PSII based biosensor for the detection of photosynthetic inhibitors

-Analysis procedure-

Amperometric measurements of photosynthetic inhibition

The inhibitory effect of herbicides on PSII activity is evaluated through the recording of the current due to the re-oxidation of the reduced form of an artificial electron acceptor , which was formed during the photosynthetic step.

Once the system has been illuminated, the photosynthetic reaction takes place releasing oxygen and reducing the electron acceptor present in the measurement buffer. This was then re-oxidised at the electrode surface by applying a suitable potential and a current peak, proportional to the photosynthetic activity, can be recorded.

When the inhibitor is present in the solution the photosynthetic activity decreases, resulting in a lower current peak height.

Reagents and instrumentation

- ✓ PSII based screen-printed based biosensors
- ✓ Measurement buffer (Required composition: MES 0.015 mol L^{-1}, mannitol 0.05 mol L^{-1}, NaCl 0.1 mol L^{-1}, $MgCl_2$ 0,005 mol L^{-1}, 0,00005 mol L^{-1} chloramphenicol, 0,0002 mol L^{-1} duroquinone)
- ✓ Methacrylate cell box
- ✓ Temporised illumination unit
- ✓ Potentiostat
- ✓ Connector for screen-printed electrodes

Preparation of testing solutions.

Prepare the buffer 10X concentrated without duroquinone (MES 0.15 mol L^{-1}, mannitol 0.5 mol L^{-1}, NaCl 1 mol L^{-1}, $MgCl_2$ 0,05 mol L^{-1}, 0,0005 mol L^{-1} chloramphenicol) Concentrated solutions of duroquinone can be prepared in MeOH and it can be added to the 1X buffer to a the final concentration of 0,0002 mol L^{-1}

- Preparation of measurement buffer (blank, 1X buffer): dilute the 10X buffer ten fold (i.e. 100 µL of 10 buffer + 900 µL of MilliQ water). Add duroquinone to the concentration above mentioned.
- Preparation of solution of standards: prepare a concentrate solution of standard in MeOH (i.e. atrazine 0,05 mol L^{-1}). Dilute this solution in the measurement buffer prepared as described above, to the desired concentration (useful range between 10^{-8}-10^{-6} mol L^{-1})
- Preparation of real samples: filter trough a 0,45 µm syringe filter an aliquot of the sample. Buffer the solution using 10X buffer (i.e. add 100 µL of 10X buffer + 900 µL real sample). Finally add duroquinone to the required concentration 0,0002 mol L^{-1}.

Experimental procedure (Fig.1)

i. Locate a screen-printed biosensor in the slot created on the bottom part of the cell
ii. Fix the top part with screws
iii. Check, through the well opening, that the position of the electrode surface resulted centred
iv. Drop 50 µL of testing solution (containing the standard solution or real sample) in the well and close it with the cap that is housing the LED.
v. Apply the working potential at the electrode (+620mV) and, at the same time, activate the light-pulsing temporisation unit by pressing the button.

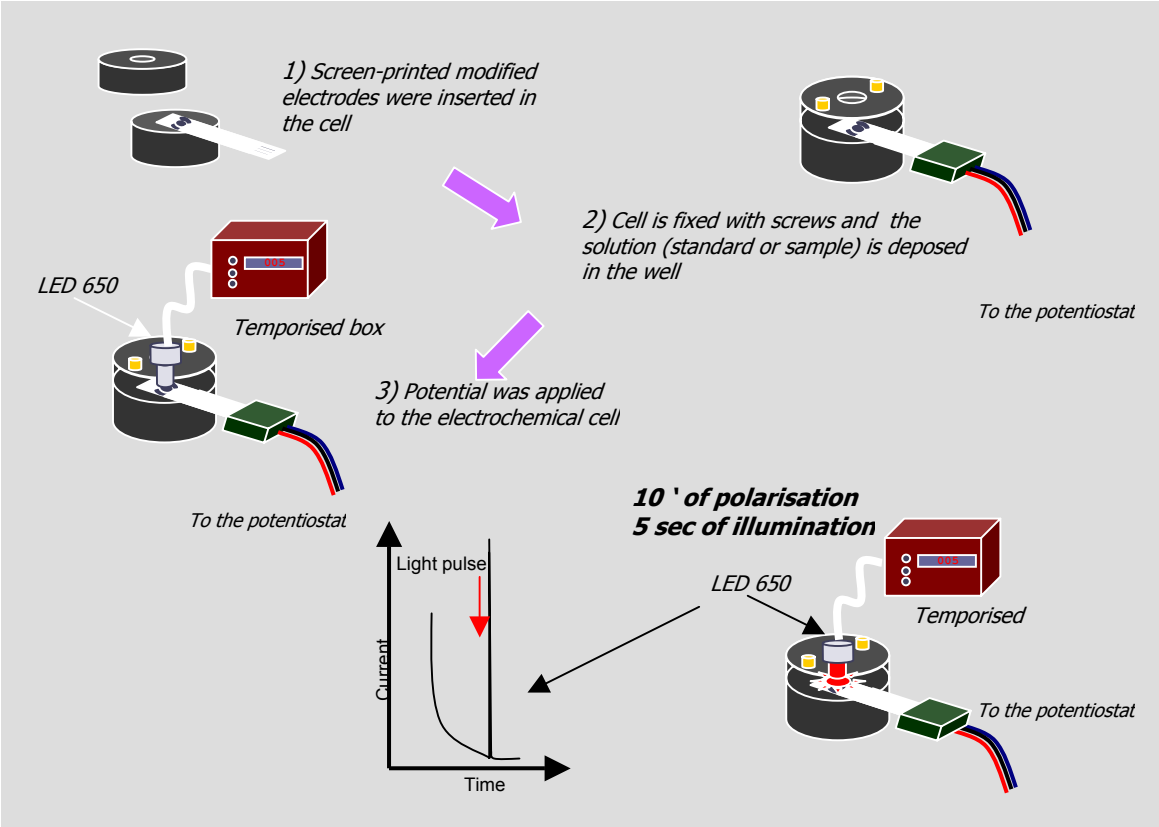

Figure 1- Schematic representation of the measurement

vi. After 10 min the light pulse will be applied; the mediator reduction will then achieved at the electrode surface and the peak current will be obtained (Fig. 2).

vii. After the measurement, wash the cell with MilliQ water and replace the biosensor for a new measurement.

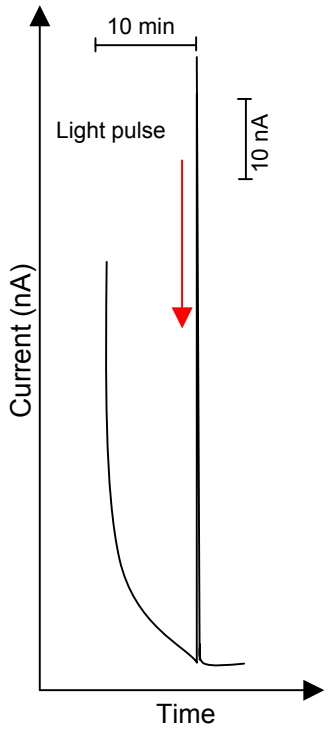

Figure 2- Amperometric signal obtained upon illumination of SPCEs modified with thylakoid membranes

Data treatment

The inhibition values can be calculated using the following formula.

$[(I_{blank}-I_{sample})/I_{blank}] * 100$

where I_{blank} is the current value obtained for a blank (test solution containing only buffer) and I_{sample} the value obtained for different inhibitors concentrations.

References

Bettazzi F., Laschi S. and Mascini M. "One–shot screen-printed thylakoid membrane-based biosensor for the detection of photosynthetic inhibitors in discrete samples", *Biosensors & Bioelectronics, Submitted*

Authors: Dr. Francesca Bettazzi, Dr. Serena Laschi

Corresponding author: Dr. Serena Laschi, e-mail address serena.laschi@unifi.it