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Synthesis of Silver Nanoparticles via Chemical Reduction and its Anti-bacterial Activities in Wastewater of Shrimp Pond

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Abstract **- A simple and facile method of synthesis of silver nanoparticles is developed in this work. The silver nanoparticles are prepared by chemical reduction method using sodium citrate as a reducing agent. The average size, size distribution, morphology, composition and structure of particles were determined by scanning electron microscopy (SEM), Transmission Electron Microscopy (TEM), Inductively coupled plasma mass spectrometry (ICP-MS), and UV/Visible absorption spectrophotometry. We found that the silver nanoparticles with size about of 20 nm and good distribution are prepared without using any surfactant or stabilizer. The antibacterial activity is surveyed with six different concentrations in 6 hours. We found that all bacterials in the shrimp effluent have been removal completely after 6 hours contact with Ag NPs 0,1 mg/L. The result demonstrated that systhesized Ag NPs in this study is obtained high treatment bacterials in the shrimp effluent effect. The results of this work suggest that the good properties of Ag NPs are synthesized by this facile method and the high anti-bacterial activities are also determined in the wastewater of shrimp pond, obtaining standard drain out the river and reuse.**

Keywords: Silver, Nanoparticles, Chemical Reduction, Sodium Citrate, Anti-Bacterial

I. INTRODUCTION

NanoSilver, in form of collodials silver which have been known in electronics, in optics, and most importantly in medical applications as antibacterials activity for a long time ago[1-3]. Silver ions are used in the formulation of dental resin composite, in coating of medical devices, as a bactericidal coating in water filters, as a antimicrobial agent in air sanitizer sprays, pillows, respirators, socks, wet wipes, detergents, soaps, shampoos, toothpaste, washing machine and may consumer products, as bone cements, and in many wound dressing to name few [4-6]. There are many ways to synthesize silver nanoparticles(Ag NPs) such as include physical, chemical and biological methods. Chemical reduction is the most frequently applied for the preparation of Ag NPs as stable, colloidal dispersions in water or organic solvents. Ag NPs was synthesized by the silver nitrate solution and by the use of different reducing agents such as ascorbic acid, hydrazine, dry methane, dimethyl formamide and sodium borohydride. The shape, size and size distributions strongly defend on the strong and weak tendency of organic substants to reduce the silver salt [7-9]. Nanosilver particle size plays an important role in silver toxicity, Avask A. et al. demonstrated that size - dependence toxicity of Ag NPs to bacterial, yeast, algae, crustaceans and mammalian cells in vitro [10-11]. The antimicrobial effects of silver (Ag) ion or salts are well known, but the Ag NPs on microorganisms and antimicrobial mechanism have not been revealed clearly. The antimicrobial activity of Ag NPs was performed yeast, Escherichia coli, staphylococcus aureus. The study shown that Ag NPs can be used as effective growth inhibitors in various microorganisms, making them applicable to divers medical devices and antimicrobial control systems[12].Li, Wen-lu, et al, studied the antibacterial activity and acting mechanism of Ag NPs on Escherichia coli. The growth, permeability and morphology of the bacterial cells were investigated following treatment with Ag NPs. The results suggested that Ag NPs may damage the structure of bacterial cell membrane and depress the activity of some membranous enzymes, which cause E.coli bacterial to die eventually [13]. The Ag NPs is small size and enormous specific surface area, faciliates more rapid dissolution of ions than the equivalent bulk materials, potentially leading to increased toxicity of nanosilver[14]. However, the previous methods have used toxic reducing agents and stabilizers. In addition, using of stabilizers would be affect to antibacterial activity of Ag NPs. To troubleshoot these problems, the simple and the most commonly used bullk – solution synthetic method for silver nanoparticle is the chemical reduction of silver nitrate and sodium citrate is used the dual role of a reductant and a stabilizer. The citrate ion agent caps the particle and prevents further growth or aggregation [16-17].

Herein, Ag NPs are prepared by chemical reduction using sodium citrate as a reductant and stabilizer. The average size, size distribution, morphology, composition and structure of particles were determined by dynamic light scattering (DLS), scanning electron microscopy (SEM), Transmission Electron Microscopy (TEM) Inductively coupled plasma mass spectrometry (ICP-MS), and UV/Visible absorption spectrophotometry. The antibacterials activities of systhesised Ag NPs are tested against with bacterials in the wastewater of shrimp pond with various Ag concentrations.

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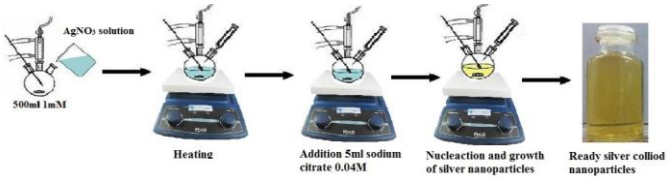
II. EXPERIMENTAL SECTION

A. Materials

Silver nitrate (>99.9% pure), sodium citrate (>99 % pure) and sodium hydroxide were purchased from Germany and used at the desired dilutions. All reagents that used in this experiment were analytical grade and were used without further purification. Throughout the procedures, double deionized (DI) water was used. And the bacterials into wastewater of shrimp pond is treated by AgNPs.

B. Synthesis of silver nanoparticles

Silver nanoparticles were synthesized by chemical reduction method through the reduction silver nitrate and sodium citrate. All chemicals were used as purity and double – distilled deionized water was used. The aqueous solution of silver nitrate 1mM and sodium citrate 0.04M were prepared. A solution of $AgNO_3$ 1mM was heated until it began to boil. Then sodium citrate 0.04M was added dropwise to the silver nitrate solution until 5 ml. It was held at intervals of one hour.



Scheme 1: The preparation of Ag NPs via chemical reduction

C. Anti-bacterial

The anti-bacterial activities of as-synthesized AgNPs were studied by using it as the anti-bacterial agents the wastewater treatment of shrimp pond. The various concentrations of Ag NPs are used to study its effect on the treatment ability the bacterial in the wastewater of shrimp pond as following Table 1. All samples are tested after 24h of incubation at 37 ^oC.

Table 1 : The experiment sample for anti bacterials by different concentrations of AgNPs

Sample	1	2	3	4	5	6
Concentration (mg/L)	0	0,02	0,04	0,06	0,08	0,1

D. Characterization of AgNPs

UV-Vis extinction spectra were recorded using a spectrophotometer (Perkin-Elmer Lambda 35, USA) in absorbance mode (range 200–800 nm) at desired dilutions of silver colloids. Powder X-ray diffraction (XRD) patterns of Ag NPs were obtained with XRD – D8 Advance – Bruker AXS (Germany) measurements using CuK□ X-ray tube emitting at 1.54 Å. The data were collected from 20 to 80 in 2θ scale in step size of 0.030 with a scan rate of 20. min^{-1}. Transmission electron microscopy (TEM) images were performed at 100 kV on a field-emission instrument of the type JEOL JEM-1010 with an ultrahigh resolution pole piece, providing a point resolution about 2 Å. The concentration of silver solution was determined by ICP measurement

III. RESULTS AND DISCUSSIONS

The formation of silver nanoparticles occurs after the reduction of aqueous silver salts with tri-sodium citrate (0.04M) within duration of 1 hour. During the addition of the sodium citrate to the aqueous $AgNO_3$ solution, a light yellow color slowly appeared in the mixture solution, suggesting the formation of Ag NPs. The intensity and color variations between the samples arise from the reaction times. This observation is explained due to when the reducing agent is added into aqueous silver salt in drop wise manner, growth of silver "seed nucleation" is controlled in nano dimension. This control growth of silver atoms lead to another phenomenon called "localized surface plasmon oscillation". Due to the citrate acted both to reduce the metal cation and stabilize the resulting nanoparticles. Also, it was believed that this reactant played a role on determining the growth of the particles. Due to this oscillation, optical property of (color-pale yellow) synthesized silver nanoparticles differ from bulk silver salt [3, 20, 18].

The formation of Ag NPs and Ag NPs size are determined by UV – Vis spectroscopy as well Figure 1. Nano silver colloids have absorption wavelength about 400 – 500nm [17 – 19]. As shown in Fig. 1, silver nanoparticles exhibit a sharp extinction peak at 425 nm wavelength for silver nanoparticles with the average sizes 30. As predicted, the absorption maxima of Ag NPs shifted to longer wavelength with increase in Ag NPs size. The full width at half maximum (FWHM) of the corresponding peaks determines dispersity of the nanoparticles, where a large FWHM is attributed to peak broadening and hence, polydispersity. This result is demonstrated that after one hour reacted, Ag NPs are formed. A chacterristic surface plasmon absorbtion band of Ag NPs were observed at 425 nm.

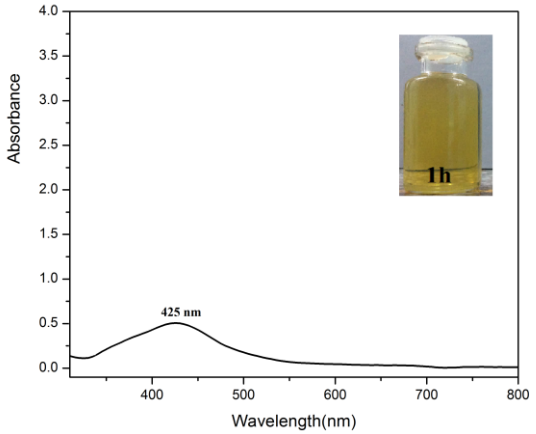


Fig 1: UV –Vis absorption spectra of silver nanoparticles

The structure of Ag NPs was characterized by XRD measurement. (Figure 3) The typical powder XRD pattern of the prepared Ag NPs with different reaction time is shown in Fig. 2. The data shows diffraction peaks at 2θ = 38.2°, 44.4°, 64.6°, which can be indexed to (111), (200), (220), planes of pure silver. All the peaks in XRD pattern can be readily indexed to a face-centered cubic structure of silver as per available literature (JCPDS, File No. 4-0783). It confirmed that the main composition of the nanoparticles was silver.

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When increasing the reaction time, the peak become narrow, suggesting the large particle size formation. This is corresponding with the crystallite size (L) of the material of thin film has been evaluated by Scherrer's formula. L =0.94λ/β cos θ where λ is wavelength (0.15418 Å) of X-rays used, b is broadening of diffraction line measured at half of its maximum intensity (in radian), and θ is Bragg's diffraction angle (in degree). The crystallite size of silver nanoparticles has been estimated to be ~35 nm for 1 hour.

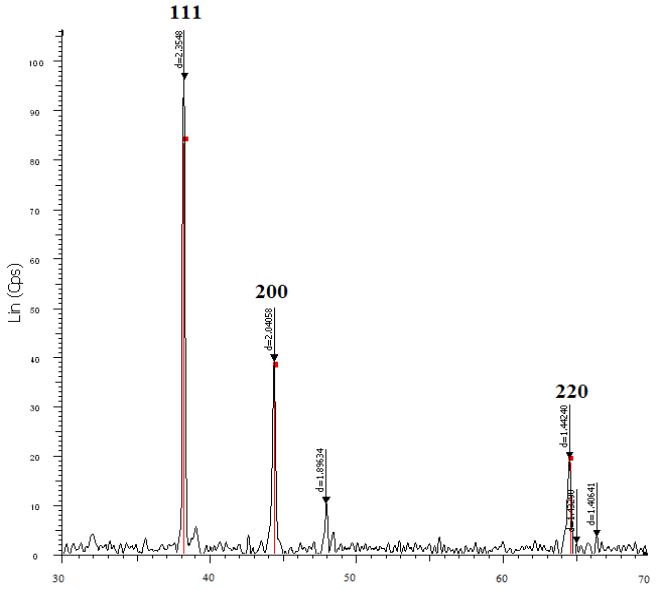


Fig 2 : XRD of silver nanoparticle is prepared by chemical reduction

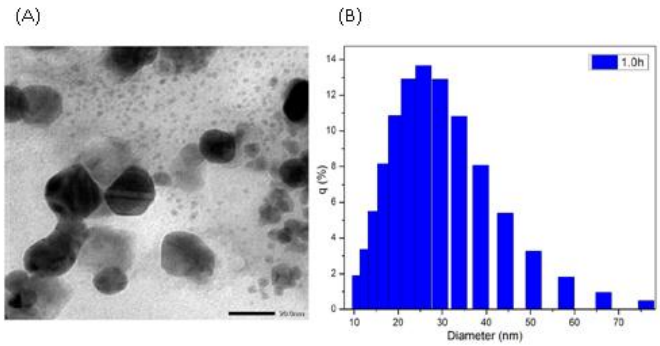


Figure 3. TEM and size distribution of Ag NPs

The size and morphology of Ag NPs are characterized by TEM on Figure 3. It is suggested that the morphology of Ag NPs is spherical particle and distributed quite uniformly. The particle size of Ag NPs is about 20 -30 nm with highly distribution. Moreover, the high resolution and overview of as-synthesized Ag NPs are shown in the Figure 4**.** We concluded that the silver nanoparticles with size about of 20-30 nm and good distribution are synthesized without using any surfactant or stabilizer via chemical reduction using sodium citrate as a reductant. The concentration of Ag solution after 1 hour reaction was also characterized by ICP/MS. It is found that its concentration is about 95.72 mg/l.

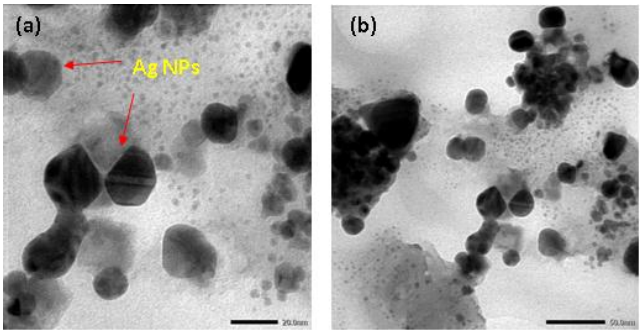


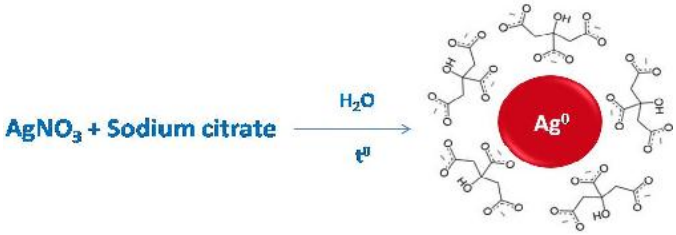
Figure 4. TEM image of Ag NPs at reaction time about 1 h for (a) scale bar 20 nm and (b) scale bar 50 nm

The formation of Ag NPs by using the sodium citrate as a reductant agent could be expressed as follow. (Scheme. 2) The mechanism of reaction:

$$4Ag^{+} + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag^{0} + C_6H_5O_7H_3 + 3Na^{+} + H^{+} + O_2 \quad (1)$$

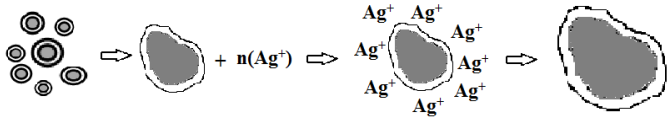
$$Ag^{+} + e_{aq} \rightarrow Ag^{0} \rightarrow (Ag)_2^{+} + citrate^{-} \quad [(Ag)_2^{-} \ldots (citrate)^{-}] \rightarrow \quad (2)$$

When Ag NPs were formed, citrate ions will cap the particles prevent them aggregation. Equilibrium 2 illustrates the formation of a complex between $(Ag)_2^{+}$ and citrate ion[20-21] .



Scheme 2: Mechanism form Ag NPs caped by citrate ions

But when reaction time was prolonged, it made the particles cluster together and form the large particle size. It's illustrated by the Scheme 3.



Scheme 3: The growth of the particle size of Ag NPs [14]

Even if, the Ag NPs have small size or large size, they were capped by the citrate ions. It is explained the reason why Ag NPs were formed and stabilized for a long time. Therefore a small particle size about 20 – 30 nm and good distribution have been obtained with the reaction time of 1 hour, resulting in enhancement the antibacterial activity as well. Because when the particle size is small, the active surface area is also increase significantly. In this work, the antibacterial effect of the prepared Ag NPs samples was studied on bacteria, namely E. coli in wastewater of shrimp pond with various concentration of Ag solution. The anti-bacterial activities of Ag solution was studied by testing the anti-bacterial activities The Ag solution was used with various concentration as described on the Table 3.

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Table 3. The result removal the bacterial of the different Ag NPs concentration

Sample	1	2	3	4	5	6
Concentration (mg/L)	0	0,02	0,04	0,06	0,08	0,1
E. coli total MPN/100ml	7500	4300	3000	2300	2300	-
Eff %	--	43	60	69	69	99,99

The reaction time for testing is fixed 6 hour for all samples. It indicated that all E. Coli bacterials in wastewater of shrimp pond were removal completed after 6 hours contact with Ag NPs 0,1 mg/L. (Fig. 5) The result demonstrated that as-synthesized Ag NPs in this study exhibited highly efficiency in treatment E.Coli bacterials in in wastewater of shrimp pond. This is explained due to the small particles size with highly distribution of as-synthesized Ag NPs was prepared without using any surfactants as PVP, CTAB that could not be covered the active site of Ag NPs, leading to highly anti-bacterial activities that others.

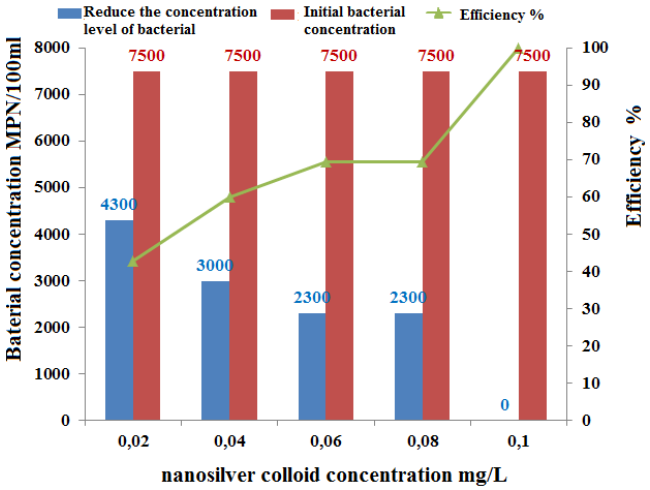

Figure 5. The antibacterial effect of the prepared Ag NPs samples on E. coli bacteria in wastewater of shrimp pond with various concentration of Ag solution

It is well-known that silver nanoparticles are harmful significantly to bacteria, due to they could bind closely to the surface of microorganisms causing visible damage to the cells, and demonstrating good self-assembling ability. These silver nanoparticles which have antibacterial effect can be used against resistant antibiotic bacteria. The mechanism for antibacterial action of silver nanoparticles is described as following:

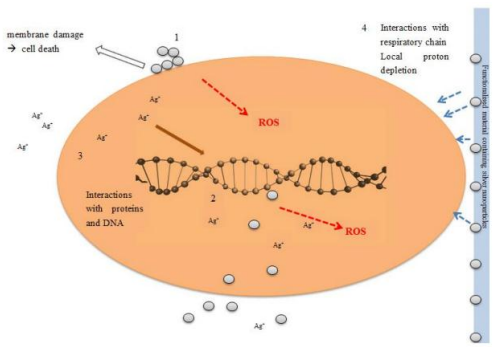

Figure 7. Schematic representation of the known mechanism of antibacterial action of Ag NPs and release ionic silvers

The antibacterial activity of Ag NPs is explained by the silver cation on the Ag NPs surface is continuous release to be a notable determinant resposible for efficient antibacterials activity Malina et al., 2010 [22]. The systhesized Ag NPs is small size it make ion silver release rapidly and extremly large surface area of nanoparticles enable them to make strong contact with the bacterials surface. And then ion silvers penetrating inside the bacterial cell, resulting in DNA damage. Dissolution of Ag NPs release antimircobial ion silvers which can interact with sulfur – containing protein in the bacterial cell wall which may lead to compromise functionality. This phenomenon is often considered as the main mechanism of the antimicrobial activity of Ag NPs [14

IV. CONCLUSIONS

A simple and facile method of synthesis of silver nanoparticles with small size is developed in this work. The silver nanoparticles are prepared by chemical reduction method using sodium citrate as a reducing agent. We found that the silver nanoparticles with size about of 20 nm and good distribution are prepared without using any surfactant or stabilizer. The antibacterial activity of silver nano-particles was studied. The results of this work suggest that the good properties of Ag NPs are synthesized by this facile method that exhibited the highly anti-bacterial activity the E.Coli bacterials in the wastewater of shrimp pond. Nano particles materials show highly active properties due its large surface area. Application of Ag NPs based on these findings may lead to valuable discoveries not only in the anti-bacterial field but also in various fields such as medical agents.

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