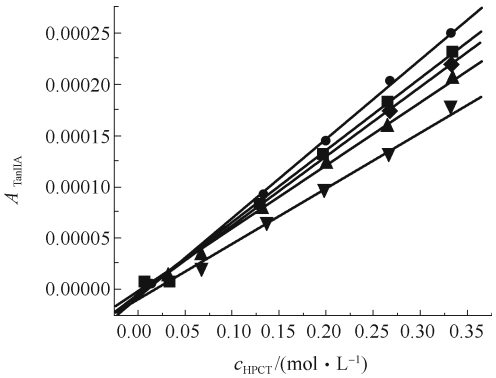
into the solution to make it into a buffer system with 1:1 methanol–phosphate ratio. A series of samples with concentrations ranging from 1 to 10 mg·L^{-1} was prepared; the reference sample was with a blank solvent. The absorbance of samples was measured at a wavelength of 269 nm. A linear regression of absorbance (A) and concentration (c) was made and the regression equation is $A = 0.1123c + 0.01642$, $r = 0.9996$. The result shows that, in the concentration range of 1–10 mg·L^{-1}, the linear relation of absorbance and concentration of Tan IIA is good. With the existence of HPCD, the absorption strength of Tan IIA is not influenced. Therefore, the standard curve could be used for the determination of Tan IIA in the coexistence system with the existence of the inclusion complex [6].

3.1.2 The drawing of equilibrium phase solubility curve

Phosphate buffers with pH of 6, 6.8, 7.4, 8, and 9 amounts to those buffers HPCD solutions 0, 6.65×10^{-3}, 13.31×10^{-3}, 33.27×10^{-3}, 66.53×10^{-3}, 133.07×10^{-3}, 199.60×10^{-3}, 266.12×10^{-3}, 332.67×10^{-3} mol·L^{-1}. Excess Tan IIA powder was added into the above solutions. Samples were under ultrasonic centrifugation for 30 minutes, and vibrated for 48 h at 25°C in the water bath vibrator (100 t/min). When the solution was stable, the supernatant fluid was obtained through a 0.22 μm millipore filter. The filtrate was diluted and the absorbance was determined at a wavelength of 269 nm. The concentration of the drug was calculated by the external standard method. The equilibrium phase solubility curve was obtained with HPCD molarity as the horizontal ordinate and drug concentration as the vertical ordinate. The result is shown in Fig. 2.



▼−pH 6; ▲−pH 6.8; ■−pH 7.4; ♦−pH 8; ●−pH 9.

Fig. 2 Phase solubility curves of tanshinone IIA in HPCD

3.1.3 Discussion

As shown in Fig. 2, the solubility of Tan IIA increases with the concentration of HPCD at different pH. It shows that HPCD has a quite good solubilization effect on Tan IIA. According to the classification of Higuchi [7], the phase solubility curve of this system is of A_L type and it shows that 1: 1 inclusion complex is formed in the system. The intensity of inclusion is indicated by formation constant K_f.

A linear regression of the solubility of Tan IIA (mol·L^{-1}) and the concentration of HPCD (mol·L^{-1}) was made to get the regression equation. The formation constant K_f was calculated according to the formula $K_f = \text{slope}/S_0(1\text{-slope})$ with data from regression equation [8]. In this formula, slope is the slope of regression equation and S_0 is the solubility of Tan IIA at a certain pH. The result is shown in Table 1.

Table 1 The stability constants K_c of tanshinone IIA at different pH

pH	Eq.	r	K_c
6.0	$Y = 5.07433 \times 10^{-4}c - 5.00869 \times 10^{-6}$	0.99753	9524
6.8	$Y = 6.17127 \times 10^{-4}c - 3.15836 \times 10^{-6}$	0.99937	6179
7.4	$Y = 6.78601 \times 10^{-4}c - 7.28468 \times 10^{-6}$	0.99866	5699
8.0	$Y = 7.17752 \times 10^{-4}c - 1.12359 \times 10^{-6}$	0.99801	3712
9.0	$Y = 7.75837 \times 10^{-4}c - 8.94324 \times 10^{-6}$	0.99852	1573

As shown by Table 1, the formation constant of Tan IIA and HPCD is larger at pH 6 and 6.8, showing that their inclusion is intense in weakly acid solutions. However, as shown in Fig. 2, the solubility of Tan IIA increases apparently with the increase of pH in the alkaline solution and the solubilization effect of HPCD is more obvious. But the increase of the concentration of [OH$^-$] also speeds up hydrolysis. According to the chemical reaction equilibrium theory, the concentration of Tan IIA decreases with hydrolysis of Tan IIA and the inclusion complex will break down. The reaction will is reversed for the inclusion reaction. Therefore, the stability of the inclusion complex drops and K_c decreases with the increase of pH.

3.2 The study of inclusion with the thermodynamic method

The inclusion force of the host molecule and guest molecule is mainly from the van der Waals force, hydrophobic interaction force and, hydrogen bond force. Therefore the thermodynamic method could be adopted to study the inclusion process for a deeper understanding of it. The formation constant of inclusion reaction changes with the temperature and it is one of the most important parameters of thermodynamics. The methods used in thermodynamics study were mainly pyrotitration, spectroscopic method, potential method, and chromatography [9,10]. This study adopted the spectroscopic method.

3.2.1 The effect of temperature on the inclusion reaction

In the inclusion reaction of cyclodextrin and the guest molecule, temperature is an important factor to influence the inclusion. Therefore, a series of experiments were done to study the inclusion process of Tan II A–HPCD at different temperatures. The formation constant K_f was obtained at different temperatures, as was shown in Fig. 3. From Fig. 3,

we could see that the formation constant K_f gradually decreases with the increase of temperature. It shows that the inclusion complex may decompose and the guest molecules enter into the water phase from the cavity of cyclodextrin and the stability of inclusion complex drops. Analyzing the mechanism of reaction, this result may be due to the increase in speedof molecular movement with the increase of temperature. The conclusion from this experiment is that the increase of temperature is of no benefit to the inclusion reaction.

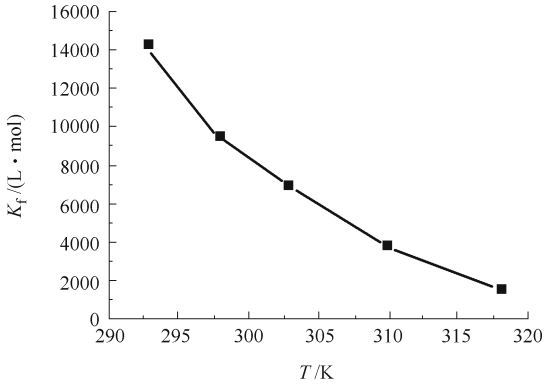


Fig. 3 Effect of temperature on the formation constants K_f of complexes of Tan IIA–HPCD

3.2.2 Calculation of the change of entropy, enthalpy, and free energy of the inclusion reaction with the thermodynamic method

According to the data in Fig. 3 and van't Hoff's equation $\ln K_f = -\Delta_r H_m^{\ominus}/(RT) + \Delta_r S_m^{\ominus}/R$, the curve of $\ln K_f$ versus $1/T$ was obtained, as shown in Fig. 4.

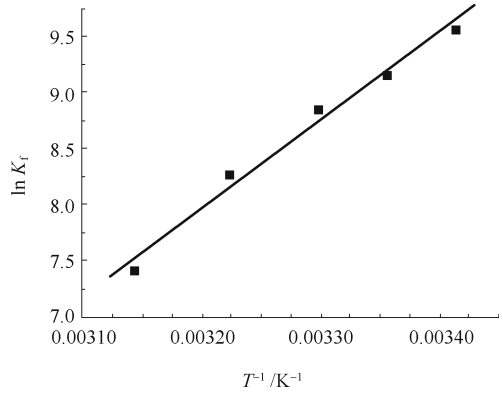


Fig. 4 The curves of $\ln K_f$ versus. $1/T$ of complexes of Tan IIA-HPCD

The change of entropy can be calculated by the intercept of the straight line. The linear regression equation for Tan IIA–HPCD is $\ln K_f = 7889.0284T^{-1} - 17.29433$ $(r = 0.994)$. The computed result is $\Delta_r S_m^{\ominus} = -143.78\ \mathrm{J \cdot mol^{-1} \cdot K^{-1}}$. According to the equation $\Delta_r G_m^{\ominus} = -RT\ \ln K$, the change of free energy at different temperatures can be obtained. Substitute $\Delta_r S_m^{\ominus}$ and $\Delta_r G_m^{\ominus}$ in the equation $\Delta_r H_m^{\ominus} = \Delta_r G_m^{\ominus} + T\Delta_r S_m^{\ominus}$ to get the change of enthalpy of the inclusion reaction at different temperatures. The result is shown in Table 2.

Table 2 Effect of temperature on K_f, $\Delta_r S_m^{\ominus}$, $\Delta_r G_m^{\ominus}$ and $\Delta_r H_m^{\ominus}$ of the inclusion complex of Tan IIA–HPCD

T/K	K_f/ ($L^2 \cdot mol^{-2}$)	$\Delta_r S_m^{\ominus}$ / ($J \cdot mol^{-1} \cdot K^{-1}$)	$\Delta_r G_m^{\ominus}$ / ($kJ \cdot mol^{-1}$)	$\Delta_r H_m^{\ominus}$ / ($kJ \cdot mol^{-1}$)
293	14192.9	−143.78	−23.289	−65.417
298	9524.28	−143.78	−22.698	−65.544
303	6913.30	−143.78	−22.272	−65.837
310	3808.92	−143.78	−21.250	−65.822
318	1661.71	−143.78	−18.386	−64.108

From Table 2, we could note that $\Delta_r H_m^{\ominus}$ in the inclusion reaction is of negative value, showing that the inclusion reaction is an exothermic process; $\Delta_r S_m^{\ominus}$ is a negative value, showing that the driving force of inclusion reaction is mainly enthalpy; van der Waals force, hydrophobic interaction force, hydrogen bond force, and the intramolecular recognition are the main source for the change of enthalpy; $\Delta_r G_m^{\ominus}$ is also a negative value, showing that the inclusion reaction is a spontaneous process.

3.3 The molecular modeling experiment [11]

3.3.1 Computational method

The molecular modeling used the software Insight II and was completed in Silicon Graphics Incorporation workstation.

3.3.2 Computed results

We studied the two inclusion modes of HPCD and Tan II A. Mode 1 is that Tan II A inserts into the HPCD through the minor port and mode 2 is that Tan IIA inserts into the HPCD through the major port. The two most optimal inclusion modes are shown in Fig. 5. The corresponding energy is shown in Table 3.

The unit of the numerical value in Table 3 is $kJ \cdot mol^{-1}$. From Fig. 3 and Table 3, we could see that the total energy of the system after the insertion of Tan IIA through the minor port and major port of HPCD are 781.744 $kJ \cdot mol^{-1}$ and 728.39 $kJ \cdot mol^{-1}$, respectively. It shows that Tan IIA selects the major port to enter HPCD and the inclusion complex formed is of the lowest in energy and stable in structure. The detailed energy analysis finds that internal is the energy related to bond. It is mainly the hydrogen bond that determines the total energy of the system. Besides this, Non-bonds like VDW and Electrostatic also play a part. This is consistent with the above thermodynamic analysis.

3.4 The study of IR spectrum [12]

Comparing the IR spectra of HPCD alone, Tan IIA alone, mixture of HPCD and Tan IIA, and inclusion complex (shown in Fig. 6), it is found that the vibration absorption peak of the carbonyl group in Tan IIA is 1670 cm^{-1}, vibration absorption

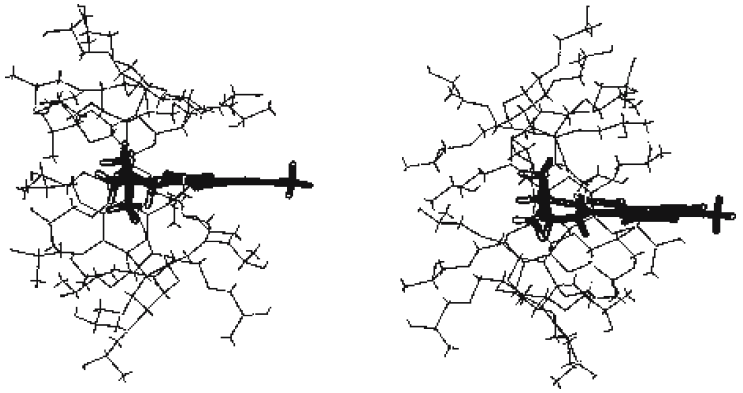


Fig. 5 The two most optimal inclusion modes of Tan IIA–HPCD (left: minor port; right: major port)

Table 3 Energy of the most optimal inclusion modes of Tan IIA-HPCD

	Total	Internal	Bond	Angle	Torsion	Out of plane	Non-bond	VDW	Electrostatic
Mode 1	781.74	701.11	72.17	352.71	275.64	0.59	80.63	77.19	3.43
Mode 2	728.39	665.63	67.45	331.33	266.44	0.42	62.76	72.59	–9.83

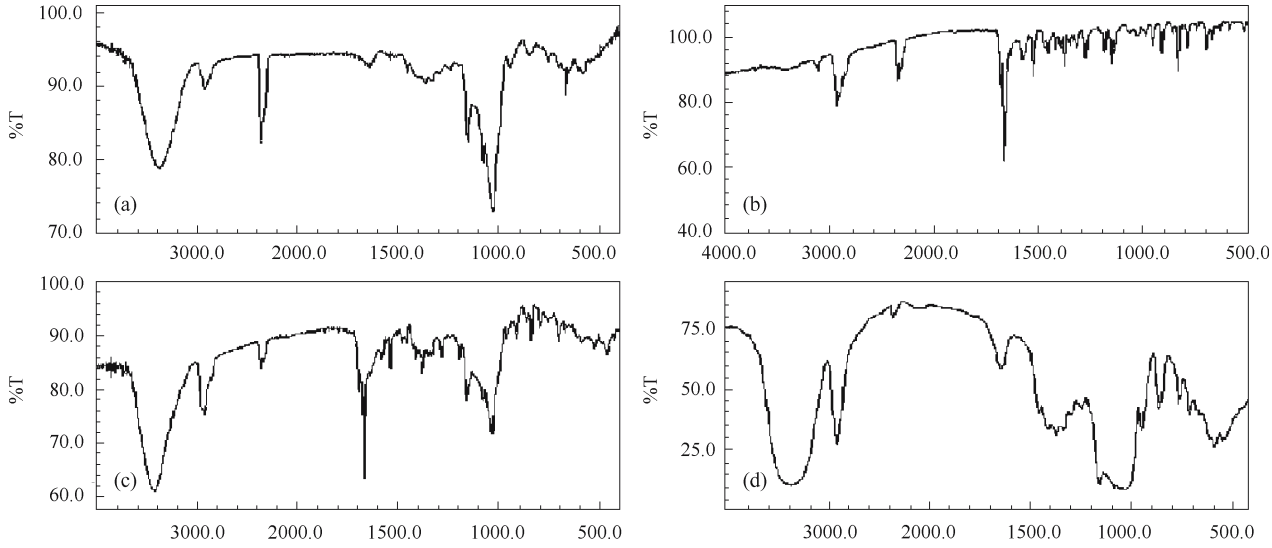


Fig. 6 IR spectra of (a) HPCD alone, (b) Tan IIA alone, (c) mixture of HPCD, and Tan IIA, (d) and inclusion complex

peak of the carbonyl group in mixture of HPCD and Tan IIA does not move, vibration absorption peak of the l carbonyl group in the inclusion complex moves to 1645 cm^{-1} in the longer wave direction. This is because the hydrogen bond is formed in the inclusion complex of Tan II A–HPCD and this result is consistent with the conclusion of the molecular modeling.

4 Conclusions

This study proved the formation of inclusion complex by phase solubility method and found that the 1: 1 complex was formed. By changing the pH value, we got to know that the inclusion constant decreases with the increase of pH. This results from the speeding of hydrolysis of Tan IIA and the decrease of stability with the increase of OH^- concentration. Besides, the effect of temperature on the reaction was studied through thermodynamics. The possible inclusion mechanism was discussed and the driving force of inclusion was discussed from the thermodynamic point of view. The thermodynamic parameters, such as $\Delta_r S_m^{\Theta}$, $\Delta_r G_m^{\Theta}$, $\Delta_r H_m^{\Theta}$, were computed by van't Hoff's equation and some energy formulas. Thus, we have the following conclusions: (i) the increase of temperature is of no benefit to the inclusion reaction; (ii) the inclusion reaction is a spontaneous process wherein heat is released; (iii) the driving force of inclusion reaction is enthalpy; (iv) intramolecular recognition (van der Waals force, hydrophobic interaction force) is the main source for the change of enthalpy. This result was verified in the

following molecular modeling test. Lastly, the IR spectrum was used to characterize the solid inclusion complex, to verify fully the formation of solid inclusion complex.

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