Self-Assembled Two-Dimensional Molybdenum Disulfide Nanosheet Geno-Interface for the Detection of Salmonella

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ABSTRACT: This report presents a novel lab-on-a-paper (LoP)-based device coupled with a molybdenum disulfide nanosheet (MoS2NS)-modified electrochemical genosensor for detecting Salmonella-specific DNA. Conductive electrodes were grafted on a paper-based substrate employing a stencil printing technique, and MoS2NS was decorated on the working electrode. MoS2NS has strong affinity toward nucleos bases, which made it a best sensing interface for the immobilization of DNA. Morphological, optical, and structural characterizations were accomplished using X-ray diffraction (XRD), high-resolution transmission electron microscopy (HR-TEM), UV–vis spectroscopy (UV–vis), and Raman spectroscopy, respectively. The current studies of an electrochemical genosensor demonstrated a good linear detection range from 100–20 nM and a low limit of detection of 20 nM toward Salmonella DNA with \( R^2 = 0.991 \). The proposed LoP-based genosensor confirmed as a better sensing podium and an effectual immobilization matrix for DNA.

INTRODUCTION

Salmonella enteritidis-based epidemics from different sources of food have been raised the public awareness due to its worldwide severity. Salmonella enterica, includes more than 2500 diverse serotypes, causes 17–21 million reported cases each year globally.1–7 Various conventional methods are available for the pathogen detection. These methods have some setbacks in regard to accuracy and co-infection with other pathogens along with; they are time-consuming process as it requires laborious preparation.8 They are time consuming such as slow growth due to which an excess time is lapsed to get the final result, which can turn out to be fatal. To overcome these drawbacks, other modern molecular biology techniques, namely, enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR), have been in use. But these methods have some traditional limitations such as prolonged multiple enrichment steps, potential for false positive, cross contaminations, and failed reactions due to the presence of inhibitory substances that leads to inconsistent results.9–12 There is an extensive need for fast and accurate diagnosis of Salmonella for the effective management of the infectious diseases caused by this pathogen.

Electrochemical biosensor promises great reliability and specificity toward pathogen detection, food safety, and quality control.13–17 Herein, a smart approach, that is, lab-on-a-paper (LoP) device, coupled with a performant biomolecular sensing system, which could assure an effective personalized management of disease related to salmonella, was used. The LoP device offers many advantages over a macroelectrode-based electrochemical technique such as allowing low consumption of the sample, device-portability, and an easy technique to overcome the complex laboratory functions. A paper substrate was used for the fabrication of microelectrodes that further encapsulated with hydrophobic barriers and functionalized with a biomolecule. Biomolecule sensors have capabilities that can be converted into a diagnostic platform, which is perfectly compatible with point-of-care testing (PoCT).17–20 For a rapid and low-cost detection of Salmonella, various biomolecules are being introduced in biosensors such as DNA, RNA, aptamer, and antibody. In the present study, we employed a DNA probe of the inv A virulent gene of Salmonella, which is responsible for the penetration into the intestinal epithelium.21–24 A genosensor offers specificity associated with DNA base pairing, sensitivity, and rapidity that is required to solve all aforementioned drawbacks.25

Many metal dichalcogenides have captivating interest in the field of sensing attributed to their higher electron transfer capacity and large surface area.26–28 MoS2NS is consist of sandwiched layers of Mo and sulfur that are bonded together by van der Waals weak forces. MoS2NS adsorb the ssDNA via van der Waals interaction.29 Furthermore, the layered structure of MoS2 has an excellent biocompatibility and a functional active site, which enhance the planar electrical transportation activity for sensing application.30–35 It was demonstrated that...
the self-assembled nanoarchitecture was easily dispersed and assembled as nanosheets on a paper-based device.

As per our knowledge, a paper-based platform for electrochemical sensing of *Salmonella*-specific DNA, using 2D nanomaterials, is yet to be studied. Furthermore, MoS$_2$ facilitates many electroactive sites, defects, and high surface areas that leads to an increase in the sensitivity and selectivity of the electrochemical paper-based LoP devices.$^{36,37}$

## RESULTS AND DISCUSSION

### Structural and Morphological Characterization of MoS$_2$

Figure 1a,b shows the morphology of the MoS$_2$ nanosheets as observed by TEM. TEM images revealed that MoS$_2$ nanosheets are composed of more than five layers with an interlayer spacing of around 0.689 nm.

The phase and crystal structure identification of the synthesized MoS$_2$ has been studied by XRD as given in Figure 2a. The presence of crystalline diffraction peaks indicates that synthesized MoS$_2$ was crystalline in nature. The peaks at 14.35, 33.31, 38.10, 44.32, 58.97, 64.56, and 77.67$^\circ$ were assigned to (002), (100), (103), (006), (110), (0111), and (0010), respectively. The XRD peaks are attributed to hexagonal phase (JCPDS card nos. 17-0744 and JCPDS-37-1492).$^{38,39}$

Raman spectra illustrated the evolution in structural parameters when the 3D bulk block material changes into a 2D van der Waals bonded layered architecture and depicted about the quality and number of layers present in a 2D-layered material. Figure 2b shows the Raman spectra of MoS$_2$ nanosheets synthesized by a hydrothermal process for 24 h. Two prominent peaks were observed at 383 and 408 cm$^{-1}$(E$_{2g}^1$ and A$_{1g}$ modes). The E$_{2g}^1$ mode can be attributed to in-plane vibration molybdenum atoms along a particular direction while the sulfur atom in the other. The A$_{1g}$ mode is out of plane vibrations of sulfur located at 408 cm$^{-1}$. The distance between these two Raman peaks is $\sim$25 cm$^{-1}$, which implies the formation of MoS$_2$ nanosheets.$^{40}$ Figure 2c shows the UV–vis spectrum of MoS$_2$ nanosheets sonicated in N-methyl-2-pyrrolidone (NMP) for 8 h. Two characteristic peaks were detected at 613 and 673 nm (2.02 and 1.84 eV, respectively) that correspond to the characteristic band gaps of MoS$_2$.$^{41}$

### Electrochemical Characterization of Different Phases of Electrodes

The electrochemical behaviors of ePAD, MoS$_2$/NS/ePAD, ssDNA/MoS$_2$/NS/ePAD, and dsDNA/MoS$_2$/NS/ePAD electrodes were detected using cyclic voltammetry (CV) in sodium phosphate buffer (0.1 M, pH = 7.4) consisting of MB solution by potential sweeping between $-1.0$ to $+1.0$ V at a voltage scanning rate of 100 mV s$^{-1}$. The bare ePAD showed a small anodic peak current of 0.43 $\mu$A (Figure 3a). The anodic peak current of MoS$_2$/NS/
ePAD electrodes increased to 6.2 μA (Figure 3a), which was assigned to the large surface area, enhanced transfer rate of electron, and redox activity of MB at an electrode/electrolyte interface, which is attributed to a unique electron transfer kinetic property of MoS2 nanosheets. MoS2NS has the capability to adsorb ssDNA with the help of various interactions, dominantly using van der Waals force for adsorption. The peak anodic current at ssDNA/MoS2NS/ePAD was 5.5 μA, which is higher than the bare and lower than the nanosheet-modified electrode (Figure 3a). When target DNA (20 nM) was introduced, there was further decrease in the current to 4.8 μA (Figure 3a) due to the MB intercalation between the DNA strands that confirmed the presence of complimentary DNA strands. The aforementioned CV results were in line with the DPV analysis as depicted in Figure 3a,b.

Various parameters related to ePAD were optimized such as the scan rate, pH, immobilization time, and probe concentrations to attain the maximum current signal. CVs of ssDNA/MoS2NS/ePAD were recorded at the scan rates from 20 to 100 mV s⁻¹. It is detected that the peak current was enhanced with increasing scan rate, which confirmed the stability of the electrode (Figure 4a–c). Figure 4b shows the variation of peak current on square root scan rates.
interfacial current with a square root of the scanning rate, which confirmed that the interfacial kinetics was diffusion controlled. Figure 4c shows the plot of log(I) versus log(V), and the linear dependence confirmed that the process is diffusion controlled with an $R^2$ of 0.98. A range of pH (6.4–8.0) was also optimized as shown in Figure 4d. MB is a basic dye and tends to form a cationic group at physiological pH, while DNA is a negatively charged molecule due to the phosphate group. Therefore, there will be more absorption of MB ions, which leads to the maximum sensing response at pH 7.4.

Calculation of Electroactive Surface Area for Modified ePAD. Calculation of the electroactive surface area of the ePAD is to depict the performance of the working region of the sensor. This working area can be determined from the slope of $I_{pa}$ versus $v^{1/2}$ as per the Randles–Sevick equation

$$I_{pa} = (2.687 \times 10^5)n^{3/2}v^{1/2}D^{1/2}AC$$

where, $I_{pa}$ is the peak anodic current, $n$ is the electrons transferred ($n = 2$), $v$ is the scan rate, $D$ is the diffusivity of MB ($D = 5 \times 10^{-10}$ m$^2$ s$^{-1}$), $A$ is the electroactive surface area (m$^2$), and $C$ is the concentration of the methylene blue solution.$^{19,25}$

The electroactive area of MoS$_2$NS-coated ePAD was calculated to be 10 times more than bare ePAD.

Different concentrations of ssDNA were immobilized onto the surface of MoS$_2$NS/ePAD to obtain the maximum sensing signal. A CV graph depicted that the interfacial current decayed with increased probe concentrations because of more organic layers of nucleic acid, which may hinder the flow of electrons. Hence, 20 μM was chosen as the optimum probe DNA concentration (Figure 5a). The electrochemical performance of the incubated electrodes with probe DNA was checked at intervals of 1 h and 40 min, 2 h and 40 min, and 3 h and 40 min. The sensor response was decreased at 2 h and 40 min as well as 3 h and 40 min because the active sites of MB were occupied by the ssDNA. Thus, the sufficient immobilization occurred at 1 h and 40 min, and further increasing the time of immobilization caused a decrease in the current response. Thus, the ssDNA was immobilized onto MoS$_2$/ePAD for 1 h 40 min in further experiments are shown in Figure 5b.

Electrochemical Sensing of Target DNA with ssDNA/MoS$_2$NS/ePAD. The electrochemical response of the ssDNA/MoS$_2$NS/ePAD electrode was studied as a function of a complementary target DNA concentration against Salmonella using the CV technique (Figure 6a,b). During this experiment, various target DNA concentrations (100–20 nM) in MB (pH 7.4) were added to the functionalized working region of ePAD. The current response decayed linearly with an increase in target DNA concentrations. A similar trend was observed in previous studies, which reported the decay of current as a
function of target DNA levels.\textsuperscript{30} This decrease in current is attributed to the generation of hybrid duplexes, which prevents the MB-ssDNA interaction. After hybridization of target DNA with ssDNA, the number of unpaired nitrogenous bases gets reduced due to base pairing of DNA strands by hydrogen bonding, which leads to weak interaction of MB molecules. Hence, there is a decrease in the anodic current as depicted from the CV curve. Figure 6b shows the sensor calibration graph of current as a function of complementary target DNA concentrations. The variation of interfacial current was linear with the target DNA concentrations (100–20 nM), and the relationship was given by the $R^2$ value, which is equivalent to 0.991.

Sensor Selectivity, Reproducibility, and Reliability. To explore the selectivity, reproducibility, and reliability of the fabricated sensor, the analyses were performed under the same conditions. The experiment was carried out using ssDNA/MoS$_2$NS/ePAD with the noncomplementary and a three base pair mismatch sequence. After hybridization of the ssDNA/MoS$_2$NS/ePAD with the noncomplementary DNA and three base pair mismatch sequence, an insignificant difference in the sensing response was observed as compared to the probe DNA-modified electrode. However, as soon as the fabricated sensor was introduced to the specific target DNA, the current decreased significantly. Such a response of the fabricated sensor showed that the above ePAD was highly specific for Salmonella DNA (Figure 7a). To check the repeatability of the fabricated genosensor, cyclic voltammograms of ssDNA/MoS$_2$NS/ePAD were obtained 5 times under the same condition. Five sets of the same electrodes were prepared with a fixed concentration of ssDNA and incorporated for the sensing of target DNA. The fabricated electrodes exhibited almost similar results that depicted the excellent reproducibility of the DNA-based biosensor (Figure 7b).

**CONCLUSIONS**

A paper-based genosensor for the sensing of Salmonella-specific DNA is demonstrated on the ssDNA/MoS$_2$NS/ePAD-modified electrode. The proposed genosensor showed higher sensitivity and specificity toward Salmonella-specific DNA, and enhanced electrochemical properties of 2D nanomaterials was also observed, thereby indicating a limit of detection of 20 nM. Furthermore, present methodology reduced the sample and analysis volume. The LoP-based device genosensor proves to be effective for the detection of Salmonella-specific DNA.

**MATERIALS AND METHODS**

**Materials.** All the reagents used were of high purity grade and utilized without treatments. Sodium molybdate dihydrate (Na$_2$MoO$_4$·2H$_2$O; 99% purity) was purchased from Fisher Scientific. Thioacetamide (CH$_3$CSNH$_2$; 99% purity) and silicotungstic acid were purchased from CDH (Central Drug House) Ltd. Chemicals should be handled carefully to avoid the direct contact as they may cause various complications. The 18-mer synthetic oligonucleotides were purchased from Integrated DNA technologies (IDT), India. The sequences are as follows:

- Capturing probe: 5′-GATGAGTATGGTGAGCCGA-3′
- Target DNA: 3′-CTACTCATAACTACGGCT-5′

Tris-EDTA (TE) buffer was used to prepare different concentrations of oligonucleotides (tris-10 mM and pH 8.0, EDTA-1 mM). DNA strands of the inv A gene of Salmonella was used as ssDNA in this work.\textsuperscript{18} Target DNA was used along with MB, and 10 s was chosen as the hybridization time for all experimental studies.

**Apparatus and Methods.** Differential pulse voltammetry (DPV) and cyclic voltammetry (CV) were carried out using the potentiostat/galvanostat (Autolab model: AUT83785). The ePADs, fabricated by screen printing, are based on a conventional two-electrode setup. The microstructural characterizations were performed by using XRD (SmartLab guidance, Rigaku) for the identification of the phase and crystallinity. HR-TEM (Technai, FEI) was done for the identification of the phase and crystallinity. Uv–vis spectroscopy (Cary series, Agilent Technology) was utilized to determine the band gap. Raman spectroscopy (HR800 JV, LabRAM HR) was performed to determine the number of layers that correspond to the peak difference in MoS$_2$.

**Synthesis of MoS$_2$ Nanosheets.** For the synthesis of MoS$_2$ nanosheets, sodium molybdate dihydrate (Na$_2$MoO$_4$·2H$_2$O; 3 mM) and thioacetamide (CH$_3$CSNH$_2$; 9 mM) were dissolved in 50 mL of distilled water, and then silicotungstic acid (2.8 mM) was allowed to mix under constant stirring. The final solution mixture was transferred to a 100 mL teflon-lined autoclave and kept at 220 °C for ~24 h. Consequently, the autoclave was cooled to room temperature. The obtained products were filtered and then washed continuously with distilled water, ethanol, and NaOH (1 M) and further dried in vacuum at 50 °C for 6 h.
Fabrication of ePAD Integrated with MoS₂ and ssDNA. Designing the method of the ePAD platform mainly relies on low-cost materials such as cellulose paper, conductive carbon ink, and solid wax to create the electrode and hydrophobic barrier. Conductive ink was employed to fabricate the ePAD to create a two-system electrode on the cellulose-based substrate: counter and working electrodes. A solid wax was grafted on the ePAD surface to illustrate the complete hydrophobic barrier (using a hot plate at 150 °C). The ePAD consists of one circular region: the immobilization area on hydrophobic barrier (using a hot plate at 150 °C) was grafted on the ePAD surface to illustrate the complete substrate: counter and working electrodes. A solid wax was grafted on the ePAD surface to illustrate the complete hydrophobic barrier, which prevents overflowing of reagents into the electrochemical readout cell, whereas otherwise, electrodes fouling would occur. Different paper-based electrodes were designed for various studies, and the conductivities of the prepared electrodes and were also checked by a multimeter to confirm that electrodes were of similar resistance.

To obtain signal amplification along with a fast detection, MoS₂ was used as a biointerface. As a signal transducer, interaction of methylene blue (MB) with DNA was exploited. The MB showed differential interaction with ssDNA and dsDNA. The signal recorded at the ePAD coated with ssDNA/MoS₂NS was due to the difference in the redox activity of MB. MB shows interaction with the free guanine bases of ssDNA-modified electrode that enhances the current signal. In case of hybridization with target DNA, MB molecules intercalate between the bulky double helix structure of the hybrid.17,42,43 The obtained peak current was evaluated for the hybridization between the base pairs of the hybrid due to which the current response decreases. Such a phenomena can be ascribed to the steric hindrance of the reducing groups of MB, which gets stacked-up between the bulky double helix structure of the hybrid.

To measure the circular region/immobilization area, the scan rate, pH, and time were optimized for ePAD measurements. First, the circular region/immobilization area is separated by printing patterns of solid wax: a stack of the bulky double helix structure of the hybrid. The MB showed differential interaction with ssDNA and target DNA. The interactions of MB with dsDNA and ssDNA were also checked by a multimeter to confirm that electrodes were of similar resistance.

The interactions of MB with dsDNA and ssDNA were probed by means of DPV and CV in the MB (1 μM) in sodium phosphate buffer (0.1 M; pH 7.4) at 100 mV s⁻¹ from −1.0 to +1.0 V (Scheme 1).

Scheme 1. Schematic Representation of Fabrication of Paper-Based Electrode

**ePAD Chip Calibration.** Each step such as the synthesis of MoS₂ nanosheet (MoS₂NS), probe concentration (ssDNA specific to Salmonella sp.), and analytical parameters such as the scan rate, pH, and time were optimized for ePAD measurements. First, the circular region/immobilization area was first decorated with MoS₂NS. Afterwards, the ssDNA specific to Salmonella was immobilized onto the circular surface of the working electrode (WE). Finally, the electrochemical readout of the assay was performed. Different concentrations of target DNA were applied to the circular area of the modified electrode (ssDNA/MoS₂NS/ePAD). The hybridization of target DNA with immobilized ssDNA was performed by subjecting various concentrations of the target molecule (100–20 nM) into 1 μM MB in sodium phosphate buffer (0.1 M; pH 7.4) at 100 mV s⁻¹ in the potential ranges from −1.0 to +1.0 V. Electrochemical studies such as CV and DPV were performed to confirm the hybridization between the ssDNA and target DNA. Different electrodes having optimized concentrations of ssDNA over MoS₂NS/ePAD were prepared and incubated for different intervals of time (1 h and 40 min, 2 h and 40 min, and 3 h and 40 min). The same protocol as above is applied at Salmonella ssDNA/MoS₂NS/ePAD for hybridization reactions of probes with noncomplementary and mismatch sequences, which were used instead of the target sequence.44

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**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

The present work was supported to one of the authors (Dr. Manika Khanuja) by Science and Engineering Research Board [SERB (no. ECR/2017/001222)].

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