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CHARACTERISTICS OF SILVER NANOPARTICLES IN DIFFERENT pH VALUES

Stability of silver nanoparticles strongly influences the potential of their application. The literature shows wide possibilities of nanoparticles preparation, which has significantly impact on their properties. Therefore, the improvement of AgNPs preparation plays a key role in the case of their practical use. The pH values of the environment are one of the important factors, which directly influences stability of AgNPs. We present a comparing study of the silver nanoparticles prepared by „bottom-up" methods over by chemical synthesis and biosynthesis using $AgNO_3$ (0.29 mM) solution. For the biosynthesis of the silver nanoparticles, the green freshwater algae *Parachlorella kessleri* and *Citrus limon* extracts were used as reducing and stabilizing agents. Chemically synthesized AgNPs were performed using sodium citrate (0.5%) as a capping agent and 0.01% gelatine as a reducing agent. The formation and long term stability of those silver nanoparticles synthesized either biologically and chemically were clearly observed by solution colour changes and confirmed by UV-vis spectroscopy. The pH values of formed nanoparticle solutions were 3 and 5.8 for biosynthesized AgNPs using extract of *Citrus limon* and *Parachlorella kessleri*, respectively and 7.2 for chemically prepared AgNPs solution using citrate. The SEM as a surface imaging method was used for the characterization of nanoparticle shapes, size distribution and also for resolving different particle sizes. These micrographs confirmed the presence of dispersed and aggregated AgNPs with various shapes and sizes.

Keywords: Ag nanoparticles, particles size, stability, pH

1. Introduction

Silver nanoparticles (AgNPs) of all the nanomaterials have become most widely used and high-demand nanomaterial for consumer products [1,2]. Silver in the nanoparticle form is extremely valuable for industrial, electrical, mechanical, and biomedical uses, because of its antimicrobial, catalytic properties and also for unique optical properties stemming from the excitation of plasmon resonances, the collective oscillation of the free electron density [3]. Currently, Ag-NPs are extensively used in medicine, medicinal devices, the food industry, pharmacology, paints, cosmetics, biotechnology, electronics, bio-sensors, engineering, energy, magnetic fields, and also in environmental remediation [4-6].

Many previous studies focused on ways of metals nanoparticles preparation and there are also lots of opportunities for their synthesis. Many chemical and physical means, such as chemical reduction, heat evaporation and electrochemical reduction have been developed. The most common way of AgNPs preparation is via reduction of a silver precursor using chemical or biological redusing agents. As it was reported [2,7] the biologically synthesized Ag nanoparticles using algae, bacteria, fungi, yeast or plants extracts appear to be more biocompatible nanomaterials. Such nanoparticles have attracted much attention and are interesting in many applications such as in nanolinear optics, spectrally selective coating for solar energy absorption, bio-labelling, intercalation materials for electrical batteries etc. [2,7,8].

The synthesis of AgNPs depends on various factors as a temperature, pH, substrate concentration, stirring and static conditions [2]. Various means of the Ag-NPs synthesis, used capping agents and also stored conditions, significantly influence vary in size, shape, surface electric charge, and in other physiological characteristics of prepared nanoparticles [9]. Because variation in nanoparticles size plays a vital role on nanoparticle activity, silver nanoparticles preparation with control over particle size, particle shape and stability is one of the main objectives during the nanoparticles preparation processes [2]. Nanoparticles agglomeration and concentration range are also two important factors affecting nanoparticles properties [5]. The aggregation is usually an irreversible process, which causes irreproducibility or complete loss of properties of nanoparticles and also nanostructured surfaces [3]. Silver nanoparticles are prone to agglomerate because of their large specific surface area and high surface energy. It is known that the size and shape of nanoparticles play an important role in various applications of nanoparticles [4].

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The present study is aimed at assessing and comparing the stability of silver nanoparticles changing pH conditions in their colloidal solutions. The biologically synthesized nanoparticles were prepared using extracts from freshwater green algae *P. kessleri* (Alg-AgNPs) and *C. lemon* (Lem-AgNPs), and chemically synthesized nanoparticles (Chem-AgNPs) were prepared using Sodium citrate as a weak reducing and capping agent with gelatin to improve stability of nanoparticles.

2. Materials and experimental procedures

Silver nitrates ($AgNO_3$ p.a.) were purchased from Mikrochem, Slovakia. Three kinds of silver nanoparticles were synthesized by bioreduction and chemical reduction method of silver salts ($AgNO_3$) as a Ag^0 precursor. $AgNO_3$ solution of 9.27 mM and 0.29 mM respectively, was prepared by dissolving of $AgNO_3$ in deionised water. In order to ensure stability of prepared AgNPs, nanoparticle solutions were stored in dark at 4°C.

2.1. Preparation of *P. kessleri* and *C. lemon* extract

The green algae strain of *P. kessleri* (LARG/1) was obtained from the Institute of Botany SAS in Bratislava. The Milieu Bristol medium was used to growth algae *P. kessleri* and it was prepared according to specifications, autoclaved (120°C for 20 mins) and dispersed into sterile Petri dishes. The plates were incubated at ambient temperature and lightning interval (12:12) for 2-3 weeks and observed for their growth.

The agar plates with cultivated algae *P. kessleri* were carefully washed with distilled water to remove algae biomass. The biomass was consequently heated up to boiling for 10 min using a water-bath. The solution obtained from algae was filtered through a membrane filter and used for silver nanoparticles preparation.

The extract from *C.lemon* was prepared from fresh fruits, which were obtained from a local market in Slovakia. The obtained juice from squeezed lemons was later centrifuged at 9,000 rpm for 15 mins due to remove any undesired impurities. This lemon extract was used for preparation of silver nanoparticles.

2.2. Synthesis of AgNPs

A biosynthesis of silver nanoparticles using algae (Alg-AgNPs) was carried out using 3 ml of extract of algae biomass, which was transferred into Erlenmeyer flasks containing 250 ml of $AgNO_3$ (9.27 mM) solution. The Erlenmeyer flasks were stored in lighting condition at the room temperature to allow reducing the silver ions into AgNPs.

A biosynthesis of silver nanoparticles using *C.lemon* extract (Lem-AgNPs), 3 ml of freshly prepared lemon juice was added to Erlenmeyer flasks with 250 ml of $AgNO_3$ (9.27 mM) solution under stirred and heated conditions.

Chemically synthesized AgNPs were performed using mixed gelatin/trisodium citrate method [10,11]. First of all, to prepare stock solution of $AgNO_3$, the gelatin (0.01 wt.%) was dispersed in 250 ml of 0.29 mM of $AgNO_3$ to prevent particle agglomeration. Silver nanoparticles were prepared by adding drop-wise of 15 ml of (0.5 wt.%) sodium citrate solution into $AgNO_3$ solution under heating conditions. The resulting solution was stirred for 30 minutes.

2.3. Characterization of synthesized AgNPs

The initial characterization of prepared nanoparticles such as particle size and state of aggregation was monitored by measuring the UV-vis spectra of the solutions in 10-mm optical path-length quartz semimicro cuvettes (UNICAM UV/vis Spectrometer UV4). The spectra were recorded in wavelength range from 300 nm to 800 nm.

The pH values were adjusted by 10% HNO_3 or 10% NaOH respectively to obtain solutions with pH value 2 and 10. Model HI 110 Series was used for pH measurements. After reduction, silver nanoparticles were centrifuged at 9000 rpm for 10 min using HETTICH, Universal 320 centrifuge.

Transmission Electron Microscope (JEOL model JEM-2000FX microscope operated at an accelerating voltage of 200 kV) was used for the nanoparticle size and morphology determination.

Infrared spectroscopy with Fourier transformation (FTIR) was used for the samples characterization in terms of functional groups qualitative analysis. FTIR measurements were performed by using a Spectrometer Alpha-T (Bruker, Germany) with ATR technique allowing the direct measurements of powder samples without KBr tablets preparation. Measurements proceeded in transmittance mode, in the range 400-4000 cm^{-1} with resolution of 4 cm^{-1}.

3. Results and discussion

3.1. Characterization of the nanoparticles

The cells free extract from freshwater green algae *P. kessleri* and extract fom *C.lemon* were used for silver nanoparticles biosynthesis.

Also commercially available sodium citrate has been used for the chemical synthesis of AgNPs. After adding the algae extract, lemon extract and sodium citrate, respectively to the silver nitrate solution, a visual colour changes from pale yellow to brown were observed within 1 h as signature to indicate the formation of AgNPs nanoparticles through reduction method of Ag^+ ions to Ag^0. The intensity of colours steadily increased with the time.

Due to the property of Surface Plasmon Resonance (SPR) as a collective oscillation on the conduction electrons in resonance with the wavelength of irradiated light, silver nanoparticles are

known to exhibit unique optical properties [12,13]. The typical maximum of absorption band for silver nanoparticles is in the region of λ = 350-450 nm, and its shift to red or blue depends on AgNPs size, morphology and also on the state of aggregation and the coating dielectric medium [14,15]. According to literature, significant broadening and red-shift of SPR band indicate the presence of various shaped and sized nanoparticles [16,17]. To follow the evolution of nanoparticle growth within solution the UV-vis spectroscopy was initially used. It is a widely used technique for occurrence confirmation and structural characterization of nanoparticles in colloidal solution [11,18]. Through the study, the minimum of 3-5 hours reaction time was required for the formation of AgNPs under laboratory conditions (Fig. 1A-C).

The pH measurement confirmed the reduction of Ag^+ to Ag^0 with varied pH ranges as 5.8 and 3 for Alg-AgNPs and Lem-AgNPs, and 7.2 for Chem – AgNPs respectively. According to the shape of absorption peaks of prepared silver colloidal solutions the broad absorption band observed in Alg-AgNPs and Lem-AgNPs indicated less uniform nanoparticles than Chem-AgNPs. UV-vis spectroscopy revealed that an absorption peak at approximately λ = 421 for Alg-AgNPs and Lem-AgNPs. Chem-AgNPs exhibited an absorption peak at approximately λ = 404 Our study corresponds with observation of the author [8], that UV-vis spectra of biogenic silver nanoparticles occurred at λ = 420 nm (Fig. 1A,B). The TEM images of prepared AgNPs under different conditions presented in (Figs. 2A,3A,4A) give clearer information about size, shape and size distribution of Alg-AgNPs, Lem-AgNPs and Chem-AgNPs. These images revealed presence of stable and roughly spherical Alg-AgNPs with averrage particle size of 11 nm, much less uniform Lem-AgNPs with average particle size of 30 nm and dispersed spherical Chem-AgNPs (average particle size of 17.5 nm).

For understanding the influence of different pH conditions on the size and stability of the experimental prepared nanoparticles within solution, the colloids solution were measured by UV-vis spectroscopy and confirmed by TEM observations.

In the colloidal solution with pH 2, where algae extract was added as reducing and capping agent, no significant red-shift of SPR band was observed (Fig. 2D). It is obvious, that decrease pH up to 2 caused no relevant changes of the size and stability of Alg-AgNPs.

In the Alg-AgNPs solution with pH 10 red-shift of SPR band (from 421 to 432 nm) was observed, but there was more visible increase of λ_{max} in the interval 0.4-0.9. Our observation was supplemented by TEM analyses (Fig. 2A-C), which showed that higher pH caused not only the increase in Alg-AgNPs size (Fig. 5.) but also formation of aggregates. According to literature [17], the intensity of absorption increases with concentration of AgNPs in colloidal solution.

The UV-vis analyses of AgNPs synthesized in lemon extract solution showed no changes after pH decreased from 3 to 2 (Fig. 3D). The TEM image of Lem-AgNPs confirmed spherical and more uniform nanoparticles in the lower pH of the solution (pH 2)(Fig. 3A and B). However, similarly as in case of Alg-AgNPs, increasing of pH values in the Lem-AgNPs solution up

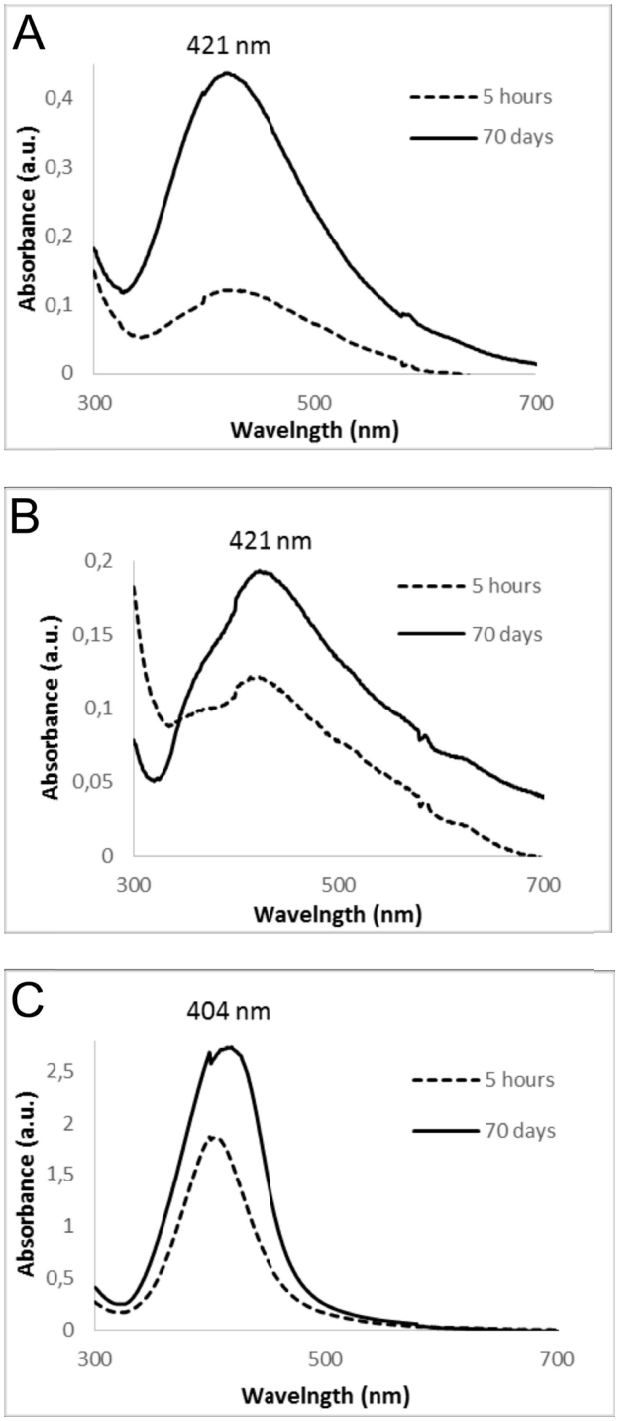

Fig. 1. The UV-vis spectroscopy of AgNPs prepared using A) algae extract (Alg-NPs), B) lemon extract (Lem-AgNPs), C) Sodium citrate (Chem-AgNPs)

to pH 10 led to gain higher intensity of λ_{max} value. In colloid solution, where pH was adjusted to alkaline value, changes shape to hexagonal particles and larger particle sized Lem-AgNPs were observed (Fig. 3C).

At chemically prepared nanoparticles (Chem-AgNPs) using sodium citrate as a weak reducing and capping agent, the symmetrical and narrow interval of SPR band was observed in the solution with pH 7.2, what indicated the presence of stable uniform particles with narrow size interval (1D). After changing

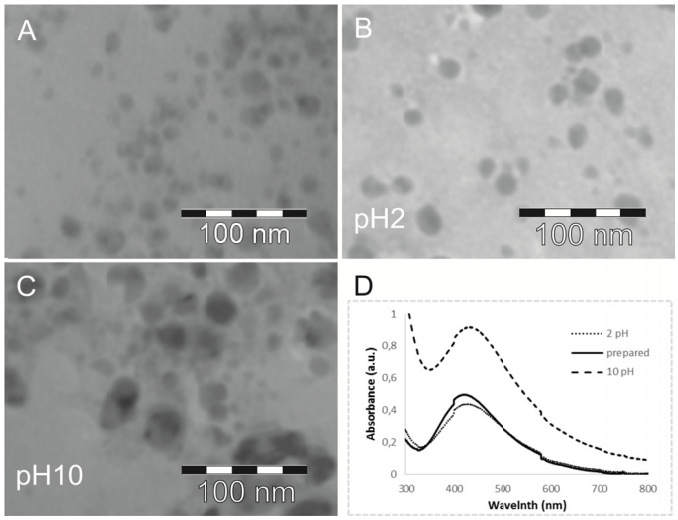

Fig. 2. The TEM images of Alg-NPs A) with resultant pH value of 5.8, B) pH value adjusted to 2, C) pH value adjusted to 10, D) UV-vis spectroscopy

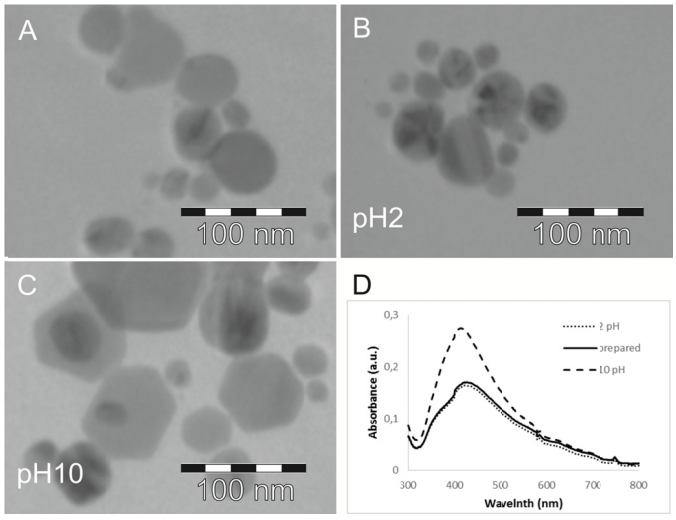

Fig. 3. The TEM images of Lem-AgNPs A) with resultant pH value of 5.8, B) pH value adjusted to 2, C) pH value adjusted to 10, D) UV-vis spectroscopy

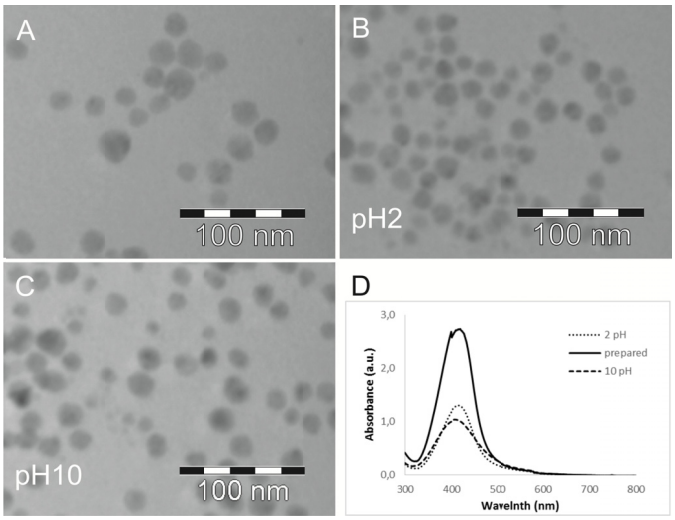

Fig. 4. The TEM images of Chem-AgNPs A) with resultant pH value of 5.8, B) pH value adjusted to 2, C) pH value adjusted to 10, D) UV-vis spectroscopy

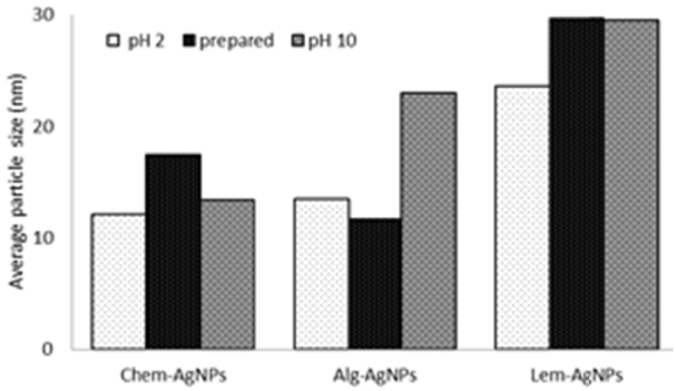

Fig. 5. Average particle size of AgNPs at different pH conditions

of pH value to 2 and 10, the values of absorbance λ_{max} changed in the intervals of 2.73 – 1.3 and 2.73 – 1.04, respectively. In this case, no red-shift of SPR band was observed (Fig. 4D). According to TEM images it is obvious that changes of pH solution do not have significant influence on the shape and agglomeration of Chem-AgNPs (Fig. 5A-D). The changes of pH solution resulted only in decreasing of particle size (Fig. 5). It is highly probable, that the presence of gelatin proteins on nanoparticles provides proper gap between the silver cores [19,20].

3.2. FTIR studies

The FTIR was used to recognize the molecules responsible for the reduction and stabilization of Ag nanoparticles. The corresponding FTIR spectra of AgNPs synthetized using reducing agents such as *P. kessleri* extract, lemon extract and sodium citrate, are shown in Fig. 6 A-C.

The spectra of dried *P.kessleri* before reduction and FTIR spectra of AgNPs synthesized with P. Kessleri extract are shown in Fig. 6A. Weak band present in the spectrum of AgNPs at 2914 cm^{-1} corresponds to asymmetric and symmetric υ(CH2) stretching. Pronounced band at 1634 cm^{-1} and 1517 cm^{-1} is associated with (C=O) stretching of amides I and υ(N–H) bending of amides II from proteins, respectively [21].

The bands at 1517 cm^{-1} and 1030 cm^{-1} may indicate the υ(C–N) stretching vibration of aliphatic amines [22,23] and υ(C–O–C) stretching of polysaccharides in carbohydrates, respectively. The bands were assigned to $\delta_s(CH_2)$ and $\delta_s(CH_3)$ bending of methyl and υ_s(C–O) stretching of COO^- groups (1318 cm^{-1}) [24]. Spectrum of *P. kessleri* before reduction showed a broad band at 3279 cm^{-1} attributed to υ(O–H) stretching of carboxyl groups [25] or phenolic compounds [13], which decreased significantly after AgNPs synthesis. According to FTIR results of Alg-AgNPs synthesized with *P. Kessleri* the presence of proteins on the surface of Alg-AgNPs as capping agents was determined. Similarly, the authors [21] confirmed

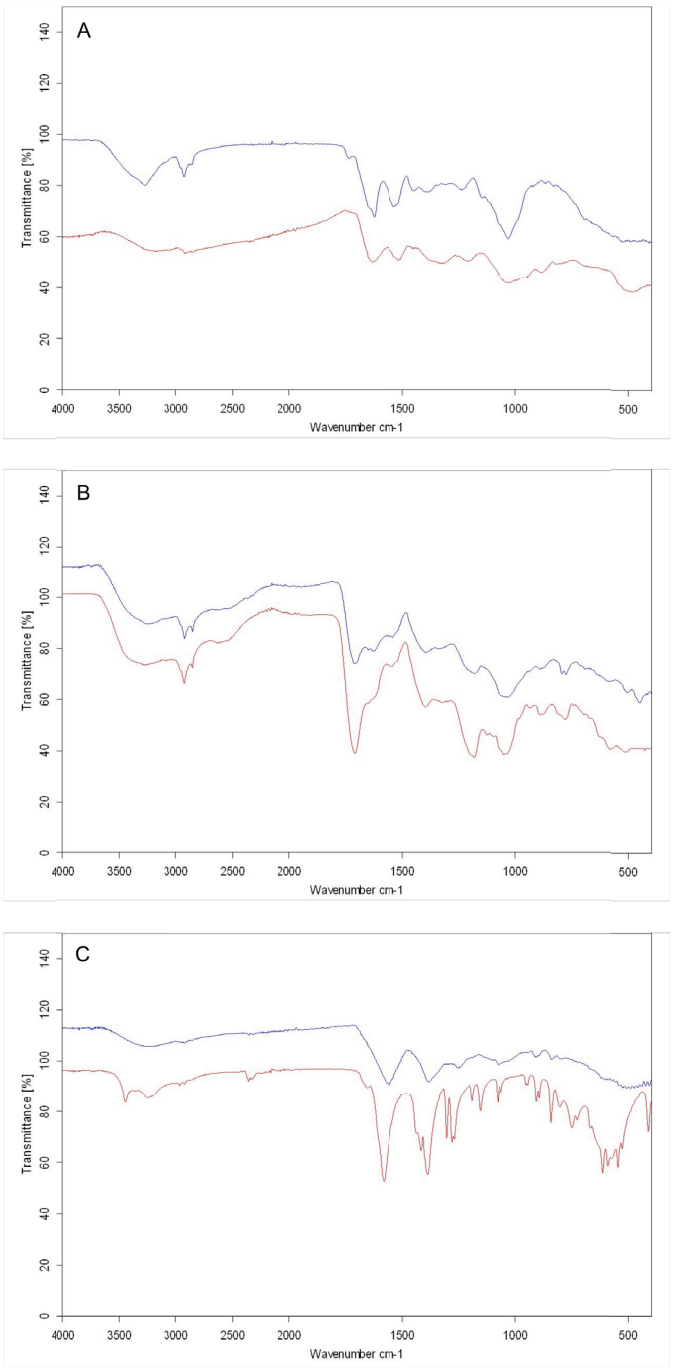

Fig. 6. FTIR spectra of AgNPs synthetized with A) *P.kessleri* extract, B) lemon extract, C) sodium citrate compared to FTIR spectra of reducing agents untreated with $AgNO_3$(control spectra)

that surface-capped proteins stabilise AgNPs and prevent their aggregation.

FTIR spectrum of Lem-AgNPs synthesized with lemon extracts (Fig. 6B) having the band at 2920 cm^{-1} denotes the presence of υ(C=O) stretching [26] or aldehydic υ(CH) stretching [27]. The less intense band of AgNPs at 1713 cm^{-1} compared to band of dried lemon extract, assigned to υ(C=O) stretching, was observed. The band at 1395 cm^{-1} and 1031cm^{-1} is attributed to the δ(C–O–C) bending vibrations [28] and υ (C–O) stretching, respectively. It is known that lemon mainly consists of citric acid, flavonoids and ascorbic acid. As observed, FTIR analysis of synthesized Lem-AgNPs indicates some functional groups, which are present in some bioorganics such as citric acid, which can be the main reducing agent in synthesis of Lem-AgNPs [27].

The FTIR spectra of Chem-AgNPs synthesized with sodium citrate are illustrated in Fig. 6C. The presence of characteristic groups of sodium citrate, such as υ(O–H) stretching of carboxyl group at 3447 cm^{-1}, δ(O–H) alcoholic at 1385 cm^{-1}, υ_{as}(O–C=O) stretching at 1581cm^{-1}, $\upsilon(CH_2)$ at 751 cm^{-1} was recorded. The reduction in the intensity of the bands at 1581cm^{-1} and 1385 cm^{-1} for characteristic groups COO^- and OH^- and their shift to 1558 $cm^{-1}$and 1384 cm^{-1}, respectively, in the case of FTIR spectrum of Chem-AgNPs confirms that citrate ions are present on the surface of Chem-AgNPs [29].

4. Conclusion

The results of ours study are from three different sources of silver nanoparticles, biologically and chemically prepared, which stability we compared in the acidic and alkaline pH conditions of the environment. From our experimental results it is clear that identified compounds in biomolecules were found to be active component responsible reduction of Ag^+ to Ag^0 and also for stabilization of AgNPs.

The changes of pH environment, resulted particularly aggregation of Alg-NPs in alkaline solution. It revealed, that the effect of stabilizing biomolecules of the algae , which cover nanoparticles are decreased in alkaline conditions. Similarly, citric acid, which is part of the biomolecules of lemon juice, loses its stabilizing ability in the basic environment, which also affects nanoparticle stability. In the case of Chem-AgNPs, despite the fact, that sodium citrate is weak reducing and capping agent, its gelatine coated nanoparticles showed to be stabilized and resistant in the acidic and alcalic pH conditions.

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