

Chapter 1

Introduction

This chapter deals with the fundamental aspects of electrochemical sensors, tracing their principles, characteristics and categorization. In addition, this chapter provides an extensive overview of the synthesis methods and applications of different materials particularly in the field of electrochemical sensing. The importance of ion beam modification in nanomaterials has also been discussed here. Lastly, the chapter elucidates the scope of the thesis along with the statement of the problem.

1.1 Electrochemical sensor

Sensors play a pivotal role in our daily lives, by providing essential information about the physical and chemical environment of a system or process. It provides a real-time information regarding the abnormalities and presence of any problem or fault in a process, allowing for timely and accurate diagnosis of the problems detected. In medical diagnosis, sensors are almost indispensable for detecting infections and disease which may be a threat to the normal functioning of the body [1,2]. Sensors are mainly classified into three types: physical sensor, chemical sensor and biosensor. Physical sensors deal with the change in physical parameters like temperature, pressure, mass, proximity, distance, wavelength, frequency, etc. In chemical sensors, the specific chemical reactions exhibited by some chemical compounds or analyte are used for their quantitative detection. While, in case of biosensors, detection is performed by recognizing the biochemical signal generated after the interaction between bio recognition molecules and their specific analytes [3]. Again, based upon the operating principles, sensors are classified as piezoelectric sensor, pyroelectric sensor, optical sensor, resistive sensor, electrochemical sensor, etc [4]. Among them, electrochemical sensors are available at affordable cost and can offer several advantages over other types of sensors. So, electrochemical sensors are widely preferred in a variety of applications including environmental monitoring, food and beverage quality control, medical diagnostics and industrial process control, etc [5,6]. Electrochemical sensor can accurately detect and measure the concentration of various chemicals/analytes present in the solution, or serum. It typically works with an electrode and an electrolyte.

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With the help of the sensing material attached to the electrode, it can transform the chemical or biochemical changes into a detectable electrical signal. Afterwards, the signal is processed via amperometric, potentiometric or conductometric techniques for monitoring and analyzing the presence and concentration of the target analyte quantitatively [7].

1.1.1 Components and types of electrochemical sensor

An electrochemical sensor basically comprised of three basic components and they are: receptor, transducer and signal processing unit. A combination of reference electrode (R.E.), counter electrode (C.E.) and working electrode (W.E.) collectively perform the transduction mechanism as shown in Figure 1.1. Receptor material is decorated over the W.E. which can selectively interact with the analyte molecules present in the solution. The electrochemical signal associated with the receptor-analyte interaction is analysed in the processing unit and monitored in the display [8]. The basic components of an electrochemical sensor are briefly discussed below:

- (a) **Receptor:** It is basically a thin layer covering the W.E. which is responsible for generating the electrical signal after interacting with the analyte. The receptor molecules can selectively detect the specific analytes, which can be of some chemical and biological substance. Based upon the types of receptors used, electrochemical sensors can be either enzymatic or non-enzymatic one [9]. In enzymatic sensors, bio recognition molecules *eg.* antibody, aptamer, etc. are used as receptor, also called bioreceptor [10]. On the other hand, in non-enzymatic sensors, transducer is decorated with certain nanostructures like metal-oxides, metal-sulfides, metal-organic frameworks (MOFs), etc. which are expected to perform selective redox activity towards specific chemicals/analytes only [11].
- (b) **Transducer:** The transduction mechanism of any sensor solely determines the working of a sensor. In case of electrochemical sensors, conducting materials are usually used as transducer. Besides, proper choice of transducer material is very crucial for fabrication of the high-performance electrochemical sensors. A transducer has to offer better electrode-electrolyte contact for smooth interfacial charge transport, also it should possess high surface-to-volume ratio with large number of active sites [12]. Again, stability is another aspect, a transducer material must be stable and exhibit consistent performance over time in the aqueous electrolyte. It has been reported that in case of enzymatic sensors conducting polymer-based nanostructures are favourable

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for transducer material due to their biocompatibility, easy surface modification strategies and high surface area [13,14]. Kim *et al.* have developed a glucose sensor by immobilizing glucose oxidase (GOx) over poly (terthiophene benzoic acid) [15]. They have reported that the benzoic acid groups located over the polymer backbones allow for better immobilization of the enzyme. A similar strategy was adopted by Tuncagil *et al.* to immobilize GOx over 4-(2,5-di (thiophen-2-yl)-1H-pyrrol-1-yl) benzeneamine polymer matrix via amide bond linkage [16]. This leads to a better performance of the biosensor with a minimum loss of biomolecules throughout the sensing experiments.

(c) **Signal processing unit:** It is responsible for handling and analyzing the signals generated by the sensor. The key goals of this unit include amplification, filtering and digitalization of the chemical or biochemical response, followed by calibration of the signals upon considering all the environmental conditions. Finally, the processed data is monitored in some recognizable graphical format for further analysis.

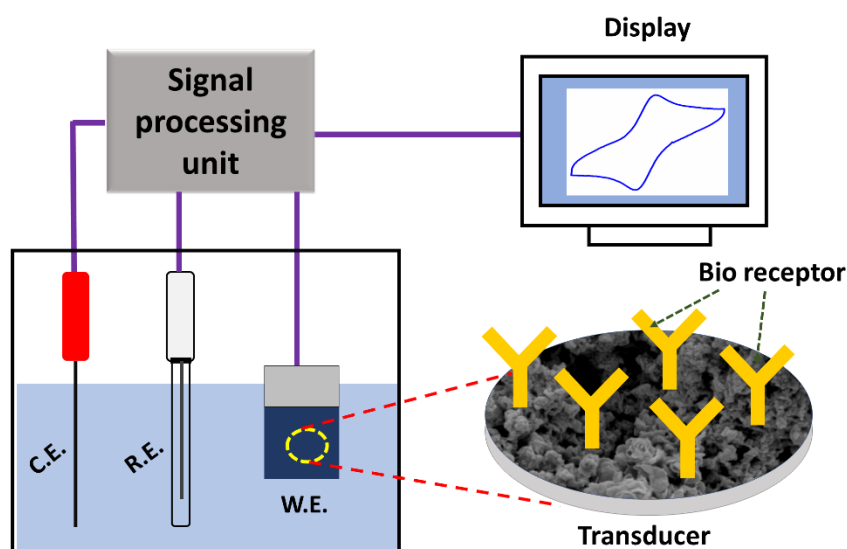


Figure 1.1: Basic components of an electrochemical sensor

1.1.2 Enzymatic electrochemical sensor

It is a type of biosensor that utilizes the biomolecules *viz.* enzyme, antibody, aptamer, DNA, RNA, etc. as bio recognition element for the detection of the specific target analytes. The specialty of these biomolecules is that they are selective to their specific analytes only. For example, glucose oxidase (GOx), is an enzyme naturally found in certain microorganisms, and this can selectively bind with glucose to produce gluconic acid and hydrogen peroxide (H_2O_2) after the enzymatic reaction [17]. Enzymes are biological catalyst known for

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speeding up a biochemical reaction without being consumed. Interaction mechanism of enzymes with the analyte was first postulated by Emil Fisher in the year of 1894 [18]. He proposed a lock and key mechanism, considering the enzyme as the lock and analyte as the key (Figure 1.2). The enzymatic reaction starts when the analyte binds to the active sites of the enzyme, and yielding the product molecules eventually. Activity of an enzyme molecule mainly depends upon the pH of the solution, substrate concentration and surrounding temperature. The pH of the solution can change the polarity of the amino groups present in the enzyme molecules, also, an optimal temperature of 30° to 60° C is preferable for better enzyme kinetics [19].

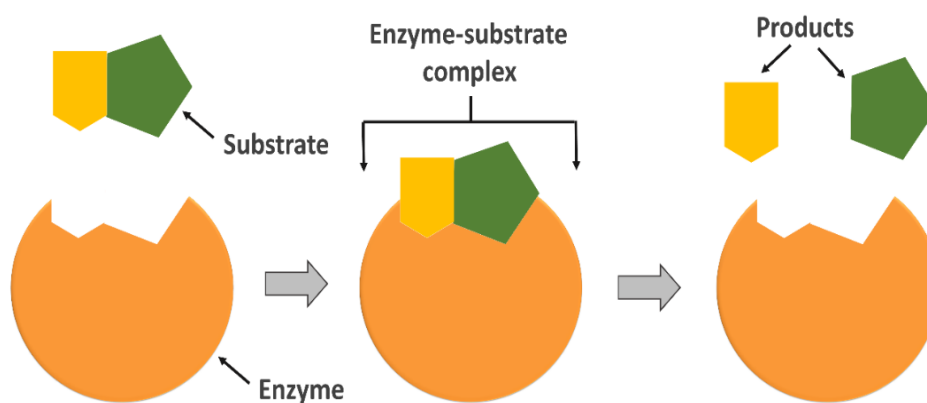


Figure 1.2: Interaction mechanism of enzyme with the target analyte

On the other hand, antibodies are specialized protein molecules composed of several amino acid groups. Antibodies, also known as immunoglobulins (*Igs*) are Y-shaped protein molecules containing two identical antigen binding sites called F_{ab} with a tail fragment F_c , as shown in Figure 1.3. The sequential order of the amino acid groups in the F_{ab} region varies for different antibodies, making them selective towards specific antigen [20]. For example, troponin is a complex protein found in muscle cells, especially in cardiac muscles, which is a biomarker for cardiac disease. For diagnosis of troponin level in blood, anti-troponin antibodies are used in the immunoassay technique [21]. To be mentioned, antibody molecules don't act as catalyst like enzymes, rather they attach non covalently with the binding site of harmful pathogens to neutralize their toxicity. The specificity and biochemical activity of an antibody largely depends upon its chemical structure. Upon close proximity, several physisorption phenomena like van Der Waals interaction, hydrogen bonding and electrostatic force arises in between antibody-antigen binding regions which is the origin of biochemical interaction of these *Igs* [22].

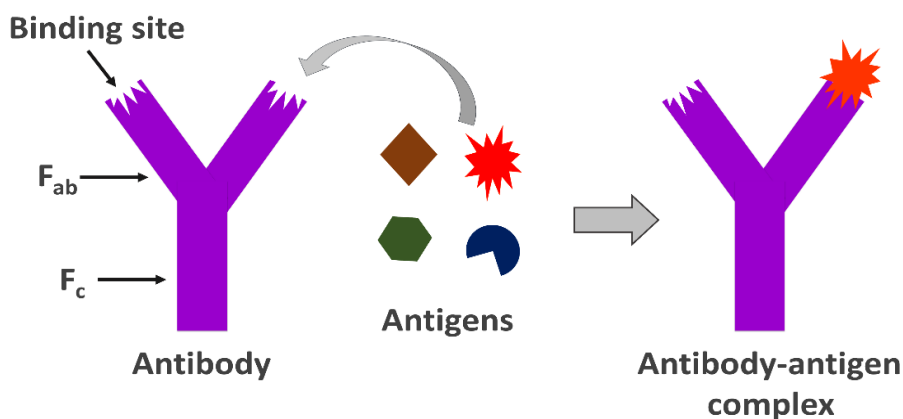


Figure 1.3: Interaction mechanism of antibody with the target analyte

The key step for fabrication of any enzymatic sensor or biosensor involves proper immobilization of the biomolecules over the transducer/electrode, and the simplest way of immobilization is via physisorption. But the physically adsorbed biomolecules may easily get wiped out from the electrode surface due to poor adhesion. Hence, immobilization via covalent linkage is the most popular strategy adopted for bioconjugation, *eg.* glutaraldehyde cross-linking method [23]. It involves the use of glutaraldehyde, a small organic molecule with two reactive aldehyde functional groups that can react with amino groups ($-\text{NH}_2$) present in the protein molecules to form covalent cross-links. This is a robust way for fabrication of biosensors with practical applications. In addition, choice of suitable transducer material also plays a vital role in antibody immobilization. It has been reported that conducting polymers usually exhibit better immobilization mechanism due to the presence of several functional groups throughout its backbone [24]. S. Chang *et al.* followed glutaraldehyde crosslinking method to immobilize monoclonal antibody of cardiac troponin I (Mab-cTnI) over silicon nanowire casted field effect transistor (SiNW-FET) for the detection of cardiac troponin I (cTnI) [25]. The fabricated immunosensor also exhibited potential application in point-of-care diagnosis.

1.1.3. Non-enzymatic electrochemical sensor

Some transition metals like nickel (Ni), copper (Cu), cobalt (Co), iron (Fe), molybdenum (Mo), zinc (Zn), etc., in their oxide form, generally exhibit electrochemical redox activity against certain biological analytes such as glucose, H_2O_2 , creatinine, uric acid, ascorbic acid and so on. This unique behaviour of these metal-oxides can be used for the enzyme-free detection of the aforesaid chemical substances. A non-enzymatic electrochemical

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sensor is a type of chemical sensor that can detect and quantify certain chemical substances present in the solution without the use of enzymes [26,27]. Although enzymatic sensors are known for their high specificity and reliability, but use of biomolecules make them vulnerable to pH, temperature and humidity. Apart from this, complex purification and production process of biomolecules come up with high fabrication cost of enzymatic sensors. In case of non-enzymatic sensors, metal-oxide nanostructures are incorporated in the transducer element for the detection of target analytes, making it a cost-effective sensing protocol [28]. Quick response time, high sensitivity, stability and durability are the added advantages associated with these types of sensors thereby offering a limitless bottom to current research. In this regard, J. Zhang *et al.* had designed CuO NP decorated Ce-metal-organic framework (Ce-MOF) via in situ precipitation method for ultrasensitive detection of glucose [29]. This CuO/Ce-MOF based sensor exhibited a very low detection limit (*LOD*) of 2 nM with a sensitivity of 2058.5 $\mu\text{A mM}^{-1}\text{cm}^{-2}$ under the linear range upto 8.6 mM. Again, A. Nisar *et al.* has fabricated nickel cobalt ($\text{Ni}_x\text{-Co}$) based bimetallic system for H_2O_2 sensing that exerted an excellent sensitivity value of 2211.4 $\mu\text{A mM}^{-1}\text{cm}^{-2}$ under the concentration range, 0.005 mM - 9 mM [30]. It is worth noting here that composite of such metal-oxides with 2D material can significantly enhance the redox kinetics as well as sensing activity of the sensor. For instance, S. Mutyala *et al.* have designed MoS_2 -interconnected porous carbon (ICPC) heterostructure via hydrothermal treatment [31]. The MoS_2 -ICPC composite material-based sensor was shown to exhibit superior activity towards sensing H_2O_2 with a low *LOD* value of 11.8 μM under a wide linear range of 20-300 μM . The non-enzymatic electrochemical detection mechanism of H_2O_2 by using NiO based sensor electrode is shown schematically in Figure 1.4. The Ni^{2+} state of the NiO exhibits a remarkable capability to reduce H_2O_2 through transfer of single electrons from Ni^{2+} to H_2O_2 , and facilitates the conversion of H_2O_2 into water and oxygen. This electron transfer process leads to the transition of $\text{Ni}^{2+} \rightarrow \text{Ni}^{3+}$ state. Afterwards, this Ni^{3+} state is reduced back to Ni^{2+} , by accepting one electron from the electron rich working electrode. This electron transfer process gives rise to the variation in reduction current in the amperometric response. Afterwards, the presence of H_2O_2 in the solution can be ascertained by monitoring the change in current.

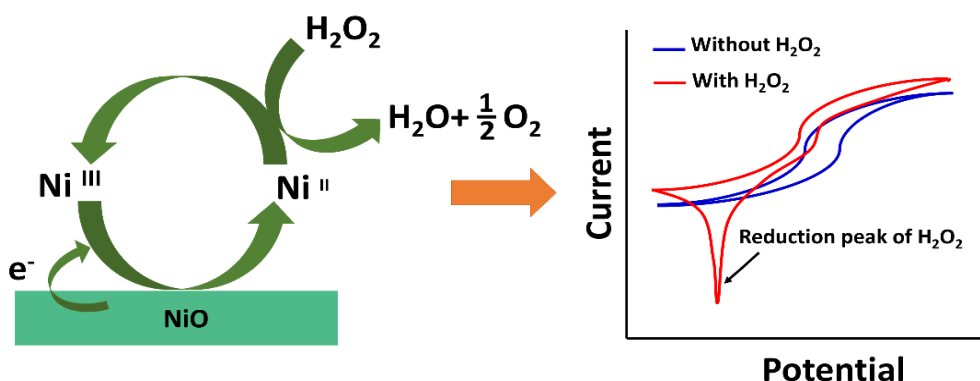


Figure 1.4: Schematic diagram of the H₂O₂ reduction mechanism by NiO based electrode

1.1.4. Sensing activity of an electrochemical sensor

The sensing activity of an electrochemical sensor refers to its ability to detect and quantify a specific analyte or target molecule in a sample through electrochemical reactions. The performance of a sensor is evaluated based upon few parameters such as linearity, sensitivity, selectivity, detection limit, response time, etc. [32].

(a) **Linearity:** The linearity of a sensor refers to how closely its output signal follows a straight line upon plotting the relationship between the added analyte concentration and respective output signal. The concentration range of analyte over which a sensor exhibits minimum deviations from the best-fit straight line is called linear range of the sensor. High linearity is desirable in many applications as it simplifies calibration and ensures accurate measurement. The equation of straight-line corresponding to the linear range is known as calibration equation. However, calibration techniques can sometimes compensate for nonlinearity also.

(b) **Sensitivity:** It indicates the effectivity of a sensor to detect and measure the presence and concentration of the specific analyte. High sensitivity indicates the ability to detect per unit concentration of the analyte with high signal amplification. Sensitivity is the ratio of the change in output signal w.r.t. per unit added concentration of analyte. It can be directly estimated from the slope of the linear calibration curve.

(c) **Selectivity:** It indicates the ability of a sensor to distinguish the target analyte from other interfering substances present in the sample. An ideal sensor should exhibit minimal response to other interfering substances, enabling precise and reliable measurements of the target analyte.

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(d) Limit of detection (*LOD*): It represents the lowest concentration of the target analyte that a sensor can detect with high precision. It can be calculated by using the relation, given as $LOD = \frac{3.3 \times \sigma_y}{m}$; where m and σ_y are the slope and standard deviation of the y-intercept in the linear calibration curve [33].

(e) Response time: It refers the time required by a sensor to provide the measurable results after getting exposed to the target analyte. It is imperative to have fast response time for rapid monitoring of the analyte concentration.

(f) Repeatability: It is the ability of the sensor to generate the identical outcomes over and over again under similar circumstances. High repeatability is preferred in case of sensing to get precise and reliable measurements. The repeatability feature of a sensor can be tested by repeating the sensing experiment while keeping all parameters uniform. In case of electrochemical sensing, multiple electrodes having the same compositions are used to perform the sensing experiment several times. The consistency in the quantitative measurements of the outcomes with low standard deviation value is considered as good repeatability.

(g) Reproducibility: It is basically the ability of a sensor to produce consistent measurements at different intervals of time during long storage by the same electrode. It provides information regarding the degradation in measured outcomes of a sensor upon storage. This feature is generally assessed for non-enzymatic sensors.

Moreover, several other factors have profound effects on the sensing activity of the electrochemical sensors *viz.* morphology and design of transducer material, kinetics of the redox process, mass transport, analyte-receptor interaction, etc [34]. By optimizing these parameters, one can certainly improve the performance of the designed sensor.

1.2 Scope of materials

The sensitivity and biochemical activity of the electrochemical sensors generally suffer from poor surface constructs of the transducer electrode. This leads to the essence of meticulous selection and modification of the material that can provide significant enhancement in mechanical, physicochemical, morphological and electrical properties of the transducer. In this section, the scopes of different materials have been discussed for their potential application as electrochemical sensors.

1.2.1. Conducting polymers

The word ‘Polymer’ has been taken from the Greek word ‘Polys-Meros’ which means ‘many-parts’. Polymers are basically organic macromolecules consisting of multiple repeating subunits called monomer [35]. Polymers were known as insulating material until the early discovery of conducting polymers (CPs) by Katon and co-workers in 1960 [36]. After wards, Shirakawa along with Heeger and Diarmid had reported the 10^9 -fold increase in conductivity of polyacetylene upon incorporation with halogen group [37]. This advancement had drawn the attention of the researchers to a new class of material called conducting polymers also known as, “synthetic metals”. Conducting polymer possesses unique electrical and optical properties along with peculiar chemical structure which allows its vital application in different fields such as super capacitors, solar cells, chemical sensors, optoelectronic devices, batteries and biosensors, [38-40] etc. The flow of electrons in CPs arises from the presence of conjugated double bonds throughout their chain. A simple 1D band of CP is postulated as an infinitely long chain consisting of carbon atoms [41]. In the ground state, carbon atom possesses the configuration of $1s^2 2s^2 2p_x^1 2p_y^1$, and that overlapping of one 2s and two 2p orbital gives rise to three sp^2 hybridized orbitals. The heads on overlapping of two sp^2 hybridized orbitals generate strong σ -bonds between two nearby carbon atoms. Whereas, the in phase and out of phase overlapping of plane perpendicular p_z orbitals produce bonding (π) and anti-bonding (π^*) molecular orbitals in CPs. The energy difference (ΔE_g) between these π (HOMO) and π^* (LUMO) lies typically in between 1.5-4 eV range, making them semiconducting in nature [42]. Large overlapping of these HOMO and LUMO energy levels eventually results in the formation of valence band (VB) and conduction band (CB) in such polymers as depicted in Figure 1.5.

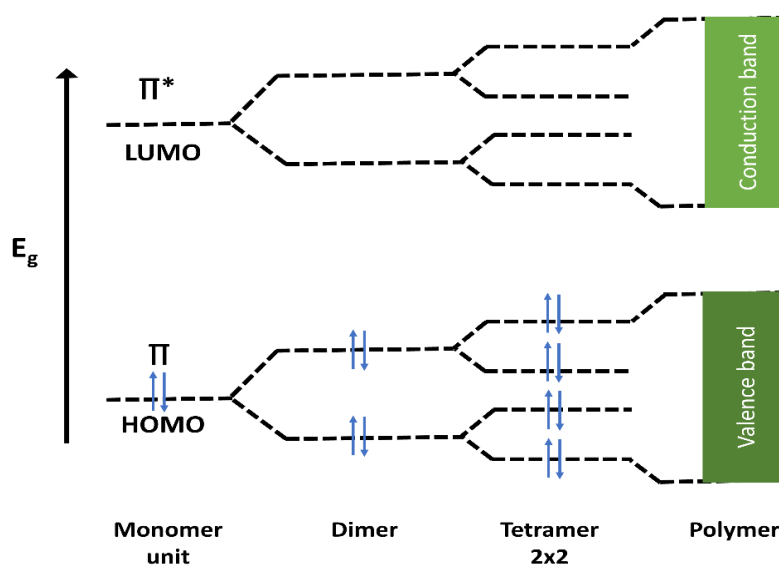
In case of CPs, transition of electrons from VB to CB takes place upon external supply of energy and become largely responsible for their electronic conductivity. Although electrical conductivity of most of the CPs ($\geq 10^2$ S/cm) lie in between the boundary of semiconductor and insulator, the conductivity of such polymer can be increased up to metallic conductivity region by doping it with oxidative/reductive substituents or by donor/acceptor radicals *eg.* ClO_4^- , BF_4^- , FeCl_4^- , Li, K, halogen groups, etc [43]. Tsukamoto *et al.* had successfully doped polyacetylene with iodine, resulting an augmented conductivity of more than 10^4 S.cm⁻¹, which is equivalent to the conductivity of lead, 4.8×10^4 S.cm⁻¹ (at T= 300 K) [44]. Some common examples of CPs are polyacetylene, polyaniline (PANi), polypyrrole (PPy), poly (3,4-ethylenedioxy thiophene) (PEDOT),

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poly-thiophene, poly-(p-phenylene vinylene) (PPV), etc. and their molecular structures are depicted in the Figure 1.5. The CPs can be synthesized by polymerizing the respective monomers through various routes, *viz.* (a) chemical, (b) electrochemical and (c) photochemical process. Table 1.1 compares the conductivity values of different CPs. Although PANi acquires a high conductivity of $\sim 10^5 \text{ S.cm}^{-1}$ making it a suitable candidate as electrode material, moderate cytotoxicity is the major drawback associated with this CP [45]. On the other hand, PEDOT is biocompatible in nature with a good conductivity range of $3 \times 10^2 \sim 5 \times 10^2 \text{ S.cm}^{-1}$. Also, PEDOT is easy to tailor due to the hydrophilic behaviour of its monomer. The better water solubility of the monomer unit enables for making composition by blending with other materials in synthetic aqueous solvent [46].

Table 1.1: Comparison of conductivity values of some common CPs [47]

Polymer type	Conductivity (S.cm^{-1})
Polypyrrole	1 ~ 50
Polyaniline	0.1 ~ 10^5
Polythiophene	$10^{-3} \sim 10^{-7}$
PEDOT	$3 \times 10^2 \sim 5 \times 10^2$
Poly (p-phenylene vinylene)	$10^{-3} \sim 10^2$



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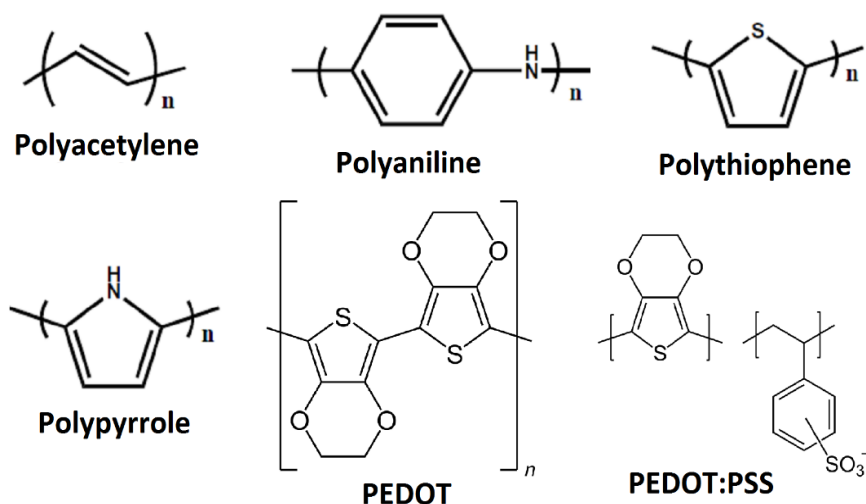


Figure 1.5: Schematic band structure of conducting polymer, and chemical structure of some common CPs [*Handbook of Nanocomposite Supercapacitor Materials I: Characteristics* (2020)]

1.2.2. Two dimensional layered nanostructures

Two-dimensional (2D) materials are known as the thinnest nanomaterial due to their atomic scale thickness. The 2D materials have attained a great deal of interest in various research fields due to their large surface area and fascinating electronic as well as optoelectronic behaviors. The strong in-plane bonds of such materials provide them high mechanical strength, whereas the weak Van der Waals interaction in between the separable stacked layers makes them suitable for designing new innovative materials with tailored properties [48]. Basically, the regular structural as well as electronic behaviour of a material deviates drastically at the surface, and the 2D nanomaterials possess highest aspect ratio of surface area to volume with a large number of surface-residing atoms [49]. For instance, the atoms of the bulk in a crystal experience isotropic environment in all directions, but surface atoms usually remain unsaturated leading to high chemical reactivity over the surface regime. In 2004, the discovery of graphene at The University of Manchester had opened up the possibility of layered 2D materials as a potential research zone [50]. Till date, extensive studies on different 2D nanostructures have been carried out all over the globe for multiple scientific applications. Some of the 2D layered nanostructures include transition metal dichalcogenides (TMDCs), layered double hydroxides (LDH), MXenes, graphitic carbon nitride (g-C₃N₄) hexagonal boron nitride (hBN), phosphorene (BP), germanene and silicenes. Few of the common 2D layered materials and their structures are shown in Figure

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1.6. These materials offer outstanding structural and physico-chemical properties which make them suitable for a wide range of applications, including catalysis, energy storage, optoelectronics, sensors, supercapacitors and biomedical research. [51,52]. Figure 1.6 depicts the structure of some common 2D materials.

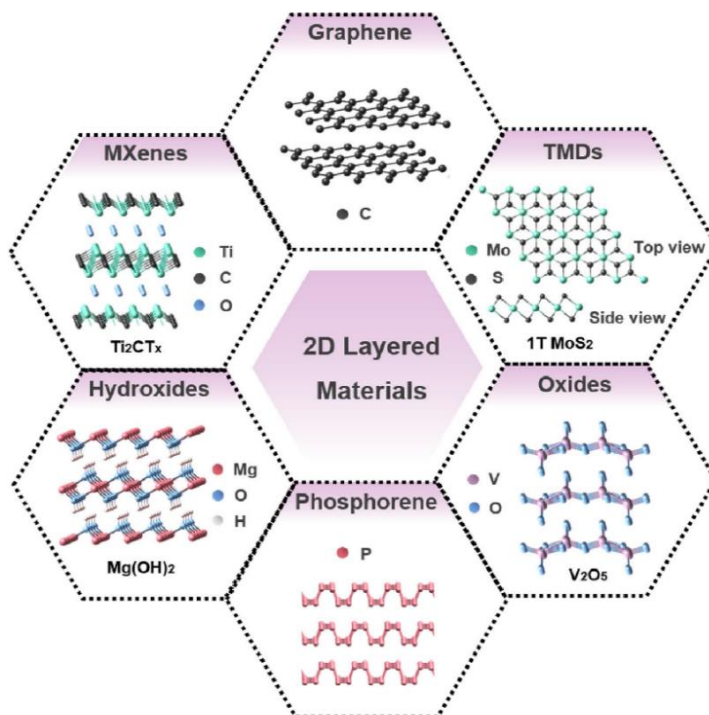


Figure 1.6: Different types of 2D layered materials and their lattice structures
[InfoMat. (2020) 2(1):3-32]

2D nanomaterials can be synthesized either by top-down or bottom-up routes. In top-down approach, bulk state of the material is converted to monolayer or few layer systems via liquid phase exfoliation or chemical reduction method [53]. Liquid phase exfoliation (LPE) method involves dispersion of the bulk into chemical solution such as to weaken their interlayer interactions followed by sonication to obtain the separated sheets. Though this sonication-based method is effective and can be optimized by using different chemical solutions and surfactants or by varying the sonication time and power, still the oxidation process occurring throughout exfoliation usually reduces the purity of the product. So, additional purification methods are required for high purity of the exfoliated nanosheets. On the other hand, exfoliation through chemical reduction is a high-yield method to obtain few layer systems which are often used for the preparation of graphene-based nanostructures. This method is popularly referred as, Hummer's method where the bulk

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graphite flakes are treated with strong oxidizing agents like sulfuric acid, potassium permanganate and catalyst like sodium nitrate to oxidize the graphite to graphene oxide (GO) nanosheets. Use of toxic reducing agents like sodium borohydride, or hydrazine hydrate is the major disadvantage of this method [54].

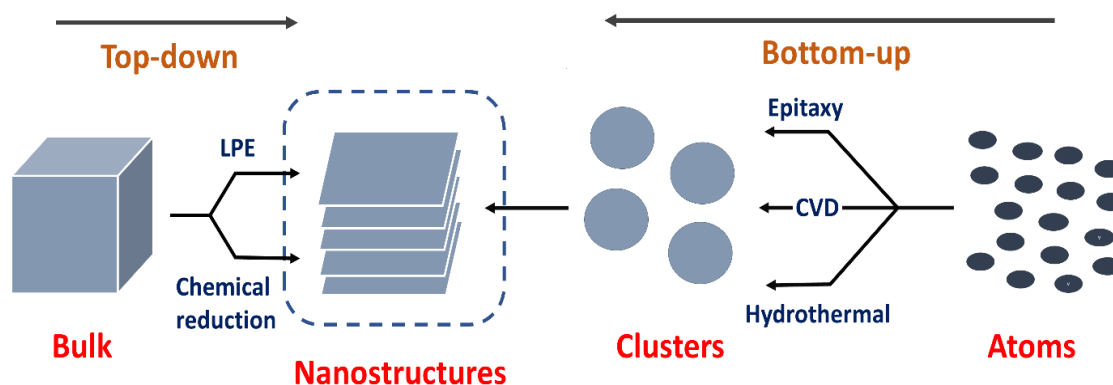


Figure 1.7: Scheme of top-down and bottom-up synthesis procedures of 2D nanostructures

Another approach of 2D nanostructure synthesis is the bottom-up technique where smaller molecules are used for the growth of bigger nanostructures. Epitaxial growth, chemical vapor deposition (CVD) and hydrothermal technique falls into this category [55]. For epitaxy growth, both liquid and gas precursors can be used. It basically involves the deposition of a monocrystalline film or monolayer over a monocrystalline substrate. In epitaxial growth, orientation and the lattice structure of the deposited film adopts the geometry of the substrate used. Being a sophisticated technique, a slight change in parameter can result in the structural disorder and of defects formation. Besides, CVD is a popular technique essentially known for the uniform growth of non-volatile 2D nanosheets over conducting metal substrate. In this method, a high temperature evacuated chamber is used containing the precursor materials in gaseous form. Afterwards, the gas mixture undergoes some chemical reaction followed by depositing the desired material over the substrate. Like the epitaxy one, this technique is also prone to the change in parameters like temperature, pressure and flow rate of the gas mixture. Lastly, in hydrothermal route, the precursors of the required 2D nanostructure are mixed together in a solution and subjected to hydrothermal treatment. The product is obtained by filtering the suspension and drying the as collected precipitate. Even if this method is an effective one, but it has very less control over the morphology and thickness of the synthesized nanosheets [56].

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The conductivity as well as electron transfer efficiency of a material largely depends upon its carrier mobility. A quantitative aspect of mobility for different 2D nanostructure is depicted in Table 1.2. The semi-metallic germanium has a very high theoretical value of mobility. Besides, high cost and complex synthesis procedure of germanene constrains its widespread use [57]. In fact, fascinating electrical behaviour, catalytic activity, higher carrier mobility and physical stability of TMDCs have offered them a special place in the family of 2D materials. The inherent properties of such 2D nanostructures have provided ample scope to the researchers for their utmost use in various fields, including electrochemical sensing [58,59]. On the other hand, GO is a derivative of graphene with fascinating physico-chemical properties which have extensive contribution in catalysis [60]. Herein, amongst other 2D materials, we shall limit our attention to GO and molybdenum disulfide, MoS₂. The advantages offered by these materials are discussed below.

Table 1.2: Electron mobility comparison of some 2D materials [61]

Material	Type	Mobility (cm ² /V. s)
GO	Insulating	< 26 [62]
Germanene	Semi-metallic	~1×10 ⁵ (Theory)
MoS ₂	Semi conducting	~200 (Mono Layer)
WS ₂	Semi conducting	100-200 (bulk)
MoSe ₂	Semi conducting	~50 (exfoliated)
WSe ₂	Semi conducting	100~500 (bulk)
BP	Semi conducting	~50 [63]

- **Graphene oxide (GO)** is an exceptional material that can be represented as a single monomolecular layer of graphite comprised of several oxygen-containing functionalities such hydroxyl (-OH), carbonyl (C=O), carboxylic acid (-COOH), alkoxy (C-O-C), and other oxygen-based functional groups (Figure 1.8). These oxygenated groups provide better solubility and surface functionalization capability to GO which allows its extensive use in nanocomposite materials. Fabrication of GO via Hummer's method results in the disruption of the *sp*² bonding orbitals of graphene that inhibit its electrical conductivity, making it electrically resistive [64]. However, a pretty high conductivity (~ 0.1 to 2.98 × 10⁴ S m⁻¹) can be attained by reducing GO to rGO upon treating with hydrazine hydrate. The GO easily gets dispersed in polymer matrix due to its hydrophilic nature making it an effective

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filler material for fabricating polymer-based nanocomposites [65]. The unique mechanical, thermal and electrical properties along with high surface area of GO enable its diverse application in energy storage devices, anti-corrosion coating and electrochemical sensing. For example, Ademar Wong *et al.* had developed rGO/PEDOT-PSS/GCE electrochemical sensor for the selective and simultaneous sensing of nimesulide and piroxicam. The modified electrode exhibited an enhanced detection mechanism for piroxicam and nimesulide, with *LOD* of 100 nM and 2.4 nM, respectively [66]. Moreover, the presence of different oxidative functional groups over the surface of GO enables better immobilization of the biomolecules via covalent coupling during the fabrication of enzymatic sensors.

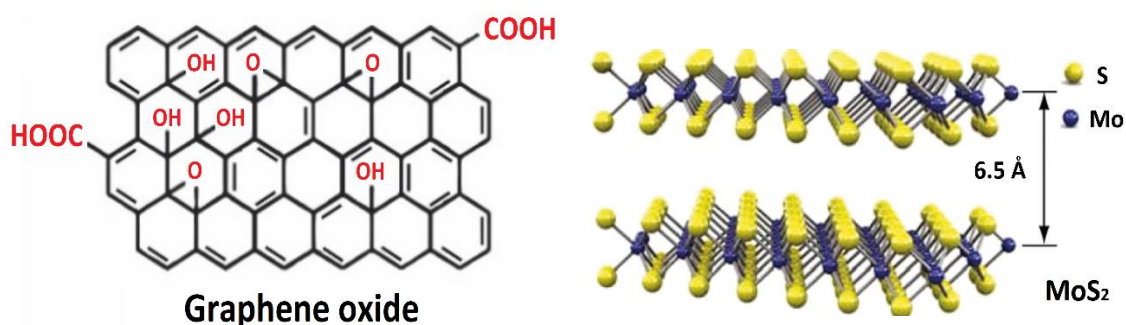


Figure 1.8: Schematic layered structure of GO [*Nanomaterials* (2018) 8(11):944] and MoS₂ [68]

- **TMDCs** exhibit a unique combination of atomic-scale thickness, direct bandgap, strong spin-orbit coupling and favourable electronic and mechanical properties, which make them interesting for fundamental studies and for several applications. TMDCs manifest multiple structural phases that arise from distinct coordination spheres of the transition metal atoms. The two common thermodynamically stable structural phases are octahedral (1T) and trigonal prismatic (2H). Some common TMDCs are MoS₂, WS₂, MoSe₂, WSe₂, etc [67]. Among them, molybdenum disulfide (MoS₂) is the most abundant one that occurs naturally in layered crystal form. Each single molecular layer of MoS₂ consists of three atomic layers where one atomic layer of molybdenum is sandwiched in between two layers of sulfur atoms. The MoS₂ layers are interconnected by van der Waals force with an interplanar separation of 6.5 Å, as shown in Figure 1.8 [68]. It can be separated into single- or few-layers by liquid phase exfoliation technique as discussed earlier. The MoS₂ possesses graphene like structure with tunable band gap. Also, large interlayer spacing, hydrophilic nature, less toxicity, decent carrier mobility ($\sim 200 \text{ cm}^2/\text{V} \cdot \text{s}$) and faster in-plane charge transfer are some of the advantages offered by this layered inorganic material which opens

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up some novel approaches in different areas like optoelectronics, catalysis, supercapacitor, electrochemical sensing, etc [69,70]. Recently, S. Yadav *et al.* had fabricated an ultrasensitive biosensor for the detection of SARS-CoV-2 nucleocapsid protein *via* immobilizing anti-SARS-CoV-2 nucleocapsid IgG antibody over the MoS₂ nanosheets decorated polydopamine (MoS₂-PDA) hybrid system [71]. It exhibited lowest detection limit of 2.80 ag/mL under the linear range of 10 ag/mL to 100 ng/mL.

1.2.3. Noble metal nanoparticles

Noble metal nanoparticles (NPs) exhibit inert behavior, displaying remarkable resistance to corrosion and oxidation in moist air. Moreover, they demonstrate low toxicity levels as compared to the transition metal NPs [72]. The unique properties of these noble metal NPs have led their widespread use from catalysis to nano-bio-sensing and plasmonic. Noble metal nanoparticles (NPs) encompass variety of elements, including Au, Pt, Ag, Rh, Ru, Ir and Pd NPs. Out of these, Au and Ag NPs are extensively studied for most of the applications. Some of the common synthesis procedures of noble metal NPs are chemical reduction, thermal decomposition, photochemical reduction and electrochemical deposition method [73]. Noble metal NPs are widely employed in the development of high-performance biosensors, because of their exceptional properties including high surface-to-volume ratio, superior conductivity, and excellent biocompatibility. These NPs can also act as immobilizing platforms for the biomolecule, resulting in a faster electron transfer pathway [74]. Generally, in basic medium antibodies are positively charged due to the presence of positively charged amino acids which can facilitate ionic interactions with the negatively charged surface of the Au NPs. The hydrophobic interaction also plays a role in non-covalent immobilization of antibody over the surface of the noble metal NPs [75]. Several groups have reported that incorporation of noble metal (Au/Ag) NPs can significantly augment the electrochemical response of the electrochemical sensors. J. Huang *et al.* had designed a high performance immunosensor by decorating graphene with Ag/Au nanoparticles and used for the detection of carcinoembryonic antigen (CEA) [76]. The proposed sandwich-like immunosensor can offer highly sensitive detection towards CEA-antigen under the linear calibration range of, 10 to 1.2×10^5 pg mL⁻¹ and with a detection limit of 8 pg mL⁻¹. Another group, N. Bohli *et al.* designed an impedimetric immunosensor which consists of glycosylated human serum albumin and antibody-functionalised AuNPs [77]. They followed an ‘upside-down’ mechanism where antigen

was used as a bioreceptor for the detection of the antibody. This innovative immunosensor exhibited a high sensitivity towards the detection of glycation level from the albumin.

1.2.4. Metal -oxide nanoparticles

Though the use of ultrasmall particles were known in ancient times, the modern concept of nanotechnology was initially introduced by Richard Feynman in 1959 [78]. Then, significant technological advancement has been realized by utilizing the distinct properties of the material that emerges at the nanoscale dimension. Metal-oxides are unique class of semiconducting inorganic solids that have captured the attention of research community due to their fascinating electrical, optical, magnetic, and catalytic properties. Metal-oxide (MO) consists of a transition metal atom covalently bonded with oxygen atom, and this spinal oxide is largely responsible for their distinctive magnetic and electrical behaviour [79]. However, the semiconducting nature of these metal-oxide nanoparticles (MO NPs) have emerged their potential use in photovoltaics, electrochemical devices, sensors, biomedical, switching application and so on. For the synthesis of metal-oxide nanoparticles (MO NPs), normally bottom-up approach is employed. Some most commonly followed synthesis routes include hydrothermal technique, chemical reduction technique, electrochemical deposition method and CVD technique [80]. Few examples of MOs include, CuO, NiO, Co₃O₄, Fe₂O₃, AgO, TiO₂, MnO₂, ZnO, etc. Metal-oxides are utilized in a diverse range of applications based upon their chemical reactivity, structural compatibility, catalytic activity, as well as their electronic and magnetic features. For instance, CuO, a transition metal-oxide, exhibits a remarkable redox activity towards glucose and has empowered its widespread use for the development of non-enzymatic glucose sensors [81]. Moreover, functionalization of these MO NPs can significantly boost several essential parameters, thereby enhancing their sensing activity. Rahim *et al.* had employed ceramic material (SiO₂/C-graphite) decorated CuO nanostructure for the detection of glucose that exhibits a low *LOD* of 0.06 μmol [82]. The as described sensor provides not only a good sensitivity of 472 $\mu\text{A mM}^{-1}\text{cm}^{-2}$, but also better selectivity and chemical stability under a linear range of 0.02–20.0 mM. Other groups, A. Elrahim *et al.* [83] and Kogularasu *et al.* [84] have developed cobalt-based H₂O₂ sensor by making composites with MoS₂ and rGO, respectively. The Co₃O₄-MoS₂ nanocomposites derived sensor exhibited a high sensitivity of 3000 $\mu\text{A mM}^{-1}\text{cm}^{-2}$ under the detection range of 0.02–1.0 mM. Whereas, the Co₃O₄-rGO composite electrocatalyst performed the efficient

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reduction of H_2O_2 in the concentration region of 0.05- 400 μM with 3450 $\mu\text{A mM}^{-1}\text{cm}^{-2}$ as sensitivity value. The unique properties exhibited by these materials have opened up numerous promising applications across diverse fields, to name a few are catalysis, energy storage and conversion, fuel cells, sensors, solar cells and other electronic and optoelectronic devices. [85].

1.3 Ion beam irradiation technique

Material modification techniques are employed for enhancing the performance of certain materials to address some specific applications. Thermal annealing, chemical functionalization, composite formation, doping, surface modification, ion implantation, etc. are some of the useful strategies adopted for effective modification of the materials. In general, several important aspects like surface tension, adsorption, wettability and catalysis take place at the surfaces and interface [86]. In fact, surface modification of the nanostructures plays a critical role in altering the physical, electrical, optical and electrochemical features of a system. There exist numerous ways for surface modification in materials, *eg.* physical and chemical vapor deposition method, plasma, etching, low and high energy ion beam irradiation technique, etc. Among these techniques, high energy ion beam irradiation, also termed as swift heavy ion (SHI) irradiation is a robust and controlled way of material modification [87]. In case of ion beam irradiation, energetic ion beams having energy ranging from few keV to a few hundreds of MeV range are bombarded on the target materials. These energetic ions lose energy during their path of traversal into the target material, giving rise to two energy loss mechanisms, *viz.* nuclear energy loss (S_n) and electronic energy loss (S_e). In nuclear energy loss process, the collisions between the ions and the target atoms are elastic in nature which is dominant in low energy (≤ 100 keV/nucleon) cases. On the other hand, electronic stopping is dominant in case of high energy ion (≥ 1 MeV/nucleon) irradiation technique where the ion-matter interaction is profoundly inelastic in nature [88]. Change in surface roughness, ion implantation, formation of defects/columnar tracks, doping and crystal phase modification are some of the probable outcomes corresponding to energetic ion irradiation [89].

1.3.1. Ion-matter interaction in swift heavy ion (SHI) irradiation

Swift heavy ion (SHI) irradiation is an ion beam modification technique where fast-moving subatomic particles or energetic heavy ions (≥ 1 MeV/amu) are bombarded on the target material. In SHI, the electronic stopping is governed by inelastic collisions between the

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ions and the target electrons, resulting excitation and ionization in the target atoms (Figure 1.9). SHI mainly produces columnar defects throughout their path in the target material, and the diameter of these cylindrical tracks typically lies in the order of several nm, depending on the composition and structure of the target. The depth of these latent tracks, also termed as the projectile range is the distance travelled by the energetic ions inside the material before they stop. Normally, it depends upon several factors, such as, initial kinetic energy of the projected ions, mass and charge of the ions, electronic and nuclear stopping powers, etc [90,91]. Apart from this, the nature and composition of the target material also play a critical role in determining the range of projectile ions. For example, the lattice structure, density, and electronic arrangement of the target material exert a significant influence on the stopping power, and thereby affecting directly on the projectile range [93]. Passage of SHI produces electronic excitation of the target atoms by depositing typical energy of around 1 to few tens of keV/Å along its path into the material. This enormous amount of energy can cause exotic changes in the irradiated specimen [93].

The electronic energy transfer from the dynamic ions to the target lattice can be explained through two competitive models: (i) Coulomb explosion model, and (ii) Thermal Spike model [94]. The Coulomb explosion model states that the target atoms get ionized via electronic excitation, along the path of heavy ions. As a result, long cylindrical zones of positively charged ions are produced due to the passage of ions. This model considers the charged atoms as point-like classical particles that are generated due to electronic excitation. The strong Coulombic repulsive forces acting among the like-charges would cause highly dynamic radial atomic displacement in the target material, until these ions are screened by conduction electrons. This is the theoretical basis to the formation of columnar tracks as per Coulomb explosion model. On the other hand, thermal spike model is another competing mechanism that explains the energy transfer mechanism during ion-matter interaction. According to this model, the kinetic energy is transmitted to the lattice by electron-phonon coupling which results in the increase in lattice temperature above its melting point. Afterwards, rapid quenching of the target lattice gives rise to amorphous tracks upon solidification of the molten phase of the material. Thermal spike model is predominant in metals due to their high electrical conductivity, largely available free electrons and rapid thermal quenching. While, Coulomb explosion model is predominant in insulators due to their lacking of conduction electrons [95]. SHI irradiation can change physical, chemical, optical, electrical and other spectroscopic properties of the target

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material by introducing defects in its structure. This ion-matter interaction can be controlled by adjusting the ion energy, ion type and fluence, such as to obtain desired material modification. SHI irradiation is a versatile tool for tailoring material properties that is popularly applied in various fields, like material science, nanotechnology and fabrication of semiconductor devices [96].

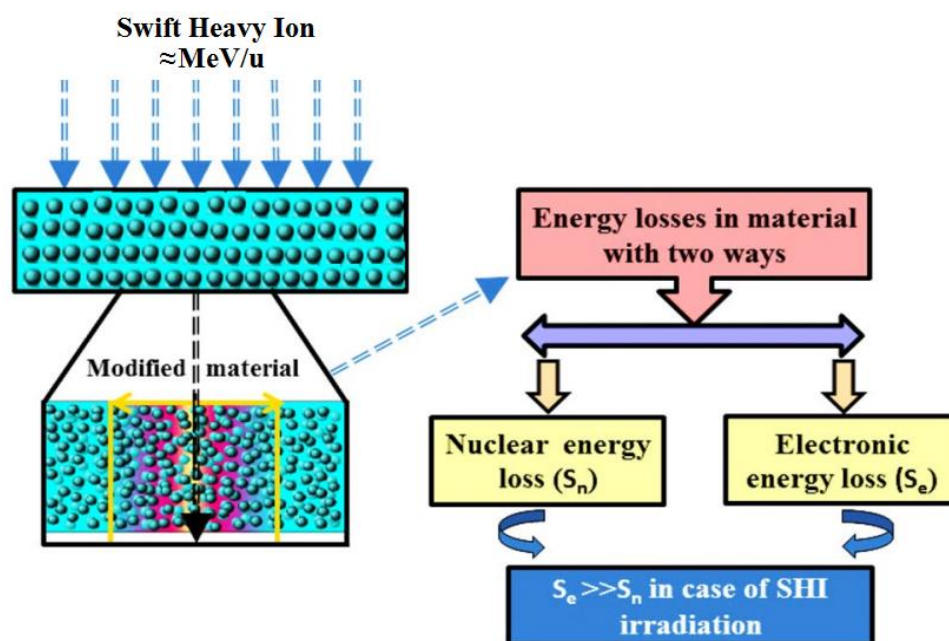


Figure 1.9: Ion-matter interaction in SHI irradiation [*Applied Physics A* (2018) 124:328]

1.3.2. Material modification via SHI irradiation

The material modification through SHI includes increase in electrical conductivity, thermal stability, change in crystallinity, enhanced electrochemical activity, structural amendment, cross-linking in polymer matrix and so on. Currently, lots of studies have focused on the SHI-based material modifications in catalysis, as the formation of randomly placed pores, vacancies, and lattice dislocations hold high potential to significantly enhance the mechanical, optical, and electrical performance of the target material [97]. The ion beam-modified systems have been reported to exhibit exceptional performance in energy storage devices, photocatalysis, electrochemical sensor and supercapacitors. For example, A. Srivastava *et al.* had irradiated 120 MeV silicon ion over polyvinylchloride-polyaniline (PVC-PANI) blends with a fluence ranging from 10^{11} to 10^{13} ions/cm² [98]. The irradiated systems exhibited significant change in conductivity with low resistance value. After

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irradiation, the polymer composite systems were found to be more sensitive towards ammonia gas and nearly 100 times rise in sensitivity value had been achieved upon increasing the fluence upto 10^{13} ions/cm². Studies have confirmed that polymeric nanostructures are highly susceptible to inter- and intra-chain modifications following SHI irradiation. Typically, a substantial deposition of electronic energy in the polymer matrix leads to a number of observable phenomena like cross-linking, chain scissioning, breaking and formation of bonds, etc. This can offer high mechanical stability to the polymer system, and could improve carrier transport through the cross-linked chains of conducting polymers, yielding high electrical conductivity [99]. The irreversible modifications of the polymeric systems are often advantageous for the fabrication of opto-electronic and electrochemical devices exhibiting superior performance. D. Sharma *et al.* had synthesized self-assembled layer by layer structure of MoS₂ and rGO, then prepared a ternary composite with polypyrrole nanotube (PPy NT) for hybrid supercapacitor applications [100]. They modified the as prepared MoS₂-rGO/PPy nanocomposite system via irradiating it with 100 MeV O⁷⁺ ion, and performed a fluence dependent electrochemical study. A high specific capacity of 1875 Fg⁻¹ was obtained for the ternary system irradiated with the fluence of 3.3×10^{12} ions/cm². The SHI irradiated hybrid supercapacitor electrode had also exhibited an improved energy density, Coulombic efficiency and cycling stability. Apart from this, extensive studies have been dedicated to 2D materials both in fundamental research and industrial applications, owing to their unique structural, optical and electrical performance along with remarkable catalytic activity. However, some 2D materials such as MoS₂, WS₂, graphene, g-C₃N₄, among others, have gained widespread utilization as filler materials to synergistically enhance the physico-chemical properties of the polymer matrix [101]. In this regard, SHI irradiation technique has immense scope for tuning properties in 2D nanostructures and their composites, as well. To exploit the effect of SHI on MoS₂, A. Ratan *et al.* had synthesized MoS₂-PVA composite system and irradiated with 80 MeV C⁶⁺ ion taking different fluences of interest [102]. They found that upon SHI treatment the conductivity of the system rises from 2.58×10^{-5} S/cm to 4.92×10^{-3} S/cm at a fluence of 3×10^{11} ions/cm². Here, the ion beam treatment was believed to have introduced adequate conducting pathways to the MoS₂-PVA system, thereby impacting the charge carrier mobility and transport properties.

1.4. Statement of problem and scope of the thesis

Immunoglobulin (*Ig*) molecules play a pivotal role in the immune response of the body, offering vital protection against pathogens and their harmful byproducts. They have remarkable ability to recognize and bind to an extensive array of antigens through their F_{ab} domains. *Ig* sensing has its unique place in biomedical applications. For many diseases, including oncological, neurological and inflammatory disease, the immune system mounts a defense against external antigens by generating specific antibodies (*Igs*). Therefore, these *Igs* can be considered as potential biomarkers for understanding the progression of several diseases and infections inside the body. As a result, the healthcare sector has experienced a growing demand for the efficient detection of these *Igs* for diagnostic purposes. In therapeutics also, a reliable quantification of these *Ig* level helps in personalized medication for the systematic treatment of several inflammatory diseases [103]. Depending upon the structural and functional characteristics, these *Igs* can be classified into five classes, *viz.* *IgA*, *IgD*, *IgM*, *IgE* and *IgG*. Among these classes, *IgG* is the most abundant one present in the blood serum. Almost 80% of the serum antibody levels comprise of *IgG*, making it suitable for assessing serological immunity [104]. Usually, most of the requisite tests performed are laboratory-based immunoassays such as thin-layer chromatography, liquid chromatography mass spectrometry, enzyme linked immunosorbent assay (ELISA), high performance liquid chromatography. These are expensive, time-consuming and require trained personnel for carrying out the experiment. To address these limitations, development of advanced biosensors with faster measurement times and lower costs is very crucial. Electrochemical detection is one of the most popular techniques, due to its simplicity, higher sensitivity, portability, lower cost and good analytical performance. Owing to these advantages, electrochemical sensing has become a method choice to deal with point-of-care application for routine health check-ups [105-107].

Across the globe, numerous groups are engaged to the development of technologically viable biosensors focusing enriched signal amplification along with excellent electrochemical stability. However, fabrication of enzyme based electrochemical sensor includes proper choice of the transducer material, prior to the antibody immobilization. Regarding this aspect, conducting polymers have drawn remarkable interest in such biomedical application due to its cost-effective processing technique, good conductivity, less toxicity and bio-compatibility [108]. Also, presence of functional moieties in the polymer backbone helps in better immobilization of different biomolecules e.g., antibody,

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DNA, enzyme, aptamers. via covalent linkage [109]. In addition, two-dimensional (2D) layered nanostructures have emerged as favourable supporting materials that can synergistically enrich the physicochemical properties of the conducting polymer based electrocatalyst. For instance, Liu *et al.* had deposited PEDOT-PSS/graphene nanoplatelets (GNPs) based composite system over fluorine doped tin oxide (FTO) coated electrode using electrospray method, and used this system as fabricated sensor probe for the selective detection of dopamine in aqueous solution [110]. The sensor exhibited a low *LOD* of 105 nM ($S/N=3$), having a sensitivity value of $27.7 \mu\text{A} \mu\text{M}^{-1}\text{cm}^{-2}$. Y. Song *et al.* have developed an electrochemical immunosensor using a composite system of reduced graphene oxide/MoS₂@polyaniline-layered nanosheets (rGO/MoS₂@PANI) for the detection of CEA [111]. The immunosensor exhibited a selective detection of CEA with a low detection limit of 0.3 pg mL^{-1} . Noticeably, the prepared immunosensor exhibited good reproducibility, high sensitivity and excellent stability. The reports show that, in situ or ex-situ incorporation of certain 2D nanostructure, such as graphene derivatives and TMDC candidates as filler material in the polymer system can enhance the sensing activity to a great extent. It will emphasize the stability aspect of the polymer matrix as well as improve the electron transfer kinetics and facilitate better electron conducting pathway to the transducer [112]. Apart from this, decoration of AuNP over the surface is also a useful approach for developing a modified electrode which can improve the performance of biosensor [113]. Nanoscale gold has been attracting much attention in electrochemical sensing due to its biocompatibility, chemical stability, superior electrical conductivity, high catalytic activity and larger surface to volume ratio. In this regard, Phongphut *et al.* presented amperometric sensor on screen printed carbon electrodes (SPCEs) based on the inkjet nanocomposite of Au/PEDOT-PSS for the detection of triglyceride (TG) with a wide dynamic range from 0–531 mg/dL, with response time of 30 s and having *LOD* of 7.88 mg/dL [114]. Similarly, Khan *et al.* had also fabricated an Au deposited PEDOT-PSS based bioelectrode for efficient detection of xanthine (XA) in real samples [115]. Thus, the inherent properties of each constituent materials of such novel hybrid nanostructure will be collectively beneficial for the improved performance of the modified transducer.

It has been discussed in section 1.1.3 that non-enzymatic sensors offer several advantages over the enzymatic ones including extended lifespan, lower cost and simplified storage measures. Several biological analytes can be detected through non-enzymatic

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approach by using certain transition metal-oxides [116]. Among them, glucose is one of the simple carbohydrates found in body essential for metabolic activity. But higher level of glucose in blood eventually leads to chronic health issue called hyperglycemia. An untreated hyperglycemia may cause tissue damage, affect kidneys, eyes and heart that would give rise to severe health problems [117]. According to World Health Organization (WHO), the global prevalence of diabetes stands at approximately 450 million presently, and this number could potentially escalate to 700 million by 2045 [118]. So, the rapid, cost effective and sensitive detection of glucose hold immense potential in the field of clinical diagnosis and routine health monitoring. Again, hydrogen peroxide (H_2O_2) is a simple peroxide found in nature which exhibits both oxidizing and reducing activity depending on the pH. It is a side product generated from some enzyme-catalyzed biochemical reactions such as glucose oxidase@glucose, lactate oxidase@lactose, cholesterol oxidase@cholesterol, etc. The concentration of H_2O_2 in urine can provide information of the entire-body oxidative stress which can be useful for monitoring renal function and diagnosing several serious diseases like atherosclerosis, Parkinson's disease, diabetes, cancer, etc. An elevated H_2O_2 level in body can also damage the DNA or protein molecules by forming reactive oxygen species [119,120]. Therefore, proper monitoring of glucose and H_2O_2 levels in body fluids is of utmost importance from both diagnosis and healthcare perspectives. As it provides essential information regarding body metabolism [121]. For non-enzymatic electrochemical detection of such analytes, careful selection and appropriate modification of the metal-oxide nanostructures is crucial for an enriching detection activity [122]. Regarding this, the essence of metal-oxide NPs for fabrication of non-enzymatic electrochemical sensors have been discussed in section 1.2.4. Interestingly, Amirzadeh *et al.* developed a non-enzymatic glucose sensor based on PEDOT-PSS/CuO/MWCNT matrix that has accomplished a very good sensitivity ($663.2 \mu\text{A}\text{mM}^{-1}\text{cm}^{-2}$), catalytic activity, as well as a wider linear range (up to 10 mM) with a low detection limit of 0.23 mM [123]. Another novel electrochemical glucose sensor was fabricated by Jae-Min *et al.*, using three-dimensional MoS_2 /graphene aerogel [124]. The *LOD* and sensitivity of MoS_2 /graphene derived sensor were found to be 0.29 mM and $3.36 \mu\text{A}/\text{mM}$, respectively, within a broad linear range of 2-20 mM. Rashed *et al.* have synthesized an electrocatalyst system by doping polythiophene with CuO (Pth-CuO) for non-enzymatic amperometric determination of H_2O_2 [125]. The sensor offered a high sensitivity of $442.25 \mu\text{A}\text{mM}^{-1}\text{cm}^{-2}$ under a dynamic linear range of 20 – 3300 μM , with a *LOD* value of 3.86

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μM . The above literature shows that incorporation of conducting polymers and 2D nanostructures with the metal-oxide NPs can potentially improve the morphology, catalytic activity and stability of the electrocatalyst system, thereby resulting a more effective non-enzymatic detection and transduction mechanism.

Apart from this, SHI based material modification possesses a higher possibility for tuning several physico-chemical properties, thus expanding their range of functionalities for specific applications. The expected modification includes change in structural, morphological and spectroscopic properties, a decrease in surface resistance of the film, formation of lattice defects and increase in sensitivity of the polymer-based sensors [126]. Regarding this, A. Ratan *et al.* reported that the electrical conductivity of MoS_2 dispersed PVA matrix had dynamically increased from 2.58×10^{-5} to 2.97×10^{-3} S/cm upon irradiation of 100 MeV Si^{9+} ion of fluence 1×10^{13} ions/cm² [127]. They proposed that SHI treatment had decreased the disorderness of MoS_2 sheets and could result in rich interfacial contact and uniform dispersion of the nanosheets in the PVA matrix. In another work, H.K. Patil *et al.* had performed the modification PANI/SWCNT by using 100 MeV oxygen ion [128]. The composite matrix irradiated with a fluence of 1×10^{10} ions/cm² had exhibited an improved electroactive nature which was further used for the detection of divalent cobalt (Co (II)) metal ions. This ion beam modified system could selectively detect Co (II) ions upto a very low detection limit of $0.355 \mu\text{M}$. A. Anitha *et al.* had spin coated thin film of WO_3 over indium tin oxide (ITO) coated glass substrate and irradiated with 150 MeV Ni^{11+} ion of different fluences [129]. To be mentioned, the film irradiated with a fluence of 5×10^{12} ions/cm² exhibited an improved electrocatalytic activity towards oxidation of guanine in 0.1 M PBS solution. They had established that the ion beam modified film can selectively detect guanine upto a low concentration limit of 2.3 nM under a wide linear range of 0.005-160 μM . Indeed, SHI based material modification can potentially reshape the electrochemical performance of filler incorporated polymer-based nanosystems.

Based on the above observation, we have drawn interest to develop an advanced transducer element capable of delivering high surface area, better catalytic performance along with efficient immobilization of the biomolecules. The literature cited in this section, as well as in sections 1.2.1, 1.2.2, and 1.2.3 demonstrate that conducting polymers and 2D nanostructures effectively meet the need for higher surface area with an effective biomolecule immobilization procedure. Additionally, the inclusion of surface-deposited AuNPs can enhance the conductivity and catalytic performance of the composite

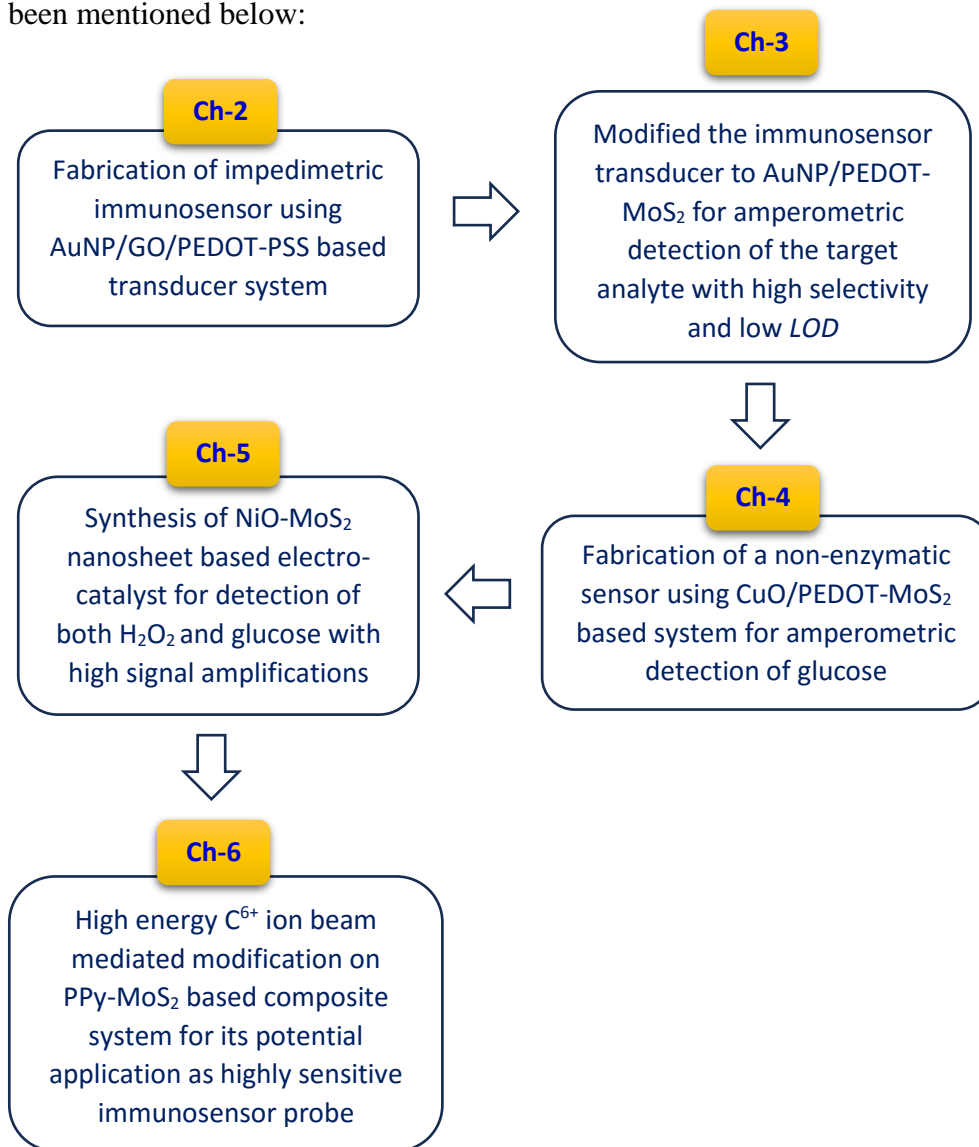
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nanosystem. Thus, a conducting polymer and 2D layered material based composite system modified with AuNPs will be a promising transducer element to design an innovative immunosensor for the enzymatic detection of *IgG*. On the other hand, literature mentioned in section 1.2.4 and current section indicate that a composite of transition metal-oxide NPs with conducting polymer and 2D layered structures will be a useful strategy for non-enzymatic detection of glucose and H_2O_2 with high sensitivity. However, proper realization of redox activity and interfacial charge transfer kinetics of such hybrid nanosystem are very essential for designing high-performance sensing probe. Lastly, we focused our study to understand the effect of ion beam driven material modification on such composite nanostructures. The effect of SHI on the sensing performance of immunosensor is an intriguing avenue that needs further exploration.

Based upon the above observations, the following objectives have been set to address the gaps and necessary modifications in the existing works. The following are the objectives of this thesis:

- 1) To synthesize conducting polymer (Polypyrrole, PEDOT, Polyaniline) by both chemical and electrochemical deposition techniques and functionalize them with 2D layered materials, like graphene, MXene, TMDCs, etc.
- 2) To immobilize antibodies, enzyme over the surface of the electrodes via covalent coupling.
- 3) To design and fabricate immunosensor with enhanced sensitivity, selectivity, reproducibility, linearity and faster response towards efficient detection of Immunoglobulin G (*IgG*).
- 4) To fabricate non-enzymatic electrochemical sensor for sensitive and selective detection of glucose and H_2O_2 .
- 5) To irradiate SHI on the synthesized electrodes and compare the sensing activity of both pristine and irradiated one.

- The flow chart of the works that have been performed throughout the thesis has been mentioned below:



1.5. Experimental tools and techniques

1.5.1. X-ray diffraction (XRD)

To exploit structural and crystallographic properties of the synthesized electrodes and nanoparticles, XRD patterns were acquired on a BRUKER AXS (D8 FOCUS) diffractometer equipped with CuK_{α} monochromatic radiation ($\lambda=1.5406 \text{ \AA}$). It is a versatile method where monochromatic X-rays of known wavelength is directed towards the target material. The crystal planes act as atomic size gratings which diffract the incident X-rays.

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The condition for constructive interference of the diffracted beams, also called Bragg's law is used for determining the interplanar spacing of the crystal structure [130]. The Bragg's law is given as,

$$2d\sin\theta = n\lambda \quad (1.1)$$

Where, d = interplanar spacing

θ = angle of incidence

λ = wavelength of X-ray used

n = order of diffraction pattern

Another important relation used in the thesis is Scherrer's formula for determination of average crystallite size of the nanostructures. The formula is given as,

$$D = \frac{k\lambda}{\beta\cos\theta} \quad (1.2)$$

Here, D is the crystallite size, k is Scherrer's constant, β is the full width half maxima of the XRD peak, θ is the position of peak in the XRD response [131].

1.5.2. Fourier transport infrared spectroscopy (FT-IR)

It is performed to investigate the presence of functional groups and vibrational modes of the chemical bonds. Every molecular bond exhibits different vibrational mode, which is the signature feature to identify the presence of that specific bond. The infrared (IR) radiation stimulates the vibration of molecules at a particular wavelength which is measured by FT-IR. This spectroscopy is usually performed under the IR range of 400-4000 cm^{-1} . This IR spectrum is splitted into two beams by using a beam splitter (semi-transparent mirror), while one part is reflected by the fixed mirror and another part of beam is reflected by a movable mirror. When these two splitted rays meet after reflection, they will give rise to interference patterns depending upon the position of the movable mirror. This overlapped beam is then transmitted through the sample where the absorption of specific wavelengths will take place depending upon the vibrational modes of the molecular bonds. Afterwards, the detector forms an interferogram pattern from the transmitted beam. For proper analysis, this interferogram is treated mathematically through Fourier transformation and represented in the form of intensity spectrum as a function of wavenumber. This whole technique is called Fourier transform infrared spectroscopy or FT-IR. Throughout the thesis work, the FT-IR spectra of the powder and peeled out film samples were recorded on a PerkinElmer Spectrum 100 instrument in Sophisticated Analytical Instrumentation Centre (SAIC), Tezpur University.

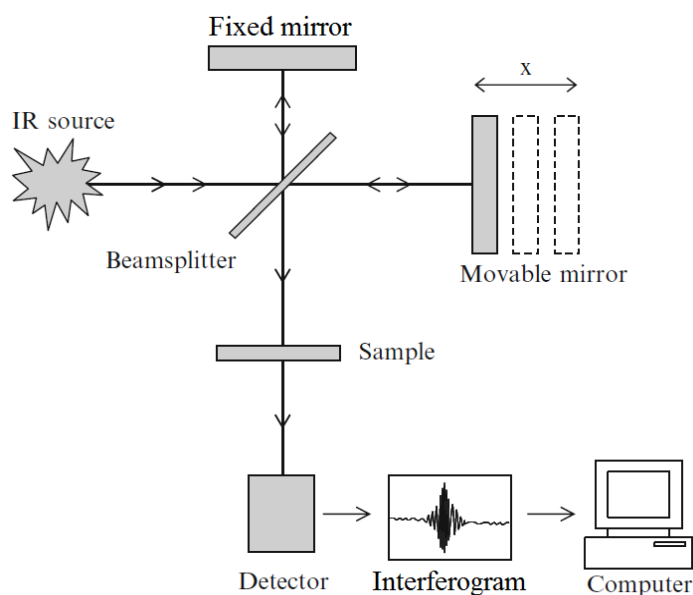


Figure 1.10: Schematic of the major components of FT-IR [*Methods and Protocols* (2012) 187-211]

1.5.3. Scanning electron microscope (SEM)

It is a very useful technique to study the morphology and composition of the thin films as well as nanoparticles. SEM consist of an electron gun made up of tungsten that can produce electrons by thermionic emission. These primary electrons are the accelerated through high potential in the form of electron beam (Figure 1.11). The electron beam is magnified at a spot size of 0.4 nm-5 nm of the target specimen with the help of magnetic lenses [132]. The bombardment of electron beam with the specimen will produce secondary electrons, characteristic X-rays and back-scattered electrons. Depending upon the density of secondary electrons emitted from different parts of the sample, the system will generate a highly magnified gray scale image. Moreover, the characteristic X-ray produced from the inner shell electronic transition of the target atom will give information about the elemental composition. In the present thesis, the morphology, elemental composition as well as elemental mapping of the synthesized nano systems were performed by SEM, JEOL-JSM-6390LV Scanning electron Microscopy at Dept. of Physics, Tezpur University.

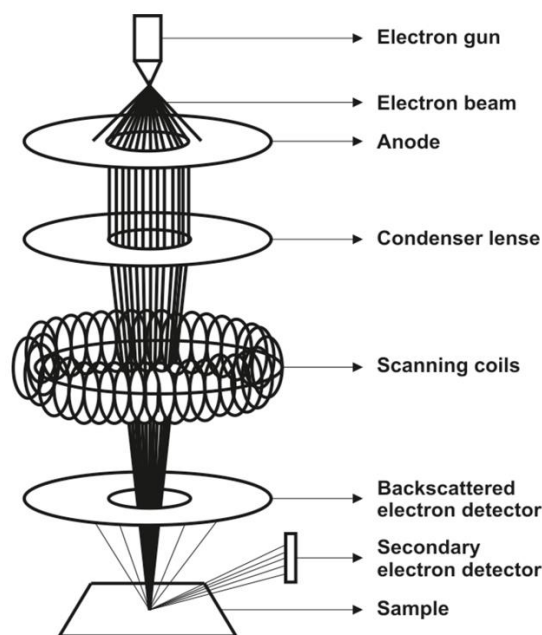


Figure 1.11: Schematic of the major components of SEM [S. Carrara, *Doctoral dissertation, Université de Strasbourg, 2017*]

1.5.4. Field emission scanning electron microscope (FE-SEM)

It is an advanced characterization technique for realizing the morphology of the specimen. The mechanism of FE-SEM is similar to SEM, while the major difference is the use of field emission electron gun source in FE-SEM. The electron gun is basically a V-shaped tungsten wire (or lanthanum hexaboride (LaB_6)) with a pointed tip [133]. A potential gradient is used for emission of electron beam, also called cold field emission mechanism which can be attained at room temperature. In both SEM and FE-SEM, the sample surface must be conductive for better image quality. Whereas, a coating of conductive material (Platinum coating) having thickness ~ 0.5 nm to 3 nm is used in case of insulating specimen [134]. Use of field emission gun allows to generate highly coherent and intense beam of electron offering better resolution and high magnification. Throughout the work, the morphologies of the synthesized nanostructures were captured by using FE-SEM, JSM-7200F instrument available at SAIC, Tezpur University.

1.5.5. Transmission electron microscopy (TEM)

In this electron microscopy technique, highly energetic electron beam is used to analyse the topography of a specimen at very high magnification. Here, the magnified electron beam is allowed to pass through the specimen. The primary electrons in the electron beam experienced elastic as well as inelastic scattering during the interaction with the target

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atoms. Then, these transmitted electron beams are further magnified and focused through magnetic lenses and incident on the fluorescent screen. The sample preparation is very crucial in case of TEM as the electron beam has to transmit through the specimen with thickness ~ 100 nm. A diluted dispersed solution of the nanoparticles drop casted over copper grid is generally used for the TEM study. It can provide information regarding topography of the target material at atomic scale offering magnification upto 300 kx times [135]. In addition, selected area electron diffraction (SAED) is another feature of this technique which can provide crystallographic information of the specimen. The TEM analysis was performed in TECNAI G2 20 S-TWIN at SAIC, Tezpur University.

1.5.6. Cyclic voltammetry (CV)

It is a popular and powerful technique for understanding the redox (reduction and oxidation) mechanism of molecular species. It is performed in an electrochemical cell consisting of working electrode, counter electrode and reference electrode. In CV, the current response of the working electrode is recorded as a function of applied potential which is a triangular waveform. The redox reaction occurs at the surface of working electrode and the respective potential is measured w.r.t. the reference electrode. The potential is swept at a constant rate in-between the initial and final value of potential window both in forward and backward directions. During the linear sweep of potential, the redox reactions take place over the working electrode due to interfacial electron transfer giving rise to current flow. The graphical representation of current as a function of potential is called cyclic voltammogram. It is very useful in understanding the electrochemical activity and redox states of various substances.

All the electrochemical deposition and measurements were performed in GAMRY (INTERFACE 1010E Potentiostat/Galvanostat/ZRA) and Frequency Response Analyzer (Biologic-SP 200) Potentiostat instruments.

1.5.7. Electrochemical impedance spectroscopy (EIS)

EIS is a versatile technique providing valuable insights into the kinetics and mechanisms of diverse electrochemical systems. It is widely applied across various area e.g. corrosion studies, energy storage, chemical and biosensing, etc. Different parameters like electrolyte resistance, charge transfer resistance, capacitive behaviour of double layer, kinetics of redox process, mass transport phenomena, etc. that arise at the electrode-electrolyte interface during an electrochemical process can be effectively studied by using EIS. It

operates by perturbing an electrochemical system by applying sinusoidal ac signal and monitoring the resultant variation in current or voltage. The EIS is normally performed in the electrochemical cell with three-electrode set up as discussed in section 1.5.6. During the study, the ac frequency is varied from few mHz to MHz range as per requirement and obtained frequency dependent measurement is simulated to an equivalent electrical circuit that consists of some passive electrical elements (e.g., resistance, capacitor, inductor). To perform this, the electrochemical analyzer software provides extension to fit the impedance data in suitable electrical model circuit. Furthermore, various interfacial parameters of the system can be estimated with the help of these fitted data. In our study, the EIS measurements are carried out in a three-electrode setup by varying the frequency from 1 Hz to 1 MHz at 0 V dc bias potential and the electrolyte solutions are chosen as per the systems under study.

1.5.8. Chronoamperometry

It is an amperometric method where the change in current in the W.E. is monitored as a function of time at a fixed dc bias potential. This method is popularly employed in the electrochemical sensing where a direct redox reaction is used for determination of the analyte concentration. This experiment is executed in a three-electrode setup similar to section 1.5.6 in which a fixed step potential is applied at the W.E. The selection of this potential depends on several factors including the nature of the transducer, mass transport and redox states of the analyte. The current in the amperometric response varies according to the concentration of analyte, thereby offering a quantitative information regarding the target analyte. In Chapter 4 of the present thesis, a potential of +0.6 V has been used as a dc bias potential along with a sample period of 0.2 s in the chronoamperometric experiment for detection of glucose.

1.5.9. Differential pulse voltammetry (DPV)

DPV is also a popular amperometric technique where a small amplitude having short pulse is superimposed with the linear sweep potential waveform and respective current response is monitored. Current is measured before the application of the pulse and at the end of each pulse and the difference between the currents is calculated. This procedure effectively reduces the background current due to the DC ramp. Thus, this procedure results in a measuring the Faradaic current free from most capacitive current. The major advantage of DPV is low capacitive current which leads to high sensitivity. The small step sizes (10-100

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mV) in DPV also lead to narrower voltametric peaks and thus DPV is often used to discriminate analytes that have similar oxidation potentials [136].

1.5.10. Transient capacitance plot

It is an effective technique where the interfacial capacitance between the electrode-electrolyte boundary is recorded as a function of time. The electrode-electrolyte interface acts as a parallel plate capacitor and the accumulated counter ions behave as dielectric interlayer. The measurement of this capacitance at a single frequency can directly reflect the interlayer characteristics. So, any perturbation in the interlayer caused by adsorption, charge accumulation, carrier transport, etc. will give rise to change in capacitive behaviour. From this change in capacitive value, the selective determination of the target analyte can be obtained. In our work, this particular experiment was performed in a three-electrode setup for impedimetric determination of goat anti-mouse *IgG*. The fabricated immunosensor was considered as W.E. and the interfacial capacitive behaviour was monitored at a low frequency of 77 Hz and at a moderate frequency of 1 kHz obtained from an ac signal of 10 mV by applying a dc bias potential of 0.8 V. This potential has been chosen through Mott-Schottky plot, as explained in section 2.3.4. This 0.8 V applied dc potential is also known as accumulation bias potential. This is because at this potential huge accumulation of counter ions takes place at the working electrode offering significant alteration in the capacitive response as well as carrier movement. Depending upon this accumulation bias potential, the fermi levels of the electrolyte and electrode align or junction formation takes place at the interface. It has high significance for accurate monitoring of the interlayer capacitance in electrochemical measurements. Besides, the value of change in capacitance due to the adsorption of antigen over the immunosensor probe can be monitored for detection of the target analyte in case of impedimetric sensing.

1.5.11. Ion beam irradiation set up

The SHI based material modification was performed in Inter University Accelerator Center (IUAC), New Delhi. The ion acceleration is executed by using a 15 UD Pelletron accelerator which can be operated at a terminal potential of upto 15 MV and can produce dc as well as pulse beam of different elements. The Pelletron is a tandem electrostatic accelerator similar to Van de Graaff generator, capable of accelerating ion beams of stable nuclei depending on the ion species. The acceleration of the ion beam is due to the electrostatic attraction between the negative ions and the high positive potential terminal.

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The ions emerging out of the accelerator have energy gain $E = V(q+1)$, where V = terminal potential, q = charge state of the positive charge. The desired energy in the ion beam is maintained with the help of an analyzer magnet. Finally, a switching magnet is used to bend and direct the ion beam into any of the six established beamlines.

We have used Material Science beamline to perform our desired experiment. For this, the as-synthesized PPy-MoS₂ nanocomposite samples (in the form of pellet and ITO supported film) were first attached in the sample holder which is a solid rectangular ladder and then the ladder was loaded in the beam chamber. A high vacuum of $\sim 10^{-6}$ bar was maintained in the beam chamber. In our work, 90 MeV C⁶⁺ ion beams with fluence in the range of 10^{10} - 10^{13} ions/cm² were used for the irradiation of samples. The projectile range of the beam was 132 μm with an electronic stopping (dE/dx) value of 1.379 keV/($\mu\text{g}\cdot\text{cm}^{-2}$). Depending upon the area of the sample pellet and spot size of the beam, the time required for each fluence per sample have been calculated by using the formula: $D = \frac{I \times t}{q \times A}$, here D , I , t , q and A signify ion beam fluence, beam current, total exposure time, charge state of ion and exposure area; respectively. The entire machine is operated by advanced, dedicated computers from the control room. The sample change process and positioning of sample holder (ladder) is also controlled remotely.

1.1.12. Chemicals and Reagents

Mouse *IgG* (Sigma-Aldrich, reagent grade, $\geq 95\%$ pure), goat anti-mouse *IgG* serum solution (Abcam and Merck, 2 mg/mL), bovine serum albumin (SRL, 10% dilute solution in PBS), phosphate buffer saline (10x PBS, SRL, 0.1 M, pH= 7.4) are used as received. 3,4-Ethylenedioxythiophene (EDOT, $\geq 97\%$ pure), Pyrrole (Sigma-Aldrich, 98% pure), Lithium Perchlorate (LiClO₄, Sigma-aldrich, 95+% pure), acetonitrile (Rankem, 99.5% pure), Graphite Fine Powder (Loba Chemicals, 98% pure), poly (4-styrenesulfonic acid) (PSS, Aldrich, M.W.~ 75,000 GPC, 18 wt% in H₂O) Potassium permanganate (KMnO₄, SRL, , $\geq 99.5\%$ pure), sodium nitrate (NaNO₃, $\geq 99\%$ pure), hydrogen peroxide (H₂O₂, EMPLURA, 30% pure), chloroauric acid (H[AuCl₄], Alfa Aesar, 99.9% pure), potassium chloride (KCl, Merck, $\geq 98.5\%$ pure), Glutaraldehyde (Merck, 25% pure) and glass electrode coated with indium tin oxide (ITO) were procured through authorized vendors. Apart from these, other chemicals that have been used throughout the work are as follows: ammonium molybdate (SRL, 99%), thiourea (Rankem, 99% pure), ethanol (EMSURE, 99% pure), copper sulfate pentahydrate (CuSO₄.5H₂O, Rankem, 99% pure), molybdenum

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disulfide (MoS_2 , Research Lab, 98% pure), N-methyl-2-pyrrolidone (NMP EMPLURA, $\geq 99\%$ pura), nickel (II) chloride hexahydrate ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, SRL, 98% pure), hydrochloric acid (HCl, Rankem, $\geq 35\%$ pure), polyvinylpyrrolidone (PVP, Loba Chemicals, M.W. $\sim 40,000$ GPC), ethylene glycol (EG, M.W. ~ 2000 , Alfa Aesar), ammonia (NH_3 , 25% pure) solution, ammonium persulfate (APS, Merck, $\geq 98\%$ pure), poly-vinylidene fluoride (PVDF, SRL, M.W. $\sim 534,000$ GPC), sulfuric acid (H_2SO_4 , EMPLURA, $\geq 98\%$ pure).