

## SYNTHESIS OF ULTRA SMALL SILVER NANOPARTICLES USING $\text{Sn}^{2+}/\text{Sn}^{4+}$ REDOX COUPLE AS STRONG REDUCTANT

Lucie HOCHVALDOVÁ, Libor KVÍTEK, Aleš PANÁČEK, Robert PRUCEK, Radek ZBOŘIL

RCPTM, Palacky University in Olomouc, Olomouc, Czech Republic, EU, [lucie.hochvaldova01@upol.cz](mailto:lucie.hochvaldova01@upol.cz)

### Abstract

Application potential of colloidal particles and nanoparticles is related to their size, shape and other physical and chemical properties. Those properties can be influenced by various ways of synthesis, while the presence of reducing agent and modifiers (polymers, surfactants) are the most vital ones. One of the strongest reducing agents is tin with the redox couple  $\text{Sn}^{\text{II}}/\text{Sn}^{\text{IV}}$  and standard potential -0.384 V. This redox system allows to change redox potential just by addition of hydrogen peroxide to the tin(II) solution, which causes its partial oxidation to tin (IV) and changes the ratio between its oxidized and reduced form. Those changes effect redox potential (which is measured by potentiometer) and influent the tailored silver nanoparticles preparation. Antibacterial activity of prepared nanoparticles, with an average size between 3 to 50 nm, were tested against pathogenic and resistant bacteria, while the smallest nanoparticles have showed high antibacterial activity with minimal inhibition concentration (MIC) in units of mg/L.

**Keywords:** Silver nanoparticles, synthesis, redox potential, stabilization, antibacterial activity

### 1. INTRODUCTION

The beginning of the 21<sup>st</sup> century is the era of nanotechnologies. [1] It is already known that many properties are changing significantly with the size decrease to nanometre scale, different shape and polydispersity of the nanoparticles. Not every size of the nanoparticles is optimal for a specific use. For instance, antibacterial [2] and catalytic activity [3] is increasing with decreasing size of the particles. [4] On the other hand, small nanoparticles are not as effective at analytical applications in biosensors, which are using interesting optical properties of nanoparticles. [5] Another reason why we need different sizes of nanoparticles is Raman spectroscopy, where each laser wavelength requires a different size for ideal enhancement of analyte's Raman signal. [6]

Thanks to different size requirements of the silver nanoparticles, a big part of research is focused on tailored methods for silver particle preparation. The most commonly used methods for metal nanoparticles synthesis are those based on reduction of soluble salts. [7] Generation of nanoparticles is associated with creation of new phase in originally homogenous system, which is related to saturation. Saturation depends on the speed of the reaction which is directly connected to the value of redox potentials. [8] Therefore, the change of redox potentials can be used to influence reaction and creation of silver nanoparticles. Unfortunately, with common reducing agents -  $\text{NaBH}_4$  [9], citrate [10], hydrazine [11], hydroxylamine [12] or sugars [13] redox potential cannot be simply changed. On the other hand, with redox system based on redox pair  $\text{Sn}^{2+}/\text{Sn}^{4+}$  redox potential can be simply changed by the change of ratio of both redox form of tin.

In this paper the influence of redox potential on the size of silver nanoparticles has been studied. Defining the change of redox potential of commonly used reducing agents is difficult, which is why reduction system based on tin(II) chloride has been used. By change of the ratio tin(II)/tin(IV) ions in the system, redox potential has been changed, which influenced the reduction of silver ions. First, the synthesis of small nanoparticles prepared by reduction of silver ions by tin(II) chloride has been optimized. The antibacterial activity of the system has been tested and eventually removal of the access of tin compounds from the system has been studied.

## 2. EXPERIMENTAL SECTION

Silver nitrate  $\text{AgNO}_3$  (Fagron), polyacrylic acid (PAA) Mr 8000 (45 % w/v, Aldrich), ammonia  $\text{NH}_3$  (28-30 %, Sigma Aldrich), tin(II) chloride  $\text{SnCl}_2$  (Sigma Aldrich), hydrogen peroxide  $\text{H}_2\text{O}_2$  (30 %, Penta) and sodium hydroxide  $\text{NaOH}$  (Lach: Ner) were used without any additional purification. Deionized water (18  $\text{M}\Omega\cdot\text{cm}$ , Millipore) was used to prepare all solutions.

Zetasizer Nano ZS (Malvern, UK) and transmission electron microscope (TEM) JEM 2010 (JEOL, Japan, 160 kV) have been used to determine size and polydispersity of prepared nanoparticles. All UV/VIS spectra have been measured on spectrophotometer Specord S 600 (Analytik Jena AG, Germany). Concentration of silver and tin in the dispersion has been measured by atomic absorption spectroscopy (contraAA300, Analytik Jena, Germany), which allowed us to recalculate obtained absorption of the sample on its concentration. Calibration solution with concentrations of 10, 20, 30 and 40 mg/l of tin have been used for tin and concentrations of 0.25; 0.5; 1; 1.5 and 2 mg/l of silver have been used for silver. Nanoparticles have been prepared with continuous stirring on electromagnetic stirrer and redox potential with pH of the solution has been measured by pH 2700 instrument (Geotech Instruments, USA). Centrifugation has been done on centrifuge (Eppendorf Centrifuge 5702) and for redispersion of silver nanoparticles has been used ultrasound generator CL-334 20100097 (Q Sonica sonicators).

## 3. RESULTS AND DISCUSSION

### 3.1. Preparation of silver nanoparticles

Colloidal dispersion of silver nanoparticles was prepared by modified Tollens method using tin(II) chloride as a reducing agent. Stock solutions were in a particular order and amount (shown in **Table 1**) added into beaker, which was cooled with ice and placed on electromagnetic stirrer. Total amount of the reaction mixture always ended up to 25 ml, while distilled water was used to fill up the missing volume.

**Table 1** The amount of the stock solutions (ml) used for preparation of 1-5 samples of silver nanoparticles

| Stock solution         | Concentration (mol/l) | 1 | 2 | 3   | 4 | 5 |
|------------------------|-----------------------|---|---|-----|---|---|
| $\text{AgNO}_3$        | 0.005                 | 5 | 5 | 5   | 5 | 5 |
| PAA 8000               | 0.008                 | 5 | 5 | 5   | 5 | 5 |
| $\text{NH}_3$          | 0.05                  | 5 | 5 | 5   | 5 | 5 |
| $\text{SnCl}_2$        | 0.25                  | 1 | 1 | 1   | 1 | 1 |
| $\text{H}_2\text{O}_2$ | 0.05                  | 0 | 1 | 1.5 | 2 | 3 |

Diamine-silver complex  $[\text{Ag}(\text{NH}_3)_2]^+$ , prepared by reaction of silver nitrate with ammonia, was reduced by tin(II) chloride (which was pre-modified by addition of hydrogen peroxide (**Table 1**)). Peroxide was added to the tin(II) chloride solution to partially oxidize tin(II) ions to tin(IV), which affected the ratio of oxidized and reduced form and influenced redox potential of the system and consequently the reduction of silver ions by itself.

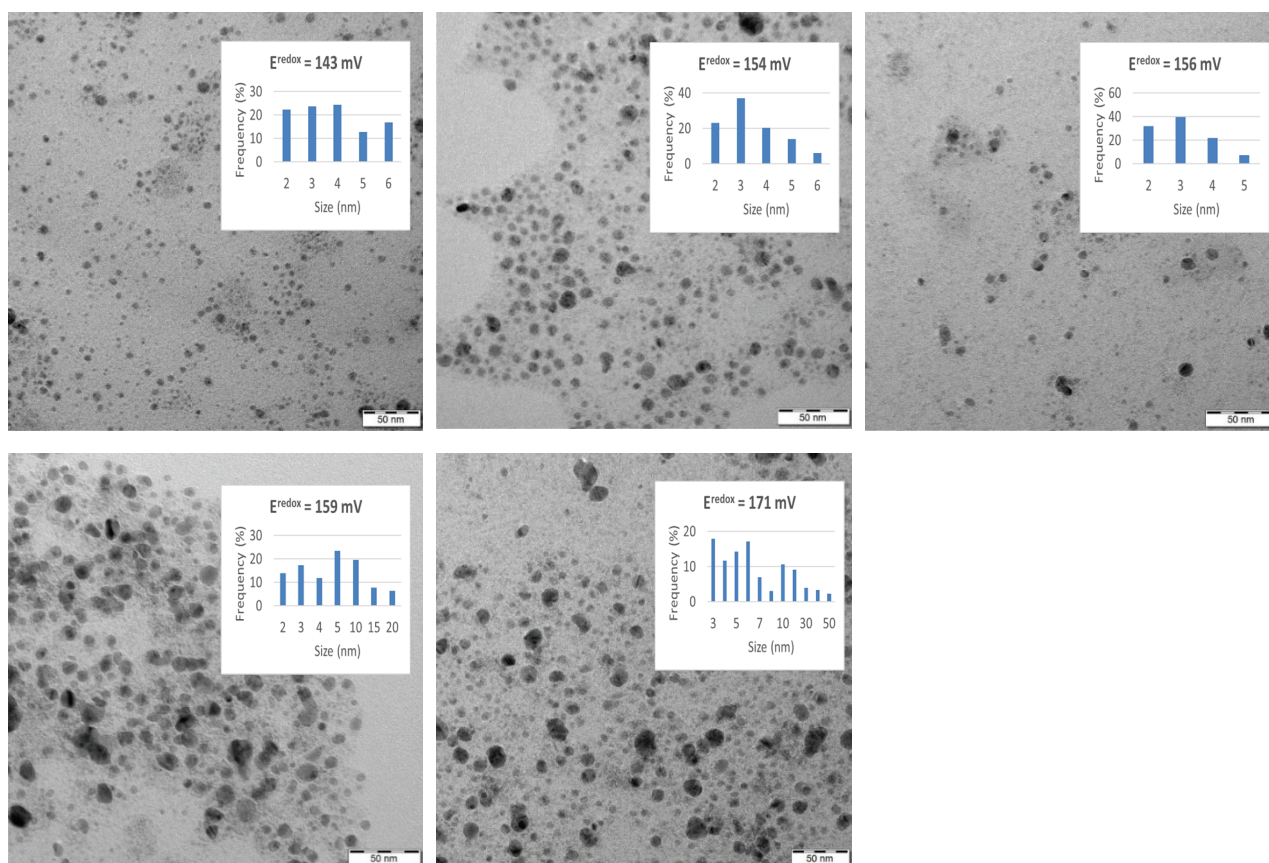
After addition of tin(II) chloride to the reaction mixture, the colour of dispersion has changed which indicated reduction of silver ions to silver nanoparticles. System prepared in this way was not stable by itself, particles started to aggregate and sediment in a while. To ensure the stability of the whole system polyacrylic acid (PAA Mr 8000) has been added to the solution.

### 3.2. Redox potential of the system

Redox potentials of each dispersion were measured by potentiometer. The values of the potential have changed with the change of the ratio tin(II) and tin(IV), which has been caused by addition of hydrogen

peroxide to the tin(II) solution. (**Table 1**) More hydrogen peroxide was added the higher amount of tin(II) was oxidised to tin(IV), which has led to the increase in redox potential (**Table 2**) and decrease of its reduction strength.

The size of the prepared silver nanoparticles was determined from TEM images (**Figure 1**). Three of the images for each sample were evaluated via program Gwyddion which in invert value mode allows to measure the size of the nanoparticles automatically. If the contrast of the nanoparticles was not good enough to ensure correct recording of the size, nanoparticles were measured manually, while all the nanoparticles presented on the image were measured. Minimally 200 particles were accounted in determination of the distribution diagram of the prepared nanoparticles.



**Figure 1** TEM images of silver nanoparticles prepared in systems with different redox potential (distribution diagrams of the prepared nanoparticles are presented in insets)

Mean diameter ( $\bar{d}$ ) of the particles, corrected sample standard deviations ( $\sigma$ ), and polydispersity (PDI) were calculated from size distributions presented on **Figure 1** and all the values are shown in **Table 2**.

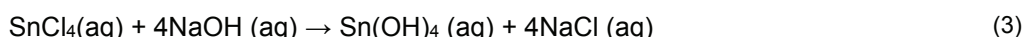
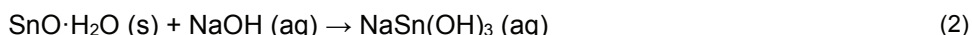
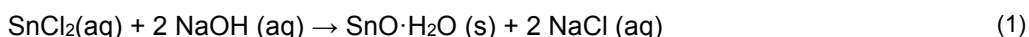
**Table 2** Average size of the nanoparticles and their polydispersity for systems prepared at different redox potentials

| Sample | Redox potential (mV) | Size (nm)          | Standard deviation (nm) | PDI       |
|--------|----------------------|--------------------|-------------------------|-----------|
| 1      | 143                  | 3.8                | 1.4                     | 0.13      |
| 2      | 154                  | 3.3                | 1.2                     | 0.14      |
| 3      | 156                  | 2.9                | 1.0                     | 0.12      |
| 4      | 159                  | 3.7/13.6 (bimodal) | 1.2/10.9                | 0.10/0.65 |
| 5      | 171                  | 4.9/23.5 (bimodal) | 1.4/23.9                | 0.09/1.03 |

As you can see in **Table 2**, small addition of hydrogen peroxide to the reducing agent does not oxidise huge amount of tin(II), so in this case tin keeps its strong reducing properties and forms small nanoparticles with sizes in the range from 2 to 6 nm. As higher amounts of hydrogen peroxide are added, higher amount of tin(II) is oxidised to tin(IV) and tin is losing its reducing properties and bigger particles are formed. After addition 2 or 3 ml of hydrogen peroxide system becomes polydisperse (bimodal) and next to the small particles (smaller than 10 nm), big nanoparticles in 10ths of nm are formed. This change of the size caused by different addition of hydrogen peroxide proves the dependency of particle size on redox potential of the system, which is controlled by different addition of hydrogen peroxide. (**Table 2**)

### 3.3. Tin removal

The last part of this research was focused on tin removal from the system. Previously described silver nanoparticles dispersion has contained gel-like precipitate of oxide-hydroxide tin compounds. More tin (IV) was present in the system, the higher amount of precipitate has been formed. At the same time the concentration of the reducing agent (tin(II) chloride) was 10 times higher than concentration of silver therefore many methods have been tested to ensure proper tin removal. The best way how to remove tin from the system has been alkalization of the system which causes aggregation of silver nanoparticles and at the same time soluble tin salts are formed. (**Reaction scheme 1-3**)



The system has been alkalized by addition of NaOH until its final concentration reached concentration 0.24 mol/l. This concentration has been established within partial study focused on influence NaOH concentration on tin compounds separation from silver nanoparticles. After the precipitation, the solid part has been separated from the liquid via centrifuge (5 min, 4,400 revolutions per minute). Afterwards the liquid part has been removed and the solid one has been diluted in the same volume of distilled water with PAA and sonicated for 1 minute with Q Sonica sonicators (E=2.062J). Nanoparticles purified this way stays thanks to PAA stable and their size stays unchanged. Concentration of tin in the system after alkalization has been determined by atomic absorption spectroscopy. The result has shown that only one alkalization is not sufficient enough to remove all tin compounds from the system and it needs to be repeated. After the second alkalization the concentration of the tin has been lower than detection limit of the device. Furthermore, with each alkalization there has been higher losses of silver and system becomes less stable. In table below are shown percentage decreases (%) of tin and silver (**Table 3**). From these data is apparent that 2 alkalizations have not caused big decrease in silver concentration and more than 97 % of original amount of tin has been removed.

**Table 3** Percentage representation of tin and silver content in silver nanoparticles dispersion after individual steps of alkalization

| Sample | Tin              |       |                 | Silver           |       |                 |       |
|--------|------------------|-------|-----------------|------------------|-------|-----------------|-------|
|        | 1 x alkalization |       | 2x alkalization | 1 x alkalization |       | 2x alkalization |       |
|        | Sn (%)           | σ (%) | Sn (%)          | Ag (%)           | σ (%) | Ag (%)          | σ (%) |
| 1      | 20.9             | 9.2   | ≤ 2.8           | 97.7             | 1.2   | 92.5            | 3.7   |
| 2      | 18.1             | 6.7   |                 | 98.1             | 1.9   | 91.7            | 1.0   |
| 3      | 15.4             | 2.2   |                 | 97.4             | 2.9   | 95.0            | 0.0   |
| 4      | 12.9             | 3.3   |                 | 98.5             | 1.0   | 91.1            | 3.7   |
| 5      | 8.0              | 1.5   |                 | 95.7             | 3.6   | 61.9            | 0.8   |



### 3.4. Biological activity of the prepared silver nanoparticles

Antibacterial activity has been tested by microdilution method, which establish minimum inhibitory concentration (MIC). MIC determines minimal concentration, which inhibits the growth of the bacteria. The testing has been carried out on microtiter plates, while all samples have been diluted with 100  $\mu$ l MH broth by geometric series from 2 to 128 times. Bacterial strain shown in **Table 4** have been injected (concentration  $10^6$  ml<sup>-1</sup> CFU (colony forming units)) and incubated for 24 hours (37 °C) and then tested for their antibacterial activity.

**Table 4** MIC of silver nanoparticles against gram-positive and gram-negative bacteria

| Bacteria                        | MIC (mg/l)                                     |  |  |   |   |
|---------------------------------|--|--|--|---|---|
|                                 | 0 ml H <sub>2</sub> O <sub>2</sub><br>(3.8 nm) | 1 ml H <sub>2</sub> O <sub>2</sub><br>(3.3 nm) | 1,5 ml H <sub>2</sub> O <sub>2</sub><br>(2.9 nm) | 2 ml H <sub>2</sub> O <sub>2</sub><br>(3.7/13.6 nm) | 3 ml H <sub>2</sub> O <sub>2</sub><br>(4.9/23.5 nm) |
| Enterococcus faecalis CCM 4224  | 6.75   | 3.38   | 3.38   | 6.75  | 13.5  |
| Staphylococcus aureus CCM 3953  | 1.69   | 1.69   | 1.69   | 3.38  | 6.75  |
| Escherichia coli CCM 3954       | 1.69   | 1.69   | 0.84   | 3.38  | 3.38  |
| Pseudomonas aeruginosa CCM 3955 | 3.38   | 3.38   | 3.38   | 6.75  | 13.5  |

**Table 4** provides evidence that silver nanoparticles synthesized with smaller addition of hydrogen peroxide (0-1.5 ml) have higher antibacterial activity than those synthesized with two or more millilitres. This phenomenon is related to the size of the nanoparticles, while for small addition of hydrogen peroxide only small particles were present, which have caused an increase of their antibacterial activity. On the other hand, for higher addition of hydrogen peroxide, bigger particles in tenths of nanometres have been formed as well, which eventually caused slight decrease in antibacterial activity of the system. Values for gram-positive (Enterococcus faecalis, Staphylococcus aureus) and gram-negative (Escherichia coli, Pseudomonas aeruginosa) bacteria are similar, which means silver nanoparticles have on them the same effect.

## 4. CONCLUSION

The synthesis of silver nanoparticles prepared by modified Tollens method using reduction of diamine-silver complex by tin compounds was studied in this paper. Reduction systems containing tin(II) chloride as a reducing agent allows easy modification of redox potential. Hydrogen peroxide was used to oxidise tin(II) to tin(IV), which changed the ratio of oxidised and reduced form. Additionally, it caused the change of redox potential, which allowed us to study its influence on the size of prepared silver nanoparticles. With a small additions of hydrogen peroxide were prepared small particles with size ranging from 2 to 6 nm. Whereas with addition of two or more millilitres were prepared bigger particles with bimodal distribution. In this case system has contained small particles which were smaller than 6 nm and also bigger particles with a size ranging in tens of nanometres. Antibacterial activity of prepared nanoparticles has been tested against pathogenic and resistant bacteria, while the smallest nanoparticles (prepared by addition of 0-1.5 ml H<sub>2</sub>O<sub>2</sub>) have shown high antibacterial activity against gram-positive and gram-negative bacteria with minimal inhibitory concentration (MIC) in units of mg/L.

The second aim of this work was to find a method for tin compounds removal (reducing agent) from the system. Many methods were tested, but the most efficient one was alkalization of the system by concentrated sodium hydroxide, which has led to the precipitation of silver nanoparticle's and dissolution of tin compounds. The liquid phase was easily separated after centrifugation, and after twice repeated alkalization more than 97 % of tin was removed from silver nanoparticle dispersions.

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