

Novel Paper-Based Electrochemical System for Detection of Anti-Spike Protein Antibodies from SARS-CoV-2

Rita M. Martins

`rita.a.martins@uc.pt`

University of Coimbra

Adélio M. Mendes

Universidade do Porto

Caglar Elbuken

University of Oulu

M. Goreti F. Sales

University of Coimbra

Research Article

Keywords: Electrochemical Biosensor, SARS-CoV-2 Immunity, Biomimetic Virus Architecture, Point-of Care Diagnostics, Paper-based Device

Posted Date: July 15th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10215707/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

A novel disposable paper-based electrochemical biosensor for detecting anti-spike protein antibodies is presented, featuring the innovative use of carbon dots derived from a biological source and a liposome-biorecognition element complex. The optimized electrochemical sensing platform is described and characterized, regarding its structural and functional properties. The sensor showed a linear response between 0.0010 and 10 $\mu\text{g mL}^{-1}$, demonstrating high sensitivity and a low limit of detection (LOD) of 1.78 pg mL^{-1} , as well as good selectivity. Real nasopharyngeal swab samples were tested for the first time using this type of paper-based electrochemical system for anti-spike antibody detection. This approach was designed to mimic the virus's response while ensuring greater sensitivity and selectivity.

Overall, a new electrochemical method for screening anti-spike antibodies was developed to monitor vulnerable populations for new SARS-CoV-2 infections and track the development of adaptive immunity within communities, contributing to the development of future point-of-care (POC) analysis without the need for specialized personnel or laboratory facilities.

Introduction

The global impact of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic has highlighted the need for rapid, accurate, and accessible diagnostic tools. While molecular-based methods targeting viral RNA remain fundamental for detecting acute infection, diagnostic assays for anti-SARS-CoV-2 antibodies are essential for understanding disease prevalence, assessing population immunity, monitoring long-term humoral immune response, and evaluating vaccine efficacy and prioritization [1]. Even now, with widespread vaccination and a return to the acute phase, the need for reliable, accurate antibody testing methods persists to monitor vulnerable populations and track the development of adaptive immunity within communities [2].

Among the various biological sample types used to detect antibodies, nasopharyngeal swabs are of particular interest because they can be self-collected by patients and used in specific testing systems. Such methodologies eliminate the need for trained personnel, laboratory facilities, and specialized equipment. However, antibody concentration in this type of sample is typically lower than in serological or plasma samples. This characteristic highlights the importance of developing new methods capable of delivering sensitive data in a point-of-care (POC) setting from self-collectible samples, such as nasopharyngeal swabs.

Currently, antibody concentration is monitored through conventional serological tests such as enzyme-linked immunosorbent assays (ELISAs), lateral flow assays, immunofluorescent assays, and chemiluminescent immunoassays [3]. However, these methods are either qualitative or require lengthy sample preparation or professional handling. In this context, electrochemical biosensors assembled on screen-printed electrodes (SPEs) have emerged as promising alternatives, offering potential as POC devices capable of providing quick responses with high sensitivity and selectivity, using small sample

volumes [4]. A review of the current literature up to May 2026 still reveals a trend towards using human serum as the primary real-sample matrix, and, often, the reported LODs are not optimal for the direct analysis of samples with inherently lower antibody concentrations. Table 1 summarizes examples of electrochemical biosensors developed for anti-SARS-CoV-2 antibody detection, highlighting the material used in the biosensor assembly, the sample type employed, and the reported LODs.

Table 1
– Electrochemical (bio)sensors for SARS-CoV-2 antibody detection in human samples.

Biosensor/Material used	Transducer	Limit of detection	Sample	Ref
C-SPE with bio-graphene	Impedimetric	$0.17 \text{ pg}\cdot\text{mL}^{-1}$	Serum	[5]
C-SPE with magnetic complexes	Voltammetric	55.6 to $92.5 \text{ ng}\cdot\text{mL}^{-1}$	Serum	[6]
Gold-Interdigitated Electrode Array	Impedimetric	$21 \text{ ng}\cdot\text{mL}^{-1}$	Serum	[7]
Gold SPE with SAMs of MSA	Voltammetric	$0.019 \text{ ng}\cdot\text{mL}^{-1}$	Serum	[8]
LIG Platforms in SPEs	Voltammetric	$560 \text{ ng}\cdot\text{mL}^{-1}$	Serum	[9]
Gold – Pt bimetallic nanoclusters in the structure of an Ig	Voltammetric	$8.22 \text{ ng}\cdot\text{mL}^{-1}$	Serum	[10]
Platform based on hydrogen bonding and Au clusters	Voltammetric	$0.03 \text{ fg}\cdot\text{mL}^{-1}$	Saliva; oropharyngeal swab	[11]
Flexible organic electrochemical transistor	Voltammetric	10 fM	Serum; saliva	[12]
Carbon WE on a PET film	Amperometric	$10.1 \text{ ng}\cdot\text{mL}^{-1}$ (IgG); $1.64 \text{ ng}\cdot\text{mL}^{-1}$ (IgM)	Serum	[13]
Phytic Acid doped PANI on graphene electrode	Impedimetric	8 nM	Serum	[14]
Gold electrode pre-modified with mix-assembly monolayer	Voltammetric and Impedimetric	2.53 nM (CV); 1.99 nM (EIS)	Serum	[15]
SPE with electrodeposited gold nanostructures	Voltammetric	0.27 nM (CV); 0.14 nM (DPV)	Serum	[16]
Graphene binding peptide in fusion with antigen	Voltammetric	$0.77 \mu\text{g}\cdot\text{mL}^{-1}$	Serum	[17]
Carboxylated SWCNTs on Carbon SPEs	Impedimetric	$0.7 \text{ pg}\cdot\text{mL}^{-1}$	Serum	[18]
PPy nanostructures with gold nanoparticles	Impedimetric	$0.386 \text{ ng}\cdot\text{mL}^{-1}$	Serum	[19]
3D nanoprinted array electrodes	Impedimetric	13 fM	Not tested	[20]

Biosensor/Material used	Transducer	Limit of detection	Sample	Ref
Aerosol Jet nanoprinted Reduced-GO Coated 3D electrodes	Impedimetric	0.0028 pM (S1) and 0.00169 pM (RBD) antibodies	Not tested	[21]
Paper-based biosensor with GO modified electrode	Voltammetric	0.96 (IgG) and 0.14 ng/mL (IgM)	Serum	[22]
LIG-based electrode	Voltammetric	20 $\mu\text{g}\cdot\text{mL}^{-1}$	Serum	[23]
Zinc oxide nanowires in paper-based electrodes	Impedimetric	0.4 pg ml^{-1}	Serum	[24]
PEDOT-AuNPs-based immunosensor	Impedimetric	$0.18 \times 10^{-19}\%$ (V/V)	Serum	[25]
Carbon SPE modified with cerium oxide-gold nanocomposite	Voltammetric and Impedimetric	2.47 pg ml^{-1}	Serum	[26]
MXene modified Carbon SPE	Voltammetric and Impedimetric	0.215 pg ml^{-1}	Serum	[27]
Paper-based C-SPE functionalized with CDs and a virus like complex	Voltammetric	1.78 pg mL^{-1}	Nasopharyngeal swab	This paper

AuNPs: Gold nanoparticles; LOD: Limit of detection; C-SPE: Carbon screen printed electrode; CDs: Carbon dots, CV: Cyclic voltammetry; EIS: Electrochemical impedance spectroscopy; GO: graphene oxide; Ig: Immunoglobulin; LIG: Laser-Induced Graphene; PEDOT: Poly(3,4-ethylenedioxythiophene); MSA: Mercaptosuccinic acid; PANI: Polyaniline; PPy: Polypyrrole; RBD: Receptor-binding domain; SAMs: Self-assembled monolayers; SPE: Screen printed electrode; SWCNT: Single-walled carbon nanotube; WE: Working electrode.

Additionally, recent progress in electrochemical POC analysis has focused on developing more environmentally friendly platforms and achieving lower LODs by using less expensive, sustainable materials for both electrode substrates and biosensor assembly. SPE platforms are typically made from ceramics with integrated printed circuits, often without consideration for the recyclability of the final system. Cellulose and printing paper emerged as alternatives for the electrode fabrication, enabling disposal of the final system through less polluting processes. Various paper-based strategies have been reported for detecting antigens and viruses [22], [24], [28]. Nevertheless, based on the reviewed literature, only one device has been reported to use this sustainable, inexpensive substrate material for the electrochemical detection of antibodies against SARS-CoV-2: the ePAD developed by Yakoh and his team [22]. However, their device requires a minimum sample exposure time of 30 min, and the LOD would need to be lower to detect positive samples with reduced antibody concentrations, as occurs in

nasopharyngeal swab samples. To decrease the LOD, electrocatalytic nanomaterials can be used to modify the working electrode (WE) surface, increasing the electroactive area and the system's conductivity.

Metal nanoparticles are the main choice for this purpose, either grown in situ or simply deposited on the WE. The risks and costs associated with handling metals have led to research on more sustainable nanoparticles. Carbon Dots (CDs) are used in the present work as an alternative to metal nanoparticles and are synthesized using a green chemistry approach. CDs are carbon-based nanomaterials with enhanced electrical and conductive properties and biocompatibility. Due to their nanometric size and graphene-like structure, CDs exhibit excellent potential to enhance biosensor sensitivity when immobilized on the surfaces of WEs [29].

Here, a disposable and highly sensitive biosensor is presented, featuring an innovative assembly approach, following appropriate characterization. For the first time, tests on real nasopharyngeal swab samples are performed using a paper-based electrochemical sensor to detect anti-spike protein antibodies, and a complex that mimics the virus structure is achieved by combining liposomes with the viral spike protein. This design approach was supported by the principle of antibody avidity, suggesting that when multiple antigen copies are spatially clustered on a membrane or particle surface, antibodies capable of engaging more than one epitope simultaneously can achieve substantially higher apparent binding strength than through a single 1:1 interaction with an isolated, surface-immobilized antigen [30]. The proposed architecture comprises the spike trimer anchored to a lipid bilayer rather than directly chemisorbed onto the electrode, intended to recreate the clustered, membrane-displayed geometry and thereby promote more effective antibody capture.

Experimental

Reagents and solutions

All chemicals were analytical grade, and ultrapure water was used (conductivity < 0.1 $\mu\text{S}/\text{cm}$). Carbon paste and dielectric ink for screen printing were obtained from SunChemical, and silver ink from RS PRO Components. Chemical reagents included potassium chloride (KCl), phosphate saline buffer (PBS) pellets, poly(vinyl chloride) (PVC), *N*-hydroxysuccinimide (NHS), *N*-ethyl-*N'* (3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC), *p*-phenylenediamine, and bovine serum albumin (BSA), all from Merck. Potassium hexacyanoferrate (III) ($\text{K}_3[\text{Fe}(\text{CN})_6]$) and cyclohexanone were purchased from Carlo Erba; and potassium hexacyanoferrate(II) trihydrate ($\text{K}_4[\text{Fe}(\text{CN})_6] \cdot 3\text{H}_2\text{O}$) from Panreac. Phospholipids 18:1 DGS-NTA(Ni) were obtained from Avanti Research. Recombinant SARS-CoV-2 Spike Trimer protein with a Histidine tag in the C-terminal and the corresponding Spike Neutralizing Antibody anti-SARS-CoV-2 were acquired from BPS Bioscience. Anti-SARS-CoV-2 nucleocapsid (N) protein antibody was obtained from RayBiotech. For ELISA reagent preparation, Tween 20 and the enzyme-conjugated secondary antibody (goat anti-human IgG with HRP) were purchased from Thermo Fisher Scientific, and

tetramethylbenzidine (TMB), citric acid, sodium citrate, and hydrogen peroxide were purchased from Merck.

Apparatus

All electrochemical experiments were conducted using homemade carbon SPEs connected to a Metrohm Autolab PGSTAT302N potentiostat/galvanostat, operated with NOVA software. Immobilization of the ST protein into the liposomes was performed in a MB-101 mixing block (Bioer, China). Size and surface charge data were obtained by Dynamic Light Scattering (DLS) and Zeta Potential analysis on Zetasizer Nano ZS from Malvern. Transmission electron microscopy (TEM) images were collected through a TEM-FEI-Tecnaï G2 Spirit Bio-Twin. Absorbance measurements in microplates for the ELISA assay were conducted in Tristar 2 LB 942 Multimode Reader Lab from Berthold Technologies.

Fabrication of carbon-SPEs

The electrodes were fabricated using a handmade screen-printing technique, which involved attaching the negative mask of the SPEs (a sticker) to the previously hydrophobized cellulose substrate, followed by the application of a conductive ink. Carbon paste from Sun Chemical was used as the conductive ink, and three layers were cast onto the SPE templates, each followed by 30 minutes of drying at 60°C in the oven. The pressure used to spread the ink was set manually and kept constant to ensure a uniform layer of consistent thickness at each step. A layer of silver ink was applied to the electrical contacts, and, after template removal, dielectric ink was also cast to insulate the three electrodes (working, reference, and counter) and left to cure overnight at 60°C.

Electrochemical measurements

All electrochemical measurements were conducted at room temperature, and solutions were prepared in ultra-pure water or PBS. The WE of the C-SPE was initially pretreated with 0.1 M KCl by performing 15 cyclic voltammetry (CV) cycles, applying a potential range of -1.0 to $+1.5$ V at a scan rate of 0.05 V s^{-1} . Squared-wave voltammetry (SWV) measurements were performed after each assembly step of the biosensor by applying a potential range from -0.3 to $+0.4$ V, with a frequency of 1 Hz, a step of 5 mV, and a modulation amplitude of 0.02 V. $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$ was used as the redox probe (0.5 mM in 0.01 mol L^{-1} PBS buffer pH 7.4).

Biosensor preparation/assembly

The procedure for assembling the biosensor on the working electrode of the C-SPEs is shown in Fig. 1. After the electrochemical pre-treatment, 20 μL of a CDs solution (synthesis adapted from [31] at 1 mg mL^{-1} , prepared in ultra-pure water) was drop-casted onto the WE of the SPE and dried for 30 min at

60°C, resulting in the SPE-CDs. The carboxylic groups on the surface of the CDs were activated with a solution of EDAC (0.025 mol L⁻¹) and NHS (0.0125 mol L⁻¹) in PBS for 90 minutes at room temperature. An amine layer was then formed on the WE to further facilitate liposome immobilization by incubating a solution of *p*-phenylenediamine (0.02 mol L⁻¹) for 60 minutes in a humid chamber. In parallel, liposomes were synthesized from 18:1 DGS-NTA-Ni phospholipids with a stock concentration of 2.5 mg mL⁻¹ in chloroform. The vial containing the phospholipids and organic solvent was kept at 60°C for 30 minutes to evaporate the chloroform, and ultra-pure water was added to achieve a concentration of 0.1 mg mL⁻¹. The solution was stirred for 30 minutes and sonicated for 15 minutes to form liposomes with an average size of 210 nm, using the hydration method. The biorecognition element was immobilized in the liposomes by combining the liposome suspension with spike trimer protein (ST), diluted in PBS to reach liposome concentrations of 0.01 mg mL⁻¹ and 0.1 mg mL⁻¹ of ST, and mixing for 1 hour at 37°C. Then the suspension was filtered through a 50 kDa ultra-centrifugal filter to remove unbound proteins. Returning to the WE of the SPE, 10 µL of liposomes with ST were incubated there for 1 hour in a humid chamber, and BSA was used to block the non-specific binding sites at a concentration of 0.5 mg mL⁻¹, also incubated for 1 hour on the WE at room temperature.

Liposomes' characterization

To determine liposome size and surface charge (zeta potential), liposome suspensions were analyzed using a Zetasizer Nano ZS. Liposome size was measured by photon correlation spectroscopy, considering the hydrodynamic diameter – the particle diameter plus the thickness of the surrounding hydration layer – and the polydispersity index (PDI), a dimensionless measure of the breadth of the particle size distribution. Surface charge was assessed by zeta potential measurements.

TEM was used to examine the surface morphology and actual size of the liposomes. The images obtained were analyzed using ImageJ and GraphPad Prism for statistical purposes.

Analytical performance of the biosensor

The analytical performance of the biosensor was first assessed in PBS buffer (0.01 mol L⁻¹, pH 7.4). The sensor's WE was incubated in PBS at room temperature for 20 min, 3–4 times, until a stable SWV signal was obtained. Increasing concentrations of anti-ST protein antibody prepared in PBS were then consecutively incubated in the WE at room temperature for 20 min and the electrochemical performance of the sensor was evaluated after each incubation. Calibration curves were plotted using the relative values to the blank from the current peaks corresponding to concentrations ranging from 0.0010 to 100 µg mL⁻¹.

To demonstrate the sensor's efficiency in a POC scenario, real nasopharyngeal swab samples were first tested by ELISA for the presence of anti-SARS-CoV-2 IgG antibodies. Three serial dilutions of a positive

sample, as well as the undiluted sample, were successively incubated on the working electrode of the sensor under the same conditions as those used in the PBS matrix.

Biosensor selectivity was evaluated by incubating the anti-N protein antibody for 20 min and comparing the sensor response with that obtained using the anti-ST protein antibody. The concentration levels used fell within the linear response range of the calibration curve for the anti-ST protein antibody.

The LOD was calculated from the linear relationship between the analytical signal and log concentration, following the IUPAC recommendations for ion-selective electrodes. In these, the LOD corresponds to the concentration of the analyte at the point where the extrapolated linear mid-range segment intersects the lowest-concentration segment of the calibration plot [32]. All electrochemical assays were performed in triplicate.

ELISA testing

The enzyme-linked immunosorbent assay (ELISA) detected the presence of IgG antibodies for the SARS-CoV-2 Spike protein in nasopharyngeal swab samples. Briefly, 96-well microtiter plates were coated overnight at 4°C with 50 µL of ST protein at 10 µg mL⁻¹ in coating buffer (PBS pH 7.4). Following 3 washing cycles with washing buffer (PBS containing 0.05% Tween-20), the plates were blocked with 50 µL blocking Solution (1% BSA in PBS) for 1 hour at 37°C. After washing the plates 3 times with washing buffer and 1 time with PBS, the primary antibody (anti-ST protein antibody) was diluted in blocking buffer and incubated in the wells of negative and positive controls, 50 µL of 0, 5 and 10 µg/mL, for 1 hour at 37°C. The nasopharyngeal swab samples were similarly incubated in the antigen-coated wells, with 3 serial dilutions in blocking buffer. Using the same washing procedure, the enzyme-conjugated secondary antibody (horseradish peroxidase (HRP)-conjugated goat anti-human IgG) was diluted in blocking buffer, at a concentration of 1:7000, as recommended by the manufacturer, and incubated for 1 hour at 37°C. The wells were then washed 3 times with washing buffer and once with PBS. Next, 50 µL of developing solution (TMB in citrate buffer with H₂O₂) was added, and the resulting mixture was incubated for 10 min at room temperature in the dark. To stop the enzymatic reaction, 50 µL of the stopping solution (1 M H₂SO₄) was added to each well and the absorbance of the microwell-plate was measured at 450 nm in a microplate reader.

A similar procedure was used to confirm the presence of the ST protein on the liposomes, with this complex serving as the antigen material. The testing wells were incubated with either the liposomes/ST complex or liposomes without the biorecognition element. Positive and negative controls with the ST protein diluted in coating buffer were incubated in the remaining wells. Subsequent washing cycles and incubation with blocking solution were performed as previously described. Primary antibody incubation was conducted over the same concentration range across all sets of conditions. The remaining steps of the ELISA assay, including absorbance measurement, were performed as in the first assay.

Results and Discussion

Biosensor assembly and characterization

The initial stage of biosensor assembly involved electrochemical pre-treatment using successive CV cycles. This pre-treatment was carried out in a KCl solution, which facilitated the permeation of ionic species at the WE surface (thereby decreasing the initial resistance to charge transfer) and improved similarity among electrode units (yielding more reproducible measurements). CV scanning was performed over a wide potential range, which also helped to remove surface impurities and oxide layers that could interfere with subsequent immobilization steps and electrochemical measurements.

Following pre-treatment, CDs were immobilized on the WE to increase the electroactive area, thereby improving the biosensor's analytical sensitivity, as previously demonstrated in the literature [33]. Carboxylic groups on CDs (-COOH) were activated via reaction with EDAC, generating a highly reactive O-acylisourea intermediate that immediately reacted with NHS. This formed an activated ester that is sufficiently unstable to react with an amine group under mild conditions [34]. The WE surface was then incubated with the diamine compound p-phenylenediamine, which bonded to the activated carboxylic surface. This generated an aminated surface, which is more suitable for immobilizing the biorecognition element, in this case, the liposomes/ST protein complex. This chemical modification results in a positively charged surface, while the biorecognition complex has an overall negative charge, thereby enhancing adsorption on the surface via ionic interactions [35]. Then, non-specific binding was prevented by incubating a BSA protein solution on the WE.

The main chemical modification steps were monitored by SWV measurements, with representative data shown in Fig. 2. After electrochemical pre-treatment, incubation with the CDs increased the conductivity of the WE, as shown by the substantial rise in the average current peaks, from 77 μA up to 150 μA , on average. The immobilization of the biorecognition element, the liposomes/ST protein complex, had the contrary effect, reducing the system's conductivity. As expected, these non-conductive biological elements decreased the current peaks by 33 μA , hindering the electron transfer on the modified electrode surface. The final assembly step further reduced the current peaks from 117 to 90 μA , as shown in Fig. 2B, confirming BSA adsorption. Overall, these results indicated that each assembly layer was successfully formed on the electrode surface.

Liposomes/ST protein complex synthesis and characterization

Liposomes containing the ST protein were developed to mimic the virus and elicit a response closer to real conditions. The liposomes consisted of a bilayer of 18:1 DGS-NTA-Ni phospholipids, whose hydrophilic head has a nickel termination capable of forming a coordination or metal-ligand complex with histidine-terminated proteins. This highly stable complex involves Lewis acid-base interactions between the imidazole nitrogen atoms of the histidine residues and the nickel ions [36].

These liposome structures were synthesized using the thin-film hydration (TFH) method, in which the phospholipids are dispersed in organic solvents. After the solvents were removed by evaporation, a thin lipidic film was formed in the flask. The hydration of the lipids occurs next, by adding an aqueous solvent under agitation. During this phase, the swollen lipids begin to form a multilamellar vesicle (MLV) suspension [37]. Sonication and/or extrusion are then essential techniques for downsizing and uniformizing liposome size.

DLS measurements were used to evaluate the hydrodynamic diameters of liposomes and the liposome/ST complex; the liposomes had an average hydrodynamic diameter of approximately 210 nm (Fig. 3A), which increased to 221 nm in the presence of the protein (Fig. 3B). This increase was predicted, as the ST protein is approximately 4.8 nm in size and is expected to be attached to the entire liposome surface. The Zeta potential of the liposomes and liposome + ST complex was also assessed, indicating that once the liposomes are functionalized with the protein, the overall net charge decreased from -35.6 mV to -48.4 mV, as shown in Fig. 3C. The ST protein has an isoelectric point (pI) of 6.3, which means that at a pH of 7.4, the protein carries an overall negative charge, thereby decreasing the net charge of the liposome + ST complex. TEM analysis confirmed the spherical morphology of the liposomes and showed an average size of approximately 205 nm (Fig. 3D, E), thereby supporting the results obtained by DLS.

An additional study of the protein and complex functionality in recognizing the anti-ST antibodies was conducted using an ELISA with either the ST protein or the liposome + ST as the antigen. Compared with the negative control, the results showed that, under both antigen conditions, the antibodies recognized the protein and the liposome + ST, as shown in Figure S3. This demonstrates not only that the ST protein formed a complex with the liposomes, but also that this complex is functional, with the protein's antibody-binding site exposed, thereby mimicking the virus's behavior. The presentation of the ST protein anchored to the liposome surface, rather than as an isolated soluble molecule, is hypothesized to enhance antibody capture through avidity effects. The clustering of multiple antigen copies on a tethered, membrane-like surface is predicted to augment the apparent binding strength of antibodies engaging more than one epitope simultaneously, both kinetically and thermodynamically, compared with monovalent engagement of a single immobilized protein [30], [38]. This can be, in fact, observed in Figure S3, since the average absorbance value for the complex liposome + ST is slightly higher than the one for the isolated ST molecules. The avidity effect has also been exploited by the recent use of a liposome-displayed antigen format to detect anti-SARS-CoV-2 antibodies in serum via an alternative immunosorbent assay, achieving a strong correlation with a reference ELISA and a pseudovirus neutralization assay [39], supporting the suitability of liposome-displayed viral antigens for antibody serology. Additionally, using structures that mimic the virus as the biorecognition element complex has been shown to provide greater stability, higher loading capacity, and enhanced signal amplification compared with single viral proteins [40]. Overall, this strategy simplified the synthesis of virus-like particles (VLPs) by employing a simple functionalized liposome structure, which is expected to increase the sensor's efficiency.

Analytical performance of the sensor in PBS and real samples

The biosensor's analytical performance was evaluated by consecutive incubation with increasing concentrations of anti-ST, diluted in PBS (0.0010 to $100\text{ }\mu\text{g mL}^{-1}$), followed by SWV electrochemical measurements. The data obtained were used to construct calibration curves by plotting the SWV peak current against antibody concentration (typical values are shown in Fig. 4A). The analytical response was assessed in four replicates, and a linear trend was observed from 0.0010 to $10\text{ }\mu\text{g mL}^{-1}$, where the relative current peak values versus the logarithm of antibody concentration showed a slope of 0.03 relative units per decade of concentration, with a squared correlation coefficient of 0.99 (Fig. 4B). At the highest concentration used, the binding kinetics changed, and the response became nonlinear, likely due to the sensors' surface saturation. To demonstrate the sensor's reproducibility, four independent calibrations were performed, yielding a relative standard deviation of the slope of less than 10% within the linear range. Overall, the sensor showed a highly sensitive response to anti-ST protein antibodies, along with an extremely low limit of detection of 1.78 pg mL^{-1} , as shown in Figure S1.

To assess its applicability, the biosensor was tested in real nasopharyngeal swab samples. An ELISA assay was first performed to verify the presence of antibodies in these samples. The typical results are shown in Figure S2. The positive sample was serially diluted three times in PBS buffer and incubated on the sensor from the lowest to the highest concentration. SWV measurements were collected after 20 minutes of incubation, as with the standard solutions. As before, the current peaks decreased after each incubation with the real samples. Figures 5A and 5B also show a linear response according to the applied dilutions, particularly in the last three samples. The most diluted nasopharyngeal swab may not contain a sufficient antibody concentration to significantly affect the electrode surface and decrease the current.

Selectivity of the sensor

The biosensor selectivity study was conducted to assess its ability to detect anti-ST antibodies and distinguish its response from that to other co-existing antibodies. This study examined the sensor's response to anti-N protein antibodies. Infected individuals also develop anti-N antibodies, which may circulate alongside anti-ST antibodies, each providing distinct insights into infection timing and immunity. Therefore, differentiating between anti-S and anti-N specific immunity is an important requirement for studying the immunological response in post-infected and vaccinated individuals.

To this end, the biosensor response was tested against individual solutions of anti-N or anti-ST protein antibodies, all prepared in PBS, over the same concentration range as in the previous calibration. The collected data consisted of current peak values from SWV measurements after each concentration incubation, plotted relative to the blank (Fig. 6). Overall, the biosensor response to anti-N was similar to the blank across all concentrations tested (relative values close to the blank), with only minor random variations, ranging from 0.939 to 0.975 , in terms of relative standard deviation. In contrast, the biosensor

signal in the presence of anti-ST antibodies was consistently more intense as the concentration increased, in line with the average values from the calibration. Comparing responses to both antibodies, the difference between anti-N and anti-ST responses increased with increasing protein concentration. Altogether, this confirmed that the biosensor provided a selective response for anti-ST antibodies, even in the presence of anti-N antibodies that are originated from exposure to the same virus. Even given that anti-N antibodies originated from the same viral exposure.

Conclusions

This work presents a ultrasensitive electrochemical biosensor for detecting SARS-CoV-2 spike protein antibodies, developed on sustainable paper-based screen-printed electrodes (SPEs). To enhance both sensitivity and biomimetic capacity, the biosensor employs a dual-innovation architecture: immobilized carbon dots (CDs) derived from natural sources, which significantly improve the sensor's electrochemical activity and signal transduction, and an innovative virus-mimicking biorecognition element, a biological complex (liposome/ST) engineered to mimic the SARS-CoV-2 virus, providing a highly favorable microenvironment for precise analyte recognition.

This approach enables direct detection of anti-spike protein antibodies in nasopharyngeal swab samples using disposable paper-based SPEs. Electrochemical characterization confirms excellent analytical performance in both buffer and real patient samples, achieving a remarkably low limit of detection (LOD) of 1.78 pg mL^{-1} and a differentiated selectivity towards anti-Nucleocapsid protein antibodies.

Overall, by using paper-based substrates and highly scalable fabrication techniques, the resulting biosensor contributes to future complex diagnostics in a cost-effective, portable format, offering new insights towards a transformative POC solution capable of screening for anti-S antibodies, monitoring vulnerable populations, and tracking community-level adaptive immunity, all without the need for specialized personnel or laboratory infrastructure.

Declarations

Author Contribution

R.M.M. was responsible for the conceptualization and conceived the study, designed and performed the experiments, analyzed the data, and wrote the original draft of the manuscript. A.M.M. , C.E and M.G.F.S. provided supervision, and critically reviewed the manuscript. M.G.F.S. contributed to the methodology related to the electrochemical platform, acquired funding, contributed to the conceptualization and design of the study. All authors approved the final version of the manuscript.

Acknowledgement

The authors acknowledge funding from the National Foundation for Science and Technology, I.P., Portugal (FCT) through the PhD. Grant, reference 2021.08391.BD. The research leading to these results also received funding from: i) FCT, Fundação para a Ciência e a Tecnologia, under projects UID/00285/2025 (DOI: 10.54499/UID/00285/2025) and LA/P/0112/2020 (DOI: 10.54499/LA/P/0112/2020); ii) FCT/MCTES (PIDDAC): LEPABE, UIDB/00511/2020 (DOI: 10.54499/UIDB/00511/2020) and UIDP/00511/2020 (DOI: 10.54499/UIDP/00511/2020) and ALiCE, LA/P/0045/2020 (DOI: 10.54499/LA/P/0045/2020).

References

1. Mink S, Fraunberger P (Dec. 2023) Anti-SARS-CoV-2 Antibody Testing: Role and Indications. *J Clin Med* 12:7575. 10.3390/jcm12247575
2. Arévalo-Herrera M et al (Jan. 2025) Longitudinal Follow-Up of the Specific Antibody Response to SARS-CoV-2 Vaccination in Colombia. *J Med Virol* 97. 10.1002/jmv.70133
3. Benzigar MR, Bhattacharjee R, Baharfar M, Liu G (2020) Current methods for diagnosis of human coronaviruses: pros and cons. *Anal Bioanal Chem* 2311–2330. 10.1007/s00216-020-03046-0
4. Rahman MM (2022) Progress in Electrochemical Biosensing of SARS-CoV-2 Virus for COVID-19 Management. 10. 10.3390/chemosensors10070287
5. Cardoso AR, Suleimenova A, Alves JF, Frasco MF, Barquinha P, Sales MGF (Feb. 2025) Ultrasound-assisted protein-enhanced graphene synthesis for rapid electrochemical antibody sensing. *Microchem J* 209:112647. 10.1016/j.microc.2025.112647
6. Yildirim-Tirgil N, Ayni E, Kaya K (Feb. 2025) Enhanced electrochemical detection of SARS-CoV-2 IgG using magnetic nanocomplexes: evaluation of preparation processes and sensor stability. *J Nanopart Res* 27. 10.1007/s11051-025-06240-2
7. Shoute LCT, Charlton CL, Kanji JN, Babiuk S, Babiuk L, Chen J (Jan. 2024) Faradaic Impedimetric Immunosensor for Label-Free Point-of-Care Detection of COVID-19 Antibodies Using Gold-Interdigitated Electrode Array. *Biosens (Basel)* 14. 10.3390/bios14010006
8. Kock BJ et al (May 2024) A Simplistic Label-Free Electrochemical Immunosensing Approach for Rapid and Sensitive Detection of Anti-SARS-COV-2 Nucleocapsid Antibodies. *ChemistrySelect* 9. 10.1002/slct.202400409
9. Martins BR et al (2024) Electrochemical Immunosensors on Laser-Induced Graphene Platforms for Monitoring of Anti-RBD Antibodies After SARS-CoV-2 Infection, *Biosensors (Basel)*., vol. 14, Nov. 10.3390/bios14110514
10. Vázquez-Díaz S, Saa L, Otaegui D, Pavlov V, Palazón A, Cortajarena AL (Feb. 2025) Dual-Mode Immunosensor for Antibody Detection: Harnessing the Versatility of Antibody-Based Nanozymes across Optical and Electrochemical Platforms. *Anal Chem*. 10.1021/acs.analchem.4c05317
11. Liv L, Yener M, Çoban G, Şevval, Can A Electrochemical biosensing platform based on hydrogen bonding for detection of the SARS-CoV-2 spike antibody, *Anal. Bioanal. Chem.*, vol. 1, p. 3. 10.1007/s00216-021-03752-3

12. Liu H et al (2021) Ultrafast, sensitive, and portable detection of COVID-19 IgG using flexible organic electrochemical transistors,, [Online]. Available: <https://www.science.org>
13. Peng R, Pan Y, Li Z, Qin Z, Rini JM, Liu X, SPEEDS (Feb. 2022) A portable serological testing platform for rapid electrochemical detection of SARS-CoV-2 antibodies. *Biosens Bioelectron* 197. 10.1016/j.bios.2021.113762
14. Drobysh M, Ramanavicius A, Baradoke A (Mar. 2023) Polyaniline-based electrochemical immunosensor for the determination of antibodies against SARS-CoV-2 spike protein. *Sci Total Environ* 862:160700. 10.1016/j.scitotenv.2022.160700
15. Towards an Electrochemical Immunosensor for the Detection of Antibodies against SARS-CoV-2 Spike Protein, (2022) 10.1149/1945-7111/ac5d91
16. Drobysh M et al (2022) Determination of rSpike Protein by Specific Antibodies with Screen-Printed Carbon Electrode Modified by Electrodeposited Gold Nanostructures, *Biosensors* Vol. 12, Page 593, vol. 12, no. 8, p. 593, Aug. 2022. 10.3390/BIOS12080593
17. Braz BA et al (Oct. 2022) Graphene-Binding Peptide in Fusion with SARS-CoV-2 Antigen for Electrochemical Immunosensor Construction. *Biosens (Basel)* 12(10):885. 10.3390/BIOS12100885/S1
18. Cardoso AR et al (Dec. 2022) An ultra-sensitive electrochemical biosensor using the Spike protein for capturing antibodies against SARS-CoV-2 in point-of-care. *Mater Today Bio* 16:100354. 10.1016/J.MTBIO.2022.100354
19. Hryniewicz BM et al (Jun. 2022) Development of polypyrrole (nano)structures decorated with gold nanoparticles toward immunosensing for COVID-19 serological diagnosis. *Mater Today Chem* 24:100817. 10.1016/J.MTCHEM.2022.100817
20. Ali MA et al (May 2022) N protein-based ultrasensitive SARS-CoV-2 antibody detection in seconds via 3D nanoprinted, microarchitected array electrodes. *J Med Virol* 94(5):2067–2078. 10.1002/JMV.27591
21. Azahar Ali M et al (Feb. 2021) Sensing of COVID-19 Antibodies in Seconds via Aerosol Jet Nanoprinted Reduced-Graphene-Oxide-Coated 3D Electrodes. *Adv Mater* 33(7):2006647. 10.1002/ADMA.202006647
22. Yakoh A, Pimpitak U, Rengpipat S, Hirankarn N, Chailapakul O, Chaiyo S (Mar. 2021) Paper-based electrochemical biosensor for diagnosing COVID-19: Detection of SARS-CoV-2 antibodies and antigen. *Biosens Bioelectron* 176. 10.1016/j.bios.2020.112912
23. Oliveira ME et al (2022) Electrochemical Biosensor Based on Laser-Induced Graphene for COVID-19 Diagnosing: Rapid and Low-Cost Detection of SARS-CoV-2 Biomarker Antibodies. 10.3390/surfaces5010012
24. Li X et al (Apr. 2021) Enhancing the performance of paper-based electrochemical impedance spectroscopy nanobiosensors: An experimental approach. *Biosens Bioelectron* 177. 10.1016/j.bios.2020.112672

25. Lorenzen AL, dos Santos AM, dos Santos LP, da Silva Pinto L, Conceição FR, Wolfart F (Feb. 2022) PEDOT-AuNPs-based impedimetric immunosensor for the detection of SARS-CoV-2 antibodies. *Electrochim Acta* 404:139757. 10.1016/J.ELECTACTA.2021.139757
26. Devi MJ, Syafira RS, Gaffar S, Irkham I, Einaga Y, Hartati YW (2026) High-sensitivity electrochemical immunosensor for anti-SARS-CoV-2 IgG detection using screen-printed carbon/cerium oxide-gold electrode. *ADMET DMPK* 14. 10.5599/ADMET.3182
27. Kanarya D, Gürbüz HN, Tirgil NY (Sep. 2026) Electrochemical SARS-CoV-2 IgG immunosensor with a high-surface-area PThi/MXene/SPE. *Appl Surf Sci* 741:167221. 10.1016/J.APSUSC.2026.167221
28. Ehsan MA, Khan SA, Rehman A (Jun. 2021) Screen-printed graphene/carbon electrodes on paper substrates as impedance sensors for detection of coronavirus in nasopharyngeal fluid samples. *Diagnostics* 11(6). 10.3390/diagnostics11061030
29. Holzinger M, Le Goff A, Cosnier S (2014) Nanomaterials for biosensing applications: A review, *Front. Chem.*, vol. 2, no. AUG, p. 63, Aug. 10.3389/FCHEM.2014.00063/BIBTEX
30. Einav T, Yazdi S, Coey A, Bjorkman PJ, Phillips R (2019) Harnessing Avidity: Quantifying the Entropic and Energetic Effects of Linker Length and Rigidity for Multivalent Binding of Antibodies to HIV-1, *Cell Syst.*, vol. 9, no. 5, pp. 466–474.e7, Nov. 10.1016/j.cels.2019.09.007
31. Shao Y et al (2020) Green synthesis of multifunctional fluorescent carbon dots from mulberry leaves (*Morus alba* L.) residues for simultaneous intracellular imaging and drug delivery, *Journal of Nanoparticle Research*, vol. 22, no. 8, p. 229-, Aug. 10.1007/S11051-020-04917-4/METRICS
32. Hibbert DB (2023) Compendium of Terminology in Analytical Chemistry. The Royal Society of Chemistry. 10.1039/9781788012881
33. Alemu YA, Louw CJ, Paolucci F, Valenti G, Baker PGL (2025) Luminescence and Electroanalytical Properties of Carbon Quantum Dots in the Context of Immunosensor Design. *ChemElectroChem* 12(6). 10.1002/celc.202400624
34. Jiang K, Schadler LS, Siegel RW, Zhang X, Zhang H, Terrones M (2004) Protein immobilization on carbon nanotubes via a two-step process of diimide-activated amidation. *J Mater Chem* 14(1):37–39. 10.1039/b310359e
35. Fujino Y et al (Dec. 2020) Electrochemical impedance spectroscopy study of liposome adsorption and rupture on self-assembled monolayer: Effect of surface charge. *J Electroanal Chem* 878. 10.1016/j.jelechem.2020.114572
36. Raghunath G, Dyer RB (2019) Kinetics of Histidine-Tagged Protein Association to Nickel-Decorated Liposome Surfaces, *Langmuir*, vol. 35, pp. 12550–12561, Sep. 10.1021/acs.langmuir.9b01700
37. Michelon M, Lombardo D, Kiselev MA (2022) Methods of Liposomes Preparation: Formation and Control Factors of Versatile Nanocarriers for Biomedical and Nanomedicine Application, *Pharmaceutics*, vol. p. 543, 2022. 10.3390/pharmaceutics14030543
38. Multivalent virus-like epitope display amplifies BCR signaling independent of avidity, *Nature Immunology* 2023 24:10, vol. 24, no. 10, pp. 1610–1611, (2023) 10.1038/s41590-023-01619-6

39. Reiner C et al (2025) Development of a Novel Homogeneous Liposome-Based One-Step Assay for SARS-CoV-2 Antibody Detection in Human Serum Based on Fluorescent Liposomes and Complement Activity, *Anal. Chem.*, vol. 97, no. 44, pp. 24543–24555, Nov. 10.1021/ACS.ANALCHEM.5C04506
40. Sun X, Lian Y, Tian T, Cui Z (2024) Virus-like particle encapsulation of functional proteins: advances and applications. *Theranostics* 14:7604–7622. 10.7150/THNO.103127

Figures

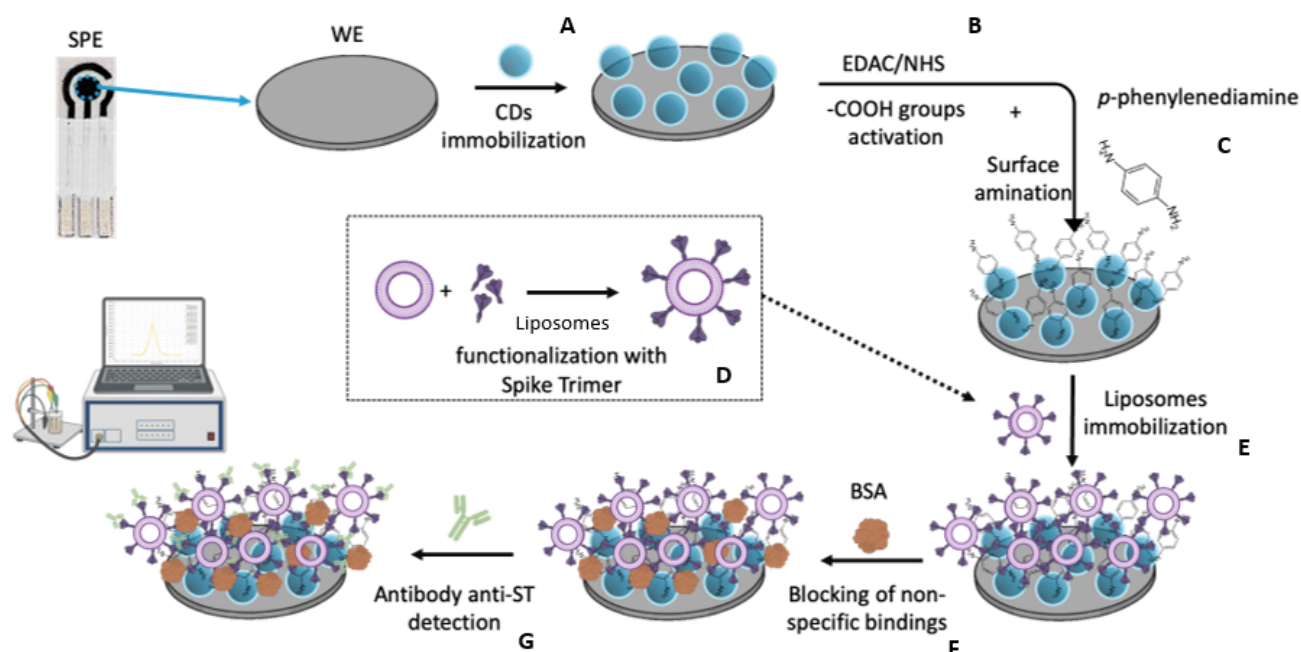


Figure 1

Schematic representation of the biosensor assembly and anti-Spike protein antibody recognition. (A) Carbon dots (CDs) immobilization in the working electrode (WE) area of the screen-printed electrode (SPE). (B) Carboxylic groups (-COOH) activation with *N*-ethyl-*N'*-(3-dimethylaminopropyl) carbodiimide / *N*-hydroxysuccinimide (EDAC/NHS). (C) Surface amination with *p*-phenylenediamine. (D) Liposomes' functionalization with the biorecognition element Spike Trimer. (E) Their immobilization on the WE. (F) Blocking of the non-specific binding sites with BSA protein. (G) Selective binding of the antibody against the Spike protein.

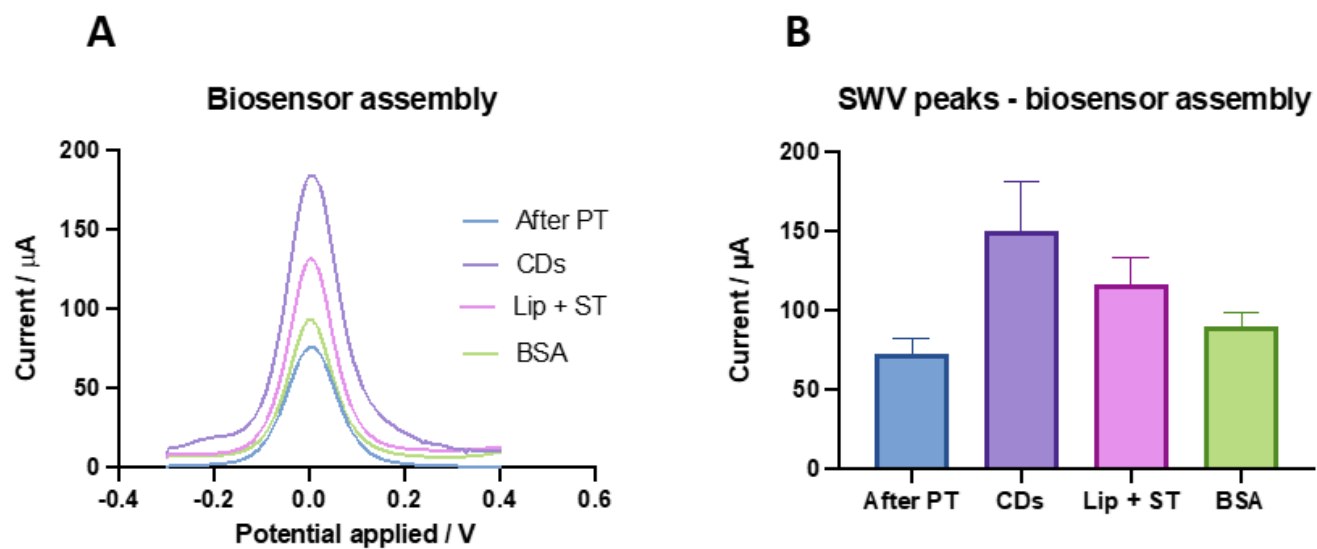


Figure 2

SWV measurements (A) and the mean current peak intensity values with the respective error bars for the sensor assembly (B), plotted for the several construction stages: after pre-treatment (PT), followed by the subsequent incubation of carbon dots (CDs), the liposomes/ST protein complex (lip +ST), and the blocking agent, the BSA protein.

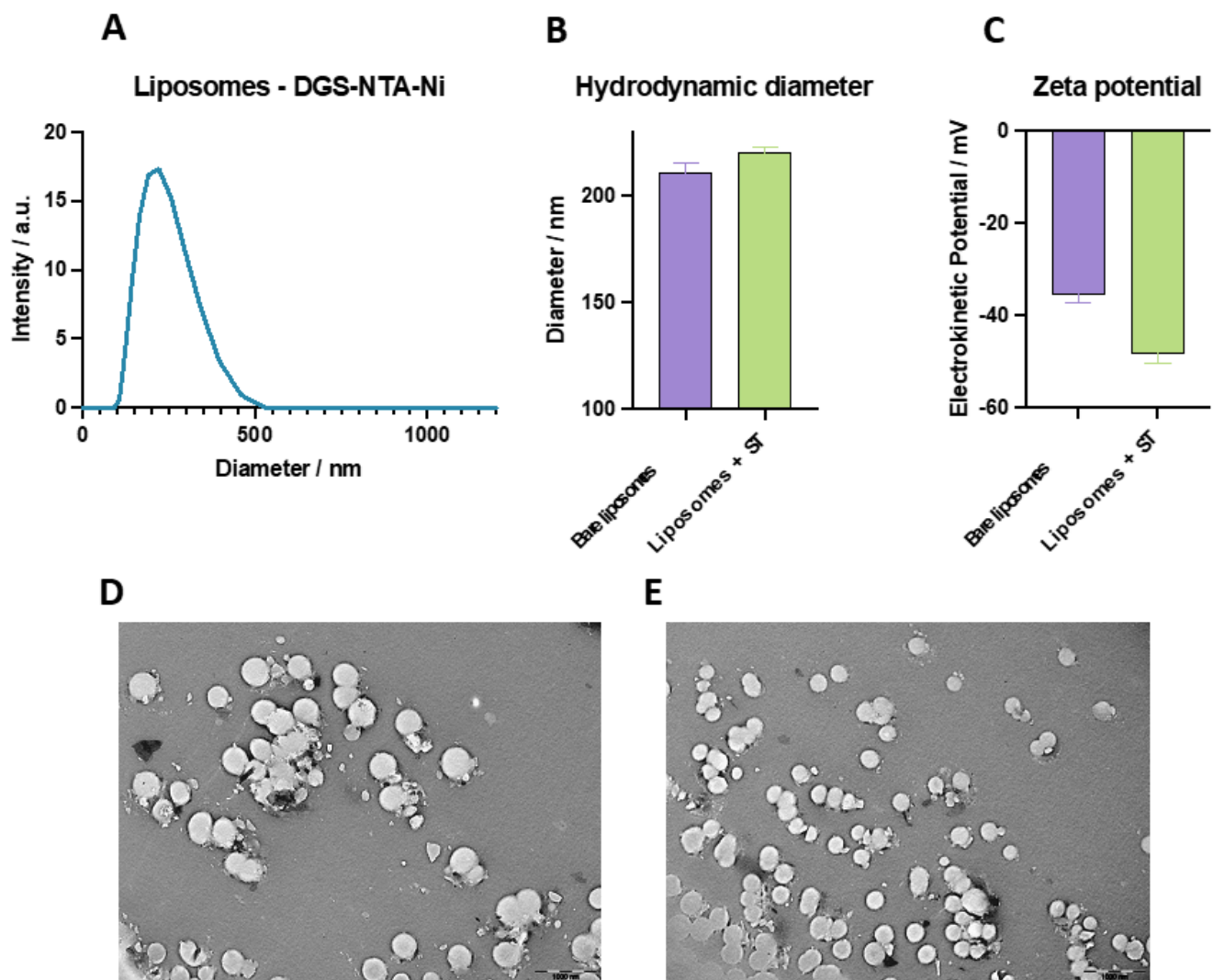


Figure 3

Characterization of liposomes. Hydrodynamic diameter and its distribution through DLS analysis (A). Liposomes with and without the ST protein characterization in terms of hydrodynamic diameter average values (B) and zeta potential (C). TEM analysis of liposomes (D), (E).

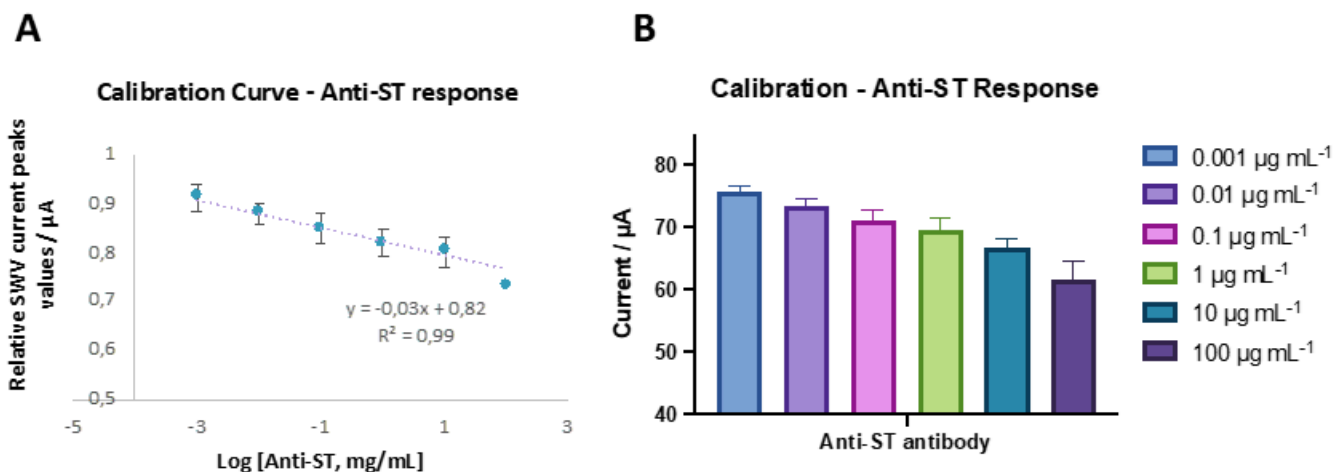


Figure 4

Calibration plot with the relative current peak current values plotted against the log concentration of antibody (A), and mean peak currents of the SWV measurements for each concentration (B).

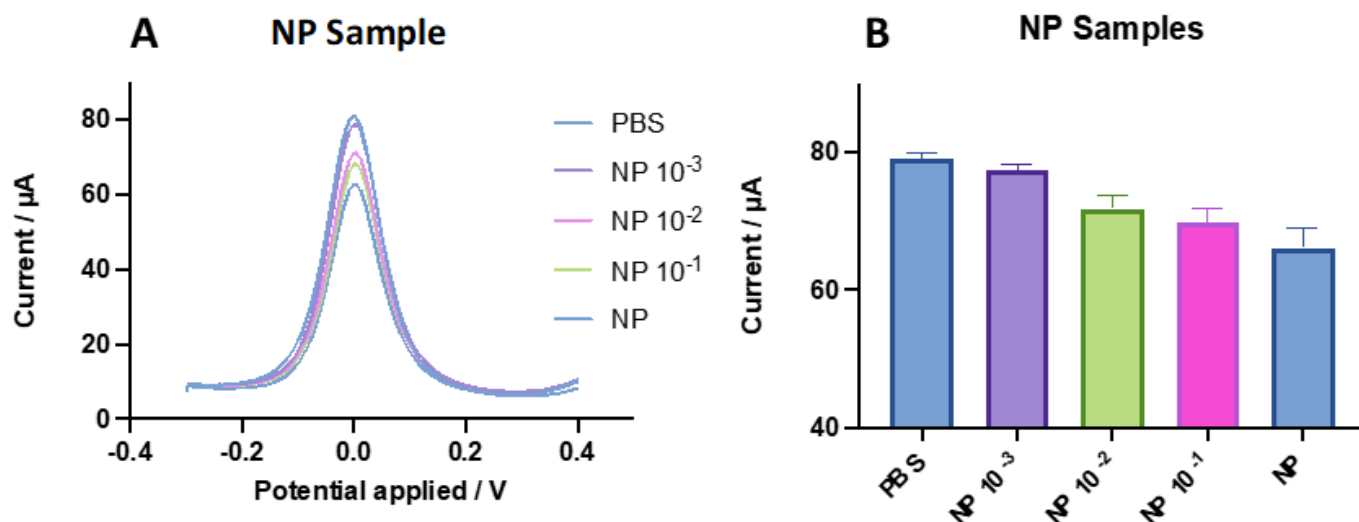


Figure 5

Voltammograms of the nasopharyngeal swab samples obtained by SWV (A), and the corresponding mean current peak values with the respective error bars (B).

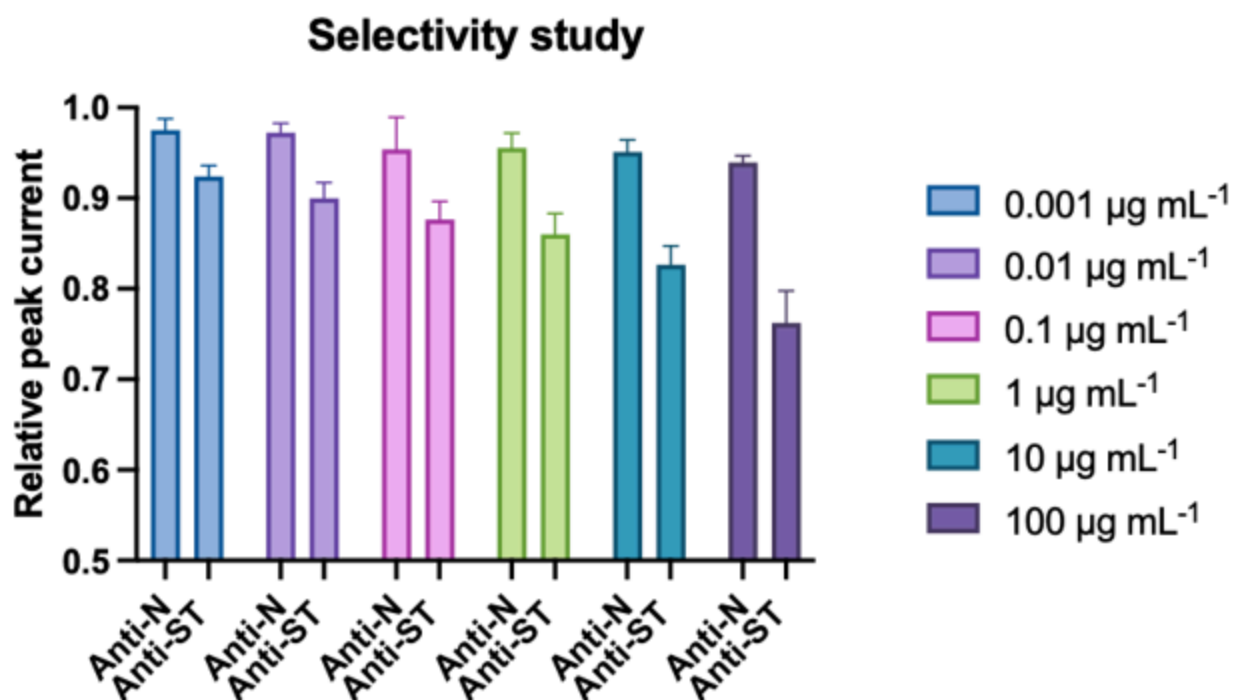


Figure 6

Relative current peaks response of the biosensor after the incubation of anti-ST or anti-N protein antibody, ranging from 0.0010 to 10 $\mu\text{g mL}^{-1}$ (SWV data).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryinformationNovelpaperbasedbiosensor.docx](#)
- [FigureS1.jpeg](#)
- [FigureS2.jpeg](#)
- [FigureS3.jpeg](#)
- [GraphicalAbstract.png](#)