An Investigation of Inclusion Complexes of Cyclodextrins with Phenylurea Herbicides by Photochemically-Induced Fluorescence. Analytical Applications

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Abstract

The effect of β-cyclodextrin (β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD) upon the photochemically-induced fluorescence (PIF) properties of four phenylurea herbicides, including linuron, diuron, isoproturon and neburon has been studied. Photochemical conversion of these nonfluorescent herbicides into strongly fluorescent photoproducts was shown to occur in β-CD and HP-β-CD aqueous media. The influence of pH, UV irradiation time and photoproduct stability on the fluorescence intensity was also investigated. In addition, the stoichiometry and formation constants of the complexes formed between herbicides and β-cyclodextrin (β-CD) or 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) were determined. The formation constant values, ranging from 184 ± 40 to 1498 ± 245 M^{-1}, were calculated by applying the iterative nonlinear regression (NLR) approach to the PIF data. Linear calibrations graphs were established in the interval 1–12 μg/mL, for diuron, linuron and neburon. The IUPAC limits of detection ranged between 580 and 700 ng/mL, according to the compound. Application to the analysis of phenylurea herbicides in spiked river water was also described.

Introduction

Cyclodextrins (CDs) are enzymatically-modified starches made up of glucopyranose units joined by α-(1,4) linkages. Three different types of CDs are known consisting, respectively, of six, seven and eight glucopyranose units (α-, β- and γ-CDs) [1]. Cyclodextrins can be considered as molecular size, torus-shaped, capsules whose empty cavity can be filled with one or several molecules of another substance (guest). As a result, inclusion complexes are formed between cyclodextrins (host) and a variety of organic molecules (guest), in which only physical forces play a role, without covalent bonding. In aqueous solution, the slightly apolar CD cavities are occupied by polar water molecules, but because of polar–nonpolar repulsive interactions, these water molecules can be replaced by less polar organic molecules. Under these conditions, the CD inclusion complexes obey a thermodynamic equilibrium, and, usually, a 1 : 1 stoichiometry is established, although, in some cases, one or more CD molecules may contain one or more organic guest molecules.

Generally, the physicochemical, photophysical and photochemical properties of the guests included in CDs are considerably modified. For instance, the fluorescence [2, 3] and room-temperature phosphorescence [4, 5] of several organic compounds are significantly increased in the presence of CDs in aqueous solutions. Also, a number of rates of chemical and photochemical reactions decrease notably

in CD inclusion complexes [6–10]. Although luminescence spectroscopy is an interesting means to characterize these organized media, very few fluorimetric studies have concerned the formation of CD complexes with pesticides [11–14]. In the case of warfarin, an anticoagulant rodenticide, and biomadiolone, the fluorescence signals, which are quenched in aqueous solutions, are restored in the presence of CD [11, 12]. Garcia-Sanchez *et al.* [13] have shown that synchronous fluorescence with variable angle scanning allows resolution of a ternary mixture of aminocarb, carbendazin and coumatetralyl in 10^{-2} M β-CD solutions. Coly and Aaron [14] have found that β-CD and hydroxypropyl-β-cyclodextrin (HP-β-CD) considerably enhance fluorescence and photochemically induced fluorescence of five aromatic pesticides in aqueous media.

Substituted ureas represent a large and important class of herbicides widely used in agriculture to control weeds in cereals, vegetables and fruit trees. The first urea compound with herbicidal properties was discovered four decades ago, and since then a large number of urea pesticides have been synthesized. The N-phenyl-N',N'-dialkylureas, including chlorotoluron, diuron, isoproturon, neburon and fluometuron, and the N-phenyl-N'-alkyl-N'-methoxyureas, such as linuron, monolinuron and metobromuron constitute the two main groups of substituted urea herbicides. Presently, urea pesticide residues are mainly determined by chromatographic methods. Gas chromatography (GC) is commonly used because of its high sensitivity and se-

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lectivity; however, the thermal instability of most phenylurea pesticides requires preparation of stable derivatives prior to detection, which complicates the analysis because of time-consuming and tedious manipulations [15–19]. Therefore, high-performance liquid chromatography (HPLC) with UV absorption or fluorescence detection is often preferred to GC [20–23].

Recently, several authors have demonstrated the usefulness of room-temperature photochemically induced fluorescence (PIF) for the analysis of aromatic pesticides and resolution of their binary mixtures [24–26] and have applied PIF detection coupled with flow injection analysis (FIA) to the determination of a phenylurea derivative (diflubenzuron) and other aromatic pesticides [27–29]. In the analysis of a variety of pesticides, including neburon, HPLC postcolumn photolysis and fluorogenic labelling with OPA-2 ME were also utilized [28]. The PIF methods are based on the direct UV irradiation of stationary liquid solutions or dynamic flow containing a nonfluorescent – or weakly fluorescent – analyte and photoconversion into strongly fluorescent photoproducts; they have been applied to clinical, pharmaceutical, biochemical and environmental analysis [30, 31]. Relative to chemical derivatization, the photochemically-induced fluorimetric methods are advantageous because of their simplicity, cleanliness, rapidity, high sensitivity and selectivity [31].

Although the cleavage of the cyclodextrin 1,4-glycosidic bonds can occur upon γ irradiation of crystalline β- and γ-CDs [32], cyclodextrins are known to be fairly resistant to irradiation by UV light, in the wavelength range used in PIF. However, until now, the effect of the presence of CDs on the photochemically-induced fluorescence signals has been investigated in very few cases [9, 14, 33, 34].

In this work, we investigate by photochemically-induced fluorescence the inclusion complexing properties of β-CD and HP-β-CD with phenylurea herbicides, including diuron, isoproturon, linuron and neburon (Scheme 1), as well as their analytical usefulness. Indeed, we have found recently that these naturally nonfluorescent compounds become strongly fluorescent after UV irradiation in organic solvents [35] and in aqueous micellar media [36]. Also, we have shown, in a preliminary communication, that phenylurea herbicides can form inclusion complexes with β-CDs [34].

Experimental

Reagents

All experiments were performed with analytical grade chemicals. Purified LC-grade water (Millipore Milli-Q-system) was used. Linuron (99%, m/m), diuron (98%, m/m), isoproturon (99%, m/m) and neburon (99%, m/m) were purchased from Riedel-de-Haën (Hanover, Germany) and used as received. Spectroscopic grade 2-propanol was used. 20% v/v buffer solutions of pH 2, 5 (0.1 M acetate/acetic acid), 7 (0.1 M monobasic potassium phosphate/ potassium hydroxide pellets) and 9 were also prepared for pH studies. β-CD and HP-β-CD were obtained from Cyclolab (Budapest, Hungary) and used as received.

Apparatus

All spectrofluorimetric measurements were carried out using a Perkin-Elmer Model LS 50 luminescence spectrometer, equipped with a xenon discharge lamp equivalent to 20 kW for 8 μs duration. The instrument was connected via a RS-232 interface to a compatible 486 PC. Data acquisition and data analysis were performed by means of Perkin-Elmer Fluorescence Data Manager software, version 2.70. Solutions were excited at different wavelengths, depending on the compound. Excitation and emission bandwidths of 4 nm were utilized for all fluorescence measurements. The scan rate of the monochromators was maintained at 350 nm. min^{-1}. All measurements were done at 20 $\pm$ 0.1 °C, using a thermostatically-controlled cell holder and a Selecta Model thermostatically-controlled water-bath.

An Osram 200 W HBO high-pressure mercury lamp with an Oriel Model 8500 power supply, was utilized for the photolysis reactions. The photochemical set-up included a light-box consisting of a fan, the mercury lamp and a quartz lens. A standard Hellma (Mullheim, Germany) 1 cm path-length quartz fluorescence cuvette was placed on an optical bench, at 30 cm from the mercury lamp. For photolysis studies, the solutions were magnetically stirred during the irradiation.

Procedure

Solutions preparation

Stock standard solutions of the pesticides (5×10^{-3} M) were prepared by dissolving the compounds in 2-propanol. Working solutions were obtained by appropriate dilutions with distilled water. All solutions were protected against light with aluminium foil. The working solutions of herbicides contained a maximum of 2% of 2-propanol.

Volumes of buffer solutions of pH 2, 5, 7 and 9 were used in order to obtain the convenient pH values. 1.4×10^{-2} M β-CD and 2.0×10^{-2} M HP-β-CD standard aqueous solutions were prepared. Solutions of lower concentration were obtained by appropriate dilution of these standard aqueous solutions.

To study the influence of HP-β-CD or β-CD in the medium, several cyclodextrin solutions were prepared, by maintaining a constant concentration of 4×10^{-4} M linuron and diuron, or 1×10^{-4} M neburon, and varying the HP-β-CD or β-CD concentration. An aliquot of the phenylurea herbicide working solution was placed in a 5-mL volumetric flask, the appropriate amount of cyclodextrin solution was added to give the final CD concentration and it was diluted with water to the final volume of 5.0 mL.

For the study of pH effects, an aliquot of the herbicide solution was placed in a 5-mL volumetric flask in order to give a final concentration of 5×10^{-5} M; 2.5 mL of 2.5×10^{-2} M HP-β-CD or 3.5 mL of 1.4×10^{-2} M β-CD (in both cases the final cyclodextrin concentration was of 1×10^{-2} M) and 1.0 mL of the corresponding pH 5 or pH 7 buffer

LINURON

DIURON

NEBURON

ISOPROTURON
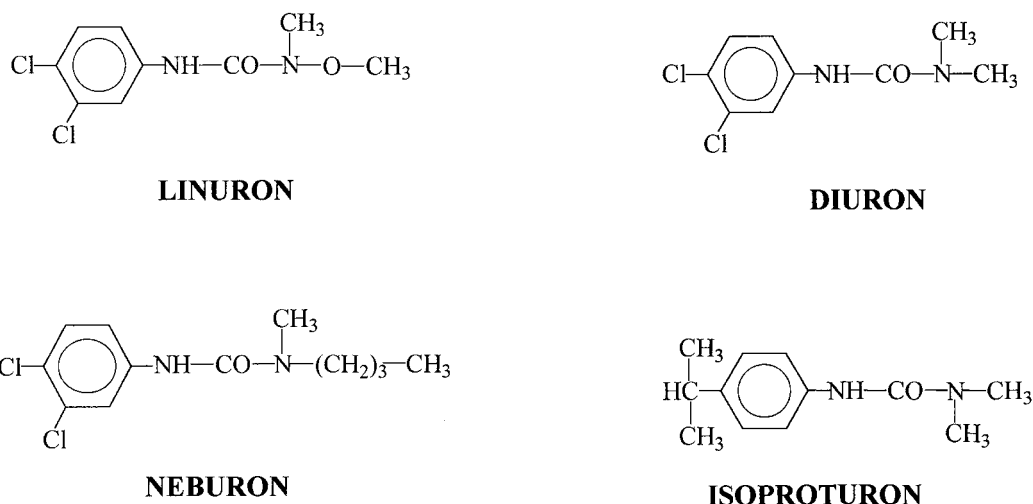
Scheme 1. Structure of the phenylurea herbicides under study.

(20% v/v) were diluted with water to the final volume of 5.0 mL.

The obtained solutions were placed in a quartz cuvette, and irradiated at room temperature with the high-pressure mercury lamp during the time required to form fluorescent photoproducts. During irradiation, the solutions were magnetically stirred. Fluorescence relative intensities of the samples were measured at $20.0 \pm 0.1\,°C$, using the photoproduct herbicide fluorescence excitation and emission optimal wavelengths.

Analytical measurements

Curves of fluorescence intensity *versus* UV irradiation time (t_{irr}) were constructed at λ_{ex} and λ_{em} values, using 5-10 min time intervals. Linear calibration curves were obtained at these λ_{ex} and λ_{em} values, by measuring the PIF signal at the optimum irradiation time (t_{irr}^{opt}), defined as the irradiation time corresponding to the maximum PIF intensity. In all cases, the PIF intensity measurements were corrected for the background signal using the appropriate blanks. To optimize the analytical results, PIF measurements were performed in triplicate and the mean values were used.

River water treatment

Guadiana river (Spain) water samples were collected in brown glass bottles previously rinsed with ultrapure water. The samples were filtered with a Whatman No. 1 filter paper in order to remove sand and other suspended solid matter, and then stored at $4\,°C$ in the dark. Before analysis, the samples were filtered successively with Millipore 0.45 μm and 0.22 μm nylon filters. A pH of a value of around 8.3 was measured for these river water samples. No fluorescent interference was detected in the river water before the spiking procedure. River water samples of 50 mL were spiked with the herbicide amount needed to give final concentrations of 0.3, 0.6 and 0.88 μg/mL^{-1}, for diuron and neburon and of 0.3, 0.6 and 0.8 μg/mL^{-1}, for linuron. A control blank was prepared without adding herbicide.

In order to improve the sensitivity of water analytical methods, some authors recommend the use of extraction procedures, including preconcentration steps [37–39]. In the present work, the solid-phase extraction (SPE) technique was utilized. Prior to extraction, the Sep-Pak Plus C18 bonded phase was conditioned with 8 mL of acetonitrile followed by 8 mL of deionized water (Milli-Q water). The disk was not allowed to dry, as recommended [40, 41]. To achieve a ten-fold pre-concentration, the following procedure was performed. The water sample spiked with the herbicide was mixed and allowed to pass through the Sep-Pak at a flow-rate of 5 mL/min. After sample extraction, the Sep-Pak was washed with 2×5 mL of Milli-Q water before elution; the herbicide trapped in the disk was collected by using 1 mL of acetonitrile as eluting solvent. The obtained fraction was evaporated to dryness in a gentle stream of nitrogen. Then, appropriate amounts of cyclodextrin and buffer solutions were added successively, and the resulting solution was diluted to a final volume of 5.0 mL.

Results and discussion

PIF spectral properties

The maximum PIF excitation (λ_{ex}) and emission (λ_{em}) wavelengths, relative PIF intensities (I_F) and t_{irr}^{opt} values (irradiation time corresponding to the photoproduct maximum fluorescence intensity) of the four phenylurea herbicides under study in β-CD and HP-β-CD solutions are presented in Table 1. As can be seen, the λ_{ex} and λ_{em} values depend significantly on the compound since they range, respectively, from 302 to 321 nm, and from 356 to 437 nm. On the other hand, they are quite similar in both cyclodextrin media. Except for linuron, all compounds exhibit a significant red-shift (8–67 nm) of their PIF emission maximum in β-CD and HP-β-CD media relative to pure water. In addition, significant enhancements of the PIF signal ranging from 2.5 to 9.0 according to the compound and the cyclodextrin, are observed in the presence of 10^{-2} M β-CD and HP-β-CD. These various CD-induced increases of PIF intensities can be attributed to the formation of inclusion complexes

Table 1. PIF spectral properties of phenylurea herbicides in cyclodextrin aqueous solutions

Compound[a]	Medium[b]	$\lambda_{ex}/\lambda_{em}$ (nm) [c]	F[d]	t_{irr}^{opt} (min) [e]
Diuron	H_2O	312/350	1.0	3
	β-CD	318/358	5.0	10
	HP-β-CD	315/358	6.0	12
Isoproturon	H_2O	330/370	1.0	8
	β-CD	302/437	–[f]	> 65[f]
	HP-β-CD	302/436	–[f]	> 65[f]
Linuron	H_2O	324/360	1.0	3
	β-CD	321/356	2.5	22
	HP-β-CD	321/357	3.4	24
Neburon	H_2O	306/350	1.0	4
	β-CD	315/358	9.0	8
	HP-β-CD	315/359	9.0	10

[a] Initial concentration: 5×10^{-5} M.
[b] β-CD and HP-β-CD concentrations: 1×10^{-2} M.
[c] PIF excitation and emission wavelengths.
[d] Relative PIF intensity corrected for the solvent signal and normalized to PIF intensity in water.
[e] Optimum irradiation time, corresponding to the photoproduct maximum fluorescence intensity.
[f] No well-defined maximum fluorescence signal in the F/t_{irr} curve, which shows a continuous increase.

between CDs and phenylurea herbicide photoproducts, with different complexing abilities. Similar results have been reported in the literature for other organic compounds [2, 3, 9, 14].

Kinetic study of the photolysis reaction in CD media

Optimum irradiation time values are much longer in the presence of CDs than in pure water, indicating that the photolysis reaction is slowed down when phenylurea herbicides are included in CD complexes.

Photochemical conversion of aromatic pesticides occurs generally in solution via a direct photolysis mechanism, in which the pesticides directly absorb the incident UV radiations. No systematic investigation of the exact nature of the fluorescent photoproducts formed during the pesticide photolysis reactions has been performed. Nevertheless, it has been suggested that the photodegradation pathways of aromatic pesticides probably involve considerable changes in the aromatic moiety of these molecules [21, 24]. In the case of the phenylurea herbicide photolysis, some authors have reported that one of the photoproducts formed is methylamine or dimethylamine [42–44]. It has also been found that aniline and substituted anilines present fluorescence spectra very similar to those of the phenylurea photoproducts, suggesting that related structures may be responsible for the fluorescence response observed for phenylurea herbicides [28].

To evaluate the kinetics of the fluorophore formation during the phenylurea herbicide photolysis, the evolution of the PIF signal with UV irradiation time was investigated in CD solutions. The shape of the curve of PIF signal *versus* irradiation time was found to depend on the type of herbicide under study, but to be independent of the cyclodextrin used

(β-CD or HP-β-CD). In the case of diuron, neburon and linuron, the PIF signal increased initially with time, reaching a maximum value and decreasing more slowly afterwards in both cyclodextrins (Figure 1a and b). The maximum PIF intensity was obtained in a shorter time for diuron and neburon than for linuron. With regard to isoproturon photolysis, the kinetic curve was characterized in both cyclodextrins by a continuous increase of the PIF signal with time and no well-defined maximum value, even after 65 min of irradiation (Figure 1a and b).

These two types of kinetic curves can be attributed to the existence of two distinct mechanisms of photolysis occurring in CDs. The first behavior corresponds to a two-step mechanism, including the rapid formation of fluorescent photoproduct(s), such as dichloro-anilines and/or dechlorinated anilines, followed by slow photodegradation into less fluorescent product(s). The second type of curve, observed only in the case of isoproturon, indicates that fluorescent photoproducts are formed much more slowly. The structure of the four herbicides supports the possibility of formation of an inclusion complex with CDs before irradiation, assuming that the photolysis process leading to fluorescent photoproducts takes place in the CD cavity. Indeed, considering that CD complexation of organic molecules generally occurs in the ground state, it can be expected that the formation of the host-guest complexes is a prior step to the photolysis reaction (Scheme 2), in agreement with other literature data [9, 10]. The longer irradiation times and the larger PIF intensities observed in β-CD and HP-β-CD relative to water are in agreement with the latter hypothesis. Comparable kinetic results were obtained previously for the photolysis reactions of phenylurea herbicides in micellar media [36] and of aromatic pesticides in CDs [14].

Interestingly, the PIF signal measured at the t_{irr}^{opt} value for diuron, neburon and linuron was kept constant for more than 1 h, when avoiding subsequent exposure to daylight or to the luminescence spectrometer xenon lamp light. It indicates that the phenylurea herbicide photoproduct(s) remain stable at least for this time period.

Effects of CD concentration

In order to evaluate the influence of CD concentration on the PIF intensity, β-CD or HP-β-CD was added in increasing amounts to aqueous solutions of the herbicides under study. Our results show significant PIF signal enhancements with increasing CD concentrations for all herbicides. Typical examples are presented for linuron and neburon in Figure 2. CD concentrations ranging between 3×10^{-4} and 1.4×10^{-2} M were investigated. As can be seen, the emission intensity increased with increasing cyclodextrin concentration and levelled off at β-CD and HP-β-CD concentrations of about 1.0×10^{-2} M, reaching a plateau value (Figure 2). In the case of linuron, the PIF enhancement was higher when using HP-β-CD than β-CD, whereas, for diuron as well as for neburon, the same increase was observed for both CDs.
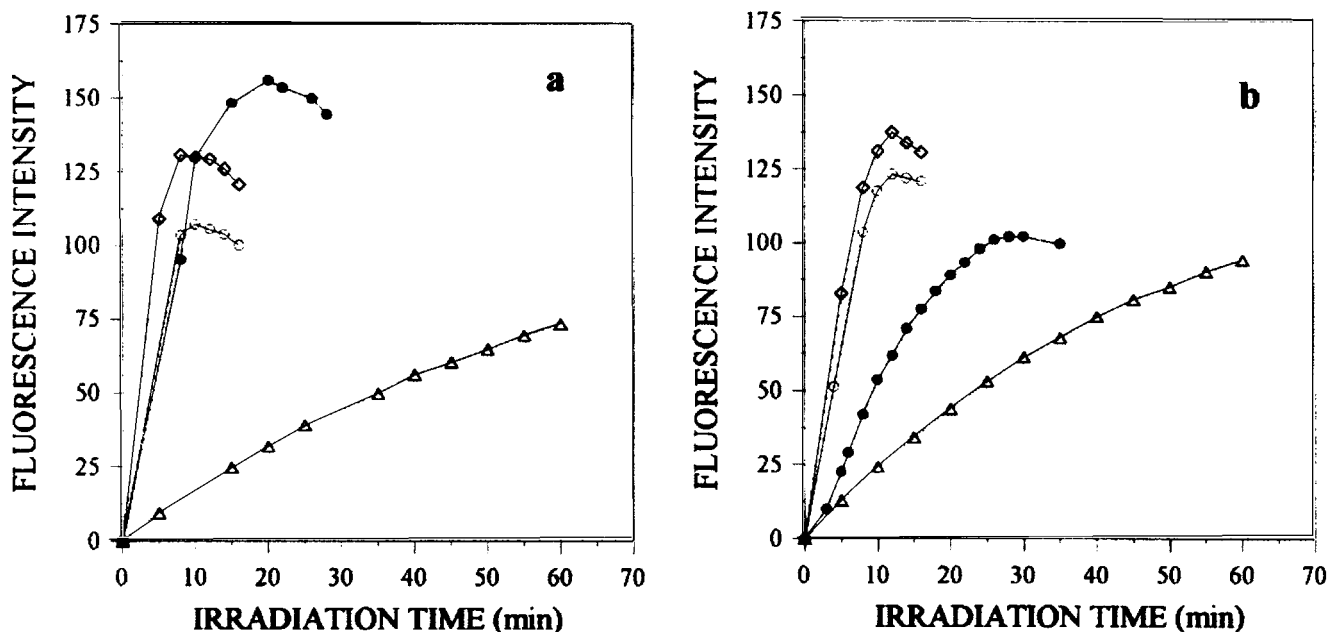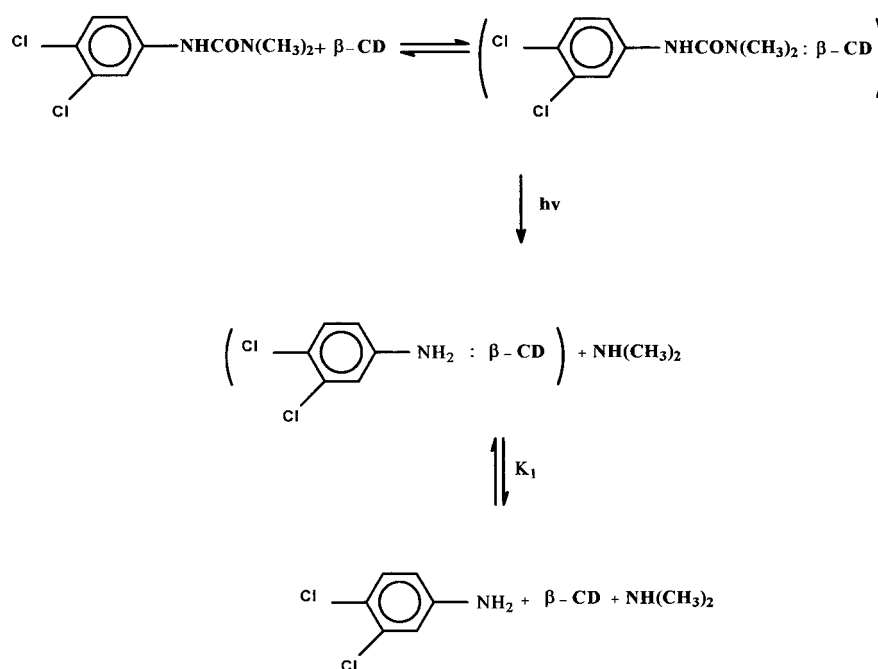
Figure 1. Effect of the UV irradiation time on the PIF intensity of 5×10^{-5} M linuron (●), diuron (○), isoproturon (△) and neburon (♦) in 1×10^{-2} M β-CD (a) and HP-β-CD (b) aqueous solution.


Scheme 2. Proposed complexation-photolysis process of diuron with β-CD. The encapsulated photoproduct was postulated to be 3,4-dichloroaniline.

Characteristics of the herbicide : CD inclusion complexes

Stoichiometry

The stoichiometry and formation constant of the phenylurea herbicide photoproduct: CD complexes were calculated as previously [9, 14] assuming a 1 : 1 stoichiometry, according to the following equilibrium:
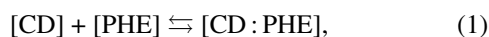
$$[CD] + [PHE] \rightleftharpoons [CD : PHE], \qquad (1)$$

where [PHE] [CD] and [CD : PHE] are, respectively, the equilibrium concentrations of the phenylurea herbicide photoproduct, cyclodextrin, and the inclusion complex. It is

assumed that the herbicide photoproduct concentration decrease due to photodegradation is negligible under the experimental conditions used.

As shown above, the PIF intensity of phenylurea photoproduct increases upon increasing CD concentration, because of the inclusion complex formation. The relation between this PIF signal increase and the CD concentration allows determination of the complex stoichiometry, according to the following equation [9]:

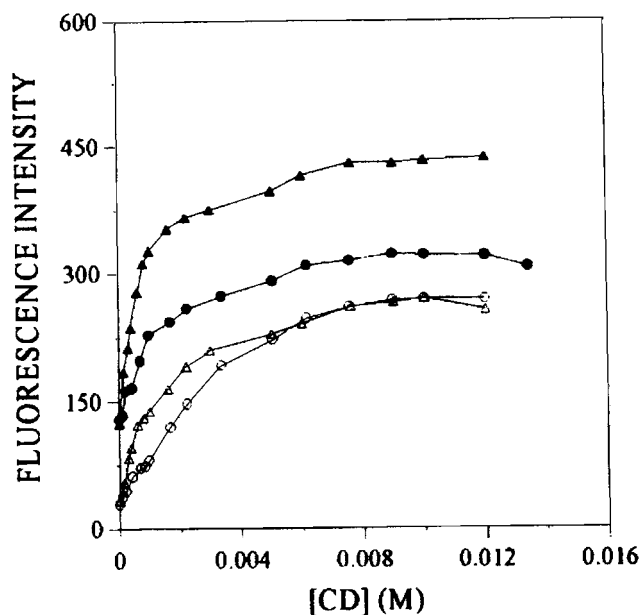$$\frac{1}{F - F_0} = \frac{1}{(F - F_\infty) K_1 [CD]_0} + \frac{1}{F_\infty - F_0}. \qquad (2)$$

Figure 2. Comparison of the effects of β-CD and HP-β-CD concentrations on the PIF intensity of linuron (4×10^{-4} M), $\lambda_{ex}/\lambda_{em} = 321/357$ nm, $t_{irr}^{opt} = 24$ min (HP-β-CD) (▲), $\lambda_{ex}/\lambda_{em} = 321/356$ mn; $t_{irr}^{opt} = 22$ min (β-CD) (●), and neburon (1×10^{-4} M), $\lambda_{ex}/\lambda_{em} = 315/359$ nm, $t_{irr}^{opt} = 10$ min (HP-β-CD) (△), $\lambda_{ex}/\lambda_{em} = 319/358$ nm; $t_{irr}^{opt} = 8$ min (β-CD) (○).

Table 2. Formation constants (K_1) of the inclusion complexes between cyclodextrins and phenylurea herbicide photoproducts

Compound	Cyclodextrin	K_1 (M^{-1})[a]	t_{irr}^{opt} (min)[b]
Diuron	β-CD	125 ± 12	10
	HP-β-CD	474 ± 69	12
Linuron	β-CD	645 ± 154	22
	HP-β-CD	1500 ± 254	24
Neburon	β-CD	184 ± 40	8
	HP-β-CD	736 ± 119	10

[a] K_1 values calculated using the nonlinear regression (NLR) analysis.
[b] Optimum irradiation time.

where F_0 denotes the phenylurea herbicide PIF intensity in the absence of CD, F_∞ is the PIF intensity when all the analyte molecules are essentially complexed with CD, F is the measured PIF intensity at each CD concentration used, $[CD]_0$ is the initial CD concentration, and K_1 is the complex formation constant.

The representation of $1/(F - F_0)$ vs $1/[CD]_0$, known as a double-reciprocal plot [45], leads to a straight line, when the stoichiometry for the complex is $1:1$. In contrast, if a plot of $1/(F - F_0)$ vs $1/[CD]_0^2$ is constructed, a downward concave curvature is obtained, confirming that the stoichiometry of the CD:herbicide complex is not $2:1$. In Figure 3, we have drawn both plots for linuron, diuron and neburon in the presence of β-CD. Similar plots were obtained for the three herbicides in the presence of HP-β-CD, indicating a $1:1$ stoichiometry in all cases.

Table 3. pH effects on the PIF intensity and other analytical parameters of phenylurea herbicides in buffered cyclodextrin aqueous solutions[a]

Compound	Medium[b]	$\lambda_{ex}/\lambda_{em}^c$	t_{irr}^{opt} (min)[d]	F^e
Diuron	**HP-β-CD(pH = 5)**	**317/356**	**12**	**1.53**
(5.0×10^{-5} M)	HP-β-CD(pH = 7)	313/362	10	1.37
	β-CD(pH = 5)	318/356	10	1.25
	β-CD(pH = 7)	318/356	8	1.00
Linuron	HP-β-CD(pH = 5)	321/357	26	1.19
(5.0×10^{-5} M)	HP-β-CD(pH = 7)	321/357	24	1.03
	β-CD(pH = 5)	**321/356**	**22**	**1.67**
	β-CD(pH = 7)	321/356	22	1.00
Neburon	HP-β-CD(pH = 5)	319/357	10	1.54
(5.0×10^{-5} M)	**HP-β-CD(pH = 7)**	**319/359**	**8**	**1.74**
	β-CD(pH = 5)	319/358	10	1.71
	β-CD(pH = 7)	319/358	6	1.00

[a] The optimal analytical conditions used to establish the calibration curves are represented in bold.
[b] Cyclodextrin concentration $[CD] = 1 \times 10^{-2}$ M.
[c] Analytical PIF excitation and emission wavelengths.
[d] Optimum irradiation time.
[e] Relative PIF intensity corrected for the solvent signal and normalized to the cyclodextrin medium giving the lowest signal for each herbicide.

Formation constants

In Benesi–Hildebrand's method, the linear plot can be used to obtain K_1, by simply dividing the intercept by the slope, but Benesi–Hildebrand plots tend to place more emphasis on lower CD concentration values than on higher ones. Therefore, the slope of the line is more sensitive to the ordinate value of the point having the smallest concentration. As a consequence, nonlinear regression (NLR) analysis provides a better estimation of K_1 [45]. Rearranging the data, we deduce the direct relationship between the observed PIF intensity, F, and the cyclodextrin initial concentration, $[CD]_0$:

$$F = F_0 + \frac{(F_\infty - F_0)K_1[CD]_0}{1 + K_1[CD]_0}. \qquad (3)$$

By applying Equation (3), the experimental data can be directly fitted, giving an exponential curve. The initial parameters of the complex needed for the NLR method were estimated from the Benesi–Hildebrand linear plots. The curve fitting procedure, based on the Marquardt algorithm, consists of calculating from Equation (3) the F values at each CD concentration through iteration, i.e., by varying the values of the initial parameters F_0, F_∞ and K_1.

Figure 4 shows an NLR plot of the experimental data for the inclusion complexes of linuron, diuron and neburon with HP-β-CD. The formation constant (K_1) values were calculated by the NLR method. As can be seen in Table 2, they range from 125 (± 12) to 1500 (± 245) M^{-1}, according to the structure of the phenylurea herbicide and to the type of CD. Isoproturon was not included in the study because it presents $F - t_{irr}$ curves without a maximum in β-CD and HP-β-CD solutions. Linuron, which displays a methoxyurea structural feature, presents a much higher K_1 value than the two other herbicides. This behavior might indicate that a
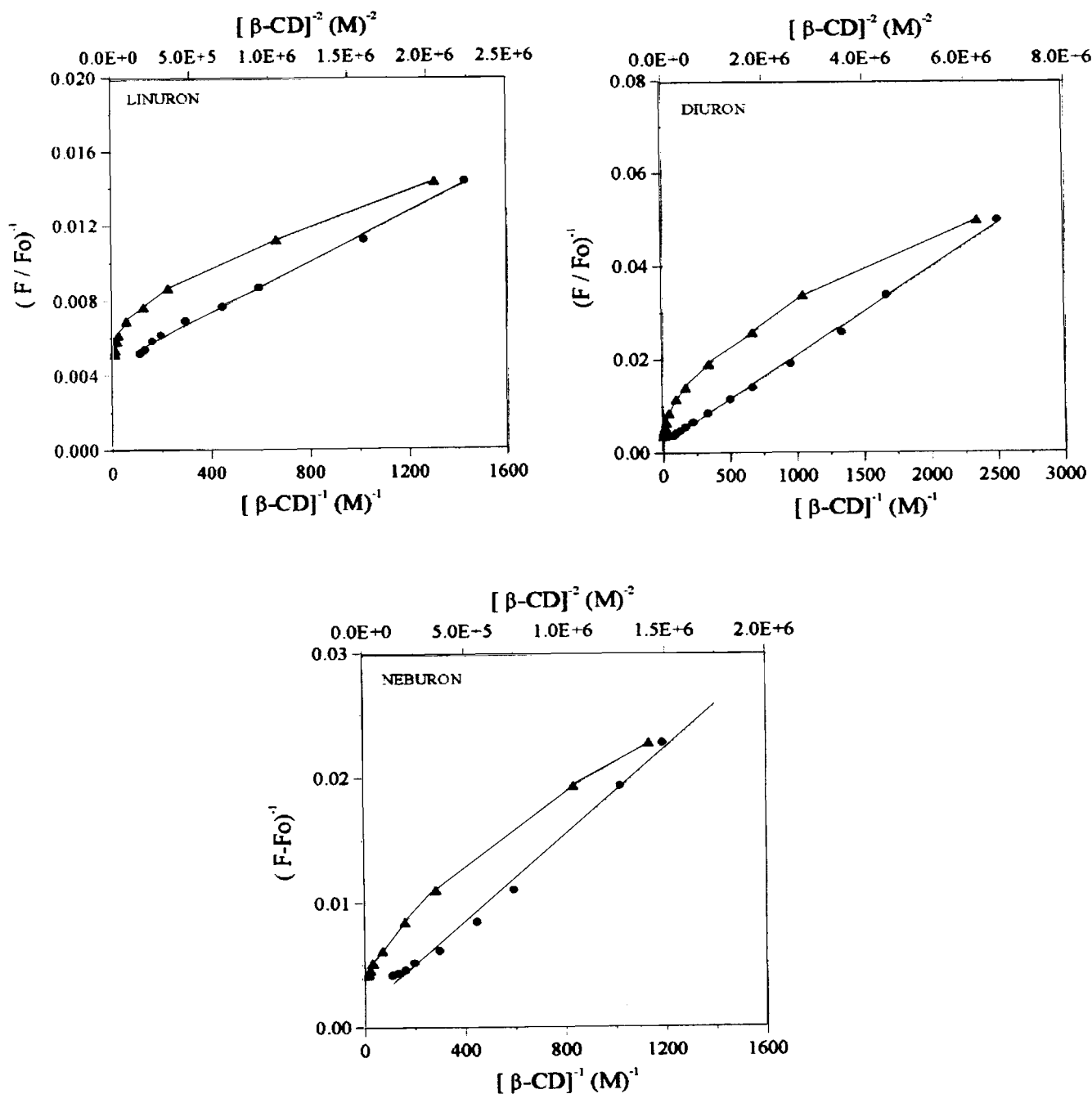
Figure 3. Double-reciprocal plots for the complexation of the different herbicides and β-CD, upon irradiation in aqueous solution. A linear relationship is obtained when the data are plotted assuming a 1 : 1 β-CD: phenylurea photoproduct stoichiometry (●) and a downward concave curvature when the data are plotted assuming a 2 : 1 β-CD: phenylurea photoproduct stoichiometry (▲). Diuron and linuron concentration 4×10^{-4} M. Neburon concentration 1×10^{-4} M.

photodechlorination (related to the presence of a N-methoxy group in the side chain) occurs during photolysis of this particular compound and/or that complexation is increased by interactions between the N-methoxy group and the cyclodextrin hydroxy substituents. As a result, encapsulation in the CD cavity would be stronger in the case of linuron than in that of diuron and neburon, as reflected by the differences in the formation constant values. Also, significantly higher association constant values are obtained with HP-β-CD than with β-CD for all compounds, which suggests that HP-β-CD possesses a better complexing ability than β-CD.

A similar behaviour has been reported in the case of aromatic pesticide complexes with cyclodextrins [14]. It is interesting to mention that we found also slightly longer irradiation times with HP-β-CD than with β-CD, showing that, when the stability of the inclusion complex is larger, longer irradiation times are required to photolyse the herbicides, because of an improved CD protection.

Concerning the geometry of the inclusion complexes, it can be expected that the phenylurea herbicides would be included in the β-CD (or HP-β-CD) cavity by the aromatic site. Indeed, in this hypothesis, the fluorophore aromatic

Table 4. Analytical figures of merit for the PIF determination of phenylurea herbicides in buffered cyclodextrin solutions

Compound : CD	Concentration range (μg/mL)	Slope	$r^{2\,a}$	LOD[b] ng/mL	LOD[c] ng/mL	Analytical sensitivity[d] ng/mL	LOQ[e] μg/mL
Diuron : HP-β-CD[f]	2.3–11.6	8.59	0.991	770	690	340	2.30
Linuron : β-CD[f]	2.5–8.7	9.72	0.991	660	580	270	1.93
Neburon : β-CD[g]	2.7–11.0	7.01	0.991	800	700	340	2.35

[a] Determination coefficient.
[b] Limit of detection according to the criterion of Clayton [50].
[c] IUPAC Limit of Detection [51].
[d] According to [49].
[e] Limit of quantification according to IUPAC [52, 53].
[f] In a 20% (v/v) pH 5.0 buffer.
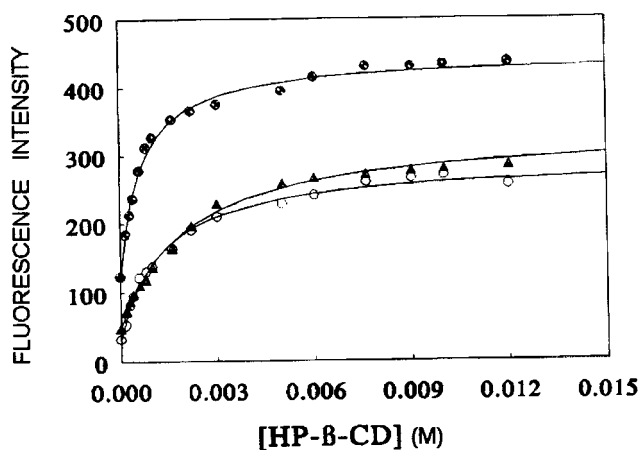[g] In a 20% (v/v) pH 7.0 buffer.

Figure 4. Application of the nonlinear regression (NLR) method to determine the variation of PIF intensity of linuron (●), diuron (▲) and neburon (○) with HP-β-CD concentration. The solid line was calculated by means of Equation (3), assuming a 1 : 1 stoichiometry and using the values of F_∞ and K_1 obtained by the NLR method. The markers are experimentally determined points.

moiety of the herbicide would directly interact with the host molecule, which is supported by the enhancement of PIF intensity observed with increasing β-CD (or HP-β-CD) concentrations, for all phenylurea herbicides under study.

pH effects

Phenylurea herbicides are known to be hydrolyzed in strongly acidic and basic media [46–48] and, as a consequence, no fluorescence signal was recorded after UV irradiation in those media. With a view to analytical applications to real environmental samples for which the pH must be fixed, the effect of pH on the analytical parameters of the herbicides under study was investigated in CD solutions. Buffer solutions with pH values of 5.0 and 7.0, close to the initial pH of the CD solutions (pH around 6) were selected. The PIF intensities of phenylurea herbicides were measured in various buffered CD media at the optimal excitation and

emission wavelengths. Our results are summarized in Table 3.

In order to choose the optimal media for establishing the calibration curves, the effect of pH on the PIF intensity (F) and t_{irr}^{opt} values in CD media was compared. In the case of diuron, the buffered CD solution providing the highest F value, i.e. pH 5 HP-β-CD medium was selected, although a slightly longer t_{irr}^{opt} value (12 min) was needed relative to a pH 7 HP-β-CD solution (10 min). For linuron, the pH 5 β-CD solution was chosen because larger F and smaller t_{irr}^{opt} values were observed in this solution than in the buffered HP-β-CD media. Finally, in the case of neburon, the highest F values were obtained in pH 5 β-CD and pH 7 HP-β-CD solutions; pH 7 HP-β-CD, which provided a smaller t_{irr}^{opt} value than pH 5 β-CD was selected. As previously, isoproturon was excluded from the study because no maximum (or plateau value) of PIF intensity could be reached after a 65-min irradiation time.

Analytical figures of merit

To determine the analytical usefulness of our method, the analytical figures of merit were evaluated under the optimal conditions (Table 4). The calibration curves were obtained by plotting the fluorescence intensity *versus* the herbicide concentration. PIF measurements were carried out in triplicate. Linear plots were established over concentration ranges of about one order of magnitude between concentrations of about 1 and 12 μg/mL. Analysis of the data by the least-squares method indicated a satisfactory precision of the linear plots, with determination coefficients (r^2) close to unity. The test of linearity on the line [49] for diuron, linuron and neburon gave, respectively, 98.2%, 97.7% and 98.0%. Moreover, the limits of detection (LOD) were calculated according to the criterion of Clayton [50] and using the IUPAC definition [51]. IUPAC LOD values obtained in buffered CD media ranged from 580 to 700 ng/mL, according to the compound. They were slightly larger than those found in micellar aqueous media [36]. The IUPAC limits of quantification (LOQ) [52,53] were between 1.93 and 2.75 ng/mL,

Table 5. Determination of phenylurea herbicides in spiked Guadiana river water samples

Compound	Concentration (μg/mL)		Recovery[b]
	added	found (E $\pm$ s)[a]	(%)
Diuron	0.30	0.31 $\pm$ 0.02	102.2
	0.60	0.60 $\pm$ 0.01	100.0
	0.88	0.91 $\pm$ 0.05	103.0
Linuron	0.30	0.28 $\pm$ 0.01	94.4
	0.60	0.57 $\pm$ 0.01	95.0
	0.80	0.77 $\pm$ 0.02	95.8
Neburon	0.30	0.24 $\pm$ 0.02	81.1
	0.60	0.52 $\pm$ 0.01	86.1
	0.88	0.75 $\pm$ 0.01	85.2

[a] E = mean concentration (average of three replicates); s = standard deviation.
[b] Corresponding average recovery value of three replicates.

depending on the compound. The relative standard deviation (RDS) values, calculated for concentrations of 3.50 μg/mL of diuron, 3.73 μg/mL of linuron and 4.13 μg/mL of neburon were, respectively, 6.7%, 4.8% and 5.1%. The Alamin application software was used for the statistical treatment of all our analytical data [54].

Analytical applications to river water samples

In order to examine the analytical applicability of the proposed PIF cyclodextrin-enhanced method to authentic samples, recovery studies were carried out on spiked river water samples, using the direct measurement procedure. Although the commission of the European Community defines the maximum amount of herbicide allowed in drinking water, no allowed maximum concentration is reported for other surface waters. Therefore, river water samples fortified from 0.3 to 0.9 μg/mL were examined.

The recoveries obtained for diuron, linuron and neburon in spiked Guadiana river water samples, using the solid-phase extraction procedure, are presented in Table 5. The recovery values ranging from about 81% to 103% can be considered as satisfactory. These recoveries are comparable to those reported in the literature [36].

Conclusion

We have demonstrated in this work that phenylurea herbicides form relatively stable inclusion complexes with β-CD and HP-β-CD. When submitted to UV irradiation for a few minutes, the phenylurea herbicides are photolysed and strongly fluorescent inclusion complexes are formed between CD and phenylurea photoproducts. These inclusion complexes present a 1:1 stoichiometry, and the association constant values indicate that the complexing ability of HP-β-CD is significantly larger than that of β-CD for all herbicides under study. Our investigation has led also to the development of a simple, reliable and relatively sensitive CD-induced photochemical-fluorimetric method, which

could be useful for the determination of several phenylurea herbicides.

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