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ELECTROSPRAY IONISATION TECHNIQUE IN BIOSENSOR DESIGN: LACCASE AS CASE STUDY

M.C. Castrovilli[1], J. Chiarinelli[1], P. Bolognesi[1], E. Tempesta[2], P. Calandra[3], A. Cartoni[1,4], A. Antonacci[5], L. Avaldi[1], F. Arduini[6], V. Scognamiglio[5]

[1]*Dipartimento di scienze fisiche e tecnologie della materia, Istituto di Struttura della Materia, Consiglio Nazionale delle Ricerche, Rome, Italy*
[2]*Dipartimento di Scienze del Sistema Terra e Tecnologie per l'Ambiente, Istituto di Geologia Ambientale e Geoingegneria, Consiglio Nazionale delle Ricerche, Rome, Italy*
[3]*Dipartimento di scienze chimiche e tecnologie dei materiali, Istituto per lo Studio dei Materiali Nanostrutturati, Consiglio Nazionale delle Ricerche, Rome, Italy*
[4]*Dipartimento di Chimica, "Sapienza" Università di Roma, Rome, Italy*
[5]*Dipartimento di Scienze Chimiche e Tecnologie dei Materiali, Istituto di Cristallografia, Consiglio Nazionale delle Ricerche, Rome, Italy*
[6]*Dipartimento di Scienze e Tecnologie Chimiche, Università di Roma "Tor Vergata", Rome, Italy*

Immobilization of enzymes on the surface of transduction systems represents a compulsory and critical phase in the development of biosensors. Indeed, by influencing orientation, loading, and mobility of enzymes by means of tailored immobilisation procedures, it is possible to tune their structure and biological activity for achieving enhanced analytical performances in terms of sensitivity, selectivity, and stability. In the specific case of the laccase enzyme, this concern was elegantly dealt by Rodríguez-Delgado and co-workers in their review asserting 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*" [1].
Herein, we described for the first time the use of electrospray ionisation (ESI) for the deposition of laccase on carbon black modified screen-printed electrodes (CB-SPEs) to design an amperometric biosensor for catechol detection. The ESI technique allows to bring large organic molecules as intact and isolated units in the gas phase, using a low-concentration solution of the molecule of interest flowed in a small capillary held at high voltage (4.5 kV) with respect to a grounded counter electrode placed 10 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, provided 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. In these conditions the deposition of the molecule can be carried out at ambient pressure [2] and automatized, with significant

reduction of costs and times of the process in comparison with vacuum depositions [3]. In particular, the recent developments in the theory of electrospray process highlighted that there is a significant contribution coming from dielectrophoretic forces in addition to the electrophoretic ones, as a result of the inhomogeneous electric field (non-zero electric field gradient) typical of the experimental setup (where needle/target geometry is adopted). This would allow the process to take place even in the absence of neat charges rendering making it less stressing towards bio-molecules.

The aim of this work is to develop a standardised and reliable immobilisation protocol that preserves normal activity of the laccase enzyme, improving its working/storage stability and peculiar features for a biosensor by a commercial point of view. Laccase enzyme was deposed by ESI technique on carbon black modified screen-printed electrodes for the development of an amperometric biosensor for catechol detection. The choice of using carbon black as nanomaterial for SPE modification relied on the increased current signal of laccase towards catechol detection, which was 3 times higher in comparison to graphite SPEs, indicating the ability of CB to provide a larger surface area for laccase deposition as well as an increased conductivity that resulted in higher signals and thus enhanced sensitivity [4,5]. The performance of the laccase CB-SPE biosensor was tested via amperometric measurements at an applied potential of 0.160 V, in the presence of increasing amounts of catechol in a concentration range from 2.5 to 150 μM. Catechol was detected at a detection limit equal to 1.35 μM within a linear range from 2.5 to 75 μM described by the equation: $y = (0.04 \pm 0.02) + (0.102 \pm 0.005)\ x$ ($R^2 = 0.956$). The working stability of the biosensor was evaluated by testing the biosensor towards the detection of catechol at a concentration of 50 μM after repeated washes of the electrode, indicating a 100 % enzyme activity up to 27 measurements. The storage stability was appraised at room temperature in dry conditions, highlighting that the enzyme retained 100 % of the activity towards 50 μM catechol within a period of 180 days. Further biosensor analytical features are under investigation including interferent studies, matrix effect and recovery studies, to underline the potential of the proposed biosensor for real agro-environmental applications.

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