

DEVELOPMENT AND CHARACTERIZATION OF A GOLD NANOPARTICLES GLASSY CARBON MODIFIED ELECTRODE FOR DITHIOTREITOL (DTT) DETECTION SUITABLE TO BE APPLIED FOR DETERMINATION OF ATMOSPHERIC PARTICULATE OXIDATIVE POTENTIAL

Maria Pia Romano¹, Maria Giulia Lionetto², Annarosa Mangone³, Anna Rita De Bartolomeo², Maria Elena Giordano², Daniele Contini⁴, Maria Rachele Guascito^{2*}.

***Corresponding Author:** maria.rachele.guascito@unisalento.it

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

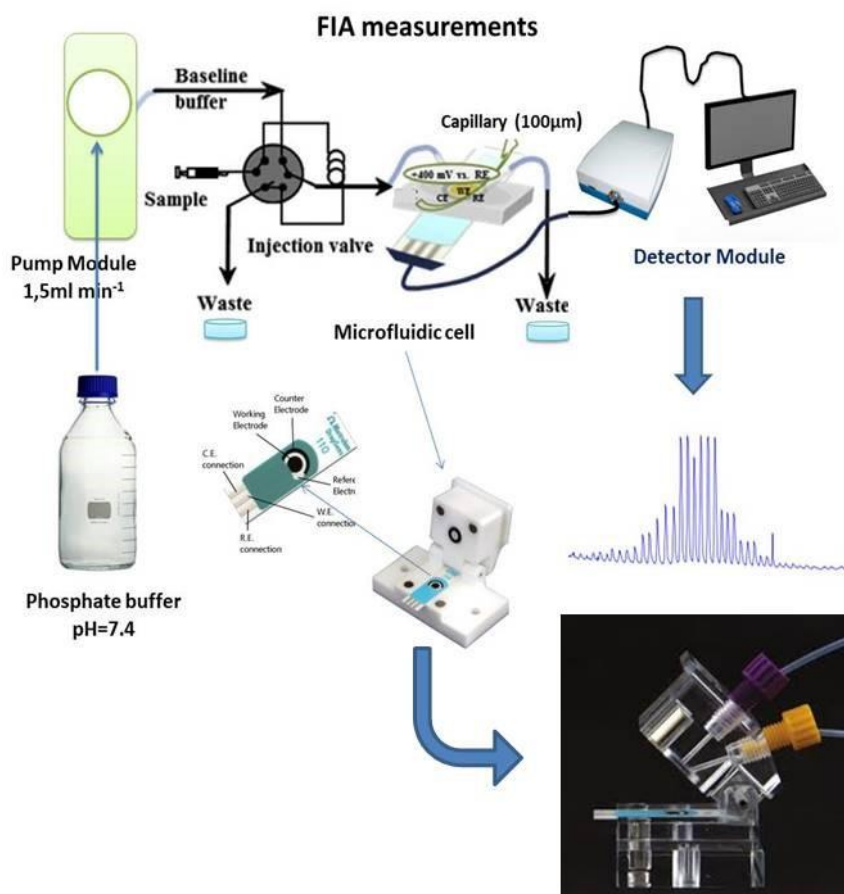
³ Dipartimento di Chimica, Università degli Studi di Bari Aldo Moro 70124 Bari, Italy

⁴ Istituto di Scienze dell'Atmosfera e del Clima, ISAC-CNR, 73100 Lecce, Italy

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1. FIA Apparatus

Concerning the flow experiments, FIA measurements have been performed by an Agilent 1260 Infinity Isocratic Pump (G1310B, Germany), flow rate 1.5 mLmin⁻¹. A six-port low pressure injection valve unit has been used (injection volume 65µL). The wall-jet Flow-Cell for screen printed electrode was a DropSens Flow-Cell (FLWCL) with an innovative open-close system (no screws needed) and an easy sensors replacement. Dimensions: 3.3 cm x 6.0 cm x 3.3 cm (Length x Width x Height). All the FIA experiments have been performed on the Dropsens DRP110 (GC) screen printed electrodes. The GC working electrode (WE area 0.1256 cm²) modified with AuNPs (SPE_GC/AuNPs) is surrounded by a silver quasi-reference electrode (Ag/AgCl), while the counter electrode (made up of GC) was the outermost ring of the device. Electric contacts are made of silver. These measurements were performed by using phosphate buffer in N₂-atmosphere. The use of N₂-atmosphere conditions could represent a problem for in field measurements; however, considering the very low flow rate needed, this problem can be easily overcome by the use of a small portable N₂ cylinder, like those generally included to generate carrier gas flow in portable field GC systems usually used in environmental monitoring.



Scheme S1: Schematic representation of FIA measurements with the DropSens Flow-Cell (FLWCL) image details.

2. PM sampling and filter extraction

The study was carried out on PM₁₀ samples collected at the Environmental-Climate Observatory of ISAC-CNR in Lecce (Southern Italy), regional station of the Global AtmosphereWatch (GAW-WMO) and ACTRIS networks, characterized as an urban background site. The observatory (40°08'N—18°07'28"E, 37 m asl) is located inside the University Campus. It could be classified as an urban background station, potentially influenced by local emissions sources (traffic and domestic heating), by short and medium-range transport from the town of Lecce and small villages located around the Campus. These samples characterized by the reported mass concentrations (mass for sampled air volume) are summarized in Table S1. A quarter of any collected filter PM₁₀ sample have been extracted with 15 mL of potassium phosphate buffer using ultra-sonication at room temperature for 30 minutes, and filtered through polypropylene syringe filters (PTFE syringe filter 25mm 0.45µm, Scharlau) to remove insoluble components (experimental details are reported in a previous work, [1]). Three of these aqueous PM₁₀ extracts (3.5 mL) have been used to make

electrochemical interference studies and six to assess the DTT rate consumption by FIA electroanalytical method. Results were compared with DTT rate consumption measured in parallel on the same samples by the spectrophotometric method to be validated [2].

	PM (μgm^{-3})	Sampling	Test
Filter 1	25.4 $\pm$ 0.3	18th July 2013	Interference
Filter 2	31.1 $\pm$ 0.5	22nd July 2013	Interference
Filter 3	28.4 $\pm$ 0.4	25th July 2013	Interference
Filter 4	24.6 $\pm$ 0.3	28th July 2013	PAD/Uv-vis
Filter 5	19.2 $\pm$ 0.3	28th July 2013	PAD/Uv-vis
Filter 6	14.6 $\pm$ 0.4	31th July 2013	PAD/Uv-vis
Filter 7	22.6 $\pm$ 0.3	3th August 2013	PAD/Uv-vis
Filter 8	16.3 $\pm$ 0.3	21st August 2013	PAD/Uv-vis
Filter 9	11.1 $\pm$ 0.4	1st July 2020	PAD/Uv-vis
Filter 10	blank	-----	PAD/Uv-vis
Buffer	phosphate	-----	PAD/Uv-vis

Table S1. Sampled filters and related measured concentrations in PM₁₀.

3. Spectrophotometric DTT assay

In the DTT assay [3], the samples of known PM masses were incubated at 37 °C with DTT (100 μM) in 0.1 M potassium phosphate buffer at pH 7.4 (5 mL total volume) for times varying from 5 to 90 min. At designated times (specifically at 5, 10, 15, 20, 30, 45, 60, and 90 min) a 0.5 mL aliquot of incubation mixture was picked up and 0.5 mL of 10% trichloroacetic acid was added to stop the reaction. Then, this reaction mixture was mixed with 2 mL of 0.4M Tris–HCl, pH 8.9 containing 20 mM EDTA, and 25 μL of 10 mM DTNB. The concentration of the formed 5-mercapto-2-nitrobenzoic acid was measured by its optical density absorption at 412 nm using a Cary 50 UV–Vis spectrophotometer (Varian Inc.). The rate of DTT consumption (δ_{DTT} , pmol/min) was determined from the slope (δ_{ABS}) and intercept (ABS_0) of linear regression of measured absorbance versus time as $\delta_{\text{DTT-UV-vis}} = \delta_{\text{ABS}}(\text{N}_0/\text{ABS}_0)$, where N_0 are the initial moles of DTT.

4. Electrochemical DTT assay in FIA

In the electrochemical assay, samples were picked up, in parallel with spectrophotometric experiments from the same reaction bath, at the same reaction times, and immediately injected in triplicate (sample loop 65 μL) for FIA analysis. The rate of DTT consumption (δ_{DTT} , pmol/min) was

now determined from the slope (δ_I) and intercept (I_0) of linear regression of measured peak currents versus time as $\delta_{\text{DTT-Electrochemical}} = \delta_I(N_0/I_0)$, where N_0 are the initial moles of DTT. Typically, a linear dependence was observed until the DTT concentration is reduced by no more 40%-50% as also observed in DTT spectrophotometric assay. Before PM real sample analysis, the sensors were validated by calibrating the electroanalytical sensor in PQN standard solutions (0.025 and 0.050 μM) used as a PM model oxidant of DTT. In all successive analysis PQN has been used as positive control [4].

In both assay typologies, the final DTT activity has been normalized in terms of collected aerosol mass (M) as $\text{OP}_{\text{DTT-M}} = (\delta_{\text{DTT}} - \delta_{\text{blank}})/M$, where δ_{blank} is the activity recorded in representative blank samples [1]. When PQN is used the $\text{OP}_{\text{DTT-M}}$ has been normalized by PQN mass. Replicates were performed to evaluate uncertainty on OP_{DTT} that was, on average, about 10% in both methods [5].

5. Au nanoparticles modified electrodes: electrosynthesis

After the drop casting, the Au electrodeposition had been conducted by chronoamperometry at the applied potential of -900 mV. The charge (Q), typically of $2200 \pm 100 \mu\text{Ccm}^{-2}$, is associated with both cathodic processes related to the reduction of Au^{3+} and H^+ to form respectively Au and H_2 , in accord to CV experiments as recorded on bare Au (see Figure S3 a). The choice to use this high cathode potential has two effects: 1) it reduces the number of active sites available for the nucleation of gold on GC, as they compete with the development of H_2 gas; 2) the development of the H_2 gas, randomly distributed on the GC surface, should not promote the formation of a homogenous 2D gold film.

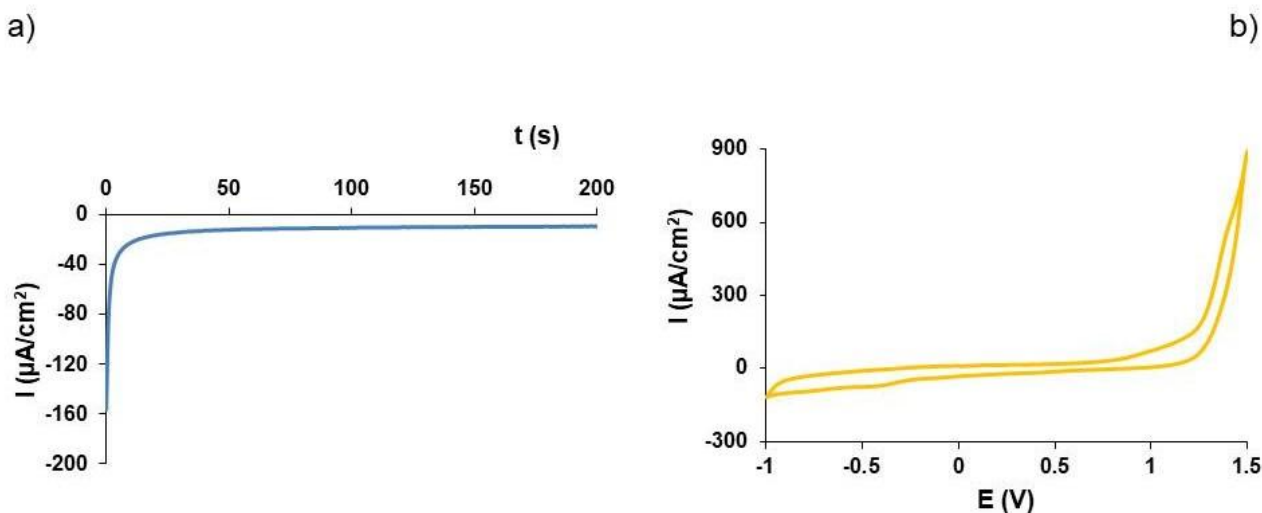


Figure S1. (a) Electrodeposition of gold by chrono-amperometry at -900 mV vs Ag/AgCl, deposition time $t=200\text{s}$, $Q= 2200 \pm 100 \mu\text{Ccm}^{-2}$; (b). CV in clean phosphate buffer solution (pH 7.4) between -1000 mV and 1500 mV of the GC gold modified electrode (yellow curve).

6. Glassy carbon plate XPS analysis

The typical example of fit of the C1s region of GC is shown in Figure S2. According to previously reported data, as obtained on electrochemical pre-treated GC electrodes, the best fit was achieved by using five components to take into account the asymmetric shape of the C1s peak signals at high binding energy [6]. The most intense peak component, at $284.5 \pm 0.1 \text{ eV}$, has been attributed to graphitic carbon. Moreover, peaks at $285.2 \pm 0.1 \text{ eV}$, $286.2 \pm 0.1 \text{ eV}$, $287.7 \pm 0.1 \text{ eV}$ and $288.9 \pm 0.1 \text{ eV}$ have been attributed to aliphatic (C-H), alcoholic (C-OH), carbonyl (C=O) and carboxylic (COOH) carbon, respectively [6]. The peak attributed to aliphatic carbon can represent also the contamination of the electrode surface (i.e. adsorbed hydrocarbon monolayers).

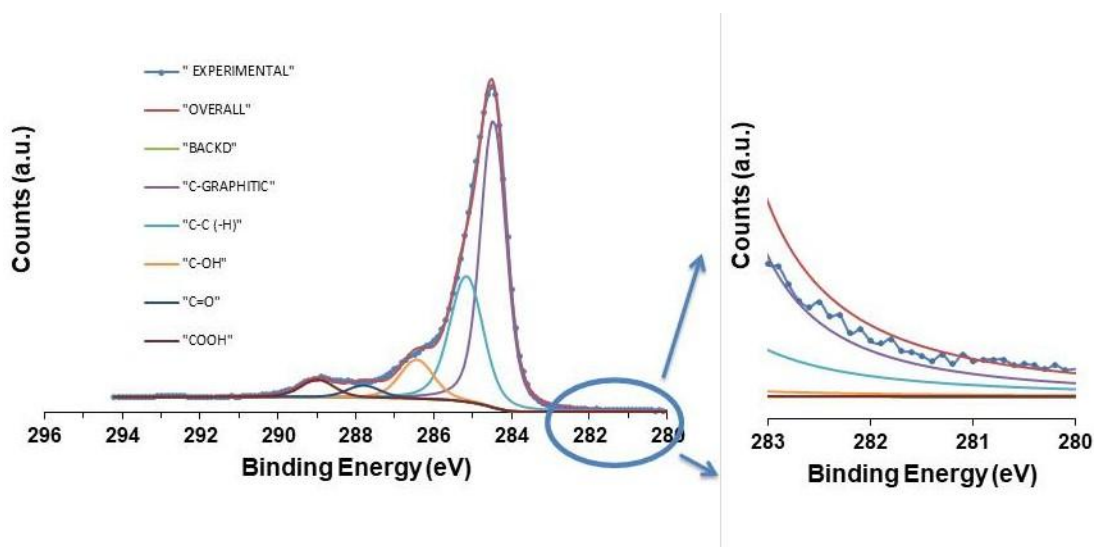


Figure S2. XPS analysis: the typical example of fit of the C1s region of GC slide.

7. The electrochemical characterization of GC/AuNPs

The electrochemical characterization of the GC/AuNPs modified electrodes was done by CV to compare the bare GC and Au electrodes (Figure S3 (a) and (b)). As expected, no electrodic processes can be observed on the GC bare electrodes between -0.400 V and +1300 mV. Otherwise, the gold electrodes base line is characterized from the typical shape that involves the gold oxidation and the reduction processes in the potential ranges between -800 mV and +1200 mV, while the water

oxidation and the hydrogen reduction start at +1300 and -900 mV, respectively. GC/AuNPs CV shape is quite different from both bulk materials. In detail, no marked red-ox processes involving gold can be observed, in the steady-state conditions. Particularly the absence of peaks related to the reduction of Au oxidized species at +440 mV, in the reverse scan, confirms that in the direct anodic scan the Au nanoparticles does not give a detectable surface oxidation process as observed on Au bulk electrode (Figure S3, b). Accordingly, even if the average electroactive surface area is reduced compared to the one of the original bare GC, an enhanced irreversible electro-catalytic activity towards water oxidation (O_2 evolution) is observed. The on-set starts at +810 mV vs +1250 mV on bare gold and vs +1400 V on bare GC, respectively.

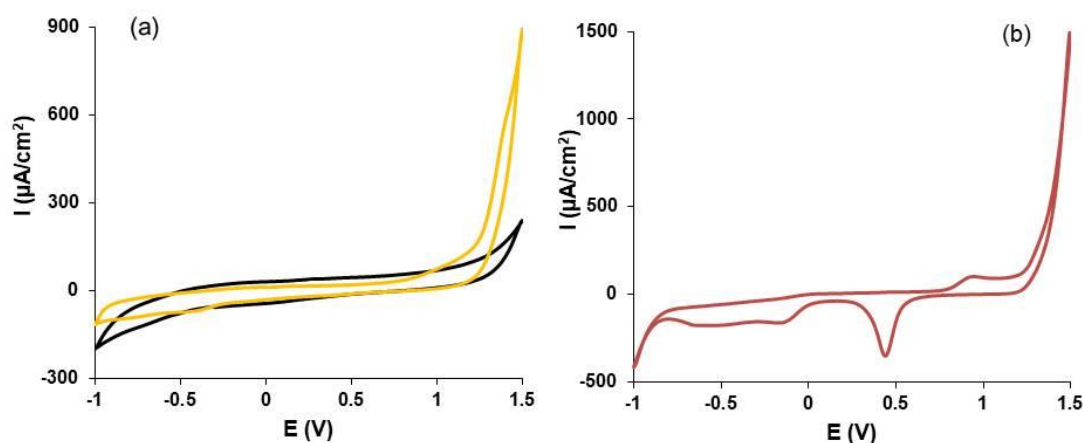


Figure S3. (a) CV in phosphate buffer solution (pH 7.4) between -1000 mV and 1500 mV for the electrodes: GC (black trace), GC/AuNPs (yellow trace) and (b) Au (red trace).

8. Chrono-amperometric DTT detection on GC/AuNPs modified electrode in batch analysis

Concerning the chrono-amperometric detection, the potentiodynamic measures at DTT concentration of 200 μ M in batch condition at different applied potentials between +200 mV and +800 mV were

performed (Figure S4). The comparison with bare gold confirmed that, in this potential range, only a small parallel Au oxide formation could interfere with the DTT oxidation signal. DTT detection at higher oxidative potentials has been avoided to exclude the oxidation of possible PM extract chemical components that could interfere with DTT oxidation in environmental samples analysis.

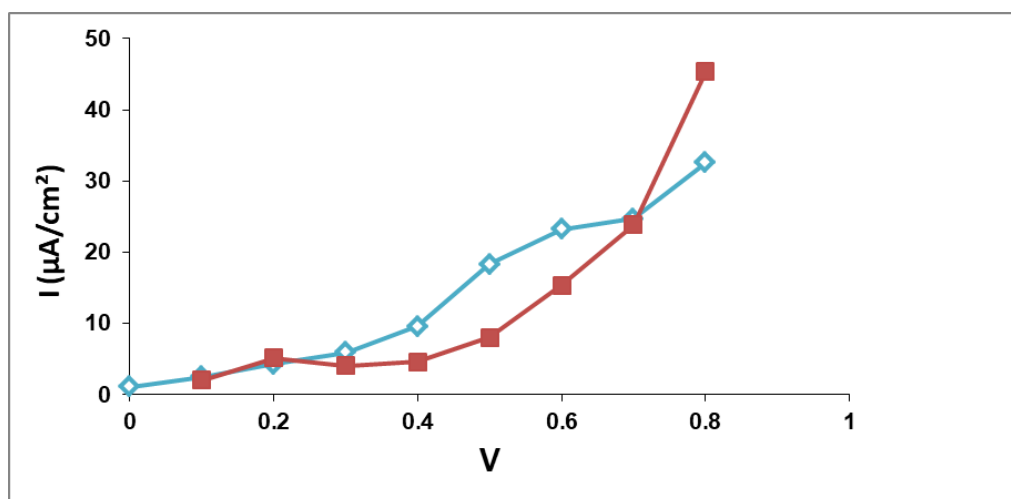


Figura S4. Amperometric responses of GC/AuNPs (blue curve), and Au (red curve) electrodes, towards DTT 200 μM in stirred and nitrogen saturated phosphate buffer (pH 7.4, $I=0.2$) at different potential values between 0.000 mV and +800 mV.

9. The Gold's amount influence on the sensor

The sensor performance is affected by the amounts of AuCl_3 (137 μM) aqueous solution casted on the electrodes to prepare the sensors. Electrodes prepared with 5 μL and 25 μL , respectively, were tested to evaluate the amperometric response at different DTT concentrations, at applied potential of +600 mV. It has to be highlighted that the sensor obtained with 25 μL showed a not reproducible response. A rapid fouling is observed (not shown). Otherwise, when 5 μL were employed the electrodes showed the same response in terms of dynamic range (from 0.750 μM to 1175 μM) as for sensors obtained by casting 15 μM , but were less performing in terms of sensitivity. The LOD was the same (750 nM) for both sensors. Amperometric responses of GC/AuNPs (15 μL) and GC/AuNPs (5 μL), towards DTT (0.75-1075 μM) in stirred and nitrogen saturated phosphate buffer (pH 7.4, $I=0.2$) are reported for comparison in figure S5 (a) and (b). The limit of DTT detection (LOD at a signal-to-noise ratio of 3) was measured being equal to 750 nM, a value that was about three times smaller than the reported in [7] [8].

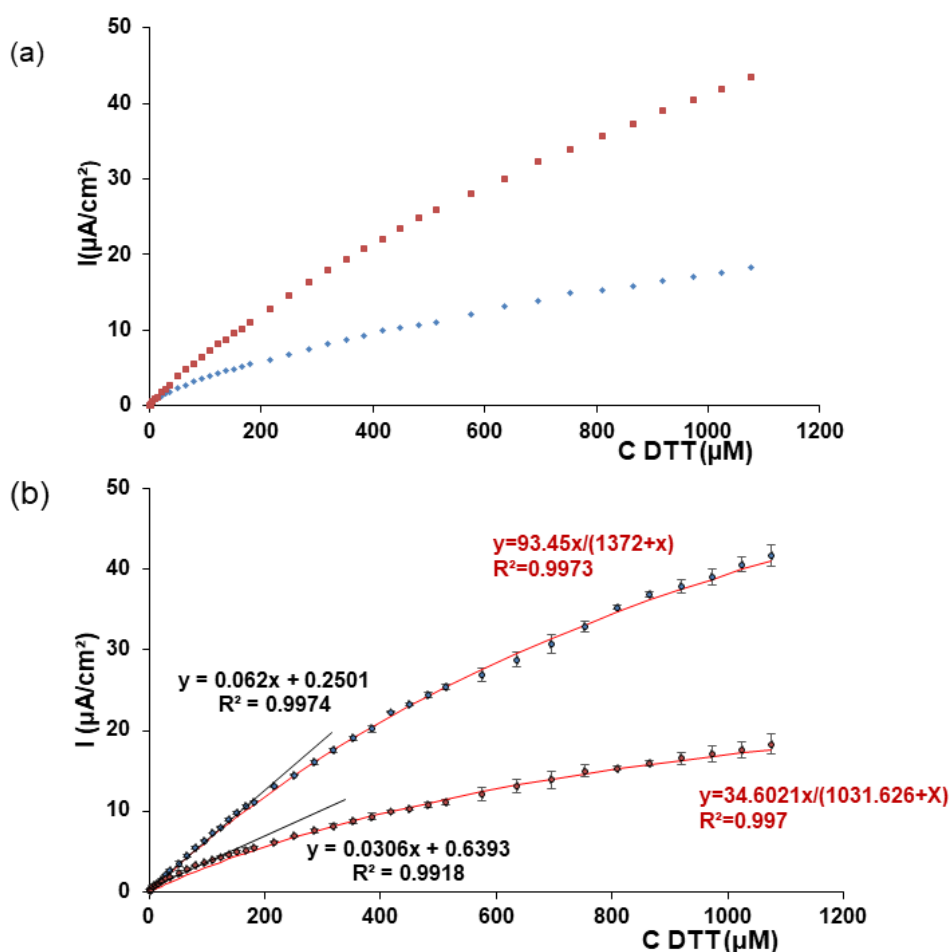


Figure S5. (a) Calibration curves related to amperometric responses of GC/AuNPs (red curve, AuCl_3 $15 \mu\text{M}$) and GC/AuNPs (blue curve, AuCl_3 $5 \mu\text{L}$) electrodes, towards DTT 0.750 - $1175 \mu\text{M}$ concentration range at $+600 \text{ mV}$. (b) Typical fits of $I_{\text{GC}/\text{AuNPs}}$ vs DTT calibration curves based on Langmuir isotherm theory (red curves). The linear fit (black curves) applies at low DTT concentrations range (0.750 - $200 \mu\text{M}$) for both sensors. Error bars represent one standard deviation about the mean ($n=3$).

10. The PM aqueous extracts potential interference in CA DTT detection.

The chrono-amperometric measurements were done to evaluate PM extract potential interferences in DTT detection. As reported in Figure S6, after a constant base line is reached in current in phosphate buffer (3 mL , $\text{pH } 7.4$, $I=0.2$), no significant amperometric response change is observed when an excess of PM aqueous extract was added (three consecutive additions of PM aqueous extract were performed). A clear response is obtained after the DTT addition, $320 \mu\text{M}$. The experiment has been repeated with two different aqueous PM extracts (filter 1 and filter 2, respectively red and green traces). Substantially, there is no significant reaction towards aqueous extract, while the current step

increment due to the DTT addition is clearly observable for both the extracts. All measurements were done as described in the experimental section.

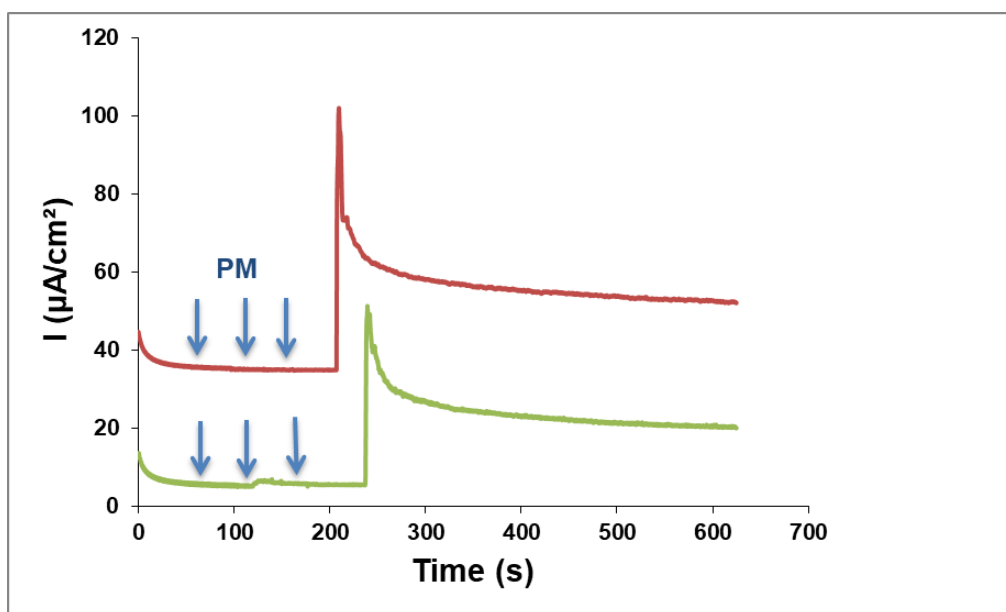


Figure S6. Amperometric responses of GC/AuNPs towards two different PM aqueous extracts (three consecutive additions of 2 mL of PM aqueous extract into a 3 mL of phosphate buffer) and then DTT 320 μ M. Filter 1 and Filter 2, respectively red and green. Measurements were performed at +600 mV.

11. Stability and PM aqueous extracts potential interferences in FIA DTT detection.

The amperometric response of the sensor to 72 successive injections of DTT in phosphate buffer solution was recorded to test the response stability. The response is reported in figure S7. A stable signal can be obtained after 15 min and kept for almost 80 min. It is evident that the stability of the signal is reached after almost 15-20 min, corresponding to a good well stabilized baseline. After, a stable signal of 2.8 μ Acm⁻² is observed for a period of time of approximatively 50 min (RSD=0.1, n=21). This stable response alongside 50 min is compatible with the measurement time necessary to study the kinetic of DTT oxidation (DTT consumption) in aqueous PM extracts due to their oxidative potential, being the required analysis time typically oscillating between 15-20 min to a maximum of 90 min [1]. It is also important to underline that these sensors are easily regenerated by activation in phosphate buffer with cyclic voltammetry (10 CVs), between -1000 and 1300 mV, thus being able to reuse them for at least 10 times or more. Tests to assess the response of the PM aqueous extracts have been done to evaluate the interference with DTT signals in three different FIA experiments using three PM samples (Filter 1, Filter 2 and filter 3 in Figure S8). In details, after 5/6 successive injection

of 200 μM DTT solutions (65 μL), an equal volume of PM aqueous extract was also injected for 5/6 successive times. In all experiments, the signals associated to PM extracts are negligible, particularly for extract filter 2. Furthermore, DTT signals intensity, in successive injections, seems unchanged confirming that the PM extract compositions don't affect negatively the response of the modified electrodes.

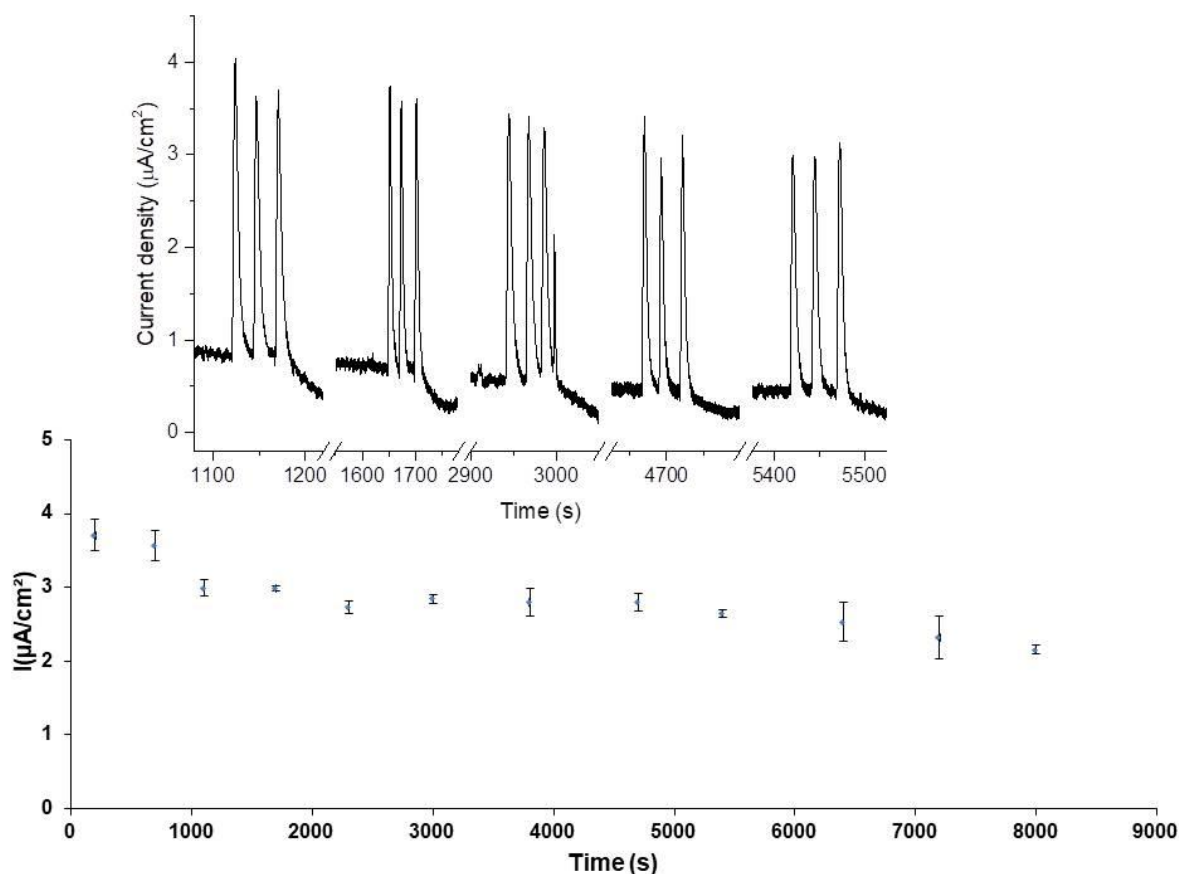


Figure S7. Evaluation of sensor stability by subsequent DTT 100 μM injection in PAD experiments in FIA analysis. (a) Peaks recorded for three successive injections of DTT repeated for 2 hours and 20 minutes. Carrier: phosphate buffer solution ($\text{pH} = 7.4$, $I=0.2$); flow rate: 1.5mL min^{-1} .

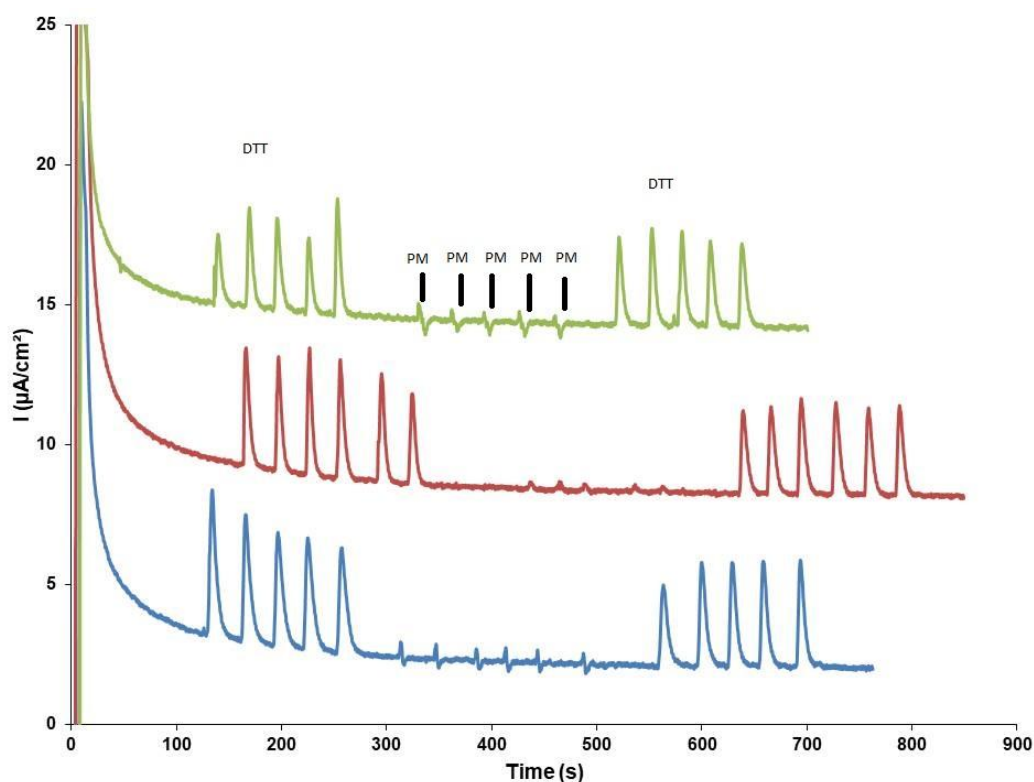


Figure S8. The response to the PM aqueous extracts to evaluate the interference with DTT signals. Three different FIA experiments have been performed using three PM samples: Filter 1-green trace, Filter 2-red trace and Filter 3- blue trace.

12. FIA analysis of DTT rate consumption in PM aqueous extracts.

Before analysing real samples, to test the method in DTT rate consumption, the electrodes were used to measure the activity of DTT in presence of PQN at known concentrations (i.e. 0.025 μM and 0.050 μM) as a positive control [9]. The linear plot of the DTT (100 μM) consumption rate current versus reaction time reported in Figure S9 (PQN concentration 0.025 μM) shows a slope of $-0.15(\pm 0.02)$ $\mu\text{A}/(\text{cm}^2 \cdot \text{min})$ and R^2 of 0.970.

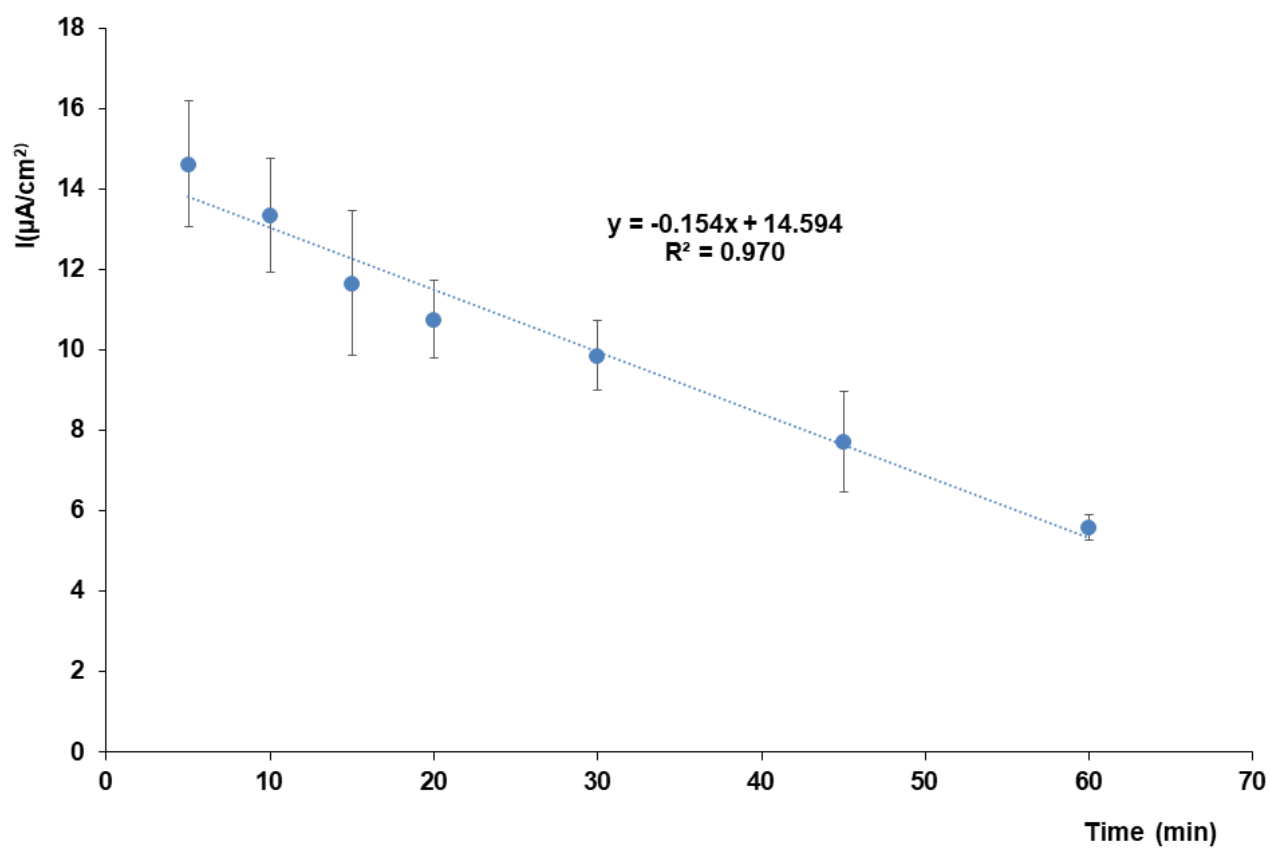


Figure S9. Peak currents related to DTT electrochemical detection in FIA analysis at reaction times of 5, 10, 15, 20, 30, 45 and 60 min, in PQN 0.025 μM . The linear regression is $y = -0.15(\pm 0.02)x + 14.6(\pm 0.5)$. Error bars represent the standard deviation about the mean ($n=3$).

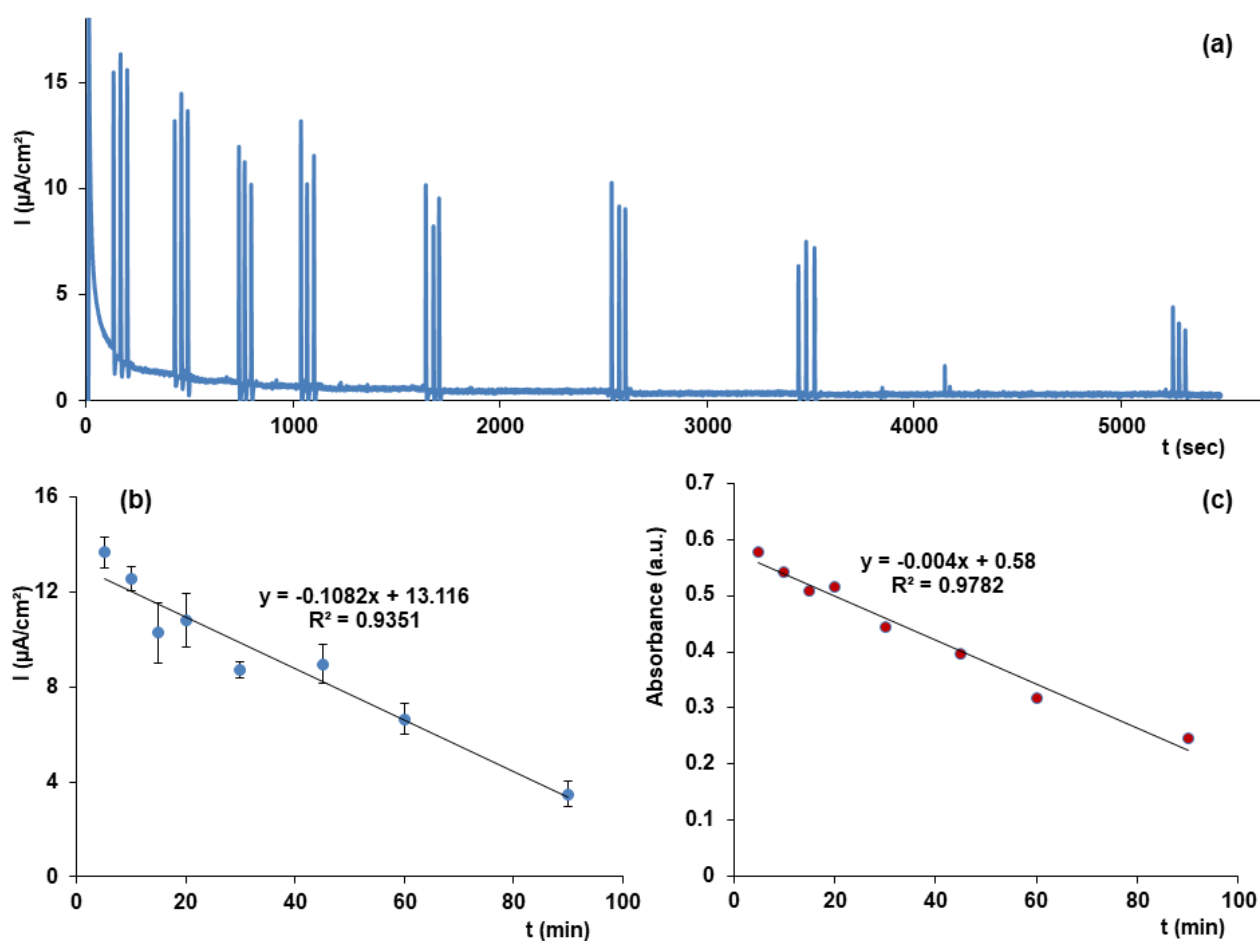


Figure S10. (a) Peak currents related to DTT ($100\ \mu\text{M}$) electrochemical detection in FIA analysis at reaction times of 5, 10, 15, 2, 30, 45 and 60 min, in in PM aqueous extract (Filter 9). (b) The related linear regression is $y(\pm 1.102) = -0.108(\pm 0.008)x + 13.17(\pm 0.36)$. Error bars represent the standard deviation about the mean ($n=3$). (c) DTT absorbance decrement versus time at reaction times of 5, 10, 15, 2, 30, 45 and 60 min obtained in parallel on the same extract used in electrochemical detection. The linear regression is $y(\pm 0.02) = -0.0040(\pm 0.0002)x + 0.58(\pm 0.02)$.

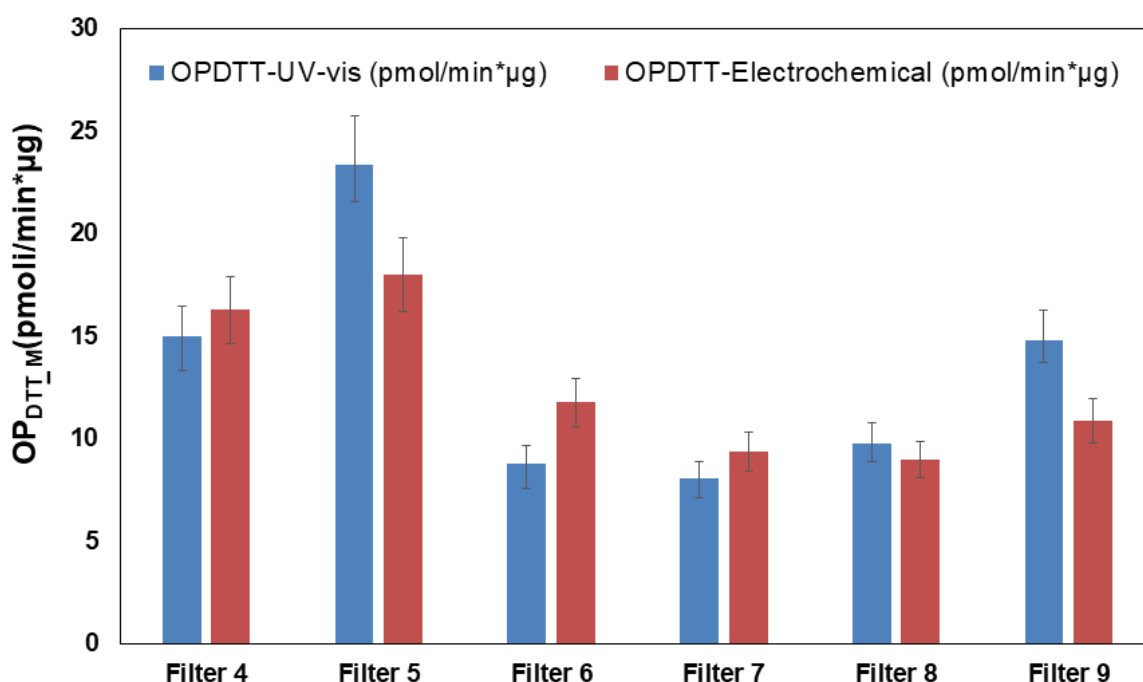


Figure S11. Comparison of OP_{DTT-M} measured in six different PM aqueous extracts samples with spectrophotometric and electrochemical FIA methods. Error bars represent the standard deviation about the mean ($n=3$).

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