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Green Synthesis of Sulphur Nanoparticles Using *Azadirachta indica* (Neem Leaf) and Its Characterization

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Nanoscience has found various applications in different biomedical fields. The synthesis of nanoparticles (NPs) has become a vast area of research due to its potential applications. These particles can be prepared by different chemical, physical and biological approaches. In recent years, green synthesis of NPs using plant extracts has gained much interest due to non-toxicity and very low cost of synthesis. The plant extracts act both as reducing or stabilizing agent as well as capping agent. Neem (*Azadirachta indica A. Juss*) is a well-known medicinal plant and has been studied for the biosynthesis of NPs. A. indica has various phytochemicals identified that can reduce

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the metal ions. The bio reduction of NPs from neem extract is an eco-friendly, low cost and green synthesis method. These NPs are reported to exhibit good antimicrobial, mainly antibacterial activity. NPs was characterized by Dynamic Light Scattering (DLS) for particle size, particle size distribution, poly- dispersity index, zeta potential, UV visible spectrophotometer and Fourier-Transform Infrared Spectroscopy (FT-IR) for functional characteristics. Dynamic light scattering measurements for particle size (nm) and Polydispersity Index showed that the particle size was in the range of 35-40 nm with Polydispersity Index of 0.280-0.290, it is the mid-range value and indicating that the particles were in disperse form in aqueous suspension. From the analysis, the zeta potential value was found to be (-5.77 mV), revealing the better stability of synthesized sulphur nanoparticles in aqueous suspension. The FT-IR spectrum showed characteristic absorption of wavenumber from 3384.73 cm^{-1} and 533.56 cm^{-1}. The absorption spectra of UV-visible spectrophotometer in between the range of 200-800 nm.

Keywords: Nanoparticles; characterization; Zeta-sizer; FTIR; UV-Spectrum.

1. INTRODUCTION

"Sulphur nanoparticles have a great potential as fertilizer carrier to control release of sulphate by the slow-release mechanism. Despite several agronomic strategies tested for improving the use S efficiency, it proved less success due to complex soil environmental factors. Sulphur use efficiency hardly exceeds 25%" [1]. "One of the innovative strategies to enhance S use efficiency is by exploiting the basic principles and concepts of nanotechnology. Smaller size of nano-sulphur and its coating will help resist unwanted environmental processes associated with conventional fertilizer, *i.e.,* leaching, evaporation, photolytic, hydrolytic and microbial degradation". [1] Nano-sulphur particle are 1000 times smaller than elemental sulphur applied as soil application releases sulphur in 3-4 weeks compared to 4-6 month in elemental. Nano-sulphur speeds up photosynthesis by 8-10 times and increase growth at first phase, increase quality and protein content, transforms the nitrates into protein (N:S ratio is 15:1), protect plants against fungus, increase shelf life of fruits and regulates soil pH [2, 3].

"There are difficulties in absorbing the nutrients from the soil and leaves in the fertilizer which was consisting of macro and micro sized fractions. Especially in the soil with high pH, the application of many sulfuric fertilizers takes a long time to reduction of sulphur to sulphate that is useful form for plants and the plant cannot make effective use of other elements. Nano S can be highly homogeneous in the distribution to the ground with an average particle size of 20 nm". [4] "Depending on the surface size, sulphur bacteria work very quickly. So it can be converted into forms that can be taken by plants within 1-2 days. Also, depending on the nano size, the absorption from the leaf is fast. Nano S allows the plants to efficiently utilize many nutrients by rapidly reducing the soil reaction (pH) and cause increase in yield and quality. The use of Nano S prevents the use of excess fertilizer that negatively affects the environment and human health. As a result, nearly 100% efficiency is obtained" [5, 6].

This study focused on synthesis of sulphur nanoparticles using neem green leaf and subsequently characterize them using Dynamic Light Scattering (DLS) for particle size, particle size distribution, poly- dispersity index, zeta potential, UV visible spectrophotometer and Fourier-Transform Infrared Spectroscopy (FT-IR) for functional characteristics.

2. MATERIALS AND METHODS

The precursor sodium thiosulphate (1M $Na_2S_4O_7$) with 5 % Neem leaf Extract was obtained from Neem (*Azadirachta indica A. Juss*). Distilled water was used throughout the experiment. 1N HCl (1 HCl:1 MQ water) was added up to yellowish colour change.

2.1 Green Neem Leaf Extract Preparation

About 10 g of green dried neem leaves were weighed and made into fine powder. Then it was dissolved in 100 ml distilled water. It was boiled in a water bath at 650°C for 3 hours. Then it was filtered using Whatman filter paper no.42 and then it was centrifuged at 5000 rpm for 15 min. Then this filtrate was stored at 5-10ºc for further experiments.

2.2 Synthesis of Sulphur Nanoparticles

Sulphur nanoparticles were prepared via direct green synthesis method in the laboratory of

Department of Nanotechnology and Centre for advanced Research in Plant Tissue Culture, Anand Agricultural University, Anand. As demonstrated by Anonymous (2017-18), first of all, extract of dry neem leaf was made by boiling leaf powder with MQ water. SNPs were prepared by adding 1M $Na_2S_4O_7$ in the neem extract (5%) pinch by pinch on stirrer. Then add 1N HCl (1 HCl:1 MQ water) up to yellowish colour change in stirrer condition. Synthesized solution was dried at 100 °C in hot air oven till the complete removal of water and dried powder obtained containing 37 percent sulphur approximately tested by turbidimetric method.

2.3 Characterization Techniques

The Synthesized sulphur NPs were characterized using Zeta sizer (ZS 90 (Malveran), UV-Vis (DU) and FTIR (Spectrum II) in Table 1.

Table 1. Characterization Instruments

Sr. No.	Name of instrument	Model
1	Zeta sizer	ZS 90 (Malveran)
2	FTIR	Spectrum II
3	UV visible spectrophotometer	DU 700

3. RESULTS AND DISSCUSSION

The synthesized sulphur NPs were yellow in colour (Plate1). “Average particle size and poly-dispersity index of the SNPs synthesized via green synthesis method, was evaluated of 35 % sulphur in suspension prepared in deionized Milli-Q water for soil application schedules using Malvern Zeta Sizer, ZS-90 instrument. Dynamic light scattering (DLS) analyse the velocity of particle movement by measuring dynamic fluctuations of light scattering intensity, caused by the random motion of the particle. This technique yields an average particle size, poly-dispersity index and counts rate of the particle present in the solution” [7, 8].

3.1 Dynamic Light Scattering

3.1.1 Particle size analysis

Data presented in Table 2 and Fig.1 revealed that the prepared SNPs for application schedules showed a particle size of 36-42 nm. The PDI (Poly-dispersity Index) scale in all the samples range from 0.494 to 0.231 indicated that it is the mid-range value and the particles remain in disperse form in all the samples. It can be considered as a good result because the particle size of synthesized SNPs is below 100 nm.

“The average particles diameter size was found to be 50 ± 4 nm. The results showed that the nanoparticles are crystalline in nature, with spherical shape” [9,10]. “The biogenic sulphur nanoparticles were spherical and poly-dispersed with particle size of 70–80 nm” [11].

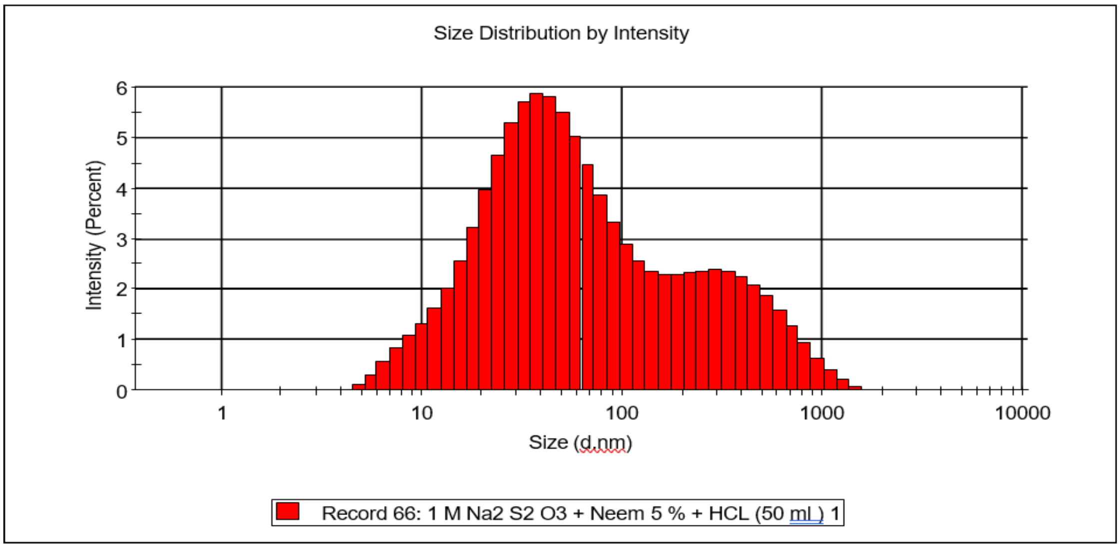
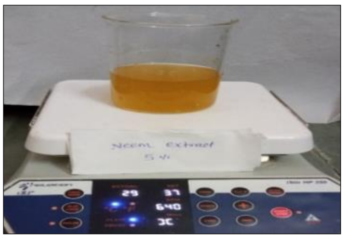
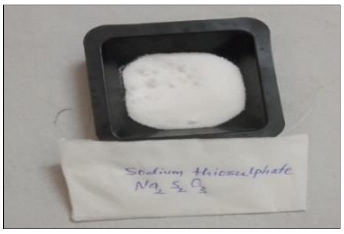


Fig. 1. Particle size distribution of synthesized SNPs using Malvern Zeta Sizer, ZS-90 instrument

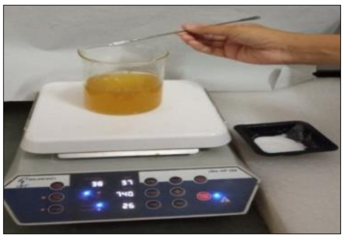
STEP-1



Neem extract

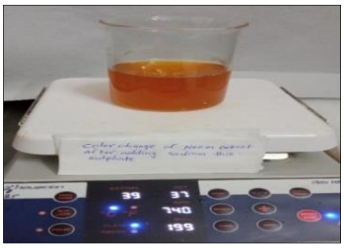


Sodium thiosulphate

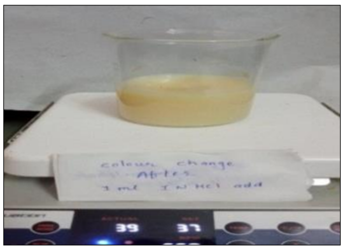


Addition of $Na_2S_4O_7$

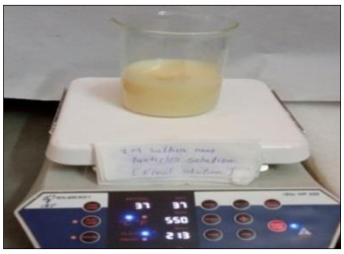
STEP-2



Color after Sodium thiosulphate



Color after HCl addition



Final solution of SNPs

STEP-3

Dried NPs powder

Crushing of powder

Final SNPs

Plate 1. Synthesis of sulphur nanoparticles

Table 2. Characterization of SNPs for particle size (nm), PdI and Kcps

Sr. No.	Sample details	Particle size (nm)	PDI	KCPs
1.	SNPs	42.19±1.72	0.494±0.023	290.2±0.2
2.	SNPs	36.18±1.54	0.231±0.026	286.5±0.9

3.1.2 Zeta potential analysis

"The surface charge of synthesized SNPs was measured following standard operating procedure using Malvern Zeta Sizer, ZS-90 instrument. Zeta potential measurement specifies the electro kinetic potential of a colloidal system" [12]. "Magnitude of the zeta potential is an indicator of repulsive forces between particles and therefore it can provide a good estimation of the suspension stability" [13,14].

"The larger zeta potential values represent lower degree of aggregation that leads to higher degree of stability of nanoparticles and smaller z-averaged hydrodynamic diameter. At lower zeta values, the nanoparticles flocculate early and the stability in nano-suspension reduces. The common dividing line between unstable and stable suspensions is taken as +30 or -30 mV; particles having zeta potentials beyond these limits are generally considered as stable" [15].

Table 3. Zeta Potential Analysis

	Mean (mV)	Area (%)	St dev (mV)
Peak	-5.77	100.0	16.2

The zeta potential values of synthesized SNPs at first synthesis are presented in Fig. 2. From the analysis, the zeta potential value was found to be (-5.77 ± 16. 2 mV) with 10.8 mS/c conductivity revealed the better stability of synthesized SNPs in aqueous suspension.

3.2 Fourier Transform Infrared Spectroscopy (FTIR)

To determine the functional groups responsible for the synthesis of SNPs, FTIR analysis was performed. The FTIR spectrum of sodium thiosulphate is showed in Fig. 3, which shows absorption bands at 3388.91, 1110.05 & 995.04 cm^{-1}. The FTIR spectrum of synthesized sulphur nanoparticles in Fig. 4, which showed that absorption bands at 3384.73, 1127.92, 996.91, 660.8, 554.21 & 533.56 cm^{-1}. The combined FTIR spectrum of sodium thiosulphate and synthesized sulphur nanoparticles is showed in Fig. 5, which shows absorption bands at 3388.91, 1110.05, 9 9 5. 0 4 cm^{-1} and 3384.73, 1127.92, 996.91, 660.8, 5554.21 & 533.56 cm^{-1} respectively same as their own spectrum. The compared FTIR spectrum of sodium thiosulphate and synthesized sulphur nanoparticles is shown in Fig. 6, which showed absorption bands at 3388.91, 1110.05, 995.04 cm^{-1} and 3384.73, 1127.92, 996.91, 660.8, 5554.21 & 533.56 cm^{-1} respectively same as their own spectrum. "The biogenic sulphur nanoparticles were spherical, poly-dispersed with particle size of 70–80 nm. FTIR spectra for control (*C. roseus* leaf extract) and experimental (sulphur nanoparticles) were recorded in the range of 4000–400 cm^{-1}" [11,16].

3.3 UV Visible Spectrophotometer

UV-Visible Spectroscopy is based on the absorption of ultraviolet light or visible light by chemical compounds, which results in the production of distinct spectra. Spectroscopy is based on the interaction between light and matter, which is used to measure the intensity of light and the intensity is proportional to the wavelength in fig.7. "UV-vis spectroscopy mentions to absorption spectroscopy in the region of UV-vis spectrum. Light frequencies of the 200–800 nm are commonly used for portraying different metal NPs in the size range of 2 nm to 100 nms. UV–vis spectroscopy is a crucial technique to ascertain the stability and formation of SNPs. UV-vis absorption measurement of the wavelength ranging from 250-400 nm is utilized to characterize SNPs" [11,17].

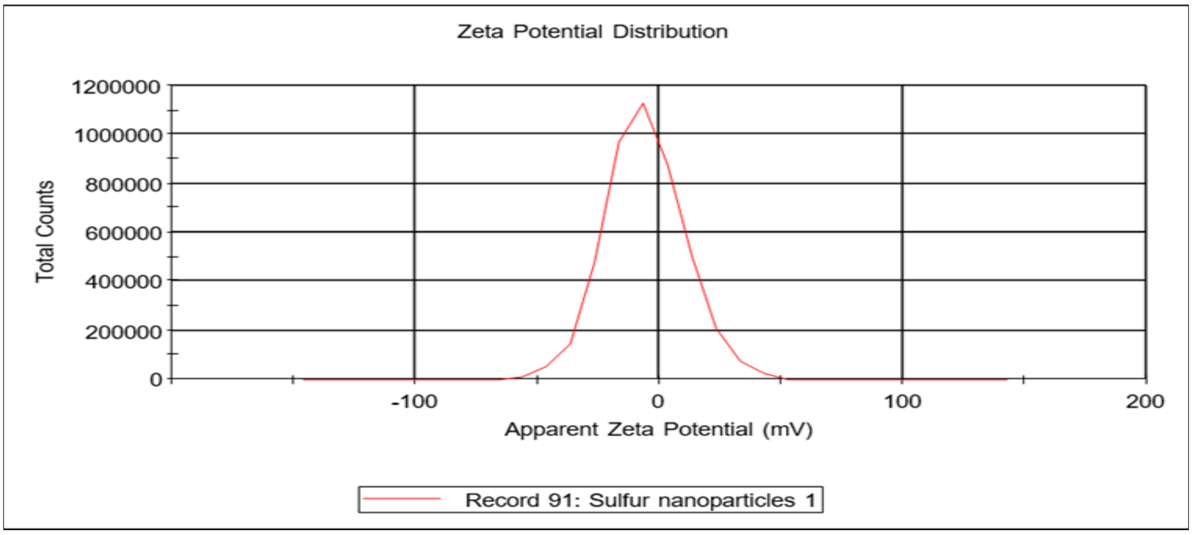


Fig. 2. Zeta potential of synthesized SNPs

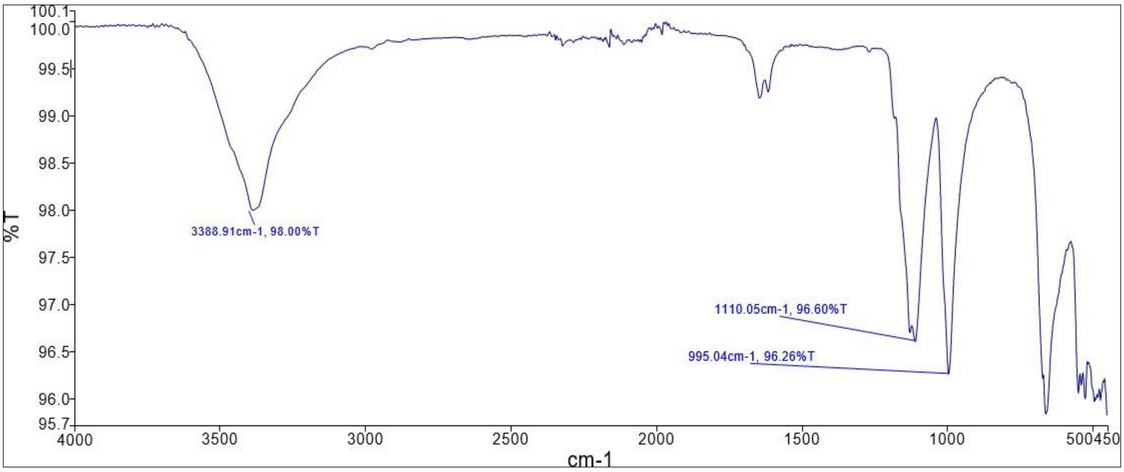


Fig. 3. FTIR spectrum of sodium thiosulphate

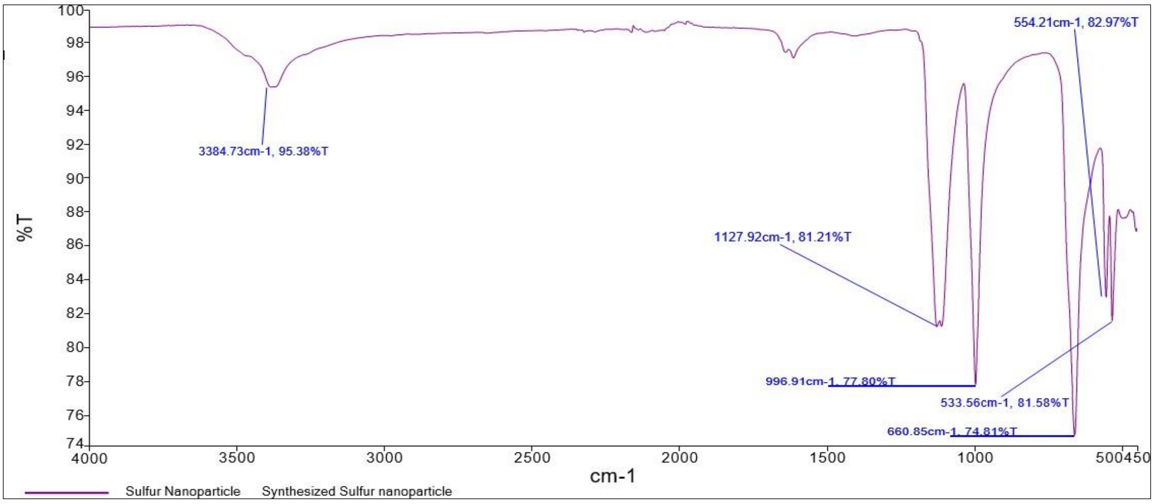


Fig. 4. FTIR spectrum of sulphur nanoparticles

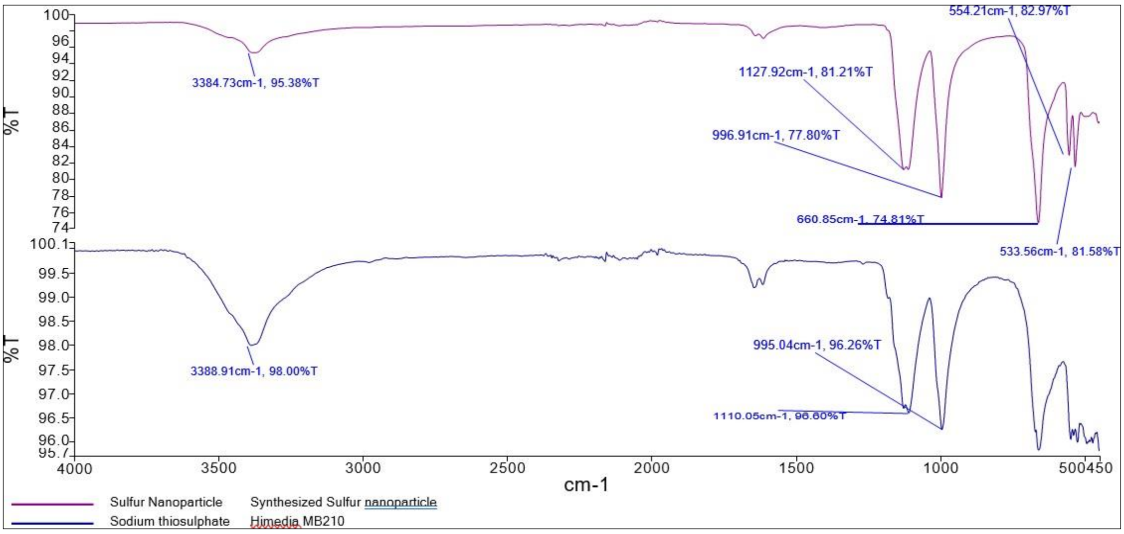


Fig. 5. Combined FTIR spectrum of sodium thiosulphate and sulphur nanoparticles

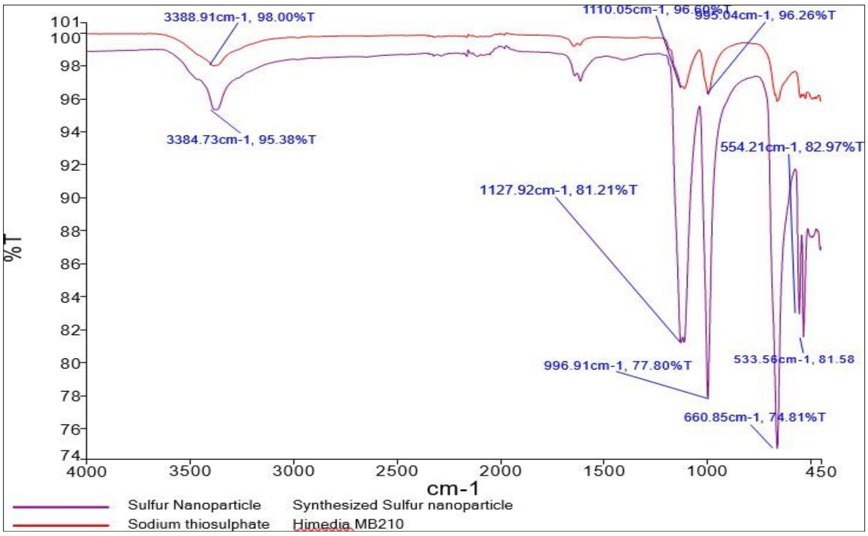


Fig. 6. Compared FTIR spectrum of sodium thiosulphate and sulphur nanoparticles

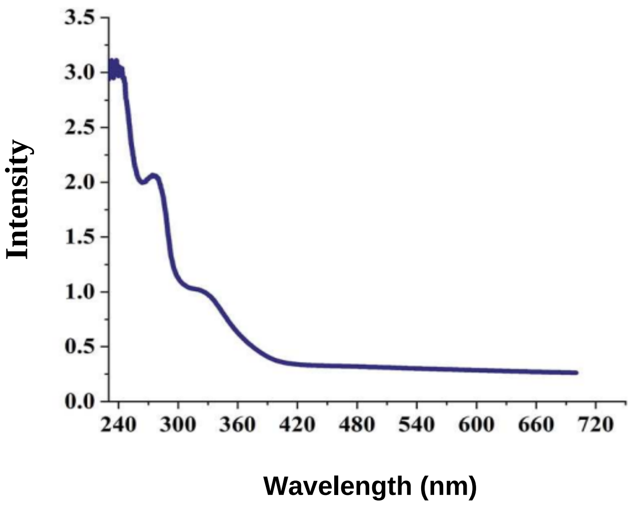


Fig. 7. UV-Vis absorption spectrum of biogenically synthesized SNPs

4. CONCLUSION

The process of using *Azadirachta indica* as a reductant to synthesize sulphur NPs is impressive for its cost-effectiveness, speed and Eco-friendliness. The synthesized sulphur NPs characterized by dynamic light scattering measurements for particle size (nm) and Polydispersity Index showed that the particle size was in the range of 35-40 nm with Polydispersity Index of 0.280-0.290, it is the mid-range value and indicating that the particles were in disperse form in aqueous suspension. From the analysis, the zeta potential value was found to be (-5.77 mV), revealing the better stability of synthesized sulphur nanoparticles in aqueous suspension. The FT-IR spectrum showed characteristic absorption of wavenumber from 3384.73 cm^{-1} and 533.56 cm^{-1}. The absorption spectra of UV-visible spectrophotometer in between the range of 200-800 nm [16].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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