

# A comparative study of capacitive immunosensors based on self-assembled monolayers formed from thiourea, thioctic acid, and 3-mercaptopropionic acid

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## Abstract

A procedure was developed for the covalent coupling of anti-alpha-fetoprotein antibody (anti-AFP) to a gold surface modified with a self-assembled monolayer (SAM) of thiourea (TU). The performance of the SAM-antibody layer was compared to those of similar layers based on thioctic acid (TA) and 3-mercaptopropionic acid (MPA) by using flow injection capacitive immunosensor system. Covalent coupling of anti-AFP on self-assembled thiourea monolayer (SATUM) modified gold electrode can be used to detect alpha-fetoprotein with high efficiency, similar sensitivity, the same linear range ( $0.01\text{--}10\ \mu\text{g l}^{-1}$ ) and detection limit ( $10\ \text{ng l}^{-1}$ ) as those obtained from sensors based on self-assembled thioctic acid monolayer (SATAM) and self-assembled 3-mercaptopropionic acid monolayer (SAMPAM). The system is specific for alpha-fetoprotein and can be regenerated and reused up to 48 times. Therefore, self-assembled monolayer using thiourea which is cheaper than thioctic acid and 3-mercaptopropionic acid is a good alternative for biosensor applications when SAMs are used.

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**Keywords:** Thiourea; Capacitive immunosensor; Thioctic acid; 3-Mercaptopropionic acid; Self-assembled monolayer; Alpha-fetoprotein

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## 1. Introduction

Immunosensors are based on binding interactions between immobilized biomolecules and the analyte of interest and their subsequent detection by appropriate detector (Mattiasson, 1984; Taylor, 1991). Several electrochemical detection principles have been used, such as potentiometric (Tang et al., 2004a; Taylor et al., 1991), amperometric (Ramanaviciene and Ramanavocous, 2004), conductimetric (Yagiuda et al., 1996), and impedimetric (Tang et al., 2004b). Capacitive measurement has also been investigated as a highly sensitive approach (Berggren et al., 1998, 2001; Berggren and Johansson, 1997; Bontidean et al., 1998; Hedström et al., 2005; Hu et al., 2002, 2005).

Capacitive immunosensor is based on the principle that for an electrolytic capacitor the capacitance depends on the thickness and dielectric behavior of a dielectric layer on the surface of a metal (Gebbert et al., 1992). It can be constructed by immobilizing biorecognition elements in a thin layer on an electrode and measuring changes in the dielectric properties when an analyte binds to the biorecognition elements on the electrode, causing capacitance to decrease.

Immobilization is an important part in capacitive immunosensor since the electrode surface has to be electrically insulated. Different immobilization techniques have been developed and biorecognition elements can be immobilized on capacitive sensors via modified semiconductor surfaces (Barraud et al., 1993; Bataillard et al., 1988), metal oxides surfaces (Gebbert et al., 1992, 1994), and self-assembled monolayers (SAMs) of sulfur compounds on gold (Berggren et al., 1998; Berggren and Johansson, 1997; Hedström et al., 2005).

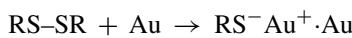
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SAMs is a particularly suitable immobilization technique for capacitive biosensor (Riepl et al., 1999), since it allows electrochemical insulation of the surface of a working gold electrode and it is an excellent immobilization technique for protein, it shields proteins from direct contact with solid surface, thus, reduces the risk of the sensing element denaturation (Wadu-Mesthrige et al., 2000). Furthermore, the proteins, use as the sensing element, are immobilized through covalent binding and they can be exposed to a high or low pH, often uses in regeneration, leading to a reusable system (Frey and Corn, 1996). SAMs can be formed at room temperature by spontaneous adsorption of alkanethiol on gold surfaces (Nuzzo and Allara, 1983; Porter et al., 1987) by the reaction of sulfide



or disulfide



The affinity between sulfur and gold atoms is extremely high, resulting in the formation of SAMs that are highly stable in air, water, and organic solvents at room temperature (Bain et al., 1989; Chaki and Vijayamohanan, 2002). They are also stable for a wide range of potential, from  $-400$  to  $+1400$  mV versus standard calomel electrode in diluted sulphuric acid solution, which is especially significant for electrochemical sensing (Finklea et al., 1987).

Capacitive biosensors have often been based on SAMs of thioctic acid (TA;  $\text{S}_2\text{C}_7\text{H}_{13}\text{-CO}_2\text{H}$ ) (Berggren et al., 1998; Berggren and Johansson, 1997; Disley et al., 1998; Hedström et al., 2005; Liu et al., 1999) and 3-mercaptopropionic acid (MPA;  $\text{HSC}_2\text{H}_4\text{CO}_2\text{H}$ ) (Disley et al., 1998; Sawaguchi et al., 2001; Vaughan et al., 1999). The carboxylic groups of the SAMs were activated with 1-ethyl-3-(3-diamino)propyl-carbodiimide (EDC) (Akram et al., 2004; Berggren et al., 1998; Berggren and Johansson, 1997; Hedström et al., 2005), and sometimes together with a succinimide, i.e. *N*-hydroxysulfosuccinimide (NHS) (Gooding and Hibbert, 1999; Staros et al., 1986; Vaughan et al., 1999). Then the activated groups were exposed to the protein solution where the activated electrophilic group attached to primary amino group of amino acid residues, forming a new peptide bond between SAM and protein. SAM containing amine-modified entities, such as 2-mercaptopethylamine (MEA;  $\text{HSC}_2\text{H}_4\text{-NH}_2$ ), was also an effective surface to which protein could be immobilized (Jiang et al., 2003). Glutaraldehyde can also be used as it was introduced to react with the self-assembled MEA monolayer on the gold electrode to covalently immobilize the protein. However, only a few studies on capacitive immunosensors were reported based on the amine-modified SAM (Mirsky et al., 1997).

Since TA, MPA, and MEA are rather expensive, an alternative cheaper thiol reagent, thiourea was investigated. Thiourea (TU;  $\text{NH}_2\text{CSNH}_2$ ) was chosen because of its low environmental impact, easier handling of reagent and the fact that it is strongly adsorbed on gold (Holze and Schomaker, 1990; Ubaldini et al., 1998). It has amino groups ( $\text{R-NH}_2$ ) that can be modified to covalently couple to the antibody. To our knowledge no one has applied it to immunosensors.

This paper reports the development of a procedure for the immobilization of antibody to a gold surfaces modified with a SAM of thiourea. The performance was compared with that of the commonly used thioctic acid and 3-mercaptopropionic acid. Alpha-fetoprotein (AFP) and anti-alpha-fetoprotein antibody (anti-AFP) were used as a model system. The evaluation of each method for immobilization was done using a flow injection capacitive immunosensor system. The comparison was done by observing several analytical parameters, such as sensitivity, linear range, limit of detection, specificity, and reproducibility.

## 2. Materials and methods

### 2.1. Materials

Anti-AFP and AFP from human fluids were obtained from Dako (Denmark). 3-Mercaptopropionic acid, *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC), and *N*-hydroxysuccinimide (*N*-hydroxy-2,5-pyrrolidinedione, NHS) were obtained from Sigma-Aldrich (Steinheim, Germany), thioctic acid 98% and 1-dodecanethiol were obtained from Aldrich (Milwaukee, USA), thiourea was obtained from BDH laboratory reagents (Poole, England). All other chemicals used were of analytical grade. All buffers were prepared with distilled water treated with a reverse osmosis-deionized system. Before use, the buffers were filtered through an Albet® nylon membrane filter (Albet, Spain), pore size 0.20  $\mu\text{m}$ , with subsequent degassing.

### 2.2. Methods

#### 2.2.1. Preparation of the gold surface

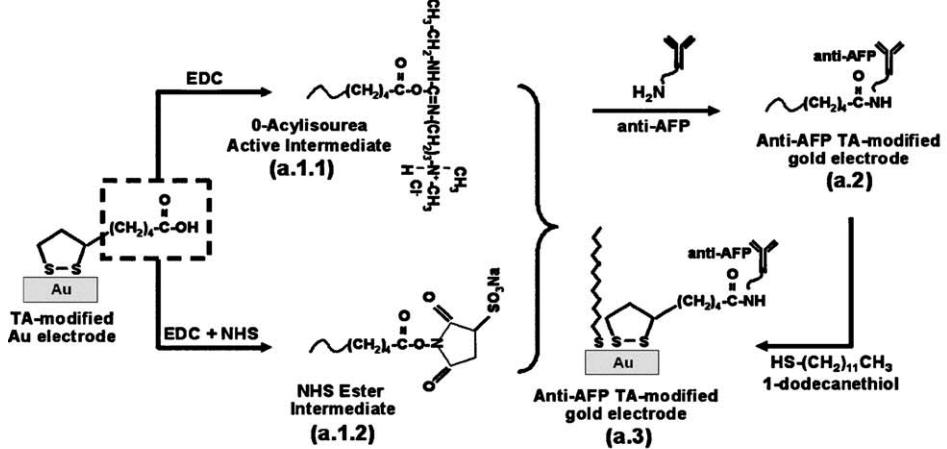
Gold electrodes ( $\varnothing$  3 mm, 99.99% purity) were polished (Gripol® 2V polishing machine, Metkon Instruments Ltd., Turkey) with alumina slurries (particle diameters 5, 1, and 0.30  $\mu\text{m}$ ) and then cleaned through sonication subsequently, 15 min each, in distilled water and absolute ethanol to remove any physisorbed multilayer (Yang et al., 1995). They were then washed in distilled water and dried with pure nitrogen gas. Each electrode was pretreated by electrochemical etching in 0.5 M  $\text{H}_2\text{SO}_4$  solution by cycling potential from 0 to  $+1500$  mV versus Ag/AgCl reference electrode with a scan rate of 0.1 V  $\text{s}^{-1}$  for 25 scans. Finally they were dried with pure nitrogen gas.

#### 2.2.2. Modification of SAMs formation

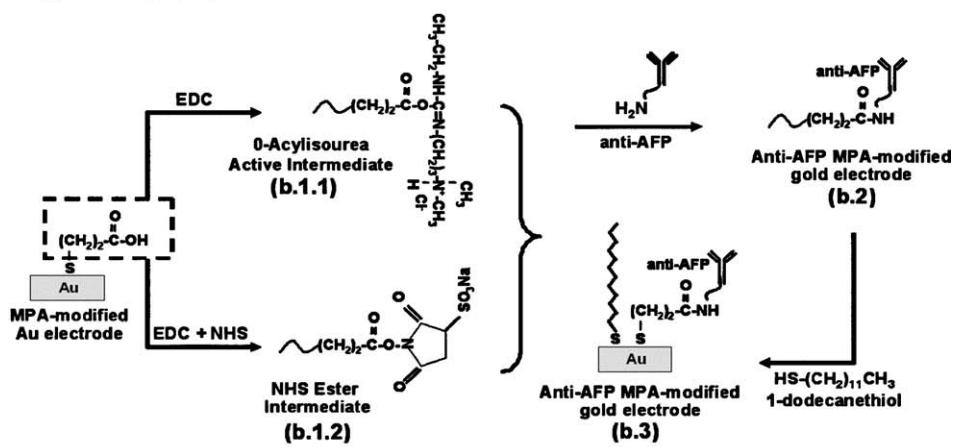
A cleaned gold electrode was immediately immersed in a thiol solution (thioctic acid, 3-mercaptopropionic acid, or thiourea) at room temperature for a period of time (see later) before being thoroughly rinsed with distilled water and dried with pure nitrogen gas. In this step self-assembled thioctic acid monolayer (SATAM), self-assembled 3-mercaptopropionic acid monolayer (SAMPAM), or self-assembled thiourea monolayer (SATUM) was formed on the gold surface.

A good formation of SAMs on gold surface depends on both the time (Dubois and Nuzzo, 1992; Kim et al., 1993; Wink et al., 1997) and concentration of thiol solutions (Kim et al., 1993; Liu et al., 1999; Wink et al., 1997). The effects of these factors were

(a) Thioctic acid, TA



(b) 3-Mercaptopropionic acid, MPA



(c) Thiourea, TU

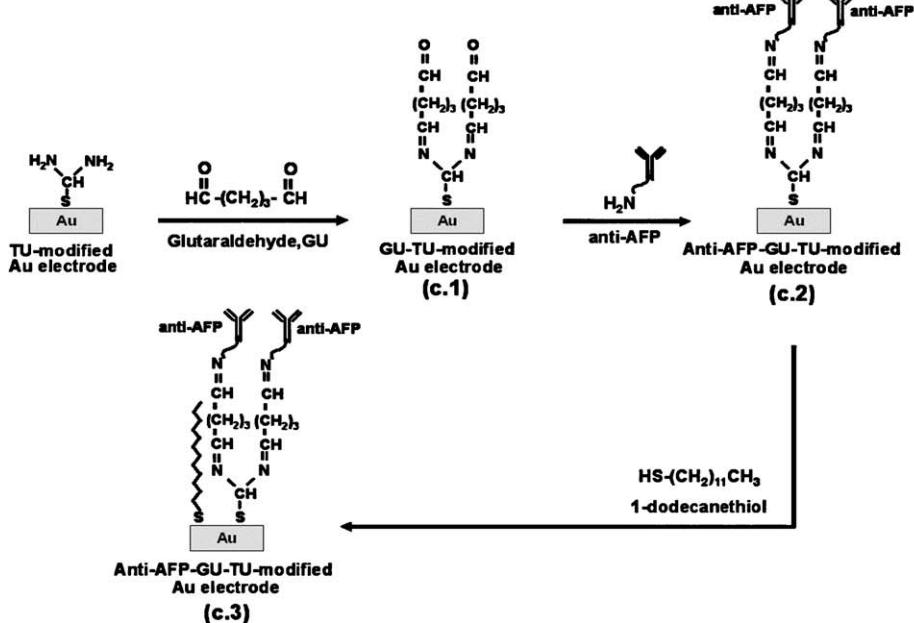


Fig. 1. Reaction mechanism for the anti-AFP immobilized on a self-assemble thiolate monolayer, (a) thioctic acid (TA), (b) 3-mercaptopropionic acid (MPA), and (c) thiourea (TU).

investigated. The optimization of the immersion time was studied by immersing cleaned gold electrodes in 100 mM of thiol reagents for 0, 0.25, 0.50, 1, 3, 6, 12, 24, 36, and 48 h. The concentrations of thiol solutions were then optimized by immersing cleaned gold electrodes in thioctic acid and 3-mercaptopropionic acid for 12 h (optimum time) and in thiourea solution for 24 h (optimum time) at concentrations of thiol solutions, 0, 10, 25, 50, 100, 250, and 500 mM.

#### 2.2.3. Immobilization of anti-*AFP*

Anti-*AFP*, used as the sensing element, was immobilized on SATAM, SAMPAM, and SATUM through covalent binding. For SATAM and SAMPAM, their carboxylic groups were activated for an amine reaction by using 0.05 M of EDC in phosphate buffer (pH 5.00) (Fig. 1(a.1.1 and b.1.1)) or 0.05 M of EDC with 0.03 M of NHS in phosphate buffer (pH 5.00) (Fig. 1(a.1.2 and b.1.2)) for 5 h (Johnsson et al., 1991; Staros et al., 1986) and then rinsed with 10 mM sodium phosphate buffer, pH 7.00, and dried. Then 20  $\mu$ l of 0.5 mg ml<sup>-1</sup> of anti-*AFP* was placed on the electrode and reaction took place overnight at 4 °C (Fig. 1(a.2 and b.2)). Finally, the electrode was reacted in a 10 mM 1-dodecanethiol ethanolic solution for 20 min (Fig. 1(a.3 and b.3)) to block the bare spots on the electrode surface.

In the case of SATUM, glutaraldehyde was introduced to react with the SATUM on the gold surface. The amine groups will be modified and free aldehyde groups will be exposed to which protein can couple. The time and amount of glutaraldehyde that were suitable for the reaction were optimized. The optimum conditions were then used to treat the surface of gold electrode (Fig. 1(c.1)), before being thoroughly rinsed with sodium phosphate buffer, pH 7.00, and dried. Then 20  $\mu$ l of 0.5 mg ml<sup>-1</sup> anti-*AFP* was placed on the electrode and reaction took place overnight at 4 °C (Fig. 1(c.2)). The electrode was then immersed in 0.1 M ethanolamine pH 8.00 for 30 min, this

step was to occupy all the aldehyde groups which did not couple to the anti-*CEA*. Finally, the electrode was reacted in a 10 mM 1-dodecanethiol ethanolic solution for 20 min (Fig. 1(c.3)) to block the bare spots on the electrode surface.

#### 2.3. Capacitance measurement

Fig. 2 shows the basic experimental set-up of the flow injection capacitive immunosensor system. Three electrodes were placed in the immunosensor flow cell (10  $\mu$ l) and connected to the potentiostat (ML 160, AD Instruments, Australia). The working electrode was the modified gold electrode. A stainless steel tube (i.d. 0.4 mm, o.d. 1.1 mm, length 25 mm) was used as the auxiliary electrode and outlet. A laboratory built Ag/AgCl reference electrode was placed opposite to the working electrode.

Continuously during the binding event between *AFP* and anti-*AFP*, potential pulses, 50 mV, are applied to the gold electrode yielding current response signals, which can be described by Eq. (1).

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

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

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

Then,  $C_{\text{total}}$  and  $R_s$  were obtained from the slope and intercept of the linear least-square fitting of  $\ln i(t)$  versus  $t$ . The measurement

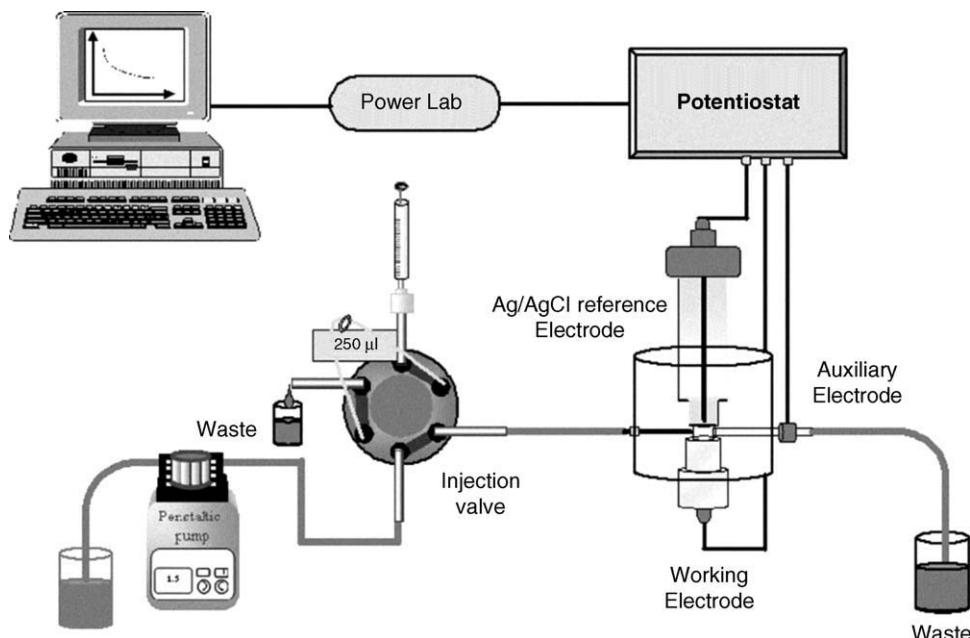


Fig. 2. Schematic diagram showing the flow injection capacitive immunosensor system.

of  $C_{\text{total}}$  was done every minute and the results were later plotted as a function of time. When the solution containing AFP was injected into the flow cell, AFP bound to the immobilized anti-AFP on the electrode causing the capacitance to decrease until it reached a stable value. The change in capacitance due to the binding was obtained by subtracting  $C_{\text{total}}$  after the binding from the  $C_{\text{total}}$  before the binding. The surface of the electrode was then regenerated with 10 mM glycine-HCl, pH 2.80 (Maupas et al., 1997) to remove AFP from anti-AFP immobilized electrode.

### 3. Results and discussion

#### 3.1. Immersion times

The effect of immersion times of thiol solutions for the formation of SAMs on gold electrode surfaces was tested by using cyclic voltammetry technique in 0.1  $\text{H}_2\text{SO}_4$  at scan rate 100  $\text{mV s}^{-1}$ . The efficiency of the formation of thiol SAMs on electrode surfaces can be described in term of surface coverage which can be estimated by comparing the area of the reduction peak of electrodorption of oxygen atom on the modified and bare gold electrode (Sabatani et al., 1987). The percent surface coverage was calculated by using Eq. (3).

$$\text{surface coverage (\%)} = \frac{(Q_{\text{MGE}} - Q_{\text{BGE}}) \times 100}{Q_{\text{BGE}}} \quad (3)$$

where  $Q_{\text{MGE}}$  is the amount of electric charge exchanged during the electrodorption of oxygen ( $\text{C cm}^{-2}$ ) of the modified gold electrode and  $Q_{\text{BGE}}$  is the amount of electric charge exchanged during the electrodorption of oxygen ( $\text{C cm}^{-2}$ ) of the bare gold electrode. In cases of TA and MPA, the percent surface coverage was found to increase with immersion up to 12 h (Fig. 3(a)).

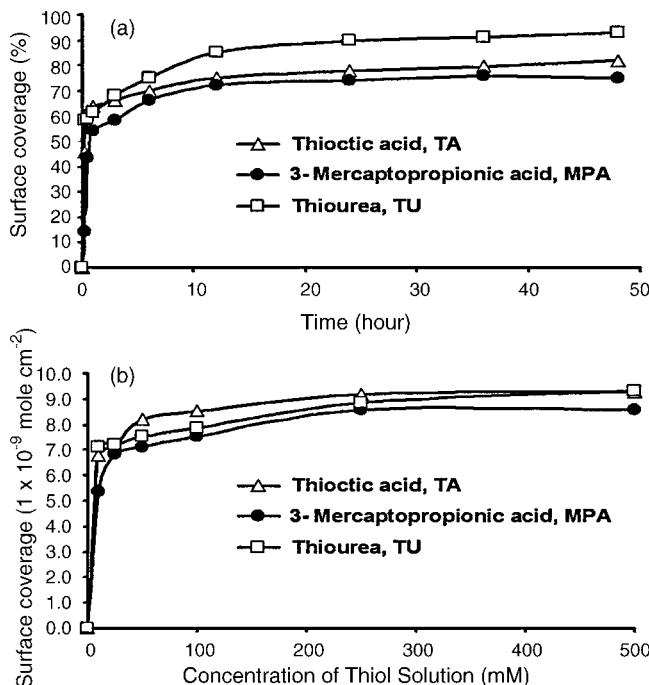


Fig. 3. The effect of (a) immersion times and (b) concentration of the thiol solutions for the formation of SAMs on gold electrode surface.

For longer immersion times, the percent coverage did not differ significantly ( $P < 0.05$ ). For the TU modified gold electrode, the insignificant difference of percent surface coverage was obtained after 24 h. Therefore, 12 h is the optimum immersion time for TA and MPA while 24 h is used for TU.

#### 3.2. Concentration of thiol solutions

The effect of concentrations of thiol solutions for the formation of SAMs on gold electrode surfaces were tested by considering the reductive desorption of these monolayers in thiol solutions via reaction (4).



At negative potentials the bond of thiol groups to gold can be reduced with consequent thiol desorption (Weisshaar et al., 1992). Thiol solutions (TA, MPA, and TU) were characterized making use of this property. Cyclic voltammograms of reduction peak of thiolate monolayer in 0.5 M KOH, indicating the desorption of thiol bound to the gold electrode surface (Weisshaar et al., 1993). The charge under desorption peak can be used to estimate the surface concentrations of the thiol solutions in monolayer as follows:

$$\Gamma = \frac{Q}{nFA} \quad (5)$$

where  $\Gamma$  is the surface coverage ( $\text{mol cm}^{-2}$ ),  $Q$  the total charge (C),  $n$  the number of electron transferred,  $F$  the Faraday's constant ( $96,485.4 \text{ C mol}^{-1}$ ), and  $A$  is the electrode surface area ( $\text{cm}^2$ ). Fig. 3(b) shows the effect of the concentration of thiol solutions for the formation of SAMs. The increase in surface coverage ( $\text{mol cm}^{-2}$ ) started to level off close to 250 mM of all thiol solutions. Therefore, 250 mM of TA, MPA, and TU were chosen for the modification of the surface of the gold electrode. At this concentration, the surface coverage for monolayers of TA, MPA, and TU were  $9.2 \times 10^{-9}$ ,  $8.6 \times 10^{-9}$ , and  $8.9 \times 10^{-9} \text{ mol cm}^{-2}$ , respectively.

#### 3.3. Immobilization of anti-AFP

##### 3.3.1. SATAM and SAMPAM

The immobilization of anti-AFP on SATAM, and SAMPAM using covalent method were done using EDC, and EDC/NHS solution for intermediate (Johnsson et al., 1991; Staros et al., 1986). The performances of these electrodes are shown in Table 1. The sensitivity of SATAM and SAMPAM activated with EDC/NHS were higher than SATAM and SAMPAM activated with EDC by about 1.5 times. This is correlated to the higher amount of the immobilized anti-AFP, which is shown as immobilization yield in Table 1, determined by detecting the amount of protein in the solution before and after immobilization by the silver binding method (Krystal, 1987).

##### 3.3.2. SATUM

The effect of glutaraldehyde was tested by determining the immobilization yield. The concentration was tested between

Table 1

Performances of anti-AFP covalently immobilized on self-assemble thioctic acid monolayer (SATAM), self-assemble 3-mercaptopropionic acid monolayer (SAMPAM), and self-assemble thiourea monolayer (SATUM)

Parameters	Performances		
	SATAM	SAMPAM	SATUM
Linear range ( $\mu\text{g l}^{-1}$ )			
Activated with EDC	0.01–10	0.01–10	NA
Activated with EDC/NHS	0.01–10	0.01–10	NA
Activated with glutaraldehyde	NA	NA	0.01–10
Limit of detection ( $\text{ng l}^{-1}$ )			
Activated with EDC	10	10	NA
Activated with EDC/NHS	10	10	NA
Activated with glutaraldehyde	NA	NA	10
Sensitivity ( $(\Delta C (-\text{nF cm}^{-2})/\log \text{AFP} (\mu\text{g l}^{-1}))$ )			
Activated with EDC	21.3	16.9	NA
Activated with EDC/NHS	32.2	24.5	NA
Activated with glutaraldehyde	NA	NA	28.7
Analysis time (min)			
Activated with EDC	13–15	13–15	NA
Activated with EDC/NHS	13–15	13–15	NA
Activated with glutaraldehyde	NA	NA	13–15
Immobilization yield (%) ( $n=3$ )			
Activated with EDC	$65.8 \pm 0.2$	$60.5 \pm 1.6$	NA
Activated with EDC/NHS	$88.6 \pm 0.8$	$88.3 \pm 0.6$	NA
Activated with glutaraldehyde	NA	NA	$88.5 \pm 0.8$
Percentage capacitance change decreasing rate within 48 times of regeneration (%/time)	–0.20	–0.19	–0.18

NA: not applicable.

0.05 and 10% (v/v). When the concentration of glutaraldehyde increased the immobilization yield also increased reaching a maximum at 5% (v/v) of glutaraldehyde and then leveled off. For the effect of incubation time (1–240 min), the highest immobilization yield is at 20 min. Therefore, 5% (v/v) glutaraldehyde and incubation time of 20 min were used for further experiments to activate the SATUM electrode in 10 mM sodium phosphate buffer pH 7.00 at room temperature. Anti-AFP was then immobilized on the activated electrode.

#### 3.4. Electrochemical performance of the process of anti-AFP immobilization

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.  $\text{K}_3[\text{Fe}(\text{CN})_6]$ ) in the electrolyte solution. Fig. 4 shows an example of these voltammograms for SATUM. At the cleaned gold surface the redox couple was oxidized and reduced according to curve a. When thiourea was self-assembled on the clean gold surface the redox peaks decreased (curve b). Then aldehyde group of glutaraldehyde was reacted with the amine and the anti-AFP was linked covalently on the electrode via reaction with the aldehyde group. The insulating property of the electrode surface was further increased (curves 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. Similar cyclic voltammograms for

SATAM and SAMPAM were also obtained indicating that all these modified surface were well insulated and were suitable for the capacitive measuring system.

#### 3.5. Linear range, detection limit, and selectivity

When AFP was injected into the flow cell, it bound to the immobilized anti-AFP on the electrode causing the capacitance

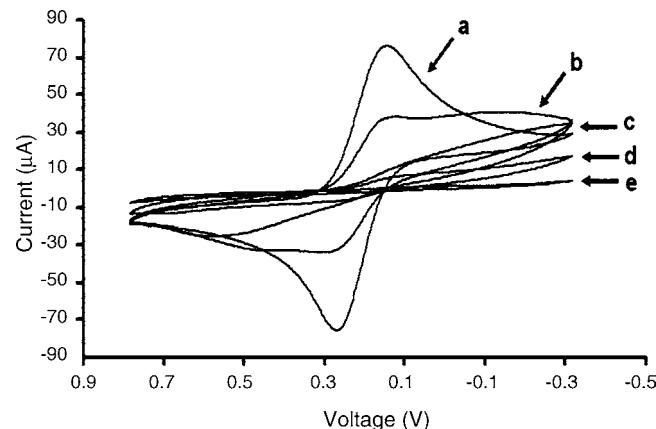


Fig. 4. Cyclic voltammograms of a gold electrode obtained in a 5 mM  $\text{K}_3[\text{Fe}(\text{CN})_6]$  containing 0.1 M KCl solution at scan rate of  $0.1 \text{ V s}^{-1}$ . All potentials are given vs. Ag/AgCl reference electrode. The voltage range was  $-0.3$  to  $0.8 \text{ V}$ . (a) Clean gold electrode, (b) self-assembled thiourea monolayer (SATUM) electrode, (c) glutaraldehyde-amine SATUM, (d) anti-AFP-glutaraldehyde-amine SATUM, and (e) as in (d) but after 1-dodecanethiol treatment.

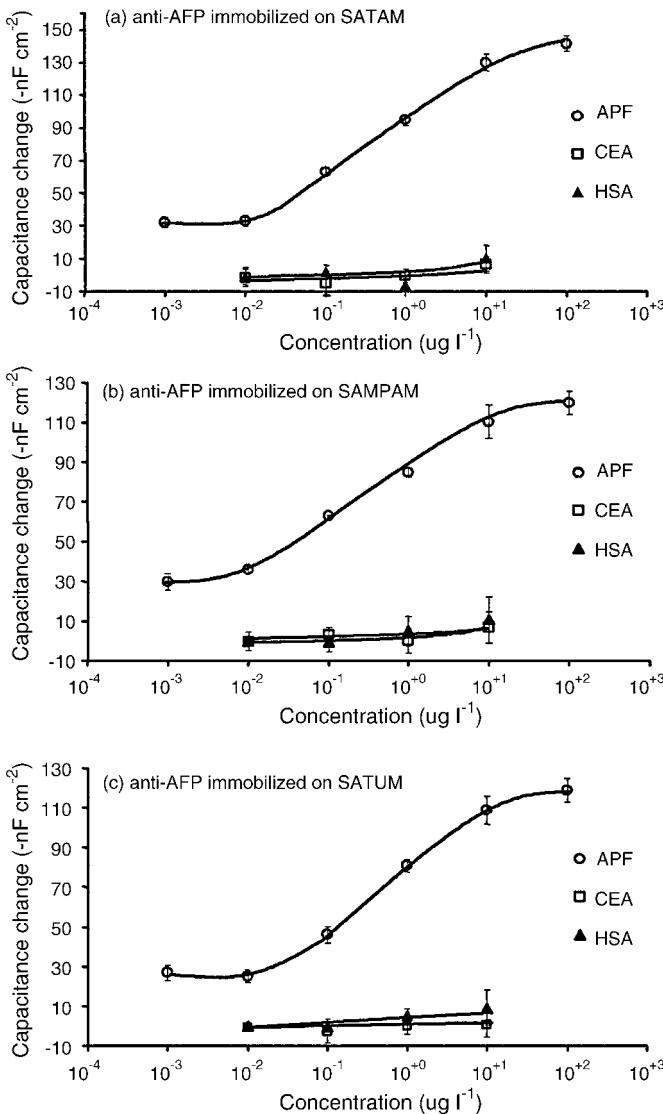


Fig. 5. Responses of the anti-AFP to alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), and human serum albumin (HSA) using (a) self-assembled thioctic acid monolayer (SATAM), (b) self-assembled 3-mercaptopropionic acid monolayer (SAMPAM), and (c) self-assembled thiourea monolayer (SATUM).

to decrease. Discrete pulse injections of AFP standard ranging from 0.001 to 100  $\mu\text{g l}^{-1}$  with intermediate regeneration steps using glycine-HCl buffer solution, pH 2.80, were performed. Fig. 5 shows the calibration curves for 250  $\mu\text{l}$  injection into 10 mM Tris-HCl buffer solution, pH 7.60, at a flow rate of 0.10  $\text{ml min}^{-1}$ . A linear relationship between the capacitance change and logarithm of AFP concentration of SATAM ( $r=0.999$ ), SAMPAM ( $r=0.999$ ), and SATUM ( $r=0.996$ ) were obtained between 0.01 and 10  $\mu\text{g l}^{-1}$ . The detection limits of SATAM, SAMPAM, and SATUM were 10  $\text{ng l}^{-1}$ , based on IUPAC Recommendation 1994 (Buck and Lindner, 1994).

The effect of substances that might interfere with the response of the AFP capacitive biosensor system was also studied. Human serum albumin (HSA) and carcinoembryonic antigen (CEA) were used to test the selectivity of the capacitive biosensor system for AFP, because the physical and chemical characteristics of albumin are similar to AFP (Bader et al., 2004) and CEA, like

AFP, is one type of the tumor markers. The capacitance changes of HSA and CEA at the concentration range 0.01–10  $\mu\text{g l}^{-1}$  on the anti-AFP immobilized on SATAM, SAMPAM, and SATUM electrodes were much lower than those from AFP (Fig. 5) and lower than the detection limit of AFP. A much higher concentration of HSA, 68  $\text{mg l}^{-1}$ , was also tested and the capacitance change was also much lower than the value at the detection limit for AFP. From these results, we suggested that these systems were specific to AFP.

### 3.6. Reproducibility

In this work, 10 mM glycine-HCl buffer solution, pH 2.80, was used as regeneration solution to break the affinity binding between AFP and anti-AFP. AFP was detected by the regenerated electrode repeatedly and reproducibility performance of anti-AFP-modified electrodes were evaluated intermittently over 6 days (12 times per day) by monitoring the change of capacitance signal at the same concentration of standard AFP (1  $\mu\text{g l}^{-1}$ ) in the flow injection capacitive biosensor system at a flow rate of 0.10  $\text{ml min}^{-1}$  Tris-HCl buffer solution, pH 7.60, and a sample volume of 250  $\mu\text{l}$ .

Percentage capacitance change decreasing rate after regeneration (%/time) of the three anti-AFP-modified electrodes are shown in Table 1. After 48 times of regeneration, the binding activity of anti-AFP immobilized on SATAM, SAMPAM, and SATUM retained more than 90% of the original capacitance change. That is, anti-AFP immobilization on SATAM, SAMPAM, and SATUM electrodes can be reused with good reproducibility up to about 48 times with the relative standard deviation (R.S.D.) of 4.2, 4.3, and 3.6%, respectively.

## 4. Conclusions

Covalent coupling of anti-AFP on self-assembled thiourea monolayer (SATUM) modified gold surface with glutaraldehyde had been proven to be a good and reliable immobilization technique for a flow injection capacitive immunosensor system. The modified electrode was sensitive and selective to the presence of AFP. The electrode can also be regenerated and reused. Comparing with the covalent coupling of anti-AFP on self-assembled thioctic acid monolayer (SATAM) and self-assembled 3-mercaptopropionic acid monolayer (SAMPAM) activated by EDC/NHS, the proposed SATUM can certainly match their performances. That is, all the systems gave the same linear range with nearly the same sensitivity and detection limits. Good stability was obtained for all the systems, i.e. they can be regenerated and reused up to 48 times with good reproducibility. Therefore, thiourea, which is cheaper than thioctic acid and 3-mercaptopropionic acid, is certainly a good alternative to be applied for the immobilization of antibodies on gold surfaces.

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