Morphology-Preferable MoSe$_2$ Nanobrooms as a Sensing Platform for Highly Selective Apt-Capturing of Salmonella Bacteria

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ABSTRACT: The present report employed nanobroom (NB)-shaped two-dimensional molybdenum diselenide (MoSe$_2$) for the preparation of a sensing matrix for the detection of Salmonella paratyphi. An aptamer specific to salmonella was immobilized onto MoSe$_2$NB-modified fluorine-doped tin oxide via glutaraldehyde cross-linking. Structural and morphological characterizations were performed using UV−vis spectroscopy, scanning electron microscopy, Fourier transform infrared spectroscopy, and X-ray diffraction techniques. Characterizations confirmed the nanobroom morphology and nanosize of the MoSe$_2$ material. Electrochemical studies revealed a good linear detection range of 10$^{-2}$−10$^{-10}$ CFU/mL with low detection limit of 1 × 10$^{-10}$ CFU/mL and with $R^2 = 0.98$. The developed preferable nanobroom-shaped sensing matrix can provide a promising platform for rapid and accurate detection of Salmonella in real samples due to its tremendous stability and sensitivity.

INTRODUCTION

Food contamination is the major problem that is posing a serious threat to the public health. Recent outbreaks trigger the public health domain for an upgrading in diagnosis and treatment methods. Among various food-borne pathogens, Salmonella is a major threat to the health.1,2 There are various serovars of Salmonella, and among them, the antibiotic-resistant species 

Salmonella enterica serovar paratyphi A (S. paratyphi A) is the causative agent of paratyphoid A fever.1,4 Food products contain different essential nutrients, which provide a compatible environment for the growth of Salmonella.1

There are various traditional techniques for the detection of Salmonella such as classical culture and enrichment methods, but these methods have some setbacks such as being lengthy, requiring expertise, and being cumbersome.5 Molecular and immunological techniques also have some associated drawbacks such as cross-reactivity and use of expensive reagents.6,7 There is need for the development of a detection system, which overcomes all of the disadvantages associated with the earlier methods. The electrochemical technique is the best alternative technique for overcoming all of the disadvantages linked with the conventional methods.8 Furthermore, in this approach, an aptamer was also used as a biological recognition element, which can directly bind to Salmonella and produce a change in the electrochemical signal. Aptamers are long stretches of DNA or RNA, which have the ability to bind with the specific protein or antigen by forming unique structural forms.9 Aptamers are the choice of researchers because of their attractive features such as lack of cross-reactivity, small size, ease of preparation, cost-effectiveness, and high specificity.10,11 Various aptamer-based sensors are developed, which satisfy all of the requirements of a diagnostic device.12−16 The aforementioned aptasensor employed a glassy carbon electrode (GCE), which is relatively expensive and for which the cleaning process is also difficult,15 but the present approach involves the use of a fluorine-doped tin oxide (FTO) electrode, which has distinct features such as cost-effectiveness, high capacitive behavior, facile cleaning, conductive behavior, and large surface area. Nanomaterials play a pivotal role in the fabrication of a biosensor due to their many advantageous intrinsic factors, which make them an ideal candidate for immobilization of biological molecules.17−19 Nowadays, two-dimensional (2D) nanomaterials are fascinating researchers owing to their characteristic features such as high surface area, increased electron transfer kinetics, and stability.20 21 Molybdenum disulfide (MoS$_2$) has gained a lot of attraction due to its large surface area, but the material has poor conductivity.22 Molybdenum diselenide (MoSe$_2$) has good electrical conductivity due to the Se that makes the material even more suitable for biosensing applications.23 Furthermore, the nanobroom-shaped platform provides a compatible environ-
ment to the biological recognition element, which ultimately leads to its increased stability and sensitivity. Thus, aptamers and MoSe2 NBs in combination made the best recognition layer for Salmonella, which leads to a lower detection limit as compared to that of earlier reported aptasensors.13,14,24 The present approach involves the development of a MoSe2-modified aptasensor for the detection of S. paratyphi. In the present study, FTO was modified with MoSe2 for providing compatible ambience to the aptamer and for promoting electron transfer kinetics. In addition, the −NH2-modified aptamer was immobilized through glutaraldehyde linkage, which provided stability and longevity to the sensing platform. The developed platform was employed for the detection of Salmonella bacteria at different dilutions. The aptasensor was characterized using electrochemical techniques, that is, cyclic voltammetry (CV) and differential pulse voltammetry (DPV) at each stage of fabrication. The developed sensor showed excellent results in terms of linearity (10⁻²–10⁻¹⁰), detection limit (10⁻¹⁰), and specificity.

■ RESULTS AND DISCUSSION

Morphological and Surface Characterizations of MoSe2. Figure 1a shows the UV–vis absorption spectrum of MoSe2 nanobrooms recorded in the range of 200–800 cm⁻¹, which was in agreement with the earlier reports.25,26 The strong absorbance peak in the visible range from 550 to 800 nm was observed, which clearly indicated that the material has high absorption in the visible region of the solar spectrum. Figure 1b shows the scanning electron microscopy (SEM) image of the MoSe2 nanomaterial. As evident from the image, microrods of length ∼10 μm and diameter ∼150 nm were homogeneously distributed. Microrods were closely packed at one end and spread out at the other end, which resulted in the formation of a “conical” broom-shaped morphology called “nanobrooms”. Figure 1c shows the Fourier transform infrared (FTIR) spectrum of MoSe2 recorded in the range of 400–4000 cm⁻¹. Peaks were observed corresponding to Mo–OH (3339 cm⁻¹); (H–O–H) and COO− (2264 cm⁻¹); COO− (2053 cm⁻¹); Se–O (1576 cm⁻¹); O–Mo–O (3572 cm⁻¹); and Se–O–Se (864, 509, and 428 cm⁻¹). Figure 1d shows the X-ray diffraction pattern of powdered MoSe2 depicting the crystal structure. The material was found to be polycrystalline, and from JCPDS file no. 17-0744, the material was confirmed as MoSe2. The peaks were not sharp, which confirmed the amorphous nature of the material. Highest peaks were observed at 14.084, 34.44, and 55.53° for the (002), (012), and (110) planes, respectively.
**Salmonella Plate Count.** At the end of the incubation period, five dilutions (10^{-2}, 10^{-4}, 10^{-6}, 10^{-8}, and 10^{-10}) of Salmonella were chosen for the plate counting method. The number of colonies forming unit (CFU) was calculated using the formula

\[
\text{CFU/mL} = (\text{number of colonies} \times \text{dilution factor})
\]

At a higher dilution (10^{-10}), growth of colonies is less, and at lower dilutions (10^{-2}), more number of colonies are formed. According to the above formula, the number of bacteria in the plate with a higher dilution (1 \times 10^{-7}) was 2.5 \times 10^{-4}. Further dilutions of the salmonella plate were calculated using the same formula as reported in Table 1.

### Table 1. Salmonella Plate Count

| dilution factor | concentration (CFU/mL) \times 10^{-9} | log (CFU/mL) |
|-----------------|---------------------------------------|--------------|
| 10^{-2}         | 250 000.00                            | −3.60        |
| 10^{-4}         | 60 000.00                             | −4.22        |
| 10^{-6}         | 10.00                                 | −8.00        |
| 10^{-8}         | 0.10                                  | −10.00       |
| 10^{-10}        | 0.08                                  | −10.09       |

**Electrochemical Characterization of Various Stages of Electrodes.** Electrochemical characterization was performed at different phases of electrodes such as bare electrode, MoSe2NBs-modified FTO electrode, aptamer/MoSe2NBs/FTO. Results depicted that the bare electrode showed insignificant electrochemical response, whereas the electrode modified with MoSe2NBs showed an increase in sensing response. MoSe2NBs provide high surface area, compatible ambience, and distinctive electron transport.27 After bioconjugation of the aptamer onto MoSe2, the electrochemical signal was decreased due to nonconductive nature of the biological molecule and the repulsion, linking the negatively charged aptamer sequence and [Fe(CN)6]^{3−/4−} (Figure 2). After incubation with salmonella, the current decreased dramatically due to the nonconductive film of bacteria specifically bound to the aptamer. All results confirmed the successful formation of different phases of electrode.

**Optimization of Experimental Variables.** Various experimental conditions such as aptamer concentration, incubation time, and temperature were optimized to obtain the maximum sensing signal. Various concentrations of the aptamer ranging from 20 to 40, 60, 80, and 100 μM were immobilized to optimize the sensing signal. Results from the DPV curve are depicted in Figure 3a, which shows that upon increasing the concentration of aptamers, the current was decreased, which was due to the more insulating layer of the biological recognition element onto the surface. The response time was also measured by incubating the modified electrode at different times. In Figure 3b, the optimal response time was also measured by incubating the salmonella/aptamer/MoSe2/FTO at different times (5, 10, 15, 20, and 25 s). DPV results concluded that upon increasing the time of incubation with Salmonella there was an increase in electrochemical response, which was due to more interaction of salmonella with the aptamer. Current decreased after 25 s because enough interaction of salmonella was already made with the aptamer. Studies at different temperatures ranging from 4 to 25, 35, and 45 °C were performed to detect the effect of temperature on the sensing response of the biosensor. DPV results as in Figure 3c proved that there was an increase in the sensing response up to 35 °C. CV of aptamer/MoSe2/FTO was detailed at the scan rate from 10 to 100, and it was observed that the peak current increased with the increasing scan rate without any potential shift. The best scan rate was selected to be 100 mV/s for subsequent studies, as shown in Figure 3d,e.

Different concentrations of salmonella were exposed to the modified electrode for 2 h. After incubation, the modified electrode was analyzed using DPV study. When the concentration of salmonella was increased, more adsorption of salmonella occurred onto the electrode surface, which ultimately hindered more electron transfer. DPV curves clearly depicted in Figure 4a that there was a decrease in the current signal with the increase in the concentrations of salmonella. The standard curve between the log concentration of salmonella and current value is also shown in Figure 4b. According to the data, we can note that as the concentration changed from 10^{-2} to 10^{-10} CFU/mL a good linear relationship could be achieved. The equation for this relationship is \( y = 4.965 \times 10^{-3}x + 6.767 \times 10^{5} \), and \( R^2 = 0.98 \). The detection limit was 10^{-10} CFU/mL.

**Selectivity, Reproducibility, and Reliability.** A control experiment was also conducted under the same conditions, using aptamer/MoSe2/FTO with an *Escherichia coli* bacterial strain. After hybridization with the *E. coli* bacterial strain, it showed a current almost similar to or larger than that of
aptamer/MoSe2/FTO. However, when the sensor was exposed to Salmonella, the current was greatly decreased. Measurements shown in Figure 5a proved that the present FTO was very specific for S. paratyphi detection. To check the repeatability of the proposed aptasensor, DPV of aptamer/MoSe2/FTO was recorded for five replicate measurements under the same conditions. Five sets of the same electrodes were prepared with the same concentration of the Salmonella aptamer and were employed for the detection of the same concentration of target Salmonella. All prepared electrodes showed similar results, which show good reproducibility of the fabricated aptasensor represented in Figure 5b.

**Application of Aaptasensor.** The developed aptasensor was also tested in a real sample (apple). The aptamer/MoSe2/FTO aptasensor was suitable for the detection of salmonella bacterial strain ($1 \times 10^{-10}$ CFU/mL) in rotten apple juice. The data obtained for the proposed aptasensor after adding in a real sample is presented in Figure 5b, indicating good accuracy of the proposed aptamer-based electrochemical bioassay for Salmonella detection.

Table 2 shows a comparative study of aptamer-based biosensors developed for the detection of food-borne pathogens. Abbaspour et al. ref 12 reported a dual-aptamer-based sandwich immunosensor for the detection of Staphylococcus aureus, where they employed a screen-printed carbon electrode for the fabrication, which is expensive, requires specific connectors, single-use, disposable, and shows limit of detection (LOD) of 1 CFU/mL, whereas others have reported refs 13, 14 the use of a glassy carbon electrode, which is relatively expensive and whose cleaning process is also difficult. Also, it is having a disadvantage of higher detection limits of 3 and 25 CFU/mL. Furthermore, the present approach is based...
on an electrochemical aptasensor for the detection of S. paratyphi, which showed excellent results in terms of detection limit ($10^{-10}$). The modified electrode, that is, the fluorine-doped tin oxide (FTO) electrode, illustrated distinct features such as cost-effectiveness, high capacitive behavior, facile cleaning, conductive behavior, and large surface area. Moreover, 2D nanomaterials (MoSe₂), owing to their characteristic features such as high surface area, increased electron transfer kinetics, and stability, have shown interesting features such as good electrical conductivity due to the Se, which make the material even more suitable for biosensing applications. This makes the present approach better than the other works.

### CONCLUSIONS

In this article, we reported the development of an aptasensor for detection of S. paratyphi using a 2D nanomaterial, MoSe₂, nanobrooms. The developed aptasensor exhibited high specificity, reproducibility, and sensitivity toward S. paratyphi. MoSe₂ exhibited fascinating features such as high surface area, increased electron transfer kinetics, and stability. The modified FTO electrode (MoSe₂/FTO) was used as a compatible matrix for the attachment of the NH₂-modified aptamer. The electrochemically prepared aptasensor responded linearly to the logarithm of the Salmonella concentration over the range of $1 \times 10^{-2}$ to $1 \times 10^{-10}$ CFU/mL and was highly efficient with a low detection limit of $10^{-10}$ CFU/mL. To make it more economical, various other economical substrates such as paper electrodes can be exploited for futuristic application. However, in the case of the biological recognition element, the present article revealed that nucleic acid aptamers are best choice in terms of sensitivity and specificity.

| modified electrodes                  | limit of detection (LOD) CFU/mL | linear range (CFU/mL) | response time | sensing interface | references |
|--------------------------------------|---------------------------------|-----------------------|---------------|-------------------|------------|
| screen-printed carbon electrode (SPCE) | 1                              | $10^{-6}$             | not reported  | AgNPs             | 12         |
| glassy carbon electrode (GCE)        | 3                              | $10^{-1}$–$10^{-8}$   | 35 min        | GO/Au             | 13         |
| glassy carbon electrode (GCE)        | 25                             | $75$–$7.5 \times 10^{4}$ | 60 min        | rGO–MWCNT         | 14         |
| 1LSRP chip                           | $10^4$                         | $10^{-3}$–$10^{-7}$   | 30 min        | AuNPs             | 28         |
| 3D-IDEA                              | $10^2$                         | $10^{-2}$–$10^{-5}$   | 30 min        | not reported      | 29         |
| gold electrode                       | 20                             | $2 \times 10^{-4}$–$2 \times 10^{-6}$ | 120 min       | AuNPs             | 30         |
| fluorine-doped tin oxide (FTO) electrode | $10^{-10}$                    | $10^{-5}$–$10^{-10}$ | 2 h           | MoSe₂ NBs         | present work |

#### EXPERIMENTAL SECTION

**Materials.** Chemicals used for the synthesis were of analytical grade, purchased from different chemical companies, and used without further purification. Sodium molybdate (Na₂MoO₄·2H₂O with 99% purity) was purchased from Fisher Scientific. Selenium (Se with 99.99% purity), hydrazine hydrate (N₂H₂·H₂O), potassium ferricyanide (K₃[Fe(CN)₆]), potassium ferrocyanide (K₄[Fe(CN)₆]), potassium chloride (KCl), and glutaraldehyde were purchased from Sigma-Aldrich, India. For the preparation of solid LB medium: solid agar, sodium chloride (NaCl), tryptone and yeast extract of Hi-Media were used. All of the other chemicals were of analytical reagent grade. Aliquots of bacterial strains were acquired from Microbial Type Culture Collection and Gene Bank CSIR-IMTECH, Chandigarh (3220-S. paratyphi). E. coli was grown at the Amity Institute of Nanotechnology under sterilized conditions in the lab. The amino-group-modified aptamer nucleotide sequence of Salmonella was synthesized by GCC Biotech (GCC Biotech India Pvt Ltd, India). The 41-mer aptamer sequence was obtained from the work of Xiaoyuan et al. (2014): 5'-NH₂-TAT GCC GGC GTC ACC CGA CGG GGA CTT GAC ATT ATG ACA-G'-3'. The aptamer probe solution of varying concentrations was prepared in Tris–ethylenediaminetetraacetic acid (EDTA) buffer solution (10 mM Tris, pH 8.0, 1 mM EDTA).

**Apparatus.** The aptamer-based electroanalysis of bacteria was carried out at different stages by recording CV and DPV in an Autolab PGSTAT-204 (Eco Chemie, The Netherlands. model: AUT83785), and the results were analyzed by NOVA 1.8 software. It consists of three-electrode systems: Ag/AgCl as a reference electrode, Pt as a counter electrode, and FTO as a...
working electrode. All of the electrochemical experiments were performed at room temperature (RT, 25 °C). UV−vis spectroscopy (Shimadzu 2600) and scanning electron microscopy (SEM) (Zeiss EVO 18 448) were performed to see the morphology of the nanostructures. The analysis of Fourier transform infrared (FTIR) spectra by a Shimadzu 8700 FTIR spectrophotometer was done to identify organic, polymeric, and, in some cases, inorganic materials. Phase identification was carried out by X-ray diffraction using a Rigaku Smart Lab X-ray diffractometer with Cu Kα radiation at 1.540 Å with 2θ in the range of 10−80°.

Preparation and Characterization of Working Electrode. The bacterial strains used in the study were S. paratyphi (MTCC: 3220) and other pathogenic strains of E. coli for the detection of specificity of the proposed aptasensor. Cultivation of both bacterial strains was performed in Luria Broth (LB) medium for 12 h, shaking in a 37 °C incubator. LB medium was prepared using tryptone (10 g), yeast extract (5 g), and NaCl (10 g). The enriched bacteria were centrifuged at 5800 rpm for 10 min (25 °C) and washed with phosphate-buffered saline (PBS) (0.1 M, pH 7.4) several times. After washing with buffer, the cell pellet was resuspended in PBS (10 mL) and was used as the original bacterial sample; further concentrations were made by diluting this in PBS. The absorbance was measured at 600 nm. The correlation between optical density and bacterial concentration (CFU/mL) was determined, at the beginning of this work, by the standard plate count method for both bacterial strains.

Synthesis of 2D Nanomaterial MoSe2 Nanobrooms. Initially, 0.02 M sodium molybdate (Na2MoO4) was dispersed in 50 mL of deionized water (DI) at RT and stirred with a magnetic stirrer for 10 min to give a clear solution. Se powder (0.04 M) was mixed with hydrazine hydrate (10 mL: N2H4·H2O) at RT and stirred with a magnetic stirrer for 15 min to give a dark brown suspension. Afterward, the selenium solution was slowly added to the clear sodium molybdate solution at RT under violent stirring for 20 min and an orange-brown solution was obtained, which was then transferred to a Teflon-lined autoclave and placed in a hot-air oven at 200 °C for 24 h. The obtained solution was washed with distilled water and acetone and then dry-vacated at 40 °C for 30 min.

The formation of broomlike nanostructures is explained on the basis of reaction kinetics and reactant concentrations. The growth of nanostructures involves two processes: nucleation and growth. The formation of nuclei is dependent on the environmental conditions, such as acidic or basic nature of the reactants; metal ion concentration; and hydrogen ion concentration, i.e., pH, etc. Depending on these conditions, formation of nuclei takes place, which further grow into corresponding nanostructures.

In the first step, Se is reduced to NaH Se by H2O with the help of N2H4 (hydrazine hydrate) and Mo O4−2 could be reduced to Mo+4. The reaction occurs between Mo+4 and Se−2, and formation of the MoSe2 nanostructure takes place in a hydrothermal setup. As per the given experimental conditions, the formation of rod-shaped nuclei takes place, which further grow into the corresponding nano/microstructures that further self-assemble themselves into broomlike nanostructures.

Preparation and Characterization of Working Electrode. FTO electrodes were diced having sizes 3 cm × 2 cm using a diamond cutter. The surface conductivity was measured using a multimeter. The bare FTO electrode was cleaned sequentially in a mild detergent, ethanol, and DI water, followed by drying at 100 °C. Subsequently, it was washed.
several times with distilled water. Afterward, prepared MoSe₂-NBs were drop-deposited homogeneously onto the surface and kept for air-drying. A chitosan solution was dropped onto the MoSe₂ electrode and was kept overnight. Then, the chitosan-modified electrode was exposed to 1 mL of glutaraldehyde solution (2.5%) for 12 h at RT to form Schiff base linkage. The aptamer sequence specific to salmonella (50 μL), ranging from 20 to 100 μM, was drop-deposited onto different glutaraldehyde-modified electrodes of same dimensions and conductivity at 36 °C for 2 h. The −CHO group of glutaraldehyde formed linkage with the −NH₂-group-modified aptamer sequence. The electrodes were washed off with ultrapure water. The electrode with the optimum concentration of the aptamer sequence was used for the subsequent experiment. When the polymer was cross-linked with the reagent such as glutaraldehyde, which is a bilinker as the glutaraldehyde molecule consists of −CHO group on both sides, where one side of the −CHO group will attach with the amino group of chitosan and form Schiff base linkage and the other −CHO group will covalently bind to the amino group of the aptamer, a strong link was established between the chitosan and the biomolecule. The capability of the developed aptasensors to detect S. paratyphi was evaluated by testing them with a solution containing various bacterial concentrations. The general procedure is summarized in Scheme 1.

**Enrichment of Bacteria.** Cultivation and plate counting of bacteria were performed according to the method by Xiaoyuan Ma, 2014. Enrichment of the bacteria was performed by inoculating the bacteria in the prepared LB broth media. The medium inoculated with the bacteria was kept under continuous shaking for 2 days. The pellet was suspended in PBS and diluted to obtain an optical density value of 0.12 at 600 nm. Different concentrations of salmonella were prepared ranging from 10⁻² to 10⁻¹⁰ using saline media. The prepared concentrations were cultured on solid media, and bacterial colonies were counted (CFU/mL).

**Electrochemical Aptsensor for the Detection of S. paratyphi.** Electrochemical characterization was done using an electrochemical setup consisting of a working electrode (ssDNA/MoSe₂/NBs/FTO), a reference electrode (Ag/AgCl), and a counter electrode (Pt wire). The working electrodes of same conductivity were exposed to different concentrations of salmonella for 2 h at room temperature to allow maximum interaction with the immobilized aptamer onto the surface of the working electrode. Afterward, electrodes were washed to remove any unbound salmonella. Subsequently, the electrode was analyzed through cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques using electrochemical workstation. The electrolyte used for electrochemical detection was potassium ferrocyanide (5 mM) containing 0.1 M KCl.

To detect the sensor specificity, the prepared electrodes were also exposed to E. coli, used as control bacteria. Experiments (preparation of aptasensors, cultivation, and enrichment) were performed using the same procedure as that for S. paratyphi. Then, a 10⁻¹⁰ dilution of E. coli bacteria was used for the electrochemical aptasensor detection in place of Salmonella, as described above.

**Real Sample Analysis.** To assess the general applicability of the proposed aptasensor, we investigated the quantification of S. paratyphi in a rotten apple. A rotten apple (5 g) was crushed separately using mortar–pestle and mixed with the help of PBS (pH 7.5). The resultant mixture was centrifuged for 10 min at 10,000 rpm, and the supernatant was taken for analysis. The real sample (1 mL) was added directly into buffer, exposed to the modified electrode, and further analyzed by electrochemical techniques.

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