# Sensitivity enhancement by gold nanoparticles with different size and origin for optical waveguide lightmode spectroscopy technique-based mycotoxin determination

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## Introduction

Mycotoxins are toxic secondary metabolites produced by a number of different fungi, and can be present in a wide range of food and feed commodities including cereal grains, oil seeds, dried fruits, apple juice, wine and meat products from animals fed contaminated meal. Because mycotoxins are resistant to food processing and do not degrade at high temperatures, they enter the human and animal food supply.

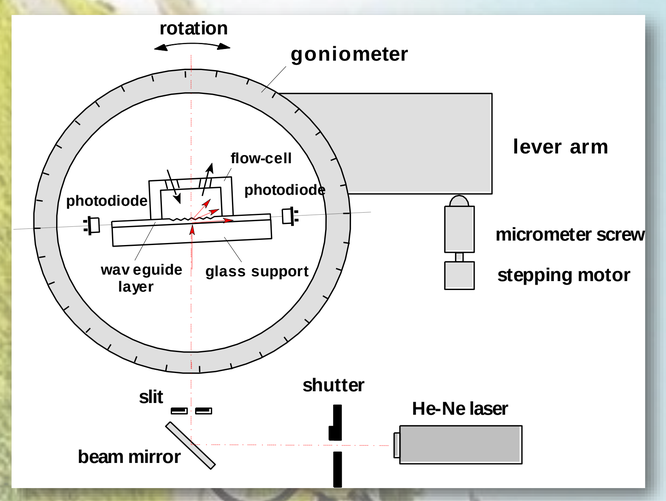
## Materials and methods

Optical waveguide lightmode spectroscopy (OWLS) technique has been used with success to detect different molecules based on the immunoreaction in competitive way by immobilization of the antigen conjugate on the sensor with covalent attachment on amino silanized (APTES) surfaces. During the measurement standard solutions containing different amount of the toxin were mixed with monoclonal antibodies of appropriate concentration, the mixture was incubated for 1 minute and injected into the OWLS system. Antibodies remained in free form in the mixture bound to immobilized antigen-BSA conjugates. The amount of antibodies bound to the surface of the chip was inversely proportional to the toxin content in the sample.

OWLS technique has been applied to the detection of mycotoxins (*e.g.* aflatoxin B1) in a competitive immunoassay format, to compare the analytical sensitivity of the developed OWLS immunosensor by enhancing the sensor surface by gold nanoparticles (AuNPs) of different size and origin (*e.g.* chemical and biotechnological synthesized) for the detection of aflatoxin B1 in raw paprika samples. BioAuNPs were produced by biosynthesis with the supernatants after shaken flask cultivation and centrifugation of the fermentation broth and immobilized with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) / n-hydroxysuccinimide (NHS). The effect of the median size of the AuNPs, the method of sensitization and conjugation were studied. The applicability of AuNPs from different sources, as commercial available ones and AuNPs produced by green biotechnological process were compared.

## Instrumentation

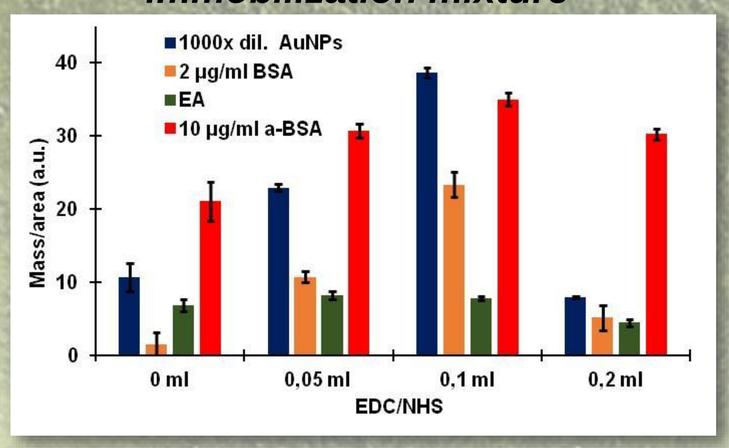

***Model OWLS 210, Microvacuum Ltd., Hungary***



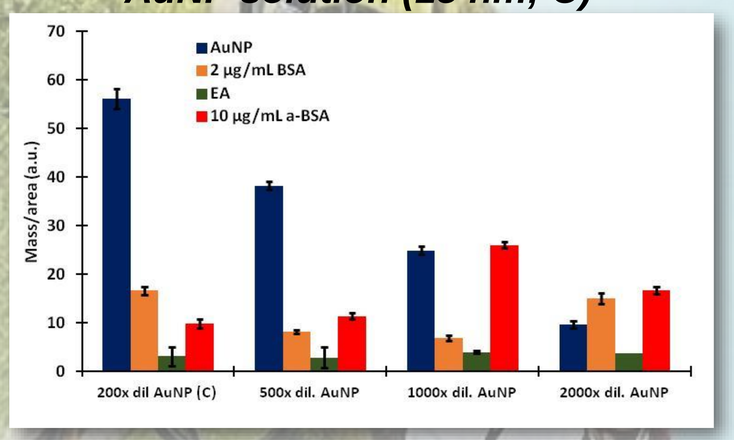

## Results

| | Origin of AuNP | Median size |
|---|---|---|
| A | Without AuNP | - |
| B | Sigma 765430 | 5 nm |
| C | Sigma 765538 | 15 nm |
| D | Sigma 765473 | 30 nm |
| E | *Humicola insolens* CBS 147.64 | 76.5 nm |
| F | *Rhizopus pusillus* WFPL 267A (ATCC 16458) | 60.1 nm |
| G | *Thermoascus aurantiacus* TUB F-43 (ATCC 58156) | 54.2 nm |
| H | *Thermomucor indicae-seudaticae* NRRL 6429 (ATCC 28404) | 69.5 nm |
| I | *Thielavia terrestris* NRRL 8126 (ATCC 38088) | 124.0 nm |

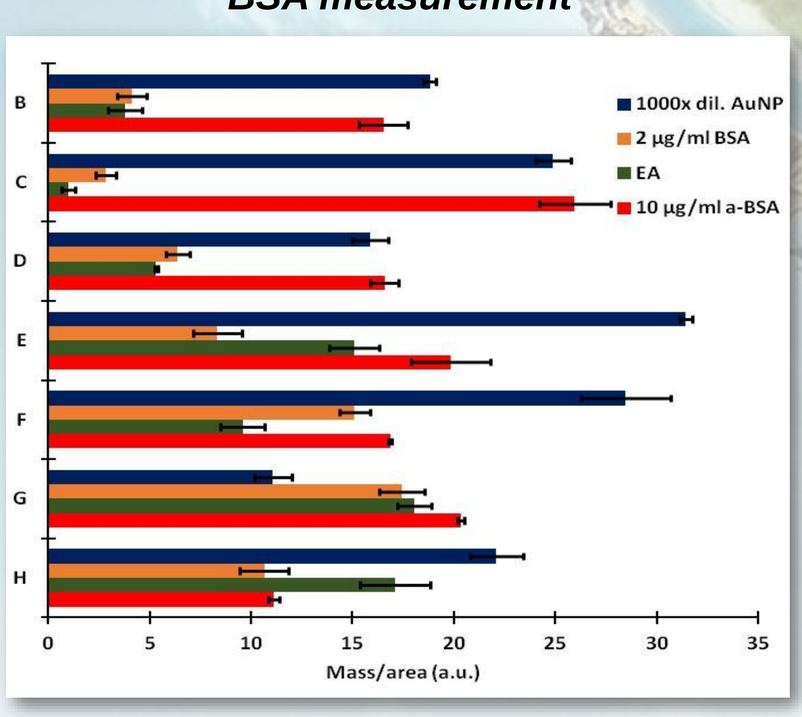
***Effect of the EDC/NHS in the immobilization mixture***



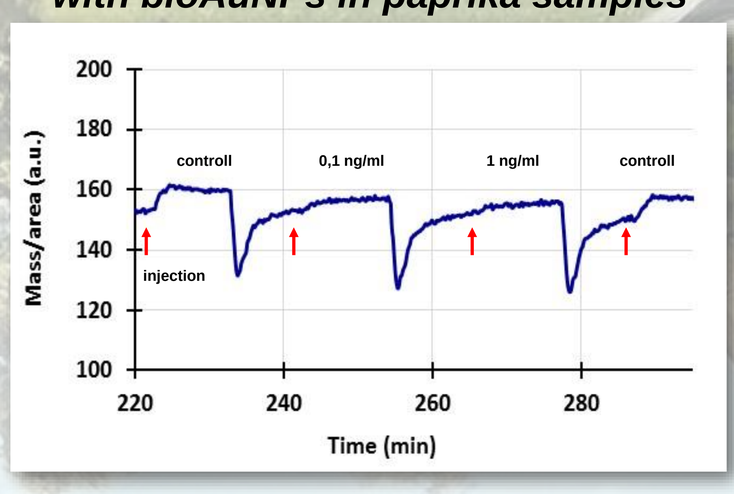

***Effect of the concentration of AuNP solution (15 nm; C)***



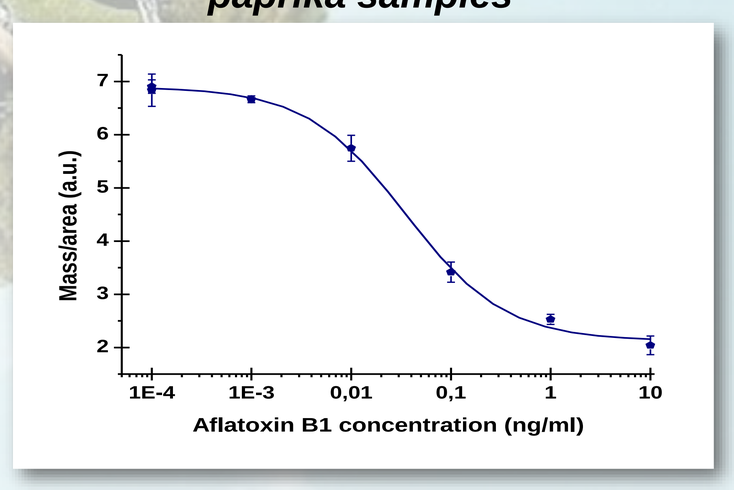

***Effect of the different AuNPs on the anti-BSA measurement***




***Measurement curve of aflatoxin B1 with bioAuNPs in paprika samples***




***Calibration curve of aflatoxin B1 in paprika samples***




## Conclusion

Measuring methods were investigated for the determination of aflatoxin B1 in paprika. Methods can be limited by the affinity and specificity of the antibodies, and detect the analytes, but not necessarily its metabolite derivatives.

Results can be achieved in real time, the sample preparation can be simplified, so the OWLS immunosensor technique has a potential for quick determination of analytes in food or in environmental samples.

## Acknowledgment

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