An innovative electrochemical sensor for thiols’ detection: a device for the measurement of OP (Oxidative Potential) of PM (Particulate Matter)

Maria Pia Romano¹*, Maria Elena Giordano², Roberto Caricato², Anna Rita De Bartolomeo², Daniele Contini³, Maria Giulia Lionetto², Maria Rachele Guascito²

1) Dipartimento di Matematica e Fisica, Università del Salento, 73100 Lecce, Italy
2) Dipartimento di Scienze e Tecnologie Biologiche e Ambientali, Università del Salento, 73100 Lecce, Italy
3) Istituto di Scienze dell’Atmosfera e del Clima, ISAC-CNR, 73100 Lecce, Italy
A new kind of electrochemical sensors (amperometric/potentiometric) operating both in batch and in flow.

The development of chemically modified electrodes, devices for the measurement of OP of PM as an alternative to the classic spectrophotometric methods, and their validation with chemical and biological assays.

Challenging task!

Reliable tools are needed for estimating the oxidant generating capacity of PM at high temporal resolution (minutes to hours) [1].

The preliminary study was carried out on a PM water-soluble fraction collected on quartz filters, as part of a greater sampling campaign performed by the Daniele Contini’s Team, ISAC-CNR Lecce. There is a long-standing partnership with DiSTeBA, Unisalento.
Aim of the study

Particulate matter (PM) air pollution has a significant impact on human morbidity and mortality!

Focus on the PM toxicity and its oxidative potential

The mechanisms by which PM impacts human health are unresolved, but evidence suggests that PM intake leads to cellular oxidative stress through the generation of reactive oxygen species (ROS). [2]

Airborne particulate matter (PM) is a prime candidate for the generation of biological oxidative stress.

Airborne PM induces harm by generating ROS in human tissues, leading to oxidative stress. [3]

Air pollution has been associated with harmful effects on human health. Small particles can penetrate deep into the lungs (the first sites of exposure to PM) and cause severe problems: DNA damage and mutations. [4]

Cytotoxicity analysis

The biological model used was the **A549 cells:** a cell line derived from human alveolar cell carcinoma of lung cancer with both characteristics of malignant tumor cells and alveolar type II cells. They are widely used in the research. [5]

The cytotoxicity and the induction of oxidative stress by the particulate have been evaluated respectively by the **MTT assay** and the use of the fluorescent probe sensitive to reactive oxygen species **CM-H$_2$DCFDA.**

The MTT assay (cell vitality test)

It measures the activity of the enzymes that catalyze the reduction of MTT bromide of 3- (MT-4,5-dimethylthiazol-2-yl)- diphenyltetrazolium, usually a yellowish salt, in formazan, which has a purple colour.

MTT A549

The MTT shows an increase in cell mortality after 24h of exposure to the PM$_{10}$ aqueous extracts.

Seven samples showed slight mortality, below 20%; sample n. 6 showed a mortality of about 20%. The particularly interesting data is that for filter N°8 (A1518Mf, dated December 8, 2017) which shows a cellular mortality of about 60%.
The **CM-H₂DCFDA probe** (chloromethyl derivative of H₂DCFDA)

It passively diffuses into cells, where its acetate groups are cleaved by intracellular esterases and its thiol-reactive CM reacts with intracellular glutathione and other thiols. Subsequent oxidation yields a fluorescent adduct that is trapped inside the cell, thus facilitating long-term studies.

**Cytotoxic effect of PM₁₀**

**Endogenous ROS production**

**A549 ROS probe PM₁₀**

Cytotoxic effect of PM₁₀ which results in an increase in endogenous production of reactive oxygen species in cells exposed to the extracts. In particular, the sample n. 8 also shows the highest % variation in the probe fluorescence intensity, suggesting a great production of ROS.
Correlation analysis and dose-dependance

Correlation analysis between the MTT test results (expressed as mortality) and the results obtained on the same samples with the CM-H₂DCFDA fluorescence. The higher the intracellular oxidative stress, the higher the mortality observed.

A dose-dependence of the fluorescence of A549 cells charged with the probe is observed, with the increasing concentration of PM. The higher the PM concentration, the greater the endogenous stress induction on A549 cells.

A549 ROS Dose-Response

\[ y = 1.4798x - 17.746 \]

\[ R^2 = 0.9124 \]
The electrochemical sensor

The operating sensor’s principle is the determination of the PO, through the measurement of the loss in dithiothreitol DTT content.

**Electrochemical sensor device for DTT detection (pH 7)**

**GC, Au, Pt** electrodes bare: to high potential! All these types of electrodes show their electroactivity, but tend to poison themselves by working at constant potential.

The electrochemical reduction of the nitro groups of 5,5dithiobis(2-nitrobenzoic acid) DTNB on GC: high sensitivity towards thiols but a very complex electrochemistry. [6]

The cobalt phthalocyanine **CoPC**-modified carbon pasta electrodes: selective detection of thiol compounds. Widely used! (R.P. Baldwin, since the 80s)

The GC and Au electrodes behaviour

Our lab tests confirmed the very high potentials of GC and Au: they cause problems. The DTT oxidation overlaps with the solvent discharge, moreover other substances different from DTT can generate some interference.

Calibration DTT different dosage Cfin 500µM

All the voltammetric and amperometric experiments have been performed in a phosphate buffer solution (pH 7.4).
The electrode preparation

GC and Au own a certain electroactivity toward DTT, now we are looking for a synergistic response  

The idea of GC/Au electrode!

The sensor is a **Glassy Carbon electrode modified by electrochemical deposition with Gold nanoparticles (GC/Au)**, useful for the dithiothreitol (DTT) detection, in order to evaluate the OP.

- Modified electrodes were prepared by **direct drop casting** of the AuCl$_3$ solution (15µL) 0.137 mM on a bare Glassy Carbon electrode.
- The Au electrodeposition had been performed by **chronoamperometry** at the applied potential of -0.9V vs Ag/AgCl for t=200s.
- **Cyclic Voltammetry** between -1.0V and 0V and then between 0V and 1.3V was performed until the steady state (20 cycles).

![Graph](image)

Q=0.178 mC/m²
The DTT detection was studied through the cyclic voltammetry and the chronoamperometric detection in the spectrophotometric concentration range (800 nM-1 mM).

Calibration DTT different dosage Cfin 857µM
Amperometric Detection with magnetic stirrer at 0.6V was performed in buffer solution on different modified electrodes. Reactive to DTT but no interference with PM aqueous extracts

Calibration curve: I(µA) function of DTT concentration (0.75-513.03 µM)
The flow rate of 1,5mL/min had been considered the best compromise to obtain more reproducible signals in a short time. After many tests, 600mV has been selected as the potential value to be applied for PAD. (E1 0,8V for t1 0,01s; E2 -1V for t2 0,02s; Ei 0,6V for t 0,5s).
Flow Injection Analysis-FIA

FIA peaks for triplicate injections of DTT and relevant calibration curve. Carrier: phosphate buffer solution (pH = 7,4); flow rate: 1,5mL min$^{-1}$; applied potential: 0.6V. DTT concentration: 10-20-30-40-50-75-100-150-200-300-400-500µM. The sensor shows a highly reproducible response as demonstrated by the very small variation of current peaks in three replicates.
Conclusions

- **Biological studies:** The MTT assay shows an increase in cell mortality after 24h of exposure to the PM aqueous extracts. The CM-CM-H₂DCFDA shows the intracellular oxidative stress induction by PM which, in turn, results in an increased citotoxicity.

- **Electrochemical studies:** The sensor operates well: it is reactive towards the DTT and shows low LOD, an high sensitivity and selectivity. The aqueous extracts of PM don't interfere with its detection.

- The electrochemical properties of the sensor are considerably influenced by the Gold nanoparticles, optimized to yield a stable and reproducible response.

- In the trend of sensitive detection, the electrochemical sensor for PM oxidative potential promote the simplicity and the highly sensitivity, with a minimal equipment.

Work in progress the miniaturization of the sensor and its technological integration for the prototype, have been developing since September 2020.
Thanks for the attention!