ANALYSIS OF DDT USING A HOME-MADE SURFACE PLASMON RESONANCE BIOSENSOR

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Environmental concern about the potential of some organic pollutants, such as pesticides and industrial chemicals, to cause adverse effects on human health and wildlife has arisen in recent years. Among these compounds, the use of DDT (Dichloro-diphenyl-trichloroethane) as organochlorine insecticide, has been widely restricted due to its persistence and accumulation in the food chain.

The utilization of SPR biosensors for the detection of environmental pollutants is based on the principles of solid-phase immunoassays. These assays require the use of antibodies (monoclonal or polyclonal), which are the key components of all immunoassays, since they are responsible for the sensitive and specific recognition of the analyte. The application of immunoassays to environmental monitoring also involves the design of hapten derivatives of low molecular weight molecules, such as DDT, to determine the antibody recognition properties. Once hapten synthesis and monoclonal antibody production have been accomplished, the use of SPR biosensing technique provides a real-time monitoring of binding interactions without the need of labelling biomolecules.

Immunoassays developed to determine DDT were inhibition tests based on the conjugate coated format, in which a 10 µg/mL concentration of BSA-DDT in 10 mM acetate buffer, pH 5.0, was immobilized on the sensor surface. The competitive heterogeneous assay required the incubation of a mixture of the analyte (DDT) and the antibody (LIB-DDT5.25), before binding of the free remaining antibody to the immobilized conjugate. As it corresponds to binding inhibition immunoassays, the SPR signal provided by the sensor was inversely proportional to the analyte concentration in the DDT-antibody mixture, and standard points fitted to a sigmoidal equation. A standard curve (see Figure 1) was obtained by averaging four individual standard curves normalized by expressing the SPR signal (SPR_{signal}) of each standard point as the percentage of the maximum response \([100 \times (SPR_{signal} / SPR_{signal\_max})]\).

**Figure 1.** Normalized average standard curve for the DDT SPR immunoassay.

The sensitivity of the immunoassay, expressed as the analyte concentration that reduces the assay signal to 50% (IC50) of the maximum signal, was 11.7 nM. The inhibition curve obtained with the application of the conjugate-coated format allowed the detection of DDT from 2.2 to 59 nM (IC80-IC20), assuming this range as the operative working range of the assay. A limit of detection of 0.8 nM was also calculated from the calibration curve, as the analyte concentration for which the normalized signal was 90% of the maximum one.

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