DETERMINATION OF THE MYCOTOXIN ZEARALENONE IN WATER BY IMMUNOFLUORESCENCE AND TOTAL INTERNAL REFLECTION ELLIPSOMETRY METHODS

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Aquafluosense
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Introduction

Through the exhaustion of our global water reserves, water protection became a strategic issue worldwide. Project Aquafluosense [1] aims to develop a new water analysis system for natural and artificial waters, allowing complex, systematic and for main parameters *in situ* fluorescence-based assessment and monitoring of water quality, including detection of several environmental xenobiotics, among others the mycotoxin zearalenone (Fig. 1). Zearalenone (ZON) is a well-known food and feed contaminant, but its occurrence as environmental contaminant in surface water is a quite new discovery [2]. Enzyme-linked fluorescent immunoassays (ELFIAs) were conducted where the label enzyme converts a substrate into a reaction product fluorescent upon excitation by light of a particular wavelength. The 96-well microplate-based fluorescent instrument developed under project Aquafluosense is capable to detect zearalenone in the concentration range of 0.4 – 400 ng/ml. The sensitivity and accuracy of the analytical methods has been demonstrated by comparative assessment with detection by total internal reflection ellipsometry (TIRE).

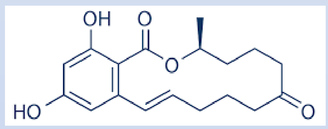

Figure 1. The chemical structure ZON

Experimental I. – ELFIA

ELFIA were conducted by the followings:
(1) coating with bovine serum albumin – ZON conjugate (1 µg/ml in carbonate buffer pH= 9.6),
(2) blocking with 1% gelatine (in phosphate buffer saline, pH=7.4),
(3) competition performed with
- ZON dilution series (0.004 pg/ml – 2 µg/ml) and
- rabbit antiserum (dilution 1:1000),
(4) labelling with goat anti-rabbit-HRP (dilution 1:7500),
(5) getting detectable fluorescence product (resorufin, Fig. 3.) and color by using QuantaRed Enhanced Chemifluorescent HRP Substrate Kit.

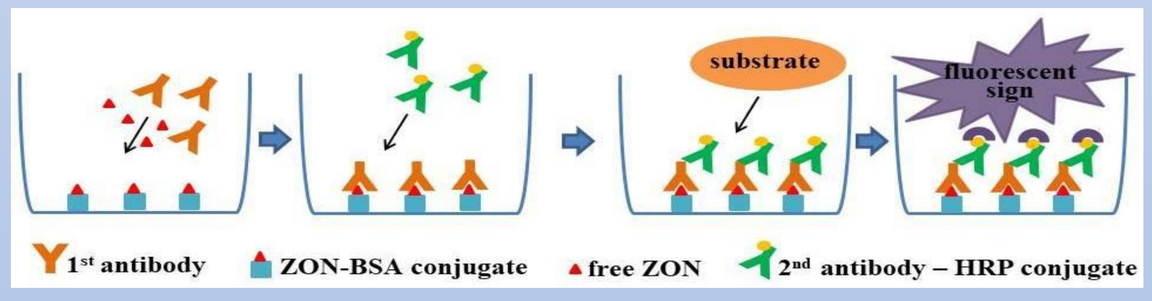

Figure 2. Schematic view of the ELFIA process

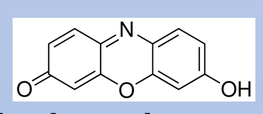

Figure 3. The chemical structure of resorufin

Experimental II. – Instrumentation

Absorbances were read by SpectraMax iD3 Multi-Mode Microplate Reader at 576 nm wavelength. Relative fluorescent signs were determined by the prototype (Fig 4. A,B) equipped with CREE XPEBGR-L1-0000-00F01 LED (520-535 nm min-max dominant wavelenght) as light source and FF01-593/40-25 output filter (peak: 593 nm, width: 40 nm) developed in Aquafluosense project.

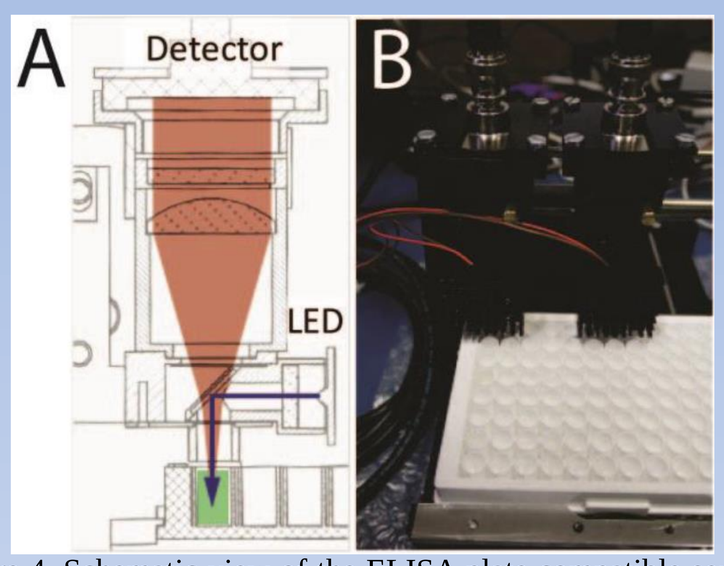

Figure 4. Schematic view of the ELISA plate compatible sensor head (A), photo of the ELISA plate compatible instrument (B)

Results

Calibration curves and LODs were determined for absorbance and fluorescence (Fig. 5). ZON at concentration of 2000 ng/ml was applied as background in determination of relative fluorescence sign where no free primary antibody remained to connect to BSA-ZON and neither fluorescent product (beside the background) nor color did not form. For comparable representation relative analytical signs are presented.

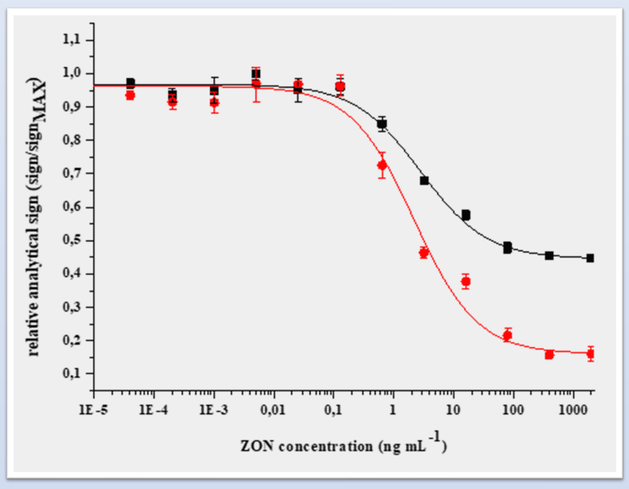

Figure 5. Competitive indirect calibration curves for ZON determined by absorbance (■) and fluorescence (●).

LOD $_{visual\ absorbance}$= 0.60 ng/ml
LOD $_{fluorescence\ detection}$= 0.40 ng/ml
LOD $_{corresponding\ ELISA}$= 0.84 ng/ml
(corresponding ELISA with o-phenylenediamine dihydrochloride, OPD)

TIRE

Sensor surfaces were prepared by a thermal evaporation of layers of chromium (Cr) – 3 nm thick and gold (Au) – 25 nm on standard microscopic glass slides. The Au-surface was modified with mercaptoethyl sodium sulfonate to enhance the negative surface change. Zearalenone-6'-carboxymethyloxime -ovalbumin conjugate (ZON-OVA) was electrostatically immobilized on the Au-surface via a polyallylamine hydrochloride layer. To block all the remaining binding sites, an additional adsorption of OVA was carried out. Then a mixture of ZON-specific antiserum and solutions of free ZON (0.01 ng/ml – 10 µg/ml) were injected (Fig. 6) [3].

LOD $_{TIRE}$= 0.01 ng/ml

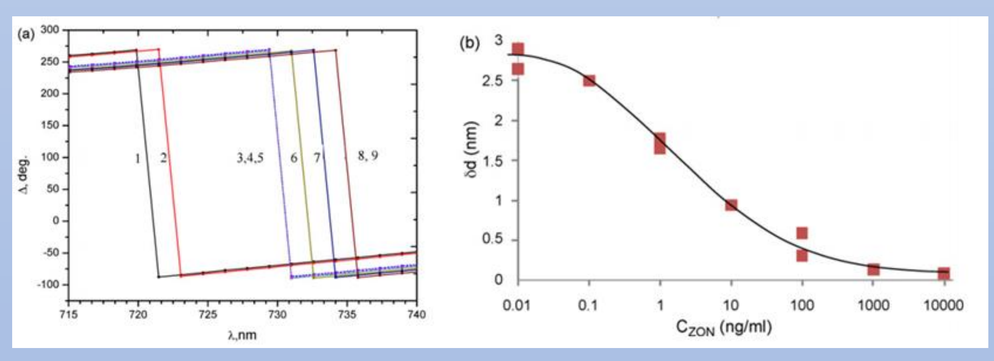

Figure 6. (a) A typical set of Δ(λ) spectra measured on bare Au surface (1), polyallylamine hydrochloride (2) ZON-OVA conjugate (3), OVA (4), Ab-ZON of from pre-incubated mixtures containing ZON: 100 (5), 10 (6), 1 (7) and 0.1 ng/ml (8). (b) Changes in the adsorbed layer thickness versus the concentration of ZON (in the mixture with Ab-ZON) obtained by fitting the TIRE data.

Discussion

Fluorescence as analytical sign in ELISA system results a more sensitive method with lower LOD value than in colorimetric assay. Total internal reflection ellipsometry, as sensor technology provide orders of magnitude lower LOD, it is not appropriate for in situ determination because of special instrument need and limit in number of samples can be measured simultaneously. 96-well microplate format allows of investigating of 25 samples in triplicates (with standard curve of 7 calibration points).

References

[1] http://aquafluosense.hu
[2] Gromadzka, K. *et al.*, Water Res. 43(4) (2009) 1051-1059.
[3] Nabok, A. et al., Sens. Actuators B 154 (2011) 232-237.

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