

Fabrication of Biodegradable Nanosensor

This chapter explores the growing importance of biodegradable nanosensor, focusing on their potential to address environmental sustainability while advancing sensor technologies. It highlights the development of a low cost fabrication of nanosensors designed for real time uric acid detection and trace level hydrogen detection. This approach combines eco-friendly materials (such as commercial paper and natural polymer based substrate) with advanced nanostructures to achieve high sensitivity and selectivity presenting a significant step forward in developing sustainable solutions for modern sensing challenges.

4.1 DEVELOP PAPER BASED NANOSENSOR FOR REALTIME URIC ACID DETECTION

This work reports the development of an inexpensive real-time sensing module for uric acid detection on simple, disposable paper substrate. Detection is based on a capacitive measurement system using functional ZnO hexagonal rods on pulse-electrodeposited Cu interdigitated electrodes (IDEs) over hydrophobic A4 paper. Further, an Arduino IDE software is used to configure the Arduino Mega Board to evaluate the change in capacitance value and to display the corresponding uric acid concentration on Liquid Crystal Display (LCD) screen. The results indicate that the developed capacitance measurement unit can be employed for the early screening of uric acid in real samples for clinical applications.

4.1.1 Introduction

Uric acid is a primary nitrogen-containing compound that is formed as a byproduct of the metabolic conversion of purines (Qiangwei Wang et al., 2019). This conversion takes place mostly in the liver, but also the muscle and the gut. Because of this, it is of the utmost importance to monitor the uric acid concentration that is excreted in human serum and urine. This is due to the fact that an increased level of uric acid in blood serum can lead to a variety of diseases, including atherosclerotic vascular disease, kidney disease, and peripheral artery disease (Chen et al., 2005). As a direct consequence of this, there is a much greater need to detect uric acid (Rashid et al., 2019). The unique bio-analyte system may be used in clinical and non-clinical labs to analyze data using biosensors. On the other hand, very few studies have been published on the development of uric acid sensor based on capacitance measuring biosensor (Dhiman et al., 2021). The capacitance measuring biosensor primarily monitors and analyses the variation in capacitance due to the introduction of an analyte, resulting in a shift in the dielectric characteristics that are determined as the sensing parameters.

Nanomaterials, with their excellent electrical, mechanical, optical, and catalytic capabilities, play a vital role in enhancing the efficiency of biosensor devices. Exceptional nanomaterials like Zinc oxide (ZnO) are used to further advance the development of biosensor devices by enhancing their biocompatibility, surface-to-volume ratio, mechanical flexibility, and charge carrier mobility. Moreover, paper-based substrates are also environmentally friendly because of the self-degradation nature of paper, which helps reduce the amount of electronic waste produced (S. Kumari et al., 2023a, 2023b; Noviana et al., 2021; Villa and Poppi, 2016; Wang et al., 2018; Y. Zhang et al., 2020). Additionally, the A4 paper has a number of advantageous characteristics such as its inexpensive nature, high flexibility, and biodegradability. Various

micro-electromechanical systems based techniques can be used to develop electrodes on the flexible substrate, such as inkjet printing, sputtering techniques, and thermal evaporation systems. However, these techniques are very expensive and required clean room facilities. Whereas electrodeposition technique uses an electrical current to reduce the metal cations in an electrolyte and deposit a thin layer of metal over the conductive surface to alter the surface properties of the substrate (Rai and Gupta, 2021). Previously uric acid is detected using various techniques, Wahyani et al. discussed the development of an electrochemical biosensor via screen-printed electrodes on a polyvinyl chloride paper substrate (Wahyuni et al., 2021). In the range of 10-80 mM, the prepared biosensor demonstrated a good sensitivity of 5 nA/M with a detection limit of 1.94 mM. Similarly, Veerla et al. reported the fabrication of a uric acid biosensor for photoresponsive sensing via the pencil drawing method on cellulose paper (Veerla et al., 2017). The biosensor exhibits an excellent sensitivity of 1.99 m/ μ M in the range of 100 mM to 450 mM. In another research, Yao et al. presented a technique for fabricating electrochemical assays with screen-printed carbon electrodes on a paper substrate for uric acid detection (Yao and Zhang, 2016).

4.1.2 Experimental Section

Commercially available A4 paper is brought from the local market of Jodhpur, India. Chemical reagents such as zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2$), slygard 184 (PDMS elastomer), and curing agent (crosslinker), isopropanol alcohol (IPA), n-heptane (C_7H_{16}), and ethanol ($\text{C}_2\text{H}_5\text{OH}$), hexamethylenetetramine (HMTA) and silver conducting paste with 200 nm (80%)-5 nm (20%) microparticles, were procured in AR grades from Sigma-Aldrich. For the electrodeposition of copper (Cu) IDEs, copper sulphate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), and sulfuric acid (H_2SO_4), were brought from Otto chemicals.

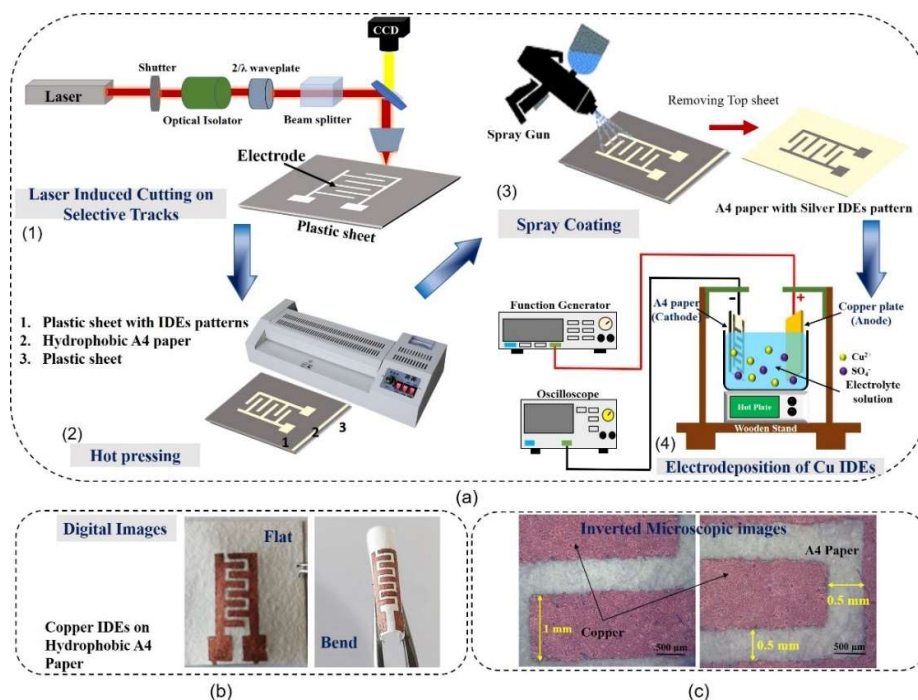


Figure 4.1: (a) Schematic representation of various steps involved in the development of Cu IDEs on hydrophobic A4 paper and (b) digital photograph of the Cu IDEs deposited on A4 paper (c) magnified images of the Cu IDEs using an inverted microscope

(a) Preparation of hydrophobic A4 paper

The hydrophobic coating is prepared on the A4 paper using the following method; Typically, slygard 184 and curing agent (crosslinker) 10:1 respectively, were added to a 50 mL C_7H_{16} solution. The solution was vigorously stirred for 20 min at ambient temperature. Following

that, the 6 cm x 6 cm A4 paper was immersed in the solution for 2 min before being removed vertically to drain the excess liquid. Finally, the coated A4 paper was cured in an electric convection oven at 100°C for 15 min and repeated two times in order to achieve the hydrophobic layer over A4 paper.

(b) Preparation of Copper Interdigitated Electrodes (IDEs)

The shape of the IDEs was laser cut on plastic sheets and then hot-pressed onto hydrophobic A4 paper. To make the hydrophobic A4 paper conductive, silver conducting paste was diluted in IPA solution and sprayed coated over the IDEs shaped structure present on plastic sheets (see Figure 4.1 (a)). Copper was electrodeposited onto A4 paper using a two-electrode in-house pulse electrodeposition setup with a polished copper (30 mm x 10 mm x 1 mm) substrate as anode and hydrophobic A4 paper substrate as a cathode. The setup contains a function generator (Siglent), an oscilloscope (Rigol), a hot plate and magnetic stirrer (Cole-Parmer, 30-550°C, 0-1100 rpm) electrodes, and an electrolyte solution. The electrolyte solution was made by mixing 0.8 M $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 100 mL DI water, and 0.1 M H_2SO_4 was used for controlling the pH and conductivity of the electrolyte. The electrolyte was magnetically stirred at 300 rpm. The bath temperature during the electrodeposition was maintained at 40°C. The mechanism of electrodeposition involves the reduction of metal ions in a solution onto a conductive surface, which is connected to the negative terminal of an external power source. When a direct or pulsed current was passed through the solution, metal ions migrate towards the electrode and undergo reduction at the surface, resulting in the formation of a solid metal layer. The digital and inverted microscopic image of the Cu electrodeposited IDEs (with finger thickness: 1000 μm and spacing between fingers: 500 μm) is shown in Figure 4.1 (b, c).

(c) Synthesis of ZnO Hexagonal rods

The ZnO hexagonal rods were synthesized via a hydrothermal method using $\text{Zn}(\text{NO}_3)_2$, HMTA, acetone, and DI water. The synthesis begins by dissolving both $\text{Zn}(\text{NO}_3)_2$ and HMTA in 2:5 in DI water, followed by placing the solution in the electric oven for 10 hours at 93°C. After the reaction, the electric oven was turned off, and the solution was allowed to gradually cool to ambient temperature before being centrifuged at 6000 rpm for 10 mins. The precipitate was then washed with DI water and $\text{C}_2\text{H}_5\text{OH}$ before being dried to yield powder ZnO hexagonal rods. Moreover, the field emission scanning electron microscopy (FESEM) technique was used to examine the morphology and dimensions of a single ZnO hexagonal rod. After dissolving the hexagonal rods in IPA solution, they were drop coated in the A4 paper-based biosensor.

4.1.3 Results and Discussion

(a) Characterization of A4 paper and ZnO hexagonal rods

The FESEM images of uncoated and coated A4 paper are shown in Figure 4.2 (a, b). The image shows the 3-D porous structures made up of cellulose microfibrils on the uncoated A4 paper. On coating the A4 paper with a polymer (PDMS)/heptane solution, the hydrophobicity of the A4 paper increases. Due to its non-reactive nature, PDMS cannot directly bond to the cellulose microfibrils of paper substrate. The addition of heptane improves PDMS adhesion to the cellulose microfibrils. This can help to increase the paper's surface energy and promote PDMS coating adhesion. Figure 4.2 (b) depicts a FESEM image of A4 paper after PDMS curing, which fills the cellulose microfibrils structure and increases the contact angle, allowing for the development of Cu electrodes via electrodeposition. Furthermore, the uniform growth of ZnO hexagonal rods is shown in Figure 4.2 (c, d). Moreover, the overall surface is not smooth due to the presence of small agglomeration of nanoparticles. The ZnO hexagonal rods are formed as a result of a series of chemical reactions. HMTA hydrolyzes in water (in the presence of heat), producing ammonia (NH_3) and formaldehyde (HCHO). The released (NH_3) promotes the formation of hydroxyl ions OH^- and prevents a significant amount of Zn^{2+} from forming in solution. NH_3 was further subdivided into NH_4^+ and OH^- . When OH^- reacts with Zn^{2+} , the complex chemical $\text{Zn}(\text{OH})_4^{2-}$ is

formed. It dehydrates to produce hexagonal rods under reaction conditions, as illustrated by the following equations (4.1 to 4.6).

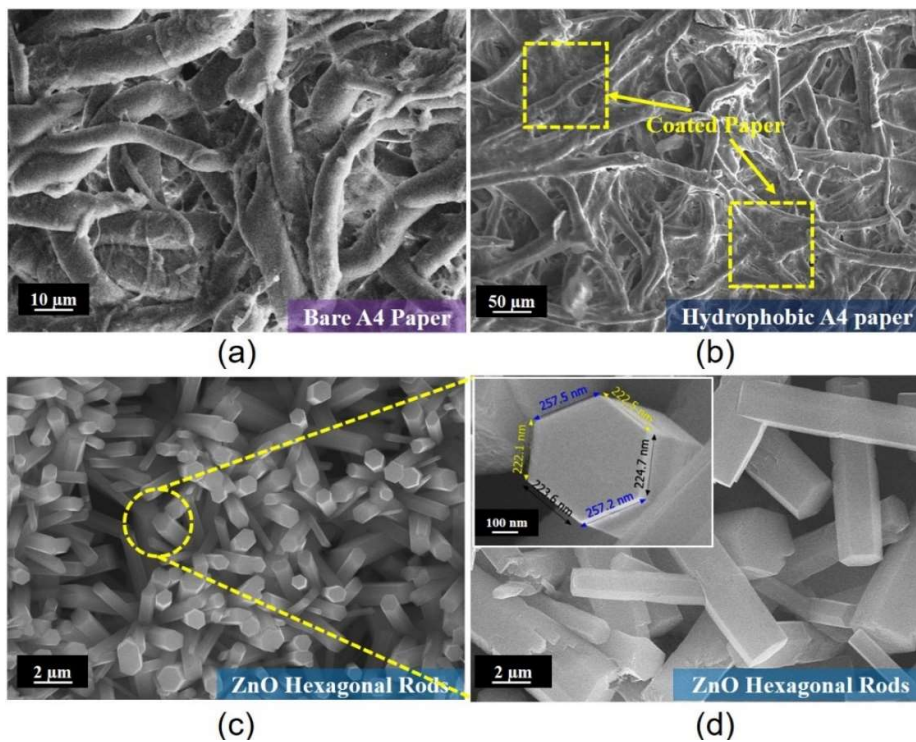
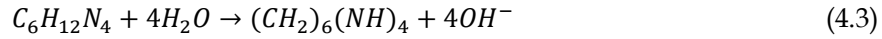
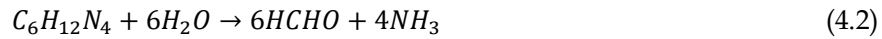
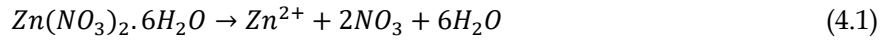


Figure 4.2: SEM images of the (a) uncoated and (b) coated A4 paper, (c, d) ZnO hexagonal rods with inset of single ZnO hexagonal rods

The energy dispersive X-ray spectroscopy (EDS) analysis of the synthesized ZnO hexagonal rods is shown in Figure 4.3 (a). The result showed that the elemental composition of the ZnO hexagonal rods contains Zn and O ratios of 54.72 and 45.28. The XRD patterns of the prepared ZnO hexagonal rod sample are shown in Figure 3.3 (b). The sharp characteristic peaks were observed at $2\theta = 31.84^\circ, 34.44^\circ, 36.23^\circ, 47.52^\circ, 56.57^\circ, 62.86^\circ, 66.48^\circ,$ and 67.95° , corresponding to (100), (002), (101), (102), (110), (103), (200), and (112) crystal planes, respectively demonstrating a high level of relevance to the standard ICDD PDF No. 79-2205. Furthermore, XRD analysis shows no evidence of the existence of other impurities such as $\text{Zn}(\text{OH})_2$ and Zn. The characteristic peak at (101) has the maximum intensity, suggesting the favored growth direction of ZnO hexagonal rods. The Raman spectrum of the prepared ZnO hexagonal rods is shown in Figure 4.3 (c). The two stronger peaks at 108 and 285 cm^{-1} have been ascribed to the Raman active, low E2 mode of nonpolar optical phonons which were previously found in the wurtzite structure of ZnO (Xing et al., 2003). Similarly, two smaller peaks at 382 and 415 cm^{-1} are ascribed to the Raman active Polar A1 mode and the non-polar E2 mode of ZnO, respectively. The peak of 696 cm^{-1} is

attributed to the high E2 mode, indicating the good crystal quality of self-assembly radial structures.

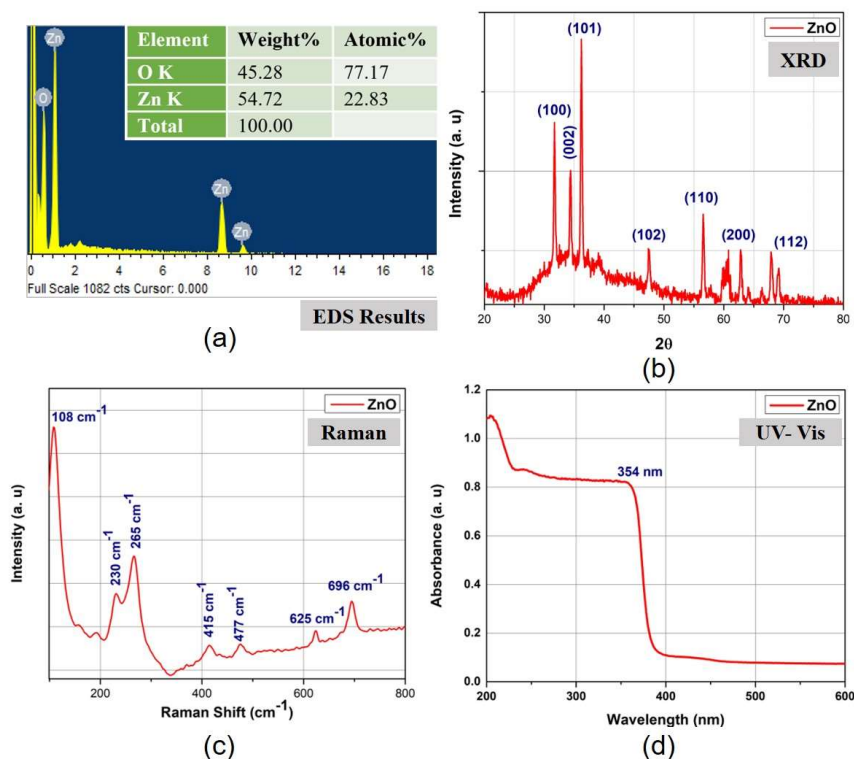


Figure 4.3: (a-d) EDS, XRD, Raman, and UV-Vis results of ZnO hexagonal rods

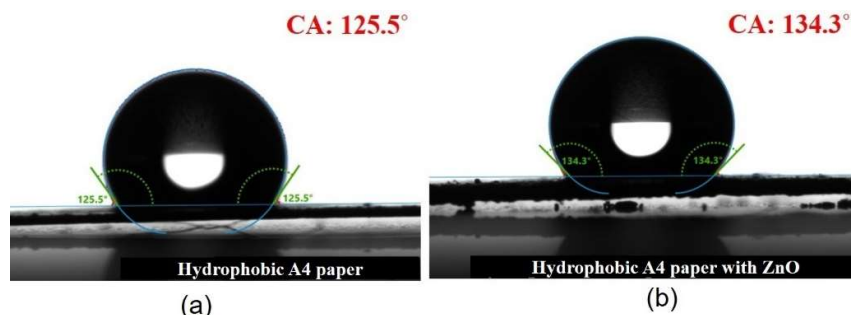


Figure 4.4: Contact angle results of (a) before and (b) after coating on A4 paper

The absorption spectra of the ZnO hexagonal rods had an absorption peak at 354 nm, which is blue-shifted in comparison to the bulk absorption peak, which exhibited bulk ZnO at 380 nm (Prasad et al., 2006) (see Figure 4.3 (d)). Furthermore, the stronger exciton effect, which is an essential feature of the quantum confinement effect in nano-semiconductors, is responsible for this noticeable and consistent change in the absorption peak. This is because the carriers are confined in a relatively limited region, allowing only electrons and holes to flow in a potential well. The surface energy of a solid surface, which is a function of the chemical nature of the surface atoms, is reflected in its wettability. Furthermore, surface energy is necessary for understanding the adhesion and wettability of protective coatings. Surface repellences in coating materials are best achieved with low surface energy. The contact angle (CA) of the hydrophobic A4 paper was 125.5°, whereas, after the drop coating of ZnO hexagonal rods, the CA increased to 134.3° as shown in Figure 4.4 (a, b). The experimentally predicted contact angle could be due to the physical roughness effect and the reduction of non-bonded (electrostatic and van der Waals) interactions as a result of the drop coating of ZnO hexagonal rods.

(b) Uric Acid Detection Using Capacitance Measurement System

A capacitance measurement unit was developed using an Arduino Mega 2560, which is a programmable microcontroller board with 54 digital input/output pins. It also has a power jack, a Universal serial bus (USB) port, an In-Circuit Serial Programming (ICSP) header, a reset (RST) button, and a 16 MHz crystal oscillator. Further, the capacitance measuring system comprises a 16 x 2 LCD screen and an A4 paper based biosensor. All three components are connected using a jumper wire. The Mega board is then attached to the laptop using a type B USB cable (see Figure 4.5). Arduino IDE software was used to program the Mega board which provides a real-time reading of uric acid solution on the 16 X 2 LCD screen. The Arduino program can measure the value of capacitance in the range of 20 pF to 1000 nF. A 0.2 μ l of the different concentrations of prepared uric acid solution were injected into the A4 paper based biosensor and the reading were recorded simultaneously on the serial monitor of Arduino IDE software as well as on the LCD screen. The device was further cleaned using the 0.1 mM phosphate-buffered saline (PBS) solution, before the next reading of uric acid.

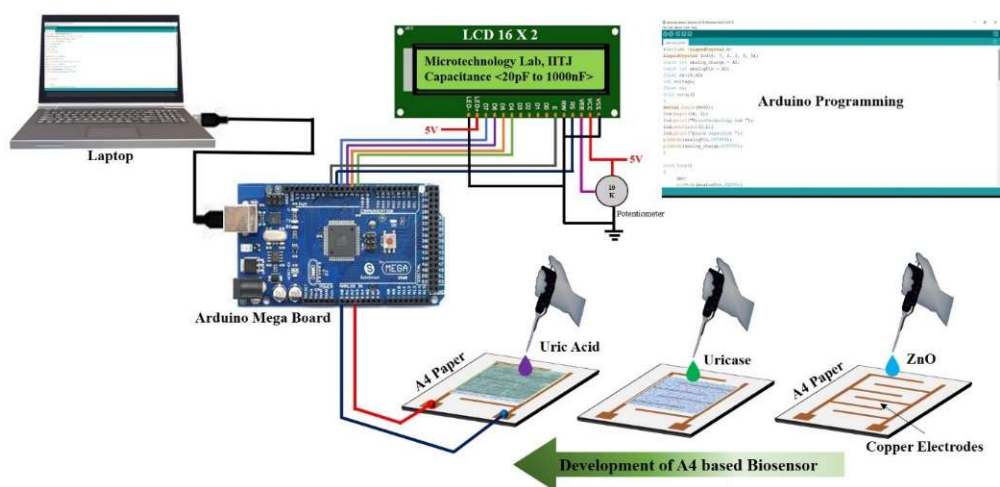


Figure 4.5: Schematic representation of Biosensor and instrumentation schemes

Figure 4.6 (a), shows the I-V characteristics of electrodeposited Cu electrodes based A4 paper biosensors are investigated for flat conditions. The inset of Figure 4.6 (a) shows the digital photograph of flat biosensors. An ohmic junction appears to have formed between the crystalline ZnO hexagonal rods and the Cu contact, as indicated by the I-V curve of the flat biosensor device. Therefore, the formation of the electrodeposited copper on A4 paper will usher in a new era of possibilities for the applications of low-cost biosensors. The response of the prepared biosensor toward a uric acid concentration ranging from 0.1 to 10 mM is seen in Figure 4.6 (b). The inset of Figure 4.6 (b) shows the calibration plot with a good linearity of 0.93 in the range of 0.1-1 mM. This curve will be used to determine the unknown uric acid concentration by comparing the capacitance values. The produced biosensor has a sensitivity of 9.00 μ F/mM cm^2 at 0.1 mM. The human serum sample was also analysed with the developed sensing unit. Firstly, plasma was separated from whole blood at 25000 rpm for 10 min (with the help of "REMI" (Revolutionary general purpose laboratory microcentrifuge) and afterwards, sample was taken for the capacitance measurement which was evaluated by spiking different concentrations (0.1-1 mM) of uric acid in the human serum sample following the standard addition method. No other pre-treatment process was performed. Figure 4.6 (c) shows the response study of uric acid based biosensors for real human serum samples. The inset of Figure 4.6 (c) shows the calibration plot with a good linearity of 0.93 in the range of 0.1-1 mM uric acid. The produced biosensor has a

sensitivity of $8.92 \mu\text{F}/\text{mM cm}^2$ at 0.1 mM with a 0.86% error. Table 4.1 shows the comparative analysis of both experiments. Figure 4.6 (d) displays the repeatability of the experiment for five consecutive measurements taken at a uric acid concentration of 0.5 mM . It is clear from looking at the graph that even after five consecutive observations, there is not a significant shift in the capacitance value. At 0.5 mM uric acid, the average capacitance value is 427.338 nF .

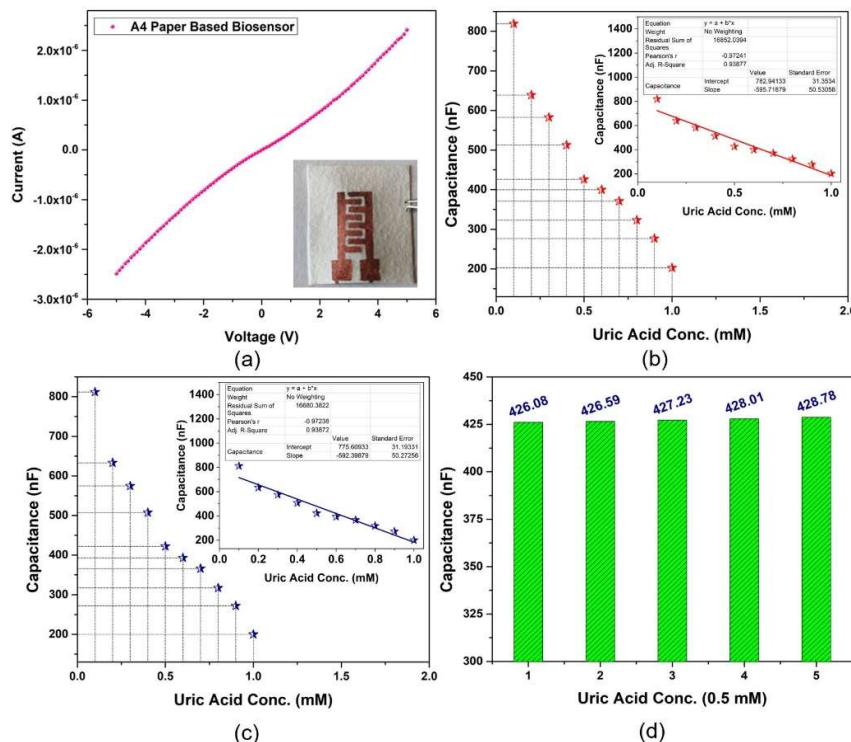


Figure 4.6: (a) The I-V characteristic curve of the A4 biosensor, (b, c) uric acid response studies for $0.1\text{-}5 \text{ mM}$ concentration for chemical and human serum based studies, inset shows the calibration curve obtained for $0.1\text{-}1 \text{ mM}$ and (d) repeatability study of the A4 biosensor for 0.5 mM uric acid concentration

Table 4.1. Comparison table for uric acid detection at different concentrations

Concentration (mM)	Capacitance Measurement (nF)		
	Prepared Uric Acid Sample	Spiked Uric Acid in Serum	Error %
0.1	819.05 ± 16	811.94 ± 62	0.86
0.2	638.96 ± 55	633.16 ± 27	0.91
0.3	582.51 ± 24	574.67 ± 43	1.34
0.4	512.71 ± 92	507.36 ± 10	1.04
0.5	426.08 ± 37	422.15 ± 38	1.38
0.6	399.87 ± 05	392.97 ± 51	1.72
0.7	371.03 ± 37	366.02 ± 99	1.35
0.8	323.15 ± 45	316.97 ± 13	1.91
0.9	276.77 ± 16	272.02 ± 47	1.71
1	202.83 ± 09	199.84 ± 72	1.47

(c) Sensing Mechanisms

The morphology of ZnO nanostructures plays a critical role in uric acid detection using capacitance measurement. Different shapes can provide unique advantages in terms of sensitivity, and response time. The level of sensitivity of the proposed uric acid biosensor is

defined by the interaction between enzyme and analyte, which is particularly specific due to the nature of uricase activity. The growing demand for the development of a mediator-free biosensor permits direct electron transport in ZnO nanostructures. This is because ZnO nanostructures have better thermal stability, are inexpensive, are biocompatible, and have antibacterial characterization. The development of an efficient biosensor relies on electron transportation (generated in the chemical reaction) from the redox center of the enzyme to the electrodes (Arora et al., 2012). In addition, because enzymes have the structure of an insulated shell, it is often necessary to utilize a mediator, which is an external redox species, to provide the electron transport path from the enzyme to the electrode. ZnO hexagonal rods were dropped cast over Cu IDEs, which tailored the electrical conductivity and redox property of the ZnO hexagonal rods. As a result, it does not require any other mediator in the system other than the bottom Cu IDEs. Figure 4.7 provides an explanation for the enzyme catalytic process at ZnO hexagonal rods for the detection of uric acid. Allantoin is produced together with CO_2 and H_2O_2 , when uric acid reacts with H_2O molecules in the presence of uricase enzyme. The high conductivity of ZnO rods could be attributed to the formation of H_2O_2 during uric acid oxidation, which then resulted in the generation of electrons, causing a shift in potential value at the surface of interdigitated electrodes on an A4 paper substrate (Ali et al., 2012). When the different concentration of uric acid surrounds the ZnO hexagonal rods, there will also be a shift in the electrode potential, which will then result in a change in the capacitance value. The high surface area-to-volume ratio of ZnO hexagonal rods provides a large contact surface for uric acid molecules to interact with. This large surface area increases the sensor's sensitivity and allows it to detect low uric acid concentrations. The comparative results of this work with previously published research articles are shown in Table 4.2.

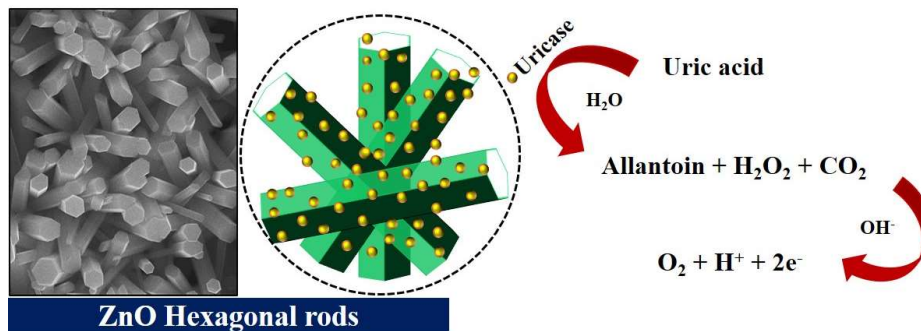


Figure 4.7 Sensing mechanisms of uric acid detection using ZnO hexagonal rods

Table 4.2: Comparative analysis of uric acid detection using various nanomaterial-based biosensors

Nanomaterial based Biosensors	Transducer Technique	Detection Range	Sensitivity
CuO nanorods (Patil et al., 2021)	Cyclic Voltammetry	0-3 mM	1017 ($\mu\text{A}/\text{mM cm}^2$)
PVA/CuO (Dhiman et al., 2021)	Capacitance	0.5-10 mM	4.72 ($\mu\text{F}/\text{mM cm}^2$)
Tetrapod ZnO (Lei et al., 2012)	Amperometry	0.8-3.49 mM	80.0 ($\mu\text{A}/\text{mM cm}^2$)
MWCNTs/PANi (Arora et al., 2014)	Cyclic Voltammetry	0.01-1.0 mM	43.2 ($\mu\text{A}/\text{mM}$)
ZnO nanoflakes (Ali et al., 2012)	Potentiometric	500-1.5 mM	66 (mV/decade)
ZnO rods (This work)	Capacitance	0.1-1 mM	9.00 ($\mu\text{F}/\text{mM cm}^2$)

4.1.4 Summary

A biosensor was fabricated by drop-coating hydrothermally synthesized ZnO hexagonal rods onto electrodeposited IDEs fabricated on hydrophobic A4 paper. Uricase was immobilized

on the ZnO hexagonal rods. Various concentrations of uric acid were applied to the paper-based biosensor, and changes in capacitance value were measured and displayed on an LCD unit in digital form using a capacitance measurement system. The experimental results demonstrate a linear relationship between uric acid concentration and capacitance value in the range of 0.01 mM to 1 mM, with a high sensitivity of 9.00 $\mu\text{F}/\text{mM cm}^2$ at 0.1 mM. The results indicate that the developed capacitance measurement unit can be employed for the early screening of uric acid in real samples for clinical applications. In short, the reported bio sensing scheme with unique approach to develop interdigitated electrodes over paper along with real-time sensing characterization has enormous potential to develop disposable and inexpensive biosensing devices for detecting variety of other diseases.

4.2 HIERARCHICAL MoS₂ NANOSTRUCTURED TUBES ON BIODEGRADABLE AND FLEXIBLE CHITOSAN BASED PLATFORM FOR LOW TEMPERATURE HYDROGEN DETECTION

The importance of flexible gas sensors is increasingly recognized for their adaptability across various applications. However, the commonly used flexible substrates are synthetic and non-biodegradable, contributing to environmental pollution. To mitigate this issue, there is a growing focus on developing low cost, environmentally friendly flexible substrates for electronic devices. In this study, we present an efficient approach aimed at fabricating a cost effective flexible chitosan platform embedded with MoS₂ nanostructured tubes synthesized via a hydrothermal method. This flexible platform, combined with MoS₂, underwent meticulous morphological characterization and comprehensive evaluation for detecting hydrogen (H₂) gas at 50°C.

4.2.1 Introduction

Gas sensing devices made from plastics and polymers offer the unique advantage of flexibility, allowing them to adapt to various structures and locations. They surpass traditional sensors by being integrated into wearable/portable devices with higher sensitivity and selectivity to target analytes (Kroutil et al., 2018). The importance of monitoring air quality and detecting flammable and explosive gases is emphasized due to environmental and workplace safety concerns (Alvarado et al., 2019). In recent years, gaseous H₂ has become widely utilized in both research and industrial settings. However, its use carries significant risks due to its highly flammable nature. This has created a pressing need for versatile H₂ sensors that offer adaptability, high sensitivity, and rapid response times to ensure safety in these applications. Further, these sensors can be incorporated into wearables for real-time monitoring of H₂ exposure, improving safety for workers in hazardous environments. Furthermore, they are ideal for portable leak detection tools, H₂-powered vehicles, and environmental monitoring systems (M. Guo et al., 2024; Gupta et al., 2016). With rapid industrialization globally, electronics waste has emerged as a significant threat to the environment. Researchers are focusing on flexible substrates because of their advantages, including being lightweight, portable, and highly flexible. Further, flexible gas sensors are increasingly important across various fields, with the potential to revolutionize sensing technology by detecting and monitoring gases and chemicals through changes in electrical properties upon exposure to target gases (B. Liu et al., 2023; Wang et al., 2024). However, commonly used flexible substrates such as polyethylene terephthalate, polyimide, etc., are synthetic and non-biodegradable, contributing to environmental pollution (Ghosh et al., 2024). To address this challenge, a new approach has emerged that emphasizes the synthesis of inexpensive, eco-friendly, and biodegradable flexible electronics (Park et al., 2022). For example, natural polymers such as chitosan, with their unique properties, show great potential as substitutes for conventional plastic substrates (P. Kumari et al., 2023a). Recently, there has been growing recognition of the remarkable sensing capabilities of one-dimensional (1D) materials, particularly in their sensing of explosive gases at low/ambient temperatures (Arafat et al., 2012).

This advancement not only solves the issue of a high power utilization of conventional sensors but also opens avenues for their integration into flexible wearable electronic devices. Recently, transition metal dichalcogenides (TMD) materials such as MoS₂ have emerged as prominent candidates for sensing applications due to their high surface area and tunable bandgap (1.2 to 1.9 eV) (Tian et al., 2023a), which offers numerous active sites for effective interaction of target analyte molecules, enhancing sensitivity at low concentrations (Jia et al., 2024; Zhao et al., 2022).

In response to the growing demand for sustainable and adaptable sensing technologies, the development of flexible and biodegradable gas sensors has emerged as a potential solution. Leveraging novel materials (such as nanostructured MoS₂ tubes) and their fabrication techniques, this chitosan based platforms hold the potential to address environmental concerns while offering robust performance in gas detection applications. Chitosan, renowned for its flexibility and biodegradability, stands out as a key candidate for sensor substrates. This research endeavors to validate the effectiveness of MoS₂ nanostructured tubes on the chitosan platform through systematic characterization and experimentation. By comparing their sensitivity, selectivity, and repeatability to conventional synthetic substrates, we further aim to demonstrate their viability as competitive alternatives for gas sensing applications. Furthermore, there is no prior literature on the fabrication of a biodegradable and flexible platform specifically designed for H₂ detection at low temperatures (50°C). Further, the versatility of the sensor makes it suitable for environmental monitoring, and healthcare settings, where there is a critical need for flexible and biodegradable sensing solutions.

4.2.2 Materials and Methods

All the chemical reagents were procured from Sigma Aldrich, including chitosan, polyvinylpyrrolidone (PvP), acetic acid (CH₃COOH, 99%), ammonium molybdate tetrahydrate (AMT; (NH₄)₆Mo₇O₂₄, 83%), thioacetamide (C₂H₅NS, 99%), tetramethylammonium bromide (C₄H₁₂BrN, 97.5%), and phosphate-buffered saline (PBS).

(a) Preparation of Flexible Chitosan Substrate

A flexible chitosan based substrate was prepared using a low-temperature solvent casting technique. Figure 4.8 illustrates the stepwise process involved in preparing this flexible substrate. Typically, 1 g of chitosan is mixed in a 1 N acetic acid solution, with 0.25 g of polyvinylpyrrolidone added as a crosslinking agent. The solution is continuously stirred at 400 rpm for 22 hours at ambient temperature, then filtered with a syringe filter to eliminate undissolved materials. Finally, the solution was poured into a petri dish and left to dry at ambient for 22 hours. The resulting dried film was then peeled off and further used for device fabrication.

(b) Synthesis of MoS₂ Nanostructured Tubes

MoS₂ nanostructured tubes were produced through a hydrothermal technique using AMT ((NH₄)₆Mo₇O₂₄·H₂O), C₄H₁₂BrN, and C₂H₅NS. In a synthesis procedure, 0.024 mol/L of AMT, 0.42 mol/L of C₂H₅NS, and 0.42 mol/L of C₄H₁₂BrN were mixed in deionized water under continuous stirring for 0.5 hours. After stirring, the dissolved mixture was used for the hydrothermal growth of MoS₂ in a 100 mL Teflon-lined autoclave, which was then placed in an oven at 200°C for 6 hours. The obtained precipitate was filtered and subjected to 12 hours of drying at 50°C in an oven, followed by calcination in an argon (Ar) environment at 600°C for 3 hours.

(c) Device Fabrication and Sensing Experiments

To fabricate a flexible resistive gas sensor, the initial step involves the deposition of a chitosan film on the PET substrate (as a supporting material), followed by depositing metal electrodes onto the prepared flexible films through a thermal evaporation process. Specifically, 300 nm of silver (Ag) metal electrodes were deposited using a shadow mask. After the electrode preparation, the chitosan film was peeled off from the substrate. Simultaneously, in a parallel process, 2 mg of MoS₂ nanostructured tubes are mixed with 2 mL of acetone and subjected to 60

min of ultrasonication. This resulting mixture was then spray-coated onto the prepared metal electrodes. Subsequently, the sensing capabilities of the developed device are analyzed by exposing it to varying concentrations of H_2 gas (1-50 ppm). The device's current-voltage characteristics and sensing response are assessed using a Keithley-2636B precision source meter. Further, humidity measurements were conducted to evaluate the sensor's response under varying RH% conditions. A mixing chamber was used to control humidity by combining dry air (zero air) regulated via a MFC with water vapor generated from a bubbler, controlled by N_2 gas flow. The mixed gases were then introduced into the sensing chamber, where RH% was continuously monitored by a humidity sensor.

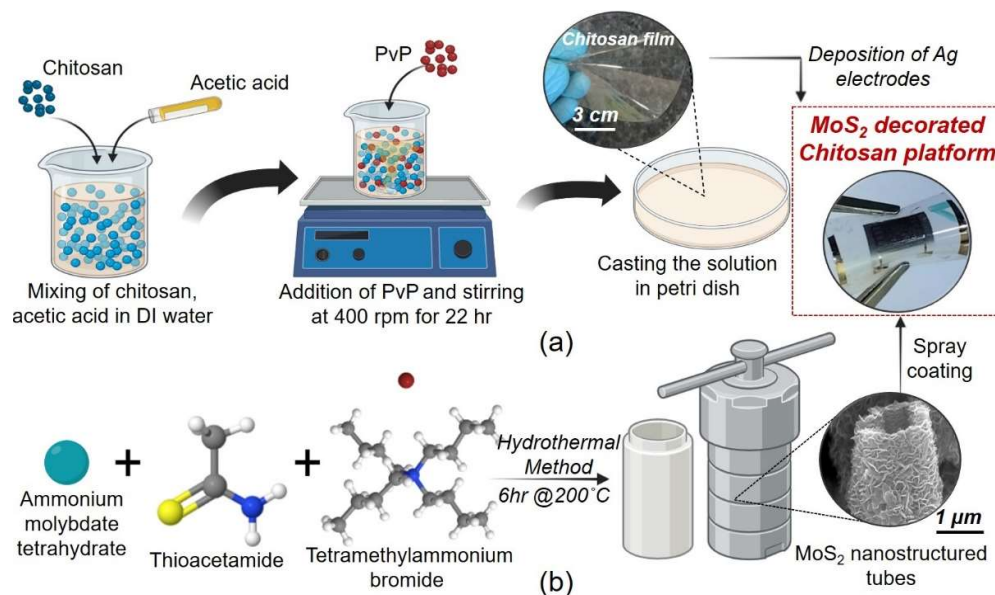


Figure 4.8: (a) Schematic view of stepwise process for preparing a flexible chitosan based sensor; inset showing a photograph of prepared chitosan film in the folding state, (b) Synthesis of MoS_2 nanostructure tubes with inset FESEM image

4.2.3 Results and Discussion

(a) Characterization of Flexible Chitosan Substrate

An emerging trend in flexible electronics involves the use of flexible polymeric and biodegradable substrates, presenting a sustainable alternative to nondegradable plastic counterparts. Here, we use a cost-effective technique to prepare a flexible using chitosan polymers. Figure 4.9 (a) displays a FESEM image of the chitosan substrate revealing a uniform and smooth surface, rendering it suitability for electronic devices with an inset of digital photograph, while Figure 4.9 (b) illustrates the bending capabilities of the substrate. To assess the film's properties, we conduct a wettability test using a contact angle goniometer, revealing a hydrophilic surface with a water contact angle of $\sim 81.65^\circ$ (see Figure 4.9 (c)). The elemental composition of the prepared substrate is further performed using EDS techniques, as depicted in Figure 4.9 (d). EDS analysis indicates the presence of C and O elements with atomic% of 70.06% and 29.94%, respectively, confirming the purity of the chitosan film (Woo et al., 2015). FTIR spectra of a chitosan film, recorded within the $800\text{--}4000\text{ cm}^{-1}$ range (see Figure 4.9 (e)). Specifically, the band observed between $3000\text{--}3600\text{ cm}^{-1}$ corresponds to the stretching vibration of free $-OH$ groups, as well as the symmetric and asymmetric stretching of $N-H$ bonds within the $-NH_2$ groups (Siripatrawan and Harte, 2010).

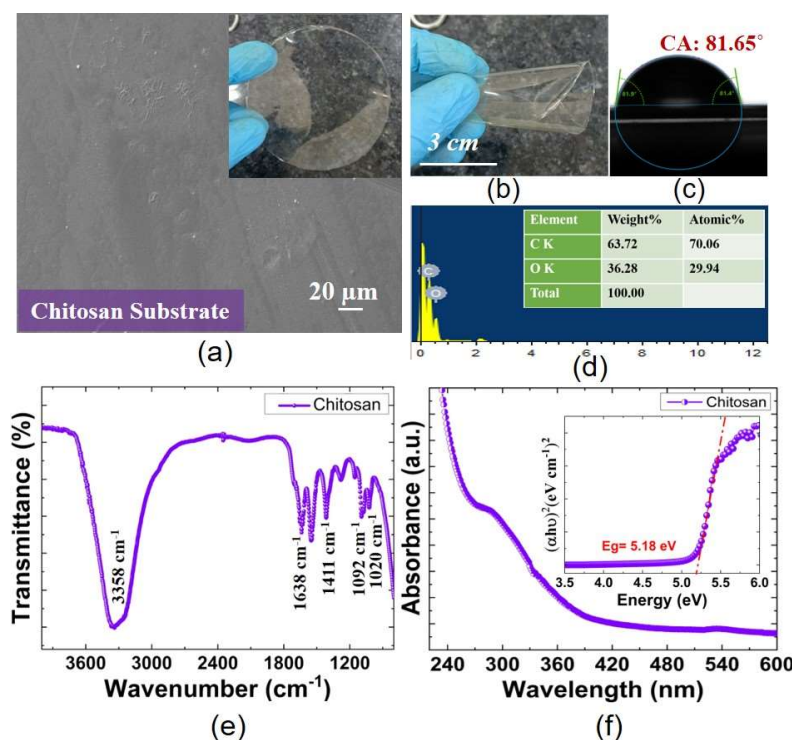


Figure 4.9: (a, b) FESEM image with inset of photograph of chitosan substrate with its bending capabilities, (c) water contact angle image, (d) EDS image, (e) FTIR spectrum, and (f) UV-Vis spectrum of chitosan film with inset Tauc plot

Additionally, the peaks at 1638 cm^{-1} and 1411 cm^{-1} correspond to vibrational modes associated with the stretching of the C=O group and the symmetric deformation of the CH_2 group (Rodrigues et al., 2020). The peaks observed at 1092 cm^{-1} and 1020 cm^{-1} are linked to vibrational modes resulting from the stretching of the -OH groups at 3rd and 5th positions in the chitosan structure (Li et al., 2010). Moreover, Figure 4.9 (f) illustrates the UV-Vis spectrum findings, revealing a broad characteristic absorption band around $\sim 280\text{ nm}$. This broad signal is due to the existence of unsaturated bonds ($\pi \rightarrow \pi$), primarily C-O within the chitosan structure (Llanos et al., 2015). Additionally, the band gap energy, measured using the Tauc plot, is found to be 5.18 eV (see inset of Figure 4.9 (f)).

The biodegradability of the prepared chitosan substrate is investigated in a PBS solution. Figure 4.10 depicts the physical transformation of chitosan film in the presence of PBS solution at 25°C . The film appears to distort within the first few seconds during the reaction with PBS medium, but it doesn't dissolve in PBS in the total experimental duration of 10 mins. However, the film breaks down into smaller grains, as shown in the inset of Figure 4.10 (f). In a subsequent experiment, the film was placed in PBS for 24 hours, resulting in the full dissolution of the film. This could be because chitosan is made up of repeating units of N-acetylglucosamine and glucosamine (Ibrahim et al., 2015). The solubility of chitosan in PBS primarily depends on the degree of deacetylation (removal of N-acetyl groups from the glucosamine units) (Fukushima et al., 2005). When chitosan is deacetylated, it becomes more hydrophilic, as the amine groups (NH_2) on the glucosamine units become protonated in PBS, forming ammonium groups. These positively charged groups can interact with water (H_2O) molecules through hydrogen bonding (Aranaz et al., 2021), making chitosan soluble in PBS solution. The observed degradation of the chitosan substrate within 24 hours when treated in PBS solution highlights its susceptibility to degradation. The biodegradability of chitosan presents a promising solution, offering the potential to replace these non-biodegradable substrates. The rapid degradation of chitosan substrates holds particular promise for the development of environmentally friendly electronic devices. Despite this, with appropriate encapsulation methods, sensors using chitosan substrates

could still find utility in prolonged applications such as wearable/flexible sensing, extending their lifespan and enhancing their practicality in various electronic applications.

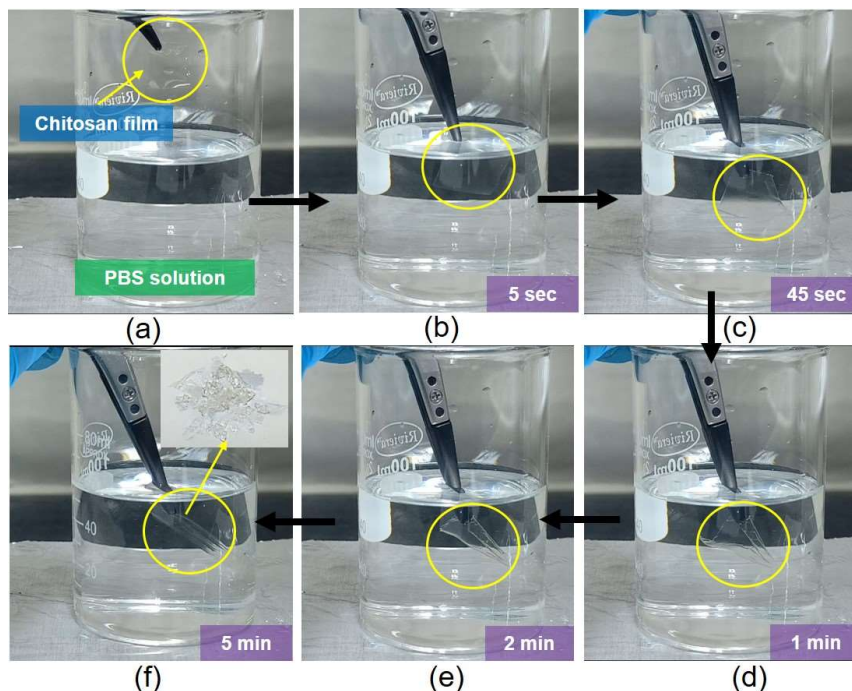


Figure 4.10: Biodegradability experiment of chitosan film in PBS solution

(b) Characterization of MoS₂ Nanostructured Tubes

The MoS₂ tubes were synthesized using a hydrothermal method using AMT ((NH₄)₆Mo₇O₂₄·H₂O) and C₂H₅NS as precursors, and C₄H₁₂BrN as additive. In Figure 4.11 (a-c), MoS₂ exhibits uniform tube-shaped structures with dimensions of $\sim 5.85 \pm 0.61$ μm in length and $\sim 1.05 \pm 0.57$ μm in width. Upon closer examination, these tubes reveal a wall thickness of ~ 240 nm. Moreover, the walls consist of numerous loosely stacked nanoflakes (~ 8 nm), arranged vertically in the radial direction. These uniformly arranged nanoflakes are expected to increase the specific surface area of the MoS₂ tubular structure, thus providing abundant active sites for target analyte during sensing experiments. Further, the TEM image provides additional confirmation that the tubular structures consist of a uniform arrangement of nanoflakes (refer to Figure 4.11 (d)). Moreover, the element mapping of the MoS₂ tubular structure is depicted in the inset of Figure 4.11 (d) indicating the existence of elements Mo and S, which further confirms the purity of the prepared sample. Furthermore, the structure characteristics of the MoS₂ tubular structure were examined using XRD. The XRD patterns of MoS₂ are illustrated in Figure 4.12 (a), revealing distinctive peaks at 14° (002), 33.2° (100), 39.6° (103), and 58.6° (110). These peaks are closely associated with the JCPDS card no. 00-006-0097, indicating successful synthesis of MoS₂ with a hexagonal crystal structure. MoS₂ exists in several crystalline phases, primarily 1T, 2H, and 3R, each with distinct structural and electronic properties. The 2H phase, characterized by a prominent peak at $\sim 14^\circ$ (002), is the most stable and commonly studied (Huang et al., 2015). It exhibits a layered hexagonal structure with semiconducting properties, particularly suitable for sensing applications. Figure 4.12 (b) depicts the Raman spectrum of the MoS₂ that reveals the presence of two prominent peaks; the in-plane vibrational mode (E_{2g}) of the Mo and S atoms at 406 cm^{-1} and the out of plane vibrational mode (A_{1g}) of the S atoms at 380 cm^{-1} . The difference between these modes, $\sim 26\text{ cm}^{-1}$, indicates that the as-synthesized MoS₂ has a multilayered structure consisting of three to four layers (Yu et al., 2015; Zhu et al., 2011).

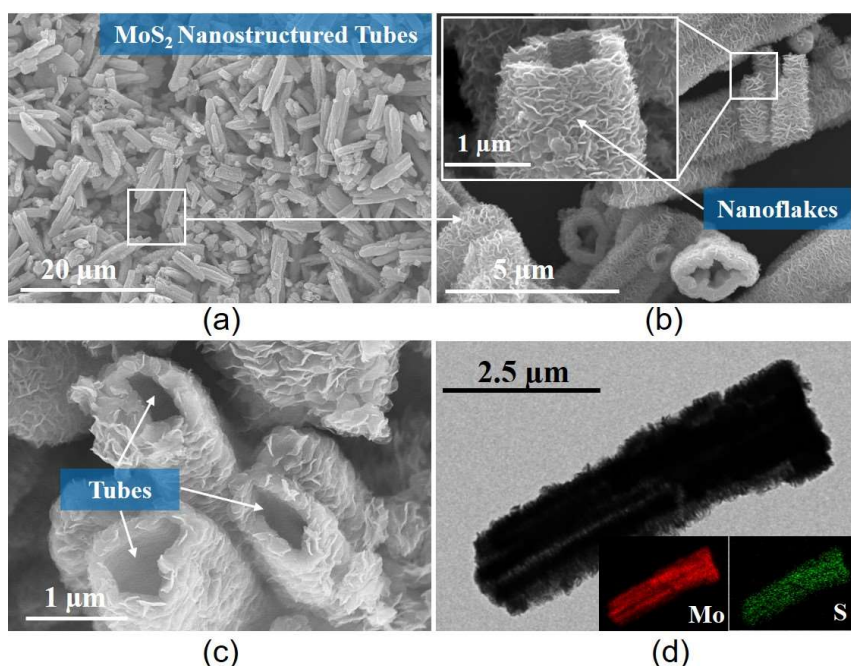


Figure 4.11: (a-d) FESEM and TEM images of the synthesized MoS₂ nanostructured tubes at different magnifications

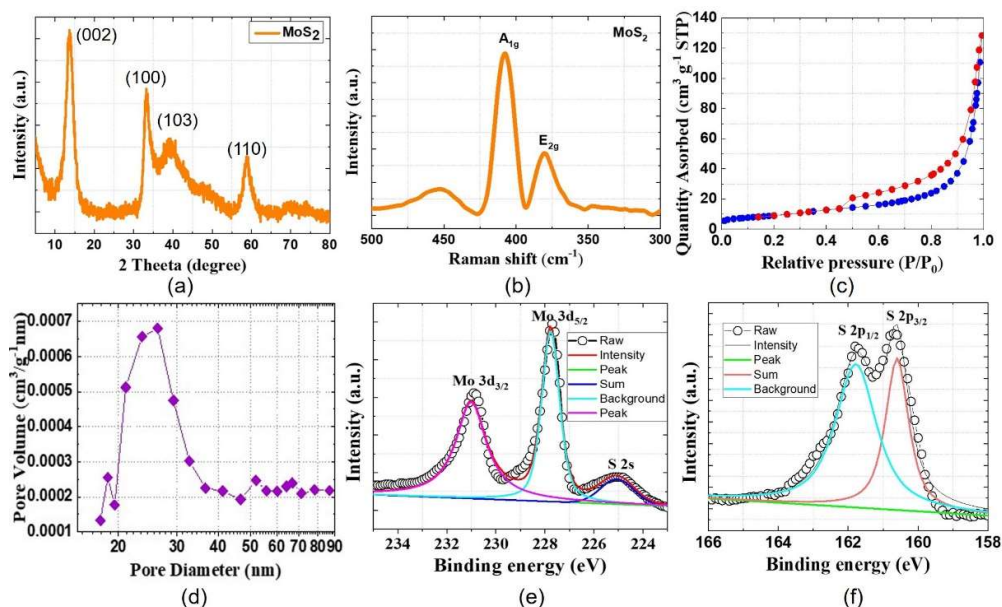


Figure 4.12: MoS₂ characterization, (a) XRD spectrum, (b) Raman spectrum, (c, d) BET results, and (e, f) XPS plot

Moreover, the nitrogen (N₂) adsorption-desorption isotherms of the MoS₂ exhibit typical type IV isothermal hysteresis loops, indicating the presence of large-sized mesopores. BET analysis revealed a specific surface area of $\sim 32.6 \text{ m}^2 \text{ g}^{-1}$ for MoS₂, with pore sizes around 26 nm (Figure 4.12 (c, d)). As a result, MoS₂ demonstrates a significantly large specific surface area and sufficient nano-porosity, which is expected to enhance its gas sensing performance by providing numerous active centers for the adsorption of target analyte on its surface. The chemical compositions and atomic valence states of the synthesized MoS₂ nanostructured tubes were analyzed further utilizing the XPS technique. Figure 4.12 (e) illustrates three discernible peaks representing Mo⁴⁺ 3d_{3/2} (230.9 eV), Mo⁴⁺ 3d_{5/2} (227.6 eV), and S 2s (225 eV) components attributed to MoS₂ (Ji et al., 2018). The presence of the S 2s peak in the Mo 3d spectra is notable, as it provides insights into the electronic interactions between S and Mo atoms within the MoS₂ lattice. The S 2s

peak overlaps with the binding energy region of the Mo 3d peaks, specifically near the Mo 3d_{5/2} peak. Despite this overlap, the S 2s peak can be distinctly resolved due to its narrow width, which allows for accurate spectral fitting and interpretation. The interaction between these peaks highlights the importance of considering the S 2s contribution when analyzing the Mo 3d spectra, as it reflects the bonding environment and electronic structure of MoS₂. Additionally, Figure 4.12 (f) depicts the S 2p spectra of MoS₂, with prominent peaks centered at 160.7 eV and 161.8 eV, attributed to S 2p_{3/2} and S 2p_{1/2}, respectively. These findings collectively demonstrate that a hydrothermal technique is useful in the large scale preparation of hierarchical MoS₂ nanostructured tubes, with well-defined electronic properties.

(c) Gas Sensing Results

To evaluate the reliability and efficacy of the synthesized MoS₂ nanostructured tubes as a sensing material, electrical measurements were performed on the fabricated sensor, as illustrated in Figure 4.13 (a). The I-V characteristic of the sensor was analyzed at 3 V, indicating an ohmic nature in the electrical contact within the device. This finding aligns with the expected behavior of pristine MoS₂, consistent with findings from prior studies (McDonnell et al., 2014). Additionally, the response (%) of the flexible sensor to 50 ppm H₂ at 25°C exhibited a drift in response, impacting the sensor's recovery to the baseline after exposure to H₂ gas. Further, the sensor demonstrates a ~5.81% response at 50 ppm. At an elevated temperature of 50°C, the devices exhibit good stability and reversibility, as depicted in Figure 4.13 (b, c), with the resistance swiftly decreasing and returning to its initial value upon exposure to 1-50 ppm H₂ concentrations. Notably, the device displays responses ranging from ~9.25% to ~17.53% under exposure to 1 to 50 ppm of H₂ gas. Furthermore, Figure 4.13 (d) illustrates the optimal curve fitting for the sensor's response to varying H₂ concentrations at 50°C. Two fitting approaches, allometric and exponential, were examined to model the sensor's data. The results demonstrate that an exponential curve provides the best fit, suggesting that the sensor's response value rises with increasing H₂ concentration until it reaches a saturation point. As depicted in Figure 4.13 (e), the sensor's behavior at 1 ppm is illustrated with τ_{res} and τ_{rec} . Various research indicates that pristine MoS₂ tends to demonstrate prolonged response and recovery periods during target gas detection, due to a consequence of its intrinsic material properties (Tian et al., 2023b). The relatively inactive surface of MoS₂ hinders its interaction with gas molecules, thereby delaying the adsorption and desorption processes. This sluggishness is exacerbated by the weak adsorption of target gas molecules on the pristine surface, which extends the necessary time for their accumulation and subsequent detection. Moreover, the diffusion of gas molecules through the pristine nanomaterial is impeded, slowing their progress toward the active sites. For instance, Liu et al. reported the fabrication of a thin film MoS₂ sensor for NO₂ gas detection (Liu et al., 2014). However, the sensor suffers from a full recovery time of long 12 h. To address these challenges and enhance MoS₂-based gas sensors, various strategies, including surface functionalization and doping, have been proposed. These methods aim to foster the creation of active sites, alter the electronic characteristics, and refine the pathways for gas diffusion, thereby improving the kinetics of the sensor's response/recovery. Figure 4.13 (f) presents a comparative analysis of the sensor's performance at 25°C and 50°C, revealing a notably higher responsiveness at the elevated temperature. Specifically, at 25°C, the sensor's relative response was ~5.8% at a concentration of 50 ppm. This response was enhanced threefold upon raising the temperature to 50°C, achieving a relative response of ~17.5% at the same concentration. The enhanced sensing behavior at the higher temperature can be attributed to the improved reactivity of H₂ molecules, which interact more effectively with the active sites on the MoS₂ nanostructured tubes.

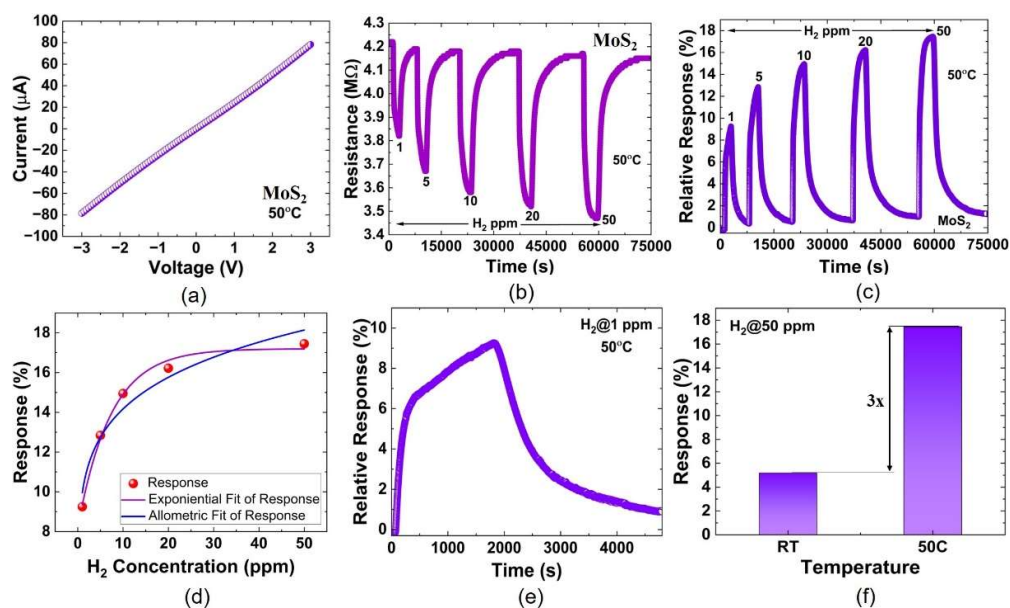


Figure 4.13: (a) I-V characterization, (b, c) Time dependent resistance and corresponding relative response curves at 50°C to different concentrations of H₂ gas, (d) Fitting of response (%) at 50°C, (e) Response of MoS₂ to 1 ppm H₂ concentration at 50°C, (f) Comparative response of the device at 50 ppm H₂ gas at 25°C and 50°C

The practical application of the developed flexible device was assessed by evaluating its performance in terms of bendability, repeatability, and selectivity. As depicted in Figure 4.14 (a), the sensor's efficacy was tested with a 50 ppm H₂ gas concentration, both prior to and following bending. Post-bending, the sensor's response experienced a slight decline from ~17.5% to ~17% at 50°C. This decrease can be ascribed to the mechanical strain imposed on the spray coated MoS₂ layer during bending, which may modify the morphology and layer orientation. Such changes have the potential to interfere with the active sites on the surface, thereby impeding the adsorption of H₂ molecules. Additionally, the physical distortion could compromise the electrical connectivity between the MoS₂ layers and the electrodes, resulting in heightened resistance and diminished sensitivity of the sensor. To evaluate the sensor's reliability, a repeatability study was conducted on the device using H₂ gas, as depicted in Figure 4.14 (b). Results indicated consistent output across three cycles of testing at 50 ppm. This uniform response underscores the stability of the device, affirming its suitability for real-world applications. Figure 4.14 (c) illustrates the sensor's response to different levels of RH (%). The observed decrease in response (%) may be attributed to the chitosan's OH and NH₂ groups, which can form weak hydrogen bonds with ambient H₂O molecules. These weak hydrogen bonds play a crucial role in enabling the reversible adsorption and desorption of H₂O molecules on the sensor's surface (P. Kumari et al., 2023b; Nasution et al., 2018).

Furthermore, in practical scenarios, low-temperature flexible devices need to exhibit selectivity in distinguishing between different gases. The sensor's selectivity was evaluated by exposing it to interfering gases such as hydrogen sulfide (H₂S), ammonia (NH₃), and nitrogen dioxide (NO₂), at a concentration of 50 ppm, as illustrated in Figure 4.14 (d). The relative response (%) for H₂S, NH₃, and NO₂ were determined to be ~11.69%, ~6.47%, and ~4.77%, respectively, which is significantly lower than the response to H₂ (~17.53%) at the same concentration. Among the investigated interfering gases, H₂ stood out as a potent reducing agent capable of effectively eliminating adsorbed oxygen from the MoS₂ device's surface, leading to superior selectivity. These findings highlight the device's versatility for a broad spectrum of applications, including environmental monitoring.

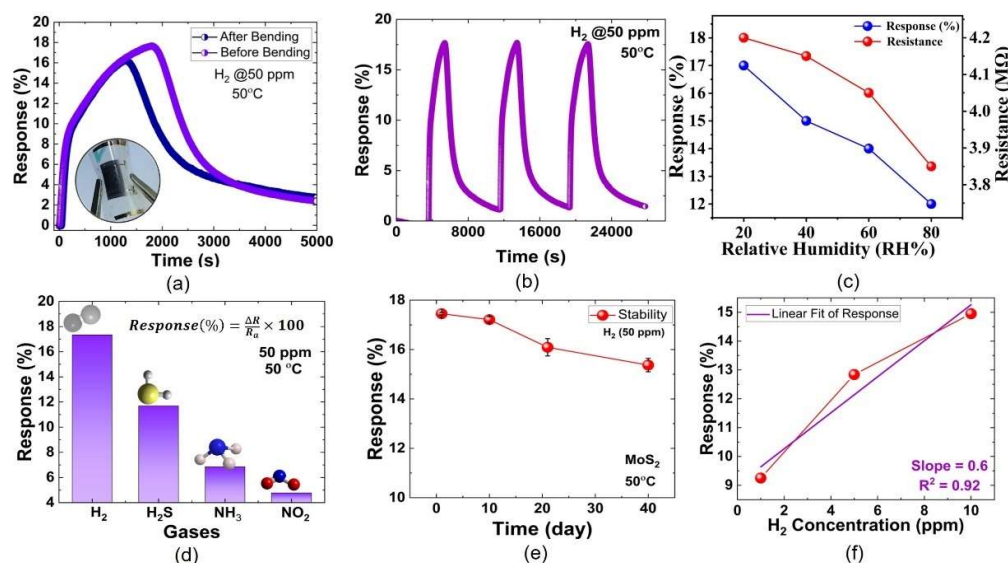


Figure 4.14: (a) Comparative analysis of the device's response before and after bending, (b) Three-cycle repeatability test conducted at 50 ppm H₂, and (c-e) Humidity, selectivity, and stability results of sensor at 50°C, (f) Linear regression analysis calculation based on response

Table 4.3 summarizes the comparison results of a previously published article on MoS₂ based gas sensor. Furthermore, Figure 4.14 (e) depicts 40 day stability results, which demonstrated that the sensor exhibited a slight decrease in response (%) when subjected to 50 ppm H₂ gas at 50°C. The sensitivity of gas sensors is quantified by the change in response corresponding to a 1 ppm alteration in the concentration of the target gas. This parameter is reflected by the slope of the linear regression line as shown in Figure 4.14 (f), which has been determined to be 0.6% ppm⁻¹. Leveraging this slope, the sensor's limit of detection (LOD = 3×(rms/slope)) is calculated. In this context, the root mean square (rms) deviation is derived from the fifth-order polynomial fit of the baseline noise, which yields a LOD of ~28 ppb.

Table 4.3: Comparative analysis of previously published articles on MoS₂ based hydrogen gas sensors

Sensing material	Temp. (°C)	H ₂ (ppm)	LOD (ppm)	Response (%)
MoS ₂ /Cs _x WO ₃ (C. M. Wu et al., 2022)	25°C	500	10	50.6
Pt/MoS ₂ (Gottam et al., 2020)	150	100	10	10
TiO ₂ /MoS ₂ (Liang et al., 2020)	25°C	200	50	4.25
MoS ₂ /Si (Liu et al., 2015)	25°C	5000	-	15.4
MoS ₂ (Yajie Zhang et al., 2018)	120	700	20	27.5
MoS ₂ Tubes (This Work)	50	50	0.028	17.53

(d) Gas Sensing Mechanisms

The functionality of the prepared MoS₂ based flexible sensor relies on alterations in resistance prompted by chemisorption of target gas molecules (Tian et al., 2023c). This leads to fluctuations in the carrier concentrations within the MoS₂ material. These changes in carrier density are predominantly influenced by the active sites on the MoS₂ surface and the nature of the target gas (whether it's oxidizing or reducing). Hence, elucidating the gas-sensing mechanism is crucial for understanding the interplay between gas molecules and charge carriers. Figure 4.15 illustrates the schematic of gas sensing mechanisms of MoS₂ nanostructured tubes when exposed to H₂ gas. In the presence of ambient air, the MoS₂ sensor interacts with oxygen molecules (O_{2(gas)}), leading to their adsorption onto the surface of MoS₂ nanostructured tubes. Consequently, these adsorbed oxygen molecules transform chemisorbed O_{2(ads)}⁻ by trapping

electrons from the conduction band (CB) of MoS₂ surface, ultimately resulting in the formation of an electron depletion layer (Neetika et al., 2021). Exposure of the device to H₂ gas enhances the effectiveness of nanostructured tubes by augmenting the accessibility to surface active sites of MoS₂, thereby facilitating gas diffusion. Moreover, H₂ molecules undergo dissociation into atoms, which then migrate to MoS₂ to react with O_{2(ads)}⁻. This dissociative adsorption of H₂ involves three sequential steps: Firstly, the adsorption of H₂ molecules onto S sites in MoS₂, secondly, the dissociation of adsorbed H₂ molecules into Mo-H and S-H pairs, and finally the diffusion of H atoms to form S-H groups at Mo edges (Park et al., 2020). Furthermore, a reduction in the concentration of O_{2(ads)}⁻ further increases the concentration of charge carriers at the interface, promoting the interaction between O_{2(ads)}⁻ H₂ atoms. This interaction results in the release of electrons back to the MoS₂, thereby enhancing the electrical conductivity of the device, as shown in the inset of Figure 3.15. Consequently, the resistance of the device decreases, which is evident in its response and recovery behavior. Additionally, the abundant porous structures and rough inner walls of the nanostructured tubes substantially enhance the specific surface area, as demonstrated by BET results. Consequently, these hierarchical nanostructured tubes offer a well-defined and aligned nano porosity that facilitates efficient gas diffusion (Yajie Zhang et al., 2018).

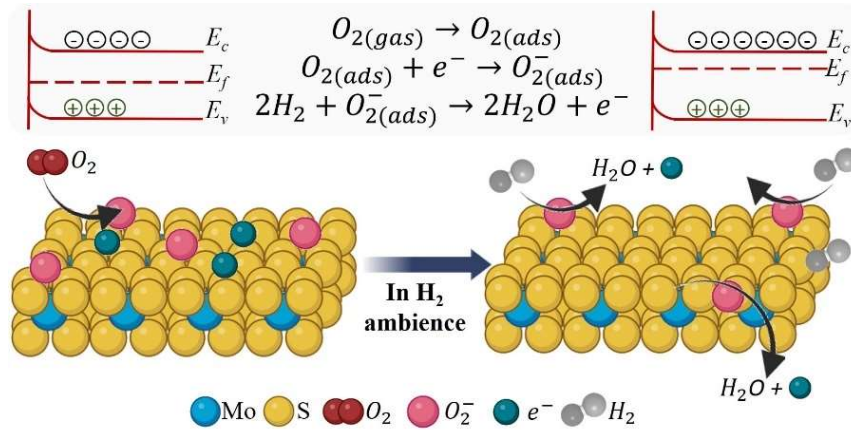


Figure 4.15: Schematic of sensing mechanism of MoS₂ based flexible gas sensor

4.2.4 Summary

This research endeavors to validate the efficacy of integrating MoS₂ nanostructured tubes onto a chitosan platform through comprehensive assessments of gas sensing performance. Thus demonstrating that the chitosan platform is a compelling alternative substrate for low-temperature H₂ gas sensing applications. The resulting sensor demonstrates a good response in detecting low concentrations of H₂ gas at 50°C. Notably, the sensor exhibits a surge of 300% higher response by proportion, reaching ~18% at 50°C, compared to a mere ~6% response at RT for 50 ppm H₂ gas. Moreover, the sensor exhibits commendable repeatability and bendability characteristics. Additionally, it displays selectivity towards H₂ over other gases such as H₂S and NO₂, achieving a LOD of ~28 ppb. These findings highlight the potential of the chitosan based platform device for monitoring trace levels of H₂ gas. This study sets a foundation for future researchers endeavoring to create a flexible and biodegradable gas sensor exhibiting high sensitivity, selectivity, and efficiency in gas performance. Such advancements are essential for environmental remediation and the accurate detection and monitoring of gases at low levels.

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