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An Investigation on Enhancement of Solubility of 5 Fluorouracil by Applying Complexation Technique- Characterization, Dissolution and Molecular-Modeling Studies

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ABSTRACT

The drug 5 fluorouracil is sparingly soluble in water. The aqueous solubility and dissolution rate of 5-fluorouracil can be increased by inclusion complexation with β-cyclodextrin. Molecular-modeling studies support the formation of stable molecular inclusion complexation of 5-fluorouracil with β-cyclodextrin monomer (1:1). Complexes were prepared by physical mixture, kneading, co evaporation and freeze drying methods. Two ratios 1:1 and 1:2 were formulated. These eight complexes were subjected to Phase-solubility study, molecular modeling and dissolution study. The complexes formed were confirmed by DSC studies. Phase solubility profile indicated that the solubility of 5-fluorouracil increased in the presence of β-cyclodextrin monomer. Results obtained by different characterization techniques clearly indicate that the freeze-drying method leads to formation of solid state complexes between 5-fluorouracil and β-cyclodextrin. The complexation of 5-fluorouracil with β-cyclodextrin lends an ample credence for better therapeutic efficacy.

INTRODUCTION

One of the major antimetabolites used in a variety of solid cancer such as stomach colon lung and breast cancer is fluorouracil (FU) (Parker et al., 1990). It is usually given intravenously, as absorption of FU from the gastro intestinal is erractic and unpredictable (Ardalan et al., 1981). FU is sparingly soluble in water. Peak plasma concentration ranged from 2-25 µ gm/ml with an elimination half life of 10 to 30 minutes following the intravenous bolus injection of fluorouracil in cancer patient (Wang et al., 1999), and Ferguson et al., 1999). Doses ranged from 9to16 mg/kg body weight. When the same dose was given by mouth to this patient plasma concentration was below 10µgm/ml and bioavailability ranged from 0to78% but was usually increased markedly if the dose was doubled. Thus it is an important to enhance the solubility and dissolution rate of FU to improve erratic and oral bioavailability to reduce the dose and the systematic side effects. The solubility of the poorly soluble drug can be altered in many ways such as modification of drug crystal form, addition of co-solvents, addition of surfactants, addition of cyclodextrin (CD) (Sangalli et al., 2001, Rajewski et al., 1996, and Londhe et al., 1999) etc. Among the possibilities the cyclodextrin approach is of particular interest. Cyclodextrin are cyclic (α-1, 4) linked oligosaccharides of α-D glycopyranose, containing relatively hydrophobic central cavity and hydrophilic outer surface. When cyclodextrin are used to solubilize water insoluble drugs, it is generally assumed that the solubilization proceeds through inclusion complex formation. In this study investigation were performed on the possibility of complexation of FU with β CD for improving the solubility and dissolution rate, thereby increasing the bioavailability and therapeutic efficacy of FU. The complexes were prepared by using different techniques like physical mixture, kneading, co evaporation and freeze drying at stoichiometric ratios.

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In vitro aqueous solubility and dissolution rate profile of the complexes were performed. Selective physiochemical determination based on differential scanning calorimetric (DSC) and molecular modeling was used to characterize the complexes.

MATERIALS AND METHOD

Fluorouracil was a generous gift from Bio-chem Pharmaceuticals ltd. β cyclodextrin was purchased from Sigma chemical co. (st. Louis,); both were used as received with no further purification. All other reagents and chemicals were of analytical grade.

Phase solubility study

FU, in amounts that exceeded its solubility, was taken into vials to which were added 15ml of water ($_{P}$H 6.8) containing various concentration of β cyclodextrin (3-15mμ). These flask were sealed and shaken at 20ºc for 5 days, the aliquots were withdrawn, using a syringe at 1hr interval and samples were filtered immediately through a 0.45μ nylon disc filter and approximately diluted. A portion of sample was analyzed by UV spectrophotometer at266nm.

Preparation of Solid Complexes

The preparation of solid complexes of FU and β Cd were performed by different technique (Bettinetti et al., 1992, Becket et al., 1999, and Blanchard et al., 2000) like physical mixture, kneading, co evaporation and freeze drying which are described below in detail. The molar ratio was kept as 1:1 and 1:2. Eight formulations were prepared.

Physical mixture was prepared by homogeneous blending of previously sieved and weighed FU and β CD in a mortar. In Kneading method β CD and distilled water were mixed together in a mortar so as to obtain a homogeneous paste. FU was then added slowly while grinding. The mixture was added to the mixture in order to obtain a suitable consistency. The paste was dried in oven at 40°c for 24 hours. The dried complex was pulverized into a fine powder. In Freeze drying method**, t**he required quantity of FU was added to aqueous solution of β cyclodextrin while mixing with a magnetic stirrer after 24hrs of agitation resulting solution was lyophilized in a freeze dryer for 24hrs. In Co evaporation method, after dissolution of β cyclodextrin in water the molar proportion of FU was added. This suspension was further kept under stirring for 24hrs.The obtained clear solution was evaporated under vacuum at a temperature of 45°c and 100 rpm in rotary evaporator. The solid residue was further dried completely at40°c for 48hrs.

Drug Excipients Compatibility Study by I.R Spectroscopy

Potassium bromide discs were prepared by pressing the 5fluoro uracil along with excipients and the spectra between 4000^{-1}cm -500^{-1}cm was obtained under the operational conditions. The absorption maxima in spectrum obtained with the substance being examined correspond in position and relative intensity to those in the reference spectrum represented.

Differential Scanning Calorimetry (DSC)

The DSC measurements were performed (Nicolazzi et al., 2002, and Uekama et al., 1998) using mettler Toledo DSC 821 DSC model, controlled by STAR software (mettler Toledo Gmbttswitzerland). All accurately wide samples were placed in sealed aluminium pans, if before heating under nitrogen flow (20ml/min) at a scanning rate of 10^{8} C min^{-1}over the temperature range of 30°c to 320°c. An empty aluminium pan was used as a reference.

Dissolution rate studies

The dissolution behavior (Croyle et al., 2001, Funasaki et al., 1999, Quaglia et al., 2001, and Dhanaraju et al., 1998) of the FU-β CD complexes were compared with pure FU. The dissolution rate studies were performed according to USP XXII rotating basket method.

The samples corresponding to 100mg of FU were placed in to hard gelatin capsules. The dissolution medium was 90oml of SGF (simulated gastric fluid)without enzymes. The stirring speed was 100rpm. The temperature was maintained at 37°C ± 1°C. The samples were withdrawn at various time intervals using a pipette filtered through 0.45μm nylon disc filter and analysed by UV spectrophotometer at 260nm.

Molecular modeling studies

To fit the 5 FU into the cavity of CD, montecarlo docking simulations were perform with refined structure. One of the low energy structures of the docking simulations of each host guest complex was subjected to molecular-dynamics (MD) simulations.

MD simulations were performed in vacuo. The MD calculations were done using the velocities verlet algorithm at constant volume with cell multiple method, for the calculations of non bonded interactions. The system was equilibrated for 100ps and the production run was done for 250ps with a time step of 1fs.

RESULTS AND DISCUSSION

Phase-Solubility Study

The phase solubility diagram for the complex formation between 5-fluorouracil and β-cyclodextrin is presented in Figure 1. This plot shows that the aqueous solubility of the drug increases linearly as a function of β-cyclodextrin concentration. It is clearly observed that the solubility diagram of 5-fluorouracil in the presence of β-cyclodextrin can be classified as the B type curve.

The host guest correlation with slope of less than 1 (0.19363) suggested the formation of a 1:1 (5-fluorouracil-β-cyclodextrin) complex with respect to β-cyclodextrin concentrations. Generally B type phase solubility behavior is typical for natural β-cyclodextrin, since the drug / CD complex is more soluble than the free drug itself but the solubility limit of the drug/CD complex is reached within the concentration range of the cyclodextrin.

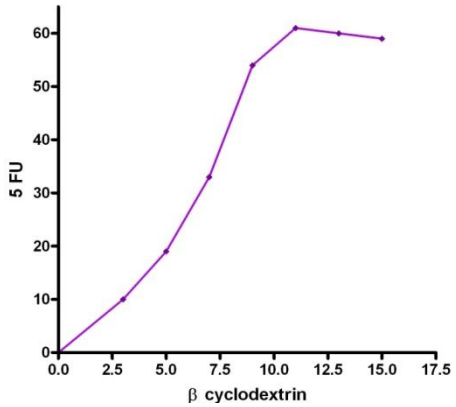

Fig. 1: Phase solubility study of the drug with β-cyclodextrin.

FTIR studies

IR spectra of fluorouracil as such and in the complexes formed by various methods were identical. Principal IR absorption peaks of 5FU at 3124 cm^{-1} (NH Stretch), 1716 cm^{-1} and 1657 cm^{-1} (C=O Stretch), 1245 cm^{-1} (CH in Plane deformation), 813 cm^{-1} (CH out of plane deformation) were all observed in the spectra of 5 FU as well as its complexes. These spectral observations thus indicated that no interaction between 5 FU and β-cyclodextrin were seen in the complex. (Fig 2)

Differential Scanning Calorimetry

The DSC thermograms for the 5-fluorouracil and the corresponding β-cyclodextrin complexes showed that, 5-fluorouracil exhibits a characteristic endothermic fusion peak at 292.46°C; hence no polymorphs of 5-fluorouracil could be found. Furthermore, β-cyclodextrin shows a broad endothermic effect at 118.34°C.

The DSC thermograms for the 5-fluorouracil-β-cyclodextrin systems show the persistence of the endothermic peak of 5-fluorouracil for the physical mixture and the kneaded product. For the freeze-dried and evaporated system, this peak is very small; this result can be explained on the basis of a major interaction between the drug and cyclodextrin. Furthermore, the characteristic endothermic effect of β-cyclodextrin is slightly shifted to higher temperatures for the freeze-dried and evaporated systems, indicating that 5-fluorouracil has complexed with β-cyclodextrin.

Dissolution Rate Studies

The dissolution profiles of 5-fluorouracil alone and the 5-fluorouracil-β-cyclodextrin complexes are reported in Figure 3 and 4. The release rate profiles were drawn as the percentage of drug dissolved vs. time. According to these results, the inclusion complexes released up to 82% of the drug in 15 minutes, and up to 86% after 40 minutes; whereas 5-fluorouracil pure drug exhibited the release of ~22% after 20 minutes and not more than 36% after 60 to 90 minutes. These quantities contrast with the markedly 2.5-fold increase in the release of freeze-dried product. It is also evident that the freeze-dried, evaporated, and kneaded systems exhibit higher dissolution rates than the physical mixture and the pure drug (Table 1). The extent of the enhancement of the dissolution rate was found to be dependent on the preparation method, since the freeze-dried and evaporated products exhibited the highest dissolution rates. The dissolution rate increase reached for the physical and kneaded mixtures is mainly due to the wetting effect of the β-cyclodextrin. In fact, this effect is more evident for the kneaded product, where the mixing process between the 2 components is more intensive. The effect of complexation with β-cyclodextrin on the solubility of 5-fluorouracil can be explained in terms of the reduction in the crystallinity of the drug caused by the freeze-drying process and the inclusion into the hydro-phobic cavity of the β-cyclodextrin. The complexes prepared by kneading technique offer a dissolution rate of approximately 65% in 60-minutes, which may be of particular interest for industrial scale preparations because of the low cost and the simple process, which involves less energy, time, and equipment.

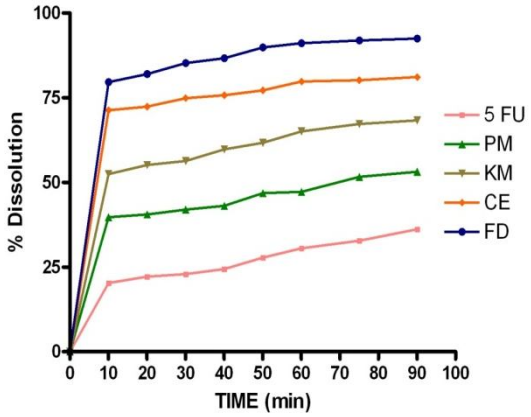

Fig. 3: Dissolution profile of 5-fluorouracil and its complexes (1:1).

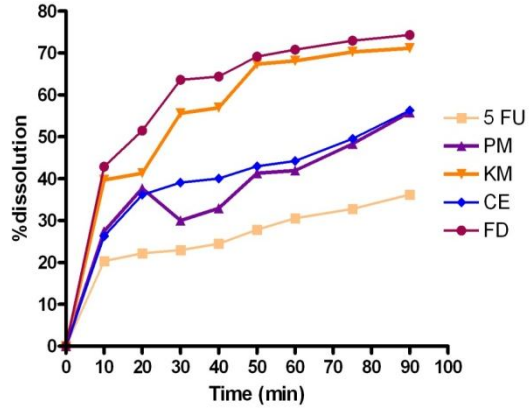

Fig. 4: Dissolution profile of 5-fluorouracil and its complexes (1:2).

Table. 1: Dissolution study of the drug in complexes.

RATIO	METHOD	SOLUBILITY mg/ml
Plain drug	-	12.1
1:2	Physical mixing	14.9
	Freeze drying	16.0
	Kneading	15.4
	Co-evaporation	13.35
1:1	Physical mixing	22.5
	Freeze drying	35.1
	Kneading	30.15
	Co-evaporation	17.24

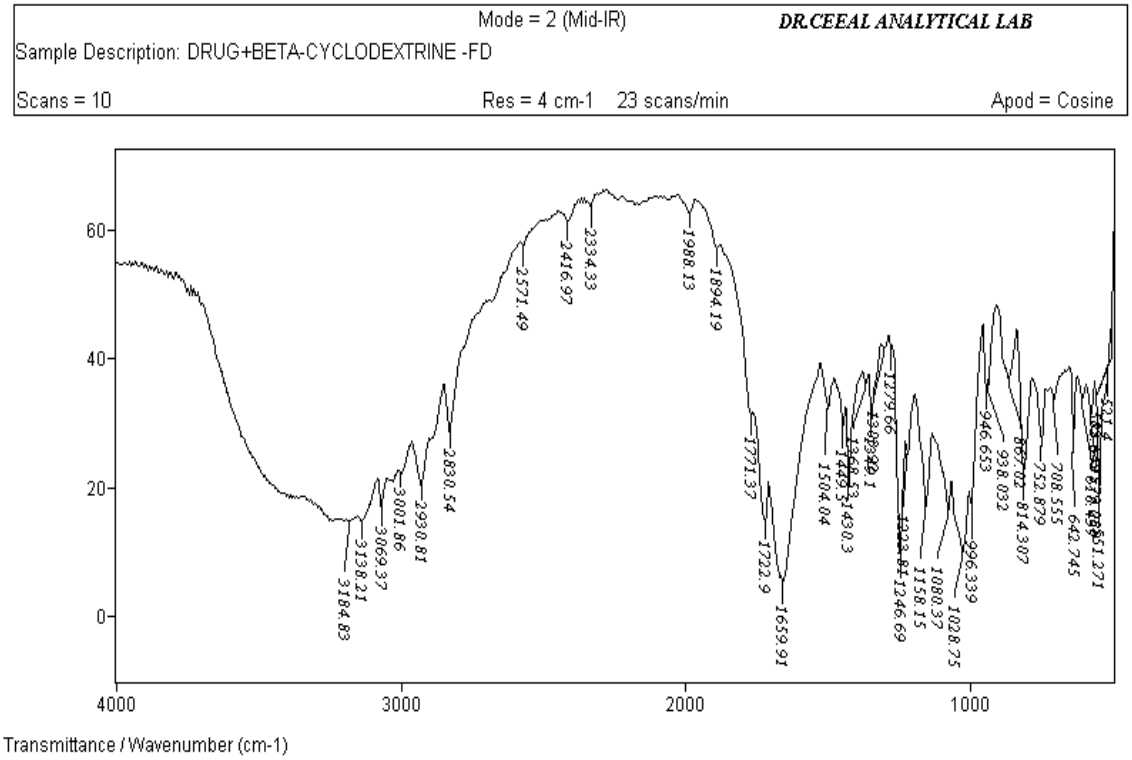

Fig. 2: IR Spectra of complex prepared by freeze drying method.

Molecular-Modeling Studies

Molecular-Modeling Studies show the structures obtained by molecular modeling according to the methods described in the experimental section. The Monte Carlo (MC) simulations showed a general tendency of inclusion complex formation and lowering interaction energy. The interaction energy was defined as the difference between the sum of the energy of individual host and guest molecule and the energy of the inclusion complex. The calculated energy value is -45.4 kcal/mol for structure 1, when 5 FU is docked through the head region of the β-cyclodextrin (ie, through the narrow rim [primary hydroxyl groups]). The energy value is -48.2 kcal/mol when 5 FU is introduced through the tail region of the β-cyclodextrin (ie, by the wider rim [secondary hydroxyl groups]). The structure obtained by molecular modeling showed, that β-cyclodextrin monomer bound 5 FU tightly. The interaction energy for the lowest energy structure showed good agreement with the MC docking simulations. The interaction energies were -57.9 kcal/mol for β-cyclodextrin monomer, -36.5 kcal/mol for structure 1 and -37.2 kcal/mol for structure 2. These results indicate the relative energetic stability of the β-cyclodextrin-5 FU as in the case of MC docking simulations. A possible molecular arrangement for the inclusion compound is that the molecule 5 FU is buried in the cavity of the β-cyclodextrin monomer. It is being held in position due to the formation of hydrogen bonds between the hydroxyl groups of the β-cyclodextrin and the fluorine of the 5 FU. The contribution due to the electrostatic interactions is very small (ie, -0.45 kcal/mol.

From the results it is clearly evident that a molar ratio of 1:1 (monomer) is suitable for β-cyclodextrin complexation of 5 fluorouracil. Results obtained by different characterization techniques clearly indicate that the freeze-drying method in the ratio of 1:1 leads to formation of solid state complexes between 5-fluorouracil and β-cyclodextrin.

CONCLUSION

The aqueous solubility and dissolution rate of fluorouracil were markedly enhanced by complexation with β cyclodextrin. The phase solubility diagram of FU-βCD was of B Type and the increase in solubility was due to the formation of 1:1 M complex. The complexes formed are adequately stable confirmed by DSC studies and Molecular modeling studies. Solid inclusion complexes of FU-βCD exhibited higher rates of dissolution than the plain FU. A 2.5 fold increase in the dissolution rate of FU was observed with FU-βCD (1:1) inclusion complexes prepared by freeze drying technique.

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