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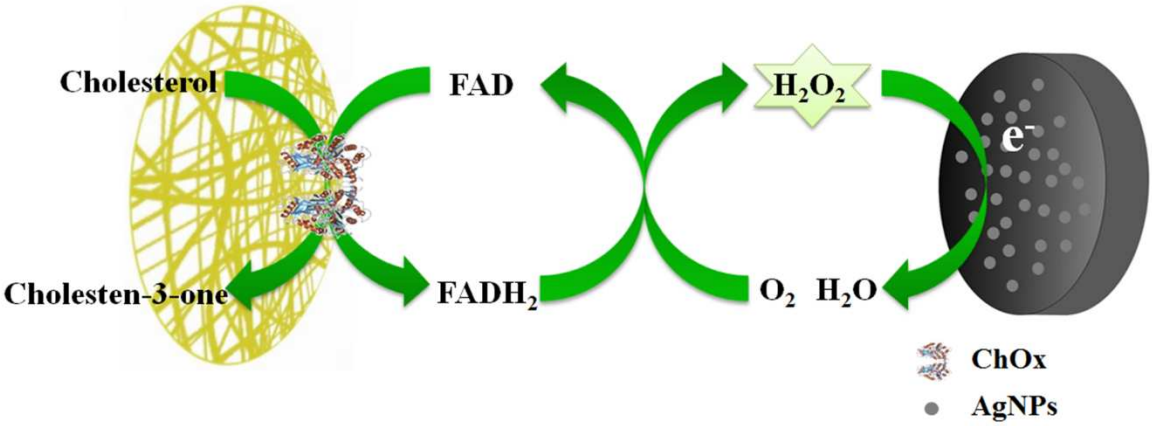

A Novel Paper-Based Device Coupled with a Silver Nanoparticle-Modified Boron-Doped Diamond Electrode for Cholesterol Detection

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Abstract

A novel paper-based analytical device (PAD) coupled with a silver nanoparticle-modified boron-doped diamond (AgNP/BDD) electrode was first developed as a cholesterol sensor. The AgNP/BDD electrode was used as working electrode after modification by AgNPs using an electrodeposition method. Wax printing was used to define the hydrophilic and hydrophobic areas on filter paper, and then counter and reference electrodes were fabricated on the hydrophilic area by screen-printing in house. For the amperometric detection, cholesterol and cholesterol oxidase (ChOx) were directly drop-cast onto the hydrophilic area, and H_2O_2 produced from the enzymatic reaction was monitored. The fabricated device demonstrated a good linearity (0.39 mg dL^{-1} to 270.69 mg dL^{-1}), low detection limit (0.25 mg dL^{-1}), and high sensitivity (49.61 µA mM^{-1} cm^{-2}). The precision value for ten replicates was 3.76 % RSD for 1 mM H_2O_2. In addition, this biosensor exhibited very high selectivity for cholesterol detection and excellent recoveries for bovine serum analysis (in the range of 99.6-100.8 %). The results showed that this new sensing platform will be an alternative tool for cholesterol detection in routine diagnosis and offers the advantages of low sample/reagent consumption, low cost, portability, and short analysis time.

1. Introduction

Cholesterol is an essential component of the mammalian cell membrane and is a precursor of bile acid and steroid hormones. However, abnormal cholesterol levels are related to several diseases, such as hypertension, coronary heart disease, arteriosclerosis, brain thrombosis, lipid metabolism dysfunction and myocardial infarction [1]. Thus, the monitoring of the cholesterol level is very important for disease control and prevention. Various analytical strategies for cholesterol assays have been developed that can be sub-classified into two groups, including a chemical and an enzymatic method [2-5]. Currently, the enzymatic method has received greater attention for cholesterol analysis because it provides greater sensitivity and selectivity than the chemical method. The well-known principle of an enzyme-based cholesterol sensor is typically based on the reaction between cholesterol and the cholesterol oxidase (ChOx) enzyme. Free cholesterol is oxidized by cholesterol oxidase to produce 4-cholestene-3-one and hydrogen peroxide (H_2O_2), and the H_2O_2 generated can be used for indirect quantification of cholesterol [6-8]. The electrochemical method, especially the electrochemical biosensor, is extremely attractive for the determination of cholesterol because the electrochemical biosensors provide high sensitivity, require inexpensive instrumentation, and are suitable for real-time analysis. Previously, several methods have been used to immobilize the enzyme onto an electrode, such as entrapment in a polymer [9], covalent linkage with glutaraldehyde [10] or a polymer [11], and electrostatic adsorption [12]. Although these sensing platforms provide good performance for cholesterol detection, limitations still remain in terms of time consumption, detection performance and the ease with which the enzyme is denatured during its immobilization. To overcome this drawback, it is necessary to design and fabricate a new cholesterol sensor that offers simple fabrication, an easy immobilization step, and a short analysis time.

Recently, filter paper has received interest as a potential material for sensor applications due to its large surface area and low cost. In 2009, electrochemical detection on a paper-based analytical device (PAD) was first developed by Dungchai and coworkers [13]. This platform became a new alternative analytical device and was applied in many application areas [14-19]. Their simplicity of fabrication, portability and disposability coupled with the small volume of reagent/sample solution required for PADs make them suitable and very appealing for the development of new clinical diagnostic tools. Moreover, the biocompatibility of filter paper makes it suitable for immobilization of enzymes or other bioreceptors [20-22]. However, the traditional paper-based electrochemical devices with a three-electrode system (consisting of a working electrode (WE), a counter electrode (CE), and a reference electrode (RE)) still have some limitations, including low sensitivity because of a small electrode area and the use of carbon ink as a working electrode [13, 23].

To solve these problems, the use of a commercial electrode coupled with PAD was considered. Boron-doped diamond (BDD) thin films are novel carbon-based materials that have very attractive properties compared to other conventional electrodes in terms of (i) wide electrochemical potential window; (ii) low and stable background current; (iii) resistance to electrode fouling; (iv) high response reproducibility and long-term response stability; and (v) biocompatibility. As advantages, we can conclude that the BDD electrodes provide the potential for electroanalysis application with high sensitivity [24-26]. To eliminate possible interference from easily oxidizable species in biological samples, cholesterol detection and measurement via the monitoring of H_2O_2 reduction is proposed in this work. Unfortunately, the reduction of H_2O_2 cannot be observed on a bare BDD electrode. Therefore, silver nanoparticles (AgNP) have been considered as the modifier for modification of the BDD surface because they have a large specific surface area, excellent conductivity and

extraordinary electrocatalytic activity. Moreover, AgNP also show an excellent electrocatalytic activity for H_2O_2 reduction [27-30].

On this basis, we have developed a novel cholesterol biosensor based on a silver nanoparticle-modified boron-doped diamond (AgNP/BDD) electrode with PAD. An electrodeposition method was used to deposit AgNP onto the BDD electrode surface. The resulting device possessed the advantages of simple fabrication, small sample volume/reagent requirements, low cost and short analysis time. In addition, the AgNP/BDD electrode exhibited a highly sensitive and selective response to detect cholesterol without disturbance from interfering compounds such as ascorbic acid, uric acid, and glucose. To the best of our knowledge, this the first report of the coupling of an AgNP/BDD electrode with PAD to fabricate a cholesterol biosensor. The results show that this device displayed excellent performance, a wide linear range, and a low detection limit for cholesterol detection.

2. Experimental

2.1 Chemicals and materials

Cholesterol and cholesterol oxidase (ChOx) from *Streptomyces* sp. (25 U/mg) and lipid cholesterol enriched from adult bovine serum were purchased from Sigma (St. Louis, MO). Potassium dihydrogen phosphate (KH_2PO_4) was purchased from Carlo Erba Reagenti-SpA (Val de Reuil, France). Hydrogen peroxide (H_2O_2), disodium hydrogen phosphate (Na_2HPO_4), potassium chloride (KCl) and boric acid (H_3BO_3) were purchased from Merck (Darmstadt, Germany). Silver nitrate ($AgNO_3$) was purchased from POCH S.A. (Poland). Glacial acetic acid (CH_3COOH) was purchased from Fisher Scientific (Pittsburgh, PA). Phosphoric acid (H_3PO_4, 85%) was purchased from Carlo Erba (Rodano, MI, USA). Carbon ink and silver/silver chloride (Ag/AgCl) ink were obtained from Gwent Group (Torfaen, United Kingdom). Filter paper grade no. 1 (size, 46 x 57 cm^2) was purchased from Whatman.

A stock solution of cholesterol was prepared daily by dissolving cholesterol in a mixture of Triton X-100 and isopropanol. This stock solution was further diluted to make different concentrations of cholesterol in 0.05 M PBS pH 7.4. The solution was stirred with a magnetic bar at 60 °C to obtain a homogeneous solution. Stock solution of ChOx was freshly prepared by dissolving in 0.05 M phosphate buffer (pH 7.0). All chemicals were used without further purification.

2.2 Sample preparation

The labeled concentration of cholesterol in adult bovine serum was used to represent the serum sample. The serum samples were prepared by dissolving adult bovine serum powder in Triton X-100 and isopropanol, and diluting with 0.05 M PBS pH 7.4. The solution was stirred with a magnetic bar at 60 °C.

2.3 Preparation of AgNP/BDD electrode

A simple one-step electrodeposition method was used for the electrode modification. A three-electrode system (Ag/AgCl (3 M KCl) electrode as the reference electrode (RE), a platinum wire as the counter electrode (CE) and a BDD electrode as the working electrode (WE)) was used in this work. Electrodeposition of AgNPs onto the BDD electrode surface was accomplished by dipping the electrode in a solution of $AgNO_3$ in Britton-Robinson buffer (pH 2) and applying a potential with stirring. The electrode was then rinsed with water to remove any physically adsorbed substances on the electrode.

The electrodeposition parameters for the modification of the electrode including deposition potentials, deposition times, and concentrations of $AgNO_3$ could affect the performance of the electrochemical response for the H_2O_2 reduction, which should be

optimized to increase the sensitivity. The ranges of each parameter optimized are shown in Table 1.

2.4 Fabrication of cholesterol sensor and analytical procedure

In this work, wax printing and filter paper (Whatman No. 1) were selected for the construction of the devices according to a previously reported method [31]. The patterned PAD was designed by the Adobe Illustrator, and then the patterns were printed onto paper using a wax printer (Xerox Color Qube 8570, Japan). The printed PAD was heated on the hot plate at 175 °C for 50 s to melt the printed wax so that it penetrated through the paper to form the hydrophobic and hydrophilic patterns. Screen-printing in-house was used to fabricate CEs and REs. Carbon ink was used for the CE. Ag/AgCl ink was used for the RE and conductive pads. The screen block was designed using Adobe Illustrator software and constructed by Chaiyaboon Co. (Bangkok, Thailand). The CE and RE were screened onto the hydrophilic area on the top of the PAD. The double-sided adhesive tape was punched to create a 1.0 cm diameter hole. This double-sided adhesive tape was used to attach the bottom of the PAD and the AgNP/BDD electrode to complete the device. For the determination of cholesterol, an optimal volume of ChOx was drop-cast and dried onto the hydrophilic zone of the PAD. Then, 10 µL of standard/sample cholesterol solution was dropped, and electrochemical measurements were performed with a model PGSTAT 101 Autolab Electrochemical System controlled with the NOVA software package (Kanaalweg 29-G 3526 KM Utrecht, The Netherlands) at room temperature (22 ± 1 °C). The overall fabrication and analytical procedure for the determination of cholesterol are shown in Scheme 1.

3. Results and Discussion

3.1 Device design

The coupling of the AgNP/BDD electrode with PAD for the fabrication of a cholesterol biosensor was designed to improve the electrochemical performance of the PAD using the BDD electrode as the WE instead of the traditional carbon screen-printed electrode. A low background current at the BDD electrode can enhance the sensitivity for the cholesterol detection. Moreover, the device that we have designed can reduce the consumption of standard/reagent solution and reduce the time required for the enzyme immobilization step by direct drop-casting of ChOx onto the hydrophilic zone of the PAD. The enzyme is immobilized on the filter paper by the adsorption process. After addition of the cholesterol solution, ChOx catalyzes the oxidation of cholesterol to generate H_2O_2 and cholest-4-en-3-one. The electrochemical reduction of H_2O_2 occurs at the modified electrode after a suitable potential is applied. The cathodic currents of H_2O_2 are recorded and used to determine the concentration of the cholesterol in the samples. The mechanism of cholesterol detection is shown in Scheme 2.

3.2 Surface morphology of AgNP/BDD electrode

Scanning electron microscope (SEM) was used to characterize the surface morphology of bare BDD electrode and AgNP/BDD electrode as shown in Fig. 1A and Fig. 1B, respectively. After electrodeposition of AgNPs, a number of nanoparticles were clearly seen. The size of AgNPs was in the nanoscale. Some bigger particles may be possibly caused by the aggregation. The distribution of AgNPs on the electrode surface led to increase the surface area and improve the electrochemical sensitivity of the modified electrode.

3.3 Electrochemical behavior of standard H_2O_2 and cholesterol on the AgNP/BDD electrode coupled with PAD

Cyclic voltammetry was initially employed to investigate the electrochemical behavior of H_2O_2 on the AgNP/BDD electrode coupled with PAD. The cyclic voltammograms (CVs) in the absence and presence of 1 mM H_2O_2, recorded at the bare BDD electrode and the AgNP/BDD electrode, corresponding to the background at the scan rate of 100 mV s^{-1} are shown in Fig. 2A. The cathodic peak of H_2O_2 did not appear at the bare BDD electrode, supporting the observation that this electrode has no electrochemical activity toward H_2O_2. For the AgNP/BDD electrode, a well-defined cathodic peak of H_2O_2 can be observed at a potential of approximately -0.61 V vs. Ag/AgCl, suggesting that the use of the BDD electrode modified with AgNPs can promote the electron transfer in the H_2O_2 reduction.

Subsequently, the electrochemical behavior of the H_2O_2 produced from the reaction between cholesterol and ChOx was investigated on an AgNP/BDD electrode coupled with the PAD. The results are shown in Fig. 2B. The cathodic peak of the H_2O_2 that was produced appeared at a potential of approximately -0.71 V. The reduction peak potential of the H_2O_2 that was produced shifted to a more negative value compared to the reduction peak potential of standard H_2O_2 because of the effect of the Triton X-100 that was used as the surfactant for dissolving the cholesterol. The results indicated that the proposed device can be used as a new biosensor for cholesterol detection. Then, the effect of the enzyme volume on the cathodic current of 38.7 mg/dL of cholesterol was investigated in the range of 0.2-1.0 µL. The optimum volume of enzyme was 0.8 µL, and this volume was used for all experiments.

In addition, the effect of dissolved oxygen in the solution was investigated by monitoring the reduction current of H_2O_2 in this system, because the effect of O_2 during the electrochemical measurements is an area of concern. From the results, the net reduction currents (after the subtraction of the background) from before and after the N_2 purge were not significantly

different, which agrees with the previous literature [32]. Thus, the cholesterol detection can be carried out in an atmospheric environment.

3.4 Effect of the scan rate

The effect of the scan rate is the most important experimental parameter for evaluating the mass transfer of the reactant toward the electrode via diffusion or adsorption control. Fig. 3 shows cyclic voltammograms of 1 mM H_2O_2 in the potential range of 0 V to -1 V vs. Ag/AgCl with different scan rates at the AgNP/BDD electrode coupled with PAD. At the scan rate from 10 to 100 mV s^{-1}, the peak current (i_p) of H_2O_2 was proportional to the square root of the scan rate ($v^{1/2}$) (inset, Fig. 2; linear regression equation: $y = -23.158x + 27.895$ with $R^2 = 0.9946$). The results suggested that the mass transfer in this system was a diffusion-controlled process.

3.5 Selection of applied potential

Due to its high sensitivity and wide applicability, chronoamperometry was selected for the electrochemical determination of cholesterol. The sensitivity and selectivity of electrochemical detection depended on the selection of the applied potential. To achieve the lowest detection limit and highest selectivity, the dependence of the current response of the fabricated biosensor on H_2O_2 reduction at applied potentials ranging from -0.5 to -1 V vs. Ag/AgCl was examined in 0.05 M PBS (pH 7.4) containing 1 mM H_2O_2. Fig. 4A shows the *i-E* curve of the reduction current of 1 mM H_2O_2 and the background current at each potential. The reduction current of H_2O_2 and the background current were significantly increased by increasing the detection potentials. On the basis of sensitivity and selectivity, a hydrodynamic voltammogram of signal-to-background (S/B) ratios was considered instead of using only reduction currents. The results are shown in Fig. 4B. The signal ratio reached a

maximum at the detection potential of -0.7 V vs. Ag/AgCl. Therefore, an applied potential of -0.7 V vs. Ag/AgCl was selected for the chronoamperometric measurement in the following experiments.

3.6 Analytical performance

The relationship between the cathodic current and the concentration of cholesterol was examined at the optimal detection potential of -0.7 V vs. Ag/AgCl and recorded at the apparent steady state current of 20 s. Fig. 5 shows the chronoamperograms of cholesterol calibration using the fabricated biosensor. The cathodic current increased with increasing cholesterol concentration due to the reduction of H_2O_2 generated by the enzymatic reaction. The linearity of the calibration curve is shown in the inset. The cathodic currents exhibited a linear correlation with the cholesterol concentration across the range from 0.39 to 270.69 mg dL^{-1} (correlation coefficient of $R^2 = 0.9967$) with a sensitivity of 49.61 µA mM^{-1} cm^{-2}, which was calculated by dividing the slope of the calibration curve by the electrode surface area. The limit of detection (LOD) was estimated as the concentration that produced three times the standard deviation of the blank sample and was found to be 0.25 mg dL^{-1}. Previously, the national cholesterol education program (NCEP) has reported that the normal level of total blood cholesterol in a human is lower than 5.18 mM (200 mg dL^{-1}) [33]. This information shows that the fabricated biosensor covers a wide range of cholesterol concentrations and can be applied for cholesterol detection in the clinical diagnostics. The apparent Michaelis–Menten constant (K_M) that gives an indication of the enzyme-substrate kinetics can be estimated based on the Lineweaver–Burk equation: $1/I_{ss} = (K_M/I_{max})(1/C) + 1/I_{max}$, where I_{ss} is the steady-state current, I_{max} is the maximum current measured under the saturated substrate conditions, and C is the cholesterol concentration. The K_M value of the system in this work was found to be 6.48 mM or 250.43 mg dL^{-1}. The performance of the proposed cholesterol

biosensor compared to the previously reported value is shown in Table 2. The results confirm that the proposed novel cholesterol biosensor exhibited excellent sensing performance. Moreover, our proposed biosensor offers the advantages of a simple and fast immobilization process.

The repeatability of the sensor was evaluated. One fabricated sensor was evaluated by repeating the measurement of 1 mM H_2O_2 ten times. The relative standard deviation (RSD) was 3.76%, indicating that the biosensor measurement was highly repeatable. Moreover, the reproducibility of the sensor was also evaluated using chronoamperometric detection of 37.8 mg/dL cholesterol. The RSD of five different sensors under the same conditions was found to be 3.5%, indicating that the fabricated biosensor had acceptable precision.

3.7 Interference study

The selectivity is an important factor for evaluating the performance of sensors. The effects of common interfering molecules that coexist with cholesterol in serum, such as glucose (Glu), ascorbic acid (AA), and uric acid (UA) were investigated. Glu (1 mM), AA (0.2 mM), and UA (0.2 mM) were added into 38.7 mg dL^{-1} cholesterol standard solution separately and then measured by the fabricated biosensor. The results showed the negligible effect of these interferences on the current response of the fabricated cholesterol sensor as shown in Fig. 6. The current ratios between the presence and absence of the interferences were found to be 1.02, 1.01, and 0.99 for glucose, AA, and UA, respectively. Thus, highly selective detection of cholesterol was obtained using the proposed method. All the above results demonstrated that the use of the AgNP-modified BDD electrode facilitates the low potential amperometric detection of cholesterol and enhances the selective ability of the biosensor.

3.8 Sample analysis

To demonstrate the possible clinical applications, the cholesterol sensor was used to determine the cholesterol level in bovine serum. The results are listed in Table 3. The cholesterol concentrations determined by the fabricated sensor are in good agreement with the concentration of the cholesterol labeled in the bovine serum sample. The values of the recoveries obtained were in the range of 99.6-100.8 %. These results clearly indicate that the proposed method can be applied to measure cholesterol in bovine serum.

4. Conclusion

In summary, we have demonstrated a novel design for an electrochemical paper-based analytical device based on an AgNP-modified BDD electrode with PAD for use as a cholesterol biosensor. This novel cholesterol biosensor offers the advantages of simple fabrication, no requirement for an enzyme immobilizing step, low consumption of reagents/sample, low cost, and short analysis time. The most significant characteristics of this cholesterol biosensor are its wide linear range, low limit of detection, high sensitivity, good reproducibility, and freedom from interference from easily oxidizable species such as glucose, ascorbic acid, and uric acid. In addition, this fabricated biosensor was successfully applied to the determination of cholesterol in bovine serum.

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References

[1] Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. The Expert Panel, Arch. Intern. Med. 148 (1988) 36-69.

[2] V. Raj, T. Johnson, K. Joseph, Cholesterol aided etching of tomatine gold nanoparticles: A non-enzymatic blood cholesterol monitor, Biosens. Bioelectron. 60 (2014) 191-194.

[3] J. Zhang, W. Wang, S. Chen, Y. Ruo, X. Zhong, X. Wu, Bi-pseudoenzyme synergetic catalysis to generate a coreactant of peroxydisulfate for an ultrasensitive electrochemiluminescence-based cholesterol biosensor, Biosens. Bioelectron. 57 (2014) 71-76.

[4] M.B. Gholivand, M. Khodadadian, Amperometric cholesterol biosensor based on the direct electrochemistry of cholesterol oxidase and catalase on a graphene/ionic liquid-modified glassy carbon electrode, Biosens. Bioelectron. 53 (2014) 472-478.

[5] R. Ahmad, N. Tripathy, S.H. Kim, A. Umar, A. Al-Hajry, Y.-B. Hahn, High performance cholesterol sensor based on ZnO nanotubes grown on Si/Ag electrodes, Electrochem. Commun. 38 (2014) 4-7.

[6] J. Shen, C.-C. Liu, Development of a screen-printed cholesterol biosensor: Comparing the performance of gold and platinum as the working electrode material and fabrication using a self-assembly approach, Sens. Actuators B 120 (2007) 417-425.

[7] N. Ruecha, W. Siangproh, O. Chailapakul, A fast and highly sensitive detection of cholesterol using polymer microfluidic devices and amperometric system, Talanta, 84 (2011) 1323-1328.

[8] M. Zhang, R. Yuan, Y. Chai, S. Chen, H. Zhong, C. Wang, Y. Cheng, A biosensor for cholesterol based on gold nanoparticles-catalyzed luminol electrogenerated chemiluminescence, Biosens. Bioelectron. 32 (2012) 288-292.

[9] J. Li, T. Peng, Y. Peng, A Cholesterol Biosensor Based on Entrapment of Cholesterol Oxidase in a Silicic Sol-Gel Matrix at a Prussian Blue Modified Electrode, Electroanalysis, 15 (2003) 1031-1037.

[10] S. Singh, P.R. Solanki, M.K. Pandey, B.D. Malhotra, Covalent immobilization of cholesterol esterase and cholesterol oxidase on polyaniline films for application to cholesterol biosensor, Anal. Chim. Acta 568 (2006) 126-132.

[11] S. Soylemez, F.E. Kanik, A.G. Nurioglu, H. Akpinar, L. Toppare, A novel conducting copolymer: Investigation of its matrix properties for cholesterol biosensor applications, Sens. Actuators B 182 (2013) 322-329.

[12] Y.J. Shin, J. Kameoka, Amperometric cholesterol biosensor using layer-by-layer adsorption technique onto electrospun polyaniline nanofibers, J. Ind. End. Chem. 18 (2012) 193-197.

[13] W. Dungchai, O. Chailapakul, C.S. Henry, Electrochemical Detection for Paper-Based Microfluidics, Anal. Chem. 81 (2009) 5821-5826.

[14] M. Santhiago, C.S. Henry, L.T. Kubota, Low cost, simple three dimensional electrochemical paper-based analytical device for determination of p-nitrophenol, Electrochim. Acta 130 (2014) 771-777.

[15] G. Sun, P. Wang, S. Ge, L. Ge, J. Yu, M. Yan, Photoelectrochemical sensor for pentachlorophenol on microfluidic paper-based analytical device based on the molecular imprinting technique, Biosens. Bioelectron. 56 (2014) 97-103.

[16] S.A. Bhakta, R. Borba, M. Taba Jr, C.D. Garcia, E. Carrilho, Determination of nitrite in saliva using microfluidic paper-based analytical devices, Anal. Chim. Acta 809 (2014) 117-122.

[17] P. Rattanarat, W. Dungchai, D. Cate, J. Volckens, O. Chailapakul, C.S. Henry, Multilayer Paper-Based Device for Colorimetric and Electrochemical Quantification of Metals, Anal. Chem. 86 (2014) 3555-3562.

[18] S.N. Tan, L. Ge, H.Y. Tan, W.K. Loke, J. Gao, W. Wang, Paper-Based Enzyme Immobilization for Flow Injection Electrochemical Biosensor Integrated with Reagent-Loaded Cartridge toward Portable Modular Device, Anal Chem. 84 (2012) 10071-10076.

[19] J. Lankelma, Z. Nie, E. Carrilho, G.M. Whitesides, Paper-Based Analytical Device for Electrochemical Flow-Injection Analysis of Glucose in Urine, Anal. Chem. 84 (2012) 4147-4152.

[20] J. Yu, S. Wang, L. Ge, S. Ge, A novel chemiluminescence paper microfluidic biosensor based on enzymatic reaction for uric acid determination, Anal. Chim. Acta 26 (2011) 3284-3289.

[21] C.S. Kuek Lawrence, S.N. Tan, C.Z. Floresca, A “green” cellulose paper based glucose amperometric biosensor, Sens. Actuators B 193 (2014) 536-541.

[22] S. Wang, L. Ge, X. Song, J. Yu, S. Ge, J. Huang, F. Zeng, Paper-based chemiluminescence ELISA: Lab-on-paper based on chitosan modified paper device and wax-screen-printing, Biosens. Bioelectron. 31 (2012) 212-218.

[23] T. Nurak, N. Praphairaksit, O. Chailapakul, Fabrication of paper-based devices by lacquer spraying method for the determination of nickel (II) ion in waste water, Talanta, 114 (2013) 291-296.

[24] E. Majid, K.B. Male, J.H.T. Luong, Boron Doped Diamond Biosensor for Detection of Escherichia coli, J. Agr. Food Chem. 56 (2008) 7691-7695.

[25] J. Sochr, Ľ. Švorc, M. Rievaj, D. Bustin, Electrochemical determination of adrenaline in human urine using a boron-doped diamond film electrode, Diam. Relat. Mater. 43 (2014) 5-11.

[26] Ľ. Švorc, J. Sochr, M. Rievaj, P. Tomčík, D. Bustin, Voltammetric determination of penicillin V in pharmaceutical formulations and human urine using a boron-doped diamond electrode, Bioelectrochemistry 88 (2012) 36-41.

[27] K. Cui, Y. Song, Y. Yao, Z. Huang, L. Wang, A novel hydrogen peroxide sensor based on Ag nanoparticles electrodeposited on DNA-networks modified glassy carbon electrode, Electrochem. Commun. 10 (2008) 663-667.

[28] J. Yin, X. Qi, L. Yang, G. Hao, J. Li, J. Zhong, A hydrogen peroxide electrochemical sensor based on silver nanoparticles decorated silicon nanowire arrays, Electrochim. Acta 56 (2011) 3884-3889.

[29] X. Li, Y. Liu, L. Zheng, M. Dong, Z. Xue, X. Lu, X. Liu, A novel nonenzymatic hydrogen peroxide sensor based on silver nanoparticles and ionic liquid functionalized multiwalled carbon nanotube composite modified electrode, Electrochim. Acta 113 (2013) 170-175.

[30] C.M. Welch, C.E. Banks, A.O. Simm, R.G. Compton, Silver nanoparticle assemblies supported on glassy-carbon electrodes for the electro-analytical detection of hydrogen peroxide, Anal. Bioanal. Chem. 382 (2005) 12-21.

[31] M.M. Mentele, J. Cunningham, K. Koehler, J. Volckens, C.S. Henry, Microfluidic paper-based analytical device for particulate metals, Anal Chem, 84 (2012) 4474-4480.

[32] S. Nantaphol, O. Chailapakul, W. Siangproh, Sensitive and selective electrochemical sensor using silver nanoparticles modified glassy carbon electrode for determination of cholesterol in bovine serum, Sens. Actuators B 207, Part A (2015) 193-198.

[33] A. Ahmadalinezhad, A. Chen, High-performance electrochemical biosensor for the detection of total cholesterol, Biosens. Bioelectron. 26 (2011) 4508-4513.

[34] M.M. Rahman, X.-b. Li, J. Kim, B.O. Lim, A.J.S. Ahammad, J.-J. Lee, A cholesterol biosensor based on a bi-enzyme immobilized on conducting poly(thionine) film, Sens. Actuators B 202 (2014) 536-542.

[35] A. Umar, R. Ahmad, S.W. Hwang, S.H. Kim, A. Al-Hajry, Y.B. Hahn, Development of Highly Sensitive and Selective Cholesterol Biosensor Based on Cholesterol Oxidase Co-Immobilized with α-Fe2O3 Micro-Pine Shaped Hierarchical Structures, Electrochim. Acta 135 (2014) 396-403.

[36] C. Wang, X. Tan, S. Chen, R. Yuan, F. Hu, D. Yuan, Y. Xiang, Highly-sensitive cholesterol biosensor based on platinum–gold hybrid functionalized ZnO nanorods, Talanta, 94 (2012) 263-270.

[37] A. Safavi, F. Farjami, Electrodeposition of gold-platinum alloy nanoparticles on ionic liquid-chitosan composite film and its application in fabricating an amperometric cholesterol biosensor, Biosens. Bioelectron. 26 (2011) 2547-2552.

[38] C. Zhao, L. Wan, L. Jiang, Q. Wang, K. Jiao, Highly sensitive and selective cholesterol biosensor based on direct electron transfer of hemoglobin, Anal. Biochem. 383 (2008) 25-30.

[39] U. Saxena, M. Chakraborty, P. Goswami, Covalent immobilization of cholesterol oxidase on self-assembled gold nanoparticles for highly sensitive amperometric detection of cholesterol in real samples, Biosens. Bioelectron. 26 (2011) 3037-3043.

[40] X. Cai, X. Gao, L. Wang, Q. Wu, X. Lin, A layer-by-layer assembled and carbon nanotubes/gold nanoparticles-based bienzyme biosensor for cholesterol detection, Sens. Actuators B, 181 (2013) 575-583.

[41] N. Ruecha, R. Rangkupan, N. Rodthongkum, O. Chailapakul, Novel paper-based cholesterol biosensor using graphene/polyvinylpyrrolidone/polyaniline nanocomposite, Biosens. Bioelectron. 52 (2014) 13-19.

List of scheme captions

Scheme 1. Schematic representation of the fabrication and analytical procedure for the cholesterol sensor based on the coupling of the AgNP/BDD electrode with PAD

Scheme 2. Schematic representation for the cholesterol detection processes on the AgNP/BDD electrode coupled with PAD

List of figure captions

Fig. 1. SEM images of (A) bare BDD electrode and (B) AgNP/BDD electrode prepared by electrodeposition at a potential of −0.5 V during 50 s from a pH 2 Britton-Robinson containing 5 mM $AgNO_3$

Fig. 2. (A) CVs of the bare BDD electrode (purple line) and the AgNP/BDD electrode (blue line) in 0.05 M PBS, pH 7.4. CVs of reduction of 1 mM H_2O_2 at the bare BDD electrode (green line) and AgNP/BDD electrode (red line). Scan rate: 100 mV s−1. (B) CVs of the AgNP/BDD electrode in the absence (blue line) and presence of 38.7 mg/dL cholesterol.

Fig. 3. CVs for 1 mM H_2O_2 in 0.05 M PBS (pH 7.4) at the AgNP/BDD electrode coupled with PAD for a series of scan rates (10, 20, 40, 60, 80, and 100 mV s-1). The relationship between cathodic current (μA) and $(\text{scan rate})^{0.5}$ is shown in the inset.

Fig. 4. (A) Hydrodynamic voltammograms of 38.7 mg/dL cholesterol (blue line) and background (blue line) for 20 s sampling time. (B) Hydrodynamic voltammogram of signal-to-background ratios extracted from the data shown in part A.

Fig. 5. Chronoamperograms of cholesterol (0, 0.4, 19.3, 38.7, 77.3, 116.0, 154.7, 193.4, 232.0, and 270.69 mg dL^{-1}) determination at -0.7 V vs.Ag/AgCl. The calibration plot of the cathodic currents at 20 s of sampling time for the determination of cholesterol is shown in the insert.

Fig. 6. The interference effect of 1 mM Glu, 0.2 mM AA, and 0.2 mM UA in the detection of 1 mM cholesterol in 0.05 M PBS, pH 7.4.

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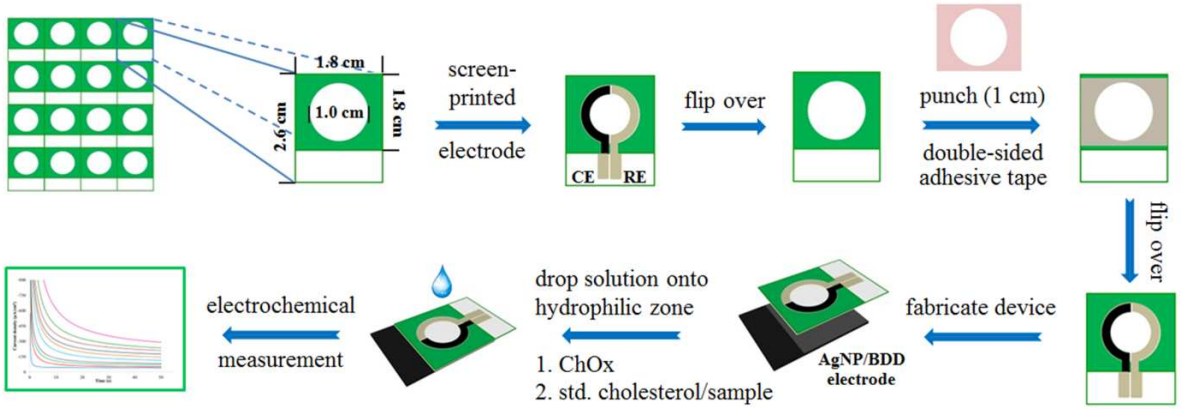
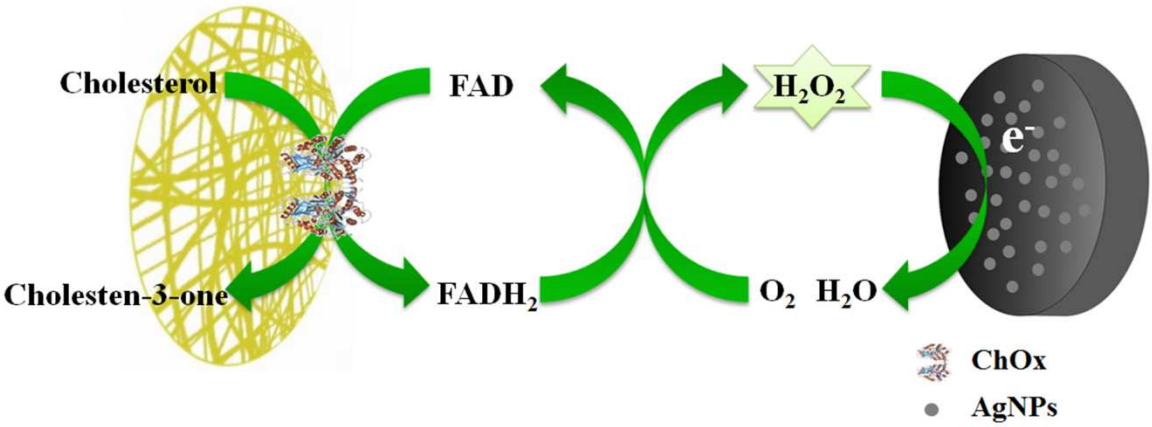
Table 1: Summary of the experimental parameters for modification of electrode

Table 2. Comparison of analytical performances of different electrochemical biosensors for the determination of cholesterol

Table 3. Determination of cholesterol in bovine serum samples



Scheme 1



Scheme 2

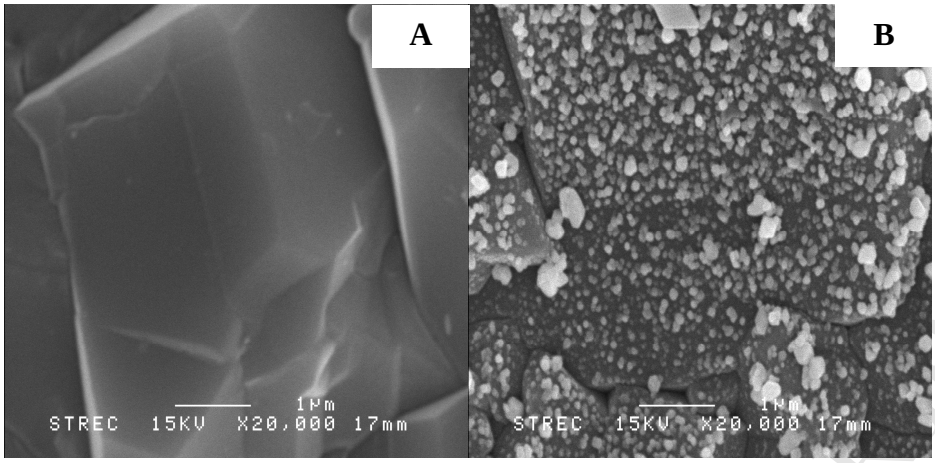


Fig. 1

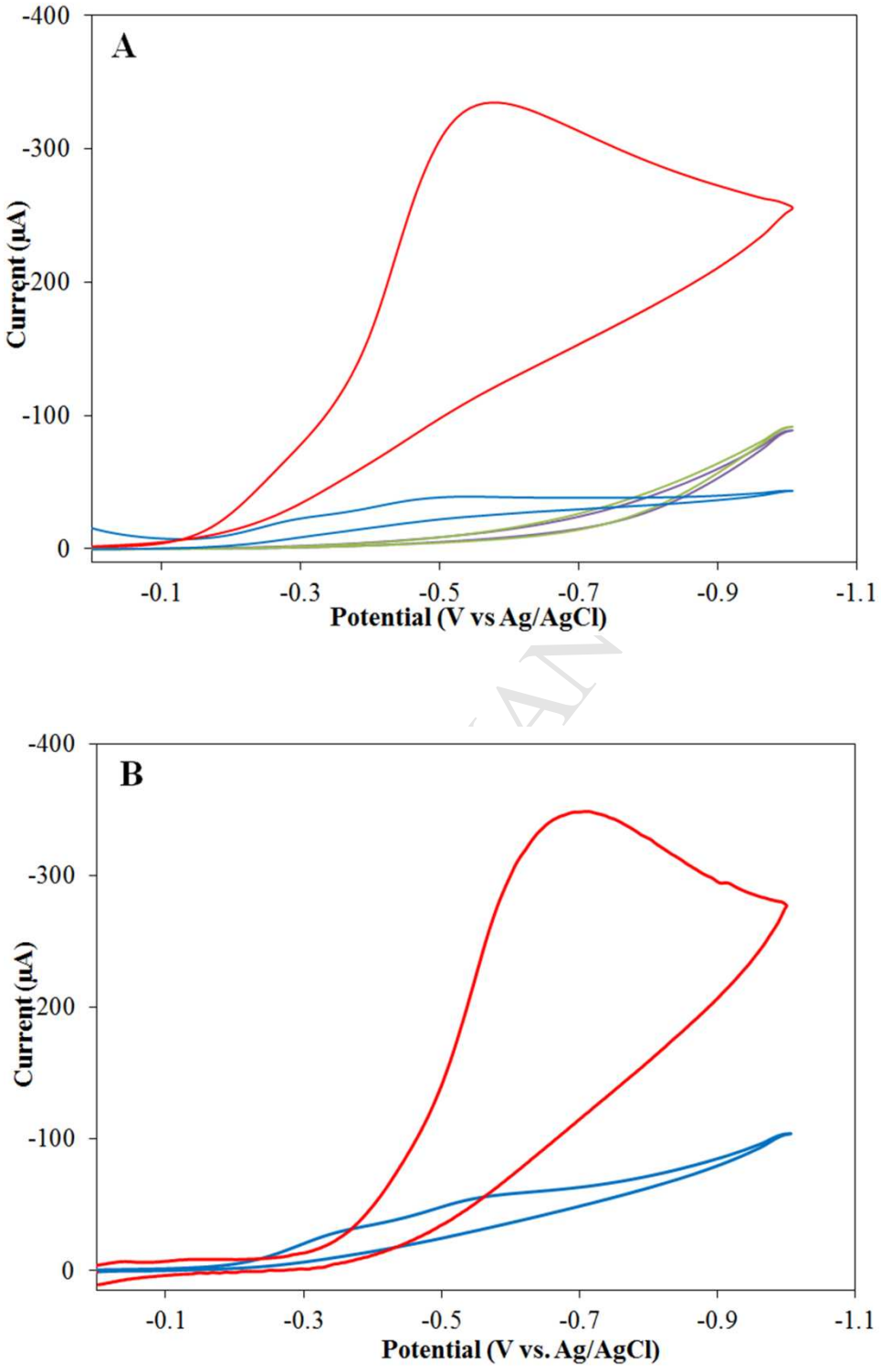


Fig. 2

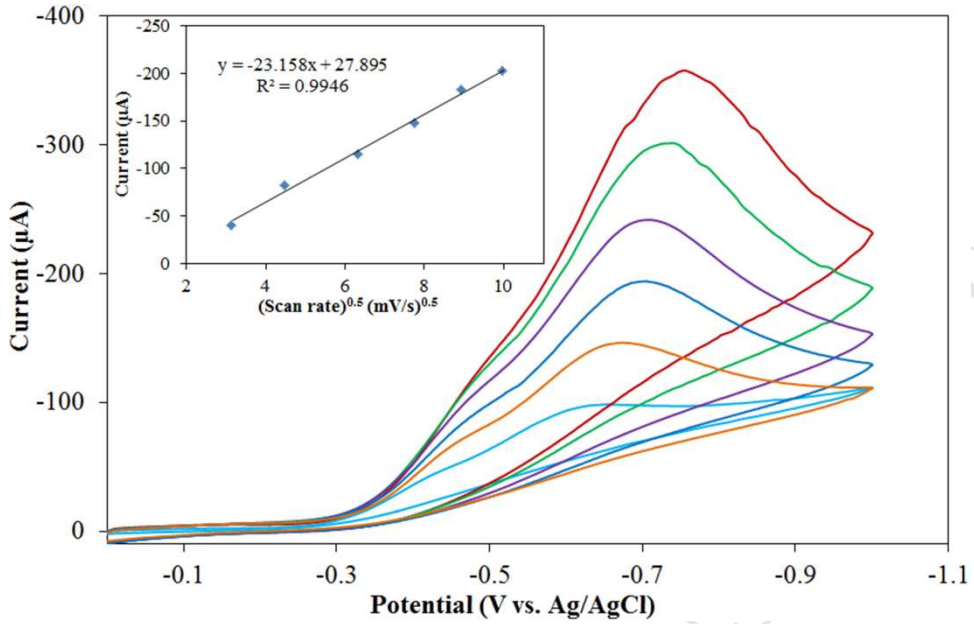


Fig. 3

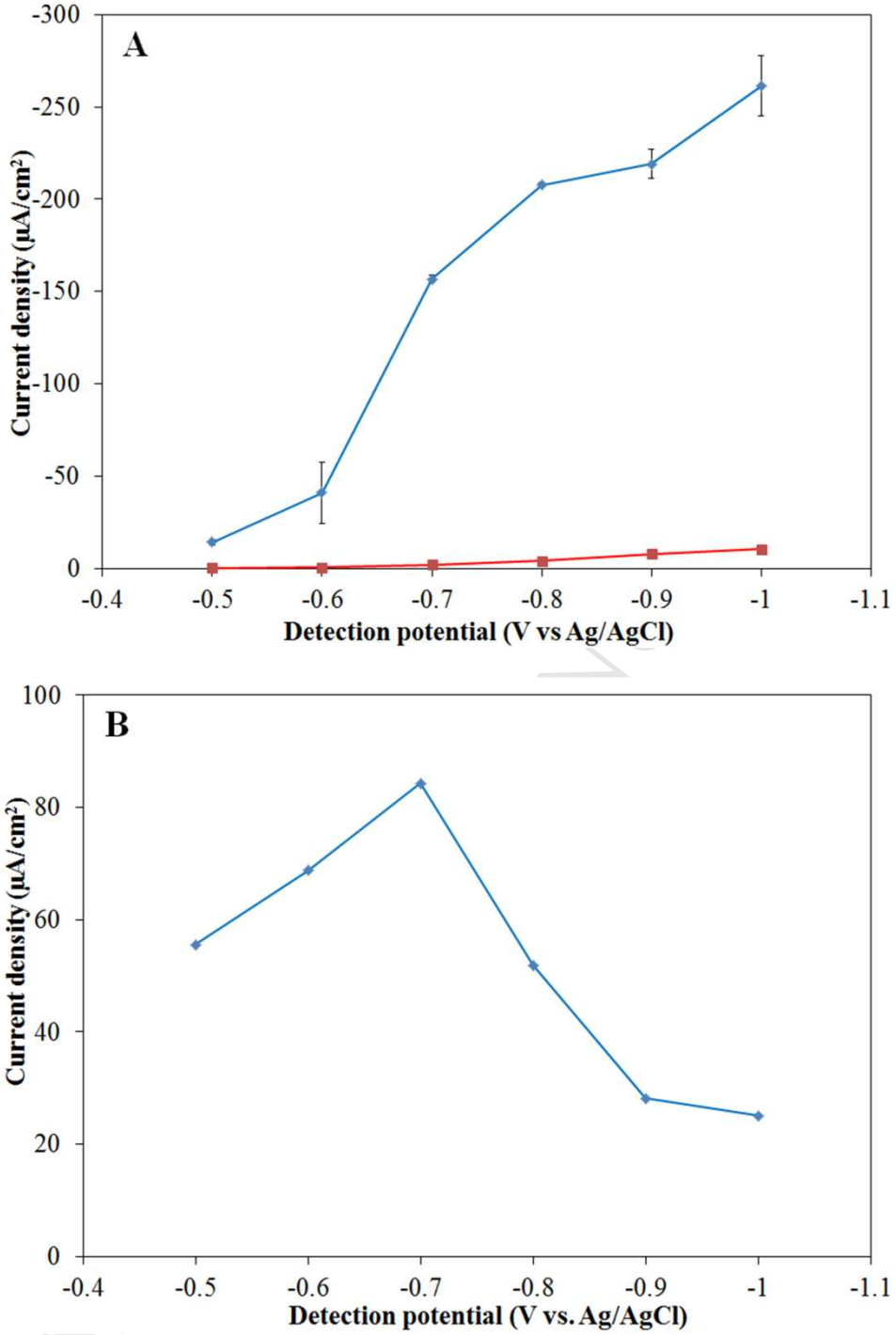


Fig. 4

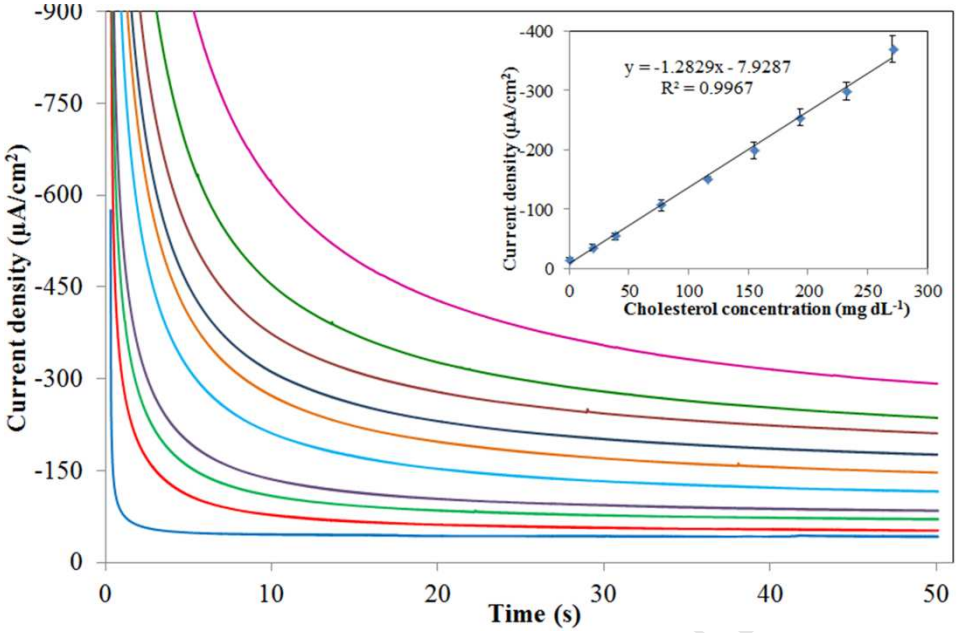


Fig. 5

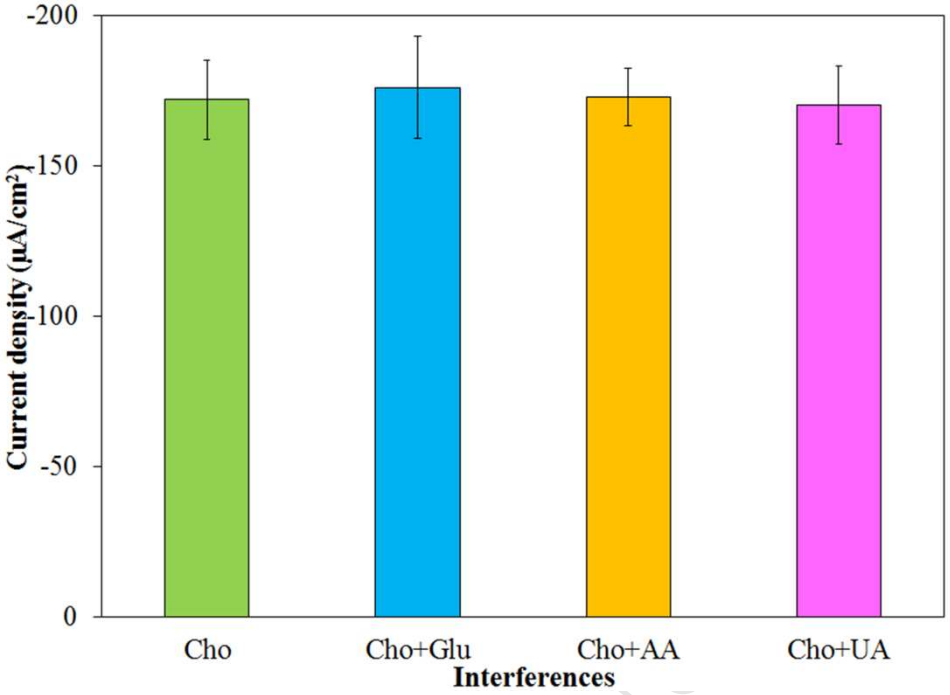


Fig. 6

Table 1

Parameters	Range tested	Optimum value
deposition potentials (V vs Ag/AgCl)	(-0.3) - (-0.7)	-0.5
deposition times (s)	25 - 300	150
concentrations of $AgNO_3$ (mM)	0.25 - 10	5

Table 2

Cholesterol biosensor	Immobilization method	Linear range (mM)	LOD (μM)
GCE/PTH/ChOx/HRP [34]	covalent linking	0.025-0.125	6.3
Nafion/ChOx/-Fe_2O_3/Ag [35]	co-immobilized with α-Fe_2O_3 micro-pine shaped hierarchical structures	0.1-8.0	18
ChOx/Pt–Au@ZnONRs/CS-MWCNTs/GCE [36]	adsorption	0.0001-0.7593	0.03
ChOx/AuPt–Ch–IL/GCE [37]	cross-linking	0.05-6.2 and 6.2-11.2	10
ChOx–CS/Hb–CS/GCE [38]	encapsulation	0.01-0.6	9.5
AuE/dithiol/AuNPs/MUA /ChOx [39]	covalent attachment	0.04-0.22	34.6
$(PAH–MCNTs–GNPs/HRP)_4/(PAH–MCNTs–GNPs/ChOx)_4$ [40]	electrostatic interaction	0.18-11	20
G/PVP/PANI nanocomposites [41]	adsorption	0.05–10	1
AgNPs/BDDE coupled with PAD (this work)	adsorption	0.39-270.69 mg dL^{-1} or 0.01-7 mM	6.5

Table 3

Sample	Labeled concentration (mg/dL)	Determined value (mg/dL)	%RSD	Relative error (%)	% Recovery
A	40	40.6±1.4	3.4	+1.5	99.6
B	60	61.3±1.8	3.0	+2.1	100.7
C	80	79.4±1.0	1.0	-0.3	100.8
D	100	99.6±1.0	1.3	-0.7	100.6

Highlights

- Novel PAD coupled with AgNP/BDDE for cholesterol determination was developed.
- Wide linear range, low detection limit and high selectivity were achieved.
- This sensor was successfully applied for cholesterol determination in bovine serum.
- This platform offers the advantages of low sample/reagent consumption and low cost.