

RBD-Modified Polyaniline-Based Label-Free Immunosensor for Sensitive Impedimetric Detection of Anti-SARS-CoV-2 Antibodies

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Electrodeposition of aniline	<ul style="list-style-type: none">• 0.1 M aniline in 0.5 M HCl,• 5 CV cycles, -0.2 V to $+0.8$ V, 100 mV s^{-1},• Electrodeposition carried out in a solution, not in a drop,• Freshly distilled aniline was used.
Crosslinking	<ul style="list-style-type: none">• 2.5% glutaraldehyde in 0.1 M PBS, pH=7.4,• 10 μL solution applied on a regular working SPCE,• 6 μL solution applied on a dual working SPCE.
Sensing element	<ul style="list-style-type: none">• Positive control: SARS-CoV-2 spike RBD ($10\text{ }\mu\text{g mL}^{-1}$) in PBS, pH=7.4,• Negative control: BSA ($10\text{ }\mu\text{g mL}^{-1}$) in PBS, pH=7.4,• Blocking with Glycine (1.0 M) in PBS, pH=7.4,• 10 μL solution applied on a regular working SPCE, and 6 μL on a dual working SPCE.
Impedimetric measurement	<ul style="list-style-type: none">• Incubation with human anti-SARS-CoV-2 IgG antibody,• Optional amplification with unlabeled secondary goat anti-human IgG antibody,• Measurements with EIS,• 10 μL solution applied on a regular working SPCE, and 6 μL on a dual working SPCE.

Scheme 1. Experimental conditions during immunosensor preparation steps and measurement.

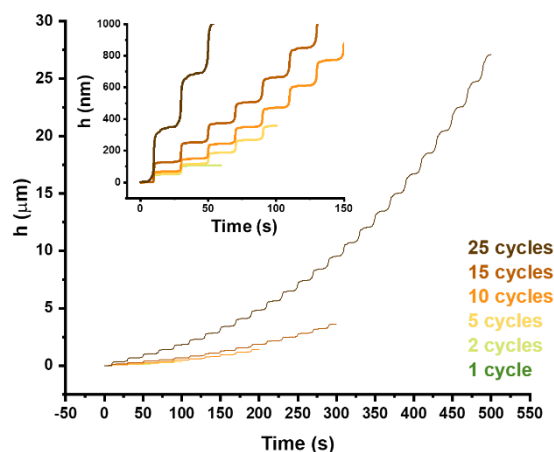


Figure S1. Polyaniline thickness growth profiles obtained for different number of electrodeposition cycles. Data were obtained using Eq. 1 presented in the manuscript.

Complementary enzyme-linked immunosorbent assay (ELISA)

The performance of the electrochemical immunosensor was compared with that of an indirect ELISA assay. A 96-well microplate (Greiner Bio-One No. 675061) was coated with RBD dissolved in PBS at a concentration of 60 ng per well and incubated for one hour at room temperature. In subsequent steps, wells were blocked with 1x ELISA/ELISPOT Diluent (Invitrogen No. 00-4202) for one hour and incubated with human anti-SARS-CoV-2 IgG antibodies and goat anti-human IgG antibodies conjugated to horseradish peroxidase (also diluted in 1x ELISA/ELISPOT Diluent) for another hour each. After each step, the plate was washed four times in a microplate washer (Tecan HydroSpeed) with a washing buffer containing 0.05% Tween-20 and 10% PBS. Finally, 50.0 μ L of the liquid 3,3',5,5'-tetramethylbenzidine substrate system (Sigma Aldrich No. T8665) was added to each well and left for ten minutes to develop a purple color. After stopping the reaction with 50.0 μ L of 1.0 M H_3PO_4 per well, the absorbances of the samples were measured at 450 nm (signal) and 620 nm (background) using a Synergy Mx multimode microplate reader (BioTek). Antibody concentration was calculated by comparing the real sample's value to the calibration standards' values after subtracting the background absorbance from the signal.