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ANALYSIS OF PESTICIDES BY FLOW INJECTION COUPLED WITH CHEMILUMINESCENT DETECTION

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Abstract

The analyses of pesticides carried out by coupling flow injection analysis with chemiluminescence (CL) detection during the last ten years are reported in this review. The review is divided into three sections devoted to the different families of pesticides, namely, organophosphorus, carbamates (together with dithiocarbamates) and other pesticides from the remaining groups. Relevant analytical data such as limits of detection, dynamic ranges, recoveries and sample pretreatments are recorded in three tables. Descriptions about the fundamentals of the CL systems used and new promising approaches as photoinduced chemiluminescence (PICL), multicommutation and molecular connectivity are also included.

Keywords: Flow Injection Analysis, chemiluminescence, pesticides,

INTRODUCTION

The contribution of pesticides to health and economy are closely interrelated. They contribute directly to our health through control of certain vector-borne disease and to the economy through increased production of food and fiber and protection of many materials during storage. Pesticides have been used to a limited degree since ancient times (1550 BC); but in the last decades, not only have the number of pesticides and their tonnage increased, but the number and variety of their uses have increased also (Hayes 1991). Their value to society is clearly reflected by the 30,000 million dollars expended worldwide in 2001 (see http://www.epa.gov/oppbead1/pestsales).

The contamination of soils and food by pesticides has caused a serious concern, to watch over the safety of our food supplies, international organizations regulate their maximum residue levels (MRLs) on foods and agricultural commodities (see http://www.fao.org). Contamination of ground and surface waters by pesticide residues was for many years generally regarded as transitory, because the focus was on organochlorine pesticides, which were of very low water solubility and with a strong tendency to attach to particulate matter. In addition, the soil profile is known to acts as a purifying filter. However, the information accumulated in the last years about organochlorine pesticides and other herbicide compounds, which were generally more water-soluble, has led to pesticide residues in both, surface and ground waters being detected. Consequently, policies have been developed to reduce contamination of ground and surface waters, and regulatory limits and guideline levels were introduced for residues in drinking water.

In the European Union, water intended for human consumption must meet minimum specified requirements, including a maximum level for each pesticide of 0.1 μg L^{-1} and a maximum of 0.5 μg L^{-1} for total pesticides, except for aldrin, dieldrin, heptachlor and heptachlor epoxide, which are each limited to maximum levels of 0.03 μg L^{-1} (Hamilton 2003). Those legal restrictions have led to a growing demand for analyses of increasing sensitivity and selectivity that combine automatability and cost-effectiveness with a high reproducibility, sensitivity and selectivity.

Analysis of pesticides in environmental, food, clinical and forensic samples is a difficult task because of the matrix complexity and low concentration values of the target compounds. Gas chromatography (GC) has been widely used for pesticide analysis in food, environmental and clinical samples due to its high selectivity and sensitivity. However, some analytes are not suitable for GC separation because of their thermal lability, low volatility or high polarity. Phenylurea, carbamate, dinitrophenol, benzimididazole and some organophosphorus, pyrethroid and quaternary ammonium derivatives are examples of such pesticides (Vasilescu 2005).

Chemiluminescence (CL) has revealed as an excellent tool for the detection of pesticides, as it can provide the high sensitivity required in those analysis, due to the high dilution of these substances in environmental samples. CL is defined as the emission of light as the result of a chemical reaction. As external light source is not necessary, CL measurements do not suffer from source fluctuation noise or light scattering. Hence, chemiluminescent reactions can sometimes afford superior detection limits compared to those achievable with fluorescence (Barnett 2005). The inherent selectivity of CL detection arises from the limited number of substances that can produce significant amounts of light. Nevertheless, in some cases selectivity can be a concern because several species can yield emission with a given reagent. In those situations it can be necessary to couple CL detection with selective chemical techniques such as immunoassay (Zhao 2009), liquid chromatography (HPLC) (Gámiz-Gracia 2009) or electrophoresis (Huang 2006); or separation procedures such as solid phase extraction (SPE) (Picó 2007) to achieve an interference-free measurement. Selectivity and sensitivity can also be improved through electrogenerated chemiluminescence (ECL), since the reagents can be generated in situ in an electrolytic process allowing a great temporal and spatial control over the reaction (Karsten 2001).

The required automatability for pesticide analysis can be provided by Flow Injection Analysis. This methodology has gained widespread use due to its immense potential for routine analysis and its high reproducibility (which makes flow injection (FI) methods reliable for use in intercomparison studies). Its great versatility has made possible its successful coupling with a variety of detection systems such as atomic spectroscopy (Anthemidis 2009), UV spectrophotometry (Tzanavaras 2007), fluorescence (Wang 2009), electrochemistry (Blasco 2007) and chemiluminescence (Wang 2009), as well as with separation methods such as electrophoresis (Lu 2009) or HPLC (González San Miguel 2009). Environmental safety is another attribute of this methodology. As a consequence of its inherent miniaturization, a considerable decrease in reagents consumption and waste disposal is achieved; moreover, the closed-system chemistry prevents operator coming into contact with hazardous chemicals. Simplicity, low cost and rapidity are other outstanding features that have been made possible the application of FI methods to environmental analysis (Wang 2009) (Xu 2005) (Miró 2004).

From all above exposed, it can be deduced that requirements for pesticide analysis can be readily accomplished by the coupling of CL detection and FI methods. CL can provide a high sensitivity in the determination, and FI methodology is particularly well suited to monitoring transient light emission from chemiluminescent reactions because of its high rapidity and reproducibility providing a high automatability to the analytical process.

Although some reviews have been recently reported on the application of CL detection to pollutants in environmental samples, they are not exclusively focused either on pesticides (Wang 2009) (Dunec 2003) or on FI techniques (Gámiz Gracia 2005). In this article an update of the applications of FI methods coupled with CL for pesticides determination in environmental samples is reported. This review is divided into three sections. The first and second sections are devoted to organic phosphorus and carbamate pesticides (including dithiocarbamates) respectively. The third section contains the pesticides belonging to the rest of chemical groups, which have been analyzed by FI-CL. Relevant analytical data (dynamic ranges, limits of detection, recoveries and sample pretreatments) are shown in three tables.

Additional information regarded to the fundamentals of the different FI-CL systems used is also included.

ORGANOPHOSPHORUS PESTICIDES

The general formula for organic phosphorus compounds is depicted in figure 1:

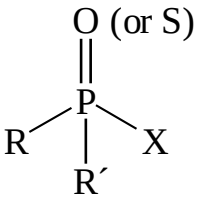


Figure 1. Structure of organophosphorus pesticides.

Most of these pesticides are used as insecticides, and only a few are used as herbicides. In about half of all organophosphorus pesticides X= CH_3O and in the next to large group of these substances X= C_2H_5O.

Most environmentally persistent organic chlorine pesticides have been replaced by the less persistent and more specific organic phosphorus pesticides, and more recently by carbamate pesticides. At present, organophosphorus pesticides are very popular in agriculture because of their high insecticidal activity. But as a result of the acetylcholinesterase (AChE) inhibition caused by these pesticides, they do have a high toxicity to mammals. Therefore, it is very important to develop sensitive, fast and reliable methods for their determination.

As it is shown in the table 1, in most of the organophosphorus pesticides the determination was carried out using the luminol reaction. In that reaction, luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) is oxidized in aqueous alkaline solution to produce the excited 3-aminophthalate anion, which emits light when it is relaxed to the ground state (Figure 2).

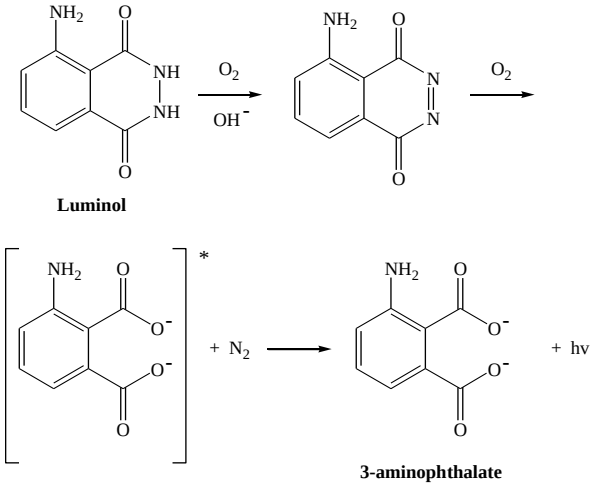


Figure 2. Mechanism of chemiluminescent reaction of luminol.

Oxidants such as permanganate, periodate or hexacyanoferrate can be used, but hydrogen peroxide is the most commonly employed. The reaction can be catalyzed by transition metal ions (Co^{2+}, Cu^{2}, Fe^{3+}, etc) or hemin and heme proteins (hemoglobin, peroxidases, catalases, etc). The optimum pH is between 8 and 11 depending upon the catalyst.

Schoenemann (Schoenemann 1994) (Frische 1980) proposed a reaction mechanism which would explain the basis of the organophosphorus pesticides determination using luminol. According to it, these compounds would be oxidized by hydrogen peroxide to

produce a peroxophosphonate and then, this substance would oxidize luminol to generate CL in alkaline solution. It would explain, for example, the basis of the determination of trace residue of dichlorvos in vegetable samples using luminol, hydrogen peroxide and the cationic surfactant cetyltrimethylammonium bromide (CTAB) as sensitizer (Wang 2001). The analysis of another three organophosphorus pesticides, namely, methyl parathion, malathion and fenitrothion, carried out by these authors confirmed the above mentioned mechanism. Only for malathion CL signal could not be observed when reacted with hydrogen peroxide. Bearing in mind the proposed mechanism, it could be explained by the fact that this pesticide contains a P-S group rather than a P-O, and thus the production of a peroxophosphonate would impossible.

Table 1. Analysis of organophosphorus pesticides.

Pesticide	CL system	DR (μg L^{-1})	LOD (μg L^{-1})	Matrix (r, %)	Sample pretreatment	Reference
Buminafos	MnO_4^-/H_2SO_4 [a]/H_2O_2 (80ºC) [b]	10-1000	5	Water (97-106) Soil (97)	Anionic exchange (Duolite A-102 D) Cationic exchange (Duolite C20)	(López-Malo et al. 2008)
Chlorpyrifos	Luminol/IO_4^-	0.48-484	0.18	Fruit (94.4-107.4)	Extraction with water	(Song et al. 2002)
Diazinon	$MnO_4^-/H_2SO_4/SO_3^{2-}$	300-1500	100	NR	NR	(Waseem, Yaqoob, and Nabi 2007)
Malathion		600-1800	300	NR	NR	
Dichlorvos	Luminol/ H_2O_2/CTAB	20-3100	8	Water (91.4-93.3) Vegetables	NR	(Wang et al. 2001)
Dichlorvos	Luminol/Molybdate/Vanadate[c]/ $S_2O_8^{2-}$ [b]	10-5000	10	Vegetables (93-113)	Extraction with water	(Baoxin et al. 2007)
Omethoate				Vegetables (92-113)		
Trichlorfon (Dipterex)				Vegetables (87-110)		
Fenitrothion	Luminol/H_2O_2/NaCl	10-200	4	Water (98-102)	NR	(Liu et al. 2007)
Monocrotophos		20-1000	7	Water (98-102)	Cationic exchange on-line	(Du et al. 2003)
Phoxim		10-1000	5.4	Vegetables (86-106)	-	(Liu et al. 2007)
Glyphosate	$Ru(bpy)_3^{2+}/H_2SO_4/PbO_2$	1.7-34	1.18	Formulation (93.0-99.4)	NR	(Adcock et al. 2004)
Methamidophos	Luminol/H_2O_2	20-13000	47	Vegetables (90-109)	SPE	(Li et al. 2006)

Parathion and parathion methyl residue determinations in rice samples were carried out by using the same CL system (Liu 2003) (Rao 2001). In the parathion analysis a non-ionic surfactant, polyethylene glycol 400 was used as sensitizer and a cationic exchange column was employed to remove interferences from cationic species. That problem was overcome in the dichlorvos determination (Wang 2001) taken advantage of the slower speed in the chemiluminiscent reaction of the pesticide compared with the matrix reaction of luminol-H_2O_2 catalyzed by metallic ions. Hence, the signal-to-noise ratio was enhanced five times by varying the flow rates from 3.9 to 0.5 mL min^{-1}. Phosphamidon in water was also analyzed using the same CL system with sodium dodecyl benzene sulfonate (SDBS) (Li 2009) or NaCl, (Hun 2005) as sensitizers. This latter substance was also employed as enhancer of the CL signal in the determination of monocrotophos (Du 2003), fenitrothion (Liu 2007b), phoxim (Liu 2007c) and methamidophos (Li 2006) using luminol/H_2O_2.

As it was previously mentioned, together with luminol, not only hydrogen peroxide, but several oxidants can be employed. For example, in the determination of chlorpyrifos (Song 2002) periodate was the oxidant chosen. It was immobilized together with luminol on

an anion-exchange resin; the method was applied to the determination of this pesticide in fruit without need of SPE systems or ionic exchange columns.

An interesting approach was developed by Baoxin et al. (Baoxin 2007). It was based on the fact that organophosphorus pesticides can be decomposed into orthophosphate with potassium peroxodisulphate as oxidant under ultraviolet radiation (Pérez-Ruiz 2001). The produced orthophosphate can react with molybdate and vanadate to form the vanadomolybdophosphoric heteropoly acid which can oxidize luminol to produce intense CL emission (Zui 2000). The decomposing kinetic characteristics of these pesticides are different and strongly dependant on their molecular structure. This fact provides the possibility for resolving their mixtures and enabling their quantitative analysis. This method was applied using a continuous flow system to the simultaneous determination of dipterex, dichlorvos and omethoate, which were model of P-C band, P-O band and P-S band organosphosphorus pesticides, respectively. Instead of eliminating the interfering species by a separation procedure, an artificial neural network, a powerful non-parametric non-linear modeling technique made possible the quantification of the analyte along with the interferences.

A fast, sensitive and cheap enzymatic method for the paraoxon determination was proposed by Danet et al. (Danet 2000). It was based on the use of a reactor with immobilized acetylcholinesterase (AChE) on controlled pore glass. The percentage of inhibition in enzyme activity after the passage of pesticide solution through the reactor was correlated to the pesticide concentration using the CL signal obtained through the reaction of thiocholine (produced from acetylcholine used as a substrate) with luminol in the presence of potassium ferricyanide.

Although most of organophosphorus determinations were carried out using luminol reaction, in a few of them, as buminafos (López Malo 2008), diazinon and malathion (Waseem 2007b), CL was obtained by the direct oxidation with permanganate in acidic medium. In the first case, the method involved the on-line photo-degradation of the analyte (stopped flow, 5 s) and its subsequent chemiluminescent oxidation by the potassium permanganate. CL from diazinon and malathion (Waseem 2007b) was sensitized by sulfite. Finally, the tris(2,2´bipyridine)ruthenium(II) system was used by Adcock et al. (Adcock 2004) for glyphosate determination.

CARBAMATE AND DITHIOCARBAMATE PESTICIDES

These compounds have the structure depicted in figure 3:

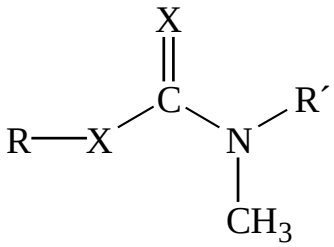


Figure 3. Structure of carbamate and dithiocarbamate pesticides.

Where R is an alcohol, oxime or phenol and R´ is hydrogen, a methyl group or N- or S-substituted moieties. Finally, X= O and S in carbamate and thiocarbamate pesticides, respectively. Carbamates inhibit acetylcholinesterase in both insects and mammals; their rapid biodegradation has resulted in a broad use as insecticides. Most of dithiocarbamates are used as fungicides and a few as herbicides (Hayes 1991).

As it is shown in table 2, many of these pesticides have been analyzed by direct oxidation with strong oxidants such as MnO_4^- or Ce (IV) in an acidic medium. In some cases the carbamate itself was directly oxidized, such was the case of carbaryl (Waseem 2007b)

(Murillo Pulgarin 2006), carbofuran (Waseem 2007b) (Xie 2005) and tsumacide (Liu 2007a). In those cases, CL emission was sensitized by the use of substances such as rhodamine 6 G or sulfite. In another carbamate determinations: carbaryl (Tsogas 2006), asulam (Chivulescu 2004), karbutylate, fenobucarb and isoprocarb (Amorim 2007); CL emission was achieved after irradiation with UV light, hence a photoproduct of them was the species which produced the analytical signal after reaction with the two above mentioned oxidants. In some cases, such as aldicarb (Palomeque 2004), ziram and zineb (López-Paz 2008) the CL emission provided by that photoproduct was very weak and it was necessary the addition of quinine in order to enhance the signal obtained.

Those photochemically induced chemiluminescence (PICL) methods have advantageous features from an analytical point of view, since the use of light as a reagent minimizes the environmental impact of analyses, avoiding the addition of large concentrations of pollutant reagents. Additionally, dilution of samples is avoided and improved sensitivity and selectivity together with greater simplicity and shorter analysis times are achieved. FI techniques are especially attractive for PICL-based methods (Catalá-Icardo 2008), since they allow irradiation time to be easily controlled. Because photoproducts are often unstable, flow methods improve reproducibility, increasing significantly throughput and automatability, while minimizing reagents consumption. The photoreactor design is of paramount importance in the manifolds; it comprises two elements, namely, the light source and the container holding the solution to be irradiated. Low-pressure Hg lamps and Teflon tubing helically coiled around them are usually chosen as light source and container respectively.

The determinations of aldicarb (Palomeque 2004), asulam (Chivulescu 2004), karbutylate, fenobucarb and isoprocarb (Amorim 2007) were carried out by using multicommutation. In that methodology the six-port rotary valve usually employed to insert the sample in FI analysis is replaced by solenoid valves. These devices, first introduced by Zagatto et al. (Reis 1994), have allowed to improve some of the inherent features of FI methods, such as low sample and reagents consumption, reproducibility and automatibility.

In the figure 4 are shown a typical FI manifold used for CL detection of pesticides using strong oxidants (A) and an assembly in which multicommutation mode is used to carry out the same determination (B). As can be observed the only differences are the replacement of the six-port rotary valve for solenoid valves and the position of the peristaltic pump, which aspirates the solutions. Solenoid valve V_1 allows to aspirate the sample solution only when necessary, which minimizes sample consumption, reducing the cost and environmental impact of the analysis. Moreover, the inserted volumes are controlled by the time the solution is allowed to circulate, which eliminates the need of changing the length of sample loop. It improves considerably the automatability of the system. Solenoid valve V_2 allows an optimum mixture between sample and oxidant by inserting small segments of both solutions; it results in a better reproducibility and sensitivity of the determination, as sample and reagent dispersion are effectively controlled.

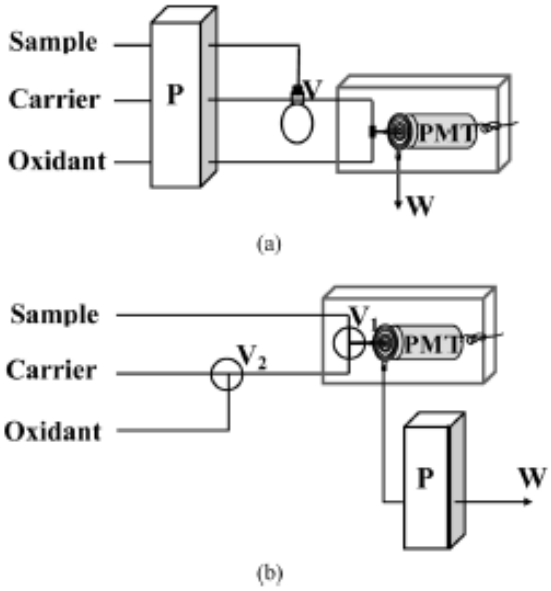


Figure 4: Typical Flow Injection manifolds with rotary (A) and solenoid valves (B).
P: peristaltic pump; V: six-port rotary valve; V_1, V_2: solenoid valves; W: waste; PMT: photomultiplier tube.

Carbaryl (Pérez-Ruiz 2003), propoxur (Pérez-Ruiz 2007), carbofuran and promecarb (Pérez-Ruiz 2002) could be analysed using the tris(2,2´bipyridine)ruthenium (II) system with a remarkable throughput of 200 injections h^{-1}. In that system, an orange emission centred at 610 nm results from $[Ru(bpy)_3^{2+}]^*$. A scheme of the followed mechanism is:

a) $Ru(bpy)_3^{2+} \rightarrow Ru(bpy)_3^{3+} + e^-$
b) $Ru(bpy)_3^{3+} + \text{analyte} \rightarrow [Ru(bpy)_3^{2+}]^*$
c) $[Ru(bpy)_3^{2+}]^* \rightarrow Ru(bpy)_3^{2+} + h\upsilon$

The determination of these pesticides was based on the on-line conversion of the pesticides into methylamine by irradiation with UV light. Methylamine was subsequently reacted with the reactive species $Ru(bpy)_3^{3+}$ generated through the on-line photoxidation of $Ru(bpy)_3^{2+}$ with peroxodisulfate. Perez Ruiz et al. (Pérez-Ruiz 2002, 2003 and 2007) used two photo-reactors in parallel for both processes; in one of them $Ru(bpy)_3^{2+}$ was generated by on-line photo-oxidation and in the other the photo-degradation of the pesticides to methylamine took place. Acetone showed a strong enhancing effect, which was investigated by changing the wavelength used. As a result of those studies it was concluded that acetone enlarged the triplet state population of the pesticides accelerating their photochemical reaction.

The oxidation of luminol in alkaline medium has been also used for carbamates determination. Hence, it has been demonstrated that carbaryl can produce a great enhancement of the CL emission from the luminol oxidation when is oxidized by potassium permanganate (Huertas Pérez 2004). That approach was also employed by the same authors to carry out carbofuran analysis (Huertas Pérez 2005). Carbaryl can also inhibit that CL from luminol when Co (II) as catalyst, and hydrogen peroxide as oxidant are present. Taking advantage of it, a fast method (120 injections h^{-1}) has been developed for its determination in water (Waseem 2007b). Apart from carbaryl and carbofuran, pirimicarb can also enhance CL from luminol, when it is present together with hydrogen peroxide and horseradish peroxidase (HRP). An analytical method based on this fact has been developed for the determination of that pesticide in fresh water (Navas-Díaz 2008).

Luminol reaction has been also employed for dithiocarbamate fungicides determination (Kubo 2003). The electron spin resonance measurements performed to explain the mechanism, showed that hydroxyl radicals, which are an important intermediate in luminol chemiluminescence, were generated from dithiocarbamate fungicides in the alkaline

solution. The use of light was proposed by Waseem et al. for the determination of the dithiocarbamates maneb, nabam and thiram (Waseem 2009). In that case, no oxidizing agent was used in the FI-CL manifold. The light was used for the deriving of the pesticide in NaOH medium, and then the photoproducts were reacted with luminol to generate CL. The authors suggest that this fact was possibly due to the generation of an excess of superoxide radicals, which increased the concentration of a hydroperoxide, intermediate required for completion of the luminol CL.

Probably due to the limited aqueous solubility and stability of bis-(2,4,6-trichlorophenyl) oxalate (TCPO) and bis-(2,4-dinitrophenyl) oxalate (DNPO), the peoxyoxalate reaction was not commonly employed to develop FI-CL methods for pesticide determinations. The only exception was the determination of carbaryl carried out by García Campaña et al. (Soto Chinchilla 2004). The procedure was based on the TCPO reaction, with previous off-line irradiation with UV light to carry out the hydrolysis of the carbaryl, yielding methylamine, which was derivatized with *o*-phthalaldehyde to form a fluorophore using imidazole as a catalyst. The method was accurate and selective.

Table 2. Analysis of carbamate and dithiocarbamate pesticides.

Pesticide	CL system	DR (µg L^{-1})	LOD (µg L^{-1})	Matrix (r, %)	Sample pretreatment	Reference
CARBAMATES						
Aldicarb	MnO_4^-/H_2SO_4/Quinine a/Fe(III)/SDS [b]	2.2-100	0.069	Water (98.1-98.7) Formulation (97.4)	Anionic exchange (IRA-900)	(Palomeque et al. 2004)
Asulam	MnO_4^-/H_2SO_4 [a]/Glycine buffer (pH 8.3) [b]	40-5000	40	Water (90.3-130)	NR	(Chivulescu et al. 2004)
Carbaryl	Ce(IV)/HNO_3/Rhodamine 6 G	50-2000	28.7	Water (93.2-96.1) Soil (97.5-98.4) Grain (94-5-98.1) Formulation (99.2-101.5)	SPE (Sep-Pak C18) Extraction with chloroform Dissolved in ethanol	(Murillo-Pulgarín et al. 2006)
	TCPO/H_2O_2/Imidazole/SDS/Acetone [b]+ O-phtaldehyde + N-acetylcisteine	26-650	31	Water (89-106) Vegetables (80.6-86.8)	NR Extraction with ethyl acetate Alumina cartridge	(Soto-Chinchilla et al. 2004)
	Luminol/H_2O_2/Co(II)	100-4024	47.7	Water (84-99)	NR	(Waseem, Yaqoob,
	$MnO_4^-/H_2SO_4/SO_3^{2-}$	100-2000	10	Water (85-106)	NR	(Waseem, Yaqoob, and
	MnO_4^-/H_2SO_4	10-1000	14.8	Water (75.0-99 .0)	NR	(Tsogas et al. 2006)

(Continued)

Table 2. *Continued*

Pesticide	CL system	DR (μg L^{-1})	LOD (μg L^{-1})	Matrix (r, %)	Sample pretreatment	Reference
	Luminol/MnO_4^-	5-100	4.9	Water (90.1-129.1)		(Huertas-Pérez et al. 2004)
				Cucumber (69.2-91.3)	Extraction with ethyl acetate Alumina cartridge	
	$Ru(bpy)_3^{2+}/S_2O_8^{2-}$/UV/ Acetone/ phosphate buffer (pH=6.5) [b]	40-4000	12	Water (90-110)	NR	(Pérez-Ruiz et al. 2003)
				Formulation (100.1)	Extraction with acetone	
				Soil (96-99)	Extraction with chloroform	
				Blood serum (98)	Extraction with ethyl acetate	
Carbofuran	$Ru(bpy)_3^{2+}/S_2O_8^{2-}$/UV Acetone/ Phosphate buffer (pH=6.5) [b]	220-11200	53	Water (99.5-102.0)	NR	(Pérez-Ruiz et al. 2002)
				Soil (99.8-102.0)	Extraction with chloroform	
	$MnO_4^-/H_2SO_4/SO_3^{2-}$	100-2000	50	Water (88-106)	NR	(Waseem, Yaqoob, and Nabi 2007)
	Ce(IV)/ H_2SO_4/SO_3^{2-}	80-10000	28.4	Cabbage (98.5-112.0)	NR	(Xie et al. 2005)
	Luminol/MnO_4^-	60-500	20	Water (93-137)	NR	(Huertas-Pérez et al. 2005)
				Lettuce (64-73)	Extraction with ethyl acetate Alumina cartridge	
Fenobucarb	MnO_4^-/Polyphosphoric acid/60 ºC [a]/NaOH [b]	NR	50	Water (99.6-105.1)	Water samples Anionic exchange (Duolite A-102D)	(Amorim et al. 2007)
				Human urine (95.1)	Cationic exchange (Duolite C20)	
Isoprocarb			30	Water (101.2-103.6)		
				Human urine (94.7)	Human urine samples SPE (Bond Elut C18)	
Karbutylate		20-20000	10	Water (100.6-102.3)		
				Human urine (99.4-100.9)		
Pirimicarb	Luminol/H_2O_2/HRP	4.25-30.75	0.12	Water (98.3-118.5)	NR	(Navas-Díaz et al. 2008)
Promecarb	$Ru(bpy)_3^{2+}/S_2O_8^{2-}$/UV/ Acetone/Phosphate buffer (pH=6.5) [b]	410-16600	85	Water (98.0-103.0)	NR	(Pérez-Ruiz et al. 2002)
				Soil (98.6-100.0)	Extraction with chloroform	
Propoxur		50-5000	5	Water (93-104)	SPE Octadecylsilica (DSC 18)	(Pérez-Ruiz et al. 2007)
				Fruit (85-92)	Extraction with dichloromethane SPE Carbon (Envicarb)	
				Vegetables (88-93)		
Tsumacide	MnO_4^-/HNO_3/Rhodamine 6 G	2-200	0.66	Vegetables (95.6-98.2)	Extraction with methanol	(Liu et al. 2007)
DITHIOCARBAMATES						
Mancozeb	Luminol/$Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$	10-10000	0.1	-	-	(Kubo et al. 2003)
Propineb			0.5			
Ziram			2			
Maneb	Luminol/NaOH [b]	10-4000	10	Water (100-103)	Chelax 100 or EDTA	(Waseem et al. 2009)
Nabam			8	Water (98-102)		
Thiram		10-1000	5	Water (98-101)		

(Continued)

Table 2. *Continued*

Pesticide	CL system	DR (μg L^{-1})	LOD (μg L^{-1})	Matrix (r, %)	Sample pretreatment	Reference
Thiram	Ce(IV)/H_2SO_4/Quinine	7.5-2500	7.5	Water (99-104)	SPE Octadecylsilica C18	(Waseem et al. 2010)
Zineb	Ce(IV)/H_2SO_4/Quinine/ NaOH[b]	5-200	1	Water (95.3-99.4)	Cationic exchange (Duolite C206A)	(López-Paz 2008)
Ziram		5-1000	1	Water (95.1-101.3)	Anionic exchange (IRA-400)	

DR: dynamic range; LOD: limit of detection.

AChE: acetylcholinesterase; $Ru(bpy)_3^{2+}$: tris(2,2´bipyridine)ruthenium (II); SDBS: sodium dodecyl bencene sulfonate; TCPO: bis-(2,4,6-trichlorophenyl) oxalate.

NR: not reported or unnecessary pretreatment; r: recovery rate; SPE: solid phase extraction.

a. Multicommutation.
b. Media used for pesticide photodegradation

OTHER PESTICIDES

In this section pesticides belonging to families other than organophosphorus, carbamates and dithiocarbamates have been included. Their structures and the chemical group they belong (see http://agrochemicals.iupac.org) are shown in table 3. They have very different chemical properties and uses, derived from those diverse chemical structures, consequently a variety of analytical methods have been applied to their determination. The fundamentals of most of them have been described in the former sections.

It is noteworthy the great number of pesticides analyzed by direct oxidation with strong oxidants. Such were the cases of bromoxynil (Pawlicová 2006) and antu (Murillo-Pulgarín 2005). For the determination of this pesticide, stopped-flow technique in a continuous flow system was used to record three quantitative parameters, namely, maximum emission intensity, total emission area and CL decay rate to define the analytical features of the method. In the analysis of 3-indolyl acetic acid (Pimentel Neves 2007), dimethylformamide and β-cyclodextrin were used as sensitizers. The role of the inclusion complexes formed between β-cyclodextrin and certain organic molecules as enhancers of luminescence quantum yield is well known (Santana-Rodríguez 2006). Sulphite and quinine were the sensitizers of choice in the dinoseb (Waseem 2007b) and diquat (López-Paz 2009) determinations respectively. In the bipiridilium herbicide analysis a remarkable throughput of 144 injections h^{-1} was achieved using a very simple instrumentation.

An important number of pesticides determination by direct oxidation with strong oxidants was carried out by Martínez Calatayud et al. coupling PICL as detection system with multicommutation flow methodology. That analytical strategy made possible the determination of the rodenticide strychnine (Alves-Ferreira 2007) and the herbicides: acrolein (Gamazo-Climent 2007), propanil and other pesticides of the same chemical family, namely: alachlor, flumetsulam, furalaxyl and ofurace (Albert-García 2006); benfuresate (Albert-García 2008), fluometuron (Sa 2007), chlorsulfuron (Mervartova 2005) and diphenamid (Czescik 2007). The PICL of this latter pesticide was predicted by applying molecular connectivity. Using this approach the structure of a molecule is mathematically described by means of topological descriptors. Their correlation with physical, chemical and biological properties can be used to predict the CL in liquid phase avoiding preliminary work with different oxidant systems, which can be time-consuming and expensive (Lahuerta-Zamora 2001) (Catalá-Icardo 2005) (Sahuquillo-Ricart 2007). Hence, after testing a large number of pesticides belonging to the substituted amide herbicides group, the equation obtained by Martínez Calatayud et al. predicted the PICL of those pesticides. Bearing in mind its relevance,

diphenamid was selected as a test-substance of the group to develop a new method of analysis.

Luminol reaction was the CL system of choice for the determination of DDT (Botchkareva 2002). *p*-Iodophenol was used together with luminol, hydrogen peroxide and horseradish peroxidase (HRP), since that substance increases significantly the intensity and duration of CL from luminol, particularly when HRP is employed as catalyst. Carbendanzim (Liao 2006) determination was carried out taking advantage of its enhancement effect on the CL emission intensity by the reaction of luminol with H_2O_2 in alkaline medium; standard addition method was used to analyze tap-water samples. A similar enhancement effect was played by the photoproduct of simetryn (Waseem 2008).

An important number of the pesticides showed in the table 3 have been determined using flow injection immunoassays (FIIA). The automatability of the immunoassays has been greatly increased thanks to the use of FI, which decreases the analysis time by reducing the cleaning steps and, at the same time, increasing the reusability of the biosensors. CL has allowed the ultrasensitive detection of labels in immunoassays (Baeyens 1998) (Roda 2004) because it provides similar, or even better, sensitivity and detectability than radioisotopes with rapid measurement and without need of lengthy incubations.

Atrazine is one of the herbicides more widely used. Because of it, a great concern about its presence in the environment has been generated, and thus it is that a great number of analytical methods have been developed for its determination. Hence, Jain et al. (Jain 2004) developed a fast and sensitive immunosensor for the determination of atrazine using a protein G modified disc composite from monolithic metacrylate and polyethylene. The sensitive HRP catalysed enhanced chemiluminescence reaction of luminol, H_2O_2 and *p*-iodophenol (PIP) was used for monitoring the HRP-label.

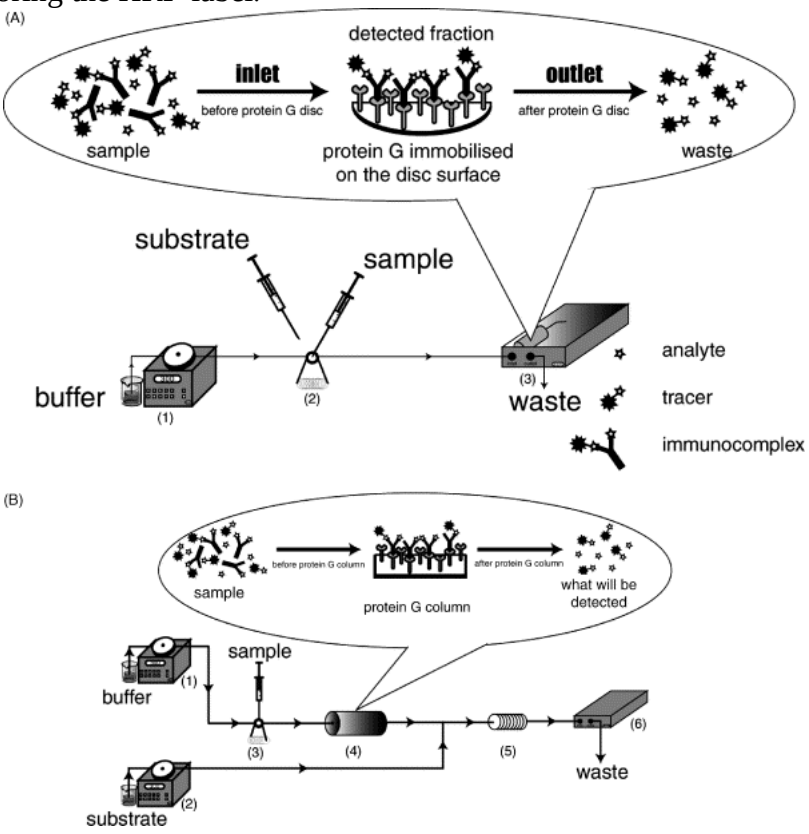


Figure 5: Flow immunoassay systems.
(A) disc-based system: (1) peristaltic pump; (2) injection valve with 36 μL loop; (3) CL detector, containing flow cell with a protein G disc.
(B) Column-based system: (1) and (2) peristaltic pumps for carrier and substrate buffers; (3) injection valve with 100 μL loop; (4) protein G column (V=200 μL); (5) reaction coil; (6) CL detector. (Jain 2004)

Two manifolds were assayed; in one of them depicted in figure 5A, a protein G disc flow immunosensor was inserted in the flow cell, and both, sample and substrate were injected via rotary valves. In the other manifold, shown in the figure 5B, that immunosensor was in a column outside of the detector, the substrate was continuously flowing and only the sample was injected. The sensitivity of the method was ten-fold higher and the selectivity was significantly improved when the immunosensor was inserted in the flow cell; although the multiple assay steps that are necessary resulted in a lower sample throughput (10 versus 15 samples h^{-1}).

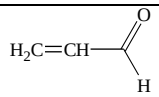
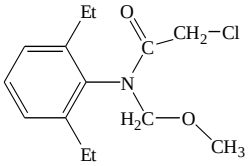
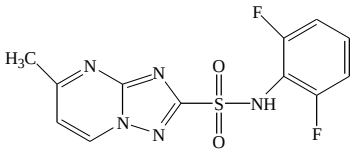
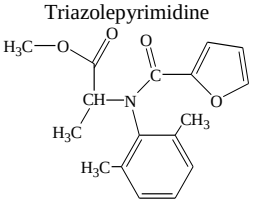
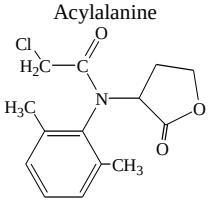
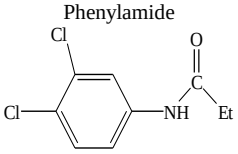
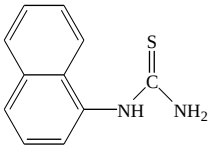
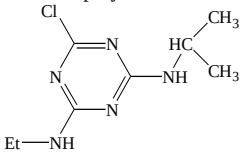
Yakovleva et al. (Yakovleva 2002) reported other semi-automated immunoassay method for atrazine analysis. A mixture of the pesticide was passed over a silicon chip surface with an anti-atrazine antibody immobilized and then an enzyme substrate was injected to quantify the tracer concentration bound on the immune active surface, which allowed the atrazine determination. In the determination of the herbicide atrazine carried out by Tudorache et al. (Tudorache 2007), the antibody immobilization on a magnetic bead together with the use of a magnetic field allowed the sensitivity to be significantly increased due to the correct orientation of the antibodies. Other advantages achieved were: more specific area for the binding of larger amounts of biomolecules and increased stability of the surface-bound antibody. Another FI immunosensor for field analysis was developed by Ciumasu et al. (Ciumasu 2005) for atrazine, diuron and TNT determination. The antibody was in that case, immobilized on a gold surface of pyramidal structures inside an exchangeable single-use chip.

The tris(2,2´bipyridine)ruthenium (II) system was used by Beale et al. (Beale 2009) for atrazine determination in waters by FI analysis; prior to analysis, SPE was necessary in order to remove natural organic matter, which caused a significant positive chemiluminescent response. The same CL system was employed for morpholine fungicides, dodemorph and tridemorph (González 2000) analyses. In the morpholines determination, two strategies of $Ru(bpy)_3^{3+}$ generation were assayed, by oxidation with cerium (IV) and electrochemically. Although ECL was the more elegant method for analysis, it was less tolerant to methanol, which supposed a serious drawback, as it was the substance employed as extractant.

Another example of the widely employed FIIA determinations is the regenerable immunobiosensor developed for the chemiluminescent analysis of the herbicide 2,4-D (Marquette 2000). The detection was obtained within 20 min, which is a low assay duration when considering immunochemical assays. The determination of this herbicide has been also carried out substituting the antibody by a molecular imprinted polymer (MIP) (Surugiu 2001) which improves considerably the stability, ease of preparation and cost of the biosensor.

Finally, imidacloprid determination (Lagalante 2007) was based on a gas-phase reaction between nitric oxide and ozone. UV photochemical dissociation of the pesticide yielded nitrite, which was reduced to nitric oxide by iodide and removed from the aqueous stream with a membrane separator before mixing with ozone. The method exhibited linear response over four orders of magnitude (results in table 3 were calculated for 1 mL of sample) with improved selectivity with regard to enzyme linked immunoassays (ELISA).

Table 3. Analysis of other pesticides.

Pesticide	Structure and chemical group	CL system	DR (μg L^{-1})	LOD (μg L^{-1})	Matrix (r, %)	Sample pretreatment	Reference
Acrolein	 Aldehyde	MnO_4^-/Polyphosphoric acid [a]/ Ethanol [b]	5-100	0.1	Water (99.2-104.3) Soil (103.6-105.4) Human urine (101.5-102.4)	Anionic exchanger (Duolite A-102D) Cationic exchanger (Duolite C20) SPE (Bond Elut C18 or SAX)	(Gamazo-Climent et al. 2007)
Alachlor	 Chloroacetamide	MnO_4^-/H_2SO_4 [a]/ Acetic-acetate buffer (pH 4.8) [b]	NR	41	Water (95-100)	Anionic exchange (Amberlite XAD-4) Cationic exchange (Duolite C20)	(Albert-García et al. 2006)
Flumetsulam	 Triazolepyrimidine			25	Water (97-105)		
Furalaxyl	 Acylalanine			34	Water (95-102)		
Ofurace	 Phenylamide			58	Water (96-102)		
Propanil	 Anilide		10-5000	8	Water (97.1-102.9) Formulation (98.8)		
Antu	 1-naphtylthiourea	MnO_4^- /H_2SO_4/Formaldehyde [c]	50-3000	5	Water (99.9-100.7) Grain (91.4-104.9)	SPE (Sep Pak C18) Extraction with acetone + SPE (Sep Pak C18)	(Murillo-Pulgarín et al. 2005)
Atrazine	 Triazine	$Ru(bpy)_3^{2+}/H_2SO_4$	2.15-2150	0.014	Water (83.9-114.9)	SPE (Bond Elut C18)	(Beale et al. 2009)
		Luminol/H_2O_2/HRP	NR	0.2	NR	NR	(Ciumasu et al. 2005)
		Luminol/H_2O_2/HRP/PIP	10^{-5}-10^{-3}	$3 \cdot 10^{-6}$	NR	NR	(Tudorache et al. 2007)
			0.8-8	0.017	Tap water	NR	(Jain et al. 2004)
			0.06-0.4	0.038	Surface water	NR	
			0.002-2.1	0.0008	NR	NR	(Jakovleva et al. 2002)

CONCLUSIONS

The FI-CL based determinations of pesticides carried out during the last ten years have been presented. The number of pesticides and variety of samples analyzed with good recoveries have demonstrated the versatility of this approach. Another relevant analytical features achieved were the high sensitivity and automatability provided by the CL detection and FI techniques, respectively.

Nevertheless, advances are still required to improve the selectivity and applicability of these methods. The use of light as reagent in the PICL methods can enlarge considerably the number of pesticides which could be analyzed. The selectivity of the proposed methods can be greatly improved by a judicious choice of SPE systems and/or ionic exchangers. Molecular connectivity and multicommutation are very promising tools. Hence, on the one hand, topological descriptors can be employed to predict CL avoiding expensive and time consuming screening procedures. On the other hand, the use of solenoid valves can increase the automation of the developed systems. Bearing in mind that all these approaches have been still scarcely used, it can be predicted a great potential for the application of FI-CL methods to environmental samples.

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