

## Ambient Electrospray Deposition: An Efficient Technique to Immobilize Laccase on Cheap Electrodes With Unprecedented Reuse and Storage Performances

**Authors :** Mattea Carmen Castrovilli, Antonella Cartoni

**Abstract :** Electrospray ionisation (ESI), a well-established technique widely used to produce ion beams of biomolecules in mass spectrometry (ESI-MS), can be used for ambient soft landing of enzymes on a specific substrate. In this work, we show how the ambient electrospray deposition (ESD) technique can be successfully exploited for manufacturing a promising, green-friendly electrochemical amperometric laccase-based biosensor with unprecedented reuse and storage performance. These biosensors have been manufactured by spraying a laccase solution of 2 µg/µL at 20% of methanol on a commercial carbon screen printed electrode (C-SPE) using a custom ESD set-up. The laccase-based ESD biosensor has been tested against catechol compounds in the linear range 2-100 µM, with a limit of detection of 1.7 µM, without interference from cadmium, chrome, arsenic, and zinc and without any memory effects, but showing a matrix effect in lake and well water. The ESD biosensor shows enhanced performances compared to the ones fabricated with other immobilization methods, like drop-casting. Indeed, it retains 100% activity up to two months of storage at ambient conditions without any special care and working stability up to 63 measurements on the same electrode just prepared and 20 on a one-year-old electrode subjected to redeposition together with a 100% resistance to use of the same electrode in subsequent days. The ESD method is a one-step, environmentally friendly method that allows the deposition of the bio-recognition layer without using any additional chemicals. The promising results in terms of storage and working stability also obtained with the more fragile lactate oxidase enzyme suggest these improvements should be attributed to the ESD technique rather than to the bioreceptor, highlighting how the ESD could be useful in reducing pollution from disposable devices. Acknowledgment: The understanding at the molecular level of this promising biosensor by using different spectroscopies, microscopies and analytical techniques is the subject of our PRIN 2022 project ESILARANTE.

**Keywords :** reuse, storage performance, immobilization, electrospray deposition, biosensor, laccase, catechol detection, green chemistry

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