SRI VENKATESWARA INTERNSHIP PROGRAM FOR RESEARCH IN ACADEMICS (SRI-VIPRA)

Project Report of 2022: SVP-2223

"Biomedical Applications of Functionalised MoS₂"

An Overview



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SRIVIPRA PROJECT 2022

Title : Biomedical Applications of Functionalised MoS₂

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Certificate

This is to certify that the aforementioned students from Sri Venkateswara College have participated in the summer project SVP-2223 titled "**Biomedical Applications of Functionalised MoS**₂".The participants have carried out the research project work under my guidance and supervision from ...21st June 2022 to 25th September 2022.

The work carried out is original and carried out in an online mode.

Signature of Mentor Dr Lalita Josyula

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• Introduction

Until now, the most well-known of two-dimensional (2D) nanomaterials, graphene, and graphene derivatives have received great deal of attention due to their fascinating and extraordinary properties. Since 2004, graphene has been studied extensively; it's nano-structures prepared, characterized and successfully applied in electronics, sensors, and energy devices.



However, it is well known that graphene has some shortcomings, such as zero energy band gap, intrinsic defects, chemical inertness, and so on, making it less appealing in some applications. In recent years, a newly emerging kind of 2D nanomaterial, two-dimensional transition metal dichalcogenides (2D TMDCs) is

garnering a lot of attention. The generalized formula is MX2, where M represents a transition metal and X represents chalcogen. M comprises transition metals from the IVB to VIIB group, including Ti, V, Cr, Mn, Zr, Nb, Mo, Tc, Hf, Ta, W, and Re; X represents the chalcogenide elements, sulfur, selenium, and tellurium of the sulfur group. The metal coordination of 2D TMDCs is generally either trigonal prismatic or octahedral.

The synthesis method is gradually gaining perfection. Currently, the synthesis methods could be divided into two categories, top-down and bottom-up methods.



(Picture showing Top-Down & Bottom up)

2D TMDCs synthesized through top-down methods are mainly used in the biomedical field, while 2D TMDCs prepared through bottom-up methods are mostly applied to photoelectric devices and the catalysis field.



Transition metal dichalcogenides (TMDCs) are an interesting family of 2D materials with an X-M-X layered structure where a plane (M) of a transition metal element (e.g., Ti, Hr, V, Ta, Mo, W, etc.) is sandwiched by two hexagonal planes (X) of a chalcogenide (S, Se, or Te). Three-atom-thin single-layers of MX2 exhibit distinctly different properties from their bulk counterparts.



Particularly, a semiconducting analog of graphene, molybdenum disulfide (MoS₂), has attracted huge attention in the last few years because of its excellent nanoelectronics, optoelectronics, and energy harvesting properties.

For example, single-layer MoS_2 exhibits a direct bandgap (~1.8 eV) and acceptable mobility for transistor applications (~200 cm² V⁻¹s⁻¹). And single-layer MoS2-based field effect transistor (FET) can exhibit a high current on/off ratio exceeding 1 x 10⁸ at room temperature, which is thus expected to be a potential candidate for electronic applications. The direct bandgap also results in photoluminescence from the MoS₂ monolayer, which provides novel opportunities for optoelectronic applications. Compared to their applications in energy storage and optoelectronic devices, the use of MoS₂ nanosheets as a sensing platform, especially for biosensing, is still largely unexplored.

Structure and Properties

 MoS_2 structures differ from 3D, 2D, one-dimensional (1D), or dot structures. Their characteristics and applications also change from one dimension to another, they can be semiconducting, metallic, or superconducting. They exist in several layers and shapes. The bulk (3D) structure can be tri-agonal (T), hexagonal (H), and Rhombohedral (R), where 2H MoS_2 means 2-layer hexagonal shape MoS_2 . The three main structures are 1T, 2H, and Crystals 2021, 11, 355 3 of 24 3R, where the 1T phase coordinates in an octahedral structure, 2H and 3R in a trigonal prismatic structure. The 1T structure is known to be metallic while the other two are semiconducting. The monolayer of hexagonal MoS_2 is also semiconducting. Both 2H and 3R are used as dry lubricants. Due to the nonlinear optical properties of the 3R phase, it is used in nonlinear optical mass sensing in quantum measurements and biomedicine. As an example, for gas sensors, the different phase materials of MoS_2 can be interesting in obtaining high sensitivity and rapid desorption.

 MoS_2 exists in different 2D structures like nanosheets, nanoribbons, 1D structures as nanowires and nanotubes, or 0D structures as quantum dots and nanoplatelets. The thickness of 2D nanoribbons was found to be of 1 to 3 layers of MoS_2 , while the thickness of 1D nanowires (NW) can have lengths from 14 to 30 nm and a width of 0.6 nm approximately. The structure of 1D nanoplatelets and their properties were investigated. The nanoplatelets are 12–30 nm with one-unit cell width. They have very high catalytic activity for hydrodesulphurization. The quantum dots range from 2 to 10 nm in size. They have a higher band gap than nanosheets and stronger bonds between Mo atoms than monolayers. The change in the band gap of MoS_2 from one dimension to another changes the photoluminescence characteristics and thus has different optical properties according to its dimension. Additionally, monolayers or other low-dimension forms are also easy to implement in optical nanostructures to enhance the photoluminescence intensities and emission rates through light-matter interactions. This is a strong motivation for MoS_2 to be included in optical applications

The absorption coefficient and refractive index are the parameters that determine the response of a material when a certain wavelength passes through it. The absorption coefficient determines the distance the spectrum pass inside the material before being absorbed. A high absorption coefficient means high attenuation to the wave applied. Semiconductors have high absorption coefficients for short wavelengths (high energy and frequency spectrum) and low absorption coefficients for long wavelengths (they do not have enough energy to excite electrons from the valence band to the conduction band). MoS_2 has a relatively large absorption coefficient for the wavelengths from 400 nm to 500 nm with a sharp decay at 500 nm.

The key factor behind the wide use of MoS_2 in optoelectronics is its tunable bandgap that changes with size and structure; Different bandgaps mean tunable photoresponsivity (R), specific detectivity, and response time, and thus, a wide range of applications. The MoS_2 multilayers and monolayers have a high refractive index of more than 2, where it can be used in the coating. Since the photoluminescence (PL) spectra are affected by the band gap, doping, and structure of the material, MoS_2 has different PL activity. It has a peak exciton in a single layer MoS_2 . The PL properties of monolayer MoS_2 are enhanced by adding an H_2O_2 solution, where it acts as a strong oxidizer without changing the crystalline structure of MoS_2 .

TMDs are known for their low PL quantum yield (QY) which is the ratio of the number of emitted photons to the number of generated electron-hole pairs and they are between 0.01 to 6%. The work in was able to raise the QY of MoS_2 to 95% using a chemical treatment of an organic superacid. The observed lifetime of MoS_2 carriers was nearly 10.8 ns, which opens the way to be used in high-performance lasers and solar cells.





A monolayer MoS_2 has high strength, less than that of graphene, and good elasticity similar to that of graphene oxide, with Young's modulus of 0.33 ± 0.07 TPa. A single layer of MoS_2 has more flexibility than bulk structures, where its Young's modulus is 0.24 TPa. Unlike other semiconductors, the flexibility of MoS_2 prevents the deformation and band gap shifts that may happen to its crystalline structure when subjected to strain. However, the mechanical strain is used to alter MoS_2 electronic characteristics, and trans- Crystals 2021, 11, 355 5 of 24 form them from semiconductors to metals. It transforms the direct band gap of MoS_2 monolayers into an indirect one and high strain values can cause structure deformation and transform MoS_2 into metal.

Multilayer MoS_2 is known to have an indirect band gap of 1.2 eV, which increases with the decrease in the number of layers until we have a direct band gap of 1.8 eV in monolayer MoS_2 . Although the MoS_2 bandgap value is good, it is still far from the 1.12 eV direct bandgap of Silicon. The mechanical strain affects the band gap of MoS_2 and changes it from a direct to indirect band gap and transfers the material from a semiconducting material to a metallic one. The 4d and 3p orbitals in Mo and S respectively determine the properties of MoS_2 . The projected density of states (PDOS) of bulk and monolayer MoS_2 are nearly the same, but there are some peaks in PDOS in the case of monolayer MoS_2 . A monolayer MoS_2 changes to an n-type semiconductor when doped with chromium, copper, and scandium (Sc) and to a p-type when doped with Nickel or Zinc. Doping with Titanium (Ti) transfers MoS_2 to a p-type or n-type semiconductor according to the levels and sites of doping.

TMDs are known to be non-magnetic, and if we managed to add magnetism to them, they can be used as tunable semiconductors. A study studied the magnetic behavior and characteristics of multilayer MoS₂ specifically. The study showed that

 MoS_2 has a long spin diffusion length of 235 nm and that an in-plane spin polarization can suppress electron spin-relaxation. The work showed that MoS_2 attains semi-metallic ferromagnetic properties when doped with Sc and a unity spin polarization value, which is favorable in spintronics.

• Processing and Preparation

MoS₂, a transition metal dichalcogenide (TMDC) material, possesses great potential in biomedical applications such as chemical/biological sensing, drug/gene delivery, bioimaging, phototherapy, and so on. Particularly, monolayer MoS₂ has more extensive applications due to its fantastic physical and chemical properties such as ultra-high surface area, easy modification, and high biodegradability. It is of great significance to prepare advanced monolayer MoS_2 with enhanced energy exchange efficiency (EEE) for the development of MoS₂based nanodevices and therapeutic strategies. In this work, monolayer MoS₂ film was firstly synthesized through the chemical vapor deposition (CVD) method, and the surface of MoS₂ was further modified via the baking process to develop p-type doping of monolayer MoS₂ with high EEE. After the baking process, the surface of monolayer MoS₂ was transferred to p-type doping, followed by confirmation by Xray photoelectron spectroscopy (XPS) and Raman spectra analysis. The morphology, surface roughness, and layer thickness of monolayer MoS₂ before and after baking were thoroughly investigated using atomic force microscopy (AFM). The results showed that the surface roughness and layer thickness of monolayer MoS₂ modified by baking were increased in comparison to those of MoS_2 without baking, indicating the higher energy conversion efficiency of p-type doping. Moreover, a photoluminescence (PL) spectrum study revealed that p-type doping of monolayer MoS₂ displayed much stronger photoluminescence ability which was taken as evidence of higher photothermal conversion efficiency. This study not only developed a novel MoS₂ with high EEE for future biomedical applications but also demonstrated that the baking process is a promising way to modify the surface of monolayer MoS₂.

At present, a variety of preparation methods including mechanical exfoliation, liquid phase exfoliation, chemical exfoliation, chemical vapor deposition, and solvothermal synthesis, have been developed to synthesize 2D TMDCs with single or few layers. These methods can be divided into two categories: top-down (get layered nanomaterials from bulk crystals through different exfoliation ways) and bottom-up approaches (use atoms or molecules as precursors to grow into layered nanomaterials under special conditions).

• Top-Down Synthesis

• Mechanical Cleavage

Mechanical cleavage is the most typical top-down method. In the mechanical cleavage process, the adhesive force of scotch tape is used to obtain monolayer or few-layer structures from bulk crystals. To date, many kinds of ultrathin 2D TMDCs have been synthesized under the mechanical cleavage method. Mechanically exfoliated ultrathin 2D TMDCs are equipped with personal advantages and disadvantages. Ultrathin 2D TMDCs prepared through this method are highly crystalline nanosheets with large sizes and few defects, which are suitable for electronic devices and fundamental studies of intrinsic physicochemical properties. However, the production rate is low, and the size and thickness are hard to control. The substrate is needed to support the nanosheet. The 2D TMDCs prepared by this method are difficult to meet the needs of biomedicine.

Liquid Exfoliation

Liquid exfoliation is another typical top-down method. Liquid exfoliation could realize successful exfoliation of bulk crystals via ultrasonication in a specific solvent. By sonicating, the weak van der Waals interaction but not strong covalent bonds in-plane could be broken down. Therefore, proper ultrasonic intensity and ultrasonic time are critical to realizing the successful exfoliation of bulk crystals. The main function of solvent molecules is to stabilize exfoliated nanosheets and inhibit their reassembly. The solvent molecules with appropriate surface energy bind to the surface of nanosheets via van der Waals interaction. Hence the matching degree of surface free energy between solvent molecules and nanosheets is very important to improve the exfoliation efficiency. At present, the common solvents are mainly organics, such as dimethylformamide (DMF) and N-methyl-pyrrolidone (NMP). To date, multiple ultrathin 2D TMDCs have been synthesized through liquid exfoliation, such as MoS₂, WS2, NbSe2, TaSe2, and NiTe2. Liquid exfoliation makes up for some deficiencies of mechanical cleavage, realizing the large-scale preparation of ultrathin 2D TMDCs with good photoelectric properties. However, the organic solvents used in the liquid exfoliation process are undesirable in the following applications, and it is difficult to produce single-layer 2D TMDCs through this method. Therefore, it is necessary to further improve the experimental conditions for the large-scale synthesis of monolayer 2D TMDCs in a non-toxic solvent.



• Chemical Exfoliation

The chemical exfoliation method is to insert intercalators into the interlayer of the bulk crystals with the help of ultrasonication in water, realizing the successful exfoliation of bulk crystals. The most common intercalators are organometallic compounds, such as butyl lithium, naphthyl sodium, etc. During the synthesis process, intercalators are firstly intercalated into interlayers of bulk TMDCs in water or ethanol. Then the bulk TMDCs are exfoliated into ultrathin nanosheets under sonication. Now the insertion of intercalators into bulk TMDCs has been realized in battery, and the amount of intercalators was regulated by controlling the voltage. Chemical exfoliation has been used to prepare a variety of ultrathin 2D TMDCs without the use of toxic organic solvents in the synthesis process. This method could meet the needs of the biomedical applications of ultrathin 2D TMDCs.

• Bottom-Up Synthesis

• Chemical Vapor Deposition

Chemical vapor deposition is a typical bottom-up method. The reaction process is to expose the reaction precursor to the substrate under high temperature and pressure. The role of reaction precursors is to provide transition metal atoms and chalcogenide atoms, respectively, and react to generate ultrathin 2D TMDCs. Finally, the reaction product was deposited on the substrate, thus the ultrathin 2D TMDCs were obtained. Ultrathin 2D TMDCs

nanosheets prepared through this method possess excellent electronic properties and high crystal quality. However, high vacuum and high temperature are necessary for the synthesis process. And the use of substrate increases the transfer process of nanosheets.



• Solvo-Thermal Synthesis

Solvo-thermal synthesis is another bottom-up method. By the solvothermal method, ultrathin 2D TMDCs could be obtained from precursors under a specific solvent and specific reaction time. The results show that after the reaction of molybdic acid or tungstic acid with thiourea at 773K for 3 h, the ultrathin MoS₂ or WS2 nanosheets can be prepared. The strength of this method is that it could realize the high-yield preparation of ultrathin 2D TMDCs at a lower cost. Hence this method will be promising for the industrial application of 2D TMDCs. The shortage of solvothermal synthesis is that single-layer nanosheet is difficult to be obtained.

• Applications

2D MoS_2 shows remarkable properties that make it advantageous for biosensing applications. When grown into planes with relatively large lateral dimensions, 2D MoS_2 planes are ended onto basal surfaces with no dangling bonds. As a result, these large planes are particularly stable in liquid and oxygen-containing gaseous media, which facilitates their efficient incorporation into biosensing structures. In nanoflake morphology, when the surface-to-thickness ratio is reduced, 2D MoS_2 edges and corners can be engineered to either molybdenum or sulfur terminations. The molybdenum termination gives the opportunity to possibly use the metallic properties of these sites when required.



Similar to graphene and other 2D materials, 2D MoS₂ offers large surface areas that enhance its biosensing performance. Due to the existence of a suitable bandgap, the overall sensitivity of devices based on 2D MoS₂ is much larger than graphene and graphene oxides which have either no or small bandgap. Many stoichiometric 2D oxides in comparison, have large bandgaps that require relatively high applied energy for their electronic band structure modulation.





As 2D MoS₂ is atomically thin, upon interactions with a target biomaterial, its whole thickness is affected. The surface properties of 2D MoS₂ are very different from those of graphene and its oxides. MoS₂ surface energy is unique and is a strong function of its sulfide deficiency. Functionalization of basal surfaces and sulfurended edges of 2D MoS₂ can be challenging.

2D MoS₂ electronic band structure characteristics give rise to certain excitonic and fluorescent properties. 2D MoS₂ PL spectrum shows two exciton peaks of A (~1.92 eV) and B (~2.08 eV) at the K point. These peaks are suggested to appear due to valence band spin-orbit splitting by ~145 meV. Two extra exciton peaks of C and D also exist. The introduction of excess electrons causes a split of the exciton A peak, which is associated with the emergence of negative trions of high binding energies. 2D MoS₂ shows strong vibrational characteristics. This material has four Raman-active modes (E1g, E12g, A1g, and E22g). It also demonstrates two IR-active modes (A2u and E1u). E12g is an in-plane mode, attributed to the opposite vibration of two S atoms concerning Mo, and A1g mode is in an out-of-plane vibration of only S atoms in opposite directions.

Reducing the number of layers results in the red and blue shifts of E12g and A1g modes, respectively. Due to its out-of-plane nature, the A1g mode is susceptible to adsorbates on the MoS_2 surface and charge exchanges. The Raman and IR active modes are also functions of lateral dimensions, the permittivity of the environment, defects, and intercalation properties.



Functionalized MoS₂ can efficiently be used for protein surface recognition. Negative MoS₂ recognizes the positively charged protein, ChT, and positive MoS₂ inhibits the enzymatic activity of the negatively charged protein, β -gal. Neutral MoS₂ does not interact with any proteins, but zwitterionic MoS₂, albeit overall neutral, inhibits ChT and enhances the activity in β -gal. Nonfunctionalized ce-MoS₂ is not suitable for biological applications as it does not effectively interact with the protein at a relevant concentration as well as not stable in biological media. The mode of inhibition reveals that both proteins are inhibited in a non-competitive manner.

By decorating MoS_2 nanosheets with hyaluronic acid (HA), these functionalized MoS_2 nanosheets have been developed as a tumor-targeting chemotherapeutic nanocarrier for near-infrared (NIR) photothermal-triggered drug delivery, facilitating the combination of chemotherapy and photothermal therapy into one system for cancer therapy.

The nanocomposites (MoS₂-SS-HA) generated a uniform diameter (ca. 125 nm), exhibited great biocompatibility as well as high stability in physiological solutions, and could be loaded with the insoluble anti-cancer drug erlotinib (Er). The release of Er was greatly accelerated under near-infrared laser (NIR) irradiation, showing that the composites can be used as responsive systems, with Er release controllable through NIR irradiation. MTT assays and confocal imaging results showed that the MoS₂-based nanoplatform could selectively target and kill CD44-positive lung cancer cells, especially drug-resistant cells (A549 and H1975).

In vivo tumor ablation studies prove a better synergistic therapeutic effect of the joint treatment, compared with either chemotherapy or photothermal therapy alone.

Functionalized $2H-MoS_2$ exhibits highly enhanced antibacterial efficiency compared to similarly functionalized metallic $1T-MoS_2$ against pathogenic bacteria. The newly synthesized functionalized $2H-MoS_2$ exhibits better hemocompatibility, which makes it suitable for in vivo applications.

Electrochemical sensing is being studied recently for ultrasensitive sensing, and MoS_2 shows sensitive electrochemical detection properties when combined with other materials where a notable change in the electrochemical impedance occurs. This technique was used in to detect food pathogens

Based on the fluorescence and quenching properties of MoS_2 , it can be used in biosensing to detect DNA, cancer biomarkers, and different amino acids. FET-based sensors are also used for biomolecular sensors like DNA, antigens, proteins, biotin, and pH values. Optical sensors are mainly based on the PL characteristics and fluorescence quenching and recovery properties of MoS_2 . It can be used in detecting DNA, H_2O_2 , and ion sensors.

Future and Challenges

In recent few years, MoS₂ nanosheets with large surface area, intriguing optical properties, and easily functionalized surface, have attracted a lot of interest in biomedical applications.

In this review, the recent progress of MoS₂ nanosheets in synthesis and modification methodologies, biomedical applications including detection, bioimaging, PTT, drug delivery, and theragnostic, as well as toxicity evaluation have been summarized and discussed.

However, for future clinical applications of MoS_2 nanosheets, there are still tremendous challenges ahead. The synthesis and modification methods still need to be optimized. The preparation of MoS_2 nanosheets with specific thickness, lateral size, and crystal phase is urgently needed for evaluating their corresponding functionalities and toxicity profiles. Besides, the reliable scale-up production of MoS_2 nanosheets is vital for their practical applications. It would also be interesting to fabricate element-doped MoS_2 or other ternary TMDC nanosheets, trying to figure out mutual influence between elements and more intriguing properties. Moreover, modification of MoS_2 nanosheets with specific groups to render them targeting ability or stimulate-responsive capability is still demanded for biomedical diagnosis and therapy.

For in vitro and ex vivo biosensing applications, although there have been many papers utilizing MoS_2 nanosheets for the detection of biomolecules and even clinical carcinoma tissues, several critical issues remain. More efforts are needed to excavate and clarify the unique superiorities of MoS_2 nanosheets, in addition to their large surface area and special physiochemical properties, for biosensing applications over conventional nanomaterials. More attention should also be paid for the verification of the repeatability and reliability of MoS_2 nanosheet-based biosensors.

For in vivo imaging or therapeutic applications of MoS₂ nanosheets, their biological behaviors and toxicity evaluation are extremely important fundamental questions. There are several preliminary studies to claim that MoS₂ nanostructures, especially with appropriate surface functionalization, are not notable toxic in vitro and in vivo.

Our recent studies also show that compared to other types of widely explored TMDCs, MoS_2 nanostructures are unique in terms of their biodegradation behaviors and relatively fast excretion, which could be further accelerated for ultrasmall MoS_2 nanodots. However, more in-depth systematic investigations are still demanded to further evaluate the toxicology profiles of MoS_2 nanostructures with different sizes and surface coatings, particularly in larger animal models, before their possible clinical translation.

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