The effect of variation of reducing agent concentration on optical properties of silver nanoparticles as active materials in surface plasmon resonance (SPR) biosensor

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Abstract. Using chemical reduction methods, silver nanoparticles have been successfully synthesized. As a precursor, silver nitrate (AgNO\textsubscript{3}) was used and as a reducing agent was trinatrium citrate. In the process of synthesis, there was a variation of concentration of the reducing agent of trinatrium citrate. Optical properties of silver nanoparticles formation and structure in a colloidal solution were studied by using a UV-Vis spectrometer. In this synthesis process, the colloid nanoparticles produced dominated by silver nanoparticle grains since the yellowish color formed in the sample. The UV-Vis spectrometer shows that there is a shift in the spectrum of silver nanoparticles colloidal absorption in the range of 429.01 nm to 433.06 nm for the variation of trinatrium citrate reducing agent concentration to the colloid formation of the silver nanoparticles. A shift in the angle of SPR is observed when a thin silver layer as the active material of the sensor is coated silver nanoparticles. Increasing the concentration of reducing substances of a thin silver nanoparticles films will shifts the angle of SPR. This SPR angle shift sharpens the reflectivity value and changes the surface plasmon wave constants due to changes in the surface plasmon constant which is caused by changes in the dielectric constant of thin silver nanoparticles films.

1. Introduction

The surface plasmon resonance (SPR) instrument as a biosensor is a variation of the incident wave angle in which the wave reflectance is observed as a function of the incident angle. The active material for generating surface plasmon polariton (SPP) is used in gold and silver metals and the test material used is a biomolecular-based dielectric material consisting of biorecognition and analyte elements. The approach is known to have produced a highly sensitive phenomenon and in particular its instrumentation has also been commercially available with an angle resolution of 0.001 [1]. However, the existing procedure still has a weakness that is the difficulty to directly detect biomolecules, especially small molecules with short and non specific changes in refractive index [2, 3]. To overcome this, several SPR biosensor detection methods have been developed, such as the SPR signal strengthening method using the addition of colloidal gold nanoparticles to the target molecule [4].

In this paper, SPR biosensor will be developed based on conventional SPR model by placing silver nanoparticle structure on top of a thin silver film in order to increase the sensitivity of SPR biosensors [5]. Various methods of preparation of silver nanoparticles such as radiolysis, ultrasonic irradiation,
electrochemical synthesis, thermal decomposition and chemical reduction of metal salts have been reported [6 - 9]. Therefore, in this research, a method of synthesis of silver nanoparticles where the growth of the nanoparticles controlled by varying the trinatrium citrate reducing agent concentration was used. UV-Vis spectroscopy was used to characterize the optical properties of silver nanoparticles.

2. Experimental Methods

2.1. Experiment.
The Attenuated Total Reflection (ATR) method in the Kretschmann configuration was used to observe the addition of silver nanoparticles to the SPR biosensor system. Silver nanoparticles thin film was deposited on the silver thin film surface using a spray method. Prism type used in this research was BK7 with refractive index 1.51. The nanoparticles used in the SPR system were synthesized with aquadest solvent using AgNO₃ 0.001M with a variation of the trinatrium citrate reducing agent. In synthesis, PVA 1.70 ppm stabilizer was used and heated at 90°C for 5 minutes with stirring time of 10 minutes. The concentration of trinatrium citrate reducing agent in the silver nanoparticles solution used for each layer was varied with variation of 0.01 M, 0.04 M and 0.05 M.

2.2. Characterization.
The reduction of metal ions was monitored by measuring the UV-Vis spectroscopy of the solution. The UV-Vis spectroscopy measurement of silver nanoparticle was recorded on Shimadzu dual beam spectrophotometer (model UV-1650 PC) operated at a resolution of 1 nm. The silver nanoparticle was measured in a wavelength ranging from 350 to 800 nm

3. Results and Discussion
Using chemical reduction methods, silver nanoparticles have been successfully synthesized. In this synthesis process, the colloid nanoparticles produced dominated by silver nanoparticle grains since the yellowish color formed in the sample.

This study focused on efforts to study the action of trinatrium citrate as a reducing agent. Information about the role as the reducing agent was obtained by analyzing the surface plasmon bands on the absorption spectrum of silver nanoparticles formed with different citrate concentrations. Trinitrate citrate can be used well as a reducing agent because it provides a drastic effect on the reduction of the size of the synthesized nanoparticles in a stable state.

Figure 1 shows the maximum absorption band of surface plasmon for a spectrum of colloidal silver nanoparticles synthesized with a PVA stabilizer at a temperature of 90°C with a variation in molar concentration of reducing agent. In Figure 1 it is shown that the colloidal spectrum of silver nanoparticles synthesized with a 0.01 M trinatrium citrate reducing concentration, the maximum absorption band is at a wavelength of 427.73 nm. The addition of molar concentration of the reducing agent during synthesis can make the maximum absorption band of the surface plasmon sharper and also make the maximum absorption band shifted toward the greater wavelength. The maximum absorption band for trinatrium citrate 0.01M becomes 427.73 nm and for concentration 0.04, 0.05 and 0.06 become 429.01 nm, 431.99 nm and 433.06 nm respectively. The surface plasmon absorption bands in the three treatments were in the range of 429.01 nm to 433.06 nm for the variation of the concentration of the trinatrium citrate reducing agent. This suggests that the silver nanoparticles produced are round or almost spherical with the particle distribution being quite dispersive despite the tendency for clumping. Such nanoparticle dispersibility levels could potentially be used as SPR biosensor active materials.
Figure 1. The absorption spectra of UV-Vis silver nanoparticles synthesized with variations in the concentration of reducing agent.

The Attenuated Total Reflection (ATR) method in the Kretschmann configuration was used to observe the addition of silver nanoparticles to the SPR biosensor system. Figure 2 shows the SPR reflectance curve for an SPR biosensor system. Figure 2 shows that when a thin layer of silver is deposited with silver nanoparticles, there will be a shift in the angle of SPR. Before being deposited with a thin film of silver nanoparticles, the SPR biosensor system has an SPR angle of 42.7° with a reflectance value of 0.337 and a surface plasmon constant of 1.0094×10^7 m⁻¹. The addition of a thin film of silver nanoparticles to the SPR biosensor system shifts the SPR angle, thus sharpening the reflectance value and changing the surface plasmon wave constants as shown in Table 1.

Figure 2 and Table 1 also show that variations in the concentration of trinatrium citrate reducing agents shift the angle of SPR upon concentration increase. This shift of SPR angle make the reflectivity value sharper and changes the surface plasmon wave constants. The surface plasmon wave constants for each layer addition can be calculated by entering the SPR angle value for each addition of a thin layer system with a laser beam frequency of 2,977×10¹⁷ Hz [10]. Changes in the surface plasmon constants are caused by changes in dielectric constants due to the addition of silver nanoparticle layers on the surface of silver thin films and the use of different concentrations of trinatrium citrate reducing substances in silver colloidal nanoparticles.

The value of the surface plasmon constant in Table 1 is the calculation of experimental results and from the table it is known that the angle of SPR will shift along with the increase in the value of the wave constant. The SPR angle is the angle where the resonance between evanescent waves and surface plasmon waves occurs when the surface plasmon constant and the electromagnetic wave constant are the same.

Shifting the SPR angle and increasing the reflectance value due to the constant in the surface plasmon changes which is caused by changes in the dielectric constant of thin silver nanoparticles films. The larger constant of the wave, the greater the angle of the SPR produced. These research results can be a
reference that placing additional layer of silver nanoparticles on the SPR surface sensing can increase its sensitivity.

![Figure 2](image.png)

**Figure 2.** The SPR curve observes the SPR phenomenon using silver nanoparticles synthesized with variations in the concentration of reducing trinatrium citrate. *Insert* shows the smoothness of the SPR curve.

**Table 1.** Value of surface plasmon wave constants ($K_x$), reflectance and resonance angle for each addition of thin films of nanoparticles synthesized with variations in the concentration of reducing trinatrium citrate.

| Parameter of Layer | angle of SPR (°) | reflectance value | $K_x (10^7 \text{ m}^{-1})$ |
|--------------------|------------------|------------------|-----------------------------|
| Silver thin film   | 42,7             | 0,337            | 1,0094                      |
| Silver nanoparticles + PVA (Naci 0,01 M) | 42,8             | 0,275            | 1,0113                      |
| Silver nanoparticles + PVA (Naci 0,04 M) | 43,0             | 0,263            | 1,0151                      |
| Silver nanoparticles + PVA (Naci 0,05 M) | 43,1             | 0,440            | 1,0170                      |

4. Conclusion

Synthesis of silver nanoparticles with chemical reduction method has been successfully carried out. In this synthesis process, the colloid nanoparticles produced dominated by silver nanoparticle grains since the yellowish color formed in the sample. UV-Vis spectrometers shows that the silver nanoparticle colloid absorption spectrum was produced in the range of 429.01 nm to 433.06 nm for variations in the variation of trinodium citrate reducing agent concentration to silver nanoparticle colloid formation. A
shift in the angle of SPR is observed when a thin silver layer as the active material of the sensor is coated silver nanoparticles. Increasing the concentration of reducing substances of a thin silver nanoparticles films will shifts the angle of SPR. This SPR angle shift sharpens the reflectivity value due to changes in the surface plasmon constant which is caused by changes in the dielectric constant of thin silver nanoparticles films. The larger constant of the wave, the greater the angle of the SPR produced. These research results can be a reference that placing additional layer of silver nanoparticles on the SPR surface sensing can increase its sensitivity.

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