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Surface-enhanced Raman Scattering (SERS) Substrate of Colloidal Ag Nanoparticles Prepared by Laser Ablation for Ascorbic Acid Detection

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ABSTRACT

Ag nanoparticles were synthesized by laser ablation using an Ag plate in distilled water. This method was performed using a laser with a wavelength of 532 nm and energy of 30 mJ for 60 min. Ag nanoparticles successfully formed, confirmed by the selected area electron diffraction (SAED) which revealed four principal crystal planes of (111), (200), (220) and (311). The size distribution of Ag nanoparticles ranged from 5 to 40 nm, as estimated from electron imaging observed by transmission electron microscope (TEM). Ascorbic acid was used as the analyte to test the characteristics of surface-enhanced Raman scattering (SERS) of colloidal Ag nanoparticles. The concentration of ascorbic acid (1.0, 0.5 and 0.25 wt%) and incubation time (0 and 6 h) were varied to determine the limit of detection and the effect of incubation time. The Raman scattering spectroscopy results showed that the colloidal Ag nanoparticle substrate improved the signals for detection of ascorbic acid.

Keywords: Laser Ablation, Nanoparticles, Silver, Ascorbic acid, SERS

INTRODUCTION

Ag nanoparticles have highly efficient and unique light absorption and distribution properties. The color of Ag nanoparticles depends on the size and shape of the particles. In general, the peak absorbance value of Ag nanoparticles is appeared at a wavelength of 400–500 nm, and as the particle size increases, the absorbance peak progressively shifts to the right (Kamat, Flumiani, & Hartland, 1998). Colloidal nanoparticles produce colors due to localized surface plasmon resonances (LSPRs). LSPR is an optical phenomenon that occurs when a light wave trapped on a conductive nanoparticle smaller than the light wavelength. This phenomenon results in the interaction between light waves and surface electrons in the nanoparticle (Petryayeva & Krull, 2011). The wavelength of the LSPR excitation can be used to determine the size of the nanoparticle (Haiss, Thanh, Aveyard, & Fernig, 2007). The size of the nanoparticles can thus be predicted through their specific LSPR wavelengths (Mulfinger et al., 2007).

Ag nanoparticles have stable properties and potential in various applications, such as in anti-viral compounds (Sharma, Frontiera, Henry, Ringe, & Van Duyne, 2012), drug delivery compounds (Dreaden, Mackey, Huang, & Kang, 2011), therapy (Austin, MacKey, Dreaden, & El-Sayed, 2014) and surface-enhanced Raman spectroscopy (SERS) (Kneipp, Kneipp, Wittig, & Kneipp, 2010).

Nanoparticles synthesis can be performed by top-down or bottom-up methods. Bottom-up techniques, such as chemical methods, have some limitations, e.g., the presence of impurities, resulting in excess precursor residues or unexpected by-products, can lead to decreased control of the size and shape and low stability of the synthesized nanoparticles. Among the various methods developed to overcome these problems, physical methods of nanoparticle synthesis, such as laser ablation, are classified as top-down techniques. Synthesis of nanoparticles with the laser ablation method can be conducted in water or an organic medium to obtain colloidal nanoparticles (Amendola & Meneghetti, 2009).

Ag nanoparticles have various applications. Colloidal Ag nanoparticles have great potential in the development of methods for detecting specific compounds in an unknown material, including SERS substrates (Guicheteau et al., 2013) to increase the sensitivity in detecting an analyte molecule in a sample. Raman scattering is a form of non-elastic interaction between light and material and has become an analytical technique for material identification (Fazio, Trusso, & Ponterio, 2013). Since its discovery in 1970, SERS has continued to develop in both research and industry fields. SERS is a signal enhancement phenomenon in Raman spectroscopy caused by analytical interaction with metal nanoparticles, such as Ag nanoparticles.

The SERS effect of Ag nanoparticle colloids can be utilized in the detection of an organic molecules (Fazio et al., 2013), such as ascorbic acid or vitamin C. Ascorbic acid is an essential compound in the human body, and is required for collagen formation, enzyme activation, and antioxidant properties (Rahmawati & Bundjali, 2012). It can be identified using a Raman spectrophotometer. The Raman spectra of ascorbic acid have several major peaks, identified by Raman shifts at 825, 1299, 1692 and 3480 cm^{-1}. These Raman spectra results were obtained using laser wavelengths of 532 nm and 488 nm (Berg, 2014). Therefore, this study investigated the utilization of the SERS activity of colloidal Ag nanoparticles prepared by laser ablation method for the detection of ascorbic acid.

EXPERIMENTAL SECTION

Ag nanoparticles were synthesized using the laser ablation method in a liquid medium by referring to a previous study (Amendola & Meneghetti, 2009). Laser ablation was performed on a 99.9% silver metal plate (ANTAM) using Nd:YAG nanosecond laser (Q-Smart 850, Quantel) at a wavelength of 532 nm, frequency of 10 Hz, ablation time of 1 h, and distilled water as the solvent. Laser energy of 30 mJ measured by a laser power/energy meter (LabMax-Top, Coherent) was used with ablation set up shown in **Figure 1**. After synthesis, Ag nanoparticles produced were studied by transmission electron microscopy (TEM FEI Tecnai G2 20 S-Twin) with the accelerating voltage of 200 kV for imaging and electron diffraction analysis.

The UV-Vis spectrum was obtained using a UV-Vis spectrophotometer (MayaPro 2000, Ocean Optics). The Raman spectrum test was carried out using a diode pumped solid state (DPSS; Elforlight) with a wavelength of around 532 nm and a detector (Maya2000, Ocean Optics) with the set-up as shown in **Figure 2**. Ascorbic acid solutions of 1.0, 0.5 and 0.25 wt% were prepared as analyte sample. L-ascorbic acid ($C_6H_8O_6$, 99%, Sigma-Aldrich) was used in this study without any prior treatment.

RESULTS AND DISCUSSION

The colloidal Ag nanoparticles used in this study were obtained from laser ablation using Nd:YAG laser. The process of synthesizing Ag nanoparticles using laser ablation methods, shown in **Figure 3**, involved several stages, such as evaporation, nucleation, nuclei growth and coalescence, and nanoparticles growth (Amendola & Meneghetti, 2009).

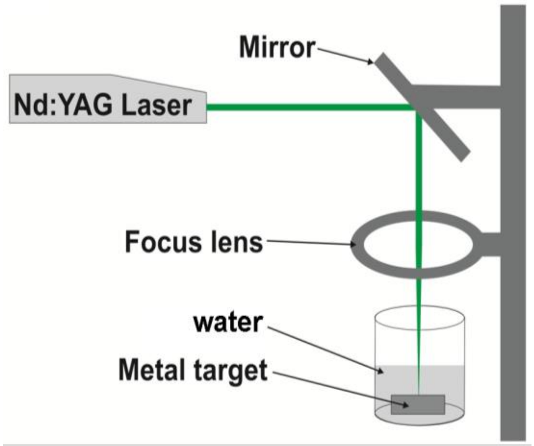

Figure 1. Experimental setup in Ag nano-particle synthesis by pulse laser ablation

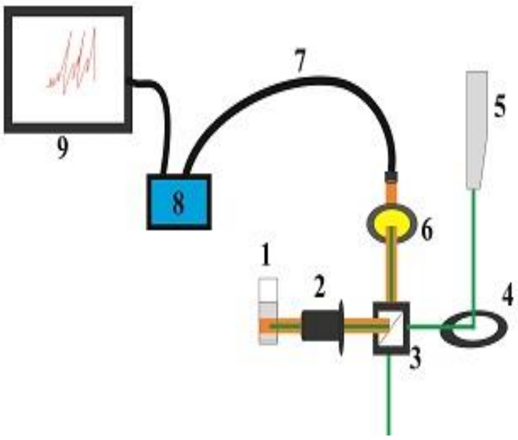

Figure 2. The experimental setup in Raman scattering study: cuvette as sample place (**1**); objective lens (20x) (**2**); beam splitter (**3**); optical mirror (**4**); DPSS laser in wavelength of 530.421 nm (**5**); notch laser filter (**6**); optical fiber (**7**); spectrophotometer (Ocean Optics Maya 2000) (**8**) and computer (**9**).

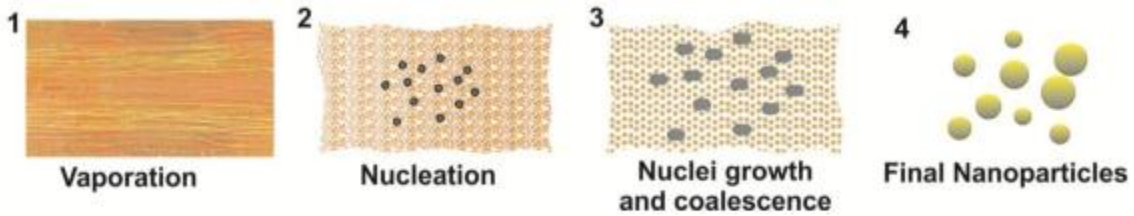

Figure 3. The stages of nanoparticle formation in laser ablation synthesis processing.

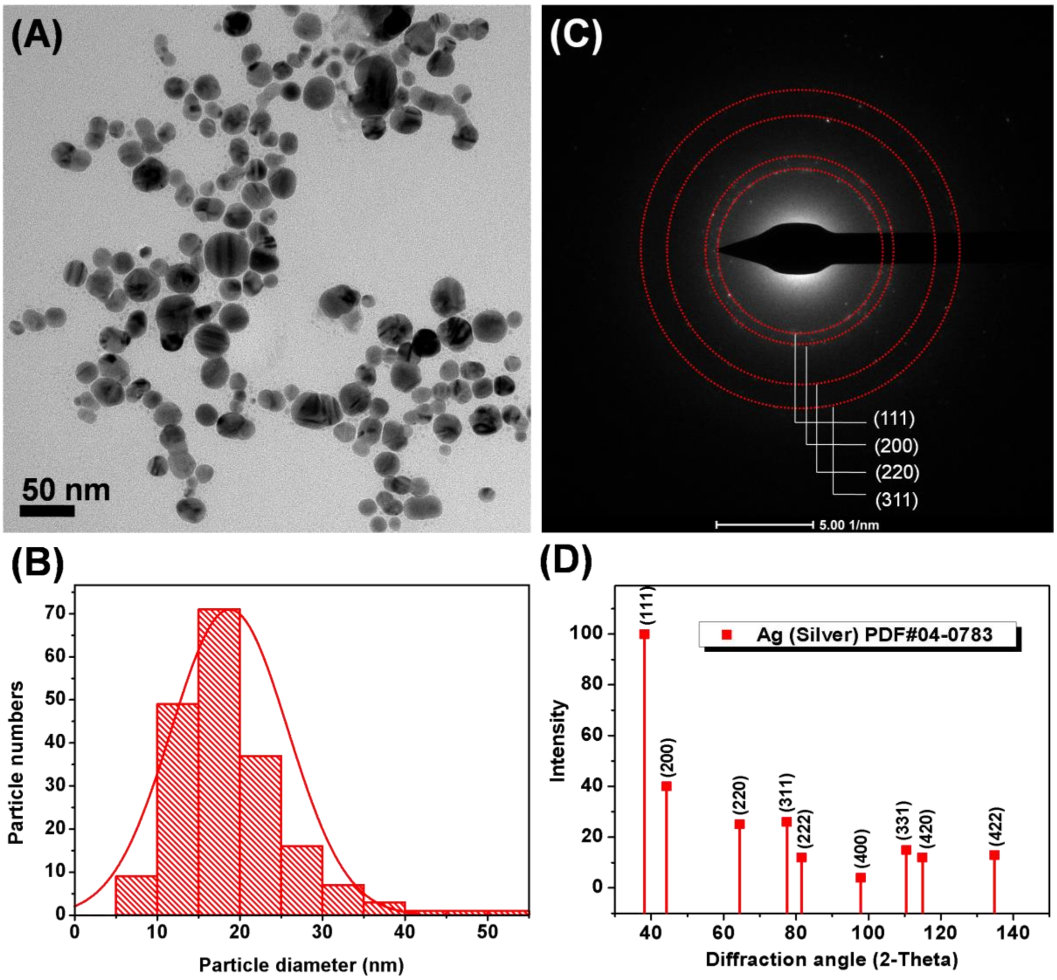

Figure 4. Ag nanoparticles imaging taken by transmission electron microscope (TEM) **(A)**; the histogram of diameter of Ag nanoparticles **(B)**; selected area electron diffraction (SAED) of the Ag nanoparticles in the represented TEM image with diffracted planes **(C)**; confirmed to the X-ray diffraction database of Ag (silver) **(D)**.

To confirm the success of the Ag nanoparticles formation in colloidal sample produced after laser ablation, we used the transmission electron microscope to observe not only the particles size but also the electron diffraction of the particles. **Figure 4** shows transmission electron microscope (TEM) imaging and the size distribution of the observed nanoparticles (**Figure 4**(**A**) and **4**(**B**)); and the selected area electron diffraction (SAED) of the represented TEM image noted by diffracted planes matched with the X-ray diffraction database of Ag (silver) (**Figure 4**(**C**) and **4**(**D**)).

According to the TEM analysis shown in **Figure 4**(**A**) and **4**(**B**), the colloidal sample produced after laser ablation was the Ag particles growth in a spherical shape of nanometer size. The histogram presented in **Figure 4(B)** shows that the Ag nanoparticles have a diameter range of 10-30 nm.

The particles produced in laser ablation were confirmed as Ag particles studied from the selected area electron diffraction (SAED), as shown in **Figure 4**(**C**) and **4**(**D**). Four diffraction ring observed in the SAED picture (**Figure 4**(**C**)), represented to the four principal diffracted planes of Ag (111), (200), (220), and (311) according to the X-ray diffraction database of Ag (**Figure 4**(**D**)). We confirmed these four crystal planes by calculating the lattice spacing (*d*) interpreted from the SAED ring pattern with a camera length of 680 mm, and electron wavelength of 0.0027 nm (at 200kV accelerating voltage). The calculated *d* spacings of the four planes were 0.2359, 0.2051, 0.1448 and 0.1219 nm for crystal planes of (111), (200), (220), and (311), respectively, which were close to the *d* spacing of Ag (silver) in X-ray diffraction database.

The confirmation of Ag nanoparticles formation was also performed by UV-Vis spectrophotometer to study their plasmon excitation spectra, as presented in **Figure 5**. The synthesized colloidal Ag nanoparticles produced after laser ablation was in a greenish fluid, shown as the inset in **Figure 5**. The UV-Vis spectra analysis showed that the colloidal Ag nanoparticle exhibited LSPR spectra with a peak wavelength of 412 nm. This result is consistent with previous research (Bakir, 2011) stating that Ag nanoparticles exhibit LSPR excitation wavelengths between 400 and 500 nm. Moreover, since the obtained peak was located at a wavelength of 412 nm, it can be predicted that the colloidal Ag nanoparticles have a diameter less than 40 nm (Mulfinger et al., 2007) which are in a good agreement with the particle size distribution estimated from the imaging analysis observed by TEM, as shown in **Figure 4**(**B**).

After Ag nanoparticles formation was well confirmed, the study was continued to the Raman scattering study. The colloidal Ag nanoparticles obtained were used as the SERS substrate for the detection of ascorbic acid. The ascorbic acid solution with Ag nanoparticles was tested using a Raman spectrophotometer, and the results revealed that the Raman scattering was more powerful when Ag nanoparticles were added to the ascorbic acid solution.

A schematic of the Raman scattering test shown in **Figure 6** illustrates a comparison of ascorbic acid detection with and without Ag nanoparticle addition. Raman scattering of ascorbic acid with Ag nanoparticles addition revealed more intense Raman scattering compared to those without Ag nanoparticles addition. Ascorbic acid will be adsorbed on the surface of Ag nanoparticles resulting in enhanced Raman scattering.

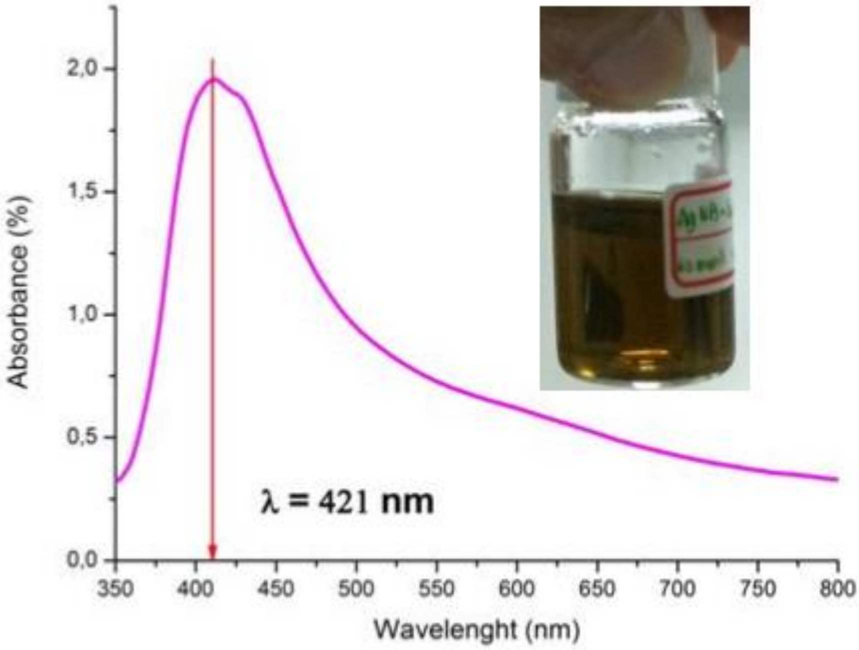

Figure 5. UV-Vis spectra of Ag nanoparticles after laser ablation; Inset: colloidal Ag nanoparticles produced by laser ablation.

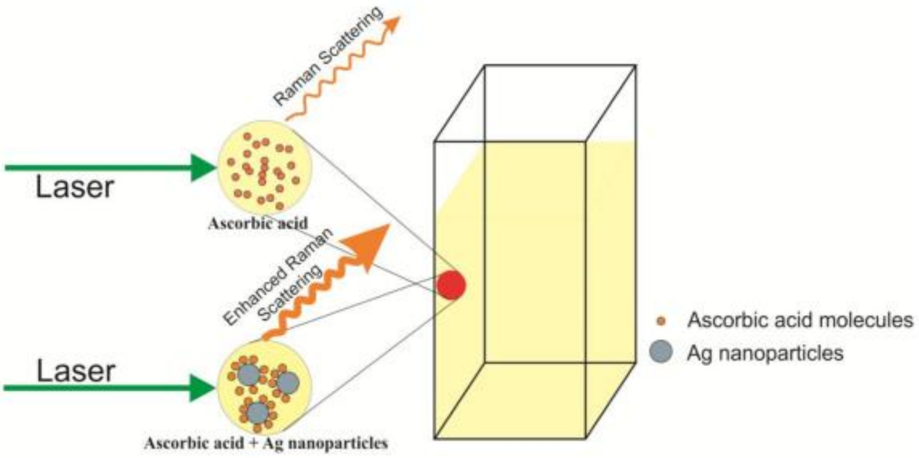

Figure 6. Illustration of the SERS effect due to the addition of Ag nanoparticles in ascorbic acid solution.

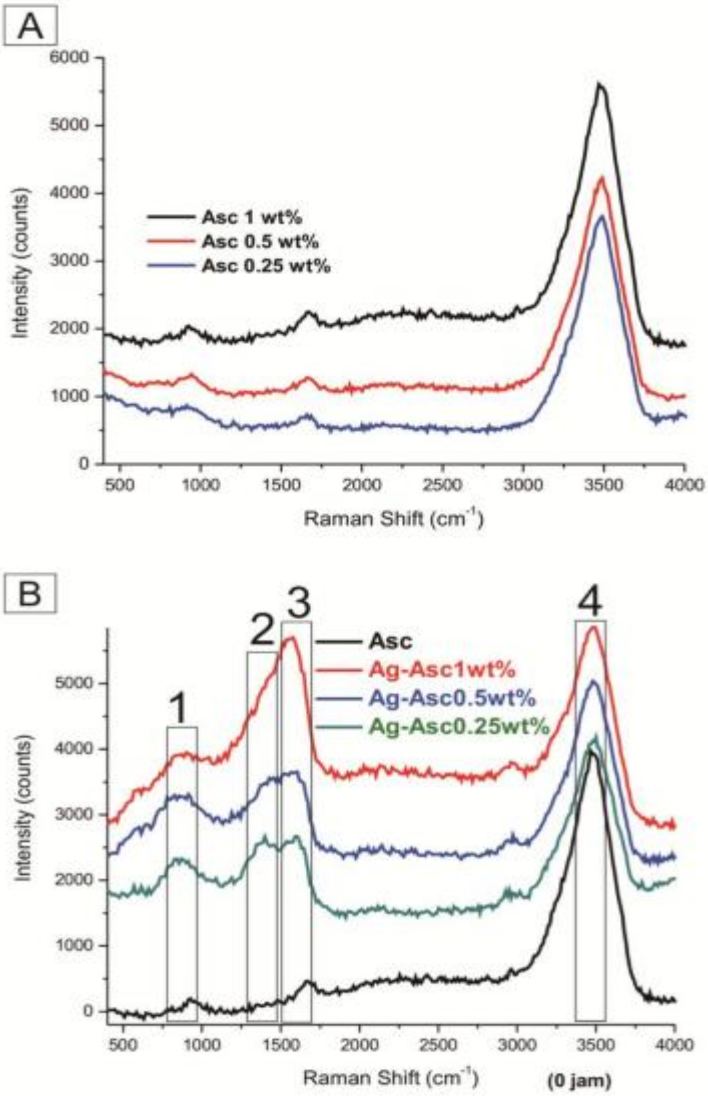

Figure 7. Raman shift spectra of ascorbic acid at various concentrations without (**A**) and with **(B)** colloidal nanoparticles added (Asc = ascorbic acid; Ag = Ag nanoparticles; numbers 1–4 indicate peaks presented in **Table 1**).

Figure 7(**A**) shows the Raman shift spectra of the ascorbic acid solution at different concentrations. Raman shifts of ascorbic acid with a concentration of 1.0, 0.5 and 0.25 wt% exhibit almost similar peaks. The peak value of each ascorbic acid solution has an almost similar value, i.e., 825 , 1299, 1692, 2915 and 3480 cm^{-1}; these values are similar to those obtained in a previous study (Berg, 2014). The highest peak in the range of ~3400 cm^{-1} was identified as the peak of the distilled water solvent. **Figure 7**(**B**) shows the Raman shift spectra of a solution of ascorbic acid at different concentrations with the addition of colloidal Ag nanoparticle colloids. The colloidal Ag nanoparticles were added at a 1:1 volume ratio of Ag nanoparticles and ascorbic acid solution for all ascorbic acid concentrations. The peak intensity of 1, 2, and 3 in the spectra shown in **Figure 7**(**B**) was significantly higher than the peak intensity of the spectra shown in **Figure 7**(**A**). This concludes that the addition of colloidal Ag nanoparticles successfully increased the ascorbic acid peaks. The observed peaks in the Raman shift spectra are shown in **Table 1**.

Based on the spectra results, there is an increase in peak intensity after addition of the Ag nanoparticles. In the ascorbic acid sample without Ag nanoparticles, the peak location is not clearly visible, and after addition of the Ag nanoparticles, the ascorbic acid peaks appeared with different intensities at various ascorbic acid concentrations. **Table 1** shows the increases of the peak intensity observed in the Raman spectra where the increase of the peak intensity reached 3 to 20 times from the initial intensity value. The significant increase of peak intensity clearly shows the Raman shift values before and after addition of Ag nanoparticles, and this is referred to as the SERS phenomenon.

Furthermore, the effect of the interaction duration of Ag nanoparticles and ascorbic acid compounds was also studied. This was done through an incubation process of the ascorbic acid solution and colloidal nanoparticle mixture in a bottle at room temperature for 6 h. The incubation effect was tested for ascorbic acid detection at concentration 0.25 wt% with colloidal Ag nanoparticle added, as shown in **Figure 8**.

As shown in **Figure 8**, the peaks of the Raman signals from the mixture of ascorbic acid (0.5 wt%) and Ag nanoparticle at incubation times of 0 h and 6 h were all matched to each other at the same location Raman shift. Both spectra of ascorbic acid with addition of colloidal Ag nanoparticles have Raman signals at 825, ~1420, and ~1560 cm^{-1} (Raman characteristic for ascorbic acid) that are more intense than the spectra in the case without Ag nanoparticles. However, when comparing the spectra with incubation times of 0 and 6 h, the intensities of the peaks in the Raman signal were not significantly different. The results suggest that the effect of the incubation duration is not significant; in other words, the addition of colloidal Ag nanoparticles can directly increase the Raman signal intensity with no an additional treatment time.

Table 1. Raman shift spectra peaks of ascorbic acid with colloidal Ag nanoparticles at various ascorbic acid concentrations.

Peak number	***Raman shift* (cm^{-1}) and the peak intensity**				**Ref. (Berg, 2015)**	**Signal**
	Asc	Ag-Asc 0,25 wt%	Ag-Asc 0,5 wt%	Ag-Asc 1 wt%		
1	810,46 (↑ 108,33)	825,32 (↑ 549)	825,32 (↑ 683,33)	825,32 (↑ 312)	825 cm^{-1}	SERS
2	-	1385,49 (↑ 545)	1427,09 (↑ 967,33)	~1420 (↑~1500)	1299 cm^{-1}	SERS
3	1659 (↑ 94)	1591, 46 (↑ 1170,67)	1564,29 (↑ 1100)	1577,88 (↑ 1826,67)	1692 cm^{-1}	SERS
4	3479	3479,08	3479,08	3479,08	3480 cm^{-1}	Distilled water

Note: ↑ indicates an increase in the peak intensity (in counts unit)

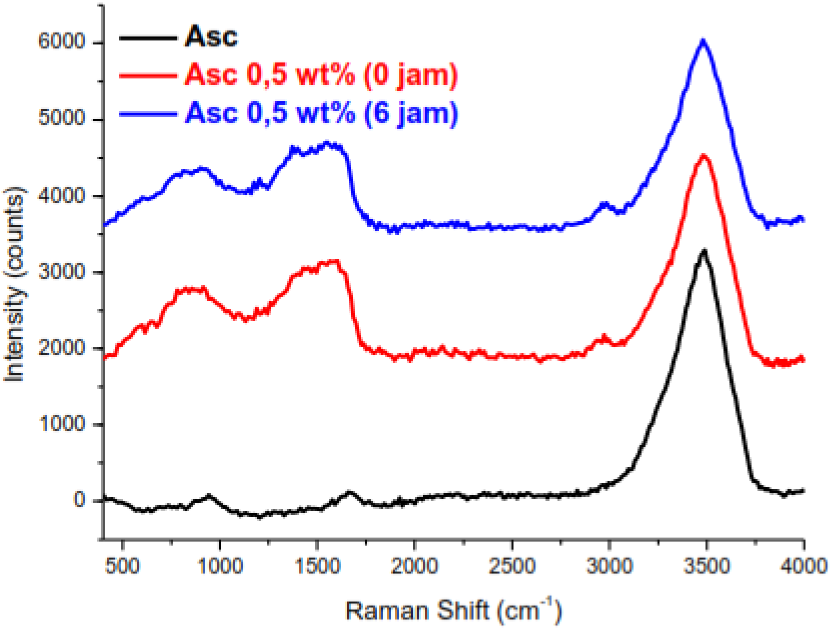

Figure 8. Raman shift spectra of the interaction of ascorbic acid (0.5 wt%) with added Ag nanoparticles for 0 and 6 h incubation time.

CONCLUSIONS

Ag nanoparticles were synthesized by laser ablation with an excitation wavelength of at 412 nm. Colloidal Ag nanoparticles increased the intensity of ascorbic acid detection signals by three- to twenty-fold, as shown by Raman spectrometer measurements. However, the incubation duration of Ag nanoparticles and ascorbic acid solution mixture did not have a significant effect on the increase in the signal intensity of ascorbic acid detection. In general, we concluded that colloidal Ag nanoparticles synthesized by the laser ablation method were potentially applied as a substrate for detection of ascorbic acid analytic compounds more sensitively and quickly using Raman spectrophotometry.

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