Immunofluorescence Sensor for Water Analysis

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Abstract

We demonstrated a bulk optical fluorescence based immunosensor capable of 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.

Introduction

Due to the large number of pollutants and their derivates 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 [1]. In addition, such methods should be able to detect a multitude of analytes in one single testcycle. In many applications the analyte spectrum changes over time, for instance, in analysing ground water where the pollutants, like pesticides, vary over time according to the time of their input. Therefore, a sensor should be as flexible as possible in the analyte spectrum which can be addressed.

Laboratory based systems like HPLC and GC for quantifying compounds are established well and are very accurate and advanced. However, they need short term maintenance, well trained personnel operating the apparatus and extensive sample pre-treatment. Any sort of analytical device overcoming these limitations would be of high value for the user. From a practical point of view this device should cover the range of operation the potential applicant needs and should be robust, simple to use and not laboratory based.

There are several optical approaches to address these considerations. Many of them are evanescent field devices relying either on label-free sensing or the use of labels for the detection method [2]. The evanescent field arises at the interface between two dielectric media if the condition for total internal reflection is complied with. It decays exponentially into the media of lower refractive index. The penetration depth is of the order of half the operating wavelength. In evanescent field sensor applications an optical waveguide is used as the transducer element. A fraction of the total guided power in the waveguide is carried by the evanescent field. The sample covers part of the waveguide surface and interacts with the guided light via the evanescent field. Among the demonstrated planar devices in the literature are grating and directional couplers [3, 4], surface plasmon resonance [5, 6],

resonant mirror [7], interferometric [8, 9] and fluorescence based sensors [10]. All of the transducers have (bio-)chemically modified surfaces to allow for specific recognition. Most of them showed good sensitivity in determining pollutants. However, there are two major drawbacks associated with these approaches. So far most of these devices have been designed to be single analyte only detectors and they are not very simple to use.

Our measurement approach puts emphasis on simple handling of the sensor combined with an integrated and rugged set-up while retaining good sensitivity and offering the possibility of multianalyte detection in one single testcycle. It is based on excitation of fluorescence by attenuated total internal reflection. 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 covalently linked to a dextran layer attached to the surface of the transducer were performed to detect the analytes. The antibodies were labelled with the red excitation and emission Cy5.5 dye. This allows the use of a small sized and low cost laser diode as excitation light source. We demonstrated the performance of the sensor for the detection of the pesticides 2,4-dichlorophenoxyacetic acid (2,4-D) and simazine in water.

Two strategies of how to enhance device sensitivity are discussed. Also presented is the detection of isoproturone by means of an auxiliary antibody system sandwiched between the sensor surface and the analyte specific antibody. This demonstrates a concept of flexible biochemistry to address a varying range of target analytes on the same transducer.

Experimental

Set-up: A schematic of the set-up is depicted in Figure 1. As the transducer element a bulk optical glass slide (60mm x 15mm x 1.5mm) with a polished 45° bevel on one end-face was used. To the sensitive surface of the transducer analyte derivatives bound to a dextran layer are attached. Light from a collimated (beam diameter = 1 mm) and modulated laser

diode (Point Source, UK) operated at 639 nm, 3 mW cw 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 with an area of 2.5 mm^2. Over an active length of 40 mm up to six different analytes could be detected. Fluorescence is collected by high numerical aperture polymer fibres (NA = 0.46) located under the sensor chip opposite the active spot. The collected light is filtered by means of a bandpass filter (Schott, FRG) to reject any pump radiation and detected by photodiodes using lock-in detection. The transducer was embedded in a flow cell made of PMMA with nominal dimensions of the flow channel of 40 mm x 1.7 mm x 0.06 mm. All reagents were delivered by a flow analysis system operated with a syringe pump (Perkin Elmer DS6 and AS 90). Fluid handling and data acquisition is fully automated and computer controlled.

Materials: Common chemicals and biochemicals in analytical grade where purchased from SIGMA, Deisenhofen / Germany (dextran, di-isopropylcarbodiimide (DIC), ovalbumin, pepsin). 3-Glycidyloxypropyl-trimethoxysilan (GOPTS) was bought from Fluka, Deisenhofen / Germany. Cy5.5 dyes for labelling the antibodies were purchased from Amersham, Braunschweig / Germany. Pesticide standard solutions were purchased from Riedel-de-Haën (Seelze, FRG). Monoclonal anti-2,4-D IgG antibodies were purchased from Veterinary Research Institute (Dr. M. Franek, Brno, CZ), polyclonal sheep anti-simazine, anti-isoproturone and anti-progesterone antibodies and the analyte derivatives were kindly supplied by Dr. Ram Abuknesha (GEC, London). Amino dextran of molecular weight of about 30.000 g/mol was prepared according to reference [11].

Surface modification: The transducer surface for the immunoassays was modified as follows. After cleaning in freshly prepared piranha solution (H_2O_2 : H_2SO_4, 2:3) for 30 min and rinsing with de-ionisized water the transducer was dried at room temperature. 30 µl of

GOPTS was applied to the dried surface and reacted for 1.5 h in a nitrogen atmosphere. The silanised surface was rinsed with dry acetone and dried under a steady flow of nitrogen. Aminodextran dissolved in water (2:1) was coupled to the silanised surface for 6 h. Analyte derivatives dissolved in DMF were attached to the remaining amino groups of the dextran using DIC. This procedure leads to a high density of specific binding sites for the antibodies at the transducer surface. Unspecific binding is lowered to a minimum due to the shielding of the glass surface by the amino dextran [11]. Spatially resolved modification was achieved by pipetting the dextran modified with the analyte derivative on the transducer surface by means of a microdrop dispensing system. Areas of about 8 mm^2 centered at the reflection spots were accessed by this technique.

Labelling of antibodies: The Cy5.5 fluorophores were attached to the antibodies by forming a peptide bond between the active ester group of the dye and free amino groups of the antibodies. The number of dye molecules attached to individual antibodies could be adjusted to values between two and four. For details see [12].

Results and discussion

Device characterisation: To get an estimate for the expected signal levels in the bulk glass slide used as transducer in our TIRF sensor the fraction of evanescent guided power was calculated as shown in Figure 2 according to [13]. The parameters chosen were as follows: refractive index of the guide, the superstrate and air: $n_{guide} = 1.52$, $n_{sup} = 1.33$, $n_{air} = 1.0$, guide thickness d = 1.5 mm and wavelength $\lambda = 633$ nm.

For practical operating conditions working with an angle of incidence of the light propagating in the waveguide of 65° approximately 0.25 ‰ of the total guided power is expected to be carried in the evanescent field. Assuming a light coupling efficiency of 75 % into the transducer this corresponds to 0.28 μW evanescent guided power contained in all

modes. For comparison, the calculated fraction of evanescent guided power for a waveguide thickness of d =10 μm is about a factor of 50 higher than for the bulk glass chip. However, for integrated optical waveguides high coupling efficiencies are much harder to achieve. Additional optical components like prisms, lenses or fibre pigtailing are needed for light coupling.

In the bulk transducer a laser beam of diameter 1 mm will produce reflection spots of an area of about 2.5 mm^2. An assumed IgG antibody monolayer coverage on the waveguide surface of 5 mg/m^2 [14] corresponds to 12.5 ng of antibody on the reflection spot. If each antibody is labelled with one dye molecule the number of antibodies corresponds to a concentration of 0.15 mmol/l dye molecules in the estimated probed volume of 0.5 nl of the evanescent field. Under real assay conditions (0.5 μg/ml antibody concentration, incubation times about 300s, and a high density of binding sites) about $1/10.000^{th}$ of this value should be resolved [15]. Therefore, a simple performance check of the transducer is the measurement of the sensor response to dye concentration excited in the bulk of the sample solution. According to the derivation from above a 15 nmol/l dye solution brings the same number of fluorophores into the probed volume as a $1/10.000^{th}$ of a monolayer of bound antibody.

The sensor response to Cy5.5 solutions in phosphate buffered saline (PBS, 10 mmol/l KH_2PO_4, 150 nmol/l in deionized water at pH = 7.4) of different concentrations is shown in Figure 3. Concentrations down to 10 nmol/l can be resolved. A detection limit of 8.8 nmol/l is obtained, calculated from three times the standard deviation of the baseline. This corresponds to about 400 fW of detected fluorescence power. Taking into account a quantum efficiency of 0.3 for the dye, a roughly estimated collection efficiency of fluorescence of 3 % and propagation losses in the polymer fibres and filters in the order of 50 %, an excited fluorescence power at the interface in the order of tens of pW is determined for a $1/10.000^{th}$ of a monolayer antibodies.

Pesticide immunoassays: The analytical performance of the device was demonstrated in obtaining calibration curves for 2,4-D and simazine. To quantify the pesticide concentrations binding inhibition immunoassays were carried out with an antibody concentration of 0.5 µg/ml. All pesticide standard solutions were prepared in PBS (pH 7.4) from a single master solution of 100 µg/ml. To 100 µl antibody solution (5 µg/ml) 800 µl standard solution and 100 µl (1 mg/ml) ovalbumin solution were added and mixed thoroughly. This mixture was left for 20 min (so called 'pre-incubation'). During this time antibody binding sites were occupied according to the concentration of pesticide. After pre-incubation the mixture was delivered to the transducer surface and incubated in a steady flow of 120 µl/min for 5 min. Anitbodies with free binding sites left are able to bind to the transducer surface. Surface bound fluorescence is excited in the evanescent field of the sensitive reflection spot. After binding the transducer was rinsed with PBS in order to remove any fluorescent material weakly bound to surface. 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. Therefore, if maximum signal levels are required it is not possible to gain information about the binding kinetics of the antibodies to the transducer surface. For the calibration of the sensor the difference of the integral of the signal trace was taken over 10 s during the background measurement and on switching on the laser after the binding process. The calibration data obtained in this way is less dependent on short term signal fluctuations than simply taking the signal difference before and after binding. Regeneration of the sensor surface was carried out with pepsin (2 mg/ml at pH 1.9) for four minutes followed by a short pulse (15 s) of acetonitrile, proprionic acid and water (50:1:50) and rinsing with PBS. The regeneration cycle was repeated two times in order to remove all antibodies left on the surface. A complete testcycle takes about 15 min for completion.

A typical fluorescence time trace during the binding of dye labelled antibodies to the transducer surface is shown in Figure 4. The actual fluorescence intensity is an overlay of fluorescence excited from labelled antibodies bound to the surface, fluorescence excited in the bulk by stray light and degradation of bound fluorophores due to photobleaching. This complex nature of the fluorescence signals makes it hard to retrieve precise data of the underlying binding kinetics. When the laser is turned on and fluorescence is excited continuously during binding, the fluorescence signal increases as long as the binding rate to the surface is higher than the photobleaching rate of the bound fluorophores. It reaches a maximum and decreases when photobleaching exceeds the rate of binding to the surface. For the sake of a high dynamic range of the sensor signal we decided to shut off the laser during incubation. This is on the expense of gaining additional information about the binding kinetics. There is significant photobleaching of bound fluorophores to be seen on turning on the laser after binding. The flurorescence signal falls below half its maximum value within the first two minutes of illumination.

Figure 5 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 and the laser turned off during incubation. On average 2.5 and 1.8 dye molecules were attached to the 2.4-D antibody and the simazine antibody respectively. The mid-point of test is 1 µg/l for 2,4-D and 0.25 µg/l for simazine. The limit of detection (lod) determined using three times standard deviation of the mean blank values are 0.035 µg/l and 0.026 µg/l respectively. These lods meet the requirements for determining pesticides in drinking water demanded by the European Union (lod = 0.1 ppb for a single pesticide). The same transducer was used for more than 100 testcycles without much loss of performance. The decrease in the average maximum signal response when no analyte was present was about 5%.

The analytical performance of the assay relies strongly on the affinity of the antibodies to the analyte in the sample and the analyte derivative bound to the transducer surface. The higher the affinity constants the lower the antibody concentration which can be applied to obtain similar signal levels. This in turn leads to lower lods. Affinity constants between the antibody and the analyte in the sample of $1 \cdot 10^9$ l/mol and $3 \cdot 10^9$ l/mol where found for 2,4-D and simazine antibodies respectively by fitting the calibration curves to a model derived from the law of mass action [16]. The model fits the data for the 2,4-D calibration very well, for simazine the goodness of the fit is poorer. This is explained by the fact that the model applies strictly only to monoclonal antibodies, like 2,4-D. The affinity constant determined for the polyclonal simazine antibody is to be understood as an averaged value for the whole antibody population.

So far no more than two analytes have been detected at the same transducer simultaneously. One limiting factor on the ability to perform more than one assay simultaneously on the same transducer is the availability of low cross-reactant antibodies combined with high affinity between the antibody and the analyte. In our experience this seems to be the bottle neck to true multi-analyte assays at present.

Flexible analyte spectrum: One approach to address a variable analyte spectrum is the introduction of an auxiliary antibody that can be conjugated with different target analyte derivatives at a time. The auxiliary antibody must not be labelled with a fluorophore. In this mode of operation the transducer surface is modified with a capture reagent being specifically recognised by the auxiliary antibody. In an assay, the auxiliary antibody is effectively sandwiched between the sensor surface and the analyte specific antibody. The assay can be conducted either sequentially or competitive. Upon changing the target analyte derivative linked to the auxiliary antibody the same sensor can be used to detect a different target analyte without changing the surface modification of the transducer. This concept is

outlined in [17]. Figure 6 shows a measured calibration curve for the detection of isoproturone with an anti-progesterone antibody used as auxiliary antibody. Applied concentrations were 0.5 µg/ml and 1 µg/ml for the primary and auxiliary antibody respectively. The surface of the transducer was modified with isoprogesterone hemi succinate. A mid-point of test of 30 µg/l was achieved. The reduced performance of the sandwich assay compared to the two calibrations without auxiliary system is due to the more complex nature inherent in this assay. It needs dedicated optimisation for the best choice of applied antibody concentration and for the choice of auxiliary antibody conjugate.

Sensitivity enhancement: It can be shown [20] that the expected mid-point of test and the limit of detection of a recorded calibration curve as described above shifts to lower analyte concentrations if the applied antibody concentration is reduced (no more than the inverse affinity constant). However, reduced antibody concentrations lead to lower fluorescence signals. This, in turn, requires more sophisticated detection schemes or more efficient and sensitive means to excite fluorescence on the transducer. Therefore, we investigated to ways how to further enhance device sensitivity by optimising the trandsducer design.

It was shown that thin high index films deposited on top of a waveguide can be employed to enhance sensitivity of integrated or fibre optical evanescent field devices. Enhancement factors of up to one to two orders of magnitude were reported [18, 19]. The high index film effectively shifts the maximum of the field distribution in the waveguide towards the interface between waveguide and sample. This leads to an increase in the proportion of power in guided modes carried by the evanescent field in the superstrate.

To investigate the effect of a thin high index film experimentally a 50 (±7) nm thick tantalum pentoxide film was deposited on the transducer surface. The refractive index of the film at 633 nm was determined to be $n = 2.1$ [19]. For different angles of incidences in the waveguide the signal difference between PBS and a 5 µmol/l dye solution was measured and

compared to a waveguide without high index overlay. The ratio of these signals were regarded as enhancement factors. An increase in fluorescence signal to a factor of up to eight has been obtained for a large angle of incidence as shown in Figure 7.

However, even though there is a significant enhancement verified experimentally for the overlaid glass slide, it is observed that the absolute signal levels decrease by more than a factor of eight as the angle of incidence grows. This is in agreement with the results shown in Figure 2. For large angles of incidence the evanescent guided power decreases considerably. Therefore, in practise this enhancement cannot be exploited to increase the overall sensitivity of the device.

As an alternative route to enhance device sensitivity, we studied the performance of the sensor with a different type of waveguide. Instead of the bulk optical 1.5 mm thick glass slide we employed an integrated optical channel waveguide as the transducer element. The basic experimental set-up remained the same. As indicated previously, for integrated optical waveguides the proportion of evanescent guided power is expected to be much higher than for bulk optical components. As the transducer a Na^+/K^+ ion exchanged integrated optical channel waveguide in a 1 mm thick Pyrex substrate was fabricated. The nominal waveguide width was 10 μm and the active area of fluorescence excitation and collection approximately 0.02 mm^2. The fluorescence signals obtained with dye solution in this device were of a similar magnitude to the ones obtained with the bulk transducer. Considering the lower coupling efficiency to the waveguide and 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.

In future work we will concentrate on the development of more sophisticated integrated optical waveguide structures. Designs incorporating high index films and waveguide designs to spread out the power in the guide in order to reduce the very pronounced rate of

photobleaching with these devices will be important issues to be addressed. Additionally, structuring the device for multianalyte detection and putting attention on ways how to simplify and enhance coupling to the device will be dealt with.

Summary and Outlook

A TIRF (total internal reflection fluorescence) immunosensor was demonstrated, which allows the detection of a multitude of analytes in one single testcycle. For the transducer we characterised and investigated a bulk optical glass slide with a bevelled and polished end-face for light coupling. The device is robust, compact and very cost effective as it does not require any additional optical components but the transducer and the laser diode.

Calibration curves for the pesticides 2,4-dichlorophenoxyacetic acid and simazine were obtained. Limits of detection of 0.035 µg/ml and 0.026 µg/ml for these pollutants were determined respectively. The observed photobleaching of the Cy5.5 fluorophore labels was considerable. An assay to address a variable range of analytes was presented. The assay employed an additional auxiliary antibody conjugated with the target analyte sandwiched between the transducer surface and the target analyte antibody. A mid-point of test of 30 µg/l was achieved.

Two ways how to enhance device sensitivity were discussed. First, a 50 nm thick Ta_2O_5 film deposited on the surface of the transducer enhanced fluorescence excitation compared to a uncoated transducer by a factor of up to eight. Unfortunately this enhancement could not be exploited to enhance the overall sensitivity of the device due to the lower overall signal levels. Second, studies of fluorescence excitation in the evanescent field of an integrated optical channel waveguide confirmed the more efficient way to fluorescence excitation with this transducer compared to the bulk optical transducer.

On the transducer side, new waveguide structures to reduce the power densities at the interface between waveguide and sample will be designed. From a practical point of view, work on simplified, more efficient and rugged light coupling techniques to integrated optical waveguides needs to be done. This will be a big step towards the use of these transducers outside a laboratory environment.

On the biochemical side, the development work on recognition structures with high affinity and low cross reactivity needs to be pushed. At present, the availability of these compounds seems to restrict the number pollutants which can be analysed in multianalyte biochemical sensors simultaneously.

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Figure Captions

Figure 1: Set-up of the bulk optical fluorescence sensor for multianalyte detection. Fluorescence excited by attenuated total internal reflection at spatially resolved reflection spots is collected by polymer fibres and detected by photodiodes (PD). A laser diode operating at 639 nm is used as excitation light source.

Figure 2: Calculated fraction of evanescent guided power for waveguide parameters λ = 633 nm, n_{guide} = 1.52 and n_{sup} = 1.33.

Figure 3: Sensor response to Cy5.5 dye solutions of different concentrations.

Figure 4: Signal traces for measurements taken with the excitation laser turned on and off during incubation (c[ab] = 500ng/ml).

Figure 5: Calibration curves for the detection of 2,4-dichlorophenoxyacetic acid and simazine respectively. The mid-point of test was 1 µg/l and 0.25 µg/l respectively.

Figure 6: Calibration curve for the detection of isoproturone employing a sandwich assay with anti-progesterone as auxiliary antibody.

Figure 7: Measured enhancement factor for the bulk optical glass transducer overlaid with a 50 nm Ta_2O_5 film. The enhancement corresponds to an increased fraction of power carried in the evanescent field for the overlaid guide.

Biographies

Albrecht Klotz graduated in Physics at the University of Tübingen in 1995. Currently he is working towards his PhD at the Institute of Physical Chemistry at the University of Tübingen. His fields of interests are in optical sensors and in fluorescence detection with optical transducers.

Claudia Barzen graduated in Chemistry at the University of Karlsruhe in 1996. Currently she is working towards her PhD at the Institute of Physical Chemistry at the University of Tübingen. Her fields of interests are TIRF (Total Internal Reflection Fluorescence), surface chemistry, immunoassays and environmental analysis.

Andreas Brecht graduated in Biochemistry at the University of Tuebingen. After working for a small company in the field of electronic cell size determination, he took a PhD on direct optical detection of affinity reactions at the Institute of Physical Chemistry, University of Tübingen. Activities and interests are biosensors and bioanalytical devices for affinity interactions. Currently he works on membrane receptors at the Swiss Federal Institute of Technology at Lausanne, Switzerland.

Günter Gauglitz is professor in chemistry at the department of Physical and Theoretical Chemistry at the University of Tübingen. He is head of a research group of 20 scientific co-workers. His main activities are in optical spectroscopy and optical (bio)chemical sensors with applications in environmental analysis and in pharmascreening projects.

Richard D. Harris received the B.Sc. degree in applied physics from the University of Bath in 1986. In 1992 he received the M.Sc. degree in applied optics from the University of Salford. He was awarded the degree of Ph.D. from the University of Southampton in 1996. He iscurrently a Postdoctoral Fellow in the Optoelectronics Research Centre, University of Southampton, studying integrated optical fluorescence sensors.

Geoffrey R. Quigley received the BSc. degree in physics from Imperial College London in 1995. He is now studying for the degree of PhD. at the Optoelectronics Research Centre, University of Southampton,

where he is working on the sensitivity enhancement of integrated optical sensors by high index films.

James S. Wilkinson received the BSc(Eng) in electronics in 1977 and the PhD degree in the field of integrated optics in 1985, both from University College London. From 1977 to 1979 he was with the GEC Hist Research Centre working on optical fibre telecommunications systems From 1983 to 1985 he was with the Department of Nephrology of St Bartholomew's Hospital, London, working on sensing and control for haemodialysis procedures. He is now Reader in Optoelectronics in the Department of Electronics & Computer Science, University of Southampton, UK. He is partially seconded to the Optoelectronics Research Centre at Southampton University, where he leads a research group investigating integrated optical devices including chemical and biochemical sensors with particular application to water quality monitoring, and planar waveguide lasers and amplifiers.

Ram Abuknesha is a senior biochemist working currently at the Division of Life Sciences, Kings College, London. His interests include immunochemical techniques for environmental analysis. Currently he works on the development of novel immunoassay formats, immunoaffinity chromatography, purification methods and development of multianalyte immunosensor systems.

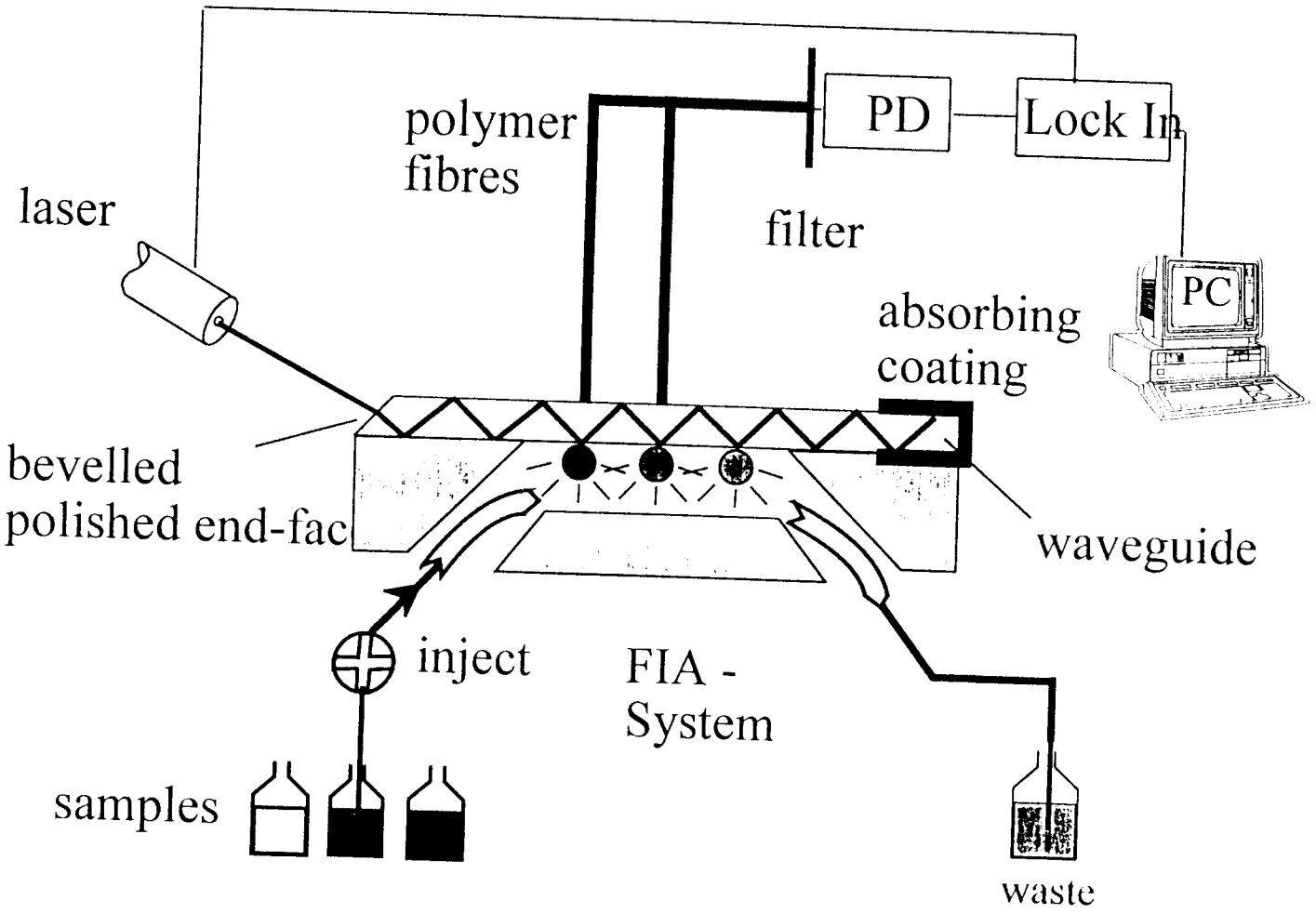
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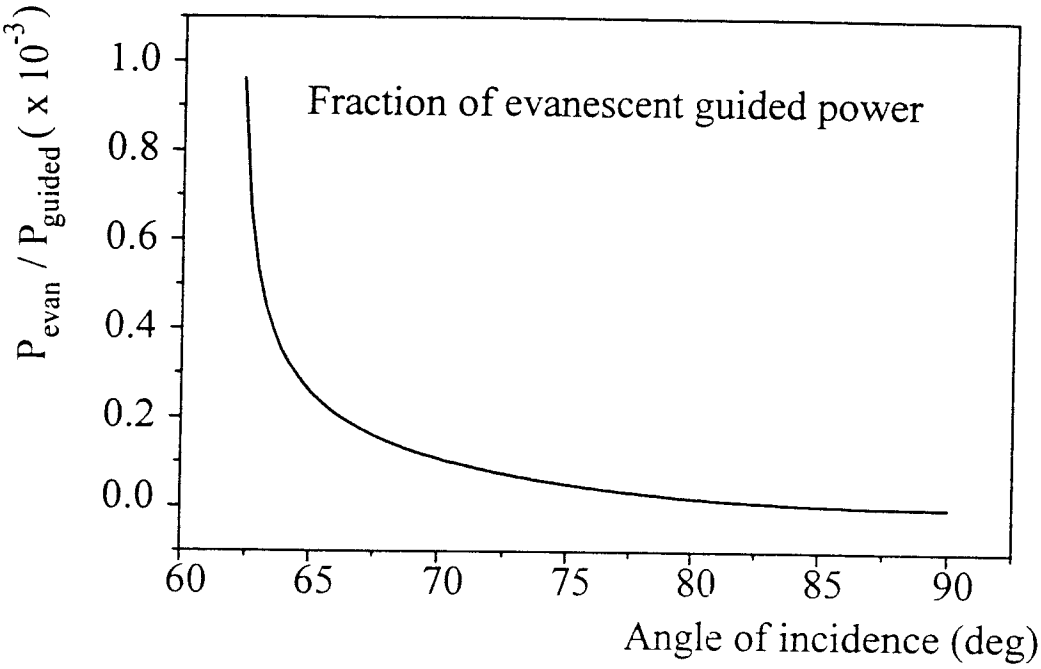
Figure 1


Fraction of evanescent guided power
$P_{evan} / P_{guided} (x 10^{-3})$
1.0
0.8
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0.4
0.2
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60
65
70
75
80
85
90
Angle of incidence (deg)

Figure 2

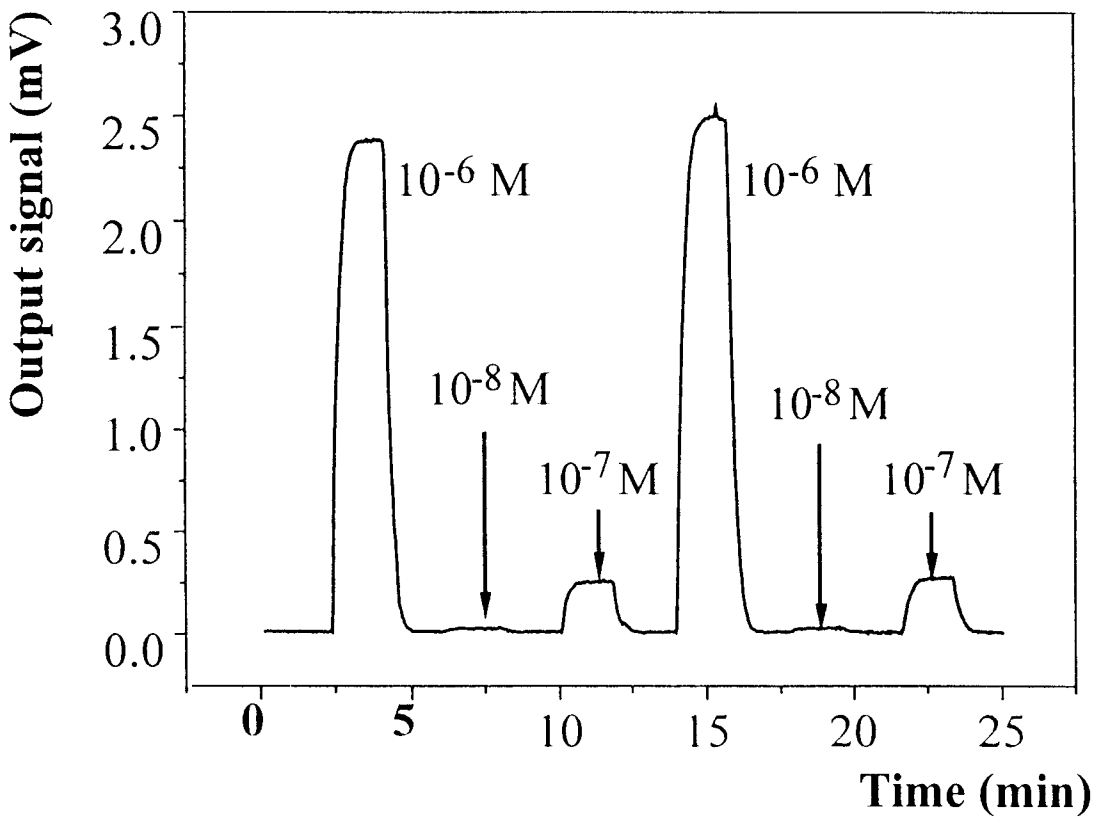
Output signal (mV)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
10-6 M
10-8 M
10-7 M
10-6 M
10-8 M
10-7 M
0
5
10
15
20
25
Time (min)

Figure 3

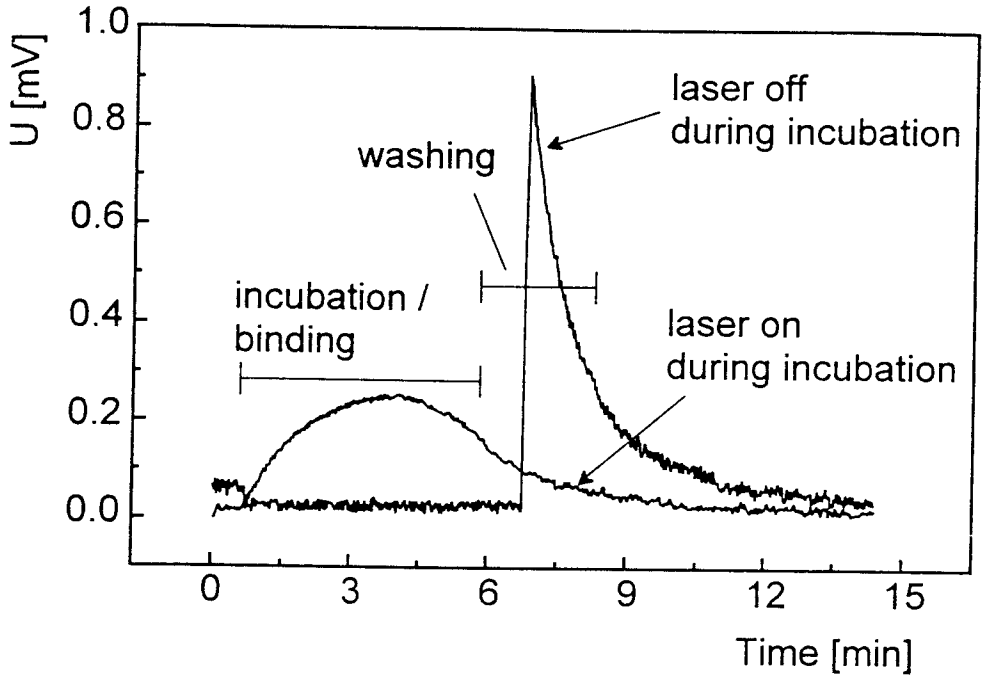
U [mV]
1.0
0.8
0.6
0.4
0.2
0.0
0
3
6
9
12
15
Time [min]
washing
laser off
during incubation
incubation /
binding
laser on
during incubation

Figure 4

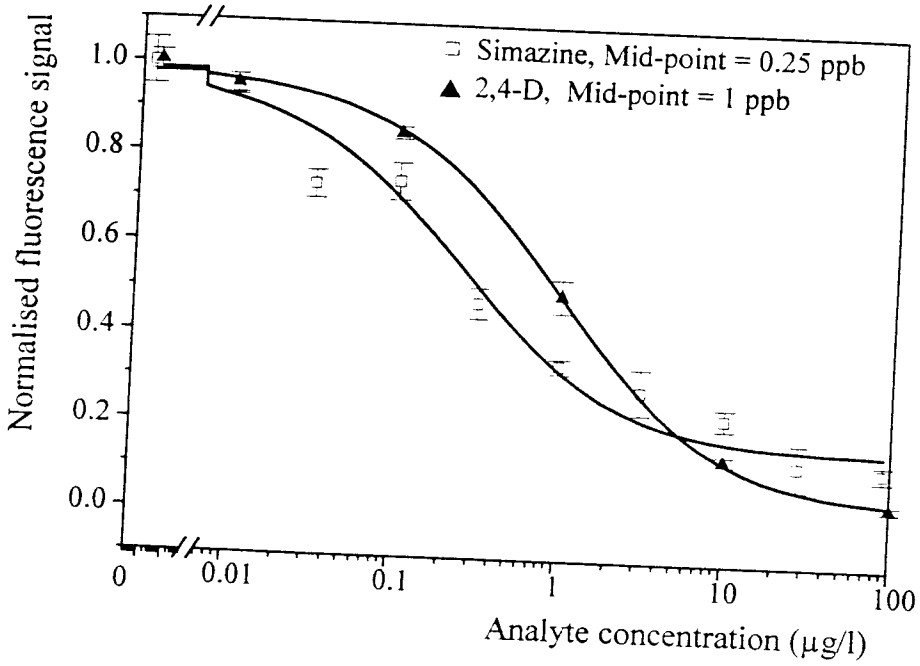
Normalised fluorescence signal
1.0
0.8
0.6
0.4
0.2
0.0
0
0.01
0.1
1
10
100
Analyte concentration (µg/l)
Simazine, Mid-point = 0.25 ppb
2,4-D, Mid-point = 1 ppb

Figure 5

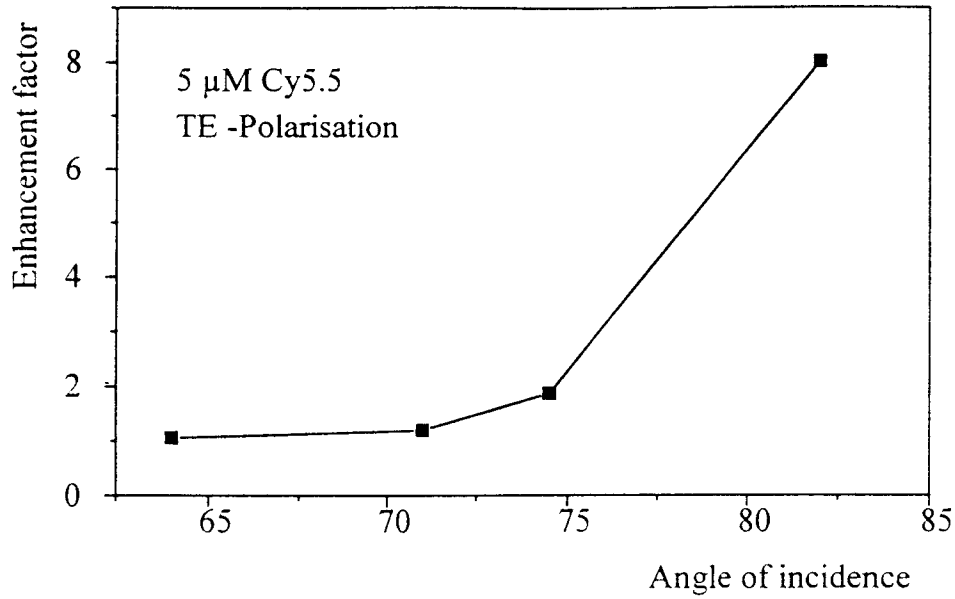
Enhancement factor
8
6
4
2
0
5 µM Cy5.5
TE -Polarisation
65
70
75
80
85
Angle of incidence

Figure 7

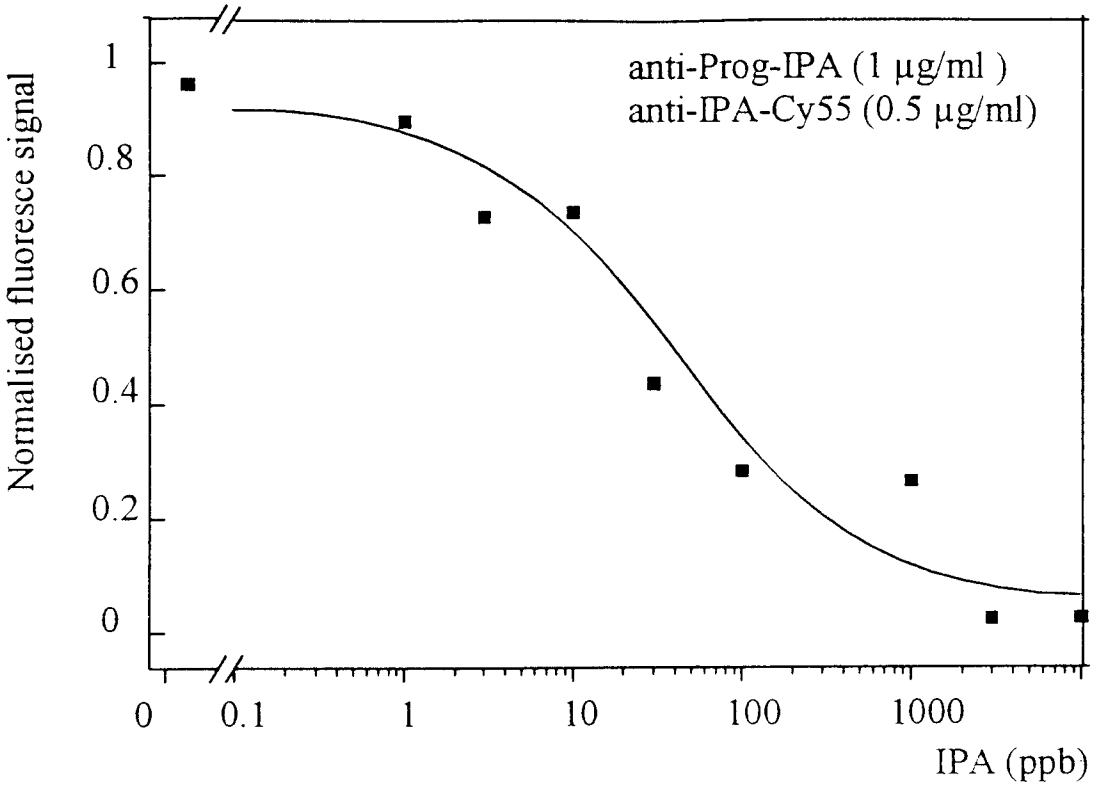
Normalised fluoresce signal
1
0.8
0.6
0.4
0.2
0
anti-Prog-IPA (1 µg/ml)
anti-IPA-Cy55 (0.5 µg/ml)
0
0.1
1
10
100
1000
IPA (ppb)

Figure 6