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Contributed Paper

# Inclusion Complexation of Indomethacin with Hydroxypropyl-β-cyclodextrin

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## ABSTRACT

This study aimed to increase the solubility of indomethacin in water by complexation it with hydroxypropyl-β-cyclodextrin (HPβCD). Phase-solubility analysis was used to investigate interactions in aqueous solution between HPβCD and indomethacin. Equimolar indomethacin-HPβCD solid systems were prepared by four different methods including physical mixtures (PM), kneading (KN), coevaporation (COE) and freeze-drying (COL) methods. The complex was characterized by infrared spectroscopy, differential scanning calorimetry, X-ray diffractometry, thin layer chromatography and dissolution rates. The complexation efficiency of indomethacin-HPβCD was determined spectrophotometrically. The solubility of indomethacin increased linearly as the concentration of HPβCD increased. This indicated a feature of the $A_L$-type complex that the water-soluble complexes existed in the solution. The average of apparent 1:1 stability constant of the complex ($K_{1:1}$) at 30°C was 340 $M^{-1}$. The KN and COE methods formed partial inclusion complexes, whereas the COL method gave complete complexation. The dissolution rates of indomethacin increased when complexed with HPβCD. HPβCD complexation of an ionized drug molecule by the COL method exhibited the highest dissolution rate of indomethacin [the dissolution efficiency after 90 min ($DE_{90}$) at 61.7±0.9% and $t_{50\%}$ of 13 min., while the uncomplexed indomethacin showed $DE_{90}$ at 15.4±0.1% and $t_{50\%}$ more than 90 min. The COL process was the best method because of the high content and dissolution rate of the drug. It is also the simple method to prepare the inclusion complexes. The result from this study has suggested the dissolution rate enhancement of indomethacin by the simple complexaion method with HPβCD.

**Keywords:** indomethacin, hydroxypropyl-β-cyclodextrin, inclusion complex, kneading, coevaporation, freeze-drying

## 1. INTRODUCTION

Indomethacin ([1-(4-chlorobenzoyl)- 5-methoxy-2-methylindol-3yl]acetic acid;

$C_{19}H_{16}ClNO_4$; mw=357.79, Figure 1) is an active non-steroidal anti-inflammatory drug (NSAID) and has been widely used in the treatment of arthritis, gout, spondylitis, bursitis, tendinitis and other inflammatory diseases because of its effective suppression of pain, fever, color, and edema. However, it is practically insoluble in water (2-7 μg/ml). The development of oral dosage forms which exhibit rapid dissolution rate is necessary to improve its oral bioavailability. Most NSAIDs cause

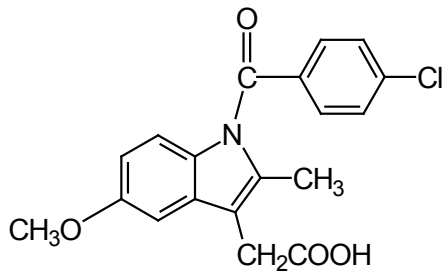


**Figure 1.** Chemical structure of indomethacin ([1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]acetic acid).

some form of gastrointestinal ulceration and bleeding. Indomethacin gave higher ulcerogenic potential than other NSAIDs [1]. Several approaches have been applied to improve the bioavailability of poorly water-soluble drugs and reduce the gastrointestinal irritation. One of the most simple and convenient approach is the application of cyclodextrins (CDs) which are cyclic torus-shaped oligosaccharides consisting of 6, 7 or 8 α-1,4-linked glucopyranose units, referred to as α-, β- or γ- cyclodextrin, respectively. Among different cyclodextrins, the beta and its hydroxyalkylated derivative, 2-hydroxypropyl-β-CD (HPβCD) are widely used in pharmaceutical industry as complexing agents. The CD molecule has hydrophobic central cavity capable of taking up a whole drug molecule or some part of it, whereas the exterior of the molecule is hydrophilic. In aqueous solution, the cavity is occupied by water molecules, and a suitably size and hydrophobic guest molecule may displace the water molecules from the CD cavity, leading to the formation of inclusion complexes [2]. Several methods have been published for druγ-CD inclusion complex formation including physical blending, kneading, co-precipitation technique, solution/solvent evaporation, neutralization precipitation, milling/co-grinding, atomization/spray drying, lyophilization/freeze-drying, microwave irradiation and supercritical technique [3]. Bandi et al. have demonstrated that indomethacin-HPβCD complexes with enhanced dissolution rate can be formed using a supercritical fluid $CO_2$ process which was single-step and organic solvent-free [4].

The objective of the present study is to investigate the possibility using of the simple process to improve solubility and dissolution rate of indomethacin by complexation with HPβCD using the three different complexation methods. The physiochemical characteristics of the solid inclusion complexes were also investigated.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Indomethacin was from Sinochem (Jiangsu Wuxi, China), HPβCD (Kleptose® HPB; average mw=1400) of molar substitution 0.6 was obtained from Roquette-Freres (Lestrem Cedex, France), hydroxypropyl methylcellulose (HPMC) with the respective substitution and viscosity types of 2910 and 50 cP was purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan). All other chemicals used were of analytical reagent grade from commercial sources.

### 2.2 Solubility Studies

An excess amount (±300 mg) of indomethacin was added to the aqueous solutions containing various concentrations of HPβCD (0 to 140 mM). The suspensions were equilibrated at 30±0.5°C under electromagnetical stirring at

120 rpm for 7 days, and then filtered through a 0.45-μm pore size membrane filter (Millipore, Bedford, MA), diluted appropriately with water and the indomethacin content in the filtrate was determined spectrophotometrically (Milton Roy Spectronic 1001 Plus spectrophotometer, Rochester, NY) at 264 and 280 nm for indomethacin and indomethacin ammonium salt, respectively. The presence of HPβCD did not interfere with the assay of indomethacin. This experiment was performed in triplicates.

The apparent stability constant of the indomethacin-HPβCD complex, assuming 1:1 stoichiometry, was calculated from the straight line portion of the solubility diagram (Figure 2), according to the equation [5-6]:

$$K_{1:1} = \text{slope}/S_o\ (1 - \text{slope})$$

where $S_o$ is the solubility of the drug in water without HPβCD.

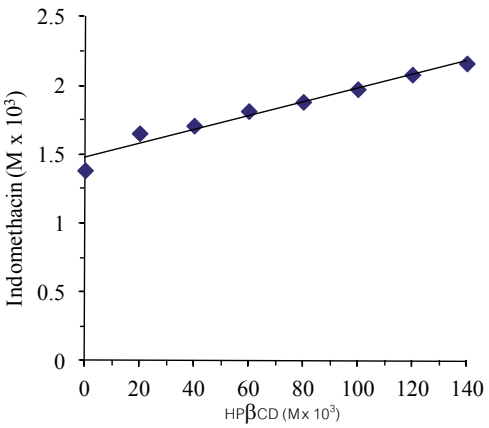


**Figure 2.** Phase solubility diagram of indomethacin in water containing various concentrations of HPβCD at 30±0.5°C ($n$ = 3).

### 2.3 Preparation of Binary Systems

Two types of binary systems were prepared [7], namely physical mixtures (PM) and solid inclusion complexes, prepared by kneading (kneaded systems, KN), coevaporation (coevaporated systems, COE) and freeze-drying (colyophilized systems, COL) methods. In all systems, druγ-HPβCD molar ratios were at 1:1 (20% w/w of indomethacin).

*Physical mixture method (PM)*: The required molar (1:1) quantities of indomethacin and HPβCD were weighed accurately and mixed together thoroughly in a mortar with vigorous trituration for about 5 min.

*Kneading method (KN):* HPβCD was suspended in an aqueous 1% (w/w) HPMC solution. Indomethacin was then added into the slurry with continuous kneading at approximately 1000 rpm for 5 h (Electrothermal Minipack, Hammant & Morgan Ltd., England). The complex was dried at 50°C for 24 h.

*Coevaporation method (COE):* Indomethacin and HPβCD were dissolved in the lowest volume of 80% (v/v) ethanol necessary to obtain a solution and continuously stirred for 30 min. Then, the solutions were evaporated under vacuum at 40°C with a rotary evaporator (Büchi Rotavapor Model R-124, Switzerland).

*Colyophilization method (COL) or Freeze-dried method:* Indomethacin and HPβCD were added into water and few drops of 28% (w/v) ammonium hydroxide solution were subsequently added until the formation of solution. The resulting mixture was frozen in a Christ FOC-1 Model K-40 equipment (Balzers-Pfeiffer GmbH, Aßlar, Germany), rotating at 75 rpm. Samples were lyophilized at -30°C in a Christ LOC-1M Model Alpha 2-4 apparatus for 24 h. No trace of ammonium ion was detected in the colyophilized product with the Nessler reagent.

*Physical mixture of freeze-dried substances (PM-COL):* Indomethacin was added into water and few drops of 28% (w/v) ammonium hydroxide solution were subsequently added until the formation of solution. HPβCD was dissolved in a minimum quantity of water. The solution of indomethacin and HPβCD were freeze-dried. Indomethacin and HPβCD dried powder were weighed accurately at the molar ratio of 1:1 and mixed gently in a vial for 5 min.

All of the dried systems were crushed, passed through sieve 315-μm mesh and stored under vacuum in a desiccator prior to use.

**2.4 Determination of Complexation Efficiency**

The complexation efficiency of indomethacin-HPβCD was determined spectrophotometrically, by measuring the total drug content and the indomethacin percentages complexed with HPβCD in the solid systems. The free-drug and the druγ-HPβCD were separated by washing out the products with diethylether through a Whatman filter paper No. 1. The residue remaining on the filter paper was air-dried and diluted appropriately with water. The total indomethacin content in the inclusion complex and the drug percentages complexed with HPβCD were determined at 264 and 280 nm for indomethacin and indomethacin ammonium salt, respectively. A calibration graph was constructed, by plotting the concentrations of either indomethacin or indomethacin ammonium salt against the absorbance values. The linearity of assay was determined from six working standard solutions of indomethacin or indomethacin ammonium salt in distilled water (concentrations: 5.0-60.0 μg/ml), prepared in triplicates. The correlation ($r^2$), intercept and slope of the calibration graph were calculated. The absorbance values of the samples were observed and compared with those obtained from the calibration graph, to determine the amount of indomethacin or indomethacin ammonium salt. The yields of KN, COE and COL products were also calculated. The experiment was performed in triplicates.

**2.5 Infrared Spectroscopy**

The IR spectra of pure indomethacin, pure HPβCD, the mixture of indomethacin-HPβCD (PM) and inclusion complexes of indomethacin were subjected to infrared (IR) characterization using FTIR-Shimadzu 8501 spectrophotometer (Shimadzu, Japan) to predict any possible interaction. Each sample was mixed with KBr powder and compressed into transparent disc under high pressure. The resulting discs were then tested within the range of 4000-500 $cm^{-1}$.

**2.6 Differential Scanning Calorimetry**

Approximately 5 mg each of pure indomethacin, pure HPβCD, the mixture of indomethacin- HPβCD (PM) and inclusion complexes of indomethacin were subjected to differential scanning calorimetry (DSC-7 Model, PerkinElmer, Inc., MA, USA). During each scan, 1-2 mg of the sample was heated in a hermetically sealed aluminum pan at the heating rate of 5°C $min^{-1}$, using an empty aluminum pan as the reference. All samples were heated in the range of 20-350°C.

**2.7 X-ray Diffractometry**

The powder X-ray diffraction patterns were obtained with a Siemens D-500 diffractometer. Samples were irradiated with monochromatized Cu-Ka radiation and analyzed between 2*q* angles of 5 and 60° at the scan rate of 1° $min^{-1}$.

Samples were dispersed in either methanol or water at an equivalent concentration of 1 mg/ml. Each sample was applied on a silica gel 60 $F_{254}$ TLC plate. The mobile phase employed in this study was the mixture of chloroform and methanol (4:1, v/v), with the development distance of 10 cm and spot detection under UV light at 254 nm with TLC scanner (Camag, Switzerland).

**2.9 Dissolution Studies**

Dissolution studies were performed using the USP type 1 basket method (100 rpm) in 900 ml of distilled water and maintained at 37±0.5°C for 90 min. Samples including pure indomethacin, PM, PM-COL and solid inclusion complexes, equivalent to 50 mg of the drug were previously sieved (particle size lower than 315 μm). At the predetermined time

intervals (10, 20, 30, 40, 50, 60 and 90 min), samples were withdrawn with a filter-syringe (pore size: 0.45 μm) and spectrophotometrically assayed for the drug content, as described in the solubility studies. Dissolution efficiency after 90 min ($DE_{90}$) was calculated from the area under the dissolution curve at 90 min (using the trapezoidal rule) and expressed as the area percentages of the rectangle described by 100% dissolution at the same time. The indomethacin percentages dissolved after 90 min ($DP_{90}$) and time necessary to dissolve 50% drug ($t_{50\%}$) were also determined. All experiments were carried out in triplicates.

## 3. Results and Discussion

### 3.1 Solubility Studies

The phase solubility study represents a basic requirement for optimizing the inclusion complex and evaluating the affinity of a certain drug to a specific CD. This process has been widely used to determine the molar ratio at which the drug forms a complex with CD. The phase solubility diagram of indomethacin with various HPβCD concentrations (0 to 140 mM) at 30±0.5°C was shown in Figure 2. The solubility of indomethacin increased linearly as the concentration of HPβCD increased. Thus, it was the Higuchi's $A_L$-type phase solubility curve indicating an improved solubility. Thus, the formation of a 1:1 complex can be assumed. The average of the apparent stability constant of the complex ($K_{1:1}$) was estimated to be 340 $M^{-1}$ ($n$ = 3). The value of this study was similar to the previous studies. Salústio et al. have demonstrated that the $K_{1:1}$ value of indomethacin in β-cyclodextrin was 366 $M^{-1}$ [8]. Xin et al. have shown that the $K_{1:1}$ values of indomethacin in β-cyclodextrin/epichlorohydrin at the molar ratio of 1:15 and β-cyclodextrin/epichlorohydrin/choline chloride at the molar ratio of 1:15:4 were 320 and 342 $M^{-1}$, respectively [9]. Therefore, it can be concluded that a stable inclusion complexes between indomethacin and HPβCD at 1:1 molar ratio was formed. This fact is well supported by the work of El-Feky et al. [10].

### 3.2 Preparation and Characterization of the Solid Inclusion Complexes

The calibration graphs of indomethacin and indomethacin ammonium salt solutions in distilled water were shown to be linear ($r^2$ > 0.9938), over the concentration range of 5.0-60.0 μg/ml. The respective regression equations for indomethacin and indomethacin ammonium salt solutions in distilled water were as follows: $y = 0.0184x + 0.0335$ and $y = 0.0206x - 0.0363$, where $y$ is the absorbance (mAU*s) and $x$ is the concentration of indomethacin or indomethacin ammonium salt (μg/ml). The yields of KN, COE, and COL products were at 69%, 87%, and 83% respectively. The respective total indomethacin contents in the PM, KN, COE and COL solid systems were at 19.8±1.8%, 20.0±2.2%, 20.0±2.1% and 21.4±1.1% (w/w), whereas the percentages of indomethacin complexed by HPβCD in PM, KN, COE, and COL products were at 29.8±0.2%, 46.8±2.4%, 53.4±1.9%, and 77.3±3.7%, respectively ($n$ = 3).

The IR spectra of HPβCD, pure indomethacin, PM and indomethacin- HPβCD inclusion complexes were shown in Figure 3. The PM and KN products gave the simple super imposition of indomethacin and HPβCD IR spectra. The characteristic absorption bands (carbonyl stretching region) of indomethacin ($n_{CO}$ = 1695 and 1715 $cm^{-1}$) were maintained in the PM and KN systems. There were no appreciable differences between the IR spectra of the PM and KN preparations. A marked modification of the absorption pattern was instead observed for the COE and COL products. In the COE products, the typical $n_{CO}$ bands at 1715 $cm^{-1}$ disappeared, whereas those of the COL products appeared strongly broader and shifted to the lower wave numbers

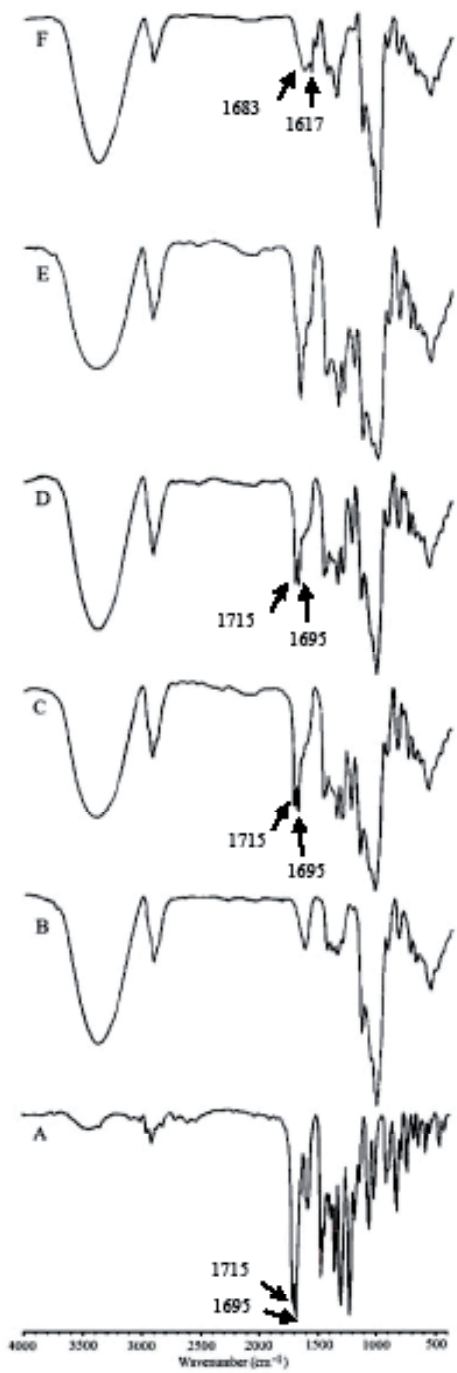


**Figure 3.** The IR spectra of A: indomethacin, B: HPβCD, C: physical mixture (PM), D: kneaded system (KN), E: coevaporated system (COE), F: colyophilized system (COL).

($n_{CO}$ = 1617 and 1683 $cm^{-1}$). For the FT-IR spectrum, the complete disappearance of the absorption bands of the drug has revealed the complete complexation, while the presence of the absorption bands of the drug has confirmed the partial complexation [11]. These results indicated that the inclusion of the drug within the HPβCD cavity can be achieved by the COE and COL methods, but not by the PM and KN processes. The disappearance of the bands due to the carbonyl group of indomethacin on the complex spectra suggested the inclusion of the two carbonyl groups into the HPβCD cavity [12-13].

Figure 4 showed the DSC thermograms of pure indomethacin, HPβCD, PM and indomethacin- HPβCD inclusion complexes. The thermogram of indomethacin demonstrated simple thermal behavior of the drug and revealed a sharp endothermic peak at 160.7°C corresponding to its melting point with an onset near 158.9°C and the melting enthalpy of 100.2 J/g. The DSC analysis of HPβCD gave rise to the curve of three endotherms. The first endothermic peak corresponds to the dehydration of HPβCD·$n$$H_2O$ and the remaining two could be assigned to the melting and the decomposition of the melted HPβCD structure. The effect of cyclodextrins on the drug DSC thermogram is observed as broadening, shifting and the appearance of new peaks or disappearance of the certain peaks [14]. The peak of PM due to the melting of the drug was found to be shifted to 153°C with the considerable decrease in intensity. The KN and COE products gave the small melting peak located in nearly the same position (147°C) of the pure indomethacin melting peak, indicating a partial inclusion complexation of the drug. On the other hand, the peak corresponding to the melting of indomethacin was totally absent in the COL system, implying drug amorphization and/or its interaction with the

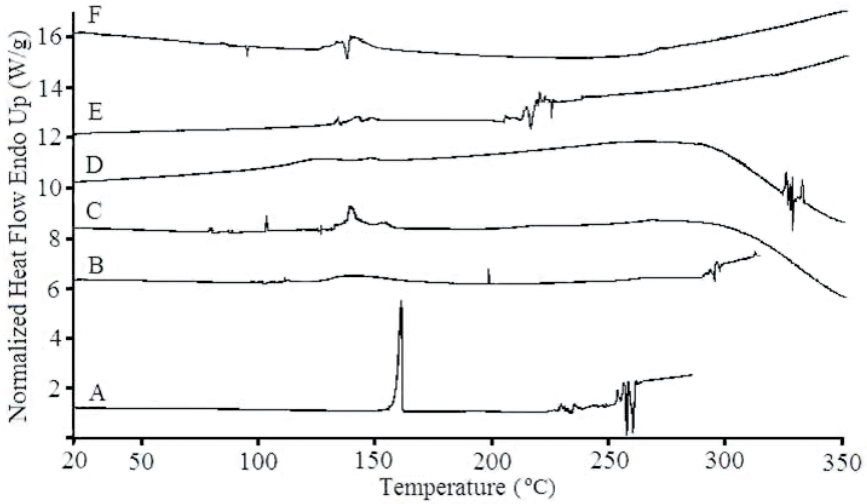


**Figure 4.** Differential scanning calorimetry thermograms of A: indomethacin, B: HPβCD, C: physical mixture (PM), D: kneaded system (KN), E: coevaporated system (COE), F: colyophilized system (COL).

amorphous carriers during the DSC scan. In the DSC thermogram, the appearance of the drug peak in the complex has indicated the partial complexation since some free drug molecules still exist in the system. The absence of the drug melting peak has exhibited the complete complexation as previously reported [15]. The last endothermic peak of the DSC scan of KN and COE preparations corresponds to the fusion of the inclusion compound. This developes into a peak showing irregular variations, which are characteristics of a thermal degradation reaction. The interaction between the drug and cyclodextrins is weak since the shift in the endothermic peak is very small. Thus, the COL system provides the formation of complete inclusion complex with strong interaction. Several studies have demonstrated that the method of complex preparation have been found to influence druγ-cyclodextrin inclusion complex formation. The DSC endotherm of Salbutamol at 158°C was shifted to 150°C in the physical mixture showing a weak interaction. But, the freeze dried complex showed no peak around 157°C indicating the formation of a true inclusion complex [16]. Formation of inclusion complex of azelaic acid with HPβCD by various methods has been evaluated by DSC. The thermogram of PM still demonstrated the melting point of azelaic acid, indicating that an inclusion complex could not be obtained by simply blending the drug with HPβCD. The COE system did not exhibit the melting endothermic peak of azelaic acid, indicating that azelaic acid was incorporated in the HPβCD cavity [17].

Further evidences of complex formation were obtained by X-ray powder diffraction, as demonstrated in Figure 5. The diffraction patterns of the pure indomethacin displayed crystallinity, whereas an amorphous pattern lacking crystalline peaks was observed for HPβCD. The diffractograms of the PM and the inclusion complexes prepared by the KN and COE methods were the superimposed figures of indomethacin and HPβCD, with the peaks exhibiting a lower intensity. No change in the crystalline structure was observed due to the KN and COE processes, since all principal peaks attributable to indomethacin crystals were still

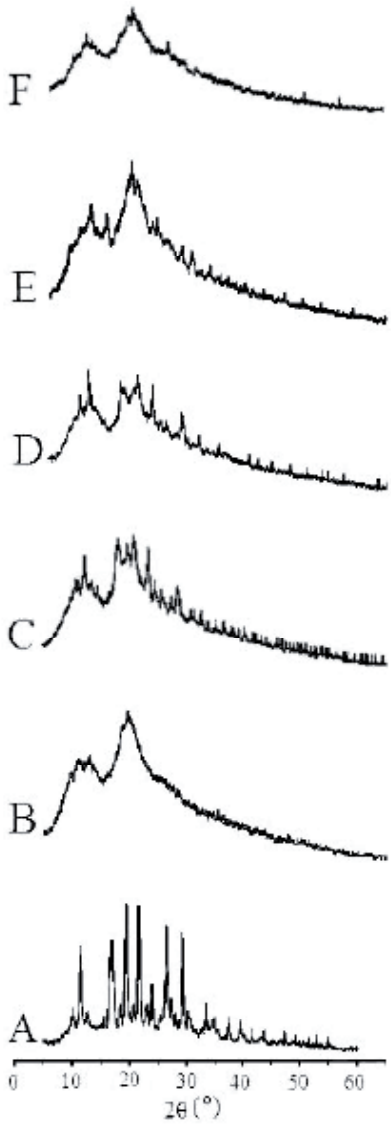


**Figure 5.** Powder X-ray diffraction patterns of A: indomethacin, B: HPβCD, C: physical mixture (PM), D: kneaded system (KN), E: coevaporated system (COE), F: colyophilized system (COL).

detectable in the X-ray profiles of the KN and COE products. However, a decrement in the intensity of the KN and COE preparations was observed due to the diminished crystallinity of the sample. The presence of the new peaks and loss of some peaks of the drug in the KN and COE systems may indicate that a change in the crystal structure has been produced due to the formation of a partial inclusion complex. The disappearance of indomethacin crystalline peaks of the COL system confirmed a strong drug amorphization affected by HPβCD. The diffractogram of the inclusion complex prepared by the COL method was practically identical to that of the amorphous HPβCD. This could be attributed to the inclusion complex formation of indomethacin and HPβCD. Thus, an equimolar amount of HPβCD was sufficient to complex all drug using the COL method. HPβCD has been reported to be useful for the conversion of the crystalline drugs to the amorphous forms with an improved dissolution [18]. The absence of the drug melting in the COL system (Figure 4) was due to an intrinsically amorphous state of indomethacin, which was prevented from nucleating in its original crystal structure during the colyophilization process. Therefore, the X-ray diffraction analysis confirmed the DSC results.

### 3.3 Dissolution of Indomethacin

The dissolution profiles of indomethacin from the pure drug and various binary systems with HPβCD in water at 37°C and the relevant dissolution data are presented in Figure 6 and Table 1, respectively. A marked increase in the dissolution rate of pure indomethacin was evident even in the PM. Hence, this might be attributed to the improved wetting and the formation of the readily soluble complexes in water [19]. This enhancement can be attributed to the higher hydrophilic characteristics of the systems due to the presence of the carrier, which can reduce the interfacial tension between indomethacin and the dissolution medium [20].

All systems with HPβCD led to an enhancement of indomethacin dissolution rate. In fact, the drug percentages dissolved after 90 min from the COL system was up to 71.5% of the dose, as compared to only 24.0% released

from the pure indomethacin (Table 1). The dissolution behavior of PM has been found to be similar to that of the inclusion complex prepared by the KN method. It suggested that the presence of indomethacin and HPβCD in water produced a rapid complexation. The Rf value difference of the free indomethacin and the complexed indomethacin on TLC has indicated the complex formation between the drug and the carrier when water was incorporated in the mixture. The IR spectroscopy has been used to characterize the complexes and also to confirm the complexation of the solid state [21]. From the TLC studies, it was found that the PM method also affected drug polarity (Rf of indomethacin in PM, KN, COE, COL products = 0.55 < Rf of pure indomethacin = 0.58), indicating that the rapid complexation of indomethacin and HPβCD occurred when water was incorporated in the mixture. The HPβCD complexation in the COL solid system exhibited the highest dissolution rate of indomethacin ($DE_{90}$ = 61.7±0.9%, $n$ = 3), while the $DE_{90}$ of indomethacin was only at 15.4±0.1% ($n$ = 3). In addition, the time necessary to dissolve 50% indomethacin from the COL products was short of only 13 min, as compared to those of the pure indomethacin (>> 90 min), PM (> 90 min), PM-COL (22

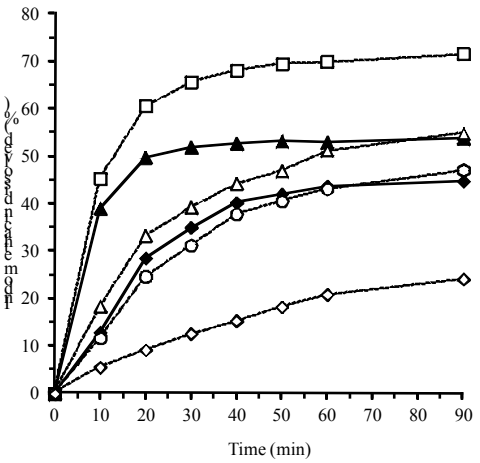


**Figure 6.** Dissolution profiles of (◇) indomethacin, (◆) physical mixture (PM), (▲) physical mixture of colyophilized indomethacin and HPβCD (PM-COL), (○) kneaded system (KN), (△), coevaporated system (COE), (□) colyophilized system (COL). The values denote the mean of three determinations.

**Table 1.** Dissolution efficiency after 90 min ($DE_{90}$), percentages of indomethacin dissolved after 90 min ($DP_{90}$) and time (min) necessary to dissolve 50% drug ($t_{50\%}$) of indomethacin and various binary systems with HPβCD.

| Sample | $DE_{90}$ (%)[a,b] | $DP_{90}$ (%)[b] | $t_{50\%}$ (min) |
|---|---|---|---|
| Indomethacin | 15.4 ± 0.1 | 24.0 ± 0.1 | >>90 |
| PM | 34.7 ± 0.7 | 44.7 ± 1.4 | >90 |
| PM-COL | 48.0 ± 0.2 | 53.8 ± 0.3 | 22 |
| KN | 33.6 ± 0.1 | 47.0 ± 1.9 | >90 |
| COE | 40.6 ± 0.3 | 54.7 ± 0.6 | 58 |
| COL | 61.7 ± 0.9 | 71.5 ± 1.4 | 13 |

Note : physical mixtures of indomethacin and HPβCD (PM), physical mixtures of colyophilized substances (PM-COL), kneaded system (KN), coevaporated system (COE) and colyophilized system (COL)

[a] Area under the dissolution curve at 90 min is expressed as the area percentages of the rectangle described by 100% dissolution at the same time.

[b] The experimental data represent the mean ± SD of three determinations.

min), KN (> 90 min) and COE (58 min) products. The PM-COL and COL exhibited the fastest $t_{50\%}$ of 22 and 13 min. This may be resulted from the indomethacin ammonium salt obtained by the colyophilization method using ammonium hydroxide. In fact, salts can improve the solubility and dissolution characteristics in comparing to the original drug [22]. Thus, the fastest dissolution rate of indomethacin from the PM-COL and COL may be from the synergistic effects of salt formation and inclusion complex. An increase of indomethacin dissolution rate could not solely be explained by ionization and amorphization of the drug, since PM-COL exhibited lower $DE_{90}$ (48.0±0.2%) and $DP_{90}$ (53.8±0.3%) values than the COL system ($DE_{90}$ = 61.7±0.9%, $DP_{90}$ = 71.5±1.4%, $n$ = 3). Therefore, this might be attributed to the complex formation as well as ionization and amorphization of the drug.

In the COL system, the entire drug was in the complexed form, while indomethacin in the KN and COE systems was partially complexed. The $DP_{90}$ value ($n$ = 3) of PM (44.7±1.4%) was lower than those of KN (47.0±1.9%), COE (54.7±0.6%) and COL (71.5±1.4%) systems. The inclusion complexes prepared by the KN and COE methods gave lower $DE_{90}$ (KN: 33.6±0.1%, COE: 40.6±0.3%) and $DP_{90}$ (KN: 47.0±1.9%, COE: 54.7±0.6%) values than the COL system ($DE_{90}$ = 61.7±0.9%, $DP_{90}$ = 71.5±1.4%, $n$ = 3). This might be due to the partial complexation in the KN and COE systems. Kneading of indomethacin with HPMC (water-soluble polymers) in the presence of small amount of water can extremely be effective to improve its apparent solubility with the maintenance of drug crystallinity to some extents. However, energy added to reduce the particle size can result in the increased van der Waal's interactions and electrostatic attraction between particles leading to the reduction of an effective surface area due to agglomeration thus decreasing dissolution rate [23]. In the KN and COE methods, HPβCDs were capable of forming inclusion complexes with indomethacin by taking up some parts of the molecule into the cavity, whereas the whole drug molecule was encapsulated into the cavity when the solid inclusion complex was prepared by the COL method. Based on the IR spectra, it was proposed that during the complex formation, the indomethacin moiety encapsulated in the cavity of HPβCD molecule was the 4-chlorobenzoyl group (Figures 1 and 3). Rossi et al. have characterized that the geometry of the indomethacin-β cyclodextrin complex is the 4-chlorobenzoyl unit inserted into the cavity of β cyclodextrin through its larger rim [24]. The dissolution enhancement of the COL systems could be attributed mainly to the formation of the soluble inclusion complexes of the drug with cyclodextrins and the increase of the drug energy due to the reduction of the crystallinity following complexation [25]. It has been reported that the significant improvement of in dissolution characteristics in case of the inclusion complex prepared by the freeze-dried method with HPβCD may be due to the formation of the solid inclusion complex in the dissolution media with better interaction of the drug and HPβCD the during the freeze-dried method [26].

The hydrogen bonds between the hydroxyl groups of the neighboring cyclodextrin molecules are mediated by water molecules [27]. In some cases, the inclusion complexes can also be formed with the ionized species of the drug. Loftsson et al. have reported that cyclodextrin complexation of the ionized drug molecules may result in the much larger total solubilization of the drug, both due to complexation and ionization [28]. A better solubilization of bropirimine in water by the addition of ammonium hydroxide has been reported [18]. Thus, a synergistic effect can be explained for the system of lyophilization of indomethacin solutions with HPβCD in

an aqueous ammonium hydroxide solution as produced the solid-state indomethacin inclusion complexes shown by the IR spectra, DSC and X-ray diffractometry.

## 4. Conclusion

The solubility and dissolution of indomethacin can be enhanced by complexation with HPβCD. The COL products exhibited the highest $DE_{90}$ and $DP_{90}$ values and the shortest time necessary to dissolve 50% of indomethacin. The yield of the COL products resulting from complexation was relatively high (83%). The actual indomethacin content in the COL products was found to be 21.4±1.1% (w/w). The indomethacin percentages complexed with HPβCD in the COL products was the highest (77.3±3.7%), as compared to the PM, KN and COE products. In the COL method, the 4-chlorobenzoyl group of indomethacin was located well inside the cavity. Therefore, the COL process was the best method because of the high content and dissolution rate of the drug. It is also the simple method to prepare the inclusion complexes. The development of oral dosage forms containing complexed indomethacin which exhibited rapid dissolution rate may improve its oral bioavailability and reduce the gastrointestinal irritation. The amount of the complex that contains 50 mg of indomethacin is 250 mg, which could be easily formulated in a capsule or tablet. The complexation method using freeze-drying is simple with great potential for industrial production.

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