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Environmental Monitoring of Mercury using Bacterial Bioreporter Microchips

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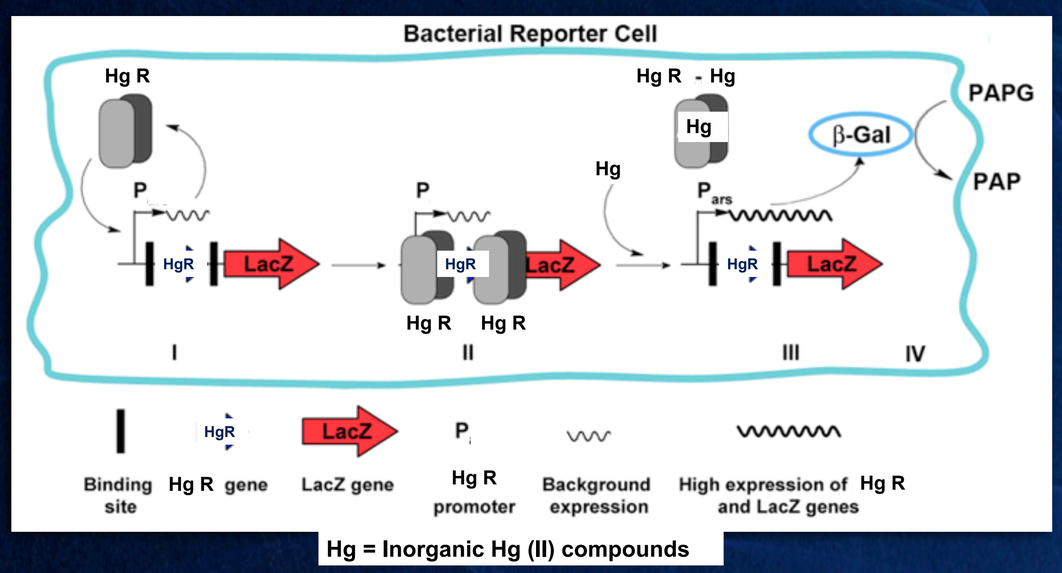
1. INTRODUCTION

Toxic metals are often discharged by various industrial processes which lead to the contamination of freshwater and marine environment. Mercury, a toxic heavy metal contamination mainly arises due to the burning of fossil fuels, oil refining, rubber processing, fertilizer, pesticides, pulp and paper industry, thermometers, dental fillings, electroplating industries, barometers, manometers, fluorescent lamps, batteries, cosmetics, and pharmaceuticals.[1] Electrochemistry has a great potential for the quantification of a wide range of contaminants since it provides fast, sensitive, non-expensive and highly reliable measurements. Thus by combining electrochemical detection strategies, cost-competitive sensors can be miniaturized and integrated into lab-on-a-chip devices with fast responses and low detection limits.[2,3]
In the present contribution we have implemented an inkjet printed microchip and commercially available microchip for the electrochemical readout of a bacterial bioreporter for the sensitive analysis of Hg (II) in tap water. With this aim, a naturally Hg (II) resistant *E. coli* bacteria has been genetically modified to produce the reporter protein β-Galactosidase (β-Gal) in the presence of Hg (II). If we use p-aminophenyl β-D-galactopyranoside (PAPG) as a substrate for β-Gal, then p-aminophenol (PAP) is produced and detected using the microchips.

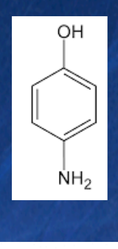
2. DETECTION MECHANISM



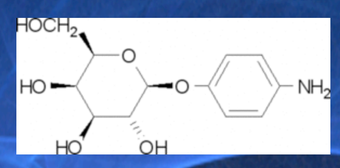
Hg = Inorganic Hg (II) compounds

Figure 1. Detection mechanism of the Hg (II) bioreporter:

I The Hg R repressor protein is synthesized from the HgR gene under control of the promoter (P).
II Hg R binds to its two binding sites on the DNA (black upright bars) and prevents expression of itself and of the reporter gene (LacZ).
III When Hg (II) is present, Hg R loses its affinity for the binding sites on the DNA and the transcription of the Hg R and LacZ genes increases, leading to the subsequent formation of beta-galactosidase (β-Gal).
IV Finally, p-aminophenyl β-D-galactopyranoside (PAPG) diffuses through the cell membrane and is cleaved by β-Gal to form p-aminophenol (PAP) that is detected in the microchips outside the cell.



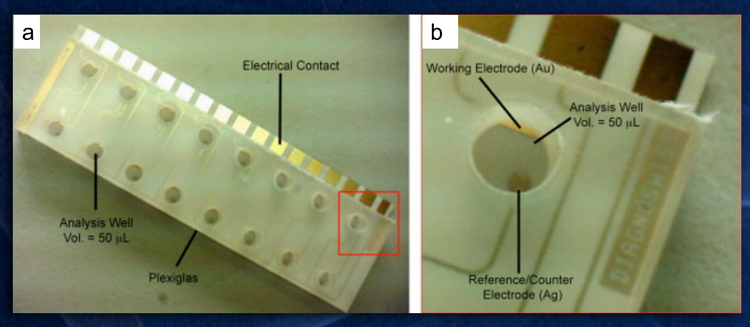
PAP



PAPG

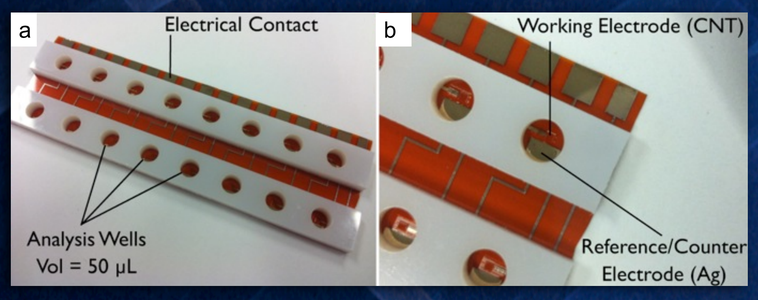
3. MICROCHIPS

DiagnoSwiss microchip



Picture 1: Diagnoswiss microchip employed for the bioelectrochemical monitoring of Hg (II). The microchip contains 16 independent electrochemical sensors (a), each one (b) consisting of a two-electrode setup (working electrode Au, counter/reference electrode Ag) and a well (50 µl) made in Plexiglas.

Inkjet printed (IJP) microchip



Picture 2: Inkjet printed microchip employed for the bioelectrochemical monitoring of Hg (II). The microchip contains 16 independent electrochemical sensors (a), each one (b) consisting of a two-electrode setup (working electrode CNT, counter/reference electrode Ag) and a well (50 µl) made in Plexiglas.

IJP microchips were prepared on a polyimide substrate by printing first an Ag layer for the electrical path and the quasi-reference electrodes. Then a carbon nanotube working electrode was printed in such a way that only a small section is in physical contact with the Ag layer. Finally, an insulating layer was printed to protect the Ag tracks and to precisely define the working electrode area.
Electrical connections for both microchips are made through a custom-made potentiostat multi-plexer (Diagnoswiss S. A., Switzerland) that applies a common potential value to all the cells.

4. RESULTS

Each microchip well was filled with assay mixture composed of: Hg (II) solution, cell suspension and PAPG solution. After all samples were loaded, a potential of 0.05 V was applied to monitor the production of (PAP) that is directly related with the recorded current. Measurements were performed after given (20 min) time intervals.

DiagnoSwiss microchip

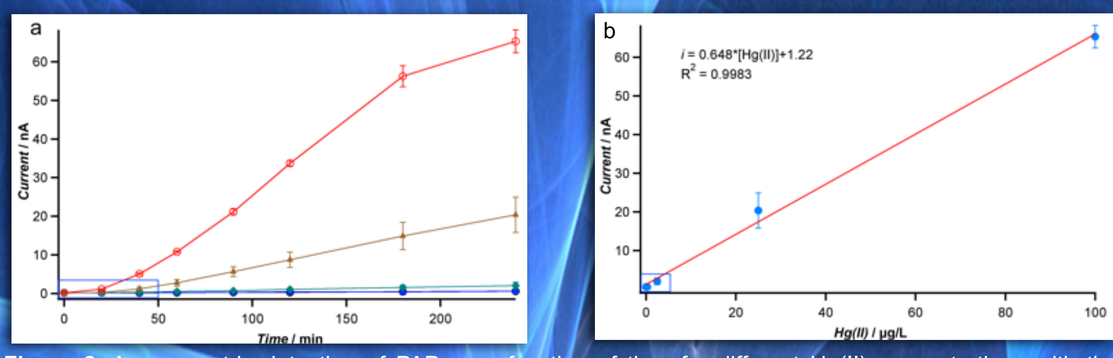


Figure 2. Amperometric detection of PAP as a function of time for different Hg(II) concentrations with the diagnoSwiss chip: 0 ppb (black circles), 0.25 ppb (filled blue squares), 2.5 ppb (filled cyan diamonds), 25 ppb (filled brown triangles) and 100 ppb (empty red circles). E_{appl} = 0.05 V vs Ag electrode. The error bars represent the calculated standard deviation from a triplicate. b) Calibration curve for Hg(II) determined with the data obtained after 240 min of induction time.

Inkjet printed microchip

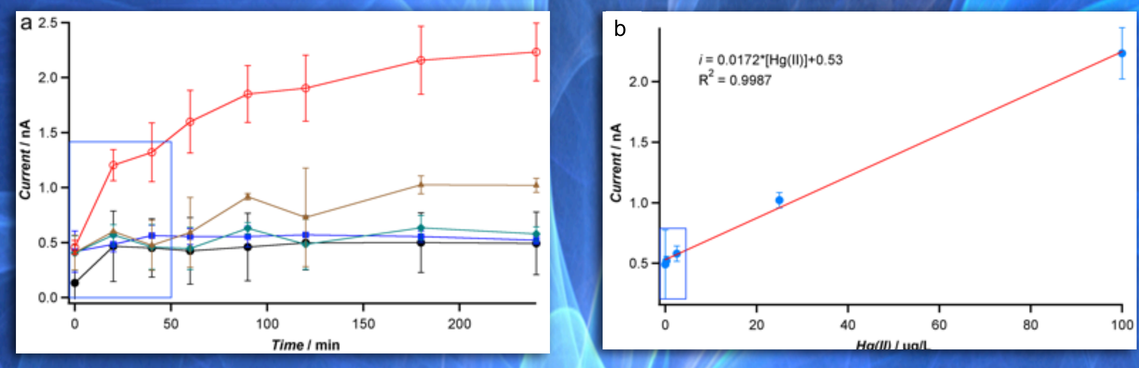


Figure 3. Amperometric detection of PAP as a function of time for different Hg(II) concentrations with the inkjet printed chip: 0 ppb (black circles), 0.25 ppb (filled blue squares), 2.5 ppb (filled cyan diamonds), 25 ppb (filled brown triangles) and 100 ppb (empty red circles). E_{appl} = 0.05 V vs Ag electrode. The error bars represent the calculated standard deviation from a triplicate. b) Calibration curve for Hg(II) determined with the data obtained after 240 min of induction time.

5. CONCLUSIONS AND PERSPECTIVES

Both, diagnoSwiss and inkjet printed microchip gave very sensitive analysis of Hg (II) in tap water, with a good signal differentiation in the range 2.5 - 100 ppb of Hg (II), and with a lowest detectable concentration of Hg (II) equal to 2.5 ppb (limit of toxicity suggested by WHO is 6 ppb). Inkjet printed chips had a lower current range which could be increased by optimizing the electrode area and electrical connections. Further work would be set on implementing magnetic beads-based immunoassay to improve signal-noise ratio. Furthermore, it is planned to implement this strategy in on-line flow-cell system with electrochemical detection for on-line monitoring of Hg (II) in an Envirobot platform.

LEPA Laboratoire d'Electrochimie Physique & Analytique

References

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