Triethylamine: a versatile ligand to control the properties of nanomaterials

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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Declaration

I certify that except where due acknowledgment has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; any editorial work, paid or unpaid, carried out by a third party is acknowledged; and, ethics procedures and guidelines have been followed. I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

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Mandeep Singh
This thesis is dedicated to my Father

Late Dr. Baldev Singh
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List of Abbreviations

AES: Atomic emission spectroscopy
UV-Vis: Ultraviolet-visible
PESA: Photoelectron emission spectroscopy in air
XPS: X-ray photoelectron spectroscopy
XRD: X-ray diffraction
TEM: Transmission electron microscopy
HR-TEM: High-resolution transmission electron microscopy
SEM: Scanning electron microscopy
AFM: Atomic force microscopy
XPS: X-ray photoemission spectroscopy
EDX: Energy dispersive x-ray spectroscopy
BET: Brunauer, Paul Hugh Emmett and Edward Telle
2D: Two-dimensional
3D: Three-dimensional
NS: Nanosheets
MPs: Microparticles
NPs: Nanoparticles
ROS: Reactive oxygen species
SPR: Surface plasmon resonance
Tin monoxide: SnO
Iron oxide: γFe₂O₃
Gadolinium hydroxide: Gd(OH)₃
Gadolinium oxide: Gd₂O₃
nm: nanometre
μm: micrometre
TMB: 3,3′,5,5′-Tetramethylbenzidine
OPD: o-phenylenediamine

ABTS: 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt

TEA: triethylamine

LED: light emitting diode L: Length

W: Width
Abstract

Triethylamine (TEA) acts as an outstanding hydrolysing agent along with certain shape-directing properties for nanomaterial synthesis. The use of TEA in this thesis for nanomaterial synthesis offers new potential in controllably tuning the underlying synthesis reaction pathways to obtain nanomaterials of different compositions and morphologies. Firstly, TEA was found to be quite effective for nanoparticle synthesis concerning the low cost of material production through high yields, the requirement of less-intensive purification steps, and facile long-term storage offered by high ambient stability of materials prepared using TEA. These characteristics are other difficult to control and therefore restricts the applications of nanomaterials at the industrial scale. This low molecular weight organic tertiary amine has certain unique characteristics that allow it to perform in diverse ways, as illustrated throughout this thesis – these include amphiphilicity (ability to dissolve both in aqueous and organic solvents), high basicity (similar to that of ammonia), and newly discovered shape-directing ability. As such, this thesis reports the development of a range of nanomaterials by establishing new synthesis protocols, while keeping TEA as the central theme as one of the reagents employed during proposed methodologies and reactions. Chapter 1 provides a state-of-the-art of the prior literature in the field, and a diverse range of instrumental techniques employed to characterize the nanomaterials prepared using TEA thoroughly are captured in Chapter 2. Chapter 3 then discusses the experimental outcomes of this thesis and is sub-divided into multiple sub-chapters, each covering a specific nanomaterial employing TEA in different ways. Chapter 3a first demonstrates the ability of the TEA to directly synthesise iron oxide ($\gamma$Fe$_2$O$_3$) nanoparticles both in aqueous and organic solvents. Secondly, it is demonstrated how TEA can be used as a dynamic amphiphilic molecule for the phase transfer of the organic ligand-stabilised iron oxide particles from an organic to an aqueous phase. The magnetic hyperthermia properties of these materials were evaluated, and the role of Brownian relaxation in magnetic
hyperthermia was highlighted. Chapter 3b demonstrates the importance of TEA in obtaining highly oxygen-deficient and photostable copper oxide (Cu₂O) nanoparticles. It is well known that Cu(I) is highly unstable in water and is prone to oxidation to Cu(II), which makes the aqueous synthesis of Cu₂O challenging. This works shows that TEA can act as a coordinating ligand to form a Cu(I)TEA complex, which on water-mediated hydrolysis produces Cu₂O particles. The application of these materials for enhanced visible light photocatalytic activity is demonstrated by establishing the importance of oxygen vacancies in photo-induced catalytic reactions. Chapter 3c demonstrates that gadolinium-based one-dimensional (1D) nanorods of Gd(OH)₃ and Gd₂O₃ can be realised using TEA as a dynamic molecule. These Gd-based nanomaterials showed inherent enzyme-mimic peroxidase-like NanoZyme activity. Such Gd-based nanorods were exploited for the first time as colourimetric sensors for rapid detection of cysteine, a biologically important molecule. In Chapter 3d, I demonstrate that TEA can be used to obtain layered tin monoxide (SnO) microspheres which can then act as a starting material to obtain high-quality 2D SnO nanosheets. In the context of the importance of this process, it may be appreciated that over the last decade, a new dimension of the material aspect, i.e. two-dimensional (2D) research has emerged where nanomaterials have shown remarkable distinctive or enhanced properties in comparison to the other dimensional materials. Although much progress has been made to explore such 2D materials using the top down approach but rather limited efforts have been made to obtain such materials using bottom up approach. This chapter fills this gap by preparing 2D SnO using a bottom-up approach. Further, we explored detailed optical characteristics of the bulk SnO w.r.t to 2D thin SnO, and our findings revealed an unprecedented opening of the bandgap from ~2.7 eV (bulk) to 4.1 eV (monolayers) due to strong quantum confinement. These 2D SnO sheets were utilized for photodetector applications.
Chapter 1

Introduction

This chapter provides a detailed literature review in the area of nanotechnology in general and material synthesis in specific. The various synthesis methods/protocols along with synthesis parameters are discussed in detail and how one can change the nanomaterials physical and chemical properties of nanomaterials by varying synthesis methods is elaborated upon. Further, the chapter discusses the various types of amines and their potential applications across various fields. With the available discussed literature, further the tertiary amine i.e. triethylamine is discussed in detail and how we can use this molecule in the field of nanomaterial synthesis is elaborated. Towards the end, a chapter-wise breakdown of the thesis is presented and a brief description of the content in each chapter is included.
Nobel laureate Prof. Richard P. Feynman in American Physical Society meeting at Caltech in 1959 introduced the field of nanotechnology in the now-famous lecture “There’s Plenty of Room at the Bottom” [1]. In 1974, Prof. Norio Taniguchi coined the term “Nanotechnology” which mainly consists of the processing of separation, consolidation, and deformation of materials by one atom or a molecule [2]. In the 1980s, Eric K. Drexler with his visionary ideas of “molecular assemblers” gave much publicity to the nanotechnology field [3, 4]. This led to the tremendous developments of a multidisciplinary field, in which basic sciences (physics, chemistry, and biology), material science and engineering field amalgamated together to demonstrate the importance of nanoscale level [5]. In general, nanotechnology refers to the manipulation of the materials at one billionth of a meter (10⁻⁹ m) level. It has been observed that materials’ well-established properties like optical, electrical, mechanical and biological properties are drastically changed with their size reaching the nanoscale regime [6]. As an example, when the size of the gold shifts from bulk to the nanoscale, it’s catalytic properties improve, where the bulk size does not show any catalytic properties and nanosize shows excellent catalytic properties [7]. Similarly, iron oxide magnetism shifts from ferromagnetic to superparamagnetic with the decrease in size [8].

Although nanotechnology and nanoscience are often taken under one umbrella, there is quite a contrast difference between the two terms. In general, nanotechnology refers to the understanding, manipulation, fabrication, design, and application of the materials on the nanoscale level (<100 nm). Such materials are often termed as nanostructured materials which include materials of a variety of shapes and sizes. Whereas on the other hand, nanoscience refers to the study of the fundamental relationships between physical, chemical properties of nanomaterials and how they are linked with size dimensions [1, 6].
1.1 Synthesis Approaches

In order to obtain these nanostructured materials, researchers generally follow two basic approaches i.e. bottom-up and top-down. The main selection of the approach depends upon the end product that is needed. The factors that control this end product include size, shape and particle distribution, composition, and agglomeration. In the top-down approach, the big bulky material is broken-down into smaller pieces, typically till one of the dimensions is less than 100 nm. To achieve this, we use various methods which can be further classified under the following categories [9]:

i) **Physical techniques**

- *Mechanical milling*
- *Wet / Dry / Reactive ion / Plasma-based etching*
- *Low and high power ultrasonication*
- *Wet / Dry sputtering*
- *Laser-based ablation*
- *Soft / Photo-based lithography*
- *Electrospraying*
- *Pyrolysis*

ii) **Liquid-phase techniques**

- *Spin coating*
- *Liquid-based sonication*

However, out of these, mechanical milling and ultrasonication are being most commonly followed due to the ease in operation. In mechanical milling, the powder is placed in a high energy mill typically containing stainless steel or tungsten carbide balls in a closed chamber. The balls are allowed to agitate at a controlled speed which impacts the bulk powder, resulting in breaking down the crystal along the easy zone axis to form nanopowder. The
quality of the obtained nanopowder is governed by many parameters such as mill type; dry or wet milling; temperature; time etc. On the other hand, ultrasonication is based on microbubble formation technology when the ultrasonic waves pass through a liquid medium, where these bubbles grow and collapse in microseconds. The collapsing of these microbubbles generates energy which helps in breaking-down of the bulk crystals into nanometer size along the various zone axes. However, it prefers to downsize along the easy zone axis, where the forces between the various layers can easily be broken down. The advantage of both the techniques is that they are economical and viable for large scale processing at industrial scale. However, the quality of the nanocrystals is not as good as those obtained using the bottom up synthesis approaches.

In the bottom-up approach, materials are prepared by a process akin to self-assembly, using atomic and molecular materials as building blocks, where these blocks turn into nanoparticles after nucleation, allowing them to aggregate to increase in size and well-desired shapes. To achieve this, various methods can be used that can be further classified under following categories:

i) **Gas / chemical vapour based methods**
   - Chemical thermal deposition
   - Chemical plasma enhanced deposition
   - Plasma arc
   - Molecule-based beam epitaxy (MBE)
   - Sputtering

ii) **Solution / wet chemical-based methods**
   - Reduction using chemicals
   - Sonochemical
   - Co- / single-precursor based precipitation
- Colloidal based gelation (Sol-gel)
- Solvothermal

iii) Self-assembly-based methods
- Template based growth
- Electrostatic based assembly
- Self-assembled mono/multilayers
- Langmuir-Blodgett thin films

In the bottom-up approach, solution phase techniques are most commonly followed especially the precipitation and sol-gel techniques, as one can easily control the size, crystal facets and many other characteristics of materials, as per their need. Secondly, a large variety of functionalization of the nanomaterials can be obtained in-parallel during synthesis. Thirdly, the quantity and the quality of the crystals along with uniformity in particle size and shape can be obtained in a small synthesis time. In Precipitation technique, the solution containing the anions and cations lead to nucleation and growth in the presence of e.g., the hydrolyzing agent (commonly used are sodium hydroxide - NaOH, ammonium hydroxide-NH₄OH etc.). This leads to the formation of the small agglomerates of the nanoparticle within the solution. These nanoparticles can be separated and cleaned from molecular precursors and other undesirable by-products to obtain them in the purest forms. The general scheme for the synthesis of nanoparticles using precipitation method is shown in Figure 1.1.

![Figure 1.1: General scheme for the synthesis of nanoparticles using the precipitation method.](image)
The Sol-gel technique leads to the formation of the inorganic gel network, where the formation of the nanoparticles involves generation of polymers by connecting the metal centers with oxo (M-O-M) or hydroxo (M-OH-M) bridges. The drying of such polymers under ambient conditions leads to form the dry powder which under subsequent thermal heating leads to polycondensation, improving the crystallinity of the material. Amorphous glass or ceramics are commonly prepared by this technique.

1.2 Nanomaterial Dimensional Aspect

Based on the above-listed techniques, one can synthesize the nanomaterials of various dimensions which can be categorized as follows and depicted in Figure 1.2:

Figure 1.2: Classification of materials by size: 0D (Zero dimensional); 1D (One dimensional); 2D (Two dimensional); 3D (Three dimensional)

1.2.1 0-Dimensional nanomaterials

Since the discovery of quantum dots, a tiny speck of material, in 1980 by Russian physicist Alexei Ekimov, there has been an immense interest in this field due to very unique properties being observed in materials once we can retain them in quantum confined states [10]. Such materials are termed as 0-D nanomaterials, where a single 0-D particle can be a few nanometers wide holding a few dozen atoms across and contain anything from perhaps a hundred to a few thousand atoms. They have well-defined energy levels according to the
well-established quantum laws and find applications in numerous areas which include solar cells, light emitting diodes (LEDs), quantum computing, targeted drug delivery, photocatalysis, photodetectors etc. They can be obtained by a number of synthesis approaches like colloidal synthesis [11]; plasma synthesis [12]; molecular beam epitaxy [13]; viral assembly [14]; electrochemical [13] etc.

1.2.2 1-Dimensional nanomaterials

In recent years one-dimensional (1-D) nanomaterials which include nanorods, nanobelts, nanofibers, nanowires, and nanotubes are constantly striving to develop new fundamental science as well as potential electronics and biological applications due to their unique properties. 1-D nanomaterial, due to linear dimensionality including size in nanoscale regime (quantum confinement) can act as building blocks for the electrical and thermal transport mechanisms or for strong mechanical properties [15]. Thus, these materials are being widely explored for fabricating electronic, optoelectronic, electrochemical and electromechanical nanodevices. The researcher has developed a variety of approaches to obtain these nanomaterials including VLS (Vapour–liquid-solid) [16], laser ablation-catalytic growth [17], oxide-assisted growth [18], template-induced growth [19] and metal-organic chemical vapor deposition (MOCVD) [20] etc.

1.2.3 2-Dimensional nanomaterials

A novel material platform in crystal research in terms of two-dimensional (2D) got highlighted in 2004, with the groundbreaking news of obtaining the thinnest and strongest carbon material for the first time with honeycomb lattice geometry, as known to be graphene [21-24]. Owing to its exceptional physiochemical properties, graphene is a huge success and till now it has been extensively used across various research fields ranging from physical science to biomedical sciences and holds a huge market share especially in the electronics
Graphene can be obtained using numerous methods including top-down methods like mechanical exfoliation; sonication; ball-milling and bottom-up methods like epitaxial growth onto the substrate; chemical vapor deposition etc. [24-26].

Recently, in parallel to graphene success, the researchers have started to explore other 2D elementals -enes with novel exciting properties [27-29]. Likewise in Group IV after graphene, in 2012, silicene was obtained by two separate research groups onto silver and zirconium diboride surface [30, 31] and in 2014, germanene has been fabricated onto gold substrate [32]. From Group V - in 2014, phosphorene was reported for the first time with its ambipolar behavior that makes it a suitable candidate for future electronic applications [33, 34]. Stanene (on bismuth telluride) and bismuthene (on silicon carbide) were first time reported in 2015 and 2017 with their 2D topological insulator characteristics [35, 36]. Similarly, antimonene has been reported which shows its ultra-stable nature, in comparison to phosphorene and stanene [37, 38]. However, in terms of freestanding 2-D materials, only three elemental -enes from group IV and V i.e. graphene, phosphorene, and antimonene have only been realized till date.

1.2.4 3-Dimensional nanomaterials

Gels are basically a network of various hydrophilic and hydrophobic molecules which are associated or bound together using special amphiphilic molecules, thus creating a kind of 3D environment of aqueous and non-aqueous regions via physical interactions (e.g. micellization, lyotropism, and crystallization) or hydrogen bonding. The gel-based products and the industry based on them like the cosmetic industry, food processing industry, the biological industry has doubled from the last decade, thus revealing their importance to the human society. Gels can be classified depending upon the solvent used [39, 40]. For example: (i) Water-based gels known as hydrogels are the most sought class of gels in the field of stimuli-responsive systems, where the stimulus is provided by the solvent composition,
solutes, pH, temperature, electric field, light, nanoparticle interaction etc. Taking the benefits of such stimuli-responsive systems, gels are being widely used in the field of drug delivery, chemical robotics, membrane separation processes, molecular separation, protein crystallization etc. [41].

(ii) *Organogels* are the gels with organic phase as the major phase and they are most often used in medicine for drug delivery. For example, gelatin is most commonly used to hold biological enzymes and bacteria for their use of them in various applications. Recently, Lecithin organogels such as Pluronic lecithin organogels (PLoS) have been used for drug delivery [42, 43].

(iii) *Xerogels* are the evaporative product left when the liquid is being removed from the 3D environment which collapses due to capillary forces and interfacial forces/bonding between different phases. This results in size reduction along with weight, thus forming the xerogel [44].

(iv) *Aerogels* is also an evaporative product which is highly porous in nature and is formed at highly supercritical levels of temperatures and/or pressures [45].

### 1.3 Synthesis Parameters

Researchers follow various synthesis parameters in order to achieve a desired size and shape of the nanomaterial. Although describing them all in detail is beyond the scope of the thesis but a concise understanding is presented in the context of co-precipitation technique that I have mostly used while synthesising materials presented in this thesis. Iron oxide nanoparticles are synthesized by a co-precipitation technique where stoichiometric 2:1 ratio of ferrous (Fe$^{3+}$) and ferric (Fe$^{2+}$) salts in an aqueous environment is taken in the presence of a hydrolysing agent (e.g. an alkali). Nanoparticles are formed via two steps with a short burst of nucleation once the species concentration reaches the critical supersaturation, followed by
a growth stage. To produce iron oxide nanoparticles with monodispersity, there should be a good separation between the nucleation and growth stages. The general chemical equation is represented as

\[
\text{Fe}^{2+} + 2\text{Fe}^{3+} + 8\text{OH}^- \rightarrow \text{Fe}_3\text{O}_4 + 4\text{H}_2\text{O} \quad \text{Equation 1.1}
\]

But the final product depends upon various parameters as listed.

**1.3.1 Salt molar ratios** - According to the thermodynamics of this reaction, complete precipitation of Fe₃O₄ should be expected with a stoichiometric ratio of 2:1 (Fe³⁺/Fe²⁺) in a non-oxidizing oxygen environment at a pH between 8 and 14. As per chemical reaction, magnetite (Fe₃O₄) would be the product formed but it is prone to oxidation if the proper inert environment is not being maintained during the pre and post-synthesis. During pre-synthesis, even the stoichiometric ratio of 2:1 (Fe³⁺/Fe²⁺) is hard to maintain due to the ease of the oxidation of ferric ions relative to ferrous ions [46]. For this not to happen, nitrogen (N₂) gas bubbling or taking the stoichiometric ratio of less than 2:1, could possibly solve the problem. However, these might affect the overall product morphology including size reduction [47, 48].

**1.3.2 Nature of solvent ionic strength** – In general, nanoparticle size and shape are highly affected by the localized environment of the precipitation medium. In the specific case of iron oxide, Vayssieres et al. [49] observed that stabilization of nanoparticles seems to result from thermodynamics rather than kinetics, where the secondary particle growth by Ostwald ripening is halted above a critical pH value (10-11). Jiang et al. [50] have found that the pH variations in a sequential manner with the addition of urea leads to narrow particle size distribution. Qiu et al. [51] observed that the presence of sodium chloride (1M) during the synthesis of magnetite nanoparticles, leads to decrease in nanoparticle size by 1.5 nm in comparison to one obtained without its presence. Similarly, Kim et al. [52] observed that
with the decrease in pH (NaOH conc. 1.5M), the magnetite nanoparticle size decreases from 3 to 6 nm.

1.3.3 Reaction temperature – Temperature is one of the critical parameters, which defines the size and shape in general and the crystallographic phase in specific of the nanomaterials. Like Shen et al. [53] observed that the $\gamma$-Fe$_2$O$_3$ (maghemite phase) were formed if the precipitation temperature was below 60 °C. Wu et al. [54] observed that magnetite nanoparticle size remained 2-3 nm at 20 °C and 80 °C, whereas size increased to ~4 nm at 40 °C and 60 °C. They believe that the temperature commanded the competition between nanocrystalline nucleation and growth, where the nucleation to growth rates decreases from 20 °C to 40-60 °C and again increase at 80 °C. Hence, the grain size changes alongside the co-precipitating temperature. Casula et al. [55] observed that the size of the iron oxide nanoparticles decreased from 35 nm to 7 nm with increase in temperature from 285 °C to 298 °C.

1.3.4 Synthesis atmosphere – This parameter is very crucial for the nanomaterials that are prone to ambient oxidation. To keep the oxygen away during the synthesis, a constant inert atmosphere (by bubbling N$_2$ or Ar) is created to eliminate any kind of oxidation effect onto the synthesized nanomaterial. Further, it has been observed as well that the bubbling not only protects the material from oxidation but also affects the final shape and size. Kim et al. [52] observed that bubbling N$_2$ gas not only protects critical oxidation but also reduces the magnetite nanoparticles size from 8 nm (in air) to 6 nm (in N$_2$ ). Liu et al. [56] observed that when synthesis proceeds under air without the protection of N$_2$ gas, the product is reddish-brown which indicates that there is a contamination of other iron oxides phases in the colloids as a result of the strong oxidation. Similar results were obtained by Shen et al. [53] and Maity et al. [47].
1.3.5 Injection flux rate – Researchers mostly follow three injection routes as: (a) Rapid precursor injection – allows rapid hydrolysis and condensation reaction, due to short burst of nucleation, leading to monodisperse nanoparticles, and has been extensively used to prepare monodisperse magnetic nanoparticles at high temperatures [55, 57]; (b) Drop-wise injection – for achieving a slow, continuous nucleation and growth process. However, most researchers find it difficult to control the hydrolysis and condensation rates to produce monodisperse nanoparticles, due to the formation of different size nuclei which leads to broad particle size distribution [57, 58]; (c) Multiple-shot injection – recently some researchers have followed this route to obtain nanoparticles with narrow particle size distribution [55, 57]. On the other hand, Babes et al. [59] have noted that during the nanoparticles synthesis, the precursor injection rate doesn’t have much influence on the final product.

1.3.6 Synthesis reaction time – it is an important parameter to be considered, particularly in cases where the material thermodynamics allow it to potentially form different phases under benign reaction conditions. Like Martinez-Mera et al. [60] observed that when aging time is less than 5 minutes, magnetite is the only phase formed. However, with aging time greater than 5 minutes, lepidocrocite phase (Fe(OH)) as impurity is formed along with magnetite phase. Li et al. [57] observed that PMAA-PTTM (polymer ligand, thiol-terminated poly(methacrylic acid) coated Fe₃O₄ nanoparticles size increased slightly from 4.4 to 5 nm with an increase of reaction time from 1 to 310 minutes. Similarly, Casula et al. [55] observed that the diameter of the iron oxide nanoparticles increases rapidly within the time scale of 1-30 minutes.

1.3.7 Role of surfactants – an important parameter in altering the shape of the nanomaterial; phase protection and to provide the stability to the nanomaterials in various solvents. Owing to the small size and corresponding large surface to volume ratio nanoparticles tend to aggregate to reduce their surface energy unless stabilized [53]. Thus, the
The colloidal stability of the nanoparticles has to be evaluated by considering all particle-particle interactions (van der Waals attractive forces and electrostatic repulsive forces), i.e. the DLVO (Derjaguin-Landau-Verwey-Overbeek) theory and this theory have to be extended in case of magnetic nanomaterials to include magnetic dipole interactions. The precise control over the nanoparticle synthesis protocol plus control of these colloidal forces is the backbone to obtain stable nanomaterial suspensions. Thus, colloidal stability can be achieved by providing the good separation between the particles via either steric (coating with bulky surfactants) or ionic (coating with opposite charges) forces [61]. Laurent et al. [46] differentiated the various surfactants used for coating the nanoparticles under following categories:

(i) **Monomeric stabilizers** – *Carboxylates* – citric acid [62], gluconic acid [62, 63], dimercaptosuccinic acid [64], phosphorylcholine [65], etc. oleic and lauric acid [47, 53]; (ii) *Phosphates* – poly(vinyl alcohol phosphate) [66].

(ii) **Inorganic materials stabilizers** – silica [67], gold [68], etc.

(iii) **Polymer stabilizers** – dextran [69], polyethylene glycol (PEG) [70], polyvinyl alcohol (PVA) [69], alginate [71], chitosan [72], polyvinylpyrrolidone (PVP) [73], polymethacrylic acid [74], poly(acrylic acid) [75], ethylcellulose [76], poly(ethylene oxide)-b-poly(methacrylic acid) [77], polyalkylcyanoacrylate [78], etc.

In context to iron oxide nanoparticles, Bee et al. [79] showed that in the absence of chelating organic citrate anions, the typical size of the maghemite nanoparticles was 8 nm and in the presence, it decreased to 3 nm. Such size reduction was attributed to the way the citrate ions prevented the overlap situation between the nucleation and the growth stages. Li et al. [57] observed that the surfactant/Fe$^{3+}$ ratio when increased from 0.7 to 3.4, the nanoparticle size decreased from 6.1 to 4.5 nm. However, further increase in the ratio to 7.2 didn’t have a significant effect on particle size. Casula et al. [55] observed that the presence of fatty acids used as coordinating surfactant, retarded the nucleation, which strongly stabilized the
monomers in solution. Thus, the presence of monodisperse nuclei and a fast growth regime were considered responsible for the formation of the nanocrystals with monodisperse size and shape. They obtained 13 nm iron oxide nanocrystals with iron precursor: surfactant ratio to be 1:1.5. Liu and Huang [62] observed that the citric acid concentration significantly affected the crystallinity of the iron oxide product, where higher the concentration, poorer the crystallinity.

**1.3.8 Role of hydrolyzing agent** – it plays an important role in the final product quality during the nanomaterial synthesis. The basic role of the hydrolyzing agent is to start the nucleation process, followed by a growth process according to Lamer diagram shown in Figure 1.3. So, for the best correlation between the nucleation and the growth stages, LaMer diagram suggested by LaMer and Dinegar [80] describes the best nanoparticle manufacturing process in a homogeneous solution. According to the model, precursor solute concentration, C increases to critical nucleation level, \( C_{\text{crit}} (C_{\text{min}}) \) and this is the onset of the nucleation stage. This \( C_{\text{min}} \) will move to \( C_{\text{max}} \) when the amount of solute added is balanced by the nucleation stage with the generation of nuclei. At this point, the curve follows a downward trend, where nucleation is still growing in number till they reach \( C_{\text{crit}} \) level again. At this point, the nucleation stage is followed up by the growth stage, and a good separation between the both is utmost important in order to obtain the monodispersed nanoparticles [80, 81]. The best suitable kinetics model that describes this process of formation of nanoparticles was given by LaMer theory and supported by the Sugimoto’s model [82]. Figure 1.3 shows the LaMer diagram as a schematic explanation for the formation procedure of monodispersed particles [80, 82, 83].
Figure 1.3: La Mer diagram for the generation of atoms, nucleation and subsequent growth of colloidal systems

Till now a number of hydrolyzing agents have been used to obtain nanoparticles. However, sodium hydroxide (NaOH) and ammonium hydroxide (NH₄OH) are widely used to synthesize a variety of nanoparticles. In most cases, however, to achieve the desired shape and size of the nanoparticles, these agents were used in the presence of a variety of surfactants. This is done in order to control the aggregation stage which leads to polydispersity of the nanoparticles. In the nanotechnology area, amines are regularly used for nanomaterial synthesis including surface functionalization, gel formations etc. Among them, ethanolamine and polyethylamine are among the most commonly used. However, the aliphatic tertiary amines, such as triethylamine have not been investigated in detail in the nanomaterials area as a shape-directing hydrolyzing agent.
1.4 Amines in general

Nitrogen is found widely distributed among plants and animals in various forms of organic compounds for the sustaining of life on this planet earth. Most of the hormones, vitamins, amino acids, proteins, nucleotides etc. contain this constituent element for the proper functioning of the cellular organisms [84, 85]. Nitrogen is also a constituent element in a variety of industrial compounds and polymers. Nylon is one of the best examples of man-made materials that has nitrogen as one of the basic constituent elements. Overall, we can say that nitrogen is one the element which is worth studying from different research aspects [84].

Nitrogen forms various bonds with other widely distributed elements, i.e. carbon, hydrogen, and oxygen. Since nitrogen has five valence electrons, it can form single, double or triple covalent bonds to hydrogen or with carbon. Methylamine (CH$_3$NH$_2$) is the simplest alkyl amine, a pyramidal shape amine with nitrogen in the center by replacing a hydrogen atom of ammonia as shown in Figure 1.4.

![Methylamine structure](image)

Figure 1.4: Structure of the methylamine [84]

Amines can undergo nitrogen inversion in which the three bonded groups can temporarily occupy a common plane as shown in Figure 1.5 [85]:

![Methylamine nitrogen inversion](image)
1.4.1 Nomenclature and Classification of amines

In general, the primary amine’s nomenclature has a suffix – amine – as here the alkyl group is directly bonded to –NH₂ group. Similarly, based on bonding, the secondary and tertiary amines are described with the prefix di and triamines, respectively. For better understanding of the conversion of the alcohols to various amines, consider the following example as shown in Figure 6 where the tertiary alcohol (tert-butyl alcohol) is converted to a primary amine (tert-butylamine) and to tertiary amine (triethylamine) by first replacing the –OH group to form primary amine or replacing the –C group in total with nitrogen to form tertiary amine.

The basic classification of the various amines depends upon the functional group of nitrogen atom with a lone pair of electrons as a characteristic of amine which classifies it as an organic compound. This can be treated as an ammonia derivative where alkyl or aryl group can substitute for one or more hydrogen atoms. These compounds can easily be distinguished because of their distinctive unpleasant smell of rotting fish [84, 85]. Basically, they are classified into four subcategories and as shown in Figure 1.6.
(i) **Primary (1°) amines** - one hydrogen atom in ammonia is substituted by an aromatic or alkyl group. Examples: methylamine, aniline etc.

(ii) **Secondary (2°) amines** - When either alkyl, aryl or a combination thereof, are bound along with one hydrogen atom to the nitrogen. Examples: dimethylamine, diphenylamine etc.

(iii) **Tertiary (3°) amines** - When three organic substituents are either alkyl, aryl or a combination thereof. Example: trimethylamine, triethylamine, EDTA etc.

(iv) **Cyclic amines** - They are either secondary or tertiary amines. Examples: aziridine; N-phenylpiperidine and some are shown in Figure 1.7.

![Structural representation of the various cyclic amines](image)

Figure 1.7: Structural representation of the various cyclic amines [84]

For better understanding of the conversion of the alcohols to various amines, consider the following example as shown in Figure 1.8 where the tertiary alcohol (tert butyl alcohol) is converted to a primary amine (tert butylamine) and to tertiary amine (triethylamine) by first replacing the –OH group to form primary amine or replacing the –C group in total with nitrogen to form tertiary amine.

![Structural representation of the conversion of alcohols to amines](image)

Figure 1.8: Structural representation of the conversion of alcohols to amines [84]
There are some complex amines as well, where the amino group is treated as a substituent in primary amines. In secondary and tertiary amines, they are named as N-alkylamino (-NHR) or N, N-dialkylamino (-NRR’) group. The few complex amines are γ-aminobutyric acid and β-(N, N-dimethylamino)caproic acid as shown in the Figure 1.9. There is one more special class of amines known as anilines with amino substituted benzene is present where the direction of the numbering is based on the location of the second group [84].

![Diagram of amines](image)

**Figure 1.9: Examples of some complex amines**

### 1.4.2 Physical properties of Amines

#### 1.4.2.1 Melting and boiling points of amines

Low molecular weight amines are gases at room temperature, whereas high molecular weight amines are liquids or solids. In general, they have a higher boiling point than alkanes with similar molecular weight, but lower than that of alcohols as shown in Figure 1.10.

![Diagram of boiling points](image)

**Figure 1.10: Structural representation of ligands along with boiling points**

Because of the more polar nature of the C-N bond in comparison to C-C bond, amines usually have higher boiling points than hydrocarbons with similar molecular weight. In
addition, primary and secondary amines can easily form intermolecular hydrogen bonds, as they can act as both hydrogen donors and acceptors [84, 85]. Just the special case is of the tertiary amines, as they have no hydrogen atoms bonded to the nitrogen atom, so it doesn’t act as hydrogen donor.

![Diagram of hydrogen bonds in amines and alcohols]

Figure 1.11: Structural representation of the change of strength of various bonds in (a) an amine and (b) an alcohol [84]

In other words, it cannot form intermolecular hydrogen bonds. This is the reason why they have low boiling points in comparison to similar molecular weight primary and secondary amines. This is because nitrogen is less electronegative than oxygen, so N-H bond is less polar than O-H bond, and the hydrogen bond in amines is weaker than the hydrogen bond in alcohols as shown in Figure 1.11. Table 1.1 enlists the boiling point of some amines.

Table 1.1: The boiling point of some amines [85]

<table>
<thead>
<tr>
<th>Name</th>
<th>Boiling point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimethylamine</td>
<td>3</td>
</tr>
<tr>
<td>Triethylamine</td>
<td>90</td>
</tr>
<tr>
<td>Diethylamine</td>
<td>56</td>
</tr>
<tr>
<td>Dimethylamine</td>
<td>7</td>
</tr>
<tr>
<td>Butylamine</td>
<td>77</td>
</tr>
</tbody>
</table>
sec-butylamine | 63  
Cyclohexylamine | 134  
Ethylmethyamine | 37  
Propylamine | 48  
Methyamine | -7  
Isopropylamine | 33  
Isobutylamine | 68  
tert-butylamine | 45  
Dipropylamine | 111  
Tripropylamine | 156  
Ethylamine | 17

### 1.4.2.2 Solubility of amines

Amines’ miscibility in water depends heavily upon the number of carbon atoms present in them, where five or fewer carbon atoms are usually miscible with water. On the other hand, as molecular weight increases, the solubility of amines deceases [85]. Because of the hydrogen bond donors and acceptors nature, the primary and secondary amines forms hydrogen bond network with water and make themselves miscible, whereas in case of tertiary amines, they get miscible with water due to the nitrogen atom’s non-bonded electron pair (acts as acceptor of a hydrogen atom of water) which forms hydrogen bond readily with water as shown in Figure 1.12 and 1.14.
1.4.2.3 Odour and Toxicity of Amines

Amines usually have a very strong fishy odour where it smells like ammonia for low molecular weight amines and fishy for high molecular weight amines. Two specific examples, where the amines smell likes decaying animal tissues are the putrescine and cadaverine. Due to physiological nature, living organisms can’t ingest them, and in the case of consumption, it can lead to poisoning and also death. Likewise, human skin contact to various amines lead to their absorption instantly and needs to be handled with care while working. Like arylamines such as benzidine and β naphthylamine shown in Figure 1.13 can be readily absorbed by the skin and hence are carcinogenic in nature.

Figure 1.12: Interaction of amines with water via the hydrogen bonds [84]

Figure 1.13: Structural representation of (a) benzidine and (b) β naphthylamine [84]
1.4.2.4 Basicity of amines

For understanding the basicity of the amines, we should know the structure of both the amine and its conjugate acid. The basicity of amines is usually represented as a $pK_b$, which is the negative logarithm of $K_b$ [85]. As an example, for the amine with $K_b = 10^{-3}$, the $pK_b$ is 3. In general, as the base strength increases, the $pK_b$ decreases. In common practice, we describe the relative base strength of amines in terms of the $pK_a$ values of their conjugate acids. For amines with large $pK_b$, its conjugate ammonium ion has a small $pK_a$. Thus, the both these values, i.e. $pK_b$ and $pK_a$ for a conjugate acid-base pair are related as equation 2:

$$pK_b + pK_a = 14$$

Equation 1.2

From this equation, it is quite evident that from a strong amine base, the conjugate ammonium ion needs to be more stable in relative to amine base and vice versa. The Table 1.2 shows the basicity of some amines.

In the case of triethylamine, it is a weaker base than the secondary amine, i.e. diethylamine because its conjugated acid is not as effectively solvated, whereas diethylamine has the proper balance of electrons between the alkyl groups and subsequent stabilization of the conjugate acid. The schematic representation in Figure 1.14 explains this phenomenon in detail.

![Figure 1.14: Structural representation of (a) diethylamine and (b) triethylamine interaction with water](image-url)
Table 1.2: Basicity of some amines [85]

<table>
<thead>
<tr>
<th>Name</th>
<th>$K_b$</th>
<th>$pK_b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimethylamine</td>
<td>$5.5 \times 10^{-4}$</td>
<td>4.26</td>
</tr>
<tr>
<td>Triethylamine</td>
<td>$5.7 \times 10^{-4}$</td>
<td>3.24</td>
</tr>
<tr>
<td>Diethylamine</td>
<td>$5.3 \times 10^{-4}$</td>
<td>3.01</td>
</tr>
<tr>
<td>Butylamine</td>
<td>$4.8 \times 10^{-4}$</td>
<td>3.22</td>
</tr>
<tr>
<td>Propylamine</td>
<td>$4.7 \times 10^{-4}$</td>
<td>3.33</td>
</tr>
<tr>
<td>Methylamine</td>
<td>$4.3 \times 10^{-4}$</td>
<td>3.37</td>
</tr>
<tr>
<td>Tripropylamine</td>
<td>$4.5 \times 10^{-4}$</td>
<td>3.35</td>
</tr>
<tr>
<td>Dipropylamine</td>
<td>$1.0 \times 10^{-4}$</td>
<td>3.00</td>
</tr>
<tr>
<td>Dimethylamine</td>
<td>$4.7 \times 10^{-4}$</td>
<td>4.26</td>
</tr>
<tr>
<td>Isopropylamine</td>
<td>$4.0 \times 10^{-4}$</td>
<td>3.40</td>
</tr>
<tr>
<td>Ethylamine</td>
<td>$4.4 \times 10^{-4}$</td>
<td>3.36</td>
</tr>
</tbody>
</table>

1.4.2.5 Solubility of the ammonium salts

The equation (3) represents how the nitrogen atom of the amine gets protonated to produce an ammonium salt when they are added to a solution of a strong acid like hydrochloric acid.

$$RNH_2 + HCl \leftrightarrow RNH_3^+Cl^-$$  

Equation 1.3

Low molecular weight ammonium salts are usually water soluble with a higher melting point than their respective amines, and less prone to oxidation due to the presence of positive charge; particularly if their hydrocarbon portion is small. This property is well exploited by the pharmaceutical industry, where the drugs are often prepared by having these ammonium salts, for their better solubility in body fluids. Another benefit is the odour where it has virtually no smell in comparison to the fishy odour of the amines. Like an example, ephedrine which is a common drug used for cold and allergy has a melting point of 79 °C with an
unpleasant fishy odour. However, its hydrochloride salt has a melting point of 217 °C with odourless as shown in Figure 1.15.

![Ephedrine hydrochloride](image)

**Figure 1.15: The basic structure of the ephedrine hydrochloride [85]**

### 1.4.3 Synthesis of amines

#### 1.4.3.1 Alkylation with alcohols

Majority of amines are prepared using ammonia by the alkylation with alcohols as shown in equation (4).

\[
ROH + NH_3 \rightarrow RNH_2 + H_2O \quad \text{Equation 1.4}
\]

The reaction of amines and ammonia with alkyl halides is as follows and basically followed up in the laboratory-based synthesis as shown in equation (5).

\[
RX + 2R'NH \rightarrow RR'NH + [RR'NH_2]X \quad \text{Equation 1.5}
\]

#### 1.4.3.2 Reductive routes

These routes are employed by using the hydrogenation process. Like nitriles are reduced to amines using hydrogen via the nickel catalyst. The reduction of nitroaromatics, lead to the synthesis of aniline. On an industrial scale, hydrogen being light element is often used as reductant, but at the laboratory scale, iron and tin are used as reductive catalysts.

#### 1.4.3.3 Specialised methods

However, in addition to the general routes, to make particular amines there are some specific methods like Gabriel synthesis using organohalide as substrate [86]; Staudinger reduction using azide as substrate [87]; Schmidt reaction using carboxylic acid as substrate [88]; Aza-
Baylis-Hillman and Birch reduction reaction using imine as substrate [89]; Hofmann degradation using amide as substrate [90]; Nitrile reduction using nitriles as substrate [91]; Delepine reaction using organohalide as substrate [92], Buchwald-Hartwig reaction using aryl halide as substrate [93]; Menhutkin reaction using tertiary amine as substrate [94]; Leukart reaction using ketone and aldehydes as substrates [95]; Hofmann-Loeffler reaction using haloamine as substrate [96]; Eschweiler-Clarke reaction using amine as substrate [97] etc.

1.4.4 Detection of amines

Spectroscopy based techniques like FTIR, Raman, and NMR are widely used for the detection of amines [85]. However, out of them, FTIR is probably the best technique to detect the presence/ type of various amines in an organic compound in less time and efforts. The C-N stretching absorption occurs in the region between 1050-1250 cm\(^{-1}\) and N-H in the range from 3200-3380 cm\(^{-1}\). In particular, primary amines have two absorption bands in this region as shown in Figure 1.16.

![Figure 1.16: FTIR of the primary amine](image)

On the other hand, secondary amines have a single absorption band as shown in Figure 1.17.
In the case of tertiary amines, there is no absorption in the region from 3200-3380 cm$^{-1}$ because of the lack of N-H bond, as depicted in Figure 1.18.

### 1.4.5 General applications of amines

The amines are widely used across a number of industries, namely paint and dye industry; pharmaceutical industry, agriculture industry in the manufacturing of fertilizers, polymer industry in the manufacture of nylon, explosive industry in the manufacture of explosives (via nitric acids) and the gas treatment industry [85]. Some of them are listed in Table 1.3.
Table 1.3: The application of the various amines

<table>
<thead>
<tr>
<th>Paint and dye industry</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl Orange</td>
<td>Used in titration and often treated as pH indicator, where it turns pink in an acidic environment and yellow in a basic environment.</td>
</tr>
<tr>
<td>Direct brown 138</td>
<td>It is a red-light brown colour dye. Highly soluble in water to give orange-brown and slight in alcohol.</td>
</tr>
<tr>
<td>Sunset Yellow FCF</td>
<td>Also known as orange-yellow is an azo dye derived from petroleum. Mostly named as FD&amp;C yellow 6 in US and E110 in Europe when added in food for human consumption. Found commonly in desserts, sauces, snacks, chocolates, caramels, preserved fruits etc. Also used in cosmetics and drugs.</td>
</tr>
<tr>
<td>Ponceaucoo</td>
<td>It is red colour azo dye and used in a variety of food products since it is least toxic in nature.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pharmaceutical industry</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpheniramine</td>
<td>Used for cold, hay fever, itchy skin, insect bites etc.</td>
</tr>
<tr>
<td>Ephedrine</td>
<td>Used as decongestants</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>Used as sedates to relieve anxiety, excitement or mental conditions</td>
</tr>
<tr>
<td>Phenethylamines</td>
<td>Used as psychedelic drugs</td>
</tr>
<tr>
<td>-----------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Lofepramine</td>
<td>Used to relieve depression</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>Used to treat depression related disorder, bipolar disorder, neuropathic pain</td>
</tr>
<tr>
<td>Clomipramine</td>
<td>Used to treat obsessive-compulsive disorder, depression, chronic pain</td>
</tr>
<tr>
<td>Amoxapine</td>
<td>Used to treat the major depressive disorder, schizophrenia</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>Used to treat blood pressure, hemorrhoids</td>
</tr>
</tbody>
</table>

**Gas treatment industry**

Monoethanolamine, diethanolamine, methyl diethanolamine | Used for carbon dioxide removal and hydrogen sulphide.

1.5 Triethylamine – a shape-directing hydrolyzing agent

Triethylamine (N(CH₂CH₃)₃) is a colourless volatile liquid with a strong fishy odour. It is commonly abbreviated as ET₃N or TEA. The basic physical and chemical properties of triethylamine are listed in Table 1.4.

Table 1.4: Some basic physical and chemical properties of triethylamine

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>0.7255 g mL⁻¹</td>
</tr>
<tr>
<td>Melting Point</td>
<td>−114.70 °C; −174.46 °F; 158.45 K</td>
</tr>
<tr>
<td>Boiling point</td>
<td>88.6 to 89.8 °C; 191.4 to 193.5 °F; 361.7 to 362.9 K</td>
</tr>
<tr>
<td>Vapour pressure</td>
<td>6.899–8.506 kPa</td>
</tr>
<tr>
<td>Henry’s law constant (Kₜ)</td>
<td>66 μmol Pa⁻¹ kg⁻¹</td>
</tr>
<tr>
<td>Property</td>
<td>Value</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>Acidity (pK_a)</td>
<td>10.75 (for the conjugate acid) (H_2O), 9.00 (DMSO)</td>
</tr>
<tr>
<td>Magnetic susceptibility (χ)</td>
<td>-81.4·10⁻⁶ cm³ mol⁻¹</td>
</tr>
<tr>
<td>Refractive index (n_D)</td>
<td>1.401</td>
</tr>
<tr>
<td>Heat capacity (C)</td>
<td>216.43 J K⁻¹ mol⁻¹</td>
</tr>
<tr>
<td>Std enthalpy of formation (at room temperature)</td>
<td>−169 kJ mol⁻¹</td>
</tr>
<tr>
<td>Std enthalpy of combustion (at room temperature)</td>
<td>−4.37763 to −4.37655 MJ mol⁻¹</td>
</tr>
</tbody>
</table>

Over the last century, triethylamine has been extensively used in manufacturing of pharmaceuticals such as ampicillin, amoxicillin, cefradine, penicillamine etc.; pesticides, polymerization inhibitors, high-energy fuels, rubber vulcanization agent, catalyst, in organic synthesis, special solvent for paint removal; enamel; anti-hardening agents; surface active agents; preservatives; bactericides; ionic exchange resins; dyes; perfumes; liquid rocket propellants etc. [98]. Considering these factors, and the huge variety of the roles previously demonstrated by TEA, in general, my research work in the Ph.D. is further elaborated with the use of TEA under nanotechnology umbrella defining the role of TEA in the synthesis of various nanomaterials for biological and electronics applications. The key focus of my work revolves around how to control the facets and dimensions of the nanomaterials ranging from one-dimensional (1D) to two-dimensional (2D) to three-dimensional (3D) using TEA.

In order to understand the basic role of the TEA in the synthesis of various nanomaterials, we have to get some insights on how TEA behaves when it comes in contact with water. In general, the triethylamine: water (TEA/W) mixture is a partially miscible system with a lower critical transition temperature (LCST) of 18.4 °C at 32.1 wt.% critical concentration of TEA.
The formation of the hydrogen bonds between the TEA and water below the LCST is responsible for its miscibility and breaking of these hydrogen bonds are responsible for their phase separations. Such type of system is termed as a structured liquid. This hydrogen bonds are short-range forces and their formation depends strongly on the relative orientation of the two molecules concerned. Thus, LCST is considered to be an optimal temperature where at least one of the components of the solution begins rotating and below LCST the hydrogen bonding between water and TEA molecules obstructs such free rotations. Recently it has been observed that below the LCST, water-TEA is not totally a homogeneous mixture and there are temporal concentration fluctuations. The hydrogen bond dominance depends upon whether it is present in the TEA rich zones or Water rich zones, where the latter had more bonds due to the presence of excess water molecules. It has been experimentally observed that breaking of hydrogen bonds happens first in TEA rich zones and this followed by the Water rich zones, which eventually leads to phase separation between the two entities [99, 100].

The self-associated water molecule chain clusters formed by the dangling protons have nitrogen site centered in the water TEA system, with the weak interaction with the methyl and methylene groups where higher the amount of hydrogen bonds within one cluster, better the stability. Thus, by modifying the water concentration of the water–TEA mixtures the cluster concentration can be adequately managed and, therefore, the dangling protons available for hydrogen-bonding. But the main idea here in this thesis is to emphasize on how we can use this molecule for the nanomaterial synthesis. Alkali molecules such as NaOH and NH4OH are commonly employed as hydrolysing agents for nanomaterials synthesis [61,69,72,73]. However, these alkali molecules have been observed to play little or no influence in controlling the morphological characteristics. Since a significant body of existing literature discusses these common alkalis, my efforts in the current thesis have focussed on
TEA as a shape-directing hydrolysing agent. Being an organic compound with basic nitrogen with a lone pair of electrons, organic amines have been widely explored in material science for the synthesis of various nanoparticles, including metals, metal oxides, quantum dots, etc. [101-109]. Due to the presence of nitrogen and basicity of amines, the affinity of these molecules to various metals can be modulated, resulting in the variations in the structural features of the end product. In general, long chain amines are used as surface-stabilising agents during nanomaterials synthesis, as they may preferentially bind to various crystal facets, thus allowing control over nanoparticle size and morphology. On the other hand, short chain organic amines have not been sufficiently exploited in material science for the synthesis of nanoparticles with only a selected few studies that do not necessarily paint the true picture around the role of such amines in materials synthesis [101, 104]. As a brief summary, various amines utilised till date in materials science include dodecylamine [101]; triethanolamine [102]; oleylamine [103, 107]; diisopropylamine [104]; arginine [105]; poly(ethylene imine) [106]; ethanolamine [106]; 2,2-(ethylenedioxy)bis(ethylamine) [106]; ethylene diamine [108]; diethylamine [109]; ethylamine [109]; trimethylamine [109]; tripropylamine [109]; tributylamine [109]; triethylamine [109] etc. for synthesis of various nanomaterials.

1.6 Objective of the Thesis

Synthesis of nanomaterials of a variety of shapes and sizes is quite well established in literature till now; however, there is always some space for the new molecules to enter into the list, as to bring new chemical activities and/or new morphologies. Here, I am introducing the new tertiary amine molecule triethylamine (TEA) which acts as a structured agent in synthesizing a variety of nanomaterials of different shapes and sizes. In my thesis, my prime focus was to control the structural and chemical composition of nanomaterials by using TEA.
as a hydrolyzing agent. Further, I demonstrate that TEA can also be used for rapid phase transfer of organically-coated nanomaterials in less than a minute and using this ligand, 2D layered materials can also be easily synthesised using a bottom-up approach. The as-obtained materials were characterized in detail before applying for the desired applications like the photoelectric effect, photocatalysis, magnetic hyperthermia and biosensing.

1.7 Thesis Outline

The work presented in this thesis describes the synthesis of several different classes of nanomaterials, while incorporating TEA as a novel hydrolysing agent and/or a phase transfer agent, with an end goal of synthesising a material which has desirable electronic, catalytic and biological properties that are enhanced with respect to different structures formed in the presented novel synthesis approach. Importantly, it has been demonstrated that using the TEA mediated synthesis approach, a multitude of nanoscale materials with unusual and functional properties can be synthesized in a rather simple, repeatable process. The chapter-wise breakdown of the thesis is as follows:

1.7.1 Chapter 2 concerns the many instruments used in this body of work for the characterization of the synthesized materials. The instruments used are UV-Visible-Near infrared absorbance spectroscopy (UV-Vis-NIR); vibrational spectroscopy techniques including Fourier transform infrared spectroscopy (FT-IR), Raman spectroscopy and surface-enhanced Raman spectroscopy (SERS); X-ray diffraction (XRD); transmission and scanning electron microscopy (TEM and SEM); elemental analysis techniques such as energy dispersive X-ray analysis (EDX) and X-ray photoelectron spectroscopy (XPS); Hydrodynamic size and Zeta potential measurements using dynamic light scattering; Atomic force microscopy; and Super Quantum Interference Device (SQUID) etc.
1.7.2 Chapter 3a discusses the synthesis of superparamagnetic iron oxide nanoparticles (SPIONs) for the magnetic hyperthermia application. Here we used TEA for one-pot synthesis of iron oxide nanoparticles which can be directly synthesised in aqueous or organic media, and also can be readily dispersible in both aqueous and organic medium after drying them in the powder forms. We further, developed a fast and rapid approach to enable phase transfer of the organic coated nanoparticles to the aqueous medium. The as-obtained nanoparticles were well characterized using various techniques and their magnetic properties were studied. It was observed that the magnetic properties of these materials improved following the phase transfer of these materials to aqueous phase, which allowed us to demonstrate their magnetic hyperthermia capabilities for the biological application. The work presented in this Chapter was published in Applied Materials Today (2018, 12, 250-259) and due to the commercial significance of this work, a patent application was lodged (M. Singh, V. Bansal, R. Ramanathan, A. Gammilonghi. Method for preparing an aqueous dispersion of metal oxide particles. Australian Provisional Patent Application No. AU2018901786).

1.7.2.1 Synthesis Methodology

Triethylamine has not been previously used for the synthesis of superparamagnetic iron oxide nanoparticles. This work for the first time reported one-pot synthesis of the iron oxide nanoparticle across the aqueous and organic medium, wherein TEA was used as a hydrolysing as well as capping agent to produce SPIONs.

Briefly, direct amine functionalized aqueous-dispersible iron oxide nanoparticles were prepared in a single precursor step approach as follows: take 2:1 molar ferric (Fe³⁺) and ferrous (Fe²⁺) ions in 40 mL of water and magnetically stir for 5 minutes at 80 °C. Then 5 mL of triethylamine is added into the solution at once, allowing the dissolved Fe ions to get hydrolyzed to form iron oxide nanoparticles according to the following equation:

\[(\text{CH}_3\text{CH}_2)_3\text{N} + \text{H}_2\text{O} \rightarrow (\text{CH}_3\text{CH}_2)_3\text{NH}^+ + \text{OH}^- \quad \text{Equation 1.6}\]
The cationic amine molecule produced during the reaction were adsorbed onto the formed iron oxide nanoparticle surface via the hydroxyl groups within the mother liquid which made them strongly aqueous dispersible. The dispersion was allowed to cool under ambient conditions at room temperature after one hour of magnetic stirring. The nanoparticles were washed 5 times with acetone: methanol (1:1) solution and dried in an oven at 60 °C overnight and are denoted as “A-SPIONs”.

Similar single precursor step approach was followed for obtaining the non-aqueous solvent dispersible iron oxide nanoparticles as follows: ferric (Fe$^{3+}$) and ferrous (Fe$^{2+}$) ions in 2:1 molar ratio was dissolved in 40 mL of water and stirred for 5 minutes in an oil bath kept at 80 °C under the oxidizing environment (air). Then 0.5 mL of oleic acid pre-dissolved in 5 mL acetone was added to the above solution and stirred further for 2 min, followed by the addition of 5 mL of triethylamine at once. Here, triethylamine will hydrolyze the iron ions to form iron oxide nanoparticles; however, the nanoparticle fresh surface will be covered by the oleic acid surfactant molecules present initially in the mother liquid. The dispersion was allowed to cool under ambient conditions at room temperature after one hour of magnetic stirring. The nanoparticles were washed 5 times with acetone: methanol (1:1) solution and dried in an oven at 60 °C overnight and are denoted as “O-SPIONs”.

The as-synthesized TEA coated nanoparticles are well-dispersed in an aqueous medium. However, for the oleic acid coated nanoparticles, we further developed a novel and rapid phase transfer protocol by utilizing additional TEA molecules to transfer particles from organic to aqueous medium. In brief, bath sonication is used where 0.1 g of O-SPIONs nanoparticles were dispersed in 1 mL of triethylamine for 1 minute. A desired amount of water is added to this, to prepare a defined nanoparticle concentration in the aqueous phase.
To remove the excess of the ligands, a 24-hour dialysis was performed with the replacement of the water after every 2 hours and these particles are denoted as “AO-SPIONs”. Overall the beauty of this phase transfer mechanism is that it is simple, rapid and very straightforward where the amphiphilic triethylamine molecules play a direct role in particle phase transfer.

1.7.2.2 Application aspect – Magnetic Hyperthermia

1.7.2.2a General Concept

Magnetic materials can be used as heat mediators in the hyperthermia applications, where the temperature rises in the medium where they are suspended or in a tumor, in the presence of a remote-controlled alternating magnetic field. The National Cancer Institute recognizes three types of hyperthermia treatments [110]:

a) Localized hyperthermia - which has its impact at a very small area like tumors, and temperature rise can be achieved using micro, radio or ultrasound-based frequencies. Gordon et al. [111], showed for the first time local heating in vivo for the dextran-coated iron oxide nanoparticles.

b) Regional hyperthermia - which has its impact on larger areas like tissues, organs or limbs, and in this case, the use of an external applicator is a must.

c) Whole body-based hyperthermia - where the whole animal body is subjected to treatment. Such clinical trials are underway in Germany for the brain and prostrate cancer [52].

Among various frequencies, radio frequency (RF) based hyperthermia is considered to be the most desirable due to its biological target selectivity under the biological permissible limits. In RF-based hyperthermia, magnetic nanoparticles can work to produce heat which can eventually be delivered in one of the four ways, i.e. via the arterial/ direct/ in-situ implant formation/ active targeting, respectively. The magnitude of the heat produced depends upon a number of factors which include nanoparticle size, shape, suspension medium, the strength of
the RF field etc. All these factors, determine the overall heating efficiencies based on the
relative contributions from the hysteresis losses, as well as the Neel, and Brownian relaxation
processes. However, out of all these relaxations, hysteresis is mainly avoided by obtaining
single domain nanoparticles which produce heat either by the thermal fluctuations due to
either Brownian fluctuations of the grain itself within the carrier liquid or internal
fluctuations of the magnetic moment with respect to the crystal lattice (Neel fluctuations).
Shorter the fluctuation time, more dominant is overall heating of the nanoparticle.
Rosensweig [112] provided the analytical relationship between the power dissipation from
the magnetic nanoparticles under the RF field. Thus, the effective power loss under adiabatic
conditions is given as:

\[ P' = \int_0^\infty P f(D) dD, \frac{\Delta T}{\Delta t} = \frac{P}{\rho C_p} \quad \text{Equation 1.9} \]

Where f(D) is a lognormal particle size distribution, Cp is the weighted specific heat capacity
and \( \rho \) is the weighted density.

1.7.2.2b Experimental Set-up

The device used to induce magnetic hyperthermia is represented in Figure 1.19. The heating
properties of the nanoparticle were measured within the span of 300 s by using a 400 kHz
generator with a nominal power output of 2.5 kW and the rise in temperature was measured
with respect to time. The ability of the nanoparticles to dissipate the heat under the alternating
magnetic field via the Brownian and Neel relaxation mechanisms were measured in terms of
specific absorption rate (SAR) using the formula [113]:

\[ \text{SAR} = \sum \frac{m_{c_i} \Delta T}{m_{NP}} \frac{\Delta T}{\Delta t} = \frac{\bar{C}_p}{\bar{W}_{NP}} \frac{\Delta T}{\Delta t} \quad \text{Equation 1.10} \]

where \( \Delta T \) is changed in temperature, \( \Delta t \) is changed in time, \( \Delta T/\Delta t \) is the heating rate of the
colloidal dispersions of iron oxide nanoparticles, as evaluated by the linear section of the
temperature rise curve; $W_{NP}$ is the mass fraction of iron oxide nanoparticles in the sample; and $C_p$ is the mean value of its specific heat capacity in the investigated temperature range.

Figure 1.19: (a) Schematic representation of the hyperthermia unit. (1) Computer to record change in temperature with 1 s resolution, (2) temperature base controller, (3) fiber optics-based thermometer, (4) sample, holder, (5) water-cooled two coil copper rings attached to an AC generator (400 kHz), and (6) a represented SPION with indicated Néel and Brownian relaxations [113].

1.7.2.2c Results and conclusions

The TEA-mediated synthesis and phase-transfer protocol reported in this work allowed us to make the direct comparison of the role of Neel and Brownian relaxations in inducing magnetic hyperthermia by evaluating the properties of different SPIONs prepared here, as heat susceptors for radiofrequency (RF)-induced heating. Also, this study has for the first time, reported the one-pot synthesis of superparamagnetic iron oxide nanocrystals of maghemite phase using TEA as a dynamic molecule that can directly produce SPIONs under mild conditions that are readily dispersible both under aqueous and organic environments. Interestingly, it was noticed that TEA molecules could not only facilitate phase-transfer of oleic acid-capped SPIONs from an organic to the aqueous phase, but the powders of oleic
acid-capped SPIONs could also be directly re-dispersed in aqueous solvents in a single step using TEA. This new phase transfer protocol is not only rapid and highly efficient; importantly, the magnetic properties of oleic acid-capped SPIONs are improved after their aqueous phase transfer. This is otherwise rarely the case, as the current phase transfer protocols typically deteriorate the magnetic properties of SPIONs. The phase-transferred SPIONs obtained from this new route revealed outstanding magnetic properties, which could then be exploited for a proof-of-concept demonstration in magnetic hyperthermia. While the materials reported in this study are likely to find use in biomedical applications, it is important to highlight the versatility of TEA molecules as unique amphiphilic tertiary molecules for a variety of applications. More details are given in Chapter 3.

1.7.3 Chapter 3b discusses the synthesis of highly oxygen-deficient and photostable Cu$_2$O nanomaterials using triethylamine and application of these materials in visible light photocatalysis. Being a p-type semiconductor, Cu$_2$O is a very important material in various applications, especially in the electronics industry. However, obtaining this material in an oxygen-deficient state is still a challenge, which once available, is expected to open up new applications of these materials. Taking this challenge into account, here a novel method was developed to obtain the highly photostable and oxygen-deficient Cu$_2$O nanoparticles using a low-temperature method in the presence of TEA with 90% yield in gram scales. These nanoparticles were well characterized using numerous techniques and it was observed that they exhibited outstanding photoactivity in visible light by hydroxyl radical generation. Overall, through this work, we could establish the importance of oxygen defects in transition metal oxides in the photocatalysis area.

1.7.3.1 Novel synthesis of Cu$_2$O nanoparticles through forming a Cu(I)-TEA complex
In this work, we reported for the first time that how triethylamine can be efficiently used in obtaining “oxygen-deficient” cuprous oxide (Cu$_2$O) nanoparticles by first synthesising a metal coordination complex between the copper(I) and TEA species in acetonitrile, followed by hydrolysis of this complex in a biphasic solvent mixture of acetonitrile and water. The synthesis procedure is a two-step procedure. First, the Cu(I)-TEA metal coordination complex was prepared by magnetic stirring (500 rpm) of copper (II) chloride (0.1M in 400 mL acetonitrile) in a round bottom flask at 80 °C for 5 minutes. Following this, 50 mL of TEA was added at once to the above stirring solution. The mild reducing ability of TEA resulted in a reduction of Cu (II) species to Cu (I), which instantaneously formed an ionic complex with TEA. The formation of this Cu(I)-TEA complex was evident from the generation of non-violent vapors on the addition of TEA to the copper salt. With continuous stirring for 1 hour, the solution phase turned to dark-red. After being cooled to room temperature under ambient conditions, the reaction product was centrifuged at 9,000 rpm for 15 minutes to remove any potentially precipitating species. The liquid phase corresponding to the Cu(I)-TEA complex was collected and subsequently used for the synthesis of Cu$_2$O nanoparticles. In the next step, for the large-scale synthesis of Cu$_2$O nanoparticles, 450 mL of the purified Cu(I)-TEA complex was brought to 80 °C in a round bottom flask. After 5 minutes, an equivalent volume of water (preheated to 80 °C) was added to this complex, which resulted in instantaneous hydrolysis of Cu(I)-TEA complex, leading to precipitation of Cu$_2$O nanoparticles. The particles were allowed to precipitate for 4 hours under non-stirring conditions. Following this, reaction contents were allowed to cool for 1 hour, and the Cu$_2$O nanoparticles were collected by centrifugation at 9,000 rpm for 15 minutes. The particles were further washed three times with acetone/methanol (1:1 v/v) to obtain a yellow pellet. The precipitate was dried under vacuum at room temperature to achieve a gram scale (2.56 g)
yield of Cu₂O particles in a single batch, which corresponds to over 90% conversion of the starting copper salt. The plausible reaction mechanism is as follows:

\[
\text{CuCl}_2 + \text{NEt}_3 \rightarrow [\text{CuCl}_2]^- [\text{NEt}_3]^+ \quad \text{Equation 1.11}
\]

\[
[\text{CuCl}_2]^- [\text{NEt}_3]^+ \leftrightarrow [\text{CuCl}][\text{NEt}_3] + \text{Cl}^- \quad \text{Equation 1.12}
\]

\[
2[\text{CuCl}][\text{NEt}_3] + \text{H}_2\text{O} \rightarrow \text{Cu}_2\text{O} + 2\text{NEt}_3 + 2\text{HCl} \quad \text{Equation 1.13}
\]

1.7.3.2 Application aspect- Photocatalysis

1.7.3.2a General Concept

In general term, any material is termed as a catalyst which enhances the rate of a reaction. And when such material does this job with the aid of light it is known as a photocatalyst. In other words, photocatalysis involves the use of light as an energy source for the chemical reaction to happen by the suitable material. In photocatalysis, the light is absorbed by the material, which creates the electron-hole (e-h) pairs within the material crystal lattices. These pairs will be responsible for the free radicals generations which can aid in secondary reactions. In nature, chlorophyll in plants acts as a natural photocatalyst which captures and uses light to turn water and carbon dioxide into oxygen and glucose. Titanium dioxide is the first ever material reported for use as a catalyst. Generally, photocatalysis can be of two types:

(I) **Homogeneous Photocatalysis** – here all the reactants and the photoactive catalyst are from the same phase like Fe ions and Fe ions/H₂O₂ system and the production of the OH radicals. The H₂O₂ can further undergo photolysis under the UV light to generate more OH radicals which can further be used for Fenton type processes as follows [114]:

\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{HO} + \text{Fe}^{3+} + \text{OH}^- \quad \text{Equation 1.14}
\]

\[
\text{Fe}^{3+} + \text{H}_2\text{O}_2 \rightarrow 2 \text{HO} + \text{Fe}^{2+} + \text{H}^+ \quad \text{Equation 1.15}
\]

\[
\text{Fe}^{2+} + 2 \text{HO} \rightarrow \text{Fe}^{3+} + \text{OH}^- \quad \text{Equation 1.16}
\]
H₂O₂ + hu →* HO ++ HO \hspace{1cm} \text{Equation 1.17}

Fe^{3+} + H₂O + hu → HO * + Fe^{2+} + H⁺ \hspace{1cm} \text{Equation 1.18}

(II) \textit{Heterogeneous Photocatalysis} – here the reactants and the catalysts are from different phases. It is most commonly used by the transition metal oxides and various semiconductors due to the presence of bandgap. Without efficient photoexcitation, the e-h pair can recombine leading to the heat generation, or they may be efficiently separated, such that the holes generally produce the OH radicals. An example is degradation of methyl orange using metal oxide, where the hydroxyl radicals are generated in both oxidative and reductive photocatalytic reactions [115]:

\begin{align*}
\text{UV} + \text{MO} &\rightarrow \text{MO} (h + e^{-}) \hspace{1cm} \text{Equation 1.19} \\
\text{h}^+ + \text{H}_2\text{O} &\rightarrow \text{H}^+ + \ast \text{HO} \hspace{1cm} \text{Equation 1.20} \\
2\text{h}^+ + 2\text{H}_2\text{O} &\rightarrow 2\text{H}^+ + \text{H}_2\text{O}_2 \hspace{1cm} \text{Equation 1.21} \\
\text{H}_2\text{O}_2 &\rightarrow 2 \ast \text{HO} \hspace{1cm} \text{Equation 1.22} \\
\text{e}^- + \text{O}_2 &\rightarrow \ast \text{O}_2^- \hspace{1cm} \text{Equation 1.23} \\
\ast \text{O}_2^- + \text{HO} \ast 2 + \text{H}^+ &\rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \hspace{1cm} \text{Equation 1.24} \\
\text{HO}_2\text{H} &\rightarrow \text{HO} \ast + \ast \text{OH} \hspace{1cm} \text{Equation 1.25}
\end{align*}

1.7.3.2b \textit{Results and conclusions}

Since \text{Cu}_2\text{O} nanoparticles are typically prone to surface oxidation, we carefully studied the photostability, crystal structure, defect states, and presence of oxygen vacancies in these nanoparticles. Further, the energy band structure and charge recombination lifetimes of nanoparticles were obtained, which was followed by assessing their ability to catalytically oxidize an eco-toxic dye under visible light illumination and simulated solar irradiation. Overall, our study not only provided a facile protocol to achieve a large-scale synthesis of photostable, oxygen-deficient \text{Cu}_2\text{O} nanoparticles but also emphasized the significance of
oxygen vacancies in semiconducting photocatalysts for enhancing their photocatalytic performance.

Under control conditions, in the absence of the catalyst, the methyl orange (MO) itself could not be degraded under visible light. However, the catalytic reaction in the presence of light followed a pseudo-first order kinetics. The Cu$_2$O nanoparticles led to near complete photodegradation of the MO molecules with an extremely high rate constant of 0.334 min$^{-1}$. In contrast, the commercial counterpart (bulk Cu$_2$O) could not promote MO degradation (rate constant of 0.519 x 10$^{-3}$ min$^{-1}$). We believe that this difference of three orders of magnitude in the photoactivity is most likely due to the poor dispersibility and low surface area of the commercial Cu$_2$O that might have further reduced during the experiments via aggregation. In comparison, our nanoparticles remained highly dispersible and offered appropriate band gap for visible photoexcitation. The stability studies of Cu$_2$O nanoparticles during multiple cycles of reaction revealed ~80% of photoactivity retention over 10 cycles. The observed gradual loss of activity (~20%) may be partially due to the loss of Cu$_2$O nanoparticles during post-reaction harvesting and re-use over multiple cycles. We further evaluated the performance of the catalyst under solar illumination conditions simulated for equator daylight. The MO molecules completely degraded within 1 hour, leading to decoloration of the resultant solution. These observations confirmed that the photostable Cu$_2$O nanoparticles offer remarkable potential as an efficient visible light photocatalyst for the practical harvesting of the solar energy.

For the validation of the recombination efficiency of charge carriers, we performed transient photoelectrochemical (PEC) measurements on Cu$_2$O nanoparticle-based devices prepared on a conducting FTO substrate. Initially, we obtained open circuit photocurrents by exciting Cu$_2$O films with different wavelengths of light. The chosen LED light sources included 470 nm (Ex > Eg), 525 nm (Ex ≈ Eg), and 623 nm (Ex < Eg) to represent various photoexcitation
scenarios. On exposure to the same light intensities (500 µW/cm²), the highest photocurrent densities were obtained when the surface was illuminated with 470 nm wavelength. As expected, the other two low energy sources could not efficiently photoexcite Cu₂O, thereby showing relatively lower responses. The current density response profiles over 10 on/off repeat cycles showed a repeatability of >98% at all wavelengths, thus reaffirming the high photostability of Cu₂O nanoparticles prepared in this study.

1.7.4 Chapter 3c discusses 1-Dimensional Gadolinium nanomaterial. Gadolinium-based materials are used in biological applications, especially as MRI contrast agents. However, their applications in the field of NanoZyme-based catalysts had not been well studied. Here we had developed a novel route for the synthesis of two Gd-based nanomaterials viz. Gd(OH)₃ and Gd₂O₃ nanorod using TEA as a hydrolyzing agent. The catalytic peroxidase-mimic NanoZyme activity was thoroughly studied for both the rods and they were employed for the colorimetric sensing of L-cysteine (an amino acid of high biomedical relevance). This was the first-time that Gd-based nanomaterials had been shown for peroxidase-like NanoZyme activity.

1.7.4.1 Gadolinium-based nanorods (1-Dimensional)

Triethylamine was used to obtain the Gd(OH)₃ and Gd₂O₃ based 1-D nanorods. In a typical Gd(OH)₃ nanoparticle synthesis, 40 mL of 1 M Gd(NO₃)₃ in deionized water (18.2 MΩ cm⁻¹ resistivity, MilliQ) was added to a round-bottom flask and placed in an oil bath at 80 °C under constant stirring. On reaching the desired temperature, 5 mL of triethylamine was added and the reaction was allowed to proceed at 80 °C under constant stirring for one hour. At the end of the reaction, the mixture was allowed to cool to room temperature and the white precipitate containing Gd(OH)₃ nanoparticles were washed in acetone: methanol mixture (1:1 v/v) and vacuum dried for further characterizations. The white precipitate containing
Gd(OH)₃ nanoparticles was further thermally annealed at 700 °C for 12 h to obtain a white powder containing Gd₂O₃ nanoparticles. The plausible reaction mechanism is as follows:

Reaction 1: Formation of the Gd(OH)₃ i.e. hydrolysis process

\[
(CH_3CH_2)_3N + H_2O \rightarrow (CH_3CH_2)_3NH^+ + OH^- \quad \text{Equation 1.26}
\]

\[
Gd^{3+} + 3OH^- \rightarrow Gd(OH)_3 \quad \text{Equation 1.27}
\]

Reaction 2: Formation of the GdOOH i.e. dehydration process 1

\[
Gd(OH)_3 \rightarrow GdOOH + H_2O \quad \text{Equation 1.28}
\]

Reaction 3: Formation of the Gd₂O₃ i.e. dehydration process 2

\[
2GdOOH \rightarrow Gd_2O_3 + H_2O \quad \text{Equation 1.29}
\]

1.7.4.2 Application aspect- Biosensing

1.7.4.2a General Concept

There is a strong need to sense and measure quantities of various species by human or machines by converting the signal using an appropriate sensor. When nanomaterials are exploited to make sensors, such sensors are known as nanosensors and in conjugation, with biological molecules, they are termed as nanobiosensors. In specific, a biosensor functions based on the type of target analyte device that can change the signal response in different formats, ranging from electrical, chemical or color signal.

Figure 1.20: A general schematic representing the working of a biosensor
Basically, such a biosensor involves 3 components as shown in Figure 1.20 and 1.21:

i) Recognition component that has high specificity for bio-based analytes produced.

ii) Detection and Transmittance components that detect specific biological entity and converts it into an appropriate signal.

iii) Amplification component that amplifies or enhanced the as produced signal into an appropriate output in the form of digital display.

Figure 1.21: The various components of the biosensors

Till now a number of biosensors have been developed depending upon the intended application. They can be classified into two types (i) Based on bioreceptor – enzyme-based, microbial-based and affinity-based biosensor. (ii) Based on transducer - potentiometric based, amperometric based, conductometric based, optical based, acoustic or piezoelectric based biosensors. In general, these biosensors have found application in food analysis, biomolecules interaction study, drug development, crime detection, medical diagnosis, environmental monitoring, quality control, industrial process control, detection for biological warfare agents etc.
1.7.4.2b Results and conclusions

The catalytic peroxidase-mimic NanoZyme activity of these Gd(OH)₃ and Gd₂O₃ nanorods was established for the first time, where the catalytic activity was suppressed specifically in the presence of L-cysteine that allowed us to develop a colorimetric sensor to detect this biologically relevant molecule among various other contaminants. This suppression, which could either be caused due to catalyst poisoning or enzyme inhibition, prompted an extensive investigation of the kinetics of this catalytic inhibition in the presence of cysteine. The NanoZyme activity was found to be dependent on the phase of the Gd particles such that Gd(OH)₃ nanorods showed superior activity in comparison to Gd₂O₃ nanorods. The catalytic activity of both nanorods was also dependent on the pH, temperature, nanomaterial concentration and substrate concentration. This revealed a competitive inhibition process, a mechanism akin to those observed in natural enzymes, bringing nanozymes a step closer to the biological systems.

1.7.5 Chapter 3d discusses 2 Dimensional SnO nanomaterials. Recently, 2D materials have gained unprecedented attention as potential candidates for next-generation optoelectronic devices due to their fascinating optical and electrical properties [116]. There are a variety of 2D materials ranging from mono-elemental ‘enes’; dichalcogenides; oxides and hydroxides [117]. Among various oxides, Tin monoxide (SnO) is a p-type semiconductor and found its application in various fields like water splitting, electronics [118] etc. As a part of this work, we reported for the first time, synthesis of 2D SnO in a “soft manner” by synthesizing the SnO microspheres of appropriate crystal orientation using TEA, followed by exfoliation of microspheres to form nanosheets. Our approach of obtaining 2D thin SnO layers is simple with low sonication power and straightforward. Further, we correlated the size-dependent opening of the bandgap from 0.8 eV (bulk) to 4.1 eV (monolayers), thus observing the quantum confined states in SnO nanosheets. It was also the first time that we
showed experimentally that SnO bandgap may open by over 1 eV through quantum confinement. Photoconductivity studies further validated our correlation that thin sheets are UV active materials in comparison to the bulk sheets. Such materials thus can be a base for well-designed next-generation optoelectronic devices.

1.7.5.1 SnO (2-Dimensional)

Through thinning of the bulk 2D layered material, one can obtain unique and interesting properties which might be useful for a number of applications especially in the field of electronics. This has seen emerging interest in producing new 2D nanomaterials. In obtaining such 2D nanomaterials, we may follow either a “top-down” or a “bottom-up” approach. This thesis has shown that triethylamine can play a significant role in producing 2D materials both via “top-down” and “bottom-up” approaches.

For instance, initially, using a “bottom-up approach” using triethylamine as a hydrolyzing agent we were able to obtain SnO microspheres of appropriate crystal orientation. Thereafter, thin 2D SnO sheets were exfoliated from the microspheres. As such, we initially developed a method for preparing layered SnO microspheres through the thermal decomposition of a tin oxide hydroxide intermediate at moderate temperatures. Briefly, a tin precursor (tin(II) 2-ethyl hexanoate) was first reacted with triethylamine at room temperature and this mixture was then injected into water, causing the instantaneous precipitation of tin oxide hydroxide (Sn₆O₄(OH)₄) as a white powder. Subsequent heating of this powder in the ambient environment caused its thermal decomposition to SnO in the form of a black powder. These SnO microspheres were then dispersed in common solvents (including ethanol and DMF), and through a simple ultrasonication treatment for a short period of time (1-5 minutes) on a standard bench top sonicator bath, a yellow colloidal suspension of exfoliated nanosheets was produced. This method is in stark contrast with most liquid-based techniques used to synthesize 2D materials, which involve lengthy and powerful sonication techniques which are
time and energy intensive. However, in the work presented in this thesis, the chemistry between water and TEA is at the cornerstone of obtaining 2D SnO.

### 1.7.5.2 Application aspect - Photoelectric Effect

#### 1.7.5.2a General Concept

When light falls on any material, the electrons will be ejected from its surface via the transfer of energy from the light to the atoms and the rate of electron ejection highly depends upon the materials characteristic bandgap. Such ejected electrons are known as photoelectrons. With size reduction to the nanoscale, one can open the bandgap which can be exploited under different energy requirements. The basic principle is shown in Figure 1.22.

![Figure 1.22: Schematic representation showing the excitation of one material w.r.t to bandgap opening](image)

#### 1.7.5.2b Results and conclusions

From this work, it was demonstrated that the properties of 2D SnO are strongly dependent on its dimensions. As verified through optical absorption and photoluminescence studies, a strong size-dependent quantum confinement effect in 2D SnO leads to substantial variation in its optical and electrical properties. This resulted in a remarkable (>1 eV) band gap widening in atomically thin SnO. Through photoconductivity measurements, we further validated a
strong correlation between the quantum-confined properties of 2D SnO and the selective photoresponse of atomically thin sheets in the high energy UV light. Such tunable semiconducting properties of 2D SnO could be exploited for a variety of applications including photocatalysis, photovoltaics, and optoelectronics in general.

1.7.6 Chapter 7 provides a summary of the research completed during this Ph.D. candidature (listed in table 1.5) and provides a scope for future work in the areas studied.

Table 1.5: Summary of the research work presented in the thesis

<table>
<thead>
<tr>
<th>Metal Oxide</th>
<th>Concept</th>
<th>Synthesis Procedure</th>
<th>physical/chemical properties</th>
<th>Applications</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron oxide</td>
<td>Utilization of TEA for synthesis and aqueous phase transfer</td>
<td>Hydrothermal approach</td>
<td>Quasi-spherical morphology of sub-10 nm diameter; amine- and oleic acid-surface functionalized particles can both form stable aqueous colloids</td>
<td>Hyperthermia</td>
<td>[113]</td>
</tr>
<tr>
<td>Copper(I) oxide</td>
<td>Utilization of TEA for the synthesis of oxygen-deficient nanomaterial</td>
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<td>Microspheres with an average diameter of 150 nm; highly photostable; role of oxygen vacancies in photocatalysis established</td>
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<tr>
<td>Gadolinium hydroxide and gadolinium oxide</td>
<td>Utilization of TEA for the synthesis of nanorods</td>
<td>Hydrothermal approach</td>
<td>Nanorods with an average width of 25 nm and an average length of 250 nm for Gd₂O₃ and an average width of 15 nm and an average length of 100 nm for Gd(OH)₃; a biomimetic nanozyme-mediated competitive inhibition process</td>
<td>Biosensing</td>
<td>[102]</td>
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</tbody>
</table>
Tin monoxide demonstrated Utilization of TEA for the synthesis of microspheres and their soft exfoliation Hydrothermal approach Porous layered microspheres synthesised that could easily exfoliate to SnO monolayers; significant widening of band gap of SnO from bulk to monolayers demonstrated Photodetector [116]

References:


88. Wolff, H.


Chapter 2

Characterisation techniques

This chapter summarises the operating principles of the different techniques used to characterise the nanomaterials synthesised in this thesis.
The thesis shows the use of TEA in the synthesis of different nanomaterials. These materials were characterised using UV Visible Near-infrared spectroscopy (UV-Vis-NIR); vibrational spectroscopy techniques including Fourier transform infrared spectroscopy (FT-IR); Raman spectroscopy and surface-enhanced Raman spectroscopy (SERS); X-ray diffraction (XRD); transmission and scanning electron microscopy (TEM and SEM); elemental analysis techniques such as energy dispersive X-ray analysis (EDX); X-ray photoelectron spectroscopy (XPS); hydrodynamic size and zeta potential; and Super Quantum Interference Device (SQUID).

2.1 UV visible absorption spectroscopy (UV-Vis)

UV-Vis spectroscopy is one of the key instrument used to characterize nanomaterials where important information such as optical bandgap, size, shape, and localised dielectric environment can be obtained. The basic components of the UV-Vis spectrophotometer include the laser beam, monochromator, signal detector and sample, which could either be a solid or liquid sample. This technique is often used as it is fast, simple and reliable way to provide vital information on the optical properties of the nanomaterial.

The unique optical property of each nanomaterial will result in a distinct UV-Visible spectrum. When a sample is exposed to a laser beam that matches the energy difference between the possible electronic transitions, some of the light energy will be absorbed by the molecule. This will result in the electrons to be promoted to a higher energy state orbital. The spectrometer essentially measures the degree of light absorption by the sample at different wavelengths. The resultant spectrum indicates the wavelengths of light absorbed by the sample, while the troughs in the spectrum shows what wavelength of light are transmitted. Given that each wavelength has a particular energy associated with it, we can describe the
correlation between the energy associated with a given fragment of the spectrum and the frequency of the incident radiation. Equation 2.1 describes this correlation:

\[ \Delta E = h\nu = \frac{hc}{\lambda} \quad \text{Equation 2.1} \]

where \( \Delta E \) is the energy change in the electronic transitions of a molecule, \( h \) being a Planck’s constant \( (6.626 \times 10^{-34} \text{ J} \cdot \text{s}) \), \( \nu \) is the frequency of the incident radiation \( (\text{s}^{-1}) \), \( c \) is the speed of light \( (\sim 3 \times 10^8 \text{ m} \cdot \text{s}^{-1}) \) and \( \lambda \) is the wavelength of the incident photon.

UV-Vis spectrum can also be used to determine the concentration of a molecule in solution according to the Beer-Lambert Law, the absorbance is directly proportional to the concentration of the molecule. Therefore, we can use the following equation 2.2:

\[ \log_{10} \frac{I_0}{I} = \epsilon cl = A \quad \text{Equation 2.2} \]

where \( \epsilon \) is the molar extinction coefficient, \( l \) is the optical path length i.e. the dimension of the cell or cuvette, \( c \) is the molar concentration, \( I_0 \) is the intensity of the incident light at a given wavelength, \( I \) is the transmitted intensity, \( \log_{10} \frac{I_0}{I} \) is the optical density which in simple terms is the absorbance \( A \).

When UV-vis spectrum is used for the characterisation of nanomaterials, the absorption of photon by the material is due to collective surface electrons oscillation and is more commonly known as Surface Plasmon Resonance (SPR). The SPR feature, especially observed in metal nanoparticles is distinct. However, the morphological characteristics of the nanomaterial can have significant influence on the SPR feature. The basic working layout is shown in Figure 2.1.

In this thesis, optical absorption spectra of the synthesised nanomaterials were acquired using an Agilent Cary 7000 spectrophotometer with a resolution of 1 nm within a range of 300--
The NanoZyme activity, photocatalysis and colorimetric biosensing were assessed using Envision multimode plate reader (Perkin Elmer) operating at a resolution of 5 nm over a wavelength range of 230-1000 nm.

![UV-vis spectrophotometer layout](image)

Figure 2.1: The basic layout of the UV-vis spectrophotometer.

### 2.2 Fourier transform infrared spectroscopy (FT-IR)

Vibrational spectroscopy is an important technique to identify molecules and surface species. This is achieved by looking at the atomic vibrations of molecules when exposed to electromagnetic radiations. Of the different vibrational spectroscopy techniques, Raman spectroscopy and Fourier transform infrared spectroscopy are the two commonly used characterisation tools employed in materials science.

Atoms are typically in a constant state of vibration creating dipole moment even at non-zero temperatures. Some of these dipole moments are infrared active (IR active) *i.e.* they can absorb energy from outside sources which are close to their own vibrational frequencies. The molecules absorb energy from only the radiation which it can coherently react *i.e.* the radiations of its own oscillation frequency. This would mean that each functional group with specific frequencies is very sensitive to its chemical environment and neighbouring species. The appearance or absence of vibrational frequencies in the IR spectrum gives important
information on the structure and orientations. The vibrational frequency can be calculated using the following equation 2.3:

\[ \nu = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}} \]  

Equation 2.3

where \( k \) is chemical bond force constant of the associated chemical bond and \( \mu \) is the reduced mass [1, 2].

In addition to the infrared spectrum, modern infrared spectrometers employ a mathematical operation of Fourier transform. This increases the signal to noise (S/N) ratio in a reduced time thereby allowing acquisition of a large number of scans. This is achieved by illuminating the sample with multiple frequencies rather than a monochromatic beam. Fourier transformation converts the time domain (intensity vs. time) signal to a frequency domain signal (intensity vs. frequency). The basic layout of an FTIR instrument is shown in Figure 2.2:

![Figure 2.2: The basic layout of the FTIR system](image)

FTIR measurements were performed using a Perkin Elmer Spectrum 100 instrument equipped with micro-ATR (diamond ATR crystal) with a resolution of 4 cm\(^{-1}\). Each spectrum represents an average of 50 scans. The background spectra consisting of the base diamond
crystal under the same experimental conditions were deducted from the respective signals obtained from the samples.

### 2.3 Raman Spectroscopy

Raman spectroscopy is a complimentary technique to FT-IR. However, Raman is more sensitive to the changes in the vibrational states. This changes their polarizability. In contrast, in FTIR there is a change in the dipole moment. There are two light scattering phenomena that can occur when a molecule is irradiated by the incident photon *viz.* elastic and inelastic scattering. If the molecule absorbs energy, the scattered photon will have a lower energy than the incident photon. This is referred to as Stokes shift (Figure 2.3). In contrast, if the molecule loses energy, the photon will have a higher energy than the incident photon. This is referred to as anti-Stokes shift (Figure 2.3).

![Figure 2.3: Mechanism of various scatterings](image)
When there is no exchange of energy between the incident photon and the molecules, their frequencies remain the same. This is known as Rayleigh scattering [3]. The basic instrument layout Fourier transformed based Raman spectroscopy is shown in Figure 2.4.

![Figure 2.4: Experimental setup of the Raman spectroscopy](image)

Raman spectra were acquired on a Horiba LabRAM HR Evolution Raman spectrometer using a 532 nm laser and power ranging between 0.5 and 50 mW (1–100% of the total laser power) using either pure Si or Si/SiO$_2$ (300 nm of thermally grown oxide) as substrates.

### 2.4 X-ray diffraction (XRD)

X-ray diffraction (XRD) is the most powerful instrument used to characterise nanomaterials. The technique provides important information on the crystal structure of the nanomaterial. XRD is based on the constructive interference of monochromatic X-rays and a crystalline sample. When a material is bombarded with the X-rays (generated by the cathode ray tube), it will produce interference patterns when Bragg’s law is satisfied. The interaction of the X-rays with the material is affected by the thickness of the material. For instance, the x-ray reflected...
from the surface travel a shorter distance than those reflected from deep inside planes of the material crystal lattice structure (as shown in Figure 2.5).

![Figure 2.5](image)

Figure 2.5: The pathway of interaction between the material crystal planes and the electron beam

W. L. Bragg was the first to recognize that the distance travelled by the electron is dependent on the spacing between atomic layers (d) and the incident angle of the electron beam (θ). Typically, when collecting the spectra, the angle of incidence and wavelength of X-rays are known. Therefore, the interplanar spacing can be calculated using the Bragg equation 2.4:

$$d = \frac{n\lambda}{2\sin\theta} \quad \text{Equation 2.4}$$

where n is the X-ray wavelength; θ is the scattering angle; λ is the integer representing the order of diffraction peak; d is the interplanar distance in the material. Thus, by calculating the d, one can deduce the information about the size, phase of the materials.

The XRD patterns presented in this thesis were obtained using Bruker AXS D4 Endeavour – wide-angle XRD with Cu Kα radiation at 40 kV.
2.5 Transmission Electron Microscopy (TEM)

While techniques such as UV-Vis spectroscopy, FTIR, Raman and XRD provide some information on the material, direct imaging of the particles in the nano dimensions is only possible using electron microscopy. The basic principle between a common light microscope and electron microscope is similar. In a common microscope, the resolution of the image is dependent on the wavelength of light. In electron microscope, a beam of electrons is used to illuminate the sample where the electrons with de Broglie wavelength inversely proportional to the voltage used accelerate them is employed. This helps the electron microscope of achieve high resolution images in the nano and sub-nano dimensions.

Transmission electron microscopy (TEM) uses electron beams emitted from a lanthanum hexaboride or tungsten or field emission gun using accelerating voltage in the range of 80 – 400 kV. The electron beam is directed to the sample through the use of magnetic condenser lenses. The interaction of the electrons with the sample can have several fates viz. (i) the electrons are undeflected i.e. electron pass through the specimen; (ii) elastic scattering or (iii) inelastic scattering of the electrons. When all three processes occur, the resulting image will have no contrast. The insertion of different apertures alters the interaction of the electrons with the sample leading to a clear image. The basic layout showing the different components of the TEM is shown in Figure 2.6.

Samples for TEM were prepared by drop-casting the solution containing the nanoparticles on a carbon-coated copper grid. TEM imaging was performed using a JEOL 1010 TEM instrument operated at an accelerating voltage of 100 kV. Samples for high resolution-TEM were prepared by drop-casting the samples on to a holey carbon support film followed by imaging using a JEOL 2100F TEM instrument operated at an accelerating voltage of 200 kV.
Figure 2.6: The pathway of interaction between the material crystal planes and the electron beam.

2.6 X-ray Photoelectron Spectroscopy (XPS)

XPS is a surface sensitive technique providing vital information on the material composition and its electronic states up to 10 nm in depth. The photoelectric effect forms the base for this working of this instrument *i.e.* single photon in/electron out process. In XPS, X-rays generated by a monochromatic source interacts with the sample in ultra-high vacuum. The X-rays interacts and is absorbed by the sample causing the atoms to ionize (Figure 2.7) and the concomitant emission of electrons from the top layer of the sample (known as photoelectrons). The kinetic energy of these electrons is measured, from which the binding energy (BE) can be calculated according to the equation 2.5 based on the 1914 Ernest Rutherford work principle:
\[ E_{\text{binding}} = E_{\text{photon}} - E_{\text{kinetic}} - \Phi \]  

Equation 2.5

where \( E_{\text{binding}} \) is the energy related to the electron ejected from the core level of an atom; \( E_{\text{kinetic}} \) is the kinetic energy of the electron ejected from the core level of an atom; \( \Phi \) is the XPS machine work function. As each element has a unique electronic configuration, the binding energy is a characteristic of the element.

Figure 2.7: Schematic demonstration the removal of core level electrons from a material on excitation with X-rays during X-ray photoemission spectroscopy

The binding energy not only depends on the level from which the photoemission occurs but it is also dependent on the oxidation state of the atom as well as the chemical and physical environment. A subtle change in any of these results in a shift in the peak position commonly referred to as chemical shifts. This makes this technique an important characterisation tool for determining the chemical states and environment of each element in a sample.

In this thesis, XPS spectra of various core levels were recorded using a Thermo Scientific K-Alpha X-ray Photoelectron Spectrophotometer instrument at a pressure lower than \( 1 \times 10^{-9} \) Torr (1 Torr = \( 1.333 \times 10^2 \) Pa). All scans were recorded with un-monochromatized Mg K\( \alpha \)
radiation (photon energy of 1253.6 eV) at pass energy of 20 eV and an electron take off angle of 90°. The overall resolution for all XPS measurements was 0.1 eV. The core level spectra were background corrected using the Shirley algorithm and chemically distinct species were resolved using a nonlinear least square fitting procedure.

2.7 Hydrodynamic Size and Zeta Potential

Both physical and dynamic size of nanoparticles has its importance for applications where the physical size is the actual size of the nanoparticle as observed in microscopy while the dynamic size is the size of the particle when suspended in a solution. It is well known that ions, surfactants and capping agents can adsorb on the surface of the nanoparticles. This can result in an increase in the hydrodynamic size of the nanoparticle as shown in Figure 2.8.

![Figure 2.8](image)

Figure 2.8: shows the hydrodynamic size of the particle

Dynamic light scattering (DLS) is a quasi-elastic scattering technique used to measure the hydrodynamic size. The technique exploits the scattering of light from the nanoparticle surface in solution having the base of Brownian motion to reveal their hydrodynamic size. To measure the diffusion rate of the particles, the Stokes-Einstein equation is followed where the
solvent viscosity should be known before measuring the particle size and follows the equation 2.6:

\[
R_h = \frac{K_B T}{6\pi \eta D_t} \quad \text{Equation 2.6}
\]

where \( K_B \) is Boltzmann constant, \( T \) is the temperature, \( \eta \) is the medium viscosity, \( D_t \) is the diffusion coefficient and is used to calculate the hydrodynamic radius of the particle suspended in the solution. The basic working the instrument is shown in Figure 2.9.

![Figure 2.9: Schematic representation of laser scattering](image)

A Malvern Zeta sizer Nano ZS analyser instrument was used to obtain the hydrodynamic size of the nanoparticles.

References:


Chapter 3

Introduction to series of papers

It is evident from the literature review (Chapter 1) that triethylamine is a promising hydrolysing agent for obtaining a variety of nanomaterials with improved properties for a wide range of applications. The nanomaterials as obtained using triethylamine are of high quality in terms of both physical and chemical states. The various nanomaterials synthesized using this amine for different applications will be presented as a series of papers in subsequent sections.

**Paper 1 (Section 3a)** introduces a new route for the synthesis of high quality superparamagnetic iron oxide nanoparticles (SPIONs) across aqueous and organic solvents using triethylamine (TEA) as a dynamic amphiphilic molecule. This paper also shows the ability of TEA molecules to act as a rapid phase-transfer agent to make organic ligand-coated particles highly water dispersible. This allowed these materials to be used for hyperthermia applications.

**Paper 2 (Section 3b)** proposes a new synthesis strategy to obtain highly oxygen-deficient and photostable copper oxide ($\text{Cu}_2\text{O}$) nanoparticles using TEA. Since $\text{Cu}(\text{I})$ ions are typically highly unstable in water and prone to oxidation, this is achieved through forming a $\text{Cu}(\text{I})$-TEA complex that on hydrolysis produces $\text{Cu}_2\text{O}$ particles. The application of these particles for enhanced visible light photocatalytic activity is demonstrated through establishing the importance of oxygen vacancies in photo-induced catalytic reactions.
**Paper 3 (Section 3c)** further shows how TEA can be used to synthesize gadolinium (Gd) based one-dimensional (1D) nanorods. As such, the TEA-mediated synthesis of Gd(OH)$_3$ and Gd$_2$O$_3$ was established. Here, for the first time, we established the inherent peroxidase like NanoZyme activity of Gd-based nanomaterials, and employed the competitive inhibition ability of cysteine towards developing a colorimetric sensor for this biologically important molecule.

**Paper 4 (Section 3d)** finally shows the role of TEA for the synthesis of high quality two-dimensional (2D) nanosheets of tin monoxide (SnO). This work laid the foundation of an important concept in the synthesis of 2D materials that the quality and the crystal orientation of the starting bulk material is extremely important, when efforts are made to exfoliate bulk materials into their 2D counterparts. The study also revealed an unprecedented widening of the bandgap of SnO from $\sim$2.7 eV (bulk) to 4.1 eV (monolayers) due to quantum confined effects.
Chapter 3a

One-pot synthesis of maghemite nanocrystals across aqueous and organic solvents for magnetic hyperthermia

We demonstrate that in a single step rapid process, triethylamine (TEA) can directly synthesise superparamagnetic iron oxide nanoparticles (SPIONs) that are readily dispersible in both aqueous and organic media. We further demonstrate the versatility of TEA in facilitating one-step phase transfer of SPIONs from organic to aqueous solvents. We then extend the applicability of this approach to demonstrate for the first time that organic molecules-capped SPIONs can also be directly dispersed in aqueous solvents. These SPIONs show remarkable potential for hyperthermia, as demonstrated through their outstanding magnetic field induced heating performance, which improves after their phase transfer to the aqueous phase. The current study establishes the importance of a suitable nanoparticle phase transfer protocol to ensure that the final product retains all the desirable application-focussed properties.


One-pot synthesis of maghemite nanocrystals across aqueous and organic solvents for magnetic hyperthermia

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A B S T R A C T

Superparamagnetic iron oxide nanocrystals (SPIONs) continue to receive intensive interest due to their significant prospects in biomedical applications. However, a critical requirement for such applications is the ability to synthesise high quality nanocrystals that are not only readily dispersible in aqueous solvents, but also retain good magnetic properties in an aqueous medium. This is not easy to achieve because, although organic solvent-based syntheses provide better control over SPIONs properties, their magnetic properties in particular, tend to deteriorate during aqueous phase transfer. In the current work, we propose a new route for the synthesis of high quality SPIONs using triethylamine (TEA) as a dynamic amphiphilic molecule. We demonstrate that in a single step, TEA can directly synthesise SPIONs that are readily dispersible in both aqueous and organic media. We further demonstrate the versatility of TEA in facilitating one-step phase transfer of SPIONs from organic to aqueous solvents. We then extend the applicability of this approach to demonstrate for the first time that organic molecules-capped SPIONs can also be directly dispersed in aqueous solvents. These SPIONs show remarkable potential for hyperthermia, as demonstrated through their outstanding magnetic field induced heating performance, which improves after their phase transfer to the aqueous phase. The current study establishes the importance of a suitable nanoparticle phase transfer protocol to ensure that the final product retains all the desirable application-focussed properties.

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1. Introduction

Magnetic materials, particularly those based on iron oxide, continue to receive intensive interest due to their dynamically tuneable magnetic properties, depending upon their size, shape, composition and surface ligands [1,2]. This makes them suitable for a diverse range of applications, including magnetic resonance imaging (MRI) [3–4], hyperthermia [5–8], targeted drug delivery [3,9,10], data storage [11], sensing [12,13], water remediation [14–16], energy production [17], as actuators for nanorobots [18], and for purification of high value products [19]. The desirable properties of magnetic materials typically include high field reversibility, high net saturation magnetisation, superparamagnetism, loop shifting, magnetic anisotropy, minimal dead layer contribution, high blocking temperatures, biocompatibility and colloidal stability [2,20].

However, a number of challenges need to be overcome to produce a magnetic material suitable for a specific application. A key challenge is, being magnets, the attractive forces among particles lead to large aggregates that inversely impact upon the magnetic properties [1,20–22]. To overcome this issue, a number of surface passivation strategies involving either surfactant molecules or a shell of inorganic material have been proposed. The resultant electrostatic and steric repulsions stabilise magnetic nanoparticles against aggregation, leading to stable dispersions [1,20,22,23]. However, the nature of the surface ligands plays one of the most important roles in defining the overall magnetic properties of these nanomaterials.

Iron oxide is a promising magnetic material for biomedical applications due to high biocompatibility of magnetite (Fe3O4) and maghemite (γ-Fe2O3) [24]. These materials can be readily prepared using a variety of aqueous and organic synthesis routes [20,24–29], however, each of these has associated benefits and limitations. For instance, aqueous phase synthesis, especially the commonly-employed co-precipitation technique, offers rapid synthesis of
nanoparticles but does not allow good control over their properties. On the other hand, non-aqueous phase synthesis, such as thermal decomposition methods offer good control over the nanoparticle size, morphology and other physico-chemical properties, but the resultant particles are heavily coated with a thick layer of oleic acid and high boiling surfactants. These coatings not only adversely affect magnetic properties; they also pose challenges in employing these high quality SPIONs for biomedical applications due to their poor aqueous dispersibility [22,24,27]. Considering the high quality of these oleic acid-capped SPIONs, efforts have been made to make them water dispersible through ligand exchange, ligand modification, silanization and surfactant/polymer coatings [20,23]. However, these additional surface coatings have consistently resulted in lower magnetisation over the parent particles due to the contributions from number of factors, like aggregation, increase in magnetic dead layer, magnetic phase change, etc. Overall, the improvement in the magnetic properties of SPIONs after their aqueous phase transfer has remained challenging, yet highly desirable from the perspective of biomedical applications [30].

As such, for clinical applications, access to better quality SPIONs that offer longer sedimentation times, high surface area, high net magnetisation, and smaller magnetic dipole interactions continues to remain in high demand. One potential application of such SPIONs is in remote controlled magnetic hyperthermia; wherein SPIONs can be used as localised heat generators to, for instance, destroy malignant tumours in the presence of an external alternating magnetic (AC) field [5–7]. Exposing SPIONs to a high frequency AC field can generate localised heat either due to rotation of the entire magnetic particle within the surrounding fluid (Brownian relaxation) and/or due to the rotation of the magnetic moment within the magnetic core (Néel relaxation). It is extremely challenging to determine the exact contribution of each of these relaxations to the net heat generated by the SPIONs and only a selected few studies have been able to distinguish relative contributions of these relaxations [5,7].

The current work provides an opportunity to fundamentally distinguish the relative contributions of Brownian and Néel relaxations in magnetic hyperthermia. This is achieved by developing a new synthesis route that can directly synthesise high quality SPIONs that are dispersible in aqueous as well as non-aqueous solvents. This one-pot synthesis strategy employs TEA as a dynamic amphiphilic ligand that can hydrolyse iron precursors irrespective of the choice of the solvent, while also simultaneously acting as a capping agent. Additionally, the amphiphilicity of TEA allows rapid and facile phase-transfer of oleic acid-capped SPIONs from organic to aqueous solvents in a single step. It is noted that the TEA-mediated phase transfer protocol is not only simple; it also allows production of a suitable SPION that have outstanding potential for hyperthermia. This suitability is particularly evident from the observed improvement in the magnetic properties of SPIONs after their phase-transfer to aqueous media. Access to a suite of materials with similar physico-chemical properties also allowed us to establish the importance of Brownian relaxation in inducing magnetic hyperthermia, while reflecting upon the importance of a suitable nanoparticle phase transfer protocol for a targeted application.

2. Results and discussion

Amine-coated aqueous-dispersible iron oxide nanoparticles were prepared in a single step using TEA as a unique amphiphilic hydrolysing as well as capping agent. When an aqueous solution containing ferric and ferrous ions in 2:1 molar ratio is exposed to TEA, the TEA promotes instantaneous hydrolysis of iron precursors to form iron oxide nanoparticles according to Eqs. (1) and (2):

\[
(CH_3CH_2)_3N + H_2O \rightarrow (CH_3CH_2)_3NH^+ + OH^- \tag{1}
\]

\[
Fe^{2+} + 2Fe^{3+} + 8OH^- \rightarrow Fe_3O_4 + 4H_2O \tag{2}
\]

The formed magnetite crystals are further oxidised in an oxygen-rich mother solvent under ambient conditions to maghemite [24], as per Eq. (3):

\[
Fe_3O_4 + 2H^+ \rightarrow \gamma Fe_2O_3 + \gamma Fe^{3+} + H_2O \tag{3}
\]

The cationic amine molecules (pK₅ ~ 10.75 for the conjugate acid in water) produced during the reaction are simultaneously adsorbed onto the surface of iron oxide nanoparticle via the surface hydroxyl groups, which makes them highly dispersible in the aqueous mother liqueur. These colloidal suspensions could be magnetically separated and vacuum dried in the form of readily aqueous dispersible powder and is denoted as “A-SPIONs”. A similar one-pot approach was followed to obtain organic solvent-dispersible iron oxide nanoparticles. However, in this case, the mother liqueur also contained oleic acid dissolved in acetone. This allowed the obtained nanoparticle powder to be covered with oleic acid, an organic solvent compatible surface ligand. This organic solvent dispersible nanoparticle powder is denoted as “O-SPIONs”.

We could not only directly synthesise SPIONs in aqueous and organic solvents, the dynamic co-solubility of TEA molecules across various solvents also allowed us to rapidly transfer organic-dispersible oleic acid-capped O-SPIONs to an aqueous phase. This was achieved by simple exposure of O-SPIONs powder to additional TEA, followed by dispersion using a simple bench-top sonicator (40W). Given that TEA is an amphiphilic molecule that can mix well both with aqueous and organic solvents, when water is introduced to this TEA-based colloidal dispersion, the particles become well-dispersed in water. This aqueous dispersion of nanoparticles phase-transfered from organic to aqueous phase is denoted as “AO-SPIONS”.

Overall, the approach presented here offers a remarkable tunability over the dispersibility of SPIONs in aqueous and organic solvents by a simple TEA-assisted approach. The outcomes are reflected from Fig. 1 that shows the potential of this approach through photographs of the as-synthesised powders of different SPIONs after their direct and selective dispersion in water (A-SPIONs) or in hexane (O-SPIONs). Further, the amphiphilicity of TEA molecules provides a next level of control, wherein the powders of organic-selective oleic acid-coated SPIONs (O-SPIONs) can also be readily dispersed in water by adding an excess of TEA (AO-SPIONs).

Fig. 2 shows the representative TEM images and corresponding SAED patterns of the as-synthesised water-dispersible A-SPIONS and hexane-dispersible O-SPIONS. The TEM images reflect particles of quasi-spherical morphology of sub-10 nm diameter. The
corresponding HRTEM images show that the obtained SPIONs are high quality single crystals of maghemite (γ-Fe₂O₃) as validated from the lattice spacing between the crystal planes [31]. The XRD patterns of A-SPIONs and O-SPIONs reveal high crystallinity of these particles with well-defined lattice planes and Miller indices corresponding to the γ-Fe₂O₃ phase (Fig. 3a). As the similarity between the XRD patterns of maghemite (γ-Fe₂O₃) and magnetite (Fe₃O₄) does not allow reliable differentiation between these two phases, we further characterised these SPIONs using FTIR spectroscopy (Fig. 3b). The FTIR spectra of both these SPIONs show broad peaks at ca. 630 and 448 cm⁻¹ that are characteristic of the stretching vibrations in the tetrahedral site of the Fe–O bond in γ-Fe₂O₃ [31]. This suggests that the TEA-mediated synthesis of iron oxide particles results into a maghemite phase.

The surface chemistry of these particles was also assessed using FTIR spectroscopy (Fig. 4a). The FTIR spectrum of A-SPIONs reveals a strong signal corresponding to the presence of hydroxyl groups on the nanoparticle surface, as evident from the –OH vibrations at ca. 3398 and 1632 cm⁻¹. Additional signatures corresponding to TEA molecules are also reflected from the –CH and –CN stretching vibrations at ca. 1360 and 1057 cm⁻¹, respectively. Thus, the FTIR spectrum of A-SPIONs supports that the surface of these nanoparticles is rich in hydroxyl groups, and is covered by amine molecules, making them highly dispersible in aqueous solvents. On the other hand, in case of O-SPIONs, the vibrational features corresponding to the hydroxyl groups are nearly absent, and the FTIR spectrum is dominated by the vibrational modes of oleic acid that coat these particles and make them dispersible in organic solvents. This is reflected from the –CH₂ stretching modes at ca. 2920 and 2851 cm⁻¹ along with asymmetric and symmetric –COO stretching modes at ca. 1519 and 1408 cm⁻¹, respectively. The mode of interaction of –COO group with the SPION surface can be further elucidated based on Δν,COO (111 cm⁻¹), which is supportive of the –COO group binding to the nanoparticle surface via a bidentate and/or bridging coordination [20,32]. Further, these oleic acid molecules form a bilayer structure onto the nanoparticle surface, as is evident from the observed free carboxylate signature at 1701 cm⁻¹. When we compare the FTIR spectrum of O-SPIONs with AO-SPIONs, the latter of which was obtained from the TEA-assisted phase transfer of O-SPIONs to water, the two FTIR
spectra remain largely unchanged. For instance, in AO-SPIONs, the oleic acid molecules remain chemisorbed on the SPION surface, as evident from the appearance of the –CO₂H stretching vibrations at 2923 and 2847 cm⁻¹ as well as the –COO vibrations at 1522 and 1405 cm⁻¹, respectively. The observed Δν =178 cm⁻¹ also supports that oleic acid remains bound to the SPIONs surface via bidentate and/or bridging coordination as in case of O-SPIONs. Further, in AO-SPIONs, the presence of C–O stretching of the free oleic acid suggests that oleic acid maintains its bilayer structure after phase-transfer, suggesting that the interactions of TEA molecules are not strong enough to remove the oleic acid from the nanoparticle surface. Another notable observation is that in the phase-transferred AO-SPIONs, the hydroxyl group population on the nanoparticle surface does not significantly increase. This indicates that the amphiphilicity of the TEA molecules might allow their interaction at the zone of bilayer structure in a manner such that the hydrocarbon chains of this tertiary amine interact with the long chains of oleic acid through hydrophobic interactions, whereas their terminal amine groups provide aqueous dispersibility to these particles.

To further understand the nature of ligand binding to the nanoparticle surface, we performed TGA-DTG analysis of SPIONs (Fig. 4b and c). The heat-induced weight loss observed from the nanoparticle surface can be categorised into three regions, wherein region I represents the weight loss due to the removal of adsorbed moisture or surface hydroxyls; region II represents the weight loss due to loosely-bound/physisorbed ligands; and region III is broadly indicative of strongly-bound chemisorbed species. The respective weight loss values are recorded in Table S1. One of the key observations is that A-SPIONs undergo significantly less weight loss (~2.6%) when heated over the whole temperature range in comparison to the other two SPIONs (22.7% for both O-SPIONs and AO-SPIONs). This is expected as the synthesis of A-SPIONs involved only TEA without any oleic acid, the latter of which is a significantly larger molecule and therefore results in overall higher heat-induced weight loss in case of O-SPIONs and AO-SPIONs. Secondly, the weight loss profiles of O-SPIONs and AO-SPIONs are largely similar, validating the FTIR results that during phase-transfer TEA molecules do not undergo ligand exchange with the oleic acid molecules. As such, in the case of A-SPIONs, the negligible weight loss observed in regions II and III is attributed to the thermal decomposition of surface-bound TEA molecules. Before we further discuss the TGA-DTG profile of O-SPIONs and AO-SPIONs, it is notable that pure oleic acid typically shows a one-step thermal decomposition at ca. 250 °C, and its binding to any surface will be expected to enhance this thermal degradation temperature. In contrast to the free oleic acid, in case of O-SPIONs and AO-SPIONs, a gradual two-step weight loss trend is observed in the regions of interest, wherein an initial weight loss in region II can be attributed to the decomposition of oleic acid molecules physisorbed on the SPIONs, followed by the loss in region III that corresponds to the loss of strongly chemisorbed oleic acid molecules that form a bilayer through hydrophobic interactions among the hydrocarbon chains of oleic acid.

To obtain further insights into the role of TEA as surface ligand in these SPIONs, we analysed these materials using XPS, a highly surface-sensitive technique with a field of depth of only a few nanometres (Fig. S1). The XPS analysis of all three SPIONs revealed Fe 2p3/2 and Fe 2p1/2 core binding energies (BEs) at ca. 711.1 and 724.5 eV, respectively (with satellite peaks at higher BEs). These features are typical of Fe³⁺ in maghemite, confirming the phase purity of SPIONs [33–35]. Further, the analysis of N 1s core levels revealed the presence of TEA only in A-SPIONs and AO-SPIONs with BE at 399.7 eV, whereas N 1s signatures were not observed in O-SPIONs [36,37]. This supports the involvement of TEA molecules in making O-SPIONs water-dispersible during their TEA-mediated phase-transfer to water to form AO-SPIONs.

To understand the quality of our phase-transfer protocol, we evaluated the hydrodynamic diameter of different SPIONs using DLS (Fig. 4d). The as-synthesised A-SPIONs and O-SPIONs revealed the average hydrodynamic radii of 8.5 and 7 nm, respectively. However, in comparison to O-SPIONs, the A-SPIONs showed a broader particle size distribution, suggesting that TEA-assisted synthesis of maghemite nanoparticles in organic solvents is more desirable. This observation is not surprising, as the synthesis of iron oxide nanoparticles in organic solvents is well-known to offer higher quality crystals in comparison to those obtained in an aqueous solvent. In the current case, the ability of TEA molecules to act as a dynamic phase-transfer agent after O-SPIONs are synthesised in an organic solvent, offers clear advantages to obtain a high quality product in an aqueous medium. This is evident from the comparison of the DLS spectra of O-SPIONs and AO-SPIONs, suggesting that the aqueous phase transferred SPIONs retain their low polydispersity, with an average hydrodynamic radius of 8.5 nm. Further, the ζ-potential measurements of SPIONs in aqueous phase revealed values of −32 and −46 mV for A-SPIONs.
and AO-SPIONs, respectively, supporting that the maghemite nanoparticles synthesised in the organic medium form a stable colloidal suspension after their aqueous phase transfer. In case of A-SPIONs, the negative surface charge arises from the surface hydroxyl groups, whereas the negative surface charge of AO-SPIONs is believed to be arising from the free –COO groups present on their surface in a hydrogen-bonded environment involving the carbonyl group, triethylamine and water molecules [38–40].

For biological applicability of SPIONs, it is important that they retain their colloidal stability under physiological conditions and do not aggregate in the presence of salts and biomolecules. Typically, salts and biomolecules neutralise the charge on the nanoparticle
surface, leading to nanoparticle aggregation and influencing their overall properties. The stability of the colloids in the presence of salts and biomolecules highly depends on the synthesis and phase transfer strategy, and as such, high colloidal stability is critical for biomedical applications. In the current study, since O-SPIONs were not water-dispersible and A-SPIONs showed a relatively broad size distribution, only AO-SPIONS with narrow particle size distribution were subjected to stability studies under biologically-relevant conditions. This involved studying the DLS profile of AO-SPIONS after exposing these particles independently to phosphate buffer saline (1 x PBS) that mimics the physiological osmolality, and to a mixture containing 1 x PBS with 10% foetal bovine serum (1X PBS + 10% (v/v) FBS) that represents the serum proteins present in the biological fluids. The hydrodynamic size obtained from DLS provides information about the effective hydrated size of the particles in solution. If particle aggregation occurs, this would result in significant broadening and associated increase in the hydrodynamic size. In contrast, if biomolecules in the surrounding solution interact with the particles and form a biomolecular corona, this will appear as a noticeable increase in the hydrodynamic size. As evident from Fig. 4e, the average hydrodynamic radius of AO-SPIONS dispersed in water (8.5 nm) does not significantly change in the presence of 1 x PBS (8.9 nm), but slightly increases in the presence of additional FBS (10.6 nm). It is also notable that the presence of FBS does not significantly affect the particle size distribution, and therefore this increase in hydrodynamic size can be attributed to the formation of a serum protein corona on the SPION surface [41–44]. These results clearly demonstrate the high stability of AO-SPIONS phase-transferred to water using TEA-mediated proposed strategy and support their potential usage for biomedical applications.

Next, we studied the magnetic properties of the SPIONs by measuring the temperature-dependent (M–T) and field-dependent (M–H) magnetisation curves (Fig. 5). In all three types of SPIONS, the M–T curves reveal that the ZFC (zero field cooled) magnetisation monotonically increases with an increase in temperature when compared to the FC (field cooled) magnetisation (Fig. 5a). In all these cases, the ZFC and FC curves overlap at or above the room temperature (blocking temperature – T{sub b}) supporting the room-temperature superparamagnetism in these SPIONs. The superparamagnetic behaviour is also revealed in the M–H curves that show higher saturation magnetisation (Ms) at lower temperature (4 K) compared to that at 300K (Fig. 5b and Table S2). The A-SPIONS, O-SPIONS and AO-SPIONS showed the room-temperature Ms values of 78.7, 50.2 and 59.3 emu/g, respectively. High Ms values correspond to high relaxivities, and are desirable for different biomedical applications, ranging from MRI to hyperthermia. As such, the values obtained in the current case are on the higher end of bulk saturation magnetisation typically observed for maghemite nanoparticles [45]. Higher Ms is expected from A-SPIONS compared to O-SPIONS and AO-SPIONS due to the lack of the bulky oleic acid molecules on the surface of A-SPIONS. Another notable observation is the ~20% increase in the Ms of AO-SPIONS once O-SPIONS are phase-transferred to water (Table S2). Considering that FTIR, TGA-DTG and XPS did not support a ligand exchange mechanism, this increase in saturation magnetisation might be attributed to a number of other factors such as surface spin canting or frustration of antiferromagnetic exchange interactions, whose effect becomes more pronounced when the particle size is in single domain regime [3,7,27,46].

Oleic acid is a commonly used surfactant for the synthesis of high quality iron oxide nanoparticles. However, most of the synthesis routes that employ oleic acid as a surface ligand require synthesis in an organic solvent. As is observed in the current study, and many other previous studies, the quality of SPIONS obtained in an organic phase synthesis is significantly better than those obtained from an aqueous phase synthesis route. However, transfer of these oleic acid-capped SPIONS to aqueous phase is critical before they can be employed for biomedical applications. Therefore, it is of no surprise that a number of phase transfer techniques have been developed to transfer SPIONS from an organic to aqueous medium. However, the literature is limited with studies where similar nanoparticles with same composition and particle size distribution in both aqueous and non-aqueous media have been evaluated for their hyperthermia performance. This is a rather important parameter, as the surrounding medium plays a great role while evaluating the field-dependent heating ability of SPIONS. The TEA-mediated synthesis and phase-transfer protocol reported here allows us to make this direct comparison by evaluating the properties of these SPIONS as heat receptors for radiofrequency (RF)-induced heating.

To investigate the heating abilities of these SPIONS for potential hyperthermia application, a fixed iron equivalent concentration was chosen to minimise the effects of magnetic field inhomogeneities, and measurements were performed using the set-up shown in Fig. 6a. For hyperthermia measurements, A-SPIONS and AO-SPIONS were directly suspended in water, while O-SPIONS were suspended in toluene as the solvent affords a high boiling point of 110.6 °C to perform hyperthermia measurements. To remove the influence of external thermal gradients, the two-turn copper coil was cooled with circulating cold water and the sample was placed in a plastic vial covered with thick polystyrene foam insulation, achieving near adiabatic conditions. After switching on the RF source (400 or 200 kHz frequency with a field amplitude of 10 kA/m{sup −1}), temperatures were recorded every 1 s for a total span of 300 s. To keep the current study relevant to biological hyperthermia, we chose these RF parameters, as 400 kHz and lower frequencies are considered biologically safe [47]. At 400 kHz, the field penetration into tissues at 15 cm depth is expected to be more than 99% [48], whereas the background non-specific heating of surrounding water and tissues due to eddy currents remains insignificant at this frequency [49]. This has been validated through in vivo experimental studies, showing that even a higher AC field of 500 kHz with an amplitude of 37.3 kA/m{sup −1} did not cause negative health impact on animals during magnetic hyperthermia [50]. These prior observations suggest that our RF parameters are relevant to the safe operational limits for biomedical hyperthermia.

The specific absorption rates (SAR) obtained from the linear regression analysis of time-dependent temperature increase profile of different SPIONS at 400 kHz reveal SAR values of 96.9 W g{sup −1} for A-SPIONS and 65.3 W g{sup −1} for O-SPIONS (Fig. 6b, calculations are detailed in experimental, and SAR under different conditions are summarised in the supplementary information Table S3). This ~50% higher SAR for A-SPIONS is consistent with the relative saturation magnetisation of these materials, in which case A-SPIONS showed ~50% higher Ms over O-SPIONS. Interestingly, when the O-SPIONS are phase transferred to water, the SAR of AO-SPIONS is enhanced by 45% to 139.9 W g{sup −1}. This remarkable SAR enhancement can be understood based on two major contributory factors responsible for magnetic field-induced heating. These include (i) Néel (τ{sub N}) relaxation that involves change in the orientation of the inherent magnetisation of a SPION, and (ii) Brownian relaxation (τ{sub B}) that involves the rotation of the SPION with respect to the surrounding dispersion medium under an applied magnetic field [5–8]. As the inorganic component (composition, crystal structure, size, polydispersity) of O-SPIONS and AO-SPIONS is exactly the same, the contribution of Néel (τ{sub N}) relaxation to the magnetic hyperthermia may be safely considered equivalent in both cases. Therefore, it is the change in the nature of Brownian relaxation that seems to play a significant role in enhancing the SAR of phase-transferred AO-SPIONS. Additional SAR measurements at lower frequency (200 kHz) reveal approximately 50% reduction in the SAR values (Table S3), which is expected considering the linear relationship between the SAR and the applied frequency.
addition, as AO-SPIONs showed narrow particle size distribution and high stability under different physiologically-relevant conditions (Fig. 4e), we also compared the SAR activity of AO-SPIONs dispersed in water with those after dispersion in either 1× PBS or in 1× PBS along with 10% FBS (Fig. 5c). The SAR activity of these particles only reduced marginally and they continued to show high SAR activity. This is attributed to the high stability of phase-transferred SPIONs in the present of salts and serum. Overall, these observations reflect upon the importance of a suitable phase transfer protocol employed for transferring high quality iron oxide nanocrystals to aqueous phase. As such, in the current case, TEA acts as a dynamic ligand that not only allows direct synthesis of iron oxide nanocrystals both in aqueous and organic solvents, but also facilitates phase transfer of nanoparticles synthesised in organic medium to an aqueous medium while ensuring that the end product is suitable for hyperthermia applications.

3. Conclusion

This study has for the first time, reported one-pot synthesis of superparamagnetic iron oxide nanocrystals of maghemite phase using TEA as a dynamic molecule that can directly produce SPIONs under mild conditions that are readily dispersible both under aqueous and organic environments. Interestingly, we notice that TEA molecules can not only facilitate phase-transfer of oleic acid-capped SPIONs from an organic to aqueous phase, but the powders of oleic acid-capped SPIONs can also be directly dispersed
in aqueous solvents in a single step using TEA. This new phase transfer protocol is not only rapid and highly efficient; importantly, the magnetic properties of oleic acid-capped SPIONs are improved after their aqueous phase transfer. This is otherwise rarely the case, as the current phase transfer protocols typically deteriorate the magnetic properties of SPIONs. The phase-transferred SPIONs obtained from this new route revealed outstanding magnetic properties, which could then be exploited for a proof-of-concept demonstration in magnetic hyperthermia. While the materials reported in this study are likely to find use in biomedical applications, it is important to highlight the versatility of TEA molecules as unique amphiphilic tertiary molecules for a variety of applications. Our recent work has shown the applicability of TEA for the synthesis of a range of difficult-to-synthesise nanomaterials, including Gd$_2$O$_3$ nanorods, 2D SnO nanosheets, highly oxygen-deficient Cu$_2$O nanospheres and photoactive CuO nanorods [13,51–53]. As such, the novel synthesis and phase-transfer modulation ability of highly functional $\gamma$-Fe$_2$O$_3$ SPIONs presented here adds a new chapter to the versatility of this unique tertiary amine.

4. Materials and methods

**Materials:** Iron(III) chloride hexahydrate (FeCl$_3$·6H$_2$O), iron(II) chloride tetrahydrate (FeCl$_2$·4H$_2$O), oleic acid (90% purity), toluene, triethylamine (99% purity), phosphate buffer saline (PBS), foetal bovine serum (FBS), acetone and methanol were purchased from Sigma–Aldrich and used as received. Deionised Milli-Q water was used in all the experiments.

**Nanoparticle synthesis:** A-SPIONs synthesis – A 40 mL of aqueous solution containing ferric (0.2 M Fe$^{3+}$) and ferrous (0.1 M Fe$^{2+}$) ions in 2:1 molar ratio was stirred for 5 min in an oil bath at 80 °C under ambient environment. This was followed by rapid injection of 5 mL of TEA into the solution, enabling the hydrolysis of iron precursors to form iron oxide nanoparticles. The reaction products were then allowed to cool under ambient conditions over one hour, accompanied with magnetic stirring. The obtained nanoparticles were washed 5 times with acetone: methanol (1:1) solution via magnetic separation and the residual solvents were evaporated overnight in an oven at 60 °C.
O-SPIONs synthesis – The synthesis protocol remained largely similar to A-SPIONs except that before injecting the TEA into the aqueous solution containing ferric (Fe³⁺) and ferrous (Fe²⁺) ions, a 5 mL of acetone solution containing 0.5 mL of oleic acid was added to iron precursors and stirred for 2 min.

**Nanoparticle phase transfer:** A-SPIONs synthesis – The O-SPIONs synthesised using the approach mentioned in the earlier section served as the starting material. 1 mL of TEA was added to 0.1 g of the O-SPIONs powder and sonicated for 1 min in a standard bench-top sonicator (Unisonics FXP4M with operating power of 40 W). Excess of TEA and oleic acid ligands were removed by dialysis with excess water over a 24 h period using a 12 kDa cut-off dialysis membrane with four changes of water. An appropriate amount of water can be added to prepare aqueous-dispersible SPIONs of a pre-defined nanoparticle concentration.

**Material characterisation:** The particle size and size distribution were evaluated from transmission electron microscopy (TEM) images, which were obtained using the JEOL 1010 TEM instrument operated at 100 kV. TEM was also employed to obtain the corresponding selected area electron diffraction (SAED) patterns of nanoparticles. High resolution TEM (HRTEM) images were obtained using JEOL 2100F microscope operated at 80 kV. Thermogravimetric analysis (TGA-DTG) was performed to quantify the extent of oleic acid coating using Pyris 1 TGA instrument (Perkin-Elmer, Inc.) with a ramping rate of 5 °C min⁻¹ in the temperature range of 30–600 °C under an inert (N₂) atmosphere. X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) studies were performed to determine the iron oxide phase and the surface chemistry of the nanoparticle surface. XRD patterns were recorded using Bruker AXS D4 Endeavour Wide Angle X-Ray Diffraction instrument with Cu Kα radiation (λ: 1.5406 Å) at room temperature. FTIR spectra of the nanoparticles were collected using the Perkin Elmer spectrum 100 spectrometer in the range of 4000–400 cm⁻¹ at 4 cm⁻¹ resolution. All presented spectra are the average of 256 scans. For X-ray photoelectron spectroscopy (XPS), samples were prepared by drop casting the sample on Si substrates coated with a 100 nm thermally-evaporated Au thin film, and measurements were carried out using Thermo Scientific K-Alpha XPS instrument using an Al Kα X-ray source (1486.7 eV) at a pressure better than 1 × 10⁻⁹ Torr (1 Torr = 1.333 × 10⁻⁶ Pa). The general scan and Fe 2p, and N 1s core level spectra for the samples were recorded at a pass energy of 20 eV and electron take off angle of 90°. The overall resolution was 0.1 eV for XPS measurements. The core level spectra were background corrected using Shirley algorithm and chemically distinct species were resolved using standard Gaussian–Lorentzian functions [54,55]. The core level binding energies (BE) were aligned with adventitious carbon binding energy of 285 eV. Hydrodynamic size of the nanoparticles was obtained from the dynamic light scattering (DLS) measurements using Malvern Zetasizer. A superconducting quantum interference device (SQUID) magnetometer MPMS-XL (Quantum Design, USA) was used for the characterising the magnetic properties. For the zero-field cooled (ZFC) measurements, the sample was cooled at 4K in the absence of an external magnetic field. A field of 100 Oe was then applied and the magnetisation was measured with increasing temperature. For the field-cooled (FC) measurement, the sample was cooled at 4K in the presence of a 100 Oe field and then the magnetisation was measured with increasing temperature. The field-dependent magnetisation was measured at 300, 100 and 4 K. The measured magnetisation was normalised with respect to the net mass of iron oxide, i.e. deducing the weight of the surfactant (determined by TGA-DTG).

**Hyperthermia measurements:** The heating properties of the nanoparticle were measured within the span of 300 s by using a 400 kHz generator with nominal power output of 2.5 kW and the rise in temperature was measured with respect to time. The ability of the nanoparticles to dissipate the heat under the alternating magnetic field via the Brownian and Neel relaxation mechanisms were measured in terms of specific absorption rate (SAR) using the formula [9]:

$$\text{SAR} = \frac{m_{\text{CL}} c_p \Delta T}{m_{\text{np}}} \left( \frac{\Delta T}{\Delta T_{\text{np}}} - \frac{c_p}{c_p} \frac{\Delta T}{\Delta T_{\text{np}}} \right)$$

where ΔT is change in temperature, ΔT is change in time, ΔT/ΔT is the heating rate of the colloidal dispersions of iron oxide nanoparticles, as evaluated from the linear section of the temperature rise curve; wnp is the mass fraction of iron oxide nanoparticles in the sample; and c is the mean value of its specific heat capacity in the investigated temperature range.

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**Appendix A. Supplementary data**

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.apmt.2018.06.003.

**References**

Supplementary Information
for

One-pot synthesis of maghemite nanocrystals across aqueous and organic solvents for magnetic hyperthermia

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**Figure S1.** Fe 2p and N 1s core level XPS spectra of different SPIONs.
**Table S1.** Percentage weight loss profile of different SPIONs as obtained from TGA-DTG.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight Loss (%)</th>
<th></th>
<th></th>
<th>Total (30-500 °C)</th>
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<tr>
<td></td>
<td>Region I (30-130 °C)</td>
<td>Region II (130-280 °C)</td>
<td>Region III (280-500 °C)</td>
<td></td>
</tr>
<tr>
<td>A-SPIONs</td>
<td>0.23</td>
<td>1.51</td>
<td>0.83</td>
<td>2.57</td>
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<tr>
<td>O-SPIONs</td>
<td>0.12</td>
<td>5.91</td>
<td>16.71</td>
<td>22.74</td>
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<tr>
<td>AO-SPIONs</td>
<td>1.81</td>
<td>5.17</td>
<td>15.77</td>
<td>22.75</td>
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</table>
Table S2. Saturation magnetization ($M_s$) values of different SPIONs as function of temperature.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Saturation Magnetisation (emu/g equivalent of iron oxide particles)</th>
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<tbody>
<tr>
<td></td>
<td>300 K</td>
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<tr>
<td>A-SPIONs</td>
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</tr>
<tr>
<td>O-SPIONs</td>
<td>50.2</td>
</tr>
<tr>
<td>AO-SPIONs</td>
<td>59.3</td>
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</table>
Table S3. SAR values (W.g\(^{-1}\)) of different SPIONs under different conditions.

<table>
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<tr>
<th>Sample</th>
<th>400 kHz</th>
<th>200 kHz</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water/ Toluene</td>
<td>1X PBS</td>
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<tr>
<td>A-SPIONs</td>
<td>96.9</td>
<td>-</td>
</tr>
<tr>
<td>O-SPIONs</td>
<td>65.3</td>
<td>-</td>
</tr>
<tr>
<td>AO-SPIONs</td>
<td>139.9</td>
<td>131.7</td>
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</tbody>
</table>
Chapter 3b

Oxygen-deficient photostable Cu$_2$O for enhanced visible light photocatalytic activity

This work highlights the importance of the oxygen vacancies in an inorganic semiconductor Cu$_2$O in the photocatalytic applications. This work is the first report on oxygen-deficient Cu$_2$O synthesised on a gram-scale through a novel approach involving hydrolysis of a Cu(I)-triethylamine [Cu(I)–TEA] complex at low temperatures. The oxygen vacancies in these Cu$_2$O nanoparticles led to a significant increase in the lifetimes of photogenerated charge carriers upon excitation with visible light. This, in combination with a suitable energy band structure, allowed Cu$_2$O nanoparticles to exhibit outstanding photoactivity in visible light through the generation of electron-mediated hydroxyl (OH•) radicals.

Oxygen-deficient photostable Cu₂O for enhanced visible light photocatalytic activity†

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Oxygen vacancies in inorganic semiconductors play an important role in reducing electron–hole recombination, which may have important implications in photocatalysis. Cuprous oxide (Cu₂O), a visible light active p-type semiconductor, is a promising photocatalyst. However, the synthesis of photostable Cu₂O enriched with oxygen defects remains a challenge. We report a simple method for the gram-scale synthesis of highly photostable Cu₂O nanoparticles by the hydrolysis of a Cu(Ⅰ)-triethylamine [Cu(Ⅰ)-TEA] complex at low temperature. The oxygen vacancies in these Cu₂O nanoparticles led to a significant increase in the lifetimes of photogenerated charge carriers upon excitation with visible light. This, in combination with a suitable energy band structure, allowed Cu₂O nanoparticles to exhibit outstanding photoactivity in visible light through the generation of electron-mediated hydroxyl (OH•) radicals. This study highlights the significance of oxygen defects in enhancing the photocatalytic performance of promising semiconductor photocatalysts.

Introduction

Semiconductor photocatalysis represents an efficient way to utilize solar energy, and as such, offers a promising avenue for next-generation green technologies, including the production of clean energy and efficient environmental management.1–4

Semiconducting titanium dioxide (TiO₂) remains the most extensively investigated photocatalyst due to its high chemical stability, low toxicity, low cost, and commercial availability.5

However, the large band gap (3.2 eV) and high recombination rates of charge carriers in TiO₂ mean that in practice, it only works well in the ultraviolet (UV) spectral range.6–8 Considering that the UV component corresponds to only ~3% of the solar irradiation reaching the earth’s surface,9,10 there has been an ongoing pursuit for new catalysts that are visible light active, possess a direct narrow band gap, and have high photochemical stability.

Cuprous oxide (Cu₂O), a p-type semiconductor, offers a narrow direct band gap (Eg of 2.1–2.6 eV) that is coupled with the appropriate positioning of its conduction band just above the reduction potential of water.11–14 These desirable semiconductor properties, in combination with high natural abundance, low toxicity, and low cost of copper, make Cu₂O a promising material for visible light-driven applications, such as photocatalysis, photovoltaics and photoelectrochemical devices.15–17 However, to realise the true potential of Cu₂O, limitations such as its stability during synthesis and storage, photostability during catalysis, and synthesis of oxygen-deficient Cu₂O need to be addressed.

Notably, the high chemical activity of Cu₂O, which is an advantage for photocatalysis, arises from the +1 oxidation state of Cu, which can either be reduced to Cu⁰ or oxidised to Cu²⁺ thereby facilitating redox reactions efficiently. However, this brings an associated drawback, i.e., Cu₂O is very prone to rapid oxidation into cupric oxides (CuO and CuO₂).18 This poses...
significant challenges not only to produce phase-pure $\text{Cu}_2\text{O}$ under mild conditions in large quantities, but also to retain the photoactive $\text{Cu}_2\text{O}$ phase without its oxidation to dramatically less-active cupric oxides during photocatalysis.

The other major challenge associated with photoactive $\text{Cu}_2\text{O}$ is that irrespective of the synthesis conditions, copper vacancies have been found to be the most stable defect states, whereas the synthesis of oxygen-deficient $\text{Cu}_2\text{O}$ remains challenging. However, the presence of oxygen vacancies in semiconductor photocatalysts has been demonstrated to offer remarkable potential in photocatalysis by suppressing charge carrier recombination processes.21–23

Herein, we demonstrate a new aqueous phase approach that allows the gram-scale synthesis of highly oxygen-deficient $\text{Cu}_2\text{O}$ nanoparticles under ambient conditions. The ease of synthesis offered by our approach provides potential opportunities to produce this important photocatalyst at an industrial scale. We overcome the synthesis challenge associated with stable phase-pure $\text{Cu}_2\text{O}$ nanoparticles via addressing the well-known problem of the instability of inorganic Cu(i) salts in water due to the higher lattice and solvation energies for the Cu$^{1+}$ ion over those of Cu$^{2+}$ ions.24 As such, this problem typically results in the rapid disproportionation of Cu$^{1+}$ ions into Cu$^{2+}$ and Cu$^{0}$ in an aqueous environment as per eqn (1):25

$$2\text{Cu}^{1+} \rightarrow \text{Cu}^{2+} + \text{Cu}^{0} \quad (1)$$

A few selected soft organic ligands, in particular, N-methylated ligands, have the ability to stabilize transition metals in low valence states through forming a hydrophobic complex with the metal ions.26 For instance, in 1963, Tollin and co-workers reported the synthesis of low melting Cu(i) complexes through a 1 : 1 melt reaction between amine-based soft ligands and copper chloride salts.27 These complexes had either a linear structure with coordination number 2 or a tetrahedral structure with coordination number 4, and it was noted that the complexes with coordination number 2 could be synthesised with strongly basic or easily polarisable ligands. Such complexes may be considered as ionic liquids as they exhibit low vapour pressures due to electrostatic interactions between the polarised ligand and the metal ion.27,28

In our investigations, we employed triethylamine (Et$_3$N, TEA) as a highly basic polarisable tertiary amine ($pK_a = 10.75$) to initially form a Cu(i)-TEA complex. The spontaneous hydrolysis reaction of this Cu(i)-TEA complex with water at 80 °C then produced spherical $\text{Cu}_2\text{O}$ nanoparticles on the gram scale (2.56 g per batch) with over 90% conversion yield from the precursor copper salts. This is important considering that the scale-up synthesis of nanomaterials is typically difficult, yielding only few micrograms to milligrams of materials per batch under standard unoptimized laboratory conditions. Furthermore, since $\text{Cu}_2\text{O}$ nanoparticles are typically prone to surface oxidation, we carefully studied the photostability, crystal structure, defect states, and presence of oxygen vacancies in these $\text{Cu}_2\text{O}$ nanoparticles. Additionally, the energy band structure and charge recombination lifetimes of $\text{Cu}_2\text{O}$ nanoparticles were obtained, which was followed by assessing their ability to photocatalytically oxidise eco-toxic environmental pollutants, such as a coloured methyl orange dye and the colourless tetracycline antibiotic under visible light illumination and simulated solar irradiation. Overall, the present study not only provides a facile protocol to achieve the large-scale synthesis of photostable, oxygen-deficient $\text{Cu}_2\text{O}$ nanoparticles but also emphasises the significance of oxygen vacancies in semiconducting photocatalysts for enhancing their photocatalytic performance.

Results and discussion

The schematic in Fig. 1 provides a summary of the gram-scale process used for the synthesis of oxygen-deficient $\text{Cu}_2\text{O}$ nanoparticles. The first critical step is to obtain a Cu(i)-TEA complex through the spontaneous reaction of copper(ii) chloride with TEA in acetonitrile at 80 °C (see Experimental section for details). While the formation of stable coordination complexes of Cu(i) species was first studied over 50 years ago,27–29 to our surprise, such complexes have not yet been explored to produce difficult-to-synthesise $\text{Cu}_2\text{O}$ nanoparticles. It has been noted that among various primary, secondary and tertiary amines, the latter form stable metal complexes with transition metal ions, due to the strong coordination bond between the metal and the tertiary amines.29 We chose TEA as a model tertiary amine to act as a coordinating agent for the formation of a Cu(i)-TEA complex. The reaction mechanism involved in the formation of the Cu(i)-TEA complex is believed to be initiated through a redox mechanism involving one electron transfer process giving rise to a dichlorocuprate(i) anion and a radical cation via a series of reaction intermediates (eqn (2) and (3)). These radical ions may co-exist in equilibrium with other copper(i) complexes of TEA.

$$\text{CuCl}_2 + \text{NEt}_3 \rightarrow [\text{CuCl}_2]^-[\text{NEt}_3]^+ \quad (2)$$

$$2[\text{CuCl}_2]^-[\text{NEt}_3]^+ \rightarrow 2[\text{CuCl}][\text{NEt}_3] + \frac{1}{2}\text{Cl}_2 \quad (3)$$

We propose that this Cu(i)-TEA complex offers a new promising approach to produce $\text{Cu}_2\text{O}$ nanoparticles in large quantities. This is because the high basicity of the TEA molecules ($pK_a = 10.75$) pre-complexed with Cu(i) should allow the

![Schematic diagram of the synthesis process](image_url)
spontaneous hydrolysis of this complex in water to form Cu$_2$O (eqn (4)).

\[ 2[\text{CuCl}][\text{NEt}_3] + \text{H}_2\text{O} \rightarrow \text{Cu}_2\text{O} + 2\text{NEt}_3 + 2\text{HCl} \]  

(4)

When the Cu(I)–TEA complex is mixed with water at 80 °C, a yellowish-orange colloid begins to form instantaneously. After 4 hours of reaction, 2.56 g of vacuum-dried Cu$_2$O powder is collected from a single batch that corresponds to a yield of over 90% of the precursor copper ions.

To understand the crystal growth mechanism of Cu$_2$O formation, time-dependent experiments were conducted at room temperature (instead of 80 °C to slow down the thermal-driven kinetics). We retrieved the colloidal product after 10 s, 30 s, 1 min, 2 min, and 4 min of the reaction and analysed their morphology by transmission electron microscopy (TEM) after rapidly drying-off the TEM samples under vacuum (Fig. 2). After 10 s, the majority of the structures were in the form of long interlaced nanotubes with an average diameter of ~5 nm and a length over 100 nm. Within 30 s, these nanotubes formed closely packed structures, some resembling quasi-spherical morphologies. As the reaction progressed, the wrapping of these interlaced nanotubes continued, such that within 4 min, only quasi-spherical particles averaging 50–200 nm in diameters, comprised of ensembles of nanotubes, were observed. High-resolution TEM (HR-TEM) imaging of the nanotubes revealed an interplanar spacing of 0.24 nm, consistent with the (111) lattice plane of cubic Cu$_2$O (Fig. S1, ESI†). Interestingly, even at this early time point of the reaction, we didn’t observe the formation of any intermediate copper hydroxide phase. This supports the notion that the hydrolysis of a Cu(I)–TEA complex by water leads to the spontaneous formation of interlaced tubular structures of Cu$_2$O, which then rapidly evolve into spherical morphologies.

The morphology, size, and crystallinity of quasi-spherical Cu$_2$O nanostructures obtained at 80 °C were investigated by electron microscopy. Fig. 3a shows a representative high resolution scanning electron microscopy (SEM) image of the Cu$_2$O nanostructures, suggesting that their overall quasi-spherical morphology formed through ensembles of multiple ultra-small particles. These ensembles showed an average diameter of 150 nm, as evident from the particle size distribution obtained from SEM (Fig. S2, ESI†). The dynamic light scattering (DLS) analysis revealed consistent results with a narrow size distribution and an average hydrodynamic diameter of 340 nm (Fig. S3, ESI†). The TEM image further confirms the quasi-spherical morphology containing an ensemble of several ultrasmall particles within a larger particle of 100–200 nm diameters (Fig. 3b). The corresponding selected area electron diffraction (SAED) pattern from these particles confirms their high crystallinity with well-defined (110), (111), (200), (220), (311) and (222) diffraction planes corresponding to the cubic phase of Cu$_2$O (Fig. 3c). The high-resolution EDX mapping further

![Fig. 2](image-url)  

Fig. 2  Schematic illustration of the morphological evaluation of Cu$_2$O nanoparticles and the corresponding TEM images over reaction times of (a) 10 s, (b) 30 s, (c) 1 min, (d) 2 min and (e) 4 min at room temperature.

![Fig. 3](image-url)  

Fig. 3  (a) SEM, (b) TEM, (c) SAED pattern, and (d–f) HR-TEM images of Cu$_2$O nanoparticles along with (g, h) the corresponding lattice fringe distribution in a defect-free highly crystalline area and a defect-rich area, respectively, as marked with yellow lines in panels (e) and (f). The inset in (e) shows a fast Fourier transform (FFT) of the red highlighted region.
affirms the presence of Cu and O in these particles (Fig. S4, ESI†).

High-resolution TEM (HR-TEM) images reveal clear lattice fringes with interplanar spacings of 0.24, 0.21 and 0.29 nm that are consistent with the (111), (200), and (110) planes of cubic phase Cu2O (Fig. 3d).30 We also noticed the presence of irregular regions in the form of discontinued lattice fringes on the surface of Cu2O crystals (Fig. 3e–h). These features suggest that a highly defect-rich Cu2O structure might have formed during crystal transformation from nanorods to nanospheres. Interestingly, the fast Fourier transform (FFT) of the region containing both ordered and discontinuous lattices (inset of Fig. 3e) showed that irrespective of the presence of defects, the overall crystallinity of the region remained as cubic Cu2O. As such, these defects within the Cu2O crystal are likely to induce localized copper and oxygen vacancies, which may have important implications for their optical and resultant photocatalytic properties, as investigated later.

While HR-TEM studies provide strong evidence that the prepared material is cubic Cu2O, in general, it is extremely challenging to stabilise pure copper(i) oxide under ambient conditions. Therefore, we employed a number of complementary techniques to investigate the oxidation state of copper in these nanoparticles. Electron paramagnetic resonance (EPR) spectroscopy offers an ideal avenue to assess the oxidation state of copper. We performed continuous wave (CW) X-band EPR measurements on Cu2O nanoparticles, and compared the EPR spectrum with that of the liquid Cu(i)–TEA complex (the precursor for Cu2O synthesis) and a Cu(ii) sulphate EPR standard (Fig. S5, ESI†). It is noted that in X-band EPR, only copper centers in the +2 oxidation state should be visible. The dominant oxidation state of copper centers in the Cu(i)–TEA complex as well as in Cu2O nanoparticles is expected to be +1. Both samples are therefore expected to be EPR silent or only exhibit weak Cu(ii) signals. In contrast, the CuSO4 standard should generate a strong Cu(ii) signal.

As expected, CuSO4 generated a typical EPR signal, representative of Cu(ii). Interestingly, the Cu(i)–TEA complex, which we expected to be EPR silent, also generated a Cu(i) signature. A comparison of the double integral EPR signal measured for the Cu(i)–TEA precursor and the CuSO4 standard demonstrates that the proportion of the precursor in the +2 oxidation state is rather low, of the order of 2%. We believe that this feeble Cu(i) signature in the Cu(i)–TEA complex is most likely due to the complex equilibrium kinetics that takes place during Cu(i)–TEA synthesis among multiple intermediates, as represented in a simplified form through eqn (2) and (3).

The EPR signal of Cu2O nanoparticles suggests that it is unlikely that the spectrum represents a single species, but instead a distribution. The overall EPR profile of Cu2O, with a positive peak ca. g = 2.4, and a more narrow feature at ca. g = 2, is characteristic of Cu(ii) in a tetraagonally elongated octahedral/square planar ligand field. A further resolved peak below g = 2 indicates a sub-population with a different coordination (trigonal/tetrahedral). This is also true for the Cu(i)–TEA precursor spectrum, although its hyperfine structure is unresolved. A comparison of the EPR signals of Cu2O nanoparticles with the CuSO4 standard after factoring in changes in the instrument settings, temperature and normalisation based on the total copper in each sample suggests that the EPR signal for Cu2O nanoparticles is less than 1% of that of the standard. These results thus support the notion that the Cu(ii) concentration in Cu2O nanoparticles is indeed very small. Since Cu(i)–TEA also showed Cu(ii) signals, and other complementary techniques did not show any features of Cu(ii) oxides, we believe that the weak Cu(ii) signals from Cu2O nanoparticles are most likely due to the unhydrolysed Cu(i)–TEA complex that may act as a capping agent to stabilise Cu2O nanoparticles during synthesis.

Furthermore, since Cu2O is generally prone to surface oxidation during storage, to assess their ambient stability over the long term, we analysed a Cu2O nanoparticle 8 weeks post-synthesis using high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM), and fine-structure electron energy loss spectroscopy (EELS) as a line profile across the particle length (Fig. 4). The O K edge from the particle shows a prominent peak at 532.5 eV, confirming the presence of the cubic Cu2O phase.31 Importantly, the peak positions of the fine structures of Cu L3 and L2 energy edges are representative of the oxidation state of this transition element.32 The observed Cu L3 edge at 933.4 eV remains consistent across various locations within a single particle (zones 1–6). This is representative of the Cu11+
oxidation state, further confirming that our nanoparticles remain stable in the pure crystalline phase of Cu2O under ambient conditions.

The crystallinity and phase purity of Cu2O nanoparticles are further evident from their powder X-ray diffraction (XRD) analysis and comparison with a commercial Cu2O nanopowder (Fig. 5a). Both materials show characteristic diffraction lines at 30.2, 37.1, 43.0, 62.1, 74.5, and 78.5° corresponding, respectively to the (110), (111), (200), (220), (311) and (222) lattice planes of cubic Cu2O (ICDD No. 05-0667). The absence of other diffraction peaks supports the lack of any potential impurity in the material. In comparison with the commercial Cu2O, the as-synthesised nanoparticles showed broader diffraction peaks, supporting that the particle domains are in the nanoscale range. The crystallite size of as-synthesised Cu2O nanoparticles (12.8 ± 4.1 nm) calculated based on the Scherrer equation was found to be over six times smaller than that of commercial Cu2O (68.2 ± 17.9 nm). The average crystallite size of 12.8 nm for as-synthesised Cu2O nanoparticles further supports that the larger spheres of ca. 150 nm diameter observed during electron microscopy are composed of ultra-small Cu2O nanoparticles. Furthermore, N2 porosimetry studies to compare the surface area of Cu2O nanoparticles with the commercial powder (Fig. S6b, ESI†) revealed that the Cu2O nanoparticles synthesised using a Cu(I)-TEA complex showed a type III N2 sorption isotherm with a high volume of N2 absorbed. In contrast, the commercial Cu2O showed a negligible amount of N2 absorption. As such, our material showed over an order of magnitude higher BET surface area (42 m² g⁻¹) compared to its commercial counterpart (4 m² g⁻¹). This higher surface area of Cu2O nanoparticles prepared in our study is likely to be advantageous through providing more number of active surface sites for photocatalytic processes.

To investigate the defect states in Cu2O nanoparticles, we simultaneously studied the confocal Raman and photoluminescence (PL) properties of the particles and performed X-ray photoelectron spectroscopy (XPS), while comparing with the commercial counterpart (Fig. 5). The Raman spectrum of commercial Cu2O nanoparticles shows a characteristic major peak at ca. 219 cm⁻¹, along with a few low-intensity unresolved features (Fig. 5a). The strong vibrational band at 219 cm⁻¹ is characteristic of Cu2O and corresponds to the second order overtone 2Γ₁₂ mode. In the case of as-synthesised Cu2O nanoparticles, the 2Γ₁₂ mode is slightly shifted to 217 cm⁻¹.

The additional Raman peaks in Cu2O nanoparticles at 92, 148, 416, 520, and 632 cm⁻¹ can be ascribed to a combination of defects, non-stoichiometry, and resonant excitations. In particular, the 148 cm⁻¹ feature is attributed to Γ₁₅ oxygen vacancies (LO), and its high intensity in as-synthesised Cu2O nanoparticles confirms the presence of a large number of oxygen defects in the Cu2O lattice. As such, the Raman spectroscopy confirms a highly defect-rich material, in which the high intrinsic mobility of oxygen and copper species within an asymmetric Cu–O stretching and an asymmetric O–Cu–O bending environment offers great promise for photocatalysis applications.

The presence of oxygen defects in our material was further confirmed by PL spectroscopy, as the relative position of the emission peak is indicative of the defect states in Cu2O (Fig. 5b). The as-synthesised Cu2O nanoparticles exhibited a predominant peak at 706 nm that is attributable to the recombination of excitons bounded to oxygen vacancies (ν₀) in the cubic Cu2O lattice. This supports the presence of intrinsic oxygen defects in our material. In contrast, the commercial Cu2O nanoparticles did not show PL signature at ca. 700 nm, but instead showed a strong feature at 928 nm that corresponds to the emission from excitons bounded by copper vacancies (ν₇₅) in the lattice.

In addition to Raman and PL spectroscopy, the O 1s core level XPS spectra of the commercial (Fig. 5c) and as-synthesised (Fig. 5d) Cu2O nanoparticles further confirm that our nanoparticles are rich in oxygen vacancies. As such, the O 1s spectrum in each case could be resolved into three distinct components with binding energies maxima at ca. 529.0, 530.1, and 531.8 eV that are attributed to the oxygen present in the Cu2O lattice, oxygen associated with an oxygen vacancy (ν₀) environment, and the ambient oxygen adsorbed on the surface of particles arising from water or carbonate species, respectively. Out of the total oxygen available within the Cu2O lattice, the relative amount of oxygen vacancy concentration in as-synthesised...
Cu$_2$O nanoparticles corresponds to ca. 73%, while only 23% oxygen vacancies were found in commercial Cu$_2$O. While commercial Cu$_2$O is predominantly rich in copper vacancies, as evident from PL spectroscopy (Fig. 5b), the co-existence of a certain degree of oxygen vacancies in the commercial material is not surprising.

Altogether, these spectroscopic investigations provide strong evidence that while commercial Cu$_2$O is highly copper-deficient, the nanostructured Cu$_2$O reported in this study is predominantly oxygen-deficient. The presence of oxygen vacancies in a semiconductor has been observed to offer remarkable potential in suppressing the electron and hole recombination processes, thereby enhancing the photo-absorption capacity of the material.\(^{41}\) As such, the enhanced formation of oxygen vacancies in nanostructured Cu$_2$O reported here could provide similar benefits in promoting photocatalytic performance.

The optical band gap of these oxygen-deficient Cu$_2$O nanoparticles was obtained from the optical absorption measurements (Fig. 6a) using Tauc’s plot (Fig. 6b)\(^{42}\) with the following eqn (5).

\[ A = \frac{k(h\nu - E_g)^{n/2}}{h\nu} \quad (5) \]

where \(A\) is the absorbance, \(k\) is a constant, \(h\) is the Planck’s constant, \(\nu\) is the frequency, and \(n\) equals 1 for direct transitions. The obtained direct band gap (\(E_g\)) value of 2.45 eV for Cu$_2$O nanoparticles is in good agreement with the literature (2.1–2.6 eV).\(^{33}\) In comparison, the commercial Cu$_2$O showed a band gap of 2.1 eV. It is likely that the larger band gap of our material is due to the presence of defects and resultant potential deviation from a perfect stoichiometry. As such, this wider energy gap is more favourable to reduce the recombination rates of the charge carriers during photocatalysis. Furthermore, this band gap edge of 2.45 eV (corresponding to 506 nm wavelength) is suitable for visible light-induced photostimulation.

Next, we evaluated the photocatalytic performance of oxygen-deficient Cu$_2$O nanoparticles through studying a model reaction involving the photo-oxidative degradation of methyl orange (MO) under visible light illumination. The pristine MO molecules show a strong absorption peak at 463 nm, and their photodegradation can be monitored by evaluating the reduction in the absorbance intensity using UV-Vis absorption spectroscopy.\(^{43-46}\) \(C_t/C_0\) was used to compare the photocatalytic performance of the spherical Cu$_2$O nanoparticles and the bulk counterpart (\(C_0\) is the initial concentration of MO, and \(C_t\) is the concentration at time \(t\)). Fig. 7a shows the photocatalytic degradation of MO over 15 min in the presence of as-synthesised oxygen-deficient Cu$_2$O nanoparticles under visible light illumination. The insert shows that within 15 min, the intense orange colour of MO completely disappears due to the high visible light photoactivity of Cu$_2$O nanoparticles. The kinetics of MO degradation by Cu$_2$O nanoparticles under visible light irradiation is further compared with the commercial Cu$_2$O nanoparticles in Fig. 7b and c. Control experiments in the absence of the catalyst show that MO itself cannot be
degraded under visible light. The reaction in the presence of light followed pseudo-first order kinetics. The as-synthesised oxygen-deficient Cu2O nanoparticles led to the near complete photodegradation of the MO molecules with an extremely high rate constant of 0.334 min⁻¹. In contrast, the commercial counterpart could not promote MO degradation (rate constant of 0.519 × 10⁻³ min⁻¹). We believe that this difference of three orders of magnitude in the photodegradation is partly due to the poor dispersibility and low surface area of the commercial Cu2O nanoparticles that might have further reduced during the experiments via aggregation. In comparison, our nanoparticles remained highly dispersible and offered appropriate band gap for visible photoexcitation. The stability studies of as-synthesised Cu2O nanoparticles during multiple cycles of reaction revealed retention of ∼80% of photoactivity over 10 cycles (Fig. 7d). The observed gradual loss of activity (∼20%) may be partially due to the loss of Cu2O nanoparticles during post-reaction harvesting and re-use over multiple cycles. In contrast, the commercial Cu2O nanoparticles showed very poor reusability with less than 15% of the original catalytic performance retained in the second cycle. This loss of activity in commercial Cu2O is due to their poor photostability and rapid oxidation to CuO and other higher oxidation state copper oxides within one cycle of photocatalysis, as evident from XRD analysis (Fig. S7, ESI†). On the other hand, the as-synthesised oxygen-deficient Cu2O nanoparticles retain their cubic phase purity even after repeated exposure to ten cycles of photocatalysis reactions (Fig. S8, ESI†). These results, in combination with long-term EELS stability studies (Fig. 4), provide convincing evidence that oxygen-deficient Cu2O nanoparticles prepared using the new Cu(I)–TEA hydrolysis route reported here have outstanding photostability under ambient as well as visible light illumination conditions.

We further assessed the prospects of oxygen-deficient Cu2O nanoparticles in degrading other organic environmental pollutants by investigating the photooxidative degradation of tetracycline in visible light (Fig. S9, ESI†). In the absence of a catalyst, tetracycline could not be degraded, indicating the importance of a suitable catalyst for its photodegradation. The commercial Cu2O could degrade only 7% tetracycline, whereas the oxygen-deficient Cu2O showed ∼90% degradation efficiency within 20 min of visible light irradiation.

Convinced with the high photostability and photocatalytic activity of oxygen-deficient Cu2O nanoparticles, we further evaluated its performance under simulated solar illumination conditions (Oriel AAA, AM1.5) (Fig. S10, ESI†). The MO molecules completely degraded within 1 hour, leading to the decolouration of the resultant solution. These observations confirm that the photostable Cu2O nanoparticles offer remarkable potential as an efficient visible light photocatalyst for the practical harvesting of solar energy.

To obtain mechanistic insights into oxygen-deficient Cu2O-driven photocatalysis, we determined the position of the conduction band (CB), valence band (VB), Fermi level (E_F), associated band gap energy (E_g), and the lifetime stability of the photogenerated charge carriers, all of which synergistically play important roles in determining the overall photocatalytic performance of semiconductors. The excitation of an electron from VB to CB of a semiconductor photocatalyst can only occur if the optical radiation energy is equal to or greater than the E_g. Once the electron (e−) is photoexcited to the CB, it leaves a hole (h+) at the VB, and the separation efficiency of these charge carriers dictates the overall photocatalytic performance. The oxygen-deficient Cu2O nanoparticles reported in this study have a direct optical band gap of 2.45 eV (Fig. 6b), which makes them photoactive under visible light. Thus, the VB and CB potentials of the Cu2O nanoparticles are measured through eqn (6) and (7).47

\[ E_{VB} = X + 0.5E_g - E_F \]  
\[ E_{CB} = E_{VB} - E_g \]

where E_{VB} is the valence band edge potential, X is the electronegativity of the semiconductor, E_F is the energy of free electrons versus hydrogen (4.5 eV), E_g is the band-gap energy of the semiconductor, and E_{CB} is the conduction band edge potential. The calculated CB and VB potential values of Cu2O nanoparticles w.r.t. to a nascent hydrogen electrode (NHE) correspond to −0.42 (E_{CB}) and 2.03 eV (E_{VB}), respectively.

Furthermore, the Fermi level position for oxygen-deficient Cu2O nanoparticles was determined to be 0.29 eV above the E_{VB} from the valence band XPS by using the intercept of the tangent to the density of states at the Fermi edge (Fig. S11, ESI†). The relevant energy band positions of p-type semiconductor Cu2O nanoparticles are presented in Fig. 8 and suggest that upon photoexcitation with visible light, electrons (e−) and holes (h+) will be photogenerated (eqn (8)). These electrons will have sufficient potential to reduce the molecular O2 to produce the superoxide anion (O2−), as the CB edge potential of Cu2O nanoparticles is located at −0.42 eV (vs. NHE), while the required O2 reduction potential is −0.28 eV.12 These O2− radicals can then undergo a series of reductive reactions to produce H2O2 (eqn (9)) and OH− radicals (eqn (10) and (11)). These OH− radicals can then participate in
the photooxidation of MO via eqn (12), due to a favourable MO redox potential (0.94 eV).\(^48\)

\[
\text{Cu}_2\text{O} + h\theta \rightarrow \text{Cu}_2\text{O}(e^-) + \text{Cu}_2\text{O}(h^+) \quad (8)
\]

\[
\text{O}_2 + 2\text{H}_2\text{O} + 2e^- \rightarrow \text{H}_2\text{O}_2 + 2\text{OH}^- \quad (9)
\]

\[
\text{OH}^- + h^+ \rightarrow \text{OH}^+ \quad (10)
\]

\[
\text{H}_2\text{O}_2 + e^- \rightarrow \text{OH}^- + \text{OH}^- \quad (11)
\]

\[
\text{MO} + \text{OH}^- \rightarrow \text{MO}_{\text{ox}} \quad (12)
\]

To validate the photo-induced production of \text{O}_2^- and \text{OH}^- radicals, EPR spectroscopy involving spin trap experiments was performed (Fig. S12, ESI†). The spin trap used was 5,5-dimethyl-pyrroline N-oxide (DMPO), as DMPO allows the detection of both superoxide and hydroxyl radicals. In such experiments, these radical species are detected by their reaction with the initially EPR-silent DMPO. It is noted that the DMPO-OH adducts give rise to two different EPR signals. To test the quality of our DMPO stock solutions and the performance of our illumination setup, control measurements of the photodecomposition of \text{H}_2\text{O}_2 into \text{OH}^- radicals were performed. In the control DMPO solution, a negligible stable population of the DMPO-OH adduct was observed. Upon the addition of oxygen-deficient Cu\textsubscript{2}O nanoparticles in the dark, a small concentration of the DMPO-OH adduct was observed. Upon the illumination of the Cu\textsubscript{2}O/DMPO reaction mixture for 15 min with white light led to a roughly 2-fold increase in the DMPO-OH adduct signal. The photo-induced DMPO-OH adduct EPR signal was observed to decay in the dark over the course of an hour. The signal could however be regenerated upon further white light illumination. The DMPO-OH adduct was not observed in any sample, although may have decayed owing to its short lifetime. The EPR spin trap experiments strongly support our above hypothesis that the \text{OH}^- radicals produced upon the photoexcitation of oxygen-deficient Cu\textsubscript{2}O nanoparticles is predominantly responsible for the observed photocatalytic activity of this material. The photo-induced production of \text{OH}^- radicals is further evident from an experiment involving terephthalic acid (TA) as a \text{OH}^- radical-specific fluorogenic probe that is readily converted into a highly fluorescent 2-hydroxy terephthalic acid in the presence of photoexcited Cu\textsubscript{2}O nanoparticles (Fig. S13, ESI†).\(^49\)

To validate the role of these oxidative species in the degradation of MO molecules, we studied the photocatalysis performance of oxygen-deficient Cu\textsubscript{2}O nanoparticles in the presence of ethylenediaminetetraacetic acid (EDTA) and tertiary butyl alcohol (TBA), which act as scavengers for electrons and \text{OH}^- radicals, respectively (Fig. S14, ESI†). Both these scavengers were able to significantly suppress the photooxidative efficiency of Cu\textsubscript{2}O nanoparticles, affirming the role of \text{OH}^- radicals in the Cu\textsubscript{2}O-driven visible light photocatalysis.

The recombination dynamics of the photogenerated charge carriers was investigated from the time-resolved photoluminescence (TRPL) spectrum of Cu\textsubscript{2}O nanoparticles using a pulsed laser and measuring the corresponding PL decay times (Fig. 9). The instrument response function (IRF) is shown in Fig. S15 (ESI†), confirming the ability of the instrument to resolve a few nanoseconds decay. The TRPL spectrum shows a multi-exponential decay behaviour with at least two very distinct fluorescence lifetimes (τ) – one of the order of few nanoseconds, and the other of the order of 40 nanoseconds. While the τ of the order of 1–5 nanoseconds is typically observed for semiconductor photocatalysts,\(^50,51\) it is uncommon to observe such extremely long-lived states leading to carrier lifetimes of an order of higher magnitude. This signifies the importance of oxygen vacancies present in Cu\textsubscript{2}O nanoparticles in acting as deep trap sites and thereby assisting in the efficient separation of the charge carriers.

To further validate the recombination efficiency of charge carriers, we performed transient photoelectrochemical (PEC) measurements on oxygen-deficient Cu\textsubscript{2}O nanoparticle-based devices prepared on a conducting FTO substrate. Initially, we obtained open circuit photocurrents by exciting Cu\textsubscript{2}O films with different wavelengths of light (Fig. 10a). The chosen LED light sources included 470 nm (\(E_{ex} > E_g\)), 525 nm (\(E_{ex} \approx E_g\), and 623 nm (\(E_{ex} < E_g\)) to represent various photoexcitation scenarios. Upon exposure to the same light intensities (500 µW cm\(^{-2}\)), the highest photocurrent densities were obtained when the surface was illuminated with 470 nm wavelength. As expected, the other two low energy sources could not efficiently photoexcite Cu\textsubscript{2}O, thereby showing relatively lower responses. The current density response profiles over 10 on/off repeat cycles showed a repeatability of >98% at all wavelengths, once again reaffirming the high photostability of Cu\textsubscript{2}O nanoparticles prepared in this study (Fig. S16, ESI†). Further experiment involving the exposure of Cu\textsubscript{2}O devices to different intensities of 470 nm wavelength showed a consistent increase in current output with increasing light density (Fig. 10b). This signifies the importance of light intensity in promoting charge carrier separation during photoexcitation. We also performed electrochemical impedance spectroscopy (EIS) at the open circuit potential of 0 V for investigating the influence of photoexcitation on charge transfer and carrier recombination at the Cu\textsubscript{2}O/solution interface (Fig. 10c).\(^52\) The

![Image](image-url)

**Fig. 9** Time-resolved photoluminescence (TRPL) decay transients for oxygen-deficient Cu\textsubscript{2}O nanoparticles along with the background instrument counts.
EIS responses are shown as Nyquist plots which involve observing the imaginary part $Z''$ versus the real part $Z'$ of the complex impedance $Z$. In Nyquist plots, the diameter of the semicircle is proportional to the resistance to charge transfer encountered by the semiconductor. The comparison of the EIS response of Cu$_2$O devices under dark and photoilluminated conditions reveals that when photoexcited with a 470 nm LED, the diameter of the loop decreases. This supports the visible light-driven photoexcitation of electrons to the CB, thereby promoting the photoactivity of oxygen-deficient Cu$_2$O nanoparticles.

Conclusions

In summary, we have for the first time, shown the ability to synthesize highly photostable and oxygen-deficient Cu$_2$O nanoparticles using a low-temperature method. This method allows the production of Cu$_2$O nanoparticles in gram scale with over 90% yield by a simple water-mediated hydrolysis of a Cu(I)–TEA complex. This material could be stored for long periods without oxidising to other copper oxides. As such, these findings are highly significant for the industrial applications of this important p-type semiconductor photocatalyst. Importantly, the presence of an appropriate energy band structure that allows the efficient generation of photocarriers upon excitation with visible light, and a highly oxygen-deficient Cu$_2$O lattice that increases the longevity of these charge carriers play a synergistic role in achieving outstanding photocatalytic activity. Overall, an effective route to synthesize oxygen defect-rich Cu$_2$O in large quantities is likely to find substantial applications in photo-driven processes such as environmental remediation and energy conversion/storage devices.

Experimental section

Materials

Copper(II) chloride (CuCl$_2$), triethylamine (TEA), acetone, methanol, ethanol, acetonitrile, methyl orange, tetracycline and 5,5-dimethyl-pyrroline N-oxide (DMPO) were purchased from Sigma-Aldrich, AR grade and used as received. Deionized water (MilliQ, 18.2 MΩ cm) was used for all the experiments.

Synthesis of a Cu(i)–TEA complex

The Cu(i)–TEA metal coordination complex was prepared by the magnetic stirring (500 rpm) of copper(II) chloride (0.1 M in 400 mL acetonitrile) in a round bottom flask at 80 °C for 5 minutes. Following this, 50 mL of TEA was added at once to the above stirring solution. The mild reducing ability of TEA resulted in a reduction of Cu(II) species to Cu(I), which instantaneously formed an ionic complex with TEA. The formation of this Cu(i)–TEA complex was evident from the generation of non-violent vapours upon the addition of TEA to the copper salt. With continuous stirring for 1 hour, the solution phase turned to dark-red. After being cooled to room temperature under ambient conditions, the reaction product was centrifuged at 9000 rpm for 15 minutes to remove any potentially precipitating species. The liquid phase corresponding to the Cu(i)–TEA complex was collected and subsequently used for the synthesis of Cu$_2$O nanoparticles.

Synthesis of Cu$_2$O nanoparticles

For the large-scale synthesis of Cu$_2$O nanoparticles, 450 mL of the purified Cu(i)–TEA complex was brought to 80 °C in a round bottom flask. After 5 minutes, an equivalent volume of water (pre-heated to 80 °C) was added to this complex, which resulted in the instantaneous hydrolysis of the Cu(i)–TEA complex, leading to the precipitation of Cu$_2$O nanoparticles. The particles were allowed to precipitate for 4 hours under non-stirring conditions. Following this, the reaction contents were allowed to cool for 1 hour, and the Cu$_2$O nanoparticles were collected by centrifugation at 9000 rpm for 15 minutes. The particles were further washed three times with acetone/methanol (1:1 v/v) to obtain a yellowish-orange pellet. The precipitate was dried under vacuum at room temperature to achieve a gram-scale (2.56 g) yield of Cu$_2$O particles in a single batch, which corresponds to over 90% conversion of the starting copper salt.
Materials characterization

The particle size, morphology, and crystallinity of Cu$_2$O nanoparticles were determined by FE-SEM, as well as TEM and HR-TEM. EDX analysis was performed on a FEG-Varios SEM instrument operated at 15 kV. TEM images were obtained on a JEOL 1010 instrument, operated at 100 kV accelerating voltage. HR-TEM images and SAED patterns were obtained on a JEOL 2100F microscope operating at 200 kV accelerating voltage. EELS was performed in STEM mode on a JEOL 2100F instrument. Multiple EELS spectra were recorded along the surface of Cu$_2$O particles to validate its oxidation state across the particle surface. A Malvern Zeta sizer Nano ZS analyser instrument was used to obtain the hydrodynamic size of Cu$_2$O nanoparticles. The crystallinity of Cu$_2$O was determined by using a Bruker D8 Discover micro-diffraction system with a Cu Kα (1.514 Å) radiation source. The 2θ values in the range of 10–80° were obtained with a 0.021° step size. The determined crystalline phases were indexed based on the International Diffraction Data-Powder Diffraction File (ICDD-PDF). The crystallite size of the particles was calculated using the Scherrer equation based on all of the diffraction peaks of Cu$_2$O. Specific surface areas ($S_{BET}$) of Cu$_2$O nanoparticles were determined by nitrogen (N$_2$) sorption isotherms at −196 °C using a Brunauer–Emmett–Teller (BET) analysis method on a Micromeritics (ASAP 2000) analyser. Raman and PL measurements were performed on a Horiba LabRAM HR evolution Raman spectrometer using a 532 nm laser source. The UV-Vis absorbance measurements of an aqueous suspension of Cu$_2$O nanoparticles were carried out using an Agilent Cary 7000 UV-Vis-NIR spectrophotometer equipped with an integrating sphere. XPS measurements were performed on a K-alpha spectrometer (Thermo Scientific) using a monochromatic Al-Kα (1486.7 eV) radiation source at room temperature under ultra-high vacuum (10$^{-8}$ Pa). TRPL experiments were performed using a custom-built scanning confocal fluorescence microscope with a 100× objective (NA = 0.9) operating at room temperature. A tunable and pulsed (6 ps fundamental pulse-width) white light laser (Fianium, WhiteLaseSC 400) was used to excite particles at 500 nm with a repetition rate of 5 MHz. Photons were collected with avalanche photodiodes (APD, Excelitas, SPCM-AQRH-14) and analysed with a correlator card (Picoquant, TimeHarp 260).

EPR measurement

CW-X-Band EPR spectra were recorded using a commercial Bruker E500 spectrometer equipped with an ER4122 SHQ resonator. Low temperature (20 K) experiments were performed using an Oxford Instruments ESR 935 cryostat and ITC503 temperature controller. Frozen solution and solid powder samples were loaded into standard X-band EPR tubes of 3.8 mm i.d. Liquid solution EPR measurements were performed using a quartz flat cell. A tungsten-halogen lamp and water filter were used to illuminate Cu$_2$O solutions. Room temperature CW X-band EPR measurements were performed for Cu$_2$O nanoparticles to characterize any copper signals that may be present. The low temperature EPR spectra of the solutions of the precursor Cu(i)–TEA (100 mM) and the EPR-standard CuSO$_4$ (100 μM) were also recorded that allowed the estimation of the proportion of Cu(n) in the Cu(i)–TEA precursor sample. To estimate the proportion of Cu(n) in the Cu$_2$O nanoparticles, we assumed that we can quantitatively compare the powder EPR spectra recorded for the Cu$_2$O sample with the CuSO$_4$ standard, factoring in changes in the instrument settings and the temperature. For spin trap experiments, 20 mg of Cu$_2$O nanoparticles was dispersed in 2 mL of water (0.07 M). 1 mL of this solution was then mixed with 1 mL of DMPO dissolved in water (4.6 mM) in the dark. The reaction mixture was continuously stirred with a small magnetic stirrer. An aliquot of this mixture was taken prior to illumination and its EPR spectrum was recorded. The reaction vessel containing the Cu$_2$O/DMPO mixture was then illuminated for 15 min using cold white light and the EPR spectrum was recorded.

Photocatalysis measurement

The photocatalytic activity of the Cu$_2$O nanoparticles was assessed by observing their capacities to degrade MO in aqueous solutions. The photocatalytic tests were performed under cold white LED under a fixed illumination of 16 mW cm$^{-2}$. In each experiment, the sample (2 mg) was suspended in 20 mL of 5 × 10$^{-5}$ M (C$_0$) MO solution. The suspension was mixed under dark conditions for 30 minutes to make sure an adsorption–desorption equilibrium was achieved before any light illumination. Afterwards, at each interval (5 minutes) of light illumination, the light-exposed solution (700 μL) was collected, followed by centrifugation at 10 000 rpm to separate the particles from the solution. The supernatant was measured using a PerkinElmer UV–Vis Lambda-365 spectrophotometer to record the MO concentration (C$_0$). Moreover, the stability of the photocatalyst was also evaluated by performing ten consecutive cycles of catalytic experiments. At each cycle, the used photocatalyst was collected, filtered, and dried at 60 °C followed by washing several times with ethanol for the next test. Similarly, to study the photocatalytic oxidation of tetracycline antibiotic, 2 mg of catalyst was suspended in 20 mL of 2.5 × 10$^{-5}$ M tetracycline solution and time-dependent photo-oxidation was recorded over 20 min using UV-Vis spectroscopy. Photoelectrochemical measurement

The Cu$_2$O nanoparticles were deposited on FTO coated glass substrates through an electrophoresis (EP) technique reported earlier. The EP process involved transferring the Cu$_2$O nanoparticles (2 mg mL$^{-1}$) in acetone media and adding 10 mg of iodine per 5 mL of acetone. The total volume of the EP solution was 7 mL. A potential of 10 V DC was applied to the working and counter electrodes (both containing FTO coated glass substrates) for a period of 3 minutes and dried at 70 °C for 30 min. The prepared substrates were dipped in such a way as to obtain a deposition of Cu$_2$O nanoparticles over a 1 cm$^2$ surface area. PEC analysis was carried out in a quartz chamber using similar conditions reported in previous studies. The PEC tests including the time-resolved photoresponse and EIS
studies were carried out on an Ivium compactstat workstation using a custom-made PEC test chamber with LED light sources that isolates the device from external light. A homemade pulse generator was employed to obtain time-resolved photoresponse signals (5 seconds on and 10 seconds off mode per cycle). The LED sources include 470 nm, 525 nm, and 663 nm, with each LED specified to an FWHM of better than 25 nm. The LEDs were used after calibrating with a PM16-140 calibrated power meter from THORLABS. An aqueous solution of 0.1 M europium[III] nitrate pentahydrate ([Eu(NO₃)₃]·5H₂O) was used as the supporting electrolyte while Ag/AgCl and Pt were used as the reference and counter electrodes, respectively. EIS was measured in an open circuit (0 V potential) with frequencies from 0.01 to 105 Hz and an amplitude of 5 mV around the tested potential.

**Conflicts of interest**

There are no conflicts of interest to declare.

**Statement of contributions**

M. S. synthesised the material and performed most of the characterisation; D. J. performed the photocatalysis studies and prepared the first draft; A. E. K., Y. M. S. and S. K. B. were involved in the PEC studies; E. D. G. and J. v. E. were involved in band gap calculations; P. R. and B. C. G. were involved in the HR-TEM, EELS and EDX measurements; R. R. was involved in the XPS studies; M. J., J. L. and N. C. performed the EPR measurements and analysis; V. B. designed the study, analysed the data with other co-authors and prepared the manuscript.

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**References**

Electronic Supplementary Information

**Oxygen-deficient photostable Cu$_2$O for enhanced visible light photocatalytic activity**

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Fig. S1 HR-TEM images of Cu$_2$O nanotubes formed after 10 sec of reaction at room temperature.
Fig. S2 Particle size distribution of as-synthesised Cu₂O nanoparticles, as determined by SEM.
**Fig. S3** Hydrodynamic diameter of as-synthesised Cu$_2$O nanoparticles in water, as determined by DLS.
Fig. S4 EDX mapping of as-synthesised Cu$_2$O nanoparticles obtained on a nickel TEM grid.
Fig. S5 (a) Low-temperature (20 K) CW-X-Band EPR spectra of the Cu(I)-TEA complex and CuSO4 standard. The spectra have been scaled for clarity. Instrument settings: microwave power – 63 μW; and field modulation amplitude – 2.0 mT; (b) Room-temperature CW-X-Band powder EPR spectra of oxygen-deficient Cu2O nanoparticles. The baseline (EPR spectrum of the empty resonator) is subtracted from the obtained trace. Instrument settings: microwave power – 6.3 mW; and field modulation amplitude – 1.0 mT.
Fig. S6 (a) Powder XRD, and (b) N$_2$ sorption isotherms of as-synthesised Cu$_2$O nanoparticles and commercial Cu$_2$O.
**Fig. S7** XRD patterns of commercial Cu$_2$O nanoparticles before and after visible light-induced photooxidative degradation of methyl orange. The appearance of CuO signatures (marked with *) after photocatalysis reaction due to oxidation of commercial Cu$_2$O are noted.
**Fig. S8** XRD patterns of as-synthesised oxygen-deficient Cu$_2$O nanoparticles after repeated cycles of visible light-induced photo-oxidative degradation of methyl orange.
Fig. S9 (a) Photo-oxidation of tetracycline using oxygen-deficient Cu$_2$O, as evident from the UV-Vis absorption spectra of tetracycline after visible light photo-irradiation over 20 minutes; (b) plots of $C_t/C_0$ vs. irradiation time depicting the photodegradation of tetracycline in the presence of different catalysts.
**Fig. S10** Absorption spectra of MO over 1 h of exposure to simulated solar light in the presence of oxygen-deficient Cu$_2$O nanoparticles. Insets show the colours of the corresponding solutions.
Fig. S11 Valence band XPS spectrum of oxygen-deficient Cu$_2$O nanoparticles.
Fig. S12 Liquid solution CW-X-Band EPR Spectra of the DPMO-OH adduct generated by white light illumination of oxygen-deficient Cu$_2$O nanoparticles. Top trace: DMPO solution with no Cu$_2$O added; middle trace DMPO/Cu$_2$O reaction mixture before exposure to light; bottom trace DMPO /Cu$_2$O reaction mixture after 15 min illumination. Instrument settings: microwave power – 200 mW; and field modulation amplitude – 0.1 mT.
Fig. S13 Fluorescence spectra of 3 mM terephthalic acid (TA) obtained in the presence of oxygen-deficient Cu$_2$O nanoparticles at the $\lambda_{ex}$ of 320 nm. Before photoexcitation, TA molecules remain non-fluorescent; however after 15 min of visible light photoexcitation, the OH' radicals generated by the photo-excited Cu$_2$O nanoparticles readily react with TA to produce highly fluorescent 2-hydroxy terephthalic acid ($\lambda_{max}$ ~425 nm).
Fig. S14 Plots of $C_t/C_0$ versus irradiation time for oxygen-deficient Cu$_2$O nanoparticles-driven MO photodegradation in the presence of different scavengers. EDTA and TBA are able to suppress the photo-oxidation efficiency to 38% and 25%, respectively, in comparison to the control reaction involving no scavenger.
**Fig. S15** Instrument response function (IRF) for the instrument used to acquire the time resolved PL spectrum of Cu$_2$O nanoparticles.
Fig. S16 The dynamic photocurrent response repeatability of the substrate over 10 exposure cycles of constant intensity of 500μW•cm⁻² for wavelengths of (a) 470 nm, (b) 525 nm, and (c) 623 nm.
Chapter 3c

Competitive inhibition of the enzyme-mimic activity of Gd-based nanorods toward highly specific colorimetric sensing of L-cysteine

Gadolinium (Gd)-based one dimensional (1D) nanorods were synthesized in a facile manner using triethylamine (TEA) as a hydrolysing agent. Gadolinium, while well known for its MRI capabilities, such materials has rarely been used for sensing applications. Here we explored a novel application of Gd$_2$O$_3$ and Gd(OH)$_3$ nanorods through assessing their ability to mimic the activity of natural peroxidase enzyme (NanoZyme activity). Considering that L-cysteine could selectively suppress this NanoZyme activity through a competitive inhibition process, these Gd-based materials could then be employed for highly specific colorimetric sensing of this amino acid of high biomedical significance.

Competitive Inhibition of the Enzyme-Mimic Activity of Gd-Based Nanorods toward Highly Specific Colorimetric Sensing of L-Cysteine

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ABSTRACT: Gd-based nanomaterials offer interesting magnetic properties and have been heavily investigated for magnetic resonance imaging. The applicability of these materials beyond biomedical imaging remains limited. The current study explores the applicability of these rare-earth nanomaterials as nanozyme-mediated catalysts for colorimetric sensing of L-cysteine, an amino acid of high biomedical relevance. We show a facile solution-based strategy to synthesize two Gd-based nanomaterials viz. Gd(OH)₃ and Gd₂O₃ nanorods. We further establish the catalytic peroxidase-mimic nanozyme activity of these Gd(OH)₃ and Gd₂O₃ nanorods. This catalytic activity was suppressed specifically in the presence of L-cysteine that allowed us to develop a colorimetric sensor to detect this biologically relevant molecule among various other contaminants. This suppression, which could either be caused due to catalyst poisoning or enzyme inhibition, prompted extensive investigation of the kinetics of this catalytic inhibition in the presence of cysteine. This revealed a competitive inhibition process, a mechanism akin to those observed in natural enzymes, bringing nanozymes a step closer to the biological systems.

INTRODUCTION

Enzyme mimicking nanomaterials, commonly referred to as nanozymes, have received extensive interest recently due to their emerging applications in biosensing, environmental monitoring, and clinical diagnostics.ⁱ⁻⁴ Comparatively low-cost, high stability, and the ability to fine-tune the catalytic performance of nanozymes by altering composition, morphology, and other properties has now fuelled interest in using nanozymes in place of natural enzymes.⁵⁻⁸ To date, a variety of metals,⁹⁻¹¹ metal oxides,¹²⁻¹⁴ metal sulfides,¹⁴⁻¹⁵ metal halides,¹⁶ and carbon based nanomaterials¹⁷⁻¹⁹ have been investigated for their intrinsic oxidase-, peroxidase-, catalase-, and superoxide dismutase-like activity.

Of the various groups of elements, lanthanide-based nanomaterials exhibit rich optical and magnetic properties due to their unique 4f electron structure.²⁰⁻²² Such materials have attracted increasing attention specifically in biomedical applications.²² It is however surprising that there are very few reports of such rare earth-based nanomaterials for their potential as nanozymes. Of the several lanthanides, gadolinium-based materials show interesting magnetic properties due to the presence of seven unpaired electrons.²² This has seen Gd-based nanomaterials to be used in magnetic resonance imaging (MRI).²₃⁻²₅ In addition, Gd-based nanomaterials have been observed to offer better biocompatibility over Gd-based chelates that are often employed for MRI applications.²⁵,²⁶ These Gd-based nanomaterials are now occupying an important place as potential therapeutic and sensing agents.²⁷,²⁸ Although the importance of Gd-based nanomaterials is well recognized for MRI applications, its applicability in sensing through exploiting its nanozyme activity has not been investigated.

Cysteine, a sulfur containing amino acid, plays a vital role in protein synthesis, metabolism, detoxification, and biological signaling.²⁹⁻³¹ Deficiencies in cysteine are known to result in growth defects, skin lesions, lethargy, fat loss, and liver damage, while elevated levels are associated with cardiovascular and neurotoxic diseases.²⁹⁻³¹ Given the high biological significance of this important amino acid, accurate quantification of cysteine concentrations is of paramount importance. Current strategies including spectroscopy,³⁰,³²,³³ capillary electrophoresis,³⁴ and high-performance liquid chromatography³⁵⁻³⁷ have shown potential for cysteine sensing. However, the need for technical expertise, complexity of operations, high cost of instrumentation, long detection times, and laborious sample preparation limits the practical applicability of such techniques.³⁸,³⁹ In
contrast, colorimetric sensors based on nanzymes address these limitations and offer significant potential in the development of sensors against a range of chemical and biological molecules.\(^3,4,7,8,40-42\)

In this manuscript, we report a facile approach to synthesize two Gd-based materials viz. Gd(OH)\(_3\) and Gd\(_2\)O\(_3\) nanorods. We assess their enzyme-mimicking characteristics and find that the Gd(OH)\(_3\) nanorods show superior peroxidase-like nanozyme activity. This is the first time that nanomaterials based on Gd are reported for their intrinsic nanozyme activity. Further, we utilize this nanozyme activity of Gd(OH)\(_3\) nanorods to develop a highly specific colorimetric “turn-off” sensor for l-Cys detection. A thorough investigation of underlying sensing mechanism provides evidence that, instead of a typical “catalyst poisoning” phenomenon, the “competitive inhibition” of the catalytic activity of the nanozymes by l-cysteine takes place, thus making the proposed sensor as a biomimic of natural inhibitors in the biological signaling pathways.\(^31\)

### EXPERIMENTAL SECTION

#### Materials.
Gadolinium(III) nitrate hexahydrate (Gd(NO\(_3\))\(_3\)·6H\(_2\)O), triethyleneamine, 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS), 3,3’5,5’-tetramethylbenzidine (TMB) and α-phenylenediamine (OPD), sodium acetate, l-cysteine (Cys, C), l-alanine (Ala, A), l-arginine (Arg, R), l-aspartic acid (Asp, D), l-asparagine (Asn, N), l-histidine (His, H), l-glutamine (Gln, Q), l-glutamic acid (Glu, E), l-lysine (Lys, K), l-leucine (Leu, L), l-phenylalanine (Phe, F), l-methionine (Met, M), l-tryptophan (Trp, W), l-tyrosine (Tyr, Y), l-threonine (Thr, T), l-serine (Ser, S), l-valine (Val, V), l-glutamine (Gln, Q), l-proline (Pro, P), l-isoleucine (Ile, I), cystine, d-p-homocystine, cystamine dihydrochloride, l-glutathione reduced (GSH or γ-glutamyl-l-cysteinylglycine), l-glutathione oxidized (GSSG or glutathione disulfide), dimethyl sulfoxide, ampicillin sodium salt, penicillin G sodium salt, streptomycin sulfate salt, sodium sulfate, sodium 2-propanethiolate, sodium ethanethiolate, 2,2’-thiodiethanol, and terephthalic acid (TA) were purchased from Sigma-Aldrich. 30% (w/w) hydrogen peroxide (H\(_2\)O\(_2\)) was purchased from Chem Supply. Three peptides with 12 amino acid residues (MSPHPHPRHHHT, RGRRRRLSCRLL, and SPPRLLPWLRMP) were custom-designed and obtained from Selleck Chemicals LLC. All chemicals were used as received without any modifications.

#### Synthesis of Gd(OH)\(_3\) and Gd\(_2\)O\(_3\) Nanorods.
In a typical Gd(OH)\(_3\) nanoparticle synthesis, 40 mL of 1 M Gd(NO\(_3\))\(_3\) in deionized water (18.2 M\(\Omega\) cm\(^{-1}\)) resistivity, Milli-Q) was added to a 500 mL three-necked round-bottom flask and placed in an oil bath at 80 °C under constant stirring. On reaching the desired temperature, 5 mL of triethyleneamine was added, and the reaction was allowed to proceed at 80 °C under constant stirring for 1 h. After the end of the reaction, the mixture was allowed to cool to room temperature, and the white precipitate containing Gd(OH)\(_3\) nanoparticles was washed in an acetone/methanol mixture (1:1 v/v) and vacuum-dried for further characterizations. The white precipitate containing Gd(OH)\(_3\) nanoparticles was further thermally annealed at 700 °C for 12 h to obtain a white powder containing Gd\(_2\)O\(_3\) nanoparticles.

#### Characterization of Gd(OH)\(_3\) and Gd\(_2\)O\(_3\) Nanorods.
Samples for transmission electron microscopy (TEM) and high resolution TEM (HR-TEM) were prepared by drop coating the samples on a holey carbon TEM grid. The samples were analyzed using JEOL 1010 and JEOL 2100F (FEG system) TEM instruments, operated at accelerating voltages of 100 and 80 kV, respectively. EDX maps and spectra were collected using a 2100F TEM instrument in STEM mode using an Oxford X-Max S 80T using a 1.5 nm probe. X-ray diffraction (XRD) was obtained using Bruker AXS D4 Endeavor, wide angle XRD with Cu Kα radiation (\(λ = 1.5406\) Å). X-ray photoemission spectroscopy (XPS) measurements were carried out using Thermo K-Alpha XPS instrument operating at a pressure better than 1 × 10\(^{-8}\) Torr (1 Torr = 1.333 × 10\(^{-3}\) Pa). The Gd 3d and O 1s core level spectra were recorded with unmonochromatized Al Kα radiation (photon energy of 1486.7 eV) at a pass energy of 20 eV and an electron take off angle of 90°. The overall resolution was 0.1 eV for all XPS measurements. The core level spectra were background corrected using the Shirley algorithm, and chemically distinct species were resolved using nonlinear least squares fitting procedure.\(^43\) The binding energies (BEs) were aligned with an adventitious carbon binding energy of 285 eV. Thermogravimetric (TGA) analysis was performed using Pyris 1 TGA instrument (PerkinElmer, Inc.) in a temperature range of 30–850 °C under atmosphere conditions with a ramping rate of 5 °C min\(^{-1}\). The Gd ions were quantified using Agilent 4200 Microwave plasma-atomic emission spectrometer (MP-AES). The absorbance spectra were obtained using Envision multimode plate reader (PerkinElmer). Raman spectra were obtained using a Horiba LabRAM HR Evolution micro-Raman system equipped with 532 nm laser (0.5 μm lateral resolution, 0.25 s exposure) and a 100× objective. Raman spectra was background corrected using an in-house developed smoothing free algorithm.\(^44\) The specific surface area of both nanorods was measured using nitrogen (N\(_2\)) adsorption isotherms at −196 °C using a Bruanauer–Emmett–Teller (BET) analysis method on a Micromeritics ( ASAP 2000) instrument. Surface charge was measured using a Malvern Nano-Zs/Zen3600 zetasizer instrument.

#### Peroxidase-Like Nanozyme Activity of Gd(OH)\(_3\) and Gd\(_2\)O\(_3\) Nanorods.
The peroxidase-like nanozyme activity of Gd(OH)\(_3\) and Gd\(_2\)O\(_3\) was investigated through its ability to oxidize peroxidase substrate (ABTS, TMB, or OPD) to colored product (green, blue, or orange) in the presence of H\(_2\)O\(_2\). These colored products were characterized by a typical absorbance signal at ca. 420, 650, and 450 nm, respectively. Nanoparticle concentration-dependent activity was performed using 0–0.8 mM Gd ion concentrations using ABTS (1 mM) and H\(_2\)O\(_2\) (10 mM) in sodium acetate buffer pH 4 (10 mM) at 37 °C; pH-dependent activity was measured in reaction buffers (pH 1–8) and temperature-dependent activity (10–80 °C) in pH 4 sodium acetate buffer. Steady state kinetic analysis was performed by varying the substrate concentration from 0.4–4 mM for ABTS and 0–500 mM for H\(_2\)O\(_2\). The obtained data were fitted to Michaelis–Menten and Lineweaver–Burk plots using OriginPro 2016 and apparent enzyme kinetic parameters were calculated. Catalyst reusability was assessed by using the Gd(OH)\(_3\) nanorods for five consecutive cycles where the nanorods were washed several times between each catalytic cycle. Post catalysis, the Gd(OH)\(_3\) nanorods were recovered using centrifugation and the morphology and phase was assessed using TEM and XRD. The stability of the nanorods in acidic media was evaluated by determining the Gd ion concentration using atomic emission spectroscopy. Samples for stability studies were prepared by incubating them in acidic media and obtaining the nanorod pellet using high speed centrifugation. The Gd ion concentration was determined from the pellet. A similar protocol was used to obtain the pellet after five catalytic cycles.

#### Cysteine Sensing Using Gd(OH)\(_3\) Nanorods.
For sensing experiments, ABTS solution (2.5 mM), H\(_2\)O\(_2\) (20 mM), and cysteine (l-Cys) in various concentrations (0–400 μM) were added to sodium acetate buffer solution (pH 4.0). Then, 0.5 mM of the Gd(OH)\(_3\) catalyst was then added. The variation in green color of the reaction mixture was monitored as a function of time and quantified using UV-Visible spectrophotometry. Important sensing parameters such as limit of detection (LoD), limit of quantification (LoQ), accuracy, and precision was calculated.\(^38\)

#### Systematic Evaluation of the Turn-Off Cysteine Sensor Using Sulfur Containing Compounds.
The cross-reactivity of the sensor was established by comparing the enzyme mimic catalytic activity in Gd(OH)\(_3\) nanorods (0.5 mM, ABTS – 2.5 mM and H\(_2\)O\(_2\) - 20 mM) in presence and absence of other commonly existing amino acids, 13 sulfur containing compounds, and 3 peptide sequences. The concentrations of the amino acids and the sulfur containing compounds were kept at 75 μM, while the concentrations of peptides were maintained at 1 mg/mL. Cross reactivity studies were also performed by evaluating the sensor response when a combination of different amino acids was used. Mechanistic understanding of the

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Cys sensing was evaluated by determining the enzyme kinetic parameters in the presence of 5 μM L-Cys concentration.

**Terephthalic Acid (TA) Studies.** To understand the competitive inhibition reaction mechanism, we employed TA as a capture probe for OH• radicals. For this, Gd(OH)₃ nanorods (1 mM Gd ion equivalent) were incubated with 0.25 mM TA and 20 mM H₂O₂ in 100 mM sodium acetate buffer at pH 4 with and without L-Cys (75 μM). Appropriate control experiments were also setup. The reaction was incubated at 37 °C for 4 h. The nanorods were centrifuged, and the supernatant was used for fluorescence measurements. The change in fluorescence was measured at an excitation wavelength of 315 nm using Fluoromax-4 spectrophotometer.

### RESULTS AND DISCUSSION

TEM images obtained from both Gd(OH)₃ and Gd₂O₃ show rod-like morphologies (Figure 1). The Gd(OH)₃ nanorods show an average width of 15 nm and an average length of 100 nm (Figure 1a1). Post thermal annealing, the Gd(OH)₃ nanorods convert to Gd₂O₃ nanorods, where TEM images show an increase in the size dimensions with an average width of 25 nm and an average length of 250 nm for Gd₂O₃ nanorods (Figure 1b1). This increase in the nanorod size during thermal annealing is well-known and is referred to as a coalescence process.45 HR-TEM images show that both Gd(OH)₃ (Figure 1a2) and Gd₂O₃ nanorods (Figure 1b2) are highly crystalline in nature. The Gd(OH)₃ nanorods show clear lattice fringes (Figure 1a3) with d-spacings of 1.81 and 3.12 Å, corresponding to the (002) and (101) planes of hexagonal Gd(OH)₃.46 In contrast, the annealed Gd₂O₃ nanorods (Figure 1b3) show lattice fringe with a d-spacing of 3.12 Å, corresponding to the (222) plane.46 EDX elemental maps and corresponding spectra show that the Gd(OH)₃ and Gd₂O₃ nanorods primarily contain Gd and O, with minor signals from C and N elements. These signals suggest that both materials may have amine bound to the surface during synthesis. The triethylamine used for the synthesis of Gd(OH)₃ and Gd₂O₃ nanorods is known for its ability to direct the crystal growth along the [001] direction, thereby providing shape control.47 In the case of Gd(OH)₃ nanorods, the surface energy of the [001] face is much lower than the other faces.48 Therefore, the growth along the [001] is likely to be significantly faster resulting in the formation of nanorods.

The crystal structures of both nanorods were examined by XRD patterns, as shown in Figure 2a. The diffraction pattern obtained from Gd(OH)₃ confirms the hexagonal phase (space group P6₃/m; JCPDS card No. 83-2037; a = b = 6.3 Å, c = 3.6 Å).46 This diffraction pattern shows three main peaks at 2θ = 16.2°, 28.2°, and 29.6° corresponding to the (100), (110), and (101) planes of the hexagonal phase.46 Thermal annealing resulted in a clear phase change as evident from the XRD pattern of Gd₂O₃ nanorods. The pattern matches well with the cubic phase (space group la-3; JCPDS card No. 3-065-3181; a = b = c = 10.82 Å) of Gd₂O₃, with a major peak at 2θ = 28.6° corresponding to the (222) plane.46 Other planes observed for both nanorods are also in good agreement with previous

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**Figure 1.** (1) Low magnification and (2) high magnification TEM images, (3) HR-TEM images, (4) EDX maps, and (5) EDX spectra of Gd(OH)₃ (series a) and Gd₂O₃ (series b) nanorods. Key for EDX spectra # = Cu, * = Gd, δ = O, and β = C.

**Figure 2.** (a) XRD patterns, (b) Raman spectra, and (c) Gd 3d core level and (d) O 1s core level XPS spectra obtained from Gd(OH)₃ and Gd₂O₃ nanorods.
The change in the crystal phase as a function of temperature was also observed by TGA analysis of the Gd(OH)$_3$ nanorods, as shown in Figure S1, in the Supporting Information. At temperatures between 25 and 250 °C, a gradual decrease in the weight is observed. This is primarily due to the removal of adsorbed CO$_2$ and H$_2$O. A further sharp weight loss is observed at ca. 315 °C due to a dehydration process resulting in the formation of GdOOH (confirmed using XRD, Figure S2, in the Supporting Information). The newly formed intermediate GdOOH further converts to form Gd$_2$O$_3$ and H$_2$O at ca. 425 °C. No further significant weight loss is observed. In total, a weight loss of about 13.5% is observed during the conversion of the hydroxide to the oxide phase.

The Gd(OH)$_3$ and Gd$_2$O$_3$ nanorods were also characterized using Raman spectroscopy (Figure 2b). The Raman spectrum of Gd(OH)$_3$ shows three distinct peaks at 309.4, 385.6, and 491.2 cm$^{-1}$, which can be assigned to A$_{1g}$, E$_{2g}$ (translatory modes predominately of the anions), and E$_{1g}$ (liberation) modes, respectively. Only a single major Raman peak is observed in the case of Gd$_2$O$_3$ at 360.2 cm$^{-1}$ that corresponds to the F$_{2g}$ mode. The presence of these modes further confirms the high quality of the Gd-based nanorods.

XPS, a highly surface sensitive technique, was then used to confirm the oxidation states of Gd in both of these nanorods through studying the Gd 3d (Figure 2c) and O 1s (Figure 2d) core level spectra. The Gd 3d core level for the Gd(OH)$_3$ nanorods shows two characteristics 3d$_{5/2}$ and 3d$_{3/2}$ splitting components (spin–orbit splitting 32.2 eV) with peaks arising at binding energies (BEs) of ca. 1185.7 and 1217.9 eV, respectively. The Gd 3d spectrum for the oxide phase shows a minor shift to higher BEs, which is in good agreement with that observed previously. The O 1s core level XPS spectrum obtained from the hydroxide phase shows a broad signature with BE maximum at 528.9 eV and can be attributed to the O in the Gd(OH)$_3$, whereas the O 1s core level for the oxide shows two distinct chemical species at 528.6 and 531.5 eV that corroborate well with the oxygen vacancies and the oxygen atoms in the Gd$_2$O$_3$ lattice, respectively.

The intrinsic peroxidase-like nanozyme activity of Gd(OH)$_3$ and Gd$_2$O$_3$ nanorods was established by evaluating their ability to oxidize different peroxidase substrates (TMB, ABTS, or OPD) in the presence of H$_2$O$_2$ as an electron acceptor. It was immediately clear that both nanorods catalyzed the oxidation of ABTS to ABTS$\bullet^+$ in the presence of H$_2$O$_2$ to produce a deep green color (Figures 3 and S3, Supporting Information), with a consistent increase in absorbance maxima as a function of time, suggesting that the hydroxide phase is a better catalyst. Brunauer–Emmett–Teller (BET) analysis suggested that the Gd(OH)$_3$ nanorods had significantly higher surface area (62.5 m$^2$/g) over Gd$_2$O$_3$ nanorods (35.8 m$^2$/g), which might be an important factor responsible for the higher catalytic activity of Gd(OH)$_3$ (Figure S5, Supporting Information). Additionally, the higher activity could also result from the significantly higher utility of the Gd-based nanorods.
positive charge (zeta potential) of the Gd(OH)$_3$ nanorods, leading to better nanorod-substrate interaction. To ascertain that the observed nanozyme activity was not due to the leaching of Gd ions, control experiments were performed wherein the solutions of the potentially leached ions were used as the catalyst (both nanorods were incubated in pH 4 buffer for 60 min and extracted through centrifugation). The absence of any catalytic activity of the potentially leached ions further emphasizes the direct role of the nanorods in catalyzing the oxidation of the peroxidase substrate (Figure S6, Supporting Information). Additional experiments showing the postreaction stability of the nanoparticles and reusability across multiple cycles of usage is shown in Figure S7, Supporting Information.

Similar to other peroxidase-mimic nanomaterials, the nanozyme activity of Gd(OH)$_3$ and Gd$_2$O$_3$ was found to be dependent on nanoparticle concentration, temperature, and pH (Figure 4b–d). Although the nanozyme activity increases as a function of nanoparticle concentration, the activity saturates at higher nanoparticle concentrations. For instance, no significant change in the activity is observed beyond the equivalent of 0.5 mM Gd ion concentration for Gd(OH)$_3$ and 0.7 mM for Gd$_2$O$_3$ nanorods (Figure 4b). Temperature-dependent nanozyme activity assessment suggests that the activity increases with temperature for both nanorods up to 60 °C (Figure 4c). However, the activity starts to reduce beyond this temperature.

The nanozyme activity of both nanorods also shows a pH dependent trend, such that the activity is maximum at acidic conditions, pH 4 (Figure 4d). This highlights the similarity between nanoparticles as enzyme mimics and natural enzymes where the catalytic activity is governed by factors such as enzyme concentrations, temperature, and pH. Based on these factors, the most optimum parameters were selected for further studies, which included nanorods containing 0.5 mM equivalent of Gd and pH 4. Although 60 °C showed maximum performance, for potential biomedical applications, such high temperatures would result in alteration/decomposition of biological samples. Therefore, a temperature of 37 °C was chosen for further experiments as both nanorods still presented reasonable nanozyme activity.

The kinetic parameters for the Gd(OH)$_3$ and Gd$_2$O$_3$ nanorods were studied according to the enzyme kinetic theory. Important enzyme kinetic parameters such as Michaelis–Menten constant ($K_m$), maximum initial velocity ($V_{max}$), and turnover number ($K_{cat}$) were determined. Typical Michaelis–Menten and Lineweaver–Burk plots were observed for Gd(OH)$_3$ and Gd$_2$O$_3$ nanorods for both ABTS and H$_2$O$_2$ concentrations (Figure S8, in the Supporting Information). These further confirmed that both nanorods show typical enzyme-like behavior. Table S1 summarizes the calculated $K_m$, $V_{max}$, and $K_{cat}$ values based on the Lineweaver–Burk plots, where $K_m$ is indicative of the binding affinity between the nanozyme and substrate (typically lower the $K_m$ higher the affinity) and $V_{max}$ reflects how fast the nanozyme catalyzes the oxidation of the substrate. It is immediately clear that both Gd(OH)$_3$ and Gd$_2$O$_3$ nanorods have higher affinity for peroxidase substrate (ABTS) than for H$_2$O$_2$, and as such, the substrate affinity of the Gd$_2$O$_3$ nanorods is higher than the Gd(OH)$_3$ nanorods. This observation is consistent with other peroxidase-mimic nanomaterials where the nanozyme is generally observed to have a higher affinity to the peroxidase substrate over H$_2$O$_2$. Lower affinity of both nanorods to H$_2$O$_2$ than to ABTS suggests that a higher amount of H$_2$O$_2$ is required to achieve maximum catalytic activity. In contrast to the trend observed for the affinity of the nanozyme to ABTS, the Gd(OH)$_3$ nanorods show better affinity to H$_2$O$_2$ than Gd$_2$O$_3$ nanorods. Further, the $V_{max}$ toward ABTS as well as H$_2$O$_2$ is higher for the Gd(OH)$_3$ over Gd$_2$O$_3$ nanorods. This confirms that Gd(OH)$_3$ is a better catalyst among the two. Additionally, the catalytic constant $K_{cat}$ also known as the turnover number (maximum number of molecules of substrate that an enzyme can convert to a product per catalytic site per unit time) was calculated as it provides a robust comparison of the nanozyme activity. The Gd(OH)$_3$ nanorods showed a higher $K_{cat}$ value for both peroxidase substrate and H$_2$O$_2$, further affirming that the hydroxide phase has superior enzyme-like characteristics in comparison to the Gd$_2$O$_3$ nanorods. Given that the Gd(OH)$_3$ nanorods show superior nanozyme activity, the Gd(OH)$_3$ nanorods was chosen as a model nanozyme to determine its ability to detect cysteine.

The introduction of l-cysteine (l-Cys) into the reaction (nanorods + ABTS + H$_2$O$_2$) suppresses the inherent peroxidase-like nanozyme activity of the Gd(OH)$_3$ nanorods. Such deactivation/suppression of the catalytic activity, more commonly referred to as ‘catalyst poisoning’, is well documented. Such poisoning may either lead to improved selectivity or render the catalyst ineffective. A classic example is the Lindlar catalyst, where the catalyst (Pd) is poisoned with different forms of lead or sulfur to specifically obtain alkenes. This poisoning is typically irreversible unless the poison is removed by extensive cleaning. In the current case, the deactivation/suppression of the catalytic activity was dependent on the concentration of l-Cys (Figure 5a).
However, a time dependent kinetic study of the catalytic activity in the presence and absence of L-Cys shows that the suppression was only temporary (Figure S9, Supporting Information). For instance, when the reaction proceeds in the presence of 100 μM L-Cys, the inherent nanozyme activity of Gd(OH)₃ nanorods is suppressed for the first 10 min, following which the nanozyme activity regains leading to the oxidation of peroxidase substrate. The retention time during which the activity remained suppressed was dependent on the concentration of L-Cys (Figure S10, Supporting Information). This interesting observation of the catalyst regaining its catalytic ability without washing is significantly different from that observed in a typical catalyst poisoning leading to selectivity. In the current study, regaining of the catalytic activity without washing suggests that a different mechanism may be involved in L-Cys mediated suppression of the nanozyme activity. A possible alternate is the competitive inhibition process seen in natural enzymes. During this process, an inhibitor, through binding to the catalytically active site of the enzyme, prevents the oxidation of the peroxidase substrate. The kinetic studies are supported by the standard reduction potentials (Table S2, in the Supporting Information) for H₂O₂, cysteine, and ABTS that suggest that the potential for L-Cys oxidation is thermodynamically more favorable than ABTS. This suggests that the L-Cys would be first oxidized by H₂O₂ to its disulfide form, which will inhibit the ABTS oxidation at earlier time points. Once a majority of the L-Cys molecules are oxidized to form cystine, the nanorods are then free to oxidize ABTS resulting in the formation of the colored product. Therefore, when the reaction proceeds in the presence of L-Cys and H₂O₂, the inherent nanozyme activity of the Gd(OH)₃ nanorods is suppressed.

To determine the nature of the L-Cys mediated inhibition of Gd(OH)₃ catalytic activity, we performed detailed nanozyme kinetic studies, as catalytic inhibition processes have a significant influence on Vₘₐₓ and/or Kₘ of the reaction. These inhibitors may be categorized as either competitive or noncompetitive. A characteristic feature of competitive inhibition is that the inhibitor blocks the catalytic ability through binding to the catalytically active site of the enzyme. In this case, when the substrate concentration increases during the reaction, the probability of the inhibitor binding to the enzyme reduces. This means that, at high substrate concentration, the catalytic ability of the enzyme remains unchanged (i.e., Vₘₐₓ remains unchanged), whereas the substrate affinity decreases (i.e., Kₘ increases). In contrast, during noncompetitive inhibition, the inhibitor binds to the enzyme away from its catalytically active site. In this case, the enzymatic activity is not completely blocked; instead, the efficiency of the catalytic activity is reduced. This means that the Vₘₐₓ is reduced but the Kₘ remains unchanged during noncompetitive inhibition.

With this understanding, we designed further experiments to determine the nature of catalytic inhibition process. To understand this inhibition mechanism, enzyme kinetic parameters were calculated for ABTS and H₂O₂ in the presence and absence of L-Cys (Figure Sb). Since the y intercepts in both cases remain unchanged, this suggests that there is no change in the Vₘₐₓ in the presence or absence of L-Cys. In contrast, a significant influence on the Kₘ values is observed when kinetic studies are compared in the presence and absence of L-Cys. The Kₘ for ABTS and H₂O₂ increased from 3.28 to 8.77 mM and 16.11 to 232.19 mM, respectively in the presence of l-Cys. This confirms that the L-Cys mediated suppression of the nanozyme activity of Gd(OH)₃ nanorods is a result of competitive inhibition between the substrates and L-Cys molecules.

The kinetic studies are supported by the standard reduction potentials (Table S2, in the Supporting Information) for H₂O₂, cysteine, and ABTS that suggest that the potential for L-Cys oxidation is thermodynamically more favorable than ABTS. This suggests that L-Cys would be first oxidized by H₂O₂ to its disulfide form, which will inhibit the ABTS oxidation at earlier time points. Once a majority of the L-Cys molecules are oxidized to form cystine, the nanorods are then free to oxidize ABTS resulting in the formation of the colored product. Therefore, when the reaction proceeds in the presence of L-Cys and H₂O₂, the inherent nanozyme activity of the Gd(OH)₃ nanorods is suppressed.

Figure 6. Systematic evaluation of the specificity of Gd(OH)₃ nanorods to detect L-Cys in the presence of other nonspecific analytes. (a) 20 natural amino acids as represented using one letter amino acid code, and (b) a number of sulfur containing contaminants. In panel b, the black bars and red bars represent the sensor response in the absence and presence of L-Cys, respectively. Legends: (1) Cys, (2) Cys + Ala, (3) Cys + Ala + His, (4) Cys + Ala + His + Glu, (5) Cys + Ala + His + Glu + Asn, (6) Cys + Ala + His + Glu + Asn + Ser, (7) Cys + 9 amino acids, (8) Cys + 14 amino acids, (9) Cys + 19 amino acids, (10) homocysteine, (11) cystine, (12) cystamine, (13) GSH, (14) GSSG, (15) dimethyl sulfoxide, (16) ampicillin, (17) penicillin, (18) streptomycin sulfate, (19) sodium sulfate, (20) sodium 2-propanethiolate, (21) sodium ethanethiolate, and (22) 2,2-thiodiethanol. Concentration of each of the analytes is 75 μM, e.g., the red bar in sample (9) containing 20 amino acids will have a total concentration of 20 × 75 μM.
ing Information). The data confirms that Gd(OH)₃ nanorods are able to produce OH• radicals in the presence of H₂O₂. As expected from an antioxidant molecule, L-Cys either standalone or in the presence of nanorods does not produce any OH• radicals. However, we noted that, in the presence of H₂O₂, L-Cys behaves as a pro-oxidant and produces a significant amount of OH• radicals. Therefore, in the presence of H₂O₂, L-Cys loses its antioxidant properties and is unable to reduce ABTS⁺⁺ to ABTS. Further, when Gd(OH)₃ nanorods are introduced to the system (nanorods + L-Cys + TA + H₂O₂), the passivation of nanorod surfaces by L-Cys molecules leads to reduced production of the OH• radicals. As such, these observations corroborate well with the kinetic studies (Figure Sb,c) that support a L-Cys mediated competitive inhibition mechanism.

Considering that the competitive inhibition of the nanozyme activity of Gd(OH)₃ nanorods shows a L-Cys concentration dependent trend, this allowed us to explore this activity for developing a cysteine sensor. The nanozyme activity could be suppressed with as low as 0.2 μM concentration of cysteine, and this suppression of the nanozyme activity continued until the concentration of L-Cys was increased up to 200 μM following which the suppression of the activity saturates. A plot of the relative nanozyme activity vs L-Cys concentration suggests that the sensor operates in a linear dynamic range of 0.2–75 μM (Figure 5d). The linear regression value of 0.998 evinces the desirable linearity of the curve, supporting the quantitative detection ability of this approach. Important parameters such as LoD, LoQ precision, and accuracy were also determined to evaluate the performance of the sensor. The cysteine sensor showed a LoQ of 2.6 μM and LoQ of 7.98 μM. When tested for 20 repeat events at 10 μM L-Cys concentration, the sensor was found to operate with a precision of 97.2% while showing 90% accuracy at 5% confidence level and 100% accuracy at 10% confidence level. These values are comparable to the previous reports.30,58,59

To understand if the suppression of the catalytic activity is specific only in the presence of cysteine, the activity was monitored in the presence of all of the 20 natural L-amino acids. Figure 6a shows that the activity is not significantly suppressed by any other amino acids including L-Met, which is another sulfur-containing amino acid. This suggests that the thiol functional group in L-Cys may have the propensity to get oxidized by the catalytic activity than the thioether functional group in L-Met.58 Even high concentrations of L-amino acids did not show the ability to suppress the catalytic activity (Figure S12, in the Supporting Information). Further, we carried out a systematic assessment of other sulfur containing compounds for their ability to potentially suppress the catalytic activity of the Gd(OH)₃ nanorods (Figure 6b). It is clear that the catalytic ability of the Gd(OH)₃ nanorods is not deactivated/suppressed in the presence of other sulfur containing contaminants. In fact, the catalytic activity of the Gd(OH)₃ nanorods is not suppressed even in the presence of all other 19 natural amino acids. However, the addition of cysteine with these contaminants resulted in the suppression of the catalytic activity. This is remarkable, as when equimolar concentrations of 20 amino acids are mixed, the L-Cys concentration corresponds to only 5% of the total analyte concentration with 95% nonspecific analytes. Another noticeable observation is that the nanozyme activity is not affected in the presence of homocysteine, which is a nonprotein α-amino acid and a homologue of cysteine but with an additional methylene (−CH₂−) bridge.58 While changes in the physio-

logical homocysteine levels have important implications in progression of a large number of diseases, the differentiation between cysteine and homocysteine remains highly challenging due to their high structural similarity.29 The high selectivity of Gd(OH)₃ nanorods toward cysteine over homocysteine offers potential toward selective differentiation between these molecules. Further, it is interesting that the proposed nanozyme sensor remains selective for cysteine in the presence of GSH (a tripeptide consisting of glutamate, cysteine and glycine). The levels of reduced and oxidized glutathione may remarkably vary in different organs, and their relative concentrations are responsible for a myriad of physiological activities. The ability of the nanozyme cysteine sensor not to be influenced by glutathione offers immense promise in utilizing this sensor for physiological cysteine monitoring.60

While highly promising, at this stage, we do not fully understand the mode of selectivity of the Gd(OH)₃ nanozyme sensor toward cysteine, particularly in the presence of homocysteine and GSH. One potential explanation might be the structural differences between these molecules that dictate their molecular interactions with the surface of Gd(OH)₃ nanorods.

Next, we evaluated the ability of the Gd(OH)₃ nanorods to specifically detect cysteine-containing biomolecules (Figure 7).

![Figure 7](image_url)

Figure 7. Evaluation of sensor response in the presence of three peptides (1 mg/mL) either without cysteine (1 and 2) or containing a cysteine residue (3). Legends: (1) MSPHPHPHRRHTT, (2) SPPRLPPWRMP, and (3) RGRRRLSCLRL.

To achieve this, three different peptide sequences were designed such that one of the peptides contained a cysteine residue while the other two were without cysteine. The two peptides without cysteine residues did not show any influence on the nanozyme activity, whereas cysteine-containing peptide behaved similar to free cysteine molecules by suppressing the catalytic activity. This further supports the potential of Gd(OH)₃ nanorods-based sensing platform for specific detection of cysteine in biological systems.

### CONCLUSIONS

This study for the first time establishes the inherent peroxidase-like nanozyme activity of Gd-based nanomaterials. The nanozyme activity was found to be dependent on the phase of the Gd particles such that Gd(OH)₃ nanorods showed superior activity in comparison to Gd₂O₃ nanorods. The catalytic activity of both nanorods was also dependent on the pH, temperature, nanomaterial concentration, and substrate concentration. Having established the superior nanozyme activity of Gd(OH)₃ nanorods based on enzyme kinetic theory, the applicability of these nanozymes as a turn-off sensor for L-Cys sensing was further established. The sensor showed a LoD
of 2.6 μM and LoQ of 7.98 μM, with a precision of 97.2% while showing 90% accuracy at 5% confidence level and 100% accuracy at 10% confidence level. The sensor showed outstanding selectivity to accurately detect L-Cys even in the presence of other amino acids and a number of sulfur containing contaminants. The debate of whether the loss of nanozyme activity in the presence of cysteine is due to catalyst poisoning or a natural enzyme inhibition process was assessed. An evidence of the latter process occurring was established by comparing the enzyme kinetic parameters of the nanozyme mediated colorimetric sensing, while emphasizing the need for detailed kinetic investigations of nanozyme activity to realize their full application potential.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.7b01926.

Figure S1: Thermogravimetric analysis of Gd(OH)₃ nanorods. Figure S2: XRD pattern of GdOOH. Figure S3: UV–vis spectra of nanozyme activity. Figure S4: Relative peroxidase-like and oxidase-like nanozyme activity. Figure S5: BET surface area analysis. Figure S6: Relative nanozyme activity in the presence of leached ions. Figure S7: Catalyst stability. Figure S8: Michaelis–Menten curves and Lineweaver–Burk plots for Gd(OH)₃ and Gd₃O₄ nanorods. Figure S9: Time dependent nanozyme activity of Gd(OH)₃ nanorods in the presence of L-Cys. Figure S10: Time dependent nanozyme activity of Gd(OH)₃ nanorods in the presence of increasing concentration of L-Cys. Figure S11: Terephthalic acid experimental results. Figure S12: Specificity for L-Cys detection using Gd(OH)₃ nanorods at high L-Cys concentration. Table S1: Steady state kinetic parameters. Table S2: Standard reduction potentials. (PDF)

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§The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. M.S., P.W., and P.D.L. contributed equally.

Notes

The authors declare no competing financial interest.

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Supporting Information

for

Competitive inhibition of the enzyme-mimic activity

of Gd-based nanorods towards highly specific
colorimetric sensing of L-Cysteine

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**Figure S1.** Thermogravimetric (TGA) analysis reflecting upon the change in crystal phase of Gd(OH)$_3$ with respect to temperature.

A gradual decrease in weight was observed in the range of 25-250 °C due to the removal of adsorbed CO$_2$ and H$_2$O from the surface of Gd(OH)$_3$ nanorods. This was followed by the sharp weight loss at *ca.* 315 °C due to the formation of an intermediate product, GdOOH. The formed GdOOH was converted to Gd$_2$O$_3$ and H$_2$O at *ca.* 425 °C as signified from another weight loss feature. No additional weight lost was observed up to 900 °C. The total weight loss during the conversion of Gd(OH)$_3$ to Gd$_2$O$_3$ was about 13.5%.
Figure S2. X-ray diffraction patterns (XRD) of GdOOH. The material (GdOOH) was obtained by annealing Gd(OH)$_3$ nanorods at 300 °C for 6 hours (JCPDS Card No. 075-3267). The broad peak seen at lower 2θ values corresponds to amorphous silica on which the GdOOH samples were drop casted for obtaining the XRD pattern.
**Figure S3.** UV-Visible spectra of (a) Gd(OH)$_3$ and (b) Gd$_2$O$_3$ in the presence of pristine nanorods (1), ABTS+H$_2$O$_2$ (2) and nanorods+ABTS+H$_2$O$_2$ (3) after 15 minutes of incubation at 37 °C.

The obtained UV-Visible spectra confirm that both nanorods were capable of oxidizing ABTS to green color product in presence of H$_2$O$_2$ with inherent maximum absorbance at *ca.* 420 nm.

Further, the control experiments reveal that the reactions do not proceed either in the absence of nanorods or H$_2$O$_2$. 
Figure S4. Relative peroxidase-like and oxidase-like nanozyme activity in the presence of different colorimetric substrates after 30 min of reaction. Peroxidase activity refers to experiments performed in the presence of $\text{H}_2\text{O}_2$, whereas the oxidase activity refers to those performed in the absence of $\text{H}_2\text{O}_2$.

The obtained results suggest that the Gd-based nanomaterials only possess intrinsic peroxidase-like nanozyme activity.
Figure S5. N$_2$ adsorption-desorption isotherms for Gd(OH)$_3$ and Gd$_2$O$_3$ nanorods.
Figure S6. Relative nanozyme activities of Gd(OH)₃ (1), Gd₂O₃ (2) nanorods, leached solution from Gd(OH)₃ (3) and leached solution from Gd₂O₃ (4) as a function of time.

The results of this study demonstrate that the observed oxidation of peroxidase substrate (ABTS) is directly related to Gd(OH)₃ and Gd₂O₃ nanorods, and not due to any potentially leached metal ions.
Figure S7. (a) Gd(OH)$_3$ catalyst stability over 5 catalytic cycles, (b) TEM image of the nanorods after exposure to five consecutive catalytic cycles, (c) XRD patterns obtained from the Gd(OH)$_3$ nanorods post-catalysis, and (d) Gd ion concentration obtained from different samples.

The results suggest that the catalytic activity of the Gd(OH)$_3$ nanorods is retained even after the catalyst is used for five cycles with no change in the morphology or phase. AES studies suggest that the Gd(OH)$_3$ nanorods remain stable in the acidic environment. To ascertain that the Gd ions do not leach, the samples were incubated in acidic media (pH 4.0) for 30 min and the samples were spun at high speeds to obtain the nanorod pellet. The Gd ion concentration was determined from the pellet. A similar protocol was used to obtain the pellet after five catalytic cycles.
**Figure S8.** Michaelis-Menten (a & b) and Lineweaver-Burk (c & d) plots for Gd(OH)$_3$ and Gd$_2$O$_3$ nanorods. The reaction was performed using 10 mM sodium acetate buffer at 37 °C and the concentration of Gd-based nanorods was maintained at 0.5 mM. The concentration of H$_2$O$_2$ was 10 mM during the variation of ABTS concentration (0-4 mM) whereas 2.5 mM ABTS concentration was kept during H$_2$O$_2$ variation (0-500 mM).

The enzyme-like behavior of Gd(OH)$_3$ and Gd$_2$O$_3$ nanorods was confirmed by fitting the obtained data in two enzyme kinetic models namely, Michaelis-Menten and Lineweaver-Burk plot.
Figure S9. Relative peroxidase-mimic nanozyme activity of Gd(OH)$_3$ nanorods as a function of time in the presence of (1) pristine nanoparticles, (2) nanoparticles + H$_2$O$_2$ + ABTS, (3) nanoparticles + H$_2$O$_2$ + ABTS + cysteine and (4) H$_2$O$_2$ + ABTS + cysteine.
**Figure S10.** Time dependent suppression in relative activity of Gd(OH)$_3$ nanorods as a function of L-Cys concentration. The concentration of Gd(OH)$_3$ nanorods, ABTS and H$_2$O$_2$ was maintained at 0.5 mM, 2.5 mM and 20 mM, respectively, while varying L-Cys concentration (0, 50, 100 and 200 µM).

The introduction of L-Cys leads to a suppression in peroxidase-like nanozyme activity in Gd(OH)$_3$. The time dependent kinetics reveals that this suppression in the activity is temporary and the retention time for suppressed activity is dependent on the concentration of L-Cys.
**Figure S11.** The change in the fluorescence (Fl) spectra of terephthalic acid (TA) under different experimental conditions.
Figure S12. Specificity of nanozyme sensor towards L-Cys at a fixed amino acid concentration of 200 µM. The concentration of Gd(OH)$_3$ nanorods, ABTS and H$_2$O$_2$ was kept at 0.5 mM, 2.5 mM ABTS and 20 mM, respectively. The relative activity was calculated by converting the absorbance value in the absence of amino acid (No AA) as 100%.

This study shows that even at higher concentration of amino acids, the developed sensor retains its specificity towards L-Cys.
Table S1. Steady state kinetic parameters for Gd(OH)$_3$ and Gd$_2$O$_3$ nanorods.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Gd(OH)$_3$</th>
<th>Gd$_2$O$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ABTS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_m$ / mM</td>
<td>3.28</td>
<td>1.27</td>
</tr>
<tr>
<td>$V_{max}$ / mM s$^{-1}$</td>
<td>$2.5 \times 10^{-5}$</td>
<td>$1.2 \times 10^{-5}$</td>
</tr>
<tr>
<td>$K_{cat}$ / s$^{-1}$</td>
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<td>$0.58 \times 10^{-4}$</td>
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<tr>
<td><strong>H$_2$O$_2$</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_m$ / mM</td>
<td>16.11</td>
<td>28.51</td>
</tr>
<tr>
<td>$V_{max}$ / mM s$^{-1}$</td>
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<td>$3.5 \times 10^{-5}$</td>
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<tr>
<td>$K_{cat}$ / s$^{-1}$</td>
<td>$4.6 \times 10^{-4}$</td>
<td>$1.7 \times 10^{-4}$</td>
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**Table S2.** Standard reduction potential for Cysteine, H$_2$O$_2$ and ABTS

<table>
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<tr>
<th>Reaction</th>
<th>$E^0$/V (vs. SHE)</th>
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<tbody>
<tr>
<td>2 Cysteine $+ 2H^+ + 2e$ $\rightarrow$ Cystine</td>
<td>-0.34</td>
</tr>
<tr>
<td>H$_2$O$_2$ $+ 2H^+ + 2e$ $\rightarrow$ 2H$_2$O</td>
<td>1.76</td>
</tr>
<tr>
<td>ABTS $+ 2H^+ + 2e$ $\rightarrow$ ABTS$^{2-}$</td>
<td>0.67-1.08</td>
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Soft exfoliation of 2D SnO with size-dependent optical properties

Tin monoxide, SnO, is an important p-type semiconductor with applications across photocatalysis (water splitting) and electronics (transistors). In this work, we devised a novel soft exfoliation strategy to produce SnO nanosheets with tunable optical and electrical properties. Our approach involved the initial synthesis of layered SnO microspheres, which could then be readily exfoliated through a low-power sonication step to form high quality SnO nanosheets, even up to monoatomic layer thickness. This resulted in a remarkable band gap widening (>1 eV) in atomically-thin SnO. As verified through in-depth optical absorption and photoluminescence studies, we demonstrated a strong size-dependent quantum confinement effect in 2D SnO that allows controllable tenability of the opto-electronic properties of this technologically-important material.

Two-dimensional (2D) materials have recently emerged as a novel class of compounds with unique electrical, optical and mechanical properties [1]. They constitute a paradigm shift in the way we conceive and design nanomaterial-based devices, and they are predicted to revolutionize current electronic device technologies [2]. Following the pioneering work on both mechanically exfoliated [3] and liquid-phase exfoliated graphene [4], many 2D materials have been synthesized, including transition metal dichalcogenides (TMDC, such as MoS$_2$, MoSe$_2$, WS$_2$, WSe$_2$, NbS$_2$) [5, 6], metal oxides and hydroxides (such as MoO$_3$, MnO, WO$_3$, Ni(OH)$_2$ and many ternary and quaternary compounds) [7, 8], metal nitrides and carbides (such as hexagonal BN and the family of compounds known as MXenes) [9], elemental compounds (including phosphorene, antimonene and silicene) [10, 11], and cesium lead halide perovskites [12].

SnO is an important $p$-type semiconductor, with very interesting optoelectronic properties, including a small indirect band gap of $\approx 0.7$ eV, a much larger direct band gap of $\approx 2.7$ eV, and high hole mobility [19, 20]. SnO is also a rare example of an oxide semiconductor.
in which ambipolar conductivity can be obtained, as it simultaneously offer reasonably large electron affinity (3.7 eV—suitable for n-doping) and reasonably small ionization potential (4.4 eV—suitable for p-doping). Due to these properties, SnO has been used to fabricate high mobility $p$-type [19, 21] and ambipolar [22] thin film transistors, efficient photocatalysts for water splitting [23], anodes for lithium ion batteries [24], and, just recently, improved anodes for sodium ion batteries [25]. Moreover, bulk SnO has a tetragonal crystalline structure which is layered along the (0 0 $z$) crystallographic direction. The layered nature of the crystalline lattice makes SnO highly promising as a 2D material [26]. However, despite its scientific and technological importance, SnO has hardly been reported in a 2D form. Only a few studies have shown the fabrication of 2D nanosheets of SnO using either time-consuming hydrothermal and intercalation treatments [21, 25], or using expensive and lengthy vacuum-based methods such as pulsed laser deposition [23]. Liquid phase exfoliation methods are promising thanks to their inherent simplicity, and as such have been employed for the synthesis of many 2D materials, although they usually require lengthy and energy-intensive ultrasonication steps [27, 28]. In this study we present the ‘soft’ liquid phase exfoliation strategy, which is both reproducible and facile. Firstly, we developed and optimized a robust method for preparing layered SnO microspheres through the thermal decomposition of a tin oxide hydroxide intermediate at moderate temperatures (see methods section). Briefly, a tin precursor (tin(II) 2-ethylhexanoate) is first reacted with triethylamine at room temperature (see FTIR in supporting information, figure S1 (stacks.iop.org/TDM/4/025110/mmedia)). This mixture is then injected into water, causing the instantaneous precipitation of tin oxide hydroxide (Sn$_6$O$_4$(OH)$_4$) as a white powder. Subsequent heating of this powder in an ambient environment at 180 °C causes its thermal decomposition into SnO in the form of a black powder (figure 1), as confirmed by thermogravimetric analysis (TGA) and x-ray diffraction (XRD). TGA shows ~4.2% weight loss which corresponds to the full dehydration and decomposition of the initial oxide hydroxide to SnO (supporting information, figure S2). XRD also shows full conversion of Sn$_6$O$_4$(OH)$_4$ (ICDD No. 84-2157) to SnO (ICDD No. 85-0423) after annealing at 180 °C (figure 1). All diffraction peaks detected in the annealed sample can be ascribed to tetragonal SnO, and no residual impurities coming from the initial oxide hydroxide phase could be detected. Scanning electron microscopy (SEM) images of the as-synthesized SnO microspheres shows a clear change in morphology from featureless powders to porous layered microspheres with diameters in the range of 5–20 μm (figures 1(b)–(e) and supporting information, figure S3). We note that a similar morphology of layered SnO microspheres was recently
obtained through a biomimetic approach [29]. SEM analysis of our SnO microspheres indeed confirms their lamellar structure, which is conducive to the formation of thin SnO nanosheets upon subsequent exfoliation.

The as-prepared SnO microspheres are then dispersed in common solvents (including ethanol and N,N-Dimethylformamide, DMF), and through a simple sonication treatment for a short period of time (1–5 min) in a standard bench-top ultrasonic bath a yellow colloidal suspension of exfoliated nanosheets is produced (figure 1(a)). This facile exfoliation is in stark contrast with other commonly employed liquid-phase exfoliation techniques currently used to synthesize 2D materials, which involve lengthy (several hours) and powerful (hundreds of Watts) ultrasonication treatments [28]. The ability of our method to easily exfoliate SnO nanosheets is ascribed to the distinctive lamellar morphology of the synthesized SnO microspheres. XRD analysis of the exfoliated sample deposited on glass substrate shows pure SnO phase, with a preferential orientation along the (002) direction, as evidenced by the increase in relative intensity of the (001) and (002) diffraction peaks, and the strong reduction in the intensity of the (110) and (220) peaks (figure 1(a) and supporting information, figure S4). This suggests that the exfoliated nanosheets lay flat on the substrate exposing their (002) surfaces parallel to the substrate. Calculation of the texture coefficient and of the alignment factor (see supporting information, table S1) shows that the extent of orientation is greater than 55% in the exfoliated sample [30]. Conversely, the as-synthesized SnO microspheres, although layered, are randomly oriented during XRD measurements, as expected.

To further analyze the morphology of the exfoliated SnO, we carried out SEM analyses of drop-casted samples of the nanosheet suspensions (figure 1(f) and supporting information, figure S5). SnO nanosheets can be clearly seen, with lateral dimension predominantly in the 0.5–1 μm range, consistent with the average lateral size of the platelets composing the initial SnO microspheres (figure 1(e)). However, both larger (2–5 μm) and smaller (<500 nm) nanosheets are also observed (as also evident later from TEM images). It should be noted that the smaller nanosheets may result from the fracture and/or further exfoliation of the SnO platelets during sonication. The different electron contrast of the nanosheets in the electron microscopy images is indicative of their different thicknesses.

As discussed earlier, XRD analysis of the exfoliated material showed the absence of additional species other than SnO (as for example, metallic Sn or SnO2). To ensure that no surface oxidation/reduction of SnO occurred during the exfoliation process, the chemical nature of the surface of exfoliated SnO was probed using x-ray photoelectron spectroscopy (XPS, figure 1(g)). The core level spectrum of Sn 3d shows the characteristic Sn 3d5/2 and Sn 3d3/2 splitting components (spin–orbit splitting of 8.5 eV) with sharp peaks observed at binding energies of 486.1 and 494.6 eV, which correspond to Sn2+ in pure tetragonal SnO [31]. Additionally, the O 1s spectrum shows two components with the lower energy feature corresponding to oxygen atoms within the crystalline lattice, while the higher energy component corresponds to surface oxygen atoms, including hydroxyl groups and adsorbed organics [32, 33]. All these values are in good agreement with literature data for crystalline SnO [31, 34].

Transmission electron microscopy (TEM) was also used to characterize the morphology and crystallinity of the exfoliated SnO. In agreement with SEM, nanosheets with lateral dimension ranging between hundreds of nanometers and few micrometers are readily observed (figure 2). In addition, both thick (with high electron contrast) and thin nanosheets (with low electron contrast) can be detected. High resolution TEM images of a single flake (figures 2(d)–(f)) show highly ordered atomic structures, as also evidenced from the extremely regular fast Fourier transform (FFT) and selected area electron diffraction (SAED) patterns. All spots observed in these patterns could be readily assigned to the [001] zone axis of tetragonal SnO, showing predominant distances between lattice planes of 2.71 Å and 1.91 Å, which can be indexed to the (110) and (200) planes respectively. Collectively, these results confirm the highly crystalline nature of the exfoliated SnO, and corroborate the XRD results showing that the nanosheets, which lay flat on the TEM grid, primarily expose the (002) family of lattice planes.

To gain further insights into the quality of exfoliated SnO, we used Raman spectroscopy (figure 3). SnO typically has two main Raman peaks, albeit weak, at 113 and 211 cm−1, corresponding to the B1g and A1g vibrational modes, respectively (see also supporting information, figure S6) [35]. These peaks are readily observed in both the as-synthesized SnO (layered microspheres) and in the exfoliated nanosheets. The absence of any additional peaks that may arise from impurities or decomposition products establishes that the soft exfoliation strategy proposed in this study does not influence the intrinsic material properties of SnO. However, Raman analysis of a number of individual SnO microspheres and exfoliated flakes revealed a change in the relative intensity of the $B_{1g}$ and $A_{1g}$ peaks between microspheres and nanosheets (supporting information, figure S7). Specifically, the $A_{1g}/B_{1g}$ ratio is greater than 1 in thin nanosheets (typically in the range of 1.5−2.4, even if sometimes the $B_{1g}$ peak is almost suppressed), while it is smaller than 1 in microspheres (typically between 0.6 and 0.9). This distinctive behavior of few-layered SnO with respect to bulk has also been observed recently for vacuum-deposited SnO [21]. This evidence may indicate that the relative intensities of these two Raman modes could be used to distinguish SnO nanosheets from bulk SnO. However, we note that Raman modes of highly anisotropic structures, and especially 2D materials, have been shown to be sensitive to light polarization and crystal orientation, and therefore the thickness of the nanosheets might not be the only fac-
observed (figures 3(f) and (g)), possibly resulting from incomplete exfoliation of the layered microspheres. In addition, we observed a progressive decomposition affecting the ratio between $A_{1g}$ and $B_{1g}$ peaks \[36\]. In order to further investigate the properties of 2D SnO according to its dimensionality, we separated different populations of nanosheets by applying controlled centrifugation conditions (see the methods section) as reported previously for other 2D materials \[39, 40\]. While it is typically difficult to precisely separate 2D materials according to their dimensions (thickness and lateral size) using centrifugation-based methods, these methods do allow a series of samples of progressively reduced dimensions to be isolated when increasing the centrifugation speed. All the samples obtained at different centrifugation speeds were initially analyzed using TEM (supporting information, figure S10). As expected, SnO nanosheets isolated at low centrifugation speeds are typically larger and thicker, as evident from their high electron contrast in the TEM images. However, very thin nanosheets (with low electron contrast) are also observed in these samples. By increasing the centrifugal force, larger and thicker, i.e. heavier flakes are progressively eliminated from the solutions until only very thin and small nanosheets remain. These are sometimes very hard to distinguish because they tend to wrinkle and wrap around each other, and their electron contrast is very similar to the underlying carbon coating of the TEM grid, which is an indication of their near-atomic thicknesses. AFM measurements on SnO flakes revealed that in general, the flakes obtained at low centrifugation speeds (1000–2000 rpm) were highly polydisperse, with thickness up to ~40 nm, while those obtained at high speeds (12 000 rpm) were predominantly within the 2–5 nm range of thicknesses, which corresponds to 4–10 atomic monolayers (additional AFM images in supporting information, figure S11).

To prove that the AFM images are indicative of SnO, and therefore to exclude the presence of contaminants that could affect the surface morphology of the samples, we performed Raman and AFM analyses on the same spot (figure S9 in the supporting information). We detected SnO Raman signals in correspondence of the nanosheets observed in the AFM, confirming that the structures observed in the AFM images are indeed SnO nanosheets.

Atomic force microscopy (AFM) was also used to characterize the SnO nanosheets. Figure 3(d) shows an AFM image of a single flake with a lateral size of approximately 0.5 μm and a height of 4.5–5 nm, as confirmed by a line scan (figure 3(e)). Considering that the theoretically expected thickness of a SnO monolayer is 0.484 nm, we can deduce that the flake is composed of ~10 layers of SnO \[26\]. However, thinner and smaller flakes can also be observed, with height below 2 nm, which correspond to ~4 layers of SnO. These thin flakes could potentially show distinctive properties due to quantum confinement, as already reported for other 2D materials including phosphorene \[37\] and MoS$_2$ \[38\]. Thicker (30–40 nm) SnO platelets are also observed (figures 3(f) and (g)), possibly resulting from incomplete exfoliation of the layered microspheres.

The prominent yellow color of the exfoliated SnO can become progressively less scattering and less colored. By increasing the centrifugation speed, the solutions obtained at low speeds (1000–2000 rpm) were highly polydisperse, with thickness up to ~40 nm, while those obtained at high speeds (12 000 rpm) were predominantly within the 2–5 nm range of thicknesses, which corresponds to 4–10 atomic monolayers (additional AFM images in supporting information, figure S11).

After confirming the reduction in size of SnO with increasing the centrifugal force applied to the sample, we assessed the optical properties of 2D SnO as a function of the centrifugation speeds (figure 4 and supporting information, figure S12). At low speeds, solutions are yellow in color and slightly scattering, corroborating the presence of large and/or thick flakes within the suspension. By increasing the centrifugation speed, the solutions become progressively less scattering and less colored. The prominent yellow color of the exfoliated SnO can be ascribed to its bulk direct band gap with an associated energy of ~2.8 eV, as confirmed by the Tauc plots presented in the Supporting Information in figure S13.

The black color of the as-synthesized powders (SnO microspheres) is due to the low indirect band gap of SnO (~0.7 eV) \[19, 20\], which is predominant for bulk,
micrometer-sized materials, and becomes negligible for dilute nano-sized materials.

By measuring the absorbance of SnO samples both in standard configuration and within an integrating sphere (see methods), and by analyzing the tin concentration in each individual solution using atomic emission spectroscopy (AES) (table S2), we obtained absorption, extinction, and scattering plots as a function of wavelength. Briefly, the extinction spectra are obtained from standard transmittance ($T$) data using the well-known Lambert–Beer law in the form $T = 10^{-c \varepsilon l}$, where $\varepsilon$ is the molar extinction coefficient, $C$ is the molar concentration of SnO, and $l$ is the cuvette path length. However, the extinction data encompasses the contribution from both absorption ($\alpha$) and scattering ($\sigma$), according to the relationship $\varepsilon(\lambda) = \alpha(\lambda) + \sigma(\lambda)$, where $\lambda$ denotes the wavelength dependence of these functions [40, 41]. By inserting the SnO samples within an integrating sphere we also measured the pure absorptive component of the samples. The scattering contribution is then evaluated by simply subtracting the absorption from the extinction. As expected, the scattering component decreases with increasing the centrifugation speed, due to the progressive removal of larger sheets from the suspensions. The direct band gap component of SnO is observed at ~2.8 eV in the samples centrifuged up to 6000 rpm, but it cannot be detected when applying higher centrifugation speeds. Interestingly, another sharp absorption edge can be identified at higher energies (~4.1 eV) in all samples, which seems rather independent from centrifugation speed. This distinctive UV absorption, which is supported by recent density functional theory (DFT) calculations, is ascribed to band gap widening in quantum confined, atomically thin SnO sheets [42].

The optical absorption characteristics as a function of centrifugation conditions are further validated by photoluminescence (PL) measurements (figures 5 and S14). All samples show two main emission peaks, one centered at 313 nm (3.97 eV, peak 1) and another one centered at 407 nm (3.05 eV, peak 2). The first peak can be ascribed to the band edge emission of quantum confined sheets, corresponding to the sharp absorption observed at 4.1 eV, while the second peak, which is much broader, is possibly due to surface defects and trap state emissions that are commonly observed in many semiconducting nanomaterials [43–45]. Similar PL spectra of nanostructured SnO showing two separate

**Figure 3.** (a) Raman spectrum of a SnO flake. (b) Raman map and (c) corresponding optical microscope image of a typical SnO flake. (d) AFM image of thin SnO nanosheets. (e) Height profile scan along the green and blue dashed lines in (d). (f) AFM image of a thick SnO nanosheet. (g) Height profile scan along the green and blue dashed lines in (f).
high energy component (4 eV), applied to our SnO samples both techniques identify a radiative relaxations and trap states. However, when they are also affected by other parameters such as non-representative of the recombination of the excitons, but and dielectric screening. Conversely, PL spectra are representative of size-dependent variation in exciton binding energies transitions in highly quantum confined materials, due to semiconductors, it fails to precisely identify excitonic band gap value. Specifically, while the Tauc analysis is range (2.75–2.9 eV), which is ascribed to the blue range —(2.75–4.1 eV), ascribed to highly quantum confined SnO, and a lower energy component (2.75–2.9 eV), which is consistent with literature values for the direct band gap transition in bulk SnO.

Overall, these optical measurements support (i) the presence of highly quantum confined SnO thin sheets in all samples, and (ii) the additional presence of thicker nanosheets and platelets in samples separated at low centrifugation speeds, which are responsible for the lower energy absorption/emission components, and for the scattering contribution.

Given the observed variations in optical properties according to centrifugation speed, we further explored the energy levels of SnO through the use of photoelectron spectroscopy in air (PESA, supporting information, figure S15). This technique enables to measure the ionization potential of materials, and to evaluate the work function and the energy level associated with the valence band maximum (for metallic and semiconducting samples, respectively) [47]. The layered SnO microspheres have a valence band maximum of 4.4 eV, in line with both theoretical [42] and experimental [20] reported values for SnO. The exfoliated SnO samples show a very similar valence band maximum at 4.4–4.5 eV. Notably, at higher centrifugation speeds the top of the valence band is seen to shift towards deeper values, especially in the samples centrifuged at 8000 rpm or above, although the signal-to-noise ratio in these samples is rather low due to the small amounts of material probed. Optical absorption measurements show that these samples do not contain thick flakes that would have otherwise been responsible for optical absorptions in the visible range, but they all possess a wide band gap associated with quantum confined sheets. In light of this, we can conclude that thick exfoliated flakes show bulk-like properties (direct band gap energy and valence band maximum similar to micron-sized SnO), while highly quantum confined sheets show a wider band gap and a deeper valence band maximum, which is consistent with recent theoretical predictions for atomically thin SnO nanosheets [42]. These thin SnO nanosheets are responsible for the distinctive optical and electronic properties observed in the samples separated at high centrifugation speeds.

To assess the potential applicability of 2D SnO as a semiconducting material for optoelectronics, and to further demonstrate the different properties of bulk-like and quantum confined nanosheets, we evaluated the electrical response of the SnO flakes under photo-excitation. Two sets of SnO nanosheets were separated at either 1000 rpm or 12,000 rpm and were deposited between two pre-patterned Au electrodes as described in the methods section. As presented earlier, samples separated at high centrifugation speeds are composed of quantum confined nanosheets which can absorb only UV light with wavelengths shorter than ~300 nm (estimated band gap >4 eV). Conversely, exfoliated SnO nanosheets separated at low centrifugation speeds also show a prominent absorption at ~2.8 eV, corresponding to the bulk direct band gap. These nanosheets can absorb light in the blue region, with wavelengths up to ~450 nm. Therefore, the photoresponse of the ‘thick’ flakes is expected to be radically different compared to that of the thin, quantum contributions—a sharp UV peak and a broad peak in the blue range—have also been observed recently [46]. Interestingly, at low centrifugation speeds the broad emission peak in the visible region shows an additional low energy contribution centered in the 425–450 nm range (2.75–2.9 eV, peak 3), which is ascribed to the bulk direct band gap discussed earlier. We note that the evaluation of the band gap using both the absorption edge and the PL peak is an approximation of the actual band gap value. Specifically, while the Tauc analysis is fairly accurate for band-to-band transitions in bulk semiconductors, it fails to precisely identify excitonic transitions in highly quantum confined materials, due to size-dependent variation in exciton binding energies and dielectric screening. Conversely, PL spectra are representative of the recombination of the excitons, but they are also affected by other parameters such as non-radiative relaxations and trap states. However, when applied to our SnO samples both techniques identify a high energy component (4–4.1 eV), ascribed to highly quantum confined SnO, and a lower energy component (2.75–2.9 eV), which is consistent with literature values for the direct band gap transition in bulk SnO.

Figure 4. (a) Digital photograph of the SnO suspensions centrifuged at different speeds (1000 rpm to 12,000 rpm, left to right). (b) Absorption, extinction and scattering plots for the sample centrifuged at 2000 rpm. (c) Absorption, (d) extinction and (e) scattering plots as a function of centrifugation speed. (f) and (g) Linear-logarithmic plots of absorption, extinction and scattering coefficient at two fixed wavelengths as a function of centrifugation speed.
Confined nanosheets. Figure 6 shows the absorption spectra of the two families of SnO nanosheets, together with the emission spectra of the UV (peak at 280 nm) and blue (peak at 455 nm) LEDs that we used to probe the optoelectronic responses of SnO devices. Thicker nanosheets separated at 1000 rpm can absorb light emitted by both LEDs, while quantum confined SnO obtained at high centrifugation speeds can only absorb the high energy UV radiation. It was observed that the current through the device comprising the thick flakes increased during exposure to both light sources (figure 6(b)), while in the case of the quantum confined nanosheets, the current increased only on exposure to UV light and no discernible response was seen under blue light excitation (figure 6(d)). Moreover, the photocurrent measured when a thick flake is exposed to UV light is higher compared to that measured when the same flake is exposed to blue light (~60% increase compared to the baseline value under UV, with respect to ~30% increase under blue light). This is in agreement with the lower energy associated with visible light compared to UV light, and the lower absorption cross section of SnO at visible wavelengths (see figure 6(a)). These results correlate well with the optical properties presented earlier and highlight the distinctive change in the intrinsic characteristics of 2D SnO when it reaches the quantum confinement regime.

Conclusion

In conclusion, we have presented a new ‘soft’ exfoliation strategy to successfully exfoliate SnO nanosheets composed of <10 atomic monolayers. This strategy relies on producing highly layered SnO microspheres using tin (II) 2-ethyl hexanoate and triethylamine. This morphology enables the fast and easy exfoliation of SnO nanosheets through simple sonication at low powers and in common solvents such as ethanol. Highly crystalline nanosheets with lateral size ranging between hundreds of nanometers and few micrometers, and thickness as low as 2 nanometers are readily achieved. Controlled centrifugation is then applied to separate different populations of nanosheets, which possess size-tunable optical and electronic properties, including widening of the band gap, indicating a strong quantum confinement effect in 2D SnO. Photoconductivity measurements demonstrate a strong correlation between the quantum-confined properties of thin SnO nanosheets and their selective photoelectrical response in the high energy UV light, therefore indicating the potential of few monolayer thick SnO for optoelectronic applications under specific UV light conditions. These results highlight the importance of carefully designing the starting materials that can then be easily exfoliated to produce their 2D counterparts. The strategy proposed here constitutes a step forward to developing reliable and scalable methods for the production of 2D materials, which can then be used for the fabrication of next-generation (opto)electronic devices.

Methods

Materials

Tin (II) 2-ethyl hexanoate (SnHex, 92.5–100%), triethylamine (TEA, >99%) and N,N-dimethylformamide (DMF, anhydrous 99.8%) were supplied by Sigma Aldrich. Ethanol (>99.5%), acetone (>99%), and diethyl ether (>99.5%) were purchased from Chem-Supply.

SnO synthesis

In a standard reaction, SnHex (5 ml) and TEA (2 ml) are mixed at room temperature and vigorously stirred for 2 min triggering a mild exothermic reaction. Separately, a vial containing 9 ml of deionized (Milli-Q) water is preheated in a water bath at 40 °C and the SnHex-TEA mixture is rapidly injected into the water under stirring.
causing the instantaneous precipitation of tin oxide hydroxide \(\text{Sn}_4\text{O}_7(\text{OH})_4\) in the form of a white powder. After 15 min the flask is removed from the water bath, letting the solid phase sediment at the bottom of the vial. The liquid supernatant is then removed, and the flask is placed in a preheated oven at 180 °C for 60 min causing the conversion of \(\text{Sn}_4\text{O}_7(\text{OH})_4\) to SnO microspheres in the form of a black powder. The resulting powder is then suspended in a mixture of acetone and ethanol and centrifuged at 4000 rpm for 5 min to remove the unreacted precursors and excess organics. This procedure is repeated 3 times, and then an additional wash cycle is done with diethyl ether. The obtained SnO powders are dried overnight in vacuum and stored in the dark.

Exfoliation of SnO powders is conducted by suspending 160 mg of the synthesized powders in 40 ml ethanol and by sonicating the suspension in a benchtop ultrasonic bath (Unisonics FXP4M with operating power of 40 W) for 5 min. Within a few seconds, the suspension becomes yellow and slightly scattering, confirming the successful exfoliation of SnO. The suspension is then centrifuged at 500 rpm for 5 min to remove any unexfoliated powder, and the supernatant is split into seven tubes and centrifuged at different speeds (1000, 2000, 4000, 6000, 8000, 10 000 or 12 000 rpm for 5 min). Each tube, only the supernatant is kept and the pellet is discarded. The obtained samples are then stored in the dark for future use.

Characterization techniques

Unless specified differently, all characterizations were performed on samples deposited on silicon substrates by simply drop-casting the SnO nanosheet solutions and letting the solvent evaporate naturally. XRD patterns were collected on samples deposited on glass substrates using a Bruker D4 Endeavor diffractometer equipped with a Cu-Kα radiation source and operated at 40 kV and 35 mA. TGA of powder samples was conducted on a Perkin Elmer Pyris 1 instrument at a heating rate of 5 °C min⁻¹. SEM images were acquired on a FEI Nova 200 NanoSEM operated at 10 kV and 30 pA. TEM images were acquired on a Jeol 1010 microscope operated at 100 kV. High resolution (HR) TEM images were acquired on a Jeol 2100F microscope operated at 80 kV. For TEM analyses, samples were prepared by depositing a drop of the nanosheet solutions on a holey carbon-coated copper grid. XPS was conducted on a Thermo Scientific K-Alpha instrument using an Al Kα x-ray source (1486.7 eV). Samples were deposited on Au-coated Si substrates and the core level XPS spectra of the target elements (Sn 3d and O 1s) were obtained and corrected for charging effect. All spectra were corrected using Shirley background correction and resolved using standard Gaussian–Lorentzian functions. Raman spectra were acquired on a Horiba LabRAM HR Evolution Raman spectrometer using a 532 nm laser and power ranging between 0.5 and 50 mW (1–100% of the total laser power) using either pure Si or Si/SiO₂ (300 nm of thermally grown...
oxide) as substrates. AFM images were collected using a Bruker Dimension Icon instrument in tapping mode. The chemical composition of the samples centrifuged at different speeds was analyzed with AES using an Agilent 4200 MP-AES instrument after digesting the solution in concentrated hydrochloric acid. Optical absorption spectra of nanosheet suspensions in the UV–Vis–NIR range were conducted on a Perkin Elmer Lambda 1050 spectrophotometer equipped with an integrating sphere. The extinction spectra of SnO suspensions were acquired in standard configuration, while the absorption component of the extinction was obtained by inserting the cuvettes with the SnO suspensions within the integrating sphere, allowing for the simultaneous collection of both transmitted and scattered radiation. The scattering component was then obtained by subtracting the absorption from the extinction. PES spectra were obtained on a Perkin Elmer LS50 fluorimeter using an excitation wavelength of 280 nm. PES was conducted on a Riken Keiki AC-2 spectrometer using 50 nW radiation on samples deposited on glass substrates. The electrical measurements were conducted on SnO nanosheets drop-casted onto pre-patterned electrodes. The patterns were created on Si substrates covered with 100 nm SiO₂: a photoresist layer was deposited by spin coating and dried at 100 °C, exposed to UV light through a mask using a mask aligner system (MA6, SÜSS Micro-Tech) which defined the patterns, and subsequently developed. The Cr/Au metal electrodes (10/100 nm) were then deposited on the developed patterns using electron beam evaporation. Finally, the lift-off in acetone was carried out to reveal the required metallic contact pads for micro-probes and electrical measurement. The electrical measurements were conducted under ambient air and at room temperature using a Keithley 4200SCS semiconductor parameter analyzer. The optical stimulation is provided by using commercial high power light-emitting diodes (LED, Thorlabs, Inc.). The illumination power was calibrated at commercial high power light-emitting diodes (LED, analytical. The optical stimulation is provided by using commercial high power light-emitting diodes (LED, Thorlabs, Inc.). The illumination power was calibrated at 2.5 mW cm⁻² using a commercial UV-enhanced silicon photodetector (Newport Corporation).

Acknowledgment

MS thanks the Commonwealth of Australia for an Australian Postgraduate Award. EDG, RR and SW thank RMIT University for the Vice Chancellor Research Fellowships scheme. The Australian Research Council is acknowledged for supporting this work through the following grants and fellowships: Future Fellowship (FT140101285, VB), Discovery Project (DP170103477, VB and RR), Discovery Early Career Researcher Award (DE170100164, EDG), Linkage Infrastructure, Equipment and Facilities grant (LE150100001). The authors acknowledge the facilities and the technical assistance of the RMIT University’s Microscopy and Microanalysis Facility (RMMF). The authors would also like to thank the CSIRO for providing access to absorption spectrophotometer and PESA.

Author contributions

MS and EDG contributed equally to this work. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Supporting Information

Soft Exfoliation of 2D SnO with size-dependent optical properties

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Figure S1
Fourier transform infrared (FTIR) spectra of triethylamine (TEA), Tin (II) 2-ethyl hexanoate (SnHex) and of the mixture of the two reacted for 2 minutes. Clear vibrational peaks distinctive of the two precursors are not present in the mixture (marked with *), and concurrently new peaks appear in the mixture of the two (marked with ^). This is indicative of a reaction occurring between TEA and the tin precursor. Specifically, the carbonyl vibration of free carboxylic acid (possibly due to excess ethyl hexanoic acid in the tin precursor) at ~1720 cm\(^{-1}\) disappears when SnHex is reacted with TEA, and at the same time a new peak emerges at ~1580 cm\(^{-1}\) due to ammonium ions vibration. However, this peak is difficult to isolate due to its overlap with the two main vibrational peaks of carboxylate ions at ~1560 cm\(^{-1}\) and 1610 cm\(^{-1}\).

Figure S2
Thermogravimetric analysis (TGA) of the as-synthesized Sn\(_6\)O\(_4\)(OH)\(_4\). a) TGA curve obtained with a constant heating rate of 5 °C/min, after stabilizing the sample at 70 °C for 30 minutes to remove absorbed moisture. b) TGA curve obtained by increasing the temperature up to 180 °C and holding the sample at this temperature for 3 hours. While the transition from Sn\(_6\)O\(_4\)(OH)\(_4\) to SnO is observed between 200 °C and 230 °C in the conventional TGA measurement (a), a constant decrease in weight is observed at temperatures above 100 °C, possibly due to the slow dehydroxylation of the Sn\(_6\)O\(_4\)(OH)\(_4\). To confirm this, we conducted isothermal TGA at 180 °C (b), which shows constant decrease in weight until the expected value for SnO is reached.
**Figure S3**
a,b) SEM images of Sn₆O₄(OH)₄ powders. c,d) SEM images of SnO microspheres obtained after annealing the Sn₆O₄(OH)₄ powders. e,f) Zoomed view of the SnO microspheres showing the layered morphology.

**Figure S4**
XRD patterns and fitted Lorentzian functions for a) as-synthesized SnO powders and b) exfoliated SnO nanosheets. A clear increase in intensity of the (00z) diffraction peaks and a concurrent decrease in intensity of the (xx0) peaks can be observed in the exfoliated sample with respect to the as-synthesized SnO powders.
**Figure S5**
SEM images of exfoliated SnO nanosheets.

**Figure S6**
Raman spectrum of an exfoliated SnO nanosheet deposited on silicon substrate, showing the two main vibrational modes for SnO and the conventional silicon peak at 520.9 cm\(^{-1}\).
**Figure S7**
Raman spectra of SnO microspheres and exfoliated nanosheets, showing the two main vibrational modes for SnO. The $A_{1g}$ peak at ~211 cm$^{-1}$ in the exfoliated sample is more intense compared to that of the microspheres sample. The ratios of the peak intensities of $A_{1g}$ to the $B_{1g}$ modes in these microspheres and exfoliated SnO nanosheets samples are 0.7 and 1.7, respectively. Spectra have been vertically translated for clarity.

![Raman spectra of SnO microspheres and exfoliated nanosheets](image)

**Figure S8**
Raman spectra of SnO microspheres using different laser powers (532 nm wavelength, with 100% power corresponding to 50 mW). At 10% power (5 mW) and below the samples show the distinctive $B_{1g}$ and $A_{1g}$ vibrational modes of SnO centered at 113 and 211 cm$^{-1}$, respectively. At slightly higher laser powers the $B_{1g}$ mode shifts to lower wavenumbers and the $A_{1g}$ mode is broadened. Concurrently, additional vibrational peaks start to appear. Further increase in laser power causes the complete disappearance of SnO modes and the appearance of new peaks, indicating the decomposition of the starting material.

![Raman spectra of SnO microspheres using different laser powers](image)
Figure S9
Combined AFM/Raman study on the same exfoliated SnO flakes, to confirm that the AFM images are indeed indicative of SnO. Both thin (highlighted by a blue box) and thick (highlighted by a red box) SnO show Raman signatures distinctive of SnO, even if the signal is weak in very thin samples. Notably, large aggregates are still composed of individual SnO sheets with thickness below ~5 nm, as highlighted by the line scans.
Figure S10
Representative TEM images of SnO nanosheets as a function of the centrifugation speed.
Figure S11
AFM images and respective line scans of various SnO flakes separated at 1000 rpm (a,b) and 12000 rpm (c, d). Scale bars are 4 μm (a), 50 nm (b) and 500 nm (c,d). The flake in (c) is composed of three flakes stacked on top of each other, as confirmed by the line scans.
**Figure S12**
Absorbance spectra in standard configuration (dashed lines) and with the cuvette positioned inside the integrating sphere (straight lines) for suspensions of SnO nanosheets separated at different centrifugation speeds. The scattering contribution (difference between the two plots) decreases with increasing the centrifugation speed.

![Absorbance Spectra](image)

**Figure S13**
Tauc plots of SnO nanosheets obtained at different centrifugation speeds. The band edge at ~4.1 eV is observed in all samples and it is ascribed to highly quantum confined sheets, while the absorption at ~2.8 eV appears only on samples obtained at low centrifugation speeds, and it is close to the expected value for the direct band gap of bulk SnO.

![Tauc Plots](image)
Figure S14
Photoluminescence spectra at varying excitation wavelengths for the sample obtained after centrifugation at 4000 rpm. Since no shift in the peak position is observed when varying the excitation wavelength, we can exclude that such peaks are related to Raman modes.

![Figure S14](image)

Figure S15
Photoelectron yield measured with PESA for as synthesized and exfoliated SnO according to the centrifugation speed. The contribution from the glass substrate is also shown as a reference. The onset of photoelectron emission is due to the ionization potential, which for a semiconductor such as SnO corresponds to the top of the valence band.

![Figure S15](image)
Table S1
Texture coefficient $P$ and alignment factor $A$ evaluated from the XRD data presented in Figure S4 for the SnO microspheres and the exfoliated SnO nanosheets.

<table>
<thead>
<tr>
<th>Reference</th>
<th>SnO microspheres</th>
<th>SnO nanosheets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$I_0$</td>
<td>$I$</td>
</tr>
<tr>
<td>001</td>
<td>60</td>
<td>26</td>
</tr>
<tr>
<td>101</td>
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<td>296</td>
</tr>
<tr>
<td>220</td>
<td>33</td>
<td>65</td>
</tr>
</tbody>
</table>

The texture coefficient $P$ of the $(hkl)_i$ diffraction is calculated according to the formula:

$$P (hkl)_i = \frac{I (hkl)_i}{I_0 (hkl)_i} \left[ \frac{1}{n} \sum_{i=1}^{n} \frac{I (hkl)_i}{I_0 (hkl)_i} \right]^{-1}$$

where $I_0$ is the expected intensity for a randomly oriented material (reference card ICDD No. 85-0423), $I$ is the experimental intensity of the specific $(hkl)_i$ peak and $n$ is the total number of peaks taken into consideration. $P$ ranges between 1 (no alignment) and $n$ (perfect alignment). If for a specific diffraction $P$ is $<1$, it means that the peak taken into consideration is suppressed because of an orientation in a complementary direction. In the exfoliated sample it is evident that $P$ is much greater than 1 when calculated in the (00Z) direction, while it is much smaller than 1 when calculated in the orthogonal (XX0) direction.

The alignment factor $A$ can be derived from the texture coefficient according to the following:

$$A (hkl)_{i (\%)} = \frac{P (hkl)_{i} - 1}{n - 1} \times 100$$

and it ranges from 0% (no alignment) to 100% (complete alignment). Similarly to what observed for $P<1$, negative $A$ values are due to crystal texturing in directions different to that taken into consideration. Consistently with the $P$ values, the alignment factor for the exfoliated SnO nanosheets is as high as 55% along the (001) direction, suggesting strong orientation of the nanosheets on the substrate.
Table S2
SnO concentration as a function of centrifugation speed for the samples used for optical absorption measurements, as evaluated using atomic emission spectroscopy.

<table>
<thead>
<tr>
<th>Centrifugation speed (rpm)</th>
<th>SnO concentration mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>85</td>
</tr>
<tr>
<td>2000</td>
<td>44.5</td>
</tr>
<tr>
<td>4000</td>
<td>26.5</td>
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<tr>
<td>6000</td>
<td>25</td>
</tr>
<tr>
<td>8000</td>
<td>23.5</td>
</tr>
<tr>
<td>10000</td>
<td>22</td>
</tr>
<tr>
<td>12000</td>
<td>21.5</td>
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</table>

Supporting References
Chapter 4

Conclusions and scope for future research

This chapter summarises the key findings of the work presented in this thesis and provides scope for potential future endeavors.
This chapter is split into two parts. The first part concludes the research being established in the previously published chapters and prime conclusions thus obtained are discussed in an elaborative manner. The second part provides the insights in the future prospects to be sketched from the present work and utilization of the materials reported in this thesis for various practical applications

4.1 Summary and Conclusions

In chapter 1, I had provided an overview of the previously established research with a context to the following fields:

i) *Synthesis approaches* – here, I discussed both the bottom and top-down approaches. Physical and liquid-based synthesis techniques were discussed. Further, mechanical milling and ultrasonication were discussed among the top-down approaches, whereas precipitation and sol-gel were discussed among the bottom-up approaches in detail.

ii) *Nanomaterial dimensional aspect* – here, I discussed the nanomaterial dimension aspect which includes 0D, 1D, 2D, and 3D materials. A detailed overview regarding the synthesis of various dimensions of nanomaterials was provided along with their application aspects.

iii) *Synthesis parameters* – here, I had included iron oxide nanomaterial as a core example and explained all about the importance of various synthesis parameters to alter the physical and chemical properties of nanomaterials. Parameters like salt molar ratios; solvent ionic strength; reaction temperature; synthesis atmosphere; injection flux rate; reaction time; the role of surfactants; hydrolyzing agent role etc. were discussed in detail.

iv) *Amines* – since the center stage of my Ph.D. research work is on using amines for obtaining various nanomaterials, here I discussed in detail about the amines; their
classification and nomenclature; types of amines; various physical properties of amines which include their melting and boiling points, solubility, odor and toxicity, basicity, etc. Various synthesis methods of amines were discussed; detection of the amines using various spectroscopic techniques and their general application in various industrial and biological fields were also covered.

v) *Triethylamine* – as a shape-directing agent was discussed in detail. Their physical properties were discussed. Since throughout my Ph.D. research work, the nanomaterial that I synthesized had water as one of the constituent systems, so a detailed discussion was provided that showed how this amine interacts with water, and the recent developments that have taken place to obtain an understanding of the interactions with water molecules till date.

Further, in this chapter, after covering the prior art, I provided a summary of the work presented in this thesis and in the context of synthesis methodology for various nanomaterials and their application aspects. Overall, the work presented in this chapter was an attempt to establish a relationship between utilization of the triethylamine for the synthesis of various nanomaterials and the fine-tuning of the crystallite facets w.r.t various reaction conditions like temperature, precursor concentrations etc.

*In chapter 2*, I provided a detailed theoretical overview of the various characterization tools that I had used to characterize my nanomaterials throughout this thesis.

*In chapter 3a*, the work focused on Iron oxide nanoparticles. The prime focus remained on the utilization of tertiary amines for synthesizes of various iron oxide nanoparticles. Apart from this, use of tertiary amines for the instant aqueous phase transfer of organic coated nanoparticles under benign conditions was shown. The synthesis method described here to make various nanoparticles employed precipitation route where the tertiary amines acted as a
hydrolyzing agent for the metal ions and became a fundamental step in obtaining various nanostructures in one step. The as-formed nanoparticles were simultaneously coated by amine molecules which protected them against aggregation and made them dispersible in the aqueous solvents.

Due to magnetic dipole interactions among magnetic nanomaterials, an ongoing challenge has been to make these materials stable in various media for a variety of applications. For this, previous work has explored a number of strategies which include the adsorption of bulky ligands onto the nanoparticle surface or coating of suitable counterions. In most of the previously-employed stabilizing conditions, the final end product compromises the net magnetic properties of the iron oxide nanoparticles which thereof affect their application capability. Thus, the results presented in this chapter addressed an important gap by developing a new highly efficient protocol that allowed enhancing the net magnetization of iron oxide nanoparticles, along with the ability to allow rapid phase transfer of these particles in various aqueous and organic media.

As such, in this research work, triethylamine was used for the first time to synthesize iron oxide nanoparticles in a “One-pot” reaction, stabilizing particles in both the organic and aqueous mediums. Further, it was shown that triethylamine can further be exploited in the “instant single step” phase transfer of the oleic acid-coated iron oxide nanoparticles from organic medium to the aqueous medium. As-synthesized oleate-capped nanoparticles in organic solvent showed remarkable magnetic properties, which were further improved by the triethylamine molecules when they are transferred to the aqueous medium.

The reported phase transfer protocol is not only rapid, it is also functionally efficient, as it improves the net magnetic properties of the oleic acid capped nanoparticles which are the backbone for better hyperthermia heating applications. Till date, most phase transfer methods
have either employed bulky molecules onto the nanoparticle surface or ligand exchange for phase transfer, but in both cases the time for transfer often ranges between 30 minutes to 2-3 days, during which simultaneous deterioration of the magnetic properties of the magnetic nanoparticles take place. In our present case, the rapid phase transfer occurs within 30 seconds along with improved magnetic properties. The phase transfer protocol could also be applied directly to oleic acid-capped iron oxide powders to make them water dispersible in a single step without compromising with their dispersibility. These scalability aspects allow the obtained particles more viable for utilization in larger scales for intended applications. To prove this, we employed the synthesized materials for the hyperthermia application, since this application relies heavily on good magnetic properties of the material.

For hyperthermia application, we exposed the as-synthesized iron oxide nanoparticles stabilized in aqueous and organic mediums to high-frequency AC fields under the biological limits to generate the remote-control heating within the medium due to (i) Brownian relaxation i.e. motion between the nanoparticle and the medium, (ii) Néel relaxation i.e. due to magnetic core fluctuations. Determination of the relative contributions of these relaxations in hyperthermia has remained extremely challenging due to lack of appropriate materials where the nanoparticle core remains unchanged during phase-transfer.

In the work presented in this Chapter, we could effectively distinguish the relative contributions of Brownian and Néel relaxations in magnetic hyperthermia. TEA being a dynamic amphiphilic ligand, allowed the net magnetization of the iron oxide nanoparticle after the phase transfer to increase ~20% from the parent nanoparticles, which allowed improving the hyperthermia efficiencies of the iron oxide nanoparticles. The specific absorption rates (SAR) of these particles increased by 45% for the same set of nanoparticles after they had been transferred to the aqueous medium from the organic phase which could well be attributed to the increase in the net magnetization of the nanoparticles while keeping
the morphological and polydispersity conditions same. Thus, here we concluded that Brownian relaxation seems to play a significant role in enhancing the SAR along with an increase in the net magnetization. Further, we also tested the hyperthermia viability of the phase transferred particles under various biological conditions.

In a nutshell, the research presented in this chapter for the first time, reported the one-pot synthesis of superparamagnetic iron oxide nanocrystals of maghemite phase using TEA as a dynamic molecule that could directly produce SPIONs under mild conditions that were readily dispersible both under aqueous and organic environments. The phase-transferred SPIONs obtained from this new route revealed outstanding magnetic properties, which could then be exploited for a proof-of-concept demonstration in magnetic hyperthermia.

**In chapter 3b**, I discussed the photocatalytic capability of the copper oxide nanoparticles. In general, harvesting of solar energy by the materials especially the semiconductors provide a promising avenue for next-generation green technologies, including the production of clean energy and efficient environmental management. Till now, titanium dioxide (TiO$_2$) is one of the most widely used photocatalysts due to high chemical stability, low-cost and low toxicity. However, this material only responds well under the ultraviolet (UV) spectral range (bandgap 3.2 eV) which accounts for only $\sim$3% of the solar irradiation reaching the earth's surface. This has persuaded researchers to hunt for other suitable materials or in more precise term the catalysts that are visible light active, possess a direct narrow band gap, and have high photochemical stability.

One of the promising materials is cuprous oxide (Cu$_2$O) which is a p-type semiconductor with a narrow direct band gap (Eg of 2.1–2.6 eV). It is an abundance on earth, low cost and with low toxicity along with appropriate positioning of its conduction band just above the reduction potential of water, thus making it suitable as a catalyst for visible light-driven
applications, such as photocatalysis, photovoltaics and photoelectrochemical devices. However, this material is prone to further oxidation and rapidly oxidizes to CuO. To avoid this, researchers have used various surfactants onto its surface to protect it from oxidation but the surfactant itself could lead to the decrease in catalytic activity of the materials. To overcome this challenge, in this chapter, I develop a novel synthesis route to obtain Cu$_2$O nanoparticles in gram scale levels with enhanced photostability over months.

The new synthesis route for the gram-scale synthesis of highly oxygen-deficient Cu$_2$O nanoparticles under ambient conditions took advantage of the stability of the Cu$^{1+}$ ion in organic medium like acetonitrile instead of water, where Cu$^{1+}$ will tend to disproportionate to Cu$^{2+}$ and Cu$^0$. In acetonitrile, by employing TEA, I could initially form a low vapor pressure ionic liquid with Cu$^{1+}$ ions known as Cu(I)–TEA complex. This complex could be stored under ambient conditions over months and one can obtain the fresh Cu$_2$O nanoparticles, just by the addition of water to this complex that allows instantaneous hydrolysis of the complex through protonation of the TEA molecules in the complex. Using this simple method, spherical Cu$_2$O nanoparticles on the gram scale (2.56 g per batch) with over 90% conversion yield from the precursor copper salts could be produced. The obtained nanoparticles were well studied in detail, including the photostability, crystal structure, defect states, and presence of oxygen vacancies by a number of characterization techniques.

I further assessed the prospects of oxygen-deficient Cu$_2$O nanoparticles toward degrading organic environmental pollutants by investigating the photooxidative degradation of dye molecules and tetracycline. In case of tetracycline, in the absence of a catalyst, tetracycline could not be degraded, indicating the importance of a suitable catalyst for its photodegradation. The commercial Cu$_2$O could degrade only 7% tetracycline, whereas the oxygen-deficient Cu$_2$O showed $\sim$90% degradation efficiency within 20 min of visible light irradiation. Convinced with the high photostability and photocatalytic activity of oxygen-
deficient Cu$_2$O nanoparticles, we further evaluated its performance under simulated solar illumination conditions. Under simulated solar illumination, the methyl orange (MO) molecules could be completely degraded within 1 hour, leading to the decoloration of the resultant solution. These observations confirmed that the photostable, oxygen-deficient Cu$_2$O nanoparticles offer remarkable potential as an efficient visible light photocatalyst for the practical harvesting of solar energy. This work also established the importance of oxygen-vacancies/defects in enhancing the photooxidative capabilities of the semiconductor catalysts.

*In chapter 3c*, enzyme mimicking gadolinium-based nanorods for the biosensing applications were reported. Gadolinium-based materials are extensively used as an MRI contrast agent. Thus, they occupy an important place in various therapeutic and sensing applications. Cysteine is an amino acid which contains sulfur and plays an important role in protein synthesis, metabolism, detoxification, and biological signaling. Its deficiencies could lead to a number of ill effects which include liver damage, fat loss, cardiovascular problems, skin lesions etc. Thus, a new material is the need of an hour to detect cysteine in a precise and efficient manner in the presence of other thiolated molecules.

Thus, we synthesised two Gd-based materials viz. Gd(OH)$_3$ and Gd$_2$O$_3$ nanorods and their enzyme-mimicking (peroxidase-mimic) characteristics were noted where hydroxide phase was found to be far superior to the oxide phase. The catalytic activity was highly dependent on the pH, nanomaterial concentration, substrate concentration, and temperature. This has been the first time that gadolinium-based materials have been reported for their intrinsic nanozyme activity. Further, the hydroxide phase nanorods could be used to make highly specific colorimetric “turn-off” sensor for L-Cys detection. The sensor showed a LoD of 2.6 μM and LoQ of 7.98 μM, with a precision of 97.2% while showing 90% accuracy at 5% confidence level and 100% accuracy at 10% confidence level. This work also opens up the possibility of combining the T1 MRI imaging capabilities of Gd-based materials with
nanozyme mediated colorimetric sensing, while emphasizing the need for detailed kinetic investigations of nanozyme activity to realize their full application potential.

In chapter 3d, a novel strategy of obtaining the two-dimensional (2D) SnO nanoflakes was discussed along with their size dependent optical and photoconductivity measurements. Recently, two dimensional (2D) materials have held prime interest due to their unique mechanical, electrical and optical properties in the quantum-confined regime in the z-direction. To obtain such materials, researchers follow both the mechanical exfoliation techniques and the liquid-phase exfoliated technique. However, the liquid phase exfoliated method has an advantage over the mechanical exfoliation due to large-scale production, control of the dimensions along various facets etc. Keeping this in mind, I had followed the liquid phase exfoliation method and develop a new “soft exfoliation” technique to obtain the thin tin monoxide (SnO) 2D layers and studied the size-dependent optical properties. SnO, a p-type semiconductor has a bulk indirect band gap of ~0.7 eV and a larger direct band gap of ~2.7 eV. SnO is in fact one of the rarest semiconductors with ambipolar characteristics. The Bulk SnO has a tetragonal crystalline structure which is layered along the (0 0 z) crystallographic direction, thus it is possible to exfoliate SnO into thin sheets along this direction.

My strategy was to initially synthesize highly layered bulk SnO microspheres, and from these appropriately-oriented particles, I was able to exfoliate thin nanosheets in just a few minutes in ethanol using a standard low-power (40 W) bench-top ultrasonic bath. This is impressive considering that most of the liquid exfoliation synthesis strategies rely on high-power ultrasonication over several hours to days. Further, size-dependent optical studies confirmed the presence of quantum-confined SnO thin sheets, where the thickness population could be controlled using the centrifugation speeds. The photoconductive measurements demonstrated a strong correlation between the quantum-confined properties of thin SnO nanosheets and
their selective photoelectrical response in the high energy UV light, therefore indicating the potential of few monolayer thick SnO for optoelectronic applications under specific UV light conditions. This provides an excellent base of using such 2D SnO thin sheets for the next-generation (opto)electronic devices.

4.2. Scope and Future prospects

A variety of amines have been explored in materials science for applications ranging from hydrolysing agents to capping agents and shape-directing agents. In this context, a major emphasis has previously been placed on primary amines, wherein the short-chain amines have been typically used as a hydrolysing agent, whereas long-chain amines are commonly used as shape-directing and stabilization/capping agents. The role of tertiary amines in materials chemistry has not been hitherto investigated in detail. A key differentiator of the work presented in this thesis is to employ triethylamine as a tertiary amine to explore its role in materials science. The work presented in this thesis, in fact provides convincing evidence that short-chain tertiary amines, such as triethylamine may occupy an important place in controlled synthesis and facile phase-transfer of various materials. I have shown that using this interesting amphiphilic tertiary amine, a number of challenging problems can be addressed – these range from, for instance, synthesis of highly oxygen-deficient Cu$_2$O to 2D SnO. The stabilisation of the lower oxidation state oxides using triethylamine in the synthesis is very promising that allows access to these difficult-to-synthesise oxides nanomaterials.

As a future work, from promising leads noted from my thesis work, it would be worth to continue to investigate the role of tertiary amines, and in particular, triethylamine for the synthesis of other challenging nanomaterials. An important aspect to study will be the mechanistic role of these unique amines in obtaining interesting nanomaterials. Some of my preliminary work not presented in this thesis indicates that the ability of triethylamine molecules to locally crystallise water molecules in a clathrate-type structure provides it with a
unique strength in materials chemistry, but these aspects need to be further investigated in
detail. Synchrotron-based high-resolution techniques such as SAXS might be useful in
shading light to such mechanistic aspects.

It would also be worth looking at the ability of triethylamine to synthesis nanomaterials of
other compositions and morphologies through control of various reaction parameters, such as
temperature, pH, salt concentrations, additives, etc. This may provide next level of control in
obtaining crystals of desirable characteristics. Based on some preliminary investigations, not
presented in this thesis, it may be commented that triethylamine is also able to synthesise
many other oxides such as ZnO, NiO, Co$_3$O$_4$, etc, in 2D morphologies. Another useful
avenue may be to investigate the role of triethylamine in synthesising complex oxides (e.g.
pervoskites) and mono-elemental ‘enes’ (e.g. graphene, phosphorene, antimonene) through
employing both bottom-up and top-down approaches.

Notably, the present research shows some progress in various types of nanomaterials
synthesis for a variety of application like catalysis, magnetic hyperthermia, biosensing, and
optoelectronics. In the future, while triethylamine continues to provide access to unique
materials, it would be worth investigating in-parallel, the relevant application of those new
materials, ranging from biosensing to opto-electronics. Such applications will of course
depend upon the unique properties that these new materials show.

Overall, a significant amount of work still needs to be performed to realize the full potential
of this unique tertiary amine in obtaining different nanomaterials with defined shape, sizes,
composition, and importantly crystal defects. As previously stated, the utilization of different
amines in nanomaterial field is still in a relatively young state, as there are still a lot of
unknowns and challenges that remain to be tackled. It is of paramount importance to explore
the full potential of tertiary amines in materials science and nanotechnology. The work
presented in this thesis focussed on TEA as a tertiary amine to control various aspects of materials synthesis. In the future, it would be interesting to study the effect of mono- and di- amines on the morphological and physico-chemical properties of nanomaterials during synthesis. This is likely to facilitate working with amines in a more controlled manner. Future work in this direction will indeed add significant value to the nanomaterial synthesis field through providing access to hitherto unknown materials with unique properties. Overall as an emerging field of research, there are many challenges to overcome and the work presented in this thesis is a small stepping stone in a new direction.
Appendices
Publication Summary:

**Patents**


**Journal Articles**


