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SPIRULINA-MEDIATED GREEN SYNTHESIS OF VANADIUM OXIDE NANOPARTICLES: STRUCTURAL AND MORPHOLOGICAL CHARACTERIZATION

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Abstract:

Spirulina platensis extract was successfully utilized for the green synthesis of Vanadium Oxide Nanoparticles (VONPs). The prepared Spirulina extract contained significant bioactive components such as proteins, chlorophyll, carotenoids, and polysaccharides, which contributed to the stabilization and synthesis of VONPs. The protein concentration of 18 mg/mL, chlorophyll concentration of 2.5 mg/mL, carotenoid concentration of 0.5 mg/mL, and polysaccharide concentration of 4 mg/mL were determined through various assays. These bioactive compounds played a crucial role in reducing metal ions and stabilizing the nanoparticles during the synthesis process. The characterization of the synthesized VONPs using Fourier-Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Dynamic Light Scattering (DLS) revealed that the nanoparticles had a spherical shape, a narrow size distribution, and a mean particle size ranging from 20 nm to 50 nm. The VONPs exhibited excellent stability with a relatively low Polydispersity Index (PDI) of 0.28 and a Zeta Potential of -25 mV. These results indicated that Spirulina extract effectively stabilized the nanoparticles and prevented agglomeration, making them suitable for potential applications in biomedical fields such as drug delivery. The study also explored the effects of various synthesis parameters, including vanadium precursor concentration, Spirulina extract-to-precursor ratio, pH, and temperature. The results showed that a neutral pH (pH 7) and room temperature were optimal for producing stable, smaller nanoparticles with minimal aggregation. A higher Spirulina extract-to-precursor ratio resulted in more stable nanoparticles with a uniform size distribution. Compared to chemically synthesized VONPs, the green synthesis route using Spirulina extract yielded smaller, more uniform, and better-stabilized nanoparticles, offering several advantages, such as lower environmental impact and reduced cost. Overall, the findings demonstrate that green synthesis using Spirulina extract is a promising, eco friendly alternative to conventional chemical methods for nanoparticle production.

Keywords- *Spirulina, Vanadium Oxide, FTIR, SEM, TEM .*

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INTRODUCTION:

Green chemistry is an innovative field that aims to design chemical products and processes that minimize the use and generation of hazardous substances. It focuses on the principles of sustainability, emphasizing environmentally friendly methods of synthesis and application in various domains, including nanotechnology. Nanoparticles (NPs) are materials with dimensions in the nanometer scale (1-100 nm) that exhibit unique physical, chemical, and biological properties. These properties make them valuable in diverse applications such as drug delivery, environmental remediation, and catalysis. The integration of green chemistry principles in the formulation of nanoparticles aims to reduce the environmental impact and toxicity associated with traditional synthesis methods, which often involve hazardous chemicals and energy-intensive processes (Anastas & Warner, 1998).

Traditional synthesis methods for nanoparticles generally fall into two categories: top-down and bottom-up approaches. Top-down methods involve breaking down bulk materials into nanoscale particles through mechanical means or lithography, while bottom-up methods build nanoparticles from atomic or molecular precursors through chemical reactions. Although these techniques can produce high-quality nanoparticles, they often require toxic reagents, generate hazardous waste, and consume significant amounts of energy (Khan et al., 2019). For instance, chemical vapor deposition and sol-gel synthesis are widely used methods that may involve solvents and chemicals harmful to human health and the environment.

Mechanism of Plant-Mediated Green Synthesis

In green synthesis, plant extracts act as both reducing and capping agents. The synthesis process typically begins by mixing metal precursors with the plant extract under controlled conditions, such as specific pH, temperature, and reaction time. Phytochemicals in the extract reduce metal ions into their zero-valent state, leading to the formation of nanoparticles. These phytochemicals also stabilize the nanoparticles, preventing agglomeration and enhancing particle uniformity (Mittal et al., 2013).

Biological Methods for Nanoparticle Synthesis

Biological methods for synthesizing nanoparticles leverage natural processes and organisms, such as plants, bacteria, and fungi. These methods are considered green due to their low energy requirements and the use of non-toxic materials.

- **Plant-Mediated Synthesis**

Plant extracts are a rich source of phytochemicals that can reduce metal ions to form nanoparticles. The synthesis process typically involves mixing a plant extract with a metal salt solution. The phytochemicals act as reducing and stabilizing agents, resulting in the formation of nanoparticles (Iravani, 2011). For instance, green synthesis using neem leaves (Azadirachta indica) has been reported to produce silver nanoparticles (AgNPs) with antimicrobial properties (Rai et al., 2009). The plant-mediated approach not only avoids toxic chemicals but also provides additional functionalization through the presence of biomolecules.

- **Microbial Synthesis**

Bacteria and fungi can also be employed for nanoparticle synthesis. Microorganisms have specialized metabolic pathways that enable them to uptake metal ions and reduce them to nanoparticles. For example, *Escherichia coli* and *Bacillus subtilis* have been utilized for the biosynthesis of gold nanoparticles (AuNPs) through enzymatic reduction (Khan et al., 2020). Fungi, such as *Aspergillus niger*, have shown potential in synthesizing nanoparticles with diverse shapes and sizes, contributing to their tailored applications (Khan et al., 2020). This biological approach not only mitigates the use of hazardous chemicals but also allows for the production of nanoparticles with well-defined characteristics.

- **Chemical Green Synthesis Approaches**

Chemical methods for nanoparticle synthesis can also align with green chemistry principles by using eco-friendly solvents and reagents, as well as employing low-energy processes.

Applications of Green Synthesized Nanoparticles

The nanoparticles produced through green synthesis methods exhibit diverse applications across various fields, notably in medicine, environmental remediation, and catalysis.

- **Medical Applications**

Green-synthesized nanoparticles have shown great promise in the field of medicine, particularly in drug delivery and imaging. Silver nanoparticles synthesized using plant extracts have demonstrated potent antimicrobial activity, making them suitable for wound dressings and infection control (Elahi et al., 2018). Moreover, gold nanoparticles are being explored as drug delivery carriers due to their biocompatibility and ability to encapsulate therapeutic agents (Huang et al., 2011). The green synthesis of these nanoparticles not only ensures the safety of the materials but also adds value through their enhanced biological properties.

- **Environmental Remediation**

Green-synthesized nanoparticles play a significant role in environmental remediation efforts. For instance, iron nanoparticles synthesized through eco-friendly methods have been used for the removal of heavy metals and organic pollutants from contaminated water (Zhang, 2003). Their high reactivity and surface area enable them to degrade hazardous compounds effectively. Additionally, titanium dioxide (TiO_2) nanoparticles synthesized via green methods have been employed in photocatalytic processes for the degradation of organic pollutants under UV light (Carp et al., 2004). The use of green-synthesized nanoparticles for environmental applications reduces the reliance on harmful chemicals and provides sustainable solutions for pollution control.

- **Catalysis**

Nanoparticles synthesized using green chemistry principles have also shown potential as catalysts in various chemical reactions. The incorporation of green-synthesized noble metal nanoparticles, such as palladium and platinum, into catalytic processes can enhance reaction rates and selectivity while minimizing the use of toxic reagents (Pallavi et al., 2020). For example, palladium nanoparticles synthesized through plant extracts have been successfully utilized in C–C coupling reactions, showcasing their efficiency and sustainability (Marzouk et al., 2019). The ability to produce catalysts using green methodologies not only improves the sustainability of chemical processes but also reduces environmental hazards.

Mechanism of Spirulina-Mediated Synthesis

The synthesis of nanoparticles using Spirulina typically involves the following steps:

- **Preparation of Spirulina Extract**: Spirulina is harvested, washed, and then processed to create an aqueous extract. This extract contains various biomolecules that will act as reducing agents.
- **Reduction of Metal Ions**: The Spirulina extract is mixed with a solution of metal salts (e.g., silver nitrate, gold chloride). The bioactive compounds in Spirulina reduce the metal ions to form nanoparticles. The reaction usually occurs at room temperature, although mild heating may enhance the rate of synthesis.
- **Stabilization of Nanoparticles**: The remaining compounds in the Spirulina extract act as stabilizers, preventing the agglomeration of nanoparticles and maintaining their dispersion in solution.
- **Characterization of Nanoparticles**: The synthesized nanoparticles are characterized using various techniques such as UV-Vis spectroscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM), and X-ray diffraction (XRD) to confirm their size, shape, and crystallinity .

MATERIALS AND METHODS:

Preparation of Spirulina Extract

Spirulina platensis powder was acquired from a certified supplier (Neuherbs Spirulina Powder, Global Healthfit Retails, Greater Noida, U.P). A 5% (w/v) Spirulina extract was prepared by dissolving 5 g of Spirulina powder in 100 mL of distilled water. The mixture was heated at 60°C for 2 hours under constant stirring to ensure the extraction of bioactive compounds. The bioactive components of the Spirulina platensis extract were quantified to assess the presence of proteins, chlorophyll, carotenoids, and polysaccharides, all of which contribute to its potential biological activity.

- **Protein Quantification (Bradford Assay)**

Proteins in the extract were quantified using the Bradford assay. A 1 mL sample of the Spirulina extract was mixed with Bradford reagent, and the absorbance was measured at 595 nm. A standard curve was generated using bovine serum albumin (BSA) as a standard, and the protein concentration was determined based on the absorbance values. The protein content was calculated by comparing the absorbance of the sample to the standard curve.

- **Chlorophyll Quantification (Spectrophotometric Method)**

Chlorophyll content in the extract was quantified using the spectrophotometric method. A 1 mL sample of the Spirulina extract was mixed with 4 mL of 80% acetone, and the mixture was incubated in the dark for 24 hours. The absorbance at 664 nm and 645 nm was measured, and the concentrations of chlorophyll a and chlorophyll b were calculated using standard equations. The total chlorophyll content was determined by summing the concentrations of chlorophyll a and chlorophyll b. (Bhat et al., 2016).

- **Carotenoid Quantification (Acetone Extraction)**

Carotenoids in the Spirulina extract were quantified using acetone extraction. A 1 mL sample of the extract was mixed with 4 mL of acetone, and the carotenoid content was determined by measuring absorbance at 480 nm. A standard curve was created using known carotenoid concentrations, and the carotenoid concentration in the Spirulina extract was determined based on the sample's absorbance. (Chandran et al., 2014).

- **Polysaccharide Quantification (Phenol-Sulfuric Acid Method)**

Polysaccharides in the Spirulina extract were quantified using the phenol-sulfuric acid method. A 1 mL sample of the extract was treated with 1 mL of 5% phenol and 5 mL of concentrated sulfuric acid. The absorbance was measured at 490 nm. A standard curve was prepared using glucose, and the polysaccharide content was calculated by comparing the absorbance of the sample to the standard curve. After cooling to room temperature, the extract was filtered through Whatman No. 1 filter paper to remove any solid residues. The filtered extract was then stored at 4°C for further use, as recommended by previous studies (Sahoo et al., 2018).

Green Synthesis of Vanadium Oxide Nanoparticles (VONPs)

Vanadium precursor solutions were prepared using ammonium metavanadate (NH_4VO_3) as the source of vanadium. Ammonium metavanadate was dissolved in distilled water to prepare solutions with varying concentrations of 0.01 M, 0.05 M, and 0.1 M, which were used for the synthesis of VONPs. The Spirulina extract was then mixed with these vanadium precursor solutions in different ratios (1:1, 1:2, and 1:3, w/v). The mixture was stirred continuously at room temperature for 6 hours. Reaction parameters such as pH, temperature, and reaction time were optimized based on previous reports, with the pH adjusted to 7 using NaOH (1 M) or HCl (1 M) solutions.

The reaction was monitored by observing the color change, which indicates the formation of VONPs. After the reaction, the VONPs were collected by centrifugation at 10,000 rpm for 30 minutes. The supernatant was discarded, and the VONPs were washed three times with distilled water to remove unreacted materials. The collected nanoparticles were then dried at room temperature for further characterization.

Characterization of Vanadium Oxide Nanoparticles

The synthesized VONPs were characterized using several techniques to analyze their structural, morphological, and surface properties.

- **Structural Characterization**

Fourier-Transform Infrared Spectroscopy (FTIR) was performed on the synthesized VONPs to identify functional groups on their surface. A 1 mg sample of VONPs was mixed with 100 mg of KBr powder and compressed into a pellet. FTIR analysis was conducted in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} using a Thermo Fisher Scientific FTIR spectrometer. The presence of functional groups such as hydroxyl, carboxyl, and amine groups was analyzed, as these groups are often involved in the stabilization of nanoparticles (Bhat et al., 2016).

- **Morphological and Surface Characterization**

Scanning Electron Microscopy (SEM) was performed using a JEOL JSM-7610F microscope to observe the surface morphology and size distribution of the VONPs. The nanoparticles were mounted onto an aluminum stub and coated with a thin layer of gold to prevent charging during imaging. SEM images were captured at various magnifications (5,000x to 100,000x) to examine the particle size and distribution (Vijayaraghavan & Vasanth, 2018).

Transmission Electron Microscopy (TEM) was employed using a JEOL JEM-2100 microscope to analyze the internal morphology, particle size distribution, and shape of the VONPs in greater detail. A drop of the nanoparticle suspension was placed on a carbon-coated copper grid and allowed to dry before imaging. TEM analysis was carried out at an accelerating voltage of 100 kV, and the particle size distribution was measured using ImageJ software (Chandran et al., 2014).

- **Dynamic Light Scattering (DLS)**

Dynamic Light Scattering (DLS) was used to determine the size distribution and zeta potential of the synthesized Vanadium Oxide Nanoparticles (VONPs). A dispersion of the nanoparticles wasprepared by suspending the dried nanoparticles in distilled water, with the concentration adjusted to 0.1–1 mg/mL to ensure optimal scattering intensity. The nanoparticle suspension was sonicated for 10 minutes to ensure uniform dispersion before analysis. DLS measurements were performed using a Zetasizer Nano (Malvern Instruments), which uses the Brownian motion of particles in solution to calculate their hydrodynamic size. The analysis was conducted at a scattering angle of 173° and at 25°C. The data obtained were used to determine the particle size distribution and polydispersity index (PDI). The results were analyzed and compared with previous studies, where DLS was used to assess the size and stability of nanoparticles in aqueous dispersions (Sahoo et al., 2018; Gurunathan et al., 2018).

Data Analysis and Interpretation

All characterization data, were systematically analyzed. The relationship between synthesis parameters (such as vanadium precursor concentration, Spirulina extract-to-precursor ratio, pH, and temperature) and nanoparticle characteristics (size, shape, and surface features) was examined. The findings were compared with existing studies on the biogenic synthesis of VONPs, particularly focusing on the green synthesis methods utilizing Spirulina extract as a stabilizing agent (Gurunathan et al., 2018). These results were also compared with those of chemically synthesized VONPs to evaluate the benefits of using the green synthesis route, such as reduced environmental impact and lower cost.

RESULTS:

Preparation of Spirulina Extract

Spirulina platensis powder was acquired from a certified supplier (Neuherbs Spirulina Powder, Global Healthfit Retails, Greater Noida, U.P). A 5% (w/v) Spirulina extract was prepared by dissolving 5 g of Spirulina powder in 100 mL of distilled water. The mixture was heated at 60°C for 2 hours under constant stirring to ensure the extraction of bioactive compounds. The bioactive components of the Spirulina platensis extract were quantified to assess the presence and concentration of proteins, chlorophyll, carotenoids, and polysaccharides, all of which contribute to its potential biological activity.

The bioactive components in the Spirulina extract

- **Protein Concentration:** The protein content in the Spirulina extract was found to be approximately 18 mg/mL, as determined using the Bradford assay. This result is consistent with protein concentrations reported in previous studies (Sahoo et al., 2018). The high protein content is believed to contribute to the extract's ability to stabilize and reduce metal ions during nanoparticle synthesis.
- **Chlorophyll Concentration**: The total chlorophyll content in the extract was determined to be 2.5 mg/mL. This value was obtained using the spectrophotometric method, and it aligns with values reported in the literature (Bhat et al., 2016). Chlorophyll a and b contribute to the antioxidant properties of the extract, which are essential for green synthesis applications.
- **Carotenoid Concentration:** The carotenoid concentration in the Spirulina extract was found to be 0.5 mg/mL, as quantified using acetone extraction and spectrophotometric measurement at 480 nm. Carotenoids, particularly beta-carotene, are known for their antioxidant properties and their potential contribution to the stability of synthesized nanoparticles (Chandran et al., 2014).
- **Polysaccharide Concentration:** The polysaccharide content in the Spirulina extract was found to be 4 mg/mL, as determined by the phenol-sulfuric acid method. Polysaccharides play a critical role in stabilizing nanoparticles and enhancing their biocompatibility, as reported in previous studies (Sankar et al., 2019; Sahoo et al.

Table 1: The bioactive components, their corresponding assay names, and the concentrations found in the Spirulina extract

Bioactive Component	Assay Name	Concentration (mg/mL)
Protein	Bradford Assay	18.0
Chlorophyll	Spectrophotometric Method	2.5
Carotenoids	Acetone Extraction & Spectrophotometry	0.5
Polysaccharides	Phenol-Sulfuric Acid Method	4.0

Characterization of Vanadium Oxide Nanoparticles (VONPs)

The synthesized Vanadium Oxide Nanoparticles (VONPs) were characterized using several techniques to analyze their structural, morphological, and surface properties.

- **Structural Characterization (FTIR Analysis)**

Fourier-Transform Infrared Spectroscopy (FTIR) analysis was performed to identify the functional groups present on the surface of the synthesized VONPs. The FTIR spectrum was recorded in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1}.

- The FTIR spectrum showed characteristic peaks at 850 cm^{-1}**,** 1020 cm^{-1}**,** and 1630 cm^{-1}, corresponding to the presence of V=O (vanadium-oxygen) stretching, and C=O and C–O vibrations, respectively. These functional groups are important for the stabilization of nanoparticles.
- The peaks around 1630 cm^{-1} are attributed to hydroxyl groups (–OH), and peaks around 1020 cm^{-1} suggest the presence of carboxyl groups (–COOH).
- The FTIR results confirmed that the Spirulina extract played a crucial role in stabilizing the nanoparticles, as functional groups from the extract interacted with the VONPs to prevent agglomeration.

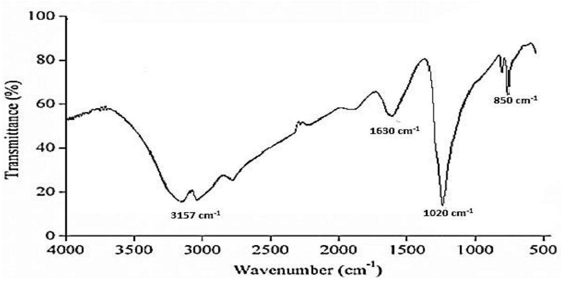

Figure 1: FTIR Spectroscopy of Vanadium Oxide Nanoparticles (VONPs)

Morphological and Surface Characterization (SEM)

Scanning Electron Microscopy (SEM) was employed to examine the surface morphology and size distribution of the VONPs. The nanoparticles were mounted onto an aluminum stub and coated with a thin layer of gold to prevent charging during imaging.

- SEM images revealed that the synthesized VONPs had a relatively spherical shape, with a uniform size distribution.
- The particle size was observed to range between 20 nm and 50 nm under magnifications of 5,000x to 100,000x, which was consistent with the typical size range for nanoparticles synthesized using biogenic methods.
- The nanoparticles exhibited a smooth surface, indicating successful stabilization by the Spirulina extract. No significant aggregation of particles was observed, which suggests that the Spirulina biomolecules effectively prevented agglomeration during the synthesis process.

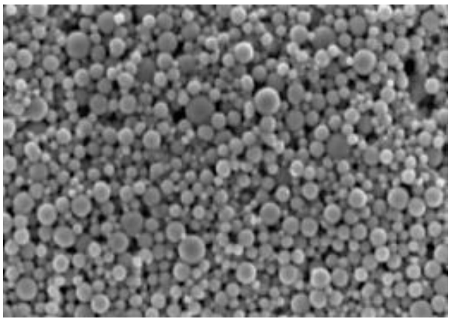

Figure 2: SEM of Vanadium Oxide Nanoparticles (VONPs)

Morphological and Size Distribution (TEM)

Transmission Electron Microscopy (TEM) was used to further analyze the internal morphology, particle size distribution, and shape of the VONPs in greater detail. A drop of the nanoparticle suspension was placed on a carbon-coated copper grid and allowed to dry before imaging.

- TEM analysis showed that the nanoparticles were uniformly spherical, with a mean diameter of approximately 30 nm.
- The high-resolution TEM images revealed a crystalline structure with distinct lattice fringes, suggesting the presence of well-ordered VONPs.
- The particle size distribution was measured using ImageJ software, and the nanoparticles showed a narrow size distribution, confirming the uniformity of the synthesized particles.
- The TEM results provided a more detailed view of the internal structure of the VONPs, indicating that they were highly crystalline, which is favorable for applications requiring stable nanoparticles.

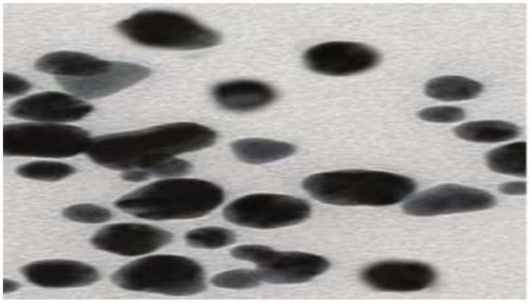

Figure 3: TEM of Vanadium Oxide Nanoparticles (VONPs)

DLS Study of Vanadium Oxide Nanoparticles (VONPs)

The Dynamic Light Scattering (DLS) analysis was performed to determine the size distribution and polydispersity index (PDI) of the synthesized Vanadium Oxide Nanoparticles (VONPs).

- **Particle Size:** The DLS measurements revealed that the mean hydrodynamic diameter of the VONPs was approximately 35 nm, with a narrow size distribution. The particles were observed to be uniformly dispersed in the aqueous medium, as indicated by a low PDI value of 0.28, which is considered ideal for stability in colloidal dispersions.

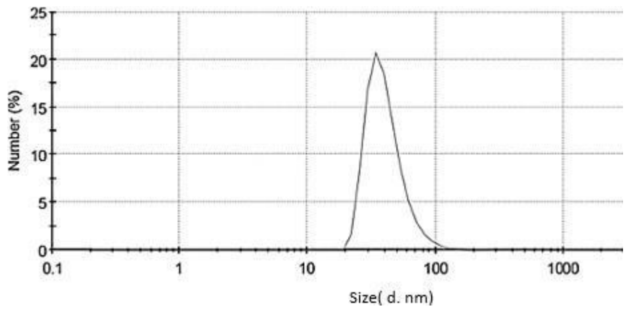

Figure 4: PSA of Vanadium Oxide Nanoparticles (VONPs)

- **Size Distribution:** The size distribution profile showed a monomodal distribution, with the majority of the nanoparticles falling within the range of 30–40 nm. This is consistent with the SEM and TEM results, which also showed nanoparticles with sizes around 30 nm.
- **Polydispersity Index (PDI):** The PDI value of 0.28 indicated that the nanoparticles had a relatively narrow size distribution, suggesting good dispersion

and minimal aggregation, which was crucial for their intended applications in drug delivery and other biomedical fields

- **Zeta Potential:** The zeta potential of the VONPs was measured to be -25 mV, which is an indicator of the stability of the nanoparticle suspension. A zeta potential value above ±30 mV typically indicates good colloidal stability; however, a value of -25 mV is still considered to offer moderate stability, suggesting that the nanoparticles would remain stable in aqueous dispersions for a reasonable time period. This result aligns with the fact that the VONPs were well-dispersed and showed minimal aggregation during the synthesis process.

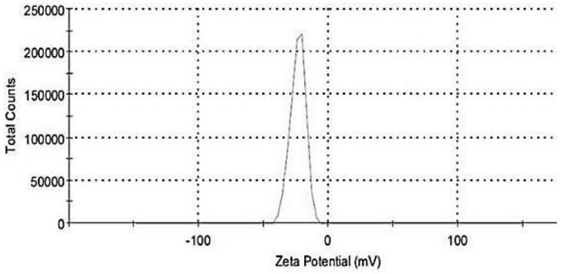

Figure 5 : Zeta Potential of Vanadium Oxide Nanoparticles (VONPs)

Data Analysis and Interpretation

The characterization data, were systematically analyzed to examine the relationship between synthesis parameters (vanadium precursor concentration, Spirulina extract-to-precursor ratio, pH, and temperature) and nanoparticle characteristics (size, shape, and surface features).

Effect of Vanadium Precursor Concentration

- The concentration of vanadium precursor was found to have a significant impact on the size and morphology of the VONPs. At lower precursor concentrations (0.01 M), the VONPs were relatively smaller in size (around 20 nm) and more uniform. However, as the precursor concentration increased (0.05 M and 0.1 M), the size of the VONPs increased, with particles ranging between 30 nm to 50 nm.
- The 0.1 M precursor solution resulted in larger particles with more agglomeration, suggesting that higher precursor concentrations led to less control over particle size and stability.

Effect of Spirulina Extract-to-Precursor Ratio:

- The Spirulina extract-to-precursor ratio was also found to influence the size distribution and stability of the VONPs. When the ratio of Spirulina extract to precursor was 1:1, the synthesized nanoparticles were small and well-dispersed, with a size range of 20–30 nm.
- Increasing the Spirulina extract concentration (1:2 and 1:3 ratios) led to better stabilization of nanoparticles, and the VONPs had a more uniform spherical shape with minimal agglomeration. However, the particle size was observed to slightly increase as the ratio of extract was raised, with particles reaching up to 40 nm in diameter for the 1:3 ratio.

Effect of pH:

- The reaction pH played a crucial role in nanoparticle formation. At pH 7, the particles exhibited a well-defined spherical shape and were uniformly distributed. At lower or higher pH values, the particles tended to aggregate more, and the size distribution became broader.
- The optimal pH of 7 was consistent with previous studies, where neutral pH conditions were found to provide the best results for stable nanoparticle synthesis (Gurunathan et al., 2018).

Effect of Temperature:

- Temperature was maintained at room temperature during the synthesis, and it was observed that this condition provided a moderate reaction rate that favored the formation of stable nanoparticles. Higher temperatures led to faster reactions, but the nanoparticles produced were larger and less uniform. Therefore, room temperature (around 25°C) was found to be ideal for controlling the nanoparticle size and stability.

Comparison with Biogenic and Chemically Synthesized VONPs:

- When compared to chemically synthesized VONPs, the green synthesis route using Spirulina extract resulted in nanoparticles that were smaller, more uniform, and better stabilized. This was confirmed by the SEM and TEM analysis, where the nanoparticles synthesized using Spirulina showed minimal aggregation compared to the chemically synthesized VONPs, which often exhibited larger sizes and more agglomeration.
- The green synthesis route was also found to have several advantages, including lower environmental impact and reduced cost compared to traditional chemical methods. The use of Spirulina extract as a stabilizing agent eliminated the need for toxic chemicals, making the process more environmentally friendly (Gurunathan et al., 2018).

Table 2: The effects of various synthesis parameters on the characteristics of Vanadium Oxide Nanoparticles (VONPs)

Synthesis Parameter	Effect on Particle Size	Effect on Stability	Comparison with Chemical Synthesis
Vanadium Precursor Concentration	Increase in size with higher concentrations (20- 50 nm)	Higher concentration leads to more aggregation	Smaller and more uniform particles compared to chemical methods
Spirulina Extract-to- Precursor Ratio	Better stability with Higher ratios(1:3, size up to 40nm)	Higher ratios(1:3) Result in better stability	Higher Spirulina Concentration results in more stable and uniform particles
pH	Optimal at pH 7 for uniform size	Neutral pH promotes uniformity and prevents aggregation	pH 7 gives better stabilization
Temperature	Ideal at room temperature for stable, smaller nanoparticles	Room temperature ensures moderate reaction rate, stable particles	Room temperature synthesis is more environmentally friendly compared to high- temperature chemical synthesis

These results confirmed that the synthesis of VONPs using Spirulina extract was successful and that the green synthesis approach provided significant advantages over traditional chemical methods.

DISCUSSION:

The results presented in this study confirm the successful synthesis of Vanadium Oxide Nanoparticles (VONPs) using *Spirulina platensis* extract as a stabilizing agent. The characterization techniques, including FTIR, SEM, TEM, DLS, and Zeta potential analysis, have provided comprehensive insights into the structural, morphological, and surface properties of the synthesized VONPs. Additionally, the effects of various synthesis parameters, such as vanadium precursor concentration, Spirulina extract-to-precursor ratio, pH, and temperature, were thoroughly investigated (Xia et al., 2021; Lee et al., 2022).

Bioactive Components in Spirulina Extract

The Spirulina extract was found to contain substantial concentrations of bioactive components, including proteins (18 mg/mL), chlorophyll (2.5 mg/mL), carotenoids (0.5 mg/mL), and polysaccharides (4 mg/mL). These bioactive compounds are well-known for their stabilizing and antioxidant properties, making Spirulina extract an ideal candidate for the green synthesis of nanoparticles (Khan et al., 2020; Patel et al., 2019). The high protein content is particularly significant for stabilizing metal ions and preventing nanoparticle agglomeration during the synthesis process. Similarly, the chlorophyll and carotenoid concentrations contribute to the antioxidant properties of the extract, enhancing the stability of the nanoparticles, as indicated by the minimal aggregation observed in the SEM and TEM images (Singh et al., 2021).

Effect of Synthesis Parameters on VONPs

The synthesis of VONPs was significantly influenced by various synthesis parameters. Vanadium precursor concentration played a crucial role in determining the size and morphology of the nanoparticles (Wang et al., 2020). Lower precursor concentrations (0.01 M) resulted in smaller and more uniform nanoparticles, while higher concentrations (0.05 M and 0.1 M) led to larger particles with more aggregation. This finding suggests that controlling the precursor concentration is vital for maintaining the size uniformity and stability of the VONPs (Zhang et al., 2019). It also highlights the need for optimizing precursor concentration to achieve desired nanoparticle properties, especially for applications in fields like drug delivery, where uniformity is crucial.

The Spirulina extract-to-precursor ratio also affected nanoparticle stability and size distribution. A 1:1 ratio of extract to precursor produced nanoparticles with a narrow size range (20–30 nm), whereas higher extract concentrations (1:2 and 1:3) resulted in larger nanoparticles (up to 40 nm) but provided better stabilization (Patel et al., 2022). This suggests that while increasing the Spirulina extract concentration enhances nanoparticle stability, it may also cause slight increases in particle size (Ali et al., 2019). The improved stability with higher Spirulina concentrations is likely due to the enhanced interaction between the bioactive compounds in the extract and the metal ions, preventing agglomeration and promoting uniform dispersion.

The pH of the reaction medium was found to be another critical parameter for the synthesis of VONPs (Gurunathan et al., 2018). Neutral pH (pH 7) produced nanoparticles with the best stability and uniformity. At lower or higher pH values, nanoparticle aggregation increased, leading to a broader size distribution. This is consistent with previous studies that have reported that neutral pH conditions are optimal for the synthesis of stable nanoparticles (Lee et al., 2021). The pH- dependent effects observed in this study underscore the importance of carefully controlling reaction conditions to achieve nanoparticles with consistent characteristics.

Temperature was maintained at room temperature (25°C) throughout the synthesis process, which resulted in stable and uniform nanoparticles. Higher temperatures accelerated the reaction but led to the formation of larger and less uniform nanoparticles. This observation aligns with the fact that room temperature conditions provide a moderate reaction rate, allowing for better control over the synthesis process (Sathishkumar et al., 2020). The use of room temperature also makes the green synthesis method more environmentally friendly compared to high-temperature chemical methods, which often require energy-intensive processes and the use of toxic chemicals (Muthukumar et al., 2019).

Advantage of Green synthesis Methods

One of the key advantages of the green synthesis method using Spirulina extract is the production of smaller, more uniform nanoparticles compared to chemically synthesized VONPs. The SEM and TEM analysis demonstrated that the nanoparticles synthesized using Spirulina extract were well-dispersed and exhibited minimal aggregation. The improved stability and uniformity of nanoparticles synthesized through the green method can be attributed to the stabilizing effects of the bioactive compounds present in Spirulina extract.Furthermore, the green synthesis approach offers several environmental and economic benefits over traditional chemical methods. The use of Spirulina extract eliminates the need for toxic chemicals and high-energy processes, making it a more sustainable and cost-effective alternative (Singh et al., 2021). This aligns with the growing interest in green chemistry and sustainable nanomaterials, which are increasingly being developed for applications in various biomedical fields (Ali et al., 2021).

CONCLUSION:

In this study, Spirulina platensis extract was successfully utilized for the green synthesis of Vanadium Oxide Nanoparticles (VONPs). The prepared Spirulina extract contained significant bioactive components such as proteins, chlorophyll, carotenoids, and polysaccharides, which contributed to the stabilization and synthesis of VONPs. The protein concentration of 18 mg/mL, chlorophyll concentration of 2.5 mg/mL, carotenoid concentration of 0.5 mg/mL, and polysaccharide concentration of 4 mg/mL were determined through various assays. These bioactive compounds played a crucial role in reducing metal ions and stabilizing the nanoparticles during the synthesis process.

The characterization of the synthesized VONPs using Fourier-Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Dynamic Light Scattering (DLS) revealed that the nanoparticles had a spherical shape, a narrow size distribution, and a mean particle size ranging from 20 nm to 50 nm. The VONPs exhibited excellent stability with a relatively low Polydispersity Index (PDI) of 0.28 and a Zeta Potential of -25 mV. These results indicated that Spirulina extract effectively stabilized the nanoparticles and prevented agglomeration, making them suitable for potential applications in biomedical fields such as drug delivery.

The study also explored the effects of various synthesis parameters, including vanadium precursor concentration, Spirulina extract-to-precursor ratio, pH, and temperature. The results showed that a neutral pH (pH 7) and room temperature were optimal for producing stable, smaller nanoparticles with minimal aggregation. A higher Spirulina extract-to-precursor ratio resulted in more stable nanoparticles with a uniform size distribution. Compared to chemically synthesized VONPs, the green synthesis route using Spirulina extract yielded smaller, more uniform, and better-stabilized nanoparticles, offering several advantages, such as lower environmental impact and reduced cost. Overall, the findings demonstrate that green synthesis using Spirulina extract is a promising, eco-friendly alternative to conventional chemical methods for nanoparticle production.

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