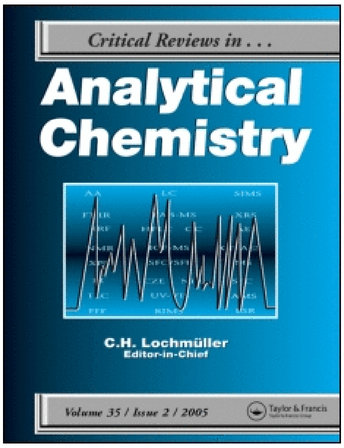

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Laser-Induced Degradation of Organophosphates and Monitoring of Their Toxicity by Cholinesterase Biosensors

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ABSTRACT: Laser-induced photo-oxidation of organophosphorus compounds in aqueous media and the applicability of an acetylcholinesterase based bioanalytical method for evaluation of organohosphate's toxicity has been investigated. Rapid decomposition has been achieved within two hours following the irradiation for the range of concentrations, limited by the solubility of pesticide (up to 40 mgL^{-1}). The compounds detected in the irradiated samples suggest that diazinon is converted directly into 2-isopropyl-4-methyl-6-hydroxypyrimidine without the formation of more toxic diazoxon. This transformation involves oxidation of the sulphur atom to the sulphate anion. A recently developed biosensor was tested for detection of toxic organophosphate and carbamate compounds. Sufficient sensitivities to different pesticides (carbofuran, propamocarb, oxydemeton-methyl, and parathion-ethyl) and LOD's, as low as 0.1 ng/mL, can be achieved in samples of salad, iceberg lettuce, and onion without time-consuming sample preparation procedures. The results show an agreement with the concentrations of pesticides (20–200 μg/kg) determined with standard GC/MS detection. The developed photothermal biosensor offers new low cost means in detecting low concentrations of pesticides with high throughput and little or no sample pretreatment.

1. INTRODUCTION

Organophosphorus compounds are mostly used in agriculture as pesticides. The pathways of their decomposition, such as hydrolysis, photolytic oxidation, microbial transformations, and other biological processes were intensively investigated recently [1]. It was demonstrated that the exposure of different organophosphorus compounds to light results in photolytic degradation [2, 3] Chemical oxidation of selected compounds can be obtained by using photo assisted Fenton reaction (Fe^{3+}/H_2O_2/UV light) [4]. This reaction involves hydrogen peroxide and Fe(III) salts as catalysts, and UV light. This is similar to some other oxidation processes, such as those involving H_2O_2/UV, O_3/H_2O_2, O_3/UV, or semiconductor photo catalysis (TiO_2/UV) [5–7].

Several scientists have utilized aqueous suspension of semiconductors in combination with UV irradiation for degradation of different organic pollutants, such as chlorinated compounds and nitrogen-containing compounds [8]. Among the applied semiconductors, titanium dioxide (TiO_2) proved to be the most efficient. The reason for this is in its capability to form electron-hole pairs under the illumination of UV light. The combinations of H_2O_2/UV, TiO_2/UV, and H_2O_2/TiO_2/UV also have been used for photo degradation of some organophosphorus compounds, including diazinon.

Recently, excimer lasers were applied as light sources for different chemical transformations, such as photopolymerization and crosslinking, particularly because they provide monochromatic radiation of high intensity [9, 10].

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Based on the examples described above, it is obvious that most reactions rely on the use of different reagents that might be problematic from the ecological point of view, unless appropriate methods are used for their removal. In addition, all the experiments require relatively long irradiation times (several hours). Recently, the possibility of performing the detoxification of organophosphates using a laser light source in combination with shorter irradiation times and without the addition of any potential pollutant to the sample, was investigated [11]. Some of the most recent results of this research will be described in this contribution.

The essential step of detoxification procedures should also be the determination of metabolite's toxicity, which requires a simple, rapid, and inexpensive screening method. Most of the conventional methods for determination of organophosphates, such as HPLC, GC, and GC/MS are labour intensive, time consuming, and require expensive instrumentation. Because of this, bioanalytical methods, such as biosensors, were recently developed and found useful as an alternative pre-screening method for toxicity testing. Among the bioanalytical methods available for the detection of organophpsphate and carbamante pesticides, enzymatic methods based on cholinesterase inhibition were widely investigated, and a detailed review on this subject has been published [12]. By applying these devices, it is possible to reliably identify potentially hazardous samples that need further analysis in laboratory testing, using more expensive and time-consuming analytical approaches. Unfortunately, most of the biosensors lack sufficient sensitivity to determine the concentrations of the pesticides present in the environmental samples and/or require long incubation times. A new approach utilizes photothermal detection based on thermal lens spectrometry (TLS) in combination with a bioanalytical FIA system to achieve low limits of detection and a relatively short time of analysis [13, 14]. In this contribution, the effectiveness of such a method in determining the toxicity of samples contaminated with organophosphate and carbamate pesticides will be demonstrated.

2. EXPERIMENTAL

Photodegradation Studies

Experiments have been carried out in a 1 cm × 1 cm quartz cuvette, using an excimer pulse laser (XeCl, 20 ns, 308 nm). The initial concentrations of diazinon and metabolite were 40 mg/L. Samples of diazinon have been irradiated by laser pulses of 40 or 50 mJ. After irradiation, the diazinon was extracted from the samples in time intervals ranging from 10 min to 24 hrs using chloroform. After the evaporation of the solvent, the residues have been dissolved in acetonitrile for HPLC analysis (Eclipse XDB-C8 25 cm × 4.6 mm column, 70:30 acetonitrile/water mobile phase, 1 mL/min flow rate, detection at 254 nm) or in hexane for GC-FID analysis (Supelco SPB-1 30m × 0.53 mm column, helium as a carrier gas). Sulphate concentrations in the reaction mixtures have been determined by ion chromatography (Anion HC column (Alltech), 2.1 mM $NaHCO_3$/1.6 mM Na_2CO_3mobile phase, 1 mL/min flow rate, suppressed conductivity detection).

Toxicity Tests

The immobilization of acetylcholinesterase (AChE) or butirylcholinesterase (BuChE), which was used in this work, to controlled-pore glass (CPG) was carried out according to the procedure published elsewhere [15]. The CPG beads with an immobilized enzyme were stored at 4°C in phosphate buffer (pH 6.0). Before being used, the immobilized enzyme was packed into a PEEK column (60 mm × 2.1 mm i.d.). To assemble the photothermal biosensor the bioanalytical column was incorporated into a FIA manifold. The carrier buffer has been continuously pumped through the system by a HPLC pump at a 0.5 mL/min flow rate. To determine the enzyme activity, 20 μL of the substrate (acetylthiocholine iodide (ASChI)) containing 5,5′′-dithio-bis-2-nitrobenzoic acid (DTNB) was injected onto the bioanalytical column to generate 5-thio-2-nitrobenzoate [16], which absorbed the excitation laser light at 488 nm. A dual-beam (pump-probe configuration) thermal lens spectrometer, similar to the instrument described elsewhere [13], was used as a transducer of the bioanalytical signal.

All the samples (10–25 g) were chopped and meshed in liquid N_2 before one mL of acetone and 9 mL of 0.05 M phosphate buffer (pH 8.0) were added. The mixtures were treated in a stomacher for 15 min. The suspensions were centrifuged (10 min, 2500 rpm), and the supernatants were directly applied onto the bioanalytical column without any extraction or preconcentration step. The sample solution was injected via 1 mL injection loop, and the remaining enzyme activity was determined as described. The pesticide's concentration was determined from the degree of inhibition, based on the

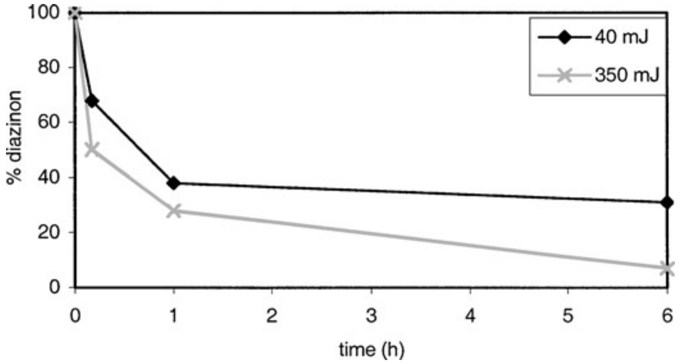

FIGURE 1. Degradation of diazinon in demineralized water following irradiation at different laser energies.

previously prepared calibration curve by injections of paraoxon standard solution (10 ppb).

The determined concentrations of the pesticides were converted to paraoxon equivalent concentrations (c_{eq}) according to the following equation:

$$c_{eq.} = c_x \times \frac{LD_{50(p)}}{LD_{50(x)}} \quad [1]$$

where $c_{eq.}$, c_x, $LD_{50(p)}$, and $LD_{50(x)}$ are paraoxon equivalent concentration, the determined pesticide concentration, the lethal dose for oral exposure of rats to paraoxon, and lethal dose for oral exposure of rats to particular pesticide, respectively.

3. RESULTS AND DISCUSSION

Within the range of concentrations, limited by the solubility of pesticide (up to 40 mg/L), rapid decomposition of the pesticide has been achieved. For 1 mL samples only, 40 mJ of irradiation energy have been needed for 60% degradation efficiency within one hour following the irradiation, as shown in Figure 1. However the isolation of diazinon from the sample 24 hours after irradiation has shown total degradation of the pesticide. By increasing the total irradiation energy to 350 mJ, over 90% degradation efficiency was achieved 6 hours after the irradiation. The efficiency of degradation was supported by the detection of IMHP in all irradiated samples, as confirmed by the GC analysis. At the same time the corresponding reduction in diazinon concentration was accompanied by the increase of the sulphate concentration in aqueous phase, as shown in Table 1.

No evidence for the presence of diazoxon was shown by the GC/MS analysis. This suggests that diazinon is converted directly into IMHP without the formation of diazoxon, which is more toxic compared to its thiono form diazinon. The sulphur atom is simultaneously oxidized into sulphate ion.

A series of experiments under the same reaction conditions as those used in studies of diazinon was additionally carried out on aqueous solution of the IMHP to detect its eventual degradation. The results of the irradiation experiment have shown that the degradation of the metabolite in aqueous solution is minimal.

TABLE 1
Changes of Diazinon and Sulphate Concentrations After Irradiation of Diazinon

Sample	GC analysis Diazinon	Ion chromatography Sulphate
Diazinon—standard solution	40 ppm	0
Sulphate standard	0	30 ppm*
Diazinon, irradiated by 350 mJ, isolation 10 min after irradiation	20 ppm	5.5 ppm
Diazinon, irradiated by 250 mJ, isolation 2 h after irradiation	12 ppm	10 ppm
Diazinon, irradiated by 250 mJ, isolation 6 h after irradiation	4 ppm	12 ppm

*Max. concentration, which can be achieved from 40 ppm of diazinon is 12.6 ppm.

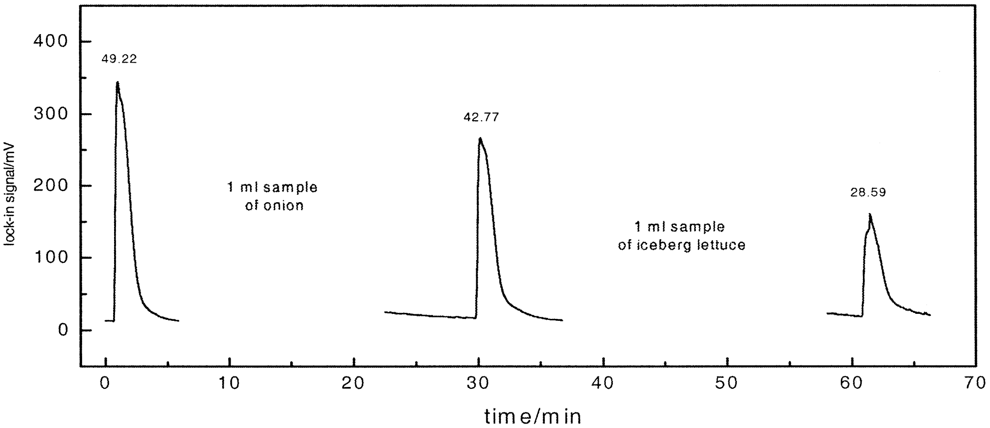

FIGURE 2. Bioanalytical TLS signals from enzyme substrate obtained before and after injection of onion and iceberg lettuce samples.

To evaluate the performance of the recently developed photothermal biosensor [13, 14], for the detection of pesticides in real samples, the determination of total organophosphate and carbamate pesticides concentration in contaminated vegetables, such as onion, iceberg lettuce, and salad was carried out. The samples of vegetables were processed as previously described and directly injected onto the bioanalytical column. The corresponding inhibitions deduced from bioanalytical signals, such as those shown in Figure 2, were 13, 33, and 26% for the onion, iceberg lettuce, and salad sample, respectively. The same samples were later analyzed by GC/MS to determine the actual concentrations of pesticides present in the samples. The paraoxon equivalent concentrations for the pesticides present in the samples were calculated from data of LD_{50} for oral exposure of rats to particular pesticide and for paraoxon [17, 18], according to Equation (1). The results were compared to the values for the paraoxon equivalent concentrations determined by a biosensor, as summarized in Table 2.

It can be shown that the results in the case of the iceberg lettuce and onion sample are in agreement with those obtained with the standard GC/MS method. The least agreement was achieved in the case of the salad sample containing parathion-ethyl. The lower result can be attributed to the insufficient oxidation of the compound (the parent compound parathion-ethyl weakly inhibits the enzyme, whereas the oxidized form paraoxon-ethyl is a strong inhibitor of the enzyme). This can be improved by the addition of a weak oxidizing agent before analyzing the sample.

Finally, it has to be stressed, that all determined concentrations of pesticides in the investigated samples are well below the levels permitted by EU regulations. This confirms the adequate sensitivity of the biosensor, which makes it suitable for simple, rapid, and accurate identification of samples that should be further analyzed by GC/MS or other techniques in confirming the concentrations of organophosphates and carbamates that exceed the permitted regulatory values.

TABLE 2
Comparison of Concentrations of Pesticides in Vegetable Samples Determined by the Bioanalytical FIA-TLS and by GC/MS

	FIA-TLS		GC/MS		
Sample	Enzyme inhibition (%)	Paraoxon equivalent (mg/kg)	Pesticides in the sample (mg/kg)	Paraoxon equivalent (mg/kg)	EU regulation (mg/kg)
			0.02 carbofuran	0.012	0.3
Onion	13.1 ± 0.4	0.013 ± 0.005	0.11 propanocarb		Not allowed
Iceberg lettuce	33 ± 1	0.021 ± 0.005	0.18 oxydemeton-methyl	0.018	0.5
Salad	25.8 ± 0.8	0.009 ± 0.005	0.05 parathion-ethyl	0.05	0.05

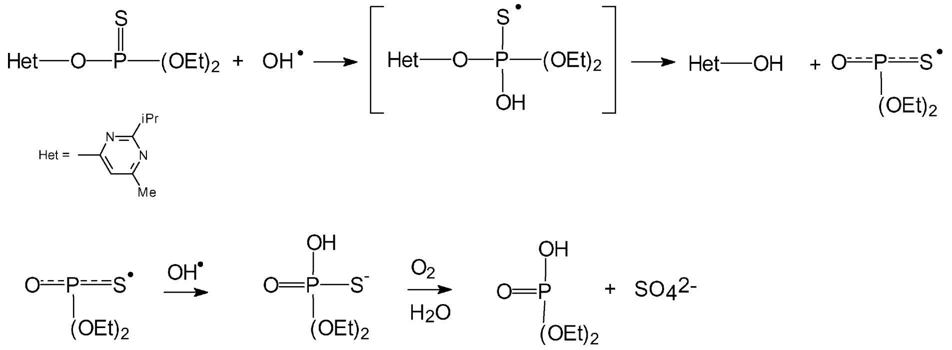

FIGURE 3. The suggested mechanism of laser induced photodegradation of diazinon.

4. CONCLUSIONS

The degradation of diazinon, during the irradiation with high energy laser light pulses, does not contribute significantly to the overall efficiency of photodegradation. However, the degradation most probably is initiated by hydroxy radicals formed during the irradiation and proceeds at relatively low reaction rates (2×10^{-6} mol/min). Over 70% of diazinon can be degraded already within the first two hours following the irradiation, with the increase in efficiency up to 95% during the next four hours. On the basis of the presented results, the mechanism for the photodegradation of diazinon was proposed (Figure 3). The oxidation of sulphur takes place which is confirmed by the ion chromatographic detection of sulphate anion in water phase. Simultaneously, the oxidation and hydrolysis of the esther bond and release of the metabolite occur.

It was additionally shown that the detection of organophosphate and carbamate pesticides is possible without time-consuming and environmentally unfriendly extraction and preconcentration steps. The contaminated samples of onion, iceberg lettuce, and salad were giving positive results for organophosphate and carbamate pesticides, when analyzed by the biosensor. Further analyses on GC/MS confirmed the presence of various organophosphates (parathion-ethyl, oxydemeton-methyl) and carbamates (carbofuran, propamocarb) in concentrations below the regulatory limits set by the EU. The agreement between the results obtained with the described bioanalytical FIA system and the GC/MS method were found to be very satisfactory for samples containing oxo-organophosphates or carbamates (carbofuran, propamocarb, oxydemeton-methyl). For the sulphur derivatives, such as parathion-ethyl, the agreement can be improved by the addition of a weak oxidizing agent before analyzing the sample. Nonetheless, the photothermal acetylcholinesterase biosensor offers a new means to screen for the presence of organophosphate and carbamate pesticides or their cholinesterase inhibiting metabolites.

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