



Development of an amperometric immunosensor for detection of staphylococcal enterotoxin type A in cheese

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ABSTRACT

This paper reports a novel electrochemical immunosensor for the sensitive detection of staphylococcal enterotoxin A (SEA) based on self-assembly monolayer (SAM) and protein A immobilization on gold electrode. Three different methods of protein A immobilization were tested: physical adsorption, cross-linking using glutaraldehyde and covalent binding after activation with N-hydroxysuccinimide (NHS)/N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) on cysteamine-modified gold electrode. The EDC/NHS method for protein A immobilization was selected to lead development of the biosensor. The coating steps of the surface modification were characterized by cyclic voltammetry and the biosensor response by chronoamperometry. The advantages of the immunosensor were exposed in its high sensitivity and specificity. The proposed amperometric immunosensor was successfully used for determination of SEA in contaminated and non-contaminated cheese samples with excellent responses.

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1. Introduction

Staphylococcal food-poisoning is an intoxication resulting from the ingestion of foods containing one or more enterotoxins produced by some strains of *Staphylococcus aureus* (Derzelle et al., 2009). Staphylococcal enterotoxins (SEs) are classified as members of the pyrogenic toxin superantigen family. They have low molecular weight proteins (26–30 kDa), are heat stable, resistant to gut proteases and stable over a wide range of pH (4–10) (Dinges et al., 2000; Le Loir et al., 2003; Omoe et al., 2005). Among the staphylococcal enterotoxins, the staphylococcal enterotoxin type A (SEA) is one of the most important enterotoxins recovered from food poisoning outbreaks followed by staphylococcal enterotoxin type B (SEB) and staphylococcal enterotoxin type D (SED) (Balaban and Rassoly, 2000). It has been estimated that the food industry spends, on average, 1.5% to 2% of its profits on quality control and appraisal (Prodromidis and Karayannis, 2002).

Conventional methods for detecting the presence of SEs are complex, requiring fully automated systems (Lancette and Bennett, 2001). Therefore, the rapid methods, which generally come in kit form, are the only practical options for routine testing laboratories, although they are not inexpensive. None of the methods mentioned are ideal for use by a small or medium sized manufacturer, considering that the methods are

either very expensive requiring specialized equipment or demand qualified people to use them.

Demands of sensitivity, specificity, easy use and low cost of analytical measurements have stimulated considerable interest in the development of biosensors as diagnostic tools in the food industry (Leonard et al., 2003; Velusamy et al., 2010). The literature reports some studies about optic biosensors for detection of staphylococcal enterotoxins in food (Strachan et al., 1997; Rassoly and Rassoly, 1999; Homola et al., 2002). However, this type of transducer is more expensive and susceptible to interferences of ambient light. Electrochemical biosensors are more attractive due to large amount existing technology of other commercial biosensors. This fact makes it easier to miniaturize the biosystem. Electrochemical immunosensors based on the specific reaction of the antibody and antigen with electrochemical transduction has been an attractive subject for the food analysis (Marquette and Blum, 2006; Ricci et al., 2007). A frequently used format in electrochemical immunosensing is of an amperometric immunosensor employing a sandwich assay, where antibodies are immobilized on a conductor surface to bind to antigens and antibodies are labeled with enzymes such as horseradish peroxidase (HRP), producing electroactive species from an added substrate, in order to obtain an analytical signal.

A diversity of immobilization platforms have been employed for the immunosensor development. Self-assembly monolayers (SAMs) are chemisorbed spontaneously on gold substrates forming well-ordered molecular assemblies (Campuzano et al., 2006; Brooksby et al., 2006). Cysteamine (HS-CH₂-CH₂-NH₂) is a thiol with a short chain length that has two functional groups that can be used as a bridge between

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the electrode and the proteins. In this study, the use of cysteamine SAM is evaluated for the optimum surface modification of gold electrode and the immobilization of the protein A for obtaining an orientation-controlled immobilization of the antibodies.

Protein A, from *Staphylococcus aureus*, is a highly stable receptor, capable of binding to the crystallizable fragment (Fc) of immunoglobulins, such as IgG from a large number of species. The fragment antigen-binding (Fab fragment) of IgG antibody are thus oriented away from the solid phase (Muzzucchelli et al., 2010). The main advantages of using protein A in the construction of immunosensors are the binding to a wide variety of IgG via the Fc fragment, the stability in a wide pH range and the dissociation of the IgG–protein A complex under controlled conditions (pH 3.5 to 4.5) (Babacan et al., 2000; Marxer et al., 2003; Chen et al., 2007).

Protein A is assembled on the cysteamine monolayer through different methods. The three main are: physical adsorption, cross-linking using glutaraldehyde and covalent binding after activation with N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) (Tili et al., 2004; Mendes et al., 2009). In general, in the construction of immunosensors only one of these methods has been explored and the performance of each one has not been compared in the detection of the target specie. In this study, we developed an immunosensor after comparing the electroanalytical response employing each method of immobilization mentioned above. The best method was selected to develop an amperometric immunosensor for detection of SEA. Finally, commercial cheese samples, previously tested with a commercial immunological kit, were evaluated by the developed biosensor.

2. Materials and methods

2.1. Reagents and apparatus

Peroxidase from horseradish (HRP) (250 U mg⁻¹), glutaraldehyde (25%), N-hydroxysuccinimide (NHS), N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and cysteamine were purchased from Sigma Aldrich. All other chemicals were from analytical-reagent grade and used as received without further purification. Brain heart infusion agar (BHI) (Difco, USA) and brain heart infusion broth (BHIB) (Difco, USA) were used for the growth of the enterotoxigenic *S. aureus* strain 722FRI (Food Research Institute Wisconsin, USA). The staphylococcal enterotoxin kit SET-RPLA test (including the standard enterotoxins SEB, SEC and SED) was acquired from Oxoid. All solutions were prepared with Milli-Q water.

2.2. Staphylococcal enterotoxin A (SEA) production

The production of SEA was assayed by using membrane-over-agar (MOA) plates (Casman and Bennett, 1963). Plates were prepared with 25 mL of BHI agar containing 1% yeast extract and 0.1% dibasic potassium phosphate. The agar layer was covered with a membrane disk made of Spectra/Pore Membrane Dialysis Tubing 600–8000 with width of 10 mm.

Previously, the culture of enterotoxigenic *S. aureus* was prepared in BHI broth and incubated at 37 °C for 48 h. Aseptically, the membrane was inoculated with 500 µL of the culture. The inoculum was homogenized on the surface and incubated at 37 °C for 24 h (Robbins et al., 1974). After the incubation period, the cultures were removed from the membranes by washing with 2.5 mL of 0.02 M Na₂HPO₄, pH 7.4. After washing, the material collected was centrifuged at 10,000 ×g for 15 min, and the supernatant fluid was collected for SEA testing (Braga et al., 2005). The optimum-sensitivity-plate (OSP) method was used to detect the presence of SEA in the culture supernatant according to Robbins et al. (1974). The purified antibodies anti-SEA were obtained from the Ezequiel Dias Laboratories (Belo Horizonte, Brazil).

In the following step, the antibodies were diluted in 1% glutaraldehyde solution with four times the concentration of peroxidase for 1 h and then,

they were dialyzed overnight against PBS pH 7.0 (Avrameas, 1969). The antibodies against SEA, after being conjugated to peroxidase were used in all remaining studies at 30 µg mL⁻¹.

2.3. Construction and assessment of the immunosensor

2.3.1. Pretreatment of the gold surface

First of all, the gold electrode ($\phi_{\text{int}} = 0.025 \text{ cm}^2$) was cleaned using four different procedures: a) mechanical polishing of the electrode surface with alumina (0.3 µm); b) cleaning in deionized water in an ultrasonic bath for 5 min; c) immersion in piranha solution (1:3 H₂O₂: H₂SO₄) for 3 min and d) electrochemical cleaning by 25 successive scans in 0.5 M H₂SO₄ aqueous solution with potential sweep from 0 to 1.5 V.

2.3.2. Immobilization procedures

All immobilization steps were performed on a pre-treated gold surface. The electrodes were immersed ethanolic solution solution of 10 mM cysteamine for 3 h at room temperature to form the cysteamine monolayer, rinsed with ethanol and water, and dried at room temperature (Mendes et al., 2008). Thereafter, three different methods were used to immobilize protein A:

- Direct adsorbing protein A on a modified gold electrode to capture the anti enterotoxin A. The modified electrode with Cysteamine was incubated in 5 mg mL⁻¹ of protein A solution diluted in 10 mM phosphate buffered saline (PBS) (pH 7.4) for 1 h.
- Immobilization of protein A through the cross-linking agent glutaraldehyde. The modified electrode with cysteamine was incubated in aqueous solution of 2.5% glutaraldehyde for 1 h. The electrode was, subsequently, incubated in 5 mg mL⁻¹ protein A solution for 1 h. After each incubation step, the electrode was washed with 10 mM PBS (pH 7.4).
- Immobilization of protein A through covalent binding after the activation of carboxy groups with EDC/NHS (Susmel et al., 2000; Tili et al., 2004). In the first step, solution of 2 mM EDC and 5 mM NHS solution was freshly prepared in an acetate buffer (pH 5.0) reacted for 30 min. Thereafter, 5 mg mL⁻¹ of protein A was added the EDC/NHS solution and left to react for 1 h. Finally, the modified electrode with cysteamine was incubated in protein A and EDC/NHS solution for 1 h. After each incubation step, the electrode was washed with 10 mM PBS. Then, the electrode was incubated in anti-SEA (100 mg mL⁻¹) overnight. The non-specified sides of the modified electrode were blocked 1% BSA for 1 h. After washing, the electrode was incubated in 30 µL of SEA (1 mg L⁻¹, pH 7.4) for 1 h.

2.3.3. Analytical response

The analytical response was performed through the exposure of the modified electrode in samples containing SEA for 1 h at room temperature. Next, the electrode was incubated overnight with polyclonal antibodies against SEA conjugated to peroxidase. After rinsing with PBS, the electrode was ready for analytical measurements. These were performed in an electrochemical system of three electrodes using Ag/AgCl as a reference and helical platinum wire as a counter electrode. Studies of cyclic voltammetry were performed at scan rate of 100 mV s⁻¹, equilibration time for 5 s, with a potential sweep: i) –0.4 to 0.7 V in 4 mM K₃[Fe(CN)₆] and 1 M KCl solution, ii) –0.2 to 0.3 V in 0.1 mol L⁻¹ PBS (pH 7.4). The amperometric response was generated by the HRP reaction with 100 µM hydrogen peroxide in the presence of 60 µM hydroquinone in an electrochemical cell (10 mL) containing 0.1 mol L⁻¹ PBS (pH 7.4). The response was determined by polarizing the gold electrode at –35 mV until a stable baseline (steady state) was reached, in approximately less than 30 s.

All measurements were conducted at room temperature in solution purged with N₂ for 15 min. Electrochemical measurements were

performed with the potentiostat/galvanostat Autolab/ PGSTAT12 and the software GPES (Eco Chemie, The Netherlands).

2.3.4. Surface characterization

A scanning electron microscope (SEM; Quanta 200 FEG System; FEI Company, USA) was employed to observe the surface of the gold surface modified with cysteamine–protein A–anti SEA. The images were obtained using a scanning voltage of 20 kV.

2.3.5. Assessment of biosensor responses in cheese sample

Samples of commercial handmade cheese type Coalho (Jaguaribe, Brazil) were conducted to laboratory under refrigerated conditions (4 °C). The samples were divided into 25 g aliquots, homogenized in 0.85% NaCl solution in the 1:1 ratio and centrifuged at 9000 ×g for 30 min at 4 °C. The supernatant was filtered through 0.45 mM membrane Millipore and purified with chloroform (1:1) and again centrifuged. The first step was the evaluation of Coalho cheese samples to verify the presence of SEA. This was conducted by enzyme immunoassay RPLA (Reversed Passive Latex Agglutination) using the SET-RPLA test kit (Oxoid®). Thereafter, the same samples were evaluated by the developed biosensor.

2.4. Statistical analysis

All reported data are means of three replicates analyses. The standard deviation (SD) was determined for each mean. Coefficient of variation which was defined as the sample standard deviation divided by the sample average and multiplied by 100 was also calculated for some of the data.

3. Results and discussion

3.1. Preparation of immunosensor

The schematic diagram of the immunosensor construction and SEA binding is shown in Fig. 1. First, a monolayer of cysteamine was formed on the electrode surface through the strong Au–thiolate bond. The next step was the protein A immobilization which bound to the crystallizable fragment (F_c) of IgG. Then, a specific binding event occurred between the immobilized antibodies and the antigens presented in the sample. Finally, the HRP-labeled antibodies bound to SEA, forming a sandwich assay.

3.2. Assessment of immobilization methods for protein A

The method of biomolecules immobilization on various conductive surfaces determines important operating characteristics of the immunosensor, such as sensitivity, selectivity and stability. The use of cysteamine on gold surface allows the stable attachment of proteins. The protein A binding to the modified electrode makes it feasible to immobilize an antibody in an oriented form and favors the antigen–antibody interaction, which can further improve the stability and sensitivity of the immunosensor. In this regard, three immobilization methods of

protein A were evaluated for the percentage of surface coverage and their influence in the biosensor response in the presence of SEA: physical adsorption, covalent binding through the activation with EDC/NHS and cross-linking using glutaraldehyde. The percentage of surface coverage for cysteamine–protein A assembly was calculated according to Furtado et al. (2012).

The biosensor response constructed from the three different methods of immobilization was evaluated by cyclic voltammetry in PBS solution containing $60 \mu\text{mol L}^{-1}$ hydroquinone and $100 \mu\text{mol L}^{-1}$ hydrogen peroxide, considering the cathodic peak current amplitude obtained from voltammograms. There was a relationship between the percentage of surface coverage and biosensor response, where the methods of protein A immobilization that showed higher surface coverage had a lower response in the presence of SEA (Table 1). This fact is associated with the electron flux involved in the reduction–oxidation (redox) reaction in the interface solution–biosensor. If the percentage of surface coverage is high, a hindrance of redox reactions close to surface occurs resulting in the loss of biosensor sensibility.

In the protein A immobilization method involving EDC/NHS a lower surface coverage electrode and higher current of cathodic peak was verified indicating that this method of immobilization favors the electron flux involved in the redox reaction and is efficient in the recognition of SEA. In this method, an efficient and fast immobilization of protein A is made possible by utilizing the electrostatic attraction forces through the concentration of the carboxy reactive groups of protein and the amine group of the cysteamine. This gives a high local concentration of the reactive protein A in the cysteamine surface, whereby covalent binding can occur, forming stable amide bonds (Löfås and Johnsson, 1990). The assembly of cysteamine–protein A–primary antibody anti SEA (first antibody) on the gold surface was characterized through SEM (Fig. 2a). According to this figure, the presence of small granules, possibly due to agglomerations of antibodies in the modified surface was verified.

According to Fig. 2b, the immobilization method employing EDC/NHS showed a clear advantage over the other methods tested: a resolute cathodic peak and low capacitive current. This last characteristic is particularly desirable for amperometric biosensor devices because they measure the Faradaic current that arises on a biosensor surface. This current depends upon the flux of the reaction product in solution (Skoog et al., 2006). The stability of the bindings employing the EDC/NHS immobilization was evaluated after 50 cycles of cyclic voltammetry. The biosensor showed a coefficient of variation of 0.33% indicating a good stability of the biosystem (Fig. 2c).

After modification of gold electrode with cysteamine–protein A–anti SEA immobilization, the SEA presented in the sample was captured by anti-enterotoxins, remaining attached to the modified electrode. Then, HRP-labeled anti-enterotoxin interacted with SEA. The addition of H_2O_2 solution to this immuno-reaction system initiated the redox reaction catalyzed by HRP.

The amperometric responses of the biosensor were generated from enzymatic reaction (HRP) in the presence of substrate (H_2O_2) using mediator. In this study, hydroquinone was used as mediator in order

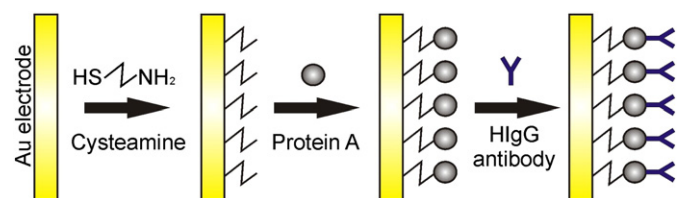


Fig. 1. Schematic illustration of the assembly cysteamine/protein A on gold electrode for immobilization of antibody anti-SEA.

Table 1

Percentage of gold surface coverage for the three methods of protein A immobilization and potential of the cathodic peak (E_{pc}) of the voltammograms in 4 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$ and 1 M KCl solution. The biosensor response was verified in the presence and absence of HRP-labeled antibody in PBS solution pH 7.4 containing $60 \mu\text{M}$ hydroquinone and $100 \mu\text{M}$ hydrogen peroxide. Concentration of SEA: 1 mg mL^{-1} .

Method	Coverage (%)	E (mV)	Anti-SEA conjugate ΔJ (A cm^{-2})
Bare surface	–	–0.037	–
Absorption	5.2	–0.022	$1.68 \times 10^{-7} (\pm 0.24)$
Glutaraldehyde	10.7	–0.050	$1.13 \times 10^{-7} (\pm 0.01)$
EDC/NHS	2.5	–0.023	$2.46 \times 10^{-7} (\pm 0.20)$

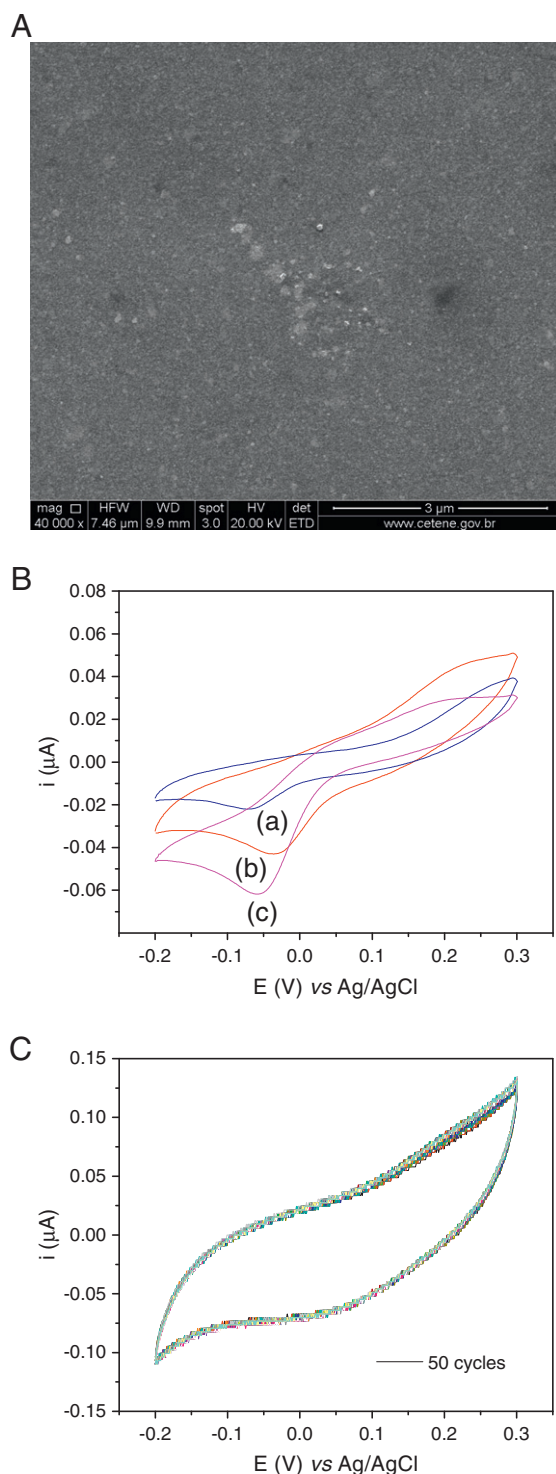


Fig. 2. (A) Scanning electron micrograph of the gold electrode with cysteamine-protein A-anti-SEA immobilized (40,000 $\times$, 20 kV). (B) Cyclic voltammetry of the biosensor for the three methods of protein A immobilization: (a) physical adsorption (b), glutaraldehyde (c), EDC/NHS in PBS solution pH 7.4 containing 60 μ M hydroquinone and 100 μ M hydrogen peroxide. (C) Cyclic voltammetry after 50 scans of the biosensor by the protein A immobilization method using EDC/NHS in PBS solution pH 7.4 at 100 mV s^{-1} of scan rate. Concentration of SEA: 1 mg mL^{-1} .

to decrease susceptibility to interfering substances and obtain low work potential, avoiding the chances of biomolecules denaturation (Chaubey and Malhotra, 2002). Hydroquinone is one of the most commonly used mediators in antibodies-labeled HRP (Lu et al., 1997; Dai et al., 2004). It is a low molecular weight redox couple, which shuttles electrons from

the redox center of the enzyme to the surface of the indicator electrode. During the catalytic reaction, the mediator reacts with the reduced enzyme and then diffuses to the electrode surface to undergo rapid electron transfer (Rosatto et al., 2001). A scheme of the reactions involved in the determination of the SEA is presented in Fig. 3.

The high increase in the current of the cathodic peak potential is associated with peroxidase activity in the biosensor that catalyzes the oxidation of hydroquinone to p-quinone in the presence of hydrogen peroxide and, subsequently, the p-quinone formed is reduced electrochemically on the surface of the biosensor (Lei et al., 2004). Thus, this potential was selected as a working potential of the biosensor for the chronoamperometric measurements.

3.3. Characterization electrochemical of the biosensor

Considering that the best method of protein A immobilization was employing EDC/NHS, this one was selected to the subsequent studies.

Cyclic voltammetry can provide useful information on the changes of the electrode behavior after each assembly step. As shown in Fig. 4a, the redox probe $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$ reveals a reversible cyclic voltammogram at a bare gold electrode. After the pretreated gold electrode was covered with cysteamine, the penetration of the redox probe close to the surface electrode was slightly reduced. Then, a decrease in the current response was observed (Fig. 4a). After the immobilization of protein A on the modified gold electrode the penetration of the redox probe was further reduced. This fact was also verified after the binding of the first antibody-SEA-HRP-labeled antibody.

Cyclic voltammograms of the modified electrode were obtained in a 1 M KCl and 4 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$ solution at a variety of scan rates. Fig. 4b shows that the relationship between the peak current (I_p) and the square root of the scan rate ($v^{1/2}$) is linear for the modified electrode, which suggests that the electrode reactions occurring on the electrode are nearly reversible and the mass transfer phenomenon in the double layer region of the electrode is mainly controlled by diffusion (Song et al., 2010).

3.4. Calibration of the biosensor

The microslide gel double diffusion technique is approved by AOAC International (AOAC, 1990) and is the current standard for evaluating new methods for detection of staphylococcal enterotoxin. Other methods used for food extracts should be at least as sensitive as the microslide technique, which requires concentrating extracts from 100 g of food in as much as 600 mL to about 0.2 mL. The microslide gel double diffusion technique requires at least 30–60 ng of enterotoxin

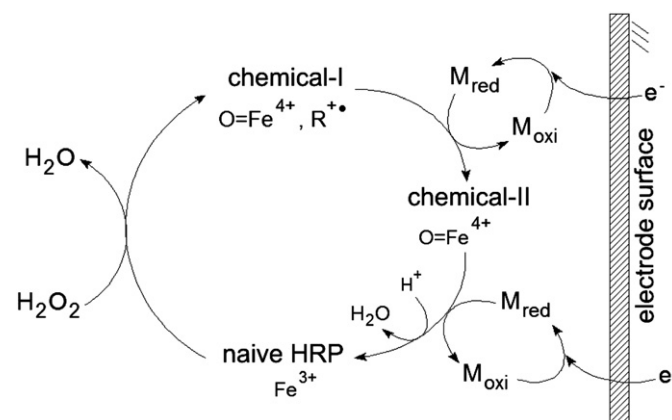


Fig. 3. Catalytic cycle of HRP immobilized on electrode and mechanism of mediated electron transfer. M = mediator, $\text{R}^{\bullet+}$ = porphyrin cation radical.

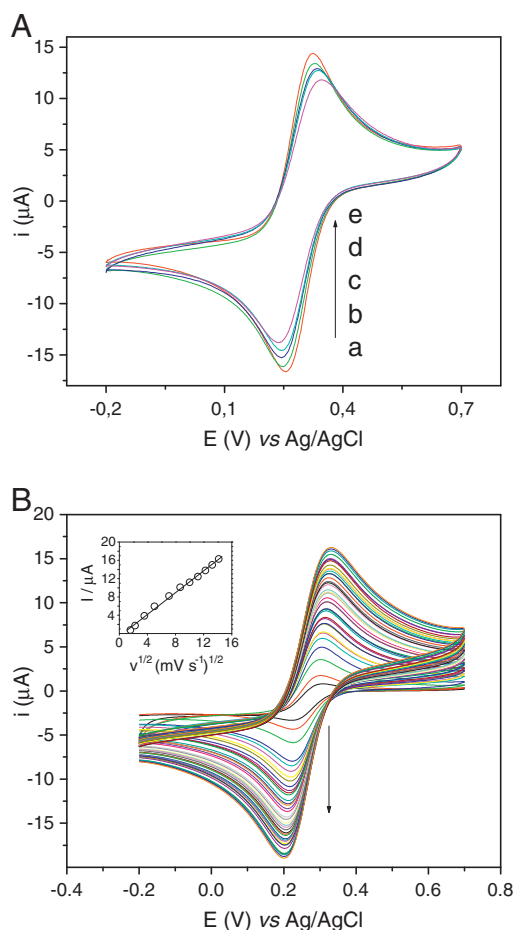


Fig. 4. (4a) Cyclic voltammograms in 4 mM $K_3[Fe(CN)_6]$ and 1 M KCl solution after each step of biosensor construction: cysteamine self assembly (10 mM); protein A immobilization (0.05 mg mL^{-1}); BSA blocking (1%); binding to SEA (1 mg mL^{-1}); binding to HRP-labeled antibody ($0.032 \mu\text{g mL}^{-1}$). (4b) Cyclic voltammograms at different scan rates of the biosensor. The scan rate is from 2.5 to 200 mV s^{-1} . Inset: Current (i) as a function of the square root of the scan rate.

per gram of food. Less sensitive methods are inadequate (Bennett and Hait, 2001). In this context, the performance of the biosensor was completely satisfactory.

The biosensor response in the presence of SEA in PBS pH 7.4 at $60 \mu\text{M}$ hydroquinone and $100 \mu\text{M}$ peroxide hydrogen is shown in Fig. 5. The calibration curve presented a good linearity ($r=0.99$) in the range from 0.016 to 0.150 mg mL^{-1} . The limit of detection of 33.9 ng mL^{-1} was estimated considering three times the standard deviation of the measurement of the blank divided by the slope of the calibration curve. The limit of quantification of 112 ng mL^{-1} was estimated considering ten times the standard deviation of the measurement of the blank divided by the slope of the calibration curve (sensitivity of the biosensor).

Immunological methods are preferred for the detection of enterotoxins. ELISA is the method of choice, because reagents are commercially available in polyvalent and monovalent formats for both toxin screening and serotype specific identification assays of *S. aureus* isolates (Aydin et al., 2011). However, the sensitivity and specificity of the assays depend greatly on the antibody used and the techniques of preparation of the sample (AOAC, 1990).

The reproducibility of the biosensor was evaluated from the amperometric response with 1 mg mL^{-1} SEA using three different modified electrodes. The relative standard deviation of these electrodes was 8.3%. Thus, the biosensor showed a good, reproducible behavior.

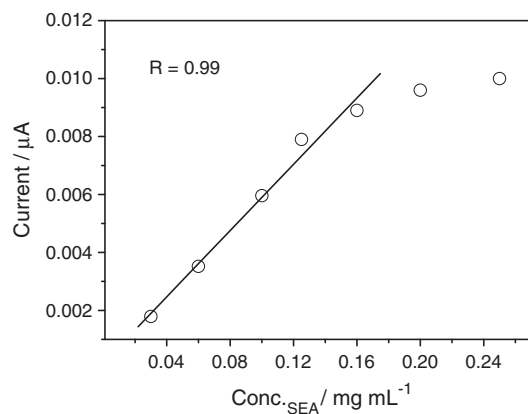


Fig. 5. Behavior obtained for different concentrations of SEA in PBS pH 7.4 containing $60 \mu\text{M}$ hydroquinone and $100 \mu\text{M}$ H_2O_2 . $E_{\text{ap}} = -35 \text{ mV}$. Insert: The calibration curve of the biosensor.

3.5. Assay of cross-reactivity to other enterotoxins

The homology between the chains of enterotoxins may reach 80% and based on this fact they are classified into two groups, one group including SEA, SED and SEE and the other covering SEB and SEC (Balaban and Rassoly, 2000). The immunosensor developed for SEA detection showed percent cross-reactivity with SEB, SEC and SED of 7.2%, 28.6% and 30.5% respectively, confirming the homology between them (Table 2).

3.6. Assessment of the biosensor response in the cheese sample

The feasibility of applying the biosensor proposed for the detection of SEA in a complex matrix was studied. First of all, the evaluation of the presence of SEA in handmade Coalho (curd) cheese samples was conducted by enzyme immunoassay RPLA using the kit SET-RPLA test (Oxoid®). The result of the test occurs after incubation for 24 h and is qualitative according to an agglutination pattern described by the manufacturer. Considering the results of the test, negative and positive samples of SEA of Coalho cheese were selected to verify the biosensor performance.

According to Fig. 6 the biosensor was capable to distinguish the contaminated and non-contaminated cheese samples. This fact depicts the potential use of the biosensor for SEA detection in food.

Finally, the biosensor showed good sensitivity and specificity for the detection of SEA. After construction of the bioreceptor surface it is possible to obtain the quantitative results in only 1 h after the incubation period with the sample. Therefore, this is a promising methodology for the development of a product to be used as a powerful tool in food safety, especially in milk and its derivatives, which have a greater possibility of SEA contamination.

4. Conclusion

The immunosensor was constructed from the best methodology tested for protein A immobilization. Through this optimization, an efficacious amperometric immunosensor was successfully constructed for detection of SEA in cheese. The electrochemical biosensor was able

Table 2
Immunosensor response to the enterotoxins B, C and D.

Enterotoxin*	Cross-reactivity (%)
SEB	28.6 (± 0.002)
SEC	7.2 (± 0.019)
SED	30.5 (± 0.115)

*Enterotoxin concentration: 1 mg mL^{-1} .

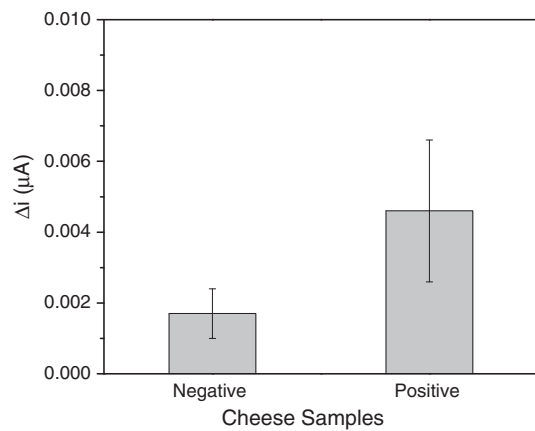


Fig. 6. Assessment of the biosensor in contaminated and non-contaminated cheese samples. Biosensor response in PBS solution pH 7.4 containing 60 μM hydroquinone and 100 μM H_2O_2 , $E_{ap} = -35$ mV.

to detect quantitatively SEA with a detection limit of 33.9 ng mL⁻¹. Moreover, the developed biosensor showed a high sensitivity and specificity and good reproducibility.

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