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ORIGINAL ARTICLE

Complexation of capsaicin with β-cyclodextrins to improve pesticide formulations: effect on aqueous solubility, dissolution rate, stability and soil adsorption

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Abstract The binary systems of capsaicin (CP) and β-cyclodextrin (βCD) or hydroxypropyl-β-cyclodextrin (HPβCD) were investigated in an attempt to improve formulations of this pesticide. UV spectral shift methods indicated guest–host complex formation between CP and the two cyclodextrins (CDs). Phase solubility analysis showed B_s type diagrams with βCD, A_L type with HPβCD indicating the formation of an inclusion complex at 1:1 stoichiometric ratio in solution state. Solubility profiles indicated a 50-fold enhancement of CP solubility could be achieved in the presence of 60 mM HPβCD with respect to CP alone. Solid co-evaporated systems (CES) with 1:0.5–1:5 molar ratios of CP/CDs were physicochemically characterized, revealing that the true inclusion complexes could be formed in the solid CP/βCD systems with 1:5 molar ratio and in the solid CP/HPβCD systems with the molar ratios more than 1:3, respectively. In contrast, crystalline drug was detectable in all other systems. Compared with corresponding physical mixtures (PMs), the CES exhibited significant enhancement with regard to CP dissolution and the protection from CP degradation under the accelerated conditions. It was also revealed that complexation of CP with HPβCD had a pronounced improved effect on the pesticide formulations and greatly reduced the amount of CP adsorbed in the soil. These results demonstrate that HPβCD may be a preferred excipient, enabling more efficient and intelligent use of CP/CDs inclusion complexes in the development of pesticide formulations.

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Introduction

Capsaicin (CP) (Fig. 1) (E)-*N*-(4-hydroxy-3-methoxybenzyl)-8-methylnon-6-enamide, is a well-known pungent ingredient of the genus capsicum, exerting major pharmacological effects on the cardiovascular, respiratory and sensory nervous systems [1–3]. For instance, CP stimulates C-fibers to produce a contractile response by releasing sensory neuropeptides such as substance P and neurokinin A. CP is also applied as a biochemical pesticide in agriculture due to its efficacy against a broad spectrum of insects [4]. However, the performance of the pesticide could be evidently limited due to its poor solubility [5] and chemical instability in dry heat [6]. On the other hand, the presence of pesticide residues in the environment poses potential risks for human health, such as causing skin irritation or diarrhea and vomiting [7]. Therefore, it is very important to introduce effective methods to improve the solubility and stability of CP, meanwhile reducing CP residues in the soil.

Complexation with cyclodextrins (CDs) has been widely used to enhance the solubility and dissolution rate of poorly soluble drugs [8–10]. The most important structural feature of CDs is the hydrophobic central cavity capable of forming stable complexes with properly sized drug molecules [11, 12]. The formation of inclusion complexes between CDs and pesticides often results in an improvement in physical, chemical, and biological properties of pesticides, such as an increase in stability of degradable pesticides, the enhancement of bioactivity, and the acceleration in degradation of pesticides [13, 14]. Pesticides are formulated not only to improve their efficacy to pests but

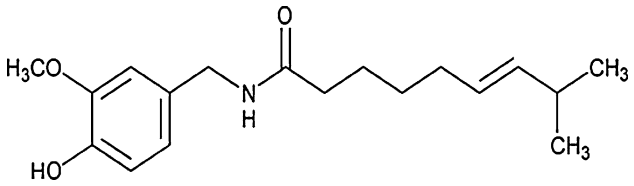

Fig. 1 Chemical structure of CP

also to reduce the environmental risk associated with overdosing. In addition, CD also has some inherent advantages which have extended its usage scope, such as being non-toxic to humans and resident microbial populations as a sugar [15], small sorption loss in soils [16], and easy removal after use [17]. Because of the beneficial effects of the formation of inclusion complexes, CDs have proven important in application of up-to-date agrochemical practice. Among the CDs, βCD and its hydrophilic derivative, such as HPβCD, are favored as a result of their suitable cavity sizes and low price.

A few of previous researches have demonstrated that CDs can improve the solubility of CP. Zi et al. [18] evaluated the ability of HPβCD to influence the percutaneous absorption of CP through isolated rat skin in liquid solution. In the study, HPβCD was employed in CP hydrogel as solubilizer and penetration enhancer in transdermal delivery. Yuan et al. [19] revealed that HPβCD was the optimal excipient for the CP hydrogel, compared with a zone. Furthermore, the inclusion compound of capsaicinoid with HPβCD has been prepared in previous report [20]. HPβCD has been proved to enhance the solubility of capsaicinoid. However, to our knowledge, no one has comprehensively studied the solubility, physicochemical characterization, stability of CP/CDs inclusion compound as a new pesticide formulation.

The aim of our study is to evaluate the effect of complexation on aqueous solubility, dissolution rate, stability and soil desorption of CP, in order to develop a new pesticide formulation. In our work the binary systems of CP with βCD or HPβCD were prepared by co-evaporation method. The UV spectral methods and phase solubility technique were used to investigate the interaction of CP with CDs in solution. The solid-state properties of CP and its binary systems were evaluated by differential scanning calorimetry (DSC), X-ray diffractometry (XRD), Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). In vitro dissolution studies of all the prepared systems were carried out to investigate the effect of the molar ratio and CD type on CP dissolution. Subsequently, modified accelerated tests were performed on the storage stability of CP. Finally, CP adsorption–desorption experiments in soil were carried out using a batch equilibration method.

Materials and methods

Materials

CP (Fig. 1) (MW = 305.4) was obtained from Wuhan Yuancheng Technology Development Co. Ltd. (Hubei Province, China) and the purity was 99.15%. βCD and HPβCD (DS = 0.8) were purchased from Wacker Chemie (Burghausen, Germany). All other chemicals and solvents were reagent grade or HPLC grade. Double-distilled water was used throughout. The soil employed to carry out the adsorption–desorption experiments was taken from the superficial horizon (0–25 cm) of the farmland. The main physicochemical properties of the soil were as follows: pH, 7.5; cationic exchange capacity, 18.5 cmol kg^{-1}; organic carbon, 16.3 kg^{-1}; sand 21.4%; silt, 57.9%; and clay, 21.7%.

UV spectroscopic measurements

UV spectroscopic measurements were used to study the complex formation between CP and CDs in distilled water [21]. The concentrations of CDs were increased from 5 to 15 mM, while the concentration of CP was kept constant at 0.04 mM. Stock solutions of CP in methanol and CDs in water were prepared in our study. Aliquots from each solution were transferred to a 50 mL volumetric flask and the volume was made up using distilled water so that the required concentrations were obtained. The prepared solutions were filtered through 0.45 μm Millipore filter after stirring for 8 h at 25 °C. A Shimadzu UV-2450 visible spectrophotometer was used to record absorption spectra from 200 to 400 nm against blank solutions containing the same concentrations of CDs. Absorbance of pure CP was also recorded for comparison.

Phase solubility studies

The phase solubility technique was carried out to establish the effects of CDs on the solubility of CP in triplicate using the Higuchi and Connors method [22]. Excess amounts of CP were added to 25 mL of either distilled water or aqueous solutions containing increasing concentrations of the CDs (0–60 mM) in a 50 mL series of screw-capped vials. The sealed vials were agitated on a rotary shaker for 48 h at 25 °C and equilibrated for further 24 h. After equilibrium attainment, the clear supernatant was filtered through a 0.45 μm Millipore filter and immediately diluted with methanol followed by HPLC assay.

The apparent stability constants (K_s) of the complexes were calculated from the slope of the phase solubility diagram according to the following equation [23]:

$$K_s = \text{slope}/S_0(1 - \text{slope})$$

where S_0 was the solubility of CP at 25 °C in the absence of CDs.

The complexation efficiency (CE), which reflected the solubilizing power of CDs toward this pesticide, was determined from the linear phase solubility diagram according to the following equation:

$$CE = \text{slope}/(1 - \text{slope}) = K_s \cdot S_0$$

Preparation of CP/CD solid systems

All the CP/CD preparations were prepared using a co-evaporation technique at molar ratios of 1:0.5, 1:1, 1:2, 1:3 and 1:5 (CP to CD). Co-evaporated systems (CES) were obtained by dissolving calculated amount of CP and CDs in 250 mL of 80% aqueous ethanol. The solution was stirred up to complete dissolution of the powders and the solvent was then removed at reduced pressure using a rotary evaporator at 40 °C. Physical mixtures (PM) were prepared by simple mixing in a mortar with pestle for 30 min at the same molar ratios for comparison. Both the obtained CES and PMs were sieved through a 250 μm sieve and placed in an oven.

Differential scanning calorimetry (DSC)

DSC analysis was performed using a PerkinElmer differential scanning calorimeter (DSC-50, PerkinElmer, Japan). The apparatus was calibrated with purified indium (99.9%). Samples (3 mg) were placed in flat-bottomed aluminum pan and heated at a constant rate of 10 °C/min, in an atmosphere of nitrogen at a temperature range of 20–400 °C.

X-ray diffractometry (XRD)

X-ray diffraction patterns were obtained from a Philips diffractometer (PW-1710), with Cu Kα radiation, voltage 30 kV, current 20 mA, scan range 3–50°, scan rate 1°/min.

Fourier-transform infrared spectroscopy (FTIR)

FTIR absorption spectra of CP and its binary systems with CDs was recorded using a spectrometer (Nexus 670, Nicolet Instrument Co.) equipped with a DTGS detector. KCl disks were prepared (2 mg sample in 200 mg KCl) and scanned over a range of 400–4,000 cm^{-1} with a resolution of 4 cm^{-1}.

Scanning electron microscopy (SEM)

SEM was performed to analyze the structural aspect of pure CP and the binary systems before and after the stability study, with a Scanning Microscope (JSM-5610LV, Japan). Samples were mounted on aluminum stubs, using double-sided sticky tabs and vacuum coated with gold for 180 s, to render them electrically conductive.

In vitro dissolution studies

In vitro dissolution studies were conducted in the distilled water using the basket method at a rotation speed of 50 ± 1 rpm using a ZRS-8G six-vessel dissolution apparatus (Tianjin University Pharmaceutical Instrument Series, China). Powdered samples containing 10 mg of CP or its equivalent prepared binary systems were added to the dissolution medium (250 mL). At predetermined time intervals (1, 3, 5, 10, 20, 40, and 60 min), aliquots (2 mL) were withdrawn following filtration (0.45 μm) and replaced by an equal volume of the same dissolution medium kept at 25 ± 0.5 °C. Concentrations of CP were determined spectrophotometrically at 280 nm using the regression equation of a standard curve developed in the same medium. The resulting dissolution curves were analyzed in terms of the dissolved drug percentage (DP_t) and the dissolution efficiency (DE_t) according to the equation:

$$DE_t = \int_t^0 DP_t / D_{100}t \times 100$$

where D is the percentage of the dissolved drug at time t and $D_{100}t$ is the area of the rectangle corresponding to a total dissolution at the same time [24]. The reported values are the arithmetic mean of six measurements ± standard deviations. The dissolution efficiency (DE) data of the binary systems were statistically analyzed using two-way ANOVA to test the significance of the effects of the CD types and molar ratios at $p \leq 0.05$. Multiple comparisons between molar ratios and CD types were then performed according to Scheffé test using SPSS software, version 7.5 (SPSS Inc., Chicago, IL).

Stability studies

Thermal and photochemical stability studies were performed to investigate the solid-state stability of CP and its binary systems with CDs. Samples for thermal stability study were stored in glass jars with lids protected from light at 55 °C under dry heat condition [6]. Aliquots were withdrawn and subjected to HPLC analysis. Samples for photochemical stability study were stored in a SYW-160B drug stability test chamber (Ningbo Science Equipment Ltd, Ningbo, China) equipped with a white fluorescent lamp (rapid-start type 20 W). The illuminance was set at 4500 lx. The photochemical stability test was carried out at 25 °C.

Pesticide adsorption–desorption experiments in soil

CP adsorption experiments in soil were carried out in triplicate in 50 mL centrifuge tubes, by mixing 5 g of each soil with 25 mL of 0.01 M $CaCl_2$ solution or 0.01 M HPβCD, containing various concentrations (0, 2, 4, 6, 8 and 10 mg L^{-1}) of CP. The samples were shaken for 48 h at 25 °C. Preliminary kinetic studies showed that 48 h of shaking was sufficient to reach pseudo-equilibrium. The supernatant was then removed after centrifugation and filtered through a 0.22 μm Millipore glass fiber membrane filter, and the CP concentration was determined in the filtrate by HPLC. Adsorption isotherms were obtained by plotting the amount of CP adsorbed by the soil (μmol kg^{-1}) versus the respective concentration in equilibrium (mg L^{-1}).

Desorption studies were carried out after adsorption equilibrium was reached, for the points corresponding to CP initial concentration of 4, 8, 10 mg L^{-1}, by removing the supernatant after centrifugation, replacing it by 25 mL of 0.01 M $CaCl_2$, allowing equilibration for an additional 24 h period, and finally following the protocol used in the adsorption experiments. Three consecutive desorption experiments were carried out.

The amount of pesticide adsorbed at equilibrium, C_s was calculated from the mass balance equation given by:

$$C_s = (C_0 - C_e)\ V/m$$

where C_0 and C_e represent the initial (mg L^{-1}) and equilibrium concentrations (μmol L^{-1}) of CP in liquid phase, V is the volume of pesticide solution used (L), and m is the mass of soil used (kg).

Sorption isotherms were fitted to the logarithmic form of the Freundlich equation:

$$\log C_s = \log K_f + n \log C_e$$

where K_f and n are constants, which related to the adsorption capacity of soil for CP and the sorption intensity, respectively.

Results and discussion

UV spectroscopic measurements

The UV absorption spectral changes of CP titrated with βCD and HPβCD were presented in Fig. 2. Spectra analysis was first performed on a set of aqueous solutions containing 0.04 mM CP and different concentrations of βCD and HPβCD (5–15 mM). The absorption maximum remained unchanged, however addition of CDs caused a visible increase of intensity which indicated that CP formed complexes with CDs in the experimental conditions [25]. The observed hyperchromic shift could be ascribed to the perturbation of the chromophore electrons of CP due to the inclusion into the CDs cavity.

Phase solubility studies

The phase solubility profiles of CP/βCD and CP/HPβCD in distilled water at 25 °C were shown in Fig. 3. The solubility of CP increased as a function of CDs due to the formation of inclusion complexes. It was revealed that solubility of CP increased with the increasing concentration of βCD in the range of 0–6 mM. When the concentration of βCD was more than 6 mM, the solubility limit of the complex is reached (0.545 mM) and further βCD addition resulted in the precipitation of the complex. Thus, a B_s type phase solubility diagram, according to Higuchi and Connors classification, was obtained. The molar ratio of the inclusion complex was calculated from the initial ascending part of the curve, a nearly straight line with a slope of 0.122 ($r = 0.9919$), indicating that a complex with a 1:1 molar ratio was formed in the solution. The apparent stability constants, K_s obtained from the slope of the phase solubility diagrams was 1187 M^{-1} and the CE was 0.138. However, the phase solubility diagram of CP/HPβCD was different from that of CP/βCD and the curve followed an A_L type, which showed a linear increase of CP solubility with a slope of 0.101 ($r = 0.9999$) during the concentration range investigated. The K_s was calculated to be 966 M^{-1} and CE was 0.112 for the CP/HPβCD complex.

The K_s and CE values suggested that CP could form a more stable complex with βCD than with HPβCD, which could be attributed to a steric hindrance of the hydroxypropyl group of HPβCD restricting the entry of the guest molecule into the CD cavity [26]. βCD at the concentration of 6 mM provided the best improvement in CP solubility (0.545 mM) due to the low solubility of βCD, whereas HPβCD could exhibit great enhancement on the CP solubility (6.240 mM) with increasing concentration of HPβCD (60 mM), which is preferred to practical application.

Differential scanning calorimetry (DSC)

DSC can be used for the recognition of inclusion complexes. When guest molecules are embedded in CD cavities or in the crystal lattice, their melting, boiling, or sublimation points generally shift to a different temperature or disappear [27]. The DSC curves for the raw materials, as well as all the binary systems studied were recorded in Fig. 4. CP showed a characteristic endothermic peak corresponding to its melting point (58 °C). In the considered temperature range, the DSC curve of the two CDs exhibited a very broad endothermal phenomenon between 70 and 120 °C due to loss of water [28]. The characteristic

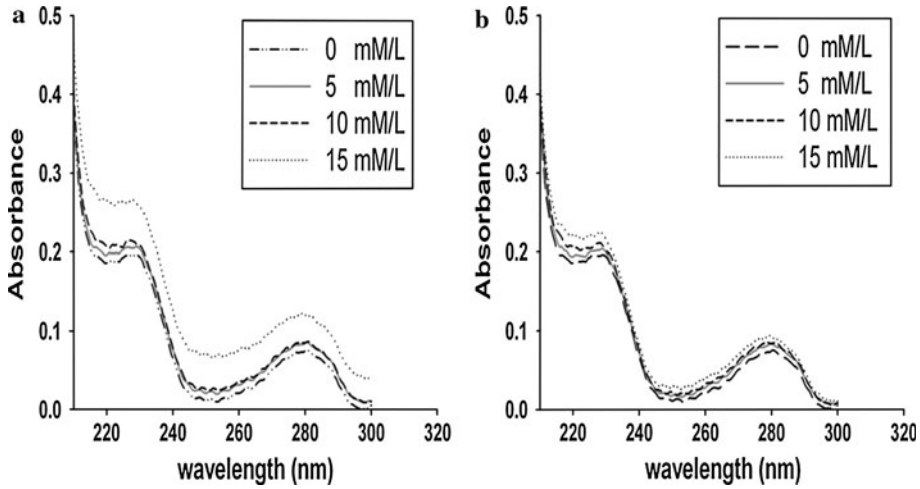

Fig. 2 Different ultraviolet absorbance spectra of CP in presence of βCD (**a**) and HPβCD (**b**)

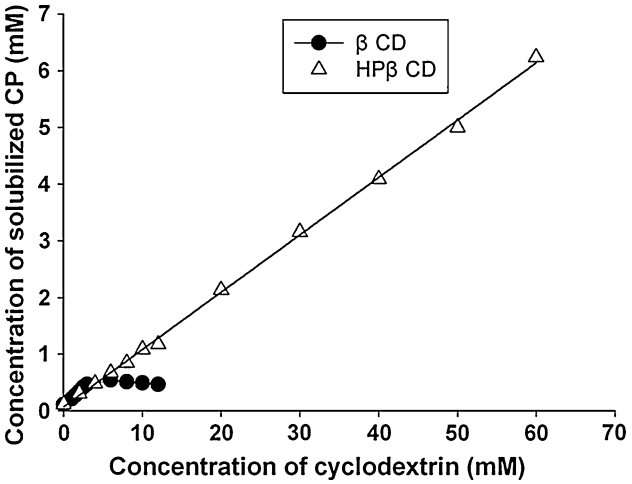

Fig. 3 Phase solubility diagrams of CP with different CDs in distilled water at 25 ± 0.5 °C

endothermic peak of CP was also evident in the analysis of PM of CP and CDs. This suggested that there was no interaction between CP and CDs on simple mixing. While turning to the CES, there was only dehydrated peak of CDs, the endothermic peak of CP at 58 °C disappeared in 1:5 (CP/βCD) molar ratio system, as well as in 1:3, 1:5 (CP/HPβCD) systems. Additionally, the drug melting endotherm was recorded in other CES, with markedly reduced intensity compared with the corresponding PMs. The results for 1:5 CP/HPBCD CES were not shown. These results indicate that a complete true inclusion complex was formed in the CP/βCD system at the molar ratio of 1:5 (CP to βCD) and in the CP/HPβCD system at 1:3 and 1:5 (CP to HPβCD).

X-ray diffractometry (XRD)

X-ray powder diffraction patterns of pure components (CP, βCD and HPβCD) and binary systems were collected (Fig. 5). In the X-ray diffractogram of CP powder, crystalline peaks at a diffraction angle of 2θ 11.8°, 19.3°, 20.1°, 23.0°, 23.9°, 24.2°, 24.8°, 25.3° and 26.9° were recorded suggesting that the drug existed as crystalline material. The XRD patterns of βCD revealed several diffraction peaks which were indicative of their crystalline character [29], whereas the absence of any peak in the HPβCD diffractogram revealed the amorphous nature of this compound. It was observed that diffractograms of PMs resulted from the combination of each component analyzed separately. Lower intensities of the diffraction peaks were found in PMs due to particle size reduction during mixing and dilution of the pure crystalline components [30]. The characteristic diffraction peaks of CP totally disappeared in the CP/βCD system at the molar ratio of 1:5 (CP to βCD) as well as in the CP/HPβCD systems at 1:3, 1:5 (CP to HPβCD) indicating that the complex constituted a new solid state. In other systems, the characteristic diffraction peaks of CP were still present or slightly decreased, suggesting that an inclusion complex was not completely formed.

Fourier-transform infrared spectroscopy (FTIR)

Further evidence of complex formation was obtained from a FTIR study, which investigated the functional groups of CP involved in the complex. The infrared spectra of different samples were presented in Fig. 6. Not all the changes of stretching frequency of CP could be observed in the binary systems because parts of the characteristic bands of CP were overlapped with CDs. However, two bands of CP appearing at 1643 cm^{-1} (C=O) and 1543 cm^{-1} (N–H bending) can be used to assess the interaction between CDs and the guest molecule (CP) in the solid state [31]. It was observed that the absorption peak at 1543 cm^{-1} disappeared in the CP/βCD system at the molar ratio of 1:5(CP

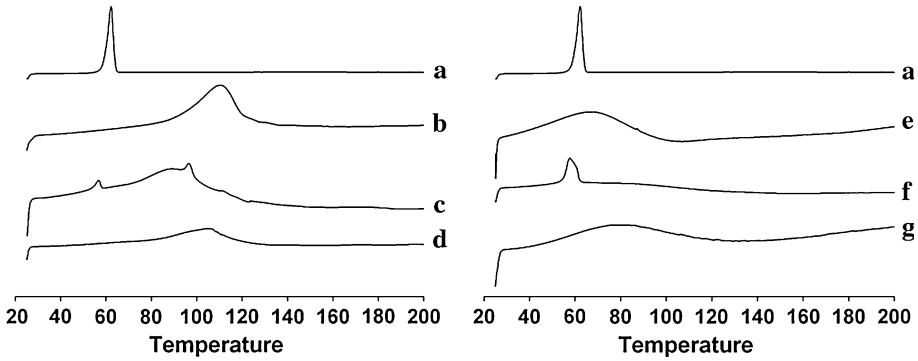

Fig. 4 DSC-thermograms of (*a*) CP (*b*) βCD (*c*) CP/βCD 1:5 PM (*d*) CP/βCD 1:5 CES (*e*) HPβCD (*f*) CP/HPβCD 1:3 PM (*g*) CP/HPβCD 1:3 CES

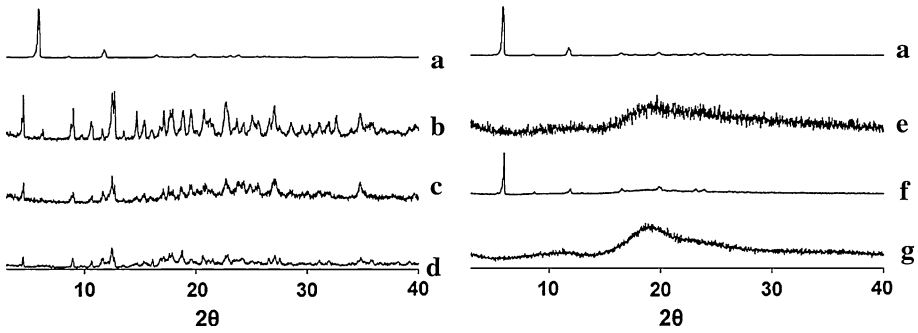

Fig. 5 XRD spectra of (*a*) CP (*b*) βCD (*c*) CP/βCD 1:5 PM (*d*) CP/βCD 1:5 CES (*e*) HPβCD (*f*) CP/HPβCD 1:3 PM (*g*) CP/HPβCD 1:3 CES

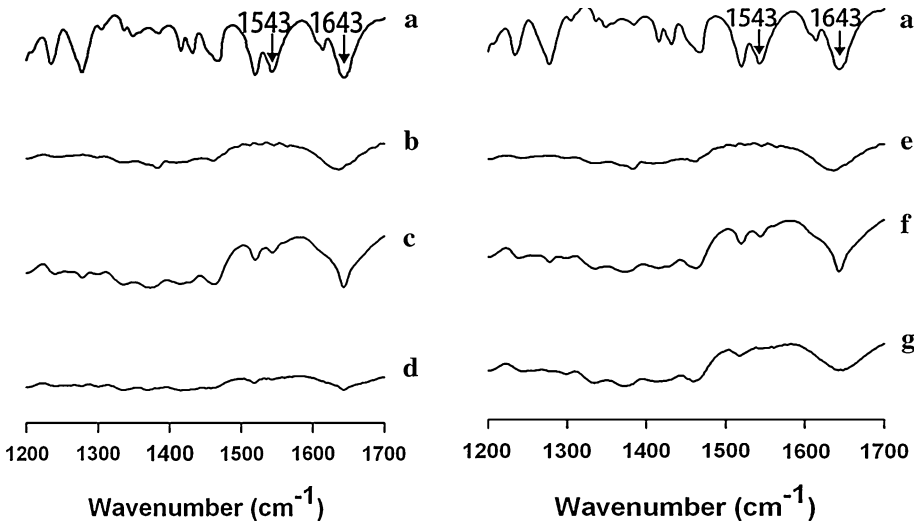

Fig. 6 FTIR spectra of (*a*) CP (*b*) βCD (*c*) CP/βCD 1:5 PM (*d*) CP/βCD 1:5 CES (*e*) HPβCD (*f*) CP/HPβCD 1:3 PM (*g*) CP/HPβCD 1:3 CES

to βCD) and in the CP/HPβCD systems at 1:3, 1:5 (CP to HPβCD). The peak intensity of 1543 cm^{-1} only decreased slightly in all the other systems prepared by co-evaporation technique. Also, the peak corresponding to the carbonyl group of CP significantly decreased in intensity in the above-mentioned systems compared to that observed in PMs. These changes in IR spectra suggest that the amino group and carbonyl group of CP were entrapped into the host cavities during solid state inclusion complexation [32]. This finding is consistent with the results obtained from DSC and XRD.

Scanning electron microscopy (SEM)

Although the SEM technique is not suitable to prove the formation of an inclusion complex, it can confirm the existence of one or several components, as well as provide information about particle size and shape. SEM analysis, shown in Fig. 7, portrayed CP was seen as needle-like crystals, partially agglomerated in bundles. βCD consisted of large crystalline particles of rather irregular size, whereas HPβCD appeared as amorphous spherical or pieces of spherical particles. In keeping with the X-ray

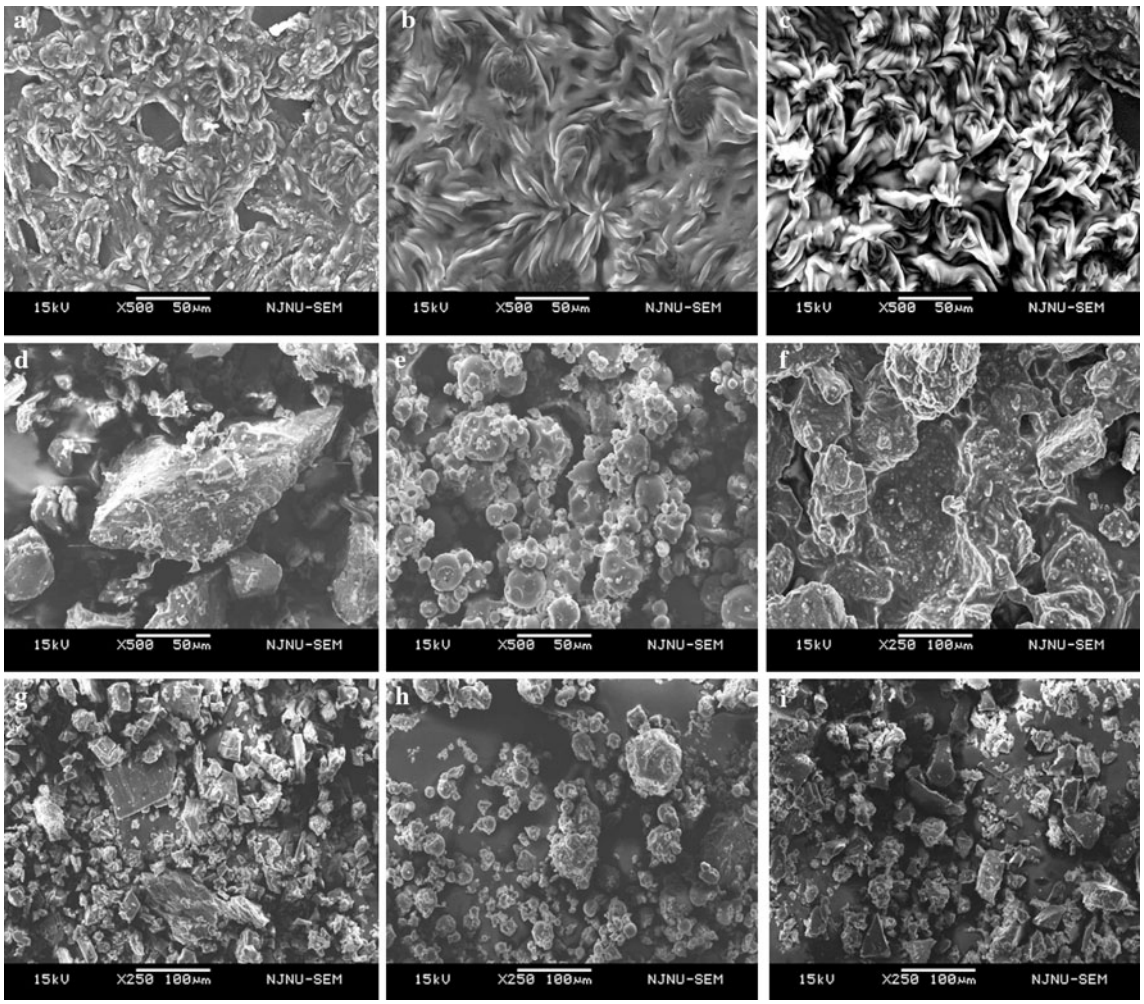

Fig. 7 SEM images of **a** CP **b** CP, light **c** CP, heat **d** βCD **e** HPβCD **f** CP/βCD 1:5 PM **g** CP/βCD 1:5 CES **h** CP/HPβCD 1:3 PM **i** CP/HPβCD 1:3 CES

analysis findings, the characteristic drug crystals, mixed with CD particles or adhered to their surface, were clearly detectable in all PMs. In contrast, uniform solid particles with smaller dimensions and homogeneous structure were observed for the co-evaporated samples. Thus, the CES could significantly promote the dissolution rate and DE of CP due to smaller solid particles.

In vitro dissolution studies

The dissolution behaviors of CP alone, and various binary systems were investigated. Their dissolution profiles, drawn as the cumulative percentage of CP dissolved versus time, were depicted in Fig. 8. Additionally, the dissolution percentage (DP) after 5 and 30 min, as well as the DE at 60 min were summarized in Table 1 in order to represent the changes of drug dissolution at different time.

It was evident that all the binary systems exhibited improved dissolution behaviors compared with the free drug. PMs showed a slight increase of the dissolution rate due to the surfactant-like properties of CDs, which reduced the interfacial tension between the water insoluble drug particles and the dissolution medium, thus improving the wettability and dissolution of the drug [30]. However, PMs with βCD dissolved less rapidly after 10 min in comparison with pure CP. This phenomenon could be explained by poor solubility of complexes forming during the dissolution study the poor solubility of formation of readily complexes or self-association of βCD and βCD complexes.

The better performance of CES, whose DEs were significantly higher than corresponding PMs on the basis of the DE60 data (Table 1), could be ascribed to the improved solubility of CP due to its enhanced deeper interactions with CDs. This is supported by DSC, X-ray and FTIR analyses. According to our results, all the CES dissolved very rapidly within the first 5 min, while the pure drug dissolved less than 30% after 30 min. The CD molar ratio upon the dissolution was clear, whereas the

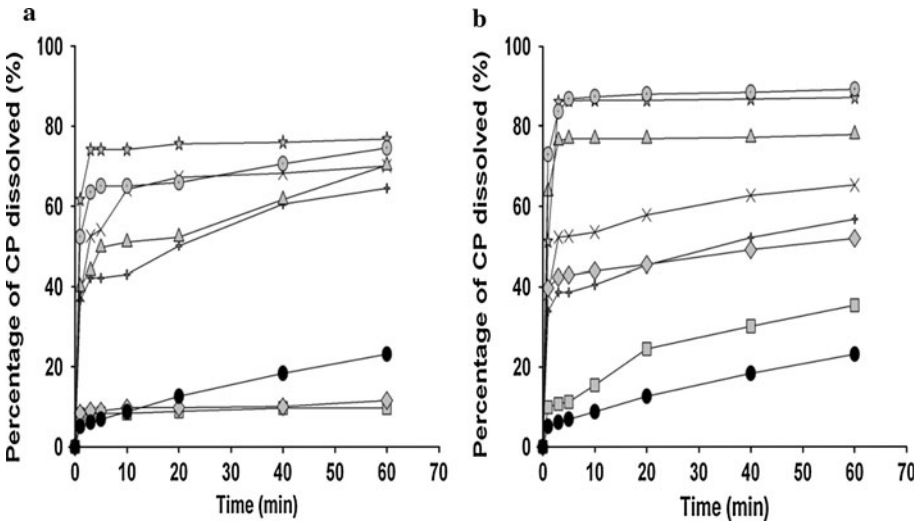

Fig. 8 Dissolution profiles of CP from CP/βCD (**a**) and CP/HPβCD (**b**) binary systems (*plus* 1:0.5 CES; *times* 1:1 CES; *triangles* 1:2 CES; *stars* 1:3 CES; *circles with dot* 1:5 CES; *squares* 1:1 PM; *diamonds* 1:5 (βCD) or 1:3 (HPβCD) PM; *filled circles* pure CP) n = 6

Table 1 Dissolution profiles of CP from CP/CD binary systems

CP/βCD system	DP.5	DP.30	DE.60	CP/HPβCD system	DP.5	DP.30	DE.60
Pure CP	6.84 ± 0.96	15.49 ± 0.46	0.65 ± 0.02	Pure CP	6.84 ± 0.96	15.49 ± 0.46	0.65 ± 0.02
1:0.5 CES	42.19 ± 1.23	55.33 ± 0.95	0.83 ± 0.01	1:0.5 CES	38.63 ± 1.63	48.75 ± 1.24	0.84 ± 0.03
1:1 CES	54.11 ± 2.01	67.78 ± 2.24	0.93 ± 0.07	1:1 CES	52.57 ± 2.12	60.26 ± 1.86	0.90 ± 0.07
1:2 CES	49.71 ± 2.40	56.96 ± 2.33	0.81 ± 0.06	1:2 CES	76.93 ± 2.06	77.07 ± 1.35	0.98 ± 0.05
1:3 CES	74.15 ± 1.52	75.82 ± 1.48	0.97 ± 0.10	1:3 CES	86.40 ± 1.54	86.65 ± 2.20	0.98 ± 0.09
1:5 CES	65.07 ± 1.36	68.25 ± 1.25	0.91 ± 0.06	1:5 CES	86.82 ± 1.68	88.25 ± 1.27	0.97 ± 0.11
1:1 PM	8.38 ± 0.65	9.29 ± 0.74	0.94 ± 0.03	1:1 PM	11.22 ± 0.89	27.35 ± 0.64	0.71 ± 0.02
1:5 PM	9.00 ± 0.86	9.92 ± 0.62	0.86 ± 0.07	1:3 PM	42.86 ± 1.10	47.29 ± 0.82	0.90 ± 0.04

drug dissolution was enhanced upon increasing the proportion of CD. This may be due to the availability of more CD molecules in the hydrodynamic layer surrounding the pesticide allowing for undergo in situ inclusion of drug molecules. However, PMs showed the least effect on the molar ratio since the enhancement in dissolution is mainly due to the wetting effect of the CDs, to which CDs contribute to an equal extent, with their different molar ratios [33, 34].

In addition to the CP/CD molar ratio, the effect of CD type was also evident on the dissolution of CP. The solid binary system with HPβCD exhibited better enhancement than those with parent βCD, revealing the importance of choosing the proper carriers. That could be mainly attributed to excellent water solubility of HPβCD so as to solubilizing and wetting CP molecule, although HPβCD achieved a little lower K_s, CE values compared with βCD according to the phase solubility study.

The results presented herein allow us to conclude that CES system is superior to PM system, and CP/HPβCD systems exhibit better performance than the CP/βCD systems with regard to CP dissolution. The results of the two-way ANOVA performed on the DE_{60} data revealed a significant difference among the different CD types, and molar ratios ($p \leq 0.05$). The computed F values indicate that the dissolution of CP from its binary system was dependent mostly on the molar ratio followed by CD type. No significant difference was found between the CP/HPβCD systems 1:3, 1:5. Therefore, the CP/HPβCD system, prepared at a molar ratio of 1:3 was chosen for further formulation due to the well-documented safety profile and low cost of HPβCD. HPβCD ability to increase the dissolution rate and hydrosolubility of this pesticide, while avoiding the use of organic solvents and excessive application of CP, may be a first step in the development of new CP formulations.

Stability studies

To investigate the influence of CDs on the thermal and photochemical behaviors of CP, various solid samples were also investigated under dry heat or light irradiation. The result was given in Fig. 9 and it was observed that the effect of light irradiation on the degradation of CP was a little greater than the degradation caused by high heat. Additionally, the color of CP changed from white to pink

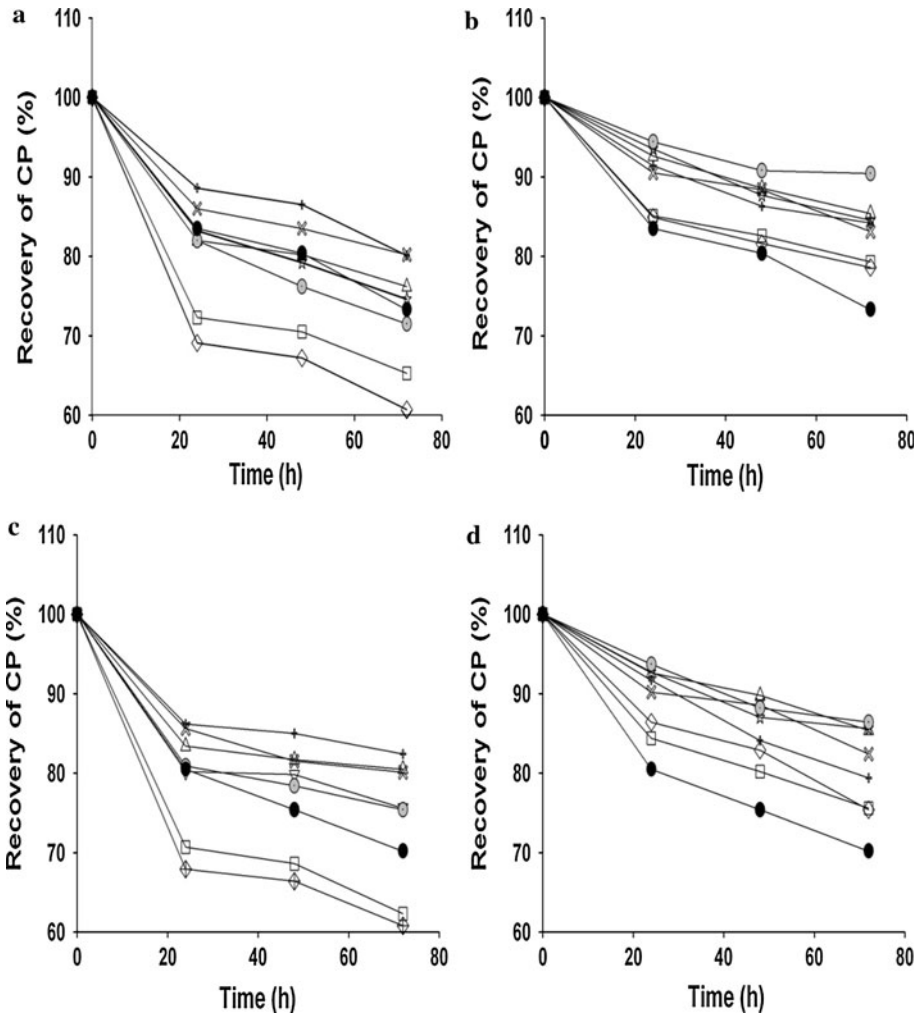

Fig. 9 Recovery of CP after heat or light irradiation **a** βCD heat **b** HPβCD heat **c** βCD light irradiation **d** HPβCD light irradiation, n = 6 (*plus* 1:0.5 CES; *times* 1:1 CES; *triangles* 1:2 CES; *stars* 1:3 CES; *circles with dot* 1:5 CES; *squares* 1:1 PM; *diamonds* 1:5 (βCD) or 1:3 (HPβCD) PM; *filled circles* pure CP) n = 3

after exposure to light irradiation. Though few reports were found in the literature on these discoveries, we presume that certain groups, such as amide group, are more sensitive to light irradiation. However, this is beyond the scope of this study and ought to be reported in more detail in the future.

In CP/HPβCD binary systems, the CES exhibited better performance than corresponding PMs in preventing CP from degradation under both high heat and light irradiation. Furthermore, it seemed that the extent of degradation of CP was inhibited with the increasing proportion of HPβCD. For instance, the CP remaining in the 1:3 CP/HPβCD CES was 10% higher than that in the corresponding PM after 72 h under light irradiation. So we presume that the CES of CP with HPβCD improves the stability of CP. Higher concentrations of HPβCD meant more CP would be complexed, causing greater protection of vulnerable groups in the hydrophobic central cavity.

Strangely, in CP/βCD binary systems, the degradation of CP increased with the proportion of βCD and CES showed better performance than corresponding PMs. A previous report suggested that some pesticides exhibited an increase or decrease in the intensity of light absorption when included in the cavity of a CD [35]. Kamiya and Nakamura [36] proposed that some of the CDs promoted the photodegradation of pesticides, while the others inhibited it. However, our results suggest that complexation of βCD with CP improved the stability of CP to some extent (observed comparison between CES at the molar ratios of 1:1, 1:5, and corresponding PMs). Additionally, the lower the concentration of CP analyzed, the easier it was degraded, just as the lower the concentration of solute employed, the more instable it was in solution. In comparison of the CES prepared by the two CD types, HPβCD was more efficient in preventing the degradation of CP. Therefore, the efficacy of the examined complexes in stability studies was not related to the corresponding complex formation constants (1187 M^{-1} for βCD and 966 M^{-1} for HPβCD).

SEM was also used to investigate the change in the morphology and shape of the solid samples after the stability tests. The crystalline aspect of CP was markedly

changed after dry heat and light irradiation. CP exhibited a strong tendency to aggregate and agglomerate whether alone, or in 1:1 CP/βCD CES. This was especially noticeable after light irradiation, whereas no notable change was observed in the 1:1 HPβCD/CP CES (figures not shown). This may be considered indicative of instability of βCD CES, compared with HPβCD CES.

According to all the results above, it is possible to conclude that both CDs are able to form true inclusion complexes with CP using the co-evaporated technique. By complexation, not only the solubility of CP was greatly improved, but also the pesticide dissolution rate and efficiency were remarkably enhanced. Moreover, it was observed that low concentration of CP could exert a promotive effect on the CP degradation, whereas complexation with HPβCD would improve the stability of CP. Concerning the practical applications, the promising results with HPβCD imply that the gained inclusion complex at the molar ratio of 1:3 (CP to HPβCD) could be of the pesticide.

Pesticide adsorption–desorption experiments in Soil

CP adsorption isotherm in soil in the presence of 0.01 M $CaCl_2$ as background electrolyte was presented in Fig. 10. It was well described by the linearized Freundich equation ($R^2 = 0.998$). The n and K_f values were calculated at 0.981 and 10.19, respectively. Pesticides soil adsorption would be related to the organic matter of the soils, and in some cases it was also related to soil pH and cation exchange capacity [37], the type of soil cation [38], or adsorbate concentration [39]. In our study, the ratio of the organic matter to the total soil used in the study was about 4.3% (much higher than common soil). Thus, it is possible to say that the affinity of the soil for CP was related to its higher organic matter, and CP had a high tendency to be adsorbed on the soil surface. Desorption experiments on soils were carried out employing 0.01 M $CaCl_2$ solutions for extraction. For $CaCl_2$ solution, the desorption behavior deviated markedly from that corresponding to the adsorption isotherms, indicating that CP adsorption on the soil was not completely reversible. Meanwhile, the total percentages of CP desorbed (%D) for each concentration in the presence of 0.01 M $CaCl_2$ were calculated and the values reached 3.5, 12.7 and 14.2% at the CP initial concentrations of 4, 8 and 10 mg/L, respectively. Our data clearly suggest CP is easier to be adsorbed on the soil surface at low concentration.

CP adsorption isotherm using 0.01 M HPβCD solution presented in Fig. 10 was also well described by the linearized Freundich equation ($R^2 = 0.989$). But the K_f value was only 2.10, quite lower than the corresponding K_f value employing 0.01 M $CaCl_2$. The reason could be the interaction of CP with HPβCD, yielding the formation of the water-soluble inclusion complexes as similar results that using CD to extract the herbicide 2,4-dichlorophenoxyacetic acid from soil [40]. This implied that the decrease in CP adsorption capacity was due to the higher tendency of CP/HPβCD complexes to remain in solution. The total percentages of CP desorbed in HPβCD experiment were calculated and the values reached 16.7, 7.8 and 7.6% at the CP initial concentrations of 4, 8 and 10 mg/L, respectively. The results indicate that the lower the amount of CP adsorbed, the higher the %D. From the adsorption–desorption experiments, it was observed that CP had a high tendency to be adsorbed on soil surfaces, leading to low bioavailability, inactivation as pesticide, and sometimes to soil contamination. However, the application of HPβCD in the pesticide formulations could effectively reduce CP adsorption from solution to soil, thereby decreasing the risk of its residual effects.

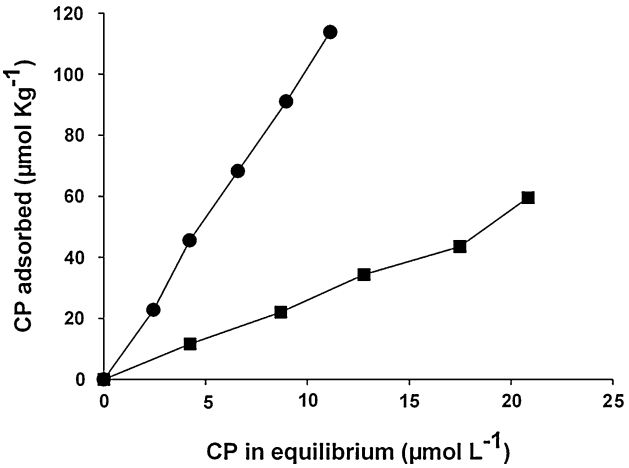

Fig. 10 Adsorption isotherms of CP in the presence of 0.01 M $CaCl_2$ (*filled circles*) and HPβCD (*filled squares*)

Conclusions

In summary, CP/βCD or CP/HPβCD binary systems, in both solution and solid state, were investigated specifically on the characterization for host/guest interaction and enhanced effects on the CP formulations. We report that the CP/HPβCD or CP/βCD inclusion complex was formed at a 1:1 stoichiometric ratio in solution state. A 50-fold enhancement of CP solubility could be achieved in the presence of 60 mM HPβCD with respect to CP alone, whereas only a fivefold enhancement of CP solubility was achieved in the presence of 6 mM βCD due to its poor solubility.

The solid CES of CP/CD improved the dissolution and stability of CP compared with PMs. In the CES CP was able to form true inclusion complexes with βCD or HPβCD

at molar ratios of 1:5 or 1:3 respectively. Moreover, the affinity of HPβCD for the pesticide observed in the conditions of this study yielded a marked reduction in the sorption of the pesticide by soil, and would be promised for in situ remediation of pesticide-contaminated soil.

Furthermore, the employment of CP/HPβCD inclusion complexes significantly enhanced various effects of CP formulation including: the solubilization, dissolution, and protection from degradation. With regard to practical applications, these advantages would not only facilitate processing of the preparations but may potentially increase the efficacy of CP, while providing an improved mechanism for mediating its removal from the environment. Pesticide granules and emulsions are popular pesticide formulations due to their physical stability and the convenience. They provide with regard to storage and transportation. The appropriate stoichiometric ratio presented here is significant for preparing the pesticide inclusion complexes, and may potentially be preferred for future development of both solid or liquid formulations.

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