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PREPARATION AND CHARACTERIZATION TERNARY INCLUSION COMPLEXES OF HYDROCHLOROTHIAZIDE IN B-CD AND POLYETHYLENE GLYCOL

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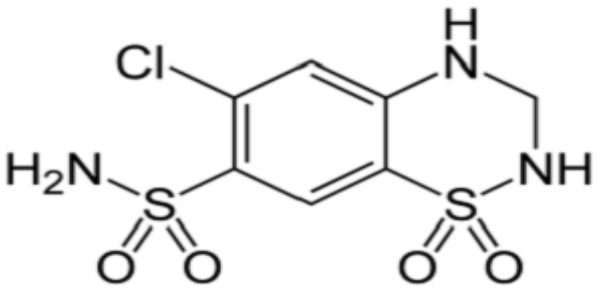
ABSTRACT

The aim of present research was to formulate and characterize inclusion complexes between β-cyclodextrin (β-CD) and Hydrochlorothiazide (HCT). Equimolar HCT/ β-CD solid system in the presence and absence of 0.2% (w/v) polyethylene glycol was prepared by kneading method. A phase solubility study was done to estimate solubility constant (KS) and complexation efficiency (CE). Improvement of KS and CE showed the additive effect of auxiliary substances (polyethylene glycol). The dissolution properties of binary and ternary systems were determined and compared with HCT alone. The ternary system has shown several times faster than the binary system of HCT. The optimized binary and ternary systems were characterized by phase solubility, FTIR, SEM, and DSC. These results showed that ternary inclusion complexes were formed.

KEYWORDS: HCT, β-CD, Kneading method, Dissolution rate, Scanning Electron Microscopy.

INTRODUCTION

Hydrochlorothiazide (HCT) is a diuretic agent, chemically described as a 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide, which is widely used in antihypertensive pharmaceutical preparations, which decrease the blood volume by acting on the kidneys to reduce sodium (Na) reabsorption in the distal convoluted tubules.[1, 2] Its molecular formula is $C_7H_8ClN_3O_4S_2$ having molecular weight 297.74 g/mole. It is practically insoluble in water, freely soluble in methanol, soluble in diluted ammonia or sodium hydroxide and Dimethylformamide. It is while crystalline powder, odorless with melting point in the range of 271-273 ^{0}C.[3, 4]



Chemical name: 6-cloro-1, 1-dioxo-3, 4-dihydro, 2H-1, 2, 4- benzothiazine-7-sulfonamide

Figure 1: Chemical structure of Hydrochlorothiazide

The cyclodextrins (CDs) are the products of enzymolysis of starch. They are a series of cyclic oligosaccharides comprises of six (α-CD), seven (β-CD) and eight (γ-CD) units of (α-1, 4)-linked α-D-glucopyranose arranged in a truncated cone-shaped structure.[5, 6] The exterior of the cavity is hydrophilic due to the large number of hydroxyl groups while the interior of the cavity is lined with skeletal carbon and ethereal oxygen moieties of the glucose residue which make it relatively a polar and creates a hydrophobic microenvironment.[7] This remarkable structure makes CDs well known for their capability to host a variety of hydrophobic drug molecules to form inclusion complexes.[8] The complex usually exhibits high stability when the dimension of the hydrophobic drug molecule is suitable for the dimension of the CD cavity.[9] The formation of an inclusion complex may greatly affect physical and chemical properties of the guest molecules, the solubility may be enhanced, the chemical reactivity and the spectroscopic and electrochemical properties may also be altered.[10] Thus, CDs are widely used in aspects relevant to the pharmaceutical, agrochemical and food industries.[11]

In the current research, it seemed of interest to investigate the rate of polyethylene glycol in improving the dissolution and solubility properties of HCT. The purpose of this work was to study the interaction of HCT with β-CD with or without presence of polyethylene glycol. In order to estimate the influence of β-CD and polyethylene glycol on HCT solubility the interaction was investigated by phase solubility. The influence of β-CD and the polyethylene glycol on physicochemical properties of HCT was characterized by formulating a solid system with equimolar quantities of β-CD and HCT in the presence and absence of 0.2 % (w/v) polyethylene

glycol by kneading method DSC, SEM, and FTIR was used to characterize the binary and ternary system.

MATERIALS AND METHODS

Materials: HCT was received as gift sample from Medispray Pharmaceuticals Pvt. Ltd., Satara. Polyethylene glycol was purchased from Signet chemicals Mumbai. β- CD procured from Gangwal Chemicals Pvt. Ltd. All other chemicals were of analytical reagent grade.

Methods

Phase solubility: Phase solubility studies were performed according to the method reported by Higuchi Connors. An excess amount of HCT was added to 10 ml distilled water containing various conc. of β-CD (0-0.01 M) with or without fixed conc. of polyethylene glycol (0.2% w/v) in stopper tubes and mixture were shaken in rotary shaker for 72 Hrs 37±0.5^{0}C at 150 rpm. After achieving equilibrium the solution was filtered through 0.45µm membrane filter paper. The sample was diluted suitably and assayed for content HCT by U.V. Spectrophotometer at 272 nm (Shimadzu 1700, Japan). The solubility constant and Complexation efficiency were calculated by using the following equations.

$$Ks = \frac{Slope}{So\ (1 - Slope)}$$

*Ks = Stability constant

*So = solubility of HCT in absence of CD

$$CE = \frac{Slope}{(1 - Slope)}$$

*CE = Complexation Efficiency

Kneading method: For preparation of ternary complex, HCT, β-CD and polyethylene glycol in required quantity, were wetted with minimum & equal quantity of water & methanol in order to obtain pasty mass. To evaluate the effect of trituration time, kneading was carried out for 30 and 60 min, respectively. Afterwards, obtained powders were kept for drying at 50^{0}C for one day, further they were crushed, sieved (60#) and stored in desiccators at 25±2^{0}C.

Aqueous solubility[12]: An excess amount of prepared binary and ternary complex added into 10 ml water in stopper tubes. They were kept and shaking to achieve equilibrium appropriate aliquots was withdrawn filtered through whatman filter paper no.41. The filtrate analyzed by UV visible spectrophotometrically at 272 nm.

Dissolution studies: Dissolution studies were performed according to the USFDA dissolution method for HCT. The dissolution rates of HCT and solid systems were measured in a dissolution apparatus (Labindia 8000) using the paddles. Dissolution studies were carried out using 900 ml of 0.1N HCl at 37±0.5^{0}C at 50 rpm. At fixed time intervals (2, 5, 10, 15, 20, 30, 45 and 60 min) 5 ml samples were withdrawn. The dissolution medium was 5ml aliquot with 5ml fresh 0.1N HCl. The solution was immediately filtered 0.45µm membrane filter suitably diluted and was determined spectrophotomerically 272 nm.

Scanning electron microscopy: The morphology of powdered samples of selected for formulation was studied by SEM using the JEOL (Jeol 5400 Japan). The powders were previously fixed on brass stub using double sided adhesive tape and then were made electrically conductive by coating in vacuum with a thin layer of gold (100-300^{0}A) for 240s photographs were taken 5-10 Kv voltage and appropriate magnification.

Fourier transformation-infrared spectroscopy (FTIR)
FTIR studies were performed to determine the compatibility study between drug and other components of microemulsions by KBR dispersion method considering % transmittance of FTIR spectrophotometer (IR Affinity1, Shimadzu, Japan). The base line correction was completed using dried potassium bromide and % transmittance analyzed at in the spectral region of 4000-400 cm-1 using a resolution of 4 cm-1 and 40 cm-1 scans.

Differential Scanning Calorimetry (DSC)
DSC has been one of the most widely used calorimetric techniques to study the solid state interaction of drug with β-CD and polyethylene glycol. The DSC curves of pure drug, cyclodextrin and binary complexes were recorded on Mettler Toledo DSC. The thermal behavior was studied by heating all samples (in the range of 1-5 mg of weight) in a sealed aluminum pan using empty sealed aluminum pan as reference, over a temperature range of 40-300^{0}C at a rate of 10^{0}C/min and under nitrogen flow. The results of pure materials binary and ternary systems were evaluated for change in endothermic peaks and interpreted for the formation of complexes.

RESULT AND DISCUSSION

Phase solubility studies[13]: Phase solubility diagram of binary mixture of HCT and β-CD and ternary complex with (0.2%) Polyethylene glycol is done by Higuchi Connors method [1]. Phase solubility diagram for binary and ternary inclusion complex is A_L type, which indicates the formation of soluble complex.

The shape of solubility curve may indicate that 1:1 molar ratio is most probable for the inclusion complex formed. According to Higuchi Connors equation the calculated solubility constants are K_s = 107.73(±5) for HCT and β-CD complex, K_s = 129.3397(±2) for ternary complex of HCT, β-CD and Polyethylene glycol. The Polyethylene glycol studied from 1:1 inclusion complex with HCT and β-CD ternary complex gives a soluble complex while in the presence of β-CD inclusion complex with limited solubility are formed.[2,3]

Equations =

1) y= 0.167x+0.0021, R^2=0. 993 (Binary).
2) y= 0212x+0.0021, R^2=0. 976 (Ternary).

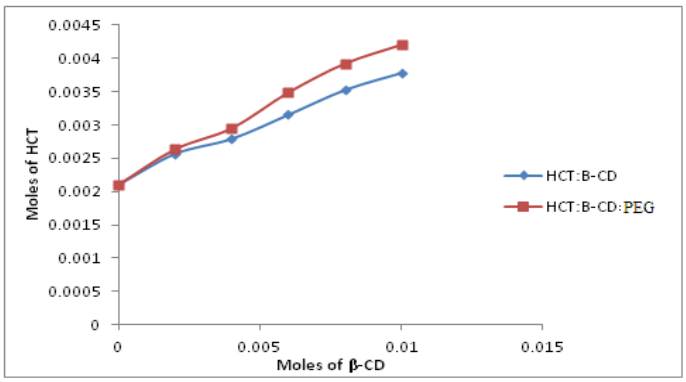


Figure 2: Phase solubility diagram of HCT: β-CD system in presence and absence of Polyethylene Glycol

Table 1: Effect of polymer, Polyethylene glycol (1% w/v) on slope of phase solubility diagrams and stability constant (Ks) for binary and ternary system of HCT with β-CD

System	Slope	r^2	Ks(M^{-1})* Mean ± SD	K_{TS}/K_{BS}	C.E
HCT- β-CD	0.167	0.993	107.73±5		0.2008
HCT- β-CD- PEG	0.212	0.976	129.33±2‡	1.2005	0.26903

*K_{TS}/K_{BS} ratio of Ks for ternary and binary complexes; * indicates mean of three readings; S.D.: Standard deviation. ‡ p value compared to HCT- β-cd (p <0.001) i.e. significant.C.E.:Complexation efficiency*

An indication of the process of transfer of HCT from pure water to aqueous solution of β-CD was obtained from the values of Gibbs free energy change. The obtained values of Gibbs free energy are shown in Table 2. $\Delta G_{tr}°$ values were all negative for β-CD at various concentrations, indicating the spontaneous nature of HCT solubilization, and it decreased with an increase in its concentration, demonstrating that the reaction became more favorable as the concentration of β-CD increased.

The negative nature of the Gibbs free energy changes ($\Delta G_{tr}°$) (shown table 2) are indicative of the spontaneity of the process. The endothermic heats of solution further explain the increase in solubility with temperature.

Table 2: Gibbs free energy of transfer ($\Delta G_{tr}°$) for solubilization process of HCT in aqueous solutions of β-CD at 37°C

Moles of β-CD	Gibbs free Energy of systems	
	HCT: β-CD	HCT:β-CD:PEG
0.002	-490.28	-557.31
0.004	-702.26	-841.51
0.006	-1002.68	-1250.47
0.008	-1285.60	-1545.05
0.01	-1456.96	-1715.77

Drug content: The percentage of drug content of binary and ternary system was found between 93.86±1.95 to 98.78±0.87 while for coevaporated binary and ternary complex the drug content was found to be 97.24±1.44 and 93.86±1.95. The drug content of spray dried complexes were found to be 98.78±0.87 and 93.90±0.91

Aqueous solubility: The solubility studies of HCT with βcd in binary and ternary system with 0.2%w/v PEG in water showed an enhancement in solubility as compared to pure drug alone. The 1:1 inclusion complex of HCT inclusion complex with or without PEG showed higher solubility than their pure drug alone, the enhancement of solubility of complex mainly attributed due to the formation of a stable inclusion complex of HCT and βCD. The stability constant suggests that β-CD and HCT have sufficient affinity towards each other to form a stable inclusion complex. In ternary system polyethylene glycol not only enhances their complex efficiency but also enhance their binding towards the β-CD.

Table 3: Solubility studies of binary and ternary complex of HCT

System	Solubility in water at 25°C mg/ml* (mean ± SD)
HCT	0.716± 0.1
BK (binary)	1.870±0.127
BK (ternary)	2.436±0.115

Dissolution studies

The comparative in vitro dissolution profiles of HCT, kneaded binary and ternary complexes in 0.1 N HCl, pH 1.2 shown in fig. 3. It is clearly shown that the dissolution profile of the pure drug sample was 45% after 60 min. It was interesting to note that in case of kneading binary complex. The dissolution complex was more than 90% after 10 min. And that of ternary complex showed the dissolution profile for kneaded complex more than 90% after 5min. The kneaded complexes showed higher dissolution rate. In the presence of β-CD the hydrophobic portion of drug molecule can be interacted with hydrophobic portion cyclodxtrin cavity to form an inclusion complex, whereas at the same time, the hydrophilic portion lowers the aqueous surface tension by acting as a surfactant towards the cyclodextrin complexes and thus increasing its wettability and dissolved.

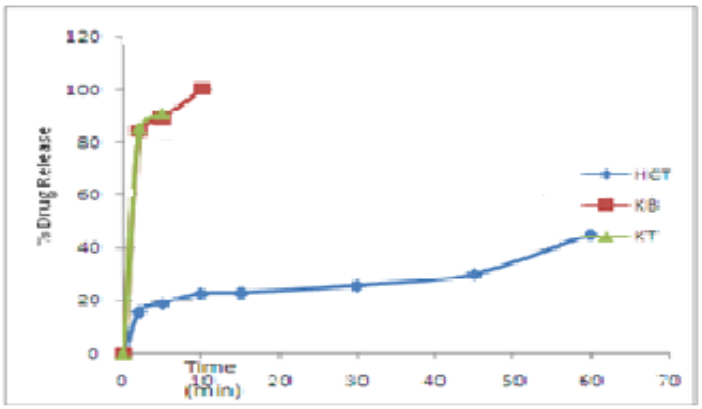


Figure 3: Dissolution profile of HCT, Binary Complex and Ternary Complex

Scanning electron microscopy (SEM) (4, 14, 15)

SEM images of the HCT and ternary complexes prepared by kneading method represented in fig.4 SEM study showed the morphology and microscopy photography of the drug and inclusion complexes. From SEM analysis it can be clearly indicated that HCT particles appeared as stone shaped irregular crystals. Microscopic examination of ternary complexes showed drasting change in the original morphology and shape. In ternary system showed small and irregular piece and like engulfment of material in cavity. Thus, change in the morphology of complex as compared to drug showed interaction between HCT and complexing agent. The result revealed that there might be increase the surface area of HCT due to complexation. Due to increased surface area, porosity of material was also increased. The improvement of porosity of material had further enhanced the wettability of material. The wettability might have accelerated the solubility as well as dissolution rate of HCT.

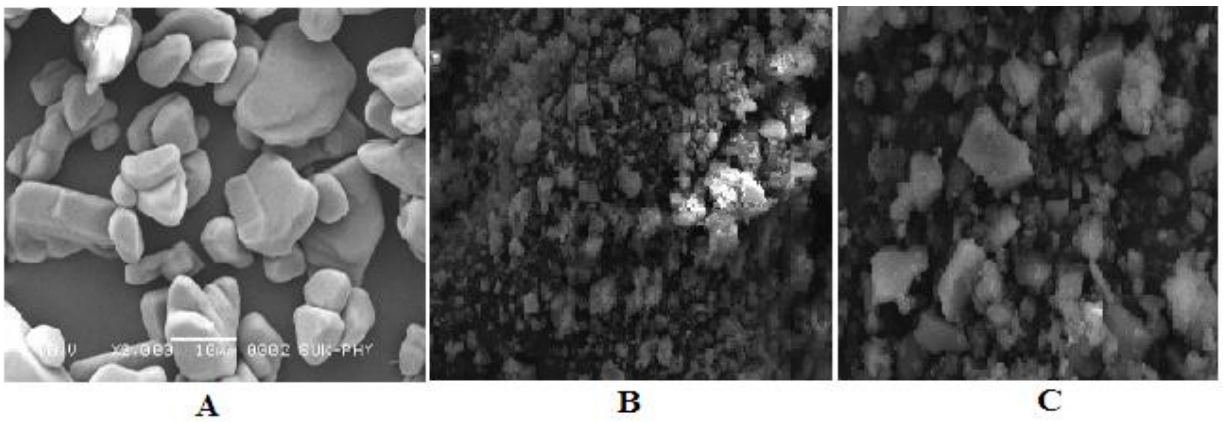


Figure 4: SEM of A- HCT, B- Binary Complex and C- Ternary Complex

FTIR Spectroscopy Study: The IR spectra of HCT, kneaded binary (KB) and ternary (KBT) systems are shown in fig. The IR spectroscopy has also been used to assess the interaction between guest and cyclodextrin inclusion complex, shifts or changes in absorption spectra. The IR spectrum of HCT showed absorption bands at 3360.87 cm-1,3265.114 cm-1,3167.094 cm-1 due to NH stretching,1597.450 cm-1 stretching of C=C aromatic ring,1317.08 cm-1 showed C=N stretching and 1243.287 cm-1 showed SO2 stretching. In binary system showed interaction with β-CD, HCT and polyethylene glycol. All binary and ternary systems exhibited significant alteration (attenuation, broadening, frequency shifts and/or disappearance) in characteristic bonds of either drug or β-CD demonstrating modifications in drug or β-CD environment. In binary system peaks of HCT

3360.876 cm-1, 3167.094 cm-1 were disappeared. FTIR of all the ternary system exhibited similar behavior as that of binary system with change peak intensities of 3265.114 cm-1 in β-CD to 3325.28 cm-1 respectively characteristics peak in β-CD at3399.36 cm-1, 2929.24 cm-1 were shifted to 3255.84 cm-1, 3172.90 cm-1. All the binary peaks in ternary and binary system were found to be smoothened and did not show new peaks.

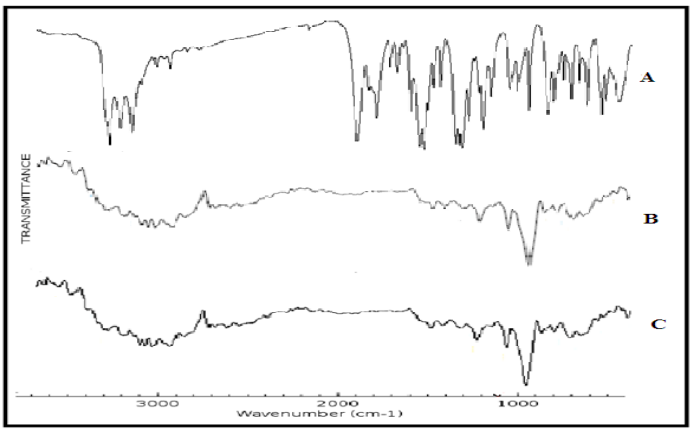


Figure 5: FITR Spectrum of A-HCT, B-Kneading Binary Complex, C- Kneading Ternary Complex

Differential scanning calorimetry (DSC)

The existence of an interaction between two components can be obtained by thermal analysis (DSC) when guest molecules are included in the CD's cavity, their melting point usually shift to different temperature or disappear.

The thermal curve of pure HCT is characterized with presence of the melting endotherm at 268.12°C. Broad endothermal peak at 90.20°C was observed for the amorphous β-CD which was related to loss of water molecule i.e. dehydration process.

An evidence for formation of inclusion complexes between HCT and β-CD was the change in shape of melting peak of HCT and its shift towards higher temp. (e.g. for complex the peaks corresponding to melting points of drug substance were observed at 286.63^{0}C as a result of inclusion of drug in to the cyclodextrin cavity).

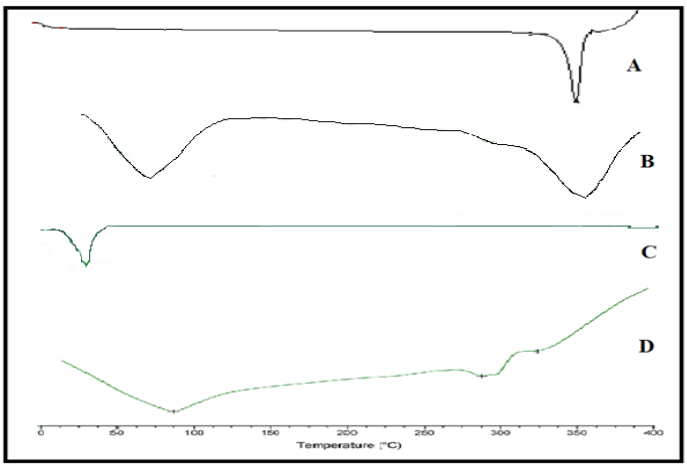


Figure 6: DSC Spectrum of A-HCT, B-β CD, C-PEG, D- Ternery Complex

CONCLUSION

This study has revealed that the improving the dissolution performance of HCT by its complexation with β-CD in presence of polyethylene glycol using kneading technique. Polyethylene glycol showed a more pronounced effect on the enhancement of aqueous solubility and faster rate than binary complex. Phase solubility study of binary and ternary systems were showed that stability constant of ternary system is higher than that of binary system. Thus addition of polyethylene glycol in ternary complexes of β-CD beneficial in terms of improvement in CE, rate of complex formation and enhancement of poorly water soluble HCT. Ternary complex system was also found to give faster drug release as compared to pure HCT.

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