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Physicochemical and molecular modeling studies of cefixime–L-arginine–cyclodextrin ternary inclusion compounds

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ABSTRACT

In an attempt to improve the physicochemical properties of cefixime (CEF), its supramolecular inclusion compounds were prepared with β-cyclodextrin (βCD) and hydroxypropyl-β-cyclodextrin (HPβCD) in presence and/or absence of ternary component L-arginine (ARG) using spray drying technique. Initially, the phase solubility studies revealed a stoichiometry of 1:1 molar ratio with an A_L-type of phase solubility curve. The stability constants of binary systems were remarkably improved in presence of ARG, indicating positive effect of its addition. The inclusion complexes were characterized by FTIR, XRPD, DSC, SEM, particle size analysis, and dissolution studies. Further, molecular mechanic (MM) calculations were performed to investigate the possible orientations of CEF inside βCD cavity in presence and/or absence of ternary component. In case of physicochemical studies, the ternary systems performed well as a result of comprehensive effect of ternary complexation and particle size reduction achieved by a spray drying technology.

1. Introduction

An inclusion complexation with cyclodextrins is an interesting and extensively used one of the techniques for solubility/dissolution enhancement of poorly water-soluble drugs (Ficarra et al., 2000; Shah, Sancheti, Vyas, Karekar, & Pore, 2010). Cyclodextrins (CDs) are cyclic torus shaped oligosaccharides able to entrap a guest molecule spatially in their central hydrophobic cavity without any covalent interactions (Fernandes, Carvalho, Pereira da Costa, & Veiga, 2003; Stella & Rajewski, 1997). The outer surface of CDs is sufficiently hydrophilic to act them as potential solubilizing agents (Loftsson, Hreinsdottir, & Masson, 2005). The inclusion complexation of drug molecules with CDs usually results in favorable changes in the physicochemical properties of the drug, such as solubility, dissolution rate, stability and bioavailability (Aleem, Kuchekar, Pore, & Late, 2008; Doiphode, Gaikwad, Pore, Kuchekar, & Late, 2008) rendering them more suitable for oral drug delivery.

Although, CDs have been proved to be versatile carriers in pharmaceutical research, a large amount of CDs is frequently required to solubilize small amounts of a poorly water-soluble drug due to their lower complexation efficiency (Ribeiro, Loftsson, Ferreira, & Veiga, 2003). However, it is possible to improve the complexation efficiency of CDs by incorporation of small amounts of certain water-soluble auxiliary substances such as polymers (Gajare, Patil, Kalyane, & Pore, 2009; Valero, Perez-Revuelta, & Rodriruez, 2003), hydroxyl acids (Pokharkar, Khanna, Venkatpurwar, Dhar, & Mandpe, 2009) and/or amino acids (Bramhane, Saindane & Vavia 2011; Mura et al., 2005; Patil, Pore & Kuchekar 2008; Shah, Karekar, Sancheti, Vyas, & Pore, 2009) to the complexation media. Such systems, which are composed of three different molecular entities (drug, CD and an auxiliary substance) are often termed as supramolecular ternary complexes in which an auxiliary substance in conjunction with the CD, improves the desired physicochemical, and transport properties of a drug molecule. In addition to that, the third component enhances the efficiency of drug delivery with simultaneous reduction in the amount of CD required, thereby making the formulation of the final product cost-effective (Kurkov & Loftsson, 2012; Loftsson & Brewster, 2012).

Thus, considering all aspects of ternary systems, the efforts were undertaken to improve the physicochemical properties of a third generation broad spectrum oral cephalosporin (Markham & Brogden, 1995; Montay et al., 1991; Ziv, Lavy, Glickman & Winkler 1995) cefixime (CEF) (Fig. 1), via ternary complexation with CDs using an amino acid L-arginine (ARG) as an auxiliary substance. CEF chemically (6R,7R)-7[(Z)-2-(2-amino-4-thiazolyl)-2-(carboxymethoxyimino]-acet-amido)-8-oxo-3-vinyl-5-thia-1-azabicyclo-(4,2,0) octa-2-ene-2 carboxylic acid is a white to slightly yellowish crystalline powder, effective against wide variety of Gram-positive and Gram-negative aerobic bacteria. The presence

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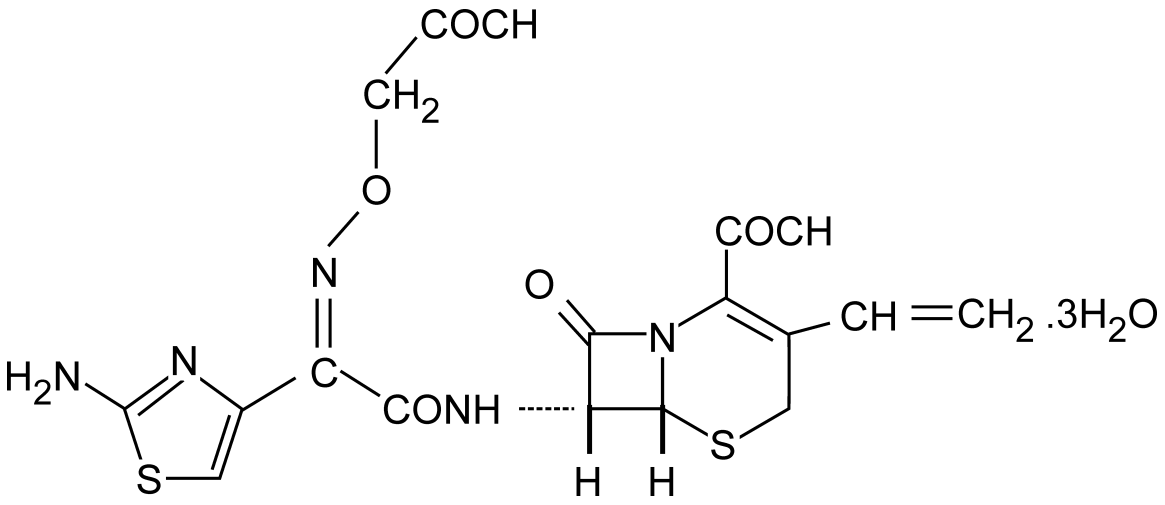

Fig. 1. Chemical structure of cefixime.

of 7-methoxyimine aminothiazole substituted cephem nucleus is a characteristic property of the third generation cephalosporins and confers excellent stability to CEF against β-*lactamases* type of enzymes (Genvresse & Carbon, 1993). However, poor aqueous solubility of CEF (Genvresse & Carbon, 1993; Markham & Brogden, 1995; Montay et al., 1991) contributes to its nearly 40–50% absorption (Genvresse & Carbon, 1993; Healy, Sahai, Sterling, & Racht, 1989) from gastrointestinal tract when administered orally. Poor aqueous solubility and hence the dissolution often result in variable bioavailability and limited therapeutic outcome of a drug.

The literature survey reported that few experiments on the effect of Captisol complexation on hydrolytic degradation of CEF in presence of polymers have been conducted where; stabilization of CEF has been shown to be achieved to some extent after Captisol complexation (Mallick, Mondal, & Sannigrahi, 2008). In this article, the authors report their investigations on preparation and characterization of inclusion compounds with cyclodextins for improvement of physicochemical properties of CEF. The spray drying technique was used to prepare inclusion complexes as particle size reduction to micron size by spray-drying technique is an adventitious approach as far as size and shape is concerned, also contributing to enhanced complexation efficiency thereby, performance of the inclusion complex (Kristmundsdottir, Gudmundsson & Ingvarsdottir 1996; Shah, Pore, Dhawale, Burade, & Kuchekar, 2012). The possible orientations of CEF inside a CD cavity in the presence and/or absence of ternary component were studied using molecular modeling and structural designing by computational chemistry methods. Further, molecular mechanic (MM) calculations were performed to optimize possible stoichiometry, geometry and stability of inclusion complex and as complementary evidences to support experimental studies. Initially phase solubility studies were conducted in distilled water to obtain the stoichiometry and the type of the solubility curve of the complex formation. The spray dried complexes were characterized by Fourier Transformation Infrared Spectroscopy (FTIR), X-ray Powder Diffractometry (XRPD), Differential Scanning Calorimetry (DSC), Scanning Electron Microscopy (SEM), particle size analysis and drug content uniformity studies. All systems including pure drug were further evaluated for their in vitro dissolution performance in 0.01 N HCl.

2. Material and methods

2.1. Materials

Cefixime was provided by Okasa Pharma, Satara, India, as a gift sample. βCD and HPβCD was kindly provided by Panacea Biotech, Chandigad, India. ARG and all other chemicals were purchased from Loba Chem, Mumbai, India, Analytical grade reagents and double distilled water were used throughout the experiment.

2.2. Phase solubility studies and interaction of CEF with CDs

The phase solubility studies in distilled water at room temperature (25 ± 2 °C) were performed in triplicate according to the method described by Higuchi and Connors (Higuchi & Connors, 1965). Excess amount of CEF (50 mg) was added to 20 mL of aqueous solution containing various concentrations of βCD or HPβCD (0–0.01 M) with or without addition of auxiliary substance ARG (0.25%, w/v). Then, the suspensions were mechanically shaken on rotary shaker for 96 hrs at 150 rpm. After equilibrium was achieved, the samples were filtered through 0.45 μm membrane filter and appropriately diluted. The concentration of CEF was determined spectrophotometrically (Shimadzu UV–vis spectrophotometer 1800, Japan) at 288.4 nm. The apparent stability constants (Kc) of the binary and ternary complexes were calculated according to the following equation:

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

where, S_0 is the solubility of CEF in absence of CDs.

The complexation efficiencies of CDs were also calculated from the following equation (Brewster & Loftsson, 2007).

$$\text{Complexation efficiency(CE)} = D_0 K_{1:1} = \frac{\text{Slope}}{(1 - \text{slope})} \quad (2)$$

where, D_0 is the intrinsic solubility of drug.

An indication of process of transfer of CEF from pure water to aqueous solution of HPβCD and βCD was obtained from the values of Gibbs free energy of transfer (ΔG_{tr}°) and it was calculated using the following equation:

$$\Delta G_{tr}^{\circ} = -2.303RT \log\left(\frac{S_c}{S_0}\right) \quad (3)$$

where, S_c/S_0 is the ratio of molar solubility of CEF in aqueous solution of HPβCD and βCD with or without auxiliary substance (0.25%, w/v) to that in distilled water in absence of HPβCD and βCD.

2.3. Molecular modeling studies

Molecular modeling was used to analyze the 3D geometry of CEF and βCD alone and in the complex by using VLifeMDS 4.1 software (VLife Sciences and Technologies, Pune, India). The MMFF program

was employed to optimize the structures. The molecular structure of βCD was taken from Pubchem Structural Database (CID444041). The CEF molecule was drawn using VLife Engine module. To fit the CEF in to βCD cavity, CEF was allowed for systematic conformational analysis with rms gradient 0.01 kcal/mol and dielectric constant 1. The most stable CEF conformer was positioned into the βCD cavity at different orientations. To βCD "Tail" and "head" denotations were used to describe the section of the cavity. According to these denotations 2-amino, 4-thiazolyl ring considered to be the reference position for CEF. In tail denotes the confirmer that has its ring next to a secondary hydroxyl group of βCD (wide rim of βCD) and "head" denotes the conformer next to a primary hydroxyl group of βCD (narrow rim of βCD).

For the optimization of stoichiometry (CEF: βCD), all stoichiometries was studied, i.e. 1:1, 1:2 and 2:1 and total energies after optimization were calculated for them. In MM calculations, relative thermodynamic relationship is considered as proportional to stability of the complex. The enthalpies of formation of inclusion compounds were calculated by using the following formula,

$$\Delta H = \Delta H_{fS:CD} - \Delta H_{fS} + \Delta H_{fCD} \quad (4)$$

where, ΔH_{fS} is an enthalpy of formation of guest, ΔH_{fCD} is an enthalpy of formation of host and $\Delta H_{fS:CD}$ is an enthalpy of formation of complex (Jesus et al., 2006).

2.4. Preparation of solid systems by spray drying technique

For the preparation of binary and ternary systems, equimolar quantities of CEF and βCD and/or HPβCD in presence and/or absence of ARG (0.25%, w/v) were transferred to a beaker containing 100 mL of methanol and sonicated for 10 min. The mixture was stirred for 4 days at 150 rpm at a room temperature 25 ± 2 °C by using a magnetic stirrer. The resultant clear solution was spray dried using Lab spray dryer (Labultima, LU222 Advanced, Mumbai, India) under following set conditions: Inlet temp – 65 °C; outlet temp – 45 °C; cool temp – 20 °C; aspirator speed – 40 mBar; feed rate – 10 ml/min. The spray dried powder was collected and stored in desiccators to prevent it from moisture.

2.5. Particle size analysis

The inclusion complexes were characterized for average particle size using a laser diffraction technique (Malvern Mastersizer 2000 SM, Malvern Instruments Corp. U.K.). The particle size distribution is given by $d(0.9)$, $d(0.5)$ and $d(0.1)$ which is the particle size diameters determined at 90th,50th,and 10th percentile of particle undersized, respectively.

2.6. X-ray powder diffractometry

The XRPD patterns of all formulations were recorded by using X-ray diffractometer (PW 1723, PHILIPS, the Netherland) with tube anode Cu over the interval 20–80°/2θ. The operational data were as follows: Generator tension (voltage) 40 kV, Generator current 30 mA, and scanning speed 2°/min.

2.7. Differential scanning calorimetry

Thermal analysis of all systems were performed using DSC analyzer (Metler Toledo Pvt. Ltd., Switzerland). A sample (5 mg) was sealed in an aluminum pan and heated under a nitrogen atmosphere at a heating rate of 10 °C/min over the temperature range of 30–300 °C.

2.8. Fourier transformation-infrared spectroscopy

Infrared spectra were obtained by using FTIR (Shimadzu, Affinity1, Japan) using KBr disks. The sample was previously ground and mixed thoroughly with KBr. The KBr disks were prepared by compressing the powder. The scanning range was kept from 4000 to 500 cm^{-1}.

2.9. Scanning electron microscopy

CEF and all systems were directly mounted on the aluminum stub and coated with a thin gold-ion layer by sputter coated unit (SEM-JOEL Instruments, JSM-6360, Japan). The surface topography was analyzed with a scanning electron microscope operated at an acceleration voltage of 5 kV and obtained micrographs were examined at ×500, ×1000, ×2000 and ×5000.

2.10. Percentage drug content study

Drug content was examined by dissolving samples equivalent to 5 mg drug in 5 mL of methanol. The volume was adjusted by 45 mL of distilled water. The solution was filtered through Whatman filter paper no. 41, suitably diluted and analyzed spectrophotometrtically at 288.4 nm (Shimadzu 1800, Japan).

2.11. In vitro drug release

The dissolution studies of the CEF and all systems were performed in triplicate in dissolution apparatus (Lab India, Model 2000, Mumbai, India) using the paddle method, according to USP Type II. Samples were placed in dissolution vessel containing 900 mL of 0.01 N HCL, maintained at 37 ± 0.5 °C at 50 rpm according to US FDA guidelines (US FDA website). 100 mg of pure drug or its equivalent amount of complexes was added to 900 mL of 0.01 N HCL. 5 ml of samples was withdrawn at appropriate time intervals. The volume of dissolution medium was adjusted to 900 mL by replacing each 5 mL aliquot withdrawn with 5 mL of fresh 0.01 N HCL. The solution was immediately filtered through 0.45 μm membrane filter, suitably diluted and analyzed spectrophotometrically at 285.4 nm (Shimadzu 1800, Japan).

3. Results and discussion

3.1. Phase solubility studies and interaction of CEF with CDs

The phase solubility curve of CEF in aqueous CDs solutions with and without the addition of 0.25% (w/v) an auxiliary substance ARG is shown in Fig. 2. The solubility of CEF increased linearly as a function of CDs concentration, featuring the A_L-type of phase solubility diagrams, indicating formation of water soluble complexes in all systems (Davis & Brewster, 2004). The possible stoichiometry assessed was 1:1 as the slopes of these phase solubility diagrams were less than unit in all cases (Higuchi & Connors, 1965).

Thus stability constants of CEF in βCD were founded to be 300 ± 6.5 (binary) and 2835 ± 11.5 (ternary) whereas, in HPβCD these were observed to be 440 ± 7.8 (binary) 1257 ± 9.2 (ternary) systems. The linear host-guest correlation coefficient (r^2) was found to be 0.928, 0.955 and 0.934, 0.905 for binary, ternary systems of βCD and HPβCD respectively.

The complexation efficiencies were found to be 0.136 (βCD binary), 1.318 (βCD ternary), 0.153 (HPβCD binary) and 0.428 (HPβCD ternary) systems. It could be seen that an addition of ARG to the complexation media increased slope, stability constant ($P < 0.001$) and complexation efficiency of both the CDs toward CEF. The results obtained from phase solubility data are clearly indicative of the positive effect of the addition of an auxiliary substance

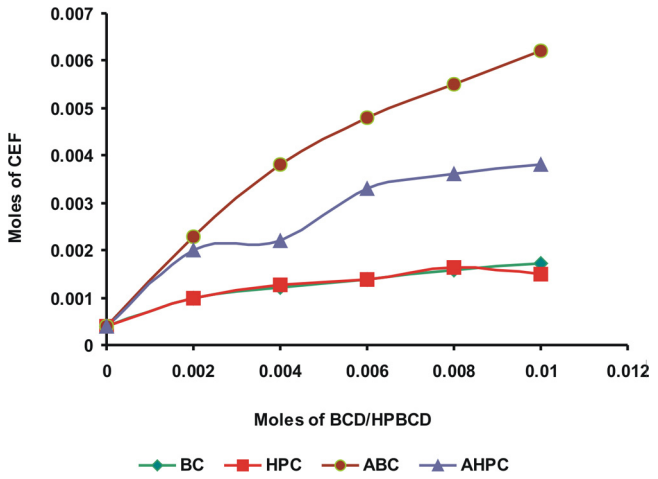

Fig. 2. Phase solubility diagram of βCD binary (BC), βCD ternary (ABC), HPβCD binary (HPC) and HPβCD ternary (AHPC) systems.

ARG in terms of improved solubility of CEF, association constant and complexation efficiency of CDs.

The positive effect of addition of ARG toward promotion of solubility parameters might be attributed to the formation of the amphiphilic structure of the CEF characterized by a strongly hydrophobic portion and a hydrophilic polar head, in the presence of ARG through electrostatic interactions and salt formation with the acidic functionality of CEF. Further, ARG also interacts with CD simultaneously through hydrogen bonding mechanisms (Berge, Bighley, & Monkhouse, 1977; Laveneziana, Speranza, Raulli, & Paredi, 1996). The hydrophobic portion of the amphiphilic structure of a drug is believed to interact with the hydrophobic CD cavity, whereas, at the same time, the hydrophilic portion can act as a surfactant toward the CD complex, reducing the aqueous surface tension, and thereby promoting its solubility (Shah et al., 2009; Mura et al., 2005).

An indication of the process of transfer of CEF from pure water to an aqueous solution of CDs was obtained from the values of Gibbs free energy change. For the concentration of βCD, 0.002, 0.004, 0.006, 0.008, 0.010, The value of ΔG_{tr}° (J/mol) in aqueous solution of βCD alone were found to be, −2266.7, −2714.2, −2912.0, −3265.5, −3574.7 and in presence of ARG, −4069.3, −5562.0, −6139.2, −6475.5, −6771.5 while, for that of HPβCD alone, −2263.8, −2714.2, −2912.0, −3486.0, −3783.7 and in presence of ARG, −3973.3, −4211.7, −5213.5, −5428.5 and −5562.0, respectively.

The ΔG_{tr}° values were all negative for CDs at various concentrations, giving idea about the spontaneous nature of CEF solubilization, and it decreased with an increase in its concentration, indicating that the reaction became more favorable as the concentration of CDs increased (Patel, Patel, Bhimani & Patel 2008). These values indicated that the system was releasing energy upon complexation undergoing Van der Waals and electrostatic interactions and became more favorable in the presence of ARG suggesting that ternary micro complexes could be prepared using this auxiliary substance.

3.2. Molecular modeling studies

The principal goal of performing molecular modeling studies was to optimize the stoichiometry of the complex formation and predict the possible orientations of CEF inside the βCD cavity. As it is difficult to define the most realistic structure of HPβCD due to mixture of its different isomeric forms; HPβCD complexes were not subjected for molecular modeling studies (Fernandes et al., 2003). The results of molecular mechanic calculations were expressed in terms of energies such as enthalpy and complexation energy (Pascal et al., 2005) by considering that entropy remains constant for the same host molecule (Jesus et al., 2006).

For the optimization of stoichiometry (CEF: βCD), the total energies were calculated for possible stoichiometries and that was found to be 2051 kcal/mol, 5798 kcal/mol, 3538 kcal/mol, for 1:1, 1:2 and 2:1 stoichiometries respectively. The stoichiometry of CEF

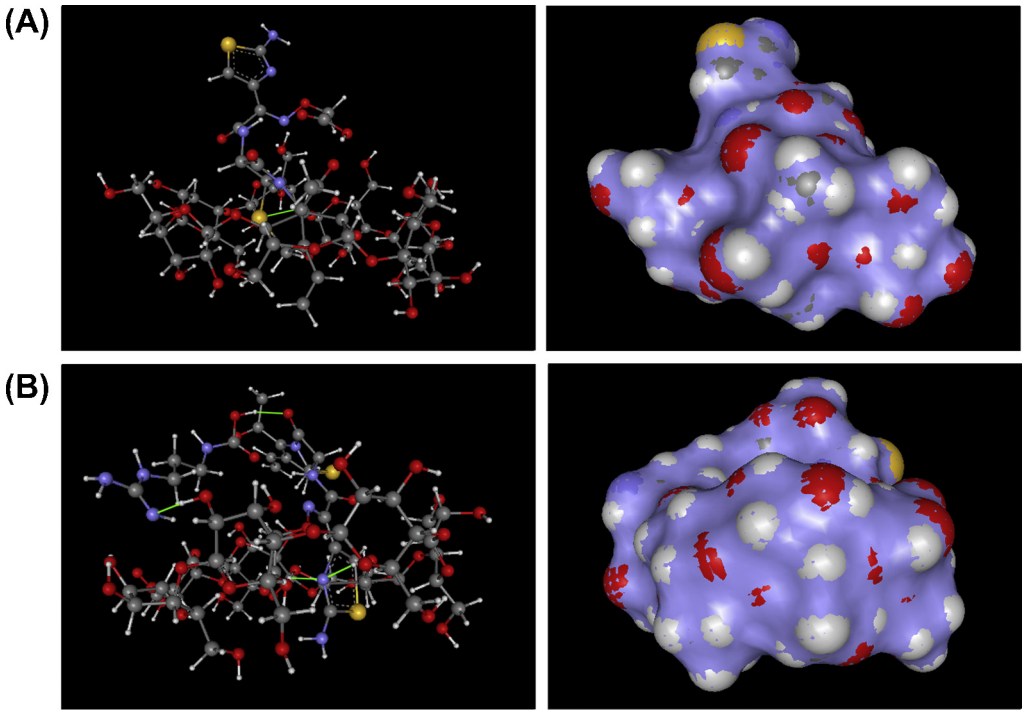

Fig. 3. Ball–sticks and space filled stabilized geometric model of the βCD binary system (A), βCD ternary system (B).

and βCD complex was found to be 1:1, showing lowest total energy amongst other stoichiometries, which was considered to be more stable. The obtained results were also supported by phase solubility studies as discussed earlier.

Fig. 3 shows the most stable geometry of CEF and βCD inclusion complex in ball and stick and space filled model for both binary and ternary systems including hydrogen bond formation in green color. The most possible modes of CEF inside βCD cavity giving the value for ΔH [kcal/mol], complexation energy [kcal/mol], and number of forming hydrogen bonds as, Thiazolyl in TAIL" ($\Delta H = -364{,}169$, 38.63, 0), Thiazolyl out TAIL" ($\Delta H = -634{,}088$, −1257.37, 1), Thiazolyl in HEAD" ($\Delta H = -5393$, 20.63, 1). Thiazolyl out HEAD" ($\Delta H = -590{,}973$, 27.63, 0), and that for ternary systems Thiazolyl in TAIL" ($\Delta H = -103{,}806$, 305.63, 1), Thiazolyl out TAIL" ($\Delta H = -51{,}168$, 85.63, 2), Thiazolyl in HEAD" ($\Delta H = -1{,}899{,}284$, −988.37, 4). Thiazolyl out HEAD" ($\Delta H = -976{,}128$, 253.63, 2).

In case of binary systems, among all possible studied orientations "2-nitro 4-Thiazolyl out TAIL" ($\Delta H = -634{,}088$ kcal/mol) showed most stable complex corresponding to the greatest absolute value of heat of formation and complexation energy with one O—H bond formation (Fig. 3A) (Jesus et al., 2006).

The ternary complex (CEF: βCD: ARG) was found to be stabilized ($\Delta H = -1{,}899{,}284$ kcal/mol) by insertion of "2-nitro 4-thiazolyl moiety in HEAD" region of βCD cavity (Fig. 3B) with the formation of four O—H bonds.

From the results, it could be concluded that βCD was able to accommodate cephem ring from wider rim inside the βCD cavity in absence of ARG while insertion of 2-nitro 4-thiazolyl moiety in βCD cavity was promoted through narrow rim in presence of ARG indicating its involvement in the hydrogen bonding interactions with βCD and CEF outside the βCD cavity.

3.3. Particle size analysis

The particle size analysis revealed that all complexes were in micron scale. The particle size of CEF was found to be 1.2702 μm. The particle size of binary system was 0.0485 μm and 0.1758 μm of βCD and HPβCD complexes respectively, whereas in case of ternary systems it was 0.861 μm and, 0.205 μm for βCD and HPβCD complexes respectively. Due to spray drying technique, the size of pure drug was significantly reduced to a greater extent in its formulations (Shoyele & Cawthorone, 2006). This has contributed to an increase in specific surface area and consequently faster dissolution for CEF particles (Shah et al., 2012).

3.4. X-ray powder diffractometry

The XRPD patterns of all samples are presented in Fig. 4. The diffractogram of CEF (Fig. 4A) exhibited peaks at 5, 5.1, 5.2, 5.3, 5.4, and 5.5 ($2\theta^{\circ}$) with peak intensities of 1376, 1396, 1266, 1257, 1227 and 1071 respectively which shows the somewhat amorphous nature of pure drug. However, some crystalline traces were still detectable. The peak intensities for βCD (Fig. 4C) and ARG (Fig. 4D) was found to be, 1474, 1387, 1358, 1262, 1226, 1284 and 1788, 1520, 1509, 1493, 1477, 908 respectively indicating their crystalline nature. A typical halo-pattern was recorded for HPβCD (Fig. 4B) which showed peaks at 1457, 1418, 1367, 1349, 1324, 1179, indicating its amorphous nature.

The peak intensities for βCD binary (Fig. 4E) and βCD ternary (Fig. 4G) were recorded to be 1361, 1393, 1350, 1288, 1219, 1165 and 245, 251, 241, 245, 250, 262, respectively. The intense peaks in the diffraction pattern of βCD and ARG were found to be completely diffused in complexes, indicating a significant reduction in the crystallinity. A complete amorphous state was achieved in the case of HPβCD binary (Fig. 4F) and ternary (Fig. 4H) complexes as indicated by their diffraction patterns with the intensities 1435, 1376, 1356, 1307, 1231, 1128 and 150, 148, 136, 162, 131, 161 respectively, which might have contributed for improved solubility and dissolution rate during physicochemical studies (Shah et al., 2012).

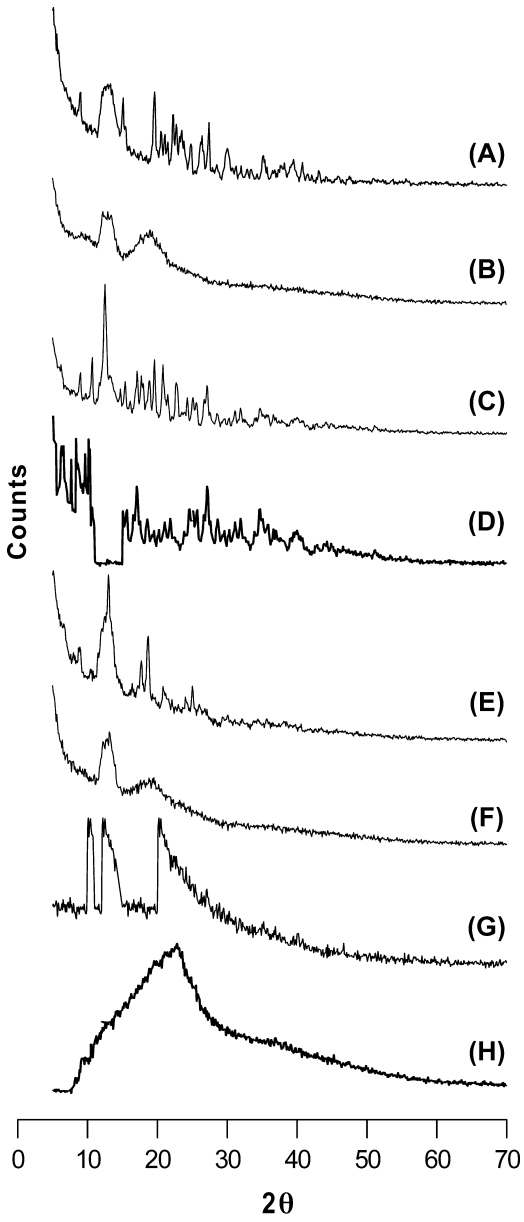

Fig. 4. XRPD pattern of CEF (A), HPβCD (B), βCD (C), L-arginine (D), βCD binary system (E), HPβCD binary (F), βCD ternary system (G), HPβCD ternary (H).

3.5. Differential scanning calorimetry

DSC is an alternative tool used to investigate the interaction between host and guest molecules. When guest molecules are included in CD cavity, their melting points usually shift to a different temperature or disappear (Shah et al., 2012).

DSC pattern of CEF and all systems are shown in Fig. 5. The DSC thermogram of CEF showed a glass transition (T_g) at 90 °C, indicating its amorphous nature (Fig. 5A). ARG exhibited a characteristic endothermic peak at 286 °C (Fig. 5D), while the thermogram of βCD (Fig. 5C) displayed a broad endotherm at 120 °C, indicating the presence of crystalline phase that was found to be diffused in its binary and ternary complexes (Fig. 5E 110 °C) and (Fig. 5G 128 °C) respectively, indicating formation of an inclusion complex with CEF.

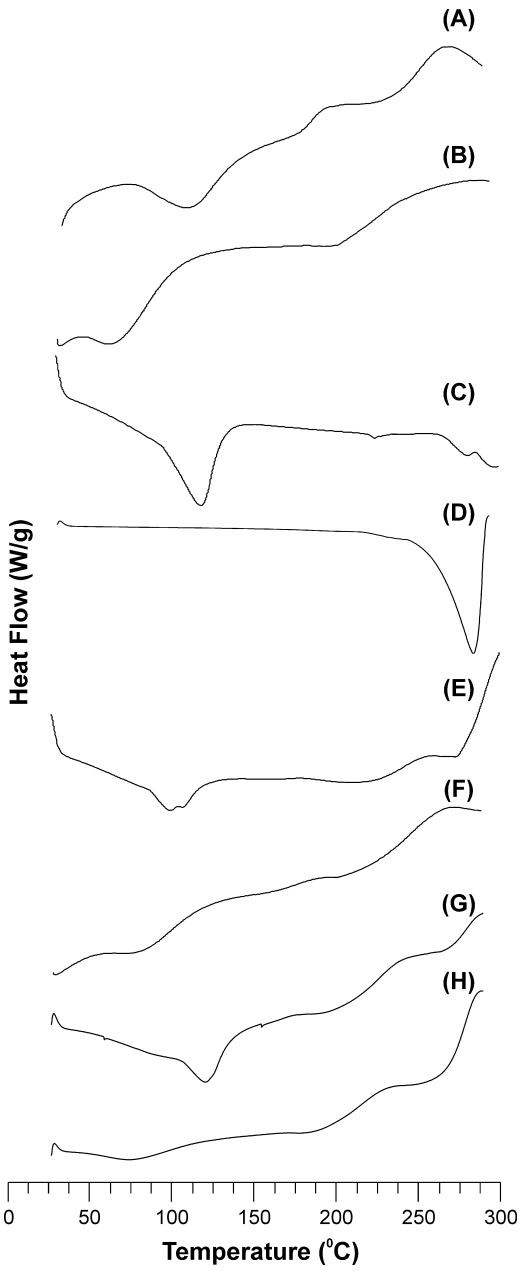

Fig. 5. DSC thermograms of CEF (A), HPβCD (B), βCD (C), L-arginine (D), βCD binary system (E), HPβCD binary (F), βCD ternary system (G), HPβCD ternary (H).

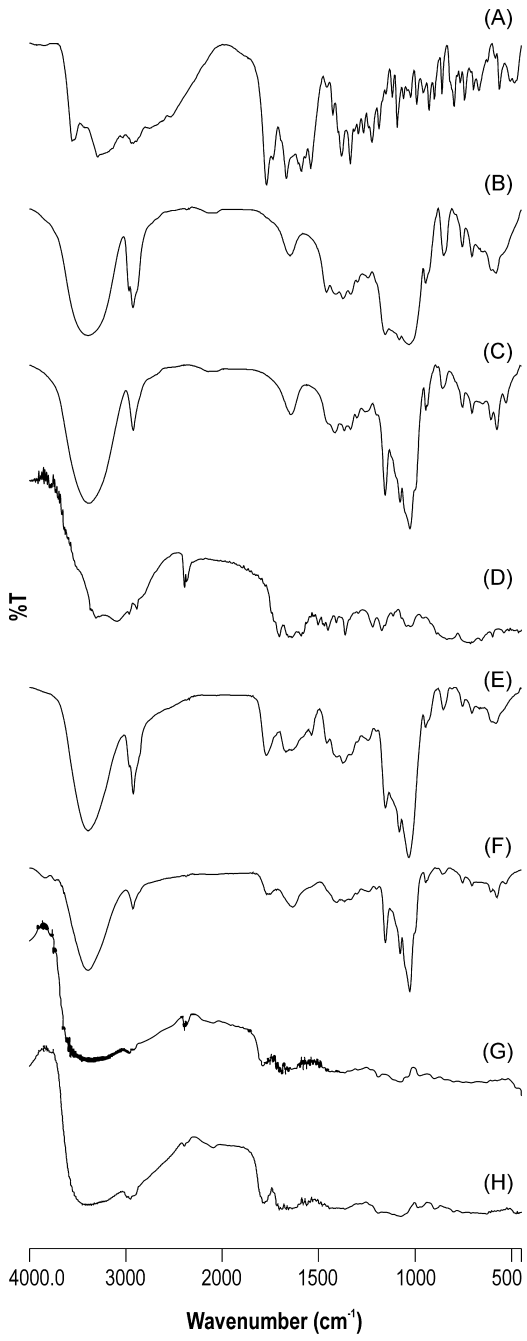

Fig. 6. FTIR spectra of CEF (A), HPβCD (B), βCD (C), L-arginine (D), HPβCD binary (E), βCD binary system (F), βCD ternary system (G), HPβCD ternary (H).

The thermal curve of pure HPβCD is characterized by a broad endothermic peak at 62.80 °C, indicating a loss of water due to dehydration process (Fig. 5B) (Sinha, Anitha, Ghosh, Nnda, & Kumaria 2005). It did not show any melting endotherm suggesting its amorphous character. In DSC thermogram of HPβCD binary (Fig. 5F), and ternary complexes (Fig. 5H), it was observed that T_g of CEF was elevated to 140 °C and 190 °C confirming existence of strong physical interaction between drug and HPβCD and formation of stable inclusion complex of CEF with HPβCD in both the systems.

3.6. Fourier transformation infrared spectroscopy

The possible interaction between drug and carrier was studied by FTIR spectroscopy. The FTIR patterns of all systems are shown in Fig. 6. The principle absorption peaks of CEF (Fig. 6A) were observed at 3295.42 cm^{-1} (N—H, stretch, amine), 2942.02 cm^{-1} (C—H, Stretch), 3563.91 cm^{-1} (O—H, stretch, COOH), 1669.09 cm^{-1} (C=O stretch CONH), 1337.88 cm^{-1} (C—N stretching, aromatic), 670.75 cm^{-1} (C—S, Stretching), 1591.68 cm^{-1} (ring stretching vibrations), 1771.79 cm^{-1} (C=O, stretch, COOH), 746.16 cm^{-1} (C—H, bending), 1542.09 cm^{-1} (C=C, stretch).

Fig. 6B displays absorption bands of HPβCD at 3399.3 cm^{-1} (O—H, stretch), 2929.2 cm^{-1} (C—H, Stretch), 1034.6 cm^{-1} (C—O—C stretch). The peaks of βCD was recorded at 3396.98 cm^{-1} (O—H, stretch), 2926.07 cm^{-1} (C—H, Stretch), 1644.87 cm^{-1} (H—O—H bending), 1028.84 cm^{-1} (C—O—C stretch) (Fig. 6C). FTIR spectrum of ARG shows (Fig. 6D) prominent peaks at 3296.3 cm^{-1} and 3068 cm^{-1} (broad OH carboxylic or NH guanidine due to intramolecular hydrogen bonding), 2864.29 (C—H stretch), 1678.0 cm^{-1}

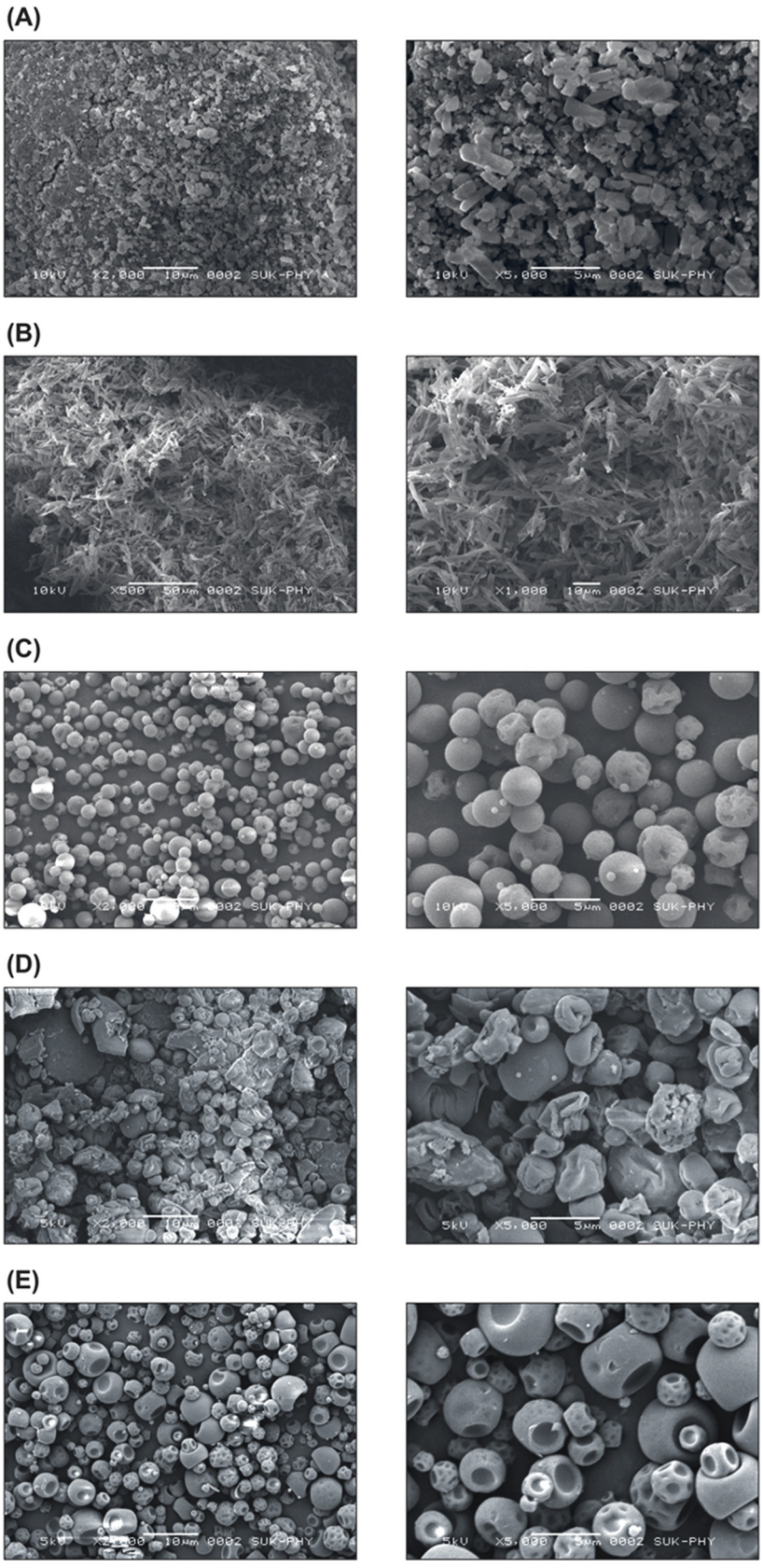

Fig. 7. SEM of CEF (A), βCD binary system (B), HPβCD binary (C), βCD ternary system (D), HPβCD ternary (E).

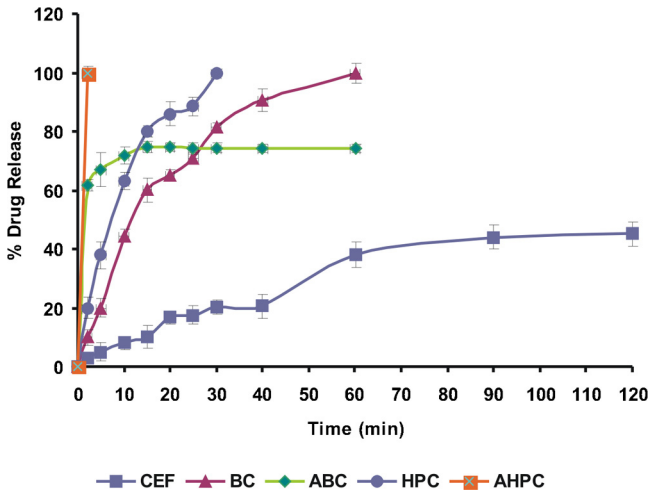

Fig. 8. The dissolution curves of CEF, βCD binary (BC), βCD ternary (ABC), HPβCD binary (HPC) and HPβCD ternary (AHPC) systems.

(C=O, amide), 1330 cm^{-1} (C—N stretch) and 1620 cm^{-1} (C=O carbonyl COOH).

In all binary and ternary systems, modifications [frequency shifts and/or appearance, attenuation, broadening] in characteristic bands of the CEF and CDs were noted. Further, the peaks in formulations were observed to be smoothened and no any new peak observed indicating non covalent interaction in inclusion complexes (Gajare et al., 2009).

3.7. Scanning electron microscopy

SEM was used to investigate the microscopic surface and morphological characteristics of pure drug and complexes (Fig. 7). Pure drug was characterized by the presence of rough, uneven, broken particles of larger size while some crystals were showing rectangular shape. The microphotographs of spray dried inclusion complexes showed the characteristic morphology as a small sized, spherical and homogeneous particles, with a significant reduction in particle size and indicating the existence of amorphous entities. As βCD is crystalline in nature, microphotographs of spray dried βCD binary complex showed the characteristics crystalline structure images. However, βCD ternary system shows small and spherical particles. In contrast, both the systems of HPβCD appeared as smooth and spherical shaped particles.

These microphotographs suggested the formation of complexes of CEF with hydrophilic CDs in the solid state. Thus, altered particle shape, reduced particle size and close contact between hydrophilic CDs in complexes might be responsible for the enhanced drug solubility and dissolution rate of CEF (Yadav, Yadav, Karekar, Pore, & Gajare, 2012). Further, spray drying technology had contributed for smoothened and round shape of micro-particles resulting rapid drug release from the complexes.

3.8. Percentage drug content

The percentage of drug content for CEF:βCD, CEF:βCD:ARG, CEF:HPβCD and CEF:HPβCD:ARG systems were found to be 75 ± 1.08% (w/w), 98 ± 1.07% (w/w), 100 ± 1.06% (w/w), 93 ± 1.07% (w/w) respectively.

3.9. In vitro drug release

The CEF release profiles through binary and ternary complexes are illustrated in Fig. 8. The release rate profiles were given as the percentage of drug dissolved (vs.) time. The values of % drug dissolved in 2 min (DP_2), and dissolution efficiency at 2 min (DE_2) were evaluated.

The % drug release at 2 min for CEF, CEF:βCD, CEF:βCD:ARG, CEF:HPβCD and CEF:HPβCD:ARG were found to be 3.0 ± 1.0, 10.00 ± 2.0, 61.78 ± 3.5, 20.00 ± 2.4 and 99.98 ± 5.1 respectively. The corresponding dissolution efficiencies of these systems at 2 min (DE_2) were found to be 0.75 ± 0.05, 2.6 ± 0.2, 15.4 ± 0.70, 5.00 ± 1.1 and 24.99 ± 2.3, respectively.

From the findings, it is evident that, dissolution rate of CEF was greater in both binary and ternary systems. A significant difference between the dissolution profile (DE_2 value) of pure CEF and all its systems ($P < 0.001$) was observed. However, CDs were thought to improve the wetting ability of drug particles and this might have prevented aggregation of particles with increased surface area, when exposed to aqueous dissolution medium (Elkordy, Essa, & Faheem, 2009). In binary systems higher hydrophilicity of the CD with its ability to form stable inclusion complex with the drug, might have responsible for the improved dissolution profile of pure drug. Further in case of ternary systems increasing stability (molecular modeling phase solubility studies) and complexing property was achieved by using ARG as a ternary component which resulted in improved aqueous solubility and dissolution rate as compared to pure drug. Indeed, again the ternary HPβCD systems have performed well as compared to βCD ternary systems, as a result of lower association constant and higher hydrophilicity which might be responsible for immediate release of CEF when exposed to dissolution medium.

The dissolution property of spray dried CEF was improved by (1) surfactant like properties of carrier, which can reduce the interfacial tension between water insoluble drugs and the dissolution medium, (2) Increasing the stability and complexing property due to ARG in ternary systems, (3) use of CDs which are able to form hydrogen bonds with drug molecules, (4) reduction in particle size achieved using spray drying technology (Takeuchi, Nagira, Yamamoto, & Kawashima, 2004). Thus it could be concluded that spray drying offers one step and adventitious technique in the formulation process (Lin & Kao, 1989).

4. Conclusion

In the present investigation, the synergistic effect of ARG in the formation of inclusion compounds of CEF with CDs resulted in tremendous improvements in physicochemical properties as compared to pure drug. An enhancement in dissolution was attributed to the comprehensive effect of complexation, micronization achieved through spray drying technology and addition of an auxiliary substance. In addition to that, the molecular modeling studies confirmed the stoichiometry, stability and geometry of CEF/βCD complex with inclusion of cephem ring of CEF inside the βCD cavity from wide rim and thiazolyl ring of CEF embedded in βCD cavity through narrow rim in binary and ternary systems respectively. The results were supported by physicochemical and characterization studies. In all formulations, HPβCD ternary system offered significant advantages through its excellent physicochemical performance over all other systems. In conclusion, CEF is able to form a stable inclusion complex with both CDs in the presence and absence of ARG with improved physicochemical properties.

Conflict of interest

Authors declare no conflict of interest.

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