Detection of triglyceride using an iridium nano-particle catalyst based amperometric biosensor

Wei-Yin Liao,[a] Chung-Chiun Liu*[b] and Tse-Chuan Chou[ac]

Received 30th January 2008, Accepted 4th July 2008
First published as an Advance Article on the web 19th September 2008
DOI: 10.1039/b801703d

The detection and quantification of triglyceride (TG) using an iridium nano-particle modified carbon based biosensor was successfully carried out in this study. The detection procedures were based on the electrochemical detection of enzymatically produced NADH. TG was hydrolyzed by lipase and the glycerol produced was catalytically oxidized by NAD-dependent glycerol dehydrogenase producing NADH in a solution containing NAD^+. Glyceryl tributyrate, a short chain triglyceride, was chosen as the substrate for the evaluation of this TG biosensor in bovine serum and human serum. A linear response to glyceryl tributyrate in the concentration range of 0 to 10 mM and a sensitivity of 7.5 nA mM^{-1} in bovine serum and 7.0 nA mM^{-1} in human serum were observed experimentally. The potential interference of species such as uric acid (UA) and ascorbic acid (AA) was assessed. The incorporation of a selected surfactant and an increase in the incubation temperature appeared to enhance the performance of this biosensor. The conditions for the determination of TG levels in bovine serum using this biosensor were optimized, with sunflower seed oil being used as an analyte to simulate the detection of TG in blood. The experimental results demonstrated that this iridium nano-particle modified working electrode based biosensor provided a relatively simple means for the accurate determination of TG in serum.

1. Introduction

Over the past few decades, it has become apparent that high serum triglyceride (TG) levels are associated with an increased risk of atherosclerotic events.[1,2] Atherosclerosis is associated with human vascular disorders such as coronary artery disease (CHD). The serum TG level is directly related to lipoprotein metabolism, which is essentially the conversion of triglyceride-rich lipoproteins into lipoproteins such as high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) through the removal of triglyceride.[3,4] When a high TG level is present in serum, LDL-C and HDL-C become small and dense, creating a highly atherogenic state.[2–6] Also, from epidemiological studies,[7–10] it is evident that the raised serum TG level is associated with the risk of developing cardiovascular disease. Therefore, a high serum TG level is a significant risk factor for CHD.[11] The American Heart Association suggests that:[12] TG levels less than 150 mg dL^{-1} (1.7 mmol L^{-1}) are normal, levels from 150 mg dL^{-1} to 199 mg dL^{-1} (2.25 mmol L^{-1}) are borderline-high, levels from 200 mg dL^{-1} to 499 mg dL^{-1} (5.65 mmol L^{-1}) are high, and levels over 500 mg dL^{-1} (5.65 mmol L^{-1}) are very high. Thus, TG levels at 0 to 150 mg dL^{-1} are focused on in this study as the foundation for developing a biosensor for individuals to be able to measure TG levels by themselves at home or for a preliminary and simple assessment in a point-of-care environment.

Several methods for the determination of TG have been developed. Generally, they are enzymatic and consist of two main reaction steps. The first step is the hydrolysis of TG into glycerol and fatty acids in the presence of lipase. The second step is to quantitatively determine the glycerol liberated, which can be carried out in diverse ways. Broadly stated, they can be divided into two types. One is to use chemical methods that involve glycerol oxidation to formaldehyde using periodate and then quantifying the formaldehyde produced spectrophotometrically. The spectrophotometric determination of formaldehyde can be achieved in a variety of ways: (1) mixing with phenylhydrazine, ferricyanide and hydrochloric acid, with spectrophotometric determination at 540 nm;[13] (2) mixing with chromotropic acid and sulfuric acid, accompanied by spectrophotometric determination at 570 nm;[14] (3) mixing with 3-methyl-2-benzothiazolinone and ferric chloride, with spectrophotometric determination at 620 nm;[14] (4) using ammonium acetate and acetylacetone with colorimetric determination at 412 nm and fluorometrically by using 400 nm primary and 485 nm secondary filters.[15]

Another approach is based on the enzymatic detection of glycerol by using a biosensor. Biosensors for the evaluation of glycerol have been based on the amperometric detection of hydrogen peroxide or nicotinamide adenine dinucleotide (NADH), both of which are products formed by the enzymatic oxidation of glycerol using different enzymes. Glycerol can be phosphorylated with glycerol kinase in the presence of adenosine 5′-triphosphate (ATP) forming glycerol-3-phosphate and adenosine diphosphate (ADP).[16] These two products can act as substrates for different enzymes and *via* different

[a]*Department of Chemical Engineering, National Cheng Kung University, Tainan, 70101, Taiwan*
[b]*Electronics Design Center and Department of Chemical Engineering, Case Western Reserve University, 44106, USA. E-mail: cxl9@case.edu; Tel: +1 216-368-2935*
[c]*Department of Chemical Engineering, Tatung University, Taipei, 104, Taiwan*

enzymatic reactions produce H_2O_2 and NADH. For instance, in the presence of glycerol phosphate oxidase [EC 1.1.3.21], glycerol-3-phosphate can be oxidized with oxygen producing dihydroxyacetone phosphate and H_2O_2.[17] Or, by using glycerol-3-phosphate dehydrogenase [EC 1.1.1.8], glycerol-3-phosphate can be oxidized, resulting in the production of NADH and dihydroxyacetone phosphate in a NAD^+ containing solution.[18] ADP can then react with phosphoenolpyruvate using pyruvate kinase [EC 2.7.1.40] to produce ATP and pyruvate, which can then be oxidized by pyruvate oxidase [EC 1.2.3.3] to produce H_2O_2.[19] Additionally, glycerol can be oxidized by using glycerol dehydrogenase [EC 1.1.1.6] with NAD^+ to generate NADH in a simple two-stage reaction.[20] These two approaches for detecting glycerol, if incorporated into a biosensor would be a practical means for the development of a commercially viable portable TG detector.

The enzyme used to measure glycerol was glycerol dehydrogenase, which will oxidize glycerol resulting in NADH in a solution containing NAD^+. Then, NADH can be directly oxidized at a conventional electrode, such as gold, platinum or carbon; however, a large overpotential is required, due to the slow charge transfer kinetics.[21,22] Numerous efforts have been made in order to improve the electron transfer between NADH and the conducting substrate by the immobilization of diverse redox mediators, which may provide a means of decreasing the overpotential.[23,24] These studies can be classified into groups that use: quinones,[25] aromatic diamines,[26] oxometalates,[27] polymetallophthalocyanines,[28] organic dyes,[29] carbon nanotubes[30] and metal complexes.[31,32] Among these approaches, we have focused on the use of iridium nano-particles as a metallic catalyst doped into a carbon paste. Using a thick film screen-printing method we have formed the working electrode as a biosensor in a cost effective manner. This approach has given us advantages both in terms of stability and in promoting the catalytic reduction of redox species. Additionally, the electrode is notable for its ability to inhibit the oxidation of interfering species.[33,34] When compared to previous designs, the disposable biosensor, which was developed in this study can be easily mass produced and used as a convenient way to assess the TG levels at home or in a point-of-care environment.

In this study, the biosensor used was an iridium nano-particle modified thick film screen-printed carbon paste electrode, formed on a disposable polyester substrate, which was designed to detect TG concentrations in serum. In the evaluation of TG, this iridium nano-particle modified working electrode revealed a lower oxidation potential with respect to the enzymatically generated NADH in serum containing lipase, glycerol dehydrogenase and NAD^+ in comparison with a conventional non-catalytic electrode. Thus, this biosensor demonstrated minimal interference when challenged with ascorbic acid (AA) and uric acid (UA) at their normal physiological concentrations. Therefore, the TG levels can be easily and directly determined by evaluating the enzymatic reaction of the TG which produced NADH using this single use, disposable and cost effective biosensor. This represents a significant advancement with respect to the field-effect transistor (FET) type and the metal (gold or platinum) modified with mediator type biosensors,[20,35–37] which are both more expensive and FET type biosensors, which are less sensitive to low concentrations of TG.

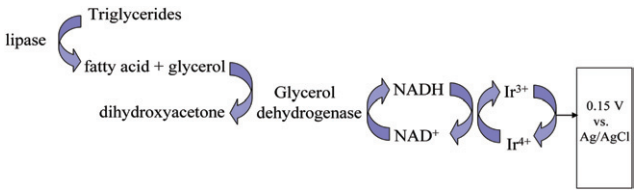

Fig. 1 Schematic diagram of the mechanism of TG determination by the iridium modified carbon biosensors.

2. Mechanism of TG sensing

The enzymatic reaction scheme for measuring the TG levels using this biosensor is presented in Fig. 1. First, TG is hydrolyzed by lipase resulting in the production of three different carbon chain length fatty acids and glycerol. The glycerol can then be directly oxidized by glycerol dehydrogenase with the participation of NAD^+ as the electron acceptor, resulting in the formation of dihydroxyacetone, NADH and hydrogen ions. The enzymatically produced NADH can be monitored by an electrochemical sensor through its reoxidation cycle to NAD^+. Therefore, the TG level can then be determined using this biosensor by measuring the output current changes from the enzymatic oxidation reaction producing NADH.

3. Experimental

3.1 Reagents

Lipase (EC 3.1.1.3) from *Candida rugosa* and glycerol dehydrogenase (GDH) (EC 1.1.1.6) from *Cellulomonas* were purchased from Sigma (St. Louis, MO). Bovine serum collected from cattle in New Zealand was bought from Invitrogen (Grand Island, NY) and human serum (catalog no. H4522) was obtained from Sigma (St. Louis, MO). Glyceryl tributyrate, β-nicotinamide adenine dinucleotide hydrate (β-NAD) from yeast, potassium phosphate, and Triton X-100 were also obtained from Sigma (St. Louis, MO). Sunflower seed oil from Supelco (St. Louis, MO) was used as a substitute for human triglyceride for evaluating the TG biosensors for reasons of cost and commercial availability. All reagents in this study were of the best quality available commercially.

The materials for fabricating the biosensor, including the insulator and Ag/AgCl inks, were from NAZDAR (Shawnee, KS) and DuPont, respectively. The chemical compounds for preparing the iridium doped carbon ink were iridium nano-particles dispersed carbon (5% Ir) from E-TEK, Inc. (Somerset, NJ), polyethylenimine (50% in water, from Aldrich (Milwaukee, WI)) and hydroxyethyl cellulose (MW 250 000, from Aldrich (Milwaukee, WI)). De-ionized water was used for the preparation of all aqueous solutions.

3.2 Fabrication of disposable mini biosensor

The biosensors used in this study were based on a three-electrode system, which comprised the working, counter and reference electrodes. They were fabricated by the standard thick-film screen-printing technique and are shown in Fig. 2. The biosensors were printed on a 787 mm × 584 mm polyester sheet substrate from DuPont (Melinex 329). The dimensions of each

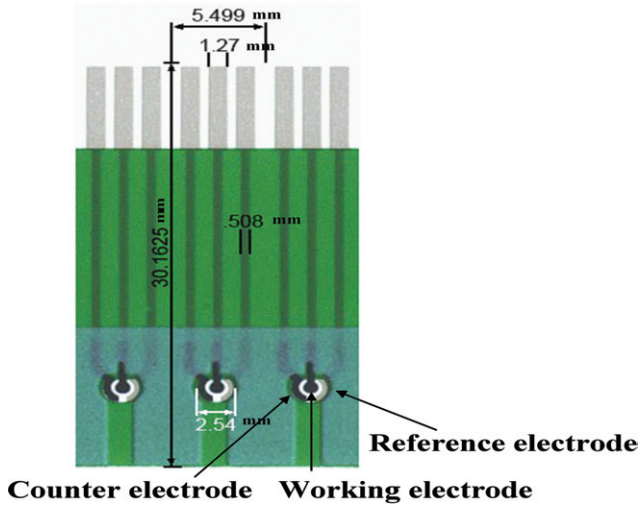

Fig. 2 Photograph of the thick-film screen-printed, single use, disposable mini iridium modified carbon biosensor.

electrode was 30 mm × 5.5 mm and the working electrode was 0.1 mm in diameter. First, the silver conductive paths were printed onto the substrate. Then, the iridium nano-particle doped carbon ink for the working and the counter electrodes was screen-printed at the designed positions to connect the silver conducting paths. The iridium doped carbon ink was formed as follows: first the viscous solution was prepared by dissolving polyethylenimine (1.36 mL) and hydroxyethyl cellulose (0.34 g) in 10 mL de-ionized water with stirring. Then, the iridium doped carbon powder (0.9 g) was added into the solution (5 g) with homogenization for 5 min, resulting in a homogenous iridium doped carbon ink. Silver/silver chloride ink was then applied in the design space and connected to the silver path as a reference electrode. The insulation ink was printed covering the substrate except for the three electrodes and the electrical conducting areas. By following this simple fabrication procedure, biosensors of this type could potentially be produced on an industrial scale. In this study, we first fabricated the sensor prototype in-house, and then these disposable biosensors were fabricated with assistance from Conductive Technologies (York, PA) in large quantity.

3.3 Procedures for measurement of TG

The assay procedures for determining the TG level in serum using the biosensor were carried out as follows: first, the biosensor was placed in a 1.5 mL centrifuge tube with a well-mixed bovine serum (1 mL) containing lipase (10 mg), GDH (8 U) and NAD^+ (0.01 M) to acquire the background cyclic voltammograms and the *I–t* curves. Then, the biosensor was removed from the tube, before adding the TG. After the TG was added into the serum solution, the solution was incubated and shaken for 2.5 min. Finally, the biosensor was re-introduced into the centrifuge tube allowing cyclic voltammograms and *I–t* curves of TG in serum to be obtained.

Cyclic voltammetry and amperometric studies were carried out using an Electrochemical Workstation, Model 660c, CH Instruments (Austin, TX).

3.4 Safety note

The reconstituted TG controlled serum samples were treated with extreme care as is appropriate to the testing of any blood sample. All pipette tips, centrifuge tubes, gloves, and used serum samples were collected in a biohazard container and disposed of properly. The laboratory bench and evaluation system, including the water bath and measuring cell were washed and scrubbed with 95% alcohol.

4. Results and discussion

4.1 Characterization of the TG biosensor

Based on the mechanism shown in Fig. 1, TG is able to be detected by measuring the enzymatically produced NADH, which can be oxidized irreversibly usually resulting in the fouling of the electrode's surface and an increase in the overpotential. Therefore, it would be desirable to immobilize the catalyst and thereby reduce the electrochemical overpotential during NADH electrooxidation. In this study, iridium nano-particles were chosen as the catalyst and were doped into the carbon ink for the fabrication of the screen-printed biosensor. The catalytic activity for NADH oxidation of the iridium modified biosensor is shown by the cyclic voltammograms in Fig. 3 (a). It is apparent that in the bovine serum without glycerol, there was no observable oxidation peak from the biosensor in a 0.01 M NAD^+ bovine serum which additionally contained 8 U GDH. On the other hand, a manifest anodic peak was revealed at about +0.15 V *versus* the Ag/AgCl printed reference electrode in serum with glycerol, which was due to the electrooxidation of enzymatically produced NADH. The intensity of the NADH oxidation peak was enhanced by an increase of glycerol concentration in the serum. This suggested that NADH formed from NAD^+, the cofactor for GDH catalyzing the oxidation of glycerol, would be directly oxidized at the electrode's surface. The developed biosensor for the detection of NADH was able to operate at a relatively low oxidation potential, +0.15 V, and also it was found that the current of the oxidation peak was proportional to the concentration of the enzymatically generated NADH.

Triglycerides are a family of lipids in which the glycerol is esterified with free fatty acids. One of simplest of the triglycerides is glyceryl tributyrate, a short chain triglyceride, which can be used as a prototype for the quantitative determination of the TG concentration by the biosensor. Fig. 3 (b) shows the cyclic voltammograms obtained by this iridium nano-particle modified biosensor in bovine serum at different concentrations of glyceryl tributyrate. When glyceryl tributyrate was added into the bovine serum, an anodic peak appeared at +0.15 V *versus* Ag/AgCl and this peak increased as a function of the glyceryl tributyrate concentration. The associated electrocatalytic oxidation of NADH is shown in Fig. 3 (a). The results in Fig. 3 suggested that this TG biosensor could be successfully used for the electrochemical detection of enzymatically generated NADH in the test medium, *i.e.* serum, when present with the substrate, the enzyme and the cofactor (NAD^+).

The cyclic voltammetry results verified that the mechanism for the detection of the TG concentration indicated that an applied potential of +0.15 V *versus* the printed Ag/AgCl reference electrode could be used for the amperometric measurements of TG

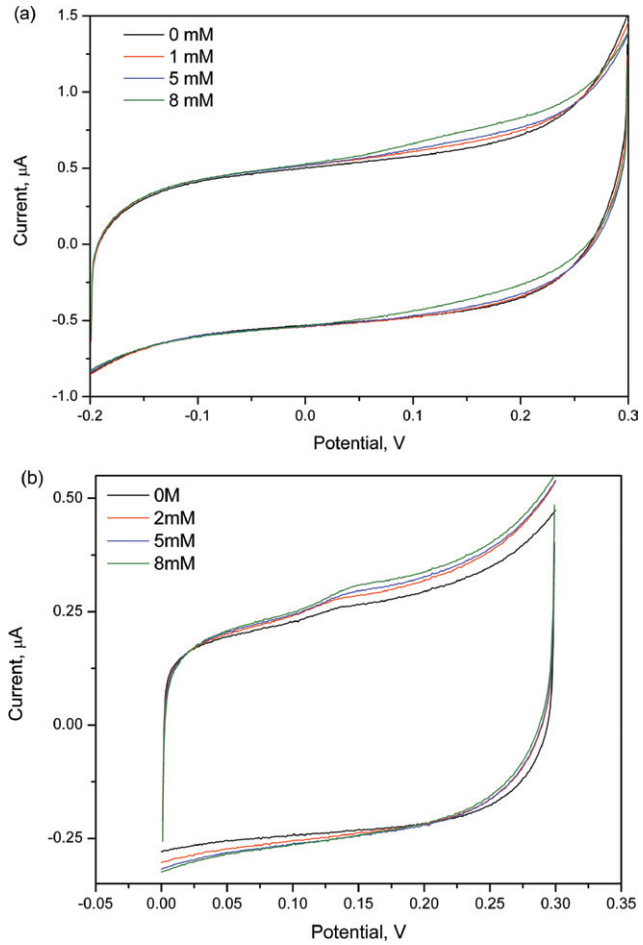

Fig. 3 (a) Cyclic voltammograms of the biosensor with different amounts of glycerol in bovine serum containing NAD^+ (0.01 M) and GDH (8 U). (b) Cyclic voltammograms of the biosensor with different amounts of glyceryl tributyrate in bovine serum with the presence of lipase (10 mg), NAD^+ (0.01 M) and GDH (8 U). The scan rates of (a) and (b) were controlled at 10 mV s^{-1}.

by this biosensor. Fig. 4 shows the amperometric responses of this biosensor to different glyceryl tributyrate concentrations. Each data point shown in Fig. 4 was determined with a new, single use, disposable biosensor. The inset in Fig. 4 is the corresponding calibration curve made by plotting the current measured at the 10th s of the current response curve after the incubation of the reactants for 2.5 min. A linear relationship was observed for the response to a glyceryl tributyrate concentration ranging from 0 to 10 mM with a sensitivity of 7.5 nA mM^{-1}. The experimental results supported the idea that the detection of TG in bovine serum can be achieved by measuring the produced NADH and also provides information for optimizing the amperometric analysis of TG levels by revealing the oxidation peak at an applied potential of +0.15 V *versus* Ag/AgCl.

4.2 Effects of AA and UA on TG sensing

The potential interference of AA and UA on this biosensor for the detection of the TG concentration in bovine serum was also assessed experimentally. AA and UA were chosen as the potential interfering species because they could be easily oxidized in the

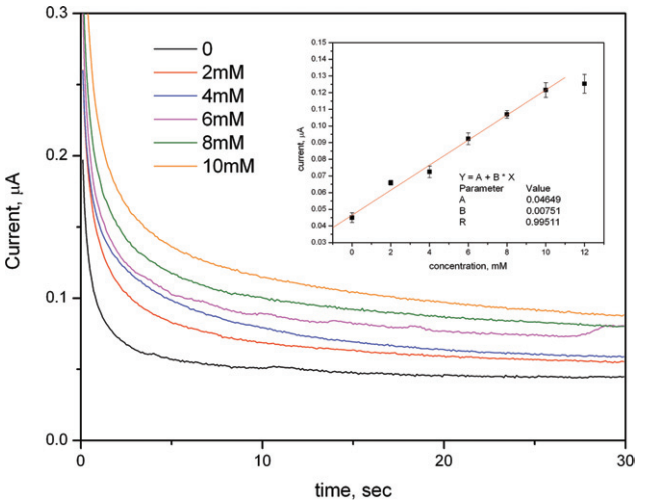

Fig. 4 The steady state current response curves of the biosensor for different concentrations of glyceryl tributyrate. The applied potential was 0.15 V *versus* Ag/AgCl. The calibration curve was plotted using the current measured at fixed time interments (10th s) *versus* glyceryl tributyrate concentrations. The serum volume was 1 mL containing lipase (10 mg), NAD^+ (0.01 M) and GDH (8 U).

NADH detection process. The interference tests were carried out using the same analytical procedures described above. The distinction was that the interference test had a constant concentration of AA and UA added into the bovine serum. The concentrations of AA and UA in the bovine serum were 86 and 416 μmol L^{-1}, respectively, based on the normal concentration ranges of these components in blood. Fig. 5 shows the results obtained in bovine serum with and without the interfering species. The linear region of the calibration curve from 0 to 10 mM glyceryl tributyrate in bovine serum was the same as that with the serum containing the interfering species. The sensitivities of the glyceryl tributyrate calibration curves in bovine serum in the presence of UA and AA were 7.5, 9.5 and 7.4 nA mM^{-1}, respectively. The experimental results showed that UA and AA did indeed increase the background current in the measurement of the glyceryl tributyrate concentration. However, when we eliminated the background current for the interference effect on the TG biosensor as shown in the insert of Fig. 5, the detecting current difference showed only a minute increase in the test serum containing UA and almost no change in the serum with AA. This indicates that the presence of UA in the concentration range given above has a minute interference effect and AA does not contribute any interference with respect to the detection of TG using this method.

Finally, this TG biosensor was tested in human serum using the operation procedures and conditions obtained from the results using bovine serum. The sensitivity of the TG biosensor when detecting glyceryl tributyrate was 7.0 nA mM^{-1}. The interference test was also carried out using UA and AA together in human serum to evaluate its performance. From the results shown in Fig. 5, the sensitivity of the TG biosensor in human serum containing UA and AA was found to be 9.2 nA mM^{-1}, which is almost the same as when using bovine serum. Thus, this TG biosensor can be used to detect TG in human serum effectively.

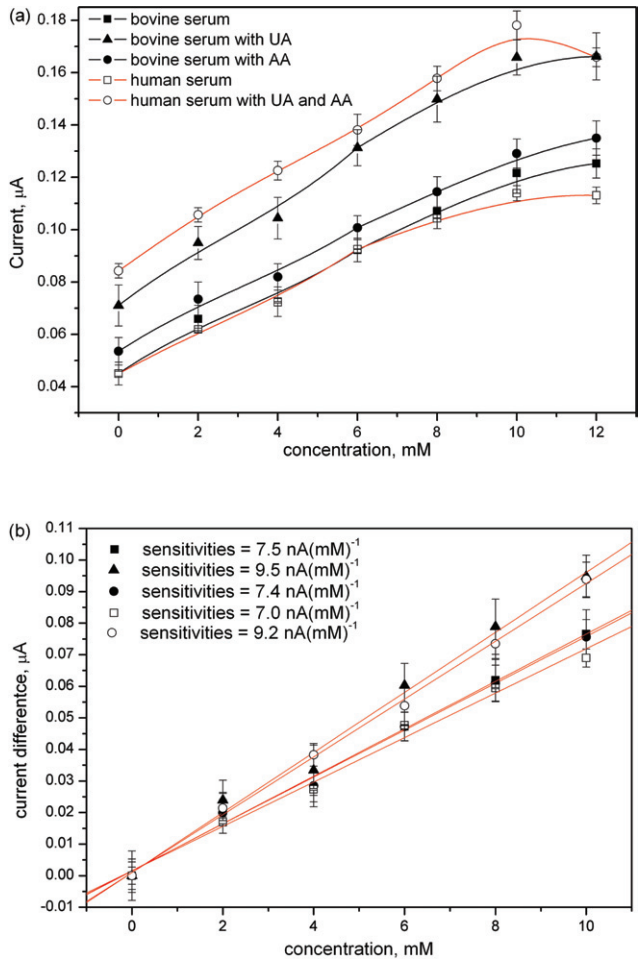

Fig. 5 (a) Effect of UA and AA on glyceryl tributyrate calibration curves. Current *versus* glyceryl tributyrate concentration curves were obtained for bovine serum (■), containing 416 μmol L^{-1} UA (▲) and 86 μmol L^{-1} AA (●) and for human serum (□), containing 416 μmol L^{-1} UA and 86 μmol L^{-1} AA (○). (b) The calibration curves of background current elimination. The serum (1 mL), additionally contained lipase (10 mg), NAD^+ (0.01 M) and glycerol dehydrogenase (8 U). The applied potential was 0.15 V *versus* Ag/AgCl.

4.3 The influences of surfactant and incubation temperature

TG is the simplest lipid constructed from the three fatty acids, which are linked to a single glycerol by an ester bond. The polar hydroxyls of glycerol and the carboxylates of the fatty acids are bonded by ester linkages, and TG is a nonpolar, hydrophobic molecule, essentially insoluble in water. Furthermore, TG has a lower specific gravity than water, suggesting that the enzymatic reaction for detecting the TG may take place at the interface between TG and the aqueous solution. Thus, lipase hydrolyzed TG by a direct interaction with an individual TG molecule at the interface and the products will then dissolve into the aqueous solution where they undergo subsequent reactions.[38,39] The mechanism in which lipase reacts with TG can be regarded as a biomolecular reaction of enzyme and the relevant surface ligand, which is a heterogeneous process and a time-consuming reaction. Therefore, in order to ameliorate the drawbacks of this enzyme reaction, two simple methods were proposed: firstly, by

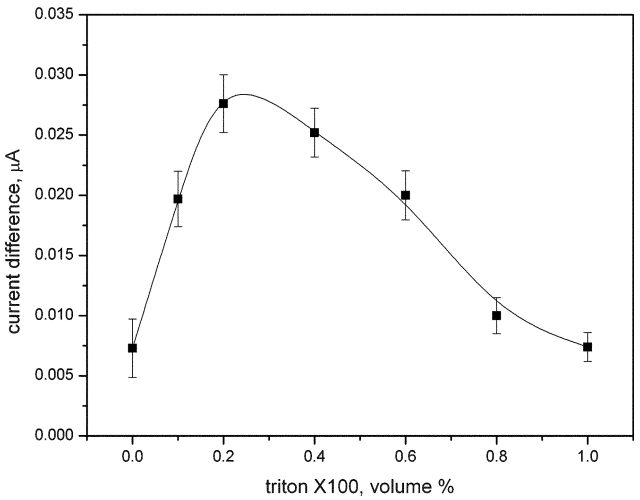

Fig. 6 Effect of the surfactant Triton X-100 quantity on the biosensor in the evaluation of 2 mM glyceryl tributyrate bovine serum. The samples for each data point contained lipase (10 mg), NAD^+ (0.01 M) and GDH (8 U). The applied potential was 0.15 V *versus* Ag/AgCl.

using a surfactant to improve the solubility in order to produce more interfaces and secondly by increasing the temperature of detection in order to promote the enzymatic conversion of the substrate. The influence of the surfactant in the measurement of the TG concentration is shown in Fig. 6. The evaluation was undertaken in bovine serum containing a constant glyceryl tributyrate concentration (2 mM), which also contained lipase (10 mg), GDH (8 U) and NAD^+ (0.01 M). Fig. 6 shows that the current difference increased when the surfactant was added into the testing medium and an optimal condition was found at 0.25% V. This result can be explained by the physical condition of the glyceryl tributyrate in the solution. In general, glyceryl tributyrate and surfactant together in a solution would form a stable state, a micelle. When the amount of surfactant in the solution increased, more glyceryl tributyrate dissolved, allowing more micelles to be produced. More micelles in the solution increased the interface surface area, which provided more possible reaction surfaces for the lipase to hydrolyze glyceryl tributyrate. Consequently, the current difference from the enzyme reaction increased by adding the surfactant into the solution. However, when more surfactant was added into the solution, the structure of the micelle became larger and more complicated structurally. This would decrease the interface area, leading to the possibility of the reduction of the glyceryl tributyrate hydrolysis by lipase, consequently the current difference decreased.

An increase in the operating temperature of this sensing system would result in an increase in the kinetic energy of the molecules in the system, which would promote the enzyme colliding with the substrate. Thus, an increase in the temperature of the system would enhance the reaction rate of the substrate being converting into product. The temperature effect on the biosensor detecting TG in the bovine serum can be observed in Fig. 7. The samples used were prepared in the same manner as those used described in Fig. 6. In Fig. 7, the current difference became larger and larger with an increasing rate of 3.93 nA per unit temperature in the range from 37 °C to 57 °C. This experimental result suggested that an increase in the operating temperature increased the rate

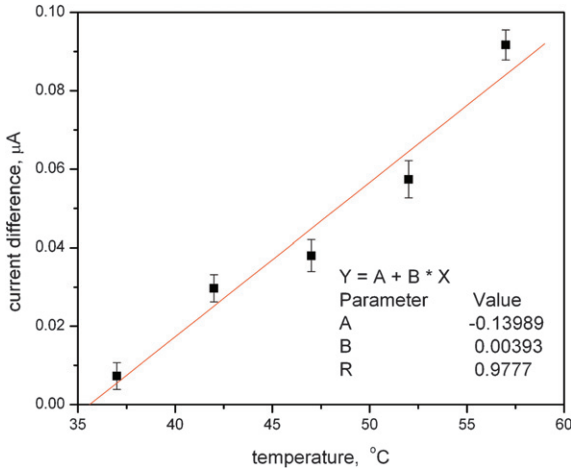

Fig. 7 Effect of the incubation temperature on the biosensor in the evaluation of 2 mM glyceryl tributyrate bovine serum. The samples for each data point contained lipase (10 mg), NAD^+ (0.01 M) and GDH (8 U). The applied potential was 0.15 V *versus* Ag/AgCl.

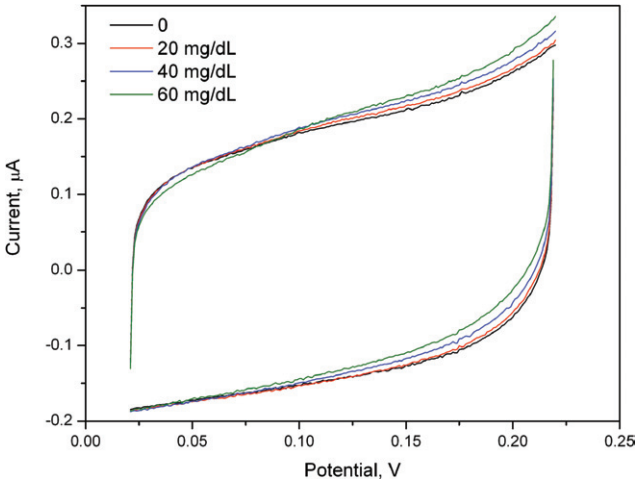

Fig. 8 Cyclic voltammograms of the biosensor with different amounts of sunflower oil in the presence of lipase (10 mg), NAD^+ (0.01 M) and GDH (8 U) bovine serum. The scan rate was controlled at 10 mV s^{-1}.

of enzyme collision with substrate, which improved the enzyme binding to the substrate at the interface. This was reflected in the increment of the current difference.

The experimental results shown in Fig. 6 and Fig. 7 suggest that the addition of an appropriate quantity of a surfactant and the increase in the operating temperature of the system enhanced the ability of this biosensor to measure the TG in the bovine serum.

4.4 Estimation of triglycerides

The most important function of a TG biosensor is to determine the total TG content in human blood. The samples used for this TG biosensor evaluation, *i.e.* to imitate and to measure the TG level in blood, were different volumes of sunflower seed oil in bovine serum. The samples were prepared as follows: In a final volume of a 1 mL bovine serum, various quantities of sunflower seed oil were added, resulting in a range of concentrations from 0 to 140 mg dL^{-1} representing the different TG levels in serum.[12] Then lipase (10 mg), NAD^+ (0.01 M), surfactant (0.25% V) and GDH (8 U) were added into the test sample and shaken in order to obtain a homogeneous mixture. The temperature of the system was controlled at 52 °C in order to improve the sensitivity of the TG biosensor. After an incubation time of 2.5 min, the biosensor was then placed in the bovine serum samples in order to evaluate its sensing ability.

Fig. 8 shows the cyclic voltammograms of the TG biosensor presented in the bovine serum with various TG concentrations. An anodic peak was observed at +0.15 V *versus* Ag/AgCl, which increased as a function of the TG concentration. This observation was identical to those shown in Fig. 3. These results confirmed the ability of this biosensor for the detection of TG within this concentration range. These results also indicated that at an applied potential, *i.e.* +0.15 V *versus* the printed Ag/AgCl, amperometric measurements of TG in bovine serum and human serum could be accomplished as shown in Fig. 9. Each curve in Fig. 9 was determined with a new, single use, biosensor and the TG concentration range chosen was based on the information available from the American Heart Association. The insert in Fig. 9 is the corresponding calibration curve derived by plotting the current measured at the 10th s of the current response curve after an incubation time of the reactions in bovine serum and human serum for 2.5 min. A linear relationship was observed for the TG concentrations ranging from 0 to 150 mg dL^{-1} with a sensitivity of 0.191 nA dL mg^{-1} (equal to 16.9 nA mM^{-1}) in bovine serum and 0.188 nA dL mg^{-1} (equal to 16.63 nA mM^{-1}) in human serum. The experimental results shown in Fig. 8 and Fig. 9 indicate that this TG biosensor can be used for the analysis of TG in bovine serum within the physiological concentration range.

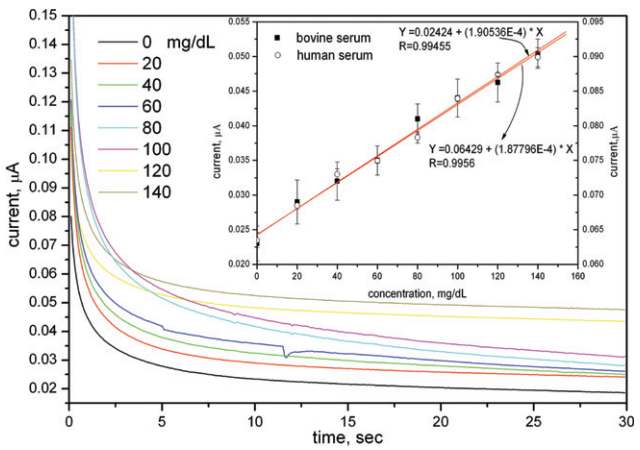

Fig. 9 The steady state current response curves of the biosensor for different concentrations of sunflower seed oil. The applied potential was 0.15 V *versus* Ag/AgCl. The calibration curve was plotted using the current measured at fixed time (10th s) interments *versus* different sunflower seed oil concentrations in bovine serum and in human serum. The samples for each curve were 1 mL of bovine serum, which additionally contained lipase (10 mg), NAD^+ (0.01 M) and GDH (8 U).

5. Conclusions

The amperometric detection of TG, using our iridium nanoparticle modified working electrode novel biosensor, was successfully achieved in this study. The detection principle was based on the electrocatalytic measurement of enzymatically produced NADH from TG decomposition. The experimental results showed that glyceryl tributyrate can be amperometrically measured with a linear region from 0 to 10 mM and with a sensitivity of 7.5 nA mM^{-1} in bovine serum. UA and AA were shown to have only a minimal interference effect on the evaluation of glyceryl tributyrate concentrations. In order to improve the efficacy of the TG biosensor's sensing performance, 0.25% Triton X-100 (v/v) surfactant was added into the bovine serum and the incubation temperature was increased to 52 °C. Both of these changes enhanced the performance of the TG biosensor for the measurement of the concentration of sunflower oil in serum as was shown by the increase of the sensitivity and also demonstrated the feasibility of using the sensor for the direct detection of blood TG levels. A final evaluation of the sensor was undertaken using TG in human serum as a natural physiological matrix. The results of this study were in good agreement with those already found for the measurement of TG in bovine serum, thereby supporting the potential of the sensor developed to be used in a laboratory or clinical setting.

In conclusion, a biosensor for the detection of the heart disease biomarker, TG, has been developed. This biosensor offers the promise of determining TG levels within the physiological concentration range of human serum with accuracy and sensitivity.

Acknowledgements

One of the authors, Wei-Yin Liao, is very grateful for the financial support from the research grants of National Science Council (project number: 095JFA09009 and 95–2221-E-036–052-MY3) and the National Cheng Kung University, Taiwan. Partial support by the NSF Grant No.0609128, a NIRT initiative grant and by the Electronics Design Center, Case Western Reserve University are also gratefully acknowledged.

References

1 B. G. Nordestgaard, M. Benn, P. Schnohr and A. Tybjaerg-Hansen, *J. Am. Med. Assoc.*, 2007, **298**, 299.
2 J. S. Cohn, C. Marcoux and J. Davignon, *Arterioscler., Thromb., Vasc. Biol.*, 1999, **19**, 2474.
3 P. N. Durrington, *Atherosclerosis*, 1998, **141**, S57.
4 M. C. Cabezas and D. W. Erkelens, *Neth. J. Med.*, 2000, **56**, 110.
5 P. Barter, *Atheroscler. Suppl.*, 2005, **6**, 15.
6 H. N. Ginsberg, *Circulation*, 2002, **106**, 2137.
7 H. Iso, Y. Naito, S. Sato, A. Kitamura, T. Okamura, T. Sankai, T. Shimamoto, M. Iida and Y. Komachi, *Am. J. Epidemiol.*, 2001, **153**, 490.
8 N. Sarwar, J. Danesh, G. Eiriksdottir, G. Sigurdsson, N. Wareham, S. Bingham, S. M. Boekholdt, K. T. Khaw and V. Gudnason, *Circulation*, 2007, **115**, 450.
9 M. Egger, G. D. Smith, D. Pfluger, E. Altpeter and P. C. Elwood, *Atherosclerosis*, 1999, **143**, 275.
10 J. Jeppesen, H. O. Hein, P. Suadicani and F. Gyntelberg, *Circulation*, 1998, **97**, 1029.
11 P. E. McBride, *J. Am. Med. Assoc.*, 2007, **298**, 336.
12 http://www.americanheart.org/presenter.jhtml?identifier=183.
13 Z. H. Liu, M. L. Wen, Y. Yao, N. H. Shi, S. Q. Liu and M. Qiao, *Collect. Czech. Chem. Commun.*, 1999, **64**, 1412.
14 A. Afkhami and F. Mosaed, *Microchem. J.*, 2001, **68**, 35.
15 L. A. Kaplan, in *Clinical chemistry: Theory, analysis, and correlation*, ed. A. J. Pesce and S. C. Kazmierczak, Mosby, St. Louis., 4th edn, 2003.
16 I. Lapenaite, A. Ramanaviciene and A. Ramanavicius, *Crit. Rev. Anal. Chem.*, 2006, **36**, 13.
17 A. Curulli, F. Valentini, S. Orlanduci, M. L. Terranova and G. Palleschi, *Biosens. Bioelectron.*, 2004, **20**, 1223.
18 D. Compagnone, M. Esti, M. C. Messia, E. Peluso and G. Palleschi, *Biosens. Bioelectron.*, 1998, **13**, 875.
19 F. Mizutani, Y. Hirata, S. Yabuki and S. Iijima, *Electrochemistry*, 2003, **71**, 414.
20 V. Laurinavicius, B. Kurtinaitiene, V. Gureviciene, L. Boguslavsky, L. Geng and T. Skotheim, *Anal. Chim. Acta*, 1996, **330**, 159.
21 J. Moiroux and P. J. Elving, *Anal. Chem.*, 1978, **50**, 1056.
22 H. Jaegfeldt, *J. Electroanal. Chem.*, 1980, **110**, 295.
23 S. A. Wring and J. P. Hart, *Analyst*, 1992, **117**, 1215.
24 M. Hedenmo, A. Narvaez, E. Dominguez and I. Katakis, *Analyst*, 1996, **121**, 1891.
25 B. W. Carlson and L. L. Miller, *J. Am. Chem. Soc.*, 1985, **107**, 479.
26 A. Kitani, Y. H. So and L. L. Miller, *J. Am. Chem. Soc.*, 1981, **103**, 7636.
27 K. Essaadi, B. Keita, L. Nadjo and R. Contant, *J. Electroanal. Chem.*, 1994, **367**, 275.
28 F. Q. Xu, H. W. Li, S. J. Cross and T. F Guarr, *J. Electroanal. Chem.*, 1994, **368**, 221.
29 M. Ishiyama, K. Sasamoto, M. Shiga, Y. Ohkura, K. Ueno, K. Nishiyama and I. Taniguchi, *Analyst*, 1995, **120**, 113.
30 C. E. Banks and R. G. Compton, *Analyst*, 2005, **130**, 1232.
31 J. Wang, G. Rivas and M. Chicharro, *J. Electroanal. Chem.*, 1997, **439**, 55.
32 M. C. Rodriguez and G. A. Rivas, *Electroanalysis*, 1999, **11**, 558.
33 J. Shen, L. Dudik and C. C. Liu, *Sens. Actuators, B*, 2007, **125**, 106.
34 Y. C. Luo, J. S. Do and C. C. Liu, *Biosens. Bioelectron.*, 2006, **22**, 482.
35 L. C. Wu and C. M. Cheng, *Anal. Biochem.*, 2005, **346**, 234.
36 R. R. K. Reddy, I. Basu, E. Bhattacharya and A. Chadha, *Curr. Appl. Phys.*, 2003, **3**, 155.
37 R. R. K. Reddy, A. Chadha and E. Bhattacharya, *Biosens. Bioelectron.*, 2001, **16**, 313.
38 O. G. Berg, M. H. Gelb, M. D. Tsai and M. K. Jain, *Chem. Rev.*, 2001, **101**, 2613.
39 R. A. Deems, *Anal. Biochem.*, 2000, **287**, 1.