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Investigating the Effect of Different Algae on the Production of Silver Nanoparticles

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Abstract

Along with the efficient antibacterial property of silver nanoparticles, algae, due to its biocompatibility, biodegradability and low cost, is taken into consideration for synthesis of nanoparticles as reducing agent. This study focuses on the biosynthesis of silver nanoparticle using different species of algae (Nannochloropsis, Chlorella, Scenedesmus). The effect of some variables including concentration of silver nitrate ($AgNO_3$), type of algae and harvesting time, were studied at three levels, via experimental design using response surface method, central composite design. The designed method resulted in 20 different experiments with the response factor of silver nanoparticle concentration. The results illustrate that *Chlorella* demonstrates the highest nanoparticle concentration at extended harvesting times, whereas *Nannochloropsis* exhibits the lowest nanoparticle concentration at shorter harvesting times. The synthesized silver nanoparticles appear spherical through the scanning electron microscope (SEM). Nanoparticles using Chlorella algae appear smaller with the mass formation, which may contribute, to their aggregation. The aggregation behavior of the silver nanoparticles synthesized with Chlorella Vulgaris can be attributed to the absence of sufficient stabilizers in the external solution, which is due to the inability of Chlorella to secrete stabilizing agents. The Fourier Transmittance Infrared Spectrum verified the presence of algae ingredients, which were responsible for the reduction of the silver ions and synthesis of the silver nanoparticles.

Keywords: Silver nanoparticles, Algae, Biosynthesis, Experimental design.

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Introduction

Nanotechnology has permeated every aspect of human life, significantly influencing various scientific and industrial fields [1]. The rapid progress of nanotechnology has led to the development of nanoparticles, which possess unique characteristics due to their small size, high surface area, and exceptional physicochemical properties.

Nanoparticles can be synthesized by physical and chemical methods. While these methods are widely employed, presence of toxic by-products remains a major challenge. To address this issue, biological methods have been proposed as an eco-friendly, simple, cost-effective and clean alternatives for nanoparticle biosynthesis [2-4].

Microorganisms play a crucial role in these environmentally compatible approaches, with various biological entities—such as fungi, bacteria, yeast, and algae—being utilized for the biosynthesis of silver nanoparticles [4-7]. Algae have garnered attention for nanoparticle synthesis due to their advantages, including low cost, availability, environmental compatibility, and high metal uptake capacity [5, 8, 9]. Vasanthakumar et al. successfully employed Nannochloropsis as bioreductant to reduce sodium selenite for biosynthesis of Selenium nanoparticles [4]. Silver has long been recognized for its antibacterial, antifungal, anti-viral and anti-inflammatory properties, making it a valuable component in medicine since ancient time. Compared to silver metal, silver nanoparticles exhibit enhanced antimicrobial properties due to their increased surface area, which enables better interaction with microorganisms [8, 10]. In comparison to silver ions, silver nanoparticles synthesized with Microcoleus, a cyanobacterium, demonstrated remarkable antimicrobial activity as reported by sudha et al. These nanoparticles were also found to be spherical and well distributed [11]. Furthermore, silver nanoparticles synthesized with Isochrysis by Gnanakani et al. exhibited potent antimicrobial activity against pathogenic bacteria [12].

Rajeshkumar et al. confirmed the role of alga in mediating silver nanoparticle synthesis and their effectiveness against certain pathogenic fungi [13]. Various micro and macro algae species have been utilized in silver nanoparticle biosynthesis, including *Sargassum cinereum* [14], *Sargassum muticum* [15], *Sargassum longifolium* [16], *Sargassum wightii* [16], *Chlorella vulgaris* [17], *Acanthophora specifera* [18], *Padina pavonia* [19], *Gracilaria corticata* and *G. edulis* [20], *Portieria hornemannii* [21], *Padina SP* [22].

Obaid et al. reported promising antibacterial efficacy in silver biosynthesized nanoparticles using *Arthrospira platensis* algae. They attributed these results to the algae's heavy metal uptake capacity and biocompatibility, highlighting its potential for nanoparticle synthesis [23].

The present study, concerns the biosynthesis of silver nanoparticles (SNPs) using three species of algae (Nannochloropsis, Chlorella, Scenedesmus). The effect of some variables ($AgNO_3$

concentration, type of algae and harvesting time), were investigated through experimental design using response surface methodology and central composite design.

Experimental

Materials

The following chemicals were used of analytical grade: silver nitrate (Sigma Aldrich, USA), Sodium hydroxide (PanReac AppliChem, Spain), hydrochloric acid (Merck, Germany), Ethanol (Merck, Germany), Agar (Merk, Germany). *Algal stock* (Nannochloropsis, Chlorella, Scenedesmus) was provided by Iranian National Algae Culture Collection (INACC).

Preparation of culturing media

The used algae are cultivated in BG-11 media. The modified BG-11 media was prepared in accordance with the presented data table in Al-Rikabey's study, under the ultraviolet light using biological safety cabinet of UV. The media was stored at 4°C and was autoclaved for 20 minutes (121°C and 1.5 bar), before each experiment [24].

Preparation of Algal Extract

20 ml of each *algal stock* (Nannochloropsis, Chlorella, Scenedesmus) was placed in 1000 ml Erlenmeyer flasks containing 400 ml of prepared culture media. Each flask *mouth* was sealed with the aluminum foil *covering* the *cotton* wool. The flasks were then put in a shaker incubator in 25^{o}C and pressure of 1 atm, with speed of 140 rpm for 24 hours. Three replicates were considered for each alga species. To ensure algae growth and to observe the growth process, optical density was measured by spectrophotometer (SHIMADZU, UV-1650PC). As microalgae have chlorophyll content, the wavelength of the device was set to chlorophyll absorption wavelength (680 nm) [25].
Harvesting of the cells was done in the exponential log phase. The cells were then washed with distilled water [24].

Preparation of Silver Nanoparticles

For nanoparticles synthesis, three different concentrations of $AgNO_3$ solution (0.001, 0.002, 0.005 M) were added to each of the designated algae cultures. The flasks were maintained under the same condition as previously. Color changes in reaction mixture indicate the formation of silver particles. Since reaction time influences the biosynthesis of nanoparticles, three distinct harvesting times were considered according to the experimental design. Nanoparticle harvesting was performed via centrifugation for 10 min (at 4°C and 5000 rpm). The resulting pellets were washed with sterile

distilled water and dried at 70°C. Bioreduction of the silver ions was monitored by UV-Vis spectrum analysis at an approximate wavelength of 430 nm, which is characteristic of AgNPs. Absorption at the mentioned wavelength indicates the reduction in silver ions and presence of SNPs. The experiment was conducted in accordance with the predetermined setup, as shown in table 1.

Statistical analysis

In the present study, the impact of some variables was investigated via experimental design using response surface method, central composite design. The design variables including $AgNO_3$ concentration, type of algae and harvesting time are denoted by A, B and C respectively in table 1 and were investigated at three levels. Level -1, level 0 and level 1 are as coded values representing the main values of design variables. The designed method resulted in 20 different experiments with the response factor of silver nanoparticle concentration. An analysis of variance was used to estimate experimental error. Finally, control factors with low importance values were observed to be part of the experimental error.

Table 1. Studied variables via experimental design (-1, 0, 1 as coded values).

design variables	**Level -1**	**Level 0**	**Level 1**
$AgNO_3$ concentration (mol/lit) (A)	0.001	0.002	0.005
Algae species (B)	Chlorella	Scenedesmus	Nannochloropsis
Harvesting time (day) (C)	2	5	8

Scanning electron microscopy analysis

The size and morphology characterization of the synthesized silver nanoparticles were analyzed by Hitachi S-4500 scanning electron microscopy machine. To prepare the samples, a drop of AgNPs solution, must be placed directly onto a carbon coated copper grids. The films were allowed to dry at room temperature.

Fourier transforms infrared spectroscopy (FTIR)

FTIR was used to recognize the potential biomolecules responsible for the reduction of the silver ions and synthesis of the AgNPs by used algae. It is also used to determine the functional groups involved in the synthesis of AgNPs. FTIR spectrum was recorded on Shimazdu IR Prestige-21 FTIR instrument.

Results and discussion

Our study has been limited to investigate the impact of only three factors (concentration of $AgNO_3$, type of algae and harvesting time) on biosynthesis of nanoparticles and was excluded the inspection of some parameter's effect such as temperature and pH.

Optical density characterization

Three kinds of algae including Nannochloropsis, Chlorella and Scenedesmus were cultivated in BG-11 media. The growth of microalgae was observed by optical density using a UV-Vis spectroscopy with a wavelength of 680 nm. The growth curves for the used algae shown in figure 1 are as we expected. Each microorganism such as microalga has a four phase growth process including lag phase, logarithmic phase, stationary phase and death phase [25-27]. High fluorescence intensity is related to a logarithmic phase of growth [28]. Lag phase is the first phase in which the microorganism adapts to the growth condition [26]. In figure 1 we observe an incremental flow in log phase of each alga. The intensity patterns are not the same.

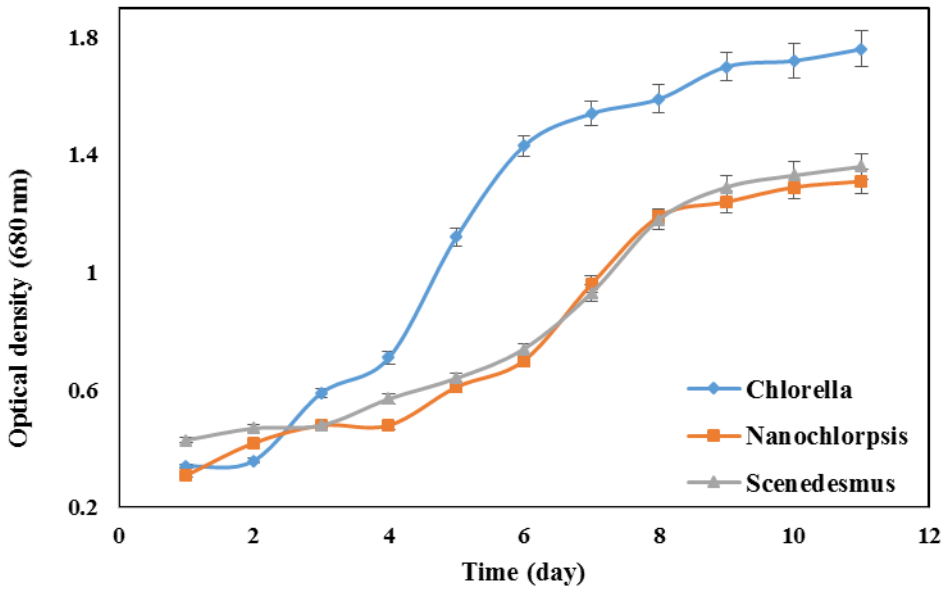

Figure 1. Graphs of the Optical Density values of algae isolates in BG-11 medium for a 12 day incubation period.

Production of silver nanoparticles

Figure 2 illustrates that silver nanoparticle production is significantly influenced by $AgNO_3$ concentration. By increasing $AgNO_3$ concentration, production of silver nanoparticles is being increased significantly. Our observation aligns with findings of Rodriguez-Leon et al. who reported that higher silver nitrate concentrations result in greater nanoparticle yields [29]. It is also consistent with Khalid et al.'s finding who reported maximum reduction of Ag^+ at 5 mM between a series of various concentrations of silver nitrate ranging from 1 to 5 mM. They also believed that the concentrations above 5 mM of silver nitrate could be toxic [30]. However, the effect of algal species on nanoparticles production is not as much as the effect of $AgNO_3$ concentration. As shown in Figure 2, at lower $AgNO_3$ concentrations, Ag production is almost the same for all three algae species. However, as the salt concentration increases, Chlorella demonstrates superior performance in silver nanoparticle synthesis compared to the other algae species. Additionally, Figure 1 indicates that Nannochloropsis exhibits the lowest nanoparticle production among the three species.

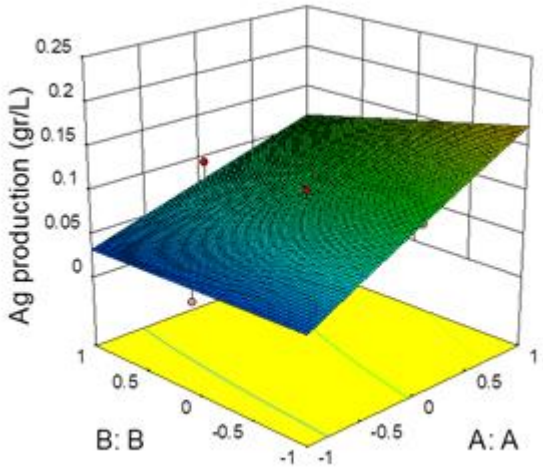

Figure 2. The effect of $AgNO_3$ concentration and algae species on silver nanoparticles production (-1, 0, 1 as coded values for design variables A &B; the significant terms of the model are specialized by $p<0.05$).

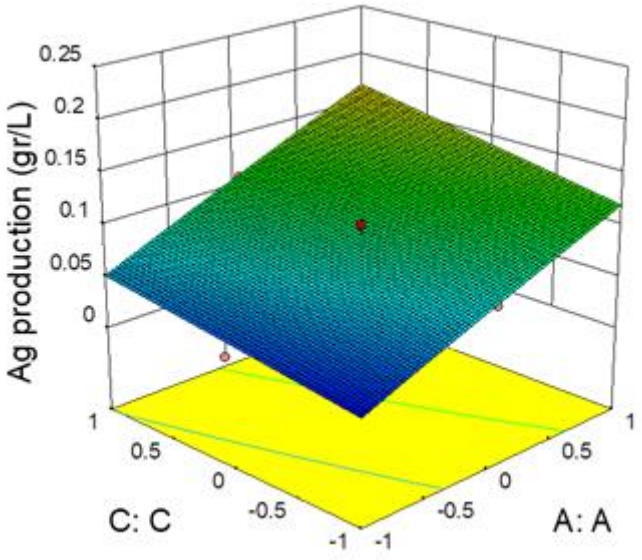

Figure 3. The effect of AgNO3 concentration and harvesting time (day) on silver nanoparticles production (-1, 0, 1 as coded values for design variables A &C; the significant terms of the model are specialized by $p<0.05$).

Figure 3 illustrates the direct impact of $AgNO_3$ concentration on nanoparticle production. It is evident that harvesting time has a comparatively smaller effect on the response than salt concentration. The chart shows a steeper slope at higher salt concentrations, indicating a more pronounced influence of $AgNO_3$ levels on nanoparticle synthesis.

At lower salt concentrations, harvesting time has minimal impact on nanoparticle production. However, at higher salt concentrations, an increase in harvesting time leads to a significant rise in silver nanoparticle production. Notably, on the eighth day of harvest (at level 1), under high $AgNO_3$ concentrations, the highest nanoparticle production is observed.

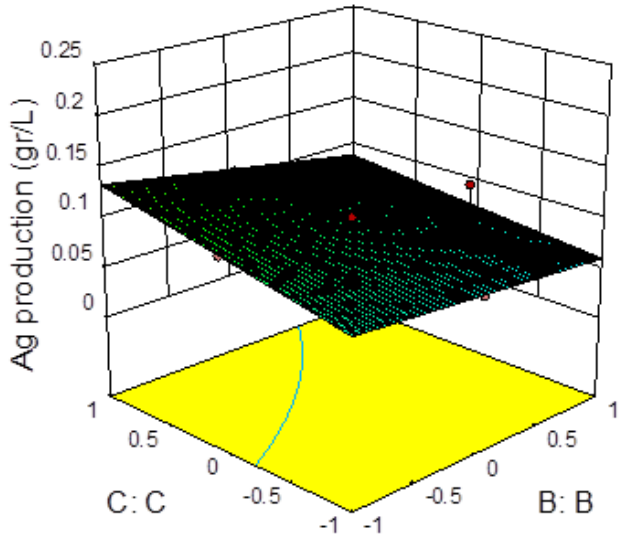

Figure 4. The effect of algae species and harvesting time (day) on silver nanoparticles production (-1, 0, 1 as coded values for design variables B &C; the significant terms of the model are specialized by $p<0.05$).

Figure 4 highlights the positive impact of harvesting time on nanoparticle production. Notably, at shorter harvesting times, the algal species have no significant effect on nanoparticles production. By increasing harvest time, the effect of algae type becomes more noticeable. This observation aligns with the findings of Chugh, who reported that nanoparticle production accelerates with longer reaction times [6]. Our results are also consistent with those of Mohandass [14].

Additionally, Chugh has discussed the role of algae in influencing reaction time [6]. As depicted in Figure 4, *Chlorella* demonstrates the highest nanoparticle production at extended harvesting times, whereas *Nannochloropsis* exhibits the lowest production at shorter harvesting times.

Scanning electron microscopy analysis

The scanning electron microscope (SEM) is utilized to analyze the morphology, size, shape, and distribution of nanoparticles. The following images correspond to nanoparticles synthesized using three different types of algae. Figure 5 presents nanoparticles produced by *Nannochloropsis*, which appear spherical, distinct and uniformly distributed. These nanoparticles measure approximately 200 nm in size and exhibit lower surface porosity compared to those synthesized from other algae. In contrast, Figure 6 displays nanoparticles derived from *Scenedesmus*. While the particles appear relatively smooth, their size distribution is highly irregular. Figure 7 illustrates nanoparticles synthesized using Chlorella algae. In this image, particle adhesion and mass formation are evident. Based on the scale provided, the nanoparticles appear smaller in size, which may contribute to their aggregation. Similar aggregation behavior has been reported in silver nanoparticles synthesized with Chlorella Vulgaris by Aldayel et al. They attributed this phenomenon to the absence of sufficient stabilizers in the external solution, suggesting that *Chlorella* lacks the ability to secrete stabilizing and capping agents into the surrounding medium [31].

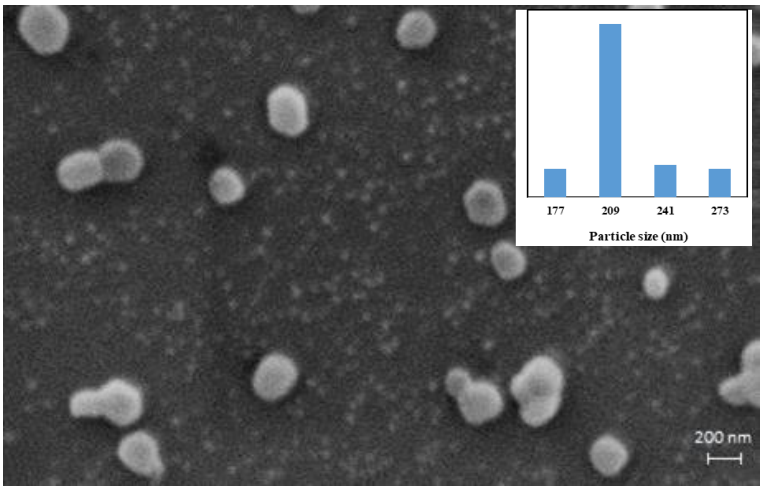

Figure 5. SEM micrographof biosynthesized Chlorella SNPs using Nannochloropsis.

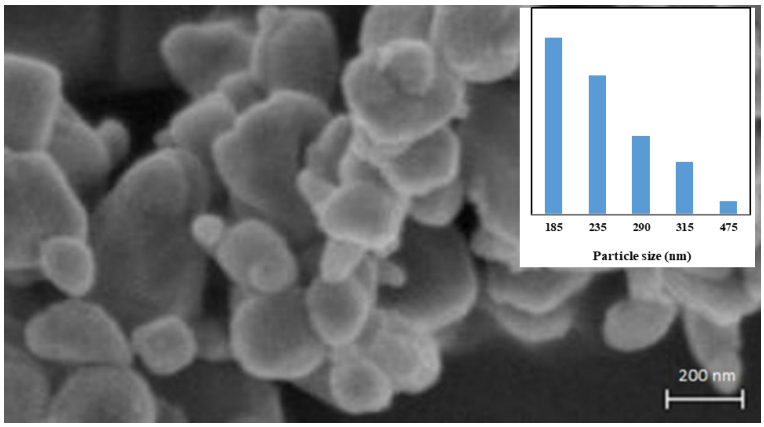

Figure 6. SEM micrographs of biosynthesized SNPs using Scenedesmus.

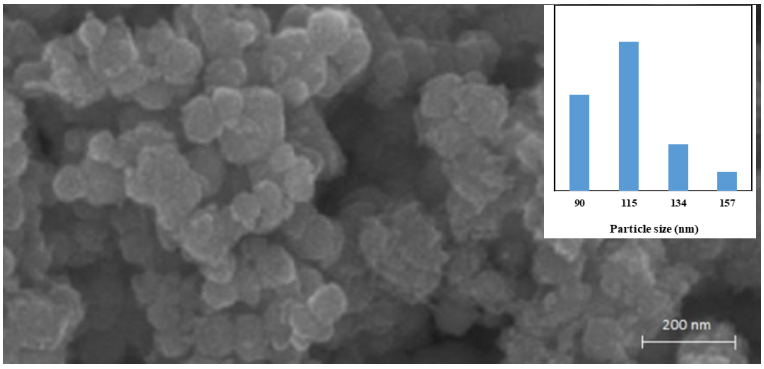

Figure 7. SEM micrographs of biosynthesized SNPs using Chlorella.

FTIR analysis

Presence of different functional groups in the synthesized product is determined by FTIR. Formation and stabilization of silver nanoparticles are confirmed by functional groups of fatty acids, lipids, proteins, polyphenols and amines through FTIR spectra [12]. Figure 8, 9 and 10 show the FTIR results for the silver nanoparticles synthesized with Chlorella, Nannochloropsis and Scenedesmus respectively.

In our FTIR results of biosynthesized SNPs, a sharp band at 3106.11 in Figure 8 and a prominent band at 3262.15 in Figure 9 are attributed to stretching vibrations of O-H bonds of alcohols. This is almost in agreement with the results of Adenigba et al., who assigned the O-H bonds of alcohols to the peak at 3272 for Chlorella-AgNPs and 3294 for Nannochloropsis-AgNPs [32]. A band at 3430 in FTIR results of Annamalai et al., who biosynthesized the silver nanoparticles using Chlorella vulgaris, corresponds to OH stretching of alcohol or phenol groups [17]. According to Jayashree et al.'s results, the broadband at 3416.14 in Figure 10 can be referred to the OH stretching of hydroxyl groups and the N-H stretching, which indicates the presence of various macromolecules, including carbohydrates, proteins, and lipids [34].

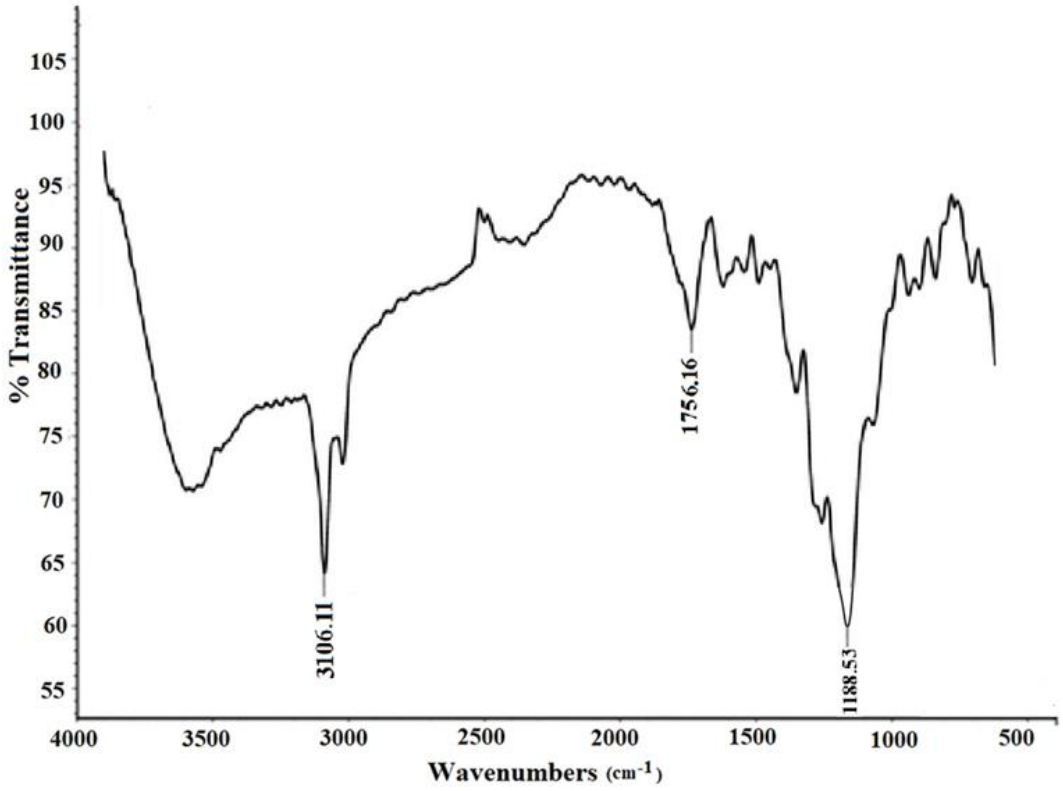

Figure 8. FTIR spectrum of biosynthesized AgNPs using Chlorella.

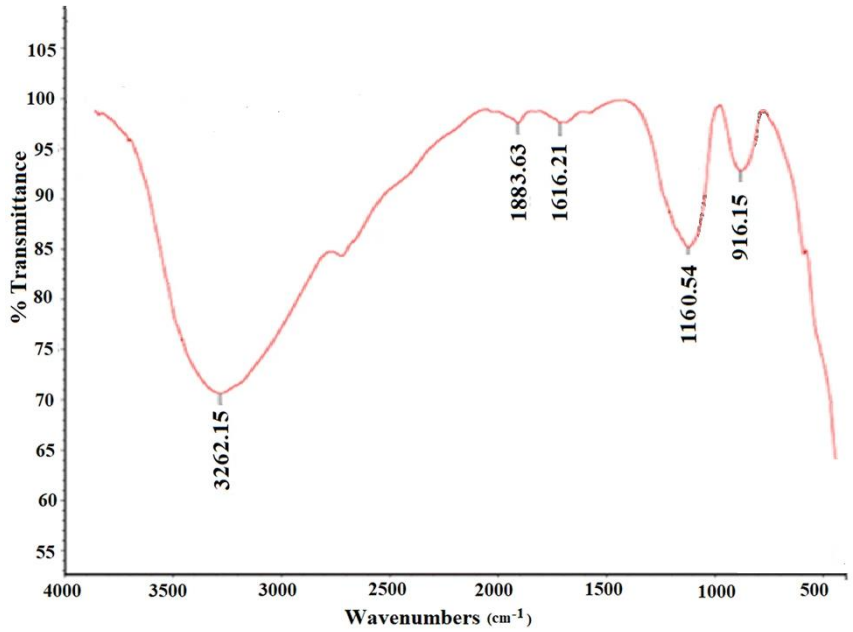

Figure 9. FTIR spectrum of biosynthesized AgNPs using Nannochloropsis.

Based on Jayashree et al.'s claim, the peak at 1375 in Figure 10 may be due to the C-N stretching vibration of aromatic amines [34]. A sharp peak observed at 1160 in Figure 9 aligns with the FTIR results of Gnanakani et al., who investigated the synthesis of silver nanoparticles using a partially purified ethyl acetate extract of Nannochloropsis sp. hexane (EAENH) fraction of microalga [35]. The mentioned peak corresponds to C–O–C stretching, signifying the presence of carbohydrates. Jayashree et al. confirmed the claim above and attributed the peak at 1155 to C-O-C stretching of carbohydrates and polysaccharides [34]. The peaks in the region 900-1020 show characteristics for C–O and C–O–C stretching of polysaccharides based on Fazelian et al.'s statement [36]. In the mentioned region, an appeared peak at 916 in figure 9 is justified based on the above explanation.

In Annamalai et al.'s opinion, bands around 1650 $cm^{-1,}$, which appeared in our FTIR results, indicate amide linkages between amino acid residues in Protein [17]. It coincides with Fazelian et al.'s report, which attributed the peaks in the region 1692–1599 to amid I band mainly C = O stretching of protein [36]. Gnanakani et al. who referred the peak 1634 to the stretching of amides [35] also confirm it.

The appearance of the peak at 1756 can be interpreted in the same way as Gnanakani et al. justified the peak at 1736, which suggested the reduction of Ag+ due to the oxidation of polyphenolic groups and verified the conversion of functional groups during AgNP synthesis [35].

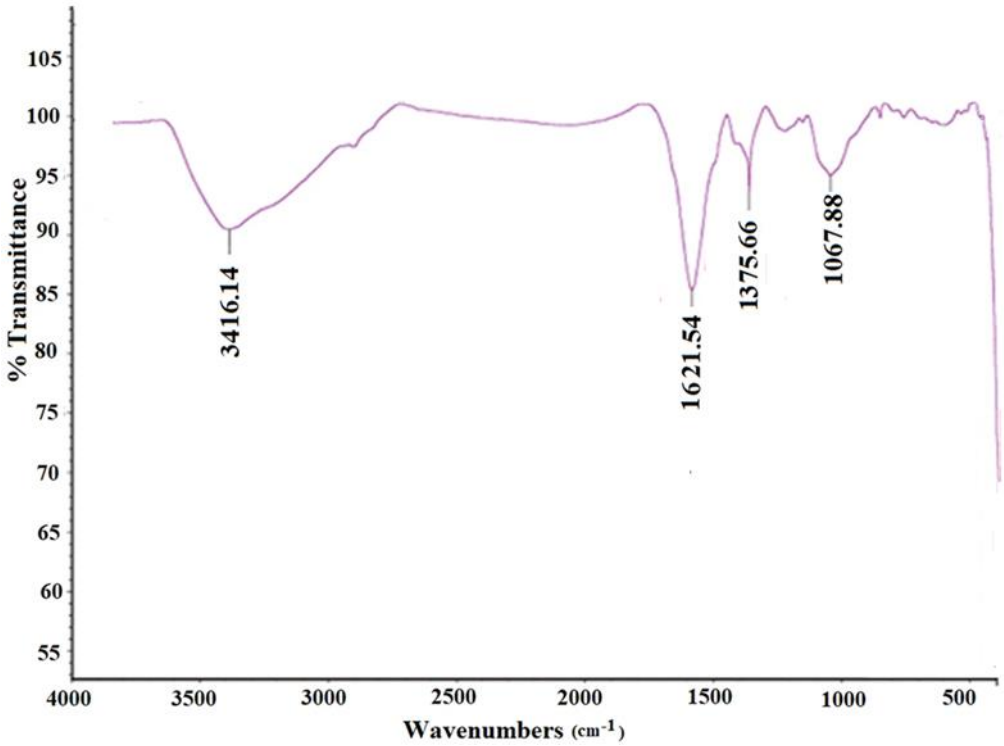

Figure 10. FTIR spectrum of biosynthesized AgNPs using Scenedesmus.

The FTIR results, revealed the presence of proteins that are very important in the biosynthesis of Ag nanoparticles. Proteins are being attached with silver ions and reduce them to nanoparticles. Proteins are also believed to increase the stability of formulation and prevent agglomeration [33].
In the previous studies, it is stated that proteins could be as a coating to cover the metal nanoparticles [17]. One of the limitations of the chemical synthesis method is the hazarding of chemical agents as a coating [33].

Conclusion

Along with the rapid advancement of nanotechnology, green synthesis of NPs is a superior method to eliminate the production of chemical by-products, which are serious threat to human life. Meanwhile algae play a significant role in these environmentally compatible approaches and are considered a proper candidate in green synthesis of silver nanoparticles not only as bioreductant agents for reduction of silver ions, but also due to some of their special features, including safety, biocompatibility and eco-friendly properties which make them valuable across diverse scientific disciplines. Many issues must be concerned in biosynthesis of nanoparticles using algae. Algae alternative, determination of algae concentration, as well as optimizing the synthesis conditions, including temperature, pH, are all points that affect the quality, structure, and morphology of the synthesized nanoparticles. Future research should focus on longitudinal studies to establish causal relationships between influential factors. Additionally the unique physicochemical properties of algae lead them to be used in different industries such as medical applications.

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