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Detection of low molecular weight toxins using optical phase detection techniques

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Abstract

Optical methods based on recording a phase shift of electromagnetic waves offer high sensitivity for detection of bio-reactions. The method of Total Internal Reflection Ellipsometry (TIRE), which records simultaneously two parameters Ψ and Δ related, respectively, to the amplitudes and phases of p- and s-components of polarized light, was exploited here for detection of mycotoxins (T-2, zearalanone, and aflatoxin). The TIRE direct label-free immunoassay enables detecting the above mycotoxins in sub-ppb concentrations in water as well as in food extracts. Further enhancement of sensitivity can be achieved with the use of $SiO_2/Si_3N_4/SiO_2$ planar waveguides operating as polarization interferometers (PI).

Keywords: total internal reflection ellipsometry; planar waveguide; polarization interferometry; mycotoxins; direct immunoassay

1. Introduction

Detection of environmental pollutants and toxins produced as a result of extensive industrial and agricultural activities is of high importance nowadays. The list of chemicals of interest is long and includes heavy metals, organic chemicals, pesticides, etc. Particular interest is currently focused on mycotoxins, products of fungal metabolism which may naturally occur in agricultural products and food stored in inappropriate conditions. Standards for maximal concentration of those toxic substances set in the environmental legislation in the EU, US and worldwide are typically in the ppb (part per billion) range. The detection of the above mentioned analytes in low concentration is a difficult task, mostly due to their low molecular weight. The use of optical sensors having high sensitivity offers a solution for this problem. The method of Total Internal Reflection Ellipsometry (TIRE) [1,2] combines the advantages of high sensitivity of spectroscopic ellipsometry and experimental conveniences of Kretschmann SPR (surface plasmon resonance). In contrast to the conventional SPR based on the monitoring the intensity of reflected p-polarized light, the method of TIRE detects two parameters Ψ and Δ related to the amplitude ratio and the phase shift of p- and s-components of polarized light. As a result the sensitivity of TIRE is about 10

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times higher that of conventional external reflection ellipsometry and SPR techniques [1,3]. The method of TIRE in conjunction with direct immunoassay was successfully utilised for detection of different low molecular weight toxins, such as herbicide active ingredients (atrazine and simazine) and mycotoxins (T-2), and achieved the sub-ppb level of detection [2-5]. The range of mycotoxins was extended in this work by including other products of fungal metabolism zearalanone and aflatoxin.

Further development and scaling down of phase sensitive optical methods for biosensing may utilise planar waveguides. A simple planar waveguide device of a $SiO_2/Si_3N_4/SiO_2$ sandwich structure on silicon wafer can operate as a polarization interferometer where the p-polarized component of light is affected by molecular absorption while the intact s-component serves as a reference. Phase shift between p- and s-components developed in the course of adsorption constitutes the sensor output. Modeling and test experiments showed further increase in sensitivity at least in two orders of magnitude [6]. The same waveguide operating in the regime of attenuated total reflection was utilised for detection of traces of heavy metals or pesticides in sub-ppb concentrations in water [7,8].

2. Methodology

The TIRE experimental set-up schematically shown in Fig. 1 was built on the basis of automatic spectroscopic J.A. Woollam ellipsometer M2000 (1) operating in the 370-1000 nm spectral range and exploiting the rotating compensator principle. The addition was a 68^0 trapezoidal prism (2) which allows coupling the white light beam to a thin gold film deposited on the BK7 glass slide (3) at the conditions close to total internal reflection similarly to conventional Kretschmann SPR systems [9]. A cell (4) of 0.2 ml in volume with the inlet and outlet was sealed against the gold coated side *via* rubber O-ring to enable performing molecular adsorption and different biochemical reactions on the gold surface. The sensitivity of Δ to small changes in refractive index and thickness of the adsorbed layers is 10 times higher than that of Ψ and thus of SPR [3], so the $\Delta(\lambda)$ spectra were used in this study.

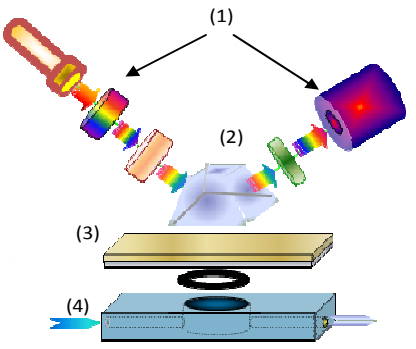

Fig. 1 TIRE experimental set-up.

The key element in the experimental set-up of Polarisation Interferometry (PI) shown in Fig. 2 is the $SiO_2/Si_3N_4/SiO_2$ waveguide structure fabricated on the surface of silicon wafer [10]. The circularly polarized light from the fan-beam laser diode is coupled into the waveguide *via* semi-cylindrical lens; the light coming out from the other side of the waveguide is going through the polarizer and collected with photodetector [6]. The sensing window is etched in the top SiO_2 layer and coated with the sensing layer; the cell attached on top allows performing the required bioreactions. In the above system, the phase shift between the p-component of electromagnetic wave (which is affected by molecular adsorption) and the s-component (serving as a reference) is developed in the course of molecular adsorption and therefore results in a multi-periodic sensor response.

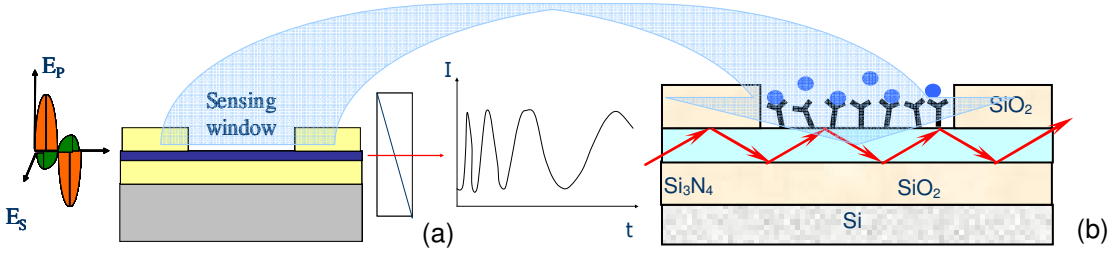

Fig. 2 Polarization Interferometer experimental set up (a); the $SiO_2/Si_3N_4/SiO_2$ planar waveguide (b).

High sensitivity of the above optical sensing methods allows the use of a cost effective direct label-free immunoassay approach, when the molecules of mycotoxins are binding to specific antibodies immobilized on the surface. The technology of electrostatic layer-by-layer deposition [6,11] was exploited in this work for the immobilisation of proteins on the surface of gold (in TIRE method) and Si_3N_4 in (in PPI method). In particular, the antibodies (Ab) specific to mycotoxins were electrostatically attached to the surface *via* the poly-cationic layer of

(poly)allylamine hydrochloride (PAH). An intermediate layer of Protein A molecules having a binding site to the second domain of IgG based Ab was used to orient the antibodies with their Fab-fragments towards the solution. The procedure of sample preparation was described in detail earlier [2-5]. All the chemicals used were either purchased from Sigma-Aldrich or supplied by our collaborators from the Ukraine and Hungary. Aqueous solutions of T-2, zeralanone, and aflatoxin were prepared by multiple dilutions of respective stock solutions in methanol. Certain experiments were carried out on grain food samples (maize, buckwheat, fodder, bread, muesli) allegedly containing T-2 mycotoxin. T-2 was extracted (using acetonitrile) from 5g portions of the above samples being fresh, stale (3 days after expiry date), and mouldy (kept at summer room temperature for up to 3 months).

3. Results and discussion

A typical set of $\Delta(\lambda)$ experimental spectra shown in Fig. 3 demonstrates the spectral shift caused by consecutive adsorption of layers of PAH, Protein A, and Ab as well as by binding different concentrations of T-2 mycotoxin to Ab (from 0.15 ng/ml up to 300 ng/ml). Ellipsometry data fitting allows the evaluation of thickness values of the adsorbed layer. Since the refractivity increments caused by adsorption of different biomolecues represent only 0.1-0.14% of the refractive index [12], the spectral changes were associated mainly with the thickness. The resulted calibration curve for T-2 mycotoxin (Fig. 4), obtained earlier [4], showed a possibility of detection of T-2 mycotoxin in concentrations down to 0.15 ng/ml (or 0.15 ppb). This time, the data points for real samples were added to the graph. The results showed that T-2 mycotoxin in dangerously high concentrations of more than 10 ng/ml can be easily generated in mouldy (or even stale) grain-products, while fresh products do not contain any traces of T-2.

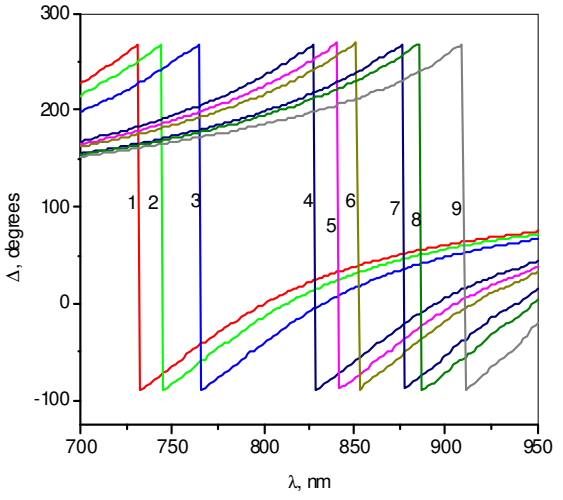

Fig. 3. Typical spectra of Δ for the bare Au surface (1), after consecutive adsorption of PAH (2), Protein A (3), antibodies to T-2 (4), and after binding T-2 mycotoxin in different concentrations of 0.15mg/ml (5), 1.5 mg/ml (6), 7.5 mg/ml (7), 75mg/ml (8), 300mg/ml (9).

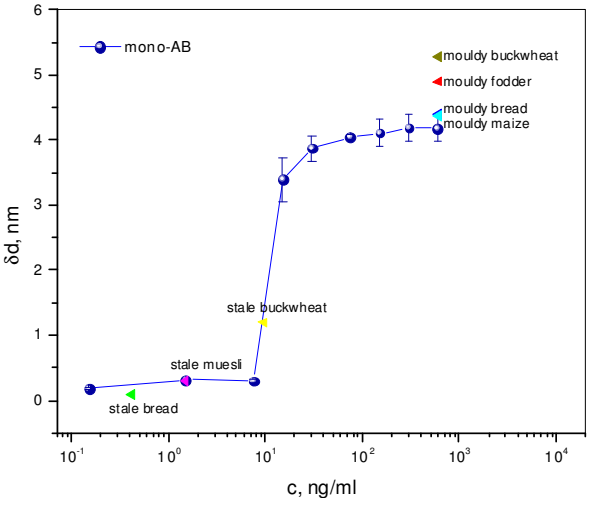

Fig.4 Calibration curve (adsorbed layer thickness vs. concentration) for T-2 mycotoxin; food samples are represented by triangular data points with respective descriptions.

The calibration curves (i.e. thickness changes vs. mycotoxin concentration) obtained from the TIRE experiments for the other two mycotoxins: zearalanone and aflatoxin are shown in Fig. 5. The response to aflatoxin appeared to be about 3 times less than that for zearalanone; and both are smaller than that for T-2 (compare with Fig. 4). This could be due to the limited concentration (or activity) of antibodies. Another explanation may be related to the hydrophobicity of the above mycotoxins and thus their abilities to form aggregates in aqueous solutions [5]. Similarly to T-2, the minimal detected concentrations for both zearalanone and aflatoxin are about 0.1 ng/ml and 0.4 ng/ml, respectively, which is quite remarkable achievement (no other optical direct immunoassays provide such high sensitivity). Further experiments on mycotoxin detection are currently underway.

The method of PI offers substantial increase in sensitivity (as compared to TIRE) due to the multiple reflections of of light in the waveguide. Considering the ratio of refractive indices of core and cladding of 2 : 1.46 for the

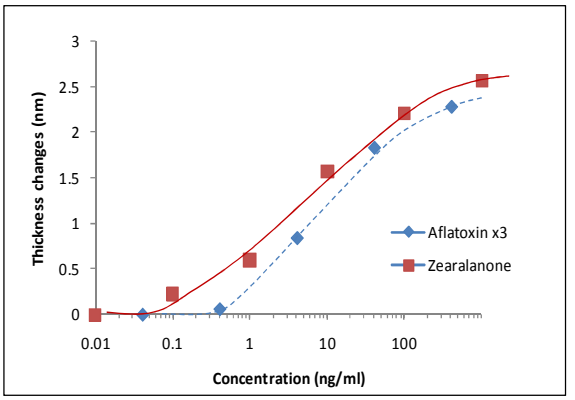

Fig. 5. TIRE calibration curves for direct immunosensors for zearalanone and aflatoxin. The thickness values for aflatoxin were three fold increased.

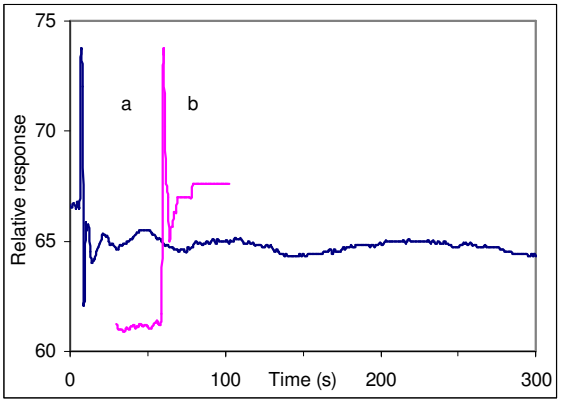

Fig. 6. Typical responses of PI waveguide sensor to immobilisation of anti-IgG from its 100μg/ml solution (a); binding of IgG from its 1 ng/ml solution (b).

waveguide used and the actual geometrical dimensions of the sensing window of 4x6 mm and thickness of Si_3N_4 core of 200 nm, the anticipated number of reflections can be about 10^4; in practice it was shown to be about 800 [7]. Therefore, we expect further decrease in the detection limit for mycotoxins for 2–3 orders of magnitude as compared to TIRE measurements, i.e. down to the ppt (part per trillion) range. The experiments reveal more than 2π phase shift upon binding of 1ng/ml of human IgG to goat-on-human anti-IgG (see Fig. 6) [6,13]. This gives more than two orders of magnitude boost of sensitivity as compared to the TIRE method, which typically shows the change in Δ of 1–2 degrees. Similarly to the above modeling, the detection limit is expected to go down to pg/ml (or ppt) level.

4. Conclusions

The method of TIRE in conjunction with the simple and cost efficient direct immunoassay approach allowed the detection of low molecular weight toxins, particularly mycotoxins T-2, zearalanone, and aflatoxin in concentrations down to 0.1 ng/ml. T-2 was found in the stale and mouldy samples of grain products. Further development of optical biosensors based on the measurements of the phase shift of electromagnetic waves may lie in the exploitation of planar waveguides.

Acknowledgements

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