Lysine-Functionalized Tungsten Disulfide Quantum Dots as Artificial Enzyme Mimics for Oxidative Stress Biomarker Sensing

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ABSTRACT: The color generating from the biochemical reaction between 3,3′,5,5′-tetramethylbenzidine and Lysine@WS2 QDs was used a signal for the detection of hydrogen peroxide. The QDs were prepared using a combination of techniques, that is, probe sonication and hydrothermal treatment. Analysis via UV−vis spectroscopy, Fourier transform infrared and Raman spectroscopy, X-ray diffraction, energy-dispersive spectroscopy, and transmission electron microscopy yielded detailed information on the nature and characteristics of these quantum dots. Furthermore, as-synthesized quantum dots were studied for their capability to mimic peroxidase enzyme using 3,3′,5,5′-tetramethylbenzidine as a substrate. Consequently, a colorimetric sensor utilizing Lysine@WS2 QDs could detect hydrogen peroxide in a range of 0.1−60 μM with a response time of 5 min. The same material was used for H2O2 detection using impedance spectroscopy, which yielded a dynamic range of 0.1−350 μM with a response time of 30−40 s.

1. INTRODUCTION

Reactive oxygen species are important messengers in signaling cascades and control the environment around a cell. Although their presence is very important for a cell, high levels of these species cause oxidative stress.1 Oxidative stress is an imbalance between the antioxidant defense system and production of reactive oxygen species.2 Reactive oxygen species cause oxidation of various biomolecules such as DNA, proteins, fatty acids, and amino acids, which leads to diseases like cardiovascular disease, neurological disorders, diabetes, and even cancer.3,4 Many biomarkers are responsible for oxidative stress, such as 4-hydroxy-2-nonenal,5 8-oxo-2′-deoxyguanosine (8oxodG),6 5-chlorocytosine,7 5-chlorouracil,8 3-nitro-tyrosine (3-NO-Tyr),9 and hydrogen peroxide (H2O2)10 to name a few. H2O2 is a well-established oxidative stress biomarker and has been found to be associated with malignancies amongst other diseases.11,12 Therefore, monitoring of oxidative stress biomarkers, especially H2O2, is of clinical relevance.

Current methods of H2O2 detection in biological systems are either based on enzymatic reactions13,14 or use fluorescent tags15,16 for signal amplification. Enzymatic reactions are sometimes difficult to monitor because the compounds formed during the reaction degrade rapidly.16 The use of fluorescent tags, though, allows the detection of H2O2 in subcellular compartments17 but the synthesis of these tags is very difficult and expensive. To overcome the limitations of such conventionally used methods, new potential substitutes must be looked for.

Nanoparticles, in the recent past, have been found to be good candidates for the enzyme mimetic activity,16−23 as explored by researchers for various applications. Platinum nanoparticles,24 iron core/carbon shell,25 cobalt oxide (Co3O4),26 silver phosphate (Ag3PO4),27 iron-doped zinc sulfide quantum dots,28 nitrogen-doped graphene quantum dots,29 and gold nanorices30 have been reported to possess peroxidase-like activity. Transition-metal dichalcogenides (TMDs) such as tungsten disulfide nanosheets (WS2 NS) have also been recently reported to possess peroxidase activity.31,32 TMDs are known to possess little or no cytotoxic effects and are therefore considered safe for in vivo biomedical applications.33 Regarding hydrogen peroxide detection using artificial enzymes, only few reports are available on combined optical and electrochemical-based systems.

In the present work, the ability of lysine-functionalized tungsten disulfide quantum dots (Lysine@WS2 QDs) to mimic peroxidase enzyme for hydrogen peroxide detection has been studied using both optical and electrochemical techniques. Results of this study indicated that the lysine-stabilized and functionalized WS2 QDs have the ability to convert 3,3′,5,5′-tetramethylbenzidine (TMB), a substrate of horseradish peroxidase (HRP) enzyme, into a blue-colored product in the presence of hydrogen peroxide (H2O2). The absorbance of
this product shows a prominent absorbance peak at 652 nm because of formation of a charge-transfer complex product. This was used as an optical signal for the estimation of hydrogen peroxide. The advantage of this method is the replacement of the peroxidase enzyme, as the enzymes generally require controlled storing conditions to keep their activity intact. The replacement of the enzyme with an artificial enzyme is advantageous in terms of its rugged usage. The current work also reports use of Lysine@WS₂ QDs for the detection of hydrogen peroxide using electrochemical impedance spectroscopy (EIS). Impedance studies were found to be more sensitive as compared to the optical detection of hydrogen peroxide.

2. RESULTS AND DISCUSSION

2.1. Characterization of Lysine@WS₂ QDs. 2.1.1. Morphological Characterization. Figure 1a–f shows EDS, transmission electron microscopy (TEM), and elemental mapping of Lysine@WS₂ QDs. The elemental mapping of Lysine@WS₂ QDs clearly shows the presence of carbon, oxygen, nitrogen, tungsten, and sulfur. The carbon content can be due to carbon-coated grids used for imaging and oxygen is attributed to the atmosphere. The nitrogen, tungsten, and sulfur contents are contributed by lysine and tungsten disulfide quantum dots. The inset of Figure 1a shows the high-resolution transmission electron microscopy (HR-TEM) image of functionalized quantum dots, which shows nearly spherical-shaped & well-dispersed quantum dots having an average particle size of 4–6 nm. Figure 1b–f shows mapping of individual elements.

2.1.2. Spectroscopic Characterization. The synthesis of Lysine@WS₂ QDs was further confirmed by studying their absorbance behavior (Figure 2a). The figure shows that little absorbance is observed in case of non-functionalized WS₂ QDs, which is expected from this class of material. However, on closer examination of the spectrum, it was found that a small peak near 280 nm was present, which matches well with the previous report. The Lysine@WS₂ QDs, however, exhibited a prominent hump at 225 nm, which can be attributed to the absorbance of the lysine molecule. Lysine, though, is known to exhibit absorbance at 270 nm not because of the presence of any chromophore, but a possible role of the −NH₂ moiety is anticipated. In literature, to date, no explanation for the signature around 300–350 nm was found. A possible explanation could be the presence of additional functional groups at the surface of the QDs, as shown by Xu et al. The blue shift evident in the present data can be explained by quantum confinement effects, which are...
Lysine@WS2 QDs, on the other hand, showed all characteristic peaks corresponding to two phonon modes of 2H-WS2, which are basically responsible for the signal in Raman spectroscopy.41 However, the Raman spectrum of functionalized quantum dots, no such peaks are observed, which can be due to absence of layer to layer interactions, which are smaller in size as compared to bare QDs.

The presence of various functional groups in synthesized material was confirmed from Fourier transform infrared (FTIR) spectroscopy and the result is shown in Figure 2b. Bare WS2 QDs showed peaks corresponding to O–H stretching and W–S stretching at 3200 and 500 cm⁻¹. The Lysine@WS2 QDs, on the other hand, showed all characteristic peaks corresponding to the presence of lysine and other probable functional groups. The peaks around 3070, 2834, 1741, 1648, 1289, and 475 cm⁻¹ correspond to O–H stretching, C–H stretching, C=O stretching, N–H stretching, C–N stretching, and W–S stretching, respectively.34,38 The peak corresponding to W–S stretching got shifted to 475 cm⁻¹, in case of Lysine@WS2 QDs.

Figure 2c shows the Raman spectra of functionalized and non-functionalized WS2 QDs. In general, the Raman spectrum of bulk WS2 shows two peaks around 363 and 429 cm⁻¹. These peaks correspond to two phonon modes of 2H-WS2, that is, E₂g and A₁g, respectively.39 The E₂g phonon mode corresponds to in-plane optical mode, whereas A₁g is an out-of-plane vibration of the sulfur atoms.40 In case of non-functionalized quantum dots, no such peaks are observed, which can be due to absence of layer to layer interactions, which are basically responsible for the signal in Raman spectroscopy.41 However, the Raman spectrum of functionalized quantum dots showed the presence of two major peaks at 1100 and 1156 cm⁻¹ corresponding to C–H bonds and N–H bonds, respectively. This confirms the presence of the amine functional group on quantum dots.2,3

2.1.3. Crystallographic and Surface Tension Measurements. The X-ray diffraction (XRD) pattern of functionalized and non-functionalized quantum dots is shown in Figure 3a. The pattern shows the polycrystalline nature of the synthesized material as is evident from the broad peak obtained at around 14°, corresponding to (002) plane. The presence of the (002) plane indicates the formation of a monolayer or bilayer WS2, which is a characteristic nature of this class of material.44,45 The XRD pattern of Lysine@WS2 QDs is also similar, which confirms that the polycrystalline behavior of QDs remains intact even after the functionalization.

The crystallite size was calculated from the Scherrer equation using the XRD pattern and was found to be around 6 nm, which is in the quantum domain. From Bragg’s law, the lattice spacing (d), lattice parameters ‘c’ & ‘a’ were also calculated for the (002) plane and found to be 5.97, 2.985, and 11.94 Å, respectively. These results are as per the hexagonal crystal of WS2, confirming the lattice parameters a = b and a = 4c.

Surface tension measurements were also performed for lysine-functionalized and non-functionalized WS2 QDs. The study is important because enzymes such as HRP show maximum activity either by modifying it appropriately or by using some organic solvents only.46 This can be dealt with by developing a system or platform, which can show HRP-like catalytic activity even in other solvents. With an assumption that functionalized WS2 QDs can mimic the HRP, their surface tension was studied with the result shown in Figure 3b. The surface tension of non-functionalized WS2 QDs was found to be 81.79 mN/m and surface tension value of lysine was 28.80 mN/m. Lysine@WS2 QDs showed surface tension value to be 48.02 mN/m, which is much less than that of WS2 QDs. The study shows a significant reduction in surface tension values after functionalization, which confirmed the development of a highly hydrophilic system. This reduction in surface tension is similar to chemical surfactants, which are generally used for synthesis of WS2 QDs. Hence, it can be concluded that lysine has a potential to be a viable replacement of surfactants, which are being used for the synthesis of WS2 QDs.

2.1.4. Electrochemical Characterization. The electrochemical behavior of sequentially modified electrodes, namely,
bare screen-printed carbon electrode (SPE), SPE/lysine, SPE/WS2 QDs, and SPE/Lysine@WS2 QDs, respectively, was also recorded using cyclic voltammetry (CV) and EIS. Figure 3c shows results of CV studies at the electrodes mentioned above. As compared to the bare electrode, the lysine-modified electrode showed a decreased current value. In another set of experiments, the quantum dots-modified electrodes showed an enhanced current value, which might be due to the activation of a fast charge transfer in presence of quantum dots.

The diffusion constant for all the above-mentioned electrochemical systems was calculated using Randles–Sevcik equation:

$$ I_p = (2.69 \times 10^5)n^{3/2}ACD^{1/2}v^{1/2} $$

where $I_p$ is the observed current, $n$ is the number of electrons, $A$ is the area of an electrode (0.071 cm$^2$), $C$ is the concentration of redox species (1 mM), $v$ is the scan rate (0.60 V s$^{-1}$), and $D$ is the diffusion constant.

The diffusion constant $D$ was calculated and found to be 4.927 × 10$^{-5}$, 5.328 × 10$^{-5}$, and 6.290 × 10$^{-5}$ and 4.206 × 10$^{-3}$ cm$^2$ s$^{-1}$ for bare, WS2 QDs, Lysine@WS2 QDs, and lysine-modified electrodes, respectively. This clearly suggests that in case of Lysine@WS2 QDs-modified electrode, the current is diffusing at a much faster pace as compared to other modified electrodes.

A Brown–Anson model was used to calculate the surface coverage of films of various materials (WS2 QDs, lysine and Lysine@WS2 QDs) on the electrode. This was calculated using the formula:

$$ I_p = n^2F^2I^*AV/4RT $$

where $n$ is the number of electrons transferred, $F$ is the Faraday constant (96 584 C mol$^{-1}$), $I^*$ is the surface coverage of the film (mol·cm$^{-2}$), $A$ is the surface area of an electrode (0.071 cm$^2$), $V$ is the scan rate (0.60 V s$^{-1}$), $R$ is the gas constant (8.314 J mol$^{-1}$·K$^{-1}$), and $T$ is the absolute temperature (298 K). The surface coverage ($I^*$) was calculated and found to be 2.828 × 10$^{-8}$, 3.306 × 10$^{-8}$, 4.608 × 10$^{-8}$, and 2.060 × 10$^{-8}$ mol·cm$^{-2}$ for bare, WS2 QDs, Lysine@WS2 QDs, and lysine-modified electrodes, respectively. Modifying the electrode with Lysine@WS2 QDs clearly gives better surface coverage, which in turn will provide more electroactive sites for the reaction to happen.

The ratio of $I_{ps}/I_{pc}$ gives an additional information on reversibility of the reaction, which was also calculated for respective electrodes. The ratio is calculated and found to be 1.106, 1.091, 1.032, and 1.090 for bare, WS2 QDs, Lysine@WS2 QDs, and lysine-modified electrodes, respectively. The value closer to 1.0 indicates that the reaction is reversible. In our case, the Lysine@WS2 QDs-modified electrode gives the value closest to 1.0 and, hence, follows a reversible reaction model.

In electrochemical impedance spectroscopy, the plot between imaginary and real impedance is referred to as the Nyquist plot. The Nyquist plot is a semicircle for electron-transfer-limited reactions and if the reaction has a diffusion-controlled process as well, then the semicircle is accompanied with a linear portion also. The diameter of the semicircle is significant for data interpretation. The greater the diameter, more is the impedance. Figure 3d shows the Nyquist plot of all electrodes. The results show that the obtained Nyquist plot has both semicircle as well as linear line, which indicates that the reaction is diffusion-controlled. Among all the Nyquist plots, the diameter of the semicircle for Lysine@WS2 QDs-modified electrodes is found to be the smallest. This ensures a fast electron-transfer.

**2.2. Peroxidase Activity of Lysine@WS2 QDs.** Before studying the peroxidase activity of synthesized material, fluorescence studies were also performed; just in case if their fluorescent properties can be useful is shown in Figure 4a. It was observed that as the excitation wavelength was increased, (from 300 to 360 nm), the photoluminescence intensity also increased, and the maximum emission was obtained at 450 nm. At higher excitation wavelengths (370–400 nm), the intensity...
2.2. Reaction Mechanism of Lysine@WS2 QDs-Catalyzed H2O2-TMB Reaction. The peroxidase activity of the Lysine@WS2 QDs was investigated in terms of its catalytic ability to oxidize the TMB (chromogenic substrate) in the presence of H2O2. Generally, the TMB gets oxidized during the enzymatic degradation of H2O2 by horseradish peroxidase (HRP). The one electron oxidation of TMB results in a radical cation that forms a charge transfer complex with unoxidized compound. This charge-transfer complex absorbs at 652 nm. The completely oxidized form (diimine) absorbs at 450 nm, which is formed by two sequential one-electron oxidation of TMB. The peroxidase activity of the synthesized Lysine@WS2 QDs can possibly be explained by formation of the charge-transfer complex between negatively charged Lysine@WS2 QDs and positively charged TMB. The schematic of chemical reaction is shown in Figure 4b.

The chemical reaction between H2O2, TMB, and quantum dots results in the formation of a blue-colored product, which is expected to be formed by charge-transfer complex formation between diimine and diimine oxidation products, as mentioned above. The quantum dots catalyze the oxidation of TMB by reacting with H2O2 and produce the color. This complex gives absorption peaks at 450 and 652 nm. In our present work also, both the peaks could be observed (Figure 4c) and the absorbance peak obtained at 652 nm has been used as a signal for monitoring the reaction. To confirm the role of quantum dots in the catalytic reaction, different sets of experiments were conducted, with different reaction mixtures, namely, only Lysine@WS2 QDs, TMB + H2O2, TMB + Lysine@WS2 QDs, TMB + H2O2 + Lysine@WS2 QDs. Except in the TMB + H2O2 + Lysine@WS2 QDs reaction mixture, the blue color did not appear (Figure 4d) and hence no peaks were observed. In the TMB + H2O2 + Lysine@WS2 QDs reaction mixture, blue color was generated as a result of which the prominent peak at 652 nm is observed (Figure 4d). This confirms the role of Lysine@WS2 QDs as a peroxidase-mimicking material. The study interestingly showed that the individual entities, namely, lysine and quantum dots did not show the peroxidase activity. The hybrid of two in the form of functionalized quantum dots however showed peroxidase activity. WS2 QDs possess intrinsic peroxidase activity, which has been reported in previous reports also. This can be due to quick transfer of the electrons in the material. Lysine however does not possess any peroxidase activity. However, the combination of lysine with WS2 QDs generated amine groups on the QDs. The lone pole of electrons in these amine groups enhanced the intrinsic peroxidase activity of the WS2 QDs and hence more color generation was observed when using TMB.

2.2.2. Colorimetric Detection of Hydrogen Peroxide. The synthesized Lysine@WS2 QDs were studied for their capability to mimic the catalytic activity of peroxidase enzyme for H2O2 estimation. For this, varying concentrations of H2O2 were added to the reaction mixture containing a 200 μL TMB (4 mM) and a 40 μL of Lysine@WS2 QDs sample. The experiment was conducted five times (n = 5). The samples were incubated for 5 min and then their absorbance recorded (Figure 5a). The inset in Figure 5a shows the color variation in the reaction solution at various hydrogen peroxide concentrations. The absorbance at 652 nm increased with increase in H2O2 concentration. The linearity is obtained in the range 0.1–60 μM with a regression value of 0.9803 from 10–60 μM (Figure 5b). Table 1 shows a comparison of linearity obtained with other catalysts as artificial or enzyme-mimicking materials. The current work shows better results in comparison to previous reports on peroxidase activity for hydrogen peroxide detection in terms of a broad linear range (0.1–60 μM) and a short response time (5 min), result discussed in coming sections.

In most of the earlier reports, the color development with the enzyme-mimicking took more time. Authors used poly[2-(3-thienyl)ethyloxy-4-butyloxysulfonate] and gold nanoparticles, respectively, as enzyme mimics. The linearity range obtained was narrow as well as the response time was longer. Although Guo et al. demonstrated that iron oxide-graphene (Fe3O4-graphene)-based nanostructures could show response in 3 min, the dynamic range reported by them is very narrow, that is, 0.2–10.0 μM. In the graphene oxide-platinum nickel hybrid (GO-
PtNi) work, the system had a working range from 80 to 1500 μM, which, although very broad, is not able to detect the concentration ranges below 1 μM.

2.2.3. Optimization of Experimental Conditions. The reaction conditions such as response time, TMB concentration, Lysine@WS2 QDs volume, temperature, and pH were optimized. For the response time study, the reaction was monitored from 0 to 1200 s and the response was recorded for both WS2 QDs and Lysine@WS2 QDs. In WS2 QDs, moreover the intensity of color generated was very less as compared to color generated with functionalized QDs. Also, non-functionalized QDs showed a response time of 700 s (approx. 12 min), whereas the functionalized QDs showed a reaction time of about 300 s only (Figure 6a). The reaction was constant up to 900 s and thereafter a decrease in the absorbance of colored compound was observed. Hence, for further reactions, the optimum reaction time was kept as 5 min.

The result of effect of TMB concentration on chemical reaction is shown in Figure 6b,c. The concentration was varied from 3 to 12 mM. From 3 to 9 mM concentration of TMB, the response increased linearly with $R^2$ value of 0.98 but after that, the response started decreasing. For final studies, 4 mM concentration of TMB was used to ensure minimum use of constituents.

The concentration of Lysine@WS2 QDs was also optimized. For this, the volume of Lysine@WS2 QDs was varied from 10 to 90 μL. The maximum absorbance was obtained with 40 μL (Figure 6d); thereafter, a decrease in absorbance was observed. Hence, 40 μL was selected as the optimum value for further studies.

Variation in the pH value results in a large change in the enzymatic activity; therefore, the effect of pH on catalytic reaction was studied. The plot of pH versus absorbance is shown in Figure 6e. The maximum absorbance was obtained at a pH value of 4.0, which might be because the equilibrium shifts toward the charge transfer complex (at 652 nm) instead of the imine complex (at 450 nm), thus leading to the formation of a more prominent charge-transfer complex than imine complex and hence a more dominant blue color, which implies that the acidic pH condition favors formation of the charge-transfer complex, whereas an alkaline pH condition leads to formation of an imine complex.67 This trend is in line with earlier reported peroxidase mimics. The previous report of WS2 nanosheets having peroxidase activity also displayed maximum activity at pH 4.0.61 Yao et al. in their work showed that iron sulfide nanowires had the potential as a peroxidase mimetic having maximum activity at a pH value of 4.0.68 Vanadium oxide-mesoporous carbon composite showed peroxidase activity for detection of glucose. This material also showed maximum catalytic activity at pH 4.0.

The change in temperature profile and its respective effect on catalytic reaction was studied to understand the dependence of reaction on temperature. The temperature was varied from 0 to 80 °C and the maximum absorbance was achieved at 37 °C (Figure 6f). All the identically prepared sets showed similar absorption values at 652 nm as shown in Figure 6g. A good reproducibility is obtained with the developed enzyme-mimicking quantum dots, which has been concluded by monitoring the absorbance of identically prepared five sets.

In the human body, various agents similar in function to H2O2 exist, which may or may not interfere in the peroxidase activity of Lysine@WS2 QDs. Thus, the interference study was also performed. For this, ascorbic acid (AA) and dopamine (DA) were used as interfering agents. AA, DA, and AA + DA were added to the reaction mixture having 200 μL of TMB (4 mM), 50 μL of H2O2, and 40 μL of the Lysine@WS2 QDs
sample. The presence of AA, DA, or both in the reaction mixture did not interfere with the peroxidase activity of the Lysine@WS2 QDs as presented in Figure 6h. Hence, the system can be reported as highly selective toward hydrogen peroxide.

2.2.4. Detection of H2O2 in Urine Samples. Real sample analysis was done using urine samples. Direct urine did not give any reaction, which might be due to the absence of H2O2 in urine. Hence, the urine sample was spiked with known concentrations of H2O2 (50, 60, 100, 110 μM) and the reaction conditions were kept as 200 μL of TMB (4 mM), 50 μL of spiked urine sample, and 40 μL of the Lysine@WS2 QDs sample. The result is shown in Table 2.

% recovery = (absorbance of H2O2 recovered /absorbance of H2O2 added) × 100

Table 2. Analysis of H2O2 Concentration in the Spiked Urine Sample (n = 4)

| Absorbance of H2O2 added (from standard curve) | Absorbance of H2O2 recovered | % Recovery |
|-----------------------------------------------|-----------------------------|------------|
| 0.396 (50 μM)                                 | 0.405                       | 102.27     |
| 0.400 (60 μM)                                 | 0.437                       | 109.25     |
| 0.441 (100 μM)                                | 0.438                       | 99.31      |
| 0.457 (110 μM)                                | 0.511                       | 111.81     |

With spiked samples also, the sensor showed a linear response with increase in spike concentration.

2.3. Electrochemical Detection of Hydrogen Peroxide. The developed Lysine@WS2 QDs were used for electrochemical detection of hydrogen peroxide also for which impedance spectroscopy was employed. For this, the quantum dots were drop-casted on SPE and air-dried. This modified electrode was then used for impedance measurements. The impedance response is recorded in the form of a Nyquist plot. Figure 7 shows the Nyquist plot recorded with Lysine@WS2 QDs/SPE electrodes as a function of H2O2 concentration (0.1−350 μM). The diameter of the semicircle decreased as the concentration of H2O2 increased, which might be due to enhanced electrochemical activity of H2O2 in the case of QDs. This trend continued from 0.1 to 350 μM. This linear range is much better than that obtained with colorimetry. In pH studies, the diameter of the semicircle decreased when moving from 2.0 to 7.0 pH in the range; thereafter, it increased dramatically. The abrupt increase in the diameter of the semicircle of the Nyquist plot can be because at alkaline pH, hydrogen peroxide decomposes into H+ and perhydroxyl. The perhydroxyl results in the formation of free radicals, which cause an obstruction to the flow of current to the electrode, thereby increasing the diameter of the semicircle in the Nyquist plot. Another assumption is that as ferro-ferri is used as the redox marker, the iron in contact with the H2O2 can form insoluble iron hydroxide, which can create hindrance in the electron flow and hence more impedance is observed. Figure 7c,d shows the Nyquist plot for reproducibility and interference study. In impedance study also, the electrodes showed good reproducibility and selectivity.

Table 3 shows comparison of linearity ranges obtained with different electrochemical techniques. All the nanostructures

| Nanostructure used | Electrochemical technique used | Linear range (μM) | References |
|--------------------|-------------------------------|------------------|------------|
| MoS2 interconnect | voltammetry, amperometry      | 0−200            | 71         |
| porous carbon      |                               | 0−300            |            |
| heterostructure    |                               |                  |            |
| MoS2 flowers grown | amperometry                   | 5−145            | 72         |
| on graphene/carbon |                               |                  |            |
| nanotubes         |                               |                  |            |
| graphene/Au nanoparticle | amperometry             | 0−130            | 73         |
| polyanionate-grafted |                               | 100−20 000       | 74         |
| graphene nanohybrid |                               |                  |            |
| Lysine@WS2 QDs   | impedometry                   | 0.1−350          | This study |

Figure 7. (a) Nyquist plot of SPE/Lysine@WS2 QDs in the presence of H2O2 (0.1−350 μM) on the Lysine@WS2 QDs-modified SPE. (b) Effect of pH variation on the sensors response. (c,d) Nyquist plot for reproducibility and interference study, respectively.
reported for electrochemical sensing of hydrogen peroxide have narrow working range as compared to the current study as shown in Table 3. In earlier reports, the researchers reveal H₂O₂ using polyoxometalate-grafted graphene nanohybrid worked in the higher concentration ranges (100–20 mM), but did not mention the utility of this nanostructure in lower hydrogen peroxide concentration values.

### 3. MATERIAL AND METHODS

#### 3.1. Materials.
Tungsten (IV) sulfide nanopowder (90 nm average particle size), TMB (≥99%), sodium hydroxide (NaOH) pellets, l-Lysine monohydrochloride were purchased from Sigma-Aldrich (India) and were used as received. Hydrogen peroxide (30%) and hydrochloric acid (HCl) were procured from Merck (India). Potassium ferrocyanide [K₃Fe(CN)₆] and potassium ferricyanide [K₄Fe(CN)₆] were purchased from HiMedia, (India). SPEs having a working diameter of 3 mm with an area of 0.071 cm² were purchased from Zensor (Taiwan) and were used for electrochemical studies. Deionized (DI) water (18.2 MΩ), obtained from Millipore Integral System (USA), was used to prepare all the aqueous solutions required during experimentation. The solutions and glassware were autoclaved prior to being used. All the solutions were kept at 4 °C to avoid its oxidation in the presence of light. Stock solution of hydrogen peroxide (1 mM) was prepared in deionized water and diluted further for experimentation purpose. Interference study was conducted by using interferents such as AA and DA obtained from Sigma-Aldrich (India). Dibasic potassium phosphate (K₂HPO₄) and potassium dihydrogen phosphate (KH₂PO₄) salts were purchased from Sigma-Aldrich (India) for preparing phosphate buffer (PB) solutions.

#### 3.2. Characterization of Lysine@WS₂ QDs.
The optical characterization and monitoring of enzyme-like catalytic reactions were performed on UV–visible spectrophotometer [Hitachi, model U3900H, Japan]. To study the photo-luminescence properties of the quantum dots, a multimode plate reader (Molecular Devices, SpectraMax i3x, USA) was used. The functional groups of the synthesized material were analyzed using FTIR spectrometer [PerkinElmer, Model Spectrum-II, USA]. The diffraction pattern was obtained using an X-ray diffractometer [Bruker, model D8 ADVANCE, Germany]. A Raman analyzer [Renishaw, UK] was used to record Raman spectra. TEM analysis was done on [FEI, Model TECNAI G²F-20, USA]. The elemental composition of the synthesized material was done using energy dispersive spectroscopy (EDS) [Hitachi, model SU8010, Japan]. Surface tension and contact angle measurements for the synthesized material were conducted using a Drop Shape Analyzer [Kruss, DSA-100E, Germany]. The electrochemical characterization and hydrogen peroxide estimation were performed on electrochemical analyzer [CH Instrument, model CHI660C, USA]. A probe sonicator [Helix Biosciences, India] was used during the synthesis of material. Remi C-24 Plus [India] was used for centrifugation purpose.

#### 3.3. Synthesis of Lysine@WS₂ QDs.
For synthesis of lysine-functionalized WS₂ QDs, the earlier reported protocol was used with some modifications. Briefly, 300 mg of WS₂ nanopowder and 150 mg of l-lysine monohydrochloride were mixed in 100 mL of deionized water. This was subjected to probe sonication for 3 h, followed by hydrothermal treatment at 200 °C for 6 h in a Teflon-lined stainless-steel autoclave. After completion of reaction, the solution was allowed to cool down naturally. The resulting solution was centrifuged at 10 000 rpm for 30 min. These QDs are termed as Lysine@WS₂ QDs in the paper.

#### 3.4. Peroxidase Activity.
The peroxidase-like activity of Lysine@WS₂ QDs was studied by monitoring the oxidation of TMB in the presence of H₂O₂. The catalytic reaction resulted in the formation of a blue-colored compound (oxidized TMB), which shows an absorbance at 652 nm in spectroscopic studies. For control experiments, a reaction was performed in the absence of H₂O₂ and Lysine@WS₂ QDs. Every reaction mixture was incubated at room temperature (RT) for 5 min prior to absorbance measurements.

#### 3.5. Colorimetric Detection of Hydrogen Peroxide.
For the colorimetric detection of hydrogen peroxide, the reaction mixtures consisting of H₂O₂, TMB, and QDs were incubated at RT for 5 min and the absorbance of reaction product was recorded. In this process, the concentrations of TMB, H₂O₂, and QDs were optimized by varying their respective concentrations. The effect of varying pH, incubation time, and temperature was also studied. The effect of pH was observed from 2 to 8. For incubation time, the reaction was monitored up to 1200 s and the effect of temperature was studied from 0 to 80 °C.

The effects of interfering species, reproducibility, and real sample analysis were also observed. For interference study, AA and DA were used. The reproducibility study was performed by monitoring the absorbance of five identical sets of reaction. For real samples study, urine sample was used. Prior to use, the urine sample was centrifuged at 10 000 rpm for 15 min at 4 °C followed by further filtration using a 0.22 μm filter (Millipore) to remove any suspension and kept at 4 °C. This urine sample was spiked with a different hydrogen peroxide concentrations at 50, 60, 100, and 110 μM.

#### 3.6. Electrochemical Detection of Hydrogen Peroxide.
CV and electrochemical impedance spectroscopic (EIS) techniques were used for electrochemical characterization of bare SPE, SPE/lysine, SPE/WS₂ QDs, and SPE/Lysine@WS₂ QDs electrodes. Ferro-ferri cyanide redox marker, prepared in PB, was used as an electrolyte and redox marker. Electrochemical detection of hydrogen peroxide was performed by using impedance spectroscopy. The measurements were recorded in the frequency range of 1.0 to 100 000 Hz with an amplitude of 0.005 V. The effect of pH, interferents, and reproducibility was also studied for impedance measurements.

### 4. CONCLUSIONS
The current study aimed at determining the ability of lysine-functionalized WS₂ QDs serving as peroxidase mimics. Although several materials have been reported to mimic peroxidase activity, to date the reported enzyme surrogates are characterized by an exceedingly slow response. The Lysine@WS₂ QDs developed in the present study provided a response time of 5 min using colorimetric read-out techniques at RT, whereas EIS yielded an even higher sensitivity at a shorter response time of about 30–40 s only. Finally, the application in real-world sample matrices confirmed the utility of the developed enzyme mimics during the detection of hydrogen peroxide in urine. Thus, it can be concluded that these QDs
may effectively be used as peroxidase-mimicking enzyme surrogates in hydrogen peroxide detection schemes that work in real-world sample matrices.

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