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Short communication

Study of the physicochemical properties of trimethoprim with β-cyclodextrin in solution

Ning Li [a], Yun-Hui Zhang [a,*], Xiao-Li Xiong [b], Zhi-Geng Li [a], Xing-Hua Jin [a], Ya-Nan Wu [a]

[a] *College of Pharmaceuticals and Biotechnology, Tianjin University, Tianjin 300072, PR China*
[b] *Chongqing Jiatong Co., Ltd., Chongqing 400052, PR China*

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Abstract

The behavior of trimethoprim (TMP) in aqueous solutions containing different concentration of β-cyclodextrin (β-CD) was characterized by the solubility method, UV spectrophotometry and differential scanning calorimetry (DSC). The UV spectra enhancement of TMP as result of complex with β-CD was investigated. Complexation with β-CD increase the TMP aqueous solubility and the phase solubility diagram was A_L type. Thermodynamic parameters of the complex process, K, ΔG, ΔH and ΔS, were determined from the phase solubility diagram at 298 and 318 K, respectively. The experimental results indicated that the complex process was an enthalpy-driven process. Mechanism of the complex of β-CD with TMP was further discussed using the molecular model program. Results showed that the 3,4,5-trimethoxybenzyl group of the TMP was partly embedded in the cavity of β-CD.

Keywords: Trimethoprim; β-cyclodextrin; Complex; Phase-solubility; Thermodynamic parameters; Mechanism of the complex

1. Introduction

Cyclodextrins (CDs) are toroidally shaped polysaccharides made up of six to eight D-glucose monomers connected at 1 and 4 carbon atoms. The cavity of CDs is relatively hydrophobic compared to water, while the external faces are hydrophilic. They are capable of discerning various types of guest molecules by selectively incorporating such molecules through size and polarity consideration [1]. Up to now, several driving forces have been proposed for the inclusion of CDs with substrates [2–4]: hydrogen binding, Van der Waals force, hydrophobic interaction and the release of 'high energy water' molecules from the cavity. As a result of complex formation, the characteristic properties of the included substance, such as solubility, chemical reactivity and spectral properties will be changed. Thus, CDs have been applied to several areas of science and technology [5–7]. In the pharmaceutical industry, CDs have been used to enhance the solubility, stability and bioavailability of drugs [8,9], β-cyclodextrin (β-CD) is the favorite encapsulation of drugs, for its lower price and higher productive rate.

Trimethoprim (TMP) [2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine, the chemical structure is shown in Fig. 1], which has been used clinically either alone or in combination with a sulfonamide (e.g., sulfamethoxazole, sulfadiazine, sulfamoxole), is a synthetic, broad-spectrum antimicrobial agent which acts as an inhibitor of bacterial dihydrofolate reductase [10], characterized by a very low aqueous solubility.

In this work, we have prepared and characterized complexes of TMP with β-CD, and investigated the various physicochemical parameters (equilibrium constants, enthalpy, entropy, et al.) of the β-CD and TMP system.

2. Experimental section

2.1. Materials

Trimethoprim (Pharmaceutical grade) was obtained from Nanjing Pharmaceutical Factory Co., Ltd., β-cyclodextrin

* Corresponding author. Tel.: +86 222 7401 186; fax: +86 222 7401 186.
E-mail address: ln_john@eyou.com (Y.-H. Zhang).

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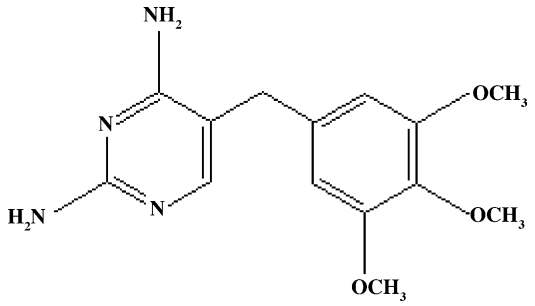

Fig. 1. Chemical structures of trimethoprim.

was purchased from Sigma. All other materials were of analytical reagent grade. These reagents were considered sufficiently well characterized by the manufacturer to be used without further purification.

2.2. UV spectrophotometric measurement

A Shimadzu UV-2450 visible spectrophotometer was used to record absorption spectra. The blank was distilled water for absorption measurements. Changes in absorbance was linear to a concentration of 10–50 mg TMP/L ($r = 0.9989$) at 280 nm.

2.3. Phase solubility studies

Solubility measurements were determined according to a modification of Higuchi and Connors [11]. Excess amount of TMP were added to aqueous solutions containing various concentration of β-CD at different temperatures, ranging from 0 to 16 mM at 25 °C, 0 to 32 mM at 45 °C. The suspensions were shaken for 10 days at 25 and 45 °C, respectively. After equilibration, the suspensions were filtered through 0.45 μm membrane filters, appropriately diluted with distilled water and the total concentration of the TMP in the filtrate was analyzed by UV absorbance spectrum.

2.4. Preparation of solid samples

The solid complexes were obtained from saturated TMP solutions in the presence of β-CD. After equilibration, the suspensions were filtered through 0.45 μm membrane filters and the filtrate was dried in hot-air oven at 105 °C. The physical mixtures were manually ground using an agate mortar and a pestle for 10 min.

2.5. Differential scanning calorimetry (DSC)

DSC measurements were performed using a Shimadzu DSC-50 (Shimadzu Co., Japan). Samples were hermetically sealed in aluminum pans and scanned over the temperature of 30–270 °C at a heating rate of 10 °C/min. The blanks were α-Al_2O_3.

2.6. Molecular model study

The most probable structure of the TMP/β-CD inclusion complex was determined using the ArgusLab (Version 3.0) program (Planaria Software). The structures of the TMP, β-CD and TMP/β-CD inclusion complex were energy minimized with AM1 (Austin Model1) algorithm.

3. Results and discussion

3.1. UV spectrophotometric analysis

Fig. 2 shows UV spectra of TMP in aqueous solution containing β-CD at various concentrations. UV spectrophotometric analysis at lambda maxima of 280 nm show a linear increase in the absorbance of TMP with a change of concentration of β-CD. A correlation between the ultraviolet absorbance and the β-CD molecular weight ($r = 0.999$ at 280 nm) was considered as an evidence of complex formation. No shifting was observed in the lambda maxima of

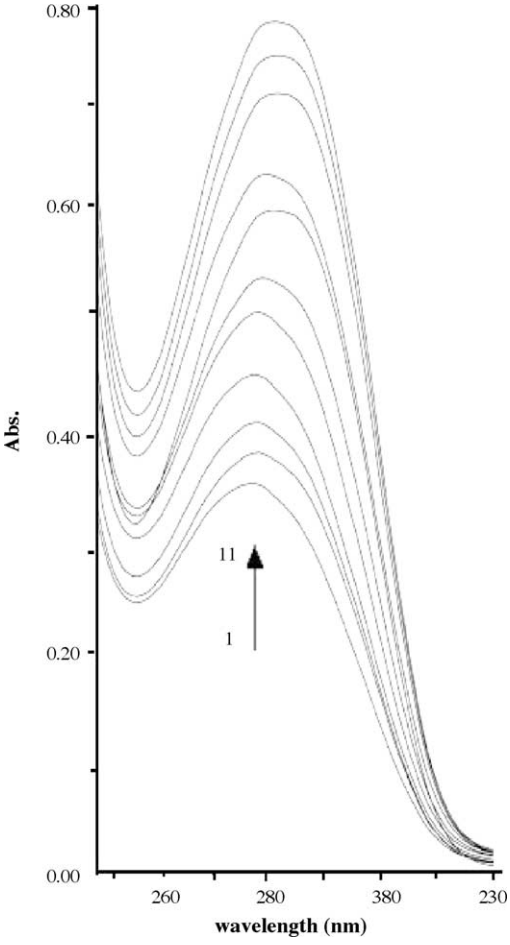

Fig. 2. UV spectra of TMP in aqueous solution containing β-CD at various concentrations at 45 °C (1) Without β-CD; (2) 2.5 mM β-CD; (3) 6.3 mM β-CD; (4) 8.9 mM β-CD; (5) 12.7 mM β-CD; (6) 15.2 mM β-CD; (7) 19.0 mM β-CD; (8) 21.6 mM β-CD; (9) 25.4 mM β-CD; (10) 27.9 mM β-CD; and (11) 31.7 mM β-CD.

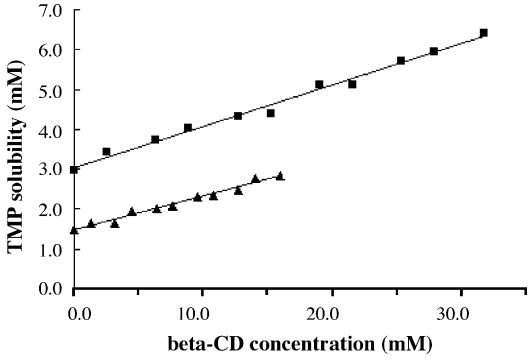

Fig. 3. Solubility diagram of TMP in the presence of different concentration of β-cyclodextrin at 25 °C(▲) and 45 °C(■).

TMP when complex with the β-CD. The β-CD showed insignificant ultraviolet absorbance.

3.2. Estimation of apparent stability constant

Fig. 3 shows the aqueous phase-solubility of TMP in different concentrations of the β-CD at 25 and 45 °C, respectively. The solubility of TMP increased with increasing β-CD concentrations. These linear phase diagrams are classified as AL-type [11] and are considered indicative of the formation of soluble complexes between the substrate (the TMP) and the ligand (the β-CD). This type of diagram indicates that the solubility of TMP increased linearity with the increase of β-CD concentration, depending on the aqueous solubility of the β-CD.

Suppose that a single complex, TMP_nCD_m, is responsible for the increase in apparent solubility of TMP. For the complex formation described by Eq. (1), the apparent stability constant K of the complex is expressed by Eq. (2):

$$\underset{(a-mx)}{mCD\ (liquid)} + \underset{(b-nx)}{nTMP\ (liquid)} \overset{K_{mn}}{\rightleftharpoons} \underset{(x)}{TMP_nCD_m(liquid)}$$
$$\Updownarrow$$
$$TMP\ (solid)\ S_0 \qquad (1)$$

$$K_{mn} = \frac{x}{(a-mx)^m(b-nx)^n} \qquad (2)$$

where m and n are stoichiometric coefficients of the reactants (β-CD and TMP), respectively, a and b are the total concentrations of the β-CD and the TMP in solution, respectively, x is the concentration of the complex TMP_nCD_m, K_{mn} is apparent stability constant, S_0 is the equilibrium solubility of TMP in the absence of β-CD.

Table 1
The effect of enhancing solubility for TMP/β-CD complexes

Temperature (°C)	S_0[a] (mM)	S[b] (mM)	S/S_0[c]	Solubility curve type[d]
25	1.5	2.9	1.9	A_L
45	3.0	6.5	2.2	A_L

[a] S_0: the solubility of TMP in the absence of β-CD.
[b] S: the solubility of TMP in 16 mM β-CD (25 °C) or in 32 mM β-CD (45 °C).
[c] Factor of enhancing solubility.
[d] According to Higuchi and Connors [11].

Eq. (2) can be given an expression for K_{mn} in terms of known concentrations, m and n. The total concentration of TMP can be expressed as Eq. (3) or (4):

$$b = S_0 + x \qquad (3)$$

$$x = b - S_0 \qquad (4)$$

A special case of Eq. (2), that in which $m = 1$ and $n = 1$, is of great interest. In this case Eqs. (5) and (6) can be derived.

$$K_{1:1} = \frac{x}{(a-x)(b-x)} \qquad (5)$$

$$b = \frac{S_0 K_{1:1}}{1 + S_0 K_{1:1}} a + S_0 \qquad (6)$$

A plot of b against a for the formation of a soluble complex TMP-CD should, therefore, yield a straight line (the Type A_L diagram). The intercept is equal to S_0 and the slope is given by

$$\text{Slope} = \frac{S_0 K_{1:1}}{1 + S_0 K_{1:1}} \qquad (7)$$

Eq. (8) results from Eq. (7):

$$K_{1:1} = \frac{\text{Slope}}{S_0(1 - \text{Slope})} \qquad (8)$$

The solubility of TMP in the presence of β-CD are presented in Table 1. A 1.9-fold increase of the apparent solubility of TMP in 16 mM β-CD at 25 °C clearly underlines the effect of complexation in the liquid state.

3.3. Thermodynamic parameters

The thermodynamic parameters of the inclusion were calculated from the K at 25 °C and 45 °C, using Eqs. (9)–(11)

Table 2
Thermodynamic parameters for interaction in aqueous solution of TMP with β-CD

Temperature (K)	$K_{1:1}$[a] (M^{-1})	ΔG_i (kJ mol^{-1})	ΔS_i (J mol^{-1} K^{-1})	ΔH_i (kJ mol^{-1})[b]
298	65	−10.3	−32.9	−20.1
318	39	−9.7	−32.7	−20.1

[a] $K_{1:1}$: apparent stability constant.
[b] Considering enthalpy (ΔH_i) as a constant.

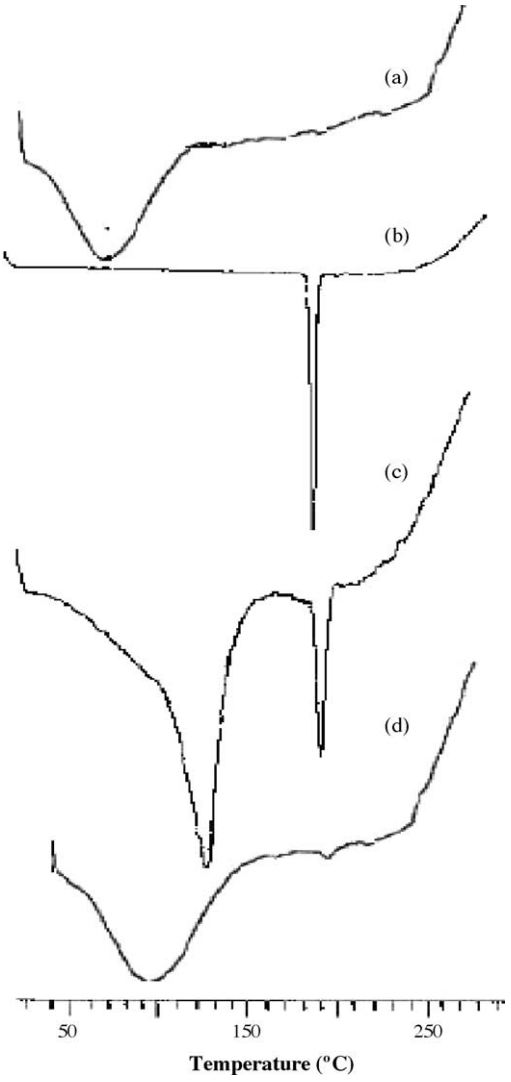

Fig. 4. DSC curves for: (a) β-CD; (b) TMP; (c) physical mixture; and (d) complex.

[12]:

$$\ln \frac{K_2}{K_1} = \frac{\Delta H(T_2 - T_1)}{RT_2T_1} \quad (9)$$

$$\Delta G_i = -RT_i \ln K_i \quad (10)$$

$$\Delta S_i = \frac{\Delta H_i - \Delta G_i}{T_i} \quad (11)$$

where K_1 and K_2 are the apparent stability constant at 25 and 45 °C, respectively, R is the gas constant (8.314 J mol^{-1} K^{-1}), ΔG, ΔH and ΔS are the standard Gibbs

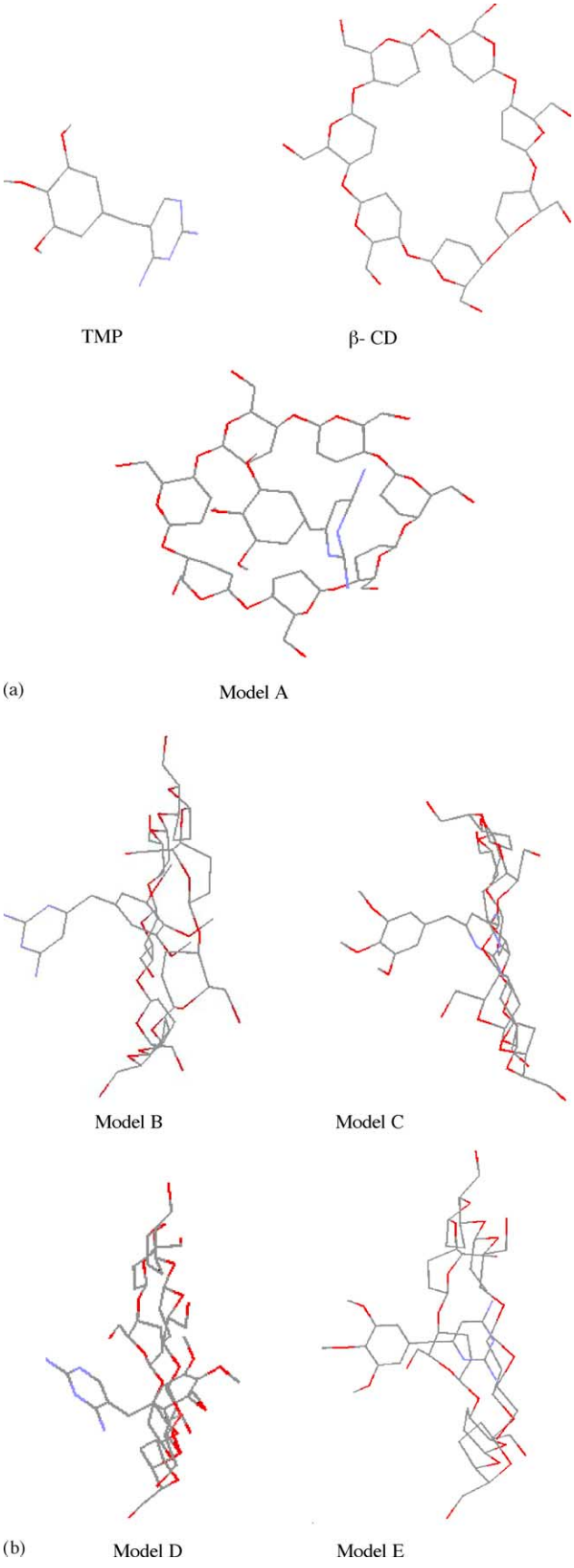

Fig. 5. Molecular models of TMP/β-CD inclusion: model A, the TMP is fully embedded in the cavity of the β-CD; Model B, only the 3,4,5-trimethoxybenzyl group of TMP is partly enclosed into the β-CD cavity; model C, only 2,4-diamino-pyrimidine group of TMP is partly interacted inside the β-CD; Model D, only the 3,4,5-trimethoxybenzyl group of TMP is fully enclosed into the β-CD cavity; Model E, only 2,4-diamino-pyrimidine group of TMP is fully interacted inside theβ-CD.

energy change, enthalpy and entropy, respectively. It should be noted that T_i is thermodynamic temperature in Kelvin (K).

The apparent stability constant (K) of β-CD-TMP system, considering formation of 1:1 complex, were calculated according to Eq. (8) and reported in Table 2. Standard thermodynamic parameters, calculated from the temperature dependency of $K_{1:1}$ values within the 25–45 °C temperature range (Table 2), are close to the ones reported earlier [13]. The results suggested that the complexation process was essentially enthalpy-controlled and that both dipolar or induced dipolar and van der Waals interactions between host and guest molecules are involved in inclusion complexation. A contribution of hydrophobic interactions involve the breakdown and displacement of the highly ordered water molecules inside the cyclodextrin cavity. The binding process is spontaneous ($\Delta G = -10.3\,\text{kJ mol}^{-1}$ at 298 K and $-9.7\,\text{kJ mol}^{-1}$at 318 K), whereas the negative entropy indicates greater order after comlexation. It is mainly due to the loss of rotational and translational freedom degree of the molecules implicated in the complexation process [14–16].

3.4. Differential scanning calorimetry analysis

Thermal analysis has been reported as a method to characterize cyclodextrin complexes [17,18]. Fig. 4 illustrates the DSC profiles of TMP, β-CD, physical mixtures and complex.

The β-CD (Fig. 4a) displays a wide and strong endothermic effect in the 70–160 °C interval, which may be ascribed to dehydration or fusion. The TMP thermal curve (Fig. 4b) is typical of crystalline anhydrous substances and is all characterized by a sharp endothermic effect (peak temperature at 201 °C), assigned to its melting. The physical mixtures (Fig. 4c) shows, after the broad endothermic effect due to the β-CD dehydration process or fusion, the TMP melting peak, which appeared substantially unaffected in its shape and area. This indicates that the drug basically maintained its original crystallinity. The DSC curve of the complex (Fig. 4d) is similar to the one of β-CD, however, a weak endothermic effect can be observed at about 201 °C, considering as a small quantity of free TMP.

3.5. Molecular-modeling studies

Assumed formation of 1:1 complex, the ArgusLab program gave five possible models (Fig. 5) for the most probable structure of the TMP/β-CD complex based on the energetic behaviors of the molecules. Model A was the less probable because the structure was energetically unfavorable. The model B was energetically most favorable in five possible models, in addition, UV spectra of TMP-β-CD aqueous solution (Fig. 2) at 280 nm shows that the chromophores of the TMP are not shielded by β-CD. A most possible arrangement for the complex seems to be model B where 3,4,5-trimethoxybenzyl group is partly embedded in the cavity of the β-CD.

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