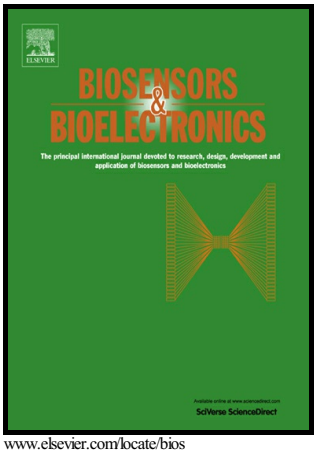

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An experimental design approach to optimize an amperometric immunoassay on a screen printed electrode for *Clostridium tetani* antibody determination

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Highlights

- An innovative amperometric immunosensor with the antigen directly attached onto a screen printed electrode for the sensitive and rapid detection of *Clostridium tetani* antibody
- A response surface methodology using a central composite design permitted a limited number of experiments for the optimization of four parameters affecting the immunoassay response
- Minimum immunosensor and sample handling for possible point of care assays.

Abstract

An immunoassay for the determination of anti-*tetani* antibodies has been developed using a screen printed electrode (SPE) as solid support for toxoid (antigen) immobilization. The assay was performed in guinea pig serum. The immunoreaction and the subsequent amperometric detection occurred directly onto the SPE surface. The assay consisted of spiking the anti-*tetani* sample directly onto the toxoid modified SPE, and then a second antibody, i.e. a HRP-labeled anti-immunoglobulin G, was deposited onto the biosensor. Subsequent amperometric detection was realized by spiking 10 µL of a hydroquinone (HQ) solution into 40 µL of buffer solution containing hydrogen peroxide. An experimental design approach was implemented for the optimization of the immunoassay. The variables of interest, such as bovine serum albumin (BSA) concentration, incubation times and labeled antibody dilution, were optimized with the aid of the response surface methodology using a circumscribed central composite design (CCCD). It was observed that two factors exhibited the greatest impact on the response, i.e. the anti-*tetani* incubation time and the dilution factor of the labeled antibody. It was discovered that in order to maximize the response, the dilution factor should be small,