WAVEGUIDE SURFACE PLASMON RESONANCE BIOSENSOR FOR SIMAZINE ANALYSIS

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We report the performance of gold-coated waveguide surface plasmon resonance biosensors for the detection of the pesticide simazine in aqueous solution. Calibration against standard simazine solutions identified a detection limit of 0.22 μg/l.

Introduction

The possible uses and market potentials of guided-wave optical biosensors are well reported[1]. Optical biosensors are generally small, light, and rugged, offering the portability required for a field monitoring system. Integrated optical sensors maybe connected by optical fibres and allow for the fabrication of multiple sensors in a single substrate using photolithographic techniques. The use of a surface plasmon resonance (SPR) to form the basis of a chemical sensor has been known for some years[2]. A surface plasmon can be formed by using a 'bulk' optical component such as a prism, and equipment employing this approach is commercially available[3]. Another option is to employ the distributed coupling between a planar waveguide and a surface plasmon in a metal-coated waveguide.In this paper we demonstrate the use of a simple gold-coated waveguide SPR biosensor to monitor the presence of simazine in aqueous solution using an antibody based non-competitive assay. This device represents a portable sensor realisation of the otherwise laboratory-based 'bulk' SPR technique.

Waveguide SPR Sensor Design and Operation

The configuration of the sensor, whose operation can be described in terms of coupled modes, is detailed in figure 1. A single TM mode is excited in the input waveguide and divided into two paths with a 3 dB y-junction splitter. The mode propagating under the gold film then couples to the surface plasmon mode,

guided by the interface between the metal film and the superstrate, if the two modes are closely phase matched. If a thin film is adsorbed to the metal surface then the coupling condition between the two modes is altered causing a change in the output intensity of the sensor. The other arm of the y-junction acts as a reference to minimise the effects of input power drifts. A rigorous model has been used to evaluate the type of sensor shown in figure 1 when monitoring the adsorption of a thin film to the surface of the gold film. This model solves for all the modes in the structure but assumes homogenous 'slab' waveguides. Extensive design work[4] has lead to optimised sensor designs for the aqueous environment.

Experimental Measurement of Simazine in Aqueous Solution

Waveguides were fabricated by potassium ion-exchange in glass substrates (n=1.471) at a temperature of 385 °C for 8.5 hours. A 53 ± 3 nm thick gold film was deposited on the waveguides by thermal evaporation. To evolve the basic SPR waveguide structure into a simazine sensor, antigens to the simazine antibody were covalently attached to the gold film. This was achieved by chemically attaching thiols to the gold film, covalently binding dextran to this modified surface, and finally binding the antigen to the simazine antibodies to the dextran layer. The experimental arrangement for the assay is detailed in figure 2. A wall-jet flow cell was clamped on top of a waveguide coated with a gold film over a length of 3 mm and attached to an ASIA-FIA system from Ismatec (Zürich). A stream of 10 mM phosphate buffer solution (PBS), pH 7.4, was passed over the modified gold film to provide a stable environment. A 10 mW, linearly polarised, He-Ne laser (λ = 632.8 nm), was end-fire coupled into the SPR sensor to excite a TM mode. After passing through the y-junction splitter the twin outputs were focussed onto silicon photodiodes and the resulting signals were captured and ratioed by a PC.

A typical test cycle, of 20 minutes duration, recorded by the sensor, is shown in figure 3. A 1 ml test sample is created from 0.33 µg of simazine antibody in 100 µl of PBS and 900 µl of PBS-buffered pesticide solution. The sample is pre-incubated for 5 minutes before being injected into the flow cell on top of the sensor where it is incubated for 8 minutes. Due to the low antibody concentration the binding rate to the sensor surface is diffusion limited and linear. Linear regression is performed to determine the slope of the binding curve during sample incubation in the flow cell. After sample incubation the sensor surface is regenerated to remove the antibodies with pepsin followed by acetonitrile/propionic acid.

Repeated test cycles were carried out using varying concentrations of simazine, from 0 µg/l to 100 µg/l (0 to 100 ppb), to calibrate the biosensor. A sigmoidal curve was then fitted to a plot of the slopes of

the binding curves measured for each test sample. These calibration curves were repeated and averaged over a 5 day period to give an indication of the stability, dynamic range, and detection limit of the sensor.

Results and Discussion

The averaged calibration curve generated by repeated sets of measurements over a 5 day period is detailed in figure 4. The mid-point of the calibration curve is at a simazine concentration of 0.44 ppb. The coefficient of variation (CV) for the blank measurements is 8 % . A detection limit of 0.22 ppb was calculated from figure (4) based on a binding curve slope 3 standard deviations below that of the blank measurement. The average CV for the calibration curve is 15 % for 0 to 1 ppb. For the antibody concentration of 0.33 μg/ml the sensor operates over a simazine concentration range of 0.22 ppb to approximately 1.0 ppb. In the case of the example test cycle shown in figure 3 the correlation coefficient of the linear regression fitted to the binding curve during sample incubation is 0.9998. The rms value of the residuals of the linear regression, expressed as a percentage deviation from the fitted line, is 0.05 %.

The baseline drift of the sensor was also evaluated over the 5 day period. The CV of the baseline over the whole period was 3 %, with a minimum/maximum ratio of 0.90. This drift includes changes in baseline due to the variability of the regeneration phase of the test cycle and is not solely a reflection on the optical stability of the sensor. Over a time period of the test cycle, 20 minutes, with only PBS flowing across the sensor surface, the CV of the baseline was calculated to be 0.1 %. A total of 266 assays were conducted over the whole trial period with this sensor.

Conclusions

Gold coated waveguide SPR sensors have been fabricated and the gold films were chemically modified to enable the detection of the pesticide simazine by employing appropriate antibodies. A detection limit of 0.22 ppb (0.22 μg/l) was achieved. Whilst lying outside of the limit imposed by the EU for individual pesticides the detection limit is sufficiently close to have demonstrated the feasibility of the waveguide SPR approach. It is expected that the application of improvements in sensor stability would increase the performance of the system to meet the EU limit for pesticides in drinking water.

Acknowledgements

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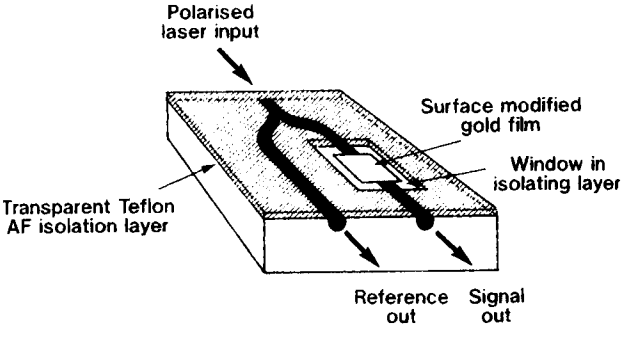

Figure (1) *Schematic SPR Biosensor Structure*

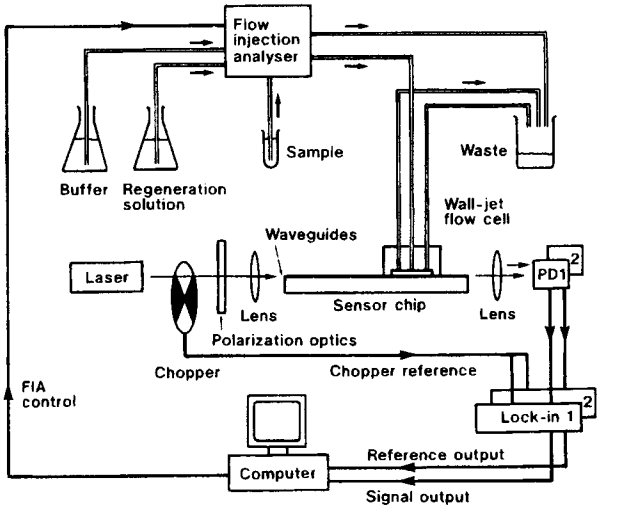

Figure (2) *Experimental Arrangement for Pesticide Detection*

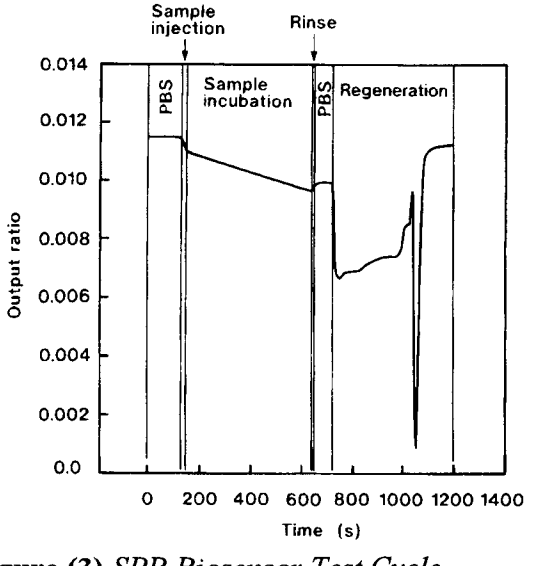

Figure (3) *SPR Biosensor Test Cycle*

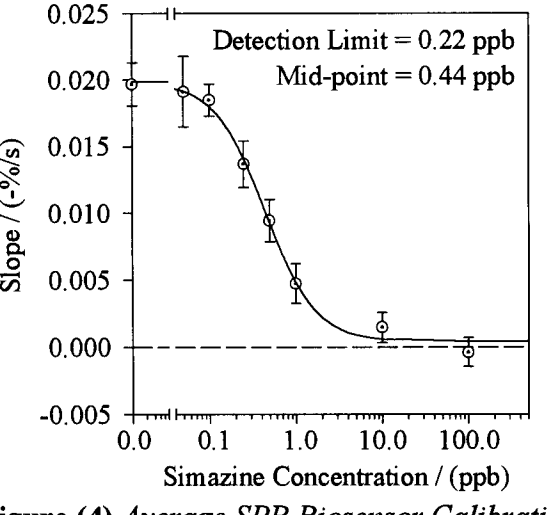

Figure (4) *Average SPR Biosensor Calibration Curves*