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Laser Ablation Technique to Synthesis AgNPs in Harsh Environment During Conjugation with OVA and their Effects on *Candida albicans*

Yasmin A. Adi[1,*], Mohammed H. Mushrif[2], S.A. Abdulateef[3]

Abstract

In this study was developed a rapid, easy, eco-friendly and free-polutions method to AgNPs synthesized which was laser ablation technique to bulk target silver in harsh environment which was SBF due to salts and ions content cause silver nanoparticle aggregated via the interactions among nanoparticles and components of the ionic solution. So, was add ovalbumin to achived long term stability of colloidal AgNPs solution because of this protien has been used to decrease ionic strength and avoid nanoparticle agglomeratioin in biofluids by attached it to the AgNPs surfaces in the mixture, to protecting them from the NaCl and preventing aggregation.then, noticed the color change on the sample and examined it with a transmission, electron microscope to see it's size and shape, as well as the zeta potential to know it's cahrge, in addition to measuring it in FTIR and uv-vis spectrophotometer over a month to prove the stabillity of the sample. And applied of the silvernanoparticle solution and their dilutions on cultured media of C.albicans which isolated from human patients with candida infection.

Keywords: AgNPs silvernanoparticle, pulse laser ablation PLAL, uv-vis, ftir, z-potential, tem, *candida albicans*.

INTRODUCTION

Nano-sized substances are, critical matter in both fundamental and technological sciences Because of their usage, in a variety of domains, including, chemistry, physics, biology, material science, medical, and catalytic. Metal nanoparticles (NPs) have been the subject of decades of practical scientific experiments. In fact, Faraday was one of the first scientists in the 1850s who studied metal NPs [1].

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Pulsee laser ablation in liquid is one of the fundamental techniques for creating nanoparticles from various substrate solvents. Under the impact of a laser beam, the metallic solution is combined with the liquid medium to generate nanoparticles that range in size from 1 to 100 nm. this technique offers an alternative to the more traditional chemical reduction techniques. this procedure is economical, environmentally friendly, and does not result in any harmful byproducts. However, The most frequently used nanoparticles by scientists worldwide are silver nanoparticles. Nanoparticles have unique properties that make them beneficial in a variety of many other businesses including agricultural, animal husbandry, aesthetics, and sanitary conditions.

These characteristics include rising conductivity, chemical resistance, antibacterial, antiviral, antifungal, anti-angiogenic, and anti-inflammatory abilities [2].

So, the AgNPs are Metal inorganic nanoparticles that contain special traits, including optical characteristics and surfaces Plasmon resonance, (SBR,). Shiny colors are created in noble metal NPs by the particle Plasmon, sometimes referred to as the resonance stimulation of a group of oscillations inside the particle's electron, conduction band, However, the strongly those metal NPs absorb SPR depends on their size, form, and content [3].

A protein known as albumin is something that may coagulate under heating, is hydrophilic, and accumulates to a modest degree in salt solutions. The statement may be redefined as follows: Serum albumin, which is the biggest protein component of blood plasma, is mostly synthesized in the livers of humans and other vertebrates as well as, Egg white is an aqueous, rich medium mostly made up of proteins that make up around 10–12 percent of the content. These proteins include ovalbumin, Ovo transferrin, lysozyme, and ovomucin. Ovalbumin is the major egg white protein synthesized in the hen's oviduct, within the magnum tissue, and is responsible for egg white formation. It accounts for about 54% of the total proteins of egg albumen. First isolated and purified by crystallization by Hofmeister in 1890 [4]. It is a glycoprotein a molecular weight of around 42.7 kDa with 385 amino acids, nearly a quarter of which are charged. Also, it's used as antimicrobial and antifungal, and antioxidant features which have aided in the development of health- and pharmaceutical-related goods [5].

Through the solvent-directed assembly, proteins are used only to create protein nanoparticles (PNPs). Proteins inside the solution condense onto nanostructures during desolvation, which involves adding an unsuitable solvent to a protein solution to promote protein-protein interactions. These PNPs differ from those that develop from a protein's identity sequences, including vaults or cage [6]. The likelihood of a non-target immune system response decreases without a self-assembling tag that was developed to produce nanoparticles on the antigen for the tag were too diminished. Albumin was first used to create desolvate PNPs. PNPs had previously been demonstrated to be capable to transport folded activated enzymes into cells Within that creation of nanoparticle vaccines, distribution of correctly folded antigen proteins is particularly desired [7].

SBF is a solution which has the ionic concentrations similar to those found in blood plasma from humans and is kept at the temperature and pH that is near to that of the body and described as noncellular, protein free, supersaturated calcium phosphate liquids that are typically buffered under physiologic temperatures (pH Equal 7.4 and 36.5 Celsius), such as 0.9 percentage NaCl aqueous solution or phosphate buffered, saline (PBS) [8].

Candida albicans is a prevalent fungus that has transformed from a normal flora does not cause disease to an opportunistic pathogen that lives in human mucosal and several surrounding habitats [9].

MATERIALS AND METHODS

Synthesis of Silvernanoparticles

A high-purity Ag target was laser-ablated while submerged in a tow beakers the first contain solution of simulated body fluid, high concentration NaCl (1 mol), and the second contain SBF and oval albumin (275 μM) at room temperature. The experimental setup design for the pulse, laser ablated in liquid, (PLAL,) technique is depicted in Figure 1.

Inside a 20 millileter glass container containing 4 mL of simulated body fluid and soluited in it 0.04 gm of ovalbumin, the bulk silver targeted which situated at the base then exposed to radiation from a Q-switched, Nd:YAG lasers with such a wavelength range of 1064,nm, a pulse period of 4 m, as well

as a repetation frequence be a 10 HZ. A colloidal solution was employed to ablate the Ag target using laser intensities of 2000 mJ/pulse, even the light laser, beams was directed towards to the Ag aim, via employing around 100 mm of,focal distance for, focal lense. The, colloidal solutions, properties, were measured using UV/Visible spectrophotometer, a transmission electron microscope, fourier transform inferared spectroscopy and z-potential.

Then, this colloidal solution applied on cultured media with *C.albicans* which isolated from saliva of human patient of candida infection in agar well diffusion method.

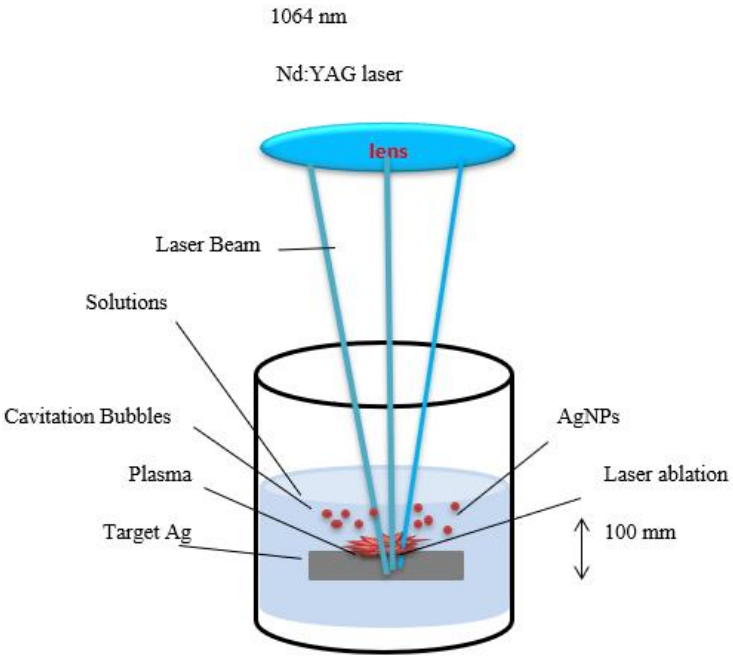

Figure 1. Diagram of PLAL system (10).

Isolation of *C.albicans*

By using diagnostic techniques, *C.albicans* was isolated from saliva samples of patients of various ages and sex. The isolates were then cultivated on Sabouraud Dextrose agar with chloramphenicol, and the plates were incubated at 37°C for 24-48 hours. incubated the tubes at 37°C for 2 to 4 hours, transferred a drop of the serum to a slide for observation after air drying, heat fixing, and finishing a staining to gram stain. Also, the results of the germ tube test were examined under a light microscope. Also, we performed diagnostic testing on all samples using the VITEK2YSTcard in the Biomerix (France) [10, 11].

Agar well Diffusion Test

When *C. albicans* suspension was cultivated using a spreader on SDA, it was compared to 0.5 108 colony forming units/ml (MacFarland standard solution). After that, create wells with tips measuring 10 mm in diameter on sabouraud dextrose agar that had already been inoculated with yeast. Then, pour the solutions into a hole one volume at a time (20 ul). As a result, agar plates were incubated in the appropriate conditions overnight at 37°C. The fungal inoculum under investigation is prevented from growing when the antimicrobial medication diffuses in the agar medium and creates a zone of inhibition [12].

Preparation of Antifungal Dilutions Solution

The AgNPs solution was separated in centrifuge to taken the precipitate of AgNPs to make ten dilutions of it in distilled water by adding 1 ml of AgNPs sedmentation into 9 ml of distile water then take one ml from first dilution to the seconed tube which has 9 ml of DW [13].

RESULTS AND DISCUSSION

Color dependence of silver nanoparticles

Tests on the samples characteristics were done to determine sample stability. Some variations in the coloring of the solutions were noticed following the laser ablation of the Ag target in theSBF,SBF+OVA solutions. In the first solution was seen agglutination of AgNPs solution resulting to interactions between nanoparticles with elements in biological medium or ionic solutions. According to a new theory, aggregates or agglomerates form once the attractive forces energies across nanoparticles are stronger than the repelling electrostatic attraction. Also, in the second solution NPs caused the solution's color to shift from pale yellow to dark brown. It's possible that the color shift is an a sign of colloidal AgNPs generation by modifying specific parameters depicted in Figure 2. The significant optical properties of metal nanoparticles are spread throughout their bright, intense colors [14].

Figure 2. The colloidal solution of AgNPs [14].

Absorabtion Spectra

In this case of just Fluid (SBF), the uv-vis spectrum showed 2 peaks at 274.5 and 201.5 nm with absorbance equal to 0.039 and 2.841 respectively note the Table 1. These peaks could be assigend to the α-α* transition of the fluid salt component show in (Figure 3) this displacement is evidence of the presence of salts and ions in the solution.

Table 1. Wavelength and absorbtion to UV-visible spectrum to SBF [15].

	Wavelength nm	Absorbance
1	274.5	0.039
2	201.5	2.841

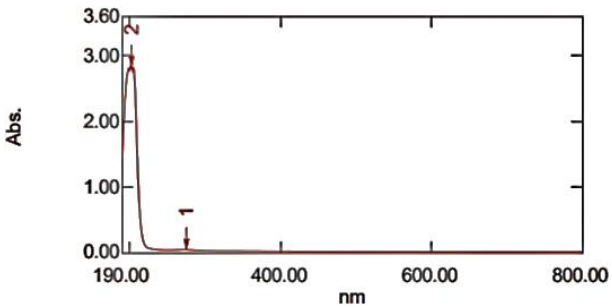

Figure 3. Diagram illustrate the UV-vis spectrum for SBF [15].

In the case of SBF with AgNPs excised by pulsed laser, the spectrum showded four peaks at (349.5, 267.0, 224.5, 202.5) nm with absorbance 0.044, 0.103, 0.143, 2.896 respectively note the (Table 2). The measurment in (Figure 4) prove that the peak of SBF was shifted from 201.5 to 202.5 nm and the second peak was shifted from 274.5 to 267.0 nm. These shifts are solid evidence to the reaction SBF with Ag and the formation of AgNPs, furthermore the spectrum give another two peaks 349.5 and 224.5, the peak at 349.5 is assigned to AgNPs which attributed to the SPR peak. However, agglomeration of nanoparticles was absorved in the solution due to forces between nanoparticles and components in biological media or ionic solutions, according to a scientific revelation, aggregates or agglomerates form when van der Waals attractive interactions between nanoparticles are stronger than electrically charged repulsions. (Figure 2) [16]. Whenever the ionic inside a biology media causes aggregation, reducing a space between particles, it results in a situation where Van der Waals forces predominate. Ions from NaCl can produce this situation. When applied immediately to liquid, sodium chloride splits into Na and Cl-. Van der Waals attractive forces take control practically immediately after the dissolved chloride ions attaches towared a AgNPs surfaces, neutralizing surfaces charges, which resulting in precipitation (Figure 4).

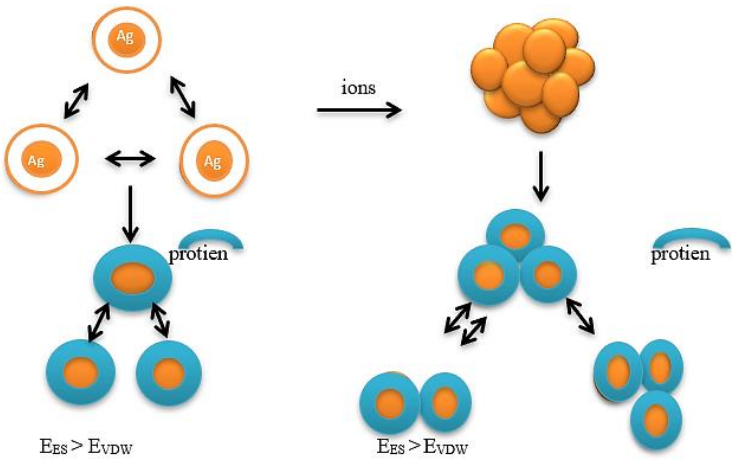

Figure 4. Diagram illustrate the Mechanism for aggregation. By neutralizing the stabilising electrostatic forces (EEs) on AgNPs produced by laser ablation, ions triggers the van der Waals forces (Evdw), which then promote aggregation formation [Figure 5]. The AgNP surfaces are bound by OVA when it is introduced to the mixture, protecting them from the NaCl and preventing aggregation [17].

Table 2. Wavelength and absorbtion to uv-vis spectrum in SBF+ AgNPs [18].

NO	Wavelength nm	Absorbance
1	349.5	0.044
2	267.0	0.103
3	224.5	0.143
4	202.5	2.896

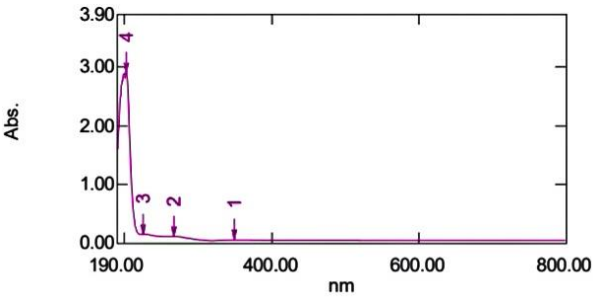

Figure 5. UV-VIS spectrum graph to Wavelength and absorbtion in SBF+AgNPs [18].

But the case of colloidal solution wich form mixture of SBF+OVA+ AgNPs, the spectrum showed peak at 343.5 nm with absorbance 1.298 and another peak at 206.5 nm with absorbance 2.032, the measurment prove that the peak of fluid was shifted from 201.5 in (Figure 1) to 206.5 nm and the second peak was shifted from 349.5 in (Figure 2) to 343.5 nm, also another peak was shifted from 244.0 in (Figure 4) to 243.5 nm. Also, the peak shrunk and changed to the short wavelength (blue shifting) a sign of formation of AgNPs with smaller sizes, This shifts are solid evidence to the reaction of fluid with silver nanoparticles and oval albumin [Table 3]. Ovalbumin has been used to lower ionic strength and stop nanoparticles from aggregating or agglomerating in biological fluids [19] The nanoparticle-protein complex can therefore be formed when the nanoparticles are solubilized in biological fluids and spontaneously coated with different biomolecules, such as a proteins. The nanoparticle-protein complexes are stable in biological fluids for a period of time ranging from hours to days. Additionally, The Nanoparticles are further prevented from aggregating or agglomerating by the proteins that coat their surface. Since a surfaces chemistries for the nanostructures play a major function to the development of nanoparticle-protein complexes, it is crucial to find out whichever surface chemical is most conducive for the creation of the compound [20].

Table 3. Wavelength and absorbtion to uv-vis spectrum in colloidal solution SBF+OVA+AgNPs (21).

No	Wavelength nm	Abs.
1	343.5	1.298
2	243.5	1.135
3	206.5	2.032

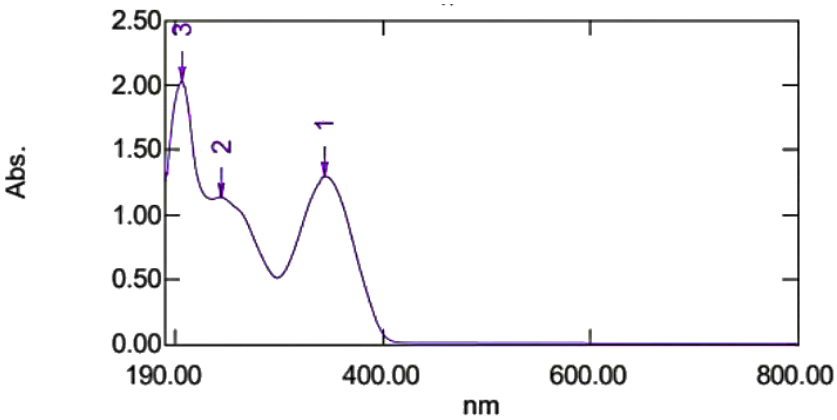

Figure 6. This diagram illustrated the UV-visible spectrum to wavelength and absorabtion in AgNPs at present SBF+OVA [21].

The measurement of the sample's wavelength (AgNPs, SBF and OVA) over a period of time for amonth. Observe that how wavelength changed slightly after 20 days, but returned to the original wavelength after 30 days, This demonstrates the sample's high degree of stability note that in the Table 4 and Figures 6–8).

Table 4. The measurment of wavelength to the AgNPs solution in a SBF with OVA for a mounth (22).

Wavelength after 10 days	Wavelength after 20 days	Wavelength after 30 days
205.5	212.0	204.0
243.0	241.5	244.0
345.0	332.0, 346.0	343.0

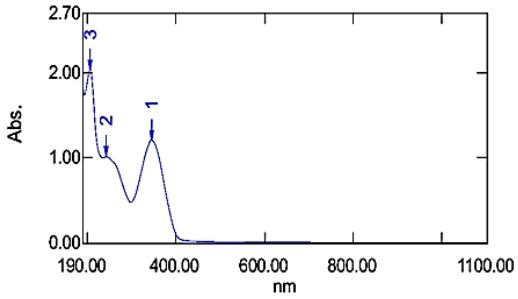

Figure 7. Illustrates the wavelengths and absorption in AgNPs after ten days at the present SBF+OVA concentration for the UV-visible spectrum [22].

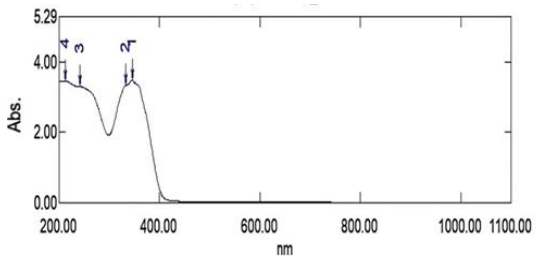

Figure 8. shown the wavelengths and absorption in AgNPs after 20 days at the present SBF+OVA concentration for the UV-visible spectrum [22].

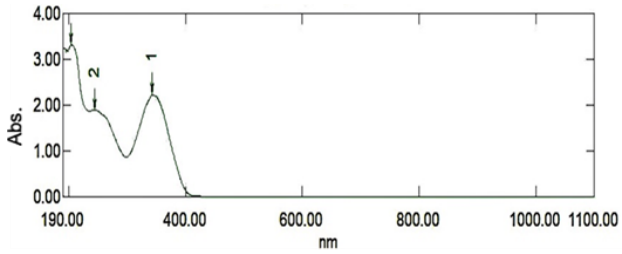

Figure 9. Appear the wavelengths and absorption in AgNPs after 30 days at the present SBF+OVA concentration for the UV-visible spectrum [22].

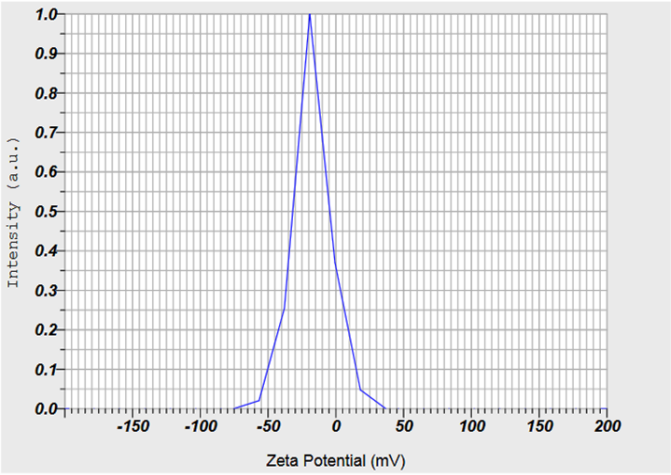

Figure 10. z_{ζ}-potentials for colloidal solution of AgNPs [23].

Zeta Potential of AgNPs

ZP proves that colloidal nanoparticles are stable. Figure 10 display the zeta potential values of AgNPs at 2000 mj laser power and 1300 pulses which was -17.2 mV the larger negative values in the colloidal solution represent more stable NPs

Fourier Transform Inferared Spectroscopy

FTIR used for studying vibrational motion of atoms and molecules, so it is used to identify functional groups. A given frequencies of IR light absorbed by each molecule depending on its characteristics. [Figure 11] Also its qualitative and quantitative method because it identifies the molecule and its amount in the sample [24] Fourier transform infrared spectroscopy of colloidal solution Ag NPs have been recorded in the range of wavenumber (400-4000) cm^{-1}, using FTIR model Shimadzu, 800 series, Japan [Figure 12]

In the just SBF the FTIR appeared a large broad peak at 3251.98 recognized that this OH^- and strong hydrogen bond. however, a presence medium sharp peak also a weak small peak at 1631.78 and 2144.84 indicate in to double and triple bound respectively, also have a single bond [25].

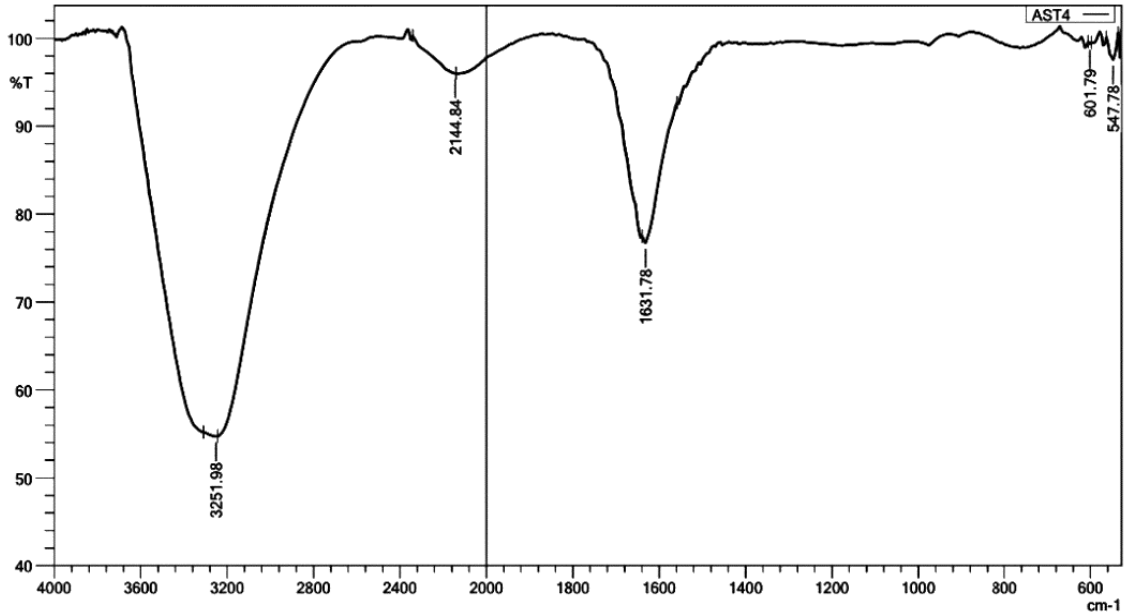

Figure 11. FTIR graph to simulated body fluid (26).

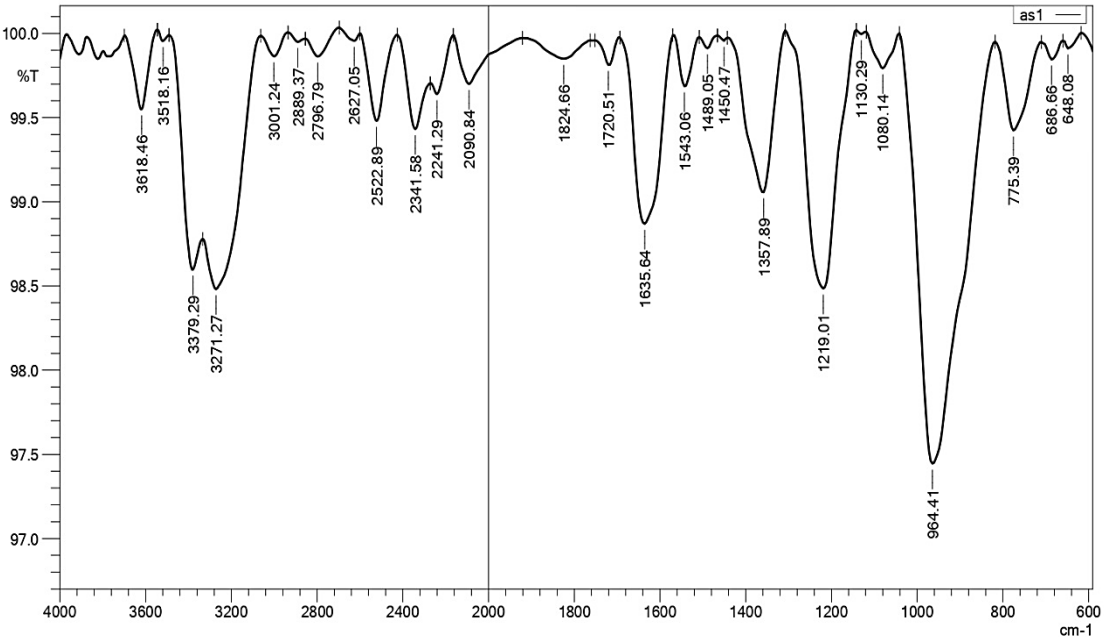

Figure 12. FTIR graph to colloidal solution of AgNPs [26].

In this case of colloidal solution to AgNPs several peaks of vibrations occurred between wave number 600-4000 cm^{-1}, This is also the solid evidence to the presence of AgNPs and their reaction it with ovalbumin and SBF. The large sharp peak at 964.41 cm^{-1}, small sharp peak at 775.39 also the medium peak and small sharp peak at 1219.01 and 1357.89 respectively indicate to single bond [26]. However, The broad peak at 3379.29 and 3271.27 indicate to the presence OH-COOH Carboxylic acid, which has OH^- a strong hydrogen bond, so there are also many small sharp and wide peaks to indicate the existence many combounds and bonds [26].

Transmission, Electron, Microscopy

The TEM microscope approach enables us to examine tiny objects (mostly on scale of a few nanometres to 100,nm) via focusing a narrow electron, beam, upon a sample. When an electrons, beam travels through, a medium, a portion of the electrons may be dispersed dependent on how dense the material was. a picture of the material would be created when the fixed electrons strike a light source in at base of the microscopy. In order to determine the sizes, size diffusion and forms of the Silver nanoparticles, TEM utilizing Image J processing, software was used. So the silver nanoparticles were round in forms, with typical dimensions lower than, 20 nm. Since these variables have a direct impact on the cavitation bubble's as well as the plasma's formed by it's process, the effect of laser fluence happens. Larger particles are produced as a result, and the duration of the cavitation bubble before collapsing is extended [27]. When compared to the AgNP generation method using pulse laser ablation in water, this behavior demonstrated good consistency (PLAL) [Figure 13]. Using PLAL the process for the production of NP was identified. It involved predictable stages of nucleation it during cooling of the plasma plume, growth, and cementation.

TEM investigation demonstrated that PLAL produced NPs with the a polycrystalline structure, confirming nucleation, growth, and cementation. Additionally, PLAL in a reacting solvent will not result in the production of pure metal NPs. Finally, PLAL in a stabilizer-containing, pure solution resulted in modest average NP sizes. For particle manufacturing, it is crucial to select appropriate solvents of which the molecules become soluble [29].

Selected area electrone diffraction patterns of the AgNPs generated in the second solution (SBF+OVA+AgNPs), demonstrating their face-centered cubic, crystal structure shows in the Figures 14–15.

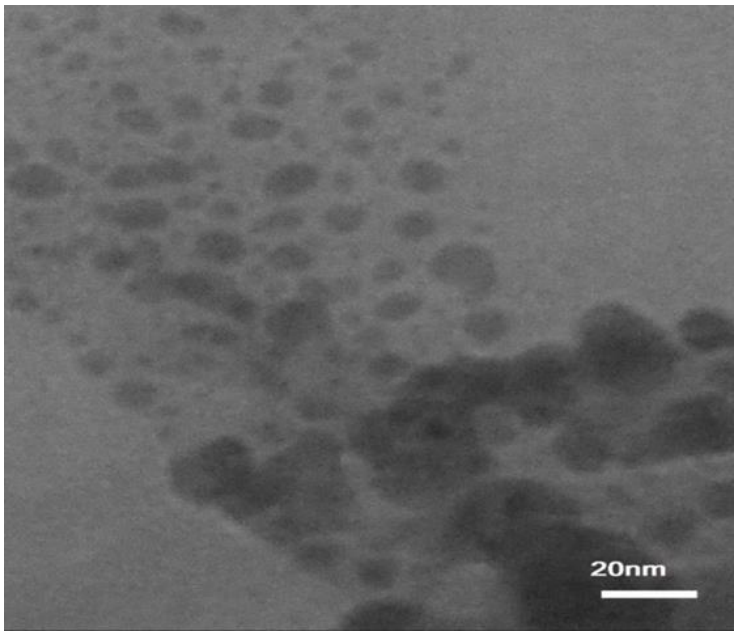

Figure 13. TEM image for AgNPs [28].

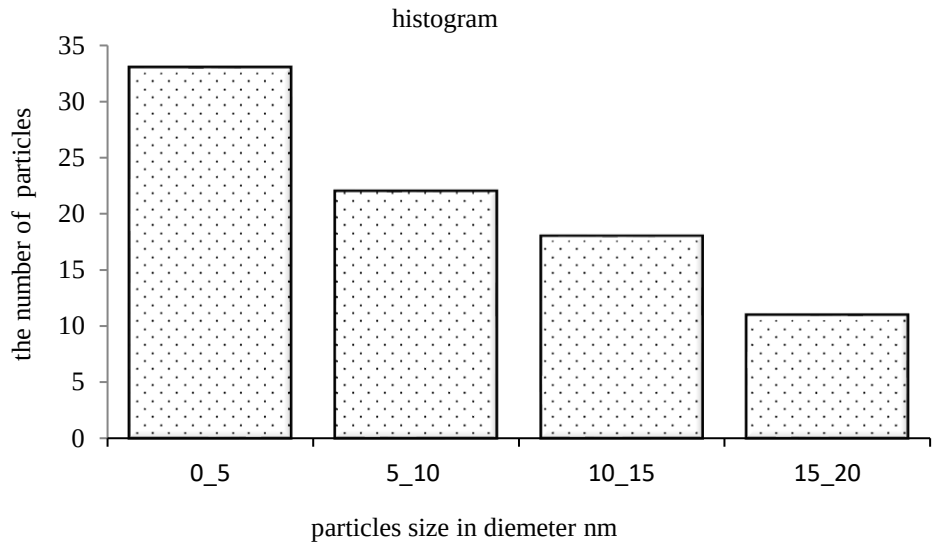

Figure 14. Illustrated transmission, electron microscopy image of AgNPs created inside of an SBF + OVA + AgNPs solutions, in addition to size, distributions of the particles [28].

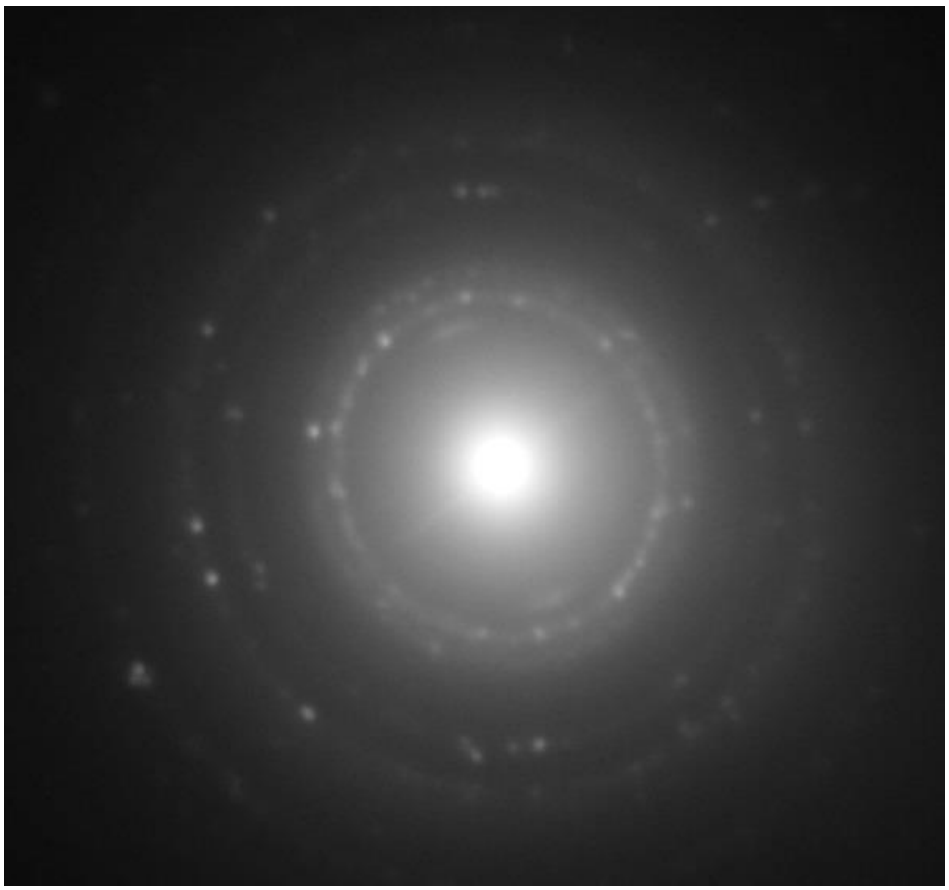

Figure 15. SAED patterns revealing a cubic face structures for AgNPs [30].

Isolation and Identification of *Candida* spp

Cultural Characteristics

As seen in Figure 16 white to cream, spherical, curvy, soft, and smooth with a distinctive yeast odor, all samples were grown on Sabouraud Dextrose agar with Chloramphenicol as a selective media [31].

Figure 16. *C.albicans* colonies were cultivated on SDA at 37°C in 24h [31].

Gram Stain

All *candida* isolates underwent microscopic inspection following gram staining to confirm the presence of *Candida albicans*. Image showed gram-positive, round to oval, with present pseudohypha and budding yeast cells, as shown in Figure 17.

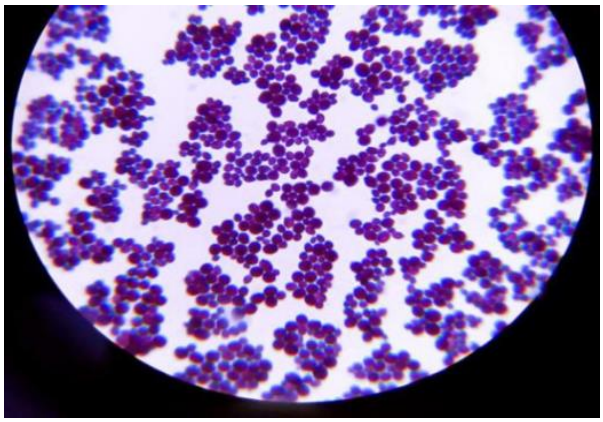

Figure 17. *C. albicans* seen via a light microscope(X40) [31].

Germ Tube Formation Test

Candida albicans isolates produced elongated, and cylinder protrusions at the site of attachment to the yeast cells, known as the germ tube, which indicated good findings (Figure 18).

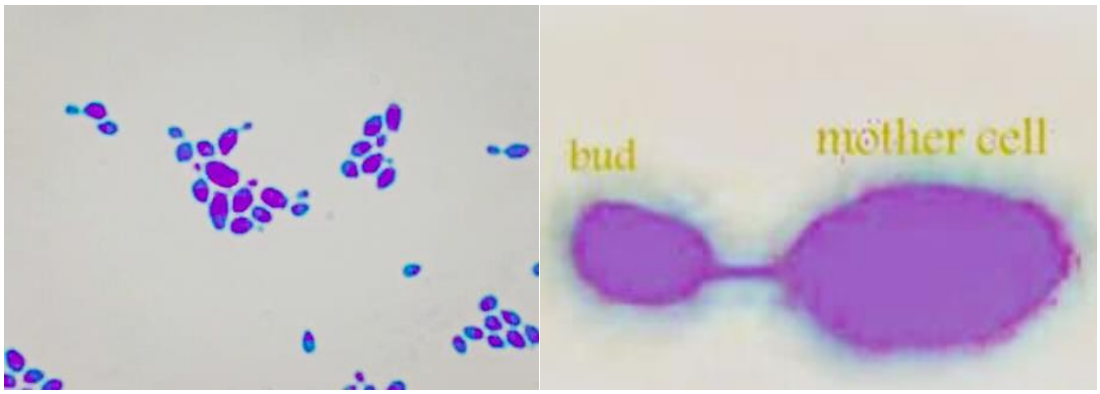

Figure 18. Illustrated germ tube formation in the *C.albicans* (X40) [31].

Well difussion assay

The outcomes of an overnight 37°C incubation demonstrated that the AgNPs solution in synergistic with ovalbumin affected its capacity to inhibit the isolated fungus. But the dilutions of only AgNPs solution no effect on *C.albicans*.

The AgNPs in synergistic with OVA have the ability to make zone inhibition about 14 -16 mm in all cultured isolates show in Figure 19.

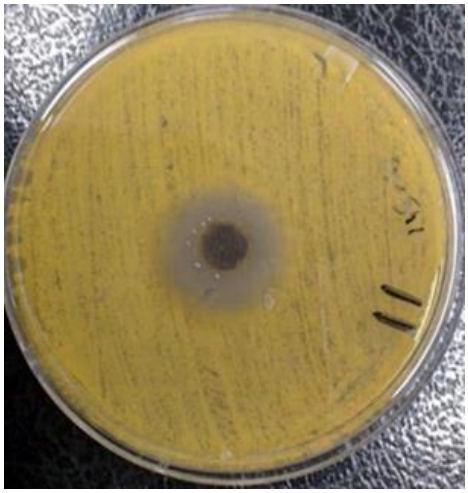

Figure 19. The colloidal of AgNPs solution with ovalbumin.

But the only AgNPs solution and their dilutions without OVA not effect on the cultured isolates of *C. albicans* show Figure 20.

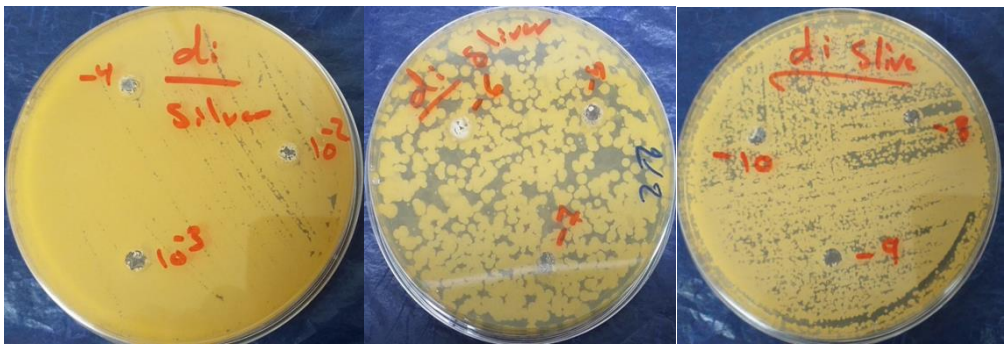

Figure 20. The dilutions of only AgNPs solution without OVA on the cultured isolates of *C. albicans*.

CONCLUSION

In this study were created AgNPs, which showed for being stable in the harsh Cl-anions environment. The outcomes of the study suggest that it is challenging to sustain colloidal AgNPs for an extended period in a solution which contains only NaCl. In these kinds of harsh environments, it was discovered that adding OVA to the NaCl solution was an efficient way to produce stable AgNPs. It is notable that when OVA was attached onto the AgNPs, a considerably increased colloidal stability was obtained. This observed activity were due to the formation of a proteins thin layer on over AgNP cores,The most important finding of the current study is that the technique suggested can produce steadiness over time for this medium, that might which will creation to slimy AgNPs which seem to be bioavailable for different components as well as utilized for biotechnology, energy science also a medical, techniques. In addition, a saline solution stopped the AgNP aggregating. Consequently, it is anticipated that the findings of this study will be useful for various biological research. To a greatest of our knowledge, this work uses laser ablation to manufacture AgNPs in a harsh environment. The

approach used in this work saves time and requires fewer preparatory procedures, resulting in the single-step production of AgNPs, which meant that the sample preparation was completed in a short period of time. Furthermore, this technique may be used with a few low-contamination substances in ambient circumstances. As a result, the suggested strategy is useful and simple to implement.

Also, concluded that the AgNPs not effect on *C.albicans* except with a synergistic effect with another solution such as OVA or any antifungal.

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