TÜBİTAK

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Fast and sensitive detection of mycotoxins with Real-time Electrochemical Profiling and nanoparticles amplification system

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INTRODUCTION

Deoxynivalenol (DON) as one of the mycotoxins persists in grains during storage and is unaffected by high temperature and pressure during processing. Its testing has a great importance in order to prevent contaminated food to reach the consumer. Conventional DON testing requires expensive equipment and time consuming procedures, hence as an alternative a new biosensing platform based on Real-time Electrochemical Profiling (REP^{TM}) has been developed for fast and easy DON detection in grain. Au electrode array has been designed and fabricated on silicon dioxide wafer and a sensor cassette has formed a fluidic channel (~10 µl) over the electrodes. The deoxynivalenol detection has been conducted according to an optimised REP^{TM} assay protocol using deoxynivalenol standards at varying concentrations and a standard curve was obtained .

Methodology and Results

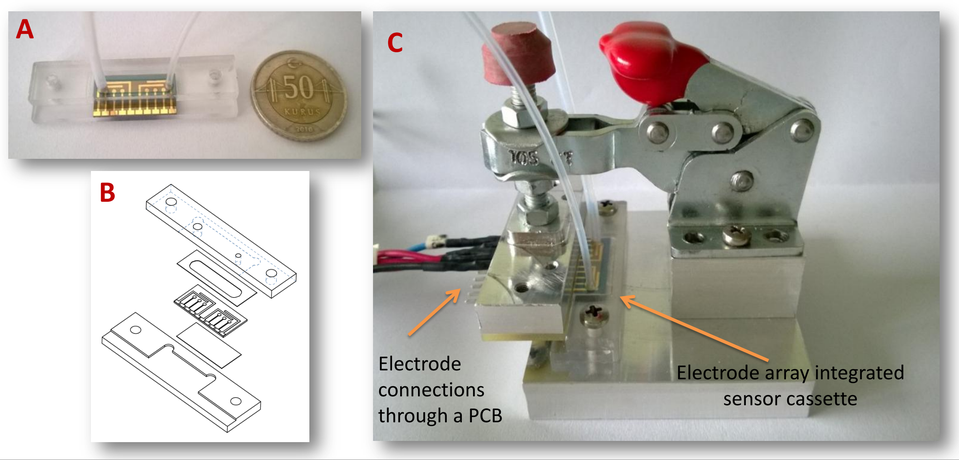

Figure 1 (A) Electrode array integrated sensor cassette. B) A flow cell is formed and the electrode array is fixed to the sensor cassette by means of a double sided sticky tape. C) For the laboratory prototype, a printed circuit board (PCB) attached clamp is used to establish electronic connections between the electrode arrays and the potentiostat device.

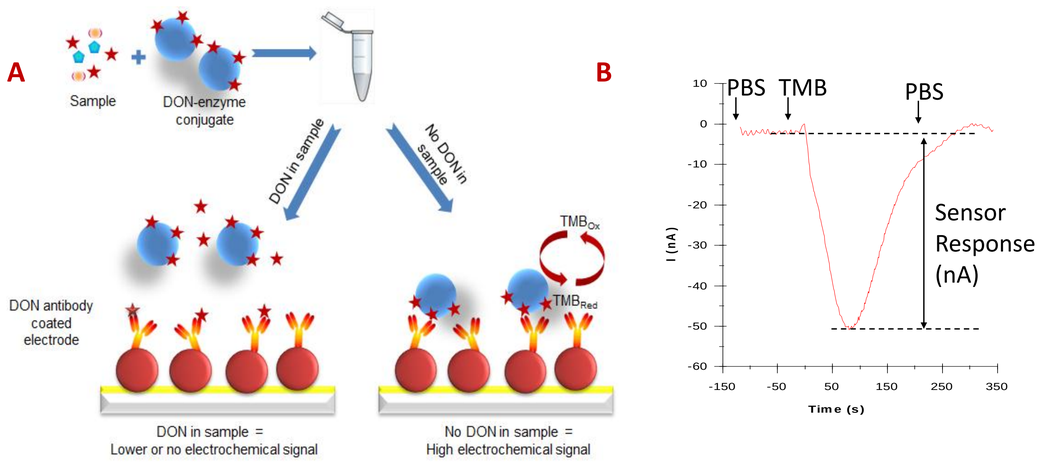

Figure 2 A) The schematics of the mycotoxin detection assay on electrodes. B) The current response of the electrodes against -0.1 V potential is measured continously during the buffer and TMB substrate injections to obtain a sensorgram. The amplitude of the obtained current is proportional to the amount of surface bound HRP enzyme.

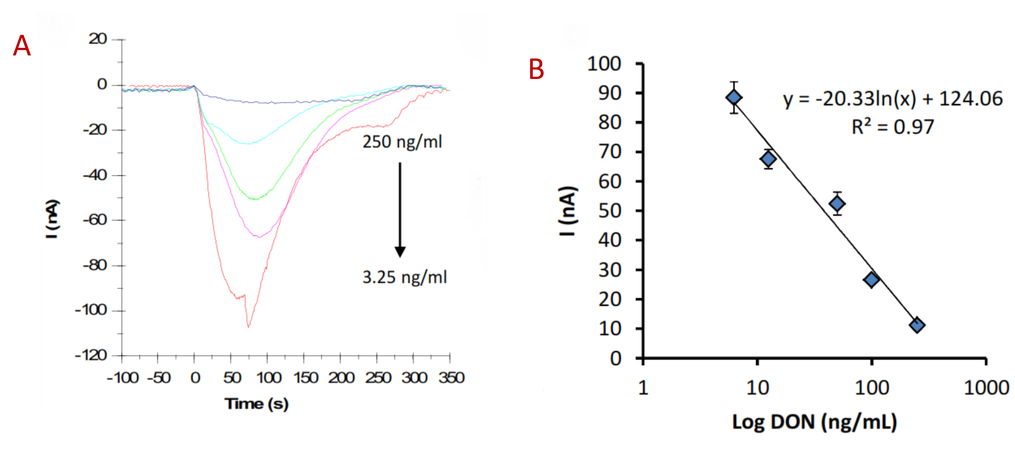

Figure 4 (A) The REP^{TM} assay has been performed for DON detection, where the amperometric measurement was taken in real time during the injection of TMB substrate (50 µL/min). The obtained current is proportional to the amount of the surface bound DON-HRP conjugate and inversely proportional to the DON concentration in sample. **(B)** Linear and **(C)** logarithmic calibration curves for the DON detection assay.

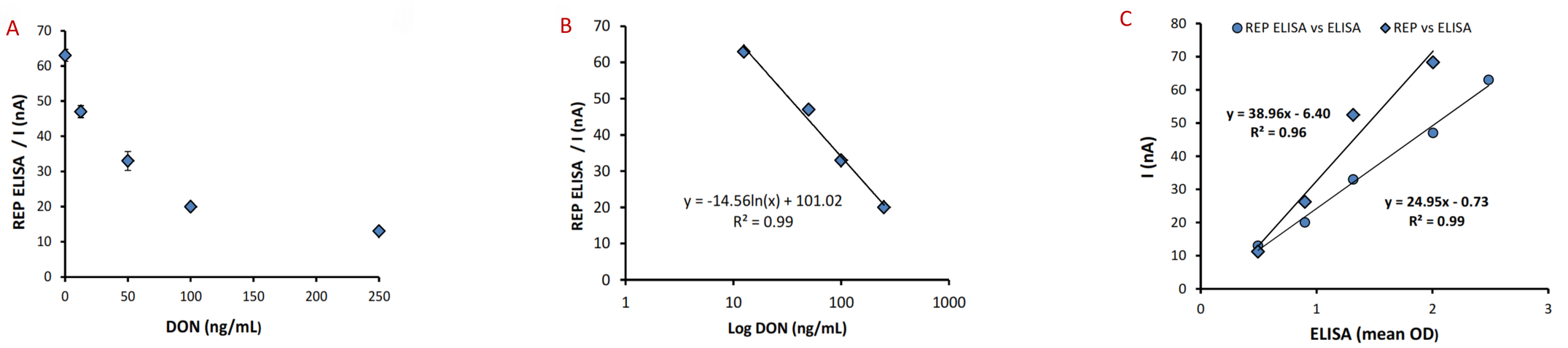

Figure 5 The REP-ELISA assay has been performed in microwells. After 5 minutes incubation of the microwells with TMB, the solution was removed with a pipette and immediately injected on to the electrode arrays (bare Au) for electrochemical measurement and the current was measured continuously for a given -0.1 V potential. **(A)** Linear and **(B)** logarithmic calibration curves for the REP-ELISA assay. (C) The comparison of the REP-ELISA and REP^{TM} assay results with respect to the conventional ELISA readings for DON detection.

CONCLUSION

Towards the realisation of an electrochemical device for on-site mycotoxin testing, it is essential to miniaturise the electrode array and integrate with a microfluidic system. Therefore, a new sensor chip has been fabricated that consists of electrode arrays with 1 mm diameter working electrodes and shared reference/counter electrodes. In addition, a sensor casette made of PMMA was designed and fabricated, that created a microfluidic channel on the electrode arrays. Initially, the electrochemical characterisation of the electrode arrays after surface modification has been investigated. Later the mycotoxin detection assay has been performed using this new sensor chip. The possible out of the laboratory, on-site detection of toxins would provide frequent and on-time detection of toxins before it spreads to whole silos and hence will prevent disposal of vast amounts of crops and provide a huge economic impact for the agricultural industry. In addition, would provide an on-site, fast and cost effective solution for testing the imported/exported crops. Therefore, the objective of the study has been the development of a new sensing platform (REPTM) for rapid, accurate and on-site detection of mycotoxins in crops. The advantages of the proposed toxin detection platform with respect to existing technologies are its ease of use, sensitivity and reliability. The proposed REPTM platform consists of novel electrode arrays that are easy to fabricate, has a small imprint allowing fluidic system integration, enables multiplexed measurements and performs well in terms of electrochemical detection as shown through the DON detection assays. Therefore, it could provide a convenient tool to fabricate portable, convenient, fast, sensitive, low cost and automatedsensing devices for a variety of applications. Our further work involves the integration of the developed electrode array and its fluidics cassette to an electrochemical analyser to obtain an automated REPTMbased detection platform for automated mycotoxin tests.

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