

**Área: ANA****Portable electrochemical immunosensor for detection of dengue NS1 protein based on alizarin-carbon nanotubes modified screen-printed carbon electrode****Mariana Booz (IC),<sup>1\*</sup> Júlia T. Eble (IC),<sup>1</sup> Daniela Z. Mezalira (PQ),<sup>2</sup> Daniela Brondani (PQ)<sup>1</sup>****[mariana.booz@grad.ufsc.br](mailto:mariana.booz@grad.ufsc.br)**<sup>1</sup>Departamento de Ciências Exatas e Educação, Universidade Federal de Santa Catarina, Campus Blumenau;<sup>2</sup>Departamento de Química, Universidade Federal de Santa Catarina, Campus Florianópolis.

Keywords: Electrochemical biosensor, Screen-printed carbon electrode, Dengue virus, NS1 biomarker.

**Highlights**

- Alizarin-CNTs and anti-NS1-AuNPs were immobilized on the SPCE to obtain the immunosensor.
- The alizarin redox current before and after NS1 attachment was monitored as a function of the NS1 concentration.

**Abstract**

Dengue is one of the key infections with worldwide public health impact, as 40% of the world's population reside in areas in which dengue is endemic.<sup>1</sup> A clinical diagnosis of dengue may also be challenging because some of the symptoms are similar to those of fellow tropical diseases.<sup>2</sup> The non-structural 1 (NS1) protein is a prognostic marker for severe dengue, and elevated levels of NS1 in the blood are correlated with a higher risk of developing Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS).<sup>3</sup>

Considering that, a portable label-free electrochemical immunosensor was developed for NS1 biomarker voltammetric detection. First, a screen-printed carbon electrode (SPCE) was produced in the laboratory with customized carbon ink. A dispersion with approximately 1 mg of alizarin and 1 mg of carbon nanotubes (CNTs) in 2 mL water:DMF (1:1) was previously prepared (overnight), resulting in efficient dye adsorption on the nanomaterial surface. An aliquot of alizarin-CNTs dispersion was dropped on the SPCE working electrode, and the solvent was evaporated under vacuum. Citrate-stabilized gold nanoparticles (AuNPs) were used to anchor the anti-NS1 monoclonal antibodies. An aliquot of anti-NS1/AuNPs was dropped on alizarin-CNTs/SPCE, and after 15 min washed with phosphate buffered saline (PBS 0.1 mol L<sup>-1</sup>, pH 7.0) to remove non-immobilized antibody. Then NS1 antigen solution was incubated on the immunosensor surface for a determined time and washed with PBS for subsequent analysis. The electrochemical measurements were done using square wave voltammetry (SWV) before and after NS1 protein incubation.

Electrochemical characterizations (cyclic voltammetry and electrochemical impedance spectroscopy) were performed at each stage of the immunosensor construction. Experimental parameters were optimized to improve the analytical performance of the immunosensor. Various conditions were investigated, including: (i) SWV technique parameters to enhance the redox probe response; (ii) alizarin and CNTs (evaluating functionalization) mass quantities to improve the formation and stability of the nanocomposite film; (iii) anti-NS1 concentration and immobilization time; and (iv) NS1 protein incubation time, seeking efficient antibody-antigen interaction with a feasible analysis time.

SWV measurements (potential range -0.2 to +0.6 V vs. graphite, amplitude of 75 mV s<sup>-1</sup>, frequency of 10 Hz, and increment of 1 mV s<sup>-1</sup>) were used for analytical purposes, with PBS as the supporting electrolyte and without the need for the addition of a redox probe solution. A proportional decrease in the alizarin oxidation peak current (*i<sub>p</sub>*) was observed with an increase in analyte NS1 concentration, allowing the quantification of this biomarker. Under the previously optimized main conditions, satisfactory results were obtained after 15 min of antigen incubation, and the NS1 protein was detected in a range of 0.05 to 7 µg mL<sup>-1</sup>. Some optimizations are still ongoing to improve this performance, as well as studies on selectivity, stability, and application in simulated blood serum.

<sup>1</sup>FEBS J., 282 (2015), pp. 3368-3394; <sup>2</sup>Nat. Rev. Microbiol., 8 (2010), pp. S30-S37; <sup>3</sup>Diagn. Microbiol. Infect. Dis., 68 (2010), pp. 50-54.**Acknowledgments**

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