ElectroSpray Ionisation Deposition for biosensor application

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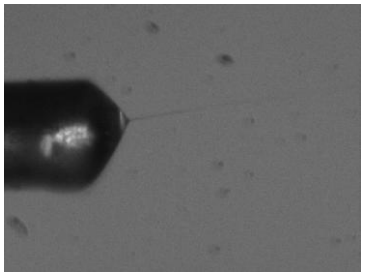
The ElectroSpray Ionization (ESI) developed by Fenn et al. [1] allows to bring large organic molecules (proteins, enzymes etc) as intact and isolated units in the gas phase. The technique is based on the use of a low-concentration solution of the molecule of interest flowing in a small capillary held at high voltage (typically a few kV) with respect to a grounded counter electrode placed some 10-15 mm away. On the tip of the emitter, the surface tension of the liquid cannot support the formed charge and therefore the liquid forms the so-called 'Taylor cone' inside which a Coulomb explosion creates a spray of charged droplets. The size of the droplets continue decreasing as the solvent evaporates by releasing a gas of molecular ions. The ESI process usually takes place in the air (Figure 1a) and then the jet can be transported into a vacuum chamber for the analysis. Originally developed for protein studies with mass spectrometry, ESI was then used with other types of systems (polymers, Nanoparticles, bacteria ...) as well as for different applications, as 'soft-landing' deposition. In these cases, the apparatus combines electrostatic transport and filter devices, to select the ions of interest and guide them towards either a spectrometer for the analysis or a surface for the deposition.

Such a device is under construction at the CNR-ISM Montelibretti. Presently, it consists of i) a heated capillary to transport the charged species in vacuum and ii) a first differential pumping stage equipped with an octupole ion guide, see Figure 1b. A vacuum chamber housing a quadrupole mass spectrometer (QMS) for mass-over-charge, m/z, selection of the charged species, followed by a quadrupolar deflector to direct them towards a deposition chamber is being designed and will soon be commissioned. All in all, an in-vacuum 'soft-landing' deposition apparatus opens-up the unique possibility to perform m/z selected deposition in UHV condition of large biomolecular species [2], with control over the kinetic energy of the deposited species. On the other hand it is a quite complex and costly set-up, suffering severe limitations due to the low fluency of the m/z selected species. The main long term goal of the project is to increase the intensity of the ESI beam by mean of a customised, home-made, apparatus in order to make 'soft landing' a competitive technique for nanotechnology applications such as the production of biosensors and organic devices.

In parallel to the design of the in-vacuum apparatus, the potentiality of the ElectroSpray Ionisation technique for the deposition of active enzymes is being tested using a simpler set-up for 'in-air' deposition. This approach provides effective removal of the solvent, with the advantages that the deposition can be carried out at ambient pressure or in controlled atmosphere, with significant reduction of costs and times of the process, which could be easily automatized and applied on large scale.



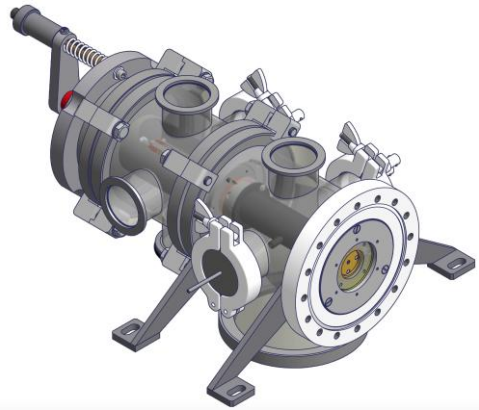


Figure 1a (top). The Taylor cone and ESI beam. The inner diameter of the needle is 0.1 mm.
Figure 1b (right). The non-commercial ESI source at the CNR-ISM, Montelibretti, made of a heated capillary and a octupole ion guide *[Design from Aarhus University]*.

Laccase, a well-known enzyme used for the detection of polyphenols in different matrices [3,4] is used for these studies. Various experimental conditions, such as needle sizes and voltages, flow rate, type and amount of solvents, pH solution, geometry of the ESI-support system, are being evaluated in order to assess the performances of the ESI technique for biosensor fabrication. The enzimatic activity of Laccase prior to deposition and after dissolution in buffer of the deposited material is verified and compared by using syringaldazine colorimetric essay [5].

Solvents play a crucial role both for the ESI process as for the preservation of enzyme activity. We observed that the presence of the ethanol or methanol solvents (20% concentration) does not affect the value of absorbance with respect to the Laccase in pure buffer solution (pH 5.5). Therefore, as a preliminary test, a concentration of 0.2 μg/μl of laccase in a solution of 80% water and 20% of ethanol or methanol (with or without the addition of 0.01 % of formic acid) has been used to deposit 6 μg of Laccase on conductive polymer (Polyethylene terephthalate, PET) substrates. The results of the spectrophotometric measurements with syringaldazine assay showed:

- negligible difference between ethanol/methanol solvents, at least at the chosen conditions, where the value of absorbance of the Laccase deposited and re-dissolved is 70% with respect to the one not undergone ESI deposition. The loss of 30% activity can be attributed to the overall effects of the ESI process and effectiveness in the extraction of the deposited material.
- negative effects due of the presence of the formic acid, even at 0.01 % concentration, with a residual value of absorbance of about 10%.

To investigate the immobilisation on screen printed electrodes (SPE) as well as the homogeneity of deposition at different scales, measurements with an Atomic Force Microscope (AFM) and a profilometer are also being performed.

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References.

[1] J.B. Fenn et al., Science 4926 (1989) 64; The Nobel Prize for Chemistry 2002.
[2] D.E. Clemmer, R. R. Hudgins, M. F. Jarrold, J. Am. Chem. Soc. 117 (1995) 10141−10142.
[3] M.M. Rodríguez-Delgado et al. Trends in Analytical Chemistry 74 (2015) 21–45.
[4] M. Verrastro et al. Talanta 154 (2016) 438–445.
[5] J.P. Ride, Physiological Plant Pathology 16 (1980) 187-196.