Piperine-Hydroxy acid-Cyclodextrin Inclusion Complexes; Physicochemical, Computational, and Proton Nuclear Magnetic Resonance Spectroscopy Studies: PART I

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Abstract

Introduction: In an attempt to improve the physicochemical properties of piperine (PIP), its inclusion complexes were prepared with β-cyclodextrin (βCD) and hydroxypropyl-β-cyclodextrin (HPβCD) in presence and/or absence of hydroxy acids by lyophilization technique. **Materials and Methods:** Primarily, the stoichiometry of the complex formation and thermodynamic parameters was accessed by phase solubility study described by Higuchi and Conners method. The molecular modeling studies were performed to elucidate the stability, possible interactions, and geometry of PIP inside the βCD cavity. The prepared lyophilized inclusion complexes were characterized by proton nuclear magnetic resonance spectroscopy (^{1}H NMR) and Two-dimensional Nuclear Overhauser Effect spectroscopy (2D ^{1}H NMR**)**, Fourier transformation-infrared spectroscopic (FTIR), scanning electron microscopic (SEM), Log P, and dissolution studies. **Results:** The phase solubility data revealed the formation of 1:1 stoichiometry with A_L type of solubility curve at 25°C. Thermodynamic studies indicated that the inclusion process was spontaneous. The molecular modeling studies were depicted the insertion of piperidine ring inside βCD cavity. As complementary evidence, ^{1}H NMR and 2D ^{1}H NMR studies predicted that whether in presence or absence of hydroxy acids, CD is able to accommodate the piperidine ring. FTIR analysis revealed scattering peaks assigned for PIP were smoothened in all lyophilized inclusion complexes. SEM images indicated modifications in morphology of PIP particles in its lyophilized complexes. Dissolution studies revealed significant improvement in dissolution efficiencies of PIP in all prepared inclusion complexes. **Conclusion:** The effectiveness of ternary hydroxy acids was found to be appreciating toward improvement in aqueous solubility and dissolution properties of PIP.

Key words: Complexation, computational chemistry, cyclodextrin, hydroxy acids, piperine

INTRODUCTION

Piperine (PIP) [(2E,4E)-1-[5-(1,3-Benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]piperidine, is a natural ingredient, possessing seasoning property which makes black pepper as a significant segment in serving of mixed greens dressing.[1] PIP acts as a bioenhancer by repressing the cytochrome P450 enzyme.[2] It likewise shows a few activities such as hypolipidemic, anticonvulsant, antimutagenic, protective effect against gastric ulcer-like activity, slight insecticidal,[3] and antibacterial property.[4] In spite of such properties, its application in food and pharmaceutical industries remains limited due to its low aqueous solubility and bioavailability.[2,5,6] Some researchers earlier reported that the inclusion complexation of PIP with β-cyclodextrins (βCD) improved its aqueous solubility and bioavailability. Few experiments such as oil in water emulsion system, formulation of nanospheres, self-emulsifying drug delivery system, and inclusion complexation

with βCD of PIP have been accounted for betterment in water solubility and bioavailability of PIP.[5,7-9] However, no any ternary inclusion complexation approach for enhancement of solubility and bioavailability of PIP has been employed yet.

CD inclusion complexation has been accounted to enhance the desired physicochemical properties of low hydrophilic compounds significantly by accommodating its hydrophobic portion into the CD cavity.[10,11] However, parent βCD has limited aqueous solubility. HPβCD; the hydroxy derivative of βCD has an amorphous nature, better water solubility, and least toxicity, making it more preferable than that of basic one.[12] CDs are having low complexation efficiency, which restrains its utilization to a limited degree.[13] Therefore, complexation efficiency of CDs can be improved by the incorporation of some low molecular weight hydroxy acids[14] to the complexation media, bringing about the formation of ternary complexes; thereby deduction in the amount of CD is required and making the formulation of final product profitable. The basic drug may form salts with hydroxy acids such as citric acid (CA) and/or ascorbic acid (AA), contributing to the enhancement in hydrophilicity of drugs as a consolidated impact of salt formation and inclusion complexation.[15]

By considering all viewpoints, the attempts have been undertaken to prepare inclusion complexes of PIP with CDs in an influence of CA and AA as ternary components to improve the physicochemical properties of PIP, which is as far as anyone is concerned has not been published at this point. The phase solubility studies were pursued to determine possible stoichiometry of all complexes. The solid-state inclusion complexes were prepared by lyophilization technique and characterized by Fourier transformation-infrared spectroscopy (FTIR), scanning electron microscopy (SEM), proton nuclear magnetic resonance spectroscopy (^{1}H NMR), and Two-dimensional Nuclear Overhauser Effect spectroscopy (2D ^{1}H NMR), Log P, and *in-vitro* dissolution studies.

MATERIALS AND METHODS

Materials

PIP (Molecular weight: 285.34 g/mol, Purity 97%) was purchased from Sigma Aldrich Chemicals Pvt. Ltd. (Mumbai, India). βCD (Molecular weight: 1135 g/mol, Purity 98%) was procured from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). HPβCD (Molecular weight: 1400 g/mol, degree of substitution −0.65, Purity 98 %) was purchased from HiMedia Laboratories Pvt. Ltd (Mumbai, India). AA (Purity 98 %), and CA (Purity 98 %) were purchased from Loba Chemie Pvt. Ltd (Mumbai, India). Analytical grade reagents and glass distilled water were used throughout the experiment. All chemicals were used without further purification.

Phase solubility and thermodynamic studies

An excess amount of native PIP was incorporated to 10 ml of an aqueous solution containing varying concentrations of CDs (0–0.005 M) with or without the addition of ternary components such as CA (0.25 % w/v) and AA (0.25% w/v). The resulted suspensions were mechanically shaken on an incubator shaker (REMI-CIS 24 plus Incubator Shaker, Mumbai, India) for 72 h at 150 rpm. After equilibration, the samples were withdrawn and filtered through Whatman filter paper no. 41 and appropriately diluted if necessary. The concentrations of PIP were analyzed spectrophotometrically (Shimadzu UV-Vis spectrophotometer 1800, Japan) at 341 nm. The apparent stability constant and complexation efficiency of inclusion complexes were determined by the following equations.[16,17]

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

S_0 is the solubility of PIP without CDs and slope is obtained by a phase solubility diagram.

$$CE = Do.K1:1 = \frac{Slope}{1-Slope} \quad (2)$$

D_0 is the intrinsic solubility of the drug and $K_{1:1}$ is the complexation constant for the interaction of one drug molecule with CD molecule.

The apparent stability constants of all systems were statistically analyzed by ANOVA (Instate GraphPad software Inc. Version 3.05)

The thermodynamic events occurring during the inclusion complexation were further examined by conducting phase solubility analysis at 20, 25, and 37±2°C. The integrated form of the Van't Hoff equation enables the determination of ΔH and ΔS, based on the modification in stability constants with temperatures.[18]

$$\ln K = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} \quad (3)$$

R is the gas constant and T is the temperature in Kelvin.

ΔG is a replication of the process of transfer of PIP from pure water to an aqueous solution of CDs, which can be estimated by the following equation,

$$\Delta Gtr° = -2.303RT\log\left(\frac{Sc}{So}\right) \quad (4)$$

Where, S_c/S_0 is the ratio of molar solubility of PIP in an aqueous solution of CDs with or without ternary substances (0.25 % w/v) to that in distilled water in absence of CDs.

Molecular modeling studies

In silico studies were carried out using VLifeMDS 4.3 software (VLife Sciences and Technologies, Pune, India)

on the Intel i3 CORE processor with an operating system of Windows 7. The VLife engine module was used to draw the molecular structures of PIP, βCD, CA, and AA. All structures were optimized by MMFF program with rms gradient 0.01 Kcal/Mol and dielectric constant 1. The most optimized PIP conformer was positioned into the βCD cavity (either from wide/narrow rim) with or without ternary substances at different orientations and again optimized. The respective complexation energies (ΔE) were determined by the following equation,

$$\Delta E = E_{fS:D} - E_{fS} + E_{fCD} + E_{fAx} \tag{5}$$

$E_{fS:D}$ is the total energy of the inclusion complex after optimization, E_{fS} is the total energy of the guest molecule after optimization, E_{fCD} is the total energy of the host molecule after optimization, and E_{fAx} is the total energy of ternary molecule after optimization.

Preparation of solid systems by lyophilization

Equimolar quantities of pure PIP (0.285 g) and both the CDs (βCD – 1.135 g and HPβCD – 1.4 g), with or without the incorporation of ternary components (0.25% w/v) were transferred to a beaker containing a mixture of 50 ml of methanol and 50 ml of distilled water. The prepared mixtures were sonicated for 15 min and then allowed to stir for 72 h at 25±2°C with 150 rpm using a magnetic stirrer (REMI-CIS 24 plus Incubator Shaker, Mumbai, India). The resulted mixtures were filtered and placed in a deep freezer (ELCOLD, Denmark) at −80°C for 24 h. After deep freezing, the obtained solutions were lyophilized (DELVAC- mini Lyodel, Chennai, India) at −80°C for 4 days. The received solid state complexes were kept in desiccators to avoid it from moisture absorption.

FTIR analysis

Infrared spectra of all samples were obtained using ATR (BRUKER – ECO – ATR – ALPHA, Germany). The samples were directly placed on a sample pan and analyzed from 600 cm^{-1} to 4000 cm^{-1} spectral range with 24 scans.

¹H NMR and 2D NMR

¹H NMR and 2D NMR analysis of PIP, βCD, HPβCD, AA, and inclusion complexes (PB, PH, PBA and PHA) were performed using a NMR spectrometer (JNM-ECZ600R/S1, JEOL, JAPAN) with a TH5 Probe operating at 600 MHz and in D$_2$O (βCD, HPβCD, AA, PH, PBA, and PHA), CDCl$_3$ (PIP) and DMSO-d$_6$ (PB) solution.

SEM

SEM was used to study the surface morphology of samples which was operated at an acceleration voltage of 20 kV.

Pure PIP and lyophilized complexes were directly mounted on an aluminum stub and coated with a thin gold-ion layer by sputter coated unit (SEM-JOEL Instruments, JSM-6360, Japan) and obtained micrographs were examined at ×500, ×1000, ×2000, and ×5000 magnifications.

Partition coefficient (Log *P*) analysis

The excess amount (equivalent to 15 mg) of pure PIP and lyophilized complexes were added to the glass tubes containing 10 ml each of octanol and water, which were previously allowed to stand for 24 h at room temperature. These tubes were placed on an incubator shaker (REMI-CIS 24 Plus Incubator shaker, Mumbai, India) and shaken for 24 h at 25°C. After equilibrium achieved, the resultant solutions were shifted to the separating funnel and allowed to stand for 6 h. The separated aqueous layer was analyzed spectrophotometrically (Shimadzu 1800, Japan) at 341 nm.[19] The Log *P* value calculated by the following equation,

$$\text{Log } P = \text{Log}\left(\frac{C_o}{C_w}\right) \tag{6}$$

C_o is the concentration of PIP in octanol, and C_w is the concentration of PIP in water.

The data of the partition coefficient were statistically analyzed by ANOVA (Instate GraphPad software Inc. Version 3.05).

In-vitro dissolution studies in 0.1 N HCl at pH 1.1

In-vitro dissolution studies of samples were performed on a dissolution test apparatus (ELECTROLAB–TST-06L/ LX, New Mumbai, India) by paddle method.[7,20] Ten milligrams of PIP or its equivalent amount of the complexes were placed in a dissolution vessel containing 900 ml of 0.1 N HCl, maintained at 37±0.5°C at 50 rpm. Five milliliters of samples were withdrawn at 2, 5, 10, 15, 30, 45, and 60 min time intervals. The volume of dissolution media was adjusted to 900 ml by replacing each 5 ml aliquot withdrawn with 5 ml of fresh 0.1 N HCl. The filtered solutions were analyzed spectrophotometrically at 342 nm (Shimadzu UV-Vis Spectrophotometer 1800, Japan).

RESULTS AND DISCUSSION

Phase solubility and thermodynamic studies

The phase solubility curves of PIP in aqueous solutions of βCD and HPβCD in an influence (PBC, PBA, PHC, and PHA) and in absence (PB, PH) of hydroxy acids are displayed in Figure 1. The water solubility of PIP increased linearly as the function of CD concentration, representing the A$_L$ type of phase solubility diagram.[21]

The phase solubility and thermodynamic parameters associated with inclusion phenomenon are indicated in Table 1, which clearly defined that all slopes were <1 depicting the formation of water soluble complexes with 1:1 stoichiometry[22] and the ternary inclusion complexes were more effective with respect to water solubility, stability constants ($P<0.001$) and complexation efficiencies than the binary complexes. PIP is the weak base and it may form weak salt with weak acids. Therefore, the addition of hydroxy acids as ternary components might have contributed to the enhancement of hydrophilicity of drugs by electrostatic/hydrogen bonding interactions with PIP.[14,15]

Van't Hoff plot displayed in Figure 2 which is the function of the log of K_S and inverse of absolute temperature. A_L type of phase solubility profiles were observed for some complexes (PB-20, 25, 37°C), while A_p (PH-37°C, PHC-25°C, PHA-37°C) and A_N (PH-20°C, PBC-20°C, PBA-20°C) type of profiles were also found. For that case, the association constant was calculated with respect to A_L type of phase solubility profile. The enthalpy (ΔH) values for some complexes were observed to be positive (PB, PHC, and PHA), while for some complexes (PH, PBC, and PBA) it found to be negative, suggesting the complexation phenomenon was exothermic for PH, PBC, and PBA complexes which might be attributed to a more favorable complexation process.[23] Positive entropy (ΔS) values indicated that a highly disordered state of complexes and the process was without extensive desolvation. From observed ΔH and ΔS
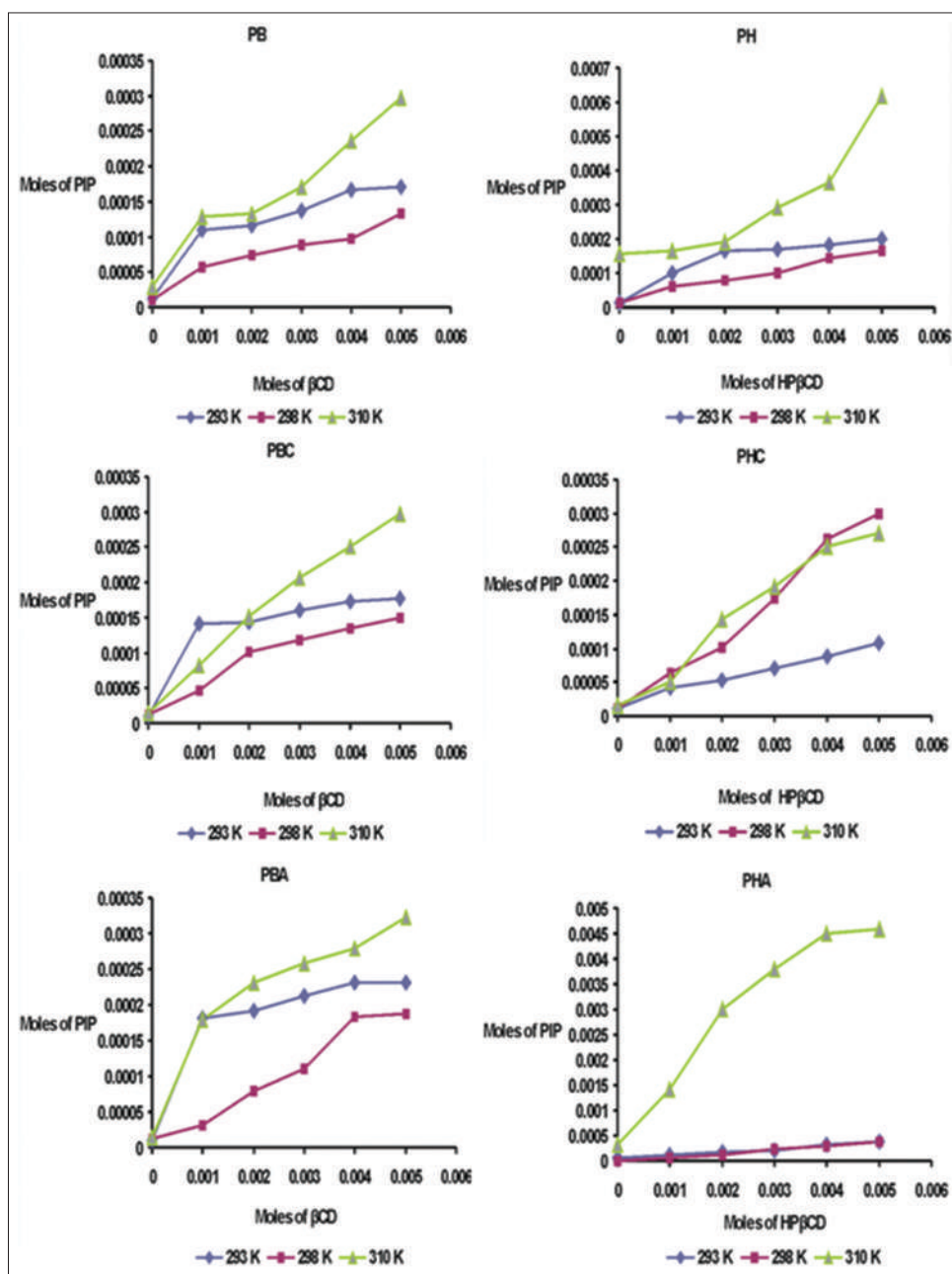

Figure 1: Phase solubility diagram of piperine (PIP) with b-cyclodextrin (βCD)/HPβCD at different temperatures. PB: PIP + βCD; PH: PIP + HPβCD; PBC: PIP + βCD + CA; PHC: PIP + HPβCD + CA; PBA: PIP + βCD + AA; PHA: PIP + HPβCD + AA

Table 1: Phase solubility data and thermodynamic parameters involved in binary and ternary inclusion complexes of pure drug with cyclodextrins

Systems	T (°C)	S_0 (Moles/mL)	Slope	r^2	K_s (M⁻¹)[a]	K_{TS}/K_{BS}	CE	ΔH (KJ/Mol)	ΔS (J/Mol/K)	ΔG (KJ/Mol)
PB	20	0.000048	0.028	0.819	597±15	-	0.0214	26	143	-17
	25	0.000022	0.021	0.934	1000±110					
	37	0.000044	0.048	0.953	1138±153					
PH	20	0.000050	0.030	0.993	7720±113	-	0.0298	-98	-266	-19
	25	0.000022	0.029	0.982	1380±89					
	37	0.000060	0.100	0.999	690±22					
PBC	20	0.000040	0.038	0.988	7720±111	1.17	0.0277	-65	-152	-20
	25	0.000024	0.027	0.935	1173±95[b]					
	37	0.000026	0.056	0.990	1498±133					
PHC	20	0.000016	0.018	0.983	1137±70	1.97	0.0599	54	229	-14
	25	0.000008	0.058	0.972	2181±230[c]					
	37	0.000014	0.055	0.973	4230±139					
PBA	20	0.000020	0.065	0.938	7826±223	1.69	0.0400	-29	-30	-20
	25	0.000025	0.039	0.966	1695±88[b]					
	37	0.000082	0.052	0.834	3586±346					
PHA	20	0.000034	0.068	0.971	2193±93	3.26	0.0810	33	179	-20
	25	0.000025	0.075	0.989	3260±131[c]					
	37	0.000010	0.749	0.906	4945±1666					

PB: PIP + βCD; PH: PIP + HPβCD; PBC: PIP + βCD + CA; PHC: PIP + HPβCD + CA; PBA: PIP + βCD + AA; PHA: PIP + HPβCD + AA; S_0: Solubility of PIP in absence of CD; r^2: Regression coefficient of phase solubility graph; K_s (M⁻¹): Association constant of complex; K_{TS}/K_{BS}: Ratio of Ks for ternary and binary system; CE: Complexation efficiency; ΔH: Enthalpy; ΔS: Entropy; ΔG: Gibbs free energy. [a]Indicates mean ± S.D. (n=3), [b]Significant difference compared to *Ks* of PB ($P < 0.001$), [c]Significant difference compared to *Ks* of PH ([p] < 0.001). PIP: Piperine, βCD: b-cyclodextrin
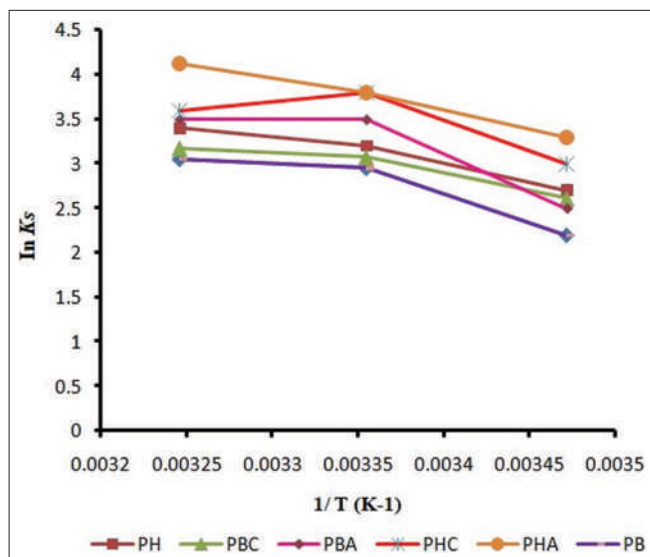

Figure 2: Van't Hoff plot (ln K vs. 1/T) for piperine: b-cyclodextrin (βCD)/HPβCD inclusion complexes

values, it could be concluded that the removal of an enthalpy rich water molecule from the CD cavity is a very important ruling force for PIP and CD complexation.[10] Standard Gibbs free energy change (ΔG) given by the change in enthalpy and entropy depicted spontaneity of the complexation process.

Molecular modeling studies

As depicted in Figure 3 and Table 2, the insertion of piperidine ring through the narrow rim of βCD was the most stable model corresponding to the least complexation energy, optimum Van der Waals and electrostatic interactions with intra CD hydrogen bonding in presence and absence of ternary components. Therefore, it could be concluded that βCD was able to accommodate the piperidine ring from narrow rim inside the βCD cavity in the presence and absence of both ternary components, suggesting their involvement in non-bonded interactions with PIP and βCD. In general, PIP metabolizes rapidly by demethylation of methylenedioxy group, glucuronidation, and sulfation reaction,[24] Therefore, accommodation of piperidine ring inside the CD cavity may prevent its metabolism occurred by glucuronidation resulting in long-term therapeutic action of the drug.

FTIR analysis

The possible interaction between PIP and CDs was studied by ATR-IR spectroscopy. The IR patterns of all systems are shown in Figure 4. The principle absorption peaks of PIP [Figure 4A] were observed at 3100 cm⁻¹ (C-H stretching aromatic), 1635 cm⁻¹ (C=C symmetric and asymmetric
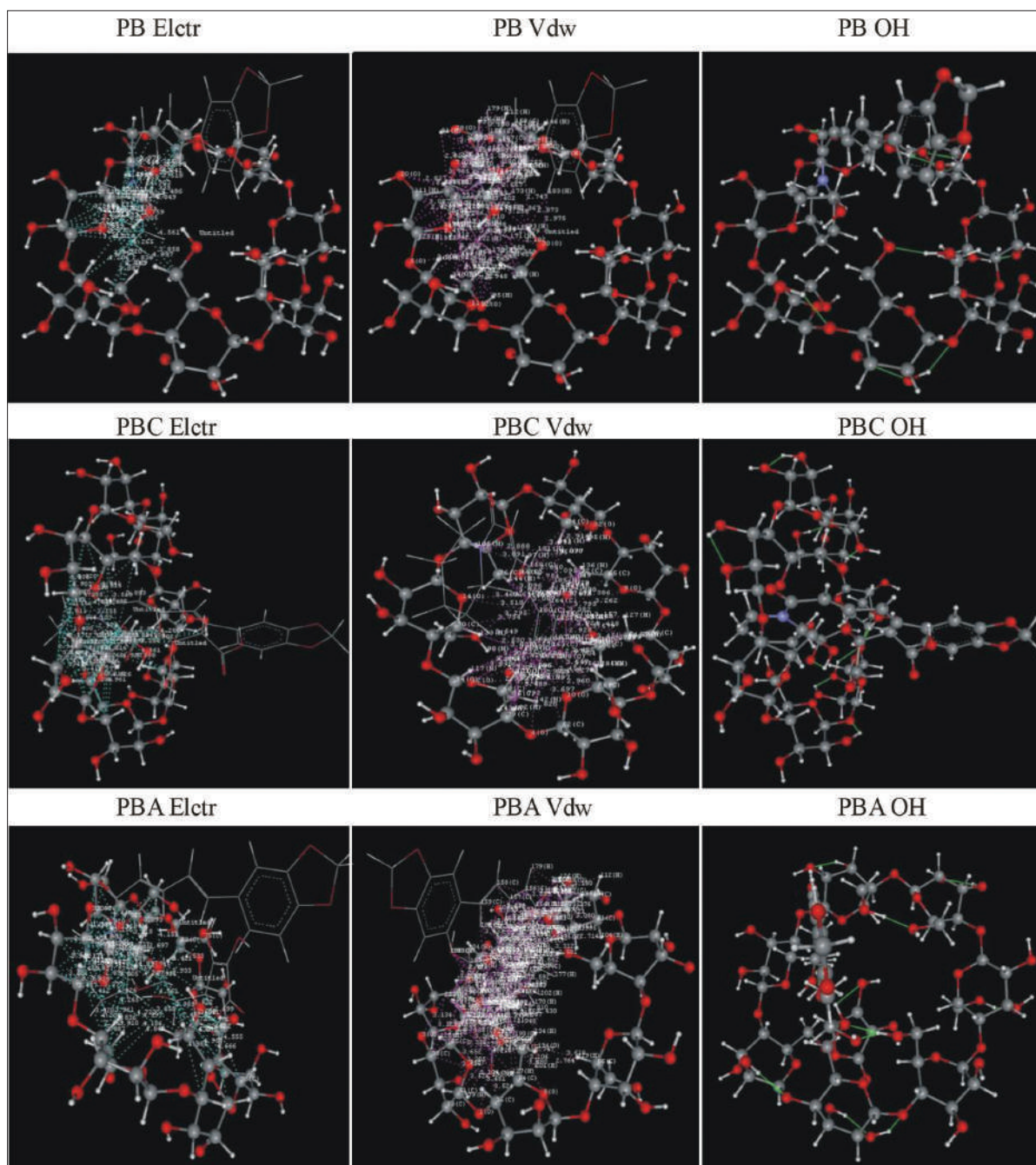
Figure 3: Optimized geometric models of PB, PBC, and PBA with electrostatic (Elctr), Van der Waals (Vdw) and hydrogen bonding (-OH) interactions in presence and absence of ternary components

Table 2: Molecular modeling data of all systems				
Systems	ΔE	Vdw	Elctr	O-H bonds
1. PB	−59343	160	32	8
2. PBC	−135866	192	−44	9
3. PBA	−409539	167	32	10

ΔE: Complexation energy (Kcal/mol); Vdw: Van der Waals force of interactions; Elctr: Electrostatic force of interactions; O-H bonds: Number of hydrogen bonds

stretching, diene), 1550 cm^{-1} (C=C aromatic stretch, benzene), 1600 cm^{-1} (-CO-N stretching), for ethylenedioxy group = 2925 cm^{-1} (C-H stretching aliphatic), 1450 cm^{-1} (CH$_2$ bending), 930 cm^{-1} (C-O stretching), and 805 cm^{-1} (out-of-plane C-H bending 1,2,4-trisubstituted phenyl).

The absorption spectra of βCD [Figure 4B] had shown prominent peaks at 3225 cm^{-1} (O-H, stretching, OH), 2933 cm^{-1} (C-H stretching aliphatic), 1653 cm^{-1} (H-O-H, bending), and 1017 cm^{-1} (C-O-C, stretching). Figure 4C displayed absorption bands of HPβCD at 3741 cm^{-1} (O-H stretch), 2922 cm^{-1} (C-H stretch), and 1024.23 cm^{-1} (C-O-C stretch). Figure 4D indicated absorption spectra of CA at 3492 cm^{-1} (O-H, stretching, OH) and 1750 cm^{-1} (C=O aliphatic, carboxylic acid) and AA

[Figure 4E] has shown prominent peaks at 3210-3786 cm^{-1} (O-H stretch), 1750 cm^{-1} (C=O stretch, 5 member lactone ring), 1650 cm^{-1} (C=C stretch), 1270 cm^{-1} (C-O-C stretch), and 1019 cm^{-1} (C-O-C bend).

In case of PB (Figure 4F) complexes, the principle absorption peaks of pure PIP at 2900 cm^{-1}, 1200 cm^{-1}, and 1050 cm^{-1} were shifted to 2889 cm^{-1}, 1153 cm^{-1}, and 1026 cm^{-1}, respectively, for PBC [Figure 4G] complexes at 2912 cm^{-1}, 1200 cm^{-1}, and 1022 cm^{-1}, respectively, and for PBA [Figure 4H] complexes at 2890 cm^{-1}, 1150 cm^{-1}, and 1077 cm^{-1}, respectively.

Furthermore, for the case of PH [Figure 4I] complexes, the peaks of pure PIP at 2900 cm^{-1}, 1200 cm^{-1}, and 1050 cm^{-1} were shifted to 2917, 1633, and 1021, respectively, for PHC [Figure 4J] system at 2911, 1720, and 1077, respectively, for PHA [Figure 4K] complexes at 2921, 1674, and 1021, respectively. In lyophilized complexes, all peaks were found to be smoothened and did not show any new peaks indicating non-covalent interaction in inclusion complexes.[25,26]

¹H NMR and 2D NMR analysis

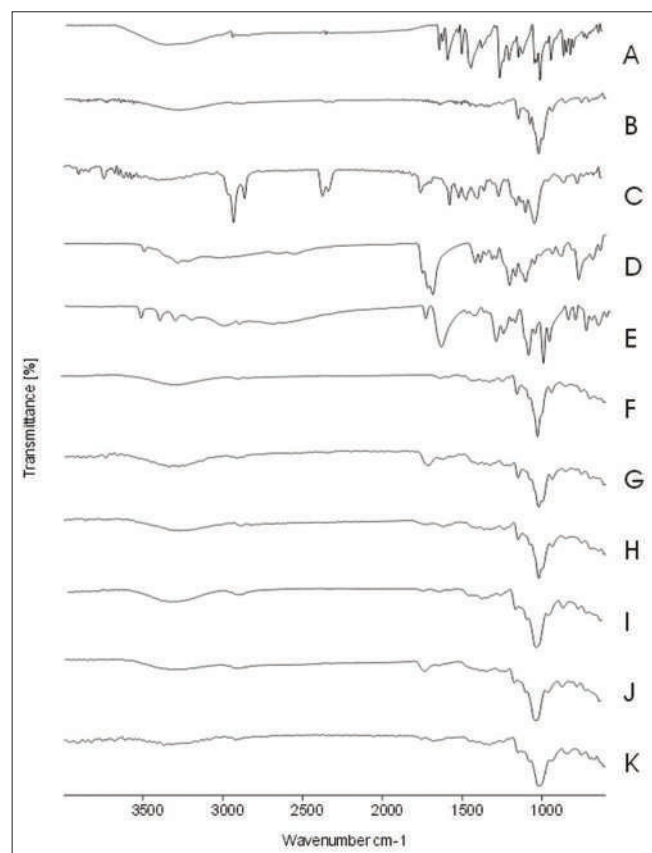The chemical structure of PIP, AA, and CDs is demonstrated in Figure 5a. The interactions between PIP and CDs have been carried out by ¹H NMR spectroscopy [Figure 5b]. Pure PIP exhibited signals at 5.82 ppm (H-1), 6.97 ppm (H-2), 6.89 ppm (H-3), and 6.77 ppm (H-4) of methylene dioxyphenyl moiety. The aliphatic groups of PIP were found at 6.74 ppm (H-5), 6.72 (H-6), 7.27 (H-7), and 6.42 (H-8). The signals of (H-9), (H-10), (H-11), (H-12), (H-13), (H-14), and (H-15) were observed at 3.58 ppm, 1.66 ppm, 1.58 ppm, 1.68 ppm, 3.58 ppm, 1.68 ppm, and 3.58 ppm, respectively. The signals for H-3' and H-5' of CD were observed at 3.67 ppm and 3.79 ppm, respectively. Pure AA exhibited signals at 4.80 ppm (H-1") of furanone, 4.22 ppm (H-2") of methine, and 3.59 ppm (H-3") of methylene.

The changes that occurred in NMR signals clearly indicated host-guest interactions. The protons of PIP located inside the CD cavity in complex, experienced upfield shift changes due to the shielding effect by the CD cavity. The chemical shift change ($\Delta\delta$) values for PIP protons are given in Table 3. In the case of PB complexes, all protons of PIP experienced upfield shift, whereas H-2, H-4, H-10, and H-12 are diffused indicating the formation of inclusion complexation. All protons of PBA complexes exhibited an upfield shift except H-8 and H-9 protons explicating the insertion of piperidine or methylene dioxyphenyl moiety inside the CD cavity. The binary and ternary systems of PIP with HPβCD displayed high upfield shift of piperidine and an aliphatic moiety of PIP, suggesting its penetration inside the HPβCD cavity.

In general, the penetration of the drug molecule (guest) takes place from the wider rim side of the cavity. The H-3' and H-5'

Figure 4: Fourier transformation-infrared spectroscopy spectrum of Piperine (A), β-cyclodextrin (βCD) (βCD) (B), HPβCD (C), CA (D), AA (E), PB (F), PH (G), PBC (H), PHC (I), PBA (J), PHA (K)

Table 3: Proton nuclear magnetic resonance spectroscopy (600 MHz) chemical shift change ($\Delta\delta$) values for various protons of piperine in the presence of β-cyclodextrin or HPβCD at 25°C

Protons	PB	PBA	PH	PHA
H-1	−0.08	−0.06	0.07	…
H-2	…	−0.01	0.02	…
H-3	−0.09	−0.03	−0.02	−0.09
H-4	…	…	−0.01	…
H-5	−0.04	…	−0.01	0.01
H-6	−0.18	0.02	0.04	0.03
H-7	−0.19	−0.04	−0.02	−0.06
H-8	−0.27	0.07	0.08	0.02
H-9	−0.01	0.01	−0.04	…
H-10	…	−0.04	−0.05	−0.11
H-11	−0.23	−0.06	−0.04	−0.03
H-12	…	−0.06	−0.07	−0.13
H-13	−0.01	−0.01	−0.04	…
H-14	−0.322	−0.06	−0.06	−0.12
H-15	−0.006	0.01	−0.03	−0.004

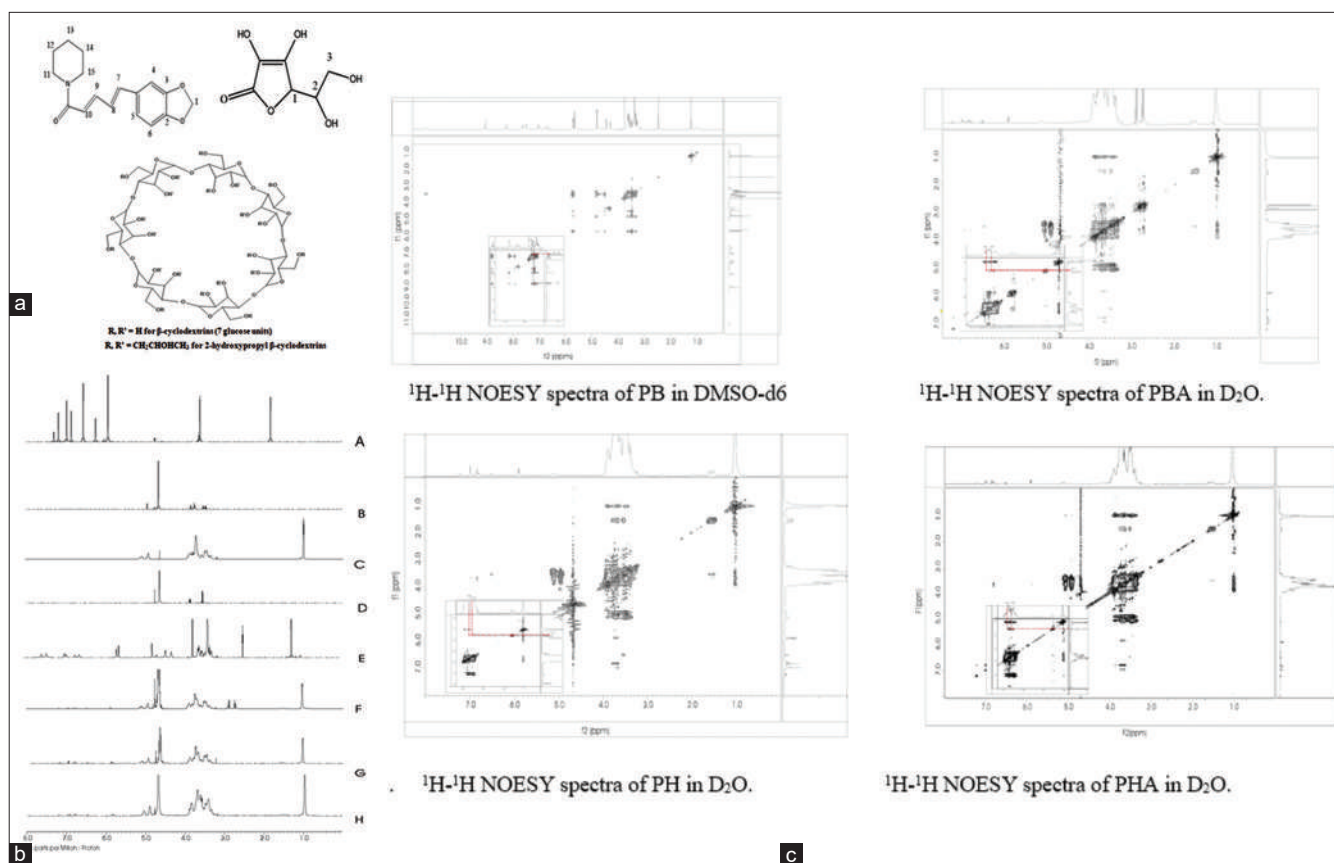Negative values indicate upfield shift

Figure 5: (a) Structure of piperine (PIP), AA, β-Cyclodextrin and Hydroxypropyl-β-Cyclodextrin (b) ^{1}H NMR spectra of (A) PIP, (B) b-cyclodextrin (βCD) (C) HPβCD, (D) AA, (E) PB, (F) PBA, (G) PH, (G) PHA (C) ^{1}H-^{1}H NOESY spectra of PB, PBA, PH, and PHA

protons situated at an inner region of CD cavity, therefore, change in chemical shifts of H-3′ and H-5′ protons of CDs in the presence of guest molecule suggests that inclusion in cavity has taken place.[27] In all the systems of PIP with CDs, the signals for H-5′ and H-3′ protons exhibited high field shifts compared to pure CDs which indicate inclusion in cavity has taken place as these protons are located inside the CD cavity.

A deep entrance of guest into the CD cavity results in the chemical shift of both the protons H-3′ and H-5′ of CD, whereas the shift in only H-3′ protons occurs when the cavity entrance is shallow.[28] The chemical shift change (Δδ) values for CD protons are explicated in Table 4. The ratio of ΔδH-5′/ΔδH-3′ gives the idea regarding depth of penetration of guest inside the CD cavity. The positive values of ΔδH-5′/ ΔδH-3′ protons might be attributed to the deep insertion of the PIP from the wider rim of CD.

^{1}H-^{1}H NOESY analysis is a very interesting tool used to estimate the relative positions of CD protons and guest protons for analyzing the structure of inclusion complex.[2] To predict and confirm the inclusion structure of PIP inside the CD cavity, ^{1}H-^{1}H NOESY analysis has been carried out in solution state [Figure 5c].

Table 4: Proton nuclear magnetic resonance spectroscopy (600 MHz) chemical shift change (Δδ) data for CD protons in the presence of piperine at 25°C

Systems	H-3'	H-5'	Δδ H-5'/ Δδ H-3'
PB	−0.064	−0.04	0.625
PBA	−0.117	−0.018	0.153
PH	−0.11	−0.023	0.209
PHA	−0.117	−0.018	0.153

Negative values indicate upfield shift

The PB system has exhibited cross peaks between piperidine ring (H-9, H-13), methylene dioxyphenyl moiety (H-1) of PIP, and H-3' and H-5' protons of βCD suggesting deep penetration of PIP inside the βCD cavity through the wider annular rim in such a way that both piperidine and methylene dioxyphenyl moiety facing toward the wider rim of βCD. In earlier studies, Ezawa et al. have reported that methylene dioxyphenyl moiety of PIP penetrates in βCD cavity through the wider rim.[5] In case of PBA complexes, the cross peaks were observed between H-10, H-11, H-12 (piperidine ring) of PIP and H-3' and H-5' protons of βCD indicating penetration of piperidine ring in βCD cavity through wider annular rim with deep penetration.

The binary and ternary complexes of PIP with HPβCD displayed a similar pattern of cross peaks. The PH and PHA complexes have shown cross peaks between H-10, H-11, H-12 (piperidine ring) of PIP and H-3' and H-5' protons of HPβCD which indicates deep penetration of piperidine ring in HPβCD cavity through wider annular rim. From observed results, it could be clear that βCD has capacity to accommodate both piperidine and methylene dioxyphenyl moiety facing toward wider rim of βCD, while HPβCD obliges just piperidine ring in its cavity through its wider annular rim. In prior investigations researchers previously reported that the incorporation of piperidine ring, aliphatic group or methylene dioxyphenyl moiety of PIP was relying upon the nature of CD which is very supported here.[2]

No alteration of signals of AA protons has been observed, indicating ternary component just gave synergistic effect to inclusion by exerting various non-bonded interactions such as hydrophobic interactions and Van der Waals force of interactions. Therefore, from observed findings, it could be concluded that both the CDs were able to accommodate the piperidine ring inside the CDs cavity in such a way that piperidine ring and aliphatic moiety facing toward the wider annular rim of CDs with deep penetration in the presence and absence of AA as a ternary component.

SEM analysis

SEM [Figure 6] was used to study the morphological characteristics of drugs and complexes. Pure PIP was characterized by the presence of sharp, smooth, and crystalline particles. The particles of PB and PBA complexes exhibited altered shape and have shown uneven, smooth
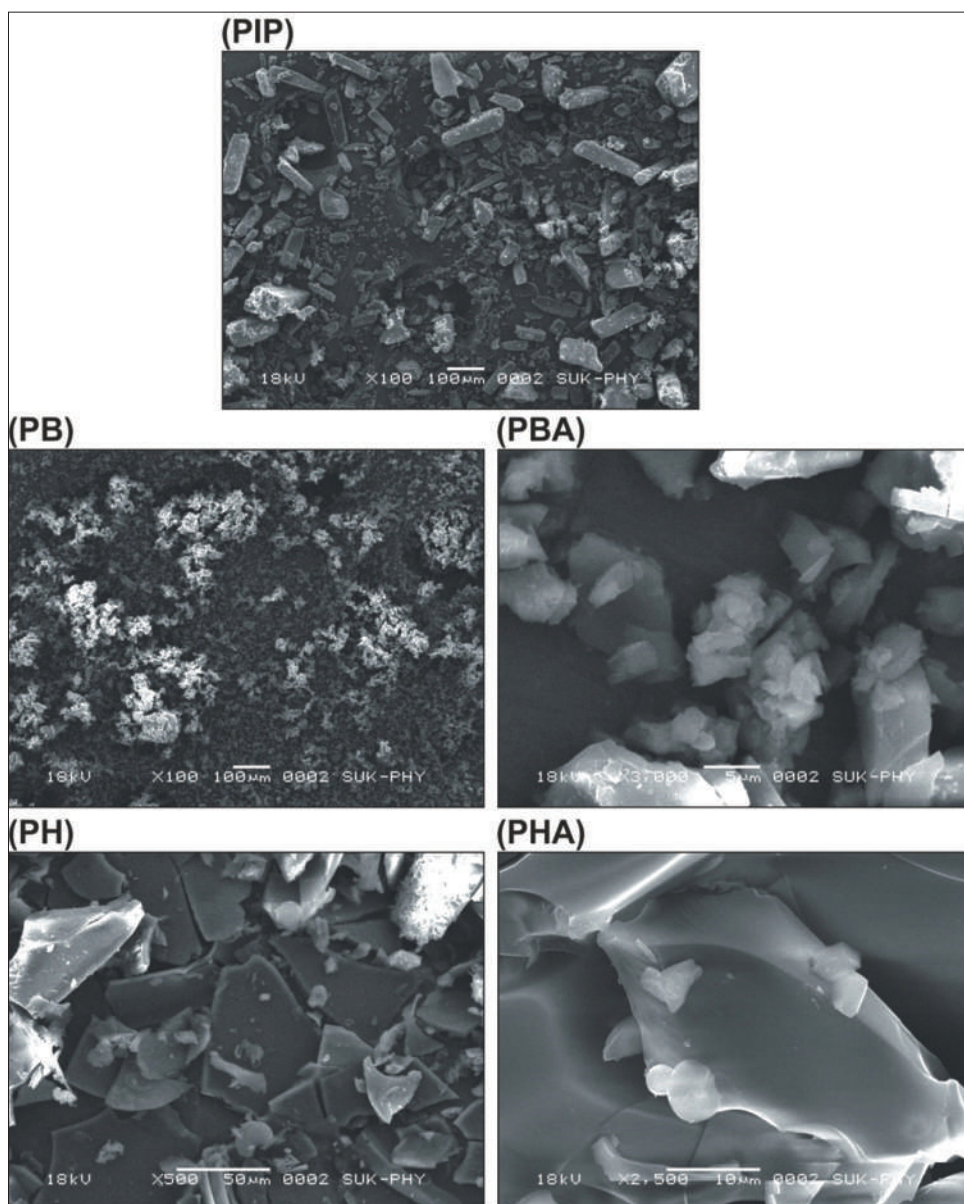

Figure 6: Scanning electron microscopic of piperine, PB, PH, PBA, and PHA

lumps type morphology. The amorphous nature of HPβCD has contributed to change in morphology of PH and PHA complexes showing irregular pieces of amorphous aggregates revealing significant modifications in the original morphology of pure PIP. The microphotographs of all systems suggested that the alteration in morphology of particles in lyophilized complexes confirmed the presence of a new solid phase which might have played an important role in the modifications of physicochemical properties of drugs to some extent.[29]

Partition coefficient (Log *P*)

The partition coefficient (Log *P*) values of PIP, PB, PH, PBC, PHC, PBA, and PHA complexes were determined as 4.03±2, 2.82±1, 0.90±0.3, 2.69±1, 0.31±0.2, 2.42±1.1, and 0.02±0.01, respectively ($P < 0.001$). It was evident from observed data that all inclusion complexes have shown lower Log *P* values than that of pure PIP, whereas HPβCD binary and ternary complexes indicated a tremendous difference in Log *P* values, indicating an improvement in aqueous solubility due to amorphous natured HPβCD and addition of hydroxy acids as ternary components.[15,29]

In-vitro dissolution studies in 0.1N HCl at pH 1.1

The *in-vitro* drug release profile in 0.1 N HCl at pH 1.1 is illustrated in Figure 7 where the percentage of drug dissolved is plotted against time. The % drug release at 10 min (DR_{10}) for PIP, PB, PH, PBC, PHC, PBA, and PHA was found to be 8±2, 17±2, 13±1, 18±2, 12±2, 22±2, and 100±5, respectively, and dissolution efficiencies of the same at 10 min (DE_{10}) were calculated as 3±0.8, 8±1, 7±1, 9±2, 6±0.3, 9±1, and 38±4, respectively. A significant difference between the dissolution efficiencies of PIP and complexes ($P < 0.001$) was observed. From the findings, it was noted that the drug release rate was rapid and complete from HPBCD binary (PH) as well as ternary systems (PHC, PHA) indicating HPβCD performed well as complexing and solubilizing agent.[21,29] The PHA complexes have shown 100 % drug release at 10 min time interval suggesting the addition of AA as a ternary component
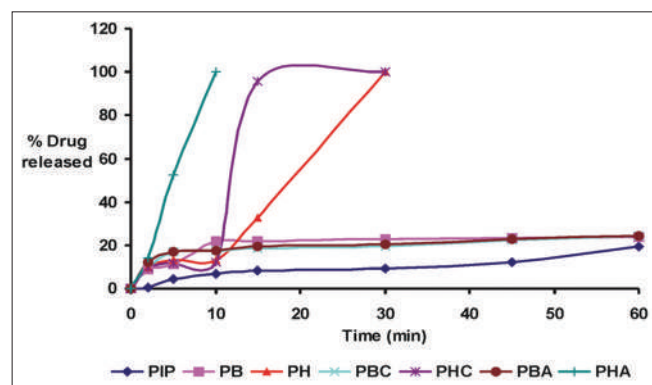

Figure 7: Dissolution profile of piperine and all inclusion complexes in 0.1 N HCl at pH 1.1

resulted in improved stability (phase solubility studies) and complexing property.

CONCLUSION

The present research work demonstrated successful formation of inclusion complexes of PIP with βCD and HPβCD in presence and absence of hydroxy acids by lyophilization techniques. The applications of hydroxy acids have significantly contributed to the improvement in physicochemical characteristics of PIP through inclusion complexation with CDs. In addition to that MM studies depicted the insertion of the piperidine ring of PIP inside the βCD cavity in the presence and/or absence of AA, which was in full agreement of NMR results. In all formulations, HPβCD ternary inclusion complexes formed with AA gave significant advantages through its excellent physicochemical properties over all other formulations. All in all, the selection of AA as a ternary component could be the best choice in terms of improvement in solubility and dissolution of PIP by inclusion phenomenon with βCD and HPβCD.

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