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Direct application strategy to immobilise a thioctic acid self-assembled monolayer on a gold electrode

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Abstract

Immobilisation of a self-assembled monolayer (SAM) onto an electrode surface is often achieved by immersing it in a solution for over 24 h. A biological or biologically derived recognition component can then be linked to the SAM in fabricating a biosensor. This time consuming immobilisation step can be a drawback in biosensor development, especially when repeated preparations of the biosensor are required. In this work, an alternative immobilisation strategy involving the direct application of a known quantity of the ethanolic solution of the alkanethiol, thioctic acid, on a gold electrode surface was studied. The solution was left to dry at room temperature for approximately 20 min. Comparable results including the relative percentage decrease in double layer capacitance, the surface coverage and the percentage of binding to the bacterial protein, Protein A, were obtained relative to those obtained with SAM formed by the immersion method. Shewhart's statistical analysis technique was applied to examine the stability in terms of the relative percentage decrease in double layer capacitance. In these tests, within 99.7% confidence control limits, only a 1% deterioration was observed over a 3-month period. Therefore, all these results have demonstrated that the direct application method yields a stable thioctic acid SAM on a gold electrode surface with characteristics similar to those obtained with an immersion method. However, formation of a SAM using direct application can be achieved within a significantly shorter period of time compared to immersion method.

Keywords: Thioctic acid SAM; Electrochemical biosensors; Immobilisation techniques

1. Introduction

There are continuing efforts to develop biosensors capable of providing a faster, simpler and more economical means of sample detection and analysis. Recently, biosensors have been developed for use under extreme environmental conditions [1]. Very often, a requirement in these developments is that a biological or biologically derived recognition component is immobilised on the surface of the biosensor for interaction with a targeted analyte to produce a measurable signal via a transducer. The immobilisation step can be achieved using a variety of substances including magnetic immunoparticles [2], lipid membranes [3–6], sol–gels [7–9], Nafion [10], conducting polymers [11–13] and self-assembled monolayers [14–17]. In particular, the simple procedure involved in immobilising self-assembled monolayers (SAMs) makes them an attractive strategy for achieving better control in the orientation and molecular organisation of biomolecules at interfaces. Alkanethiols and disulfides are among the molecules known to form a stable SAM on a gold surface owing to the strong S–Au covalent bond [18–23]. For example, exploitation of the SAMs of 3-mercaptopropionic acid has facilitated proteins and enzymes to communicate electrons directly with the electrode surface in the absence of mediators, while maintaining their physiological activities [24]. Similarly, a well-defined recognition layer of 3-mercaptopropionic acid SAM has enabled the fabrication of a spatially ordered bi-enzyme sensor resulting in an amplified response [25]. Further, SAMs of 12-mercaptododecanoic(8-biotinylamide-3,6-dioxaoctyl)amide and 11-mercaptoundecanol were easily crosslinked with the Fc portion of an antibody (via a biotin–streptavidin system) to optimally align the antibody on an immunosensor for attachment to its specific antigen [26]. A distinct advantage offered by the highly ordered SAM in such work is

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the uniform amount of antibody attaching to each of its terminals.

The adsorption kinetics of alkanethiols on a Au(1 1 1) surface are often described as a two-step process [27,28]. Initially, there is a fast growth of the film thickness to 80–90% of the final value, typically within a few minutes, followed by a slower process in which both the thickness and wettability approach an equilibrium value in approximately 10–20 h. Hence, in forming a SAM-coated surface, the biosensor is frequently incubated in a SAM solution of concentration 10–100 mM for an appreciably long period of time ($\geq$24 h) to achieve a high-quality SAM with as complete a surface coverage as possible [29–31]. However, in a study of the experimental parameters affecting the preparation of a SAM-coated electrochemical enzyme biosensor, Campuzano et al. [32] reported that these incubation times would vary depending on the SAMs used. In their work, a period of 15 h was required when a 40 mM mercaptopropionic acid solution was used to achieve a dense SAM. Conversely, a period of 3 h was found to be sufficient when a 40 mM solution of mercaptoundecanoic acid was employed. Nonetheless, such a time consuming step can often be a drawback in the fabrication of biosensors, especially when repeated preparations of a biosensor are needed. This has prompted us to investigate the effectiveness of a more efficiently prepared SAM, exemplified by the alkanethiol, thioctic acid (6,8-dithiooctanoic acid), in immobilising a protein on the surface of a gold electrode. In this respect, several experimental parameters including the immobilisation technique, the concentration of thioctic acid in an ethanolic solution, its exposure time to the gold surface, stability on the surface and the extent of active binding with proteins will be examined. In conjunction with such a study, we aim to provide a systematic evaluation of the experimental conditions used in coating a thioctic acid SAM on a gold electrode.

2. Experimental

2.1. Reagents

Thioctic acid, Protein A, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), and a bicinchoninic acid (BCA) protein determination kit were purchased from Sigma–Aldrich (Sydney, Australia). Reagent grade KNO_3 (Ajax Chemicals, Sydney, Australia), H_2SO_4, $K_4Fe(CN)_6$, NaCl (all from BDH) were used as received. HPLC gradient grade acetonitrile (Aldrich) was dried over 4 Å molecular sieves, and ethanol (Selbys) was glass distilled before use. Aqueous solutions were prepared using Milli-Q water (Milli-Q Reagent Water System). In all voltammetric experiments, the analyte solution was purged with nitrogen for 10 min. After deaeration, a blanket of nitrogen was kept over the solution throughout the experiment. A further 1 min was allowed for the solution to become quiescent prior to the commencement of each experiment.

2.2. Instrumentation

A 10 ml electrochemical cell accommodated with a three-electrode system, consisting of a 3 mm diameter gold disc working electrode (BioAnalytical Systems, West Lafayette, IN, USA), a Ag|AgCl reference electrode (BioAnalytical Systems) and a platinum coil auxiliary electrode, was used throughout. All electrochemical experiments were performed using a MacLab/4e potentiostat (ADInstruments, Castle Hill, Australia) interfaced with a Macintosh computer via an EChem v 1.3-1 and a Chart 4.2 software (ADInstruments). An Eppendorf Biophotometer was used for the spectrophometric determination of protein. The geometric area of gold electrodes was determined by measuring the diameter under an Olympus SZ10 Research microscope.

2.3. Electrode preparation

Prior to the deposition of SAM, the gold electrodes were polished sequentially in a slurry of alumina powder of 1.0, 0.3 and 0.05 μm to a mirror finish. The electrodes were then sonicated for 5 min to remove any alumina remnants and rinsed thoroughly with Milli-Q water. The electrodes were placed in hot ($\sim$60 °C) concentrated H_2SO_4 for 5 min and in aqua regia for 30 s, followed by a further 30 s polishing in the 0.05 μm alumina slurry. The electrodes were sonicated again for 2 min, thoroughly rinsed with Milli-Q water and then ethanol before being allowed to air dry for 10 min. Cyclic voltammetry at the gold electrodes in freshly prepared, deoxygenated 0.5 M H_2SO_4 was performed between +1600 and −200 mV until a votammogram characteristic of a clean gold electrode was obtained. Only such electrodes were used in subsequent experiments.

2.4. Formation and determinations involving SAMs

For comparison, a conventional technique adopted by many researchers [33–35], and one employed in our laboratory, were carried out to immobilise a thioctic acid SAM on a gold electrode surface. In the former, the gold electrode was immersed in 0.5 ml of thioctic acid solution (in ethanol) for 24 h. In our own procedure to assemble a monolayer, 20 μl of a thioctic acid solution (in absolute ethanol) was left to dry on a clean gold surface at room temperature. All these electrodes were washed twice, each time in 1 ml of fresh ethanol to remove any unbound thioctic acid and were then air dried for a further 2–3 min.

In determining the double layer capacitance associated with either a bare or a SAM-coated electrode, cyclic voltammetry in 0.1 M KNO_3 solution was conducted between −400 and +400 mV, and the capacitance was evaluated based on the scan rate and the average of the cathodic and anodic currents.

The surface coverage of the thioctic acid SAM was estimated using a method reported by Weisshaar et al. [36]. In this method, linear scan voltammetry in 0.5 M KOH between −400 and −1800 mV was performed at a scan rate of 100 mV s^{-1}. Based on the relation $Q = nFA\Gamma$ (where Q is the total charge (C), A is the electrode surface area (cm^2), Γ is the surface coverage (mol cm^{-2}), n and F have their usual electrochemical meaning), the charge under a desorption wave was then used to provide a measure of the surface coverage of thioctic acid. Also, the thickness of SAM, d (Å), was estimated using the relation $C_p = \varepsilon\varepsilon_0/d$, where C_p is the capacitance per unit area, ε the relative dielectric constant (2.6 for alkanethiols [37,38]), ε_0 is the vacuum permittivity (8.85×10^{-14} C^2(J cm)$^{-1}$).

2.5. Attaching Protein A on SAM

As part of the evaluation of the immobilised thioctic acid SAM, the extent of binding of the bacterial protein, Protein A, linked to the thioctic acid on the electrode was also investigated. To facilitate the binding of Protein A, a thioctic acid-coated gold electrode was initially immersed in a 1% (w/v) solution of EDC in acetonitrile for 5–6 h. This is to activate the free carboxylic groups of thioctic acid by forming an *o*-acylurea intermediate [39]. After rinsing twice with acetonitrile the electrode was dried in a nitrogen atmosphere. Upon drying, 30 μg of Protein A solution (in 0.9% (w/v) NaCl) were spread over the surface of the electrode. All electrodes were kept in a refrigerator at 4 °C overnight for the reaction to take place.

Following protein immobilisation, the electrode was washed by immersing in 1 ml of 0.9% (w/v) NaCl for 10 min with occasional shaking. The wash solution was then assayed for total protein using a BCA protein assay reagent kit. In this assay, a working reagent was initially prepared according to the instructions provided by the manufacturer. Briefly, 50 ml of BCA were mixed with 1 ml of 4% (w/v) $CuSO_4$ solution. Then, 800 μl of this reagent were mixed with 400 μl of the blank solution (prepared using Milli-Q water), standard solutions and electrode wash solution, respectively. A period of 30 min was allowed for the reaction to go to completion at 40 °C. All the reaction vessels were then placed in chilled water to quench the reaction. Absorbance of solutions was measured against a blank at 562 nm. All determinations were conducted in duplicate.

3. Results and discussion

3.1. Characterising a clean Au electrode

Prior to modification with SAMs, cyclic voltammetry at polished and pretreated Au electrodes in 0.5 M H_2SO_4 was performed. Fig. 1a depicts a typical cyclic voltammogram obtained with oxygen adsorption occurring on the gold surface between 1.2 and 1.6 V, and oxygen desorption between

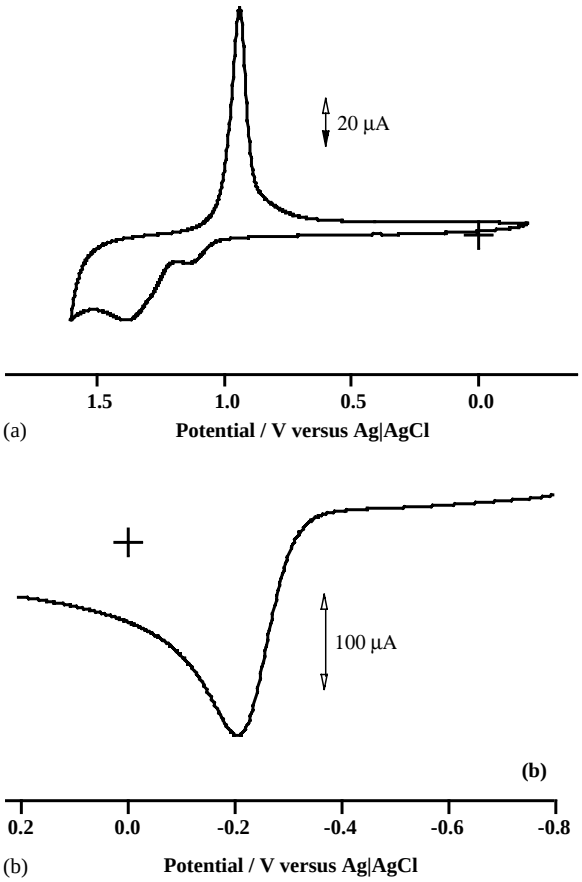

Fig. 1. (a) Cyclic voltammogram of 0.5 M H_2SO_4 at a clean gold electrode; scan rate = 500 mV s^{-1}. (b) Linear scan voltammogram of 5.0 mM $Fe(CN)_6^{4-}$ in 1.0 M of KNO_3 as supporting electrolyte; scan rate = 50 mV s^{-1}. In each voltammogram, the cross represents zero current position.

0.8 and 1.0 V. This result is in agreement with those reported by others [40] and is used as an indication of a clean gold surface. Next, based on the Randles–Sevcik equation:

$$I_{pa} = (2.69 \times 10^5) n^{3/2} A D^{1/2} \upsilon^{1/2} C_{ox} \quad (1)$$

where υ is the potential scan rate, and C_{ox} the bulk concentration of an oxidant, the electrochemical surface area (A) was estimated from the anodic peak current (I_{pa}) obtained in the linear scan voltammogram of 5.0 mM $Fe(CN)_6^{4-}$ in 1.0 M KNO_3 as supporting electrolyte (shown in Fig. 1b). Eight determinations of this parameter at a scan rate of 50 mV s^{-1} and using a D value of 6.5×10^{-6} cm^2 s^{-1} at 20 °C [41] gave a mean electrochemically active area of (0.1215 ± 0.0027) cm^2 (where the error here represents the standard deviation). On the other hand, a geometric surface area of 0.07085 ± 0.0008 cm^2 was determined. Hence, a surface roughness factor of 1.71 ± 0.04 arising from repeated polishing of electrodes was estimated from these areas. Note that this surface roughness factor is comparable to that (between 1.3 and 1.5) obtained by Hoogvliet et al. [42] using a triple-potential pulse (at +1.6, 0.0 and −0.8 V) pretreatment of gold in pH 7.4 phosphate buffer. The relatively low

surface roughness factor obtained in this work is most likely the result of chemical etching of bulk polycrystalline gold with aqua regia, which is known to reduce microscopic surface roughness [42]. Notably, a low surface roughness factor is desirable for the reproducible formation of high-quality SAMs on gold [43].

3.2. Evaluating the thioctic acid SAMs on Au electrodes

3.2.1. Double layer capacitance and surface coverage measurements

In general, SAMs produced from alkanethiols with different chain lengths and degrees of branching were reported to exhibit different characteristics [44]. For example, owing to stronger Van der Waals forces among longer-chain alkanethiols, more stable SAMs are obtained [45,46]. However, within these longer chain lengths, there is an associated delay for an electrons to travel over a longer distance to the SAM-electrode interface for redox reactions. Relatively high detection limits are thus usually achieved using longer-chain alkanethiols compared to those of a shorter chain length. For example, a detection limit of approximately 15 nM was obtained using a biosensor prepared with SAM of 3-mercaptohexadecanoic acid [47], whereas a shorter-chain alkanethiol such as thioctic acid yielded a detection limit of 15 fM [48]. The present work will thus focus on thioctic acid, which consists of five-carbon chains, to compromise between stability and low detection limits.

SAMs are conventionally immobilised on the surface by immersing a biosensor in a SAM solution for over 24 h to accommodate a two-step process (a fast growth and then a slower equilibrium step) during its formation. In our laboratory, we have investigated an alternative strategy in which a known quantity of an ethanolic solution of thioctic acid is directly applied on a gold electrode surface, and then allowed to air dry at room temperature, in order to enable a more efficient immobilisation method for biosensor fabrication. To compare the characteristics of the SAMs obtained by these two methods, we have initially assessed the double layer capacitance arising from the immobilised monolayer and its surface coverage. Fig. 2 shows the cyclic voltammograms obtained at a gold electrode in 0.1 M KNO_3 before and after being coated with a thioctic acid SAM. In Fig. 2a, the thioctic acid SAM was obtained following the direct application and air drying of 20 μl of 250 mM of thioctic acid in ethanol on the electrode for 20 min. On the other hand, in Fig. 2b, the gold electrode was immersed in 250 mM thioctic acid in ethanol for 24 h. As expected, only background charging currents were observed in these voltammograms. However, owing to a decrease in the electrode surface area exposed to KNO_3, the charging current obtained at a passivated thioctic acid-coated electrode is diminished compared to that at the bare electrode. Note that two separate electrodes were employed in Figs. 2a and b, giving rise to the different magnitudes of current observed in these voltammograms. In conjunction with the potential scan rate, the charging currents between −0.2 and 0.2 V were used to estimate the double layer capacitance. In Fig. 3a, we have plotted the relative percentage change in double layer capacitance (to normalise the variations arising from the electrodes used) at the coated gold electrode (refer to the left ordinate), compared to that before it was coated with SAM, over the range of 0.5–500 mM thioctic acid. Initially, in both immobilisation methods, a higher thioctic acid concentration used in the immobilisation experiment was observed to lead to a sharp percentage drop in the double layer capacitance (as indicated by the negative values). This is expected because of the formation of a more densely packed thioctic SAM per

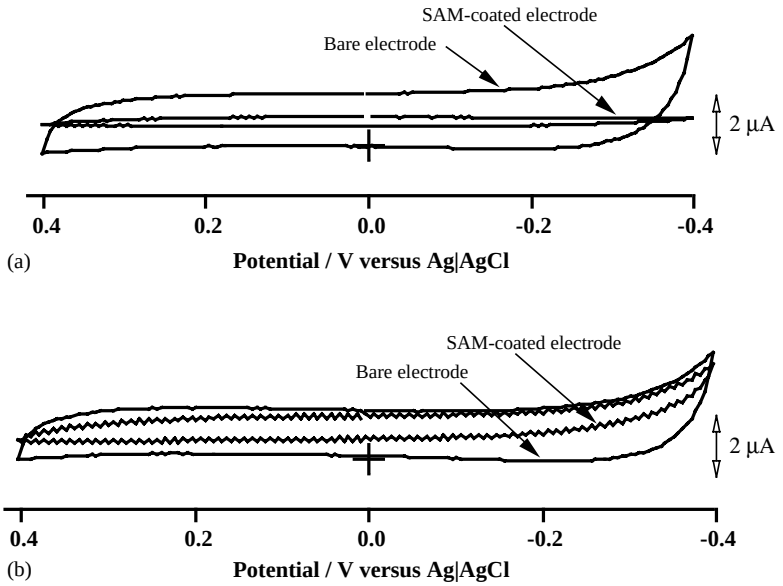

Fig. 2. Comparison of cyclic voltammograms of 0.1 M KNO_3 at a bare gold electrode and a thioctic acid SAM-coated gold electrode using: (a) a direct application method and (b) an immersion method; scan rate = 500 mV s^{-1}.

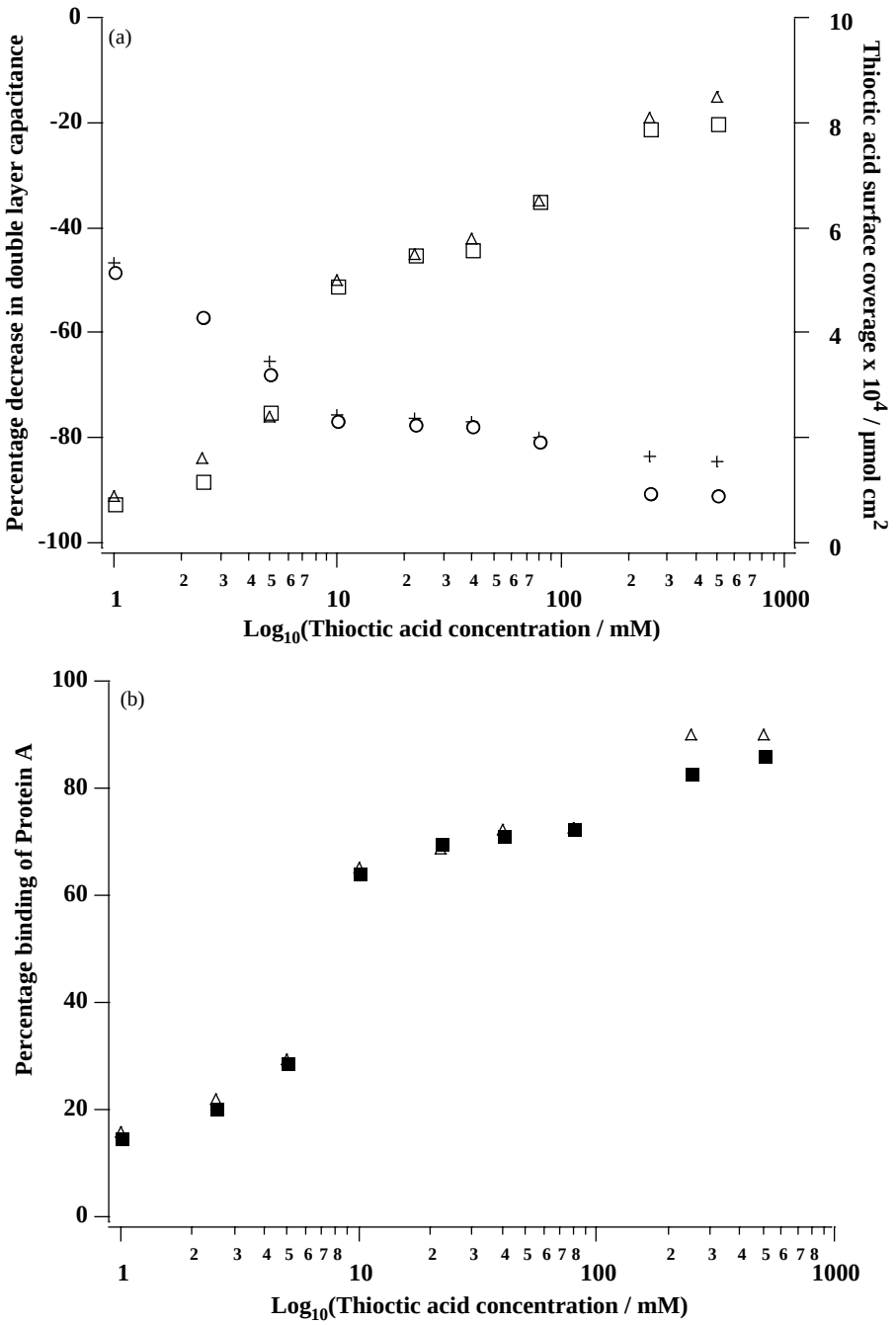

Fig. 3. Using the direct application (△ and +) and immersion method (□ and ○): (a) the percentage change in double layer capacitance (+ and ○) of a thioctic acid SAM-coated electrode relative to the bare electrode (left-hand abscissa), and the thioctic acid surface coverage (△ and □) obtained (right-hand abscissa), are plotted as a function of $\log_{10}$(thioctic acid concentration). (b) A plot of percentage binding of Protein A on a thioctic acid SAM over the same concentration range used in (a).

unit surface area in a thioctic acid solution of higher concentration, giving rise to a smaller double layer capacitance and hence a larger percentage drop value. However, even at a thioctic acid concentration as high as 250 mM, a reduction of approximately 85–90% in the double layer capacitance was observed, suggesting that there was still a small portion of gold surface uncovered by SAM and was readily permeable to KNO_3. Moreover, very similar percentage decreases were obtained at electrodes prepared using 250 and 500 mM solutions of thioctic acid, indicating likely saturation of SAM on the electrode surface at concentrations higher than 250 mM. However, no further investigations beyond this concentration range were conducted because thioctic acid began to precipitate out of the ethanolic solutions at concentrations higher than 500 mM. Hence, a concentration of 250 mM was used in our subsequent investigations.

Interestingly, we have discovered that the percentage decrease in double layer capacitance values at SAM-coated electrodes obtained in the direct application method were very similar to those in the 24 h immersion method. According to Fenter et al. [49], thiols self assemble onto gold surfaces initially in a lying-down phase, which subsequently rearranges to a standing-up phase. Indeed, they observed a lying-down phase at low surface coverage values where one sulfur from the thioctic acid was directly bound to the Au^{3+} surface with no S–Au bond yet existing for the other sulfur head groups. In the direct application method, as the thioctic acid was deliberately placed on top of an electrode surface, gravity would have readily facilitated a direct lying-down phase in the formation of a strong Au–S covalent bond. This was followed by an upright alignment of carbon chains due to Van der Waals forces between the carbon chains in the standing-up phase, resulting in a more rapid self-assembling of the thioctic acid. On the other hand, in the immersion method, the electrode was usually suspended vertically in a thioctic acid solution. An appreciable period of time was required for the attachment of randomly moving thioctic acid in solution onto the gold surface during the lying-down phase of the process, followed by the standing-up phase. Hence, the direct application method is capable of forming thioctic acid SAMs with a comparable relative percentage decrease in double layer capacitance but within a shorter period of time. In addition, a relatively high concentration of 250 mM of thioctic acid was used in our work. This condition would be likely to promote a more densely packed thioctic acid assembly on the electrode surface (see below). Coupled with gravitational forces, Van der Waals forces were expected to have aligned rapidly the carbon chains in an upright position away from the gold surface. Based on the double layer capacitance values obtained, the corresponding thicknesses of SAM formed on the electrode surface were estimated. As tabulated in Table 1, over the concentration range between 0.5 and 250 mM, the thioctic acid thicknesses varied from 6.4 to 10.0 Å, which are similar to the reported value of 8 Å [50]. These results further support the validity of the formation of SAM using the direct application method within a much shorter time compared to the immersion method.

Table 1
Thickness of thioctic acid SAM obtained at different concentrations

Thioctic acid (concentration (mM))	Thickness of thioctic acid (SAM ± standard deviation (Å))
0.5	6.4 ± 0.3
1.0	7.7 ± 0.1
2.5	8.2 ± 0.4
5.0	7.7 ± 0.1
10.0	10.0 ± 0.3
22.0	6.8 ± 0.5
40.0	7.9 ± 0.3
80.0	7.7 ± 0.2
250.0	6.6 ± 0.2

Each standard deviation was based on the results obtained on eight electrodes.

In this work, we have also estimated the surface coverage of SAM on the electrode prepared by both the direct application and immersion method. This is based on the amount of thioctic acid desorbed from the electrode in 0.5 M KOH as Au^{3+} was voltammetrically reduced to Au. The values obtained (see the right ordinate) are also plotted in Fig. 3a over the same range of thioctic acid concentrations used in the double layer capacitance measurement experiments. Clearly, a higher thioctic acid concentration was expected to yield a higher surface coverage, leading to a corresponding decrease in the double layer capacitance.

Based on the surface coverage obtained in 250 mM thioctic acid solution and the electrode surface area, approximately 9.3×10^{-11} mol of thioctic acid would form a monolayer on the electrode surface. However, in both the direct application and immersion method, an excess amount of thioctic acid was available throughout the concentration range covered in this study (e.g. 1.0×10^{-8} mol when 0.5 mM thioctic acid was used). Hence, there was always more than sufficient thioctic acid present to form a SAM on the electrode surface, giving rise to a similar percentage change in double layer capacitance (refer to the left ordinate) as the thioctic acid concentration was increased in both the direct application and immersion method, as shown in Fig. 3a. Meanwhile, the percentage change in double layer capacitance obtained in these two methods was observed to be less variable in the higher concentration range ($\geq$10 mM). As explained previously, we attribute this to both the effect of gravitational force and enhanced Van der Waals forces arising from the increasingly large excess of thioctic acid present to align the molecules on the electrode surface. Consequently, a densely packed thioctic acid SAM structure was formed, yielding slightly larger, but similar, reductions in double layer capacitance in the $\geq$10 mM concentration range.

3.2.2. Attaching Protein A to the thioctic acid SAM

In constructing a biosensor, a biological recognition component can be immobilised on its surface using a SAM. As a biological molecule candidate, we have immobilised the bacterial protein, Protein A, on the surface of thioctic acid-coated gold electrodes. Specifically, Protein A is a protein component isolated from the cell wall of more than 90% of strains of the bacterium, *Staphylococcus aureus*, and has a molecular mass of 42 kDa. In this work, the free terminals of thioctic acid on the electrode surface were activated by incubation in 1% (w/v) EDC in acetonitrile for 5 h to promote the formation of a link with Protein A via an amide bond. Following Protein A attachment, the electrode was thoroughly rinsed in 0.9% (w/v) NaCl. An indirect quantification of Protein A linked to thioctic acid was performed by a spectrophotometric determination of the amount of Protein A in the wash solution. The results obtained using the direct application and immersion methods for thioctic acid immobilisation are shown in Fig. 3b. In this way, we can see that approximately 90% of Protein A was bound to the

thioctic acid-coated surface obtained by the direct application method, and 83% at the thioctic acid-coated surface using the immersion method. In contrast, based on the direct application method alone, binding values of $3.3 \pm 0.8\%$ ($N = 16$ and the error represents standard deviation) and $4.7 \pm 2.1\%$ ($N = 8$) were obtained when Protein A was attached onto a bare gold electrode surface and when the free terminals of thioctic acid carbon chains were not activated, respectively, indicating a relatively insignificant degree of binding in both cases. Hence, the high percentage binding values of protein with the SAM suggest that not only the gold surface was populated by a densely packed monolayer, but the free terminals of thioctic acid were also in the appropriate orientation for binding. This is in agreement with work reported by Fenter et al. [49] that a high surface coverage of thiols was only obtained in a standing-up phase.

3.3. Effect of air drying time

In the direct application method, a period of 20 min was allowed for the evaporation of ethanol during the immobilisation of SAM after delivering 20 μl of 250 mM thioctic acid on a gold electrode surface at room temperature (~20 °C). Note that we have limited the present study to room temperature as this is the most convenient experimental condition in practice. By maintaining the thioctic acid concentration at 250 mM, incomplete drying was visually observable on the electrode surface when an air drying period shorter than 20 min was permitted and these electrodes were not useful for further experiments. Additional experiments were then conducted by varying the drying time between 20 and 300 min and < 1% in the relative double layer capacitance was determined over this range of air drying times. Therefore, there did not seem to be a difference in the quality of thioctic acid SAM on the electrode as long as sufficient time was allowed for the evaporation of ethanol. An air drying time of 20 min was thus subsequently adopted.

3.4. Stability test of thioctic acid-coated gold electrodes

In this work, we have also studied the stability of a thioctic acid-coated gold electrode prepared using the direct application method. Here, gold electrodes were modified by allowing 20 μl of 250 mM ethanolic thiotic acid solution to air dry for 20 min at room temperature. Five determinations of the relative percentage drop in double layer capacitance of the modified electrodes were carried out daily in 0.1 M KNO_3 for 20 days during April and July 2002, respectively. In between measurements, cyclic votammetry at these SAM-coated electrodes in 0.1 mM solution of $Fe(CN)_6^{4-}$ (in 0.1 M KNO_3 supporting electrolyte) was performed to investigate the effect on the stability of SAM. The electrodes were stored with the electrode surface directed upward in a refrigerator at 4 °C when they were not in use. Fig. 4 compares the cyclic voltammograms obtained on Day 1 and Day 20 in both the April and July 2002 sets of results. Similar to Fig. 2a, no oxidation currents were observed in these voltammograms due to a blocking effect arising from the thioctic acid SAM on the electrode surface. Shewhart's statistical analysis (based on the sample range values in the relative percentage drop in double layer capacitance) was then employed to examine the stability of the SAM. A control chart with 99.7% confidence control limits is shown in Fig. 5. The results indicate that the percentage drop in the double layer capacitance at the SAM-coated electrodes were relatively invariable within each of the April and July 2002 sets of data, with only an average of 1% difference observed in the relative double layer capacitance. Therefore, there

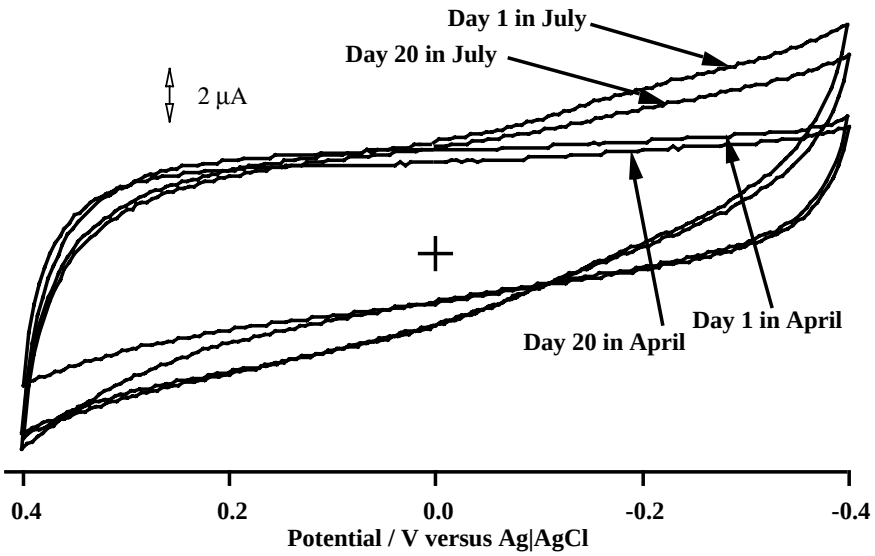

Fig. 4. Comparison of cyclic voltammetric response at thioctic acid SAM-coated gold electrodes in 0.1 M KNO_3 in Day 1 and Day 20 over a 3-month period; scan rate = 500 mV s^{-1}.

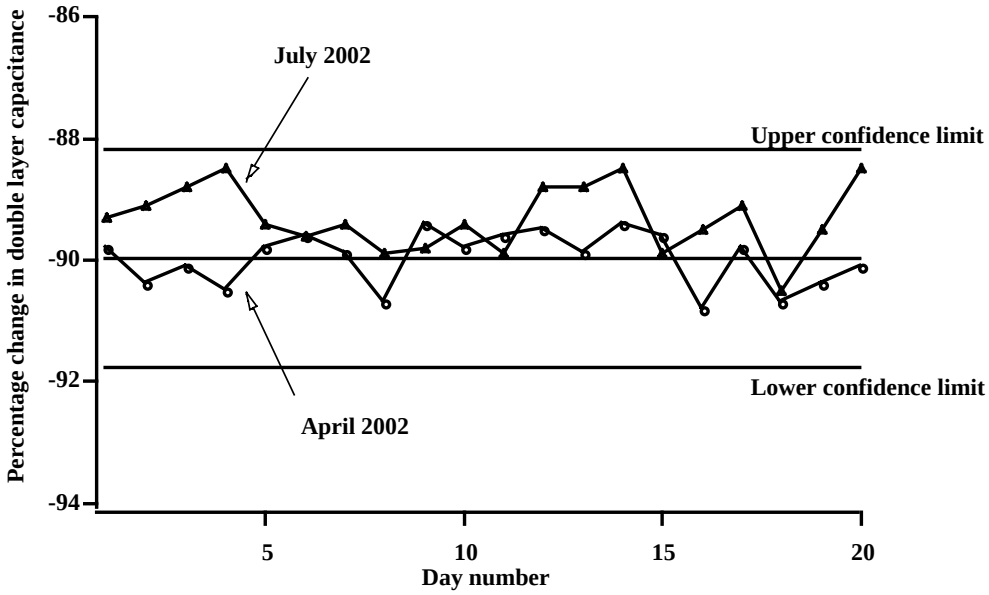

Fig. 5. A control chart with 99.7% confidence limits showing the percentage change in double layer capacitance of two electrodes over a 20-day period.

was merely a very slow deterioration of the thioctic acid SAM under the present experimental conditions over the 3-month period. Moreover, this decrease is still well within the 99.7% limits set in the control chart, reflecting the acceptable degree of stability of thioctic acid SAM obtained.

4. Conclusion

A direct application method was studied in order to facilitate a more efficient strategy for the immobilisation of a thioctic acid SAM on gold electrodes. In this method, as the ethanolic solution of thioctic acid was deliberately left to dry in air, we speculate that gravity would have aided in expediting the lying-down phase and, perhaps, to a lesser degree, the standing up phase involved in the formation of SAM on an electrode surface. The SAM obtained was shown to exhibit similar characteristics in terms of the relative percentage drop in double layer capacitance, surface coverage and the percentage of binding of Protein A, compared to that prepared using a more conventional immersion method. Therefore, the results indicated that a shorter time period in the direct application method (an air drying duration of 20 min was used in this work) provides a faster, more convenient method for the preparation of SAM-coated gold electrodes. Moreover, the thioctic acid SAM formed using the direct application method was shown to display acceptable stability well within the 99.7% limits over a 3-month period.

References

[1] S. Dong, B. Wang, Electroanalysis 14 (2002) 7.
[2] J. Gascón, E. Martinez, D. Barceló, Anal. Chim. Acta 311 (1995) 357.
[3] Z. Wu, B. Wang, Z. Cheng, X. Yang, S. Dong, E. Wang, Biosen. Bioelectron. 16 (2001) 47.
[4] D.P. Nikolelis, G. Theoharis, Electroanalysis 14 (2002) 1661.
[5] D.P. Nikolelis, S.E. Petropoulou, G. Theoharis, Electrochim. Acta 47 (2002) 3457.
[6] D.P. Nikolelis, S. Pantoulias, Anal. Chem. 73 (2002) 5945.
[7] A. Taurino, S. Capone, C. Distante, M. Epifani, R. Rella, P. Siciliano, Thin Solid Films 418 (2002) 59.
[8] S.S. Rosatto, P.T. Sotomayor, L.T. Kubota, Y. Gushikem, Electrochim. Acta 47 (2002) 4451.
[9] A. Walcarius, Electroanalysis 13 (2001) 701.
[10] A.F. Chetcuti, D.K.Y. Wong, M.C. Stuart, Anal. Chem. 71 (1999) 4088.
[11] M. Santandreu, F. Céespedes, S. Alegret, E. Martinez-Fàbregas, Anal. Chem. 69 (1997) 2080.
[12] A. Mulchandani, S. Pan, Anal. Biochem. 267 (1999) 141.
[13] R. Dobay, G. Harsányi, C. Visy, Anal. Chim. Acta 385 (1999) 187.
[14] M.C. Leopold, J.A. Black, E.F. Bowden, Langmuir 18 (2002) 978.
[15] J.J. Gooding, P. Erokhin, D. Losic, W. Yang, V. Policarpio, J. Liu, F.M. Ho, M. Situmorang, D.B. Hibbert, J.G. Shaoter, Anal. Sci. 17 (2001) 3.
[16] J.J. Gooding, V.G. Praig, E.A.H. Hall, Anal. Chem. 70 (1998) 2396.
[17] H.O. Finklea, in: A.J. Bard, I. Rubinstein (Eds.), Electroanalytical Chemistry–A Series of Advances, vol. 19, Marcel Dekker, Basel, 1996, p. 110.
[18] A.M. Smith, M.W. Ducey Jr, M.E. Meyerhoff, Biosen. Bioelectron. 15 (2000) 183.
[19] N.K. Chaki, K. Vijayamohanan, Biosen. Bioelectron. 17 (2002) 1.
[20] C.A. Widrig, C. Chung, M.D. Porter, J. Electroanal. Chem. Interfacial Electrochem. 310 (1991) 335.
[21] R.G. Nuzzo, F.A. Fusco, D.L. Allara, J. Am. Chem. Soc. 109 (1987) 2358.
[22] R.G. Nuzzo, B.R. Zegarski, L.H. Dubois, J. Am. Chem. Soc. 109 (1987) 733.
[23] J.J. Tupper, R.J. Colton, D.W. Brenner, Langmuir 10 (1994) 2041.
[24] S. Dong, J. Li, Bioelectrochem. Bioenerg. 42 (1997) 7.
[25] J.J. Gooding, L. Pugliano, D.B. Hibbert, P. Erokhin, Electrochem. Commun. 2 (2000) 217.
[26] T. Kaiser, P. Gudat, W. Stock, G. Pappert, M. Grol, D. Neumeier, P.B. Luppa, Anal. Biochem. 282 (2000) 173.
[27] W. Pan, C.J. Durning, N.J. Turro, Langmuir 12 (1996) 4469.

[28] M. Kawasaki, T. Sato, T. Tanaka, K. Takao, Langmuir 16 (2000) 1719.
[29] J.J. Gooding, E.A.H. Hall, D.B. Hibbert, Electroanalysis 10 (16) (1998) 1130.
[30] Q. Cheng, A. Brajter-Toth, Anal. Chem. 68 (1996) 4180.
[31] J. Li, Ding, E. Wang, S. Dong, J. Electroanal. Chem. 431 (1997) 227.
[32] S. Campuzano, R. Gálvez, M. Pedrero, F.J.M. de Villena, J.M. Pingarrón, J. Electroanal. Chem. 526 (2002) 92.
[33] D.M. Disley, D.C. Cullen, H. You, C.R. Lowe, Biosen. Bioelectron. 13 (1998) 1213.
[34] P. Krysinski, M. Brzostowska-Smolska, Bioelectrochem. Bioenerg. 44 (1998) 163.
[35] K. Wadu-Mesthrige, N.A. Amro, G. Liu, Scanning 22 (2000) 380.
[36] D.E. Weisshaar, M.M. Walczak, M.D. Porter, Langmuir 9 (1993) 323.
[37] M.D. Porter, T.B. Bright, D.L. Allara, C.E.D. Chidsey, J. Am. Chem. Soc. 109 (1987) 3559.
[38] C.E.D. Chidsey, D.N. Loiacono, Langmuir 6 (1990) 682.
[39] C. Duan, M.E. Meyerhoff, Mikrochim. Acta 117 (1995) 117.
[40] C. Hinnen, N.V. Houng, A. Rousseau, J.P. Dalbera, J. Electroanal. Chem. 95 (1979) 131.
[41] R.V. Bucur, A. Bartes, V. Mecea, Electrochim. Acta 23 (1977) 641.
[42] J.C. Hoogvliet, M. Dijksma, B. Kamp, W.P. van Bennekom, Anal. Chem. 72 (2000) 2016.
[43] S.E. Creager, L.A. Hockett, G.K. Rowe, Langmuir 8 (1992) 854.
[44] C. Berggren, B. Bjarnason, G. Johansson, Biosen. Bioelectron. 13 (1998) 1061.
[45] J.J. Gooding, D.B. Hibbert, Trends Anal. Chem. 8 (1999) 525.
[46] M. Dijksma, B. Kamo, J.C. Hoogvliet, W.P. van Bennekom, Langmuir 16 (2000) 3852.
[47] V.M. Mirsky, M. Riepl, O.S. Wolfleis, Biosen. Bioelectron. 12 (1997) 977.
[48] C. Berggren, G. Johansson, Anal. Chem. 69 (1997) 3651.
[49] P. Fenter, F. Schreiber, L. Berman, G. Scoles, P. Eisenberger, M. Bedzyk, Surf. Sci. 412/413 (1998) 213.
[50] J.F. Hicks, A.C. Templeton, S. Chen, K.M. Sheran, R. Jasti, R.W. Murray, J. Debord, T.G. Schaaff, R.L. Whetten, Anal. Chem. 71 (1999) 3703.