



A smartphone-integrated aptasensor for SARS-CoV-2 S1 protein using multi-walled carbon nanotube–ionic liquid modified electrodes

Huseyin Senturk¹ · Arzum Erdem¹

Received: 4 December 2025 / Accepted: 7 February 2026 / Published online: 4 March 2026
© The Author(s) 2026

Abstract

An aptamer-based electrochemical biosensor was developed for the highly sensitive detection of SARS-CoV-2 S1 protein using electrodes modified with a multi-walled carbon nanotube–ionic liquid (MWCNT–IL) composite. Comprehensive electrochemical and microscopic characterizations of the MWCNT–IL material were conducted, followed by the optimization of experimental parameters including ionic liquid concentration and surface modification time. Critical parameters for aptasensor construction, aptamer concentration, immobilization time, and aptamer–protein interaction time, were also optimized. Under optimal conditions, the biosensor exhibited a wide linear response from 1 fg/mL to 1 ng/mL in buffer, with a remarkably low limit of detection (LOD) of 0.11 fg/mL. Selectivity evaluations against hemagglutinin antigen (HA) and MERS-CoV-S1 (MERS) protein confirmed the sensor's high specificity for SARS-CoV-2 S1 protein. The sensor's applicability in artificial saliva was also successfully demonstrated, yielding accurate quantification and low detection limits (LOD: 0.16 fg/mL). Recovery studies in artificial saliva demonstrated satisfactory accuracy, with recoveries ranging from 108% to 120% and high reproducibility. Notably, the aptasensor maintained strong selectivity even in the presence of matrix interferences. Furthermore, the potential for point-of-care use was assessed via a smartphone-connected portable potentiostat. This handheld system enabled a linear detection range of 1 fg/mL to 1 ng/mL with a LOD of 0.83 fg/mL. Collectively, these results highlight the MWCNT–IL-based aptasensor as a robust, low-cost, and portable platform for rapid COVID-19 diagnostics, offering considerable promise for deployment in point-of-care and resource-constrained environments.

Keywords Multi-walled carbon nanotube · Ionic liquid · SARS-CoV-2 S1 protein · Aptasensor · Differential pulse voltammetry · Smartphone-integrated aptasensor

Introduction

COVID-19 is a disease caused by the SARS-CoV-2 virus, which continues to pose a significant global threat despite the widespread implementation of vaccines and therapeutic interventions [1]. The emergence of new variants has sustained the demand for effective diagnostic tools [2, 3]. As of May 2025, the World Health Organization (WHO) has reported over 777 million confirmed COVID-19 cases and more than 7 million related deaths worldwide, highlighting the urgent need for rapid and accurate diagnostic methods

[4]. SARS-CoV-2 is an enveloped, single-stranded RNA virus belonging to the Coronaviridae family [5, 6]. Primarily targeting the respiratory system, the virus is known for its high transmissibility and pathogenicity. Among its structural proteins, the spike (S) protein plays a pivotal role in viral entry into host cells. Consequently, extensive research has focused on this protein for both diagnostic and therapeutic purposes [5–7]. Accurate and timely detection of COVID-19 remains critical for controlling the spread of outbreaks. The presence of asymptomatic carriers and the continuous evolution of viral variants further emphasize the need for rapid and reliable diagnostics. However, current diagnostic techniques also present certain limitations. For example, although the reverse transcription polymerase chain reaction (RT-PCR), recognized as the gold standard, offers high sensitivity, it is time-consuming and requires specialized laboratory equipment. These limitations have driven the

✉ Arzum Erdem
arzum.erdem@ege.edu.tr

¹ Department of Analytical Chemistry, Faculty of Pharmacy, Ege University, 35040 Izmir, Türkiye

development of alternative systems that are low-cost, portable, and capable of delivering rapid results [8–10].

Electrochemical biosensors offer promising solutions to address these challenges. These devices convert specific biological interactions on the sensor surface into measurable electrical signals, enabling the sensitive and selective detection of target analytes at low concentrations [11]. Their key advantages, including portability, rapid response time, and cost-effectiveness, make them highly suitable for point-of-care diagnostics. Furthermore, electrochemical biosensors outperform conventional techniques in several aspects, such as simplified sample preparation, ease of use, miniaturization potential, and short analysis times. Their ability to integrate with portable devices also enhances their applicability in on-site and bedside diagnostics [12–15].

To further enhance the analytical performance of electrochemical biosensors, various nanomaterials have been integrated into electrode interfaces. Recently, two-dimensional (2D) nanomaterials have attracted significant attention for SARS-CoV-2 detection due to their large surface area and unique electronic properties. For instance, Gutierrez-Galvez et al. [16] and Enebral-Romero et al. [17] demonstrated the efficacy of few-layer bismuthene and bismuthene-DNA nanoconjugates for highly sensitive viral sensing. Similarly, molybdenum disulfide (MoS_2) and its heterostructures with graphene have been successfully employed to develop robust sensing platforms for pathogen detection [18, 19]. While these 2D materials offer exceptional planar properties, one-dimensional (1D) carbon nanotubes continue to offer distinct advantages in terms of creating conductive 3D networks and ease of functionalization.

In this context, to leverage the advantages of carbon-based nanomaterials, a composite of multi-walled carbon nanotubes (MWCNTs) and ionic liquid (IL) was employed as the electrode modification platform (MWCNT-IL). Unlike ensuring only conductivity, the MWCNT-IL composite provides a synergistic effect where the IL prevents the aggregation of MWCNTs, creating a homogeneous and stable 3D sensing interface with enhanced electron transfer kinetics. MWCNTs facilitate faster electron transfer, thereby enhancing the sensitivity of the biosensor [20, 21]. Meanwhile, IL contributes to the stability of the electrode by promoting homogeneous surface modification [22]. The synergistic combination of these materials enables the aptasensor to detect low concentrations of the target analyte with high precision. Moreover, the high surface area of MWCNTs allows for efficient immobilization of aptamers, while IL enhances ionic conductivity, leading to more stable redox probe signals [22]. This facilitates the detection of even subtle signal variations upon analyte binding. Additionally, the thermal and chemical stability of IL improves the durability of the aptasensor and ensures reliable performance in long-term

analyses [23]. The uniform surface modification provided by IL also improves the reproducibility of the biosensor, contributing to consistent analytical performance. Owing to these features, the MWCNT-IL composite was selected in this study as an ideal platform for the sensitive detection of the SARS-CoV-2 S1 protein. In this context, pencil graphite electrodes (PGEs) were specifically selected as the transducer platform due to their widespread availability, disposable nature, and significantly lower cost compared to conventional screen-printed electrodes, making them ideal candidates for developing affordable diagnostics for resource-limited settings.

Aptamers are single-stranded DNA or RNA molecules that bind to target molecules with high specificity. Their chemical synthesizability, low production cost, and high stability have made them increasingly attractive components for biosensor design in recent years [24, 25]. Due to their strong affinity toward specific target analytes, aptamers enable highly sensitive and selective detection [24–26]. Leveraging these advantages, this study aims to develop an aptamer-based electrochemical biosensor for the detection of the SARS-CoV-2 S1 protein. Several studies in the literature have employed different techniques to design aptasensors for SARS-CoV-2 S1 protein detection [27–37]. In the study by Curti et al. [31], an electrochemical aptasensor was developed by immobilizing a redox-labeled DNA aptamer specific to the S1 protein onto a screen-printed carbon electrode modified with multi-walled carbon nanotubes (MWCNTs). The detection was based on signal reduction resulting from aptamer–protein binding. The sensor exhibited high selectivity for the target protein, with a reported detection limit of 7 nM. The aptasensor was successfully applied in both buffer solutions and viral transport media commonly used for nasopharyngeal swab collection, with no cross-reactivity observed with other viral proteins. In another study, Wang et al. [29], developed an aptasensor based on helicase-dependent isothermal amplification (HRCA) for S1 protein detection. The detection strategy relied on a sandwich complex consisting of antibody–target–aptamer immobilized on a microplate surface, with fluorescence signals amplified via HRCA. The method achieved a detection limit of 89.7 fg/mL within a concentration range of 100 fg/mL to 1 $\mu\text{g/mL}$. In diluted artificial saliva samples, the detection limit for SARS-CoV-2 spike pseudovirus was calculated as 51 TU/ μL . Adeel et al. [28], developed a label-free electrochemical aptasensor for the rapid detection of the SARS-CoV-2 spike protein. The sensing platform was constructed on a porous carbon fabric modified with gold nanoparticles to enhance conductivity and electrochemical performance. Thiol-functionalized DNA aptamers were immobilized on the electrode surface for specific spike protein recognition. Using $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as the redox probe,

the sensor demonstrated detection via differential pulse voltammetry (DPV) and chronopotentiometry (CP). The calculated limits of detection were 0.11 ng/mL (DPV) and 37.8 ng/mL (CP). In diluted human saliva, detection limits were 0.167 ng/mL (DPV) and 46.2 ng/mL (CP). Cennamo et al. [38], designed an optical aptasensor targeting the receptor-binding domain (RBD) of the SARS-CoV-2 spike glycoprotein. A specific aptamer was immobilized on a short polyethylene glycol interface coated onto a gold nanofilm deposited on a D-shaped plastic optical fiber. The aptamer–protein interaction was monitored using the surface plasmon resonance (SPR) technique, enabling highly sensitive detection in the 25–1000 nM range, with a detection limit of 37 nM. Selectivity was validated against interferents such as BSA, AH1N1 hemagglutinin, and MERS-CoV spike proteins. Furthermore, the aptasensor was successfully tested in diluted human serum samples.

In this study, a novel electrochemical aptasensor based on the synergistic MWCNT-IL composite was developed for the ultrasensitive detection of the SARS-CoV-2 S1 protein. To the best of our knowledge, this is the first report utilizing the MWCNT-IL platform for S1 protein detection, distinguishing it from existing 2D nanomaterial-based or standard carbon-based sensors. The electrochemical characterization of the fabricated aptasensor was performed using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Various experimental parameters, including modification time with MWCNT-IL, IL concentration, aptamer concentration, incubation periods for aptamer and protein interactions, and comparison of interaction procedures, were systematically optimized. Under optimized conditions, the analytical performance of the aptasensor was evaluated. The sensor's selectivity was confirmed, and its applicability was demonstrated in artificial saliva. To further assess its suitability for point-of-care testing, the detection of S1 protein was also performed using a portable potentiostat integrated with a smartphone. The proposed MWCNT-IL-based aptasensor offers a low-cost, portable, and highly sensitive alternative for COVID-19 diagnostics. With its rapid analysis time and broad linear detection range, this aptasensor holds strong potential as an effective alternative to conventional diagnostic methods for SARS-CoV-2.

Materials and methods

Chemicals and apparatus

Electrochemical measurements were performed using an AUTOLAB PGSTAT302N potentiostat equipped with the FRA 2.0 module, operated via NOVA 1.11.1 software (Eco Chemie, The Netherlands). Additionally, a

portable potentiostat (GalvanoPlot, SolarBiotec, Türkiye) was employed for its implementation to the point-of-care application. The laboratory-based measurements were conducted inside a Faraday cage (Eco Chemie, The Netherlands) to minimize external electrical noise. A conventional three-electrode configuration was employed, consisting of a pencil graphite electrode (PGE) as the working electrode, an Ag/AgCl reference electrode (BAS, Model RE-5B, W. Lafayette, USA), and a platinum wire as the counter electrode.

The optimized aptamer sequence specific to the SARS-CoV-2 S1 protein was purchased from Aptamer Group (York, United Kingdom). Due to proprietary considerations, the manufacturer did not disclose the complete sequence details. In this study, two different buffer systems were used: acetate buffer solution (ABS, 0.5 M, pH 4.80) and phosphate-buffered saline (PBS, 50 mM, pH 7.40). For the aptamer–target interaction, a binding buffer recommended by the manufacturer was employed. This buffer contained MES monohydrate, MgCl_2 , CaCl_2 , NaCl, KCl, Na_2SO_4 , Tween 20, and bovine serum albumin (BSA). Aptamers were prepared and diluted using this binding buffer to preserve their structural and functional integrity. The recombinant SARS-CoV-2 S1 protein was obtained from Sino Biological. A stock solution was prepared in ultrapure water at a concentration of 250 $\mu\text{g/mL}$ and stored at -80°C until use. Working solutions of the S1 protein were freshly prepared by dilution in PBS (50 mM, pH 7.40). For selectivity experiments, MERS-CoV S1 protein (also purchased from Sino Biological) was prepared as a 150 $\mu\text{g/mL}$ stock solution in ultrapure water and stored at -80°C . Working dilutions were also made using PBS. Influenza hemagglutinin (HA) antigen was purchased from Sigma-Aldrich and prepared as a 5 mg/mL stock solution in ultrapure water, stored at -20°C , and similarly diluted with PBS for experimental use. The ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate (IL) and carboxylic acid-functionalized multi-walled carbon nanotubes (MWCNTs) were both purchased from Sigma-Aldrich.

Artificial saliva was prepared based on the formulation reported by Vozgirdaite et al. [39]. The artificial saliva solution contained 4 mg/mL urea, 4 mg/mL mucin (from porcine stomach), 0.6 mg/mL Na_2HPO_4 , 0.3 mg/mL NaHCO_3 , 0.6 mg/mL CaCl_2 , 0.4 mg/mL KCl, and 0.4 mg/mL NaCl, all dissolved in ultrapure water. The solution was homogenized by ultrasonication for 20 min and adjusted to pH 7.2 using 0.1 M NaOH. The prepared artificial saliva was stored at 4°C and diluted in PBS (50 mM, pH 7.40) at various ratios for experimental use.

All reagents, including sodium chloride (NaCl), sodium hydroxide (NaOH), dipotassium hydrogen phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4),

potassium ferricyanide [$K_3Fe(CN)_6$], potassium ferrocyanide trihydrate [$K_4Fe(CN)_6 \cdot 3H_2O$], acetic acid, and *N,N*-dimethylformamide (DMF), were of analytical grade and obtained from Sigma-Aldrich. The *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) were also purchased from Sigma-Aldrich. All aqueous solutions were prepared using ultrapure water obtained from a Milli-Q® purification system.

Preparation of multi-walled carbon nanotube-ionic liquid (MWCNT-IL) composite

To prepare the MWCNT-IL composite, carboxylic acid-functionalized multi-walled carbon nanotubes (MWCNTs) were first dispersed in DMF at a final concentration of 500 $\mu\text{g/mL}$. The suspension was subjected to ultrasonic treatment in a bath sonicator for 30 min to ensure uniform dispersion. In parallel, a solution of the IL was prepared in DMF at a specified concentration and sonicated for 30 min. Subsequently, a defined volume of the IL solution was added to the MWCNT dispersion, and the resulting mixture was further sonicated for an additional 30 min to promote homogeneous integration of the components. Following sonication, the composite suspension was stored at 4 °C until use. Prior to electrode modification, the composite was re-sonicated for 10 min to ensure uniformity, after which it was applied directly for electrode surface modification.

Procedure

The step-by-step procedure employed for the development of the electrochemical aptasensor for SARS-CoV-2 S1 protein detection is described below:

- (i) Activation of the pencil graphite electrode (PGE) surface: The electrochemical activation of the PGE surface was performed in acetate buffer solution (ABS, 0.5 M, pH 4.80) by applying a constant potential of + 1.2 V for 30 s.
- (ii) Modification of the PGE surface with the MWCNT-IL composite: Following electrochemical activation, the PGE surface was modified with the previously prepared multi-walled carbon nanotube-ionic liquid (MWCNT-IL) composite. The electrodes were immersed in the MWCNT-IL dispersion and incubated for 60 min to allow for surface modification. The modified electrodes were then left to dry at room temperature for 30 min.
- (iii) Immobilization of the SARS-CoV-2 S1-specific aptamer on the electrode surface: After surface modification, the immobilization of the aptamer specific to the SARS-CoV-2 S1 protein was carried out. Initially, the

electrodes were activated chemically by immersion in a freshly prepared solution containing 5 mM EDC and 8 mM NHS in 50 mM MES buffer (pH 6.0) for 30 min. Subsequently, the activated electrodes were immersed in an aptamer solution of defined concentration and incubated for 30 min to allow aptamer immobilization. After immobilization, the electrodes were rinsed with binding buffer to remove weakly adsorbed aptamer strands from the surface.

- (iv) Interaction between the immobilized aptamer and the SARS-CoV-2 S1 protein: The interaction between the surface-immobilized aptamer and the SARS-CoV-2 S1 protein was then performed. Aptamer-immobilized electrodes were immersed in SARS-CoV-2 S1 protein solutions prepared at various concentrations in PBS (50 mM, pH 7.40) and incubated for 30 min to allow for aptamer-protein binding. Following incubation, the electrodes were thoroughly rinsed with PBS to remove unbound protein. It is important to note that a separate surface blocking step was not required in this assay. The binding buffer used during the aptamer-protein interaction contains BSA and Tween 20, which effectively function as blocking agents to minimize non-specific binding during the incubation period. Additionally, previous studies [32] indicated that a separate blocking step reduced signal sensitivity, whereas the antifouling properties of the MWCNT-IL composite naturally suppress non-specific adsorption.
- (v) Differential pulse voltammetry (DPV) measurements: DPV measurements were conducted in a solution of 2.5 mM $[Fe(CN)_6]^{3-/4-}$ prepared in 0.1 M KCl. The potential range was set from − 0.1 V to + 0.8 V, with a scan rate of 50 mV.

A schematic representation of the developed electrochemical aptasensor for SARS-CoV-2 S1 protein detection is illustrated in Fig. 1.

Results and discussion

In this study, an aptasensor was developed for the detection of the SARS-CoV-2 S1 protein using electrodes modified with a composite of multi-walled carbon nanotubes (MWCNTs) and an ionic liquid (IL). The study initially focused on the characterization of the MWCNT-IL composite to evaluate its properties and suitability for biosensor applications.

Microscopic characterization studies were conducted to investigate the surface morphology of the developed electrochemical aptasensor for SARS-CoV-2 S1 protein detection. For this purpose, scanning electron microscopy (SEM)

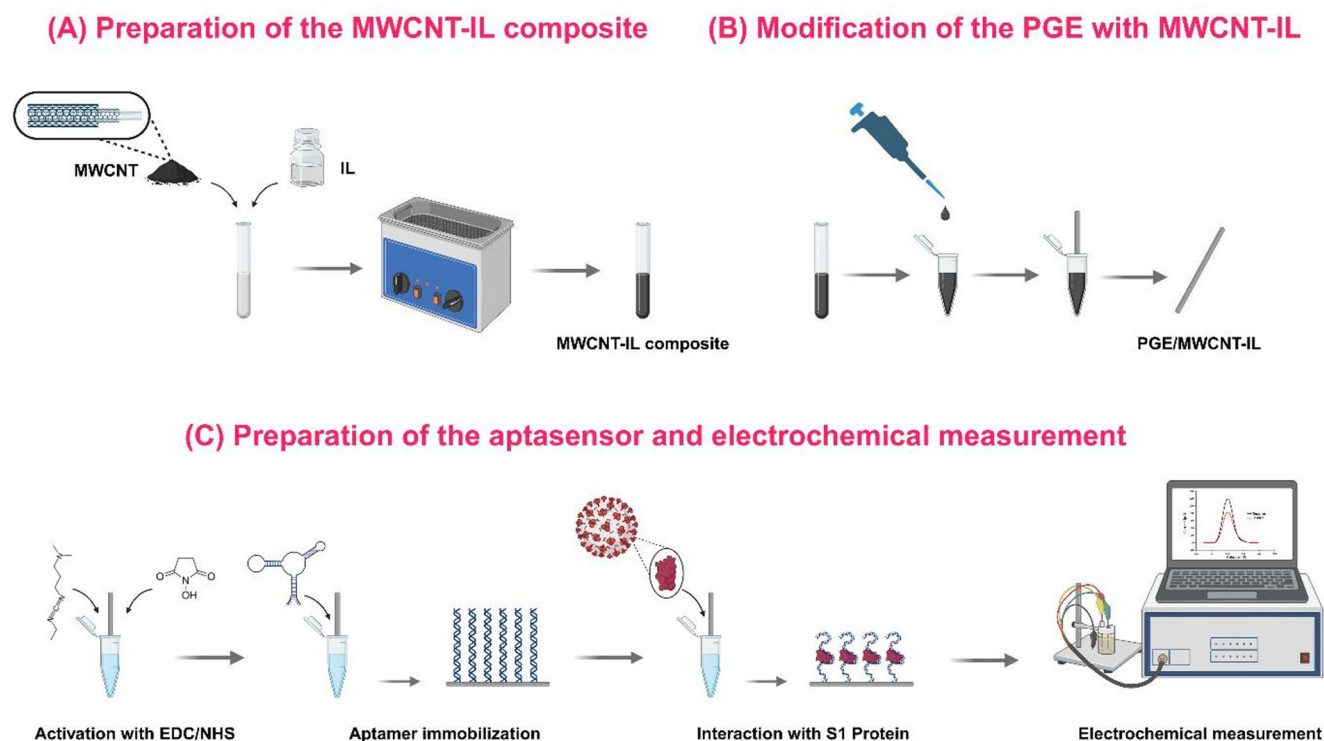


Fig. 1 Schematic illustration of the experimental procedure steps for the electrochemical aptasensor developed for the detection of SARS-CoV-2 S1 protein. **(A)** Preparation of the MWCNT–IL composite, **(B)**

Modification of the electrode surface with the prepared MWCNT–IL composite, **(C)** Construction of the aptasensor and the electrochemical measurement process. The figure was created using BioRender

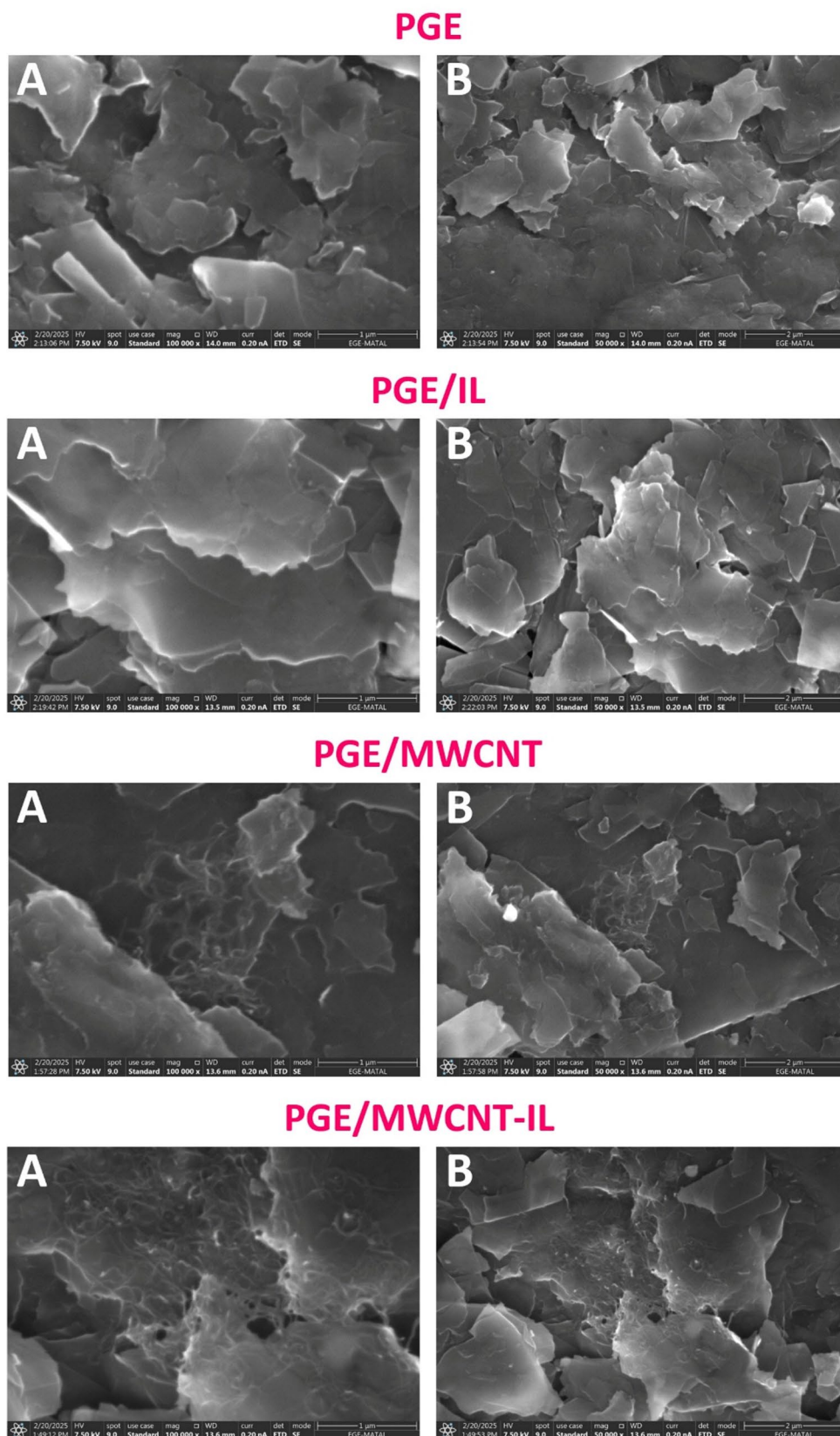
analyses were performed for unmodified and modified pencil graphite electrodes (PGE, PGE/IL, PGE/MWCNT, and PGE/MWCNT–IL). SEM imaging was carried out at the Central Research Test and Analysis Laboratory Application and Research Center of Ege University. The resulting SEM micrographs are presented in Fig. 2.

The SEM images obtained from surface characterization revealed distinct morphological differences among the PGE, PGE/IL, PGE/MWCNT, and PGE/MWCNT–IL electrodes. In the bare PGE electrodes, the characteristic layered morphology of the graphite structure was clearly observed, reflecting the inherent structural features of the material. In the case of PGE/IL electrodes, a more homogeneous and smoother surface morphology was noted, indicating that the IL formed a uniform coating over the electrode surface, resulting in a more even topography. For the PGE/MWCNT group, the typical web-like structure of carbon nanotubes was observed; however, these structures appeared to be concentrated in localized regions on the electrode surface. In contrast, PGE/MWCNT–IL electrodes exhibited a more uniform distribution of the nanotube network, with the distinctive fibrous structure of the carbon nanotubes clearly visible across a larger portion of the surface. This improved dispersion is attributed to the presence of IL, which facilitated the homogeneous integration of MWCNTs onto the electrode surface (Fig. 2). The observed web-like morphology aligns

well with previous reports in the literature [40, 41]. The widespread presence of this network across the modified surface underscores the synergistic effect of the MWCNT–IL composite and confirms the successful surface modification of the electrode. It should be noted that further morphological characterization (e.g., EDAX) for the aptamer layer was not feasible due to the high phosphorus content of the ionic liquid (BMIM–PF₆), which interferes with the detection of the DNA backbone. Consequently, the successful immobilization of the aptamer was verified using electrochemical technique, which provide higher sensitivity for tracking surface interface changes.

Following the fabrication of the aptasensor for SARS-CoV-2 S1 protein detection, electrochemical characterization was conducted using cyclic voltammetry (CV). In this context, the PGE surface was individually modified with MWCNT, IL, and MWCNT–IL composite materials by immersing the electrodes in the respective dispersions for 1 h. The effect of each modification on the electrochemical response was evaluated via CV measurements. The experiments were performed in 2.5 mM [Fe(CN)₆]^{3–/4–} redox probe solution, prepared in 0.1 M KCl, over a potential range of – 0.5 V to + 1.0 V at a scan rate of 50 mV/s. The resulting voltammograms are presented in Fig. S1. The electrochemical data demonstrated that surface modification with 500 µg/mL MWCNT, 10% IL, and the combined

Fig. 2 SEM images of PGE, PGE/IL, PGE/MWCNT, and PGE/MWCNT–IL surfaces obtained at magnifications of (A) 1 μm and (B) 2 μm



500 $\mu\text{g/mL}$ MWCNT-10% IL composite led to current enhancements of 9.32%, 11.17%, and 24.17%, respectively, compared to the bare PGE control. Control experiments performed with the same electrode configurations (PGE, PGE/IL, PGE/MWCNT, and PGE/MWCNT-IL) in 0.1 M KCl without the redox probe yielded no detectable signals (Fig. S1), confirming the specificity of the redox probe response. These results clearly indicate that the MWCNT-IL composite provides superior electrochemical performance not only compared to the unmodified electrode but also when compared to electrodes modified with MWCNT or IL alone. The enhanced performance of the composite is attributed to the synergistic interaction between the two components. MWCNTs contribute to efficient electron transfer due to their high surface area and excellent electrical conductivity. Meanwhile, the ionic liquid facilitates charge transport by providing an ionically conductive environment and enhances the stability of electrochemical reactions. Furthermore, the integration of IL into the MWCNT matrix promotes a more homogeneous dispersion of the nanotubes across the electrode surface, resulting in a well-organized and accessible conductive network. This synergistic combination not only improves electron transfer kinetics but also provides a favorable interfacial environment for analyte–electrode interactions, thereby yielding a significant increase in current response. Therefore, the MWCNT-IL composite offers a distinct advantage in terms of sensitivity and electrochemical performance over electrodes modified with either component alone [42–49].

To evaluate the effect of IL concentration on the electrochemical performance of the MWCNT-IL composite, an optimization study was carried out. In this study, composite dispersions were prepared using different IL concentrations (2.5%, 5%, 10%, and 15% v/v), and the PGE surfaces were modified with each composite. The impact of IL concentration on sensor response was then assessed using CV. The results are shown in Fig. 3A. According to the results, the highest current response was obtained when the IL concentration was increased to 5%. Beyond this point, further increases in IL concentration led to a gradual decrease in current enhancement. Compared to the unmodified control electrode, current enhancements of 19.66%, 38.71%, 24.17%, and 14.60% were observed for composites containing 2.5%, 5%, 10%, and 15% IL, respectively (Fig. 3A). Based on these findings, the composite containing 5% IL yielded the highest and most reproducible current increase and was therefore selected as the optimal IL concentration. The significant enhancement in current observed at 5% IL concentration is likely due to an optimal balance between ionic conductivity and accessible active surface area. At this concentration, it is proposed that IL forms a uniform and thin layer over the MWCNT surface, promoting efficient

electron transfer while maintaining adequate exposure of active sites. In contrast, at higher IL concentrations, the excessive accumulation of IL may lead to partial coverage of the MWCNT active regions, impeding electron transfer. Moreover, the formation of a thicker IL layer may hinder analyte diffusion to the electrode surface, resulting in decreased current response [50].

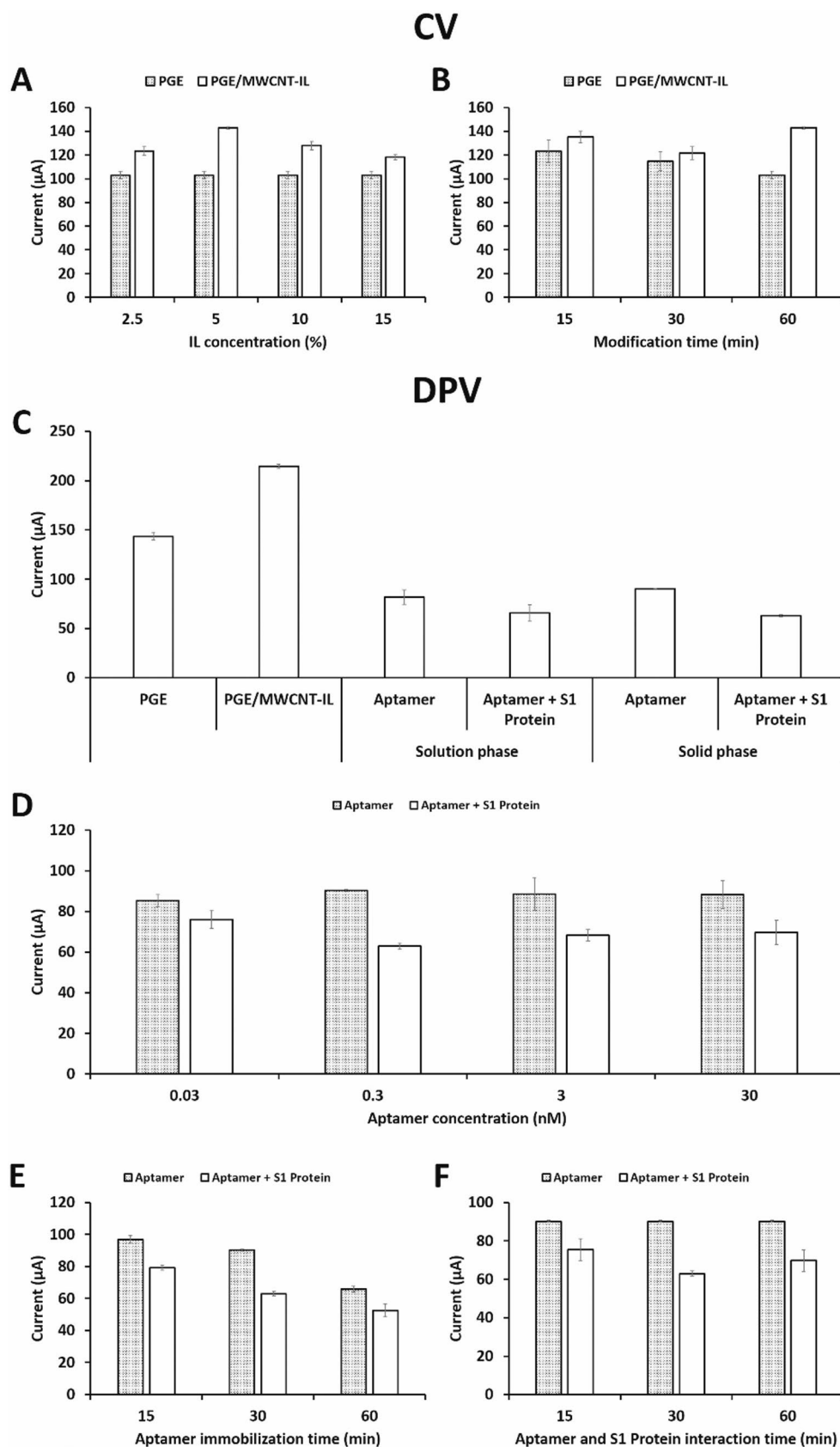
Following the optimization of IL concentration in the MWCNT-IL composite, further studies were conducted to investigate the effect of different modification durations on the sensor response. For this purpose, PGE surfaces were modified with the optimized composite (containing 5% IL) for various durations (15, 30, and 60 min), and the resulting electrochemical responses were evaluated by CV (Fig. 3B). Compared to the control group, current enhancements of 9.53%, 5.92%, and 38.71% were observed for 15, 30, and 60 min of modification, respectively (Fig. 3B). The highest and most reproducible current increase was achieved with a modification time of 60 min, which was therefore selected as the optimum modification duration.

The effect of scan rate on the current response of the electrodes modified with the MWCNT-IL composite was investigated using CV in 2.5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox probe solution at various scan rates (10, 25, 50, 75, 100, 150, and 200 mV/s). The resulting voltammograms are presented in Fig. S2. As illustrated, a linear relationship was observed between the square root of the scan rate and the peak current (Fig. S2), indicating that the electron transfer process at the electrode–solution interface is governed by a diffusion-controlled mechanism.

Additionally, the electrochemical behavior of the sensor constructed with the MWCNT-IL composite was further evaluated by electrochemical impedance spectroscopy (EIS). EIS measurements were performed in 2.5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox probe solution using both unmodified and MWCNT-IL modified PGE electrodes. The corresponding Nyquist plots are shown in Fig. S3. A significant decrease in charge transfer resistance (R_{ct}) was observed for the modified electrodes compared to the bare PGE (Fig. S3), suggesting improved conductivity and enhanced electron transfer efficiency at the electrode–electrolyte interface. This reduction in R_{ct} is attributed to the high electrical conductivity of MWCNTs and the ionic nature of IL, which together exhibit a synergistic effect that accelerates charge transport and minimizes resistance losses. Moreover, the homogeneous distribution of IL across the MWCNT surface is believed to facilitate the accessibility of the redox probe to the electrode surface, thereby further contributing to the improved electrochemical performance [42–44, 47–49].

Following the optimization and characterization of the composite material, further studies were carried out to optimize experimental parameters of the aptasensor. As an

Fig. 3 Histograms showing (A) the effect of IL concentration in the MWCNT-IL composite and (B) the effect of modification time during electrode surface modification with the MWCNT-IL composite on the sensor response. In the aptasensor developed for the detection of SARS-CoV-2 S1 protein, histograms obtained from optimization studies related to (C) the interaction procedure, (D) aptamer concentration, (E) aptamer immobilization time on the electrode surface, and (F) the interaction time between the aptamer and S1 protein on the electrode surface



initial step, two different aptamer–target interaction procedures were investigated to determine the most effective approach. In the solution-phase interaction procedure, 0.3 nM of the aptamer was mixed with 80 ng/mL of SARS-CoV-2 S1 protein under gentle agitation for 5 min. The resulting aptamer–protein complex was then immobilized onto the PGE/MWCNT-IL surface (pre-activated with EDC/NHS) by incubation for 15 min [32]. In the electrode surface interaction procedure, 0.3 nM aptamer was first immobilized onto the EDC/NHS-activated PGE/MWCNT-IL electrode by incubation for 30 min. Subsequently, the modified electrodes were immersed in an 80 ng/mL S1 protein solution and incubated for another 30 min to facilitate aptamer–protein binding at the electrode surface. Following both procedures, differential pulse voltammetry (DPV) measurements were performed in 2.5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox probe solution. The current responses before and after aptamer–protein interaction were compared at + 0.2 V, and the results are presented in Fig. 3C and Fig. S4. According to the findings, the solution-phase interaction procedure led to a 19.58% decrease in current relative to the control group, while the electrode surface interaction procedure resulted in a more pronounced 30.21% current decrease (Fig. 3C). These results clearly indicate that the electrode surface interaction procedure yields a more effective signal suppression and was thus selected as the optimum strategy for subsequent experiments.

The electrochemical detection principle is based on the modulation of the redox probe's electron transfer efficiency at the electrode interface. Initially, the modification of the PGE with the MWCNT-IL composite significantly enhances the electroactive surface area and conductivity, facilitating rapid electron transfer. Following this, the decrease in current following aptamer immobilization can be explained by electrostatic repulsion. DNA aptamers carry negatively charged phosphate backbones, and the redox probe $[\text{Fe}(\text{CN})_6]^{3-/4-}$ is also anionic in nature. Upon immobilization, repulsive electrostatic forces arise between the aptamer layer and the redox species, which hinders their approach to the electrode surface and consequently impedes electron transfer. Furthermore, the aptamer layer alters the surface charge distribution, rendering the surface more negatively charged and further restricting access of the redox probe. This phenomenon accounts for the reduction in current observed post-immobilization and is consistent with findings reported in previous studies [30, 51–53]. Additionally, the observed decrease in current after aptamer–S1 protein binding is attributed to steric hindrance caused by the formation of an aptamer–protein complex on the electrode surface. This complex creates a larger molecular structure that physically obstructs the access of the redox probe to the electrode interface, reducing the efficiency of electron

transfer and thereby decreasing the electrochemical signal. These findings are also in agreement with previous literature [30, 51–53]. In conclusion, the electrode surface interaction procedure was determined to be the most effective strategy for aptamer–target binding and was employed in all subsequent experiments. To validate the specificity of the sensor response, a control DPV measurement was performed using the MWCNT-IL modified electrode in the absence of the aptamer. Upon incubation with 80 ng/mL S1 protein, a high current response of $132.30 \pm 14.54 \mu\text{A}$ ($n = 3$) was maintained. In contrast, the aptamer-functionalized electrode (baseline signal: $90.23 \pm 0.61 \mu\text{A}$; $n = 3$) exhibited a significant decrease to $62.97 \pm 1.41 \mu\text{A}$ ($n = 3$) under the same conditions. This comparison confirms that the S1 protein does not cause significant non-specific blocking of the electrode surface and that the observed signal suppression is due to the specific recognition by the immobilized aptamer.

Following the determination of the optimal interaction procedure, further optimization studies were conducted to evaluate the effect of aptamer concentration on sensor performance. Based on our previous work [32] and relevant literature [34], different concentrations of aptamer (0.03, 0.3, 3, and 30 nM) were immobilized on the PGE/MWCNT-IL-modified electrode surface. Subsequently, the electrodes were incubated with SARS-CoV-2 S1 protein to allow for aptamer–target interaction at the surface. DPV measurements were then performed, and the results are presented in Fig. 3D. Among all tested concentrations, the most pronounced and reproducible current decrease (30.21%) compared to the control was observed with 0.3 nM aptamer, with a relative standard deviation of 2.24% ($n = 3$), as shown in Fig. 3D. Therefore, 0.3 nM was selected as the optimal aptamer concentration for further experiments.

Subsequently, the effect of aptamer immobilization time on sensor performance was investigated. For this purpose, 0.3 nM aptamer was immobilized onto the electrode surface for varying durations (15, 30, and 60 min), followed by incubation with S1 protein to facilitate aptamer–target interaction. DPV measurements were conducted after each interaction step, and the results are shown in Fig. 3E. A gradual decrease in both the baseline and post-interaction current responses was observed with increasing immobilization time. The greatest current decrease (30.21%) relative to the control group was obtained after 30 min of immobilization (Fig. 3E). Hence, 30 min was determined to be the optimal immobilization duration.

Finally, the effect of aptamer–target interaction time on the electrochemical response was examined. After immobilization of the aptamer onto the electrode surface, the electrodes were incubated in S1 protein solution for varying durations (15, 30, and 60 min). DPV measurements were recorded following each interaction period, and the results

are depicted in Fig. 3F. The most significant and reproducible current decrease (30.21%, RSD=2.24%, $n=3$) relative to the control was achieved after 30 min of incubation with the S1 protein (Fig. 3F). Accordingly, 30 min was selected as the optimal interaction time.

Following the optimization of experimental parameters, the analytical performance of the developed aptasensor for SARS-CoV-2 S1 protein detection was evaluated under optimal conditions. For this purpose, S1 protein solutions were prepared in the concentration range of 1 pg/mL to 10 ng/mL. Aptamer-immobilized electrodes modified with the MWCNT-IL composite were immersed in these solutions to allow interaction between the aptamer and the S1 protein at the electrode surface. DPV measurements were then performed to monitor the sensor response across increasing concentrations. The resulting response profile is illustrated in Fig. S5. To determine the limit of detection (LOD) within the linear response range, S1 protein solutions were prepared in the range of 10^0 to 10^6 fg/mL. Aptamer-immobilized electrodes were incubated in these solutions, followed by DPV measurements conducted in 2.5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox probe solution. The corresponding DPV voltammograms and the calibration plot of the average current response ($n=3$) are shown in Fig. 4A and B.

As the concentration of S1 protein increased, a progressive decrease in current was observed. This phenomenon can

be attributed to the increasing formation of aptamer-protein complexes on the electrode surface, which imposes steric hindrance. At higher target concentrations, more binding events occur, resulting in the formation of larger molecular assemblies that physically block the access of redox probe molecules to the electrode surface. This steric effect limits electron transfer and thus leads to a reduction in the electrochemical signal [30, 51]. The proportional relationship observed between the decreasing current and increasing S1 protein concentration demonstrates the aptasensor's capability for quantitative analysis of the target analyte.

A calibration curve was constructed over the linear range of 10^0 to 10^6 fg/mL (Fig. 4B). The regression equation was obtained as: $I/\mu\text{A} = -6.61 \log(C_{\text{S1 Protein}} / \text{fg mL}^{-1}) + 81.94$ with a correlation coefficient of $R^2 = 0.99$. The calibration curve was plotted on a semi-logarithmic scale to maintain linearity across the wide concentration range ($10^0 - 10^6$ fg/mL). This approach allows for a meaningful visualization of the sensor response and a more accurate estimation of analytical parameters. The LOD was calculated according to the IUPAC method [54] using the equation:

$$S_{\text{LOD}} = S_{\text{blank}} - k \times \sigma_{\text{blank}}$$

where S_{blank} is the average electrochemical signal of the blank (zero concentration), σ_{blank} is the standard deviation

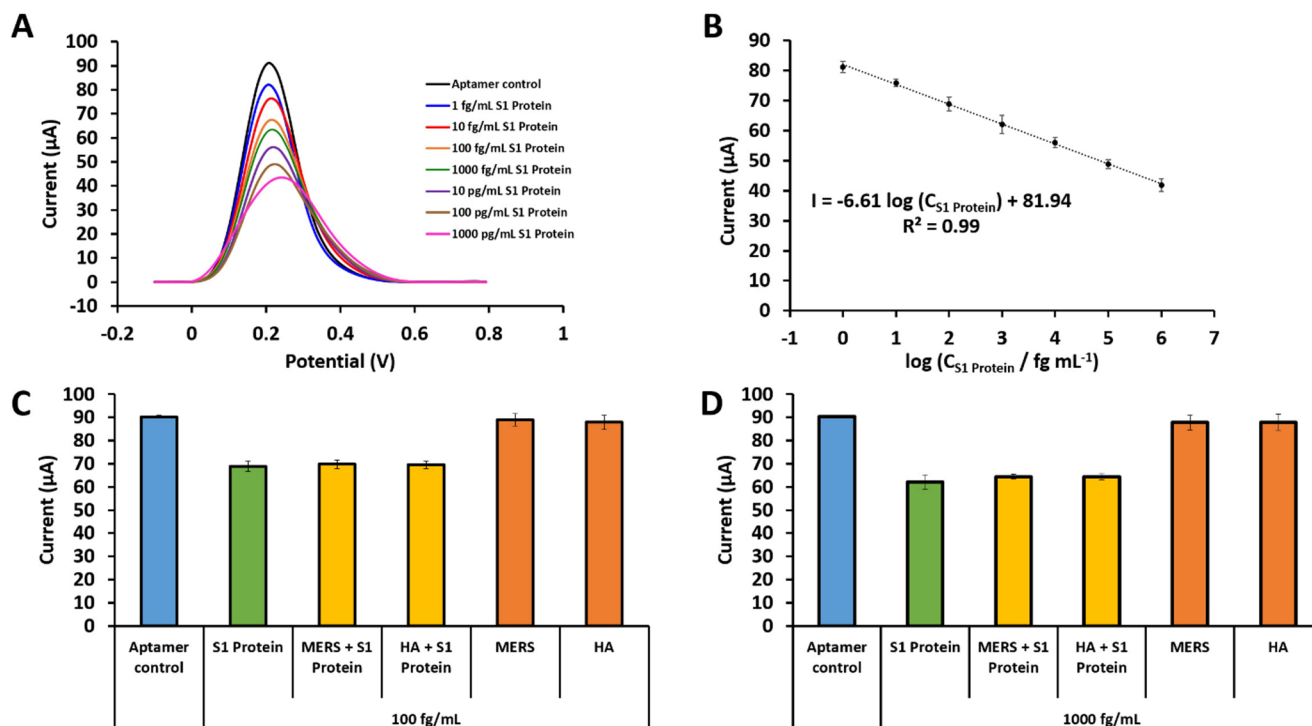


Fig. 4 (A) Differential pulse voltammograms and (B) the corresponding calibration curve obtained in the concentration range of 10^0 – 10^6 fg/mL in buffer medium using the developed aptasensor for the detection of SARS-CoV-2 S1 protein. (C) and (D) Histograms showing the

selectivity results against HA and MERS at concentrations of 100 fg/mL and 1000 fg/mL, respectively. DPV measurements were performed in a 2.5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox probe solution

of the blank signal ($n = 3$), and k is the numerical factor chosen according to the desired confidence level ($k = 3$). The signal value corresponding to S_{LOD} was then projected onto the semi-logarithmic calibration equation ($I/\mu\text{A} = -6.61 \log (C_{\text{S1 Protein}} / \text{fg mL}^{-1}) + 81.94$) to determine the corresponding S1 protein concentration. According to this method [54], the LOD was calculated to be 0.11 fg/mL. Furthermore, the sensitivity of the biosensor in buffer solution was determined by dividing the slope of the calibration curve (6.61) by the effective surface area of the electrode (0.2796 cm^2), yielding a value of $23.64 \mu\text{A mL fg}^{-1} \text{ cm}^{-2}$.

A comparative overview of previously reported aptasensors for COVID-19 diagnosis is provided in Table 1.

In the literature, various electrochemical aptasensors have been developed for the detection of COVID-19 biomarkers, targeting different viral proteins such as the receptor-binding domain (RBD), S1 subunit, full-length spike (S) protein, and nucleocapsid (N) protein. In the present study, an electrochemical aptasensor based on a MWCNT-IL composite-modified electrode was developed for the detection of the SARS-CoV-2 S1 protein. When compared to previous reports [27, 28, 30, 31, 36, 37], the proposed aptasensor exhibited a significantly lower detection limit over a broader linear concentration range. This enhanced analytical performance is attributed primarily to the unique properties of the composite material employed. The high electrical conductivity of the MWCNT-IL composite improved electron transfer kinetics at the electrode interface, thereby increasing the sensitivity of the aptasensor and facilitating the

detection of ultra-low analyte concentrations. Moreover, the large surface area provided by the nanomaterial enabled the immobilization of a higher density of aptamer molecules, further enhancing the biosensor's responsiveness. In addition, the ionic liquid contributed to a homogenous modification of the electrode surface, which not only improved the sensor's signal stability but also enhanced its repeatability and operational robustness. Collectively, these features allowed the aptasensor to surpass the performance metrics of several previously reported systems and establish itself as a promising and practical alternative for the electrochemical detection of COVID-19 biomarkers.

In addition to analytical performance, the reproducibility of the developed aptasensor was also evaluated. Reproducibility was assessed by recording the sensor response to 1 pg/mL SARS-CoV-2 S1 protein over three separate days, using two independently prepared aptasensors per day. The results are summarized in Table S1. According to the data presented, intra-day reproducibility (expressed as %RSD) was calculated as 0.80% for Day 1, 6.11% for Day 2, and 4.00% for Day 3. Inter-day reproducibility, based on six independently fabricated aptasensors, was determined to be 3.79%. These findings demonstrate the aptasensor's high level of reproducibility and confirm its reliability for consistent detection of the S1 protein across multiple measurements.

The selectivity of the developed aptasensor was further assessed using HA and MERS protein as potential interfering agents. HA was chosen due to the symptomatic overlap

Table 1 Electrochemical aptasensor studies reported in the literature for COVID-19 detection

Analyte	Method	Linear range	Limit of detection	Application	Ref.
Receptor binding domain	Electrochemical impedance spectroscopy	10 pM–25 nM	1.30 pM	SARS-CoV-2 pseudovirus	[27]
	Electrochemical impedance spectroscopy	0.01–64 nM	7.0 pM	Human saliva samples	[30]
	Differential pulse voltammetry	20–100 nM	7 nM	Artificial viral transport medium	[31]
	Square wave voltammetry	0.5–250 ng/mL	0.36 ng/mL	Saliva samples	[37]
S1 protein	Square wave voltammetry	0–1000 nM	0.75 fM	Pharyngeal swab samples	[33]
	Electrochemical impedance spectroscopy	10^1 – 10^6 ag/mL	8.80 ag/mL	Artificial saliva samples	[32]
	Electrochemical impedance spectroscopy	-	-	Clinical samples	[34]
Spike protein	Square wave voltammetry	0.1 fg/mL–1.2 $\mu\text{g/mL}$	0.03 fg/mL	Saliva and viral transport medium samples	[35]
	Differential pulse voltammetry (DPV) and chronopotentiometry (CP)	0–1000 ng/mL	DPV: 0.11 ng/mL CP: 37.8 ng/mL	Diluted human saliva samples	[28]
Nucleocapsid protein	Square wave voltammetry	5–100 nM	2.40, 1.95, 10 and 1.75 nM for A15, A58, A48 and A61 aptamers, respectively	Artificial saliva samples	[36]
S1 protein	Differential pulse voltammetry (DPV)	1 fg/mL–1 ng/mL in buffer and artificial saliva medium	0.11 fg/mL in buffer medium and 0.16 fg/mL in artificial saliva medium	Artificial saliva samples	This study

between influenza and COVID-19, while MERS-CoV-S1 was selected because of its structural similarity to the SARS-CoV-2 S1 protein. Selectivity tests were conducted at two concentrations (100 fg/mL and 1000 fg/mL), both in the presence and absence of the target S1 protein. The results are shown in Fig. 4C and D. In the absence of S1 protein, the relative current responses at 100 fg/mL were calculated as 2.60% for HA and 1.41% for MERS. At 1000 fg/mL, the responses were 2.63% for HA and 2.75% for MERS. In contrast, when S1 protein was present in the system, the relative responses in the presence of HA and MERS at 100 fg/mL were 99.05% and 98.70%, respectively. At 1000 fg/mL, the corresponding values were 96.25% and 96.30%. These percentage values were calculated by considering the signal obtained from 100 fg/mL and 1000 fg/mL S1 protein as 100%, respectively. Overall, the low background signals from HA and MERS and the strong target response in mixed conditions confirm the high selectivity of the developed aptasensor toward SARS-CoV-2 S1 protein, highlighting its suitability for application in complex biological matrices and in the presence of potential interferents. To validate the statistical differences between the S1 protein and other interfering agents such as HA and MERS, Student's *t*-test analysis was performed. The analysis was conducted to determine whether the average current responses ($n=3$) obtained from each sample group were significantly different, under the assumption of equal variances among the groups. At a 95% confidence interval, the obtained *p*-values for 100 fg/mL HA-S1 protein, 100 fg/mL MERS-S1 protein, 1000 fg/mL HA-S1 protein, and 1000 fg/mL MERS-S1 protein were found to be 0.006, 0.007, 0.017, and 0.013, respectively. Since all *p*-values were below the significance threshold of 0.05, these results confirm that there are statistically significant differences between the groups analyzed. Collectively, these findings demonstrate that, compared to other potentially interfering agents, the aptamer specific to the S1 protein exhibits a high binding affinity toward its target and that the developed biosensor displays excellent selectivity for the S1 protein.

To evaluate the applicability of the developed aptasensor in complex biological matrices, its performance was tested in artificial saliva. The artificial saliva solution was prepared as described in the Material and methods section. Artificial saliva was diluted at a ratio of 1:20 in PBS based on the optimization studies reported in our recent work [32]. In that study, dilution ratios ranging from 1:5 to 1:50 were evaluated, and the 1:20 ratio was determined to be optimal, providing the highest signal response and reproducibility while effectively minimizing matrix interference. Although the direct analysis of undiluted saliva is desirable for practical POC applications, our preliminary investigations indicated that the use of undiluted samples resulted

in poor reproducibility (high %RSD) and signal instability. This is primarily due to the high viscosity of the matrix and the rapid passivation of the electrode surface by salivary proteins. Therefore, the 1:20 dilution step was essential to minimize these matrix effects and ensure the reliability of the assay. S1 protein was spiked into this matrix at varying concentrations ranging from 1 fg/mL to 10 ng/mL. Aptamer-immobilized and MWCNT-IL-modified electrodes were immersed in these solutions to allow aptamer-target binding at the electrode surface. The sensor response to increasing S1 concentrations was evaluated using DPV in redox probe solution (Fig. S6). To determine the LOD within the linear range, S1 protein solutions were prepared in the concentration range of 10^0 to 10^6 fg/mL using 1:20 diluted artificial saliva. Aptamer-immobilized electrodes were incubated in these solutions and DPV measurements were conducted in the redox probe solution. The resulting voltammograms and calibration curve (mean current values, $n=3$) are presented in Fig. 5A and B. The calibration equation was determined as: $I/\mu\text{A} = -5.19 \log(C_{\text{S1 Protein}} / \text{fg mL}^{-1}) + 78.96$ with a correlation coefficient of $R^2 = 0.99$. According to the IUPAC method [54], the limit of detection was calculated as 0.16 fg/mL. Furthermore, the sensitivity of the biosensor in artificial saliva medium was determined by dividing the slope of the calibration curve (5.19) by the effective surface area of the electrode (0.2796 cm^2), yielding a value of $18.56 \mu\text{A mL fg}^{-1} \text{ cm}^{-2}$. It is worth noting that the current response obtained in artificial saliva (intercept: 78.96 μA) was comparable to that in the buffer medium (intercept: 81.94 μA), showing only a minimal signal suppression. This high signal retention is attributed to the physicochemical properties of the MWCNT-IL composite. The IL not only enhances the electrical conductivity but also acts as an antifouling interface, reducing the non-specific adsorption of salivary proteins (e.g., mucins) that typically passivate the electrode surface. Consequently, the electron transfer efficiency of the redox probe is preserved even after incubation in the complex saliva matrix.

The selectivity of the aptasensor was also assessed in 1:20 diluted artificial saliva using HA and MERS proteins as potential interferents. These experiments were performed at two concentrations (100 fg/mL and 1000 fg/mL), both in the absence and presence of S1 protein. The results are illustrated in Fig. 5C and D. In the absence of S1 protein, the current responses were minimal: 0.01% for HA and 0.58% for MERS at 100 fg/mL, and 0.57% for HA and 0.26% for MERS at 1000 fg/mL. When co-incubated with S1 protein, the relative signal responses at 100 fg/mL were 98.08% for HA and 98.61% for MERS, while at 1000 fg/mL they were 99.98% and 99.87%, respectively. These percentages were calculated by setting the response to 100 fg/mL and 1000 fg/mL S1 protein as 100%. These findings demonstrate that

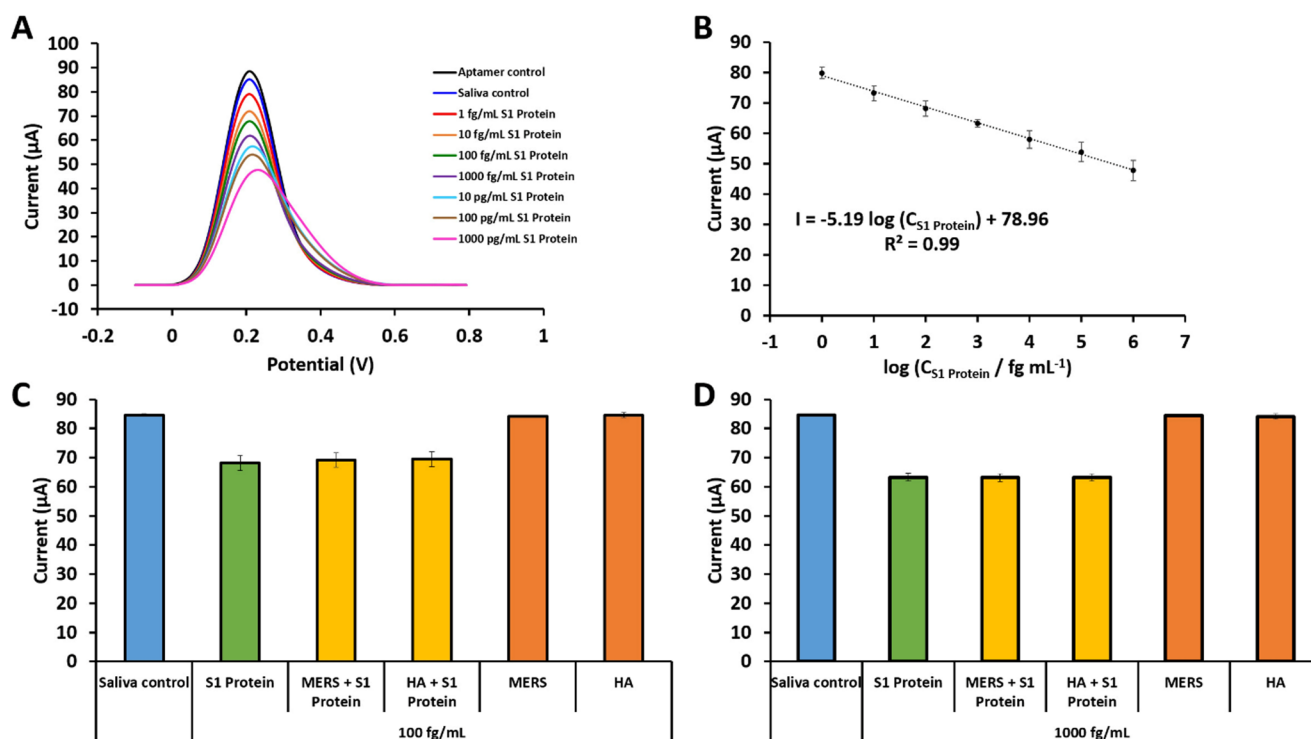


Fig. 5 (A) Differential pulse voltammograms and (B) the corresponding calibration curve obtained in the concentration range of 10^0 – 10^6 fg/mL in 1:20 artificial saliva medium using the developed aptasensor for the detection of SARS-CoV-2 S1 protein. (C) and (D) Histograms

showing the selectivity results against HA and MERS at concentrations of 100 fg/mL and 1000 fg/mL, respectively. DPV measurements were performed in a 2.5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox probe solution

the developed aptasensor retains high selectivity for S1 protein even in a diluted artificial saliva matrix, indicating its strong potential for practical application in real biological fluids. To further confirm the statistical differences between the S1 protein and other interfering agents such as HA and MERS, Student's *t*-test analysis was conducted based on the results obtained from samples prepared in artificial saliva. The analysis aimed to assess whether the average current responses ($n=3$) of each group differed significantly, under the assumption of equal variances across the sample sets. At a 95% confidence level, the *p*-values obtained for 100 fg/mL HA–S1 protein, 100 fg/mL MERS–S1 protein, 1000 fg/mL HA–S1 protein, and 1000 fg/mL MERS–S1 protein were 0.006, 0.008, 0.002, and 0.001, respectively. Since all *p*-values were found to be below the statistical significance threshold of 0.05, these results indicate that the differences between the tested groups are statistically significant. Collectively, the findings confirm that the aptamer specific to the S1 protein exhibits a strong binding affinity toward its target molecule. Moreover, the developed biosensor maintains high selectivity for the S1 protein even in a complex biological matrix such as artificial saliva.

To further evaluate the applicability of the aptasensor, recovery studies were conducted in 1:20 diluted artificial saliva by spiking known concentrations of S1 protein (0.1,

1, and 10 pg/mL). The recovery results are summarized in Table S2. Recovery values ranged from 108% to 120%, with relative standard deviation (%RSD) values below 6%. These results confirm that the developed aptasensor is accurate, reproducible, and suitable for detection of SARS-CoV-2 S1 protein in simulated saliva samples, highlighting its potential for non-invasive diagnostics.

To demonstrate the point-of-care applicability of the developed aptasensor, electrochemical measurements were conducted using a portable potentiostat (GalvanoPlot). The GalvanoPlot device was connected to a smartphone via a Type-C interface, and electrochemical measurements were performed using a standard three-electrode configuration. A photograph of the experimental setup, illustrating the connection between the smartphone and the portable potentiostat during the measurement, is provided in Fig. S7. S1 protein solutions in the range of 1 fg/mL to 10 ng/mL were prepared in 1:20 diluted artificial saliva. Aptamer-immobilized and MWCNT-IL composite-modified electrodes were immersed in these solutions to facilitate aptamer–target interaction at the electrode surface. DPV measurements were then carried out in redox probe solution to evaluate the sensor response to increasing analyte concentration (Fig. S8).

To determine the LOD within the linear response range, S1 protein solutions were prepared at concentrations

ranging from 10^0 to 10^6 fg/mL in 1:20 diluted artificial saliva. Aptamer-immobilized electrodes were incubated in these solutions, and subsequent DPV measurements were performed using the smartphone-integrated portable potentiostat. The resulting voltammograms and the calibration curve based on average current responses ($n = 3$) are presented in Fig. S9. The calibration equation was determined as: $I/\mu\text{A} = -7.64 \log(C_{\text{S1 Protein}} / \text{fg mL}^{-1}) + 107.06$, with a correlation coefficient of $R^2 = 0.99$. Based on the IUPAC method [54], the limit of detection was calculated to be 0.83 fg/mL. Furthermore, the sensitivity of the biosensor in artificial saliva medium was determined by dividing the slope of the calibration curve (7.64) by the effective surface area of the electrode (0.2796 cm^2), yielding a value of $27.32 \mu\text{A mL fg}^{-1} \text{ cm}^{-2}$.

Conclusion

In this study, an aptamer-based electrochemical biosensor was developed for the detection of SARS-CoV-2 S1 protein using a MWCNT-IL composite-modified electrode. To the best of our knowledge, this represents the first electrochemical aptasensor reported for SARS-CoV-2 S1 protein detection based on an MWCNT-IL composite platform. The composite material was systematically characterized and optimized, followed by the optimization of various experimental parameters related to aptasensor construction and operation. Under optimized conditions, the aptasensor demonstrated a wide linear response range ($1 \text{ fg/mL} - 1 \text{ ng/mL}$) in buffer, with an ultra-low limit of detection calculated as 0.11 fg/mL . Selectivity studies confirmed that the aptasensor exhibited high specificity for the S1 protein in the presence of potentially interfering proteins such as HA and MERS. The practical applicability of the developed aptasensor was successfully demonstrated in artificial saliva samples, where both sensitivity and selectivity were retained. Compared to previously reported aptasensors for COVID-19 detection [27, 28, 30, 31, 36, 37], the proposed sensor achieved a lower detection limit over a broader concentration range. Taken together, these findings suggest that the MWCNT-IL-based aptasensor offers a low-cost, portable, and highly sensitive alternative for SARS-CoV-2 diagnostics. In addition to its low detection limit and high specificity, the sensor's short analysis time and broad linear range position it as a promising platform with strong potential to complement or even surpass existing diagnostic methods for COVID-19. While the results obtained in artificial saliva demonstrate the robust performance of the aptasensor in a complex matrix, it is acknowledged that real clinical samples may present additional challenges due to biological variability and the presence of specific enzymes. Therefore,

further clinical validation using nasopharyngeal swabs or saliva samples from COVID-19 patients is planned as the next stage of this research to fully establish the point-of-care utility of the proposed device.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00604-026-07918-7>.

Acknowledgements A.E. would like to express her gratitude to the Turkish Academy of Sciences (TÜBA) as the full member for its partial support. The authors acknowledge BioRender for assistance in creating the graphical abstract and Fig. 1.

Author contributions Huseyin Senturk: Investigation, Methodology, Data acquisition, Writing – original draft– review & editing. Arzum Erdem: Investigation, Conceptualization, Methodology, Data acquisition, Supervision, Funding acquisition, Writing – original draft– review & editing.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK). The authors declare that no funding was received for this study.

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval Not applicable.

Competing interests The authors declare no competing interests.

Clinical trial number Not applicable.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

1. Zhu N, Zhang D, Wang W et al (2020) A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 382:727–733. <https://doi.org/10.1056/nejmoa2001017>
2. Mahshid SS, Flynn SE, Mahshid S (2021) The potential application of electrochemical biosensors in the COVID-19 pandemic: A perspective on the rapid diagnostics of SARS-CoV-2. *Biosens Bioelectron* 176:112905. <https://doi.org/10.1016/j.bios.2020.112905>
3. Pascarella G, Strumia A, Piliago C et al (2020) COVID-19 diagnosis and management: a comprehensive review. *J Intern Med* 288:192–206. <https://doi.org/10.1111/joim.13091>

4. WHO (2025) WHO COVID-19 dashboard. In: WHO. <https://data.who.int/dashboards/covid19/cases>. Accessed 11 May 2025
5. Walls AC, Park YJ, Tortorici MA et al (2020) Structure, Function, and antigenicity of the SARS-CoV-2 Spike glycoprotein. *Cell* 180:281–292. <https://doi.org/10.1016/j.cell.2020.02.058>
6. Wang Q, Zhang Y, Wu L et al (2020) Structural and functional basis of SARS-CoV-2 entry by using human ACE2. *Cell* 181:894–904. <https://doi.org/10.1016/j.cell.2020.03.045>
7. Krammer F (2020) SARS-CoV-2 vaccines in development. *Nature* 586:516–527. <https://doi.org/10.1038/s41586-020-2798-3>
8. Kevadiya BD, Machhi J, Herskovitz J et al (2021) Diagnostics for SARS-CoV-2 infections. *Nat Mater* 20:593–605. <https://doi.org/10.1038/s41563-020-00906-z>
9. Kumar N, Shetti NP, Jagannath S, Aminabhavi TM (2022) Electrochemical sensors for the detection of SARS-CoV-2 virus. *Chem Eng J* 430:132966. <https://doi.org/10.1016/j.cej.2021.132966>
10. Ravi N, Cortade DL, Ng E, Wang SX (2020) Diagnostics for SARS-CoV-2 detection: A comprehensive review of the FDA-EUA COVID-19 testing landscape. *Biosens Bioelectron* 165:112454. <https://doi.org/10.1016/j.bios.2020.112454>
11. Grieshaber D, MacKenzie R, Vörös J, Reimhult E (2008) Electrochemical Biosensors - Sensor principles and architectures. *Sensors* 8:1400–1458. <https://doi.org/10.3390/s80314000>
12. Wang J (2006) Electrochemical biosensors: towards point-of-care cancer diagnostics. *Biosens Bioelectron* 21:1887–1892. <https://doi.org/10.1016/j.bios.2005.10.027>
13. Singh A, Sharma A, Ahmed A et al (2021) Recent advances in electrochemical biosensors: Applications, challenges, and future scope. *Biosensors* 11:1–31. <https://doi.org/10.3390/bios11090336>
14. Erdem A, Senturk H, Yildiz E, Maral M (2022) Amperometric immunosensor developed for sensitive detection of SARS-CoV-2 Spike S1 protein in combined with portable device. *Talanta* 244:123422. <https://doi.org/10.1016/j.talanta.2022.123422>
15. Erdem A, Senturk H, Yildiz E, Maral M (2022) Impedimetric detection based on Label-Free immunoassay developed for targeting Spike S1 protein of SARS-CoV-2. *Diagnostics* 12:1–15. <https://doi.org/10.3390/diagnostics12081992>
16. Gutierrez-Galvez L, García-Fernandez D, Barrio M, del et al (2024) Free PCR virus detection via few-layer bismuthene and tetrahedral DNA nanostructured assemblies. *Talanta* 269:125405. <https://doi.org/10.1016/j.talanta.2023.125405>
17. Enebral-Romero E, García-Fernandez D, Gutierrez-Galvez L et al (2024) Bismuthene - Tetrahedral DNA nanobioconjugate for virus detection. *Biosens Bioelectron* 261:116500. <https://doi.org/10.1016/j.bios.2024.116500>
18. Romero EE, Perinán EM, López D et al (2025) MoS₂ - DNA tetrahedral bioconjugate for high - performance DNA biosensors: application in viral infection diagnostics. *Microchim Acta* 192:1–12. <https://doi.org/10.1007/s00604-025-07084-2>
19. Enebral-Romero E, Gutierrez-Galvez L, Cano R, Del et al (2023) Pathogen sensing device based on 2D MoS₂ / graphene heterostructure. Manuel V a ennifer G o. *Sens Actuators B Chem* 392:134105. <https://doi.org/10.1016/j.snb.2023.134105>
20. Wang J (2005) Carbon-nanotube based electrochemical biosensors: A review. *Electroanalysis* 17:7–14. <https://doi.org/10.1002/elan.200403113>
21. Vashist SK, Zheng D, Al-Rubeaan K et al (2011) Advances in carbon nanotube based electrochemical sensors for bioanalytical applications. *Biotechnol Adv* 29:169–188. <https://doi.org/10.1016/j.biotechadv.2010.10.002>
22. Shiddiky MJA, Torriero AAJ (2011) Application of ionic liquids in electrochemical sensing systems. *Biosens Bioelectron* 26:1775–1787. <https://doi.org/10.1016/j.bios.2010.08.064>
23. Singh VV, Nigam AK, Batra A et al (2012) Applications of ionic liquids in electrochemical sensors and biosensors. *Int J Electrochem* 2012:1–19. <https://doi.org/10.1155/2012/165683>
24. Song S, Wang L, Li J et al (2008) Aptamer-based biosensors. *TrAC Trends Anal Chem* 27:108–117. <https://doi.org/10.1016/j.trac.2007.12.004>
25. Iliuk AB, Hu L, Tao WA (2011) Aptamer in bioanalytical applications. *Anal Chem* 83:4440–4452. <https://doi.org/10.1021/ac201057w>
26. Yoo H, Jo H, Oh SS (2020) Detection and beyond: challenges and advances in aptamer-based biosensors. *Mater Adv* 1:2663–2687. <https://doi.org/10.1039/d0ma00639d>
27. Abrego-Martinez JC, Jafari M, Chergui S et al (2022) Aptamer-based electrochemical biosensor for rapid detection of SARS-CoV-2: nanoscale electrode-aptamer-SARS-CoV-2 imaging by photo-induced force microscopy. *Biosens Bioelectron* 195:113595. <https://doi.org/10.1016/j.bios.2021.113595>
28. Adeel M, Asif K, Alshabouna F et al (2022) Label-free electrochemical aptasensor for the detection of SARS-CoV-2 Spike protein based on carbon cloth sputtered gold nanoparticles. *Biosens Bioelectron X* 12:100256. <https://doi.org/10.1016/j.biosx.2022.100256>
29. Wang Z, Zhang C, He S, Xu D (2023) An ultrasensitive fluorescence aptasensor for SARS-CoV-2 antigen based on hyperbranched rolling circle amplification. *Talanta* 255:124221. <https://doi.org/10.1016/j.talanta.2022.124221>
30. Amouzadeh Tabrizi M, Acedo P (2022) An electrochemical impedance Spectroscopy-Based aptasensor for the determination of SARS-CoV-2-RBD using a carbon Nanofiber–Gold nanocomposite modified Screen-Printed electrode. *Biosensors* 12:142. <https://doi.org/10.3390/bios12030142>
31. Curti F, Fortunati S, Knoll W et al (2022) A Folding-Based electrochemical aptasensor for the Single-Step detection of the SARS-CoV-2 Spike protein. *ACS Appl Mater Interfaces* 14:19204–19211. <https://doi.org/10.1021/acsami.2c02405>
32. Erdem A, Senturk H, Yildiz E, Maral M (2024) Optimized aptamer-based next generation biosensor for the ultra-sensitive determination of SARS-CoV-2 S1 protein in saliva samples. *Int J Biol Macromol* 281:136233. <https://doi.org/10.1016/j.ijbiomac.2024.136233>
33. Jiang Y, Chen X, Feng N, Miao P (2022) Electrochemical aptasensing of SARS-CoV-2 based on triangular Prism DNA nanostructures and dumbbell hybridization chain reaction. *Anal Chem* 94:14755–14760. <https://doi.org/10.1021/acs.analchem.2c03401>
34. Lasserre P, Balansethupathy B, Vezza VJ et al (2022) SARS-CoV-2 aptasensors based on electrochemical impedance spectroscopy and Low-Cost gold electrode substrates. *Anal Chem* 94:2126–2133. <https://doi.org/10.1021/acs.analchem.1c04456>
35. Rahmati Z, Roushani M, Hosseini H, Choobin H (2022) Label-free electrochemical aptasensor for rapid detection of SARS-CoV-2 Spike glycoprotein based on the composite of Cu(OH)₂ nanorods arrays as a high-performance surface substrate. *Bioelectrochemistry* 146:108106. <https://doi.org/10.1016/j.bioelectchem.2022.108106>
36. Siu RHP, Jesky RG, Fan Y-J et al (2024) Aptamer-Mediated electrochemical detection of SARS-CoV-2 nucleocapsid protein in saliva. *Biosensors* 14:1–11. <https://doi.org/10.3390/bios14100471>
37. Amouzadeh Tabrizi M, Acedo P (2022) Highly sensitive aptasensor for the detection of SARS-CoV-2-RBD using aptamer-gated methylene blue@mesoporous silica film/laser engraved graphene electrode. *Biosens Bioelectron* 215:114556. <https://doi.org/10.1016/j.bios.2022.114556>
38. Cennamo N, Pasquardini L, Arcadio F et al (2021) SARS-CoV-2 Spike protein detection through a plasmonic D-shaped plastic

- optical fiber aptasensor. *Talanta* 233:122532. <https://doi.org/10.1016/j.talanta.2021.122532>
39. Vozgirdaite D, Ben Halima H, Bellagambi FG et al (2021) Development of an ImmunoFET for analysis of tumour necrosis Factor- α in artificial saliva: application for heart failure monitoring. *Chemosensors* 9:26. <https://doi.org/10.3390/chemosensors9020026>
 40. Gowda JI, Buddanavar AT, Nandibewoor ST (2015) Fabrication of multiwalled carbon nanotube-surfactant modified sensor for the direct determination of toxic drug 4-aminoantipyrine. *J Pharm Anal* 5:231–238. <https://doi.org/10.1016/j.jpha.2015.01.001>
 41. Sengiz C, Congur G, Eksin E, Erdem A (2015) Multiwalled carbon Nanotubes-Chitosan modified Single-Use biosensors for electrochemical monitoring of Drug-DNA interactions. *Electroanalysis* 27:1855–1863. <https://doi.org/10.1002/elan.201500107>
 42. Abo-Hamad A, Alsaadi MA, Hayyan M et al (2016) Ionic Liquid-Carbon nanomaterial hybrids for electrochemical sensor applications: A review. *Electrochim Acta* 193:321–343. <https://doi.org/10.1016/j.electacta.2016.02.044>
 43. Ajayan PM (1999) Nanotubes from carbon. *Chem Rev* 99:1787–1799. <https://doi.org/10.1021/cr970102g>
 44. Armand M, Endres F, MacFarlane DR et al (2009) Ionic-liquid materials for the electrochemical challenges of the future. *Nat Mater* 8:621–629. <https://doi.org/10.1038/nmat2448>
 45. Fukushima T, Kosaka A, Ishimura Y et al (2003) Molecular ordering of organic molten salts triggered by single-walled carbon nanotubes. *Sci* (80-) 300:2072–2074. <https://doi.org/10.1126/science.1082289>
 46. Park S, Kazlauskas RJ (2003) Biocatalysis in ionic liquids - Advantages beyond green technology. *Curr Opin Biotechnol* 14:432–437. [https://doi.org/10.1016/S0958-1669\(03\)00100-9](https://doi.org/10.1016/S0958-1669(03)00100-9)
 47. Polo-Luque ML, Simonet BM, Valcárcel M (2013) Functionalization and dispersion of carbon nanotubes in ionic liquids. *TrAC - Trends Anal Chem* 47:99–110. <https://doi.org/10.1016/j.trac.2013.03.007>
 48. Torimoto T, Tsuda T, Okazaki KI, Kuwabata S (2010) New frontiers in materials science opened by ionic liquids. *Adv Mater* 22:1196–1221. <https://doi.org/10.1002/adma.200902184>
 49. Wardak C (2009) Ionic liquids as new lipophilic additives to the membrane of lead ion-selective electrodes with solid contact. *Int J Environ Anal Chem* 89:735–748. <https://doi.org/10.1080/03067310902887642>
 50. Nie J, Xiao S, Tan R et al (2019) New insights on the fast response of poly(Ionic liquid)s to humidity: the effect of free-ion concentration. *Nanomaterials* 9:1–15. <https://doi.org/10.3390/nano9050749>
 51. Erdem A, Senturk H (2024) Smartphone-Controlled aptasensor for voltammetric detection of patulin in Apple juice. *Sensors* 24:754. <https://doi.org/10.3390/s24030754>
 52. Senturk H, Eksin E, Işık Ö et al (2021) Impedimetric aptasensor for lysozyme detection based on carbon nanofibres enriched screen-printed electrodes. *Electrochim Acta* 377:138078. <https://doi.org/10.1016/j.electacta.2021.138078>
 53. Senturk H, Erdem A (2024) Levan modified patulin specific impedimetric aptasensor with its application in food and biological fluids. *Microchem J* 199:109987. <https://doi.org/10.1016/j.microc.2024.109987>
 54. IUPAC (1976) Nomenclature, symbols, units and their usage in spectrochemical analysis—iii. Analytical flame spectroscopy and associated non-flame procedures. *Pure Appl Chem* 45:105–123. <https://doi.org/10.1351/pac197645020105>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.