



Electrochemical Detection of S-RBD Protein for Point-of-Care SARS-CoV-2 Monitoring Using Platinum-Black-Based Sensor Array

Zeeshan¹ · Sathish Panneer Selvam¹ · Jaehwan Park¹ · Sungho Park¹ · Hee-Eun Kim² · Sungbo Cho^{1,3} 

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Abstract

The COVID-19 pandemic has led to a substantial increase in the advancement of point-of-care (POC) diagnostic tools due to their potential utility in detecting and managing the spread of the disease. Currently, many diagnostic techniques necessitate advanced laboratory equipment and specialized expertise to deliver dependable, cost-effective, specific, and sensitive POC tests for COVID-19 diagnosis. Herein, we report a highly sensitive electrochemical sensor array that features S-RBD protein, covalently anchored on the surface-engineered Pt-black-coated microdisk gold electrodes to monitor severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Computer simulations were performed using different electrode gaps to optimize and fabricate the gold microdisk electrode array. The high sensitivity was ensured by decreasing the electrode gap as well as by depositing Pt-black nanoparticles on the microdisk gold electrodes, by means of chronopotentiometry. The electrical readout depends on monitoring changes in the cyclic voltammograms at the electrode/electrolyte interface as a result of the competitive interaction between monoclonal COVID-19 antibodies and varying antigen concentrations. Overall, the developed electrochemical sensor array exhibits promising electroanalytical capabilities by displaying an excellent linear response ranging from 100 to 1 µg/ml with a detection limit of ~ (0.23 ng/ml). In addition, as a proof-of-concept application, the developed electrochemical sensor array was employed as a sensing platform for the detection of heat-inactivated SARS-CoV-2. Such accomplishments highlight the advantages of low-cost localized electronic devices with high sensitivity and rapid multiple samples detection capabilities to play a crucial role in controlling the spread of infectious diseases like COVID-19.

Keywords S-RBD protein · Pt-black (platinum black) · Heat-inactivated SARS-CoV-2 · Monoclonal COVID-19 antibodies · Microdisk electrochemical sensor array · Chronopotentiometry · Cyclic voltammograms

1 Introduction

The outbreak of the coronavirus disease, caused by the SARS-CoV-2 virus was first seen in the city of Wuhan, China, on December 2019. On February 11, the World Health Organization (WHO) officially designated the disease caused by SARS-CoV-2 as COVID-19 (Corona Virus Disease 2019), and later, WHO declared COVID-19 to be

a global pandemic, affecting regions all around the world. Because of its rapid transmission via direct contact, the total number of cases worldwide surpassed 346 million, resulting in approximately 5.5 million deaths by January 2022 [1]. Controlling the spread of the COVID-19 disease has hinged on the critical element of early detection. Early detection has played a pivotal role in preventing further health, social, and economic consequences, allowing for the implementation of appropriate countermeasures in a timely manner.

To date, several diagnostic and detection techniques have been developed to combat the virus's spread. One of the most popular methods is reverse transcriptase real-time polymerase chain reaction (RT-PCR), which offers rapid and dependable quantification of mRNA transcription [2]. It is known for its speed and effectiveness, allowing the precise quantification of gene quantities in target sequences with a high degree of specificity and sensitivity [3–6]. However,

✉ Sungbo Cho
sbcho@gachon.ac.kr

¹ Department of Electronic Engineering, Gachon University, 1342 Seongnamdaero, Seongnam 13120, Gyeonggi, Korea

² Department of Dental Hygiene, Gachon University College of Medical Science, Incheon 21936, Korea

³ Department of Health Science and Technology, GAIHST, Gachon University, Incheon 21999, Korea

PCR tests require controlled laboratory environments, specialized protocols, and sophisticated equipment, making them impractical for applications in the resource-limited settings of developing countries [7]. Also, it is so sensitive that even trace amounts of residual DNA from previous tests or impurities can lead to inaccurate results [8, 9].

On the other hand, immunologic assays use antibody–antigen interactions to detect viral antigens, such as the nucleocapsid (N) protein or spike (S) protein as well as to detect the immune response (antibody) against SARS-CoV-2 [10, 11]. When an antigen binds to a capture antibody on a solid substrate, the detection antibody linked to suitable reporters binds to an additional epitope on the target antigen. This interaction leads to the generation of electrochemical or optical signals. Immunoassays have been adapted into various formats suitable for point-of-care (POC) diagnosis, including lateral flow assays [12–14]. However, these devices either require long analysis periods exceeding 1 hour or susceptible to produce false-positive results [15]. False-positives often result from non-specific interactions between the target and immobilized proteins, representing a significant contributor to inaccuracies [16]. As a result, there is still a high demand for a rapid, highly sensitive, specific, and precise approach to COVID-19 diagnosis in point-of-care testing to effectively manage the pandemic.

Electrochemical biosensors are widely utilized analytical instruments for transducing enzymatic reactions related to a diverse array of protein and nucleic acid analytes [17–23]. Furthermore, due to their scalability, high sensitivity, selectivity, rapid response time, relatively lower costs, and ease of use, electrochemical sensors have recently gained attention as a promising diagnostic tool for COVID-19 [24–30]. These exceptional attributes make these sensors essential tools for the precise monitoring of biomarkers and early detection of various diseases. However, a critical challenge in electroanalytical methods is the interference caused by biomolecule adsorption on the sensor surface, limiting their potential applications. Since, the sensor's immobilization capacity, sensitivity, conductivity, and specificity depend upon the surface area and material composition of the electrode [31, 32]. One effective solution to tackle this issue involves designing efficient electrochemical sensors modified with specific nanomaterials or molecules, thereby enhancing their efficiency and improving the analytical performance.

Noble metals like gold and platinum possess excellent properties that make them highly valuable for various biosensing applications [33, 34]. Pt shares similar characteristics with gold, including excellent conductivity, chemical stability, high surface area, biocompatibility and a strong interaction with thiol groups [35–38]. However, one key advantage of Pt is its superior hydrophilicity particularly in the form of Pt-black compared to gold. This enhanced hydrophilicity facilitates better wetting and adsorption of

biomolecules onto the electrode surface, promoting stronger binding interactions and improved sensitivity. Furthermore, Pt-black's high electrochemical active sites and robust catalytic activity facilitate enhanced electron transmission, thereby accelerating electrochemical reactions, resulting in fast response times and improved detection limits [39, 40].

Among the various proteins found in coronaviruses, a significant focus for vaccines and antibody development is the spike glycoprotein (S-protein). This protein plays a crucial role in recognizing receptors and facilitating the fusion of the viral and host cell membranes, enabling the virus to enter the host cell [41, 42]. The S-protein comprises two subunits: S1 and S2. The S1 subunit contains a receptor-binding domain (RBD), facilitating the attachment of the virus to the host cell surface. Whereas, the S2 subunit is responsible for facilitating the fusion of the viral and host cell membranes. The S-RBD protein of SARS-CoV-2 displays a higher binding affinity for hACE2 (human angiotensin-converting enzyme 2) compared to the S-RBD of SARS-CoV, leading to increased transmissibility [43].

Considering all the aforementioned information, we report a comprehensive study based on the development, detail characterization, assay optimization, and assessment of heat-inactivated samples using a highly sensitive electrochemical sensor array for detecting S-RBD protein. The selection of the S-RBD protein was based on its clinical significance in COVID-19 diagnostics. The developed sensor utilizes a gold disk electrode (AuDE) array modified with Pt-black nanoparticles. Initially, computer simulations were performed using different electrode gap configurations to optimize and fabricate the gold microdisk electrode array. The heightened sensitivity (in terms of current density and capacitance) was attained through the reduction of the gap distance between the working and counter electrodes within the gold microdisk electrode array. In addition, the deposition of Pt-black nanoparticles on the microdisk gold electrodes contributed to the enhanced analytical sensitivity in detecting the S-RBD protein. Scheme 1 illustrates the fabrication process and the operational mechanism of the developed electrochemical sensor. The combination of a large surface area, superior conductivity, and a greater number of electrochemically active sites provided by the Pt-black nanoparticles enables rapid and precise detection. The electroanalytical approach is based on the competitive interaction between a constant concentration of monoclonal COVID-19 antibodies and S-RBD protein immobilized on the electrode surface (biomarker). The electrical responses associated with varying antigen concentrations were observed using cyclic voltammograms, specifically by tracking changes in the electron transfer rate at the interface between the electrode and electrolyte. The developed electrode array demonstrated the capability to detect S-RBD protein at concentrations as low as 100 pg/ml in buffer saline. Furthermore, as a

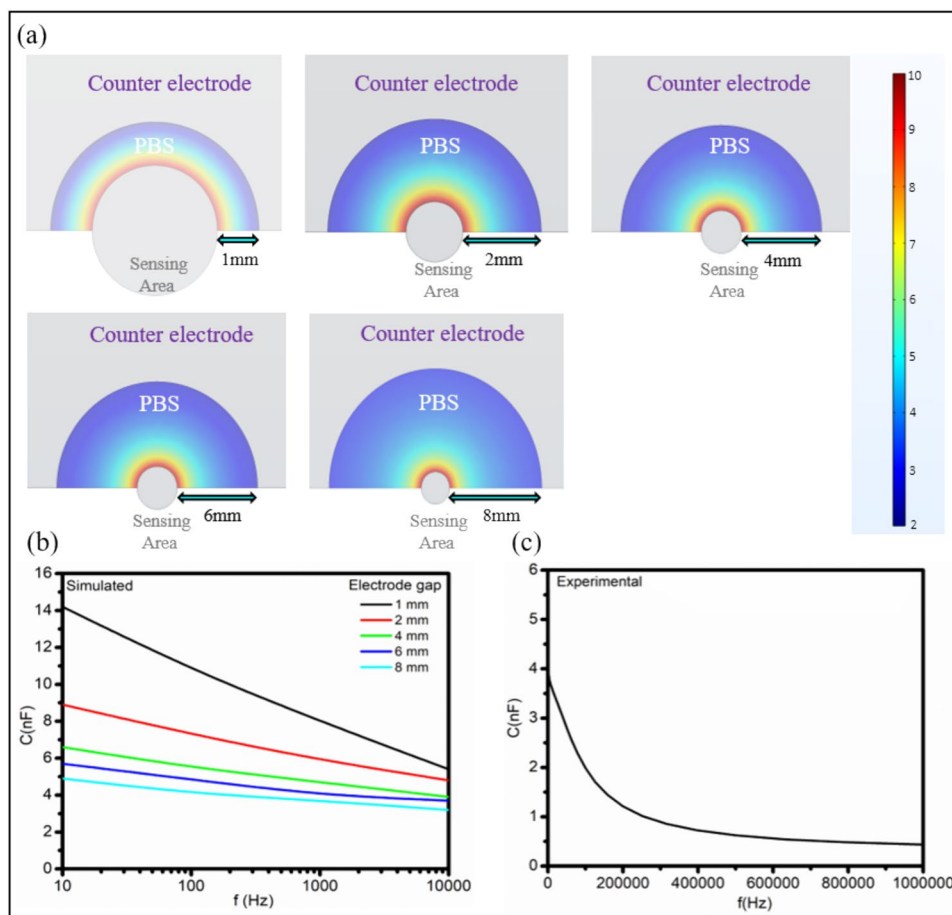
spin coater (SPIN-1200 D) at 1500 rpm for 40 s, followed by a soft bake at 95 °C for 90 s. Subsequently, the photoresist-coated substrate was aligned with a photomask using a mask aligner (Model: MDA-400M) for an exposure duration of 18 s under light radiation. After exposure, the substrate underwent post-exposure baking at 110 °C for 2 min and 30 s. The next step involved immersing the substrate in a developer solution (AZ 300 MIF) for 10–15 s to eliminate the unexposed photoresist areas. Unexposed gold regions were then removed at room temperature using etchant GE-301G. Subsequently, the remaining DNR-L300-30 photoresist was eliminated using acetone, resulting in the desired pattern on the gold-coated substrate. Following this, the glass substrate underwent a 15-min ultrasonic cleaning process, and an insulation layer of SU-8 2002 was applied upon completion of the deposition process. As a first prototype, all the surface engineering related to the fabrication of the microdisk electrode array was adapted from the optimized electrical properties of the microdisk electrode array based on the finite element approach by using COMSOL™ Multiphysics software as shown in Fig. 1a. To facilitate electrochemical deposition and store the solvents for immuno-reaction and electrochemical measurements, a polystyrene

8-well chamber was attached to the microdisk electrode array with eight reaction chambers. Polystyrene was chosen due to its advantageous properties, such as being non-toxic, cost-effective, and capable of securely holding solvents. This ensures that the solvents remain in close proximity to the electrode surface, thereby facilitating efficient reactions.

2.3 Preparation of Pt-Black-Enhanced Electrochemical Sensor

Pt-black was electrodeposited using a three-electrode configuration after the gold microdisk electrode array was cleaned with ethanol and DI water. The deposition was carried out in the sensing area chamber on the gold microdisk electrode, which contains 2.5% hydrogen hexachloroplatinate (IV) hexahydrate, lead diacetate trihydrate (0.05%), and HCl (0.01 M). Lead diacetate trihydrate decreases the over potential and accelerates the conversion of PtCl_6^{2-} to platinum metal Pt(0), which is the key step to deposit platinum black on the electrode surface [44]. Pb ions, when adsorbed onto the electrode surface, serve as potential nucleation sites for the formation of Pt islands. In addition, the presence of Pb ions results in a reduction in the conversion of Pt(IV) to Pt(II) during the

Fig. 1 Computer simulations to assess electrode gap influence for microdisk sensor optimization. **a** Finite element simulations of current distributions for different 3D models of microdisk electrode configuration. **b** Output performance based on simulation results for different electrode gaps. **c** The experimental results of the optimum electrode, which was fabricated based on the simulation results



electrodeposition process, thus effectively lowering the energy barrier required for the reduction of Pt(IV) to Pt(0). Pt-black electrodeposition was conducted using a constant current density of -2.5 mA per square centimeter for a duration of 400 s, with reference to the external Ag/AgCl reference electrode, in a three-electrode configuration. The developed Pt-black electrode was cleaned before being incubated for 12 h at room temperature in a solution of 6-mercaptohexanoic acid (100 mM). Following the modification with a self-assembled monolayer (SAM), the electrode surface was washed with ethanol and deionized water. To anchor antibodies onto the SAM-modified electrode (DE), the carboxyl groups of MHA were activated within an aqueous solution of 200 mM EDC and 50 mM NHS for a duration of 15 min. Next, 10.0 μ l of antibody solution (10 μ g/ml, prepared in PBS) was applied onto the electrode surface to immobilize the antibody for 30 min. Following that, PBS was used to wash the electrode to remove unattached materials from the electrode surface. Subsequently, the electrode surface was incubated using 0.5% BSA (bovine serum albumin) to block the non-specific sites. Finally, the developed Pt-black-based electrochemical sensor was washed with PBS before being used for S-RBD protein detection experiments.

2.4 Electrochemical Signal Readout

To assess the analytical range of the SARS-CoV-2 electrochemical sensor, 10.0 μ l of different concentrations (ranging from 1 pg/ml to 1 μ g/ml) of the S-RBD protein were prepared in PBS and then allowed to incubate for 5 min on the developed microdisk electrode configuration. Subsequently, the electrodes were washed with PBS buffer, and cyclic voltammetry measurements (CV) were conducted. In order to perform the electrochemical signal readout, the designed sensor array must be integrated into the measuring setup both electrically and fluidically, which is accomplished via a specially designed chip holder. The setup used here consists of a polystyrene-made 8-well chamber to hold the solvent of virus samples, a potentiostat (Emstat Pico MUX) for signal readout, and a laptop for displaying the acquired signal. The sensing array was connected to the Emstat Pico MUX system (PalmSens, Netherlands), which can monitor the electrical readouts of the eight-channel sensing array simultaneously using PSTrace (PalmSens, Netherlands) software. The real-time data (cyclic voltammograms) were collected for each sensor by applying a sweep starting from -1.0 V and stopping at 1.0 V.

3 Results and Discussions

3.1 Computer Simulation of Electrode Properties

The optimum electrical characteristics of the gold microdisk electrode array were calculated using COMSOL™

Multiphysics software. For finite element simulations, a 3D geometry of the fabricated microdisk electrode array was designed and simulated in a frequency domain electric current module. The model consists of a buffer layer (PBS: $\sigma = 1.6$ S/m, $\epsilon = 80$), a counter electrode, and a microdisk-working electrode (Gold: $\sigma = 4.11 \times 10^7$ S/m, $\epsilon = 1.143$). The simulations were performed at a fixed voltage in the frequency range of 10 Hz– 10^4 Hz. This is because the capacitance change was most sensitively measured at this frequency. The electrode gap parameters were controlled to determine the optimum electrical properties for the gold microdisk electrodes:

The electrode gap (1–8 mm) between the working and counter electrode was analyzed and the current distributions of different microdisk electrode models were derived as shown in Fig. 1. The following equations were used to determine the capacitance based on the current data:

$$J = \sigma E + j\omega D + J_e, \quad (1)$$

$$Z = \frac{V}{I}, \quad (2)$$

$$Z = j\omega L + (i\omega C)^{-1}, \quad (3)$$

$$C = -i(2\pi f \cdot Z)^{-1}, \quad (4)$$

where J stands for current density, E represents the electric-field intensity, D for electrical permittivity and J_e stands for externally produced current. Equation (1) was used to determine the current for each frequency from the software, while the impedance (Z) value may be determined from the current and voltage value set at (200 mV) using Eq. (2). Since the inductor (L) parameter in the equivalent circuit is almost zero, therefore the imaginary part of impedance and frequency were used to calculate the capacitance as illustrated in Fig. 1b using Eq. (4) [45]. In Fig. 1b, it is evident that as the gap distance between the working and counter electrodes decreases, the capacitance response or the sensitivity of the sensor increases. This increase in capacitance response or heightened sensitivity can be attributed to several electrochemical factors including enhanced electric-field intensity, improved double layer formation, reduced diffusion distance and decreased inter-electrode resistance, all of which contribute to high charge transfer and mass transport, ultimately leading to more effective detection and response to analytes molecules.

Furthermore, the comparison of different electrode configurations in Fig. 1 demonstrates that the electrode with a 1 mm gap distance between the working and counter electrodes outperformed the others with higher gap distances. This suggests that a smaller electrode gap leads to superior sensor performance, reinforcing the significance

of considering this parameter. Based on the simulation results and the expected high performance indicated by the observed trends, we proceeded to fabricate a prototype of an electrode array with eight working disk electrodes and a common counter electrode for the detection of the S-RBD protein. The empirical data obtained from the fabricated electrodes aligned well with the simulation results, further validating the effectiveness of the optimized electrode configuration in achieving high sensitivity. Hence, our computational analysis clarifies not only the relationship between electrode configuration and sensor sensitivity but also acts as a crucial guiding principle in the design and optimization of sensor platforms for biomedical applications.

3.2 Formation of the Self-Assembled Monolayer (SAM) on the Pt-Black Electrode

In the process of developing an electrochemical sensor, the deposition of a SAM layer on the electrode surface is a fundamental step as it provides an organized framework for controlled and precise interaction between the biosensor and target molecules [46]. Prior to the formation of the SAM on the Pt-black-based electrode, the electrode surface was thoroughly cleaned to remove impurities. Following the cleaning step, the electrode was then immersed in a solution of 6-mercaptopentanoic acid (100 mM) for 12 h at room temperature.

To verify the presence of a SAM on the electrode surface, AFM (atomic force microscopy) was used to assess the degree of roughness and surface topologies for both the unmodified and SAM-modified electrode surfaces. In Fig. 2a, d, you can observe the surface topology and the associated height profile of the AuDE in its pristine condition. Figure 2b, e display the surface topology and height profile of the Pt-black electrode, while Fig. 2c, f present the surface topology and corresponding histogram height profile of the Pt-black electrode after modification with the SAM. Following the SAM modification, the electrode's surface exhibited a noticeable smoothing effect, resulting in a reduced roughness of ($R_q = 3.026 \mu\text{m}$) in contrast to the ($R_q = 3.051 \mu\text{m}$) roughness observed before the SAM modification. This difference in roughness ($\sim 25 \text{ nm}$) provides clear evidence for the successful formation of the SAM on the Pt-black-based electrode.

Furthermore, EDS analysis was carried out to confirm the elemental composition of the SAM modification on the electrode's surface. Following the SAM modification, sulfur (S) was found with an atomic percentage of 11.17%, as seen in Fig. 2i. This finding validates the effective creation of the SAM layer on the electrode surface through the robust bonding between Pt-black and sulfur.

The re-confirmation of the deposition of the SAM on the Pt-black electrode surface was successfully performed by the treatment with NaBH_4 solution to remove SAM from

the electrode surface. The SAM-modified electrode was immersed in 0.5 M NaBH_4 solution for 20 min to completely remove the SAM layer, and to restore the original electrochemical characteristics. The CV results demonstrate a significant increase in current intensity after the removal of the SAM layer as shown in Figure S1(b). This increase is nearly equivalent to the current observed in the CV results for the pristine Pt-black electrode as shown in Figure S1(a). The outlined procedure for removing the SAM layer from the electrode surface serves as a verification step to confirm the successful formation of the SAM on the Pt-black electrode surface.

3.3 Electrochemical Characterization of the (AuDE/Pt-black/SAM/EDC-NHS/anti-SARS-CoV-2 spike RBD) Sensor

The stepwise functionalization process of the developed sensor was evaluated by assessing its electrochemical performance in a 10 mM phosphate-buffered saline (PBS) solution at a scan rate of 80 mV/s. Figure 3a, b illustrates the cyclic voltammograms (CV) and capacitance profiles obtained from the following electrodes: bare AuDE, Pt-black-deposited gold disk electrode (Pt-black/AuDE), SAM/Pt-black/AuDE, anti-SARS-CoV-2/SAM/Pt-black/AuDE and BSA/anti-SARS-CoV-2/SAM/Pt-black/AuDE. Initially, the bare AuDE displayed a nearly flat voltammetric response due to their poor conductivity. However, upon depositing Pt-black, the electrodes displayed the appearance of a pair of redox peaks and a significant increase in the peak current (I_p). This increase in the peak current (I_p) can be attributed to both the expanded surface area and the inherent conductivity of the Pt-black-NPs [47]. In the next step, the electrode surface was modified with the SAM, leading to a significant decrease in the peak current. The sharp decline in peak current suggests the non-conductive nature of both the hydrocarbon chains and carboxylic acid groups within the SAM, hindering the movement of the electron transfer toward the electrode surface. Finally, the antibody was immobilized on the sensor surface to complete the fabrication of the electrochemical sensor. After the antibody immobilization, the current intensity in the CV exhibited a further reduction. This reduction was attributed to the formation of a supramolecular inclusion complex, which hindered the electron transfer at the electrode/solution interface. These findings provide confirmation of the successful attachment of anti-SARS-CoV-2 antibodies to the electrode surface, which is responsible for recognizing RBD spike protein after immobilizing the surface with the non-specific blocking agent (BSA). The developed sensor was employed for electrochemical sensing of RBD spike protein at different concentrations, which were prepared in phosphate-buffered saline (PBS).

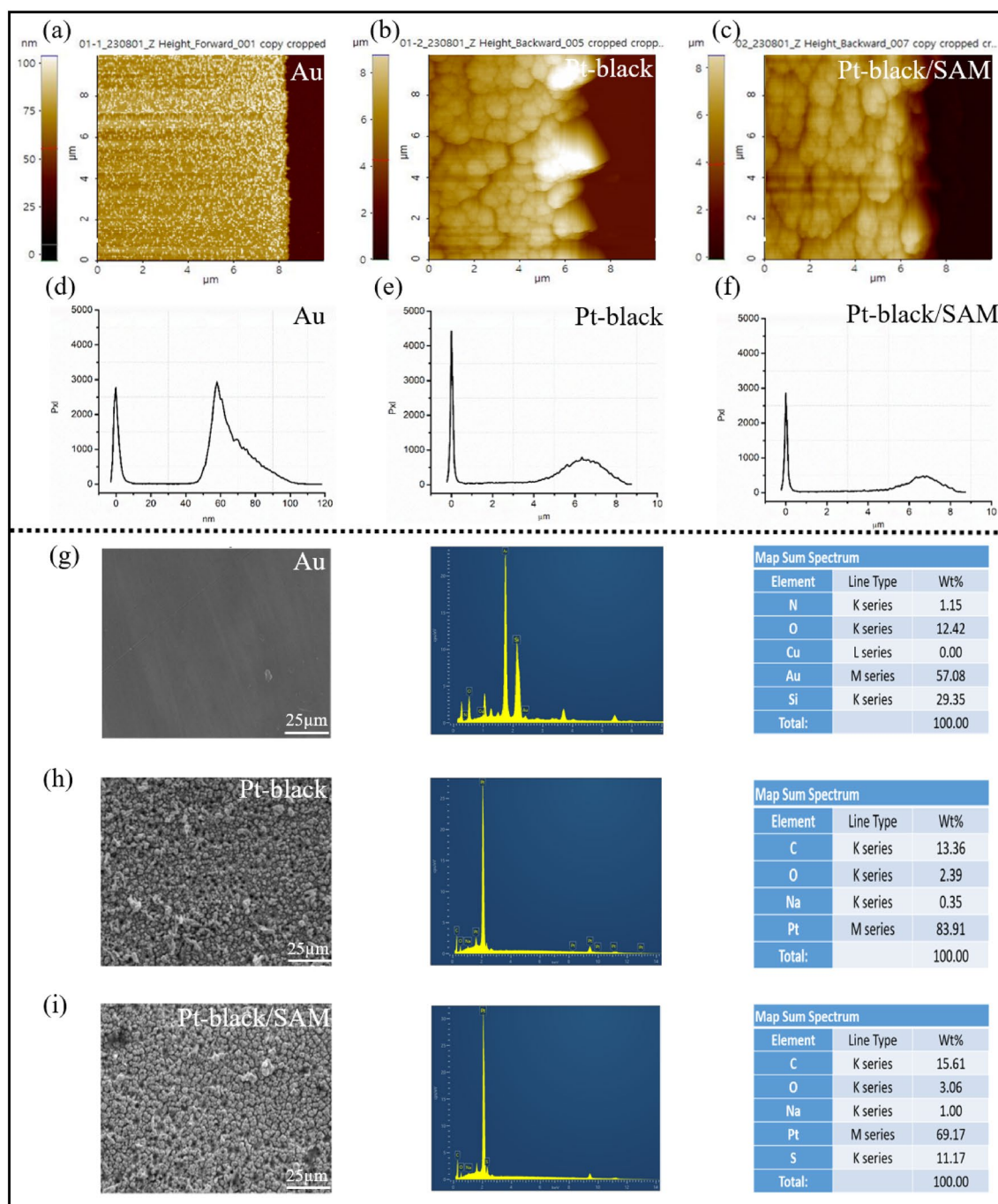


Fig. 2 AFM surface topologies for **a** Bare Au electrode, **b** Pt-black deposited Au electrode **c** SAM-modified Pt-black electrode. Histogram height profiles of **d** Bare Au electrode, **e** Pt-black deposited Au

electrode, **f** SAM-modified Pt-black electrode. SEM and EDS results for **g** Bare Au electrode, **h** Pt-black deposited Au electrode, **i** SAM modified Pt-black electrode

Furthermore, the developed sensor was employed for capacitance detection in a phosphate-buffered saline (PBS) solution. The reduction in electrode capacitance following the deposition of SAM, anti-SARS-CoV-2, and BSA can be attributed to the creation of a sequence of dielectric layers at the electrode/solution interface. The sensor's capacitance at the interface between the electrode and the

solution is characterized by a series of multiple capacitors as depicted in Fig. 3b [48]. The decrease in electrode capacitance observed after the sequential deposition of SAM, anti-SARS-CoV-2, and BSA serves as evidence of S-RBD spike protein detection through a capacitance-based sensing mechanism.

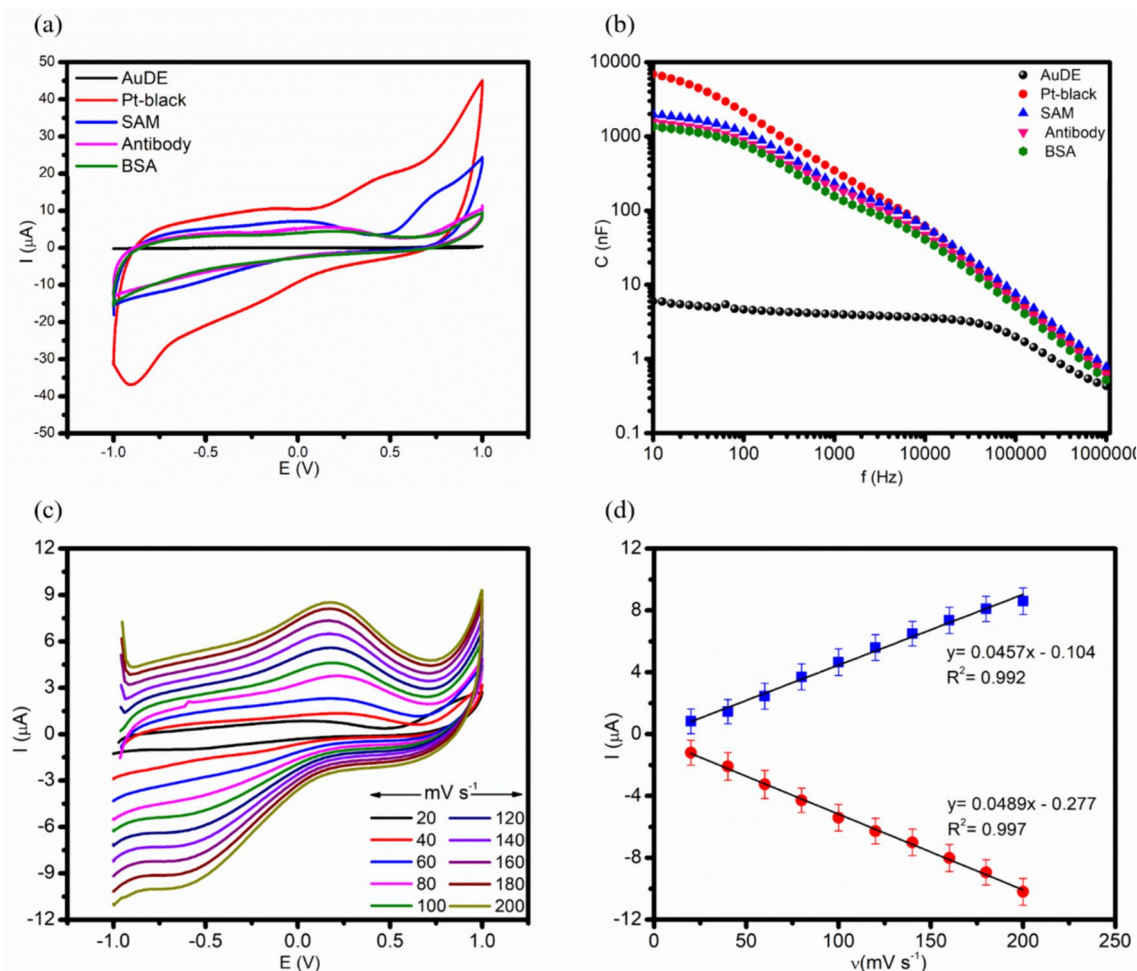


Fig. 3 **a** CV response and **b** Capacitance of the development of sensor in 10 mM PBS obtained at each modification step of AuDE, Pt-black deposition, SAM immobilization, antibody anchoring and immobilization of non-specific blocking agent (BSA) at a scan rate

of 80 mV/s. **c** The cyclic voltammograms of 10 mM PBS containing 1 ng/ml of spike RBD protein at various scan rates 20–200 mV/s. **d** The plot of peak current vs scan rate relative to electro-oxidation of 10 mM PBS

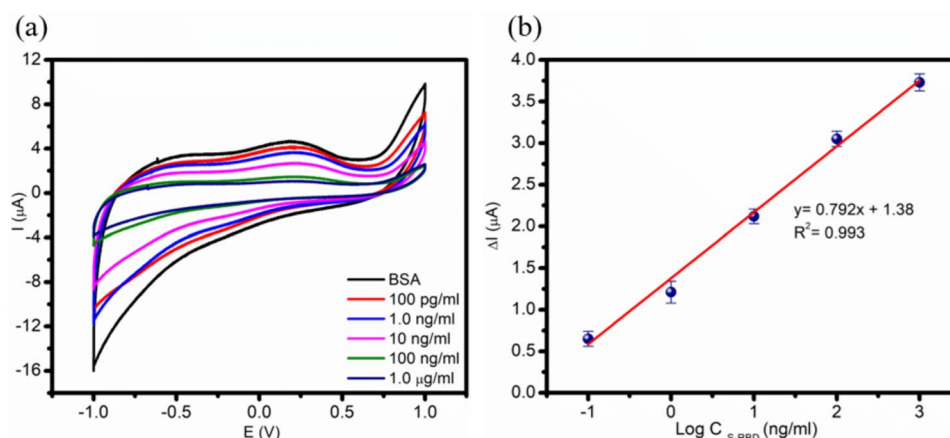
3.4 Scan Rate Study

Using CV, we examined how different scan rates affect the oxidation peak currents of protein in PBS solution containing 1 ng/ml of S-RBD protein as depicted in Fig. 3c. As the scan rate increased from 20 to 200 mV/s, the oxidation peak currents exhibited a linear increase that was proportional to the scan rate. Figure 3d illustrates the relationship between the peak current and the scan rate. The linear relationship observed between oxidation and reduction peak currents and the scan rate yielded linear equations represented as $Y = 0.0457x - 0.104$ and $Y = 0.0489x - 0.277$ with correlation coefficients (R^2) of 0.992 and 0.997 respectively. Based on these findings, it is confirmed that the oxidation reaction of protein followed an adsorption-controlled process and is likely to be dependent on oxide groups at the electrode's surface [49].

3.5 Analysis of S-RBD Protein Adsorption on Electrode Surface

The electrochemical response of the fabricated sensor toward various S-RBD protein concentrations was evaluated in the presence of a fixed concentration (10 $\mu\text{g/ml}$) of monoclonal COVID-19 antibody and PBS. Under optimized parameters, CV voltammograms were used to examine different protein concentrations as shown in Fig. 4a. Following the incubation of protein, the sensor exhibited a progressive decline in its current response from low to high concentrations (100 pg/ml to 1 $\mu\text{g/ml}$). It is because, the specific binding of S-RBD protein and COVID-19 antibody facilitates the formation of an insulating layer on the electrode surface, which hinders the electron transfer between the electrode and electrolyte. As illustrated in Fig. 4a, the sensor exhibited a significant fluctuation in current as protein concentrations varied, and

Fig. 4 **a** The cyclic voltammograms obtained at various concentrations of S-RBD protein. **b** The plot of logarithmic concentration against ΔI



there was no apparent saturation even at a concentration as high as 1 $\mu\text{g/ml}$. Furthermore, calibration experiments were conducted to extract favorable electroanalytical results from the calibration curve. This includes an excellent linear regression ($R^2 = 0.993$) from 100 pg/ml to 1 $\mu\text{g/ml}$ with a detection limit (LOD) of ~ 0.23 ng/ml as shown in Fig. 4b.

Furthermore, the analytical performance of the developed electrochemical sensor was evaluated in comparison to similar SARS-CoV-2 detection electrochemical sensors reported in the literature, as summarized in Table S1. In this context, the developed platinum-black-based sensor array exhibited electrochemical performance that was either comparable or superior to that of other electrochemical sensors designed for the detection of SARS-CoV-2. In particular, we can assess the superiority of our research over previous studies in four specific sections: (1) High sensitivity and long-term stability of the developed electrochemical sensor were achieved by covalent immobilization of antibodies on the metal surface (Pt-black). This is in contrast to physical adsorption, which is known to have drawbacks such as low stability, poor reproducibility, and limited control over the orientation of immobilized molecules [50]. (2) The multi-channel electrochemical readout led to a significant improvement in the accuracy of diagnosing SARS-CoV-2. (3) Low detection limit accompanied by a rapid response time of 5 min. (4) The numerous electrochemically active sites offered by Pt-black nanoparticles serve as signal amplifiers, thereby facilitating heightened sensitivity and precise detection.

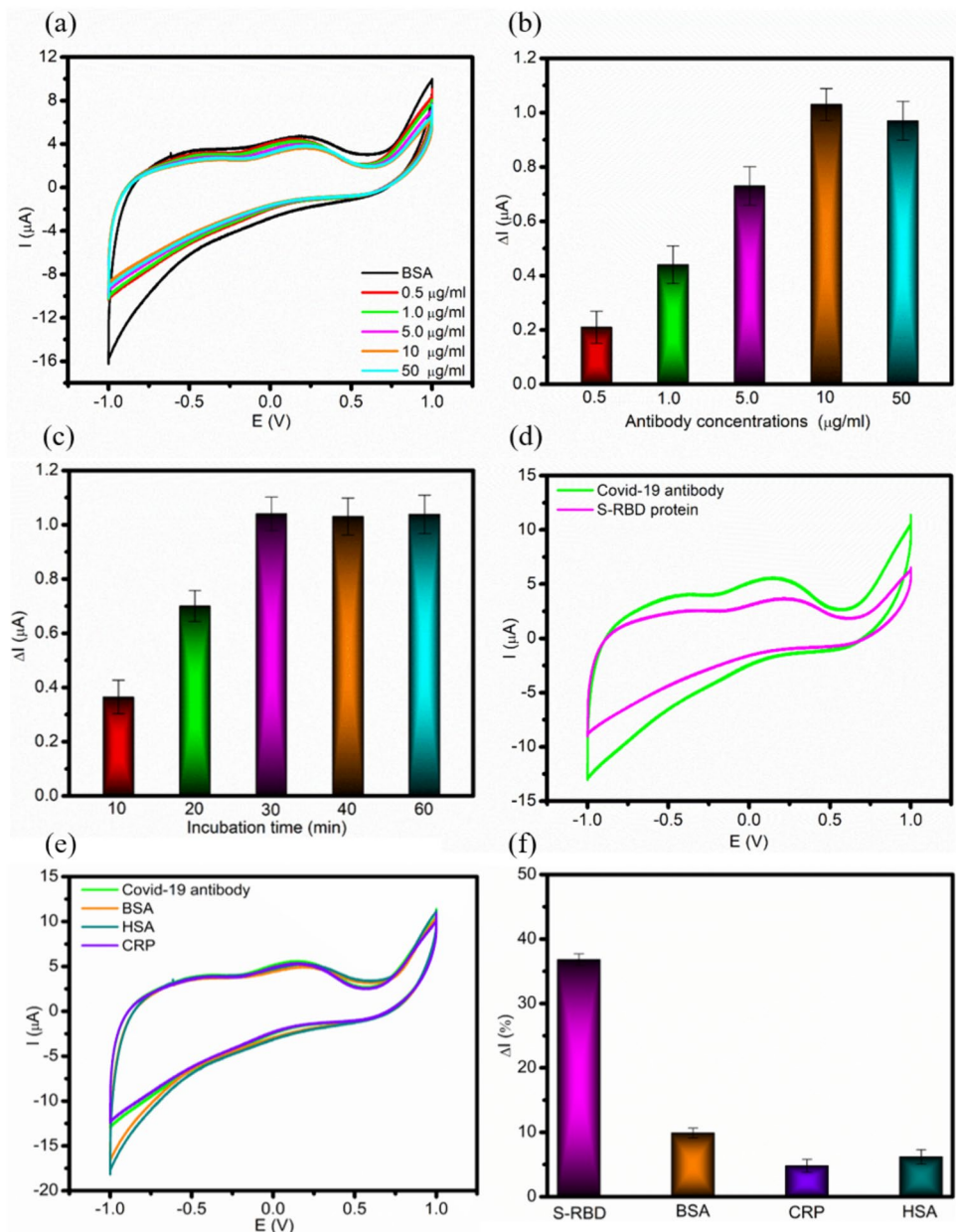
3.6 Optimization of Antibody Concentrations

The experimental parameters that hold significant influence over the sensor performance primarily include the concentration of antibodies and incubation time. Hence, various concentrations of COVID-19 antibody, specifically 0.5, 1, 5, 10, and 50 $\mu\text{g/ml}$, were investigated to determine the concentration that produces the highest signal value when interacting with the biomarker/antigen (S-RBD protein) as shown in

Fig. 5a. The experiment revealed that lower concentrations of antibodies were insufficient to effectively bind all the proteins present, leading to a correspondingly low current signal. However, the current response exhibited a noticeable increase with the high antibody concentration, reaching a plateau at 10.0 $\mu\text{g/ml}$ as depicted in Fig. 5b. Beyond this point, the response remained nearly unchanged even as the antibody concentration increased further, suggesting that the surface had reached a state of saturation with the antibody. Therefore, a concentration of 10.0 $\mu\text{g/ml}$ was selected as the ideal antibody concentration for constructing the electrochemical sensor. Furthermore, the impact of incubation time was evaluated, which reveals that a duration of 10 and 20 min did not produce satisfactory sensitivity. Therefore, 30 min was chosen as the optimal incubation time, as it represents the minimum period required for the effective formation of antibody–antigen–antibody as shown in Fig. 5c.

In addition, the specificity of the monoclonal COVID-19 antibody in binding to the S-RBD protein was investigated in comparison to non-specific proteins. This control experiment was conducted to clarify the essential function of the monoclonal COVID-19 antibody in identifying specific biomarkers. Therefore, the electrochemical response of the developed electrochemical sensor was analyzed in the presence of a fixed concentration (10 $\mu\text{g/ml}$) COVID-19 antibody, using a coexistence matrix consisting of proteins such as BSA, human serum albumin (HSA) and c-reactive protein (CRP). BSA (5 mg/ml), HSA (10 $\mu\text{g/ml}$), and CRP (5 $\mu\text{g/ml}$) were chosen to individually assess potential cross-reactivity with each protein, evaluate sensor specificity in the presence of common serum proteins, and identify any interferences affecting accurate detection of the specific protein (S-RBD) in biologic samples. As shown in Fig. 5e, no significant changes were observed in the current response after the interaction of non-specific proteins (BSA, HSA, and CRP) exposed to the monoclonal COVID-19 antibody. Notably, significant changes in current response occur when the specific protein (S-RBD) interacts with the same concentration

Fig. 5 CV results to optimize the important parameters of the developed electrochemical sensor including **a**, **b** Concentration of monoclonal COVID-19 antibody and **c** Incubation time. Selectivity of the electrochemical sensor. **d** Changes in the CV response carrying the specific protein (S-RBD) as biomarkers and **e** changes in the CV response carrying the non-specific proteins (BSA, HSA, and CRP) as biomarkers. **f** Percentage change in the current response achieved by the electrochemical sensor in the presence of specific and non-specific biomarkers. The selectivity study was conducted after being incubated with 10 $\mu\text{g/ml}$ COVID-19 antibody for 30 min



of monoclonal COVID-19 antibody, as depicted in Fig. 5d, resulting in a ΔI value of 38% for S-RBD protein, compared to ΔI values of 9% for BSA, 4.8% for CRP, and 6.2% for HSA. These results indicate two important findings (1) the selectivity of the antigen–antibody interactions at the developed SARS-CoV-2 electrochemical sensor, emphasizing that the monoclonal COVID-19 antibody did not interact with the non-specific protein. (2) the specificity of the SARS-CoV-2 electrochemical sensor, as no non-specific adsorption was detected in the absence of the specific biomarker (COVID-19 S-RBD protein). Importantly, these findings align with the inherent nature of the monoclonal COVID-19 antibody used in the study. Monoclonal antibodies offer superior specificity compared to polyclonal antibodies because they

target a single epitope, thus preventing cross-reactions with non-specific proteins [51]. Furthermore, monoclonal antibodies enhance reproducibility as they maintain consistent characteristics across different batches, making them a popular choice for clinical diagnosis [52].

3.7 Heat-Inactivated SARS-CoV-2 Variant (B.1.1.7)

To evaluate the reliability and accuracy of the developed electrochemical sensor, we conducted an analysis to diagnose a heat-inactivated sample of the SARS-CoV-2 variant (B.1.1.7) with an unknown concentration. The fabricated electrochemical sensor quantifies the concentration of the RBD spike protein in a SARS-CoV-2 sample using a

standard addition approach. Therefore, five different solutions (100ul each) were prepared in PBS with the same concentration and volume (10ul each) of the unknown (heat-inactivated) sample but varying the amount of the known (S-RBD protein) samples at concentrations of 0, 2, 4, 6, and 8 ng/ml for calibration. This process guarantees uniformity in the samples, ensuring that each of them contains an identical amount of the unknown sample, that may potentially interfere with the assay. The electrochemical sensor's response was measured for all the samples, including those with known concentrations of the S-RBD protein and those without known concentrations of the protein as shown in Fig. 6b. A calibration plot depicting the relationship between peak current and the standard additions of the known samples resulted in a straight-line graph as shown in Fig. 6c. The S-RBD protein concentration was determined within the SARS-CoV-2 variant (B.1.1.7) by identifying the point on the x-axis where the extended linear regression line intersects the concentration axis with a signal value of zero.

3.8 Repeatability and Reproducibility

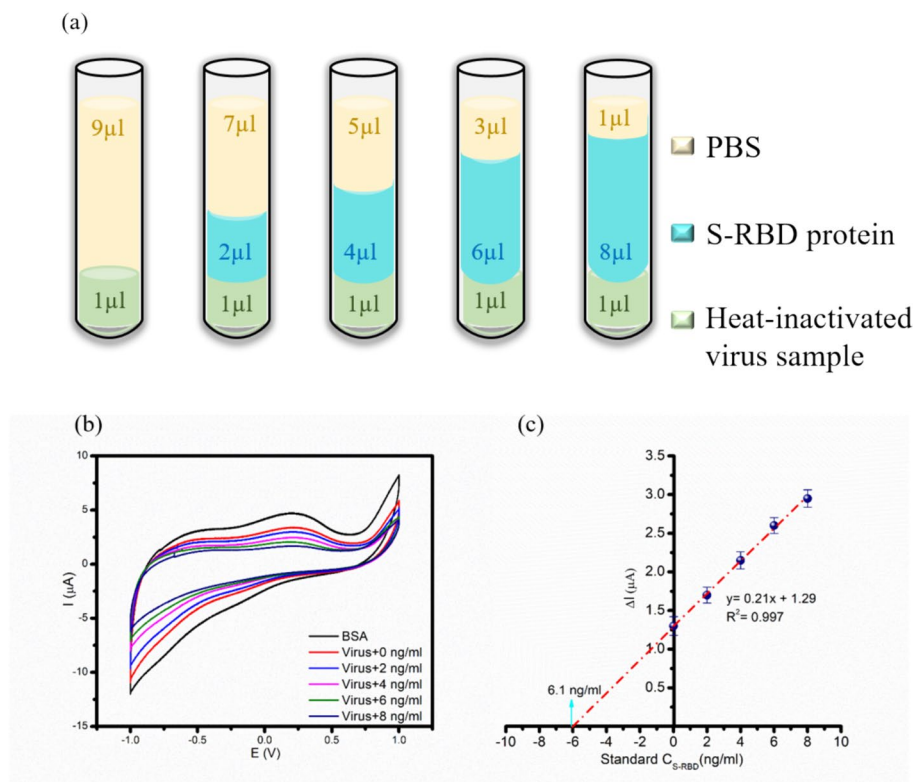
The repeatability and reproducibility of the developed electrochemical sensor were investigated as these are critical considerations for their potential use in clinical applications. To assess the sensor's repeatability, the identical electrode was employed seven times to detect 1 ng/ml of S-RBD protein, with a 30-min gap between each trial, and the changes

in cyclic voltammograms were monitored. To achieve high reproducibility and repeatability, maintaining uniformity and precision during fabrication, along with stable experimental conditions, was crucial. Optimization of antibody concentrations, incubation times, and washing steps, coupled with accurate sensor fabrication, minimized impedance mismatch between electrodes and ensured consistent performance. Figure S2(a) displays the response current from eight measurements conducted with a single electrode. The relative standard deviation (RSD) for the recorded current value was determined to be approximately 3.7%, which ensures the consistency and reliability of measurements. In addition, five identical electrodes were analyzed to monitor 1 ng/ml of S-RBD protein under the same conditions to evaluate the reproducibility of the developed sensor as shown in Figure S2(b). The RSD of the recorded current value was found to be 1.45%, signifying good reproducibility of the developed electrochemical sensor.

4 Conclusion

Considering the continuous rise in COVID-19 cases and the emergence of highly transmissible and infectious variants of concern, the demand for a highly sensitive, speedy, users friendly, and customizable production of personal protective equipment, without the requirements of advanced laboratory techniques or specialized expertise has become more

Fig. 6 **a** Application of the standard addition method to spike S-RBD protein into the heat-inactivated virus sample. **b** Cyclic voltammograms of the obtained relative response. **c** The concentration of the S-RBD protein in the virus sample is estimated by extrapolating the standard curve and determining the x-intercept



urgent than before. To achieve this goal, we provide the foundation for the development of the first prototype of an electrochemical sensor array utilizing Pt-black nanoparticles for point-of-care detection. The primary benefits (high sensitivity and rapid detection) of the developed SARS-CoV-2 electrochemical sensor array are supported by the use of a nanostructured sensor platform modified with pt-black nanoparticles. These nanoparticles offer exceptional conductivity, a high number of electrochemical active sites, strong catalytic properties, and chemical stability. In addition, to guarantee high sensitivity, the impact of different electrode gaps on the sensor's performance was thoroughly assessed using commercial simulation software (COMSOL). The simulation results correspond with empirical data, confirming that as the electrode gap decreased, there was an increase in the capacitance change caused by the analyte adsorption. The COVID-19 diagnosis was achieved by monitoring changes in the voltammetric current upon competitive interaction between anti-S-RBD-SARS-CoV-2 and the virus. This sensor configuration demonstrated excellent sensitivity for both heat-in-activated virus samples and purified S-RBD protein with detection limits of (0.40 ng/ml) and (0.23 ng/ml) respectively. The developed Pt-black-based electrochemical sensor array enables the simultaneous detection of distinct biomarkers, opening the door to a new era of cost-effective and highly sensitive devices for clinical diagnosis of COVID-19 with fast and timely analysis. Furthermore, to expand upon the future direction of this aspect, we aim to enhance the Pt-black-based electrochemical sensor's potential in biomarker detection. This involves integrating it with mobile health technologies, platform miniaturization, exploring new biomarkers, and rigorous clinical validation. By exploring these pathways, we believe that the sensor array has the potential to transform COVID-19 diagnostics and make substantial contributions to public-health initiatives.

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Data availability Not applicable.

Declarations

Conflict of interest The authors declare no conflict of interest.

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