A White Light Reflectance Spectroscopy label-free biosensor for the determination of fungicide Carbendazim

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Introduction

Fungal pathogens cause significant damages to the food crops every year resulting in poor yield, deficient food quality and huge economic loss; therefore, the use of fungicides gains ground to circumvent these problems [1].

Carbendazim (methyl 1*H*-benzimidazol-2-ylcarbamate) is a low molecular weight (191.19), systemic, broad-spectrum, benzimidazole-type fungicide (***Figure 1***), used worldwide as pre- and post-harvest treatment to control fungi that compromise the quality of various vegetables, fruits, cereals and seeds [2-3]. Despite its potential usefulness, carbendazim constitutes a major environmental pollutant, being hazardous for humans and animals; therefore, reliable determination of carbendazim levels in water, soil and food samples remains a highly desirable analytical goal.

In the current work, a label-free sensor based on White Light Reflectance Spectroscopy (WLRS) was designed, developed and applied for the fast and sensitive determination of carbendazim.

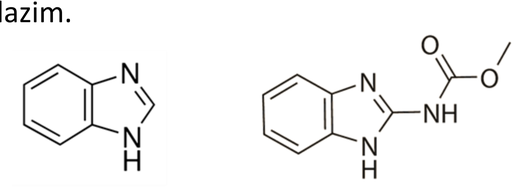

Figure 1. *Chemical structures of benzimidazole core-moiety (left) and carbendazim (right).*

Experimental/Methods

WLRS instrumentation/Detection principle

WLRS methodology involves a visible/near infra-red light source (ThetaMetrisis SA) and a miniaturized USB controlled spectrometer (Ocean Optics Inc.), operating in the corresponding spectral range (***Figure 2***). The white light emitted from the light source is guided to a reflection probe (ThetaMetrisis SA), which delivers the incident light to the biomodified surface and collects the reflected light directing it to the spectrometer. The surface consists of a transparent SiO_2 film over a Si reflecting substrate covered by a custom designed microfluidic cell (Jobst Technologies GmbH) providing the fluidic connections to the solutions. The particular sensor allows the label-free and real-time monitoring of the biomolecular reactions by recording the shift in the interference spectrum caused by the increase of the effective thickness of the biomolecular layer on the sensing surface during immunoreaction.

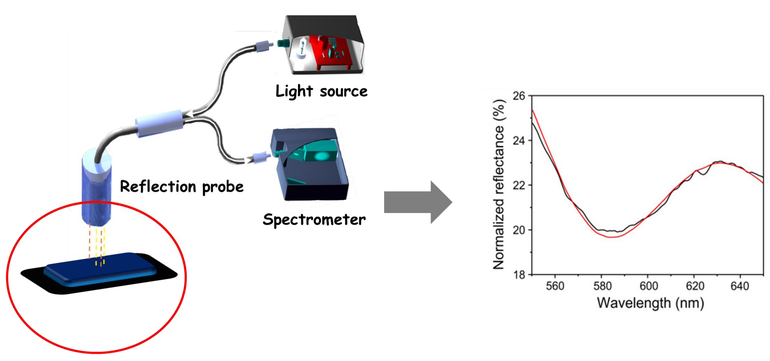

Figure 2. *Schematic representation of WLRS instrumentation and detection principle*

Biochip preparation and assay performance

SiO_2/Si chips were cleaned with piranha solution, activated by APTES, biofunctionalized through deposition of a suitable benzimidazole conjugate and then blocked with bovine serum albumin. For the assay, 1:1 v/v mixtures of an in-house developed anti-carbendazim antibody [4] with the calibrator/samples is pumped over the chip, followed by biotinylated secondary antibody and unlabeled streptavidin. The whole assay (***Figure 3***) is completed within 28 min.

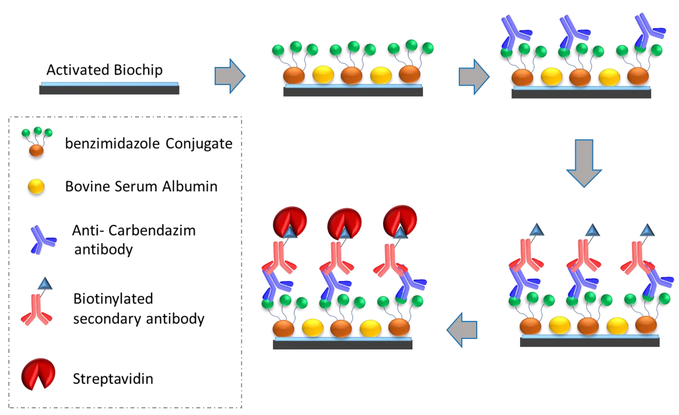

Figure 3. *Schematic representation of assay procedure for the label-free detection of Carbendazim with the WLRS biosensor.*

Results

For carbendazim detection, a competitive immunoassay format was adopted, due to the low-molecular weight of the analyte. After proper optimization, the WLRS platform allowed for carbendazim detection within 28 min total analysis time (***Figure 4***), 18 min of which correspond to the primary immunoreaction, 7 min to the secondary immunoreaction and 3 min to the biotinylated secondary antibody/streptavidin reaction. A typical calibration curve obtained from the WLRS biosensing system for carbendazim is shown in ***Figure 5***, and the main characteristics of the assay are collected in ***Table 1***.

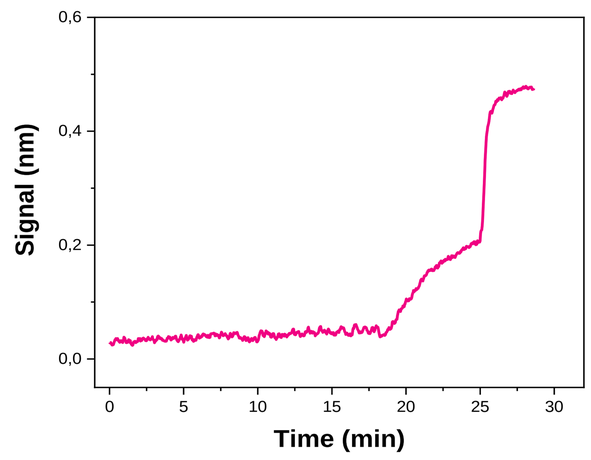

Figure 4. *Real-time response curve obtained from a biochip in the presence of zero calibrator, applying the optimized immunochemical protocol*

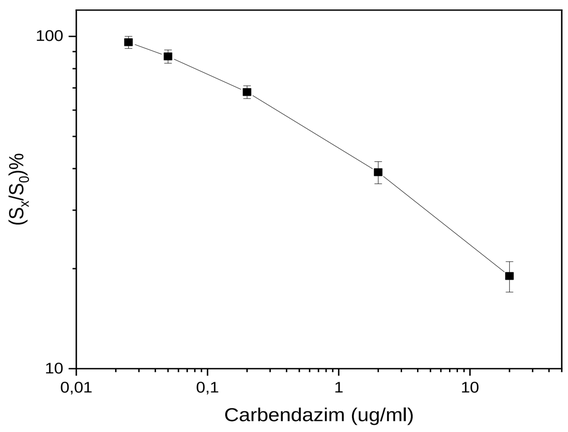

Figure 5. Typical calibration curve obtained with carbendazim calibrators prepared in assay buffer. Each point represents the mean value of three independent runs±SD. S_0=zero-calibrator signal; S_x =calibrator signal

Table 1. *Immunochemical detection of carbendazim using the WLRS biosensing platform: Assay characteristics*

Assay characteristic	Value
LoD (ng/mL)	20
Dynamic range (ng/mL)	up to 20000
Intra-assay CVs (%)	≤6.9
Inter-assay CVs (%)	≤9.4
Duration	28 min

Conclusion/Perspectives

In this work, a WLRS-based biosensing platform for the **label-free** and **real-time** immunochemical determination of carbendazim was successfully developed. The proposed sensor allowed for the **sensitive** and **fast** quantification of carbendazim levels down to 20 ng/mL within less than 30 min. The proposed biosensor is planned to be applied for the determination of carbendazim in samples of interest, e.g. citrus fruit juices. Taken it altogether, excellent analytical characteristics and short analysis time combined with small size of the analytical device render the proposed WLRS biosensor ideal for future **point-of-need** determination of carbendazim in food and environmental samples.

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