DEVELOPMENT OF COMPETITIVE ELECTROCHEMICAL IMMUNOSENSOR BASED ON GOLD NANOPARTICLE-SANDWICHED STRUCTURE FOR SARS-COV-2 DETECTION

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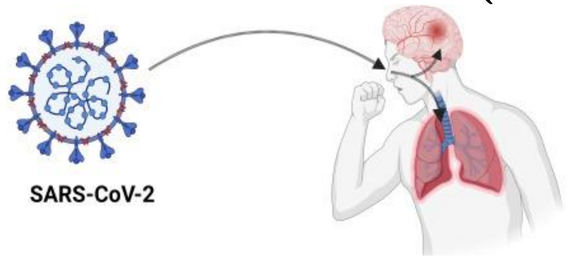
Abstract

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, has significantly impacted global public health and economies, highlighting the urgency for fast, sensitive, and reliable diagnostic tools. Rapid and precise detection of viral antigens is key to controlling the spread of infections and enabling swift treatment. In this study, we successfully developed a competitive electrochemical immunosensor for detecting the SARS-CoV-2 antigen, utilizing a gold nanoparticle-sandwiched structure composed of polyethyleneimine-coated gold nanoparticles (PEI-AuNPs) and nanotags. A 40-fold dilution of PEI-AuNPs is optimal for modifying a screen-printed carbon electrode (SPCE), offering a high specific surface area, rapid electron transfer, excellent electrical conductivity, and good biocompatibility for antigen loading. Subsequently, the spike protein S1+S2 is immobilized onto the modified electrode, resulting in the formation of spike protein S1+S2/PEI-AuNPs/SPCE for specific recognition of antibody. In parallel, gold nanotags were prepared by conjugating PEI-AuNPs with an anti-spike protein S1+S2 antibody, forming PEI-AuNPs/anti-spike protein S1+S2. In the presence of the target, immunocomplexes form through antigen-antibody interactions in the gold nanotag solution. Furthermore, the interaction between spike protein S1+S2/PEI-AuNPs/SPCE and gold/antibody nanotags forms competitive immunocomplexes, facilitating the sensitive detection of the SARS-CoV-2 antigen. Under optimal conditions, the sensor achieved sensitive detection of SARS-CoV-2 antigen within a range of 0.10 to 100 ng/mL with a limit of detection (LOD) of 0.09 ng/mL. The electrochemical immunosensor exhibits high selectivity, reliable reproducibility, and exceptional stability. In addition, the proposed competitive immunosensor can detect SARS-CoV-2 in human serum samples with a good recovery. The outstanding performance offers an important tool for the early screening and detection of the SARS-CoV-2 antigen, especially in combating the ongoing COVID-19 pandemic.

Introduction

➢ Coronavirus disease 2019 (COVID-19)



Symptoms of COVID-19
- **Fever**
- **Dry cough**
- **Fatigue**
- **Loss of taste or smell**

Fig. 1 COVID-19 infection [1]

➢ Electrochemical immunosensor

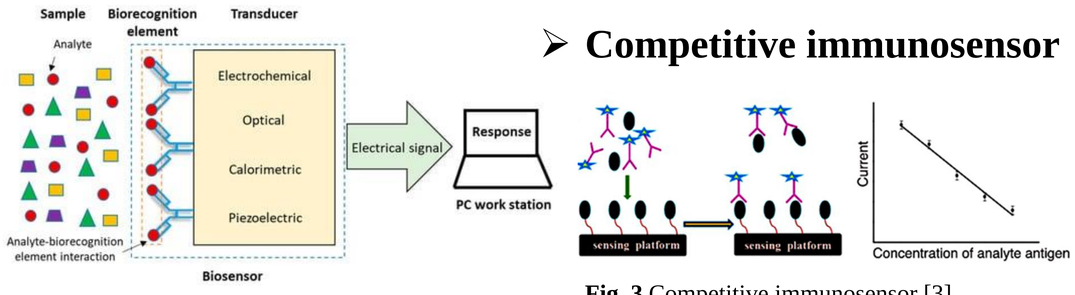


Fig. 2 Electrochemical immunosensor [2]

➢ Competitive immunosensor

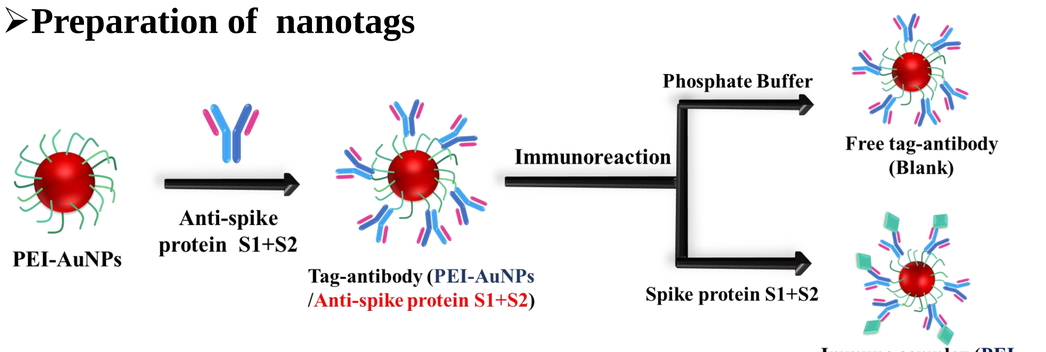
Fig. 3 Competitive immunosensor [3]

Objective

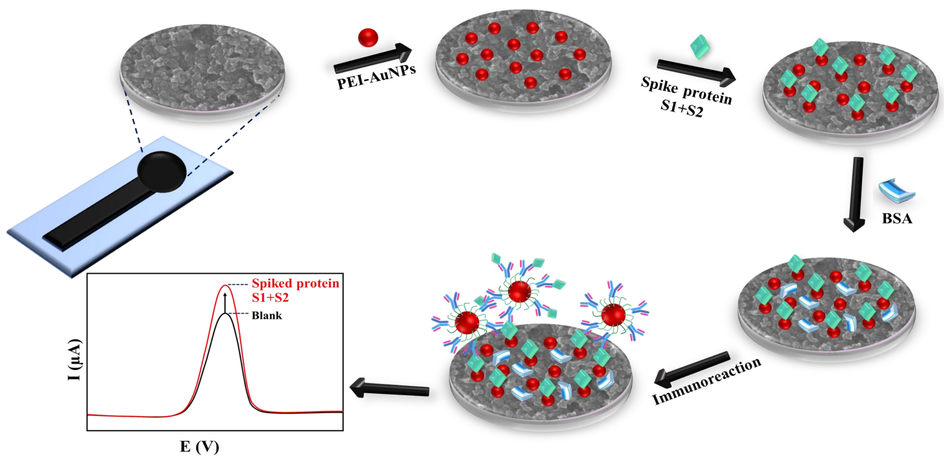
To develop a competitive immunosensor utilizing PEI-AuNPs-modified SPCE and gold nanotags for the sensitive and specific detection of SARS-CoV-2 antigens.

Methodology

➢Preparation of nanotags



➢Fabrication of the immunosensor



Results and discussion

➢ Characterization of modified electrodes

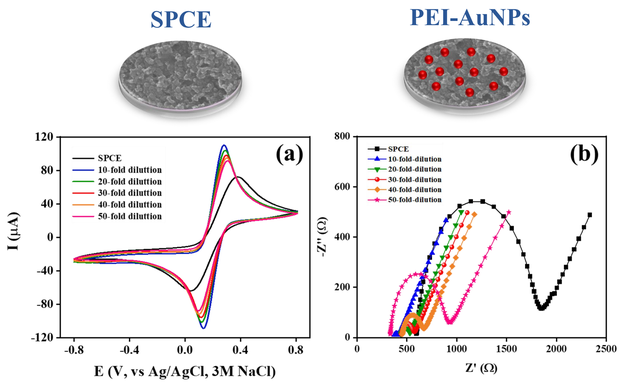


Fig. 4 (a) CVs and (b) EIS spectra of unmodified and modified electrodes with different dilution of PEI-AuNPs at 10-, 20-, 30-, 40-, and 50-fold, measured in 0.010 M PBS (pH 7.4) solution containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$.

➢ Fabrication of the immunosensor

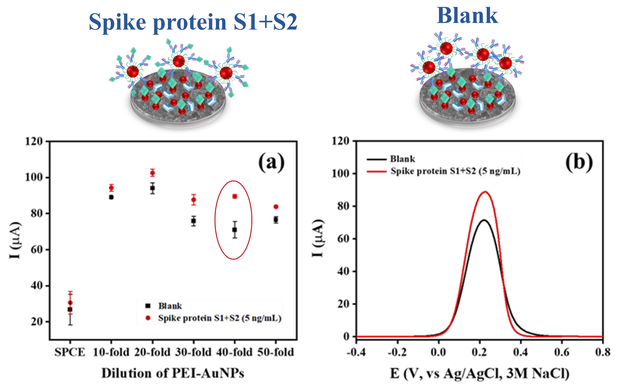


Fig. 5 (a) DPV peak currents after incubation with 0 ng/mL (black) and 5.0 ng/mL (red) spike protein S1+S2 on unmodified and PEI-AuNPs-modified electrodes at various dilutions.(b) DPV curves for stepwise device fabrication in 0.010 M PBS (pH 7.4) with 5.0 mM $[Fe(CN)_6]^{3-/4-}$.

➢ Optimization of fabrication conditions

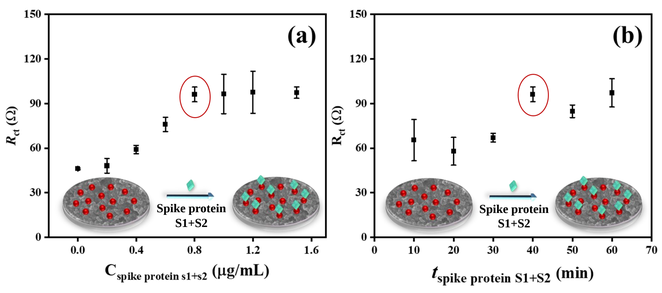


Fig. 6 Optimization of spike protein S1+S2 concentrations (a) and incubation time (b) by employing EIS responses for the immobilized spike protein S1+S2 on 40-fold dilution of PEI-AuNPs-modified SPCE in contact with 0.010 M PBS (pH 7.4) solution containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$.

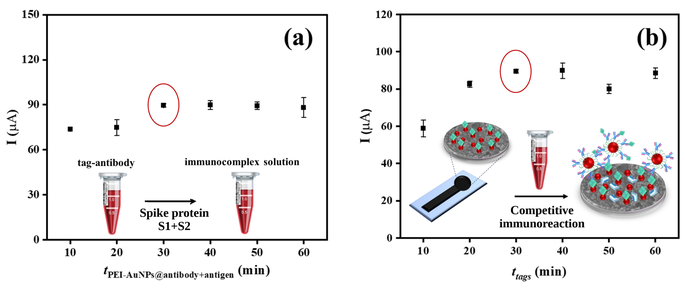


Fig. 7 DPV current responses of (a) immunoreaction time of tags-antibody with antigen and (b) incubation time of tags-antibody-antigen on fabricated electrode (spike protein S1+S2/PEI-AuNPs/SPCE), measured in 0.010 M PBS (pH 7.4) solution containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$.

➢ Analytical performances

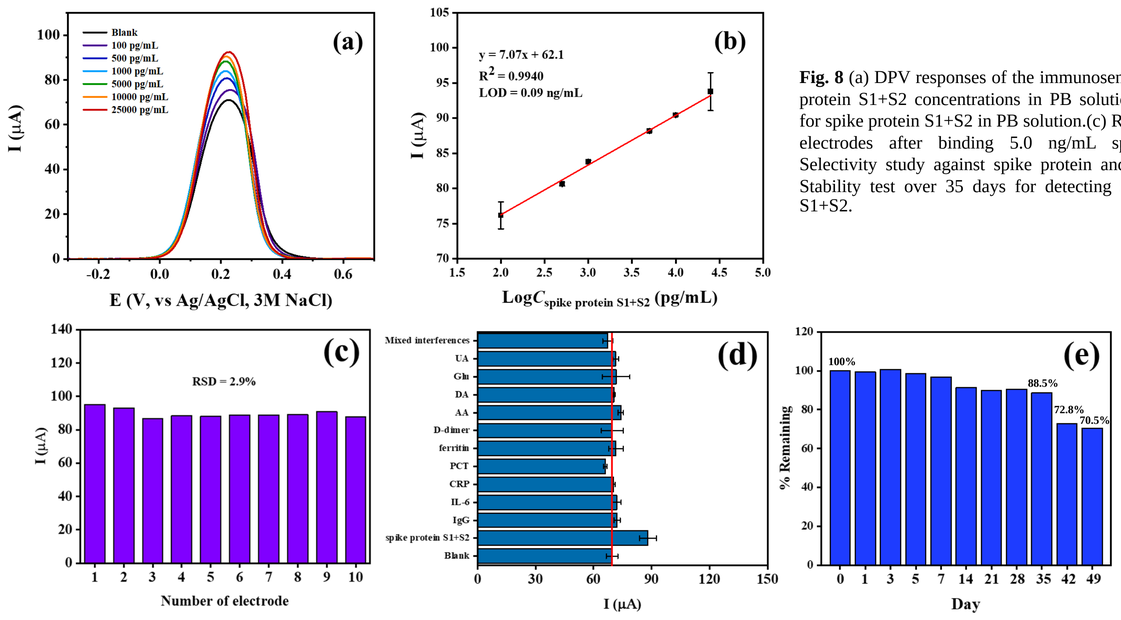


Fig. 8 (a) DPV responses of the immunosensor with increasing spike protein S1+S2 concentrations in PB solution.(b) Calibration curves for spike protein S1+S2 in PB solution.(c) Reproducibility test for ten electrodes after binding 5.0 ng/mL spike protein S1+S2.(d) Selectivity study against spike protein and mixed interferences.(e) Stability test over 35 days for detecting 5.0 ng/mL spike protein S1+S2.

Conclusions

- The 40-fold dilution of PEI-AuNPs provides a large specific surface area, fast electron transfer, and good electrical conductivity, offering sufficient active sites for the immobilization antigens.
- The competitive immunosensor exhibits excellent analytical performance for spike protein S1+S2 detection with a linear logarithmic range of 0.1-25 ng/mL and a low limit of detection (LOD) of 0.09 ng/mL .
- The proposed competitive immunosensor can be applied for the detection of spike protein S1+S2 in human serum samples with good recovery.

References

[1] Awatade, N.T.; Wark, P.A.B.; Chan, A.S.L.; Mamun, S.A.A.; Mohd Esa, N.Y.; Matsunaga, K.; Rhee, C.K.; Hansbro, P.M.; Sohal, S.S.; on behalf of the Asian Pacific Society of Respirology (APSR) COPD Assembly. The Complex Association between COPD and COVID-19. J. Clin. Med. 2023, 12, 3791.

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[3] Chen, H.; Zhang, J.; Huang, R.; Wang, D.; Deng, D.; Zhang, Q.; Luo, L. The Applications of Electrochemical Immunosensors in the Detection of Disease Biomarkers: A Review. Molecules 2023, 28, 3605. https://doi.org/10.3390/molecules28083605

Acknowledgment

Department of Chemistry, Faculty of Science, Chiang Mai University.