**“****PHOTOCATALYTIC DEGRADATIO OF ORGANIC DYE BY SILVER NANOPARTICLE FROM *AKCALYPHA WILKESIANA* VIA GREEN ROUTE”**

A DISSERTATION

 SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE

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IN

### BIOTECHNOLOGY

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**CANDIDATE’S DECLARATION**

I hereby certify that the work which I presented in the Major Project-II entitles

“**Photocatalytic degradation of organic dye by silver nanoparticle synthesized from *Acalypha wilkesian*a via green route**” in fulfilment of the requirement for the award of the degree of Master of Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi is an authentic record of my own, carried out during a period from 7-Jan-2021 to 28-May-2021, under the supervision of **Dr. Navneeta Bharadvaja.**

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other University. The work has been communicated in Scopus indexed journal with the following details:

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CERTIFICATE

I hereby certify that the Project dissertation titled “Photocatalytic degradation of organic dye by silver nanoparticle from *Acalypha wilkesiana* via green route” which is submitted by Neha, 2K19/MSCBIO/19, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is a record for the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

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**ABSTRACT**

Silver nanoparticle (AgNP) was synthesized using a medicinal plant Acalypha wilkesiana leaf extract through bottom-up approach. synthesis of nanoparticle via green route is simple, easy to handle and inexpensive in comparison of physical and chemical methods. Phytochemical screening of Acalypha wilkesiana determines the presence of coumarins, flavonoids, sterol, tannin, phenol and saponin. Synthesis of nanoparticle were detected at regular intervals with proper characterization technique majorly include UV-Vis spectroscopy. Further characterization involves the Scanning Electron Microscopy/ Transmission Electron Microscopy (SEM/TEM) which determines the size and shape of the nanoparticle. Fourier transform infrared spectroscopy helps in finding the biochemical function group of plant extract which serves as reducing and capping agent in nanoparticle synthesis. And crystal nature of nanoparticle was analysed via X-ray diffraction (XRD). Here we aimed the remediation of polluting aqueous solution via green route of silver nanoparticle synthesis and study the photocatalytic activity of organic dyes and ability to detect the toxic metal like Pb, As, Ni, Hg, Cr, Co, Fe, Mn, Pd and Se.

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**CHAPTER 1**

**INTRODUCTION**

Nowadays contaminated water and soil have become the great threats in our day to day lives and affecting the environment. according to a report only 30% of wastewater is treated rest all the wastewater has been discharged into industrial sewage which include heavy metal, toxic chemical and textile dyes. These toxic chemical on surface water creates hurdle many ways [1].The supply of fresh water for agriculture production is decreasing. hence treatment of wastewater is becoming very necessary. Heavy metal like lead, zinc, chromium and iron along with inadequate level of cadmium and pesticide accumulated in river and makes the ground water polluted [2]. Heavy metals in soil can also be accumulated with long term irrigation with contaminated wastewater even in low amount [3]. These heavy metals accumulate in food chain and reaches to human caused various severe diseases like cancer, malnutrition, neurological problem, reproductive diseases gastrointestinal upset with compromised immune system [4]. Naturally occurring heavy metals like arsenic, radon and fluoride etc., above significant concentration act as contaminant. For example, river Yamuna has high concentration above permissible limits of nickel, magnesium and lead which pollute the water bodies [3]. Water and soil pollution are co related with each other. Agricultural ruff, chlorine and chloramine which are used for controlling growth of microbes when present above international standard of water control leads to water pollution. Formation of by product like halo acetic acids and trihalomethane and chlorite causes cancer, anemia and neurological diseases respectively [5]. Other causes of water pollution include air pollution, temperature associated to cause algal blooms in water bodies which enhances the level of nitrogen and phosphorus, automobile emission, leakage of underground storage system, radiation leakage from various nuclear power plant, sewers overflow, improper functioning of wastewater treatment system and many more[6].

 Here we are dealing with the dye which also responsible for water contamination. We can define dye by its solubility and reactivity with organic compounds[7]. Removal of dye in water by conventional method is very difficult due to its increase solubility. The unique property of dye is its colored impartation which is due to chromophoric group in molecular structure. This fixation of color related to auxotrophic group which is highly polar in nature and only bind to the polar group [8]. Exposure of dye in water bodies result in penetration of light due to which the rate of photosynthesis decreases affecting the growth of the plants [9]. 15-50% of azo dye is found in water bodies because it does not bind to fabric. This wastewater incorporated with dye used in irrigation as agricultural practices by affecting the soil microbial communities, enzyme activity and growth of plant [10]. Although there are many ways for dye removal from water bodies such as physical methods which is associated with the use of biosorbents, coagulants and filtrations. Biosorbents include activated carbon, chitin, chitosan, silica gel, zeolite, rice husk, clays, red mud, fly ash etc. [11]

Physical methods of nanoparticle synthesis include mechanical milling, laser ablation, ultra-sonication, etc. [12]. In mechanical milling, nanoparticle formed in a mechanical device refers to “mill” which helps in reduction of particle size by imparting energy to the sample. Sample is placed between two colloiding mechanical ball which result in formation of nano size powder with respect to time [13]. Laser ablation uses light to vaporize material. ablating the solid target that lied in gaseous or liquid environment and collection of nanoparticles in the form of nano power or colloidal solution. The colloidal solution must be ultrapure free of counter ion or by-product [14]. For example, in silver nanoparticle synthesis we need high purity of silver slice at the bottom of the glass vessel containing appropriate amount of water. after that we apply 1000nm pulsed Nd:YAG laser which vaporize the metal and target particle is collected in the aqueous solution. the yellow-colored nanoparticle is obtained with respect to time [15].

Chemical methods include ionization, electrochemical oxidation Fenton’s oxidation, irradiation oxidation and ultra-chemical oxidation[16]. For chemical method synthesis we require three components that are metal salt, reducing agent like sodium citrate, sodium borohydride, ascorbic acid etc., and capping agent like thiols citrate and polymer like PVA, PVP. [17]. Basic principle is, metal salt reduces by reducing agent and converted into metal nanoparticle which are highly unstable and it will be placed intactly and formed the agglomeration or aggregation. For stabilization of nanoparticle, we add stabilizing agent or capping agent that will prevent the agglomeration and aggregation. Synthesis of nanomaterial involves two major steps that are nucleation and growth. In nucleation, the initiation of formation of first nanocrystal takes place in the solution which are capable of growing [18]. Nucleation is of two types, homogenous nuclei forms monodispersed particle while in heterogenous nuclei, nucleation occurs at different time and give polydisperse particle. Nucleation contributes to controlling property of final product and size distribution. slow growth are preferred for making the highly monodispersed nanoparticle [19]. Metal salt reduce to form metal nanoparticle and these metal particle form cluster to form crystallites. These crystallites come into the form of primary particle and starts growing, we need to stop this growth to prevent agglomeration. Arrested precipitation method requires to stop the growth, it involves mixing of nanoparticle vigorously for more numbers of nucleation [20]. Also, to prevent it from Oswald ripening, stabilizers are used. Tunning of the size of nanoparticle highly depends upon nucleation and growth rate, fast nucleation result in smaller nanocrystal whereas slow nucleation gives larger particle. The role of stabilizing agent is very important, it helps us to prevent uncontrollable growth of particle, particle aggregation, control growth rate, particle size, allows particle solubility in various solvents. Stabilizing agent are classified as electrostatic and steric stabilization. Electrostatic stabilization includes the uniform particle charge which result in coulombic repulsion of force between individual particle. In steric stabilization, metal is covered by layer of material that is are sterically bulky (polymer, surfactants) [21]. Metal nanoparticle shows different color after the synthesis because incident light creates oscillation in conduction electron on surface of nanoparticle and electromagnetic radiation is absorbed, this is called surface plasmon resonance. Reductant concentration, stirring rate, temperature, filtered solution contributes to average size and size distribution of nanoparticle [22]. Strong reducing agent such as sodium borohydride and weak reducing agent like positively charged bilayer stabilizer CTAB result in seed formation and nanorod growth respectively. For example, synthesis of silver nanoparticle involves addition of appropriate amount of silver nitrate and sodium borohydride act as reducing and capping agent respectively. The obtained yellow color represents the synthesized silver nanoparticle which can be further characterize by UV-Vis spectrophotometer[23].

The biological synthesis of nanoparticle is similar to chemical method. Here instead of using chemical reducing agent, we use microbial enzyme from microorganism and phytochemical compound from plant which act as reducing agent [24]. The following prepared biomass is further added to metal salt to form metal nanoparticle followed by purification and proper characterization and use for various further application. There are many advantages of using biological method for synthesis of nanoparticle like it is an eco-friendly way without using toxic chemical, require low cost and easy, by varying culture, pH, temperature and concentration of biomass we can alter the shape and size of nanoparticle [25].

There are various nanoscale materials that are found in nature for example magneto tactic bacteria which is a heterogenous group of prokaryotes and they migrate along geomagnetic field lines as they don’t have flagella. Membrane bound magnetic particle called magnetomers helps in facilitating the migration of magneto tactic bacteria that help in production of magnetite nanoparticle [26]. Diatoms synthesized siliceous materials and several others multicellular organism. Bacteria can be use intra cellularly as well as extracellularly. *Pseudomonas* stuntzeri AG259 has been isolated from silver mines successfully produce silver nanoparticle within periplasmic space [27] Similarly, near metal industry soil sample contain the same bacteria which can convert the metal into metal nanoparticle.

It is required to grow the bacteria in agar plate the transfer it to pure inoculum that is test tube followed by further transfer to LB broth in conical flask for 24 hours. Metal precursor added after the growth of the bacteria and incubation done at optimize condition. Silver nanoparticle synthesized from *Corynebacterium* sp SH09[28]. *Bacillus flexus* mediated silver nanoparticleis triangular shaped as well as face centered cubic which exhibit antibacterial property [29]. Silver nanoparticle synthesis by using *Serratia nematodiphila* show ,antibacterial activity [30]. Morgnella sp. RP-42 isolated from insect midgut synthesize silver nanoparticle extracellularly [31].

 Nanoparticle synthesize from yeast is simple and economically viable. Enzyme present in yeast grow in presence of metal to form nanoparticle. Yeast strain exhibit various cellular activities which control size and shape of nanoparticle. *Candida grabrata* and *schizosaccharmyces* used to synthesize cadmium sulphide nanoparticle [32]. Fungi secrete higher amount of protein which result in higher production of nanoparticle, silver nanoparticle are synthesize by using nitrate reductase from *Fusarium oxysporum* [33], *Phaeneroechaete chrysosporium* (fungus) [34], *Aspergillus flavus*[35]*, Aspergillus fumigatus*[36]*, Fusaricum oxysporium* [37]and *Fusarium semitectum*[38]*.* Bimetallic nanoparticular Ag/AgCl are synthesize from *Azadirachta indica* exhibit antimicrobial property and potentially degrade methyl blue dye [39]. The biological synthesis of nanoparticle is an ecofriendly way. The source could be bacteria, yeast, algae and plant which act as reducing and capping agent to provide stability of nanoparticle. Synthesis of nanoparticle from plant is discussed in the project.

**CHAPTER 2**

**REVIEW OF LITERATURE**

Plant mediated nanoparticle synthesis are eco-friendly and inexpensive way to contribute the to phytoremediation of organic and inorganic pollutants. Plant act as reducing as capping agent in synthesis of nanoparticle. The phytocompounds like polyphenols, flavonoids, terpenoids, sterol, alkaloids, glycosides, tannins, etc. determined by various qualitative phytochemical tests. The particular functional group of which acts in reduction metal detected by Fourier transform infrared microscopy (FTIR). The synthesized nanoparticle is very unstable due to which we use capping agent like sodium borohydride, CTAB etc.

**Figure 1** Representation of plant mediated nanoparticles synthesis

Silver nanoparticle is majorly used in purification of water because it exhibits strong antimicrobial properties. Silver nitrate is most commonly used as a metal ion source. The synthesis of silver nanoparticle from various plant extract takes place at various optimum condition. Temperature, pH, metal ion concentration, concentration of biomass, contact time, agitation speed, etc. are some parameters which need to optimized. Silver nanoparticle synthesize by using Panax Ginseng root extract and silver nitrate as metal precursor in following 4 ratios in addition of 1:1, 2:1, 3:1 and 4:1 along with barium chloride successfully detect mercury in the aqueous solution. The prepared silver nanoparticle characterized by UV-Vis spectroscopy showing absorbance at 404nm. and of spherical shape with 4-20 nm [40]. *Dahlia pinnata* leaf extract and silver nitrate were used to synthesize silver nanoparticle. The synthesis is further characterized by 460 nm and FTIR result shows the peak at 1064cm-1, 3265cm-1, 2916cm-1, 1423cm-1, 673cm-1 and 1595cm-1 indicating stretching and bending vibration of haloalkenes. It exhibits Face centered cubic structure showing 2 theta angles at 27.5, 37.75, 46.25, 54.65, 57.25, 76.68 degrees corresponding to (220), (122), (111), (331), (241), (311) planes on X-ray diffraction studies. Colorless solution changes to dark yellow is the visual indication of formation of nanoparticle [41]. Silver *Nigella sativa* mediated silver nanoparticle used shows absorption at 426nm and are 10.88nm in size. The white color changes to light brown indicates the formation of silver nanoparticle which used in degradation of Congo red dye from aqueous solution [42]. Green synthesis of silver nanoparticle from *Calendula offcinalis* in 1:9 with silver nitrate show absorbance at 436nm on UV-Vis spectrophotometer. The synthesized nanoparticle were 50-60 nm and crystal size were 14.32 nm showing face center cubic center which was analyzed by Scanning election microscopy and X-ray Diffraction used to degrade of methyl blue and methyl orange dye [43]. There is various other nanoparticle synthesized using plant extract given in table.

Photocatalysis degradation of dye uses a light source to due to which electron get excited from valence band to conduction band and generation electron hole pair which is further results in oxidative photodegradation that is formation of hydroxyl radical which comes in contact in oxygen, converting toxic dye to harmless substance like carbon dioxide, water [44]. Absorbance recorded for silver nanoparticle synthesize from *Morinda tinctoria* was 420nm. After addition of dye, the absorbance increases to 420nm by resulting in degradation of 95.3% of methyl blue dye at pH 8.6 [45]. *Amaranthus gangetic* derived silver nanoparticle potentially able to degrade Congo red dye with help by using a strong stabilizing agent that is sodium borohydride [46]. Silver nanoparticle synthesized from *Nigella sativa* seed extract degrade 98.5% of Congo red dye up to five consecutive cycles within 13 minutes on addition of sodium borohydride [42]. *Zanthoxy armatum* leaf extract potentially synthesized silver nanoparticle and able to degradation several dyes including safranine O, methyl red, methyl orange, and methyl blue which were showing absorption at 519nm, 415nm, 460nm and 664 nm with better degradation rates [47]. Gold nanoparticle synthesized from *Capsicum annum* successfully able to remove toxic metals like Pd, Cd, Cu, Zn, Ni, Co, Fe, Pb and removal percentage were 63.46%, 60.20%, 51.50%, 68.20%, 42.18%, 23.47%, 21.62%, 35.37% and 75.75% respectively under optimize pH conditions [48]. Green synthesis of SnO2 synthesized from *Vitex agnuscastus* able to degrade 91.7% of rhodamine B dye within 190 minutes and successfully able to remove 93.6% of Cobalt within 60 minutes [49]. Zero valent iron nanoparticle synthesized from *Eucalyptus globules* potentially able to adsorb hexavalent chromium. Absorption rate observed in first 30 minutes was 98.1% but it reduces to 71/9% as time increases to 90 minutes and reaction takes place upon addition of oenothein B which provide stability [50]. Zero valent silver nanoparticle synthesize from *Ficus benjamina* leaves extract successfully able to remove 75.5.% - 85% of chromium within 40 minutes at pH 6 [51].

**Table 1: Nanoparticle synthesis from various plants, their characterization, and potential applications**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Metal precursor** | **Plant** | **Characterization techniques** | **observation** | **Applications** | **Reference** |
| ***UV-vis*** | ***FT-IR*** | ***SEM*** | ***TEM*** | ***EDX*** | ***Initial*** | ***final*** |
| Copper nanoparticleCopper sulphate pentahydrate | *Piper Retrofractum Vahl* | 234-255nm | 550-570cm | Spherical shape high Cu content-70.3% | 2-10nm | Crystallinity phase -26.4% | Yellow  | Greenish black | Inhibit E. coli and staphylococcus aureus | [52] |
| Copper nanoparticleCopper sulphate pentahydrate  | *Cedrus deodara*  | 205nm | 607cm | Agglomerated and form large particle | Spherical  | No impurities  | Greenish | Dark brown | Inhibit against pathogenic strains  | [53] |
| Copper nanoparticleCopper acetate monohydrate | *Punica granatum* peel extract(anaar) | \_ | Broad spectrum at 3400 for OH present at surfaces of CuO | Spherical shape, diameter= 12.5mm | \_ | **XRD**- crystalline size = 35.80 nm monoclinic phase | Blue  | brown | Antibacterial activity against E. coli | [54] |
| Copper nanoparticleCopper sulphate pentahydrate (5mM) | *Celastrus paniculatus* leaf extract | 269nm |  | Spherical diameter=5nm | 2-10nm | **DLS-** zeta potential =-22.2mVZeta deviation=3.61mVEDS- purity (79.87%) | Yellow | green | Antifungal agent against plant pathogenic fungi Fusarium oxysporum- 76.29+/-1.52 max mycelial inhibitionDegradation efficiency on organic dye MB under sunlight  | [55] |
| Copper nanoparticleCopper sulphate pentahydrate  | Neem flower extract | 550-560nm |  | Tightly packed nanocrystal  | Spherical (5nm) | **Antibacterical study**Max. efficenicy against proteus mirabilis = 40mg/ml | Light blue = light green | Dark yellow = brown ppt  | Antimicaribial study  | [56] |
| Copper nanoparticleCopper sulphate pentahydrate  | Red extract cabbage(Brassica oleracea var. capitata f. rubra extract)  | 255nm | 3100-3600cmBand energy gap = 2.75 eV | Spherical  | Particle size = 77.5nm | **XRD-** crystalline nature, fcc structure, crystalline size= 78nm | \_ | White ppt | Good catalyst in Hantzsch reaction to form 1,4 dihydropyridine derivative Antibacterial agent against E. coli and S. aureus | [57] |
| Copper nanoparticleCopper sulphate pentahydrate | Seedless dates | 576nm | 624.57cm | \_ | Spherical  | **XRD****DLS** **Particle size distribution**= mean diameter 78nm**Zeta potential**=+41mV | Pale yellow | Red brown color | Cost effective, more feasible than chemical method in which ascorbic acid is used as reducing agent  | [58] |
| Copper nanoparticleCopper chloride  | *Tinospora cardifolia*  | 248nm | 3329cm- OH stretching 1620-C=O stretching1395-OH bending1319-C-O bending 1049- C-O-H stretching  | Spherical  | **XRD-** crystalline phase -metallic copper and copper oxide | **PSD**= 63.5nm**Polydispersity index**=0.26**Zeta potential**= -33.98mV**AFM**=178nm height | Sky blue  | Dark green | NP and nanocoated fabric possessed antimicrobial activityEfficacy of coated cotton against gram+ =65% gram- =50.5% | [59] |
| Zinc oxide nanoparticleZinc nitrate hexahydrate  | *Cassia fistula* plant extract | 370nm | Photolytic degradation- (batch reactor) =5ppm dye conc. Gives 90% degradation; ph-2 & 4 96.26% & 98.71% photodegradation efficiency Antioxidant (DPPH) Ic50=54Mg/ml | Hexagonal wurtzite structure | 5-15nm | XRD- 100,002,101 plane – increases purity of particles | - | - | Methylene blue dye degradation under UV &sunlight illumination.Antioxidant activity exhibited by NP through scavenging DPPH free radicles Bactericidal activity shown on klebsiella aerogene, E. coli, S. aureus | [60] |
| Silver nanoparticleSilver nitrate  | *Morinda tinctoria* leaf extract | 420nm | 3296 cm – carboxylic group3436-3220cm – O-H stretching, H bonded phenol and alcohol group.1634 cm – N-H bending primary amines 1672 cm – C=O stretching | Spherical rod shaped  | 79-96nm | 4 peaks of 2 theta 38.26 degree= (111)44.44= (220) 64.58 = (220)77.67 = (311) Fcc and crystalline in nature | Pale yellow | Dark brown | Degradation of methyl blue dye  | [45] |
| Silver nanoparticleSilver nitrate  | Neem leaf Neem bark Mango leaf Green tea Pepper seeds  | 420 | - | Spherical | - | - | Pale yellow  | Brownish yellow | Calorimetric sensing of toxic metal (Hg2+, Pb2+, Zn2+,Cr 3+, Cd2+, Ca2+. Cu2+,Mg2+,Ni2+,Fe2+) | [61] |
| Silver nanoparticleSilver nitrate  | *Amaranthus gangeticus* Linn leaf extract  | 416nm | 1635cm – C=O stretching 3441cm – OH & NH2  | Globular shaped  | 11-15nm | - | - | brown | Antifungal and antibacterial activity ( inhibitory activity towards gram +ve & -veDegrade congo red dye  | [46] |
| Silver nanoparticleSilver nitrate  | *Nigella Sativa* seed extract  | 426nm | 3423- 2880cmIndicates CH, -OH, -NH, C=C acting as reducing & stabilizing/caping agent | Spherical  | 10.88nm  | XRD-2theta = 38.14 degree- (111);44.36 degree(200); 64.71 (220)77.40 (311)  | White  | Light brown  | Congo Dye degradation  | [42] |
| Silver nanoparticleSilver nitrate  | *Zanthoxylum armatum* leaves | 419nm | 3431cm- N-H stretching,O-h hydroxyl grp2922cm- C-H stretching 1744cm – carbonyl stretching 1630cm- N-H bond1375 & 1238cm – N=O symmetry1045cm- C-N amines  | Spherical shape | Diameter = 15-20nm | **Zeta potential** = -21.2mVAvg size- 36nm**XRD** Fcc2 theta = 38.23 degree(111); 46.45(200); 66.65 (220);77.55 (311)Avg crystallize size – 22nm | colourless | brownish | Dye degradation – safranineO Methyl red ,Orange , blue  | [47] |
| Silver nanoparticle Silver nitrate  | *Viburnum opulus* fruit extract  | 513nm; 415nm (350-450nm) | 3398cm- stretching of O-H 1733cm- C=O stretching2931cm – C-H stretching1380cm-C-Ostretching1030cm- C-O bending  | Spherical  | 7-26nmAvg size- 16nm | **XRD-**2 theta 38.33 degree (111); 44.56 (200); 64.62 (220); 77.44(311);82.41 (222); 32.2- organic compound in sample (fcc)**TGA-** 35.14% bioactive compounds present at AgNP surface | Faint red  | Yellowish brown | Dye degradation Brilliant blue Tartrazine carmoisine | [62] |
| Silver nanoparticleSilver nitrate  | *Calendula officinalis* | 436nm | 2 theta –665cm; 3438cm; 1635cm- stretching vibration of carbonyl grp; 3338cm- O-H stretching  | Uniform and spherical  | 50-60nm140-150nm | **XRD-**Fcc, crystal size= 14.37nm | Yellow  | brown | Methyl blue and methyl orange degradation | [43] |
| Silver nanoparticleSilver nitrate  | *Dahlia pinnata* leaf extract  | 460nm | 1064cm- C-N bond 3265cm – O-H stretching 2916cm- C-H stretching 1423cm- C-H bending 673- stretching vibration of haloalkene1595cm- C-H present | Almost spherical  | Diameter- 15nm | **XRD-**Face center cubic structure, 27.5- (220); 32.75- (122); 46.25(111); 54.65(331); 57.25(241); 76.68(311) | colorless | Dark yellow  | Rapid colorimetric detection of Hg2+ | [41] |
| Silver nanoparticleSilver nitrate  | *Panax Ginseng* root extract  | 404nm | 3640cm- O-H stretching 1740- carbonyl stretching vibration of aldehyde1640- symmetri-COO stretching 1407- assymetri-COO stretching 1065- bending vibration of C-C-O & C-C-OH | spherical | 4-20nm | **-** | - | Dark brown | Detection of Hg2+ | [40] |
| Gold nanoparticleGold(lll)chloride trihydrate  | *Capsicum annum* | 335.26nm | 3324- 3329cm-=-OH grp2100cm- -CN grp1636cm- C=O grp  | Triangular  | - | **XRD-**111, 200, 220, 311 degreesCrystal size = 13.71nm | Yellow  | Dark red | 1.Water pollution removal 2.Show strong antimicrobial activity at low concentration | [48] |
| SnO2 nanoparticle (SnCl2) | *Vitex agnus-castus* fruit extract | 373.45nm | 3434.27 1633.021027.50647.45Anti-symmetric Sn-O-Sn; Sn-O symmetric | Spherical  | 4-13nmAvg size= 8nm | **XRD**26.9 degree- (110);34- (101); 38-(200); 52-(211); 58-(002); 62.1-(310); 65.2-(301); 71.4(202) | - | Light grey  | 1.Photocatalytic degradation of organic dye RhB, under UV irradiation 2.Removal of heavy metal Co+2 | [49] |
| Iron nanoparticle (FeSO4.7H2O) | *Eucalyptus globules* leaf  | `graphn given =above 420nm | 3346.25cm; 1635.58; 524.68 cm (poluphenolic content present. | spherical | 50-80nm | **XRD**peak at 2 theta 46.40 indicates zerovalent iron | Brown | black | Adsorption of Cr (VI) | [50] |
| Maghemite nanoparticle 2M FeCl3+ 1 M FeSO4.7H2O  | *Tridax* plant  | 210nm | 454 cm, 569cm,632cm, attributed toFe-O;563 cm – gamma attributed to FeO 1443-1600 attributed to C=C/C=N bond326, 402, 500, 706 are peaks of maghemite  | Cubic spinal structure (spherical and cuboidal shape) | 9.59-15.42nm | **XRD** 18.22, 29.3,30.02,32.08,43.14,57.48 degree with miller indices 011,210,220,310,330 | - | Blackish brown ppt  | Removal of heavy metal Pb and Cd  | [63] |
| Zinc NanoparticleZinc acetate (0.1M)  | *Sphagneticola trilobata* (Leaf, stem and root extract) | - | 3144cm-C=O stretching 1665cm-N-H stretch 1640cm-N-H bending in amine & amide grp. | Irregular and complex | 65-80nm | **EDS-** zinc 41.4% synthesized in Nanomaterial**XRD-**200,111,221 planes correspond to 2 theta angles. Face center cubic structure | - | Yellow color | Removal of Chromium by DPC method  | [64] |
| Cupric Oxide nanoparticle | *Calotropis procera* |  | 3444cm – OH grp; 1636cm; 1231cm;1084– protein latex519; 598cm – Cu-O band | Spherical  | 10-15nm; diameter = 15.06nm standard deviation- 5.17 | **XRD**Peak on range 20<2 theta< 80 degree Monoclinic symmetry | Green ppt  | Black ppt  | Removal of Cr (VI) from aqueous solution | [65] |
| Iron based NPFeSO4.7H2O | *Eucalyptus leaf*  | - | 3388cm – O-H stretching;2932 &2840 - C-H stretching;1720 – carbonyl grp; 1605& 1515 -C=C aromatic skeletal vib.;1330-CH3 asymmetric vib.;1048- C-N stretchingNP rxn with Cr(VI) & Cu(II) show band at 460 & 546cm -Fe-O stretch  | Polydisperse  | 20-80nm | **EDS-**Element % of Fe= 16.3;C=47.4O= 36.3**XRD**2 theta at 44.8;24 degree show biomolecule capped with Fe NP. | - | - | Removal Cr (VI) and Cu (II) when existed together or separated  | [66] |
| Zero valent silver NP Silver nitrate | *Ficus Benjamina* | 420nm | 3461- O-H stretching; 1632 cm – amide grp | Dendritic structure | 60-105nm | **-** | - | brown | Cadmium removal from aqueous solution | [51] |
| Zero valent Iron NP  | *Rose damascene, Thymus vulgaris, Utrica dioica* leaf extract | - | 3400 & 3430 cm for polyphenols; 1126-1190cm for carbonyl grp; 1628 &1640cm corresponds to C=C in akene grp; 615-617 indicating aromatic compounds of alkanes  | Non uniform exhibit differed space & void space. | 100nm**BET**Surface area=1.63(TV); 2.42(UD); 1.42 (RD)m3/g total pore vol=4.52\*10-2(TV)2.97\*10-2 (UD);2.08\*10-2(RD) | **XRD** Perfectly index crystalline Fe | - | - | Removal of Cr (VI) from aqueous solution | [67] |
| Silver nanoparticle Silver nitrate | Plant gum of- *Araucaria heterophylla**Azadirachta indica**Prosopis chilensis* (bark of tree) | 400-500nm411nm405nm415nm | 3411- O-H stretching;1645-COO- stretcing;1446 –COO- bending 1300-1450- CH3,CH2 bending 3419- O-H stretching; 2936 – OH; 1590- COO-3416- O-H stretching;2911- OH; 1689- COO- | spherical | 30nmDLS=50nm35nm; DLS=50nm50nmDLSDiameter= 75nm | **Zeta potential****(EDX)**-20.87mV-23.65mV-16.41 | - | - | 1) Effective against gram positive and gram-negative bacteria 2) not much effective against cell lines, but still exhibit anticancer anti-cancer activity 3)chromium removal efficacy was not efficient. | [68] |

**CHAPTER 3**

**MATERIALS AND MEHODS**

* 1. **Material**

The fresh leaves of *Acalypha wilkesiana* wereharvested in February 2021 from Delhi Technological University, Delhi. Chemicals such as 70% ethanol, 99% silver nitrate and sodium borohydride were used from the lab. Distilled water (DW), ionized water and glassware have been used during the experiment.

* 1. **Plant extract** **preparation**

Leaves of *Acalypha wilkesiana* were washed twice with distilled water before processing. leaves were dried in oven at 30 degrees Celsius for overnight or air dried for two days. The dried leaves were grinded with the use of motor pestle and converted into powder. An amount of 1.5g of fresh leaves of *Acalypha wilkesiana* was added to 50 mL distilled water. The obtained mixture was heated at 60-70 degree for 1 hour. Then mixture allowed cooled and then filtered using whattmann paper no. 41 to get desired extract.

Figure Purified Akcalypha wilkesiana extract

* 1. **Qualitative Phytochemical analysis of *Acalypha wilkesiana***
		1. **For alkaloid detection-**
1. Mayer’s test was performed by adding 2ml of concentrated HCl into 2 ml plant extract into a test tube, and then few drops of mayer’s reagent were added. White precipitate or green color solution indicates the presence of alkaloids.
2. Hager’s test was performed by adding few drops of Hager’s chemical agent into a pair of metric capacity unit of plant extract. Bright yellow precipitate confirmed the presence of alkaloids.
	* 1. **Detection of Betacyanin and Anthocyanin**

 2 ml of plant extract was taken in a test tube and of 1ml of NaOH (2N) was added and then heated at 100 degrees Celsius in presence of anthocyanin and betacyanin. Blue-green color and yellow color indicates the presence of anthocyanin and betacyanin respectively.

* + 1. **Anthraquinone detection**
1. 2 % HCl was prepared and added few drops into 2 ml of plant extract. Red precipitate confirms the presence of anthraquinone
2. 2 ml of plant extract become taken right into a check tube after which 1 ml benzene and 10% ammonia answer become brought to it. Indication of pink precipitates verify the presence of anthraquinone.
	* 1. **Coumarins detection**

In 1 ml of plant extract , 1 ml of 10% NaOH become added. Yellow colour suggests the presence of coumarins withinside the test sample.

* + 1. **Flavonoids detection**
1. Alkaline regent test- In 1 ml of plant extract followed by addition of 1 ml of NaOH (2N). yellow precipitates confirmed the presence of flavonoids.
2. FeCl3 test- few drops of FeCl3 had been delivered into 1 ml of plant extract. Black-pink precipitation at the lowest of take a look at tube suggests the presence of flavonoids in sample.
3. Zinc dust reduction test- 2ml of plant extract, zinc dirt and few drops of focused HCl had been added. Formation of purple color suggests the verification of flavonoids.
	* 1. **Glycosides detection (Keller Killani’s test)**

In 1ml of plant extract then 1 ml of glacial acetic acid is delivered into after the ones 2 drops of FeCl3 and focused H2SO4 along partitions of test tube. Red brown ring fashioned among of layers confirms the presence of glycosides in plant extract

* + 1. **Saponin detection**

2ml plant extract was taken into the test tube and added 2ml distilled water into it. Then the mixture was vigorously shaken up to 15 min. layer of foam around 1cm or thicker indicates the presence of saponins.

* + 1. **Tannin detection-**
1. FeCl3 test- 1ml of plant extract was added then followed by addition of 2ml of 5% FeCl3. Green-black or dark blue color confirm tannin existence.
2. Alkaline reagent test- 2 ml of NaOH (1N) was added with 2 ml of plant extract in a test tube. Color change from yellow to red confirms the presence of tannin.

* + 1. **Terpenoid detection**

2 ml plant extract introduced to 1ml of 1% HCl. After 5–6-hour 1 ml of Trim Hill reagent changed into introduced and the received answer changed into boiled in water tub for 5-10 minutes. Presence of inexperienced shadeation confirms terpenoids.

* + 1. **Phenol assessment**
1. Ellagic acid test- 1 ml plant extract added into 5% glacial acetic acid followed by the addition of few drops of 5% NaOH in a test tube. Muddy brown color confirms the presence of phenol.
	* 1. **Sterol detection**
2. Salkowski’s test- five mil of chloroform was another in two ml plant extract further addition of focused H2SO4 to walls of tube take place. Formation of red brown colour lower layer confirms the presence of sterol.

**3.3.11. Steroid and Phyto steroid detection**

1ml of plant extract turned into delivered along side 1 ml of chloroform and few drops of focused H2SO4 into test tube. The brown color rings and bluish brown rings shows the presence of steroids and phytosterol respectively.

* + 1. **Phlobatannins detection**

Few drops of 10% ammonia answer introduced to one ml of plant extract. Formation of crimson precipitate at the lowest of take a look at tube suggests the presence of phlobatannins.

* + 1. **Detection of Quinones**

 1 ml of plant extract taken right into a take a look at tube. Further addition of 1ml focused HCl takes place. Formation of purple shadeation confirms the presence of quinones.

* + 1. **Acid detection**

Sodium bicarbonate added to 1ml of plant extract. Effervescence’s formation confirms the presence of acid in the test sample.

* + 1. **Protein detection**
1. Xanthoproteic test- Few drops of focused nitic acid have been brought in 1 ml of plant extact. Yellow shadeation shows the presence of protein withinside the sample.
2. Biuret test- 0.5mg of 40% NaOH solution was added to 0.5mg of plant extract solution along with few drops of CuSO4 (1%). Formation of violet color indicates the presence of protein.
	* 1. **Carbohydrate detection**
3. Benedicts’ test -Add a few drops of Benedict reagent to 2 ml of plant extract. The tube is heated in a boiling water bath. Red brown precipitates confirms the presence of carbohydrate.

**Table 2:** Phytochemical analysis of *Acalypha wilkesiana*

|  |  |
| --- | --- |
| Chemical components  | Plant extract  |
| Anthraquinone | Negative  |
| Coumarins  | Positive  |
| Flavonoids 1. FeCl3 test
2. Alkaline reagent test
 | Negativepositive |
| Glycosides  | Negative  |
| Saponin  | Positive |
| Tannin 1. FeCl3 test
2. Alkaline reagent test
 | positivepositive |
| Phenol assessment  | - |
| Sterol  | Positive |
| Steroid and Phyto steroid | Negative |
| Phlobatannin | - |
| Quinone detection  | Negative |
| Protein 1. Xanthoprotein test
2. Biuret test
 | -negative |
| Carbohydrate  | - |
| Alkaloids 1. Mayer’s test
 | -negative |



**Figure 2:** Test tube marked 1 represent anthraquinone;2 represent coumarins; 3(i) represent FeCl3 test and 3(ii) represent alkaline reagent test for flavonoid; 4 represents glycosides; 5 represent saponin; 8(i) represents FeCl3 test for tannin; 10 represents sterol 11 represent steroid & phytosteroid; 14 represent quinone; 15(ii) represent biuret test for protein; 17 represent mayer’s test for alkaloids

* 1. **Silver nanoparticle synthesis using *Acalypha wilkesiana* leaf extract**

The silver nanoparticle synthesis was done effectively by eco-friendly way and apply the reducing capacity of phytochemical found in *Acalypha wilkesiana* leaf extract. 1mM solution of silver nitrated was prepared after that, 10ml of plant biomass were added to 90 ml aqueous silver nitrate solution(1mM) under continuous stirring at 120 rpm. The reduction of silver ions visually observed as the colour changes from yellow to brown within 5 minutes. centrifugation at 10,000rpm for 20 min to obtain the silver nanoparticle which were present in aqueous form. Supernatant was discarded and the obtained pellet was purified. The pellet was dried in the hot air oven at 60 degree and then we can use the silver nanoparticle for various characterization.

Figure synthesized silver nanoparticle

* 1. **Characterization of silver nanoparticle**

**3.5.1. Determination of silver nanoparticle formation by UV-Vis spectroscopy**

The photon energy of visible region lies between 36 -72 Kcal/mole which increases to 143 Kcal/mole near UV region [69]. The sample molecule when exposed to the light absorbed same amount of energy to the electronic transition that is from higher orbital to lower orbital. Spectrophotometer measure the absorbance to find the concentration of different molecule in the solution. The absorbance spectrum tells us about how much light is absorbed at a particular wavelength which can be demonstrated with a graph of absorbance vs wavelength [70]. Absorbance of sample will to molar concentration and path length of light in cm. This working explains by the Lambert-beer [71]. Light intensity depends upon up the kind of chemical or concentration the is present in the sample helps in determining wavelength at which light is absorbed. absorbance is inversely proportional to the transmittance. Detector only detect the transmittance. From literature studies, the UV-vis spectrophotometer ranges from 200 – 800 is used and wavelength is recorded near 400nm for corresponding silver nanoparticle [72].

**3.5.2. Fourier Transform Infrared Spectroscopy analysis for silver nanoparticle**

FTIR used to determine the structure of molecule which is unique for every compound following absorption on infrared region that is 12800 cm-1 to 10 cm -1. The characteristic feature of infrared spectroscopy is change in dipole moment of the sample when it absorbs radiation at particular wavelength upon exposure to IR [73]. Frequency of absorption analyzed by energy gap of vibrational energies which is generated by transfer of electron. Mostly every molecule can be characterized by infrared except some di-nuclear homo molecule due to their zero-dipole change. Infrared spectroscopy is advantageous because it can analyze all type of sample that is solid, liquid or gas within common infrared region[74]. After first generation where prism is used in 1950s followed by second generation with addition of monochromator 1960s, the FTIR came under third generation which used Michelson interferometer. FTIR involves radiating source, interferometer, sample molecule, detector, amplifier, A/D converter and a computer. the radiating source generates radiation that reaches to detector through interferometer which helps in splitting of light into two equal parts so that they travel different path [75]. The corresponding two beam of light is detected reaches to the detector which get amplified with use of amplifier shows the signal to the software. Interferometer comprises of two mirrors placed perpendicular to each other. In between these two, a beam splitter is which split the coming from the source and transmit it equally to moveable mirror which transmit the light and stationary mirror which reflect light. If the light travels same distance between both the mirror and beam splitter the, it is said to be zero path difference. When light falls on the movable mirror when it is shifted away from beam splitter, it travels larger distance as compared with the stationary mirror and this Optical path difference (extra distance) can be measured as 2 Delta. When optical path difference is in the multiple of wavelength and addition of multiple of wavelength to the half of wavelength result in constructive and destructive interference respectively [76]. These represent the extreme cases; the common situation lies between the constructive and destructive interference where intensity can be maximum or minimum depending upon the back-and-forth movement of the movable mirror and ultimately the plot is defined by interferogram. The Vibrational energy corresponds to reduction of mass and bond spring constant [77]. Change in wavenumber of any particular atom is highly related to bond spring constant instead of reduced mass. Vibrating energy is unique for every molecule. We can determine the functional group present as it shows molecular fingerprint of that particular molecule by matching their peaks which shows bending and stretching to the comprehensive database. From literature studies of silver nanoparticle, the vibration bands are attributed to O-H, C-H, C=C, -CH3- and C-N functional group [72].

**3.5.3. SEM/TEM and EDS Analysis**

Scanning Electron Microscopy (SEM) deals with the focusing of beam of electron at the surface of atom resulting in a more providing composition of the sample and gives better resolution of the image [78]. It is used to analyze the surface of the element which includes contaminants identification, corrosion, flaws, etc. and provide a much better resolution image that help in determining the size and shape of particle. SEM only give qualitative result of the particle [79]. The interaction of secondary electron whose energy is very low emitted on excitation by electron beam detected by the detector. Transmission Electron Microscopy (TEM) gives much higher resolution as compared to SEM. Here electron beam is transmitted at the surface of nanoparticle and provide us highly focused and magnified image.

Energy Dispersive X-Ray is attached to SEM or TEM. It tell us about the surface of the particle like quantitative elemental composition, and helps to find foreign particle which are coating on metal [80]. Elastic scattering result in back scattered electron which is located beneath the secondary electron and has much higher energy related to the atomic number. The replacement of electron facilitated by X-ray emission and change in energies is measured by EDS [81]. It identifies the composition of element in the sample and indicate it in the form of peak.

* 1. **Determination of catalytic property of silver nanoparticle upon dye degradation**

We can determine the dye degradation through UV- vis spectroscopy by evaluating the optical density of silver nanoparticle. The electron transfer from donor to acceptor held on the surface of nanoparticle thus, it acts as a catalyst for the reaction[82]. The reaction follows pseudo first order kinetics where the absorbance value with respect to time is equal to the rate constant of reduction at that particular time. The dye degradation of dye is dependent upon the morphology of synthesized nanoparticle and target dye chemical structure [83]. The dye was prepared by adding suitable amount of distilled water. In some cases, we add inorganic oxidant like sodium borohydride, Oenothein B etc. For providing stability as well as can be used as strong reductant [84]. Further addition of appropriate amount of synthesized silver nanoparticle under optimized condition. The color of the dye is fainted with respect to time and after some time the solution become colorless.

**Table 2:** Phytonanoparticles showing removal efficiency of heavy metals and dye degradation from aqueous solution

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Nanoparticle** | **Heavy metal / dye** | **UV- Vis of dye/ NP****(obs.)** | **Reaction time** | **Additives** | **pH** | **Percentage removal / observation** | **References** |
| Silver nanoparticle  | Methylene blue dye | 660nm / 420nm | 0-72 hours | - | 5.66.67.68.6 | 95.3% at 72 hours at 8.6 pH | [43] |
| Silver nanoparticle  | Congo red dye  | SPR band – 498 (pie-pie)338(n-pie) | At interval of 1.5 minutes up to 15 min | Sodium borohydride | - | Not given  | [54] |
| Silver nanoparticle  | Congo red dye  | 496nm dye/ 426 AgNP | 0-13mintues  | Sodium borohydride | - | 98.5 up to 5 cycles  | [55] |
| Silver nanoparticle  | Safranine O Methyl red,Methyl Orange, Methyl blue(10mg/l) | 519nm415nm 460nm664nm(AgNP- 419nm) | 0-24 hours  | - | - | **Degradation rate constant**1.02X10-3/min1.03X10-31.86X10-31.44X10-3 | [56] |
| Silver nanoparticle  | Brilliant blue Tartrazine Carmoisine | 629nm420nm504nm | **Reaction rate constant (k)**0.20970.00760.0496 | Sodium borohydride  | - | - | [57] |
| Silver nanoparticle  | Methyl blue Methyl orange  | 500-700350-550nm | 1mM -5 min2mM- 5min1mM-5min2mM-5min | Sodium borohydride  | - | 27.12%18.08%69.79%42.11% | [58] |
| Silver nanoparticle  | Hg2+ detection  | Not mentioned (given a graph)  | - | - | pH- 3-8(no effect of pH) | Dark yellow changes to colourless  | [59] |
| Silver nanoparticle  |  Detection of Hg2+ | Not mention (graph given) | - | - | - | Dark brown changes to colourless  | [60] |
| Gold nanoparticle | Toxic metal removal PdCd CuFeNiCoMnZnPb  | Absorption capacity of adsorbent used in aqueous soln. of toxic metal was determined by batch method. | 120 min | - | 566.5 | Removal%63.4660.2051.5068.2042.1823.4721.6235.3775.76 | [61] |
| SnO2 nanoparticle  | Photodegradation of RhBRemoval of heavy metal Co+2 | 553.5/370-375nm  | 190 min60 min | - | 7>7>8 | 91.7%Adsorption capacity93.6%Cobalt hydroxide started to ppt from aq. Sol. & adsorption was impossible | [62] |
| Zero valent iron nanoparticle  | Adsorption of Cr(VI) | - | 30min90min | Oenothein B | - | Adsorption efficiency98.1%71.9% | [19] |
| Maghemite nanoparticle  | Removal of heavy metal in fly ashPbCd  | - | 1 hours24 hr1 hr24 hr | - | 7 | 85.56%90.85%67.8%  | [21] |
| Iron based nanoparticle  | Removal of Cr (VI) and Cu (II) when co-existed together Cr (VI) and Cu (II) not present togetherRemoval efficiency of CrCu (II)Pb (II)Zn (II) | -- | 1 hrT=288KT=308K | - |  5 | Cr (VI)- 58.9%Cu (II)-33%Cr (VI)=74.2%; Cu (II)=45.2%Cu-26.8%Cr-50.7%Cu-40.8%Cr-62.6%Cr= from 75.1% to 50.8%Cu 28.3% to 64.2%44.821.531.410.8 | [33] |
| Zero valent Silver nanoparticle  | Cadmium removal from aqueous solution | - | 40 min  | - |  6 | 85%75.5% | [20] |
| Zero valent iron nanoparticles  | Removal of Cr (VI) from aqueous solution**TV-Fe** (higher% removal)UD-FeRD-Fe | Adsorption capacities 466462453.7 | 1-101-1030 min | - | 2-9 | 91.75%60.95%97.5%97.1%96.94% | [66] |
| Silver nanoparticles  | Chromium removal  | 350nm | - | Activated carbon | - | Absorbing pattern was irregular | [72] |

* 1. **Removal efficiency of metal nanoparticle for toxic metal**

Adsorption capacity depends upon various synthesizing factors. It meanly indicated to acts as adsorbent. Metal oxides presence in the soil highlights the metals present in the soil. For examples oxide of iron and iron are useful for the phosphorous removal as well as arsenic adsorption. Binding of arsenic with iron oxide nanoparticle is five times greater than any binding with large particle. Magnetite nanoparticle also favorable for the phosphate adsorption in soil. Immobilization of stabilized nanoparticle with carboxymethyl cellulose penetrate more into the soil column as compared to non-stable nanoparticle because they agglomerated from nanoparticle to microparticle. Stable nanoparticles are negatively charged as the soil particle so there is repulsion between then due to which they travel a distance of incorporating in the soil. It was observed that surface modification of nanoparticle increases its stability.

**3.7.1 Adsorption isotherm studies**

Adsorption isotherms helps us to know about the amount of adsorb metal on the surface of adsorbate that is nanoparticle at equilibrium concentration. Although there are five types of isotherms in which first three are non-porous and remaining two are porous. The three porous isotherms are Freundlich, Langmuir and BET models.

**3.7.1.1. Freundlich isotherm**

It is a type of monolayer adsorption where the adsorption is directly proportion to the pressure at a constant temperature. In addition, adsorption increases at low temperature and decreases at low temperature. Adsorption is reversible process and is accompanied with desorption. At initial state, the rate of adsorption is very high and with increasing the pressure, the desorption start. At the equilibrium point, the rate of adsorption and desorption become the same and therefore the number of adsorbed gases or heavy metals does not increase to metals or gases being adsorbed Freundlich isotherm equation is given as, x/m=KP1/n where, x is the active sites, m is the total number of places present on the surface, K and n are Freundlich constants and P is the pressure of the gases.

At low pressure, 1/n=1, that means the adsorption is increasing very rapidly with increasing pressure. At high pressure, the value of 1/n=0, from this we can conclude that the value of x/m is not dependent upon pressure. And at intermediate pressure, n>1 this implies that x/m value will not increasing very rapidly with pressure. By taking log on sides of Freundlich equation, we get

log x/m=log K +1/n log P. this represents the equation of straight line. Values of K and n can be calculated from the intercept and slope of the plot. This isotherm works only at low pressure and only gives qualitative results.

**3.7.1.2. Langmuir isotherm**

Langmuir isotherm assumes that the surface of solid if solid is homogenous with same affinity, only monolayer is formed on the surface with no lateral interaction and absorbed molecules are fixed at their space. here adsorption and desorption take place simultaneously. The rate of adsorption is directly proportional to the pressure and the number of vacancies, which is equal to N (1-theta), where N is the total number of sites present and theta is the fraction of the surface area covered by metals. as the number of adsorption sites occupied by the total number of available adsorption sites [85]. The adsorption rate is reported as Ka PN (1-theta), where ka is the adsorption rate constant, P is the pressure, and N (1-theta) is the empty spaces on the surface. The rate of desorption is reported as Kd N theta, where Kd is the rate constant for desorption.

At equilibrium, rate of adsorption and desorption becomes equal. Therefore, theta =KP/1+KP indicating the Langmuir adsorption isotherm equation. The Langmuir constant K can by determine by the slope as it also resembles the equation of straight line. At low pressure, KP<<1 then theta = KP and reaction follows the first order kinetics. At high pressure, KP>>1, theta =1 i.e., independent of pressure. And at intermediate pressure, theta = KPn.

**3.7.2. BET theory of multilayer Adsorption**

This model is given by three scientists namely Brunaer, Emmett and Teller in 1938. this assumes that adsorption at one site does is free from the adsorption at other sites, also the surface is uniform and sites are localized, multilayers adsorption takes place at the surface, heat of adsorption in succeeding layer is equal to energy of liquification and surface area for nth layer is directly proportional to thetan-1 [86]. The BET equation is given as P / Vtotal (P0-P) = 1 / Vmono C + C-1 / Vmono C (P / P0), where Vtotal is the volume of gas adsorbed at pressure P and Vmono is the volume absorbed at volume die The surface is covered with a monolayer and C is a constant velocity, the value of which can be determined by e (E1-E2) / RT, where E1 is the heat of adsorption for the 1st layer and E2 is the heat of liquefaction [87]

**3.7.3. Removal of heavy metal under various condition**

**3.7.3.1. Effect of Ph**

At low pH, H+ ions are occupied in the active sites of adsorption, with increases in pH these sites become free and available for the heavy metal on adsorbent. Removal of heavy metals carried out properly at alkaline condition. In the pH range of 3-7 there is slight increase in removal process of heavy metal like Cr(VI) removal favored at low pH due to the positive charged surface of nanoparticle at low pH attract the negative charge anions as a result an electrostatic attraction occurred[66]. Also, at some point adsorption sites become independent of pH change indicating saturation point forms hydroxyl, depends upon the type of heavy metal that is to be removed and the surface of nanoparticle.

**3.7.3.2. Temperature**

Generally, with increase in temperature the rate of adsorption decreases according to the Le Chatelier’s principle as with increase in temperature absorbent surfaces boundaries get thinner due to which the metal ion escape from the adsorbent. But in some cases, with increase in temperature from 288 K to 308 K the adsorption of Cr(VI) and Cu(II) on the surface of iron nanoparticle also increased[66].

**CHAPTER 4**

**4.1. RESULT**

We successfully synthesize silver nanoparticle using *Acalypha wilkesiana* leaf extract. By visual observation, colour change occurs from yellow to dark brown. further isolation of synthesised silver nanoparticle was done by centrifugation at 10000 rpm for 20 minutes to obtain the silver nanoparticle from aqueous solution.

Figure Colour change from light blue to brown gives the indication of formation of nanoparticle

The following silver nanoparticle under goes various characterization like UV-Vis spectroscopy, Fourier Transform Infrared Microscopy, TEM, followed by EDX Spectroscopy. The expected absorbance was approximately 400 nm that is determined by UV – vis spectroscopy.

* 1. **DISCUSSION**

Nowadays, the release of various hazardous dyes from the industries and presence of heavy metal in water bodies creating many problems related to human and animal health and affecting the environment. Nanomaterials are used in many different fields like in pharmaceutical including drug delivery, cosmetics etc, and environmental preservation. water can be polluted by presence of large quantity of heavy metals and dyes.

 We discussed the removing mechanism of these pollutant with nanoparticle synthesis with biological component. Here we aimed the remediation of polluting waste water by eco-friendly synthesis of silver nanoparticle. the synthesis of nanoparticle by *Acalypha wilkesiana* is challenging but cost effective and simple. Synthesis of nanoparticles involves proper characterization including the absorbance of metal ion, size, shape and crystalline structure of nanoparticle. After synthesis of nanoparticle of appropriate size can be used for the dye degradation as well as in removal of heavy metal from aqueous solution following reduction and adsorption kinetics. We explored the biotechnological prospects of nanoparticle synthesis applying this to industrial scale which not only remediate the environment but also proved to be helpful in health sector.

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