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RESEARCH ARTICLE

Solid-State Characterization and Dissolution Properties of Reserpine – Hydroxypropyl-β-Cyclodextrin Inclusion Complex

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ABSTRACT

The objectives of this research were to prepare and characterize inclusion complex of Reserpine with hydroxypropyl-β-cyclodextrin and to study the effect of complex on the dissolution rate of Reserpine, a water insoluble drug. Phase-solubility profile indicated that the solubility of Reserpine was significantly increased in the presence of hydroxypropyl-β-cyclodextrin and was classified as A_L-type, indicating the 1:1 stoichiometric inclusion complexes. Gibbs free energy ($\Delta G_{tr}°$) values were all negative at different concentration of hydroxypropyl-β-cyclodextrin, indicating the spontaneous nature of Reserpine solubilization, and they decreased with increase in the β-cyclodextrin concentration, demonstrating that the reaction conditions became more favorable as the concentration of hydroxypropyl-β-cyclodextrin increased. The equimolar inclusion complex of Reserpine and hydroxypropyl-β-cyclodextrin was prepared by various methods such as kneading, coevaporation and physical mixing. The molecular behaviors of drug in all samples were characterized by Fourier-transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC), and powder X-ray diffraction (PXRD) patterns, SEM. The results of these studies indicated that complex prepared kneading and co-evaporation methods exhibited the amorphousness as well as the successful inclusion of the Reserpine molecule into the cyclodextrin cavity. The complexation resulted in a marked improvement in the solubility 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 and physical mixture and from pure Reserpine.

KEYWORDS

Reserpine, Hydroxypropyl-β-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.

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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[1]. The mechanism of reserpine's toxic effects is similar to the mechanism of it's

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[2,3].

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[4].

Cyclodextrin (CD) is a cyclic (α-1, 4)-linked oligosaccharide built up of α-D-gluco-pyranose units as shown in Fig 1. Hydroxypropyl-β-cyclodextrin (HPβ-CD) is more water-soluble than the parent molecule and has hydroxypropylester groups attached to the hydroxyl groups in position 2. The molecule has a cone-alike configuration, with a hydrophilic surface and a lipophilic cavity, where hydrophobic interactions to lipophilic molecules without formation of any covalent bonds can cause formations of so-called inclusion-complexes, increasing the water-solubility and stability of the drug-substance[5-7]. 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[8]. Inclusion complex of Rofecoxib/ HPβ-CD (1:1 molar ratio) has been prepared by Baboota et al using kneading method with a subsequent improvement in dissolution due to amorphization[9]. Many other drugs such as ganciclovir, nimesulide, itraconazole, tolbutamide, etc. have been tested for CD inclusion to enhance solubility[10-13].

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. 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[14].

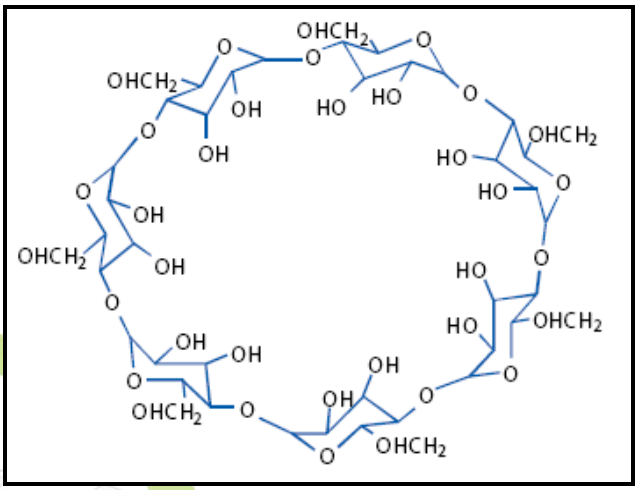

Figure 1: Structure formula for the β-Cyclodextrin molecule

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[15]. 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 (EMEA, 1999) as a criterion for the assessment of similarity between two dissolution profiles[16,17]. 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[15]. 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[18]. It is accurate expression for drug release rate. A higher MDT value indicates greater drug retarding ability[19].

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

MATERIAL AND METHODS

Materials

Reserpine (RES) was purchased from Chemdye Corp Chemco, Gujarat. HPβ-CD, Sodium acetate and mannitol was procured from Divya scientific Ltd, Ahmedabad, Gujarat. Potassium bromide (IR grade) were procured from Jay Chemicals, Gujarat. 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). 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[20]. RES, in amounts that exceeded its solubility, were transferred to screw capped vials containing 25 ml of aqueous solution of HPβ-CD (molecular weight = 1500) in various molar concentrations (0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0 and 14.0 mM/L). The contents were stirred on electromagnetic stirrer (Remi, India) for 36 h at 37°C±0.1°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 μm membrane filter, suitably diluted and analyzed spectrophotometrically for drug content at the wavelength of 268 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 2:1 stoichiometric ratio of complexes, was calculated from the phase-solubility diagrams using the following equation.

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

Where slope is obtained from the initial straight-line portion of the plot of RES concentration against HPβ-CD concentration, and so is the equilibrium solubility of RES in water.

Preparation of Inclusion Complexes

Complex of HPβ-CD and RES were prepared in the molar ratio of 2:1 (on the basis of phase solubility study) by different methods like physical mixture, kneading, spray drying and lyophilization. For ease in discussion, these samples will be designated as physical mixture (PM), kneading (KN), spray drying (SP) and lyophilization (LY) throughout the manuscript.

Physical Mixture

PM of HPβ-CD and RES was prepared by simply mixing powders with a spatula for 15 minutes.

Kneading Method

The required quantities of HPβ-CD 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°C for 24 hours. The dried complex was pulverized and then sieved through 120 #.

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, Shimazdu-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, HPβ-CD, PM, KN, SP and LY 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, HPβ-CD, PM, KN, SP and LY 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°C to 300°C, at a scanning rate of 10°C /min under dry nitrogen flow (100 ml/min).

Scanning Electron Microscopy (SEM)

The surface morphology of free powdered samples of RES, HPβ-CD, PM, KN, SP and LY 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, HPβ-CD, PM, KN, SP and LY 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(A), PM(B), KN(C), SP(D) and LY(E) 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 ± 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 230 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(A), PM(B), KN(C), SP(D) and LY(E)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 PM, KN, SP and LY. Preliminary tests demonstrated that there was no change in the λmax of RES due to the presence of HPβ-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 λmax 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^2 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[15] 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[15]

$$f_2 = 50X\log\left\{\left[1+\left(\frac{1}{n}\right)\sum_{t=1}^{n} w_t\left(R_t - T_t\right)^2\right]^{-0.5} X100\right\}$$

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[15].

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[21,22].

The solubility of RES in water at 25°C is 0.4 µg/ml; therefore, RES can be considered to be water insoluble drugs. The phase solubility diagram of RES in the presence of HPβ-CD was obtained by plotting the apparent equilibrium concentration of RES against various molar concentrations of HPβ-CD (Fig 2). This representation gives direct information about the complexation efficiency. 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 at 37°C & 72.3-fold at 25°C at 200 mM/L concentration of HPβ-CD, respectively. Increased solubility may be due to improved dissolution of RES particles in water by HPβ-CD.

Stoichiometric ratio at which optimum complexation occurs was confirmed by phase solubility analysis. The phase solubility plot was A_P type for HPβ-CD, which indicated that 2:1 (HPβ-CD – RES) inclusion complex was formed in solution. The values of apparent stability constants (Ks) for the complexes at 25ºC and 37°C, assuming a 2:1 stoichiometry, calculated from the slope of the initial straight portion of the phase solubility diagram were 323.78 M^{-1} at 25ºC and 491.22 M^{-1} at 37ºC for HPβ-CD:RES which indicated a suitable and stable complex formation. It is reported that cyclodextrin-drug complexes with the values of Ks in the range of 200 to 5000 M^{-1} show improved dissolution properties and hence better bioavailability[20].

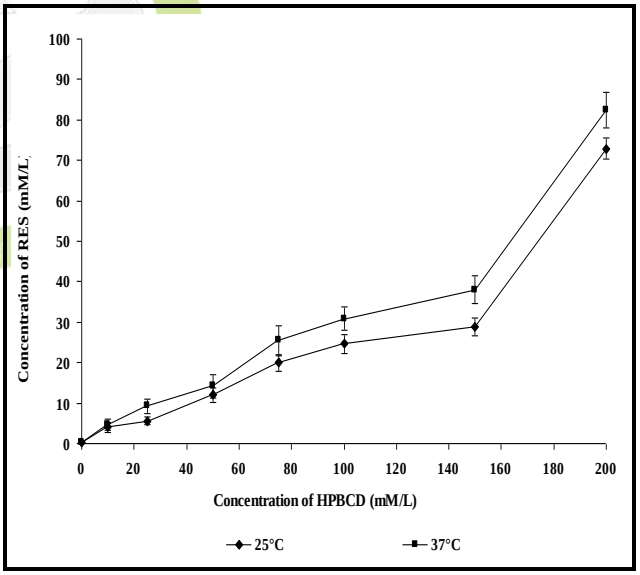

Figure 2: Phase solubility curve of RES in aqueous solution of HPβ-CD at 25°C and 37°C

The values of Gibbs free energy change ($\Delta G_{tr}°$) were calculated to understand process of transfer of RES from pure water to aqueous solution of HPβ-CD. The values of $\Delta G_{tr}°$ of RES from pure water to aqueous solutions with different concentrations of HPβ-CD at 37°C were calculated using equation[23]

Table 1: Thermodynamic parameters for solubilization process of RES in aqueous solutions of HPβ-CD at 25°C and 37°C (Mean ± SD, n = 3)

Concentration (mM/L)	ΔG(KJmol^{-1})		ΔH (KJmol^{-1})	ΔS(Jmol^{-1}k^{-1})
	25°C	37°C		
10	-4.12±0.03	-5.12±0.04	-7.17±0.14	-0.0055±0.0003
25	-5.08±0.04	-6.46±0.08	-9.27±0.15	-0.0062±0.0005
50	-6.73±0.10	-7.55±0.12	-11.44±0.26	-0.0071±0.0004
75	-7.97±0.11	-8.13±0.13	-12.87±0.31	-0.0087±0.0004
100	-9.11±0.14	-9.11±0.19	-14.07±0.34	-0.0090±0.0005
150	-10.07±0.16	-11.37±0.24	-15.34±0.42	-0.0095±0.0006
200	-11.21±0.22	-12.23±0.29	-18.29±0.43	-0.0104±0.0006

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

Where So/Ss = the ratio of molar solubility of RES in aqueous solution of HPβ-CD to that of the pure water. The obtained values of Gibbs free energy are shown in Table I. The $\Delta G_{tr}°$ 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. $\Delta G_{tr}°$ values were all negative for HPβ-CD at various concentrations, indicating the spontaneous nature of RES solubilization. Furthermore, these values decreased with increased concentration of HPβ-CD, demonstrating that the reaction became more favorable as the concentration of HPβ-CD increased.

The enthalpy of transfer ($\Delta H_t°$) and entropy (ΔS) can be calculated from a modification of the van't Hoff equation[24]:

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

Rearranging and solving for $\Delta H_t°$ yields

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

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

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

Usually complex formation with CD results in a relatively large negative ΔH and ΔS, which can be either positive or negative. Negative ΔH 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 ΔS 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[24].

Drug Content

The drug content of the PM, KN, SP and LY were found out to be 91% (±12.27), 96.03% (±7.48), 98.12% (±6.09) and 99.22% (±4.56)

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, SP and LY. This may be due to insufficient mixing which may be due to simple mixing of powders without applying pressure (HPβ-CD and RES). Final moisture content of the pure drug, PM, SP and LY was 2.13%, 1.59%, 0.78% and 0.24%, 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)[25,26]. The thermograms for pure RES, HPβ-CD, PM, KN, SP and LY 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 thermogram of the HPβ-CD 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.

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 SP 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.

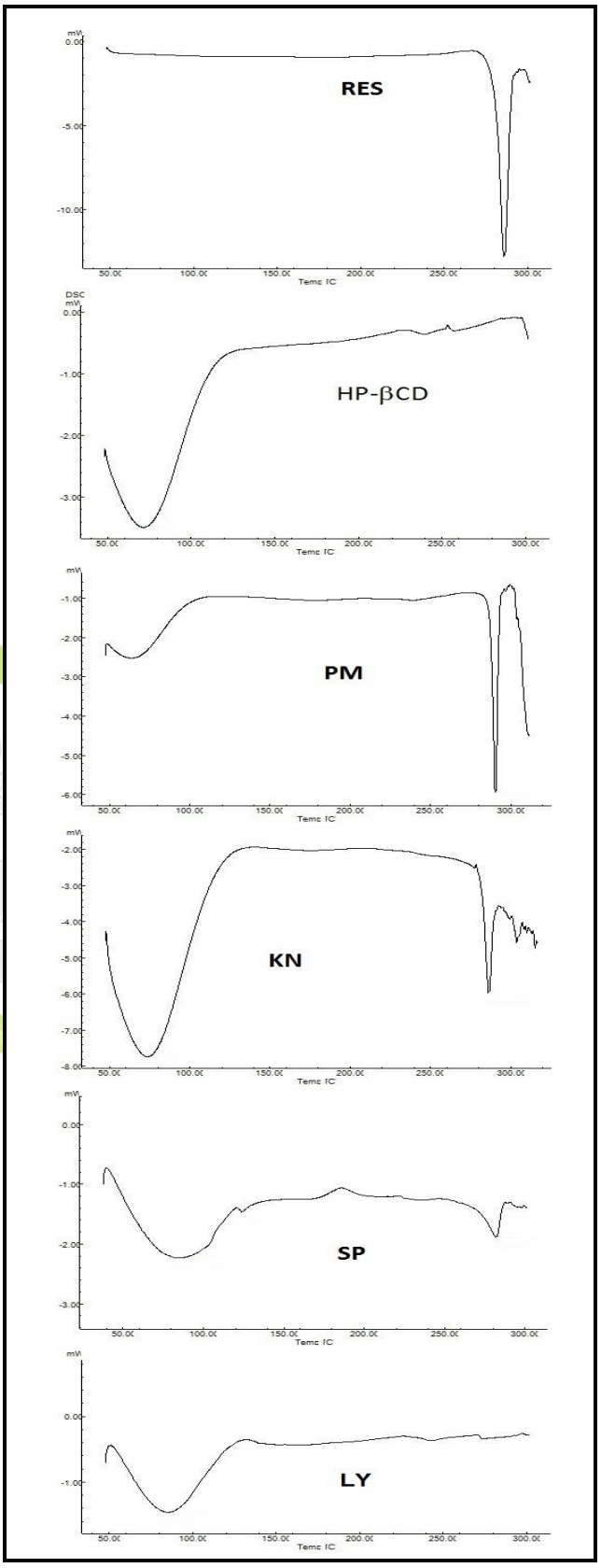

Figure 3: DSC thermograms RES (A), HPβ-CD (B), PM (C), SP (D) & LY (E)

Infrared (IR) Spectroscopic Analysis

Fourier transform infrared spectroscopy (FTIR) 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[27].

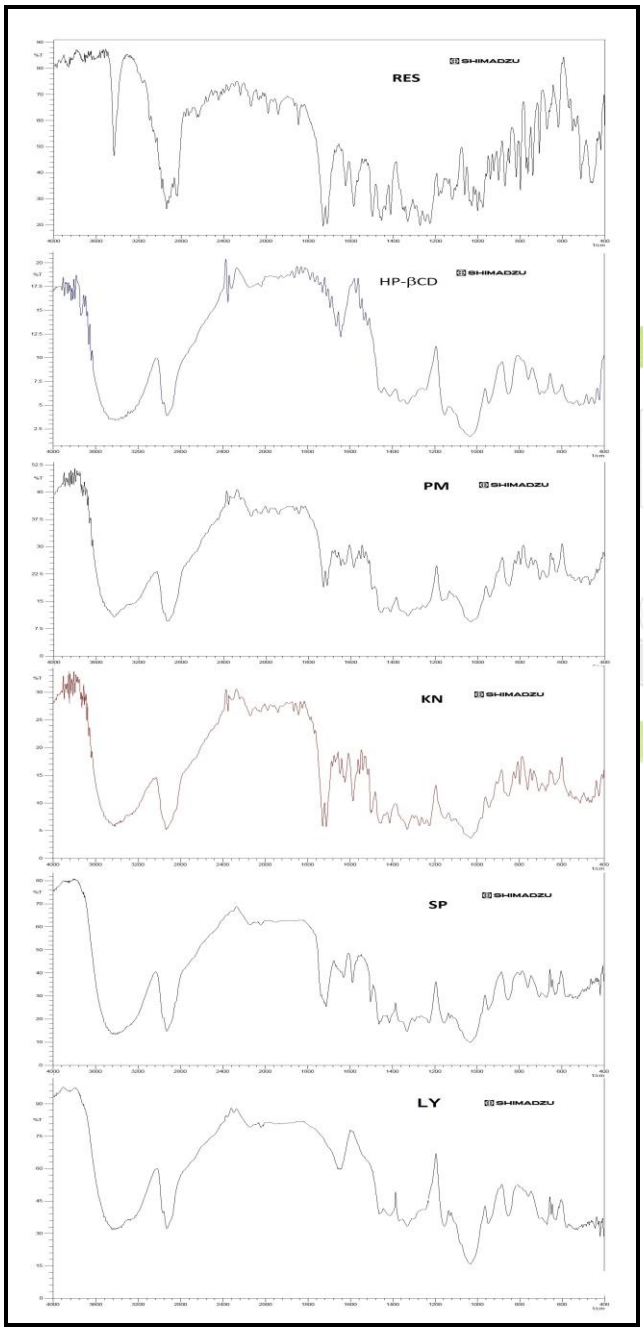

Figure 4: FTIR Spectrograms of RES (A), HPβ-CD (B), PM (C), SP (D) & LY (E)

The IR spectras of PM, KN, SP and LY were compared with spectrum of HPβ-CD and RES (Figure 4). The FTIR spectras of PM, KN, SP and LY showed no peaks other than those of CDs and RES. These results indicated aromatic ring with free H included in the CDs cavity whereas remaining part of RES oriented toward the upper exterior part of CDs cavity. Moreover, The FTIR spectras of PM, KN, SP and LY 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, HPβ-CD, PM, KN, SP and LY are reported in Figs. 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.

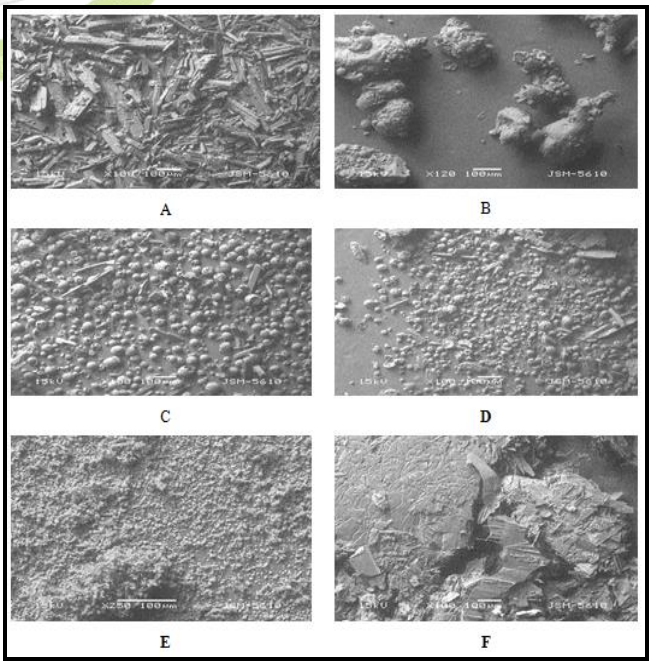

Figure 5: SEM of RES (A), HPβ-CD (B), PM (C), SP (D) and LY (E)

In SP 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.

Wettability and Dissolution Studies

The improvement in wettability of RES by physical mixing and complexation with HPβ-CD is presented in Figure 6. SP and LY with HPβ-CD showed highest wettability in water (57.1% and 68.9%, respectively), as compared to plain RES (18.9%) at 45 min. Even PM of RES with HPβ-CD enhanced wettability of RES in water significantly as compared to plain RES. Thus, the results of wettability studies indicated that HPβ-CD improved wettability of RES in water both in complex as well as in PM form due to its hydrophilicity.

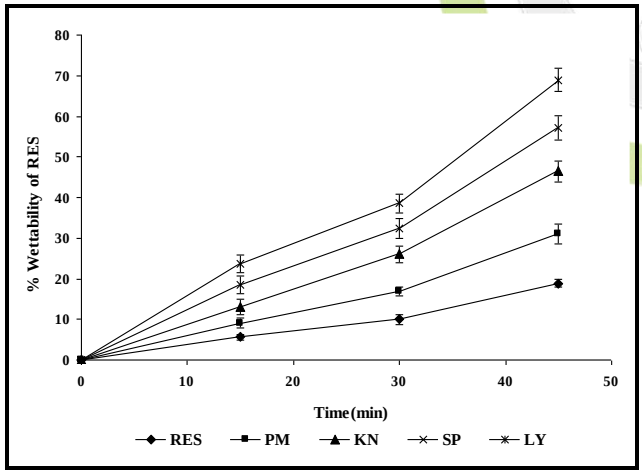

Figure 6: Wettability study of pure RES, HPβ-CD, PM, SP and LY in distill water (n=3)

Dissolution of pure RES and all other prepared systems (complexes and physical mixture) was carried out in distill 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 LY 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 HPβ-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, SP and LY significantly enhanced dissolution rate of RES significantly (65-100% in within 4 hrs). Possible mechanisms of improved dissolution rates of complexes include[14] 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.

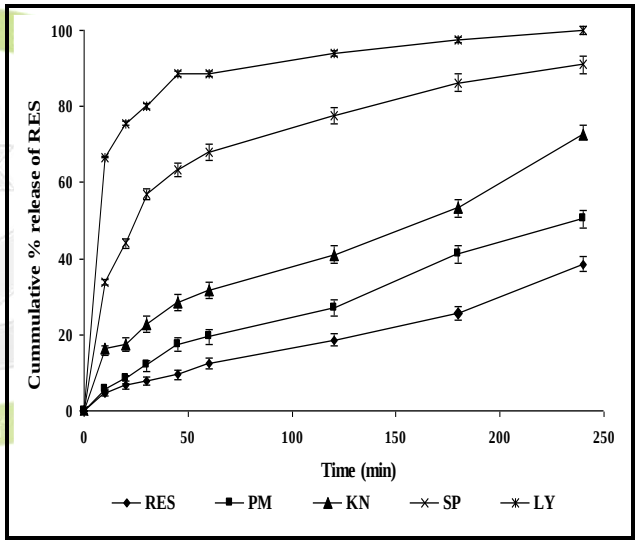

Figure 7: *In vitro* dissolution profiles of pure RES, and its physical mixture and complexes distill water (n=3)

The dissolution rate of RES from PM was higher (50 – 60 % in water) than that of pure RES (39.0%) within 4 hrs. Physical mixing of RES with HPβ-CD brings the drug in close contact CD. The increased dissolution rate observed in case of PM can be attributed to several factors such as a solubilization effect of HPβ-CD, 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 (15). The release profile of LY is highly different from pure RES (f_2 values 7.40). Even release profiles of pure RES from KN, SP and LY are also significantly different from pure RES in dissolution medium (Table 2).

Table 2: Similarity Factor (f_2) for Release Profiles of Reserpine

Sample	RES	PM	KN	SP	LY
RES	--	53.08	33.57	14.77	7.40
PM	--	--	43.87	18.37	9.70
KN	--	--	--	25.74	14.24
SP	--	--	--	--	32.10
LY	--	--	--	--	--

Formulation Studies

The complexes prepared by kneading, spray drying and lyophilization method (KN, SP 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[28].

During *in vitro* dissolution studies, complexes of RES with HPβ-CD exhibited more than 50% drug release within 25 to 30 min in water, whereas tablets prepared by compressing PM, KN and SP 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 SP and LY with HPβ-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, SP and LY.

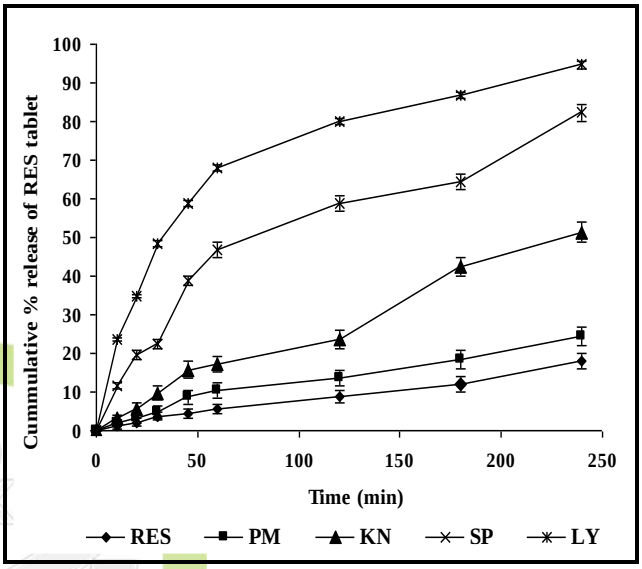

Figure 8: Comparative drug release profiles of conventional tablets containing RES and tablets containing PM and complexes of RES (n=3)

CONCLUSION

There was a significant, linear increase in the aqueous solubility of Reserpine with increasing concentration of HPβ-CD. Maximum studied concentration of HPβ-CD (200 mM/L) resulted in 87.4-fold improvement in the saturation solubility of Reserpine at 37°C. An inclusion complex of Reserpine and HPβ-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 HPβ-CD were characterized by FTIR, DSC, and SEM analysis. When compared to the pure drug, the dissolution profile of the Reserpine/HPβ-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 HPβ-CD cavity and absence of unentrapped 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 HPβ-CD. These findings suggested that the drawback of poor dissolution profile of Reserpine can be overcome by preparing its inclusion complex with HPβ-CD.

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