Catalytic Degradation of Methylene Blue as a Model Dye using Silver Nanoparticles

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CERTIFICATE

This is to certify that dissertation entitled **"Catalytic Degradation of Methylene Blue as a Model Dye using Silver Nanoparticles"** submitted by Ms. Kashmi Nirmohi in partial fulfilment of the requirement for the award of the degree of **Master of Science** in Department of Biotechnology, Thapar University, Patiala (India) is the record of the candidate's own independent and original research work carried out under our supervision and guidance. The matter embodied in this dissertation has not been submitted in part to any other University/Institute for the award of any degree or diploma in India.

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DECLARATION

I hereby declare that the work which is being presented in dissertation entitled **"Catalytic Degradation of Methylene Blue as a Model Dye using Silver Nanoparticles"** submitted by me for the award of the degree of **Master of Science** in Department of Biotechnology, Thapar University, Patiala is true and original record of my own independent and original research work carried out under the supervision of Dr. Shekhar Agnihotri. Further, I declare that no part of this dissertation has been submitted to any other University/Institute for the award of any degree in India or abroad.

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ABBREVIATIONS

MB	Methylene Blue
NP	Nanoparticle
Au	Gold
Ag	Silver
Fe	Iron
Cu	Copper
ZnO	Zinc Oxide
TiO_2	Titanium Dioxide
Nm	Nanometre
FTIR	Fourier Transform Infra-red Spectrum
TEM	Transmission Electron Microscopy
$AgNO_3$	Silver nitrate
Ag^+	Silver Ions
PVA	Poly Vinyl Alcohol
OD	Optical Density
ppm	parts per million
pH	Potential of hydrogen
$NaBH_4$	Sodium borohydride
UV	Ultra- violet
hrs.	Hours

Dedicated To

My

Beloved Parents

Abstract

For processing of dyes the textile industries uses large amount of water which leads to increase the undesirable toxicity into the water bodies. This toxicity in water bodies is dangerous to humans; decrease the activities of aquatic plants. To get rid of this toxicity there is an advanced oxidation process which is helpful in mineralizing a wide range of dyes in water systems. For removal of dye toxicity, the photo catalytic degradation activity is a practical method of interest. In this work, AgNPs were synthesised by a chemical reduction method and biogenic synthesis. AgNPs bear the desired physicochemical properties in terms of structural, optical, thermal and photo catalytic properties. Now a days, plant mediated synthesis of nanoparticles has great interest and achievement due to its various properties like it is eco-friendly, cheap, non-toxic and has low time consuming properties. In this study, the leaf and fruit extract of *Aegle Marmelos* (bael) were used for the synthesis of silver nanoparticles for removal of toxicity of dyes and also by chemical methods. The aqueous leaf and fruit extract was put into the silver nitrate solution results in the change of colour form yellow to reddish brown which indicates that there is a reduction of silver ions in silver nitrate solution. Thus various forms of synthesized AgNPs were characterized by UV-Vis spectrophotometer and FTIR spectrum, which was used to identify the functional group responsible for the reducing and capping of synthesized AgNPs. AgNPs have catalytic redox properties for dyes. The photo catalytic activity of the various forms of synthesized AgNPs was observed by degradation of methylene blue (a toxic dye) under sunlight. Synthesized silver nanoparticles formed by plant extract were effectively helps in degrading the MB dye nearly 84.4% at 6 hrs of exposure time under sunlight.

Chapter 1: Introduction

The major and most hazardous threats to the modern world is a pollution which causes by the industries, for e.g. textiles, pulp and paper mills, distilleries and tanneries, where the processing of chemicals leads to generation of large quantities of coloured wastewater, which is eventually discharged into waterways. The colour of wastewater is attributed due to the presence of toxic dyes, which contains a chromophore, *i.e.* colour causing group (Mahmoodi *et al.*, 2011). These industries however may differ in the composition of colored compound present in wastewater, where even a trace amount of dye is enough to impart colour to it. After China, Our country is the second largest in World which exports the dyeing stuffs, where 80% of the total dyes is used in textile industries alone (Ghaly *et al.*, 2014). Previous studies say that worldwide, out of $1x10^6$ tons of dye produced per year, nearly 1-1.5 x 10^6 tons of dye is discharged into ecosystem through wastewater (Zollinger, 1987). Among all industries, the textile industry contributes towards the highest discharge of dye effluents to the aqueous ecosystem which should not be acceptable among ecological level (Wang *et al.*, 2007). The effluents from industries containing dyes in reduces the sunlight penetration in water bodies and also affects the photosynthetic activities of aquatic flora and in turn affecting the food source of aquatic life forms. The presence of even minute amounts of dye in water causes deterioration in water bodies also reduces the solubility of dissolved oxygen there by increasing the biological oxygen demand of water, enhances the turbidity of water and destroys the food web of that particular area (Suteu *et al,* 2012).

Moreover, the effluent of dye components from industries forms a thin layer on the surface of water bodies thereby deteriorating the aquatic flora and fauna. Since dyes are highly complex, stable toxic pollutants in the environment over a longer duration, there is a high scope for exploring and developing various methods for their degradation.

Till date, there are various techniques which are used for the removal of toxic and unnatural dyes from water bodies so as to minimize the risk of toxicity to the ecosystem. These techniques comprise the physical method such as membrane-filtration process (Nano filtration, electro dialysis, reverse osmosis) and sorption techniques. Limited life spans of membrane, fouling of membrane, cyclic replacement of membrane are the major drawbacks of membrane filtration process.

It was reported that adsorption is an effective and best equilibrium process for the removal of decontaminants from the wastewater. The chemical method involves coagulation or flocculation, often integrated with filtration, ozonation, irradiation, adsorption, photolysis, ion pair extraction. These chemical methods are useful but uneconomical for the degradation of dyes from the water bodies, so they are not in much used and also having one drawback of discarding problem. Due to these techniques there is an aggregation of the sediment particles. These techniques are used for waste water treatment purposes having different pollutants in them, but are not that much in use because of high energy demand and large utilization of the chemical reagents. While the microbiological methods can be used such as aerobic as well as anaerobic degradation, and with the help of pure enzymes. But these biological methods take so much time to degrade the dyes partially or fully and cannot degrade complicated dyes (Can *et al.,* 2006). All of above methods have positive and negative effects. These methods are very expensive and demands high set-up costs and unable to meet the strict effluent colour standards. Biodegradation process requires strict conditions to be maintained and sometimes become difficult to operate at large scale. (Bhole *et al.,* 2003).There is an urge to degrade the presence of dyes by using emerging technology i.e., Nanotechnology shows promising potential for catalytic degradation of dyes.

The current research involves use of metallic nanoparticles, i.e., silver nanoparticles for the degradation of methylene blue as a model dye for assessing its photo catalytic potential. The silver nanoparticles were synthesized by two routes, one using chemical reduction approach and another through biogenic synthesis, involving extracts of *Aegle Marmelos (*Bael). The synthesized AgNPs were characterized through techniques, which includes i.e. UV- Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Transmission electron microscopy (TEM). The catalytic activity in the presence of sunlight of silver nanoparticles was studied at different conditions and finally the rate and extent of decolourisation and degradation was modelled using existing equations.

Chapter 2: Review of literature

2.1. Nanotechnology

The first ever concept was presented in 1959 by famous prof. of physics Dr. Richard Feynman. The term "Nanotechnology" was coined by an eminent scientist Prof. Norio Taniguchi in 1974. "Nanotechnology" is a technology of applied science which works in the field of theoretical as well as experimental changes at molecular level of compound. The word "nano" derived from the Greek word "nano", which means extremely small at nano level. The prefix "Nano" means a size of 10^{-9}. It allows the arrangement of small structures with accuracy, intelligibility and in expensive. "K. E. Drexler" is known as the father of Nanotechnology. He is the man who explains the nanotechnology in depth and popularized the subject. He is an American engineer known for increasing the value of molecular Nanotechnology. Nanotechnology has a breakthrough as a multidisciplinary scientific field and is undergoing uncontrollable development (Williams *et al.,* 2004; Chan *et al.,* 2006). The products of Nanotechnology are nanoparticles, nanotubes, and Nano rods, Nano spheres that have size below 100nm and have high surface area to volume ratio. The minimization of size brings about marked changes in their morphological properties with respect to those properties observed in massive materials. The products of nanotechnology can be of metallic nature, having mineral and polymer-based materials (Rana and Kalaichelvan, 2013). Nanotechnology is a big tree of research, having branches in every dimension and touches different fields like cosmetics, electronics, packaging, bio-sensor, medicine and paints, automobile, bio-engineering and catalysts. In Canada, there are 80 companies that make 150 products that use 88 different nanomaterial.

Nanotechnology has the power which contributes to check the long-term water quality issues and also uses the advanced filtration materials that enable greater water reprocess, and desalination. There are other water treatment technologies which have made an immense enhancement in recent past by using nanotechnology for handling the contamination problems and making advancements in future (Sushma *et al.,* 2015). Among waste water treatments, the catalytic degradation of dyes and other hazardous organic compounds is yet another domain where the application of nanotechnology has shown a great success.

2.2. Nanotechnology in Wastewater Treatment

Nanotechnology has emerged as one of the leading new innovative technologies with a great potential for treating wastewater in a more effective and efficient manner than methods that have been previously used. In terms of wastewater treatment and water purification, methods involve high costs and are not completely effective in purifying the water. Nanotechnology can help to overcome this issue. Heavy metal pollution poses as a serious threat to environment because it is toxic to living organisms, including humans, and non-biodegradable. In the area of water purification, nanotechnology offers the possibility of an efficient removal of dye effluents and germs. Today, nanoparticles, nano membrane and nano powder are used for detection and removal of chemical and biological substances. Nano materials reveal good result than other techniques used in water treatment because of its high surface area to volume ratio (Tiwari *et al.*, 2008).

2.2.1 Catalytic degradation of dyes

Sunlight is a natural energy source that is abundant and can be exploited for degradation of dye effluent. If we compared to other techniques, then solar light is the best and fast in decolorizing of dye in the presence of metal catalyst (Kansal *et al.,* 2008)**.** Nano catalysts are widely used for the active removal of dye contaminants. Different type of nano materials like TiO_2, ZnO and metallic nanoparticles (Au, Ag and Cu) are used for the catalytic degradation of dyes.

TiO_2: Titanium dioxide (TiO_2) or titanium is a well-known photo catalyst material. TiO_2 micro particles are less toxic, less resistance and less corrosive than other photo catalysts (Lee *et al.*, 2009). TiO_2 oxidation is also well- known as a type of "green" or “environment friendly” method.

TiO_2 nanoparticles are used broadly, because they have high intensity as well as very high refractive index. Dopants like Zn and Cu are used to observe the efficiency of TiO_2 micro particles on degradation of dyes. Doping technology is used to organize metal ions to enhance photo catalytic effects of TiO_2 nanoparticles. This photo catalytic reaction takes place at room temperature and the intermediate products show less toxicity because they are completely converted to CO_2.

Titanium dioxide have excellent photo catalytic property and catalyst are designated to be

Capable substrates for photo degradation of dyes water pollutants as they exhibit the acceptable activity in the range of ultraviolet radiation (Mondal *et al.,* 2013). The photo activated reactions triggered by the photon interaction by the mechanism of free radical at a proper energy level with TiO2 acting as a semiconductor catalyst (Turchi *et al.,* 1990).

ZnO: ZnO nanoparticles showed superior photo degradation efficiency and having recycling potential, having economic value and it is a highly sunlight-active material used to treat wastewater contaminated with azo dyes. One dimensional nanostructure like nano-wires, nano-rods, nano-spheres and nano fibres are preferable materials photo catalysis because they are having very high surface area to volume ratio (Wu *et al.,* 2004). Zinc oxide is a best natural n-type semiconducting material with wide band gap and having binding energy (60 meV). These ZnO are in abundance, and its nontoxicity nature helps in photo catalytic activity (Jin *et al.,* 2008). It can absorb maximum range of spectrum of radiations which makes it more useful for dye sensitized solar cells and solar photovoltaic applications. The n-type ZnO nanostructured material makes a multifunctional membrane and is very helpful in removing water contaminants by amplifying the photo-catalytic activity under the presence of visible light. It also showed water purification by exhibiting the anti-bacterial activity. With these unique characteristics, zinc oxide is one of the most significant nanomaterial and has wide range of applications.

Metallic nanoparticles:

Ag, Au and Cu are some metallic nanoparticles which plays important role in the dye degradation in which solution is decolorized. The metal nanoparticles have wide range of properties like magnetic, electronic and optical properties, which are used in different fields like agriculture, medicine and electronics (Rai *et al.,* 2012). Metal nanoparticles possess a large surface area in which it acts like a substrate for the electron transfer reaction. Just before the electron transfer reaction, both reactants are adsorbed upon the surface of the metal. Consequently, there will be gain of an electron by reactants and then reduced. Thus, through the electron transfer process silver nanoparticles will act as an efficient catalyst (Ghosh *et al.,* 2002; Wu *et al*., 2010).

Silver nanoparticles are extensively used in water filters (Jain and Pradeep, 2005) and biosensors (Chen *et al.,* 2007). In recent times, metal nanoparticles were reported as effective photo catalysts for degrading the chemical complexes, under the surrounding temperature with visual light ray (Mohamed *et al.,* 2012). This can be obtained by increasing the optical path of photons which leads to a larger rate of absorption of the nanoparticles under the influence of electric field (Garcia, 2012).

These nanoparticles have unique properties as compared to the bulk materials i.e. nanoparticles are based upon on their morphological structures and characteristics (Nathan *et al.*,2009). There is study by Wang *et al.,* (2008) in which he stated that AgNPs are superior, have great efficiency and durable photo catalysts under the atmospheric temperature under the visible light rays for the degradation of dyes and organic compounds. Furthermore, scientists have shown the great interest in the photo catalytic degradation of dyes by using nanoparticles. Biosynthesis of silver nanoparticles by marine plants and sea-weeds has been found to be cost effective, reliable and eco-friendly (Kumar *et al.,* 2012). Altogether, the photo catalytic properties of silver nanoparticles under the influence of visual light may be well because of excitation of surface plasmon resonance (SPR), which is nothing but it is a oscillation having a charge density that can proliferate at the junction between the dielectric medium and metal surface (Garcia, 2012).

Metal nanoparticles also help in the transfer of electrons from the donor to acceptor. Thus, nanoparticles acquire a large surface area, which acts like a substrate for the electron transfer reaction. Both of the reactants are adsorbed on the metal surface before the electron transfer reaction. More significantly, AgNPs showed very fast and rapid degradation with sustained reactivity compared to other degradation technique like biodegradation, and making them potential candidates for dye degradation technologies.

2.3. Mode of Synthesis of nanoparticles

The synthesis of metallic nanoparticles is an active area of applied research in nanotechnology and it is academically significant. Various methods have been implied for the synthesis of the nanoparticles. These technologies used "top-down" and "bottom-up" approaches.

2.3.1. Top-Down approach

In Top-Down approach, the nanoparticles are broken into small particles. Attrition or milling is a typical top-down method in making nanoparticles. In the top-down approach micro fabrication methods were used, in which apparently composed tools are used to mill , cut and also shape the materials into a desired model and system.

Micro patterning is a technique which includes inkjet printing and photolithography. There is a best example of this approach in which through aerosol techniques it shows the formation of powder components (Wu *et al.,* 1993) and then the completion of the components into the end product. Therefore, these techniques have been used largely in the formation of shape composite materials. One of the "top-down" approaches originates with a suitable starting material and then shape the functionality from the inner material. This method is very close to the method which is used in the semi-conductor industry for the formation of devices out of an electronic substrate like silicon, utilizing the pattern formation such as electron beam lithography and other is pattern transfer method such as reactive ion etching that have their quite spatial resolution to obtain the formulation of various structures at the nano-scale level.

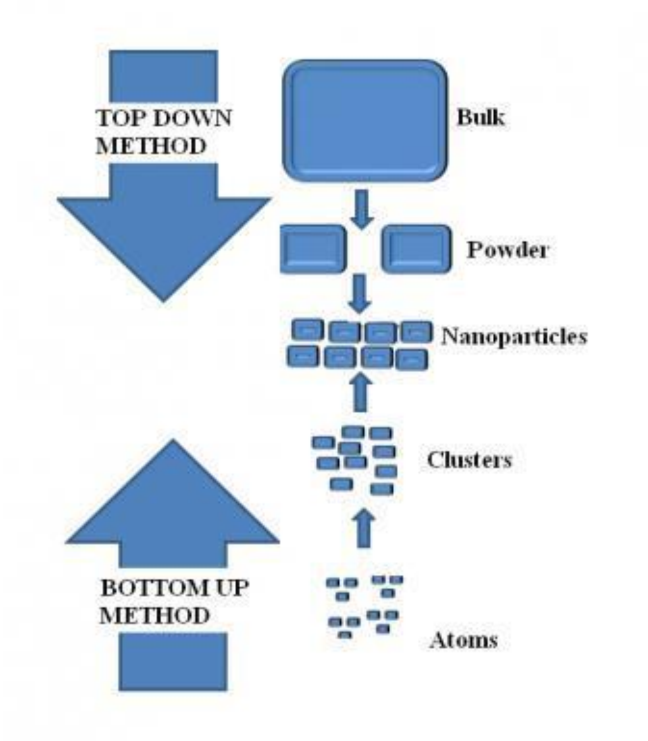

Fig. 1: Different approaches for synthesis of nanoparticles

There is a tremendous scope in the area of nanostructure formation, warranting in its own separately study, having driving issue in the electronics industries, and also will not be a major theme of this work. Another method in top-down approach is “ball-milling” in which the creation of building blocks of nanostructure through controlled, mechanical grinding of the starting of the bulk material (Koch *et al.1989*). Those formulated blocks of nano building are then consequently put together into a newly formed bulk materials.

2.3.2 Bottom-Up approach

The “Bottom-Up” approach refers to the formation of material by connecting atoms by atoms, molecules by molecules and clusters by clusters. There is a best example of this method in which the formulation of powder constituents through the aerosol technic (Wu *et al.,* 1993) and then the compression of the components into the end product. It involves different methods such as spinning, use of templates, biogenic synthesis and supercritical fluid synthesis. These methods have been used broadly in the forming of structural composite material. Spinning is another process which is used to produce supreme molecules based upon the principle in which spinning the liquefy polymer solutions which acts under the high-rise voltage electric field from the aqueous solution which are generally in micro or nano scale (Luther *et al.,* 2004). Natural biomass is also used as a reducing agent and stabilising agent, for the biogenic method for the synthesis of different metallic nanoparticles like gold, silver, metal oxides, etc. Bottom-Up method in comparison, uses the chemical properties of individual molecule to cause individual molecule unit to (1) self-assembled or self-organized into a some proper structure and (2) confide on areal accumulation. The concepts of molecular self- assembled as well as molecular recognition both are utilized in these approaches. Like in Bottom-Up approach should be able to produce the devices side-by-side is inexpensive as compared to the Top-Down approach however, they are also potentially get affected as the complexity and area of the desiderate assembly expanded.

2.4 Chemical Reduction Method

In this method chemicals are used for the synthesis of nanoparticles. The best method for the preparation of silver nanoparticles is chemical reduction method, in which nano-particles get more stability as well as easily get dispersed in water. Citric acid, Borohydride, Ascorbate, is the chemical reducing agent. In chemical reduction method, silver ions get reduced in a liquid solution mainly collects the colloidal silver (Ag) with particles having width of different nano-metre. To synthesize stable AgNPs with different shapes there are some reducing agents like, citric acid, ascorbic acid, , hydrazine ,sodium borohydride and ethanol etc. (Wang *et al.*, 2010, Khan *et al.*, 2009, Solomon *et al.*, 2007, Pastoriza *et al.*, 2000)which help in reduction of silver ions to form silver.

To reduce the silver ions weak and strong organic substrates are used which is totally depends upon the size distribution and shape. During the process of metal nanoparticle preparation, it is necessary to use the stabilizer to stabilize the NPs that can be consumed on and can bind up on to the surface of NPs and help in avoiding the clusters (Oliveir *et al.*, 2005)

There are some useful stabilizing agents like PVA (poly vinyl alcohol), PVP (poly vinyl pyrrolidone), citrate and PEG (polyethylene glycol) to stabilize the nano-particles. Some changes in nano-particle size distribution width, stability and structures are due to the changes in the artificial agent is reported by Brust *et al* (2002).

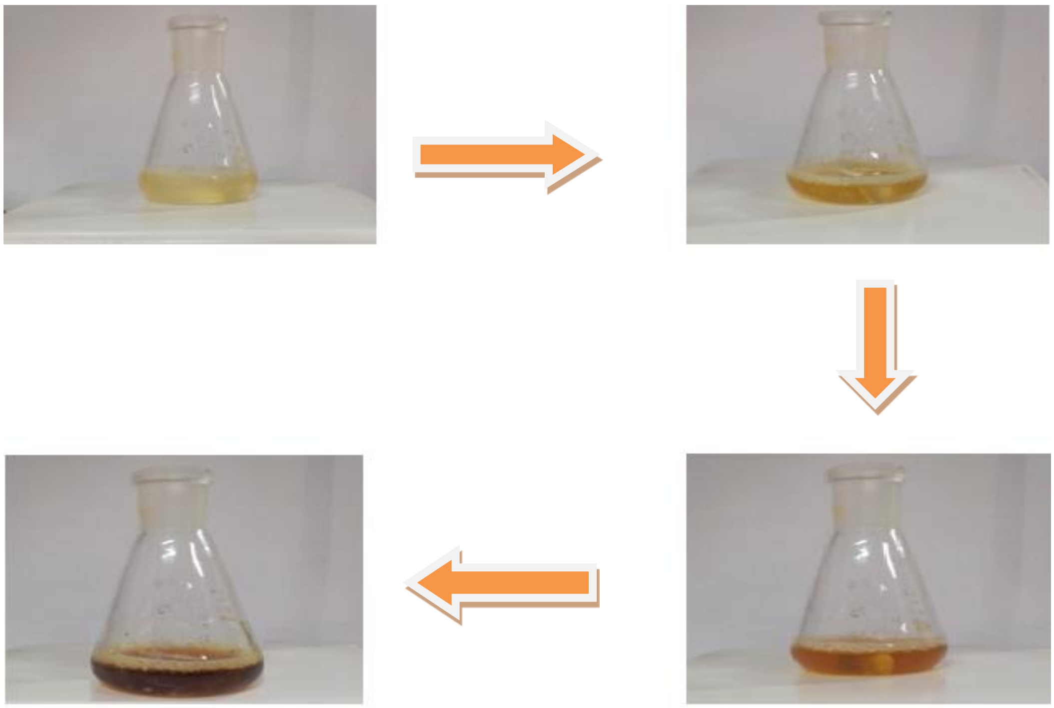

Fig 2: Process of synthesis of AgNPs by chemical method

2.5 Biogenic synthesis of nanoparticles

"Green Nanotechnology" is the new term arisen with a lot of consideration that reduces or eradicates toxic substances to be released into environment (Moghaddam *et al.,* 2010). For the synthesis of nano-particles, plants are good source for this process. Biologically synthesized nanoparticles are non-toxic, clean, environmentally acceptable, doesn't need high temperature and hazardous chemicals. As we compare to the chemical and physical method their yield is high (Rai *et al.,* 2008). Metals were used to synthesize particles of nano size. Biological synthesis can be done by plant mediated synthesis (bark, roots, stem, fruit, leaves and seeds.) and microbial synthesis (bacteria, fungi and algae).

BACTERIA: This has been explored in the synthesis of AgNPs. For the biosynthesis of nano-particles within the bacterial cells, there is strain *Lactobacillus* which helps to exposed to silver ions for the synthesis process (Korbekandi *et al.,* 2012; Nair *et al.,* 2002). It has examined that to grow nano particles of silver; there should be the direct contact of lactic acid bacteria which is present in the whey of buttermilk to solution of silver ions. The nucleation of AgNPs present on the surface of cell through the enzymes in the cell wall and then the metal nuclei were transferred in the cell, where they formed into clusters and also grew to large-sized particle. The researchers have been stated that bacteria *Lactobacillus sp*. was used for the production of AgNPs, but they were not able to determine the current process. Silver nano particles which are biosynthesized were found to be mostly spherical in shape, single i.e.25-50 nm and may be in clusters i.e. 100 nm, joined to the surface of a biomass and also were present in outside and inside of the cell. During the enzymatic process, the synthesis of AgNPs was verified by metal ion reduction as well as stabilization. Through Electron microscopy search, shows that the nano particles of silver were arranged upon the cytoplasmic cell membrane surface, outside of the cells and inside the cytoplasm, likely because metal ions get reduced by the enzymes ,which are when present on the membrane of cytoplasm and also inside the cytoplasm (Korbekandi *et al.,* 2012).

FUNGI: *Aspergillus flavus* is a fungus which is used to form more stable nano particles of silver. The nano particles formed by this were found to be very stable in aqueous minimum for 2 months with no cluster formation because of the surface binding of stabilizing the materials which is secured by the fungus only (Vigneshwaran *et al.,* 2007). In the biosynthesis of AgNPs size 5-25 nm by using *Aspergillus fumigatus* has also been examined. Mostly the shape NPs were found to be spherical in nature but some time it may be in triangular shapes (Bhainsa *et al.*, 2006).

ALGAE: *Chaetoceros calcitrans*, *Tetraselmis gracilis***,** *Chlorella salina* and *Isochrysis galbana* are the marine algae which helps in the reduction of Ag+ and therefore in the synthesis of nano particles of silver (Shahverdi *et al.*, 2007). Silver Nitrate liquid solution was incubated with properly rinsed marine cyanobacteria which get turned to yellow in colour from 72 hrs. onwards, this shows the formation of Ag nano particles. When *Spirulina platensis* biomass was exposed to 10^{-3} M aqueous $AgNO_3$ then extracellular formation of spherical AgNPs size of 7-16 nm has been identified around 120 hrs. at temp.37 °c and pH 5.6 (Govindaraju *et al.*, 2008). For the reduction and stabilization of the nano particles, proteins play the major role in this process.

Plant mediated:

This is reported that the plant extracts contain several biomolecules such as ascorbic acid, flavonoids, polyphenol, sterols, triterpenes, alcoholic compounds, polysaccharides, alkaloids, saponins, β-phenylethylamines, fructose, glucose and proteins/enzymes these can be used as a reductant to react with Ag + and however is a best platform for the synthesis of silver nano particles in a aqueous solution (Sharma *et al.*, 2007). Plant extracts may be performing as both reducing and stabilising agent for the formation of nano particles. Plants help to produce metal nano particles which are much stable as compare to the other organisms. Plant extracts can able to reduce the metal ions faster than that of bacteria and fungi. In the biosynthesis process solid spherical Ag NPs having diameter 0.5 nm to 100 nm have been produced at various concentration of silver nitrate. Commonly silver ions get reduced in aqueous solution and produce different nano-meter diameter of colloidal silver. Silver shows the highest surface plasmon resonance band intensity than any other NPs like copper and gold. For synthesis of Ag NPs various kinds of approaches using extract of plant is being used as shown in Table 1.

Herein, the silver nanoparticles were synthesized by using plant *Aegle Marmelos* commonly known as *Bael*; it is an important medicinal plant of India. It has anti-microbial, anti-fungal, Anti-inflammatory, anti-cancer, anti-diabetic and catalytic properties. A large amount of components has been taken out from different parts of Bael tree and some of them are used for study for their biological activity Table 2.

Leaf:

The leaves of *Aegle Marmelos* contain several bioactive compounds including essential oils, phenolic, alkaloids, condensed tannins, anthocyanin and flavonoid, marmesinine, glycoside, skimmianine, aegelie, cineol, citral, lupeol, citronella, eugenol and cumin-aldehyde (Maity *et al.*, 2009). s. The leaves are astringent, laxative and an expectorant are useful in treatment of inflammations, diarrhoea, dysentery, heart palpitation, and asthmatic complications (Rastogi *et al* 1991).

Fruit:

On the other hand bioactive compounds present in Bael fruits are tannins, marmelosin, aurapten, psoralen and maemelide (Krupa *et al.,* 2014**).** Marmelide is very effective against viruses and is found to influence the early stages of replicative cycle such as adsorption, penetration, etc. (Badam *et al.,* 2002). Tannin, present in the unripe fruit of this plant, has astringent property and is best remedy for diarrhoea (Vidhya *et al.*, 1991)

Bark:

Among the bioactive compounds isolated from bark are fagarine and marmin. Fagarine is present in the mature bark and possesses abortifacient activity (Takase *et al.*,, 1999). Marmin is found in the immature bark of the tree (Duke e*t al.*, 1992).

Table 1: Biogenic synthesis of metallic nanoparticles and their application

Species	Plant part	Nanoparticle	Applications	References
Trigonella foenumgraecum	Seeds	Ag	Catalytic property	Vidhu *et al.,* 2014
Helicteres isora	Leaf	Ag	Catalytic property	Bhakya *et al.,*2015
Coriandrum sativum	Leaf	Au	Antibacterial property	Narayanan *et al.,* 2008
Boswellia serrata	Gum	Ag	Antibacterial property	Kora *et al.,* 2012
Morinda tinctoria	Leaf	Ag	Catalytic property	Vanaja *et al.,* 2014
Kalanchoe pinnata	Leaf	Ag	Catalytic property	Phatak *et al.,*2015
Rosa hybrida	Petal	Au	Antibacterial	Noruzi et al.,2011
Parthenium	Leaf	Au	Antibacterial activity	Parashar et al.,2009
Paederia foetida	Leaf	Ag	Anti-inflammatory	Mollick *et al.,*2012
Murraya koenigii	Leaf	Ag	Antibacterial property	Bonde*et al.,* 2012
Ulva lactuca	Seaweed	Ag	Catalytic property	Kumar *et al.,*2012
Terminalia chebula	Fruit	Ag	Catalytic property	Jebakumar *et al.,*2012
Cinnamon zeylanicum	Bark	Ag	Antimicrobial property	Kumar *et a*l.,2009
Cycas	Leaf	Ag	Antimicrobial property	Jha *et al.,* 2010
Euphorbia hirta	Leaf	Ag	Antibacterial property	Elumalai *et al*.,2010
Mentha piperita	Leaf	Ag	Antibacterial property	Ali *et al.,*2011
Terminalia chebula	Fruit	Ag	Catalytic property	Jebakumar *et al.,*2012
Tangerine	Peel	Ag	Catalytic property	Alzahrani, 2015
Casuarina equisetifolia	Leaf	Ag	Catalytic property	Saranya *et al.,*2016
Azadiracta indica	Leaf	Fe	Catalytic property	Elavarasi*et al.,*2015

Table 2: Bioactive compounds isolated from various parts of *A.Marmelos* (bael).

Bael Compound	Plant part	Property	Reference
Marmelosin	Fruit	Antibacterial	Ghosh *et al.*, 2003
Tannin	Unripe fruit	Anti-diarrhoea	Ghosh *et al.*, 2003
Marmelide	Fruit	Antiviral	Jagetia et al 2005
Marmin	Immature bark	Antiulcer	Hajra *et al.*, 1997
Citral	Leaf	Anti allergic	Rao *et al.*, 2001
Cuminaldehyde	Leaf	Antibacterial	Jagetia *et al.*, 2004
Eugenol	Leaf	Antioxidant	Geetha *et al.*, 2001
Aurapten	Fruit	Heart beat inhibitor	Rastogi *et al.*, 1995
Luvangetin	Fruit	Antiulcer	Lapasso *et al.*, 2000
Aegelin	Leaf	Cardio active	White *et al.*, 2004
Lupeol	Leaf	Anti-inflammatory	Rastogi *et al.*, 1998

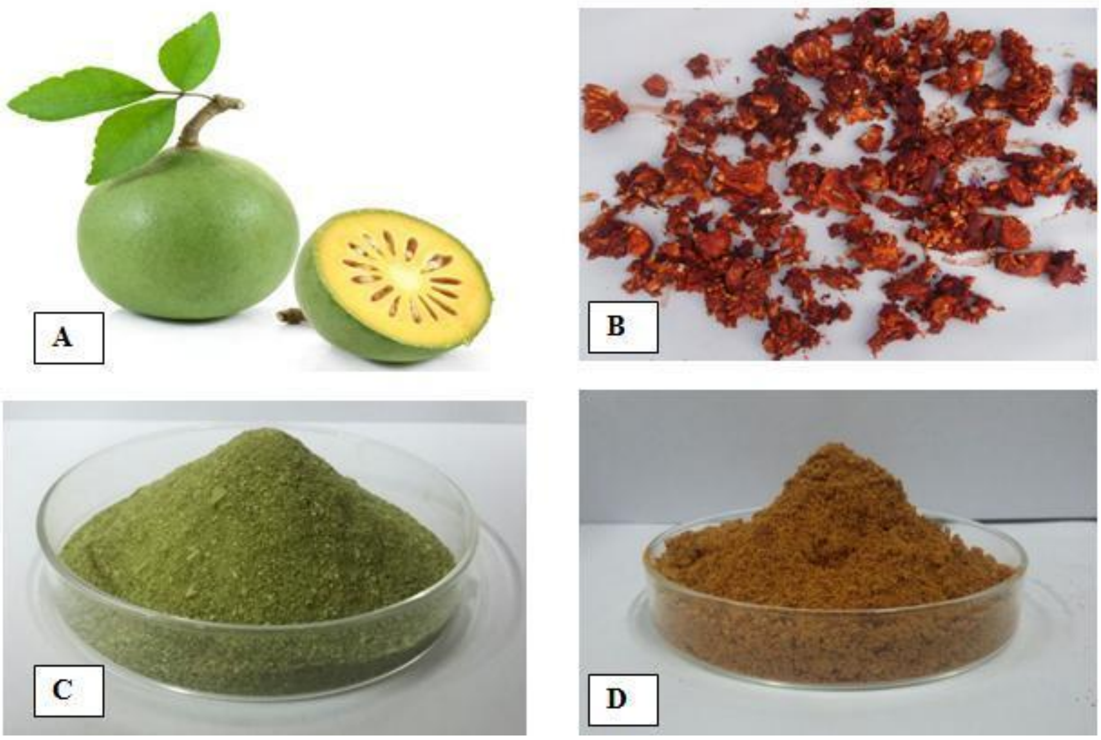

Fig. 3 Bael fruit and leaves (A) Fresh Bael fruit with leaves (B) Oven dried pulp of Bael fruit (C) Bael leaf powder (D) Bael fruit powder. (Image (A) source: http://easyayurveda.com/2012/11/26/bael-benefits-full-ayurvedic-description-about-root-fruit-and-stem/)

2.6. Characterization of Nanoparticles

The technological application of nano particles usually depends upon on their surface. It is then difficult to manage the surface and thus the properties of individual particle. There will be the quantitative as well as qualitative analysis of the surface of individual nano particle (Borm *et al.*, 2006). The following methods are employed to characterize the nanoparticle

UV-vis Spectroscopy:

In this type of spectroscopy, the electronic structure of atoms, , molecules ,ions or crystals through excitation of electrons from the ground state to the excited state i.e. Absorption and resting from the excited state to ground state i.e. emission are used for the persistence in UV-vis Spectroscopy. This is the study which deals with electronic transitions between orbital or band of atoms, liquid and solid states, ion or molecule in gaseous state (Jorgensen, 1962). Hence, unique optical properties of nano particles can be studied by UV-Vis spectroscopy (Link and El-Syed, 1999; Burda *et al.*, 2000).

It is used to confirm the reduction and formation of AgNP of Ag ions the aqueous fruit and leaves extract of *A. marmelos* treated with 1mM $AgNO_3$solution was monitored by UV-Vis absorption spectrum. Light wavelength from 200–800 nm is usually used for identification of different metal nano particles having size ranges from 2 - 100 nm reported by Feldheim and Foss 2002. An absorption peak centred at 405 nm characteristic of AgNPs was observed.

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FTIR: In Fourier transform infrared spectroscopy; the vibrational technique includes the interplaying of photons with the species in a given sample which shows transfer of energy to or from the samples through de-excitation or vibrational excitation is exploited for characterization (Nakamoto *et al.*, 1997). These vibrational frequencies provide the facts of chemical bonding in the sample. According to this, in a molecule the vibration of chemical bonds vibrates at different frequencies which depend upon the elements as well as types of bonds. FTIR spectrum of a synthesized AgNPs which shows the possibility of bio-molecules present in the fruit and leaves extracts which are totally responsible for the reduction of Ag+ and its interaction with nano-particles of silver. Thus, from the FTIR spectrum, it may be verified that the biomolecules have major role in bio reduction and in stabilization of nano particles of silver. This type of spectroscopy is helpful for recognising the surface chemistry (Chithrani *et al.*, 2006). The Organic functional group (e.g. Hydroxyls, carbonyls) connected to the surface of the nano particles as well as another surface chemical residual can be identified by using the FTIR.

2.7. Advantages of the study:

This study provides many advantages in view of eco- friendly approach:

1. Green synthesis may be helpful to be pollution free, eco-friendly by avoiding the use of hazardous chemicals to prepare silver nanoparticles.
2. Application of solar irradiation acts as a catalysing agent in the photochemical reaction of plant extract and silver nitrate solution which is cheap.
3. Photo catalytic studies of methylene blue by photosynthesized silver nanoparticles will be envisaged to degrade organic toxins in wastewaters through photo catalytic reaction.

2.8. Objectives

1. To assess the Catalytic degradation of Methylene Blue dye using AgNPs.
2. Characterization and Synthesis of AgNPs synthesized through chemical as well as biological routes
3. Compare the catalytic potential of nanoparticles synthesized using chemical and biogenic routes.
4. Compare the photo catalytic performance of silver nanoparticles in presence and absence of sunlight.

Chapter 3: Material and Methods

3.1. Materials required

Silver Nitrate ($AgNO_3$) was obtained from Sigma-Aldrich chemicals; polyvinyl alcohol (PVA) was procured from Hi-media laboratories India, Sodium borohydride ($NaBH_4$) was taken from Sigma-Aldrich chemicals, and Methylene Blue dye supplied from Loba chemie Pvt Ltd India. The healthy fruit and leaves of *Aegle marmelos* (Bael) were collected from New Shimla, sector-IV (Himachal Pradesh). All the glassware was cleaned using Aqua regia solution, followed by rinsing thrice using distilled water. The glassware were dried in oven at 60ºc for 2 hours and then stored in a dry container.

3.2. Preparation of Leaf extract:

Freshly leaves were collected and washed thoroughly with tap water and then by distilled water in order to wipe out the dust particles after this, they were subsequently dried for 2-3 days at room temperature. Leaves were crushed into fine powder using mixer grinder. About 4grams of powder of leaves was weighed and mixed with100ml of Milli-Q water. The mixture is boiled for 30 min under stirring condition at 250 rpm using magnetic stirrer at temperature 65°C. After boiling for desired duration, mixture was cooled and filtered using with Muslin cloth followed by Whatman paper (125mm).The filtrate was finally collected and then stored at 4^0C till further analysis.

3.2.1. Synthesis of AgNPs using *A. marmelos* Leaf extract:

Biogenic synthesis of silver nanoparticles using *A. marmelos* was performed using a method given by John Samuel *et al.*, 2015with a few modifications. In brief, 10ml of aqueous leaves extracts were added in a cleaned 250ml of a dry conical flask. An aliquot (10ml) of 10^{-3} M $AgNO_3$ solution was added drop wise into the leaf extract solution and the entire solution was kept in incubator shaker at 37˚C for 72 hrs under dark atmosphere for bio reduction process to be continued. After 3 days, the synthesis of AgNPs was identified by visual change in colour i.e., from yellow to dark brown.

3.3. Preparation of fruit extract:

For the isolation of fruit extract, 60 g of fresh unripened *A. marmelos* fruit was surface-cleaned firstly using tap water and then through double-distilled water. The fruit pulp was taken out carefully with the help of spatula and was subsequently was totally dried in oven at 60°c for next 48hrs. After this, dried fruit pulp material was crushed into powder using mixer grinder. The aqueous extract was prepared by introducing 5g of bael fruit powder in 100ml of Milli-Q water at 60°c for 30-40minutes under stirring condition at 250 rpm using magnetic stirrer. After boiling for the desired duration, mixture was cooled and filtered using with Muslin cloth followed by Whatman paper (125mm).The filtrate was finally collected and reserved at temp. 4°c for other search.

3.3.1. Biogenic synthesis of AgNPs using *A. marmelos* fruit extract:

Briefly, 14 ml of aqueous fruit extract was introduced in a cleaned 250ml of conical flask. 20 ml of 10^{-3} M $AgNO_3$ stock solution was prepared. With the help of pipette, 6ml of $AgNO_3$ solution was taken and was added drop wise in the fruit extract solution for bio reduction process. The biogenic formation of AgNPs was carried out at 37^{0}c by keeping it on an incubator shaker for the next 3-4 days under dark atmosphere. The formation of nanoparticles was verified everyday by evaluating a visual change in the colour of mixture.

3.4. Synthesis of silver nanoparticles using chemical reduction approach:

For chemical reduction method, sodium borohydride $NaBH_4$ and polyvinyl alcohol (PVA) were employed as reducing agent and stabilizer, respectively. Silver nitrate solution was used as a silver precursor. In atypical synthesis protocol following Agnihotri *et al.*, 2013, the required amount of $NaBH_4$ and PVA was dissolved in 23 ml of DI water in a conical flask. The above mixture was kept under stirring (250 rpm) at 65°C for 5 minutes in order to achieve a homogenous suspension. Afterwards, 2 ml of $AgNO_3$ solution was introduced in the above mixture drop wise with a rate of 1 drop/second and the colour change was observed. Stirring was continued for next 15 minutes till no further change in colour was observed. The final concentration of $AgNO_3$, $NaBH_4$, and PVA in synthesis was 1x 10^{-3} M, 2x 10^{-3}M, 6 x 10^{-3} M such that the molar ratio was found to be 1:2:6.

3.5. Preparation of methylene blue solutions

Methylene blue (MB) dye solution of 40 ppm concentration was prepared, both as a stock as well as working solution. For this, 2 mg of MB was weigh and mixed in 50ml double distilled water.

Various working solutions (40ppm, 30ppm, 20ppm, 10ppm, 5ppm, 2ppm, 1ppm, and 0.1ppm) were made in triplicates as per the desired dilution.

3.6. Spectrophotometric analysis of catalytic activity of silver nanoparticles

In 1st set of dilution 500µl of chemically synthesized silver nanoparticles were introduced in each test tube, similarly in 2nd set 500µl of bael leaf silver nanoparticles were introduced in each test tube and then in 3rd set bael fruit silver nanoparticles were introduced in each test tube. Observed the colour changed and after this O.D was taken at 613nm and 664nm.

3.7. Photo-Catalytic degradation of MB (Methylene Blue) dye

Methylene blue (MB) dye was weighed 0.9mg and mixed in 90ml of distilled water act as a stock solution. About 1ml of various forms of silver nanoparticles was mixed to 14 ml of MB solution. Then control was prepared in similar manner without adding silver nanoparticles. Before bringing to sunlight, the reaction suspensions were properly mixed on magnetic stirrer. Afterwards, these samples were put under the sunlight and monitored in interval of time period of 60min.At specific time intervals; these suspensions were measured against absorbance at 613nm and 664nm to evaluate the photo catalytic degradation of dye.

Percentage (%) degradation of dye was calculated by the given by formula:

% Decolourization = (Co-C)/Co X100

Where Co= Initial concentration of dye.

C = Concentration of dye after photo catalytic degradation.

3.8. Degradation kinetics of methylene blue using various forms of AgNPs Under visible light:

100ml conical flask was taken and washed with distilled water properly. Methylene blue stock solution was prepared according to 10 ppm for 45 ml of distilled water (0.45 mg of methylene blue was dissolved in 45ml of water). Mix the methylene blue solution on magnetic stirrer. This solution was made in duplicates. Test tube was taken and rinsed with distilled water. 500µl of methylene blue solution was taken out from the stock and 4.5 ml of distilled water was added in the test tube. Vortex it properly and then readings were taken at different time interval (0, 5, 30, 60, 90, 120, 180, 210, 240, and 270 mins). Thereafter taking readings at time 0 min, 5ml of chemically synthesized silver nanoparticles were added in stock solution. Now this stock solution was putted on stirring condition on the magnetic stirrer. After 5min reading was taken as above process up to 240 min. Similarly repeat the process for bael leaf AgNPs and bael fruit AgNPs. O.D was taken at 613nm and 664nm.

3.9. Degradation kinetics of MB dye using various forms of AgNPs under Sunlight at different pH:

Methylene blue solution was prepared according to 10 ppm. From this stock solution 14ml of MB was taken out in a 50ml beaker. After this p H (6, 8, and 10) was adjusted by p H meter. 1ml of various forms of synthesised silver nanoparticles was introduced in a beaker containing MB solution of different p H (6, 8 and 10). The solution was kept on stirring condition i.e. magnetic stirrer under the sunlight. Observed the visual colour changed and O.D was taken at 613nm and 664nm at different time interval i.e. 0-360 min.

3.10. Process Optimisation:

Time:

The optimization of the time was done to check the stability of the various forms of synthesized Silver nanoparticles. For this purpose different reaction periods (0, 5, 30, 60, 90, 120, 180, 240, and 300 min.) was chosen to check the % degradation of MB dye. The Absorbance of experimental process was measured by UV-VIS Spectrophotometer.

Temperature

Concentration of silver nitrate was optimized upon earlier parameter as 1m M. Reaction test tubes containing plant extracts (bael fruit and leaves) mixed with 1mM $AgNO_3$ were placed in the incubator shaker at temperature ranging from 25-30°C.

Concentration:

Test tubes containing different conc. of methylene blue dye i.e. (40, 30, 20, 10, 5, 2, 1, 0.1 ppm) were prepared. Plant extract AgNPs and chemically synthesised AgNPs were added to this test tubes. So as to check the degradation of dye by various forms of synthesised silver nanoparticles. Visual colour changed was observed and O.D was taken.

pH:

The reaction mixture pH was maintained at various ranges, such as 6, 8 and 10 individually. The pH was fix up by applying 0.1N HCl and 0.1N NaOH. Absorbance was recorded using UV-Visible spectrophotometer.

3.11. Characterisation studies

UV-vis spectroscopy

In a Bio reduction of silver ions in aqueous solution was monitored by UV-vis Spectrophotometer (Shimadzu UV-1800 spectrophotometer, Japan). The UV-VIS spectral assay of various forms of AgNPs was done by using at room temperature with a scan range of 200-800nm. The degradation kinetics of methylene blue dye by using various forms of silver nanoparticles was also optimised by UV-VIS spectrum with range 613nm and 664nm.

Fourier Transform Infra-Red spectroscopy (FTIR)

In FTIR analysis, various forms of silver nanoparticles were carried out through using Nicklet 380 Thermo, US Fourier Transform Infrared spectrometer. The range of scanning was set as 500-3600 cm^{-1} for absorption maxima. The KBr pellet of all forms of synthesized silver nanoparticles was used for spectrum to detect the functional molecules responsible for capping.

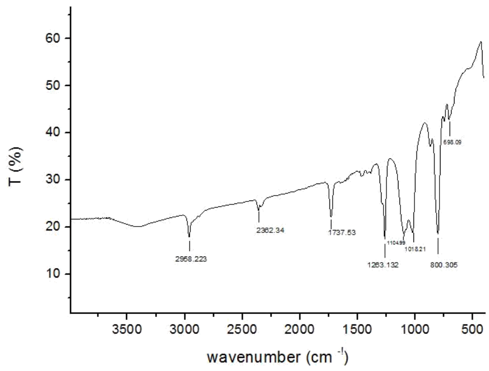
Chapter 4: Results and Discussion

4.1. UV-Vis Spectrophotometer:

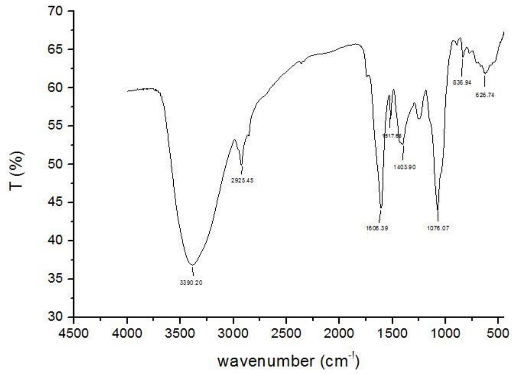
UV-Vis spectrophotometer serves as important device for characterizing AgNPs, with a help of the Surface plasmon resonances and optical absorption spectra produced by capping agents and metal nanoparticles. Chemically synthesized silver nanoparticles and plant extract (bael fruit or leaves)was confirmed by determining the absorption peak maxima at proper time-interval applying in UV–VIS spectrophotometer at range of 400–500 nm. Throughout the experiment, during the synthesis or optimization of AgNPs synthesized applying extracts of *Aegle marmelos,* maximum absorbance lies between the wavelengths 400nm to 430 nm.

4.2. Fourier Transform Infra-Red Spectroscopy (FTIR)

FTIR is an instrument which is used for the measurements to recognize the bio molecules responsible for the capping as well as stabilization of the metal NPs synthesised by chemicals and aqueous fruit and leaves extract of *Aegle marmelos* .The band at 3415.27 cm^{-1} corresponds to 1° and 2° amines. The peak at 2923.52 cm^{-1}, 2852.17cm^{-1}, 1384.62 cm^{-1}, and 2958.23cm^{-1} corresponds to internally bonded C-H stretch. The peak corresponds to 1614.10 cm^{-1}corresponds to C-C bend. The peak corresponding to 2362.34cm^{-1}, 800.305cm^{-1}, 698.09 cm^{1}, corresponds to alkanes frequency group. The presence of aromatic ring corresponds to the peak 800.305cm^{-1}. The C-O ester group, secondary amine CN stretch, Phenol stretch peaks found in FTIR analysis of aqueous fruit and leaves extract of *Aegle marmelos* was found to reduce and stabilize the nanoparticles formed. Thus, the phytochemicals present in the plant extract proves to serve as powerful stabilizing as well as reducing agent that are totally responsible for the reduction of Ag^{+}.



(A)



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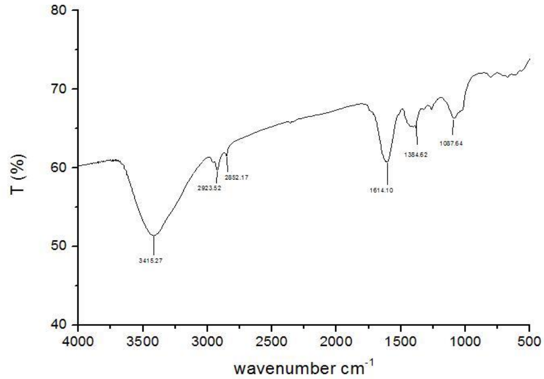


(C)

Fig 4: FTIR spectra of AgNPs (a) Chemically synthesised (b) Fruit extract (c) Leaf extract.

4.3. Evaluation of catalytic activity of Silver Nano-particles

Photo-catalytic action of AgNPs was evaluated in a 250 mL batch reactor operated under both under dark atmosphere and in the presence of sunlight, as shown in Figure 1. Methyl Blue (MB, Figure 4.1) is a representative azo dye contaminant in textiles and other industrial dying stuffs. MB was used as an ideal composite, to evaluate catalytic reductive deterioration efficacies about biogenic and chemically synthesized silver nanoparticles. In a typical protocol, the desired amount of silver nanoparticles were added to MB solution while stirring the entire mixture in a magnetic stirrer at 250 rpm for 30 min to establish the adsorption-desorption equilibrium prior to initiation of the catalytic process. Magnetic stirring was continued for the entire process so as to inhibit aggregation of silver nanoparticles and maintaining a homogenous environment. The colour of the suspension faded along with reaction time, which is an indicative of the degradation of MB dye.

UV-Vis spectrometry was employed to determine the degradation behaviour of MB and is manifested by decolourization of MB solution. The characteristic wavelength for determining MB concentration using UV-Vis spectroscopy is routinely measured at 464 nm. The absorbance was converted to concentration by Lambert-Beer"s law using a calibration curve which demonstrated a linear fit with following equation;

$$A = C \times 0.067 - 0.004 \qquad \text{Eq. (1)}$$

The percentage of MB degradation, r % can be calculated by the given equation:

$$r(\%) = \frac{C_0 - C_t}{C_t} \times 100 \qquad \text{Eq. (2)}$$

Where, $C_0$and C_t represent the concentration of MO solution after at t=0 and t=t min, respectively.

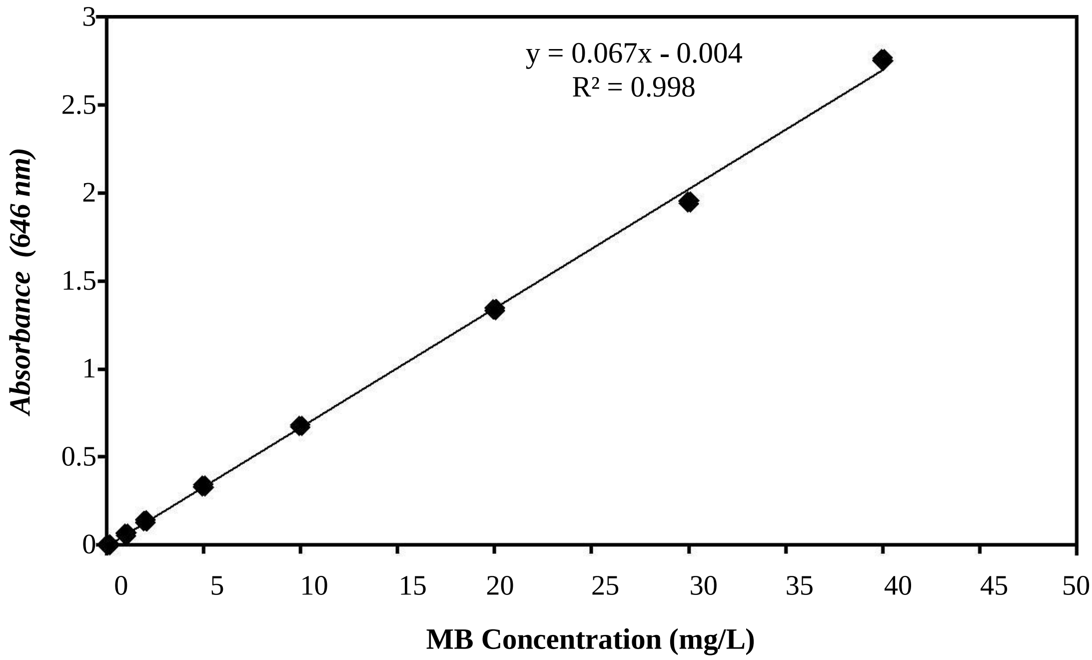


Fig.5: Standard calibration curve of MB solution at various concentrations

Results show that the percentage degradation of chemically synthesized and biogenic silver nanoparticles was observed to be 66.5 ± 2.3% and 76.8 ± 3.6 %, respectively under sunlight illumination. However, under dark conditions, the extent of degradation was lowered up to 42.3± 5.2 % and 60.1 ± 2.8 % for chemical and biogenic mediated silver nanoparticles. Irrespective of AgNP source, the presence of sunlight always enhanced MO degradation which can be responsible for photo-catalytic activity of AgNPs (Vanaja *et al.,* 2014). Interestingly, leaf mediated silver nanoparticles demonstrated higher potential to degrade MB than silver nanoparticles synthesized through chemical reduction approach, under both sunlight illumination and dark conditions. This indicates that AgNPs synthesized through leaf extract is not only eco-friendly and minimizes toxic effects; they also enhance their catalytic efficiency for dye degradation. Earlier (Kumar *et al.,* 2013; Kansal *et al.,* 2008) have demonstrated the daylight as an effective technique for degrading dyes than any other irradiation sources. The mechanism of dye degradation can be explained in the following series of photochemical reactions. When exposed to sunlight, the energized photons hit the surface of nanoparticles and excite their surface electrons (Yu *et al.*, 2012). The molecules of dissolved oxygen which are present in the dissolving medium acquire the excitation of electrons and then are transformed toward free radical species such as HO, HOO, or O_2^- radicals. The generation of these radicals at the catalytic surfaces are actually responsible to break the complex organic moieties of dye into simpler organic molecules manifested as both decolourisation and degradation of the dye (Ameta *et al.*, 2013). As a result the bio synthesized silver nanoparticles may be performed as a stable as well as efficient photo catalyst for the methylene blue dye degradation under the influence of visual light rays was reported by Vanaja *et al.*,2014.

Table 3: The Effect of a sunlight on the catalytic performance of silver nanoparticles synthesized through different routes

	% Degradation	
Source of AgNPs	**In presence of Sunlight**	**In absence of Sunlight (in dark)**
Chemically synthesized	66.5 ± 2.3	42.3 ± 5.2
Leaf extract (Biogenic)	76.8 ± 3.6	60.1 ± 2.8

* MB concentration: 20 ppm; reaction time: 6 hours

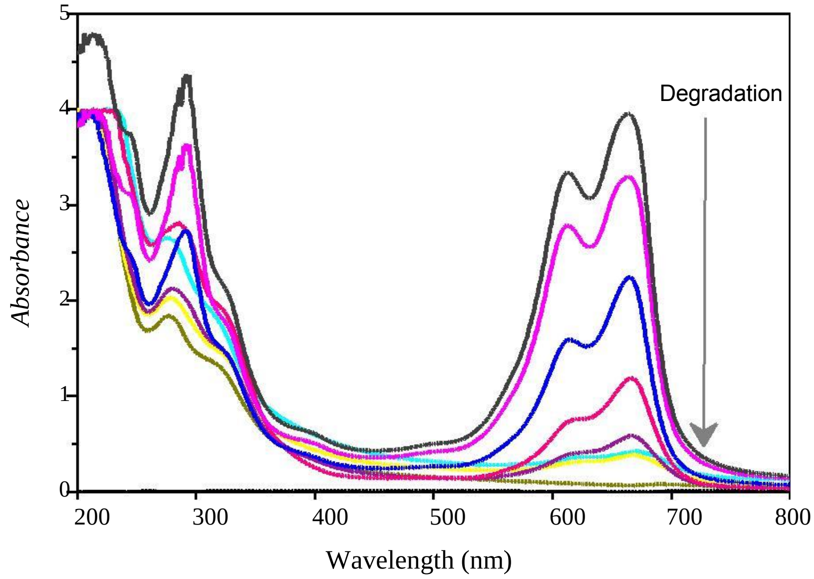


Fig 6: Methylene blue degradation with leaf extract mediated silver nanoparticles.

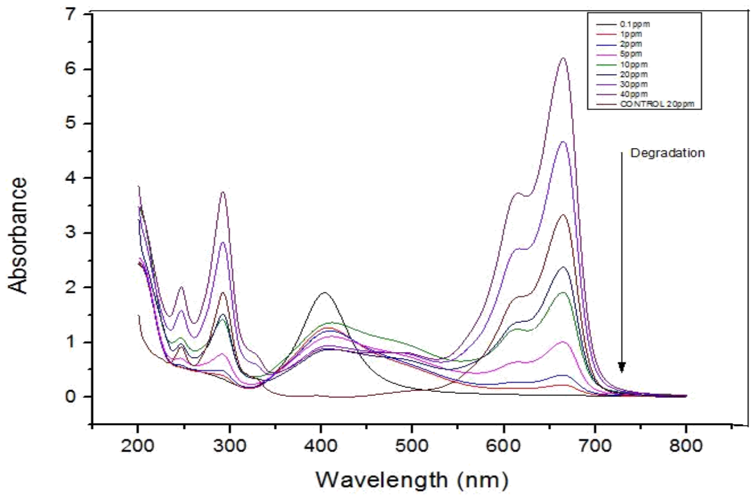


Fig 7: Methylene blue degradation with chemically synthesised silver nanoparticles.

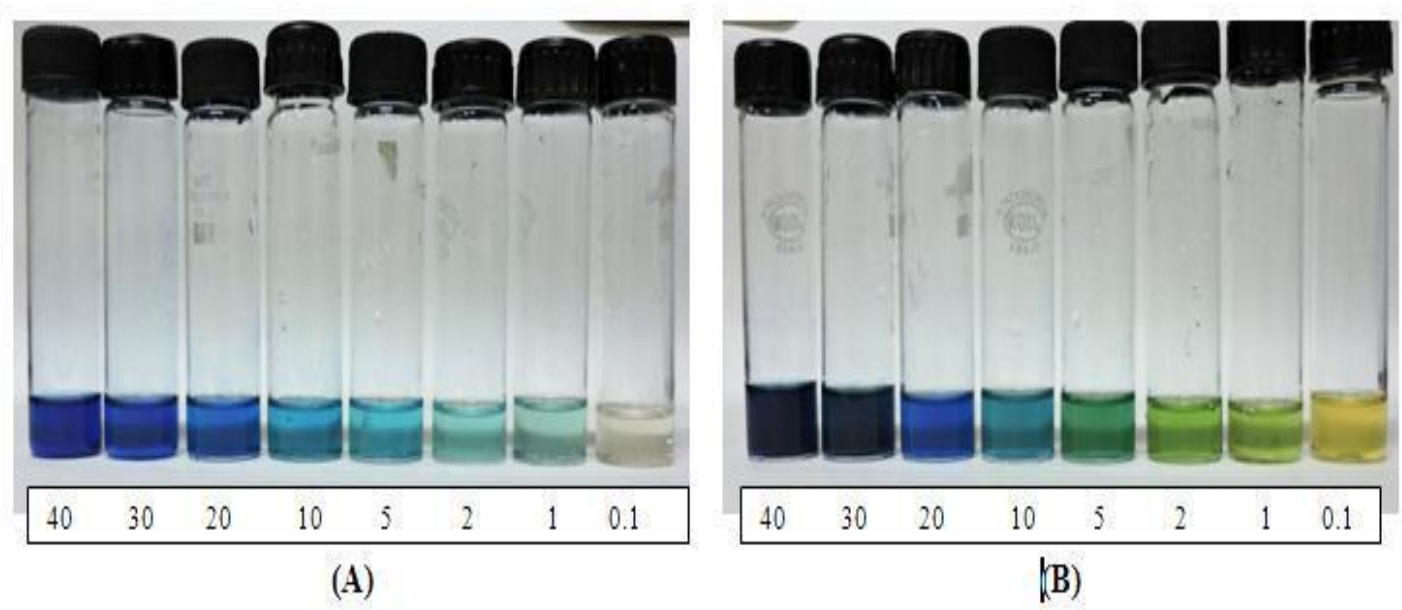
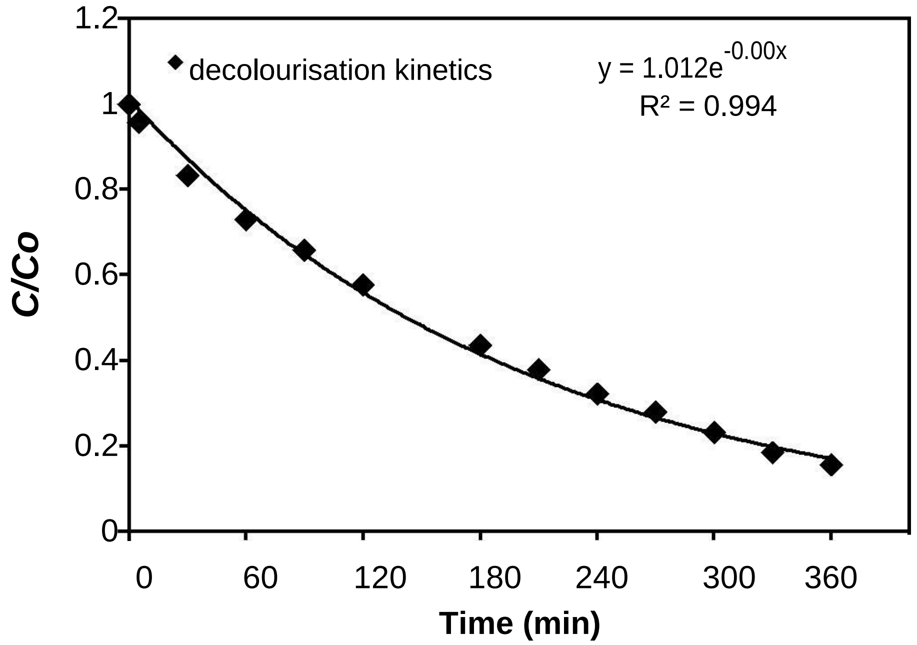


Fig 8: Degradation of Methylene blue at different concentrations (ppm) using (A) leaf AgNPs and (B) Synthetic AgNPs



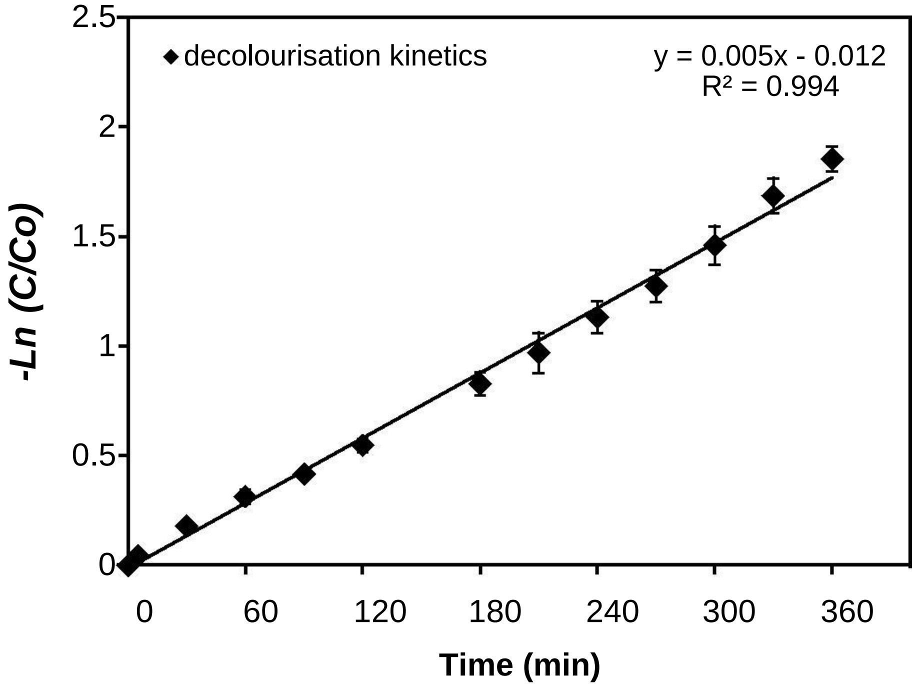
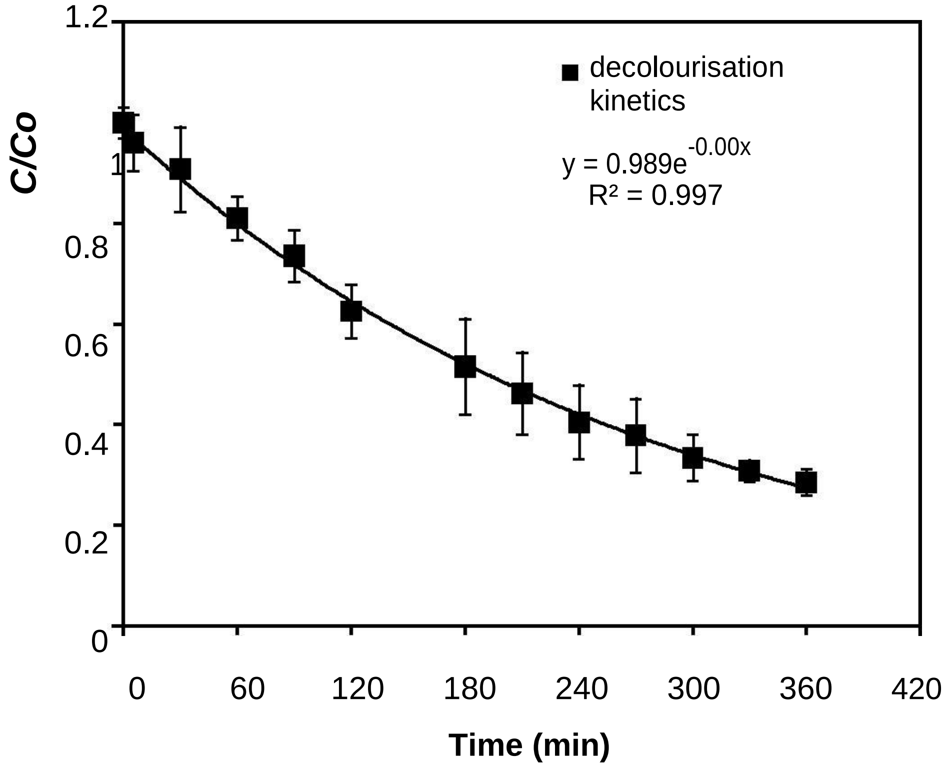


Fig.9: Decolourisation of Methylene Blue treated with biogenic (leaf extract) silver nanoparticles following pseudo-first order kinetics



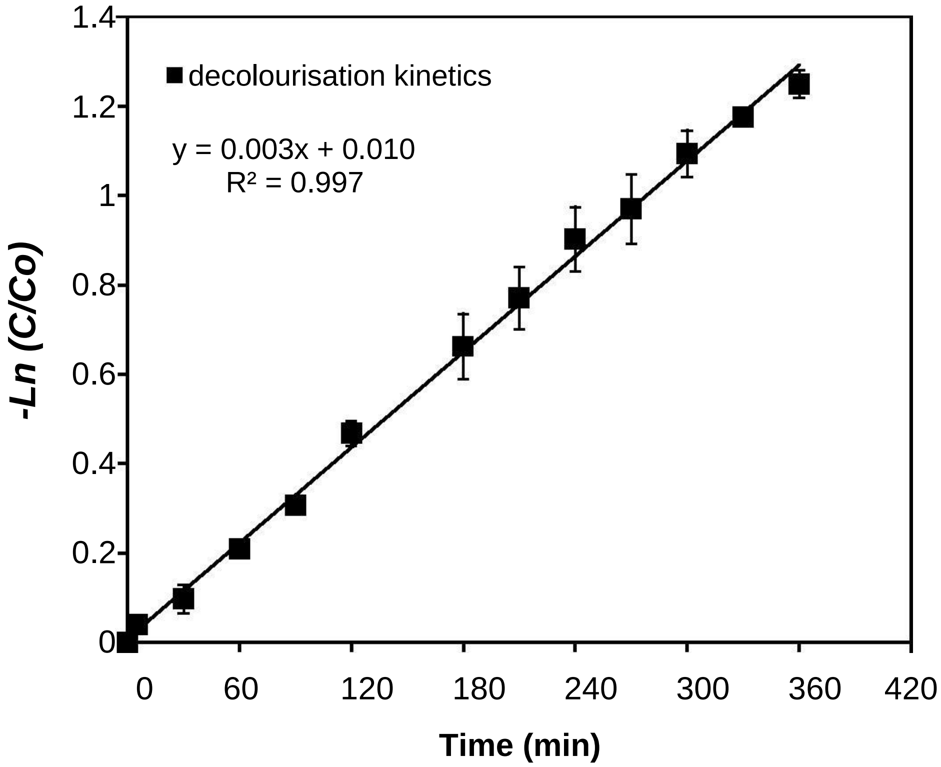


Fig. 10: Decolourisation of Methylene Blue using chemically synthesized silver nanoparticles follows pseudo-first order kinetics

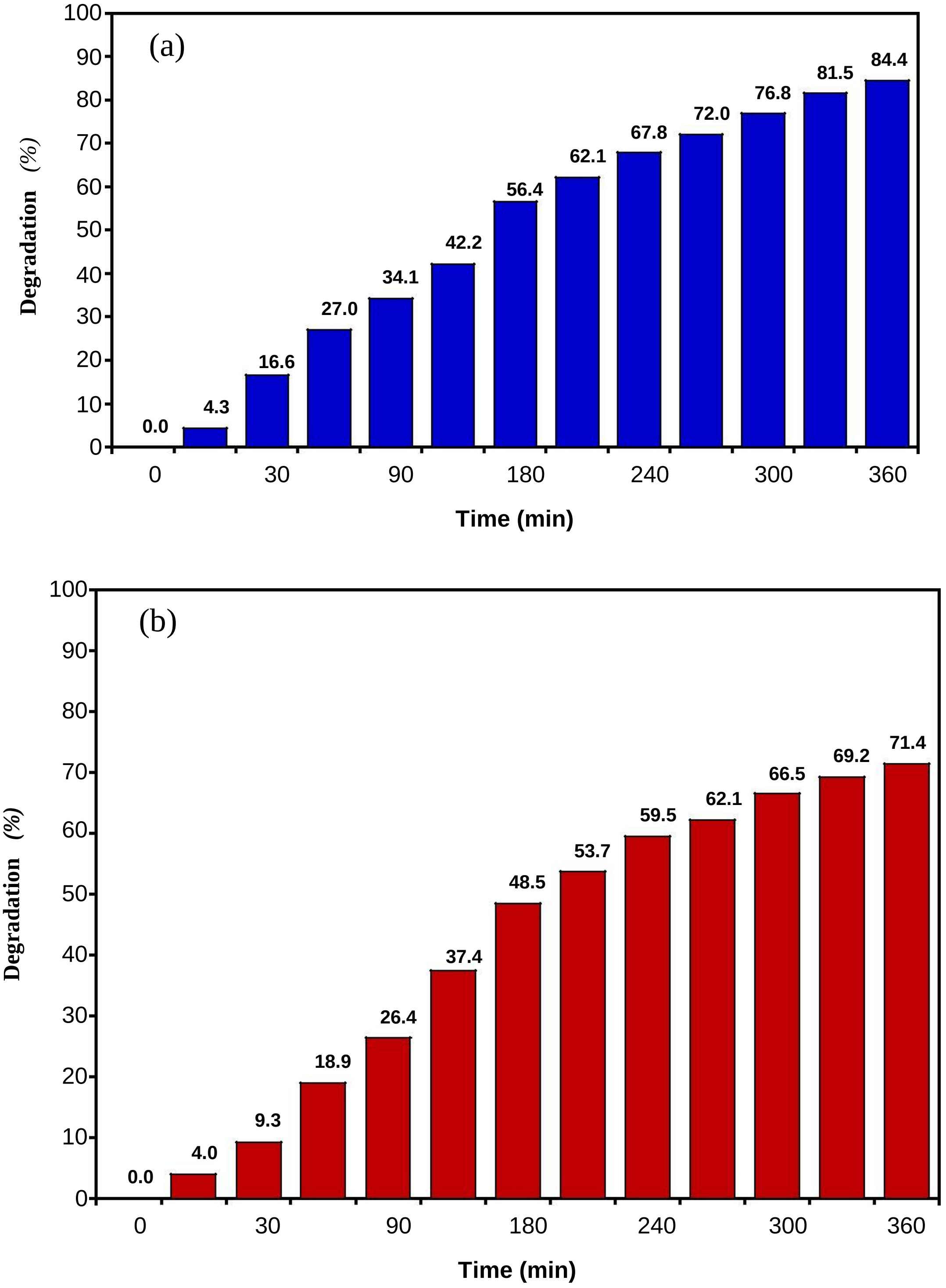


Fig. 11: Extent of degradation using (a) leaf extract silver nanoparticles (b) chemically synthesized silver nanoparticles

4.4. Effect of Catalyst dose

Since, Methylene blue dye degradation is dependent on the efficacy of photo catalytic activity of AgNPs, the effect of a catalyst conc. on photo degradation potential were evaluated to one side varying the dose about AgNPs in a 50 ml, 20 mg/L MB solution. The concentration range of silver nanoparticles was chosen from 50-400 mg/L. It was observed that the degradation efficiency was significantly increased from 52% to 90% as the AgNP dose was upgrade from 50 - 200 mg/L respectively. However, the conc. of AgNPs was further increased to 300 and 400 mg/L, the degradation efficiency was reduced to 82% and 69%, respectively. It can be explained that up to 200 mg/L, an increase in AgNP concentration may increases the corresponding number of active sites at AgNP surface, such that more MB molecules may interact at the surface and hence, the degradation efficiency of MB was improved. Further increase in AgNP concentration may lead to solution being more turbid thereby preventing the penetration of sunlight within the dye solution and cause photo catalytic activities (Akpan and Hameed, 2009). Rather an increase in AgNP conc. merely increased the scattering of light. Therefore, the optimized concentration of AgNPs for MB degradation was found to be 200mg/L.

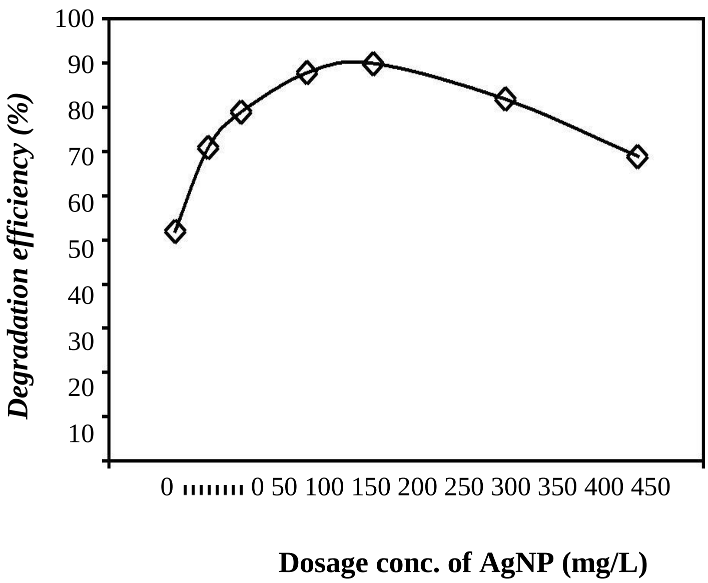


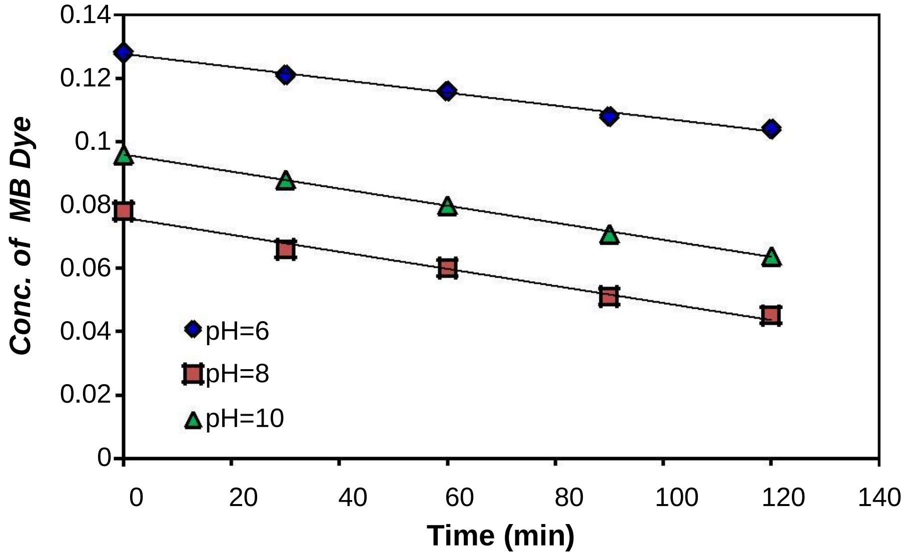
Fig12: Effect of concentration of silver nanoparticles on the photo catalytic degradation of MB. All experiments were performed at 20 ppm MB concentration at 7 pH under sunlight illumination.

4.5. Effect of pH

Isoelectric point is the pH at which a molecule exist zero charge at its surface. The isoelectric point of colloidal silver lies within a range of 3-7, which implies, that if the pH of the system falls within this range, dispersion would be unstable (Windt *et al.*, 2013). The surface of AgNPs would consist sufficient charge to confer stability only for solution having a pH either less than 3 or higher than 7. In the current study, for silver nanoparticles with negative surface charge (due to PVA stabilizer), the point of zero charge generally lies at pH 6 (the isoelectric point). Being a cationic dye, the interaction between AgNPs and methylene blue would be more if AgNP surface would bear more negative charge. Therefore, the pH of the solution was increased by adding alkali so that AgNPs would acquire more negative charge at their surface.

Since the pH of dye solution has a significant role in affecting its degradation process, the photo catalytic experiments were designed and compared at three different pH, lightly acidic like ph-6 , lightly basic like pH-8 and more basic (pH=10) at 4ml/50ml AgNP and 20 ppm dye solution. Increasing the pH value resulted in enhanced extent of degradation and the percentage degradation was found as 74.3 ± 4.2%, 87.4 ± 2.9% and 89.96 ± 1.8 % at pH values 6, 10.4 and 12.6, respectively. It is therefore justified that increasing pH may increase electrostatic absorption between negatively charged AgNPs surfaces and cationic dye, methylene blue. As the pH of the solution was decreased, there would be a significant decay into the no. of various active sites because of decrease in the net negative charge on AgNP surface. As a result, the active sites on silver nanoparticles do not tend to attract methylene blue dye to carry out photo catalytic activities and the extent of photo degradation was decreased at lower pH values.

For various studies, the degradation followed first order kinetics up to 2 hours followed by a pseudo-first kinetics for next four hours with varying pH (Fig.10). The rate constant was increased from 0.001 to 0.004 as the pH of system was increased from 6 to 8 and was then decreased to 0.003 at pH=10. Therefore, a pH of 8 was considered as the optimized value for obtaining the best degradation rate under the given operating conditions.



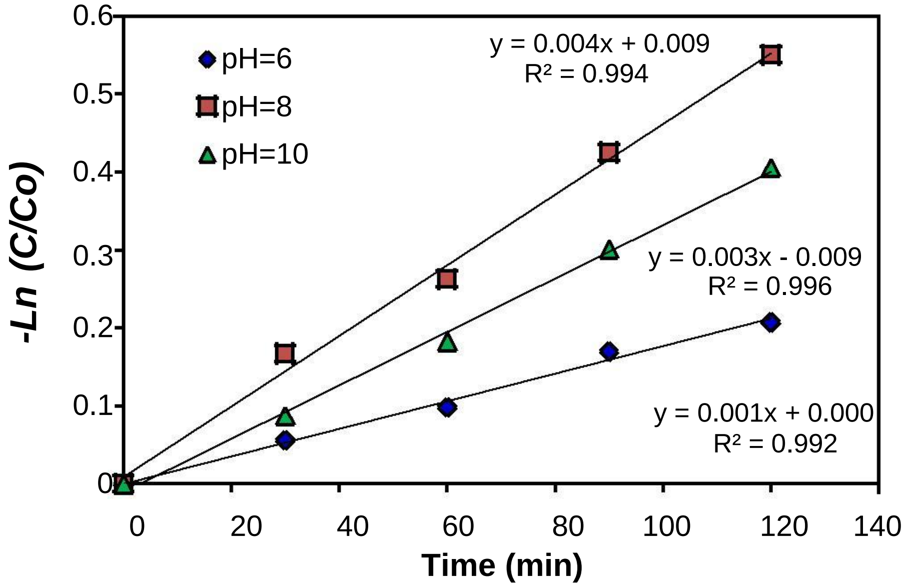


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Fig 13: Effect of pH on photo catalytic activity of chemically synthesized silver nanoparticles

Conclusion

For a better upcoming of nanotechnology, biosynthesis and green synthetic method should be used for the synthesis of nanoparticles by saving the environment to avoid the use of dangerous chemical reducing agents and organic solvents. Colloid based nanotechnology has been developed to control nanomaterial size, shape, uniformity and functionality. In the present article, we have demonstrated the synthesis of AgNPs with varying sizes using *Aegle Marmelos* leaf and fruit extract as well as using chemical route. The present synthesis method proved to be helpful in controlling the size of silver nanoparticles, thereby tuning their catalytic properties. In a reduction reaction of methylene blue dye, bio reduced AgNPs shows unique size dependent catalytic properties. AgNPs synthesised biologically shows a very high rate of degradation under the presence of sunlight. The present study, it is found that the use of natural eco-friendly and renewable will react as a reducing agent for the synthesis of AgNPs exhibit very good photo-catalytic activity against molecules of dye and can be used in waste water treatment plant. Therefore the utilization of biogenic process helps in designing of ultimate catalyst which helps to show the utmost stability as well as activity.

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