SPR BIOSENSOR PLATFORMS FOR IN-SITU ENVIRONMENTAL ANALYSIS

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Aquatic ecosystems can be contaminated by a wide variety of pollutants from industrial and agriculture activities. Among environmental pollutants, pesticides are of relevant concern due to their toxicity and their prevalence use. In the last years, we developed a portable optical-affinity biosensor based on the Surface Plasmon Resonance technique and we demonstrated its use to determine: organochlorine persistent pollutant DDT, chlorpyrifos organophosphate insecticide and carbaryl carbamate pesticide.

SPR immunoassays developed to determine DDT, chlorpyrifos and carbaryl were binding inhibition tests based on the conjugate coated format, consisting of the competitive immunoreaction of the unbound antibody present in an analyte-antibody mixture with the hapten derivative immobilized at the sensor surface. We have developed two different analytical protocols to determine the target pesticides: individual and multianalyte detection.

The multi-analyte determination approach is based on the multiple and combined spatial controlled immobilization of up to three analyte recognition elements on the same sensing surface. In this format, analyte determinations were carried out using sequential modes by flowing chlorpyrifos, carbaryl or DDT samples over the same channel. Due to the different in hapten to protein molar ratio the optimum concentration for each analyte conjugate to be immobilized should be previously determined.

The optimal combination of multiconjugate concentrations were obtained for 15, 10 and 5 $\mu g.mL^{-1}$ dilutions of DDT, carbaryl and chlorpyrifos conjugates, respectively. Limits of detection of 18 $ng.L^{-1}$ and IC50 value of 0.44 $\mu g\ L^{-1}$ were attained for DDT detection in this multianalyte format. Chlorpyrifos inhibition binding curves also reached an extremely similar linearity, and low detection limits (52 ng L^{-1}) and IC50 (1.76 $\mu g\ L^{-1}$) values. Finally, the assay sensitivity obtained for carbaryl shows a significant improvement of the detection limit when comparing the multi-analyte format (0.05 $\mu g\ L^{-1}$) to the single analyte one (1.41 $\mu g\ L^{-1}$).

The SPR biosensor reached the lowest detection limit (LOD) at sub-nanogram per litre levels within a response time of only 10 minutes and different water types (groundwater, tap and river water) were evaluated without observing matrix effects. The reusability of the sensor chip was demonstrated after approximately 200 regeneration cycles, depending on the immunoassay format, by a negligible reduction of the SPR maximum signal.

This SPR immunosensor enables on-site and real-time detection of DDT, chlorpyrifos and carbaryl via the automation of measurements. In addition, the SPR multi-surface approach has shown to be effective for determining the target analytes in a highly sensitive, simple and rapid manner, without the need of labelling biomolecules or spatially resolved surface modification. These are inherent advantages over chromatographic and fluorescence-based multi-biosensors. In addition, this method could be extended to the detection of more than three analytes by simply modifying the sensing surface with a higher number of compounds. The multi-biosensing system offers a versatile, regenerable, robust, fast and cost-effective field-analytical method for the monitoring of other hazardous or restricted compounds, from endocrine disrupters to pharmaceuticals, whenever immunoreagents were available.

The SPR sensor can also be used as an on-line immunoanalytical method for the evaluation of pesticide metabolites which can be present in the

human body. We have detected the metabolite from chlorpyrifos pesticide (3,5,6-trichloro-2-Pyridinol (TCP)) from its primary via of elimination (urine). This metabolite can be detected in urine during several days after exposure to the pesticide. Binding inhibition tests were performed in the untreated urine samples and compared to those obtained in distilled water and PBS used as control. In all cases, similar detection limits, in the micrograms per litre level (0.1-0.24 $\mu g.L^{-1}$), were attained for TCP assays independently of the dilution buffer. The reproducibility of measurements was studied throughout more than 130 regeneration cycles, allowing the repeated use of the same immunosensor surface without significant variation of the SPR signal. All measurements were developed in real-time in only 10 minutes.

The immunoassay format has shown to be a sensitive detection of TCP in human urine without the need of previous clean-up and preparation of samples. Our SPR direct detection approach to TCP quantification in urinary samples could be easily transferred to other dangerous relevant substances, drug residues, veterinary products or exposure markers susceptible to be found in body fluids (blood, urine). The simplicity, low time of response and real-time determination are inherent advantages over chromatographic and immunochemical-based methods. The SPR-sensor platform is commercialised by the company SENSIA, SL (Spain).