Supporting Information

Improved reuse and storage performances at room temperature of a new environmental-friendly lactate oxidase biosensor made by ambient electrospray immobilization

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1. Quantification of LOX immobilized during the ESD process

The amount of deposited enzyme has been evaluated by substituting the screen printed electrode (SPE) with the resonator of a quartz crystal microbalance (QCM). The custom QCM is composed of two gold electrodes with a diameter of 6 mm obtained by vapor deposition on a quartz crystal disk of 14 mm diameter [1,2] and a resonance frequency f0 = 10 MHz. The resonance frequency variation is proportional to the amount of deposited material [3]. It also depends on the viscoelastic properties of the deposited material, the adhesion on the gold material, and among different layers. As such, the response frequency versus mass had to be calibrated specifically for LOX.

To calibrate the QCM response, controlled amounts of LOX were deposited by drop-casting on the resonator. The range of units considered goes from 0.18 to 2.43 U and the linear fit obtained is reported in Fig. S1. Then the QCM has been used to measure the amount of the LOX deposited during the ESD process, by performing three depositions at the standard condition: by spraying the working LOX solution (1 µg/µL) at a flow rate of 1 µl/min, for 40 minutes on the QCM. The average frequency value obtained was interpolated on the calibration line, revealing a quantity of LOX deposited equal to 2.24±0.2 U.

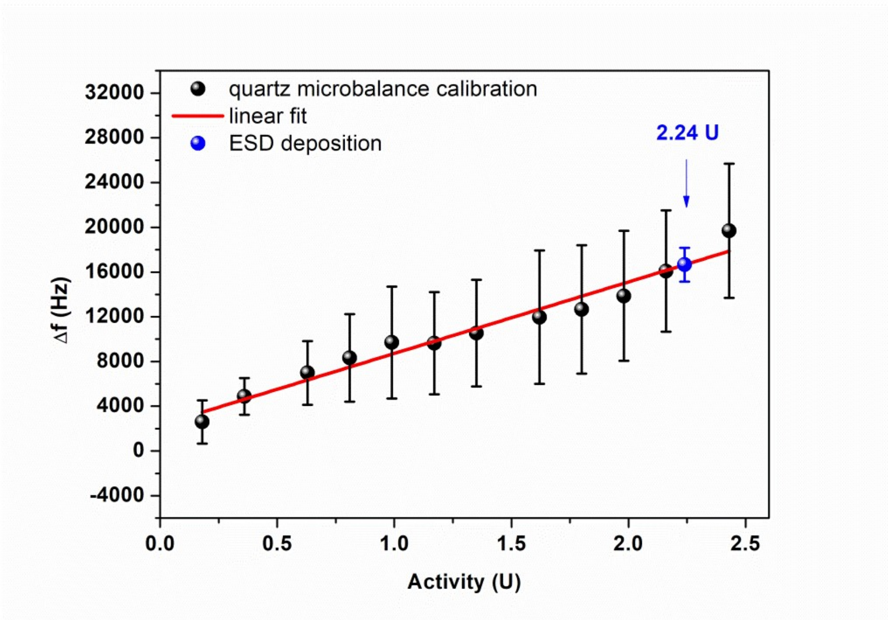

Fig. S1. Calibration curve of the amount of deposited LOX on the QCM electrode by drop-casting (black dots). The blue dot indicates the amount of LOX deposited on QCM during the ESD process. The red line is the linear regression fit (y = 6405.56 (±410.38) x + 2312.55 (±601.05), with an R^2= 0.96). The voltage settings and geometrical parameters for the deposition are the ones in Figure 1 in the main text.

2. Ciclic Voltammetry

In order to assess the correct working potential, the electrochemical characterization through cyclic voltammetry (CV) was assessed prior to conducting the chronoamperometric measurements. Fig. S2 presents CV sweeps carried out at 100 mV/s between 0.5V and -0.5 V. The measurements were performed by dropping 100 µl of PBS buffer 0.1 M pH7 on PB/C-SPEs and recording the current in the presence of only buffer (red curve) and 0.46 mM of L-Lactic acid (blue curve). These curves are compared with the CV performed on e-LOX/PB/C-SPE in the presence of 0.46 mM L-Lactic acid (black curve).

The reduction peak of Prussian Blue (PB) in Prussian White (PW) and the oxidation peak of PW in PB are shown at -0.1V and 0.26 V respectively on the black curve. The potential of -0.1V has been chosen for the subsequent chronoamperometric measurements.

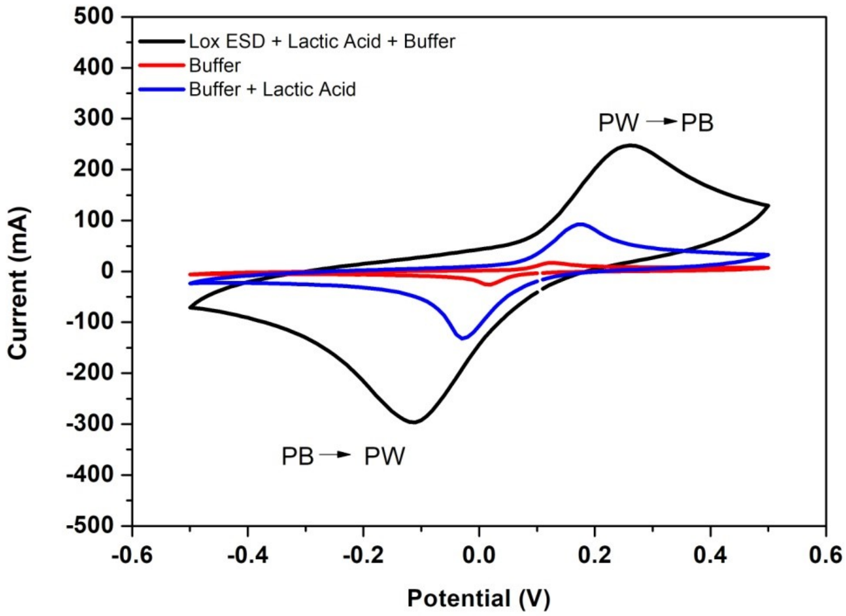

Fig. S2 CV sweeps performed at 100 mV/s between 0.5V and -0.5 V on bare PB/C-SPEs in the presence of 100 µl of PBS buffer 0.1 M pH7 (red curve) and 0.46 mM of L-Lactic acid (blue curve). CV sweep performed at the same condition on e-LOX/PB/C-SPE in presence of 0.46 mM of L-Lactic acid diluted in 100 µl of buffer (black curve).

2. Comparison of Hazardous Chemicals

In Table S1, all the main hazardous chemicals, used for the manufacturing of the biosensors listed in Table 2 of the main article, are shown. The most dangerous chemical for the environment are marked in red. They have been identified according to the Regulation (EC) No 1272/2008 and the GHS Classification[4] as well as the nomenclature, for some of them, identified by the CHEM21 selection guide.[5] As can be seen, all the biosensors mentioned in Table 2 use a large number of chemical agents that are dangerous for both human and environment. The present work demonstrates a greener approach thanks to the use of only isopropanol and water for the ESD spray solution and Prussian Blue for the sensor surface. Isopropanol is recommended by the CHEM21 selection guide and Prussian Blue has not been considered hazardous by the Regulation (EC) No 1272/2008.

Table S1. List of the main hazardous chemicals used for the manufacturing of the biosensors listed in Table 2 of the main article.

Hazardous Chemical	**Hazards identification according to Regulation (EC) No 1272/2008**	**Hazards identification according to GHS Classification [4]**	**CHEM21 selection guide[5]**	**ref**	**Present work**
Triphenylmethane isocyanate	-	**H319** Causes serious eye irritation (Category 2) **H315** Causes skin irritation (Category 2) **H334** Skin sensitization (Category 1) **H332** Acute toxicity, Inhalation (Category 4)	-	6	Absent
Dimethylsulfoxide	-	**H315** Causes skin irritation **H319** Causes serious eye irritation **H335** May cause respiratory irritation	Problematic (after discussion)	6	Absent
Potassium ferricyanide	**H319** Causes serious eye irritation (Category 2). **H411** Long-term (chronic) aquatic hazard (Category 2). Toxic to aquatic life with long lasting effects. **EUH032** Contact with acids liberates very toxic gas.			6,7	Absent
Hexachloroplatinic acid	**H290** Corrosive to Metals (Category 1). **H300** Fatal if swallowed. **H301** Acute toxicity, Oral (Category 3). **H314**, Skin corrosion (Category 1), Toxic if		-	6	Absent

	swallowed **H317** Skin sensitization (Category 1), Causes severe skin burns and eye damage. **H318** Serious eye damage (Category 1), May cause an allergic skin reaction. **H334** Respiratory sensitization (Category 1). **H372** May cause allergy or asthma symptoms or breathing difficulties if inhaled. **H373** Specific target organ toxicity - repeated exposure (Category 2), Kidney. **H400** May be corrosive to metals. **H410** Fatal if swallowed. **H411** Causes severe skin burns and eye damage, Long-term (chronic) aquatic hazard (Category 2).				
Tetrabutylammonium bromide	**H302** Acute toxicity, Oral (Category 4). **H315** Skin irritation (Category 2). **H319** Eye irritation (Category 2). **H361fd** Reproductive toxicity (Category 2). Suspected of damaging fertility. Suspected of damaging the unborn child. **H412** Long-term (chronic) aquatic hazard (Category 3).		-	8	Absent
4-Acetamidophenol	**H302** Acute toxicity, Oral (Category 4)		-	8	Absent
Ferrocene	**H228** Flammable solids (Category 1). **H302** Acute toxicity, Oral (Category 4). **H332** Acute toxicity, Inhalation (Category 4). **H360FD** Reproductive toxicity (Category 1B). **H373** Specific target organ toxicity - repeated exposure, Inhalation (Category 2), Liver. **H410** Long-term (chronic) aquatic hazard (Category 1).		-	8	Absent
Nafion ™ 117 containing solution	**H225** Flammable liquids (Category 2). **H318** Serious eye damage (Category 1). **H336** Specific target organ toxicity - single exposure (Category 3), Central nervous system. May cause drowsiness or dizziness. **H225** Highly flammable liquid and vapor. **H315** Causes skin irritation. **H319** Causes serious eye irritation. **H335** May cause respiratory irritation.		-	8,9,10	Absent
m-xylene	**H226** Flammable liquids (Category 3). **H332** Acute toxicity, Inhalation (Category 4). **H312** Acute toxicity, Dermal (Category 4). **H315** Skin irritation (Category 2). **H319** Eye irritation (Category 2). **H335** Specific target organ toxicity - single exposure (Category 3), Respiratory system . **H304** Aspiration hazard (Category 1). **H412** Long-term (chronic) aquatic hazard (Category 3).		Problematic	8	Absent
Pyridine	**H225** Flammable liquids (Category 2). **H302** Acute toxicity, Oral (Category 4). **H332** Acute toxicity, Inhalation (Category 4). **H312** Acute toxicity, Dermal (Category 4). **H315** Skin irritation (Category 2). **H319** Eye irritation (Category 2).		Hazardous	8	Absent
Argon gas	**H280** gas under pressure; may explode if heated.		-	8	Absent
Hydrogen gas	**H220** Extremely flammable gas. **H280** gas under pressure; may explode if heated.		-	8	Absent
Ammonia gas	**EUH071** Corrosive to the respiratory tract. **H221** Flammable gas (Category 2). **H280** Contains gas under pressure; may explode if heated. **H314** Skin corrosion (Sub-category 1B). Causes severe skin burns and eye damage. **H318** Causes serious eye damage (Category 1).		-	8	Absent

	H331 Acute toxicity, Inhalation (Category 3). **H400** Contains gas under pressure; may explode if heated. Short-term (acute) aquatic hazard (Category 1). **H410** Causes severe skin burns and eye damage. **H411** Long-term (chronic) aquatic hazard (Category 2). Toxic if inhaled**.**				
Ethanol	**H225** Flammable liquids (Category 2). **H319** Eye irritation (Category 2).		Recommended	8, 9, 10	Absent
Sulfuric acid	**H290** Corrosive to Metals (Category 1), **H314** Skin corrosion (Sub-category 1A), **H318** Serious eye damage (Category 1),		-	9	Absent
Potassium hydroxide	**H290** Corrosive to Metals (Category 1). **H302** Acute toxicity, Oral (Category 4). **H314** Skin corrosion (Sub-category 1A). **H318** Serious eye damage (Category 1). Harmful if swallowed. **H319** Causes severe skin burns and eye damage.		-	9	Absent
Glutaraldehyde solution	**H302** Acute toxicity, Oral (Category 4), **H331** Acute toxicity, Inhalation (Category 3), **H314** Skin corrosion (Sub-category 1B), **H318** Serious eye damage (Category 1), **H334** Respiratory sensitization (Category 1), **H317** Skin sensitization (Category 1), **H335** Specific target organ toxicity - single exposure (Category 3), Respiratory system. Very toxic to aquatic life with long lasting effects. **H400** Short-term (acute) aquatic hazard (Category 1), **H411** Long-term (chronic) aquatic hazard (Category 2). Fatal if inhaled. **EUH071** Corrosive to the respiratory tract. **H225** Highly flammable liquid and vapor. **H330** Fatal if inhaled.			9	Absent
Dococyltrimethylammonium chloride	-	**H302** Harmful if swallowed **H312** Harmful in contact with skin **H314** Causes severe skin burns and eye damage	-	10	Absent
Sodium silicate	**H290** Corrosive to Metals (Category 1). **H314** Skin corrosion (Category 1). **H318** Serious eye damage (Category 1). **H335** Specific target organ toxicity - single exposure (Category 3), Respiratory system.		-	10	Absent
Tetraethyl orthosilicate	**H226** Flammable liquids (Category 3). **H332** Acute toxicity, Inhalation (Category 4). **H319** Eye irritation (Category 2). **H335** Specific target organ toxicity - single exposure (Category 3), Respiratory system.		-	10	Absent
Cobalt phthalocyanine	**H351** Carcinogenicity (Category 2).		-	10	Absent
Adipoyl dihydrazide	-	**H320** Causes eye irritation. **H361** Suspected of damaging fertility or the unborn child. **H400** Very toxic to aquatic life. **H410** Very toxic to aquatic life with long lasting effects**.**	-	10	Absent
Hydrochloric acid	**H290** Corrosive to Metals (Category 1). **H314** Skin corrosion (Sub-category 1B). **H318** Serious eye damage (Category 1). **H335** Specific target organ toxicity - single exposure (Category 3), Respiratory system. **H319** Causes serious eye damage.		-	10	Absent
Ammonia solution 25%	**H314** Skin corrosion (Sub-category 1B). **H318** Serious eye damage (Category 1). **H335** Specific target organ toxicity - single exposure (Category 3), Respiratory system. **H400** Short-term (acute) aquatic hazard (Category 1).		-	10	Absent

	H411 Long-term (chronic) aquatic hazard (Category 2).				
Potassium ferrocyanide	**H412** Long-term (chronic) aquatic hazard (Category 3). **EUH032** Contact with acids liberates very toxic gas.		-	7	Absent
i-PrOH	**H225** Flammable liquids (Category 2). **H319** Eye irritation (Category 2). **H336** Specific target organ toxicity - single exposure (Category 3), Respiratory system.		Recommended	-	10%
Prussian Blue	**Not a hazardous substance or mixture according to Regulation (EC) No 1272/2008.**	**H302** Harmful if swallowed **H312** Harmful in contact with skin **H332** Harmful if inhaled			
Water	**Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008 GHS Not Classified**		Recommended		90%

3. References

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