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Research Article

PREFORMULATION STUDY OF THE INCLUSION COMPLEX CANDESARTAN CILEXETIL - HPβCD AND ITS COMPARISION WITH βCD

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INTRODUCTION

Candesartan cilexetil belongs to the class Angitoensin II Receptor antagonist which is used in the treatment of hypertension. Candesartan cilexetil is soluble in ethanol, methanol or DMSO. Candesartan cilexetil is included in BCS Class II which is practically insoluble in water and has partition coefficient of 9.8 at pH 7.4 (According to BCS classification developed by Amidon in conjugation with FDA guidance) [1]. This poor aqueous solubility of drug leads to difficulties in the pharmaceutical formulation. To overcome this drawback, there are many strategies to enhance the solubility in which complexation is one of the techniques which utilizes complexing agent HPβCD and its comparison with the βCD[2].

Cyclodextirns are macrocyclic oligosaccharides composed of glucopyranoside units in the $^4C_1$ conformation. The average structure is truncated cone with a cavity lined with H3 and H5 protons and lone pair of glycosidic oxygen atoms in the plane thus endowing hydrophobic character, while the bases formed by the primary and secondary OH groups bestow a hydrophilic character[3]. In β CD the second hydroxyl group is substituted with groups like alkyl, hydroxyl alkyl, amine etc., the aim of substitution of hydroxy propyl group at second position is to improve solubility and to improve fitting. Hydroxylpropyl β Cyclodextrin a hydroxyl derivative with imporved water solubility than Cyclodextrins**[4]**. HPβCD are prepared by alkylation of βCD with propylene oxide in low alkaline concentration which favors alkylation at O-2[5]. The complex forming ability of HP β CD is highly influence by the degree of substitution (DS), both size of the cavity and the reactivity of CDS are altered when hydroxyl groups are substituted. On the other hand the guests themselves influence these interactions by their size and configuration[6]

Candesartan cilexet has a bioavailability of 15%. It is weakly acidic nature[7]. To change the behavior of drug from BCS II to BCS class I inclusion complexation was opted with complexing agent HP β CD. The objectives of the present study are: To characterize the inclusion complexation of Candesartan cilexetil in the liquid state: phase solubility studies of Candesartan cilexetil with hydroxy propyl β-cyclodextrin and compare with the βCD and calculate the stability rate constants of the complexes, to prepare various of complexes with three different methods such as Dry mixing method (physical mixtures), Slurry or paste formation (kneading method), Co-evaporation method and the formation of complexes in the solid state was confirmed by Melting point, FTIR, X-ray diffraction analysis and finally to study dissolution improvement of Candesartan cilexetil with cyclodextrins: The dissolution studies were conducted according to USP guidelines.

MATERIALS

Candesartan cilexetil (MW: 610.67gms/mole) is a gift sample provided by Dr.Reddy's Lab, Hyd. HP-β-CD was supplied by Gangwal Chemicals Pvt. Ltd. Mumbai. All the chemicals and solvents used in this study are of Analytical grade. All the solutions are prepared using Millipore water.

Methods

Preparations of solid binary mixture[8]

Candesartan cilexetil – HP-β-CD binary mixture are prepared in 1 molar ratio (0.2:0.8, 0.4:0.6, 0.5:0.5, 0.6:0.4, and 0.8:0.2) as described below.

Physical mixture

Physical mixture method is simplest method. The calculated and exactly amount of Candesartan cilexetil and HP-β-CD were prepared by simply pulverizing and mixing for 15 min.

Kneading method: To HPβCD small quantity of water is added in a mortar and mixed it until a uniform paste is obtained to this Candesartan cilexetil was slowly added. Kneading is continued with the aid of the ethanol as co solvent until a homogenous paste of the mixture is formed. The slurry was kneaded for 30min and then dried in hot air oven for 40^{o}c for 24 hrs and the dried complex was pulverized in to a fine powder and passed through sieve no 60 and stored in an air tight containers.

Co-evaporation method

Co-evaporated product was obtained by dissolving equimolar amount of HP-β-CD and drug in 100ml of 50% ethanol. The powders were completely dissolved with the aid of ultra sonicator and the solvent was evaporated using a water bath at 45^{o}c. Repeat the same procedure with 150ml of solvent. The obtained solid was ground, sieved through a sieve no. 60 and store in air tight containers.

Phase solubility studies[9]

Phase Solubility studies were performed by preparing samples of 25ml solution of Millipore water with Candesartan cilexetil and HPβ-CD, 25ml of solution of PBS 7.4 with Candesartan cilexetil andHPβ-CD, 25ml of solution of 0.1N HCl with Candesartan cilexetil and HPβ-CD.

Approximately 5mg of drug were added to each 25 ml of solvent in test tube. Increasing amounts of Cyclodextrin (0.3, 0.6, 0.9, 1.2, 1.5 mM) is added to determine the change in the solubility of the Candesartan cilexetil. To form complexes these test tubes were placed in inorbital shaker for 72hrs to attain the equilibrium between the drug and the cyclodextrins. the samples were then filtered using whatman filter, The filtered samples were analyzed by UV spectrophotometer at λ_{max} of 256nm, 270nm, 257nm in Millipore water, 0.1NHCl, Phosphate buffer solution pH 7.4 respectively.

A graph is plotted with x-axis as concentration of HPβ-CD and on y-axis concentration of Candesartan cilexetil. From the graph, slope and the intercept (s_o) are used to calculate the apparent stability constant (K). (Higuchi and Connors)

$$K = \frac{slope}{so(1 - slope)}$$

Melting point

The melting point of a pure substance is a characteristic of the substance being studied and any pure sample of the substance shall have same melting point. Therefore the melting point is a physical

constant which can be used as a measure of purity and identity. Melting point range was studied by using a gallenkamp 220/240 volt melting point apparatus.

Fourier transform spectroscopy [10,11]

Fourier transform Infrared Spectrophotometer (FTIR) is employed as a important tool to identify drug excipient interactions. FTIR from Alpha Bruker, consisting of Opus software is used. Using KBr pellet technique, a scan range of 200-4000 cm $^{-1}$, the bands are identified. In these reports, the use of FTIR spectroscopy is to provide important information regarding the confirmation of inclusion complex formation of CD's with drug molecules.

X-ray diffractometery (xrd)[12]

The powder X ray diffraction patterns of raw materials and binary mixtures were obtained at room temperature using Bruker D8 Advance powder diffractometery, Japan, of Cu K_α radiation which is operated at voltage of 40Kv, current of 30 mAmp, with a 2θ value of 2º-40º, Scan rate of 2º per minute, incrementing with 0.1º.

Dissolution studies

The dissolution is performed for active ingredient, marketed drug and different binary mixtures prepared by different methods using Lab India dissolution apparatus. USP II apparatus is used for dissolution with 50rpm. Dissolution is performed in different dissolution media (Millipore water, 01NHCl- pH 1.2, Phosphate buffer solution- pH 7.4) which is thermostated at 37º C. Powder Samples containing suitable amount of Candesartan cilexetil for sink condition were added to the surface of 900ml of the dissolution medium. Samples are withdrawn at 5, 10, 15, 30, 45, 60 minutes which are filtered using whatman filter paper and samples are analyzed using UV at λ_{max} of 256nm, 270nm, 257nm in Millipore water, 0.1NHCl-pH 1.2, Phosphate buffer solution pH 7.4 respectively. The percentage drug release is calculated using after 60min. Dissolution studies are performed three times for each binary mixture in different media.

RESULTS AND DISCUSSION

Phase solubilitystudies

Phase solubility studies were performed in different media (Millipore water, 0.1NHCl- pH1.2, PBS -7.4). Phase solubility diagram shown in Fig 1 & 2 has a linear increase in the solubility of the candesartan cilexetil as the amount of complexing agents was increased. The plot was found to be linear indicating A_L type, and slope was found to be less than 1(From table 1), it could be assumed that stoichiometry of the formed binary systems was 1:1 (according to Higuchi and Connors)

Complexation constants were calculated for Candesartan cilexetil with HPβCD using the slope and the intercepts of the corresponding regression analysis data of the regression lines. Analytical parameters of the phase solubility diagrams are presented in the table. From the K_s values it indicates that Candesartan cilexetil complexation with β CD was stronger than HPβCD. As the pH of the solvent is altered K_s values are altered this can be due to ionzation of the drug in media. Ionic form of drug shows low hydrophobicity and hence weak interactions with the Cyclodextrin cavity which implies a change in the K_s value. It can be concluded that the pH had an impact on the complexation of the Candesartan cilexetil and cyclodextrins.

Table 1: Analytical parameters of Phase solubility study in Millipore water, 0.1NHCl, PBS 7.4

Cyclodextrin	Medium	S_o*10^{-5}	Equation	K M^{-1}	r^2
HPβCD	Millipore Water	1.17±0.22	y=0.002x + 1*10-[05]	171.25	0.999
	pH 1.2 Buffer	0.402±0.37	y = 0.0014x + 4*10^{-6}	174.25	0.998
	pH 7.4 Buffer	1.48± 0.12	y= 0.0111x + 1*10-[05]	797.24	0.999

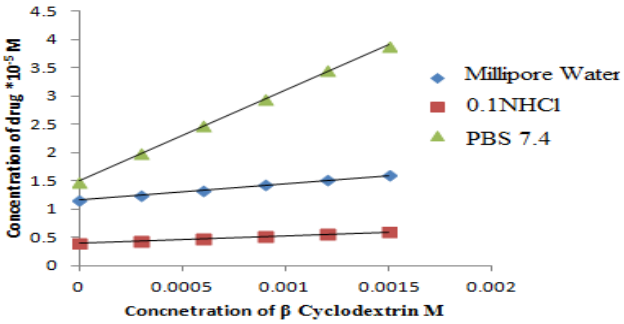

Fig. 1: Phase solubility diagram with β-CD

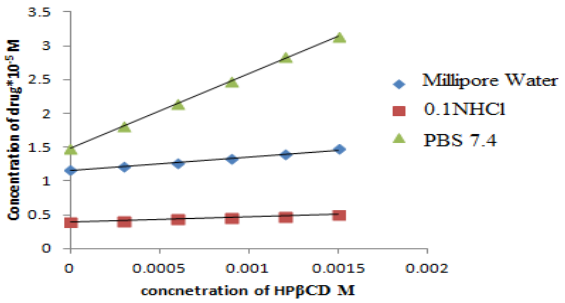

Fig. 2: Phase solubility diagram with HPβCD

Characterisation in solid state

Melting point[13]

The basis for melting strategy is that in order to dissolve, molecules must be removed from the crystal lattice. Any modification which reduces this crystal lattice energy hence reduces the melting point, would tend to increase solubility.

Table 2: Melting point of Pure drug, βCD, HPβCD and various binary mixtures

Candesartan cilexetil: 169ºC		
HPβCD: 278ºC	Inference	
1:1 Kneaded Mixture	276 ºC	Confirms Complexation
1:1 Coevaporated Mixture	262 ºC	Confirms Complexation

XRD

The XRD of powder samples complexed with βCD, HPβCD are shown in below figures. The characteristic and strong diffraction peaks of Candesartan cilexetil indicate the crystalline nature of drug. βCD has less diffraction peaks so less crystalline while HPβCD are amorphous as evidenced from the diminution of diffraction peaks . The characteristic Candesartan cilexetil peaks are altered in the inclusion complexation. The characteristic peaks are completely decreased in the co evaporation method indicating that the Candesartan cilexetil-CDs inclusion complex constitutes a new solid state. Where as in the kneading method there are some evident peaks of the Candesartan cilexetil. The prominent peaks of Candesartan cilexetil are comparatively more in the HPβCD than βCD mixtures. The HPβCD contain less prominent peaks of drug in co evaporation method when compared to the Kneading method. The reduction in the

height of the diffraction peaks indicate that decrease in the crystalline of the compound[14]

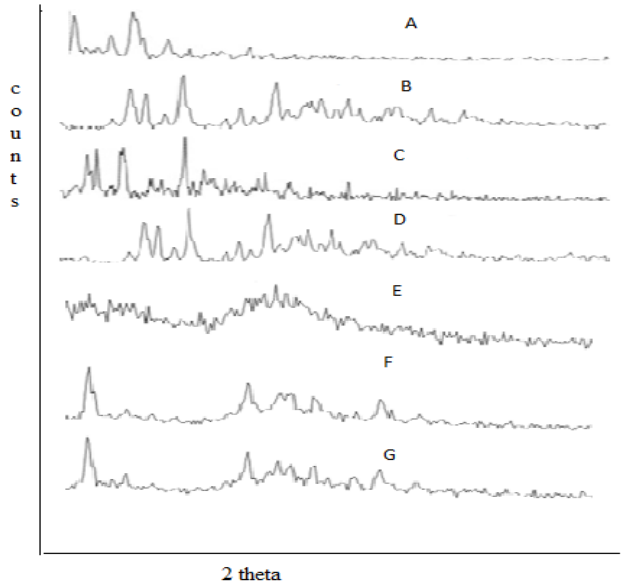

Fig. 3: XRD of the (A) Pure drug, (B) βCD, (C) 1:1 Kneaded mixture using βCD, (D) 1:1coevaporated Mixture βCD, (E) HPβCD, (F) 1:1 Kneaded mixture using HPβCD, (G) 1:1coevaporated Mixture HPβCD

FTIR

Infrared spectroscopy was obtained using shimadzu FTIR spectrophotometer. The samples were analyzed using KBr pellet technique

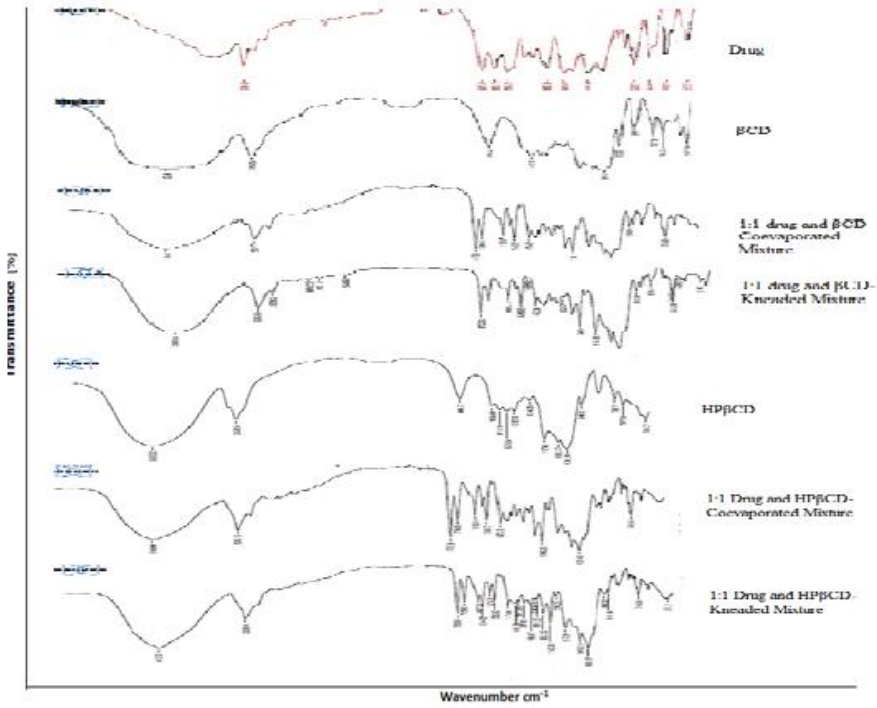

Fig. 4: FTIR drug, βCD, 1:1 Coevaporated Mixture using βCD and 1:1 Kneaded Mixture using βCD, HPβCD, 1:1 Coevaporated Mixture using HPβCD and 1:1 Kneaded Mixture using HPβCD

The prominent peak of the drug appears at 1728cm^{-1} due to the carbonyl group and methyl group at 2982cm^{-1}

The IR spectrum of β-CD is characterized by intense bands at 3300–3500 cm^{-1} (3384.45 cm^{-1}), associated with the absorption of the hydrogen bonded –OH groups of β-CD.

CH-CH vibrations appear in the 2800–3000cm-1 region (2925.54 cm^{-1}).

C-OH bending appears as broad band at 1416cm^{-1}.

C-O stretching in alcohol appears at 1026.47cm^{-1}.

C-H out of plane bending appears at 900-690cm^{-1}

The IR spectrum of HPβ-CD is characterized by intense bands at 3406 cm^{-1}associated with the absorption of the hydrogen bonded –OH groups of β-CD.

The vibrations of the C-H groups appear in the 2928 cm^{-1}.

H-OHbending appears as band at 1640cm^{-1}.

C-O-C stretching in alcohol appears at 1030cm^{-1}.

The C=O streching of the drug disappears in the binary mixtures prepared by Kneading and coevaporation technique using HPβCD, βCD indicating that there is a complex formation between CD and candesartan cilexetil.

DISSOLUTION STUDIES:

Dissolution studies on various Candesartan cilexetil- Cyclodextrin system were conducted to demonstrate the influence of the type of Cyclodextrin, and the complexation method on dissolution profile and the total amount of drug in solution. It is generally assumed that the complexes show higher dissolution as compared to that of pure drug.

But the objective is to achieve higher solubility which is characteristic of inclusion complexes. Dissolution profiles of Candesartan cilexetil and various binary systems of β CD/ HPβCD are presented in the following tables and graphs.

Table 3: The dissolution profile of pure drug in Millipore water, 0.1NHCl, PBS- 7.4

Time (Min)	Millipore water Percentage release	0.1N HCl Percentage release	PBS Percentage release
5	29.6±0.3	29.47±0.2	31.27±0.2
10	30.6±0.24	29.97±0.1	31.47±0.1
15	31.575±0.5	30.8±0.7	33.67±0.4
30	32.675±0.3	31.12±0.4	34.72±0.5
45	33.55±0.4	31.3±0.6	35.05±0.7
60	34.3±0.1	31.72±0.5	36.3±0.3

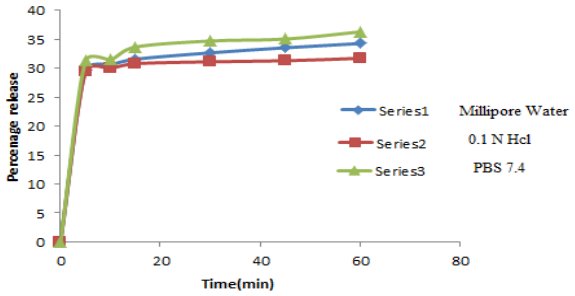

Fig 5: Time Vs Percentage of drug release of Pure drug in Millipore water, 0.1NHCl, PBS-7.4

Table 4: Percentage drug release after 60min from 1:1 ratio of binary mixtures using βCD prepared by various methods in Millipore Water, 0.1NHCl, PBS 7.4

Dissolution media	Physical Mixture		Kneading Mixture		Coevaporated mixture	
	βCD	HPβCD	βCD	HPβCD	βCD	HPβCD
Millipore water 49.8 71.2 73.67	49.8	49.72	71.2	69.89	73.67	71.82
0.1N HCl 48.47 58.05 61.55	48.47	48.17	58.05	57.32	61.55	59.43
PBS 7.4 58.56 79.05 81.75	58.56	56.86	79.05	71.05	81.75	74.68

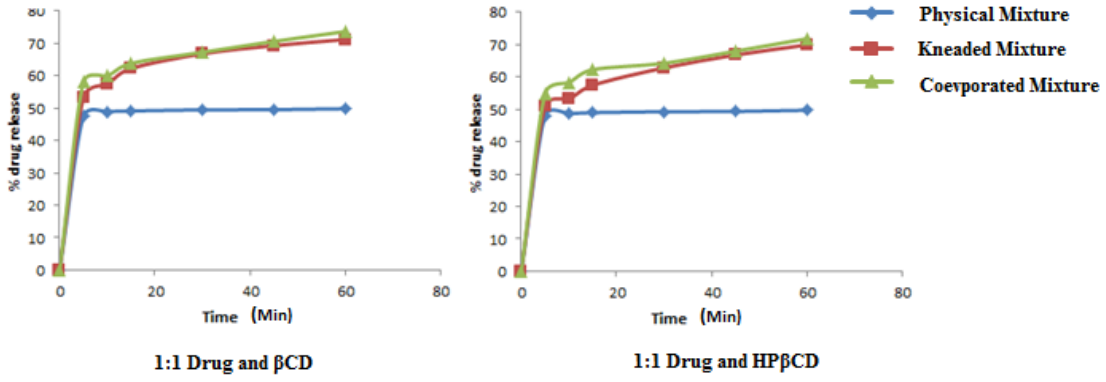

Fig. 6: Time Vs Percentage drug release of the 1:1 ratio of drug and βCD, HPβCD in Millipore Water

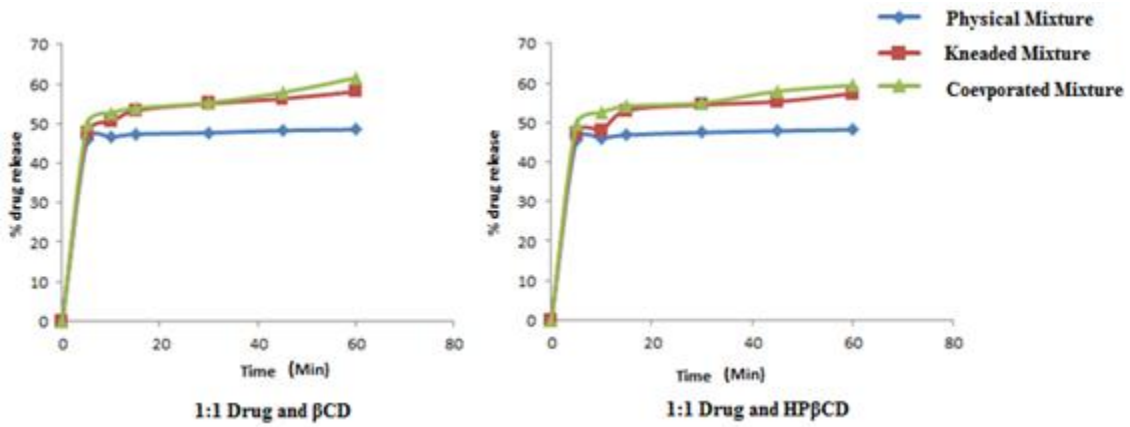

Fig. 7: Time Vs Percentage drug release of the 1:1 ratio of drug and βCD, HPβCD in 0.1N HCl

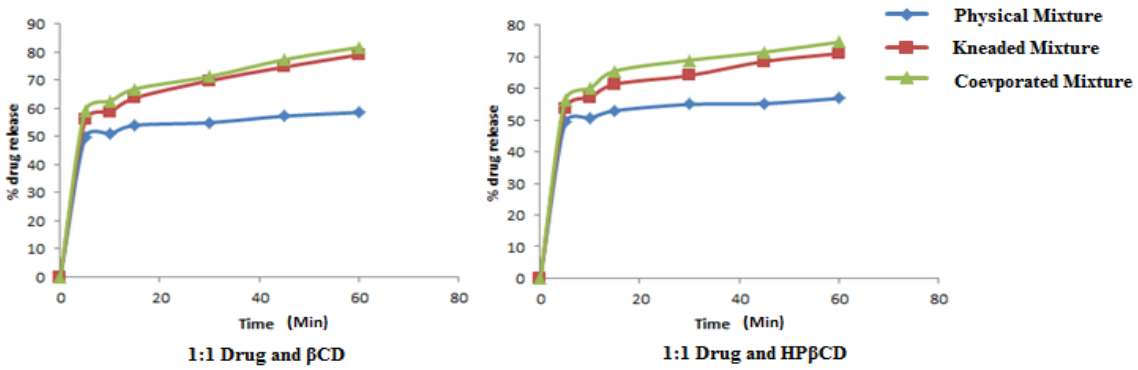

Fig. 8: Time Vs Percentage drug release of the 1:1 ratio of drug and βCD, HPβCD in PBS 7.4

- The percentage drug release from the binary mixture with different CDs falls in the following said pattern: **PBS 7.4 > Millipore Water > 0.1N HCl**
- The percentage drug release from different mixtures, Marketed product and API is given as follows: **Co evaporated Mixture > Kneaded Mixture > > Physical Mixture≈ Marketed >API**
- By comparing the dissolution profiles of 1:1 drug: βCD and 1:1 drug: HPβCD in different dissolution media 1:1 drug: βCD showed the maximum dissolution **1:1 drug: βCD > 1:1 drug : HPβCD**

CONCLUISON

This methodology has provided an important tool in predicting the bioavailability studies which in turn depends on the solubility of drug. Using this concept a BCS Class II drug can be changed to a BCS Class I drug.

βCD and HPβCD has shown the complexation of the Candesartan cilexetil. The Inclusion complexation of the drug is influenced by the preparation methods of binary mixtures and also the pH. The stability constants are varied in different media of phase solubility studies which indicates that pH influences the ionization and alters the complexation. Among the different ratio of 1 molar 0.5:0.5 showed the maximum drug release. The drug release was maximum in case of Co evaporation method followed by Kneading method. The solid state characterization was performed using Melting point (where the melting point of complexation is decreased when compared to β CD indicating the complexation is formed), FTIR, XRD. From the results it indicates that compared to parent β-CD the HPβCD has lesser ability to form complexes with large spheriform structures.

REFERENCES

1. Amidon G, Lennarnas H, Shah V, Crison J., " A theoretical basis for a biopharmaceutics drug classification: the correlation of invitro drug product dissolution and in vivo bioavailability". Pharm Res. 1995;12:413–29.
2. FDA/CDER, Guidance for industry, waiver of in vivo bioavailability and bioequivalence studies for immediate release solid oral dosage forms based on a biopharmaceutics classification system. Washington D.C., August 2000.
3. Dr. Helena Dodziuk ,institute of physical chemistry "Cyclodextrin and their complexes" published 2006, Pg No1-3.
4. Uekama K, Hirayama F and Irie T., "Cyclodextrins drug carrier system". Chemical Reviews, 2045-2076, 1998.
5. Pitha J & Rao C T, "Distribution of substituents in 2-hydroxypropylcyclo ethers of cyclomaltoheptaose. "Carbohydrate research,429-435, 1990
6. Zhengyu Jin, Chao Yuan, Xuehong Li, "Evaluaiton of complex forming ability of hydroxypropl –β-cyclodextrins", Food chemistry 106 ,2008, 50-55
7. Thomas L. Lekme, David A.Williams, Victoria F. Roche, S.William Zito "Foye's principles of medicinal chemistry" 6th edition 2008, 75.
8. F.Veiga, J.J.C Teixeria-Dias, F. Kedzierewicz, A.Aousa, P.Maincent, "Inclusion complex of tolbutamide with β-CD and hydroxy propyl β-CD", International journal of pharmaceutics 129, 1996, 63-71.
9. T.Higuchi and K.A. Connors ,Phase solubility technique in Advances in analytical ahemistry and instrumentation,Vol-4,1965; 117-212
10. Hsiue, G.-H, Liao, C-M., and Lin, S.-Y "Effect of Drug-Polymer Interaction on the Release characteristics of Methacrylic Acid Copolymer Microcapsules Containing Theophylline", Artif Organs 22 (8), 1998,651-656.

11. Sarisuta, N Lawanprasert, P., Puttipipatkhachorn, S., and Srikummoon, K., "The Influence of Drug-Excipient and Drug-Polymer Interactions on Adhesive Strength of Ranitidine Hydrochloride Film-Coated Tablets", Drug DevInd Pharm **32** (4),2006, 463-471.
12. Hirokazu Matsunaga "Solid-State Characterization of Candesartan Cilexetil (TCV-116): Crystal Structure and Molecular Mobility", Chem. Pharm. Bull. 47(2),1999, 182—186
13. Gordon l.Amidon, "Drug derivatization as a means of solubilisation: Physicochemical and biochemical strategies" Pg no187.
14. M. Narender Reddy,Tasneem Rehana,S. Ramakrishna,K. P. R. Chowdary, and Prakash V. Diwan " β-Cyclodextrin Complexes of Celecoxib: Molecular-Modeling, Characterization, and Dissolution Studies",AAPS pharmsci 2004;6(1) Article 7