Research Article

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Plasmonic Biosensor Field-Effect Devices for Virus Detection by Carbon Nanomaterials

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ABSTRACT

The predominance of viruses is a real threat to human safety as they cause severe infections. The high prevalence of viral diseases can be attributed to inadequate detection techniques. Various types of biosensors have been developed and commercialized with the intention of detecting dangerous viruses. However, they have many drawbacks. These problems are resolved by nanotechnology, which makes it possible to instantly recognize molecular targets directly. The special physical, chemical, electrical, and optical properties of nanomaterials offer advantages to biosensors. Sensors made of carbon nanotubes were initially utilized in field-effect devices for virus detection. Carbon nanoparticles show enhanced sensing capabilities. Optical biosensors with localized Surface Plasmon Resonance (SPR) can improve the performance of viral detection. The proposed method performs better than the existing SPR devices. The most often used nanomaterials are those made of metal and carbon, as well as their hybrid composites, which are used for various amplification techniques.

Keywords: Carbon nanomaterials; Surface Plasmon Resonance; Localized SPR; Biosensor; Virus detection.

1. INTRODUCTION

One of the primary causes of disease in both humans and animals is virus exposure. Viruses can also be used in bioterrorism as weapons. To stop outbreaks and the consequent threats, quick and accurate diagnosis is necessary. Viruses are responsible for a number of diseases including flu, chicken pox, AIDS, SARS, Ebola, and others. In contrast to other microbes, viruses are intracellular parasites. The bulk of these agents employ diverse enzymes produced by the host cell, and only a small subset of their genes is capable of producing new viruses (Onyancha *et al.* 2021). A timely and precise identification of many illnesses is essential due to the dearth of effective treatments. The specialized methodology and equipment required for virus identification include cell culture, polymerase chain reactions, gene sequencing, and antibody or antigen detection utilizing hemagglutination assays (Choi *et al.* 2017). A biosensor is a sensing device that transforms an observable biological event into a quantifiable signal. Typically, a transducer that transforms biological data into a quantifiable signal and a biological recognition component are integrated (Zamzami *et al.* 2022). The biological recognition component of a biosensor must be focused on a specific biomolecule, biological process, or chemical reaction in order to function properly. The biosensors may distinguish between a variety of biological elements, such as nucleic acids, antibodies, enzymes, bacteria, and viruses, depending on the type of recognition elements they use. The effectiveness of the biosensors has so far been tested using samples from humans, the environment and food (Kazemi *et al.* 2021). On the surface of a transducer with high bioactivity for targeting, biosensors are often placed. The following attachment techniques are some of them: adsorption, encapsulation, entrapment, covalent binding, and cross-linking. When using identification components, the electrochemical, electrical, and optical approaches stand out for their responsiveness and adaptability.

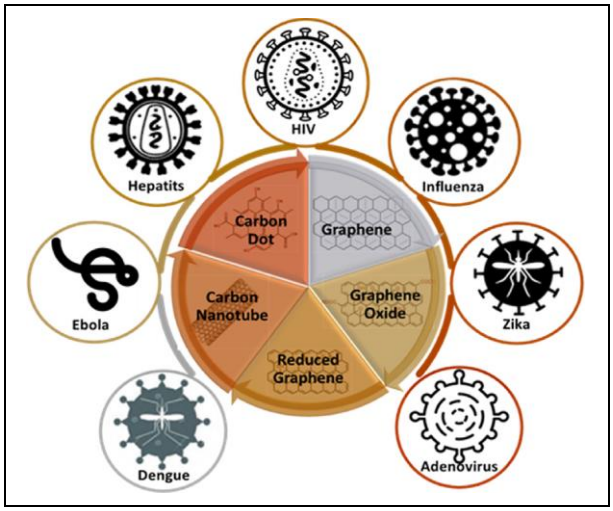

Fig. 1: Applications of carbon nanomaterials for the detection of various human viruses (Ehtesabi, 2020)

Carbon is essential for many technical applications, from pharmaceuticals to catalysts, and can

be found in a variety of forms, including nanoparticles (NPs), polymers, and supramolecular materials. In addition to the well-known carbon allotropes of diamond and graphite, the recently developed carbon nanomaterials such as carbon nanoparticles, carbon nanotubes (CNTs), graphene and its derivatives, and carbon dots (CDs) have all been actively exploited in the field of nano electronics. Figure 1 demonstrates the significance of biosensor components having a high degree of selectivity depending on certain binding sites. For the detection of influenza virus as well as the measurement of drugs and their metabolites, optical systems based on field-effect biosensors and nanomaterial-based biosensors are used. Not to be forgotten are the evaluation and measurement of analysts in biological samples and the use of quick tests to spot early illness.

In clinical and environmental samples, it is essential to identify viruses using quick, inexpensive, and simple procedures. When virus symptoms are identified, prompt control measures can be implemented. The speed at which viral contamination is identified generally affects the success of treatment and control measures. Techniques for identifying and detecting viruses are frequently found in laboratories, but it is difficult to find field-use methods that do not require huge equipment. Some of the aforementioned problems will be fixed by applying our recommended strategy. This investigation looks at how nanotubes interact with suspended viruses and their response. We have designed highly specialized nanotube electrical sensors for virus detection by chemically functionalized nanotubes that selectively interact with antiviral antibodies. These sensors may be beneficial for the environment, humans and animals, and the early detection and treatment of viral diseases transmitted via food, water, and air.

2. RELATED WORK

Recent developments that maximize the unique qualities of gold nanoparticle-based bio sensing platforms have been very significant. For instance, in an immunological chromatographic biosensor, the addition of gold nanoparticles (AuNPs) with extensive near infrared light absorption capabilities has made it possible to detect foodborne pathogens thermally and colorimetrically (Bezuneh *et al*. 2022). With the aid of wide variety of biomaterials, including silicon, chitosan, collagen, metal nanoparticles, graphene, and carbon-based nanomaterials, numerous biosensors have been developed. Metal nanoparticles are used in physical and chemical processes as they require less substrate and resources (Millon *et al*. 2022). Some examples are gold, rhodium, platinum, silver, and palladium. Numerous diseases have been successfully identified by combining immunology- and nucleic acid sequence-based methods, including polymerase chain reaction (PCR) and real-time PCR, lateral drift tests, and enzyme linked immunosorbent assays (ELISA) (Hasanzadeh *et al.* 2018). Additionally, the label-dependent or label-free technique can be implemented for use with the analytical technique. The evaluation is marked with a marker in the label-dependent method, which modifies the colorimetric or fluorescence characteristics of the signal. The analyte binds to a molecule in the label-free approach, changing the microsystem and causing impedance variations (Hamed *et al.* 2020). The AuNPs are in high demand in this field among the many different types of metal nanoparticles that are now accessible because of their excellent binding ability, stability, and signal amplification abilities (Gupta *et al.* 2021). Microorganisms like bacteria, fungus, and protozoa can spread to their hosts in a number of ways, but primarily by air, water, and food. The pathogens that cause infection most frequently are viruses like influenza and norovirus as well as bacteria like Staphylococcus aureus and Escherichia coli (Cesewski *et al*. 2020). Detection of pathogens must overcome a number of obstacles as it advances (Zhan *et al*. 2018). In order to ensure the safety of food, to prevent and diagnose disease and to monitor the environment, it is essential to find these infectious organisms early (Chen *et al*. 2020).

Clark and Lyons designed an oxidase enzyme electrode in 1962 to detect glucose. This was the first attempt towards biosensors (Castillo-Henríquez *et al*. 2020). Since then, numerous attempts to replace conventional pathogen detection techniques with biosensors have been made. A sensitive transducer element and a specific bio-recognition element are directly combined to make biosensors. Traditional detection techniques are time-consuming, have a poor limit of detection (LOD), require skilled employees, a working area, and expensive equipment. Biosensors are very dependable, efficient, and sensitive (Karakuş *et al*. 2021). The presence of the SARS-CoV-2 spike antigen caused gold nanoparticles to change colour from red to purple, as was clearly visible with the naked eye or with UV-Vis spectrometry.

3. PROPOSED MODEL

Gold nanoparticles (AuNPs) are currently leading the field of biosensing for viral infections, surpassing other metal nanoparticles. This is because they have excellent biocompatibility, superior bio-analytical properties, strong electrocatalytic activity, and a high loading capacity. The specific characteristics of AuNPs make them highly beneficial for clinical applications, where accuracy and dependability are of utmost importance. Moreover, carbon nanomaterials have exhibited substantial effectiveness in identifying viral antigens in human samples, providing prompt outcomes within a timeframe of 30 minutes. These results are in line with the guidelines of the World Health Organization, emphasizing their potential for being widely accepted and used in clinical practice. AuNPs,

when incorporated into diagnostic platforms, improve accuracy and precision by utilizing their localized surface plasmon resonance (SPR) characteristics. This method enables the accurate identification of viral particles at low concentrations, which is essential for early detection and efficient disease control. The localized surface plasmon resonance (SPR) technique not only enhances the signal but also offers a sturdy framework for real-time surveillance of viral infections, rendering it an exceedingly dependable technology for clinical environments. The suggested diagnostic technique utilizes the quick identification of carbon nanoparticles for antigens and the accurate and sensitive detection abilities of AuNPs. This combination provides a sturdy, effective, and dependable solution for clinical diagnostics, capable of delivering consistent and precise findings. Incorporating these sophisticated nanomaterials into diagnostic systems is a notable advancement in the timely identification and treatment of viral infections, leading to enhanced patient outcomes and more effective public health interventions.

3.1 Materials and Sample Preparation

Recombinant SARS-CoV-1 spike, SARS-CoV-2 spike and S1 subunit, and MERS-CoV spike His tag proteins were developed for the study in addition to Monoclonal rabbit anti-spike S1 antibodies with the assistance of Sino Biological Inc. Beijing, China. However, the latter one is not recommended to utilise blood or saliva from a patient whose test results for SARS-CoV-2 were negative to verify the specificity of the antibodies. The polyvinylidene difluoride (PVDF) exchange membrane (Immobilon P) was promoted by the Korean Merck Millipore Ltd. The buffer and washing options were made using deionized (DI) water (18.2 M/cm) produced by an MDM Wellix Plus water purification device. The remaining chemicals used in this study were of high standards, analytically pure, and so were used directly.

3.2 Instruments

All Localized Surface Plasmon resonance (LSPR) investigations used quartz micro cuvettes (700 L, Hellma) and a UV-1900i spectrophotometer (Shimadzu). To reveal modifications in particle dimension and distribution, the LSPR band of pure AuNPs and functionalized AuNPs were measured between 300 and 800 nm. While the hydrated particle sizes were investigated using a Malvern Zetasizer Nano ZS-3600 Zetasizer with dynamic light scattering (DLS), the surface functionalization of AuNPs was examined using a micro–Fourier Transform Infrared (FTIR) spectrometer, the Bruker Alpha II. The morphologies of monodispersed AuNPs were examined using high-resolution transmission electron microscopy (HRTEM) at 200 kV with a JEOL JEM 2100 HRTEM.

3.3 Carbon Nanotube-based Biosensors

Carbon nanotubes (CNTs) are cylindrical structures with exceptional mechanical, electrical, and thermal properties that can be used to create biosensors. They are produced from graphene sheets of a few nanometer in diameter. The recommended technique produced a rapid identification system that immediately recognized the virus utilizing a near-infrared mechanism (Ferrier et al. 2021). Due to the metal properties displayed by CNTs, the CNT-based sensors also show excellent promise for eliminating issues in graphene-based sensors (Dai *et al*. 2022). The durability and reproducibility of CNT-based sensors can also be increased by covalently functionalizing them with metal nanoparticles or small aromatic molecules.

3.3.1 Cnt-based Field-effect Devices for Virus Detection

Today's technology uses a Field-Effect Transistor (FET), a semiconductor component, to enable current to move from an electrode on one side (the source) to an electrode on the other (the drain). The electric field produced by a voltage applied to the gate electrode, a 0.33-µm capacitive electrode coupled by a thin dielectric layer, controls the semiconductor channel between the supply and drain. The electric FET properties of biosensors may change depending on how strongly biomolecules cling to it. Depending on the helicity, CNTs can either be metallic or semi conductive (Shariati *et al.* 2022). The FET-based biosensors can be made using semi-conductive CNTs. The adherence of biomolecules to the CNTs can affect the electric CNT-FET parameters. Graphene-based FET biosensors have attracted a lot of attention recently because of their high carrier mobility and high sensitivity to electric fluctuations. A variant of FETs are chemical resistive field-effect transistors, which are simply FETs without the gate electrode. Field-effect based CNT biosensors have been fabricated because CNTs are great candidates for field-effect devices due to their high surface area and higher conductivity.

Examining the nucleic acids, host antibodies (Abs) made against particular viral proteins, antigen (Ag), and host antibodies (Abs) are the main methods for identifying viruses. Since it is a vital part of the virus and can elicit an immune response in the host that is infected, antigen is a possible biomarker for the precise and reliable detection of viruses. For the sensitive and precise quantitative detection of viruses, the technique known as CNT-based Field-Effect devices for virus detection has been widely used (Jin *et al.* 2019). Comparing CNT-based electrochemical immune sensors to other types of immune-sensors, they have demonstrated excellent competitiveness in detecting viral antigens with better analytical capabilities.

3.3.2 CNT-FET Device Fabrication

Single Walled Carbon Nanotubes (SWCNTs) may make the essential semi-conducting channel in field-effect biosensors. Semiconductors of the p-type are SWCNTs. Between the sources and drain electrodes of field-effect devices, these CNTs are either placed evenly between the electrodes or in networks with random orientations. When creating carbon nanotube network devices, CNTs are dispersed in a solvent like dimethylformamide before being drop-cast onto the substrate. Traditional photolithographic methods can be used to generate the electrodes, either directly onto a substrate or in-between already-fabricated electrodes. Devices with aligned CNTs are frequently made via di-electrophoresis and drop-casting CNT dispersion between electrodes (Kumar *et al*. 2023). Chemical vapour deposition can also be used to produce CNTs.

3.4 Antibody Immobilization

An oblique fluorescent antibody check yielded a titer of 1:1024 for the anti-aMPV antibody. By incubating the Poly-L-Lysine (PLL)-coated system with different antibody concentrations in a 20 mM PBS solution (pH 7.4) for a set amount of time (12 h) at 4 °C, an antibody dilution of 1:300 was adsorbed on the system. The device was covered with the particular antibody to avoid non-specific binding. Bovine serum albumin was used to block all other potential adsorption sites (BSA). When compared to techniques that rely on absorbance, fluorescence shows better sensitivity, selectivity, and faster detection times. It is, however, reliant on the environment. Since fluorescent compounds display a variety of emission spectra, their sensitivity is 100 times greater than that of absorbance. This increased sensitivity can be attributed to the interactions between surface Plasmons and fluorophores in metallic nanostructures. Compared to colorimetric, SPR, reflecting, and luminescent transducers, this SR transducer has a lower LOD.

3.4.1 Preparation of AuNPs

Citrate reduction was used to synthesize AuNPs. To an aqueous solution of 5 mM $HAuCl_4$ of ultrapure water (2.5 mL), 1% sodium citrate solution was added and boiled. Another 10 minutes of vigorous stirring while boiling resulted in the colour change of the solution from pale yellow to vivid scarlet. After naturally cooling for an additional 10 min while being continuously agitated, the resulting colloidal gold solution was filtered through a 0.45-m membrane and stored at 4 °C until use. The concentration of these particles was calculated to be 2.4 nM based on the extinction coefficient of AuNPs with a diameter of approximately 15 nm, which is 3.6 $\times 10^8$ cm^{-1} M^{-1}. In order to preserve the final colloidal gold solutions at 4 °C in the dark until usage, a 0.45-m membrane filter was used. Then, while allowing it to naturally cool, the mixture was continuously stirred for an additional 10 minutes. The concentration of these particles was estimated to be 2.4 nM based on their extinction coefficient, which is 3.6 $\times 10^8$ cm^{-1} M^{-1} for AuNPs with a diameter of roughly 15 nm.

3.5 Virus Detection using Carbon Nanomaterial

Carbon nanomaterial is used to detect viral infections. The genome encodes three structural proteins of the virus: the capsid protein (C), envelope protein (E), and membrane protein (M). According to a report, the dengue virus II E proteins may also be detected using a bio-functionalized optical fibre-based sensor. Using anti-DENV II E protein IgG antibodies, the tiny area was functionalized after GO extraction to see how it would respond to various DENV II E protein concentrations. The sensor was selective and E protein-affinitive. The biosensor was coated with an antibody using sprays containing stock aMPV solutions. The viral concentration was varied in order to assess the sensitivity of the devices. A variety of dilutions of the stock virus (1:10, 1:100, 1:500, and 1:1000) were used to evaluate the sensitivity of the device. It was possible to gain insight by monitoring the current-voltage trends both before and after the antigen delivery. Each concentration required to have a minimum of 5 units tested. Improvements were made to a localized SPR sensor and a self-assembled monolayer/rGO-polyamidoamine dendrimer (SAM/NH2rGO/PAMAM) thin film optical biosensor for the detection of DENV II E proteins. Then, the sensitivity, specificity, binding affinity, and selectivity of the optical biosensors used in the localized SPR sensor were assessed.

By varying the sensing layer due to various spin rates, the connection between the sensing and analytical layers may be enhanced. The DENV II E protein-specific selectivity of the device was also tested. When Vero cells and hepatocytes are infected, heparins, an analogue of heparin sulphate proteoglycans, serve as virus receptors. Thus, the work proposes an optical biosensor network with a field-effect transducer functionalized with heparin to recognise the virus.

4. EXPERIMENTAL RESULTS

4.1. Characterization of CNT-FET Surface

The presence of PBASE and the surface modification of the CNT-FET biosensor were confirmed using X-ray photoelectron spectroscopy (XPS). It is possible to determine whether functionalized PBASE is present on the CNT surface by examining the N1s top at 400.2 eV in XPS differential spectra.

Table 1. Linear range of biosensors

Biosensor Classifications	Detection target	Linear range
Immune-sensor	Anti-H5N1	1-3.5× 10^3pg mL^{-1}
Immune-sensor	Antibody HA	51.5 pg mL^{-1}
Immune-sensor	Virus solution	3.6 PFU mL^{-1}

4.2 Scanning Electron Microscopy

The precise LBL assembly utilized for detection was constructed for SEM on a glass slide. Glass slides were prepared with a combination of 96% H_2SO_4 and 30% H_2O_2 (3:1 v/v) at 60 ℃ for 30 minutes before assembling. The next step was a 10-minute rinse in DI water. Pretreatment was done to clean the glass surface. The glass slides were treated to make them hydrophilic. The slides were then exposed to ethanol concentrations (v/v) of 50%, 70%, and 80% twice before being submerged for five minutes in 95% ethanol (v/v). After that, the slides were immersed twice for five minutes each in 100% ethanol (v/v). The samples were then prepared for the essential factor drying process since by this time, the water molecules in the samples maybe altered by the ethanol molecules. The slides were dried using a critical-point dryer that had previously been filled with 100% ethanol to remove the liquid CO_2. After drying the slides completely, they were exposed under Hitachi S-4700 SEM and sputter-coated with 2 nm platinum.

The SEM images depict the shapes of CNTs (Fig. 2). In its purest form, SiO_2 is transparent (Fig. 2A). The tight network of twisted CNT is visible in SEM images (Fig. 2B). A network with open pores that is connected and intricately entangled at the nanoscale level was produced by the CNTs. The SEM reveals aligned CNT bundles with a range of varied diameters and an average diameter of 30 nm. The SARS-CoV-2 S1 antigen attached to AuNPs is visible as a sphere-like structure in Fig. 2C. The diameters of the AuNP range from 19.3 to 23 nm, based on the SEM images in Fig. 2D. The densely glycosylated SARS-CoV-2 S1 antigen increased the growth of AuNPs when it was bound. According to the appearance of the antibody-antigen aggregation localized via AuNPs (shown in Fig. 2C), the SARS-CoV-2 S1 antibody was efficiently immobilized on the biosensing region in SEM images (Fig. 2B).

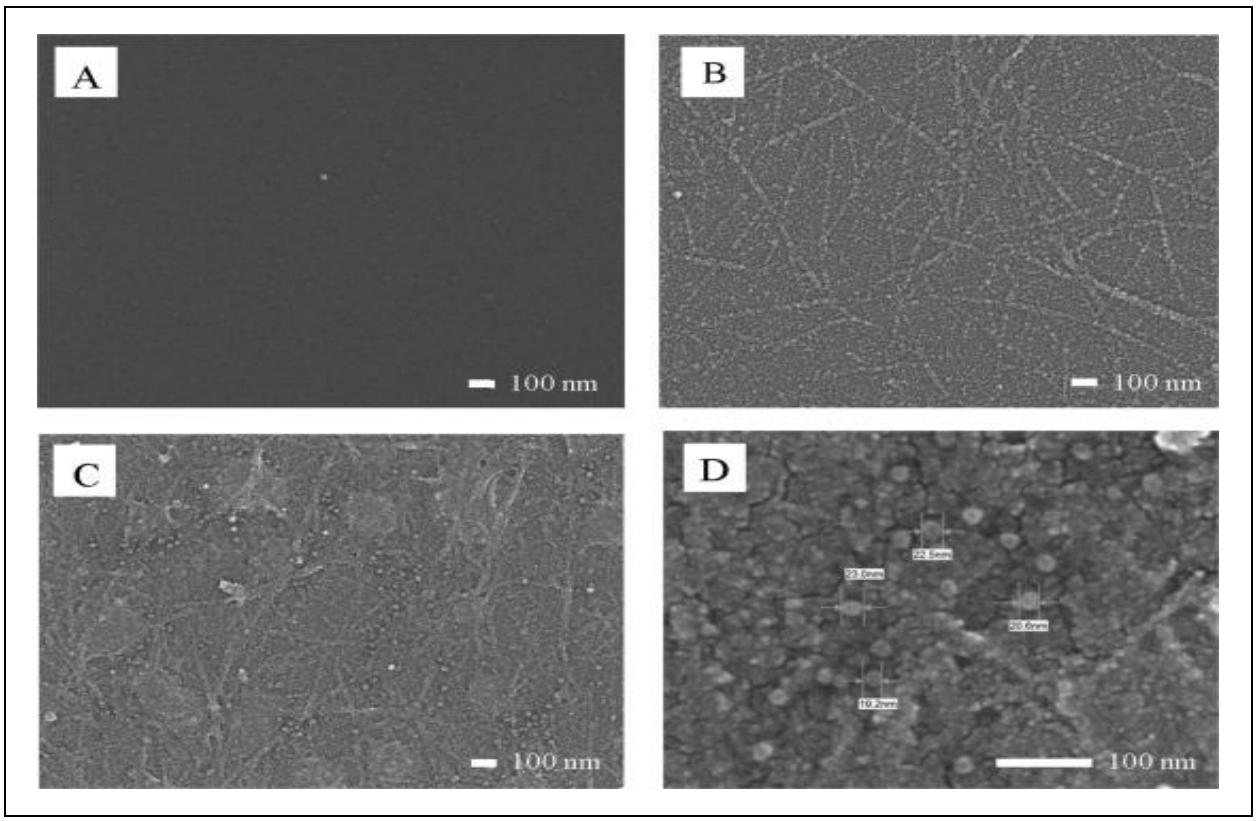

Fig. 2: Scanning electron scans of the channel region reveal the (A) SiO_2 surface as bare SiO_2, following (B) CNT coating, PBASE modification, (C) anti-SARS-CoV-2 S1 immobilization, and (D) *binding of AuNPs-conjugated SARS-CoV-2 S1 antigen.

* Image that has been 200,000 times enlarged to show the nm size of a gold nanoparticle.

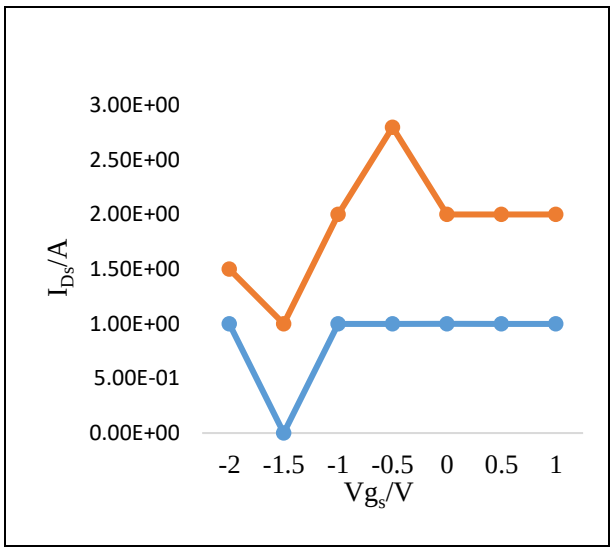

Fig. 3: (a) Source-drain current (I_{DS}) vs. supply gate voltage (V_{GS})

Source-drain current (I_{DS}) vs. supply gate voltage (V_{GS}) at a fixed 1.5 V are plotted on the exposed CNT-FET surface in Fig. 3A. The fabricated device exhibits p-type FET functionality as I_{DS} keeps rising when V_{GS} moves. A linker molecule (PBASE), which joins the antibody and CNT surface for the CNT-FET biosensor, is necessary. According to SEM images (Fig. 2B), after two mM of PBASE treatment, the electrical behavior of the PBASE-functionalized CNT-FET biosensor changed, demonstrating that the chosen linker molecule successfully altered the CNT surface.

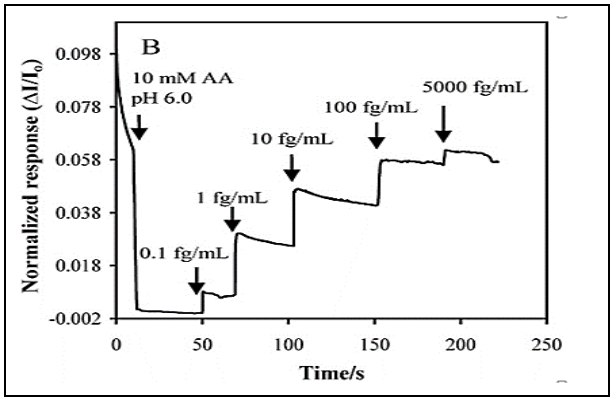

Fig. 3: (b) Normalized response vs Time

The properties of the CNT-FET device at each stage of the organic approach are shown in Fig. 3(b and c). The CNT-FETs have properties like source-drain current (I_{DS}) and gate voltage (Vg). The I-V characteristics of the CNT-FET were studied after two mM PBASE modification. SARS-CoV-2 S1 antibody was already immobilized on the CNT-FET surface. SARS-CoV-2 S1 was generated in 10 mM AA buffer with a pH of 6.0 at a concentration of 100 fg/mL. The performance of each switch was evaluated from SEM images captured under natural light (Fig. 2b).

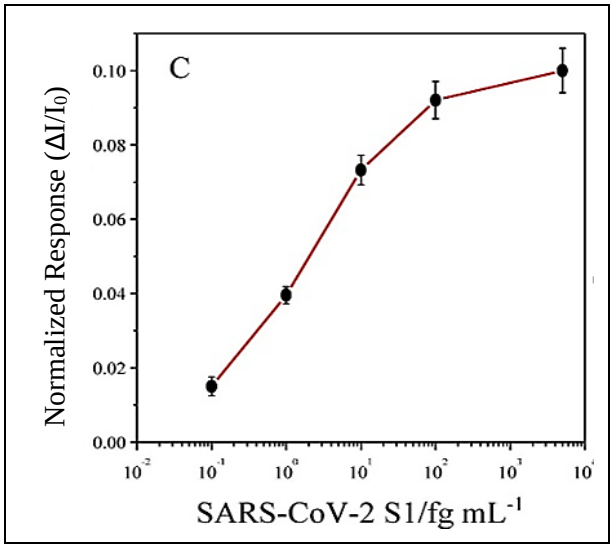

Fig. 3: (c) Normalized response vs SARS-CoV-2 S1

4.3 Synthesis and Characterization of AuNPs

Transmission electron microscope scans revealed that AuNPs were homogeneous in size, had a low aspect ratio and were monodispersed. On the UV-Vis spectrum, the characteristic absorption peak was seen at 520 nm, and the small spectral bandwidth further proved the monodispersed state of AuNPs (Fig. 4).

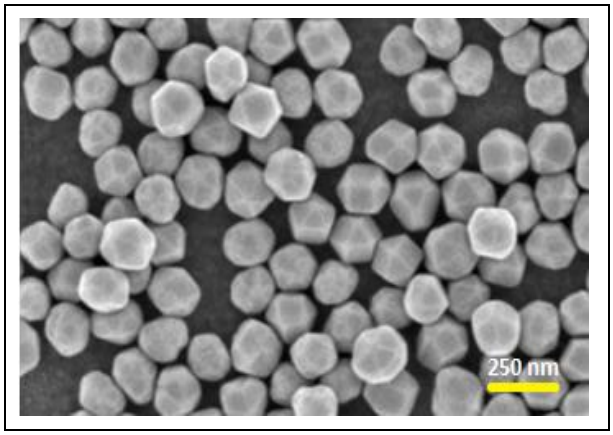

Fig. 4: Size and distribution of the AuNPs

Scale bars: 200 and 50 nanometres

Citrate reduction was used to create AuNPs that were regulated in size. Normally, Van der Waals forces mediate interactions between AuNPs over short distances. Although AuNPs are negatively charged, electrostatic repulsion caused by citrate ions on their surface inhibits AuNPs from aggregating. The synthesised AuNPs in the current work (Fig. 5) were 16

nm in size as determined by DLS and TEM, similar to earlier work.

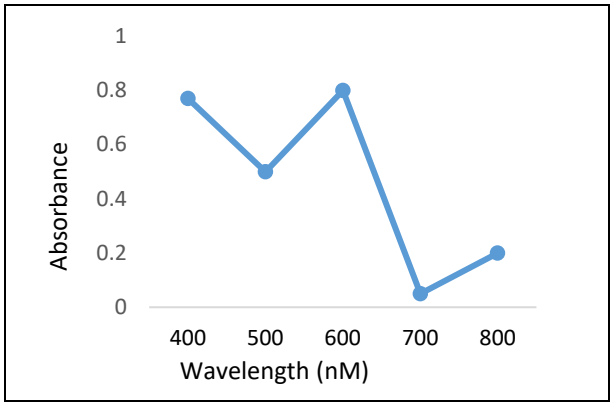

Fig. 5: UV-vis spectral range for AuNPs

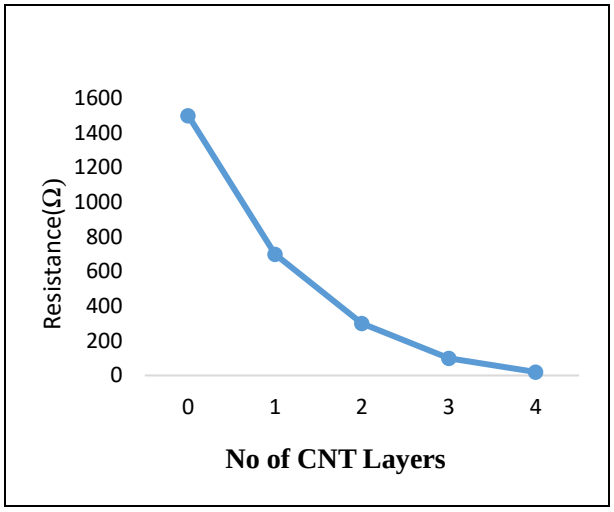

Fig. 6: Resistance in (PDDA/PSS) 2 + (PDDA/SWNT) as a function of the quantity of SWNT layers that have been deposited (n)

The electrical properties of the devices made by LBL were assessed. The conductivities depend on how the current or charge diffuses via erratically dispersed conducting filler scattered throughout an insulating matrix. Fig. 6 illustrates how the resistance varies with the number of layers.

5. CONCLUSION

In this work, carbon nanotube-based sensors for field-effect virus detection devices are proposed. Attention is drawn to carbon nanostructures by the improvement of sensor and identification functionality. The effectiveness of identifying the virus is then improved by optical biosensors with localized SPR. The proposed methods present certain unique challenges for quantitative measurements that call for careful study. Understanding of these materials is growing as a result of improved nanomaterial characterization.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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