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ARTICLE *in* TALANTA · MAY 2007

Impact Factor: 3.55 · DOI: 10.1016/j.talanta.2006.10.048 · Source: PubMed

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Theoretical prediction of the photoinduced chemiluminescence of pesticides

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Received 3 May 2006; received in revised form 10 October 2006; accepted 20 October 2006
Available online 8 December 2006

Abstract

Although it is relatively easy to find chemiluminescent (CL) molecules working on the field of direct liquid phase (especially employing strong oxidants), the molecules found as chemiluminescent are normally very weak CL compounds for developing suitable analytical CL-procedures. Therefore, it is mandatory to develop new strategies to enhance in a simple way the native chemiluminescence of such a compounds, and even to increase the number of compounds to be determined by direct chemiluminescence. Photoinduced chemiluminescence (Ph-CL) results in a simple and easily on-line accessible strategy to solve these disadvantages. In the present paper**,** molecular connectivity, a topological method which allows an unique mathematical characterization of molecular structures by the so-named topological descriptors and their correlation with physical, chemical and biological properties of molecules was applied to predict the Ph-CL in liquid phase. Molecular connectivity calculations and discriminant analysis was applied to 72 pesticides for which either a Ph-CL or non Ph-CL behaviour was observed in an experimental screening. The screening test is based on the on-line photodegradation of pesticides by using an automated multicommutation based flow asssembly provided with a photoreactor consisting of 150 cm × 0.8 mm PTFE tubing helically coiled around a 20 W low-pressure mercury lamp. Photodegraded pesticides are detected by direct chemiluminescence of the resulting photo-fragments and their subsequent reaction with potassium permanganate in sulfuric acid medium as oxidant. The screening comprised pesticides with different molecular structures and relevant members of the most important families of pesticides were tested (oxime carbamates, sulfonylcarbamates, thiocarbamates, 1,3,5-triazines, organophosphorous, hydroxybenzonitrile, sulfonylureas, phosphonic acid derivatives, imidazolinones, carboxamides, aryloxyalkanoic acids, 1,2,4-triazinones, etc.). The theoretical predictions agree with the empirical results obtained by means of the screening test performed in the multicommutation flow-assembly.

Keywords: Pesticides; Multicommutation; Molecular connectivity; Chemiluminescence; Photodegradation

1. Introduction

Chemiluminescence research is in continuous expansion by virtue of the search on new processes allowing the direct chemiluminescence-based determination of substances of pharmaceutical, clinical or environmental interest [1,2]. In this sense, direct chemiluminescence methods based on strong oxidant such as potassium permanganate have found a wide range of analytical applications [3,4].

Luminescence techniques for organic pesticides residue analysis have been limited by the fact that, relatively few of these compounds are strongly luminescent. This can be overcome converting the weakly luminescent pesticide into a luminescent compound by means of a chemical derivatization using fluorogenic labeling [5–7]. A more recent and relatively widely employed approach based on UV irradiation of non-fluorescent analytes into strongly fluorescent photoproducts has demonstrated the viability of photochemically induced fluorimetry in pesticide quantitative analysis [8]. On the other hand, it

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doi:10.1016/j.talanta.2006.10.048

has been established that the irradiation of photoreactive analytes leads to the formation of species that can be detected by CL providing very sensitive procedures [9–11]. Between the luminescent techniques it is remarkable the scarcely use of the chemiluminescent (CL) detection in the determination of pesticides. It is also to emphasize that in spite of the well demonstrated approach UV irradiation-photochemical reaction of pesticides [12], only a few works dealing with photodegradation and chemiluminescent detection have been published [13–16].

On the other hand, molecular topology and particularly molecular connectivity has widely demonstrated its ability for an easy and efficient characterization of molecular structure through the so-called topological indices. In this mathematical formalism a molecule is assimilated to a graph, where each vertex represents one atom and each axis one bond. Starting from the interconnections among the different vertexes, an adjacency topological matrix can be built whose elements *ij* take the values 1 or 0, depending whether the vertex *i* is connected to the vertex *j* or not, respectively. The manipulation of this matrix gives origin to a set of topological indices or topological descriptors. When these indices are selected adequately, it is possible to have a very specific characterization of each chemical compound [17–21]. Molecular connectivity has been tested on several properties of different classes of organic, bioorganic and inorganic compounds. This topological method has been applied to the determination of biological activities [22] and physical [23] and chemical properties [24].

Molecular connectivity has been successfully employed in the design of drugs, bronchodilator [25] and antimalarial compounds [26], hypoglycaemic agents [27], anti-neoplastics [28], cytostatic agents [29], antibacterial agents [30] and new antihistaminic compounds [31,32] have been found by means of molecular connectivity. Physico-chemical properties such as soil adsorption coefficients [33], water solubility and boiling points [34] have been tested by molecular connectivity. Also, molecular connectivity has been applied to the prediction of analytical properties, basically to chromatographic processes [35–38] and recently to liquid-phase chemiluminescence [39–41].

The work presented in this paper was mainly focused to put into the analytical chemistry fields the molecular connectivity as a new and useful tool to enhance the yield of the analytical research. The immediate purpose of the present work was to develop a simpler assay for determination of pesticides using a multicommutation flow system coupled to photochemically induced CL. The method allows on the basis of the UV irradiation and chemiluminometric detection of photoproducts the determination of pesticides which present very weak or null native chemiluminescence. The viability of this purpose was supported by molecular connectivity calculations. The appropriated selection of topological descriptors allows the prediction of pesticides properties, namely, tendency to photodegradation and chemiluminescent behaviour of photofragments. A theoretical discrimination of the Ph-CL activity of a pesticide is possible, and consequently, to develop new and suitable Ph-CL analytical procedures.

2. Experimental

2.1. Reagents

All reagents were analytically pure unless stated otherwise and prepared in deionized water (18 MΩ cm) using a Sybron/Barnstead Nanopure II water purification system. Pesticides were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Mineral acids and alkalis, $KMnO_4$, H_2O_2, tested in preliminary assays (all from Panreac, Barcelona, Spain), $Fe(NO_3)_3{\cdot}9H_2O$ (Probus, Barcelona, Spain), $FeSO_4{\cdot}7H_2O$ (Fluka, Buchs, Switzerland).

2.2. Apparatus

The flow manifold used (see Fig. 1) comprised a PTFE coil of 0.8 mm i.d. and a Gilson Minipuls 2 (Worthington, OH, USA) peristaltic pump. For the fully automated manifolds three Model 161T031 solenoid valves (NResearch, Northboro, MA, USA) were used. The solenoid valves were connected to a laboratory-made interface type KSP. Its actuation was programmed using a home made Solenoid Valves software running on a Pentium-type computer in Microsoft Windows 98. The programme and interface allow an independent control of the solenoid valve, the sequence of insertions and the number of cycles according to the number of samples, reagent solutions or standards to be inserted. The photoreactor consisted of a 150 cm length and 0.8 mm i.d. PTFE tubing helically coiled around a 20 W low-pressure mercury lamp (Zalux) for germicidal use. The flow cell was a flat-spiral quartz tube of 1 mm inner diameter and 3 cm total diameter backed by a mirror for maximum light collection. The photodetector package was a P30CWAD5F-29 Type 9125 photomultiplier tube (PMT) supplied by Electron Tubes operating at 1280 V and was located in a laboratory-made light-

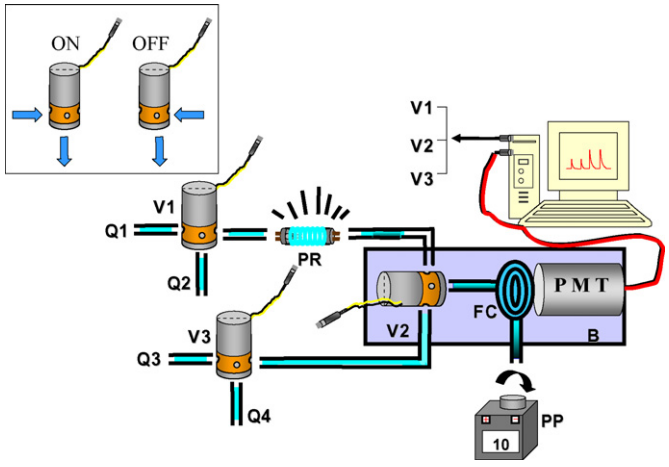

Fig. 1. Solenoid valve flow-assembly. Q_1: pesticide aqueous solution; Q_2: medium of photodegradation; Q_3:oxidant ($KMnO_4$ 7×10^{-4} M in H_2SO_4 2 M); Q_4: H_2O. V_1, V_2 and V_3: solenoid valves; PR: photoreactor; PP: peristaltic pump (flow-rate 10 mL min^{-1}); PR: photoreactor (150 cm length and 0.8 mm i.d. PTFE tubing helically coiled around a 20 W low-pressure mercury lamp (Zalux) for germicidal use); PMT: photomultiplier tube (1280 V); FC: spiral flow-cell (flat-spiral quartz tube of 1 mm inner diameter and 3 cm total diameter backed by a mirror for maximum light collection); B: light-tight box.

tight box. The output was fed to a computer equipped with a counter-timer, also supplied by Electron Tubes.

2.3. Procedures

2.3.1. Stock solution preparation

Stock standard solutions of pesticide (50 μg mL^{-1}) were prepared by exactly weighing and dissolving the pesticide in deionized water. The working standard solutions were freshly prepared by diluting the stock standard solution in the appropriate volume of deionized water. All solutions of pesticides were protected against light.

2.3.2. Analytical measurements and solenoid valve flow-assembly

The peristaltic pump was placed after the detector and the sample and reagent streams were driven to the detector flow-cell by aspiration at a flow-rate of 10 mL min^{-1}. The manifold is constituted of a set of three solenoid valves, each one acting as an independent commutator (see Fig. 1). The way of work of a valve can be described as follows: $N(t_1, t_2)$, where t_1 is the time of valve in ON, t_2 the time of valve in OFF, and N is the number of cycles ON/OFF. Changes in the manifold configuration affected only the number and length of pulses (time ON/time OFF) applied to each solenid valve. The reconfiguration of the flow system was thus a logical reconfiguration via software. The insertion profile for obtaining a typical transient analytical signal in the screening procedure is depicted in Fig. 2. First, 50 alternated microinsertions of pesticide and medium of photodegradation were performed. During each microinsertion V_1 remains activated during 0.4 s (valve ON, the pesticide Q_1 is aspirated), and deactivated during 0.2 s (valve OFF, medium of photodegradation Q_2 is aspirated). During the 30 s that the sample insertion takes place, V_2 remains in ON, allowing that the photoreactor fills of the mixture pesticide-medium of photodegradation. This loading time also is used for washing the inner walls of the photoreactor avoiding contamination between samples. Then V_1 and V_2 are switched simultaneously and remained OFF during 150s of stopped-flow (time of UV photoirradiation). Previously to the chemiluminescent reaction (t = 165 s, see Fig. 2), chemiluminescent reagent ($KMnO_4$ (Q_3)) is flowing by switching ON valve V_3. After the stopped flow, V_2 is activated and 30 alternated microsegments of photodegradated pesticide and chemiluminescent reagent are inserted. A chemiliminometric response is obtained and the transient analytical signal returns to the base line ready for a new cycle.

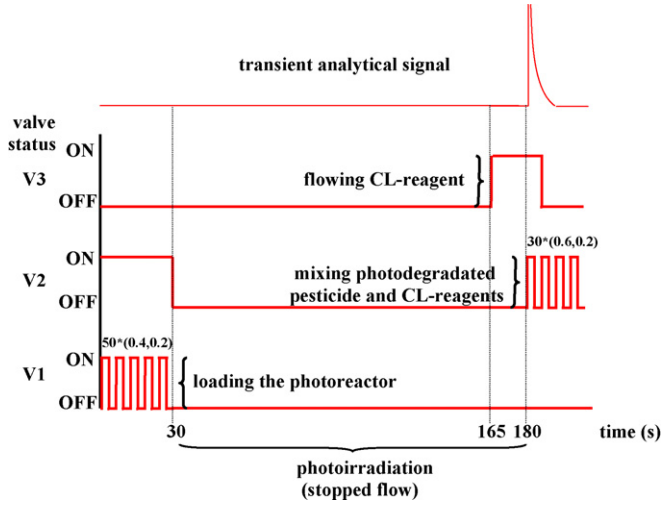

Fig. 2. Schematic profile of a multi-insertion cycle. $N(t_1,t_2)$. N: number of insertions. In each insertion the solenoid valve is t_1 seconds ON and t_2 seconds OFF. [V_1 = 50(0.4,0.2); V_2 = 30(ON), 165(OFF), 30(0.6,0.2); V_3 = 165(OFF), 39(ON)].

2.3.3. Molecular connectivity and topological descriptors

Molecular connectivity has demonstrated to be an excellent tool for a quick and accurate prediction of many analytical, physicochemical and biological properties. One of the most interesting advantages of molecular connectivity is the straightforward calculation of molecular descriptors to work with.

In this mathematical formalism a molecule is assimilated to a graph, where each vertex represents one atom and each axis one bond. Starting from the interconnections among the different vertexes, an adjacency topological matrix can be built up, which elements ij, take the values either 1 or 0, depending upon the vertex i is connected to the vertex j or not, respectively. The manipulation of this matrix gives origin to a set of topological indices or topological descriptors, which characterize each graph and they can be used to perform QSAR studies as well.

In this work we use several graph-theoretical descriptors like the connectivity indices χ up to the order 10—including descriptors type path, cluster, path-cluster and chain [42], topological charge indices G_i, J_i until order 5 [43] tom type electrotopological state indices 49 [44,45] other simple descriptors such as the number of atoms and ramifications, the Wiener index, etc. All these descriptors, more than 140, were obtained for each compound, using the programs Molconn [46] and Desmol11 [47].

Table 1 depicts the indices used in this work, definition and the references describing their calculation in detail. All descriptors were computed from the adjacency topological matrix obtained from the hydrogen depleted graph.

2.3.4. Linear discriminant analysis

Stepwise linear discriminant analysis, SLDA, is a useful technique for finding discriminant functions. It is a pattern-recognition method, which permits (facilitates), by combining variables, the evaluation of the ability to distinguish among two or more groups of populations. In our work the independent variables were the topological indices and the discrimination property was the chemiluminescent activity. The SLDA study is performed with two groups of compounds, the training group, which includes compounds with photoinduced chemiluminescence (active), and not chemiluminescent compounds even by on-line UV-photoirradiation (inactive), facilitating the discovery of the discriminant function, and the test group (also with active and inactive structures, randomly chosen from the training group), which facilitates the evaluation of the quality of the discriminant function obtained. Election of connectivity functions was performed with the BMDP Biomedical package [50]. The method used for selection of the descriptors was the F-Snedecor

Table 1
Symbols and definitions of topological indices used in this work

Symbol	Name	Definition	References
${}^{\kappa}\chi_{\tau}$ (k = 0–4; t = p, c, pc)	Randić-like indices of order k and type path (p), cluster (c) and path-cluster (pc)	${}^{k}\chi_{t} = \sum_{j=1}^{{}^{k}n_{t}} \left(\prod_{i \in S_j} \delta_i \right)^{-1/2}$ δ_I, number of bonds, σ or π of the atom i to non-hydrogen atoms. S_j, jth sub-structure of order k and type t	[42]
${}^{k}\chi_{t}^{v}$ (k = 0–4; t = p, c, pc)	Kier–Hall indices of order k and type path (p), cluster (c) and path-cluster (pc)	${}^{k}\chi_{t}^{v} = \sum_{j=1}^{{}^{k}n_{t}} \left(\prod_{i \in S_j} \delta_i^{v} \right)^{-1/2}$ δ_i^{v}, Kier–Hall valence of the atom i. S_j, jth sub-structure of order k and type t	[42]
${}^{k}D_{t}$ (k = 0–4; t = p, c, pc)	Connectivity differences of order k and type path (p), cluster (c) and path-cluster (pc)	${}^{k}D_{t} = {}^{k}\chi_{t} - {}^{k}\chi_{t}^{v}$	[42]
G_k (k = 1–5)	Topological charge indices of order k	$G_k = \sum_{i=1}^{N-1} \sum_{j=i+1}^{N} \lvert \mathbf{M}_{ij} - \mathbf{M}_{ji} \rvert \delta(k, \mathbf{D}_{ij})$ $\mathbf{M} = \mathbf{AQ}$, product of the adjacency and inverse squared distance matrices for the hydrogen-depleted molecular graph. $\mathbf{D}$, distance matrix; δ, Kronecker delta	[43]
G_k^{v} (k = 1–5)	Valence topological charge indices of order k	$G_k^{v} = \sum_{i=1}^{N-1} \sum_{j=i+1}^{N} \lvert \mathbf{M}_{ij}^{v} \mathbf{M}_{ji}^{v} \rvert \delta(k, \mathbf{D}_{ij})$ $\mathbf{M}^{v} = \mathbf{A}^{v}\mathbf{Q}$, product of the electronegativity-modified adjacency and inverse squared distance matrices for the hydrogen-depleted molecular graph. $\mathbf{D}$, distance matrix; δ, Kronecker delta	[43]
J_k, J_k^{v} (k = 1–5)	Pondered topological charge indices of order k	$J_k = \frac{G_k}{N-1}$, $J_k^{v} = \frac{G_k^{v}}{N-1}$	[43]
${}^{m}\kappa$	Kappa index	${}^{m}\kappa = m \frac{{}^{m}P_{\max} {}^{m}P_{\min}}{({}^{m}P)^2}$, defined in terms of the number of graph vertices A and the number of paths ${}^{m}P$ with length m = 1, 2 and 3	[48]
S_i	Sum-electrotopological indexes type	$S_i = I_i + \Delta I_i$	[44,45]
V_i	V_3 and V_4	Number of graph vertices with topological valence 3 or 4	[49]

Topological descriptors were calculated for each compound by using Molconn-Z [46] and DESMOL11 [47] programs.

parameter, and the classification criterion used was the minimum value of the Mahalanobis distance. The quality of the discriminant function is evaluated through Wilk's U-statistical parameter.

By applying the topological pattern to the whole group, a distribution pattern [51] can be constructed to represent the expectancy for each classification group in each function interval. In general, the expectancy for a group A over an interval x is defined mathematically as:

$$E_{\mathrm{a}} = \frac{\text{percentage of A in } x}{\text{percentage if non-A in } x + 100}$$

E_{a} and E_{i} denoting activity expectancy and inactivity expectancy, respectively, in our case.

3. Results and discussion

3.1. Experimental screening in a multicommutation flow-assembly

Aldicarb[13] was employed as a test substance and prior to a new screening session the system was checked with the aid of a 50 μg mL^{-1} aqueous solution of aldicarb and NaOH as medium of photodegradation. First, the homogeneous-phase photodegradation of pesticides in different media and combined with chemiluminescence detection of the photoirradiated analyte was studied. The study of media was focused on chemical species forming after UV irradiation hydroxyl radicals, which react with organic pollutants in a non selective manner and lead to an efficient photocatalyst for pesticide degradation [52]. NaOH, Fe(II) and H_2O_2 employed in the photo-Fenton reaction, and Fe(III) aquacomplexes described as an efficient photocatalytic system for the mineralisation of pesticides by sunlight irradiation [12,53] were tested.

The tandem-flow assembly used is depicted in Fig. 2. The sample solution Q_1 (blank or pesticide aqueous solution, 50 μg mL^{-1}) was mixed with the photodegradation medium solution Q_2 (H_2O, 10^{-3} NaOH, 0.05% H_2O_2, Fe(III) 6×10^{-5} M or Fe(II) 6×10^{-5} M). The mixture was obtained by means of V_1 (50(0.4, 0.2)). After the alternated microinsertions of pesticide and medium, V_1 switched OFF and the flow was stopped 150 s. Then, V_2 was activated (30(0.6, 0.2)) and 30 alternated insertions of photoirradiated pesticide (V_2 ON, 0.6 s) and oxidant solution Q_4 ($KMnO_4$ 5×10^{-4} M + H_2SO_4 2 M, V_2 OFF, 0.2 s) were performed. V_3 worked as follows: (165,39). Water (Q3) was aspirated the first 165 s of the cycle, then V_3 switched ON and the last 39 s of the cycle (Q4) was aspirated according to V_2 configuration. All pesticides were tested with the lamp OFF and ON. All solution were aspirated at a flow rate of 10 mL min^{-1}.

The criteria for selecting the suitable chemical and physico-chemical variables for the screening were established employing aldicarb[13] as test substance.

The effect of the time of exposure to UV light was studied by changing the time of stopped-flow (time of V_2 in OFF). The analytical signal was found to increase with increasing irradiation time as a result of an increased photodegradation yield. The increase in irradiation time resulted in an increase in the output up to around 120 s; then, the increase was small tending to a plateau. The signal was found to level off after 180 s. A irradiation time of 150 s was chosen for the screening.

The selected insertion profile was: $V_1 = 50(0.4,0.2)$; $V_2 = 30(ON),165(OFF)$, $30(0.6,0.2)$; $V_3 = 165(OFF)$, $39(ON)$ (see symbols in Section 2.3.2). The insertion of a large sequence of sample–medium segments (N) was essential due to the large length of the photo-reactor (150 cm) and to ensure an effective mixture of the photo-reactor effluent with the mixture of the oxidant for the chemiluminometric process. Values of $t_1/t_2 > 1$ in V_1 and V_2 avoided the excessive dilution of the pesticide in the flow system. Excellent reproducibility was obtained for times of insertion as short as 0.2 s, equivalent to the insertion of 32 μL of solution.

Flow rates lower than 6 mL min^{-1} were discarded due to the fast kinetic of the chemiluminescent reaction. The increase in flow rate resulted in an increase in the output up to around 9.5 mL min^{-1}; then, a plateau was obtained. The selected flow rate for aspirating sample and reagent solutions was 10 mL min^{-1}.

Acidic permanganate system gives rise to light emission for many compounds and was selected for the present screening. In fact, it is presented as the most efficient oxidant for direct liquid phase chemiluminescent processes. A search employing the analytical abstract data base (1980–2005) and the key words chemiluminescence and the oxidants $KMnO_4$, Ce(IV), $Fe(CN)_6^3$, *N*-bromosuccinimide yielded as conclusions that 42% of the published papers used potassium permanganate as strong oxidant. The reason of this behaviour is associated to the mechanism of chemiluminescence generation by potassium permanganate. Several authors [4] have contributed to explain it. According to Barnett, it seems the emitter responsible for acidic potassium permanganate chemiluminescence is an excited manganese (II) species of unknown constitution. This hypothesis seems to be verified by enhancing effect of polyphosphates on the chemiluminescent emission using KMO_4 as oxidant. The excellent behaviour of potassium permanganate associated to direct chemiluminescence procedures should be due to an unusual case of phosphorescence at room temperature.

On the other hand, the behaviour of permanganate requires special care in selecting its concentration. The signal shows a parabolic profile increasing sharply with concentration up to a maximum value beyond which it decreases abruptly. Attending to the robustness of the procedure, 7×10^{-4} mol L^{-1} $KMnO_4$ in 2 M H_2SO_4 was selected for the screening.

Attending to the observed Ph-CL response, pesticides can be divided into two groups: (a) compounds which do not present chemiluminescence with lamp OFF and ON; (b) CL-pesticides which either increased dramatically the CL-response with lamp ON, or turns into chemiluminescent ones after irradiation.

From an analytical point of view the most interesting Ph-CL effects are included in group (b) (see Tables 1 and 2) and they are suitable for developing new and sensitive chemiluminescent analytical procedures. Following comments are focused on this group.

In general, a significant increase in the chemiluminescent emission intensity was observed with lamp ON [I(ON)/I(OFF) ratios] for reference compounds in different media were: asulam (46.7), ethoprophos (43.4), imazamethabenz-methyl (45), all in 10^{-3} M NaOH medium; aldicarb (87.5), ethoprophos (500), MCPA (125) and propoxur (150), all in 6×10^{-5} M Fe(III); azamathiphos (50), bromoxymil (300), monolinuron (117), imazamethabenz-methyl and imazapyr (60), in 0.05% H_2O_2; aldicarb (58), ethoprophos (300) and ametryn (36) in aqueous solution; aldicarb (70), EPTC (57), ethoprophos (40), MCPA (125) and oxadixyl (45), all 6×10^{-5} M in Fe(II). Only aldicarb in H_2O_2, methomyl in NaOH and H_2O_2 media, and chlorpropham in H_2O_2 and aqueous media yielded negative results with lamp ON and OFF. The only pesticide for which a diminution of the chemiluminescence behaviour was observed after irradiation was azamethiphos in NaOH medium. Ametryn, aminocarb, carbetamide, chlorfenac, dichloprop, endothal, hymexazole, imazapyr, isocarbamide, metalaxyl, metazachlor, monolinuron, nicotine, phosphamidon, 2,4,5-T, thiofanox and tribenuron did not presented native chemiluminescence in at least four of the tested media, but turned into strong chemiluminescent compounds in all media after irradiation (Table 3).

Attending the media of photodegradation, H_2O_2 provided the best analytical signals (number of counts) after irradiation with the low-pressure mercury lamp. Nevertheless, the effectiveness of this medium on the chemiluminescent response was also confirmed with lamp OFF; only 25% of the pesticides tested with H_2O_2 did not present chemiluminescence with lamp OFF and they did with lamp ON. This percentage increased remarkably in the rest of media. About 65, 68, 75 and 57% of pesticides which did not present chemiluminescence or very weak chemiluminescence behaviour (close to zero) with lamp OFF, were clearly chemiluminescent after UV-irradiation in NaOH, Fe(III), aqueous solution and Fe(II) medium, respectively. The best results (compromise between chemiluminescence intensity and effective conversion of non-chemiluminescent pesticides into chemiluminescent ones) were obtained employing Fe(III) as photocatalyst.

Although the Ph-CL study was applied to a hetereogeneous group of pesticides, some families of pesticides are widely represented, so that the influence of the chemical structure on the Ph-CL behaviour of pesticides can be studied by comparing the analytical signal for structurally related compounds (*e.g.* carbamates, ureas, organophosphorus, 1,3,5-triazines and imidazolinones). Imidazolinone pesticides imazapyr, imazaquin and imazamethabenz presented good Ph-CL behaviour; nevertheless imazethapyr, differing from imazapyr in a ethyl radical bonded to the *N*-heterocyclic ring, yielded a negative response against UV-irradiation. Opposite to this behaviour are the 1,3,5-triazines. The non-chemiluminescent character of atraton, prometon

Table 2
Results obtained in the LDA study and classification of the compounds from pattern of induced chemiluminescence activity proposed

Compound	DF[a]	Probability[b]	Class[c]
Training group—active			
Aminocarb	5.77	0.997	+
Benzatone	7.35	0.999	+
Carbetamide	8.03	1.000	+
Cinosulfuron	3.26	0.963	+
Dichlorprop	4.71	0.991	+
Endothall	2.00	0.881	+
2,5,4-T	3.18	0.962	+
Hymexazol	−0.40	0.400	−
Imazapyr	2.10	0.891	+
Isocarbamide	−4.15	0.016	−
MCPB	3.33	0.965	+
Metalaxyl	6.72	0.999	+
Metsulfuron methyl	3.45	0.969	+
Nicotine	4.67	0.991	+
Phosphamidon	−0.82	0.306	−
Azamethiphos	2.72	0.938	+
Bromoxynyl	4.01	0.982	+
Chlorpropham	5.20	0.995	+
Cloprop	6.26	0.998	+
Dioxacarb	4.60	0.990	+
Ethoprophos	9.28	1.000	+
Fenuron	8.21	1.000	+
Imazamethabenz A	4.24	0.986	+
Imazaquin	2.66	0.934	+
MCPA	4.49	0.989	+
Mecoprop	5.09	0.994	+
Metamitron	9.75	1.000	+
Monolinuron	6.15	0.998	+
Oxadixyl	5.00	0.993	+
Thiofanox	1.85	0.864	+
Training group—inactive			
Aldisuf	−5.37	0.995	−
Amitrole	−1.60	0.831	−
Chloralose alphe	−7.59	0.999	−
Cyromazine	−3.53	0.972	−
DNOC	−0.01	0.503	−
Ethylene thiourea	−8.92	1.000	−
Glufosinate ammoniun	−6.25	0.988	−
Monocrotophos	−3.61	0.974	−
Propanocarb	−5.52	0.996	−
Secbumeton	−2.81	0.943	−
Trichlorfon	−7.61	1.000	−
Allidochlor	−4.15	0.984	−
Butoxycarboxim	−3.45	0.969	−
Crimidine	−1.02	0.736	−
Dichlorvos	−4.66	0.991	−
Ethephon	−9.88	1.000	−
Fosetyl Al	−8.42	1.000	−
Imazethapyr	−0.17	0.543	−
Prometon	−3.09	0.957	−
Propiconazole	0.79	0.312	+
Terbacil	−2.44	0.920	−
Triflumizole	−2.72	0.938	−
Test group—active			
Aldicarb	0.36	0.589	+
Ametryn	3.66	0.975	+
Asulam	4.00	0.982	+
Chlorfenac	4.07	0.983	+
EPTC	1.77	0.854	+
Imazamethabenz B	3.79	0.978	+
Metazachlor	6.65	0.999	+
Methomyl	2.46	0.921	+
Propoxur	6.01	0.998	+
Tribenuron	4.63	0.990	+
Test group—inactive			
Bronopol	−8.24	1.000	−
Daminozide	−4.84	0.922	−
Dikegulac	−13.20	1.000	−
Ethidimuron	−0.40	0.598	−
2,4-D	4.10	0.016	+
Flupropanate	−8.12	1.000	−
Methyl isothiocyanate	−8.10	1.000	−
Pirimicarb	−1.28	0.783	−
TCA	−8.38	1.000	−
Thiazafluron	−4.07	0.983	−

Training active and inactive group and test active and inactive group.
[a] Value of the DF (discriminant function) for each compound.
[b] Probability of activity.
[c] The compounds are classified either as active (+) or inactive (−) according to the value of column DF.

and cyromazine was not affected by UV-irradiation; however, ametryn, containing a sulfur group (easily oxidisable under experimental conditions) bonded to the monocyclic heterocycle, is a Ph-CL active compound. In general, carbamate pesticides can be included in the active group presenting Ph-CL. This was the case for aldicarb, asulam, carbetamide, chlorpropham, dioxicarb, methomyl, propoxur and thiofanox. Butoxycarboxim and propanocarb were exceptions to the observed tendency. Organophosphorus and urea pesticides are not clearly classified. Ureas are not significantly represented in the screening test and organophosphorus were found to be non-chemiluminescent and Ph-CL compounds in similar proportion.

The Ph-CL of pesticides is strongly dependent on the chemical structure. Structurally related compounds presenting scarcely differences in their structure show very often different chemiluminometric behaviour.

In order to check the present strategy as an effective way for increasing and improving the number of substances and suitable analytical procedures based on direct chemiluminescence detection, a search employing the analytical abstract data base (1980–2005) and the key words chemiluminescence and the name of pesticide included in group (b) was performed. The search yielded negative results (no previous reported works related to the direct chemiluminescence behaviour of these compounds were published) for aminocarb, azamethiphos, bentazone, carbetamide, chlorfenac, cloprop, dichlorprop, dioxicarb, endothall, EPTC, ethoprophos, fenuron, hymexazol, imazamethabenz-methyl, imazapyr, imazaquin, isocarbamid, MCPA, metalaxyl, metazachlor, metsulfuron-methyl, monolinuron, oxadixyl, phosphamidon, 2,4,5-T, thiofanox and tribenuron. Nevertheless, the positive response with lamp ON was confirmed by the screening test.

3.2. Molecular connectivity calculations

In this work, a set of 72 structurally heterogeneous pesticides was analyzed. These comprise an inactive group (pesticides,

Table 3
Theoretical classification and experimental results for tested compounds

Compound	DF[a]	Probability[b]	Theoretical class[c]	Experimental result	Medium[d]
Clopyralid	1.48	0.805	+	+	All tested media
Cycluron	−5.43	0.995	−	+	Fe(III)/Fe(II)/H_2O_2
Cymoxanil	−2.47	0.920	−	−	H_2O_2[e]
Dimethenamid	3.40	0.968	+	+	All media
Fosamine	−6.12	0.986	−	−	Fe(II)
Glyphosate	−8.07	1.000	−	+	All media
Mecoprop	5.09	0.993	+	+	Fe(III)/Fe(II)/H_2O/H_2O_2
Mepiquat	−1.28	0.783	−	+	NaOH/Fe(III)/Fe(II)/H_2O_2
Metolcarb	6.25	0.998	+	+	All media
Picloram	0.21	0.541	+	+	Fe(III)/Fe(II)/H_2O/H_2O_2
Trinexapac-ethyl	2.77	0.940	+	+	All media

[a] Value of the DF (discriminant function) for each compound.
[b] Probability of activity.
[c] The compounds are classified either as active (+) or inactive (−) according to the value of column DF.
[d] Media with chemiluminescent response after UV-irradiation.
[e] Very weak chemiluminescent behaviour.

which do not present chemiluminescence with lamp OFF and ON, and an active group (CL-pesticides, which either increased dramatically the CL-response with lamp ON, or turns into chemiluminescent ones after irradiation). From an analytical point of view the most interesting Ph-CL effects are included in group (b) and these pesticides are suitable for developing new and sensitive chemiluminescent analytical procedures. Each group was separated in two groups, the training and the test group. Based on this, the obtained discriminant functions were validated.The discriminant function (DF) chosen was:

$$\mathrm{DF} = 1.061\, S^T(\mathrm{aCHa}) + 3.374\,\mathrm{SS} + 7.867 J^2 - 15.014^{10}\chi_{\mathrm{P}} + 1.346^{1}\chi^{v} - 9.604^{4}\chi_{\mathrm{C}} + 102.62^{6}\chi^{v}_{\mathrm{CH}} - 12.740$$

$$N = 72, \qquad F = 18.37, \qquad U\text{-statistics (Wilks' } \lambda) = 0.238$$

Charge indices *Ji*, ${}^{m}\chi_t$ connectivity indices and electrotopological indices used as topological descriptors in the discriminant function are related to the distribution of intramolecular charge and pure structural features, respectively.

Table 2 shows the classification of the results obtained with the DF for each group. A pesticide will be considered active if $DF_i > 0$, or inactive if $DF_i < 0$. In the training group (active and inactive group) an accuracy of 90 and 95.5%, respectively was observed. Moreover, a success of probability of 100% and 90% was obtained respectively with the test groups (active and inactive).

Distribution diagrams can be constructed representing the expectancy for each classification group in any range of DF. The overlapping region of the graphs is related to the discriminant power of the DF function. The discriminant ability of the DF function is associated to a small or nonexistent (in the limit) overlapping region. Distribution diagrams for training and test groups are depicted in Fig. 3.

The discriminant power of the proposed DF was confirmed by the correct classification of closed structurally related compounds. Imidazolinone pesticides imazapyr, imazaquin (training active group) $DF_i > 0$ and a probability higher than 0.89. Imazamethabenz (actually a mixture of imazamethabenz A (2-(4-isopropyl-4-methyl-5-oxo-4,5-dihydro-1*H*-imidazol-2-yl)-5-methyl-benzoic acid methyl ester) and imazamethabenz B (2-(4-isopropyl-4-methyl-5-oxo-4,5-dihydro-1*H*-imidazol-2-yl)-4-methyl-benzoic acid methyl ester) could be emphasized. Both of the them (imazamethabenz A and B) were classified as active compounds, the first one in the training group and the other one in the test group, with a probability higher than 0.97. In the 1,3,5-triazine family cyromazine and prometon were inactive but ametryn was correctly included in the

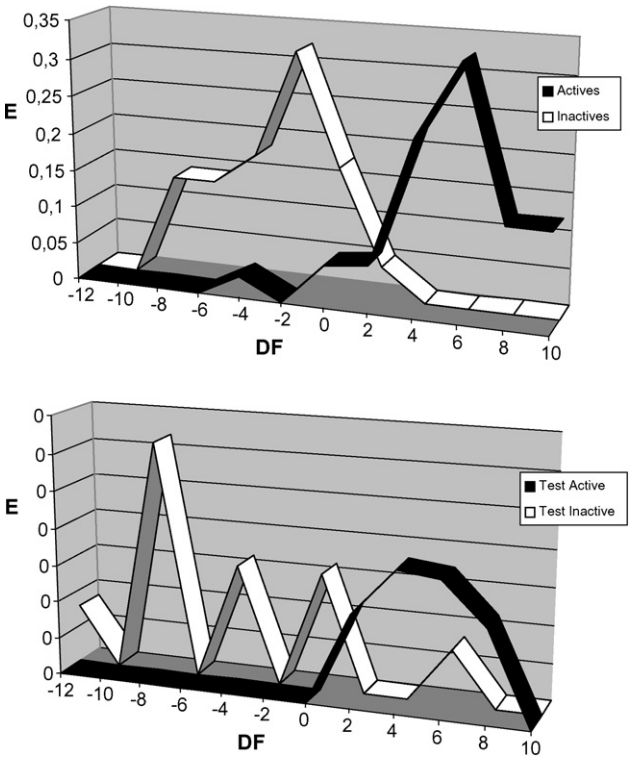

Fig. 3. Distribution diagrams for photoinduced chemiluminescence activity. White line: non-photoinduced chemiluminescence pesticides. Black line: photoinduced chemiluminescence pesticides. Upper: training group. Lower: testing group.

active group. In general, carbamate pesticides presented $DF_i > 0$. This was the case of aldicarb, aminocarb, asulam, carbetamide, chlorpropham, EPTC, methomyl, propoxur and thiofanox. Nevertheless, propamocarb, pirimicarb and butoxycarboxim were classified as inactive compounds.

Finally, the discriminant function was applied to structures not employed in the development and validation of the DF function. The criterion $DF > 0$ was chosen for selecting new pesticides as potential Ph-CL compounds. The viability of the theoretical prediction of the Ph-CL of pesticides was experimentally confirmed by testing the theoretically active pesticides in the multicommutation flow assembly. Good correlation was found for the tested compounds (see Table 3).

4. Conclusions

An automated procedure based on the multicommutation approach for the on-line photodegradation-direct chemiluminescent determination of pesticides is presented.

The photolysis provided by low-pressure mercury lamps of germicidal use permits to increase the number of compounds of environmental interest to be determined by direct chemiluminescence (even compounds without or very weak chemiluminescent behaviour) thanks to the chemiluminescence properties of the resulting photofragments. Although the short time of photoirradiation (150 s), dramatic differences were observed for many pesticides.

The use of low pressure mercury lamps in photodegradation processes allows the determination of compounds which do not present "native" chemiluminescence by using the appropriated medium of photodegradation (e.g. chlorpropham, dichloprop, endothall, hymexazol, methomyl, nicotine, phosphamidon, etc.).

The proposed systems led to strong chemiluminescence for aldicarb, aminocarb, bromoxymil, ethoprophos, imazapyr, imazaquin, imazamethabenz methyl, monolinuron, propoxur, etc. In general, a great improvement of the sensitivity was obtained.

Molecular connectivity is a effective molecular topological tool for the prediction of photoinduced chemiluminescence. It is possible to predict the Ph-CL tendency for a pesticide and, therefore, to develop suitable direct chemiluminescent analytical methods. From six compounds whose Ph-CL behaviour was predicted, six were found experimentally as chemiluminescent after UV-irradiation with a low-pressure mercury lamp. The obtained results corroborate the goodness of the selected descriptors for a topological characterization of pesticides.

LDA provides a pattern for the classification of pesticides (active and inactive against Ph-CL) attending their topological similarity.

Moreover, Ph-CL behaviour was found for pesticides (e.g. aminocarb, bentazone, carbetamide, chlorfenac, dichloprop, EPTC, hymexazol, imazamethabenz-methyl, imazaquin, isocarbamid, MCPA, metazachlor, metsulfuron-methyl, oxadixyl, 2,4,5-T, etc.) for which no previous reported works related to the direct chemiluminescence behaviour of these compounds were published.

Acknowledgments

This research was supported by the Spanish Government (Ministerio de Ciencia y Tecnología, Proyect BQU2002-04248-C02-02). Inma Sahuquillo Ricart also gratefully acknowledges FPI program scholarship from the Spanish Ministerio de Ciencia y Tecnología.

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