



Encapsulation of plumbagin using cyclodextrins to enhance plumbagin stability: computational simulation, preparation, characterization, and application

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Abstract

Encapsulation of plumbagin using cyclodextrins (CDs), including α -cyclodextrin (α CD), β -cyclodextrin (β CD), and γ -cyclodextrin (γ CD) to form inclusion complexes was investigated to prevent the loss of plumbagin in pharmaceutical and nutraceutical products. Computational simulations and phase solubility studies suggest that the complex formations of plumbagin with CDs are possible. β CD is chosen, as it has the lowest price and can form the complex in a wide concentration range with 1:1 host–guest molar ratio. Two techniques of the complex formation, co-precipitation and freeze-drying, were evaluated for both pure plumbagin and extracted plumbagin from *Plumbago indica* root to represent lab-scale and industrial-scale productions, respectively. The complexes from these two techniques can prevent the loss of plumbagin up to three-folds better than plumbagin in free form. The preservation by encapsulation can increase the remaining plumbagin from 22.68 to 60.26% after exposure at 50 °C for 6 weeks.

Keywords Encapsulation · Inclusion complex · Plumbagin · Cyclodextrins

Introduction

Active compounds derived from plants have various applications across multiple industries, including foods, cosmetics, and pharmaceuticals. However, the instability of these compounds when exposed to sunlight, moisture, temperature, and oxidizers is a major obstacle for commercial utilization. The tendency of active compounds to degrade leads to a short shelf life and inconsistent product quality. Plumbagin is a natural naphthoquinone found in *Plumbago indica* [1, 2] with multiple pharmacological properties, including anti-bacterial, anti-inflammatory, and anti-cancer activities [3–7], but it is unstable when exposed to temperatures above 4 °C [4]. Plumbagin also has a hypolipidemic activity, as it

can reduce the low-density lipoprotein form of cholesterol while increase the high-density lipoprotein form to prevent accumulation of cholesterol in blood vessels [8]. In order to utilize plumbagin in commercial products, a preservation technique of plumbagin from environmental conditions requires to be implemented. Encapsulation is an effective technique which can preserve active compounds in host molecules. This technique can prevent the degradation of active compounds from exposure to sunlight, high temperature, oxidizing agent, and moisture [9–14]. Since the applications of plumbagin are mainly in the pharmaceutical and food products, a suitable non-toxic host molecule is used in encapsulation.

Several compounds, such as phospholipid micelle, liposome, niosome, and cyclodextrin (CD) have been introduced as the host molecules for plumbagin encapsulation to enhance the anticancer efficacy and aqueous solubility [15–19]. Micelle and liposome were reported for plumbagin encapsulation for slow release applications in drugs. There is no report about the preservation of plumbagin in the solid form in these studies [15, 19]. Niosome was used for preservation of plumbagin. However, the preservation efficiency of plumbagin in niosome is low, especially in first

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7 days [18]. Relative to other host molecules, CD is an interesting host for plumbagin preservation in pharmaceutical and food products due to the effectiveness of the cost and preparation methods. The computer simulation results from our previous study show multiple hydrogen bonds between plumbagin and CD in the form of inclusion complex that provides the potency of the preservation in the form of solid complex [20]. CD, a non-toxic cyclic oligomer of glucose [21–23], is commonly used as an encapsulating host molecule in pharmaceutical, food, cosmetic, and textile applications to increase solubility, preserve odor and color, and prevent the degradation of active compounds [24–31]. CD has a truncated cone shape with a hydrophilic outer surface and a hydrophobic inner cavity. The hydrophilic surface allows CD for preparation in an aqueous solution and the hydrophobic cavity provides the ability to encapsulate a hydrophobic active compound to form an inclusion complex [32]. Primary cyclodextrin is available in three forms: α -cyclodextrin (α CD; 6 glucose units), β -cyclodextrin (β CD; 7 glucose units), and γ -cyclodextrin (γ CD; 8 glucose units) with cavity sizes of 0.47–0.83 nm [11, 33]. Several works have reported the inclusion complex preparation using β CD and its derivatives to increase the efficacy of pure plumbagin [16–18].

Computer simulations can be employed to investigate the stability, host–guest interactions, and proper conditions for plumbagin inclusion complex formation with α CD, β CD, and γ CD. Our previous work reported the molecular interactions of plumbagin inclusion complexes with β CD and its two derivatives in aqueous phase using computer simulations [20]. There are two different conformations of plumbagin inside the β CD cavity with 1:1 host–guest molar ratio. However, there is no report of simulations and experiments on the inclusion complexes of plumbagin using α CD and γ CD.

In pharmaceutical and food industries, the solid inclusion complex of plumbagin provides the ease in product storage and transportation. The solid complex can be formed by co-precipitation and freeze-drying. Co-precipitation is a widely used technique in lab-scale to obtain small amount of a solid complex, which can be used to evaluate complex formation and characterization. However, this technique is not efficient in industrial-scale production due to a large amount of solvent and wastewater disposal [34]. Freeze-drying is one of the attractive techniques for industrial-scale production of the inclusion complex, as it utilizes low temperatures to minimize the loss of heat-labile guest molecules during the process [24]. In this study, co-precipitation was used for lab-scale production using pure plumbagin while freeze-drying was used for industrial-scale production using extracted plumbagin from *Plumbago indica* root, which contains other compounds besides plumbagin. The conditions of these two techniques were also evaluated along with the

economic considerations for the feasible industrial production. Moreover, there is no report on the differences in encapsulation efficiency and preservation capability between the inclusion complex formation from pure plumbagin and extracted plumbagin from *Plumbago indica* root.

Therefore, the objectives of this study are to: (1) investigate the possibility and host–guest molar ratio of the plumbagin/ α CD, plumbagin/ β CD, and plumbagin/ γ CD inclusion complex formations using computer simulation, (2) evaluate the complex formation of pure plumbagin with CDs using co-precipitation, (3) evaluate the complex formation of extracted plumbagin from *Plumbago indica* root by freeze-drying for scaling up and industrial production, and (4) investigate the preservation capability of the complexes using pure plumbagin and extracted plumbagin. The encapsulation technique in this study can effectively preserve plumbagin from storage environment. Other active compounds of interest to the pharmaceutical and food industries may also be preserved by utilizing the techniques outlined in this study.

Materials and methods

Computational simulation method

Molecular structure construction

The crystal structures of plumbagin, α CD, β CD, and γ CD were obtained from Cambridge Crystallographic Data Centre [35] with the CSD entries: PVVAQS01 [36], BANXUJ [37], BCDEXD03 [38], and CIWMIE10 [39], respectively. Hydrogen atoms were added into the structures, and the structures were fully optimized by the semi-empirical quantum mechanical PM6 method using the Gaussian 16 software package [40].

Molecular docking calculation

The AutoDock 4.2.6 software [41] with the Lamarckian Genetic algorithm [42] was used to generate the possible conformations of plumbagin/CD inclusion complexes. The first step was to prepare of the guest (plumbagin) and host (CDs) molecule coordinate files, delete the non-polar hydrogen atoms, and merge their charges with the carbon atoms. The rotatable bonds of the guest molecule were defined while the host molecules were kept fixed. The systems were investigated in a three-dimensional volume divided into many small grid boxes with a grid spacing of 0.375 Å. The grid center of the boxes was set at the center of the host molecules. The boxes have ($x \times y \times z$) dimensions of (12.75 Å \times 12.75 Å \times 7.50 Å), (13.50 Å \times 13.50 Å \times 7.50 Å) and (16.50 Å \times 16.50 Å \times 7.50 Å) for α CD, β CD, and γ CD,

respectively. One hundred docking calculations were performed on each host–guest complex. The results were clustered to identify similar conformations based on all-atom root mean square deviation within 2 Å. At the end of the molecular docking calculations, the representative docked conformations with the lowest energy from molecular docking were selected for further full geometry optimization.

Complex formation energy calculation

The possible conformations from docking calculations of plumbagin/CD inclusion complexes were then constructed and fully geometry optimized by the PM6 method, in which all atoms were allowed to move freely. The most stable conformation of the complexes was evaluated by the complex formation energy (ΔE) using Eq. (1).

$$\Delta E = E_{PL/CD} - (E_{PL} + E_{CD}) \quad (1)$$

where $E_{PL/CD}$, E_{PL} , and E_{CD} are the heat of formations of the complex, plumbagin, and CD, respectively.

Materials

Plumbagin (99.9%, Acros Organics, Geel, Belgium) was used as the guest molecule, α CD (98.0%, Tokyo Chemical Industry, Tokyo, Japan), β CD (97.0%, Sigma-Aldrich, Missouri, USA), and γ CD (99.0%, Tokyo Chemical Industry, Tokyo, Japan) were used as the host molecules in this study. Ethanol (99.9%, Merck, Massachusetts, USA) was used as the co-solvent in the inclusion complex preparation. *Plumbago indica* root powder (TPC Herb, Bangkok, Thailand) was used to obtain the crude extract by maceration in ethanol (95.0%, Spread Business, Pathum Thani, Thailand).

Phase solubility studies and thermodynamic parameters of inclusion complex formation

The phase solubility study [43] was performed to investigate the effect of α CD, β CD, and γ CD to the plumbagin solubility. CDs were dissolved in deionized water to prepare the solutions with concentrations of 0 to 16 mM in sealed conical flasks. Ten milligrams of plumbagin (0.05 mmol) was added into 10 mL of each CD solution to form the inclusion complex. The mixtures were shaken in a thermostatic incubator (Model: NB-205V, N-Biotek, Gyeonggi-do, Korea) protected from light at 25 °C and 150 rpm for 48 h to attain the equilibrium. The mixtures were then filtered to separate undissolved plumbagin from the solution. The amount of dissolved plumbagin in filtrate was determined by HPLC. The phase solubility of plumbagin/ β CD was also studied at 35 °C and 45 °C with the same procedure to determine the stability constant (K_C). The value of K_C of the complex can be calculated from the slope of the linear portion of the

phase solubility profile at different temperatures using the Eq. (2).

$$K_C = \frac{\text{Slope}}{S_0(1 - \text{Slope})} \quad (2)$$

where S_0 is the intrinsic solubility of plumbagin in the absence of CD.

The thermodynamic parameters, including enthalpy change (ΔH) and entropy change (ΔS) of the complex formation were calculated using the van't Hoff equation shown in the Eq. (3) [14, 44, 45].

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

Determination of host–guest molar ratio by Job's method

The continuous variation method (Job's method) was used to investigate the changes in absorbance of an inclusion complex solution for determination of an inclusion complex host–guest molar ratio. Plumbagin and β CD solutions were prepared in deionized water and mixed at different molar ratios (R) to make up the total solution of 0.3 mM. The absorbance of plumbagin was recorded at 254 nm and 25 °C using an UV/Visible spectrometer (Model: Genesys 10S, Thermo Fisher Scientific, Massachusetts, USA). The changes in absorbance multiplied by molar ratio, i.e., $\Delta A \cdot R$, were plotted with R to obtain the Job's plot.

Preparation of plumbagin/CD inclusion complexes

Inclusion complex formation of pure plumbagin with CDs by co-precipitation

The plumbagin/CD inclusion complexes were prepared by mixing 400 mg of CDs in 50 vol% ethanol–water solution and plumbagin with the host–guest molar ratio of 1:1. The mixture was shaken in an orbital shaker (Model: NB-101M, N-Biotek, Gyeonggi-do, Korea) at 25 °C for 48 h. The solution was then kept in a refrigerator overnight to precipitate the plumbagin/CD inclusion complexes. The solid complexes were filtered, dried in an oven at 40 °C for 24 h, and then kept at –20 °C prior to the characterization.

Effects of solvent in complex formation

Plumbagin dissolves well in ethanol but poorly dissolves in water. In order to encapsulate plumbagin by β CD in the form of inclusion complex, the effect of solvent (ethanol–water system) at various concentrations was evaluated. β CD (400 mg) was dissolved in 20 mL of 0, 30, 50, and 70 vol% of ethanol–water solutions. Plumbagin with the host–guest

molar ratio of 1:1 was added to each β CD solution. The solution was then filtered to get rid of undissolved plumbagin. The solution was then kept in a refrigerator overnight to obtain the inclusion complex. The solid complex was filtered, dried in an oven at 40 °C for 24 h, and then kept at –20 °C to prevent the loss of plumbagin prior to the composition analysis.

To determine the amount of plumbagin in the complex, plumbagin in the solid complex was extracted by 99.9% ethanol at 40 °C for 60 min using sonication (Crest Ultrasonics, New Jersey, USA) in a closed system. The solid β CD, which is not dissolved in pure ethanol, was filtered out. The amount of plumbagin in the solution was determined by using HPLC. The encapsulation efficiency was calculated using the Eq. (4).

$$\text{Encapsulation efficiency} = \frac{\text{Amount of plumbagin encapsulated}}{\text{Amount of plumbagin initially added}} \quad (4)$$

The percentage of undissolved plumbagin and encapsulation efficiency are summarized in Table 1. Hence the appropriate concentration for complex formation in this study is 50 vol% of ethanol–water solution.

Inclusion complex formation of extracted plumbagin with β CD by freeze-drying

The *Plumbago indica* extract was prepared by maceration of the *Plumbago indica* root powder in ethanol with a 30-min sonication twice a day for 3 days. The filtered solution was concentrated and dried using a rotary evaporator (Model: R3, Büchi, Flawil, Switzerland) at 45 °C and 175 mbar with cooling water at 4 °C. The *Plumbago indica* extract was kept at –20 °C before use. The yield was 0.37 mg of plumbagin/g of root.

The inclusion complex formation of extracted plumbagin was performed by an addition of extracted plumbagin to β CD in 50 vol% of ethanol–water solution. Since the *Plumbago indica* extract contains other compounds [46], the host–guest molar ratio was varied as 1:1, 3:1, and 5:1 to investigate a proper molar ratio. The mixture was shaken at 25 °C for 48 h. The obtained solution was concentrated

by using a rotary evaporator prior to freeze-drying (Model: Freeze Dryer –55 °C, Operon, Gyeonggi-do, Korea). The freeze-dried complex was kept at –20 °C. The amount of encapsulated plumbagin after extracted by ethanol was determined by HPLC. The encapsulation efficiency was calculated using the Eq. (4).

Characterization of plumbagin/CD inclusion complexes

X-ray diffractometry (XRD)

The crystal structures of plumbagin/ α CD, plumbagin/ β CD, and plumbagin/ γ CD inclusion complexes were characterized by a X'Pert PRO diffractometer (PANalytical, Almelo, the Netherlands). Pure plumbagin, α CD, β CD, γ CD, and physical mixtures were used to make comparisons. The physical mixtures of plumbagin and CDs were prepared by mixing plumbagin directly into each CD at 1:1 molar ratio. XRD was operated with a Ni-filter, a Cu $K\alpha_1$ radiation, a voltage of 40 kV, and a current of 45 mA at 25 °C. The XRD patterns of the samples were recorded between 2 θ of 3° and 40° with a step size of 0.02° and a step time of 0.5 s.

Fourier-transform infrared spectroscopy (FTIR)

The functional groups of the inclusion complexes were analyzed by a Nicolet iS50 FTIR spectrometer (Thermo Fisher Scientific, Massachusetts, USA). Solid samples for FTIR were prepared by mixing 1 wt% of each sample in spectral grade potassium bromide (Specac, Pennsylvania, USA) and pressed into thin disks. The spectrograms were recorded at wavenumbers between 400 and 4000 cm^{-1} with 16 scans and a resolution of 4 cm^{-1} in the transmission mode.

Differential scanning calorimetry (DSC)

The energy changes of the inclusion complexes were characterized by a DSC 1 (Mettler-Toledo, Ohio, USA). Each solid sample of 5 mg was placed in an aluminum crucible. The samples were heated from 30 to 200 °C at 10 °C/min under N_2 with a flowrate of 50 mL/min.

Thermogravimetric analysis (TGA)

The thermal analysis of the inclusion complexes was performed on a TGA/DSC 3+ HT/1600/219 (Mettler-Toledo, Ohio, USA). The samples were weighed and placed in alumina crucibles. The samples were heated from 30 to 500 °C at 10 °C/min under N_2 with a flowrate of 50 mL/min.

Table 1 Percentage of undissolved plumbagin and encapsulation efficiency at different ethanol concentrations

Ethanol concentration (vol%)	Undissolved plumbagin (%)	Encapsulation efficiency (%)
0	97.2	0.78 ± 0.18
30	78.3	15.10 ± 0.31
50	0.0	53.54 ± 0.43
70	0.0	16.62 ± 0.16

High performance liquid chromatography (HPLC)

The amount of plumbagin was determined by a 1260 Infinity II LC system (Agilent Technologies, California, USA) equipped with a quaternary pump (Model: G7111A), a vial sampler (Model: G7129A), a multicolumn thermostat (Model: G7116A), and a variable wavelength detector (Model: G7114A). The separation was performed on a Luna 5 μm C18(2) 100 \AA , 250 mm \times 4.6 mm column (Phenomenex, California, USA) controlled at 25 $^{\circ}\text{C}$ with an isocratic elution using 60 vol% of HPLC grade acetonitrile (RCI Labscan, Bangkok, Thailand) in aqueous solution at 1 mL/min flow rate. The injection volume was set at 10 μL and the separated compound was detected at 254 nm.

Evaluation of preservation capability of inclusion complex

The plumbagin/ βCD inclusion complex and plumbagin in free form were exposed at 25 $^{\circ}\text{C}$ and 50 $^{\circ}\text{C}$ with a duration of 4 weeks in order to evaluate the plumbagin stability of both forms. Each sample of 10 mg was kept separately in batch mode. The remaining plumbagin after exposure was extracted by 99.9% ethanol (Merck, Massachusetts, USA) and determined by HPLC. The stability of extracted plumbagin from the *Plumbago indica* root in both inclusion complex and free form were also carried out using the same procedure with a duration of 6 weeks.

Results and discussion

Computational simulations of plumbagin/CD inclusion complexes

Molecular docking of inclusion complexes

The molecular docking technique was used to determine the possibility of binding between plumbagin and CDs by considering the complex binding energy. The docking was performed by fixing the host molecules and allowing the guest molecule to be flexible in the specified grid box. The binding energy of the complexes is presented in Table 2.

The calculations indicate three possible conformations of 1:1 host–guest molar ratio for all CD systems, as shown in Fig. 1. In conformation **I**, the hydroxyl phenolic group of plumbagin was placed in the CD's cavity and located near the narrow-side of the host molecule. In conformation **II**, the methyl quinone group of plumbagin was placed in the cavity of CD and located near the narrow-side of the host molecule. Another model, conformation **III**, the naphthoquinone ring was inserted into the CD cavity, where its hydroxyl phenolic group located near the narrow-side of CD. However, the

Table 2 Free binding energy (ΔG) of plumbagin/CD inclusion complexes and number of conformations in each cluster (frequency) obtained from molecular docking calculations at 25 $^{\circ}\text{C}$

Host	Cluster	Conformation	Frequency (%)	ΔG (kJ mol $^{-1}$)	
				Lowest	Average
αCD	1	II	100	−21.63	−21.51
βCD	1	I	59	−21.09	−21.05
	2	I	4	−21.00	−20.92
	3	II	25	−20.96	−20.92
	4	I	12	−20.75	−20.75
γCD	1	III	100	−21.92	−21.88

naphthoquinone ring in conformation **III** was oriented in different angles, compared with that in conformation **I** as shown in Fig. 1.

According to the size of CD, only conformation **I** was favorable for plumbagin/ αCD , both conformation **I** and **II** were found in plumbagin/ βCD , and only conformation **III** was favorable for plumbagin/ γCD . However, the rigidity of the host molecules in the docking calculations was not similar to reality. Therefore, the semi-empirical PM6 calculation was further used to investigate the molecular interactions between plumbagin with three different CDs.

Energy of complex formation

The inclusion complex of plumbagin with each CD systems was suggested by docking calculations. Both of conformation **I** and **II** were constructed for αCD and βCD . Plumbagin was possible to form the complex with γCD in all conformations due to the large cavity diameter of γCD . All complex conformations were then fully optimized by the PM6 method which provides the free motions for both host and guest molecules as shown in Fig. 2.

From the PM6 results, Tables 3 and 4 list the heat of formation and complex formation energy of minimized free compounds and inclusion complexes in this study. The negative values of the energy indicate possible formations of the plumbagin inclusion complexes with all CDs in this study. The lower value of complex formation energy indicates more favorable inclusion complex formation. The results show that the favorable formation is related to the cavity size of CDs, where the larger cavity leads to more favorable inclusion complex formation as follows: $\gamma\text{CD} > \beta\text{CD} > \alpha\text{CD}$. Moreover, the simulation shows that the favorable formation can occur at 1:1 host–guest molar ratio of plumbagin with three different CDs.

Table 5 presents the distance of intermolecular hydrogen bonds which are found in the PM6 minimized inclusion complex structures. Three types of hydrogen bonds were established between plumbagin and CD at the

Fig. 1 Schematic illustration of possible conformations of inclusion complex

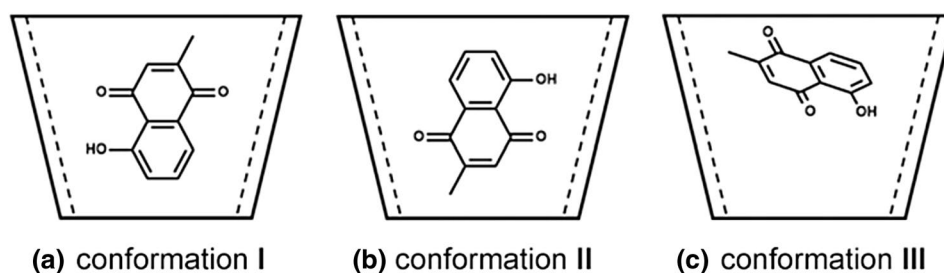


Fig. 2 Energy-minimized structures of plumbagin/CD inclusion complexes

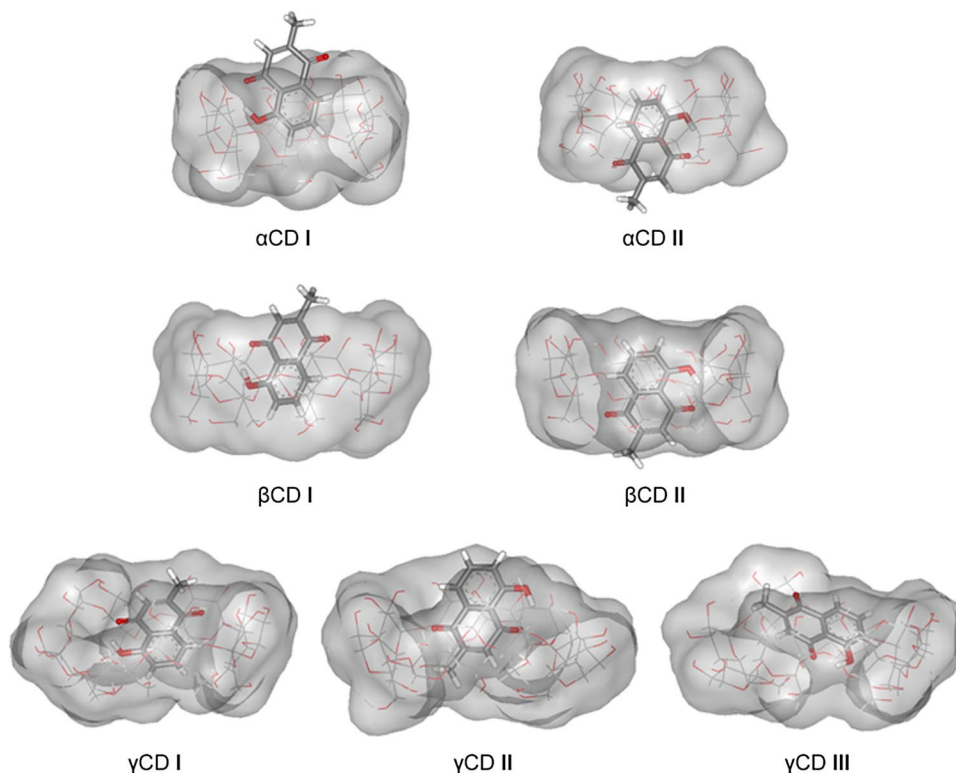


Table 3 Heat of formation (E) of minimized free compounds from PM6 method

Compound	E (kJ mol ⁻¹)
Plumbagin	-332.65
αCD	-5598.96
βCD	-6578.55
γCD	-7459.44

positions assigned in Fig. 3. The first type of hydrogen bonds, which is often found in the inclusion complex systems, occurred between an ether-like anomeric oxygen atom of CD and a hydrogen atom of plumbagin's hydroxyl group ($O_{4(CD)} \cdots H_{(OH-PL)}$). The second type occurred between an oxygen atom of plumbagin's carbonyl group and the hydrogen atom of the secondary hydroxyl group at O3 of CD ($O_{(CO-PL)} \cdots H_{(O3H-CD)}$) or the hydrogen atom of the primary hydroxyl group at O6 of CD ($O_{(CO-PL)} \cdots H_{(O6H-CD)}$). The last type occurred between

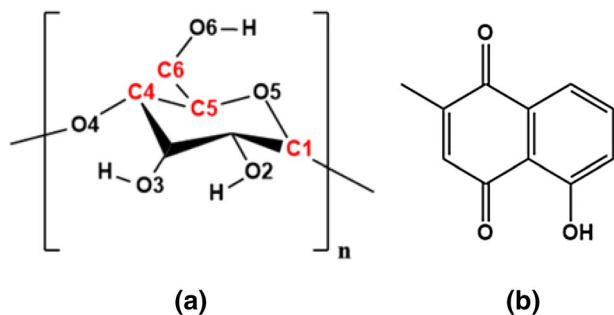
Table 4 Heat of formation (E) and complex formation energy (ΔE) of minimized plumbagin/CD inclusion complex structures from PM6 method

Inclusion complex	E (kJ mol ⁻¹)	ΔE (kJ mol ⁻¹)
αCD I	-5966.53	-34.92
αCD II	-5956.56	-24.95
βCD I	-6956.10	-44.89
βCD II	-6971.16	-59.96
γCD I	-7890.75	-98.65
γCD II	-7892.48	-100.38
γCD III	-7910.02	-117.92

an oxygen atom of plumbagin's hydroxyl group and the hydrogen atom of the secondary hydroxyl group at O3 of CD ($O_{(OH-PL)} \cdots H_{(O3H-CD)}$) or the hydrogen atom of the primary hydroxyl group at O6 of CD ($O_{(OH-PL)} \cdots H_{(O6H-CD)}$).

Table 5 Distance of hydrogen bonds between plumbagin (PL) and α CD, β CD, and γ CD host molecules from PM6 minimized inclusion complex structures

Inclusion complex	Distance (Å)
αCD I	
$O4_{(\alpha CD)} \cdots H_{(OH-PL)}$	1.99
$O_{(CO-PL)} \cdots H_{(O3H-\alpha CD)}$	2.08
αCD II	
$O4_{(\alpha CD)} \cdots H_{(OH-PL)}$	1.92
βCD I	
$O4_{(\beta CD)} \cdots H_{(OH-PL)}$	1.90
βCD II	
$O4_{(\beta CD)} \cdots H_{(OH-PL)}$	2.03
$O_{(CO-PL)} \cdots H_{(O6H-\beta CD)}$	1.93
γCD I	
$O_{(OH-PL)} \cdots H_{(O6H-\gamma CD)}$	2.04
γCD II	
$O_{(OH-PL)} \cdots H_{(O3H-\gamma CD)}$	2.03
$O_{(O3H-\gamma CD)} \cdots H_{(OH-PL)}$	2.20
γCD III	
$O_{(CO-PL)} \cdots H_{(O3H-\gamma CD)}$	2.09
$O_{(OH-PL)} \cdots H_{(O6H-\gamma CD)}$	2.06

**Fig. 3** Structures of **a** glucose unit of CD with atomic numbering (where n is 6, 7, and 8 for α CD, β CD, and γ CD, respectively) and **b** plumbagin

In this work, the theoretical configuration and molecular interaction of plumbagin/ β CD inclusion complexes in gas phase, calculated using the PM6 method, agree with the previous work, which was calculated using the PM6 method in aqueous phase [20]. Both results suggest that conformation I and conformation II are favorable, with the complex formation energy (ΔE) differences of $15.07 \text{ kJ mol}^{-1}$ and 0.13 kJ mol^{-1} , in gas phase and aqueous phase, respectively.

Phase solubility of plumbagin/CDs

The phase solubility of plumbagin/CDs was conducted to validate the simulation results. Figure 4a shows that CDs

increase the plumbagin solubility in aqueous solution. However, α CD slightly increases the plumbagin solubility, compared to β CD and γ CD. The results correspond to the simulation prediction ($\alpha\text{CD} < \beta\text{CD} < \gamma\text{CD}$) at low concentration of CDs below 1 mM. The behavior agrees well with simulations, which was performed in gas phase. At high concentration, the phase solubility diagram of plumbagin/ γ CD does not follow the simulations and shows a reduction of plumbagin solubility due to the self-aggregation of γ CD [47] as evidenced by the precipitate formation in the experiment. The phase solubility diagram in Fig. 4a also shows that β CD exhibits the complex formation in a wide range of β CD concentration. The phase solubility diagram of plumbagin/ β CD at 25°C is linear with the slope less than one, which can be classified as A_L type indicated 1:1 host–guest molar ratio [43]. The phase solubility of plumbagin/ β CD was also performed at 35°C and 45°C as shown in Fig. 4b, which can be also classified as A_L type at these given temperatures.

The slope and y-intercept (S_0) of the phase solubility diagram were used to determine the stability constant (K_C) at different temperatures as listed in Table 6. The value of K_C was used to evaluate the thermodynamic properties of the complex formation.

Figure 5 shows the van't Hoff plot from K_C at different temperatures, in which the slope and y-intercept were used to calculate ΔH and ΔS of the complex formation. The calculated ΔH of $-14.85 \text{ kJ mol}^{-1}$ shows the exothermic complex formation and indicates the weak interaction between plumbagin and β CD, such as hydrophobic interactions, van der Waals interactions, and hydrogen bonds [14], which correspond to the modeled complex in Table 5. The ΔS value of $-10.70 \text{ J mol}^{-1} \text{ K}^{-1}$ indicates that the plumbagin movement in β CD cavity is restricted by the host–guest interactions. The value of ΔG at 25°C of $-11.66 \text{ kJ mol}^{-1}$ indicates that the plumbagin/ β CD inclusion complex formation is spontaneous.

Determination of host–guest molar ratio by Job's method

Figure 6 shows the Job's plot of the plumbagin/ β CD inclusion complex system. The Job's plot of plumbagin/ β CD inclusion complex system exhibits the highest point on the curve at the molar ratio of host and guest (R) is 0.5. According to the Job's method, the host–guest molar ratio of the complex is determined by the molar ratio (R) at the highest point of $\Delta A \cdot R$. The results suggest that the host–guest molar ratio of plumbagin/ β CD inclusion complex system is at 1:1, which corresponds to the phase solubility results.

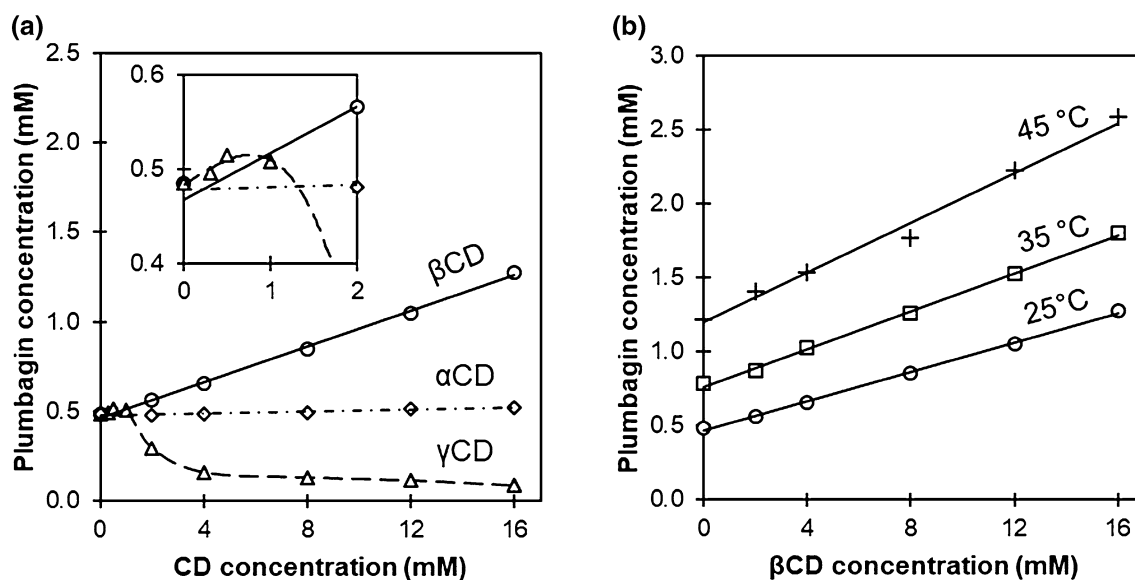


Fig. 4 Phase solubility diagrams of **a** plumbagin/CDs at 25 °C and **b** plumbagin/ β CD at 25 °C, 35 °C, and 45 °C

Table 6 Phase solubility parameters and K_C at 25 °C, 35 °C, and 45 °C

T (°C)	S_0 (mM)	Slope	R^2	K_C (M^{-1})
25	0.467	0.0495	0.9977	111.32
35	0.763	0.0641	0.9982	89.74
45	1.201	0.0840	0.9890	76.41

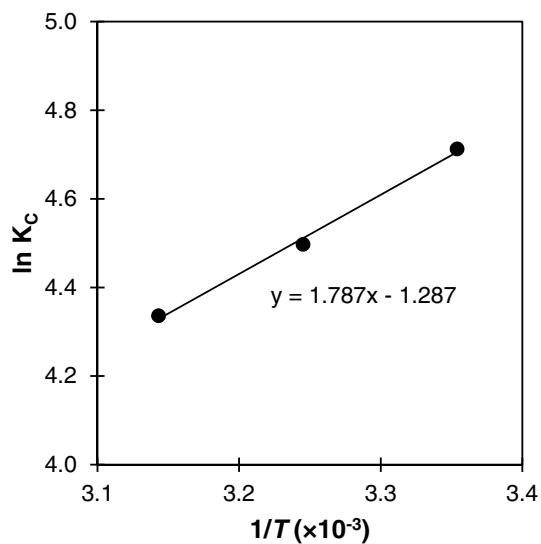


Fig. 5 van't Hoff plot of plumbagin/ β CD inclusion complex formation

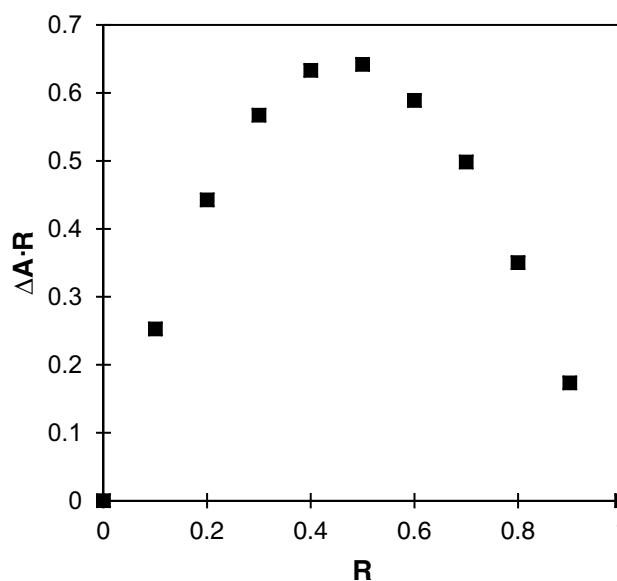


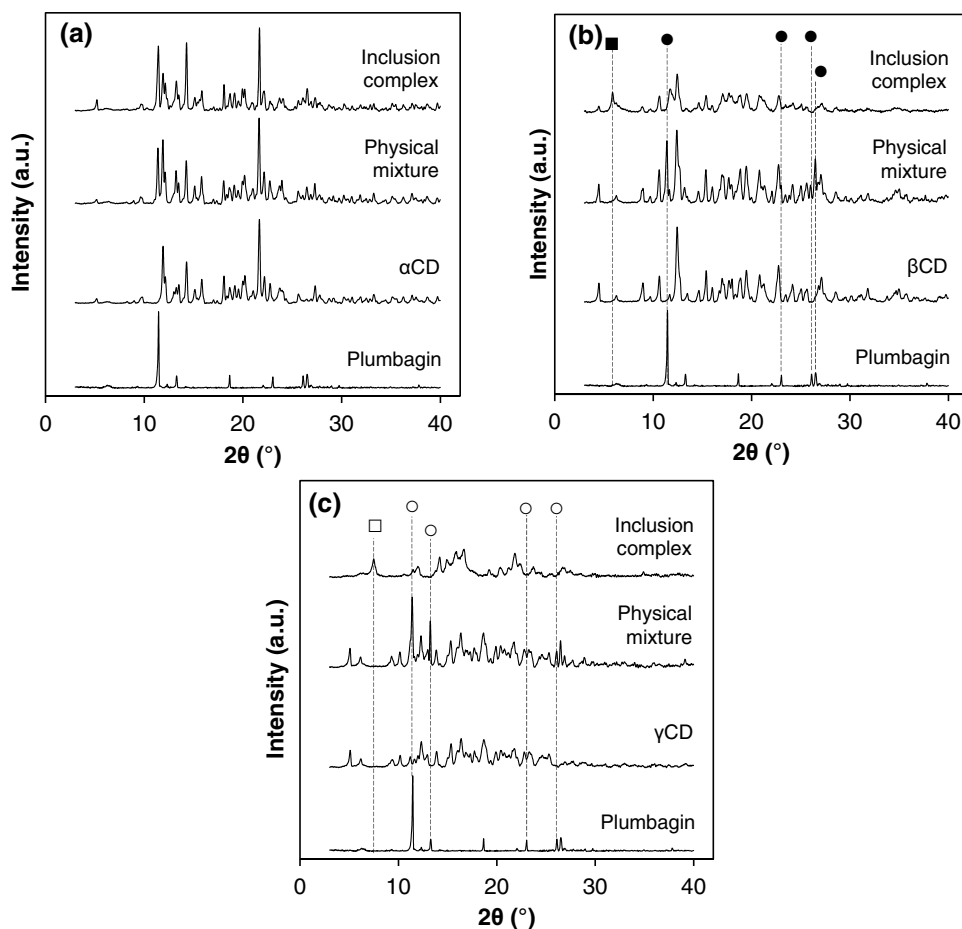
Fig. 6 Job's plot of plumbagin/ β CD inclusion complex

Characterization of plumbagin/CD inclusion complexes

X-ray diffractometry (XRD)

Figure 7 illustrates the XRD patterns of the plumbagin/ α CD, plumbagin/ β CD, and plumbagin/ γ CD inclusion complexes, compared to plumbagin, CDs, and their physical mixtures. The symbols (filled square, filled circle, open square, open circle) notify the changes of XRD pattern

Fig. 7 XRD patterns of inclusion complexes: **a** plumbagin/ α CD, **b** plumbagin/ β CD, and **c** plumbagin/ γ CD



between inclusion complex and physical mixture to indicate the complex formation.

The XRD patterns of plumbagin and CDs contain peaks that indicate the crystalline nature of the compounds. The characteristic peaks of plumbagin locate at 2θ of 11.45° , 13.27° , 18.65° , 23.03° , 26.09° , and 26.51° . The XRD patterns of the physical mixtures show the peak combination of plumbagin and CDs in free form. The XRD patterns of plumbagin/ β CD and plumbagin/ γ CD complexes exhibit the different patterns, compared to those of physical mixtures. The plumbagin/ β CD complex pattern shows the disappearance of plumbagin's peaks at 11.45° , 23.03° , 26.09° , and 26.51° , indicated by the filled circle symbols. A new peak, indicated by the filled square symbol, was found at 5.87° . For the plumbagin/ γ CD complex, the disappearance of plumbagin's peaks at 11.45° , 13.27° , 23.03° , 26.09° also occurred as indicated by the open circle symbols, and a new peak was also found at 7.53° , indicated by the open square symbols. The XRD results indicate the differences in the crystal structure of plumbagin/ β CD and plumbagin/ γ CD inclusion complexes, compared to their physical mixture. In contrast, the plumbagin/ α CD

complex and the physical mixture show the same XRD patterns which indicate the same crystal structure.

Fourier-transform infrared spectroscopy (FTIR)

Figure 8 illustrates the FTIR spectra of the inclusion complexes, physical mixtures, and pure compounds. The characteristic peaks of plumbagin were observed as follows: (1) ring stretching (1258 cm^{-1}), (2) combined modes of ring stretching, O–H in plane bending, and aromatic C–H in plane bending (1455 cm^{-1}), (3) C=C stretching (1608 cm^{-1}), (4) combined modes of C=O stretching and C=C stretching (1644 cm^{-1}), and (5) combined modes of C=O stretching and C=C stretching (1663 cm^{-1}) [48]. The physical mixtures of plumbagin and CDs exhibit the combination of peaks corresponding to both plumbagin and CDs in free form without any significant peak shift.

The IR spectra of plumbagin/ β CD and plumbagin/ γ CD complexes reveal the shift of peak from their physical mixtures. The plumbagin/ β CD complex exhibits peak shifts from the spectrum of physical mixture at 1262 , 1461 , 1615 , 1646 , and 1675 cm^{-1} . The plumbagin/ γ CD

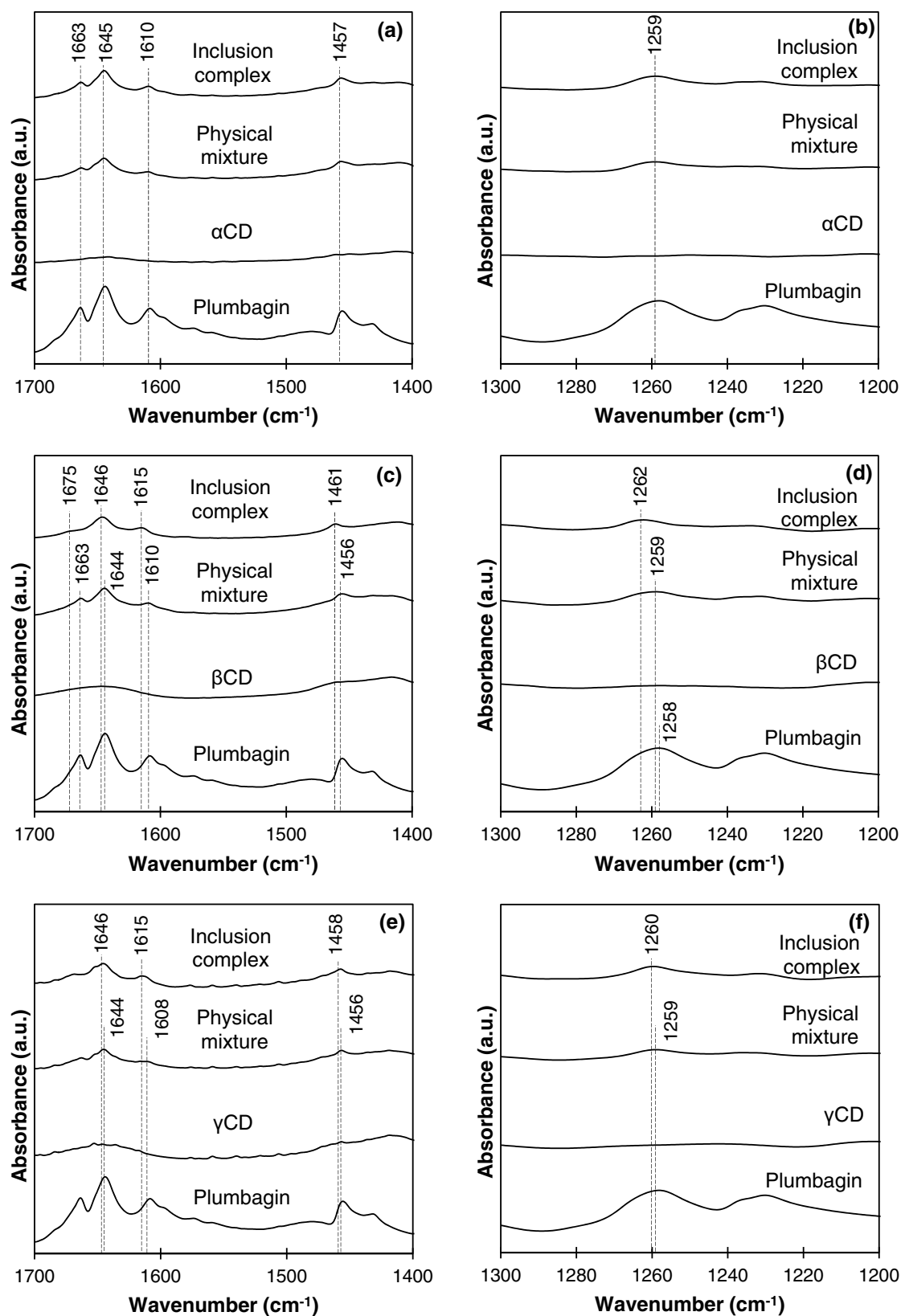


Fig. 8 FTIR spectra of inclusion complexes: **a, b** plumbagin/ α CD, **c, d** plumbagin/ β CD, and **e, f** plumbagin/ γ CD in selected ranges (1400–1700 cm^{-1} and 1200–1300 cm^{-1})

complex also shows the peak shifts at 1260, 1458, 1615, and 1646 cm^{-1} . The plumbagin/ β CD and plumbagin/ γ CD complexes show the differences in the interactions between plumbagin and CDs, compared to the physical mixtures. For the plumbagin/ α CD complex, there is no peak shift observed in the spectrum of the complex. The FTIR results agree with the XRD results as the plumbagin/ α CD complex shows no difference from the physical mixture.

Differential scanning calorimetry (DSC)

Figure 9 shows the DSC curves of plumbagin/CD inclusion complexes. For the pure compound, plumbagin shows an endothermic peak from its melting point at 79 °C. The DSC curves of the physical mixtures and the plumbagin/ α CD complex show the peaks of plumbagin's melting point at the same temperature as the pure compound. In contrast, the plumbagin/ β CD and plumbagin/ γ CD complexes show no plumbagin's melting point peak at 79 °C. The missing peak of plumbagin's melting point indicates the encapsulation of plumbagin by β CD and γ CD.

Thermogravimetric analysis (TGA)

Figure 10 illustrates the TG and DTG curves of the compounds in this study. All CDs have two steps of weight loss from water and thermal decomposition. The weight loss of pure plumbagin starts to be observed at 110 °C and continues up to 240 °C. The DTG curves of physical mixtures show the same temperatures of weight loss as observed in pure CDs and plumbagin. DTG curve of plumbagin/ α CD complex is similar to that of physical mixture which shows no evidence of complex formation as shown in Fig. 10b.

In contrast, plumbagin/ β CD and plumbagin/ γ CD complexes show no weight loss of plumbagin as shown in Fig. 10d, f, respectively. The missing weight loss of plumbagin indicates the encapsulation of plumbagin by β CD and γ CD.

The XRD, FTIR, DSC, and TGA results indicate that plumbagin can form the inclusion complexes with β CD and γ CD but not with α CD. From the phase solubility results, the plumbagin/ γ CD complex suffers from agglomeration of the complex in water at high concentration. The plumbagin can form the inclusion complex

Fig. 9 DSC curves of inclusion complexes: **a** plumbagin/ α CD, **b** plumbagin/ β CD, and **c** plumbagin/ γ CD

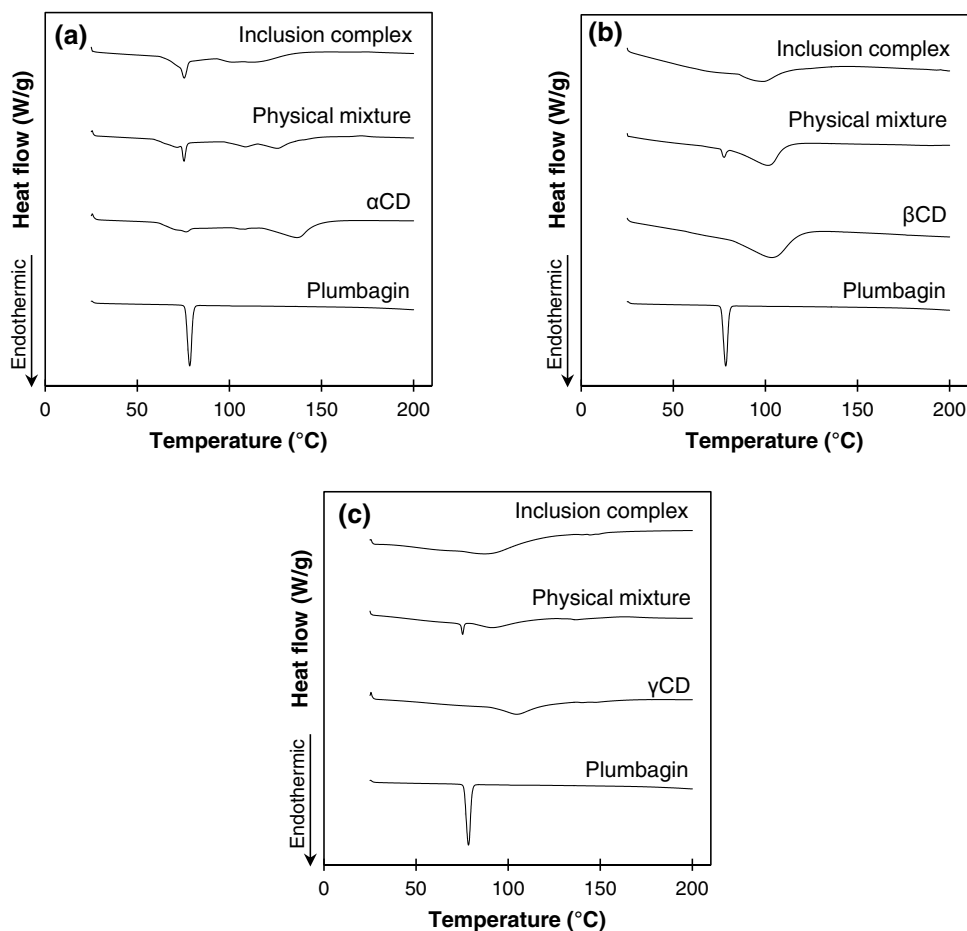
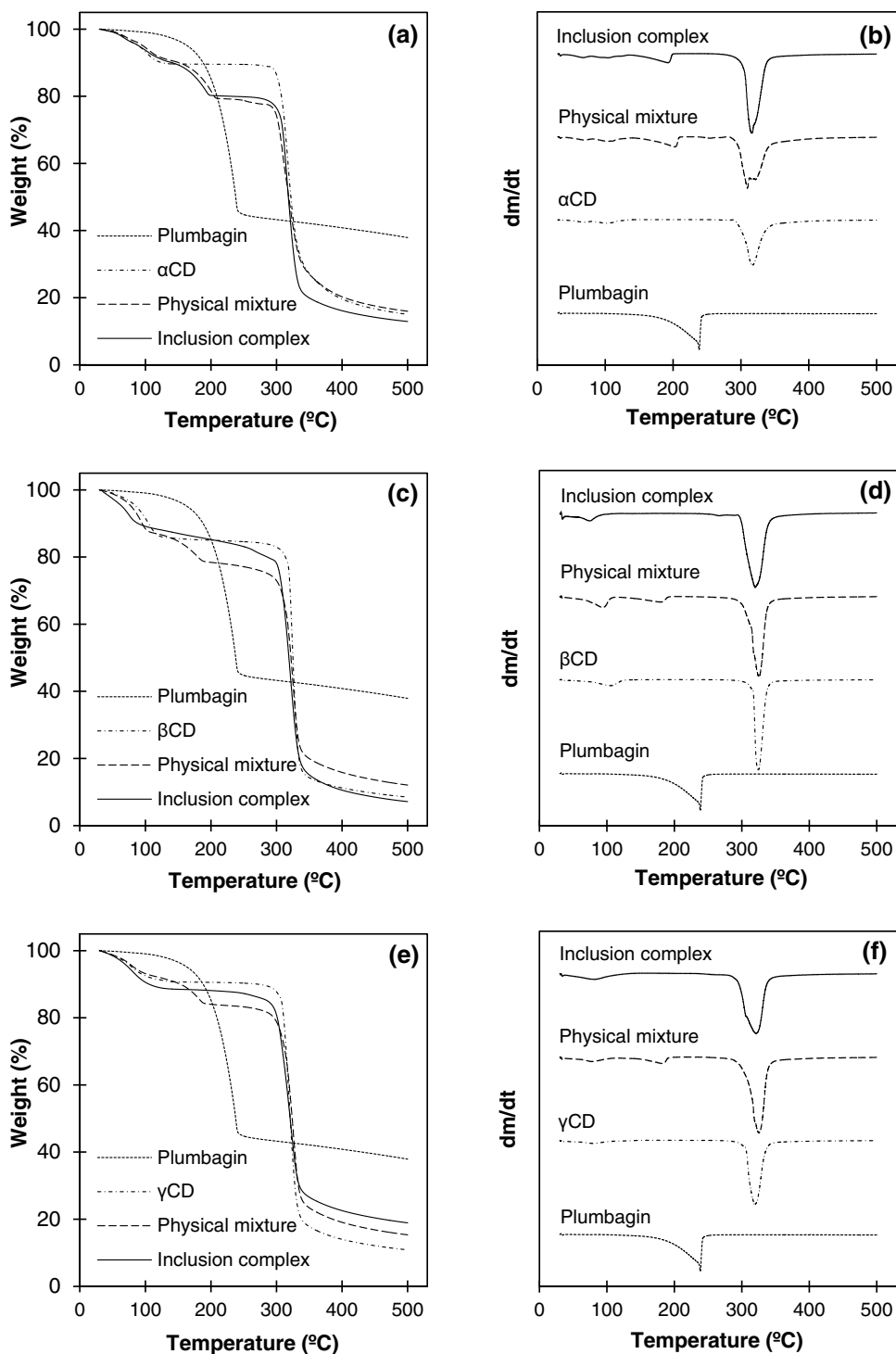


Fig. 10 Thermal gravimetric (TG) and differential thermal gravimetric (DTG) curves of inclusion complexes: **a, b** plumbagin/ α CD, **c, d** plumbagin/ β CD, and **e, f** plumbagin/ γ CD



with β CD at wide range of concentration and β CD is relatively cheap, compared to other CDs. Therefore, β CD was selected as the proper host to form the inclusion complex with pure plumbagin and extracted plumbagin from *Plumbago indica* root in this study.

Preservation capability of pure plumbagin/ β CD inclusion complex prepared by co-precipitation

The plumbagin/ β CD inclusion complex and plumbagin in free form were exposed to the surrounding at 25 °C and 50 °C for 4 weeks to evaluate the preservation capability of

Fig. 11 Preservation capability of plumbagin in free form and inclusion complex at **a** 25 °C and **b** 50 °C

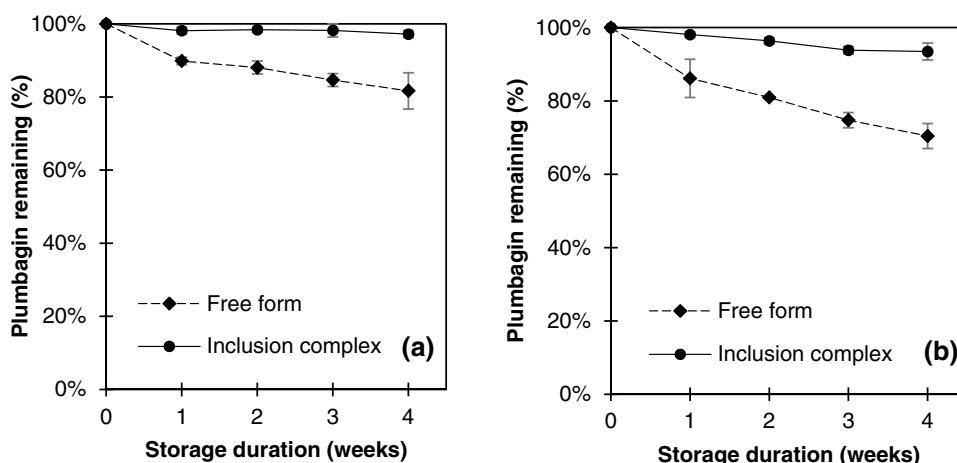


Table 7 Encapsulation efficiency of complex formation prepared by different host–guest molar ratios

Host–guest molar ratio	Encapsulation efficiency (%)
1:1	18.18 ± 0.52
3:1	30.26 ± 0.57
5:1	38.54 ± 0.25

the complex. Figure 11 shows the comparison of the preservation capabilities between plumbagin in free form and complex. Plumbagin in free form decreases as the storage duration increases. After 4 weeks, plumbagin remained at 81.67% at 25 °C and 70.43% at 50 °C. The results show that plumbagin in free form is not stable.

The inclusion complex shows a significant increment in stability as evidenced by a much slower plumbagin losing rate relative to that of plumbagin in free form. After 4 weeks, plumbagin in the complex remained at 97.20% at 25 °C and 93.46% at 50 °C. The encapsulation of plumbagin by β CD can be used to increase the stability of plumbagin in lab-scale. In real production, it is very difficult and costly for the purification of active compound. Therefore, the scale-up process of complex formation from *Plumbago indica* extract using freeze-drying was also investigated.

Preparation of plumbagin/ β CD inclusion complex from *Plumbago indica* extract prepared by freeze-drying

Table 7 lists the encapsulation efficiency of complex prepared from the *Plumbago indica* extract by different host–guest molar ratios. The ratios were based on the amount of extracted plumbagin from *Plumbago indica* root. The encapsulation efficiency increases as the host–guest molar ratio increases up to 5:1. Unlike the complex formation of pure plumbagin, various compounds in the extract [46] can also compete with plumbagin to form the complex

with β CD. The complex of extracted plumbagin might not follow the A_L type (1:1 host–guest molar ratio) as the complex of pure plumbagin. Therefore, the host–guest molar ratio for plumbagin/ β CD inclusion complex from the extract is an important factor to be considered for the industrial scale-up.

Preservation capability of plumbagin/ β CD inclusion complex from *Plumbago indica* extract prepared by freeze-drying

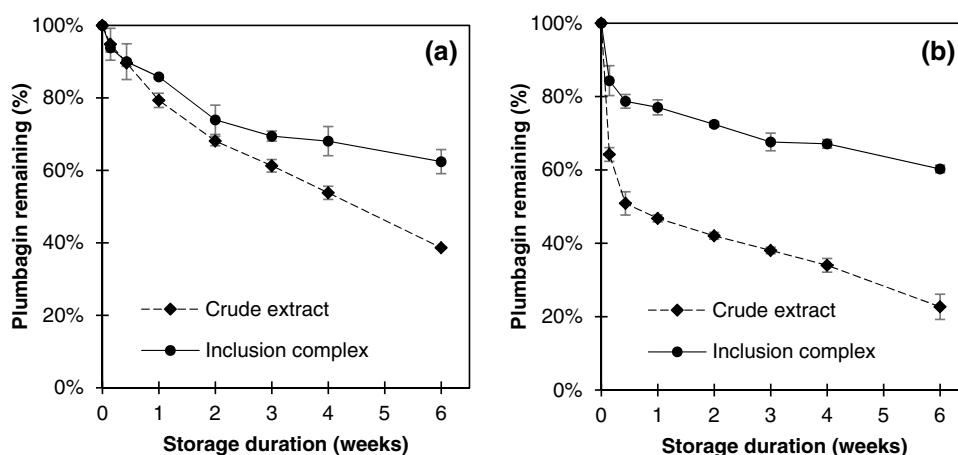
Preservation of extracted plumbagin from *Plumbago indica* root in form of inclusion complex was evaluated at 25 °C and 50 °C for 6 weeks, compared to that of crude extract as shown in Fig. 12. The extracted plumbagin in form of crude extract remained at 38.64% at 25 °C and 22.68% at 50 °C after 6 weeks. The results show that the extracted plumbagin is not stable.

The inclusion complex shows an increment in the stability of plumbagin. Plumbagin decreases at a slower rate, compared to that of extracted plumbagin. After 6 weeks, plumbagin in form of complex remained at 62.41% at 25 °C and 60.26% at 50 °C. The encapsulation of plumbagin by β CD can be used to increase the stability of extracted plumbagin from *Plumbago indica* root in industrial-scale production using freeze-drying.

Conclusion

Encapsulation of plumbagin using α CD, β CD, and γ CD were investigated, based on the simulation results of 1:1 host–guest molar ratio. The inclusion complexes of plumbagin using CDs were performed and found that plumbagin can form the complex only with β CD and γ CD but not with α CD, evidenced by XRD, FTIR, DSC, and TGA results. The phase solubility study shows that β CD can form the complex

Fig. 12 Preservation capability of plumbagin in form of extract and inclusion complex at **a** 25 °C and **b** 50 °C



formation in a wide range of concentration without agglomeration. The Job's method confirms 1:1 host–guest molar ratio and agrees well with the simulation results of the spontaneous formation of plumbagin/ β CD inclusion complex. The inclusion complex of pure plumbagin and extracted plumbagin from *Plumbago indica* root with β CD were performed by co-precipitation and freeze-drying, respectively to evaluate the preservation capability of plumbagin. The inclusion complexes can increase the preservation capability of plumbagin up to three-folds of the pure compound at a high storage temperature of 50 °C. Therefore, the encapsulation technique using CDs can serve as the preservation technique for the active compounds from plants in pharmaceutical and food products to maintain the quality and increase the shelf life of the products.

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