Chapter 5
Capacitive Biosensor for Direct Detection of Protein Affinity Reaction

5.1 Introduction

A biosensor is an analytical device that integrates a biorecognition element on a solid-state surface, enabling a reversible biospecific interaction with the analyte, and a signal transducer. Enzyme, cell, tissue, receptor, single-stranded DNA, antibodies, or antibody fragments can all be used as biorecognition elements. Affinity biosensors are one type of biosensor that based on binding interaction between the immobilized biorecognition element and the analyte of interest (Mattiasson, 1984; Taylor, 1991). Affinity biosensor had been successfully applied in clinical and pharmaceutical chemistry as well as contaminants in the environmental area, where high selectivity and sensitivity were required (Byfield and Abuknesha, 1994; Jiang *et al.*, 2003).

Affinity biosensors for label-free detection are based on the detection of the physical changes during the affinity complex formation. It had been performed with surface plasmon resonance, SPR (Calakos *et al.*, 1994; Chapple *et al.*, 1998; Corr *et al.*, 1994; Pei *et al.*, 2000; 2001; Polymenis and David Stollar, 1995; Rao *et al.*, 1999), piezoelectric (quartz crystal microbalance, QCM (Chu et al., 1995; Konig and Gratzel 1994; 1995; Lu et al., 2000; Shen *et al.*, 2005; Su *et al.*, 1999; Suri *et al.*, 1995; Tajima *et al.*, 1998; Yang and chen, 2002)) and electrochemical methods, e.g. potentiometric (Bush and Rechnitz, 1987; Fu *et al.*, 2004; Keating and Rechnitz, 1984; Tang *et al.*, 2004b; 2004c; Yuan *et al.*, 2004), amperometric (Ramanaviciene and Ramanavicius, 2004; Sargent and Sadik 1998; Tang *et al.*, 2005; Zhang *et al.*, 2005), conductimetric (Kanungo *et al.*, 2002; Yagiuda *et al*, 1996), impedimetric (art *et al.*, 2005; Geant *et al.*, 2005; Vagin *et al.*, 2003) and capacitive (Bataillard *et al.*, 1988; Berggren and Johansson, 1997; Berggren *et al.*, 1998; Billard *et al.*, 1991; tidean *et al.*, 1998; Hedström *et al.*, 2005; Hu *et al.*, 2002; 2005; Jiang *et al.*,

2003; Wu *et al.*, 2005a; 2005b; Yang *et al.*, 2005). Among these, capacitive affinity biosensor had attracted more interest recently for its higher sensitivity and less complicated instrumentation (Berggren *et al.*, 1998; 2001; Berggren and Johansson, 1997; Bontidean *et al.*, 1998; Hedström *et al.*, 2005; Jiang *et al.*, 2003; Hu *et al.*, 2002; 2005; Wu *et al.*, 2005a).

The principle of the capacitance transducer is based on the electrical double-layer theory. A working electrode is immobilized with the biological sensing element and has a stable capacitance response. Binding of the target analyte to the biorecognition element on the electrode surface causes the capacitance to decrease. The immobilization of the biorecognition element is the most important part for the construction of an affinity biosensor based on a capacitive transducer because the electrode surface has to be electrically insulated. The formation of self-assembled monolayers (SAMs) of several compound, via spontaneous adsorption of sulfur-containing molecules on gold electrode surface, are highly stable (in air, water, and organic solvesnts at room temperature), and well insulating (Bain *et al.*, 1989; Chaki and Vijayamohanan, 2002). Thus, capacitive affinity biosensor was often fabricated by coupling biorecognition element covalently on the electrode surface of the SAMs-modified electrode (Bain *et al.*, 1989; Berggren and Johansson, 1997; Berggren *et al.*, 1999; Bontidean *et al.*, 1998; 2000; 2003 Hedström *et al.*, 2005; Jiang *et al.*, 2003; Mirsky *et al.*, 1997).

This project reports the initial investigation of the use of a flow injection capacitive biosensor system for the direct detection of protein by affinity binding. Two affinity binding pairs, human serum albumin (HSA) and anti human serum albumin antibody (anti-HSA), and crystallizable fragment (Fc-fragments) from IgG (anti-HSA) and protein A, were used as models to test the system (Figure 5.1).

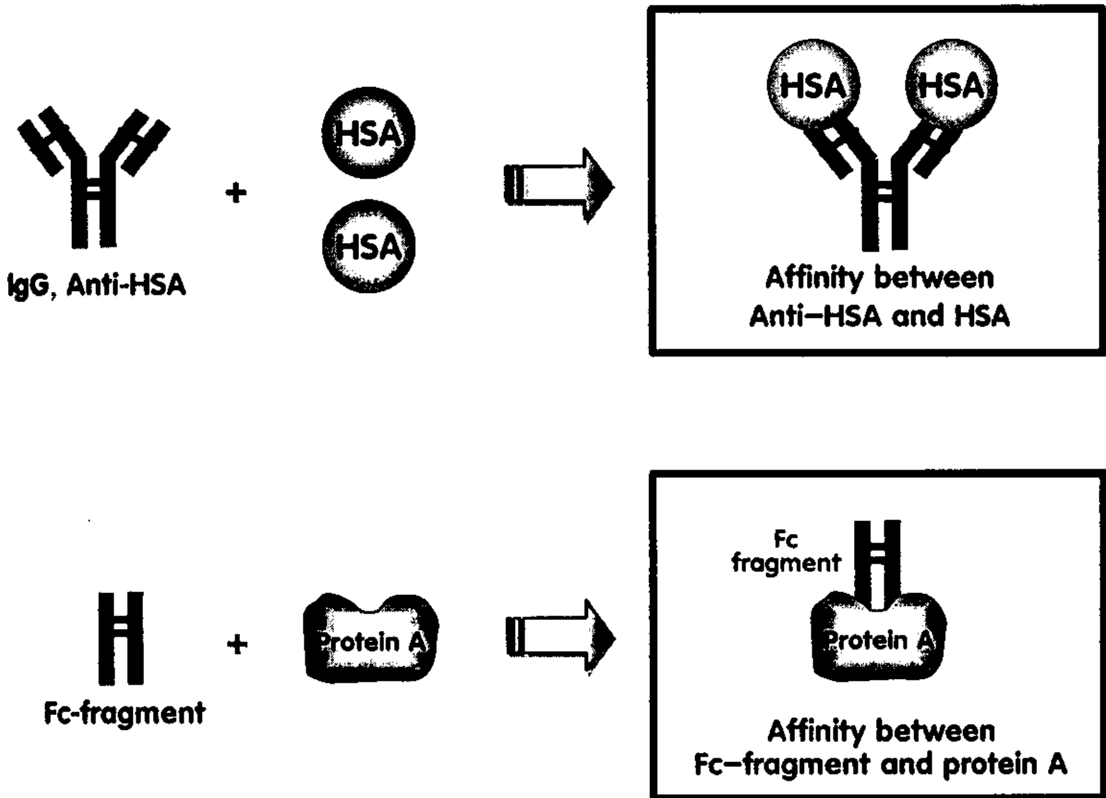


Figure 5.1 Two affinity binding pairs, human serum albumin (HSA) and anti human serum albumin antibody (anti-HSA), and crystallizable fragment (Fc-fragments) from IgG (anti-HSA) and protein A.

5.2 Materials

Anti human serum albumin (anti-HSA, IgG) and human serum albumin (HSA) were obtained from Dako (Denmark). Bovine serum albumin (BSA), thioctic acid and *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC) were obtained from Sigma (St. Louis, USA), 1-dodacanthiol was obtained from Aldrich (Deisenhofen, Germany). All other chemicals used were of analytical grade. All buffers were prepared with water treated with a Milli-Q system, preceded by a reverse osmosis step, both from Millipore (Bedford MA, USA). Before use, the buffers were filtered through a Millipore filter with pore size 0.22 μm with subsequent degassing.

5.3 Methods

5.3.1 Preparation of Fc-fragments from anti-HSA (IgG)

5.3.1.1 Papain-digestion of anti-HSA IgG

The IgG of anti HSA was digested with papain (activity > 1,750 U.S.P./mg, MP Biomedicals, USA) as shown in Figure 5.2. Papain digestion was performed overnight using an enzyme to antibody ratio of 1:20 in 20 mM potassium phosphate buffer saline (PBS), pH 7.4 with 100 mM L-cystein and 20 mM EDTA at 37 °C in a shaker water bath. Then 20 mM iodoacetamide was added and the mixture was put into an ice bath in the dark for 1 h to stop the reaction (Aybay, 2003). The digested sample solution consists of Fab (50 kDa) and Fc (50 kDa) fragments, papain (23 kDa) and the remainder of undigested IgG (150 kDa).

5.3.1.2 Purification of Fc-fragments

To obtain the Fc-fragments the digested sample solution was purified by using gel filtration column (Figure 5.3a) and protein A affinity column (Figure 5.3b).

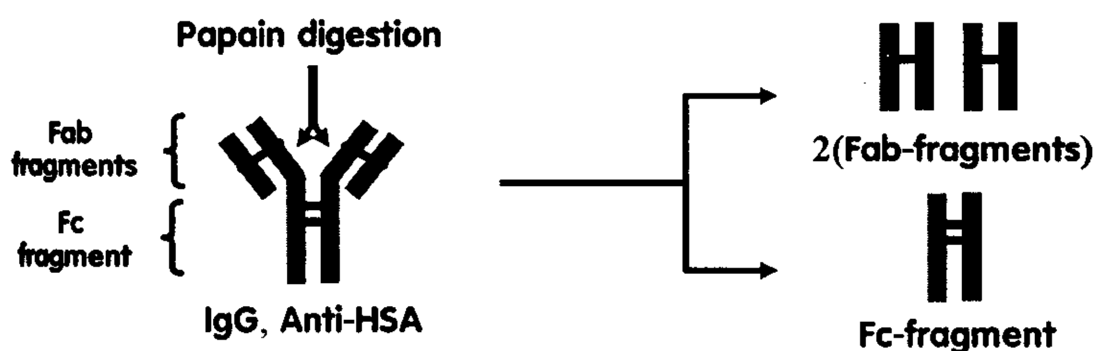


Figure 5.2 Schematic diagram of digestion of IgG molecule by papain resulting in two Fab-fragments and one Fc-fragment.

5.3.1.2.1 Gel filtration column (Sephadex G-50)

Gel filtration is the simplest of all chromatography techniques. It separates molecules on the basis of differences in size. It is used to remove small molecules from a group of larger molecules. Digested sample solution, 1.9 ml, was applied to a gel filtration column at a flow rate of 0.3 ml min^{-1}. PBS passes continuously through the column bringing the molecules with it. Molecules that are larger than the pores are unable to enter into the pores and pass through the column. Smaller molecules diffuse into the pores and are delayed in their passage down the column. So, large molecules leave the column first followed by smaller molecules, the order of which relates to their sizes as show in Figure 5.3a. In this step, the fraction of peak 2 (Fc-fragment and Fab-fragment) was collected to be purified using protein A affinity column.

5.3.1.2.2 Protein A affinity column

Protein A is derived from a strain of *Staphylococcus aureus* and contains five regions that bind to the Fc region of IgG. As an affinity ligand, protein A is coupled to Sepharose so that these regions are free to bind. One molecule of coupled protein A can bind to at least two molecules of Fc region of IgG. Protein A column was used to purify Fc-fragment from Fab-fragment. The separated sample solution (fraction of peak 2 in section 5.3.1.2.1) was passed through protein A column that was equilibrated with 20 mM PBS, pH 7.00 at a flow rate of 135 μl min^{-1}. Only the Fc-fragment bound to protein A and Fab-fragment left the column first. Fc-fragment was then eluted with 0.1 M sodium citrate, pH 3.00 with the same flow rate. This fraction was collected in a tube containing neutralization buffer (1 M Tris-HCl, pH 9.00).

5.3.2 Determination of Fc-fragments

Amount of Fc-fragments in the purified sample solution (fraction tubes in section 5.3.1.2.2) was analysed by using bicinchoninic acid (BCA) protein assay (Bicinchoninic Acid Protein Assay kit, BCA-1, Sigma). The principle of the bicinchoninic acid (BCA) assay is based on the reaction of protein with alkaline Cu^{2+} to form Cu^{1+} that can be monitored using the intense purple color formed with the

specific Cu^{1+} chelating compound bicinchoninic acid. The amount of reduction is proportional to the protein present.

BCA working reagent (Bicinchoninic Acid Protein Assay kit, BCA-1, Sigma), 2.0 ml, was added to 0.1 ml of each BSA protein standard (0.20, 0.40, 0.60, 0.80, 1.00 mg ml^{-1}), blank, and unknown sample (the purified sample solution). The tubes, was sealed with parafilm, and incubated at 37 °C for 30 min. The absorbance of each solution was measured at 562 nm. Protein concentration was determined by comparing the absorbance of the unknown samples to the standard curve prepared using the BSA protein standards (Figure 5.4).

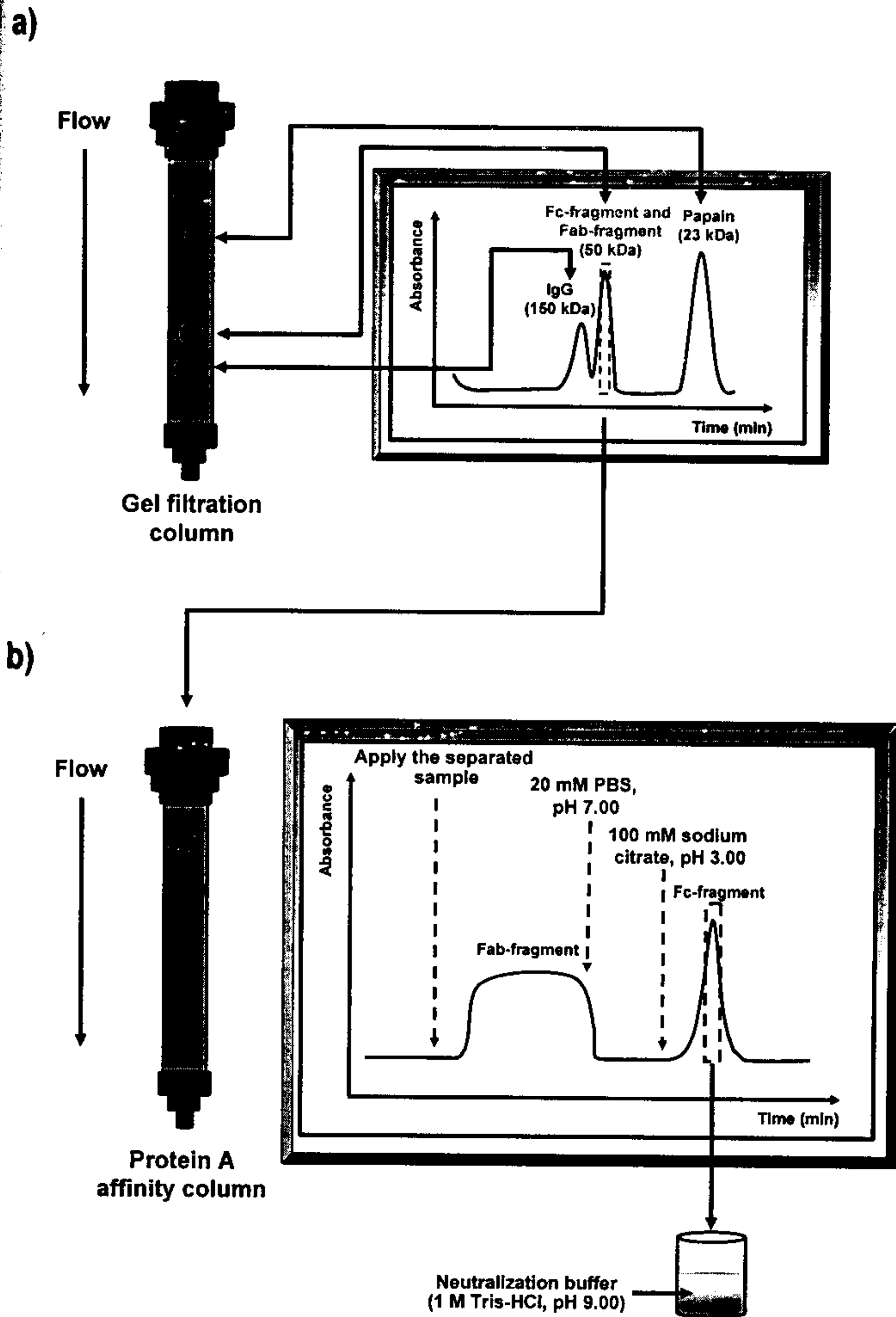


Figure 5.3 a), Separation of Fc-fragment and Fab-fragment from the digested sample solution on gel filtration column. b), Purification of a Fc-fragment from Fab-fragments by protein A affinity column.

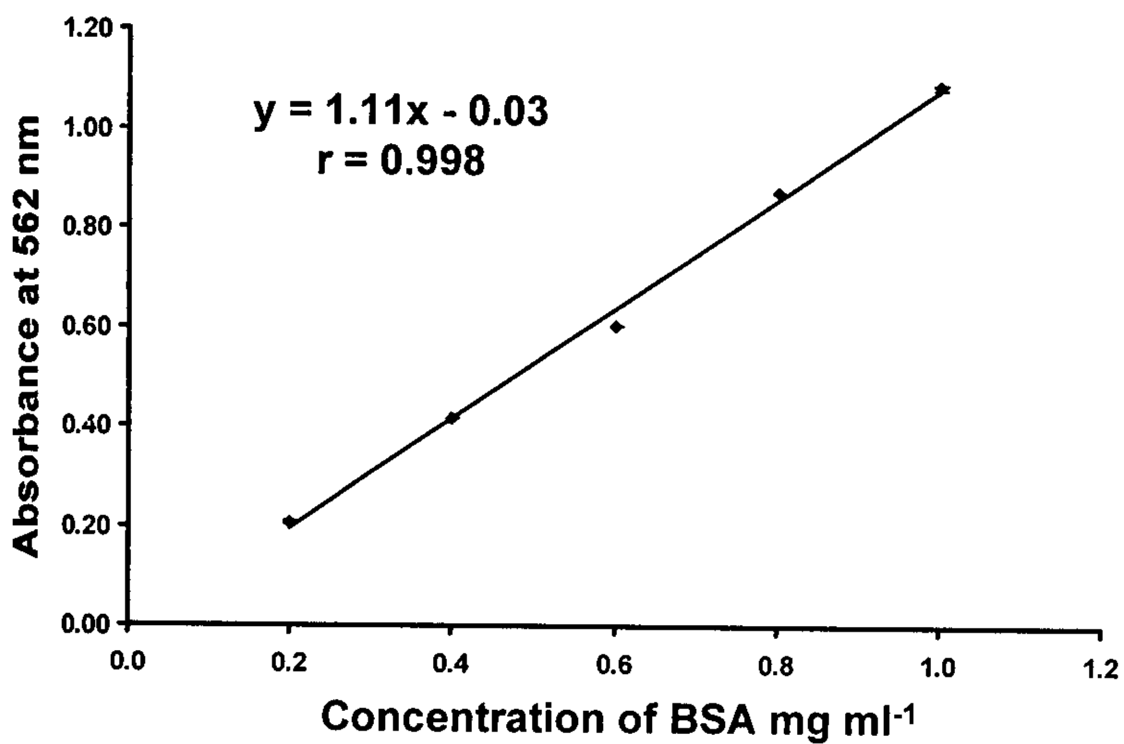


Figure 5.4 Standard curve of absorbance (562 nm) *versus* protein sample concentration.

5.3.3 Immobilization

5.3.3.1 Pretreatment of gold surface

For the formation of a well ordered and packed SAM, both the structure and the state of the gold surface are important (Dijksma *et al.*, 2000; Troughton *et al.*, 1988). Thus, pretreatment procedures of gold surfaces prior to SAM are necessary. The procedure applied to gold surface in this work was a sequential polishing treatment "until a mirror surface is obtained". Gold rod electrodes (Ø 3 mm, 99.99% purity) were used as transducer in the biosensor method. The gold surface was polished using alumina slurries with particle diameter 0.1 and 0.05 μm (Struers, Denmark) and then thoroughly rinsed with distilled water. In Figure 5.5, two optical microscopy images of the same electrode are shown: (a) before and (b) after polishing with alumina.

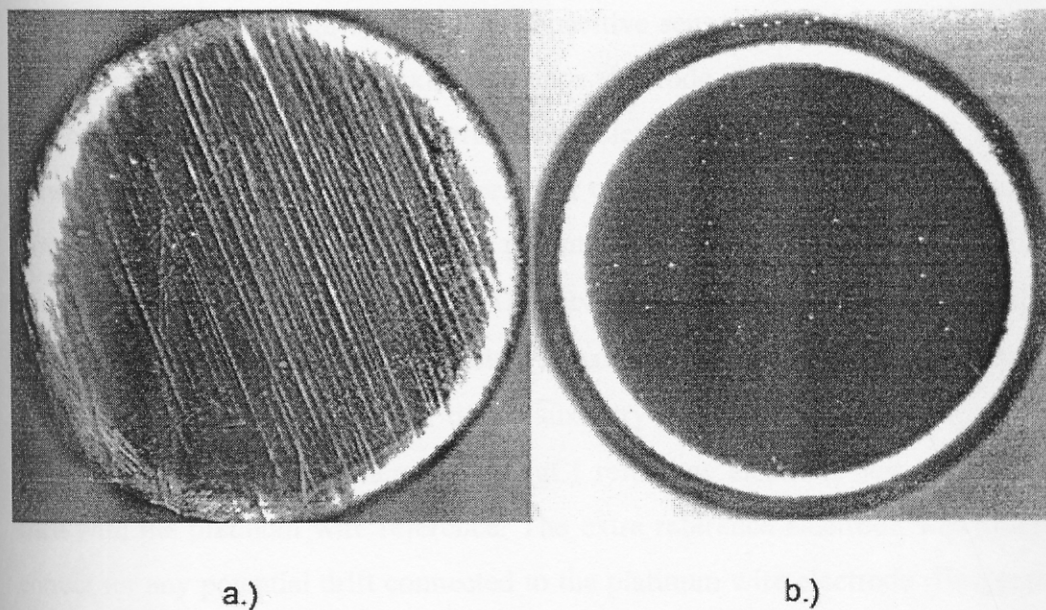


Figure 5.5 Gold electrode surface (Ø 3 mm) under optical microscopy before (a) and after polishing with alumina (b).

5.3.3.2 Immobilization of anti-HSA or Fc-fragment

The cleaned gold electrodes were positioned in Teflon holders and placed in a plasma cleaning device (Mod. PDC-3XG, Harrich, NY) for 10 minutes. The plasma-cleaned electrodes were immediately put in a thioctic acid solution (2% (w/w) in absolute ethanol). After reaction overnight, the electrodes were thoroughly rinsed with absolute ethanol and dried under vacuum. Thereafter, the gold electrodes were put in EDC solution (1% w/w in dry acetonitrile) for 5 h and then rinsed with 100 mM potassium phosphate buffer (PPB), pH 7.20 and dried with chemically pure nitrogen gas. The electrodes were then immersed in 1.0 mg ml^{-1} anti-HSA or Fc-fragment in 0.1 M borate, pH 8.50 overnight at 4°C. Finally, before placing in the biosensor flow-cell, the electrodes were reacted in a 10 mM 1-dodecanethiol ethanolic solution for 20 min to block any pinholes or bare spots on the electrode surface.

5.3.4 Capacitance measurement

Figure 5.6 shows the basic experimental set-up of the flow-injection based capacitive biosensor system. The capacitive sensor was part of a potentiostatic three electrode system with an extra reference electrode. A Keithley 575 measurement and control system (Keithley Instruments, Cleveland, OH, USA) was connected between a potentiostat and a computer. Four electrodes were placed in the biosensor flow cell (10 µl) and connected to the potentiostat. The working electrode was made from a modified gold rod (Ø 3 mm). The auxiliary electrode was a disc shaped piece of platinum foil with a hole in the center. The reference electrode was a platinum wire (Ø 0.5 mm) situated in the center of the auxiliary electrode in close proximity to the transducer surface. A commercial Ag/AgCl reference electrode was concomitantly used with the platinum wire reference. The extra reference electrode was utilized to correct for any potential drift connected to the platinum wire electrode (Berggren and Johansson, 1997; Berggren *et al.*, 1998; Bontidean *et al.*, 2003).

Continuously during the binding event between biorecognition element and analyte, 50 mV potential pulses are applied to the gold electrode yielding current response signals, which can be described by equation 1.

$$i(t) = \frac{u}{R_S} \exp\left[\frac{-t}{R_s C_{Total}}\right] \qquad (1)$$

Where i(t) is the current in the circuit as a function of time, u is the pulse potential applied, R_s is the dynamic resistance of the recognition layer, t is the time elapsed after the potential step was applied, and C_{total} is the total capacitance measured at the working electrode/solution interface. Taking the logarithm of equation (1) the relationship becomes

$$\ln i(t) = \ln \frac{u}{R_S} - \frac{t}{R_s C_{Total}} \qquad (2)$$

The logarithm of current gives a linear relationship with respect to time from which R_s and C_{total} can be calculated. The resistance (R_s) and total capacitance (C_{total}) values were evaluated from equation (2) directly in the program (Berggren *et al.*, 1999). The total capacitance was shown and stored as a function of time on the computer (Berggren and Johansson, 1997; Berggren *et al.*, 1998; Bontidean *et al.*, 2003). Consequently, affinity binding of analyte to the immobilized bioreconition molecules on the working electrode will cause the C_{total} to decrease and the change in capacitance (ΔC) was calculated and processed (Figure 5.7). Since the interactions between analyte (Ag) and biorecognition molecules (Ab) on electrode surface are via non-covalent bonding, the analyte was dissociated from the biorecognition element on the electrode surface by using regeneration solution.

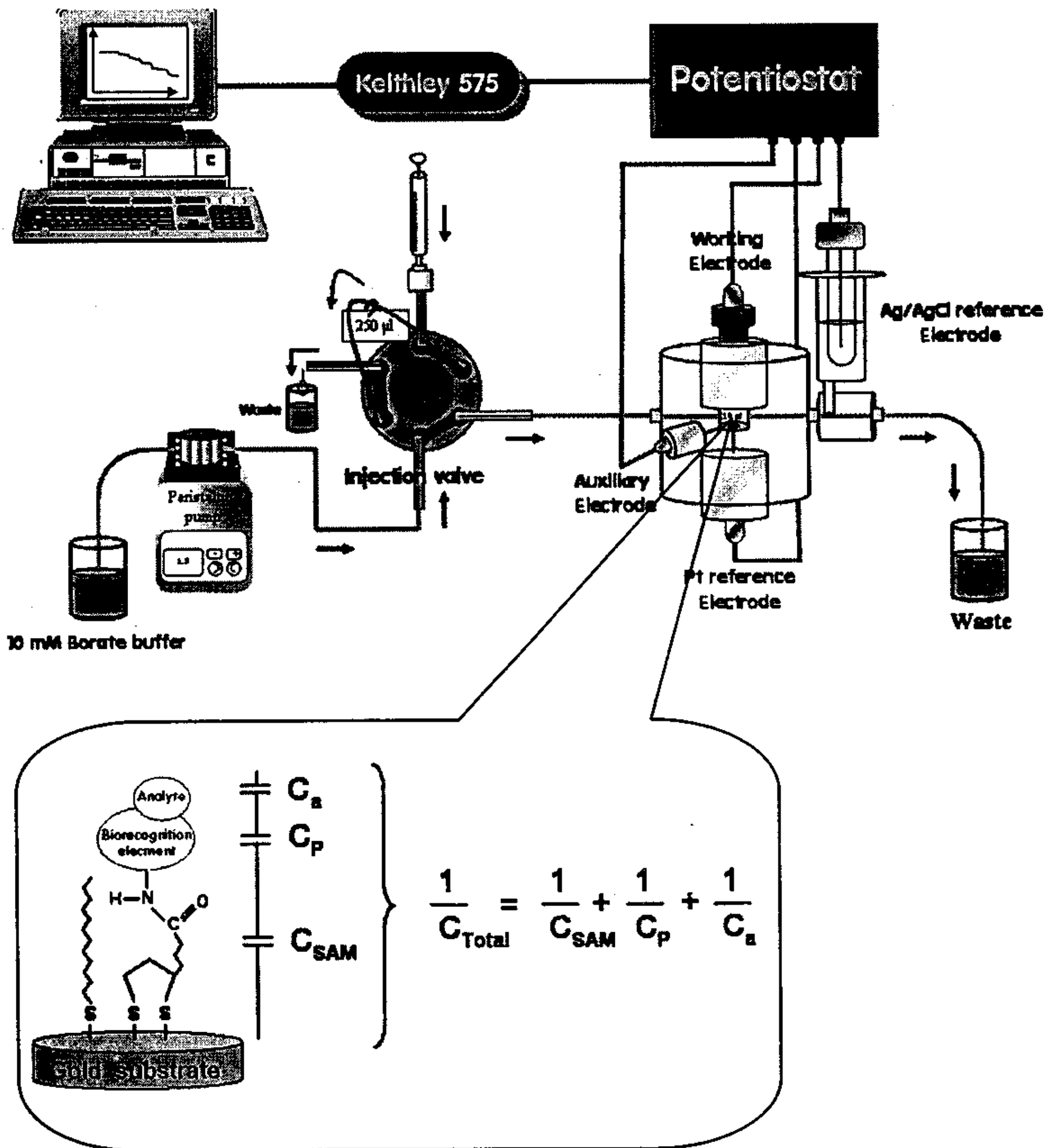


Figure 5.6 Schematic view of the flow injection capacitive biosensor system. The capacitive properties of the tranducer surface where C_{SAM}; the capacitance of self-assembled thioctic acid monolayer, C_P; the capacitance in protein layer, C_a; the capacitance of analyte interaction and C_{Total}; the total capacitance measured at the working electrode/solution interface.

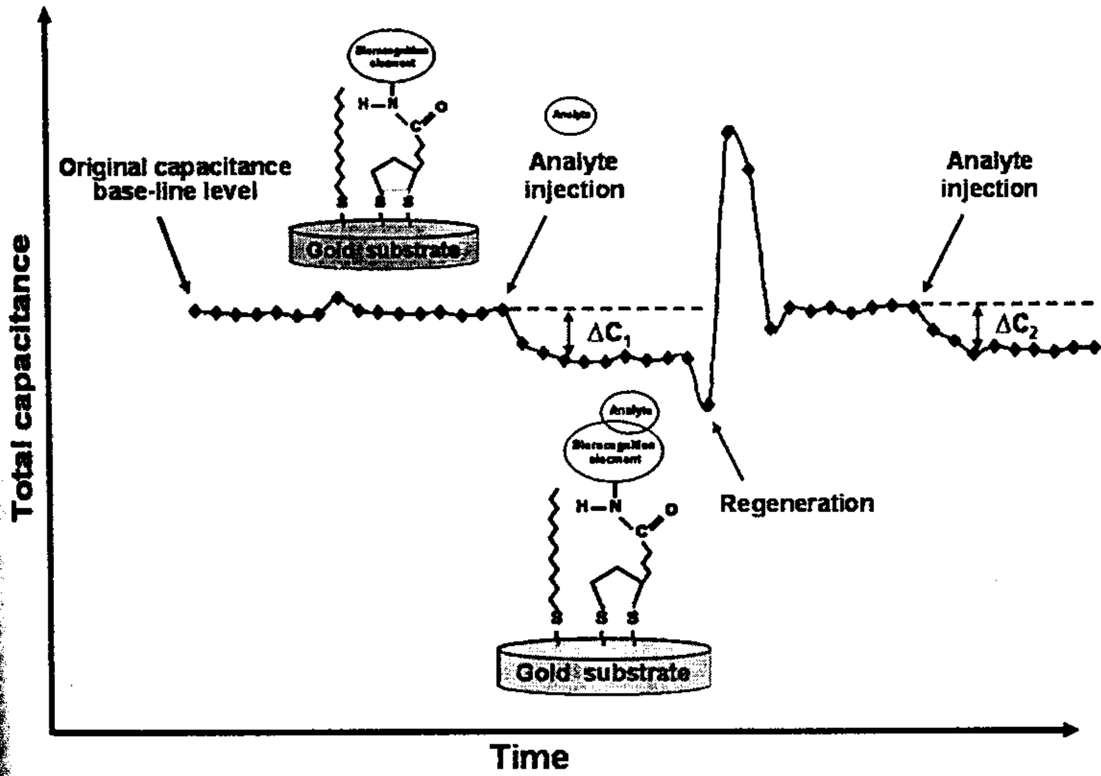


Figure 5.7 Schematic diagram showing the change in capacitance (ΔC) as a function of time caused by binding between anlyte and biorecognition element with subsequent signal increase due to dissociation under regeneration conditions.

5.3.5 Capacitive biosensor for HSA

The detection of HSA is investigated initially to test the capacitive system, therefore, the effect of only two parameters were tested, *i.e.*, type of running buffer and concentration of anti-HSA used in the immobilization. The running conditions were flow rate 250 μl min^{-1}, sample volume 250 μl (Hedström *et al.*, 2005), and the running buffer used throughout the experiments was a 10 mM borate buffer, pH 8.65 (Hermanson *et al.*, 1992) (except when the effects of buffer types were tested).

5.3.6 Capacitive biosensor for protein A

For the capacitive biosensor for protein A, the tested parameters were type, pH and concentration of the regeneration solution, sample volume, and flow rate. The running buffer used throughout the experiments was a 10 mM borate buffer, pH 8.50. The effects of type, pH and concentration of regenerate solution were tested with conditions; 250 μl min^{-1} flow rate of buffer, 250 μl sample volume. The effect of the sample volume was tested at a flow rate of 100 μl min^{-1}.

5.4 Results and discussion

5.4.1 HSA

The flow injection capacitive biosensor system was used to test the efficiency of the affinity biding pair of HSA and anti-HSA.

5.4.1.1 Effect of type of buffer solution

The influence of the type of buffer used in the flow injection capacitive biosensor system; 10 mM borate buffer, pH 8.65 (Hermanson *et al.*, 1992), 10 mM citrate buffer, pH 7.40 (Berggren and Johansson, 1997), 10 mM Tris-HCl, pH 7.40 (Quist *et al.*, 2004), and 10 phosphate buffer, pH 7.40 (Hedström *et al.*, 2005; Navrátilová *et al.*, 2001) were studied (Figure 5.8). Borate buffer was chosen to be used in further analysis because it gave the highest sensitivity. This corresponded well with the fact that this buffer is normally used as a running buffer in the process of purification of HSA (Hermanson et al., 1992).

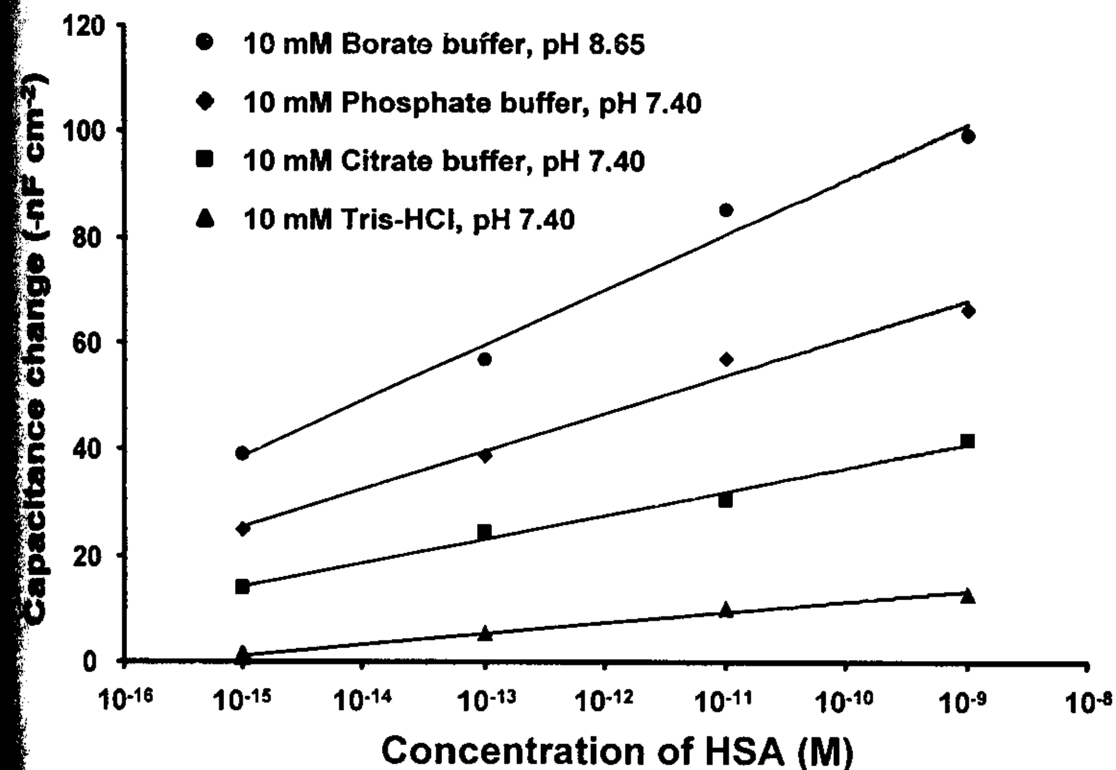


Figure 5.8 Responses of the flow injection capacitive biosensor system for HSA using different buffer solutions.

4.1.2 Effect of concentration of anti-HSA

The effect of the concentration of anti-HSA used in the immobilization, 0.10, 0.25, 0.50, and 1.00 mg ml^{-1}, was studied. The sensitivity (slope of calibration curve) resulting from injections of HSA standard solutions (10^{-15} to 10^{-7} M) increased with concentration (Figure 5.9) with a trend of leveling off at 1.00 mg ml^{-1}. Therefore, 1.00 mg ml^{-1} of anti-HSA was chosen for later work.

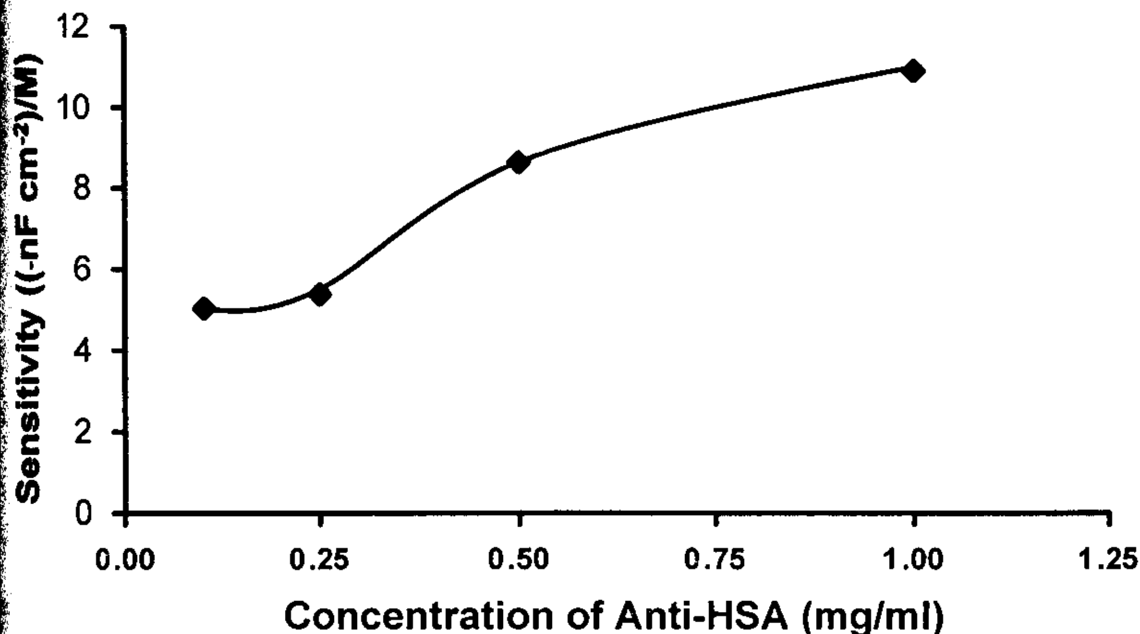


Figure 5.9 Sensitivity of the flow injection capacitive biosensor system for HSA using different concentration of anti-HSA in the immobilization.

5.4.1.3 Linear dynamic range and detection limit

When HSA was injected into the flow cell, it bound to the immobilized anti-HSA on the electrode causing the capacitance to decrease. Discrete pulse injections of HSA standard ranging from 10^{-15} to 10^{-5} M with intermediate regeneration steps using 100 mM glycine-HCl buffer solution, pH 2.20 were performed. Figure 5.10 shows the calibration curves for 250 µl injection into 10 mM borate buffer solution, pH 8.65 at a flow rate of 250 µl min^{-1}. The plot between capacitance change and logarithm of HSA concentration showed two linear ranges with different sensitivities, *i.e.*, from 1×10^{-15} to 1×10^{-9} M and 1×10^{-7} to 1×10^{-5} M. The detection limit was 1 fM based on IUPAC Recommendation 1994 (see section 4.2) (Buck and Lindner, 1994).

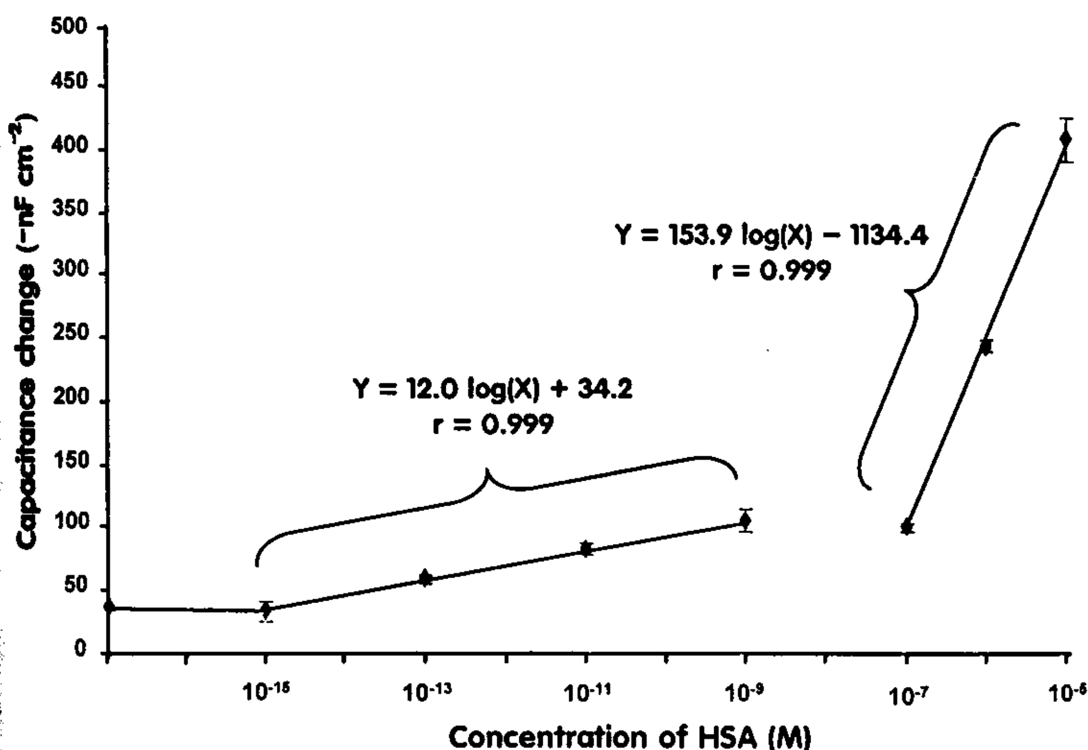


Figure 5.10 Capacitance change *vs.* the logarithm of HSA concentration for a transducer surface with immobilized anti-HSA under optimized conditions (250 μl min^{-1} flow rate, 250 μl sample volume, 10 mM borate buffer, pH 8.65).

5.4.1.4 Selectivity

To test the selectivity, 1×10^{-5} M bovine serum albumin (BSA) was injected into the flow injection capacitive biosensor system for HSA. The capacitance change of BSA (1×10^{-5} M) on the anti-HSA immobilized on electrode was lower than the detection limit of HSA (Figure 5.11). This suggested that the observed capacitance change was selective to HSA and was not caused by an unspecific adsorption of protein to the sensor surface. After nineteen regenerating experiments, the capacitance change, when injected with 1×10^{-5} M of HSA, was approximately the same as the first measurement.

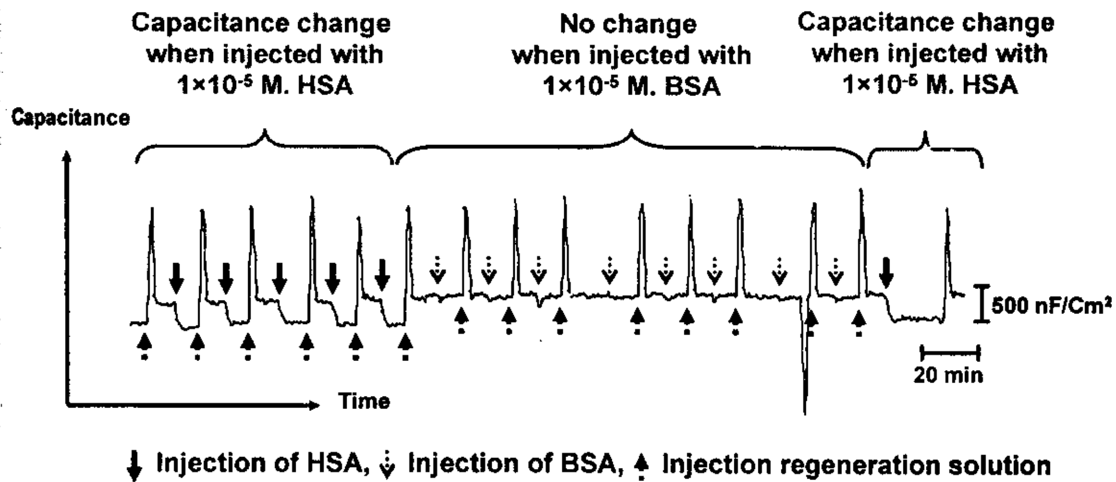


Figure 5.11 Capacitance measurement showing the specificity of the HSA-anti-HSA affinity binding system.

5.4.2 Protein A

Protein A is a cell wall component produced by several strains of *Staphylococcus aureus*. It consists of a single polypeptide chain of molecular mass 42,000 daltons and contains little or no carbohydrate. Protein A is able to bind specifically to the Fc region of immunoglobulin molecules, especially to those of the IgG class (Ey *et al.*, 1978; Hermanson *et al.*, 1992; Lindmark *et al.*, 1983). Thus, affinity chromatography on protein A column is widely used for the purification of monoclonal or polyclonal antibodies (Hahn *et al.*, 2005; Hahn *et al.*, 2006). Protein A may sometime leak from the column and contaminate in the final product (Steindl *et al.*, 2000). Normally, the production of pure monoclonal or polyclonal antibodies will be used for immunological assays. If protein A is a contaminant in the product it will bind to the Fc region of the antibody. In some immunological assays, Fc region is used, if it is already bound to protein A this may cause false results. So, it is important to determine the trace amount of protein A in final product. The conventional method for estimating protein A is "protein A ELISA for sample containing IgG". But this method has disadvantages, such as, expensive, require skill, complexity, need time to get analytical results, and can not analyte at very low concentration (George *et al.*,

1997). The work presents in this part describes the development of label-free capacitive biosensor for protein A at very low concentration.

5.4.2.1 Effect of regeneration solution

If the binding interaction of affinity pair is non-covalent bound, it can be separated by using the suitable regeneration solution. Since the interaction between protein A and the immobilized Fc-fragments is via hydrophobic interaction together with some hydrogen bonds and two salt bridges (Hahn *et al.*, 2003; Li *et al.*, 1998), therefore, protein A can be removed from Fc-fragments by using regeneration solution. Ideally, regeneration of the working electrode should remove any non-covalently bound protein A (analyte) without disrupting the activity of the Fc-fragments 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 Fc-fragments electrode was calculated from the capacitance change (ΔC) resulting from the binding between protein A (*i.e.* 0.1 ng ml^{-1} of protein A standard) and Fc-fragments before (ΔC_1) and after regeneration (ΔC_2) (Figure 5.7) as follows;

$$\text{\% Residual activity} = \frac{\Delta C_2 \times 100}{\Delta C_1} \qquad (2)$$

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). The parameters of the regeneration solution tested were type, pH, and concentration of regeneration solution.

First, three types of regeneration solution, *i.e.*, glycine buffer (Hahn *et al.*, 2003), citrate buffer (Amercham biosciences, 2002), and urea solution (Sigma®, 2003) were tested. Results in Table 5.1 show that for the type of regeneration agent, 25 mM citrate buffer, pH 3.00 and 25 mM urea were ineffective with low percent residual activity values (47 ± 4 and 55 ± 11 %), indicating that some protein A

molecules were still bound to Fc-fragments after the regeneration step. However, for 25 mM glycine buffer, pH 3.00 it was shown to be effective with a percent residual activity of 68 ± 2 %. So, glycine buffer was chosen for further testing.

The pH and concentration of glycine buffer solution were then investigated. Influence of pH of glycine buffer solution, ranging from 4.00 down to 2.00 was first studied (Table 5.1). Generally, the percent residual activity of the Fc-fragments electrode increased as the pH decreased. Good residual activity values were obtained (89 ± 1 to 87± 17 %) at pH 2.50 and 2.00. Since low pH may lead to the production of pinholes on the electrode surface due to the removal of SAM (Jiang *et al.*, 2003), therefore, pH 2.50 was chosen to avoid deterioration of the insulting SAM layer.

To further optimize the dissociation of the protein A-Fc-fragments complex, the influence of concentration of glycine from 10 to 200 mM was studied (Table 5.1). The values of percent residual activity increased form 71 ± 3 to 91 ± 10 when concentration of glycine increased form 10 to 100 mM. At 200 mM the percent residual activity was lower than at 100 mM. This may be because at 200 mM the SAM layer on the electrode can also be destroyed by a high ion strength solution (Jiang *et al.*, 2003). Since at 50 mM residual activity above 90 % was achieved, therefore, 50 mM glycine buffer, pH 2.50 was used as the regeneration solution in the continued experiments.

Table 5.1 Assayed values of the type, pH and concentration of regeneration solution. The efficiency of protein A removal from the Fc-fragments immobilized on the electrode was studied by injecting 10^{-12} M of protein A standard solution.

Regeneration solution	Percentage of average residual activity
Type	
25 mM Glycine buffer pH 3.00	68 ± 2
25 mM Citrate buffer pH 3.00	55 ± 11
25 mM Urea	47 ± 4
pH	
25 mM Glycine buffer pH 2.00	87 ± 17
25 mM Glycine buffer pH 2.50	89 ± 1
25 mM Glycine buffer pH 3.00	74 ± 3
25 mM Glycine buffer pH 3.50	65 ± 9
25 mM Glycine buffer pH 4.00	48 ± 3
Concentration	
10 mM Glycine buffer pH 2.50	71 ± 3
25 mM Glycine buffer pH 2.50	89 ± 1
50 mM Glycine buffer pH 2.50	90 ± 4
100 mM Glycine buffer pH 2.50	91 ± 10
200 mM Glycine buffer pH 2.50	77 ± 5

5.4.2.2 Flow rate

In a flow injection capacitive biosensor system, the flow rate of the buffer passing through the capacitive flow cell is the main factor affecting the yield of interaction between protein A and immobilized Fc-fragments on the electrode surface. So optimization of flow rate is necessary. The changes in capacitance registered when varying the flow rates in the range 50 - 500 µl min^{-1} increased when the flow rate decreased from 500 to 100 µl min^{-1} (Figure 5.12). However, for 50 and 100 µl min^{-1} the capacitance changes did not differ significantly ($P < 0.05$). So, 100 µl min^{-1} was chosen.

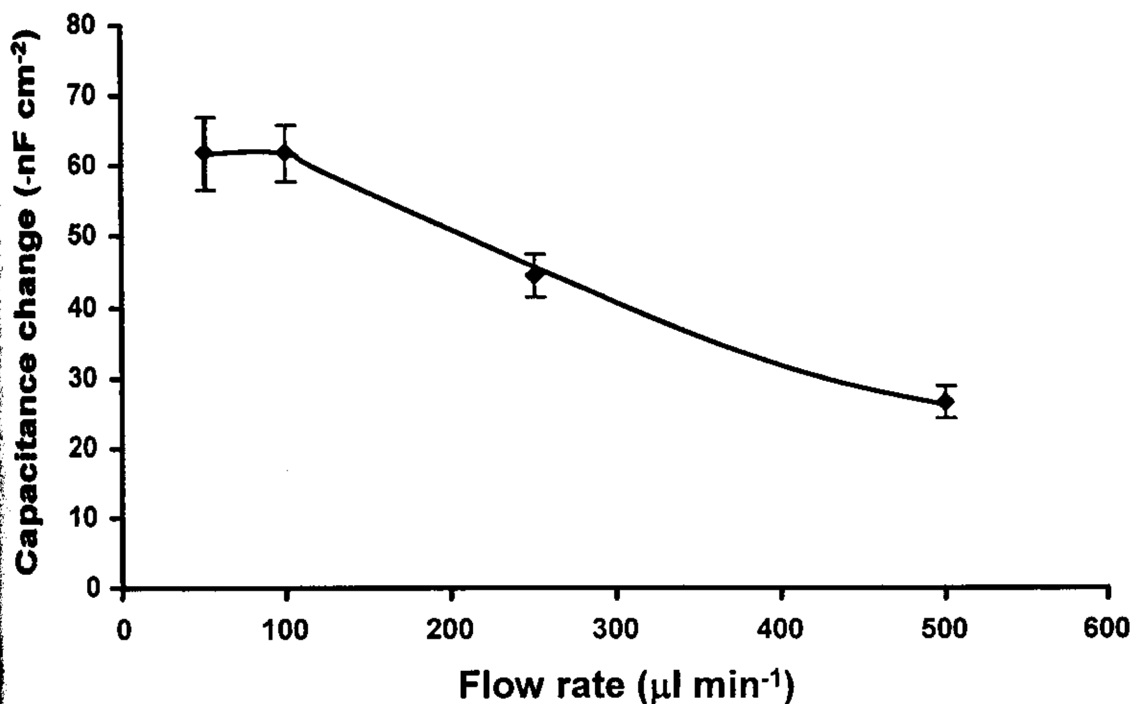
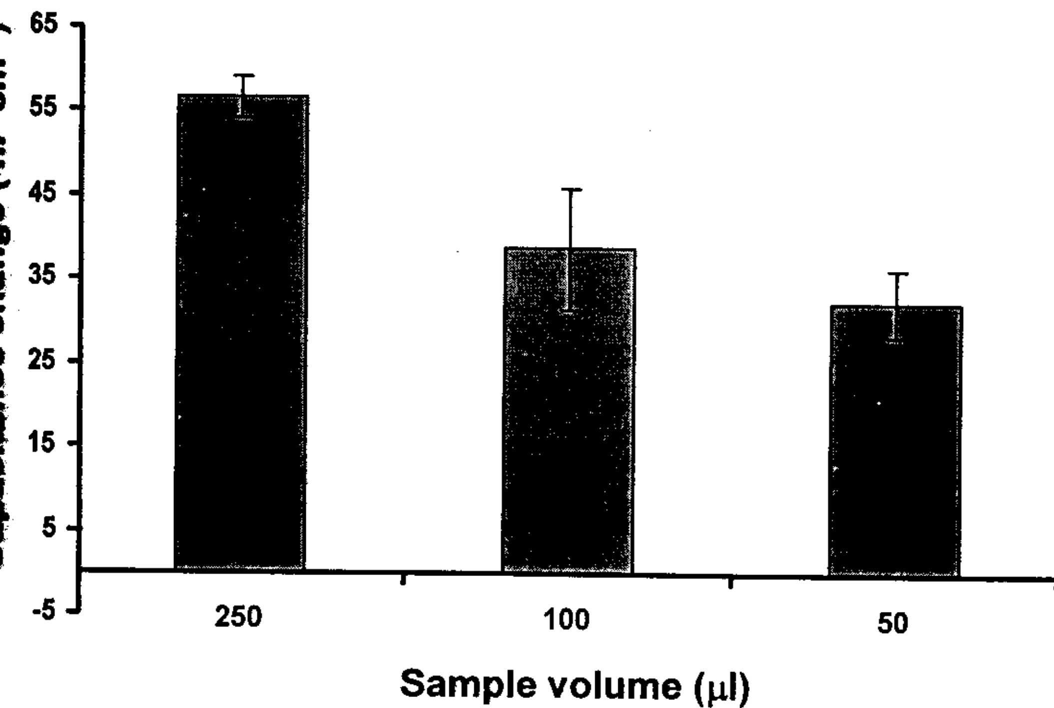


Figure 5.12 Responses of the flow injection capacitive biosensor system for protein A at different flow rates.

.4.2.3 Sample volume

Figure 5.13 shows the responses of the capacitive biosensor system at ree different sample volumes. The change in capacitance signal (-nF cm^{-2}) increased the sample volume increased from 50 to 250 μl. Although the capacitance change creases with the sample volume the analytical time also increase. Since at a sample lume of 250 μl the response was high and the analytical time was already 12-17 in, larger volume was not tested and 250 μl was used for further experiments.



gure 5.13 Responses of the flow injection capacitive biosensor system for protein A at different sample volume.

5.4.2.4 Linear dynamic range and detection limit

Discrete pulse injections of protein A standard ranging from 10^{-15} to 10^{-9} M with intermediate regeneration steps using 50 mM glycine buffer solution, pH 2.50 were tested. Figure 5.14 shows the calibration curves for 250 μl injection into 10 mM borate buffer solution, pH 8.50 at a flow rate of 100 μl min^{-1}. The plot between capacitance change and logarithm of protein A concentration showed a linear range from 1.0×10^{-14} to 1.0×10^{-10} M. The linear regression equation was ΔC (-nF cm^{-2}) = $10 \cdot \log$ protein A (M) + 177, with a correlation coefficient of 0.992. At 10^{-9} M the capacitance change is lower than at the 10^{-10} M, this may due to the life-time of the electrode. At this concentration, the electrode has already been used for more than 36 times. The detection limit was 10 fM based on IUPAC Recommendation 1994 (see section 4.2) (Buck and Lindner, 1994).

5.5 Conclusions

In this study it has been shown that protein affinity reactions can be directly detected using capacitive detection principle. The capacitive affinity biosensor system is clearly suitable for direct assay. It had a very low detection limit of 1 fM and 10 fM for HSA and protein A, respectively. It requires relatively short analysis time (12-17 min), is selective and sensitive to target analyte, and can be developed for other affinity binding pairs. Furthermore, the regeneration of the immunologically active surface on the electrode is possible. Therefore, several assays can be done using the same electrode.

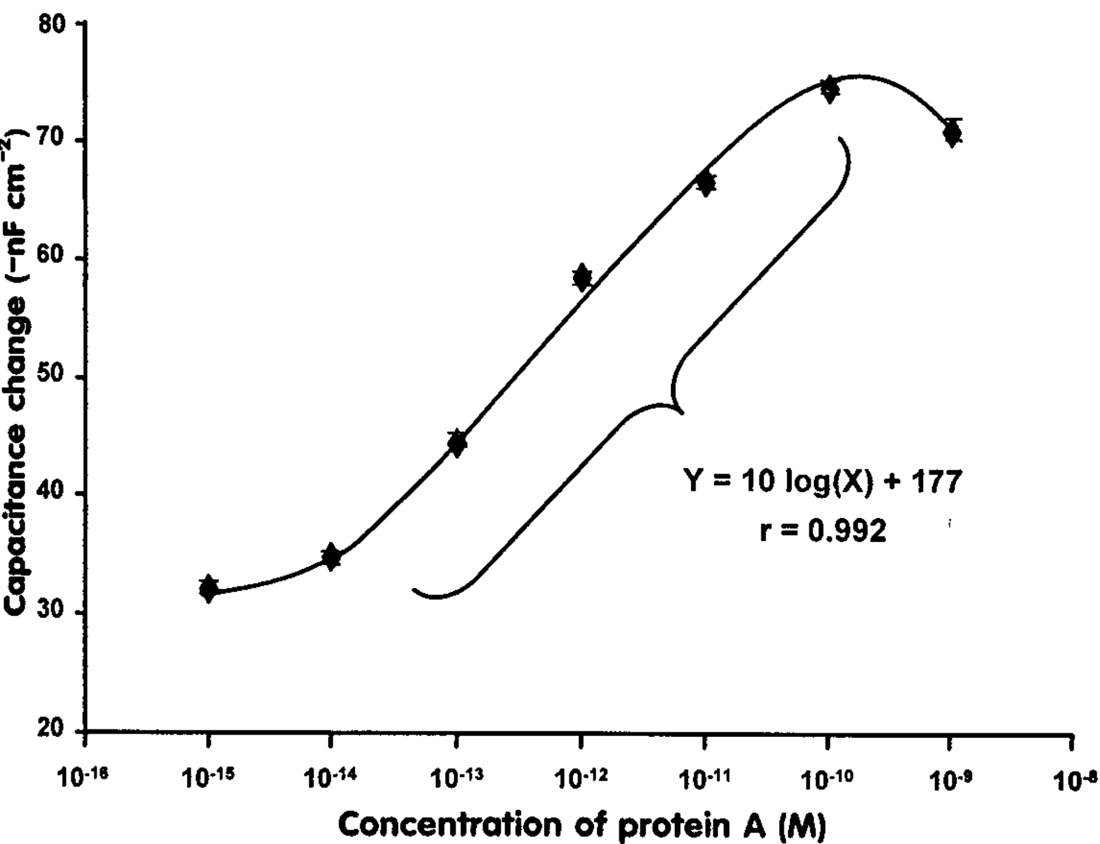


Figure 5.14 Capacitance change *vs.* the logarithm of protein A concentration for a transducer surface with immobilized Fc-fragments under optimized conditions (100 μl min^{-1} flow rate, 250 μl sample volume, 10 mM borate buffer, pH 8.50).