Talanta 116 (2013) 569–574

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Determination of phenylurea pesticides by direct laser photo-induced fluorescence

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ARTICLE INFO

Article history:
Received 11 April 2013
Received in revised form
3 July 2013
Accepted 5 July 2013
Available online 11 July 2013

Keywords:
Photo-induced fluorescence (PIF)
Pesticides
Photoproducts
UV
Laser

ABSTRACT

A direct Laser Photo-Induced Fluorescence (DL-PIF) method is developed for the determination of two phenylurea pesticides, namely fenuron and diflubenzuron. The DL-PIF method uses a tunable Nd:YAG-OPO Laser to obtain the photoproduct(s) and to simultaneously analyse their fluorescence in a short acquisition time on an intensified CCD camera. Compared to classical PIF methods, the use of a tunable laser improves the selectivity (by choosing the suitable excitation wavelength), increases the sensitivity (due to the high energy of the beam) and also reduces the time of analysis. The analytical performances of this method for the determination of both pesticides are satisfactory in comparison to other classical PIF methods published for the determination of phenylurea pesticides. The calibration curves were linear over one order of magnitude and the limits of detection were in the ng mL^{-1} range. Satisfactory recoveries were obtained in the analysis of both pesticides in river and sea water spiked samples.

1. Introduction

Pesticides are widely used in agriculture to improve productivity and, consequently, they can produce residues in crops, soils and surface water. Their persistence is an important matter of concern due to their toxicity and possible carcinogenicity. The presence of pesticides could affect ground water or disrupt water treatment plants. Consequently, a European Union Directive (98/83/EC, 1998) advice for surface waters a maximum value of 5 μg L^{-1} for the total concentration of all pesticides and 1 μg L^{-1} for the maximum concentration of an individual pesticide.

Substituted ureas are an important group of pesticides that are used as herbicides (phenylureas and sulfonylureas) and insecticides (benzoylureas). In this work, we investigated fenuron, i.e., a phenylurea pesticide that acts as a photosynthesis inhibitor herbicide, and diflubenzuron, i.e., a benzoylurea that acts as a chitin inhibitor insecticide (Table 1).

Both pesticides under study are naturally non-fluorescent, but they can be photolysed into rapidly-formed strongly fluorescent photoproducts upon UV irradiation. This Photo-Induced Fluorescence (PIF) property can be used to determine these compounds by fluorescence detection. This corresponds to the classical PIF methods and it has been widely applied for the determination of pesticides and drugs [1,2]. As an alternative to classical PIF, we have developed a different method based on Direct Laser Photo-Induced Fluorescence (DL-PIF) [3], which is for the first time applied for the determination of phenylurea pesticides. We have used a tunable Nd:YAG-OPO Laser [4] to obtain the photoproducts and to simultaneously analyse their fluorescence in a short acquisition time on an intensified CCD camera. Here, we determine the fluorescence characteristics and the kinetic formation of the photoproducts obtained by DL-PIF for both pesticides, we expose the analytical performances obtained and we conduct an interference study.

2. Material and methods

2.1. Absorption and fluorescence spectrophotometer

UV–visible absorption spectra are recorded on an Eclipse UV–visible spectrophotometer (Varian). Excitation and emission fluorescence spectra are obtained on a Cary Eclipse Fluorescence spectrophotometer (Varian) with an arc-xenon lamp pulsed at 80 Hz as excitation source.

2.2. Laser system and detection device

For laser induced fluorescence measurements, the light source is a Powerlite Precision 9010 (Continuum, Santa Clara, USA) pulsed Nd:YAG pump laser beam at a 10 Hz repetition rate, with a Sunlite

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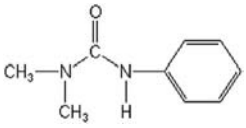
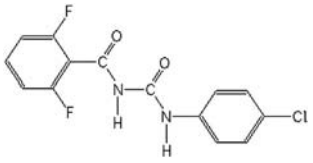
http://dx.doi.org/10.1016/j.talanta.2013.07.014

Table 1
Chemical properties of fenuron and diflubenzuron.

Fenuron	Formula	$C_9H_{12}N_2O$	
	Molecular weight	16,420 g/mol	
	Water solubility (20 °C)	3850 mg/L	
Diflubenzuron	Formula	$C_{14}H_9ClF_2N_2O_2$	
	Molecular weight	31,068 g/mol	
	Water solubility (20 °)C	0.08 mg/L	

EX OPO and FX-1 UV frequency extension system from Continuum, which permits continuous wavelength scanning from 225 to 1750 nm (LYOPO programme). The available energy in the UV domain ranges from 2 mJ at 225 nm to 10 mJ per pulse at 275 nm. It can be lowered by positioning a divergent lens in the optical path [5,14]. The detection device includes a spectrometer and an intensified CCD camera. The fluorescence was collected at a 90° angle from the excitation beam and focussed with a f/8 cm lens. The SpectraPro-550i spectrometer (Acton Research Corporation, Acton, MA, USA) has a 550 mm focal length and is equipped with a triple grating turret. The ICCD-MAX intensified CCD Camera (Princeton instruments, Trenton, NJ, USA) has a 512×512 pixel array optimised for the UV–visible domain. A 0.2 nm pixel resolution is reached with the 150 g mm^{-1} grating. The camera is operated with a ST-133 controller (RS Princeton Instruments, Trenton, NJ, USA) for data acquisition. Timing control is achieved with a DG 535 digital delay/pulse generator (Stanford Research System Inc., Sunnywale, CA, USA). The WINSPEC 32-bit Windows software package (Roper Scientific Inc., Trenton, NJ, USA) provides acquisition, display and processing functions. Fluorescence cells (10 mm light path), quartz Suprasil, are from Hellma.

2.3. Reagents

Fenuron and diflubenzuron were purchased from Sigma-Aldrich. Ethanol and methanol were obtained from Sigma. All the reagents were of analytical reagent grade. Ultrapure water (Millipore Mro-MQ System) was used for the experimental work.

Stock standard solutions of the pesticides (10^{-3} M) were prepared by dissolving the compounds in methanol. An ultrasonic bath is used to o obtain complete dissolution; then, solutions are stored in the dark. Serial dilutions were performed to obtain the working standard solutions. The working solutions were prepared either in ultrapure water (for fenuron) or in a 50/50 (v/v) methanol–water mixture (for diflubenzuron).

3. Results and discussion

3.1. Photo-induced fluorescence properties

Fenuron exhibited no native fluorescence, while the laser irradiation yielded the formation of strongly fluorescent photoproducts. The UV absorption spectrum of fenuron showed a main band in pure aqueous solution at 240 nm. Consequently, the laser beam was initially set at 240 nm so that the formation of the photoproducts could be maximised.

Fig. 1 shows the excitation–emission fluorescence matrices of a diluted aqueous solution of fenuron (EEFM) obtained, after 20 min (panel A) and 40 min (panel B) of laser irradiation at 240 nm. As can be seen, three photoproducts are formed. Their maximum excitation and emission wavelengths are shown in Table 2. Fig. 2 shows the evolution of the photoproducts fluorescence intensity with the irradiation time. PIF1 was quickly formed and showed a fluorescence intensity which remained practically constant over 40 min. The fluorescence intensity of PIF2 increased slowly with time and was found to be higher than that of PIF1 after 20 min of UV irradiation. Finally, the third photoproduct, PIF3 was also formed slowly, and showed the highest fluorescence intensity of all photoproducts after about 40 min of irradiation.

Some interpretation of the three PIF compounds spectra from fenuron has been conducted based on their fluorescent characteristics and on bibliographical data. The main photodegradation processes of fenuron correspond to the loss and the oxidation of its alkyl-chains and, as a secondary process, the hydroxylation of the aromatic ring [6]. Based on its fluorescent characteristics, PIF1 could be attributed either to benzene ($\lambda_{ex}=253$ and $\lambda_{em}=280$ nm, pure compound), or to phenol ($\lambda_{ex}=220$ and $\lambda_{em}=302$ nm, pure compound). PIF2 corresponded to aniline ($\lambda_{ex}=231$ and $\lambda_{em}=343$ nm, pure compound), and was confirmed after the analysis of an irradiated sample of fenuron by HPLC chromatography (C18 reverse phase, 30 °C column oven, methanol/water 60/40 v/v, 1.0 mL min^{-1}) followed by fluorescence detection. The analysis of the irradiated sample by GC–MS also indicated the presence of aniline. The photodecomposition of fenuron into aniline has been already demonstrated by Mazzochi and Rao [7]. Unfortunately, PIF3 could not be identified.

Similar to fenuron, diflubenzuron exhibited no native fluorescence, while laser irradiation yielded the formation of two fluorescent photoproducts. First, the UV absorption spectrum of diflubenzuron was measured. Since diflubenzuron did not absorb in water, all the experiments were conducted in a 50/50 (v/v) methanol–water mixture where it presented a strong absorbance band with a maximum at 260 nm. Consequently, the laser beam was initially set at 260 nm in order to maximise the formation of photoproducts. Fig. 1 shows the excitation–emission fluorescence matrix of the two photoproducts (PIF1 and PIF2) obtained, respectively, after irradiation times of 1 min and 7 min at 260 nm. The maximum excitation wavelengths for both photoproducts, PIF1 and PIF2, were respectively of 230 nm and 220 nm (Table 2).

The kinetic formation of the diflubenzuron photoproducts was studied by monitoring the evolution of the fluorescence of the photoproducts with irradiation time at an excitation wavelength of 240 nm and an emission wavelength of 342 nm (for PIF1) and 422 nm (for PIF2). Fig. 3 shows that the first photoproduct, PIF1, rapidly appeared and decreased after an 1-min irradiation time, while the second one, PIF2, continuously increased, becoming preponderant after an irradiation time of 6 min, and then stabilized.

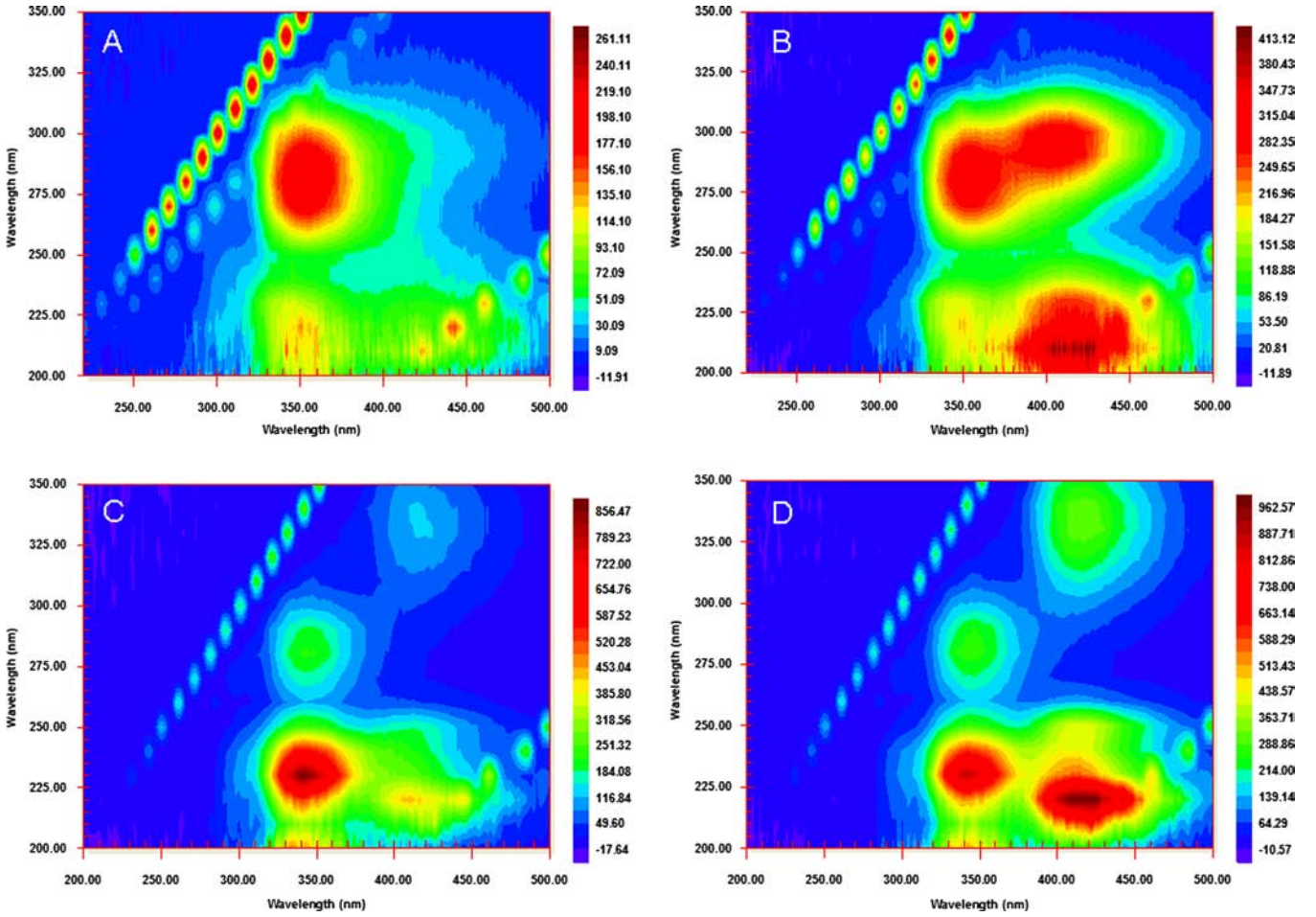


Fig. 1. Evolution of the excitation/emission fluorescent matrix with laser irradiation at 240 nm: for fenuron after 20 min (A) and 40 min (B) of irradiation and for diflubenzuron after 1 min (C) and 7 min (D) of irradiation.

Table 2
UV absorption of fenuron and diflubenzuron and fluorescence properties of their photoproducts.

Pesticide		Absorbance	Fluorescence	
		λ_{max} (nm)	λ_{ex} (nm)	λ_{em} (nm)
Fenuron	Pure water	240		
	PIF 1		**225**	308
	PIF 2		230/**280**	342
	PIF 3		230/**295**	420
Diflubenzuron	50:50 v-v methanol–water	260		
	PIF 1		**230**/285	342
	PIF 2		**220**/340	422

Bold correspond to the main wavelength

This evolution can also be seen on the excitation/emission fluorescent matrix recorded after 1 min (Fig. 1C) and 7 min (Fig. 1D) of irradiation.

Some interpretation of the two PIF compounds spectra from diflubenzuron has also been conducted. As for fenuron, PIF1 has the same fluorescent characteristics as aniline, and therefore could correspond either to aniline or to an aniline derivative such as 4-chloroaniline (which has the same excitation and emission wavelengths as aniline). 4-chloroaniline and N-methyl-4-chloroaniline have been found to be some of the main photoproducts arising from the degradation of diflubenzuron [8]. Unfortunately, PIF2 could not be identified.

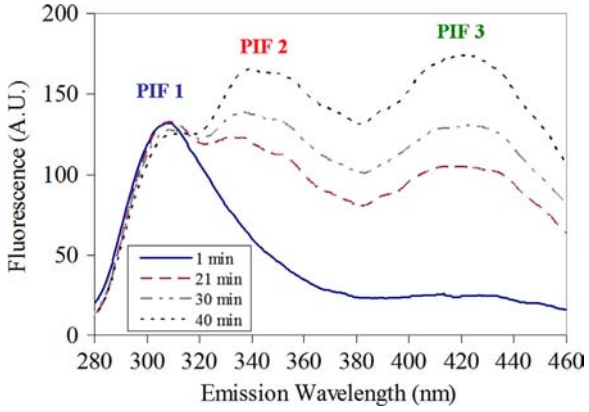


Fig. 2. Evolution of the photoproducts DL-PIF emission spectra of a fenuron aqueous solution (initial concentration = 2.5 μg mL^{-1}) vs. the irradiation time. Laser beam: 240 nm, 1 mJ, 10 Hz.

3.2. Direct laser photo-induced fluorescence analysis

Based on the kinetic formation of the photoproducts, an analytical application has been undertaken to determine the fenuron concentration in water by measuring the fluorescence of the photoproducts with DL-PIF spectrometry. Although PIF3 showed the highest fluorescence values in the kinetic studies, we decided to measure the fluorescence of PIF1 in order to minimise the time of analysis. However, since the DL-PIF method used only one laser, the

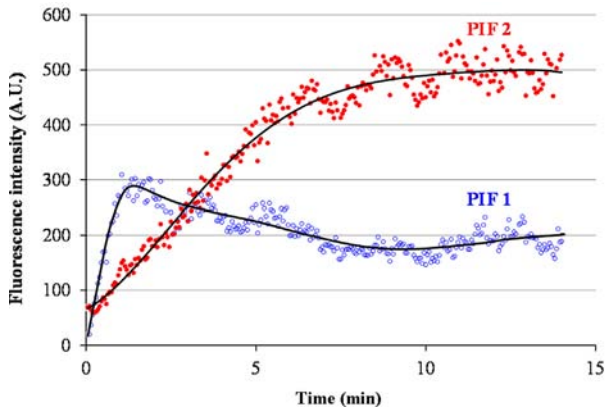


Fig. 3. Evolution of the photoproduct(s) DL-PIF1 (λ_{em}=342 nm) and PIF2 (λ_{em}=420 nm) of a diflubenzuron aqueous solution (initial concentration=10 μg mL^{-1}) vs. the irradiation time. Laser beam: 240 nm, 1 mJ, 10 Hz.

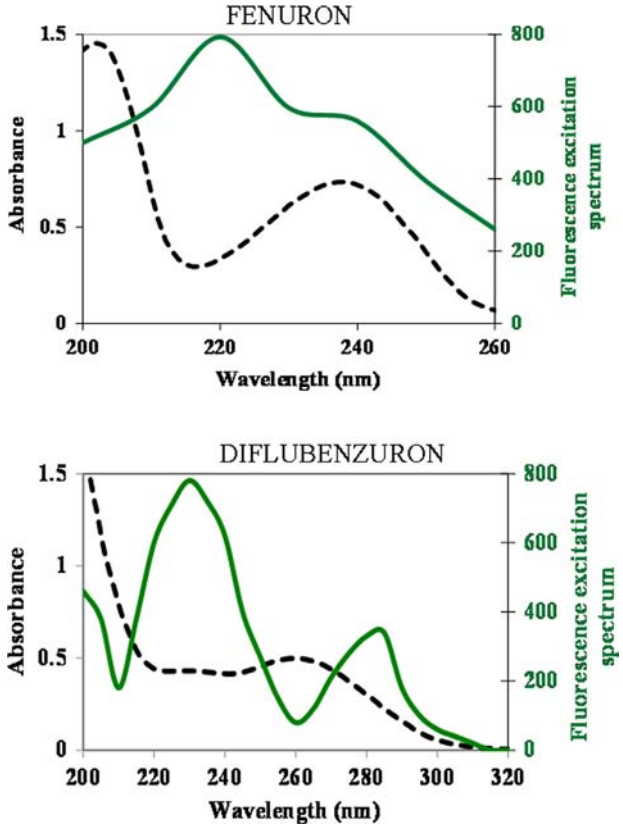


Fig. 4. Comparison of the absorbance spectrum of the pesticide (left *y*-axis) and the excitation–emission spectrum (right *y*-axis) of its first photo-induced compound (PIF1) for fenuron (upper panel) and diflubenzuron (lower panel).

same wavelength had to be set to form the photoproducts, as well as to excite the formed photoproducts, in order to measure their fluorescence in the same time. Consequently, a compromise between both wavelengths had to be found. For this purpose, we have drawn together in Fig. 4 (upper panel) the absorbance spectrum of fenuron (left *y*-axis) and the excitation fluorescence spectrum of the PIF1 compound (right *y*-axis). For fenuron, it can be seen that the best excitation wavelength to maximise the PIF1 fluorescence is 220 nm. However, at this wavelength the fenuron absorption is much lower and consequently the PIF1 formation is not favored. On the other hand, at 240 nm the fenuron absorption is maximum, which induced the maximal PIF1 formation. Since at 240 nm the excitation wavelength of PIF1 was still high, the 240-nm value appeared to be the best compromise to conduct analytical applications, even if at this wavelength the fluorescence of PIF1 is not at its maximum.

Since the fluorescence of fenuron PIF1 was already maximal after one minute of irradiation, the water samples were directly irradiated in the cell for one minute at 240 nm and the PIF1 fluorescence signal at 308 nm was simultaneously acquired and integrated by an ICCD camera. Linear calibration curves were established by plotting the PIF1 intensity vs. the fenuron concentration. The fluorescence intensity of PIF1 was measured in triplicate for each concentration. The linear dynamic range was spread over about one order of magnitude. The limits of detection (1.5 ng mL^{-1}) and quantification (5 ng mL^{-1}) were relatively low (Table 3).

Diflubenzuron was determined by measuring the PIF 1 fluorescence in order to minimise the time of analysis. PIF1 showed much lower fluorescence when it was excited at 260 nm, as can be seen in Fig. 1, and therefore, an optimisation of the excitation wavelength was also necessary in this case. So we decided to consider a new wavelength that would be not too far from the maximum of absorption of diflubenzuron, but at the same time as close as possible to the PIF1 maximum excitation wavelength (Fig. 4, lower panel). To maximise the fluorescence emission the best wavelength appears to be 230 nm, but at this wavelength the energy delivered by the laser beam decreases significantly in comparison to 240 nm. Consequently, the best compromise is 240 nm; at this wavelength the laser energy is still high, and even if the absorbance of diflubenzuron is a little bit lower, the fluorescence of PIF1 is much higher.

Since the fluorescence of the first photoproduct, PIF1, decreased after 1 min of irradiation, the samples, prepared in a 50/50 (v/v) methanol–water mixture, were directly irradiated for one minute at 240 nm, and the PIF1 fluorescence signal at 342 nm was

Table 3
Analytical performance of DL-PIF method for the determination of fenuron and diflubenzuron.

	Fenuron	Diflubenzuron
Solvent	Water	50/50 (v/v) methanol–water
$\lambda_{ex}/\lambda_{em}$ (nm)	240/308	240/342
Linear range (μg mL^{-1})	0.02–10	0.08–8
R^2 (%)	99.52	99.91
b	85.2	54.5
s_b	2.4	1.1
s_{res}	2.2	2.6
Detection limit (μg mL^{-1})[a]	0.0015	0.0048
Quantification limit (μg mL^{-1})[b]	0.005	0.016
RSD (%) (for 1 μg mL^{-1})[c]	2.2	3.6
Standard addition calibration curve in river water		
	Fenuron	Diflubenzuron
b	90.3	54.8
s_b	1.3	1.52
t_{cal}	1.87	0.13
p-value (%)	8.4	89.9
Standard addition calibration curve in sea water		
	Fenuron	Diflubenzuron
b	91.2	58.1
s_b	2.7	1.53
t_{cal}	1.6	1.88
p-value	12.3	8.2

b, slope; s_b, standard deviation of the slope; R^2, determination coefficient; s_{res}, regression standard deviation.

[a] Calculated as the concentration of analyte giving a signal-to-noise (S/N) ratio of 3.

[b] Calculated as the concentration of analyte giving a signal-to-noise (S/N) ratio of 10.

[c] Relative standard deviation (n=10).

simultaneously acquired and integrated by an ICCD camera. The fluorescence of PIF1 was measured in triplicate for each concentration. Table 3 shows that the calibration curves were linear over one order of magnitude. The limits of detection (4.8 ng mL^{-1}) and quantification (16 ng mL^{-1}) were satisfactory, although higher than those obtained for fenuron.

For both pesticides, the detection limits are better than those obtained by classical PIF methods applied to phenylurea pesticides in direct analysis without preconcentration step (i.e., about 650 ng mL^{-1} for diuron, linuron and neburon [9]) and even better than the detection limits obtained with the use of miscellar medium to enhance fluorescence (i.e., about 600 ng mL^{-1} for diuron, linuron and neburon [9] or 450 ng mL^{-1} for monolinuron, diuron, linuron and neburon [10]).

3.3. Interference study of other pesticides

Since the PIF compounds of fenuron and diflubenzuron are very similar, we have studied whether fenuron interferes with diflubenzuron and, inversely, whether diflubenzuron interferes with fenuron. In addition, we have studied the interferences potentially induced by the presence of other pesticides. We have determined a tolerance limit for the interfering pesticides which is defined as the concentration of the interfering for which the percentage of variation of the PIF signal does not exceed $\pm$5% (Table 4).

First, we have determined fenuron and diflubenzuron in water samples that contain simultaneously both pesticides. For a solution of 1 μg mL^{-1} of fenuron, we have added increasing concentrations of diflubenzuron (up to 2 μg mL^{-1}). Even if the PIF compounds of diflubenzuron have almost the same fluorescent behaviour as the ones from fenuron, the addition of diflubenzuron does not affect the PIF spectra of fenuron (see Table 4). This is because the photo-products of diflubenzuron are only formed in a water/methanol mixture but not in pure water. We have also evaluated the effect of increasing concentrations of fenuron (up to 2 μg mL^{-1}) in the determination of a solution of 1 μg mL^{-1} of diflubenzuron. Table 4 shows that fenuron produces a high interference effect, since a concentration as low as 0.04 μg mL^{-1} increases the PIF signal of diflubenzuron above the tolerance limit. To overcome this interference, we have corrected the PIF signal obtained in the water/methanol mixture by the fluorescence of fenuron obtained from the PIF measurements in pure water. Moreover, a correction factor was applied to take into account that the fluorescent quantum yield of the photo-products of fenuron is higher in water/methanol mixtures than in pure water. After this correction, the determination of diflubenzuron was not affected by the presence of fenuron.

Secondly, for the interference with other pesticides, we have chosen some commonly used in Senegal. These pesticides (i.e., carboxin, linuron, propanyl and 2,4-D) exhibit no native fluorescence but, as for fenuron and diflubenzuron, they can be also photolysed in fluorescent photoproducts. We have evaluated the effect of increasing concentrations of carboxin, linuron, 2,4-D and propanyl (i.e., from 0 to 2 μg mL^{-1}) on the fluorescence of a solution of 1 μg mL^{-1} of either fenuron or diflubenzuron. For fenuron, the PIF spectra behaviour is almost not affected by the presence of the pesticides under study. Only the addition of linuron (a phenylurea pesticide) at concentrations higher than 2 μg mL^{-1} increases the PIF signal above the tolerance limit. However, for diflubenzuron the PIF spectra is affected by all the pesticides under study since all of them have a similar structure and similar fluorescent photo-products (aniline derivatives) with high fluorescence quantum yields in water/methanol mixtures. A concentration of linuron of 0.2 μg mL^{-1} interfere in the determination of diflubenzuron. The other pesticides have higher tolerance limits but they increase also the PIF signal of diflubenzuron since their photo-products have a very similar behaviour.

Other interferences could come from other pesticides or other pollutants with a native fluorescence corresponding to the excitation and emission wavelengths of the PIF photoproducts. However, these species does not interfere with the PIF signal since the background fluorescence can be measured before irradiating the water samples and subtracted from the fluorescence of the PIF photoproducts.

Table 4
Effect of other pesticides in the PIF signal of fenuron and diflubenzuron.

Pesticide	Type	Tolerance limit (μg mL^{-1})	
		Fenuron	Diflubenzuron
Fenuron	Phenylurea	–	0.04 (ndi after correction)
Diflubenzuron	Phenylurea	ndi	–
Linuron	Phenylurea	2	0.2
Carboxine	Phenylamide	ndi	0.8
Propanyl	Phenylamide	ndi	0.6
2,4-D	Chlorophenoxyacid	ndi	1.4

Tested concentration range of the pesticides: 0–2 μg mL^{-1}.
ndi: no detected interference.
Tolerance limit: concentration of the interfering for which the percentage of variation of the PIF signal (of a solution of 1 μg mL^{-1} of either fenuron or diflubenzuron) does not exceed $\pm$5%.

3.4. Analytical applications

In order to evaluate the usefulness of the DL-PIF method, recovery experiments were performed on river water (Sénégal river valley, Sénégal) and sea water (Atlantic ocean, Dakar seaside, Sénégal). The Senegal River irrigates one of the most important agricultural areas of Senegal and can be submitted to some pesticide pollution. Moreover, the Senegal River is also used to produce drinking water for Saint Louis, the regional capital. The water samples were collected in 0.5-L amber glass bottles samples and filtered through a filter disk (45 μm) in order to eliminate the organic suspended matter. The water samples under study were found to be free of any fluorescent species. The filtered water samples were initially fortified at 0.2 μg mL^{-1}. Then, several aliquots of 2.5 mL of this spiked water samples were introduced in 5 mL flasks, and increasing concentrations of either fenuron or diflubenzuron (i.e., from 0.08 μg mL^{-1} to 7 μg mL^{-1}) were added and the flasks were completed to the mark. The percentage of the water samples utilised for the analysis was of 50% (v/v).

For fenuron, the recovery values were estimated between 0.08 to 6 μg mL^{-1}. In all cases, we obtained satisfactory values, ranging from 94% to 106% for river water and 88 to 113% for sea water (Table 5). The mean recoveries obtained for river water (101%) and for sea water (103%) were very close to 100%. A *t*-test was carried out in order to check if these recoveries were significantly different from 100%. The *t* statistic was calculated as: $t=(R_m-100)/(s(R)/n)$ (where R_m is the mean recovery, $s(R)$ the recovery standard deviation and n the number of analyses). In both cases, the mean recoveries were not significantly different from 100% (at a probability $\alpha=0.05$).

To check the absence of matrix effects, the standard addition method was carried out with water samples initially fortified at 200 ng mL^{-1}. The standard addition plots obtained were linear in river water and in sea water and the slope values of the calibration and standard addition curves were practically identical (see Table 3). A *t*-test was also carried out in order to compare the slope values obtained for the calibration and the standard addition curves. The difference between the slopes was found to be not significant (i.e., for all the cases the *p*-value was higher than 5%) This confirmed the absence of any significant matrix effect in river and sea water samples.

Table 5
Recovery values obtained in spiked river water and sea water samples for the determination of fenuron and diflubenzuron.

Added ($\mu g\ mL^{-1}$)	Found ($n=3$) ($\mu g\ mL^{-1}$)	Recovery, R (%)	Mean recovery, R_m (%)	Recovery standard deviation, $s(R)$ (%)
River water				
Fenuron				
0.08	0.08	106	101	4.8
0.5	0.50	100		
1	0.94	94		
2	1.95	98		
4	4.23	104		
6	6.29	105		
Diflubenzuron				
0.1	0.11	110	97	10.9
0.5	0.53	106		
1	0.81	81		
2	1.75	88		
5	5.03	101		
7	6.71	96		
Sea water				
Fenuron				
0.08	0.09	113	103	9.1
0.5	0.48	97		
1	0.88	88		
2	2.20	110		
4	4.03	101		
6	6.44	107		
Diflubenzuron				
0.1	0.08	82	95	11.8
0.5	0.56	112		
1	0.86	86		
2	1.74	87		
5	4.96	99		
7	7.28	104		

For diflubenzuron, the recoveries were estimated between 0.1 and 7 $\mu g\ mL^{-1}$. To check the absence of matrix effects, the standard addition method was carried out in the same way as for fenuron. In the case of river water, an important difference between the slopes was obtained for the standard addition and the calibration curves, due to the presence of matrix effects. In order to eliminate these matrix effects, 20 mL of the river water samples spiked with diflubenzuron were extracted with 20 mL of chloroform. The chloroform extract was evaporated to dryness and the residue was then dissolved in 20 mL of a 50/50 (v/v) methanol–water mixture. After the extraction, the difference between the slopes of the standard addition and the calibration curves were found to be not significant at $\alpha=0.05$. The same extraction procedure was carried out for the sea water samples spiked with diflubenzuron. In this case, this liquid–liquid extraction was necessary because the addition of methanol in sea water samples induces salt precipitation, making impossible the fluorescence measurement. After the extraction, the standard addition curves obtained for the sea water samples showed also no matrix effects. The recovery values of diflubenzuron were comprised between 81% and 106% for river water; 82% and 112% for sea water. The mean recoveries obtained for river water (97%) and for sea water (95%) were also satisfactory and not significantly different from 100% (at a probability $\alpha=0.05$).

4. Conclusion

It is worthwhile to note that the analytical performances of the DL-PIF method are better than those obtained with the classical PIF method for phenyl urea pesticides [9,10]. The calibration curves were linear over one order of magnitude and the limits of detection were in the $ng\ mL^{-1}$ range. Moreover, the recovery values obtained for the determination of both pesticides in river water and sea water were satisfactory. Therefore, the DL-PIF method can be easily applied for a fast, preliminary screening of these pesticides in environmental samples.

In another study, we have already worked on the determination of naturally-fluorescent pesticides by laser induced fluorescence [11], but compared to this work and to classical PIF methods, the DL-PIF method offers important advantages. Indeed, concerning the instrumental part, the use of a tunable laser improves the selectivity, since it is possible to choose a specific excitation wavelength in order to photochemically form and excite the PIF compound. The sensitivity is also improved thanks to a higher energy radiation (about 1 mJ at 240 nm), and also to a sensitive detection with an ICCD camera. Concerning the analytical procedure, the DL-PIF method brings important simplifications. Only one step is needed to create the PIF compound and excite it at the same time, which reduces the sample handling and contamination risk. Moreover, a flow analysis system, which is often used in the classical PIF methods [12,13] and sometimes complex to handle, is not required in the DL-PIF method.

Acknowledgements

PA Diaw thanks the French Embassy of Dakar for a Ph.D. grant in support of this work and the University of Brest for its mobility scholarship.

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