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Food Safety

ELECTROCHEMICAL CHARACTERIZATION OF AN IMMUNOSENSOR FOR DETECTION OF SALMONELLA SPP.

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Abstract

The absence of Salmonella spp. in foods is a requirement of health authorities worldwide. However, conventional detection methods are laborious and especially time consuming, requiring several days to confirm the result. In this context, biosensors for detection of pathogenic bacteria represents a faster alternative diagnosis. This study was conducted with the objective of developing and evaluating the performance of an electrochemical immunosensor for detection of Salmonella spp. In the assembling of the sensor one gold surface was modified by self-assembled monolayers technique using thiol cysteamine (10 mM) for 3 h at room temperature. The protein A was used for the directed immobilization of the primary antibody after activation of carboxyl groups with EDC/NHS (2 mM/ 5mM). Optimization studies were conducted to determine the pre-treatment step, and the primary antibody and protein A ideal concentrations by applying the cyclic voltammetry technique.The electrochemical characterization studies of speed scan and stability of bindings were also made by using the same technique. The analytical response of the immunosensor was obtained using a secondary antibody labeled with enzyme peroxidase, and the signal was evaluated by applying the chronoamperometry technique. The immunosensor was developed based on the results of the optimization studies. The response curve was prepared by exposing the device to different concentrations of bacteria. Salmonella Typhimurium, strain ATCC 51812 was used as a standard. Bacteria were grown in nutrient broth for 24 h, and after were centrifuged at 4000 rpm for 30 min. The cells were suspended in phosphate buffer solution, and serial dilutions were prepared in the same solution. The quantification of cells was determined by spread plating 0.1 mL of each dilution in nutrient agar. The polishing suface with alumina followed by sonication in 96% ethanol was quite efficient for the pre-treatment. In the optimization step of the protein A and primary antibody, higher analytical signal was obtained for concentrations of 7.5 and 75.0 mg mL^{-1}, respectively. The electrochemical characterization showed decrease in the current electric of the voltammograms in 5 mM $K_3[Fe(CN)_6]$ and 0.1 M KCl solution confirming the efficiency of the process and reached a total coverage of 51.8 % of the electrode surface. The stability of the biomolecules bindings was evaluated after 30 cycles of cyclic voltammetry. The immunosensor showed a coefficient of variation of 17% indicating a good stability. The device had presented a qualitative behavior in diferent concentrations of Salmonella with a detection limit of 10 CFU mL^{-1}. The immunosensor requires at least 125 min for the results proving to be a more rapid detection of Salmonella spp., as well as the possibility of application to assurance food safety in the production chain. Further, tests has been conducted in milk to assess the effectiveness of the immunosensor in a food matrix.