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Research Article

SPECTROSCOPIC INVESTIGATION FOR PHOTOSTABILITY OF HYDROCHLOROTHIAZIDE COMPLEXES WITH β-CYCLODEXTRIN AND HPMC

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ABSTRACT

Objective: Hydrochlorothiazide (HCT) is a poorly water-soluble drug. The aim of this study was to determine whether inclusion complexes between β-cyclodextrin (βCD) and HCT are formed and also studied effect of auxiliary substance hydroxypropyl methyl cellulose (HPMC) on HCT and photostability of HCT and to characterize these.

Methods: Equimolar HCT/βCD solid systems in the presence or absence of 0.2% (w/v) of HPMC were prepared by kneading method. The systems were characterized by phase solubility, dissolution study, Fourier transmission infrared, differential scanning calorimetry, and the photostability test followed by ultraviolet spectroscopy. The results suggest the true binary and ternary inclusion complex.

Result: The test results were proved that binary and ternary inclusion complex of HCT enhance the water solubility and reduce the photo degradation rate.

Conclusion: Kneading method is suitable for preparation of binary and ternary inclusion complex of HCT enhancement of solubility and reduce the photo degradation rate.

Keywords: Hydrochlorothiazide, β-cyclodextrin, Hydroxypropyl methyl cellulose, Phase solubility profile, Characterization.

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INTRODUCTION

Hydrochlorothiazide (HCT) is a diuretic agent, chemically described as a 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide, which is widely used in antihypertensive pharmaceutical preparations, reduce active sodium reabsorption, and peripheral vascular resistance. Its molecular formula is $C_7H_8ClN_3O_4S_2$ having molecular weight of 297.74 g/mole. It is insoluble in water, freely soluble in methanol, soluble in diluted ammonia or sodium hydroxide. According to the Biopharmaceutics Classification System, aqueous solubility and permeability are the most important variables affecting drug bioavailability. HCT is classified as Class IV, where the drugs have low solubility and low permeability characteristics after oral administration. Cyclodextrins (CDs) are one of the available pharmaceutical strategies to circumvent these drawbacks (Fig. 1) [1,2].

CDs are cyclic (α-1,4)-linked oligosaccharides of D-glucopyranose containing a relatively hydrophobic central cavity and a hydrophilic outer surface. CDs are able to form inclusion complexes with poorly water-soluble drugs. These inclusion complexes have been shown to improve stability, solubility, dissolution rate, and bioavailability. This improvement in hydrophilicity may be attributed either to the formation of inclusion complexes or the highly homogeneous assembly between CDs and drugs in the solid state. In most cases, this association increases the solubility of poorly soluble drugs [3,9].

The aim of this work was to investigate the photostability of HCT in its binary and ternary system with βCD and to improve its dissolution rate profile. The effect of polymer, hydroxypropyl methyl cellulose (HPMC) 0.2% (w/v) on phase solubility curve of HCT:βCD was also investigated. The stability constants of the complexes were established. The result obtained from phase solubility.

Studies served as proper choice of HPMC (0.2% w/v) as a carrier in ternary systems [11]. The inclusion complexes of HCT with βCD were prepared by spray drying method. The dissolution properties of inclusion complexes were studied and compared with pure HCT. Differential scanning calorimetry (DSC) and Fourier transmission infrared (FTIR) were used to characterize the solid state properties of HCT and inclusion complex. The solubility study and dissolution behavior of binary and ternary system were further evaluated.

MATERIALS AND METHODS

Chemicals

HCT was obtained as a gift sample from Okasa Pharmaceutical Pvt. Ltd. Satara, India, and βCD M/s Loba Chemie Ltd. Mumbai. All other ingredient and solvent used were of analytical grade.

Methods

All experiments were carried out under subdued light to prevent photodegradation of HCT.

Phase solubility study [4-6]

Phase solubility studies were carried out in triplicate in water according to the method described by Higuchi and Connors [4]. Excess amount of HCT (50 mg) was added to 10 ml of aqueous solution containing various concentrations of βCD (0-0.01 M) with or without fixed concentrations of HPMC (0.2% w/v). Then, the suspensions were shaken on a rotary shaker at 25±2°C for 72 hrs. After equilibrium was achieved, the samples were filtered through 0.45 µm membrane filter and appropriately diluted. The concentration of HCT was determined spectrophotometrically (Shimadzu 1700, Japan) at 271 nm. The apparent 1:1 stability constant were calculated from the phase solubility diagrams, according to the following equation:

K_c=Slope/S_0 (1-slope)

S_0 is solubility of HCT in the absence of βCD

Solubility studies [5]

Solubility studies were performed according to the method reported by Higuchi and Connors. Excess of pure drug and inclusion complex for binary and ternary systems was added to 10 ml of distilled water taken in stopper conical flasks and shaken for 24 hrs in rotary flask shaker at room temperature. After shaking to achieve equilibrium, appropriate aliquots were withdrawn and filtered through Whatman filter paper no. 41. The filtrate so obtained was analyzed spectrophotometrically at 271 nm.

Drug content estimation [5]

Equivalent amount of 10 mg binary and ternary complexes were taken in 100 ml volumetric flask and added 10 ml of methanol and further volume is make up with distilled water. The solution was filtered and suitably diluted with distilled water. The drug content was determined using ultraviolet (UV) spectrometer at 271 nm.

Dissolution studies [5-7]

The dissolution rate studies of HCT alone, inclusion complex of binary and ternary systems were performed in a dissolution apparatus (Laboratory India) using the paddle method, according to USP Type II apparatus. Dissolution studies were carried out using 900 ml of 0.1 N HCl at 37±0.5°C at 50 rpm. 50 mg of HCT or its equivalent amount of HCT-βCD binary and ternary complex was added to 900 ml of 0.1 N HCl. 5 ml of samples were withdrawn at time intervals of 2, 5, 10, 15, 20, 30, 45 and 60 minutes. The volume of dissolution medium was adjusted to 900 ml by replacing each 5 ml aliquot withdrawn with 5 ml of fresh 0.1 N HCl. The solution was immediately filtered through 0.45 µm membrane filter, suitably diluted and the concentrations of HCT in samples were determined spectrophotometrically at 272 nm.

FTIR spectroscopy

IR spectrum drug was recorded as potassium bromide pellet at a resolution of 4 cm^{-1} over a range 4000-650 cm^{-1} for authentication and to study principle peaks using a Jasco-700 FTIR (Japan) spectrophotometer. The identified peaks were compared with the principle peaks of reported IR spectrum and the sample was authenticated.

DSC

DSC has been one of the most widely used calorimetric techniques to study the solid-state interaction of drug with βCD and HPMC. The DSC curves of pure drug, CD, and binary complexes were recorded on Mettler Toledo DSC. The thermal behavior was studied by heating all samples (in 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°C at rate of 10°C/minutes and under nitrogen flow. The results of pure materials binary and ternary systems were evaluated for change in endothermic peaks and interpreted for formation of complexes.

Photostability study [14]

The photostability of pure HCT and kneaded binary-ternary complex were assessed; the clear solution of different ratios prepared from the inclusion complexes was positioned 50 cm away from a UV light source. They were exposed to UV light for five different time intervals - 0, 2, 4, 6, and 12 hrs. HCT concentrations were determined by UV spectroscopy.

RESULT AND DISCUSSION

Phase solubility study

The solubility study plots obtained with βCD and in the presence and absence of HPMC (0.2% w/v) are shown in Fig. 2. Polymers are known to interact with outer surface CDs and with drug-CD complex, forming co-complexes or aggregates that show higher stability constants (K_S) values than those in the binary drug-CD system. The equilibrium phase solubility for binary and ternary complex showed that the solubility of HCT increased linearly as function of βCD. The phase solubility curve obtained as A_L type according to Higuchi and Connors. The increase in HCT solubility seems to be related to inclusion ability of a βCD molecule in water. The apparent stability constant, complexation efficiency of binary and ternary complex from slope, and linear phase solubility diagram were calculated and are shown in Table 1.

The higher value of K_S indicated that the ternary complex was more stable than binary complex. A synergistic effect on HCT solubility observed in the presence of HPMC. The addition of HPMC did not show any change in type of phase diagram but showed changes in K_S and complexation Efficiency. A solubility constant of binary system K_S value of 276.4926±5.33 M^{-1} indicated that interaction was weak unstable between components, while in case of ternary complex, the auxiliary substance poloxaer188 increase wettability and enhance the hydrogen bonds between drug and carrier and also decrease crystallinity of product [2,3].

For the binary system, host guest correlation coefficient r^2=0.974 with a slope of 0.216 suggested the formation of 1:1 complex with βCD concentrations. For a ternary system with 1% (w/v) HPMC, r^2=0.964 with slope 0.284 suggested the formation of 1:1 complex. The line equations from the linear regression analysis of these systems were as follows:

y=0.216x+0.001 (binary system)

y=0.284x+0.001 (ternary system with 1% w/v HPMC)

Solubility studies

The solubility study of HCT with βCD in binary and ternary system with 0.2%w/v HPMC in water showed enhancement in solubility as compared to pure drug alone. The 1:1 inclusion complex of HCT inclusion complex with or without HPMC showed higher solubility than their pure drug alone, the enhancement of solubility of complex mainly attributed due to formation of stable inclusion complex of HCT and

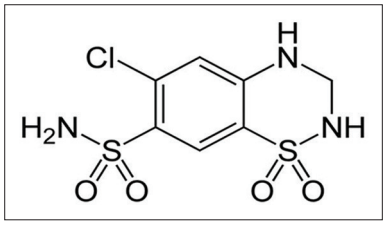

Fig. 1: Chemical structure of hydrochlorothiazide

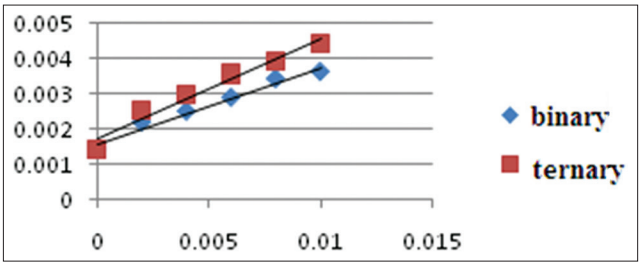

Fig. 2: Phase solubility diagram of hydrochlorothiazide-β cyclodextrin system in the presence and absence of hydroxypropyl methyl cellulose

Table 1: Effect of polymer, HPMC (1% w/v) on slope of phase solubility diagrams and stability constant (K_S) for binary and ternary system of HCT with βCD

System	Slope	r^2	K_S (M^{-1})* Mean±SD	K_{TS}/K_{BS}
Drug-βCD	0.216	0.974	276.49±5.33‡	1.4497
Drug - βCD-HPMC	0.284	0.964	399.52±2.92‡	

KTS/KBS ratio of Ks (stability constant) for ternary and binary complexes, *indicates mean of three readings, SD: Standard deviation, ǂp value compared to HCT-BCD (p <0.001), i.e., significant

βCD. The stability constant suggest that βCD and HCT having sufficient affinity toward each other to form stable inclusion complex. In ternary system, HPMC not only enhances their complex efficiency but also enhances their binding towards the βCD (Table 2).

DRUG CONTENT

The drug content in all complexes found is noted in Table 3.

Dissolution studies

The dissolution curve of HCT, kneading complex for binary and ternary system in 0.1 N HCL in 900 ml, 37±0.5°C is shown in Fig. 3. The release rate profile was expressed as % of drug released vs. time. Ternary inclusion complex showed higher dissolution rate as compared to binary complex and pure drug. The ternary kneaded complex showed increased in % drug release within 5 minutes. and pure drug showed 15% release whereas binary complex of kneaded complex showed 98.90% drug release within 20 minutes. The ternary inclusion complex containing HPMC kneaded complex showed 97.99% drug release within 10 minutes. The enhancement of dissolution was due to formation of inclusion in solid state with reduction in crystallinity of HCT as confirmed by FTIR studies, leading to increased hydrophilicity higher effect and increase contact between drug and carrier [5,6,12].

FTIR spectroscopy

HCT, βCD, HPMC interaction in inclusion complex is mediated weak forces between molecules such as hydrogen bond and hydrophobic interaction (Fig. 4) present FTIR absorption spectra for HCT, βCD, HPMC, binary complex (B), and ternary complex (C).

Comparison among the HCT, βCD, HPMC showed loss of resolution in typical HCT bands observed at 3360.876 cm^{-1}, 2835.328 cm^{-1}, 1597.450 cm^{-1}, 1317.08 cm^{-1}, 1243.287 cm^{-1}, 820 cm^{-1} shows N-H stretching, C-H stretching, C=C stretching of aromatic ring, C-N stretching, SO_2 stretching C-H bend. Host:guest interaction of HCT, βCD, and HPMC observed in spectra. It showed disappearance of OH deformation band of water molecule of CD cavity [5,6].

In B and C, spectra showed the shifting of spectral band and also be observed that inclusion compounds spectra are similar to each other implying the B and C kneading technique used to obtained in binary and ternary inclusion complex.

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 shifts to different temperature or disappear.

Table 2: Solubility study of pure HCT and binary and ternary complex in water

System	Solubility in water at 25°C mg/ml* (mean±SD)
HCT	0.716±0.1
BK (binary)	1.756±0.135
BK (ternary)	2.2423±0.315

*Indicates mean of three readings, SD: Standard deviation,
HCT: Hydrochlorothiazide, BK: Kneading complex

Table 3: Estimation drug content of BK and BKT inclusion complex of HCT

S. No.	System	Drug content* (mean±SD)
1	BK (binary)	97.88±1.44
2	BK (ternary)	96.80±1.52

*Indicates mean of three readings: SD: Standard deviation, βCD: β-cyclodextrin, BK: Kneading complex, HCT: Hydrochlorothiazide

The thermal curve of pure HCT is characterized by the presence of the melting endotherm at 268.12°C [13]. Broad endothermal peak at 85.95°C, 281.65°C was observed for the βCD which was related to the loss of water molecule, i.e., dehydration process (Fig. 5).

Photostability study

For confirmatory studies, samples were exposed to light providing an overall illumination of not <1.2 million lux hrs and integrated near UV energy of not <2000 watt hrs/m^2 to allow direct comparison to be made between HCT and binary-ternary complexes. The analysis of photostability was performed with the help of UV spectroscopy. From UV spectroscopy, the concentration of drug and drug content were observed and observation showed that the UV light for 5 different time intervals 0, 2, 4, 6, 12 hrs. Each sample responded differently to these conditions. It decreased with the increasing time interval and UV

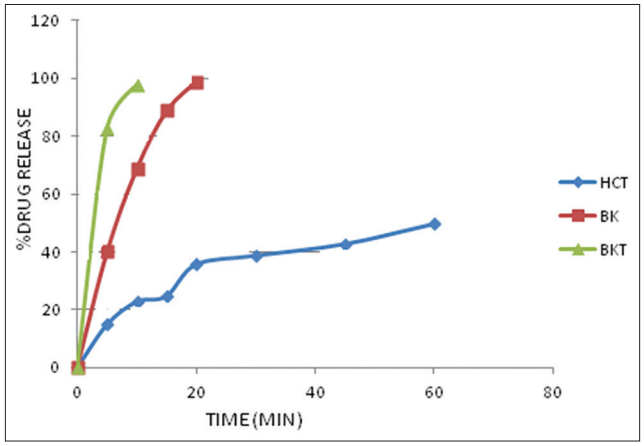

Fig. 3: Dissolution profile study of pure hydrochlorothiazide, binary and ternary complex. HCT: Hydrochlorothiazide; BK: Binary kneaded complex; BKT: Binary kneaded ternary complex

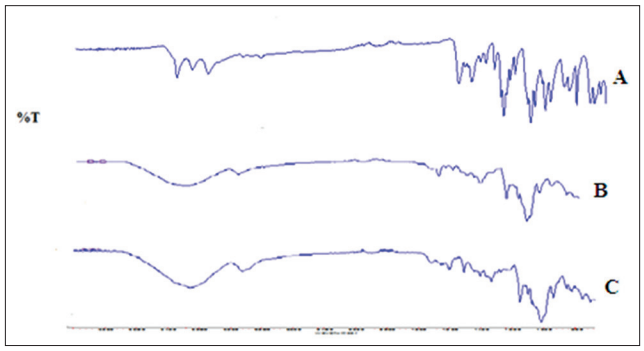

Fig. 4: Fourier transmission infrared of pure hydrochlorothiazide (A), binary (B), and ternary (C) complex

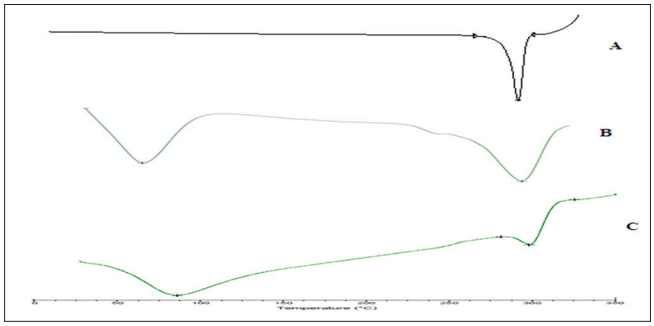

Fig. 5: Differential scanning calorimetry of pure hydrochlorothiazide (A), binary (B), ternary (C) complex

Table 4: Photostability study of pure HCT, BK, and BKT complex

Sample/time	0 hrs	2 hrs	4 hrs	6 hrs	12 hrs
BK (% drug release)	98.90	60.04	52.89	54.98	58.24
BKT (% drug release)	93.98	70.12	61.80	68.90	78.09

BK: Binary kneading complex; BKT: Binary kneading ternary complex, HCT: Hydrochlorothiazide

exposure. The values for HCT are also decreasing with increasing time interval and UV exposure which indicates that HCT degraded when exposed to light, but in case of binary and ternary complexes values decreased at first but then increased at the end. This was because HCT stabilized due to βCD and HPMC. The amount released after 6 hrs was more than that after 4 hrs which are clearly indicated the photostabilization (Table 4).

CONCLUSION

On the basis of solubility, stability, and dissolution study results, one can conclude that kneading method is highly useful for the preparation of solid inclusion complex for HCT:βCD complex. The stability constant of HCT, βCD, complex in the presence and absence of HPMC was increasing significantly.

All ternary inclusion complexes were shown better dissolution performance than binary inclusion complex indicating proper choice of HPMC as carrier. Thus, addition of HPMC in HCT binary inclusion complexes can be beneficial of improvement in complexation efficiency, rate of complex formation, and enhancement of dissolution properties of poorly water-soluble HCT. In case of binary and ternary complexes, values decreased at first but then increased at the end. This was because HCT stabilized due to βCD and HPMC. The amount released after 6 hrs was more than that after 4 hrs which are clearly indicated the photostabilization.

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