



## PREPARATION AND EVALUATION OF RESERPINE – CYCLODEXTRINS INCLUSION COMPLEXES

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### ABSTRACT

The focus of this investigation was to confirm the potential of chemically modified cyclodextrins (hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD/Kleptose® HPB) and sulfobutylether- $\beta$ -cyclodextrin (SBE- $\beta$ -CD/Captisol®)) to improve the dissolution rate of Reserpine in oral delivery. Native and modified cyclodextrins were screened via phase solubility studies in order to select the most efficient cyclodextrin in formation of stable inclusion complexes.  $A_p$ -type phase solubilization resulted from phase solubility diagrams of all cyclodextrin indicated formation of 2:1 stoichiometric inclusion complexes between CDs and Reserpine. Gibbs free energy ( $\Delta G_{tr}^\circ$ ) values were all negative, indicating the spontaneous nature of Reserpine solubilization, and they decreased with increase in the cyclodextrins concentration, demonstrating that the reaction conditions became more favorable as the concentration of cyclodextrins increased. The inclusion complexes of Reserpine and both cyclodextrins were prepared in 2:1 molar ratio by various methods such as physical mixing, kneading, spray drying and lyophilization. The complexes characterized by Fourier-transform infrared (FTIR) spectroscopy differential scanning calorimetry (DSC) and Scanning electron microscopy (SEM) studies delineates amorphousness as well as the successful inclusion of the Reserpine molecule into the cyclodextrin cavity. The complexation exhibited marked improvement in the solubility and wettability of Reserpine. These complexes exhibited substantially higher and faster rates of dissolution compared to that of Reserpine and physical mixture. Physical mixture also showed significant improvement in the dissolution rate compared to pure Reserpine. Mean dissolution time (MDT) of Reserpine decreased significantly after preparation of complexes and physical mixture. Similarity factor ( $f_2$ ) indicated significant difference between the release profiles of Reserpine from complexes, physical mixture and from pure Reserpine.

**KEYWORDS:** Reserpine, hydroxypropyl- $\beta$ -cyclodextrin, sulfobutyl- $\beta$ -cyclodextrin, inclusion complexation, *in-vitro* dissolution studies.

### INTRODUCTION

Reserpine (RES) is produced by several members of the genus *Rauwolfia*, a climbing shrub indigenous to southern and southeast Asia. Extracts of *Rauwolfia serpentina* have been used medicinally in India for centuries. They were used in traditional Hindu medicine for a variety of conditions, including snakebite, hypertension, insomnia, and insanity. Reserpine has also been used as a tranquilizer and sedative in animal feeds.<sup>[1]</sup>

The mechanism of reserpine's toxic effects is similar to the mechanism of its pharmacologic effects. Reserpine inhibits normal sympathetic activity in both the CNS and peripheral nervous system by binding to catecholamine storage vesicles. This prevents the normal storage of

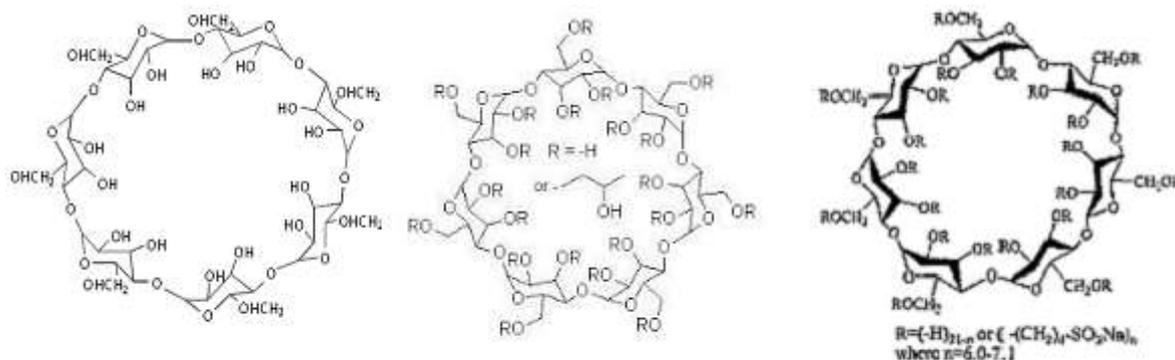
catecholamines and serotonin in the nerve cell, with the result being catecholamine depletion. Reserpine has also been described as inhibiting catecholamine synthesis by blocking the uptake of dopamine into the storage vesicle.<sup>[2-3]</sup>

Reserpine a biologically active naturally occurring alkaloid that exists at room temperature as a white or pale-buff to yellow odorless powder. It is practically insoluble in water; freely soluble in chloroform, methylene chloride, and glacial acetic acid; soluble in benzene and ethyl acetate; and slightly soluble in methanol, ethanol, acetone, ether.<sup>[4]</sup>

Cyclodextrin (CD) is a cyclic ( $\alpha$ -1, 4)-linked oligosaccharide built up of  $\alpha$ -D-glucopyranose units as

shown in Figure 1. Hampered aqueous solubility of native  $\beta$ -CD had given advantage for chemical modification by substitution with alkyl groups like methyl, hydroxypropyl and sulfobutyl ether resulting in enormous amplification in solubility with good safety profile.<sup>[5]</sup> Modified  $\beta$ -CD have gained wider applications in delivering the drugs (BCS class II and IV) via different routes of administration due to their increased solubilizing effect resulting in stable inclusion complexes and improved permeation across biological membranes.<sup>[6,7]</sup> Complexation with CDs has been

reported to enhance the solubility, dissolution rate and bioavailability of poorly water soluble drugs. CDs first came to the fore in marketed products as drug delivery technologies that enabled the development of various prostaglandins.<sup>[8]</sup> Inclusion complex of Rofecoxib/ HP $\beta$ -CD (1:1 molar ratio) has been prepared by Baboota et al using kneading method with a subsequent improvement in dissolution due to amorphization.<sup>[9]</sup> Many other drugs such as ganciclovir, nimesulide, itraconazole, tolbutamide, etc. have been tested for CD inclusion to enhance solubility.<sup>[10-13]</sup>



**FIGURE 1: Structure formula for the  $\beta$ -Cyclodextrin, hydroxypropyl- $\beta$ -cyclodextrin and sulfobutylether- $\beta$ -cyclodextrin molecules**

$\beta$ -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  $\beta$ -CD - cholesterol complex in the kidney and thereby causing nephrotoxicity. HP $\beta$ -CD and SB $\beta$ -CD, a chemical derivative of  $\beta$ -CD, similarly improves the aqueous solubility of many drugs, but it is more hydrophilic than the  $\beta$ -CD, forms a less stable complex with cholesterol, and is therefore less toxic.<sup>[14]</sup>

In this study an attempt was made to compare the similarity between *in vitro* dissolution profiles of Reserpine from complexes, physical mixture and pure Reserpine. Dissolution profiles can be compared by calculating similarity factor ( $f_2$  values) and mean dissolution time (MDT). The method for calculating similarity factor was first reported by Moore and Flanner, 1996.<sup>[15]</sup> It has also been adopted by the Center for Drug Evaluation and Research (US FDA, 1997) and by the Human Medicines Evaluation Unit of The European Agency for the Evaluation of Medicinal Products (EMA, 1999) as a criterion for the assessment of similarity between two dissolution profiles.<sup>[16, 17]</sup> 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.<sup>[15]</sup> 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.<sup>[18]</sup> It is accurate expression for drug release rate. A higher MDT value indicates greater drug retarding ability.<sup>[19]</sup>

The present study was planned to improve the aqueous solubility and dissolution rate of RES by preparing its complexes with HP $\beta$ -CD employing various methods such as kneading, coevaporation and physical mixing. The study further aimed to characterize the interaction between RES and HP $\beta$ -CD.

## MATERIALS AND METHODS

### Materials

Reserpine (RES) was purchased from Chemdye Corp Chemco, Gujarat. HP $\beta$ -CD and SB $\beta$ -CD were procured from Divya scientific Ltd, Ahmedabad, Gujarat. Directly compressible lactose, microcrystalline cellulose, talc, and magnesium stearate were received as gift samples from Maan Pharmaceuticals Ltd., (Ahmedabad, India). Necessary glassware for present research works was purchased from Durga scientific Ltd., Vadodara. 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, 1965.<sup>[20]</sup> RES, in amounts that exceeded its solubility, were transferred to screw capped vials containing 25 ml of aqueous solution of HP $\beta$ -CD (average MW  $\approx$  1460) and SB $\beta$ -CD

(average MW  $\approx$  1425) in various molar concentrations (0, 10, 25, 50, 75, 100, 150 and 200 mM/L). The contents were stirred on electromagnetic stirrer (Remi, India) for 36 h at  $37^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$  and 350 rpm (this duration was previously tested to be sufficient to reach equilibrium). After reaching equilibrium, samples were filtered through a  $0.22 \mu\text{m}$  membrane filter, suitably diluted and analyzed spectrophotometrically for drug content at the wavelength of 268 nm using spectrophotometer (Shimadzu-1601, UV/Vis spectrophotometer, Shimadzu Corp, Kyoto, Japan). Solubility studies were performed in triplicate ( $n = 3$ ). The apparent stability constant ( $K_s$ ), according the hypothesis of 1:1 stoichiometric ratio of complexes, was calculated from the phase-solubility diagrams using the following equation.

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

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

#### Preparation of Inclusion Complexes

Complex of HP $\beta$ -CD and SB $\beta$ -CD with RES were prepared in the molar ratio of 1:1 (on the basis of phase solubility study) by different methods like physical mixture, kneading, and lyophilization. For ease in discussion, these samples will be designated as physical mixture (PMHP and PMSB), kneading (KNHP and KNSB), spray drying (SDHP and SDSB) and lyophilization (LYHP and LYSB) for HP $\beta$ -CD and SB $\beta$ -CD, respectively throughout the manuscript.

#### Physical mixture

PM of HP $\beta$ -CD and SB $\beta$ -CD with RES was prepared by simply mixing powders with a spatula for 15 minutes.

#### Kneading method

The required quantities of both CDs and distilled water were mixed together in a motor so as to obtain a homogeneous paste. RES was then added slowly; while grinding, a small quantity of methanol was added to assist the dissolution of RES. The mixture was then ground for 1 hour. 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 oven at  $45 - 50^{\circ}\text{C}$  for 24 hours. The dried complex was pulverized and then sieved through 120 #.

#### Spray Drying

The inclusion complex of RES and HP $\beta$ -CD and SB $\beta$ -CD were prepared by spray drying method Briefly, accurately weighed quantity of RES was dissolved separately in methanol and then added drop by drop to the aqueous solutions of HP $\beta$ -CD and SB $\beta$ -CD, respectively. The resultant solutions were spray dried using a spray dryer (LU-222, Advanced, Labultima, Mumbai). The spray drying was done at the

following sets of conditions; air flow rate at 400 NI/h, spray nozzle with a diameter 0.7 mm under the atomization pressure of 2 kg/cm<sup>2</sup> with a feed rate of 4ml/min. The inlet temperature was kept at  $120^{\circ}\text{C}$  and out let temperature  $90 \pm 2^{\circ}\text{C}$ . The vacuum in the system was 60 mmWc and aspiration rate was 40 m Bar. The product thus obtained was collected, packed, doubly wrapped in an aluminum foil and stored in a desiccator till further use.

#### Lyophilization method

Based on phase solubility graphs an  $A_L$ -type means that the complexation ratio CD: Reserpine will be on 1:1 molar basis. Briefly, accurately weighed quantity of RES was dissolved separately in methanol and then added drop by drop to the aqueous solutions of HP $\beta$ -CD and SB $\beta$ -CD, respectively. The resulting clear solutions were frozen at  $-160^{\circ}\text{C}$  under liquid nitrogen and subsequently dried at  $-45^{\circ}\text{C}$  in a freeze dryer (VirTis BenchTop, K series, NY, USA) under vacuum (pressure of  $185 \times 10^{-3}$  Mbar) in order to obtain a dry powder of inclusion complexes of RES.

#### Drug Content

The complexes prepared by different methods were assayed for RES content by dissolving a specific amount of the complex in methanol and analyzing for the RES content spectrophotometrically at 268 nm on spectrophotometer (U.V. visible spectrophotometer, Shimadzu-1800). Final moisture content of all samples was measured by electronic moisture balance (Sartorius, model MA-45, Germany).

#### Characterization of Complexes

##### Fourier Transform Infrared (FTIR) Spectroscopic Analysis

The FTIR spectrums of moisture free powdered samples of RES, HP $\beta$ -CD and SB $\beta$ -CD and its physical mixtures and complexes with CDs were obtained using a spectrometer (FTIR-8300, Shimadzu Co., Kyoto, Japan) by potassium bromide (KBr) pellet method.

##### Differential Scanning Calorimetry (DSC) Analysis

DSC scans of the powdered sample of RES, HP $\beta$ -CD and SB $\beta$ -CD and its physical mixtures and complexes with CDs were recorded using DSC- Shimadzu 60 with TDA trend line software. The samples (6–7 mg) were accurately weighed in crimped aluminum pans and heated from  $50^{\circ}\text{C}$  to  $300^{\circ}\text{C}$ , at a scanning rate of  $10^{\circ}\text{C}/\text{min}$  under dry nitrogen flow (100 ml/min).

##### Scanning electron microscopy (SEM)

The surface morphology of free powdered samples of RES, HP $\beta$ -CD and SB $\beta$ -CD and its physical mixtures and complexes with CDs were examined by means of a scanning electron microscope (Philips, LC ESEM). The samples were fixed on a brass stub using double-sided tape and then made electrically conductive by coating in a vacuum with thin layer of copper. The photographs were taken with a Pentax (model MZ-10) camera at an

excitation voltage of 10 kV and magnification factors of 200 and 3500.

#### Wettability and *In-vitro* Dissolution Studies

Wettability study was performed using open tubes containing RES, HP $\beta$ -CD and SB $\beta$ -CD and its physical mixtures and complexes with CDs were placed 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. Each test was repeated four times and the mean was calculated.

Dissolution studies of RES, HP $\beta$ -CD and SB $\beta$ -CD and its physical mixtures and complexes with CDs in powder form were performed to evaluate *in vitro* drug release profile. Dissolution studies were carried out using USP dissolution apparatus type II with 100 ml dissolution medium (distilled water) at 37°C  $\pm$  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) and then assayed for RES content by measuring the absorbance at 268 nm using spectrophotometer. Fresh medium (5 ml), which was replaced into the dissolution medium after each sampling to maintain its constant volume throughout the test.

RES and its physical mixtures and complexes with CDs were evaluated for *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 RES and its physical mixtures and complexes with CDs. Preliminary tests demonstrated that there was no change in the  $\lambda_{max}$  of RES due to the presence of HP $\beta$ -CD and SB $\beta$ -CD 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 RES. Tablets containing 4 mg of RES were made by direct compression using different formulation excipients like microcrystalline cellulose, talc and magnesium stearate. Tablets containing complexes prepared by KN, SP and LY methods equivalent to 4 mg RES were made similarly but quantity were adjusted with lactose to prepare a tablet with equal weight. 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<sup>2</sup> hardness and 3.3 to 3.6 mm thickness. For the assay, three tablets were crushed and a blend equivalent to 4 mg of RES was weighed and dissolved in dissolution mediums. The tablets were studied in triplicates (n = 3) 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<sup>[15]</sup> for calculating a similarity factor  $f_2$  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<sup>[15]</sup>:

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

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 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.<sup>[15]</sup>

## 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.<sup>[21, 22]</sup>

The phase solubility diagram of RES in the presence of both CDs was obtained by plotting the apparent equilibrium concentration of RES against various molar concentrations of HP $\beta$ -CD and SB $\beta$ -CD (Figure 2 (a) & (b)). The solubility of RES in water at 25°C is 11  $\mu$ g/ml; therefore, RES can be considered to be water insoluble drugs. From this curve, it can be seen that the apparent solubility of RES increases due to the formation of an inclusion complex between RES and CDs. A linear increase of solubility of RES was observed with an increase in concentration of CDs in water. Increasing amounts of CDs increased the amount of RES going into water, improving the aqueous solubility of RES. Solubility of RES was increased by 87.4-fold & 109.7-fold at 37°C and 72.3-fold & 85.6-fold at 25°C at and 200 mM/L concentration of HP $\beta$ -CD & SB $\beta$ -CD, respectively. Increased solubility may be due to improved dissolution of RES particles in water by CDs.

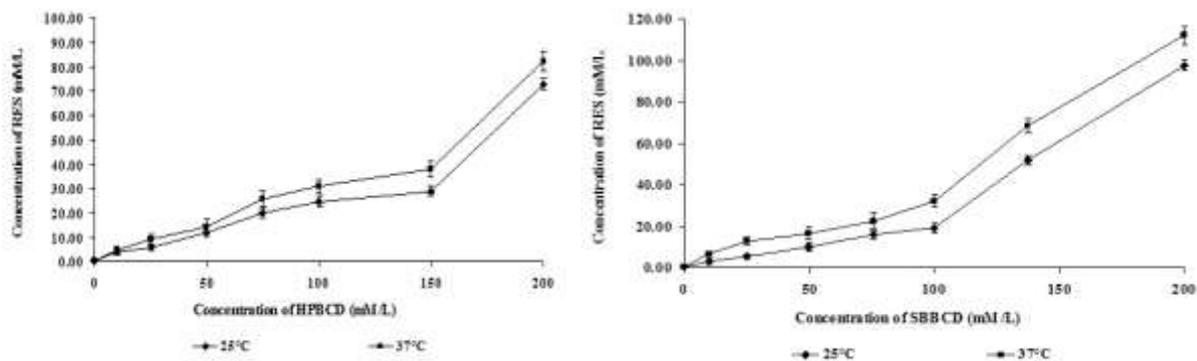


FIGURE 2: Phase solubility curve of RES in aqueous solution of HPβ-CD and SBβ-CD at 25°C and 37°C

Stoichiometric ratio at which optimum complexation occurs was confirmed by phase solubility analysis. The phase solubility plot showed an A<sub>p</sub> type solubility curve for both CDs, which indicates that 2:1 HPβ-CD – RES and SBβ-CD – RES inclusion complex was formed in solution. The values of apparent stability constants (K<sub>s</sub>) 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 257.73 M<sup>-1</sup> & 323.78 M<sup>-1</sup> at 25°C and 311.54 M<sup>-1</sup> & 491.22 M<sup>-1</sup> at 37°C for HPβ-CD:RES & SBβ-CD:RES, respectively which indicated a suitable and stable complex formation. It is reported that cyclodextrins-drug complexes with the values of K<sub>s</sub> in the range of 200 to 5000 M<sup>-1</sup> show improved dissolution properties and hence better bioavailability.<sup>[20]</sup>

The values of Gibbs free energy change (ΔG<sub>tr</sub><sup>o</sup>) were calculated to understand process of transfer of RES from pure water to aqueous solution of CDs. The values of ΔG<sub>tr</sub><sup>o</sup> of RES from pure water to aqueous solutions with

different concentrations of both CDs at 37°C were calculated using equation<sup>[23]</sup>

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

where S<sub>o</sub>/S<sub>s</sub> = the ratio of molar solubility of RES in aqueous solution of CDs to that of the pure water. The obtained values of Gibbs free energy are shown in Table 1 (a) & Table 1 (b). The ΔG<sub>tr</sub><sup>o</sup> 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. ΔG<sub>tr</sub><sup>o</sup> values were all negative for CDs at various concentrations, indicating the spontaneous nature of RES solubilization. Furthermore, these values decreased with increased concentration of CDs, demonstrating that the reaction became more favorable as the concentration of CDs increased.

Table 1(a): Thermodynamic parameters for solubilisation process of RES in aqueous solutions of HPβ-CD at 25°C and 37°C

Conc. of (mM/L)	ΔG (KJmol <sup>-1</sup> )		ΔH (KJmol <sup>-1</sup> )	ΔS (Jmol <sup>-1</sup> K <sup>-1</sup> )
	25°C	37°C		
10	-4.12±0.03	-5.12±0.04	-7.17±0.14	-0.0055±0.003
25	-5.08±0.04	-6.46±0.08	-9.27±0.15	-0.0062±0.005
50	-6.73±0.10	-7.55±0.12	-11.44±0.26	-0.0071±0.004
75	-7.91±0.11	-8.13±0.13	-12.87±0.31	-0.0087±0.004
100	-9.11±0.14	-9.11±0.19	-14.07±0.34	-0.0090±0.005
150	-10.07±0.16	-11.37±0.24	-15.34±0.42	-0.095±0.006
200	-11.21±0.22	-12.23±0.29	-18.29±0.43	-0.0104±0.006

TABLE 1(b): Thermodynamic parameters for solubilisation process of RES in aqueous solutions of SBβ-CD at 25°C and 37°C

Conc. of (mM/L)	ΔG (KJmol <sup>-1</sup> )		ΔH (KJmol <sup>-1</sup> )	ΔS (Jmol <sup>-1</sup> K <sup>-1</sup> )
	25°C	37°C		
10	-5.21±0.02	-5.63±0.04	-9.87±0.13	-0.0346±0.002
25	-6.63±0.06	-7.37±0.07	-11.42±0.15	-0.0130±0.004
50	-8.76±0.09	-9.66±0.11	-13.28±0.21	-0.0116±0.005
75	-9.71±0.09	-10.71±0.14	-15.23±0.27	-0.0145±0.006
100	-10.37±0.14	-11.45±0.17	-16.26±0.29	-0.0155±0.008
150	-11.55±0.18	-12.70±0.23	-16.99±0.33	-0.0138±0.007
200	-12.63±0.24	-13.88±0.26	-18.85±0.36	-0.0144±0.006

The enthalpy of transfer ( $\Delta H_t^\circ$ ) and entropy ( $\Delta S$ ) can be calculated from a modification of the van't Hoff equation<sup>[24]</sup>:

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

Rearranging and solving for  $\Delta H_t^\circ$  yields

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

Linear regression of  $\ln(S_c/S_o)$  versus  $1/T$  for CDs concentrations of 10.0, 25.0, 50.0, 75.0, 100.0, 150.0 and 200.0 mM/L gives a slope equal to  $-\Delta H_t^\circ/R$ . This treatment assumes that  $\Delta H_t^\circ$  is reasonably constant over the temperature range studied.

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

Usually complex formation with CD 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 CDs, 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.<sup>[24]</sup>

#### Drug Content

The drug content of RES in PMHP, KNHP, SDHP, LYHP, PMSB, KNSB, SDSB and LYSB were found out to be 91% ( $\pm 12.27$ ), 96.03% ( $\pm 7.48$ ), 97.83% ( $\pm 5.48$ ), 99.22% ( $\pm 4.56$ ), 92% ( $\pm 11.55$ ), 98.66% ( $\pm 8.07$ ), 98.84% ( $\pm 6.32$ ), and 99.46% ( $\pm 3.01$ ), respectively, which approximately corresponds to stoichiometric ratio of the

complex and indicate chemical stability and content uniformity of RES in its complex form. Content uniformity for the PM is lower than KN, SD and LY. This may be due to insufficient mixing which may be due to simple mixing of powders without applying pressure (CDs and RES). Final moisture content of the pure drug, PMHP, KNHP, SDHP, LYHP, PMSB, KNSB, SDSB and LYSB was 2.13%, 1.59%, 0.87, 0.24%, 2.01%, 1.27%, 0.73%, and 0.19%, respectively.

#### Characterization Of Complexes

##### Differential Scanning Calorimetry (DSC) 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).<sup>[25,26]</sup> The thermograms for pure RES, HP $\beta$ -CD, PMHP, KNHP, SDHP, LYHP, SB $\beta$ -CD, PMSB, KNSB, SDSB and LYSB are presented in Figure 3. DSC curve of RES displays a sharp endotherm at 286.13°C, which is due to drug melting, characteristic of an anhydrous crystalline substance. In the thermograms of the both CDs peak near to 100°C was due to loss of water from CDs molecules.

In the PM systems is clearly distinguishable the drug endothermic peak. This indicates that in such systems the drug has basically maintained its original crystallinity. In KN systems, there are substantial size reduction, and slight shift to lower temperatures of the drug melting point. This shift can be due to the decrease in the crystallinity and increase in the amorphousness of the KN samples. Comparing with that of PM systems it could be ascribed to some drug-cyclodextrin interaction.

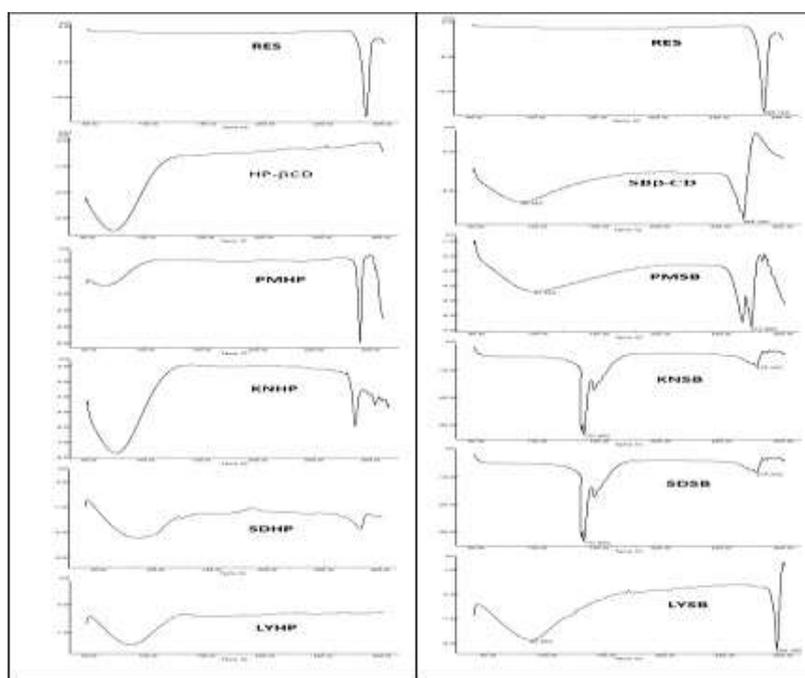


FIGURE 3: DSC thermograms RES, HP $\beta$ -CD, PMHP, KNHP, SDHP LYHP, SB $\beta$ -CD, PMSB, KNSB, SDSB and LYSB

Disappearance of the fusion peak of the drug is often interpreted as evidence of an inclusion complex formation. The disappearance of the RES melting peak from the thermogram of SD and LY might be due to the crystalline RES being included within the central cavity of the CDs suggests the formation of a true inclusion complex. This also confirmed that spray drying and lyophilization methods were the best methods for the preparation of inclusion complexes.

#### ***Infrared (IR) Spectroscopic Analysis***

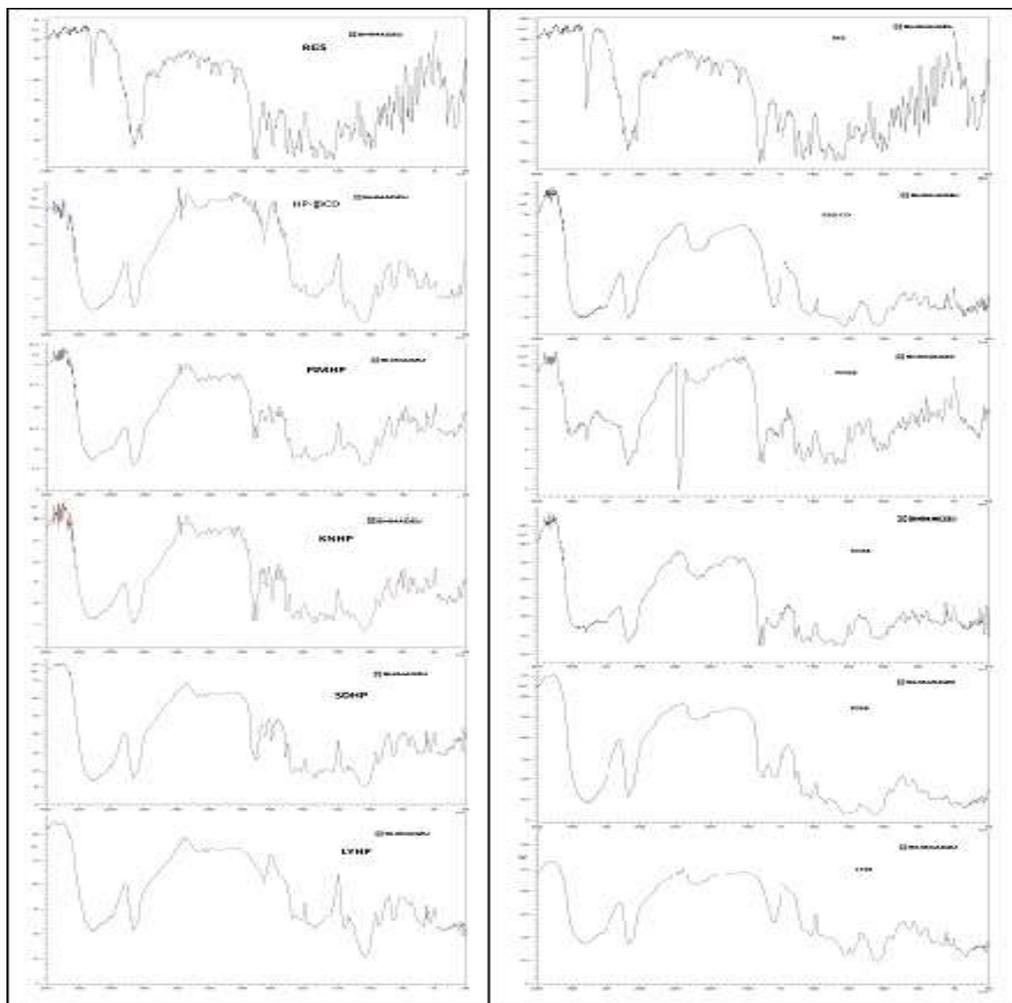
Fourier transform infrared spectroscopy (FTIR) has been used to assess the interaction between  $\beta$ -CD and guest molecules in the solid state. The chemical interaction between the drug and the carrier often leads to identifiable changes in the infrared profile of complexes. However, some of the changes are very subtle requiring careful interpretation of the spectrum.<sup>[27]</sup>

The IR spectras of PMHP, KNHP, SDHP, LYHP, PMSB, KNSB, SDSB and LYSB were compared with spectrum of HP $\beta$ -CD, SB $\beta$ -CD, and RES (Figure 4). The FTIR spectras of PMHP, KNHP, SDHP, LYHP, PMSB, KNSB, SDSB and LYSB showed no peaks other than

those of CDs and RES. These results indicated aromatic ring with free H included in the CDs cavity where as remaining part of RES oriented toward the upper exterior part of CDs cavity. Moreover, The FTIR spectras of PMHP, KNHP, SDHP, LYHP, PMSB, KNSB, SDSB and LYSB were equivalent to the addition spectrum of CDs and RES which suggested absence of well defined chemical interaction between CDs and RES during preparation of complex by lyophilization, spray drying and kneading method.

#### ***Scanning electron microscopy (SEM)***

SEM microphotographs of RES, HP $\beta$ -CD, PMHP, KNHP, SDHP, LYHP, SB $\beta$ -CD, PMSB, KNSB, SDSB and LYSB are reported in Figure 5. RES is characterized by regular shaped crystals; CDs are composed of spherical particles with amorphous character. In PM, the characteristic RES crystals, which were mixed with excipient particles or adhered to their surface, were clearly detectable, thus confirming the presence of crystalline drug. In the KN, it was possible to distinguish RES crystals agglomerated on the surface of CDs particles that had lost their original shapes and in this case crystal sizes were smaller.



**FIGURE 4: FTIR Spectrograms of RES, HP $\beta$ -CD, PMHP, KNHP, SDHP LYHP, SB $\beta$ -CD, PMSB, KNSB, SDSB and LYSB**

In SD products the original morphology of raw materials disappeared, and it was not possible to differentiate the two components (drug and cyclodextrin). The SP systems showed amorphous and homogeneous aggregates of spherical particles, a particular aspect

characteristic of this type of systems. Finally, LY products appeared to be of a lesser crystalline structure with a soft and fluffy appearance and again, crystals of the single components (drug & cyclodextrin) were still not distinguishable.

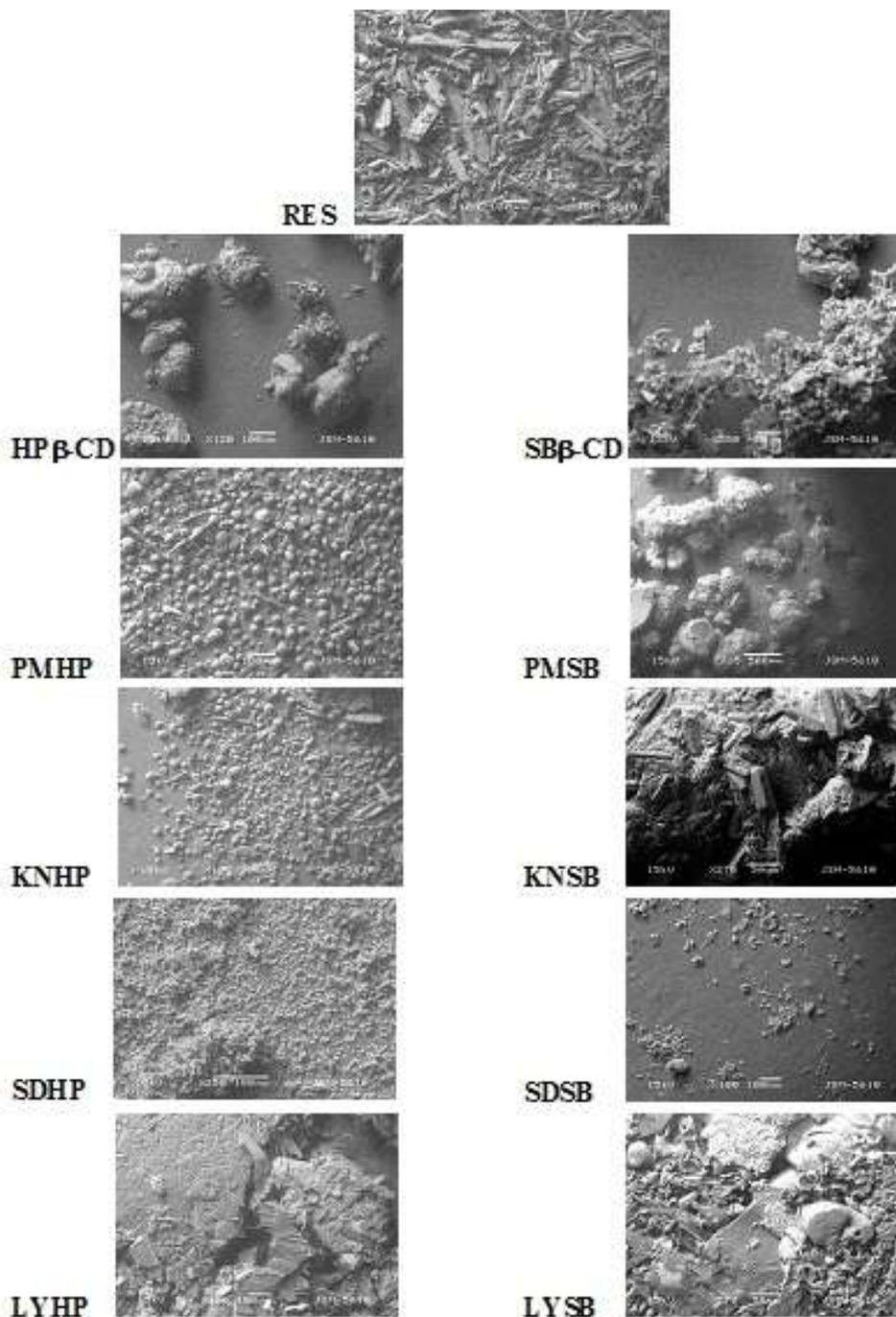


FIGURE 5 (a): SEM of RES, HP $\beta$ -CD, PMHP, KNHP, SDHP, LYHP, SB $\beta$ -CD, PMSB, KNSB, SDSB and LYSB

#### Wettability and Dissolution Studies

The improvement in wettability of RES by physical mixing and complexation with CDs is presented in Figure 6. LY with HP $\beta$ -CD and SB $\beta$ -CD showed highest wettability in water (68.9% and 95%, respectively), as compared to plain RES (18.9%) at 45 min. Even SD and

PM of RES with HP $\beta$ -CD and SB $\beta$ -CD enhanced wettability of RES in water significantly as compared to plain RES. Thus, the results of wettability studies indicated that both CDs improved wettability of RES in water both in complex as well as in PM form due to its hydrophilicity.

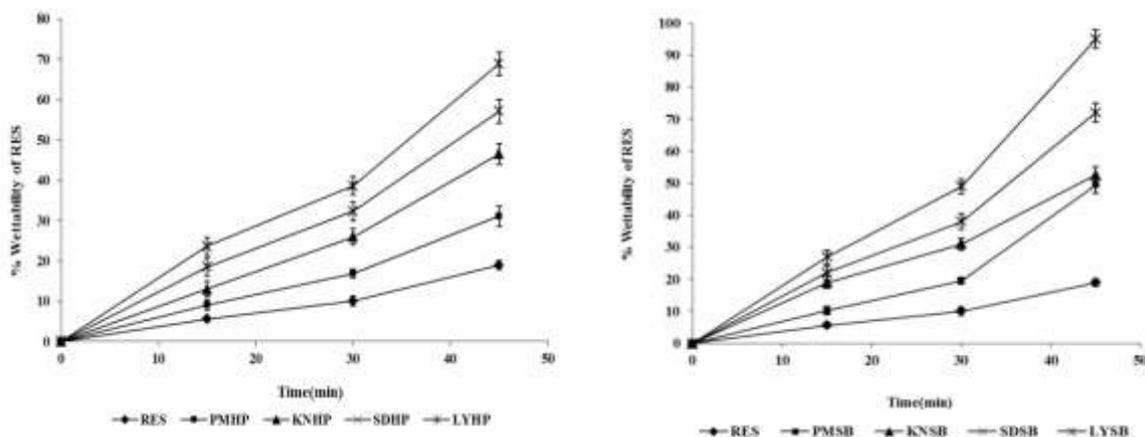


FIGURE 6 (a): Wettability study of RES, PMHP, KNHP, SDHP, LYHP, PMSB, KNSB, SDSB and LYSB (n=3)

Dissolution of pure RES and all other prepared systems (complexes and physical mixture) was carried out in distilled water. The reported values are arithmetic means of three measurements. From this data, it is evident that onset of dissolution of pure RES is very low in dissolution medium (7.9% within 30 min). KN, SP and LP considerably enhanced dissolution rates within 30 min compared to pure RES and PM. The graphical presentation of the dissolution profile of pure RES, its PM and complexes with HP $\beta$ -CD and SB $\beta$ -CD in water over a period of 4 hrs is shown in Figure 7. It is evident

that the dissolution rate of pure RES is very low in water, about 39.0% of the drug being dissolved in 4 hrs. KN, SD and LY significantly enhanced dissolution rate of RES significantly (65-100% in within 4 hrs). Possible mechanisms of improved dissolution rates of complexes include<sup>[14]</sup> 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.

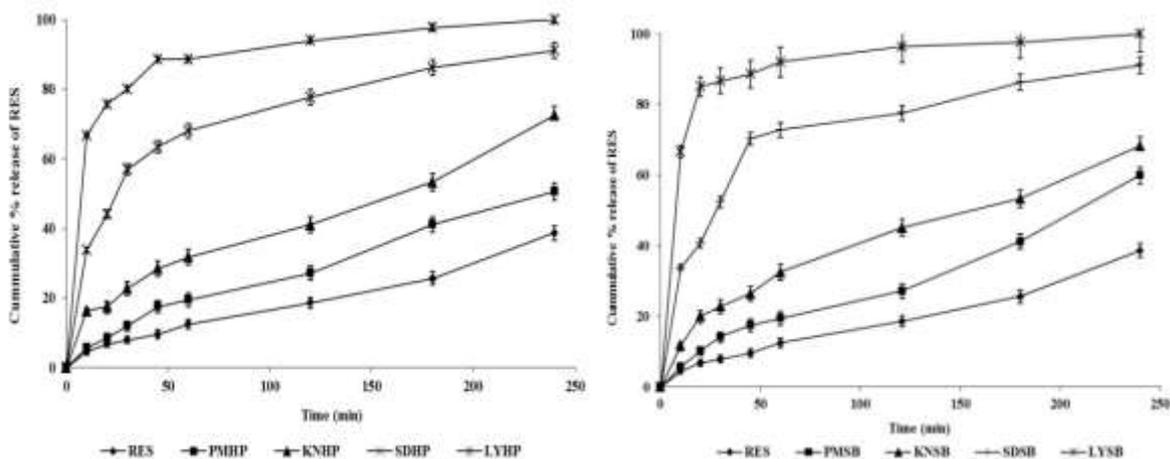


FIGURE 7: *In vitro* dissolution profiles of RES, its Physical Mixture & Complexes with HP  $\beta$ -CD and SB  $\beta$ -CD in distilled water (n=3)

The dissolution rate of RES from PM was higher (50 – 60 % in water) than that of pure RES (39.0%) within 4 hrs. PM of RES with HP $\beta$ -CD and SB $\beta$ -CD brings the drug in close contact with 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.

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.<sup>[15]</sup> The release profile of LY is highly different from pure RES ( $f_2$  values 7.40). Even release profiles of pure RES from KN, SD and LY are also significantly different from pure RES in dissolution medium (Table 2(a) & (b)).

**TABLE 2 (a): Similarity Factor ( $f_2$ ) for Release Profiles for *in-vitro* dissolution profiles of RES, its PM & Complexes with HP $\beta$ -CD**

Sample	RES	PMHP	KNHP	SDHP	LYHP
RES	--	53.08	33.57	14.77	7.40
PMHP	--	--	43.87	18.37	9.70
KNHP	--	--	--	25.74	14.24
SDHP	--	--	--	--	32.10
LYHP	--	--	--	--	--

**TABLE 2 (b): Similarity Factor ( $f_2$ ) for Release Profiles for *in-vitro* dissolution profiles of RES, its PM & Complexes with SB $\beta$ -CD**

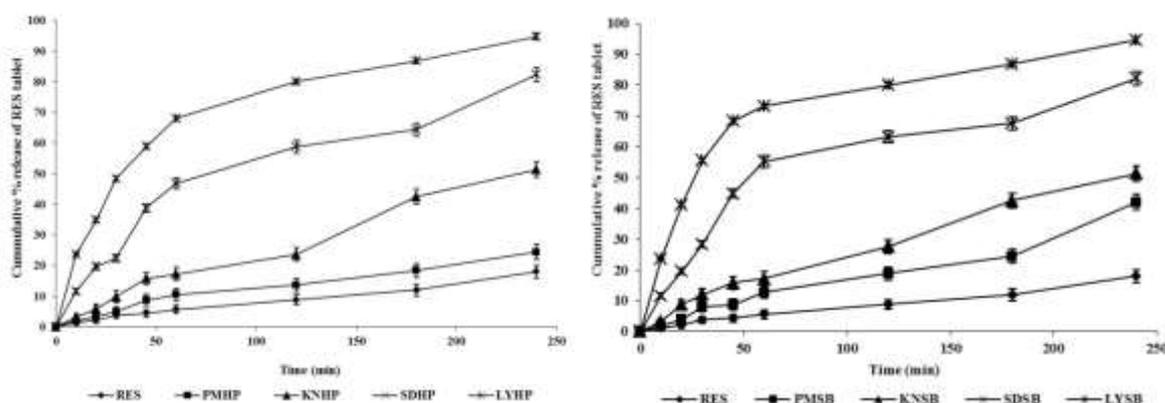
Sample	RES	PMSB	KNSB	SDSB	LYSB
RES	--	58.22	38.02	13.59	6.60
PMSB	--	--	47.96	16.02	7.91
KNSB	--	--	--	21.23	12.21
SDSB	--	--	--	--	30.77
LYSB	--	--	--	--	--

### Formulation Studies

The complexes prepared by kneading, spray drying and lyophilization method (KN, SD and LY) were studied for 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.<sup>[28]</sup>

During *in vitro* dissolution studies, complexes of RES with HP $\beta$ -CD and SB $\beta$ -CD exhibited more than 50% drug release within 25 to 30 min in water, whereas tablets prepared by compressing PM, KN, SD and LY provided same drug release within 80 to 100 minutes.

The tablets prepared using complexes showed faster and reproducible release as compared to the tablets containing pure RES. Tablets prepared using SD and LY with HP $\beta$ -CD showed 82.32% and 94.7 % release in 4 hours in water (Figure 8). Tablets prepared using KN also showed improvement in dissolution profiles of RES. This confirmed the advantage of improved aqueous solubility of RES in its complex form, which can be formulated as tablets with better dissolution characteristic. Release profiles of RES from conventional tablets containing RES alone are significantly different from tablets containing KN, SD and LY.



**FIGURE 8: Release profiles RES from conventional tablets containing only RES and tablets containing PM, KN, SD and LY with HP  $\beta$ -CD and SB  $\beta$ -CD in distilled water (n = 3)**

### CONCLUSION

There was a significant, linear increase in the aqueous solubility of Reserpine with increasing concentration of HP $\beta$ -CD and SB $\beta$ -CD. Maximum studied concentration of HP $\beta$ -CD and SB $\beta$ -CD (200 mM/L) resulted in 87.4-fold and 109.7-fold improvement in the saturation solubility of Reserpine at 37°C. An inclusion complex of

Reserpine and HP $\beta$ -CD and SB $\beta$ -CD in a molar ratio of 2:1 was prepared successfully by kneading, spray drying and lyophilization method. The prepared complexes and physical mixture of Reserpine and HP $\beta$ -CD and SB $\beta$ -CD were characterized by FTIR, DSC, and SEM analysis. When compared to the pure drug, the dissolution profile of the Reserpine/HP $\beta$ -CD and Reserpine/SB $\beta$ -CD

complex is dramatically improved, which proved its suitability to develop an oral form. Inclusion complex prepared by lyophilization method showed highest improvement in *in-vitro* drug release which may be due to presence of entrapped drug inside the CDs cavity and absence of untrapped drug which was also well characterized by DSC, FTIR, and SEM studies. The *in-vitro* drug release of the physical mixture improved too but to a lesser extent compared to complexes prepared by kneading and physical mixing method. Tablet prepared using complex prepared by lyophilization method showed highest dissolution profile compared to tablets prepared using complexes of Reserpine prepared by spray drying, kneading, physical mixing method and without HP $\beta$ -CD and SB $\beta$ -CD. These findings suggested that the draw back of poor dissolution profile of Reserpine can be overcome by preparing its inclusion complex with cyclodextrins.

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