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Multicomponent cyclodextrin system for improvement of solubility and dissolution rate of poorly water soluble drug [☆]

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ABSTRACT

The purpose of the present study was to investigate the interaction of Cinnarizine (CIN) with Hydroxypropyl- β -Cyclodextrin (HP β CD) in the presence of Hydroxy Acids (HA). Various binary and ternary systems of CIN with HP β CD and HA were prepared by kneading and coevaporation methods. For the ternary systems, HA were tried in three different concentrations. The interaction in solution phase was studied in detail by the phase solubility method, and the solid phase interactions were characterized by Fourier Transform Infrared (FTIR) spectroscopy, Differential Scanning Calorimetry (DSC), X-Ray Diffractometry (XRD), Scanning Electron Microscopy (SEM) and Proton Nuclear Magnetic Resonance (¹H-NMR). Phase solubility revealed the positive effect of HA on the complexation of CIN with HP β CD. Solid phase characterization confirmed the formation of inclusion complex in the ternary systems. Solubility and dissolution studies illustrated that out of three different concentrations tried, HA were most effective at the 1 M concentration level. Ternary systems were very effective in improving the solubility as well as dissolution profile of CIN than the CIN-HP β CD binary systems. FTIR, ¹H-NMR and Molecular docking studies gave some insight at molecular level that actually which part of CIN was interacting with the HP β CD. Molecular docking and free energy calculation even enlighten the role of tartaric acid in increasing solubility of CIN in the ternary system.

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1. Introduction

Cinnarizine (CIN) [1-(diphenylmethyl)-4-(3-phenyl-2-propenyl) piperazine] is an antihistaminic drug, which is mainly used for the control of vomiting due to the motion sickness. It acts by interfering with the signal transmission between vestibular apparatus of the inner ear motion receptor and the vomiting center of the hypothalamus. The disparity of signal processing between inner ear motion receptors and the visual senses is abolished, so that the confusion of brain whether the individual is moving or standing is reduced.

According to Biopharmaceutical Classification System (BCS), CIN falls under Class II [1]. It is practically insoluble in water but has a log P value of 5.8, which suggests the high lipophilicity and high permeability of CIN. Bioavailability of CIN is dissolution rate limited because of its poor aqueous solubility. Therefore, it is possible to improve the bioavailability of CIN by improving its intrinsic solubility and dissolution rate. Tokumura et al. reported the inclusion complexation of CIN with β -cyclodextrin (β CD), which was confirmed by the solubility method, powder X-Ray Diffractometry (XRD), Differential Scanning Calorimetry (DSC) and Proton Nuclear Magnetic Resonance ($^1\text{H-NMR}$) spectroscopy [2]. Tokumura et al. also reported the enhancement of bioavailability of CIN from its β CD complex on oral administration with L-Phenylalanine as a competing agent [3]. Furthermore, Jarvinen et al. reported the improvement in the oral bioavailability of CIN when complexed with β CD, Hydroxypropyl- β -Cyclodextrin (HP β CD) and Sulphobutylether- β -Cyclodextrin (SBE-4- β CD) [4]. However, to the best of our knowledge, the solid states of the various CIN-HP β CD complexes have not been previously characterized.

Cyclodextrins (CDs), cyclic oligosaccharide with a hydrophilic outer surface and hydrophobic central cavity, can form a stable complex with a variety of drugs [5,6]. CD complexation has been established as an effective method for the improvement of solubility and bioavailability of the many hydrophobic drug molecules. From various CDs, β CD is the most frequently used CD to modify the physicochemical properties and improve the solubility and bioavailability of many drugs due to its low cost [7]. However, its low intrinsic solubility and nephrotoxicity have confined the application of β CD. Many chemical derivatives of β CD are available in the market that possess high solubility and low toxicity than that of its parent. One of those derivatives is HP β CD, which shows a 50 fold improvement in the solubility [8] and low toxicity when given parenterally [9]. It is highly biocompatible and pharmacologically inactive, which is why many researchers have used HP β CD as a safe and effective material to improve the solubility and bioavailability of the hydrophobic drug [10–13].

From the work of many researchers, it can be observed that the addition of a suitable auxiliary substance significantly improves cyclodextrin solubilizing and complexing abilities by multicomponent complex formation [14]. For example, a small amount of water soluble polymer [15,16] or co-solvent [17] can positively influence the solubility of nonpolar solutes. On the other hand, low molecular weight Hydroxy Acids (HA) have been reported to intensify the cyclodextrin's solubilizing power towards basic drug. Hydroxy acids showed synergistic mutual enhancement in the solubility of host as well as cy-

clodextrin where low solubility cyclodextrin such as β CD is used. This can be explained by the specific interaction of HA with hydrogen bond system of host or by alteration of hydrogen bond network of surrounding water molecules [18–24]. Wang et al. reported the use of lecithin for improving the solubility and stability of dihydroartemisinin [25].

The objective of the present study was to investigate the interaction of CIN with HP β CD in the presence and absence of HA such as anhydrous Citric Acid (CA) and anhydrous Tartaric Acid (TA) as a co-complexing agent. Formation of an inclusion complex was investigated by a phase-solubility method. Solid state interactions of the binary and ternary systems were characterized by Fourier Transform Infrared Spectroscopy (FTIR), DSC, XRD, Scanning Electron Microscopy (SEM) and $^1\text{H-NMR}$.

2. Materials and methods

2.1. Materials

CIN was kindly gifted by Hikal Ltd. (Mumbai, India); HP β CD (MW-1380) was gifted by the Signet Chemical Corporation (India). Anhydrous citric acid (CA) and anhydrous tartaric acid (TA) were obtained from Sigma-Aldrich (India). These chemicals were used as received without further treatment. All other reagents were of analytical reagent grade purity. Double distilled water (DW) was used throughout the study.

2.2. Phase solubility studies

Phase solubility studies of CIN with HP β CD in the absence and presence of HA were performed in distilled water according to the Higuchi and Connors method [26]. The experiments were carried out in triplicate. An excess amount of CIN was added to 5 ml of DW or 1% HA solution (CA and TA solutions) containing various concentrations of HP β CD (0–0.1 M) in glass vials that were subsequently tightly closed and mechanically shaken at $25 \pm 2^\circ\text{C}$ for 48 h. The suspensions were filtered using a 0.45- μm membrane filter. The filtrate were suitably diluted and spectrophotometrically analyzed at 254 nm for CIN content. The presence of HP β CD did not interfere with the spectrophotometric assay of the drug. The phase solubility diagrams were constructed by plotting graph of the concentration of CIN against the concentration of HP β CD. The apparent stability constant (K_s) and complexation efficiency (C_{eff}) were calculated from the slope of the linear plot of the phase solubility diagram according to Eqs. (1) and (2),

$$K_s = \frac{\text{Slope}}{S_0(1 - \text{Slope})} \quad (1)$$

where S_0 is the solubility of drug in absence of CDs

$$C_{eff} = \frac{\text{Slope}}{(1 - \text{Slope})} \quad (2)$$

2.3. Job's plot

Job's plot otherwise known as the method of continuous variation was used to ascertain the stoichiometry for CIN:HP β CD

Table 1 – Ratios of CIN, HP β CD and HA for various binary and ternary systems.

System	Molar concentration			
	Drug	HP β CD	CA	TA
A1 (Binary)	1	1	–	–
A2 (Ternary)	1	1	0.5	–
A3 (Ternary)	1	1	1	–
A4 (Ternary)	1	1	1.5	–
A5 (Ternary)	1	1	–	0.5
A6 (Ternary)	1	1	–	1
A7 (Ternary)	1	1	–	1.5
A8 (Binary)	1	–	1	–
A9 (Binary)	1	–	–	1

complexation. Varying moles of CIN were added to the solution for increasing the molar concentration of HP β CD. The total moles of CIN and HP β CD were kept constant for all the solutions. Solutions were kept at 37 °C for 48 h on an orbital shaker. After 48 h, samples were centrifuged at 10,000 rpm for 10 min to settle the free drug, and the supernatant was filtered through a 0.45- μ m filter and analyzed using UV–vis spectroscopy for the amount of CIN solubilized, which was then plotted against the mole fraction of HP β CD.

2.4. Preparation of solid systems

Various binary and ternary systems were prepared as per Table 1 by the following methods. A1 is a binary system of CIN and HP β CD, A2–A4 are CIN–HP β CD–citric acid ternary systems, A5–A7 are CIN–HP β CD–tartaric acid ternary systems, whereas A9 and A10 are CIN–citric acid and CIN–tartaric acid binary systems, respectively.

2.4.1. Physical mixture (PM)

PM was prepared by homogeneously mixing the exactly weighed components previously sieved through sieve no. 80.

2.4.2. Kneading (KN) method

Components were weighed and dry triturated in a mortar for 15 min. The mixture was then kneaded with 76% (v/v) Ethanol for about 45 min. During this process, an appropriate quantity of the solvent was added in order to maintain a suitable consistency required for kneading. The product was dried at 50 °C and kept under vacuum for 24 h. The dried mass was then passed through sieve no. 80.

2.4.3. Co-evaporation (CE) method

Weighed amount of components was added in a required amount of solvent (76% v/v ethanol) and sonicated for 5 min on a bath sonicator. This solution was subjected to coevaporation at temperature 60 °C using a rotary vacuum evaporator till all the solvent got evaporated. The product thus obtained was further dried at 50 °C and kept under vacuum for 24 h. After drying, the product was sieved through a 80 mesh sieve.

In addition, physical mixtures of CIN with equimolar concentration of CA (A8) or TA (A9) were prepared for a comparison purpose.

2.5. Drug content determination

Amounts of binary and ternary systems equivalent to 50 mg of CIN were weighed. The individual samples were added into 50 ml of methanol and suspensions were sonicated for 45 min. Volumes were made up to 100 ml with DW and filtered. These samples were further diluted appropriately with DW and analyzed spectrophotometrically at 254 nm. The drug content of all the systems was determined.

2.6. Evaluation and characterization of solid systems

2.6.1. Aqueous solubility studies

Excess amount of solid system was added in 5 ml DW in a vial. The suspension was shaken at room temperature for 48 h on a mechanical shaker. The suspension was centrifuged at 10000 rpm for 10 min, and the supernatant was filtered through the 0.45 μ m membrane filter. The dissolved amount of drug was assayed spectrophotometrically at 254 nm.

2.6.2. Fourier Transform Infrared (FTIR) spectroscopy study

FTIR spectra of CIN, HP β CD and all solid systems were recorded on Jasco-700 FT-IR spectrophotometer using KBr discs. The instrument was operated under dry air purge and the scans were collected at scanning speed of 2 mm/s with resolution of 4 cm^{-1} over the region of 4000–400 cm^{-1} . The scans were evaluated for the presence of principle peaks of drug, the shifting and masking of drug peaks due to cyclodextrin and the appearance of new peaks.

2.6.3. Differential scanning calorimetric (DSC) analysis

The DSC curves of pure materials and binary systems were recorded on SII EXSTAR DSC 6220 model of differential scanning calorimeter. The thermal behavior was studied by heating all samples (10 mg) in sealed aluminum pans, using alumina powder as reference, over a temperature range of 30–300 °C at a heating rate of 10 °C/min. Dry nitrogen was used as a purge gas. The results of pure materials and solid systems were evaluated for shift and change in the intensity of peaks.

2.6.4. X-ray diffraction (XRD) studies

Powder X-ray diffraction patterns were recorded using Phillips P Analytical X'Pert PRO powder X-ray diffractometer. The scanning rate employed was 1°/min, and the samples were analyzed between 2 θ angles of over 7–45°. The powder diffraction patterns of CIN, HP β CD and solid systems were recorded.

2.6.5. Scanning Electron Microscopy (SEM)

The surface morphologies of the drug and various solid systems were examined by a Philips 500 scanning electron microscope.

2.6.6. Proton nuclear magnetic resonance ($^1\text{H-NMR}$) spectroscopy

H^+ spectra were taken at 25 °C on a Variant Mercury Plus model operating at a proton frequency 400 MHz using 5 mm sample tubes. DMSO was used as a solvent. Chemical shifts were expressed in ppm downfield from the signal (0 ppm) of TMS.

2.6.7. Molecular modeling studies of binary and ternary systems

The molecular modeling studies of CIN with HP β CD in the presence and absence of TA were carried out using the Schrodinger software suite (Schrodinger, LLC, New York) in the Maetsro module (version 11.1).

Structure collection: CIN and TA structures were drawn and optimized using Ligprep module. Finally, the geometry optimization was carried out using the OPLS2005 force field. HP β CD structure was drawn by adding 2-hydroxy propyl chain to native β CD structure imported from PDB (PDB ID: 1BFN). Geometry of HP β CD was optimized using Macro model module.

Generation of supramolecular inclusion complex models: The Glide module was used for generating HP- β -CD inclusion complexes. The grid was generated using the Glide Grid Generation panel in Glide. For generating HP β CD binary supramolecular inclusion complex, CIN was docked with standard precision (SP) mode on HP β CD. The ternary supramolecular inclusion complex was generated by docking the binary inclusion complex with TA in SP mode.

Binding affinity calculation: The binding affinity " ΔG " was calculated using the Prime MM-GBSA module (version 4.5, Schrodinger), which calculates the free energy change upon formation of the complex in comparison to total individual energy based on change in the solvent accessible surface area [27].

2.6.8. Dissolution studies

The dissolution rate studies of CIN alone and from various solid systems were performed using a USP XXIII dissolution apparatus type-II (6 stations VDA-6DR Veego Scientific, India) at 37 ± 0.5 °C stirring at 75 rpm. In total, 25 mg of CIN or its equivalent amount of solid system was added to 900 ml of DW. The aliquots of 5 ml were withdrawn at time intervals of 10, 20, 30, 45, 60, 90, 120 and 180 min, diluted appropriately and analyzed spectrophotometrically at 254 nm.

3. Results and discussion

3.1. Phase solubility studies

Result for the phase solubility of CIN with HP β CD shown in Fig. 1 revealed that the solubility of CIN was increased as a function of HP β CD concentration. Phase solubility diagram of CIN with HP β CD gave a curve that could be misinterpreted as A_p type of curve, but Loftsson et al. classified phase solubility diagram of CIN with HP β CD as A_L^- type of curve [28]. Yalkowsky explained that CIN, being very lipophilic and water insoluble drug with large aromatic planar regions, promotes self-association in aqueous solution [29]. These characteristics of CIN leads to A_L^- type phase solubility profile. Stability constant (K_s) was found to be 558.35 M^{-1} , which was calculated using the slope of terminal linear portion. The phase solubility diagrams of CIN with HP β CD in 1% HA (CA or TA) solution shown in Fig. 2 were of A_L type. However, the addition of HA improved the solubility of CIN and the complexation efficiency (Table 2); the stability constant (K_s) was found to be reduced. This decrease in stability constant was explained

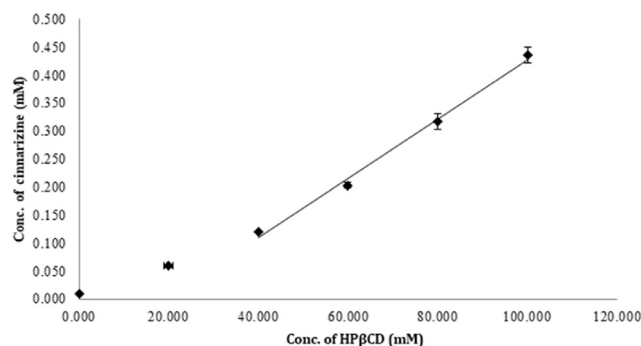


Fig. 1 – Phase solubility diagram of CIN-HP β CD binary system. Data are represented as mean \pm SD ($n = 3$).

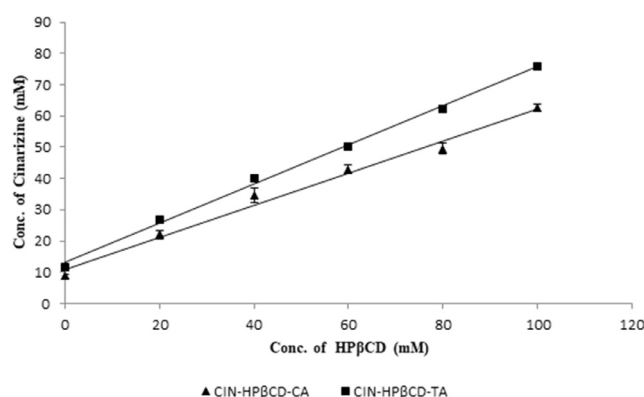


Fig. 2 – Phase solubility diagram of CIN-HP β CD-HA ternary systems. Data are represented as mean \pm SD ($n = 3$).

Table 2 – Values of the stability constant (K_s) and complexation efficiency (C_{eff}) of different binary and ternary systems.

System	C_{eff}	$K_s \text{ (M}^{-1}\text{)}$
CIN-HP β CD binary system	0.00503	558.35
CIN-HP β CD-CA ternary system	1.058	96.14
CIN-HP β CD-TA ternary system	1.68	127.73

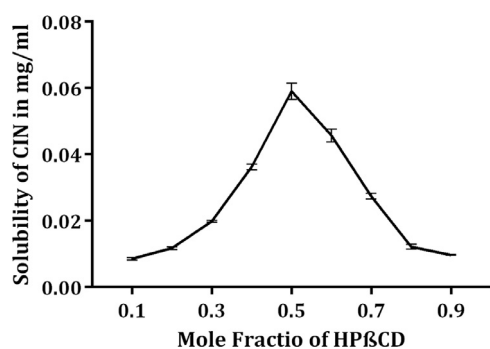
on the basis of the higher initial drug solubility due to an increased ionization of CIN in the presence of HA with consequent less affinity to the apolar cavity. Although the stability of CD complexes of un-ionized drugs is usually better than those of their anionic counterparts, the achieved total solubility (free ionized drug + free un-ionized drug + ionized drug complex + unionized drug complex) usually increases [18].

3.2. Job's plot

The graph of mole fraction of HP β CD vs. amount of CIN solubilized (mg/ml) in Fig. 3 shows a bell shape curve with maxima at 0.5 mol fraction of HP β CD. This represents that maximum CIN is solubilized when CIN:HP β CD ratio is 1:1. This con-

Table 3 – Aqueous solubility of pure drug and various solid systems.

Pure Drug (CIN) Solid Systems	Drug content	Aqueous solubility of CIN (mg/ml)		
		PM	KN	CE
Pure Drug (CIN)	99.98 ± 0.11%		0.0056	
A1 (Binary)	98.65 ± 0.21%	0.0585	0.2373	0.2907
A2 (Ternary)	99.32 ± 0.19%	1.256	2.587	3.403
A3 (Ternary)	101.65 ± 0.2%	2.203	5.251	6.117
A4 (Ternary)	99.65 ± 0.16%	2.352	5.395	6.035
A5 (Ternary)	98.23 ± 0.19%	1.184	3.197	3.368
A6 (Ternary)	101.93 ± 0.22%	2.520	6.304	6.851
A7 (Ternary)	98.92 ± 0.32%	2.568	6.405	6.891
A8 (Binary)	99.39 ± 0.29%	0.835		
A9 (Binary)	100.39 ± 0.13%	0.949		

**Fig. 3 – Job's plot for CIN:HPβCD complex.**

firm the stoichiometry of CIN:HPβCD, which was determined by the phase solubility curve.

3.3. Drug content determination

The results of the assay indicated that the content in the various solid systems prepared by different methods was in the range of 98%–102%. The drug content of various binary and ternary systems is presented in Table 3.

3.4. Evaluation and characterization of solid systems

3.4.1. Aqueous solubility studies

From the results of solubility studies (Table 3), it was evident that HPβCD helped in improving the aqueous solubility of CIN. PM of A1 binary system showed 10.44 times increment in the solubility compared to the pure drug; this can be attributed to the enhanced wettability of CIN by HPβCD. However, the A1 binary system formed by the CE method showed 51.91 times increment in the solubility in contrast to 42.38 times increment in the solubility by the KN method. On the contrary, ternary systems showed an enormous increase in the aqueous solubility of CIN compared to pure drug as well as binary systems. Among the ternary systems prepared with various concentrations of HA (0.5, 1 and 1.5 mol), the system with 1 mol of HA showed optimum results. Increasing HA concentration in the ternary system from 1 mol to 1.5 mol did not

show any drastic improvement in the solubility as it was seen when the concentration of HA was increased from 0.5 mol to 1 mol. In ternary systems, even PM was observed to increase the solubility of the drug due to ionization, but KN and CE showed better results than PM, suggesting the complex formation. PM of A8 and A9 binary systems showed solubility lesser than all ternary systems (Table 3), which concluded that solubility enhancement was not only because of the ionization of the drug but HPβCD also played an important role in the enhancement of the solubility. CE product of A6 ternary system showed an optimum result among all ternary systems, giving 1223.39 times increment in the aqueous solubility of CIN.

Because it was evident from the aqueous solubility studies that the ternary system containing 1:1:1 molar ratios of CIN, HPβCD and HA was giving optimum results, further characterization was done for the binary system and for A3 and A6 ternary systems.

3.4.2. Fourier Transform Infrared (FTIR) spectroscopy study

FTIR spectra of CIN, HPβCD and A1 binary systems are presented in Fig. 4A. The FTIR spectrum of CIN showed principle absorption peaks at 702.04 cm⁻¹ and 748.33 cm⁻¹ (C–H bending of aromatic ring), 962.41 cm⁻¹ and 997.13 cm⁻¹ (C–H bending of an alkene), 1135.99 cm⁻¹ (C–N bending of a piperazine ring) 2958.60 cm⁻¹ and 2806.23 cm⁻¹ (C–H stretching of an alkane). The IR spectrum of the PM of A1 binary system was found to be a superimposition of the two parent compounds with the peaks of both the substances appearing with some peaks of lower intensities. Slight shifts were observed in certain peaks, mainly 702.04, 748.33 and 2806.23 cm⁻¹ of CIN, indicating an interaction between the CIN and cyclodextrin. In the case of A1 binary systems prepared by the KN and CE methods, there was even further decrease in the intensities of the peaks of the drug, but the peaks were still visible, indicating an incomplete complex formation. No new peak was observed in the FTIR spectrum of binary systems, confirming that there is no chemical interaction and no new covalent bond has formed between the CIN and CDs. Fig. 4B and C shows the FTIR spectra of A3 and A6 ternary systems. In both the ternary systems, PM was just the superimposition of individual component, whereas the KN and CE products showed shift in the peaks, which was similar to the binary systems pointing out that HA was not causing any chemical change in the drug.

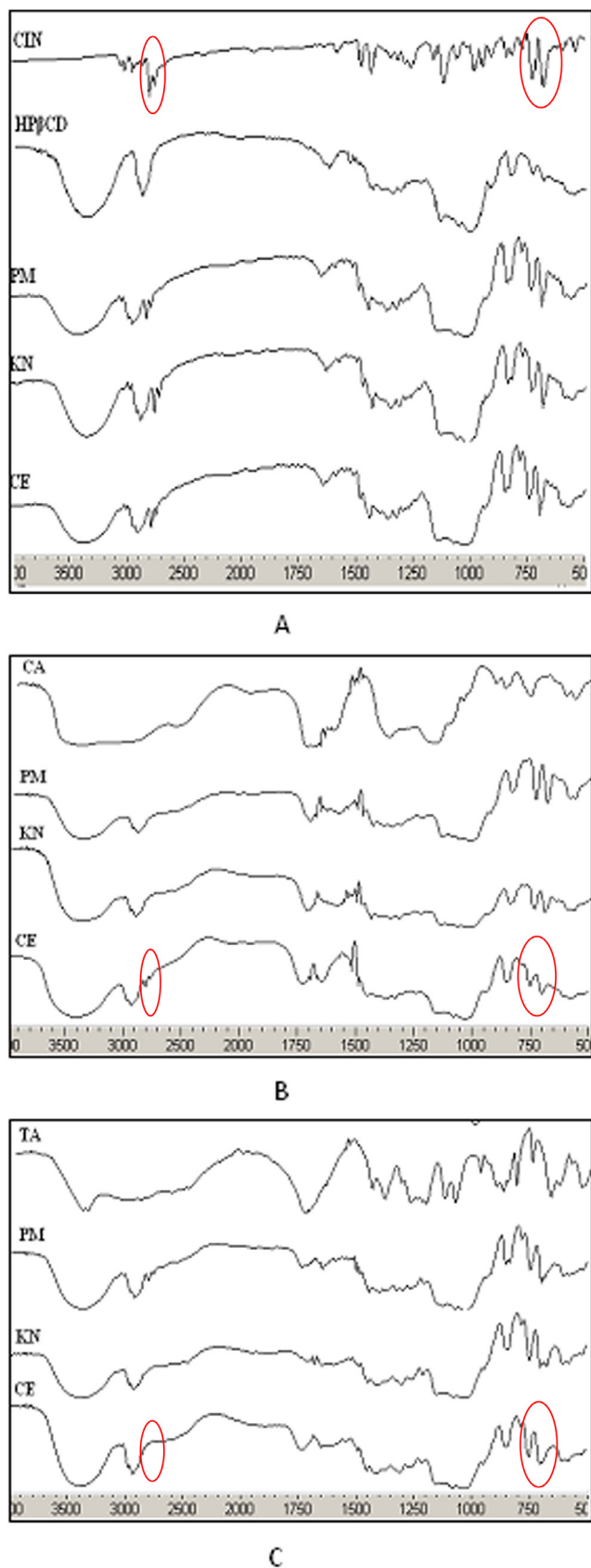


Fig. 4 – FTIR spectra of A1 binary systems (A), A3 ternary system (B) and A6 ternary system (C).

Peaks at 702.04 and 748.33 cm⁻¹ were shifted, and the intensities of these peaks were much reduced, stating the involvement of an aromatic ring in the inclusion process. The peak at 2806.23 cm⁻¹ corresponding to the C–H stretching of the alkane was missing, which can be attributed to the restricted rotation of CIN inside the HPβCD cavity.

3.4.3. Differential scanning calorimetric (DSC) analysis

DSC has been proven to be a much powerful analytical tool in the characterization of solid-state interaction between the drug and cyclodextrin. Fig. 5A shows the DSC thermogram of CIN, HPβCD and A1 binary systems. DSC thermogram of CIN showed a sharp endothermic peak at 121.1 °C, representing its melting point, which was in accordance with the observation of Kalava et al. [30]. A broad endothermic peak at about 122 °C was observed for HPβCD, indicating the loss of a water molecule. The thermograms of PM, KN and CE products in case of A1 binary system were just the superimposition of the starting material with a decrease in the peak intensity because of the dilution of the drug with HPβCD and slight shifting of CIN peaks to 120.4, 121.5 and 120.3 °C. This may be the indication of some drug–CD interaction, but it did not represent the complete inclusion of CIN in CD cavity and thus the formation of a true complex. Enthalpy of fusion for CIN has also been reduced in the order of CE > KN > PM for binary systems. However, in the case of both A3 and A6 ternary systems (Figs. 5B and 4C), PM retained small and slightly broader peaks for CIN, while those were completely disappeared in the KN and CE products. Inclusion of CIN in cyclodextrin cavity forming a true inclusion complex prevented the recrystallization of CIN and kept it in an amorphous form, which explains the disappearance of the characteristic melting point of CIN in KN and CE products of A3 and A6.

3.4.4. X-ray diffraction (XRD) studies

The XRD pattern of CIN showed many characteristic intense peaks that represented the crystalline nature of the drug; on the other hand, HPβCD showed a diffused pattern. Fig. 6 shows the XRD spectra of various solid systems. Diffraction pattern for PM of A1 binary system as well as A3 and A6 ternary systems retained most of the peaks of CIN with a slight change in the peak location and reduction in the peak intensity due to the dilution of the drug with HPβCD. On the other hand, diffraction patterns of KN and CE products of A1 binary system were more diffused and the intensities of the characteristic peaks of CIN were further reduced but still visible, which indicated the partial amorphization of the drug. However, KN and CE products of A3 and A6 ternary systems showed a completely diffused diffractogram, implying complete amorphization of the drug. Similar results were obtained by Fernandes et al. while working with nicardipine–cyclodextrin complexes [31].

3.4.5. Scanning Electron Microscopy (SEM)

Fig. 7 illustrates the SEM micrograph of the CIN, HPβCD and various solid systems. CIN (7A) was observed to have defined parallelogram shaped crystals that can be easily identified, whereas HPβCD (7B) showed shrunken spherical particles resembling a bowling ball [7]. The PM of A1 binary system (7C)

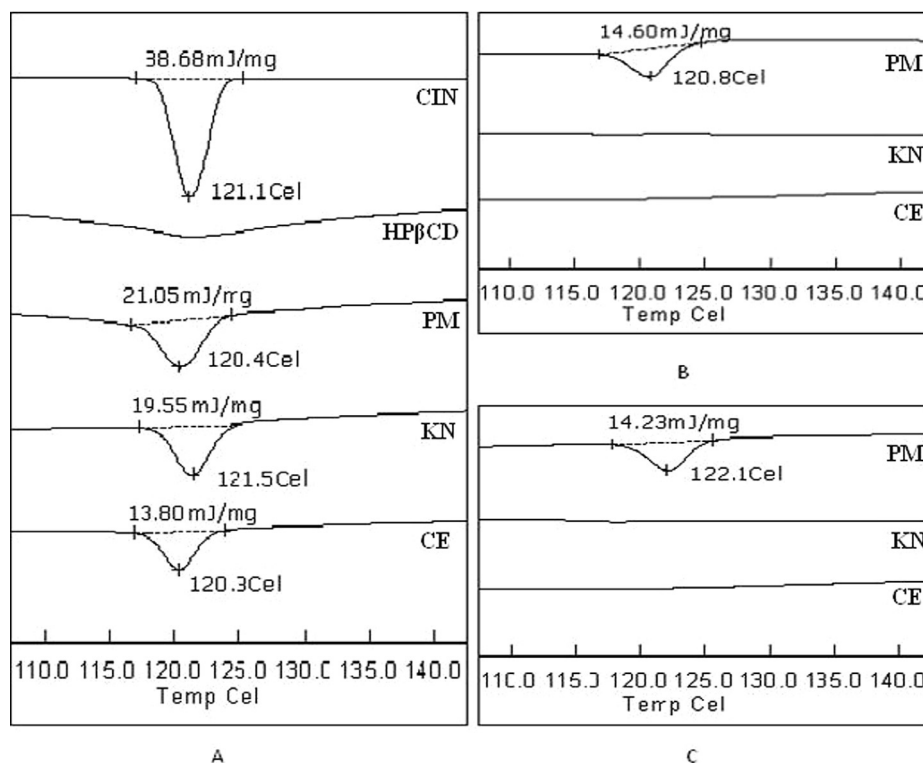


Fig. 5 – DSC thermogram of A1 binary systems (A), A3 ternary system (B) and A6 ternary system (C).

showed the presence of CIN crystals mixed with the particles of HP β CD. The size of crystals has reduced because of the processing. Comparing PM of A1 binary system with pure CIN and HP β CD crystals, it can be noted that there is no apparent interaction between drug and CD in the solid state. In the CE product of A1 binary system (7D), some agglomerates were seen with sparse unmodified CIN crystals. This suggested that though there was some interaction between the CIN and CD after co-evaporation, complete complexation was lacking. PM of A3 and A6 ternary systems (Fig. 7E and F, respectively) showed the presence of unmodified CIN crystals, which proved that there was no interaction between the components in PM. However, in CE product of A3 and A6 ternary systems, (Fig. 7G and H, respectively), the parallelogram crystal structure of CIN was missing and more agglomerated and the amorphous structure was identifiable, concluding the formation of complexes.

3.4.6. Proton nuclear magnetic resonance ($^1\text{H-NMR}$) spectroscopy

The formation of inclusion complexes can be proved from changes in the chemical shifts of the drug and the CD protons in $^1\text{H-NMR}$ spectra. In the present case, the induced shifts were calculated by the following equation: $\Delta\delta = \Delta\delta(\text{free}) - \Delta\delta(\text{complex})$. In this convention, positive and negative signs show upfield and downfield shifts, respectively. Inclusion of drug moiety inside the HP β CD cavity changes the chemical shift values of protons H3 and H5 of HP β CD, which are located inside the CD cavity. Sometimes, H6 proton is also affected due to its presence on the rim of the CD cavity.

The $^1\text{H-NMR}$ spectra of pure CIN, HP β CD, co-evaporated products of CIN-HP β CD binary system, CIN-HP β CD-citric acid ternary system and CIN-HP β CD-tartaric acid ternary system are shown in Fig. 8 with specific protons assigned to CIN (protons 1, 2a, 2b, 3a, 3b, 4 and 5) and HP β CD (protons H1, H3, H5 and H6), which were affected in the process of the formation of a true inclusion complex. The chemical shift values of various HP β CD protons in the free and complexed forms are shown in Table 4. The upfield changes in the chemical shifts of the H3 and H5 protons are noted for CE product of A1 binary system, whereas protons H1 and H6 showed minimal shifting and the peaks of protons H2 and H4 were indistinguishable due to the overlapping. In CE products of A3 and A6 ternary systems also, there was an upfield shift noted for protons H3, H5 and H6, confirming the inclusion of CIN in hydrophobic cavity of HP β CD. Chemical shift of proton H1 was not much affected, whereas peaks of protons H2 and H4 were indistinguishable due to overlapping.

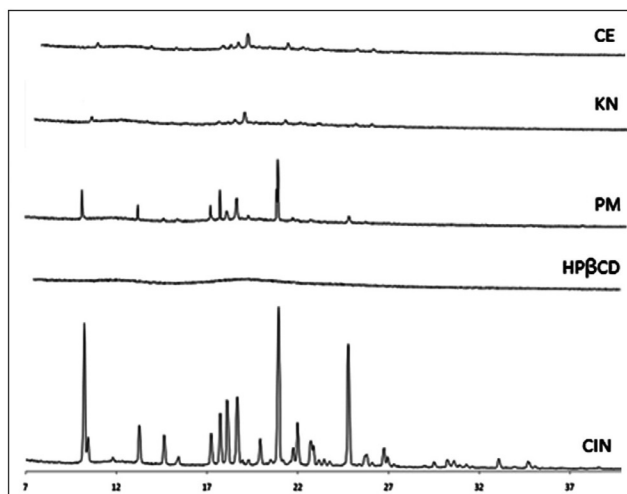
The structure of CIN with the proton numbering used is presented in Fig. 8. Table 5 enlists the chemical shift values of various CIN protons in free and complexed forms. For CE product of A1 binary system, from the $\Delta\delta$ values, it is clear that there are very minute changes in the chemical shift of CIN protons that are listed here, and other protons that are not listed did not experience any change at all. This negligible change in chemical shift suggested that there might be a presence of free drug. $^1\text{H-NMR}$ studies confirmed that in the A1 binary system, some interaction did take place, but the complete complexation was not achieved. In contrast to the A1 binary system, CE product of A3 and A6 ternary systems showed

Table 4 – Chemical shift values of HP β CD protons in free and complexed forms.

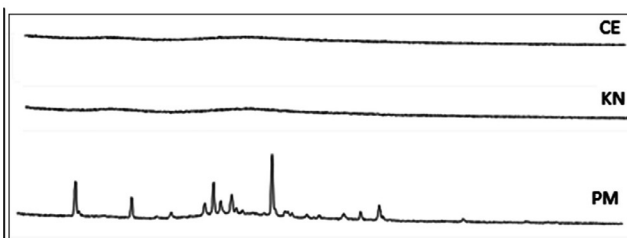
HP β CD proton no.	δ_{free}	A1 (CE) $\delta_{\text{complexed}}$	$\Delta\delta$ (ppm)	A3 (CE) $\delta_{\text{complexed}}$	$\Delta\delta$ (ppm)	A6 (CE) $\delta_{\text{complexed}}$	$\Delta\delta$ (ppm)
H1	4.839	4.842	-0.003	4.838	0.001	4.833	0.006
H3	3.615	3.603	0.012	3.603	0.012	3.599	0.016
H5	3.435	3.348	0.087	3.321	0.114	3.363	0.072
H6	3.462	3.468	-0.006	3.486	-0.024	3.472	-0.01

Table 5 – Chemical shift values of CIN protons in free and complexed forms.

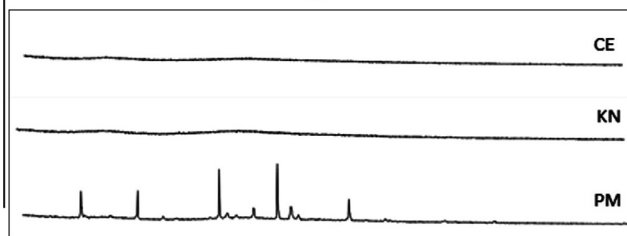
CIN proton no.	δ_{free}	A1 (CE) $\delta_{\text{complexed}}$	$\Delta\delta$ (ppm)	A3 (CE) $\delta_{\text{complexed}}$	$\Delta\delta$ (ppm)	A6 (CE) $\delta_{\text{complexed}}$	$\Delta\delta$ (ppm)
1	7.161	7.165	-0.004	7.184	-0.023	7.18	-0.019
2a, 2b	7.273	7.279	-0.006	7.293	-0.02	7.287	-0.014
3a, 3b	7.398	7.401	-0.003	7.436	-0.038	7.409	-0.011
4	6.479	6.484	-0.005	6.619	-0.14	6.537	-0.058
5	6.295	6.299	-0.004	6.313	-0.018	6.3	-0.005



A



B



C

Fig. 6 – X-ray diffractogram of A1 binary systems (A), A3 ternary system (B) and A6 ternary system (C).

significant changes in the chemical shift of CIN protons. Considerable downfield shift in the proton of the benzene ring (1, 2a, 2b, 3a, 3b) and the proton of alkene (4, 5) of CIN in the CE product of A3 and A6 ternary systems confirmed the inclusion of “3-phenyl-2-propenyl” side chain of the CIN molecule in the CD cavity. Results of FTIR, XRD, DSC and SEM supported the formation of an inclusion complex.

3.4.7. Molecular docking studies

Docking studies gave a better insight about the molecular interactions during complex formation. As shown in Fig. 9, in binary complex formation, “3-phenyl-2-propenyl” side chain of CIN entered the HP β CD cavity and formed an aromatic hydrogen bond with 2-OH group of HP β CD with a bond length of 2.7 Å. Diphenyl ring also formed an aromatic H-bond with ethereal oxygen in glucopyranose unit of HP β CD with a bond length of 2.9 Å; this confirms the results of $^1\text{H-NMR}$ studies. In ternary complex, tartaric acid stabilized the complex by forming an additional salt bridge (3.14 Å) and an H-bond (2.62 Å) between the carbonyl group and hydroxyl group of TA respectively and NH^+ in piperazine ring of CIN. Binding energy calculations gave ΔG (free energy for binding) for binary complex as -23.484 kcal/mol and for ternary complex as -52.57 kcal/mol. This clearly indicates that the ternary complex is more stable than the binary complex. There was a drastic change in the electrostatic energy in binary (-7.587 kJ) and ternary (-658.662 kJ) complexes. This may be due to the electrostatic attraction between TA and CIN.

3.4.8. Dissolution studies

PM of CIN-HP β CD binary system showed no improvement in the dissolution profile of CIN, but the KN and CE products of A1 binary systems showed an enhanced dissolution profile than the pure drug. Binary system of CIN-HP β CD prepared with the KN and CE methods released 18% and 20% drug, respectively; in contrast, a 5% drug release was obtained from plain drug and PM of CIN-HP β CD binary system, at the end of 180 min. Data of dissolution studies for the ternary systems revealed an

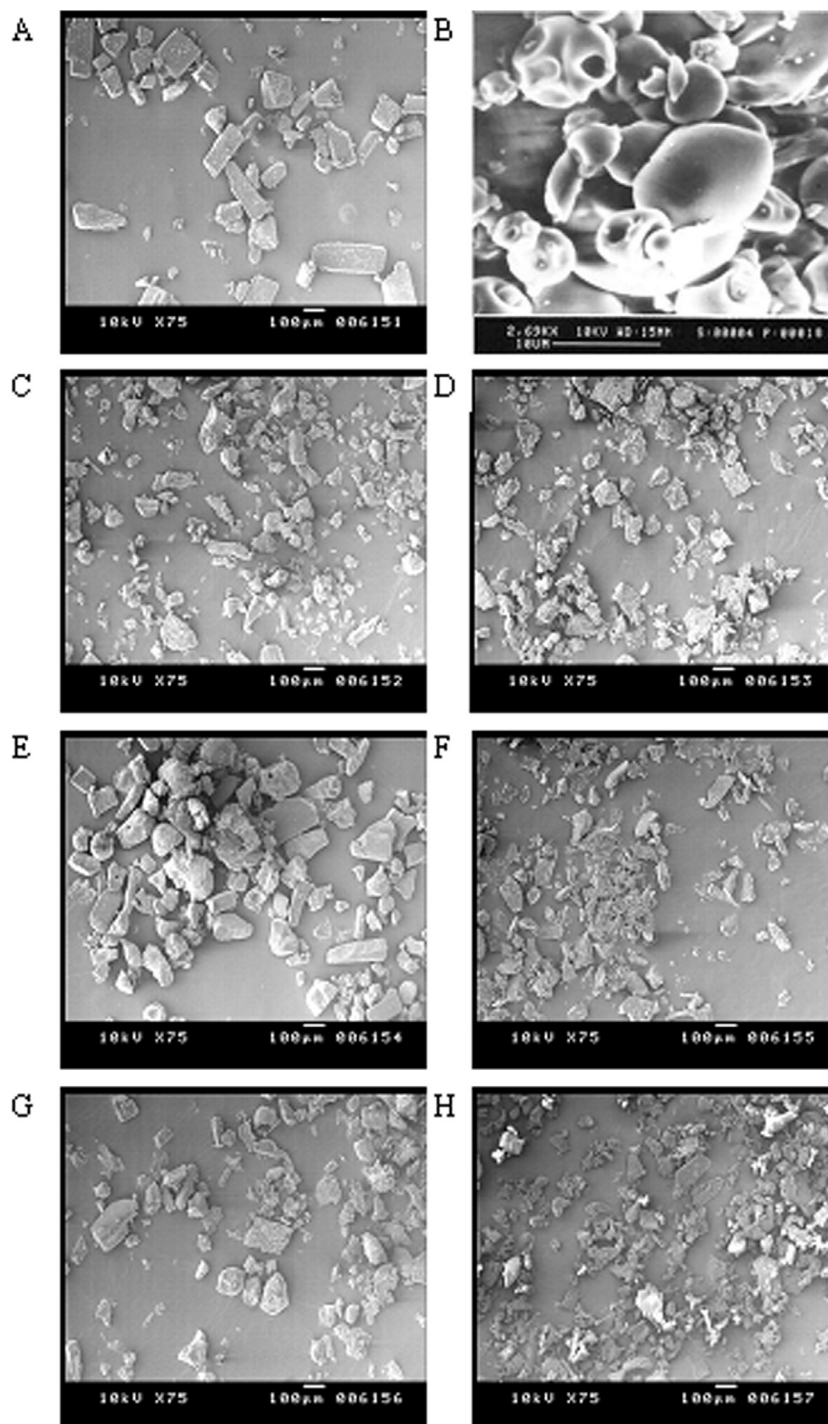


Fig. 7 – SEM of CIN-HP β CD solid systems: CIN (A), HP β CD (B), CIN-HP β CD PM (C), CIN-HP β CD co-evaporated (D), CIN-HP β CD-CA PM (E), CIN-HP β CD-CA co-evaporated (F), CIN-HP β CD-TA PM (G), CIN-HP β CD-TA co-evaporated (H).

enhancement in the dissolution of CIN compared to the binary systems. Dissolution studies bolster the findings of solubility studies that HA gave optimum results at the 1 M concentration levels. Table 6 gives % cumulative release of CIN at the end of 20 min for various binary and ternary systems. From Table 6, it is evident that CE gives better results than the PM and KN.

The dissolution profiles of various binary and ternary systems are shown in Fig. 10. Dissolution of drug from the ternary systems was more rapid and higher than the pure drug and binary system. In the ternary systems, TA was seen to be much more effective than CA; moreover, CE of the A6 ternary system showed rapid and complete dissolution of

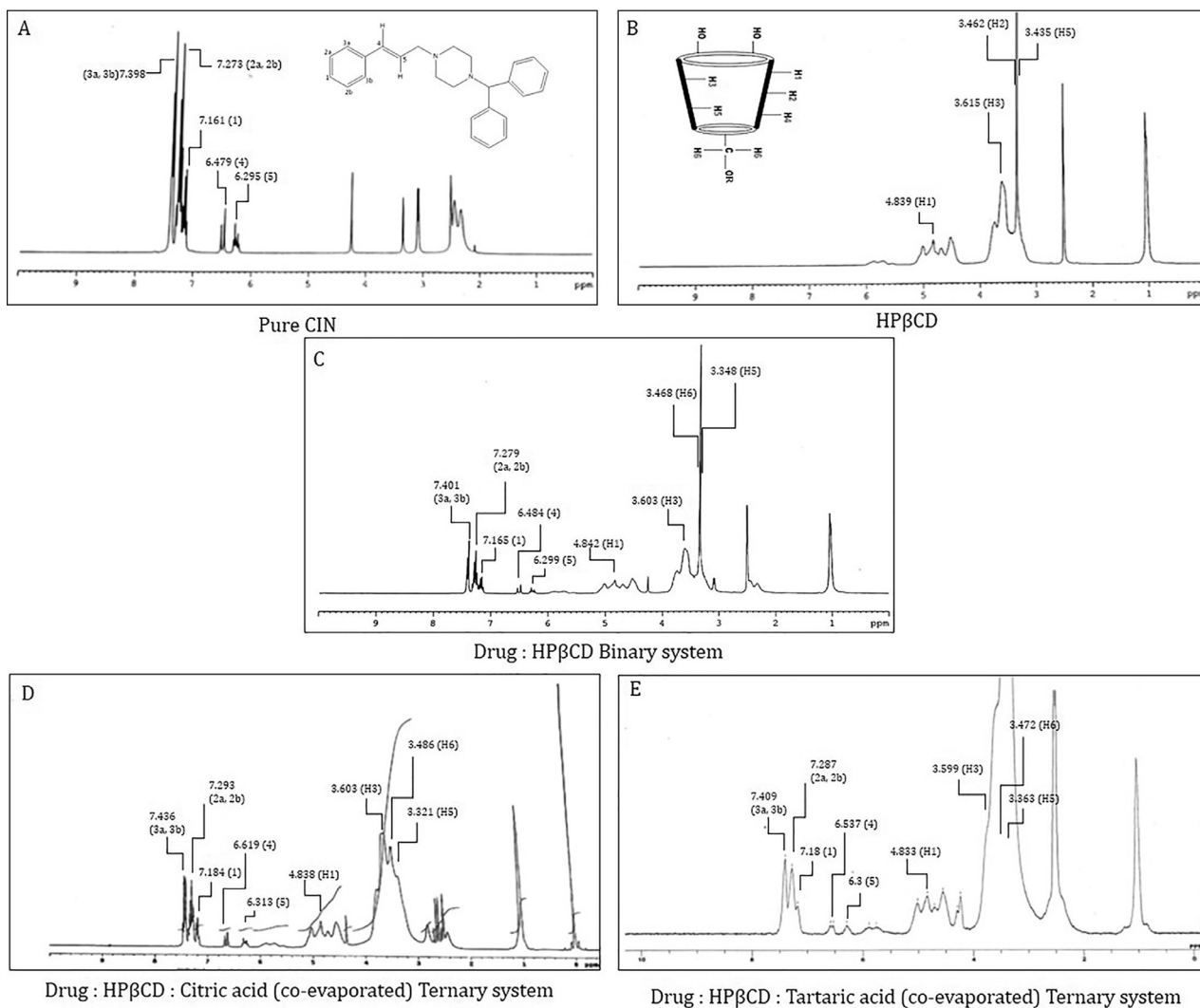


Fig. 8 – $^1\text{H-NMR}$ for (A) pure CIN, (B) HP β CD, (C) CIN:HP β CD binary system, (D) CIN:HP β CD:CA ternary system and (E) CIN:HP β CD:TA ternary system with peak representation of CIN and HP β CD protons.

Table 6 – % cumulative release of CIN–HP β CD–HA ternary systems.

Formulation code		% Cumulative release after 20 min	Formulation code		% Cumulative release after 20 min
A2	Physical mixture	24.335	A5	Physical mixture	25.905
	Kneading	49.143		Kneading	49.460
	Co-evaporated	53.899		Co-evaporated	51.651
A3	Physical mixture	26.319	A6	Physical mixture	36.818
	Kneading	52.015		Kneading	86.729
	Co-evaporated	63.860		Co-evaporated	99.802
A4	Physical mixture	32.395	A7	Physical mixture	36.671
	Kneading	60.663		Kneading	40.987
	Co-evaporated	62.065		Co-evaporated	81.106

CIN in 20 min. Slow and incomplete dissolution of CIN from the PM of CIN–HA (A8 and A9) binary systems implied that HA alone was slightly effective in improving the dissolution rate of drug, but HA in the presence of CD enhanced the drug release efficiently, giving complete drug release

within 20 min from CIN–HP β CD–TA ternary system. This confirmed that the improvement in the dissolution and solubilization of CIN was not only because of a favorable pH change due to HA but also due to the synergistic effect of HP β CD and HA.

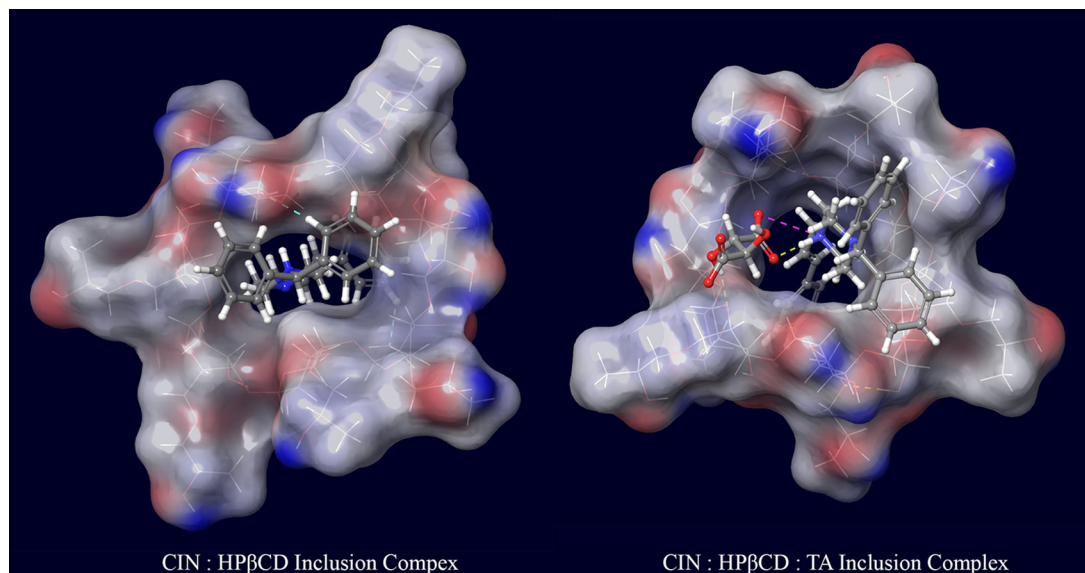


Fig. 9 – Docking pose for CIN:HPβCD binary and CIN:HPβCD:TA ternary inclusion complex.

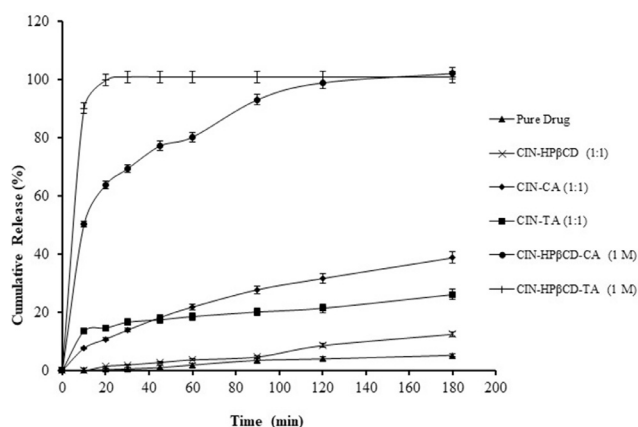


Fig. 10 – Dissolution profile of various binary and ternary systems (▲) pure drug (CIN), (×) CIN-HPβCD (1:1), (◊) CIN-CA (1:1), (■) CIN-CA (1:1), (●) CIN-HPβCD-CA (1:1:1), (+) CIN-HPβCD-TA (1:1:1) in distilled water. Data are represented as mean \pm SD ($n = 6$).

4. Conclusion

In conclusion, phase solubility studies clearly indicated the effectiveness of HA in improving the complexation efficiency of HPβCD towards CIN. The physicochemical characterization of CIN-HPβCD binary system suggested the incomplete inclusion complexation, whereas in CIN-HPβCD-HA ternary system, a new solid amorphous phase was observed, which confirms the complete inclusion complexation. FTIR, Molecular docking studies and $^1\text{H-NMR}$ studies elucidated the inclusion of “3-phenyl-2-propenyl” side chain of CIN in the hydrophobic cavity of HPβCD. Solubility and the dissolution rate of the CIN were superior for ternary systems than for the binary systems; the plain drug and molecular studies illustrated that the additional hydrogen bond and salt bridge formed between the

drug and tartaric acid may be the reason for the improvement in the solubility of CIN. The improved physicochemical properties and dissolution rate by complexation may in turn improve the drug bioavailability and its onset of action.

Conflicts of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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