

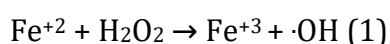
NECESSITY OF THE RESEARCH WORK

I.1. OBJECT, SCOPE AND APPLICATIONS

In recent era nanomaterials explore a wide-application in various field of science such as Physics, Chemistry, Biomedical and Materials science. Nanomaterials are the particles having the sizes 1 to 100 nm, such smaller size of the particles explore the hidden surface and consequently increases the surface by volume ratio and tuning the electrical, photochemical, mechanicals, optical and magnetic properties from the bulk materials [1]. Due to their unique property, nanomaterials are used in drug delivery, biosensor, photonics, Nano medicine, electronics and industries. Recently Nano medicines grew a huge attention for their tremendous applications in cancer therapy [2-4]. Nanoparticles based medicines are safe and successful as they entered the biological cancer cells easily without disturbing the normal cells it brings the ability of targeting the cancer cells and low cytotoxicity. Knowledge of cancer cell membrane displayed that membranes are positively charged and counter negatively charged nanoparticles are easily entered this cell by a unique endocytic mechanism consequently ruptured the cell membrane and showing cytotoxicity in cancer cells. Another vast application of nanomaterials in textile industry for their unique semiconducting property, they are used as photo catalysts for degradation of environmental harmful textiles waste organic dyes. Recently Nano-photo catalyst is the main focused of researcher due to easy to production, low cost, environmental friendliness, abundance of their raw materials in earth crust. In addition, they have some drawbacks in photocatalytic processes; they can easily recombine their charge carriers and employ low amount of light during the process [5-7]. So it is the challenge for researcher to improve the electron hole separation efficiency for better utilization of light in order to getting high quantum efficiency. Recent study reveals that doping of substances to the semiconducting nanomaterials improves the charge separation. Doping could induce the formation of defect by creating oxygen vacancies and trapped state which captures only electron to inhibit the electron-hole recombination thereby increasing the photocatalytic quantum yield. Environmental friendly nanomaterials preparation is very much important for

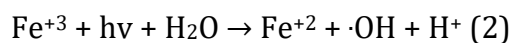
researcher as their production requires low cost, simple green method and less environmental hazardous. Other convenient methods like as solvothermal, hydrothermal method, aqueous precipitation method, sol-gel method is more effective and we can easily control their shape and size however all these methods are costly and produced hazardous pollutants [8-9].

Nanomaterials have been investigated as promising tools for the advancement of drug and gene delivery, diagnostic biosensors and biomedical imaging. Nanomaterials of specific morphologies and sizes can be synthesized readily using both chemical and physical methods. Most of these methods are reported as extremely costly, hazardous and great threat for environment. Water pollution by emergent pollutants like as pesticides, pharmaceuticals and hazardous chemicals is a serious problem for environmental aspect, so removal of these is a challenging task [10]. In particular organic dyes are frequently used in industrial perspective which is a wide group of chemical compounds, treated as hazardous organic pollutants due to their retention property in water over a long time and these are accumulated as sedimentation in water [11]. Basically, these are misbalance the eco-system and left a bad impact on aquatic bodies. Among all approach to removal of contaminants of waste water, AOP (advanced oxidation process) attracted more to researchers due to their sustainable green approach for illuminating the contaminants as the left minimum side effect in the environment [12-13]. In this process the contaminants are removed by highly reactive radical's species, such as $O^{\cdot-}$, $O_3^{\cdot-}$ and $\cdot OH$, in particular AOP photocatalytic, Fenton and photo Fenton reaction are mostly studied [14-15]. In photocatalytic process the organic contaminates are illuminated by irradiation of UV-Visible light with certain amounts of catalyst in order to generation of photoelectrons(e^-) and photo-holes (h^+) followed by production of highly reactive $\cdot OH$ and $O^{\cdot-}_2$ radicals which are then attacked organic pollutants to degrade into small fragments. Fenton process involves the production of highly reactive short lived $\cdot OH$ radicals in a redox cycle involving the change of oxidation state in between reaction of iron salt (Fe^{2+}) and hydrogen peroxides (H_2O_2). [16]



The regeneration of initial oxidation state of metal ions accelerates the rate of Fenton reaction and enhancing the overall rate of removal of organic contaminates. In photo

Fenton process both of hydrogen peroxide and UV -visible light are assisted the reaction. In this process the newly generated photoelectrons are initiated the regeneration of initial oxidation state (active Centre), and a high-rate constant in degradation process are observed.



Metal oxide nanozymes are a class of nanomaterials that exhibit enzyme-like catalytic activity due to their unique structural and compositional properties. A type of nanomaterials known as metal oxide nanozymes exhibits enzyme-like catalytic activity as a result of special structural and compositional characteristics. Metal oxide nanozymes are inorganic substances, generally metal oxides, as unlike to natural enzymes, which are often made up of proteins [27–28]. Due to their potential uses in a variety of domains, such as biomedicine, environmental remediation, and industrial processes, these nanozymes have attracted a lot of attention recently [29–30]. The following are some of the main features and benefits of metal oxide nanozymes: The catalytic functions of natural enzymes are mimicked by metal oxide nanozymes. By providing surfaces for reactant molecules to contact, they can speed up chemical processes by lowering the activation energy necessary for the reaction to take place. As opposed to enzymes made of proteins, which can be sensitive to variations in pH, temperature, and other environmental factors Metal oxides are relatively inexpensive compared to natural enzymes, which can be difficult and expensive to produce and purify [31-33]. Metal oxide nanozymes' catalytic activity can be tailored by changing elements such particle size, composition, and surface shape. Researchers can create nanozymes with particular features for specific applications thanks to their tunability. Some metal oxide nanozymes are biocompatible and safe to employ in biomedical settings without having any negative effects. Metal oxide nanozymes have been investigated for a number of biomedical uses, including cancer treatment, drug transport, and bio sensing. For example, they can be utilized as synthetic enzymes to speed up bodily processes [34-35]. For the oxidation of contaminants in water and the atmosphere, metal oxide nanozymes can be utilized. They can degrade organic pollutants and change dangerous compounds into less damaging ones. Despite their many benefits, metal oxide nanozymes may not have the same level of substrate specificity as natural enzymes, which are highly specialized to their substrates [35-38].

Metal oxide nanozymes' design and optimization are difficult due to the poorly known mechanisms behind their catalytic activity. With regard to potential toxicity and regulatory considerations, the usage of nanomaterials—including metal oxide nanozymes—needs to be carefully considered. In conclusion, metal oxide nanozymes are promising nanomaterials with catalytic activity resembling an enzyme and a wide range of applications [39-41]. Their potential to transform businesses and address significant issues is becoming more and clearer as research advances. Metal oxide nanozymes of various sorts have been created and investigated for their catalytic characteristics. Here are some instances of different metal oxide nanozymes and some possible uses for them.' MnO_x nanozymes are useful for anti-inflammatory and antioxidant applications because they have the potential to scavenge reactive oxygen species (ROS). Due to their catalytic activity in producing chemiluminescence, they can also be used in bio sensing and bio imaging. Ceria nanozymes can imitate the function of genuine peroxidase enzymes because they exhibit peroxidase-like activity [27, 42-45]. They have been investigated for a number of biomedical uses, including ROS scavenging, medication administration, and enzyme-linked immunosorbent tests (ELISAs). Mn₃O₄ nanozymes are effective for environmental cleanup since they have demonstrated catalytic activity in the degradation of organic contaminants and antibiotics in water. Due to their potential as a cancer therapeutic, they have also been researched. Due to their capacity to produce reactive oxygen species, they have also been investigated for their potential in the treatment of cancer [27, 28, 29, 45-47]. Iron oxide nanozymes can be used in bio sensing applications since they exhibit peroxidase-like activity. They have been researched for their potential in targeted drug delivery and cancer theranostics (therapy and diagnostics). Both oxidase- and peroxidase-like activity are displayed by these nanozymes. For detecting glucose, cholesterol, and other analyses, they have been utilized in colorimetric assays [48-51]. The peroxidase-like activity of ZnO nanozymes has been studied because it can be used in bio sensing and water purification. They are also being researched for possible medicinal uses. Several Co₃O₄ nanozymes have been demonstrated to peroxidase and catalase like property [52-56]. They have been utilized in organic dye degradation as well as colorimetric assays. These are but a few instances of metal oxide nanozymes and some of their possible uses. Researchers are constantly coming up with new materials and uses for these catalytic nanoparticles as the field of nanozymes quickly develops. Metal oxide nanozymes are useful tools for many

disciplines, including biomedicine, environmental research, and materials engineering, due to their distinct characteristics and tenability.

I.2 Importance of carbon and metal oxide based nanomaterials;

NPs are usually classified based on their dimensions, morphology, shape, composition, agglomeration and uniformity. NPs are classified into four categories based on their electron movement of dimension: Zero dimension(0D), One dimension (1D), including thin films which are mainly used in electronic devices and sensor mechanisms; Two dimension (2D), which contains second-generation NPs for example carbon nanotubes, with high absorption and stability; and Three dimension (3D), NPs which includes dendrimers and quantum dot. Based on the chemical compositions the NPs are also classified into inorganic, organic, and carbon-based NPs

I.2.1 Carbon based materials:

Fullerene

Growing the demand of nanomaterials carbon contributes a huge, carbon nanomaterials have the key composition is carbon and other element like oxygen and hydrogen may be present as a functionalization [56]. Carbon nanomaterials including fullerene, carbon nanotubes (CNT), Graphene, graphene oxide and fluorescent carbon quantum dots (CQDs). These carbon based nanomaterials have attracted huge attention to the researcher and engineers due to their tremendous applications biomedical and engineering perspective and also they have extraordinary physical, chemical, optical, mechanical, thermal properties [57]. Fullerene is the example of zero dimension nanomaterials which consist of carbon only and form a Bucky ball like structure tubular and ellipsoidal form. Oswa first predicted the fullerene and kroto first experimentally discovered the Buckminster fullerene and owing to this discovery he was awarded by noble prize in 1985. Many of characterization technique tells that C₆₀ (buck minister fullerene) is consist of 12 pentagonal and 20 hexagon rings and symmetrically filling of the rings looks like graphite. Since then many others fullerene have been discovered for example C₄₀, C₅₆, C₇₀, C₈₀, and C₈₄. They all have unique properties and different densities some of them are chiral the inherent properties made them a brilliant candidate in materials science and engineering [58].

Carbon Nano tube (CNTs)

Carbon Nano tube (CNTs) was theoretically described by Radushkevich and Lukyanovich in 1952 when it was first detected and [59]. After 24 years later, scientist discovered SWCNTs prepared by pyrolysis of a mixture of benzene and hydrogen [60]. In recent time Iijima's method for preparing MWCNT is well accepted method and it is obtained arc evaporation of C_{60} . The CNTs are the example of 1-D nanomaterials and each carbon atom is sp^3 hybridized. According to their structure the carbon nanotube are classified as SWCNTs and MWCNTs. If graphene sheets rolls on someone get The SWCNTs and if several SWCNT are co-exist then MWCNTs are obtained. Out of many fundamental properties the Chirality and optical properties are the most common intrinsic property the CNTs [61]. The small change in the roll on angle θ might have the greatest influence on optical and electronic properties. Because it tune the bandgap of that materials therefore it resulting in the semiconductor or conductor. Fortunate to its physical and chemical property CNTs are used in several optoelectronic and biomedical applications.

Graphene

The concept of graphene was first introduced by Hanns-Peter Boehm in 1961-62 after publishing the paper where he coined the term graphene that extremely thin hypothetical single layer graphite structure [62]. After than many attempts has been done finally in 2004 Andre Geim and Konstantin Novoselov[63] first isolated and properly characterize, This work resulted in the two winning the Nobel Prize in Physics in 2010 "for ground breaking experiments regarding the two-dimensional material graphene. Graphene is an example of 2D-nanomaterials which are consist of single-atom-thick planar sheet of sp^2 covalently bonded carbon atoms looks like a honeycomb crystal lattice and the neighboring atoms of carbon hexagons separated by a bond length of 1.42\AA and layer thickness of 3.3\AA (Figure 7) [64-65]. Basically, three of the four valence electrons are allowed to bond to their next neighbors (σ -bonds), and the fourth electron (π -electron) is free (delocalized) and oriented perpendicularly to the sheet as shown in Figure 8(a). Since the ground breaking discovery many other form of graphene also discovered for example graphene oxide, reduced graphene oxide and graphene quantum dot are common[66]. Among them graphene quantum dot recognize

as a popular among the scientist because it's tremendous optoelectronic and semi-conductor properties. GQDs are the example of 0-D nanometer-sized carbon materials which shown the features of both graphene and carbon quantum dot and mixture of them. In general graphene quantum dot is smaller than graphene sheet if the size in between 1-20 nm then one called it graphene quantum dot. A conversion of 2-D graphene into 0-D GQD leads to an exhibition of innovative phenomena owing to the quantum incarceration and edge effects. These materials portray excellent photo stability, biocompatibility, and low toxicity.

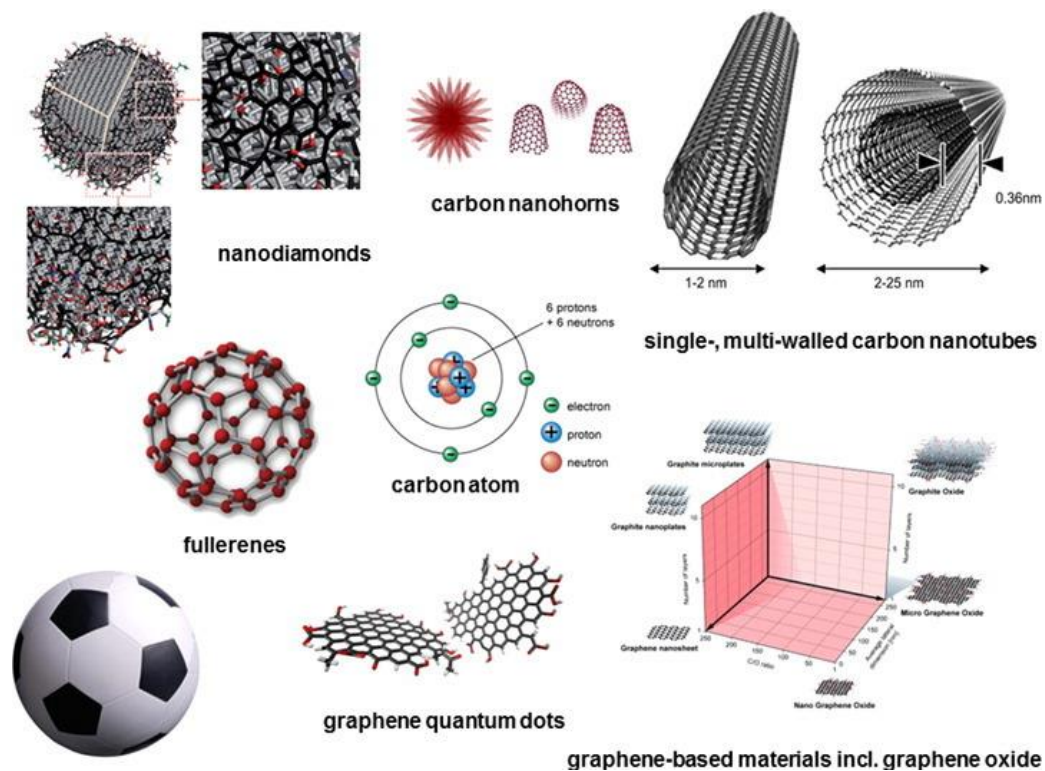


Fig.I.1 different kinds of carbon based materials

Graphitic Carbon nitrides

Beyond graphene and carbon nanotube, carbon nitrides are another class of materials carbon based contains carbon and nitrogen in its back bone. Carbon nitrides are the polymeric compound and successfully achieved by substitution of nitrogen atom into carbon backbone and became a most appealing candidates in recent for diverse application in numerous field. Long back in 1834 Berzelius 1st introduced the traces of carbon nitride named melon that contains tries triazine unit [67]. However the potential value were recognized in recent year. Graphitic carbon nitrite is the allotropes of carbon

nitride which was prevalent for their chemical inertness, high environmental stability, and insoluble in acidic and basic solution. The ideal graphitic carbon nitride are example of 2-d materials that consist of triazine unit. All carbon and nitrogen in graphitic carbon nitride is sp^2 hybridized like as graphene recently graphitic carbon nitride has been attracted the research hers because of its tremendous optoelectronic property [68]. It easily absorb the visible light thanks to its band gap. It has a moderate band gap 2.7 eV (460) nm, which can harvest the visible light as well as a suitable CB and VB edge position for both water reduction and oxidation [69]. Therefore, the graphitic carbon nitride are widely used in metal free photo catalyst the low photocatalytic activity is due easy of charge carrier recombination. For that, g-C₃N₄ promptly becomes the shining star as a green photo catalyst in the field of photo catalysis. Moreover, g-C₃N₄ could be synthesized from cheap and available precursors in a very simple synthesis method are the main reason for the widespread applications as visible-light-driven photo catalyst

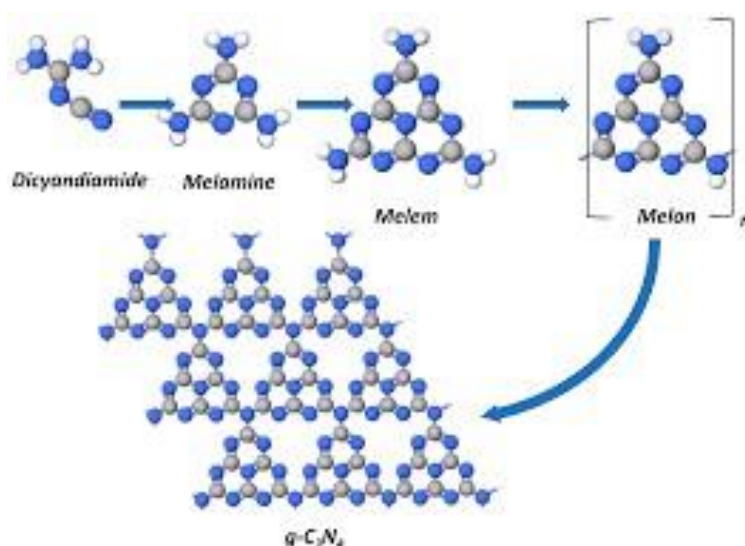


Fig.I.2 3D structure of graphitic carbon nitride

Polyaniline

Polyaniline is another class of carbon based materials. Historically it is an old materials in the by F. Ferdinand Range, Carl Fritzsche, John Lightfoot, and Henry Letheby in the different time periods studied that oxidation of aniline. And later Lightfoot isolated the aniline black He successfully developed the first commercially route to synthesis of Aniline black [68].- And after many years later in 1862 the first electrochemical method

has been report for the determination of small quantities of aniline. Shirakawa in 1977 first introduced the conductive polymer polystyrene and after then an overview of some metal-free carbocatalysts PANI is a class of such a nanoparticle that has the features of a conjugated system and rich heteroatoms on its backbone [69]. For several years, PANI served as a conductive polymer. Previously polyaniline was known as black aniline formed by simple coupling of aniline later for simplicity the black aniline is named as polyaniline. Polyaniline is recognized for its tremendous environmental stability and electrical conductivity. Depending on the oxidation state polyaniline is classified into three category emeraldine base emeraline salt leucoemeraldine (white/clear) pernigraniline (blue/violet) [70]. If PANI is fully oxidized then it known as pernigraniline if half oxidized then emeralidne and fully reduced form is known as leucoemeraldine. Among them emeralidine are wide used in optoelectronic and recently in bio materials field [71]. Both of emeralidine base and salt are highly stable and conductive having the conductivity range of $10-10 \text{ s cm}^{-1}$ and 30 s cm^{-1} respectively. The conductivity is easily controlled by synthetic procedure by submerging and treating with of picric, phosphoric, or camphor sulfonic acid.

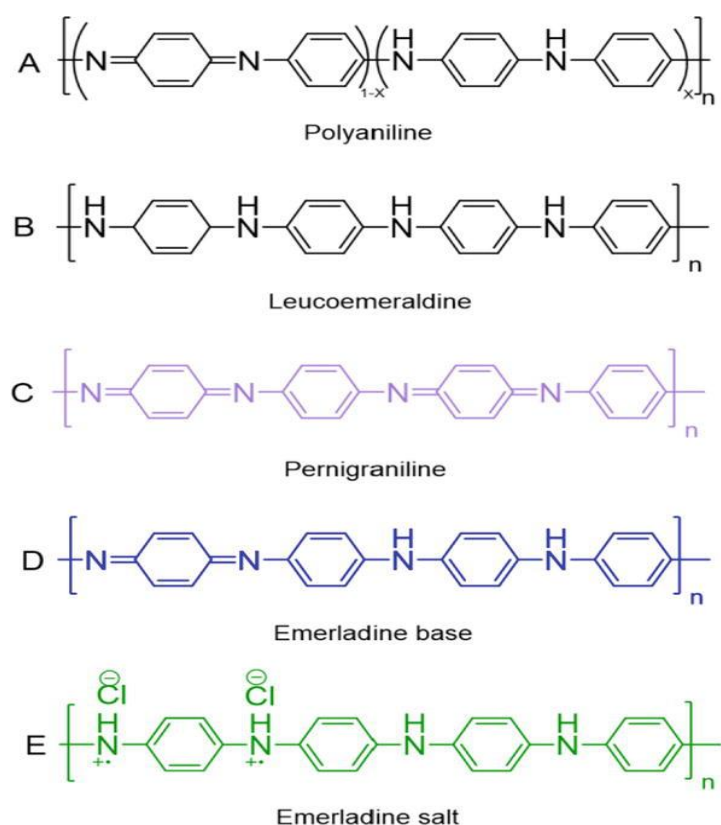


Fig.I.3 different kinds of PANI

I.2.2 Metal oxide based nanomaterials

The synthetic dyes are generally used as a colouring agent in food pharmaceutical and textile industries. The main constitute of these organic dyes is phenolic backbone that cause the environmental hazards. Most of the time these waste dyes are effluent by drain and causes water and soil pollution [72]. In maximum cases, these unused harmful dyes are thrown into the rivers and tunnels as wastage without any processing, which is the reason for water pollution by misbalancing the aquatic eco-system. Having many of aromatic groups in dye they shown tremendous environmental stability and remains in the environment large time. The conventional method has been proven a failure to degrade of that dyes. Therefore, researchers choose semiconductor materials having wide-bandgap they easily absorb the visible light and degrade the most of these toxic dyes. Till date there are many of semiconductors materials such as TiO_2 , CdS , ZnO , and WO_3 etc [73] have been studied as photo catalysts. In recent years, the AOP (advanced oxidation process) believe one of the most promising candidate for pollutant degradation caused by dyes. Traditional Fenton reaction ($\text{Fe}^{+2}/\text{Fe}^{+3}$) is one of the most useful method in homogeneous solution in the advanced oxidation process. For the degradation of organic pollutants. But simultaneously, sludge formation, low reusability and low applicability of wide pH range make it a secondary pollutant, and backlog its practical applications. Fortunately heterogeneous catalyst nanoparticles ($\text{Fe}_2\text{O}_3/\text{Fe}_3\text{O}_4$), easily overcome all these problem therefore, they are proven as better alternative for Fenton reaction [74]. However, the instability due to rapid aggregation of magnetic nanoparticles have pushed the researchers to discovery another Fenton agent. Apart from Ferrous/Ferric ions, there are some metal ions pair, including $\text{Co}^{+2}/\text{Co}^{+3}$, $\text{Cu}^{+1}/\text{Cu}^{+2}$, $\text{Cr}^{+3}/\text{Cr}^{+6}$, $\text{Ce}^{+3}/\text{Ce}^{+4}$, $\text{Mn}^{+2}/\text{Mn}^{+3}$, and $\text{V}^{+3}/\text{V}^{+5}$, also exhibited the Fenton and Fenton like reaction due to their inherent variable oxidation states [75].

Among the rare earth materials, Cerium-based nanomaterials have attracted most to the researcher because of its unique properties and low toxicity So, the CeO_2 is technologically important and its application spreads in various applications, including catalysts, sensors, and photonics. Recently, that particular materials CeO_2 are widely focused for its high antioxidant nature, therefore CeO_2 is therapeutically very important for Nano medicine and cancer biology. The CeO_2 possessed the mixed-valence states in the surface of nanomaterials which induce oxidation reduction process and thereby the

production of various reactive oxygen species. CeO₂ nanoparticles be present in two form in the surface of CeO₂ one is CeO₂ where Ce in +4 oxidation state and Ce₂O₃ where Ce is in +3 oxidation state, so the mixed valance state and surfaces oxygen vacancy makes CeO₂ nanoparticles as an excellent Fenton catalyst. Recently, [76-78] studied the Fenton's like degradation process of the organic pollutant catalysed by CeO₂ nanoparticle. Martínez et al., 2011[80] discover the degradation mechanism which is the result of the activation of H₂O₂ in initial step and subsequently form surface peroxide species and then highly reactive hydrogen peroxide radical. Later on the consecutive mechanism going on and organic pollutant degrade into small fragment. Cyclodextrins are class of water-soluble polysaccharides having hydrophobic interior and hydrophilic exterior which make them superior host in supramolecular chemistry. Here the cyclodextrin, used as a stabilizer for synthesis of CeO₂ nanoparticles [79]. The conventional technique for synthesis of nanomaterials is environmental unfriendly due to its hazardous condition. So the researcher explore the alternative environment friendly green method to synthesise novel nanoparticles. Here a small sized CeO₂ nanoparticles have been synthesise via an environment friendly green method using Neem leaves extract as a reducing agent and β-cyclodextrin as a stabilizer. As a green precursor neem leaves extract act as a good reducing agent which contains a numbers of polyphenols compounds including Nimbin, azadirachtin, and Nimbidine (Mandali et al., 2009) [81]. Polyphenols are the compounds having antioxidant and reducing ability recently, with neem leaves extract the silver nanoparticles were synthesized successfully. The formations of nanoparticles' success are further investigated by different characterization methods like XRD, UV, DLS, FTIR, SEM, and Fluorescence, etc. Here we successfully synthesized the rod like CeO₂ using β-cyclodextrins as a stabilizer and neem extract (green chemicals) as a reducing agent first-time. Our current studies' objectives are to reduce the harmful effect of conventional chemicals in preparation of Nano catalyst and check its applicability in the Fenton-like degradation reaction of organic pollutants. We study the degradation of major textile cationic dye (MB, Rh-B) and anionic dye (EBT) with H₂O₂ and synthesized CeO₂ Nano-catalyst. Taking the MB as a model dye, we further study its (degradation) dependencies in catalytic doses.

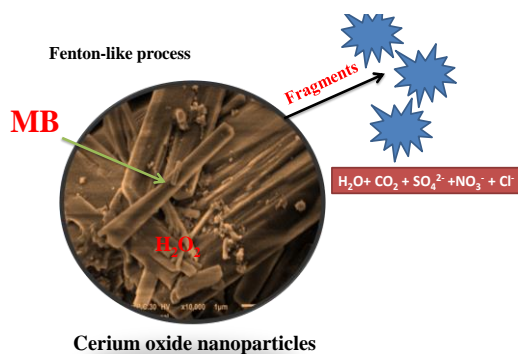


Fig.I.3 Mechanism of MB degradation by Fenton like mechanism with CeO₂

Natural enzymes, known as the bio molecular catalysts, have high bio-catalytic activity while maintaining high specificity towards substrates and offers a number of biological processes in living system [82]. Beyond catalysing the biological process some of the biocatalyst can specifically catalyzed some chemical reactions also under ambient condition. Among them oxidase, horseradish peroxidase (HRP), glucose oxidase, superoxide dismutase and catalase are important for various analytical uses [83]. Though their high application in the field in medical, analytical and environmental science they have certain disadvantages too, including low operational stability, vulnerability to a harsh environment, high expenses, and easily denaturized in wide range of pH and temperature[84-85]. So, in call of replacement for bio enzyme there should be an artificial enzyme for same working ability while breakdown the limitation of natural enzyme. The time came in 2007 when Yan and co-workers[82] at first broke the long-believed allegory of biological inactivity of inorganic nanomaterials named as nanozyme which could make up for the shortcomings of natural enzymes. From this time the nanozyme have received widespread attraction to the researchers for their high application in medicinal science, sensing application, and bio-electro-catalysis. Semiconductor nanomaterials are also studied for their superoxide dismutase (SOD), peroxidase (POD), catalase, and glucose POD-like property some common nanozyme includes Fe₃O₄ NPs, Co₃O₄ NPs, V₂O₅ NPs, and MnO₂ NPs[86-87] were well studied. In spite of single nanomaterials there are numerous nanocomposites have been already for multi enzyme mimic activity, the nanomaterials includes Mn₃O₄/Pt NC, NiO₂/C, MoO₃/C Nano rods, and CoO₃/C nanocomposites[88]. Oxidase (OD) and peroxides (POD) are two enzyme of oxidoreductases family studied extensively for their high application in bio-analytical and biomedical science. Oxidase enzyme reduced the molecular oxygen in

order to oxidize the substrates whereas for peroxidase a supplementary oxidant H_2O_2 needed for oxidation. The common substrates for oxidase and peroxidase are including 3,3,5,5-tetramethyl benzidine (TMB), ophenylenediamine(OPD), dopamine hydrochloride (DOPA), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and luminal. In recent year Oxidase (OD) and POD nanozymes have attracted the researcher most because they are related to the ROS generation therefore important in CDT and antimicrobial activity. Oxidase and peroxidase nanozyme induced ROS generation the biological system as a synergistic way, oxidase increase the peroxide level and the peroxidase dissociate these peroxide to produced destructive hydroxyl radical and after then these hydroxyl radical attract the carcinoma cells and microbes disrupt the chemical redox state intercellular level and ruptures the cancer cells[89]. Most of CDT (chemo dynamic therapy) is accomplished by mixed metal oxide possess a redox pair (Mn^+/Mn^{+1}) which are catalyzed Fenton or Fenton like reaction in order to produced highly destructive. Which catalyzed the decomposition of intercellular H_2O_2 and produced destructive hydroxyl radical, generally the mixed redox state on the surface of nanomaterials catalyzed the both Fenton and enzyme mimicking reaction. Which possess a redox pair (Mn^+/Mn^{+1}) and produced highly reactive hydroxyl radicals, and having a powerful oxidation capability causes chronic oxidative damage in carcinoma cells [90].

Cerium oxide (Nano ceria) has gained enormous importance in recent years as nanocatalysts because of its exclusive physical and chemical properties [91-92]. Nano ceria has been extensively applied in a variety of fields, such as bioassays, catalysis, and antioxidant therapy. The CeO_2 nanostructure shows a high catalytic activity in a range of applications owing to the existence of mixed valence states of Ce^{3+} and Ce^{4+} , and also the presence of oxygen vacancies. The explanation to this catalytic action is that the redox couple can control between each state in a $CeO_2 \leftrightarrow CeO_{2-x} + x/2O_2$ ($Ce^{4+} \leftrightarrow Ce^{3+}$) recycle system [93]. The catalytic performance of Nano ceria originates from the surface oxygen content, and increasing the $Ce^{3+}/(Ce^{3+} + Ce^{4+})$ ratio enhances the surface oxygen imperfection in the structure, leading to upgrading in catalytic properties [94].

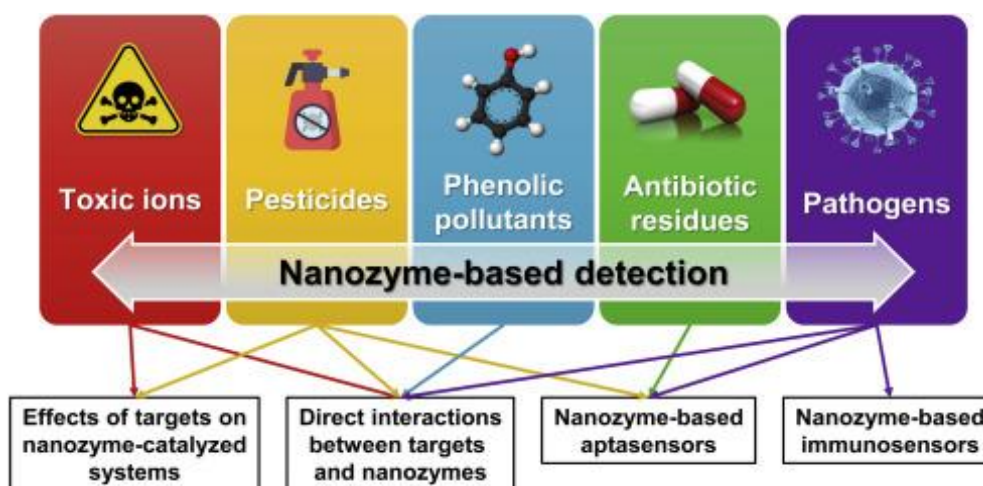


Fig.I.4 Applications of nanozyme in different detection system

Mn_3O_4 NPs are another material that has drawn the attention of researchers due to its wide range of applications in the biological sector and semiconductor characteristics. Currently, the properties of CeO_2 , MnO_2 , NiO , and V_2O_5 are thoroughly investigated for their ability to mimic enzymes like SOD, CAT, POD, and oxidases. Notably, Mn_3O_4 NPs have demonstrated some enzyme mimicking characteristics, albeit there have been very few studies of this activity compared to other catalysts. Particularly for diverse reactions including the oxidation of organic molecules and the breakdown of reactive oxygen species (ROS), Mn_3O_4 nanozymes have demonstrated remarkable catalytic abilities [95]. Mn_3O_4 nanozymes' unique surface characteristics, which offer active sites for interactions with substrates and enable them to speed up chemical reactions, are what give them their catalytic activity. Mn_3O_4 nanozymes are appealing for applications such as due to these qualities. Mn_3O_4 nanozymes have been researched for their potential in medicinal uses, such as the treatment of cancer. They have the ability to catalyse the production of ROS, which can cause cancer cells to undergo apoptosis (programmed cell death) [96]. To prevent harming healthy cells, their catalytic activity must be carefully controlled. It is possible to employ Mn_3O_4 nanozymes to digest air and water contaminants. They can support efforts to clean up the environment by catalyzing the degradation of organic pollutants into less dangerous compounds. Mn_3O_4 nanozymes can be used in sensors to find different ions or compounds. Their catalytic activity can be used to provide sensitive and focused detection techniques for use in environmental monitoring and healthcare. The significance of Mn_3O_4 nanozymes in energy-related applications. For instance, they can be used as catalysts in

electrochemical processes like fuel cells to improve the effectiveness of energy conversion [97]. Mn_3O_4 nanozymes' capacity to produce ROS, which can harm bacterial cell membranes and prevent bacterial development, has made them promising candidates for use as antimicrobial treatments. It's vital to remember that research and development on the characteristics and uses of Mn_3O_4 nanozymes is still ongoing. Studies are now being conducted with the goal of improving the stability, biocompatibility, and catalytic effectiveness of nanomaterials for a variety of applications. Recently, it was demonstrated that a Mn_3O_4 -based nanozyme mimicked the action of peroxidase and ascorbate oxidase. In general, the metal oxide-based peroxidase system functions most effectively in the pH range of 3 to 4, although at neutral pH, its activity completely disappears. For catalytic and medicinal applications, nanoenzyme work on physiological pH is crucial. Numerous techniques, such as surface modifications of nanoparticles to function in neutral pH conditions, have already been devised. The direction of the materials is towards applications.

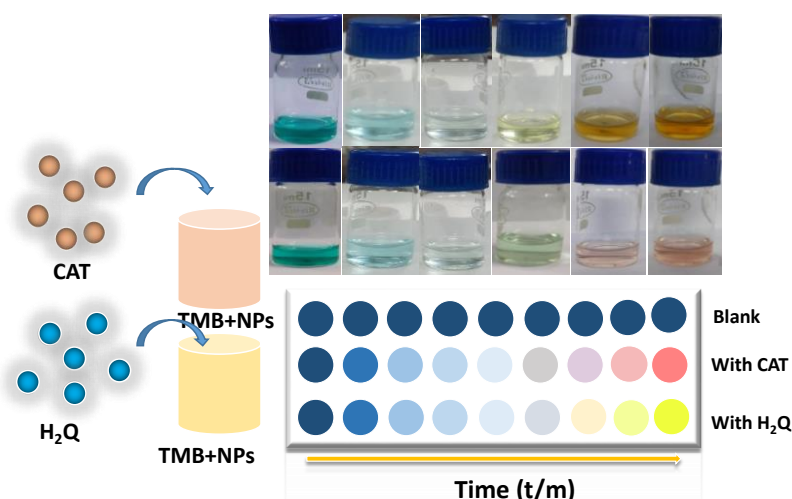


Fig.I.5 Colorimetric detection of catechol and hydroquinone by Mn_3O_4 nanozyme

CuS nanozymes, often referred to as copper sulphide nanozymes, are a subset of nanomaterials that have catalytic activity that is intrinsically enzyme-like. Nanozymes are artificial nanomaterials that can replicate the catalytic properties of enzymes, in contrast to natural enzymes, which are protein-based catalysts.

Due to their distinct characteristics and prospective uses in a variety of industries, such as biomedicine, environmental cleanup, and sensing, copper sulphide (CuS) nanozymes have drawn attention. CuS nanozymes can replicate the catalytic activity of natural

enzymes including peroxidase, catalase, and oxidase, which is one of their remarkable properties. The oxidation of organic contaminants and the production of reactive oxygen species (ROS) for medicinal purposes are just a few of the reactions they can speed up. Compared to natural enzymes, CuS nanozymes are often more robust and stable. They are suitable for usage in a range of situations since they can endure a greater range of pH and temperature conditions. CuS nanozymes' catalytic activity can be adjusted by adjusting their size, shape, and surface characteristics. Researchers can tune their performance for particular applications thanks to this tenability [98]. CuS nanozymes have demonstrated promise in medicinal fields like cancer treatment and imaging. They are candidates for photodynamic therapy because they can produce ROS, which are harmful to cancer cells when exposed to light. Their catalytic activity can also be used for diagnostic and biosensing purposes. CuS nanozymes can be used to breakdown organic pollutants and eliminate impurities from water and soil in environmental applications. Through their catalytic activity, contaminants can be better broken down, leading to healthier ecosystems. The antibacterial potential of CuS nanozymes has been studied. They have the ability to produce ROS that are bactericidal, perhaps providing a fresh method of battling microbial diseases [99]. It's important to note that the field of nanozyme research is quickly developing, and new uses and discoveries are continually being developed. CuS nanozymes were a topic of current research as of my most recent update in September 2021, however advancements after that time might not be covered in my expertise. Always refer to the latest scientific literature for the most up-to-date information on this topic.

I.3 Advantages of colorimetric detection of analytes:

A common method in analytical chemistry for detecting the presence of particular analytes based on changes in colour is colorimetric detection. This approach is common for many applications because of its many benefits. Here are a few benefits of colorimetric analyte detection. Colorimetric assays can be carried out quite easily and quickly. They are usable by a variety of users, including non-experts, and do not require complicated equipment or in-depth training. It is not necessary to use specialised equipment or complex analysis to detect colour changes because they may be seen with the unaided eye. Particularly helpful for point-of-care or field-based applications is this visual component. Results from colorimetric assays are frequently available quickly,

usually in a few minutes. This short turnaround time is useful in circumstances when timely detection is essential, such as in environmental monitoring or medical diagnostics. Comparing colorimetric assays to other analytical methods that demand pricey equipment or materials, they are frequently more economical. The assay design's simplicity may result in lower total costs. High degrees of sensitivity can be attained by contemporary colorimetric assays, enabling the identification of analytes even at low concentrations. They are therefore appropriate for applications requiring the detection of traces of the target analytes. For a variety of analytes, such as ions, small molecules, proteins, nucleic acids, and more, colorimetric assays can be created. Due to their adaptability, they can be used in a variety of industries, including forensic research, environmental monitoring, food safety, and medicine. Colorimetric detection can offer quantitative and qualitative data regarding the presence and concentration of the analyte, depending on the assay's design. To link colour intensity to analyte concentration, calibration curves can be created. The development of portable test kits that may be utilised in the field, isolated locations, or environments with limited resources is made possible by the simplicity of colorimetric assays. Without the requirement for a laboratory, these kits can be utilized for on-site analysis. Colorimetric assays frequently only call for a few simple steps in sample preparation, saving time and effort in getting samples ready for analysis. When working with complicated matrices or when a quick screening is required, this is extremely helpful. Liquids, solids, and gases are just a few of the sample types that can be used with colorimetric assays. They are useful for analysing a variety of materials found in various applications because to their versatility. The interpretation of results is made simpler by the qualitative and visual nature of colorimetric assays. When complex data analysis may not be possible, this can be useful. The simultaneous detection of many analytes by multiple colorimetric assays in a single sample enables the detection of complicated mixtures or the simultaneous screening of numerous targets. Overall, colorimetric detection is a useful tool in a variety of scientific, medical, and industrial situations because it strikes a balance between ease of use, sensitivity, and affordability.

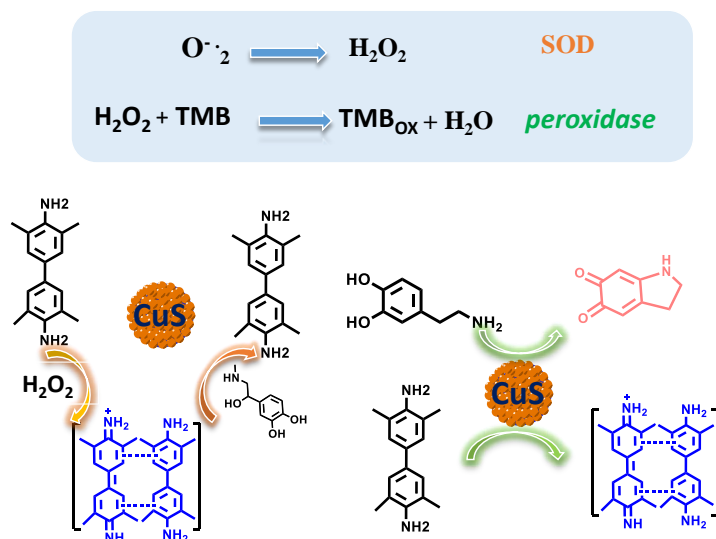


Fig.I.6 Various substrates oxidation by CuS nanozyme

I.4 Nanozyme with antioxidant property:

Antioxidant-rich nanoparticles have drawn a lot of interest recently because of their potential uses in a variety of industries, including health, cosmetics, food, and materials science. Antioxidants are molecules that can help shield cells against oxidative stress, which is brought on by an imbalance between the body's capacity to neutralise reactive oxygen species (ROS) and its ability to produce them. The ageing process and a number of disorders are linked to oxidative stress [100]. There are numerous ways that nanoparticles can exhibit antioxidant properties: Some nanoparticles are chemically capable of directly scavenging and neutralising ROS due to their inborn chemical characteristics. For instance, it has been investigated how well free radicals and ROS may be absorbed by nanoparticles made of metals like gold, silver, and cerium oxide [101]. Recently, there has been a lot of interest in antioxidant-rich nanoparticles due to their potential applications in a number of fields, including materials science, food, cosmetics, and health. The compounds known as antioxidants can protect cells from oxidative stress, which is brought on by an imbalance between the body's ability to produce and neutralise reactive oxygen species (ROS). Oxidative stress is connected to the ageing process and a number of illnesses. Nanoparticles can display antioxidant capabilities in a variety of ways: Antioxidant properties refer to a group of compounds that are often present in foods such as fruits and vegetables and which have the capacity to counteract the damaging effects of molecules in the body known as free radicals. These free radicals are connected to several health problems and have the capacity to

harm cells [102]. Antioxidants neutralize these harmful substances, which helps to prevent diseases like cancer and heart issues. Minerals like selenium and the vitamins C and E are examples of known antioxidants. Although more research is required to completely understand how antioxidants function, it is believed that they have health benefits. Popular tests used to assess antioxidant capacity include nitric oxide, DPPH (2, 2-diphenyl-1-picrylhydrazyl), ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), and superoxide radical scavenging assays [103].

1. DPPH assay: This test is often used to evaluate a nanoparticle's capacity to scavenge free radicals. When in its radical form, DPPH is a purple-colored free radical that is stable. An antioxidant's donation of an electron to the radical results in DPPH changing from purple to yellow when they interact. The antioxidant's capacity to scavenge is reflected in the degree of color change.

2. ABTS assay: ABTS is a different stable free radical that has the ability to change color. When ABTS is exposed to antioxidants or nanoparticles in this test, the radicals are dispersed and the material changes color from blue-green to colorless. The decolorization is an indication of the nanoparticles' scavenging ability.

3. Assay for Nitric Oxide: Nitric oxide (NO), a signaling molecule, can turn into a harmful free radical when it is produced in excess. Nitric oxide radicals are created under strictly controlled circumstances for this assay. By interacting with antioxidant nanoparticles, these radicals can be neutralized and the production of dangerous compounds can be stopped. A decrease in nitric oxide concentration signifies scavenging activity.

4. Superoxide radical scavenging assay: Superoxide radicals, which are generated in biological systems, are the source of oxidative stress. This test assesses the nanoparticles' capacity to scavenge superoxide radicals. Usually, a material such as NBT (nitro blue tetrazolium) is used, which changes color when it comes into contact with superoxide radicals. The scavenging activity of the nanoparticles is indicated by the drop in color intensity.

Comparing these characteristics to bulk materials, they may result in increased antioxidant activity. It is possible to create nanoparticles that are biocompatible, which enables them to interact with biological systems without having negative consequences. Particularly for prospective biological uses, this is significant. Antioxidant nanoparticles

may be used for several purposes, such as: Antioxidant nanoparticles may be utilised to create treatments for diseases like cancer, cardiovascular disease, and cancer that are brought on by oxidative stress [104]. They might potentially contribute to drug delivery techniques. Antioxidant-rich nanoparticles could be added to skincare products to shield skin cells from harm from toxins and UV radiation. By halting oxidation and spoiling, antioxidant nanoparticles may be employed to increase the shelf life of food goods. They might also assist in preserving the food's nutritional value. Antioxidant nanoparticles could be added to materials to increase their toughness and resistance to deterioration from the environment. It's crucial to remember that while antioxidant nanoparticles may have positive effects, there are also worries about their safety, potential toxicity, and environmental effects [105]. To completely comprehend their behaviour in biological systems and the larger environment, much research is needed. Regulatory bodies and researchers continue to investigate these aspects to ensure the responsible development and application of antioxidant nanozyme. We developed CuS, Mn₃O₄@g-C₃N₄ nanocomposites which shown the multi enzyme mimicking activity includes oxidase, peroxidase, SOD and CAT mimicking activity therefore we have successfully applied them as artificial antioxidant system.

Pathogenic bacteria that have been found in food that are dangerous to humans include *Bacillus subtilis*, *Salmonella sp.*, *Salmonella typhimurium*, *Escherichia coli*, and *Staphylococcus aureus*. Metal oxide nanoparticles are an effective antibacterial pathogen remover. The antibacterial capabilities of metals may not be present in their oxide or salt forms, but they may be present in their Nano form. Metal oxide nanoparticles interrupt the electron transport chain, which stops microbial development and results in cell death. They do this by having strong links to microbial DNA and protein. Thus, we use a precise synthesis of metal oxide nanoparticles in our research that can be used to stop the growth of bacteria. Reactive oxygen species and the toxicity of free metal ions are two ways that nanoparticles can harbor bacteria. Gram-positive bacteria have a higher IC₅₀ value than Gram-negative bacteria due to their thicker peptidoglycan covering. The growing need for novel antimicrobials is met by synthesized nanocomposites, which may also reduce healthcare costs associated with the rise in antibiotic resistance in bacteria. Because synthetic antimicrobials are used in so many different industries—cosmetics, medicine, textiles, and the environment—the need for novel synthetic antimicrobials has grown dramatically.