# Progetto : **DESIR**

## Deposizioni per ElectroSpray Ionization e biosensoRi

**WP1** : **REALIZZAZIONE ED OTTIMIZZAZIONE DI UN SISTEMA DI DEPOSIZIONE PER ELECTROSPRAY IONIZATION (ESD) DI ENZIMI IN ARIA.**

Responsabile : A. Cartoni (Università La Sapienza).

**FINALITA':**

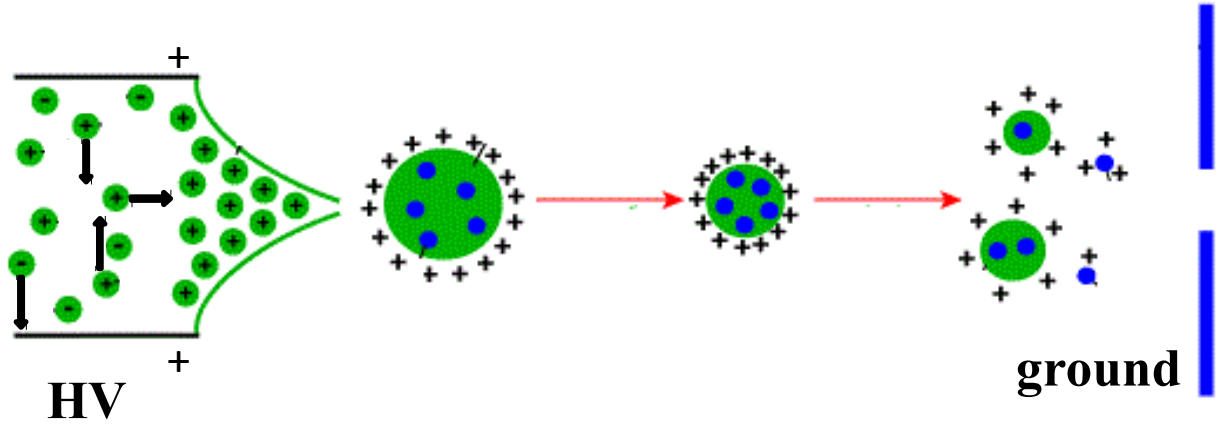
- definire le condizioni sperimentali per effettuare una deposizione per ESD di enzimi attivi
- ottimizzare le tecniche di analisi chimico-fisica per valutare l'efficacia della tecnica e la preservata attività del materiale depositato
- valutare l'applicabilità della tecnica ESD "in aria" per la produzione di biosensori

Attività 1.1.: Studio dell'effetto di solventi e additivi utilizzati nella soluzione del sistema da depositare

Attività 1.2.: Misura quantitativa del materiale depositato e analisi morfologica.

# WP1

# Perché ESI




- The technique uses a low-concentration solution of the molecule of interest flowing in a small capillary held at high voltage (4.92 kV) with respect to a grounded counter electrode placed 14 mm away.

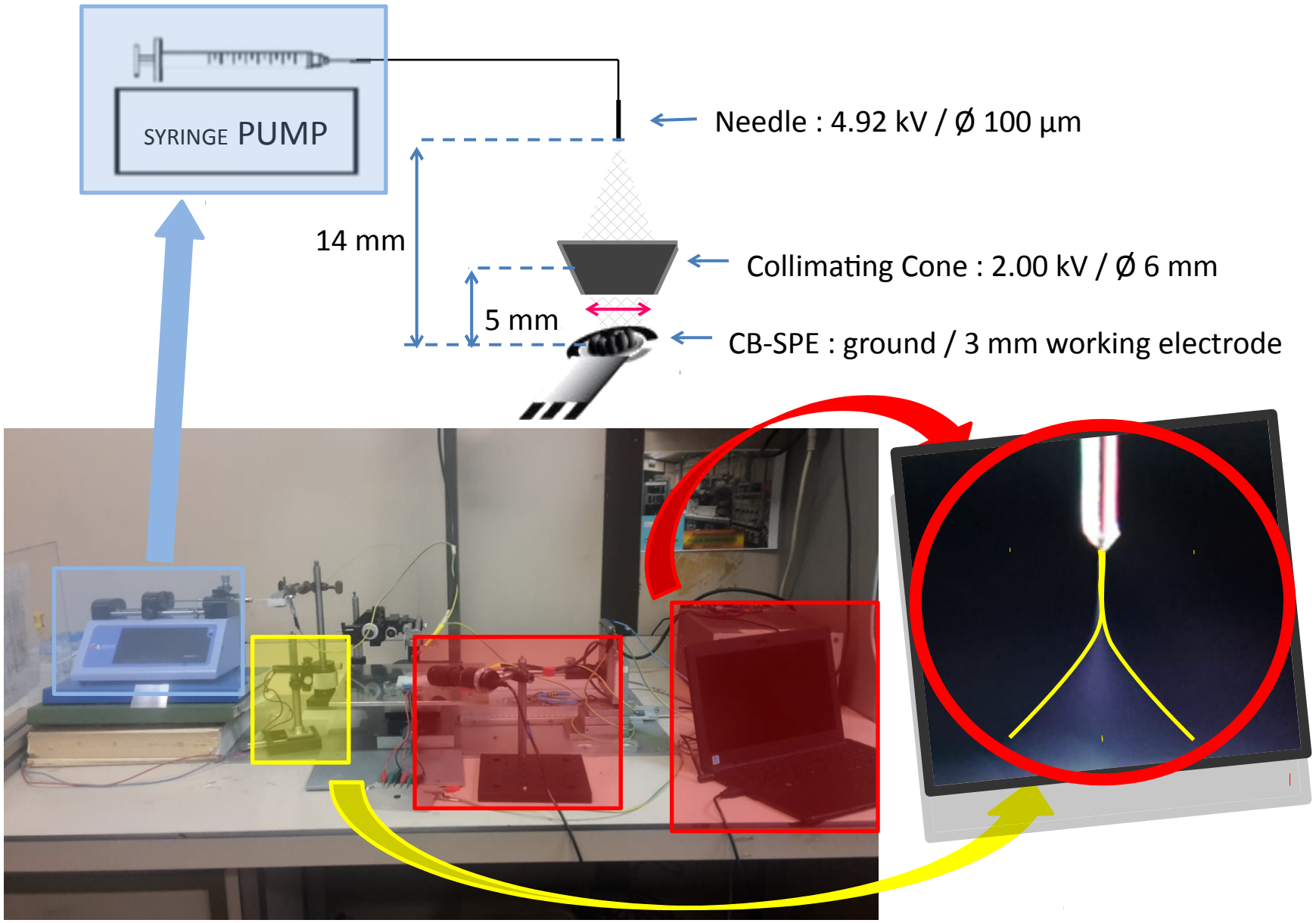
- The charges on the liquid surface at the end of the capillary repel each other and expand at the solution/gas interface into a Taylor cone. The electrostatic force is counter-balanced by the surface tension of the liquid

- When the surface tension cannot stand anymore the charges a Coulomb explosion creates a spray of charged droplets whose size decreases as the solvent evaporates to form a gas of molecular ions.

This approach provides effective removal of the solvent, with the advantages that the deposition can be carried out at ambient pressure or in controlled atmosphere, with significant reduction of **costs** and **times** of the process, which could be easily automatized and **applied on large scale**.

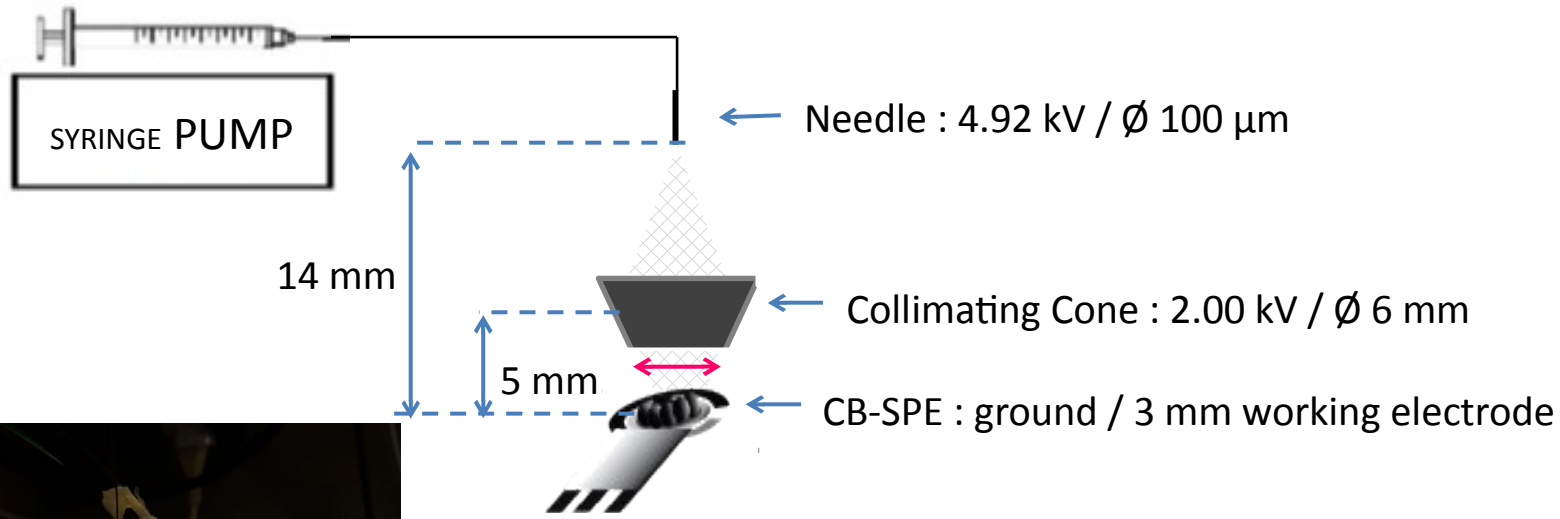
# WP1

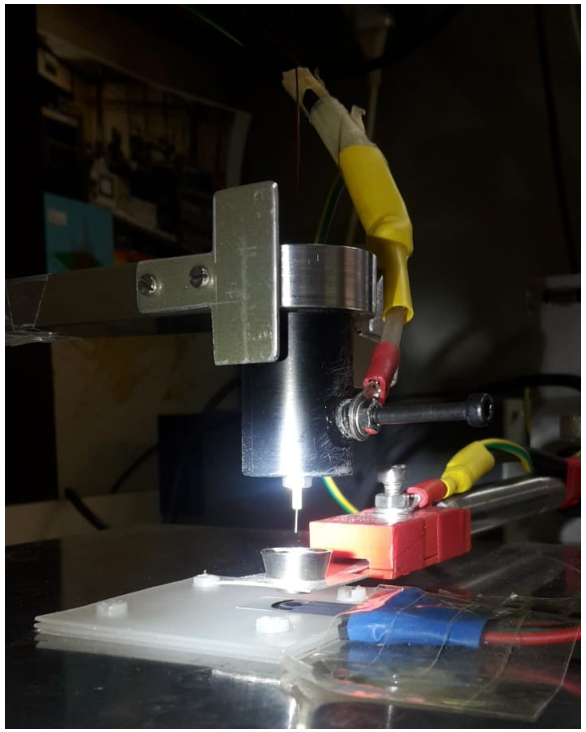
# Experimental set-up

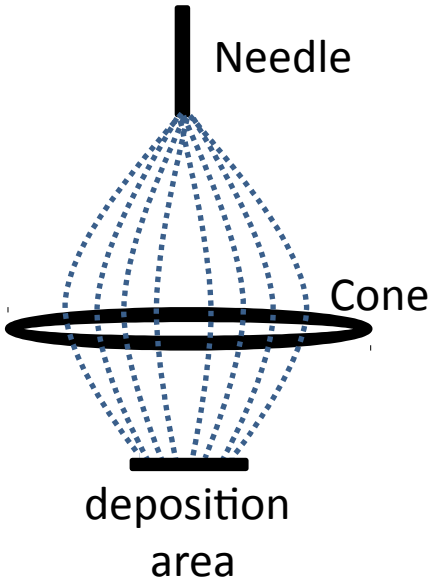


# WP1

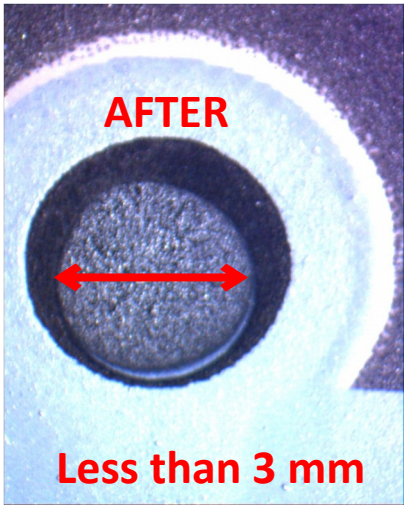
# Experimental set-up









# WP1

Attività 1.1.: Studio dell'effetto di solventi e additivi utilizzati nella soluzione del sistema da depositare

## Laccase from *Trametes versicolor 10 U/mg*

### pH analysis

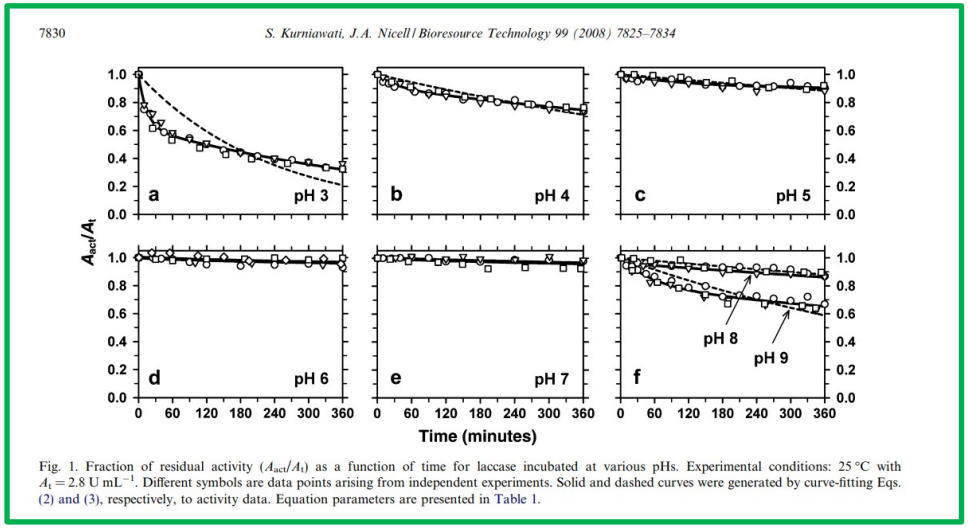
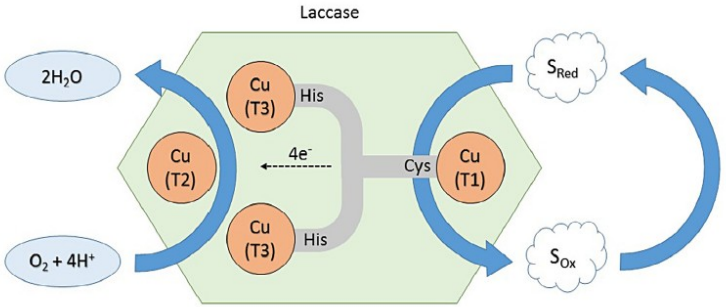


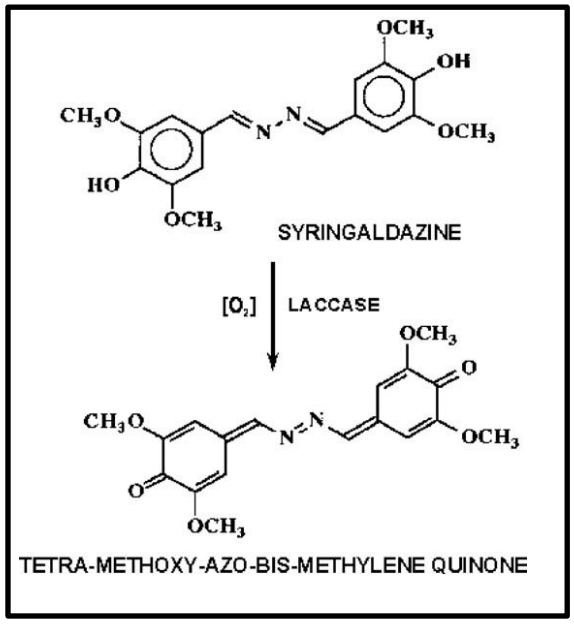

Fig. 1. Fraction of residual activity ($A_{act}/A_t$) as a function of time for laccase incubated at various pHs. Experimental conditions: 25 °C with $A_t = 2.8$ U mL$^{-1}$. Different symbols are data points arising from independent experiments. Solid and dashed curves were generated by curve-fitting Eqs. (2) and (3), respectively, to activity data. Equation parameters are presented in Table 1.

TEST:

- 1485 µl of buffer (acetic acid and sodium acetate) solution at pH 5.5
- 150 µl of syringaldazine solution (syringladazine and absolute methanol)
- 15 µl of laccase solutions: *buffer* (blanc), *a* and *b*

The activity of the enzyme has been measured with the syringaldazine test. abs: 530 nm



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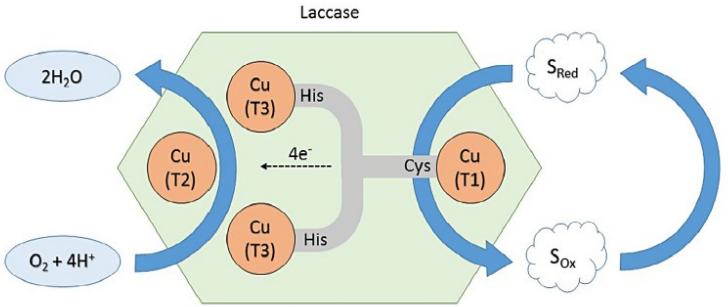
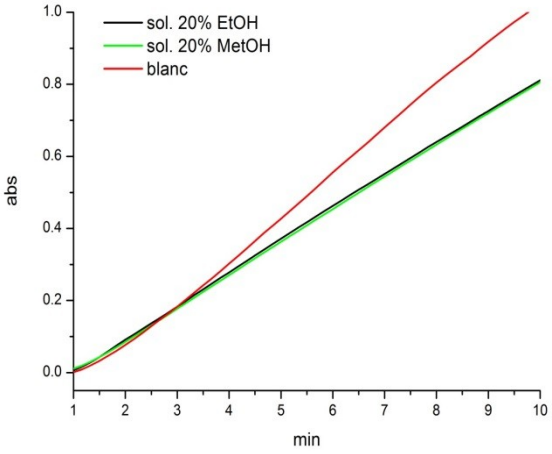


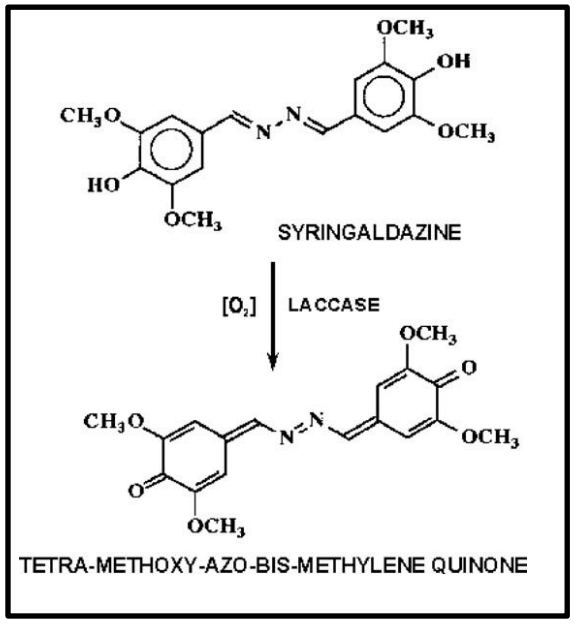




The best condition has been found for a solutions of laccase (10U) 0.2 µg/µl:

- **20% of MetOH in $H_2O$**
- **20 % of EtOH in $H_2O$**

Flow rate: 1 µL/min
Deposition Time: 15 min

Hypothetical deposed amount: 3 µg – 0.03 U

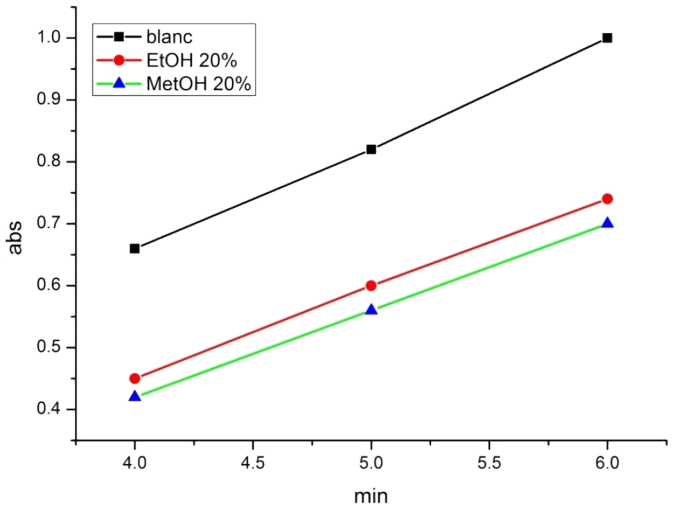


# WP1 Attività 1.1.: Studio dell'effetto del processo elettrospray sull'attività dell'enzima

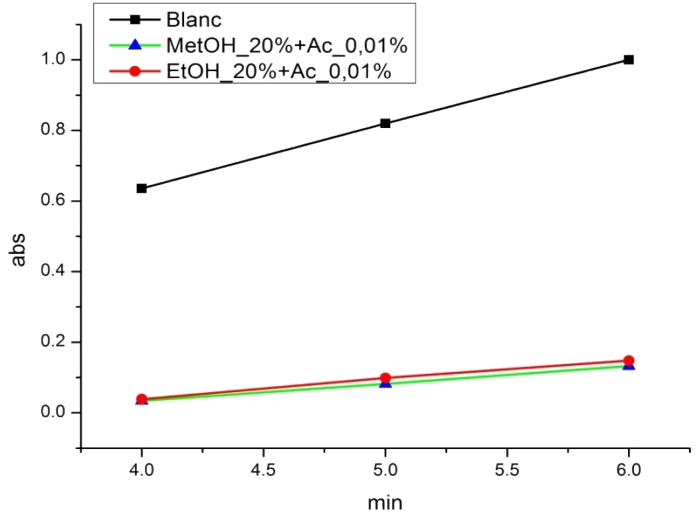
## Laccase from *Trametes versicolor*

•The preservation of the enzyme activity dissolved in different solutions (0.2 µg/µl) and subject to the ES process has been checked via the syringaldazine test [4] and spectrophotometric absorbance measurements.

• The results show that less than 30% of the enzymatic activity is lost, when laccase is dissolved in an aqueous solution at 20% of EtOH or MetOH without protonating agents.




| Solution | 20% EtOH | 20% MetOH | 20% EtOH + 0.01% formic acid | 20% MetOH + 0.01% formic acid |
|---|---|---|---|---|
| Absorbance | 74% | 70% | 15% | 13% |



# WP1

## Progetto Desir : GANTT *(legenda Mix milestone; Dx Deliverable)*

| | Attività | M 1 | M 2 | M 3 | M 4 | M 5 | M 6 |
|---|---|---|---|---|---|---|---|
| | *Coordinamento* | ***Mi1*** | | ***Mi4*** | | | ***Mi6 Mi7*** |
| WP1 | *Sistema ESD per enzimi in aria* | | | | | | |
| Attività 1.1 | *Studio effetto solventi e addittivi nella soluzione* | | ***Mi3*** | ***Mi4*** | | | ***D3*** |
| Attività 1.2 | *Misura quantitativa analisi morfologica.* | | | | | | |

Mi3: Set-up per ESD in aria operativo Mese 2

D3: Report/pubblicazione su condizioni ottimali per ESD in aria e test su laccasi

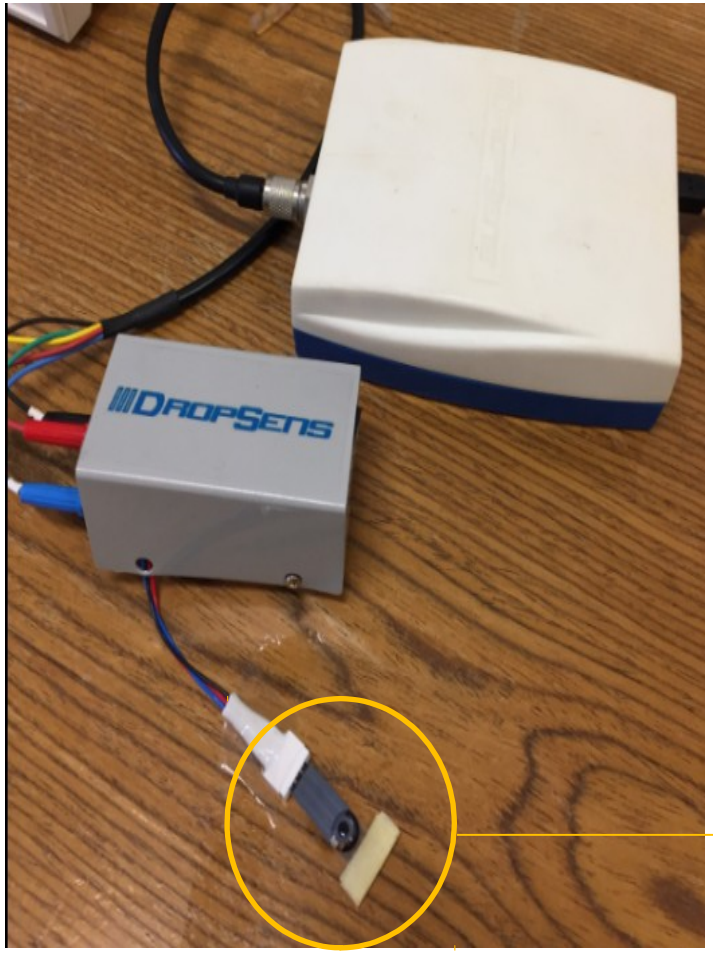
# WP1

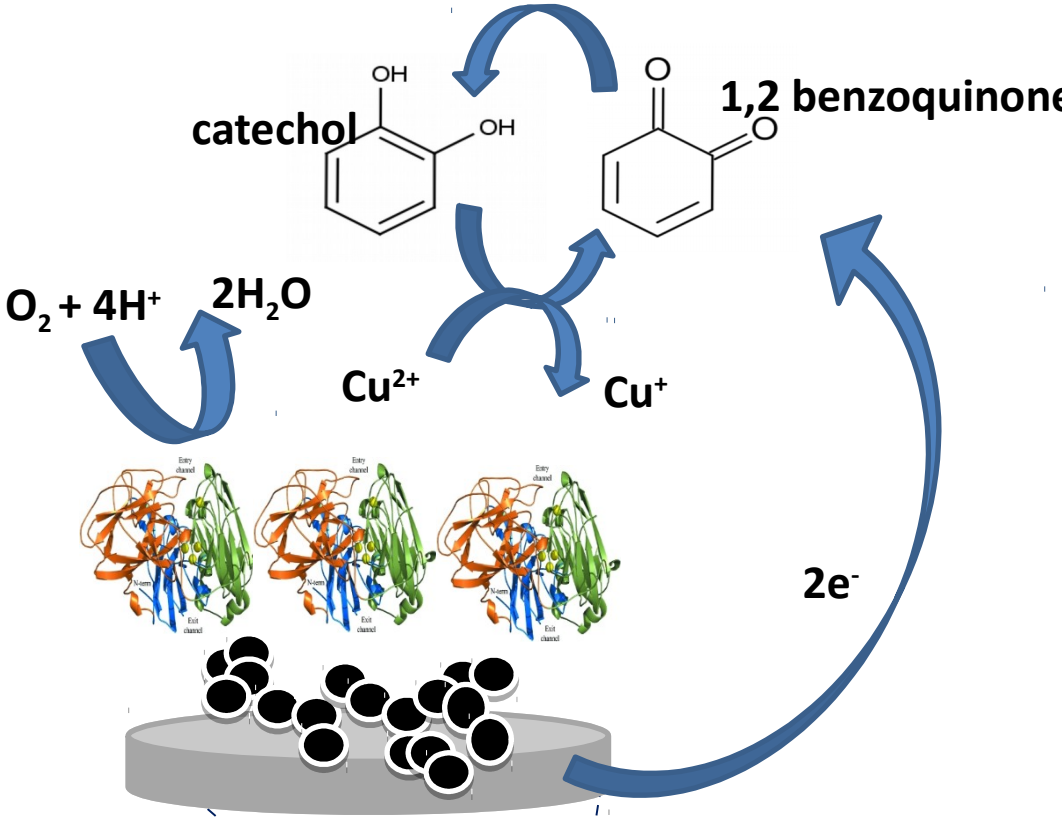
## Biosensor set-up

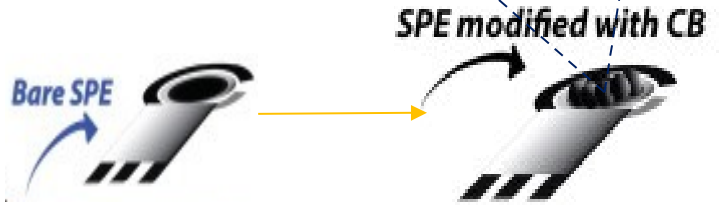
Laccase enzyme

Carbon black

Graphite working electrode



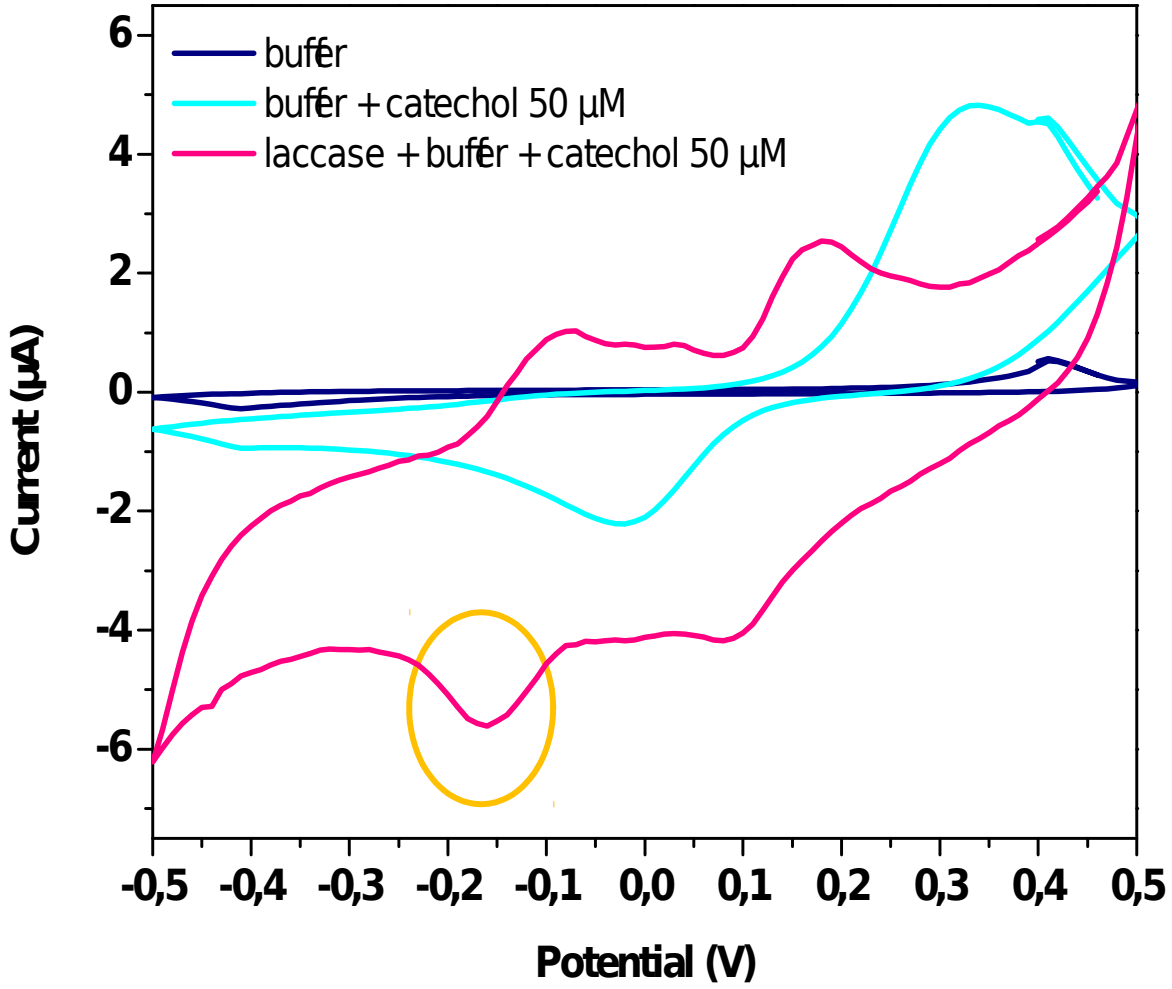




Electrodes screen printed at Tor Vergata University (Prof. Arduini)

# WP1

## Cyclic voltammetry

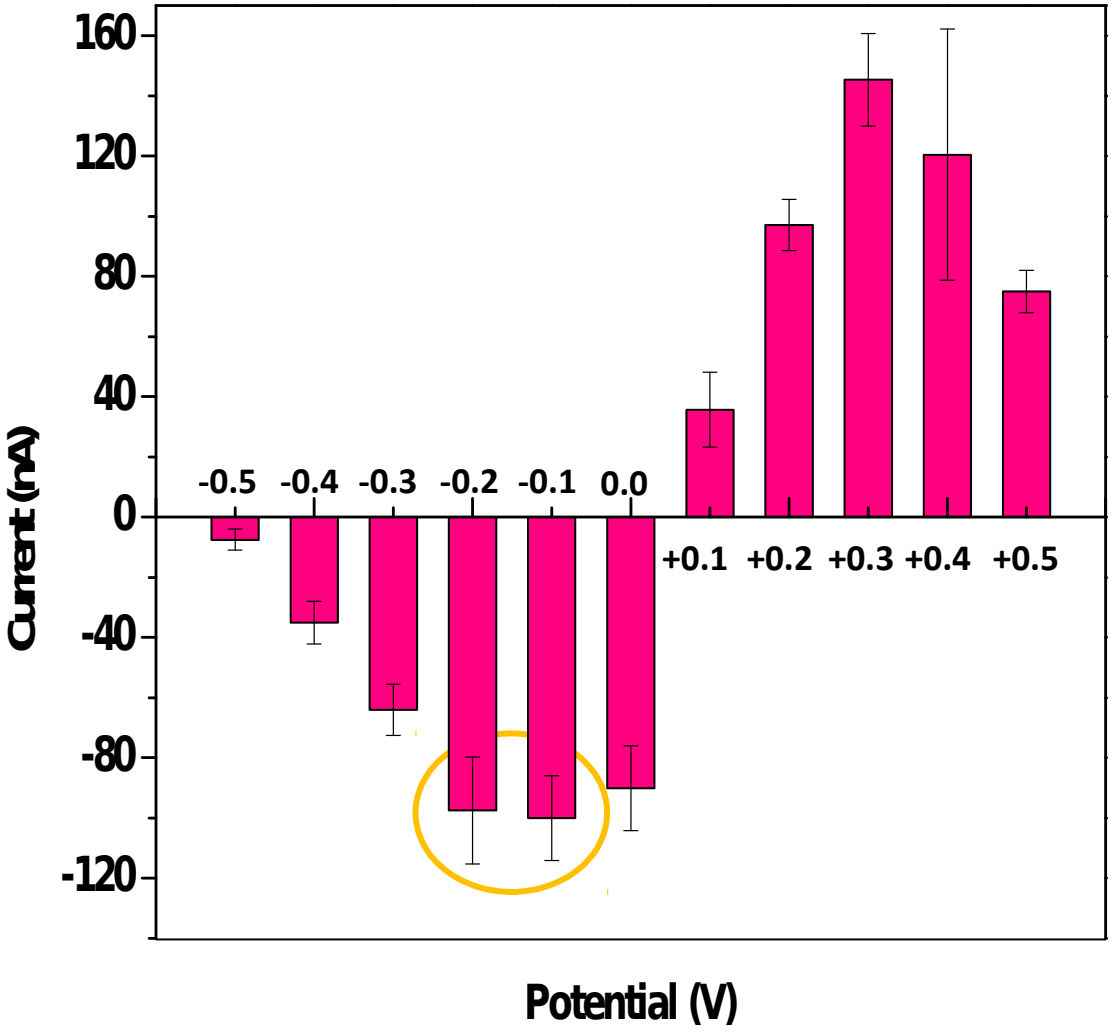
## of laccase enzyme in the presence of catechol deposed by ESI on carbon black modified screen-printed electrode




Potential range = +0.5 / - 0.5 V
Scan rate V/s = 0.1
n° scan = 3
Reaction volume = 100 µL
Catechol = 50 µM
Buffer = 0.1 M citric acid/sodium citrate pH 4.15

# WP1

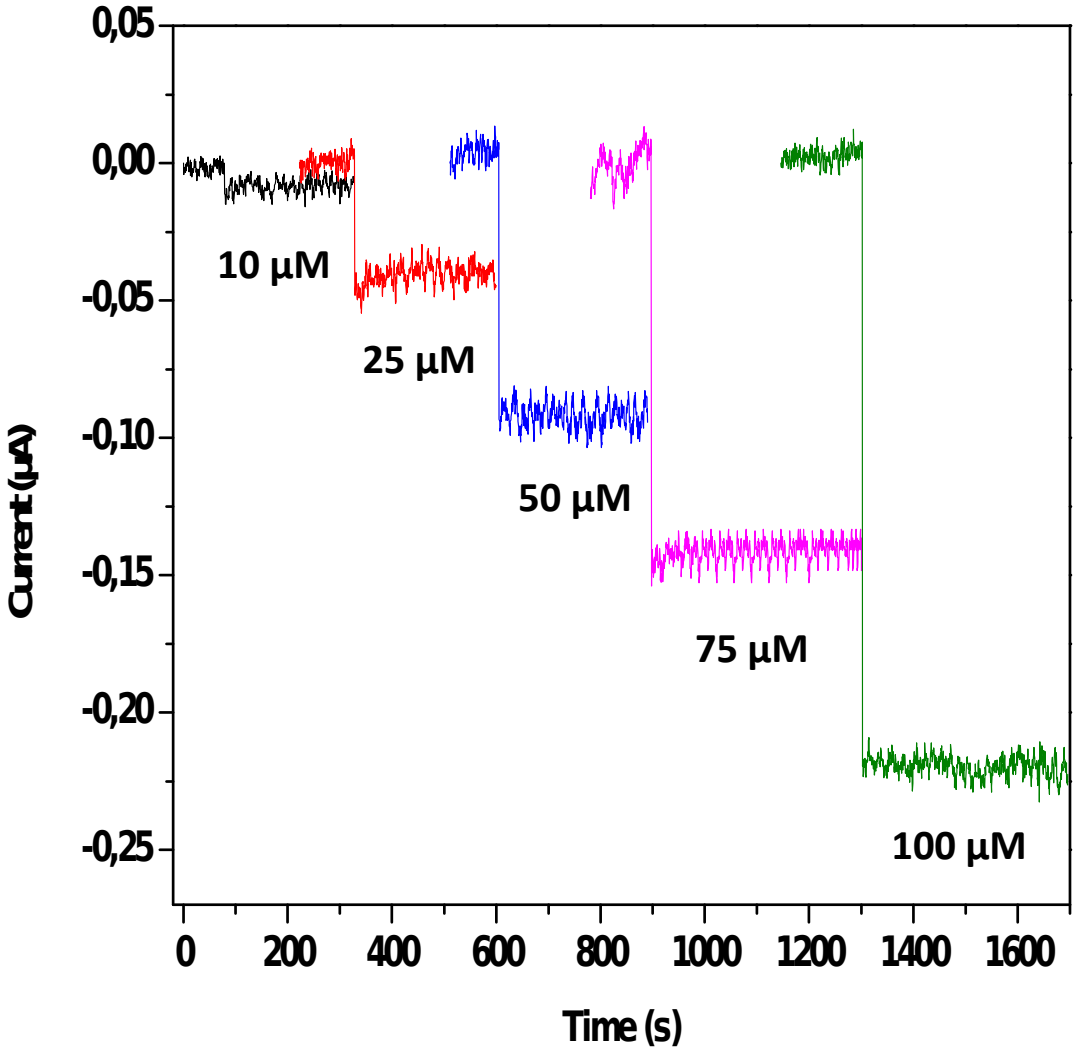
## Potential study of laccase enzyme in the presence of catechol deposed by ESI on carbon black modified screen-printed electrode




Potential range = +0.5 / - 0.5 V
Interval = 0.5 s
Reaction volume = 100 µL
Catechol = 50 µM
Buffer = 0.1 M citric acid/sodium citrate pH 4.15

# WP1

## Amperometric response of laccase enzyme deposed by ESI on carbon black modified screen-printed electrode in the presence of increasing amount of catechol

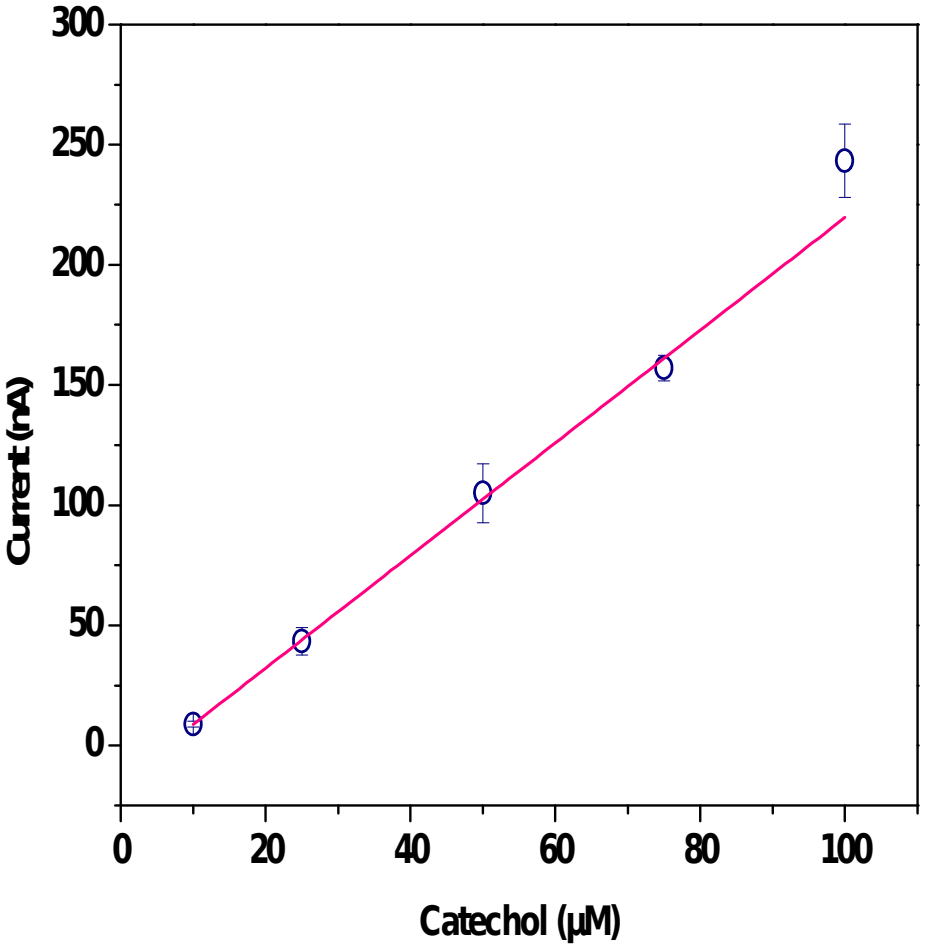



Potential = **- 0.160 V**
Interval = 0.5 s
Reaction volume = 100 µL
Buffer = 0.1 M citric acid/sodium citrate pH 4.15
Catechol range = 10 - 100 µM

# WP1

## Calibration curve

## of laccase enzyme deposed by ESI on carbon black modified screen-printed electrode in the presence of increasing amount of catechol

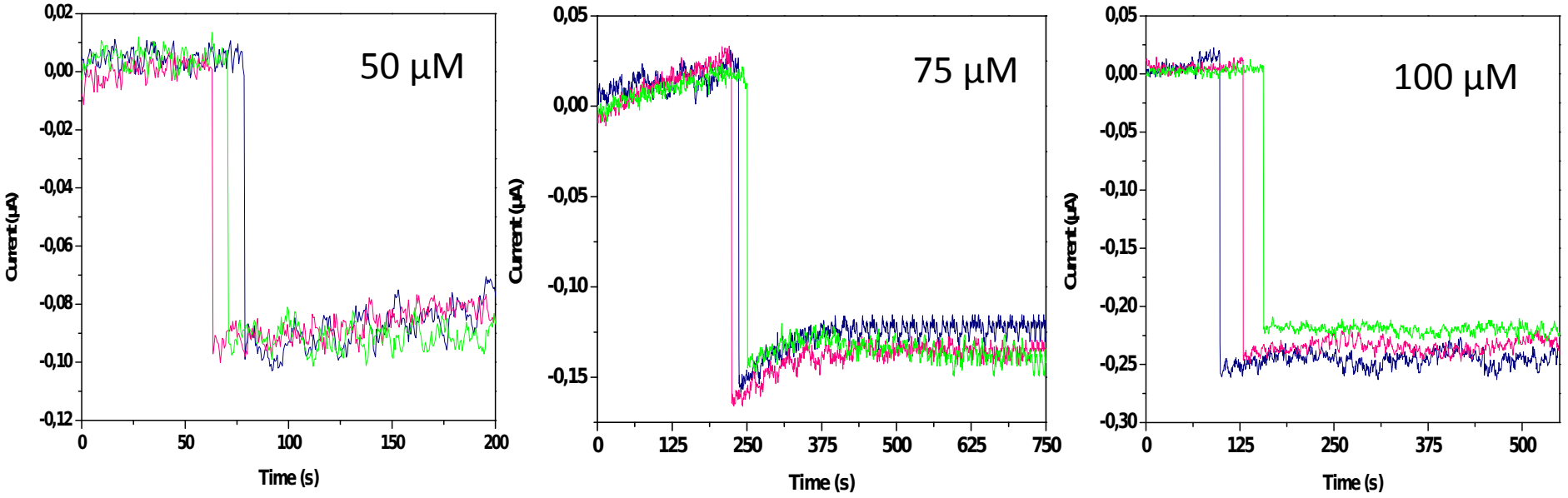



Potential = - 0.160 V
Interval = 0.5 s
Reaction volume = 100 µL
Buffer = 0.1 M citric acid/sodium citrate pH 4.15
Fit equation y = 2.35 ± 0.07 x - 14.7 ± 1.6
$R^2$ = 0.9959

# WP1

## Inter-electrode repeatability

## Amperometric signals of laccase enzyme deposed by ESI on carbon black modified screen-printed electrode in the presence of catechol

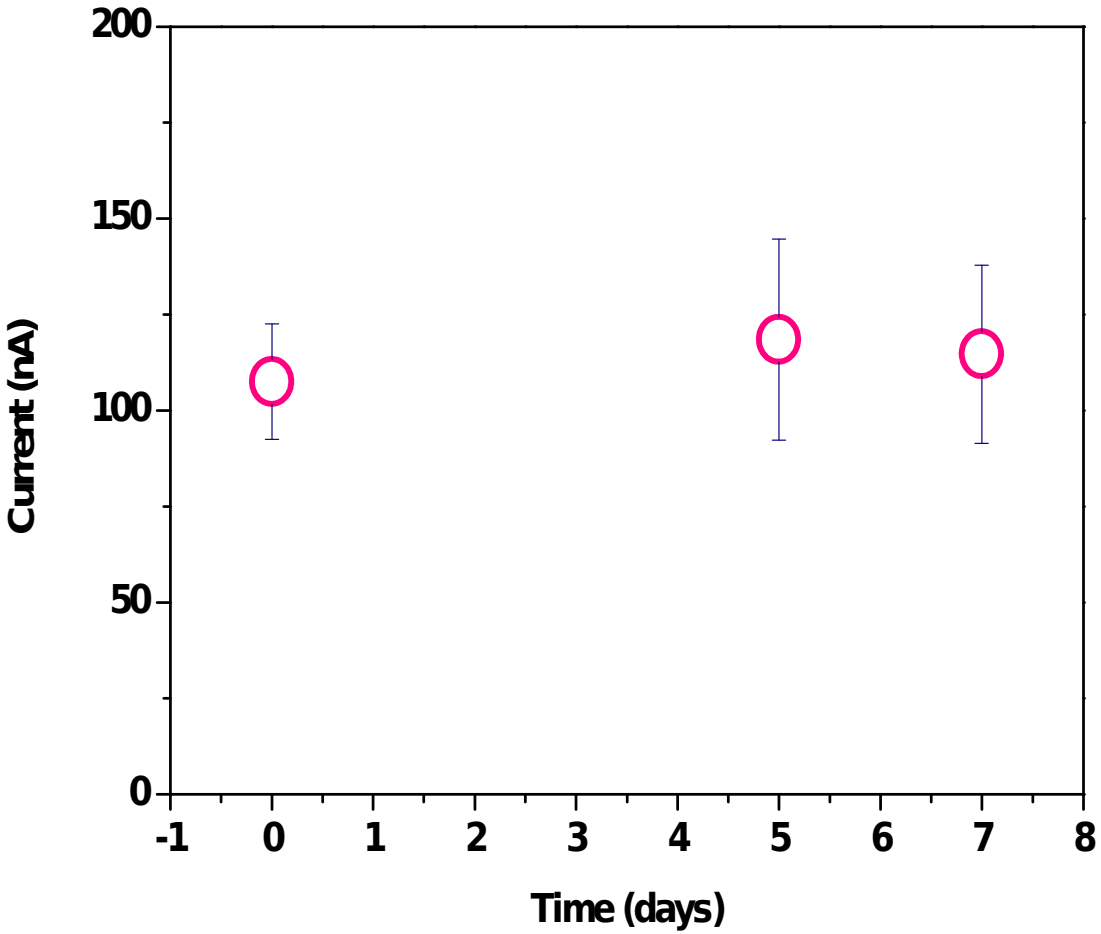



Potential = - 0.160 V
Interval = 0.5 s
Reaction volume = 100 µL
Buffer = 0.1 M citric acid/sodium citrate
pH 4.15
Catechol 50 – 75- 100 µM

# WP1

## Storage stability

## of laccase enzyme in the presence of catechol deposed by ESI on carbon black modified screen-printed electrode

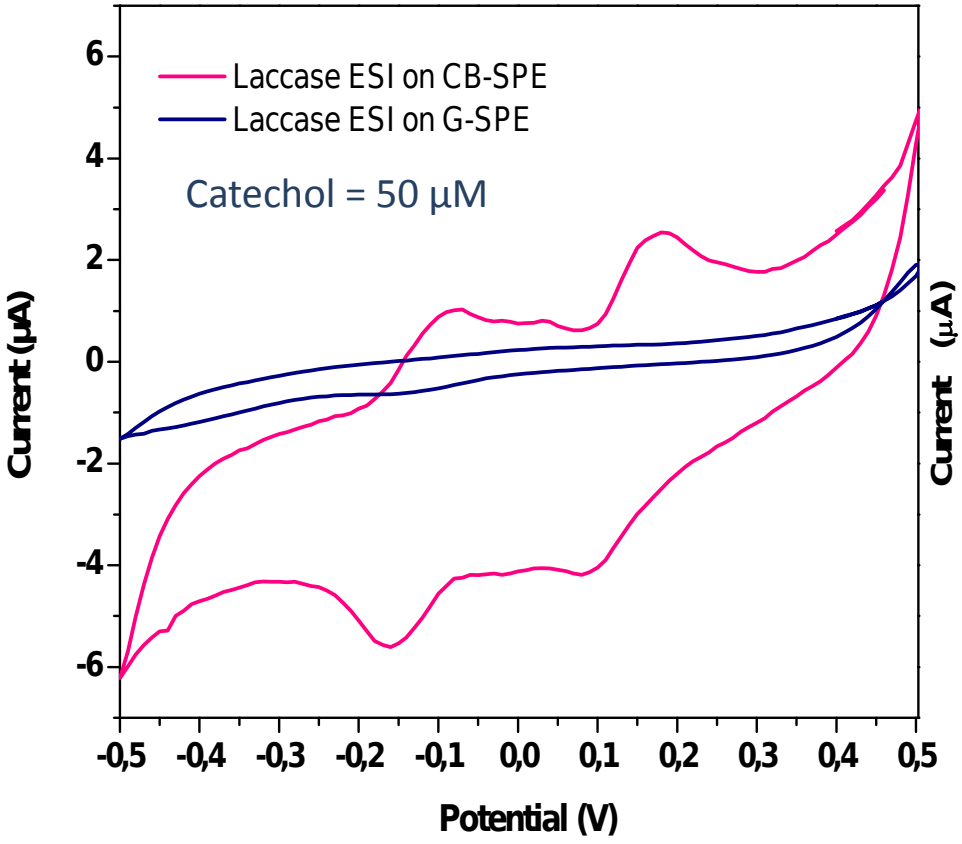


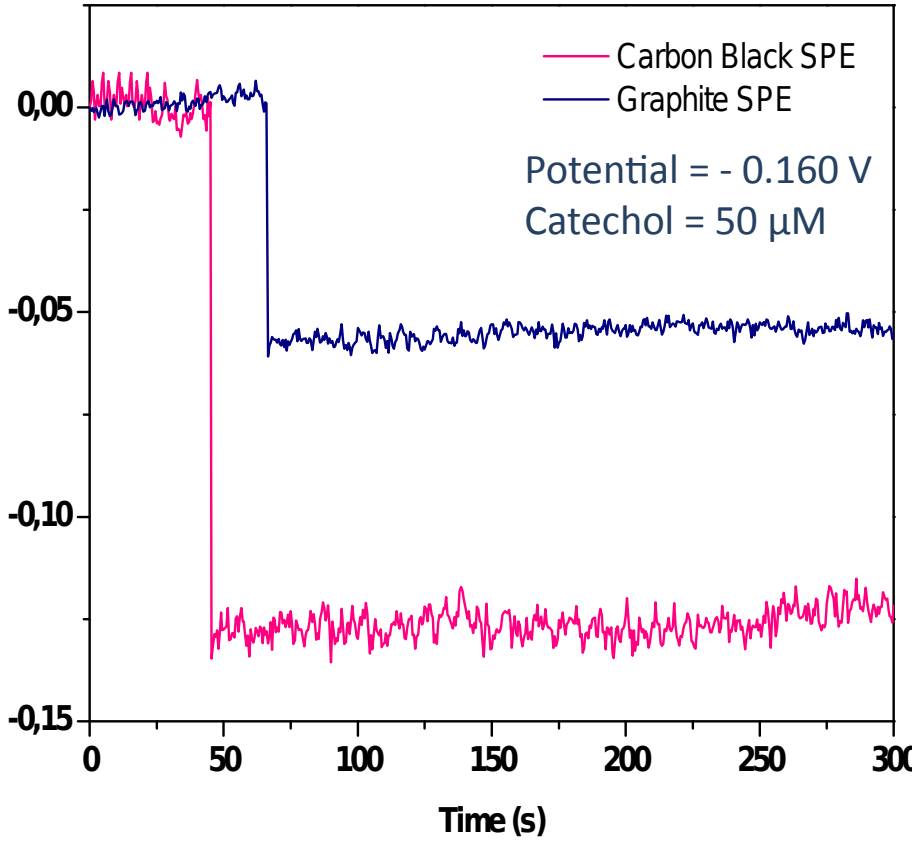

Potential = - 0.160 V
Interval = 0.5 s
Reaction volume = 100 µL
Catechol = 50 µM
Buffer = 0.1 M citric acid/sodium citrate pH 4.15

# WP1

## Carbon black advantages

## Comparison between laccase enzyme deposed by ESI on graphite and carbon black modified screen-printed electrode



# Further perspectives

*Analytical study*

- Intra-electrode repeatability
- Interference studies (e.g. copper, arsenic, phenolic compounds, ascorbic acid)
- Matrix effects (surface water)
- Recovery studies

*Morphological study*

- SEM
- Raman

*……ideas?*

*Paper writing is in progress!*