Multi-analyte biosensing of environmental priority pollutants in real water samples

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Abstract

Real-time multi-analyte detection of pollutants within the three main types of pesticides: organochlorine, organophosphate and carbamate have been performed using a two-channel Surface Plasmon Resonance (SPR) optical immunosensor. Two different immunoassay approaches have been tested to detect the target analytes by using individual and simultaneous immobilization formats. Sensor reusability was assured via the formation of alkanethiol Self-Assembled Monolayers (SAMs) allowing the regeneration of the same immunosurface throughout 200 assay cycles. Detection limits reached ppt levels (18-32 ng L^{-1}) when using both individual and multi-analyte protocols. No significant matrix effects were found for different water types and the correlation with conventional chromatographic methods showed an excellent agreement between assays. The high sensitivity, low-time of response (20 minutes) and portability of the SPR biosensor platform, would allow its application as a field analytical method for environmental waters.

1. Introduction

Amongst environmental pollutants, pesticides are of relevant concern due to their toxicity and the prevalence of their use. The European Union has strictly limited the emissions, discharges and losses of several compounds and has adopted different methods and strategies to control pollution in environmental matrices in the field of water policy (Water Frame Directive: 2000/60/EC).

Monitoring efforts for emerging and priority pollutants are conducting to the development of fast, low-cost, field analytical devices that can determine several analytes simultaneously operating unattended. Multi-analyte detection of pesticides has been commonly performed by complex analytical chromatographic methods that reach low detection limits for the target analytes. However, these methods are expensive and time-consuming and require solvent extraction, pre-clean up and pre-concentration of samples to detect low amounts of pesticides.

In this work, an optical-affinity biosensor is used to determine DDT organochlorine persistent pollutant, chlorpyrifos organophosphate insecticide and carbamate carbaryl pesticide. This sensor is based on Surface Plasmon Resonance principle and detects, as increases in the refractive index, immuno-binding reactions occurring at the sensor surface. Using solid-phase immunoassay principles, this biosensor is capable to detect the selected pesticides as sensitively as other immunochemical methods. Furthermore, the SPR biosensor advantages ELISA and other biosensor formats allowing pesticide determinations more rapidly and without labelling requirements.

Two different approaches were followed to determine the target pesticides individual and simultaneously by using modified supports with a single analyte derivative or both of them immobilized at the same sensing surface. The SPR device works under reversible conditions allowing the continuous monitoring of a great number of samples. Therefore, it could be applied as an on-site method for the multi-analyte, simple and fast detection of DDT, chlorpyrifos, carbaryl and other priority substances in environmental waters of early warning stations [1].

2. SPR instrumentation

SPR analysis was performed by using the β-SPR sensor system (SENSIA S.L., Spain) shown in Fig.1. It works in the Kretschmann configuration to achieve the resonant condition by total reflection. The thin gold layer used as sensor chip is coupled to the prism structure via matching oil with the same refractive index. A p-polarised light is emitted by a 3 mw laser diode source, operating at 670 nm. The laser beam, once divided into two equal beams, passes through the high refractive index prism and is made incident onto the backside of the gold-film glass slide. The distance between the two beams is 3.1 mm, the same as the separation of the two flow cells. The flow cells (volume=300 nL; surface area=3 mm^2) come into contact with the gold-coated sensing surface and allow the analysis of two independent measurements simultaneously and/or the use of one of the channels as reference. SPR measurements are carried out at a fixed angle of incidence to

monitor shifts in mass at the sensing surface in real time. Samples are pumped over the SPR sensor chip, at a constant speed of 30 μL min^{-1}.

a) b)

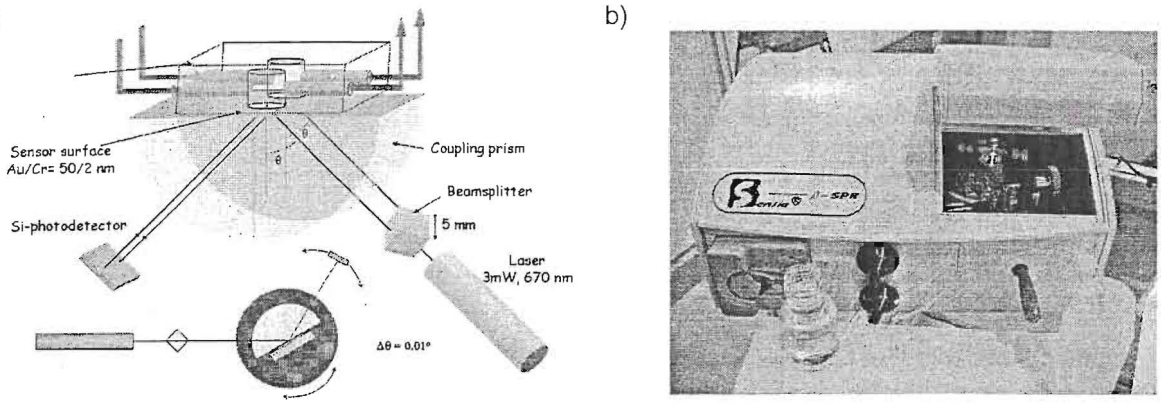

Figure 1. Schematic set up of the β-SPR sensor system from SENSIA, S.L., including sensor, optics, electronics and flow delivery system: a) scheme of the SPR device and b) SENSIA β-SPR platform

3. SPR immunoassay format

An alkanethiol monolayer was formed onto the gold substrate to achieve a reusable sensing surface while preserving the chemical properties of the immobilized molecule. The covalent attachment of the recognition element via carbodiimide coupling ensured the stability and selectivity of the biosensing surface through repeated use.

For the detection of low-molecular weight compounds like DDT, chlorpyrifos and carbaryl, binding inhibition immunoassays on the conjugate-coated format were required. Binding inhibition assay formats consist of antibody-analyte mixtures solutions containing a fixed concentration of monoclonal antibody and different concentrations of analyte. After incubating the mixture for a short period, the sample is pumped over the biosensing surface and only antibodies with free binding sites can couple to the immobilized haptens.

The antibody-analyte association was disrupted using HCl 0.1 M as regeneration agent. Standard calibration curves were obtained by averaging three individual curves.

4. Multi-analyte detection

DDT, chlorpyrifos and carbaryl measurements were carried out using two different approaches based on inhibition binding assays. First, single analyte determinations were developed for simultaneous pesticide monitoring by flowing DDT, chlorpyrifos and carbaryl samples separately over each channel of the β-SPR system. For instance, DDT immunoassays were performed on the flow cell with the gold layer coated with the DDT conjugate whilst carbaryl inhibition tests were monitored in the channel wherein the corresponding carbaryl recognition element had been previously immobilized at the optimal concentrations.

The second approach was based on a multi-analyte protocol in which two or three analyte derivatives were immobilized on the gold-coated surface of one individual flow cell. In this format, DDT; chlorpyrifos and carbaryl analyses were carried out using sequential and additive modes. The sequential protocol is based on the performance of the whole analysis cycle of a pesticide, including regeneration, followed by the corresponding cycle of the other two pesticides. A complete assay cycle takes 60 minutes. For the additive protocol the three pesticides are analysed successively and all bound analytes are removed in a single regeneration step. Therefore, the analysis time is reduced to 40 minutes.

The assay sensitivity was estimated by the assessment of limits of detection (LODs), I_{50} and dynamic range values. Detection limits were experimentally determined as the analyte concentration giving a 10% reduction of the maximal SPR signal. Following these criteria, results for the different analysis formats achieved similar sensitivity values. The comparison between calibration graphs and sensitivity values are shown in Fig. 2. Limits of detection of 32 and 18 ng L^{-1},and I_{50} values of 1.06 and 0.44 μg L^{-1} were found for DDT individual and simultaneous immobilization formats, respectively. Chlorpyrifos inhibition binding curves also reached an extremely similar linearity and low detection limits (52-58 ng L^{-1}) and I_{50} (1.96-1.84) μg L^{-1} values for both assay protocols [2]. Finally, the assay sensitivity obtained for carbaryl single and multi-analyte approaches were in the same range for the I_{50} values (3.12-2.45 μg L^{-1}) whilst a slight improvement of the detection limit

was observed when comparing the multi-analyte format (0.47 μg L^{-1}) to the single analyte one (1.41 μg L^{-1})[3].

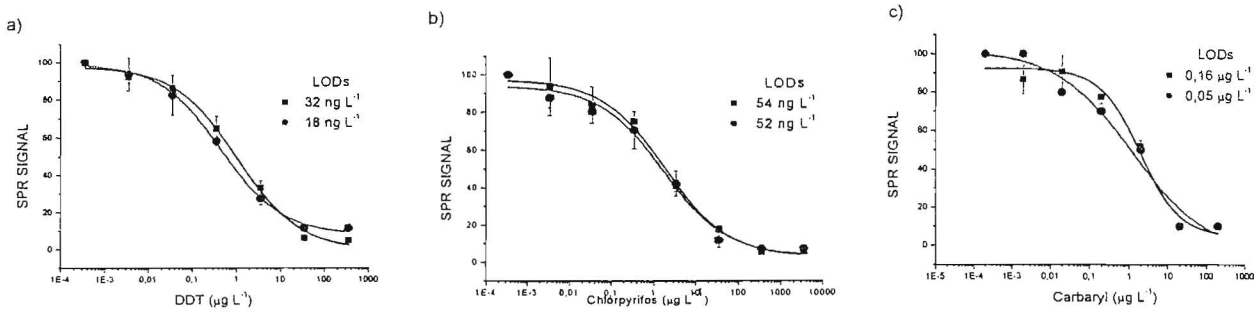

Fig. 2. Standard calibration curves for DDT a), chlorpyrifos b) and carbaryl c) single (■) and multi-analyte (●) immunoassays.

5. Effect of water matrices

To evaluate the possible interference of the different composition of real water types on the SPR measurements, single-analyte standard curves were prepared in water samples from different sources (drinking, river and ground water) and in distilled water as a control (Fig. 3). The assay sensitivity and recovery (between 80 and 120%) obtained with real water samples proved the feasibility of this immunosensor for the precise determination of pesticides in environmental waters without any sample pre-treatment.

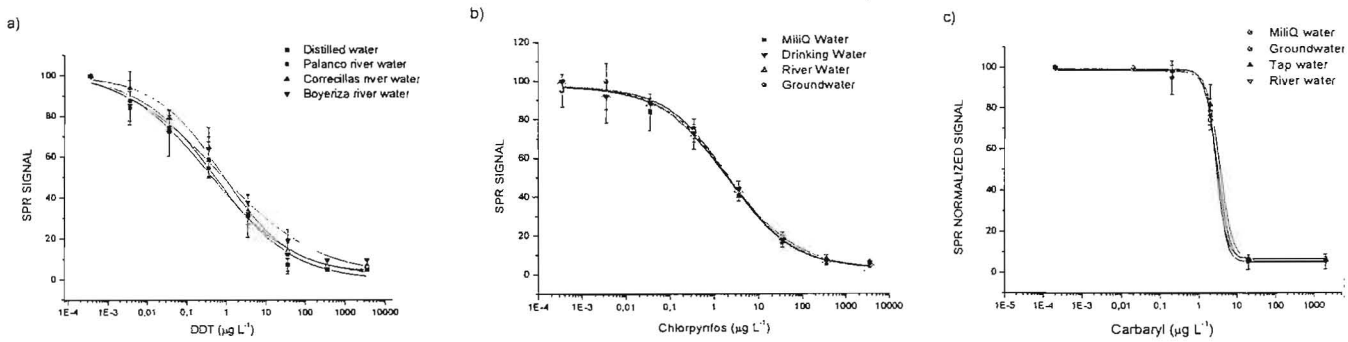

Fig. 3. Standard calibration curves of: a) DDT, b) chlorpyrifos and c) carbaryl in different water types: groundwater, drinking and river water.

6. Validation of the method

The validation of SPR immunoassays was performed by using gas chromatography coupled to mass spectrometry (GC-MS) for DDT and chlorpyrifos measurements and high performance liquid chromatography (HPLC-MS) for carbaryl determination as the reference method. The correlation data indicated an excellent agreement between assays (r^2: 0.99). Contrary to other biosensing techniques, wherein immunosensor results are commonly overestimated with respect to the chromatographic analysis, no significant discrepancies were found when comparing the SPR and chromatographic methods, as shown by the slope of the linear regression analysis (Fig. 4).

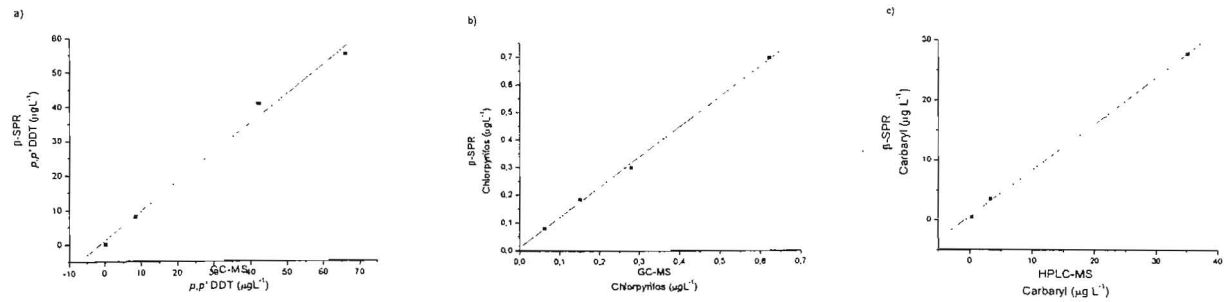

Fig.4. Correlation between SPR and chromatographic methods for four concentrations of: a) *p,p'* DDT (y = 0.853x + 1.025), r^2=0.995; b) chlorpyrifos (y = 1.094x + 0.01), r^2=0.999; c) carbaryl (y = 0.907x + 0.887), r^2=0.998.

Conclusions

The development of a commercialised SPR sensor system (SENSIA S.L) is presented as a remarkable worthy method for the detection of potentially hazardous pesticides. The sensitivity attained by using both single-analyte derivative and multi-conjugate immobilization approaches, together with the simplicity and fastness of the analysis are inherent advantages over routinely used chromatographic methods. The β-SPR biosensor reached the lowest detection limit at sub-nanogram per litre levels within a response time of 20 minutes. The β-SPR immunosensor enables the on-site real-time detection of DDT, chlorpyrifos and carbaryl via the automation of measurements. Therefore, this SPR immunosensor could be a useful and reliable method for the screening of environmental pollutants from real contaminated locations.

References

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