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Preparation and Characterization of the Inclusion Complex of Chlorpyrifos in Cyclodextrins To Improve Insecticide Formulations

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The chemical control of crops by organophosphate insecticide treatment is usually limited because the insecticides do not maintain their efficiency for long periods for several reasons, including environmental conditions or rapid degradation of the active ingredient. Chlorpyrifos is an organophosphate insecticide used worldwide to control a variety of soil insects and arthropods in a wide range of crops. It is easily soluble in organic solvents but shows poor water solubility. The inclusion of chrorpyrifos in cyclodextrins (CDs) improves its water solubility, bioavailability, and insecticidal activity and helps prevent overdosing, leading to more cost-effective and more environmentally friendly agricultural practices. Solubility studies of chlorpyrifos in the presence of different types of CDs show G_2-β-CDs to be the most effective CDs in the complexation process, giving 1:2 complexes, with complexation constant (K_c) values of 12.34 $\pm$ 3.1 M^{-1} for K_1 and 3895 $\pm$ 183 M^{-1} for K_2. These complexation constant values were corroborated by applying a fluorimetric method.

KEYWORDS: Chlorpyrifos; complexation; cyclodextrin; fluorimetric method; solubility method; 1:2 complexes

INTRODUCTION

Chlorpyrifos is a broadly active nonsystemic insecticide used to control a variety of soil insects and arthropods in a wide range of crops, including citrus fruits, peaches, nectarines, vegetables, cereals, maize, and tobacco. In addition, postharvest application prior to storage is indicated for pest protection. It is moderately toxic to mammalian species (*1*) but extremely toxic to a wide range of nontarget aquatic biota (*2*). It is effective by ingestion and contact and/or inhalation, because it is absorbed through skin, gut, and pulmonary membranes. Chlorpyrifos acts by phosphorylating acetylcolinesterase both at the synapse of neurons and in the plasma (*3, 4*).

Chlorpyrifos is actually one of the most widely used insecticides in the world: more then 50 000 kg/year in are used in Europe (*5*), while in the U.S.A., more then 5 million kg/year are used in agriculture and 4 million kg/year are used for nonagricultural purposes (*6*). Because of its widespread use, persistency, and toxicity, chlorpyrifos has been included in priority lists of pesticides within the EU (*7*).

The chemical control of crops by organophosphate insecticide treatment represents an efficient means of pest control, but their use is limited because the insecticides do not maintain their efficiency for long periods. This may be due to different factors, including environmental conditions or rapid degradation of the active ingredient. However, regardless of the physical properties of the insecticide, methods are needed to improve the progressive release of the compound, so that plant organs can be protected longer.

The molecular inclusion of pesticides offers several advantages over conventional agrochemical formulations: for examples, a constant level of the active ingredient is released, providing enhanced efficacy and reduced mammalian toxicity and phytotoxicity, while the solubility of water-insoluble compounds is increased. The latter aspect is particularly important, because it allows more effective penetration of the active compound.

Microencapsulation of biologically active compounds into biopolymers using the molecular inclusion technique promises a variety of applications in drugs as well as in the agrochemical industry, because biologically active compounds can be slowly released from the biopolymer for a specified period of time (*8*−*10*). However, despite the important benefits that the technique offers for dispensing active agents, there are several disadvantages related to biopolymeric matrices, such as cost, water solubility, the use of organic solvents, and general

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problems concerning the molecular encapsulation of drugs and agrochemicals (*11*−*13*).

Cyclodextrins (CDs) are cyclic oligosaccharides, containing 6 (α-CD), 7 (*β*-CD), or 8 (*γ*-CD) α-(1,4)-linked glucose units, formed by the enzymatic degradation of starch by bacteria. The most important structural feature of these compounds is their truncated cone shape, with a hydrophobic interior cavity and hydrophilic surfaces (*14*). It is well-known that they are capable of forming inclusion compounds both in solution and in solid state with a variety of guest molecules, which are placed in their hydrophobic interior cavity (*15*). CDs are widely used in pharmaceutical science (*16*) to improve the chemical stability, absorption, bioavailability, and controlled release of some drugs and the dissolution of nonpolar compounds and in food technology to protect active ingredients against oxidation (*17, 18*). However, in the past decade, CDs have aroused considerable interest in many other fields (agriculture, nanocomposite technology, chromatography, biotechnology, etc.), because of the low cost of production. Many synthetic pesticides can form inclusion complexes with CDs (*19*), often resulting in improvements in their chemical and physical properties: these include enhanced solubility and bioavailability (*20*−*23*), the increased stability of photodegradable and/or unstable pesticides (*24, 25*), catalytic effects on the degradation of pesticides (*26*), the diminution of unpleasant taste, odor, and controlled release (*27*), a reduction of contact toxicity to humans (*28*), improvements in pesticide adsorption, and inhibition of their leaching in soils (*29*−*31*). Several publications predict a rapid development in the application of CDs to pesticide formulations in forthcoming years (*32*−*34*). In addition, CDs, as biodegradable enzyme-modified starch derivatives, are natural, nontoxic compounds, harmless to microorganisms, and hence, not noxious for the environment (*35, 36*).

Current commercial chlorpyrifos is formulated and sold as an emulsifiable concentrate. Enhanced solubilization by complexation may increase chlorpyrifos efficiency and help prevent overdosing, resulting in more cost-effective and environmentally friendly agricultural practices. The aim of this work was to investigate the possibility of obtaining inclusion complexes of chlorpyrifos in CDs, as a first step to obtaining formulations that head to a more rational use of this insecticide by improving its water solubility, bioavailability, and insecticidal activity.

MATERIALS AND METHODS

α-, HP-*β*-, and *γ*-CDs were purchased from Tokyo Chemical Industry U.K. Ltd. (TCI). G_2-*β* and *β*-CDs were kindly supplied by Amaizo, American Maize-Products Company, Hammond, IN. All other chemicals used were of analytical grade. Chlorpyrifos was obtained from Indalva, S.L. (Alicante, Spain).

Phase solubility diagrams were constructed according to Higuchi and Connors (*37*). Excess amounts of chlorpyrifos were added to aqueous solutions of increasing concentrations up to 100 mM of α-, *γ*- G_2-*β*-, and HP-*β*-CDs and 15 mM in the case of *β*-CDs, in 10 mL of water at 25 °C. The samples were maintained in an ultrasonic bath for 60 min to reach equilibrium. The aqueous solutions were filtered through a 0.2 *μ*m cellulose membrane filter and diluted in 80% ethanol−water. The chlorpyrifos concentration was determined using a GC instrument (HP-5980 series II) equipped with a flame photometric detector. A DB-1701 capillary column (30 m × 0.25 mm i.d., 0.25 *μ*m thickness) (J. and W. Scientific, Folsom, CA) was used for chlorpyrifos determinations. The GC conditions were as follows: column temperature, 60 °C (1 min), 60−120 °C at 30 °C min $^{-1}$, 120−220 °C at 5 °C min $^{-1}$ (held for 3 min), and 15 °C min $^{-1}$ to 280 °C (held for 24 min); the carrier gas was helium; injection temperature, 250 °C; injection volume, 2 *μ*L with HP7673 autosampler (HP); injection mode, splitless; detector temperature, 300 °C (*38*).

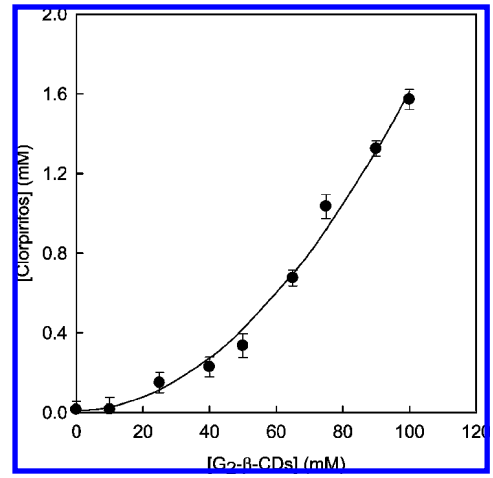

Figure 1. Phase solubility diagram of chlorpyrifos with G_2-*β*-CDs (●). The line shows the best fit to eq 7.

Steady-state fluorescence measurements were performed with a Shimadzu RF 5301 PC spectrofluorimeter. Emission fluorescence spectra were acquired in the 330−600 nm intervals, at a fixed excitation wavelength of 350 nm. The reaction medium contained 1.43, 0.953, and 0.715 *μ*M chlorpyrifos and increasing concentrations of G_2-*β*-CDs (from 0 to 80 mM) prepared in water to reach a final volume of 1 mL at 25 °C.

All determinations were carried out by triplicate.

RESULTS AND DISCUSSION

The aqueous solubility of chlorpyrifos was studied in the presence of increasing concentrations of different types of CDs (α-, *β*-, *γ*-, HP-*β*-, and G_2-*β*-CDs). Only in the case of G_2-*β*-CDs did chlorpyrifos solubility increase with an increasing CDs concentration, indicating that G_2-*β*-CDs trap the insecticide within their hydrophobic cavity. **Figure 1** shows the phase solubility diagram obtained for chlorpyrifos with G_2-*β*-CDs in water. The solubility curve obtained can be classified as A_P type (*37*), indicating that the stoichiometry of the complex was higher than 1:1.

Assuming that the stoichiometry of the complexes is 1:2, the equilibrium constants between free and complexed chlorpyrifos were determined using a model involving the sequential binding of two CD molecules to one chlorpyrifos molecule (*39*)

$$\text{Chlorp} + \text{CD} \overset{K_1}{\longleftrightarrow} \text{Chlorp}-\text{CD}$$

$$\text{Chlorp}-\text{CD} + \text{CD} \overset{K_2}{\longleftrightarrow} \text{Chlorp}-\text{CD}_2$$

where the complexation constants K_1 and K_2 are defined as

$$K_1 = \frac{[\text{Chlorp}-\text{CD}]}{[\text{Chlorp}]_f[\text{CD}]_f} \quad (1)$$

$$K_2 = \frac{[\text{Chlorp}-\text{CD}_2]}{[\text{Chlorp}-\text{CD}][\text{CD}]_f} \quad (2)$$

The mass balance for the chlorpyrifos and CD in aqueous solution may be represented by the equations

$$[\text{Chlorp}]_t = [\text{Chlop}]_f + [\text{Chlorp}-\text{CD}] + [\text{Chlorp}-\text{CD}_2] \quad (3)$$

$$[\text{CD}]_t = [\text{CD}]_f + [\text{Chlorp}-\text{CD}] + [\text{Chlorp}-\text{CD}_2] \quad (4)$$

where subscripts f and t stand for free and total, respectively.

Table 1. Stability Constants (K_1 and K_2) Values for 1:2 Chlorpyrifos G_2-β-CDs, Calculated by Solubility and Fluorescence Methods

	K_1 (M^{-1})	K_2 (M^{-1})
phase solubility diagram	12.34 ± 3.1	3895 ± 183
fluorescence	15.28 ± 3.3	4068 ± 195

Reorganizing eqs 1 and 2, [Chlorp−CD] and [Chlorp−CD_2] can be expressed as

$$[\text{Chlorp}-\text{CD}] = K_1[\text{Chlorp}]_f[\text{CD}]_f \quad (5)$$

$$[\text{Chlorp}-\text{CD}_2] = K_1K_2[\text{Chlorp}]_f[\text{CD}]_f^2 \quad (6)$$

Then, substituting these two equations into eq 3 the following relationship was obtained:

$$[\text{Chlorp}]_t = [\text{Chlorp}]_f + K_1[\text{Chlorp}]_f[\text{CD}]_f + K_1K_2[\text{Chlorp}]_f[\text{CD}]_f^2 \quad (7)$$

$[CD]_f$ can be deduced from eq 4 following substitution of eqs 5 and 6 and rearrangement

$$[\text{CD}]_f = \frac{\sqrt{((1 + K_1[\text{Chlorp}]_f)^2 + (4K_1K_2[\text{Chlorp}]_f[\text{CD}]_t))}}{2K_1K_2[\text{Chlorp}]_f} \quad (8)$$

Fitting the data of the phase solubility diagram presented in **Figure 1** by a nonlinear regression using Sigma Plot (Jandel Scientific) to eq 7 but where $[CD]_f$ is substituted by eq 8, S_0 is 0.01 mM, and taking into account that $[Chlorp]_f$ always refer to the maximum aqueous solubility of chlorpyrifos ($S_0 = 0.01$ mM), the values of the complexation constant for the formation of 1:1 complexes (K_1) and the complexation constant for the formation of 1:2 complexes (K_2) were obtained (**Table 1**). The K_2 value (3895 ± 183 M^{-1}) was significant higher than the K_1 value (12.34 ± 3.1 M^{-1}), suggesting that the 1:2 inclusion complexes are the most predominant species in the described conditions.

To corroborate that the complexes formed between chlorpyrifos and G_2-β-CDs were 1:2 and that the proposed model involving the sequential binding of two CD molecules to one chlorpyrifos molecule is correct, the inclusion complexing properties were also investigated by photochemically induced fluorescence (PIF).

Fluorescence spectroscopy is a very sensitive technique that allows for the complexation of many compounds with CDs in aqueous media to be measured in a very short time (*40*−*45*). Moreover, several authors have demonstrated the usefulness of room-temperature PIF for the analysis of aromatic pesticides (*46*−*49*).

To evaluate the influence of CD concentration on the PIF intensity of chlorpyrifos, G_2-β-CDs was added in increasing amounts to the aqueous solutions of chlorpyrifos. **Figure 2** shows the significant PIF signal enhancements, obtained with increasing G_2-β-CDs concentrations (from 0 to 80 mM) for all of the chlorpyrifos concentrations studied (0.715, 0.953, and 1.43 μM). In all cases, the emission intensity increased with the G_2-β-CD concentration and leveled off when all of the chlorpyrifos has been entrapped in the hydrophobic cavity of the CDs. Therefore, at chlorpyrifos concentrations of 0.715, 0.953, and 1.43 μM, the PIF plateau level was reached with 10, 20, and 40 mM of G_2-β-CDs, respectively (**Figure 2**).

As shown above, the PIF intensity of chlorpyrifos increases with increasing G_2-β-CD concentrations because of inclusion

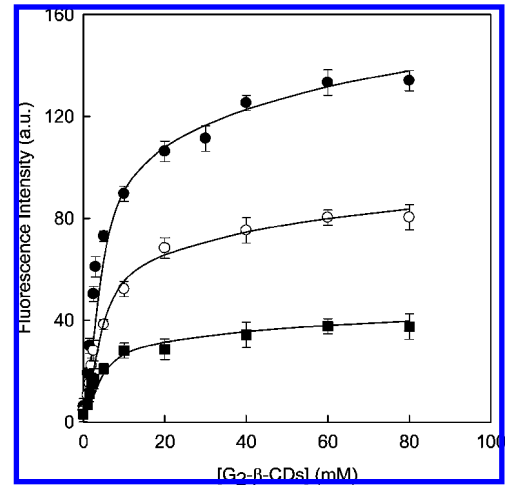

Figure 2. Fluorescence intensities of chlorpyrifos in the presence of increasing concentrations of G_2-β-CDs: (■) 0.715 μM chlorpyrifos, (○) 0.953 μM chlorpyrifos, and (●) 1.43 μM chlorpyrifos. The lines show the best fits to eq 9.

complex formation. The relation between the observed PIF intensity (F) and the total CD concentration $[CD]_t$ is

$$F = F_0 + \frac{(F_\infty - F_0)K_1[\text{CD}]_t}{1 + K_1[\text{CD}]_t} + \frac{(F_\infty - F_0)K_1K_2[\text{CD}]_t^2}{1 + K_1K_2[\text{CD}]_t^2} \quad (9)$$

where F_0 denotes the chlorpyrifos PIF intensity in the absence of CDs, F_∞ is the PIF intensity when all of the chlorpyrifos molecules have been complexed with CDs, F is the measured PIF intensity at each CD concentration used, $[CD]_t$ is the total CD concentration, K_1 is the complexation constant for the formation of 1:1 complexes, and K_2 is the complexation constant for the formation of 1:2 complexes.

The above eq 9 shows a nonlinear relationship between F and $[CD]_t$ as in all of the curves in **Figure 2**. Fitting the data by nonlinear regression using Sigma Plot (Jandel Scientific), values of 15.28 ± 3.3 and 4068 ± 195 M^{-1} were obtained for K_1 and K_2, respectively, between chlorpyrifos and G_2-β-CDs (**Table 1**). These values are quite similar to those obtained by the solubility method (**Table 1**).

To corroborate the presence of 1:2 complexes, the inverse of eq 9 was calculated

$$\frac{1}{(F - F_0)} = \frac{2}{(F_\infty - F_0)} + \frac{1}{(F_\infty - F_0)K_1[\text{CD}]_t} + \frac{1}{(F_\infty - F_0)K_1K_2[\text{CD}]_t^2} \quad (10)$$

Equation 10 showed a quadratic relation between $1/(F_0 - F)$ and $1/[CD]_t$. The Benesi−Hildebrand (B−H) equation for 1:2 complexes (*49, 50*) is a simplified form of our eq 10, in which the complexation constants K_1 and K_2 are simplified in a unique constant (K). This simplification permits us to obtain a linear relationship between $1/(F_0 - F)$ and $1/[CD]_t^2$ when the complex stoichiometry is 1:2. The B−H equation for 1:2 complexes is (*50*)

$$\frac{1}{(F - F_0)} = \frac{1}{(F_\infty - F_0)} + \frac{1}{(F_\infty - F_0)K[\text{CD}]_t^2} \quad (11)$$

The representation of $1/(F - F_0)$ versus $1/[CD]_t^2$, which is a double-reciprocal plot (*51*), leads to a straight line for all of the

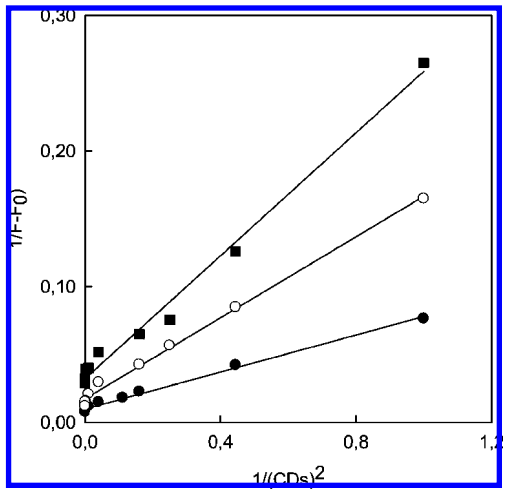

Figure 3. (A) Benesi−Hildebrand plot for chlorpyrifos/G_2-β-CDs (eq 10): (■) 0.715 μM chlorpyrifos, (○) 0.953 μM chlorpyrifos, and (●) 1.43 μM chlorpyrifos.

chlorpyrifos concentrations studied (**Figure 3**), confirming that the stoichiometry of the chlorpyrifos/G_2-β-CDs complex is 1:2.

CDs may be used as potential additives for commercial chlorpyrifos formulation because they are inexpensive and environmentally safer than conventional additives. They are capable of forming complexes with chlorpyrifos, not only enhancing its solubility for more efficient delivery but also preventing its hydrolysis and, thus, facilitating its insecticidal action. Of the tested CDs, G_2-β-CDs appears to be the best complexation agent for chlorpyrifos, because the cholrpyrifos/G_2-β-CDs complex formed shows higher stability constants with a stoichiometry of 1:2.

In addition, because of the great importance of chlorpyrifos insecticide in plant protection strategies, the inclusion of this compound in G_2-β-CDs should be regarded as contributing toward its sustainable and more economic use. The significant increase in its bioactivity provides an effective approach for a more rational application of chlorpyrifos, diminishing the amount of organic solvents and chlorpyrifos used and increasing its efficacy.

LITERATURE CITED

(1) Racke, K. D. Environmental fate of chlorpyrifos. *Rev. Environ. Contam. Toxicol.* **1993**, *131*, 1–151.

(2) Ward, S.; Arthington, A. H.; Pusey, B. J. The effects of a chronic application of chlorpyrifos on the macroinvertebrate fauna in an outdoor artificial stream system: species responses. *Ecotoxicol. Environ. Saf.* **1995**, *30*, 2–23.

(3) Goldstein, A.; Aronow, L.; Kalman, S. M. *Principles of Drug Action: The Basis of Pharmacology*; Wiley: New York, 1974.

(4) Grob, D.; Harvey, J. C. Effects in man of the anticholinesterase compound sarin (isopropyl methyl phosphonofluoridate). *J. Clin. Invest.* **1958**, *37*, 350–368.

(5) Barceló, D. EPA and other methods for the determination of priority pesticides and their transformation products in water. A review. *J. Chromatogr.* **1993**, *643*, 117–143.

(6) United States Environmental Protection Agency (U.S. EPA). Pesticide industry sales and usage: 1992 and 1993 market estimates. Office of Pesticide Programs, Washington, D.C., 1994.

(7) Lacorte, S.; Lartiges, S. B.; Garrigues, P.; Barceló, D. Degradation of organophosphorus pesticides and their transformation products in estuarine waters. *Environ. Sci. Technol.* **1995**, *29*, 431–438.

(8) Anderson, J. M.; Kim, S. W. *Advances in Drug Delivery Systems*; Elsevier: Amsterdam, The Netherlands, 1986.

(9) Bruck, S. D. *Controlled Drug Delivery*; CRC Press: Boca Raton, FL, 1983; Vol. 1−2.

(10) Tyle, P.; Ram, B. P. *Targeted Therapeutic Systems*; Marcel Dekker: New York, 1990.

(11) Teixeira, M. A.; Paterson, W. J.; Dunn, E. J.; Li, Q.; Hunter, B. K.; Goosen, M. F. A. Assessment of chitosan gels for the controlled release of agrochemicals. *Ind. Eng. Chem. Res.* **1990**, *29*, 1205–1209.

(12) Kenawy, E. R.; Sherrington, D. C. Controlled release of agrochemical molecules chemically bonded to polymers. *Eur. Polym.* **1992**, *28*, 841–862.

(13) Hirayama, F.; Uekama, K. Cyclodextrin-based controlled release systems. *Adv. Drug Delivery Rev.* **1999**, *36*, 125–141.

(14) Szejtli, J. *Cyclodextrins and Their Inclusion Complexes*; Akadémiai Kiadó: Budapest, Hungry, 1982.

(15) Nakai, Y.; Yamamoto, K.; Terada, K.; Watanabe, D. New methods for preparing cyclodextrin inclusion compounds. I. Heating in a sealed container. *Chem. Pharm. Bull.* **1987**, *35*, 4609–4617.

(16) Duchêne, D.; Wouessidjewe, D. Pharmaceuticals uses of cyclodextrins and derivates. *Drug Delivery Ind. Pharm.* **1990**, *16*, 175–182.

(17) López-Nicolás, J. M.; Pérez-López, A. J.; Carbonell-Barrachina, A. A.; García-Carmona, F. Use of natural and modified cyclodextrins as inhibiting agents of peach juice enzymatic browning. *J. Agric. Food Chem.* **2007**, *55*, 5312–5319.

(18) López-Nicolás, J. M.; Pérez-López, A. J.; Carbonell-Barrachina, A. A.; García-Carmona, F. Kinetic study of the activation of banana juice enzymatic browning by the activation of maltosyl-β-cyclodextrin. *J. Agric. Food Chem.* **2007**, *55*, 9655–9662.

(19) Szejtli, J. Cyclodextrins in pesticides. *Starch/Staerke* **1985**, *37*, 382–386.

(20) Dailey, O.; Bland, J.; Trask-Morrell, B. Preparation and characterization of cyclodextrin complexes of the insecticides aldicarb and sulprofos. *J. Agric. Food Chem.* **1993**, *41*, 1767–1771.

(21) Pérez-Martínez, J. I.; Arias, M. J.; Ginés, J. M.; Moyano, J. R.; Morillo, E.; Sánchez-Soto, P. J.; Novák, C. 2,4-D-cyclodextrin complexes. Preparation and characterization by thermal analysis. *J. Therm. Anal.* **1998**, *51*, 965–972.

(22) Lezcano, M.; Al-Soufi, W.; Novo, M.; Rodríguez-Núñez, E.; Vázquez, J. Complexation of several benzimidazole-type fungicides with α- and β-cyclodextrins. *J. Agric. Food Chem.* **2002**, *50*, 108–112.

(23) Ginés, J.; Pérez-Martínez, J.; Arias, M.; Moyano, J.; Morillo, E.; Ruiz-Conde, A.; Sánchez-Soto, P. J. Inclusion of the herbicide 2,4-diclorophenoxyacetic acid (2,4-D) with β-cyclodextrin by different processing methods. *Chemosphere* **1996**, *33*, 321–334.

(24) Kamiya, M.; Nakamura, K. Cyclodextrin inclusion effects on photodegradation rates of organophosphorus pesticides. *Environ. Int.* **1995**, *21*, 299–304.

(25) Kamiya, M.; Nakamura, K.; Sasaki, C. Inclusion effects of cyclodextrins on photodegradation rates of parathion and paraoxon in aquatic medium. *Chemosphere* **1994**, *28*, 1961–1967.

(26) Ishiwata, S.; Kamiya, M. Cyclodextrin inclusion: Catalytic effects on the degradation of organophosphorus pesticides in neutral aqueous solution. *Chemosphere* **1999**, *39*, 1595–1600.

(27) Szente, L. Stable controlled-release organophosphorous pesticides entrapped in β-cyclodextrin. *J. Therm. Anal.* **1998**, *51*, 957–963.

(28) Loukas, Y.; Antoniadou-Vyza, E.; Papadaki-Valiraki, A.; Machera, K. β-Cyclodextrin inclusion complex of a new organophosphorus insecticide. Determination of stability constant with HPLC. *J. Agric. Food Chem.* **1994**, *42*, 944–948.

(29) Morillo, E.; Pérez-Martínez, J.; Ginés, J. Leaching of 2,4-D from a soil in the presence of β-cyclodextrin: Laboratory columns experiments. *Chemosphere* **2001**, *44*, 1065–1069.

(30) Fuoco, R.; Colombini, M. Electrochemical and spectral evidence of the inclusion of the herbicide difenzoquat by cyclodextrins in aqueous solution. *J. Electroanal. Chem.* **1994**, *368*, 149–154.

(31) Pérez-Martínez, J.; Ginés, J.; Morillo, E.; Arias, M.; Moyano, J. Improvement of the desorption of the pesticide 2,4-D via complexation with HP-β-cyclodextrin. *Pestic. Sci.* **2000**, *56*, 425–430.

(32) Wang, X.; Brusseau, M. Solubilization of some low-polarity organic compounds by HP-β-CD. *Environ. Sci. Technol.* **1995**, *29*, 2632–2635.
(33) Manolikar, M.; Sawant, M. Study of solubility of isoproturon by its complexation with β-cyclodextrin. *Chemosphere* **2003**, *51*, 811–816.
(34) Pérez-Martínez, J.; Ginés, J.; Morillo, E.; Rodríguez, M.; Moyano, J. 2,4-Dichlorophenoxyacetic acid/partially methylated-β-cyclodextrin inclusion complexes. *Environ. Technol.* **2000**, *21*, 209–216.
(35) Jiradecha, C. Removal of naphthalene and 2,4-dinitrotoluene from soils by using carboxymethyl-β-cyclodextrin. *Nat. Sci.* **2000**, *34*, 171–178.
(36) Bardi, L.; Mattei, A.; Steffan, S.; Marzona, M. Hydrocarbon degradation by a soil microbial population with β-cyclodextrin as surfactant to enhance bioavailability. *Enzyme Microb. Technol.* **2000**, *27*, 709–713.
(37) Higuchi, T.; Connors, K. A. Phase solubility techniques. *Adv. Anal. Chem. Instrum.* **1965**, *4*, 107–212.
(38) Gabaldón, J. A.; Maquieira, A.; Puchades, R. Development of a simple extraction procedure for chlorpyrifos determination in food samples by immunoassay. *Talanta* **2007**, *71*, 1001–1010.
(39) López-Nicolás, J. M.; Bru, R.; Sánchez-Ferrer, A.; García-Carmona, F. Use of "soluble lipids" for biochemical processes: Linoleic acid−cyclodextrin inclusion complexes in aqueous solutions. *Biochem. J.* **1995**, *308*, 151–154.
(40) Enoch, I. M. V.; Swaminatha, M. Inclusion complexation of 2-amino-7-bromofluorene by β-cyclodextrin: Spectral characteristics and the effect of pH. *J. Fluoresc.* **2004**, *14*, 751–756.
(41) Enoch, I. M. V.; Swaminatha, M. Dual fluorescence and photoprototropic characteristics of 2-aminodiphenylsulphone-β-cyclodextrin inclusion complex. *J. Inclusion Phenom. Macrocyclic Chem.* **2005**, *53*, 149–154.
(42) Enoch, I. M. V.; Swaminatha, M. Fluorimetric study on molecular recognition of β-cyclodextrin with 2-amino-9-fluorenone. *J. Fluoresc.* **2006**, *16*, 501–510.
(43) Panja, S.; Chakravorti, S. Dynamics of twisted intramolecular charge transfer process of 4-*N*,*N*-dimethylaminocinnamic acid in α-cyclodextrin environment. *Chem. Phys. Lett.* **2001**, *336*, 57–64.
(44) Ma, L.; Tang, B.; Chu, C. Spectrofluorimetric study of the β-cyclodextrin−dapsone−linear alcohol supramolecular system and determination of dapsone. *Anal. Chim. Acta* **2002**, *469*, 273–283.
(45) Iglesias, E. Inclusion complexation of novocaine by β-cyclodextrin in aqueous solutions. *J. Org. Chem.* **2006**, *71*, 4383–4392.
(46) Coly, A.; Aaron, J. J. Photochemical−spectrofluorimetric method for the determination of several aromatic insecticides. *Analyst* **1994**, *119*, 1205.
(47) Eremin, S.; Laasis, B.; Aaron, J. J. Photochemical−fluorimetric method for the determination of total chlorophenoxyacid herbicides. *Talanta* **1996**, *43*, 295–301.
(48) Luis Vilchez, J. L.; El-Khattabi, R.; Blanc, B.; Navalón, A. Photochemical−fluorimetric method for the determination of the insecticide imidacloprid in water samples. *Anal. Chim. Acta* **1998**, *371*, 247–253.
(49) Mahedero, M. C.; Muñoz de la Peña, A.; Bautista, A.; Aaron, J. J. An investigation of inclusión complexes of cyclodextrins with phenilurea herbicides by photochemically fluorescence. Analytical applications. *J. Inclusion Phenom. Macrocyclic Chem.* **2002**, *42*, 61–70.
(50) Benesi, H. A.; Hildebrand, J. H. A spectrophotometric investigation on the interaction of iodine with aromatic hydrocarbons. *J. Am. Chem. Soc.* **1949**, *71*, 2703–2707.
(51) Connors, K. A. *Binding Constant, The Measurement of Molecular Complex Stability*; Wiley: New York, 1987.

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