SUPPORTING INFORMATION

Delayed Sensor Activation Based on Transient Coatings: Biofouling Protection in Complex Biofluids

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Materials and Methods

Reagents and solutions

All of the reagents used were of the highest available grade. The commercial polymer (Eudragit® L100) was obtained from Evonik Industries (Germany). Glucose oxidase (GOx) from *Aspergillus niger*, Type X-S (EC 1.1.3.4), chitosan, potassium hexacyanoferrate (II) trihydrate, D(+)-glucose, bovine serum albumin (BSA), gelatin from bovine skin (type B), human serum type AB (male, from human AB plasma, sterile-filtered), sodium dodecyl sulfate (SDS), phosphate buffer solution (1.0 M, pH 7.4), and fluorescein isothiocyanate (FITC, $\lambda_{em} = 514$ nm) were obtained from Sigma-Aldrich (St. Louis, MO). Triton X-100, 2-propanol and potassium ferricyanide were obtained from Fisher Scientific. Sodium hydroxide (NaOH) pellets were obtained from Mallinckrodt Chemicals. Acetic acid and hydrochloric acid (HCl) were obtained from EMD Chemicals Inc. (Gibbstown, NJ). Human whole blood was obtained from Innovative Research. Saliva was collected from a volunteer in fasting state. The collected saliva was used without dilution and/or filtration. Conductive carbon ink (E3449) and silver/silver chloride ink (E2414) were obtained from Ercon Inc. (Wareham, MA). Prussian blue (PB)/graphite ink (C2070424P2) was obtained from Gwent Inc. (Torfaen, UK).

All buffer solutions were prepared with deionized water obtained from a Millipore Milli-Q purification system (18.2 MΩ cm at 25 °C): 0.1 M phosphate buffer (PBS) solution, pH 6.5; 0.1 M acetic acid; 5 mM $[Fe(CN)_6]^{4-/3-}$ consisting of potassium hexacyanoferrate/potassium ferricyanide mixture solution (5 mM each in PBS, pH 6.5); 4, 8, or 16 % (w/v) Eudragit® L100 polymer in isopropanol supplemented with 0.05% (w/v) SDS; 0.5% (w/v) FITC-dye in 16% coating (prepared in isopropanol, 0.05% (w/v) SDS); 50 mg L^{-1} gelatin in PBS, pH 6.5; 5,000 mg L^{-1} Triton X-100 in PBS, pH 6.5, and 1 M glucose in PBS, pH 6.5.

Equipment and instruments

Cyclic voltammetry (CV) and chronoamperometric measurements were performed at room temperature with a CHI1230A potentiostat (CH Instruments, Austin, TX) controlled by a CHI1230A software. The fluorescent characterization of the polymeric coating was displayed using an Evos FL Imaging system coupled with a GFP

filter. A Maxi-Mix (Type 16700 Mixer) vortex, pH meter (Seven Easy, Mettler-Toledo, Switzerland) and semiautomatic MMP-SPM printer (Speedline Technologies, Franklin, MA) were also used.

Sensor preparation

The design of the screen-printed electrodes used in this work was an array composed of four working electrodes (bare carbon or GOx-PB-graphite, diameter of 3-mm each one), one counter electrode, and one Ag/AgCl pseudo-reference electrode to perform simultaneous multi-sensing (Figure S2 a).

The screen-printed fabrication process was similar to our earlier work.[1, 2] The customized multi-electrode sensor template was developed using AutoCAD software (Autodesk, San Rafael, CA) and produced by Metal Etch Services (San Marcos, CA), using stainless steel stencils. In the printing process, a sequence of Ag/AgCl conductive ink was used to print the conductive current collector, and then conductive carbon ink or PB/graphite ink was used to print the working and counter electrodes. Finally, the printed layers were cured at 85 °C for 20 min after each printing step.

GOx-PB-electrodes preparation

For the preparation of the glucose biosensors, the PB/graphite screen-printed working electrode surfaces were modified by drop casting 3 µL of a GOx solution (40 mg mL^{-1} containing 10 mg mL^{-1} BSA stabilizer in PBS 0.1 M, pH 6.5) mixed in a 1:1 (v/v) ratio with a chitosan solution (0.5% (w/v) in 0.1 M acetic acid). The electrodes were then allowed to dry overnight, at 4 °C, before use.

Sensor modification with the dissolvable coating

In order to obtain sensors with anti-biofouling properties and delayed activity, the working electrode surface of bare carbon or GOx-PB-electrodes were coated with the polymer Eudragit® L100, which dissolves at pH > 6.0. A single layer (3 µL of 4, 8 or 16% (w/v)) or consecutive layers (3 µL of 16% (w/v)) of the polymeric solution were drop casted onto the electrode surface. After each modification, the isopropanol was evaporated at room temperature.

Coating dissolution experiments

After coating the different working electrodes, the dissolution of the coating layers was evaluated at different times by the incubation of the different sensors in: 0.1 M PBS, pH 6.5; gelatin or Triton X-100 solutions (prepared in 0.1 M PBS, pH 6.5), or directly in undiluted human serum (pH≈6.5), whole blood (pH≈7.0) and whole saliva (pH ~ 8.0) samples. The pH value of all these solutions, greater than 6.0, ensured the complete dissolution of the polymeric coating and the consequent exposure of the working electrode surface.

Electrochemical measurements

CV was selected as the electrochemical technique to characterize the activation or fouling processes over the surface of the carbon working electrodes. Such characterization was evaluated at room temperature using CV scanning from −0.4 to 0.7 V (*vs.* Ag/AgCl) at a rate of 100 mV s^{-1} in 5.0 mM $[Fe(CN)_6]^{4-/3-}$ (0.1 M PBS, pH 6.5).

Chronoamperometric measurements, at GOx-PB-electrodes, were used to monitor the glucose levels in PBS, undiluted blood and saliva samples. The chronoamperometric responses were recorded at room temperature every 30 min in the sample solution, using a potential step of −0.2 V (*vs.* Ag/AgCl) for 60 s.

Biosensing-model application

The percentage of GOx-PB sensor response determined in undiluted saliva and whole blood samples at uncoated or single 16 % coated GOx-PB-electrodes was calculated every 30 min. At each testing time, the corresponding chronoamperometric intensities measured in unsupplemented samples were subtracted from those obtained in 40 mM glucose-spiked samples. Then, the corresponding glucose % signals were calculated considering the 100% signal as the sensor response obtained at time 0 min or 120 min for uncoated or coated electrodes, respectively.

Supporting Tables

Table S1. Estimation of the polymer mass loading deposited over the electrode depending on the number of layers and the % of polymer in the precursor solution used for the coating. Working electrode diameter, 3-mm; polymer drop volume, 3 µL.

Eudragit L100		
Number of layers	**%**	**Mass loading, $\cdot 10^{-4}$ g/cm^2**
	4	17.0
1	8	34.0
		67.9
2	16	135.8
3		203.7

Supporting figures

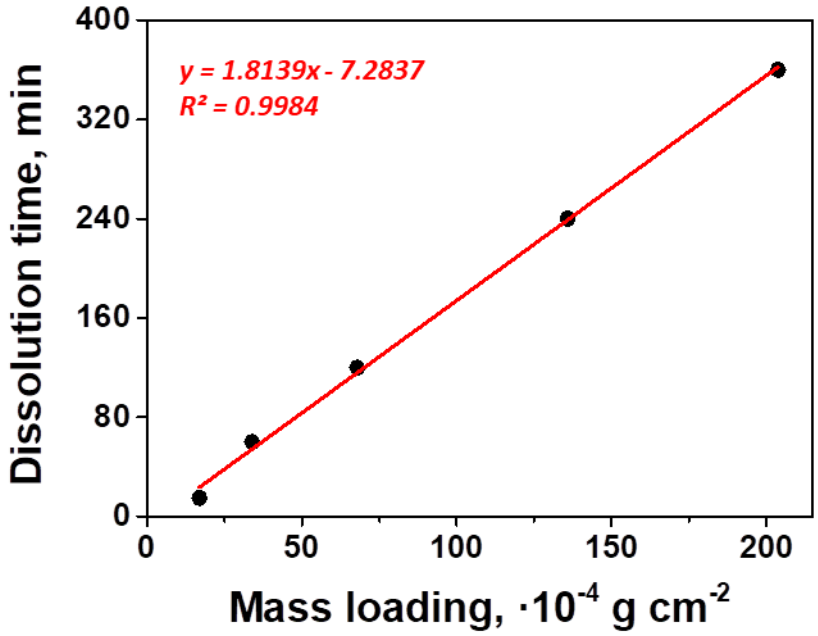

Figure S1. Dependence of the coating dissolution time (results shown in Figures 2a and 2b in the main text) with the polymer mass loading deposited over the electrode.

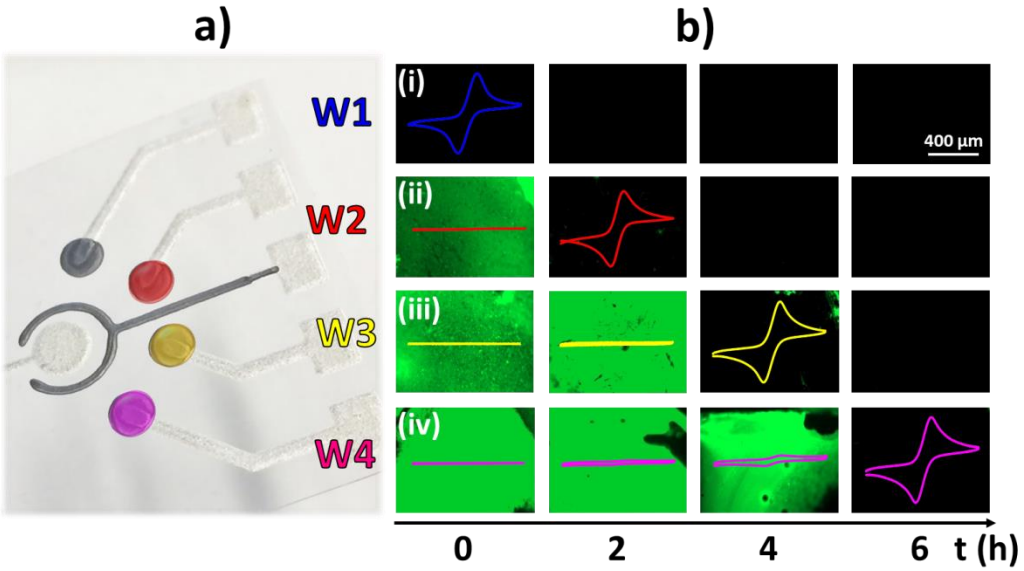

Figure S2. Fluorescence characterization of the polymeric coating at different times over the multiple sensors. a) Image showing the design of the 4-electrode array, each electrode coated with different number of layers of 16% coating. b) Fluorescence images and CVs obtained at the 4 electrodes coated with different layers (from (i) to (iv): 0, 1, 2, and 3 layers) of a FITC-dye labeled coating along with the corresponding CV curves obtained for each electrode at specific activation times. CV: 5 mM $[Fe(CN)_6]^{4-/3-}$ in 0.1 M PBS pH 6.5, v= 100 mV s^{-1}.

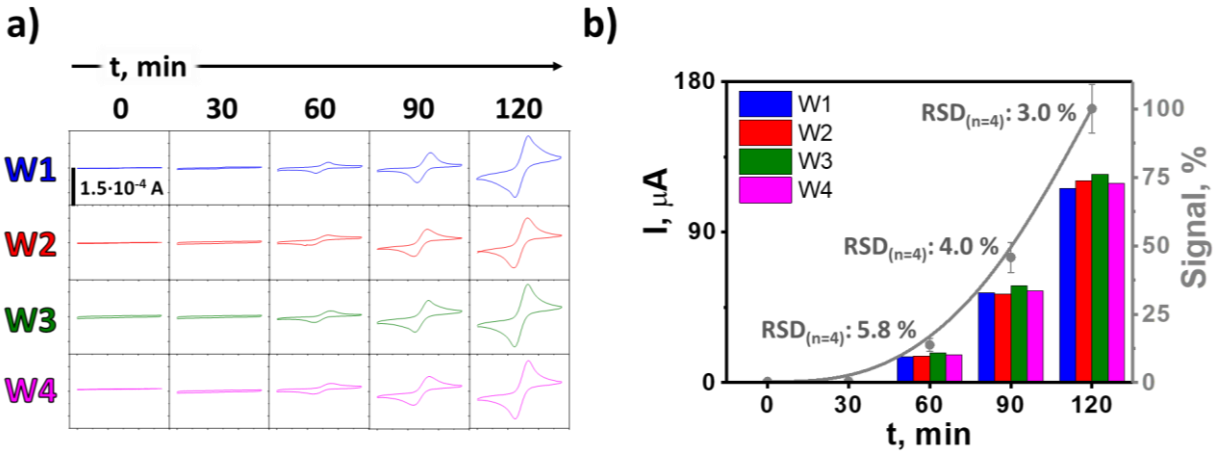

Figure S3. Reproducibility of the delayed sensors. CVs (a) and oxidation peak currents (b) obtained from four different working electrodes, each coated with a single layer of the 16% coating, at specific activation times. RSD values corresponding to the 60, 90, and 120 min activation times are displayed in (b). CV: 5 mM $[Fe(CN)_6]^{4-/3-}$ in 0.1 M PBS pH 6.5, v= 100 mV s^{-1}.

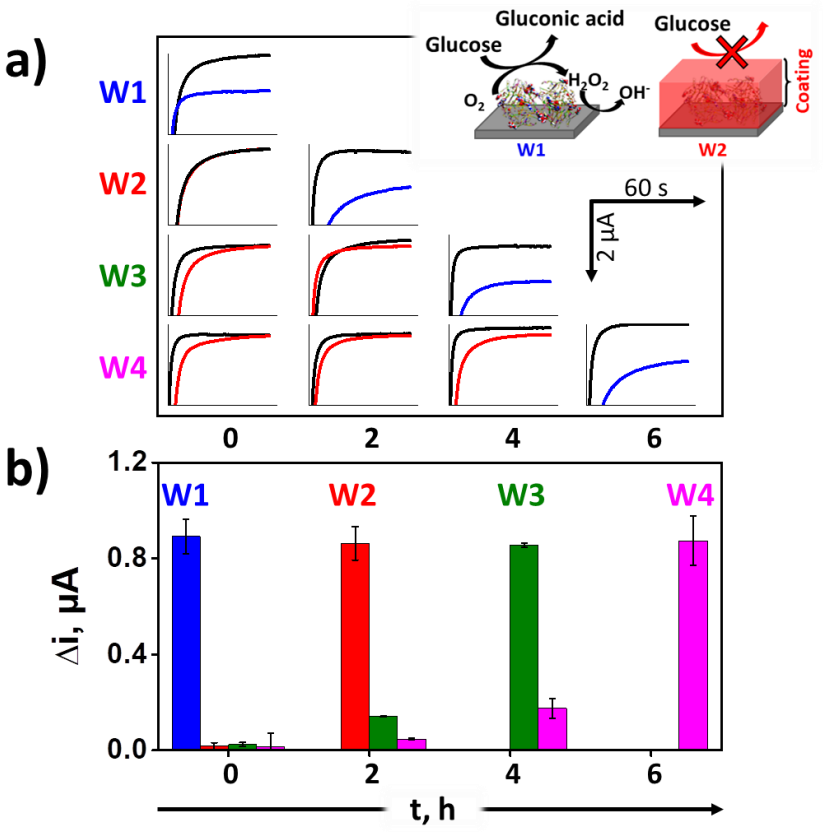

Figure S4. Delayed biosensors for glucose detection. Chronoamperograms (a) obtained in absence (black curves) or presence of 3 mM glucose (blue: activated electrode; red: deactivated electrode) at specific activation times (W1-W4: uncoated or coated with 1-3 layers of the 16 % coating, respectively) and corresponding currents obtained in the presence of 3 mM glucose (b). Inset in (a): schematic concept of the coated-delayed biosensor for glucose detection. E_{app}= −0.2 V (*vs.* Ag/AgCl), 60 s, in 0.1 M PBS pH 6.5.

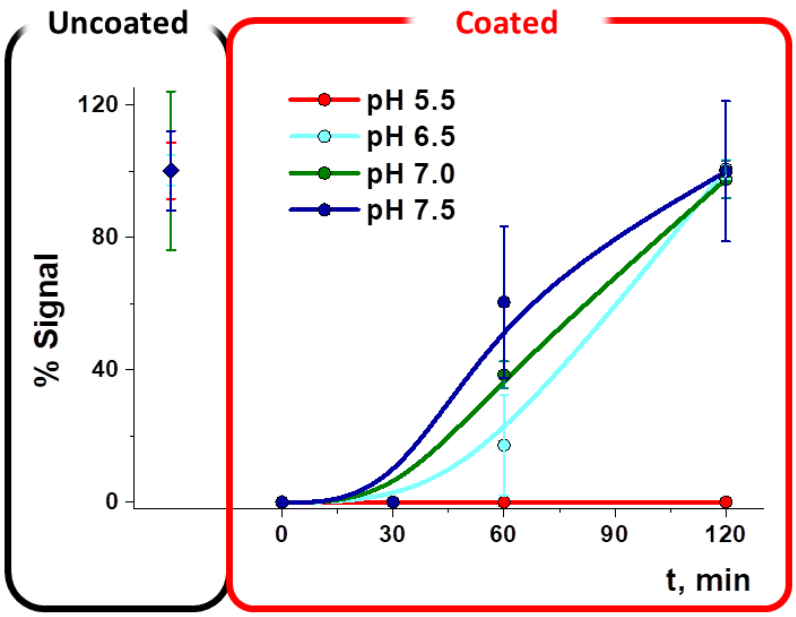

Figure S5. Relative (%) response of GOx-PB sensors (10 mM glucose) in 0.1 M PBS adjusted to different pH values (5.5, 6.5, 7.0 and 7.5, red, cyan, green and blue, respectively), corresponding to uncoated (left side) or coated (single layer with 16%; right side) electrodes at specific exposure times (0, 30, 60 and 120 min).

References

(1) Sempionatto, J. R.; Nakagawa, T.; Pavinatto, A.; Mensah, S. T.; Imani, S.; Mercier, P.; Wang, J. *Lab Chip* **2017**, *17,* 1834−1842.

(2) Bandodkar, A. J.; Jia, W.; Yardımcı, C.; Wang, X.; Ramirez, J.; Wang, J. *Anal. Chem.* **2015**, *87,* 394−398.