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The Inclusion Complex of Piromidic Acid with Dimethyl-β-cyclodextrin in Aqueous Solution and in the Solid State

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Abstract. Inclusion complex formation of piromidic acid (PA) with dimethyl-β-cyclodextrin (DM-β-CD) in aqueous solution and in the solid state was confirmed by the solubility method, differential scanning calorimetry (DSC) and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. The apparent stability constant, K_c, of the complex was estimated to be 244 M^{-1}. The stoichiometry of the complex was given as the ratio 1:2 of PA to DM-β-CD. The dissolution rate of the PA/DM-β-CD complex was much greater than that of intact PA.

Key words: Piromidic acid/DM-β-CD inclusion complex, phase-solubility, DSC, ^{1}H-NMR, dissolution profile.

1. Introduction

The α-, β-, and γ-cyclodextrins are cyclic oligosaccharides consisting of six, seven, and eight glucose units respectively. Although they have found many applications [1] they have some undesirable properties. The limited application of cyclodextrins in the pharmaceutical field seems to be related to their relatively low aqueous solubility [2]. Recently, the chemically modified cyclodextrins have received considerable attention because their pharmaceutical properties and inclusion behaviors are different from those of natural cyclodextrins. For example, the methylated β-cyclodextrins, such as heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD) and heptakis-(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-CD), are extremely soluble in water (more than 30 w/v % at 25 °C), and they interact with a variety of drug molecules [3].

Piromidic acid (PA), 5,8-dihydro-8-ethyl-5-pyrrolidopyrido(2,3-*d*)pyrimidine-6-carboxylic acid is a pyrido pyrimidine derivative, a congener of nalidixic acid. PA is widely used for its antibacterial activity against several gram negative pathogens. PA is very slightly water soluble [4, 5].

Cyclodextrin complexation has been extensively applied to enhance the solubility, dissolution rate, membrane permeability and bioavailability of slightly soluble drugs [6–9].

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Thus, this investigation was carried out with the aim of improving the dissolution characteristics of piromidic acid.

2. Experimental

2.1. MATERIALS

Piromidic acid and the cyclodextrins (α, β, γ, DM-β-CD) were supplied from Dainippon Pharmaceutical Co., and Nippon Shokuhin Kako Ltd., respectively. All other chemicals and solvents were of analytical reagent grade.

2.2. METHODS

2.2.1. *Solubility Studies*

The phase solubility diagram was obtained according to the method described by Higuchi, *et al.*, [10]. Excess amounts of PA were added to buffer solutions at pH 7.4 (0.02 M Na-acetate) containing various concentrations of DM-β-CD in a measuring flask. These were shaken at 37 ± 0.5 °C for 4 days. Following equilibration, the contents of the measuring flask were filtered through a Toyo TM-2 membrane filter (0.45 μm). A portion of the sample was adequately diluted and analyzed using a Hitachi 323 spectrophometer at 273 nm to determine the concentration of piromidic acid.

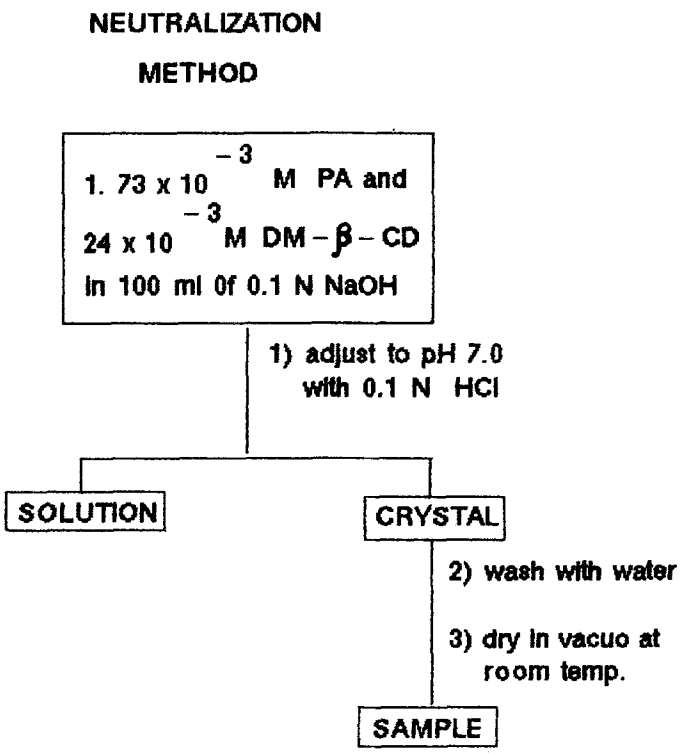

Chart 1. Method for mass preparation of inclusion complex.

2.2.2. *Preparation of Inclusion Complex*

The preparative method is shown in Chart 1. The stoichiometry of the complex prepared by the neutralization method was then analyzed and was found to be 1:2 (PA:DM-β-CD).

The physical mixture of PA with DM-β-CD in a 1:2 molar ratio was prepared by simple blending in a ceramic mortar (10 min).

2.2.3. *Differential Scanning Calorimetry Study*

The differential scanning calorimetry was carried out using a Perkin-Elmer model 1B differential scanning calorimeter at a scanning speed of 8 °C/min over the temperature range of 400 to 600 K.

2.2.4. *^{1}H-NMR Spectroscopy Study*

The ^{1}H-NMR spectra were recorded in deuterium oxide and sodium hydroxide-d_1 solution (about 40% NaOD in D_2O) using a JNM-FX 100 spectrometer operating at ^{1}H–99.65 MHz. The ^{1}H-chemical shifts are given relative to external tetramethysilane within ±0.002 ppm.

2.2.5. *Dissolution Studies*

Dissolution rates of piromidic acid from the inclusion complex and the physical mixture were measured by the method of Nogami *et al.* [11]. The powdered sample (150 mesh) of the drug or its equivalent amount of the complex was put into 50 ml of buffer solutions at pH 7.4 in a dissolution cell which was kept at 37±0.5 °C and the dissolution medium was stirred. At appropriate intervals, 1 ml samples were filtered through a membrane filter (0.45 μm), diluted and assayed spectrophotometrically.

3. Results and Discussion

3.1. PHASE SOLUBILITY DIAGRAM

The formation of the complex between PA and DM-β-CD was studied by a solubility method. Figure 1 shows an equilibrium phase solubility diagram obtained for the PA/DM-β-CD system in buffer solution at pH 7.4. The plot shows a typical B_s-type solubility curve. The initial rising portion is followed by a plateau region and then decreases in total concentration of PA with precipitation of a microcrystalline complex at a high DM-β-CD concentration. The stoichiometry of the complex was found to be 1:2 (PA:DM-β-CD) from the PA content.

The apparent stability constant, K_c, of the DM-β-CD complex was calculated to be 244 M^{-1} from the initial straight line portion of the solubility diagram (Figure 1).

3.2. EVIDENCE OF INCLUSION COMPLEX FORMATIONS

As shown in Figure 2 the thermograms of PA and its physical mixture with DM-β-CD show an endothermic peak at around 316 °C corresponding to the melting

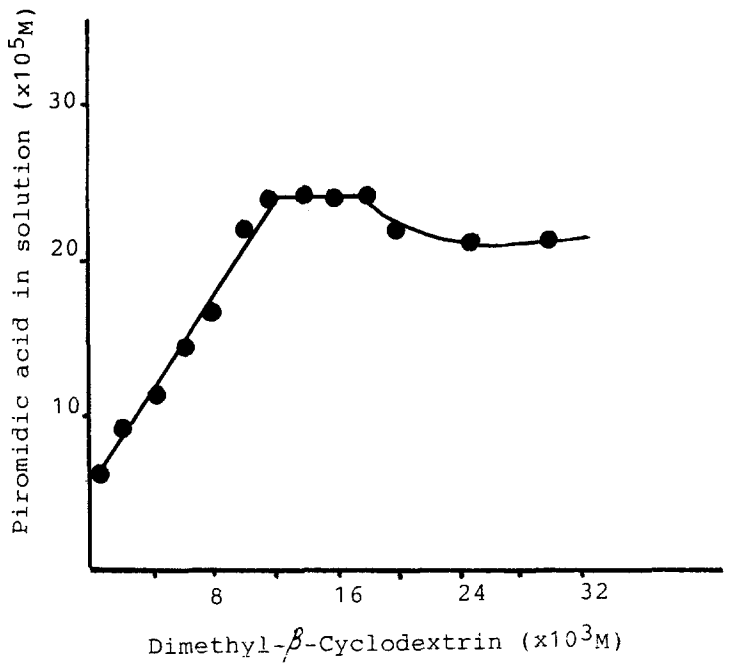

Fig. 1. Phase solubility diagram of piromidic acid/DM-β-CD system in buffer solution (0.02 M Na-acetate) pH 7.4 at 37 °C.

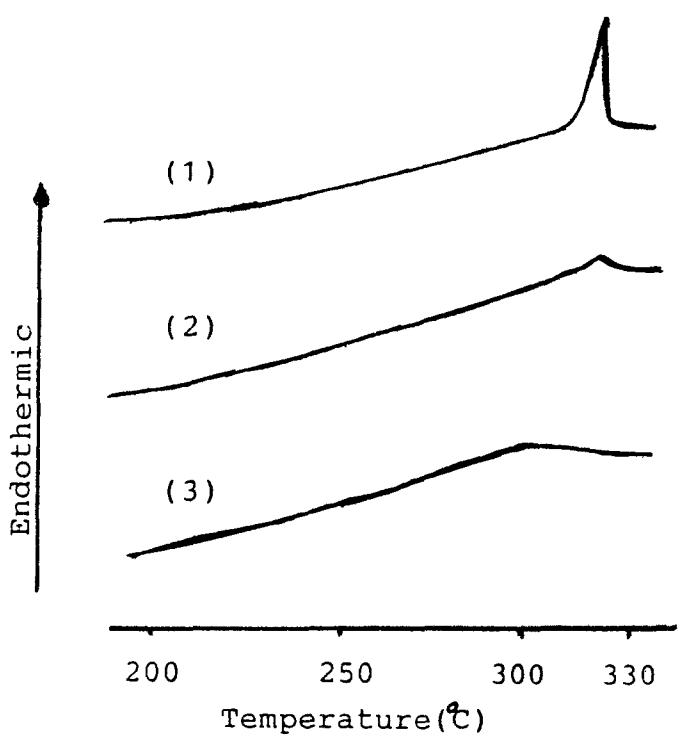

Fig. 2. Differential scanning calorimetry curves at a scanning speed of 8 °C/min. 1, Intact PA; 2, Physical mixture of PA and DM-β-CD; 3, PA/DM-β-CD inclusion complex (1:2 molar ratio).

point of the drug. In contrast, the endothermic peak was not observed in the case of the assumed inclusion complex.

Figure 3 shows the effect of DM-β-CD on the ^{1}H-NMR spectrum of PA in D_2O. All of the proton signals of PA shifted to lower field with increasing amounts of DM-β-CD. Similar chemical shift changes have been observed for other drug-CD systems [12, 13, 14]. The chemical shifts of PA to low field might be induced by the diamagnetic anisotropy of particular bonds of DM-β-CD and Van der Waals shifts [15].

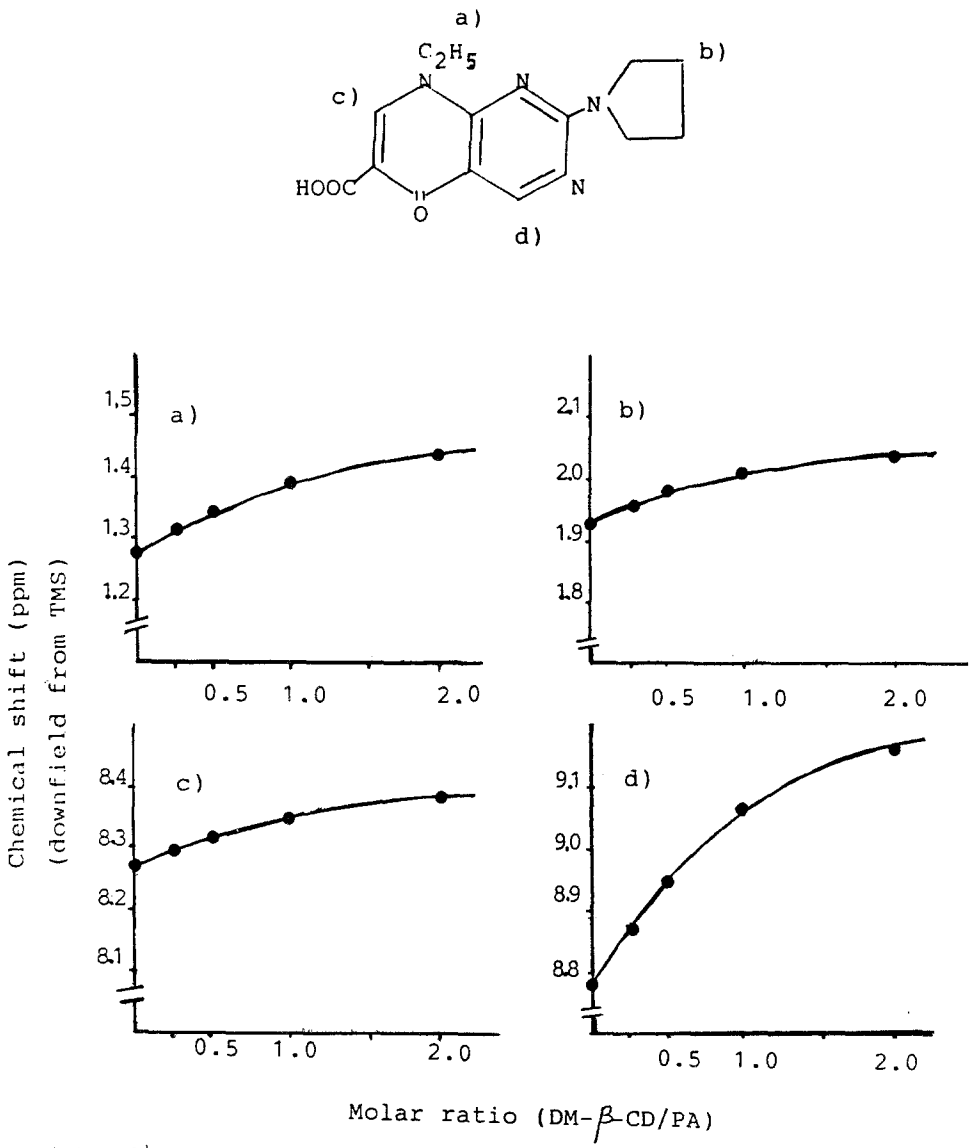

Fig. 3. Variation of ^{1}H chemical shifts of 0.01 PA with concentration of DM-β-CD in 0.1 N NaOD at room temperature.

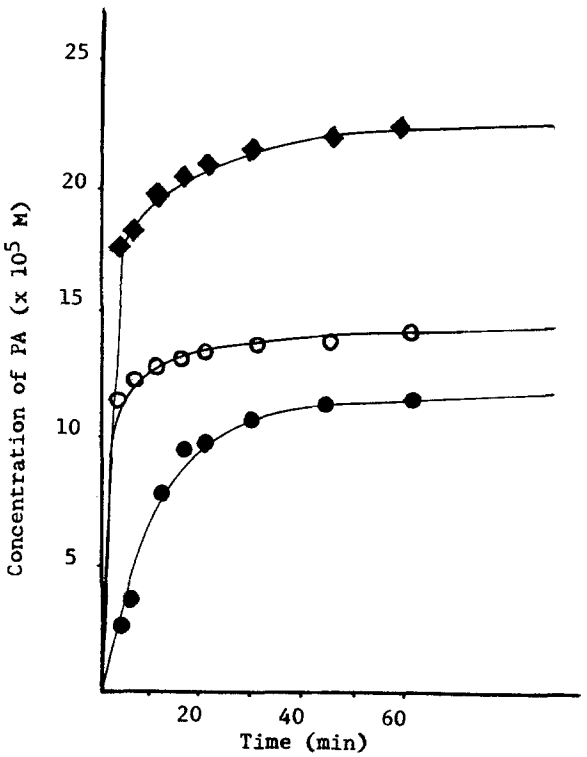

Fig. 4. Dissolution profiles of piromidic acid and its DM-β-CD complex in pH 7.4 buffer solution at 37 °C. ●, Intact PA; ○, Physical mixture of PA and DM-β-CD; ◇, PA/DM-β-CD inclusion complex (1:2 molar ratio). Each point is the mean of five determinations.

3.3. DISSOLUTION BEHAVIOR OF PIROMIDIC ACID AND ITS COMPLEXES

The relative rates of PA and its DM-β-CD complex in powder form are shown in Figure 4. It is evident that the PA/DM-β-CD complex dissolved much more rapidly than intact PA. The enhancement of the dissolution characteristics of PA by DM-β-CD inclusion complexation may be due to improvements in solubility and wettability.

4. Conclusion

The above results suggest that complex formation with DM-β-CD may enhance the bioavailability of PA. Thus, the increased dissolution rate suggests that the PA/DM-β-CD complex may have utility in the development of fast dissolving dosage forms with improved bioavailability.

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