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Short communication

# Inclusion complex of trimethoprim with β-cyclodextrin

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**Abstract**

The complexation of trimethoprim (TMP) with β-cyclodextrin (β-CD) was studied. UV-spectrophotometry and phase-solubility techniques were employed to investigate the complexation behaviour in liquid medium, and to demonstrate that the aqueous solubility of TMP increased 2.2-fold due to complexation with β-CD. Solid samples prepared by co-evaporation (in 2 wt% acetic acid solution) have also been studied, using differential scanning calorimetry (DSC) and powder X-ray diffraction (XRD), to assess the formation of the inclusion complex. The water content of the complex and β-CD was determined using thermo gravimetric analysis (TGA). In vitro dissolution analysis indicated that dissolution properties of TMP/β-CD complex were superior compared to both pure TMP and the corresponding physical mixture of TMP and β-CD.
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*Keywords:* Inclusion complex; Trimethoprim; β-cyclodextrin; Solubility; Dissolution properties

## 1. Introduction

β-Cyclodextrin (β-CD) consists of seven amylose units, connected at the 1 and 4 carbon atoms, formed by enzymatic cyclization of starch. The molecule is a truncated cone-shaped molecule with a hollow, tapered cavity of 7.9 Å depth. The top and bottom diameters of the cavity are 6.0 and 6.5 Å, respectively. The cavity of β-CD is relatively hydrophobic compared to water, while the external faces are hydrophilic. β-CD is capable of discerning various types of guest molecules by selectively incorporating such molecules through size and polarity considerations [1]. Several driving forces have been proposed for the inclusion of β-CD with substrate including hydrogen bonding, Van der Waals forces, hydrophobic interaction and the release of 'high energy water' molecules from the cavity [2–4]. 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, β-CD was extensively studied by various experimental techniques [5–7] and in the pharmaceutical industry β-CD has been used to enhance the solubility, stability and bioavailability of drugs [8–11]. β-Cyclodextrin is the favorite cyclodextrin for the encapsulation of drugs because of its lower price and higher production rate.

Trimethoprim (TMP) [2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine], 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, characterized by bitter taste and very low aqueous solubility (approximately 1.4 mM in water at 25 °C) [12,13]. It is important to study the inclusion complex of TMP with β-CD, for it may decrease the bitter taste, enhance the absorption of TMP and can provide better understanding of drug complextion. In this investigation TMP was complexed with β-CD in an attempt to improve its aqueous solubility and dissolution properties.

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## 2. Experimental

### 2.1. Materials

Trimethoprim (pharmaceutical grade) was obtained from Nanjing Pharmaceutical Factory Co., Ltd., β-cyclodextrin 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. Preparation of solid samples

The solid complex of TMP with β-CD in 1:1 molar ratio was prepared by co-evaporation. TMP (5.80 g) was dissolved in 130 ml water (at 80 °C, containing 2 wt% acetic acid) to achieve the solution of the drug, then β-CD powder (22.70 g) was slowly added to the solution. The resulting mixture was stirred for 10 h at 80 °C and was dried to obtain a white powder. The powder was washed three times with absolute ethanol and the solvent was eliminated by vacuum evaporation at 80 °C. The final product was pulverized and sieved (75–150 μm).

The physical mixture of TMP and β-CD in 1:1 molar ratio was obtained by mixing individual components, that had previously been sieved (75–150 μm), together with a mortar and spatula for 10 min.

### 2.3. Acetic acid and water assay

The acetic acid content of the complex was determined by titration with sodium hydroxide. Samples were prepared by dissolving 1 g of complex in 100 ml of water, using phenolphthalein as indicator. The resulting solution was titrated with 0.01 M sodium hydroxide [14].

The water content of the complex and β-CD was determined using thermo gravimetric analysis (TGA) with the TGA/SDTA851e (Mettler Toledo, Swiss) Thermal Analyzer. Samples were desiccated over phosphorous pentoxide for 2 days prior to assay to remove surface absorbed water. Samples were prepared by placing about 15 mg of sample into a platinum boat. Analysis was performed by computing the percent weight loss from the thermal transition occurring from the room temperature to 130 °C to yield the percent water content. The nitrogen flow rate was 100 ± 10 ml/min and the heating rate was 5 °C/min.

### 2.4. UV spectrophotometric measurement

A UV spectrophotometric measurement was developed to quantitatively measure TMP and its complex. A Shimadzu UV-2450 visible spectrophotometer was used to record absorption spectra. The path length of the quartz cell was 10 mm and the scan range was from 340 to 240 nm with 1 nm intervals, the blank was distilled water. Firstly, the linear range was calibrated by using five different concentrations of aqueous solutions of TMP (in 10–50 mg/l range) to establish a TMP calibration curve. Secondly, powder of β-CD was dissolved in the above TMP solutions (the concentration of β-CD in solutions approximately was 500 mg/l) then the solutions were shaken at room temperature. After 10 days, the samples were analyzed.

### 2.5. 2.5Differential scanning calorimetry (DSC)

DSC was used as a quantitative measure of crystallinity of TMP and developed to characterize the formation of a true inclusion complex. DSC measurements were performed using a Shimadzu DSC-50 (Shimadzu Co., Japan). Pure TMP, β-CD, the physical mixture (1:1 molar ratio) and the equimolar complex were desiccated over phosphorous pentoxide for 2 days prior to assay to remove surface absorbed water. Samples (10–15 mg) were placed into pierced aluminum pans with a perforated lid under static air and scanned over the temperature of 30–270 °C at a heating rate of 5 °C/min. The blanks were α-$Al_2O_3$.

### 2.6. Powder X-ray diffraction (XRD)

XRD was used as another quantitative measure of crystallinity of TMP and developed to characterize the formation of the inclusion complexation. The powder X-ray diffraction (XRD) patterns of pure TMP, β-CD, physical mixture and corresponding complex were recorded using an automated Rigaku D/MAX 2500V/PCX X-ray diffractometer (Rigaku Co., Japan) with monochromatized Cu Kα radiation and analyzed between 2$\theta$ angles of 5–50°. The voltages, current, step size and scanning rate were 40 kV, 200 mA, 0.02° (2$\theta$) and 2°/min, respectively.

### 2.7. Solubility studies

Samples were prepared by adding 25 ml aqueous solutions to a series of 50 ml stoppered flasks each containing increasing quantities of β-CD as follows: 0, 3, 6, 9, 12, 15, 19, 22, 25, 28 and 32 mM. Excess TMP (50 mg) was added into each flask, then the suspensions were shaken for 10 days at constant temperature (45 ± 1 °C). 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. The phase-solubility diagram was constructed by plotting the total dissolved TMP concentration against the total β-CD concentration. The association constant ($K_{1:1}$) was calculated as follows from the slope of phase-solubility diagram, where $S_0$ is the solubility of TMP in absence of β-CD [15]:

$$K_{1:1} = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (1)$$

### 2.8. Dissolution studies

In vitro dissolution studies of pure drug (TMP), the physical mixture and the solid complex were carried out in 900 ml of deionized water at 37.0 ± 0.5 °C, using a USP XXI Type 2 Dissolution Rate Test Apparatus by powder dispersed amount method (powder samples were spread over the dissolution medium). The sample powder of TMP (200 mg) or an equivalent amount of the physical mixture or complex was added to the dissolution medium (deionized water) and a speed of 50 rpm was used in each test. A 5 ml aliquot was withdrawn at different time intervals (5 or 10 min) with a filter-syringe (hybrid cellulose ester film, pore size 0.45 μm) and replaced with 5 ml fresh dissolution medium. The filtered samples were suitably diluted, if necessary, and assayed for TMP by measuring the maximal absorbance at 280 ± 2 nm. Each test was repeated three times. The dissolution profiles were constructed by plotting the cumulative percent drug dissolved against time.

## 3. Results and discussion

### 3.1. Acetic acid and water content

The acetic acid content was about 0.04% for the complex, which suggests that the acid had no significant effect on the following analysis. The water content of the complex and the β-CD was 8.03% and 13.26%, respectively. The decrease in water content of complex may be mainly attributable to the water in the β-CD cavity being replaced by the drug.

### 3.2. UV spectrophotometric analysis

The UV analysis results showing changes in maximal absorbance was linear for a concentration of 10–50 mg TMP/l ($r = 0.9989$) at 280 ± 2 nm. The maximal absorbance of the solutions of TMP and β-CD was almost equal to that of the same concentration of TMP solutions (Fig. 1b and c). The β-CD showed insignificant ultraviolet absorbance (Fig. 1a). No shift was observed in the lambda maxima of TMP when mixed with the β-CD. In the following analysis, the β-CD concentration was lower than that of present analysis and its ultraviolet absorbance could be neglected. Therefore, the determination of the TMP content of the physical mixture and complex may be calculated using the TMP calibration curve.

### 3.3. Differential scanning calorimetry analysis

DSC has been shown to be a very powerful analytical tool in the characterization of solid-state interactions between drugs and cyclodextrins [16–18]. Thermograms were analyzed qualitatively by examination both the peak temperature and the endothermic transition contour. The relative crystallinity of drug ($drug_{RDC}$) in physical mixture and complex

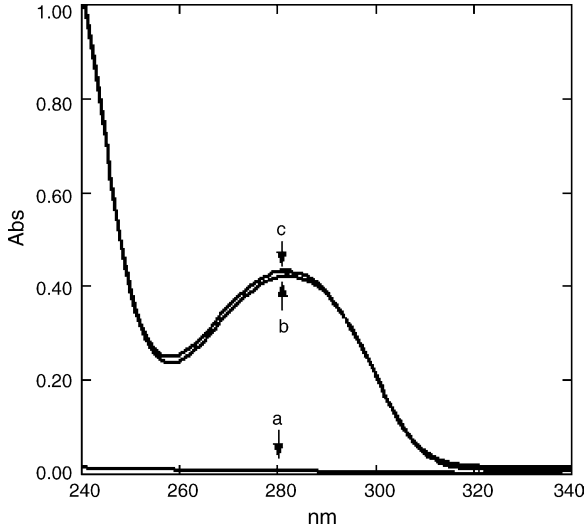


Fig. 1. UV spectra: (a) 500 mg/l β-CD; (b) 25 mg/l TMP; (c) 500 mg/l β-CD and 25 mg/l TMP.

was estimated by the ratio between the melting enthalpy of the drug calculated in the sample and that of the pure drug, according to the Eq. (2):

$$\text{drug}_{\text{RDC}} = \frac{\Delta H_{\text{sam}}}{\Delta H_{\text{d}}} \quad (2)$$

where $\Delta H_{\text{sam}}$ is the melting enthalpy of drug calculated in the physical mixture or complex and $\Delta H_{\text{d}}$ is the melting enthalpy of the pure drug sample [19,20]. Fig. 2 illustrates the DSC profiles of β-CD, TMP, the physical mixture and corresponding complex. Quantitative data for TMP crystallinity were extracted from DSC curves in Fig. 2 and summarized in Table 1. The decrease of in relative crystallinity of TMP in the physical mixture was likely due to the weak interaction between the TMP and β-CD or other reasons.

The curve of β-CD (Fig. 2a) displays a wide and strong endothermic effect in the 100–130 °C interval (peak $T_{\text{max}} = 117.6$ °C), which may be ascribed to dehydration. The TMP thermal curve (Fig. 2b) is typical of crystalline anhydrous substances and is characterized by a sharp endothermic effect (peak temperature at 200.8 °C), assigned to its melting. The thermogram of the physical mixture (Fig. 2c) shows the broad endothermic effect due to the β-CD dehydration process. The TMP melting peak was slightly shifted to lower temperature and become wider which may be explained by the weak interaction between the TMP and β-CD in the

Table 1
DSC data and the relative degree of crystallinity of drug ($drug_{RDC}$)[a] for TMP, physical mixture and complex

| Sample | Peak temperature ($T_{\text{max}}$, °C) | $\Delta H$ (kJ/mol) | $Drug_{RDC}$ |
|---|---|---|---|
| Pure TMP | 200.8 | 6.217 | 1.00 |
| Physical mixture | 198.0 | 5.843 | 0. 94 |
| Inclusion complex | – | – | – |

[a] Mean of two determinations; $\Delta H$: calculated by peak area integral.

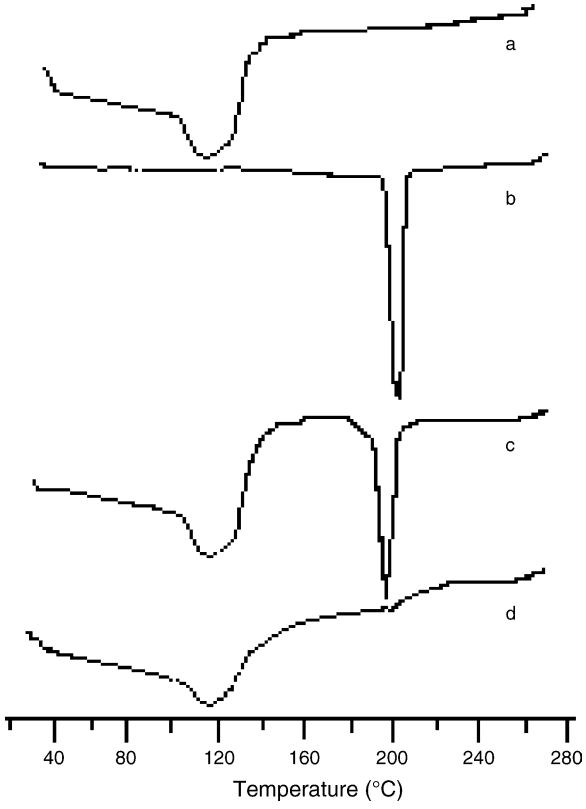


Fig. 2. DSC curves for: (a) β-CD; (b) TMP; (c) physical mixture; (d) complex.

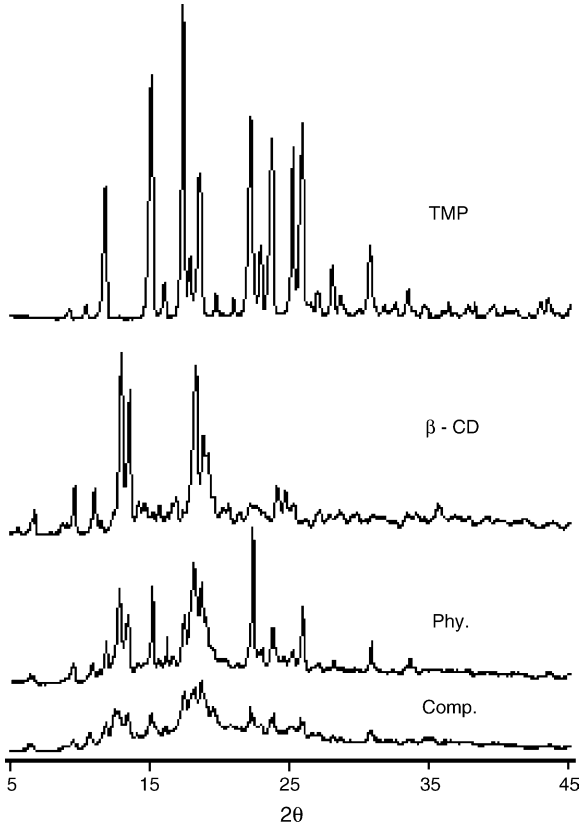


Fig. 3. Powder X-ray diffraction patterns of TMP, β-CD, physical mixture (phy.) and equimolar complex (comp.).

physical mixture at high temperature. The thermal curve of the complex (Fig. 2d) is similar to that of β-CD and dissimilar to the physical mixture. Furthermore, the characteristic endothermic effect of β-CD slightly shifting to higher temperature for the complex ($T_{max}$ = 118.7 °C), could be explained by the fact when a guest molecule enters the β-CD cavity and some of the cavity water is lost, thus the energy of the remaining water changes. These observations indicated the formation of an amorphous solid dispersion, i.e., the TMP molecule inside the β-CD cavity and formation of a true inclusion complex.

### 3.4. X-ray diffraction

X-ray diffractometry is a useful method for the detection of inclusion complexation in powder or microcrystalline states. The diffraction patterns of the complex should be clearly distinct from that of the superimposition of each component if a real inclusion complex has been formed. The diffraction patterns (Fig. 3) show TMP and β-CD were polycrystalline and the physical mixture was the superimposition in a certain proportion as each component. The diffractogram of the complex differed from that of the corresponding physical mixture, where the characteristic peaks of TMP, particularly at 22.3° (2$\theta$), nearly disappeared, indicating the formation of a true inclusion complex. These observations were in accordance with the results of the DSC analysis.

The relative intensity (RI) of diffraction peaks was estimated by comparing some representative peak heights in the diffraction patterns of samples with that of the pure drug, according to the Eq. (3):

$$\mathrm{RI} = \frac{H_{\mathrm{sam}}}{H_{\mathrm{d}}} \tag{3}$$

where $H_{sam}$ is the peak height of the physical mixture or complex under representative angle, and $H_d$ is the peak height of the pure drug at the same angle [11,21]. The RI values of physical mixture and complex are given in Table 2. The decrease in the intensity of the physical mixture (as evidenced by peak heights) compared with pure TMP was likely due to their different composition since this was a pure substance being compared to a physical mixture of two substances with different diffraction patterns [11].

Table 2
The RI values of physical mixture and complex

| Sample | 2$\theta$ | | | | |
|---|---|---|---|---|---|
| | 15.2° | 16.2° | 22.3° | 25.9° | 30.8° |
| RI (phy.) | 0.26 | 0.82 | 0.67 | 0.33 | 0.50 |
| RI (comp.) | 0.06 | 0.15 | 0.06 | 0.12 | 0.16 |
| RI (comp.)/RI (phy.) | 0.23 | 0.18 | 0.09 | 0.36 | 0.32 |

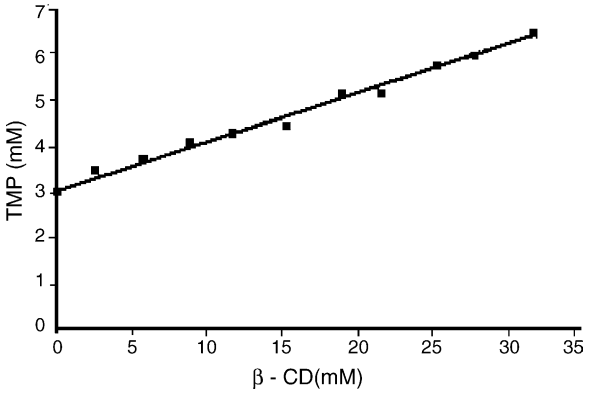


Fig. 4. Phase-solubility diagram for the TMP–β-CD system at 45 ± 1 °C.

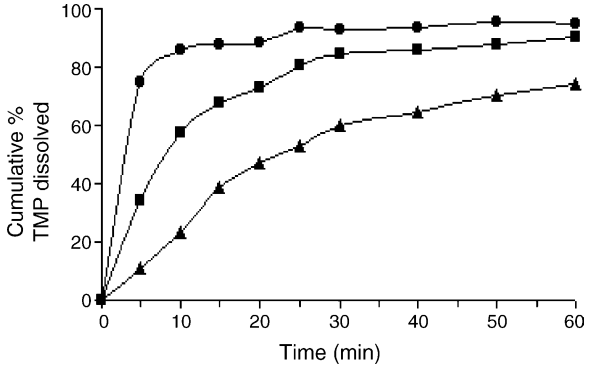


Fig. 5. Dissolution curves of trimethoprim (TMP) (▲) TMP alone: (●) TMP/β-CD complex; (■) TMP–β-CD physical mixture.

### 3.5. Solubility studies

UV spectrophotometric analysis at 280 ± 2 nm showed a linear increase in the maximal absorbance of TMP with a change of concentration of β-CD, i.e., the solubility of TMP increased linearly with an increase in the concentration of β-CD, giving $A_L$ type solubility diagram (Fig. 4) [15]. The correlation between the solubility of TMP and the concentration of β-CD ($r = 0.999$ at 280 ± 2 nm) was considered as an evidence of complex formation. The apparent solubility of TMP increased by 2.2-fold in 32 mM β-CD solution at 45 ± 1 °C. The formation of an inclusion complex between TMP and β-CD in aqueous solution can be assumed by the $A_L$ type solubility diagram [15], i.e., by the linear relationship between dissolved TMP concentration and the amount of β-CD. The association constant ($K_{1:1}$), considering the formation of a 1:1 complex, was calculated as $K_{1:1} = 39\ M^{-1}$. The relatively low $K_{1:1}$ is probably due to steric hindrance of TMP and indicates that β-CD interact weakly with TMP.

### 3.6. Dissolutions studies

When a drug–CD inclusion complex is dispersed in a dissolution medium, a very rapid dissolution is often observed. Dissolution rate tests are based on the observation in order to characterize the inclusion complexation between a drug and a cyclodextrin. Fig. 5 shows the dissolution profiles of TMP, TMP/β-CD complex (1:1 molar ratio) and the corresponding physical mixture. The faster dissolution rate of the complex could be explained by both the greater surface areas of contact between TMP and β-CD and decrease in TMP crystallinity as a consequence of specific drug–carrier interactions, as demonstrated by DSC and X-ray diffraction analyses. The increase in TMP dissolution rate observed for physical mixture may be mainly attributable to the hydrophilic effect of the carrier, which can reduce the interfacial tension between the poorly soluble drug and the dissolution medium, thus leading to a high dissolution [11,22].

## 4. Conclusions

The inclusion complex was prepared successfully with the insoluble drug trimethoprim and the water-soluble complexing agent β-cyclodextrin. The properties of the TMP/β-CD complex were characterized by UV spectrophotometry, thermo gravimetric analysis, differential scanning calorimetry, powder X-ray diffraction, phase-solubility techniques and dissolution analysis. The dissolution properties of TMP/β-CD complex were superior to those of pure TMP and the corresponding physical mixture. Thus, the pharmaceutical properties of aqueous solubility and dissolution rate of TMP can be partially improved by complexation with β-CD. In the present work, for the low price and high production rate of ß-CD, the TMP/β-CD complex can be considered as a promising way to enhance the physicochemical properties of TMP. However, in order to obtain even higher solubility, better dissolution character and much less bitter taste, the authors are investigating the complexes of TMP with derivatized CDs.

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