Development of Chitosan/Graphene oxide/nano-silver Hydrogel for Photocatalytic Dye Degradation and Antibacterial Action

A

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CERTIFICATE

This is to certify that the work which is being presented in the dissertation entitled **"Development of Chitosan/Graphene oxide/nano-silver Hydrogel for Photocatalytic Dye Degradation and Antibacterial action"**, in partial fulfillment of the requirements for the award of degree of Master of Technology in Biotechnology submitted in Department of Biotechnology of Thapar University, Patiala, is an authentic record of candidate's (Ms. Tamanna Mahajan) own work carried out under the supervision of Dr. Shekhar Agnihotri and refers other researcher's work which are duly listed in the reference section. The matter embodied in this dissertation has not been submitted in part to any other university or institute for the award of any degree in India.

This is to certify that the above statement made by the candidate is correct and true to the best of my knowledge.

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DECLARATION

I hereby declare that the dissertation work entitled "**Development of Chitosan/Graphene oxide/nano-silver Hydrogel for Photocatalytic Dye Degradation and Antibacterial Action**" is an authentic record of my own project work carried out at Department of Biotechnology, Thapar University, Patiala under the guidance of **Dr. Shekhar Agnihotri**. The matter presented in this dissertation has not been submitted in part or full to any other university or institute for the award of any degree in India or Abroad.

Tamanna Mahajan

Place: Patiala

Date: 4-9-2017

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LIST OF SYMBOLS AND ABBREVIATIONS

%	percentage
µl	micro litre
Ag	Silver
Ag^+	Silver ions
$AgNO_3$	Silver nitrate
AgNP's	Silver nanoparticles
CFU	Colony forming units
CNT's	Carbon nanotubes
COD	Chemical oxygen demand
CS	Chitosan
Cu	Copper
Fe	Iron
FTIR	Fourier transform infra-red spectroscopy
gm	grams
GO	Graphene oxide
hrs.	Hours
MB	Methylene blue
mg	mili grams
ml	mililitre
mM	micromolar
NaBH4	Sodium borohydride
nm	nanometre
NP	Nanoparticle
ºC	degree centigrade
OD	Optical density
pH	Potential of hydrogen
ppm	parts per million
rpm	revolutions per minute
SEM	Scanning electron microscope
TiO_2	Titanium dioxide
UV	Ultra-violet
w/v	weight by volume
ZnO	Zinc oxide

ABSTRACT

Water pollution, due to human activities has emerged as one of the biggest challenge in 21st century creating an inevitable hazard for environment and human health. Majority of water contamination occurs due to the discharge of industrial waste from textile, leather tannery, paper and pulp containing a copious amount of colored dye which is released directly into the water resources creating an ecological imbalance. Being complex in nature, the dye molecules are difficult to degrade and therefore persist over a long duration in water systems resulting in severe health hazards. It is unfortunate that the existing treatments (physical, chemical, and biological) or technologies have few limitations for being universally employed in water treatment purposes, which in turn restrict their applicability for complete removal of dye pollutants from water sources. Therefore, there is an utmost need to develop some effective, cheap and eco-friendly methods for dye removal and minimizing the ecotoxicological implications.

In recent past, nano-driven technologies have provided a few promising solutions for using nanomaterials in water purification applications and enhancing the environmental sustainability. In the present study, a biogenically-derived polymeric nanocomposite of nano-silver/chitosan/graphene oxide hydrogel was synthesized in order to evaluate its catalytic performance to degrade methylene blue (MB) as a model dye under sunlight illumination. A porous hydrogel nanocomposite with semi-interpenetrating network of chitosan (CS) and graphene oxide (GO) was fabricated, which acted as a nanoreactor for *in-situ* synthesis of silver nanoparticles (AgNPs) and their subsequent immobilization. The hydrogel nanocomposites were characterized using electron microscopy which indicated a stable formation of nanocomposite with immobilized AgNPs onto polymeric networks. The synthesized hydrogels showed remarkable swelling capacities with swelling ratio of 83.17% and 80.23% for GO-CS and GO-CS-Ag respectively. Experiment conducted under sunlight (natural source) with GO-CS hydrogel showed a 99.7% degradation of MB (initial concentration, 40 ppm) within 5 hrs. The introduction of silver into gel although delayed its degradation efficiency, a dye degradation of 98.7% was still achieved after 7 hrs of its exposure to sunlight. Modelling experiments indicated that dye removal follows second order model kinetics using these nanocomposites. The CS/GO hydrogels (pristine and Ag-

loaded) were also examined for its disinfection property against *Bacillus subtilis* (Gram- positive) and *E. coli* (Gram-negative). The antibacterial studies done under liquid culture and solid media indicated that the pure GO-CS hydrogels could not demonstrate any significant antibacterial effect. On the contrary, nano-silver immobilized GO-CS hydrogel showed an unprecedented biocidal action where a complete disinfection (intial bacterial count, 10^3-10^5 CFU/ml) was achieved even after 60 minutes and 180 minutes against *Bacillus subtilis* and *E.coli* strains respectively. This study therefore provides a novel tool to use hydrogel based system for minimizing water contamination with dual performance attributed its photocatalytic dye removal activity and antibacterial action.

CHAPTER-1

INTRODUCTION

Environmental pollution has emerged as the major problems of the moderen world today. In recent years industrialization and urbanization have grown extensively and has affected biodiversity that imposes a great threat to human existence (Singh *et al*., 2009). Among diverse array of environmental pollutions i.e. pollution of air, water, food, and soil, water is elixir of life and the most influential media so its pollution directly affects the global health. There has been an enormous increase in the demand of water in the past few decades with the use of about 70, 22 and 8% of the available fresh water in the area of agriculture, industry and household respectively. This resulted in the generation of an enormous amount of wastewater with a number of hazardous pollutants present in it (Helmer *et al*., 1997; Nemerrow, 1978). There has been a widespread awareness about the toxicity and carcinogenicity of many polluting chemicals with the expansion in industrialization (King *et al*., 1997). Pathogenic microorganisms that live in such contaminated environments cause many infections and can also be fatal to human beings. According to a report by WHO/UNICEF 2010, millions of children die every year worldwide due to diarrheal infections caused by contaminated water. Unlike the natural substances that are biodegradable, synthetic chemicals show resistant to biodegradation (Fernando and Aust 1994) and therefore many synthetic compounds like pesticides, wood preservatives, organochlorines, polycyclic aromatic hydrocarbons, man-made polymers, munitions waste and synthetic dyes are the major environment pollutants (Pointing, 2001). The complex structure of synthetic dyes is the reason behind their resistance to biodegradation.

WH Perkins discovered the use of synthetic dyes in 1956 (Kant, 2012). Many industries, such as paper, plastics, textile, foodstuffs, leather tannery and dyestuffs consume a considerable amount of water and dyes to color their products (Crini *et al*., 2008). Worldwide, the total annual production of synthetic dyes is about 10^6 tons of which about 1×10^5 - 1.5×10^5 tons are released into water bodies (Pereira *et al*., 2012). Textile sector is the chief contributor of dye effluents and the second largest polluter of clean water globally. It has been estimated by the World Bank that of the total industrial water pollution, about 20% is caused by the textile industry. About 72 toxic chemicals from textile dyeing reaches our water supply, many of which cannot

be filtered or removed (Nachiyar *et al*, 2014). After China, India is the second largest exporter of dyeing stuffs where 80% of the total dyes are used in textile industries alone (Ghaly *et al.,* 2014). As a result, an enormous amount of polluted wastewater is generated which leads to various ecological and health problems if discharged untreated.

Dyes are complex organic compounds containing an aromatic structure that brings long lasting color to other substances (Mondal, 2008). Dyes can be categorized into natural and synthetic types. Natural dyes are those which are obtained from natural materials like plants, animals and mineral matters whereas synthetic dyes are obtained from petroleum compounds (Gurses *et al.,* 2016). Dyes possess a color bearing group known as chromophore which absorb light in the visible range and give dye its color and exhibits resonance of electrons due to the presence of a conjugated system (Abrahart, 1977). The color of the dye is strongly dependent on these components and the color is lost if any of these is missing. Auxochromes, the color helpers are also present in some of the dyes along with chromophores (Zollinger, 2003). On absorption of light by dye molecule, electronic excitation takes place from a lower to a higher energy level and then releases energy in the form of photons of different frequencies which is responsible for different colors. Azo dyes have widespread applications in industries like food, leather, textile and pharmaceuticals. The characteristic property of an azo dye is the presence of N=N bond in its structure (Chudgar, 1991).

The colloidal particles, colors and oily scum present in the waste water increase its turbidity and give it a very foul smell along with a bad appearance. Scum accumulated hinders the sunlight penetration into the water which is the utmost requirement for the process of photosynthesis (Kant, 2012). Oxygen transfer mechanism is thus affected at air water interface and the dissolved oxygen in water gets depleted. Disruption of oxygen transfer is the most deleterious effect that could take place because of textile effluents and as dissolved oxygen is a requisite for marine life; its depletion can adversely affect the aquatic fauna. Apart from affecting aquatic flora and fauna , synthetic dyes also affect the water resources from aesthetic point of view since many dyes can be noticeable even at very low (>1ppm) concentrations (Ali *et al.,* 2010) . These effluents when leach into the fields can reduce the soil productivity by clogging the soil pores. The soil becomes harder in

texture and penetration of roots into the soil is not permitted. These dyes are also mutagenic, carcinogenic, and toxic to life. Dyes if ingested by the body are metabolized by beneficial intestinal micro flora which reduces these dyes into carcinogenic colorless amines (Fonovich, 2013). Severe allergic reactions such as respiratory tract infections, contact dermatitis, irritation in eyes, skin and mucous membrane can also be caused due to textile dyes. A number of cases of dye workers with kidney, liver and urinary bladder cancers have been been reported. There is an urgent challenge to eradicate these dyes from industrial wastewaters due to their carcinogenicity, mutagenicity and toxicity (Akarslan *et al*., 2015). Studies in the past have concluded that due to the complex structure of dyes, these show resistance to oxidizing agents and aerobic digestion and hence become very difficult to degrade (Crini *et al*., 2008; Wang *et al*., 2007). The use of chemicals and physical methods for water disinfection can result in the formation of disinfection bi-products which can prove to be toxic. It is very challenging for an engineer to effectively remove dyes as well as microorganisms from waste waters as some of the processes are not economical and others are not effective (Zhang *et al.*, 1995). There is no universal method to remove dye from colored waste water. Various physical, chemical and biological techniques have been used for dye removal. Some of the physical and chemical methods includes floatation and ozonation (Lin *et al*., 1993), electro floatation (Ogfitveren *et al*., 1994), anion exchange resins, precipitation, coagulation, filtration, electrolysis, photo degradation, electrochemical destruction, irradiation, adsorption and the use of activated carbon (Pala *et al*., 2002). Many biological and microbiological methods have also been employed for dye degradation as many microorganisms have been found to have the ability of removing dyes either by degradation or by bio sorption (Miao, 2005). Although all the above mentioned techniques are useful, but dye removal using these techniques ends up producing hazardous bi-products which needs further processing. In practical, no single process has the potential to effectively remove dyes and microbes from waste waters and therefore there is a need to use a series/blend of different techniques to get the desired quality of water. Adsorption using low cost adsorbents has been recognized to be an economic and effective method for water purification. In recent times, an emerging technology i.e., Nanotechnology has come up as a promising solution for catalytic degradation of dyes. Adsorption followed by catalytic degradationion of dyes and microbes using nanomaterials can prove to be an excellent approach for dye

removal. Liquid-phase adsorption is considered as the most effective method for removing pollutants from waste water owing to its advantage of being economically feasible, simple, flexible and easy operating procedures (Crini *et al.*, 2008 ; Mondal *et al*., 2008 ; Ghaedi *et al*., 2012).

Nanotechnology provides a very good opportunity for developing techniques in order to eradicate pollutants from water bodies. The inherent distinguishable properties of nanomaterials like high aspect ratio for adsorption, photosensitivity, catalytic activity and antimicrobial properties make them a very good choice to be used in waste water treatments. Various nanomaterial like TiO_2 (Rauf *et al.*, 2011) , Fe_3O_4 (Zang *et al*., 2011), ZnO_2 (Daneshvar *et al*., 2007), nano silica , nano silver (Das *et al*., 2013), graphene oxide (Liu *et al*., 2012), Carbon Nano Tubes (CNT'S) (Gupta *et al*., 2013) have the capability of removing dyes and microbes either by adsorption or by photocatalytic degradation. Metal nanoparticles have remarkable properties like high ordered crystalline structure, large surface area, large number of vacant reactive surface sites and high mechanical strength which makes them an ideal choice for eradication of toxic materials and harmful microbes (Ghaedi *et al*., 2012). Silver nanoparticles have given promising results for degrading dyes photo catalytically in visible light due to Surface Plasmon Resonance effect (Rauf *et al*., 2011) and destruction of microorganisms by releasing metal ions or reactive oxygen species which disturbs cell wall integrity. These nanoparticles act like a catalyst and facilitate a large surface area for electron transfer reaction and for bacterial contact. When catalyst is exposed to light, electrons jump from valence band to conduction band forming an electron – hole pair which then migrates to the surface of catalyst to initiate redox reaction. Reactive oxygen species such as O_2 •, HO_2 •, H_2O_2 and •OH are generated as a result of redox reaction which can then react with the dye molecules adsorbed on the catalyst surface to form other species and thus the decolorisation of the dye occurs (Rauf *et al*., 2011). But there are certain problems in using silver nanoparticles as water purification agent as these can form aggregates in the absence of any substrate material in aqueous phase which can gradually decrease their efficiency in long term use (Gupta *et al*., 1998; Morones *et al*., 2005). Also, these nanoparticles can't be loosely held in aqueous systems due to their high cost and due to the problem of being leached out in the aquatic system which can cause

adverse effects on life. Therefore, there is a need to develop an immobilization matrix for nanoparticles to form nanocomposites and make the best out of it.

The immobilization matrix can be either metal, ceramic or polymer based (Camrago *et al.*, 2009). Polymeric gels are typically soft versatile materials with extensive range of potential applications. These types of gels can be easily prepared using covalent or non-covalent bonding. Natural materials are becoming an attractive choice for hydrogel preparation due to their reusability, renewability, biocompatibility and biodegradability. Chitosan is a classic bio macromolecule derived by deacetylation of a natural biopolymer chitin (Han *et al*., 2013). It has been explored by several scientists as a bio sorbent and as an antimicrobial agent for elimination of dye molecules and microorganisms. Its economic feasibility, excellent chelation property and polyfunctionalty due to which it tightly binds the pollutants in the water makes it a very good choice for being used in water treatments (Crini *et al.*, 2008). However, chitosan hydrogels have a very poor mechanical strength in water which is a major disadvantage for a material to be used for water treatment purposes. This disadvantage can be overcome by different techniques like physical blending, chemical modifications, interpenetrating polymer networks and crosslinking methods (Agnihotri *et al*., 2012).

In current study, an immobilizing matrix of chitosan and graphene oxide for *in situ* synthesis of AgNPs was designed. The gels were then studied for their swelling ratios, porosity, mechanical strength and dye removal capacity for checking their potential in water treatments. The characterization of these gels was then done using UV- visible spectroscopy, SEM (Scanning Electron Microscopy).

Objectives of the Work

Following objectives have been designed to complete this study

1. Synthesis and characterization of graphene oxide using modified Hummer's approach
2. Development of a highly stable and cross-linked network of chitosan– graphene oxide hydrogel which would act as immobilization matrix for *in-situ* synthesis of silver nanoparticles.

3. Determination of photocatalytic removal of methylene blue (MB) as a model dye under various process parameters such as effect of catalytic dosage, pH, and contact time using Ag/Chitosan-GO hydrogel nanocomposite.
4. Evaluating the antimicrobial activity of these nanocomposites against Gram positive (*B. subtilis*) and Gram negative (*E.coli*) bacterial strains.

CHAPTER-2

REVIEW OF LITERATURE

2.1 Nanotechnology: A Brief Overview

Nanotechnology, a multidisciplinary branch of science has gained a lot of interest in research areas like chemistry, biochemistry, medical, environment remediation and material science (Joo *et al.*, 2006). According to a definition given by National Nanotechnology Initiative "Nanotechnology can be defined as the understanding and manipulation of matter at dimensions between 1 and 100 nanometers where unique phenomena enable novel applications" (Karn *et al.*, 2009). Extensive research is going on to apply nanomaterials for environment remediation, including improvement in the current manufacturing procedures to reduce pollution and creating alternative energy sources which are more economical.

Nanotechnology is not a new concept. People have been subconsciously engaged with nanotechnology and nanosized objects since ancient times without proper understanding of the reason behind their unique properties and applications (Tolochko, 2009). The art of manipulating matter at its atomic and molecular level for creation of novel properties and applications might appear to be a modern concept but craftsmen of the past have been controlling matter at most minuscule levels. There are numerous examples of ancient art works which were designed using nanocomposites. One of the famous examples is the 'Lycurgus cup' from Roman era which changes its color when light is reflected or passed through it. It is made up of a glass which contains gold and silver nanoparticles and these are distributed in such a way that the glass appears red when light passes through it but change its color from red to green in the reflected light (Schaming, 2015). People used to put silver coins in water storage vessels for water disinfection and purification purpose (Melaiye *et al*., 2005). Another example goes back to ancient Egypt where dying hair in black was a common practice. They used a paste of lime, lead oxide and water to dye their hair black. The Egyptians were able to form lead sulfide nanoparticles (galenite) by reacting dye paste with sulfur and obtained a uniform, long-lasting hair color (Tolochko, 2009).

Richard Feynman gave a visionary talk "*There's Plenty of Room at the Bottom*", in 1959 and introduced the concept of ‘‘nanoscience’’ in it (Feynman 1960). The word ‘‘nanotechnology’’ was introduced by Norio Taniguchi in 1974 and defined it as the ‘‘production technology which helps to obtain ultra-small sizes of approximately 1 nm with an ultrahigh precision’’ (Taniguchi 1974). Development of nanotechnology took a pace after the invention of Scanning Tunneling Microscope in 1981 and Atomic Force Microscope in 1986. These tools opened the doors of ‘‘nanoworld’’ for scientists by revolutionizing imaging system which makes the manipulation of objects easier (Schaming *et al*., 2015).

2.2 Nanotechnology in Water Treatment

Nanotechnology proves to be an effective, efficient and economical solution for achieving water purification which has been contaminated by organic and inorganic matter, toxic metals and pathogenic microorganisms flown into the water streams by the industrial and domestic wastes (Theron *et al*., 2008). Variety of nanomaterials like nanosorbents and nanocatalysts are being employed for water purification. The use of nanomaterials has an advantage over conventional materials as the former has larger aspect ratio as compared to the bulk material .This distinctive property makes them a very good adsorbent and catalyst as adsorption is a surface phenomenon and catalytic activity is also increased due to their increased surface area. This property also explains their antibacterial activity, high surface area of nanoparticles make them more interactive with bacteria and kill them. Also, the adsorption capacity of nanosorbents is very high & specific and increase in the catalytic activity of nanocatalysts increases the reactivity and accelerates contaminant degradation. Examples of commonly used nanocatalysts are semiconductor and metallic nanoparticles like titanium, silver, gold, copper etc. which has been employed for the degradation of contaminants like halogenated herbicides , organochlorine pesticides, azo dyes, polychlorinated biphenyls and nitro aromatics (Qu *et al., 2013*).

2.2.1 Nanomaterials as Adsorbents for Dye Removal

Amongst various techniques of water purification, adsorption is the most commonly employed technique as it is cheap, easy to handle, can be utilized for elimination of various pollutants and it also produces a high-quality treated effluent (Jain *et al*., 2003). Conventional adsorbents for water purification include materials like

molecular sieve carbon, silica gel, alumina, activated carbon and polymeric adsorbents. US Environmental Protection Agency has cited activated carbon as one of the best control technologies but its extensive application is constrained due to economical reasons (Derbyshire *et al*., 2001). Numerous researches are being carried out to develop cheap and effective adsorbents in order to cut down the cost of water purification. Many researchers have proposed non-conventional inexpensive adsorbents such as industrial waste products like waste carbon slurries, agricultural waste products like coconut shell, rice husk, maize cob and bagasse pith, clay, silica, fly ash, biosorbents like chitosan (Crini *et al*., 2006). However, the saturation of surface area or active sites, lack of specificity limits the efficiency of all these adsorbents. Nano-adsorbents overcome all these limitations and offer substantial improvement by providing extremely high specific surface area, short intraparticle diffusion distance and large number of adsorption sites (Qu *et al*., 2013). Extensive research is now going on to use nanomaterials like chitosan, graphite, graphene oxide, reduced grapheme oxide, carbon nanotubes for water purification.

Chitosan Biopolymer

Chitosan is formed by deacetylation of chitin which is a natural biopolymer. (Dutta *et al.,* 2004). Factors like biocompatibility, low cost and non-toxic nature makes chitosan widely popular in application areas like environment remediation, food technology and medical fields. In food technology, chitosan has been used to make edible coating to keep food products fresh and to increase their shelf life. In medical field, the antimicrobial and film forming ability of chitosan is used to fabricate wound dressings and scaffolds for bone and tissue engineering (Goy *et al*., 2009). Its use as a biosorbent in water treatment is justified by two important reasons. One being its low price in comparison to activated carbon and other is its polyionic nature contributed by numerous functional groups present in its structure which gives it an outstanding chelation property and the ability to tightly bind pollutants like dyes and metal ions (Crini *et al*., 2008, Vakili *et al*., 2014). Dotto *et al*., (2011) investigated the dye adsorption capacity of acid blue 9 and food yellow 3 by chitosan obtained from shrimp wastes. Adsorption capacity was calculated to be 210 mg/g for acid blue and 295 mg/g for food yellow. The effect of stirring rate, contact time and pH was checked and results depicted an increase in adsorption capacity with increasing time and decreasing pH. A study conducted by Annadura (2002) investigated the effect of

factors such as dosage, temperature, pH and particle size on adsorption capacity of methylene blue by chitosan. Adsorption increased with increase in temperature from 30°C to 60°C, increasing pH from 6.7 to 9.5 and on decreasing particle size from 1 .651 mm to 0 .177 mm. Maximum dye adsorption occurred at 60°C (29 .9 mg/g); at pH 9 .5 (29 .8 mg/g) and with particle size of 0 .177 mm (30 mg/g).

Graphene oxide

Graphene Oxide is the oxidized form of graphene, commonly synthesized by chemical oxidation of graphite. Oxidation process results into graphene sheets which contain carboxylic acid groups at their edges and hydroxyl and epoxy groups in their basal planes (Ramesha *et al*., 2011). It has an arrangement of sp^2 carbon atoms in its layer of two dimensional honeycomb structures and has many unique properties such as flat and large surface area, very good mechanical strength and non-toxicity (Hosseinabadi *et al*., 2015). Potential of graphene oxide as an adsorbent of environment pollutants such as dyes and metal ions is due to their and strong $\pi-\pi$ interaction on the surface and ultra large surface area (Liu *et al*., 2012). Hosseinabadi *et al*., 2015 prepared GO using modified Hummers method and checked the dye adsorption capacity of graphene oxide against Basic blue, basic red 46 and basic red 18 dyes. He concluded that dye adsorption on graphene oxide followed Langmuir adsorption isotherm and showed pseudo-second-order kinetics. Dye removal increases as the dosage of adsorbent increases with maximum adsorption of 89% with 0.01g of GO for BB41; 94% adsorption with 0.02g of GO for BR18 and 81% with 0.03g of GO for BR46. The increase in adsorption and dye removal with increasing amount of adsorbent may be due to an increase in adsorbent surface area which provides more adsorption sites. Liu *et al*., 2012 used a three-dimensional graphene oxide sponge generated from a GO suspension to remove both the methylene blue (MB) and methyl violet (MV) dyes. The effect of factors like contact time, stirring speed, temperature and pH on speed and efficacy of dye adsorption by graphene oxide was investigated. The study showed 99.1% removal of MB and 98.8% removal of MV within 2 min of the reaction. An adsorption capacity of 397 and 467 mg/g for MB and MV dye respectively was displayed by three dimensional GO sponges and adsorption process of MB and MV showed pseudo-second order kinetic model. Yang *et al*., (2011) also investigated the adsorption capacity of graphene oxide (GO) methylene blue (MB) removal from its aqueous solution and concluded that at concentrations lower than

250 ppm, dye adsorption is very fast and efficient with 99% of dye removal. Removal process gives best results at higher pH and low temperature showing a huge absorption capacity of 714 mg/g.

Carbon Nanotubes

CNTs consist of sheets of graphite or graphene oxide rolled up into a cylindrical shape which exhibits a special sidewall curvature and possesses a π-conjugative structure with a highly hydrophobic surface. Due to their properties like well-defined hollow and layered structure, hydrophobic wall, high porosity, large surface area easily modified surfaces, carbon nanotubes are evolving as very good adsorbents. The hexagonal arrangements of carbon atoms in sheets of CNT allow them to interact with other molecules through π–π electronic and hydrophobic interaction (Gupta *et al*., 2013).

Mishra et al. (2010) used functionalized multi walled carbon nanotubes for the adsorption of three azoic dyes namely Congo red, golden yellow and reactive green HE4BD. Adsorption capacity was maximum in case of Congo red (148mg/g) followed by reactive green (152 mg/g) and golden yellow (141 mg/g). In a work by Madrakian et al. (2011) magnetically-modified multi-walled carbon nanotubes were used for removal of four cationic dyes crystal violet, thionine, methylene blue and janus green B from water samples. The effect of pH was studied and it was concluded that the adsorption capacity for all cationic dye chosen was maximum at pH 7.0. The data for dye adsorption was analyzed by the Langmuir adsorption model and maximum adsorption capacity was obtained for JG, CV, MB and TH dyes as 250.0, 227.7, 48.1 and 36.4 mg/g, respectively.

2.2.2 Nanoparticles for Photocatalytic Degradation of Dyes

Advanced oxidation processes (AOPs), including photo-catalytic degradation of dyes using semiconductor and metal nanoparticles is considered as an efficient treatment for dye pollution. This method uses sunlight in presence of a catalyst to speed up the process of removal of environmental and aquatic organic contaminant and for destruction of highly toxic molecules. When catalyst is irradiated with UV or visible light, it catalyzes redox reaction in presence of air or water. The type of radiation to be irradiated depends upon the type of catalyst which is to be used for remediation (Ajmal *et al*., 2014).

Nanoparticles are gaining importance for waste water treatment via. photocatalytic mechanism due to its superiority over conventional techniques such as quick oxidation of pollutants and no formation of polycyclic products (Bhakya *et al*., 2015). It is advantageous over other methods as it includes low temperature, radically low level of energy consumption and fewer expenses which make it feasible to be used at commercial scales (Ehrampoosh *et al*., 2011). Various oxides including TiO_2, ZnO and others have engrossed attentions for photocatalytic degradation of dyes among which TiO_2 is of great interest because of its low cost and high stability. However, TiO_2 has a comparatively large energy band-gap due to which it absorbs light only in UV region. This is a big drawback in its application of being used as a photo catalyst as visible light contribute to 50% of the total sunlight whereas UV light contributes to only less than 10% of total solar energy. In order to overcome this limitation scientists have carried out many researches to increase the catalytic activity by incorporation of metal particles like gold, silver and iron to expand the range of solar energy absorption. Nanoparticles of some transition metals can be used as an alternative catalyst in place of semiconductors for dye degradation. The band gap energy for electronic excitation lies in visible region in case of metals which allows redox reaction and thus photo catalysis in presence of visible light and makes the degradation process very efficient (Bhakya *et al*., 2015).

General Mechanism for Photocatalytic Degradation

Various mechanisms have been proposed for photocatalytic degradation of dyes using nanomaterials. Most of the studies claim that the photocatalytic effect of nanomaterials is attributed to the generation of reactive oxygen species (ROS). As per the hypothesized mechanism, when light is irradiated over nanocatalysts, the electrons in the valence band get excited to the conduction band by absorbing energy. This shifting of electron gives an extra electron in conduction band creating a hole in valence band and hus creates an electron-hole pair which is responsible for redox reaction (Fig 1). The reaction mechanism is as follows:

$$\textbf{Catalyst} + h\nu \longrightarrow e^- + h^+$$

As a result of this excitation, valence band gains a positive potential which generate hydroxyl free radicals at catalyst surface and the conduction band gains negative potential due to extra electrons which is high enough to reduce molecular oxygen.

$$e^- + O_{2\,(ads)} \longrightarrow {}^{\bullet}O_{2\,(ads)}{}^-$$

$$e^- + H_{(ads)}{}^+ \longrightarrow {}^{\bullet}H_{(ads)}$$

$$h^+ + OH_{(ads)}{}^- \longrightarrow {}^{\bullet}OH_{(ads)} \quad \text{(in alkaline solutions)}$$

$$h_{VB}{}^+ + H_2O_{(ads)} \longrightarrow H^+ + {}^{\bullet}OH_{(ads)} \quad \text{(in neutral solutions)}$$

Hydroxyl free radical acts as an oxidizing agent and can attack the organic matters like dye present at the catalyst surface. It also attacks the bioresistant toxic compounds and degrades them into harmless non-toxic products like CO_2, H_2O. This decomposition reaction is explained by the following: (Khataee *et al*., 2010)

$$h_{VB}{}^+ + OM \longrightarrow OM^{\bullet +} \longrightarrow \textbf{oxidation of OM}$$

$$OH_{(ads)} + OM \longrightarrow \textbf{degradation of OM}$$

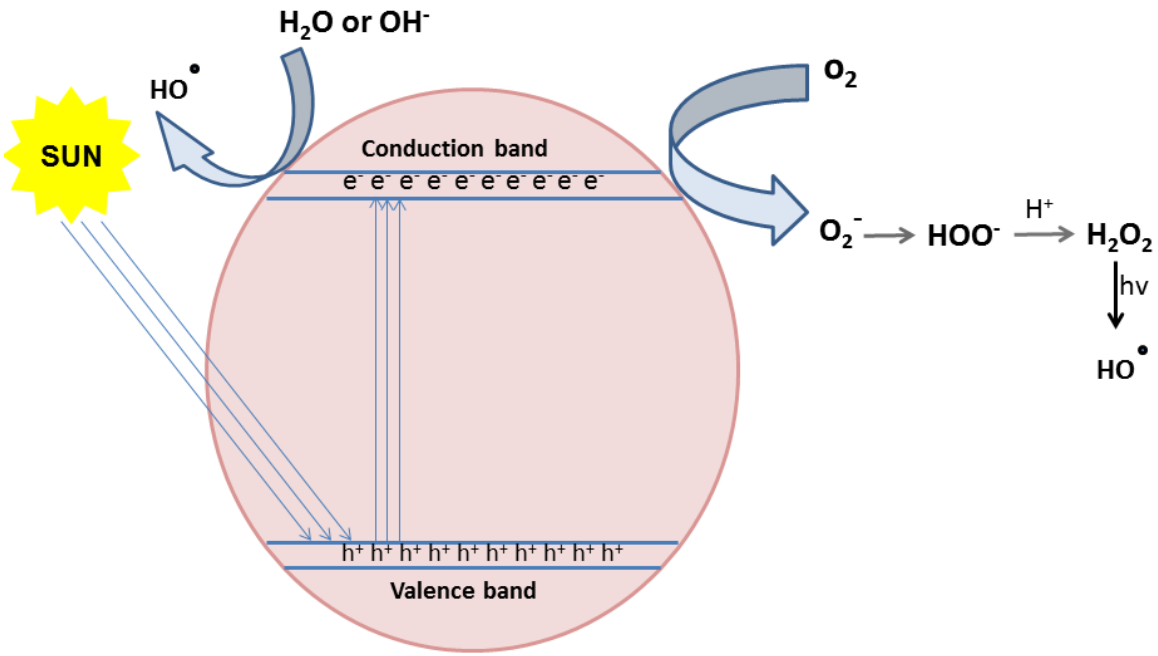


Fig 1: General mechanism for photocatalytic degradation dyes

Titanium dioxide (TiO_2)

In recent years TiO_2 based photodegradation of dyes have gained great interest owing to its ability to completely remove organic pollutants from wastewater and its various other merits such as low-cost, non-toxicity and chemical stability (Rauf *et al*., 2011). Many studies have been carried out to use TiO_2 along with dopants like Zn and Cu to enhance photo catalytic effects of TiO_2 nanoparticles in photo degradation. Ehrampoosh et al. (2011) examined the degradation of methylene blue solution in a tubular quartz reactor using varying concentrations of TiO_2 nano-particles and the reactor was irradiated with ultraviolet C with a constant intensity of 2.8 mW/cm^2. Different dosage of catalyst ranging from 0.3g/L to 1.2 g/L was used to see the effect of catalytic dosage on dye degradation. Effect of different pH conditions ranging from 3 to 9 and different dye concentration ranging from 15 to 60 mg/L was examined. The results showed that TiO_2 irradiated with UV light is very efficient in removal of methylene blue. Best results were shown when 15mg/L of dye solution was treated at pH 7 and removes about 98% of dye in less than 2hrs. In an experiment performed by Rashed et al. (2007) the effect of three sources of irradiation Halogen lamp, fluorescent lamp, and sunlight for TiO_2 mediated photocatalytic degradation of methyl orange was evaluated. Different concentrations of dyes were used and it was analyzed that degradation was maximum in case of initial concentrations of methyl orange. Among the three irradiation sources used sunlight gave the best results during decolorisation of methyl orange. Decolorisation of dye was irreversible and the reaction followed pseudo-first order kinetics.

Zinc Oxide

ZnO has come up as another promising semiconductor for photocatalytic degradation of dyes. It has a wide energy band gap in ultraviolet region of spectrum and also a large free-excitation binding energy due to which the excitonic emission can take place even at room temperature (Zhai *et al*). These have gained importance due to their abundant availability, nontoxic nature, ability to operate at high temperatures and in harsh conditions, diverse processing technologies and are an environmental friendly semiconductor. It is suggested for water treatment because of its large surface-to-volume ratio (Jin *et al*., 2008). Chakrabarti et al. (2004) explored the efficiency of ZnO as a catalyst for photo degradation of two dyes Eosin Yellow and Methylene Blue in a batch reactor with illumination of a 16 W lamp. Results showed

that ZnO was very effective in photocatalytic degradation of dyes from aqueous solution. Along with the removal of color, a considerable reduction in Chemical oxygen demand (COD) depicted the partial oxidation of dissolved organics matter by ZnO nanoparticles. The dye degradation rate equation fits into Langmuir–Hinshelwood model and it followed pseudo-first order kinetics. The effects of factors like, catalyst dosage, concentration of dye, intensity of UV-radiation and pH of the water sample were also checked. It was analyzed that with increase in catalytic dosage an increase in the degradation capacity was achieved. Dye degradation showed best results at low concentration of dyes and Methylene Blue was degraded faster as compared to Eosin Yellow. When catalyst was recycled for one more time, its efficiency was reduced to 58.9% as compared to the previously used catalyst. Nagaraja et al. (2012) synthesized nano crystalline ZnO powder (size 12-50 nm) by solution combustion method to carry out the photo degradation of Rhodamine B (RB) model dye. The study aimed to check the effect of factors like crystallite size, concentration of the dye, amount of catalyst and pH on photocatalytic dye degradation. The results revealed that more than 95% of dye decolorisation occurred within 8 min when the solution was continuously stirred under sunlight at basic pH. Along with color degradation, chemical oxygen demand (COD) was also decreased which concluded that ZnO is a very good photo catalyst for water treatment.

Metallic Nanomaterials

Metal nanomaterials are flexible materials that have wide range of applications in environmental remediation, energy production, water treatment, medical science and personal care products (Jyoti *et al.,* 2016). Metal nanoparticles possess some unique optical properties due to which they have a strong absorbance band in the visible region, which is not the case for bulk metals. The presence of this absorbance band in visible region of the spectrum is due to the Surface Plasmon Resonance effect i.e. oscillation of conduction electrons. Due to Surface Plasmon Resonance, metals nanoparticles, particularly of gold, silver and copper have received significant interest. Secondly, metal nanoparticles have very large surface to volume ratio due to which they are found as promising adsorbents and catalysts as both adsorption and photocatalysis are surface phenomena and increases with an increase in the surface area of the adsorbent and catalyst respectively. Many studies have been carried out for degradation of harmful dyes with metal nanoparticles owing to their unique

physiochemical and electronic properties which are absent in bulk metals. Some metal nanoparticles like that of gold and silver have shown remarkably active and selective catalysis for several important reduction reactions (Jyoti *et al*., 2016). Nadaf et al. (2016) described a greener approach for synthesizing gold nanoparticles extracellularly using *Bacillus marisflavi* YCIS MN 5. Gold nanoparticles were synthesized by addition of gold chloride solution into a cell-free extract of *B. marisflavi* at room temperature within 96 h. Gold nanoparticles thus synthesized were checked for their ability to catalytically reduce two organic dyes Congo red and methylene blue. Results of the study concluded that gold nanoparticles were very efficient in degradation of dyes in aqueous solution and degraded about 88% of methylene blue within 2 minutes and 98% of Congo red within 20 min. Dye degradation followed pseudo-first order kinetics with a rate constant of 0.2192 and 0.2484 min^{-1}.

Though Gold and Silver both act as very good catalyst for photocatalytic degradation of dyes and a lot of literature is available supporting their role in catalytic application but silver is chosen over gold as it is less expensive as compared to gold and cut the cost of the reaction.

Silver Nanoparticles

Extensive researches are being carried out to evaluate AgNPs for their photocatalytic ability to reduce dyes from waste water. AgNPs are a material of choice due to their advantages of having high surface to volume ratio, cost effective, non-toxic, and also it is a novel way of treatment of several dye pollutants. A study carried out by Jyoti et al. (2016) analyzed the degradation capacity of silver nanoparticles to reduce safranine orange and Methyl red. A decrease in the dye concentration was observed by a decrease in UV peak intensity within 24 h of incubation. Dye degradation rate constant of safranine orange and Methyl red was calculated to be 1.02×10^{-3} min^{-1} and 1.03×10^{-3} min^{-1} respectively. The literature research in catalysis field reveals that catalytic activity is dependent on the shape, size, structure and morphology of nanoparticles. Giri et al. (2016) used a green chemical method by mixing tannic acid with silver nitrate and then stirring the mixture for 1 minute at room temperature for synthesizing silver nanoparticles (AgNPs). Synthesized AgNPs were then used to degrade Disperse Orange 3 in the presence of sodium borohydride. Dye degradation

was analyzed using sodium borohydride, silver nanoparticles and a mixture of silver nanoparticles & sodium borohydride. The results monitored using spectrophotometer showed silver nanoparticles alone were found to be very slow but the addition of $NaBH_4$ in silver nanoparticles significantly increased the degradation rate indicating the catalytic behavior of silver nanoparticles.

2.3 Multi-functional Nanomaterials

The use of silver nanoparticles, graphene oxide and chitosan has been witnessed in several studies for their intended role of either being acted as a photocatalyst, an adsorbent or an antimicrobial material. However, to the best of our knowledge, a very few literature has been reported which explored their multiple functionalities altogether. It has been stated earlier that graphene oxide and chitosan are very good candidates for being used as adsorbents for dye removal and silver act as a photocatalyst for dye degradation. Apart from their above mentioned applications these nanomaterials can also be used as antimicrobial agents for water disinfection.

Both chitosan and chitin have shown antimicrobial activity against a wide range of organisms like bacteria, algae, yeasts and fungi when interacted *in vivo* and *in vitro* with different forms of chitosan like that of solutions, films and composites (Goy *et al*., 2009). The antibacterial activity of chitosan has been explained by various proposed models, the most acceptable of which was investigated by Raafat et al. (2008) which states that the positive charge of the polycationic chitosan interacts with negative charge of microbial cell membranes. This electrostatic interaction causes changes in the cell membrane permeability which leads to internal osmotic imbalance and also causes the hydrolysis of the peptidoglycans in the cell wall, as a result of which intracellular electrolytes leak out and this inhibits the growth of microorganisms. Raafat et al. (2008) observed changes in the structure of *S. simulans* cells under transmission electron microscope when these cells were reacted with chitosan. It was observed that "vacuole-like" structures are created beneath the cell wall due to local detachment of cell membrane from the cell wall at the points of attachment of chitosan. This results in the efflux of electrolytes from cell causing a decrease in the internal bacteria pressure. Zheng *et* al. (2003) conducted a study to see the antimicrobial effect of different concentrations and molecular weights of chitosan on *E. coli* and *Staphylococcus aureus*. In this study the MW of chitosan was kept below 305 KDa. It was analyzed that increase in the concentration of chitosan

increases antimicrobial effect and microbial inhibition reaches to 100% when the concentration is increased to 1% for both *E. coli* and *S. aureus.* But the antimicrobial effect showed different behaviors with different molecular weights in case of *E. coli* and *S. aureus a*s antimicrobial activity increases with increasing molecular weight of chitosan in case of *S. aureus* but in case of *E. coli* the decrease in MW of chitosan increased the antimicrobial effect. Zheng *et al* proposed that the main reason could be that in case of *S. aureus* which is a gram positive bacteria, chitosan of higher MW forms a film around the bacteria and inhibits nutrient adsorptions whereas low MW chitosan easily enters into the microbial cell of *E.coli*, gram negative bacteria and disturbs cell metabolism.

In recent few years, graphene oxide (GO) nanowalls have been reported to exhibit strong antibacterial activity against both Gram-positive as well as Gram-negative bacteria. The mechanism supporting their antimicrobial activity could be that wide range of aggregated graphene oxide sheets may intertwine bacterial and fungal spores which results into the local perturbation of their cell membrane. Inactivation of the cells is thus caused due to the electrolyte leakage from fungal spores and decrease in membrane potential of bacteria. This means that GO acts as an antimicrobial by mechanically wrapping the cells and damaging them locally which leads to lysis of cell ultimately leading to its death (Chen *et al*., 2014). A study conducted by Liu et al. (2011) was aimed to compare the effects of four different graphene based materials as antibacterial agents. The four material chosen for the study were graphite, graphite oxide, graphene oxide and reduced graphene oxide. Test was conducted against *E. coli* and results showed that graphene oxide dispersion with concentration of 40 mg/mL had the best antibacterial activity among four with 89.7% of viable cell reduction. A particular investigation carried by Chen et al. (2014) reveals the toxic effect of GO against two bacterial species *X. campestris pv. undulosa* and *P. syringae* and two fungal species *F. oxysporum* and *F. graminearum*. Results proved that GO is a powerful antimicrobial agent as it was effective against all the four pathogenic strains. It killed about 90% of the bacteria and 80% of fungus when 500mg/L of GO was used.. Bykkam et al. (2013) used modified Hummers approach to synthesize graphene oxide and checked its antibacterial activity against two bacterial species *Klebseilla* and *Staphylococcus.* The antimicrobial efficiency of graphene oxide was tested by the analysis of zone of inhibition which appeared after an incubation of 24

hours. All these experiments proved that graphene oxide has a powerful antimicrobial effect.

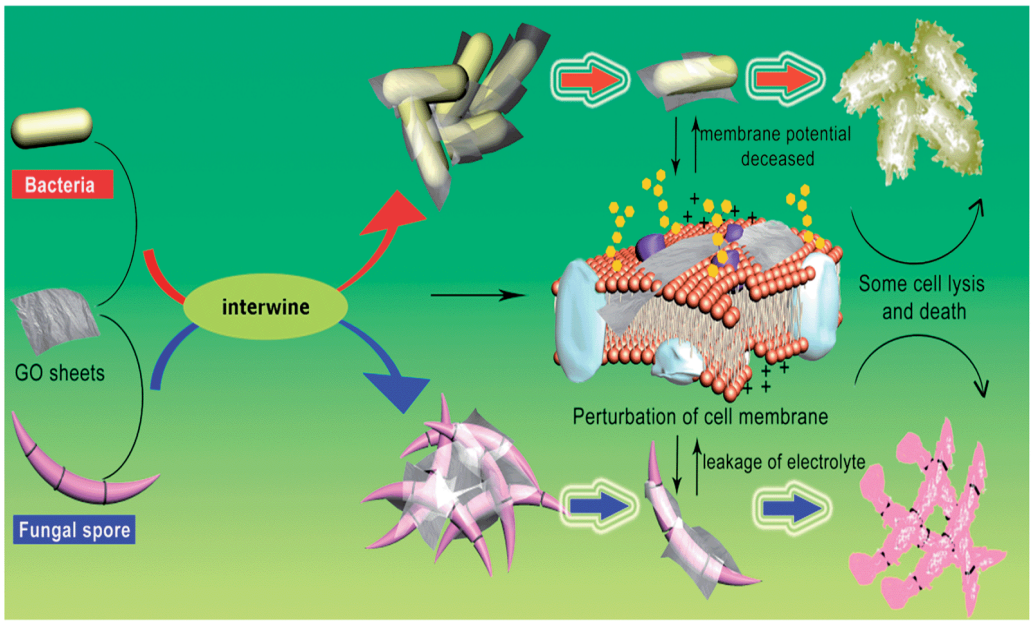


Fig 2: Mechanism describing the antibacterial activity of graphene oxide (Chen et al. 2014)

Silver has been used in the field of human healthcare and medicine since ancient times. Though, it does not have any nutritional value in body but human body contains about 2.3 µg/l of silver. There are numerous examples from past where silver was used as an antimicrobial agent. Vessels made of metallic silver were used as a disinfectant for storage and purification of water in ancient times. Alexander the Great (335 BC) used to drank and store water in vessels made up of silver during his journey for many campaigns. Silver has also been used for storing water in Apollo spacecraft, the MIR space station and even in the NASA space shuttle. It was used as an antiseptic for preventing post-surgical infections, is extensively used in dentistry and other medical devices. Silver nitrate was used for treatment of various infectious diseases long before the concept of disease causing microorganisms came into picture (Melaiye *et al*., 2005). Silver nanoparticles have been exploited for various applications like environmental remediation and in medical science due to their unique properties. Among most of the medical applications, its antimicrobial property is majorly explored (Prabhu *et al*., 2012).

Several models explain the mechanism behind the antimicrobial action of silver nanoparticles. One of the model states that silver nanoparticles are capable of adhering to bacterial cell wall and then gradually penetrates inside the wall, accumulates and form 'pits' on the cell surface (Sondi *et al*., 2004). This leads to distortion in the cell wall and its permeability and thus cell death. Another mechanism could be the free radical formation silver nanoparticles on coming in contact with the bacteria, which are responsible for making the cell membrane porous and ultimately leads to the death of the cells (Kim *et al*., 2007). Another model states that nanoparticles can release silver ions when in contact with bacteria which then react with the thiol group of enzymes and inactivates them (Matsumura *et al*., 2003, Feng *et al*., 2000). When these ions interact with respiratory enzymes they produce reactive oxygen species which are fatal to cell. Also, silver is a soft acid and thus it reacts with soft base. Silver nanoparticles when penetrates inside the cells reacts with sulfur and phosphorus which are soft bases and also a major component of DNA. Nanoparticles when react with S and P of DNA can damage DNA and leads to cell death (Hatchett *et al*., 1996, Morones *et al*., 2005). Apart from all these effects, nanoparticles also show their effect on bacterial signal transduction pathway. It is a well-known fact that protein substrate needs to be phosphorylated for the progress of signal transduction pathway. Silver nanoparticles inhibits the signal transduction by dephosphorylating the protein substrates on tyrosine residues and thus the stopped the growth of microorganisms (Shrivastava *et al*., 2007).

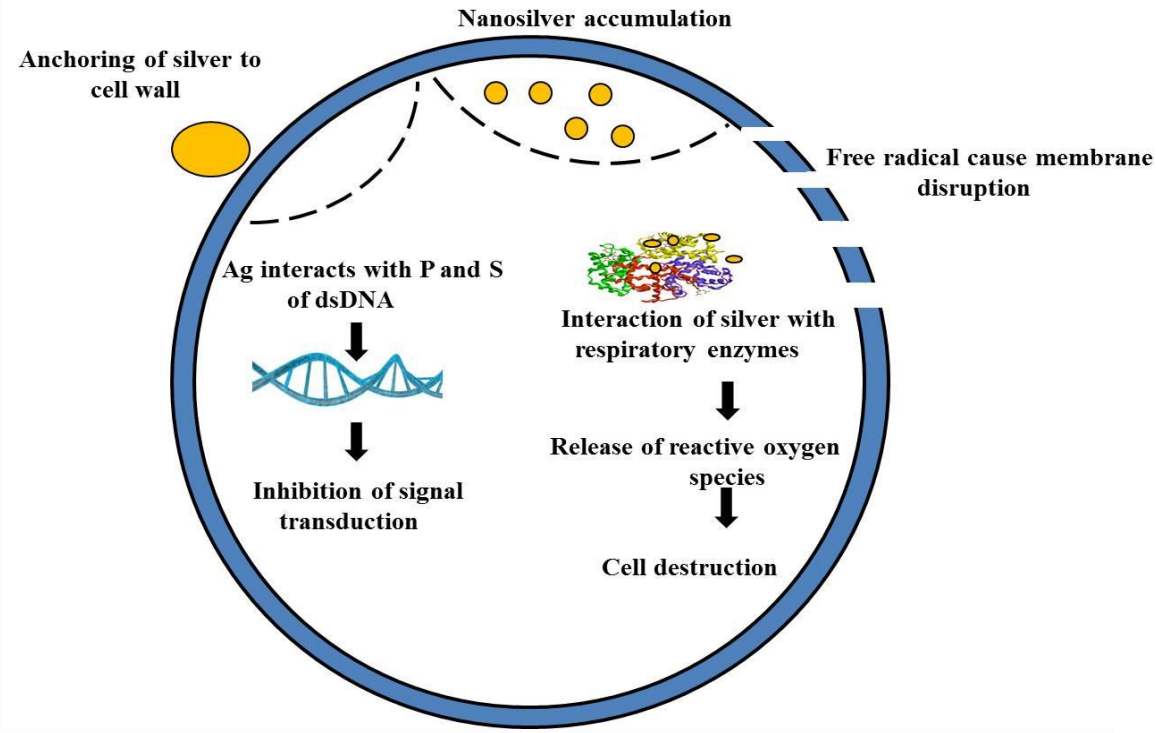


Fig 3: Different mechanisms explaining the antimicrobial action of silver nanoparticles

A lot of study needs to be done in order to claim the true mechanism behind the antimicrobial action of AgNPs. Sondi et al. (2004) used *E.coli* as a model microorganism for evaluating the antimicrobial property of silver nanoparticles. Different flasks containing *E.coli* culture were incubated with increasing concentrations of silver nanoparticles and then this was spread on solid agar plates. The nanoparticles under study were shown to have very good bactericidal effect. Electron microscopy (Scanning and Tunneling) was used to find the mechanism behind their bactericidal effect. Results showed the formation of pits in the bacterial cell wall due to the sticking of silver nanoparticles which leads cell wall damage and its death. Kim et al. (2007) investigated the silver nanoparticles for their antimicrobial activity against yeast, *Escherichia coli* and *Staphylococcus aureus*. Muller Hinton agar plates were used to check the antimicrobial activity by supplementing the culture plates with Ag nanoparticles of various concentrations. Inhibition of yeast and *E. coli* occurred at low concentration whereas the inhibitory effects were not very profound in case of *S. aureus*. Reason behind this low efficacy against *S. aureus* could be its thick peptidoglycan layer as compared to gram positive bacteria which inhibits silver nanoparticles to penetrate into the cells.

Despite of the remarkable properties of silver nanoparticles in water purification and dye degradation, their use is still limiting in aqueous solutions because of certain limitations associated with it. Silver nanoparticles if present in aqueous dispersion tend to form aggregates and undergo extensive oxidation which makes them unstable and this can affect their antimicrobial and dye degradation efficacy. Shape and size of nanoparticles cannot be controlled if present in aqueous phase. Also, these nanoparticles are not recoverable and reusable if present in aqueous phase and this adds to the cost associated with the usage of silver nanoparticles. Their presence in water system can affect the aquatic ecosystem. In order to reduce the risk associated with the use of silver nanoparticles, a new technique of immobilizing silver nanoparticles inside a polymeric matrix has been suggested which ensures that there will be minimum leaching of silver nanoparticles from the matrix. Extensive research is going on in order to find suitable polymeric materials for incorporation of silver nanoparticles. Various polymers have been investigated in different forms like that of multilayered films, microparticles, nanofibers, and hydrogel structures (Zhang *et al*., 2004). Nanoparticles gave good results when they were immobilized in the carriers but sincere research is still being made to synthesize new hydrogel nanocomposite system where all the components of the nanocomposite retain their individual properties and the potential of these components could be exploited in the combined system (Hamidi *et al*., 2008).

2.4 Hydrogel as Immobilization Matrix

Hydrogels are three- dimensional, hydrophilic polymeric networks that and can swell in water, holding a large amount of water. Hydrogels maintain their structure while it swells in water and this resistance to dissolution while adsorption is because of the cross-links between its network chains. The water absorbing ability of hydrogel is due to the presence of hydrophilic functional groups such as –OH, — COOH, CONH,–SO_3H, and –NH_2 on their crosslinking polymers (Dwivedi *et al*., 2011). Hydrogels have become a material of choice due to their unique properties like softness, smartness, and the ability to store large amount of water (Okay. 2009). First synthetic hydrogel was developed by Wichterle and Lim in 1954 and after this development hydrogel technologies was thought to be applied to a wide range of application like in hygienic products , in drug delivery systems , diagnostics , wound dressing, food additive, environmental remediation , water purification, biomedical applications;

tissue engineering, materials to regulate biological adhesions and Biosensors (Ahmed, 2015).

2.4.1 Method and Mechanism of Network Formation in Hydrogel

Initially the macromolecular chains of preliminary material are linked together leading to the formation of soluble, branched polymer which is called as 'Sol'. The linking of polymeric chains goes on and the size of branched polymer increases with a decrease in the solubility. Final form of this branched polymer is known as the 'gel'/network. The point at which gelling of polymer occurs is called as the 'gel point'. This conversion of a linear polymer to its branched form is known as 'sol-gel transition/sol-gel gelation' which can be of two types: physical or chemical gelation (Rubinstein *et al*., 2003).

A number of methods are being employed for making cross-linked networks of natural or synthetic polymers for making hydrogels. Physical, chemical, graft polymerization and radiation crosslinking are used to make hydrogel networks. Polymeric chains are linked via chemical reaction or ionizing radiations to generate main-chain free radicals which when recombine can act as cross-linking junctions for network formation. Physical method of network formation which includes electrostatic interactions and entanglements are also being used. But of all the methods used, chemical cross-linking using a cross-linking agent is the most preferred technique in order to generate permanent hydrogels. Chemical cross linkers such as glutaraldehyde are used and crosslinking is done by the functional groups (–OH, -COOH,-NH_2) present on the polymers (Ahmed, 2015).

There are numerous characteristics which make hydrogel an attractive choice to be used in water purification. First and the most important is its water adsorption capacity. The particle size and size of pores can be controlled while synthesis of hydrogel and this is required in order to achieve desired absorption rate depending on the requirement of application. It is very economical to use hydrogels if made of natural polymers and also they are reusable. It has very high stability and durability in swelling environment and can be easily stored with no deterioration in their properties. Smart hydrogels can absorb/desorb or swell/deswell in the solution on change of environmental factors like pH, temperature etc. (Ahmed *et al*., 2009).

All these properties of hydrogels makes them a very good choice to be used as an immobilizing matrix for nanoparticles and making hydrogel nanoparticulate system for dye removal and water disinfection purpose. Thus, the development of silver-nanoparticles based non-toxic hydrogel nanocomposite is an active area of research.

2.5 Hydrogel Based Nanocomposite

At present many polymeric hydrogels are being used in wide range of applications in our surroundings. Examples being the refrigerants (coolers), super-absorbent polymers which are used in diapers and sanitary napkins, contact lenses, gels used for electrophoresis, wound dressing. Many food items like tofu, jelly, elephant's foot are also examples of hydrogels. Apart from contact lenses and super-absorbent polymers, there is no extensive development in the field of hydrogel till date from the viewpoint of chemical products. This slow pace in the growth of hydrogels is because of the disadvantages associated with synthetic polymer hydrogel as chemically cross-linked hydrogels are fragile and are inadequate to functions as smart gels. The introduction of the concept of nanocomposites helped to overcome this problem. Hydrogel nanocomposites are the substances in which two or more organic and inorganic components with quite distinct properties are compounded into polymer hydrogels on a nanometer scale. As a result of this a new type of polymer hydrogel i.e. nanocomposite hydrogel with extraordinary swelling and mechanical properties was created. Its various advantages include improved mechanical properties such as ultra-high elongation, high tensile strength, very high fracture energy, controlled modulus, high compressive strength, high and rapid swelling-deswelling. Nanocomposite hydrogels have also been reported with unique porous morphologies and self-healing properties (Haraguchi *et al*., 2007).

The most appealing property of hydrogel due to which it has been chosen as a matrix is that the hydrogel polymer will hold water together in its swollen state and provides free space inside its porous network which can act as a nanoreactor for the synthesis of nanoparticles and also it allows the free diffusion of some solute molecules (pollutant or organic molecules like dye) inside this network which is very important for photocatalytic dye degradation of dye and water disinfection (Okay. 2009). Jiao et al. 2015 prepared nanocomposite hydrogels containing reduced graphene oxide and silver nanoparticle for removing organic dyes like Rhodamine Blue and Methylene blue from wastewater. The nanocomposite hydrogel was prepared by *in situ* co-

reduction of graphene oxide as well as silver acetate within hydrogel matrix to form reduced graphene oxide and silver nanoparticles composite hydrogel. Hydrogels were found to be very effective in dye degradation as 100% of RhB and MB were degraded within 70 minutes and 30 minutes respectively. Moon et al. (2013) tested the efficacy of PVA/PAAc/TiO_2/GO hydrogel nanocomposite for wastewater treatment where the resulting nanocomposite was synthesized through radical polymerization and condensation reaction. In this study, the semi-interpenetrating network of PVA/Pac hydrogel was prepared followed by uniform dispersion of TiO_2/GO nanoparticles within the matrix. The introduction of TiO_2 into GO sheets resulted in an enhanced photocatalytic activity for pollutant decomposition as GO acts as a very good adsorbent due to interaction between GO and pollutants. At pH 10, 88% reduction of Coomassie Brilliant Blue was achieved after 200 min but this reduction was decreased to 20% when experiment is performed at pH 2. This decrease in reduction capacity of hydrogel is due to the pH-sensitive swelling property of PVA/PAAc/TiO2/GO nanocomposite hydrogels. In a different study, a chemically cross linked, porous, chitosan–PVA hydrogel synthesized by repeated freeze–thaw treatment was evaluated as an immobilization matrix for *in situ* synthesis of silver nanoparticles (Agnihotri et al. 2012). The characterization of the prepared gel was done using FE-SEM and FE-TEM which indicated that AgNPs were uniformly distributed on the surface as well as inside the gel. These nanocomposite hydrogels showed effective antibacterial activity when used against *E. coli* and showed a reduction of 83.5 % and 24.4 % in case of pure and silver loaded hydrogel respectively within 12 h of incubation.

Recently, a few studies have encouraged using chitosan based nanocomposites employing silver nanoparticles and graphene oxide for photocatalytic degradation of dyes as well as antibacterial activity. For instance, a study evaluated the use of reduced graphene oxide/chitosan/silver hydrogel nanocomposites for photocatalytic degradation of dyes in waste water. The 3-dimensional porous GO-CS hydrogel was synthesized via a self-assembly process and was characterized using techniques like SEM and TEM to study the surface and internal morphology while EDX was done to analyze the chemical components present in the prepared hydrogels. Dye degradation rates reached up to 100% for MB and Rhodamine blue within 70 minutes of its exposure to hydrogels when experiment was carried in presence of UV light but

degradation capacity of same nanocomposite was significantly reduced in the absence of UV light. Degradation of both Methylene blue and Rhodamine blue were in accordance with the pseudo-second-order model (Jiao et al. (2015). In another study, chitosan beads embedded with silver and graphene oxide nanocomposites were exploited for their antibacterial performance (Chook et al. 2016). Initially, the silver and graphene oxide composite was made using microwave radiation method and then GO/CS/Ag nanocomposite was formed by dispersing Ag/GO composite solution into the chitosan/acetic acid suspension. The mixture was stirred properly and then beads of GO/CS/Ag composite were prepared by dripping the suspension in 0.1M NaOH which were then characterized using FE-SEM. The beads were then checked for their antibacterial activity against *E. coli* and *S. aureus* which effectively inhibited their growth as compared to the chitosan beads alone. Conclusively, the chitosan hydrogel based nanocomposite with AgNP and GO have shown good potential to be used as an effective approach for photocatalytic degradation of dye as well as antibacterial activities in waste water treatment. A substantial lack in research data to the concerned domain thus forms a basis of current research plan.

CHAPTER-3

MATERIALS AND METHODS

3.1 Materials Required

Chitosan (purity 99%; deacetylation degree 85%; pH 5-6; MW 100,000–300,000) was procured from Nano Wings Pvt. Ltd., Telangana (India) while silver nitrate was obtained from Sigma-Aldrich (India). A fine graphite powder (purity 98%, particle size -60 mesh) and sodium borohydride were obtained from Loba Chemie Pvt. Ltd. Hydrogen peroxide (30%) was obtained from Rankem and Glutaraldehyde (25% in water) was obtained from Spectrochem Pvt. Ltd. For antimicrobial studies, the bacterial strains *E.coli* and *Bacillus subtilis* were taken from Department of Biotechnology, Thapar University, Patiala. Double deionized water was used thoroughly for all the experiments related to nanomaterial synthesis and photocatalytic studies.

Initially, glassware were washed with detergent and then was kept in oven at 50-60 °C for drying. After drying, glassware were treated with aqua regia for 15-30 minutes to dissolve metals and other organic impurities and subsequently washed thrice with DI water. Finally cleaned glassware was kept in distilled water for 15 minutes, oven dried and stored in an air tight container till use in future.

3.2 Preparation of Graphene oxide:

Graphene Oxide was prepared using modified Hummer's approach following Chen *et* al., (2013). Specifically, 70 ml of sulfuric acid was taken in a flask and was kept in ice bath (0°C) for 10 minutes. 3 gm of graphite powder was mixed with 1.5 gm of sodium nitrate and this mixture was added in sulfuric acid which was kept in ice bath (0°C).The mixture was then stirred at 400 rpm for 1 hr and 9 gm of potassium permanganate was then added in fraction of 25 mg, every time while stirring was continued and the temperature of the mixture was maintained below 20°C until the entire potassium permanganate was introduced into the reaction mixture. Afterwards, the next temperature of the reaction mixture was raised to 35-40°C and kept on stirring for 12 hrs. After 12 hrs, 500 ml of water was added with constant stirring in a fraction of 100 ml each time at a time interval of 10 min such that a temperature of 65°C is maintained. 15 ml of 30% hydrogen peroxide was added drop-wise with

constant stirring till a brownish yellow color is obtained which marks the completion of the oxidation reaction. The solution was then centrifuged to separate graphene oxide from the reaction mixture. Washing was done initially three times with 5% HCl and distilled water in an alternative manner and then washed with distilled water until pH of resulting solution is approached to 7. It is then kept overnight at 50°C for drying and is then grinded into a fine powder.

3.3 Preparation of Chitosan / Graphene oxide Hydrogel

Chitosan / graphene oxide hydrogel was prepared using procedure given by Sun *et al*., 2015 with certain modifications. Chitosan solution (3%) is prepared by dissolving 3 gm of chitosan in 100 ml of 2.5% acetic acid solution at 40°C while stirring overnight at 700 rpm. Graphene oxide suspension was prepared by dissolving 50 mg of graphene oxide in 2 ml of 2.5% acetic acid solution. For preparation of hydrogel composites, chitosan and graphene oxide solutions were mixed overnight at 40ºC to obtain a homogeneous suspension. Gel formation was achieved by adding 500 µL of glutaraldehyde drop-wise into chitosan-GO solution at room temperature and was mixed for 15 minutes before casting into the plastic syringes. Curing is done by keeping these syringes at 60ºC for 2 hrs . Gel was kept in oven at 40- 50 °C for curing until the gel becomes solid. Hydrogel was then precipitated by adding 12% NaOH and was left overnight at room temperature to remove hydrogel from the syringes. Removed hydrogel was washed thrice with distilled water to remove NaOH. Finally, repeated freeze-thaw cycles at -20 °C for 6 hrs and 25 °C for 2 hrs respectively for 4 days resulted in the formation of porous semi-interpenetrating networks were developed. In this way porous chitosan/ GO hydrogel was formed and kept for further characterization.

3.4 *In situ* Synthesis of Silver Nanoparticles in Chitosan/Graphene oxide Hydrogel

Chemical reduction was used for *in situ* synthesis of silver nanoparticles in the gel. Chitosan-GO hydrogels were cut into circular discs of desired length with the help of sterilized blade. These circular discs were introduced into 10 mM silver nitrate solution and were kept in dark for 24 hrs. Silver loaded gels were washed twice with distilled water to remove the unbound silver ions and for reduction these hydrogels were placed in 10 mM of chilled sodium borohydride ($NaBH_4$) solution for 10

minutes. Finally the gel discs are washed with distilled water and kept for further characterization.

3.5 Swelling Studies

For calculating the swelling ratio, two discs each of diameter 5mm for GO-CS and GO-CS-Ag were taken and were vacuum dried for 72 hrs in order to remove all the moisture present in it. Dry weight of both the gels was taken and gels were then dipped into water. Swollen gels were removed from water after 1 min, wiped with tissue and again weighed. This was repeated after every 1 minute till the weight of swollen gel become constant. Swelling ratio was then calculated using the following equation:

$$Swelling\ ratio = \left(\frac{W2-W1}{W1}\right) * 100 \ldots\ldots\ldots\ldots\ldots (1)$$

where, W1 and W2 represent the weight of dried and swollen hydrogel respectively.

3.6 Porosity Measurements of GO-CS and GO-CS-Ag Hydrogels

Porosity is defined as the ratio of pore volume to bulk volume. Bulk volume of cylindrical hydrogel disc is calculated using formula $V1 = \pi r^2 h$, where r is the radius of the gel and h is the height of the gel. Known volume of hydrogel (V1) is then immersed in known volume of water (V2) and final volume of water is noted (V3). Porosity is then calculated using the following equation:

$$Porosity = \left(\frac{V1+V2-V3}{VI}\right) * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

where, V1 represents weight of hydrogel, V2 represents initial volume of water and V3 represents the increased volume of water after immersion of hydrogel

3.7 Characterization

UV-Visible Spectrum of graphene oxide formed was recorded using a spectrophotometer (SHIMADZU, UV-2600, Japan). Graphene oxide dispersion of concentration 1mg/ml was sonicated and its absorbance was taken between 200 to 800 nm range. Morphology and topography of graphene oxide, pristine GO-CS and silver loaded GO-CS-Ag was characterized using Scanning Electron Microscope (SEM)(Hitachi S-3400N, Japan). Hydrogels were broken into small pieces and

vacuum dried to remove any moisture if present. Gold coating was done using a sputter for making the samples conductive for image generation and analysis.

3.8 Preparation of Methylene blue solutions

Methylene blue (MB) dye solution of different dilutions was prepared for making a standard curve and a working solution of 40ppm was prepared for carrying out dye degradation experiments. A stock solution of 100 ppm was prepared by dissolving 10 mg of dye in 100 ml of distilled water. Various dilutions (50 ppm, 40 ppm, 20 ppm, 10 ppm, 5 ppm, 2 ppm, 1 ppm, 0.5 ppm) were made in duplicates as per the desired dilutions. Absorbance at 664 nm is taken and a standard curve of dye dilutions vs. absorbance was made.

3.9 Dye Adsorption by Graphene oxide

Dye adsorption capacity of graphene oxide was evaluated using the following procedure.2 mg of graphene oxide was added in 100 ml of 40 ppm methylene blue dye solution and the mixture was stirred at 200 rpm for 30 min. After stirring, the mixture was centrifuged at 5000 rpm for 10 min to settle the graphene oxide with dye adsorbed on it and absorbance of the supernatant was then recorded at 664 nm. The same was repeated with varying concentrations of graphene oxide (4 mg, 8 mg, 10 mg and 20 mg) and absorbance of their supernatant was recorded.

3.10 Dye Removal by Nanocomposite Hydrogels

For evaluating the dye removal capacity of prepared hydrogels, 0.4 gm of dried hydrogel was put into 100 ml of 40 ppm methylene blue dye solution and was kept in sunlight. 1ml of sample was drawn out of this solution at different time intervals till complete degradation is achieved and absorbance was checked at 664 nm in order to find the dye degradation rate.

Percentage dye degradation was calculated using the given formula

$$\%\ Removal = \left(\frac{A_0 - A}{A_0}\right) * 100 \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

where, A_o is the initial absorbance of dye solution and A is the absorbance after degradation. The effect of catalytic dosage and pH was checked by repeating the experiment at varying concentration of hydrogel (0.4 gm, 1.2 gm, 2.0 gm, 2.8 gm, 4.0 gm) and at various pH (5,7,9).

3.11 Antibacterial Activity of Hydrogel

The gels formed are checked for their antibacterial activity using colony forming assay against *Bacillus subtilis* and *E. coli*. The sterilized nutrient broth medium was inoculated with 10 µl of microbial culture and was kept at 37ºC, 120 rpm for 18 hrs. Culture was centrifuged when cells reach their mid-log phase and pellet was suspended in phosphate buffer solution after washing thrice with the same. The cell pellet is diluted with phosphate buffer saline till its absorbance reaches one at 600 nm which corresponds to $1*10^9$ CFU/ml. Antibacterial activity of prepared hydrogels was then tested by incubating the culture at 37ºC, 120 rpm with hydrogels (2 hydrogels of 10 mm diameter) immersed in it. Culture samples were withdrawn and plated at different time intervals to determine the antibacterial action of hydrogels. Culture sample with no hydrogels immersed was kept as a control.

CHAPTER-4

RESULTS AND DISCUSSIONS

4.1 Formation of Graphene oxide

Graphene oxide was synthesized using modified hummers method with certain modifications as stated earlier. Oxidation of graphite using H_2SO_4, $NaNO_3$ and $KMnO_4$ resulted in thick brown colored slurry which was a mixture of graphene oxide, residues of un-oxidized graphite powder and unreacted oxidizing agents. This solution gave a bright yellow color on adding H_2O_2 which is an indicative of successful oxidation of graphite to graphene oxide (Huang *et al*., 2011). Repeated centrifugation was done to remove the unwanted salts, ions and un-oxidized graphite particles to obtain a pure GO suspension. Figure 4(b) showed the image of synthesized graphene oxide powder which exhibited a brown color and had certain toughness as it was very difficult to grind.

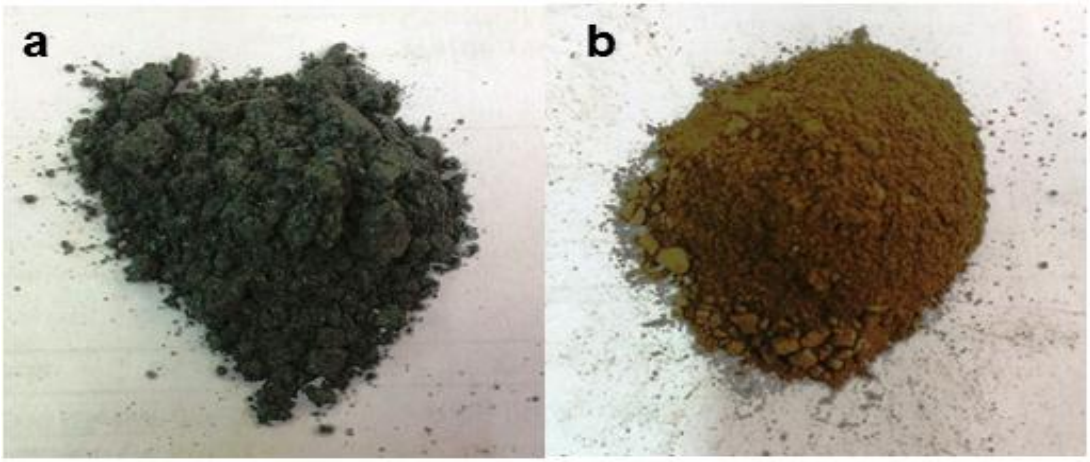


Fig 4: Photographical images of (a) Graphite powder (b) synthesized graphene oxide

4.2 UV-Visible Spectrum of Synthesized Graphene oxide

The formation of graphene oxide and the yield was determined through UV-Vis spectroscopy. The spectrum corroborates with earlier reports where a main absorption peak at 235 nm and a shoulder around 305 nm was obtained (Fig 5). The peak at 235 nm is attributed to π-π* transition of C=C bond while a shoulder around 305 nm is credited to n- π * transition of C=O bonds (Chen *et al*., 2013). On comparing with

pure graphite powder, no such peaks were observed at respective wavelengths indicating a stable transformation of graphite into graphene oxide.

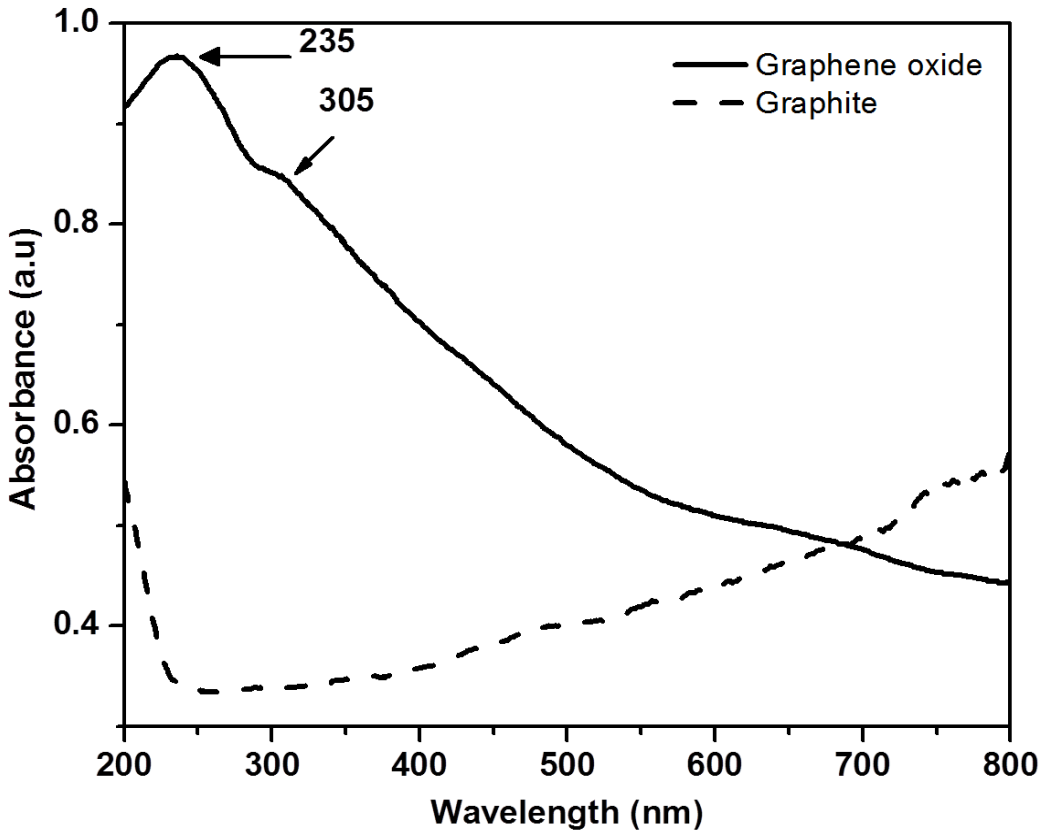


Fig 5: UV-Visible spectrum of aqueous GO dispersion

4.3 Formation of GO-CS Hydrogel

Figure 6 (a) and (b) showed the photographic images of GO-CS hydrogels before and after freeze thaw treatment respectively. It is quite evident from the figure that freeze thaw cycles carried for four days resulted in the formation of pores within the hydrogel. The semi-interpenetrating network of chitosan and graphene oxide showed macro-porous structure after freeze thaw cycles for four days which gave an advantage in dye adsorption due to an effective increase in the surface area of the hydrogel.

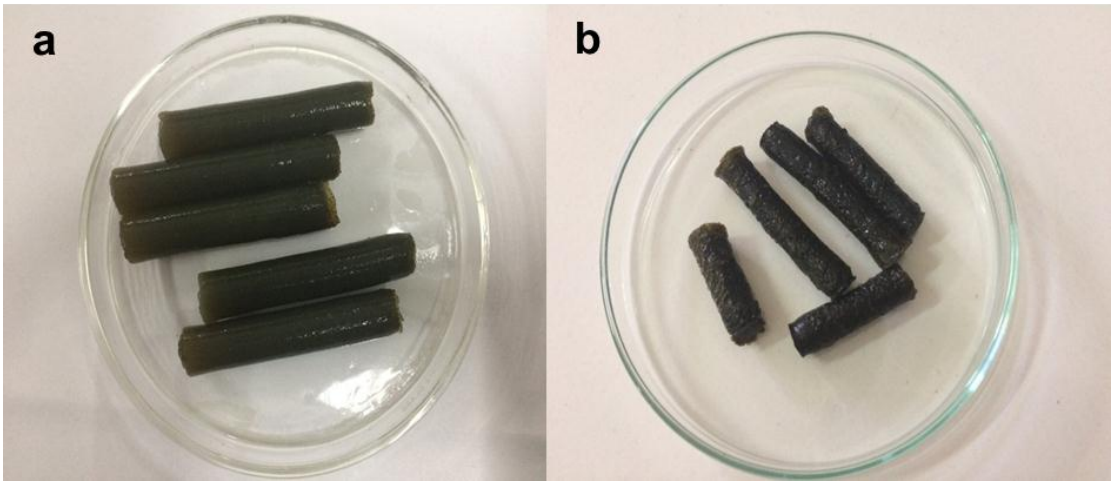


Fig 6: Synthesized GO-CS hydrogel (a) before freeze thaw (b) after freeze thaw cycles

4.4 *In situ* Synthesis of Silver Nanoparticles in Hydrogel

After loading of silver into hydrogels by soaking them in silver nitrate solution, no visible change in color was observed. But these gels changed their color from greenish black to brownish black within 8-10 minutes of their contact with sodium borohydride solution (Figure 7). This color change indicates the synthesis of silver nanoparticles inside the hydrogels. The reason behind the synthesis of silver nanoparticles inside the hydrogel could be that the porous structure of completely swollen hydrogels provides a large free space between its cross-linked polymeric networks which can act as a nanoreactor for nucleation as well as growth of the nanoparticles (Mohan *et al*., 2007). These gels when kept in silver nitrate solution take up the Ag^{+} ions in the free space within their gel networks which are then converted to silver nanoparticles after their reduction with $NaBH_4$ (Mohan *et al*., 2010). A uniform dispersion of nanoparticles is achieved as a result of non-covalent interactions between the functional groups of polymers (–OH, –NH2, –C=O) and silver ions (Bozanic *et al*., 2010). The stabilization of nanoparticles inside the gel is achieved due to their attachment with large polymer chains which cover the nanoparticles (Mohan *et al*., 2006).

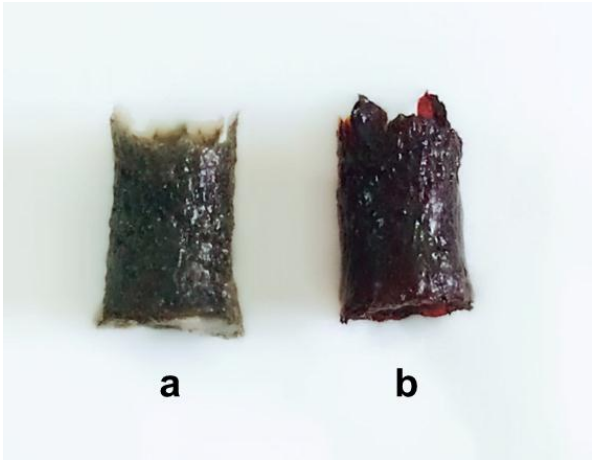


Fig 7: Silver loaded hydrogels (a) before reduction (b) after reduction

4.5 SEM - Scanning Electron Microscope

The surface morphology of as-prepared graphene oxide, GO-CS and GO-CS-Ag hydrogel was studied using SEM (Scanning Electron Microscopy). SEM images of the hydrogel indicated mesh like porous nanostructures formed by the crosslinking of graphene oxide sheets and chitosan chains (Fig 8: b,c). GO-CS hydrogel doesn't show the presence of silver nanoparticles. However, a uniform dispersion of aggregated silver nanoparticles is clearly visible in case of GO-CS-Ag hydrogels indicating the successful reduction of silver ions by sodium borohydride and the formation of ternary nanocomposite. Morphology of the hydrogel remains same even after the incorporation of silver nanaoparticles which depicts that the stable immobilization of AgNP's inside the polymeric network of chitosan and graphene oxide doesn't have any effect on the morphology of the hydrogel.

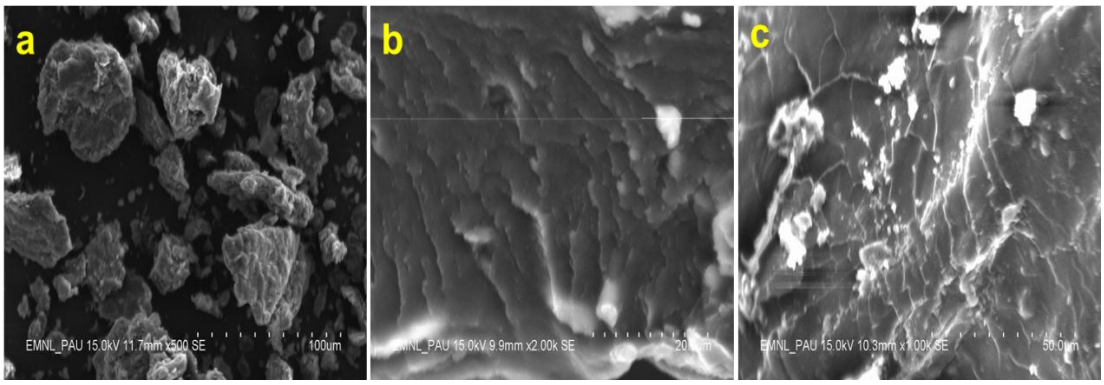


Fig 8: SEM images of (a) Graphene oxide (b) GO-CS hydrogel (c) GO-CS-Ag hydrogel

4.6 Swelling Studies of GO-CS and GO-CS-Ag Hydrogels

Swelling capacity of both GO-CS and GO-CS-Ag hydrogel was determined in order to see the effect of silver nanoparticles on the swelling capacity of the hydrogel. The results of swelling capacity are depicted in Figure 9. In case of GO-CS hydrogels swelling ratio was calculated to be 83.17 ± 0.63 %. Swelling equilibrium was reached within 5 minutes after which no significant changes were observed in the swelling ratio of the gel. This fast absorption capacity of GO-CS hydrogels can be attributed to the plenty of functional groups, attached to GO sheets. Functional groups such as −COOH, −C=O, −OH and −C−O−C− are attached on graphene oxide surface and these could increase the density of the hydrophilic groups on hydrogel polymer networks (Huang *et al*., 2012). It is evident that silver incorporated hydrogels (GO-CS-Ag) have lower swelling ratio as compared to the pristine gels. An equilibrium swelling ratio of 80.23 ± 0.48% is reached after 10 minutes. The reason behind this could be that the surface pores of the hydrogels are blocked with the incorporation silver particles which decreased the uptake of water (Lee *et al*., 2006). The other possible reason could be the tendency of silver nanoparticles to crosslink with the functional groups (-COOH, -NH_2) of polymeric chains due to which these functional groups are masked and decreases the diffusion of water inside the hydrogels.

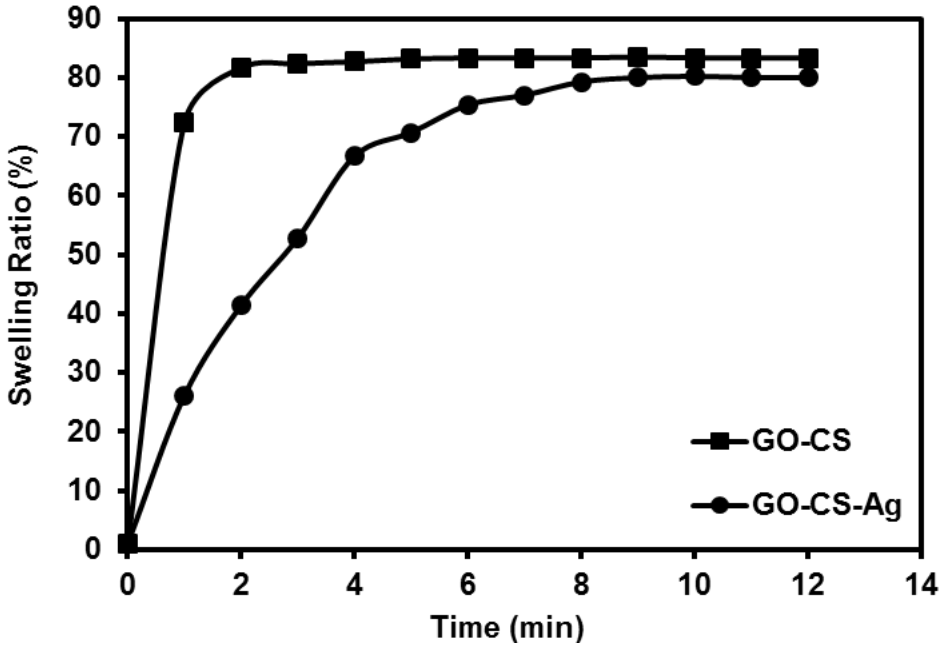


Fig 9: Swelling kinetics of GO-CS and GO-CS-Ag hydrogel at room temperature

4.7 Porosity Measurements

Solvent replacement method was used to calculate the porosity of synthesized hydrogels. Dried hydrogel was immersed in a known quantity of water till it reaches swelling equilibrium and then the rise in water level was calculated. Porosity is calculated using the following equation:

$$Porosity = \left(\frac{V1 + V2 - V3}{VI}\right) * 100$$

where, V1 represents weight of hydrogel

V2 represents initial volume of water

V3 represents the increased volume of water after immersion of hydrogel

All the hydrogels obtained yielded a very high porosity lying between 92.96 to 95.23% in case of GO-CS and between 87.23 to 90.1% in case of GO-CS-Ag. This high porosity along with extraordinarily high swelling ratio could be the reason behind the easy and fast diffusion of dyes into the hydrogels and thus for the high adsorption ability (Wang *et al*., 2015). A little reduction in the porosity of silver loaded hydrogel could be due to the blockage of surface pores by silver.

4.8 Mechanical Strength

The synthesized GO-CS hydrogel (3 wt % chitosan and 0.05 wt % graphene oxide) exhibited a very good compressive strength as compared to pristine chitosan hydrogel (Fig 10). Similar results were found by Han et al., 2013 where the calculated values for both storage moduli (G') and loss moduli (G") were greater for GO-CS hydrogel as compared to pristine chitosan hydrogel. This increase can be attributed to excellent mechanical properties (tensile strength = 125 GPa and Young's modulus = 1.1 TPa) of graphene oxide due to which it is added as a filler in polymer composites to increase their mechanical strength (Abdullah *et al*., 2015). Also, the strong electrostatic interactions along with hydrogen bonds between the functional groups of chitosan and graphene oxide make them mechanically strong (Chen *et al*., 2013).

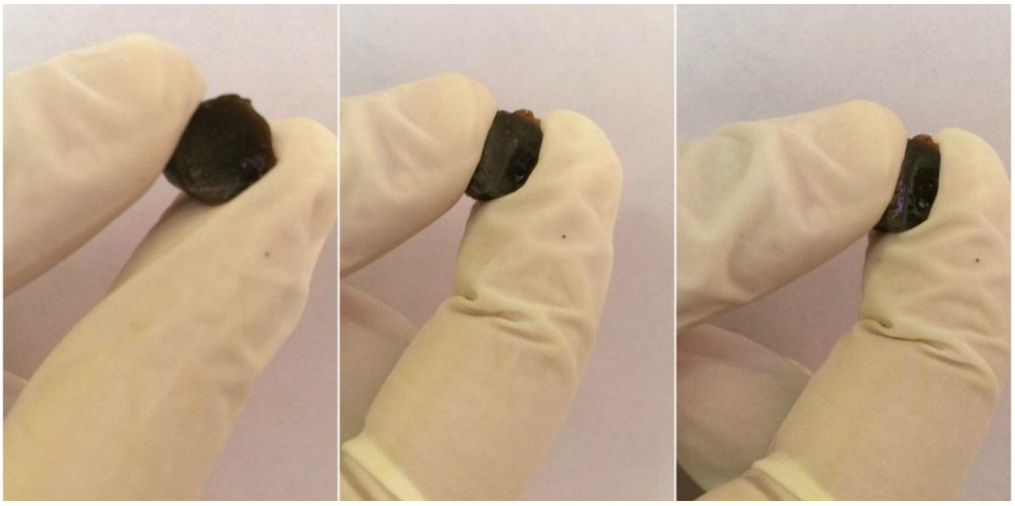


Fig 10: Compressive strength of GO-CS hydrogel

Besides their excellent mechanical properties, the synthesized hydrogels show self-healing property. The parts of hydrogel obtained after cutting with knife (Fig 11: b) were able to join again when stacked together for 2 minutes (Fig 11: c). Reason behind the self-healing of hydrogel could be the reconstruction of strong electrostatic and hydrogen bonds between chitosan and graphene oxide. The healed gel exhibits similar mechanical property as that of the original one.

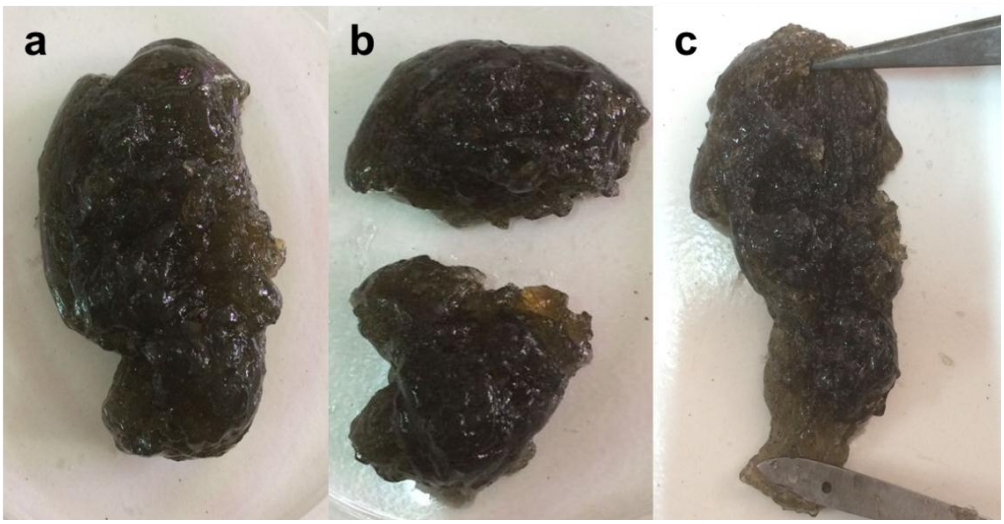


Fig 11: Self - healing property of hydrogel (a) synthesized hydrogel (b) gel cut by knife (c) self-healed hydrogel

4.9 Evaluation of Catalytic Activity of Synthesized Hydrogel Nanocomposites

4.9.1 Standard Callibration Curve of Methylene Blue

Different concentrations of methylene blue ranging from 0.5 ppm to 50 ppm were prepared and characterstic wavelength for determining their concentration using UV-Visible spectrometry was measured at 664 nm (Fig 12).

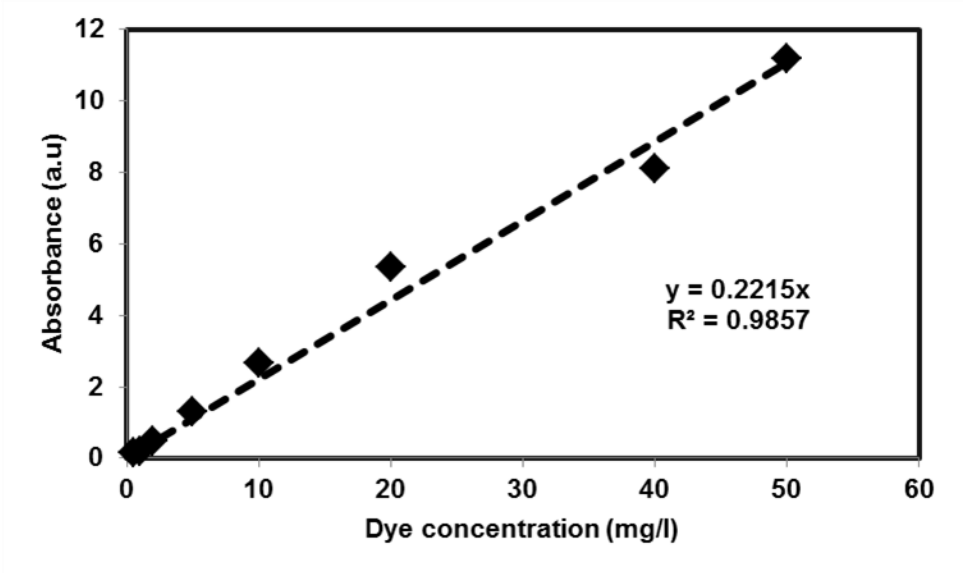


Fig 12: Standard callibration curve of methylene blue using different concentrations ofdye ranging from 0.5 ppm to 50 ppm.

4.9.2 Dye Degradation using Graphene oxide

A working solution of 40 ppm methylene blue is used in the entire study for conducting dye degradation experiments. The effect of varying concentrations of graphene oxide ranging from 2mg/100ml to 20mg/100ml dye was checked on adsorption of methylene blue. Results showed that the adsorption increases with increase in the concentration of graphene oxide. An MB dye solution of 40 mg/l changes its color from dark-blue to colorless within two minutes of stirring after addition of 20 mg graphene oxide at room temperature. Figure 13 and 14 depicts that graphene oxide is a very good adsorbent of methylene blue as 99.12 % reduction is achieved at 20mg/100ml concentration of GO. Reason behind this could be the structure of methylene blue containing cationic atoms such as S+ and rich aromatic

rings which gets easily adsorbed on the polyanionic graphene oxide via π–π stacking and ionic interaction (Liu *et al*., 2012).

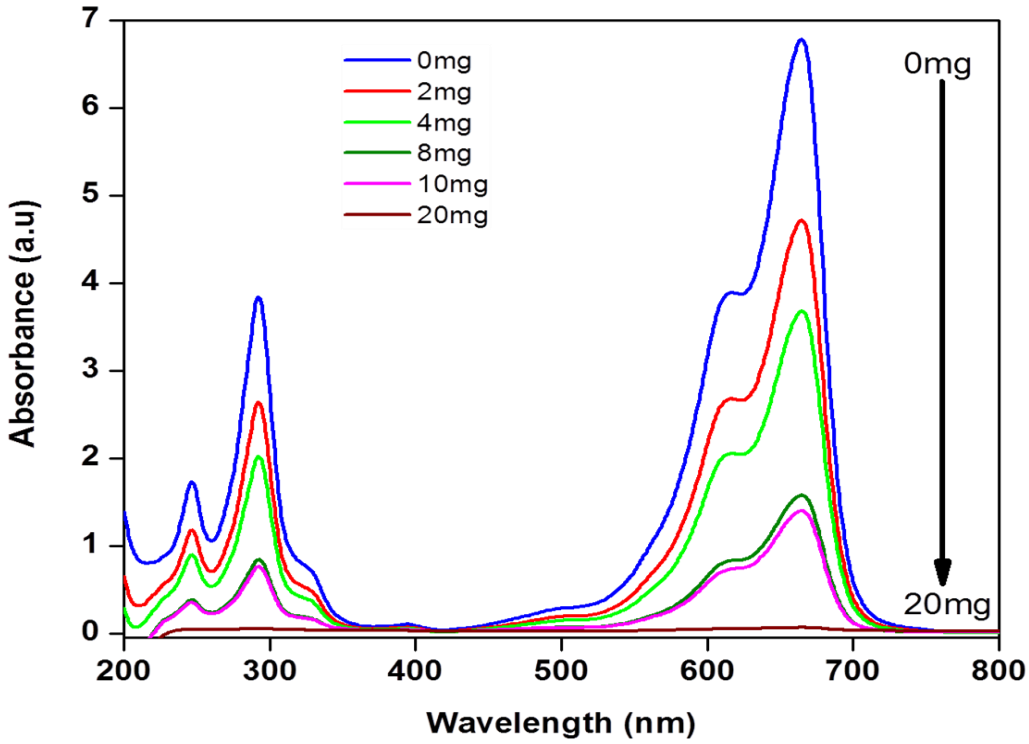


Fig 13: UV-Visible spectra showing dye degradation at different concentrations of GO ranging from 2 mg/100 ml to 20 mg/ 100 ml

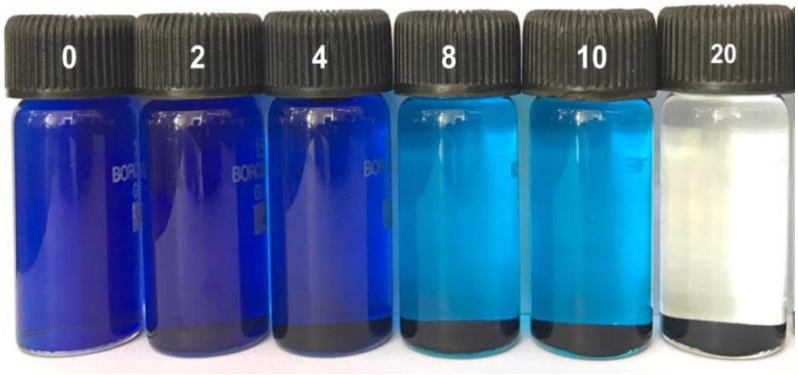
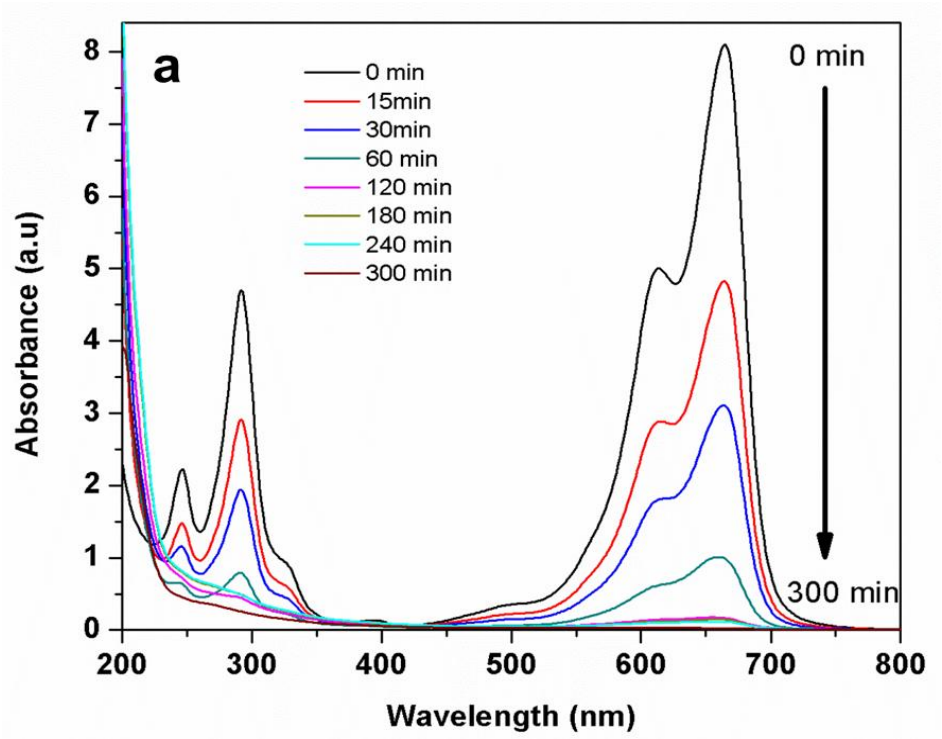


Fig 14: Methylene blue degradation at different concentrations of GO ranging from 2 mg/100 ml to 20 mg/ 100 ml

4.9.3 Dye Removal Capacities of Prepared GO-CS and GO-CS-Ag Hydrogel

The dye removal capacity of prepared GO-CS and GO-CS-Ag nanocomposite was evaluated in sunlight by putting these hydrogels into aqueous methylene blue solution. Initial dye concentration was 40 ppm and samples were withdrawn at different time intervals for their spectrophotometric analysis till the adsorption reaches its equilibrium. Figure 15 and 16 clearly depicts the good efficiency of both GO-CS and GO-CS-Ag hydrogels for dye degradation. Dye removal gradually increases with an increase in the contact time and almost all of the dye is degraded within 5-7 hours of its exposure to sunlight. Percentage dye removal was calculated using equation (3) given in section 3.11. Figure 17 shows the graph plotted between percentage dye removal and time for both GO-CS and GO-CS-Ag hydrogels. GO-CS hydrogel showed a 99.7% reduction of methylene blue within 5 hrs of its contact time. The introduction of silver into gel although delayed its degradation efficiency, a dye degradation of 98.7% was still achieved after 7 hrs of its exposure to sunlight. This excellent adsorption capacity can be attributed to the plenty of functional groups on the edges and basal plane of graphene oxide (carboxyl, hydroxyl and epoxy groups) and various chelating groups (amino and hydroxyl groups) on chitosan. These groups can easily bind to the charged organic compounds and other contaminants via electrostatic interaction (Chen *et al*., 2013) and hence are able to effectively remove methylene blue from waste water. In case of silver loaded hydrogels, silver nanoparticles are also responsible for photocatalytically removing dyes from aqueous solution via the mechanism explained earlier.



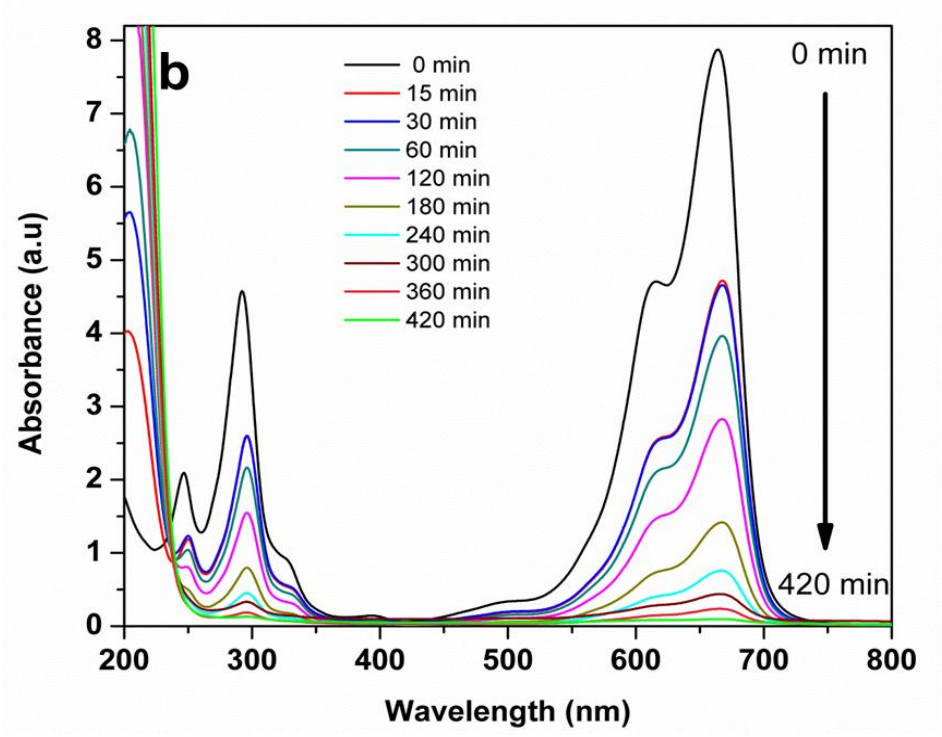


Fig 15: Methylene blue degradation at different time intervals using (a) GO-CS (b) GO- CS-Ag

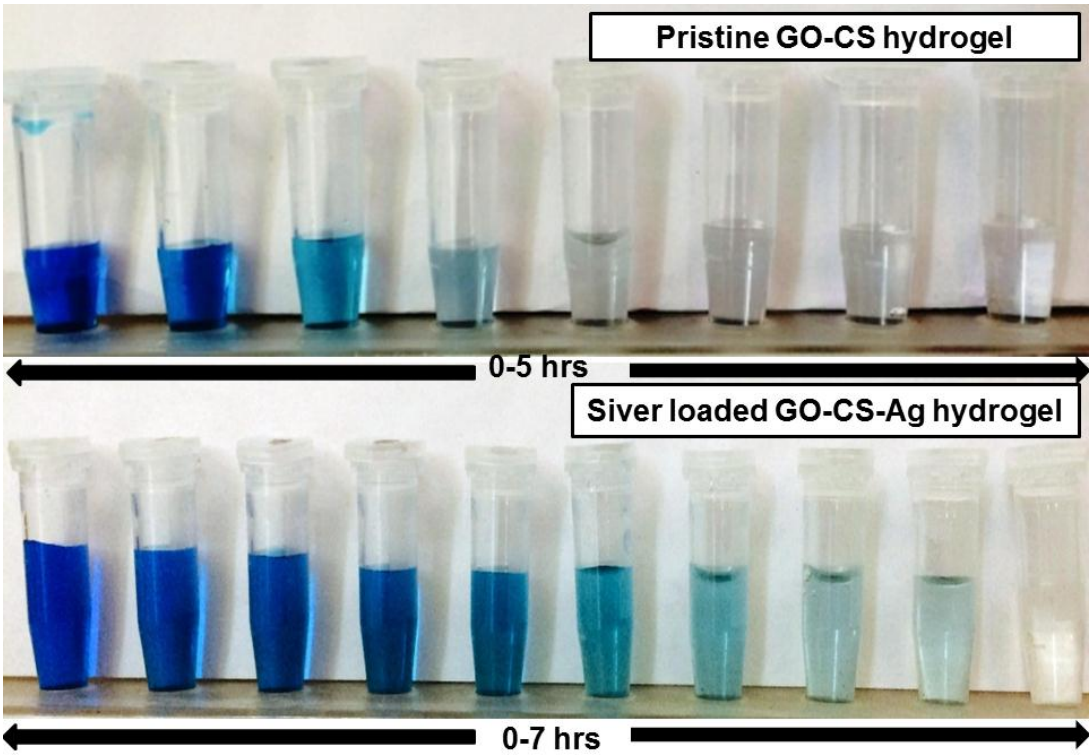


Fig 16: Dye degradation using GO-CS and GO-CS-Ag hydrogel with time

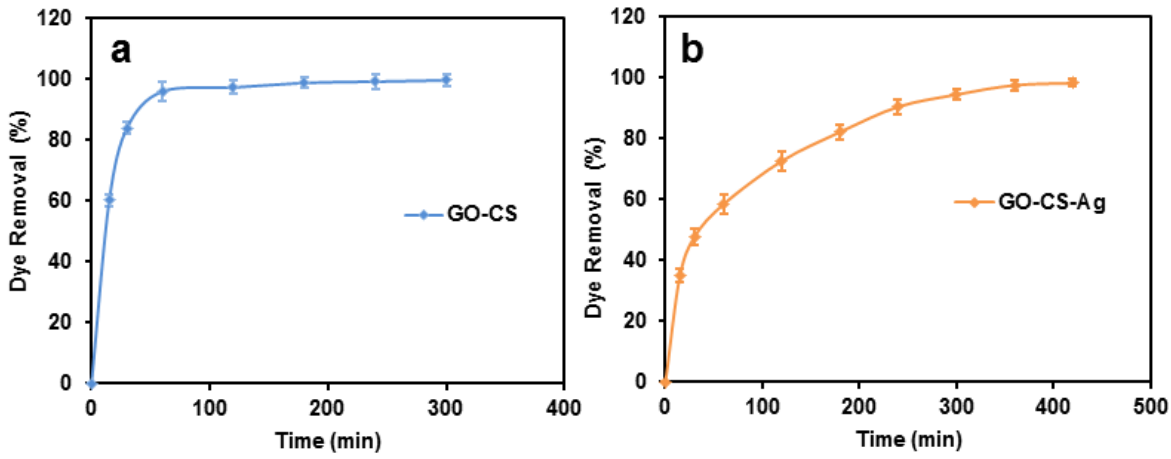


Fig 17: Percentage removal of methylene blue using (a) GO-CS (b) GO-CS-Ag.

4.9.4 Dye Degradation Kinetics

Test for dye degradation kinetics was conducted in order to determine the dye degradation rate and dye degradation process was explained using classic kinetic models. First and second order models can be described by using equations (4) and (5) respectively.

First order equation:

$$log(q_e - q_t) = \log q_e - \left(\frac{k}{2.303}\right) * t \quad \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Equation (1) is simplified as

$$\frac{q_e - q_t}{q_e} = \exp(-kt)$$

and a graph is plotted between $\frac{q_e - q_t}{q_e}$ vs t for first order model kinetics (Figure 18)

Second order equation:

$$\frac{t}{q_t} = \left(\frac{1}{k\prime * {q_e}^2}\right) + \left(\frac{t}{q_e}\right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

A graph is plotted between $\frac{t}{qt}$ vs t for second order model kinetics (Figure 17)

where, q_t represents the amount of dye degraded at any time t

q_e represents the amount of dye degraded at equilibrium

k and *k'* represents the rate constants for first and second order respectively

Table 1. shows the first order and second order kinetic data of methylene blue degradation using GO-CS and GO-CS-Ag. Interestingly, the data fitted into both first as well as second order kinetic models with their correlation coefficient being 0.999 and 0.996 for first order and 0.992 and 0.981 for second order in case of GO-CS and GO-CS-Ag respectively. Although, there is only a marginal difference between the correlation coefficients of both models, but still first order kinetic model is regarded as the most appropriate fit to describe methylene blue degradation kinetics due to its higher correlation coefficient and degradation rate.

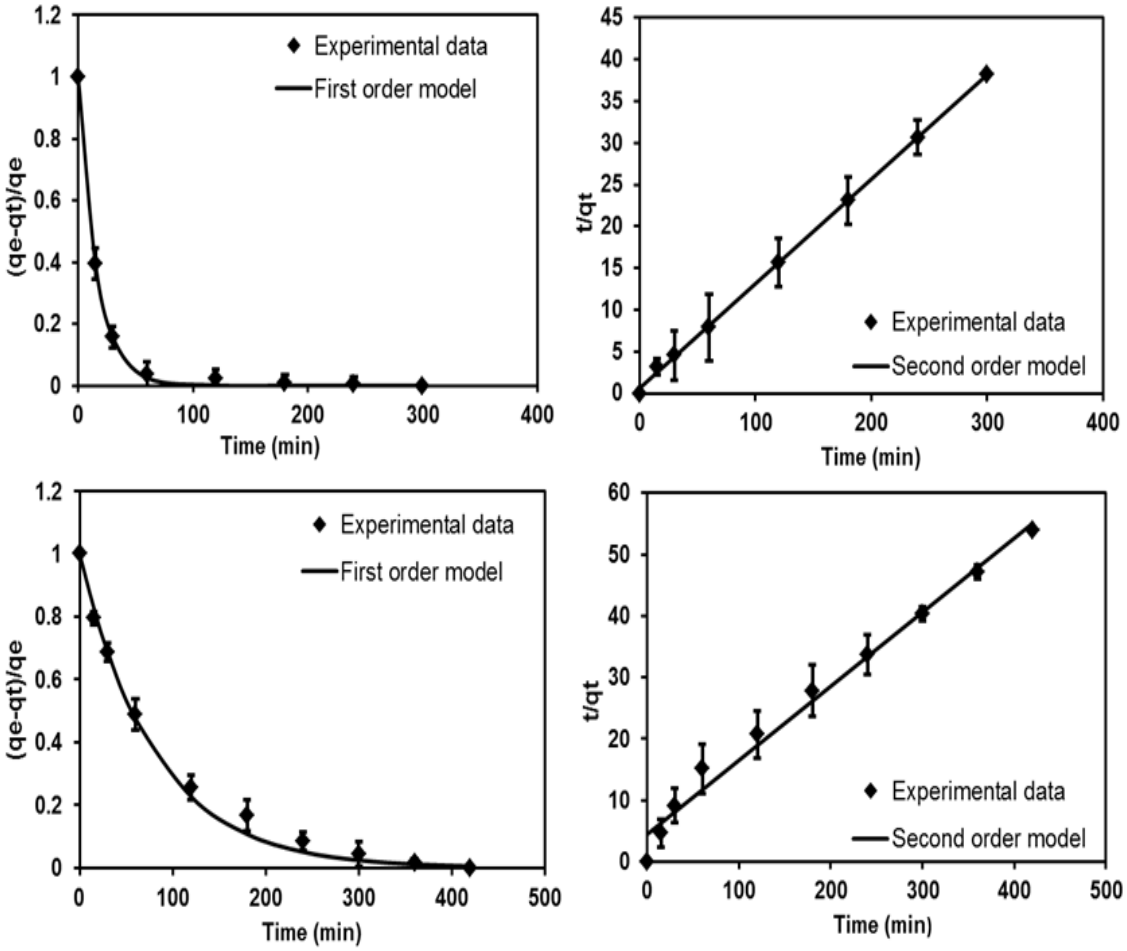


Fig 18: Degradation kinetic models of Methylene blue using GO-CS (a,b) and GO-CS-Ag (c,d)

Table 1: Kinetic parameters of methylene blue degradation using GO-CS and GO-CS-Ag nanocomposites

Sample	First-order model		Second-order model	
	k (min^{-1})	R^2	k' (gm/mg.min)	R^2
GO-CS	0.06	0.999	0.02	0.992
GO-CS-Ag	0.012	0.996	0.003	0.981

4.9.5 Effect of Catalytic Dosage on Dye Removal

Methylene blue degradation in this study is dependent on two mechanisms; one of those being adsorption and the other is photocatalytic degradation. Both of these are surface phenomena and are dependent on the surface area of the adsorbent or catalyst. Therefore, the effect of catalytic dosage on adsorption and photocatalytic degradation was checked by varying the dosage of hydrogel in 100 ml, 40 mg/L of methylene blue solution. Dose was varied from 0.4 gm to 4 gm/100 mL and it was observed that dye removal efficiency increases with an increase in the catalytic dosage.

Table 2: First order kinetic parameters of methylene blue degradation using GO-CS and GO-CS-Ag nanocomposites.

Catalytic Dose (in grams)	**GO-CS**		**GO-CS-Ag**	
	***k* (min^{-1})**	**R^2**	***k* (min^{-1})**	**R^2**
0.4	0.02	0.902	0.007	0.993
1.2	0.03	0.951	0.004	0.963
2.0	0.02	0.979	0.005	0.942
2.8	0.03	0.996	0.008	0.985
4	0.06	0.999	0.012	0.996

Degradation efficiency increases from 92.8 % to 99.7 % in case of pristine hydrogel (GO-CS) and from 51.6 % to 98.7% in case of silver loaded (GO-CS-Ag) hydrogel on increasing the dosage from 0.4 gm to 4 gm. Table 2 shows the first order kinetic parameters of methylene blue degradation using GO-CS and GO-CS-Ag nanocomposites. The degradation rate is highest when 4 gm of hydrogel is used with rate constants being 0.06 min^{-1} and 0.012 min^{-1} for GO-CS and GO-CS-Ag respectively. The increase in the dye removal efficiency with increasing amount of hydrogel may be due to an increase in the active sites on the surface which allows more dye molecules to interact and hence improves the degradation efficiency of methylene blue (Hosseinabadi *et al*., 2015).

4.9.6 Effect of pH on Dye Removal

pH can significantly affect adsorption process, especially when dealing with charged species. In the current research, both adsorbent and adsorbate are charged molecules. Chitosan is polycationic in nature while graphene oxide is polyanionic due to the presence of ionizable functional groups on their polymeric chains. Methylene blue is a cationic dye, so the effect of its adsorption on GO-CS hydrogel was checked by varying the pH of dye solution from 5 to 9. The results show that adsorption increases with an increase in pH. Reduction increased from 96 % to 99.8 % in case of GO-CS and from 73.5 % to 95.2 % in case of GO-CS-Ag when pH was increased from 5 to 9.

The results can be justified as: At low pH, the concentration of H^+ ions increases in the solution which can compete with cationic dye molecules (MB^+) for adsorption on hydrogel. Also the functional groups like –COOH and -OH remain in their un-ionized state at low pH, making adsorption very poor. On the other hand, increasing the pH will decrease the number of H^+ ions in the solution and also the groups like –COOH and –OH begin to ionize yielding –COO- and O^- , preferentially allowing cationic dye molecules to bind them and hence improves adsorption (Bajpai *et al*., 2012).

4.10 Antibacterial Assays

The antibacterial effect of pristine GO-CS and silver loaded GO–CS-Ag hydrogels was tested against *E.coli* and *Bacillus subtilis* using colony forming assay. Reduction in the number of colonies with time was studied along with growth inhibition kinetics of bacterial cells.

4.10.1 Colony Forming Assay

The effect of both GO-CS and GO-CS-Ag hydrogels is quite evident from figure 19 and 20 respectively. Figure 19 shows nutrient agar plates with bacterial colonies grown as a function of their incubation time (0 min to 3hrs) with GO-CS hydrogels. The results showed no decrease in the growth of both bacterial colonies even after their treatment with GO-CS hydrogels. Despite of a lot of literature available reporting the antibacterial property of chitosan and graphene oxide, they showed no antibacterial effect against both *Bacillus subtilis* (Gram-positive) and *E.coli* (Gram-negative) (Ali *et al*., 2011). These results are consistent with the studies conducted by Chook et al, (2012). Figure 20 shows nutrient agar plates grown with bacterial colonies grown as a function of their incubation time with GO-CS- Ag hydrogels.

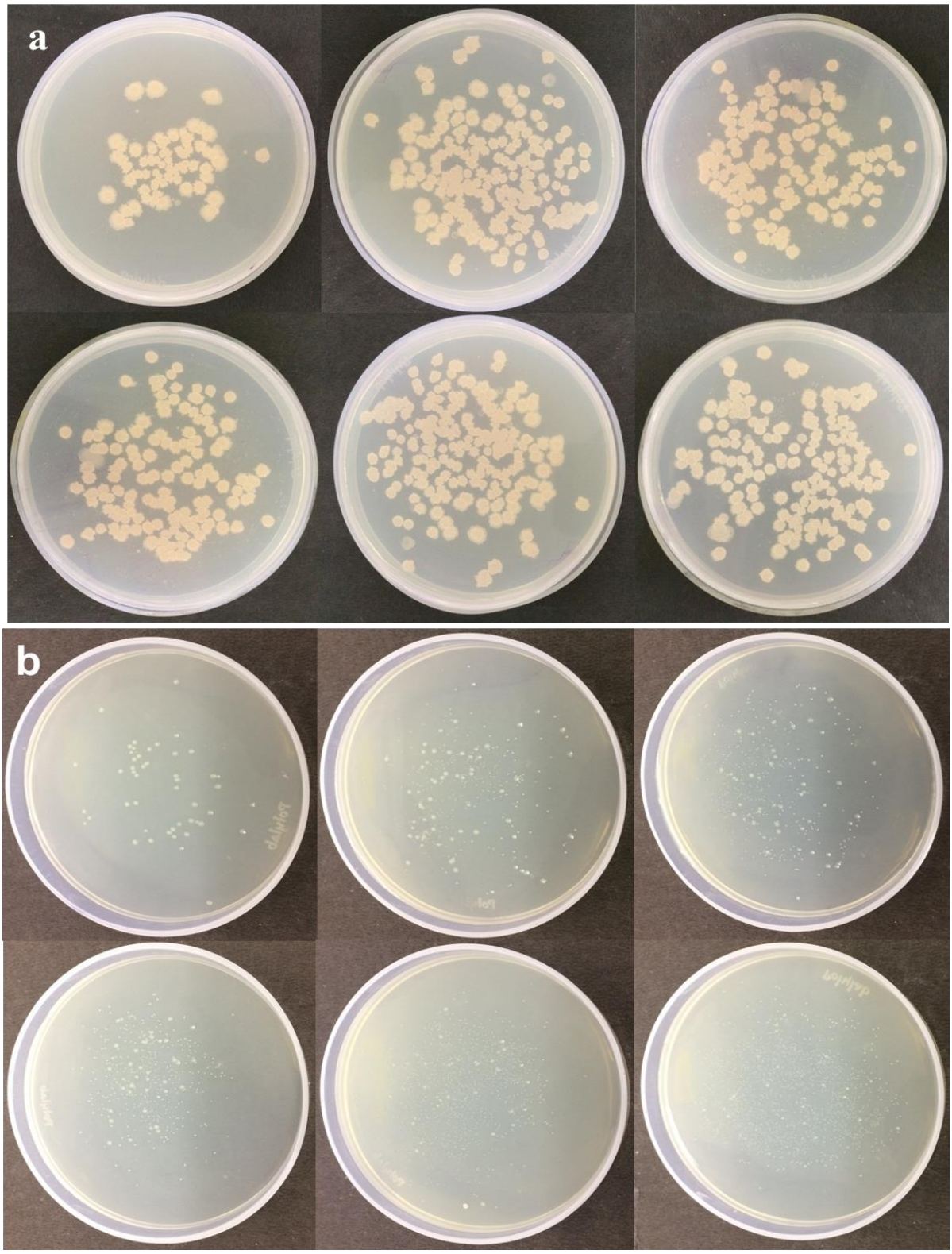


Fig 19: GO-CS treated culture of (a) *Bacillus subtilis* and (b) *E.coli* plated at (L→R) 0 min, 15min, 30min, 60 min, 120 min, 180 min

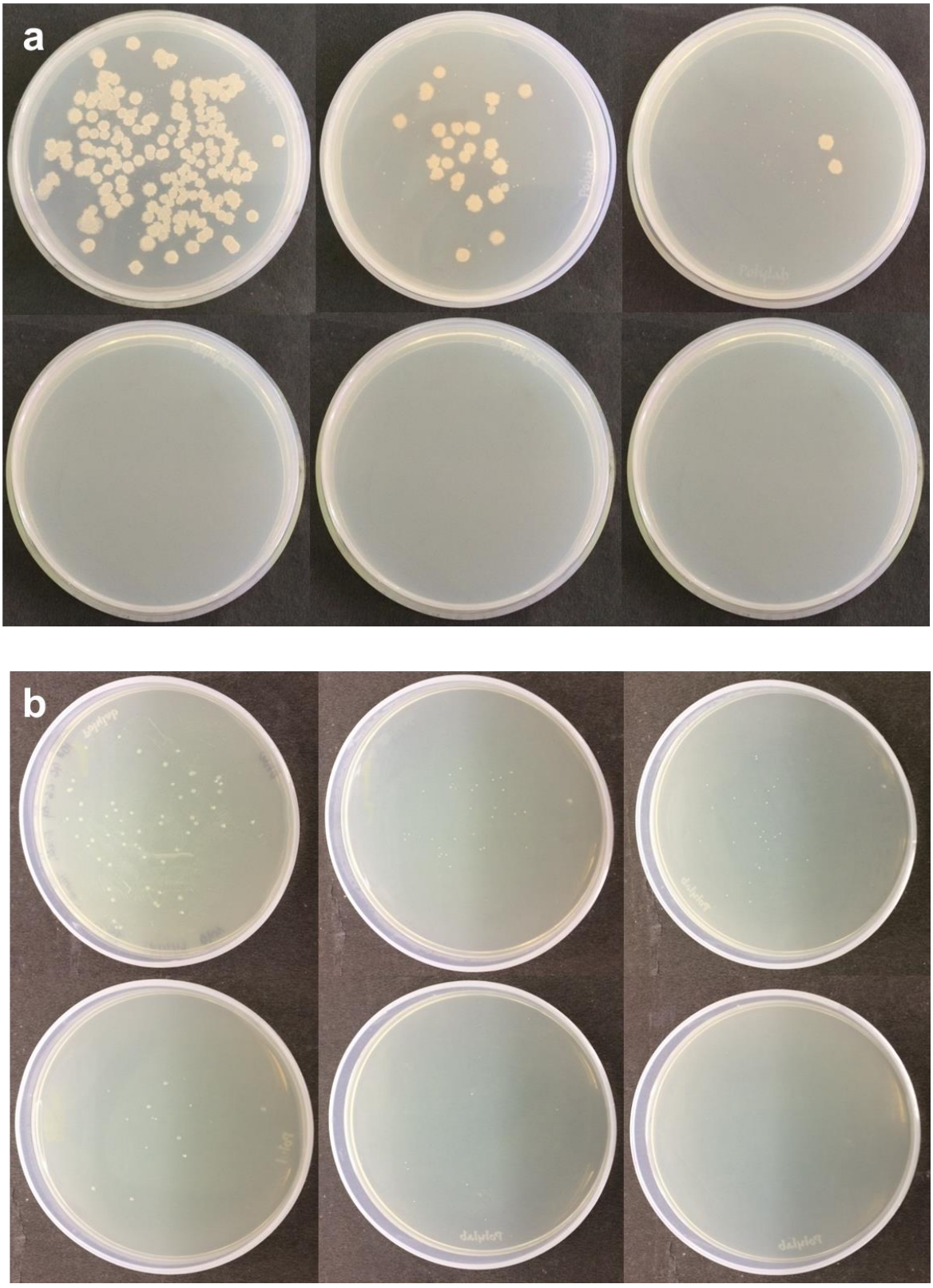


Fig 20: GO-CS-Ag treated culture of (a) *Bacillus subtilis* (b) *E.coli* plated at (L→R) 0 min, 15 min, 30 min, 60 min, 120 min, 180 min

Figure 20 clearly depicts that there is a significant reduction in the growth of colonies with increase in their incubation time with silver loaded hydrogels (GO-CS-Ag).This shows that silver nanoparticles are very effective antimicrobial agents against both gram negative as well as gram positive bacteria. Silver nanoparticles wrapped inside the hydrogel may come out gradually into the medium (Mohan *et al*., 2007) and reduce the growth of microorganisms via mechanism explained earlier. These results are in accordance with the study conducted by Agnihotri et al, (2012).

4.10.2 Growth Inhibition Kinetics

A growth inhibition curve was made in order to draw an inference about the inhibition in the growth of colonies with respect to the time of incubation of microbial solution with both pristine as well as silver loaded hydrogels. A graph is plotted between –Ln N/N_o where N is the number of colonies at time t and N_o is the number of colonies after 0 min of their incubation with hydrogels.

It is quite evident from the growth inhibition curves that GO-CS doesn't show antibacterial effect against any of the two strains tested as the number of colonies increase even after their treatment with GO-CS hydrogel. On the contrary GO-CS-Ag hydrogel act as an effective antimicrobial agent against both *Bacillus subtilis* and *E.coli*. Complete disinfection was achieved after 60 minutes in case of *Bacillus subtilis* and after 180 minutes in case of *E.coli* (Fig 21). The reason behind this low efficacy in case of *E.coli* (gram negative) as compared to *Bacillus subtilis* (gram positive) could be the thick lipopolysaccharide layer present outside the plasma membrane of E.coli which makes it harder for silver nanoparticles to penetrate and kill the cells.

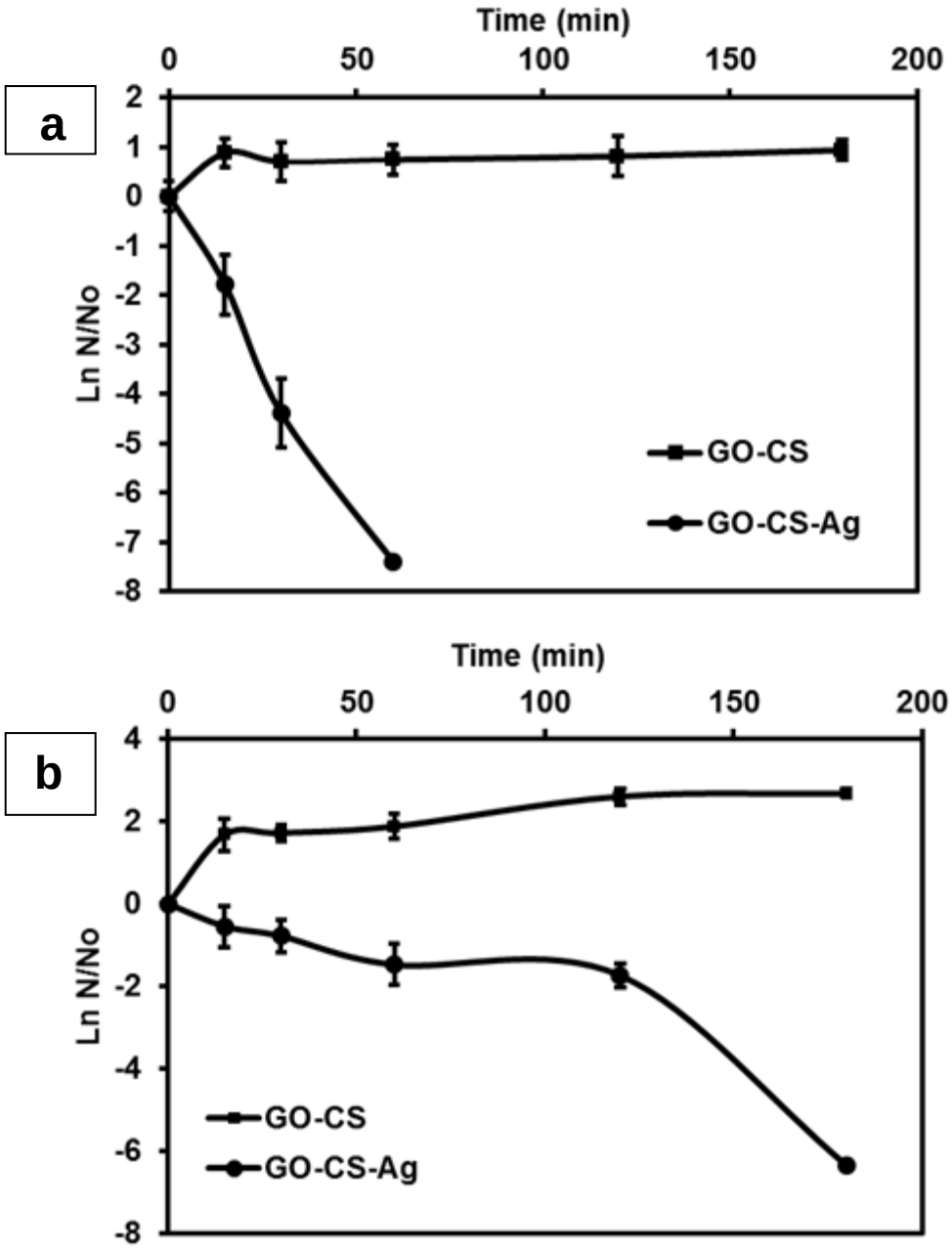


Fig 21: The effect of GO-CS and GO-CS-Ag hydrogels on the growth of (a) *Bacillus subtilis* (b) *E.coli* (Initial bacterial count 10^3 CFU/ml, 100 ml batch reactor)

CONCLUSION AND FUTURE SCOPE

A highly porous hydrogel of graphene oxide and chitosan was synthesized using chemical crosslinking and freeze thawing. *In situ* synthesis of silver nanoparticles was done by chemical reduction of $AgNO_3$ using $NaBH_4$ as a reducing agent. The synthesized hydrogels have excellent swelling capacity with swelling ratios of 83.17 ± 0.63 % and 80.2 ± 0.48% for GO-CS and GO-CS-Ag respectively. The equilibrium swelling is reached in 5 minutes in case of pristine gel but it takes about 10 minutes for silver loaded hydrogel (GO-CS-Ag) to reach swelling equilibrium. Pristine (GO-CS) and silver loaded (GO-CS-Ag) hydrogels were characterized using SEM (Scanning Electron Microscopy) which indicated the formation of stable nanocomposites with silver nanoparticles immobilized in its porous structure. The as-prepared GO-CS and GO-CS-Ag hydrogels exhibited good dye removal property when checked for the removal of methylene blue (model dye). GO-CS hydrogel showed a reduction of 99.7% in 40 ppm methylene blue within 5 hrs of its exposure to sunlight. The introduction of silver into gel although delayed its degradation efficiency, a dye degradation of 98.7% was still achieved after 7 hrs of its exposure to sunlight. Dye removal follows first order model kinetics in both the cases. GO-CS and GO-CA-Ag, both were checked or their antibacterial activity against *Bacillus subtilis* (gram positive) and *E.coli* (gram negative). GO-CS-Ag showed excellent antibacterial activity against both the strains with complete disinfection in short time interval of 60 minutes and 180 minutes respectively. However, GO-CS doesn't show any significant antibacterial activity. By combining the merits of graphene oxide, chitosan and silver nanoparticles a novel approach was presented to obtain a simple, effective and environmental friendly nanocomposite for the treatment of dyes and water disinfection purpose.

This study concluded that the prepared hydrogels can remarkably remove methylene blue from waste water. Further study can be done to see the effect of these hydrogels against various other dyes and industrial waste water containing hazardous pollutants. Also, the prepared hydrogels can be checked for their antifungal properties. Furthermore, a detailed study can be carried out in order to find the reason behind the reduction in dye removal efficiency of hydrogel after silver immobilization which was otherwise expected to increase due to the photocatalytic activity of silver for dye removal

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