

**Área: ANA**

## 3D-printed carbon black/PLA electrochemical immunosensor for detection of dengue NS1 antigen

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### Highlights

- A portable 3D-printed electrodes system was employed in the development of a label-free immunosensor.
- Anti-NS1 antibody was immobilized on cysteamine-AuNPs modified CB/PLA electrode.

### Abstract

In the past few years, 3D-printing has evolved from a mere concept into a valuable tool in bioanalytical research laboratories, enabling the fabrication of diverse analytical devices [1]. Herein, we report the development of a three-electrode system consisting of a working electrode (WE), a counter electrode (CE), and a pseudo-reference electrode (p-RE), fabricated using a conductive filament composed of carbon black and poly(lactic acid) (CB/PLA), for label-free detection of the dengue virus NS1 antigen. Following the printing process, the electrodes underwent mechanical, chemical, and electrochemical treatments to efficiently remove the surface PLA layer, directly improving their electrochemical performance [2]. Mechanical treatment involved polishing the electrode surface with sandpaper (1200 grit); chemical treatment consisted of immersion in 1.0 mol L<sup>-1</sup> NaBH<sub>4</sub> for 2 h; and electrochemical treatment was performed in 1.0 mol L<sup>-1</sup> NaOH for 900 s at +1.2 V (vs. CB/PLA). After the activation process, the WE was modified with a gold nanoparticle (AuNP) film via chronoamperometric electrodeposition, carried out for 200 s at -0.9 V using chloroauric acid (HAuCl<sub>4</sub>, 5.0 mmol L<sup>-1</sup>) in hydrochloric acid solution (HCl, 0.1 mol L<sup>-1</sup>). Subsequently, to produce the immunosensor, a CB/PLA/AuNP electrode was initially treated with a 100.0 mmol L<sup>-1</sup> cysteamine solution for 1 h to introduce amine groups on the AuNP surface, resulting in an amine-functionalized AuNP film. An aliquot of solution containing anti-NS1 (5.0 µg mL<sup>-1</sup>), EDC (5.0 mmol L<sup>-1</sup>), and NHS (8.0 mmol L<sup>-1</sup>) was then applied on the modified surface and kept for 1 h to enable covalent immobilization of the antibodies. To avoid non-specific adsorption, the electrode surface was blocked with bovine serum albumin (BSA) after anti-NS1 immobilization. All steps were performed at room temperature, and after each process, the electrodes were carefully rinsed with phosphate-buffered saline (PBS, pH 7.4). Cyclic voltammetry, electrochemical impedance and scanning electron microscopy were performed at each stage of immunosensor production for its characterization. To improve the analytical performance of the immunosensor, experimental parameters (DPV technique, reagent/biomolecule concentrations, immobilization and incubation times, among others) are being optimized. DPV analyses were performed before and after incubation of the immunosensor in a sample containing NS1 antigen, using a 1.0 mmol L<sup>-1</sup> K<sub>4</sub>[Fe(CN)<sub>6</sub>] solution in 0.2 mol L<sup>-1</sup> KCl. The proposed immunosensor has already demonstrated the ability to detect the NS1 antigen at a nanogram per milliliter level (0,4 ng mL<sup>-1</sup>) in a simulated sample, indicating its potential for early detection of the biomarker.

[1] ZHANG, C. et. al. *Bioanalysis*, v. 9, n. 4, p. 329-331, 2017.[2] PRADELAFILHO, L. A. et. al. *Analytical and Bioanalytical Chemistry*, v. 416, n. 21, p. 4679-4690, 2024.

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