Original Article

# Solid-state characterization and *in vitro* dissolution behavior of lorazepam: Hydroxypropyl-β-cyclodextrin inclusion complex

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**ABSTRACT: The objectives of this research were to prepare and characterize inclusion complexes of lorazepam with hydroxypropyl-β-cyclodextrin and to study the effect of complexation on the dissolution rate of lorazepam, a water-insoluble drug. The phase solubility profile of lorazepam with hydroxypropyl-β-cyclodextrin was an AP-type, indicating the formation of 2:1 stoichiometric inclusion complexes. Gibbs free energy values were all negative, indicating the spontaneous nature of lorazepam solubilization, and they decreased with an increase in the cyclodextrin concentration, demonstrating that the reaction conditions became more favorable as the concentration of cyclodextrins increased. Complexes of lorazepam were prepared with cyclodextrin using various methods such as physical mixing, kneading, spray-drying, and lyophilization. The complexes were characterized by differential scanning calorimetry, Fourier-transform infrared, scanning electron microscopy, and powder X-ray diffraction studies. These studies indicated that a complex prepared by lyophilization had successful inclusion of the lorazepam molecule into the cyclodextrin cavity. Complexation resulted in a marked improvement in the solubility and wettability of lorazepam. Among all the samples, a complex prepared with hydroxypropyl-β-cyclodextrin by lyophilization had the greatest improvement in the *in vitro* rate of lorazepam dissolution. The mean dissolution time for lorazepam decreased significantly after preparing complexes and physical mixtures of lorazepam with cyclodextrin. The similarity factor indicated a significant difference between the release profiles of lorazepam from complexes and physical mixtures and from plain lorazepam. Tablets containing complexes prepared with cyclodextrins had significant improvement in the release profile of lorazepam as compared to tablets containing lorazepam without cyclodextrin.**

***Keywords:*** Lorazepam, hydroxypropyl-β-cyclodextrin, inclusion complexation, *in vitro* dissolution studies

## 1. Introduction

Lorazepam (LRZ), a potent benzodiazepine, is used for the short-term relief of symptoms of anxiety or anxiety associated with depression (*1*). This drug is often used for preanesthetic medication; it is useful for managing status epilepticus, chemotherapy-induced nausea, and vomiting (*1*). LRZ binds to central benzodiazepine receptors that interact allosterically with γ-aminobutyric acid (GABA) receptors (*1*). This potentiates the effects of the inhibitory neurotransmitter GABA, increasing the inhibition of the ascending reticular activating system and blocking the cortical and limbic arousal that occurs following stimulation of the reticular pathways (*1*). LRZ is an almost white powder that is nearly insoluble in water (0.08 mg/mL) and oil (*2*). Because of its poor water solubility and extensive metabolism in the liver into pharmacologically inactive glucuronides, LRZ oral therapy is associated with a slow onset of drug action (*3*). Generally, compounds with very low aqueous solubility are considered to have dissolution rate-limited absorption and hence poor absorption, distribution, and target organ delivery (*4*). Improving aqueous solubility in such cases is a worthwhile way of improving therapeutic efficacy. In this respect, a fast-dissolving form of the compound with a high level of aqueous solubility is desirable for rapid absorption of the drug during oral benzodiazepine therapy. Complexation of such drugs with cyclodextrins (CDs) represents one way to achieve that goal.

CDs form a group of structurally related oligosaccharides with cylinder-shaped cavities that have the capacity to form inclusion complexes with many drugs by taking a whole drug molecule, or a

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part of it, into the cavity (*5*). Because of the large number of hydroxyl groups on CDs, they are water-soluble (*5*). They are known for their ability to molecularly encapsulate a wide variety of drugs into their hydrophobic cavity without the formation of any covalent bonds (*5*). The binding forces within these inclusion complexes may involve hydrophobic, van der Waals, hydrogen bonding, or dipole interactions (*5*). CDs have widespread pharmaceutical applications mainly because of their effect on enhancing the solubility and bioavailability of many drug formulations. Complexation with CDs has been reported to enhance the solubility, dissolution rate, and bioavailability of poorly water-soluble drugs (*6-9*). CDs first came to the fore in marketed products as drug delivery technologies that enabled the development of various prostaglandins (*10*).

Complexation of pharmaceuticals with β-CD causes enhancement of their solubility and bioavailability as well as stabilization against oxidation, decomposition, hydrolysis, *etc*. (*11*). Hydroxypropyl-β-cyclodextrin (HP-β-CD), a chemical derivative of β-CD, is the most accepted representative of hydroxyalkylated derivatives as a hydrophilic drug carrier because of its amorphousness, high water solubility and solubilizing power, and low cost and toxicity (*12*). Hydroxyalkylated CD derivatives have proven to be very useful in intravenous and other parenteral preparations because of their low hemolytic activity and irritation compared to β-CD and its alkylated forms (*12*). An inclusion complex of albendazole/HP-β-CD (1:1, molar ratio) has been prepared using coprecipitation and freeze-drying in order to increasing its aqueous solubility (*13*). Many other drugs such as artemisinin, etodolac, cilostazol, nimesulide, and piroxicam have been tested for CD inclusion to enhance their solubility (*14-18*).

*In vitro* dissolution testing provides an easy and convenient means of evaluating the performance of pharmaceutical preparations (*19*). The *in vitro* dissolution profile is a reliable index to accurately predict a preparation's *in vivo* performance (*19*). In the current study, an attempt was made to compare the similarities of the *in vitro* dissolution profiles of LRZ from complexes, physical mixtures, and pure LRZ. Dissolution profiles can be compared by calculating the similarity factor ($f_2$ values), an index that was first reported by Moore and Flanner in 1996 (*20*). This index has also been adopted by the Center for Drug Evaluation and Research, Food and Drug Administration (US FDA, 1997) and by the Human Medicines Evaluation Unit of the European Agency for the Evaluation of Medicinal Products (EMEA, 1999) as a criterion for the assessment of the similarity of two dissolution profiles. The similarity equation is given in the US FDA guidelines for industry for dissolution testing of immediate-release products (*21,22*). A value of 100% for the similarity factor ($f_2$) suggests that the test and reference profiles are identical (*20*). Values between 50 and 100 indicate that the dissolution profiles are similar whilst smaller values imply an increase in dissimilarity between release profiles (*20*).

Mean dissolution time (MDT) reflects the time for the drug to dissolve and is the first statistical moment for the cumulative dissolution process that provides an accurate drug release rate (*23*). It is an accurate expression of the drug release rate. A higher MDT value indicates greater drug-retarding ability (*24*).

The objective of the present study was to prepare inclusion complexes of LRZ with HP-β-CD using various methods such as kneading, coevaporation, and physical mixing to improve its aqueous solubility and dissolution rate. The study further aimed to characterize the prepared inclusion complexes by methods such as differential scanning calorimetry (DSC), Fourier transform infrared (FTIR), scanning electron microscopy (SEM), and powder X-ray diffraction (PXRD) studies.

## 2. Materials and Methods

### 2.1. *Materials*

HP-β-CD was donated by Roquette Frères, France. LRZ was donated by Astron Research Center (Ahmedabad, India). Directly compressible lactose, microcrystalline cellulose, talc, and magnesium stearate were donated by Maan Pharmaceuticals Ltd. (Ahmedabad, India). All chemicals and solvents used in this study were of analytical reagent grade. Freshly distilled water was used throughout the work.

### 2.2. *Phase solubility study*

Phase-solubility studies were performed according to the method reported by Higuchi and Connors in 1965 (*25*). Excess LRZ was transferred to screw-capped vials containing 25 mL of aqueous solution of HP-β-CD (molecular weight = 1,500) in various molar concentrations (10, 25, 50, 100, 150, and 200 mM for HP-β-CD). The contents were stirred at 400 rpm on an electromagnetic stirrer (Remi Instruments Ltd., Mumbai, India) for 48 h at 25 ± 0.1°C and at 37 ± 0.1°C (this duration was previously tested to be sufficient to reach equilibrium). After reaching equilibrium, samples were filtered through a 0.22-µm membrane filter, suitably diluted, and analyzed spectrophotometrically for drug content at a wavelength of 230 nm using a spectrophotometer (Shimazdu-11700, UV/Vis spectrophotometer; Shimadzu Corp, Kyoto, Japan). Solubility studies were performed in triplicate ($n$ = 3). The apparent stability constant (Ks), in accordance with a theoretical 1:1 stoichiometric ratio of complexes, was calculated from the phase-solubility diagrams using the following equation:

$$Ks = \frac{slope}{S_o(1 - slope)} \quad \text{----- Eq. 1}$$

where the slope is obtained from the initial straight-line portion of the plot of LRZ concentration with respect to the CD concentration and $S_o$ is the equilibrium solubility of LRZ in water.

2.3. *Preparation of inclusion complexes*

Complexes of HP-β-CD with LRZ were prepared at a molar ratio of 2:1 (on the basis of the phase solubility study) by different methods like physical mixing, kneading, spray-drying, and lyophilization. For ease of discussion, the samples were designated with different abbreviations as shown in Table 1.

2.3.1. *Physical mixing*

Physical mixtures (PMs) of CDs and LRZ were prepared by simply mixing powders with a spatula for 15 min.

2.3.2. *Kneading*

To prepare complexes by kneading, the required quantities of CDs and distilled water were mixed together in a mortar to obtain a homogeneous paste. LRZ was then added slowly; during grinding, a small quantity of methanol was added to assist the dissolution of LRZ. The mixtures were then ground for 1 h. During this process, an appropriate quantity of water was added to the mixture in order to maintain a suitable consistency. The paste was dried in an oven at 45-50°C for 24 h. The dried complexes were pulverized and then sieved through a #100 sieve.

2.3.3. *Spray-drying*

For spray-drying, LRZ was dissolved in methanol and HP-β-CD was dissolved in distilled water. Mixtures of both solutions were stirred at room temperature for 24 h and then spray-dried using a Labultima LU222 Advanced Laboratory Spray Dryer under the following conditions: inlet temperature of 70°C, outlet temperature of 45-50°C, flow rate of the solution of 400 mL/h, airflow rate of 40-50 $m^3$/h, and atomizing air pressure of 1.0-1.1 bar.

**Table 1. Abbreviations used to designate samples of LRZ prepared with HP-β-CD using different methods**

| Type of CDs | Method of preparation | Name of sample |
|---|---|---|
| HP-β-CD | Physical mixing | PM |
| HP-β-CD | Kneading | KN |
| HP-β-CD | Spray-drying | SP |
| HP-β-CD | Lyophilization (freeze-drying) | LP |

2.3.4. *Lyophilization*

For lyophilization, LRZ was dissolved in methanol and HP-β-CD was dissolved in distilled water. Mixtures of both solutions were stirred at room temperature for 24 h and then freeze-dried in a VirTis BenchTop Freeze Dryer to yield an amorphous powder. The product was sieved through a #100 sieve.

2.4. *Drug content*

The complexes prepared by physical mixing, kneading, spray-drying, and freeze-drying were assayed for LRZ content by dissolving a specific amount of the complex in methanol and spectrophotometrically analyzing it for LRZ content at 230 nm in a spectrophotometer (U.V. visible spectrophotometer, Shimazdu-1700).

2.5. *Characterization of complexes*

2.5.1. *DSC analysis*

DSC scans of the powdered samples of LRZ, HP-β-CD, and LRZ in PMs and complexes with HP-β-CD were recorded using a DSC-Shimadzu 60 with TDA trend line software. The samples (6-7 mg) were accurately weighed in crimped aluminum pans and heated from 50°C to 300°C at a scanning rate of 10°C/min under an air flow of 100 mL/min.

2.5.2. *FTIR spectroscopic analysis*

FTIR spectra of moisture-free powdered samples of LRZ, HP-β-CD, and LRZ in PMs and complexes with HP-β-CD were obtained by the potassium bromide (KBr) pellet method using a spectrometer (FTIR-8300, Shimadzu Co.).

2.5.3. *SEM*

The surface morphology of free powdered samples of LRZ, HP-β-CD, and LRZ in PMs and complexes with HP-β-CD were examined using a scanning electron microscope (Philips, LC ESEM). The samples were fixed onto a brass stub using double-sided tape and then rendered electrically conductive by coating them with a thin layer of copper in a vacuum. Photographs were taken with a Pentax (model MZ-10) camera at an excitation voltage of 10 kV and magnification of ×200 and ×3,500.

2.5.4. *PXRD analysis*

PXRD patterns of LRZ, HP-β-CD, and LRZ in PMs and complexes with HP-β-CD were determined using an Expert Phillips IW 1830 generator with a CuK α anode at 40 kV and 30 mA and a scan rate of 1° $min^{-1}$ from 2θ

in a range from 1° to 40°. Analysis was conducted by SICART, Vallabh Vidyanagar, India.

2.5.5. *Wettability and dissolution studies*

A wettability study was performed using open capillary tubes filled with LRZ, HP-β-CD, and LRZ in PMs and complexes with HP-β-CD in which the lower capillary ends were dipped into colored water (0.01% eosin in water). The upward migration of the colored front was recorded over time (*26*).

Dissolution studies of LRZ, PM, KN, SP, and LP in powder form were performed to evaluate their *in vitro* drug release profile. Dissolution studies were carried out using a type II USP dissolution apparatus with 100 mL dissolution medium (distilled water) at 37 ± 0.5°C and 50 rpm for 4 h. At different time intervals, 5 mL aliquots were withdrawn, filtered, suitably diluted with distilled water:methanol (50:50, v/v), and then assayed for LRZ content by measuring the absorbance at 230 nm using a spectrophotometer. Fresh medium (5 mL) was replaced after each sampling to maintain a constant volume of dissolution medium throughout the test.

LRZ, PM, KN, SP, and LP were evaluated *via in vitro* dissolution rate studies. Dissolution studies were performed three times, and calculated mean values of cumulative drug release were used while plotting the release curves. MDT values were calculated to compare the extent of improvement in the dissolution rate of PM, KN, SP, and LP. Preliminary tests determined that there was no change in the $\lambda_{max}$ of LRZ due to the presence of HP-β-CD dissolved in the dissolution medium.

2.6. *Formulation studies*

Formulation excipients were selected on the basis of preliminary tests which found no interference by these excipients with the $\lambda_{max}$ of LRZ. Tablets containing 4 mg of LRZ were made by direct compression using different formulation excipients like directly compressible lactose, microcrystalline cellulose, talc, and magnesium stearate. Tablets equivalent to 4 mg LRZ that contained complexes prepared by kneading, spray-drying, and freeze-drying were similarly produced but the quantity was adjusted with lactose to prepare a tablet of equal weight. The blend was compressed on an eight-station single rotary machine (Cadmach Machinery Co., Ahmedabad, India) using round-shaped flat punches to obtain tablets with a hardness of 4 to 6 kg/cm$^2$ and thickness of 3.3 to 3.6 mm. For the assay, three tablets were crushed and a blend equivalent to 4 mg of LRZ was weighed and dissolved in the dissolution medium. The tablets were studied five times ($n$ = 5) to determine the release profile of the drug using the same methodology as described in the *in vitro* dissolution studies.

2.7. *Statistical analysis*

A model-independent mathematical approach as proposed by Moore and Flanner (*20*) for calculating the similarity factor, $f_2$, was used to compare the dissolution profiles of different samples. $f_2$ is a measure of similarity of two dissolution curves in terms of the percentage dissolution and is defined by following equation (*20*):

$$f_2 = 50 \times \log \left\{ \left[ 1 + \left( \frac{1}{n} \right) \sum_{t=1}^{n} w_t (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\}$$

---- Eq. 2

where $n$ is the number of samples taken, $R_t$ is the percentage of the reference formulation dissolved at time point t, and $T_t$ is the percentage of the test formulation dissolved at time point t.

A value of 100% for $f_2$ suggests that the test and reference profiles are identical. Values between 50 and 100 indicate that the dissolution profiles are similar whilst smaller values imply an increase in dissimilarity between release profiles (*20*).

## 3. Results and Discussion

3.1. *Phase solubility study*

Phase solubility analysis has been among the preliminary requirements for optimizing the development of inclusion complexes of drugs as it permits the evaluation of the affinity between a CD and drug molecule in water. This process has been used by many researchers to determine the exact molar ratios at which drugs can form complexes with CDs (*27-29*).

The phase solubility curve of LRZ in the presence of CDs is shown in Figure 1. This curve indicated an increase in the solubility of LRZ with an increase in the concentration of the CD in water. Increasing the amount of the CD increased the amount of LRZ entering the water, improving the aqueous solubility of LRZ. The solubility of LRZ increased by 420-fold at 37°C and 322-fold at 25°C at a 200 mM concentration of HP-

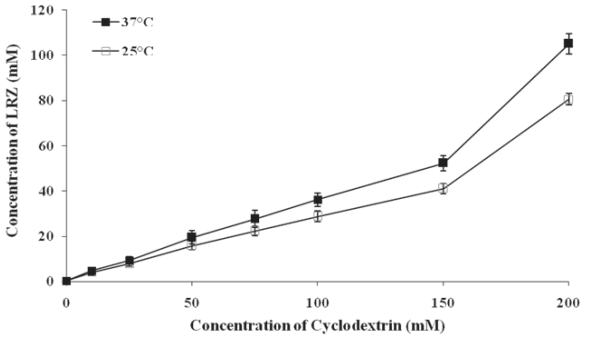


**Figure 1. Phase solubility curve of LRZ in aqueous solution of HP-β-CD at 37°C and 25°C.** Open circle, 37°C; Closed circle, 25°C.

β-CD. Increased solubility may be due to improved dissolution of LRZ particles in water due to the presence of CDs.

An indication of the process of transfer of LRZ from pure water to the aqueous solution of CDs was obtained from the values for the Gibbs free energy change. The Gibbs free energy of transfer ($\Delta G_{tr}°$) of LRZ from pure water to aqueous solutions of CDs was calculated using the following equation (*30*):

$$\Delta G_{tr}° = -2.303RT\log(\frac{S_o}{S_s}) \quad \text{----Eq. 3}$$

where $S_o/S_s$ is the ratio of molar solubility of LRZ in an aqueous solution of HP-β-CD compared to that in pure water. The obtained values for Gibbs free energy are shown in Table 2. These data provide information regarding the increased solubility of LRZ in the presence of HP-β-CD. In other words, the Gibbs free energy values provide information on whether the reaction conditions favor or disfavor drug solubilization in the aqueous carrier solution. Negative Gibbs free energy values indicate favorable conditions. $\Delta G_{tr}°$ values were all negative for HP-β-CD at various concentrations, indicating the spontaneous nature of LRZ solubilization, and the values decreased with an increase in concentration, indicating that the reaction was favored more as the concentration of HP-β-CD increased.

The enthalpy of transfer ($\Delta H_t°$) and entropy ($\Delta S$) can be calculated from a modification of the van't Hoff equation (*31*):

$$\frac{d\ln(S_c/S_o)}{dT} = \frac{\Delta H_t°}{RT^2} \quad \text{----Eq. 4}$$

Rearranging and solving for $\Delta H_t°$ yields

$$\Delta H_t° = -R\,\frac{d\ln(S_c/S_o)}{d(1/T)} \quad \text{----Eq. 5}$$

Linear regression of ln ($S_c/S_o$) versus $1/T$ for HP-β-CD concentrations of 10, 25, 50, 75, 100, 150, and 200 mM gives a slope equal to $-\Delta H_t°/R$. This treatment assumes that $\Delta H_t°$ is reasonably constant over the temperature range studied.

$$\Delta S = (\Delta H - \Delta G)/T \quad \text{----Eq. 6}$$

Usually, complex formation with HP-β-CD results in a relatively large negative $\Delta H$ and a $\Delta S$ that can be either positive or negative (*31,32*). Negative $\Delta H$ values suggest that either dipolar or induced dipolar and Van der Waals interactions between the cavity and the substrate are involved in inclusion complexation. The negative change in $\Delta S$ observed with HP-β-CD can be attributed to greater order after complexation. This is mainly due to the loss of rotational and translational freedom for the molecules involved in the complexation process (*31-34*).

The stoichiometric ratio at which optimum complexation occurs was confirmed by phase solubility analysis. The phase solubility plots revealed an $A_P$ type for HP-β-CD (data not shown), which indicated that a 2:1 (HP-β-CD-LRZ) inclusion complex was formed in solution. The values of apparent stability constants (Ks) for the complexes at 25°C and 37°C, assuming a 2:1 stoichiometry, calculated from the slope of the initial straight portion of the phase solubility diagram were 258 $M^{-1}$ at 25ºC and 491 $M^{-1}$ at 37ºC for HP-β-CD: LRZ, indicating suitable and stable complex formation (data not shown). CD-drug complexes with values of Ks in the range of 200 to 5,000 $M^{-1}$ are reported to exhibit improved dissolution properties and hence better bioavailability (*26*).

### 3.2. *Drug content*

The drug content of PM, KNB, SP, and LP was found out to be 91.2 ± 10.3%, 95.6 ± 8.5%, 98.5 ± 6.2%, and 99.0 ± 5.0%, respectively (data not shown), which roughly corresponds to the stoichiometric ratio of the complex and indicates chemical stability and content uniformity of LRZ in its complex form.

### 3.3. *Characterization of complexes*

#### 3.3.1. *DSC analysis*

DSC enables the quantitative detection of all processes

**Table 2. Gibbs free energy of transfer (ΔG), standard enthalpy change (ΔH), and entropy (ΔS) for the solubilization of LRZ in aqueous solutions of cyclodextrins at 37°C and 25°C**

| Concentration of cyclodextrins (mM) | ΔG (KJmol$^{-1}$) | | ΔH (KJmol$^{-1}$) | ΔS (Jmol$^{-1}$K$^{-1}$) |
|---|---|---|---|---|
| | 25°C | 37°C | | |
| 10 | –6.9 ± 0.04 | –1.3 ± 0.05 | –10.2 ± 0.20 | –0.00860 ± 0.0004 |
| 25 | –8.5 ± 0.06 | –2.3 ± 0.10 | –10.9 ± 0.25 | –0.00508 ± 0.0005 |
| 50 | –10.3 ± 0.10 | –3.2 ± 0.14 | –12.8 ± 0.31 | –0.00507 ± 0.0004 |
| 75 | –11.1 ± 0.12 | –3.5 ± 0.17 | –13.4 ± 0.38 | –0.00424 ± 0.0003 |
| 100 | –11.7 ± 0.20 | –4.4 ± 0.20 | –14.7 ± 0.42 | –0.00615 ± 0.0005 |
| 150 | –12.7 ± 0.26 | –5.1 ± 0.26 | –15.4 ± 0.45 | –0.00514 ± 0.0006 |
| 200 | –14.4 ± 0.29 | –15.6 ± 0.30 | –17.3 ± 0.47 | –0.00552 ± 0.0005 |

Data are shown mean ± S.D. ($n$ = 3).

in which energy is required or produced (*i.e.*, endothermic or exothermic phase transformations) (*35*). The thermograms for pure LRZ, HP-β-CD, and LRZ in PMs and complexes with HP-β-CD are presented in Figure 2. The DSC curve of LRZ displayed a sharp endotherm at 184.92ºC that was due to drug melting, characteristic of an anhydrous crystalline substance. In the thermogram of HP-β-CD, the peak between 90ºC and 120ºC was due to loss of water from CD molecules.

In PM systems, the drug endothermic peak was clearly distinguishable (Figure 2). This indicates that in such systems the drug has basically maintained its original crystallinity. In KN systems, there was a substantial size reduction and a broadening and a shift to lower temperatures of the drug melting point (175.61°C) (Figure 2). This shift may be due to the decrease in crystallinity and increase in amorphousness of the KN sample. This shift may be ascribed to some drug-CD interaction not found in PM systems.

Disappearance of the fusion peak of the drug is often interpreted as evidence of an inclusion complex formation (*36*). The disappearance of the LRZ melting peak from the thermogram of SP and LP might be due to the crystalline LRZ being included within the central cavity of the HP-β-CD, suggesting the formation of a true inclusion complex. The disappearance of this peak also confirmed that spray-drying and lyophilization were the best methods of preparing inclusion complexes.

3.3.2. *FTIR spectroscopic analysis*

FTIR spectroscopy has been used to assess the interaction between β-CD and guest molecules in their solid state. Chemical interaction between the drug and the carrier often leads to identifiable changes in the infrared profile of complexes. However, some of the changes are very subtle, requiring careful interpretation of the spectra (*37*).

The IR spectra of PM, KNB, SP, and LP were compared to the spectra of HP-β-CD and LRZ (Figure 3). The spectrum of pure LRZ had characteristic peaks at 3,100-3,250 $cm^{-1}$ (N-H stretching), 3,076 and 3,056 $cm^{-1}$ (aromatic C-H stretching), 1,696 $cm^{-1}$ (carbonyl stretching), 1,615 and 1,582 $cm^{-1}$ (aromatic ring), 1,540 $cm^{-1}$ (asymmetric $NO_2$ stretching), 1,339 $cm^{-1}$ (symmetric stretching $NO_2$ stretching), 750 $cm^{-1}$ (4 adjacent free H's, aromatic C-H out of plane bending), and 844 $cm^{-1}$ (2 adjacent free H's, aromatic C-H out-of-plane bending) (Figure 3). The FTIR spectra of HP-

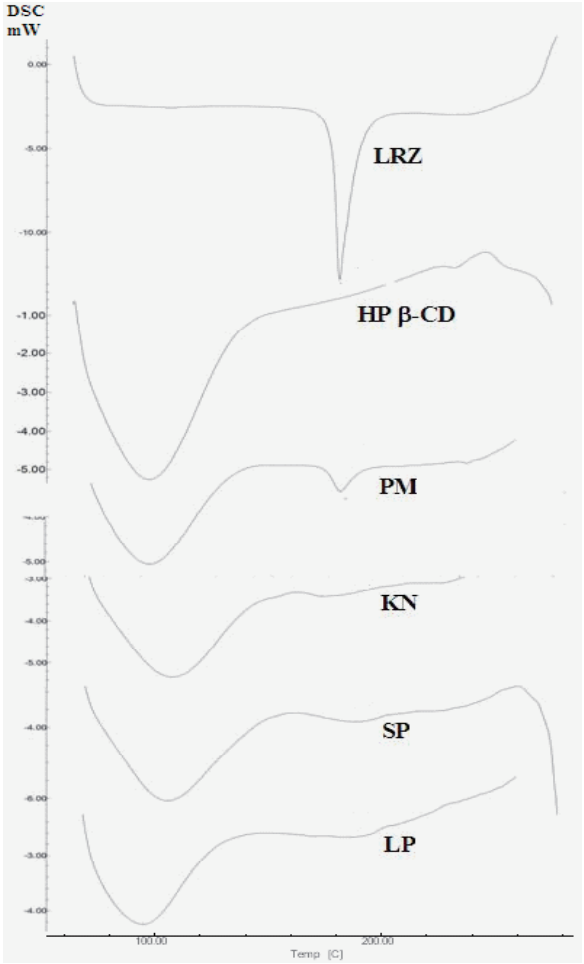


**Figure 2. DSC spectra of LRZ, HP-β-CD, PM, KN, SP, and LP.**

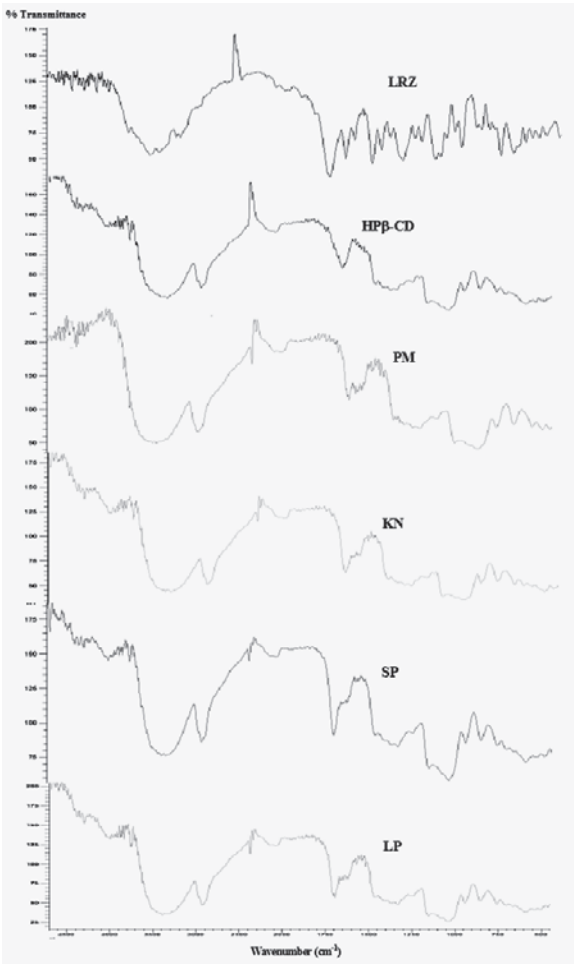


**Figure 3. FTIR spectra of LRZ, HP-β-CD, PM, KN, SP, and LP.**

β-CD are characterized by intense bands at 3,300-3,500 $cm^{-1}$ due to O-H stretching vibrations (Figure 3). The vibration of the –CH and $CH_2$ groups appears in the 2,800-3,000 $cm^{-1}$ region. The presence or absence of characteristic peaks associated with specific structural groups of the drug molecule was noted. Any sign of interaction would be reflected by changes in the characteristic peaks of LRZ, depending on the extent of interaction.

The FTIR spectra of PM, KN, SP, and LP showed no peaks other than those of CDs and LRZ. Characteristic peaks of LRZ at 1,696, 1,615, 1,582, 1,540, and 1,339 $cm^{-1}$ remained present, whereas peaks due to the aromatic ring with free H's at 750 and 844 $cm^{-1}$ were absent in the FTIR spectra of PM, KN, SP, and LP (Figure 3). These results indicated that the aromatic ring with free H's was included in the CD cavity while the remaining portion of LRZ oriented toward the upper exterior portion of the CD cavity. Moreover, the FTIR spectra of PM, KN, SP, and LP were equivalent to the addition spectrum of CDs and LRZ, which suggested the absence of well-defined chemical interaction between CDs and LRZ during preparation of complex by lyophilization, spray-drying, and kneading.

3.3.3. *SEM*

SEM microphotographs of LRZ and HP-β-CD and binary solid systems are shown in Figure 4. LRZ is characterized by regularly shaped crystals and HP-β-CD consists of spherical particles with an amorphous character. In PMs, the characteristic LRZ crystals, which were mixed with or adhered to the surface of excipient particles, were clearly detectable, thus confirming the presence of crystalline drug (Figure 4). With KN, LRZ crystals clustering on the surface of CD particles were apparent although they had lost their original shapes and were smaller in size (Figure 4).

In SP products, the original morphology of the raw materials disappeared, and discerning the two components (drug and CD) was not possible (Figure 4). The SP systems had amorphous and homogeneous aggregates of spherical particles (Figure 4), a particular aspect characteristic of this type of system (*38*). Finally, LP products appeared to have a less crystalline structure with a soft and fluffy appearance and again, crystals of the single components (drug and CD) were no longer distinguishable.

3.3.4. *PXRD analysis*

PXRD spectroscopy has been used to assess the degree of crystallinity of a given sample. When complexes of drug and polymer are formed, the overall number of crystalline structures decreases and the number of amorphous structures increases. Therefore, the final product samples have fewer and less intense peaks.

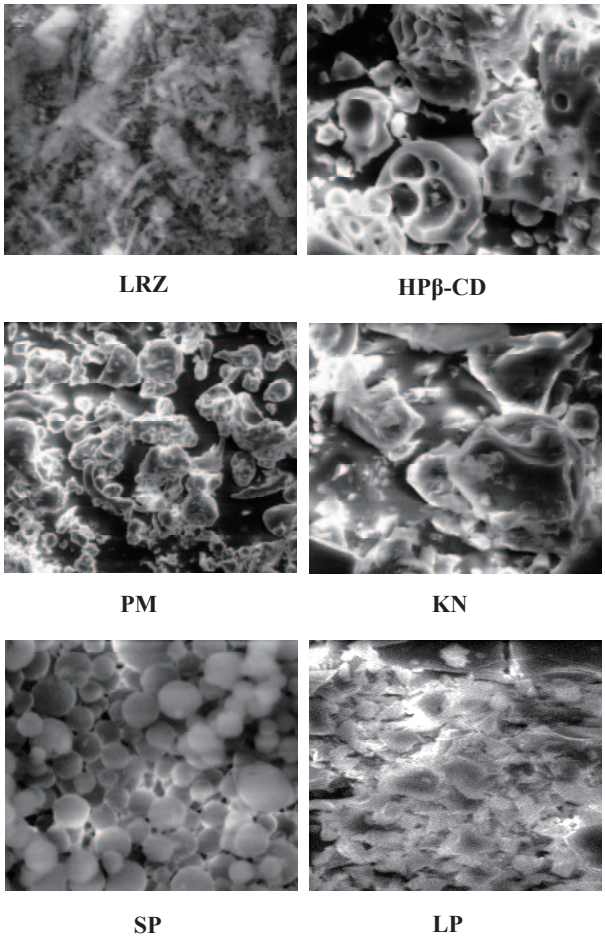


**Figure 4. Typical SEM images of LRZ, HP-β-CD, PM, KN, SP, and LP.**

This suggests that the crystallinity of complexes has decreased overall and, due to their more amorphous nature, the solubility of those complexes has increased (*39,40*).

The PXRD spectra of LRZ, HP-β-CD, PM, KN, SP, and LP are shown in Figure 5 and relative degree of crystallinity (RDC) values of these systems are presented in Table 3. In the X-ray diffractogram for LRZ powder, sharp peaks at a diffraction angle (2θ) of 6.77, 12.20, 13.25, 16.12, 17.95, 20.37, 24.52, 25.15, 27.75, 30.13, 37.01, and 38.53 were present (Figure 5), suggesting that the drug is present as a crystalline material. The amorphous structure of HP-β-CD was evident given the absence of any peak in PXRD spectra (Figure 5). The XRD spectra of complexes indicated that the degree of crystallinity decreased with the addition of a polymer, *i.e.*, HP-β-CD. RDC values decreased for all of the prepared inclusion complexes (Table 3). Only one peak related to LRZ was observed for the PM complex and no peaks were observed for KN, SP, and LP (Figure 5). The decrease in RDC values means improvement in the amorphousness of the sample. These results confirm that LRZ is no longer present as a crystalline material and its solid complexes are in an amorphous state.

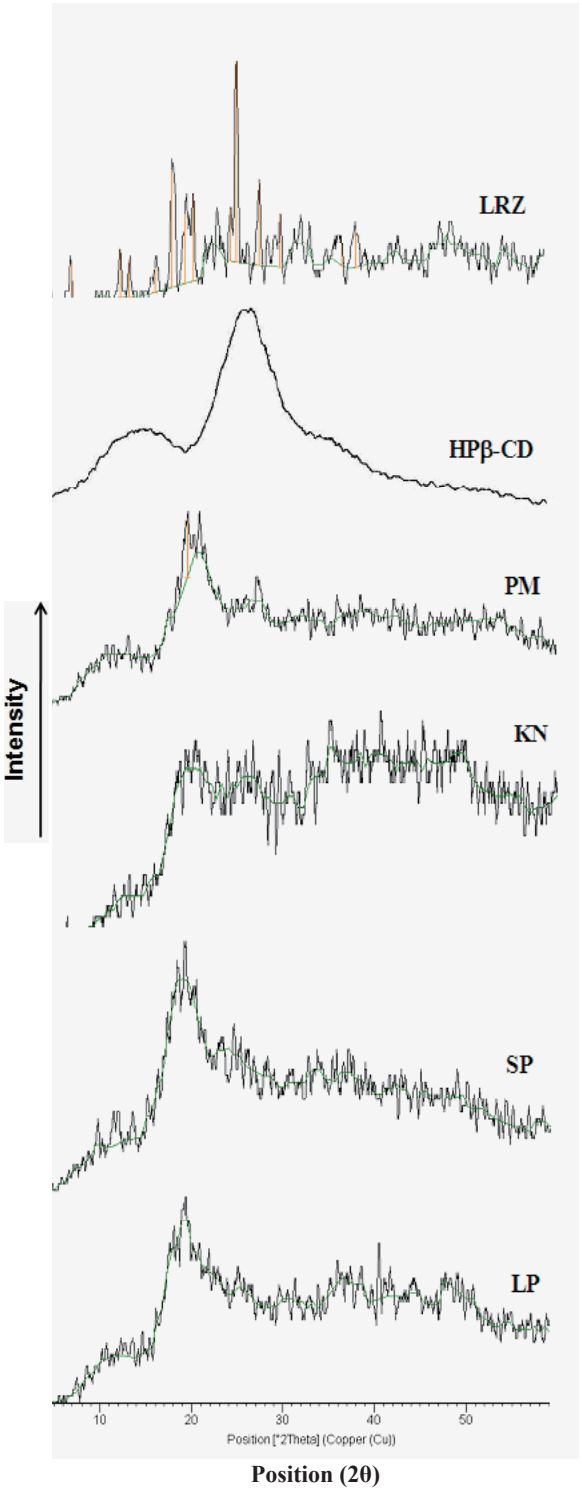


**Figure 5. PXRD Spectra of LRZ, HP-β-CD, PM, KN, SP, and LP.**

**Table 3. RDC values at 25.15° 2θ for LRZ, its physical mixtures, and complexes with HP-β-CD**

| Samples | RDC values |
|---|---|
| LRZ | 153 |
| PH | 0.91 |
| KN | 0.52 |
| SP | 0.72 |
| LP | 0.65 |

3.4. *Wettability and dissolution studies*

The improvement in wettability of LRZ by physical mixing and complexation with HP-β-CD is shown in Figure 6. SP and LP had higher wettability in water (82.4% and 98.6%, respectively) than did plain LRZ (28.0%) at 45 min (Figure 6). Even PMs of LRZ with HP-β-CD had significantly enhanced wettability of LRZ in water compared to plain LRZ. Thus, wettability studies indicated that HP-β-CD improved the wettability of LRZ in water both in complexes as well as in PMs due to its hydrophilicity.

Dissolution of pure LRZ and all other prepared systems (complexes and PMs) was carried out in distilled water. The reported values are arithmetic means of three measurements. $DP_{30\ min}$ (percent drug dissolved within 30 min) and $T_{50\%}$ (time to dissolve 50% of drug) in water are summarized in Table 4. These data reveal that dissolution of pure LRZ occurred at a very low level in dissolution medium (11.2% within 30 min). KN, SP, and LP considerably enhanced dissolution rates within 30 min compared to pure LRZ and PMs (Table 4). The dissolution profiles of pure LRZ and its PMs and complexes with HP-β-CD in water over a period of 4 h are shown in Figure 7. As is evident, pure LRZ had a very low dissolution rate in water, with about 39.0% of the drug being dissolved in 4 h (Figure 7). KN, SP, and LP significantly enhanced the dissolution rate of LRZ (65-100% in within 4 h) (Figure 7). Possible mechanisms of improved dissolution rates of complexes include reduction of crystallite size, a solubilization effect of the carrier, absence of aggregation of drug crystallites, improved wettability, dispersibility of the drug from dispersion, dissolution of the drug in the hydrophilic carrier, conversion of the drug to an amorphous state, and finally, combinations of the above mechanisms (*41*).

The dissolution rate of LRZ from PM was higher (50-60% in water) than that of pure LRZ (39.0%)

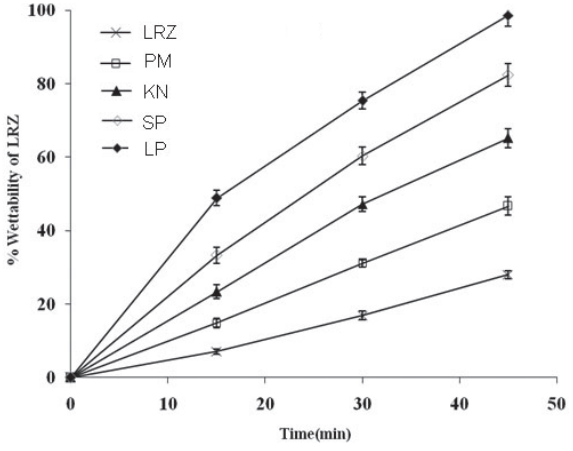


**Figure 6. Wettability study of LRZ, HP-β-CD, PM, KN, SP, and LP in distilled water.** Open circle, LRZ; Closed circle, HP-β-CD; Open triangle, PM; Closed triangle, KN; Open square, SP; Closed square, LP.

**Table 4. Dissolution data for pure LRZ and various prepared systems in water as a dissolution medium**

| Samples | % LRZ release | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $DP_{30\ min}$ (%) | | $T_{50\%}$ (min) | | MDT (min) | | $f_2$ value (*vs*. LRZ) | |
| | Complex | Tablet | Complex | Tablet | Complex | Tablet | Complex | Tablet |
| LRZ | 11.2 ± 0.6 | 5.5 ± 0.3 | > 360 | > 360 | 90.4 ± 3.3 | 99.2 ± 8.7 | – | – |
| PM | 23.5 ± 1.1 | 10.3 ± 0.5 | 192.6 ± 9.8 | > 360 | 73.0 ± 3.3 | 87.0 ± 0.2 | 44.8 | 50.2 |
| KN | 36.5 ± 1.2 | 20.8 ± 0.3 | 91.0 ± 8.7 | 73.4 ± 0.2 | 68.1 ± 3.0 | 63.8 ± 0.2 | 27.3 | 29.3 |
| SP | 68.5 ± 1.3 | 50.9 ± 0.6 | 9.0 ± 3.8 | 28.7 ± 7.4 | 31.3 ± 2.9 | 46.0 ± 5.7 | 12.6 | 17.0 |
| LP | 98.2 ± 1.6 | 65.2 ± 1.0 | 5.3 ± 1.0 | 17.2 ± 3.3 | 6.5 ± 2.0 | 33.3 ± 4.2 | 6.2 | 11.0 |

Data are shown as mean ± S.D. ($n$ =3). Abbreviations: $DP_{30\ min}$, % drug dissolved within 30 min; $T_{50\%}$, time to dissolve 50% of drug; MDT, mean dissolution time; $f_2$, similarity factor.

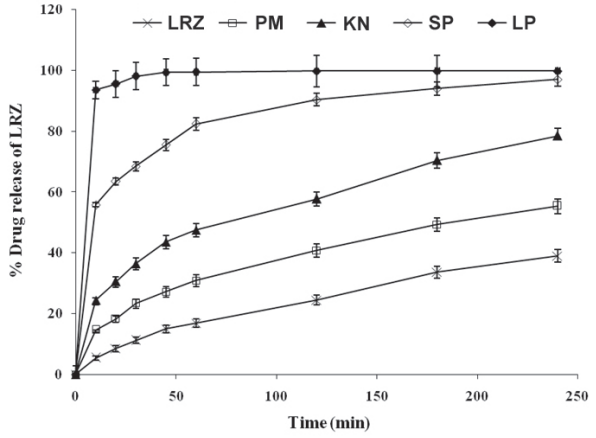


**Figure 7. *In vitro* dissolution profiles of LRZ, its physical mixtures, and complexes in distilled water.** Open circle, LRZ; Closed circle, HP-β-CD; Open triangle, PM; Closed triangle, KN; Open square, SP; Closed square, LP.

within 4 h (Figure 7). Physical mixing of LRZ with HP-β-CD brings the drug in close contact to the CD. The increased dissolution rate observed with PMs can be attributed to several factors such as the solubilization effect of the CD, improved wettability of the drug, and prevention of particle aggregation.

In order to understand the extent of improvement in the dissolution rate of LRZ from its complexes and PMs, the obtained dissolution data for pure LRZ and its PMs and complexes with CDs were fitted to the following equation (*20*):

$$MDT_{in\ vivo} = \frac{\sum_{i=1}^{n} t_{mid}\,\Delta M}{\sum_{i=1}^{n} \Delta M} \qquad \text{----Eq. 7}$$

Here, i is the number of dissolution samples, n is number of dissolutions, $t_{mid}$ is the time midway between times $t_i$ and $t_{i-1}$, and ΔM is the amount of LRZ dissolved (μg) between times $t_i$ and $t_{i-1}$. MDT reflects the time for the drug to dissolve and is the first statistical moment for the cumulative dissolution process, thus providing an accurate drug release rate (*23*). It is an accurate expression of the drug release rate. A higher MDT value indicates greater drug-retarding ability (*24*). In order to calculate the MDT for pure LRZ and its PMs and complexes with HP-β-CD, the mean ($n$ = 3) cumulative drug release (μg) was used. The obtained MDT values for pure LRZ, PM, KN, SP, and LP are presented in Table 4. The MDT for LRZ was 90.4 min in water. The MDT values for LRZ decreased to a greater extent after complexation of LRZ with CDs, *i.e.* 73.0, 68.1, 31.3, and 6.5 min for PM, KN, SP, and LP, respectively, in water (Table 4). Complexes prepared by lyophilization exhibited the best dissolution profile and the lowest MDT value.

A value of 100% for the similarity factor ($f_2$) suggests that the test and reference profiles are identical. Values between 50 and 100 indicate that the dissolution profiles are similar whilst smaller values imply an increase in dissimilarity between release profiles (*20*). The release profile of LP differed substantially from that for pure LRZ ($f_2$ values 6.23) (Table 4). Release profiles of pure LRZ from PM, KN, SP, and LP also differed significantly from that of pure LRZ in the dissolution medium.

### 3.5. *Formulation studies*

Physical properties of the complexes prepared by kneading, spray-drying, and lyophilization (KN, SP, and LP, respectively) were studied to judge their suitability for tabletting. In general, compressibility index values up to 15% and an angle of repose between 25° and 30° results in good to excellent flow properties (*39*). Compressibility, the angle of repose for complexes, and the physical properties of tablets made using these complexes are shown in Table 5. These values indicated good compressibility and flow properties, meaning that these samples are suitable for tabletting.

During *in vitro* dissolution studies, tablets prepared by compressing complexes of SP and LP exhibited more than 50% drug release within 15 to 30 min in water, whereas tablets prepared by compressing KN and PM provided the same drug release within 80 to over 360 min (Table 4, Figure 8). The tablets prepared using complexes has faster and reproducible release as compared to the tablets containing pure LRZ (Table 4, Figure 8). Tablets

**Table 5 Physical properties of complexes and tablets**

| Physical properties | LRZ | KN | SP | LP |
|---|---|---|---|---|
| Complex | | | | |
| % Compressibility | 13.7 | 13.5 | 12.4 | 13.3 |
| Angle of repose (°) | 27.6 | 26.1 | 25.3 | 24.3 |
| Tablet | | | | |
| Hardness (kg/cm$^2$) | 4.3 | 4.7 | 4.6 | 4.8 |
| Friability (%) | 0.50 | 0.60 | 0.90 | 0.70 |
| Diameter (mm) | 7.0 | 6.9 | 6.9 | 7.1 |
| Thickness (mm) | 4.0 | 4.1 | 4.1 | 4.0 |

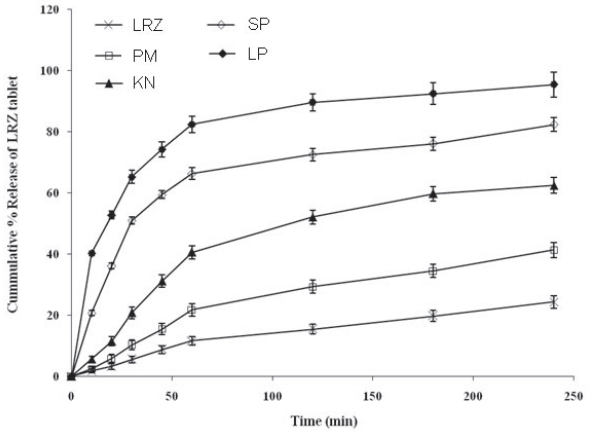


**Figure 8. Release profiles of LRZ from conventional tablets containing only LRZ and tablets containing PM, KN, SP, and LP in distilled water.** Open circle, LRZ; Closed circle, HP-β-CD; Open triangle, PM; Closed triangle, KN; Open square, SP; Closed square, LP.

prepared using SP and LP had 82.3% and 95.5% release in 4 h, respectively (Figure 8), with a respective $T_{50\%}$ of 28.7 and 17.2 min in water (Table 4). Tablets prepared using KN also showed improvement in the dissolution profiles of LRZ (Table 4, Figure 8). This confirmed the advantage of improving the aqueous solubility of LRZ in complex form since this form can be formulated into tablets with better dissolution characteristics. Release profiles of LRZ from conventional tablets containing LRZ alone differed significantly from those of tablets containing KN, SP, and LP as the $f_2$ values are 29.3, 17.0, and 11.0, respectively (Table 4). MDT values of LRZ from tablets containing SP and LP (46.0 and 33.3 min, respectively) were significantly lower than those of conventional tablets containing only LRZ (99.2 min) (Table 4).

## 4. Conclusion

Solubility studies revealed a significant increase in the aqueous solubility of LRZ with an increase in the concentration of HP-β-CD. The maximum concentration of HP-β-CD studied (200 mM at 37ºC and 25ºC) resulted in 420-fold and 322-fold improvement in the saturation solubility of LRZ. An inclusion complex of LRZ with HP-β-CD at a molar ratio of 2:1 was successfully prepared by kneading, spray-drying, and lyophilization. This was confirmed by DSC, FTIR, SEM, and XRD studies. The greatest improvement in solubility and *in vitro* drug release was observed with an inclusion complex prepared with HP-β-CD by lyophilization. The solubility and *in vitro* drug release of a physical mixture improved to a lesser degree than did that of complexes prepared by kneading, spray-drying, and lyophilization. These findings are extremely important from a commercial point of view as the prepared complex remedies the drawback of LRZ's poor dissolution profile.

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