SYNTHESIS, CHARACTERIZATION, AND MODIFICATION OF QUANTUM DOT BASED NANOGELS FOR FORENSIC APPLICATIONS

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

(FORENSIC SCIENCE)

By

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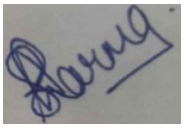
Lovely Professional University, Punjab 2025

DECLARATION

I, hereby declared that the presented work in the thesis entitled "Synthesis, Characterization, And Modification of Quantum Dot Based Nanogels for Forensic Applications" in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision of Dr. Tejasvi Pandey, working as Assistant Professor, in the Department of Forensic Science of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of another investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.



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CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled "Synthesis, Characterization, And Modification of Quantum Dot Based Nanogels for Forensic Applications" submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the Department of Forensic Science, is a research work carried out by Aditi Sharma, Registration No. 12021142, is Bonafide record of his/her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

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Abstract

The development of advanced materials through green chemistry has become a pivotal focus in materials science and engineering. Emphasizing sustainability and environmental friendliness drives the search for innovative methods that minimize the use of harmful substances and reduce ecological footprints. In this context, quantum dots (QDs) have drawn attention due to their exceptional opto-electric properties, making them suitable for a wide range of applications, including photovoltaic devices, biomedical imaging, and environmental sensing. Among various types of QDs, iron sulfide (FeS_2) quantum dots have emerged as promising candidates due to their low toxicity, earth abundance, and cost-effectiveness compared to traditional heavy metals based QDs.

Chitosan, which is a natural polysaccharide derived from chitin, is widely recognized for its biocompatibility, biodegradability, and versatile chemical functionality. Its ability to form films, fibers, and hydrogels makes it an ideal candidate for developing composite materials. The integration of FeS_2 QDs with chitosan could potentially create a composite material that combines the unique properties of both components, resulting in enhanced functionality and broader application potential. The synthesis of nanocomposites through environmentally benign methods has gained traction in recent years. Traditional synthesis routes often involve toxic solvents, high temperatures, and complex procedures that pose risks to the environment as well as to human health. In contrast, green synthetic strategies aim to utilize non-toxic reagents, mild reaction conditions, and sustainable practices. Gel chemistry, a versatile and relatively simple approach, offers a promising pathway for the green synthesis of nanocomposites. By employing gel chemistry, it is possible to control the nucleation and growth of nanoparticles within a gel matrix, leading to uniform distribution and stabilization of the nanoparticles.

FeS_2 QDs are semiconductor nanoparticles that exhibit unique size-dependent optical properties. Their narrow bandgap and high absorption coefficient make them suitable for various optoelectronic applications. Additionally, FeS_2 QDs have shown potential in catalytic applications due to their ability to facilitate redox reactions. The biocompatibility and non-toxicity of FeS_2 QDs further enhance their suitability for biomedical applications,

including bioimaging and drug delivery.

The integration of FeS_2 QDs into a biopolymer matrix such as chitosan can further enhance their properties and expand their application potential. Chitosan provides a supportive and stabilizing environment for the QDs while also imparting additional functionalities such as antimicrobial activity and film-forming ability. This combination can lead to the development of multifunctional materials with applications ranging from medical devices to environmental remediation.

Chitosan is derived from the deacetylation of chitin, which is the second most abundant natural polymer after cellulose. It possesses several advantageous properties, including biocompatibility, biodegradability, and antimicrobial activity. Chitosan's ability to form hydrogels, films, and fibers makes it a versatile material for various applications in biomedical, pharmaceutical, and environmental fields. The presence of amino groups in chitosan allows for easy chemical modification, enabling the development of tailored materials with specific functionalities. The combination of chitosan with nanoparticles, such as FeS_2 QDs, can result in composite materials that leverage the strengths of both components. Chitosan can provide a matrix that stabilizes and protects the QDs, while the QDs can enhance the optical, electronic, and catalytic properties of the chitosan matrix. This synergy can lead to the development of advanced materials with superior performance and multifunctionality.

Gel chemistry involves the use of gel matrices to control the synthesis and stabilization of nanoparticles. This method offers several advantages, including simplicity, versatility, and the ability to operate under mild conditions. In gel chemistry, a gel-forming agent, such as chitosan, is used to create a three-dimensional network that can trap and stabilize nanoparticles. The gel matrix provides a confined environment for the nucleation and growth of nanoparticles, leading to uniform size distribution and preventing aggregation. Gel chemistry is used to synthesize FeS_2 QD-chitosan composites, offering an eco-friendly and scalable approach. By employing aqueous solutions and avoiding the use of toxic solvents, this method aligns with the principles of green chemistry. Additionally, the mild reaction conditions help preserve the inherent properties of both the QDs and the chitosan

matrix, resulting in a composite material with enhanced functionality. The specific objectives of the study include the synthesis of FeS_2 QDs through a green synthesis route utilizing non-toxic reagents and mild reaction conditions, the preparation of a chitosan gel matrix to effectively trap and stabilize FeS_2 QDs, the fabrication of FeS2 QD-chitosan composites by integrating FeS_2 QDs into the chitosan gel matrix, and comprehensive characterization of the synthesized composites to confirm their structural, optical, and physicochemical properties. Additionally, the study aims to evaluate the potential applications of the FeS_2 QD-chitosan composites in biomedicine and environmental remediation, focusing on their biocompatibility, antimicrobial activity, and catalytic efficiency. The study aims to demonstrate the feasibility of using gel chemistry as a green synthetic route for producing functional nanocomposites. By leveraging the unique properties of FeS_2 QDs and chitosan, the developed composites are expected to exhibit enhanced performance and multifunctionality, paving the way for their application in various fields.

The development of FeS_2 QD-chitosan composites through a green synthetic strategy holds significant promise for advancing materials science and engineering. The study addresses the need for sustainable and environmentally friendly approaches to material synthesis, contributing to the broader goals of green chemistry and sustainable development. The successful integration of FeS_2 QDs into a chitosan matrix can lead to the creation of multifunctional materials with applications in biomedicine, environmental remediation, and beyond. The application of these sensor-based devices can be used for preparing sensor-based devices.

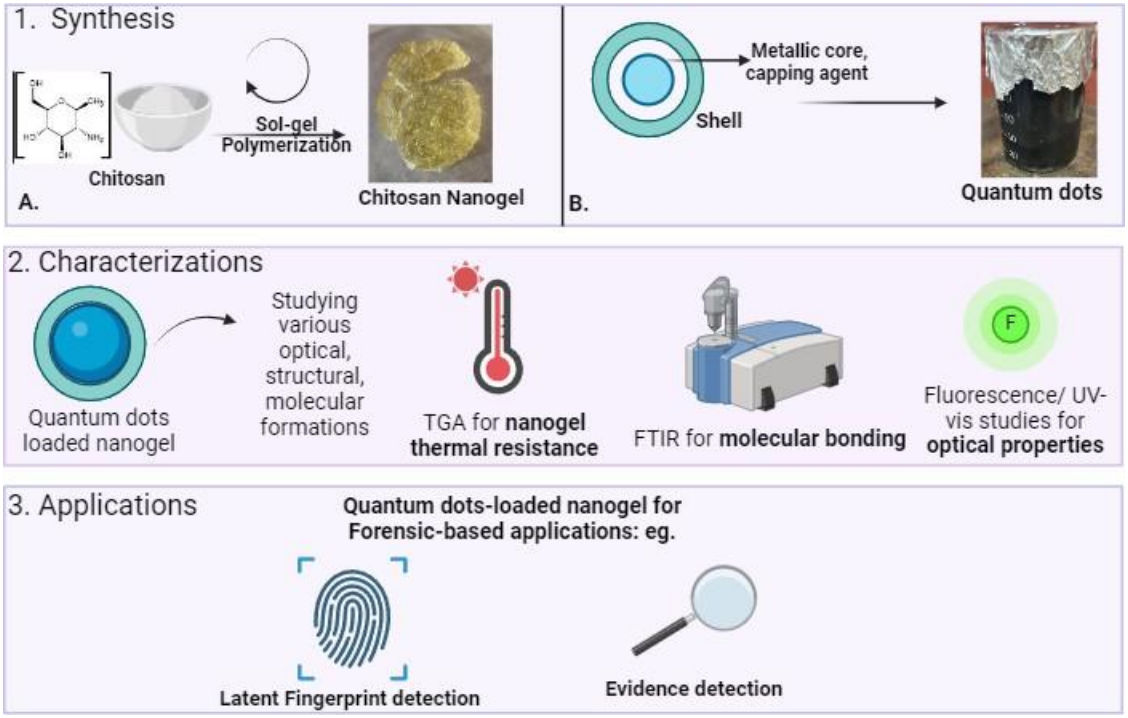

Figure: Graphical abstract of the work.

Acknowledgments

First and foremost, I express my deepest gratitude to **Mahadev** for guiding me throughout this journey. I also pay my heartfelt respects to my grandparents—**the Late Mr. Ravi Dutt Sharma, whose blessings have shaped who I am today; the Late Mrs. Chander Kanta Sharma; and the Late Mrs. Mohini Gautam**, who continue to watch over me from heaven. Their presence and blessings have been a constant source of strength and inspiration.

I sincerely appreciate my supervisor, **Dr. Tejasvi Pandey**, whose invaluable guidance has helped me grow professionally and personally. Her wisdom has been crucial in shaping my research and refining my scientific perspective. I have special thanks to Dr. Vivek Pandey, Mr. Vikas Bhargav, and Late Mr. Hemant Rana, whose mentorship has been instrumental in my academic journey.

I am also immensely grateful to **Dr. Ashok Kumar Mittal (Chancellor), Mrs. Rashmi Mittal (Pro-Chancellor), and Dr. Lovi Raj Gupta (Pro-VC) of Lovely Professional University** for providing an excellent research environment and infrastructure that enabled me to flourish.

I also extend my appreciation to **Professor Neeta Raj Sharma (HOS, School of Bioengineering and Biosciences)** for her consistent guidance and support throughout this work. I am deeply thankful to my thesis committee members, **Dr. Manpreet Kaur, Dr. Kiran, and Dr. Amandeep Kaur**, for their valuable feedback, encouragement, and time, which have significantly contributed to the enhancement of my research. My sincere gratitude also goes to **Mr. Rajesh Bains**, whose unwavering support in the laboratory has been invaluable.

Words fall short of expressing my profound gratitude to my **parents, Mr. Aditya Sharma, and Mrs. Rajni Sharma**, for their unconditional love, sacrifices, and unwavering belief in me. Their constant encouragement has been my greatest source of strength. My deepest appreciation also goes to my **brother, Vinayak Sharma**, whose encouragement has been a pillar of support in my life.

I would also like to extend my heartfelt thanks to my grandfather, **Mr. Raghubir Gautam;**

my uncles, **Adish and Abhishek;** my aunts**, Bhawna, Pooja, Pallavi, and Parveen; and my sisters, Akshita, Anshika, and Mokshika**, for their love, support, and well-being. Their presence in my life has been truly invaluable.

I want to express my gratitude to my dear friends Simran Kauts and Anuradha Sandhu for their companionship and encouragement, which have made this journey even more enjoyable. I also extend my gratitude to my best friends **Parul, Nancy, Shreya Gupta, Dr. Vikas Kundu, Mayank Sharma and Ankush,** whose support has meant the world to me during challenging times.

I hold a special place in my heart for my **family away from home**—my guardians, **Er. Mr. Rajeev Sharma and Mrs. Neeraj Sharma**, my loving sister, **Mrs. Kaveri Soni**, and my brothers, **Manvendra Yadav, Rahul Sandhu, and Shubham Sahoo**, for always encouraging and motivating me. Their unwavering support has been a source of immense strength. Finally, I extend my heartfelt appreciation to everyone who has contributed to my academic and personal growth, directly or indirectly. This achievement would not have been possible without your support.

Aditi Sharma

22-06-2025

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Chapter 1:

Introduction

1. Exploring the chitosan-based Nanogels with a greener methodology

Nanogels are polymeric structures prepared from natural or synthetically obtained monomers, which are polymerized into polymers. Natural polymers, such as gums, leaf-based extracted polymers, and animal-based polymers, are plant-based, like Chitosan. Chitin (monomer) is a widespread polymer found abundantly in the exoskeleton of crustaceans, microorganisms, and insects. Chitosan, which is a polysaccharide of chitin, is obtained from the monomer units of β-(1- 4)-2-amino-D-glucose (D-glucosamine or GlcN) and β-(1-4)-2-acetamido-D-glucose (N-acetyl- D-glucosamine or GlcAC) (Brunel et al., 2009). However, the dissolution of chitosan is not possible below or near the alkaline pH due to the hydrophobic nature and the neutrality of amino groups, and thus, the formation of intra- and inter-molecular hydrogen bonds. Thus, it is soluble at an acidic pH of 3-6. This makes chitosan comparatively less favorable for biomedicine applications; to overcome these challenges, various cross-linking agents and chitosan with various water solubilities are formed using micro-emulsion techniques. Changes in chitosan, like chemical modifications or chitosan with a lower molecular weight, could also be preferred. Apart from these properties, chitosan is biodegradable, biocompatible, anti-microbial, has good absorption capacity, has high sustainability, low toxicity, and promotes adhesion (Pujana et al., 2013). It is not carcinogenic, has high anti-microbial activity, and does not cause an immune effect (Manuel Laza, 2016). Chitosan is used in various biomedical, environmental, and nanotechnological applications by the formation of gel, especially hydrogel or nanogel.

2. Synthesis and surface modification of quantum dot-chitosan-based nanogels.2

Quantum dots are semiconductor nanoparticles in 0-dimension. “Quantum” means a discrete and diminutive unit of any physical property (Jacak et al., 2013). They are a few nanometers in size (2-20 nm). The size of quantum dots is what defines their fluorescent properties. The bandgap of the electrons is less if the size is increased, and if the bandgap is less, the electrons absorb and emit more energy, thus producing more fluorescence (Bera et al., 2010). As the size of the Quantum dots decreases, the color of the light emitted changes from red to blue. Each color of the light represents a different wavelength. They possess electronic properties that are different from larger particles due to quantum mechanics.

Quantum dots have three layers: QD core, QD shell, and a cap. The core provides semiconductor properties; the shell enables optical properties, and the cap enables or improves solubility in an aqueous buffer (Vasudevan et al., 2015). The synthesis of Quantum Dots is done by two approaches: the top-down and bottom-up approaches.

Top-down approaches to quantum dots include molecular beam epitaxy, ion implantation, e-beam lithography, and X-ray lithography, whereas bottom-up approaches include self-assembly of particles with either wet chemical methods or vapor-phase methods.

Quenching is an important term while preparing Quantum Dots and usually means the process used to decrease the fluorescence capacity of a substance. This depends on the pressure and temperature. Quenching may result in excited-state reactions, energy transfer, collisional quenching, and complex formation (Garcia de Arquer et al., 2021). The quenching can be static quenching or collisional quenching.

Iron sulfide quantum dots (FeS QDs) possess captivating optical and magnetic properties, primarily attributed to the effects of quantum confinement. The adjustability of the bandgap in FeS QDs allows for efficient absorption and emission across the infrared spectrum, rendering them highly important for applications in photodetectors, solar cells, and bioimaging (Liu et al., 2017).

Leveraging the magnetic attributes derived from the presence of iron, FeS QDs present good opportunities in fields such as magnetic resonance imaging (MRI) and magnetic hyperthermia (Wu et al., 2014). In addition to their optical and magnetic prowess, FeS QDs showcase promising chemical stability, ensuring their resilience in diverse environmental conditions. Their compatibility with biological systems enables functionalization with biomolecules, facilitating targeted delivery in various biomedical applications (Luo et al., 2020). The size-dependent quantum effects within FeS QDs contribute to their distinctive electronic characteristics, making them well-suited for applications in electronics and optoelectronics (Tufani et al., 2021)). The versatile nature of FeS QDs has spurred exploration across a spectrum of applications. In the realm of energy, these quantum dots exhibit promise in photovoltaics and photocatalysis, capitalizing on their efficient light-harvesting capabilities (Xie et al., 2019). In the field of medicine, the biocompatibility and

magnetic properties of FeS QDs make them appealing for theranostic applications, seamlessly integrating diagnosis and therapy (Gao et al., 2007). Furthermore, the potential of FeS QDs extends to environmental remediation and catalysis in chemical reactions (Shetty et al., 2023)). Capping agents in Iron sulfide are crucial in reducing the size of nanoparticles, altering surface chemistry, compactness, stability, and morphology (Javed et al., 2022). Surfactants are "surface-active agents" that are amphiphilic compounds due to the presence of both hydrophobic and hydrophilic groups in the chemical structure (Miyazawa et al., 2021). Where they state the absorption or many other properties of FeS. Thiol group interaction and biological molecule interaction of Iron sulfide; these interactions give rise to the formation of a nanoparticle protein. They play a major role in influencing cellular uptake, inflammation, accumulation, clearance, and degradation of Iron sulfide nanoparticles (Saptarshi et al., 2013).

3. Characterization of Quantum dot-chitosan-based Nanogels

Characterization of Nanogels

A. Size and zeta potential of Nanogels:

The Zeta potential of Nanogels is described as the charge developed at the interface of the solid and liquid surfaces. It is measured in millivolts and is determined by Electrophoretic Light Scattering and electroacoustic determination. It refers to the stability of the nanogels (Amanlou et al., 2019).

B. Sorption studies:

The sorption studies are done when the Nanogel is loaded with dye or another chemical. The formula for sorption is as under:

Qe (Co-Ce) *V/m; where Co & Ce are the initial and equilibrium dye concentration, Qe is the uptake of adsorbate into the solid phase, and V(l) is the volume of dye added (Pereira et al., 2016).

C. Morphological analysis of Nanogels:

Electron microscopy (scanning and transmission) studies the morphology of nanogels. It helps identify the sample's chemical, morphological, and crystalline structures.

D. Thermal studies

Thermogravimetric analysis is used for the thermal analysis of Nanogels. Also, Differential scanning calorimetry can be performed (Luckanagul et al., 2018).

E. Instrumentation of Nanogels

The X-ray diffraction spectrometry technique is used for the size, characterization, and structure of Nanogels. FTIR enables to study of the surface adsorption functional groups. NMR spectroscopy of Nanogels determines the molecular characterization of Nanogels (Farag et al., 2012).

F. Other characterizations

Other techniques include hemocompatibility studies, in vitro studies, and cytotoxicity studies.

Characterization of quantum dots

I. X-Ray Diffraction: XRD helps determine the crystal structure of the quantum dots.

II. Scanning Electron Microscope (SEM)/ Transmission Electron Microscope (TEM): Microscopes help identify the sample's chemical structure, morphology, and crystalline structure (Sathiyaraj et al., 2020).

III. Fourier transform infrared spectroscopy: FTIR helps in determining the molecular structure and bonds in the methodology.

IV. UV-visible spectroscopy: It is used for the determination of quantity & quantity, chemical kinetics, optical characterization, examination of polynuclear hydrocarbons, and molecular weight determination.

V. Fluorescence spectroscopy: Fluorescence spectroscopy helps to determine the optical properties of the quantum dots. It also helps to determine the quantum yield (Cho et al., 2014).

4. Applications of quantum dots incorporated nanogel-based sensor

Quantum dots (QDs)-based nanogels are advanced tools offering significant potential in crime prevention, evidence tracking, and forensic analysis, as shown in Figure 1.1. These nanogels leverage the unique fluorescence properties of QDs for detection and tracking purposes. When integrated into GPS chips or injected into individuals, they enable real-time tracking of criminals, thereby aiding in theft prevention and ensuring a robust chain

of custody for forensic evidence (Zareef et al., 2024).

Beyond tracking, QD-based sensors can detect a wide range of chemical and biological agents, including pesticides, toxins, drugs, amino acids, ions, pH levels, sugars, nucleic acids, cells, and tissues (Datta et al., 2023). Their applications extend to in vivo and in vitro drug delivery, incorporation in security documents, and use as fluorescent markers in inks and powders to prevent forgery.

Additionally, DNA can be conjugated with quantum dots to enhance drug detection, offering targeted and selective biosensing. QDs are also valuable in fingerprint detection, capable of visualizing blood-splattered fingerprints on non-porous surfaces and imaging sweat-based latent prints. These systems can detect binding events, bodily fluids, and other forensic cues (Fakayode et al., 2024).

QD-based sensors must be robust, highly sensitive, and stable for these applications, with distinct fluorescence visible under appropriate detection systems.

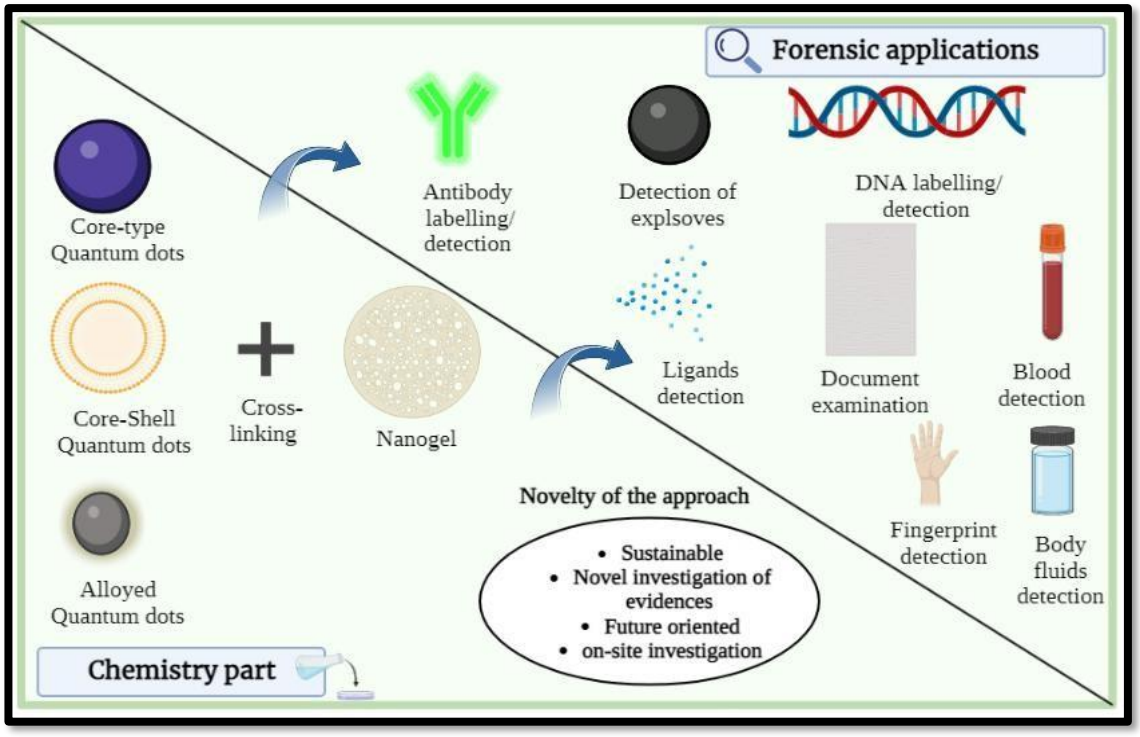


Figure 1.1: Forensic applications of the Quantum dot-based nanogel.

Chapter 2: Review of Literature

Review of literature

2.1 Nanogels

Nanogels are a 3-dimensional macromolecular polymer network that swells in the presence of a solvent. It is a nano-particle-sized gel that has high absorptivity and high drug loading capacity due to a large pore size, and can be formed by various methodologies into various shapes and sizes as desired. These nanogels are used to deliver target-specific delivery, drug delivery, and therapeutic applications. Apart from these applications, surface modifications of these nanogels can enhance physical stability for increased time in blood circulation and decrease the efficiency to be elimination and optical stability for the fluorescent activity of the nanogel-optical sensor. There are many ways to modify the surface of nanogels. Because of its antibacterial and non-toxic qualities, chitosan has found widespread usage in biomedical, medicinal, and nanotech applications (Wu et al., 2016). Hydrogels and nanogels offer a wide range of uses in nanotechnology, including wound healing, medication delivery, and cell targeting. They have a straightforward synthesis approach and may be utilized in conjunction with optical sensors such as quantum dots to detect a variety of analytes (Zha et al., 2011). Nano is a Greek term meaning “dwarf”. They are processed by synthesizing a hydrogel and a cross-linked hydrophilic polymer or a nanoparticle. Nanogels were first synthesized by a group of researchers by cross-linking polyethylene glycol (PEG) & polyethyleneimine (PEI) (Kabanov et al., 2009). Nanogels are commonly defined as aqueous dispersions of polymeric particles. These nanoparticles consist of amphiphilic or hydrophilic polyionic polymers, which could be natural (dextran, dextrin, pullulan, chitosan, hyaluronic acid, etc.) or synthetic (polymethacrylate, polyglycolic acid, and 1-lactic-co- glycolic acid) in origin. These Nanogels are very small in size (1-100 nm or one billionth of a meter). These nanogels have different properties like charge, solubility, in vivo transfer, amphiphilicity, and shape (softness). Nanogels may also show configurational and structural changes in the presence of various stimuli like pH, redox conditions, temperature, magnetic field, the concentration of enzymes, and light etc.

Nanogels may be inorganic or organic; Inorganic nanogels have properties like optical activity, electrical conductivity, and magnetic properties. In Nanodevices, the architecture could be divided into two parts: the coating and the core (Sultana et al., 2013). The coating consists of the interaction of Nanoparticles with the environment and their behavior; colloidal stability and hydrophobicity, and the core depend on the size, shape, porosity, addressability, or traceability, and physical methods.

Due to their properties like drug loading capacity, degradability, softness, etc., Nanogels are used in various chemical, biochemical, pharmaceutical, and forensic applications. In this review, we summarized some of the key features of Nanogels, including their properties, synthesis, applications, and applications.

A greener methodology for preparing nanogels is often used as it helps reduce waste production, uses minimum solvents, and uses economical processes (Warner, 2000). This process also produces fewer harmful by-products and uses less harmful reagents. The use of energy is also less, and it is sustainable to work with. Due to these rules, they also follow principles of nanocchemistry, i.e., economics, catalysis, reducing derivatives, use of renewable chemicals, use of less hazardous methodology, and safer and auxiliary solvents, which must help in solving real- time pollution problems and must be energy efficient (Andraos et al., 2022).

2.1.1 Properties of Nanogels

- Synthesis of Nanogels: The making of a homogeneous product by a combination of components or elements, i.e., synthesis, is an important feature of Nanogels. The characteristics feature of Nanogels synthesis are colloidal solubility, high surface area, biocompatibility, biodegradability, loading capability, versatility in administration route, the release of hydrophilic and hydrophobic bioactive compounds, reduction of drug payload, reduced nanogel elimination by the mononuclear phagocytic system, & low immunogenicity (Theune et al., 2019; Mauri et al., 2021).

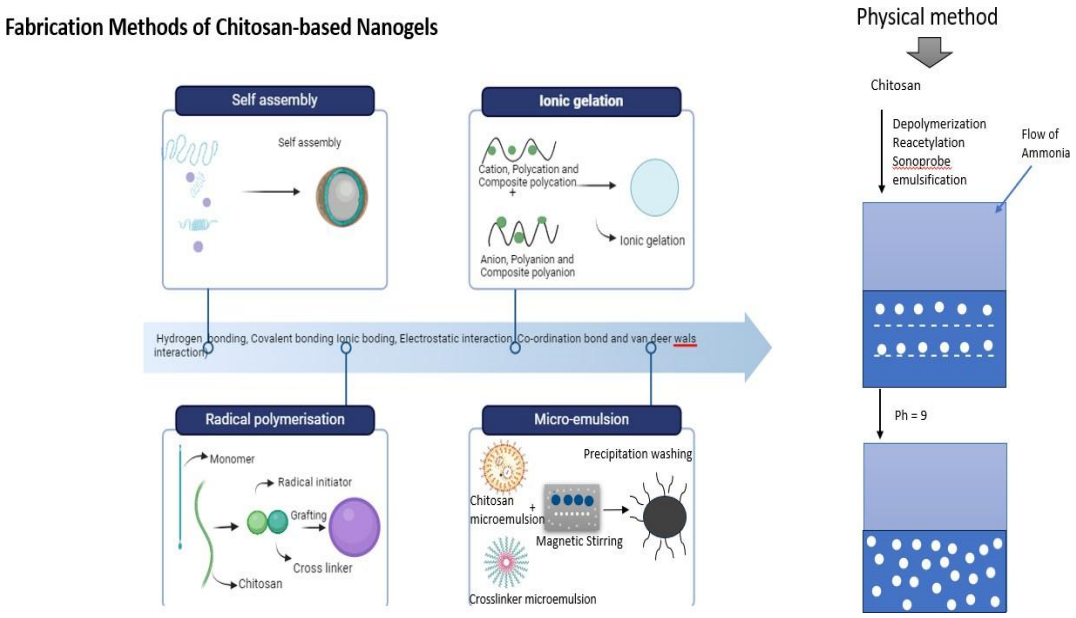


Figure 2.1: Illustration of fabrication methods of nanogels prepared from chitosan (Adapted from Wang et al., 2017)

- Stimuli-responsive behavior: Nanogels show different structural and behavioral changes when exposed to stimuli like pH, redox conditions, temperature, concentration of enzymes, light, magnetic field, etc. The nanogels responsive to pH are used in cancer therapy due to hypoxia- induced production of lactic acid in tumor cells, because of pH of normal tissue and blood is higher than that of normal cells. Poly (N-isopropyl acrylamide) (PNIPAAm) based nanogels show good temperature sensitivity (Gao et al., 2019).
- In vivo behavior: Nanogels are used for the transport of medicines and drugs to a particular site due to their high loading capacity. Due to this property of Nanogels, they show versatile properties in pharmaceutical and biochemical sciences (Shah et al., 2020).
- Colloidal nature of gels: gels swell up in the presence of solvent; they become Nanogels due to their size. Then, like colloids, they can crystallize at a high-volume fraction.
- Tunable size: Nanogels can be molded, cut, or deformed into different shapes.
- High drug loading capacity: 3-D hydrogel, i.e., Nanogel, has a submicron particle size. These are fabricated by various chemical and physical intercrossing of the polymer

(Zha et al., 2011). This cross-linking helps nanogels to maintain their structure despite absorbing fluid without disintegration. Nanogels are also used for drug delivery applications due to their tunable size, hydrophilicity, biocompatibility, and stimulus response (Shah et al., 2020; Muniraj et al., 2019).

2.1.2 Classification of Nanogels

Nanogels are classified based on their various properties, like origin or source, polymeric composition, linkage types, and responsive behavior.

Based on polymer: Nanogels could be of acrylic acid, polyesters and polyethers, polypeptides, and polysaccharides. Polyacrylates are derivatives of acrylic acid, which are a result of the alterations of vinyl and carboxyl hydrogen (Thomas, 2019). Polyethers and polyesters are formed by the covalent linkage of ethers and esters, respectively. Polypeptides are polymers that are biodegradable and are linked by amide bonds. Polymers linked by a long chain of monosaccharides or by glycosidic bonds are polysaccharides. Polymers could be homo-polymers or heteropolymers based on the presence of a monosaccharide unit, i.e., if they are based on the same units, they are homopolymers, e.g., starch, pullulan, cellulose, etc., or based on different polymer heteropolymers, e.g., heparin, chitosan, dermatan sulfate, hyaluronic acid, etc. (Muniraj et al., 2019)

Based on the structure of nanogels: based on structure, nanogels could be hollow, multi-layered, core cross-linked, and hairy. Hollow nanogels possess a hollow cavity inside a gel matrix. Hollow nanogels were synthesized by poly (N, N-dimethyl aminoethyl) (Shah et al., 2020). Multi-layered nanogels have multiple layers of single or multiple polymers. In the categorization, which is based on structure, core cross-linked nanogels or core-shell nanogels are the parents. Hairy nanogels have thin hair-like projections (Theune et al., 2019). These nanogels are prepared using RAFT or radical polymerization.

Based on responsive behavior, Nanogels in the presence of the stimulus may or may not show changes. These stimuli cause physicochemical changes in the nanogels. Some of these stimuli are light, temperature, and pH.

Based on types of linkages, nanogels could be covalently or non-covalently linked. The nanogels linked by non-covalent bonding are self-assembled by physical interactions and

do not require chemical cross-linking (Chiang et al., 2012; Gao et al., 2019). Other than this, various forces used for non-covalent linkage are ionic interaction, Van der Waals interaction, adsorption, and surface/interface interactions. On the other hand, covalently linked nanogels are linked by covalent, rigid bonds.

Chitosan nanogels

Natural polymers such as chitosan are very biocompatible and biodegradable. Chitin (polymer) is a widespread polymer found abundantly in the exoskeleton of crustaceans, microorganisms, and insects. Chitosan, which is a polysaccharide of chitin, is obtained from the monomer units of β-(1-4)-2-acetamido-D-glucose (GlcAC) and β-(1-4)-2-amino-D-glucose (GlcN). Chitosan is a sugar from shellfish, crab, lobster, and shrimp. Glucosamine (deacetylated monomer) and N-acetyl glucosamine (acetylated sugar) are linked by β-4 glycosidic bonds (Sacco et al., 2018). Chitosan is non-toxic, biocompatible, biodegradable, anti-microbial, and polycationic. It is said to dissolve in a solvent below a pH of 6. This naturally occurring hydrophobic polymer is added to dilute acetic acid. Chemical cross-linking prepares nanogels and cross-linkers (glutaraldehyde) and mixes them accordingly (Wolf et al., 2025). The gelling process starts as soon as the chitosan is dissolved.

The dissolution of chitosan is not possible below or near the alkaline pH due to the hydrophobic nature and the neutrality of amino groups, and thus, the formation of intra- and inter-molecular hydrogen bonds. Thus, it is soluble at an acidic pH of 3-5. This makes chitosan comparatively less favorable for biomedicine applications; to overcome these challenges, various cross-linking agents and chitosan with various water solubilities are formed using micro-emulsion techniques. Changes in chitosan, like chemical modifications or chitosan with a lower molecular weight, could also be preferred. Apart from these properties, chitosan is biodegradable, biocompatible, anti-microbial, has good absorption capacity, has high sustainability, low toxicity, and promotes adhesion. It is not carcinogenic, has high anti-microbial activity, and does not cause an immune effect. Chitosan is used in various biomedical, environmental, and nanotechnological applications by the formation of gel, especially hydrogel or nanogel.

Chitosan nanogels have evolved over several decades, driven by advancements in nanotechnology, biomaterials, and drug delivery systems. The development of chitosan-based nanogels stems from the broader history of chitosan as a biopolymer and its applications in medicine, pharmaceuticals, and biotechnology.

1. Discovery of Chitosan (19th Century - Early 20th Century) (Morin-Crini et al., 2019)

- 1859: French chemist Charles Rouget first discovered chitosan by treating chitin (found in crustacean shells) with an alkaline solution.
- 1930s-1950s: Chitosan was further studied for its biocompatibility and chemical properties, particularly in biomedical applications.

2. Emergence of Hydrogels and Nanogels (Late 20th Century) (Chirani et al., 2015)

- 1894: The Term hydrogel was coined and was used to describe a colloidal gel (Thakur et al., 2018).
- 1960s-1980s: Hydrogels, including those based on natural polymers like chitosan, gained attention for biomedical applications such as wound healing and controlled drug release.
- 1990s: The concept of nanogels (hydrogels at the nanoscale) emerged, offering enhanced drug encapsulation, stimuli responsiveness, and improved bioavailability. Researchers began exploring chitosan nanogels for their biocompatibility, biodegradability, and ability to form stable colloidal dispersions.

3. Development of Chitosan Nanogels (2000s-Present) (Wang et al., 2017)

- Early 2000s: Chitosan nanogels were first reported for drug delivery, particularly in targeted and controlled-release systems. Scientists explored various crosslinking methods (ionic, covalent, and physical) to improve stability and functionality.
- 2005-2015: Chitosan nanogels were widely investigated for applications in cancer therapy, gene delivery, and wound healing. Researchers optimized particle size, charge, and surface modifications to enhance cellular uptake and therapeutic efficiency.
- 2015-Present: Advancements in green synthesis, stimuli-responsive nanogels, and multifunctional nanogels (such as those incorporating quantum dots, magnetic nanoparticles, or bioactive compounds) have expanded their applications to forensic

science, biosensors, and regenerative medicine.

2.2 Quantum dots

2.2.1 Properties of quantum dots

Quantum dots, also known as “artificial atoms,” show various energy levels due to their semiconductor properties, group I to IV (Valizadeh et al., 2012). Properties of quantum dots arise from size-dependent optical and electronic features due to quantum confinement effects. The tunable bandgap of QDs allows for absorption and emission across a broad spectrum, making them valuable for imaging, sensing, and solar cells (Ekimov et al., 1981). Small-sized QDs give a special quantum confinement effect, exhibiting unique electronic transitions (Ghosh et al., 2015). Also, QDs possess notable chemical and photochemical stability, making them good for long-term applications. In the drug delivery field, their high surface-to-volume ratio enables functionalization with various ligands, facilitating targeted delivery in biomedical (Yong et al., 2009). Additionally, QDs demonstrate superior photochemical stability as compared to traditional fluorophores, leading to their crucial role in increased use in bioimaging and sensing applications (Michalet et al., 2005). The unique properties of QDs have propelled their widespread use across diverse fields. QDs are employed for bioimaging, diagnostics, and targeted drug delivery in medicine due to their superio photostability and brightness (Zhang et al., 2004)). In electronics and technology, QDs contribute to efficient solar cells and light-emitting devices owing to their excellent charge transport properties (Gur et al., 2005). Furthermore, QDs show catalysis, sensing, and quantum computing applications.

2.2.2. Iron Sulfide Quantum Dots

Iron sulfide quantum dots (FeS_2 QDs) possess captivating optical and magnetic properties, primarily attributed to the effects of quantum confinement. The adjustability of the bandgap in FeS_2 QDs allows for efficient absorption and emission across the infrared spectrum, rendering them highly important for applications in photodetectors, solar cells, and bioimaging (Liu et al., 2017). Leveraging the magnetic attributes derived from the presence

of iron, FeS_2 QDs present good opportunities in fields such as magnetic resonance imaging (MRI) and magnetic hyperthermia (Wu et al., 2014). In addition to their optical and magnetic prowess, FeS2 QDs showcase promising chemical stability, ensuring their resilience in diverse environmental conditions. Their compatibility with biological systems enables functionalization with biomolecules, facilitating targeted delivery in various biomedical applications (Jiang et al., 2016). The size-dependent quantum effects within FeS_2 QDs contribute to their distinctive electronic characteristics, making them well-suited for applications in electronics and optoelectronics (Tufani et al., 2021). The versatile nature of FeS_2 QDs has spurred exploration across a spectrum of applications. In the realm of energy, these quantum dots exhibit promise in photovoltaics and photocatalysis, capitalizing on their efficient light-harvesting capabilities (Cao et al., 2017). In the field of medicine, the biocompatibility and magnetic properties of FeS2 QDs make them appealing for theranostic applications, seamlessly integrating diagnosis and therapy (Gao et al., 2019). Furthermore, the potential of FeS_2 QDs extends to environmental remediation and catalysis in chemical reactions (Shetty et al., 2023)). Capping agents in Iron sulfide are crucial in reducing the size of nanoparticles, altering surface chemistry, compactness, stability, and morphology (Javed et al., 2022). Surfactants are “surface-active agents” that are amphiphilic compounds due to the presence of both hydrophobic and hydrophilic groups in the chemical structure (Miyazawa et al., 2021). Where they state the absorption or many other properties of FeS_2. Thiol group interaction and biological molecule interaction of Iron sulfide; these interactions give rise to the formation of a nanoparticle protein. They play a major role in influencing cellular uptake, inflammation, accumulation, clearance, and degradation of Iron sulfide nanoparticles (Saptarshi et al., 2013).

1. Early Foundations of Quantum Dots (1980s-1990s) (Reddy et al., 2024)

- 1981: Louis E. Brus at Bell Labs discovered quantum dots while studying small semiconductor particles, showing size-dependent optical properties.
- 1993: Alivisatos and Bawendi developed synthetic methods for II-VI semiconductor QDs (CdSe, CdTe), leading to their widespread application in bioimaging and

optoelectronics.

- 1990s: Research focused on cadmium and lead-based QDs, but concerns over toxicity led to the exploration of non-toxic, earth-abundant alternatives like iron sulfide.

2. Initial Development of Iron Sulfide Nanoparticles (2000s) (Chun-Rong et al., 2017)

- Early 2000s: FeS nanoparticles were synthesized for catalysis, energy storage, and environmental remediation, but their quantum confinement properties were not widely studied.
- 2005-2010: Researchers began investigating FeS nanocrystals for their stability, tunable bandgap, and potential in photovoltaics. However, challenges in controlling size, surface passivation, and quantum yield limited their applications.

3. Emergence of FeS Quantum Dots (2010s-Present) (Yuan et al., 2020)

- 2012-2015: Researchers successfully synthesized FeS quantum dots with controlled size and bandgap, demonstrating their application in photocatalysis, sensors, and bioimaging.
- 2016-2020: Green synthesis approaches, ligand engineering, and surface modifications improved FeS QD stability and photoluminescence. Studies explored their potential as eco- friendly alternatives to cadmium-based QDs in biomedical and forensic applications (Paca et al., 2017).
- 2020-Present: FeS QDs have been investigated for solar cells, hydrogen evolution reaction (HER) catalysts, biosensing, and fluorescent markers. Their non-toxic nature makes them a promising candidate for bioimaging and forensic science applications.

2.3 Synthesis

Synthesis of nanogels includes different methodologies for the polymerization of monomers. This technique includes simple polymerization of monomers by an initiator, emulsion techniques, physical self-assembly, chemical or physical cross-linking (Mauri et al., 2021), Radical-assisted precipitation polymerization, and fabrication of a template. In cross-linking, a polymer precursor is chemically or physically cross-linked through a cross-linker (Wu et al., 2016). Nanogels can be fabricated by printing the template. This technique

is used in the medical field for 3-D printing of various organs like heart valves, ovaries, ears, and bones (Cho et al., 2018). Multiple approaches for the preparation of Nanogels (Mauri et al., 2021):

- Linkage-based synthesis: In this approach, nanogels are produced using hydrogen, van der Waals, ionic, and covalent bonding. Hydrogen bonding is used to prepare physical nanogels and is affected by pH and solvent type.

Network chemistry: Network chemistry is a method that includes simple reactions, is easy to perform, requires no pre-purification, and provides higher yields. These simple reactions include cyclo-additions, carbonyl chemistry of the non-aldol type, nucleophilic ring-opening, and additions to the carbon-carbon multiple bonds. This approach is comparatively much more efficient for the synthesis of Nanogels and consists of copper-catalyzed azide-alkaline cycloaddition. This technique is necessary for biocompatible Nanogels due to the use of photo- induced linkage (Jiang et al., 2014). It was introduced by K.B. Sharpless in 2001.

- Top-down approach: This approach is used to produce Nanogels from large materials using mechanical or physicochemical forces. The photolithography method could be used for the incorporation of drugs by fabrication methods. Micro-coating or micro-molding processes could be used for spreading cells over the hydrogel (Top-Down BUA).
- Bottom-up approach: This approach produces Nanogels by physically or chemically cross- linking precursors for the growth of precursors. However, Synthesis using monomers is used as preparation by monomers is more useful than preparation by precursors.

These methods are detailed and explained below:

1. Physical or chemical cross-linking: Nanogels or microgels preparation includes physical or chemical cross-linking of macromolecules having natural or synthetic origin. Physical cross- linking is the formation of a bond between polymer chains by weak interactions due to the presence of ionic bonding, whereas chemical cross-linking is formed by polymer chains by covalent chemical bonds (Mauri et al., 2021). Before physical cross-linking,

self-assembly of different polymers in solution for the preparation of amphoteric nanogels results in cluster formation and lysosomes in the aqueous phase, and physical cross-linking of polymer chains by heating-induced gelatin. Microfluidic techniques/devices followed by cross-linking or physical cross-linking are used for the synthesis of microgel particles of dimensions from 1 to 30 nm. In chemical cross- linking, cross-linking is done between monomers and polymer precursors (Wu et al., 2016). The size of nanogels formed by physical cross-linking can be affected by changes in environmental conditions like temperature, pH, ionic bonds, and the concentration of polymer as well. The physical incorporation of an insoluble molecule into a cross-linked network is described by the semi-interpenetration method (Fu et al., 2017).

2. Chemical cross-linking involves various methods like emulsion polymerization, click chemistry cross-linking, reversible addition-fragmentation chain transfer, and photo-induced cross-linking. However, for the preparation of amino acids, amino acid cross-linking is used. In chemical cross- linking, cross-linking is done between monomers and polymer precursors.

3. 3-D fabrication of Nanogels: Nanogels could be printed in a structure of drugs/photo-initiator- loaded nanoparticles, liposomes, or nanoemulsions suspended in hydrogels. 3-D printing could be used in tissue engineering & organ transplantation. Hydrogels could provide an excellent center for cells & are used as cell carriers for 3-D bioprinting. Using 3-D printing, the fabrication of heart valves, urethra, ovaries, bionic ears, bones, and cartilage is done (Cho et al., 2018). Microfluidics fabrication methods offer advanced nanogel design, highly controllable, and large-scale production yields. These methods work by customizing the nanosystem by changing the microfluidics conditions (chip design, fluid rheology, and flow rates).

4. Free radical precipitation polymerization: In this process, a free radical initiator is used to initiate the reaction, whereas to stabilize the growing polymer globule, a surfactant is used (Fu et al., 2017). As the concentration of initiator and surfactant increases, particle size reduces. An example of a free radical initiator could be Ammonium persulfate, and that surfactant is sodium dodecyl sulfate (SDS). Surfactant is used for stabilization.

5. Core/Shell Nanogel synthesis: This process allows the making of various varieties of particle sizes (Blackburn et al., 2008). Although it is synthesized by free radical

precipitation polymerization. The process involves the preparation of core-shell Nanoparticles, a core, a shell, a core, and then a core again (Gan et al., 2001).

6. Emulsion-based methods: emulsion could be direct polymerization, i.e., dispersion of organic droplet polymers in an aqueous medium (oil-in-water emulsion) or indirect polymerization by dispersing aqueous droplets in an organic medium (water-in-emulsion method). These methods are based on the production of monodisperse droplets in a continuous phase.

Quantum dots are among the first nanotechnologies integrated into the biological sciences. QDs are nanoparticles also described as artificial atoms. They became promising nanomaterials within this category, demonstrating distinctive properties that enhance their utility, exhibiting discrete energy levels, and allowing their bandgap to be precisely modulated by varying size (Klimov et al., 2007; Valizadeh et al., 2012). Several synthesis methods include both top-down processing methods and bottom-up approaches. Within the bottom-up category, they are further divided into wet-chemical and vapor-phase methods (Valizadeh et al., 2012). Wet-chemical methods generally include microemulsion, sol-gel (Bera et al., 2008), hot-solution decomposition, sonication, or microwaves. A few of the methods for synthesizing iron sulfide include hydrothermal synthesis, microwave production, co-precipitation, high-temperature chemical synthesis, sono-chemical synthesis, and other chemical methods (Wang et al., 2022). Hydrothermal synthesis is an important and widely utilized technique for preparing FeS_2 QDs, involving the reaction of iron and sulfur precursors in an aqueous solution under selected temperature and pressure. This method allows for control over the shape and size of the FeS2 QDs by manipulating reaction parameters such as reaction time, precursor, and temperature. Microwave-assisted synthesis provides an efficient and rapid alternative, utilizing microwave irradiation for the nucleation and growth of FeS_2 QDs, which can enhance size uniformity (Yuan et al., 2020). In addition to these precursor methods, iron sulfide nanoparticles can also be prepared using date seed extract, which forms ds-FeS, useful for water treatment by removing pollutants such as hexavalent chromium and ciprofloxacin (Bhattacharjee et al., 2021).

2.4 Surface modifications

Green chemistry is the practice of environmental protection through the use of solvents, chemicals, time, and methodologies that save the environment (Duan H et al., 2015). Green chemistry principles should be followed when developing Quantum dot-based Nanogels. Although quantum dots are toxic, they can be interchanged by using green chemicals, methodology, and capping agents (Raveendran et al., 2003).

2.4.1 Agent of capping: Capping agents are used to keep nanoparticles stable. Polysaccharides, polymers, long-chain hydrocarbons, small molecules, and dendrimers are common capping agents (Dumur et al., 2011).

Polymers stabilize nanogels due to their lower binding affinity. Although the weaker interaction between polymers and nanoparticles hinders nanoparticle morphology, it also aids in energy savings and the use of green solvents in the manufacturing of capping agents (Paul et al., 1998).

Block copolymers, which provide size-controlled nanoparticles, are another type of green capping agent.

Long-chain hydrocarbons aid in the control of nanoparticle dispersibility and size. Although these nanoparticles are used in industrial processes, they are expensive and harmful to the environment because they are irritants and toxic.

2.4.2. Reducing agents: The synthesis of nanoparticles necessitates the use of toxic reducing agents, which could be replaced with less hazardous and environmentally friendly alternatives. A few commonly used reducing agents are listed, along with their toxicity.

As previously stated, toxic reducing agents could be used and replaced with environmentally friendly reducing agents. Polysaccharide-reducing agents and biological agents, such as microbial synthesis and photosynthesis, may be considered. Non-toxic polysaccharide-based reducing agents are known as "green reducing agents." Polysaccharide-reducing agents include -D-glucose, heparin, and starch (Dumur et al., 2011).

Microbial and photosynthesis are examples of biological methods. Peptides and proteins can be used in the process of synthesis and fabrication, providing options for size,

morphology, and composition control. These proteins and peptides can be derived from two sources: microbial organisms such as fungi, bacteria, actinomycetes, viruses, yeasts, and photosynthesis. Microbial synthesis is biologically compatible and has applications in medicine, cosmetics, and health care. Photo methods are thought to increase the rate of synthesis. Plant material aids in the detoxification and accumulation of heavy metals, which aids in the synthesis of nanoparticles. Plant extracts' reactive components include enzymes, amino acids, proteins, and polysaccharides.

2.4.3 Solvent: Solvent accounts for nearly 80% of the materials. However, solvents are used in the synthesis of nanoparticles for transferring heat and reactants, dispersing resulting nanoparticles, and dissolving precursors. Solvents can be toxic while remaining organic; however, environmentally designed solvents should be used to reduce environmental risks. Water, supercritical fluids such as supercritical water, ionic liquids, and supercritical carbon dioxide are among the many environmentally friendly solvents (Henderson et al., 2008). Supercritical fluids are solvents that, when heated above a critical temperature, transform into supercritical fluids. The properties of this critical state are between the gas phase and the liquid state and can be changed to these states with a small change in temperature and pressure (Sheldon, 2005). By varying the temperature, pressure, solvent type, time, and reagent concentration of the fluids, these fluids can help change the size, morphology, composition, architecture, and structure of nanoparticles (Desimone, 2002).

2.4.4 Methodologies: In addition to selecting reducing agents, solvents, and capping agents, methodologies should be chosen carefully, as conventional methods may be inefficient in terms of energy, reaction efficiencies, and reaction rates. They also use heat from outside sources such as furnaces, heating mantles, water/oil baths, and so on. Microwave and sonochemical synthesis are two environmentally friendly techniques (Wu et al., 2014).

Microwave Synthesis: Microwave synthesis is based on microwave adsorption by polar molecules. Metals, oxides, chalcogenides, and phosphates are all synthesized using microwaves. This method is relatively eco-friendly and is used in industrial and technological applications. The reaction time is reduced in this synthesis, and solvents such

as DMSO and DMF are used to create heating effects (Baig et al., 2013). Ionic liquids that are strong adsorbing agents should be used to improve the performance of microwave synthesis. Microwaves influence the penetration of reaction mixtures and thus the heating rates. As a result, this regulates the nucleation and growth of nanoparticles.

Sonication synthesis: The waves in sonication synthesis are generated by cavitation. The oscillation of primary bubbles is also caused by waves. These primary bubbles grow by absorbing ultrasonic waves and collide as a result. This collision generates a large amount of energy, and hotspots with temperatures as high as 5000K and pressures as high as 1,000 bar are formed, motivating nanoparticle synthesis. Sonication has advantages over traditional methods because it is faster, uses water or green solvents, operates under ambient conditions, and uses a small amount of reducing agents. Sonication is a "green" technique because of these benefits (Tang et al., 2004).

2.5 Characterizations

Characterization of Quantum dot composites is a pivotal aspect of research in this field (Figure 2.2). It provides insights into the composites' structural, morphological, and optical properties (Park et al., 2021). Spectroscopic techniques, including UV-Vis, FTIR, and XPS, offer valuable information about the surface chemistry and electronic states of the composites. Scanning Electron Microscopy (SEM) & Transmission Electron Microscopy (TEM) provide high-resolution images, enabling researchers to discern particle size, distribution, and morphology (Zoghi et al., 2023). Furthermore, rheological analysis aids in understanding the mechanical properties and stability of the composites (Chen et al., 2022).

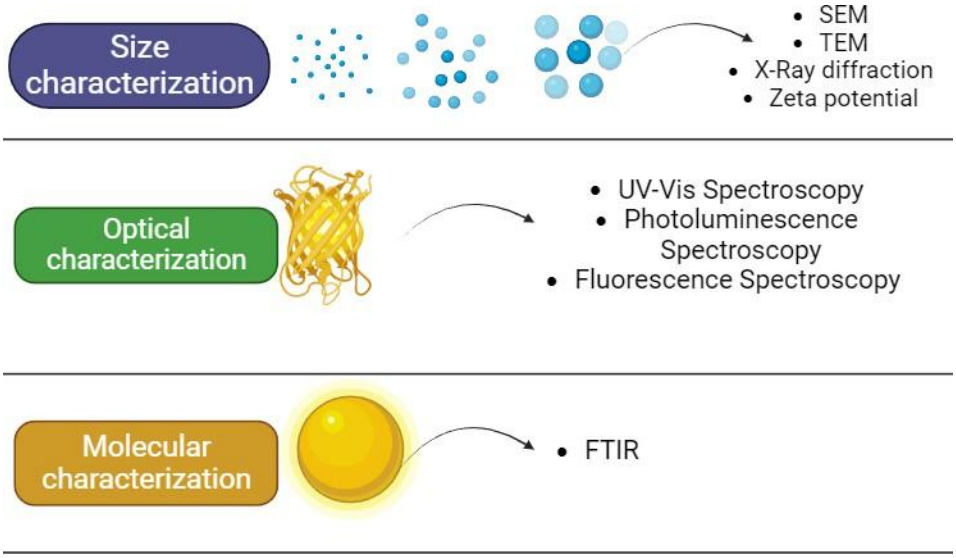


Figure 2.2: Characterization techniques of Quantum dots.

While current characterization techniques have provided a robust foundation for understanding QD-nanogel composites, there is room for improvement to gain a deeper understanding of the composite's structure-property relationships (Ramalingam et al., 2020). Emerging techniques such as super-resolution microscopy and in-situ spectroscopy promise to offer finer resolution and real-time monitoring capabilities (Schneider et al., 2017). Moreover, the integration of computational modeling and simulation techniques can provide a deeper understanding of the interplay between QDs and nanogels at the atomic and molecular levels (Pina et al., 2022). However, there is a pressing need for continued innovation to refine existing methods and explore novel approaches that afford greater control and precision in composite fabrication (Chern et al., 2019; Moon et al., 2019). Additionally, advancements in characterization techniques will be instrumental in unraveling the intricate details of QD-nanogel interactions and furthering our understanding of these composites' potential in sensing applications (Tribandpaya et al., 2021).

Although instrumental techniques provide size, surface modification, and molecular and structural details of the material produced, absorption studies, thermal analysis, and other

biomedical studies provide insight into the biocompatible characteristics of the sensor so produced. Sorption studies are done when the Nanogel is loaded with dye or another chemical (Chen et al., 2022). The formula for sorption is as under:

Qe (Co-Ce) *V/m; where Co & Ce are the initial and equilibrium dye concentration, Qe is the uptake of adsorbate into the solid phase, and V(l) is the volume of dye added.

Thermal analysis of the nanogel is done to check the temperature resistance of the nanogel. It is done through Thermo-gravitational analysis or TGA. Biomedical studies include hemocompatibility studies, in vitro studies, and cytotoxicity studies to study if the material is compatible with cells for human studies (Muniraj et al., 2020).

U.V. visible spectroscopy

UV-Visible spectrophotometer works by passing a beam of light through the sample and measuring the amount of light that is absorbed at each wavelength. The principle of UV spectrophotometry states that the amount of light absorbed is proportional to the concentration of the sample. In Figure 2.3 below, FeS_1, FeS_2, and FeS_3 exhibit wavelengths from 235 nm to 255 nm, approximately, with different absorbance, suggesting likely the same concentration for the three compounds.

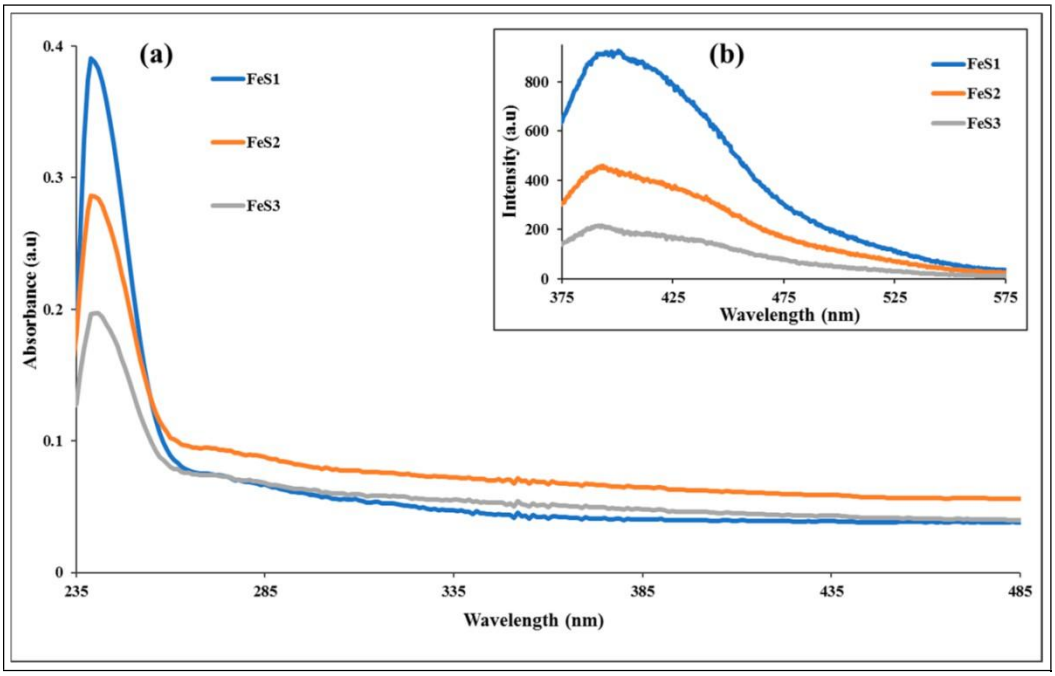


Figure 2.3: Absorption and emission spectra of the FeS_1, FeS_2, and FeS_3 iron sulfide

nanoparticles (a & b, respectively) from different Fe (II) dithiocarbamate complexes (Paca et al., 2018).

XRD spectroscopy

XRD techniques are based on Bragg's Law, which states that X-rays scatter from structures with long-range order. XRD finds the geometry or shape, and size of a molecule. In the Figure 2.4 below, we can observe peaks at different degrees, especially a sharp, long peak that is seen between 40 and 50 degrees. These peaks are similar in shape and size.

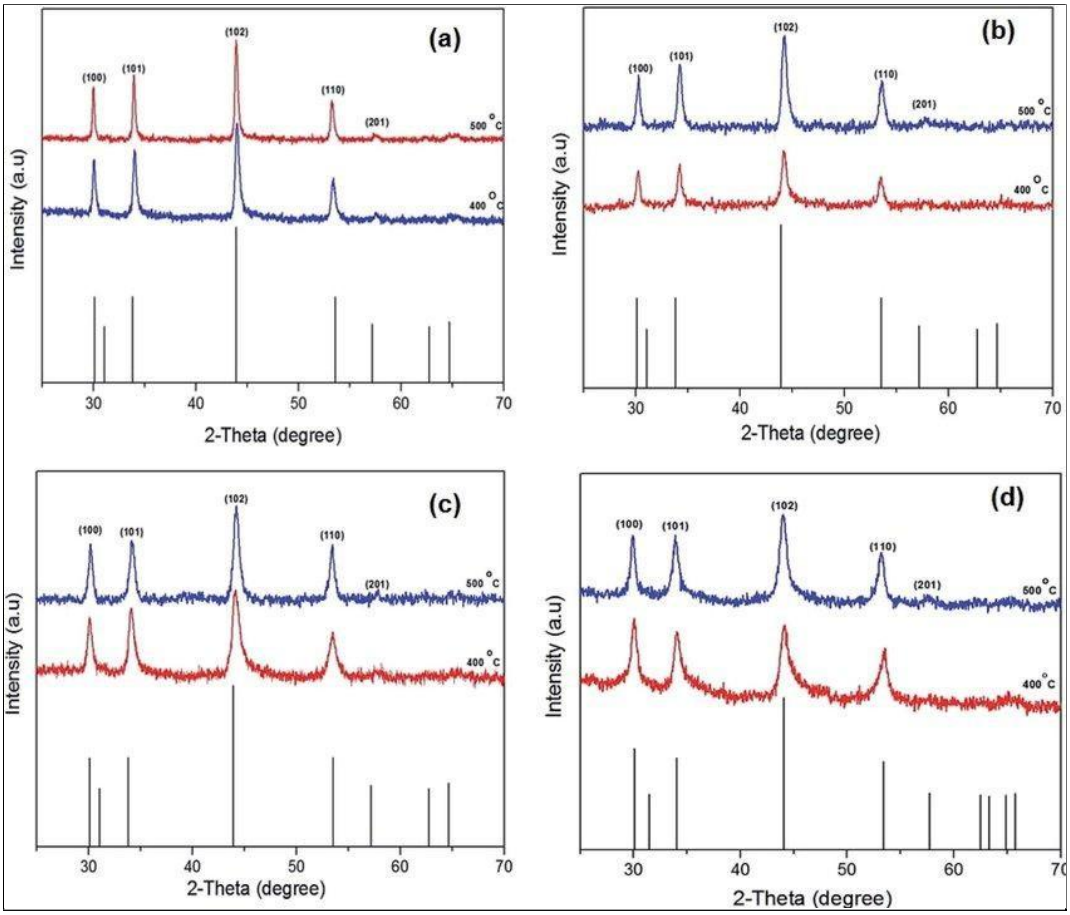


Figure 2.4: Powder X-ray diffraction patterns of the iron sulfide nanostructure obtained by pyrolysis. The black sticks represent hexagonal pyrrhotite (Almanqur et al., 2018).

FTIR

FTIR spectroscopy offers simple, nondestructive, and reliable sample analysis both

qualitatively and quantitatively. It takes advantage of how IR light changes the dipole moments in molecules. In the image below, Figures 2.5 & 2.6, Iron sulfide exhibits different peaks with monosulfide, disulfide, and trisulfide compounds.

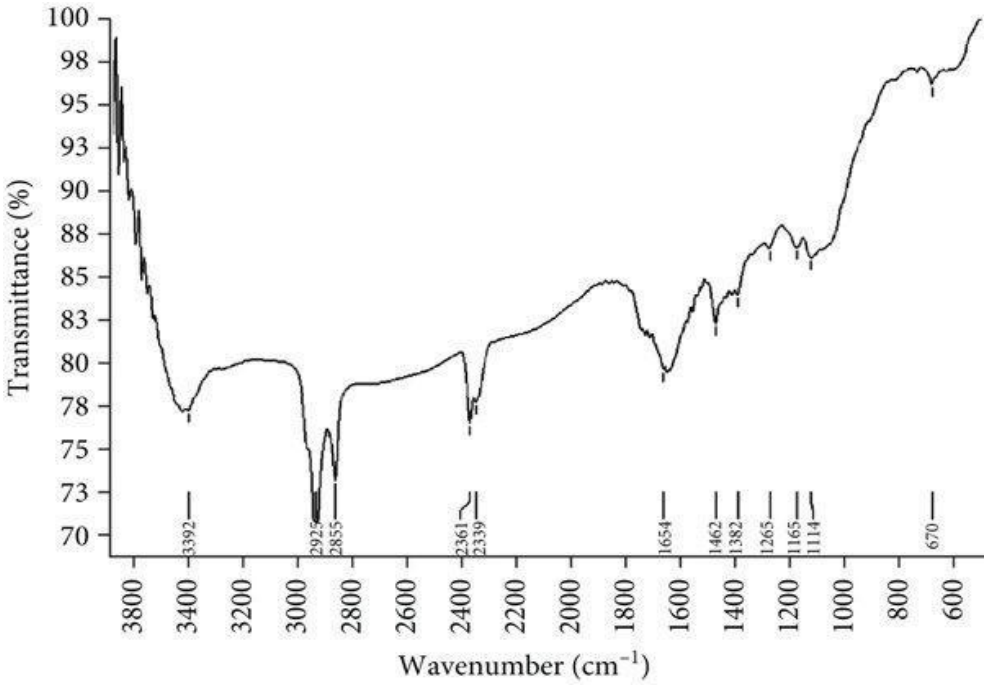


Figure 2.5: Characterization of chitosan nanogel through FT-IR spectra. (Adapted from Ashoori et al., 2020).

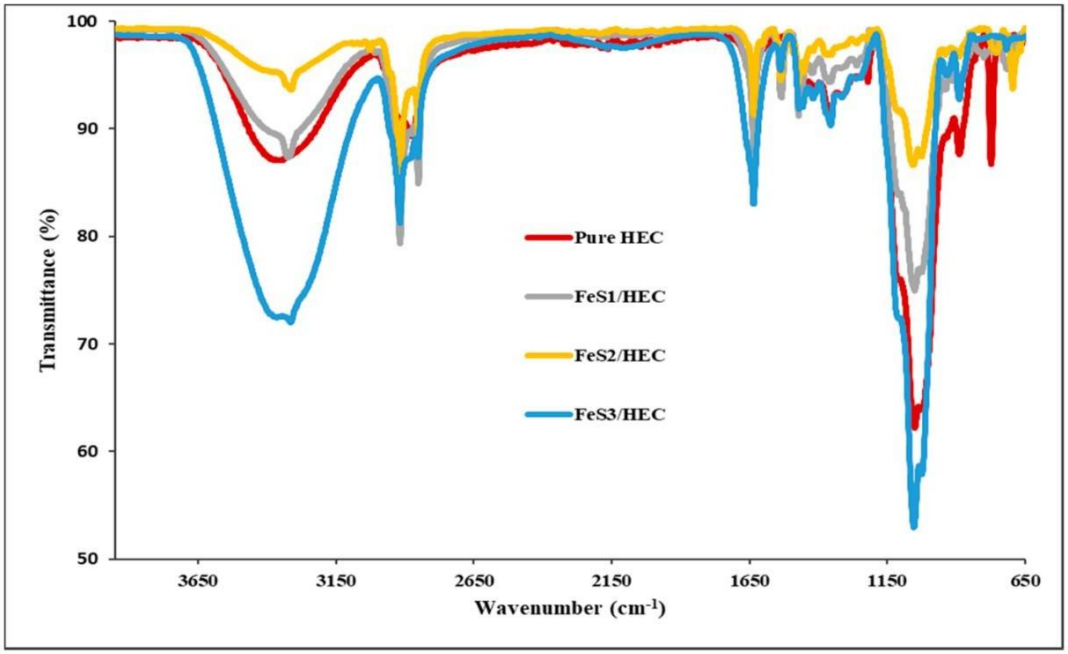


Figure 2.6: FTIR spectra of pure Hydroxyethyl Cellulose and iron sulfide/HEC nanocomposites prepared from FeS (1, 2, and 3) nanoparticles. (Paca et al., 2018).

Electron microscopy

SEM scans the beam in a raster-like pattern and collects the scattered electrons. EM plays a vital role in biomedical research in many investigations in many other fields, as it obtains high- resolution pictures. It can even take the resolution down to the nanometer scale. SEM majorly focuses on the Morphology and size of the particles. Figure 2.7 and Figure 2.8 below show SEM of chitosan & FeS thin films, respectively. High-resolution transmission electron microscopy (HRTEM) and field scanning electron microscopy (FESEM) are used in the characterization of Iron sulfide.

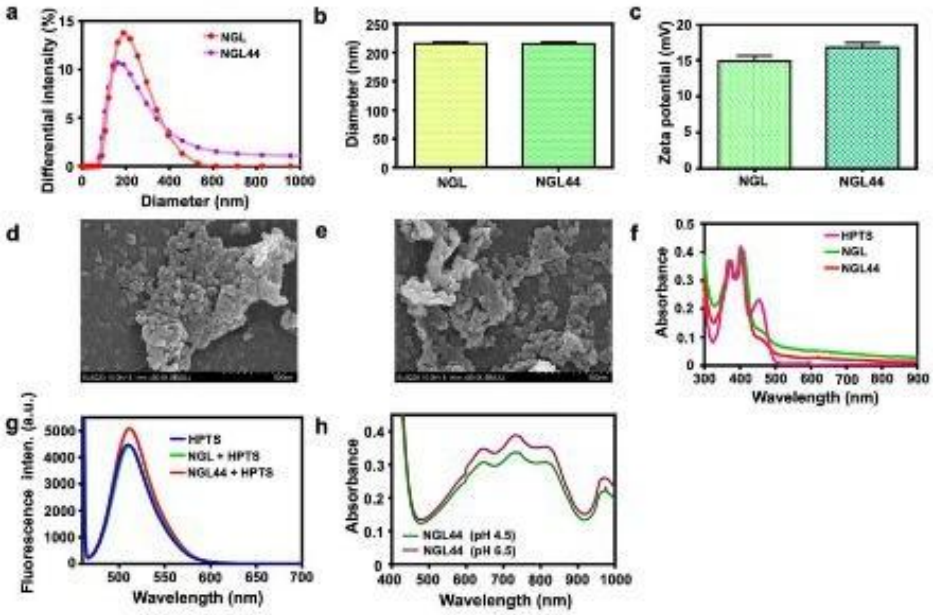


Figure 2.7: Physicochemical characteristic analysis of the chitosan nanogels (NGL & NGL 44):

a) DLS or Dynamic Light Scattering.
b) Hydrodynamic diameter evaluation;
c) zeta potential;
d) SEM of nanogel;
e) SEM of NGL44;
f) absorption spectra of the NGL, NGL44, and free HPTS;
g) fluorescence emission spectra of NGL and NGL44 at an excitation wavelength of 455 nm;
h) pH-dependent alkyl radical release (determined by ABTS assay) from NGL44. (Nirmal et al., 2023)

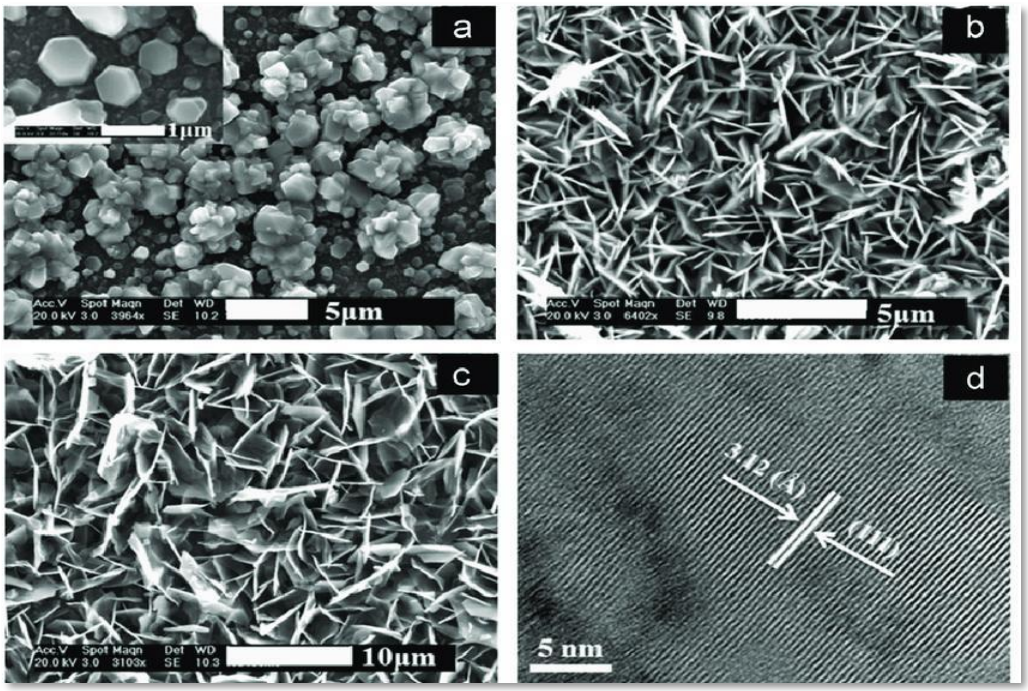


Figure 2.8: SEM images of iron sulfide thin films from precursor deposited at (a) 350 °C, (b) 400°C, and (c) 450 1C and (d) HRTEM at 400 1C showing d-spacing (3.12 ˚ A) corresponding to the (111) plane of the pyrite phase. (Akhtar et al., 2011).

2.6 Applications

Quantum dot-based nanogels are devices that aid in the tracking of objects and the prevention of theft. They are crime-prevention tools. When these Quantum dot-based nanogels are injected into criminals, they help to track them down. These quantum dot-based nanogels could be used in GPS tracking chips. This may aid in the detection and location of evidence, ensuring a proper chain of custody. Pesticides, toxins, drugs, amino acids, ions, pH, sugar, nucleic acids, and cells and tissues are also detected using quantum dot-based sensors (Duan et al., 2022). In-vivo and in-vitro drug transfer, as well as the use of trackers in security documents, are also applications. Quantum dots' fluorescence properties can also be used in inks and powders for security documents.

The use of DNA in nano-structured quantum dots is also used in drug detection. Another use for quantum dots is the detection of fingerprints. Quantum dots are used to detect blood-splattered fingerprints on non-porous surfaces. These quantum dots are used in the detection

and imaging of sweat fingerprints. Quantum dots could also be used to detect binding processes, bodily fluids, and other senses (Zhu et al., 2013).

Sensors based on quantum dot-based nanogels should be robust, sensitive, and stable. Their fluorescence-based activity should be visible enough for detection. Applications of quantum dot- based nanogels are displayed in Figure 2.9.

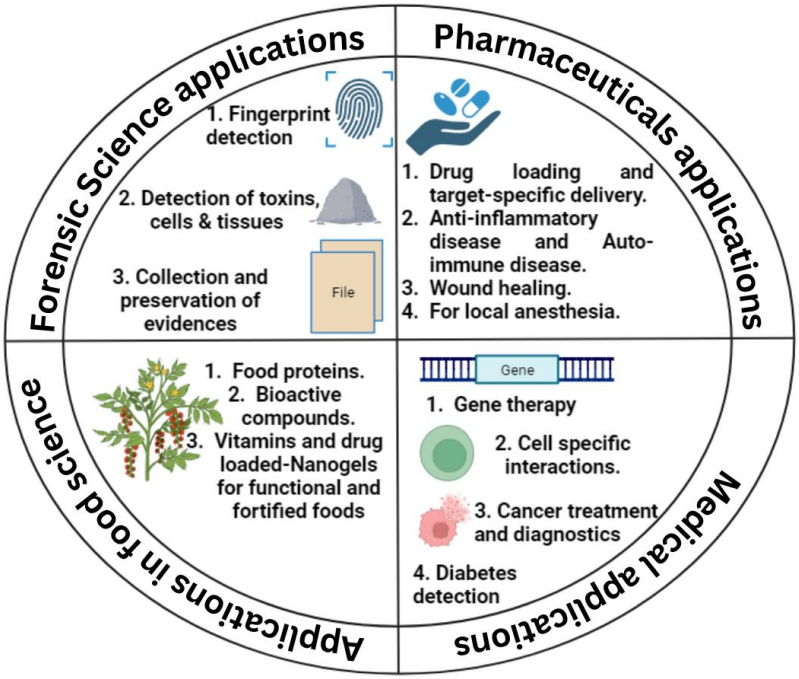


Figure 2.9: Illustration of applications of Quantum Dots in Forensic Science and other fields.

Detection of latent fingerprints: Detection of latent fingerprints has always been an important aspect of a person's individualization. It is used to connect a person, whether a suspect, victim, or perpetrator, to the crime scene. The fluorescence properties of quantum dots are used in the detection of fingerprints on porous, non-porous, and other surfaces.

Ballistics for forensic purposes: TNT and other dangerous explosives produce high sound and toxicity after an explosion, which is harmful to both biotic and abiotic factors. Various assays were used to detect TNT (Yi, 2016). Quantum dots, on the other hand, are quick and

precise. GSR, like many explosives and post-blast residues, can be detected.

Forensic document examination: Security documents are important from both an economic and forensic standpoint. These documents are susceptible to forgery and must be identified as the correct identification of documents. Quantum dots can be embedded in security threads and used in inks. This reduces the possibility of counterfeiting. Similarly, the identification of fingerprints by quantum dots on currency notes could help to prevent cases of corruption.

Forensic physics: The use of quantum dots as probes to detect lead ions in oily products such as hair dyes, masks, nail, and oil paints. This probing technique can also be used to identify paint and paint chips. These paint chip and oily product analyses aid in the investigation of various blending and blending techniques.

Forensic chemistry: Quantum dots nano-sensors are used in forensic chemistry to detect toxins, poisons, and drugs. They are also used for biomolecule and chemical detection. Their detection is quick, selective, and sensitive, and it takes less time. Anionic drugs, metallic drugs, phenolic drugs, pesticide poisoning, and drug overdose are all detected using QDs. Taggant technology employs them (Bhatt et al., 2020).

DNA forensic: DNA-based forensic analysis is used in biological, chemical, pharmaceutical, environmental monitoring, clinical diagnosis, and homeland security. Quantum dot biosensors can detect DNA, detect diseases, sequence GMOs, and detect fluorescence in situ hybridization (FISH).

Serology and forensic biology: Forensic biology typically deals with fluids, cells, and parts of various living organisms, such as flora, fauna, and other living organisms. Forensic serology is concerned with blood identification, grouping, and species identification. Fluorophores in quantum dots aid in the detection of various fluids, such as blood. A specific study demonstrates the synthesis of carbon quantum dots for the detection of 17-estradiol (Cheng et al., 2022).

Evidence location and chain of custody: With "green" solvents, methodologies, and capping agents, the fluorescence properties could be pliable. They are used to detect various biological, chemical, and physical agents due to their fluorescence and

photoluminescence properties. Quantum dots' luminescence properties ensure the proper chain of custody, as do the quantum dot- based nanogels and chips present. They help to maintain the integrity of evidence and the location of the evidence.

Research gap

While nanotechnology has transformed many areas of science, its use in forensic investigations is still developing. Most existing nanomaterials are produced using chemical methods that rely on toxic reagents and energy-intensive processes, which pose environmental and safety challenges. Although researchers have explored nanogels and quantum dots for various analytical and detection purposes, very few studies focus on producing them through green, sustainable methods that are safe for both users and the environment.

Moreover, previous work often treats material synthesis and forensic application as separate goals rather than integrating them into a single, practical framework. Important aspects such as large-scale production, long-term stability, and consistent performance under real forensic conditions have not been adequately addressed. These gaps highlight the need for developing an environmentally friendly nanomaterial that is not only efficient and reproducible but also truly applicable to real-world forensic analysis.

Hypothesis

Hypothesis 1: Green nanogels can be effectively synthesized through optimized variations in concentration, pH, and preparation methodology, and these nanogels will demonstrate significant degradation capacity under controlled conditions.

Hypothesis 2: Novel iron-based L-cysteine-capped quantum dots can be successfully synthesized, and it is hypothesized that these quantum dots will exhibit distinct and enhanced optical and electrical properties suitable for advanced analytical applications.

Hypothesis 3: Quantum dots, nanogels, and their synthesized nanocomposites can be effectively characterized for their surface morphology, particle size, composition, and related physicochemical properties, providing insights into their structural and functional attributes.

Hypothesis 4: The synthesized nanocomposite can be effectively applied to forensic principles, demonstrating its potential for enhancing detection, identification, and analytical procedures in forensic investigations.

Objectives of the research work

1. Exploring the chitosan-based nanogels with a greener methodology.

2. Synthesis and surface modification of Quantum dot-chitosan-based Nanogel.

3. Characterization of synthesized Quantum dot chitosan-based Nanogel.

4. Application of Quantum dot-based gel as optical sensors.

Chapter 3:

Materials and Methodology

3.1 Material for chitosan nanogels

Sigma Aldrich provided chitosan (deacetylated). Acetic acid was diluted from 99.5% to 75% using water and sodium hydroxide. Sigma Aldrich also provided glutaraldehyde (99%) in solution form.

3.1.1 Synthesis of Chitosan nanogels

Chitosan is a natural copolymer that has gel-forming capabilities. The amide groups of chitosan bond with the aldehyde group of glutaraldehyde to produce imide bonds via resonance with nearby double ethylene bonds. Glutaraldehyde reacts with primary amine groups to produce covalent glutaraldehyde (Mauri et al., 2021). Cross-linking of 1 glutaraldehyde molecule with 2 chitosan units forms 2 Schiff's bases (Pandey et al., 2024). Chitosan nanogels are prepared by manually stirring the chitosan soaked with dilute acetic acid (solvent) and a cross-linker. This nanogel embeds quantum dots as an optical part for detection. The mechanism of nanogel synthesis is shown in Figure 3.1. Chitosan is mixed with dilute acetic acid in the ratio of 1:10 and stirred. While stirring, add 1 ml of glutaraldehyde. This process immediately starts gelation (Monterio et al., 1999).

3.1.2 Mechanism

Chitosan is a natural copolymer with unique properties that help develop gels. The amide groups of chitosan interact with the aldehyde group of glutaraldehyde to form imide bonds due to resonance established with adjacent double ethylene bonds (Hong et al., 2024). Glutaraldehyde reacts with primary amine groups to produce covalent glutaraldehyde. This reaction could be possible in 3 propositions:

1) Formation of one Schiff's base (one aldehyde group of glutaraldehyde and one aldehyde group free).

2) Cross-linking of 1 glutaraldehyde molecule with 2 units of chitosan results in the formation of

3) Schiff's bases.

4) Cross-linking by polymerization of glutaraldehyde, which forms a cross-linking chain.

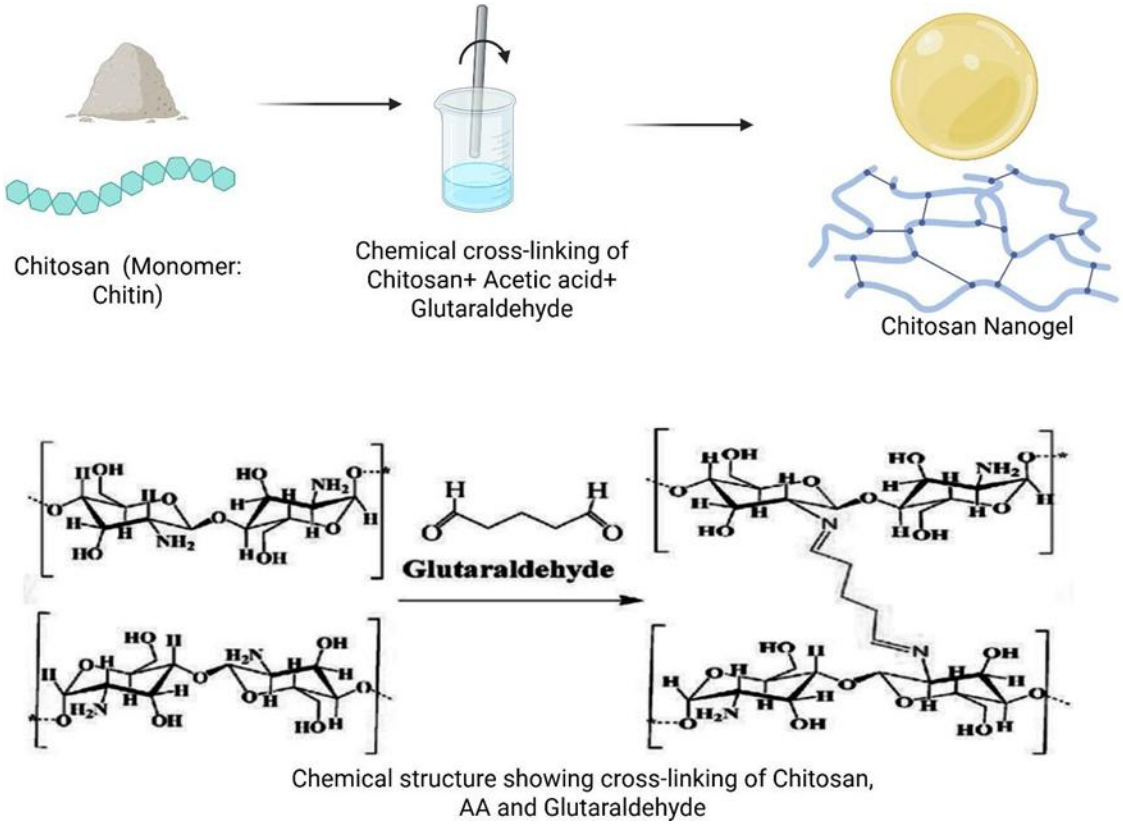


Figure 3.1: Synthesis of Chitosan nanogels and their mechanism

3.2 Material used for L-cystine-capped iron sulfide quantum dots formation

Ferric chloride, Sodium Sulphide, and L-Cysteine were procured from Sigma Aldrich chemicals. Distilled water was used as a solvent in the synthesis. The capping agent, L-Cysteine, was obtained from Sigma Aldrich chemicals. The study involves synthesizing FeS_2 quantum dots prepared using the hydrothermal technique with proper temperature control. Hydrothermal synthesis is used for its additional benefits in controlling the shape and size of the quantum dots (Mondal et al., 2016). Also, it allows the form of solution to be made under controlled temperature and pressure (Yuan et al., 2020).

3.2.1 Synthesis of Iron Sulphide Quantum Dots

0.1 M of Ferric Chloride and 0.1 M Sodium Sulphide were prepared, each of 500 ml

(Figure 3.2). The 200 ml (1:1) solution of Ferric Chloride (blue) and 200 ml of Sodium Sulphide (yellowish orange) was mixed by a magnetic stirrer into a round bottle flask, and the color changed to black. The temperature of the magnetic stirrer is kept constant. The flask is to be covered by aluminum foil with some holes to let the gases pass out. Approximately an hour later, 1.21 g of L-cysteine is added to the mixture. At this point, the color changes to greyish-white. The stirring is done for 3- 4 hours. The mixture is then left to cool down. The solution is washed and centrifuged with ethanol to clean any further impurities about 3-4 times at 5000 rpm. Let the sample dry and crystallize through heat at about 110 °C. The prepared quantum dots are sent for characterization.

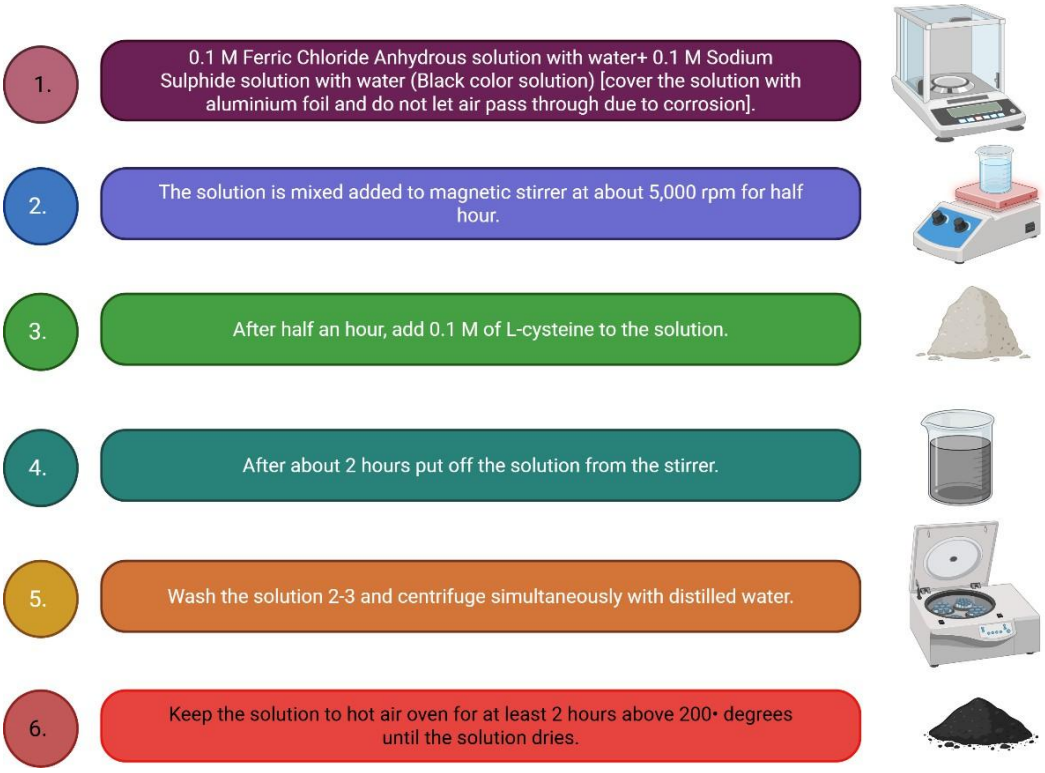


Figure 3.2: Methodology of the synthesis of Iron Sulphide Quantum dots

3.3 Surface modifications

For incorporating the Quantum dots in nanogels, three methodologies are tried:

1. Incorporating the chitosan along with the Quantum dots while preparing.
2. Incorporating the chitosan into pre-prepared quantum dots.
3. Introduction of Quantum dots into nanogels.

3.4 Characterization of chitosan nanogels

Nanogels are 3-dimensional structures that uphold swelling capacity and show excellent target- specific delivery (Wang et al., 2021). For studying the said characteristics, various studies are characterized. For studying the temperature-resistance of nanogel, TGA or thermogravimetric analysis is done (Alejo et al., 2021). This methodology tells us at what temperature the nanogel survives above 100 degrees. TGA was done in the Central Instrumentation Facility, LPU. Apart from thermal resistance, to study the surface area of the nanogels, SEM is used. Zeta potential is studied to study the potential and affinity of nanogels (Ashoori et al., 2020). Various other studies, like degradation studies, drug delivery studies, and biocompatibility studies, are done along with other cellular studies (Benamer Oudih et al., 2023).

3.5 Characterization of iron-based quantum dots

The quantum dots formed possess optical and physical properties that could be checked through various microscopic and spectroscopic techniques (Abdellatif et al., 2022). Characterization of nanogels was done for the thermostability and swelling properties of nanogels. TGA or thermogravimetric analysis was done by PerkinElmer Thermogravimetric analyzer (Model: TGA4000). FeS_2 Quantum dots can be analyzed through Fluorescence microscopy and spectroscopy (Liu et al., 2023). However, UV-VIS spectroscopy is used to check the absorption of Quantum dots at a particular wavelength (~Near IR range). UV-VIS spectroscopy was done using Perkin-Elmer UV spectroscopy (Model Ll-2800 Ex) (Shinde et al., 2020). Ethanol was used as a control, and the reading was noted from 300-900 nm. Fluorescence spectroscopy was performed by the Perkin-Elmer instrument (Model: FL 6500) at 250-300 nm and using an air filter. Optical characterizations were done at the Central Instrumentation Facility, LPU, Punjab, India.

Power XRD based on Bragg's law ($n\lambda=2dSin\Theta$, and electron microscopy (SEM and TEM) are used for studying various morphological and structural details of the quantum particles (Ullah et al., 2021). These provide the particle size for the conformation of the formation of quantum dots (2-10nm). XRD is also used to study the crystalline structure of the Quantum dots (Lee et al., 2022). FTIR is used for the compositional formation of Quantum dots (Tkachenko et al., 2022). SEM was computed by a JEOL Field Emission Scanning Electron Microscope with EDS and EBSD Sensors (Model: JSM-7610F Plus EDS: OXFORD EDS X-MaxN). TEM instrumentation was Hitachi (H- 7500) 120 kV equipped with CCD Camera. XRD results were computed using Bruker XRD (Model: D8 Advance). PerkinElmer Spectrum IR (Model: Spectrum 2) Version 10.6.1 was used for the compositional formations for studying FTIR from the Central Instrumentation Facility, Jalandhar.

3.6 Applications of Iron Sulphide Quantum dots based nanogel (Figure 3.3 & 3.4)

3.6.1 Latent Fingerprints on Non-Porous Surfaces

Latent (invisible) fingerprints are left on surfaces like tables, glass, and other smooth, non-porous materials during human contact. These prints are often invisible to the naked eye and require special techniques to be made visible for forensic investigation.

1. Synthesis of the Detection Material

   Iron Sulphide Quantum Dots (FeS QDs) are embedded into Chitosan Nanogels, which are biocompatible, biodegradable, and eco-friendly. Chitosan helps in easy dispersion and adherence to fingerprint residues. FeS QDs provide luminescent properties, crucial for visualization under UV light.

2. Preparation of Powder for Application

   The FeS QDs-loaded Chitosan Nanogels are dried and crushed into a fine powder. This powder is then applied using a brush onto the surface suspected to have latent fingerprints.

3. Fingerprint Development and Visualization

   Once applied, the powder adheres to the fingerprint residues (like oils and sweat). When

exposed to UV light, the Quantum Dots' fluorescence reveals detailed fingerprint patterns.

4. Advantages of This Technique

-Better Visualization: Due to the nanometric pore size, the material adheres well even to minute ridges of the fingerprint.

-Luminescence in Darkness: Quantum dots provide bright luminescence, allowing the fingerprint to be visible even in low-light or dark environments.

- Eco-Friendly and Biocompatible: The use of Chitosan makes the entire system safer and more sustainable than traditional fingerprint powders.

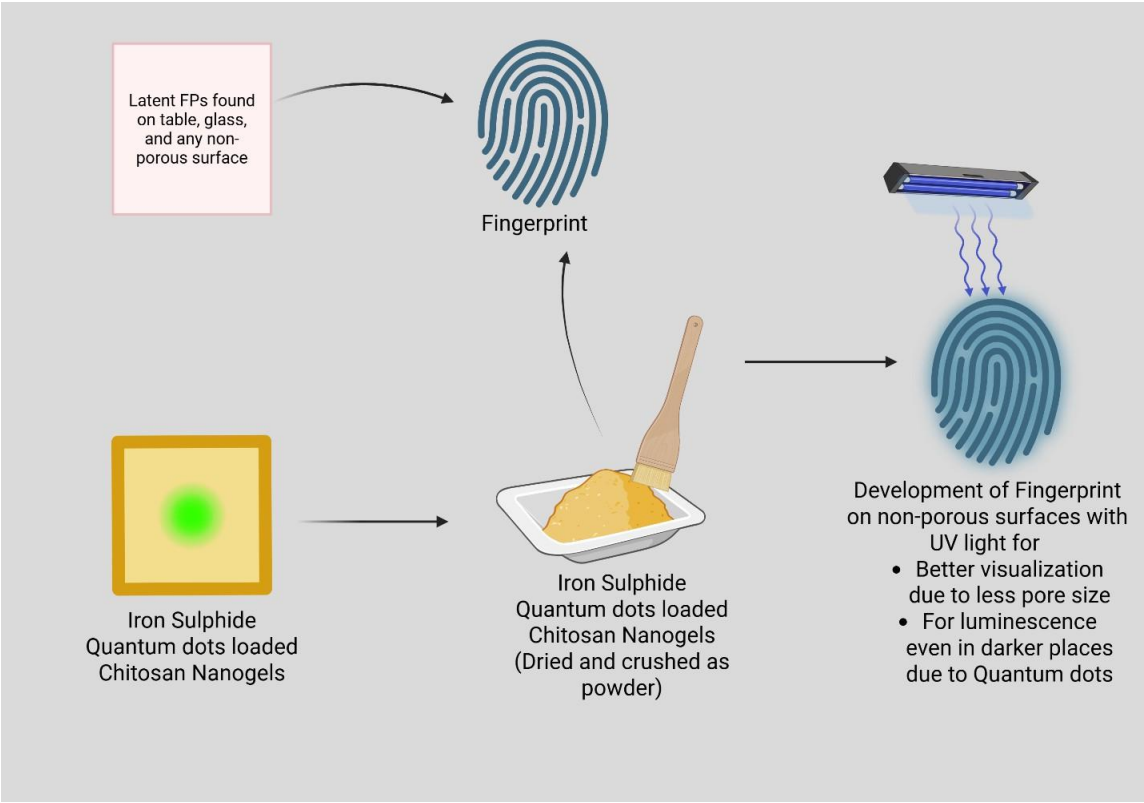


Figure 3.3: Illustration of a novel forensic application of Iron Sulphide Quantum Dots (FeS QDs) loaded into Chitosan Nanogels for the detection and visualization of latent fingerprints (FPs) on non-porous surfaces such as glass, tables, or metal.

3.6.2 Absorption Study of the common drug in the nanogel-quantum dot to study the absorptive properties of the nanogel.

1. Common Drugs (Paracetamol): These are standard over-the-counter medications used for pain relief and fever reduction.

2. Absorption in the Body: After oral intake (depicted by the pill and digestive system), these drugs get absorbed through various routes, such as the gastrointestinal tract. The image shows this with a focus on the gut, where the drug molecules pass through the intestinal lining into the bloodstream.

3. Drug Delivery via Nanogels and Quantum Dots: Instead of just conventional delivery, the drug can be encapsulated in nanogels. Quantum dots are used here for detection purposes—they fluoresce under specific conditions, allowing researchers to track the drug's presence and distribution.

4. Analysis Using UV Absorption Spectrometer: The drug concentration is then analyzed using a UV-Vis spectrophotometer, which measures how much UV light the sample absorbs. This helps determine how much drug is present at a given time.

5. Graph: Absorption vs. Time: The data collected is plotted on a graph showing drug concentration over time. This helps in understanding the pharmacokinetics—how quickly the drug is absorbed.

Calculation of absorption using statistics:

- Mean Absorption: Total of various absorptions over time versus total no. of absorptions taken.
- Standard deviation: It measures variation in the absorption values between the absorptions at each concentration.
- ANOVA (Analysis of Variance): It tells the significance of the difference between mean absorption values along different concentrations at different times.

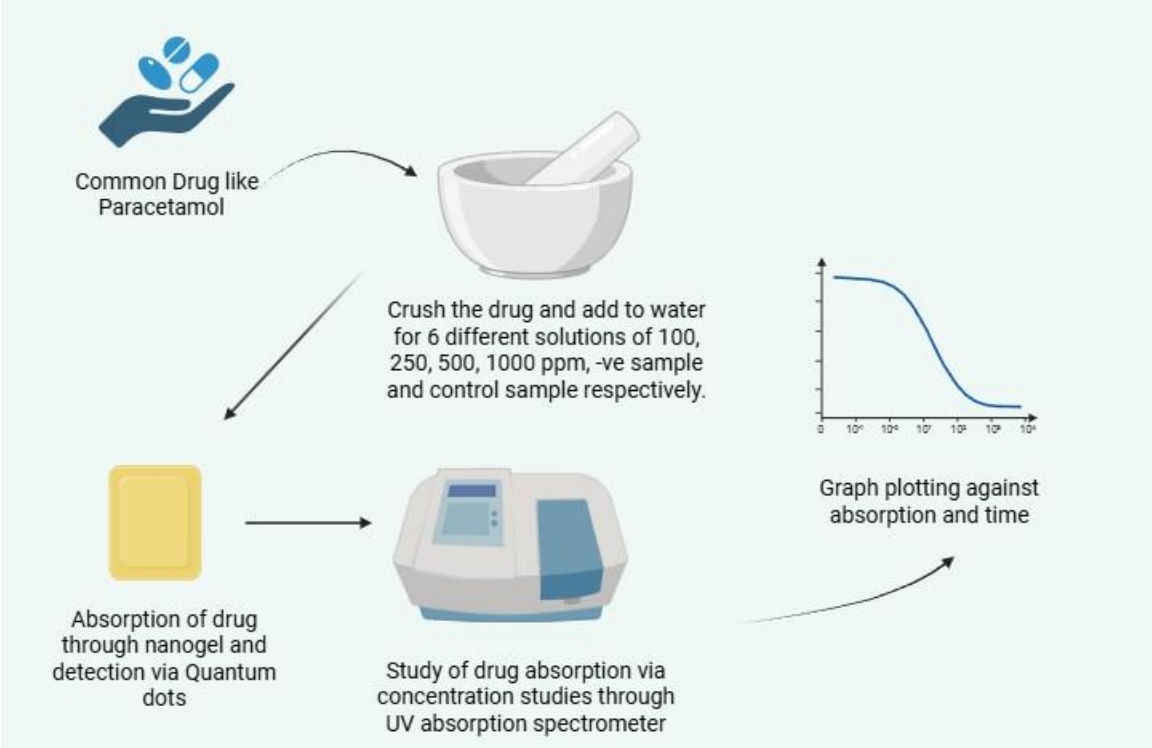


Figure 3.4: Illustration of the process of drug absorption and analysis, on common drugs like Paracetamol, and their monitoring using nanogels, quantum dots, and UV spectrophotometry.

Chapter 4: Results and Discussions

Objective 1: *Exploring the chitosan-based nanogels with greener methodology.*

The synthesis of nanogels is based on the various concentration-based optimizations of template, solvent, and cross-linker. Chitosan dissolves at a pH of less than 6. So, it is important to maintain the pH around 6 to dissolve the chitosan for the formulation of nanogels. Three different temperatures, i.e., 3, 5.75, and 7, are taken, but at pH 3, it doesn't dissolve at this pH. So, the optimal value was taken as 5.75. Chitosan: dilute acetic acid ratio is maintained for the proper dissolution of chitosan and the formation of nanogels (Figure 4.1). The quantity of chitosan is fixed according to the quantity of acetic acid used and vice versa. The ratio of chitosan dissolved in acetic acid is 1:3. The amount of solvent in a reaction ensures proper mixing and no lumping in a solution. Dilute acetic acid not only dissolves the chitosan but also provides a proper and unsaturated formation of nanogels.

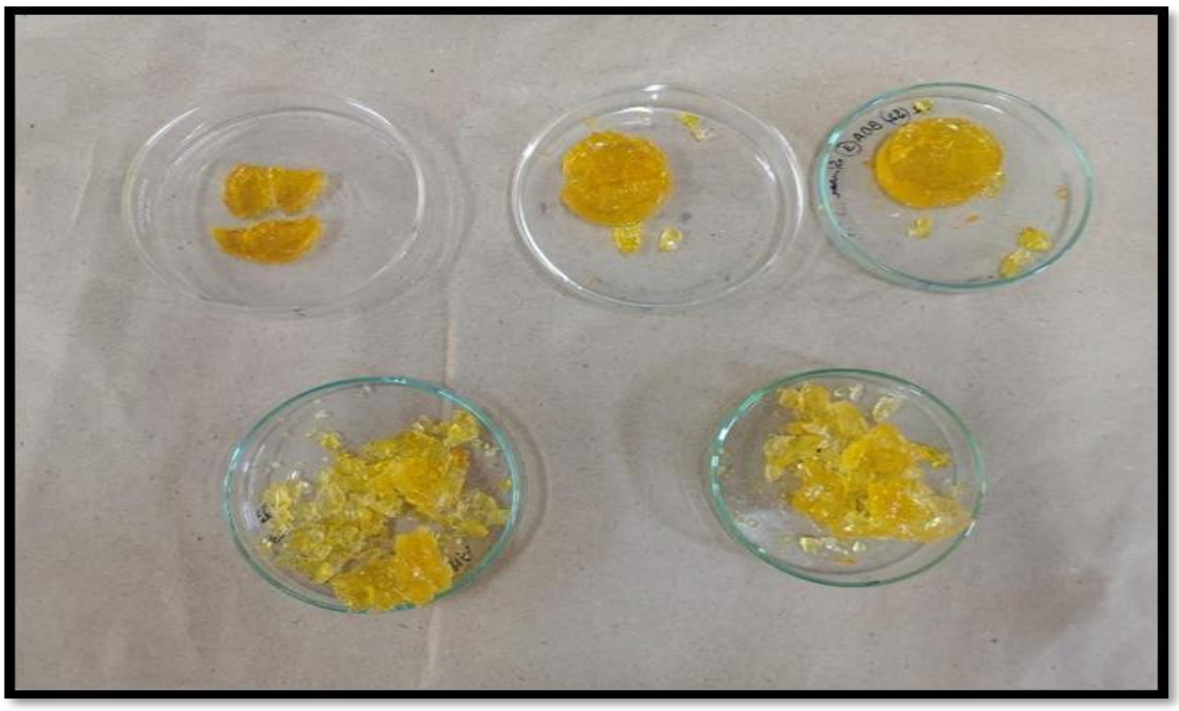


Figure 4.1: Optimizations of nanogels.

Table 4.1: Effects of pH on nanogels

S.R. No.	Chitosan (mg)	Dil. Acetic Acid (mL)	Dil. Glutaraldehyde (mL)	pH	Temp.	Results
1.	0.2	2	1	<3	35	Chitosan does not dissolve.
2.	0.2	2	1	**5.75**	35	Chitosan dissolves in the solvent.
3.	0.2	2	1	7	35	Nanogel disintegrates fast.

Chitosan dissolves at a pH of less than 6. So, it is important to maintain the pH around 6 to dissolve the chitosan for the formulation of nanogels. In the above table, three different temperatures, i.e., 3, 5.75, and 7, are taken, but at pH 3, it doesn't dissolve at this pH. So, the optimal value was taken as 5.75 (Table 4.1).

Table 4.2: Effects of chitosan amount on nanogels

S.R. No.	Chitosan (mg)	Dil. Acetic Acid (mL)	Dil. Glutaraldehyde (mL)	pH	Temp.	Results
1.	0.1	2	1	5.75	35	Nanogel does not form due to a greater amount of solvent.
2.	**0.2**	2	1	5.75	35	Nanogel survives

3.	0.3	2	1	5.75	35	Chitosan cannot be dissolved in a smaller amount of solvent.

Chitosan: Dilute Acetic acid ratio is maintained for the proper dissolution of chitosan and the formation of nanogels. The quantity of chitosan is fixed according to the quantity of acetic acid used and vice versa (Table 4.2).

Table 4.3: Effect of dil. acetic acid on nanogel

S.R. No.	Chitosan (mg)	Dil. Acetic Acid (mL)	Dil. Glutaraldehyde (mL)	pH	Temp.	Results
1.	0.2	1	1	5.75	35	Nanogel does not form due to the smaller amount of solvent.
2.	0.2	**2**	1	5.75	35	Nanogel survives
3.	0.2	3	1	5.75	35	Nanogel does not form due to a greater amount of solvent.

The amount of solvent in a reaction ensures proper mixing and no lumping in a solution. Dilute acetic acid dissolves the chitosan and provides a proper and unsaturated formation of nanogels. The amount of acetic acid used is first diluted (Table 4.3).

Table 4.4: Effect of dil. Glutaraldehyde on nanogel

S.R. No.	Chitosan (mg)	Dil. Acetic Acid (mL)	Dil. Glutaraldehyde (mL)	pH	Temp.	Results
1.	0.2	2	**1**	5.75	35	Nanogels survive
2.	0.2	2	2	5.75	35	Nanogel leaks solvent
3.	0.2	2	3	5.75	35	Nanogel leaks solvent

Glutaraldehyde is used as the cross-linker in the reaction. Once the chitosan is dissolved in the acetic acid, glutaraldehyde is added, and the cross-linking occurs. Cross-linking is a method of synthesizing nanogels. A cross-linker is added for the cross-linking process (Table 4.4.)

Objective 2: *Synthesis and surface modification of Quantum dot-chitosan based Nanogel.*
The prepared QDs are from the precipitation of Fe from Ferric Sulphide and S from Sodium sulfate. The addition of L-cysteine as a capping agent enhances the optical properties. The temperature control for the synthesis of QDs was optimal. The maintenance of temperature ensures homogeneous nucleation and speeds up the reaction process. The results of the study have been concluded through the following instrumental analysis. The synthesis protocol of L-cysteine- capped Iron sulfide-based quantum dots is explained in Figure 4.2.

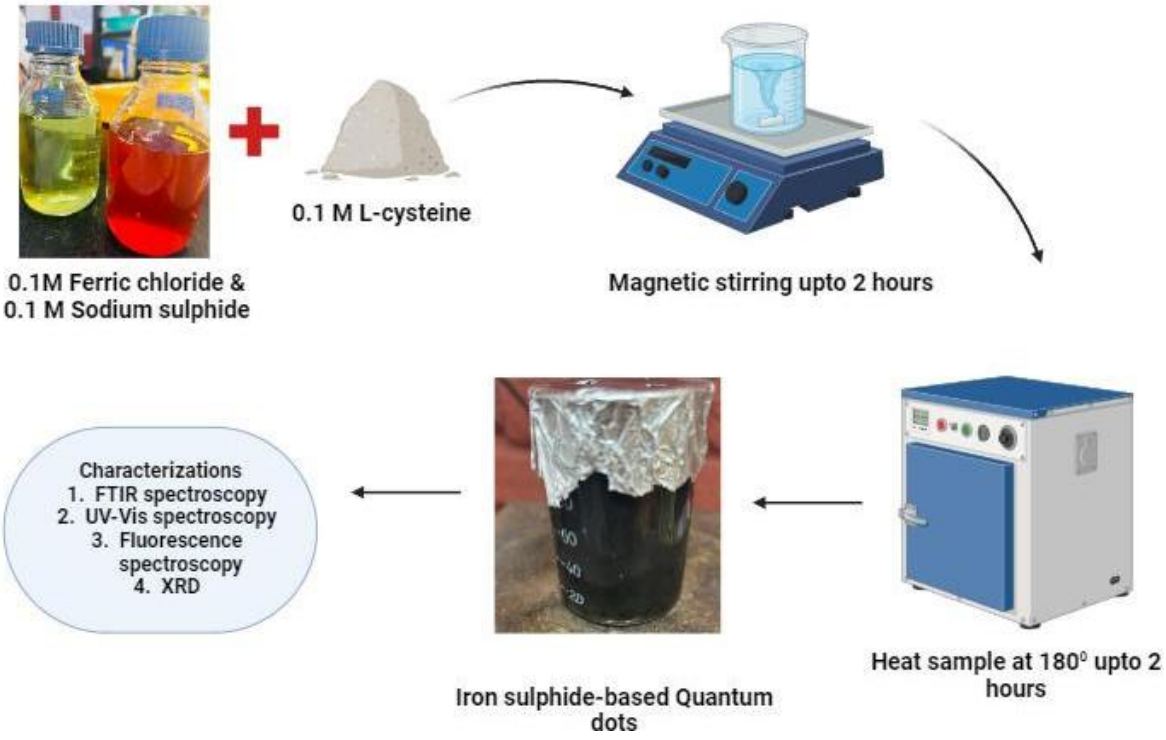


Figure 4.2: Synthesis methodology of Quantum dots

Objective 3: *Characterization of synthesized Quantum dot chitosan-based Nanogel.*

Characterization of Nanogels

Characterization of nanogels was done through absorption studies, thermogravimetric analysis, and FTIR analysis.

Characterization of Quantum Dots (Sharma et al., 2025)

Characterization of Quantum dots for size, shape, and morphology through FTIR, XRD, UV-vis, and fluorescence spectroscopy.

FTIR

Figures 4.3, 4.4, & 4.5 show standard IR interpretation standards for interpreting the data of FTIR analysis and the results. For the presence of chitosan groups, interpretation peaks at 3384 $cm^{-1,}$ which corresponds to primary amine and hydroxyl groups stretching vibrations; 1702 $cm^{-1,}$ corresponding to NH_2 hydroxyl groups; 1377 $cm^{-1,}$ corresponding to

C-N stretching vibrations of amine groups; 1154 $cm^{-1,}$ interpreting C-O-C bonds; and 613 cm^{-1}. The presence of thiol groups and the biological interaction of Iron sulfide indicate the presence of Iron sulfide (Figure 4.4). L- cysteine shows a characteristic peak around 2980 cm^{-1}. Moreover, the presence of the amine group at 1123 cm^{-1} and 1088 cm^{-1}. At 872 cm^{-1}, benzenes are present. Presence of amide groups at 1650 cm^{-1}. These confirm the presence of the interaction of Iron and Sulphide precursors and the capping of L-cysteine (Table 4.5). The presence of alkanes, amide bands, aliphatic groups, and aromatics is confirmed, which shows the presence of amide groups of chitosan, phenol, and amine groups of glutaraldehyde, and carboxyl groups in acetic acid. The presence of phenolic and alkyl halide groups confirms the presence of iron chloride; the presence of thiol groups in sodium sulfide and the sulfhydryl group, a thiol group, and a carboxylate group confirms the presence of L-cysteine.

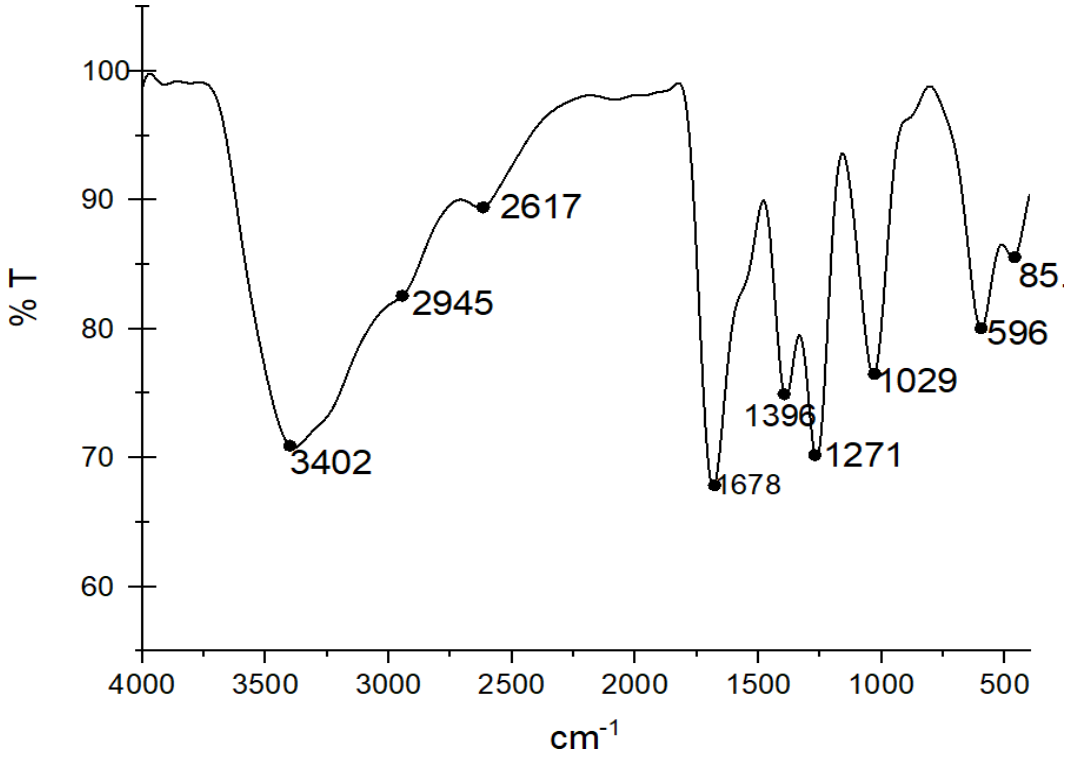
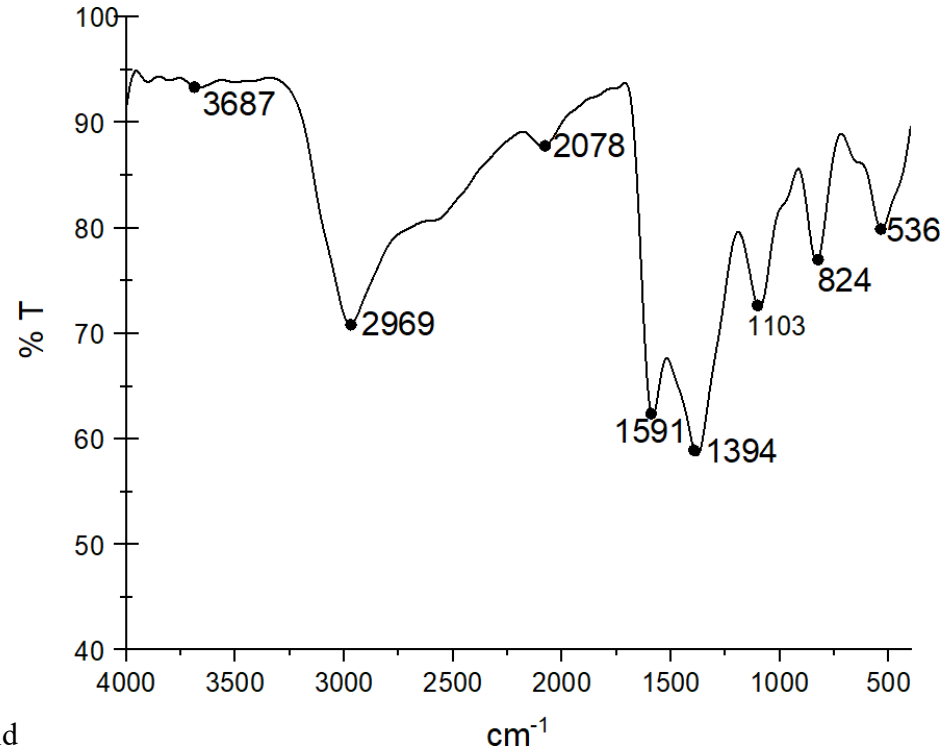


Figure 4.3: FTIR data of Chitosan nanogels. The presence of alkanes, amide bands, aliphatic groups, and aromatics is confirmed, which shows the presence of amide groups of chitosan, phenol, and amine groups of glutaraldehyde, and carboxyl groups in acetic



acid

Figure 4.4: FTIR data of FeS_2 quantum dots. The presence of phenolic and alkyl halide groups confirms the presence of iron chloride; the presence of thiol groups in sodium sulfide and the sulfhydryl group, a thiol group, and a carboxylate group confirms the presence of L-cysteine.

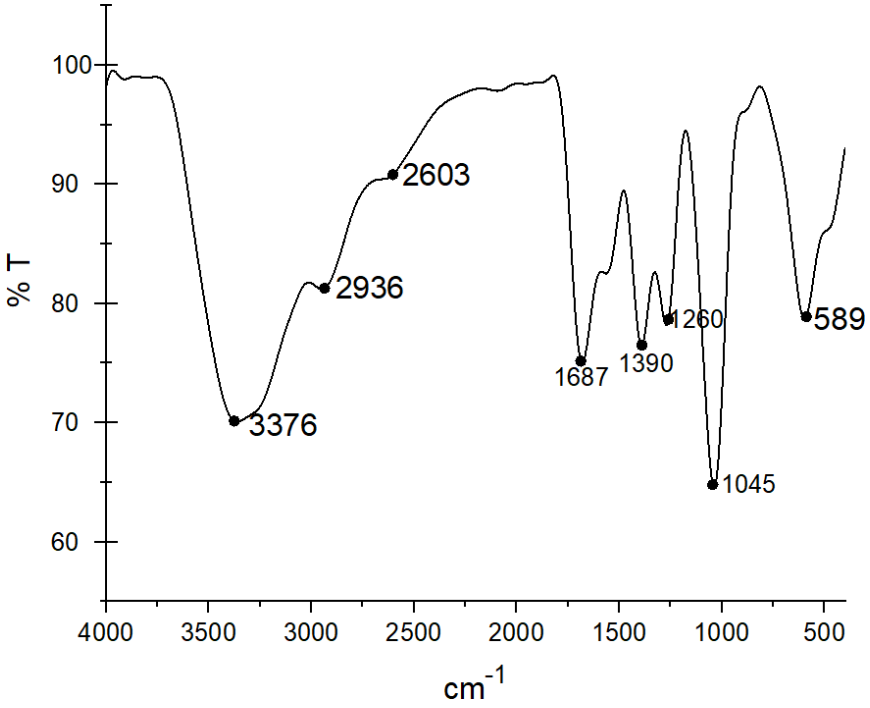


Figure 4.5: FTIR data of Nanogels containing Quantum dots. The standard IR spectrum interpretation table interprets the above.

Table 4.5: Interpretation of FTIR peaks

S.R. No.	Peaks	Interpretation
1.	3300-3350 cm^{-1}	Amine and hydroxyl groups
2.	1650-2000 cm^{-1}	NH_2 hydroxyl groups (Aromatics)
3.	1340-1250 cm^{-1}	C-O stretching vibrations
4.	2500-3000 cm^{-1}	O-H (Alcohols, phenols, and carboxylic acids)
5.	3000-2840 cm^{-1}	Alkanes (C-H stretching)
6.	1350-1000 cm^{-1}	Amine groups (C-N stretching)
7.	Below 900 cm^{-1}	Presence of aromatics
8.	1650-1580 cm^{-1}	Amide groups

TGA

Thermogravitational analysis, or TGA, is studied for the temperature-based degradation of nanogels. In various studies, nanogels have been prepared by using various heating or sonic waves. To differentiate between the nanogels prepared from microwave synthesis and those without microwave synthesis, thermogravimetric studies are conducted. Figure 4.6 represents the data of nanogels prepared from microwave (A) and without microwave synthesis (B). The findings are that the nanogels prepared without microwave synthesis are more temperature-resistant and degrade later than the ones prepared by using microwave synthesis. The final residue at the end of the test describes the material that is not decomposed or does not volatilize due to the test, as the loss of mass occurs in stages.

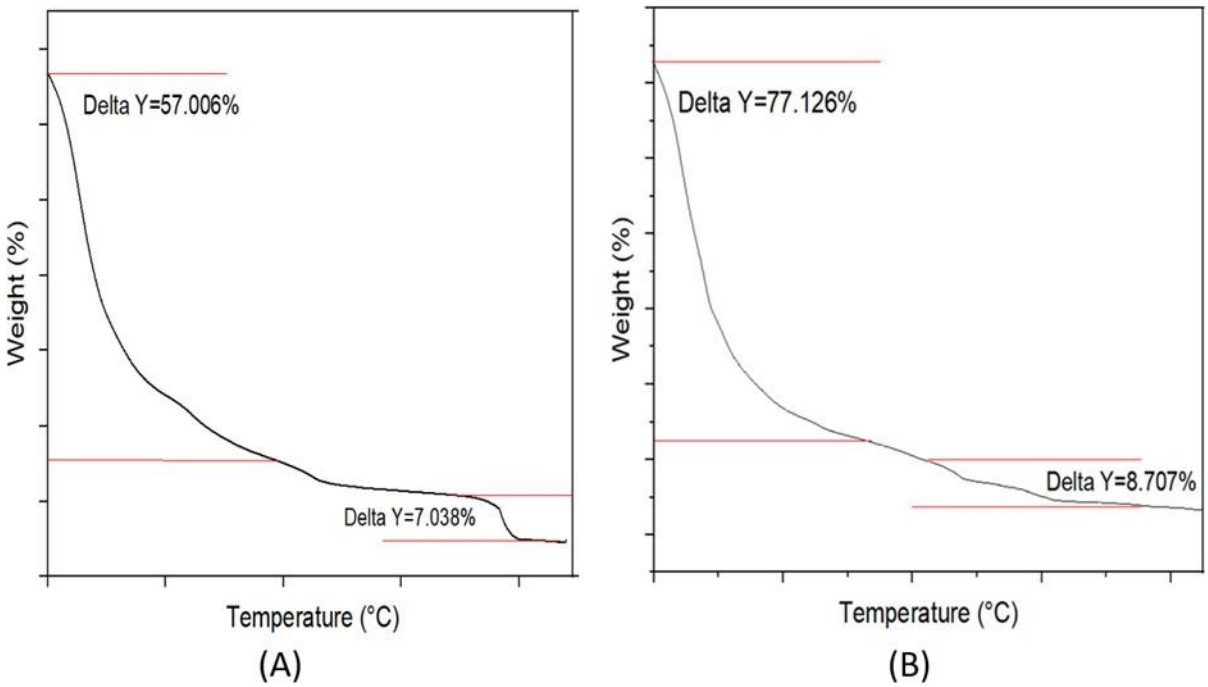


Figure 4.6: TGA of nanogels: (A) Nanogels prepared by microwave synthesis; (B) Nanogels prepared by cross-linking without microwave synthesis.

XRD

In the XRD pattern, the characteristic peaks were observed at 28.7°, 33.3°, 34.6°, and 38.2° corresponding to planes (111), (200), (211), and (220), respectively, that feature the crystallinity of the FeS_2 Quantum dots. The crystal formed at 31 degrees is an orthorhombic

crystal (31-33 degrees), and around 45 degrees is a tetragonal crystal. However, the elemental analysis of these crystals will be studied by JCPDS data. The graph data corresponds to the data provided in Figure

4.7. The phase structure of FeS_2 and the morphology of the FeS_2 thin film studied by XRD graph were analyzed by card indexed to the standard iron pyrite (FeS_2, ref card no. 961534894). This analysis is reported as a Fe-shaped lattice with S ions embedded. The fine structure of Fe ions in a divalent state corresponds to the disulfide anions, which are closed entities bonded by one Fe and two S.

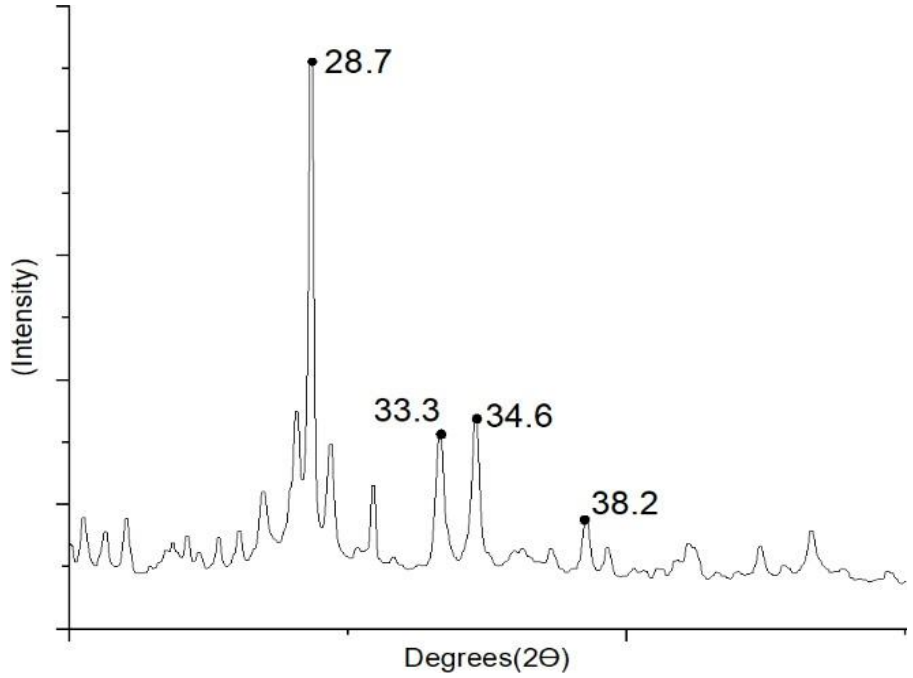


Figure 4.7: XRD data of FeS_2 Quantum dots

To calculate size from XRD data

To calculate the crystallite size from the XRD data, we use the Debye–Scherrer equation:

Debye–Scherrer Formula: $D=K\lambda/\beta\cos\theta$

Where:

- DDD = crystallite size (in nm or Å)
- K = shape factor (typically 0.9)
- Lambda λ = X-ray wavelength (for Cu Kα, it's 1.5406 Å)
- Beta β = Full Width at Half Maximum (FWHM) of the peak (in radians)

- Theta θ = Bragg angle (in radians, half of 2θ)

From our XRD Peak at 2θ = 28.7°:

A sample FWHM (β) = 0.5° = 0.00873 radians. Final Answer (with assumed FWHM = 0.5°): Crystallite size D≈16.4D approx 16.4D≈16.4 nm

UV-VIS Spectroscopy

UV-visible spectroscopy measures the optical properties of quantum dots and tells the analyte's absorption at a particular wavelength. Figure 4.8 represents the sample's excitation band in the UV section. These bands are detected at 250-275 nm. It corresponds to the excitation of the FeS_2 quantum dots that occurs in the UV range.

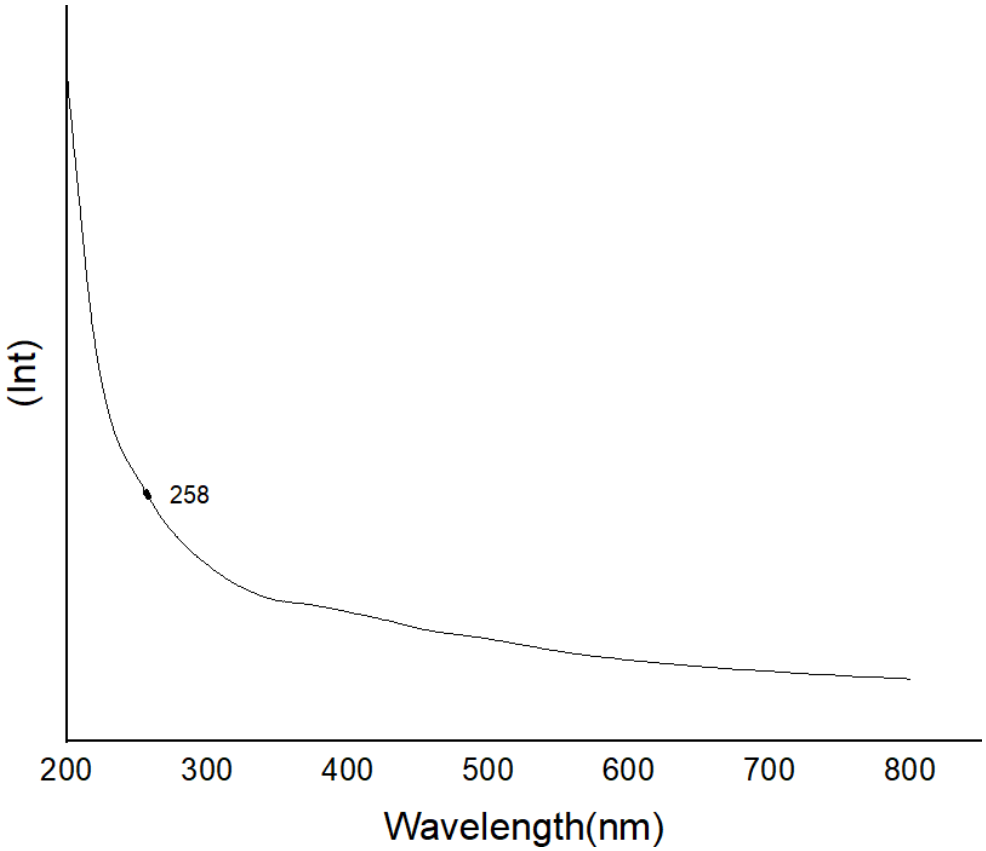


Figure 4.8: UV-visible data of FeS_2 Quantum

Fluorescence spectroscopy

Fluorescence spectroscopy is done to study the excitation and emission wavelengths

of the optically active substance at a particular intensity. Based on the UV data of the quantum dots, fluorescence scans for excitation and emission bands were studied. Fluorescence data of quantum dots of Iron Sulphide at an excitation of 200-700 nm is shown in Figure 4.9. At an excitation wavelength of 258 nm, an emission band of 568 nm was obtained.

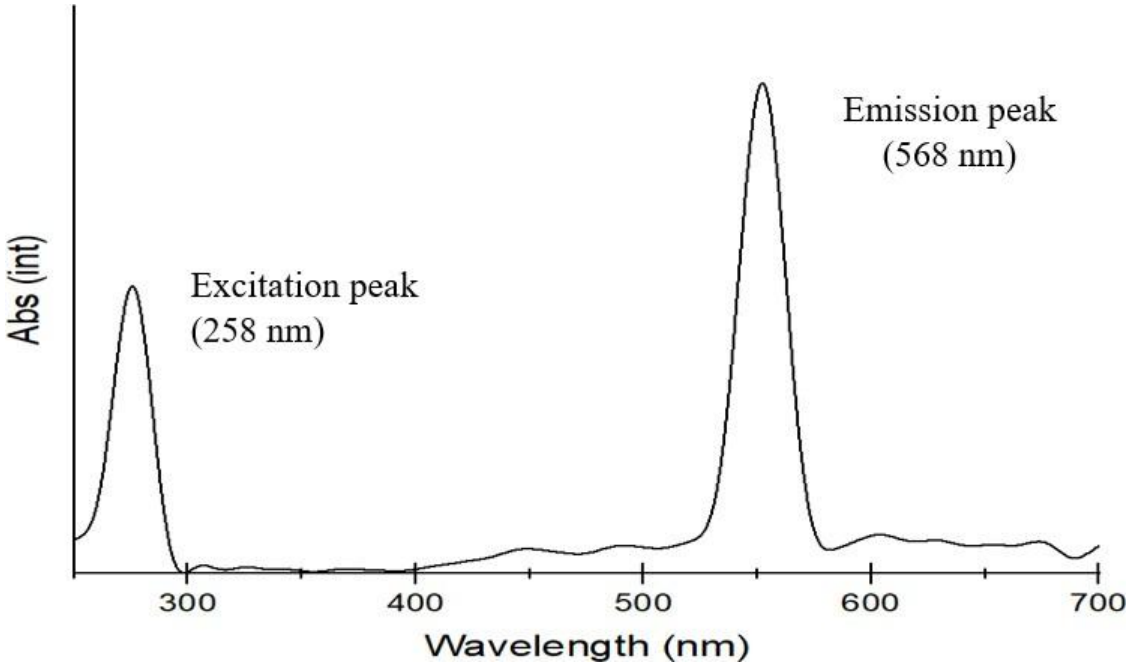


Figure 4.9: Fluorescence spectroscopy data of Quantum dots

Electron microscopy

Scanning microscopy reveals the presence of dots sized particles at 100 nm range as shown in. Figure 4.10.

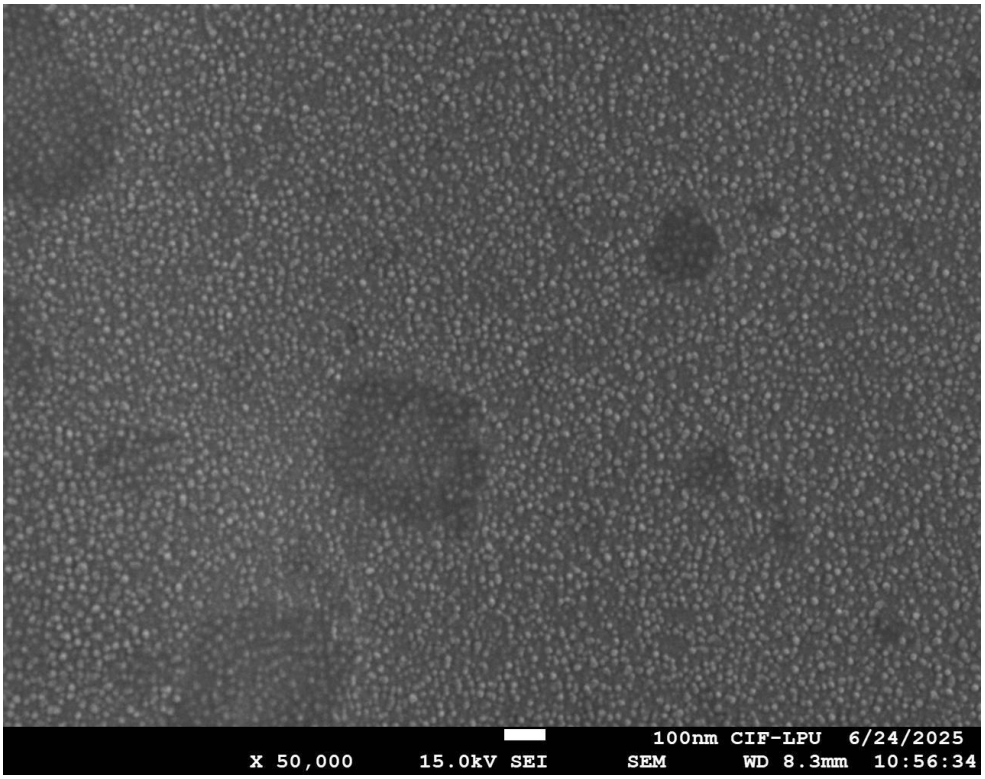


Figure 4.10: SEM image showing quantum dots particles.

Transmission electron microscopy and SAED patterns confirms that the analyzed material is polycrystalline or nanocrystalline possessing a well-defined crystal structure analyzed by sharp rings.

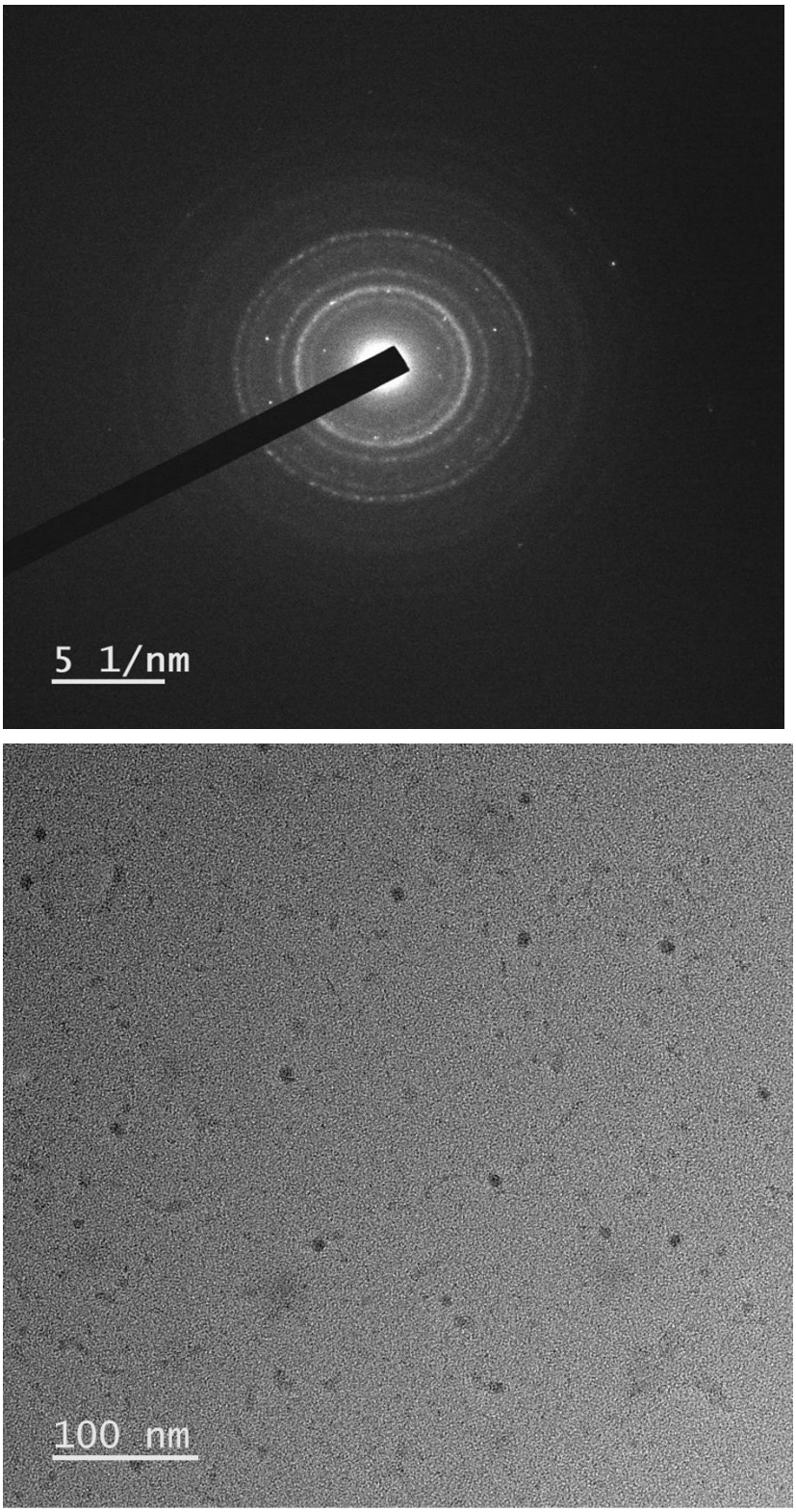


Figure 4.11: A. SAED pattern of analyzed material B. TEM image at the scale of 100 nm.

Objective 4: *Application of Quantum dot-based gel as an optical sensor.*

Latent Fingerprint detection using the powder-based nanogel-sensor

Latent fingerprints were developed on non-porous surfaces such as wooden tables, glass, and UV light was used for the detection of these prints (Figure 4.12). The fingerprints were developed by using a camel hair brush, which is usually used to develop fingerprints. The developed prints showed a high level of specificity of tertiary-level details. Also, the use of UV light brings out the fluorescence of the quantum dots.

Figure 4.12: Latent Fingerprint Detection using quantum dots

Advantages of the development of latent prints using fluorescent Nanomaterials

The problem with traditional methods of fingerprint development includes low contrast, low selectivity, toxicity, and low sensitivity (Wang et al., 2017). The problem with low contrast being similar to signal-to-noise ratio, is changed by either improving the signal or reducing the background. Signal improvement is done by enhancing the fluorescence of the material, and the background is reduced by avoiding the color distraction and decreasing the interference. In traditional methods, the color and contrast affect the development of fingerprints. Quantum dots emitting high fluorescence in the UV and NIR

regions enhance the signal and reduce the distraction of background color. Low sensitivity due to the particle size and shape of the fingerprint powder causes the low visibility and clarity of the ridge details. The particles of fluorescent nanomaterials are nano-sized (Not more than 100 nm), which could also be altered during synthesis. Also, in these traditional fingerprint powders, selectivity is very low, adhering to the papillary ridges of the prints and even humid substrates, thus developing low-quality prints. The binding affinity of the nanomaterials could be altered depending on the electric charge and specific residues in fingerprints.

Drug absorption of Paracetamol based on various concentrations and time gaps. Paracetamol is the most common over-the-counter drug used to treat pain and allergies. The drug is crushed and then dissolved into distilled water for proper absorption into the nanogel-quantum dots. The absorption was noted at different time intervals and at various concentrations (C1, C2, and C3, which are 250, 500, and 1000 ppm, respectively, along with a negative and control sample). The time versus absorption graph of multiple concentrations was plotted and is described below in Figure 4.13.

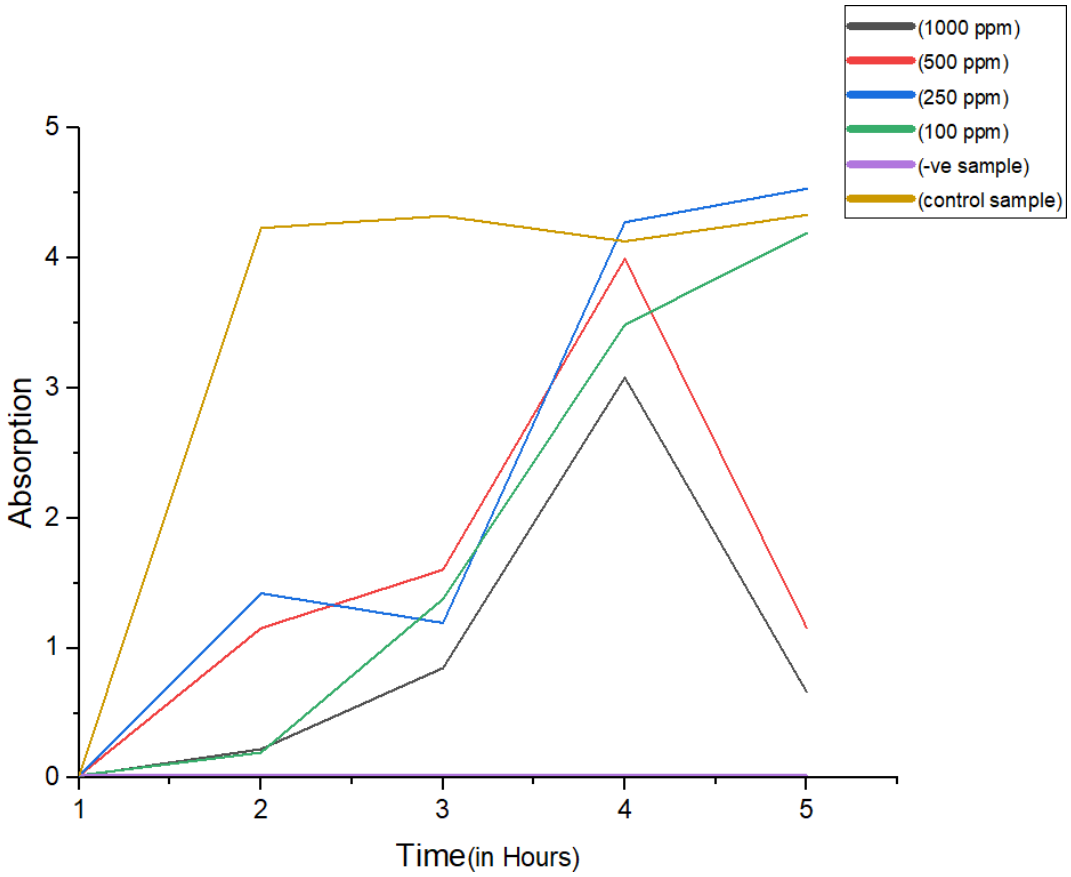


Figure 4.13: Application of Quantum dot-based nanogels for the adsorption studies of drugs (Paracetamol). The curve shows the retention time of drugs inside the nanogel before detention at different concentrations. (Peng et al., 2021).

The drug at concentration 250 ppm shows high absorption along with the control sample. The negative sample shows no or minimal absorption due to presence of quantum dots. At 4^{th} hour, the absorption is maximum.

Mean absorption of the drug concentrations

The mean absorption calculates the mean absorption at each concentration at different times, divided by the total number of absorptions taken (5) as shown in Table 4.6.

Table 4.6: Mean absorption of the samples.

S. R. No.	Sample	1st hour	2nd hour	3rd hour	4th hour	5th hour	Mean Absorption
1.	1000ppm	0.016	0.223	0.846	3.080	0.663	0.9656
2.	500 ppm	0.019	1.153	1.603	3.993	1.154	1.5844
3.	250 ppm	0.0220	1.421	1.194	4.274	4.532	2.2882
4.	100 ppm	0.0220	0.197	1.378	3.488	4.189	1.8548
5.	-ve sample	0.0220	0.0220	0.0220	0.0220	0.0220	0.0220
6.	Control sample	0.023	4.231	4.321	4.127	4.330	3.4068

Standard deviation of the absorption

The deviation measures the variation in absorption over time at each concentration. High SD values suggest the variation in absorption dynamics, especially at higher concentrations and longer durations. Table 4.7 gives the standard deviation of absorption over time at various concentrations.

Table 4.7: Standard deviation of the drug absorption studies

S.R. no.	Sample	$\bar{x}$	Sum of squared differences	Divided by (n-1)	Square root
1.	1000 ppm	0.9656	0.9006+0.5511+0.0143+4.4710+0.0915= 6.0285	1.5071	1.2278
2.	500 ppm	1.5844	2.4515+0.1861+0.0003+5.8044+0.1853=8.62 76	2.1569	1.4683
3.	250 ppm	2.2882	5.1537+0.7515+1.1979+3.9416+5.0362=16.0 810	4.0203	2.0045
4.	100 ppm	1.8548	3.3687+2.7455+0.2273+2.6727+5.4488=14.4 630	3.6157	1.9007
5.	-ve sample	0.0220	0+0+0+0+0=0	0	0
6.	Control sample	3.4068	11.4279+0.6790+0.8370+0.5192+0.8543=14. 3174	3.5794	1.8934

Discussions

Synthesis of nanogels

Chitosan nanogels are formed by dissolving chitosan (acetylation at 75%) in dilute acetic acid (75%) in the ratio of 1:10. The dissolved mixture of chitosan is then poured with 1 ml of cross- linker (glutaraldehyde), which starts the gelation process. Adding more or less of the cross-linker might result in the leaching of the nanogel. Based on this study, swelling studies of nanogel were carried which implies that the nanogel swelled up to 85% of its original weight within 1 hour.

Synthesis of quantum dots

Iron Sulphide quantum dots are synthesized by the hydrothermal method. The Iron core enhances the functionality of quantum dots. It improves the optical and electronic properties of quantum dots. The Sodium-based shell has a wider bandgap than the core and thus acts as a protective layer. It enhances the stability of the quantum dot and also provides photoluminescence efficiency and reduces surface defects. The L-cysteine capping agent acts as a protective layer for corrosion and can be used as a tunable agent for optical properties and biocompatibility.

Fourier Transform Infrared Spectroscopy (FTIR)

Figure 4.3 Interpretation: The strong broad band at 3402 cm-1 suggests the presence of hydroxyl groups-possible due to water content, alcohol, or phenolic OH groups, which is common in nanogels based on natural polymers like chitosan. The presence of aliphatic groups is visible around 2945 cm-1, characteristic of methylene or methyl groups present in organic polymer backbones. The C=O stretches are visible around 1678 cm-1, showing the presence of carboxylic acids present in cross-linked polymeric networks and biopolymers. Possible esters, ethers, and amine linkages are seen at 1271 cm-1 and 1029 cm-1, suggesting the formation of Schiff's bases. The frequency bands at 596 cm-1 and 85 cm-1 hint at inorganic interactions, which are seen in nanomaterials such as nanogels.

Figure 4.4 Interpretation: The presence of O-H stretching is seen at 3687 cm-1. C-H

stretching, basically from residual organic stabilizers or capping agents, is present at 2969 cm-1. Other peaks showing amine groups and thiol-based capping agents are present at 2078 cm-1 (C≡N stretching in case of nitrile group). Also, it suggests a possible weak overtone of the Fe-S overtone. The peak at 536 cm-1 generally appears in Fe-S stretching. The peak at 536 cm-1 gives a good match for the Fe-S stretching vibration.

Figure 4.5 Interpretation: The peak at 3376 cm-1 shows absorption due to water molecules (OH stretching), hydroxyl functionalization. Characteristic of the alkyl chain and C-H due to the presence of an organic capping agent (L-cysteine), surfactant, or polymeric stabilizers is present at 2936 cm-1. At peak 2603 cm-1, a possible S-H bonding or overtone is present due to L-Cysteine, which is a thiol-containing capping agent. The presence of strong carbonyl groups (aldehydes, ketones, carboxylic acids, or amines) is seen at 1687 cm-1, present due to bioorganic material or nanocomposite. Polysaccharide-based coatings, or bio-stabilizers, may be due to alcohol or ether functionalities are seen at 1045 cm-1. The peak at 589 cm-1 represents a signature peak of metal- based nanoparticles or Quantum dots.

Thermogravimetric Analysis (TGA)

Thermogravimetric Analysis (TGA) provides critical insights into the thermal stability and degradation profile of the synthesized nanogels. Figure 4.6 illustrates the TGA profiles of nanogels prepared with and without microwave synthesis. The TGA results reveal that chitosan nanogels prepared without microwave synthesis exhibit higher thermal stability compared to those synthesized using microwave methods. The weight loss of chitosan in the first stage is higher, but is higher in the nanogels prepared with the microwave method as compared with the manual stirring. The degradation temperature is higher for the non-microwave synthesized nanogels, indicating that the cross-linking process without microwave results in more thermally stable structures. This enhanced thermal stability could be attributed to the more uniform and extensive cross-linking that occurs in the absence of microwave-induced rapid heating, leading to a more stable nanogel network.

In contrast, the nanogels synthesized via microwave methods degrade at lower temperatures, suggesting that the rapid heating might lead to less stable cross-linking,

resulting in earlier degradation. These findings are crucial for potential applications where thermal stability is a significant consideration.

X-ray diffraction (XRD)

The XRD analysis (Figure 4.7) provides comprehensive information regarding the crystalline structure of the synthesized FeS quantum dots. The characteristic peaks observed at 28.7°, 33.3°, 34.6°, and 38.2° correspond to planes (111), (200), (211), and (220), respectively. These peaks are indicative of the crystalline nature of FeS2 quantum dots and align well with the standard iron pyrite (FeS2) phase. The presence of these peaks confirms the successful synthesis of crystalline FeS2 quantum dots. The XRD pattern indicates that the Fe ions are in a divalent state, forming a lattice structure with disulfide anions embedded in a close-packed arrangement. This structural arrangement is significant for the stability and functionality of the quantum dots, particularly in their application in nanogels for various biomedical and environmental applications.

UV-Visible Spectroscopy

UV-visible spectroscopy was utilized to evaluate the optical properties of the synthesized FeS2 quantum dots. The absorption spectra (Figure 4.8) show distinct excitation bands in the UV range (250-275 nm). These bands are characteristic of FeS2 quantum dots and confirm their successful synthesis. The absorption peaks in the UV range are indicative of the quantum dots' electronic transitions, which are essential for their application in optical and electronic devices. The specific absorption at these wavelengths also suggests that the quantum dots have a narrow size distribution and uniform morphology, which is crucial for their consistent performance in applications.

Fluorescence Spectroscopy

Fluorescence spectroscopy was employed to investigate the optical activity of the synthesized FeS2 quantum dots. The excitation-emission profile (Figure 4.9) reveals a significant emission band at 568 nm when excited at 258 nm. This fluorescence behavior

is characteristic of FeS2 quantum dots and confirms their optical activity. The emission peak at 568 nm indicates that the quantum dots are capable of emitting light in the visible spectrum, making them suitable for applications in bioimaging and sensing. The sharp and distinct emission peak also suggests that the quantum dots have a high quantum yield and minimal defect states, which are essential for their efficiency in optoelectronic applications.

Electron microscopy

Scanning microscopy confirms the presence of dot shaped particles of quantum dots and along with the size study of transmission electron microscopy and SAED analysis.

Latent Fingerprint Development

While the traditional methods are less sensitive, less selective, and have low contrast. quantum dots with their tunable size and shape can be modified as per the substrate and used to develop prints which are highly visible and get attached to ridges and furrows of the latent fingerprints present on the non-porous surfaces.

Drug absorption studies

Nanogel, due to its high pore size, is an excellent absorber for any drug. Paracetamol (An OTC drug) is absorbed into the nanogel-quantum dot nanocomposite to study the absorption properties of the nanogel. The absorption of the drug is studied over time (up to 5 hours) using various concentrations (100 ppm, 250 ppm, 500 ppm, and 1000 ppm). Also, negative and blank samples are studied together. Standard deviation measures the dispersion of the absorption values at different points from their mean absorption. A higher standard deviation value means greater variability, whereas a lower SD indicates more consistent absorption readings over time. In the absorption study, where absorption changes over 5 hours at different concentrations, SD tells the stable and erratic behavior of the sample.

Chapter 5:

Conclusion

Conclusion

In this thesis, the main objective was the preparation of chitosan-based nanogels that are prepared by keeping in mind the principles of green chemistry. The term “Green Chemistry” signifies the preparation of a product that is prepared by using minimal chemicals, which are non-toxic, and are efficient, and can be easily biodegraded. The methodology used to make such a product should be efficient, time-saving, money-saving, and produce a product that is compatible with nature and gives maximum output. This “green nanogel would be used to incorporate a non-toxic, optically- active, and gives a good quantum yield, which would be used to classify and detect evidence in the Forensic domain and other biomedical or medical applications. The study successfully demonstrated that the preparation of chitosan nanogel is green indeed and a one-step synthesis. The template or backbone of the nanogel, which is chitosan, is a non-toxic, animal-derived polymer that is a polysaccharide, and as per the literature review, is also biocompatible and biodegradable. The quantum dots prepared from Iron and Sodium precursors capped by L-cysteine provide extra optical and surface properties to the nanogel. The nanogel, having a high pore size, makes it easy for the absorption of solvent or any analyte into the nanogel. The characterizations of nanogel done by the thermogravimetric analysis, or TGA, interpret the production of nanogel that is durable (up to 3 days to a week) and can sustain higher temperatures, which can further make it biodegradable. The characteristics of the quantum dots studied by XRD, FTIR, UV-vis, and Fluorescence spectroscopy help in the assessment of the optical nature of these quantum dots and the crystallinity of the particles, along with the presence of bonding among the particles. Further, the nanogel can be used as an optical powder for fingerprint detection or as a sponge-like object for absorption of any drug or toxins, or analytes. In this study, paracetamol was successfully absorbed in the nanogel-quantum dot nanocomposite. These findings indicate that the nanogel- quantum dot nanocomposite synthesized demonstrated an excellent encapsulation efficiency along with crystalline size and optical properties. While the research achieved its aims, certain limitations, like preparing the nanogel on an industrial level for experimenting on a large

scale, blinking, and leaching properties of the quantum dots, can hinder the preparation and should be considered. Future studies could explore various microfluidic or paper-based devices based on the nanocomposite sensor. Overall, this work contributes to various other applications in biomedical, medical, agricultural, pharmaceutical, chemical, and nanotechnological applications, apart from Forensic biological, chemical, and toxicological applications.

The present study, while demonstrating successful synthesis and characterization of an eco-friendly nanomaterial, has certain limitations. The characterization was restricted to a selected range of analytical techniques, which may not fully capture long-term stability, surface charge dynamics, or aggregation behavior under varied environmental conditions. Additionally, large-scale synthesis and real forensic case validations were not undertaken within the current scope. These aspects may influence reproducibility and the broader applicability of the material in diverse forensic scenarios.

Future research will focus on expanding the analytical validation of the synthesized nanomaterial by incorporating advanced characterization methods such as XRD, zeta potential, and DLS. Further studies will explore large-scale, green synthesis optimization to assess scalability and environmental safety. The nanomaterial's performance will also be evaluated using real forensic samples to determine its sensitivity, selectivity, and stability under field conditions. Additionally, efforts will be directed toward integrating the developed material with modern forensic detection platforms such as fluorescence-based and spectrometric systems to enhance its practical applicability.

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List of publications

1. Sharma, A., Tharuny, S., Pandey, T., & Pandey, V. (2025). Eco-Friendly Fabrication of FeS_2 QD-Chitosan Biopolymer Composites: Green Synthetic Approach. *Biopolymers*, *116*(2), e70002.

2. Pandey, T., Sharma, A., Sandhu, A., & Pandey, V. (2024). Controlled polymerization in microfluidics: Advancement in nanogel synthesis. *SPE Polymers*, *5*(2), 113-126.

3. Pandey, T., Sandhu, A., Sharma, A., & Ansari, M. J. (2023). Recent advances in applications of sonication and microwave. Ultrasound and microwave for food processing, 441-470.

4. Sharma, A., Sandhu, A., & Pandey, T. (2023). A mini-review on carbon quantum dots and its applications. *International Journal of Medical Toxicology & Legal Medicine*, *26*(1 and 2), 163- 166.

List of conference presentations

1. Presented an oral presentation in "International Conference on Forensic, Security and Law (ICFSL-2024)" held at Amity University, Noida on April 3 & 4, 2024.

2. Presented Oral Presentation in "Recent Advances in Fundamental and Allied Sciences (RAFAS- 2024) held at Lovely Professional University, Phagwara on April 19-20, 2024.

3. Presented Oral Presentation in "Recent Advances in Fundamental and Allied Sciences (RAFAS- 2023) held at Lovely Professional University, Phagwara on March 24-25, 2023.

4. Presented Oral Presentation in the "25th All India Forensic Science Conference" held at NFSU, Gandhinagar on February 2-4, 2023

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ARTICLE

Eco-Friendly Fabrication of FeS_2 QD-Chitosan Biopolymer Composites: Green Synthetic Approach

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Received: 21 November 2024 | **Revised:** 28 December 2024 | **Accepted:** 15 January 2025

Funding: The authors received no specific funding for this work.

Keywords: nanocomposite | nanogels | non-toxic | quantum dots | synthetic | versatile

ABSTRACT

In this paper, we offer a unique green synthetic approach for producing iron sulfide quantum dots (FeS_2 QD)-chitosan composites using gel chemistry. The technique uses the environmental features of chitosan, a biocompatible and biodegradable polysaccharide, and the excellent electrical properties of FeS_2 QDs. By sustainable chemistry principles, the synthesis process is carried out under gentle settings, using aqueous solutions and avoiding hazardous solvents and strong chemicals. The resulting FeS_2 QD-chitosan composite has superior structural integrity, homogeneous QD distribution, and improved physicochemical characteristics. Comprehensive characterization techniques, such as X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), and photoluminescence spectroscopy, confirm the successful integration of FeS_2 QDs into the chitosan matrix while preserving their quantum properties. This work demonstrates the viability of gel chemistry as a green synthetic technique for generating functional nanocomposites, providing a scalable and environmentally responsible option for advanced material development.

Received: 27 November 2023 | Revised: 11 December 2023 | Accepted: 15 December 2023

DOI: 10.1002/pls2.10116

REVIEW ARTICLE

SPE POLYMERS WILEY

Controlled polymerization in microfluidics: Advancement in nanogel synthesis

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Abstract

This comprehensive review provides a detailed examination of controlled polymerization in the development of nanogels-based microfluidic devices. Here, we have explored the integration of atom transfer radical polymerization (ATRP) and reversible addition-fragmentation chain transfer (RAFT) techniques within microfluidic platforms for the synthesis of nanogels. The synergistic combination of ATRP and RAFT with microfluidics has emerged as a powerful tool for precise control over polymerization reactions, enabling the fabrication of well-defined, multifunctional nanogels with tailored properties. The review begins with a thorough introduction to the principles of ATRP and RAFT, highlighting their respective advantages in controlled radical polymerization. Subsequently, it elucidates the principles of microfluidics and its profound impact on polymerization processes. The merging of these techniques enables precise control over reaction kinetics, monomer conversions, and molecular weight distributions, facilitating the synthesis of nanogels with unprecedented precision and reproducibility. Furthermore, this review delves into the chemical mechanisms underlying ATRP and RAFT reactions in microfluidic environments, emphasizing the role of various initiators, catalysts, and chain transfer agents. Special attention is given to the impact of microscale flow conditions on reaction kinetics and polymer structure. In addition, the review discusses emerging trends in the field, such as the incorporation of novel monomers and the exploration of environmentally benign reaction conditions.

Highlights

- The controlled polymerization is the mechanism by which we can obtain tailormade material.
- The nanogel synthesis must ensure the gelation to be in confined dimensions here microfluidics plays key role.

KEYWORDS

ATRP, microfluidics, nanogels, polymers, RAFT

Tejasvi Pandey and Aditi Sharma contributed equally to this study.

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Ultrasound and Microwave for Food Processing

Synergism for Preservation and Extraction

2023, Pages 441-470

Chapter 17 - Recent advances in applications of sonication and microwave

Tejasvi Pandey [a], Anuradha Sandhu [a], Aditi Sharma [a], Mohammad Javed Ansari [b]

https://doi.org/10.1016/B978-0-323-95991-9.00003-5

Abstract

The innovations in sonication and microwave technology have earned recognition over the years. Nowadays, people are demanding greener technologies that can give a sustainable future. The wide applications of ultrasonication and microwave in various domains have made them a household name. Ultrasonication is a nonthermal technique that utilizes sound energy or ultrasonic frequency to extract cells, fibers, and particles.

International Journal of Medical Toxicology & Legal Medicine
Year : 2023, Volume : 26, Issue : 1and2
First page : (163) Last page : (166)
Print ISSN : 0972-0448. Online ISSN : 0974-4614.
Article DOI : 10.5958/0974-4614.2023.00026.8

A mini-review on carbon quantum dots and its applications

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Online Published on 28 July, 2023.

Abstract

Quantum dots are semiconductor sensor-based particles with optical properties that rely on the quantum confinement effect. These optical properties serve as the foundation for analyte detection and can also be used for doping. Quantum dots are classified into several types based on how they are created: Core-type quantum dots, alloyed quantum dots, and core-shell quantum dots are examples of these types. Quantum dots of the core type are made up of a single component with consistent compositions. Multicomponent systems are used in alloyed quantum dots to provide tunable optical and electronic properties. Internal structures of these quantum dots are homogeneous and gradient. Core-shell quantum dots have advantages such as efficiency and high quantum yield. This property is due to the use of a high bandgap semiconductor shell. Carbon as a material exhibits quantum effects in 0-dimensions (quantum dots), 1-dimensions (nanotubes), 2-dimensions (graphene), and 3-dimensions (graphene) (diamond and graphite). These 0-D materials are used for sensing and various analytical tasks. The mechanisms, preparations, and analytical applications of 0-D carbon materials are discussed in this study.

Top

Non-Invasive optical sensors for forensic applications: A mini-review

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Material Science is a revolutionary field that revolves around the synthesis of various novel materials and polymers for different applications. Forensic science is the developing area where polymer science and material advancements have paved the way in investigating, detecting, and analyzing various substances on-spot and on the field. Technological advancements in crime and criminal investigations have always piqued the interest of forensic scientists. The novel one-step synthesis and preparation methodology for nanostructure preparation is always a growing technological advancement. Optical sensors are devices that detect an analyte based on its optical properties, such as luminescence, photoluminescence, chemiluminescence, and bioluminescence. An analyte, a detector, a signal, an analyzer, and a response comprise a sensor. These sensors include miniature devices, paper-based sensors, microfluidic devices, and on-chip sensors. Optical devices are nanomaterials like nano-biosensors, nano-codes, nano-films, nano-coatings, quantum dots, and composites that provide biocompatibility and biodegradability, tunable optical properties, colloidal-like nature, variable particle size, and in-vivo transport. Even nanotechnological devices show a quantum confinement effect to improve optical and semiconductor properties. These devices are used in Forensic biological, chemical, physical, and trace evidence detection. This review will look at some of the most revolutionary optical advances in forensic science owning its credibility to material advancements.

1.1. Introduction

Technological advancements in crime and criminal investigations have always piqued the interest of forensic scientists. As technology advances, so do scientific discoveries. One of these

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Date of Issue : 20-05-2024
Place : Phagwara (Punjab), India

Prepared by
(Administrative Officer-Records)

Organizing Secretary
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Date of Issue: 12-04-2023
Place: Phagwara (Punjab), India

Prepared by
(Administrative Officer-Records)

Organizing Secretary
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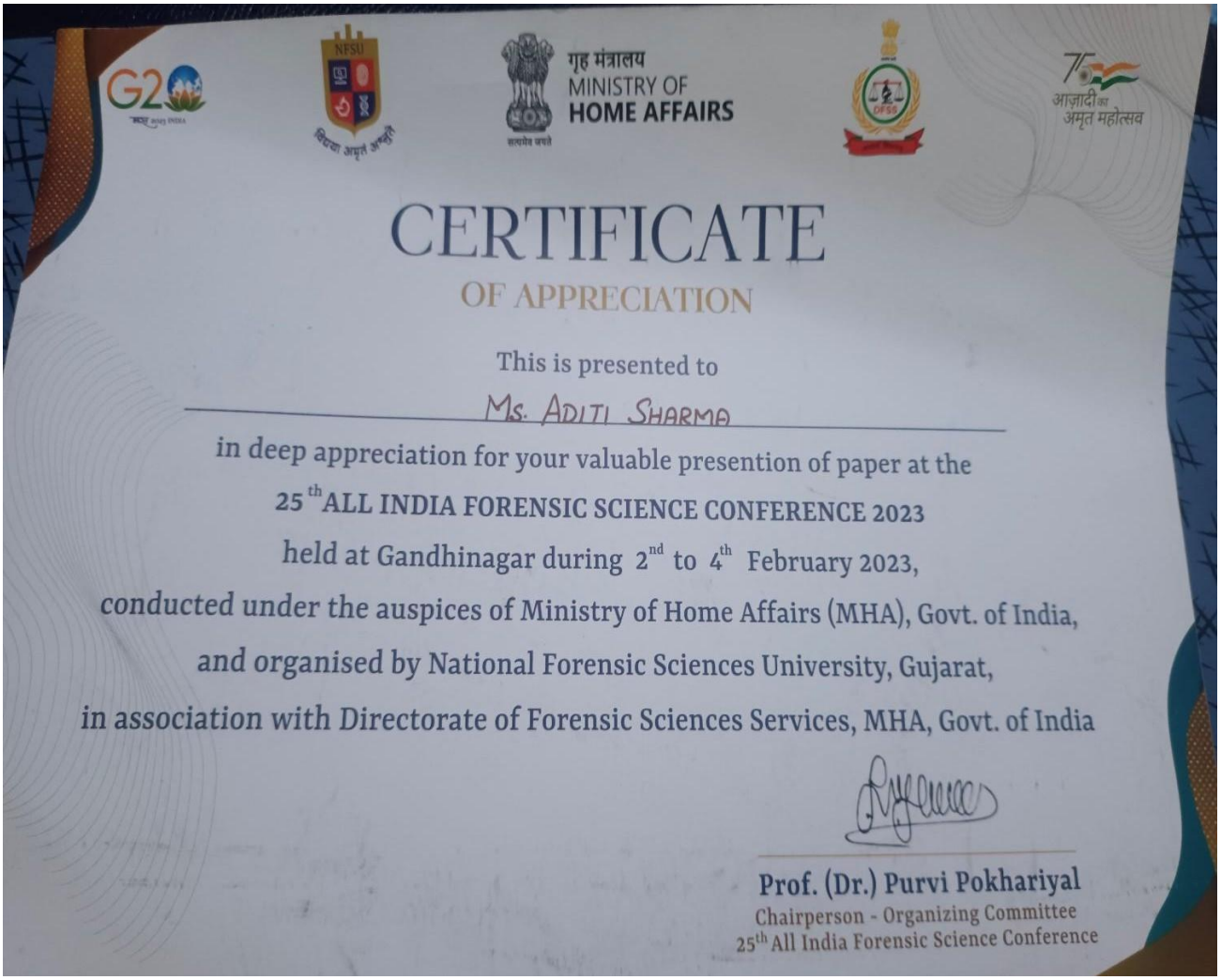

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