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Releasing of Silver From Fabrics Containing Nano Ag^0 During Washing And Toxicity Tests of Ag^+, Nano Ag^0, Nano Ag_2o, and Wash Water on *Moina Macrocopa*

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***Abstract*:** *The objectives of this research were to study the content of total silver that was detached from fabrics containing nano Ag^0 after washing and to determine toxicity test of Ag^+, nano Ag^0, nano Ag_2O, and wash water on water fleas (Moina macrocopa), a common freshwater zooplankton in a tropical region. The average content of silver nanoparticles in commercial T-shirt was 212 mg/kg of fabric. In addition, silver nanoparticles were detached from fabrics after washing in a washing machine. The maximum content of total silver in the wash water was 122.6 µg/L. The results of toxicity test showed the following order of toxicity on water flea; Ag^+ > nano Ag^0 > nano Ag_2O. The LC_{50} (Lethal Concentration) of Ag^+, nano Ag^0, nano Ag_2O were 0.045±0.13, 1.59±0.59 and 18.94±1.45 mg/L, respectively. The toxicity test of the wash water from fabrics containing silver nanoparticles revealed that the mortality of water fleas was not significantly different from the control treatments and this could be due to very low concentrations of silver nanoparticles in the wash water.*

Keywords: *silver ions, silver nanoparticles, silver oxide nanoparticles, water flea (Moina macrocopa).*

1. Introduction

Silver nanoparticles are widely used in everyday life products. For examples, they are used as antimicrobial agents as well as being used in vacuum machines, air conditioners and refrigerators. They are also added in paints, fibers or plastic and food products, bandages, surgical instruments, and various coatings to increase the removal of odors and bacteria [1,2]. In Thailand, several manufacturers have imported silver nano particles to use in mixed product types such as detergent, textiles or food packaging and so on. In particular, textile industry has commonly used silver nanoparticles in two main forms, Ag fresh (nano Ag^0) and silver oxide (nano Ag_2O). Nano Ag_2O is added into polyester because it is photocatalytic that can prevent ultraviolet ray as well as having the ability to remove undesirable odor and harmful pathogens that might lead to problems on human body and skin caused by Staphylococcus aureus, Pneumococcus spp. and Escherichia coli [3]. Nano Ag^0 also has the ability to anti bacteria. These particles have the size of > 4 nm but not exceeding 100 nm. They are inserted in the cellulose fibers and have the ability to be bound with silver particles in fabrics for long times [4].

A few researches have been studied about the release of silver nanoparticles from different textiles [5]. The release of silver nanoparticles from the fabric depended on techniques that have incorporated silver nanoparticles into fabrics [6]. Silver nanoparticles incorporated in the fabrics are detached when the fabrics are washed in the water. At first, they will appear in the form of nano Ag^0 suspended in the water and in the form of free silver ion (Ag^+). Then the nano Ag^0 will get together rapidly to form the larger aggregates. Geranio et al. [7] indicated that Ag released from the textiles into washing water presented in form of particles larger than 450 nm, and that the level of dissolved Ag was very low for most textile. Environmental conditions such as pH and dissolved oxygen in the water also influence transformation of silver nanoparticles to oxide form or they may incorporate with certain organic matter [1]. For instance, in the pelagic zone of the water body that lacks dissolved oxygen, silver nanoparticles may attach with organic sulfides such as cysteine and glutathione [8]. Silver nanoparticles in any forms in the water are known to be toxic to aquatic flora and fauna such as phytoplankton, zooplankton, aquatic macrophytes, invertebrates and vertebrates [9]-[12]. In general, municipal wastewater treatment is not capable of removing silver nanoparticles [13] and as a result they can go directly to water bodies and may cause ecological effects. The levels of toxicity of silver nanoparticle in plants and animals are different depending on certain forms of such particles. Silver nanoparticles appear to be more toxic than normal and larger sizes of silver particles because of having unique properties that can be easily got into cells of living organisms [14].

Although the use of silver nanoparticles is expanding rapidly in many products and nanotechnology is beneficial to the development of industries, the questions about their environmental impact of the loss of silver nanoparticles from fabrics to aquatic ecosystem are not yet clearly answered. Silver nanoparticles might be harmful to the organisms that live in the water. This is simply because such particles may be easily ingested or absorbed into cells of living things that will accumulate and transfer through aquatic food chain and eventually to human beings. Therefore, the main objectives of this study were to investigate the loss of silver nanoparticles from fabrics during normal washing by a washing machine since this could lead to water pollution. We also studied toxicity tests of different forms of silver nanoparticles (nano Ag^0, Ag^+ and nano Ag_2O) on water fleas (*Moina macrocopa*; *M. macrocopa*). *M. macrocopa* plays a key role in freshwater ecosystem and is regarded as the primary consumer and a food source of other omnivorous and carnivorous animals in higher trophic levels. The results of this study will lead to better understanding of fates of silver nanoparticles as well as toxicity of silver nanoparticles on aquatic organisms.

2. Material and Methods

2.1. Physical and Chemical Properties of Silver Nanoparticles and Fabrics

Before starting experiments, physical and chemical properties of nano Ag^0, and nano Ag_2O obtained from business venders were examined by using Transmission Electron Microscopy (TEM), X-Ray Diffraction (XRD) and Atomic Absorption Spectrophotometer (AAS).

We also studied the silver nanoparticle content and its characteristics in fabrics that are currently on the market. Two types of fabrics (long-sleeve male shirts) were chosen; the fabrics that had silver nanoparticles incorporated into the100% cotton and the same fabrics that did not have silver nanoparticles bound. Pieces of each fabric type were cut into a circular shape with a diameter of about 0.5 cm. Samples of fabrics were subsequently analyzed by using Scanning Electron Microscope (SEM) and Electron Dispersive X-ray Spectroscopy (EDS) (JSM-5410LV). Furthermore, we investigated silver nanoparticle contents from different parts of shirts. Shirts laced with silver nanoparticles were cut from 3 parts (front, back and sleeves). Each sample was 10x10 centimeters (average weight of 2.11 g). Fabric samples were then dried in furnace at temperature of 550 ^{o}C for 2 h. Ash obtained from furnace was subsequently dissolved in concentrated hydrochloric and nitric acid solution. Measurement of concentrations of silver nanoparticle content was done by Atomic Absorption Spectrophotometer (AAS).

In addition, we tested whether silver nanoparticles incorporated in fabrics were detached and contaminated in the water after normal washing conditions. We used 10 kg of fabrics that contained silver nanoparticles. The fabrics were washed in 60 L of deionized water for 15 minutes and then spin-dried for 5 minutes by using a Toshiba washing machine (AW 8990 ST). Washing instruction was strictly followed as showed below;

- Round I, fabrics were washed for 15 minutes and spin-dried for 5 minutes. Subsequently, fabrics were taken outside the room for air drying. Water samples were collected after washing for further measurement of total Ag content.
- Round II, dried fabrics from round I were again washed for 15 minutes and spin -dried for 5 minutes. Samples of water were again collected after washing to quantify the total Ag content.

Samples of water obtained from round I and II were analyzed for physical and chemical properties based on Standard Methods for Examination of Water and Wastewater, 21st edition of American Public Health Association, Water Environmental Federation, and American Water Work Association. Concentrations of total Ag were conducted by using Inductively Coupled Plasma (ICP).

2.2. Effects of Silver Nanoparticles on Zooplankton

Silver nanoparticles in the form of nano Ag^0 were prepared by using 2 g of silver nanoparticles powder poured in 400 mL of deionised water. Then the water samples were sonicated and stirred for 30 minutes (brand Elma, TRANSSONIC 460/H) and was filtered through a filter paper No. 1. Subsequently, 69% hydrochloric acid was added into the samples and concentrations of nano Ag^0 were quantified by Atomic Absorption Spectroscopy (AAS) [15,16]. Preparation of free silver ion (Ag^+) at concentration of 1,000 mg/L was done by dissolving silver nanoparticle powder with concentrated hydrochloric and then adding deionized water to make up to 1,000 mL (approx. pH 7). Nano Ag_2O solution (5,000 mg/L) was prepared by using 2 g of silver oxide powder obtained from commercial grade dissolved in 400 mL deionized water. Then the solution was sonicated and stirred for 30 minutes by sonicator (Elma, TRANSSONIC 460/H).

M. macrocopa was chosen in this study since it is common in freshwater ecosystems in Thailand. *M. macrocopa* was raised in 100 test tubes (16 x 150 mL) in the laboratory at constant room temperature (approx. 32 ^oC). *M. macrocopa* (neonate stage) at the age of less than 24 hr from second generation were used in the experiment. Toxicity test was based on OECD : *Daphnia* sp., Acute Immobilisation Test 202 [17]. We recorded the number of times of death and also observed movement and abnormal behavior of *M. macrocopa* after 24 hr and 48 hr once placed in water samples containing silver nanoparticles compared with control treatments. Five replicates were used for validity. We also measured water properties (dissolved oxygen, pH, concentrations of silver nanoparticles before and after experiments). Calculation of LC_{50} at 24 hr was performed by plotting data of *M. macrocopa*'s death against concentrations of silver nanoparticles in each form (unit : mg/L) by using Probit analysis from Probit table (Finney's table) [18]. We also tested statistics of LC_{50} among different forms of silver nanoparticles using One way ANOVA analysis. Lastly, we also used water samples obtained from washing (experiment I) to study toxicity test on *M. macrocopa* [17].

3. Results and Discussion

3.1. Physical and Chemical Properties of Silver Nanoparticles in Fabrics

Investigation of morphological characteristics of nano Ag^0 and nano Ag_2O using Transmission Electron Microscopy (TEM) revealed that both had diameter less than 100 nm as showed in Fig. 1(a) and Fig. 2(a), respectively.

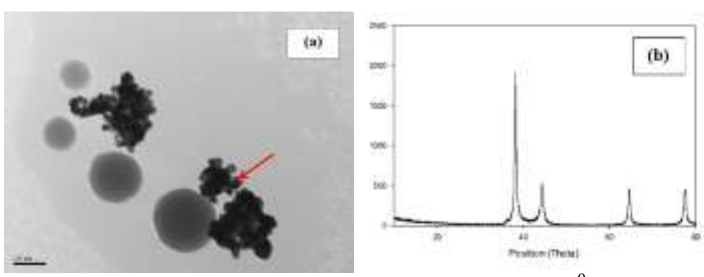

Fig. 1: TEM (a) and XRD (b) of nano Ag^0

The results of chemical properties of both using X-Ray Diffraction (XRD) are also presented in Fig. 1(b) and Fig. 2(b). The analysis of XRD results revealed that nano Ag^0 and nano Ag_2O show high-purify without any contamination of other elements. Most shapes of both nanoparticles were circular and were consistent with the study of Hackenberg et al. [19].

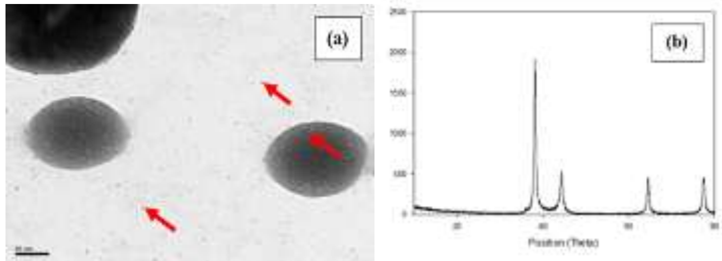

Fig. 2: TEM (a) and XRD (b) of nano Ag_2O

Nano Ag_2O particles were suspended in the water, while nano Ag^0 particles aggregated in the water. The aggregation of nano Ag^0 came from its magnetic properties. The data of Jo et al. [20] had show that the nano sized effect of Ag nanoparticles on the magnetic properties is ferromagnetic, instead of a diamagnetic component of the Ag bulk. The ferromagnetic component strongly depends on the surface of Ag nanoparticles.

In addition, the surface morphology of two types of fabrics was studied by SEM-EDS. The SEM picture also revealed that nano Ag^0 particles were incorporated into commercial fabrics as indicated by red arrows (Fig. 3(a)). Analysis of EDS also identified Ag element in fabrics. In contrast, Fig. 3(b) shows normal fabrics that did not use silver nanoparticles with total absence of silver nanoparticles.

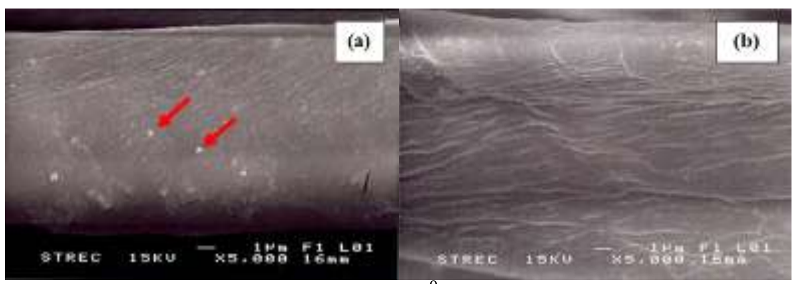

Fig. 3: SEM showing morphology of nano Ag^0 in fabrics (a) and without nano Ag^0 (b)

Perera et al. [21] revealed that the distribution and deposition of nanoparticles on the fabric depends on the interaction mechanism of nanoparticles and the fabric. Ag nanoparticles can be deposited or trapped between the warp and waft yarns, inside the nanoscale interstices of cellulose fibers or sometimes in the central lumen, depending on their size and concentration [22]. The result of total silver content from T-shirts that were cut from 3 parts (front, back and sleeves) was presented in Table I.

TABLE I: Contents of nano Ag^0 laced in fabrics

Areas of fabric (T-shirt) studied	Concentration (mg/g of fabric)
Front side	0.109±0.129
Back side	0.208±0.032
Sleeve	0.318±0.124
Average	0.212±0.104

The average content of silver nanoparticles in commercial T-shirt was 212 mg/kg of fabric. The initial silver content of the textiles was generally between 1.5 and 2,925 mg Ag/kg of fabric [5]. Water samples obtained from washing was analysed by TEM and the result shows that the main contents of wash water were suspended solid from fabrics itself as well as silver nanoparticles indicated by red arrows (Fig. 4).

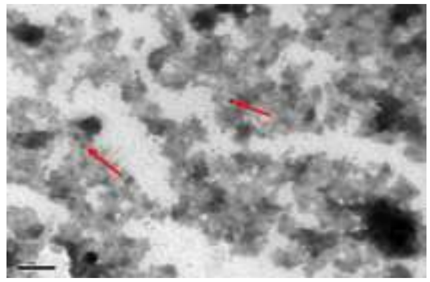

Fig. 4: TEM shows particles of nano Ag^0 in washing water

This indicated that nano Ag^0 particles in the form of solid phase can release from the fabric to wash water. The silver nanoparticles are stable up to two or three months and remains in zerovalent state, but they convert partially into oxidized state (Ag^+) when they expose to the water or air [23]. The physical and chemical of wash water characteristics is shown in Table II

TABLE II: Physical and chemical properties of the wash water (Round I and Round II)

Water quality analysis	Washing	
	Round I	Round II
Color	transparent	transparent
Odor	absence of odor	absence of odor
Temperature (C)	28.5	28.8
pH	6.68	6.82
Conductivity (mV)	19.6	11.5
Total Dissolved Solid (TDS) (mg/L)	110	82.9
Suspended Solid (mg/L)	<5	<5
DO (mg/L)	8.56	5.56
COD (mg/L)	196.8	116.8
Ag (mg/L)	122.6	67.12
Cr (mg/L)	<1	<1
Zn (mg/L)	<1	<1
Ni (mg/L)	<1	<1
Pb (mg/L)	<1	<1
Cd (mg/L)	<1	<1

After washing using a washing machine (Round I), silver nanoparticles were detached in higher quantities (122.60 µg/L) than in round II washing (67.12 µg/L). The same trend was found by Pasricha et al. [23]. They found that the silver nanoparticles in cotton fabric leaching after 1st wash, 2nd wash, and 3rd wash were 10%, 1.59% and 0.804% respectively. They also indicated that cotton released the most silver during the first wash, while nylon steadily increased its release of silver throughout subsequent washes. Thus, the fabric types have different longevities in which the Ag nanoparticles continue to function as an antimicrobial agent. Beside the effect of fabric types, the release of Ag nanoparticles from the textile depends on the one hand on the form and amount of Ag in the textiles, but also on the medium that is used for washing [5]. Benn and Westerhoff [24] reported that upto 86% of Ag released from the fabric washed by distilled water as dissolved Ag^+, whereas, Geranio et al. [7] revealed that Ag released by washing solution was much lower.

3.2. Effects of Silver Nanoparticles on Zooplankton

We studied toxicity test of nano Ag^0, Ag^+ and nano Ag_2O on M. *macrocopa*. Analysis of LC_{50} is presented in Table III.

TABLE III: LC_{50} analysis on water fleas at 24 hr

Different forms of nano silver	LC_{50} (mg/L)
nano Ag^0	1.59±0.58
Ag^+	0.045±0.12
nano Ag_2O	18.94±1.45
wash water (Round I)	-*
wash water (Round II)	-*

*At 24 hours, all water fleas were still alive.

The results revealed that Ag^+ were more toxic to water fleas as indicated by the lowest LC_{50}, followed by nano Ag^0 and nano Ag_2O, respectively. LC_{50} concentrations of Ag^+, nano Ag^0 and nano Ag_2O were 0.045±0.126, 1.59±0.589 and 18.94±1.45, respectively. Statistical analysis indicates that that there were significant differences of LC_{50} among silver nano forms (P-value < 0.05) (Table IV).

Table IV: Statistical analysis of LC_{50} of nano Ag^0, Ag^+ and nano Ag_2O on water fleas (P-value < 0.05)

Form of silver nanoparticles	nano Ag^0	Ag^+	nano Ag_20
nano Ag^0	-	7.53×10^{-5}	4.39×10^{-6}
Ag^+	7.53×10^{-5}	-	8.77×10^{-4}
nano Ag_20	4.39×10^{-6}	8.77×10^{-4}	-

We further used water obtained from washing of fabrics laced with silver nanoparticles to study toxicity test. The concentration of silver nanoparticles in water however was very low (122.6 µg/L and 67.12 µg/L) (Table II) and as a result, silver nanoparticles in the wash water did not have any negative effect on water fleas. All water fleas were still alive in wash water for 24 hr.

Ag^+ were the most toxic form to M. *macrocopa* and this may be linked to the fact that such ions can easily get inside water fleas through direct feeding and absorption through cells compared with other forms of silver nanoparticles. This study is consistent with Griffitt et al. [25] who indicated that silver ion solution was more toxic to D. pulex than silver particles sized 20-30 nm. The study of Bielmyer et al. [8] reported that no observable effect concentration (NOEC) of free silver solution on Daphnia spp. was 0.001 µg/L. Water fleas get free silver solution into the body through feeding of phytoplankton rather than direct consumption of silver nanoparticles [8]. Free silver solution can cause reproductive problems and can cause death. Oberdorster et al. [26] studied toxicity of silver nano particles on Daphnia magna and discovered that LC50 of D. magna at 24 hr was 125 mg/L. Silver nanoparticles were accumulated in gut and antennae of D. magna. His study was similar with our results that silver nanoparticles were present on external body and internal system of water fleas (Fig. 5).Toxicity of silver nanoparticles on macro invertebrates may come releasing of silver ions that affect the growth of cell [27].

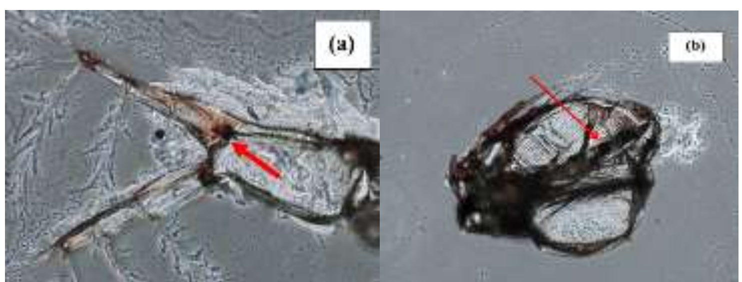

Fig. 5: Nano Ag^0 appearing outside (a) and inside (b) the bodies of water fleas

At high concentration of nano Ag^0, nano Ag^0 aggregated rapidly in water. Aggregated silver nanoparticles showed the lowest toxicity compared with the particle form because surface areas were less than particle forms. This may be explained that aggregation of silver nanoparticles was a bulky form floating on the surface of water thus, having lower ability to get into cells and body of water fleas. This study was in agreement with other studies that toxicity of silver oxide on *Daphnia* spp. was higher than aggregated silver nanoparticles. Griffitt et al. [25] studied toxicity of nano Ag_2O (20-30 nm) and found that LC_{50} of *D. magna* and *D. pulex* at 48 hr was 0.04 and 0.067 mg/L, respectively. However, LC_{50} of *M. macrocopa* in this study at 24 hr was in average 18.94 mg/L higher than the previous study. Lower toxicity of this study may be explained that the size of nano Ag_2O was larger than that of Griffitt et al. [25] and therefore causing less effect on *M. macrocopa*.

4. Conclusion

Silver nanoparticles can be detached from fabrics after washing and release to the environment. This can lead to water pollution and may affect aquatic organisms especially if the content of silver nanoparticle is high. Toxicity test of silver nanoparticles can clearly affect zooplankton. In the future, uses and development of silver nanoparticles should be with care and further investigation of effects of silver nanoparticles in freshwater ecosystems should be carried out.

5. Acknowledgements

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