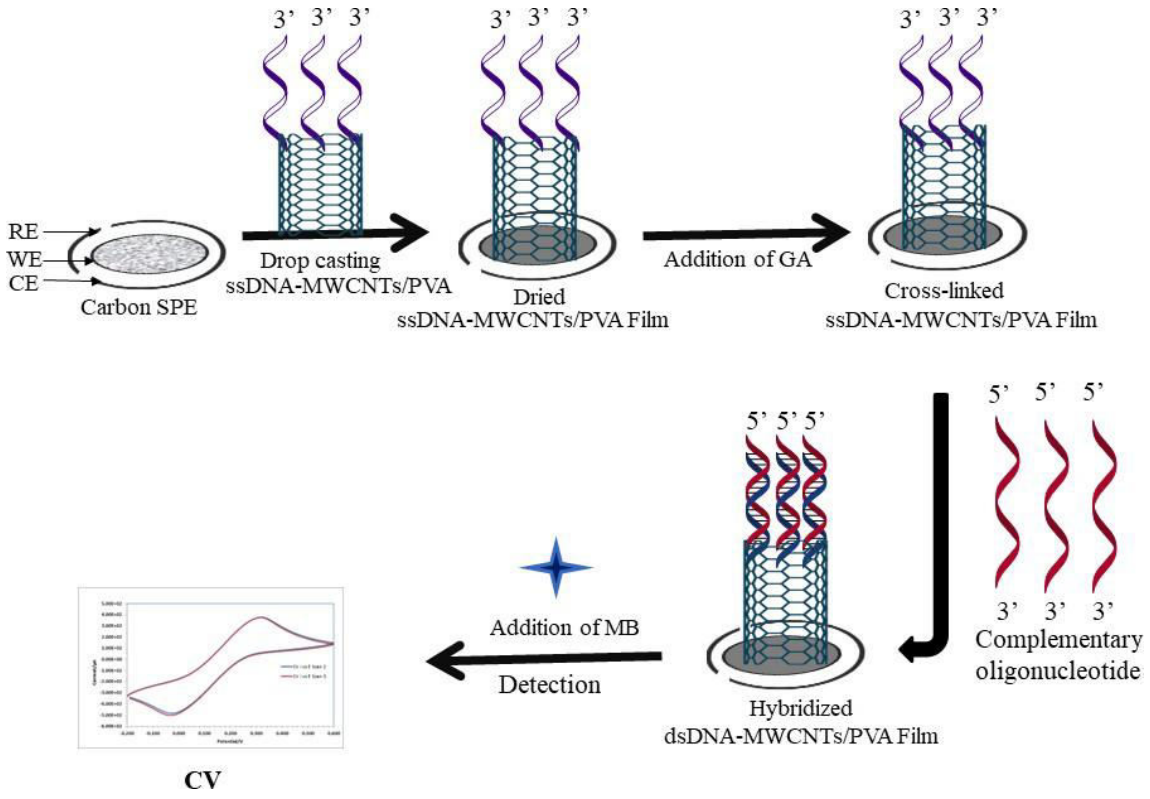
CHAPTER 5

TESTING OF COVID-19 REAL SAMPLES OF Nsp3 GENE OF SARS-CoV-2 USING DNA CHIP

Research highlights:

- Real sample analysis was successfully done using previously made chip.
- Successfully detected SARS-CoV-2 in real samples.

Graphical Abstract:



5.1 Introduction:

World Health Organization (WHO) declared the COVID-19 as a pandemic January 9, 2020 (Mojsoska et al., 2021). COVID-19 is caused by the novel coronavirus SARS-CoV-2. By the end of 2023, it has affected over 77.1 million people worldwide, resulting in approximately 6.9 million reported deaths. Early diagnosis of COVID-19 is crucial to the control the spread of the virus and to isolate and treat infected.

COVID-19 was caused by the severe acute respiratory syndrome coronavirus 2 (SARSCoV2), which was first identified in Wuhan, China in December 2019. The genome of the virus was sequenced in January 2020 (Bhalla et. al., 2020). Developing rapid and easy diagnostic technique is the first step to control the spread of the virus and provide the right medication to the infected. Previously, a DNA chip-based sensor was developed in our laboratory. In the present study, the DNA chip-based sensor was used successfully to detect COVID-19 positive real samples.

Testing methods, such as RT-PCR, ELISA, and CT scans, are available to identify COVID-19. However, CT scans have a low specificity but a high sensitivity. The gold standard for a conclusive diagnosis is RT-PCR, yet it can produce inaccurate results. ELISA is cost-effective and rapid but has low accuracy (Mojsoska et al., 2021). All these approaches need specialised facilities and knowledgeable specialists.

Developing methods for onsite point-of-care (POC) tests resolves the problems listed and increases the pace of detection. The majority of the more than 140 commercial tests available for SARS-CoV-2 detection are RT-PCR-based. One-step RT-PCR kits are available from certain sources, however their poor amplification rates result in subpar accuracy.

Using electrochemical DNA chips is the best way for cutting down the test delay times. Reverse transcriptase PCR can be used to create cDNA from samples, and after that, the cDNA can be immediately assessed using electrochemical biosensors. This process can yield results in a matter of minutes, doing away with the necessity for cDNA amplification via qPCR. The testing procedure can be greatly accelerated with this method. Although this principle's

electrochemical biosensor was built in our laboratory previously, real sample validation was still pending.

To test the feasibility of the concept, an electrochemical oligonucleotide-chip has been developed for the detection of the Nsp3 gene of SARS-CoV-2. SARS-CoV-2 is a single-stranded positive-sense RNA virus (29.9 kb) with four structural proteins (S, E, M, N) and 14 open reading frames (ORFs). ORF1a and ORF1b encode 16 non-structural proteins (Nsp1-16), with Nsp3 being the largest. Nsp3 plays a critical role in viral replication, forming the transcription complex, separating translated proteins, increasing virulence, and promoting inflammatory responses in the lungs (Lavigne et al., 2021; Udugama et al., 2020; Wang et al., 2020).

The chip was coated with oxidized MWCNTs functionalized with a ssDNA oligonucleotide, dispersed into a PVA polymer matrix, and tested on a screen-printed electrode. The previously made DNA-chip demonstrated high specificity and replicability, offering cost-effectiveness, faster analysis time, and on-site testing comparable to qPCR. This genosensor successfully recognised complementary target ssDNA of the Nsp3 gene in the concentration range of 1×10^{-8} to 1×10^{-15} M, with the linear range of 1×10^{-10} to 1×10^{-15} M. Excellent specificity was demonstrated by the genosensor towards its complementary target sequence. The electrochemical response of the sensor after incubation with the non-complementary ssDNA (PupE ssDNA) was measured, and the results showed a decrease in current at both the anodic and cathodic peaks. This decrease indicates non-specific adsorption of the non-complementary ssDNA at the electrode surface. Since MB could not intercalate when the two strands are not complementary to each other, the current response was hindered, which demonstrates that the sensor does not bind with random sequences of ssDNA. This further supports the high specificity of the sensor for its complementary target sequence. The sensitivity and specificity of the chip-based sensor were comparable to PCR based methods (Jinal Thakkar's Thesis 2022). It was still necessary to validate the electrode's performance using real samples.

5.2 Materials and methods:

Potassium ferricyanide (K_3 [Fe $(CN)_6$] -26664) was purchased from Qualigens Fine Chemicals, India. Sodium chloride (NaCl-33205), sodium phosphate dibasic anhydrous (Na_2HPO_4-

1944143), sodium phosphate monobasic anhydrous (NaH_2PO_4-59443), ethylenediaminetetraacetic acid disodium salt (EDTA, disodium salt-054448), Tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl-2044123), Sodium hydroxide pellets (NaOH-1949181), and Sodium dodecyl sulfate (SDS-54468) were bought from SRL

Pvt. Ltd. India. Hydrochloric acid (HCl) was obtained from Qualikems Fine Chem Pvt. Ltd., India. Polyvinyl alcohol (PVA-0531500500) and glutaraldehyde (GA-0396500500) were purchased from Loba Chemie Pvt. Ltd., India. Raw MWCNTs were procured from Ad Nanotechnologies, India. Methylene blue (MB-M4159) was puchased from Sigma Aldrich, India. Single-stranded DNA (ssDNA) probes for the Nsp3 gene (5'-GTGCCACTTCTGCTGCTCTT-3') was synthesized by Eurofins, India, which meets HPSF purification standards. The screen-printed carbon electrode was from PalmSens BV, Netherlands. Every reagent used in this investigation was purchased, with the highest purity and conformed to molecular biology grade criteria. All the reagents were used exactly as supplied, which required no further purification, except for the multi-walled carbon nanotubes (MWCNTs).

5.3 Results and Discussion:

To confirm the application of the biosensor in the detection of SARS-CoV-2 positive samples was investigated. Serum samples of Covid-19 were purchased and isolation of RNA was done. Reverse transcriptase PCR was carried out with RNA from SARS-CoV-2 as the template, generating complementary DNA (cDNA). For the validation of the DNA chip sensor, double-stranded cDNA samples were used at 58.3°C the melting temperature of cDNA samples of 1 fM & 10 fM concentrations and CV peak current (Ip) was monitored with respect to the oligonucleotide probe. The change in Ip with respect to probe, negative control (aptamer free coated electrode) confirms that the hybridization occurred between the DNA sequences on the electrode and in the reverse transcribed sample. The change in Ip was observed in both the positive samples with margin of error ±6.41 and ±5.28 for 1fM and 10 fM respectively.

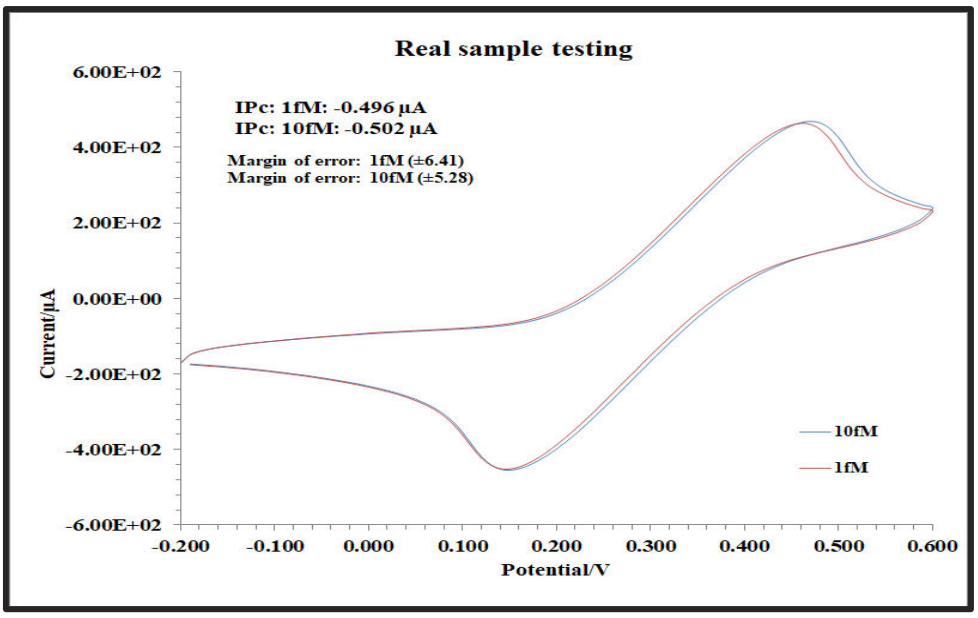


Figure 5.1 Testing of real positive samples of Covid-19 at 2 concentrations 1 fM and 10 fM.

5.4 Conclusion:

In summary, DNA-chip based biosensor was tested against the real samples of Covid- 19 for its practical application and successfully identified them.