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*Research Article*

# Physico-Chemical Characterization and *In Vitro* Dissolution Assessment of Clonazepam—Cyclodextrins Inclusion Compounds

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***Abstract.*** The objectives of this research were to prepare and characterize inclusion complexes of clonazepam with β-cyclodextrin and hydroxypropyl-β-cyclodextrin and to study the effect of complexation on the dissolution rate of clonazepam, a water-insoluble lipid-lowering drug. The phase-solubility profiles with both cyclodextrins were classified as $A_P$-type, indicating the formation of 2:1 stoichiometric inclusion complexes. Gibbs free energy $(\Delta G_{tr}{}^{o})$ values were all negative, indicating the spontaneous nature of clonazepam solubilization, and they decreased with increase in the cyclodextrins concentration, demonstrating that the reaction conditions became more favorable as the concentration of cyclodextrins increased. Complexes of clonazepam were prepared with cyclodextrins by various methods such as kneading, coevaporation, and physical mixing. The complexes were characterized by Fourier transform infrared spectroscopy and differential scanning calorimetry studies. These studies indicated that complex prepared kneading and coevaporation methods showed successful inclusion of the clonazepam molecule into the cyclodextrins cavity. The complexation resulted in a marked improvement in the solubility and wettability of clonazepam. Among all the samples, complex prepared with hydroxypropyl-β-cyclodextrin by kneading method showed highest improvement in *in vitro* dissolution rate of clonazepam. Mean dissolution time of clonazepam decreased significantly after preparation of complexes and physical mixture of clonazepam with cyclodextrins. Similarity factor indicated significant difference between the release profiles of clonazepam from complexes and physical mixture and from plain clonazepam. Tablets containing complexes prepared with cyclodextrins showed significant improvement in the release profile of clonazepam as compared to tablet containing clonazepam without cyclodextrins.

**KEY WORDS:** β-cyclodextrin; clonazepam; hydroxypropyl-β-cyclodextrin; inclusion complexation; *in vitro* dissolution studies; mean dissolution time.

## INTRODUCTION

Clonazepam (CLZ) belongs to a class of anticonvulsants that enhances gamma-aminobutyric acid (GABA) receptor responses. Anticonvulsants used for several types of seizures, including myotonic or atonic seizures, photosensitive epilepsy, and absence seizures. CLZ exerts its action by binding to the benzodiazepine site of the GABA receptors, which causes an enhancement of the electric effect of GABA binding on neurons resulting in an increased influx of chloride ions into the neurons. This results in an inhibition of synaptic transmission across the central nervous system (1,2). CLZ is a light yellow crystalline powder which is practically odorless. It is freely very soluble in methanol, ethanol, and acetone, and practically insoluble in water (at 25°C<0.1 mg/ml). It is generally considered that compounds with very low aqueous solubility will show dissolution rate-limited absorption and, hence, poor absorption, distribution, and target organ delivery (3). Improvement of aqueous solubility in such a case is a valuable goal to improve therapeutic efficacy.

Cyclodextrins (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 part of it, into the cavity (4,5). Because of the large number of hydroxyl groups on CDs, they are water-soluble. 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. CDs have widespread pharmaceutical applications mainly because of their effect on enhancing the solubility and bioavailability of many drug formulations. Complexation with cyclodextrins 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).

β-cyclodextrin (β-CD) has ideal dimensions to complex a range of commonly used drugs. Unfortunately, it has a limitation of high affinity for cholesterol, which may lead to crystallization of poorly water-soluble β-CD–cholesterol complex in the kidney, and thereby causing nephrotoxicity.

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Hydroxypropyl-β-cyclodextrin (HPβ-CD), a chemical derivative of β-CD, similarly improves the aqueous solubility of many drugs, but it is more hydrophilic than the β-CD, forms a less stable complex with cholesterol, and is therefore, less toxic (11). HPβ-CD is more water-soluble than the parent molecule and has hydroxypropylester groups attached to the hydroxyl groups in position 2. Mass spectrometry and molecular modeling studies on the inclusion complexes between α, β-cyclodextrins, and simvastatin were done (12). Inclusion complex of Rofecoxib/HPβ-CD (1:1 molar ratio) has been prepared using kneading method with a subsequent improvement in dissolution due to amorphization (13). Many other drugs such as ibuprofen, tolbutamide, ganciclovir, nimesulide, itraconazole, etc. have been tested for CDs inclusion to enhance solubility (14–18).

*In vitro* dissolution testing provides an easy and convenient means to evaluate the performance of pharmaceutical preparations. The *in vitro* dissolution profile is a reliable index to predict the *in vivo* performance accurately. In this study, an attempt was made to compare the similarity between *in vitro* dissolution profiles of CLZ from complexes, physical mixture, and pure CLZ. Dissolution profiles can be compared by calculating similarity factor ($f_2$) values. The method was first reported by Moore and Flanner (19). It has also been adopted by the Center for Drug Evaluation and Research (United States Food and Drug Administration (US FDA), 1997) and by the Human Medicines Evaluation Unit of The European Agency for the Evaluation of Medicinal Products ( European Medicines Agency, 1999) as a criterion for the assessment of similarity between two dissolution profiles. The similarity equation is given in the US FDA guidelines for industry for dissolution testing of immediate-release products (20,21). A value of 100% for the $f_2$ 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 (19).

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 (22). It is an accurate expression for drug release rate. A higher MDT value indicates greater drug retarding ability (23).

The objective of the present study was to prepare inclusion complexes of CLZ with β-CD and HPβ-CD using various methods such as kneading, coevaporation, and physical mixing to improve its aqueous solubility and dissolution rate. The study was further aimed to characterizations of prepared inclusion complexes by methods such as Fourier transform infrared (FTIR) and differential scanning calorimetry (DSC) studies.

## MATERIALS AND METHODS

### Materials

HPβ-CD and β-CD were generous gifts from Roquette Frères, France. Clonazepam was received as a gift sample from Centure Chemicals Pvt Ltd, (Mumbai, India). The sample sodium lauryl sulfate (SLS) was procured from S.D. Fine Chemicals, (Vadodadra, India). Directly compressible lactose, maize starch, sodium starch glycollate, colloidal silicon dioxide, and magnesium stearate were received as gift samples from 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.

### Phase-Solubility Study

Phase-solubility studies were performed according to the method reported by Higuchi and Connors (24). CLZ, in amounts that exceeded its solubility, were transferred to screw capped vials containing 25 ml of aqueous solution of β-CD (molecular weight=1,135) or HPβ-CD (molecular weight=1,500) in various molar concentrations (0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, and 14.0 mM/L, each for β-CD and HPβ-CD). The contents were stirred on electromagnetic stirrer (Remi, India) for 48 h at 37°C±0.1°C and 400 rpm (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 the wavelength of 310 nm using spectrophotometer (Shimazdu-1601, UV/Vis spectrophotometer, Shimadzu Corp, Kyoto, Japan). Solubility studies were performed in triplicate ($n$=3). The apparent stability constant (Ks), according the hypothesis of 1:1 stoichiometric ratio of complexes, was calculated from the phase-solubility diagrams using the following equation:

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

where, slope is obtained from the initial straight line portion of the plot of CLZ concentration against CDs concentration, and $S_0$ is the equilibrium solubility of CLZ in water.

### Preparation of Inclusion Complexes

Complexes of β-CD and HPβ-CD with CLZ were prepared in the molar ratio of 2:1 (on the basis of phase-solubility study) by different methods like physical mixture, coevaporation, and kneading. For ease in discussion, the samples designated with different abbreviations are shown in Table I.

#### *Physical Mixture*

Physical mixture (PM) of CDs and CLZ were prepared by simply mixing powders with a spatula for 15 min.

#### *Coevaporation Method*

For preparation of complexes by coevaporation method, methanol and water were used as solvents. The required quantities of CLZ and CDs were dissolved in the same quantities of methanol and water, respectively. Both the solutions were mixed, and solvents were evaporated by controlled heating at 45–50°C. The resultant solids were pulverized and then sieved through 120 #.

**Table I.** Abbreviations Used to Designate Samples of CLZ Prepared with β-CD and HPβ-CD by Different Methods

| Type of CDs | Method of preparation | Name of sample |
|---|---|---|
| β-CD | Physical mixture | PMB |
| β-CD | Coevaporation | CEB |
| β-CD | Kneading | KNB |
| HPβ-CD | Physical mixture | PMH |
| HPβ-CD | Coevaporation | CEH |
| HPβ-CD | Kneading | KNH |

*Kneading Method*

For preparation of complexes by kneading method, the required quantities of CDs and distilled water were mixed together in a motor so as to obtain a homogeneous paste. CLZ was then added slowly; while grinding, a small quantity of methanol was added to assist the dissolution of CLZ. 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 pastes were dried in an oven at 45–50°C for 24 h. The dried complexes were pulverized and then sieved through 120 #.

## Drug Content

The complexes prepared by kneading, coevaporation, and physical mixture method were assayed for CLZ content by dissolving a specific amount of the complexes in methanol and analyzing for the CLZ content spectrophotometrically at 310 nm on spectrophotometer (U.V. visible spectrophotometer, Shimazdu-1601).

## Characterization of Complexes

*Fourier Transform Infrared Spectroscopic Analysis*

FTIR spectrums of moisture free powdered samples of CLZ, CDs, its PMs and complexes with β-CD and HPβ-CD were obtained using a spectrometer (FTIR-8300, Shimadzu Co., Kyoto, Japan) by potassium bromide pellet method.

*Differential Scanning Calorimetry Analysis*

DSC scans of the powdered sample of CLZ, CDs, its PMs and complexes with β-CD and HPβ-CD were recorded using DSC-Shimadzu 60 with TDA TrendLine 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 air flow (100 ml/min).

*Powder X-ray Diffraction Analysis*

Powder X-ray diffraction (PXRD) patterns of CLZ, CDs, its PMs and complexes with β-CD and HPβ-CD, were determined using Phillips PW 3710 scanner, IW 1830 generator with a CuK α anode at 40 kV and 30 mA, and at a scan rate of $1°min^{-1}$ from $2\theta$ range from 1° to 40° conducted by Zydus Research Center, Ahmedabad, India.

## Wettability and Dissolution Studies

Wettability study was performed using open capillary tubes filled with CLZ, CDs, its PMs and complexes with β-CD and HPβ-CD with their lower capillary ends dipped into colored water (0.01% eosin in water). The upward migration of the colored front was registered as a function of time (25).

Dissolution studies of CLZ in powder form, its PMs and complexes with β-CD and HPβ-CD were performed to evaluate *in vitro* drug release profile. Dissolution studies were carried out using US Pharmacopeia dissolution apparatus type II with 500 ml dissolution medium at 37°C±0.5°C and 50 rpm for 4 h. 0.1 N HCl and distilled water containing 0.25% w/v of SLS were used as different dissolution mediums. At fixed time intervals, 5-ml aliquots were withdrawn, filtered, suitably diluted, and assayed for CLZ content by measuring the absorbance at 310 nm using spectrophotometer. Equal volume of fresh medium at the same temperature was replaced in to the dissolution medium after each sampling to maintain its constant volume throughout the test. Pure drug, its PM and complexes, with β-CD and HPβ-CD were evaluated for dissolution rate studies. Dissolution studies were performed in five replicates ($n=5$), and calculated mean values of cumulative drug release were used while plotting the release curves. MDT values and $f_2$ values were calculated to compare the extent of improvement in the dissolution rate of CLZ from pure drug, its PM and complexes with β-CD and HPβ-CD. Preliminary tests demonstrated that there was no change in the $\lambda_{max}$ of CLZ due to the presence of CDs dissolved in the dissolution medium.

## Formulation Studies

Formulation excipients were selected on the basis of preliminary tests which demonstrated no interference of these excipients with the $\lambda_{max}$ of CLZ. Tablets containing 10 mg of CLZ were made by direct compression using different formulation excipients like directly compressible lactose, colloidal silicon dioxide, and magnesium stearate. Tablets containing complexes prepared by kneading method equivalent to 10 mg CLZ were made similarly, but using less quantity of lactose. The blend was compressed on an eight-station single rotary machine (Cadmach, India) using round-shaped, flat punches to obtain tablets of 4 to 6 $kg/cm^2$ hardness and 3.3 to 3.6 mm thickness. For the assay, three tablets were crushed and a blend equivalent to 10 mg of CLZ was weighed and dissolved in dissolution mediums. The tablets were studied in five replicates ($n=5$) for release profile of drug using the same methodology as described in *in vitro* dissolution studies.

## Statistical Analysis

Model-independent mathematical approach proposed by Moore and Flanner (19) for calculating $f_2$ was used for comparison between dissolution profiles of different samples. The similarity factor $f_2$ is a measure of similarity in the

percentage dissolution between two dissolution curves and is defined by following equation (19):

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

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

A value of 100% for the $f_2$ 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 (19).

## RESULTS AND DISCUSSION

### Phase-Solubility Study

Phase-solubility analysis has been among the preliminary requirements towards the optimization of the development into inclusion complexes of the drugs as it permits the evaluation of the affinity between cyclodextrin and drug molecule in water. This process has been used by many researchers for the determination of the exact molar ratios in which the drugs could make complexes with CDs (26,27).

The phase-solubility curve of CLZ in the presence of CDs is shown in Fig. 1. This curve indicated an increase in solubility of CLZ with an increase in concentrations of CDs in water. Increasing amounts of CDs increased the amount of CLZ going into water, improving the aqueous solubility of CLZ. Solubility of CLZ was increased by 10.2-fold at 37°C, 8.6-fold at 25°C, 16.1-fold at 37°C, and 13.2-fold at 25°C at 14 mM/L concentration of β-CD and HPβ-CD, respectively. Increased solubility may be due to improved dissolution of CLZ particles in water by CDs.

An indication of the process of transfer of CLZ from pure water to aqueous solution of CDs was obtained from the values of Gibbs free energy change. The Gibbs free energy of transfer ($\Delta G_{tr}{}^{o}$) of CLZ from pure water to aqueous solutions of CDs was calculated using equation (28).

$$\Delta G_{tr}{}^{o} = -2.303RT \log\left(\frac{S_o}{S_s}\right) \quad (3)$$

where, So/Ss = the ratio of molar solubility of CLZ in aqueous solution of CDs to that of the pure water. The obtained values of Gibbs free energy are shown in Table II. This data provide the information regarding the increased solubility of CLZ in the presence of CDs. In other words, the Gibbs free energy values provide the information whether the reaction condition is favorable or unfavorable for drug solubilization in the aqueous carrier solution. Negative Gibbs free energy values indicate favorable conditions. $\Delta G_{tr}{}^{\circ}$ values were all negative for CDs at various concentrations, indicating the spontaneous nature of CLZ solubilization, and it decreased with an increase in its concentration, demonstrating that the reaction became more favorable as the concentration of CDs increased. These values also indicated that the extent of improvement in solubility was more with HPβ-CD as compared to β-CD.

The enthalpy of transfer ($\Delta H_t{}^{o}$) and entropy ($\Delta S$) can be calculated from a modification of the van't Hoff equation (29):

$$\frac{d\ln(S_c/S_o)}{dT} = \frac{\Delta H_t{}^{o}}{RT^2} \quad (4)$$

Rearranging and solving for $\Delta H_t{}^{o}$ yields:

$$\Delta H_t{}^{o} = -R\frac{d\ln(S_c/S_o)}{d(1/T)} \quad (5)$$

Linear regression of ln (*Sc*/*So*) *versus* $1/T$ for both CD concentrations of 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, and 14.0 mM/L gives a slope equal to $-\Delta H_t{}^{o}/R$ . This treatment assumes that $\Delta H_t{}^{o}$ is reasonably constant over the temperature range studied.

$$\Delta S = (\Delta H - \Delta G)/T \quad (6)$$

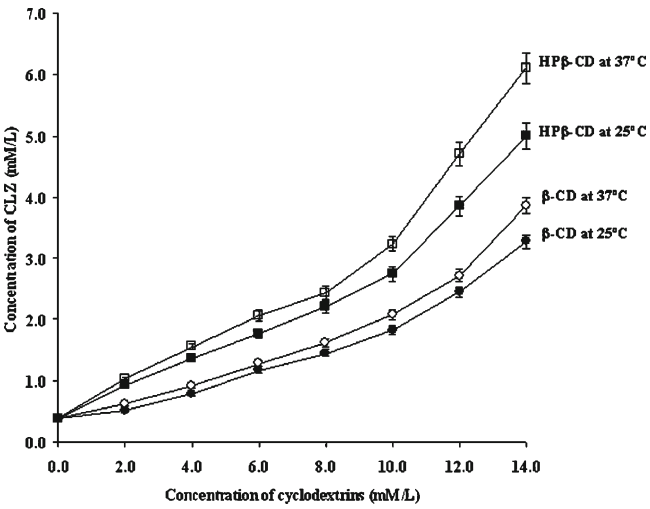


**Fig. 1.** Phase solubility curve of CLZ in aqueous solution of β-CD and HPβ-CD at different temperature ($n$=3)

Usually, complex formation with CDs results in a relatively large negative $\Delta H$ and $\Delta S$ that can be either positive or negative. Negative $\Delta H$ values suggested 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 of $\Delta S$, observed with β-CD and HPβ-CD, can be attributed to greater order after complexation. It is mainly due to the loss of rotational and translational freedom degrees of the molecules implicated in the complexation process (29–32).

Stoichiometric ratio at which optimum complexation occurs was confirmed by phase-solubility analysis. The phase-solubility plot were $A_P$ type for both CDs, which indicated that 2:1 (β-CD–CLZ) and (HPβ-CD–CLZ) inclusion complex was formed in solution. The values of apparent Ks for the complexes at 37°C, assuming a 2:1 stoichiometry, calculated from the slope of the initial straight portion of the phase-solubility diagram were 678 $M^{-1}$ at 37°C for β-CD: CLZ and 878 $M^{-1}$ at 37°C for HPβ-CD:CLZ which indicated a suitable and stable complex formation. It is reported that

**Table II.** Gibbs Free Energy of Transfer ($\Delta G$), Standard Enthalpy Change ($\Delta H$), and Entropy of ($\Delta S$) for Solubilization Process of CLZ in Aqueous Solutions of Cylodextrins at 37°C and 25°C ($n=3$)

| Concentration of cyclodextrins (mM/L) | ΔG (KJmol$^{-1}$) | | | | ΔH (KJmol$^{-1}$) | | ΔS (Jmol$^{-1}$ K$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|
| | β-CD | | HPβ-CD | | β-CD | HPβ-CD | β-CD | HPβ-CD |
| | 25°C | 37°C | 25°C | 37°C | | | | |
| 2 | −0.8±0.03 | −1.3±0.04 | −2.3±0.08 | −2.5±0.09 | −5.7±0.15 | −5.8±0.18 | −0.014±0.0003 | −0.010±0.0003 |
| 4 | −1.9±0.07 | −2.3±0.09 | −3.3±0.12 | −3.6±0.13 | −6.0±0.16 | −8.3±0.26 | −0.012±0.0003 | −0.015±0.0003 |
| 6 | −2.9±0.12 | −3.2±0.13 | −3.9±0.16 | −4.3±0.18 | −6.3±0.18 | −10.2±0.32 | −0.010±0.0003 | −0.019±0.0004 |
| 8 | −3.5±0.14 | −3.5±0.17 | −4.5±0.19 | −4.8±0.22 | −7.6±0.21 | −10.6±0.39 | −0.013±0.004 | −0.019±0.0004 |
| 10 | −4.1±0.19 | −4.4±0.21 | −5.1±0.24 | −5.5±0.26 | −8.3±0.26 | −10.9±0.40 | −0.013±0.0003 | −0.017±0.0005 |
| 12 | −4.8±0.24 | −5.1±0.25 | −5.9±0.30 | −6.4±0.33 | −8.3±0.28 | −11.4±0.42 | −0.010±0.0004 | −0.016±0.0005 |
| 14 | −5.7±0.28 | −5.9±0.31 | −6.6±0.35 | −7.2±0.37 | −10.2±0.34 | −13.4±0.61 | −0.014±0.0004 | −0.020±0.0006 |

cyclodextrin-drug complexes with the values of Ks in the range of 200 to 5,000 $M^{-1}$ show improved dissolution properties and, hence, better bioavailability (25).

## Drug Content

The drug content of the PMB, PMH, CEB, CEH, KNB, and KNH were found out to be 92.21% (±10.23), 93.78% (±9.83), 97.35% (±6.17), 97.45% (±5.82), 97.14% (±4.07), and 98.01% (±5.02), respectively, which approximately corresponds to stoichiometric ratio of the complex and indicate chemical stability and content uniformity of CLZ in its complex form.

## Characterization of Complexes

### *Fourier Transform Infrared Spectroscopic Analysis*

Fourier transform infrared spectroscopy has been used to assess the interaction between β-CD and guest molecules in the solid state. The 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 spectrum (33).

The IR spectra of PMB, CEB, KNB, PMH, CEH, and KNH were compared with spectrum of β-CD, HPβ-CD and CLZ (Fig. 2). The spectrum of pure CLZ presented characteristic peaks at 3,100–3,250 $cm^{-1}$ (N-H stretching), 3,076, 3,056 $cm^{-1}$ (aromatic C-H stretching), 1,696 $cm^{-1}$ (carbonyl stretching), 1,615, 1,582 $cm^{-1}$ (aromatic ring), 1,540 $cm^{-1}$ (asymmetric $NO_2$ stretching), 1,339 $cm^{-1}$ (symmetric stretching $NO_2$ stretching), 750 $cm^{-1}$ (four adjacent free Hs and aromatic C-H out of plane bending), and 844 $cm^{-1}$ (two adjacent free Hs and aromatic C-H out of plane bending), respectively. The FTIR spectrums of the β-CD and HPβ-CD are characterized by intense bands at 3,300–3,500 $cm^{-1}$ due to O–H stretching vibrations. 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 CLZ, depending on the extent of interaction.

The FTIR spectra of PMB, CEB, KNB, PMH, CEH, and KNH showed no peaks other than those of CDs and CLZ. Characteristic peaks of CLZ at 1,696 $cm^{-1}$, 1,615, 1,582 $cm^{-1}$, 1,540 $cm^{-1}$, and 1,339 $cm^{-1}$ were remain present where as peaks due to the aromatic ring posses free H's at 750 $cm^{-1}$ and 844 $cm^{-1}$ were absent in the FTIR spectra of PMB, CEB, KNB, PMH, CEH, and KNH. These results indicated aromatic ring with free H included in the CD cavity where as remaining part of CLZ oriented toward the upper exterior part of CD cavity. Moreover, the FTIR spectra of PMB, CEB, PMH, and CEH were equivalent to the addition spectrum of CDs and CLZ which suggested absence of well-defined chemical interaction between CDs and CLZ during kneading, coevaporation, and mixing.

### *Differential Scanning Calorimetry Analysis*

Differential scanning calorimetry enables the quantitative detection of all processes in which energy is required or produced (i.e., endothermic or exothermic phase transformations). The thermograms for pure CLZ, CDs, its PM and complexes with β-CD and HPβ-CD are presented in Fig. 3. The CLZ showed a melting peak at 242.46°C. In the thermogram of the β-CD and HPβ-CD peak, between 90°C–120°C was due to loss of water from CDs molecules.

In the thermograms of all samples, peaks due to β-CD and HPβ-CD was observed at the same position, i.e., between 80°C–120°C. Peak of CLZ at 242.46°C was present at the same position, i.e., near to 241.62°C in PMB, CEB, PMH, and CEH. In case of KNB and KNH, peak due to CLZ is almost disappeared, which may be due to trapping of CLZ in the CDs cavity. This also confirmed that kneading method was the best method for the preparation of inclusion complexes.

### *Powder X-ray Diffraction Analysis*

Powder X-ray diffraction spectroscopy has been used to assess the degree of crystallinity of the given sample. When complexes of drug and polymer are formed, the overall number of crystalline structure is reduced and the number of amorphous structures is increased. So, the final product sample shows less number as well as less intensity of peaks. This shows that overall crystallinity of complexes is decreased

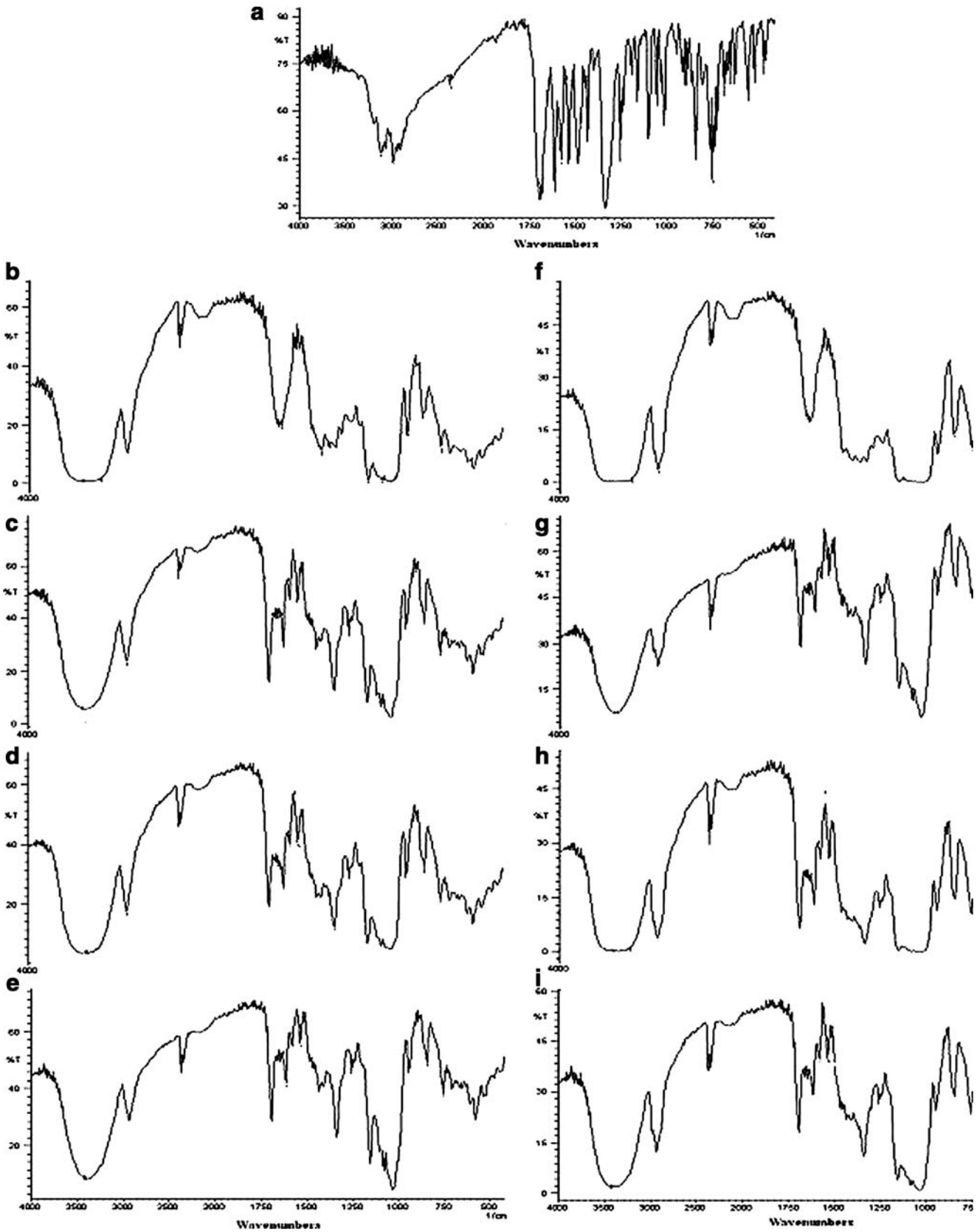


**Fig. 2.** FTIR spectra of CLZ **a**, β-CD **b**, PMB **c**, CEB **d**, KNB **e**, HPβ-CD **f**, PMH **g**, CEH **h**, and KNH **i**

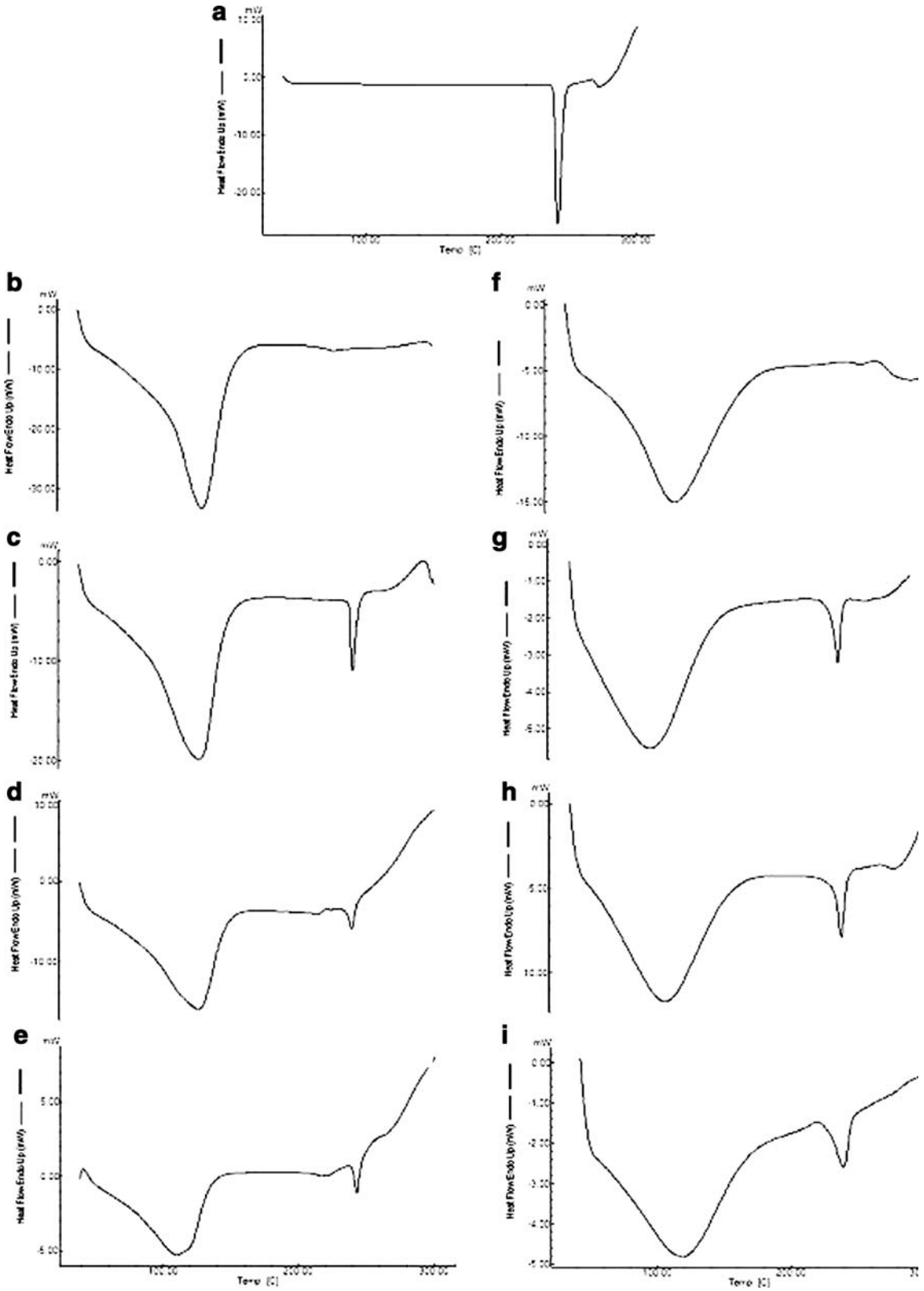


**Fig. 3.** DSC spectra of CLZ **a**, β-CD **b**, PMB **c**, CEB **d**, KNB **e**, HPβ-CD **f**, PMH **g**, CEH **h**, and KNH **i**

and due to more amorphous nature, the solubility is increased.

The PXRD spectra of CLZ, β-CD, PMB, CEB, KNB, HPβ-CD, PMH, CEH, and KNH are shown in Fig. 4. CLZ shows major peak at 2$\theta$ values for drugs (CLZ) are 11.84, 14.75, 14.98, 18.22, 18.50, 20.03, 20.45, 22.98, 23.91, 24.32, 26.07, 27.14, 27.38, 27.80, and 30.22. In β-CD, major peaks showed at 2$\theta$ values are 4.39, 8.87, 10.55, 12.40, 15.29, 19.46,

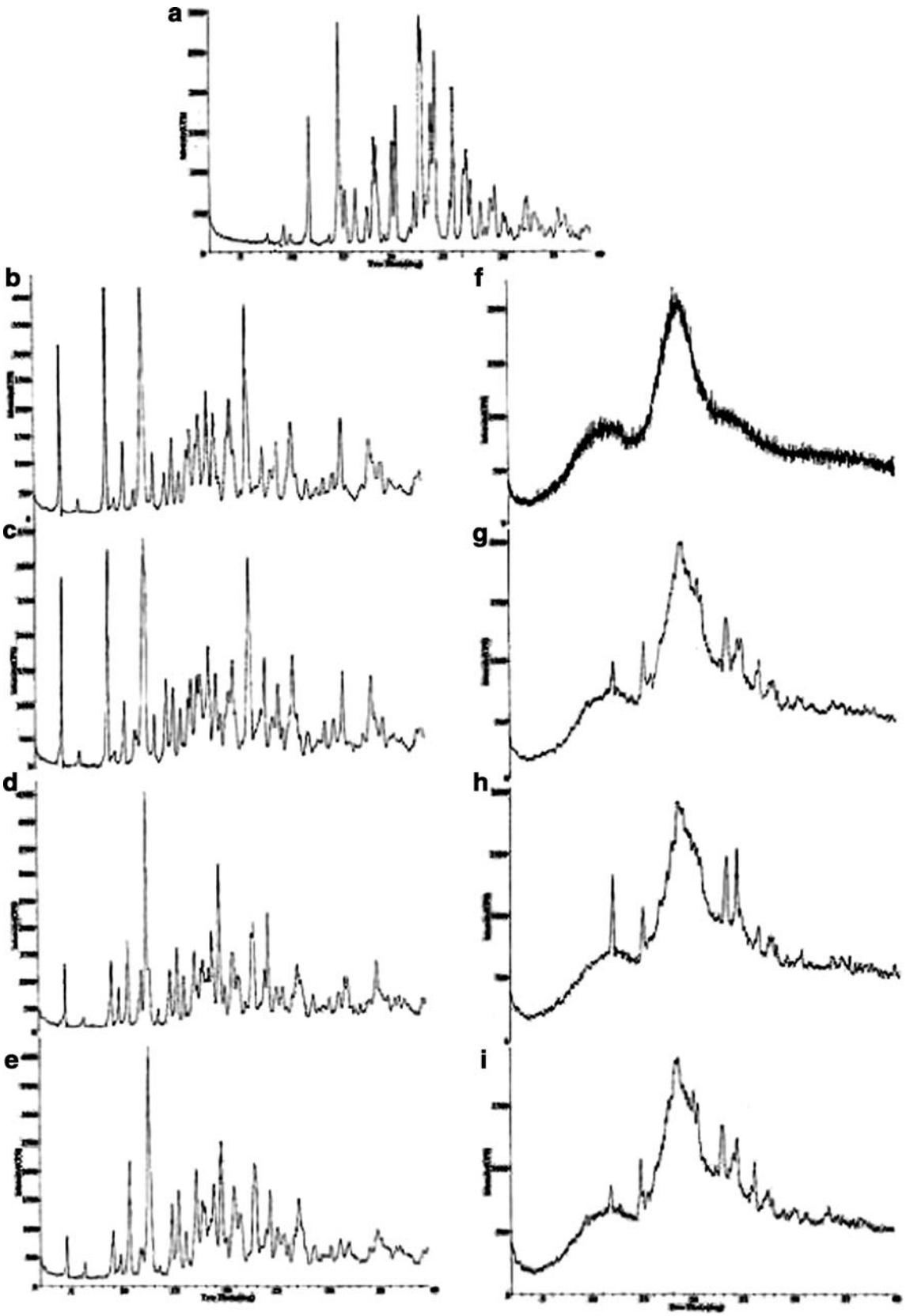


**Fig. 4.** Powder X-ray diffraction spectra of CLZ **a**, β-CD **b**, PMB **c**, CEB **d**, KNB **e**, HPβ-CD **f**, PMH **g**, CEH **h**, and KNH **i**

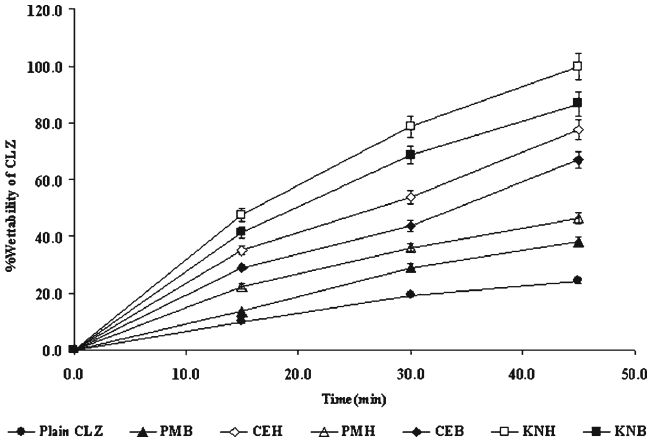


**Fig. 5.** Wettability study of plain CLZ, its physical mixtures, and inclusion complexes in water ($n$=5)

21.03, 22.56, 27.01, 31.81, and 34.64. In HPβ-CD, the structure is more amorphous so there are no major peaks shown. The study of these spectra indicated that degree of crystallinity was decreased by addition of polymers, i.e., β-CD and HPβ-CD. Degree of crystallinity was decreased to maximum extent in case of KNH. Complexes prepared using HPβ-CD showed more decrease in degree of crystallinity (only one or two peaks related to CLZ) as compared to that of β-CD. In case of complexes with β-CD too, KNB showed maximum decrease in degree of crystallinity. The decrease in degree of crystallinity means improvement in amorphousness of the samples. Hence, from the above discussion, it can be concluded that kneading method leads to amorphous complex of CLZ with CDs which will result in more improvement in solubility as compared to other samples.

## Wettability and Dissolution Studies

The improvement in wettability of CLZ by physical mixing and complexation with CDs is presented in Fig. 5. KNH and KNB showed highest wettability in water (99.9% and 86.6%, respectively), as compared to plain CLZ (24.1%) at 45 min. Even PMs of CLZ with CDs enhanced wettability of CLZ in water significantly as compared to plain CLZ. Thus, the results of wettability studies indicated that both CDs improved wettability of CLZ in water both in complex as well as in PM form due to its hydrophilicity.

The dissolution of poorly soluble drugs requires dissolution media that are different from those normally used for water-soluble drugs. One of the techniques that have been used is the incorporation of a small amount of surfactant in the dissolution medium (34). The use of surfactants in the dissolution systems may be physiologically more relevant, due to presence of natural surfactants like bile salts in the gastrointestinal tract. The mechanisms of surfactants to enhance the *in vitro* dissolution of poorly water-soluble drugs may be wetting, micellar solubilization, and/or deflocculation. It is necessary that a biorelevant medium will require similar surface activity as bio-fluids. Studies on SLS solutions indicate that surface tension of SLS solutions decreased dramatically above the critical micelle concentration (0.023%), and it reached a minimum surface tension at 0.2% with no significant change at higher concentrations (34,35). This suggested that a biocomparable surface activity can be achieved at low surfactant concentrations (0.2%). SLS was selected as a suitable surfactant in the present dissolution studies because preliminary experiments confirmed that SLS exhibited higher solubilization for CLZ than other surfactants. Based upon these findings, dissolution of pure CLZ and all other prepared systems (complexes and physical mixture) was carried out in aqueous SLS solution (0.25% w/v). When the CLZ was dispersed on the surface of the aqueous surfactant solution, CLZ rapidly left the surface and was dispersed in the bulk of solution which clearly indicate wetting of CLZ, unlike pure water. Since the principal objective of this work was to improve the dissolution rate of CLZ, dissolution studies were carried out for initially 4 h.

$DP_{30\ min}$ (percent drug dissolved within 30 min) and $T_{50\%}$ (time to dissolve 50% drug) in water are reported in Table III. From this data, it is evident that onset of dissolution of pure CLZ is very low in dissolution medium (9.15% within 30 min). CEB, KNB, CEH, and KNH considerably enhanced dissolution rates within 30 min compared to pure CLZ, PMB, and PMH. The graphical presentation of the dissolution profile of pure CLZ, its PMs, and complexes with β-CD and HPβ-CD in water over a period of 4 hrs are shown in Fig. 6. It is evident that the dissolution rate of pure CLZ is very low in water, about 36.7% of the drug being dissolved in 4 hrs. CEB, KNB, CEH, and KNH significantly enhanced dissolution rate of CLZ significantly (65–100% in within 4 hrs).

**Table III.** Percent of Drug Dissolved Within 30 min ($DP_{30\ min}$), Time to Dissolve 50% Drug ($T_{50\%}$), Mean Dissolution Time (MDT), and $f_2$ from Pure CLZ, PMB, PMH, CEB, CEH, KNB, and KNH in Water as Dissolution Medium ($n$=5)

| | Percent of clonazepam release | | | | | | $f_2$ value | |
|---|---|---|---|---|---|---|---|---|
| | $DP_{30\ min}$ | | $T_{50\%}$ min | | MDT (min) | | CLZ VS | |
| Sample | Complex | Tablet | Complex | Tablet | Complex | Tablet | Complex | Tablet |
| CLZ | 9.15±0.43 | 4.11±0.23 | >360 | >360 | 90.61±3.34 | 179.83±8.71 | – | – |
| PMB | 17.04±1.02 | – | 216.46±9.76 | – | 88.5±3.31 | – | 48.72 | – |
| PMH | 18.14±1.02 | – | 188.72±8.71 | – | 78.72±3.02 | – | 42.91 | – |
| CEB | 23.94±1.09 | 7.39±0.36 | 95.19±3.76 | 179.98±7.43 | 68.62±2.89 | 157.19±5.73 | 31.45 | 38.77 |
| CEH | 28.09±1.12 | 15.86±0.54 | 76.24±3.32 | 108.74±4.40 | 67.72±2.56 | 118.34±4.41 | 27.53 | 25.58 |
| KNB | 39.19±1.07 | 11.29±0.42 | 42.97±1.18 | 154.39±6.91 | 58.16±2.42 | 148.22±5.54 | 18.78 | 33.89 |
| KNH | 48.09±1.43 | 20.62±0.89 | 32.57±1.04 | 87.45±3.27 | 45.04±2.03 | 102.70±4.23 | 14.17 | 21.80 |

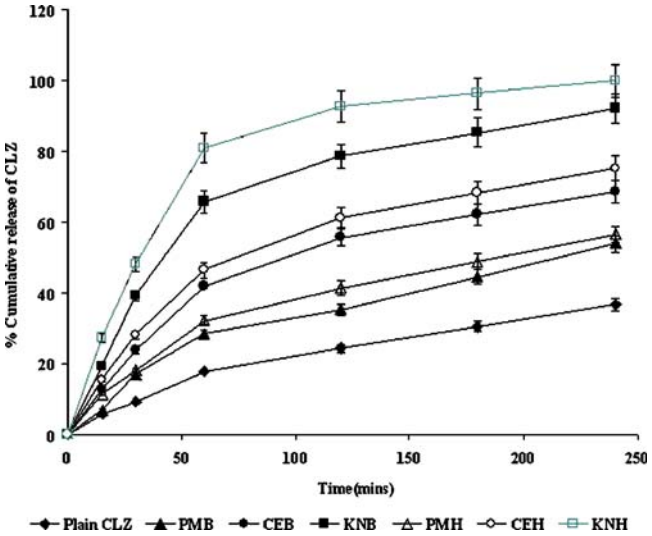


**Fig. 6.** *In vitro* dissolution profiles of plain CLZ and its inclusion complexes in water ($n$=5)

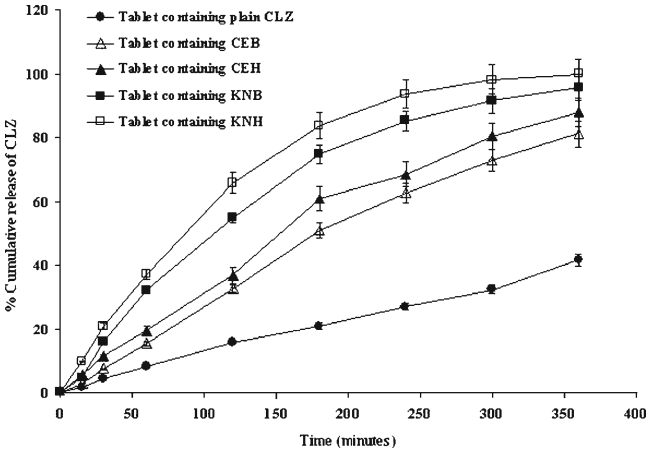


**Fig. 7.** *In vitro* dissolution profiles of tablets containing plain CLZ and its inclusion complexes in water ($n$=5)

Possible mechanisms of improved dissolution rates of complexes include (36) reduction of crystallite size, a solubilization effect of carrier, absence of aggregation of drug crystallites, improved wettability, dispersibility of a drug from dispersion, dissolution of the in the hydrophilic carrier, conversion of drug to amorphous state, and finally, the combination of the above methods.

The dissolution rate of CLZ from PMB and PMH was higher (50–60% in water) than that of pure CLZ (36.7%) within 4 hrs. Physical mixing of CLZ with CDs brings the drug in close contact CDs. The increased dissolution rate observed in case of PM can be attributed to several factors such as a solubilization effect of CDs, improved wettability of drug, and prevention of particle aggregation.

In order to understand the extent of improvement in dissolution rate of CLZ from its complexes and physical mixture, the obtained dissolution data of pure CLZ, its PM and complexes with CDs were fitted into equation (19)

$$MDT_{invitro} = \frac{\sum_{i=1}^{n} t_{mid}\Delta M}{\sum_{i=1}^{n} \Delta M} \quad (7)$$

Here, $i$ is dissolution sample number, $n$ is number of dissolution times, $t_{mid}$ is time at the midpoint between times $t_i$ and $t_{i-1}$, and $\Delta M$ is the amount of CLZ 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 that provides an accurate drug release rate. It is an accurate expression for drug release rate. A higher MDT value indicates greater drug retarding ability. In order to calculate MDT of pure CLZ, its PM and complexes with β-CD and HPβ-CD, the mean ($n$=3) of cumulative drug release (μg) was used. The obtained values of MDT for pure CLZ, PMB, PMH, CEB, CEH, KNB, and KNH are presented in Table III. The MDT of CLZ is 90.61 min in water. The MDT values of CLZ decreased to greater extent after preparing complex of CLZ with CDs, i.e., 68.62 min and 67.72 min for CEB and CEH and 58.16 min and 45.04 min for KNB and KNH in water. Even MDT values of PMB and PMH were sufficiently lower than pure CLZ. Complexes prepared by kneading method, which exhibited the best dissolution profile and lowest MDT values, were used for the formulation studies.

A value of 100% for the $f_2$ 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 (19). Calculated $f_2$ values are presented in Table III. From this table, it is evident that the release profile of KNB and KNH is highly different from pure CLZ ($f_2$ values 18.78 and 14.17). Even release profiles of pure CLZ from CEB, CEH PMB, and PMH are also significantly different from pure CLZ in dissolution medium.

## Formulation Studies

The complexes prepared by kneading and coevaporation method (KNB, CEB, KNH, and CEH) were studied for

**Table IV.** Physical Properties of Complexes and Tablets

| Physical properties | | CLZ | CEB | CEH | KNB | KNH |
|---|---|---|---|---|---|---|
| For Complex | % Compressibility | 13.74 | 13.52 | 12.37 | 13.28 | 11.92 |
| | Angle of repose (°) | 27.61 | 26.11 | 25.30 | 24.34 | 23.48 |
| For Tablet | Hardness (Kg/cm$^2$) | 4.3 | 4.7 | 4.6 | 4.8 | 4.9 |
| | Friability (%) | 0.50 | 0.60 | 0.90 | 0.70 | 0.60 |
| | Diameter (mm) | 7 | 6.9 | 6.9 | 7.1 | 7.1 |
| | Thickness (mm) | 4 | 4.1 | 4.1 | 4 | 4 |

physical properties to judge its tableting ability. In general, compressibility index values up to 15% and angle of repose between 25° to 30° results in good to excellent flow properties (37). Compressibility, angle of repose for complexes, and physical properties of tablets made using these complexes are shown in Table IV. These values indicated good compressibility and flow properties, making these samples suitable for tableting.

During *in vitro* dissolution studies, complexes of KNB and KNH exhibited more than 35% drug release within 25 to 30 min in water, whereas tablets prepared by compressing KNB and KNH provided same drug release within 80 to 100 min. The tablets prepared using complexes showed faster and reproducible release as compared to the tablets containing pure CLZ and no CDs. Tablets prepared using KNB and KNH showed 84.99% and 93.59% release in 4 h with $T_{50\%}$ of 154.39 min, and 87.45 min in water, respectively (Fig. 7). Tablets prepared using CEB and CEH also showed improvement in dissolution profiles of CLZ. This confirmed the advantage of improved aqueous solubility of CLZ in its complex form, which can be formulated as tablets with better dissolution characteristic. Release profiles of CLZ from conventional tablets containing CLZ alone are significantly different from tablets containing KNB and KNH as the $f_2$ values are 33.89 and 21.80. MDT values of CLZ from tablets containing KNB and KNH (148.22 and 102.70 min) are significantly lower than that of conventional tablets containing only CLZ (179.83 min).

## CONCLUSION

Solubility studies showed a significant increase in the aqueous solubility of clonazepam with increasing concentration of β-CD and HPβ-CD. At maximum, studied concentration of β-CD and HPβ-CD (14 mM/L at 37°C) resulted in 10.2-fold and 16.1-fold improvement in the saturation solubility of clonazepam. An inclusion complex of clonazepam with β-CD and HPβ-CD was prepared successfully by kneading and coevaporation method in a molar ratio of 2:1. This was confirmed by FTIR and DSC studies. The highest improvement in solubility and *in vitro* drug release were observed in inclusion complex prepared with HPβ-CD by kneading method. More improvement in solubility and *in vitro* drug release of clonazepam was observed with HPβ-CD as compared to β-CD. The solubility and *in vitro* drug release of the physical mixture, when compared to that of the complexes prepared by kneading and coevaporation method, was improved to a lesser degree. These findings are extremely important from a commercial point of view as the prepared complex removes drawback of poor dissolution profile of clonazepam.

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