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Complexation of carbendazim with hydroxypropyl-β-cyclodextrin to improve solubility and fungicidal activity

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ABSTRACT

The effect of hydroxypropyl-β-cyclodextrin (HPβCD) on the improvement of the solubility and fungicidal activity of carbendazim (MBC) has been investigated. The inclusion complexation of HPβCD with MBC has been prepared and characterized by phase solubility diagram, fluorescence, ^{1}H NMR, ROESY and FT-IR spectra. The stoichiometric ratio and stability constant were determined by Job's plot and phase solubility studies, respectively. The inclusion complex MBC·HPβCD has exhibited different properties from MBC. The obtained inclusion complex was found to significantly improve the water solubility of MBC. In addition, the biological activity indicated that the complex displayed the better fungicidal activity than MBC. The present study provided useful information for a more rational application of MBC.

1. Introduction

Cyclodextrins (CDs), a class of macrocyclic compounds, are well known to have a hollow truncated cone with a hydrophobic cavity and a hydrophilic wall. CDs are able to form host-guest complexes with the guest molecules that possess suitable polarity and dimension. After formation of the complexes, the physical, chemical and biological properties of guest molecules can be significantly improved (Szejtli, 1998). β-Cyclodextrin (βCD) is commercially available in large quantities and cheaper, so it was applied in a large area such as agriculture, food, toiletry, and pharmaceutical industries (Schirra et al., 2002). However, due to formation of intramolecular hydrogen bonds between C_2, C_3 hydroxyl groups in βCD molecule, its water solubility is relative poor (1.85 g/100 mL H_2O) (Qian, Guan, & Xiao, 2008), which restricting its wide range of applications. Hydroxypropyl-β-cyclodextrin (HPβCD) is a hydroxyalkyl derivative of βCD. The introduction of hydroxypropyl groups in structure of HPβCD interrupts intramolecular hydrogen bonds of βCD, which have higher water solubility and a larger hydrophobic cavity compared with native βCD. In addition, toxicological studies pointed out that HPβCD has a higher security, therefore it is considered to be promising alternative of βCD (Gould & Scott, 2005).

Carbendazim (MBC), methyl-2-benzimidazolecarbamate (Fig. 1), is a benzimidazole fungicide. It appears as the active substance of benomyl and thiophanate-methyl and moreover it is the main degradation product of these two compounds (Delp, 1987). MBC can be absorbed by plants and transferred to every part, to interfere mitosis of bacterial cell and inhibit its growth (Ben-Aziz & Aharonson, 1974). It is a widely used systemic fungicide against different fungi affecting fruits, vegetables and cereals (Hutson, Roberts, & Jewess, 1999; Reyes, Barrales, & Diaz, 2003). However, MBC has poor water solubility (8 mg/L, 25 °C) (Worthing & Hance, 1991), which have become a major constraining factor on its application of fungicidal activity. It was reported that the water solubility of MBC can be improved by formation of complex with CDs (Lezcano, Al-Soufi, Novo, Rodríguez-Núñez, & Tato, 2001; Schmidt, von der Eltz, & Kaluza, 1996). M. Lezcano (Lezcano, Al-Soufi, Novo, Rodríguez-Núñez, & Tato, 2001) has used the phase solubility diagram to study βCD on the solubilization effect of MBC, but the effect was little.

Therefore, the aim of this work was to establish a possibility of obtaining inclusion complex of MBC with HPβCD, as a first time to obtain formulation that supplied a more rational use of MBC, improving its water solubility, bioavailability and fungicidal activity. Herein, to explore the host-guest interaction, the complex was systematically characterized by phase solubility diagram, fluorescence, ^{1}H NMR, ROESY and FT-IR spectra. Additionally, the fungicidal activity on *T. viride* of MBC and its complex was compared.

2. Materials and methods

2.1. Materials

MBC (Aldrich) was recrystallized twice from ethanol with active carbon. HPβCD (DS = 8.64) was purchased from Shandong Xinda

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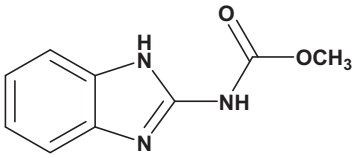

Fig. 1. The molecular structure of MBC.

Fine Chemical Co., Ltd., which was used without further purification. *Trichoderma viride* (*T. viride*) was obtained from Biology Institute of Shandong Academy of Sciences. Other chemicals used were of analytical reagent grade. The redistilled water was used throughout.

2.2. Methods

2.2.1. Preparation of inclusion complex

A kneading method was carried out to prepare the complex. MBC (0.1912 g, 1.0 mmol) and HPβCD (1.6370 g, 1.0 mmol) were wetted with ethanol and kneaded thoroughly. During the kneading, drops of ethanol were added to the solid mixture in order to maintain its consistency as a paste. Then the obtained solid was washed sequentially with redistilled water and ethanol, respectively. At last, it was dried to constant weight in a vacuum desiccator.

2.2.2. Preparation of physical mixture

MBC (0.1912 g, 1.0 mmol) and HPβCD (1.6370 g, 1.0 mmol) were ground into powder, respectively, and then the two compounds were mixed slightly till the powder was even. Finally, the physical mixture was obtained and dried in vacuum.

2.2.3. Phase solubility studies

The phase solubility studies were performed according to the method reported by Higuchi and Connors (Higuchi & Connors, 1965). An excess amount of MBC (10 mg) was added to aqueous solutions (10 mL) containing different concentrations of HPβCD (0, 10, 20, 30, 40 and 50 mM). The flasks were shaken at 20 °C for a week. Then equilibrium was reached, the suspensions were filtered with syringe through a 0.45 μm hydrophilic membrane filter, and the concentration of MBC in the filtrate was properly diluted and analyzed at 285 nm using a UV-Vis spectrophotometer (Varian Cary-100). The presence of HPβCD did not interfere with the spectrophotometric assay of MBC, and the linear regression equation was $A = 15.254C$ (mM) + 0.0170 ($R = 0.9994$). The apparent stability constant K_c was calculated from the straight line obtained in the phase solubility diagram, where S_0 is the intrinsic solubility of MBC in redistilled water in the absence of HPβCD:

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

2.2.4. Fluorescence measurements

The fluorescence measurements were carried out on Shimadzu RF-5301PC spectrofluorimeter. A 1.0×10^{-2} M HPβCD standard solution, a 1.0×10^{-3} M MBC standard solution and a phosphate buffer solution (0.20 M, pH 7.0) were prepared. Then the solutions of 5.00 mL HPβCD, 0.50 mL MBC, 5.00 mL HPβCD + 0.50 mL MBC were transferred into three 10 mL color comparison tubes, respectively. All the color comparison tubes were then placed with 2.00 mL of phosphate buffer solution and the mixed solutions were diluted to 10 mL with redistilled water, then the fluorescence intensity of the solutions were measured at 310 nm with excitation at 280 nm.

A continuous variation method (Job's plot) was performed in order to confirm the stoichiometry of the complex (Job, 1928). The sum of the concentrations of both components was kept constant ([MBC] + [HPβCD] = 1.0×10^{-4} M) and the molar fraction of MBC (r = [MBC]/([MBC] + [HPβCD])) varied from 0.0 to 1.0. In order to calculate the stoichiometry, the fluorescence emission intensity variations (ΔF) of MBC were plotted versus the molar fraction (r).

2.2.5. NMR

One-dimensional ^{1}H NMR spectra were recorded at room temperature on Bruker AVANCE III 400 NMR spectrometer (Germany) using a 5 mm probe and a simple pulse-acquire sequence. Acquisition parameters consisted of spectral width 4000 Hz, acquisition time 3.98 s, number of scans of 8, and relaxation delay of 1 s. Equimolar HPβCD and the complex MBC·HPβCD were respectively dissolved in D_2O (Aldrich). The signal at 4.67 ppm of HOD was used as an internal reference.

Rotating-frame overhauser effect spectroscopy (ROESY) experiments were acquired in the phase sensitive mode with the same spectrometer and Bruker standard parameters (pulse program roesyphpr). For ROESY spectra, the time domain data was zero filled to 1024 points in F2 and 256 points in F1. The ROESY data was acquired with a spin-lock mixing time of 200 ms, and a relaxation delay of 2 s.

2.2.6. FT-IR spectra

The IR spectra of HPβCD, MBC, physical mixture and complex were recorded by using Thermo Nicolet AVATAR 360 FT-IR spectrophotometer in region 4000–400 cm^{-1}. Potassium bromide pellets were used for all samples.

2.2.7. Bioassay

The fungicidal activities of MBC and the complex MBC·HPβCD against *T. viride* in vitro were tested according to previously reported literature (Zhu, Wang, Chen, Yang, & Yang, 2007). Five different concentration gradient solutions of MBC and the complex were prepared with sterilized water. The solutions (1 mL) were mixed rapidly with thawed potato glucose agar culture medium (9 mL) under 50 °C. The mixtures were poured into Petri dishes. The final solutions of pure drug and the complex contained MBC were 0.025, 0.05, 0.10, 0.20 and 0.50 μg/mL, respectively. After the dishes were cooled, the solidified plates were incubated with 4 mm mycelium disk, inverted, and incubated at 28 °C for 48 h. The mixed medium without sample was used as the blank control. Three replicates of each test were carried out. The mycelial elongation diameter (mm) of fungi settlements was measured after 48 h of culture. The growth inhibition rates were calculated with the following equation:

$$I(\%) = \frac{C - T}{C} \times 100 \quad (2)$$

Here, I is the growth inhibition rate (%), C is the control settlement diameter (mm), and T is the treatment group fungi settlement diameter (mm). The growth inhibition rates were then converted into probability values, and plotting the growth inhibition probability values against logarithm of the concentration of MBC. The EC_{50} values (the concentration to give 50% inhibition) of pure MBC and the complex were obtained from a concentration–inhibition ranking relationship.

3. Results and discussion

3.1. Phase solubility studies

Phase solubility diagram has been extensively used in investigating the solubility of some drugs and agrochemicals in the presence of CDs (Rajabi, Tayyari, Salari, & Tayyari, 2008; Villaverde,

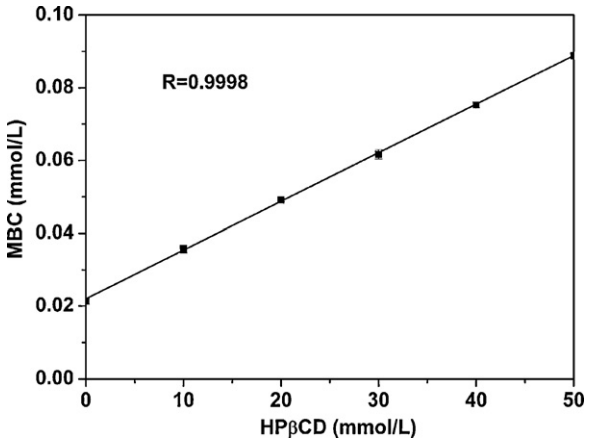

Fig. 2. Phase solubility diagram of MBC in the presence of HPβCD.

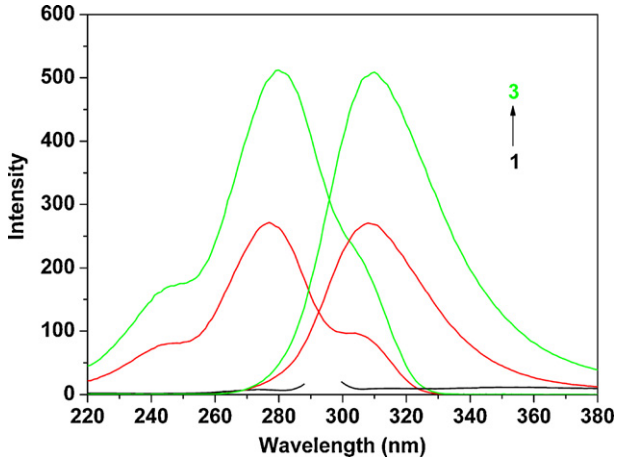

Fig. 3. The fluorescence spectra of (1) 5.0×10^{-3} M HPβCD; (2) 5.0×10^{-5} M MBC; (3) 5.0×10^{-5} M MBC + 5.0×10^{-3} M HPβCD (λ_{ex} = 280 nm, λ_{em} = 310 nm, $Slit_{ex/em}$ = 15 nm).

Morillo, Pérez-Martínez, Ginés, & Maqueda, 2004). The phase solubility diagram of MBC in the presence of different concentrations of HPβCD was presented in Fig. 2. It was observed that within the range of the concentrations of the HPβCD aqueous solutions used, the solubility of MBC increased linearly as a function of HPβCD concentration. According to Higuchi and Connors, the phase solubility diagram of MBC and HPβCD was classified as type A_L and indicated that the formation of a 1:1 inclusion complex between MBC and HPβCD. The apparent stability constant K_c was calculated according to Eq. (1) and the value was 61.07 M^{-1}. As compared with the water solubility of free MBC, there was a 4.2-fold increase in the presence of 50 mM HPβCD.

3.2. Fluorescence studies

Fig. 3 displayed the fluorescence spectra of MBC in aqueous solutions in the absence and presence of HPβCD. An obvious

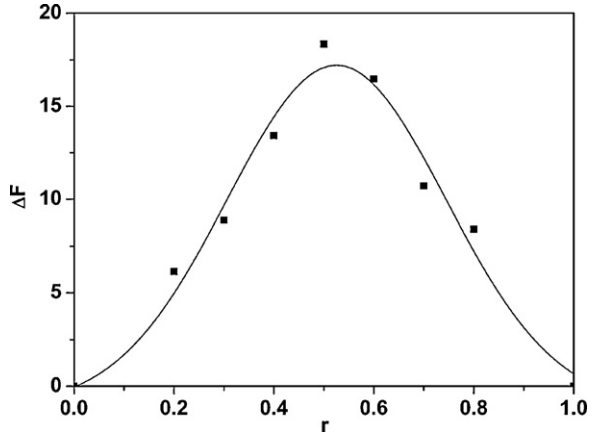

Fig. 4. Continuous variation plot (Job's plot) for the complex MBC·HPβCD (λ_{ex} = 280 nm, λ_{em} = 310 nm, $Slit_{ex/em}$ = 15 nm).

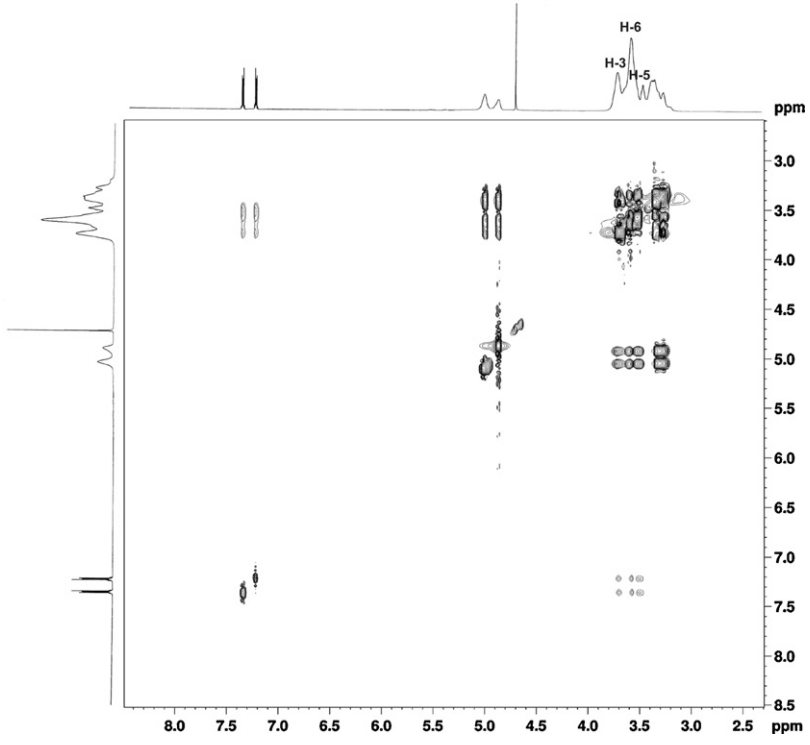

Fig. 5. Portion of the ^1H–^1H ROESY spectrum for inclusion complex MBC·HPβCD.

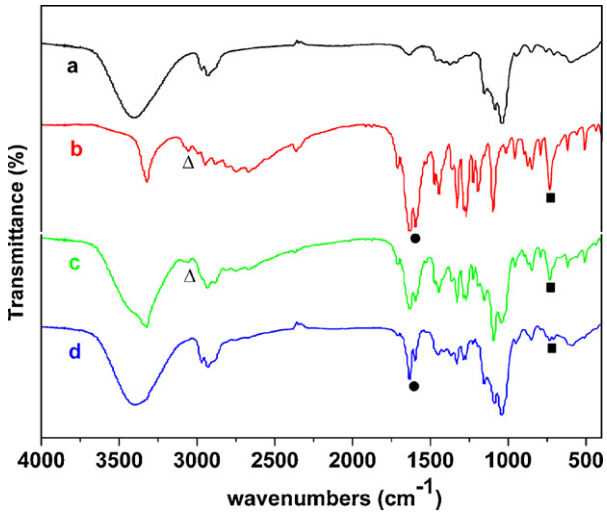

Fig. 6. FT-IR spectra of HPβCD (a), MBC (b), physical mixture MBC/HPβCD (c), and inclusion complex MBC·HPβCD (d).

increase in fluorescence intensity of MBC was observed which showed the formation of supramolecular complex between HPβCD and MBC. The reason of HPβCD sensitizing fluorescence intensity was that the guest with no geometrical change happening in a twisted intramolecular charge transfer (TICT) excited state (Ayman Ayoub, 2007; Somes Kumar, 2002) and the decrease of fluorescence quenching induced by the oxygen in aqueous solution (Du, Zhang, Jiang, Huang, & Chen, 1997).

The Job's method was performed in order to confirm the stoichiometry of the complex. The plot observed in Fig. 4 showed the maximum at a molar fraction of about 0.5, indicating that the stoichiometry of the complex MBC·HPβCD was 1:1 in agreement with the phase solubility studies.

3.3. NMR spectra

NMR spectra are one of the most direct evidence for the formation of the inclusion complex (Misiuk & Zalewska, 2009). Chemical shift variations of selected host and guest protons reflect the formation of a complex between them. H-3 and H-5 protons of HPβCD are positioned inside the cavity, H-3 closer to the wider rim and H-5 on the opposite. Table 1 showed the ^{1}H NMR chemical shifts (δ) and changes ($\Delta\delta$) of HPβCD protons upon complexation of MBC in D_2O. After the complex was formed, the δ of H-3 and H-5 protons shifted for −0.035 and −0.029 ppm, respectively. The upfield shifts are due

Table 1
^{1}H NMR chemical shifts (δ/ppm) and changes ($\Delta\delta$/ppm) of HPβCD protons upon complexation of MBC.

HPβCD protons	δ_{free} (ppm)	$\delta_{complex}$ (ppm)	$\Delta\delta$ (ppm)
H-1	4.862	4.858	−0.006
H-2	3.393	3.399	0.006
H-3	3.799	3.764	−0.035
H-4	3.230	3.233	0.003
H-5	3.504	3.475	−0.029
H-6	3.648	3.618	−0.030

to the anisotropic magnetic effect induced by the presence of the aromatic group of the guest molecule (Veiga, Fernandes, Carvalho, & Geraldes, 2001). It was suggested that the benzene ring of MBC was included into the HPβCD cavity. At the same time, H-3 protons shifted upfield most significantly, indicating that MBC inserted into the HPβCD cavity from the wider rim.

Afterwards, the mode of the inclusion complex was further conformed by 2D NMR spectroscopy since cross-peaks in ROESY spectra are expected for protons that are closer than 4 Å in space (Schneider, Hacket, Rüdiger, & Ikeda, 1998). The ^{1}H–^{1}H ROESY spectrum for the inclusion complex MBC·HPβCD (Fig. 5) showed the benzene ring protons (m, 4H, δ 7.350–7.201 ppm) of MBC have cross peaks to the H-3, H-5 and H-6 protons of HPβCD, indicating the deep insertion of the benzene ring into the host cavity (Kwon et al., 2009; Yang et al., 2011).

3.4. FT-IR spectra

The variation of the shape, shift, and intensity of the FT-IR absorption peaks of the guest or host can provide enough information for the occurrence of the inclusion (Szente, 1996). FT-IR spectra of HPβCD (a), MBC (b), physical mixture MBC/HPβCD (c), and inclusion complex MBC·HPβCD (d) were presented in Fig. 6. It can be seen that the spectrum of (c) was essentially the combination of (a) and (b), indicating that the physical mixture MBC/HPβCD did not lead to inclusion. However, there were obvious changes in FT-IR spectra after the complex MBC·HPβCD was formed (Fig. 6(d)). The band at 3056 cm^{-1} (Δ) corresponding to C—H stretching vibration of the benzene ring disappeared in the complex. The band at 1630 cm^{-1} and 1595 cm^{-1} (●) corresponding to C=C stretching vibration of the benzene ring shifted to 1635 cm^{-1} and 1599 cm^{-1}, respectively, and the sharpening of the bands were observed. Meanwhile, the intensity of C—H bending vibration at 732 cm^{-1} (■) of the benzene ring decreased. Therefore, FT-IR spectra confirmed the inclusion complex was formed, and the benzene ring of MBC

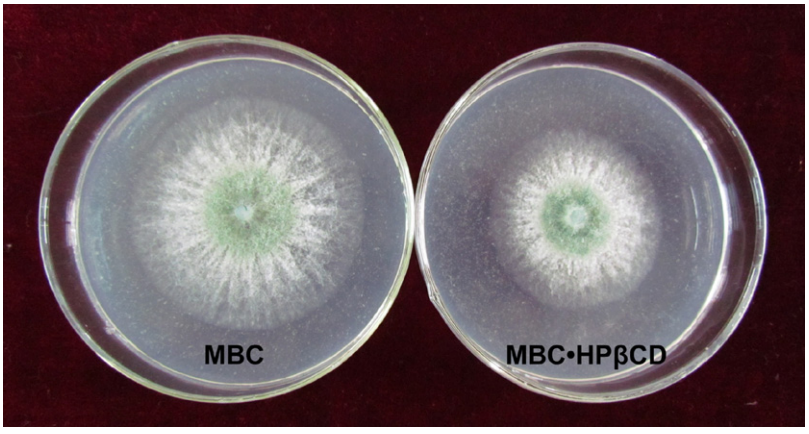

Fig. 7. The fungicidal activity to *T. viride* of MBC and inclusion complex MBC·HPβCD (C_{MBC} = 0.10 μg/mL).

Table 2
The toxicity equations and EC_{50} of MBC and inclusion complex MBC·HPβCD against *T. viride*.

	Toxicity equation	Correlation coefficient (R)	EC_{50} (μg/mL)	Improved times
MBC	$y = 0.9395x + 6.1340$	0.9447	0.305	–
MBC·HPβCD	$y = 0.7765x + 6.2971$	0.9629	0.190	1.61

was included into the HPβCD cavity which was in accordance with the NMR results.

3.5. Fungicidal activity

After complexation with HPβCD, the mycelial elongation diameter (mm) of fungi settlements for MBC was significantly inhibited (Fig. 7), indicating that the complexation of MBC with HPβCD significantly improved the bioavailability of MBC due to the improvement of the water solubility.

Fungicidal activity as shown in Table 2 revealed that the EC_{50} value of MBC against *T. viride* was 0.305 μg/mL, whereas the EC_{50} value of the complex against *T. viride* was 0.190 μg/mL. As compared with the fungicidal activity of free MBC, there was a 1.61-fold increase in the complex.

4. Conclusions

In this work, the inclusion complex MBC·HPβCD with host–guest ratio 1:1 has been prepared. Phase solubility studies, fluorescence spectra, NMR and FT-IR spectra were applied to characterize the formation of the complex. The apparent stability constant of the MBC·HPβCD complex of 61.07 M^{-1} was calculated according to the phase solubility diagram, and the stoichiometry was conformed by the Job's plot. One-dimensional ^1H NMR and ROESY experiments indicated the mode of the complex in which the benzene ring of MBC was encapsulated with HPβCD cavity.

The complexation of MBC with HPβCD significantly improved the bioavailability of MBC and, therefore, resulted in about two-fold increase of the fungicidal activity. The significant increase of the bioactivity of MBC in the presence of HPβCD provides an effective approach for a more rational application of MBC, diminishing the use of organic solvents and the amount of MBC and increasing its efficacy.

Acknowledgements

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References

Ayman Ayoub, A. S. (2007). Inclusion complex of 2-naphthylamine-6-sulfonate with β-cyclodextrin: Intramolecular charge transfer versus hydrogen bonding effects. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *66*, 1228–1236.

Ben-Aziz, A., & Aharonson, N. (1974). Dynamics of uptake, translocation, and disappearance of thiabendazole and methyl-2-benzimidazolecarbamate in pepper and tomato plants. *Pesticide Biochemistry and Physiology*, *4*(2), 120–126.

Delp, C. J. (1987). Benzimidazole and related fungicides. In H. Lyr (Ed.), *Modern selective fungicides: Properties, applications, mechanisms of action* (pp. 233–244). New York: Wiley.

Du, X. Z., Zhang, Y., Jiang, Y. B., Huang, X. Z., & Chen, G. Z. (1997). Study of room-temperature phosphorescence of 1-bromonaphthalene in sodium dodecylbenzene sulfonate and β-cyclodextrin solution. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *53*, 671–677.

Gould, S., & Scott, R. C. (2005). 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD): A toxicology revie. *Food and Chemical Toxicology*, *43*, 1451–1459.

Higuchi, T., & Connors, K. A. (1965). Phase-solubility techniques. *Advances in Analytical Chemistry and Instrumentation*, *4*, 117–212.

Hutson, D. H., Roberts, T. R., & Jewess, P. J. (1999). Insecticides and fungicides: Carbendazim. In D. H. Hutson, & D. H. Roberts (Eds.), *Metabolic pathways of agrochemicals* (pp. 1120–1124). United Kingdom: Royal Society of Chemistry, Cambridge.

Job, P. (1928). Job's method of continuous variation. *Annali di Chimica*, *10*, 113–203.

Kwon, S., Lee, W., Shin, H. J., Yoon, S. i., Kim, Y. t., Kim, Y. J., et al. (2009). Characterization of cyclodextrin complexes of camostat mesylate by ESI mass spectrometry and NMR spectroscopy. *Journal of Molecular Structure*, *938*, 192–197.

Lezcano, M., Al-Soufi, W., Novo, M., Rodríguez-Núñez, E., & Tato, J. V. (2001). Complexation of several benzimidazole-type fungicides with α- and β-cyclodextrins. *Journal of Agricultural and Food Chemistry*, *50*, 108–112.

Misiuk, W., & Zalewska, M. (2009). Investigation of inclusion complex of trazodone hydrochloride with hydroxypropyl-β-cyclodextrin. *Carbohydrate Polymers*, *77*, 482–488.

Qian, L., Guan, Y., & Xiao, H. (2008). Preparation and characterization of inclusion complexes of a cationic β-cyclodextrin polymer with butylparaben or triclosan. *International Journal of Pharmaceutics*, *357*, 244–251.

Rajabi, O., Tayyari, F., Salari, R., & Tayyari, S. F. (2008). Study of interaction of spironolactone with hydroxypropyl-β-cyclodextrin in aqueous solution and in solid state. *Journal of Molecular Structure*, *878*, 78–83.

Reyes, J. F. G., Barrales, P. O., & Diaz, A. M. (2003). Gel-surface enhanced fluorescence sensing system coupled to a continuous-flow assembly for simultaneous monitoring of benomyl and carbendazim. *Analytica Chimica Acta*, *493*, 35–45.

Schirra, M., Delogu, G., Cabras, P., Angioni, A., D'Hallewin, G., Veyrat, A., et al. (2002). Complexation of imazalil with β-cyclodextrin, residue uptake, persistence, and activity against penicillium decay in citrus fruit following postharvest dip treatments. *Journal of Agricultural and Food Chemistry*, *50*, 6790–6797.

Schmidt, A., von der Eltz, H., & Kaluza, K. (1996). Cyclodextrin-biocide complex. US patent: US5506216.

Schneider, H. J., Hacket, F., Rüdiger, V., & Ikeda, H. (1998). NMR studies of cyclodextrins and cyclodextrin complexes. *Chemical Reviews*, *98*, 1755–1786.

Somes Kumar, D. (2002). Inclusion complexation of 2-(4′-N,N-dimethylaminophenyl)-1H-naphth[2,3-d]imidazole by β-cyclodextrin: Effect on the twisted intramolecular charge transfer emission. *Chemical Physics Letters*, *361*, 21–28.

Szejtli, J. (1998). Introduction and general overview of cyclodextrin chemistry. *Chemical Reviews*, *98*, 1743–1754.

Szente, L. (1996). Cyclodextrins. In J. Szejtli, & T. Osa (Eds.), *Comprehensive supramolecular chemistry* (pp. 253–278). Oxford, UK: Pergamon.

Veiga, F. J. B., Fernandes, C. M., Carvalho, R. A., & Geraldes, C. F. G. C. (2001). Molecular modelling and ^1H-NMR: Ultimate tools for the investigation of tolbutamide: β-Cyclodextrin and tolbutamide: Hydroxypropyl-β-cyclodextrin complexes. *Chemical & Pharmaceutical Bulletin*, *49*, 1251–1256.

Villaverde, J., Morillo, E., Pérez-Martínez, J. I., Ginés, J. M., & Maqueda, C. (2004). Preparation and characterization of inclusion complex of norflurazon and β-cyclodextrin to improve herbicide formulations. *Journal of Agricultural and Food Chemistry*, *52*, 864–869.

Worthing, C. E., & Hance, R. J. (1991). *The pesticide manual*. Surrey: British Crop Protection Council.

Yang, L. J., Chen, W., Ma, S.-X., Gao, Y.-T., Huang, R., Yan, S. J., et al. (2011). Host–guest system of taxifolin and native cyclodextrin or its derivative: Preparation, characterization, inclusion mode, and solubilization. *Carbohydrate Polymers*, *85*, 629–637.

Zhu, X. L., Wang, H. B., Chen, Q., Yang, W. C., & Yang, G. F. (2007). Preparation and characterization of inclusion complex of iprodione and β-cyclodextrin to improve fungicidal activity. *Journal of Agricultural and Food Chemistry*, *55*, 3535–3539.