



Tandem Synthesis of High Yield MoS₂ Nanosheets and Enzyme Peroxidase Mimicking Properties

Suresh Thangudu ^{1,*}, Mu Tzu Lee ¹ and Sami Rtimi ^{2,*}

- ¹ Department of Chemistry, National Tsing Hua University, Hsinchu 30013, Taiwan; mercylee@gapp.nthu.edu.tw
- ² Powder Technology Laboratory, École Polytechnique Fédérale de Lausanne (EPFL), Station 12, CH-1015 Lausanne, Switzerland
- * Correspondence: suresh120689@gmail.com (S.T.); Rtimi.sami@gmail.com (S.R.)

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Abstract: Molybdenum Sulfide nanosheets (MoS₂ NSs) have unique properties that allow its use in a wide range of applications. Unfortunately, a lack of green synthesis methods to achieve a high yield remains a challenge after decades. Herein we report a simple, ecofriendly, green and cost-effective approach to synthesize water soluble MoS₂ NSs via probe/Tip sonication method. The sequential batch manner pathway allows us to attain a high yield of $MoS_2 NSs$ (~100%). The prepared MoS_2 NSs were characterized using up-to-date surface science techniques. UV-visible-NIR spectroscopy allowed us to visualize the doublet peaks of pristine MoS_2 at 610 and 680 nm concomitant with the inter-band transitions at 394 nm and 460 nm. Using Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS), the crystallites' sizes were estimated. X-ray diffraction (XRD) and Raman Spectroscopy were performed with respect to the bulk MoS₂. The energy difference between the Raman peaks revealed that our NSs are formed of 5–6 layers. Further, we explored enzyme peroxidase mimetic properties of the synthesized MoS_2 NSs. Results showed that the present MoS_2 NSs offer excellent peroxidase mimicking properties. Most importantly, we observed that the optical properties and characteristics of MoS₂ NSs synthesized by the current green method are similar to those of MoS₂ NSs synthesized using conventional harsh methods reported in the literature. So that we strongly assume that the present method is a green alternative for the existing low yield and harsh experimental procedures to achieve water soluble MoS₂ NSs in high yield. The synthesized soluble NSs are promising catalysts for the detection of toxic chemicals in the environment and/or for following enzymatic chromogenic reactions.

Keywords: MoS₂ nanosheets; probe sonication; optical properties; enzyme mimicking properties; green synthesis

1. Introduction

After the graphene era, there has been significant attention on two dimensional transition metal chalcogenide nanomaterials (TMCs) [1], in particular for MoS₂, because it has a structural analogue to graphene where two sulfur atoms are sandwiched with one molybdenum atom and these layers are held together by Van der Walls forces [2]. As well, its unique physio-chemical properties make it a promising candidate for various applications, such as catalysis [3], electronics [4], nanomedicine [5], energy and environmental applications [6], beyond graphene. As an advancement of nanotechnology, nano-sized MoS₂ has many more advantages than the bulk MoS₂ because of its enhanced surface area, good optical absorption and extreme flexibility [7,8]. To this end, the vast utilization of MoS₂ NSs in several emerging fields has drawn scientific attention towards synthesis of MoS₂ NSs with high yields using different approaches. Therefore, several methods have been reported in the literature



for the synthesis of MoS₂ NSs, including mechanical exfoliation (ME) [9], chemical vapor deposition (CVD) [10], liquid exfoliation [11], and sonication assisted metal intercalation; however, these methods have their own limitations [12], The ME method can produce large area and nearly defect-free MoS_2 NSs flakes, but it lacks scalability [13]. The CVD method can result in wafer sized monolayer MoS_2 NSs, but higher temperatures and transfer processes are needed [14]. Liquid phase chemical exfoliation is one of the most widely used methods to synthesize MoS₂ NSs from the bulk MoS₂ and involves the intercalation of the lithium (Li) atoms and reaction of the intercalated Li with water [15]. However, this process requires the use of strong intercalating agents, such as n-butyl lithium (n-BuLi) and sodium napthalenide. Additionally, the incomplete insertion of ions and post exfoliation steps to restore the MoS₂ structure are still limiting factors for safe and effective scale-up. Thus, in recent years, many research pioneers have been more focused on the green synthesis of MoS₂ NSs. In this regard, the ball milling method was reported as a good way for scaling up the production of MoS₂ NSs. However, it destroys the crystal structure and offers lower yields of NSs formation of about 45% [16]. On the other hand, the simple sonication method can offer a simple and rapid production of MoS₂ NSs. However, unfortunately, utilizing toxic solvents such as N-methyl 2-pyrrolidone (NMP) [17,18] and N, N-dimethylformamide (DMF) [19], and low yields of MoS_2 NSs are still the limiting factors to scale up the synthesis. Sonication assisted exfoliation was achieved on different solvent systems, such as house-hold detergent (Ultra Tide) + water [20], Ethanol + water [21], DMSO + water [22]. Even though it offers a greener way to produce NSs, but it still suffers from lower yields. Therefore, there is still a significant scope to develop an efficient green and ecofriendly approach to prepare water soluble MoS_2 NSs in large scale with higher yields is highly desired. Table 1 summarizes the literature overview of various synthesis methods of MoS₂ NSs and some remarks characterizing these preparations.

Method	Reagents	Remarks	Ref.
Hydrothermal	(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O, Sulfocarbamide, oxalic acid	Low yield Micro particles Time consuming	[23]
Liquid exfoliation	MoS ₂ powders, N-vinyl-2-pyrrolidone	Toxic solvents Micro sheets	[24,25]
Microwave synthesis	(NH ₄) ₂ MoS ₄ , DMF	Toxic solvents	[26]
Mechanical exfoliation	High temperature, transfer process	High temperature, transfer process	[9]
Chemical Vapor Deposition (CVD)	MoO ₂ , SiO ₂ , Sulfur		[10]
Ionic liquid assisted grinding exfoliation	MoS ₂ powder, BMIMPF ₆ , DMF	Toxic solvents	[27]
Ball milling and chemical intercalation	MoS ₂ powders, DMF, NMP	Toxic solvents, Disordered NSs, lower yields	[16]
Morrison method	MoS ₂ Bulk, Hexane, n-buthyllithium, N2 atmosphere	Toxic solvents, inert condition	[28]
Ultra-sonication followed by hydrothermal	$((NH_4)_2MoS_4, DMF and water$	Toxic solvents	[29]
Probe sonication (tandem process)	MoS_2 powder, PAA and water	High yield, water soluble NSs, batch manner	Present work

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In this work, we report a simple and green tip/probe sonication approach to synthesize water soluble MoS_2 NSs. The sequential batch manner/tandem synthesis allowed us to achieve MoS_2 NSs in high yield (estimated yield is ~100%) than the conventional methods reported in literature. Further, we assessed the optical and enzymatic peroxidase mimicking properties of the synthesized MoS_2 NSs. To the best of our knowledge, this is the first report on the synthesis of MoS_2 NSs via the batch manner pathway to accelerate the yields.

2. Results and Discussion

2.1. Characterization of MoS₂ NSs

Figure 1 illustrates the batch synthesis method of MoS_2 NSs via probe sonication method. Further, the morphology of the as-synthesized B1/B2/B3-MoS₂ NSs was examined by TEM analysis. As shown in Figure 2, low and high resolution TEM images revealed that B1-MoS₂ NSs were uniformly distributed with the clear morphology of nanosheets. After probe sonication, all the MoS₂ NSs synthesized in different batches (B1/B2/B3-MoS₂ NSs) exhibited less size as compared to the bulk MoS₂, showing 2–3 μ m size (as provided by the manufacturer). These sizes are further discussed from the DLS results in Section 2.2. HR-TEM images of MoS₂ NSs show the clear lattice fringes resulting from a good crystalline nature. The same features, such as clear morphology and crystalline nature, were also observed for B2-MoS₂ NSs and B3-MoS₂ NSs (Figure 2). EDS analysis revealed the presence of both elements (Mo and S) in the synthesized MoS₂ NSs as seen in Figure S1.



Figure 1. Schematic representation of batch manner synthesis of MoS₂ NSs.



Figure 2. Low and high resolution TEM images of batch manner synthesized MoS₂ NSs, such as B1-MoS₂, B2-MoS₂ and B3-MoS₂ NSs.

The changes in size of MoS₂ during the synthesis were monitored using Dynamic Light Scattering (DLS) analysis. Bulk MoS₂ is approximately ~2–3 μ m, which is in agreement with the size provided by Sigma-Aldrich. After the probe sonication for 3 h, the size of B1-MoS₂ NSs was dramatically reduced to 235 ± 5 nm. Similarly, the size of B2-MoS₂ NSs and B3-MoS₂ NSs were reduced to 189 ± 6 nm and 185 ± 5 nm, respectively, as shown in Figure 3A. However, the size of the residual content R1-MoS₂ and R2-MoS₂ were 850 ± 70 nm and 535 ± 10 nm, respectively (Figure S2). Further, we postulated the yields of B1/B2/B3-MoS₂ NSs by vacuum drying the resulting NSs. Weighing the dry powder allowed for the estimation of the yields by using amount/weight of starting precursor at each batch. The approximate yields are as follows: ~50%, ~30% and ~20% of MoS₂ NSs were achieved for B1-MoS₂ NSs, B2-MoS₂ NSs and B3-MoS₂ NSs, respectively. Overall, the present batch manner pathway offers a high yield (100%) water soluble MoS₂ NSs in a green manner within three tandem cycles.



Figure 3. (A) DLS and (B) UV-visible absorption spectra of synthesized MoS₂ NSs.

2.2. Optical Properties of MoS₂ NSs

Light absorption properties of as-synthesized MoS₂ NSs were studied by Ultraviolet-visible-near infrared (UV-vis-NIR) spectrophotometry. As shown in Figure 3B, B1-, B2- and B3-MoS₂ NSs exhibited good light absorption in the spectral region 200–1000 nm. Commonly, the spectral absorption of MoS₂ NSs exhibits four kinds of excitonic/electronic transitions, which are assigned as A, B, C, and D excitons. Briefly, the peaks are positioned at 680 nm and 610 nm and correspond to the doublet peaks of pristine MoS₂, and are assigned as A and B excitonic inter-bands transitions. Spin-orbit splitting transition is responsible for the arising doublet peaks at the K peak. The C and D transitions at 460 nm and 394 nm originated from the inter-band transition between occupied dz2 orbital and the unoccupied dxy, dx2–y2, and dxz,yz orbitals [30]. In addition, two other peaks appeared at 394 nm and 460 nm, which can be attributed to the optical absorption of smaller sized nanosheets due to the quantum effects in MoS₂ NSs [31]. Moreover, the absorption spectra of the synthesized MoS₂ NSs are in agreement with spectral properties reported in the literature [20,27].

Additionally, we used PAA as a biocompatible/water soluble ligand, which is believed to be surface coordinated with MoS₂ NSs. To confirm the presence of PAA on MoS₂ NSs, we carried out Zeta potential measurements. As shown in Figure 4A, the synthesized B1-, B2- and B3-MoS₂ NSs displayed negative surface charge corresponding to the carboxylic group (COO⁻) in PAA, which was surface bonded on MoS₂ NSs. Besides zeta potential measurements, we also performed the FTIR analysis to confirm the presence of PAA on MoS₂ NSs. As shown in Figure S3, absorption bands of PAA on B1/B2/B3-MoS₂ NSs were identified at 2951, 1714, and 1453 cm⁻¹, which correspond to the vibrational modes of CH₂, C=O, and C-O, respectively. Whereas for the FT-IR spectra of bulk MoS₂, we did not observe these characteristic peaks corresponding to the PAA. The successful surface attachment of PAA greatly offered a water soluble nature to MoS₂ NSs. The optical images of water soluble B1-, B2- and B3-MoS₂ NSs are shown in Figure 4B. It is readily seen from Figure 4B that the three prepared NSs exhibit high dispersibility in water.



Figure 4. (A) Zeta potential analysis and (B) optical images water soluble MoS₂ NSs.

2.3. XRD and Raman Spectra Analysis of MoS₂ NSs

Figure 5A shows the crystallinity and phase purity of the synthesized MoS_2 NSs by X-ray diffraction (XRD). Further, the synthesized MoS₂ NSs XRD patterns were compared with the bulk MoS₂. The XRD results clearly revealed that the formation of nanosheets results from reducing the (002) diffraction plane in all synthesized MoS_2 NSs as compared to the bulk MoS_2 . All the synthesized B1/B2/B3-MoS₂ NSs presented diffraction peaks of (002), (100), (103), (104), (105) and (008), which reflected the pure hexagonal phase of MoS₂ NSs (magnified XRD spectra shown in Figure S4). These results are in alignment with the reported literature [20,27]. It is worth mentioning that we did not observe any peaks corresponding to impurities, indicating that as synthesized MoS₂ NSs possessed good crystallinity with high purity. Likewise, Raman spectra of the as-synthesized B1-MoS₂ NSs were recorded. Two peaks corresponding to the E2g (the in-plane vibration mode) and the A1g (out-of-plane vibration mode) were observed for the MoS₂ Nanosheets. These peaks were observed for the as-prepared B1/B2/B3-MoS₂ NSs at 381 cm⁻¹, 384 cm⁻¹, 382 cm⁻¹ (for E2g) and 406.8 cm⁻¹, 408.7 cm⁻¹, 406 cm⁻¹ (A1g), respectively, as shown in Figure 5B. These values correspond to the characteristic peaks of MoS₂. Moreover, based on the Raman spectra, the reduction in bulk MoS₂ to NSs clearly shows that the distance between two peaks E2g and A1g is shorter than bulk MoS₂ [32]. The energy difference between the two peaks is in the range $\sim 24.2-25.8$ cm⁻¹ which corresponds to the presence of 5–6 layers in the synthesized $MoS_2 NSs$ [20,33]. Lee et al. worked on the weakness of the van der Waals interlayers interaction and how these forces can affect the bonding and the lattice vibrations of few layered MoS₂ [33]. Compared to graphene, MoS₂ presents highly stable layers that do not present complexities associated to the non-adiabatic electron-phonon coupling as it is in the case of graphene. Further, we compared our results with the existing ultra-sonication methods. As shown in Table S1, most of the ultra-sonication assisted methods extensively utilize toxic organic solvents, such as NMP/Isopropanol (IPA)/ethanol, for the exfoliation of bulk MoS₂ to MoS₂ NSs. In some other reports, even though offering a greener pathway, longer exfoliation times and lower yields are still considered as limiting factors. Consequently, the present Tandem process method can significantly overcome the mentioned limitations and produce high yield water soluble MoS₂ NSs.



Figure 5. (A) XRD analysis and (B) Raman spectra of synthesized MoS₂ NSs and bulk MoS₂.

2.4. Peroxidase Mimetic Properties of MoS₂ NSs

Various nanomaterials were successfully explored in environmental and biomedical applications [34–36]. Recently, more research was focused on nano-enzymes—particularly enzyme peroxidase mimicking properties-due to its great promise in biomedical applications, such as excellent substrate specificity and superior catalytic power. However, unfortunately, low sensitivity, low operational stability, and high costs of catalyst preparation and purifications limit their practical usage [37,38]. Therefore, herein we examined the enzyme peroxidase-like activities of present green and water soluble B1-MoS₂ NSs by catalytic oxidation of TMB to oxidized TMB (ox-TMB) in the presence and absence of H_2O_2 at room temperature. TMB is a chromogenic peroxidase probe which can react with \cdot OH radicals to form its oxidation product during the enzymatic degradation of H₂O₂ [39]. Further, ox-TMB is blue, and was monitored by measuring the absorbance at 652 nm. The detailed schematic illustration of peroxidase activity on MoS₂ NSs is shown in Figure 6A. As shown in Figure 6B, the buffer + TMB + H_2O_2 + MoS_2 NSs group exhibited excellent peroxidase properties and resulted in the radical enhancement detected by monitoring the absorption peak of ox-TMB at 652 nm. In contrast, other groups, such as buffer + TMB, buffer + TMB+ $MoS_2 NSs$, buffer + H_2O_2 + $MoS_2 NSs$, did not show significant absorption enhancement. Notably, in the buffer + TMB + H_2O_2 group, a small absorption enhancement was observed that might be due to the slight decomposition of H₂O₂ at room temperature. Subsequently, we also measured the time dependent peroxidase activity of MoS₂ NSs at standard condition (buffer + TMB + H_2O_2 + MoS₂ NSs). Figure 6C shows that the absorption of oxidized TMB (ox-TMB) at 652 nm gradually increased with prolonged reaction time. The excellent peroxidase activity can be visualized by the blue color. As shown in Figure 6D, the buffer + TMB + H₂O₂ + MoS₂ NSs group displayed bright blue color compared to other control groups, confirming the formation of ox-TMB product. Besides B1-MoS₂ NSs, we also evaluated the peroxidase activity of B2- and B3-MoS2 NSs at identical conditions (i.e., same concentration of NSs, 100 µL of MoS2 NSs (~500 μ g mL⁻¹)). Our results show that B2- and B3-MoS₂ NSs also exhibit an excellent peroxidase activity, similar to B1-MoS₂ NSs (Figure S5).



Figure 6. Peroxidase activity of B1-MoS₂ NSs. (**A**) Schematic representation of peroxidase mimetic activity. (**B**) TMB oxidation absorption spectra at various conditions. (**C**) Time dependent peroxidase activity of B1-MoS₂ NSs. (**D**) Color visualization of TMB oxidized product on peroxidase mimic B1-MoS₂ NSs.

2.5. Peroxidase Activity Mechanism

To further understand the peroxidase activity mechanism of the present MoS₂ NSs in the oxidation of TMB to ox-TMB in the presence of H_2O_2 , we carried out some mechanistic controls. One possible mechanism for the peroxidase-like property of the MoS_2 NSs might be its ability to decompose H_2O_2 species to produce hydroxyl radicals (-OH). To further confirm the generated -OH, here we used Terephthalic acid (TA) assay [40]. As shown in Figure 7A, TA can react with -OH and produce highly fluorescent 2-hydroxy terephthalic acid (2-TAOH), which shows an emission intensity around 430 nm after excitation at 315 nm. As shown in Figure 7B, the emission intensity increased in the presence of TA + H_2O_2 + MoS_2 NSs; whereas other control groups, such as TA alone and TA + MoS_2 NSs, did not exhibit any significant enhancement in emission intensity. These results clearly indicate that the generation of hydroxyl radicals in the presence of MoS₂ NSs are key species to decompose the H_2O_2 to water and oxygen. Notably, the TA + H_2O_2 group also shows a small enhancement in the emission intensity. This can be attributed to the H_2O_2 decomposition at room temperature, which is well aligned with our TMB oxidation assay results. Overall, from TMB oxidation and terephthalic acid assay results, we strongly believe that the present synthesized water soluble MoS₂ NSs exhibits an excellent peroxidase enzyme, mimicking by itself without coupling it with other co-catalyst as is the actual case. Thus, present MoS_2 NSs can be useful to detect environmentally toxic chemicals, such as H_2O_2 , via naked eye detection, as well as in biomedical cancer diagnostic applications.



Figure 7. (A) reaction scheme of Terephthalic acid assay. (B) Photoluminescence spectra of Terephthalic acid assay at various conditions.

3. Materials and Methods

3.1. Material

MoS₂ powder was purchased from Sigma-Aldrich (powder, <2 μ m, 98%, St. Louis, MO, USA). PAA, Poly (acrylic acid), 35% H₂O₂ aqueous solution were purchased from SHOWA chemical (Tokyo, Japan) and 3,3',5,5'-Tetramethylbenzidine (TMB) and Terephthalic acid (TA) were obtained from Alfa Aesar (Haverhill, MA, USA). All chemicals were used without further purification.

3.2. Synthesis of MoS₂ NSs

Commercially available bulk MoS_2 with an average diameter of 2–3 µm (Sigma-Aldrich, St. Louis, MO, USA) was used as a starting precursor. To attain MoS_2 NSs with more biocompatible/water soluble, Poly (acrylic acid) (PAA) was added prior to the probe sonication. First, aqueous solution of bulk MoS_2 (300 mg) and PAA (100 mg) were subjected to probe sonication for a period of 3 h (QSONICA probe sonicator, 25 W, 31 amplitude). Subsequently, the solution was centrifuged (5000 rpm, 3 min), and the supernatant solution containing MoS_2 NSs and the residue were collected separately and noted as batch-1 MoS_2 NSs (B1- MoS_2 NSs) and residue 1 MoS_2 (R1- MoS_2), respectively. Successively, we added the appropriate amount of PAA to the R1- MoS_2 NSs in supernatant and the residue 2 MoS_2 (R2- MoS_2). Finally, R2- MoS_2 NSs were subjected to sonication for 3 h by adding PAA and

achieved a batch 3 MoS_2 NSs (B3- MoS_2 NSs). Overall, the present successive batch manner process significantly offered a high yield of MoS_2 NSs (100%) within three tandem cycles of probe sonication. Finally, samples were stored in water for further applications.

3.3. Characterization

The morphology and elemental analysis of synthesized MoS₂ NSs was examined by using HR-TEM and Elemental Dispersive Spectroscopy (EDS) (JEOL JEM-2100, 200 kV, Tokyo, Japan), respectively. MoS₂ NSs sample droplets were dried on a Lacey carbon grid for TEM analysis. A UV–visible-NIR analysis of MoS₂ NSs aqueous solution was carried out by using a UV-vis-NIR spectrophotometer (JASCO, Tokyo, Japan). DLS Nanotrac Wave (Microtrac Inc., Philadelphia, PA, USA) was used for particle size analysis and zeta potentials of MoS₂ NSs. Raman spectra were measured by using micro-Raman spectrometer (HORIBA, LabRAM, HR800, Kyoto, Japan). FT-IR data were recorded on a Bruker Tensor 27 FT-IR (DTGS detector and KBR beam splitter, Bruker, Vertex 80v and Tensor 27, Billerica, MA, USA).

3.4. Enzyme Peroxidase Activity Measurement

Enzyme peroxide mimetic properties of MoS_2 NSs were evaluated by using TMB assay. Briefly, 50 µL of 50 mM H₂O₂ and 100 µL of 50 mM TMB were mixed in 3.0 mL of sodium acetate buffer (0.2 M, pH 4). To this solution, 100 µL of MoS_2 NSs (500 µg mL⁻¹) solution were added and incubated for 30 min at room temperature. Afterwards, samples were centrifuged (10,000 rpm, 5 min) and measured the absorbance at 652 nm by using UV-visible spectrophotometer. In a similar way control experiments were carried out such as buffer + TMB, buffer + TMB+ MoS_2 NSs, buffer + H_2O_2 + MoS_2 NSs.

3.5. Identification of Hydroxyl Radicals (•OH)

To further identify the -OH formation on MoS₂ NSs, TA assay was performed. Briefly, to the buffer solution (pH = 5), 50 mM H₂O₂, 1 mM TA and 100 μ L of MoS₂ NSs (500 μ g mL⁻¹) added. Further, the reaction mixture was incubated for 1 h at room temperature and then after the catalyst was centrifuged. The final solutions were subjected to the fluorescence measurements at excitation 315 nm.

4. Conclusions

In summary, high yield water soluble $MoS_2 NSs$ were successfully synthesized from bulk MoS_2 material by using a simple probe sonication method. The sequential batch/tandem process could significantly improve the yield of $MoS_2 NSs$ and reach up to 100% in a green manner. Subsequently, synthesized B1/B2/B3-MoS₂ NSs were well characterized. Moreover, the optical properties of present synthesized $MoS_2 NSs$ still remains the same as the $MoS_2 NSs$, which were synthesized by reported harsh condition methods. Thus, it is a clearly evident that the present method is a good and green alternative for the existing low yield and non-green methods. Furthermore, $MoS_2 NSs$ exhibited excellent intrinsic peroxidase mimetic enzyme activities at ambient conditions. Mechanistic studies revealed that the $MoS_2 NSs$ playing a key role in generation of hydroxyl radicals by decomposing the H_2O_2 efficiently. Overall, we strongly envision that the present cost effective, green and eco-friendly approach is able to seize significant attention to fabricate a high yield synthesis of metal chalcogenide nanosheets for the future energy, environment and biomedical applications.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4344/10/9/1009/s1, Figure S1: Energy dispersive spectroscopy (EDS) elemental analysis of B1-MoS₂ NSs, Figure S2: DLS size distribution analysis of bulk, R1 and R2-MoS₂, Figure S3: FTIR spectra of bulk MoS₂ and as synthesized B1, B2 and B3-MoS₂ NSs, Figure S4: XRD spectra of as synthesized B1, B2 and B3-MoS₂ NSs, Figure S5: TMB oxidation assay for evaluating the peroxidase activity of B2 and B3-MoS₂ NSs at identical conditions and Table S1: Literature summary of methods for synthesizing the water soluble MoS₂ nanosheets via sonication assisted methods by using bulk MoS₂.

Author Contributions: S.T. and S.R. designed the experiments. S.T. and M.T.L. performed the experiments and analyzed the results. S.T. wrote the first draft of the manuscript. S.R. reviewed the results and final draft. For the final version, all the authors have given their approval. All authors have read and agreed to the published version of the manuscript.

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