Journal of Fluorescence, Vol. 16, No. 2, March 2006 (© 2006)
DOI: 10.1007/s10895-005-0044-x

Laser–Induced Fluorescence Detection of Carbamates Traces in Water

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Received September 25, 2005; accepted November 29, 2005
Published online: March 17, 2006

The absorption and fluorescence spectra of carbaryl (CB), carbofuran (CF) and carbendazim (MBC) have been studied. Fluorescence lifetime and fluorescence quantum yields are also reported as well as the influence of pH, solvent and presence of humic acids on fluorescence. The limit of detection (LD) of the three compounds has been measured by direct analysis by laser-induced fluorescence (LIF) using a pulsed YAG laser with an Optical Parametric Oscillator (OPO) as excitation source and an Intensified Charged Coupled Device (ICCD) camera for the fluorescence detection. Instrumental LD found for CB, for MBC and for CF are respectively 4, 50 and 1000 ng L^{-1}. In tap water, the LD obtained is 800 ng L^{-1} for MBC and 20,000 ng L^{-1} for CF. For CB, the use of a time shift between excitation and emission allows to reach a LD of 20 ng L^{-1} in tap water.

KEY WORDS: Carbaryl; carbofuran; carbendazim; laser-induced fluorescence; temporal resolution.

INTRODUCTION

Pesticides are extensively used in agriculture to improve the productivity. Among them, carbamates are an important family whose general structure is $R_1OCONR_2R_3$. They have received an increasing popularity in recent years because of their wide range of biological activity. *N*-Methyl carbamates are used as insecticides, whereas benzimidazole carbamates are used as fungicides.

In the present paper, we studied three carbamates (Fig. 1): two insecticides, carbaryl (CB) and carbofuran (CF); and a fungicide carbendazim (MBC), which is also the major degradation product of benomyl.

These compounds are toxic for humans, animals and plants, and they contaminate surface waters and aquifers. Consequently, a European Union Directive (98/83/EC, 1998) has set a maximum admissible concentration of 0.5 μg L^{-1} for the sum of all pesticides and 0.1 μg L^{-1} for an individual compound in drinking waters, whereas the values for surface waters are 5 and 1 μg L^{-1}.

Environmental Protection Agency (EPA) methods exist for carbamates that employ gas chromatography (EPA method 515.1) and liquid chromatography (EPA method 531.1, 632 and 8318). Gas chromatography has some drawbacks for these compounds because of their thermal degradation and the need of pre-treatment. Liquid chromatography is often preferred with UV, fluorescence or electrochemical detection. Both chromatographic methods require extraction and concentration steps which are time- and solvent consuming.

The aim of this study was to develop methods based on laser-induced fluorescence temporally resolved for the direct determination or screening of CB, CF and MBC in raw and tap water. Fluorescence is generally very sensitive, but it is influenced by pH, solvents, quenchers, degradation, etc. Consequently, an analytical method based on fluorescence should take into account these factors.

In the first part of this paper, we present the UV-Visible absorption and excitation–emission fluorescence spectra in water at different pH and in methanol. The

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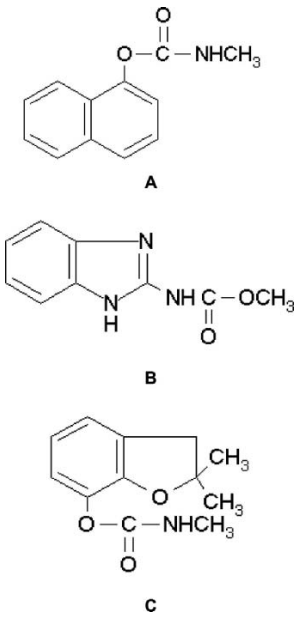

Fig. 1. Formula of (A) carbaryl (CB), (B) carbofuran (CF), and (C) carbendazim (MBC).

fluorescence lifetime and quantum yields have been measured, and the influences of the solvent on these parameters are detailed as well as the influence of humic acids in water.

The second part concerns the sensitivity and selectivity of the LIF detection method we used to determine these analytes and to achieve low detection limits.

EXPERIMENTAL

Apparatus

Absorption and Fluorescence Spectrophotometer

UV-Visible absorption spectra are recorded on a Eclipse UV-Visible spectrophotometer (Varian), excitation and emission fluorescence spectra on a Cary Eclipse Fluorescence spectrophotometer (Varian).

Laser System and Detection Device

For laser–induced fluorescence measurements the light source is a Powerlite Precision 9010 (Continuum Santa Clara, CA) pulsed Nd:YAG pump laser beam at a 10 Hz repetition rate, with a Sunlite EX OPO and FX-1 UV frequency extension system from Continuum, which allows continuous wavelength scanning from 225 to 1750 nm. The available energy in the UV domain ranged from 2 mJ at 225 nm to 10 mJ per pulse at 275 nm [1]. It can be lowered by positioning a divergent lens in the optical path. For our experiments the energy received by the sample at any wavelength was less than 800 μJ per pulse.

The detection device included a spectrometer and an intensified CCD camera located 50 cm away from the sample cuvette. The fluorescence was collected at 90° from the excitation beam and focused with a *f*/8 cm lens.

The SpectraPro-750i spectrometer (Acton Research Corporation, Acton, MA) had a 750-mm focal length and was equipped with a triple grating turret.

The ICCD-MAX intensified CCD Camera (Princeton instruments, Trenton, NJ) had a 512×512 array optimised for the UV-Visible domain. A 0.2 nm per pixel resolution is reached with the 150 g mm^{-1} grating.

The camera was operated with a ST-133 controller (RS Princeton Instruments, Trenton, NJ) for data acquisition and 16-bit digital conversion. Timing control was achieved with a DG 535 digital delay/pulse generator (Stanford Research System Inc., Sunnywale, CA). The WINSPEC 32-bit Windows software package (Roper Scientific Inc., Trenton, NJ) provided acquisition, display and processing functions.

Chemicals

CF, CB and MBC were purchased from Sigma. Spectroscopic grade methanol was from Merck (Uvasol grade). Stock solutions of carbamates were prepared in methanol and stored in dark at 3°C.

Humic acids are from Riedel de Haën, stock solutions were prepared at pH 8 and working solutions were daily prepared. Experiments were carried out under atmospheric conditions at 19°C.

RESULTS AND DISCUSSION

Spectroscopic and Photo Physical Properties

Absorption Spectra

The absorption spectra of CB, CF and MBC have been recorded at room temperature in methanol (Fig. 2) and in aqueous solution at different pH. In methanol, the three compounds have a wide absorption band between 260 and 300 nm but the shape is different. The main peak is at 280 nm for CB, 287 nm for MBC and 281 nm for CF. In pure water, the shape of the absorption spectra of CB and CF are unchanged, whereas those of MBC is slightly modified. Its maximum is slightly blue-shifted (284 nm), whereas the absorption band at 290 nm increases.

The absorption spectra for CB and for CF do not vary from pH 3 to 9. For MBC, the absorption spectrum

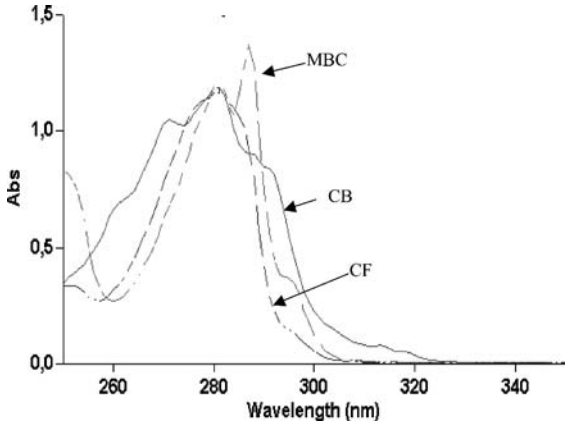

Fig. 2. Absorption spectra of carbofuran (CF), carbaryl (CB) and carbendazim (MBC) in methanol.

changes depending on the pH. Three isobestic points are observed at 277, 280 and 284 nm. The spectrum recorded at pH 7 is in good agreement with the one observed in methanol. In acidic solution at pH 3, an other spectrum is observed with two maxima at 283 and 273 nm. These spectra are in agreement with those published by Boudina *et al*. [2] for the neutral MBC and its protonated form $MBCH^+$. Our observations are consistent with the published pK_a value (4.5) [3, 4].

Fluorescence

CB, CF and both the protonated and neutral forms of MBC are fluorescent. The maxima of fluorescence are 290 and 390 nm for the protonated form of MBC and 305 for its neutral form. The maxima for CB and CF are 335 and 310 nm, respectively, in pure water.

In methanol, fluorescence maxima are unchanged for CB and MBC, whereas that of CF is blue-shifted (from 310 in water to 304 nm in methanol) (Fig. 3).

The quantum yields of fluorescence of the three neutral compounds have been determined in methanol upon excitation at 270 nm. Phenol has been taken as reference (φ^0=0.07 in water [5]). We measured its quantum yield of fluorescence in methanol and found φ_F=0.09 in that solvent using relation (1):

$$\varphi_M = \frac{I_M}{I_0} \times \frac{A_0}{A_M} \times \frac{n_M}{n_0} \times \varphi^0 \qquad (1)$$

where I_M and I_0 are the integrated fluorescence intensities in methanol and water, respectively, A_M and A_0 the respective absorbances, and n_M and n_0 the respective refractive index.

The absorption at excitation wavelength is lower 0.12 to avoid inner filter effects. For each compound, the fluorescence intensity increases with the absorption (Fig. 4), giving a straight line at low absorbance values in

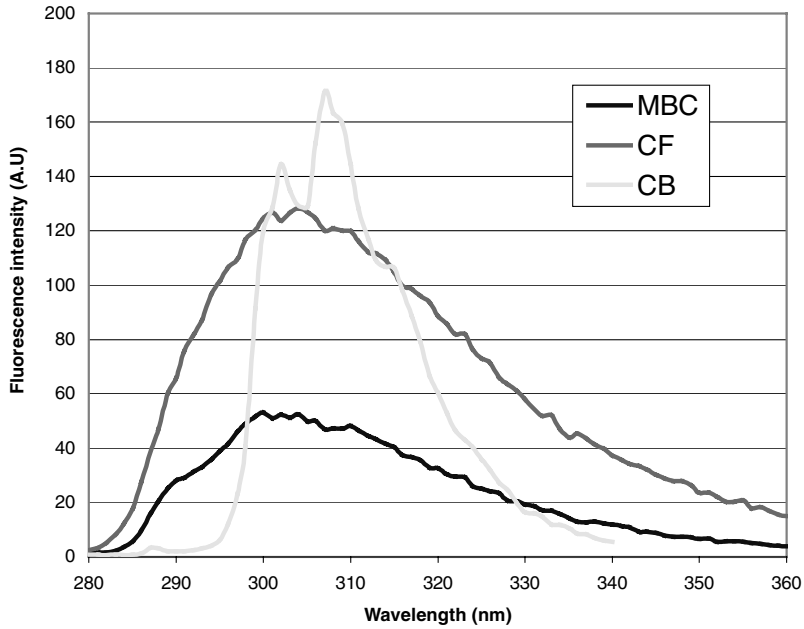

Fig. 3. Fluorescence spectra of carbofuran (CF), carbaryl (CB) and carbendazim (MBC) in methanol.

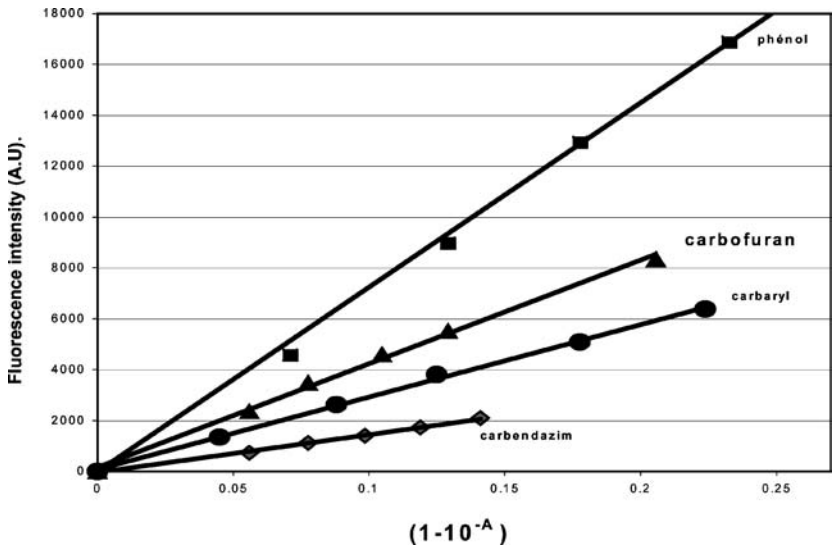

Fig. 4. Determination of fluorescence quantum yields in methanol. Intensity of fluorescence versus $(1–10^{-A})$.

agreement with relation (2):

$$I_F = \varphi_F I_{exc}(1 - 10^{-A}) \qquad (2)$$

where I_F is the integrated fluorescence, φ_F the fluorescence quantum yield, A the absorbance at 270 nm and I_{exc} the intensity of the excitation. The following results were found in methanol:

Carbaryl $\varphi_F = 0.036$

Carbaryl $\varphi_F = 0.018$

Carbaryl $\varphi_F = 0.050$

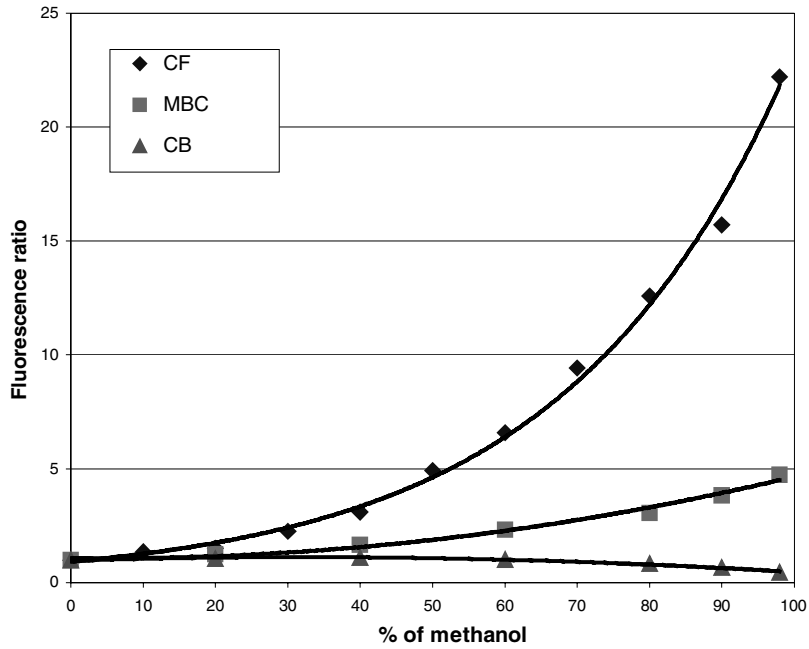

Fig. 5. Influence of the percentage of methanol in methanol–water mixtures on the fluorescence intensity IF of carbofuran (CF), carbaryl (CB) and carbendazim (MBC). Fluorescence ratio = IF (x% methanol)/IF (100% methanol).

From these measurements, CF is the most fluorescent compound in methanol. The fluorescence lifetime calculated from time-resolved fluorescence decays are 3, 5 and 25 ns, respectively, for MBC, CF and CB. These results are in good agreement with the literature data [6]. In pure water, the fluorescence quantum yields are clearly changed compared to that in methanol.

We studied the influence of the percentage of methanol in methanol–water mixtures of the compounds. Absorption and emission spectra were recorded. For the three compounds there is no neat change in the absorption and emission spectra, whereas the fluorescence intensity is drastically modified.

For CB, the quantum yield of fluorescence is 2.5 times lower in methanol than in water, whereas those of MBC is five times higher in methanol. For CF, the fluorescence strongly increases in methanol (Fig. 5). Compared to quantum yields in methanol, the following quantum yields can be estimated in water:

$$\begin{aligned} \text{CB} \quad & \varphi_F = 0.090 \\ \text{MCB} \quad & \varphi_F = 0.004 \\ \text{CF} \quad & \varphi_F < 0.002 \end{aligned}$$

MBC value is in good agreement with the Mazellier *et al*.'s result [4].

CF was studied in various organic solvent–water mixtures in order to better understand the influence of solvent properties on its fluorescence quantum yield. In acetonitrile, methanol, ethanol and propanol the same emission spectrum is observed and the fluorescence intensity is close to that measured in methanol. For each solvent, water was gradually added and the same shape of the $I = f(\%$ water) curve was observed (Fig. 6).

The evolution is reversible: the fluorescence increases on addition of solvent and decreases on addition of water, excluding the hypothesis of a degradation in water. The reason of the drastic decrease of CF fluorescence in water may be an aggregation of CF in the ground state because of its low solubility. Nevertheless, it seems improbable because of the low concentration used in this study compared to the published solubility of the compound (700 mg L^{-1}) [7]. Moreover, the absorption spectrum in unchanged in the range 0.2–2 mg L^{-1} and the Beer–Lambert law is verified. Additionally, the length of the hydrocarbon chain of alcohols (Fig. 6) has no effect on the $I = f(\%$ water) curve, whereas such an effect is expected in the case of aggregation [8,9]

We have not been able to establish any correlation between the fluorescence intensity and the Taft *et al*. [10] solvatochromic parameters α, β, π^* representing the ability of a solvent to give a proton (α), its ability to accept a proton (β) or its polarizability (π^*).

The neat effect of water on the CF fluorescence may depend on its high polarity and its small size compared to other solvents. Water may form intermolecular hydrogen bonds with CF in the excited state, in competition with CF intramolecular hydrogen bonds. These water–CF hydrogen bonds may lead to a non-radiative decay of CF excited state.

The same effect may be responsible for the decrease of MBC fluorescence quantum yield in water.

Influence of Humic Acids

Because the association of carbamates with humic acids has been reported at high concentration [11], we studied the fluorescence properties of the three carbamates in the presence of humic acids at 1 mg L^{-1} and pH 8 for carbamate solutions around 200 μg L^{-1}. No significant change of the fluorescence intensity was observed, and we can conclude that there is no significant association of carbamates with humic acids leading to a quenching of fluorescence for such concentrations.

Analytical Results

We attempted to lower the limits of detection (LD) of each carbamates in water samples. We used a laser excitation in order to excite more molecules and the fluorescence was collected through a spectrometer connected to an intensified camera (ICCD) to achieve a better sensitivity.

The pulsed YAG laser was connected to an OPO which allows one to choose the best excitation wavelength regarding both the selectivity and the sensitivity of analysis. It had a pulse width of 4 ns and the camera allowed to measure the fluorescence decay. The choice of a delay between excitation and fluorescence acquisition is a way to eliminate the fluorescence of contaminants with short lifetime and, if necessary, the Raman diffusion. It can consequently improve the analysis selectivity.

The LD have been computed following the IUPAC definition.

Carbaryl Analysis

CB was analysed in permuted and tap water as well as in the presence of 1 mg L^{-1} of humic acids. It was excited at 277 nm. The energy of the excitation beam was optimised in order to get the best fluorescence intensity and a photo-degradation of the compound lower than 10%

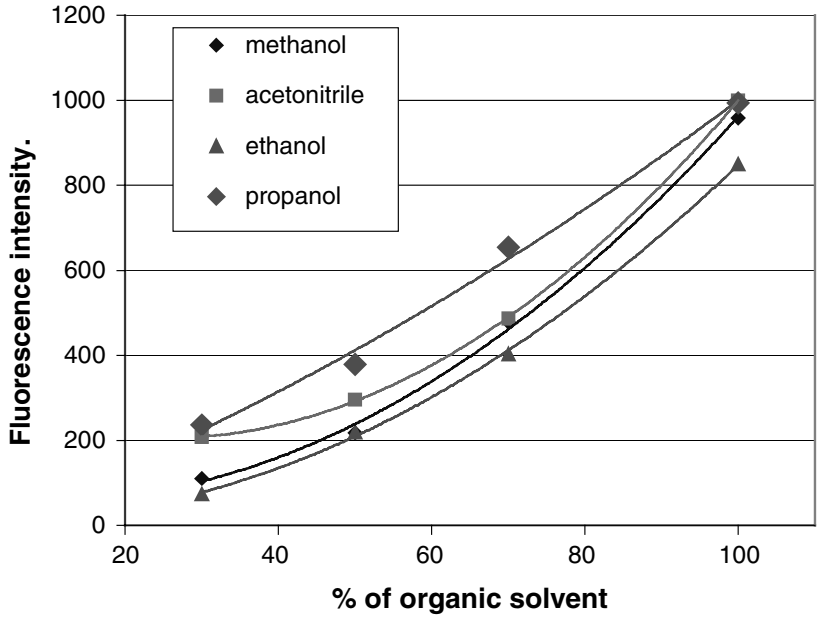

Fig. 6. Influence of the percentage of solvent in solvent–water mixtures on the fluorescence intensity of carbofuran (CF), carbaryl (CB) and carbendazim (MBC).

during the measurement. The photo-degradation depends on the energy absorbed by the sample which itself depends not only on the pulse energy but also on the absorption at the excitation wavelength [12–14]. So, the pulse energy should be optimised for each absorption, therefore for each concentration. We followed the photo-degradation of a 2 μg L^{-1} CB solution by measuring the averaged fluorescence intensity for 10 pulses during the first 300 pulses. From that experiment we chose a 500 μJ per pulse energy for solutions whose concentration was lower than 2 μg L^{-1}.

As the fluorescence lifetime of CB is relatively long (25 ns), it is interesting to use a pulse delay between excitation and emission in order to avoid the fluorescence of contaminants. The best signal–to–noise ratio was obtained for a delay of 20 ns, and we get in this case a LD of 20 ng L^{-1} in tap water. The value of the delay has a strong influence on the sensitivity. Whereas 20 ng L^{-1} is found for a 20 ns delay, it is 150 ng L^{-1} for an 8 ns delay.

We studied CB in a raw water containing humic acids (1 mg L^{-1}). Humic acids present a fluorescence maximum at 470 nm but the emission band is wide and the fluorescence intensity is very strong at this concentration so that even at 335 nm it can be observed. As their fluorescence lifetime is short, a delay of 30 ns between excitation and emission was used to eliminate it. By this way, we measured a LD of 30 ng L^{-1} in that medium.

Carbofuran Analysis

As shown previously, the fluorescence quantum yield of CF in water is very low and its fluorescence lifetime is shorter than 5 ns. The maximum excitation wavelength is 275 nm, but if that wavelength is used to analyse the sample, the Raman diffusion is superposed to CF emission around 305 nm and the LD is decreased. Consequently, we have tested two other excitation wavelengths (245 and 255 nm). The best LD obtained is 20 μg L^{-1} in tap water at 245 nm without delay and for an excitation energy of 800 μJ.

This LD is higher than the CB one because of the low quantum yield and the short fluorescence lifetime of CF which does not allow to use a delay to decrease the blank fluorescence.

Carbendazim Analysis

The fluorescence of MBC in water is stronger than that of CF but its fluorescence lifetime is also shorter than 5 ns, and we cannot excite MBC at the excitation maximum because of Raman diffusion. With an excitation at 245 nm and energy of 700 μJ, we got an 800 ng L^{-1} LD in tap water.

The fluorescence quantum yields measured and the detections limits obtained for the three pesticides are summarised in Table I.

Limit of detection: $LD = 3\sigma \frac{C}{H}$, where σ is the standard deviation of 10 blank intensity measurements, H the

Table I. Fluorescence Quantum Yield Compared to the Detection Limits Obtained for the Three Pesticides Studied in Tap Water and in Raw Water Containing Humic Acids

	Fluorescence quantum yield (φ_F)	$LD_{instrumental}$ (ng L^{-1})	$LD_{tap\ water}$ (ng L^{-1})	$LD_{raw\ water}$ (ng L^{-1})
Carbaryl (CB)	0.090	4	20	30
Carbendazim (MBC)	0.004	50	800	Too short lifetime
Carbofuran (CF)	0.002	1000	20,000	Too short lifetime

fluorescence intensity of the carbamate solution minus the fluorescence of the blank and C the carbamate concentration. All intensity measurements have been averaged on 10 nm around the emission maximum of the carbamate under study. To obtain the instrumental limit of detection, LD_I, σ is replaced by the instrumental standard deviation σ_I measured on 10 nm average instrumental signal.

CONCLUSION

For tap water, interesting limits of detection can be reached by direct analysis. In the case of CB, without pre-concentration step, we succeeded in obtaining a LD lower than the European Union limit for drinking water. The LD of MBC and CF analysis are higher because of their low fluorescence quantum yield in water and of their short fluorescence lifetime. Consequently, to reach the legal limit for drinking water, these compounds should be extracted by an organic solvent and concentrated before analysis.

For direct analysis of natural samples we have shown that humic acids do not influence the fluorescence of carbamates at low concentrations. The carbamates under study have absorption maxima at short wavelength where humic acids also absorb. As humic acids fluorescence lifetimes are around 4 ns, only compounds with longer lifetime ($>$15 ns) can be easily measured. Among the three carbamates studied, only CB can be quantified in a 1 mg L^{-1} humic acid solution.

From a general point of view, it appears that direct determination or screening in tap water using our experimental set-up may be interesting for many compounds even with very low fluorescence quantum yields.

For raw water (in the presence of humic substances) the direct screening of compounds requires a fluorescence lifetime longer than 15 ns. Only in this case low LD can be obtained, even when quantum yields are relatively weak, thanks to a few nanoseconds time shift between excitation and emission detection.

Nevertheless, regarding the diversity of the fluorescent compounds that may be present in a polluted raw water and their wide range of concentration, only the absence of the analyte at a concentration higher than the calculated LD for that medium can be announced by direct analysis. For quantification purposes, separation and concentration steps may be required.

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