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Electrospray deposition as a smart technique for laccase immobilisation on carbon black-nanomodified screen-printed electrodes

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Abstract

Enzymes immobilization represents a critical issue in the design of biosensors to achieve standardization as well as suitable analytical performances in terms of sensitivity, selectivity, and stability. In this work electrospray deposition (ESD) has been exploited as a novel technique for the immobilisation of laccase enzyme on carbon black modified screen-printed electrodes. The aim is to fabricate an amperometric biosensor for phenolic compound detection. The electrodes produced by ESD have been analysed by scanning electron microscopy and characterised electrochemically to prove that this immobilisation technique is suited to manufacture high performance biosensors. The results show that the laccase enzyme maintains its activity after undergoing the electrospray ionisation process and deposition and the fabricated biosensor has improved performances in terms of storage (up to 3 months at room temperature) and working (up to 25 measurements on the same electrode) stability. The laccase-based biosensor has been tested for phenolic compound detection, with catechol as target analyte, in the linear range 2.5-50 μM , with 2.0 μM limit of detection, without interference from lead, cadmium, atrazine, and paraoxon, and without matrix effect in drinking, surface, and wastewater.

Keywords: electrospray deposition, laccase enzyme, carbon black, screen-printed electrodes

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1. Introduction

Immobilization of enzymes [Mohamad et al., 2015] on the surface of the transduction system represents a compulsory and critical phase in the development of biosensors. Indeed, via tailored immobilisation procedures, which influence orientation, loading, and conformational mobility of the enzymes, it is possible to tune their structure and biological activity in order to achieve enhanced analytical performances in terms of sensitivity, selectivity, and stability.

The selection of an immobilization method must consider several parameters, in order to maximise the bioreceptor activity and to provide the best performance of the device, in terms of stability and reusability. For example, the immobilisation of the enzyme on the screen-printed electrodes (SPEs) is a critical factor to establish an efficient electron transfer between the enzyme and the working electrode, in the fabrication of third generation biosensors. In the specific case of laccase enzyme, this concern was elegantly dealt with by Rodríguez-Delgado and co-workers in their review [Rodríguez-Delgado et al., 2015], where they state that *“to become viable industrial catalysts, laccases need to be subject to treatments in order to make them robust, recyclable, or heterogeneous. One of the most studied treatments is immobilization, defined as attachment of an enzyme to an insoluble support. The benefits of an efficient protocol of immobilization are very important, namely prolonged use of the sensor and anticipated extended storage and working stability”*.

Many papers describe the immobilization of enzymes with a plethora of different protocols [Sassolas et al. 2012]. The various immobilization strategies for laccase envision both physical and chemical methods, spanning from classical procedures like covalent binding, adsorption, encapsulation, and cross-linking [Cincotto et al., 2015; Chen et al. 2015; Gill et al., 2018; Azarikia, F., 2015] to the more recent approaches like jet printing [Virtanen et al., 2014] and laser printing technologies (e.g. Laser Induced Forward Transfer, LIFT) [Touloupakis et al., 2014]. The new trends point towards the exploitation of promising nanomaterials for enzyme immobilisation, including graphene, nanotubes, nanoparticles and nanocomposites among others. Indeed, these nanomaterials demonstrated to provide larger enzyme loading surface areas as well as an amplified electron transfer, thus further enhancing the analytical performances of laccase as bioreceptor [Castrovilli et al., 2019]. In the last few years, novel strategies are gaining momentum for laccase immobilisation. Among them, the soft plasma jet technique exploits a continuous wave helium corona plasma discharge to polymerize laccase on bioactive glass [Malinowski et al., 2018], showing good coating performance in term of activity (44% of retained activity over a period of 8 days despite daily washing and testing) and durability under water wash; the Matrix Assisted Pulsed Laser Evaporation (MAPLE) described by Verrastro and co-workers for immobilising laccase enzyme on graphite SPE has shown a working

stability of 5 measurements and a storage stability of about 100 days for electrodes preserved at 4 °C in dry conditions [Verrastro et al., 2016]. Among all the procedures used so far in the production of laccase-based biosensors, those that lead to the manufacture of highly sensitive sensors are based on covalent attachment or adsorption as immobilization technique. Despite promising great sensitivity, these manufacturing procedures show the disadvantage of a decreased enzyme activity over time and a constraint in the maintenance conditions, which is usually in drying at 4°C.

In this work we explore the possibility to use Electrospray Ionisation (ESI), a soft ionisation technique, to deposit complex biomolecular species and to fabricate competitive biosensors. First introduced by J.B. Fenn [Fenn et al., 1989; Fenn, 2003; Fenn, 2002] and widely used for analytical purposes, the ESI technique allows to bring large molecules, like enzymes, proteins, oligonucleotides, DNA strands or nanoparticles of molecular mass up to the kDa - MDa range, as intact and isolated units in the gas phase at ambient pressure. Since the early works on biomolecules, where ESI sources have been coupled with high vacuum set-ups for analytical purposes in mass spectrometry (ESI-MS) [Banerjee and Mazumdar, 2012] and ‘soft landing’ deposition of mass-over-charge selected species [Deng et al., 2012], it was understood that the electrospray is a ‘soft’ ionization method that does not decompose these complex molecules [Fornari et al., 2015; Morozov et al., 1993]. Currently the Electrospray Deposition (ESD) of biomolecules at ambient pressure and temperature conditions [Morozov, 2010] is used to effectively deposit protein films and microarrays [Morozov and Morozova, 2017, 1999; Morozov and Morozova, 2015, 1999;] but, to the best of our knowledge, it has never been implemented to produce functionally active protein layers on a surface for biosensor application.

A typical ESI source [Banerjee and Mazumdar, 2012] uses a low-concentration solution of the analyte of interest flowing in a small capillary held at a few kV high voltage with respect to the counter electrode placed several mm away. At the tip of the emitter, the (multiply) charged analyte molecules on the liquid surface repel each other and expand at the solution/gas interface into the so-called ‘Taylor cone’, where the electrostatic repulsive forces are counter-balanced by the surface tension of the liquid. It is commonly stated that, 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 travelling towards the counter electrode, driven by the electric field generated by the high voltage at the emitter. In these conditions the electrospray deposition can be carried out at ambient pressure [Morozov, 2010; Morozov and Morozova, 2015, 1999] and automatized, with significant reduction of the costs in terms of equipment and processing time compared to vacuum depositions [Deng et al., 2012]. However, the physico-chemical principles at the basis of ESI is still under discussion [Aliotta et al., 2014].

Herein, we describe for the first time the use of the ESD technique for the immobilisation of laccase enzyme on carbon black modified screen-printed electrodes (CB-SPEs) to realise an amperometric biosensor for catechol detection. The choice of laccase enzyme for this first pilot experiment is also driven by the need to assess the ESD as immobilisation technique on the solid ground of a stable bioreceptor of well-known catalytic properties. The exploitation of carbon black modified screen-printed electrodes relies on the capability of this nanomaterial to provide a large surface area for enzyme loading and to enhance the electroanalytical detection of enzymatic by-product [Arduini et al., 2020]. Both properties should result in an improved sensitivity of the biosensor. Through this technique a new efficient laccase-carbon-black biosensor has been realized. The retaining of enzyme activity after ESD immobilisation, as well as the analytical performances in terms of linear range of the amperometric response, detection limit, working and storage stability, repeatability, selectivity, and accuracy have been demonstrated.

2. Materials and Methods

2.1. Chemicals and instrumentations

Fungal Laccase from *Trametes versicolor* (TvL) (E.C. 1.10.3.2, activity: 0.5 U/mg), ethanol, methanol, syringaldazine, catechol and bisphenol A were purchased from Sigma Aldrich (Merck Group). All the solutions were prepared using double-distilled water (Milli-Q system, Millipore). The carbon black -modified electrodes [Arduini et al., 2012] were supplied by SENSE4MED, Italy. The electrochemical measurements were performed using the portable potentiostat PalmSens®4 (Palm Instrument, The Netherlands), connected to a personal computer. Scanning Electron Microscopy (SEM) micrographs were acquired with a field-emission gun scanning electron microscope (Zeiss EVO MA10).

2.2 Laccase preparation for electrospray ionisation immobilisation

In order to achieve an efficient electrospray ionisation, the spray solution normally contains a low concentration of well dissolved analyte in pure water and organic solvents as acetonitrile and methanol. However, considering the effect of organic solvents on protein conformation and denaturation [Iavarone et al., 2000; Chowdhury et al., 1990], solutions of different concentrations of ethanol or methanol in water have been tested and the ideal compromise that allows good stability of the electrospray (see section 3.1) as well as preservation of the laccase enzymatic activity has been adopted using a solution of 2 µg/µl of laccase at 20% of methanol in water. Syringaldazine

assay was exploited to evaluate laccase activity in different conditions [Leonowicz and Grzywnowicz, 1981].

2.3 Electrochemical characterisation of the electrospray deposited laccase on carbon black modified screen-printed electrodes (e-Lac/CB-SPE)

Electrochemical experiments were carried out at room temperature by amperometric analysis with an applied potential of -0.160 V vs Ag/AgCl reference electrode in a total volume of 100 μ L, recording the current signals every 0.5 sec. The study of the laccase activity versus catechol was performed using a 0.1 M buffer solution of citric acid/sodium citrate at pH 4.5.

2.4 Catechol detection in real water sample

River water was collected from Sebou river (Morocco), wastewater from Regional Depuration Plant (Lazio, Italy), while drinking water from a commercial drinking water bottle. Real water samples were diluted 1:2 with 0.2 M buffer solution of citric acid/sodium citrate at pH 4.5 and loaded onto the sensor for the analysis, without any pre-treatment.

3. Results and Discussions

3.1 Electrospray deposition set-up

The ESD operational conditions depend on various properties of the enzyme solution, such as electrical conductivity, density, dielectric constant, surface tension, and viscosity. Furthermore, parameters such as flow rate, applied voltages, capillary diameter, substrate-to-capillary distance, substrate shape and electrode configuration also play an important role. All these parameters were optimised to reach the best spray conditions, and then maintained and monitored for all the performed depositions. In particular, ethanol, methanol, and formic acid at different percentage were tested as possible solvents. A 20% methanol solution was found as the best compromise for achieving both high quality of the electrospray process and a high enzyme activity (data not shown). The concentration of 2 μ g/ μ L has been found as the limit concentration at which a good spray is obtained without clogging the needle of the set-up. A conical electrode placed 9 mm from the spray needle and polarised to 2.0 kV has been used to focus the beam into a spot size of 3 mm, fully covering the working electrode of the CB-SPEs. The voltages of the needle and the cone were varied by monitoring the shape of the Taylor cone by mean of a 6x16 mm² 10° monocular (SPECWELL) coupled to a BRESSER MikrOkular Full HD digital camera until a stability was

reached. The final values of 4.9 kV (needle) and 2.0 kV (cone) correspond to a stable spray regime (Figure 1). Once investigated all the parameters, a laccase spray solution of 2 $\mu\text{g}/\mu\text{l}$ in 20% of methanol, a flow rate of 1 $\mu\text{l}/\text{min}$, and a positive voltage of 4.9 kV applied to the spray needle (100 μm internal diameter) with respect to the grounded counter electrode placed 14 mm away, resulted as the final optimal conditions. The entire set-up, depicted in Fig. 1, was located in a protected environment in order to avoid jet fluctuations and external contamination. . An amount of 13 μg of laccase has been deposited on the working electrode, as verified by an in house-developed quartz crystal microbalance (QCM) with a resonance frequency (f_0) of 10 MHz (Macagnano et al., (2008); Bearzotti et al., 2017). The QMC has been specifically calibrated for laccase response by drop casting known amounts (concentration and volume) of laccase and reading resonant frequency variation after solvent evaporation. The QCM sensor has been washed in a mixture water:ethanol (1:1) between tests. Values of 'QCM frequency' vs 'amount of deposited laccase' has shown a frequency variation of 1.4 KHz/ μg and a linear response in the range 1-40 μg , with an uncertainty of 15% estimated by different repetitions of the drop cast. No difference between consecutive additions and a single deposition was observed within the range of linearity. Having calibrated the response of the QCM to laccase by drop cast, the enzyme amount deposited by ESD has been measured by performing a deposition of laccase on the QCM sensor in identical experimental conditions as used for the ESD depositions on the CB-SPE electrodes.

3.2 SEM analysis of the e-Lac/CB-SPE

The working electrode of the CB-SPE was observed by SEM before and after laccase enzyme immobilisation by ESD (Fig. 2). At a magnification of 10 μm , the CB-SPE shows carbon black nanoparticles completely and uniformly covering the surface of the working electrode (Fig. 2A) while the e-Lac/CB-SPE (Fig. 2B) highlights the presence of a regular film on top of the CB nanoparticles, with no evident signs of cracks or formation of agglomerates. The morphology of the deposited film, which smooths the roughness but still resembles the structure of the underlying CB substrate, suggests that the laccase film has been deposited in a thin layer.

3.3 Electrochemical study of e-Lac/CB-SPE

The laccase sensing mechanism for catechol detection is based on the electrocatalysis of catechol oxidation to its corresponding 1,2 benzoquinone, as schematised in Fig. 1. This reaction is coupled with the electrocatalytic reduction of oxygen to water on the working electrode surface, where the

presence of CB nanoparticles enhances the current signal. Indeed, as largely demonstrated in literature, this nanomaterial shows significant advantages for electroanalytical measurement due to the nano-sizes, the onionlike carbon structure, and the high number of defect sites as reported in literature [Arduini et al., 2020].

The e-Lac/CB-SPEs works with the highest laccase activity at pH 4.5, and with an applied potential of -0.160 V (Fig. S1). The analytical performances of e-Lac/CB-SPEs, including the range of linear response, the detection limit and working/storage stability, were then evaluated at these fixed optimal conditions. These parameters strictly depend on the immobilization method as well as on the used SPE and nanomaterials, i.e. CB contributes to the enhancement of the current signals in comparison with bulk SPEs (Fig. S2). For example, sensitivity can be affected by the diffusion constrains of the target analyte and depends on laccase immobilisation on the surface of the working electrode. More uniform is the laccase film on the surface of the working electrode, more homogeneous will be the diffusion of the substrate.

3.3.1 Analytical features

The detection capability of the e-Lac/CB-SPEs has been tested towards catechol. To perform the amperometric measurements, 100 μ L of 0.1 M citric acid/sodium citrate buffer pH 4.5 were dropped on a printed electrochemical cell and increasing concentrations of catechol were incrementally added on the same electrode in the range from 2.5 to 75 μ M recording the current signals every 0.5 sec. As described in Fig. 3A the recorded current signal increases linearly with the catechol concentration. This measurement at increasing catechol concentrations was repeated at least five times on different electrodes produced in various batches, and for each case the stable current reading after catechol addition was background subtracted.

The average current value and the standard deviation of these measurements are reported vs catechol concentration in Fig. 3B. concentration range. The calibration curve using the linear regression given by $y = 12.4(\pm 0.3)x - 19(\pm 7)$, with an $R^2 = 0.996$, where y is the measured current in nA and x the concentration in μ M, is shown in Fig. 3B. A limit of detection (LOD), calculated as signal to noise ratio ($S/N=3$) equal to 2.0 μ M was obtained.

3.3.2 Working and storage stability studies

The operational stability of the e-Lac/CB-SPEs has been investigated by repeated amperometric measurements on the same electrode, alternating washes with 0.1 M citric acid/sodium citrate buffer

at pH 4.5 and tests in the presence of 50 μM catechol, to observe if enzyme leaching occurs. As reported in Fig. 4A, 100 % of laccase activity was retained up to 25 consecutive measurements with a progressive decrease of the current signal to about 50 % after 28 measurements and to about 30 % after 33 measurements. This gradual decrease of the current signal may be ascribed to the enzyme leaching out of the electrode, probably due to the weakening of the interaction between laccase and the electrode surface.

To evaluate the storage stability, e-Lac/CB-SPEs deposited from different batches of preparation were preserved at room temperature and tested after a variable time from few days to 3 months, with a solution of 50 μM catechol in a 0.1M citric acid/sodium citrate buffer at pH 4.5 (Fig. 4B). Each measurement was repeated at least three times. The results in Fig. 4 report an excellent storage stability up to 90 days, achieved without any particular care in the storage of the electrodes.

Therefore, the stability tests on the e-Lac/CB-SPEs reported in Fig. 4A and 4B highlight, respectively, the valuable working and storage stability of the biosensor that retains 100 % of its performances up to 25 washes and can be preserved at room temperature for at least 90 days.

3.3.3 Interferences study and matrix effect

Laccase biosensors can suffer from interferents and/or electroactive species present in natural environmental samples of catechol contamination. Among the possible interferents present in real matrices as tap and surface waters as well as in wastewater, heavy metals, herbicides, and insecticides were considered. In particular, the following interfering species were tested at safety limits provided by the EU Directive 98/83/EC for drinking water and 2008/105/EC for surface water: lead 7.2 $\mu\text{g/L}$, cadmium 5 $\mu\text{g/L}$, atrazine 0.6 $\mu\text{g/L}$, paraoxon 0.1 $\mu\text{g/L}$. These concentrations of metals and pesticides did not provide any significant response with respect to a corresponding catechol signal of 2.5 μM , both if added before and after the catechol (Fig. 5A). On the other hand, bisphenol A was also tested as possible interferent. This compound provided current signals as expected (data not shown), due to the low selectivity of laccase towards phenolic compounds, being this enzyme active toward ortho- and para-diphenol groups, including mono-, di-, and poly-phenols, aminophenols, methoxyphenols [Rodríguez-Delgado et al., 2015].

With the aim to challenge the implemented biosensor in real samples, e-Lac/CB-SPEs were tested by the analysis of catechol in tap water, river water, and wastewater (diluted 1:2 with 0.2 M sodium citrate buffer pH 4.5) by using the standard additions method and the calibration curve from catechol standard solutions. As reported in Fig. 5B, the obtained results confirm the absence of any

matrix effect, with slope ratios between standard solution and real samples of 0.98, 0.97, and 0.90 for tap water, river water, and wastewater, respectively.

3.3.4 Comparison with other laccase biosensors

Table 1 compares the performance of e-Lac/CB-SPEs, in terms of LOD, linear range of response, storage and working stability, with the ones of other biosensors produced by different immobilization techniques reported in the literature.

It can be immediately noticed from Table 1 that the most noticeable performance of the e-Lac/CB-SPEs lies in good working and storage stabilities at room temperature. Among laccase-based biosensors exploiting SPEs, none of them has ever preserved the 100 % working stability after 25 consecutive measurements, joined to an activity retention of 100 % after 3 months of storage at room temperature. These peculiar and interesting characteristics can be attributed to the novel immobilisation technique which combines the use of electrospray deposition for the laccase immobilisation and the enhancing features of nanomaterials. Indeed, the electrospray ionisation technique forms a gas of charged enzymes, whose conformation depends on the charge state [Deng et al., 2012], that are accelerated by the field towards the CB-SPE. Thus, one may speculate that some produced conformations result in favourable conditions for the biosensing application and the energetic impact of the bioreceptor onto the surface of the CB-SPE electrode favours a stronger link between the two, compared to ‘softer’ deposition techniques.

Whatever the microscopic reason, the interaction of the electrospray deposited laccase with the carbon black nanoparticles seems to ensure a very good anchorage on the surface with consequent difficult leaching. It can be proposed that the soft carbon black nanoparticles create a sort of cavity when the enzyme reaches the electrode surface with some impact energy acquired by the accelerating electric field, thus protecting it from leaching and preserving it for long periods.

As far as the LOD is concerned, the performance of the e-Lac/CB-SPE is comparable with the other sensors operating in the range of a few tents of μM . In this respect, it is important to emphasise that in this work we employ Laccase enzyme of relatively low activity, i.e. 0.5 U/mg to be compared, for example, with the 13.6 U/mg deposited by MAPLE [Verrastro et al., 2016]. It is therefore likely that the use of enhanced laccase activity would lead to a decrease in the LOD.

4. Conclusions

The experiments and tests described in this work have demonstrated the ability of the electrospray technique to immobilise enzymes of preserved activity on carbon black modified screen-printed electrodes, fabricating a competitive electrochemical biosensor for phenolic compound detection. These e-Lac/CB-SPEs, that have been characterized with the detection of catechol and amperometric measurements, show a LOD (2.0 μM) and a range of linearity (5-50 μM) which are comparable to other biosensors reported in literature. On the other hand, the e-Lac/CB-SPEs show remarkable anchorage of the enzyme on the substrate, demonstrated by good working stability (25 measurements) and storage stability up to 90 days at room temperature and humidity, experimental conditions that highlight the potential of this biosensor. The response to interferent species (lead, cadmium, atrazine, paraoxon) as well as the matrix effects were tested and proved to be negligible. In relation to already existing immobilisation methods the obtained results indicate crucial advantages, such as a good reproducibility and stability at room temperature, as well as room for improvement, particularly in terms of LOD. This could be effectively faced by the use of enzyme at higher activity. Overall, the results of this work demonstrate that electrospray deposition can be considered an effective immobilization technique for complex biomolecules of well-preserved activity/functionality. The combination of ESD technique and SPE functionalized by nanomaterials has shown the potential to become a new and effective approach for the fabrication of robust biosensors. Indeed, the use of ESD technique for enzyme deposition has been already described to form bioactive coatings for different purposes (e.g. to inhibit bacterial adhesion and enhance cell growth) (de Jonge et al., 2009), or to immobilize protein for in-situ analysis with other techniques (Pompach et al., 2016). Here, ESD technique has been exploited for the deposition of molecules (in this case the laccase enzyme) for a direct and customised production of biosensors.

Figure captions

Fig. 1. On the left handside, schematic representation of the ESD set-up used for laccase immobilisation on CB-SPE; in the inset, a picture of the Taylor cone recorded by the camera during a deposition run. On the right handside, the catalytic action of laccase leading to catechol oxidation and dioxygen reduction is highlighted by the arrows.

Fig. 2. SEM images of A) CB-SPE and B) e-Lac/CB-SPE.

Fig. 3. A) e-Lac/CB-SPE amperogram after addition of increasing amounts of catechol and b) corresponding calibration plot. Applied potential is -0.16 V, number of repetitions is n=5. Measurement volume: 100 μ L 0.1 M acid citric/sodium citrate buffer at pH 4.5. The current in the 2.5 to 50 μ M range of catechol concentration, is fitted by the linear function: $y = 12.4(\pm 0.3) x - 20(\pm 7)$ ($R^2 = 0.996$).

Fig. 4. Working stability (A) and storage stability (B) of e-Lac/CB-SPE. Applied potential is -0.16 V. Measurement volume: 100 μ L of 0.1 M citric acid/sodium citrate buffer at pH 4.5.

Fig. 5. A) Interferent study in the presence of lead 7.2 μ g/L, cadmium 5 μ g/L, atrazine 0.6 μ g/L, paraoxon 0.1 μ g/L. B) e-Lac/CB-SPE matrix effect. Applied potential -0.16 V, n=3. Measurement volume: 100 μ L 0.1 M sodium citrate buffer pH 4.5. Real samples were diluted 1:2 with 0.2 M sodium citrate buffer pH 4.5.

Table 1. Comparison of laccase-based amperometric biosensors realised using different immobilisation methods.

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Immobilisation technique	Biosensing configuration	LOD	Linear range	Storage stability	working stability	Ref.
Adsorption	Laccase immobilized on a reduced graphene oxide supported Pd-Cu alloyed nanocages platform	1.5 μ M	5-1155 μ M	<u>Stored at 4 °C</u> : after 35 day the catalytic currents still remained 98.90 %	n.d.	[Mei et al., 2015]
Adsorption	Laccase dropped on gold nanoparticles-crosslinked zein ultrafine fibres attached on glassy carbon electrode	0.166 μ M	Up to 7 μ M	<u>Stored at 4 °C</u> for 30 days: the biosensor still maintained 87.1 % of activity	<u>up to 10 measurements</u> , RSD equal to 1.74%	[Chen et al. 2015]
Covalent attachment	Laccase bound to grapheme sheets doped with a Sb ₂ O ₅ thin film	0.011 μ M	0.025 -1.03 μ M	<u>Stored at 4 °C under dry conditions</u> : the activity decreased to 52 % after 60 days	n.d.	[Cincotto et al.,2015]
LIFT	Laccase on graphite screen printed electrodes.	0.150 μ M	Up to 0.75 μ M	<u>Stored at 4 °C</u> : 90 % activity retention after 35 days	<u>up to 4 measurements</u> for the detection of 300 nM catechol solution	[Touloupakis et al., 2014]
MAPLE	Laccase on screen printed carbon electrodes	1 μ M	5-60 μ M	<u>Stored at 4 °C under dry conditions</u> : after 100 days sensitivity RSD% 11.6 %	<u>up to 5 measurements</u> for the detection of 30 μ M catechol solution.	[Verrastro et al., 2016]
ESD	Laccase electrosprayed on carbon black modified screen printed electrodes	2.0 μ M	2.5-50 μ M	<u>Stored at room temperature</u> : 100 % activity retention after 90 days.	100 % of activity retention <u>up to 25</u> consecutive measurements	Current work

Table 1.

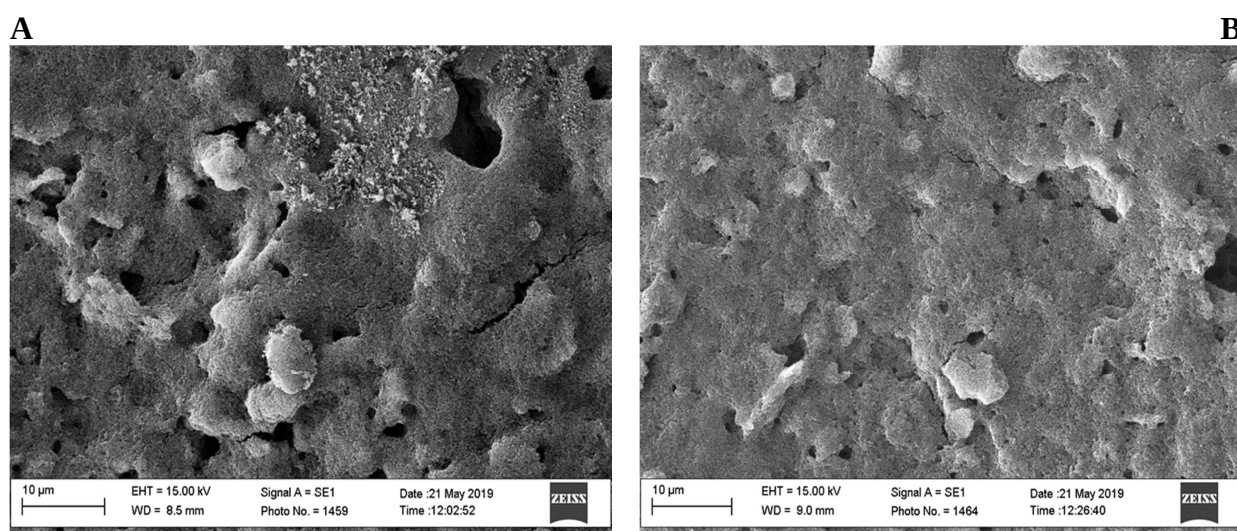


Figure 2.

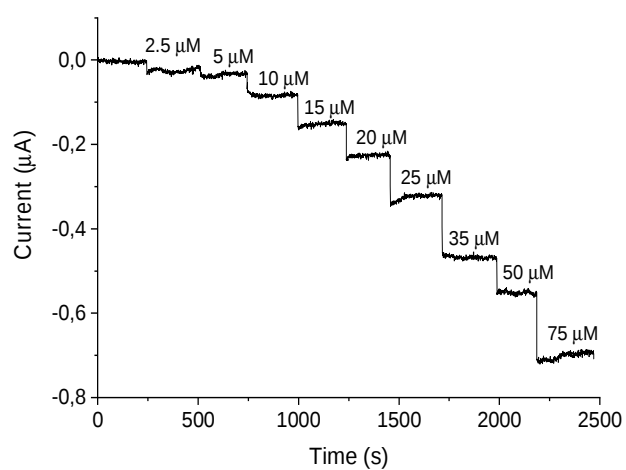
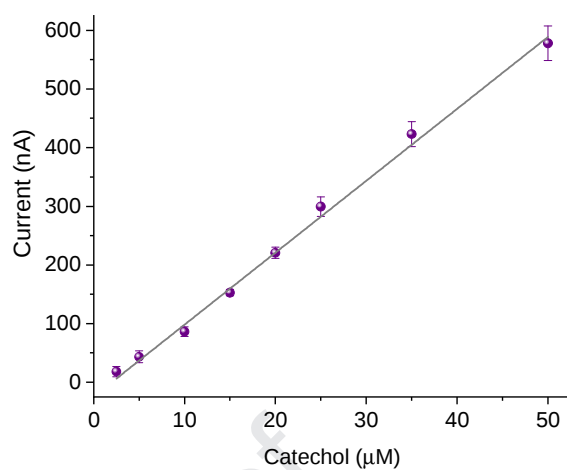
A**B**

Figure 3.

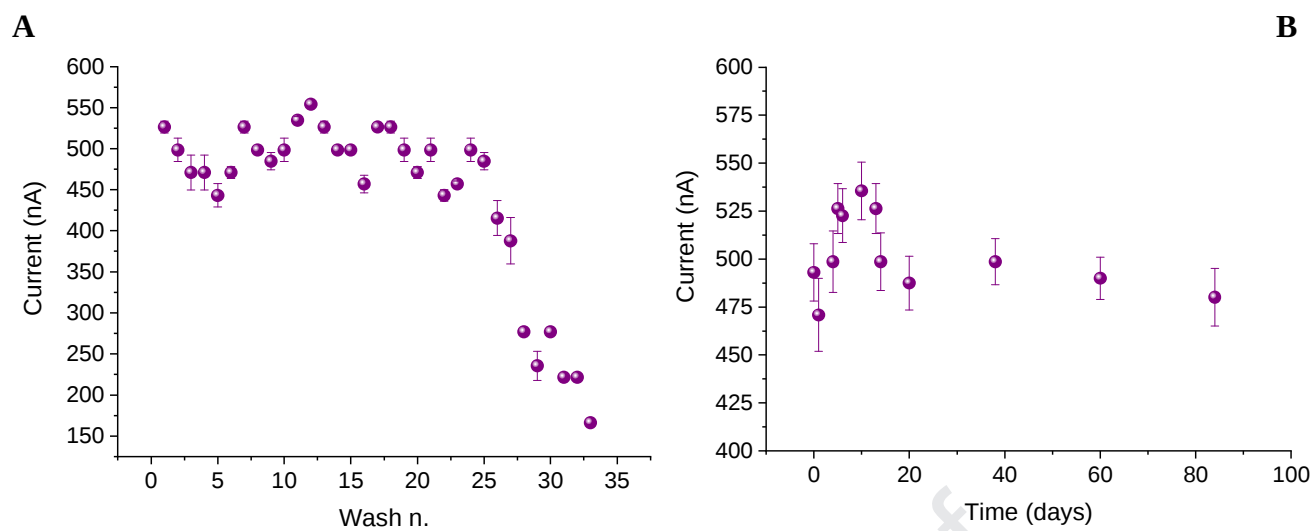


Figure 4.

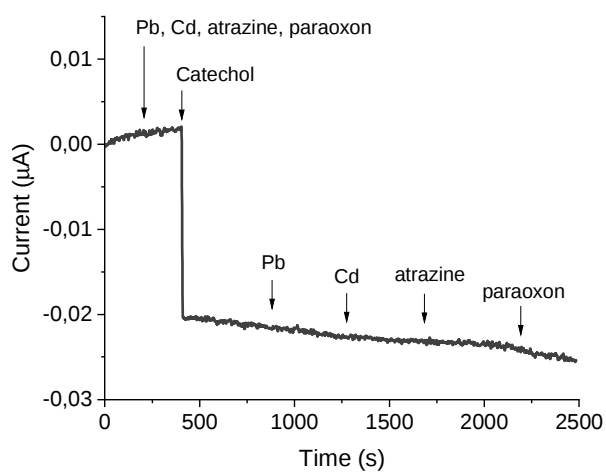
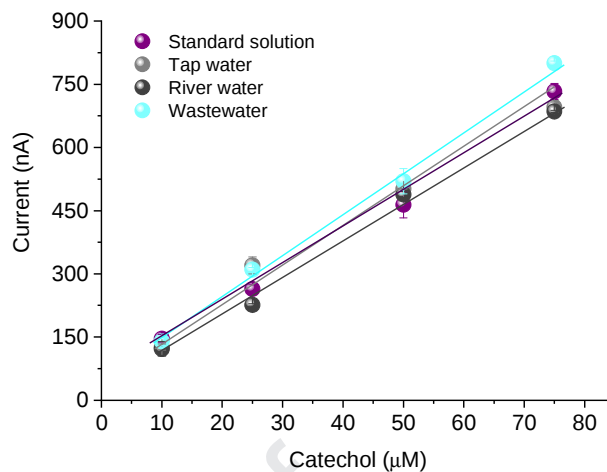
A**B**

Figure 5.

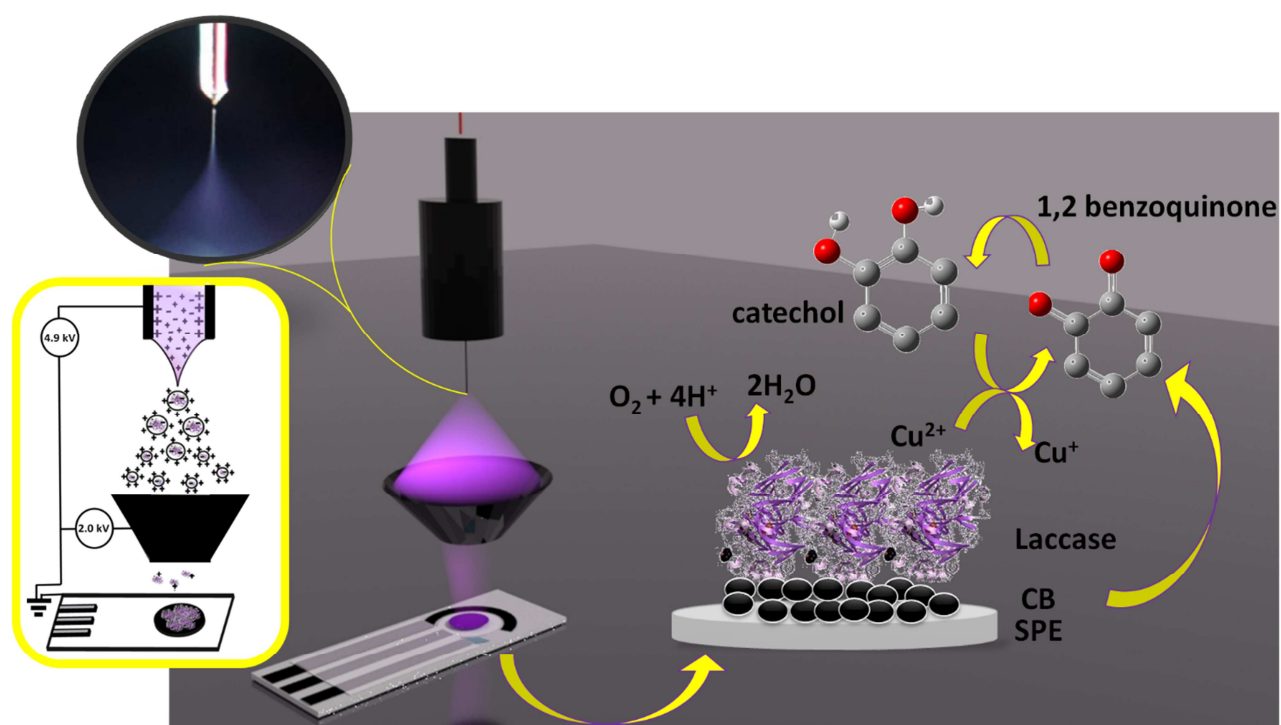


Figure 1.

Highlights

Electrospray deposition as a novel technique for enzyme immobilisation on carbon black nanommodified screen-printed electrodes.

Laccase enzyme as a case study to fabricate an amperometric biosensor for phenolic compound detection.

Electrospray ionisation process and bioreceptor deposition to improve biosensor analytical performances in terms of storage and working stability.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.