

BINARY AND TERNARY INCLUSION COMPLEXES OF HYDROCHLOROTHIAZIDE IN HP- β -CD AND POLOXAMER188, PREPARATION AND CHARACTERIZATION

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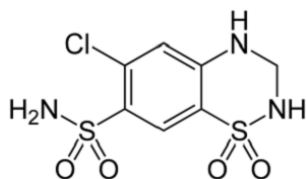
ABSTRACT

The aim of this study was to determine whether inclusion complexes between Hydroxypropyl-beta-cyclodextrin (HP β CD) and Hydrochlorothiazide (HCT) formed, and characterize these. Equimolar HCT/ HP β CD Solid system in the presence and absence of 0.2% (w/v) poloxamer188 was prepared co-evaporation and spray drying methods. A phase solubility study was done to evaluate solubility constant (KS) and complexation efficiency (CE). Improvement of KS and CE showed the additive effect of auxiliary substances (poloxamer188). 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, XRD, FTIR, SEM, DSC. These results showed that binary and ternary inclusion complexes were formed.

Keywords: HCT, HP β CD, Poloxamer188, Stability constant, Dissolution rate.

INTRODUCTION

Hydrochlorothiazide (HCT) $C_7H_8ClN_3O_4S_2$ belongs to the thiazide class of diuretics. It reduces the blood volume by acting on the kidneys to reduce sodium (Na) reabsorption in the distal convoluted tubules[1]. It is white crystalline powder, odorless with melting point 271-273°C[2]. It is practically insoluble in water and soluble in Dimethylformamide and Sparingly soluble in Methanol, soluble in Dil. Ammonia and Aq. NaOH[3].



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

Fig. 1: Chemical structure of Hydrochlorothiazide

Cyclodextrins served as a versatile carrier for poorly water soluble drugs increasing its solubility and dissolution rate through the formation of inclusion complex[4]. They are capable of alleviating the undesirable properties of drug molecule through the formation of inclusion complex. Hydroxypropyl-beta-cyclodextrin is mainly selected for its higher solubility than other cyclodextrins and this generally results in more extensive solubilization ability towards lipophilic molecule, with good safety [4,6].

In the present work, it seemed of interest to investigate the rate of poloxamer188 in improving the dissolution and solubility properties of HCT. The objective of this work was to study the interaction of HCT with HP β CD with or without presence of poloxamer188. In order to evaluate the influence of HP β CD and poloxamer188 on HCT solubility the interaction was investigated by phase solubility. The influence of HP β CD and the poloxamer188 on physicochemical properties of HCT was evaluated by preparing a solid system with equimolar quantities of HP β CD and HCT in the presence and absence of 0.2 % (w/v) poloxamer188 by co-evaporation and spray drying XRPD, DSC, SEM, FTIR was used to characterize the binary and ternary system.

MATERIALS AND METHODS

Materials

HCT was supplied by Okasa Pharmaceuticals Pvt. Ltd. Poloxamer188 was supplied by Signet chemicals Mumbai, HP β CD supplied by Gangwal Chemicals Pvt. Ltd. All other materials were of analytical reagent grade.

Methods

All experiments were carried out under subdued light to prevent photo degradation of HCT

Phase solubility

Phase solubility studies were performed according to the method reported by Higuchi Connors[5]. An excess amount of HCT was added to 10 ml distilled water containing various conc. of HP β CD (0-0.01 M) with or without fixed conc. of Poloxamer188 (0.2% w/v) in stopper tubes and mixture were shaken in rotary shaker for 72 Hrs $37 \pm 0.5^\circ\text{C}$ at 150 rpm. After achieving equilibrium the solution was filtered through 0.45 μm membrane filter paper. The sample was diluted suitably and assayed for content HCT by U.V. Spectrophotometer at 271.60 nm (Shimadzu 1700, Japan). The solubility constant and Complexation efficiency were calculated by using the following equations

$$K_s = \frac{\text{slope}}{S_o(1 - \text{slope})}$$

K_s = Stability constant

S_o = solubility of HCT in absence of CD

$$CE = \frac{\text{slope}}{1 - \text{slope}}$$

CE = Complexation Efficiency

Coevaporation method[12,13]

Equimolar amount of HCT dissolved separately in methanol and HP β CD with presence and absence of Poloxamer188 (0.2% w/v) dissolved in water. The solutions were mixed and stirred for 4 hours at room temp[7]. The solvent was removed at vacuum oven 40°C to get solid residue. The prepared binary and ternary systems were pulverized and passed to store in desiccator till further use.

Spray drying [7,8,16]

Equimolar amount of HCT and HP β CD were dissolved in methanol and water respectively. The two solutions were mixed and stirred for 4 hours at room temp[7]. The resultant solution spray dried using Lab Spray dryer (Labultima LU 222 Advanced, Mumbai, India) under following condition, inlet temp- 90°C outlet temp- 40°C , flow rate of solution 15 ml/min.

The ternary system was prepared in the same way but 0.2% w/v poloxamer188 was added in aq. HP β CD solution

Aqueous solubility[9]

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 were withdrawn filtered through whatman filter paper no.41. The filtrate analyzed spectrophotometrically 271.60 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) using the paddles[10]. Dissolution studies were carried out using 900 ml of 0.1N HCl at $37 \pm 0.5^\circ\text{C}$ at 50 rpm. At fixed time intervals (2, 5, 10, 15, 20, 30, 45 and 60 min) 5ml samples were withdrawn[3]. The dissolution medium was 5ml aliquot with 5ml fresh 0.1N HCl. The solution was immediately filtered 0.45 μm membrane filter suitably diluted and were determined spectrophotometrically 272 NM

X-ray powder diffractometry

An x-ray powder diffraction pattern was collected using Philips Analytic X-ray-PW 3710 (Holland) diffractometer with tube anode Cu over the interval $5-80^\circ/2\theta$

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 \AA) for 240s, photographs were taken 5-10 Kv voltage and appropriate magnification.

Fourier transformation-infrared spectroscopy (FTIR)

Infrared spectra were obtained using a Perkin-Elmer Spectrum one FTIR spectrometer or Jasco FTIR 4100 (Japan) using KBr disks. The samples were previously ground and mixed thoroughly with KBr. The KBr disks were prepared by compressing the powder. The scanning range was kept from 4000-650 cm^{-1} .

Differential scanning calorimetry (DSC)

DSC has been one of the most widely used calorimetric techniques to study the solid state interaction of drug with HP β CD and poloxamer188. 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 $^\circ\text{C}$ at a rate of 10 $^\circ\text{C}/\text{min}$ 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]

The solubility study plots obtained with HP β CD and in the presence and absence of poloxamer188 (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 (Ks) values than those for 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 HP β CD. The phase solubility curve obtained as A_L type according to Higuchi Connor. The increase in HCT solubility seems to be related to inclusion ability of HP β CD 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 Ks indicated that the ternary complex was more stable than binary complex. A synergistic effect on HCT solubility observed in presence of poloxamer188. The addition of poloxamer188 did not show any change in type of phase diagram but showed changes in Ks and C.E. A solubility constant of binary system Ks value of $276.4926 \pm 5.33 \text{ M}^{-1}$ indicated that interaction was weak unstable between components while in case o ternary complex the auxiliary substance poloxamer188 increase wettability and enhance the hydrogen bonds between drug and carrier and also decrease crystallinity of product[13].

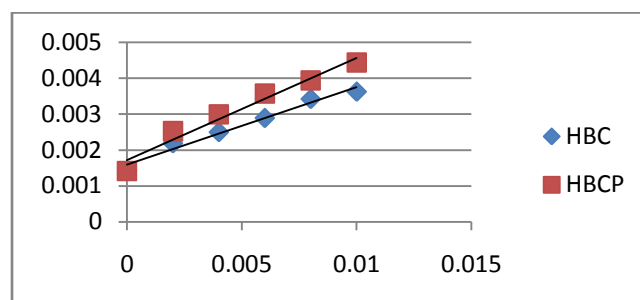


Fig. 2: Phase solubility diagram of HCT-HP β CD system in presence and absence of Poloxamer188

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 HP β CD concentrations. For a ternary system with 1% (w/v) Poloxamer188 $r^2 = 0.964$ with slope 0.284 suggested the formation of 1:1 complex. The line equations from the linear regression analysis of these systems were

$$y = 0.216x + 0.001 \text{ (Binary system)}$$

$$y = 0.284x + 0.001 \text{ (Ternary system with 1% w/v Poloxamer188)}$$

Table 1: Effect of polymer, Poloxamer188 (1% w/v) on slope of phase solubility diagrams and stability constant (Ks) for binary and ternary system of HCT with HP β CD

System	Slope	r^2	KS (M^{-1}) * Mean \pm SD	K_{TS}/K_{BS}
Drug- HP β CD	0.216	0.974	276.49 ± 5.33	---
Drug-HP β CD- Poloxamer188	0.284	0.964	$399.52 \pm 2.92^{\dagger}$	1.4497

K_{TS}/K_{BS} ratio of Ks for ternary and binary complexes; * indicates mean of three readings; S.D.: Standard deviation. \dagger p value compared to HCT- HP β CD ($p < 0.001$) i.e. significant.

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 aq. Solubility of binary and ternary complexes by said methods table 2 were indicated that there is an enhancement of aq. A solution which was high in ternary complex solubility of coevaporated ternary complexes were high.

Table 2: Aqueous solubility study of HCT with HP β CD in binary system and ternary system with 0.2% (w/v) Poloxamer188

Product code	HCT	HBC	HBCP	HBS	HBSP
Aqueous solubility (mg/ml)* mean & SD	0.69 ± 0.19	1.76 ± 0.13	4.83 ± 0.94	2.55 ± 0.17	3.09 ± 0.11

* indicates mean of three readings; S.D.: standard deviation; HCT: Hydrochlorothiazide; HBC: coevaporated binary complex; HBCP: Coevaporated ternary complex; HBS: Spray dried binary complex; HBSP: Spray dried ternary complex

Dissolution studies

The comparative in vitro dissolution profiles of HCT, coevaporated and spray dried binary and ternary complexes in 0.1 N HCl, P^H1.2 shown in fig. It is clearly shown that the dissolution profile of the pure drug sample was 59.68% after 60 min. It was interesting to note that in case of coevaporated and spray dried binary complex. The dissolution complex was more than 90% after 15min. And that of ternary complex showed the dissolution profile for coevaporated complex and spray dried complex more than 90% after 5min. Both the spray dried and coevaporated complexes showed higher dissolution rate. In the presence of HP β CD the hydrophobic portion of drug molecule can be interacted with hydrophobic portion cyclodextrin 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. Due to decrease of its crystallinity and high degree of interaction between HCT and HP β CD it displayed highest degree of dissolution coevaporated complex were characterized by XRD, FTIR, SEM and it was compared with pure HCT[7,8].

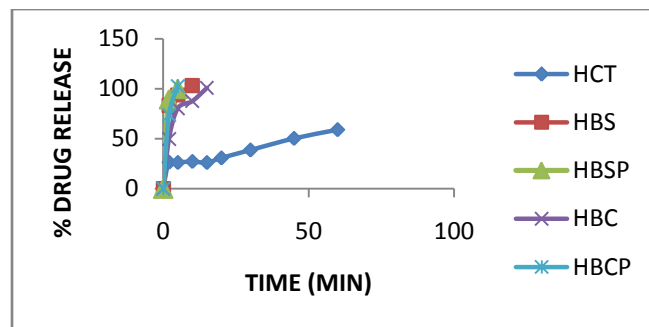


Fig. 3: The dissolution diagram of HCT- HP β CD binary system and with Poloxamer188 ternary system at 37 \pm 0.5 $^{\circ}$ C

HBC-Coevaporated binary complex, HBCP-Coevaporated ternary complex, HBS- Spray dried binary complex, HBSP- Spray dried ternary complex

Table 3: The dissolution time of HCT in 0.1N HCl at 37 \pm 0.5 $^{\circ}$ C from binary and ternary system

Systems	DP ₂ (%)	DP ₅ (%)	DP ₁₀ (%)	DE ₂	DE ₅
HCT	26.43 \pm 1.2	26.22 \pm 2.3	27.221.32	13.21 \pm 1.10	18.44 \pm 1.1
HBC	49.78 \pm 2.2	80.34 \pm 3.2	87.65 \pm 3.2	24.89 \pm 1.11	44.01 \pm 2.22
HBS	70.31 \pm 1.8	100 \pm 3.8	-	41.62 \pm 0.98	64.26 \pm 2.1
HBCP	73.68 \pm 1.46	100 \pm 2.8	-	36.84 \pm 1.32	60.27 \pm 2.7
HBSP	89.43 \pm 1.77	99.93 \pm 1.98	-	44.71 \pm 1.2	65.75 \pm 1.5

DE-Drug efficiency, DP-Percentage drug release, HCT- Hydrochlorothiazide, HBC- Coevaporated binary complex, HBCP- Coevaporated ternary complex, HBS- Spray Dried binary complex, HBSP- Spray dried ternary complex

X-ray powder diffractometry(XRD)study

The XRD pattern of pure HCT, HP β CD & poloxamer188 and their corresponding binary and ternary coevaporated complexes with HCT shown in fig. It is useful method for the detection of complexation of powder of crystalline nature. The XRPD pattern of HCT showed its crystalline nature. These intense and sharp peaks were comparing with binary and ternary coevaporated complexes. The lowered intensity band diffraction peaks and overlapping of same peaks with HP β CD(fig.4) It was clearly showed that reduction

of particle sizes and during preparation of solid complexes by of coevaporated method. Semi crystalline nature showed in binary system. In ternary system it was clearly showed that the crystallinity of the drug is decreased and amorphous nature is increased. The diffraction pattern of HCT & HPCP intense and highest peaks are disappeared and XRPD pattern were diffused indicating amorphous were state reached by ternary complex by coevaporated method SEM studies also supported the same hypothesis which was confirmed by X-ray powder diffraction.[7,8,9],[15]

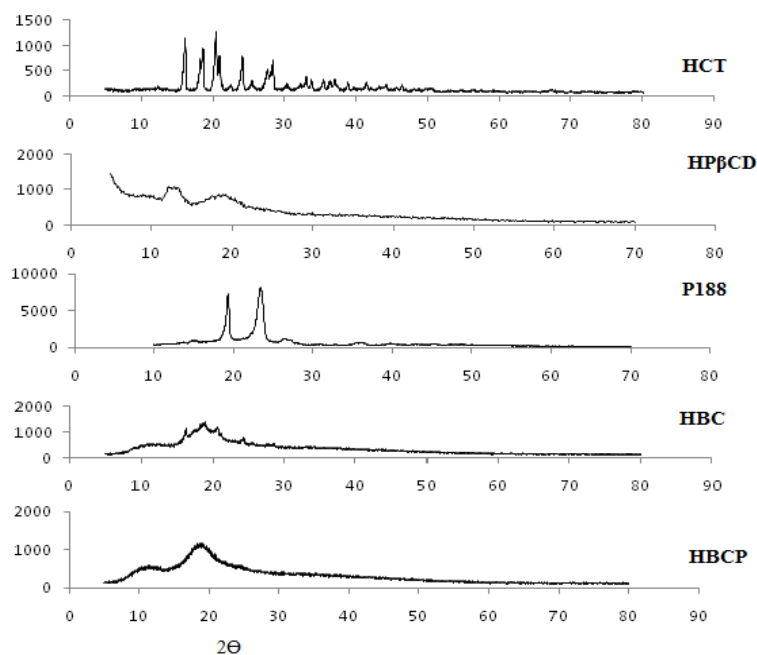


Fig. 4: XRD pattern of HCT, Coevaporated binary (HBC) and ternary (HBCP) system

HCT: Hydrochlorothiazide; HP β CD: Hydroxypropyl-betacyclodextrin; P188: Poloxamer188; HBC: Coevaporated binary complex; HBCP: Coevaporated ternary complex

Scanning electron microscopy (SEM)[7,8,15]

SEM images of the HCT, binary and ternary complexes prepared by co-evaporation 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 coevaporated binary and ternary complexes showed drastic change in the original morphology and shape. In binary system (HBC) it was clearly indicated there was no differentiation between drug and HP β CD but on the surface of complex some crystalline structure were found. In ternary system (HBCP) it was found that smooth amorphous surface of drug and lost of their original shape.

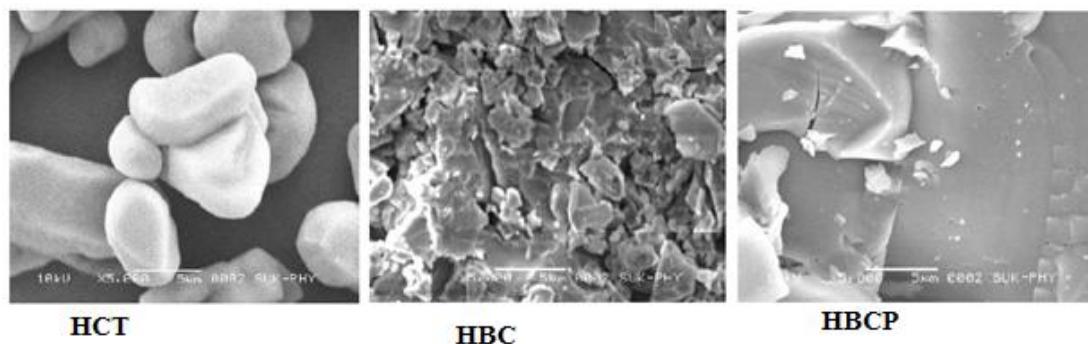


Fig. 5: Scanning electron microscopy of HCT, coevaporated binary(HBC) and ternary(HBCP) system

FTIR Study

The IR spectra of HCT, HP β CD, Poloxamer188, coevaporated binary (HBC) and ternary (HBCP) systems are shown in fig.5. 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 band at 3360.876 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[7,15]. The IR spectrum HP β CD shows prominent peaks at 3399.36 cm^{-1} (O-H), 2929.24 cm^{-1} (C-H), 1650.74 cm^{-1} (H-O-H bending), 1034.69 cm^{-1} (C-O-C)[9,13].

Host-guest interaction HCT aromatic moiety and HP β CD cavity observed in spectra. In binary system showed interaction with HP β CD, HCT and Poloxamer188. All binary and ternary systems exhibited significant alteration (attenuation, broadening, frequency shifts and/or disappearance) in characteristic bonds of either drug or HP β CD demonstrating modifications in drug or HP β CD environment.

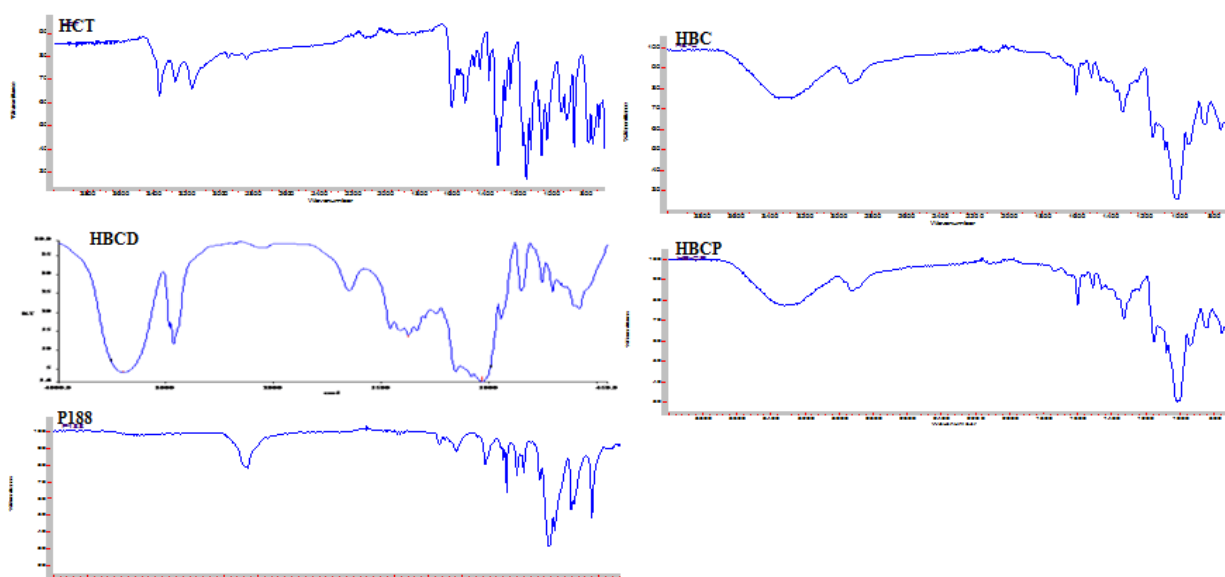


Fig. 6: FTIR spectra of HCT, HP β CD, Poloxamer188, Coevaporated binary(HBC) and ternary(HBCP) system

In binary system peaks of HCT 3360.876 cm^{-1} , 3167.094 cm^{-1} were disappeared and peak at 3266.114 cm^{-1} found to shifted 3272.012 cm^{-1} . The broad peaks of HP β CD at 3399.36 cm^{-1} was disappeared while peak at 2929.24 were shifted to 2923.598

FTIR of all the ternary system exhibited similar behavior as that of binary system with change peak intensities of 3265.114 cm^{-1} in HP β CD to 3281.916 cm^{-1} respectively characteristics peak in HP β CD at 3399.36 cm^{-1} , 2929.24 cm^{-1} were shifted to 3343.943 cm^{-1} ,

2928.749 cm^{-1} . All the binary peaks in ternary and binary system were found to be smoothed and did not show new peaks

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 endothermic peak at 90.20°C was observed for the amorphous HP β CD which was related to loss of water molecule i.e. dehydration process. Pure Poloxamer188 at 55.97°C indicates the presence of separate crystalline phase that found to be diffused in their ternary microcomplexes.

An evidence for formation of inclusion complexes between HCT and HP β CD was the change in shape of melting peak of HCT and its shift towards higher temp[7,9],[11,14,15]!. (e.g. for complex HBC,HBCP: the peaks corresponding to melting points of drug substance were observed around 275.93°C,274.17°C as a result of inclusion of drug into the cyclodextrin cavity)

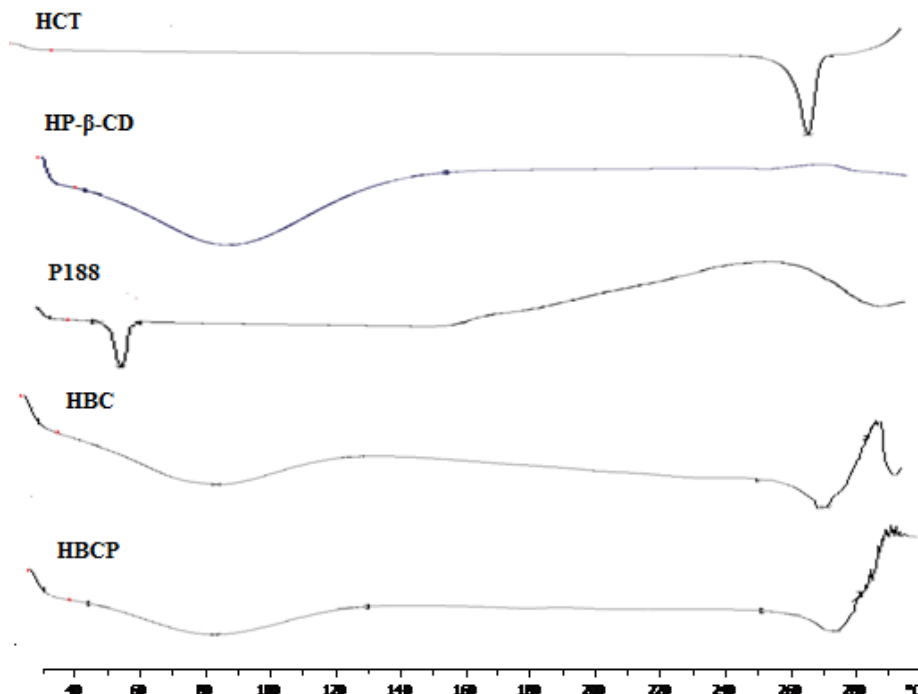


Fig. 7: DSC thermograms of HCT, HP β CD, Poloxamer188, Coevaporated binary(HBC) and ternary (HBCP) complexes.

CONCLUSION

This study has demonstrated improving the dissolution performance of HCT by its complexation with HP β CD in presence of poloxamer188 using co-evaporation and spray drying technique. Poloxamer188 showed a more pronounced effect on the enhancement of aqueous solubility and faster rate than binary complex (HPC). 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 poloxamer188 in ternary or co-complexes of HP β CD beneficial in terms of improvement in CE, rate of complex formation and enhancement of poorly water soluble HCT

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