Hue University Journal of Science: Techniques and Technology; pISSN 2588-1175 | eISSN 2615-9732

Vol. 133, No. 2B, 2024, 5–15; DOI: 10.26459/hueunijtt.v133i2B.7366

Enhancing the Self-Assembled Monolayer Formation for Protein Detection Platform through L-Cysteine Utilization

Linh Huynh Thi Thuy [1, 2*], Chun-Ping Jen [3], Guan-Da Syu [4], Nha Vo Quang [1], Cuong Ngo Xuan [1], Loc Do Quang [5], Phu Nguyen Dang [2], Tung Bui Thanh [2], Trinh Chu Duc [2]

[1] School of Engineering and Technology, Hue University, Hue, Vietnam
[2] University of Engineering and Technology, Vietnam National University, Hanoi, Vietnam
[3] Department of Mechanical Engineering, National Chung Cheng University, Chia-Yi, Taiwan
[4] Department of Biotechnology and Bioindustry Sciences, National Cheng Kung University, Tainan, Taiwan
[5] University of Science, Vietnam National University, Hanoi, Vietnam

Abstract. Electrochemical immunosensing has emerged as a contemporary sensing strategy based on the principles of specific antigen-antibody recognition, offering exceptional specificity, remarkable sensitivity, and seamless integration. In this study, we present a rapid, three-step, and cost-effective modification process to establish an immunosensing platform using a self-assembled monolayer (SAM) of L-Cysteine. This approach was experimentally implemented through quantitative detection of Bovine Serum Albumin (BSA) protein, spanning a concentration range from 0.5 μM to 8.0 μM. Optical signals, along with observable changes in electrical signals from cyclic voltammetry (CV), square wave voltammetry (SWV) and electrochemical impedance spectroscopy (EIS) confirmed the formation of monolayers on the electrode surface and detection signals for BSA protein. The characteristic curve, employing the change in charge transfer resistance (ΔR_{ct}) as a function of BSA protein concentration, was plotted with a coefficient of determination (R^2) value of 0.95136. These findings underscore the potential of L-Cysteine-based SAMs in electrochemical biosensing applications for highly sensitive and cost-efficient protein detection.

Keywords: antigen-antibody recognition, immunosensing, L-Cysteine.

1 Introduction

Self-Assembled Monolayers (SAMs) have been fundamental components of biosensors for immobilizing biorecognition elements, such as antibodies, antigens, DNA, enzymes, or aptamers [1–3]. They provide a stable and functional surface, offer control over surface properties, reduce non-specific binding, enhance sensitivity, and enable compatibility with various transduction methods. These attributes have made SAMs indispensable in advancing biosensors with heightened sensitivity and specificity, applicable in fields ranging from medical diagnostics to environmental monitoring, and beyond [4]. (R)-2-Amino-3-mercaptopropionic acid, also known as L-Cysteine, which plays a crucial role in the formation of SAMs in surface

** Corresponding: thuylinh@hueuni.edu.vn*

Received: 25–11–2023; Accepted: 10–01–2024

chemistry applications, is a naturally occurring amino acid found in biological systems. Its biocompatibility makes it suitable for SAMs used in biomedical and biotechnology applications [5], enabling surface functionalization for bioconjugation. Additionally, L-Cysteine contains a thiol (sulfhydryl) group, which is essential for SAM formation due to its strong and stable binding to many metal surfaces [6], especially gold substrates [7, 8].

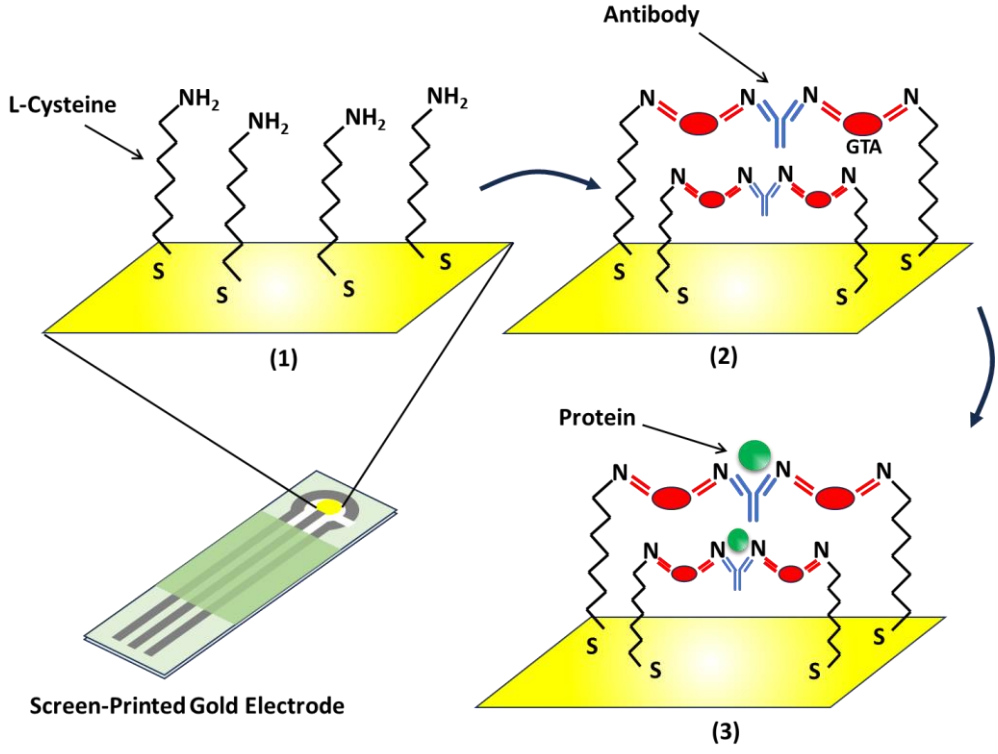

Fig. 1. Schematic diagram of the proposed process: Step (1) Self-assembled monolayer formation; Step (2) Antibody immobilization; Step (3) Protein capture

Protein detection has primarily relied on immunoassay techniques, including immunohistochemistry (IHC) [9, 10], enzyme-linked immunosorbent assay (ELISA) [10, 11], flow cytometry [12], and protein microarray [13]. Additionally, electrochemical sensors have been increasingly developed in the field of electrochemistry to enhance analytical efficiency [14]. Possessing characteristics such as flexibility, miniaturization, and affordability [15], these sensors have broadened their range of applications, particularly in the monitoring and detection of biomarkers [16]. Thanks to their high sensitivity and selectivity, electrochemical biosensors have received substantial investments in both research and manufacturing, primarily based on the widely accepted mechanism of specific antibody-antigen pairing [17–19]. However, these biosensors often depend on intricate, multi-step detection and fabrication techniques that required advanced equipment platforms.

In research conducted by Yan *et al.* [20], a SAM of L-Cysteine was utilized to modify a gold electrochemical sensor, enabling the detection of copper ions in environmental samples with a low limit of detection of less than 5 ppb. Similarly, Moccelini *et al.* [5] employed a biosensor modified with an L-Cysteine SAM on a gold electrode to detect dopamine. In their study, dopamine concentrations ranging from 9.91×10^{-6} to 2.21×10^{-4} mol L^{-1} were successfully detected through square wave voltammetry, achieving a remarkable limit of detection of 4.78×10^{-7} mol L^{-1}. The presence of L-Cysteine SAM on the gold electrode formed the essential framework for immobilizing the biological probe to detect the necessary object.

In this study, an immunosensor using L-Cysteine as a SAM was developed for the electrochemical detection of target proteins, employing a straightforward three-step process as described in Figure 1. In the first step, a SAM was formed on the screen-printed gold electrode surface using (R)-2-Amino-3-mercaptopropionic acid (L-Cysteine). This crucial layer served as an interface between the gold electrode surface and the bio-probe [21–23], facilitated by the robust bond between SAM's thiol functional group and the gold electrode surface, and the linking of its amino group with the bio-probe's amino group. The second step involved the immobilization of the anti-Bovine Serum Albumin antibody (anti-BSA) as a bio-probe, onto the electrode surface by crosslinking between its amino groups and L-Cysteine's amino groups using glutaraldehyde (GTA) [24, 25]. Bio-probe immobilization is also a critical step for various sensing and diagnostic applications, as each bio-probe can only pair with one specific target. In the third step, the detection of the Bovine Serum Albumin-fluorescein isothiocyanate conjugate (BSA) protein was performed. This detection was based on observing an increase in optical signals resulting from the specific binding of the bio-probe (anti-BSA) and the target protein (BSA), corresponding to an increase in BSA concentration ranging from 0.5 μM to 8.0 μM. Combined with the evident changes in electrical signals of cyclic voltammetry (CV), square wave voltammetry (SWV), and electrochemical impedance spectroscopy (EIS), the formation of monolayers on the electrode surface and the detection of the BSA protein were confirmed.

2 Experimental

2.1 Materials

(R)-2-Amino-3-mercaptopropionic acid (L-Cysteine) for SAM formation was obtained from Sigma–Aldrich. Glutaraldehyde 50%, also from Sigma–Aldrich, was used for crosslinking the amino groups. Anti-Bovine Serum Albumin antibody (anti-BSA) from Sigma–Aldrich was used as a bio-probe to detect the target protein. Bovine Serum Albumin-fluorescein isothiocyanate conjugate (BSA) from Sigma–Aldrich was used as the target protein at different concentrations. 10X Phosphate Buffered Saline (PBS) at pH 7.4 was from Gibco, USA. Deionized (DI) water (18.2 MΩ·cm) from a PURIST® Ultrapure Water Systems, Rephile, Singapore was used to clean electrodes five times and a light stream of nitrogen was used to dry the chip surface. Potassium

Chloride (KCl) and Potassium Ferricyanide ($K_3Fe(CN)_6$) and Potassium Ferrocyanide ($K_4Fe(CN)_6$) purchased from Choneye Pure Chemicals, Taipei, Taiwan were used for electrodes scanning in all electrochemical measurements. Screen-Printed Gold Electrode (SPAuE) chips of BioDevice Technology Ltd., Japan were connected to the Palmsens4 Potentiostat, Galvanostat for Electrochemical Impedance Spectroscopy of PalmSens BV, The Netherlands to find out the necessary signal. The surface modification steps for protein detection were applied to modify the working electrode surface of these chips. Figure 2 shows the connection of the Palmsens4 measurement device to SPAuE chip and PS Trace 5.9 software on a computer.

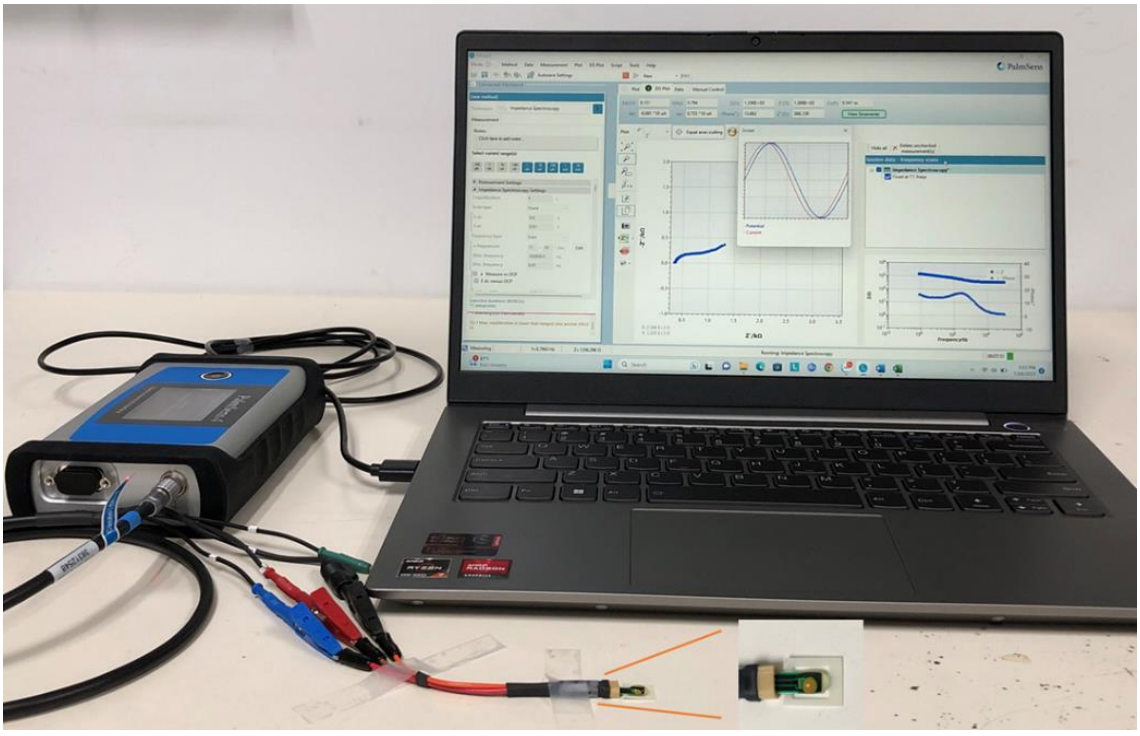

Fig. 2. Connection of the Palmsens4 measurement device to SPAuE chip and PS Trace 5.9 software on a computer

2.2 Bio-probe immobilization

In preparation for the experiments, the SPAuE chips were cleaned with an ethanol solution, then recleaned with DI water and dried with a nitrogen stream. For step 1 in Figure 1, the L-Cysteine SAM was formed on the working electrode surface of the SPAuE chip. This step involved immersing the chip overnight in a 10 mM L-Cysteine solution diluted in 1X PBS at room temperature. After cleaning with DI water and nitrogen, the SPAuE chip was labeled as SPAuE/SAM. For step 2 in Figure 1, a 3.0 µL solution containing anti-BSA at 15 µM and glutaraldehyde at 5%, both diluted in 1X PBS at a 1:1 volume ratio, was dropped on the working electrode surface. The electrode was then incubated for 2 hours at 4°C. During this

step, crosslinking occurred between L-Cysteine's amino groups and anti-BSA's amino groups. The chips were then cleaned again, labeled as SPAuE/SAM/antiBSA and stored at 4°C in preparation for the detection of BSA protein at different concentrations.

2.3 Protein detection

For step 3 in Figure 1, the working electrode surface of the SPAuE/SAM/antiBSA chip was incubated with 3.0 μL of different concentrations of BSA protein, diluted in 1X PBS, for 1 hour at 4°C. During this step, antibody-antigen pairing between anti-BSA and BSA occurred. The chip was then cleaned to remove the unspecific binding and labeled as SPAuE/SAM/antiBSA/BSA. Thanks to the fluorescent conjugation of BSA, the pairing of anti-BSA and BSA could be observed under an inverted microscope. Besides, this pairing created a non-conductive layer coating the working electrode surface, which sharply increased the impedance corresponding to R_{ct} (charge transfer resistance) in EIS signal and significantly decreased the current peak in SWV, and CV signals. To determine these electrochemical characteristics, this chip's three electrodes were covered with 35 μL of an electrolyte solution (5 mM of each $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ in 0.1 M KCl, diluted in DI water) and scanned at a rate of 50 mV/s with an Ag/AgCl reference electrode.

3 Results and discussion

3.1 Investigation of optical signal

An inverted microscope system integrated with a camera was used to observe and capture fluorescence images of the BSA fluorescein isothiocyanate conjugate. A desktop computer was connected to the camera for data acquisition. After modification process for detecting BSA protein at different concentrations, the SPAuE/SAM/antiBSA/BSA chips were placed on the inverted microscope system and the images of optical signal were collected. Figure 3 shows the images of SPAuE/SAM/antiBSA/BSA working electrode surface at BSA protein concentrations ranging from 0.5 μM to 8.0 μM. These optical images show that as the concentration increases, the fluorescence intensity becomes stronger. Figure 4 shows the fluorescence intensity measured within the same specific area at different concentrations of BSA protein using the rectangle tool in ImageJ software. The fluorescence intensity was automatically quantified and averaged at 11.722, 16.319, 20.861, 24.456 and 27.421 corresponding to concentrations of 0.5 μM, 1.0 μM, 2.0 μM, 4.0 μM and 8.0 μM, respectively. The greater the concentration, the clearer the optical signal. This result demonstrates the success in capturing and detecting BSA protein at different concentrations.

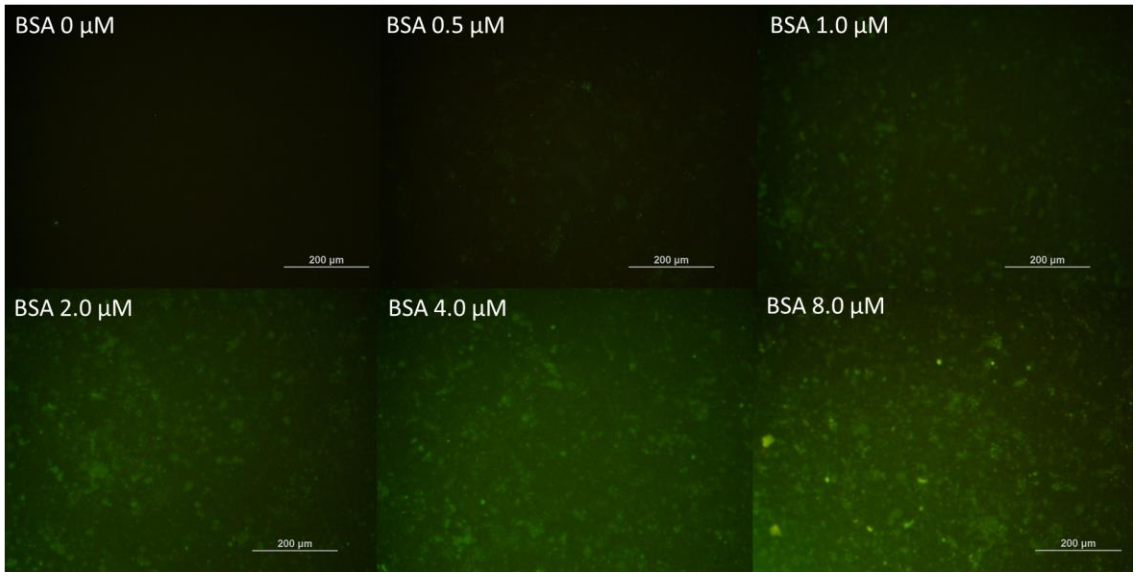

Fig. 3. Images of BSA protein on the SPAuE/SAM/antiBSA/BSA working electrode surface at BSA protein concentrations ranging from 0.5 µM to 8.0 µM

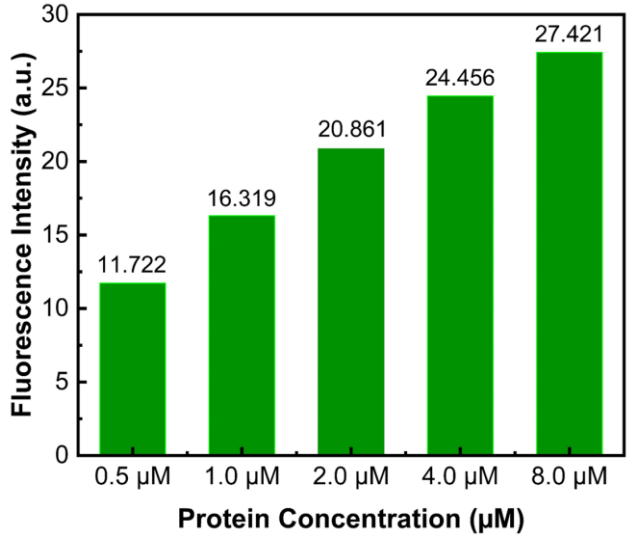

Fig. 4. Fluorescence intensity measured within the same specific area at different concentrations of BSA protein

3.2 Investigation of electrical signal

In addition to optical signals, electrochemical signals were also studied to confirm the formation of monolayers on the electrode surface and the detection of BSA protein. Figure 5a shows the cyclic voltammetry (CV) signal before and after formation of L-Cysteine SAM on the chip surface. The increase in current peaks after the SAM formation indicates an enhancement in electron transfer dynamics [26, 27]. The improved electrical conductivity of the electrode after

SAM formation can be considered an advantage of L-Cysteine compared to other SAMs, which are typically characterized by increased impedance [28, 29]. Figure 5b shows the CV signal of the chip after SAM formation, anti-BSA immobilization, and at different concentrations of BSA protein. The current peaks decrease after the immobilization of the bio-probe and the capture of the target protein at different concentrations, the higher the BSA concentration, the lower the current peak signal. To further confirm the successful capture and detection of the protein at different concentrations, the square wave voltammetry (SWV) signal was investigated, as illustrated in Figure 6. The current peaks decrease sharply after anti-BSA immobilization and with increasing concentrations of BSA protein, ranging from 0.5 μM to 8.0 μM.

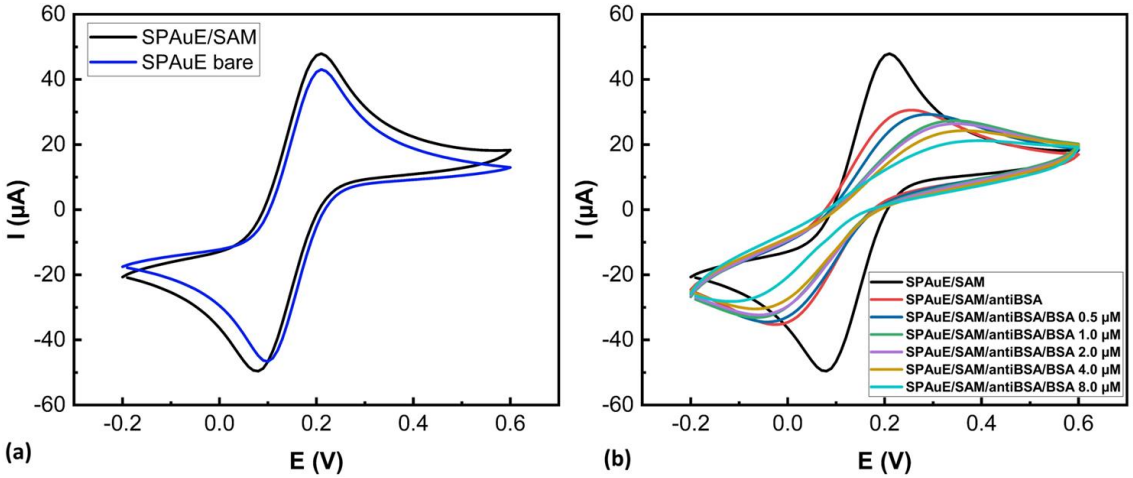

Fig. 5. (a) CV signal before and after formation of L-Cysteine SAM on the chip surface. (b) CV signal of the chip after SAM formation, anti-BSA immobilization, and at different concentrations of BSA protein

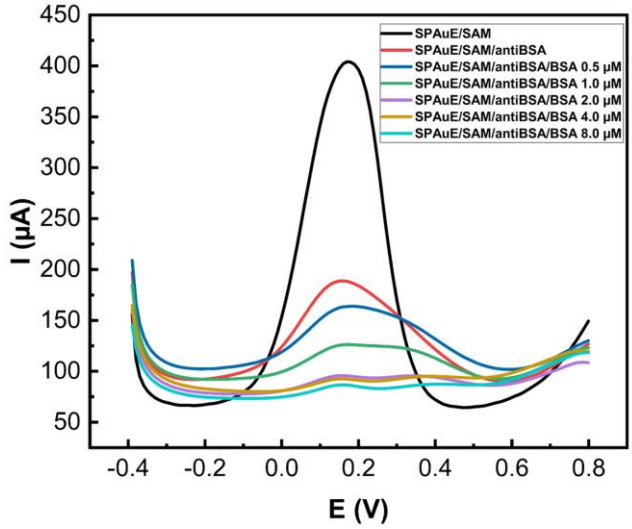

Fig. 6. SWV signal of the chip after SAM formation, anti-BSA immobilization, and at different concentrations of BSA protein

The electrochemical impedance spectroscopy (EIS) method involves analyzing total impedance of the electrochemical system by applying a small amplitude AC voltage (5 to 10 mV) over a wide frequency range, varying from high to low (MHz to mHz). The interface between the electrode and the electrolyte is presented by Randles equivalent circuit which includes R_s, C_{dl}, R_{ct} and W. R_{ct}, which characterizes the charge transfer, depends on both the morphology and the conductivity of the electrode surface [30]. Therefore, to investigate the change in conductivity of the electrode surface after SAM formation, anti-BSA immobilization, and at different concentrations of BSA protein, the chips were scanned in the electrolyte solution over a frequency range from 0.05 to 10^5 Hz with an applied voltage of 10 mV. The measured signals were automatically matched to Randles equivalent circuit using PS Trace 5.9 software to calculate R_{ct}. Figure 7a shows an increasing Nyquist plot semicircle diameter corresponding to the increasing R_{ct} after bio-probe immobilization (SPAuE/SAM/antiBSA) and BSA protein capture (SPAuE/SAM/antiBSA/BSA) at different concentrations. The Figure 7b inset shows the Randles equivalent circuit automatically matched using PSTrace 5.9 software for calculating the R_{ct} values. Figure 7b presents a linear fit with a coefficient of determination R^2 value of 0.95136, representing the change in R_{ct} (ΔR_{ct}) as a function in response to changes in BSA concentration (C) ranging from 0.5 µM to 8.0 µM as follows:

$$\Delta R_{ct}\ (k\Omega) = 0.94301 \times C\ (\mu M) + 4.81667 \quad (1)$$

The significant difference in both current and impedance between SPAuE/SAM/antiBSA and SPAuE/SAM indicates the successful immobilization of anti-BSA on the electrode surface, enabling the detection of BSA protein.

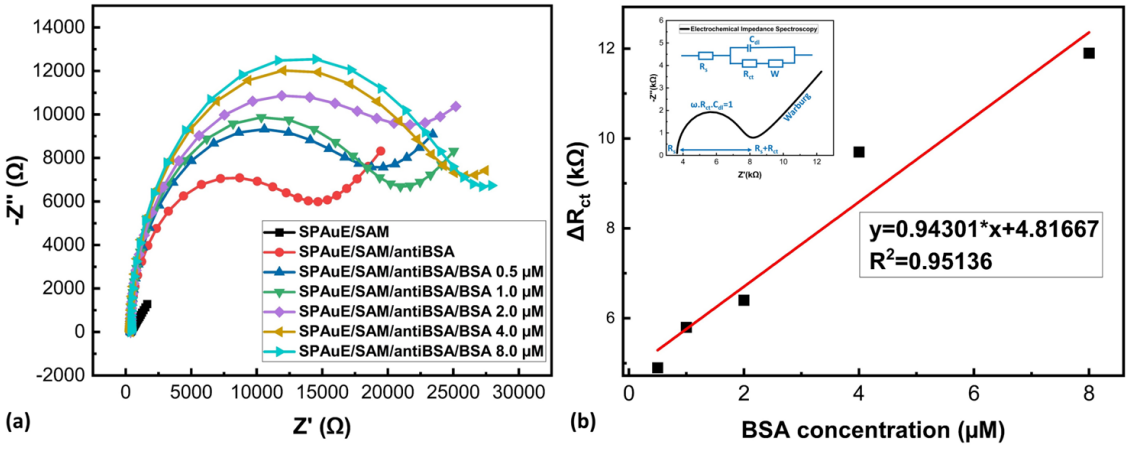

Fig. 7. (a) EIS signal of the chip after SAM formation, anti-BSA immobilization, and at different concentrations of BSA protein. (b) ΔR_{ct} plotted as a function of BSA protein concentration

4 Conclusion

In this study, we proposed a rapid, three-step and efficient immunosensor electrode modification process for detecting BSA protein. The successful formation of a SAM using L-Cysteine on the surface of gold electrode laid the foundation for electrode functionalization with a bio-probe, enabling the detection of protein within a concentration range from 0.5 μM to 8.0 μM. The proposed process induced significant changes in cyclic voltammetry, square wave voltammetry, electrochemical impedance spectroscopy, and optical signals at different concentrations. The current peaks in CV and SWV measurements decreased in correspondence with the increase in charge transfer resistance R_{ct} observed in EIS measurements as the concentration increased. This was consistent with the optical results, where fluorescence intensity, automatically calculated by ImageJ software, showed that the optical signal became more pronounced with increasing concentration. The ΔR_{ct} signal, used as a measure of BSA protein concentration, exhibited a high coefficient of determination (R^2) at 0.95136. This finding establishes a robust sensing platform with great potential for selective BSA detection. Our future work will involve deploying the immunosensor in real samples to further assess its selectivity and enhance its applicability for protein detection in research and analysis.

Acknowledgement

This research was funded by Hue University under grant No. DHH2022-18-02 and supported by International Internship Pilot Program in Taiwan.

References

1. Chaki Nirmalya K and K Vijayamohanan, "Self-assembled monolayers as a tunable platform for biosensor applications," *Biosensors and Bioelectronics*, vol. 17, no. 1–2, pp. 1–12, 2002, doi: 10.1016/S0956-5663(01)00277-9.
2. Arya Sunil K., Pratima R. Solanki, Monika Datta, and Bansi D. Malhotra, "Recent advances in self-assembled monolayers based biomolecular electronic devices," *Biosensors and Bioelectronics*, vol. 24, no. 9, pp. 2810–2817, May 2009, doi: 10.1016/j.bios.2009.02.008.
3. Samanta Debasis and Amitabha Sarkar, "Immobilization of bio-macromolecules on self-assembled monolayers: Methods and sensor applications," *Chemical Society Reviews*, vol. 40, no. 5, pp. 2567–2592, Apr. 2011, doi: 10.1039/c0cs00056f.
4. Wink Th, S J Van Zuilen, A Bult, and W P Van Bennekom, "Self-assembled monolayers for biosensors," *Analyst*, vol. 122, no. 4, pp. 43R-50R, 1997, doi: 10.1039/A606964I.
5. Moccelini Sally Katiuce, Suellen Cadorin Fernandes, and Iolanda Cruz Vieira, "Bean sprout peroxidase biosensor based on l-cysteine self-assembled monolayer for the determination of dopamine," *Sensors and Actuators, B: Chemical*, vol. 133, no. 2, pp. 364–369, Aug. 2008, doi: 10.1016/j.snb.2008.02.039.

6. Wang Sheng-Fu, Dan Du, and Qi-Chao Zou, "Electrochemical behavior of epinephrine at L-cysteine self-assembled monolayers modified gold electrode," *Talanta*, vol. 57, pp. 687–692, 2002, doi: 10.1016/S0039-9140(02)00072-3.

7. Arrigan Damien W.M. and Loïc Le Bihan, "A study of L-cysteine adsorption on gold via electrochemical desorption and copper(II) ion complexation," *Analyst*, vol. 124, no. 11, pp. 1645–1649, 1999, doi: 10.1039/a905370k.

8. Wang Wenjing, Zhijian Yi, Qiongxin Liang, Junjie Zhen, Rui Wang, Mei Li, Lingwen Zeng, and Yongfang Li, "In Situ Deposition of Gold Nanoparticles and L-Cysteine on Screen-Printed Carbon Electrode for Rapid Electrochemical Determination of As(III) in Water and Tea," *Biosensors*, vol. 13, no. 1, Jan. 2023, doi: 10.3390/bios13010130.

9. Yaziji Hadi, Clive R Taylor, Neal S Goldstein, David J Dabbs, Elizabeth H Hammond, Bryan Hewlett, Alton D Floyd, Todd S Barry, Alvn W Martin, Sunil Badve, Frederick Baehner, Richard W Cartun, Richard N Eisen, Paul E Swanson, Stephen M Hewitt, Mogen Vyberg, and David G Hicks, "Consensus Recommendations on Estrogen Receptor Testing in Breast Cancer By Immunohistochemistry," *Applied Immunohistochemistry & Molecular Morphology* , vol. 16, no. 6, pp. 513–520, 2008, doi: 10.1097/PAI.0b013e31818a9d3a.

10. Veskimäe K., S. Staff, A. Grönholm, M. Pesu, M. Laaksonen, M. Nykter, J. Isola, and J. Mäenpää, "Assessment of PARP protein expression in epithelial ovarian cancer by ELISA pharmacodynamic assay and immunohistochemistry," *Tumor Biology*, vol. 37, no. 9, pp. 11991–11999, Sep. 2016, doi: 10.1007/s13277-016-5062-6.

11. Manfred Schmitt, Alexandra S. Sturmheit, Anita Welk, Christel Schnelldorfer, and Nadia Harbeck, "Procedures for the Quantitative Protein Determination of Urokinase and Its Inhibitor, PAI-1, in Human Breast Cancer Tissue Extracts by ELISA," in *Methods in Molecular Medicine*, vol. 120, 2006, pp. 245–265. doi: 10.1385/1-59259-969-9:245.

12. Grimwade Lizz, Emma Gudgin, David Bloxham, Mike A. Scott, and Wendy N. Erber, "PML protein analysis using imaging flow cytometry," *Journal of Clinical Pathology*, vol. 64, no. 5, pp. 447–450, May 2011, doi: 10.1136/jcp.2010.085662.

13. Kuo Ho Chang, Kuang Che Kuo, Pin Xian Du, Batuhan Birol Keskin, Wen Yu Su, Tzong Shiann Ho, Pei Shan Tsai, Chi Ho Pau, Hsi Chang Shih, Ying Hsien Huang, Ken Pen Weng, and Guan Da Syu, "Profiling Humoral Immunity After Mixing and Matching COVID-19 Vaccines Using SARS-CoV-2 Variant Protein Microarrays," *Molecular and Cellular Proteomics*, vol. 22, no. 4, Apr. 2023, doi: 10.1016/j.mcpro.2023.100507.

14. Serrano V M, I S P Silva, A R Cardoso, and M G F Sales, "Carbon electrodes with gold nanoparticles for the electrochemical detection of miRNA 21-5p," *Chemosensors*, 2022, doi: 10.3390/chemosensors10050189.

15. Kim Hye Jin, Dongsung Park, Yejin Park, Dae-Hyeong Kim, and Jinsik Kim, "Electric-Field-Mediated In-Sensor Alignment of Antibody's Orientation to Enhance the Antibody–Antigen Binding for Ultrahigh Sensitivity Sensors," *Nano Letters*, vol. 22, no. 16, pp. 6537–6544, 2022, doi: 10.1021/acs.nanolett.2c01584.

16. Albareda-Sirvent Miquel, Arben Merkoci, and Salvador Alegret, "Configurations used in the design of screen-printed enzymatic biosensors. A review," *Sensors and Actuators B: Chemical*, vol. 69, no. 1–2, pp. 153–163, 2000, doi: 10.1016/S0925-4005(00)00536-0.

17. Holford Timothy R J, Frank Davis, and Séamus P J Higson, "Recent trends in antibody based sensors," *Biosensors and Bioelectronics*, vol. 34, no. 1, pp. 12–24, 2012, doi: 10.1016/j.bios.2011.10.023.

18. Olkhov Rouslan V and Andrew M Shaw, "Label-free antibody–antigen binding detection by optical sensor array based on surface-synthesized gold nanoparticles," *Biosensors and Bioelectronics*, vol. 23, no. 8, pp. 1298–1302, 2008, doi: 10.1016/j.bios.2007.11.023.

19. Sadrjavadi Komail, Mojtaba Taran, Ali Fattahi, and Alireza Khoshroo, "A microelectrode system for simple measurement of neuron specific enolase with photolithography technique," *Microchemical Journal*, vol. 182, p. 107889, 2022, doi: 10.1016/j.microc.2022.107889.

20. Yang Wenrong, J Justin Gooding, and D Brynn Hibbert, "Characterisation of gold electrodes modified with self-assembled monolayers of L-cysteine for the adsorptive stripping analysis of copper," *Journal of Electroanalytical Chemistry*, vol. 516, pp. 10–16, 2001, doi: 10.1016/S0022-0728(01)00649-0.

21. Kim Dong Chung and Dae Joon Kang, "Molecular recognition and specific interactions for biosensing applications," *Sensors*, vol. 8, no. 10, pp. 6605–6641, 2008, doi: 10.3390/s8106605.

22. Ignat Teodora, Mihaela Miu, Irina Kleps, Adina Bragaru, Monica Simion, and Mihai Danila, "Electrochemical characterization of BSA/11-mercaptoundecanoic acid on Au electrode," *Materials Science and Engineering: B*, vol. 169, no. 1–3, pp. 55–61, 2010, doi: 10.1016/j.mseb.2009.11.021.

23. Ahmad Azrilawani and Eric Moore, "Electrochemical immunosensor modified with self-assembled monolayer of 11-mercaptoundecanoic acid on gold electrodes for detection of benzo [a] pyrene in water," *Analyst*, vol. 137, no. 24, pp. 5839–5844, 2012, doi: 10.1039/C2AN35236B.

24. Mattei Giorgio, Ludovica Cacopardo, and Arti Ahluwalia, "Engineering gels with time-evolving viscoelasticity," *Materials*, vol. 13, no. 2, Jan. 2020, doi: 10.3390/ma13020438.

25. Barbosa Oveimar, Claudia Ortiz, Ángel Berenguer-Murcia, Rodrigo Torres, Rafael C. Rodrigues, and Roberto Fernandez-Lafuente, "Glutaraldehyde in bio-catalysts design: A useful crosslinker and a versatile tool in enzyme immobilization," *RSC Advances*, vol. 4, no. 4. pp. 1583–1600, 2014. doi: 10.1039/c3ra45991h.

26. Feliciano-Ramos Ileana, Miguel Caban-Acevedo, M. Aulice Scibioh, and Carlos R. Cabrera, "Self-assembled monolayers of l-cysteine on palladium electrodes," *Journal of Electroanalytical Chemistry*, vol. 650, no. 1, pp. 98–104, Dec. 2010, doi: 10.1016/j.jelechem.2010.09.001.

27. Qingwen Li, Gao Hong, Wang Yiming, Luo Guoan, and Ma Jie, "Studies on Self-Assembly Monolayers of Cysteine on Gold by XPS, QCM, and Electrochemical Techniques," *Electroanalysis*, vol. 13, no. 16, pp. 1342–1346, 2021, doi: 10.1002/1521-4109(200111)13:16%3C1342::AID-ELAN1342%3E3.0.CO;2-B.

28. Topolovsek P., F. Lamberti, T. Gatti, A. Cito, J. M. Ball, E. Menna, C. Gadermaier, and A. Petrozza, "Functionalization of transparent conductive oxide electrode for TiO2-free perovskite solar cells," *Journal of Materials Chemistry A*, vol. 5, no. 23, pp. 11882–11893, 2017, doi: 10.1039/c7ta02405c.

29. Samanman Saluma, Proespichaya Kanatharana, Wilaiwan Chotigeat, Panchalika Deachamag, and Panote Thavarungkul, "Highly sensitive capacitive biosensor for detecting white spot syndrome virus in shrimp pond water," *Journal of virological methods*, vol. 173, no. 1, pp. 75–84, 2011, doi: 10.1016/j.jviromet.2011.01.010.

30. Magar Hend S, Rabeay Y A Hassan, and Ashok Mulchandani, "Electrochemical impedance spectroscopy (EIS): Principles, construction, and biosensing applications," *Sensors*, vol. 21, no. 19, p. 6578, 2021, doi: 10.3390/s21196578.