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CYCLODEXTRIN-BASED SOLUBILITY ENHANCEMENT OF POORLY WATER-SOLUBLE DRUG INDOMETHACIN

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ABSTRACT

Indomethacin, a non-steroidal anti-inflammatory drug (NSAID), suffers from low aqueous solubility, limiting its oral bioavailability and therapeutic effectiveness. Cyclodextrins, particularly hydroxypropyl-β-cyclodextrin (HP-β-CD), are widely employed to enhance solubility and dissolution of poorly water-soluble drugs through inclusion complexation. This study focuses on the preparation and evaluation of indomethacin–HP-β-CD inclusion complexes using co-precipitation and kneading methods. The complexes were characterized using Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), and X-ray diffraction (XRD) to confirm complex formation. Solubility and in vitro dissolution studies demonstrated a significant increase in the aqueous solubility and dissolution rate of indomethacin when complexed with HP-β-CD. These results indicate that cyclodextrin-based inclusion complexes effectively enhance the physicochemical properties of indomethacin, providing a promising strategy to improve its oral bioavailability.

I. INTRODUCTION

Indomethacin ([1-(4-chlorobenzoyl)- 5-methoxy-2-methylindol-3yl]acetic acid; C19H16ClNO4; 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

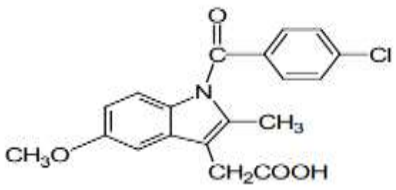

Figure 1. Chemical structure of indomethacin ([1-(4-chlorobenzoyl)-5-methoxy-2-methylindol3-yl]acetic acid).

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 druγCD inclusion complex formation including physical blending, kneading, co-precipitation technique, solution/solvent evaporation, neutralization precipitation, milling/co-grinding, atomization/spray drying, lyophilization/freezedrying, microwave irradiation and supercritical technique [3]. Bandi et al. have demonstrated that

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indomethacin-HPβCD complexes with enhanced dissolution rate can be formed using a supercritical fluid CO2 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.

II. 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 So is the solubility of the drug in water without HPβCD.

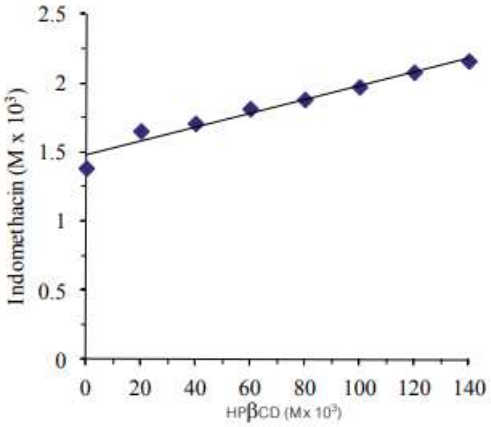

Figure 2. Phase solubility diagram of indomethacin in water containing various concentrations of HPβCD at 30±0.5°C (n = 3).

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 Freezedried 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 indomethacinHPβCD 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 airdried 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 indomethacinHPβCD (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

III. 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 AL-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 (K1:1) 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 K1:1 value of indomethacin in β-cyclodextrin was 366 M−1 [8]. Xin et al. have shown that the K1:1 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 (nCO = 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 nCO bands at 1715 cm-1 disappeared, whereas those of the COL products appeared strongly broader and shifted to the lower wave numbers

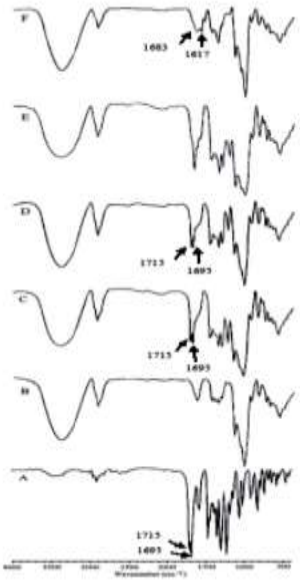

Figure 3. The IR spectra of A: indomethacin, B: HPβCD, C: physical mixture (PM), D: kneaded system (KN), E: coevaporated system (COE), F: colyophilized system (COL)

nCO = 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·nH2O 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

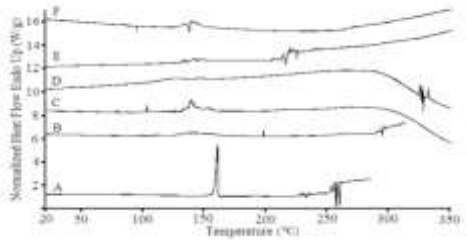

Figure 4. Differential scanning calorimetry thermograms of A: indomethacin, B: HPβCD, C: physical mixture (PM), D: kneaded system (KN), E: coevaporated system (COE), F: colyophilized system (COL).

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

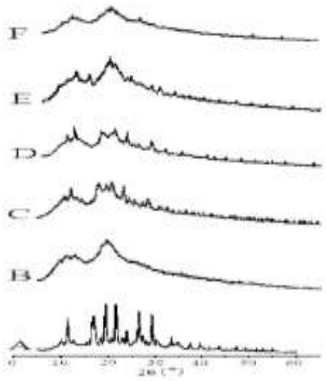

Figure 5. Powder X-ray diffraction patterns of A: indomethacin, B: HPβCD, C: physical mixture (PM), D: kneaded system (KN), E: coevaporated system (COE), F: colyophilized system (COL).

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.

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

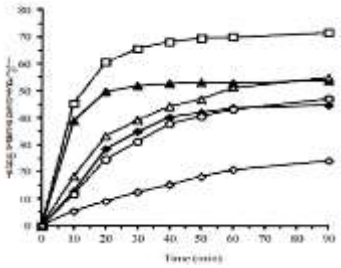

Figure 6. Dissolution profiles of () indomethacin, (◆) physical mixture (PM), (▲) physical mixture of colyophilized indomethacin and HPβCD (PM-COL), (○) kneaded system (KN), (△), coevaporated system (COE), (□) colyophilized system (COL). The values denote the mean of three determinations.

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 (DE90 = 61.7±0.9%, n = 3), while the DE90 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

Table 1. Dissolution efficiency after 90 min (DE90), percentages of indomethacin dissolved after 90 min (DP90) and time (min) necessary to dissolve 50% drug (t50%) 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 (PMCOL), 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.

In the COL system, the entire drug was in the complexed form, while indomethacin in the KN and COE systems was partially complexed. The DP90 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 DE90 (KN: 33.6±0.1%, COE: 40.6±0.3%) and DP90 (KN: 47.0±1.9%, COE: 54.7±0.6%) values than the COL system (DE90 = 61.7±0.9%, DP90 = 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 freezedried 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 freezedried method [26].

4. CONCLUSION

The inclusion of indomethacin in hydroxypropyl-β-cyclodextrin successfully enhances its solubility and dissolution rate compared to the pure drug. Characterization techniques confirmed the formation of stable inclusion complexes, while solubility studies validated their superior performance. Cyclodextrin-based complexation offers a practical and efficient approach for improving the bioavailability of poorly water-soluble drugs such as indomethacin. This strategy holds potential for developing more effective oral formulations, and further in vivo studies

are recommended to assess pharmacokinetic and therapeutic benefits.

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