SYNTHESIS AND ANTIMICROBIAL STUDY OF CHITOSAN COATED IRON OXIDE INCORPORATED MESOPOROUS SILICA NANOPARTICLES

DISSERTATION SUBMITTED TO THE UNIVERSITY OF KERALA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN ANALYTICAL CHEMISTRY

Subject code: CL243

Exam code: 63620401

MSc. Analytical Chemistry

ABSTRACT

This study demonstrates a facile strategy for the synthesis of chitosan coated iron oxide incorporated mesoporous silica nanoparticle. The synthesized mesoporous silica (MSN)was characterized by FTIR and TEM analysis. From the FTIR it was confirmed that the surfactant CTAB has been completely removed leading to the formation of mesoporous structure. The mesoporous structure of MSN was also confirmed from TEM data. CS, Fe_3O_4, and Fe_3O_4-CS were also characterized by FTIR. All the characteristic peaks of CS and Fe_3O_4 were present in the spectrum of Fe_3O_4–CS which indicates that the ironoxide nanoparticles were successfully coated with CS. The antibacterial and antifungal study of synthesized Fe_3O_4-CS-MSN was conducted. The result shows that the synthesized product shows antibacterial property against *Stephylococcus* and *E.coli* and antifungal property against *Candida albicans*.

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LIST OF ABBREVATIONS

CS	Chitosan
MSN	Mesoporous Silica Nanoparticles
Fe_3O_4 -CS	Chitosan coated Iron Oxide
Fe_3O_4 -CS-MSN	Chitosan coated Iron Oxide incorporated Mesoporous Silica Nanoparticles
FTIR	Fourier Transform Infrared Spectroscopy
HR-TEM	High Resolution Transmission Electron Microscopy
CTAB	Cetyl trimethyl ammonium bromide
TEOS	Tetraethyl orthosilicate
GPTMS	3-Glycidoxypropyltrimethoxysilane

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CHAPTER 1

INTRODUCTION

1.1 NANOPARTICLES

A nanoparticle or ultrafine particle is usually defined as a particle of matter that is between 1 and 100 nanometre in diameter. The term is sometimes used for larger particles, up to 500 nm, or fibers and tubes that are less than 100 nm in only two directions. Nanomaterials can be classified based on the dimension of the entity which is nano sized, that is the classification is based on the number of dimensions. The four types of nanomaterials based on the dimensions are 0D(quantumdots),1D(nanotubes,nanorods),2D(nanofilms,nanolayers)3D(multinanolayers)nano materials. Nanoparticles exist in several different morphologies such as spheres, cylinders, platelets, tubes etc. The surface modifications of nanoparticles help to meet the needs of specific applications for which they are going to be used for.

Nanonoparticles may be created using several methods.There are two approaches for synthesis of nanomaterials and the fabrication of nanostructures.They are Top-Down and Bottom-Up approach.Top down approach refers to the slicing or successive cutting of a bulk material to get nano sized particle.In bottom up approache refers to the buildup of materials from the bottom;atom by atom,molecule by molecule.The important methods of synthesis of nanomaterials are hydrothermal methad,sol gel method,etc.

Nanoparticles (NPs) are used as an alternative to antibiotics to target bacteria. Nanotechnology has great importance in treating bacterial infections. NPs are being used as antibacterial coatings for implantable devices and medicinal materials to prevent infection and promote wound healing, in antibiotic delivery systems to treat disease, in bacterial detection systems to generate microbial diagnostics, and in antibacterial vaccines to control bacterial infections. The antibacterial

mechanisms of NPs against bacteria and the factors that are involved has great importance now a days[1].

Major fields where nanomaterials finds application:

- Biomedical and Health care
- Renewable Energy
- Construction materials
- Electronics and Energy Storage
- Environmental Remediation
- Food packaging , processing and agriculture

1.2 Iron oxide Nanoparticles

The two main forms of ironoxide nano particles are magnetite and its oxidized form maghemite. As these are magnetic nanoparticles they show superparamagnetism. Superparamagnetic iron oxide (SPION) nanoparticles can be used to deliver peptides, DNA, chemotherapeutics and more. It is used in computed tomography as a contrast agent. In some scans such as CT scan and MRIs you may need a contrast agent to make veins, organs etc more visible. It also find application as nanozymes.the benefits of ironoxide nanoparticle nanozymes include easy modulation and high stability despite varying conditions. They can also be used to enhance nanozyme function. It is also useful in tissue repair and cancer treatment. Studies have been done for preparing pH responsive chitosan coated iron oxide nanoparticles and its characterization for use as pH sensitive drug carriers [2]

1.3 Mesoporous Silica Nanoparticles

Mesoporous materials are materials which have their pore size in between micro and macro porous materials. For mesoporous material their pores dimeters is of the size 2nm-50nm. MSNs contain the porous honeycomb-like structure of silica (SiO_2). They offer advantages of the porous structure, tunable size, biocompatibility, and larger surface area. The high surface area of pores allows the attachment of the functional groups on the MSNs. MSNPs with their unique mesoporous structure offer advantages that may allow clinically applicable nanoformulations for disease diagnosis and therapy. MSNPs possess easily tunable particle size, uniform pore size, high pore volume, greater surface area and simple mesoporous or hollow structure. MSNPs provide excellent nanoplatforms to design smart drug delivery systems for biomedical applications. With respect to drug delivery systems, they have an extraordinarily high drug loading capacity and stimuli-responsive drug release profiles.

1.4 Methods For MSN Synthesis

Sol-Gel process is a wet chemical method for the synthesis nanoparticles. In this method, the molecular precursor like metal alkoxide is dissolved in water and converted to gel by heating and stirring by hydrolysis. The formation of MSNs first began by Stöber with the formation of monodispersed spherical silica particles in the micron range. In Stöber synthesis water, base, alcohol and silica source were the main components being used. The formation of MSNs was first achieved by Grun et al., by modifying the Stöber method through the introduction of surfactant. The general process of synthesizing MSNs through the modified Stöber method consists of four steps as follows:

- Mixing water, template and base together

- Addition of silica source to the mixture and stirred. During this step the hydrolysis and condensation process take place, and silica sol was formed.
- The aging process of sol , and the gel was formed.
- MSNs powder was obtained. By calcination or acidic solvent extraction process the template was removed. Based on the process, the modified Stöber method in obtaining MSNs is considered as sol–gel method. Through this discovery, there are even more modifications made to achieve the desired MSNs.

Hydrothermal synthesis is one of the most commonly used methods for preparation of nanomaterials . In hydrothermal synthesis, the formation of nanomaterials can happen in a wide temperature range from room temperature to very high temperatures.It is a method of synthesis of single crystals that depends on the solubility of minerals in hot water under high pressure. The hydrothermal synthesis of MSN involves:

- Mixing of water, template and catalyst together.
- Addition of silica source.
- The mixture will undergo aging process in some cases. While in other cases the mixture was stirred for a short period then transferred to a Teflon-lined autoclave.
- The autoclave was heated for required time and temperature.
- MSNs powder was obtained, and a template removal process was conducted.

1.5 Green Method

Green synthesis is required to avoid the production of unwanted or harmful by-products through the build-up of reliable, sustainable, and eco-friendly synthesis procedures. Works have been done to synthesize mesoporous silica nanoparticles from coconut husk [3]. The synthesis of MSN by using hexafluoro silicic acid, waste from the fertilizer industry, was done by Abburi et

al[4]. It should be noted that although the method is considered green, the process may not obey the green chemistry principle. For example, the extraction of (silica source) from wastes such as rice husk are complicated and conducted under extremely high or low pH conditions.

1.6 MSN as Drug Delivery Vehicle

The medicines used for cancer treatment not only acts on the infected cells but it also damages healthy cells so it is necessary to make the drug reach the exact infected location. For increasing chemotherapeutic efficacy and reducing adverse effects the tumour targeted delivery and controlled release of antitumour drug is necessary. The use of smart drug delivering agents, which release the drug at the target diseased tissue only, would permit to reduce severe side effects of the chemotherapeutics. Researchers are focusing on nanomaterials as smart carriers for targeted drug delivery and stimuli-responsive systems, useful for the treatment of cancer. MSN are promising and novel drug vehicle due to their mesoporous structure and hence help in the controlled release and targeted delivery of a variety of drugs. Because of their large pore volume, high surface area, narrow pore size, easily modified surface MSN have received considerable attention. The large surface area and pore volume enables MSN to accommodate large amount of drug molecules and protect them from premature degradation and promote controlled and fast release. MSN are biocompatible accumulates in tumour and effectively deliver drugs to tumor and suppress tumour growth. All these features make MSN a very promising drug delivery material. Now adays we can see recent progress in the synthesis and surface functionalization of MSNs for drug delivery [5].pH-responsive MSN are good anticancer drug delivery agents. Cancerous cells are acidic than normal cell. The exploitation of pH values in most tumours was done to develop such systems. In order to get controlled drug release for cancer therapy Yanqin xu and coworkers prepared a pH and redox dual responsive MSN-S-S-CS was prepared via an

amide reaction of dithiodipropionic acid with amino groups on the surface of MSN and amino group on the surface of chitosan (CS)[6]. GSH(glutathione) is presents in normal cells, but in cancerous cells, It was found that the concentration of GSH is significantly higher .A work reported on the utilization of redox potential so as to produce a redox responsive system with chitosan as a way to trap the drug in the MSNs pore. A fluorescent agent which binds the chitosan with MSNs is used here. This fluorescent agent is sensitive to GSH. Once the carrier entered the cancer cell, this fluorescence agent will get cleaved, and chitosan will be released from the carrier. Therefore, the drug will be released to treat the cancerous cell.

In passive targeting allows the accumulation of nanoparticles in the solid tumours as a drug delivery agent through passive targeting, It is necessary to control the particle size, particle shape and surface properties so as to use MSN as a drug delivery agent through passive targeting. Polyethylene glycol (PEG) functionalized with MSNs can reduce RES uptake and improve the overall stability of MSNs, which can help to enhance the EPR effect.

Active targeting helps in the active uptake of nanoparticles by the tumor cells themselves. Exploiting the tumour cell-specific receptors is being done here. The MSNs surface is functionalised with a targeting ligand which can interact with the receptors, thus improving the uptake and EPR effect as more carrier accumulate at the tumour site and thereby increasing the efficacy of the treatment.

CHAPTER 2

REVIEW OF LITERATURE

The role of MSN in biomedical field is gaining importance day by day. Due to its porous structure the surface area of mesoporous materials is significantly large. MSN has many applications because of its structural features. They also shows a high biocompatibility ,ease for surface modification, and capability to load and deliver drugs, genes and diverse chemicals. For future clinical applications it is necessary to study the biological safety aspect of MSNs. Sepanta and et al tried to study the bio safety of MSN by examining its biodegradation, effect on immune cells, and how these vary depending on the size shape.[7]

Bowen et al worked to study the synthesis, biological effect and biomedical application of MSNs and MONs. They attempted to elucidate the advancement on the construction of MSNs/MONs-based theranostics nanoplatforms for diagnostic and therapeutic application.[8]

Jing Zhang et al worked to synthesize a redox responsive and CD44 targeted drug delivery system by immobilizing hyaluronic acid onto the surface of MSN via disulfide bond. This study has gained much importance in tumour therapy in clinical application.[9]

The small sizes of NPs are responsible for the bacterial effects because of their ability to easily penetrate the cell membrane .Studies done by Enik N Taylor et al revealed that the concentration of nanoparticles antimicrobial activity was a key factor in promoting antimicrobial activity.[10]

Behera SS et al carried out a work to characterize and evaluate the antibacterial activities of chemically synthesize iron oxide nanoparticles. Here the iron oxide nanoparticles was synthesized by co-precipitation method using aqueous solution of ferric and ferrous ions with sodium salt. The antibacterial effect of iron oxide nanoparticles was evaluated against ten pathogenic bacteria which showed that the nanoparticles have moderate antibacterial activity against both Gram positive and Gram negative pathogenic bacterial strains and it could be

considered as a future scope in pharmaceutical and biomedical industries. This study also concludes that the appropriate external magnetic field may be directed to the NPs so as to kill the bacteria inside the body.[11]

Ming Kongand et al worked to study the antimicrobial effect of Chitosan, Chitosan is a natural antimicrobial agent found in the shells of crustaceans like crab shrimp etc. The deacetylation of chitin also yields the biopolymer Chitosan. It is a biocompatible, biodegradable, linea polysaccharide and consisting of amino, and primary and secondary hydroxyl groups. It finds application in bio imaging, gene delivery, wound healing etc. antimicrobial activities against wide varieties of microorganisms and are having negligible toxicity towards human cells.[12]

Iron oxide nanoparticle are good in showing antibacterial properties. Chitosan polymers shows both antibacterial and antifungal properties. Nehra P et al analyzed the antibacterial and antifungal property of chitosan coated iron oxide nanoparticle against five organisms , E-coli, Bascillus subtilus, Candida albicans, Aspergillus niger and Fusarium solani. In this work iron oxide nanoparticles were synthesized by co precipitation and surface coating was done using chitosan polymer. Antimicrobial properties of NPs was tested by agar well diffusion and the measuring of the diameter of the inhibition zones. This work was able to conclude the fact that CS-Fe_3O_4 NPs are good anti microbial agents and it could be developed as a microbial resistant coating for biomedical devices.[13]

Afseneh Najafi et al recently studied, the successful formulation, characterization, and evaluation of an MSN-NH_2 nanocarrier for the delivery of cefepime (CEF) and meropenem (MEP) was done and it was evaluated for antimicrobial efficacy against MDR A. baumannii. Higher was the efficacy of drug loaded MSN-NH2 when compared to free drug. The localized

drug release lead to efficient bacterial death. Therefore the role of MSN–NH_2–CEF and MSN–NH_2–MEP as nanocarriers showed to improve the antibacterial activity of CEF and MEP respectively and reducing side effects and toxicity.[14]

Li-li Li et al worked on the scope of enzyme coated MSN as effective antibacterial agents. The increased use of antibiotics is leading to the emergence of drug resistance in pathogenic bacteria. The abuse of antibiotics is causing multidrug resistance which is a major challenge to global public health. Because of the rise in death rate caused by pathogenic bacterial infection the need for effective antibiotic alternatives is gaining importance. Lysozyme-coated mesoporous silica nanoparticles (MSNs⊂Lys) are reported as antibacterial agents that exhibit efficient antibacterial activity.[15]

Valentina Nairi et al reported the synthesis of silica carriers by sol–gel from tetraethyl orthosilicate (TEOS) as precursor of silica and cetyltrimethylammonium bromide (CTAB) as pore generating agent. The reaction conditions were modified by changing the molar ratio of water/TEOS, NH3/TEOS and amount of CTAB. The results show that the addition of CTAB and the amount of H2O strongly affected the specific surface area and the porosity of silica. The chief aim of their work is to study the particle size morphology and dispersion of mesoporous silica nanoparticles prepared by sol-gel by simultaneously varying the anount of water and CTAB.[16]

Amirali Popat et al discussed the non-surfactant induced synthesis of mesoporous silica nanoparticles. This method is gaining increasing interest Because of their low toxicity and simple purification compared to conventional surfactant-based methods this method is gaining

importance. Here they used tannic acid (TA) and four structurally related polyphenols (gallic acid, ethyl gallate, eudesmic acid and quercetin) so as to study the effect of the chemical structure and properties of polyphenols on the templating ability.[17]

Sahern Rahmani et al showed that MSN and MSNR particles were efficient in killing cancer cells. Here the synthesis and application of mesoporous silica nanoparticles (MSN) and mesoporous silica nanorods (MSNR) for drug delivery was analysed. The addition of cosolvent to the sol-gel solution helped to yield MSN and MSNR. The particle shape and structure of the mesoporosity was controlled by ethanol addition. The loading of MSN and MSNR with doxorubicin followed by its incubation with MCF-7 breast cancer cells was performed.[18]

Sen karamand et all studied the molecular basis of action of silica NPs against Gram negative and Gram positive. Bacterial invasion is the main reason for many infectious diseases. Starting with minute skin infection the range of bacterial infection extends to acute diarrheal disease .From their findings it was concluded that both shape and surface engineering contributes to bacterial killing, and the silver-ion doped and chitosan coated MSN has significant potential as an antimicrobial nanomaterial. This work provides a future scope to the development of new nanobiotics having good design and less toxicity.[19]

A recent work done by Amir Hasanzadeh et al reports the biosynthesis of AgNPs onto the urea-based periodic mesoporous organosilica (AgxNPs/Ur-PMO) for antibacterial and cell viability assay .Aqueous Euphorbia leaves were used in the synthesis of (AgxNPs/Ur-PMO)as it serves as a good reducing agent . The various characterization techniques such as XRD, SEM,

TEM, and FT-IR helped in gathering information about the physical and chemical properties of organosilica produced . An in vitro cytotoxicity test on NIH-3T3 15 cells showed that AgxNPs/Ur-PMOs have good biocompatibility. This work is expected to play an important role in biomedical applications. The use of these compounds in hospital environments can reduce nosocomial infections as well as reduce antibiotic-resistant bacter.[20]

Donya Jamshidi et al synthesized a novel bio composite chitosan/graphite based on zinc-grafted mesoporous silica nanoparticles (CGZM-bio).The antibacterial activities of this compound along with that of Zn-MSN nanoparticles Was also studied. The colloidal solutions of the Zn-MSN nanoparticles and CGZM exhibited the antibacterial activity against gram-positive (*S. aureus*) and gram-negative (*E. coli*) microbes.[21]

Kai Sun et al worked to create a novel approach to gradually promote production of ROS and combine with chemotherapy for enhancing anticancer efficacy. ROS(Reactive oxygen species)-induced cell death has been a wide strategy for tumor therapy .Here the synthesis of iron oxide core-shell mesoporous silica nanoparticles (Fe3O4@MSN) were done through the hydrolysis of tetraethyl orthosilicate on the surface of the Fe3O4 nanoparticles, and further conjugation was done using folate (PEG-FA) and mitochondrial targeting triphenylphosphonium (TPP) to form Fe3O4@MSN-TPP/PEG-FA.[22]

Maria Martinez et al conducted green synthesis method to obtain metallic nanoparticle using eucalipthus extract as reducing agent. New magnetic hybrid materials were developed by the encapsulation of iron oxide nanoparticles into a chitosan matrix. Well-formed iron oxide

nanoparticles were successfully synthesized. Under precise conditions, the encapsulation of the synthesized iron oxide nanoparticles in chitosan beads leads to the formation of magnetic hybrid organic/inorganic materials.[23]

Igor I et al tried to focus on the developments of a series of surface-functionalized mesoporous silica nanoparticle (MSN) materials as efficient drug delivery carriers. The progress on using MSN to penetrate various cell membranes in animal and plant cells is being discussed here. The work conclude that the MSN based systems has a significant role for a drug delivery applications.[24]

Qi Gan et al synthesized a pH responsive chitosan-functionalized MSNs dual-delivery system for targeted delivery of BMP-2 and Dex to enhance bone regeneration. Here GPTMS was used to link Chitosan and MSN. Bone morphogenetic protein-2 (BMP-2) and Dexamethasone (Dex) accelerate bone regeneration. The dual-delivery strategy for osteogenic protein delivery maintains the bioactivity and optimized release of the drug/protein.[25]

CHAPTER 3

OBJECTIVES

- Preparation of mesoporous silica nanoparticles (MSN) using ionic surfactant CTAB
- Preparation of Fe_3O_4 nanoparticles
- Preparation of chitosan coated iron oxide Fe_3O_4-CS
- Preparation of Fe_3O_4-CS-MSN nanoparticles
- Characterization of the prepared samples using FTIR and TEM
- Antibacterial and Antifungal analysis of Fe_3O_4-CS-MSN nanoparticles

CHAPTER 4

MATERIALS AND METHOD

4.1 MATERIALS

Chemicals used: -

- Cetyltrimethylammonium bromide/ cetrimonium bromide (CTAB)
- Sodium Hydroxide (NaOH)
- Ethanol (C_2H_5OH)
- Tetraethyl orthosilicate (TEOS)
- Hydrochloric Acid (HCl)
- Distilled water
- Chitosan(CS)
- Ironoxide Nanoparticles(Fe_30_4)
- Acetic acid
- Deionised Water
- Mesoporous Silica(MSN)
- (3-Glycidyloxypropyl)trimethoxysilane(GPTMS)
- Iron(II)chloridetetrahydrate $FeCl_24H_20$
- Iron(III)chloridehexahydrate $FeCl_24H_20$
- Agar
- Clotrimazole(Fungal strain)
- Candida albicans
- Methanol
- E.coli,Staphylococcus(bacterial strain)
- Amoxycillin

Apparatus used: -

- Round bottom Flask
- Conical flask
- Glass beakers
- Measuring jars
- Filter paper
- Magnetic stirrer
- Sterile Petriplate
- Incubator
- Glass rod

4.2 EXPERIMENTAL METHODS

4.21 Synthesis of MSN

To a solution containing 3.5 ml 2M NaOH, 3.5 ml ethanol and 50 ml of distilled water 1.0484g Cetyltrimethylammonium ammonium bromide (CTAB) was added . The mixture was heated up to 60ºC for 30 min with constant stirring, using magnetic stirrer with rpm of 500. To this 2ml of tetraethyl orthosilicate (TEOS) was added drop by drop for about 15 minutes and the mixture was stirred vigorously for 2hrs at 60ºC . The solution turned opaque. The white precipitate was washed several times with hot water and then with 9:1 ethanol: HCl. To eliminate the surfactant the precipitate was heated on a Bunsen burner for about 4 hrs, and then was kept in a muffle furnance for 5hrs at 550^{o}C .

(a) Stirring with heating. (b)MSN.

Fig. 1; Synthesis of MSN

4.22 Synthesis of Fe_3O_4 nanoparticles

The procedure was based on an already reported work with some modifications [13].

About 250mL solution containing 2.534g $FeCl_2.4H_2O$ and 6.488g $FeCl_3.6H_20$ was taken in a 500 ml beaker. The solution was made homogenous using magnetic stirrer. Later this solution was heated at 65^0C in a water bath for 20 minutes followed by the addition of 28 mL 25% NaOH. After the completion of reaction the colour changes from brown to black due to precipitate formation. Precipitate was filtered washed with DI and dried.

(a)Brown solution (b)Black solution (c)Fe_3O_4 NPs

Fig. 2; Synthesis of Fe_3O_4 nanoparticles

4.23 Synthesis of Fe_3O_4-CS nanoparticles

The procedure was based on an already reported work with some modifications [13].

About of 0.5080g chitosan was mixed in 200ml of DI water containing 12ml of acetic acid .To this 0.1328 g of Fe_3O_4 were added and was placed in a magnetic stirrer for 15-20hrs. During this process the black colour solution turned brown gelatinous and was collected in a sample bottle.

(a)Black solution (b) Brown solution (c) Fe_3O_4-CS

Fig. 3; Synthesis of Fe_3O_4-CS

4.24 Synthesis of Synthesis of Fe_3O_4-CS-MSN

About 20ml of above CS+Fe_3O_4 solution was mixed with 0.5ml of 20% GPTMS in ethanol solution and was kept in plastic close container and allowed to stand for atleast 24 hr . To this solution 0.2504g of MSN was added and stirred for 8 hrs. The final solution was collected in a sample bottle.

(a)Solution kept for 24hrs. (b)Stirring. (c) Fe_3O_4-CS-MSN.

Fig. 4; Synthesis of Fe_3O_4-CS-MSN

4.3 Antibacterial Assay by Agar Well Diffusion Method

20 ml of sterilized Muller Hinton Agar was poured into sterile petriplate, after solidification, 100 µl of test organism(*Escherichia coli* and *Stephylococcus sp*.)were swabbed on the respective plates. Four wells of 6 mm diameter were punched into the agar medium and filled with 100 µL of extracts (of 100 µl /mL and 50 µl /mL concentration), antibiotic solution (Amoxycillin) (positive control) and solvent blank-methanol (negative control).The plates were incubated for 24 hours at 37˚C. After incubation the diameter of inhibitory zones formed around each discs were measured in cm and recorded.

4.4 Antifungal Assay by Agar Well Diffusion Method

Antifungal activity of the test sample was performed by agar well diffusion method. The well diffusion method was performed using Potato dextrose agar. 20ml of sterile medium was poured into assay plate and allowed to solidify. Once the medium had solidify,four wells, each of 9mm in diameter were cut out of the agar, a sterile swab was used to evenly distribute the fungal strain(*C.albicans*) over the agar surface. The test volumes 50µl and 100 µl from 1 µl / ml of the sample were added to the desired wells. Clotrimazole was used as the positive control and the solvent(methanol) used for sample dilution was used as the negative control. The plates were incubated at room temperature for 24 hours. The zone of inhibition was measured.

4.5 CHARACTERISATION TECHNIQUES

4.51 FTIR ANALYSIS

The chemical composition of the synthesized mesoporous silica nanoparticles was studied by using FTIR spectrometer Perkin-Elmer LS-55- Luminescence spectrometer. FTIR works on the basis of IR spectroscopy, which involves the measurement of interaction of infrared radiation with matter by adsorption, emission or reflection. It is powerful technique to study and identify chemical substances or functional groups in a given sample. Since each different material is a unique combination of atoms, no two compounds produces the exact same IR-spectrum. IR spectroscopy exploits the fact that molecules adsorb frequencies that are characteristic of their structure. The size of the peak in the spectrum is an indication of amount of material present in the sample. The spectra was recorded from 500-4000 cm^{-1}.

4.52 HR-TEM ANALYSIS

High resolution transmission electron microscopy is a powerful analytical tool for studying particle structure, interphases,and crystal defects. A beam of electrons is transmitted through a specimen for image formation. The phase contrast imaging is the basis of image formation. Ultrathin samples are used for this analysis so as to facilitate the electrons to pass through the sample easily. The surface morphology of MSN is studied using this technique. The surface morphologies of mesoporous silica nanoparticles were probed using JOEL 3010-HR-TEM model operated at a voltage of 300 kV.

CHAPTER 5

RESULTS AND DISCUSSION

5.1. FTIR ANALYSIS

The FTIR spectra of pure CTAB, MSN synthesised using CTAB as surfactant , is showed in Fig.5and 6. Two bands appeared at around 1080 cm^{-1} and 799 cm^{-1} in all samples corresponding to the asymmetric and symmetric stretching vibration of Si-O-Si respectively. The band observed around 960 cm^{-1} was due to surface Si-OH groups. The peaks at 3012cm^{-1},2914cm^{-1} & 2848cm^{-1} which are characteristic peaks of CTAB, disappeared in the spectra of MSN, which shows that the surfactant has been effectively removed from MSN.

From the FTIR spectrum of Fe_3O_4(fig.7),the peak at 466cm^{-1} corresponds to Fe–O which confirmed that the synthesized particles are iron oxide .The peaks positioned at 1063 cm^{-1} and 2345 cm^{-1} appeared due to absorption of moisture by nanoparticles from the environment and O–H stretching mode .

From the FTIR spectrum of chitosan (CS)(fig.8),the peak as 3445cm^{-1} corresponds to N–H stretching vibration, 1654cm^{-1} corresponds to NH_2 group bend scissoring,1373cm^{-1} and 1085 cm^{-1} show C–O stretching vibration.

All the characteristic peaks of CS and Fe_3O_4 were present in the spectrum of Fe–CS (fig.9) which indicates that the iron oxide nanoparticles were successfully coated with CS.

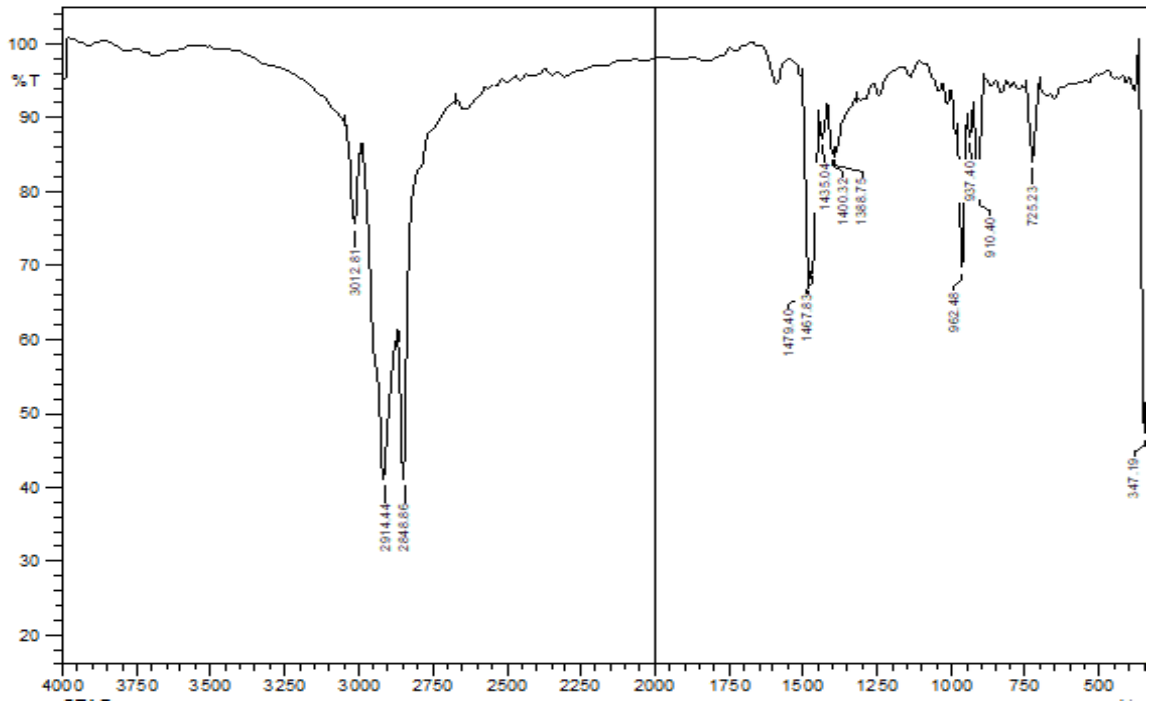

Fig. 5; FTIR of CTAB

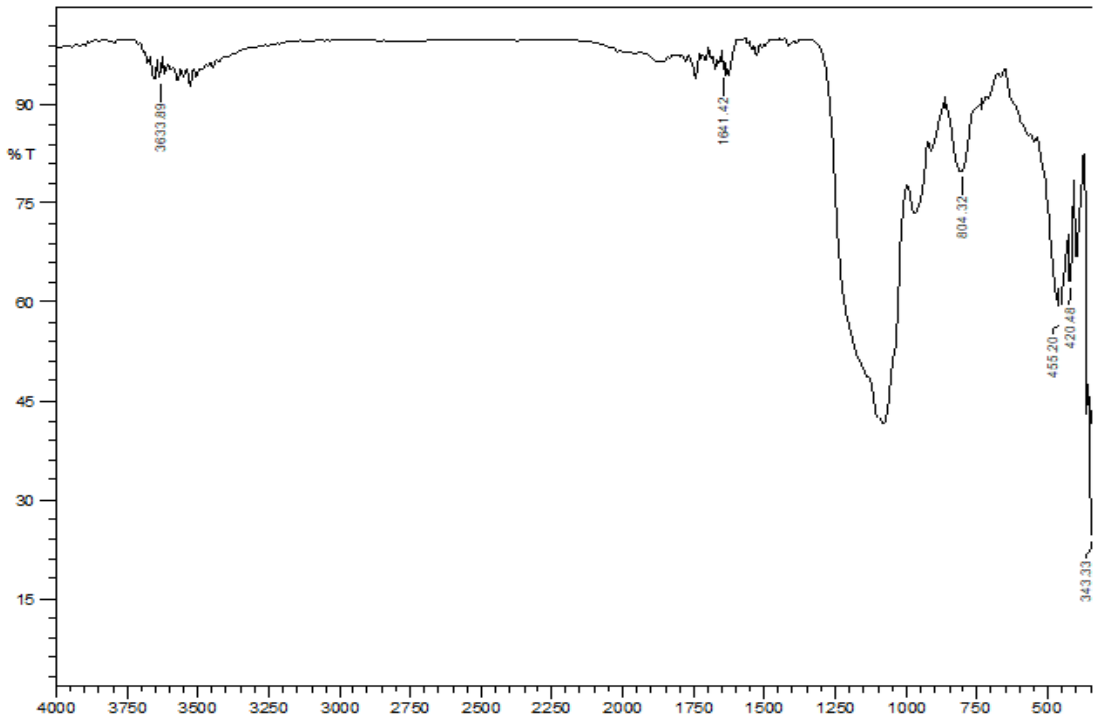

Fig. 6; FTIR of MSN synthesized using CTAB surfactant

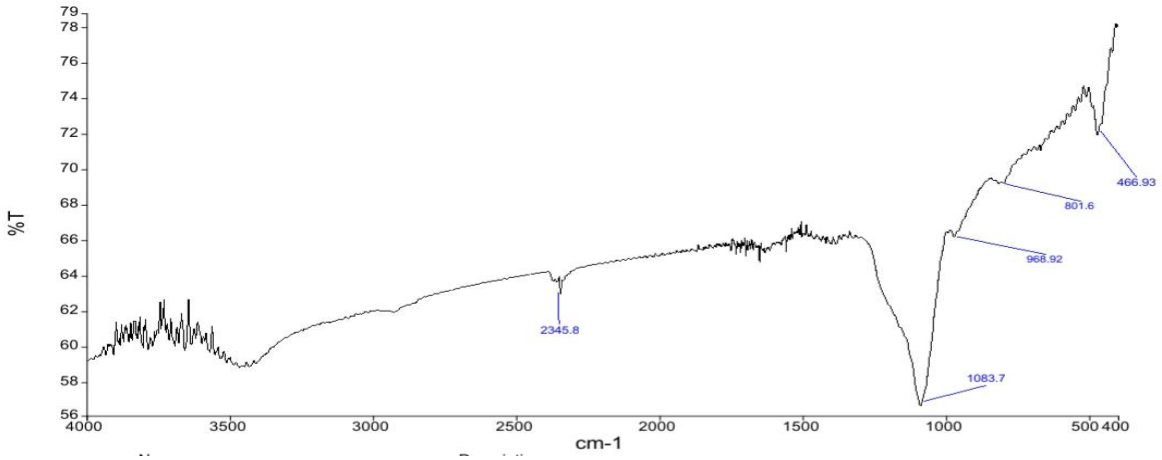

Fig. 7; FTIR of Fe_3O_4

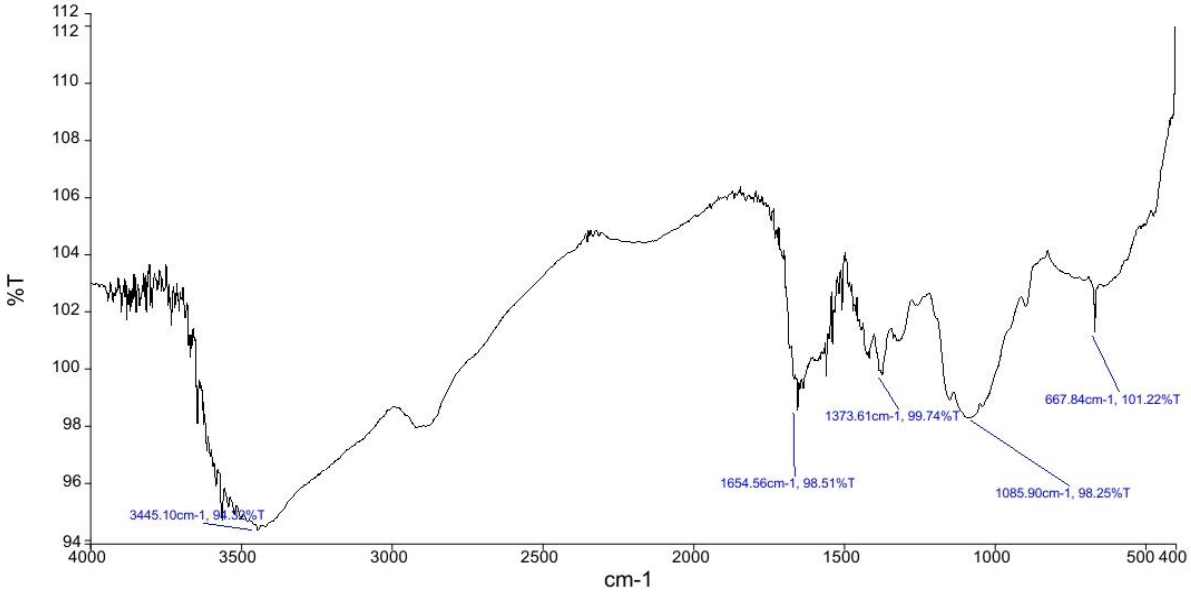

Fig. 8; FTIR of CS

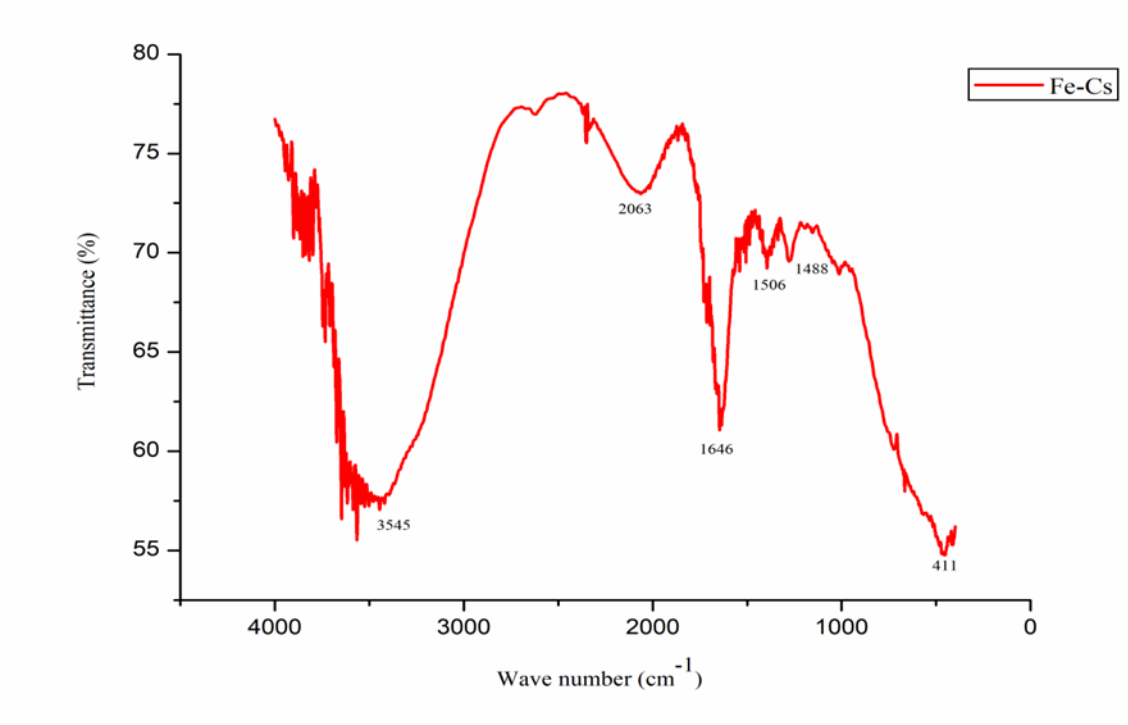

Fig. 9; FTIR of Fe_3O_4-CS

5.2 HR-TEM ANALYSIS

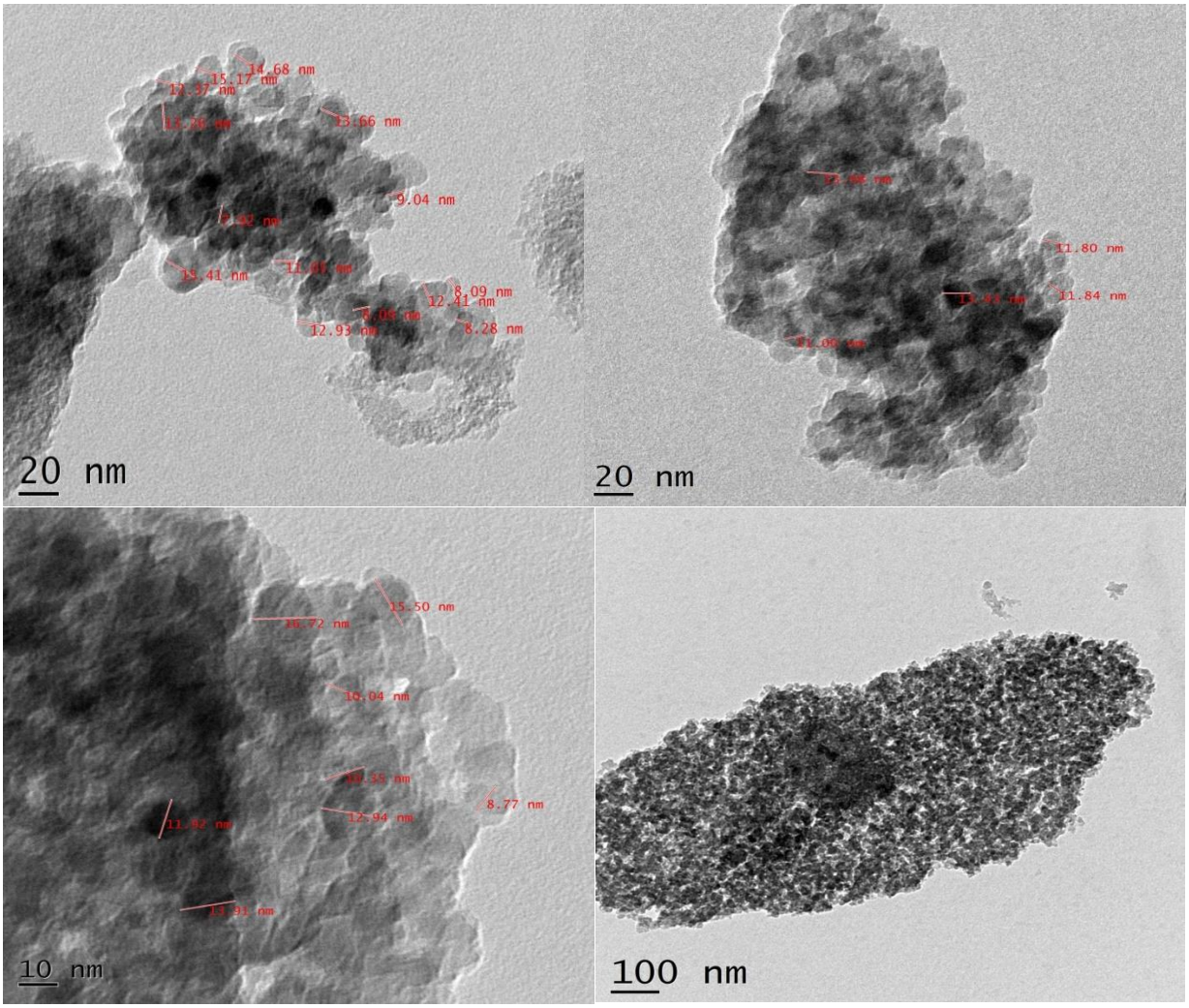

Fig. 10; HR-TEM images of MSN.

HR-TEM analysis was done to study the surface morphology of MSN. The HR- TEM images indicate a round grain like structure for the silica particles. It appears like stacking of several round grain like structures together. From these images small pore formation in the synthesized silica particles can be observed and the pore size confirms the mesoporous nature

5.3 ANTIBACTERIAL AND ANTIFUNGAL ANALYSIS

The antibacterial activity of Fe_3O_4-CS-MSN Solution produced was investigated using agar diffusion method against selected human pathogens such as and *Stephylococcus and Escherichia coli*. For antifungal study of of Fe_3O_4-CS-MSN *Candida albicans* was used as the pathogen. These different pathogens have also tested with commercially available antibiotic (Amoxycillin-for antibacterial study) and (Clotrimazole-for antifungal study). Antibiotic solution is the positive control and solvent blank is the negative control. The extracts used against the pathogenic organisms have showed varied degree of antibacterial and antifungal activity against the pathogens. The zone of inhibition got for 50 µl and 100 µl sample solution were not same. It was for the 100 µl sample solution we got the maximum zone of inhibition. The results were indicated in Table 1 and 2 respectively.

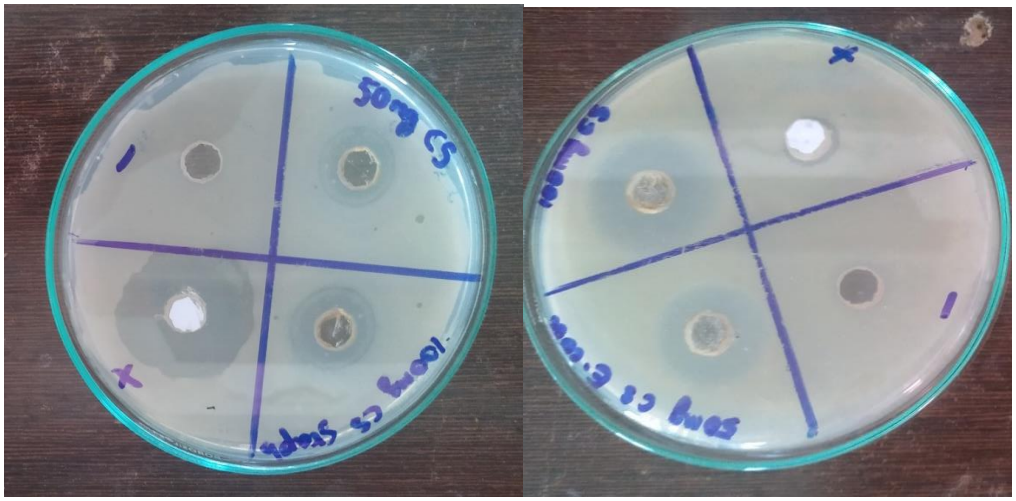

Fig. 11; Antibacterial analysis of Fe_3O_4-CS-MSN using *Stephylococcus* and *E.coli*

Sl No	Organism	Positive Control	Negative control	Test 1 (T_1) (50 µl)	Test 2 (T_2) (100 µl)
1	*Stephylococcus*	3cm	-	1.6cm	1.9cm
2	*E coli*	2.7cm	-	2.3cm	3.1cm

Table 1

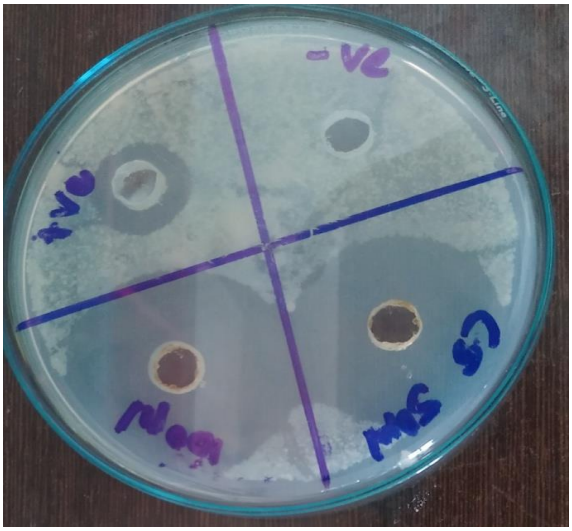

Fig. 12; Antifungal analysis Fe_3O_4-CS-MSN using *Candida albicans*

Sl No	Organism	Positive Control	Negative control	Test 1 (T_1) (50 µl)	Test 2 T_2) (100 µl)
1	*Candida.albicans*	3.3cm	-	1.6cm	1.9cm

Table 2

CHAPTER 6

CONCLUSION

This study reports the synthesis and antimicrobial study of chitosan coated iron oxide incorporated mesoporous silica nanoparticle. The synthesis of Fe_3O_4, and Fe_3O_4-CS were confirmed using FTIR analysis.The synthesis of MSN is confirmed by FTIR analysis and surface morphology studied using TEM analysis. This study also focuses on checking the antibacterial and antifungal property of synthesised Fe_3O_4-CS-MSN.The result obtained confirmed that the synthesized Fe_3O_4-CS-MSN do shows antibacterial and antifungal properties. These findings suggest that the as prepared Fe_3O_4-CS-MSN might be used as a good drug delivery agent for biomedical applications.

CHAPTER 7

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