Journal of Hazardous Materials xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Detection of ochratoxin A in beer samples with a label-free monolithically integrated optoelectronic biosensor

Varvara Pagkali[a,b], Panagiota S. Petrou[a], Alexandros Salapatas[b], Eleni Makarona[b], Jeroen Peters[c], Willem Haasnoot[c], Gerhard Jobst[d], Anastasios Economou[e], Konstantinos Misiakos[b], Ioannis Raptis[b], Sotirios E. Kakabakos[a,*]

[a] Immunoassays-Immunosensors Lab, INRaSTES, NCSR "Demokritos", Aghia Paraskevi 15310, Greece
[b] Institute of Nanoscience & Nanotechnology, NCSR "Demokritos", Aghia Paraskevi 15310, Greece
[c] RIKILT Wageningen UR, Akkermaalsbos 2, 6708 WB, Wageningen, The Netherlands
[d] Jobst Technologies GmbH, Freiburg, Germany
[e] Analytical Chemistry Lab, Department of Chemistry, University of Athens, Panepistimiopolis Zografou, 15771, Greece

HIGHLIGHTS

- Optical immunosensor for label-free detection of ochatoxin A in beer.
- The sensor consists of ten integrated on silicon Mach-Zehnder interferometers.
- Ochratoxin A is determined following a competitive immunoassay format.
- The assay could be employed for ochatoxin A determination in different beer types.

ARTICLE INFO

Article history:
Received 29 January 2016
Received in revised form 4 March 2016
Accepted 6 March 2016
Available online xxx

Keywords:
Ochratoxin A
Monolithically integrated Mach-Zehnder interferometers
Label-free detection
Beer

ABSTRACT

An optical biosensor for label-free detection of ochratoxin A (OTA) in beer samples is presented. The biosensor consists of an array of ten Mach-Zehnder interferometers (MZIs) monolithically integrated along with their respective broad-band silicon light sources on the same Si chip (37 mm^2). The chip was transformed to biosensor by functionalizing the MZIs sensing arms with an OTA-ovalbumin conjugate. OTA determination was performed by pumping over the chip mixtures of calibrators or samples with anti-OTA antibody following a competitive immunoassay format. An external miniaturized spectrometer was employed to continuously record the transmission spectra of each interferometer. Spectral shifts obtained due to immunoreaction were transformed to phase shifts through Discrete Fourier Transform. The assay had a detection limit of 2.0 ng/ml and a dynamic range 4.0–100 ng/ml in beer samples, recoveries ranging from 90.6 to 116%, and intra- and inter-assay coefficients of variation of 9% and 14%, respectively. The results obtained with the sensor using OTA-spiked beer samples spiked were in good agreement with those obtained by an ELISA developed using the same antibody. The good analytical performance of the biosensor and the small size of the proposed chip provide for the development of a portable instrument for point-of-need determinations.

1. Introduction

Ochratoxins are a group of mycotoxins produced as secondary metabolites by several fungi of Aspergillus and Penicillium species [1]. Amongst them, Ochratoxin A (OTA) is the most toxic member of the group [2] and can be found in a large variety of foods, such as, wheat, maize, barley, coffee, cocoa, wine, and beer [3–5]. OTA is a potent teratogen, immune suppressant, nephrotoxic and carcinogen [6–8], and was classified by the International Agency for Research on Cancer (IARC) in group 2B as a possible carcinogen to humans [9]. In order to protect consumers from risks related to mycotoxins, the European Commission has established regulatory limits for OTA levels in raw cereal grains and roasted coffee (5 μg/kg), in cereals and cereal products intended for human con-

* Corresponding author at: Immunoassay/Immunosensors Lab, Institute of Nuclear & Radiological Sciences & Technology, Energy & Safety, NCSR "Demokritos", GR-15310 Aghia Paraskevi, Greece.
E-mail addresses: skakab@rrp.demokritos.gr, skakab@hotmail.com (S.E. Kakabakos).

http://dx.doi.org/10.1016/j.jhazmat.2016.03.019
0304-3894/

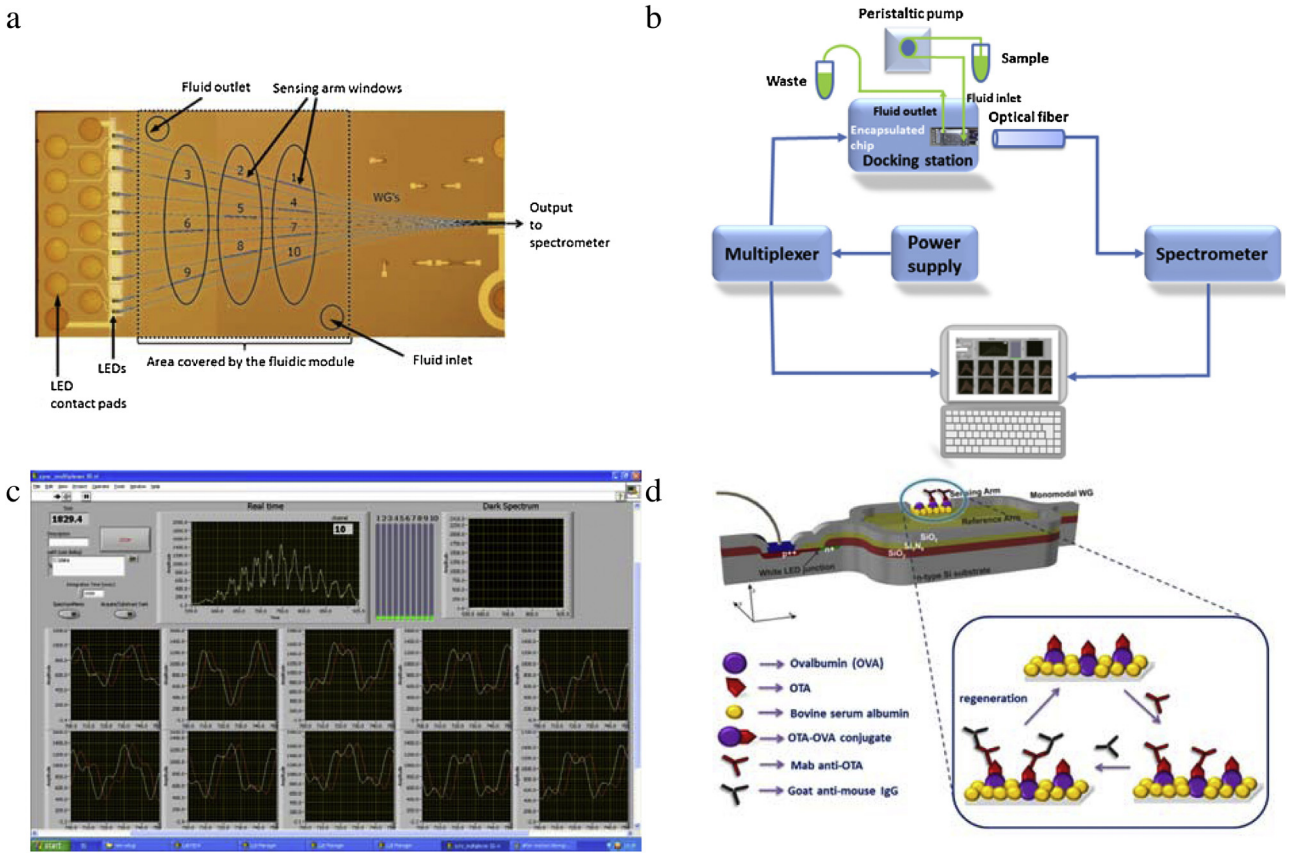

Fig. 1. (a) Microscope image of the chip overlapped by a schematic of the waveguides (not visible in the image) and an outline of the area covered from the fluidic module. (b) Schematic of the instrumentation used. (c) Screenshot showing the real-time monitoring of the output spectrum shifts of all ten BB-MZIs. A 50-nm spectral area (700–750 nm) has been isolated so as to monitor more clearly the changes in the transmission spectra. Red lines correspond to initial spectrum and white lines to spectrum after reaction. (d) Schematic of the on-chip OTA assay. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sumption (3.0 μg/kg), in wine and grape juice (2 μg/kg), as well as in baby food and cereal-based food intended for young children (0.5 μg/kg) [10].

The occurrence of OTA in beer was first described in 1983 [11] and since then many studies have been performed [12–15] indicating the need for detecting OTA in beer. Although the higher OTA values reported for most European beers do not exceed 1.5 ng/mL, values as high as 2340 ng/mL have been determined in traditionally brewed South Africa beers [14]. OTA is transferred to beer mainly from contaminated barley and/or other adjuncts used in the brewing process since it is not destroyed during the brewing procedure [12–15]. Therefore, various analytical methods have been developed for the quantitative determination of OTA in beer, cereals and other food commodities. The most frequently used methods are chromatographic ones, such as high-performance liquid chromatography coupled with fluorescence [16,17] or mass spectrometry detection [18,19], as well as immunochemical methods including enzyme-linked immunosorbent assays (ELISA) [20–22], fluorescence-based immunoassays [23], lateral flow immunoassays [24–26], cytometric microsphere immunoassays [27–29], or flow-through immunoassays incorporated in microsystems with signal recording capabilities [30–32]. Chromatographic techniques offer sensitivity and specificity, but require time-consuming sample preparation, skilled operators and expensive equipment. Immunochemical methods usually do not require complicated sample clean-up other than filtration and dilution for application to liquid samples such as beer. Nevertheless, both analytical techniques require bench-top laboratory instrumentation and are difficult to be used at the point-of-need.

Biosensors, on the other hand, represent an upcoming trend for mycotoxins detection in a wide range of food matrices [33] due to their potential for incorporation to portable devices. With respect to OTA detection, electrochemical [34–39], piezoelectric [40], and optical biosensors [41–43] have been reported. Optical biosensors are considered more appropriate for applications in complex matrices compared to more abundant electrochemical ones since they are galvanically isolated from the sample and therefore lees prone to interferences from the sample; at the same time are capable of higher detection sensitivities than the piezoelectric ones. There are few reports for OTA determination using optical biosensors; one based on optical waveguide lightmode spectroscopy (OWLS) [41] and two based on surface plasmon resonance (SPR) [42,43]. All three approaches were label-free; the reported detection limits for calibrators prepared in buffer were: 0.5 ng/mL for the OWLS-based sensor, and 1.5 ng/mL (improved to less than 0.5 ng/mL by implementation of gold nanoparticles for signal enhancement) [42], and 0.05 ng/mL [43] for the two SPR-based sensors, respectively. Despite their excellent sensitivity, both detection principles rely on external optical components and especially laser sources for excitation that make the instruments quite bulky and expensive, appropriate for laboratory use only.

In fact, the need for external optical components is considered one of the reasons that optical sensors have not reached the point of transition from lab to the field as opposed to more flexible, in terms of instrumentation, electrochemical ones. As a solution to this bottleneck, we have developed a technology for integration onto the same substrate arrays of silicon nitride waveguides along with their respective self-aligned silicon avalanche diodes

Please cite this article in press as: V. Pagkali, et al., Detection of ochratoxin A in beer samples with a label-free monolithically integrated optoelectronic biosensor, J. Hazard. Mater. (2016), http://dx.doi.org/10.1016/j.jhazmat.2016.03.019