Luminescence methods in pesticide analysis. Applications to the environment

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Current luminescence-based methods for determining pesticides in different sample matrices are reviewed. The paper is devoted mainly to fluorimetric techniques with emphasis on the description of direct and indirect fluorimetric methods, including chemical and photochemical derivatization. The use of fluorescence detection in TLC, HPLC and FIA as well as applications to environmental samples is described. The potential of phosphorimetry for pesticide analysis is also presented. The main advantages and drawbacks of luminescence detection for pesticide determination are discussed.

Introduction

Because of their widespread use in modern agricultural production, pesticides contribute significantly to the contamination of the environment, particularly of soil, and surface and/or ground waters. Presently, the worldwide consumption of pesticides is difficult to estimate since the number of different pesticides used for agricultural purposes exceeds several thousands [1-4]. Therefore, the input of agrochemical pollutants in the environment has increased proportionally and consequently, very sensitive and more selective analytical methods are needed for residue analysis of these pollutants.

Important progresses have occurred in the development and applications of various analytical tools including separation methods such as gas chromatography (GC) and high performance liquid chromatography (HPLC), and detection techniques such as electrochemistry, spectrophotometry, spectrofluorimetry and mass spectrometry. Nowadays, these techniques have allowed the identification and determination (at low concentration levels) of pesticide residues in a number of food and environmental samples [1,2,4-7].

Currently, GC remains the main analytical method used for pesticide residue analysis because of its excellent separation and detection potential, especially when combined with mass spectrometry [1,2]. However, it has some drawbacks such as the need for pretreatment procedures in the case of weakly volatile and/or thermally labile pesticides (*e.g.*, carbamates, phenylureas and sulfonylureas). The second most utilized method is HPLC, with UV-visible absorption detectors; it remains the technique of choice for screening work.

The use of luminescence techniques (especially fluorescence) for organic pesticide residue analysis has been limited by the fact that, relatively few of these compounds are strongly luminescent. However, many compounds do possess the necessary degree of aromaticity and may be converted into luminescent species using a variety of methods. Hence, two distinct cases have to be considered. (i) If the pesticide under study is luminescent, then it can be determined directly; (ii) if the pesticide is non or weakly luminescent, it can be converted into a luminescent compound using various physicochemical means, including chemical and/or photochemical reactions.

Derivatization reactions constitute an important aspect of pesticide luminescence analysis since many pesticides have one or more functional groups and can be converted into more stable derivatives to successfully ensure environmental analysis. The number of papers, especially reviews [8-13] and books [14-17], in which this subject is discussed, demonstrates this increase of the derivatization use.

In this review, the most important publications concerning significant advances in the luminescence methodology, instrumentation and applications to environmental analysis of pesticides will be reported. We will describe the recent available literature on direct and indirect fluorimetric methods, photochemically-induced fluorescence, photosensitized fluorescence and phosphorimetric methods used for pesticide analysis. As it will be shown, recent instrumentation, including sophisticated electronics and precise optical systems, makes luminescence a highly sensitive and selective tool for pesticide residue detection.

Fluorimetric methods

An early work on the fluorescence of pesticides has been performed in stationary solutions, (*i.e.*, batch procedure) by

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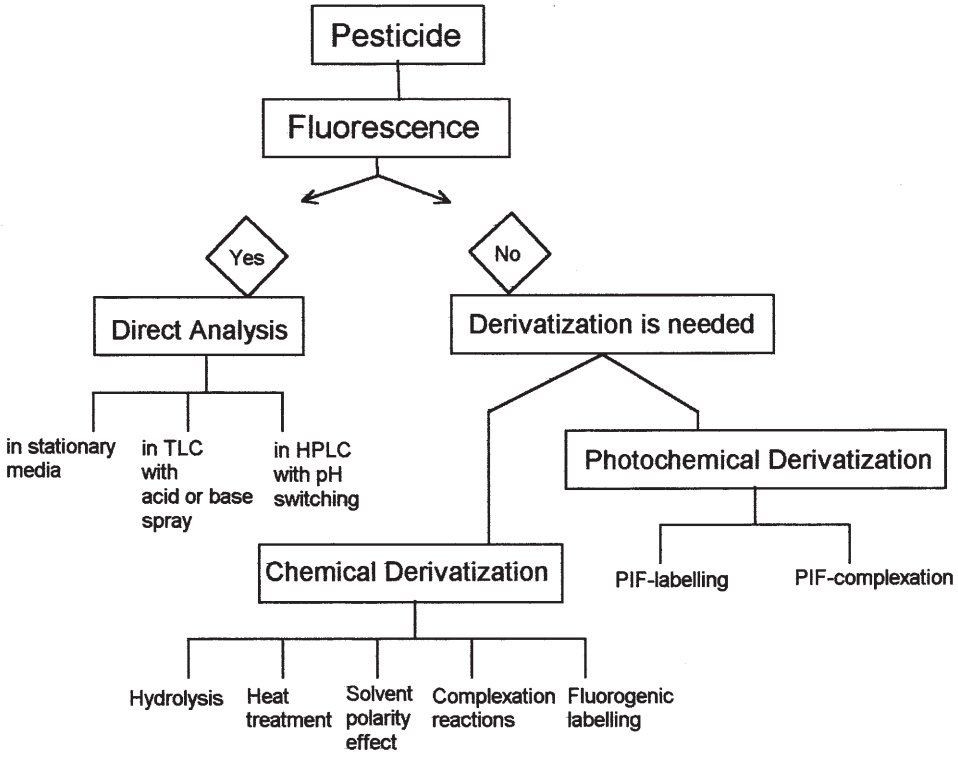

Figure 1. Schematic view of the various fluorescence techniques for pesticide analysis (reprinted from ref. [13]; copyright 1998 Elsevier science B.V.).

Hornstein [18], but it has been limited to naturally-fluorescent pesticides such as guthion, potsan, warfarin and piperonyl butoxide. The aim of this early fluorimetric assay was to investigate the possibility of quantifying pesticide residues. The promising results of this first attempt have led later to develop a number of direct fluorimetric methods and derivatization techniques allowing transforming non-fluorescent pesticides into fluorescent derivatives (Fig. 1). In the following paragraph, these various methods will be presented.

Direct fluorimetric methods

The direct methods based on the fluorescence detection of analytes are essentially applied to those pesticides exhibiting an intrinsic fluorescence strong enough to be analytically useful. Therefore, the fluorescence properties of anticoagulant rodenticides including indanediones [19-21] and coumarins [19-29], carbamate insecticides such as aminocarb [30], bendiocarb [19,21,31], benomyl [31,32], carbaryl [31,33-36] and carbofuran [35,36], and some organophosphorus insecticides, including coumaphos [31] and pirimiphos-methyl [19,21], and other classes of pesticides [31] have been investigated. For example, Krause [31] has selected 75 pesticides, metabolites and a number of industrial chemicals for HPLC studies, based on their known fluorescence or on chemical structures indicating possible fluorescence. Herbicides [37-39], and fungicides [40-43] have been also analysed by direct fluorimetry. In most cases, the pesticide fluorimetric detection has been performed on solid substrates by thin layer chromatography (TLC) [32,44] or in combination with dynamic systems including HPLC [20,22-31,33-37,40-42,45-48] and flow injection analysis (FIA) [21,43]. For instance Garcia and Aaron [43] applied FIA with spectrofluorimetric detection for determining fungicides such as thiabendazole and fuberidazole at the ng ml^{-1} level in river waters (Fig. 2). Specific techniques based on synchronous and/or derivative spectra have been also used in batch procedures [38,39].

Fluorimetric detection on solid substrates

Nowadays, this method is not frequently used for pesticide analysis. Among the various existing solid substrates, silica gel plates [32,44] are the most utilized. TLC is inexpensive and rapid, but lacks sensitivity and selectivity.

Recently, the substrate quality (involving layers prepared from particles with narrow size distributions and mean diameters between 5-15 µm) has been improved [49] and has led to the development of high performance TLC (HPTLC) for pesticide analysis [50]. Another technique called solid phase spectrofluorimetry (SPF) has allowed to determine aromatic pesticide residues in water samples; limits of detection (LODs) were in the range 0.04-0.15 ng ml^{-1} according to the pesticide molecular structure [32].

Fluorimetric detection in combination with HPLC

Fluorimetric detection has been widely used in HPLC since it is generally more sensitive than the classical UV absorption detection. Moreover, fluorescence detectors are very

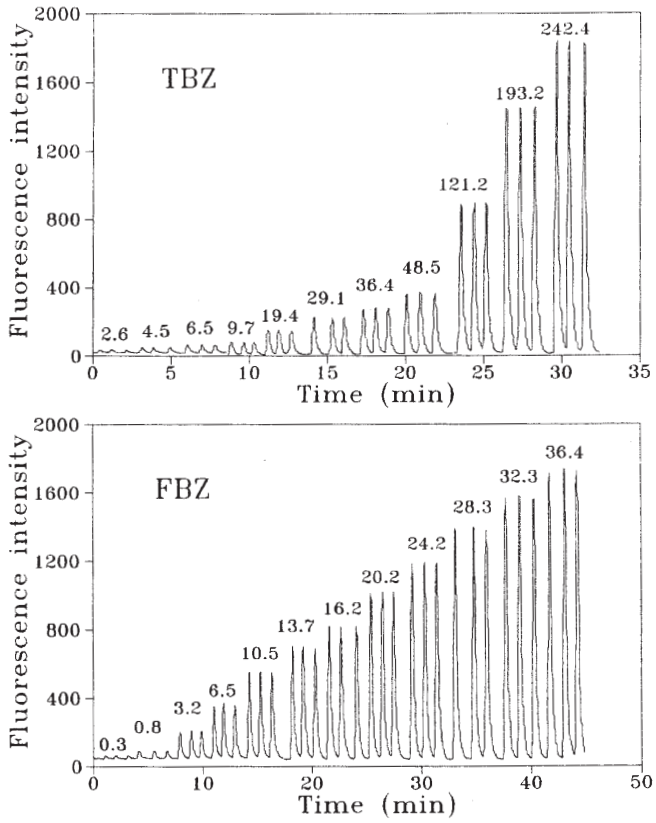

Figure 2. Typical recorded output for both series of thiabendazole (TBZ) and fuberidazole (FBZ) standards, obtained by FIA with spectrofluorimetric detection. Numbers above the peaks are concentrations in ng ml^{-1} (reprinted from ref. [43]; copyright 1996 Springer Verlag).

selective, eliminating matrix interference in real samples. HPLC is particularly useful for separation purposes because of the effectiveness of the various solid phases used in columns. Several types of HPLC systems with fluorescence detection have been reported for the determination of pesticides. For instance, normal phase HPLC, reversed phase HPLC and ionic HPLC provided LOD values in the 2-20 ng ml^{-1} for the same series of rodenticides [20,23,24,27,31]. In other HPLC applications, fluorimetric detectors have been connected in series with UV or electrochemical detectors [37,40,48].

Fluorimetric detection in stationary media

Fluorimetry in bulk solutions presents an interest for pesticide residue analysis because of enhanced selectivity brought about by the use of first and/or second derivative spectra and synchronous spectrofluorimetry [38,39]. Garcia-Sanchez and co-workers [51-54] have demonstrated the satisfactory sensitivity and selectivity of this approach. The simplification of the spectral profile and the band-narrowing effect coupled with the derivative spectral technique (very useful for the reduction of band overlapping) make both identification of weak bands and magnification of the overall fluorescence signal feasible. For instance, a LOD value of 1 ng ml^{-1} was achieved using the first derivative approach in the determination of naphthylacetic acid, a plant growth regulator, and recoveries from spiked apple samples ranged from 97 to 99 % [38].

The potential of variable angle synchronous spectrometry (VASS) for the resolution of fluorescent mixtures has been also evaluated in the case of the cyclodextrin-enhanced fluorescence determination of ternary mixtures of aminocarb, carbendazim and coumatetralyl [55]. The method was compared to the rank annihilation method (RAM). Fluorescence spectral distribution exhibits a great overlap that precludes the direct determination of this type of mixture. VASS provided better analytical recoveries (99-104 %) than RAM (84-130 %), due to better spectral resolution of the mixtures.

The use of organized media *viz.*, cyclodextrins [56], micelles [57] and liposomic vesicles [58] for resolution of mixtures of analytes [55], enhancement of fluorescence detection [59] and reduction of interferences from impurities [60] has developed rapidly. However, in most cases, the application of such media in spectrofluorimetry involves organic aromatic compounds other than pesticides [55,59,61].

In conclusion, the direct fluorimetric detection of pesticides in stationary media or in combination with TLC or HPLC techniques has proved to be highly sensitive and selective with LODs in the low ng or ng ml^{-1} range. Since the number of pesticide structures exhibiting an intrinsic fluorescence is rather limited, several derivatization reactions have been proposed.

Pretreatment fluorimetric methods

Chemical derivatization is based on the transformation of non or weakly fluorescent compounds into strongly fluorescent species following a pretreatment. The type of chemical derivatization reaction applied to the analyte depends essentially on the pesticide family under study [8,10,12,62-65]. In all cases, the method consists to generate a fluorophore by means of a chemical reagent or a thermal treatment. Hydrolysis, heat treatment, complexation reactions and fluorogenic labelling are the most widely applied derivatization techniques in pesticide fluorimetric analysis.

Hydrolysis

Hydrolysis is the simplest pretreatment method. It is performed in a strongly alkaline aqueous medium (NaOH) and at relatively high temperature (50-100 °C), leading to the formation of fluorescent anions. The method has been successfully applied to the determination of carbamates such as carbaryl [32,66-67], benomyl [32] and organophosphorus insecticides such as guthion [68] and quinalphos [69], with LODs in the low ng range. For instance, a LOD value of 6 ng has been found for carbaryl hydrolyzed into α-naphthol

on a TLC plate by spraying with 1M NaOH, and using scanning fluorimetry [66].

The sensitivity of the method can be also improved after heating treatment of the pesticide hydrolysis products.

Heating treatment

In this procedure, the pesticide sample is thermally decomposed on a chromatoplate during a specific time at a given temperature (75-225 °C) and fluorescence is monitored *in situ*. Although the mechanism of thermally induced fluorescence (TIF) is not completely established, it is probable that decomposition or rearrangement of the original molecule into more stable and/or more conjugated species takes place leading to a number of fluorescent products. Generally, TIF requires a certain degree of aromaticity within the structure of the compound under investigation.

This technique has been applied to the analysis of non-fluorescent organophosphorus insecticides [70] and other pesticides exhibiting native fluorescence on TLC plates [70,71]. Significant changes in the excitation and emission spectra occurred for the naturally fluorescent compounds as well as increase or decrease in their fluorescence signal upon heating. The effect of a variety of external reagents such as acids, bases [71] and inorganic salts [72] on a number of pesticides heated on silica gel thin layers has been investigated. The fluorescence intensity was more increased upon spraying alkali reagents than acid reagents. No background fluorescence was observed, which ensured more reproducible results. LODs were in the low ng range.

Complexation reactions

Complexation reactions have been essentially applied to the determination of organotin compounds, generally employed in agriculture as fungicides and insecticides. The approach is based on direct complexation, which leads to the formation of a strongly fluorescent pesticide-ligand complex [65,73-77].

Inorganic and organic tin compounds can form fluorescent complexes with flavonol, morin and quercetin [65,73,74]. The morin-organotin complexes are the most convenient, because they are formed rapidly at room temperature, leading to a very sensitive assay, and they are stable for long time periods in organic solvents, (*n*-hexane, ethylacetate) [73]. These complexes are poorly soluble in aqueous media [74]. Morin is especially sensitive to dialkyltin compounds. In stationary medium, LODs are 10^{-9} M for dialkyltins, 10^{-7} M for monoalkyltins, 5×10^{-7} M for trialkyltins, and 10^{-7} M for triphenyltins [65]. The recoveries of the same organotin compounds in spiked human urine and various rat organs at 1-100 nmol levels range from 91.0 to 99.7 %, depending on the organotin species. A simultaneous analysis of dialkyltin compounds and their possible metabolites was conducted by HPLC on a cyanopropyl-bonded column after post-column morin derivatization. LODs between 0.1 and 1 ng and recoveries of 91-99 % were found for dialkyltin compounds added to animal tissues at the 5 nmol level [73]. Langseth [74,75] has described a method requiring only a single HPLC pump by incorporating the ligand in the mobile phase. This approach based on an eluent containing morin or another complexing agent allows determining various organotin compounds at low pg level. Furthermore, the use of an epifluorescence microscope as an imaging detector [76] and of micellar solutions [77] permits to lower the detection limits and to increase the solubility of organotin-morin complexes in aqueous media.

Although these complexation techniques are rapid and simple, they are not selective, and they are rather susceptible to interferences. Alternatively, fluorogenic labelling can be used: a fluorophore is fixed to the analyte, which can be determined fluorimetrically, using TLC or HPLC rather than batch procedures.

Fluorogenic labelling

Because of its high sensitivity, fluorogenic labelling represent the most widely used pretreatment method for pesticide analysis [8,12]. In this method, a fluorophore molecule is chemically bonded to a non-fluorescent analyte; only a hydrogen atom or another atom of the analyte is replaced by the fluorophore moiety. Originally, Lawrence and Frei [78,79] determined pesticides using fluorogenic labelling with dansyl chloride (dans-Cl). Presently, it exists a number of labelling reagents, including dans-Cl, NBD chloride (NBD-Cl), fluorescamine and orthophthalaldehyde with 2-mercaptoethanol (OPA/2-ME) or with 3-mercaptopropionic acid (OPA/3-MP) [8-10,80]. They react easily with compounds such as alkylamines, arylamines, thiols and phenols, which are widely found among the pesticide degradation reaction products. For instance, carbamates are hydrolyzed into amines and phenols [79,81], urea herbicides lead to aniline derivatives [82,83], many organophosphorus insecticides yield thiols and phenols upon hydrolysis [84], and the *s*-triazine herbicides are hydrolyzed into alkylamines [85]. Very often, the labelling reaction mechanism is a simple, rapid, and selective two-step process. In the first step, the pesticide is hydrolysed in an alkaline medium, and in the second step, the fluorogenic labelling of the hydrolysis products takes place. Dans-Cl and NBD-Cl are generally used as pre-column derivatization reagents, while fluorescamine and OPA/2-ME are more suitable for post-column derivatization. Several examples of labelling reaction mechanisms are given, for several classes of pesticides, in the following paragraphs.

Labelling with dansyl chloride

Dans-Cl readily reacts with primary and secondary amines, at slightly higher pH with phenols, some thiols and to a lesser extent with aliphatic alcohols leading to products more fluorescent than dans-Cl [78,79,86-88].

The procedure employed by Lawrence and Frei [78,79] *i.e.*, the hydrolysis of carbamate into phenol and methylamine is followed by the reaction of dans-Cl with both hydrolysis products. Generally, the phenol derivative

(λ_{ex} = 365 nm, λ_{em} = 520 nm) is quantified since it is characteristic of each carbamate [79]. The dans-Cl technique has been also applied to the detection of carbamates in water and soil samples without additional clean-up procedure (LODs in the low nanogram range) [86], of phenylurea herbicides (LOD = 1 ng) [82], organophosphorus insecticides (LOD = 5-10 ng) [84] and *s*-triazine herbicides (LOD = 10-25 ng) [85].

Solvents have also a significant effect on both the dansylation reaction and the extraction processes [88]. Apolar solvents such as hexane, cyclohexane and benzene generally increase the fluorescence intensity of dansyl-phenol derivatives and shift the emission wavelength towards the blue, whereas polar, hydrosoluble solvents reduce the fluorescence signal, producing a red-shift of the emission maxima.

Labelling with NBD chloride

NBD-Cl only reacts with primary and secondary aliphatic amines under weakly basic conditions to form highly fluorescent derivatives. It is more selective than dans-Cl since it does not form fluorescent derivatives with phenols, thiols, alcohols or anilines. Unlike dans-Cl, the NBD-Cl hydrolysis product is not fluorescent, yielding no interference. In addition, the NBD-Cl fluorescence excitation ($\lambda_{ex} \approx$ 470-480 nm) and emission ($\lambda_{em} \approx$ 530-550 nm) maximum wavelengths are located in the visible region of the spectrum, where naturally fluorescent compounds that may be present as co-extractives do not interfere generally. Therefore, a quantitative labelling method based on NBD-Cl has been developed for determining N-methyl and N,N-dimethylcarbamate pesticides in aqueous solutions [89]. The reaction rates as well as fluorescence and TLC properties were studied to optimize the technique, and subnanogram quantities of pesticides were detected.

NBD-Cl labelling method is also very selective, since only one fluorescent derivative is produced with most carbamates, which eliminates possible interferences. However, it cannot be used to distinguish between carbamates of different classes.

Labelling with fluorescamine

Fluorescamine, a powerful fluorogenic reagent, which was synthesized by Weigele *et al.* [90], reacts directly with primary aliphatic amines in aqueous media (pH 8-10) at room temperature within less than one second, yielding a highly fluorescent derivative. Fluorescamine itself is non-fluorescent and the excess of reagent is hydrolyzed in a few seconds, producing non-fluorescent products [91]. Because of large reaction rates and a good selectivity, this labelling procedure is convenient for post-column HPLC detection of primary amines. Moreover, fluorescamine can react with primary aromatic amines in aqueous media [92]. The aliphatic amines produce in alkaline conditions (pH 8-10) a fluorophore characterized by maxima at λ_{ex} = 395 and λ_{em} = 475 nm, while the primary aromatic amines react more easily at acidic pH = 3-5 yielding a fluorescent derivative with λ_{ex} = 405 and λ_{em} = 485-495 nm.

Fluorescamine has been used essentially to label amine-generating pesticides [93-95]. The reaction mechanism is similar to dans-Cl labelling of amines and has been carried out on amines obtained either by hydrolysis of carbamates [93,94], or by reduction of nitrosubstituted pesticides such as fenitrothion and parathion methyl [95]. LODs were between 7-43 ng ml^{-1} [93,94].

Labelling with orthophthalaldehyde/2-mercaptoethanol (OPA/2-ME)

OPA selectively reacts with primary aliphatic amines. Originally, it was applied to the HPLC analysis of 7 N-methylcarbamate insecticides [80]. The reaction mechanism includes two steps: the alkaline hydrolysis of carbamate into methylamine and alcohol, and the derivatization of the released methylamine with OPA in the presence of 2-mercaptoethanol (2-ME) [80,96-118] or 3-mercaptopropionic acid (3-MP) [112,119] to form a highly fluorescent isoindole derivative identified by NMR and mass spectrometry [120]. Most studies using OPA and 2-ME or 3-MP are based on post-column HPLC derivatization, since the reagents can be easily introduced in the reaction system by successive pumps.

HPLC post-column reaction derivatization parameters have been optimized by Krause [121,122] and Engelhardt *et al.* [123]. OPA/2-ME HPLC, which is recognized by the US Environmental Protection Agency (EPA) [124], is a widely used multiresidue method for N-methylcarbamate and carbamoyloxime insecticides determination in food [102,103,122] as well as in a variety of media including water [98-100,106,110,114,117,118,125], soils [98,115,118], and plant tissues [97,101,104,105,107,109,115,116,123]. De Kok *et al.* [107] extended the method to others N-methylcarbamates, and developed a simple clean-up method for various types of crop samples.

Nondek *et al.* [125,126] significantly improved the OPA/2-ME method, by hydrolyzing carbamates at 100-120 °C, in a catalytic solid-phase reactor packed with a strong anion exchanger resin. One of the post-column reagent delivery pumps is eliminated, which avoids possible mixing problems and flow pulsations. Moreover, it suppresses band broadening, due to dilution of the analyte in the mobile phase, thus improving the sensitivity and selectivity of the method: as little as 0.1 ng of aldicarb and 0.3 ng of methomyl can be detected [126]. 22 N-methylcarbamates and their major metabolites in crop samples have been also determined using solid-phase catalysis with magnesium oxide instead of the anion exchanger resin, which minimizes band broadening [113]. Furthermore, Jansen *et al.* [127] miniaturized the catalytic solid-phase reactor using 1-mm i.d. narrow-bore HPLC columns, and obtained LODs in low ng range.

In another useful simplification, the hydrolysis and derivatization steps were combined by means of a single reagent (OPA/2-ME in 0.01 M KOH) delivered by a single post-column pump [112], which eliminates the need for both an alkali post-column pump and solid-phase or photolytic

reactors. This single-step technique has been applied to the analysis of 11 N-methylcarbamates (LOD ≈ 0.1 ng) [112], of oxamyl in potatoes [128] and of oxamyl and methomyl in crops and water samples (LOD = 1 ng) [129]. It appears more convenient than the two-step conventional technique, since sensitivity is increased, the hardware and cost are reduced and the daily procedure is simplified.

OPA/2-ME labelling remains the most sensitive and practical derivatization method for the analysis of pesticides yielding primary amines under hydrolysis or photodegradation. N-methylcarbamate and carbamoyloxime insecticides, herbicides [130], plant growth regulator [131] and phenylureas [96,122,127] have been determined using this technique.

Other labelling reagents

It exists other labelling reagents for pesticides [62,64], which have not yet gained widespread acceptance. For example, 9-anthryldiazomethane was used as labelling reagent to detect about 500 pg of chlorophenoxyacid herbicides in ground water [62]. A rapid determination of glufosinate (in less than 15 min) was also performed in environmental water samples containing about 0.25 ng ml^{-1} of this pesticide by pre-column derivatization with 9-fluorenylmethoxycarbonyl [64].

Advantages and drawbacks of fluorogenic labelling

Fluorogenic labelling constitutes a sensitive, but rather poorly selective analytical method. Relative to other derivatization techniques (hydrolysis, heat treatment, etc.), it offers a number of advantages, including simplified optimization procedures, dual or multiresidue methods, wide ranges of labels and accessible experimental variables with excellent sensitivity. However, it exists limitations due to problems associated with the simultaneous detection of individual components in mixtures. The development of HPLC has significantly improved the selectivity of the labelling methods relative to originally used TLC [78,79] and stationary solution procedure [88].

Photochemically-induced fluorimetric (PIF) methods

Photochemically-induced fluorimetry (PIF), based on the conversion upon UV irradiation of non-fluorescent analytes into strongly fluorescent photoproducts is a more recent approach than chemical derivatization and has been much less applied to pesticide quantitative analysis. Several book chapters have been devoted to PIF methods, which present several advantages relative to chemical derivatization and some drawbacks [15-17].

The main advantages are as follows: (i) the use of photons for analyte conversion instead of a chemical reagent does not require a mixing system, and therefore, the analyte must not be diluted; (ii) since most photochemical reactions take place *via* free radicals, the reactions rates are generally fast, resulting in short conversion times; (iii) the use of room temperature compared to higher temperature in thermally-initiated derivatization; (iv) the technique requires low cost equipment and is suitable to various experimental conditions such as stationary liquid solutions [132-141], dynamic systems, including flow injection analysis (FIA) [21,142-144], HPLC [145-151] and TLC [152].

Obviously, PIF provides a selective fluorogenic transformation of non-fluorescent compounds since it does not require the usual separation of fluorescent photoproduct from other photoproducts in the reaction medium. From an analytical standpoint, another advantage is that it is not necessary to identify the structure of the fluorescent compound(s) formed after UV irradiation when reproducible PIF signals are obtained. In some cases, it is possible to perform PIF simultaneous determination of a mixture without a prior separation step by using differences in optimum analytical conditions (*i.e.*, t_{irr}^{opt}, pH, solvent), characteristic of each compound [132,135,138], or by applying specific techniques, such as derivative PIF spectra [141], and/or partial least square (PLS) multivariate method [153].

PIF has some drawbacks such as the formation of non-fluorescent photoproducts; secondary thermal reactions and/or photoreactions leading to non-stable photoproducts, particularly those responsible of the fluorescence emission [17].

PIF optimal analytical conditions

Generally, PIF methods are considered as efficient fluorophore-generating systems for stationary media [132-141] as well as for flowing devices such as HPLC post-column photoreaction [142,145-149] or FIA [21,142-144]. Among the various parameters controlling the analyte conversion photoreaction and method sensitivity, two are particularly important, namely the optimum UV irradiation time (t_{irr}^{opt}, corresponding to the maximum PIF signal) [132,135,138] and the type of solvent [132,135,138,142,149]. The t_{irr}^{opt} and PIF intensity values vary significantly with the solvent polarity and its protic or aprotic character. For analytical purposes, the solvent selected should be the one giving the shortest t_{irr}^{opt} and the largest PIF signal.

PIF application to pesticide analysis

Relative to other fluorogenic derivatization techniques, PIF appears as a very useful technique for pesticide analysis because of its simplicity, shorter analysis time, enhanced sensitivity and selectivity.

PIF combined with HPLC

For the first time, Werkhoven-Goewie *et al.* [145] applied PIF to liquid chromatographic analysis of chlorophenol pesticides in effluent water samples and biological fluids. The fluorescence signals of photolysed chlorophenols were linearly related to the amounts of analyte over 2-3 orders of magnitude, with LODs in the low nanogram range, and

recoveries of 100 ± 4 % from spiked river water samples were obtained.

PIF-labelling combined with HPLC

In the OPA/2-ME labelling of phenylurea herbicides [122], Luchtefeld [146] replaced the inefficient chemical hydrolysis step by photolysis degradation. As a result, linear dynamic ranges between 2 and 48 ng, were obtained from these herbicides. Herbicides with N,N-dimethyl group were found to give a greater response than those with an N-methyl, N-methoxy moiety. In HPLC, this PIF labelling technique allows also to eliminate the need of the originally used alkali reagent post-column pump [80].

The applicability of the amine-generating photolysis reaction of pesticides OPA/2-ME derivatization was extended by Moye *et al.* [142,147-149] to a broad range of classes of nitrogeneous pesticides including carbamates, carbamoyloximes, carbamothioic acids, dithiocarbamates, organophosphorus, sulfonylureas, thiadiazolureas, thioureas, bipyridiniums, triazines, dinitrophenols, amides, acetamides and amines. PIF detection could be used with OPA/2-ME [142,146-150] and without it [142,147,148].

Phenylcarbamates, phenylamides and phenylurea herbicides were determined by HPLC using a post-column photolysis fluorescence detector [148]. Linear calibration curves were obtained over two orders of magnitude, with LODs in the low nanogram range. HPLC post-column photolysis and fluorogenic labelling with OPA/2-ME was also applied to the analysis of several pesticides including aldicarb sulfoxide, aldicarb, propoxur, thiram and neburon, in fortified ground water samples, at 10-50 ng ml^{-1} level [148]. Using the same HPLC or FIA apparatus, Patel *et al.* [142,149] measured the PIF signal of several nitrogeneous pesticides; the use of surfactant as photosensitizing agent was found to increase the PIF response of most pesticides [142]. This post-column PIF detector gave satisfactory results with and without OPA/2-ME. Many pesticides that gave a good PIF response did not give any signal when the alkaline hydrolysis OPA/2-ME detection system was used [96]. However, some aromatic carbamates were found to be rather slowly photolysed [19,154], thus leading to a low yield of methylamine photoproducts. Therefore, in the latter cases as well as for non-aromatic pesticides, sensitizing agent such as acetone [142,149] was proposed to enhance the photoconversion ratio.

PIF-complexation combined with HPLC and TLC

Stab *et al.* [151] combined post-column photoconversion and morin complexation with a normal-phase HPLC, on a cyanopropyl-bonded silica stainless steel column, to determine low nanogram amounts of triorganotin pesticides in surface water, sediments and soils. An advantage of this PIF complexation technique was the very short reaction time (t_{irr}^{opt} = 9 s). Also, Brown *et al.* [152] proposed a PIF approach based on complexation with morin and combined with TLC to screen phenyltin fungicides in potato extracts down to 10 ng ml^{-1}.

PIF combined with FIA

Aaron *et al.* [21,143,144] have also reported PIF detection coupled with FIA for the determination of several types of aromatic pesticides. This system allows improving significantly the sampling rate. Indeed, as many as 56-80 samples can be analysed per hour, with satisfactory recoveries (88-109 %) and LOD values ranging from the lower nanogram to the upper picogram levels.

PIF in stationary media

PIF has also been used in stationary media, yielding fluorescent photoproducts from several families of naturally non-fluorescent pesticides, including aromatic pesticides, chlorophenoxy acids, sulfonylurea and phenylurea herbicides [132-141]. The PIF properties of these compounds were investigated in various solvents. The LODs are in the low nanogram range. The use of aqueous micellar media [137-141] and cyclodextrins solutions [135,136] improve the sensitivity and the selectivity of PIF in a number of cases. Generally, the plots of pesticide PIF intensity *versus* the logarithm of surfactant concentration present a sudden increase of PIF signal with increasing the surfactant concentration close to the critical micellar concentration (cmc) value (Fig. 3). As a result, the LODs of pyrethroid insecticides or sulfonylurea herbicides are several times lower in sodium dodecyl sulfate (SDS) or cetyltrimethylammonium chloride

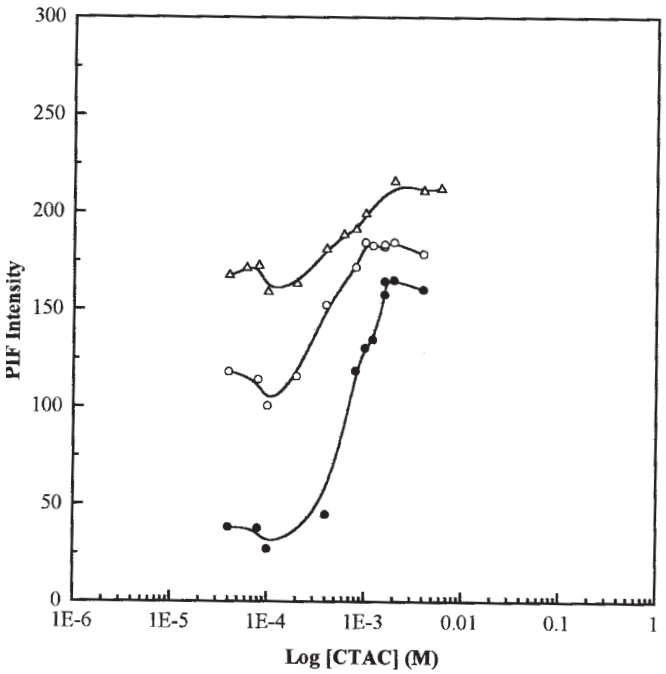

Figure 3. Influence of CTAC concentration on the PIF intensity of several herbicides: (●) 2 × 10^{-6} M chlorsulfuron; (Δ) 10^{-6} M 3-rimsulfuron; (O) 10^{-5} M sulfometuron methyl (reprinted from ref. [138]; copyright 1999 Elsevier Science B.V.).

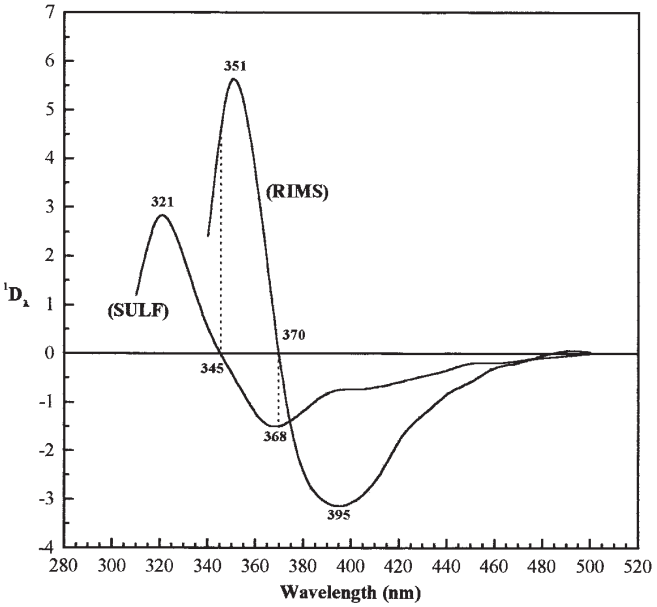

Figure 4. Simultaneous determination of binary mixture of sulfometuron methyl (SULF) and 3-rimsulfuron (RIMS) in a CTAC micellar aqueous solution by means of the first derivative PIF spectra using the zero-crossing point measurement, (λ_0 = 370 nm and 345 nm for SULF and RIMS, respectively) (reprinted from ref. [141]; copyright 2000 John Wiley & Sons, Ltd.).

(CTAC) micelles than in the common organic solvents [137,138]. Also binary mixtures of sulfonylurea herbicides have been resolved, using their first derivative PIF spectra in micellar aqueous solution (Fig. 4) [141].

We can conclude that PIF is very useful for quantifying several classes of pesticides with good sensitivity and selectivity. The combination of PIF detection with dynamic systems (HPLC, FIA) is specially attracting for pesticide residue analysis.

Photosensitized fluorimetric (PSF) methods

When the analyte is weakly or non-fluorescent and its irradiation does not lead to fluorescent photoproducts, photosensitized photoreaction can be considered. In these case, anaerobic photoreduction reactions of sensitizers such as aromatic ketones by hydrogen atom donating (HAD) compounds like aliphatic alcohols, aldehydes, saccharides, glycosides, amines and steroides are generally used [155-159]. Aromatic ketones including quinone [156,157] and anthraquinone derivatives [155,158,159] have been utilized as sensitizing agents. The mechanism of photosensitizing process is known to occur *via* the sensitizer triplet state [155]. An advantage of the photosentized fluorimetric (PSF) method is that it can be applied either to the detection of HAD compounds [155,158] or to that of quinones themselves [156,157].

However, this method has not been very much applied in pesticide residue analysis until now. Traore and Aaron [158] have sensitized the fluorescence of four naturally non-fluorescent dinitroaniline herbicides, using anthraquinone. The PSF signal was found to be proportional to the herbicide concentration. The LODs ranged from 0.3 to 4 µg ml^{-1}, and the optimal irradiation times were short (t_{irr}^{opt} < 5 min), resulting in a decrease of the analysis time. PSF constitutes a rapid and precise method for analysis of dinitroaniline herbicides.

Phosphorimetry

Several workers [160-165] have also investigated the room-temperature phosphorescence (RTP) properties of pesticides. Since RTP emission from the triplet state in fluid solution is very weak, enhancement of the RTP signal is generally achieved by incorporating the analyte into either a solid substrate (filter paper, cellulose, silica, alumina…) [166,167] or organized media (micelles [168], cyclodextrin [169]). Generally, low-temperature (liquid nitrogen, 77 K) and heavy atom effects induce strong phosphorescence signal. However, despite their usefulness, RTP and low-temperature phosphorescence (LTP) have been much less applied to the determination of pesticides than fluorimetric methods.

Aaron *et al.* [160,161] have described the RTP analytical characteristics of 32 pesticides adsorbed on filter paper. A detailed comparative study of the LTP and RTP properties of the pesticides under study show the analytical interest of LTP and RTP techniques for determination of these compounds. The obtained LODs were in the low nanogram range (10-50 ng for RTP and 0.05-35 ng for LTP) [160]. The improved sensitivity of RTP, when applied to the analysis of the same compounds in the presence of external heavy atom perturbers was also demonstrated [161].

We can conclude that phosphorimetry is a sensitive method for pesticide analysis, but its use has been limited by the rather inconvenient and often time-consuming sampling conditions required. Indeed, it is necessary either to immobilize the sample in a low temperature glass or to deposit it on an inert substrate such as filter paper. The later technique does allow the observation of phosphorescence at room temperature, but has the disadvantage of rather cumbersome sample preparation, critical drying requirement, and high phosphorescent background signal from the filter paper. An alternative to these phosphorimetric techniques would be to work in fluid solution at room temperature by means of organized media, *viz*., cyclodextrins and micelles [168,169]. Unfortunately, no data was found in the literature for pesticide phosphorimetric analysis in these media.

Conclusions and future trends

Clearly, pesticide residues from crops and environmental samples can be handled and analysed using a number of techniques. Pesticide analysis requires selective as well as sensitive detectors. Due to its great sensitivity, fluorescence detection is very useful for pesticide analysis, but because of its irregular selectivity, it cannot be recommended for pesticide screening or early-warning procedures. Nevertheless fluorescence detection is of interest in environmental analysis, especially when monitoring specific pesticides and, occasionally, classes of compounds such as carbamates and chlorophenoxy acids. Moreover, derivatized fluorescence and photochemically-induced fluorescence (PIF) are adaptable to a variety of experimental conditions such as TLC, bulk solutions and flow systems, including HPLC and FIA. As a result of its simplicity, PIF appears more convenient and rapid than fluorogenic labelling derivatization for pesticides analysis. In addition, the inability of direct or indirect fluorimetric methods for the determination of pesticides containing neither chromophore nor fluorophore might be overcome in some specific cases by using photosensitized fluorimetry. Because of complicated sampling conditions, low temperature phosphorescence (LTP) and room temperature phosphorescence (RTP) are not yet developed for pesticides analysis as it could have been expected.

Moreover, the use of organized media, (cyclodextrins, micelles, liposomic vesicles), special spectral techniques (synchronous, derivative spectra, variable angle scanning spectra...), and/or statistical treatments of the spectral data such as partial least squares regression (PLS), should help fluorescence, PIF and phosphorescence to become valuable complementary approaches for pesticide environmental analysis.

We can conclude that due to these recent technological advances and their inherent sensitivity, selectivity, and versatility, luminescence methods will certainly continue to be widely used in pesticide residue analysis.

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