Chapter 8

A Reusable Capacitive Immunosensor for Carcinoembryonic Antigen (CEA) Detection Using Thiourea Modified Gold Electrode

8.1 Introduction

Carcinoembryonic antigen (CEA) is a highly glycosylated cell surface glycoprotein (180 kDa) belonging to a group of substances known as the tumor-associated antigens (TAA) (Martin *et al.*, 1976). It is an important tumor marker responsible for clinical diagnosis of over 95 % of all colon tumors, 50 % of breast tumors, as well as tumors of the lung, pancreas, ovaries, and others of epithelial tissue origin, especially of the gastrointestinal tract (Shively and Beatty, 1985). Various commonly available methods were developed for CEA, such as enzyme linked fluorescent assay (ELFA) (Biomerieux® sa, 2003) and micropartical enzyme immunoassay (MEIA) (Abbott Laboratories, 1999). However, these methods are expensive, time consuming procedures requiring potentially dangerous materials (Shen *et al.*, 2005). Therefore, development of a new method with high sensitivity and specificity for the direct detection of CEA is highly desirable and an immunosensor registering the direct binding between antibody and antigen may be the answer.

This project reports the development of a capacitive immunosensor for the direct detection of carcinoembryonic antigen (CEA) using anti-carcinoembryonic antigen (anti-CEA) immobilized on a self-assembed thiourea monolayer (SATUM) on gold electrode. System performance of the capacitive immunosensor was tested for the determination of CEA level in human serum samples and the results were compared with a commercial method (enzyme linked fluorescent assay (ELFA), VIDAS® CEA).

8.2 Materials

Monoclonal anti-human carcinoembryonic antibody (anti-CEA) and carcinoembryonic antigen (CEA) from human fluids were obtained from Sigma (St Louis, USA), thiourea was obtained from BDH laboratory reagents (Poole, England) and 1-dodecanethiol was obtained from Aldrich (Milwaukee, USA). All other chemicals used were of analytical grade. All buffers were prepared as described in section 7.2.

8.3 Methods

8.3.1 Immobilization of anti-CEA

The conditions used for immobilization of anti-CEA to a gold surface modified with self-assembled monolayer of thiourea have been optimized prior to this work (see Chapter 7). The immobilization of anti-CEA on gold surface is a three-step procedure. The initial step is to prepare the gold electrodes (Ø 3 mm, 99.99% purity) and this follows the same procedure as described in section 7.3.1. The next step is to modify the gold electrode with thiourea. The cleaned electrode was immersed in a 250 mM thiourea solution at room temperature for 24 h, it was thoroughly rinsed with distilled water and dried with pure nitrogen gas. In this step self-assembled thiourea monolayer was formed on gold surface (Figure 8.1(a)). The final step is the coupling of anti-CEA to the modified gold electrode. This was done by treating the modified gold electrode with 5% (v/v) glutaraldehyde in 10 mM sodium phosphate buffer pH 7.00 at room temperature for 20 min, thoroughly rinse the electrode with sodium phosphate buffer before drying, this step was to activate the aldehyde groups (Figure 8.1(b)). Then 20 µl of 1.0 mg ml^{-1} of anti-CEA was placed on the surface of the electrode and reaction took place overnight at 4°C (Figure 8.1(c)). The electrode was immersed in 0.1 M ethanolamine pH 8.00 for 30 min, this step was to occupy all the aldehyde groups that did not couple to the anti-CEA. Before placing the biosensor in the flow-cell, the electrodes were treated in a 10 mM 1-dodecanethiol ethanolic solution for 20 min to block any pinholes or bare spots on the electrode surface (Figure 8.1(d)).

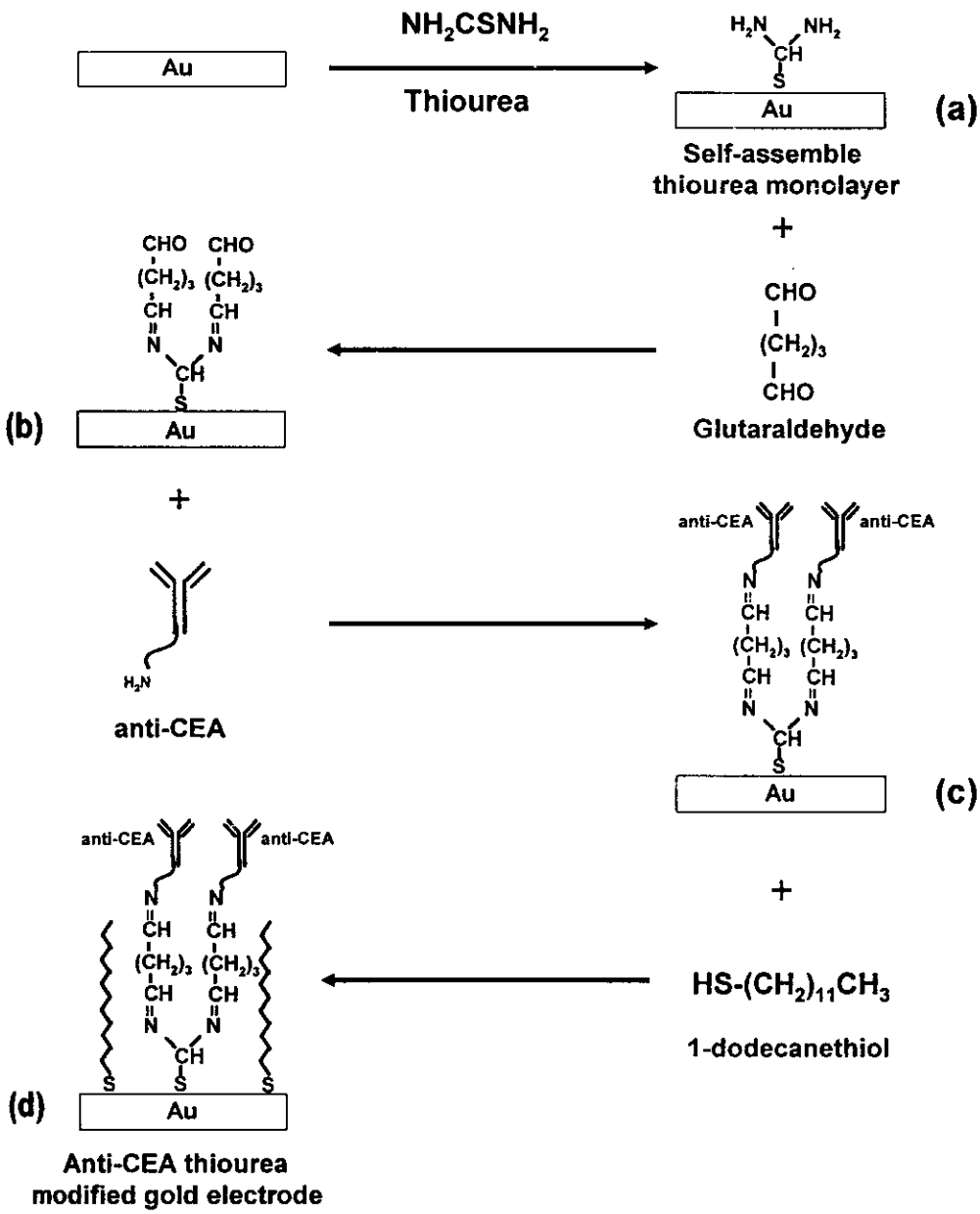


Figure 8.1 Reaction mechanism for anti-CEA immobilized covalently on a self-assemble thiourea monolayer (SATUM) modified on gold electrode. (a) Gold surface was modified with thiourea, (b) Amino groups of SATUM are activated by glutaraldehyde, (c) Covalent binding between carbonyl groups of the activated SATUM and free amino groups of anti-CEA, (d) Block any pinholes or bare spots on electrode surface with 10-dodecanethiol.

8.3.2 Capacitance measurement

The basic experimental set-up of the flow-injection based capacitive immunosensor system is as described in section 7.3.5 (Figure 7.5). Anti-CEA was immobilized on gold working electrode (WE) surface via self-assembled thiourea monolayer (SATUM). When CEA was injected into the flow cell it bound to the anti-CEA causing the total capacitance at the working electrode/solution interface to change (C_{Total}) (Figure 8.2). The measurement of C_{total} was done every minute and the results were later plotted as a function of time. Figure 8.3 shows the change in capacitance due to affinity binding between CEA and anti-CEA on the working electrode. When CEA was injected into the flow cell it bound to the anti-CEA immobilized on the electrode causing C_{total} to decrease and the capacitance change (ΔC) could be determined.

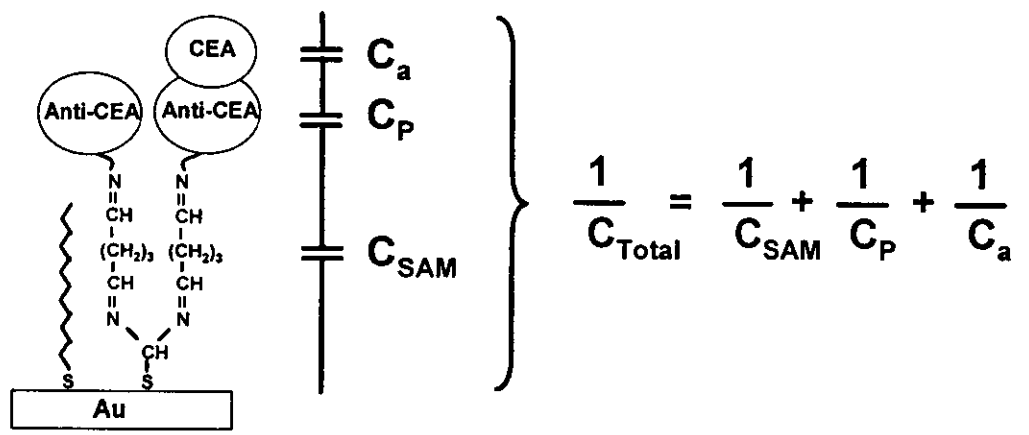


Figure 8.2 Schematic representation of the different layers on the electrode surface showing a series of capacitances determining the total capacitance; (C_{SAM}) the capacitance related to the self-assembled thiourea monolayer (SATUM), (C_P) the capacitance of protein layer, (C_a) the capacitance as a result of analyte interaction, (C_{Total}) the total capacitance measured at the working electrode/solution interface.

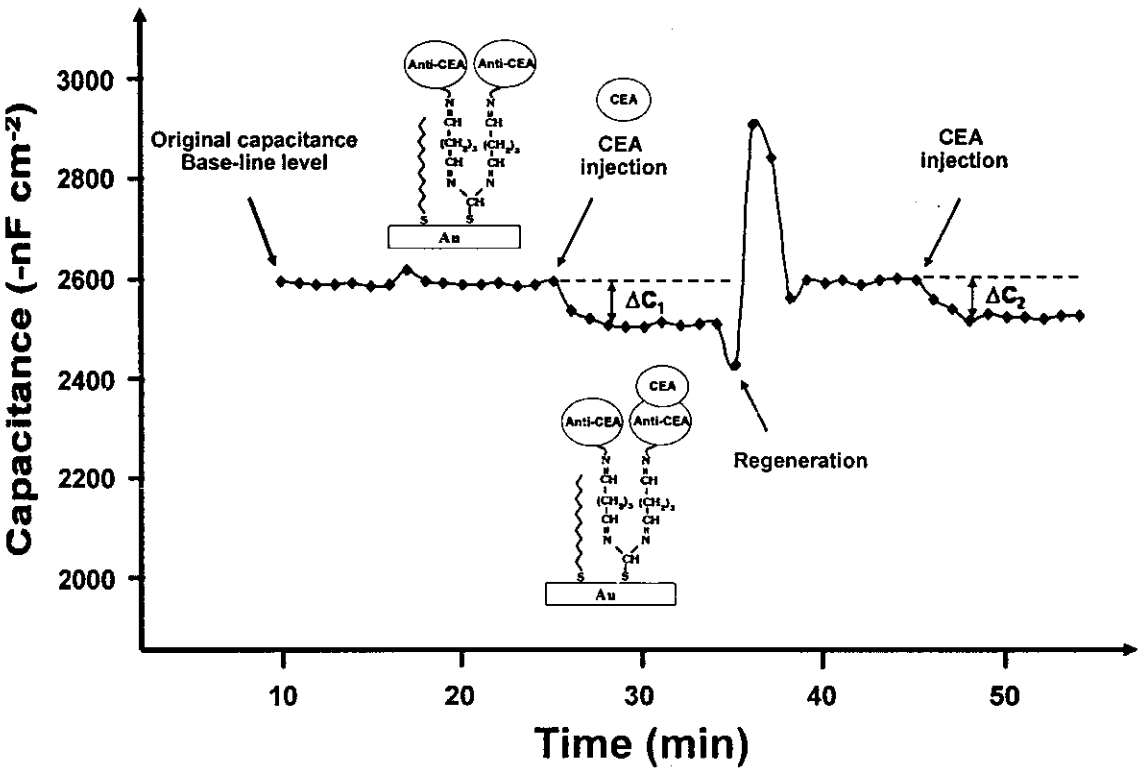


Figure 8.3 The decrease in capacitance (ΔC_1) resulting from the binding between CEA and anti-CEA with subsequent signal increase due to dissociation under regeneration conditions.

8.3.3 Optimization of the flow injection capacitive immunosensor

The operating conditions of the capacitive immunosensor in the flow-injection system were optimized for type and pH of the regeneration solutions, sample volume, flow rate and type and pH of running buffer. The running buffer used throughout the experiments was a 10 mM Tris-HCl, pH 7.00 (except when the effects of buffer types were tested). The effects of type and pH of regenerating solution were tested with conditions; 100 μl min^{-1} flow rate of buffer, 250 μl sample volume. The effect of the type of buffer solutions was tested at a sample volume 200 μl. The optimization of each parameter was performed by changing a single parameter while keeping the other parameters constant. The operating conditions were considered by balancing between the sensitivity and the time needed for one analysis.

8.3.4 Determination of the amount of CEA in serum samples

Serum samples were collected from Hat Yai Hospital and stored at -20 °C until analysis. CEA in serum samples were analysed by the ELFA technique, VIDAS® CEA method (the results obtained by Hat Yai Hospital) and the flow injection capacitive immunosensor system under optimum conditions; 100 μl min^{-1} flow rate of running buffer (10 mM Tris-HCl buffer pH 7.00) and 200 μl sample volume.

8.3.5 Comparison between the results obtained from the capacitive immunosensor system and ELFA technique (VIDAS® CEA)

The flow injection capacitive immunosensor system was validated by comparing the results to those obtained using an enzyme linked fluorescent assay (ELFA) method. In making such a comparison, the principle interest will be whether the proposed method gives results that are significantly higher or lower than the established methods. So, the analysis using the regression line (Miller and Miller, 1993) and the Wilcoxon signed rank test (Triola, 1998) were used in this work.

8.3.5.1 Regression line analysis

The regression line (Figure 8.4) can be used to compare two methods by plotting one axis of the regression graph using the results obtained by the proposed method and the other axis the results obtained from the comparison method of the same samples. Each point on the graph, thus, represents a single sample analyzed by two separate methods. The slope (m), the intercept (C), and the production moment correlation coefficient (r) of the regression line are then calculated. It is clear that if each sample yields an identical result with both analytical methods the regression line will have zero intercept and a slope and a correlation coefficient of 1 (Figure 8.4 (a)). In practice this never occurs even if systematic errors are entirely absent, random errors ensure that the two analytical procedures will not give results in exact agreement for all the samples (Figure 8.4 (b–f)). The most common tests to be done is to test whether an intercept (C) differs significantly from zero, and a slope (m) differs significantly from 1. Determining the errors in the slope (S_m) and intercept (S_c) of the regression line at 95 % significant level performs such tests. If $m \pm S_m$ cover 1 and $C \pm S_c$ cover zero, there are no systematic errors and the result are then accepted (Miller and Miller, 1993).

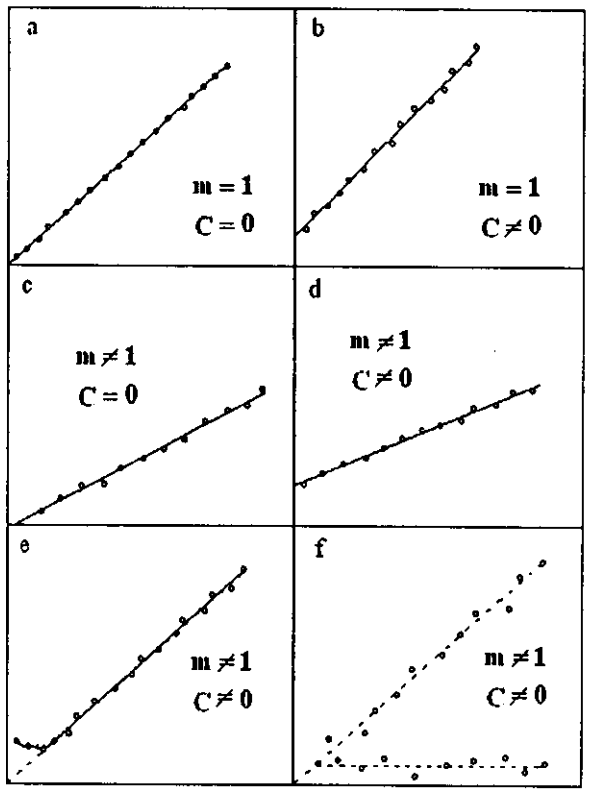


Figure 8.4 The use of a regression line to compare two analytical methods; (a) shows perfect agreement between the two methods for all the samples; (b)–(f) illustrate the result of various type of systematic errors of the slope and/or the interception. (Redrawn form Miller and Miller, 1993).

8.3.5.2 Wilcoxon signed rank test

The principle of this test has already been described in section 6.3.6.

8.4 Results and discussion

8.4.1 Electrochemical performance of the immobilization process

In the capacitive immunosensor system the insulating property of the self-assembled monolayer on the electrode surface is of vital importance. The degree of insulation was examined using cyclic voltammetry with a permeable redox couple (*i.e.* $K_3[Fe(CN)_6]$) in the electrolyte solution as shown in Figure 8.5. At the clean gold surface the redox couple was oxidized and reduced (Figure 8.5(a)). The redox peaks decreased when thiourea was self-assembled on the clean gold surface (Figure 8.5(b)). When glutaraldehyde was reacted with the amine and then anti-CEA was linked covalently on the electrode via reaction with aldehyde groups, the insulating property of the electrode surface was further increased (Figure 8.5(c) and (d)). A final capping of the electrode surface was achieved by the treatment with 1-dodecanethiol, as can be seen from the disappearance of the redox peaks in curve e. Figure 8.5.

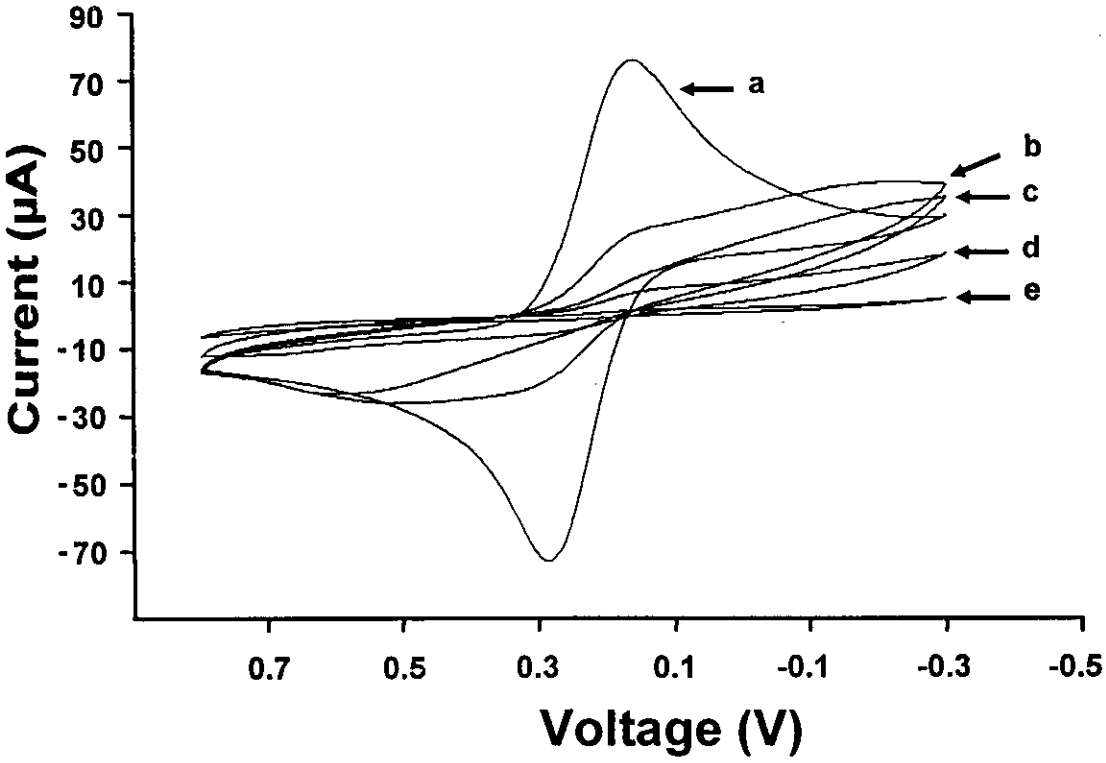


Figure 8.5 Cyclic voltammograms of a gold electrode obtained in a 5 mM $K_3[Fe(CN)_6]$ containing 0.1 M KCl solution at a scan rate of 0.1 V s^{-1}. All potentials are given *vs.* Ag/AgCl reference electrode. The voltage range was -0.3 to 0.8 V. (a) Clean gold electrode, (b) self-assembled thiourea monolayer (SATUM) electrode, (c) Glutaraldehyde-amine SATUM, (d) Anti-CEA-glutaraldehyde-amine SATUM, and (e) as in (d) but after 1-dodecanethiol treatment.

8.4.2 Optimization of the flow injection capacitive immunosensor

8.4.2.1 Regeneration solution

The interaction between CEA and the immobilized anti-CEA is via electrostatic force (Boehm, *et al.*, 2000) and CEA can be removed from anti-CEA by using regeneration solution. Ideally, regeneration of the working electrode should remove any non-covalently bound CEA analyte without disrupting the activity of the anti-CEA molecules immobilized on the electrode. Regeneration allows surfaces to be reused many times, saving both time and money. To evaluate the performance of the regeneration solution, the residual activity of the anti-CEA electrode was calculated from the capacitance change (ΔC) as a consequence of the binding between CEA (*i.e.* 0.1 ng ml^{-1} of CEA standard) and anti-CEA before (ΔC_1) and after regeneration (ΔC_2) (Figure 8.3) as follows;

$$\% \text{ Residual activity} = \frac{\Delta C_2 \times 100}{\Delta C_1} \qquad (3)$$

The criteria for regenerating the electrode surface is "if post-regeneration binding remains above 90 % compared to the binding efficiency before regeneration, the used conditions should be seen as adequate" (van der Merwe, 2000). A common approach for CEA-anti-CEA regeneration is to use different types of regenerating agents of three categories, that is high ionic strength, low pH, and high pH and the results are shown in Table 8.1.

High ionic strength and high pH regeneration agents were shown to be ineffective with very low percent residual activity values (15 ± 3 to 42 ± 5 %), indicating that many anti-CEA molecules were still occupied by CEA after the regeneration step. However, at low pH, *i.e.* 50 mM Glycine-HCl buffer, pH 2.50 and HCl, these were shown to be effective with a percent residual activity of 59 ± 16 and 67 ± 5 %, respectively. So, in this case HCl solution was chosen for further optimization.

Influence of pH of HCl solution, ranging from 3.00 down to 1.50 were studied (Table 8.1). The values of percent residual activity increased form 9 ± 6 to 94 ± 2 % when pH decreased form 3.00 to 2.00. At pH 1.50 the percent residual activity was lower than at pH 2.00. This may be because at pH 1.50 some parts of SAM were destroyed by the very low pH (Jiang *et al.*, 2003). Since at pH 2.00 residual activity values above 90 % were achieved, HCl pH 2.00 should be used as the regeneration solution in the continued experiments.

To make sure that the SAM layer would not be destroyed during long term analysis, two modified electrodes were tested. One was prepared with only the SAM layer and 1-dodecanethiol, the other with anti-CEA immobilized on the SAM layer and 1-dedecanethiol. Fifty regeneration cycles were applied. Cyclic voltammograms were obtained before regeneration and after every 10 cycles. All cyclic voltammograms were the same for both electrodes (similar to Figure 8.5(e)). This indicates that the SAM was still attached to the gold substrate. The stability of the SAM in this system was probably due to its short exposure time, only a few minutes at a time, to the regeneration solution. In most batch systems (Jiang *et al.*, 2003; Hu, *et al.*, 2002; Tang *et al.*, 2004) the acid solution is usually used to regenerate modified electrode for 5-20 min. In this work a flow injection system was used (flow rate 100 μl min^{-1}, sample volume 200 μl), and the acid solution was retained on the modified electrode for only 2-3 min/cycle.

It should be noted that the capacitance of the system increased during regeneration (Figure 8.3). This is because the capacitance also depends on the ionic strength of the solution. Higher ionic strength solution will give a higher value of capacitance (Berggren *et al.*, 1998; Jiang *et al.*, 2003). When the regeneration solution was injected into the system to dissociate the binding between CEA and anti-CEA, the capacitance signal is higher than the base-line capacitance because the ionic strength of the regeneration solution (HCl, pH 2.00) is higher than the ionic strength of the carrier buffer (10 mM Tris-HCl).

Table 8.1 The efficiency of CEA removal from the anti-CEA immobilized on the electrode studied by injecting 0.1 ng ml^{-1} CEA. The efficiency is given as capacity (in per cent of initial value) of the sensor to respond to a new pulse of CEA.

Regeneration solution	Percentage of average residual activity
High ionic strength	
1 M NaCl	29 ± 13
4 M KCl	33 ± 11
2 M $MgCl_2$	42 ± 5
Low pH	
50 mM Glycine-HCl, pH 2.50	59 ± 16
HCl, pH 2.50	67 ± 5
High pH	
5 mM NaOH	15 ± 3
50mM NaOH	17 ± 6
Low pH	
HCl, pH 3.00	9 ± 6
HCl, pH 2.50	67 ± 5
HCl, pH 2.00	94 ± 2
HCl, pH 1.50	86 ± 9

8.4.2.2 Flow rate

In a flow injection capacitive immunosensor system, the flow rate of the buffer passing through the capacitive flow cell is the main factor affecting the yield of interaction between CEA and immobilized anti-CEA on the electrode surface. So optimization of flow rate is necessary. The changes in capacitance registered when varying the flow rates in the range 25 - 800 µl min^{-1} increased when the flow rate decreased from 800 to 100 µl min^{-1} (Figure 8.6). However, for 25 and 50 µl min^{-1} the capacitance changes did not differ significantly from that observed at 100 µl min^{-1} ($P < 0.05$). So, 100 µl min^{-1} was chosen.

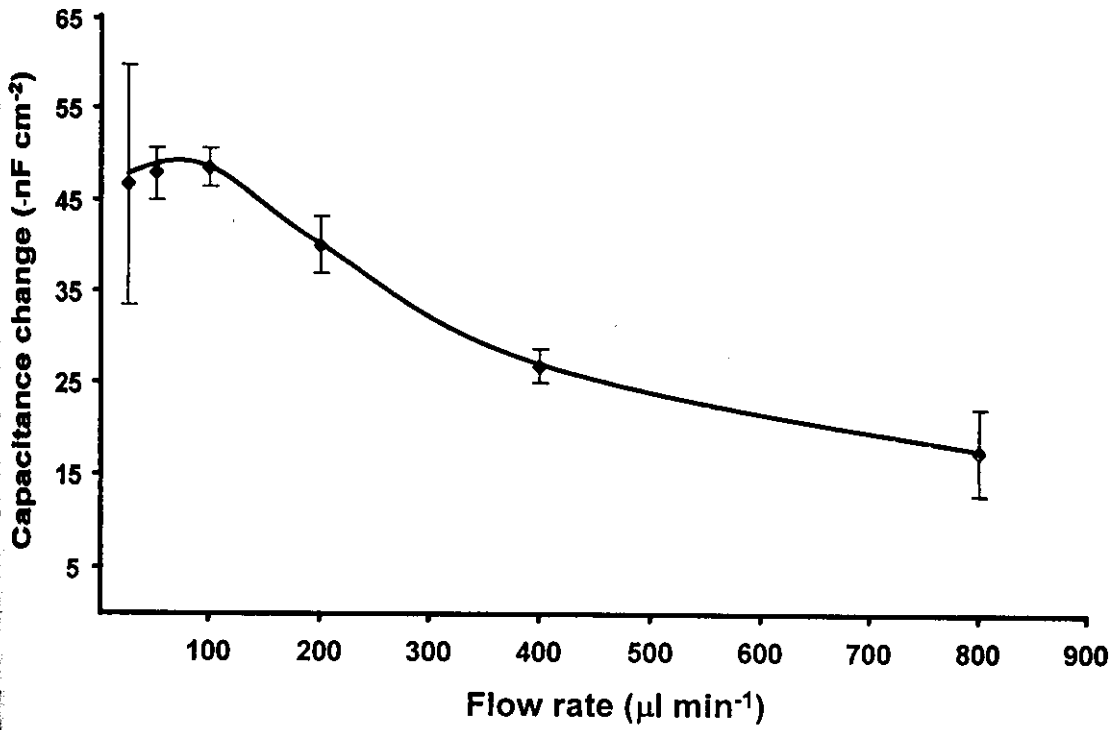


Figure 8.6 Responses of the flow injection capacitive immunosensor system at different flow rates.

8.4.2.3 Sample volume

The change in capacitance signal (-nF cm^{-2}) increased as the sample volume increased from 50 to 200 µl (Figure 8.7). At sample volume 200, 250 and 300 µl the capacitance change reached a maximum plateau. So, 200 µl was chosen because it has lower analysis time than at 250 and 300 µl.

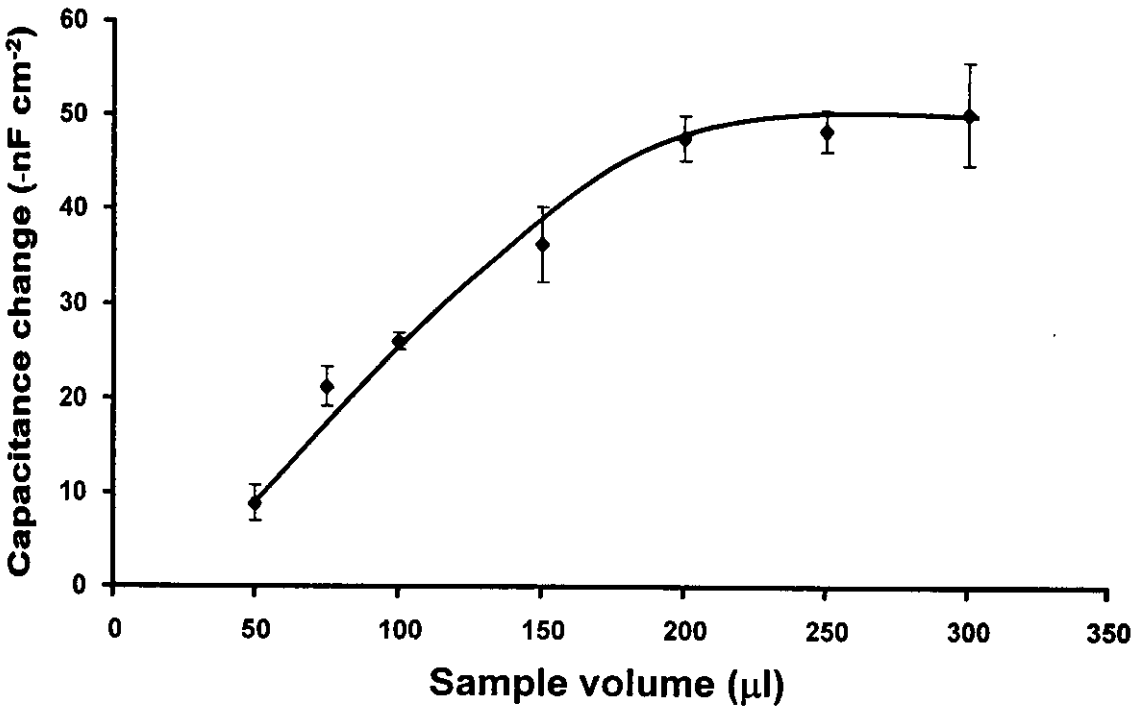


Figure 8.7 Responses of the flow injection capacitive immunosensor system at different sample volume.

8.4.2.4 Buffer solutions

8.4.2.4.1 Type

The influence of the type of buffer used in the flow injection capacitive immunosensor system was investigated (Figure 8.8) and 10 mM tris-HCl buffer pH 7.00 was chosen to be used in further analysis because it gave higher responses and sensitivity. This is because capacitance base-line level depends on the ionic strength of the buffer solution. Since the ionic strength of 10 mM phosphate buffer, pH 7.00 is higher than 10 mM tris-HCl buffer pH 7.00. Therefore, the baseline of phosphate buffer, pH 7.00 is higher than 10 mM tris-HCl buffer pH 7.00. When the analytes bind to the antibody on the electrode the capacitance change, measures from the baseline, in the tris-HCl buffer system is, therefore, higher than the phosphate buffer system.

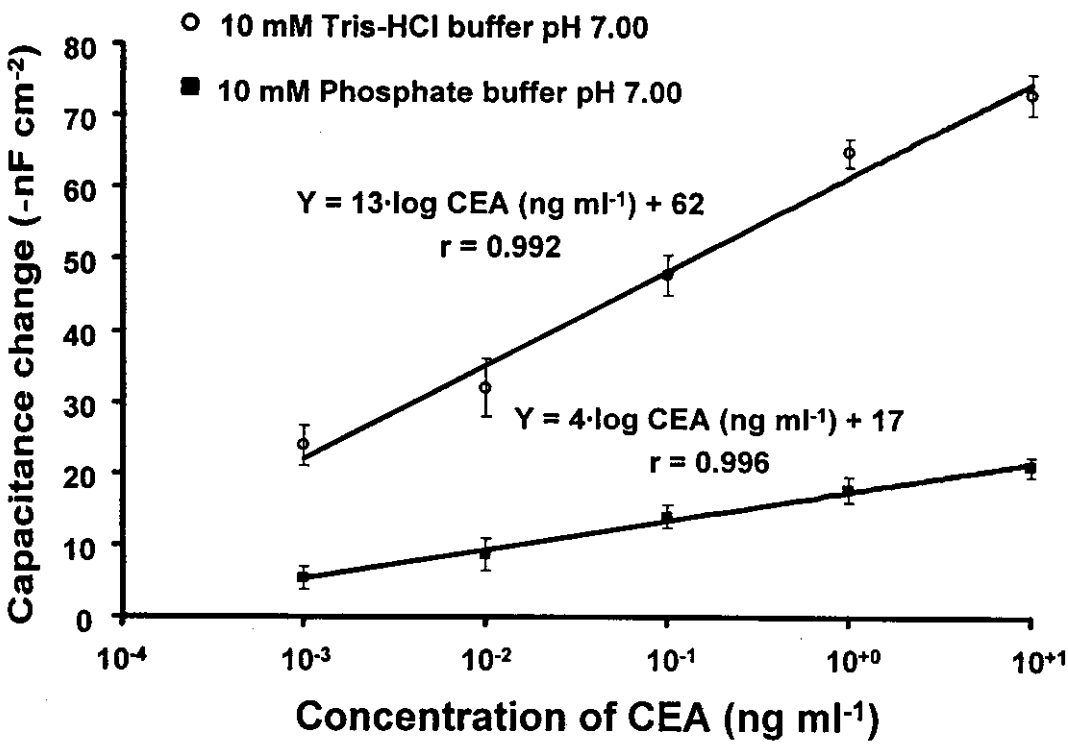


Figure 8.8 Responses of the flow injection capacitive immunosensor system at different of buffer solution.

8.4.2.4.2 pH

The influence of pH during the binding reaction was studied between 6.60 and 8.20 for the same concentration of CEA (0.1 ng ml^{-1}) in 10 mM Tris-HCl buffer solution. (The buffering capacity of Tris-HCl is low at pH values below 7.00, but still the same buffer was used). The change of capacitance increased with increasing pH from 6.60 to 7.00 and then decreased as the pH increased further (Figure 8.9). This result shows that the maximum change in capacitive signal occurs at pH 7.00. This may be explained as follows. One of the forces of binding this affinity pair is electrostatic (Boehm, *et al.*, 2000), therefore, it depends on the charges on the antibody and antigen. The isoelectric point of CEA is 3 - 4 (ShinJin Medics, 2002) and the isoelectric point of anti-CEA is 6.7 - 7.5 (Pritchett, 1994). Therefore in pH 7.00 CEA had the negative charge and anti-CEA had positive charge and this help with the binding. It is possible that at this pH the different charges on each side of the affinity binding pair enable maximum binding compare to other pHs. Therefore, Tris-HCl pH 7.00 was used as the buffer in the binding reaction.

The optimized conditions of the flow injection system are summarized in Table 8.2.

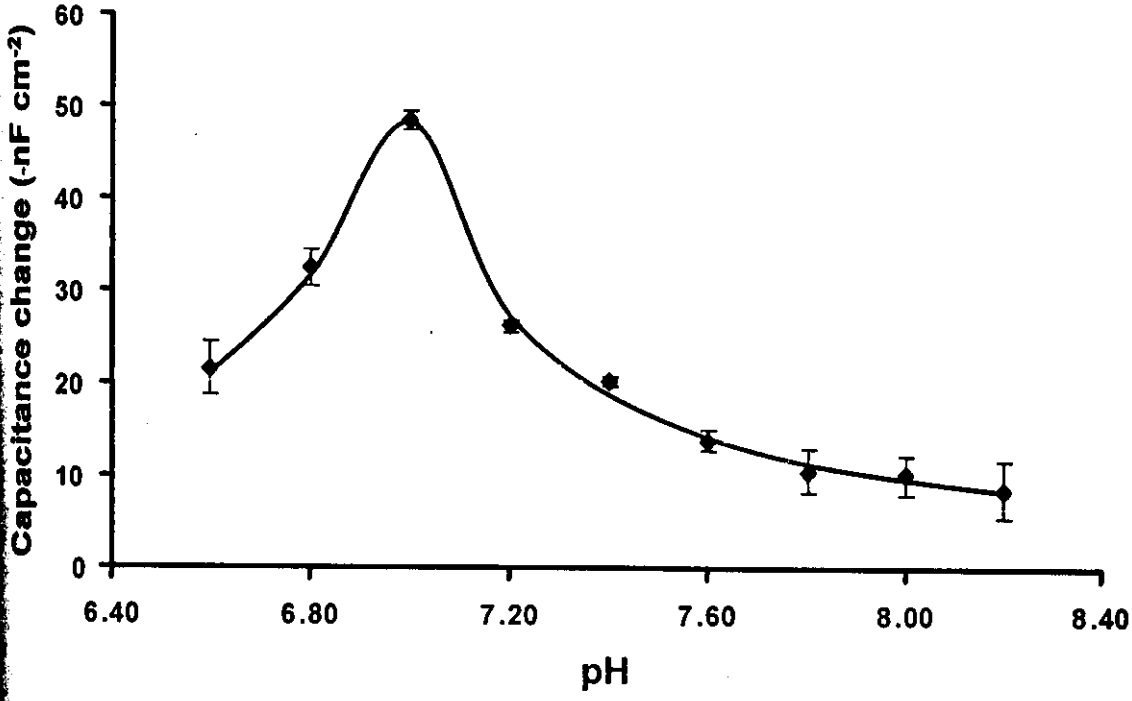


Figure 8.9 Effect of the pH of Tris-HCl buffer solution.

Table 8.2 Assayed and optimized values used in the study of the flow injection capacitive immunosensor system.

Parameters	Investigated values	Concentration of CEA (ng ml^{-1})	Optimum
Flow rate (µl ml^{-1})	25, 50, 100, 200, 400, 800	0.1	100
Sample volume (µl)	50, 75, 100, 150, 200, 250, 300	0.1	200
Buffer solutions			
Type	10 mM Tris-HCl buffer, pH 7.00	10^{-3} to 10^{-1}	10 mM Tris-HCl buffer, pH 7.00
pH	10 mM Phosphate buffer, pH 7.00	0.1	7.00
	6.60, 6.80, 7.00, 7.20, 7.40, 7.60, 7.80, 8.00, 8.20		

8.4.3 Reproducibility

After regeneration, to remove CEA analyte from the anti-CEA molecules immobilized on the electrode, CEA in the standard solution was repeatedly detected by the regenerated electrode to test the reproducibility. The performance of the anti-CEA-thiourea modified electrode was evaluated intermittently over 5 days (15 times per day) by monitoring the change of the capacitance signal at the same concentration of standard CEA (10 ng ml^{-1}).

Figure 8.10 shows the percentage capacitance change (%) versus the cycles of regeneration. The binding activity of anti-CEA immobilized by the self-assembled thiourea monolayer method retained about 91 % of the original capacitance change signal after 3 days (45 times of regeneration) The result indicated that the anti-CEA on SATUM electrode can be reused with good reproducibility for up to 45 times with an RSD lower than 3.4 %.

To test the reproducibility of the electrode preparation techniques, three different preparations of the modified electrode were tested by using the flow injection capacitive immunosensor system under optimum condition; 100 µl min^{-1} flow rate, 200 µl sample volume, 10 mM Tris-HCl buffer, pH 7.00 current buffer. The

sensitivity (slope) of the linear range (0.01 to 0.07 ng ml^{-1}) obtained from three different preparations were used to evaluate the reproducibility performance of this system. The sensitivity of each preparations (Figure 8.11) indicated that there is good reproducibility between different preparations the differences between the sensitivity of the three preparation were 5.8-6.3 % of the first preparation.

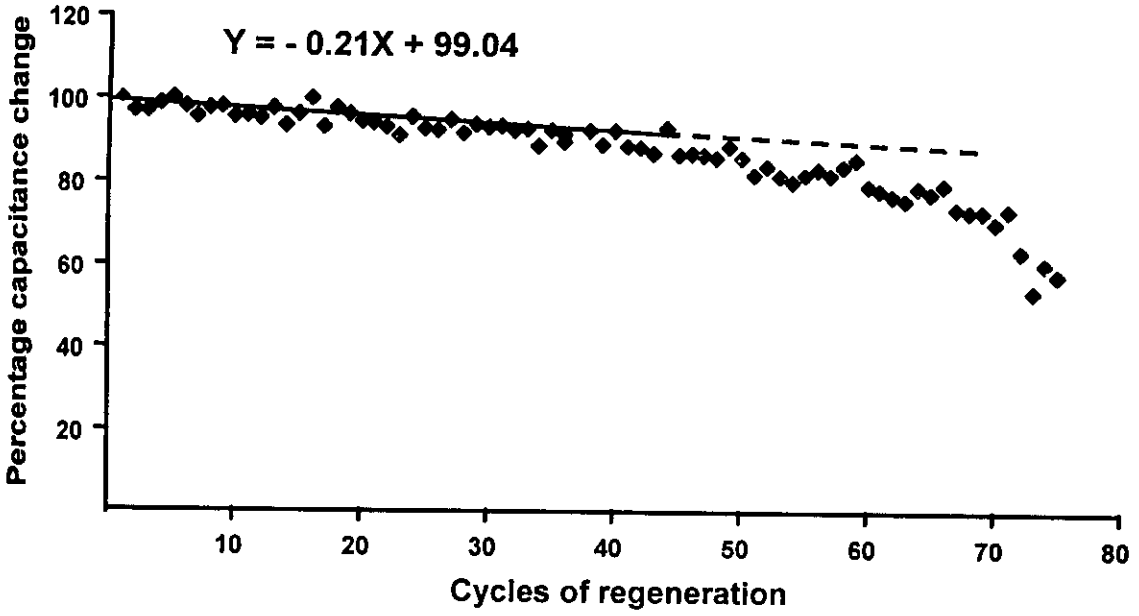


Figure 8.10 Reproducibility of the response from the anti-CEA modified electrode to injections of a fixed volume of a standard solution of CEA (10 ng/ml) with regeneration and reconditioning steps between each individual assay.

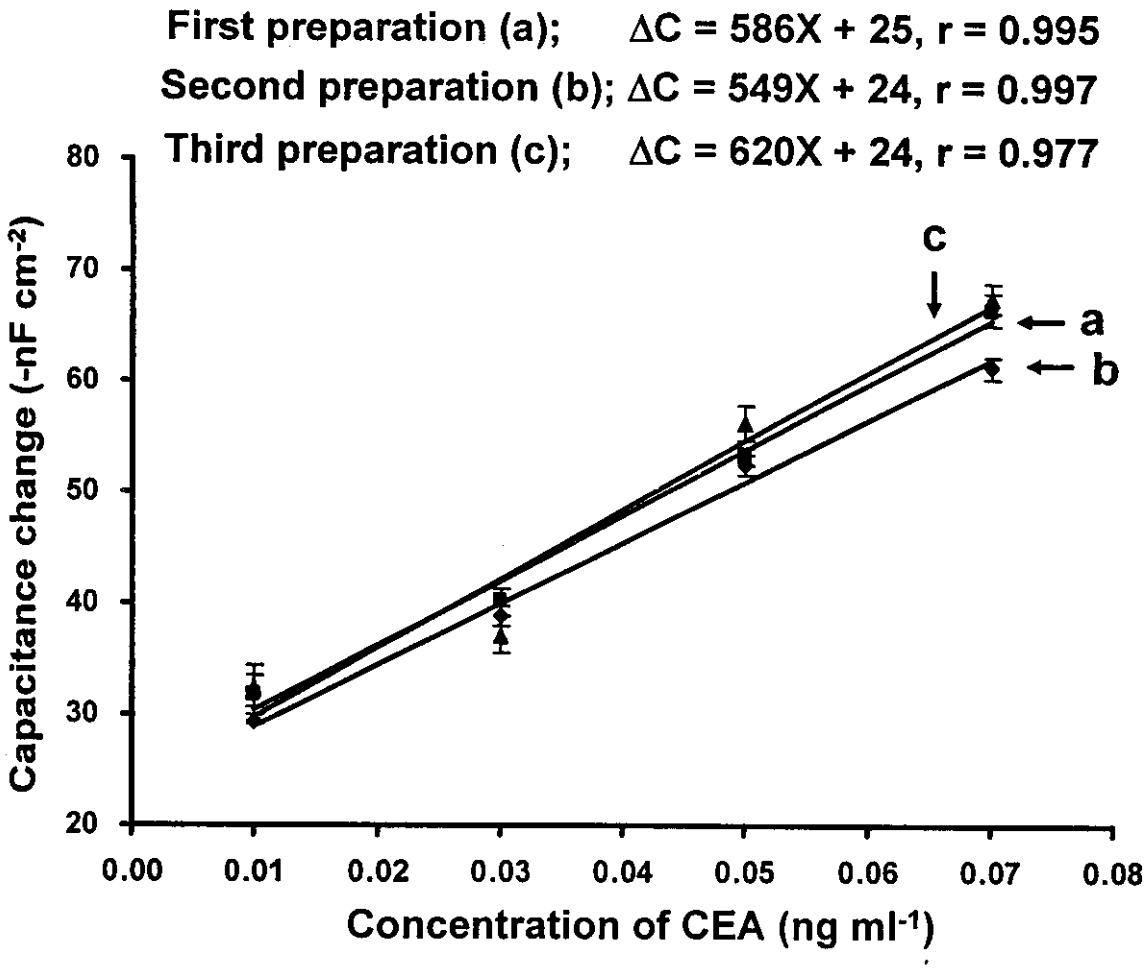


Figure 8.11 Capacitance change *vs.* the concentraion of CEA for a transducer surface with immobilized anti-CEA under optimized conditions (100 μl min^{-1} flow rate, 200 μl sample volume, 10 mM Tris-HCl buffer, pH 7.00); (a) first preparation, (b) second preparation, (c) third preparation.

8.4.4 Possible effect of non-specific binding

When analysing real samples, apart from the analyte, other types of molecule will also be presented and these may cause non-specific binding on the electrode resulting in the response. Figure 8.12 shows the response curves of standard solution and diluted serum sample in the flow injection capacitive immunosensor for CEA. After injection, the response of standard solution decreased and reached a steady level after 5-6 min (Figure 8.12a). For serum sample, the response also decreased but then increased slightly to reach a steady value (Figure 8.12b). This negative overshoot may cause by interference from other compounds in the serum, but was rapidly washed away in the flow system. It is possible that this system does not suffer from non-specific binding of other compounds contain in the serum sample. This is probably because in this system, the preparation of the modified electrode is via self-assembled monolayer that has an insulating property. This helps to prevent non-specific binding on the immunosensor surface (Luppa et al., 2001). The use of a flow system also means that non-specific compounds can not spend much time at the surface. To confirm this, future investigation can be performed with two modified electrode, one with only SAM and 1-dodecanethiol, the other with anti-CEA immobilized on SAM and 1-dedecanethiol. Serum sample can then be applied to both modified electrodes in a flow injection capacitive biosensor system. The hypothesis is that if the system does not suffer from non-specific binding the responses would only come from the electrode with anti-CEA.

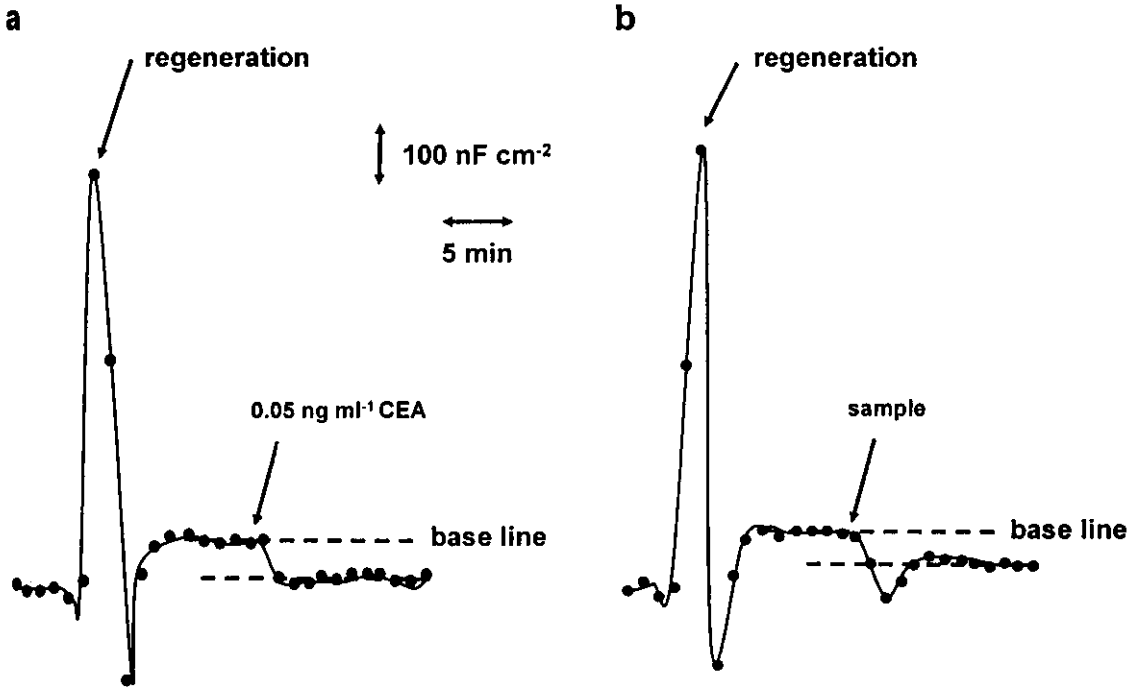


Figure 8.12 The decrease in capacitance resulting from the binding between CEA and anti-CEA; (a) standard CEA (0.05 ng ml^{-1}), (b) serum sample.

8.4.5 Linear dynamic range, detection limit

Discrete pulse injections of CEA standard solutions of concentrations ranging from 1×10^{-3} to $1\times10^{+2}$ ng ml^{-1} with intermediate regeneration steps using HCl solution, pH 2.00 were performed. Figure 8.13 shows the calibration curve for CEA under optimal conditions. A linear relationship between the capacitance change and the logarithm of CEA concentration was obtained within a dynamic detection range of 10^{-2} to 10 ng ml^{-1}. The linear regression equation was ΔC (-nF cm^{-2}) = 31 · log CEA (ng ml^{-1}) + 96, with a correlation coefficient of 0.999. The detection limit was 10 pg ml^{-1} based on IUPAC Recommendation 1994 (see section 4.2) (Buck and Lindner, 1994).

8.4.6 Selectivity

The effect of substances that might interfere with the response of the CEA capacitive biosensor system was also studied. Alpha-fetoprotein (AFP) was used to test the selectivity of the capacitive biosensor system for CEA, because it is another types of tumor marker. The capacitance changes of AFP at the concentration range 1×10^{-3}-$1\times10^{+2}$ ng ml^{-1} on the anti-CEA immobilized on SATUM electrode was lower than the detection limit of CEA (Figure 8.13). These results suggested that the system was selective to CEA.

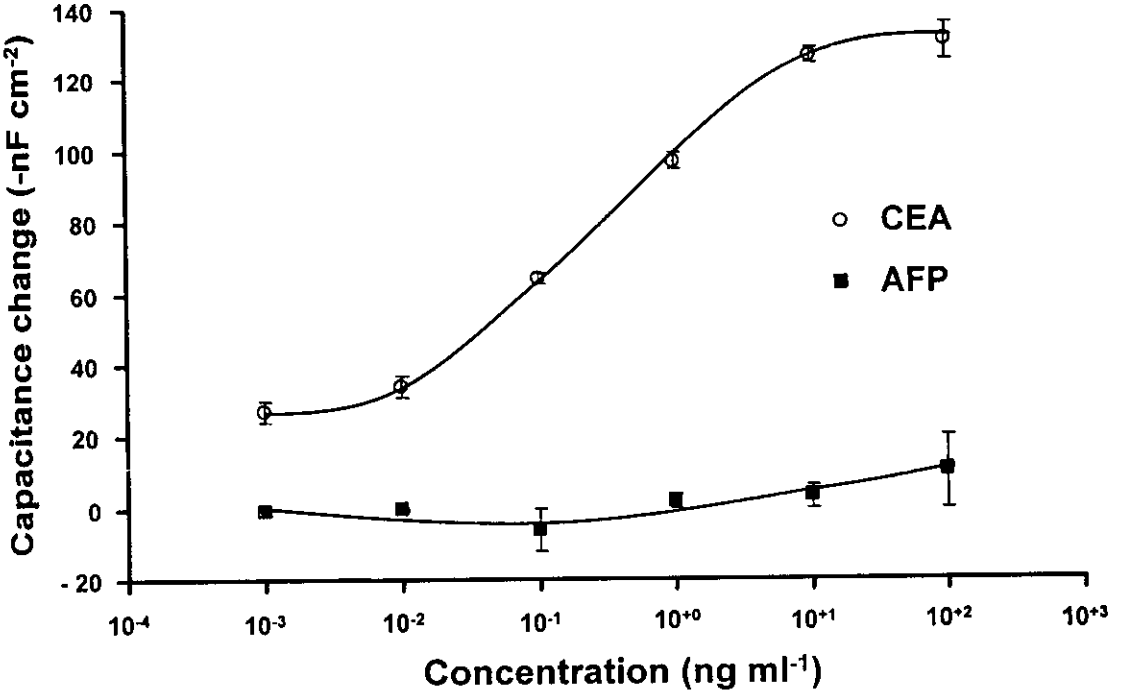
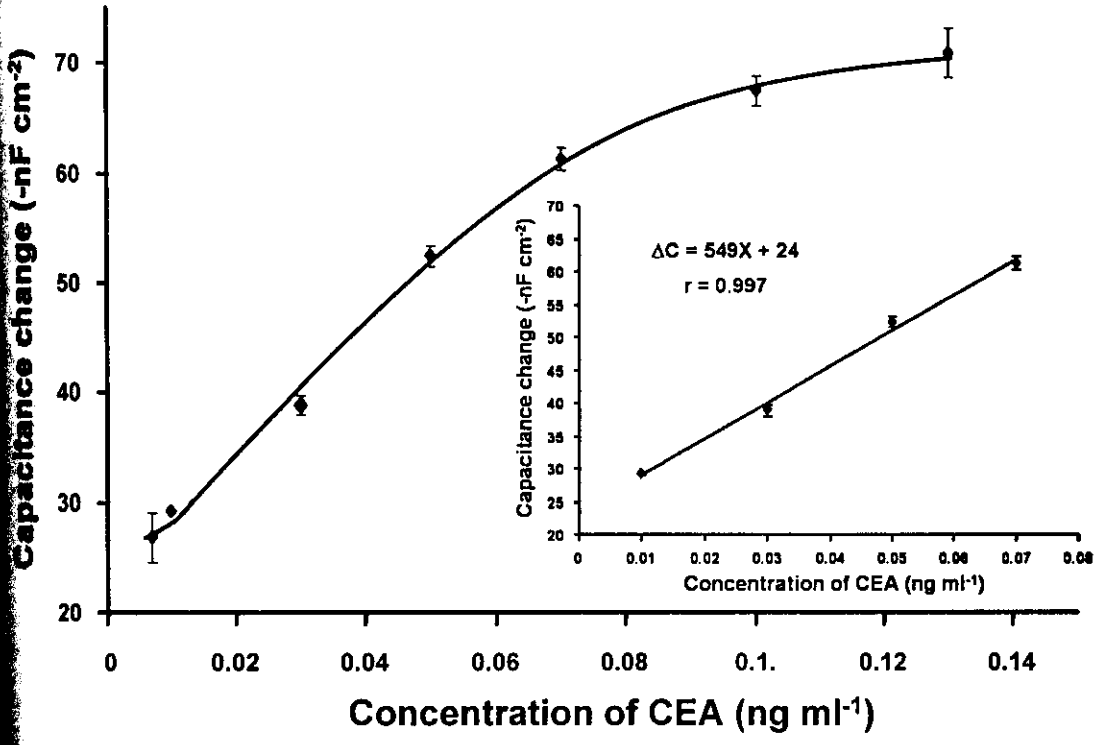


Figure 8.13 Capacitance change *vs.* the logarithm of CEA and AFP concentration for a transducer surface with immobilized anti-CEA under optimized conditions (100 µl min^{-1} flow rate, 200 µl sample volume, 10 mM Tris-HCl buffer, pH 7.00).

8.4.7 Comparison between the results obtained from the capacitive immunosensor system and ELFA technique (VIDAS® CEA)

The linear relationship in section 8.4.5 is suitable for an "order of magnitude" test of CEA. For a good quantitative analysis of CEA in serum samples, a calibration curve between the response and concentration of CEA standard would provide a more accurate result. Since the concentration of CEA in blood of healthy humans is < 2.5 ng ml^{-1} (Kakizaki *et al.*, 1998) and in the analysis the serum will be diluted about 100 times to reduce the matrix effect. The linear concentration calibration was investigated in the range between 0.007 and 0.13 ng ml^{-1} (Figure 8.14) that would cover the concentration range of CEA in diluted serum. In this case linearity was found between 0.01 and 0.07 ng ml^{-1}, *i.e.* ΔC (-nF cm^{-2}) = 549 × concentration (ng ml^{-1}) + 24.



g. 8.14 Capacitance change *vs.* concentration of CEA. The insert shows the relationship between the capacitance change and the concentration of CEA in the concentration range from 0.01 to 0.07 ng ml^{-1}.

The analysis of CEA using the capacitive immunosensor system and ELFA technique were done on the same serum samples. The capacitance change from the capacitive system was used to calculated the concentration of CEA from the calibration equation done prior to the analysis. The results of the ELFA technique were obtained from Hat Yai Hospital. Ten samples were analysed and the concentrations were found in the range of 0.58 to 5.28 ng ml^{-1} (Figure 8.15). Comparisons between the two analysis techniques was done by the regression line method (Miller and Miller, 1993) and the Wilcoxon signed rank test (Triola, 1998). For the regression line method, the regression equation of the concentration of CEA obtained from the capacitive immunosensor system (y) and ELFA technique (x) is $y = 1.00 \pm 0.05\ x + 0.02 \pm 0.09$ with a correlation coefficient of 0.996. The results showed that the slope and the intercept did not differ significantly ($P < 0.05$) from the ideal values of 1 and 0, respectively, thus, there is no evidence for systematic differences between the methods. To verify the reliability, the Wilcoxon signed rank test was also used. In this test, the null hypothesis (there is no difference between the two methods) is rejected at a significance level ($P < 0.05$) if the experimental value is less than or equal to the critical values (see Table 6.1, chapter 6). The result in Table 8.3 showed that the null hypothesis is retained, that is, there is no evidence for systematic differences between the results obtained from the capacitive immunosensor system and the ELFA technique ($P < 0.05$). That is, the concentrations determined by the capacitive immunosensor system are in good agreement to the ELFA technique.

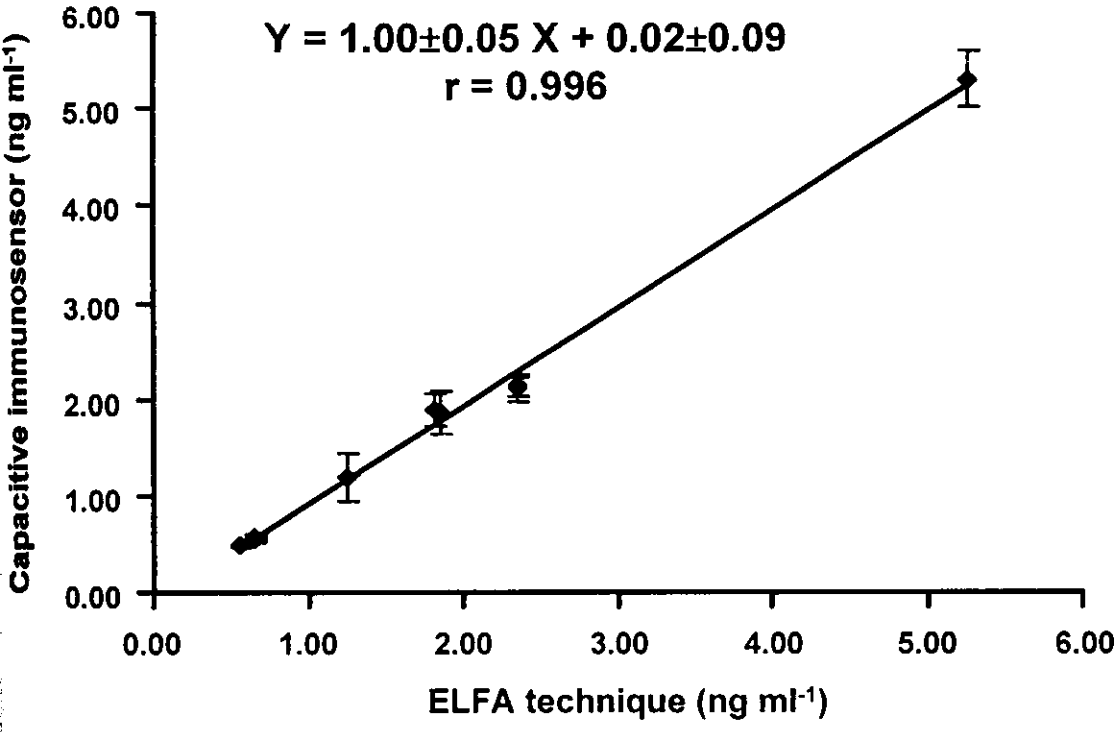


Figure 8.15 Comparison between the results obtained from the capacitive immunosensor system and ELFA technique (VIDAS® CEA) in serum samples.

Table 8.3 The Wilcoxon sign rank test for the comparison of the concentration of CEA in sample from the capacitive immunosensor system and ELFA technique (VIDAS® CEA). (The null hypothesis (there is no difference between the two methods) is rejected if the test statistic T (the lower of the sum of positive rank or negative rank-shown as italic) is less than or equal to the critical value. The null hypothesis can not be rejected if the test statistic T is greater than the critical value).

No. Sample	CEA concentration (ng/ml)		The difference of two method (ng/ml)	Rank
	ELFA technique	Capacitive immunosensor		
1	0.67	0.63	0.04	5.0
2	2.36	2.20	0.16	8.0
3	5.28	5.31	-0.03	-4.0
4	1.82	1.96	-0.14	-7.0
5	0.58	0.57	0.01	1.5
6	0.67	0.65	0.02	3.0
7	1.86	1.92	-0.06	-6.0
8	1.26	1.27	-0.01	-1.5
9	2.36	2.17	0.19	9.0
10	0.67	0.67	0.00	-
n				9
Sum of positive ranks				26.5
Sum of negative ranks				**-18.5**
Test statistic at p value < 0.05, n = 9, tabulate value				6

8.5 Conclusions

Self-assembled thiourea monolayer is suitable for the immobilization of anti-CEA via covalent binding. The modified electrode, which is simple to prepare, when incorporated in a capacitive immunosensor system could provide high sensitivity, low detection limit, good selectivity and specificity for the assay of CEA. Using the appropriate regenerating solution, good reproducibility was obtained. The electrode can be reused up to 45 times and this helps to reduce the cost of analysis. The reproducibility of the sensitivity of different electrode preparations means that recalibration is not required when changing the electrode. It is possible that the immobilization of the antibody via SAM and its operation in a flow injection system help to eliminate the effect of non-specific binding. From these properties the capacitive immunosensor system is potentially useful for direct assay of the interaction between CEA and anti-CEA on self-assembled thiourea monolayer without multiple washing and separation steps. This technique can be applied for the quantitative analysis of amount of CEA in human serum.