Immunofluorescence Sensor for Water Analysis

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Abstract

We demonstrated a bulk optical fluorescence based immunosensor capable for multianalyte water analysis. Calibration curves obtained for 2,4-dichlorophenoxyacetic acid (2,4-D) and simazine had detection limits of 0.035 µg/l and 0.026 µg/l respectively. The sensor is reusable due to its regenerability and cost effective due to the use of components customary in the trade. Ways to further enhance device sensitivity by means of a high index film deposited on the sensor surface or by employing an integrated optical waveguide as transducer are presented. A concept for the detection of a varying range of analytes on the same transducer is discussed.

Due to the large number of pollutants and there derivatives present in surface and ground waters and due to stricter regulations for the detection of these pollutants set out by the legislative bodies there is a growing need for sensitive, cost effective and fast methods to quantify these compounds. In addition such methods should be able to detect a multitude of analytes in one single testcycle and possibly should be flexible in the analyte spectrum. To address these requirements we developed a regenerable TIRF (Total Internal Reflection Fluorescence) sensor and demonstrated its performance for the detection of the pesticides 2,4-dichlorophenoxyacetic acid (2,4-D) and simazine in water. Two strategies how to enhance device sensitivity are presented. Discussed is also the detection of atrazine caproic acid by means of an auxiliary antibody system sandwiched between the sensor surface and the analyte specific antibody. This demonstrates the concept of the flexible biochemistry involved to address a varying range of target analytes on the same transducer.

As transducer element a bulk optical glass slide (60mm x 15mm x 1.5mm) with a polished 45° bevel on one end-face is used. To the sensitive surface of the transducer analyte derivatives bound to a dextran layer is attached. Light from a collimated and modulated laser diode operated at 639 nm, 3 mW output power is directly coupled into the waveguide via the bevelled end-face and is guided by total internal reflection. Fluorescence is excited in the evanescent field of the waveguide at distinct and spatially resolved reflection spots of an area of 4 mm^2. Over an active length of 40 mm up to six different analytes could be detected. Fluorescence is collected by a polymer fibre located under the sensor chip opposite of the active spot and detected by photodiodes using lock-in detection technique.

The specific recognition of the target analytes relies on the high affinity between the analytes and the anti-analyte antibodies. Binding inhibition assays, with analyte derivative attached to the modified surface of the transducer and with Cy5.5 dye labelled antibodies were performed and calibration curves obtained. The rather strong rate of photobleaching of the surface bound dye molecules makes it necessary to shut off the excitation laser during the incubation of the labelled antibodies to the transducer surface. For the calibration of the sensor the signal difference between the averaged signal over 10 s after and before the binding process was evaluated. Regeneration of the sensor surface was carried out with pepsin and a mixture of acetonitrile, proprionic acid and water. Each transducer was reused more than one hundred times without much loss of sensitivity. Fig (1) shows the average of three calibration curves for

2,4-D and simazine. Both calibration curves were obtained with an analyte specific antibody concentration of 0.5 µg/ml. The mid-point of test is 1 µg/l for 2,4-D and 0.2 µg/l for simazine. The limit of detections are 0.035 µg/l and 0.026 µg/l respectively.

As a means to further enhance device sensitivity we followed two routes. First, a 50 nm thick tantalum pentoxide film deposited on the transducer surface increased the maximum fluorescence signal of a bulk dye solution by a factor of up to eight compared to an uncoated transducer as indicated in Fig (2). This depends on the state of polarisation and the angle of incidence of the incoupled light. The enhancement is addressed to the shift of the maximum of the electromagnetic field distribution in the transducer towards the high index overlayer. As net effect, more power is carried in the evanescent field.

Second, we employed a Na^+/K^+ ion exchanged integrated optical channel waveguide in a 1 mm thick Pyrex substrate as transducer element. The nominal waveguide width was 10 µm and the active area of fluorescence excitation and collection about 0.02 mm^2. The fluorescence signals obtained with this device were similar to the ones obtained with the bulk transducer. Considering the approximately 100-fold smaller excitation area, corresponding to a 100 times smaller number of excited dye molecules, this can be regarded as a more efficient way to excite surface bound fluorescence. Effectively this can be seen as an enhancement and is addressed to the higher fraction of power guided in the evanescent field of the integrated optical waveguide and to the higher collection efficiency.

Ways how to exploit high index films and alternative waveguides designs within the sensor system will be discussed.

Following the notion of a highly flexible target analyte spectrum to be detected on the same transducer we investigated an assay which allows for different analytes to be quantified without changing the transducer. The flexibility relies on an auxiliary antibody conjugated with the target analyte derivative which is sandwiched between the transducer surface and the analyte specific antibody. A variable range of analytes can be addressed by changing the auxiliary antibody conjugation. We demonstrated this concept with the calibration of atrazine caproic acid taking advantage of anti-progesterone as the auxiliary antibody.
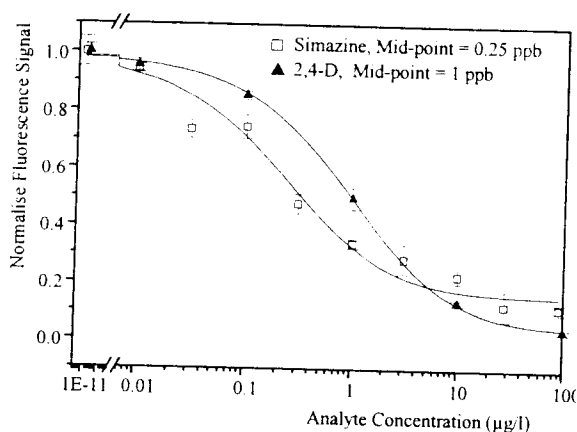

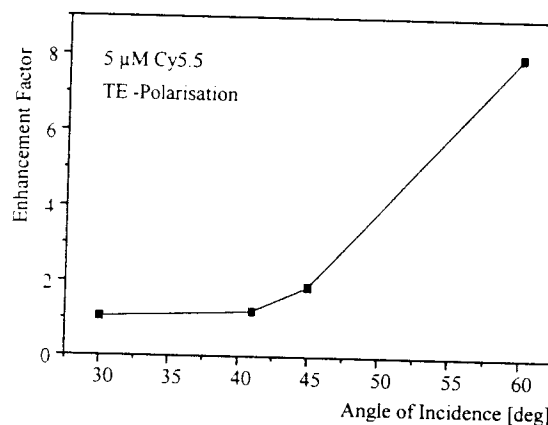Fig. (1): Averaged calibration curves for simazine and 2,4-Dichlorophenoxyacetic acid

Fig. (2): Enhancement in fluorescence excitation of a high index film deposited on the transducer surface.