

## Comparative Effects of Aloe Vera (*Aloe Barbadensis*) Water vs Ethanol Extracts on the Physicochemical Properties and Stability of Silver Nanoparticles Synthesize

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### Abstract

*Production of AgNP using different biological methods is gaining recognition due to their multiple applications. It was hypothesized that ethanol Aloe Vera (AV) extract will produce stable nanoparticles within the 100 nm range. The biological extract and AgNO<sub>3</sub> were blended and heated to synthesize AgNP. The reaction process was monitored using UV-Visible spectroscopy and FTIR was used for the characterization of biological compounds and their substituent groups before and after the reaction process. Dynamic Light scattering method was used to characterize particle size of AgNP and their bio-molecular stability. Results showed that biological compounds such as aliphatic amines, alkenes (=C-H), alkanes (C-H), alcohol (O-H) and unsaturated esters (C-O) has an average particle size of 109 and 215.8 nm and polydispersity index of 0.451 and 0.375 for ethanol and water extract, respectively. The results suggested that ethanol derived AgNP contained higher yield of organic compounds and better solubility power than water.*

**Keywords:** Silver nanoparticles, Aloe Vera, Particle size, Zeta potential, FTIR, Polydispersity, Dynamic Light scattering, Extract, physicochemical

### Introduction

Nanotechnology is a field of modern research and scientific innovation that deals with the synthesis and manipulation of particle structure within the range of 1-100 nm. Continuous miniaturization of information technology and other biological components have provided an impetus for future research strategy for technological advancement leading to innovation in the broader field of Nanotechnology (Groves, Lee, Frater & Harper, 2010). Over the last decade, research in this field has increased dramatically resulting in the fabrication of numerous forms of nano-sized matter due to anticipated applications in medical therapeutics, diagnostics, food safety, energy production, structural materials and etc. (Duncan, 2011). The use of Nanotechnology in every segment of the food industry has been identified by researchers from production to processing, packaging, transportation and storage (Lin, Li, Song & Wu, 2011).

Metallic nanoparticles (NP) have unique optical, catalytic and magnetic properties based primarily on their size and high surface to volume ratio. Silver Nanoparticles (AgNP) are of interest because of their unique properties (e.g., size and shape depending on optical, electrical, and magnetic properties), and have drawn attentions of many researchers due to their suitability of applications in different fields such as, electronics, material science and medicine (Kotthaus, G'unther, Haug, & Schafer, 1997; Klaus-Joerger, Joerger, Olsson & Granqvist, 2001). Silver NP has potential antimicrobial effects against infectious organisms such as *Escherichia coli*, *Bacillus subtilis*, *Vibrio cholerae*, *Pseudomonasaeruginosa*, *Syphilis typhus*, and *Staphylococcus aureus* (Cho, Park, Osaka & Park, 2005; Duran, Marcato, de Souza, Alves & Esposito, 2007) which are critical in food safety.

Various physical and chemical methods have been used for the synthesis of stable AgNP. The physical methods include evaporation-condensation and laser ablation of metallic bulk materials in solution (Kabashin, & Meunier, 2003; Mafune, Kohno, Takeda, Kondow, & Sawabe, 2000) while chemical methods involves chemical reduction using organic and inorganic reducing agents, electrochemical techniques, radiolysis and physicochemical reduction (Merga, Wilson, Lynn, & Milosavijeric, 2007; Wiley, Sun, Mayers, & Xi, 2005; Evanoff, & Chumanov, 2004),

There is a growing attention to biosynthesize metal NP using microorganisms and plant based substances. This has recently been referred to as green chemistry; this method is gaining importance because of its cost effectiveness, does not involve the use of toxic chemical and is considered eco-friendly (Vigneshwaranm, Ashtaputre, Varadarajan, Nachane, Paralakar, & Balasubramanya, 2007). Plant substances are suitable to produce more stable NP with various shapes and sizes, and the rate of synthesis is much faster than microorganisms. Furthermore, plant extracts are more suitable for large scale biosynthesis of NP compared to other organisms (Iravani, 2011; Korbekandi, Iravani, & Abbasi, 2009). Several researchers reported the synthesis of stable AgNPs using medicinal plants such as *Aloe vera* (Chandran, Chaudhary, Pasricha, Ahmad, & Sastry, 2006), *Magnolia Kobus*, (Song, Jang & Kim, 2009), *Basella alba*, *Oryza sativa*, *Zea mays* (Leela & Vivekanandan, 2008) and *Medicago sativa* (Alfalfa) (Gardea-Torresdey, Parsons, Gomez, Peralta-Videa, Troiani, & Santiago, 2002)

Aloe Vera has medicinal properties (Barcroft, & Alasdair, 1999). Several researchers have reported Aloe Vera plant (Figure 1) contained over 150 nutritional ingredients with wide ranges of mechanism to act in synergy or individually. It contain about ten major chemical constituents such as amino acids, anthraquinones, enzymes, minerals, vitamins, lignin, monosaccharide, polysaccharides, salicylic acid, saponins, and sterols (Barcroft, & Alasdair, 1999). In addition, Aloe vera contains vitamins A, C, and E and minerals such as zinc, and selenium which helps in boosting the immune system and combat free radicals in the body (Barcroft, & Alasdair, 1999).

Okafor, Janen, Kukhtareva, Edwards & Curley (2013) applies kinetic model to track the formation of NP over time. They detected that the formation of NP at absorption peak of approximately 413 nm for AgNP while Mewada, Pandley, & Oza (2013) at 420 nm. Particle size and surface properties are parameters of significance to the understanding the structure and stability of NP. Kuponiyi, Kassama, & Kukhtareva (2014) used the Dynamic light scattering (DLS) method to characterize the hydrodynamic characteristics of AgNP. Result suggested that ethanol derived AgNP contained higher yield of organic compound, because they are easily dissolved in organic solvents due to it high solubility power. It has a lower particle size and better polydispersity index than water. Although, the polydispersity index of ethanol is 0.451 and 0.375 for water, both are less than 0.5 which falls within the acceptable limits (ISO, 1996; 2008; Dahneke, 1983).

Characterization of biological compounds and its substituent groups with Fourier Transform Infrared Spectroscopy (FTIR) provide profile of functional groups in AgNP solution (Rajendran, Natrajan, Siva Kumar, & Selvaraj, 2010). This technique has been used to characterize silver and gold NP and their associated molecules from plant derivatives from various studies (Duran, Marcato, de Souza, Alves, & Esposito, 2007; Chandran, Chaudhary, Pasricha, Ahmad, & Sastry, 2006; Shankar, Ahmad & Sastry, 2003; 2004; 2005; Armendariz, Herrera, Peralta-Videa, Jose-Yacaman, Troiani, & Santiago, 2004; Gardea-Torresdey, Parsons, Gomez, Peralta-Videa, Troiani & Santiago, 2002; 2003). The objective of this study was to compare the effect of AgNP using Aloe vera water against ethanol extract used as a reduction agent in the synthesis of stable NP and to characterize their biological and physicochemical properties.

## **Materials and Methods**

### **Sample Preparation**

Aloe Vera leaves were collected from Alabama A&M University Greenhouse in July, 2014. The leaf surfaces were cleaned thoroughly with ethanol to remove traces of soil, dirt, and other debris. The parenchymatous (skin) of the leaves were separated from the gel of the plant by using an ethanol-sterilized surgical blade. Subsequently, 10 grams of the skin was weighed on a scale for the water and ethanol extraction. Samples were ground to increase the surface area using a small coffee grinder.

### **Ethanol and Water Extraction Biological Components**

Ethanol (50 mL) was added into a 250 mL of Erlenmeyer flask for preparation of ethanol extract and another 50 mL of W5-4 HPLC water grade was added to another 250 mL of Erlenmeyer for the preparation of the water extract. Each weighed sample was added to each flask containing water or ethanol. The flask with water and ground leaves was boiled for 5 min. Both flasks were allowed to sit overnight to complete extraction process.

### **Biosynthesis and Characterization of AgNP**

The extracts were filtered using Whatman filter paper 125 mm. The filtered extract was used as a reducing agent for the synthesis of the AgNP. The AgNP synthesis was optimized by stirring a mixture of 0.011 grams of silver nitrate, 50 mL of W5-4 HPLC water grade and 3 mL of ethanol extract and heated at 75°C. The same procedure was applied to the water extract. The synthesis was done in a dark room to avoid Ag from absorbing light. The AgNP synthesized with ethanol extract is referred to as AVEE and water as AVWE.

### **Size Reduction via Laser Illumination**

Concentration of the produced AgNP were varied at different ppm by exposing the solution to laser illumination (Continuum Electro-optics Inc. USA) at different times which produced a more sharper absorption peak with a UV-Vis. Furthermore, laser was used in an attempt to increase uniform particle sizes and particle size distribution.

### **Physicochemical Characterization of AgNP**

#### **Reaction Kinetics UV-Vis Spectrophotometer**

The resultant AgNP were characterized with conventional UV-Vis spectroscopy (Cary 3E UV-Vis, Varian Pty Ltd. Australia). Samples of 2 mL of the AgNP synthesized were pipetted in a quartz cuvette to measure the absorption of colloidal suspension (hydrosol) of AgNP and detection and the measurement of the surface Plasmon resonance property was recorded.

#### **FTIR Spectra Determination of Functional Groups**

Approximately (0.0016 g) of water and ethanol extract were dropped on FTIR disc (Real Crystal IR Card, 9.5 mm aperture, International Crystal Labs, NJ). The cards were inserted into the Nicolet FTIR spectroscopy. Scanned samples passed through an infrared beam, with the detector connected to a computer (Thermo Fisher Scientific Smart Omni transmission, Madison, Wisconsin, USA) that displays the sample spectrum, hence the absorbance plot against the wave number.

#### **Particle Size Distribution and Stability Measurements**

Dynamic Light Scattering technique was used to gain an insight on the particle size distribution of the produced AgNP. The Zetasizer Nano Series (ZEN 3690, Malvern Instrumentations Ltd, Worcestershire, UK) is the premium system in the Zetasizer range. AgNP synthesized were centrifuged to remove excess reducing agents before performing particle size and stability test. Three mL of the AgNPs synthesized was placed in a quartz cuvette, and measurements were taken by intensity and by volume. The zeta potential surface charge of AgNP was measured to determine the stability of AgNP's using Zetasizer (ZEN 3690, Malvern Instrumentations Ltd, Worcestershire, UK).

### **Results and Discussion**

Biological compound from AV was extracted with solvents (ethanol and water) for this research. Water and Ethanol are protic polar solvents and have a dispersion, polar and hydrogen bonding index of 15.5, 16, 43.3 and 15.8, 8.8, 19.4, and a specific gravity of 1 and 0.789, respectively.

Although, both are polar, ethanol has better solubility and selectivity, hence had better solvent extraction capacity of fresh AV leaves. The extracts were used as the reduction agent for silver (Ag<sup>+</sup>) into neutral nanoparticles AgNP.

## Physicochemical Properties

### Optical properties

The effect of AVEE and AVWE as a reduction agent for the synthesis AgNP was by visual observations of the nanosolution as shown in Figure 2. The color of the solutions indicates the formation and presence of NP in the emulsion. The optical properties alters with increase concentrations of NP, hence the color of the nanosolution turned to yellowish brown and then to dark brown. The shift in the optical profile is an indication of the formation of AgNP, thus agrees with other studies of many researchers (Balaji, Basavaraja, Deshpande, Mahesh, Prabhakar & enkataraman, 2009; Mukherjee, Ahmad & Mandal, 2001). The appearance of dark-brown color seems to be due to the excitation of surface Plasmon resonance in the NP. Ethanol extracts gave the best results of AgNP production compared to the water extracts. The absorption peak of AVEE was higher than the AVWE and likewise the color of the synthesized AgNP was more distinct, which is indicative of high NP concentration as will be shown in the later discussions.

### Kinetics of NP Formation

The kinetics of reaction during the formation of nanoparticles was monitored with a UV-Vis spectroscopy. The optical absorption of colloidal silver was used as an indicator for the formation of AgNP in the nano-emulsions. Many researchers have used it as a marker for the synthesis AgNP (Kuponiya et al., 2014; Kpiravani, 2011; Korbekandi, Iravani, & Abbasi, 2009). A UV-Vis absorption of colloidal Ag occurs at wavelength (430 nm), hence was used to verify AgNP spectrum as shown in Figure 3ab. The reaction kinetics test shows reaction was complete after 10 and 60 min hence gives the best absorption peak, while the peak offset reflects variation in concentration. Thus, the resulted shift in absorbance was observed at 0 and 5 min reaction time for AVEE, and the variation of absorbance was observed in the AVWE peaks at 5, 10 and 60 min and are more unified compared to the AVEE. During the reaction period, an increase in absorbance was observed at 413 nm wavelength, thus attributed to the increase in the concentration (30 ppm) of colloidal AgNP synthesized with AVEE, while the AVWE showed a lower concentration (18 ppm) (Figure 4b). Ahmad, Senapati & Khan (2003); Basavaraja, Balaji, Lagashetty, Rajasab, & Venkataraman (2008) reported similar observations. The reverse was the case, i.e., reaction rate and absorption decreases with time. Although, the spectrum indicated that AVEE synthesized AgNP were fully formed in 10 min, but a peak shift was observed. In contrast AVWE was fully developed in 5 min, although AVEE resulted to higher concentration of AgNP. This is an indication that AVWE may not be a good reducing agent for synthesizing silver nitrate into high concentration of AgNP.

### Fourier Transform Infrared-red (FTIR) Characterization of Functional Group

The FTIR measurements were carried out to determine the bio-molecular profile of the AgNP, and the FTIR spectrum is shown in Figure 4ab and also compiled Table 1&2. The FTIR bands were used to characterize the spectrum peaks that are associated to different biological compounds. Phytochemicals such as aliphatic amines, carbonyl, *alkenes* (=C-H), *alkanes* (C-H), *alcohol* (O-H) and *unsaturated esters* (C-O) are biological compounds present in the inmost part of plant leaves. Fourier Transform Infrared spectrum obtained from the AgNP synthesized with AVEE and the AVWE is shown in Figure 4a. The FTIR profile of AVEE synthesized AgNP solutions show similar peaks as the AVWE thus indicative of consistent biological compounds. Rajendram, Natrajan, Siva Kumar & Selvaraj (2010) detected the functional compounds in AV skin at the following wavenumber 611.4; 717.5; 1051.1; 1398.3; 1623.9; 1730.0; 2912.3; 3155.3 and 3398.3 cm<sup>-1</sup> using FTIR. The stretch of AVEE synthesized AgNP were found ca 500-550 cm<sup>-1</sup> (Table 1). Although AVWE shows similar trend as shown in Figure 4b and Table 2, however, no band stretch was observed in the 500-550 cm<sup>-1</sup> region, thus confirming the absence or non-pronounced nanoparticles.

The AgNP were surrounded by proteins and metabolites which are present in AVEE. The FTIR analysis confirmed that the carbonyl groups from the amino acid residues and proteins has the stronger ability to bind metal and therefore the proteins could be responsible for the capping of AgNP to prevent agglomeration and hence formed a stable emulsion. This suggests that the biological molecules could possibly perform dual functions thus contribute to the formation and stabilization of AgNP in aqueous medium (Tamasa & Suman, 2013). The presence of the carbonyl groups proved that flavonoids or terpenoids are absorbed on the surface of metal NP. This could be verified by further fractionating the AVEE to identify the individually assayed for reduction of the metal ions.

### **Particle Size Distribution and Stability of NP**

The particle size distribution (PSD) by volume of AVEE and AVWE synthesized AgNP are shown in the Figures 5ab. Figure 5a shows that about 94% by volume of the measured colloidal solution of AgNP contains particles of different sizes, the hydrodynamic diameter ranges from 15 to 100 nm and 100 to 1000 nm for AVWE synthesized AgNP. In comparison, the AVEE based AgNP produced NP within the size range of 1-100 nm while AVWE based AgNP shows relatively low concentrations of AgNP or most likely the absence of AgNP. Figure 5ab show a Polydispersity index of AVEE as 0.451 and 0.375 for the AVWE, both are less than 0.5 which falls within the acceptable limits (Pecora, 1985). The polydispersity index is a measure of the width of molecular weight distributions (MWD), it explains the degree of aggregation in the particles. The larger the value, the more cluster formation is expected and the closer the value to zero denotes monodispersity behavior. Polydisperse particles in solution have greater tendency to aggregate than monodisperse.

### **Laser Illumination of AgNP**

The AVEE and AVWE synthesized AgNP solutions were exposed to laser illumination (LI) for 5min and results of the effect on the PSD are shown in Figure 6ab. The effect of LI on the AVEE shown in Figure 6a appears to introduce bimodal behavior, indicative of the formation of a cluster. The PSD shows two ranges of aggregations from 9 to 30 nm and 30 to 100 nm (Figure 6a). While the AVWE shows a shift 60 to 200 nm and 200 to 1000 nm (Figure 6b). The post laser treatment seems to reduce the bigger particle molecular bonds thus reducing the particle sizes as shown by the values extrapolated from Figure 5 and 6.

### **Zeta potential analysis**

Nanoparticles are relatively small in sizes for which they are energetically very unstable. Therefore, as the particles undergo Brownian motion, colloids tend to balance between the attractive van der Waals' forces and the electrical repulsion due to surface charge. If the zeta potential falls below a certain level, the colloids tend to agglomerate/aggregate due to the attractive force. The potential charges on the surface of the particles reflect their stability. The electric potential at the boundary of the double layer is known as the Zeta potential, which was determined and shown in Figure 7ab. The Zeta potential measurements of AgNP synthesized with AVEE and AVWE were 11 and 12 mv, respectively. The values obtained in this research does not fall within the range of +15 and -40mV, thus according to Malvern (2011) is not stable. They reported zeta potential of stable NP with the range of +25 or -25 mV for typical high degrees of stability. This is a grey area that requires further investigations

### **Transmission Electron Microscope Analysis**

Transmission Electron Microscopy (TEM) provided further insight into the morphology and size details of the AgNP. The comparison of microgram showed that the diameters of AVEE and AVWE synthesized AgNP solution sizes varies in aggregation of clusters (2, 6, 8, 10, and 27 nm) and (5, 147 and 181nm), respectively as shown in Figure 8ab. The smallest sizes of NP were observed in the AVEE synthesized AgNP solutions compared to AVWE. Hence, the size variation in the nanosolution is shown in the DLS spectrum by the polydispersity index.

### **Conclusion**

The rapid biological synthesis of AgNP using AV leaves extract provides a simple and efficient route for synthesis of benign NP. The synthesized NP was of spherical shape and the AVEE synthesized AgNP produced smaller particle sizes compared to AVWE AgNP. Characterizations using UV-vis spectrophotometer, TEM, DLS, Zeta Potential Analyzer, and FTIR techniques have proved that AVEE gave the best AgNP in regards to particle sizes (2 nm), functional group (band stretch of 500-550 $\text{cm}^{-1}$ :C-O stretch (aliphatic esters)), formation time (10 min), higher concentration (30 ppm) and stability of particles. The PSD shows two clusters of aggregations from 9 to 30 nm and 30 to 100 nm for AVEE synthesized AgNP and 60 to 200 nm and 200 to 1000 nm for AVWE. The post laser treatment is proven to reduce the bigger particle molecular bonds thus reducing the particle sizes, hence this methods is opened to further investigation.

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**Table 1: Data of FTIR Characterization of AgNP Synthesized with AVEE**

Aloe Vera Ethanol Extract		AgNP Aloe Vera Ethanol Extract	
Wave number	Functional Groups	Wave number	Functional Groups
535.5	C≡C-H:C-H (alkynes)	586.1	C≡C-H:C-H (alkynes)
		688.1	C≡C-H:C-H (alkynes)
1005.9	C-O stretch (aliphatic esters)		
		1043.9	C-N stretch (aliphatic amines)
1158.5	C-N stretch (aliphatic amines)	1158.5	C-N stretch (aliphatic amines)
1247	C-N stretch (aliphatic amines)		
1323.7	N-O symmetric stretch (nitroalkane)		
		1399.6	C-N stretch (aliphatic amines)
1412.3	O-H bend (carboxylic acid)		
1603.6	N-H (amines)	1628.9	N-H (amine)
1743.5	C-O stretch (aliphatic esters)		
2162.5	-C≡C- stretch (alkynes)	2111.9	-C≡C- stretch (alkynes)
2353	C-N???		
2862.1	C-H stretch (alkanes)		
2925.4	C-H stretch (alkanes)		
3357.8	N-H (amines)		
		3433.7	N-H (amine)

**Table 2. Data of FTIR Characterization of AgNP Synthesized with AVWE**

AgNP's Aloe Vera H <sub>2</sub> OExt		Aloe Vera H <sub>2</sub> OExt.	
Wave number	Functional Groups	Wave number	Functional Groups
1043	C-N (aliphatic amine)	1043	C-N (aliphatic amine)
1374.3	O-H bend (carboxylic acids)C-H bending?	1399.6	O-H bend (carboxylic acids) C-H bending?
1641.5	N-H (amine)	1641.5	N-H (amine)
2048.7	Overtone (weak)	2048.7	Overtone (weak)
		2340.4	C-N??
3433.7	N-H (amine)	3433.7	N-H (amine)

**Figure 1: Aloe Vera Plant used for the Extraction**



Figure 2: Images of AgNP solution synthesized with (1) AVWE and (2) AVEE

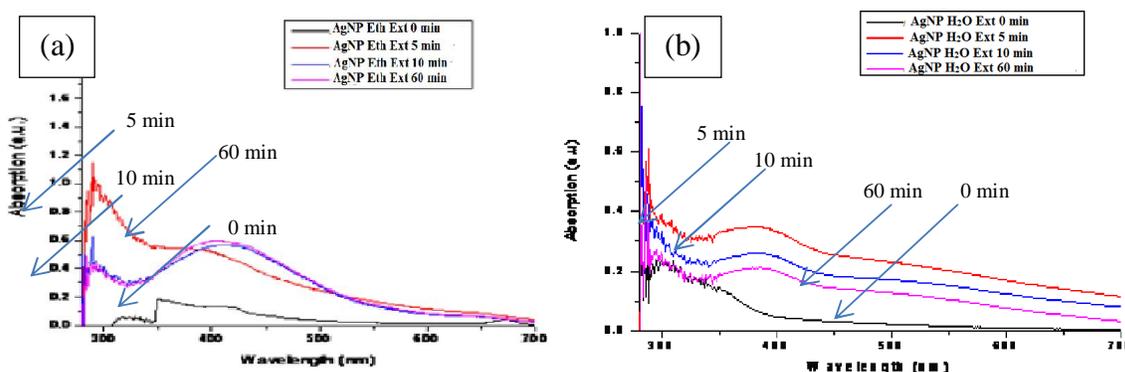


Figure 3: Kinetic of Reaction Process during the Formation of AgNP using AVEE(a) and AVWE (b) of Parenchymatous of Leaf as Reduction Agent

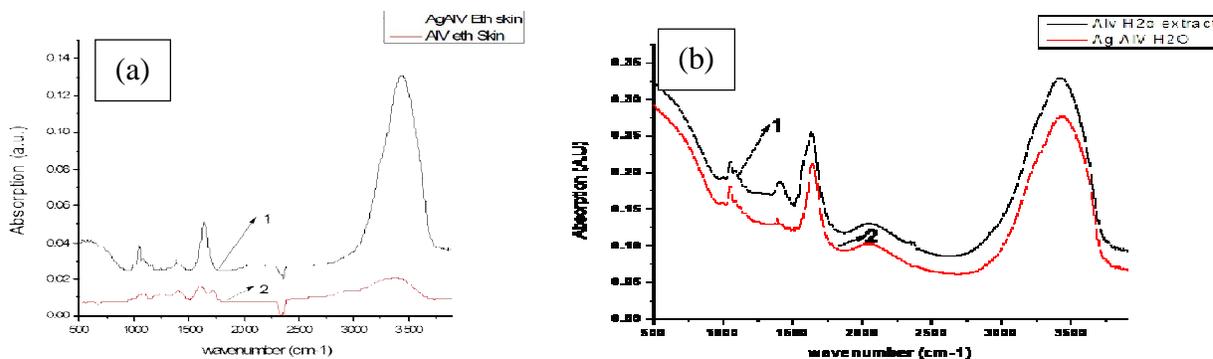


Figure 4: FTIR Spectra of 1. AgNP Nanosolution and 2. Plant Extract Synthesized with AVEE (a) and AVWE (b)

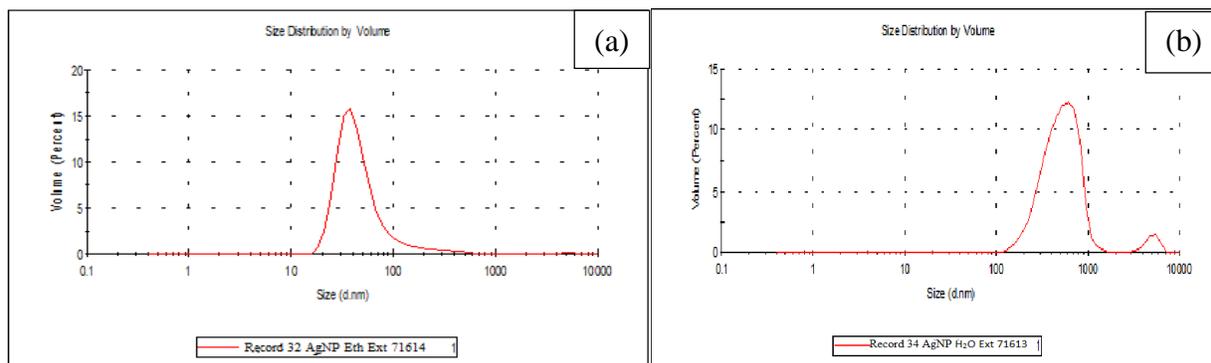
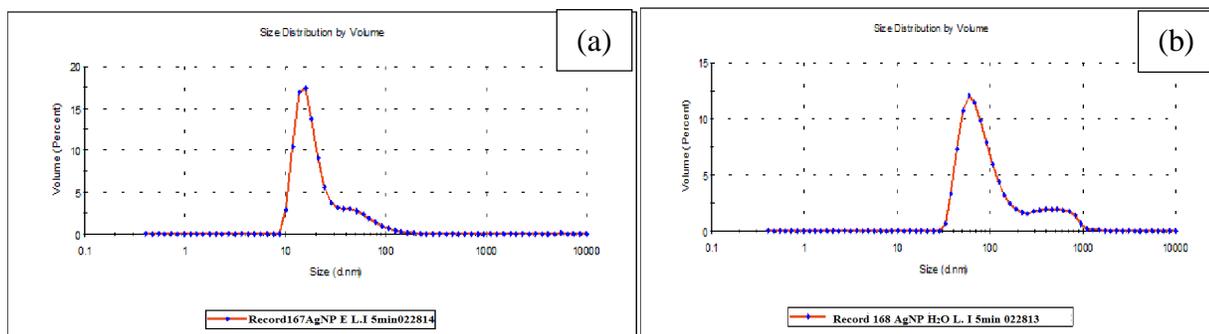
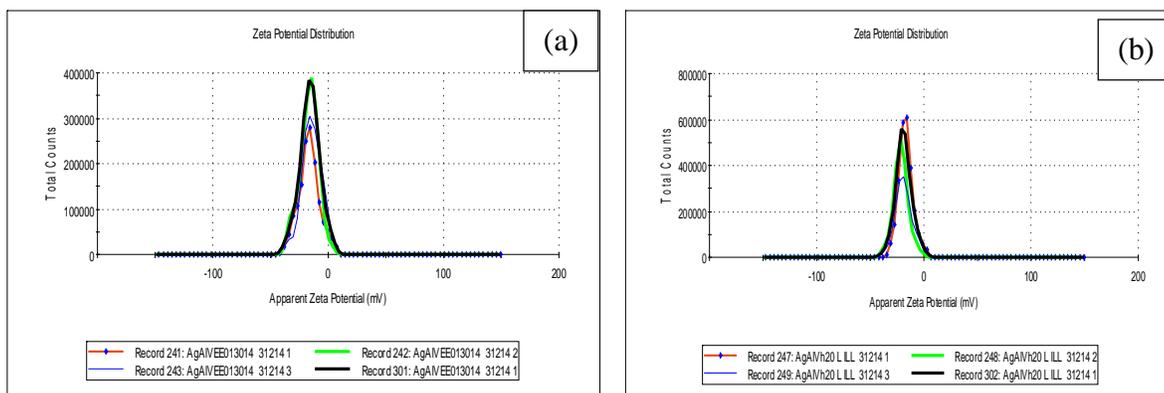


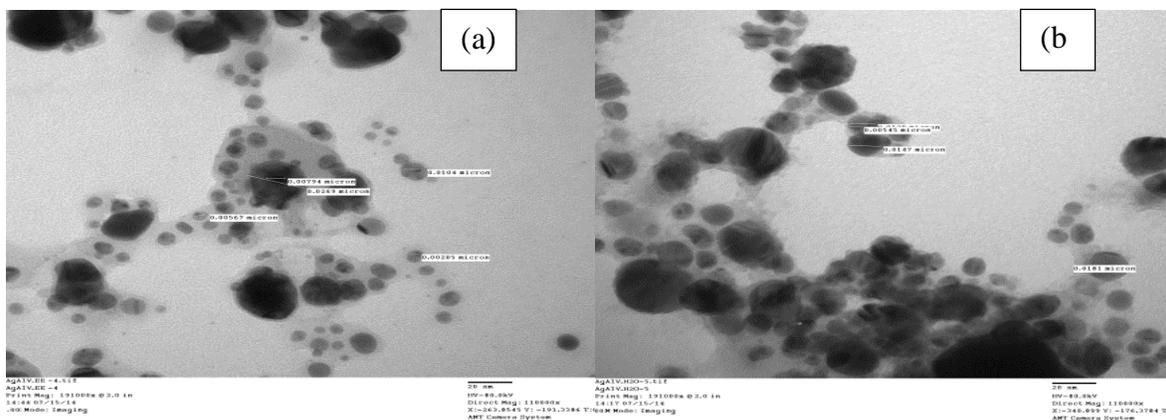
Figure 5: DLS Spectrum Hydrodynamic Diameters for AgNP Synthesized with AVEE (a) and AVWE (b)



**Figure 6: Post-Laser Treatment DLS Spectrum of Hydrodynamic Diameters for AgNP Synthesized with AVEE (a) and AVWE (b)**



**Figure 7. DLS Spectrum of Zeta Potential for AgNPs synthesized with AVEE (a) and AVWE (b)**



**Figure 8: Microgram of TEM image for AgNP Synthesized with AVEE (a) and AVWE (b)**