OWLS Based Nanosensors for Agro-Environmental and Food Safety

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***Abstract*—With globalized trade of food commodities, food safety and related environmental safety issues have become a central concern not only at national/regional levels, but all over the world. Consumers are concerned about food-borne pathogens and their toxic substances, including mycotoxins, as well as chemicals, including residues of pesticides and veterinary drugs. Environmental pollution may also lead to contamination of food/feed commodities grown in affected land. For the quick and reliable detection of hazardous substances, new types of sensors based on evanescent wave optical techniques on the rise to detect food-borne contaminants. Our results, obtained by Optical Waveguide Lightmode Spectroscopy (OWLS) based label-free immmunosensors used for determination of different chemical contaminants in food responsible for the risk of food poisoning, are presented. Measuring methods were investigated for the determination of herbicide active ingredient trifluralin and biomarker protein vitellogenin for monitoring the pollution with endocrine disrupting chemicals, as well as for different mycotoxins (aflatoxin, zearalenone, deoxynivalenol) and microbials. High specificity/selectivity of the sensitised surface coupled with high sensitivity of OWLS detection gives the possibility to develop immunosensors and microbial sensors in most cases with definitely lower limits of detection than those in traditionally used immunoassays in direct/competitive formats.**

***Index Terms*—optical waveguide lightmode spectroscopy, nanobiosensor, agro-environmental safety, food safety**

I. Evanescent Wave Optical Techniques

Nanotechnology has a multiple and rapidly expanding role in food industry. Nanomaterials are used in food production or inspection with their unique, nano-sized dispersity, or as novel methodologies utilizing nanostructures in analytical or technological processes. A great interest has focused on the development of selective and sensitive sensors for the detection of very low levels of chemical and biological substances, and for the direct measurement of molecular interactions in situ and in real time. These processes can be followed with optical sensors based on evanescent wave principles [1]. At boundaries between thin layers of different materials with negligible absorption, radiation is partially reflected and transmitted. These evanescent fields are not spatially limited in their interaction to the waveguide surface, but are allowed to penetrate to the sample overlayer, while they are affected by the characteristics and conditions of the surface. The evanescent signal is modified by the proper surface processes, with the possibility to detect surface changes with outstandingly high sensitivity. Evanescent wave optical devices can measure the interaction between complementary molecules in real time, without any need of labels [2], [3] and provide information on concentration changes detected in chemical or biological binding processes or the formation of microbial films. Most techniques discussed are assumed to be governed by changes in optical density [4]. Most important measurement setups include different optical waveguides, interferometer based sensors, Reflectometric Interference Spectroscopy (RIfS), Total Internal Reflection Fluorescence (TIRF), Total Internal Reflection Ellipsometry (TIRE), ring resonator, etc. Different detection techniques reckon among the label-free evanescent field based sensors, as the optical waveguide based sensor structures, reflectance based sensors, grating based biosensors (incoupling mode and outcoupling mode sensors interferometer based biosensors, optical ring resonator based biosensors and optical fiber based biosensors.

Great interest has been focused on the utilization of affinity-based recognition elements, as antibodies,

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binding proteins, molecularly imprinted polymers or aptamers. These techniques provide high specificity and sensitivity, good stability and cost-efficacy [5], [6]. Antibody based immunosensors, using monoclonal antibodies (mAbs) or polyclonal antibodies (pAbs) remain to provide different strategies for immunosensing both in direct or competitive methods.

II. Optical Waveguide Lightmode Spectroscopy

OWLS measurements were carried out using amino-functionalised integrated optical waveguide sensors (chips) type OW 2400 (MicroVacuum, Budapest, Hungary) containing a fine optical grating made in the waveguide on top of a glass support. The sensor output was read with an OWLS100 instrument controlled by software BioSense 2.2 (MicroVacuum, Budapest, Hungary). All experiments were performed in flow-injection analyzer (FIA) system containing a peristaltic pump (Minipuls3, Gilson, Middletown, WI, USA) with a flow rate of 80 µl min^{-1} and an injector (Rheodyne, Rohnert Park, CA, USA) equipped with an injector loop at a volume of 200 µl. The sensor holder was thermostatically controlled by an OWLS TC heater/cooler unit (MicroVacuum Ltd., Budapest, Hungary, Fig. 1).

The sensor is constructed of an optical waveguide layer on top of a glass support and of a fine optical grating made in the waveguide (Fig. 1). When polarized laser beam (typically He–Ne laser with 632.8 nm wavelength) reaches the grating, it is diffracted. The angle of diffraction depends not only on the optical parameters of the sensor, but also on the refractive index of the covering medium. The waveguide is gradually rotated around its rotation axis, and when the diffracted beam is incoupled into the waveguide, it is propagating toward the edge of the sensor through multiple internal reflections. The intensity of the incoupled light is measured with a photodiode.

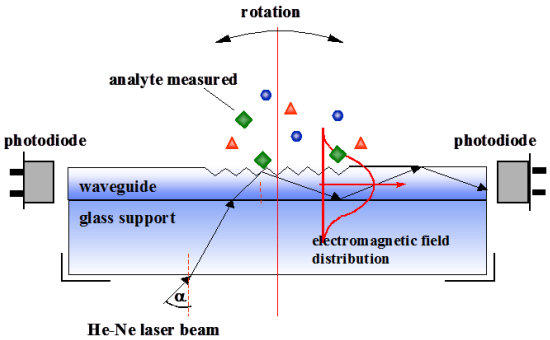

Figure 1. The schematic arrangement of an optical waveguide lightmode spectroscopy immunosensor.

III. Nanobiosensors for Pesticide and Synthetic or Natural Drug Residues and Their Indicator Proteins

OWLS based immunosensors were applied both in direct and in indirect formats for detection of the pre-emergence herbicide active ingredient trifluralin. The immobilized antigen-conjugate based OWLS system, allowed the detection of trifluralin in the concentration range of 2×10^{-7} to 3×10^{-5} ng ml^{-1} (Table I, Fig. 2) [7].

TABLE I. Detection of Trifluralin in Spiked and Field Surface Water Samples by OWLS, Elisa and GC-MS [7]

spike [ng ml^{-1}]	OWLS [ng ml^{-1}]	ELISA [ng ml^{-1}]	GC-MS [ng ml^{-1}]
0	< 0.0001	< 0.02	< 0.01
2.5	1.81 ± 0.30	1.79 ± 0.38	2.42 ± 0.08
5	5.43 ± 0.30	4.16 ± 1.16	4.32 ±1.01
25	36.3 ± 1.11	34.1 ± 4.01	24.6 ± 3.76
-- [a]	3.08 ± 1.02	1.29 ± 0.53	1.94 ± 0.53

[a] Field sample with trifluralin content. Source: Main Eastern Irrigation Channel (Keleti Főcsatorna), Hungary, sampled at July 18, 2001.

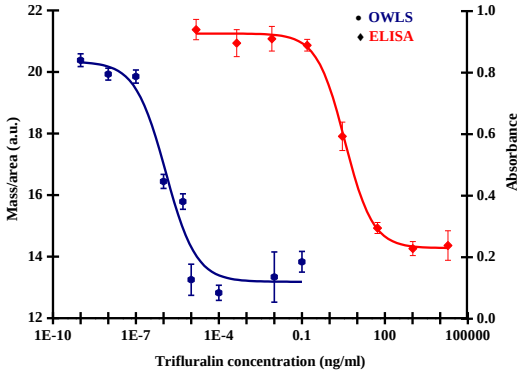

Figure 2. Calibration curves for trifluralin measured with OWLS and ELISA as a reference method [7].

Environmental and food safety is also affected by residues of veterinary drugs or even pharmaceuticals. Chloramphenicol antibiotic was measured in standard solutions with immobilization of anti-chloramphenicol antibodies onto the surface of OWLS chips, and the signal measured was proportional to the analyte concentration in the range of 32 to 320 µg ml^{-1} [8]. Detection of pharmaceutical active ingredients of natural origin in foodstuffs or food supplements is also of high concern, not only for product quality assurance, but also for food safety concerns, particularly in cases of active ingredients not registered to be present in given commodities. Thus, an OWLS based immunosensor was developed for the detection the presence of artemisinin, a biologically effective substance in various types of sweet wormwood plant Artemisia annua (Fig. 3, Table II) [9].

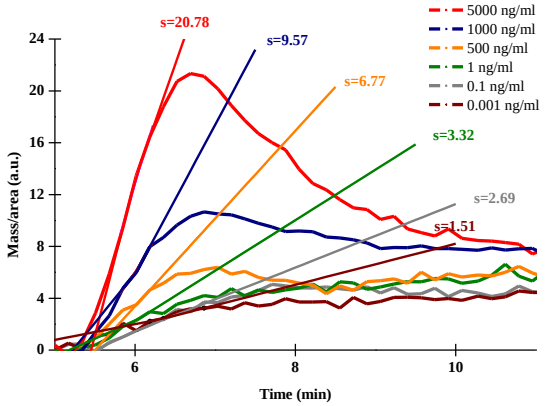

Figure 3. Evaluation of the signals of the artemether standard solution (1.0 µg ml^{-1} monoclonal artemether antibody immobilized on the sensor surface) [9].

TABLE II. ARTEMISININ CONCENTRATION FOUND IN ARTEMISIA SAMPLES [9]

	OWLS, mg g^{-1} d.w.	HPLC, mg g^{-1} d.w.
A. vulgaris commercial	0.12±0.02	>0.1
A. annua (leaves)	2.62±0.31	0.74±0.04
A. annua (shoot)	1.83±0.09	2.31±0.11
A. annua (stalk)	0.16±0.01	>0.1
A. annua (flowered shoot)	1.47±0.06	1.63±0.02
A. annua (plant)		
A. alba (leaves)	n.d.	n.d.
A. princeps (leaves)	n.d.	n.d.
A. absinthium (leaves)	0.11±0.01	
A. absinthium (shoot)	0.14±0.02	>0.1
A. vulgaris deep red (leaves)	n.d.	n.d.
A. vulgaris green l.	0.29±0.07	n.d.
A. annua Anhui	0.88±0.05	0.49±0.01
A. annua Shandong	1.19±0.07	0.86±0.05

A growing concern has been expressed in recent years regarding the impact of endocrine disrupting chemicals (EDCs), thought to mimic natural hormones. EDCs are often detected by analyte-specific immunoanalytical methods like vitellogenesis, and in turn, vitellogenin (Vtg) has become a key biomarker for assessing exposure to environmental EDCs. A label-free sensor for Vtg from carp (*Cyprinus carpio*) was developed, where purified pAbs raised in rabbits against lipovitellin (Lpv) purified from carp ovary was used (Fig. 4) [10].

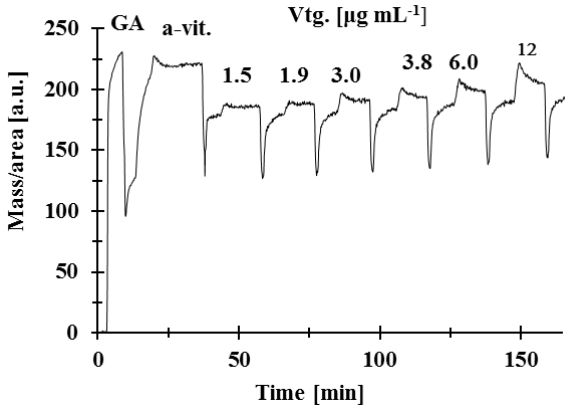

Figure 4. Immobilisation process of carp a-Lpv (43 µg ml^{-1}) and measuring cycles of different concentrations of Vtg standards (1.5-12 µg ml^{-1}) [10].

The competitive immunosensor allowed a sensitive detection range for Lpv between 3 and 100 ng ml^{-1}. To monitor the EDC pollution of the specific environmental regions, an OWLS based immunosensor was also developed for the determination of Vtg from the Oriental fire-bellied toad (*Bombina orientalis*) [11].

IV. NANOBIOSENSORS FOR MYCOTOXINS AND TOXINS IN FOODSTUFF

Mycotoxins are toxic secondary metabolites produced by fungi, and these biochemical compounds may contaminate various agricultural products, and can be present in a wide range of food and feed commodities [12].

As aflatoxins produced by Aspergillus species, particularly aflatoxin B1 (AFB1) pose substantial health risks to society, their routine monitoring and control in foodstuffs is a definite requirement. The OWLS technique has been applied for the detection of AFB1 in both competitive and in direct methods. Determination from barley and wheat flour samples well correlated with those with ELISA (Table III) [13], [14].

TABLE III. WHEAT AND BARLEY SAMPLES SPIKED WITH AFLATOXIN B1 [13]

Assigned value aflatoxin B1 (µg kg^{-1})	OWLS (µg kg^{-1})
Wheat 2.20	2.29 ± 0.61
5.50	6.53 ± 1.04
0.55	0.58 ± 0.18
1.10	3.42 ± 1.19
Barley 1.80	1.26 ± 0.28
4.50	3.08 ± 0.57
0.45	0.54 ± 0.14
0.90	0.84 ± 0.17

By using gold nanoparticles (AuNP), especially bio-AuNP-s, the sensitivity and the reusability were enhanced for the analysis of spiced paprika samples [15].

AFM1 contamination of milk occurs after the metabolism of AFB1, but AFM1 still belongs to the most toxic mycotoxin class. An immunosensing method was investigated for the detection of AFM1 using indirect (competitive) immunoassay method [16].

Contamination by deoxynivalenol (DON) occurs in cereals worldwide; therefore, an OWLS technique based label-free method was developed for detection of DON in both competitive and in direct immunoassay formats using DON-specific pAbs (Table IV) [17].

TABLE IV. THE AVERAGE VALUE OF THE MEASURED DON CONTENT [17]

Spiked DON (mg g^{-1})	Measured DON (mg g^{-1})	Recovery (%)
0.0005	0.00062±0.00018	123.0
0.001	0.00114±0.00044	114.0
0.005	0.0046±0.0006	92.0
0.01	0.0108±0.0017	108.2
0.05	0.0473±0.0048	94.5
0.1	0.109±0.006	109.3
0.5	0.465±0.134	93.1
1.0	1.11±0.05	111.0
5.0	4.58±0.36	91.6

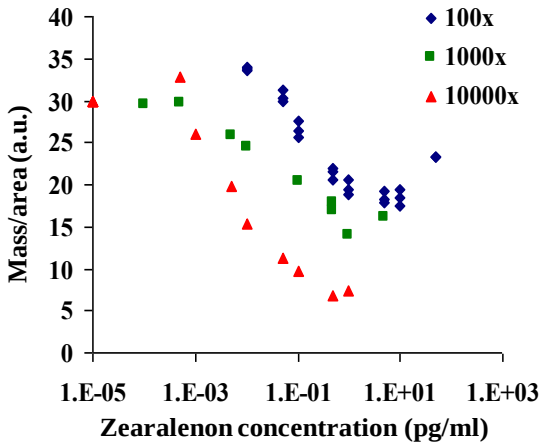

Figure 5. Zearalenon in maize samples (samples diluted 100-, 1000-, 10000-fold) [18].

As Fusarium mycotoxin zearalenone (ZON) is also a widely occurring estrogenic pollutant of fungal origin, a competitive immunosensor was investigated with outstanding sensitivity as compared to the corresponding ELISA. In their common detectable concentration range (1-100 ng ml-1, 0.1-10 µg kg-1) the two methods, OWLS and ELISA, detected closely similar toxin contents (Fig. 5) [18]. In addition to pAbs, mycotoxin-specific aptamers as molecular recognition elements were used for sensoric determination of mycotoxins ochratoxin A and zearalenone [19], [20].

V. Nanotechnology Based Microbial Sensors

Selective discrimination of bacterial strain types and detection of actual cell concentrations is of great importance also in food safety and environmental science [21]. The OWLS technique was also used for the direct measurement of Escherichia coli by immobilizing anti-E. coli pAbs onto the surface of the amino silanized waveguide sensor by covalent coupling with glutaraldehyde. The measuring range was found to be between $3*10^4$ and $3*10^7$ cfu ml^{-1} (Fig. 6) [22].

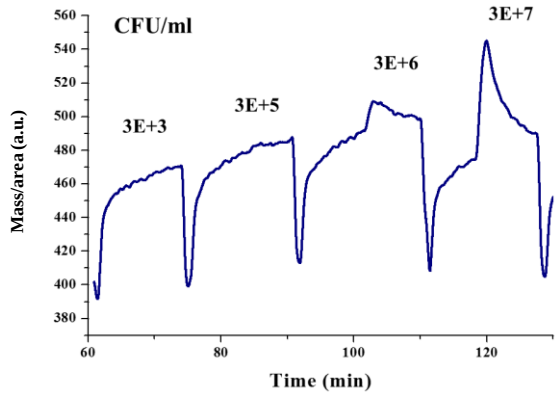

Figure 6. Concentration-dependent responses of *E.Coli* by OWLS sensor.

In the last years, a new group of enzymes, so-called silicateins, have been identified and characterized, which form the axial filaments of the spicules of the siliceous sponges, consisting of amorphous silica. Silicateins are able to catalyze the polycondensation and deposition of silica at mild conditions, hereby silica nanostructures are produced. Silicatein was expressed in E. coli and the recombinant protein resulted the formation of silica shell around the bacterial cells providing a novel technology for microbial biosensors, and the new microbial sensor-platform could be used for the detection of the inhibitory effect of stressors/environmental pollutants as hydrogen peroxide, antibiotics (CAP and penicillin G) and insecticide carbofuran in real time (Fig. 7, Fig. 8) [23], [24].

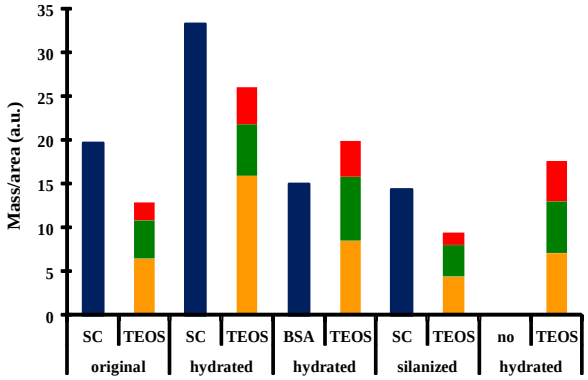

Figure 7. Effect of surface pre-treatment on biosilica formation (SC 4.8 µg ml^{-1}, TEOS 0.9 mmol l^{-1} repeated three times) [24].

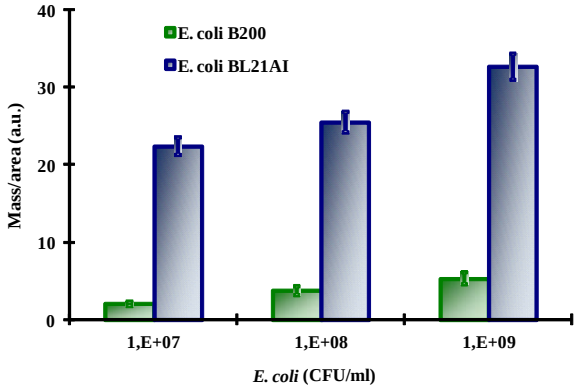

Figure 8. Signal response of *E. coli* B200 cells and pre-treated *E. coli* BL21AI recombinant cells. (2000-fold diluted anti-silicatein antibody was immobilized on the sensor surface) [23].

VI. Summary

Different applications of nanotechnology are summarized, especially the application of evanescent wave based optical techniques and their role in food industry. In the last decades a great interest has focused on the development of selective and sensitive biosensors for the detection of molecular interactions in situ and in real time followed with label-free optical sensors based on evanescent wave principles. A few measuring methods are reviewed for agro-environmental and food safety based on one of these techniques, namely with Optical Waveguide Lightmode Spectroscopy.

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