A Thesis Report

On Topic Undertaken:

Synthesis and Characterization of Porous Materials to Perform PL and UV Studies of their Interaction with Organic Dyes

Submitted in partial fulfilment of the requirement for the award of degree of
Master of Technology
In
Bio-Medical Engineering

Under the Guidance of
Dr. JAI GOPAL SHARMA
Associate Professor

By
SHUBHRA SHARMA
(Roll No. 2K13/BME/17)
Department of Bio-Technology

Delhi Technological University (Formerly Delhi College of Engineering) Shahbad Daulatpur, Main Bawana Road, Delhi - 110042, INDIA
2013-2015

DECLARATION

I hereby declare that this submission is my own work and that to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgement has been made in the text.

SHUBHRA SHARMA
(Roll No. 2K13/BME/17)

CERTIFICATE

Certified that **Ms. Shubhra Sharma** (Roll No: 2K13/BME/17) has carried out the research work presented in this thesis entitled **"Synthesis and Characterization of Porous Materials to Perform PL and UV Studies of their Interaction with Organic Dyes",** for the award of the degree of **Master of Technology (Bio-Medical Engineering)** from **Delhi Technological University, Rohini, Delhi,** under my supervision. The thesis embodies results of the original work and the studies are carried out by the student herself at **University of Delhi, Delhi** and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

Date: Signature of Guide

Dr.Jai Gopal
Associate Professor
Department of Bio-Technology
Delhi TechnologicalUniversity.

Prof. D. Kumar
Professor,Head of Department
Department of Bio-Technology
Delhi Technological University

ACKNOWLEDGEMENT

**“Expression of Gratitude is the least one
can do in response to the favours received”**

At the completion of my post-graduation, I would like to use this prospect to extend my honest appreciations and deepest regards to every person who guided and supported me in both direct and indirect ways, throughout the course of this M.tech project. I am grateful for their aspiring assistance, invaluably productive comments and remarks during the entire project work and for introducing me to the topic. I am sincerely obliged to them for contributing their ingenuous views and illuminating opinions on a number of subjects associated to the project.

I express my warm gratitude to my project guide **Dr. Jai Gopal Sharma, Associate Professor** for the invaluable support and guidance at **DTU, Delhi**. I am also grateful to **Prof. D.Kumar, Head, Department of Biotechnology,** for providing the experimental facilities in various labs of the Department.

I also extend my sincere regards to **Prof. P. D. Sahare**, Department of Physics & Astrophysics, **University Of Delhi, Delhi**. I also thankfully acknowledge the co-operation of the Ph.D. students, the Department of Physics & Astrophysics and the USIC at **University Of Delhi, Delhi** for their valuable support in carrying out various characterizations featured in this thesis.

Lastly above all, I would wish to acknowledge the undefeated faith and confidence of my loved ones, my friend Ms. Preeti Kushwaha and my family, bestowed upon me as they kept me going through all the good and rough times.

Thank you,

SHUBHRA SHARMA
(Roll No. 2K13/BME/17)

ABSTRACT

Synthesis and Characterization of Porous Materials to Perform PL and UV Studies of their Interaction with Organic Dyes

With the facts demonstrated through various applications, Zinc Oxide Nanoparticles have proved to own great potential for high porosity and high surface area to volume ratio. The synthesis of mesoporous zinc oxide nanoparticles by hydrothermal and sol-gel methods is achieved in the present work. By following these methods we have obtained two types of nanospheres different in their porosity of the same metal oxide. The modifications are created based on the interactions of the reacting species by following diverse chemical mechanisms. We have performed distinct characterization techniques like XRD, SEM, TEM and Raman to carefully examine their morphologies. As zinc oxide is known to possess optical properties, we have performed its UV studies to explore its interactions with the organic dyes. We have also analysed its photo-degradation behaviour.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

- NPs- nanoparticles
- XRD- X-ray diffraction
- SEM- scanning electron microscopy
- TEM- transition electron microscopy
- BET- Brunauer–Emmet–Teller
- PL- photoluminescence
- UV-vis- ultraviolet –visible spectroscopy
- TFTs- thin film transistors
- PMT- photo multiplier tube
- MEMs- micro electro mechanical devices
- SAW- surface acoustic waves
- PMMA- polymethylmethacrylate
- PEG- polyethylene glycol
- FS- fluorescein sodium
- Rb- rhodamine b
- ST- stilbene 420

Chapter 1:

Introduction

1.1 Introduction to Thesis

Introduction to Nanoparticles

In recent times, nanotechnology is functioning in diverse fields of physics, chemistry, biology and materials science through its action for resources and devices using a variety of techniques at nano dimensional scale. Nanoparticles are a fragment of materials that fall in the particle scale of 1–100 nm diameters.

Latest decades have witnessed nanaomaterials as a simple and popular form of material substance for a wide field of applications like sensor-making, electronics & communication, energy and data storage, optical signalling, saving environment, data transfer, cosmetic products, pharmaceuticals, biotechnology and medicine. This is because they possess suitable properties for such uses, made possible by their requisite optical, electrical and magnetic attributes in physical nature with smaller size and morphology which make them compatible with various biomolecules such as proteins, enzymes, nucleic acids, etc. Even a wide range of metal compounds can be reduced to nanoparticle forms by few strategic synthesis methods.

Consistency of Morphology

Synthetic treatment and formation of high-quality technological constituents for various application sectors requires a highly stable and pure polymeric, glass and composite substance. In concentrated bodies made from minute powders, the non-consistent particle size and arrangements in a characteristic fine particle form frequently resulted to irregular an packing shape that consequently ends in packing density variety in powder packets.

Van der Waals forces due to their attraction properties lead to accumulation of particles in an irregular form resulting in small dimensional heterogeneities. Various forms of stresses that exist in spatial arrangement lead to non-regular drying contraction proportional to the rate of solvent reduction and thus results in highly reliant in the dissipation of porosity. These stresses lead to transform elasticity to rigidity in amalgamated figures, and thus lead to crack promulgamation in the unheated body if not released.

Any instabilities in the manufactured phase of the produced particle in the furnace causes the increase in the heterogeneity of the resulting phase during the synthesising stage. Porosity differences and morphological irregularities cause any irregular phase concentration and leads to a negative impact on the synthesized product and thus not very advantageous as far applications are concerned. Density differences, different stresses, give rise to irregularities in the synthesized product and develop breaks, non-uniform pores, and more property regulating defects. Noble gas vaporisation and surface adsorption are free from constituency effects like phase change due to the purity achieved after condensation, they are suitable to make single crystals though, can also form nano-particle which are uniformly distributed, logarithmically sized and not agglomerated together. Even some other gases apart from noble gases, can give these kinds of logarithmic size distribution if we control the synthesis time and other parameters. Monitoring and managing the internal forces of stress acting on the particle ensures uniformity more than focussing on using the inert gases for the particle size-distribution. It is more favourable for us to have consistent particle size distribution rather than having to use random sized particles. Homogenous nanoparticles and colloidal suspensions provide this consistency.

As for example, homogenous particles of silica suspensions can be stabilized to ensure a greater unit of order in the suspended crystals or polycrystalline suspensions formed by agglomeration. Unit of order can be restricted by time and space permitted for higher-range associations to be formulated. Such flawed polycrystalline suspensions structures seem to be the main elements of sub-micrometric unit suspension materials strategy and thus give the initial step in forming a more severe understanding of the techniques indulged in morphological evolution of micrometric structures to provide efficient and beneficial performance substance and constituents.

Nanoparticles have proven to be of greater importance in applications as compared to large size particles, as nanoparticles will weigh less in comparison to a bigger unit particle to provide the same surface area. A large material will have almost no variety in physical features as one may get with nanoparticles which have huge variations in physical attributes. Size-related properties arise when a particle is reduced to nano-size. Thus, when a particle is reduced to nano-scale, the amount of it being adsorbed on the surface of the material starts altering things on a noticeable level. While dealing with larger particles i.e. of size in microns, the percentage of particles adsorbed on the surface are less significant as compared to the percentage of them in the material itself. Thus, nanoparticles turn important. More so, because at such a small size, the higher surface area of the material plays a pivotal role as the overall weight of the material is less. For example, 1 kg of silicon in bulk or larger scale has 1 mm3 of surface area while only 1 mg of its nano form has a surface area of 1 nm3.

Now, when reaching a smaller scale on the size chart, these nanoparticle attain some optical properties as well, due to confinement of electrons in small size and production of quantum effects. For example, observing gold nanoparticles in solution, imparts deep-red to black color; nanoparticles of gold and silicon reflects red colour to the eye while originally they are yellow and grey in reality. Thermal properties are affected too, large gold bricks take 1064 ºC of heat while their nano-counterparts need only 300 ºC (nanoparticles of 2.5 nm size). Radiation adsorption is much higher and uniform in nano-forms of materials than their larger-selves. Because homogeneity maintenance is possible. Thus, when constructing solar devices, if one can control the size, morphology and the material type, solar adsorption amount can be regulated too. Other features like, quantum packing, Surface Plasmon Resonance (SPR), paramagnetism can also be proportionated in semiconductors, metals and magnetic materials, respectively. While not all proeprties can be altered in a suitable manner, as Ferro magnets usually cause fluctuations in their magnetic directions as they absorb thermal energy, this instability causes a poor storage of memory. Colloids formation is easy or solution –forms of nanoparticles is easy to achieve as they can be finely mixed in solvent due to overcoming phase differences which are usually attained by dipping the bulk material completely or partially int the solvents otherwise, thus avoiding wastage.

Diffusion causes a high drifting force due to a huge surface area to volume ratio. Sintering can be possible at cooler temperatures than at higher temperatures while working with bulky sizes. Though, agglomeration and flow dynamics alteration happen which acts as a disadvantage to their synthesis uniformly. But their small size gives more advantages to them as compared to the negatives, that they are certainly more used often in daily lives. Titanium dioxide can clean itself, as the smaller particle size escapes eye observation and we presume them working by themselves. Zinc oxide nanoparticles block UV radiations more efficiently as compared to their bulky scale size. This explains their use in skin lotions for protection against sunlight and yet being skin friendly and stable.

Properties like elasticity change too, as smaller size in polymeric forms increase attachments in surface particles thus making tougher plastics like in clay objects. Nanoforms of materials provide more strength and exhibits good plastic behaviour. Textile industry uses nanotechnology as well when making clever and purposeful clothing.

Materials like metals and semi-conducting metals can also be converted into nanoparticles or hybrids as the need be. Semi-conducting nanoparticles are termed quantum dots if their size falls a slow as 10 nm as they start exhibiting electronic energy. They can be utilised in biotechnology and biomedical uses serving for drug delivering or contrasts in imaging.

Nanoparticles can be as rigid as solid, semi-solid and amorphous soft forms. Their soft semi-solid forms are termed as liposomes. They too serve in targeted drug delivery for treating cancers and transferring vaccines.

Nanopartciles in suspension forms or emulsifier colloids are termed Janus particles, if they are both hydrophilic as well as hydrophobic in nature in nature. They may align at oil/water interfacial boundaries showing surfactant behaviour.

Naming Morphologies

Researchers have always named their discoveries and inventions according to their surroundings for relativity and understanding. Nanoparticles may take any shape, to describe and understand them , researchers name them accordingly, as nanospheres if spherical shapes, nanoreefs if coral shapes, nano tubes or nanorods if tube or rod shapes, etc. Surfactants in colloidal suspensions or catalysts used in synthesis processes or the type of material used as the substrate affect the morphology of the end product. Different shapes serve different purpose, as carbon nanotubes are very commonly used for bridging electrical joints in scientific devices.

Powders or amorphous materials usually acquire sphere shape as they are isotropic, while anisotropic crystals often have rough outer surfaces alike having whiskers around the particle. If they agglomerate, then they are often termed as clusters.

Function Depiction

Nanoparticles in their suspension forms can be used for coating a surface, to evaluate their behaviour. Surface-coating may lead to stability of the material, increase or decrease solubility or focussing target. A coating that makes the surface attribute multivalency, then it increases stability. Surface coating a material help modify a substance and make it ready for catalytic activity in many reactions.

Surface-coating for catalysis

Reactions which are biological or organic in nature need polar solvents or aqueous solutions to prevent agglomeration of particles. Cells in blood serum need charged surface coats to promote surface binding. PEG or polyethylene glycol attached to functional groups like methoxy or hydroxyl to promote repelling.

These modifications of surfaces by coating specific substances help in moving the objected material in the target direction as address tags. Once they reach the target cell or organelle our work is done, we may also make them to go after a specific cell by tracing the path of related proteins or nucleotides. Common binders are antibodies (monoclonal), peptides, or aptamers. These targeting agents should be in controlled attendance per nanometric scale. Multivalency increase their chances of binding thus good cell signalling can be initiated. While targeting a single protein, one may use monovalent nanoparticle instead of multivalent particle for tracing its path. Immune response can be avoided by coating red blood cells as surface layer.

Nanoparticle–Biomolecule Hybrid

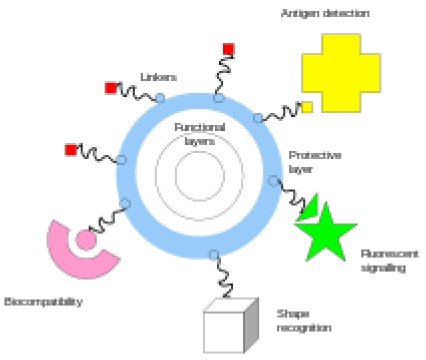


Figure1.1: A hybrid of nano-particle-biomolecule to make it biocompatible

A hybrid combining a nanoparticle and biomolecule can be used in biotechnology at various scenes. Figure 1.1 shows a picto-graphical representation **of a nano-particle-biomolecule to make it biocompatible.** Hence, also termed as nano-biotechnology. This is done to duplicate their effects as actual biomolecules. So as to achieve higher surface area to volume ratio to make potentially efficient medicines, therapy methods, etc.

When using in drug designing or making medicines, nanoparticles are often referred as nanomedicines. Even biosensor making is a form of nanomaedicine approach as they are, but electronic devices which are used for biomedical applications. This helps in in-situ and ex-situ researches. It is aimed to make valuable examination equipments and medically useful devices in future. The NNI (National Nanotechnology Institute) hopes novel commercial utilities made devoted to the pharmaceutical field, which may include therapeutic techniques, drug transferring strategies and imaging. Several research institutes are funded by the National Institutes of Health to promote fresh and effective approaches. This field brings a large revenue which supports the economy of running these research centres. Data says $6.8 billion sales had been made in the year 2004 with almost half of it being invested. In the year 2006, Nature Materials calculated about 130 nano-technological drugs and transferring equipments have been manufactured across the world. This industry promises a prominent growth in the revenue.

Drug Targeting

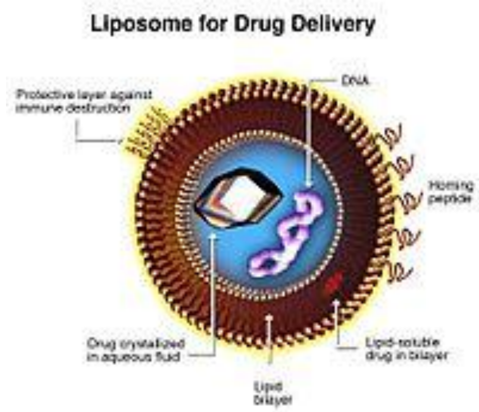


Figure 1.2: A liposome is like nanoparticle-bio conjugate hybrid for biocompatible drug targeting

Nanomaterials are existing in the world of nanomedicines as nanoparticles, dendrimers and liposomes being consistently studied to bring out effective inventions as shown in figure 1.2, a liposome is like nanoparticle-bio conjugate hybrid for biocompatible drug targetings. Nanotechnology has proven of great help in drug targeting to desired cells. In drug-related therapies where higher doses are required, it is wanted to lessen the side-effects of its consumption, so it is considered an efficient approach if the drug directly reaches the target region instead of disturbing the other surrounding regions. This prevents side-effects and wastage by lowering consumption and reducing treatment costs.

Drug-targeted delivery aims at increasing its availabity at a target organ or specific point in the body through a required duration of time. At molecular level, it is found possible by using nanoparticles specially designed for the job. Finding that specific point or bioavailabity is a tough task indeed and has already wasted a lot of cost spent on it. Nano devices are found to be less harmful due to minute size and usually placed inside body and reaction time is lesser. This placement technique is faster and more accurate than any other drug delivery method. Now the controlling factors one need to focus on while doing it are- 1) drug encapsulation, 2)drug delivery to the target and 3) drug release.

Improving the time of drug delivery and its distribution can be improved by efficient designing of drug delivery methods or liposomes or any biomolecule conjugate nanoparticles. This may differ from patients to patients. Nanoparticles can be designed to evade living system and escape an immune response. Drug can be made to reach the cell protoplasm or membrane. There they initiate a response to favour their reaction mechanism. Once they reach inside, they are activated by a specific metabolic activity that they are designed to respond to. As for example, a less soluble drug may be modified to have good solubility by having both hydrophilic and –phobic surroundings. They can avert tissue degradation through controlled drug discharge, reduce the drug discharge time, dispersion volume may be decreased, lesser effect on a non-target. Though, bio-distribution of these particles may still depend on the body more than the controlled factors thus, making it tough to reach a specific organ. There are researches conducted to overcome this shortcoming. But still, nanoparticles pose a threat to the biological system due to increasing toxicity. Nanoparticles may be used in combined treatments for altering antibiotic or antimicrobial features and even, multidrug resistance methods can be evaded.

Liposome

Liposomes as the name suggests, are made of phospholipid biomolecule, with traces of other molecules. They are surrounded by ligands to their surface. This helps them to evade the immune system when needed to reach pathological areas in treatments. They can be synthetically prepared to send nutrition and drugs to target cells or organelles. They are composed of bio molecular ligands to reach diseased organs. Actual biomolecular membranes can be broken by ultrasonication. These kinds of nanoparticles often act as surfactant molecules.

Oxide

A chemical substance with at least one single oxide ion and any other element bound to share a bond is termed an oxide. They can with a metal or a non-metal. Metals usually have an oxidation no. -2 on its oxygen anion. For example Al2O3, ZnO, MgO, etc. while some ions of metal have multiple oxidation nos. like ferrous oxide and ferric oxide. With silicon and water as the most frequent oxide form occurring in nature. Metal oxides are usually basic, non-metallic oxides are acidic while an amphoteric form of oxides with both acidic and basic nature because of elements on border of metal and non-metal region (metalloids). Depicting the nature of oxides helps in their extraction from ores.

Role of Oxidation Number

High oxidation numbered metals is considered to be more covalent in nature as compared to those with lower oxidation number. Non-metals, also covalently attached, form molecular compounds. Oxides reacting with water show same characteristic nature on periodic table variation. Metal oxides with ionic nature form hydroxides (OH^- ion) with water with the other group forming basic solution. Reverse is true with non-metals forming acids (H^+) in an acidic resulting solution. Metal oxides exist in solid forms at room temperature while non-metals as gases.

Let us concentrate **further on metal oxides**….

For naming metal oxides, we combine two (binary) words. The first being the metal name (ones with variable oxidation nos. are differentiated by writing the oxidation no. in Roman numeric form in brackets) and following word is “oxide”. They dissolve in water to form bases while in acids dissolution they form salts. Exist in crystalline forms. Metals in periodic table are classified as alkali metals and alkaline earth metals.

Accordingly they form three types of binary forms (oxidation number -2) upon reaction with oxygen; a) oxides (oxide ions, O^{2-}); b) peroxides (oxygen-oxygen single covalent bond, O_2^{2-}); and c) superoxides (similar as peroxides but with one less negative charge). Alkali metal ions have +1 oxidation number, thus if M represents metal ion and O represents the oxide then, 3 types of oxide will be chemically represented as M_2O, M_2O_2 and MO_2, respectively. Alkaline Earth metals have +2 oxidation number so only two types of oxides are possible, they will be represented as MO for oxides and MO_2 for peroxides.

Metal nitrates on heating form metal oxides of alkali metals while metal carbonates on heating form metal oxides of alkaline earthen metals. While both alkali metals and alkaline earthen metals, due to ionic bonding, form metal hydroxides of respective metals on reacting with water. Hence they are termed basic oxides. This basic behaviour causes them upon reacting with acids to undergo neutralization reaction to form respective salt and water.

MgO is the most important oxide with good thermal conduction and electrical insulation. CaO is often found useful in purifying water and making of steel. Following the periodic table the bond switches from ionic nature to covalent sharing thus properties swing form strongly basic to weakly basic to amphoteric to weakly acidic to strongly acidic.

General instructions to their nature may be followed as- basic nature increases down a group while acidic nature increases with rise in oxidation number. Manganese is the best example in this situation, with five oxidation numbers in its nature, MnO (+2 oxidation number) is least acidic while Mn_2O_7 (+7 oxidation number) is most acidic.

Metalloids (or transition metals) with +3, +2 and +1 oxidation numbers are ionic bonds in metal and oxide ions, making them exhibit basic nature. They will neutralize with acids reaction to form water and salts, example CoO. While with +7, +6, +5 and +4 oxidation numbers, transition metals happen to bond covalently when forming oxides, exhibiting acidic nature. They neutralize with basic solutions to reduce into salt and water, example CrO_3.

Now there is a glitch in the nature of +4 oxidation number as they are on the boundary of non-metallic and metallic behaviour thus, they form amphoteric compounds of oxide, equally likely to behave as acids and bases. They are soluble in both acids as well as basic solutions. Example VO_2 dissolves in acids to form vanadyl ion and with base it dissolves to form hypovanadate ion. This amphoteric nature is exhibited by metalloids on borders of metallic and non-metallic behaviour i.e. mostly by +4 oxidation number and sometimes even their neighbours (+3 or +5) .

Synthesis Approaches of Nanomaterials

There can be two major kinds of synthesis methods:

A) Creating pores in rigid materials (Top-Down method)
B) Creating small size materials with pores inside (Bottom-Up method)

Metal Oxide Nanoparticles

Oxides of metals like zinc, silicon, iron, lead, etc can also be made in nanoscales i.e. from 1 to 100 nm diameter. They can used for making sensors, medical imaging, catalysis, diagnosis, therapies, data storage, solar cells, etc.

Nanoaparticles can form dendrimeric froms too. Dendrimer is a symmetrical form often in 3D-sphere shapes, figure 1.3 displays a **Figure 1.3:** Dendrimer polymer crystal. A central molecule surrounded by others like a snow-flake.

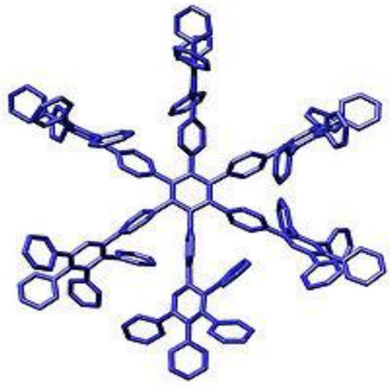


Figure 1.3: Dendrimer polymer crystal

Nanomaterials can be Classified based on their Pore Sizes

An effective way to classify small dimensional particles like nanoparticles is by the help of calculating their diameters of pores, as they are the main attributes for initiating adsorption and diffusion. As nano-is prefixed for dimensions of nano-scale measurements. The attributes provided by size is very important as the very small dimensions can drastically introduce a lot of desired uses. Pore diameter can help determine what other molecules can be diffused inside the particles and a comparison of these two can help in exploring the properties of these particles to a greater extent. If the two dimensions measured by these interactions are equivalent then we find them important as far as wall-molecule relations are concerned. If the diffused molecuels are smaller as compared to the porous nanoparticles then the wall-molecule interactions are higher than before. Hence IUPAC defined some standard measurements for this pore size dimensions, they are as follows:

1) Microporous materials: pore diameter < 2 nm

As the diameter is very small hence, only minute particles can be diffused inside them. Example linear molecules, gases, etc. and diffusion rate is also very slow but interaction attributes are high. They are utilised for cleaning gas filter membranes, gas purifiers, gas storage containers, etc.

2) Mesoporous materials: 2 < pore diameter < 50 nm

These diameters are big enough to occupy large sized molecules like large monomeric polymers or aromatic molecules. The capillary action causes diffusion, initially interactions start then filling begins. They can utilised for adsorbing vapours or liquid molecules, carrying polymeric reactions at nano-scale.

3) Macroporous materials: pore diameter > 50 nm

Particles larger than mesoporous materials' host size can be occupied by these materials like biomolecules or polyaromatic molecular units. Interaction with walls is very small as compared to other molecules. They act as matrices for functional groups' storage, functional group scaffolds like catalyst centres and as sensors as the diffusion rate is high.

Introduction to Luminescence

Luminescence

Certain substances exhibit light emitting activity when they turn a little cooler after experiencing a high temperature. It is observed in florescent and neon material lamps, television (light emission), X-ray fluoroscope monitors, luminol or luciferins, pigments, lightning, rainbows, etc. these items do not emit light on heating unlike incandescent objects (heated iron, wires conducting current or burning coal). Hence, luminescence is often considered as cold light. They have the capability of bringing visible light from invisible forms.

Chemical changes, ultraviolet radiations, beam of electrons, X-ray radiations, etc. often provide energy to certain substances which make them emit light. Electrons of these substances absorb energy and rise to higher energy state, as excited electrons changing their states are bound to return to their original states, they do so by emitting light (may be accompanied with heat) on coming back. The outer shell electrons are excited to jump during this transition. Accuracy of luminescence is based on the amount of transformation of loss of excitation energy emitting light or heat or both and only few substances are efficient enough.

Comparison of Luminescence and Incandescence

Transitioning electrons emit light on returning from excited states to quantum states emit light for luminescence. While in incandescence hot materials release light as the electrons undergo agitation. Although electrons are already vibrating at normal temperatures but on heating this vibration is increased and thus go beyond the infrared spectrum region and shifts into visible spectrum region. This to and fro causes collisions of electrons and emits light.

Pigments and dyes

Colours are an illusion of the eye which they perceive as white light is absorbed by few dyes and pigments and reflected. While with luminescent pigments and dyes reflection of a colour along with light is made on absorbing daylight. Electronic activities in the pigments and dyes turn UV light into visible light. These are often used as brighteners in advertising industry against a contrast background.

Early examinations

Aurora borealis, glowworms, fungi emitting light raised curiosity around the phenomenon, when in 1603 Vincenzo Cascariolo, an Italian started proper investigations to study it. He heated barium sulphate and coal. The resulting powder glowed in blue colour light. Later on, if exposed to sunlight this light coul be restored. Superstitions gave some assumptions to the alchemists that it might covert base metals to gold and named it “sunstone”. Some intellects also named it phosphorus which means “bearer of light”. This name was given to any material that used to glow when in dark. Phosphors is the term used to address luminescent materials while phosphorus is the name for the chemical element. Cascariolo produced barium sulphide as phosphor. Balmain produced calcium sulphide as phosphor in 1870 commercially. Though in 1866 stable zinc sulphide phosphor was produced, used in modern technology as most important phosphor available. Robert Boyle in 1672 described the properties of bioluminescent systems (burning wood or glowworms) emitting light, unaware of their biochemical origin, that ot was a cold light and can be inhibited or prevented by the action of alcohol, acid of gas like ammonia and that it needs air for emission. In 1885- 1887, it was experimented by mixing *pyrophorus* (West Indian fireflies) and *pholas* (clam)to emit light as a resultant. One was luciferase (cold-water extract, unstable with heat) and the other was luciferin (hot-water extract, stable with heat). Mixing them together helped in concluding that all luminescent reactions are examples of luciferin-luciferase reactions. Since then there have been many studies to identify the luciferin component of bioluminescent reactions because of their high accuracy.

First accurate chemiluminescent substances are synthetic compounds like luminol, blue light emitted on oxidation recorded in 1928.

Phosphorescence and fluorescence: definition

Organic molecules when undergoing electronic transitions among different multiplicity levels i.e. phosphorescence while when transitions are among same multiplicity i.e. fluorescence. They might have heat raised reactions or not. While with inorganic molecules, situation can be far more complex. Phosphorescence is a longer time phenomenon while fluorescence is just instantaneous.

Chemiluminescence and Bioluminescence

As oxidation reactions release heat because of electron transition in between energy states, in case of fluorescent molecules chemiluminescence occurs. They are very frequent occurrences but sensitive and precision sensors are required to detect them. Examples of such materials are lucigenin and lophine. While Bioluminescence is a form of chemiluminescence where enzymes play the role of catalyst, where instead of emitting heat, only light is emitted i.e. 100 percent luminescence. Best reactions are where air is the source of heat.

Triboluminescence

When certain crystals of biomolecules or biochemical components are grounded, their crystal powder shines with sparkle. Adhesive tapes emit blue light; strontium bromate, etc. emit light as well. This is due to mechanical stripping of these bulk crystals.

Types of luminescence:

- **Thermoluminescence**

Advancement of excited electrons by heat is thermoluminescence. Example minerals and crystal phosphors often on excitation by light exhibit thermoluminescence.

- **Photoluminescence**

It occurs when electromagnetic radiation fall on matter substance to emit light through a wide spectrum of gamma radiation, ultraviolet and X-rays. Luminescence may be equal or more in wavelength of the falling light. Hence, it is a result of vibration energy. In rare scenarios, light may be of lower wavelength due to anti-Stokes radiation. Ultraviolet light to visible light transformation is very important. While gamma rays and X-rays excite by ionization method with recombining electrons and ions to emit light visible to us. Scintillation counter and fluoroscope use this technique with a photomultiplier.

Electroluminescence

Emission of light by electrical discharge in the three phase of matter i.e. solid, liquid and gas is electroluminescence. In 1752, Benjamin Franklin where he associated luminescence with the existence of lightning where occurs an electrical discharge in the sky. Electroluminescence and photoluminescence are used together to build modern fluorescent flash lamps. Where electrical discharge excited the mercury and a visible light is produced. Sometimes electrolysis reactions show electroluminescence at the electrodes (a result of chemiluminescence). Some materials produce luminescence by accelerating electrons by cathode rays like in diamond, platinum, ruby, etc. Cathodoluminescence is the phenomenon where high-energy possessing electrons when coming back to normal energy states emit light. It is similar to fluorescence. Some materials like zinc sulphide exhibit both of these phenomenon and CRT or cathode ray tubes also show cathodoluminescence. In scanning electron microscopy, light coming from the specimen is used to study it.

Radioluminescence

Beta-elements or radioactive elements emit out helium particles, gamma rays, electrons, electro-magnetic radiations which can excite some luminescent substances. On bombarding or colliding with the material, alpha particles emit light. radium and tritium are often used in watches. Rainbow or aurora borealis is produced by Sun.

Luminescent Substances and Phosphor Reaction

Barium sulphide was first ever produces as a phosphor with a small efficiency for luminescence but degrades in moisture present in air. While in 1866, zinc sulphide was produced, which was found to be more stable than barium sulphide. Then later on, it was found that with the presence of an activator metal as an impurity allows sulphides to exhibit phosphor-like behaviour. Some metal oxides, phosphates and silicates on modification may produce luminescence as well.

In some metal oxides when activator like irradiation materials were introduced into the crystal structure, they luminescened. While some of them might emit light without irradiation too. The activator molecule is surrounded by metal crystal molecules or ions, which make exhibit phosphor nature. Only a small bit of activator must be added inside the crystal for maximum luminance. In high quantity, they can be poisons or killers, and reduce the efficiency of emitting light.

1.2 Motivation of the thesis

Metal oxides are very useful nano-particles usually because according to the structure of their bulky crystals, we can get a good estimate of the resulting nano-scaled particle. That is what make them fit into for many applications. ZnO can be utilised into many ways:

Bio-medical applications

It helps in the development of quantum dots for better imaging because of its high sensitivity, high stability, it has good luminescence for its use as a fluorescence probe. It can be in dentistry because of its antiseptic nature. It can be used in making silk fibre which further can be used for doping purposes of the created product. It can stimulate cell growth and proliferation. It can be drawn into nanosheets for many biomedical applications. Zinc doped hydroxyapatite can be used in osteo-grafts and dentistry and as an enzyme co-factor. Drug-delivery is an excellent use in the bio-medical field.

Photo-catalytic activity

It can be used for calcinations of flower-like ZnO-TiO_2 for photo-leaching activity.

Solid-state sensor for gas detection

Thick film technology can be used for creating gas sensing equipment and can detect gases like NO_2, CO, even vapours of organic liquids.

Photo-voltaic applications

Formation of ITO-coated glass for photo-degradation and photoluminescence can help in producing a voltage potential. The flower rod core shell ZnO can be coated on ITO (indium tin oxide) for creating a high sensitivity photovoltaic cell.

1.3 Objective of the thesis

We have proceeded with the preparation of nanoparticles. Nanoparticles have gained importance in the recent times due to their characteristics of both physical and chemical nature. Their property of being modified into desired morphology built at minute sizes of micro- or nano- scales promises a great deal of applications coming into light. As far as characterisation studies tell us we can regulate their growth from around a nucleus to their break down from a bulky material. They can be prepared by various methods as at nanoscale the physical environment to be provided during the synthesis can be controlled very efficiently. Usually the substrate tells a lot about the product to be synthesised due to a synonymous physical nature. We select metal oxide nanoparticles to be the root of the study as they due to their semiconductor nature have proven to be very helpful in forming sensors and bio-sensors, cosmetics and medicines, food products, rubber and polymers, cement, solar cells, catalysis and remediation. They are huge revolution in the healthcare industry due to their low cost. There is no hard and sole rule for the preparation techniques to adopt while creating them. Techniques like solvo-thermal method, precipitation and co-precipitation method, metallurgical method, sol-gel method, hydrolysis, etc. are some of the few preparation approaches available. The shapes like nanosheets, nanoflowers, nanorods, nanospheres and nanocombs are some identified morphologies they exhibit. These shapes provide them high surface-area to volume ratio with lesser amount of weight as compared to their bulky counterparts. Dentistry and orthopaedics are the fields where we can find its use due to the presence of zinc in trace amounts in our own biological system.

Characterisation of these nanoparticles by physical techniques like XRD, SEM, TEM, BET and Raman spectroscopy can help us find out the morphology of the manufactured product. Optical characterisation is also required so we adopted luminescence activity for this purpose. Luminescence activity of phosphors provides good measurement techniques to determine the presence of different constituents in a specimen. It uses fluorescence of light as the indicators which is a cold reaction and does not involve the use of heat hence pose no alteration to the media they are added to. The role of organic dyes in helping to conduct optical characterisation of these nanoparticles is to give an idea as to where to fit them in the vast field of their applications. The UV-Vis spectroscopy gives an idea of the interaction of organic dyes with the nano-sized catalyst into leaching the organic dyes at a faster rate than in leaching without them in the presence of a UV source or sunlight. Their porosity is a valuable asset to the lysing of the dyes from a mixture.

1.4 Proposed solution and Contribution

The solution proposed and its contribution to the present thesis can be generalised in the following subjects:

- To synthesis nano-particles of consistent shape and size and porous nature by adopting hydrothermal and sol-gel chemical methods for the reaction under controlled temperature and pressure values.
- To characterise the synthesised porous materials for learning their physical and optical nature in order to use them for appropriate applications.
- To study the photo-luminescence spectra of the activity of organic dyes upon their interaction with toxic gases.
- To study the UV-Vis spectroscopy, of the solutions formed by mixing the porous catalyst and the organic dyes after photo-catalysis.

1.5 A brief layout of the thesis

The essence of thesis can be comprised into six chapters as follows:

- Chapter 1 is the introduction to the essence of the thesis with motivation and objective
- Chapter 2 offers an insight to the contextual work and theory of the thesis background as a review of literature;
- Chapter 3 provides the characterization techniques;
- Chapter 4 covers the features of luminescence technique helpful for optical characterisation;
- Chapter 5 elaborates the experimental setup involved and the synthesis processes adopted;
- Chapter 6 provides the results and analyses based on the performance of proposed methodologies and then comparing their results;
- Chapter 7 summarises the conclusion drawn from the results and analyses of the proposed work future work.

Chapter 2:

Literature Review: A Descriptive study of Zinc Oxide Nanoparticle

2.1 Methods of Synthesis

C. Physical Approach

- **Metallurgical or Ore processing method**

D. Chemical Approach

- **Mechanochemical Procedure**
- **Controlled Precipitation Procedure**
- **Sol-Gel Approach**
- **Solvothermal and Hydrothermal Approach**
- **Emulsion or Microemulsion-Environment Approach**
- **Other Methods of Obtaining Zinc Oxide**

2.1 Methods of Synthesis

There are a wide assortment of approaches for producing ZnO, viz. vapor collection method, precipitation method, hydrothermal method, sol-gel method, microemulsion precipitation method and mechanochemical method. These different approaches help in creating particles in varying sizes, shapes and dimensional geometry. Widely two main approaches come into role, such as physical and chemical.

A. Physical Approach

- **Metallurgical or Ore processing method**

Zinc ore is heated to obtain ZnO and zinc compounds. According to standards methods can be a.) direct heating or/ the American method termed type A Zinc Oxide or b.) indirect heating or/ the French method termed type B zinc Oxide. In the American method, zinc ore is combined with coal and heated to reduce it. The zinc vapours are oxidised in the same heat reactor or furnace simultaneously in the same reaction loop. Samuel Wetherill invented this American method to occur in a reactor where there exists two layers, one layer consist of a coal bed which is ignited by a previous reaction spark. The second layer is over this burning coal consisting of a mixture of zinc ore and coal. An air passage is made under these layers so as to pass heat to the two respective layers and discharge monoxide of carbon to reduce zinc into vapours. They combine to form a stable oxide of zinc along with some contaminations like oxides of other metals example manganese, lead, iron and cadmium which were present in the zincite. These contamination oxides are converted to sulfates, which in return imparts white colour to the Zinc Oxide produced. The shape can be sphere, or spike-like. Controlling the acidity of the product is done by allowing more water-soluble substances in the product mixture in order to make the whiteness of product durable or last long. This product is of lower quality.

When ZnO is produced indirectly i.e. by French method, a graphite cauldron is heated over 907 °C or above 1000 °C containing zinc metal. At this temperature heated air combines with zinc vapors and zinc oxide is formed. This combining extracts heat out of the reaction mixture and brings the overall temperature down thus exhibiting luminescence. The product is transferred or conveyed into a cooling duct and accumulated in a bag by filtration. LeClaire in 1844 invented this method and its commonly known as the French method. The agglomerates range from 0.1 to some micrometres in size, with spheroidal shape. The vertical assembly of the furnaces, charge, refining column, vaporizer and rotary chamber makes it far more stable, pure and high quality product than the American method.

B. Chemical Approach

- **Mechanochemical Procedure**

It is a simple and lesser expensive procedure of obtaining nanoparticles on a vast scale. It accompanies dry milling on a high-energy level instigating a ball mill grinding at lower temperature. A solid thinner is added as a part of the reaction mixture to prevent agglutination of the nanoparticles. Uniformity in the grinding of the powder and breaking the particles to a desired size is a challenging task as the efficiency decreases with elating energy and time of the mill and thus, impurities increase in the resulting product. Highly homogenous crystalline particle structure and morphology, small-sized particle, least agglomeration and low production costs are the chief advantages of this method. Anhydrous ZnCl2 and Na2CO3 are the initiating reactants of thie mechanochemical method. NaCl serves as the “thinner” in the reaction mixture and keep the particles separated. $ZnCO_3$ serves as the precursor of ZnO, and is calcined or thermally treated at a temperature of 400-800 °C. The procedure starts as the following reactions,

(1) $ZnCl_2 + Na_2CO_3$ □□ $ZnCO_3 + 2NaCl$
(2) $ZnCO_3$ □□ $ZnO + CO_2$

This procedure was invented by Ao *et al.*[1], producing an average size of 21 nm crystal. The crushing of $ZnCO_3$ as the ZnO predecessor was carried out for 6h at about 600 ºC producing ZnO hexagonal structure. Heating temperatutre and grinding time determines the crystal size. Further increasing crushing time from 2 to 6 h, helps reduce size to 21.5-25 nm while increasing temperature from 400 to 800 ºC increases crystal size to 18-35 nm. Tsuzuki and McCormick [2] used the same reagents. They observed that 4h of crushing time was enough to start the reaction between the reactant substrates to produce $ZnCO_3$ precursor, which if further heated at 400 ºC produced nano crystals of 26 nm size. They showed that crushing the reactants without thinner caused agglomerates of 100- 1000 nm. Thus using $ZnCl_2$ as a thinner was confirmed. A crushing method of $ZnCl_2$ and Na_2CO_3 was also performed by Moballegh *et al.*[4] and by Aghababazadeh *et al.*[5] Moballegh *et al.* studied the effect of heating temperature on particle size. An elation in the temperature of the procedure from 300 to 450 ºC caused increase in ZnO particle size from 27 to 56 nm. Aghababazadeh *et al.* acquired ZnO with particle size of about 51 nm and a 23 m2/g of surface area, after performing the procedure at 400 °C. Stanković *et al.* [6] stretched their previous study to inspect mechanical-thermal synthesis (MTS) or mechanical instigation backed up by thermal instigation of ZnO from $ZnCl_2$ and dihydrate oxalic acid as substrates with the objective of acquiring pure ZnO nano particles. The study focussed to explore the properties of oxalic acid as a carbon-based PCA, and different grinding times, on the crystallite construction, mean element size and morphology of ZnO nanoparticles. The combination of primary substrates were crushed from 30 min to 4 h, and galvanized at 450 °C for 1 h. Qualitative evaluation of the produced powders was carried using X-ray diffraction (XRD) and Raman spectroscopy methods. The XRD examination showed flawless long-ranged and the wholesome wurtzite structure of the manufactured ZnO particles. Raman spectroscopy shows a different middle-ranged ZnO particles. Their SEM images showed that the shape of the powders toughly depends on the crushing time of the substrate mixture used, irresepective of the advanced heat behaviour. An increased time of crushing directed to a small particle size.

- **Controlled Precipitation Procedure**

A broadly used method of preparing zinc oxide, is controlled precipitation method, as it makes it conceivable to acquire a product with recurring properties. This process includes quick and impulsive drop of zinc salt mixture with a reducing agent, to bind the development of elements with detailed magnitudes, tailed by precipitation of a predecessor of ZnO from the mixture solution. At the following phase this predecessor undergoes heat treatment, backed up by crushing to remove contaminants. It is very tough to disrupt the accumulates that form, so the heated powders have a large level of agglutinated particles. The procedur of precipitation is regulated by properties such as pH, temperature and time of reaction. Zinc oxide has been prepared out of aqueous solutions of chloride and acetate of zinc. Regulated features in this procedure combined the absorption of the substrates, the rate of inclusion of reagents, and the reactor temperature. Zinc oxide was produced with a homogenous particle size and large surface area. Hong *et al* .[7] also produced zinc oxide. The procedure of preparing zinc oxide was done by precipitating zinc acetate monohydrate and ammonium carbonate. These mixtures were added into a robustly mixed aqueous PEG i.e. poly(ethylene glycol) with a mean molecular mass of 10,000. The obtained precipitates were annealed by two different methods. In the first, heating at 450 °C for 3 h and in the second, heating took place followed by heterogeneous azeotropic concentration of the predecessor; obtained zinc oxides were labelled as “powder A” and “powder B” respectively. Structural morphology were analysed by XRD and TEM which showed that powder A particles were of diameter 40 nm, and powder B particles were of diameter 30 nm. Heterogeneous azeotropic concentration entirely drops the existence of accumulates and declines the ZnO particle size.

Lanje *et al.* [9] included the cost effective and simple procedure for the manufacture of zinc oxide. A one-step protocol with a huge amount of production without undesirable contaminants is required to make a method cost-competitive. As a result, the low cost predecessors such as zinc nitrate and sodium hydroxide are used to form the ZnO nanoparticles (avg diameter 40 nm) were used. In order to drop the accumulation of the smaller particles, the starch molecule was used due to its O-H functional groups which form a micelle around the nanoparticles thus separating them. Wang *et al.* [10] too carried a controlled precipitation technique where ammonium bicarbonate and zinc sulphate hepta hydrate were used. A membrane reactor with two plate of polytetrafluoroethylene (PTFE), with stainless steel as a vessel were used. The ZnO retained, had a small range of particle sizes of 9 to 20 nm. XRD evaluation showed that ZnO had a wurtzite structure entirely. The particle size was modified by temperature, heating time, controlling rate of flow and absorption of the supply phase. Jia *et al.*[11] showed *in situ* crystallization modification from Zn(OH)2 to ZnO. X-ray diffraction (XRD) and scanning electron microscopy (SEM), two possible mechanisms from Zn(OH)2 to ZnO were taken into account. The production method of ZnO was observed in a time-monitored study by raising temperature to 85 ºC of an aqueous solution of zinc compounds and ammonium hydroxide. Slowly and steadily by forming reaction intermediates, pure ZnO is formed as the result. Dissolve-reprecipitate and crystallization occur in between to form ZnO. These studies assure the researchers of plans involving controlled reaction of Zinc compounds in aqueous medium. In precipitation reactions, surfactants surround the reacting species to prevent them from agglomerating them and thus, help the nanoparticles to form in uniformity. Frothing and fluting is kept at bay and desired particles morphology is observed in the end. Wang *et al* formed nano scaled zinc oxide from $ZnCl_2$ and NH_4OH using CTAB (cetyltrimethylammonium bromide) as chelating agent. The experimental setup was processed at room temperature, and the product was heated at 500 °C to eradicate remainders of the surfactant to remove impurities. The resulting product was found to be very crystalline wurtzite ZnO with little, properly-dissipated spherical nanopowders of 50 nm size. CTAB controlled the formation of chelation and development of crystals throughout the reaction, and also helped to avoid the making of agglomeration. Li *et al.* [12] formed zinc oxide with different shapes with the reaction of zinc nitrate hexahydrate and sodium hydroxide with sodium dodecyl sulfate (SDS) and triethanolamine (TEA) as chelators. The use of surfactant was to control the morphology of the product i.e. ZnO nano particles. His studies showed that the modification might occur because of recrystallizing of ZnO.

- Sol-Gel Approach

A very effective and wanted approach is sol-gel technique to produce ZnO nanoparticles. It's very easy to follow, less expensive, consistent, repeatable, and minor reaction set-up parameters of manufacturing with the freedom to modify the surface of ZnO inorder to increase porosity, makes it a popular method. Different shapes and sizes of desired use make it important in the role of applications it is worth of. A variety in morphology makes it to possess good amount of optical properties thus increasing the scope of research further in many fields like pharmaceuticals and bio-sensors. Reaction may occur by forming films deposited in the form of froth over sol-gel layers or formation of gel over an emulsion.

Benhebal *et al.* [13] combined zinc acetate dehydrate and oxalic acid together by dissolving in ethanol. The resulting product was studied various techniques like nitrogen adsorption, XRD, SEM, UV-Vis spectroscopy, FT-IR , etc. The produced zinc oxide was found to have a hexagonal wurtzite form with the sphere-shaped nanoparticles. BET shows a surface area of 10 m2/g which is common for a material with low porosity or crystalline nature. Ristić *et al.* [14] also prepared nano particles of ZnO by sol-gel technique using zinc 2-ethylhexanoate (ZEH) in propan-2-ol with tetramethylammonium hydroxide (TMAH) solution. After 30 min- 24 h of leaving the suspension still, it was washed with ethanol and water. TMAH is stronger than NaOH due to a high basic pH of around 14. High pH ensures that the product will not be contaminated with any cationic impurities of the base which might cause changes in the conductance calibration of the ZnO. Quantity of ZEH (though it had no effect on the particles size) and maturation time are monitored to mind the shape and size of the nano crystals. TEM showed 20-50 nm sized particles. Yue *et al.* [15] also produced ZnO by the emulsion technique. He used ultrathin AAO membrane to produce high quality, uniform-stuctured nano tubes of the metal oxide with large capacity of filling which further increased its scope of applications in the research and industrial arena.

- **Solvothermal and Hydrothermal Approach**

It does not require using organic solvents or milling or heating to further modify the product, thus, it turns out to be environmentally safe and easy to follow approach. The reactants are mixed together in a solvent after vigorous stirring for about 6h and then put in a Teflon-coated non-reactive vessel under a certain pressure in an autoclave, at high temperature of 100-300 ºC to help the synthesis to occur and then left for some days to combine the components. After simulataneous heating and cooling, nucleated crystals are formed which grow further. It is also possible to carry this reaction at lower temperatures and pressure as well. The different shapes, crystal size, purity and stability of the end product are the key advantages of this technique. Chen *et al.* [16] used $ZnCl_2$ and NaOH in the proportion of 1:2 with water as a solvent in a hydrothermal reaction. $Zn(OH)_2$ is obtained in the form of white powder in the bottom, which is washed and filtered maintaining a pH of 5-8 using HCl as the acidic source. In a temperature-programmed vacuum oven, the mixture is kept in an autoclave Teflon-layered vessel where the synthesis occur. ZnO is formed in the end. XRD and TEM are usewd to evaluate the shape and size of the powder. pH, temperature and pressure play a key role in the morphology of the crystalline particles. High pH decreases the accuracy of the technique. Ismail *et al.* [17] used zinc acetate and NaOH with hexamethylenetetramine (HMTA) at room temperature to form ZnO. $Zn(OH)_2$ intermediate was washed several times with water and then autoclaved. HMTA acts as a chelator in forming ZnO without agglomerating. SEM was used to observe the structure of ZnO.

With an high reaction time, temperature and chelator concentration, particle size is increased. Hydrothermal treatment of the predecessor, proceeded bydrying, resulted in spherical particles of ZnO with 55–110 nm size, subjected to the reaction synthesis parameters. A different particle shape and sized nanoparticle of ZnO were also prepared by Dem'Yanets *et al.* [18] used zinc nitrate or zinc acetate with any hydroxide like KOH, LiOH, NH4OH to produce ZnO nanoparticles hydrothermally. An autoclave containing the mixture was kept at stable temperature or variable temperatures of 120-250 ºC. the predecessor undergoes dehydration and recrystallizes to form hexagonal ZnO crytals with 100 nm to 20 μm size range. Increasing the hydration time and temperateure by 50-70 °C increases particle diameter thus favouring the synthesis.

Musić *et al.* [19] also showed the consequence of chemical synthesis on the morphological properties of ZnO nanoparticles. A mixture obtained from a solution of zinc acetate dihydrate with ammonium hydroxide was kept in an autoclave at 160 ºC for hydrothermal reaction. pH changed the particle size and shape. Development of the actual aqueous suspension of pH 10 for a time of 7 months at room temperature resulted in the growth of agglomerates of ZnO particles of ~20 and ~ 60 nm sizes. They also produced ZnO by sol-gel method, using sudden hydrolysis of zinc 2-ethylhexanoate with propan-2-ol. The synthesised nanoparticles caused unique variations in the standard Raman spectrum of zinc oxide.

A number of experiments showed the use of microwave reactors in hydrothermal approach gives substantial remunerations. Microwaves help in calcination of solutions from which the synthesised particles are acquired, while escaping energy loss on heating the whole reactor unit. Several chemical manufacturing succeed with higher speed and yield while using microwaves as compared to other old-style methods. Equationally fast, rapid and higher quantity heating of the reactants may be acquired by electrical current passing over the reactants. Strachowski *et al.* [20] devised a methodical approach comparing the ZnO acquired with diverse techniques of reaction incentive. The experiment was carried in such a way that the synthesis being observed occurred at the same power phases (external supplies) and with the same reaction reactor dimensions. Vessels were used with experimental energy provided using microwaves, electrical energy, heating, increased voltage pulsations and heating of the entire autoclave vessel. They observed that the nanoparticles with phase conformation closest to pure ZnO were acquired by using microwave and in the traditionally made autoclave. the powders resulted in the other vessels showed heterogenous phases like simonkolleite and hydroxyzincite alongwith zinc oxide. Utilising a microwave reactor has helped to reduce the synthesis time by numerous fold, and also gave the purest product. Schneider *et al.* [21] also used microwaves for synthesising nano particles. Zinc acetylacetonate and a zinc oxime complex mixed in alkoxyethanols were mixed together and heated to produce zinc oxide.

Schneider *et al.*, presented that the structural properties and aggregation of ZnO powders depends highly on the predecessor used. Produced Zinc oxide was evaluted using techniques such as dynamic light scattering, BET surface area, SEM, TEM, XRD, TG, PL spectra and Electron Paramagnetic Resonance. The particle size of the end product is about 40–200 nm, depending on the type of predecessor and alcohol utilised. Complex of zinc oxime in methoxyethanol gave the smallest sized product on heating. With an elevation in the concentration alcohol with a long carbon chain, the particle size is made to increase. The BET surface area of the resulting ZnO exists in the range of 10–70 m2/g. Thermal degradation of both zinc acetyl acetonate and zinc oxime made it possible to obtain a product of desired features.

Zhang *et al.* [22] used a solvothermal approach to obtain hollow and solid spheres of ZnO nanoparticles by the help of ionic liquid imidazolium tetrafluoroborate. diameters of 2–5 μm were observed in hollow spheres and channels of about 10 nm diameter with a wall thickness of 1 μm. The reaction may be a hybrid combination of ionothermal and solvothermal method. It may be assumed that this hybrid combo of ionothermal and solvothermal methods can be used to produce novel materials with distinct and unique features and dimensions.

A solvothermal approach was also used by Chen *et al.*, who produced nano crystals of ZnO free from the presence of hydroxyl groups. Zinc powder was treated with trimethylamine *N*-oxide and 4-picoline *N*-oxide in the presence of organic solvents like EDA (ethylenediamine), TMEDA *(N,N,N′,N′*-tetramethylenediamine) and toluene autoclaved at 180 ºC.

The oxidizing mediators used and the synchronizing capabilities of the solvents modified the shape and size of the ZnO nanowires. They also showed the consequence of the attendance of water in the experimental mixture. It was observed that the attendance of a hint of water catalyzed the combination of zinc powder and 4-picoline *N*-oxide and modified the size of the ZnO nanoparticles. The obtained Zinc oxide had the diameters of 24–185 nm, depending on the reaction parameters.

- **Emulsion or Microemulsion-Environment Approach**

An emulsion can be described a two phase dispersion combining liquid and semi-solid. They are often mixed at a high speed in order to create foamy liquid form. They can be two forms called oil-in-water and water-in-oil emulsion.

- **Other Methods of Obtaining Zinc Oxide**

Methods other than the ones explained above are also present wchich may be effective in producing zinc oxide nanoparticles. They can be done by using microwaves, genereating from gas phase, pyrolysis, sonochemical, etc.

Chapter 3:

Characterisation

3.1 Characterisation Analysis Methods

C. **Structural Characterisation Techniques**

D. **Optical Characterization**

3.1 Characterisation Analysis Methods

A. Structural Characterisation Techniques

Every material needs to be characterised to understand it physical properties better when we come across one. Role of characterisation becomes vital when we are dealing with materials of nano-scales as they have undergone a huge change of dimensions i.e. from bulk to minute size and it calls for an entirely new set of properties and behaviour. When dealing with nanoparticles of metal oxides, complex structures may be involved and characterisation can be a tough job. Many different types of techniques are available to characterise many aspects of nano metal oxides to understand the role of physical attributes they possess. This can help in focussing on the modifications we can offer to the synthesis process as well as to develop new ones.

Structural Analysis Practices

The construction and attributes of the manufactured porous substances in the current work have been analysed by various characterization methods. The very popular techniques often utilised for this goal are x-ray crystallography (XRD), scanning and transmission electron microscopy (SEM and TEM) and nitrogen adsorption/desorption. SEM gives details about the particle shape and size, TEM gives details about morphology at a higher order, adsorption of nitrogen gives the idea about its porosity and XRD tells about its crystal make-up. The principles and instruments involved in performing these is explained as follows:

X-Ray Diffraction

To analyse or study a crystal's atomic and molecular aspects by passing a beam of X-rays through (after filtering) it we perform X-ray crystallography. By evaluating the intensities and angles of these beams (after diffraction) a three dimensional image can be procured by viewing the electron cloud inside the crystal. We can find the mean positions of the atoms from this electron cloud and also determine their angles, flaws and various other aspects.

Many substances in all the three phases can be found with crystal forming nature in biological, organic and inorganic forms in the research field which needs to be identified. An unknown substance can be identified efficiently by this method to help categorise it under metals, alloys, biological or any other chemical field. They can help in designing medicines to cure several diseases.

X-ray powder diffraction analysis or simply XRD analysis is used to determine crystallinity of a sample usually solid. It helps to differentiate crystalline materials and amorphous materials, identify polymorphic forms like fingerprints, identify crystalline material during and after growth, measure amount of crystallinity, etc. it is highly important to identify the crystal properties as we usually opt for a crystal during several reactions. As compared to an amorphous form, a crystal possesses unique attributes like solubility and melting point which are crucial in providing an idea of the final product. XRD is shown on a diffractogram; a plot between intensity and 2θ (diffraction angles). We can identify the synthesised materials by comparing with the reference plots of the original materials. The Figure 3.1 displays an X-ray Diffraction pattern of water molecules in ice (solid crystalline phase) showing the hydrogen bonds (1) to pack the water molecule in solid form.

Nicolas Steno, a Danish scientist was the first to found crystal symmetry in 1669 and demonstrated that the angles of the lattice are the same in every crystal form. Later in 1784, René Just Haüy demonstrated stacking arrangement of blocks in same morphological objects.

While describing these three planes of the crystal William Hallowes Miller in 1839 gave integer values to represent these planes each individually. Later on many scientists described the crystal symmetries in 19th century too.

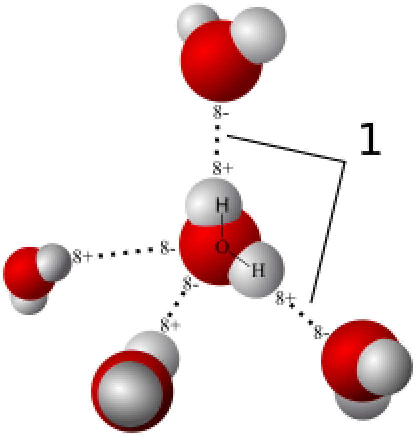


Figure 3.1: X-ray Diffraction pattern of water molecules in ice (solid crystalline phase) showing the hydrogen bonds (1) to pack the water molecule in solid form

Wilhelm Conrad Röntgen discovered X-rays in 1895, which helped in the ongoing studies of the crystal symmetry. They discovered the electromagnetic nature of these radiations. Some scientists thought that the electromagnetic nature was a false idea but Max von Laue in 1912 proved that X-rays are electromagnetic forms of energy.

The Bragg's Equation

Reflection of X-rays from one plane to another is, $x = d\sin\theta$

The difference of paths of two waves is twice the reflection path = $2\ d\sin\theta$

Thence, Bragg's equation: $n\lambda = 2d\sin\theta$

The Bragg's equation is clearly elaborated in figure 2.2 through a pictorial form.

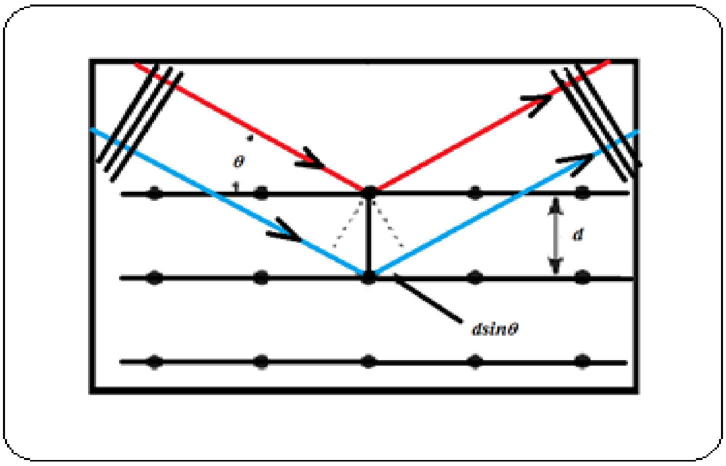


Figure 3.2: Diffraction pattern of rays between crystal layers

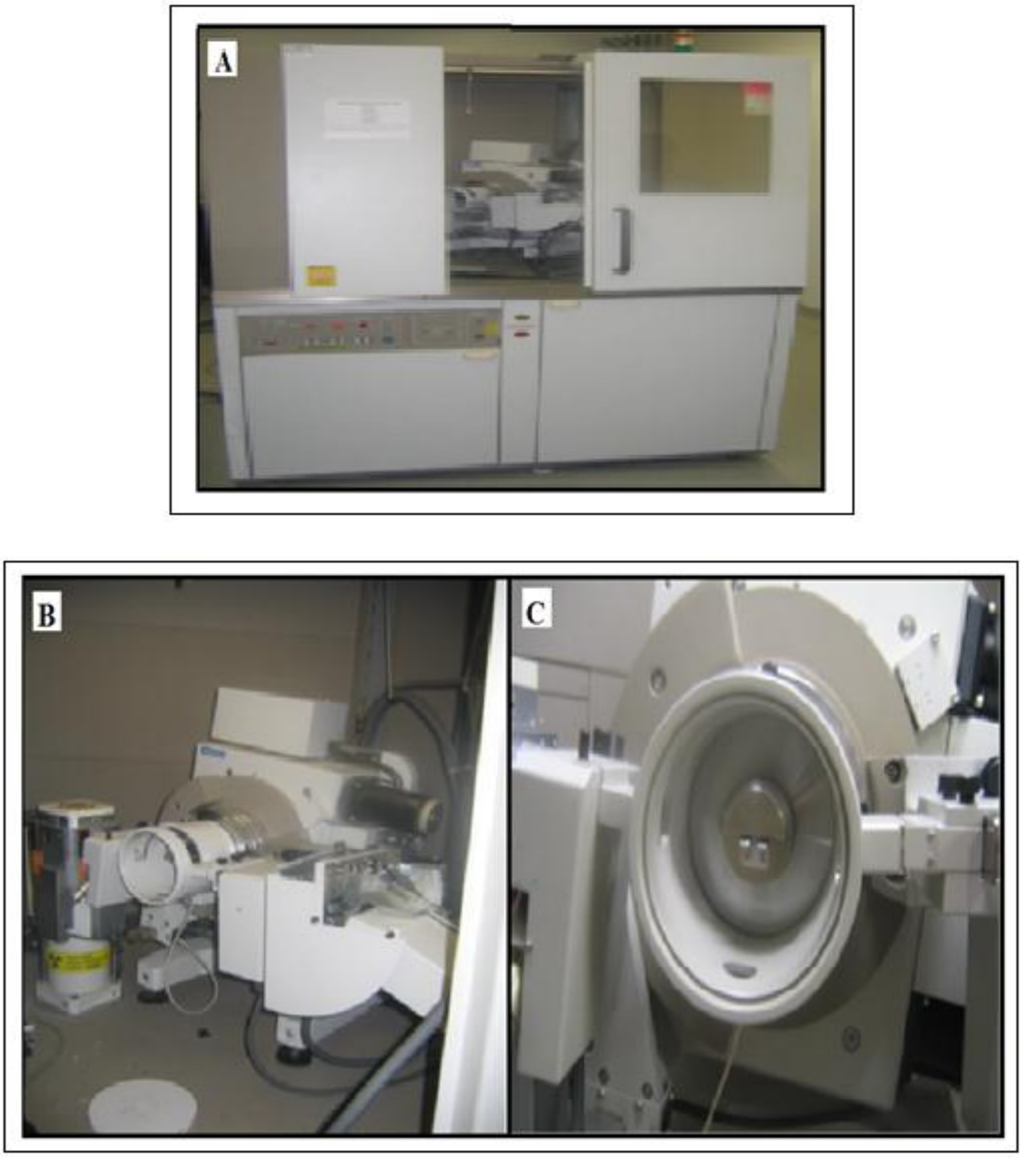


Figure 3.3: A) an XRD instrument B) Side-view of XRD Chamber C) Front-view of XRD Chamber

Instrument details and measurement principle

As shown in figure 3.3 a) an XRD instrument b) Side-view of XRD Chamber c) Front-view of XRD Chamber. Monochromatic X-rays are combined on interring with each other after passing through the crystalline material. A CRT (cathode ray tube) produces X-rays which are filtered further to produce a monochromatic beam combined to concentrate and focussed on the sample. As the X-rays incident upon the crystal it distributes and recombines to constructive intrusion which is found to follow Bragg's law. It relates wavelength of electromagnetic radiation and the path length of the diffracted ray from the lattice spacing in the target crystal. The plot thus produced by the help of the X-ray diffraction method acts like a fingerprint of the crystal's nature. We keep the data of the known crystals saved to use it for comparing of the new or desired crystal with for explaining its nature. In table 3.1 shows the instrument characteristics of an XRD Diffractometer.

Table 3.1: Instrument Characteristics of an XRD Diffractometer

Instrument	(For XRD): Bruker 3 X-ray diffractometer (For HRXRD): Bruker D8 Discover X-ray diffractometer with CuKa radiation, at 40kV/40mA
Operating range	2-70 °2Theta
Slit	0.3 mm to 10 mm
Scatter slit	2/3 °
Temperature	Room temperature
Humidity	Ambient (humidity chamber available)
Sample Amount	Approx. 10 mg

Crystal Properties

The more symmetry between the angles and axis, the simpler it becomes to index the pattern with very few lines in it. The table 3.2 depicts the possible shapes of a crystal with their respective combination of axis and the axis angles.

Table 3.2: Crystal shapes with their axis and axis angles

S.No.	Shapes	Axis	Axis Angles
1.	Cubic	A=B=C	α=β=γ=90
2.	Tetragonal	A= B≠C	α=β=γ=90
3.	Orthorhombic	A≠B≠C	α=β=γ=90
4.1	Hexagonal	A=B≠C	α=β=90, γ=120
4.2	Rhombohedral	A=B=C	α=β=γ≠90<120
5.	Monoclinic	A≠B≠C	α=γ=90, β =120
6.	Triclinic	A≠B≠C	α≠β≠γ≠90

Miller Indices

We draw a cube or cuboid with 3axes. A plane is drawn to cut it in such a way that the x-axis, b-axis and c-axis are cut at a/2, b, and c/3 respectively. The inverse of these fractional points i.e. (2,1,3) are termed as Miller Indices. As two parallel lines are assumed to intersect each other somewhere at infinity hence the plane parallel to any axis is supposed to meet it at infinity. Hence, the reciprocal of infinity is zero i.e. the 100 plane and parallel to b and c meets at 1; while the origin is set at (0, 0, 0).

Indexing Patterns

It is the method of evaluating the unit cell geometry from studying the peak positions. Two types exist, viz.

- Manual indexing: it is very time consuming but useful
- Auto indexing: it is a computer software based pattern matching method like JADE

Information provided by XRD

- Lattice attributes
- Phase intensity and purity
- Detection of crystalline structure pattern
- Identification of crystal structure
- Percent phase composition

SEM: SCANNING ELECTRON MICROSCOPY

Scanning Electron Microscopy, may also termed as SEM microscopy or SEM analysis, is used for analysing micro materials and draw inferences on the cause of failure of solid materials. It can carry very efficient scanning at high magnifications. High-resolution image graphics are generated by it, which help in evaluating even minute features and objects. Energy Dispersive X-Ray Spectroscopy (EDS) can also be carried along with SEM. In 1937 Manfred von Ardenne invented an actual microscope by scanning a small raster with a high energy beam electrons to produce a high magnifying image. He discarded the chromatic aberration too. Later on many groups have reported such works with the "stereoscan" surfacing as the commercial equipment in 1965.

SEM and EDAX EDS

The EDS constituent unit is equipped with a EDAX EDS. It helps in qualitative and quantitative analysis like to determine on and in the substance under investigation, component make-up of the samples and finding out the material foreign to the organic nature and films on metal. With few more important components, viz. 1) a varying pressure unit to hold a non-conductive and/or wet sample with least bit of preparation activity, 2) capacity of a 200 mm wide to 80 mm long sample holding chamber and 3) images of magnification ranging from 5X to 300,000X as its high-resolution SEM images.

The SEM analysis is based on the signals generated as two-dimensional image and tells us about morphology, chemical constituents (with EDS) and material alignment in the sample.

It operates by focussing a high-energy electron beam to produce different signals at the specimen surface. Data of these signals is collected to result in a 2D image of the sample specimen explaining the spatial differences in its attributes including chemical properties, surface consistency and alignment of materials. It can also analyse selected points of the sample. Thus it pays attention on the quantitative and qualitative contents to determine its chemical nature, crystal lattice arrangement, etc. separating energy spectrums of a variety of elements of their characteristic X-rays and feeding it to the software to determine the specific elements. It is displayed as a graph between X-ray counts vs. energy (in keV). Energy peaks thus generated compares sample elements corresponding to specific elements. Analysis of a spot of few microns is possible and it helps in making a composition map. Hence. EDS and SEM finding vast information about the sample analysed.

Principles of working and capabilities

SEM ids based on 1) secondary electron beam, 2) specific X-rays, 3) back-scattered electrons, 4) cathodoluminescence, 4) sample charge and 5) transmitted beam of electrons. A type of electron microscope that generates images of a specimen by scanning it using a focused electron beam is SEM (scanning electron microscopy). Interaction of specimen atoms with electrons from beam produce signals which are detected to gain information about the constituency of the sample. Raster scan pattern helps detect the signal produced by electron beam together with the beam's position. A good resolution of better than one nanometer can be produced by it. Samples can be detected in wet conditions, in low as well as high vacuum and cryogenic to high temperature ranges. Detecting secondary electrons left by the excited beam is mostly captured which depends on the angle of beam and sample spatial arrangements to produce an image. Low or narrow beam of electrons causes 3D appearing SEM image which helps to understand better the constituents of samples. Magnifications ranging from 10 times to 500,000 time, i.e. almost 250 times the best light microscopy.

Reflection of electron beam by samples by elastic scattering is termed back scattering, is used to generate SEM images with spectrum developed from X-rays.

Specific X-rays emitted by the electron beam by removing an inner shell electron from specimen, causes a higher-energy of electron beam to fill the gap to release energy. Specific X-rays are helps identify the constitution and evaluate the element abundance in the specimen sample. Specimen must be of appropriate dimensions to fit the chamber holder for a tough mounting termed as specimen stub. Angle of 45° tilt to a 15 cm semiconductor chip, very minute antiquities can be analysed with efficiency.

Electrical conductance is required as a sample property for SEM but should be kept grounded to reduce charge. Metal samples do not require cleaning and mounting while non-conductive samples require them. They usually charge by the electron beam by secondary beam of electrons, which cause flaws in images. Hence to avoid that, we coat them with electrically conductive substances (like gold, palladium, platinum, tungsten, alloys, etc.) by sputtering or by vacuum evaporation. For biological samples we need improvements of these methods. We employ a high pressure chamber for an environmental SEM where short working distance and vacuum pumping of optical electron column in the electron gun. Charge is neutralized by high pressure maintenance. Field emission guns help in low voltage SEMs which helps in producing more brightness and small spot size. Resin embedding with polishing is needed for both material samples as well as biological ones.

Transmission SEM (TSEM)

Using SEM in transmitting method by using an appropriate detector unit kept below the sample. Dark and bright fields can both be used to record images with contrast by using low electron for even biological samples at higher resolutions or magnifications with an FEG (field emission electron gun) beam.

Equipment composition

- Electron gun
- Lenses
- Sample platform
- Detectors for receiving signals and arranging them according to requirement
- Display outlet devices

Organization Requirements:

- Power supply system
- Vacuum creating system
- Cooling system

Preparing biological samples

Dry samples are required but biological samples can be wet nature too, hence they need chemical fixations to stabilise them and not hinder their properties too. Incubating in a buffer (formaldehyde, gluteraldehyde, etc.) with osmium tetroxide for post fixing is done then dehydrating them by ethanol or acetone and further removing them by liq. $CO_{2.}$ Then by removing it too. Mounting this dried sample by adhering it by epoxy, sputtering with palladium or gold and then examining. They can be cryo-fixed as well.

Preparing material samples

Surfaces are required to be polished ultra-smoothly. For EDS analysis, carbon coating is done too. Even fractures in samples can be read too on a light microscope. Chemical polishing is done on some metals, earthen samples, and ICs. High-resolution coatings are required for high-magnification imaging of thin films.

Transmission electron microscopy

A technique for studying unknown or known sample for the purpose of exploration, an electron beam can be transmitted past an ultra-thin sample which upon its transmission collaborates with the specimen and a result produces a magnified image on a display monitor (eg. a sensor, a fluorescent screen or a photographic film). It uses a high focus on the examined specimen. It is an advanced form of SEM where we can view a very minute colony or even a column of an atomic unit. de Broglie's electron wavelength is used for getting a high resolution image. It is very useful in the research field of medicines, material science, cancer, viruses and even nanoparticles and pollutants. Both small and high magnification images can be obtained from TEM imaging depending on the sample thickness. With thicker compositon material an expert view is required to understand the in=mage after higher magnification.

Max Knoll and Ernst Ruska invented the first ever TEM in 1931, with the commercial one surfacing in 1939 after developing a magnification more than light in 1933. Initially the need for resolution of images for proper investigation of the required samples provided a goal to develop TEM. The energy source varied from using ultraviolet rays to cathode rays to electron beams. When the use of electron beam proves to be evolutionary in the construction of the electron microscopes, the focus went on improving the resolution of the obtained images. Improvements went on coming its way even after World War II. Researchers at both academic and commercial level kept improvising the instrument to increase efficiency of the resulting images.

Principle of Operation

Electrons

The maximum resolution, *d*, depends on the photon wavelength, λ used to visualize the specimen and the numerical aperture.

$$d = \frac{\lambda}{2n\sin\alpha} \approx \frac{\lambda}{2\,\mathrm{NA}}$$

Visible light as a source of energy has some shortcomings hence scientists replaced it with electrons. Beam of electrons can also be of wave-like nature with wavelengths depending on their kinetic energy. Hence an improvisation of the equation was made as follows:

$$\lambda_e \approx \frac{h}{\sqrt{2m_0E\left(1+\frac{E}{2m_0c^2}\right)}}$$

where, h represents Planck's constant, m_0 represents the mass of a resting electron and E represents the accelerated electron energy. By thermionic emission generating from a tungsten filament, an electron beam is created. It is then accelerated by a voltage and concentrated by lenses (electrostatic and electromagnetic) to incident upon the specimen. Then an image is generated.

Source formation

TEM equipment is comprised of an energy source (tungsten filament) which is attached to a high potential source to generate enough current to start an electron beam to pass through vacuum (Wehnelt cylinder) to avoid wastage. This electron cloud can later be widened according to need to focus on a spot on the specimen as provided in the figure 3.4 below is a TEM sample grid with a goniometer

Optical arrangements

For the converging of the beam of electrons, we need lenses which can be arranged at variable angles. Image magnification can be done by altering the electric current through coils. By arranging these coils one can have a quadrupole or hexapole lensing. By arranging at the square vertices we can get a quadrupole lens and by increasing the coils form four to six, we get a hexapole lens.

Three stage of lensing is required namely a condenser lens, an objective lens and a projector lens. First comes the condenser lens comes into play by converging or condensing the electron beam. Second is the objective lens which focusses it after it passes through the sample. The last is the projector lens into role by enlarging the beam size to fit the fluorescence screen or other films or sensors. The distance ratio of specimen and objective lens controls the amount of magnification. The specialized of coil grouping helps correcting the asymmetry of the beam. Filters can also be used to refine the image and minimize or reduce aberrations.

Display Image

A phosphor screen made of zinc sulfide or any fine particles can be used to act as a screen to receive the electron beam after its interaction with the specimen. Current coupled diodes may also be used instead of using films. These display screens can be arranged in the beam path as the user requires.

Equipment compositon

An electron source like tungsten filament or any other specialised source

A vacuum system to provide a passage to the source generated electron beam

Specimen platform which is made of a grid-like section upon a liquid sample solution is poured (after homogeneous particle distribution) and allowed to dry. It is sectioned into ultramicrotomy divisions which are inserted into a sample holding tube. This can be rotated in a TEM goniometer.

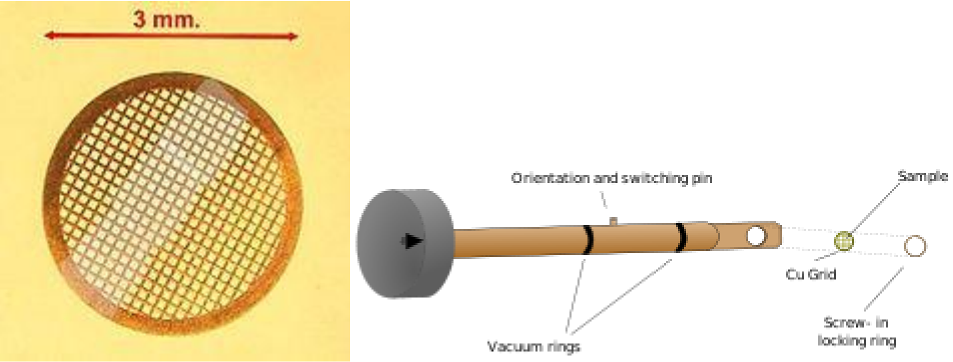


Figure 3.4: A TEM sample grid with a goniometer

Electron gun

It is an assembly of a filament, a Wehnelt cap, a circuit and an anode for extraction, as provided in the figure 3.5 a sketch of an electron gun.

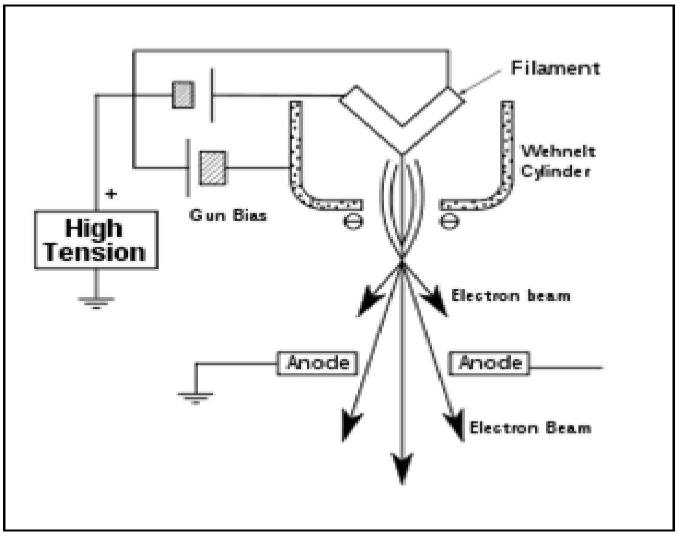


Figure 3.5: Sketch of an electron gun.

Electron lens

Used for concentrating parallel electron rays to act as an optical lens.

Apertures

Like the ones present in a camera which open to the sample a glimpse of the electron beam.

Imaging technique

The wave functions are studied after the optical lens passes the beam on specimen exposure to provide a result.

Contrast creation

Usually enrolls a bright field contrasting method to bring clarity in the obtained image.

- Diffraction contrast: On Bragg scattering a diffraction contrast can also be obtained.
- Electron energy loss
- Phase contrast: provides HRTEM (high resolution TEM)

Sample preparation methods

a. For biological samples:

- Tissue sectioning should be done in a way to cause no or very least damage to the structure
- Straining of the sample to create contrast in the morphology.

- Mechanically ground the sample to break it into minute particle size.

b. For metallic samples:

- Chemical etching
- Ion etching: by sputtering

Raman Spectroscopy Data Analysis and Monitoring

Raman spectroscopy method involves a vibrational molecular approach to form an inflexible scattering of light. A target sample's molecule undergoes energy exchange when a photon beam is incident upon it. When energy is found to be lost from wavelength, the incident photon beam is found on low end. This indicates bonding of specific kind in then sample molecule.

Like XRD, Raman also provides us with an accurate fingerprint specific to a molecule and gives an idea of its structural morphology. Similarity to FTIR is also observed in a Raman spectrum. Though, it is more useful in comparison.

An already sequenced data is used to align the new sample against it in a fixed arrangement and with a definite point gap (spatial resolution). This fixed data is showcased as a view appearance where portions of high intensity is displayed in one colour (red, most commonly) and portions of lower intensity as displayed in a distinct colour (blue, most commonly). This colour dissimilarity may reflect an alteration in phase concentration of the identical substance across the target or may display alteration in chemical constituents. Every point or spot of the image is divided into divisions like pixels with which the Raman spectrum is linked with that spot on the sample.

The Raman spectrum software permits the operator to show these single spectra and produce chemical dissipation photographs of the sample constituents. These images are captured by a built in camera and an electronically controlled platform. Any research field which requires knowing the composition of the sample can use Raman Spectroscopy. Like in medicine research, mineral and composites study.

Points to keep in mind to separate from XRD and FTIR

- Unlike powder sample in XRD, liquid aqueous samples can also be viewed as no huge aqueous absorption effects are present.
- Direct proportionality exists between spectral intensities and concentration of samples
- React vigorously to temperature alterations
- Sample can be used in the form already available, needs no particular kind of modification.
- LabRAM can be used to get a high degree of resolution between space and depth.

These points separate a Raman spectrum XED and FT-IR and play a vital role in determining chemical composition. As it is not compulsory to use only a dried powder form, two other forms of phases can be used as well i.e. solid, liquid and any solution. It is proven to helpful in telling about the physical characteristics and properties like density, lattice shape, polymorphs and stresses.

Sample Requirement for Raman Spectroscopy

At molecular level samples can be slurries, solids, gels, liquids, films, powders, etc. of who's a Raman Spectra can be obtained. Even metals can be studied by Raman spectrum. Even gaseous phase can be evaluated by Raman spectroscopy. The only condition required mandatory for gases is that as they have low molecular concentration so they need cells of high path lengths. An N=N peak can be obtained for nitrogen gas in some cases.

Water or any aqueous sample can also Raman viewed but the light scatter can be weak as the molecules undergo scattering. Example a mixture of water and alcohol will be separated into different samples i.e. water and alcohol both. The spectrum length for both the units is extended to the same length. Surface Enhanced Raman spectroscopy can even find the trace amounts of water.

Raman Spectroscopy on Heterogeneous phases

As Raman compares every sample with a pre-defined database hence a heterogeneous mixture can be observed separating each constituent spectrum-wise. The final spectrum is the coincidence of the separate spectra of all the components overlapped together. The peaks thus obtained give an idea about the composition details (of known units) of the mixture. Software tools like spectral separation and database searching can be utilised to find out and match them with known values. Even where resolution is not notable with human eye, we can utilise these software techniques to reduce the complexity. For example the Raman spectrum of o-, m- and p-xylene can be resolved from the mixture and a demonstration of their concentrations can be done separately to reveal 33% o-xylene, 33% m-xylene and 33 % p-xylene from a glass vessel.

Quantitative and Qualitative Values

Both of these values can be obtained from a Raman Spectrum. By performing or carrying out a fractional comparison of the band intensities we find a direct correlation between them to the compound concentration. Chemometric techniques can be used too if required. Sample compounds with high concentration like 90% to 100% can also be quantitatively examined to the smallest range of ppb order as well.

As it is kind of fingerprint-like unique value for each and every unique sample so the spectral database library can come in handy for comparing and finding the position of the sample among the already discovered ones. This is the qualitative examination technique.

Impacts of Purity on the spectrum

Purity of the samples is not a limiting factor as the charge coupled detectors possess a long range of data and even exposure time of the laser can be varied according to requirement.

Time taken for Raman recording

Usually a few seconds' duration time is required to obtain Raman plot but the time can depend on the sample nature.

Sample Preparation

No hard and sole rule applies to preparing a sample for Raman examination be it anything from a paper to polymers to tablets or generic forms like solids or liquids, etc. light may be passed through any medium like Pyrex glass, blister packets, plastics, bags, etc.

Physical Environment requirement

A wide range of temperature unit cells are available and fibre optics for sensing temperature (like probes) is suitable at increased temperature values and pressure values. Example 500 °C and 3000 PSI also can be accommodated rather easily. We may also adjoin other equipmental parts for assistance. Too much laser power can destroy the sample or if it is too much focussed on a fixed spot. As we need a high range of power for focussing a beam for Raman spectrum, we must pay attention not to damage the sample. While dispersive Raman lasers are of low intensity so they do not pose any danger to the sample. By increasing the area of the sample spot or by reducing the laser intensity one can expect to minimise the damage to the sample.

For example, Perkin Elmer Raman lasers equip a low power diode which h deliver a high performance. It reduces the fluorescence and gives high quality accuracy to the spectrum obtained. Lasers have 0.03 mm line width. It involves a greater than 40 dB signal to noise ratio. We can adjust the software at desired power, relative intensity, etc.

Density grating in Raman Spectroscopy

There is an Echelle image or spectrograph available with good diffraction grating which is entirely different from the general grating available. Example the Perkin Elmer uses an Echelle spectrograph for this purpose which uses a grating of 601 per mm of grating. In the path of the incident beam a box-like grater is kept at the edge of the highest available angle. It is partnered with a secondary grater which cross dispersive in nature to give a dispersion og 18001 per mm almost equal to the old spectrograph of ¼ M. In a general arrangement an Echelle grater can be fixed and still be made to produce a high resolution between the spectra. Data can be collected in a single go at random and the can be sorted. For example a Raman 200 series uses a traditional style (Czemey Turner) meter to give an image of good quality. A 6001 per mm grating is used.

Rayleigh Filtering

Very sharp peaks can be observed by these instruments, as highly efficient filters are used to clean the reflected lasers after passing through the sample. Optical density of up to 6 can be blocked by these filters with an optimisation of 95 cm^{-1}. These filters are long life filters and can be sustained for a long time not like the holographic filters. And Rayleigh filters also have 2 times the optical density regulating capability. The charge coupled detectors are used which provide a high range of grading.

Differentiation of polymorphic forms

Morphology of different forms can be detected by latest Raman instruments easily and efficiently. The differences recorded are sometimes very small to notice and hence confuse the similarities over a large range of spectra. Wave number from 100 to 3000 cm^{-1} is available with a high range of resolution possible that can notice any difference in the peaks. A multi-well plate is also present in these new machines which can observe a multiple number of unknown samples.

Raman Instruments

Bench-top, fibre optic and Raman microscope spectroscope are the different types of instruments used for measuring Raman Spectrum. Latest spectrometers for Raman are Raman Station, Raman Flex, Raman Micro, etc. the user is provided with full regulation over recording data involving procurement arrangement, starting gaining, observing background, etc. The three axes can be tuned to regulate according to requirement.

Defaults caused by Fluorescence

Raman spectra are faulted by fluorescence. To avoid fluorescence we use near infrared (785 nm) photon beam but it does not solve the problem completely. Due to photo-catalysis the fluorescence defects can be reduced in cosmetic levels and even the base-line can be amended. Perkin-Elmer Spectrum software provides all these arrangements.

Surface area and Porosity measurements

Adsorption

When a solid is interacted with a gas or vapour, the solid often adsorbs it on its surface. It is usually physical adsorption and does not change the properties of the solid permanently. As it just involves Van der Waals forces between the solid surface and adsorbate.

Characteristics of Physical Adsorption

- Low heats of adsorption has lower heat energy, no extreme or destructive structural changes.
- Multi layers of adsorption might take place for measuring pore diameter
- High temperatures are not good for the activity of adsorption.
- It is quicjk and random process as no threshold energy is needed.
- It happens only at physical level involving quick adsorption and desorption.

Adsorption and Desorption Isotherms

Adsorption isotherm is acquired by calculating the quantity of gas adsorbed through wide assortment of relative pressures operated at a persistent temperature (usually liquid nitrogen is used at 77 K). Likely desorption isotherm measurements involve calculating the amount of gas removal as pressure is lowered.

Types of Isotherms

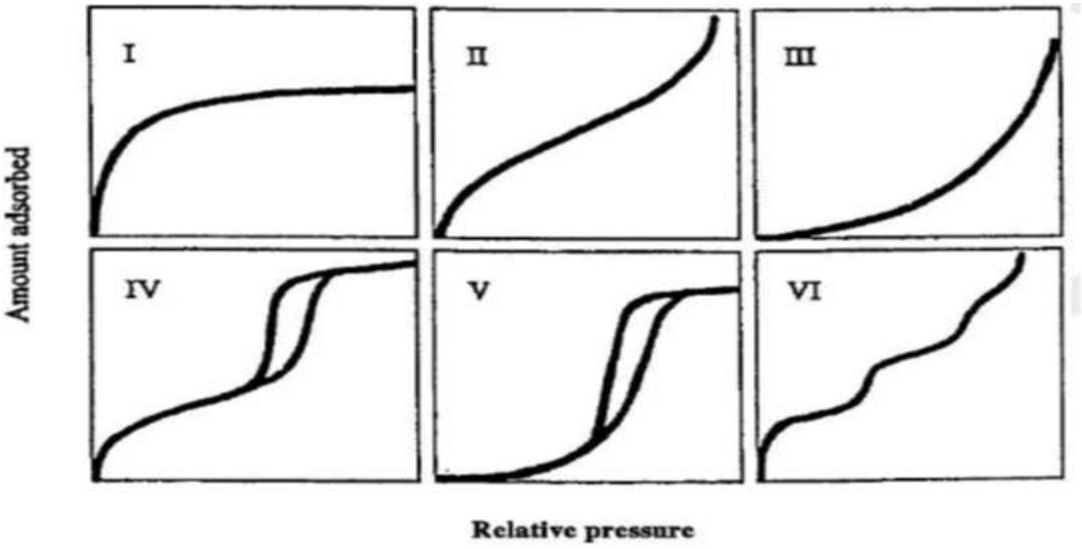


Figure 3.6: Isotherm types as described by Brunauer, Deming, Deming and Teller.

Description of these types is as follows and provided clearly through figure 3.6.

Type I

Microporous pores with the area to be adsorbed in present entirely inside these pores which have no surface left unadsorbed by the adsorbate once they come in contact.

Type II

Non-porous substance larger than microporous size limit shows type 2 isotherms. Saturation point is reached after only one layer adsorption.

Type III

It is a heat based adsorption when the adsorption heat is less than the heat of condensation. The process of adsorption is increased by increase in the interaction of the adsorbed layer with more adsorbate rather than the porous surface.

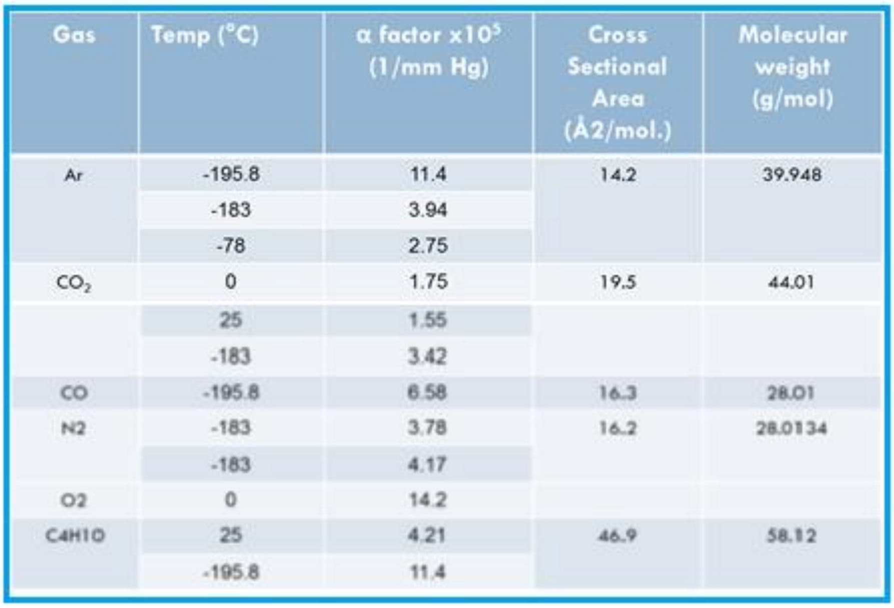
Type IV

With pore size of range 1.5 nm to 100 nm, this type of isotherm is formed. As we elevate the pressure tha adsorption is found to increase and fills the pore cavities. Also saturates once one layer is formed.

Type V

Same as type 3 but with the size range of type 4. Figure 3.7 details the types of adsorbate (gas or vapour)

Figure 3.7: Type of adsorbate (gas or vapour)



Gas	Temp (°C)	α factor x10^5 (1/mm Hg)	Cross Sectional Area (Å2/mol.)	Molecular weight (g/mol)
Ar	-195.8	11.4	14.2	39.948
	-183	3.94		
	-78	2.75		
CO_2	0	1.75	19.5	44.01
	25	1.55		
	-183	3.42		
CO	-195.8	6.58	16.3	28.01
N2	-183	3.78	16.2	28.0134
	-183	4.17		
O2	0	14.2		
C4H10	25	4.21	46.9	58.12
	-195.8	11.4		

BET

It was given by Brunauer, Emmett and Teller (BET), as a technique to evaluate and explain the pore surface area specific to the specimen. Follows the BET equation:

$$\frac{1}{W\left(\left(P_0/P\right)-1\right)} = \frac{1}{W_m C} + \frac{C-1}{W_m C}\left(\frac{P}{P_0}\right)$$

Where the letters represent the followings:
W= weight of gas adsorbed, P/P0 =relative pressure, Wm = weight of adsorbate (per monolayer) and C = BET constant

BET equation gives a linear graph between 1/[W(P/P0)-1] versus P/P0 and s represents slope and I represents intercept

$$s = \frac{C-1}{W_m C} \qquad i = \frac{1}{W_m C}$$

Weight of monolayer is calculated by:

$$W_m = \frac{1}{s+i}$$

S_t represents total surface area of the weight of porous substance present and is evaluated by:

$$S_t = \frac{W_m N A_{cs}}{M}$$

Where the equation parameters are represented as :

N = Avogadro's number

M = molecular weight of adsorbate used

Acs = adsorbate cross-sectional area

Two types of BET are derived for example like in figure 3.8:

- Single point BET: it is calculated by focussing a single point on the graph.
- Multipoint BET: it is calculated by focussing at least three points on the graph.

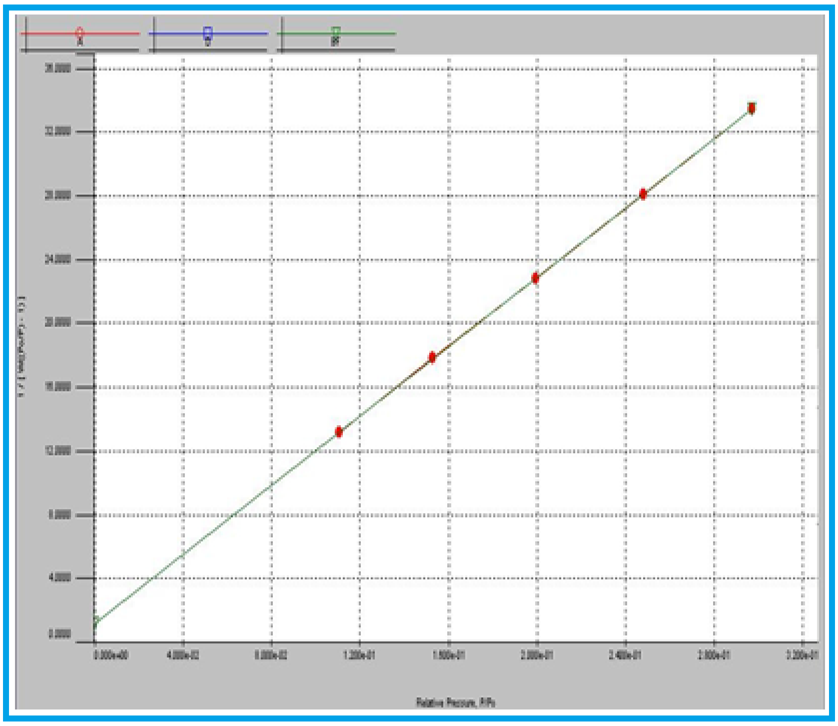


Figure 3.8: Example of a Multipoint BET plot

Langmuir Equation:

It explains the microporus substance displaying type I isothermic nature. It helps to know the monolayer adsorption properties like calculating the monolayer weight.

Porosity - pore volume measurement:

By calculating the total quantity of adsorbed gas or vapour at relative temperature approx. equal to 1, where we presume that the pores are completely filled with the liquefied adsorbate.

Porosity - pore radius measurement:

Pore volume helps to determine the mean pore diameter; figure 3.9, shows an example of adsorption/desorption isotherm

.

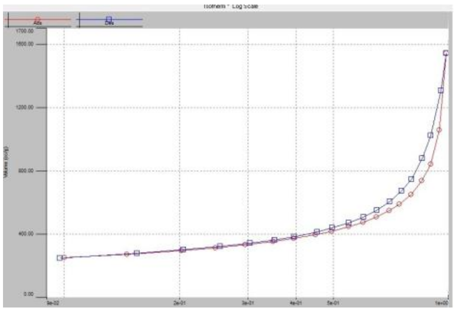


Figure 3.9: An example of adsorption/desorption isotherm

Requirements for measuring the pore parameters

We need some steps to treat the sample before doing any calculations. We need clean surfaces of platform to get rid of any impurity present as vapours before beginning the analysis. There must be dry inert gas used for the purpose of adsorption which does not react with or alters the sample's nature. In figure 3.10 gives a preview of a sorpometer by Micromeritics ASAP-2010, USA.

Figure 3.10: BET surface area measuring instrument (sorptometer)

B. Optical characterisation

Optical Characterization Techniques

UV-Vis absorption spectroscopy

We used Thermo scientific Evolution 300 UV-VIS equipped with Toshiba TCD 1304AP linear CCD-array detector (for optical resolution as accurate as 0.02 nm (FWHM)) for recording UV-Vis spectroscopy of the synthesised samples. The image of the UV-VIS instrument is shown in Figure 3.11. The optical spectra have been recorded from 220 nm to 700 nm range in a 10 mm path quartz cuvette.

Figure 3.11: Image of UV-Vis spectrophotometer instrument

On the light energy spectrum the range for UV region is stretched from 100 nm to 400 nm while visible and near ultraviolet region is stretched from 750-350 nm. While crossing absorption energies from 10^4 to 10^5 cm^{-1} by an absorbing molecule, existing in the near infrared regions to visible regions to ultraviolet regions, has its outer-shelled electrons excite for transition from an occupied orbital. A plot of absorbance (A) versus wavelength of light at each value is drawn by the instrument. The solvent of the solution should be transparent like water, ethanol, etc. allowing the dissolving species have their own colour effects. To analyse a dry unit like powder or some solid entity, we need to first dissolve it in a suitable solvent. They might be semi-soluble or insoluble in the solvent; hence we use possible techniques like diffuse reflectance and specular reflectance. Specular reflectance is used for reflective materials and diffuse reflectance is used for amorphous compounds and coarse solids. Since UV does not pass through glass efficiently thus we use quartz crystal cell for pouring sample.

For the wavelengths below 350 nm we require a hydrogen gas discharge lamp and for the wavelengths above 350 nm we require tungsten filament lamp. The spectrum of UV-Vis absorption is based on two main philosophies. One is that beyond a particular frequency an extreme absorption happens since the transmittance is reduced to zero at the absorption verge. The other is that above this absorption verge, the reflectance methods should be used.

Charge relocating spectra (motion of an electron from restricted energy band of one atom to a higher energy band but still located on the restricted energy band on the adjacent atom) and the other spectra made by the motion of an electron from a restricted energy band on one atom to derestricted energy band i.e. the conduction band specific in solids are accountable of absorption limit or verge. Absorption edge for non-conducting solids of polar nature (electronically) form in ultraviolet region while for conducting as well as semiconducting materials they form in visible region or near infrared region.

As powder samples lie in the size range of micrometres show diffuse reflectance spectroscopy (DRS) with wavelength in the same range, by following Kirchhoff's law of absorbance (α), reflectance (ρ), transmittance (τ):
$\alpha + \rho + \tau = 1$.

Here reflected beam of light is measured by the joining sphere detector (photodiode fixed below the integrating sphere) and integrating sphere. Reflection occurs when the sample neither transmits the beam nor it is absorbed by the sample. Sphere detector operates in photovoltaic mode, filters the output and magnifies it prior for suitability with the mechanism of detection.

$$R\infty = -\frac{1 - \sqrt{\frac{K}{K} + 2S}}{1 + \sqrt{\frac{K}{K} + 2S}}$$

With R∞ as reflectance relative,

K as linear extinction coefficient

and S as scattering coefficient, generally not depending on wavelength and concerns the grain size.

After measuring them, the ordinate can be transformed to Kubelka Munk function to calculate the band gap by:

$$F(R\infty) = \frac{(1 - R\infty)2}{2R\infty} = \frac{K}{S}$$

This shows the independence of S from wavelength, the scattering coefficient. The maxima and minima coincide in the absorption spectra of optical density.

Ultraviolet–visible spectroscopy

While measuring a liquid’s absorption capacity, we often evaluate its transmittance or absorbance. In UV-Vis we use an ultraviolet source for reference. It is similar to fluorescence in principle of measuring or detecting an electrons position changing or excitation activity among various energy levels. It affects the colour of the chemicals by throwing UV light on them. It measures the electrons jump from quantum states to their excited states.

Beer-Lambert stated that the absorption capacity of a solution is directly related to the amount of absorbing units present in it and the length of light travel (path length).

$A = \varepsilon bc$, where ε is a constant of proportionality, called the *absorbtivity*, A is absorbance.

Thus, if we fix the path length then we can measure the amount of absorbing units in the solution. A calibration curve can be made to focus on the rate of concentration. High pressure liquid chromatography studies can also be done by UV-Vis spectrophotometric unit

Principle of operation

Usually concerns the spins of electrons. Electrons which are free or not bonding are at the liberty to absorb energy they receive on exposure form visible light, UV or any spectral energies. The more easy the target electron to excite, the higher the wavelength. The electrons have vibrational, rotational or both the energies and these can be discrete too. The outer shell electrons undergo these transitions. They can be:

- π, σ, and *n* electrons
- charge-transfer electrons
- *d* and *f* electrons

Absorbing species: π, σ, and *n* electrons

Chromophores are composed of electrons easiest to excite, hence they absorb UV and visible lights to go for longer wavelengths. The spectrum thus obtained is complicated. As the overlapping of energy spectrum happens because of the presence of both rotational and vibrational energy bands. It is perceived as persistent line. In the figure, the transitions are represented by E_0. Figure 3.12: (a) shows electron excitation between energy orbitals and (b) different types of electron transitions.

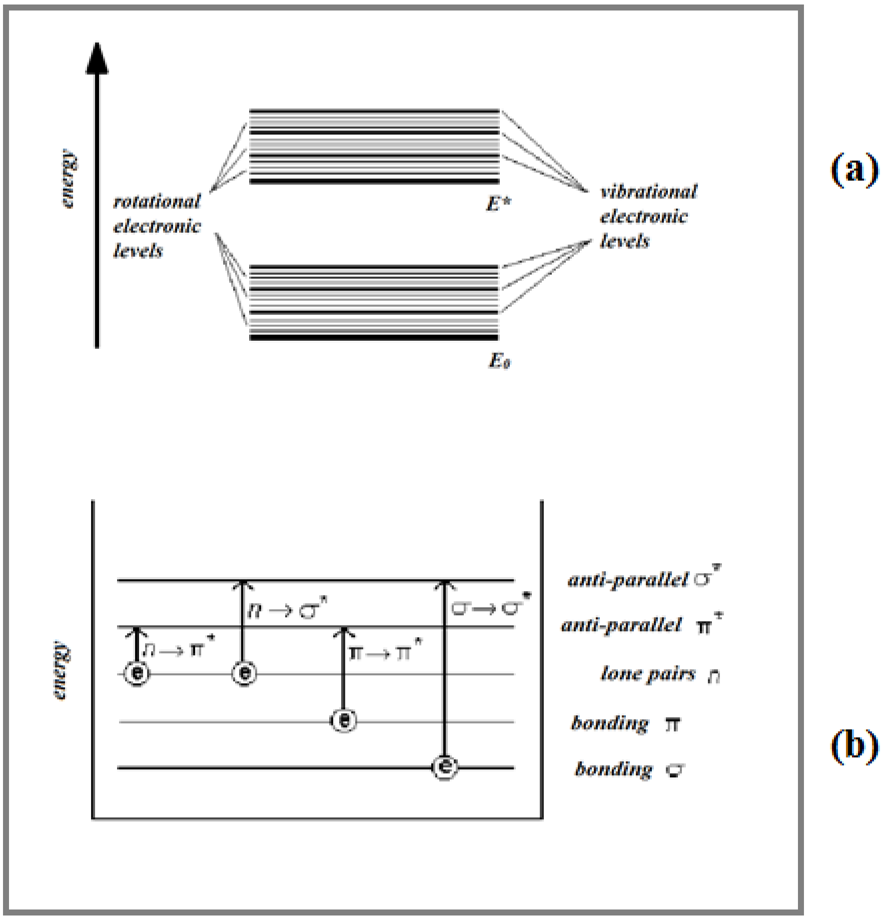


Figure 3.12: (a) shows electron excitation between energy orbitals and **(b)** different types of electron transitions.

$\sigma \rightarrow \sigma^*$ Transitions

It represents bonding between sigma and its anti-parallel orbital. Cannot be viewed by a UV-Vis spectra, as they fall too low (200 nm to 700 nm).

$v \rightarrow \sigma^*$ Transitions

Concentrated compounds are filled with non-bonding electrons which undergo this category of transitions. They require lesser energy then the above contemporaries. Their wavelengths lie within 150 nm to 250 nm. Hence the peak range is low.

$n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ Transitions

Their wavelengths lie within 200 nm to 250 nm. Hence the peak range is high. For the pi bonding, unsaturated groups are required.

Molar absorptions are less from $n \rightarrow \pi^*$ with 10 to100 L mol^{-1} cm^{-1}. Blue shift is observed with the. $\pi \rightarrow \pi^*$ give molar absorptions in the range of 1000 and 10,000 L mol^{-1} cm^{-1}. Red shifts are observed here. This is due to the polarisation among the absorber and solvents. This results in lowering of energies.

Absorption by charge-transfer

This is done by the formation of charge-transfer complexes. But the charge entities must be present and must be eager to under accepting and donating of charges. When this is assisted by an electron transfer, they release energy.

Molar absorptions are huge mostly greater than that of 10,000 L mol^{-1} cm^{-1}.

Ultraviolet-visible spectrophotometer

The equipment used for evaluating ultraviolet-visible spectroscopy is referred as UV/Vis spectrophotometer. It calculates and compares the intensity of light before and after passing through a sample. (With I_0 as the initial value of light intensity and I as the light intensity after it passing thorugh the target sample). The ratio of I/I_0 is termed as transmittance (can be calculated in percentage too). The absorbance *A* is just the opposite of transmittance *T*, calculated by:

$$A = -\log(\%T/100\%)$$

We can also use it to calculate reflectance too. The change is that here the light intensities of incident and reflected rays are compared by calculating ratios (I/I_0). The percentage of reflectance can also be measured. Figure 3.13 shows the Schematic of UV-Vis Spectrophotometer.

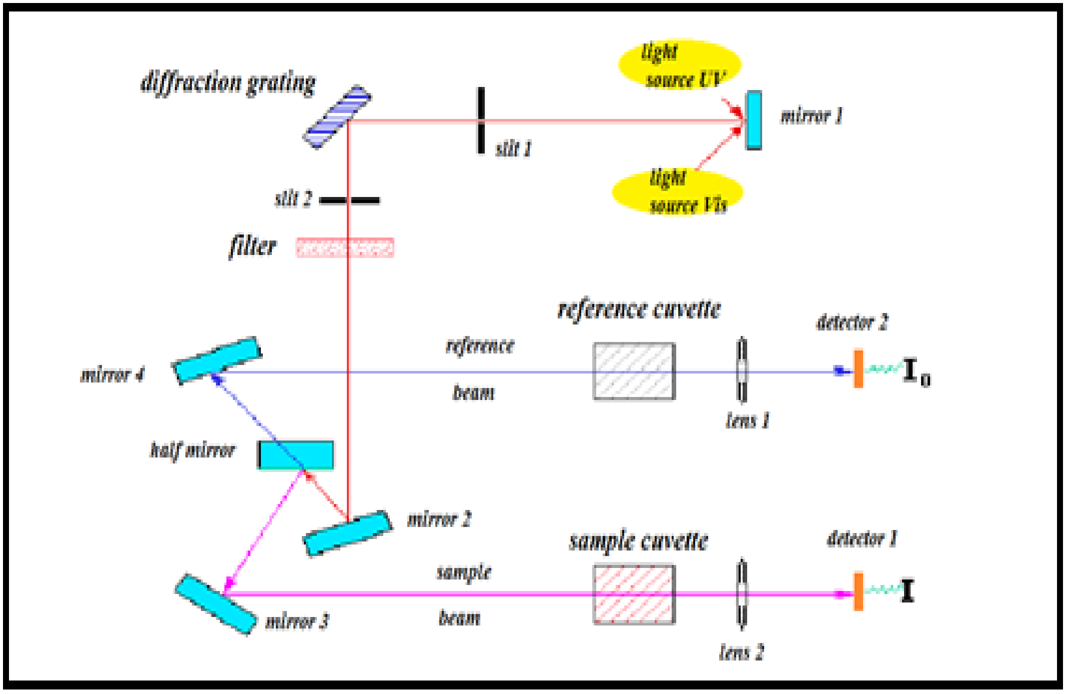


Figure 3.13: Schematic of UV-Vis Spectrophotometer.

The components present are as follows:

- A light source (UV as well as Visible light)
- A sample holder
- Monochromator with a diffraction grating (or a prism with diffraction grating arrangements)
- A detector

Light source used for radiation of energy is a tungsten filament (300 nm to 2500 nm), a D_2 arc lamp with continuous UV (190 nm to 400 nm) range, Xenon flash lamp continuous over a range of 160 nm to 2000 nm. Nowadays, even LEDs are also used for the visible light along with a PMT (photo-multiplier tube) and charge-coupled device. Scanning monochromators may combine with PMTs and photodiodes to filter the output light and thus making it monochromatic. The diffraction grating helps record the intensity at different wavelengths. Monochromators can be used with both of them as they both possess many detecting units to record different lengthen lights on different dpi at the same time.

It contains a group of detectors to collect light of several wavelengths on pixel variety by a single or double beam. I_0 must be measured without the sample to set an initial value in a single beam instrument. While in a double beam light is split in two beams and then crosses the sample. One beam is considered the reference beam and the second is the passed through sample beam. Reference beam is the initial wavelength beams i.e. the one with no absorbance. And the second one is relatively calculated as a ratio of the first beam. Both the beams are measured simultaneously by two detectors.

Uses

In diagnostic chemistry, we use UV/Vis spectroscopy to calculate the presence of various analytes or additives in the desired solution (sometimes solids and liquids as well). These analytes can biomolecules, polymeric organic compounds, transition metals, etc. Adding additives in the pure quantity alters the colour of the overall mixture like if any gas is added to it magnifies the colour of the resulting mixture thus, proving its presence. Even organic compounds have the ability to impart colour on absorption of light.

It also helps to monitor the kinetics of a reaction. The solution constituents require to electron shifts by changing color from initial reactants' color to a new color of the product. For example mercury derivative changes color on exposure to visible light. Some reactions may not be permanent and revert to original colors. We can also put a tab on the timeline of reaction's step by step follow-up by observing it spectroscopy at different time intervals. Like in the above example of mercury dithizonate, the initial color is yellow, on absorbing light it changes to blue. We take a gap of a few seconds then again observe a data, then allow it turn yellow and compare the concentrations of the solution. In temporary color change reactions we can even locate the point of equilibrium by plotting the data.

Photoluminescence Spectrophotometer

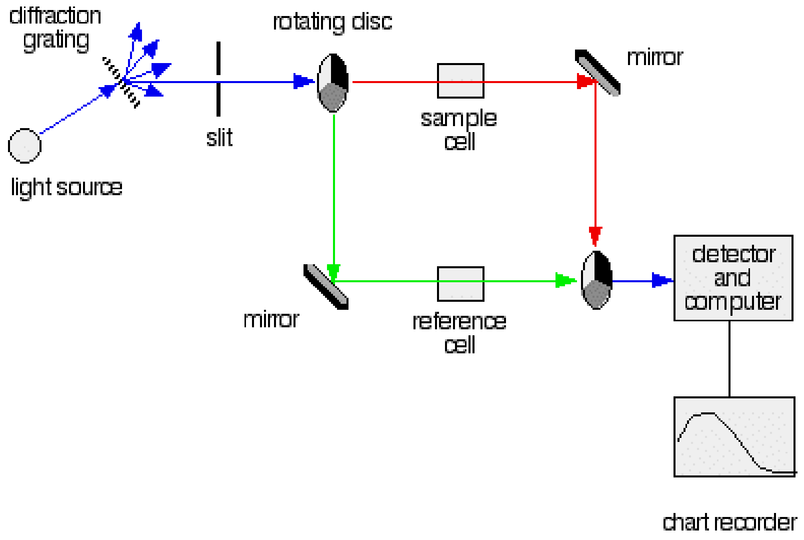


Figure 3.14: Working of a PL spectrophotometer

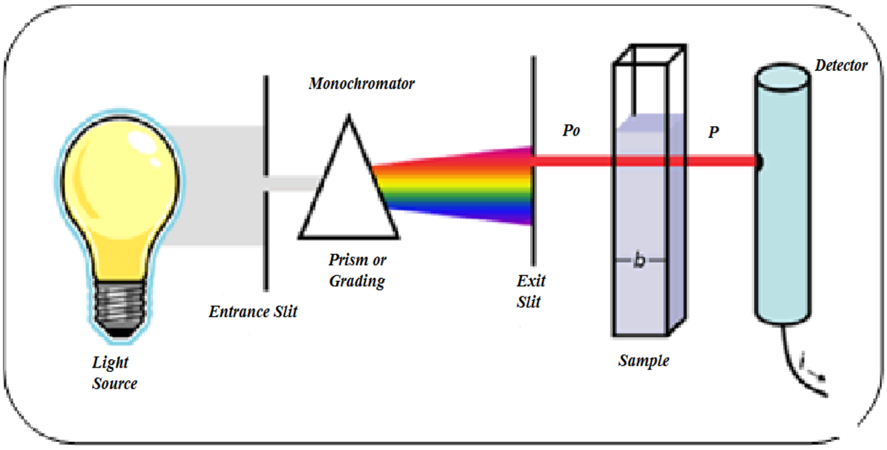


Figure 3.15: Schematic of a spectrophotometer

It is an instrument with a Xenon flash lamp which can be varied according to the choice of luminescence type, in figure 3.14 shows a brief working of a PL spectrophotometer and in figure 3.15 shows a schematic of a spectrophotometer. It can measure both emission and excitation spectra of all the three kinds of phases at the scan rate of the 24000 nm/min without any peak shift. Eclipse Bio Software is installed to operate the hardware. It contains a sample holder where a quartz crystal can be kept with sample filled in it. There are slit arrangements present to switch the incident angles.

Chapter 4:

Photo luminescence Spectroscopy and Organic Dyes

Chapter 4: *Photo luminescence Spectroscopy and Organic Dyes*

This chapter discusses about the organic dyes, their nature, importance in reaction and applications. The solvents they are compatible with to form solutions and method of mixing. Fluorescence significant results of various dye solutions by measuring their PL (photoluminescence spectra) have been presented. To measure concentrations of dyes after mixing gas like NH3 to the prepared dye solutions also, we perform PL. thus, demonstrating that the photo reactive nature of these dyes can be helpful in detecting presence of toxic gases in a specific surrounding.

4.1 Luminescent materials

It is an observation that inorganic luminescent materials are produced synthetically in large scale than the organic luminescent materials. But the organic luminescent materials are more popular in general applications. Like advertising industry where paints are used to create effects of glow-in-dark theme, example eosin, fluorescein, rhodamine, stilbene compounds, etc. They often cannot withstand light and degrade on exposure hence used to indicate matters other than durability. Organic laminators can be used as markers for invisible indications in laundry, ID cards (identification cards), stamps and banknotes, fluorescence of tissues in biotechnology and medicinal objects. They stay invisible by not absorbing any light and thus do not reflect it. This invisible marker can be viewed by throwing UV light over it.

4.1.1 Organic Dyes

Carbon atoms combined with hydrogen, oxygen or nitrogen atoms form organic dyes or pigments. They can be classified into two groups:

- **Natural Dyes**

Dyes originated form plants and animals come under natural category of pigments. They are rarely used for experimental purposes as they possess poor light withstanding nature. They were important historical dyes mostly used in textile colouring, decorating ornaments and added to cosmetics. They were environment friendly colours.

- **Synthetic Dyes**

They have carbon basis and are mostly derived from petroleum compounds. Except for *carbon black* these types of dyes often wear-out with time due to exposure by light and any reactions with them. Petroleum, chemicals and some acids often under extreme pressure produce coloured compounds that we call as pigments or dyes. Table 4.1 give the examples of natural organic and synthetic organic dyes. Figure 4.1 show different dye solutions.

Table 4.1: Examples of natural organic and synthetic organic dyes

Natural Organic Dyes	Synthetic Organic Dyes
Carbon black, Rose-madder	Perylenes, metal comple dyes like New gamboge, Dioxazsine in Danthrone

Figure 4.1: Dye solutions prepared in the lab

Popularity of Synthetic Organic Dyes

Carbon has four vacancies to form bond which render it quite flexible to combine with other molecules. The combining group can be carbon itself or any other elements present in nature. They can form boundless forms of compounds; in fact, whole organic chemistry is based on this flexibility of Carbon. While forming compounds, some atoms might possess some colour creating properties which result into creating synthetic pigments. They are usually non-toxic (some may be toxic but only least in quantity) and very promising economically. Example azo dyes are manufactured form water-soluble pigments. Some specific elements help in creating synthetic dyes like carbon, chlorine, oxygen, nitrogen and hydrogen.

Some examples are chromophores which are mainly responsible for colour creating effect. They are specific groups of molecules. They cloud or surround in an intense and complex manner within a particular shell of electrons in one or more atoms.

Intensity of Fluorescent Dyes

Fluorescent dyes are mixed with a binding agent to create fluorescence materials. Light emitting intensity depends on the quality improvement of these pigments.

They can alter UV light to visible light due to which a standardised form of resulting colours can be expected. They can be used as optical brighteners. They can be used to create different colorants. They bring eye-catching effect. They are sensitive to light reducing colour, strength and brightness hence helps in measuring photo-degradation effects in the presence of UV light.

Uses of such fluorescent dyes:

Dissolved in water or semi-soluble solvents for using in following fields:

- As solvent base for paper coats
- Silk screen inking
- Paints or spray injections
- Textiles

Intensity of Phosphorescent Dyes

These dyes possess the ability to emit light even in dark, commonly called phosphorescence. It is specifically related to fluorescence. This glow can exist for few hours in the darkness (from 2 to 4 hours which depend on the dye type and the amount of light absorbed). Other factors can be:

a) Perception ability of individual's eyes,
b) Distance with the object and surrounding environment,
c) Light present around it,
d) Types and
e) size of the pigment

Uses of such phosphorescent dyes:

- *Plastic Resins*
  Non-opaque resins which are either translucent or transparent are suitable for these.

- *Inks*
  They can be bound to pigments to create phosphorescent inks used for printing on silk screens.

- *Paints*
  Phosphorescent dyes can be bound to specific binding mediums for using in machine coating, spray painting, etc. They are of light stabilising quality.

There are several types of dyes present which are frequently used in the research field. Like xanthenes (500 nm to 700 nm), scintillators (320 nm to 400 nm), polymethines (700 nm to 1500 nm) and fluorescein (400 nm to 500 nm).

Photoisomerisation during excitation by Cyanine dyes and its lasing effects can be observed in long wavelengths. Polymethine dyes can be used in photographic films for sensitization. Rhodamine dyes are derived from Xanthenes, they have high luminescence efficiency.

Fluorescein dyes are also from this family but their efficiency is lower than Rhodamine dyes. Oxazine dyes are efficient then Rhodamine dyes. In the Infrared region they are close to Cyanine but they have high chemical stability. Aromatic dye compounds have low lasing efficiency. Stilbene and Anthracene derivatives hold the maximum seats in the aromatic class with 400 nm to 500 nm wavelength. They are stable but perform a little less than the Coumarin class.

4.2 Dyes used in the present study

4.2.1 Fluorescein

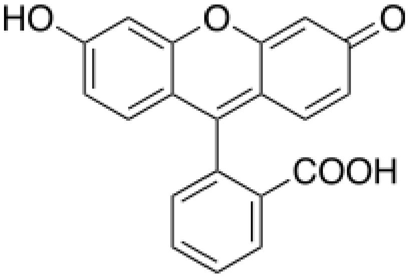


Figure 4.2: Structural diagram of fluorescein

Dark orange or red powder of synthetic organic dye called fluorescein is mildly soluble in alcohol and water. It can used in areas where fluorescent tracing is required, detecting hidden blood stains in forensics and serology, as a laser dye, in microscopy as a fluorophore; figure 4.2 features the structural diagram of fluorescein.

Maximum absorption recorded at 494 nm and maximum emission at 521 nm when water is the solvent. FAM phosphoramidite and fluorescein isothiocyanate are its derivatives. At 460 nm it shows isosbestic point which indicates that it absorbs all pH values equally. It is a colour additive and its disodium salt is termed uranine. When mixed in water it gives an orange or green colour reflection. If concentration is high it can reflect red colour too. This property can be used to view or trace the movements of an air bubble; figure 4.3 shows the fluorescence spectra of fluorescein

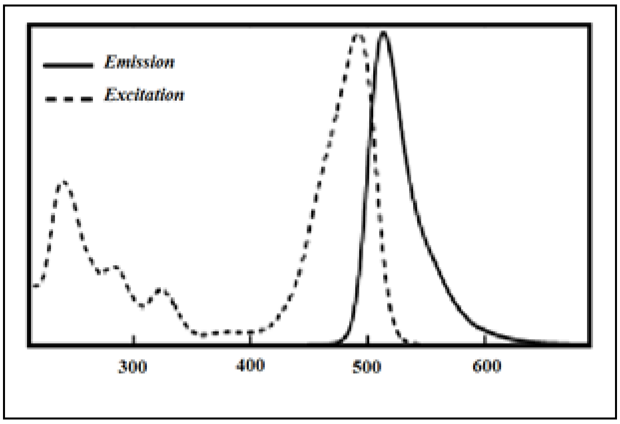


Figure 4.3: fluorescence spectra of fluorescein

Spectrum shows strong excitation peak at 494 nm and emission peak at 521 nm. An ionization potential of 6.4 makes its emission and absorption dependant on ph of 5 to 9. Their fluorescence dies in about 3 to 4 ns.

Uses

- **Healthcare benefits**

Optometry and ophthalmology uses fluorescein sodium as a diagnostic tool. It can detect corneal abrasions, corneal infections and corneal ulcers. It is also used in contact lenses of rigid gas permeable nature to find the tear layer beneath the lens. Available for one-time use in packets containing non-fluffy paper soaked in fluorescein sodium. It can also be used in diagnosing and researching retinal diseases caused by diabetes, intraocular inflammation and macular degeneration and retinal tumors. It can be used in operating on brain tumors. In open heart surgeries, the diluted fluorescein can be used to find residual defects and pinpointing multiple muscular ventricular septal defects.

- **Research in Biochemistry**

At cellular level, derivatives of fluorescein are used for cell labelling and tracing during microscopy by fluorescence. Biomolecular ligands can be attache do this dye molecule for tracing its path to specific cell addresses. Fluorescein dye can be attached to nucleoside triphosphates and merged in enzyme probe for in situ hybridisation. Labelling in FISH or immunohistochemistry is also another application of fluorescein.

- **Air-sea rescuing**

Air forces use fluorescein vessels which help in creation of markings whenever there is crew downed after the parachute releases it into the water. This technique was first used by the German air force during World War II.

- **In river systems**

It was used to colour the Chicago River green on St. Patrick’s Day in 1962. But due to its harmful effects, environmentalists later had it replaced with natural organic dyes in 1966. It can trace water leaks by dissolving in rain water and in New Zealand and Australia as a liquor dye.

- **In oilfields**

To detect leaking in subsea oil and gas pipelines; and few more subsea infrastructures, 15 % active fluorescein is uses. Divers detect it by carrying UV lights with them.

4.2.2 Rhodamine B

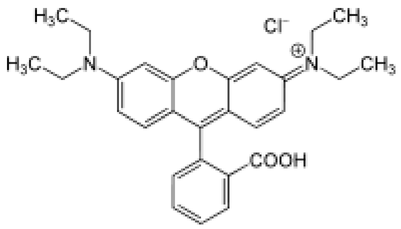


Figure 4.4: Structural diagram of rhodamine B

Rhodamine B is both a dye and a chemical compound. It is used for tracing in water to evaluate the rate of flow and direction during its transport. Fluorometers are used to detect its presence by tracing its flourescence behaviour. Very beneficial to the field of biotechnology in flow cytometry, microscopy based on fluorescence, correlational spectroscopy also based on fluorescence and performing ELISA. It can also be used in laboratories for staining, usually combined with auramine O to study acid-fast organisms like mycobacterium. Its peak is observed at 610 nm. It efficient luminescence is observed at 0.49 in normal ethanol, 0.65 in alkaline ethanol 0.68 and 1.0 in 94 % ethanol. The fluorescence alters with temperature.

Its IUPAC name is [9-(2-carboxyphenyl)-6-diethylamino-3-xanthenylidene]-diethylammonium chloride. Figure 4.4 shows the structural diagram of rhodamine B

It is more soluble in acetic acid than water. Solution of rhodamine b reacts with plastic bottles hence, it should be stored in glass vessels. Water containing chlorine degrades this dye.

Uses

Rhodamine B is found to be carcinogenic in some places; hence its products are labelled for caution. It is put under testing for its suitability to be used as a biomarker in oral vaccines in animals. It is entered through their teeth and whiskers. It can be conjugated with other molecules to form other colours like Opera rose. It is used for tracing herbicides use.

4.2.3 Stilbene

Antioxidant forms of it are commonly referred to as stilbenoids. Has two isomers by the name:

- (E)-Stilbene (trans-isomer)
- (Z)-Stilbene (cis-isomer)

Its IUPAC name is 1,2-diphenylethene.

Isomers

The cis isomer is less stable due to steric hindrance between the two aromatic rings. Both of the compounds possess diverse physical nature. For example, e-stilbene melts at a very low temperature of 5 to 6 °C while z-stilbene melts at a higher temperature of 125 °C.

(*E*)-Stilbene

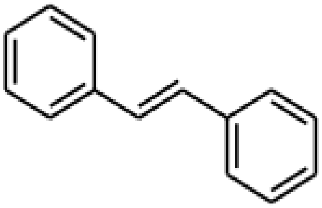


Figure 4.5: Structural diagram of *e*-stilbene

It is an organic compound containing a *trans* ethene with two phenyl groups attached on the two carbon atoms (hence also called diarylethene). Its name itself means in Greek figure 4.5 shows the structural diagram of *e*-stilbene.

(*Z*)-Stilbene

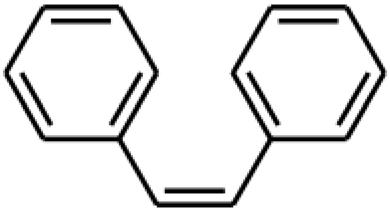


Figure 4.6: Structural diagram of *z*-stilbene

(*Z*)-Stilbene

It is an organic compound containing a *cis* ethene with two phenyl groups attached on the two carbon atoms (hence also called diarylethene). Its name itself means in Greek figure 4.6 shows the figure 4.5: structural diagram of *z*-stilbene.

Reaction chemistry

It is a conjugated alkene. The two isomeric forms undergo isomerisation between its two forms i.e. –*cis* and –*trans* when exposed to sunlight (UV light). Phenyl groups in stilbene often under cyclization reaction which is intramolecular. Electrocyclic reactions are a common site in *z*-stilbene.

Uses

- It is used in the production of optical brighteners and pigments and also used as a scintillator or a phophorescence material.
- In laser dyes
- As antioxidants found in plants (stilbenoids)
- *e*-stilbene can also be used manufacturing non-steroidal artificially produced estrogens like dienestrol.

Solvents for Organic Dyes

Stock solutions of dyes required for measuring their photoluminescence are very dilute. Hence, the dye is present in trace amounts in the dilutions of 10-3 to 10-6 M. thus, their properties and toxicity should be kept in mind. As most dyes are polar in nature so during the electron excitation from quantum levels to higher, results in higher dipole moments. Hence, polar or non-polar nature also rotates the fluorescence ability. The polar nature may help in shifting the wavelength from about 20 to 60 nm. Polarity increases the dye dissolving capability.

But if we find that solvents polar nature may interfere strongly with the dyes' fluorescence efficiency then it should be avoided as it alter the basic approach of the study and the results thus obtained. Solvents like water and alcohol are mostly used but not with Infrared dyes due to the presence of hydroxyl groups in both the solute as well as solvent. With them it is advisable to use DMSO or aromatic compounds which lack hydroxyl groups. But they are toxic solvents and prove poisonous if contacts skin, hence when using them is essential, safety measure must be used like wearing rubber gloves, ventilation must be done in the lab and cover the face.

Choosing an appropriate solvent

Dye properties make it specific for dissolving in different solvents. Solvent choice may also depend on the experimenter's need to shift the wavelength according to his desired approach. Some conditions to be kept in mind are:

- Transparent solvent must be used to allow maximum absorption during PL measurements.
- Dyes must be soluble to some extent in the solvent to render their colour to the resulting solution.
- They should not undergo degradation or any change in colour on exposure to the light at a rapid rate.

Solvent Purity

Photoluminescence efficiency is intensely proportional to the solvent purity. Flaws in the form additives and impurities the solvent purity causes dyes to quench faster or lose intensity before their maximum lifetimes. This is required to be done during photo-degradation studies of the dye solutions with catalysts added. When photosensitivity is to be studies, we pay special attention on the purity of dye solutions to assume that the measurements done so far are flawless or absolute. For making dye solutions, 500 ml (or more as required) of the solvent bottles are taken which are usually brown coloured to avoid the light from entering and maintaining dark conditions inside. Appropriate weight of dye is measured and mixed with the solvent taking care not to spill the solution. The solution is then subjected to ultra-sonication for uniform mixing of dye molecules in the solvent. Now by taking 10 % of the solution from the starting stock solution, we add more solvent to acquire 100 % solution volume. Thus, we get different molar stock solutions.

4.3 Photo-degradation Reaction

4.3.1 Types of photo-degradation

- **Homogeneous photo-degradation**

As the name suggests that the catalyst and the substrates are in the same phase, i.e. homogeneous catalysis. Ozone and photo-Fenton together account for the most generally used systems. The hydroxyl free radical comes into work here for lysis. Ozone on the presence of heat combines with water molecules to lyse it into hydroxyl free radicals. Same way in the presence of ferric ions, hydrogen peroxide breaks down into hydroxyl free radicals in the presence of UV light.

This Fenton system is beneficial as one can use sunlight as a UV source and controlling some obvious properties like pH of the solution. This way we can cut the high cost of installing electrical and UV source. The only drawback with these is that the maintaining low pH values is necessary the process to occur.

- **Heterogeneous photo-degradation**

The substrates of the solution are in different phases i.e. heterogeneous catalysis. Reaction proceeds in the same as the homogeneous degradation involving oxidations, removal of hydrogen atoms, oxygen transformation, water lysis, pollutant removal, etc. Semiconductors and metal oxides are filled with voids which are required to be filled with transition electrons. During this transfer the process of photocatalysis occurs as the electron to release energy needs to combine with the substrate molecule or the catalyst. The objective is to produce a reduced electron which in turn produces an oxidised reactant species. This can be explained as a redox reaction where dye molecules are lysed in the end which indicates the degradation aided by the catalyst at an elevated scale. This complete process happens at the porous surface of the catalyst.

4.3.2 **Applications** of photo-degradation

With the increase of reduction in clean water, we require an easy and economic method of cleaning the water bodies for reuse and recycling. Photo degradation with the use of catalysts like metal oxides has proven to be very effective and promising. Some highlights of its important functions can be:

- Water molecules can be hydrolysed by various metal oxides
- Making of self-cleaning glasses by titanium dioxide
- Cleaning of water to get rid of infection
- Coating of metal oxides for self-sterilization for avoiding microbial growth from initiating
- To oxidise impurities
- Breakdown of CO2 gas by metal oxides
- Cleaning and washing surgical instruments
- Degrading crude oil with the help of metal oxide nanoparticles
- Decontaminating water sources.

Chapter 5:

Experimental Setup and Characterization Scenario

5.1 Structural Characterization

a) XRD

b) SEM

c) TEM

5.2 Optical Characterization

5.1 Structural Characterization

a) XRD

Sample Preparation for XRD

It must be a powder and pure in form. We need 0.5 g of it though lesser amounts can also be measured. It must be packed properly in tight condition to observe good intensities which may be poor if not tightly done.

Collection of Data

The scattering reduces by 1/2 (1+cos ^{2}2θ). It shows that beyond 70° 2θ, nothing much can be observed. A nice oattern can be observed at 10° 2θ to 70° 2θ. A general study may take 30 min to 60 min while with Rietveld refinement it need 12 hrs to 18 hrs. Rietveld refinement method needs to calculate least squares for its matching algorithm to compare with already studies samples standard forms. It is highly dependent on the sample properties and instrumental properties. We have used Bruker D8 Discover machine for recording the X-ray graphs.

Data analysis

We can compare the observed data with JCPDF or JCPDS data base. They have approximately 100,000 saved patterns. For identifying any new material to match pattern.

b) SEM

As information collected from low angle powder XRD is found to be insufficient information to dtermine definite conclusions on the morphology of the materials, two more unique electron microscopy techniques can be used. The first one is Transmission Electron Microscopy (TEM) and the second one is Scanning Electron Microscopy (SEM). Scanning electron microscopy (SEM) uses a low voltage beam of electrons (<20 kV) to evaluate exterior particle shape and size to up to about 50,000x magnification. The morphology of the nanoparticles are readily observed by SEM. The SEM for these explorations is done by using SEM, JEOL, JSM-6610LV system**.** The sample was coated on a sample holder with carbon foil for adhesion and sputtering is done with gold. The photograph of the SEM instrument is shown in Figure 2.6.

c) TEM

TEM incorporates a stronger beam of electrons (~300 kV), and can help us look for the visualization of pores. TEM is not another form of XRD, as only a little sampling is acquired. The magnification given TEM is of the order 300,000x. TEM provides information which supports the powder XRD regarding the porosity and surface area.

The bright field electron microscopy of the samples is done by TECNAI TEM machine with FEI TEM model TECNAI G2 T30, U–TWIN description combined with an electron source of Tungsten or LaB_6 filament at the increasing voltage of 300 keV. To observe the measurements, we mix 10 mg of sample in 1 mL of ethanol and sonicate it for about 10 min (to get uniform dispersion of the particles as the prepared sample may not be soluble in ethanol completely). A drop of this solution is poured on carbon coated copper TEM mesh grid and allowed for the ethanol to evaporate to let the grid dry and then images are collected taken using the TECNAI TEM equipment.

d) ZnO synthesis

Synthesis procedure:

Materials Used for synthesis

- **Mesitylene**

2D skeleton

3D skeleton

Figure 5.1: 2D and 3D skeleton of Mesitylene

Heating concentrated sulphuric acid with acetone was done by Robert Kane in 1837 to make mesitylene (with chemical formula C_9H_{12}). IUPAC named it 1,3,5-trimethylbenzene. He named his new substance mesitylene because acetone is named “mesit” in Greek (the mediator) which was dehydrated to alkene, thus mesitylene also shown in figure 5.1, the 2D & 3D skeleton. Kane had determined the formula incorrectly, which was corrected by August W. von Hofmann in 1849. Later in 1866 Adolf von Baeyer demonstrated it to be consistent with 1,3,5-trimethylbenzene, a benzne deerivative with 3 methyl ($–ch_3$) groups symmetric to a ring. It is a colourless liquid form, coal tar constituent, with sweet aroma. It is predecessor to many chemical.

It is used here as a ligand for metal centers in the sol-gel preparation of ZnO nanoparticles in organometallic chemistry. It is also used a special solvent in these reactions for colloid formation. It also helps in making aerosols and ozone formation.

- **CTAB (cetyl trimethyl ammonium bromide)**

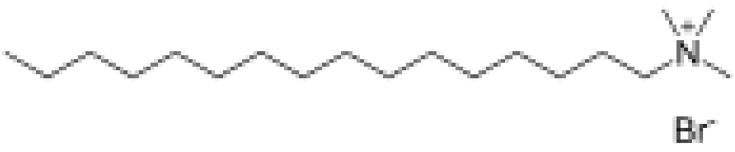


Figure 5.2: Structural diagram of CTAB

Also known as cetrimonium bromide with chemical formula $C_{19}H_{42}BrN$, CTAB has IUPAC named it as hexadecyltrimethylammonium bromide. CTAB is a surfactant with amine based cation group. Finds uses as antiseptic agent against microbes, preparing gold nanoparticles and mesoporous silica like MCM-41, DNA extraction and hair conditioners and cosmetics.

Formation of micelle around the reaction intermediates to keep them from agglomerating is done by surfactants. Surfactants are very important in sol-gel reactions where we need to minimise particle aggregation to produce desired shape and size.

- **UREA**

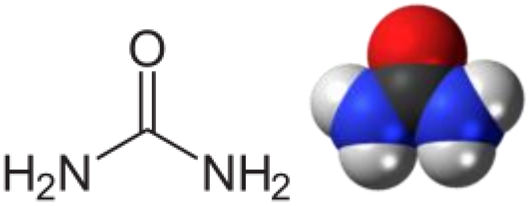


Figure 5.3: Structural diagram and 3D skeleton of Urea

It is also known as carbamide with chemical formula $CO(NH_2)_2$ and IUPAC name amino methanamide which is supported through figure 5.3. With common names as carbonyl diamide, carbonyldiamine, diaminomethanal, diaminomethanone, the urea molecule has two $-NH_2$. It is colourless and odourless water soluble liquid. Reported in 18th century by Hermann Boerhaave in urine evaporates. Its crystals were obtained by evaporating urine nad filtering with alcohol in 1733 by Hilaire Rouelle. In 1799 Antoine François, comte de Fourcroy and Louis Nicolas Vauquelin discovered nitrated crystals similar to Rouelle's and coined the term "urea." These crytals can be boiled in charcoal and filtered for preparing urea nitrate. Mixed with barium chloride and water, urea is reformed. Water evaporates and ethanol washes help urea extraction.

Metabolism of nitrogen compounds by animals is helped by urea. It serves as a nitrogen source in fertilizers and chemical industries. It can be prepared artificially as well.

Used in synthesis reactions like hydrothermal and sol-gel as surfactants. In the hydrothermal reaction here, it helps trap the organic intermediates in its porous network of hydrogen bonds between urea molecules. It also acts as a surfactant to separate intermediates and prevent aggregation of ZnO to ensure fine particle formation in the end.

- **Sodium hydroxide (NaOH)**

Lye or caustic soda named sodium hydroxide by IUPAC has NaOH chemical formula. It is a white inorganic solid compound. It is an alkali salt manufactured in pellets, granules or flakes form which are water soluble. Also dissolves in organic liquids like ethanol and methanol. It is deliquescent in nature.

Used as a strong base in the synthesis of pulp & paper, soaps & detergents, drain cleaners, textiles and drinking water.

Zinc nitrate

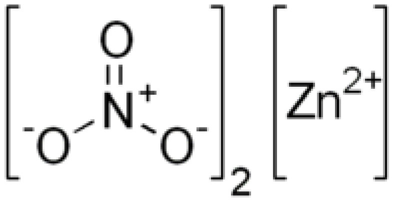


Figure 5.4: Structural diagram of Zinc nitrate

Zinc nitrate, a white deliquescent inoragnic compound usually in crystals is named by IUPAC. It exists with hexahydrate crystalline form ($Zn(NO_3)_2•6H_2O$), a structural diagram of zinc nitrate given in figure 5.4, is soluble in ethanol and water. Adding zinc to nitric acid helps form zinc nitrate. It is taken a substrate to prepare zinc oxide here, in many structural forms like nanowires. Also used in coordination polymers manufacturing, in dyes as mordant. Here it is used a zinc source.

- **Zinc Acetate**

Colorless white crystals and anhydrous form of zinc acetate (IUPAC name), exists in dihydrate forms as well with chemical formula $Zn(CH_3COO)_2$. $2(H_2O)$. has many applications like used as a food additive, medicines, etc. For example its use in lozenges to cure common cold as it releases zinc free from acetate. Thus, helps to reduce the time of cold. This duration reduction varies according to the type of zinc lozenge. The amount of zinc present in the lozenge proportionately reduces the cold's time as zinc combines with the citric acid. It is also used to overcome zinc deficiencies. When taken orally it helps stimulate copper acceptance for treating Wilson's disease. Other uses may include as an astringent, lotion, antibiotic, anti-itch, mouth freshener, plaque reducer.

Commercially used apart from cosmetics, to preserve wood, zinc compounds, mordant in dyes, nuclear plants and an analyser. Here used as a zinc source.

- Water
- Ethanol

Techniques used are those two which have been proven to give a high yield and more purity and control over the getting a desired shape.

a. By hydrothermal method

We can use a step-wise protocol to use this technique with maintain precautions for safety. We are advised to use gloves at every step of the reactions.

- 0.002mol CTAB + 0.003mol $Zn(NO)_3.6H_2O$
- Added 10ml deionised water and 40 ml anhydrous ethanol and give it a 10 min supersonic treatment
- Then addition of 0.015mol $CO(NH)_2$ under vigorous stirring is done
- Transferred this mixture to a Teflon-coated autoclave of 80 ml capacity for 2-24 hr at 150 degree cel.
- After cooling to room temperature, a white powder was obtained and collected followed by washing a number of times with distilled water and ethanol to remove the impurities, then dried at low temperature.
- Post-annealing of obtained pre-cursors or white powder was carried out at 673K for 2 hr in a temp-programmed Muffle furnace.

b. By sol-gel method

In this method, one needs to be careful as the suspension will be foamy after allowed to stand for some time (few hours to two days max.).

- We start by 2.7mmol CTAB dissolved in sol of 480 ml water along with Zinc acetate dihydrate
- Then Mesitylene (48.8mmol) is added to the solution
- It is vigorously mixed at 80 degree cel for 2 hr and addition of 3.5ml of 2M NaOH (aq) dropwise at rate of 1ml/min to this solution.
- Reaction mix is stirred vigorously at 80 degree cel for another 2 hours by reflux method.
- The resulting white precipitate are isolated by centrifuging them and washing with abundant ethanol and dried under vacuum at 100 degree cel for 12 hr
- Structure templating CTAB and mesitylene molecules were removed from composite material via acidic medium
- A suspension of the synthesized material was stirred for 6 hr at 50 degree cel in a 100 ml ethanol sol with 0.75 ml conc. HCl
- Template removed solid product was then isolated via filtration and dried under vacuum at room temp for 12 hr at 80 degree Celsius.

5.2 Optical Characterization

Organic dyes solution prep.

Experimental Method

Requirements
GR grade dyes
Fluorescein sodium
Stilbene 3 or stilbene 420
Rhodamine B
Solvent used: water (double distilled)

Methodology

Without further purification, the dyes and components of the solution were used as they were obtained from the manufacturer. Fluorescein Sodium (obtained from Spectrochem, India) gives a fluorescent yellowish green solution on mixing with water. Stilbene 420 (obtained from Exciton, USA) on dissolving in water gives a transparent and non-fluorescent solution. Rhodamine B (obtained from Exciton) gives a deep orange or red coloured solution on mixing with water.

We perform their fluorescence evaluation for studying emission and excitation spectra of each on Cary Eclipse Spectrofluorometer (Varian model) and a Xenon flash bulb as the source. Slit width selected for recording emission and excitation spectra were kept 2.5 nm for both and 5nm for both for one dye. Rate of scanning was limited to 600 nm per minute with interval duration of data recording at 1 nm in the entire experiment with a mean time of 0.1 sec. The dilution concentrations of the dye were prepared at 10^{-3} to 10^{-6} M to obtain different stock solutions for each dye. The data was recorded carefully for each concentration for a fixed wavelength. The resultant dyes prepared in the lab are shown in figure 5.5.

For Fluorescein Sodium excitation wavelength was fixed at 200 nm to 500 nm to record corresponding emission spectra and emission wavelength of 350 nm to 800 nm to record corresponding excitation spectra.

For Stilbene 420 excitation wavelength was fixed at 200 nm to 500 nm to record corresponding emission spectra and emission wavelength of 350 nm to 800 nm to record corresponding excitation spectra.

For Rhodamine B excitation wavelengths were fixed at 200 nm to 520 nm to record its corresponding emission spectra and emission wavelengths were fixed at 500 nm to 800 nm to record their corresponding excitation spectra. With the split width fixed at 5 nm.

Figure 5.5: Shows dyes stocks prepared in the lab

Photo-luminescence Spectrophotometer sample preparation

Fluorescence spectrophotometer is measured by Varian Cary Eclipse Spectrofluorometer. This instrument is made with adjoining Xenon flash lamp to allow the user have a choice of the collection mode like: fluorescence, phosphorescence or chemi- bioluminescence. This instrument uses two monochromators, excitation and emission, and an extended range photomultiplier tube detector (PMT). It is able to scan at the rate of 24000 nm/min with no or least peak shifts. The automation of the instrument is done by using the (Eclipse Bio Software) software. The spectrophotometer could be used to record the excitation and emission spectra of liquid as well as powder.

Reaction with Ammonia

For recording emission and excitation spectra of these dye solutions on their individual reactions with ammonia, we use ammonia bubbled water. It is completely saturated with ammonia. The three prepared dye solutions were used corresponding to their highest peaks obtained. Coincidentally all the three dyes have their highest peaks obtained at 10^{-4} M concentration. The slit widths were kept the same as kept while recording their normal emission and excitation spectra of the individual gases. About 3-4 ml of the dye solution mixed with ammonia solution is poured in quartz cuvette. This cuvette is cleaned form the sides with tissue to wipe off any marks made by holding it to avoid any obstacle to the light passing through the quartz. Spectrofluorometer has Teflon chamber with a Teflon lid to cover and close the chamber after placing the cuvette in the sample holder. Then the light from the Xenon flash lamp is passed, closing of the lid ensures that it is the only source of light present inside which is used to record the emission and excitation spectra of the respective dye samples. Room temperature is maintained inside the chamber.

The selected stock of 10-4 M is done to avoid evade self-quenching of the dye. Microliter units are used to slowly and steadily increase the foreign material solution (i.e. ammonia solution here) inside the chamber.

Photodegradation Activity of Catalysts

We use an in-house photochemical reactor for this process. The dye and mixture (100 ml) was poured in a pyrex container with inner and outer transparent walls, over which is attached a quartz crystal in which we assemble a UV lamp (wavelength < 400 nm) over a magnetic stirrer and made arrangements for water inlet and outlet so as to avoid heating effects of the light source. We collected a 5ml solution of this mixture and keep it aside. The we have dissolved 0.05 g of the synthesised catalysts along with the a $5X10^{-5}$ M dye solution. We continued to take 5 ml solution after a definite time interval. Later we measured their UV-vis spectra on the spectrophotometer.

UV-Vis sample preparation

The UV-Visible absorption were recorded on Thermo scientific Evolution 300 UV-VIS adjoined with Toshiba TCD 1304AP linear CCD-array detector that allows optical resolution as apt and accuarte as 0.02 nm (FWHM). The optical spectra have been recorded from 200-800 nm range in a 10 mm path quarz cuvette.

Chapter 6:

Data analysis and interpretation

6.1 Structural Characterizations of synthesised nanoporous metal oxides

- **6.1.1 Data interpretation by result analysis of SEM**
- **6.1.2 Data interpretation by result analysis of TEM**
- **6.1.3 Data interpretation and result analysis of XRD**
- **6.1.4 Data interpretation and result analysis of Raman**
- **6.1.5 Data interpretation by result analysis of N_2 adsoption and BET**

6.2 Optical Characterizations of synthesised nanoporous metal oxides

- **6.2.1Data interpretation by result analysis of photoluminescence**
- **6.2.2 Data interpretation by result analysis of UV-vis**
- **6.2.3 Data interpretation by result analysis of photodegradation**

6.1 Structural Characterizations of synthesised nanoporous metal oxides

6.1.1 Data interpretation by result analysis of SEM

SEM images of uniformly shaped and size and finely distributed ZnO microspheres shows that they are of porous nature as the external surface seems like nest-like particle. The mean diameter of the spheres is 15 mm. Figure 6.1 yields a SEM image of sample A and Figure 6.2 yields a SEM image of sample B, captured at different magnifications, A) at 1 um, B) at 2um, C) at 5 um and D) at 10 um

The nest-like appearance is the result of interweaved mesh like nanoparticles of ZnO. A messy organization of these nanoparticles provides porosity to the synthesized ZnO. It can be understood from the images that the nucleation of the particles started around a central molecule.

These ZnO nanoparticle mess around one point gives rise to the collection of 2D planar to a 3D figure to the nanoparticles making them sphere shaped rather than nanosheets or nanorods.

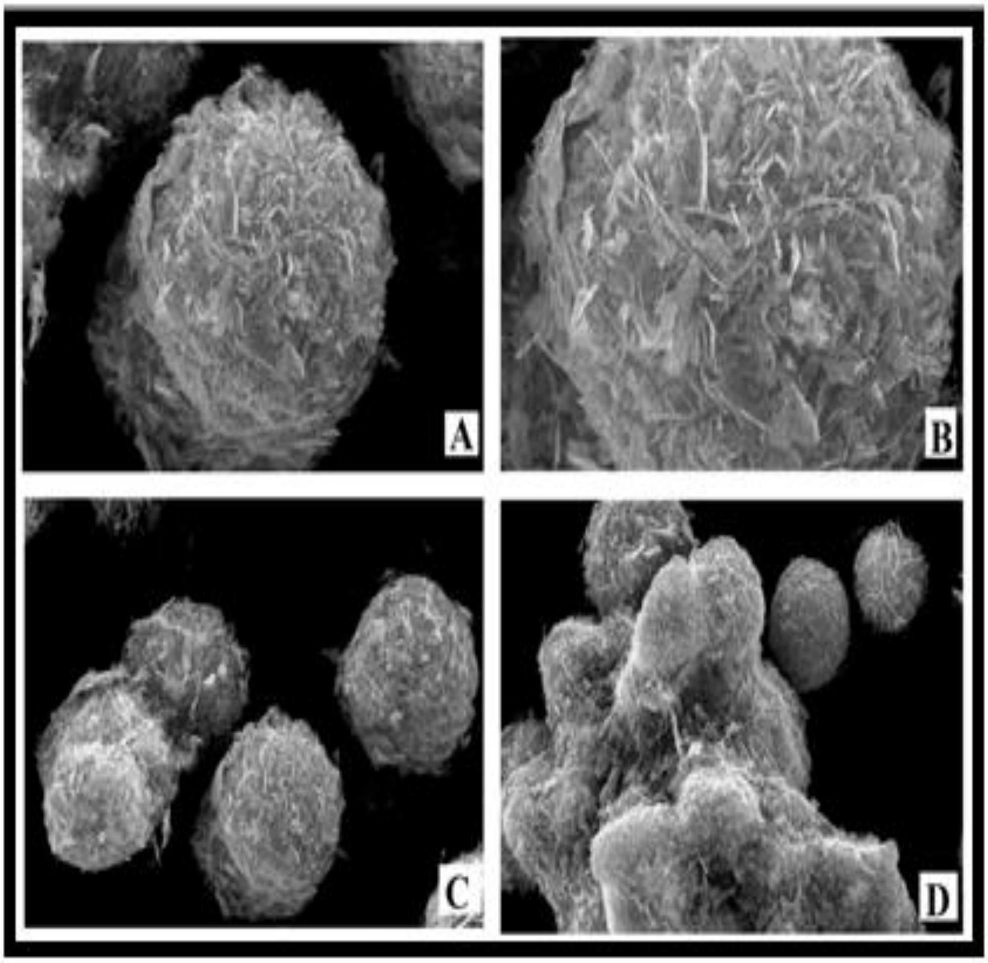


Figure 6.1: SEM image of sample A captured at different magnifications, A) at 1 um, B) at 2um, C) at 5 um and D) at 10 um

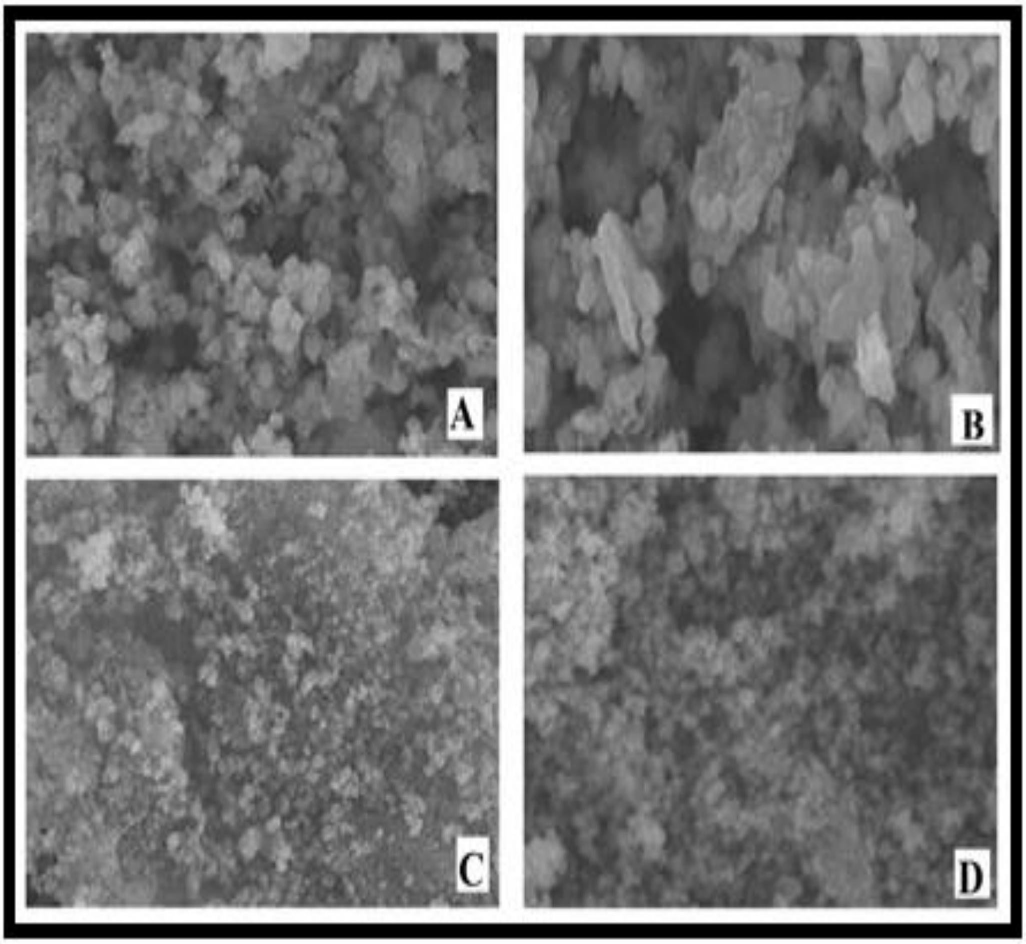


Figure 6.2: SEM image of sample B captured at different magnifications, A) at 3 um, B) at 4um, C) at 5 um and D) at 10 um

6.1.2 Data interpretation by result analysis of TEM

As the porosity started developing as the backbone of the sphere shape, we used TEM as well as High Resolution TEM (HRTEM) as produced in figure 6.3, a TEM image of sample A where we can see the surface of the particle as A) and C) show HRTEM, B) and D) show TEM and in figure 6.4, a TEM image of sample A where we can see the surface of the particle as A) and B) show HRTEM, C) and D) show TEM, for the contour viewing to understand better the morphology of the as-prepared zinc oxide. There can be holes viewed from the images which make it evident that these particles are hollow from inside though the inner surface morphology is of porous texture too. The HRTEM has shown that the outer and surrounding surface fringe is thicker the centre fringe. Thus we can assume a botton-up approach was the key to the formation of these nanospheres.

We have measured porosity and surface area too of these particles.

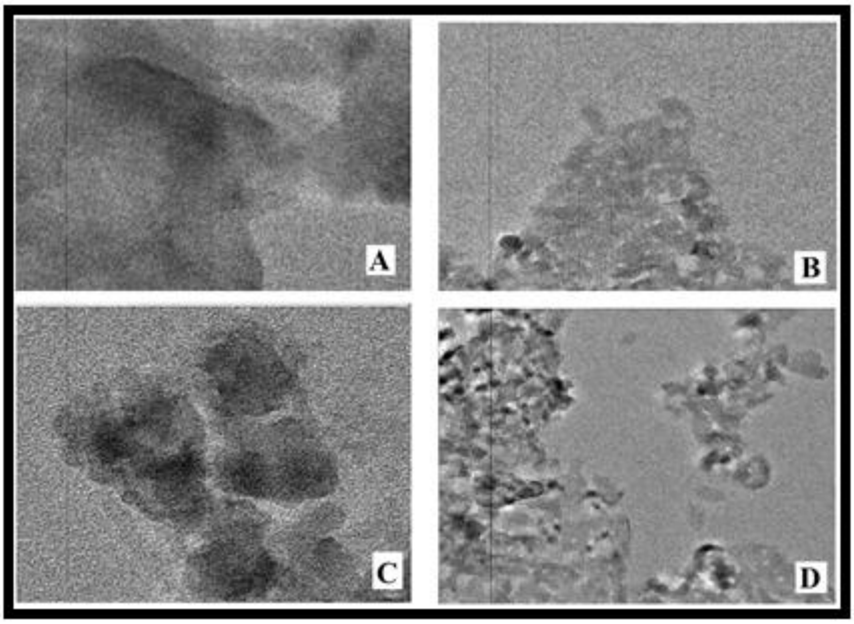


Figure 6.3: TEM image of sample A where we can see the surface of the particle as A) and C) show HRTEM, B) and D) show TEM

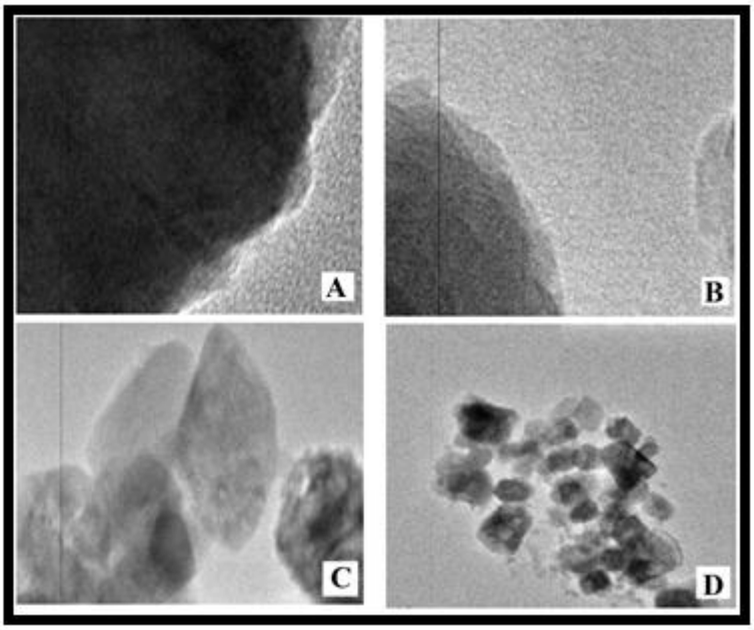


Figure 6.4: TEM image of sample A where we can see the surface of the particle as A) and B) show HRTEM, C) and D) show TEM

6.1.3 Data interpretation and result analysis of XRD

Sample A XRD plot

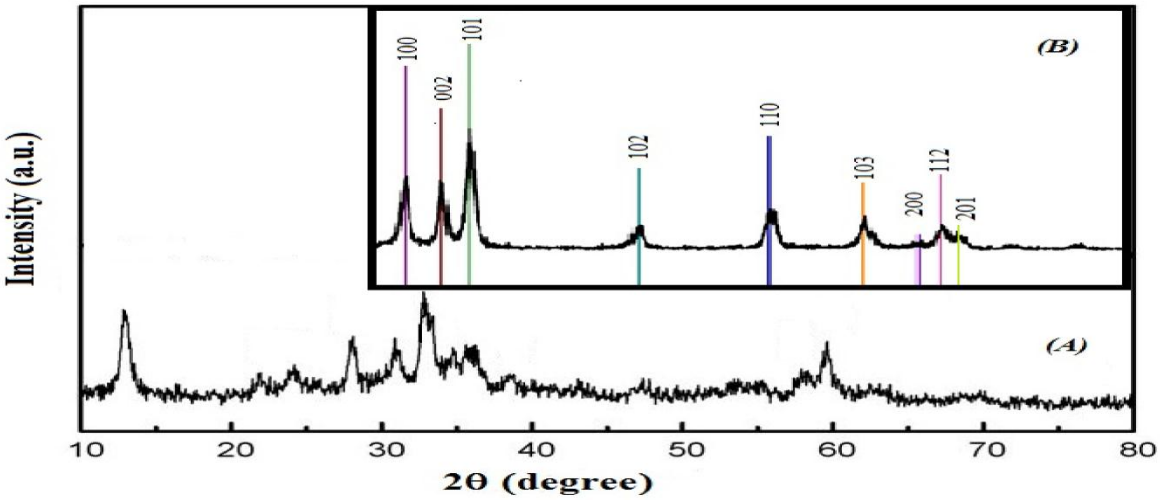


Figure 6.5: Shows the XRD pattern of sample A ZnO in comparison to the zinc substrate

Sample B XRD plot

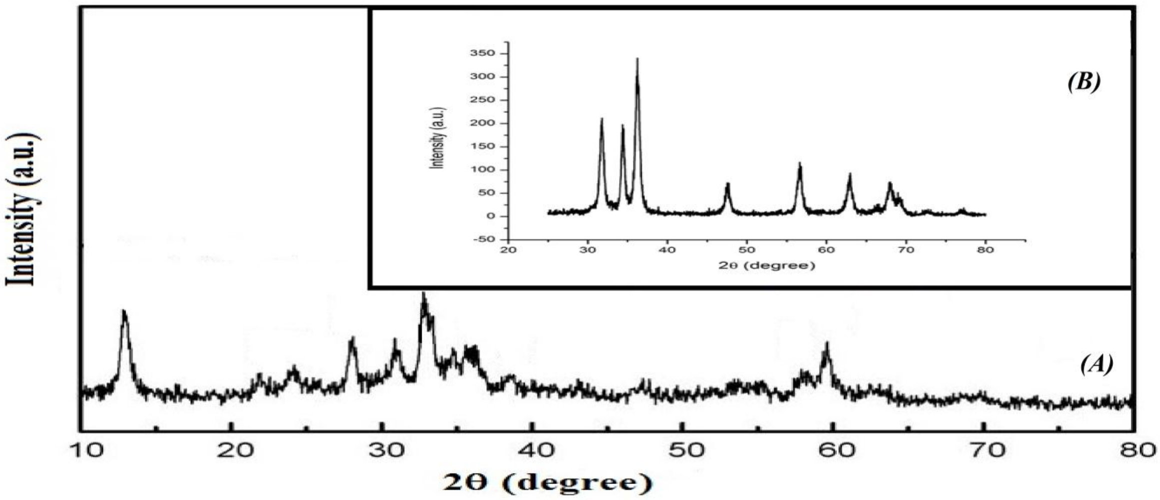


Figure 6.6: Shows the XRD pattern of sample B ZnO in comparison to the zinc substrate

The figures in 6.5 and 6.6 show the resultant XRD pattern of sample A and sample B ZnO in comparison to the zinc substrate. We can draw a clear comparison of the prepares zinc oxide sample by the two adopted methods of hydrothermal and sol-gel approaches. The peaks are compared with that of zinc oxide by JCPDF database and it determines the samples thus produces are definitely zinc oxide nanoparticles.

6.1.4 Data interpretation and result analysis of Raman

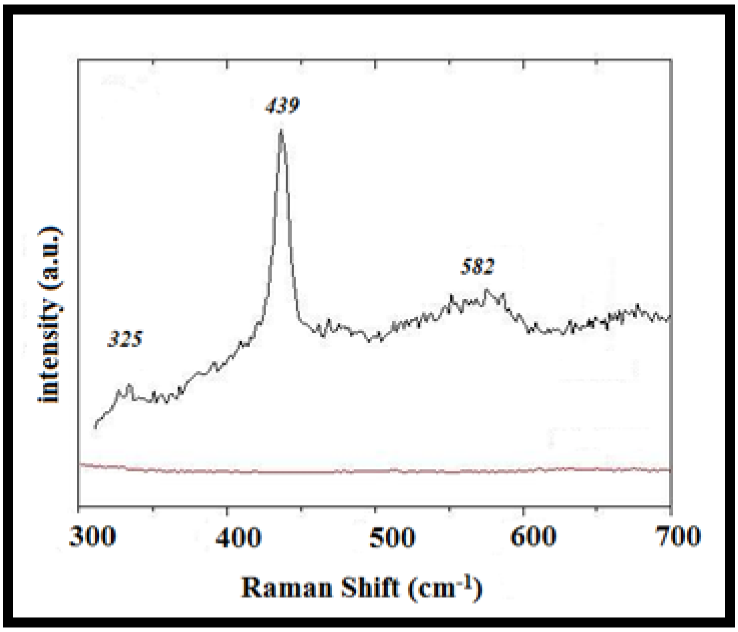


Figure 6.7: Shows Raman shift spectrum of the prepared ZnO

The Raman spectrum acts a fingerprint and it determines here that the samples thus prepared are zinc oxide as depicted from the peaks obtained in figure 6.7 which depicts the Raman shift spectrum of the prepared ZnO.

6.1.5 Data interpretation by result analysis of N_2 adsoption and BET

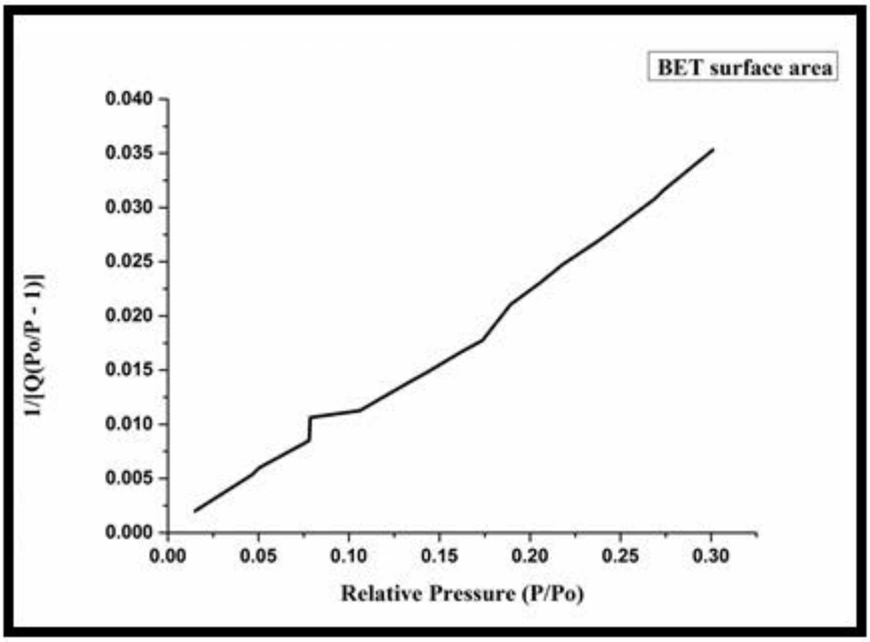


Figure 6.8: BET surface area plot of sample A

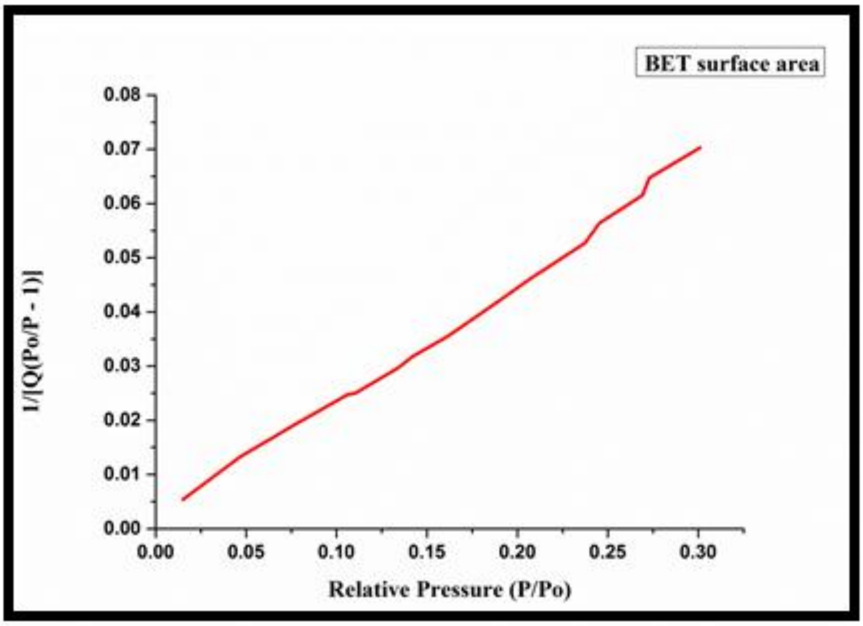


Figure 6.9: BET surface area plot of sample B

BET measures the surface area of the samples. We can derive a surface area to volume ratio by its help and get an idea of the pore diameter as performed in figure 6.8 and 6.9 separately for sample A and B.

Isotherm plots of N_2 adsorption

We can obtain the isotherm plots of the gas adsorption which shows that they are linear for both the samples of zinc oxide. They give an idea of the porosity of the samples. Figures 6.10 and 6.11 shows the N_2 adsorption isotherm linear plot of sample A and sample B.

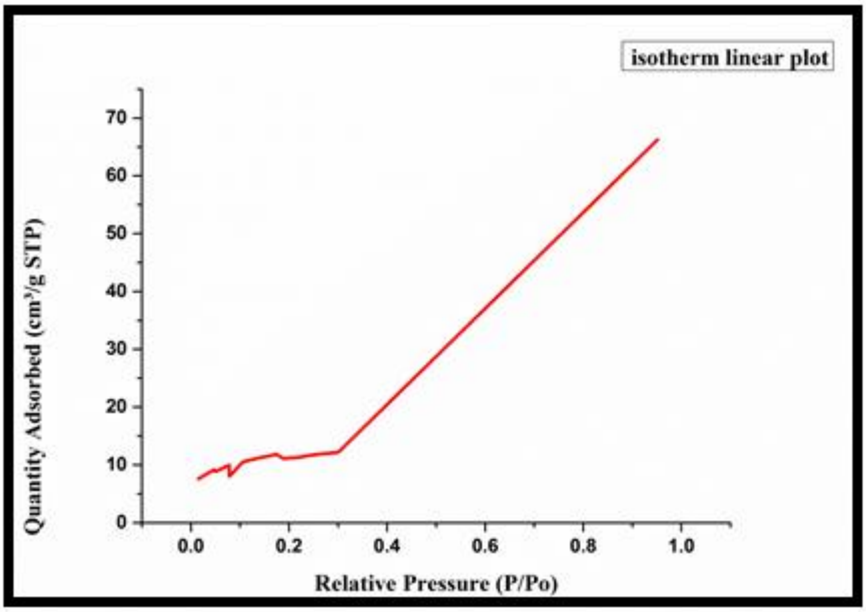


Figure 6.10: N_2 adsorption isotherm linear plot of sample A

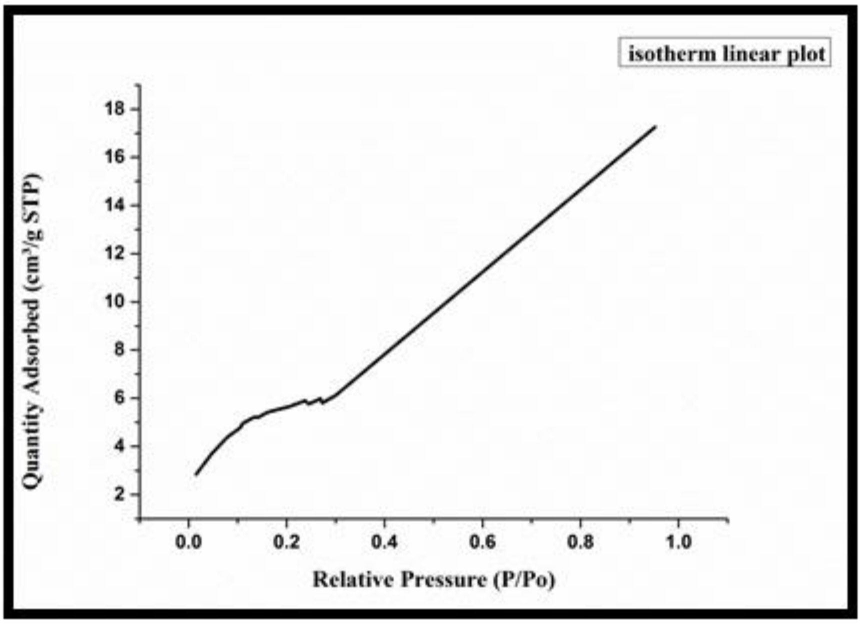


Figure 6.11: N_2 adsorption isotherm linear plot of sample B

It shows the values obtained to describe the BET surface area of the two samples prepared by two different methods i.e hydrothermal (sample A) and sol-gel (sample B). The metal oxide nanoparticles prepared by hydrothermal method have a larger surface area because of the small size and highly rough surface formed by the interweaved ZnO nanopieces. While during sol-gel no matter how careful we are during the synthesis process but the nanoparticles are of a metal hence when when the foaming stage of the reaction comes then the metal lattice being of crystal nature cannot exceed in surface area. The range can be between 12 m^2/g to 20 m^2/g. Hence the results obtained determines that they are high on porosity as far as the synthesis method is concerned though they are lower in comparison to sample A. when we focus on purity we should prefer sol-gel technique over hydrothermal technique while when we need more surface area we need to prefer hydrothermal synthesis over sol-gel method.

Table 6.1: Showing the results of the gas adsorption and BET analysis

Sample material	Surface area (m^2/g)	Pore volume (cm^3/g)	Mean pore size (nm)
A (hydrothermal method)	38.1133	0.10247	1075.524
B (sol-gel method)	19.2983	0.026703	553.479

6.2 Optical Characterizations of synthesised nanoporous metal oxides

6.2.1 Data interpretation by result analysis of Photoluminescence

1. **Fluorescein Sodium**
   A. **In dark**
      a. **Emission and excitation of bare dye**

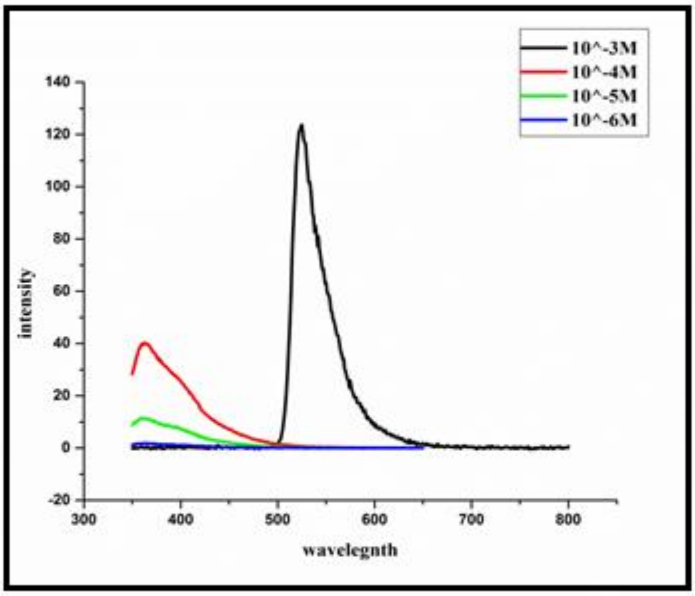


Figure 6.12: Shows the PL emission spectra of bare FS in dark environment

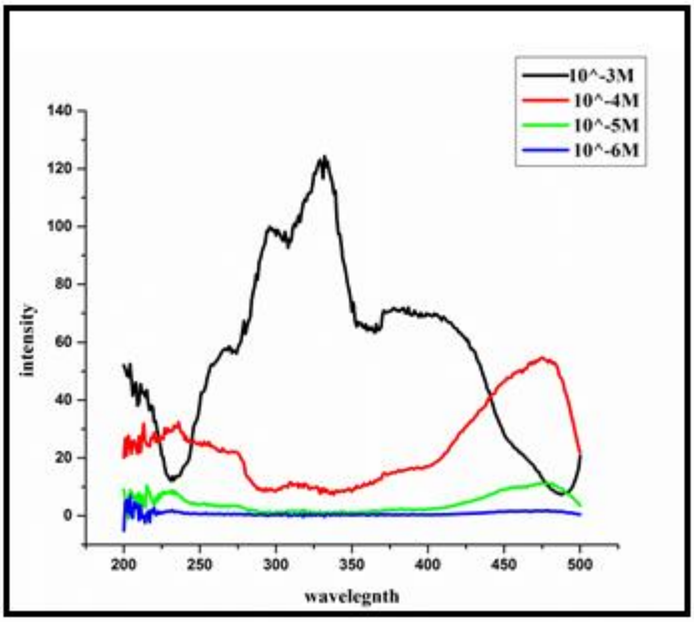


Figure 6.13: Shows the PL excitation spectra of bare FS in dark environment

b. Emission and excitation of dye with NH_4

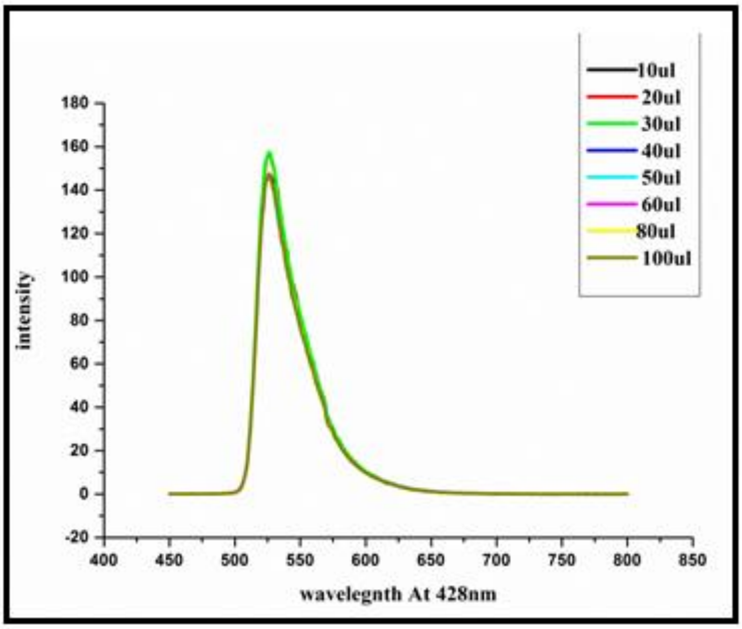


Figure 6.14: Shows the PL emission spectra of FS and ammonia in dark environment

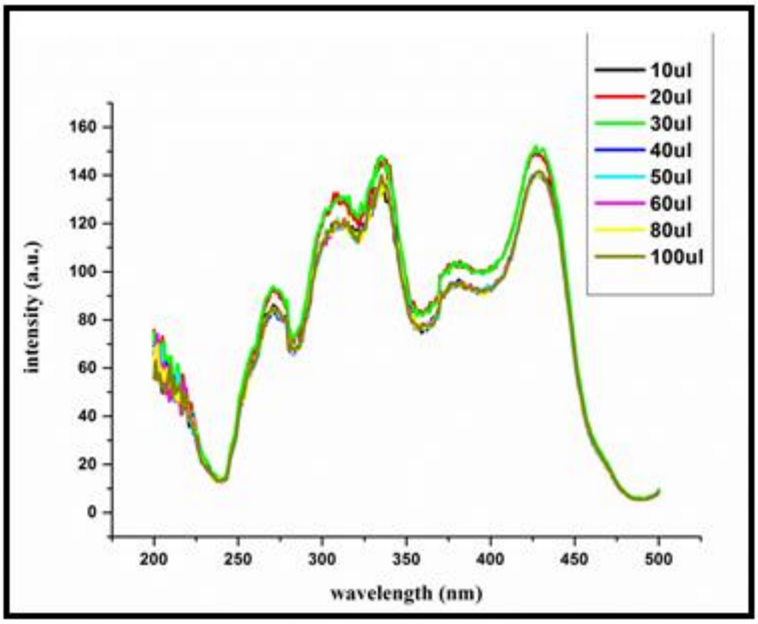


Figure 6.15: Shows the PL excitation spectra of FS and ammonia in dark environment

B. In Light

a. Emission and excitation of bare Fluorescein dye

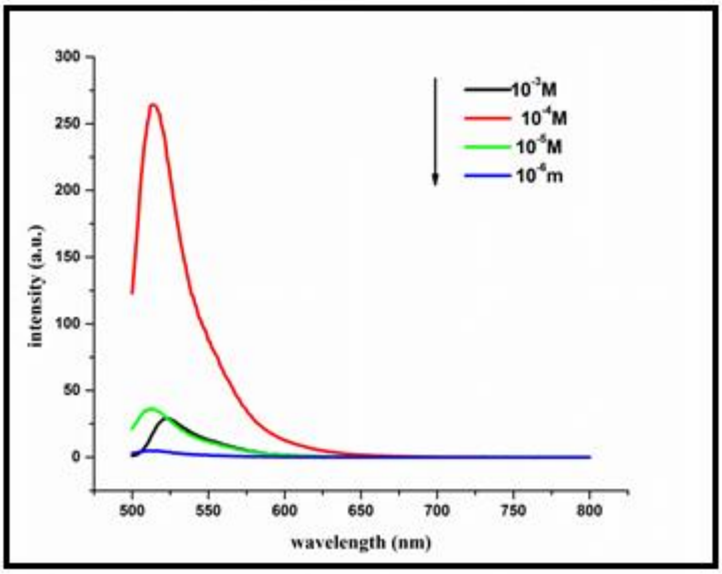


Figure 6.16: Shows the PL emission spectra of FS in the presence of light

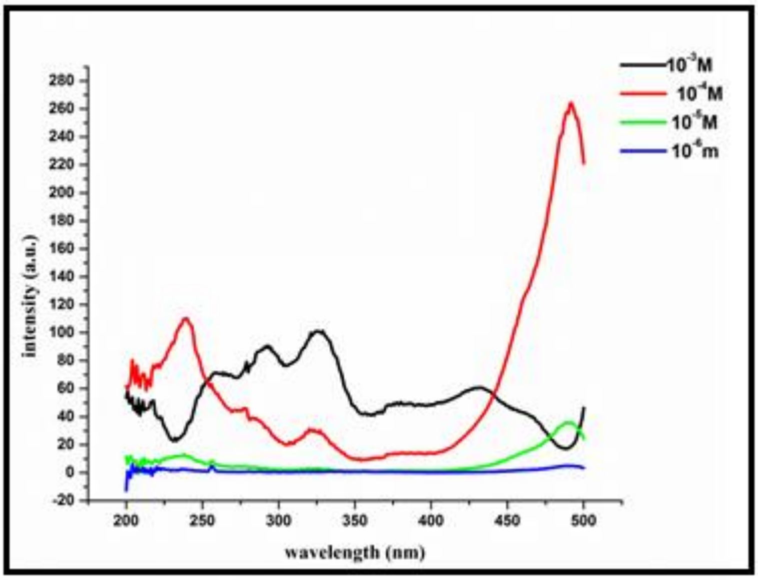


Figure 6.17: Shows the PL excitation spectra of FS in the presence of light

b. Emission and excitation of Fluorescein dye in the presence of NH_4

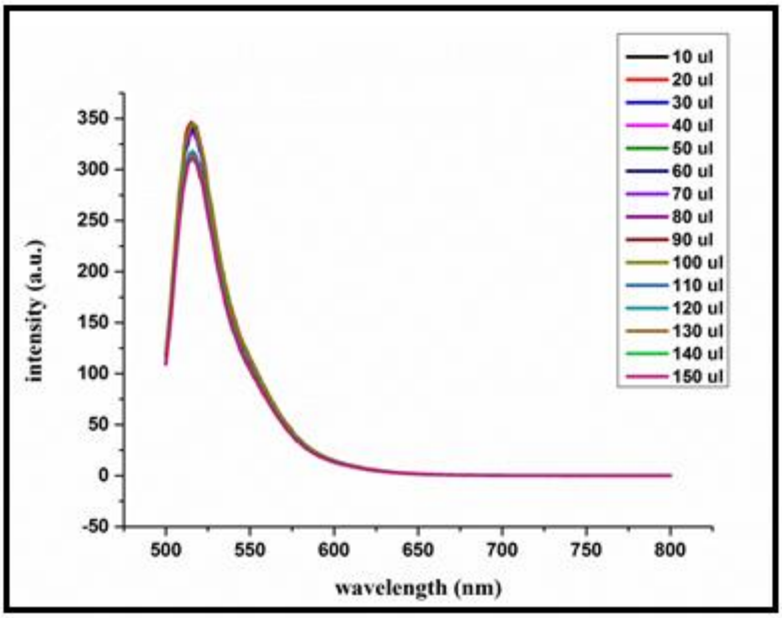


Figure 6.18: Shows the PL emission spectra of FS and ammonia in the presence of light

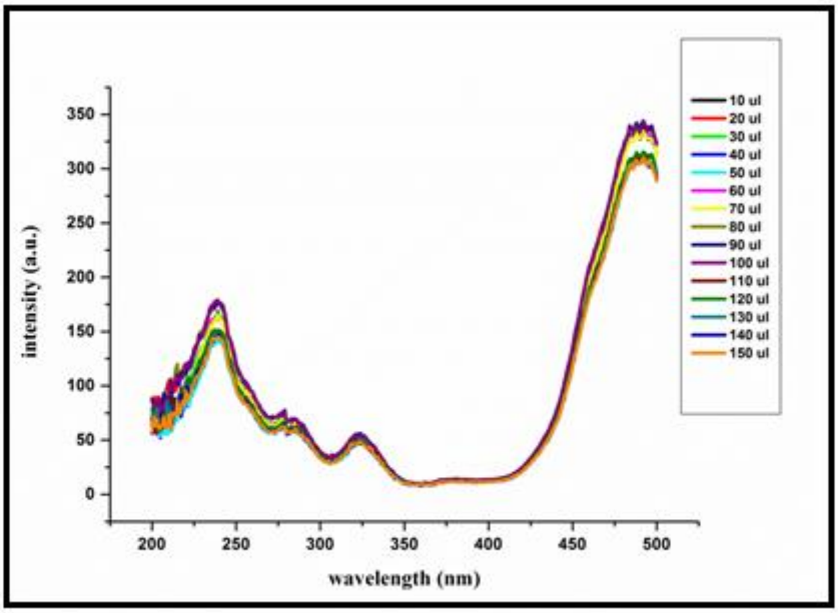


Figure 6.19: Shows the PL excitation spectra of FS and ammonia in the presence of light

2. **Stilbene**
   - **A. Emission and excitation of bare dye**

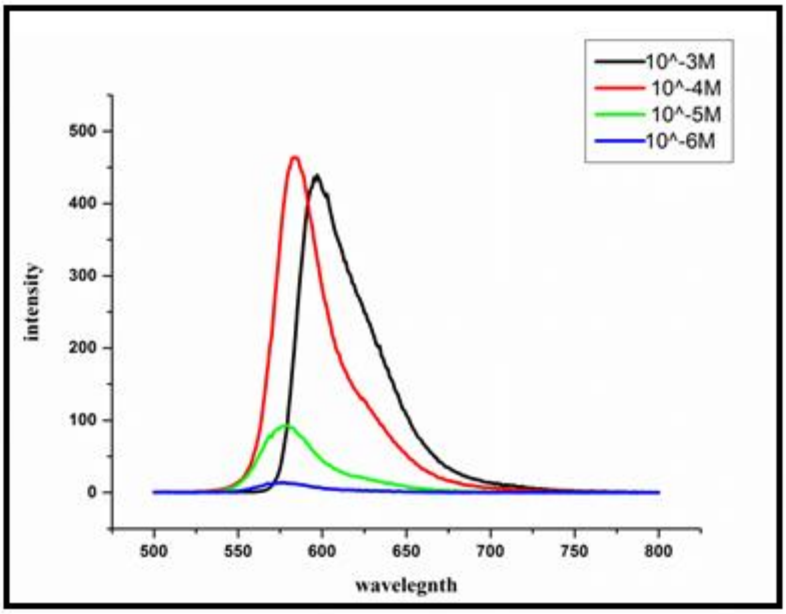


Figure 6.20: Shows the PL emission spectra of bare stilbene dye

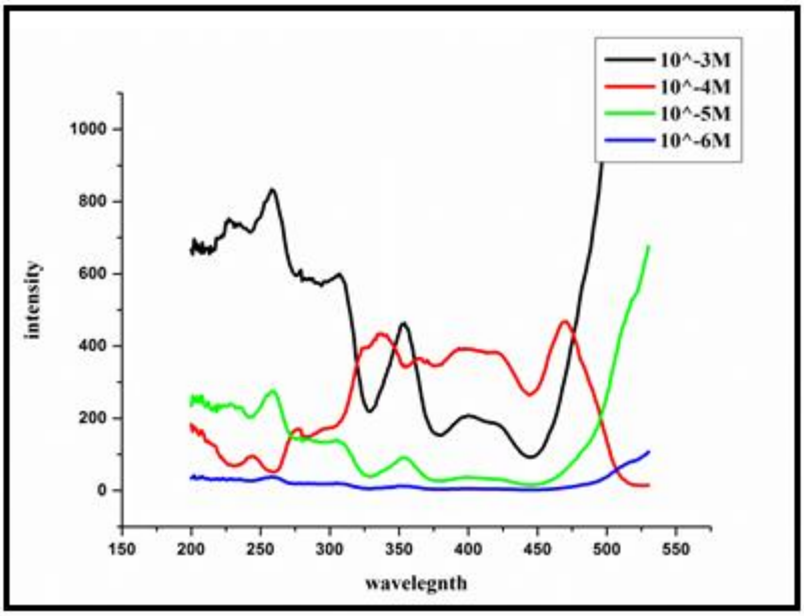


Figure 6.21: Shows the PL excitation spectra of bare stilbene dye

B. Emission and excitation of dye with NH_4

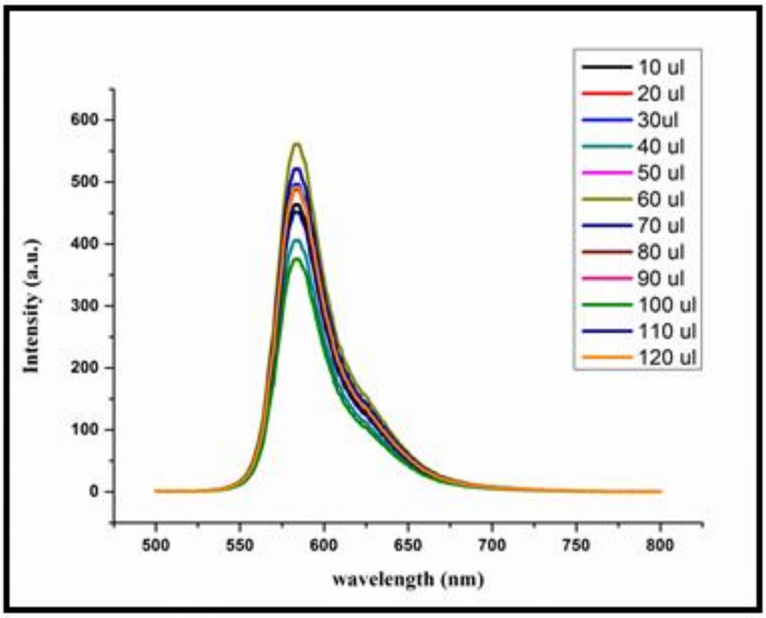


Figure 6.22: Shows the PL emission spectra of stilbene dye with ammonia

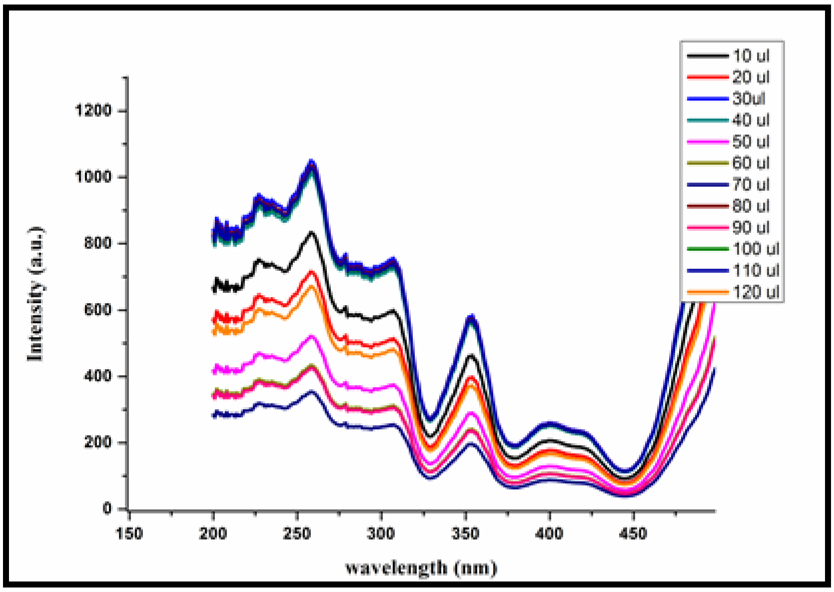


Figure 6.23: Shows the PL excitation spectra of stilbene dye with ammonia

3. Rhodamine B

A. Emission and excitation of bare dye

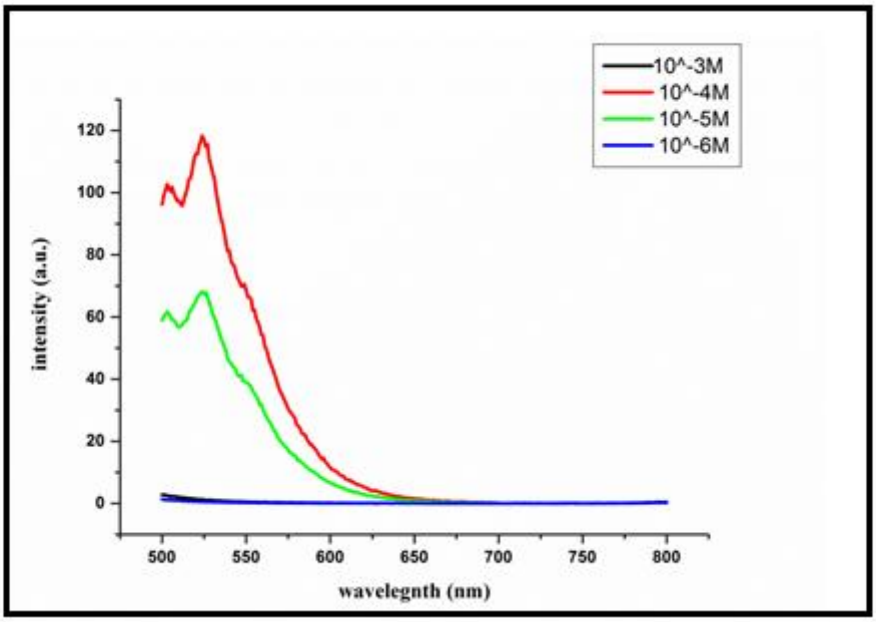


Figure 6.24: Shows the PL emission spectra of bare Rhodamine dye

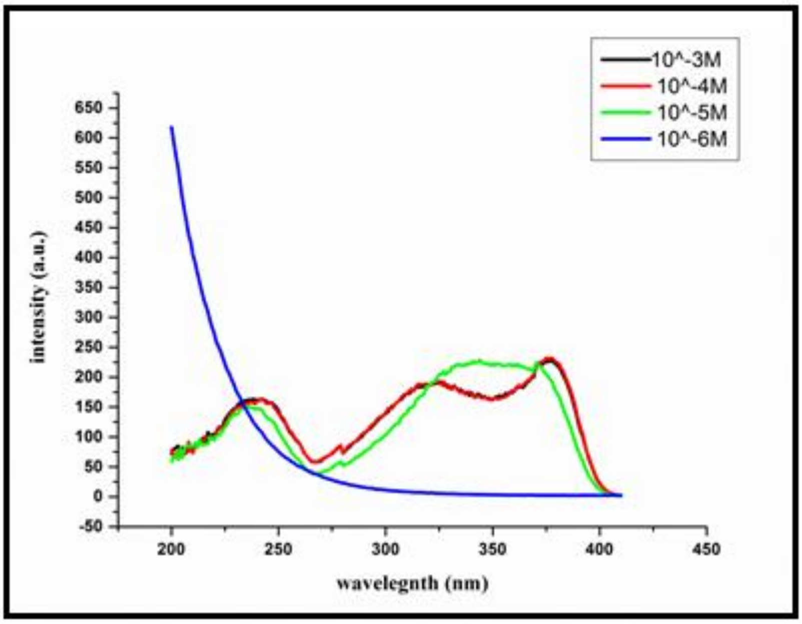


Figure 6.25: Shows the PL excitation spectra of bare Rhodamine dye

B. Emission and excitation of dye with NH_4

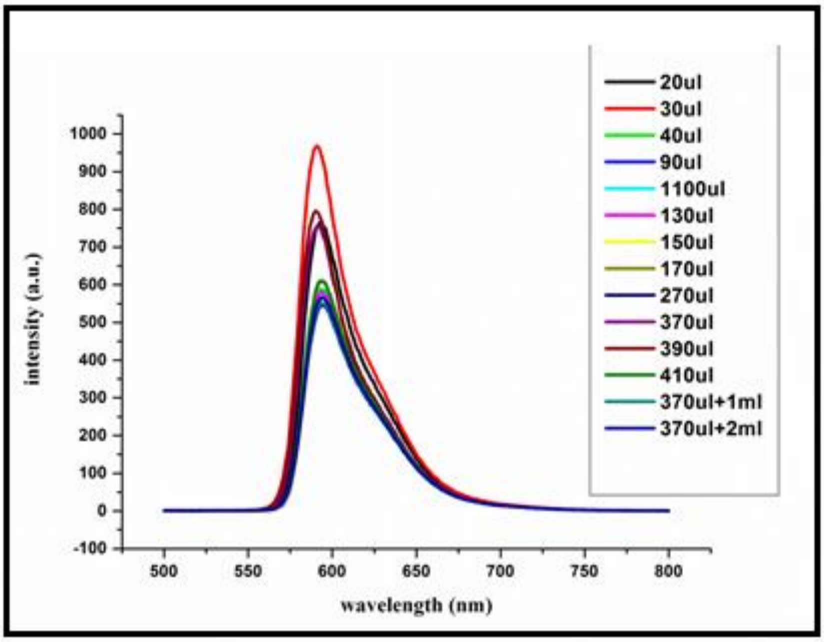


Figure 6.26: Shows the PL emission spectra of Rhodamine dye with ammonia

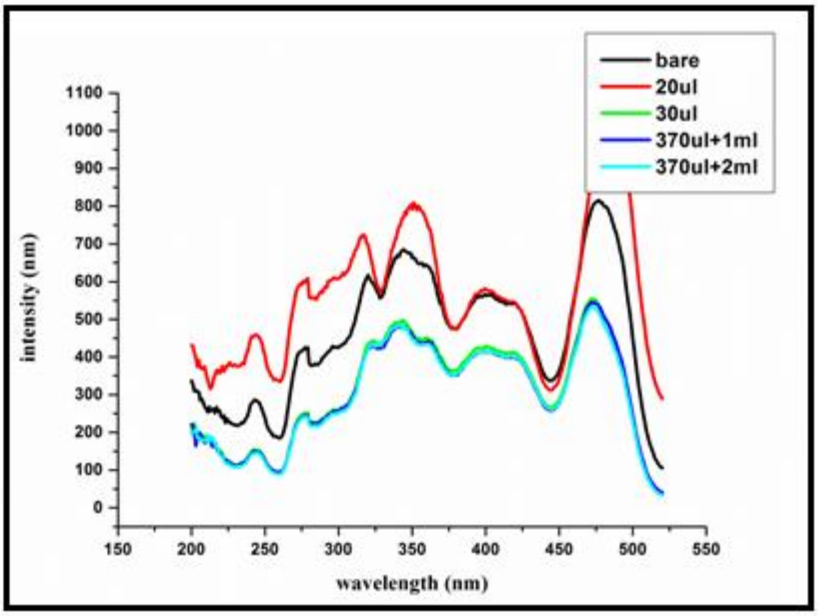


Figure 6.27: Shows the PL excitation spectra of Rhodamine dye with ammonia

We have selected the molar stocks by observing the highest peaks of each bare dyes in both the emission and excitation spectra of their photoluminescence. Coincidentally it was 10^{-4} M for all of them.

Then we have mixed ammonia at increasing concentrations in order to see the gas' effect on each dye at their highest emission and excitation peaks. Good variations are observed with stilbene and rhodamine b while in case of fluorescein sodium not much variation can be observed even after mixing with ammonia or in dark/ light environments.

6.2.2 Data interpretation by result analysis of UV-vis

A. UV-vis of sample A with Rhodamine B

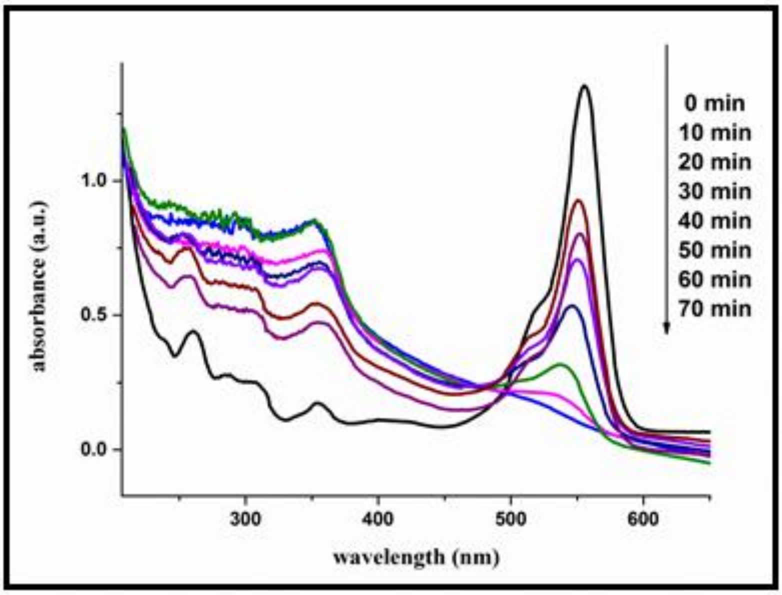


Figure 6.28: Shows the UV-Vis spectra of Rhodamine dye sample A as catalyst after photodegradation

B. UV-vis of sample B with Rhodamine B

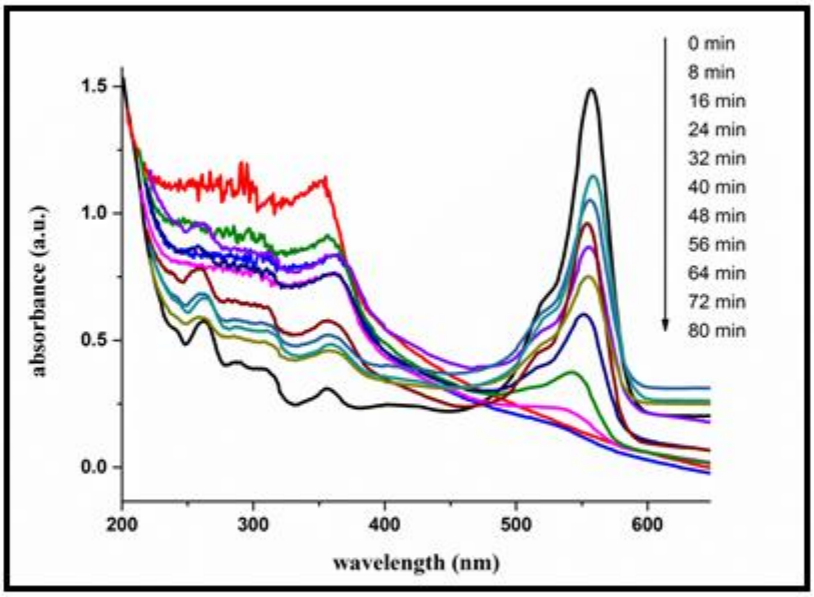


Figure 6.29: Shows the UV-Vis spectra of Rhodamine dye sample B as catalyst after photodegradation

It can be observed from the UV-vis spectra of both the sample catalysts that they degrade the dye completely after their addition to the unit solution of rhodamine b separately. From figures it can be assumed that Sample A took less time to erode the dye completely than sample B.

6.2.3 Data interpretation by result analysis of photodegradation

Comparison plot of the amount of photodegradation relative to time

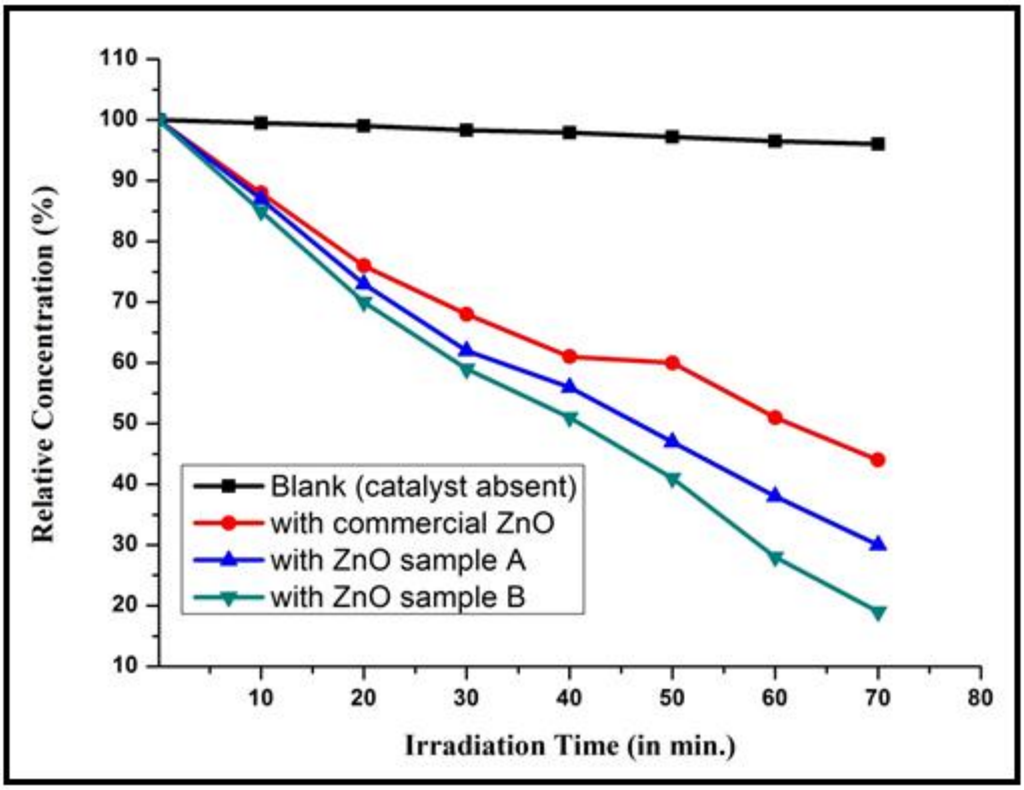


Figure 6.30: Shows a the relative percentage of dye degradation comparison between the samples

We selected rhodamine b dye for exploring the catalytic activity of the prepared ZnO samples as its photoluminescence was found to be very sensitive as compared to the other two dyes. Hence it was chosen to and added to the samples individually and monitored. The percentage of lysing can be compared by the dyes. Sample B eroded the dye a little sooner than sample A.

Chapter 7:

Conclusion and Scope for Further Study

7.1 Congregated Conclusion

7.2 Scope for further study

Chapter 7: *Conclusion and Scope for Further Study*

7.1 Congregated Conclusion

This chapter presents summary of the work done and scope for further study in this field. The biomedical field has advanced in many aspects of bringing solution to many problems through constant research being done in the field. The most attractive quality of science is that all its branches can inter-relate to each other at any point of time. Here in this study, material sciences plays a vital role in understanding a metal oxide nanoparticles' importance in creating a sensor for detecting toxicity presence by showing leaching of dye from the mixture. Photoluminescence and UV-vis spectrometry can study the changes in the dyes behaviours. The property of luminescence is helpful as it needs least amount of temperature changes while used in a reaction to emit light and thus poses least harm to the sample under detection.

The making of nanoparticles is all together a vast field of study to bring about great innovations in the world of pharmaceuticals, medicines, sensing, catalysing, cement, cosmetics, reaction mixtures, chemical industries, doping, imaging, lotions, etc. their most important property is the small size they possess and even then have all the properties of their bulky counterparts. The idea of reducing the size with increasing surface area is very helpful in the places of like drug delivery syustems where we mean to cause no harm to the living target while have our drug suffer minimum digestion and reach its destination in almost all its concentration. This promises to help treat many diseases especially cancers, where already the incorporating of doped materials will be harmful to the surrounding cells of the targeted tumours. One cannot always be too careful and always pose a threat to the person undergoing treatment. Thus to solve this problem the birth of nanotechnology or more correctly calling it the discovery of nanotechnology has been a boon.

The synthesis of these nanoparticles by the least wasteful methods and the use of the cheap and commonly available items that are non-toxic, bio-compatible both medically and environmentally, non-cytotoxic, etc. natures should be kept in mind while choosing any method. As nanoparticles due to their small size can be very dangerous as they can enter even cells from their membranes. They can be duplicated once they enter Golgi bodies or nucleus of the cells.

Selection of metal oxides for the present work was because of their conducting, radiative, elastic and other important properties. We selected zinc oxide for the purpose because of it adequate presence, economic price, easy availability, solubility, compatibility and stability. It is also found to be anti-microbial, anti-fungal, anti-bacterial and anti-septic properties. The reason is not known yet behind this property. It possesses good optical qualities too which can be used for constructing optically Pumped Lasers: Surface-Emitting ZnO DFB Laser, light emitting diodes, transparent conducting oxides and TFTs (thin film transistors), etc. they also possess piezoelectric properties which can use them for making thin film piezoelectric devices such as microelectromechanical systems (MEMS), bulk acoustic wave and SAW resonators, SAW filters, sensors, to be used in TV filters and wireless communications systems. ZnO nanoparticles can be used in making sensors and solar cells as well. They can be used in these fields because of their photo catalytic nature as they attain porosity in being prepared by various available methods hence the construction of gas sensors, bio-sensors and solar cells is a good use. They are acoustic as well as optic qualities of zinc oxide. The various shapes it possesses by different available methods help them into forming rods, sheets, flower-like bodies, petals, spheres, etc.

Various synthesis methods like metallurgical (physical technique) and the chemical techniques like sol-gel, hydrothermal, solvothermal, polymerisation, emulsion and micro-emulsion, precipitation and co-precipitation, etc. help us create desired morphologies and sizes which can vary from a range of micrometers to nanometers (with three possible porosities microspheres, macroporous and mesoporous).

The uses of luminescence techniques require an adequate knowledge of its various available types and their roles in the reactions with the help of organic dyes. Its various forms help us in generating their spectra by various devices. They help in constructing a planned approach towards bio-sensing.

7.2 Scope for further study

Synthesis of materials like nanoparticles from resources which are non-toxic, cheap and readily available by various controlled methods provide an opportunity of meeting our day to day needs in every field of life like research, home-front, pharmaceuticals and chemical industries, etc. They can help in studying the luminescence and UV studies to determine the effects of toxic gases on a specimen that can be a living or a non-living source. We can synthesis nanoparticles with dye molecules incorporated into them at the manufacturing time to ensure they get adsorbed to the nano-catalyst completely. Desired morphologies can be achieved by following different synthesis methods available, according to need.

Photodegradation: an important property of nanoparticles

A photoreaction can accelerate by the addition of a catalyst to the reaction mixture. The substrate present in the mixture has a dye present (which is adsorbed) on its surface, which absorbs the light fallen onto it. The falling light generates the catalysis of the dye by the formation of free radicals due to the electron-hole creation. It was developed after titanium oxide was used for electrolysis of water. Nowadays an advanced oxidation technique can also be used which does not necessarily include TiO_2 or UV light for degrading the dye. These generally involve the creation of hydroxyl radical. This property of the catalysts can be used to develop bio-sensors to help diagnose diseases, aid in their treatment, control environmental pollution, remediation, making pharmaceuticals, etc.

REFERENCES

REFERENCES

[1] Ao, W.; Li, J.; Yang, H.; Zeng, X.; Ma, X. (2006) Mechanochemical synthesis of zinc oxide nanocrystalline. Powder Technol. **168**, 128–151.

[2] Tsuzuki, T.; McCormick, P.G.(2001) ZnO nanoparticles synthesis by mechanochemical processing. Scr. Mater. **44**, 1731–1734.

[3] Tsuzuki, T.; McCormick, P.G. (2004) Mechanochemical synthesis of nanoparticles. J. Mater. Sci.**39**, 5143–5146.

[4] Moballegh, A.; Shahverdi, H.R.; Aghababazadeh, R.; Mirhabibi, A.R. (2007) ZnO nanoparticles obtained by mechanochemical technique and optical properties. Surf. Sci. **601**, 2850–2854.

[5] Aghababazadeh, R.; Mazinani, B.; Mirhabibi, A.; Tamizifar, M.(2006) ZnO nanoparticles by mechanochemical processing. J. Phys. Chem. Solids **26**, 312–314.

[6] Hong, R.; Pan, T.; Qian, J.; Li, H. (2006) Synthesis and surface modification of ZnO nanoparticles. Chem. Eng. J. **119**, 71–81.

[7] Hong, R.; Qian, J.; Miao, C.; Li, H; (2005) Synthesis and surface modification of ZnO nanoparticles. Spec. Petrochem. **2**, 1–4.

[8] Stanković, A.; Veselinović, L.J.; Skapin, S.D.; Marković, S.; Uskoković, D. (2011) Controlled mechanochemically assisted synthesis of ZnO nanopowders in the presence of oxalic acid. J. Mater. Sci., **46**, 3716–3724.

[9] Lanje, A.S.; Sharma, S.J.; Ningthoujam, R.S.; Ahn, J.S.; Pode, R.B.(2013) Low temperature dielectric studies of zinc oxide (ZnO) nanoparticles prepared by precipitation method. Adv. Powder Technol. **24**, 331–335.

[10] Wang, Y.; Ma, C.; Sun, X.; Li, H. (2002) Preparation of nanocrystalline metal oxide powders with the surfactant-mediated method. Inorg. Chem. Commun. **5**, 751–755.

[11] Jia, W.; Dang, S.; Liu, H.; Zhang, Z.; Yu, Ch.; Liu, X.; Xu, B. (2012) Evidence of the formation mechanism of ZnO in aqueous solution. Mater. Lett. **82**, 99–101.

[12] Li, P.; Wei, Y.; Liu, H.; Wang, X.K. (2005) Growth of well-defined ZnO microparticles with additives from aqueous solution. J. Solid State Chem. **178**, 855–860.

[13] Benhebal, H.; Chaib, M.; Salomon, T.; Geens, J.; Leonard, A.; Lambert, S.D.; Crine, M.;Heinrichs, B. (2013) Photocatalytic degradation of phenol and benzoic acid using zinc oxide powdersprepared by sol-gel process. Alex. Eng. J. **52**, 517–523.

[14] Ristić, M.; Musić, S.; Ivanda, M.; Popović, S. (2005) Sol–gel synthesis and characterization of nanocrystalline ZnO powders. J. Alloy. Compd. **39**, L1–L4.

[15] Yue, S.; Yan, Z.; Shi, Y.; Ran, G. (2013) Synthesis of zinc oxide nanotubes within ultrathin anodic aluminum oxide membrane by sol-gel method. Mater. Lett. **98**, 246–249.

[16] Chen, S.J.; Li, L.H.; Chen, X.T.; Xue, Z.; Hong, J.M.; You, X.Z. (2003) Preparation and characterization of nanocrytalline zinc oxide by a novel solvothermal oxidation route. J. Cryst. Growth **252**, 184–189.

[17] Ismail, A.A.; El-Midany, A.; Abdel-Aal, E.A.; El-Shall, H. (2005) Application of statistical design to optimize the preparation of ZnO nanoparticles via hydrothermal technique. Mater. Lett. **59,** 1924–1928.

[18] Dem'Yanets, L.N.; Li, L.E.; Uvarova, T.G. Zinc oxide: (2006) Hydrothermal growth of nano- and bulk crystals and their luminescent properties. J. Mater. Sci. **41**, 1439–1444.

[19] Musić, S.; Dragčević, D.; Popović, S.; Ivanda, M. (2005) Precipitation of ZnO particles and their properties. Mater. Lett. **59**, 2388–2393.

[20] Strachowski, T.; Grzanka, E.; Palosz, B.; Presz, A.; Ślusarski, L.; Łojkowski, W. (2003) Microwave driven hydrothermal synthesis of zinc oxide nanopowders. Solid State Phenom. **94**, 189–192.

[21] Schneider, J.J.; Hoffmann, R.C.; Engstler, J.; Klyszcz, A.; Erdem, E.; Jakes, P.; Eichel, R.A.; Pitta-Bauermann, L.; Bill, J. (2010) Synthesis,characterization, defect chemistry, and FET properties of microwave-derived nanoscaled zinc oxide. Chem. Mater. **22**, 2203–2212.

[22] Zhang, J.; Wang, J.; Zhou, S.; Duan, K.; Feng, B.; Weng, J.; Tang, H.; Wu, P. (2010) Ionic liquid-controlled synthesis of ZnO microspheres. J. Mater. Chem. **20**, 9798–9804.

[23] Kołodziejczak-Radzimska, A.; Jesionowski, T.; Krysztafkiewicz, A. (2010)Obtaining zinc oxide from aqueous solutions of KOH and Zn(CH3COO)2. Physicochem. Probl. Miner. Process. **44**, 93–102.

[24] Pyskło, L.; Parasiewicz, W.; Pawłowski, P.; Niciński, K. (2007)Zinc Oxide in Rubber Compounds; Instytut Przemyslu Gumowego: Piastow, Poland.

[25] Mahmud, S.; Abdullah, M.J.; Putrus, G.A.; Chong, J.; Mohamad, A.K. (2003) Nanostructure of ZnO fabricated via French process and its correlation to electrical

properties of semiconducting varistors. Synth. React. Inorg. Met. Org. Nano Met. Chem. **36**, 155–159.

[26] Djurišcić, A.B.; Chen, X.Y.; Lung, Y.H. (2012) Recent progress in hydrothermal synthesis of zinc oxide nanomaterials. Recent Pat. Nanotechnol. **6,** 124–134.

[27] Tsuzuki, T.; Dawkins, H.; Dunlop, J.; Trotter, G.; Nearn, M.; McCormick, P.G. (2002) Nanotechnology and cosmetic chemist. Cosmet. Aeorosol Toilet. Aust. **15**, 10–24.

[28] Ma, J.; Liu, J.; Bao, Y.; Zhu, Z.; Wang, X.; Zhang, J. (2013) Synthesis of large-scale uniform mulberry-like ZnO particles with microwave hydrothermal method and its antibacterial property. Ceram. Int. **39**, 2803–2810.

[29] Bondioli, F.; Ferrari, A.M.; Braccini, S.; Leonelli, C.; Pellacani, G.C.; Opalinska, A.; Chudoba, T.; Grzanka, E.; Palosz, B.; Łojkowski, W. (2003) Microwave hydrothermal synthesis of nanocrystalline pre-doped zirconia powders at pressures up to 8 MPa. Solid State Phenom. **94**, 193–196.

[30] Goldstein, J. (2003) Scanning electron microscopy and x-ray microanalysis. Kluwer Adacemic/Plenum Pulbishers, 689.

[31] Reimer, L. (1998) Scanning electron microscopy : physics of image formation and microanalysis. Springer, 527.

[32] Egerton, R. F. (2005) Physical principles of electron microscopy : an introduction to TEM, SEM, and AEM. Springer, 202.

[33] Clarke, A. R. (2002) Microscopy techniques for materials science. CRC Press (electronic resource).

[34] Argast, Anne and Tennis, Clarence F., III. (2004) A web resource for the study of alkali feldspars and perthitic textures using light microscopy, scanning electron microscopy and energy dispersive X-ray spectroscopy, Journal of Geoscience Education **52#3**, 213-217.

[35] Beane, Rachel. (2004) Using the Scanning Electron Microscope for Discovery Based Learning in Undergraduate Courses, Journal of Geoscience Education, **52#3**, p. 250-253.

[36] Moecher, David. (2004) Characterization and Identification of Mineral Unknowns: A Mineralogy Term Project, Jour. Geoscience Education, **52 #1**, p. 5-9.

[37] Umar, A.; Hahn, Y.; Metal Oxide Nanostructures and Their Applications.Edited by **5**, 1–36.

[38] Kumari,S.; Sahare, P.D. (2013) Optical Studies of Fluorescent Mesoporous Silica Nanoparticles, Journal of Materials Science & Technology, Elsevier **29(8)**, 742-746.

[39] Zhu, Q.; Chen, J.; Cui, Q,; Liu, L.; Li, B.; Zhou, X.; (2010)Monodispersed hollow microsphere of ZnO mesoporous nanopieces: Preparation, growth mechanism and photocatalytic performance, Materials Research Bulletin, Elsevier, **45,** 2024-2030.

[40] Radzimska. A.K.* and Jesionowski, T. (2014) Zinc Oxide—From Synthesis to Application: A Review. Materials, **7**, 2833-2881.