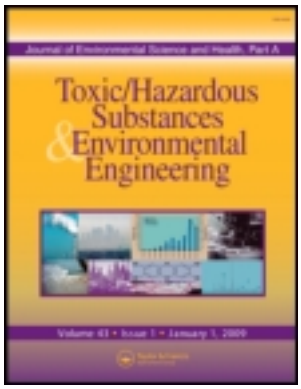

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Detection of staphylococcal enterotoxin A (SEA) at picogram level by a capacitive immunosensor

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This work presents the use of a flow injection capacitive immunosensor to detect staphylococcal enterotoxin A (SEA). The study was based on the direct detection of a capacitance change due to the binding between SEA and anti-SEA immobilized on a gold electrode. The optimal regeneration solution, flow rate, sample volume and buffer conditions were studied. Under the optimum conditions, this label-free biosensor provided linearity between 1×10^{-12} g L^{-1} and 1×10^{-8} g L^{-1} of SEA and the limit of detection was 1×10^{-12} g L^{-1} which was much lower than the infectious dose (0.5×10^{-6} – 1×10^{-6} g L^{-1}). Using the regeneration solution of, 15.0 mM glycine-HCl pH 2.20, to break the binding between SEA and the immobilized anti-SEA enabled the electrode to be reused up to 39 times. This technique was applied to analyze SEA in liquid and solid food samples. Any matrix effect can be eliminated by simple dilution. SEA contamination was found in three samples, iced tea with milk (28 ± 1 ng L^{-1}), orange juice (113 ± 6 ng L^{-1}) and fried chicken (1.1 ± 0.2 ng g^{-1}); however, the concentrations were much lower than the infectious dose. The proposed method would be useful for rapid screening of SEA in various matrices.

Keywords: Staphylococcal enterotoxin A, high sensitivity, capacitive, immunosensor, label-free detection.

Introduction

Staphylococcus aureus is a facultative anaerobic Gram-positive bacterium, that grows over a wide range of temperatures with an optimum of between 30 and 37°C and a pH from 7.00 to 7.50. The main species of staphylococcus, *Staphylococcus aureus*, is a well-known human pathogen that produces a number of staphylococcal enterotoxins (SEs) and is one of the most common causes of food poisoning. These SEs are single-chain proteins with molecular weights of between 27,000 and 34,000 Dalton.[1] Ten staphylococcal enterotoxins have been identified, i. e., A, B, C1, C2, C3, D, E, H, I and G. In children, ingestion of food contaminated with SEs, at concentrations as low as 0.5 μg L^{-1} can produce nausea, vomiting and diarrhea within 12 h while in adults similar effects occur at higher infectious doses.[2] Tests on human volunteers revealed that 20 to 25 μg (0.4 μg per kg of body weight) led to vomiting.[3]

In tropical countries, the temperature is always high, and thus, suitable for the growth of *Staphylococcus aureus* and the pyrogenic exotoxins that they produce can lead to outbreaks of foodborne disease due mainly to their SEs. Many methods for detecting SEs have been reported. These include chromatography, mainly liquid chromatography with various detectors,[2,4,5] radioimmunoassay,[6] and enzyme-linked immunosorbent assay (ELISA).[7] These methods are relatively sensitive with a limit of detection in the range of μg L^{-1}. However, liquid chromatography requires sample pretreatment steps, that increase the time for analysis, while radioimmunoassay is undesirable because of the use of radioactive materials. Although ELISA is non-radioactive, it needs a few hours for its incubation and washing steps.[8] Moreover, all these 3 methods are relatively expensive and may not be suitable for routine analysis. To overcome these drawbacks, immunosensors have been investigated as a possible alternative method for detecting SEs.

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Several detection principles have been studied for SEs immunosensors. Both indirect and direct detection have been applied using optical, mass sensitive and electrochemical detectors. For the indirect format, studies have been based on the detection of labeling agents in a sandwich or competitive assay. The use of a quartz crystal microbalance,[9] surface plasmon resonance,[10,11] fiber-optics[12] and conductometry[13] have also been reported. Indirect detection methods can provide a limit of detection down to μg L^{-1}. However, they require a number of steps and a long analysis time. Therefore, direct detection of the affinity binding that requires fewer steps has been studied based on the detection of optical,[14] mass[15] and electrochemical changes.[16,17] These direct techniques provide limits of detection in the range of ng L^{-1} and μg L^{-1}. However, when dealing with real samples from a complex matrix, signal interference generally occurred. One way to reduce this effect is by dilution,[18] but the amount of analyte is also reduced. Therefore, the lower the detection limit the better. In addition, when the analyte is a toxin, a system with the lowest detection limit is preferred. One of the systems that has proven to be very sensitive is the detection of a capacitance change based on the potentiostatic step method.[19]

Among the SEs, a number of immunosensors for SEB have been reported.[2,9,15,16] However, the SEs that is most commonly found in food poisoning is SEA.[11] So, we are interested in the development of a highly sensitive capacitive immunosensor for SEA. To the best of our knowledge, detection of SEA in food samples based on this sensitive system has not been reported. In this work the binding between SEA and monoclonal anti-SEA immobilized on an electrode surface was directly measured by detecting the capacitance change using a potentiostatic step method. Food and drink that is uncovered and laid out for sale in open air areas may be contaminated by *S. aureus*. Some of these samples were collected and analyzed by this new flow injection capacitive immunosensor.

Materials and methods

Chemicals

Rabbit anti-staphylococcal enterotoxin A (anti-SEA), staphylococcal enterotoxin A (MW 27,100 Dalton) from *Staphylococcus aureus*, thiourea and glutaraldehyde were from Sigma-Aldrich (Steinheim, Germany). 1-dodecanethiol was obtained from Aldrich (Milwaukee, USA). All other chemicals were analytical grade. Buffer solutions used in the capacitive immunosensor were prepared with deionized water. Before use, the buffers were filtered through an Albet® nylon membrane filter (Albet, Spain), pore size 0.2 μm, with subsequent degassing.

Immobilization of Anti-SEA

Gold electrodes (diameter 3 mm, 99.99% purity) were polished (Gripo® 2V, Metkon Instruments Ltd., Turkey) with an alumina slurry (Metkon, Turkey), with particle sizes of 5 μm, 1 μm and 0.3 μm, respectively. The electrodes were cleaned by sonication in ethanol for 1 min and deionized water for 10 min, followed by electrochemical etching in 0.5 M H_2SO_4 between 0 V and 1.5 V against an Ag/AgCl reference electrode at a scan rate of 0.1 V s^{-1} for 25 scans.

Gold electrodes were then modified with thiourea[20] by first immersing a cleaned electrode in 250 mM thiourea solution where a self-assembled monolayer was allowed to form on the surface at room temperature for 24 h. The surface was then thoroughly rinsed with deionized water and treated with 5% (v/v) glutaraldehyde for 20 min. This was to modify the amino groups of thiourea and expose the aldehyde groups of glutaraldehyde to couple with the anti-SEA. Twenty μL of 3 mg mL^{-1} anti-SEA was placed on the modified electrode for 24 h at 4°C. The aldehyde derivatives react with amino groups on the anti-SEA to form a Schiff's linkage. The modified electrode was then washed with 10.0 mM Tris-HCl buffer pH 7.00. The electrode was finally immersed in 1 M ethanolamine pH 8.50 for 7 min followed by 10 mM 1-dodecanethiol ethanolic solution for 20 min to block any pinholes on the electrode surface.[21]

The electrochemical behavior of each immobilization step was studied by cyclic voltammetry (CV). Electrochemical measurements were carried out using a potentiostat (Microautolab type III, Metrohm Autolab B.V., The Netherlands) coupled to an electrochemical cell containing the modified working electrode, an Ag/AgCl reference electrode (Metrohm, Switzerland) and a platinum auxiliary electrode (Metrohm, Switzerland).

Capacitance measurements

The modified gold electrode (working electrode), a stainless steel tube (auxiliary electrode) and a custom made Ag/AgCl (reference electrode) were placed in a flow cell with a dead volume of 10 μL. This was incorporated into a flow injection system (Fig. 1). Potential pulses (50 mV, pulse width 6.4 ms, one pulse per min) were applied to the working electrode with the immobilized anti-SEA (Fig. 1a) through the potentiostat (Powerlab, AD Instrument, Australia). The current response from each pulse was sampled with a frequency of 40 kHz and the capacitance of the electrode surface layer was calculated from the regression equation of the logarithm of the current versus time as described by Limbut et al.[20] The transient current from each pulse decayed exponentially (Fig. 1b and Fig. 1c) according to the following equation (1).

$$i(t) = \frac{u}{R_s} \exp\left(\frac{-t}{R_s C_{\text{total}}}\right) \quad (1)$$

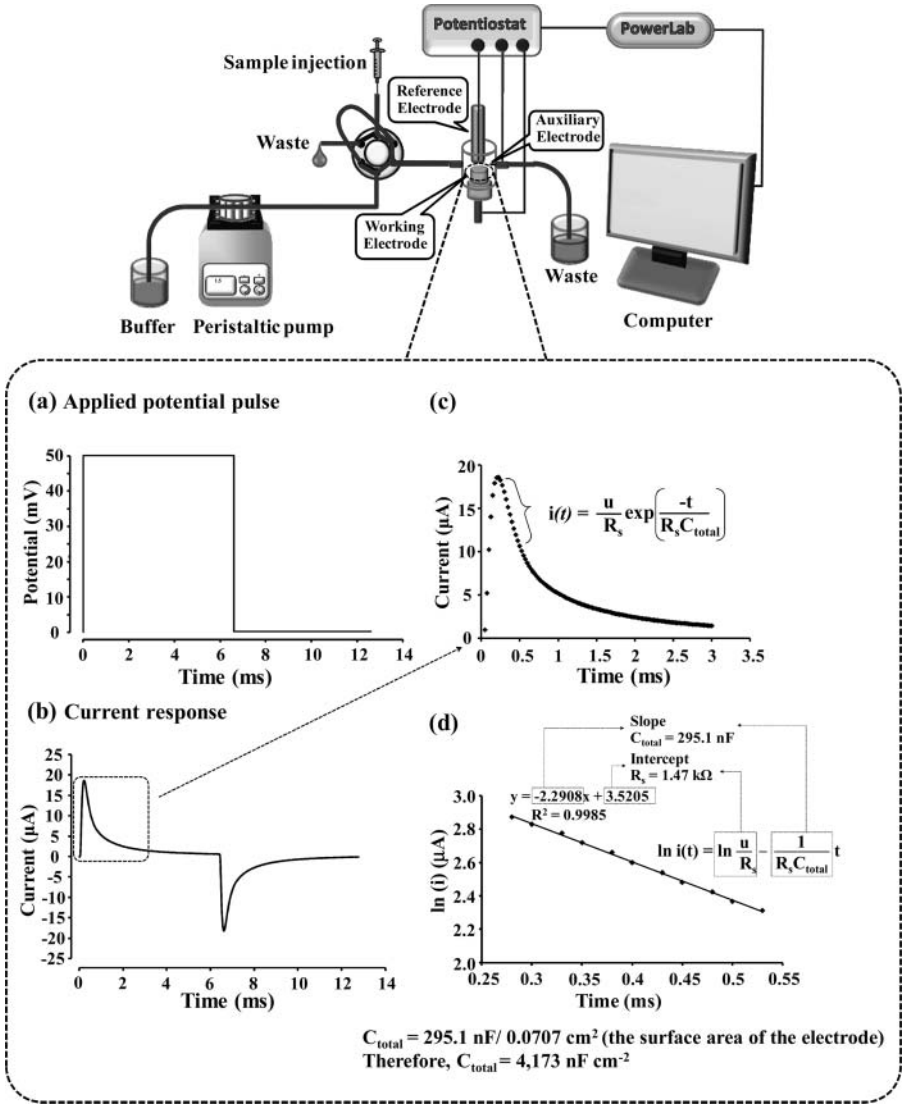

Fig. 1. Schematic diagram showing the flow injection capacitive immunosensor for staphylococcal enterotoxin A. (a) The applied potential pulse, (b) and (c) the transient current response from each pulse and (d) the plot of ln(i) vs. time, the resistance was obtained from the intercept and the capacitance was obtained from the slope.

where $i(t)$ is the current in the circuit as a function of time, u is the applied pulse potential, R_s is the dynamic resistance of the biorecognition layer, t is the time elapsed after the potentiostatic pulse was applied and C_{total} is the total capacitance measured at the working electrode/solution interface.[22] By taking the logarithm of equation (1), equation (2) was obtained.

$$\ln i(t) = \frac{\ln u}{R_s} - \frac{t}{R_s C_{\text{total}}} \tag{2}$$

C_{total} and R_s were then obtained from the slope and the intercept of the linear least-square fitting of ln $i(t)$ vs t[26] (Fig. 1d). The binding between SEA and the immobilized anti-SEA caused an increase of dielectric layer thickness and/or a change of dielectric properties. The layer of SAM, immobilized anti-SEA and SEA contributed to the total capacitance according to equation (3).

$$\frac{1}{C_{\text{total}}} = \frac{1}{C_{\text{SAM}}} + \frac{1}{C_{\text{antiSEA}}} + \frac{1}{C_{\text{SEA}}} \tag{3}$$

Optimization

Parameters that affected the binding between SEA and anti-SEA were optimized. The parameters studied were: the regeneration solution, carrier buffer and concentration of $ZnCl_2$ added to the carrier buffer solution, flow rate and sample volume. Three replications were carried out of each tested value. The optimum value of the flow system was obtained by balancing between capacitance change and analysis time. Using the optimum conditions, the performance of the capacitive immunosensor was studied.

Determination of SEA in real samples

Liquid and solid food samples that were prepared and laid out for sale without being covered were obtained from local stores. Liquid samples included fresh orange juice and ice tea with milk. Bottled drinking water was also analyzed for comparison purposes. The solid food samples used were fried chicken and fried sausage. For solid food, SEA was simply extracted: 10 g of the solid sample was homogenized in 10 mL of 10 mM PBS (phosphate buffer, 2.7 mM KCl, 138 mM NaCl) for 20 min. The suspension was centrifuged for 10 min and the supernatant was used for analysis.[10] All samples were analyzed by the flow injection capacitive immunosensor under its optimum conditions.

Results and discussion

Electrochemical characterization of the modified gold electrode

The electrochemical characteristics of the modified gold electrode were studied after each step of modification (Fig. 2). This was done by evaluating the electron transfer kinetics of $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ on the modified electrode in the electrolyte solution by cyclic voltammetry. The voltammogram of a clean gold electrode presented large oxidation and reduction peaks (Fig. 2a), which decreased when a self assembled monolayer (SAM) of thiourea was formed (Fig. 2b), hence, the ability for electron transfer was reduced. A further reduction of the redox peaks was observed when glutaraldehyde was reacted with SAM (Fig. 2c) and anti-SEA was then immobilized on the surface (Fig. 2d). Ethanolamine was added to react with access aldehyde groups, before the electrode was completely insulated with 1-dodecanethiol ethanolic solution (Fig. 2e), and this was indicated by obtaining a completely flat voltammogram.

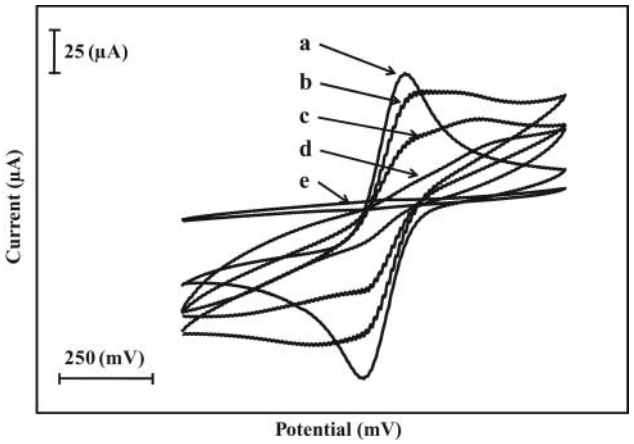

Fig. 2. Cyclic voltammograms of the modified gold electrode obtained with 5 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl solution at a scan rate of 0.1 V s^{-1} vs. an Ag/AgCl reference electrode. The voltage range was −0.3 to 0.8 V showing the voltammogram of (a) a clean gold electrode, (b) with a self assembled monolayer of thiourea, (c) after treatment with glutaraldehyde (d) after immobilization with anti-SEA and (e) after blocking with 1-dodecanethiol.

Optimization of capacitive immunosensor

Fig. 3a shows an example of the sensorgram obtained from the capacitive immunosensor. The total capacitance of the immobilized surface was first obtained ($C_{anti\text{-}SEA}$). The binding between SEA and anti-SEA resulted in a decrease of the total capacitance to a new steady state signal, which required 15-20 min. The total capacitance measured before ($C_{anti\text{-}SEA}$) and after ($C_{anti\text{-}SEA,SEA}$) the binding were

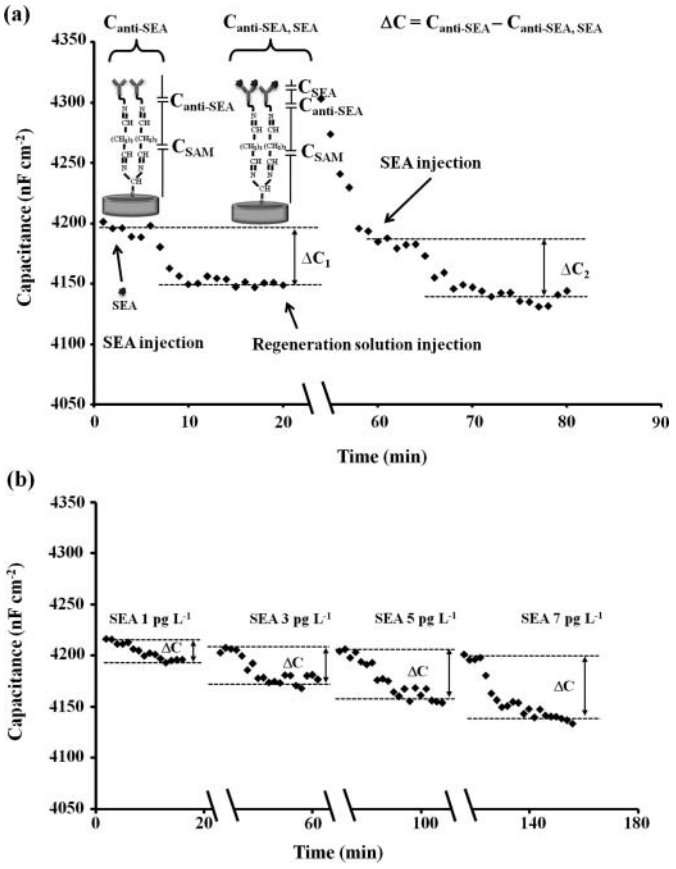

Fig. 3. (a) An example of a sensorgram obtained from the capacitive immunosensor injected with 7 pg L^{-1} of SEA. The binding of SEA to the immobilized anti-SEA caused the capacitance to decrease (ΔC_1). Regeneration solution was then injected to remove SEA from anti-SEA followed by the continuous flow of carrier buffer. When the baseline signal was recovered, the same concentration of SEA was injected and the capacitance decreased (ΔC_2). (b) An example of a sensorgram showing the change of capacitance when 1, 3, 5 and 7 pg L^{-1} of SEA was injected into the system. The measurement was carried out under the optimum conditions 10.0 mM Tris-HCl containing 0.8 mM $ZnCl_2$ pH 7.00 of carrier buffer, 50 μL min^{-1} of sample flow rate, 300 μL of sample volume and 15.0 mM glycine-HCl pH 2.20 of regeneration solution.

used to calculate the capacitance change $\Delta C = C_{anti\text{-}SEA} - C_{anti\text{-}SEA,SEA}$. Dissociation of anti-SEA and SEA was carried out using a regeneration solution (300 μL of 15.0 mM glycine-HCl pH 2.20) to remove SEA from the sensor surface. The increase of signal after injection of the regeneration solution was due to the regeneration solution having a higher ionic strength than the carrier buffer. The signal then returned to the original baseline after a continuous flow of carrier buffer. The sensor surface was then ready for the next analysis. Fig. 3b shows examples of the capacitance change after the injection of 1, 3, 5 and 7 pg L^{-1} of SEA. The capacitance changes were proportional to the concentrations of SEA.

Optimization of the flow injection capacitive immunosensor was studied to obtain the best performance for SEA analysis. The experiments were carried out using 10^{-10} g $L^{-1}$of SEA. The initial conditions were 50 μL min^{-1} of sample flow rate and 300 μL of sample volume.

Regeneration solution

The affinity binding interaction between antibody and antigen is non-covalent and it can be dissociated with an appropriate solution. Generally, a change of pH or ionic strength can be used to remove the antigen from the antibody and regenerated the surface of the electrode for the next analysis.[23] Regeneration solutions of low pH (HCl and 10.0 mM glycine-HCl pH 2.60), high pH (NaOH pH 9.00) and high ionic strength (25.0 mM $MgCl_2$) were evaluated. The efficiency of each solution type was determined by the residual activity of anti-SEA to SEA before (ΔC_1) and after (ΔC_2) regeneration (Fig. 3a) as follows:

$$\%\text{Residual activity} = \frac{\Delta C_2}{\Delta C_1} \times 100 \qquad (4)$$

When $MgCl_2$ was used (300 μL followed by carrier buffer), the regeneration time (the time taken for the signal to return to a steady baseline) was more than an hour and the capacitance change was not observed after SEA was injected again indicating that SEA could not be removed by $MgCl_2$. The long regeneration time was probably due to the high ionic strength of $MgCl_2$, i.e., a larger volume of carrier buffer was required to wash the modified electrode surface. Residual activity after NaOH, HCl and glycine-HCl were 90 ± 3%, 97 ± 5% and 95 ± 6%, respectively (Fig. 4a). The regeneration time with NaOH was 27 min while it took 18–25 min for the other 2 low pH solutions. Since the low pH provided higher residual activity and a shorter regeneration time, it was then selected as the normal regeneration solution. Between glycine-HCl and HCl, the former was selected because it is a buffer, compared to HCl and a more stable baseline was observed after the regeneration.

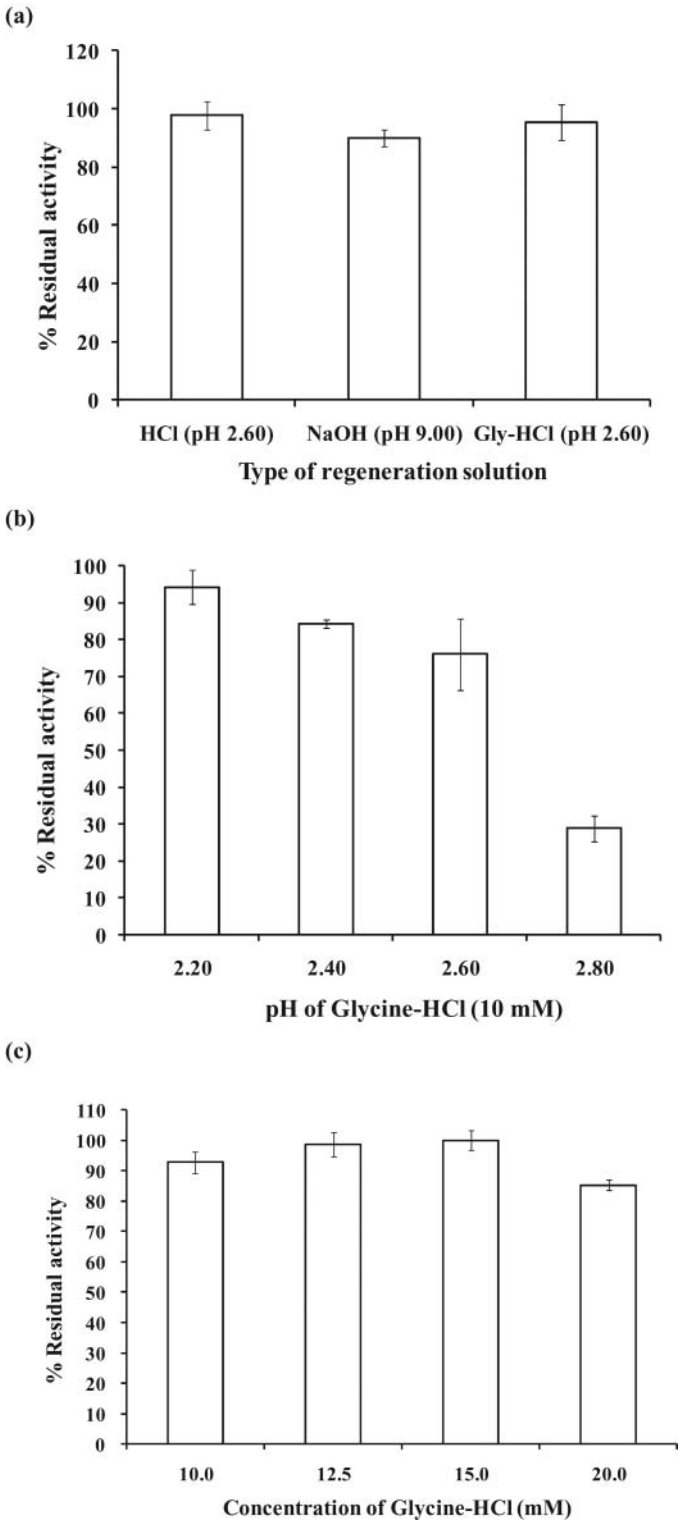

Fig. 4. Conditions of the regeneration solutions tested by comparing the percentage of the residual activity of the capacitive immunosensor for SEA between (a) types, (b) pH values and (c) concentrations of regeneration solution.

The concentration and pH of the glycine-HCl were then studied. The percentage of residual activity increased with a decrease of pH and the highest value was obtained at pH 2.20 (Fig. 4b). pH values lower than 2.20 was not studied since such a low pH solution may destroy the SAMs.[24] For the effect of concentration, 15.0 mM was found to be the optimal value (Fig. 4c).

Carrier buffer

Tris-HCl buffer was used as the carrier buffer since it has been shown to provide a stable baseline and a high capacitance change in potentiostatic systems for various analytes.[21,25,26] However, initial testing using Tris-HCl as a carrier buffer provided a low response. It was later found that the binding between SEA and anti-SEA needs Zn^{2+} as a binding metal.[27,28] So, a low concentration of $ZnCl_2$ was added in the carrier buffer. The capacitance change increased with $ZnCl_2$ concentration and leveled off at 0.8 mM and this was used in the ongoing experiments.

The concentration and pH of the Tris-HCl buffer were optimized at the same time. The studied concentrations were 7.5, 10.0 and 15.0 mM all with 0.8 mM $ZnCl_2$. Each concentration was tested at pH 7.00, 7.20 and 7.40. A buffer pH lower than 7.00 was not studied because the pH range of Tris-HCl buffer was between 7.00 and 9.00. Ten millimolar Tris-HCl buffer pH 7.00 provided the highest capacitance change (Fig. 5a). Therefore, this was selected as the optimal concentration and pH of the carrier buffer.

Flow rate and sample volume

The yield of interaction between SEA and immobilized anti-SEA can be affected by the flow rate and the amount of SEA. Therefore, the sample flow rate and sample volume were studied. The capacitance change decreased with increased flow rate (sample volume = 300 μL) due to the shorter interaction time between SEA in the solution and the immobilized anti-SEA (Fig. 5b). The highest capacitance change was observed at 50 μL min^{-1} (analysis time = 20 min). A flow rate lower than 50 μL min^{-1} was not studied due to the longer analysis time.

For the sample volume at a flow rate of 50 μL min^{-1}, the capacitance change increased when the volume increased from 100 μL and reached a plateau at 300 μL and this was selected as the optimum value (Fig. 5c). The flow rates and sample volumes at optimal and nearby values were then reinvestigated together. The result confirmed the above optimum values. Therefore, a flow rate of 50 μL min^{-1} and 300 μL sample volume were selected as optimum values.

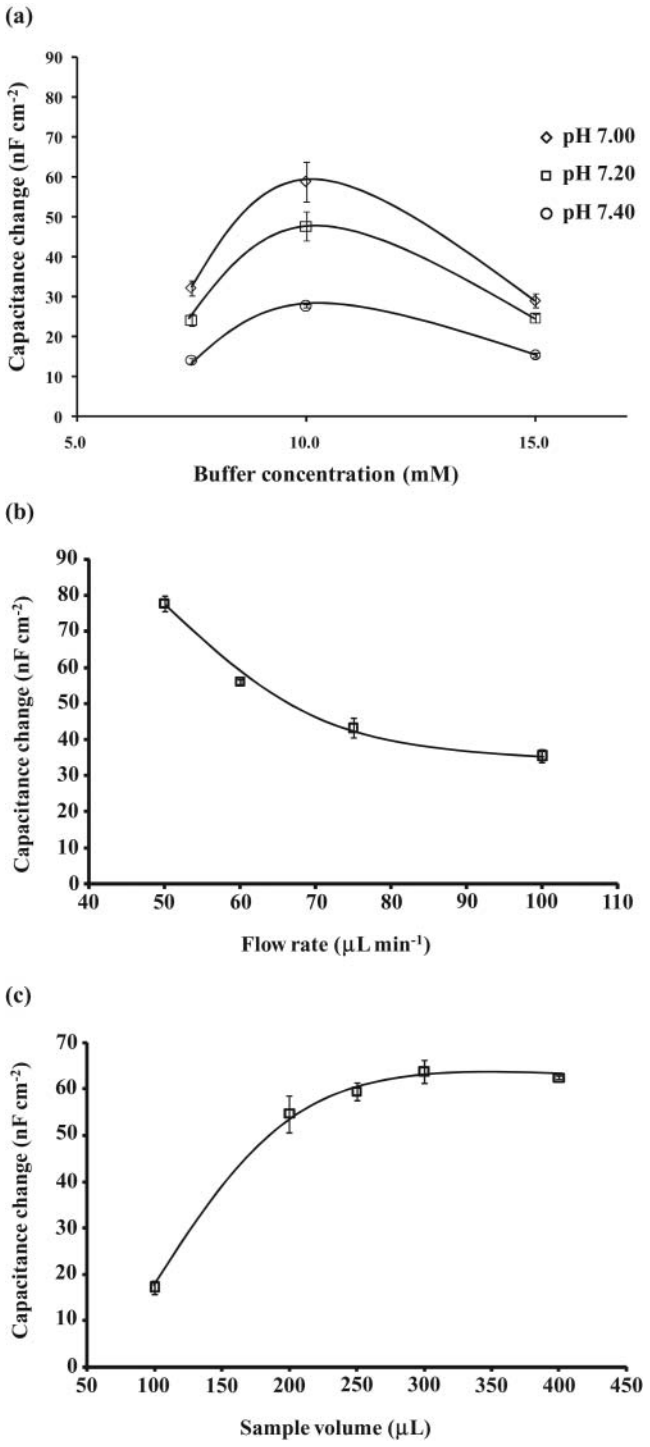

Fig. 5. Responses of capacitive immunosensor for SEA (a) effect of concentrations and pH values of carrier buffer (Tris-HCl + 0.8 mM $ZnCl_2$), (b) effect of flow rate and (c) effect of sample volume.

Reproducibility

Reproducibility was tested by analyzing the same concentration of standard SEA (1×10^{-10} g L^{-1}) 11–12 times per day for 4 days including a regeneration step using 15.0 mM glycine-HCl buffer (pH 2.20) after each measurement. During the first 39 regeneration cycles the average residual activity was 98 ± 5% (RSD = 5.1%). The residual activity then reduced rapidly to 78% on the 4th day. Cyclic voltammetry was then used to test the modified electrode after the

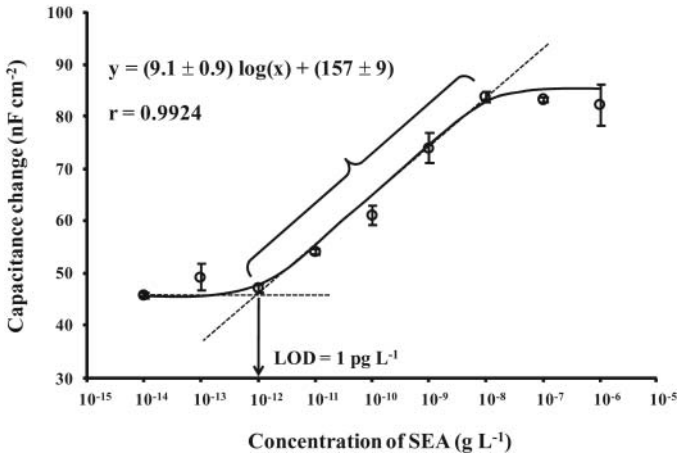

Fig. 6. Calibration curve for SEA obtained from the capacitive immunosensor under optimum conditions.

reduction of residual activity. A flat voltammogram similar to the one obtained just after the electrode preparation (Fig. 2e) was observed. That is, SAM still remained on the modified electrode and the decrease in residual activity was probably caused by the loss of anti-SEA and/or its activity after being used numerous times.

Linear dynamic range and limit of detection

Standard SEA solution of between 1×10^{-14} and 1×10^{-6} g L^{-1} were analyzed by the capacitive immunosensor under the optimum conditions. Each concentration was analyzed for 3 replications. The calibration plot (Fig. 6) provided a linear response between 1×10^{-12} and 1×10^{-8} g L^{-1}. Since the shape of the curve is similar to the response of an ion selective electrode, the limit of detection was determined following the IUPAC Recommendation 1994.[29] The limit of detection was 1×10^{-12} g L^{-1} (Fig. 6). This was extremely low compared to the 0.5×10^{-6} g L^{-1}, which is an infectious dose for children.[2] This limit of detection was much better than the detection of SEA by SPR immunosensors that were 100×10^{-6} g L^{-1}[14], 10×10^{-6} g L^{-1}[10] and 1×10^{-6} g L^{-1}.[11] Due to the very low limit of detection, real samples can be diluted by several orders of magnitude before analysis. This can reduce the matrix effect and it would be possible to analyze complex real samples with the proposed technique.

Matrix effect

Three liquid samples, orange juice, ice tea with milk, bottled drinking water, were diluted with carrier buffer. Two solid samples, fried chicken and fried sausage, were simply extracted by homogenization in PBS and subsequently diluted with carrier buffer. These 5 samples were then analyzed using the flow injection capacitive immunosensor. To study the effect of the matrix, each sample was spiked with SEA from 1 to 7 pg L^{-1} which was the concentration of the limit of detection and the nearby concentration. The calibration curve of the spiked sample (spiked curve) and carrier buffer (standard curve) were constructed. The slopes of the spiked curve and standard curve were compared using two-way ANOVA (analysis of variance), calculated by R software.[30] If there is no difference between the two curves, it means that there is no matrix effect. The slopes of the standard and spiked curves were significantly different ($P \leq 0.1$) for all samples, indicating matrix interference. To reduce the matrix effect, samples were diluted at different dilutions and tested until there was no significant difference between the two curves. Iced tea with milk, fried sausage and fried chicken needed to be diluted 10,000 times while bottled drinking water and orange juice needed 100 and 100,000 times, respectively. Therefore, diluted samples were analyzed and a standard curve was used to calculate the concentration of SEA in real samples, then multiplied by the dilution factor.

Recovery

To validate the method, a recovery test was studied by spiking different concentrations of standard SEA into liquid and solid food samples to obtain the final concentration range after dilution between 1 and 7 pg L^{-1} (LOD and the nearby concentrations). Iced tea with milk, fried chicken and fried sausage were spiked with SEA from 10 to 70 ng L^{-1}. Orange juice was spiked from 100 to 700 ng L^{-1} and bottled drinking water was 0.1 to 0.7 ng L^{-1}. Then the spiked samples were analyzed by the capacitive immunosensor. The percentage of recovery was calculated by

$$\%\,\text{Recovery} = \frac{C_1 - C_2}{C_3} \times 100 \qquad (5)$$

Where C_1 = the concentration determined in the fortified sample, C_2 = the concentration determined in the unfortified sample and C_3 = the concentration of fortification.[32] The results showed an average percentage of recovery of between 80 and 123% (Table 1) with an RSD of between 0.7 and 11%. These percentages of recovery and the RSD were within the acceptable range (For concentration of analyte at 1 μg L^{-1}, percentage of recovery is allowed to be 40–120% and 30–45.3% for an RSD).[32]

Real sample analysis

A standard curve was used to determine the SEA concentrations in diluted real samples. The obtained capacitive immunosensor signal (nF cm^{-2}) was used to determine the concentration from the standard curve and multiplied by the dilution factor. Out of the 5 samples, SEA was detected in 3 samples, iced tea with milk (28 ± 1 ng L^{-1}), orange juice (113 ± 6 ng L^{-1}) and fried chicken (1.1 ± 0.2 ng g^{-1}).

Table 1. Recoveries from spiked food samples obtained from the flow injection capacitive immunosensor (mean ± SD, n = 3).

	Recovery (%)			
	Spiked concentration (ng L^{-1})			
Samples	*10*	*30*	*50*	*70*
Ice, tea with milk	82 ± 9	111 ± 6	99 ± 2	100.9 ± 0.9
Fried chicken	92 ± 10	113 ± 5	108 ± 6	103 ± 5
Fried sausage	101 ± 3	112 ± 8	106 ± 2	101 ± 3
	Spiked concentration (ng L^{-1})			
	100	*300*	*500*	*700*
Orange juice	91.7 ± 0.6	80 ± 1	97 ± 6	98 ± 3
	Spiked concentration (ng L^{-1})			
	0.1	*0.3*	*0.5*	*0.7*
Bottled drinking water	123 ± 8	97 ± 4	100 ± 7	97.2 ± 0.8

All these detected concentrations were lower than the infectious dose of SEs in children (500 ng L^{-1}).

Conclusions

This paper presents a highly sensitive technique for the detection of SEA in liquid and solid food samples. The proposed immunosensor directly detects the binding between antigen and antibody using capacitance measurement. Both liquid and solid samples can be analyzed without complicated sample preparation procedures. The immobilized anti-SEA electrode can be reused up to 39 times. This can reduce the analysis costs. The capacitive immunosensor required only 20 min for each analysis and 25 min for regeneration about 10 samples/normal 8 h working day and 3 consecutive days for each new electrode.

The proposed capacitive immunosensor for SEA showed an extremely low limit of detection (1 pg L^{-1}). With this limit of detection, real samples can be well diluted to reduce matrix interference and still be detected. The limit of detection was also much lower than the capacitive immunosensor for SEB,[16] by three orders of magnitude. This may be because the conditions used in their work were not optimized. Also, the use of phosphate buffer as a carrier buffer gave a high capacitance baseline and subsequently the capacitance change was smaller.

For real sample analysis, liquid samples were diluted with carrier buffer while solid food samples were simply extracted by homogenizing the samples in phosphate buffer and then diluted. Quantitative analysis of SEA showed SEA contamination in ice tea with milk, orange juice and fried chicken at the level of ng L^{-1}. The study of recovery within the ng L^{-1} concentration range provided an acceptable percentage of recovery and RSD, to demonstrate that the proposed capacitive immunosensor was reliable. Thus, the proposed method would be very useful for a rapid screening of SEA in both liquid and solid real samples since a very minimal sample preparation is required.

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