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GREEN NANOTECHNOLOGY APPROACH FOR DYE-CONTAMINATED WASTEWATER TREATMENT: ADSORPTIVE POTENTIAL OF SILVER NANOPARTICLES DERIVED FROM ABIES PINDROW CONES

Muhammad Suleman (Corresponding Author)
Institute of Chemical sciences, University of Swat, KP, Pakistan
Email: dr.mschemist@gmail.com
Waqar Ahmad
Department of Chemistry, University of Science and Technology Bannu, Pakistan
Email: waqarahmad7272kundi@gmail.com
Zeeshan Ahmed
Department of Water Resources Management (WRM), Faculty of Engi Sci. Tech. (FE&ST), Lasbela University of Agriculture, Water and Marine Sciences (LUAWMS), Uthal.
Email: zeesmile1999@gmail.com
Saqib Mehmood
Department of Chemistry, Kohat University of Science and Technology Kohat, Pakistan
Email: saqibchemist48@gmail.com
Muhammad Shayan
Department of Chemistry, Kohat University of Science and Technology Kohat, Pakistan
Email: muhammadshayan929@gmail.com

Burhan Ishaque
School of Pharmacy, Applied Sciences and Public Health, Robert Gordon University, Garthdee House, Garthdee Rd, Aberdeen AB10 7AQ, Scotland.
Email: burhanishaque@gmail.com
b.ishaque@rgu.ac.uk

ABSTRACT

Dye is used to color different products in many industries, such as cosmetics, pigments and textiles. Approximately one hundred thousand commercial dyes are produced by industries and annually global production of associated dyes exceeding 7×10^{-5} tons. The purpose of present study is use of silver nanoparticles derived from *Abies pindrow* cone (APC) as an adsorbent for the removal of methylene blue dye from aqueous solution. The prepared *Abies pindrow* cone Ag-NPs (APC-Ag-NPs) were characterized using FTIR, SEM and EDX for surface functional groups and morphology and elemental analysis. The zeta potential and average size of APC-Ag-NPs) were verified *via* ELS and DLS. Furthermore, statistical analysis was incorporated to validate adsorption efficiency. Some factors being analyzed in the study include adsorbent dosage, initial dye concentration, variable particle size and temperature. It was found that increasing the adsorbent dose led to reduced dye removal due to underutilization of available sites. Particle sizes below 3.0 also increased dye removal because the smaller size allowed a large surface area and more

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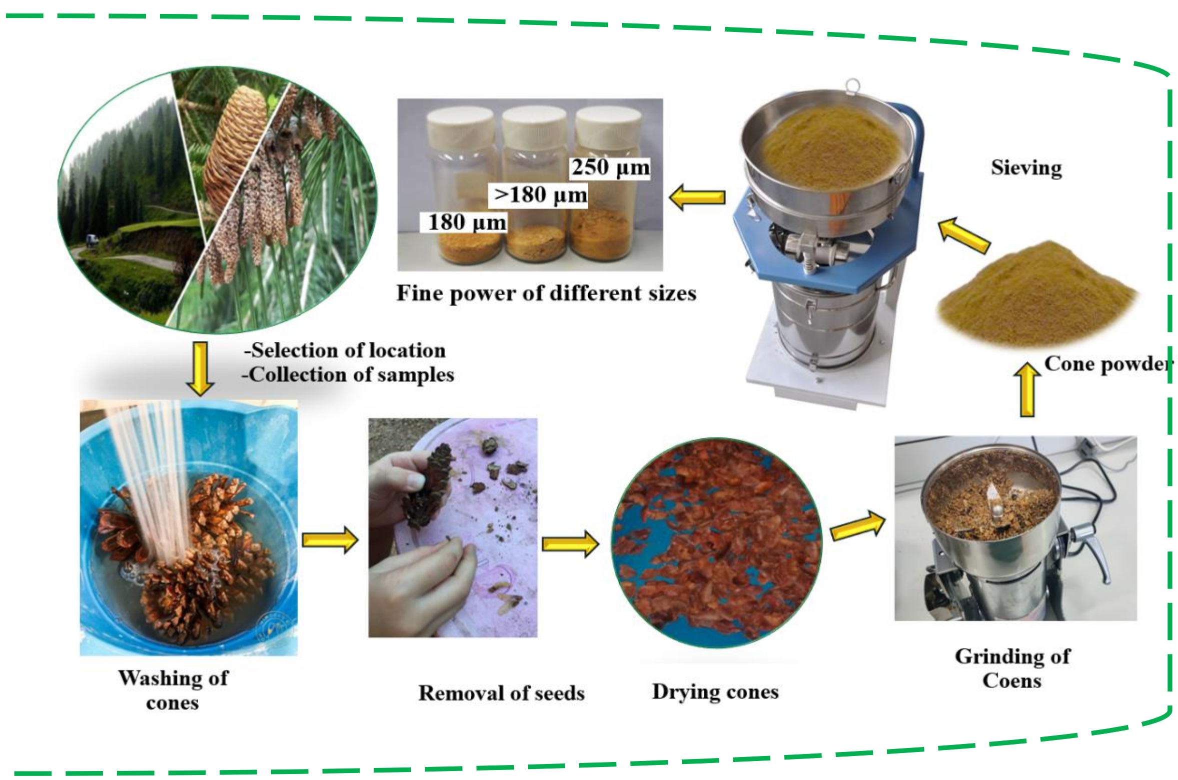
available attachment sites. The removal efficiencies increased with the increase in initial dye concentration, due to the concentration gradient. Increasing temperature led to reduced removal of dye, presumably because adsorbate-adsorbent interactions are relatively poorer and dyes become soluble. Statistical analysis was undertaken to test the importance of the adsorption trends. The mechanism of adsorption was also examined using Langmuir and Freundlich isotherm models to allow gain of further information regarding the process of adsorption. Our findings reveal that, the APC-Ag-NPs have improved efficacy of removing dye by efficient modes and can be utilized as an absorbent of eliminating dye in wastewater.

Graphical Abstract



Keywords: Methylene Blue Dye, Abies Pindrow Cone, Silver Nanoparticles (Ag-Nps), Adsorption, Wastewater.

Introduction

The ever-growing industries, including the textile industry, the paper mill industry, and the dyeing industry have played a major role in the pollution of large quantities of waste water by synthetic dye [1,2]. Being chemically complex and highly stable structures, such dyes are exceptionally inert to biological and chemical modes of degradation, thus wastewater handling is one of the most

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important environmental concerns. Methylene blue is one of the most popular dyes found among these contaminants, yet it is rather popular in the textile industries due to the purpose of dyeing the fabrics. A significant percentage of dyes that are used in the course of the industrial applications which is approximately 20 percent does not adhere to the cloth but are rather discharged into effluent stream [3]. This unutilized dye not only alters the natural color of water bodies, causing aesthetic pollution, but also clogs drainage systems and wastewater channels, creating operational problems. Beyond the visible pollution, dyes in aquatic environments exert severe ecological consequences by interfering with photosynthetic activity, impairing the growth and reproductive cycles of aquatic organisms, reducing light penetration in water bodies, and decreasing dissolved oxygen levels essential for aquatic life [4]. They also alter water chemistry and can be toxic or cause bioaccumulation in aquatic life, leading to biodiversity loss. It has been reported that methylene blue in wastewater can cause cytotoxic effects and alter microbial ecology in aquatic systems. Recent studies indicate that persistent organic pollutants in dyes exhibit long-term environmental and health hazards, necessitating effective remediation strategies. By product produced during dyeing process affecting both aquatic and human, Dyes are very dangerous for central nervous system, can cause cancer and give rise to mutation in animal. Nausea, vomiting, itchiness, eye burn, mental problems and cancer can be caused by blue methylene. It is important to eliminate dye from wastewater. Waste from industries that utilize dye must be adhered to required standards [5-7].

Similarly, natural minerals like bentonite and halloysite have been investigated for their ability to eliminate synthetic dyes, which constitute a significant portion of the dyes used in the textile industry[8-10]. Agricultural waste has also emerged as potential sustainable adsorbents. The utilization of such agricultural by-products aligns with sustainable waste management practices[11, 12]. Their abundance, low cost, and minimal treatment requirements make them attractive options for large-scale applications [13, 14].These are organic materials and have the benefits of degradation, low on toxicity and recyclable over synthetic adsorbents [16] [15]..

It is a large evergreen conifer of western Himalayan region of Afghanistan, Pakistan and northern India, found from 2000 to 3500 meters of altitude [17,18, 19]. It contains many uses to high altitude biosphere, it contributes to the formation of diverse habitat, plays an important role in carbon storage, and helps in soil consolidation [20, 21]. This species was an ecological, economic and cultural resource and is a habitat to wildlife [22]. Economically, people use it for timber in construction and making of papers and the resin used in traditional systems of medicines and other small-scale industries [23]. Abies Pindrow can grow up to 60 meters tall with a straight and cylindrical stem; it possesses dark green needle-like leaves with arrangement of two ranks [24]. It has a variety of uses, although it has not been investigated or utilized as much as do the other parts of the tree. The most obvious use of these cones is in décor They are also important in décor and there is no doubt that they have been widely used especially in decorating events such as the Christmas. Their fine and beautiful looks make them suitable for use in decoration purposes such as festive decorations or when used in natural environment. These have an important function in the reproductive process of the tree since they form in the natural habitat of Abies pindrow. There is recent research demonstrating the viability of Abies pindrow cones as a very effective bio-adsorbent in wastewater treatment. As in most plants they have to dehisce and shed seeds that play

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an important role in the survival and spreading of the plants [25].

While not specifically studied, the cones of Abies pindrow may have potential as a natural adsorbent due to their lignocellulosic structure, making them useful in environmental applications like wastewater treatment. This application is based on the success of other plant-based materials in similar roles. Agricultural and forestry waste products like these cones offer an environmentally friendly solution for wastewater treatment. However, this potential application requires further research and experimentation to determine its viability and efficiency [26]. To bridge this research gap, the present study investigates the efficiency of APC-Ag-NPs in dye adsorption, focusing on its characterization, adsorption capacity, and environmental implications.

There is development in nanotechnology into different study disciplines such as medical chemistry. When nanoparticles green synthesis is compared with microbe mediated synthesis it become clear that green synthesis is more economical and environmentally friendly operation [27, 28]. Green synthesis is known as the ideal method for the formation of nanoparticles. Sliver was of particular significance due to particular physical and chemical characteristics. [29]. These metal particles like Ag, Au, Fe, se, Zn having unique properties in nanotechnology and are important as therapeutic agent [30, 31]. The APC can easily be approachable and have no other productive objective [32]. APC can be used as an adsorbent to remove contaminants from waste stream. The main focus of current research is to utilize APC to remove dye from wastewater [33, 34]. It evaluates the adsorption efficiency and potential of APC-Ag-NPs natural material to determine its suitability for real-world applications. By optimizing factors like adsorbent dose, temperature and particle size, the research aims to contribute to sustainable water purification methods using natural resources.

Material and Methods

Synthesis of APC-Ag-NPs Adsorbent

The preparation of silver nanoparticles (Ag-NPs) from Abies pindrow cones (APC) was carried out using a green synthesis approach, which is recognized as a cost-effective and eco-friendly alternative to chemical synthesis. The procedure involved four sequential steps.

In the **first stage**, raw cone materials were prepared. This process included careful site selection for cone collection, ensuring optimal maturity of the cones, followed by seed removal, thorough washing, drying, and mechanical crushing to obtain clean plant material (Figure 1a). The cones were collected during their natural peak harvesting season, which ensured maximum retention of bioactive constituents essential for nanoparticle formation.

The **second stage** involved the extraction of phytochemicals from the cones. The cleaned and processed material was subjected to boiling at 100 °C for 2 hours, after which the extract was filtered and centrifuged at 5000 rpm for 15 minutes. This procedure ensured the removal of larger residues and provided a purified extract enriched with reducing agents.

The third step of the synthesis involved the actual fabrication of silver nanoparticles (Ag-NPs). In this stage, a carefully measured 3 mL volume of Abies pindrow cone (APC) extract was blended with 40 mL of a freshly prepared 1 mM aqueous silver nitrate ($AgNO_3$) solution. The reaction was carried out under ambient room temperature conditions without the need for external heating or additional chemical reducing agents. A noticeable visual change was recorded during the process:

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the initially pale-yellow mixture gradually shifted to a dark brown coloration. This transformation served as a clear preliminary indicator of the successful reduction of silver ions (Ag^+) into their metallic nanoscale form (Ag^0). Such a color transition is a hallmark of nanoparticle formation and is typically associated with surface plasmon resonance phenomena that arise due to the collective oscillation of conduction electrons within the metallic nanoparticles.

Following the synthesis, a purification stage was conducted to isolate high-quality nanoparticles suitable for subsequent experimental use (Figure 1b). The suspension containing newly formed APC-Ag-NPs was subjected to repeated washing cycles using deionized water to remove excess ions and residual biomolecules from the plant extract. Each washing step was followed by centrifugation at a speed of 13,000 rpm for 15 minutes, which allowed efficient separation of nanoparticles from unreacted materials and other impurities. The purified nanoparticles were collected in a dry state and kept away for carrying out the further evaluation.

To evaluate the adsorptions capacity of the synthesized APC-Ag-NPs, batch adsorptions were conducted. In this work, the variation of adsorbent dose, dye concentration, particle size, and operating temperature were the variables manipulated in designing the experiments to investigate their effects on the removal of the dye. The adsorption data was then fitted to traditional and well-known adsorption isotherm models, such as Langmuir and Freundlich equations, to calculate adsorption capacity, binding affinity and overall mechanism of interaction between dye molecules and the nano particle surface. This holistic methodology provided a synthesis of APC-Ag-NPs and the performance assessment of such material as possible adsorbent in wastewater treatment activities

Synthesis of Raw Abies Pindrow Cone (APC) Powder

Raw powder of Abies pindrow cones was obtained by a standard procedure by pulverising the cones to obtain a particle size within different ranges. The cones used in this research were taken on healthy trees in Sudan Gale when the normal period of the cones bearing comes in between 1 st May and 15 th July every year. To ensure sample integrity,. cones were placed immediately into clean plastic storage under natural light into the laboratory. Manipulation was done with sterile gloves in order to limit microbial contamination and samples stored at regulated environmental conditions.

For drying, cones were spread in a shaded, well-ventilated space for about two weeks. Periodic turning was carried out to ensure uniform drying and to prevent fungal growth. Once adequately dried, the seeds were manually separated by rubbing, leaving the cone tissues for further preparation. These dried cone segments were washed first with tap water and then with distilled water to remove any dirt or surface contaminants.

The cone material was then pulverized into a fine powder using a laboratory blender, operating for approximately two minutes per batch. The ground powder was fractionated into distinct particle size categories using standard mesh sieves, resulting in three size ranges: 180 µm, >180 µm, and 250 µm. To maintain stability, all powdered samples were stored in airtight glass containers to prevent moisture absorption before being used in subsequent extraction and nanoparticle synthesis steps.

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Synthesis of Abies Pindrow Cone Extract

In the second stage of the synthesis process, an aqueous extract of Abies pindrow cones (APC) was prepared from the previously dried and powdered cone material. Approximately 200 mL of sterile nanopore water was mixed with 50 mL of ethanol, and the powdered cones were boiled in this solution at 100 °C for two hours. This thermal treatment facilitated the extraction of bioactive phytochemicals, such as flavonoids, tannins, and polyphenolic compounds, which act as reducing and stabilizing agents in the subsequent formation of silver nanoparticles. Continuous stirring at 200 rpm during the boiling process ensured uniform extraction and improved interaction between the solvent mixture and the plant matrix.

After the heating step, the extract was allowed to gradually cool to room temperature. The same extraction protocol was applied to powders of all particle size categories prepared earlier to ensure consistency. Once cooled, the liquid extract was filtered through Whatman No. 1 filter paper to remove coarse particulate matter, thereby yielding a clear solution suitable for nanoparticle synthesis. This filtrate served as the primary reducing medium in the preparation of APC-based silver nanoparticles

Synthesis of APC- Silver Nanoparticles

Following extraction, the green synthesis of silver nanoparticles was carried out by combining 3 mL of the cooled cone extract with 40 mL of a 1 mM silver nitrate ($AgNO_3$) solution in a clean Erlenmeyer flask (Figure 2a). The mixture was continuously stirred at 150 rpm under ambient laboratory conditions to facilitate the reaction. A gradual shift in color from light yellow to dark brown with a yellowish hue was observed during the reaction, which served as a visual confirmation of silver ion (Ag^+) reduction into metallic silver nanoparticles (Ag^0).

To prevent photoreduction or decomposition, the mixture was kept in darkness and allowed to react for 24 hours. By the end of this period, the distinct dark brown coloration of the suspension indicated successful nanoparticle formation (Figures 2b and 2c). This step completed the preliminary biosynthesis of APC-Ag-NPs, which were subsequently subjected to purification for further characterization and adsorption studies

Purification of APC Silver Nanoparticles

Upon completion of APC-Ag-NPs synthesis, an isolation procedure was carried out to eliminate any presence of leftover biomass particles, unreacted silver ions, and side-products that would form during the reaction. Such purification is necessary because the nanoparticles were to be of high purity and fit to be used in other experiments.

The raw nanoparticle suspension was centrifuged at 13,000 rpm intending to set aside the nanoparticles as well as the supernatant with impurities. The pellet which was a mixture of silver nanoparticles was very carefully collected and re-dispersed in nanopore water. Centrifugation and its resuspension was carried out a number of times to further increase purity and to increase the colloidal consistency of the suspension.

After several purification steps, the nanoparticle pellets were freeze-dried at -80 o C This was an important step to retain the structural integrity, morphology, and surface chemistry of the

Annual Methodological Archive Research Review
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Volume 3, Issue 10 (2025)

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nanoparticles and simultaneously retain functional properties. The resulting dried APC-Ag-NPs were kept under suitable conditions until their further characterization and adsorption studies were made. On the whole, the nanoparticles of high quality were obtained in this multi-step purification procedure and therefore could be used in actual practical application in wastewater treatment and environmental remediation studies

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Batch Adsorption Experimentations

In order to determine the efficiency of the adsorption of APC-Ag-NPs in dye methylene blue removal adsorption tests were conducted under a controlled laboratory environment. Stock solution of methylene blue (1000 ppm) was prepared by dissolving the dye in distilled water (Figure 2d). Based on this stock solutions, working solutions were made to conduct experiments.

In a normal adsorption experiment, 25 mL of the dye solution was pipetted into a 50 mL conic flask where the initial dye concentration is measured at 30 ppm. The solution was added to 1 g/L APC-Ag-NPs adsorbent. The flask then was placed in a shaking orbital incubator where agitation was done at preset intervals such that there was sufficient interaction of dye molecules with the adsorbent surface.

Major parameters were investigated in a systematic way to examine the influence on the adsorption performance. These were initial dye concentration (20, 30, 40, 50, 70 and 100 ppm), dosage of the adsorbent (0.5, 1, 2, 3 and 4 g/L), size of the adsorbent particle (180 and >180 and 250 m), and temperature of the solution (25, 30, 35, 40 and 45o C). After incubation and agitation, samples were centrifuged, concentrate of remaining dye in the supernatant being measured by UV-visible spectrophotometry.

The methylene blue removal percentage and nanoparticles adsorption capacity were calculated according to equations (1) and (2), respectively. In these formulas, Ci corresponds with Cf is the initial and final dye concentration (mg/L), qe is the equilibrium adsorption capacity, m is the mass of adsorbent employed (g) and V is the volume of solution (mL). Such a methodological procedure gave insanely important information about the adsorption principles of APC-Ag-NPs depending on various environmental and experimental conditions

.

$$\% \text{ Removal} = \frac{\text{Ci}-\text{Cf}}{\text{Ci}} \times 100 \text{ --- (1)}$$

$$\text{qe} = \frac{\text{Ci}-\text{Cf}}{\text{m}} \times \text{v} \text{ -- (2)}$$

In these equations, Ci and Cf denote the initial and equilibrium (or final) concentrations of methylene blue in solution, expressed in milligrams per liter (mg/L). The term qe refers to the adsorption capacity of the material at equilibrium, representing the amount of dye adsorbed per unit mass of the adsorbent. The variable m corresponds to the mass of the APC-Ag-NPs used in each batch experiment (grams), while V indicates the total volume of the dye solution employed during adsorption (milliliters). Together, these parameters provide a quantitative assessment of the adsorption efficiency and the ability of the nanoparticles to capture and retain dye molecules from

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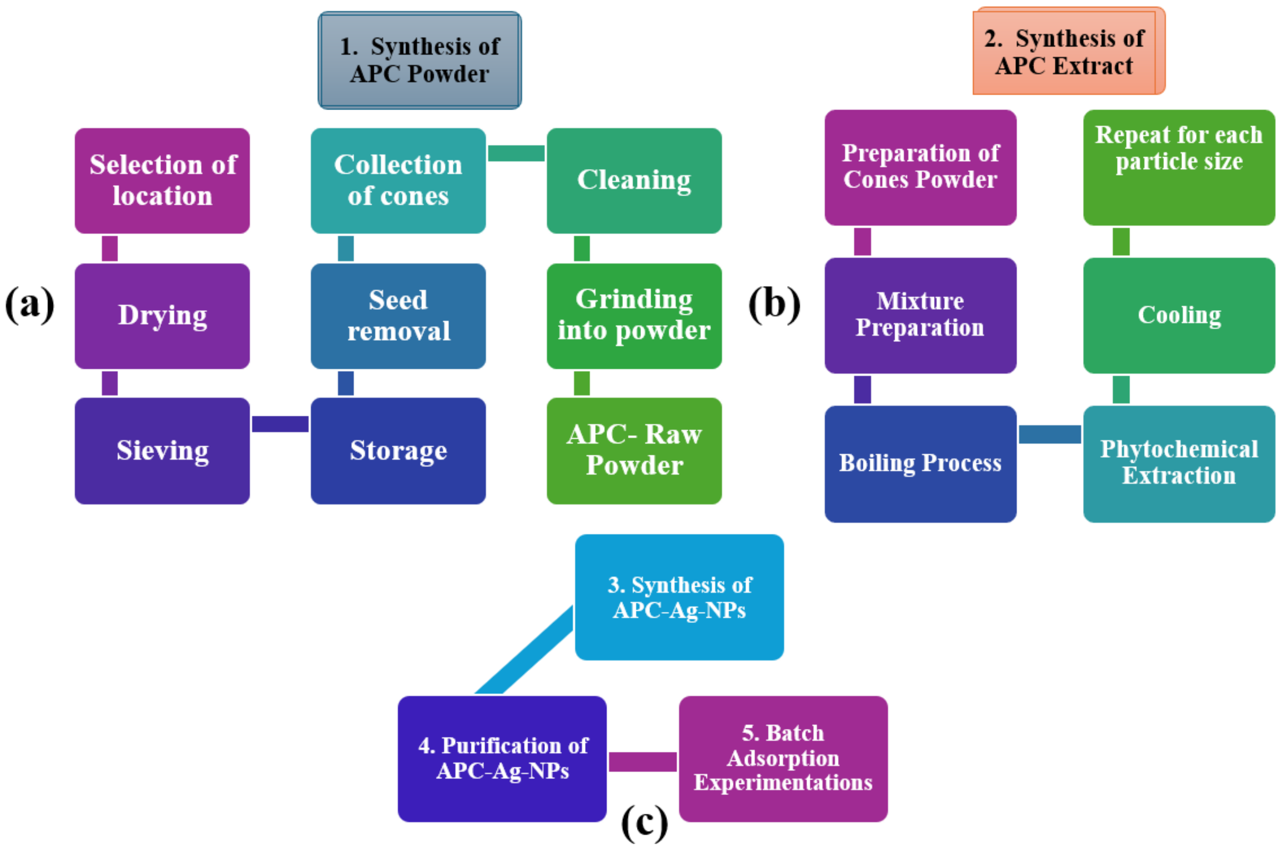
aqueous systems[35].

Figure 1 (a) Illustration of Steps involved synthesis of raw APC powder, **(b)** Step involved from synthesis of plant extract to Adsorption study.



Annual Methodological Archive Research Review
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Volume 3, Issue 10 (2025)

Online ISSN 3007-3197
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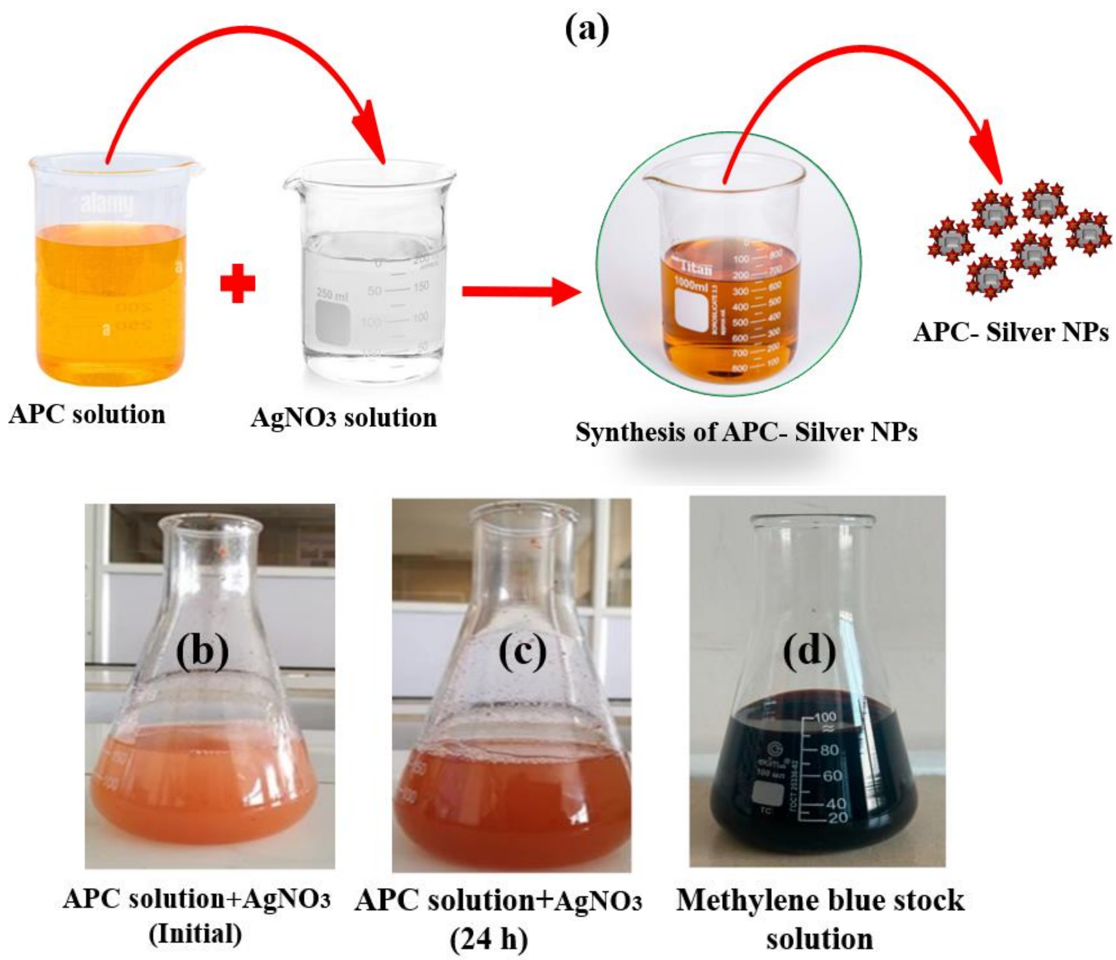

Figure 2. (a) Green synthesis using APC for Silver Nanoparticles **(b)** Initial Aqueous solution of APC-$AgNO_3$ **(c)** Aqueous solution of APC- $AgNO_3$ after 24 h, **(d)** Stock solution.
Characterizations Techniques

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared (FTIR) spectroscopy was employed to analyze the structural and chemical features of APC-Ag-NPs, using a Nicolet Magna IR 550 spectrometer. This technique is widely recognized for its ability to identify functional groups and chemical bonds present within complex biological and nanomaterial systems. For direct and accurate sample measurement, the Attenuated Total Reflectance (ATR) mode was used, which minimizes the need for extensive sample preparation and reduces potential artifacts.

TThe FTIR spectra were obtained in the mid-infrared region, i.e., in the wavenumber range between 4000 and 400 cm -1. All spectra consisted of 64 scans, sufficient to provide an acceptable signal to noise ratio. The instrument can be used to obtain a high-spectral resolution, the maximum

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http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 10 (2025)

Online ISSN 3007-3197
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of which is 0.125 cm -1, and thus subtle changes in vibrational frequencies can be detected in detail. These spectra were helpful in indicating us the molecular composition of APC-Ag-NPs and the presence of biomolecules in the cone extract that acted as reducing and stabilizing reagents in the synthesis of nanoparticles.

The recorded ATR-FTIR spectra were relatively non-destructive and allowed the identification of surface functional groups without renovation of the sample, thereby facilitating the identification of chemical interactions and surface modifications that transpired during nanoparticles manufacture. The reduction in silver ions confirmed the interaction of phytochemicals, and it also gave substantial evidence of the contribution it has on nanoparticle stability and bioactivity

.

Scanning Electron Microscopy and Energy Dispersive X-ray Spectrometer (EDX)

SEM/EDX was used to further characterize APC-Ag-NPs. The surface morphology and topographical characteristics of synthesized nanoparticles were obtained using the LEO 1530 microscope, thus, SEM analysis. The high resolution image analysis method enables visualization of an individual particle shape, distribution and extent of aggregation in the sample.

The SEM machine had attained an Energy Dispersive X-ray Spectrometer (EDS) detector that allowed complex mapping of elements along with imaging. DX selects X-ray usages, which are projected through the sample by the interaction on electrons, which enable the qualification and quantification of elemental components. Its detection range is up to uranium and thus allows full elemental mapping.

The spatial resolution and image quality in SEM-EDX analysis depends on several factors; those are energy of primary electron beam, the atomic number of the sample elements and the specimen thickness. In this work, the high voltage electron source used was between 0.2 and 30 kV, to ensure best resolution under varied sample conditions.

Combining SEM and EDX offered twin benefits that provided the reversible morphology, e.g. particle size, shape and distribution based on SEM and elemental identity, together with purity of the synthesized nanoparticles; especially as the dominant element was silver. Such thorough structural and elemental characterisations were very important to ascertain the correlation between the surface features of APC-Ag-NPs and their properties. The concept of this integrated mechanism also facilitated that the synthesized nanoparticles did not just achieve an adequate state but could also be utilized practically, i.e., in the adsorption-based wastewater treatments.

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Results and Discussion

Fourier Transfer Infra-Red Spectroscopy (FTIR)

FTIR-spectroscopy is a common and used analytical instrument in terms of molecular characterization, where its main use is to identify functional groups of the natural plant extract and investigate the role they play in the synthesis of nanoparticles. It helps offer distinct spectral fingerprints of the samples in line with their molecular structure, so researchers can trace the chemical associations during nanoparticle formation [36].

The FTIR spectra of the biosynthesized APC-Ag-NPs (Figure 3 and Table 1) indicated various absorption peaks related to various functional groups bound on the surface of the nanoparticles. Background subtraction, normalization, and baseline corrections were done to reduce experimental

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http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 10 (2025)

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http://amresearchreview.com/index.php/Journal/about

errors, and enhance the reliability of spectra prior to the assignment of the peaks.

The main peaks identified are 649 cm -1 and 818 cm -1 assigned to C-Br and C-Cl, respectively, which has associated alkyl halides. A high absorbance peak at 995 cm -1 was assigned to =C-H bending vibrations of alkenes and a medium absorptivity peak at 1143 cm -1 was related to C-N stretching of aliphatic amines. Furthermore, using the Fourier transform infrared, showed a pointy wave at 1437 cm -1 due to C-C stretching in aromatic structures and a large band at 1547 cm -1 that corresponded with N-O asymmetrical stretching in nitro compounds.

The occurrence of these functional groups implies that a wide pool of phytochemicals of the cone extract was successfully introduced on the surface of the nanoparticles. These biomolecules are thought to play a dual role: they sequester silver ions (Ag+) to metallic silver nanoparticles (Ag 0) and they also stabilize the nanoparticles to prevent aggregation and nanoparticle dispersion. This observation highly favors the efficacy of the green synthesis method in which naturally occurring compounds are of great importance in the fabrication of nanoparticles [37].

The general finding of FTIR abdominal test was that the phytoconstituents in Abies pindrow beaks played a direct role in the nanoparticle formation as well as stabilization. Their presence does not only add structural stability but it can also increase the potential bioactivity of APC-Ag-NPs. This observation presents new opportunities to apply such phytochemical-coated nanoparticles in a wide range of areas such as environmental clean-up, medical treatment, and agricultural practices. Future research can further optimize such a natural process of capping to enhance the functionality of the nanoparticle and increase applicability of nanoparticles in sustaining nanotechnology

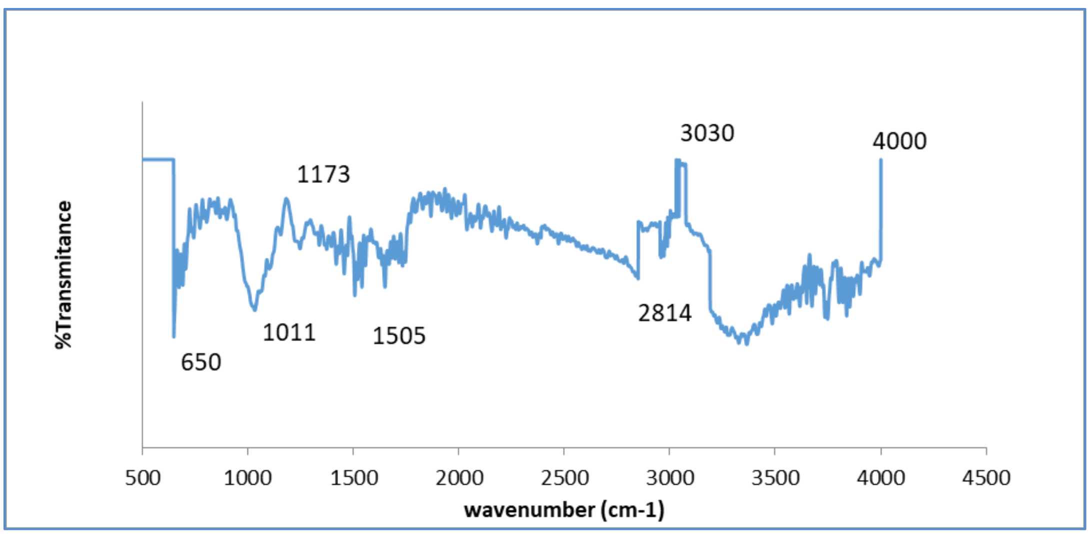

Figure 3 FTIR Analysis of ACP-Ag-NPs

Table 1 FTIR peaks and possible functional groups in APC-Ag-NPs

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 10 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

FTIR Peak (cm^{-1})	Intensity/Shape	Type of Bond	Likely Functional Group
658	Medium band	C–Br stretching vibration	Alkyl halide compounds
818	Medium band	C–Cl stretching vibration	Alkyl halide groups
995	Strong band	=C–H bending mode	Unsaturated alkenes
1143	Moderate band	C–N stretching vibration	Aliphatic amines
1437	Medium band	C–C stretching within aromatic ring	Aromatic structures
1547	Strong band	Asymmetric N–O stretching	Nitro derivatives

Electrophoretic Light Scattering (ELS) and Dynamic Light Scattering (DLS) techniques

To determine particle size and the colloidal stability of the APC-Ag-NPs synthesized, Electrophoretic Light Scattering (ELS) and Dynamic Light Scattering (DLS) were used (Figure 4a). The indicative techniques give important information of the behaviors of nanoparticles in suspension. The zeta potential was measured using LS to detect surface charge/stability of nanoparticles and the average hydrodynamic diameter of the particle by using DLS.

The ELS analysis showed that the APC-Ag-NPs had a zeta potential of -22.3 mV suggesting that the nanoparticles had an overwhelmingly negative surface charge. A negative value of the zeta potential means that there exist electrostatic repelling forces amongst the particles and therefore the aggregation and settling does not take place in the aqueous medium. These disgusting contacts play a very critical role in the consistency of a colloidal suspension, to ensure that the nanoparticles remain suspended and not clumping. This is especially significant in applications due to the higher adsorption capacity in wastewater, and performance of biological systems.

Further details of the zeta potential analysis are presented in Table 2. The dominant peak (Peak 1) demonstrated an average zeta potential of –18.5 mV with a standard deviation of 6.84 mV. This relatively narrow variation suggests consistent surface charge distribution across the nanoparticles, which indicates a stable colloidal system. The data showed that this peak accounted for 100% of the measured area, highlighting the predominance of uniformly charged nanoparticles within the sample. In contrast, a secondary minimum was recorded at 18–20 µM with an average zeta potential of 0.00 mV and 0% contribution to the overall distribution. Since this peak had negligible representation, it was considered insignificant in describing the colloidal behavior of APC-Ag-NPs.

These findings confirm that the synthesized nanoparticles possess adequate stability, as indicated by their negative zeta potential values. While highly stable colloids are usually associated with zeta potential values lower than –30 mV, the obtained –22.3 mV still suggests moderate electrostatic stability, which is sufficient for most adsorption-based applications

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Table 2 Zeta potential of APC-Ag-NPs

Zeta potential (mv)	Mean (mv)	Area (%)		St Dev. (mv)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

-19.5	**Peak1: -18.5**	**100.0**		**6.84**
16.1	**Peak 2: 0.00**	**0.0**		**0.00**

This method is particularly important because nanoparticle dimensions strongly influence their functional properties, including adsorption efficiency, antimicrobial potential, and suitability for biomedical applications such as targeted drug delivery.

The measurement obtained from DLS corresponds to the **hydrodynamic diameter**, which is generally larger than the actual core size of the nanoparticles. This discrepancy occurs because DLS accounts for not only the metallic nanoparticle core but also the surrounding hydration shell and any surface-bound biomolecules from the plant extract that move with the particles in solution. Therefore, the hydrodynamic size provides a realistic estimate of how nanoparticles behave in aqueous environments, which is essential for assessing their stability and application potential.

The particle size distribution was further evaluated using the **Polydispersity Index (PDI)**. A median PDI value of 0.206 was obtained, which falls within the range of 0.1–0.25. This indicates a relatively narrow size distribution and suggests that the nanoparticles are fairly homogeneous in suspension [38]. Samples with PDI values below 0.2 are typically regarded as monodisperse, while values up to 0.25 still indicate acceptable uniformity. Hence, the APC-Ag-NPs synthesized in this study can be considered well-dispersed and suitable for consistent performance in adsorption and biological applications.

The combined results from ELS and DLS analyses support the conclusion that APC-Ag-NPs possess moderate colloidal stability. Although the zeta potential of –22.3 mV is slightly lower than the threshold for optimal long-term stability (generally < –30 mV or > +30 mV), it is still sufficient to prevent aggregation in the short term. The presence of a non-zero negative charge ensures adequate electrostatic repulsion between nanoparticles, preventing sedimentation and maintaining their uniform suspension. This level of stability makes the synthesized nanoparticles highly applicable in environmental remediation processes and potentially valuable in biomedical fields, where controlled particle dispersion is critical for functional efficiency

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(a)

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http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 10 (2025)

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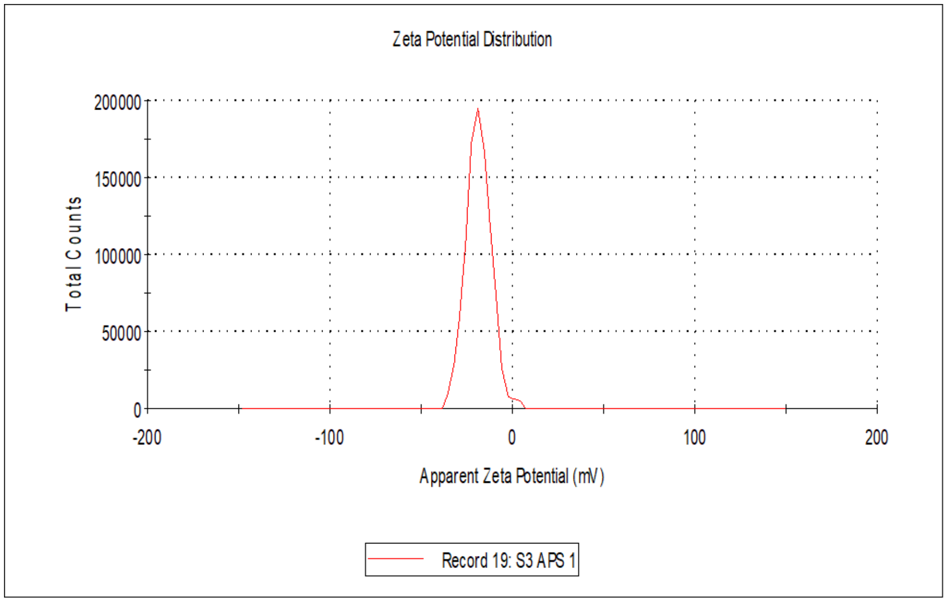

(b)

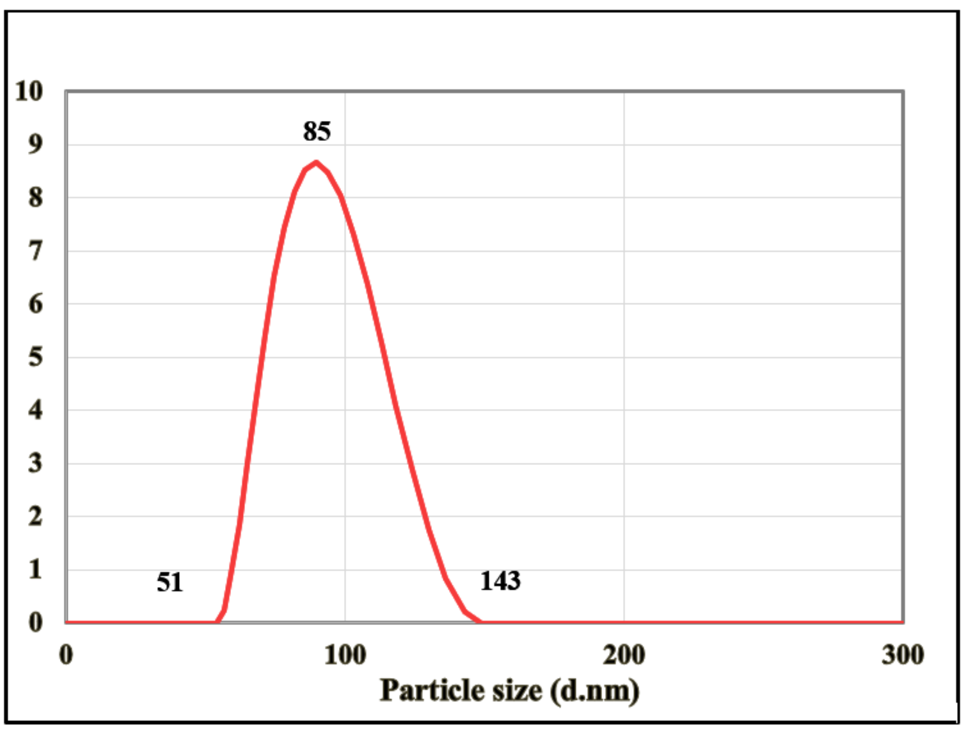

Figure 4 (a) Zeta potential and (b) Particle sizes of APC- Ag-NPs.

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 10 (2025)

Online ISSN 3007-3197 Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

SEM (Scanning Electron Microscopy)

Scanning Electron Microscopy (SEM) was employed to analyze the morphology, size, and distribution of APC-Ag-NPs synthesized using Abies pindrow cone extracts. This technique is considered one of the most effective methods for nanoscale imaging as it provides high-resolution details of particle structure and surface characteristics. SEM observations of silver nanoparticles synthesized from plant extracts frequently reveal predominantly spherical morphologies; however, irregular geometries such as triangular, tetragonal, pentagonal, and hexagonal structures are often observed as well.

In the present study, SEM micrographs (Figure 5a–d) showed that APC-Ag-NPs exhibited a range of shapes, with spherical nanoparticles being the most common. The average particle size was generally within the range of 5–100 nm, which aligns with previously reported green-synthesized silver nanoparticles. Several particles were observed in the range of 15–25 nm, confirming the nanoscale nature of the synthesized product. In addition, SEM images revealed both well-dispersed individual nanoparticles and clusters of aggregated particles, suggesting that phytochemicals present in the extract played a dual role in controlling particle growth while preventing excessive agglomeration.

The resolution of SEM imaging depends on magnification and detector settings. Images captured at varying scales (1 µm, 2 µm, 5 µm, and 20 µm) provided a comprehensive view of the nanoparticle distribution across the sample. These multi-scale observations allowed for detailed morphological characterization, offering insights into nanoparticle stability and uniformity [37,39,40]. Overall, SEM analysis confirmed the successful biosynthesis of nanoparticles, demonstrating controlled particle size distribution and surface morphology suitable for adsorption applications. [39, 40].

Energy Dispersive X-ray Spectroscopy (EDX)

To confirm elemental composition of APC-Ag-NPs, the Energy Dispersive X-ray Spectroscopy (EDX) analysis was performed (Figure 5e). The EDX spectrum showed high energy scattering surface peaks that matched silver with peak concerns noted at 3 keV. This maximum is a long-recognized property of metallic silver nanoparticles and is due to surface plasmon resonance [41,42]. The fact that the most prominent peak appeared sharp and intense stipulated that silver was the major component in the synthesized material.

Interestingly, intensity of the silver peaks was more intense in APC-Ag-NPs prepared using the Abies pindrow cone extract as compared to values recorded in other biosynthesis sources. This indicates that the extract of the cone was able to provide optimal ion reduction and stabilization, thus making nanoparticles that have greater elemental purity. Such results correlate with those of previous publications, including those making use of Curcuma longa extracts, in which similar and sharp absorption peaks at 3 keV were observed in Ag-NPs [43].

Also, any background peaks in the spectrum were related to residue or trace phytochemicals in the extract. Their presence is also a confirmation of the role played by biomolecules as capping and stabilizing agent in nano particle synthesis. As a whole, EDX data prove that the APC-Ag-NPs were successfully synthesized with a high purity of elements and stability exhibited that could be used in the wastewater treatment and other environmental remediation processes. [37].

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 10 (2025)

Online ISSN 3007-3197
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http://amresearchreview.com/index.php/Journal/about

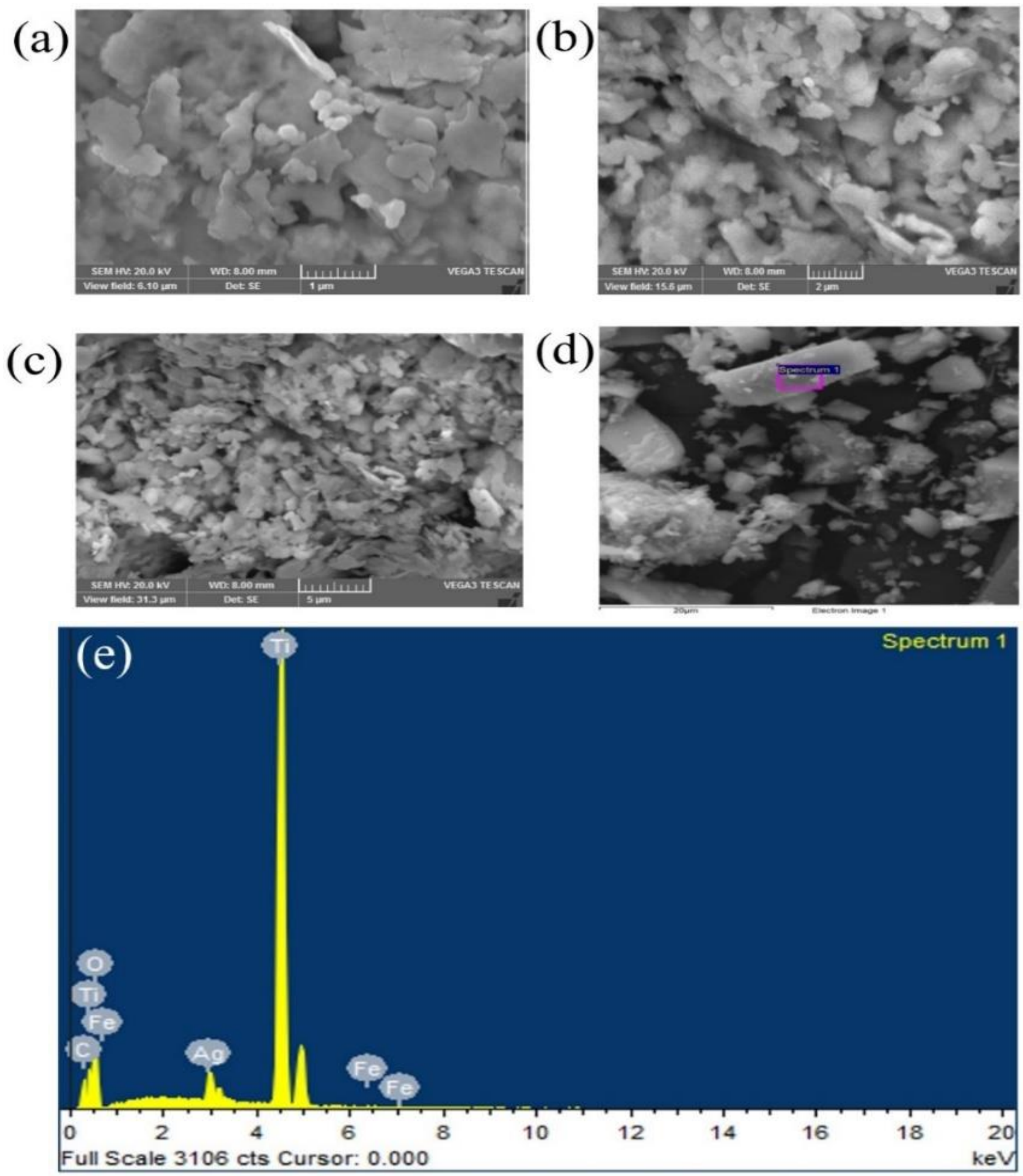

Figure 5 SEM Images at different (a) 1µm, (b) 2µm, (c) 5µm, and **(c) 20µm, (e) EDX results for Ag-NPs.**

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 10 (2025)

Online ISSN 3007-3197 Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

Adsorption Experiments

Adsorbent Dose

UV–visible spectrophotometry was used to monitor the concentration of dye in solution both before and after treatment with APC-Ag-NPs. This allowed for the calculation of two critical adsorption parameters: percentage dye removal and adsorption capacity. The results showed a clear trend in how adsorbent dosage influences performance (Figure 6a). At lower dosages, dye molecules effectively interact with available active sites, leading to high removal efficiencies. However, when the adsorbent dose is excessively increased, a decline in adsorption capacity per unit mass of adsorbent is often observed. This is because at higher dosages, although more surface sites are present, many remain unsaturated due to limited dye molecules available for interaction, leading to reduced adsorption efficiency.

This behavior highlights that an optimum adsorbent dose exists, beyond which the efficiency of dye removal decreases In practice, excessive adsorbent not only reduces adsorption capacity but also complicates recovery and handling in wastewater treatment. Furthermore, external parameters such as pH, temperature, and ionic strength significantly affect adsorption capacity. Therefore, adsorption studies under controlled variations of these factors are necessary to establish optimal conditions for maximum dye removal. By understanding these interactions, more efficient and economical treatment strategies for dye-polluted water can be developed. [44].

Particle size

The particle size of the Abies pindrow-derived adsorbent was another critical factor influencing dye removal efficiency. In this study, three particle size fractions were examined: 250 µm, 180 µm, and >180 µm (Figure 6b). Results indicated that smaller particle sizes exhibited significantly higher adsorption efficiencies compared with larger ones. This can be attributed to the higher surface area-to-volume ratio in smaller particles, which provides more active binding sites for dye molecules. Additionally, reduced void spaces in smaller particles allow dye ions to come into closer contact with adsorption surfaces, thereby improving removal rates.

Conversely, larger particles (250 µm) showed reduced performance despite possessing internal pores. This reduction in efficiency may be explained by poor pore accessibility and disconnected structures within larger particles, which limit effective dye diffusion. As a result, fewer active sites are utilized, reducing overall adsorption efficiency.

Thus, particle size optimization is essential in designing adsorbents for dye removal applications. Smaller particles not only improve removal efficiency but also allow for faster adsorption kinetics. Based on the findings of this work, the use of fine APC powders is recommended for wastewater treatment processes, as they maximize dye adsorption while minimizing adsorbent usage

Initial Dye Concentration

The initial concentration of dye plays a decisive role in determining adsorption performance. Results from this study (Figure 6c) demonstrated that dye removal efficiency does not always scale linearly with dye concentration. At low concentrations, the availability of active sites on the

Online ISSN 3007-3197 Print ISSN 3007-3189
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adsorbent surface is high relative to dye molecules, leading to effective adsorption. However, at higher concentrations, although more dye molecules are present to interact with the adsorbent, competition for binding sites increases.

This effect is explained by the concentration gradient, which acts as the driving force for adsorption. As dye concentration increases, the stronger gradient enhances diffusion toward the adsorbent surface and increases collision frequency between dye ions and active sites. Consequently, higher initial concentrations can, in some cases, result in greater adsorption rates. However, this trend reaches a limit once active sites are saturated, after which no further increase in removal efficiency is observed.

The study also highlights that the interaction between concentration and adsorption efficiency depends on both the physicochemical properties of the dye and the structural characteristics of the adsorbent [45]. A PDI-controlled distribution of nanoparticles ensures consistent adsorption behavior, but real-world applications may require careful adjustment of adsorbent dosage relative to dye concentration. Thus, understanding concentration-dependent adsorption is critical for scaling up dye removal processes in wastewater treatment plants, particularly under conditions of high pollutant loads

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Effect of Temperature on dyes removal

Temperature is one of the most critical environmental parameters influencing adsorption efficiency. The adsorption trend observed in this study (Figure 6d) revealed that dye removal efficiency initially remained relatively stable with increasing temperature, followed by a notable decline at higher temperatures. The maximum removal efficiency was recorded at approximately 96.57% under optimal temperature conditions, after which the efficiency decreased significantly.

Two primary factors explain this behavior. Firstly, increasing the temperature weakens the physical and chemical interactions between dye molecules (adsorbates) and the active binding sites on the APC surface. These interactions, often involving van der Waals forces or hydrogen bonding, become less stable at elevated temperatures, reducing the ability of the adsorbent to retain dye molecules effectively. Second, the increased fluids temperature increases the solubility of the dyes in the water phase, so they are favored to be in the dissolved rather than adsorbed states. This greater solubility decreases the attraction between the dye molecules and the adsorber surface, decreases adsorption effectiveness.

In the results, dye removal by APC-Ag-NPs appeared to be more effective at lower and moderately higher temperatures because there is a greater binding capacity of dye molecules to adsorption sites and at this stage, dye solubility is also relatively low. Consequently, optimization of temperature is compulsory in attaining maximum adsorption potential of APC-derived adsorbent in real wastewater treatments. Additionally, the findings emphasize the need to include issues of thermodynamics parameters like enthalpy and entropy difference as a more effective approach in comprehending the adsorption process and in the future application of adsorption in environmental clean up.

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The impact of APC-Ag-NPs on dye concentration and percentage removal

Another interesting effect to be found in this study was the correlation between the concentration

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 10 (2025)

Online ISSN 3007-3197 Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

of APC-Ag-NPs and the dye removal percentage (Figure 6e). It was found that the percentage removal of dye increased with increase in the concentration of nanoparticles. One can explain this trend on the higher surface area and hence more active sites to be available to capture a dye molecule in case of a large number of nanoparticles being present increasing the chances of dye molecules being captured.

This direct proportionality suggests that APC-Ag-NPs function as highly effective adsorbents, capable of trapping large quantities of dye molecules when provided in sufficient amounts. The strong adsorption performance may also be explained by the role of phytochemical residues acting as stabilizing agents, which contribute to the binding of dye molecules on nanoparticle surfaces. However, despite this positive trend, quantitative data specifying exact removal percentages at varying nanoparticle concentrations remain limited. The lack of detailed experimental evidence regarding saturation limits and adsorption kinetics represents a gap in current literature.

The findings therefore underscore two key aspects: first, that APC-Ag-NPs are promising nanomaterials for dye removal due to their scalable efficiency; and second, that further systematic studies are needed to quantify their maximum adsorption capacity, adsorption isotherms, and kinetics under different environmental conditions. Addressing these research gaps will provide a clearer understanding of their adsorption mechanisms and will support their potential deployment in large-scale wastewater treatment processes

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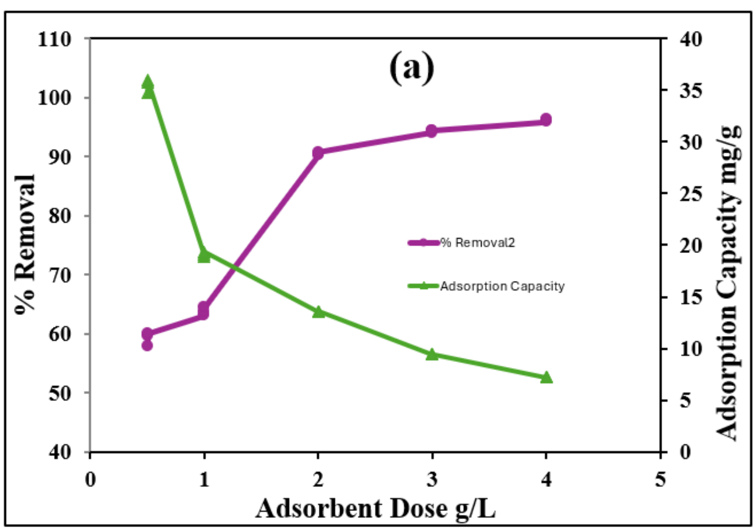

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 10 (2025)

Online ISSN 3007-3197
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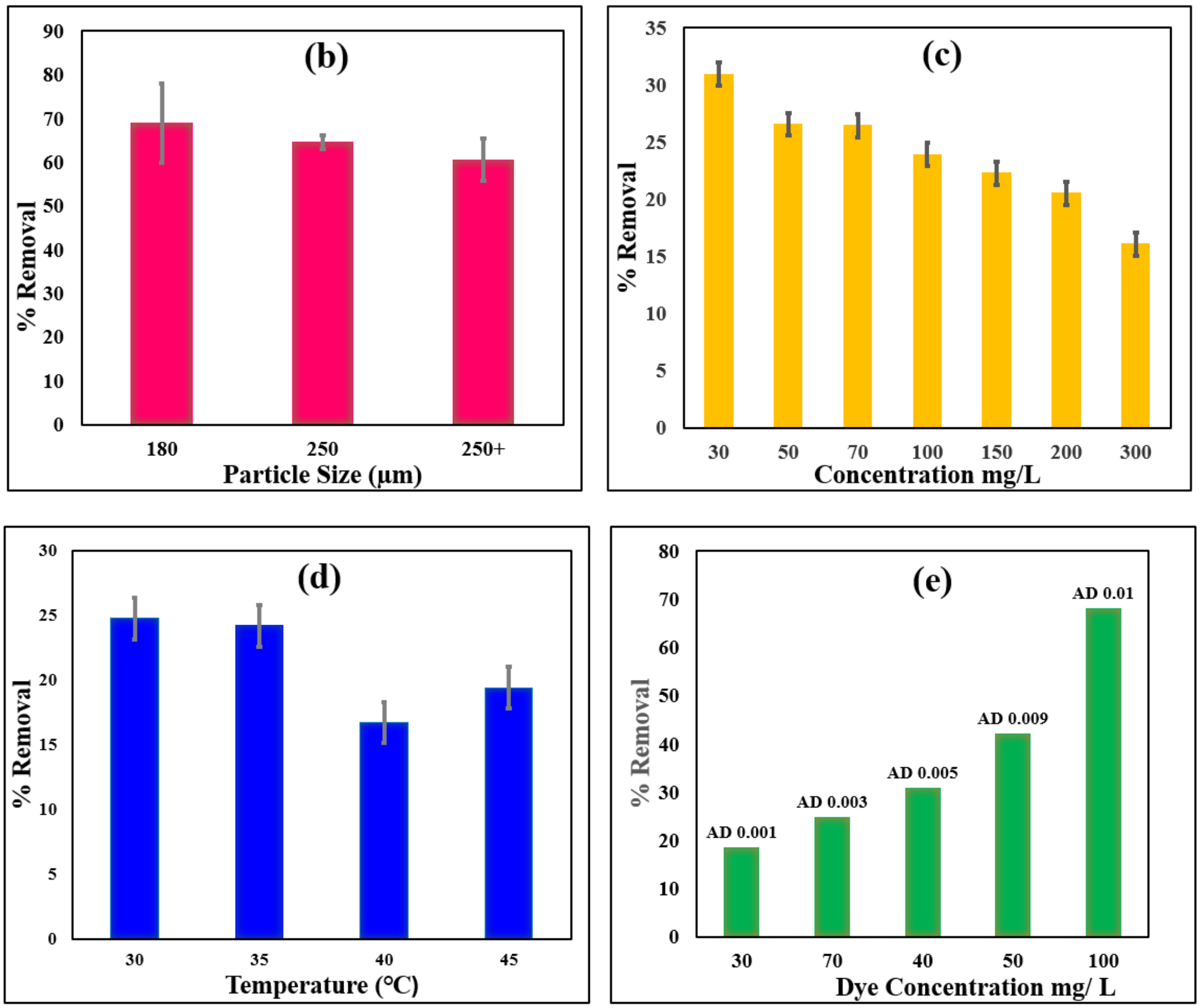

Figure 6. (a) Influence of adsorbent dosage on adsorption capacity and percentage removal of dye, **(b)** influence of particle size on adsorption capacity & % removal of dye Initial dye concentration. **(c)** influence of dye concentration on adsorption capacity & % removal of dye **(d)** influence of temperature on % removal, **(e)** influence of Ag-NPs on % removal.

Discussion

The present study demonstrates that silver nanoparticles synthesized using Abies pindrow cones (APC-Ag-NPs) have strong potential as an eco-friendly material for wastewater remediation, specifically for the removal of synthetic dyes such as methylene blue. The results of FTIR, SEM, EDX, DLS, and adsorption studies collectively provide important insights into the physicochemical properties and adsorption behavior of the nanoparticles.

FTIR analysis confirmed the presence of diverse functional groups on the surface of APC-Ag-NPs, such as amines, alkenes, aromatics, and nitro compounds. These groups originate from the

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

phytochemicals of the cone extract and act as both reducing and stabilizing agents during nanoparticle synthesis. Their presence also enhances adsorption efficiency, as they provide active binding sites for interaction with dye molecules. This dual role of phytochemicals highlights the importance of green synthesis methods, which not only reduce harmful chemical inputs but also yield nanoparticles with improved functionality.

The morphological observations from SEM further indicated that the nanoparticles were predominantly spherical but also showed a distribution of other shapes. The particle sizes fell in the nanometer range, aligning with earlier reports for green-synthesized silver nanoparticles. The formation of small and uniformly dispersed particles is advantageous, as a higher surface-to-volume ratio typically increases adsorption capacity. The EDX spectra confirmed the elemental purity of silver in the samples, with strong peaks near 3 keV, a characteristic signature of metallic silver.

Zeta potential analysis indicated a negative surface charge on the synthesized nanoparticles, suggesting colloidal stability. This electrostatic repulsion prevents agglomeration and maintains the particles in a dispersed state, which is crucial for effective interaction with contaminants in aqueous systems. Additionally, DLS analysis revealed an average hydrodynamic diameter of about 85 nm with a relatively low polydispersity index (PDI). This confirms that the particles were fairly monodispersed, which is desirable in adsorption studies.

The batch adsorption experiments provided clear evidence of the parameters influencing dye removal. Increasing adsorbent dose enhanced the percentage removal of methylene blue, but adsorption capacity decreased at higher doses due to unsaturated active sites. Similarly, particle size played a significant role, with smaller particles exhibiting better adsorption efficiency because of higher accessible surface area. The initial dye concentration was also critical, as higher concentrations increased the driving force for diffusion of dye molecules onto the nanoparticle surface. However, beyond certain thresholds, saturation effects limited efficiency. Temperature variation showed an inverse relationship with removal efficiency, indicating that the adsorption process was exothermic in nature.

Overall, the data align with both Langmuir and Freundlich isotherm models, confirming the suitability of APC-Ag-NPs for monolayer and heterogeneous adsorption. The study highlights that biosynthesized nanoparticles can serve as low-cost, sustainable adsorbents capable of addressing water pollution caused by dyes. Importantly, the combination of adsorption performance with environmentally benign synthesis places APC-Ag-NPs ahead of conventionally synthesized nanomaterials. Future work should focus on scaling up synthesis, regeneration studies to ensure reusability, and pilot-scale treatment systems to confirm the industrial potential of this material.

Conclusion

This research highlights the urgent challenge posed by dye-contaminated wastewater and demonstrates the potential of Abies pindrow cone-derived silver nanoparticles (APC-Ag-NPs) as a sustainable solution. Industrial effluents containing synthetic dyes such as methylene blue are difficult to degrade and pose ecological risks. In this study, APC extracts were successfully employed to synthesize silver nanoparticles in an eco-friendly manner, and their performance as adsorbents for dye removal was systematically investigated.

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The findings reveal that APC-Ag-NPs possess functional groups originating from phytochemicals, which not only facilitated nanoparticle formation but also enhanced stability and adsorption capacity. Structural and morphological characterization confirmed the nanoscale nature, elemental purity, and stable dispersion of the synthesized particles. Adsorption studies demonstrated that parameters such as adsorbent dose, particle size, initial dye concentration, and temperature strongly influence removal efficiency. Smaller particles and higher doses enhanced dye adsorption, while elevated temperatures reduced efficiency, confirming an exothermic adsorption process.

Importantly, the application of Langmuir and Freundlich isotherm models verified the capacity and mechanism of adsorption, indicating both monolayer and heterogeneous surface interactions. The overall results suggest that APC-Ag-NPs are efficient, low-cost, and environmentally friendly adsorbents that can be utilized for wastewater treatment applications.

In conclusion, the study establishes APC-Ag-NPs as promising candidates for the removal of toxic dyes from contaminated water. Their green synthesis not only avoids the use of hazardous chemicals but also enhances functional properties through natural phytochemical capping. With further optimization and scale-up, APC-Ag-NPs could be developed into a cost-effective and sustainable technology for industrial wastewater treatment, contributing to environmental protection and improved water quality

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Volume 3, Issue 10 (2025)

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