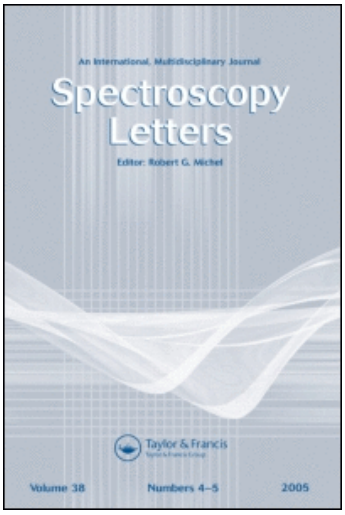

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Multi-commutated Flow-through Multi-optosensing: A Tool for Environmental Analysis

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Multi-commutated Flow-through Multi-optosensing: A Tool for Environmental Analysis

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Abstract: Multi-commutation, which refers to the use of solenoid valves to construct the flow network, has been widely used for providing automation in flow injection analysis. In this paper, the coupling of multi-commutation and multi-optosensing is developed for the analysis of two pesticides in environmental water samples, fuberidazole and *o*-phenylphenol. In optosensing, the use of the solid support allows the discrimination between the analytes and other compounds that, if measuring in solution, would interfere in the analysis; in addition, the sensitivity needed when facing environmental samples is obtained. The two analytes are separated by using C_{18} silica gel as solid support, taking into account their different kinetics of sorption/desorption when interacting with the solid support microbeads; the separation is performed in the same flow-cell where the sensing detection is carried out (by using an additional amount of solid support in the cell itself above the irradiated microzone), so both separation and determination are integrated in the cell. The native fluorescence of fuberidazole and *o*-phenylphenol was simultaneously measured at 314/356 and 250/345 nm, respectively. The detection limits obtained were 0.18 and 6.1 ng mL^{-1} for fuberidazole and *o*-phenylphenol respectively, with a sampling frequency of

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about 12 samples per hour. A recovery study was performed in waters obtained for wells and rivers, with satisfactory results.

Keywords: Automation, luminescence, multi-commutation, natural water, optosensor

INTRODUCTION

In environmental analysis, the development of the current analytical chemistry tends more and more toward the use of faster, cheaper, simpler, and more reliable automatic techniques. Flow injection analysis (FIA) is a valuable tool for the monitoring of environmental parameters and for the determination of polluting compounds in environmental samples.[1,2] Some of the limitations of flow injection analysis determinations performed in solution are solved by the coupling of FIA and solid-phase spectroscopy (SPS). The coupling of these methodologies has been called optosensing and its advantages previously described.[3,4] When using the solid support, both the selectivity (only those species retained on the solid microbeads will perform their signals) and sensitivity (preconcentration achieved by the solid support) of the system are increased.

Traditionally, the development of optosensing analysis has been performed by using six-port rotary valves.[5] This kind of manifold has several disadvantages, including difficult miniaturization and an uneasy handling of reagents, but the main problem associated with using these valves is that they are not automatic. This means that the developed methods would require continuous human intervention, so making them difficult to use in the routine analysis.

Some alternatives have arisen to avoid this handicap, such as sequential injection analysis (SIA),[6] multi-syringe flow injection analysis (MSFIA)[7] or multi-commutation.[8,9] Although they are different in terms of the kind of automation involved, the low-cost of the multi-commutation system has made us choose this kind of flow network.

The term *multi-commutation* is usually used to describe the flow system that comprises the use of solenoid valves, automatically controlled by appropriate software. Although different kinds of commercial solenoid valves are available, three-way solenoid valves are usually used. They are automatically switched on or off by means of an electric pulse. Their low cost, low weight, and easy handling make them optimum for the development of portable equipment, suitable for the *in situ* analysis of environmental samples. In addition, by simply changing the connection between the valves or the sequence of reagents and sample introduction, a completely dissimilar determination may be carried out. The valve switching is automatically controlled by means of appropriate homemade software. The characteristics of multi-commutation have been widely reviewed elsewhere.[10,11]

Therefore, the coupling of multi-commutation and optosensing has been developed in our laboratory, and satisfactory results have been already

described for the determination of one[12] or two analytes.[13,14] No handicap has been observed when coupling these methodologies, and the advantages of both are maintained, so the contribution of multi-commuted optosensing has been proved useful in the development of automatic analytical methods.

Although the determination of pesticides in environmental samples, such as natural waters, is complex and is nowadays carried out by chromatographic methods and mass spectrometry detection,[15,16] monitoring a particular compound or *in situ* analysis demands the development of new rapid and low-cost methods. In this paper, we report a multi-commuted biparameter fluorescence optosensor for the simultaneous determination of two widely used pesticides: fuberidazole (FBZ) and *o*-phenylphenol (OPP). They have been separated online by using an additional amount of solid microbeads (C_{18}) placed just above the sensing zone, in the flow cell itself, as previously described.[14] This additional solid support is used instead of a precolumn placed before the flow-through cell, so simplifying the manifold. This sensor has been applied to the determination of the target analytes in environmental waters, showing good tolerance to other potentially present pesticides; a recovery study has been carried out, obtaining excellent results, so demonstrating the suitability of the system for water monitoring of these pesticides.

MATERIALS AND METHODS

Reagents and Solutions

Analytical reagent grade chemicals were used in all the experiments.

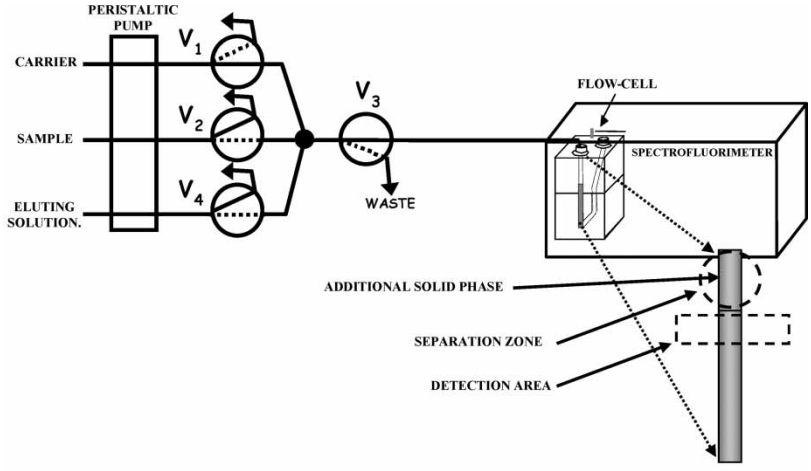
FBZ [2-(2′-furyl)benzimidazole)] (Riedel de Haën) and OPP (Sigma, Madrid, Spain) stock standard solutions of 200 mg L^{-1} were prepared in ethanol (Panreac, Barcelona, Spain). Both solutions were kept in a refrigerator protected from light and remained stable for at least 1 month in these conditions.

Working standard solutions containing FBZ and OPP were prepared in 0.5 M HCl (Panreac) solution. Methanol (Panreac) was used as carrier (30% v/v) and eluting solution (60% v/v). Deionized water was used in all cases for the preparation of the required solutions.

C_{18} bonded phase silica gel microbeads (Waters, Milford, MA, USA) with 55–105 μm average particle size was used as solid support.

Apparatus and Software

The flow setup is depicted in Fig. 1. It comprises a four-channel Gilson Minipuls-3 (Villiers Le Bell, France) peristaltic pump with rate selector and pump tubes type Solvflex (Elkay Products, Shrewsbury, MA, USA). An electronic interface based on ULM 2803 integrated circuit was employed to generate the electric potential (12 V) and current (100 mA) required to



Step	V_1	V_2	V_3	V_4	Time (s)	Description
1º	1	1	0	0	120	Sample introduction
2º	0	0	0	0	60	Elution of FBZ
3º	1	0	0	1	120	Elution of OPP
4º	1	1	1	0	5	Cleaning step 1
5º	0	0	1	0	5	Cleaning step 2

Figure 1. Multi-commutated flow configuration: V_1, valve 1 (etc.). Scheme of the valves switching: 1 means "on" and 0 means "off".

control the four 161T031 NResearch three-way solenoid valves (Neptune Research, Stow, MA, USA). The software for controlling the system was developed in Java. PTFE tubing of 0.8-mm internal diameter and methacrylate connections were also used.

A Cary-Eclipse Luminescence Spectrometer (Varian Inc., Mulgrave, Australia) equipped with a Hellma flow cell 176.052-QS (25 μL of inner volume and a light path length of 1.5 mm) (Jamaica, NY, USA) was used for all the measurements. The spectrofluorimeter was connected to a computer with a Cary-Eclipse (Varian) software package for data collection and treatment. Multi-wavelength fluorescence detection mode, available in the instrument, was used; continuous signal monitoring was simultaneously recorded at two pairs of excitation/emission wavelengths (314/356 for FBZ and 250/345 for OPP). The fluorescence emission intensity was measured as the height of the peak of the transitory signal.

The flow cell was filled with C_{18} solid support microbeads, as a slurry suspension in methanol, with the aid of the peristaltic pump; the cell was blocked

at the outlet with glass wool to avoid the displacement of the solid support. An additional amount of solid support in the flow-cell was used as a strategy to separate the analytes.

Samples Preparation

Several water samples were analyzed, two obtained from rivers and two from wells. The samples were filtered through a cellulose acetate filter (0.45-μm pore size; Millipore, Bedford, MA, USA) and the required volume of HCl was added in order to achieve the 0.5 M concentration. They were inserted into the flow system and analyzed by triplicate.

General Procedure

The flow diagram of the system is shown in Fig. 1. In the initial status, all valves are switched off and the carrier, 30% MeOH:H_2O (v/v), is flowing through the system while all other solutions are recycling to their vessels. The sample is introduced by simultaneously switching the valves V_1 and V_2 on for 120 s.

The separation of both analytes was possible due to their different retention/desorption kinetics when interacting with the solid phase in the flow-through cell (an additional amount of solid support is placed just above the irradiated area, so performing the separation of both analytes). OPP is strongly retained on the upper part of the solid support, while FBZ passes through the solid support, developing its analytical signal when reaching the irradiated area and being eluted by the carrier itself. After FBZ has developed its signal, by switching valves V_1 and V_4 the eluting solution (60% MeOH:H_2O) is introduced into the flowing system for 120 s. After the signal from OPP is recorded, the portion of tubing placed between valves V_2 and V_3 was cleaned with the next sample in order to avoid any possible contamination. The switching procedure is detailed in Fig. 1.

RESULTS AND DISCUSSION

Spectral Characteristics and Instrumental Variables

The spectral features of FBZ and OPP were recorded both in aqueous solution and solid-phase media. When they were retained on the C_{18} solid support, the maximum excitation/emission wavelengths were 314/356 nm for FBZ and 250/345 nm for OPP. As it can be observed in Fig. 2, there is a strong overlapping in the emission wavelengths, so the simultaneous determination of both analytes without performing their separation would not be possible; in addition, a slight overlapping in the excitation wavelengths is produced due to the broad excitation bands of both pesticides.

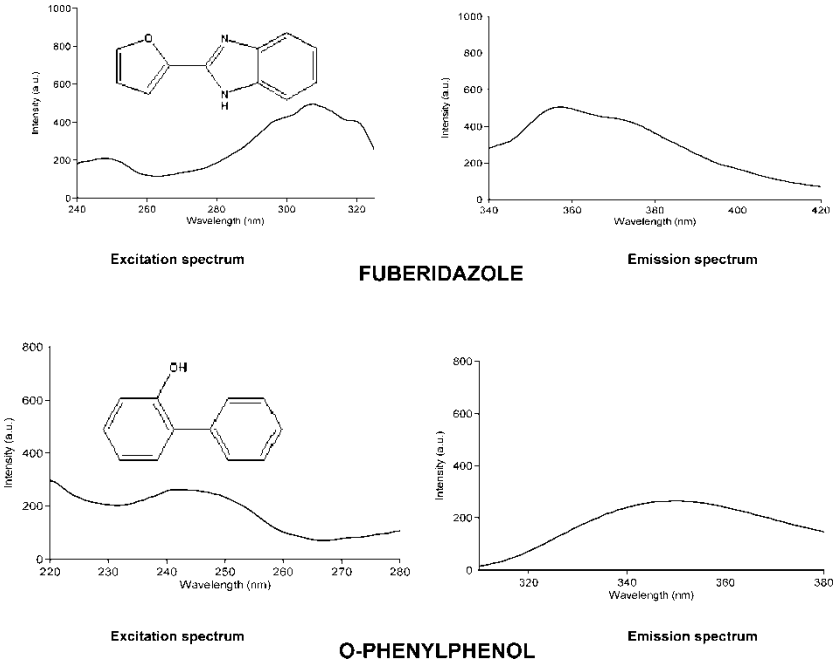


Figure 2. Excitation and emission fluorescence spectra of FBZ and OPP in solution. Structures of fuberidazole and *o*-phenylphenol.

In order to obtain the best analyte/background signal ratio, the optimum excitation and emission slit widths of the fluorimeter were established as 5/20 nm, respectively.

The voltage of the photomultiplier tube was also studied. When increasing the voltage, the sensitivity improves, but the background signal is also increased (so diminishing the linearity range of the method). Therefore a compromise between sensitivity and linear dynamic range had to be achieved. A 600 V value was finally selected.

Solid Support

Taking into account both the structure of the analytes (Fig. 2) and previous studies carried out in our laboratory, a C_{18} silica gel was selected for performing the separation. Both FBZ and OPP provide a high signal when retained on the solid support. Taking into account that FBZ can be protonated in acidic medium, but not OPP, this characteristic was studied as a tool for separating the compounds. The analytes could be separated inside the flow-through cell; instead of using a precolumn placed before the cell, we introduced an additional amount of solid support (just above the irradiated

area of the cell), which provides the separation. The cell had to be completely filled with the solid support, 2 cm above the irradiated area, in order to achieve a proper separation of the pestides; this can be observed in Fig. 1.

Chemical Variables

Sample Solution

As has been previously described, in order to separate the analytes, the exploited characteristic was the protonation of a nitrogen atom of FBZ. Different HCl concentrations were tested, and an HCl concentration of 0.5 M was chosen as optimum for obtaining a proper separation of the analytes. By using this acidic solution, OPP, which has no protonable atoms, is strongly retained on the additional solid support, while FBZ (which becomes protonated) passes through the solid support, developing its signal in the irradiated area, and it is eluted by the carrier itself. After that, by using an appropriate eluting solution, OPP is eluted from the upper part of the cell and develops its transitory signal in the sensing zone.

Therefore, all standard and sample solutions were prepared in 0.5 M HCl for further experiments.

Carrier and Eluting Solution

When using C_{18} silica gel as solid support, different percentages of hydroalcoholic solutions are usually used as carrier and eluting solutions. In this case, we performed the study of the carrier from 20% to 40% MeOH:H_2O (v/v), choosing a 30% value as optimum, obtaining the rapid complete separation of the analytes. As eluting solution, several MeOH percentages were tested, ranging from 50% up to 70%. Finally, 60% was selected, as the OPP signal diminished for higher values, and the elution was not complete for values lower than 60%.

Flow Variables

Both the flow rate and the sample introduction time have to be taken into account. The higher possible flow-rate without obtaining overpressures is selected: for the same sample insertion time, higher amounts of analytes are introduced when increasing the flow rate, so increasing the sensitivity of the system. The sampling frequency is also increased when using high flow-rates. In this case, approximately 1.1 mL min^{-1} was selected.

The study of the sample insertion time was performed from 20 up to 200 s, using a solution containing 6 and 200 ng mL^{-1} of FBZ and OPP, respectively. When increasing the time, higher volumes of sample were

introduced into the flow system, so increasing the amount of analytes. In the case of FBZ, the signal increased linearly up to 160 s, but for OPP the signal increased linearly only up to 120 s. Although the signal increases for higher volumes (but not linearly), the sample frequency becomes smaller as the sampling insertion time increases. So a compromise between sample throughput and sensitivity has to be achieved. In this case, we decided to use 120 s.

The regression equations obtained for the influence of the sample insertion time on the relative luminescence intensity (RLI) were

$$\text{FBZ: RLI} = 3.1t(s) - 13 \qquad r = 0.9982$$
$$\text{OPP: RLI} = 3.8t(s) - 14.5 \qquad r = 0.9990$$

Analytical Parameters

Following the optimized conditions previously described, the calibration graphs for both analytes were obtained; each standard was inserted in triplicate. Both the calibration graphs and the rest of the analytical parameters are detailed in Table 1. Good detection limits were obtained, 0.18 and 6.1 ng mL^{-1} for FBZ and OPP, and in both cases the RSD% was below 4%. A sample frequency of approximately 12 samples per hour was obtained, but it could be increased if lower sample insertion times were used (although the sensitivity would also decrease).

Study of Foreign Species

In order to test the usefulness of this method for analyzing real samples, the study of the possible effect caused by foreign species (common in real

Table 1. Analytical parameters

Parameter	FBZ	OPP
Linear dynamic range (ng mL^{-1})	0.6–13	20–350
Calibration graph		
Intercept	−11	−9
Slope (mL ng^{-1})	61	2.32
Correlation coefficient	0.9979	0.9998
Detection limit (ng mL^{-1})	0.18	6.1
Quantification limit (ng mL^{-1})	0.6	20
RSD (%) (n = 10)	2.3[a]	3.9[b]

[a]For a concentration level of 6 ng mL^{-1}.
[b]For a concentration level of 200 ng mL^{-1}.

Table 2. Interference study

Foreign species	Tolerated interferent/analyte (w/w) ratio	
	FBZ[a]	OPP[b]
F^-, CO_3^{2-}, NO_3^-, PO_4^{3-}, SO_4^{2-}, Na^+, K^+, NH_4^+	10,000[c]	1000[c]
Imazalil, simazine, morestan, chlorsulfuron	1000[c]	100[c]
Bendiocarb, aminocarb	1000[c]	80
Propoxur, quinmerac, imazaquin	200	100[c]
Carbaryl	100[c]	9
Carbofuran	100	50

[a] 10 ng mL^{-1}.
[b] 100 ng mL^{-1}.
[c] Maximum ratio tested.

samples) on the analytical signal was carried out. This effect was performed by adding known amounts of foreign species to a solution containing 10 and 100 ng mL^{-1} of FBZ and OPP, respectively. The tolerance level was defined as the amount of foreign species that produced an error not exceeding $\pm 5\%$ in the determination of each target compound. Both common ions in natural waters and other potentially present pesticides were tested as possible interferences of the method. As can be seen in Table 2, very good tolerance levels were obtained for all the tested ions and compounds, so showing the robustness of the proposed determination method

Applications

The usefulness of the proposed method for the analysis of real samples was demonstrated by analyzing several environmental water samples, that is, two water samples obtained from rivers and two obtained from wells. A recovery study was performed by spiking the samples with FBZ and OPP concentrations within the linear dynamic range 2–12 ng mL^{-1} and 40–300 ng mL^{-1} for FBZ and OPP, respectively. Each water sample was spiked at three concentration levels of both analytes and their concentrations determined by using external calibration (after checking the absence of matrix effect). The recoveries obtained ranged from 94% up to 106%, so showing the suitability of the method.

The proposed method, due to the low detection limits, could also be suitable for the determination of the pesticides in drinking water (maximum residue level of 0.1 ng mL^{-1}) if a preconcentration technique, such as solid-phase extraction, was used.

CONCLUSIONS

The need to develop miniaturized, rapid, simple, and reliable equipment for routine analytical determinations has made the use of multi-commutation a very useful tool for handling sample and reagents, not only in optosensors, but also in any molecular or atomic spectrometry technique. In this paper, we have introduced the possibility of applying multi-commuted optosensors to the analysis of environmental pollutants, such as pesticides, in natural waters. But this approach would be suitable for any kind of analyte or sample, just by optimizing the reagents and extraction or clean-up if necessary, as has been previously described in other published articles.

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