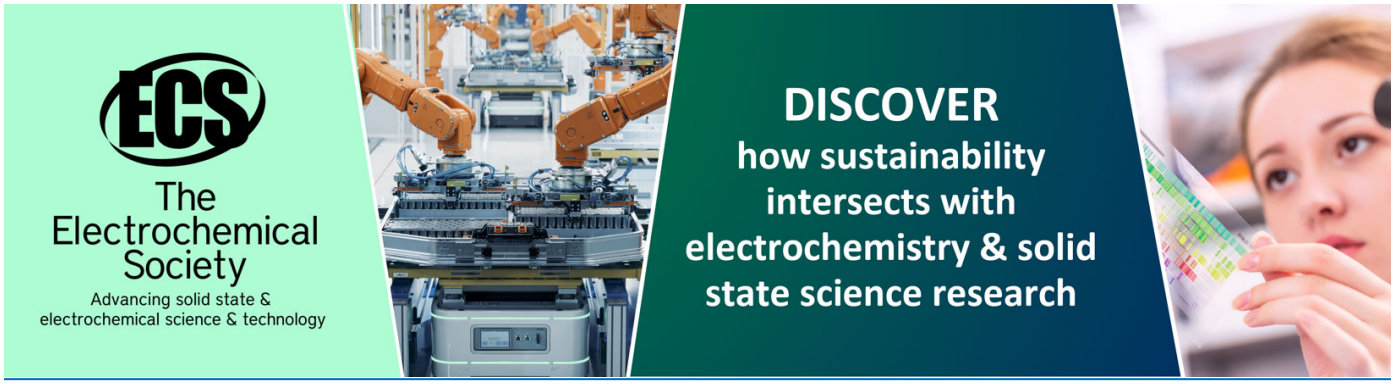
Copper Nanoparticles: Synthesis and Biological Activity



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Copper Nanoparticles: Synthesis and Biological Activity

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Abstract. By means of XRD and FESEM analysis, it is established that copper nanoparticles with sizes less than 10 nm are formed during the chemical reduction, which form aggregates mainly with spherical shape. Presence of gelatin during the chemical reduction of copper induced formation of smaller size distribution nanoparticles than that of nanoparticles synthesized without gelatin and it can be related to formation of protective layer. Synthesized Cu nano-powders have sufficiently high activity against the *Erwinia amylovora* bacterium, and the bacterial growth inhibition depends on the Cu nanoparticles concentration. At a concentration of 5 mg / ml of Cu nanoparticles, the exciter growth inhibition zone reaches a maximum value within 72 hours and the lysis zone is 20 mm, and at a concentration of 1 mg / ml this value is 16 mm, which also indicates the significant antibacterial activity of this sample.

1. Introduction

Synthesis and prediction of the properties of metal nanodisperse systems open wide opportunities for the creation of highly effective new drugs with biological activity for use in medicine and agriculture [1]. Metal nanoparticles show a pronounced biological activity including bacteriostatic and bactericidal effects [2]. Therefore, the study of the regularities in the manifestation of the biological activity of nano-disperse metals, depending on the method of their production, shape, size and physicochemical properties, is an urgent task.

The purpose of this study is to prepare Cu nanoparticles by chemical reduction method and to study their biological activity.

2. Experimental

2.1. Synthesis and characterization of Cu nanoparticles

Aqueous solutions containing Cu ions were prepared from copper sulphate crystal hydrate ($CuSO_4 \cdot 5H_2O$), 64% solution of hydrazine hydrate ($N_2H_4 \cdot H_2O$) was served as a reducing agent. As a stabilizer for Cu nanoparticles food gelatin was used, which was purified by washing three times in distilled water.

Nanosized Cu- powder synthesis is based on the chemical reduction of Cu (II) ions by hydrazine. Reducing activity of hydrazine is enhanced at pH> 10, therefore Cu nanoparticles were synthesized in alkaline and ammonia medium at pH of 11.

To synthesize Cu nanopowder in an alkaline or ammonia medium concentrated sodium hydroxide solution or a 10% ammonia solution was added into a 50 ml of an aqueous solution of copper (C_{Cu}^{2+} = 0.05 M) a until the solution pH reached to 11. The solution is then heated to 50-60°C in a water bath and a hydrazine solution is added into the heated solution with an amount exceeding (in moles) 10 times the excess of copper ions. The solution with continuous stirring with a magnetic stirrer was held at this temperature for 30 minutes. The reduction product is then separated from the liquid using a centrifuge, washed with water until neutral (pH=) and ethanol. Obtained product was dried in drying oven at 55-60°C. During the synthesis, the gelatin solution was added to the copper salt solution in such amount that its content in the resulting mixture is 0.2%.

The phase composition of the products obtained from chemical reduction was established by X-ray diffraction (XRD) analysis. The diffractograms were taken on a RINT-2500 HV X-ray diffractometer (Kumamoto University, Japan) using copper-filtered radiation. Field emission scanning electron microscopy (FE-SEM) was used to determine the dispersion and morphology of the nanopowders. Micrographs of finely dispersed copper particles were taken on FE-SEM, JOEL JSM-7600F (Kumamoto University, Japan).

2.2. Biological activity of Cu nanopowders

A causative agent of fruit tree diseases – the gram-negative bacterium *Erwinia amylovora* (strain BK) was used as a test-object for the biological activity of copper nanoparticles. The bacterial strains were taken from laboratory collection bacteria from phytopathology laboratory of the Kyrgyz-Turkish "Manas" University.

The metal nanoparticles antibacterial action evaluation was carried out by the agar block method; for this, the culture of the test bacterium was grown on the surface of a solid nutrient medium, then holes with diameter of 5 mm were made using a sterile metal drill. 1 ml of an aqueous solution of copper nanoparticles was added to the wells at various concentrations and the Petri dishes with the cultures were further incubated at a constant temperature (27-28° C). The lysis zone of bacterial colonies around the agar blocks was observed and measured within 72 hours.

The biological activity of all Cu nanopowder samples (synthesized in alkaline and ammonia media, with and without gelatin) was investigated. Cu nanopowder solutions were prepared with a concentration of 5, 1, 0.5 and 0.1 mg per 1 ml.

3. Results and Discussion

3.1. Crystal structure, morphology and size of the nanoparticles

The reduction of Cu ions by hydrazine was carried out in an alkaline and ammonia medium with no use of stabilizer, and in second experiment with gelatin as a stabilizer. XRD diffractions of the obtained products are presented in figures 1 and 2.

The results show that the content of oxide phases is high in an alkaline medium. Along with metallic Cu, the product contains oxides of Cu_2O and CuO (figure 1a). In an ammoniacal medium, Cu^{2+} ions are mainly reduced to a metal, and univalent copper oxide is present in a small amount (figure 1b).

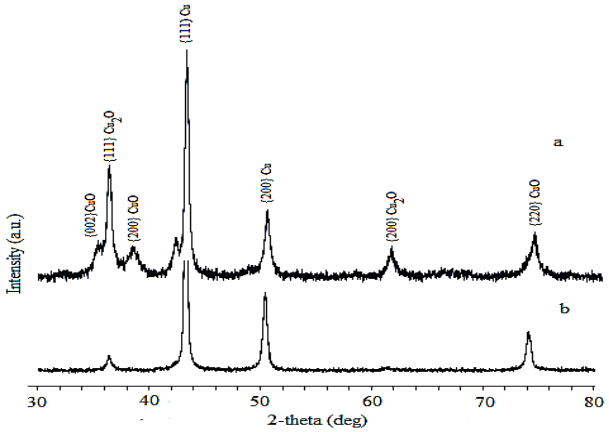

Figure 1. XRD patterns of Cu reduction with hydrazine products, in alkaline (a) and ammonia (b) media.

The size of the coherent scattering regions (CSR) of Cu is estimated from the broadening of the diffraction profiles by the Scherrer-Selyakov equation [3]. The sizes of Cu particles obtained in alkaline medium ranged from 20 to 43 nm, in ammonia medium they have sizes from 26 to 30 nm. Cu nanoparticles obtained in an ammonia medium have a narrower size distribution, compared to particles obtained in an alkaline medium.

The reaction medium composition effect can be explained by the fact that the reduction of Cu occurs from various forms of Cu ions. In alkaline medium at pH 10-11, Cu^{2+} ions are transferred to sparingly soluble hydroxide $Cu(OH)_2$, and in the ammonia medium a complex ion $[Cu(NH_3)_4]^{+}$ is formed. These forms of Cu are different in the dissociation constant and in the electrode potentials value. This quantities influence the recovery process and, accordingly, on formed products [4].

To improve the Cu nanoparticles aggregative stability, their synthesis was carried out in the presence of gelatin.

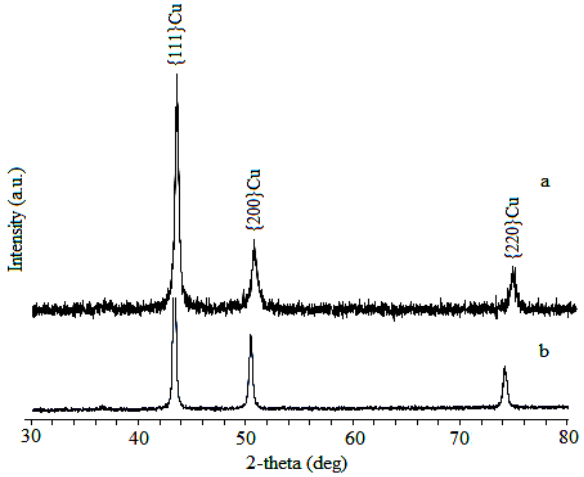

Figure 2. XRD patterns of Cu reduction with hydrazine products in the presence of gelatin in alkaline (a) and ammonia (b) media.

In the presence of gelatin in both alkaline and ammonia media, Cu^{2+} ions are almost completely reduced to metal (figure 2).

By the broadening of the diffraction profiles, the dimensions of the Cu nano-crystallites obtained in the alkaline medium in the presence of gelatin are ranged from 20 to 25 nm, and for Cu particles obtained from the ammonia with gelatin the size ranged from 22 to 33 nm. Such a narrow distribution of Cu particles obtained in the presence of gelatin in comparison with particles obtained without stabilizers is due to the fact that gelatin, forming a protective film on the surface of Cu nanoparticles, prevents aggregation and reduces an average size of the nanoparticles.

The nanopowders dispersion was studied by electron microscopy (figures 3 and 4).

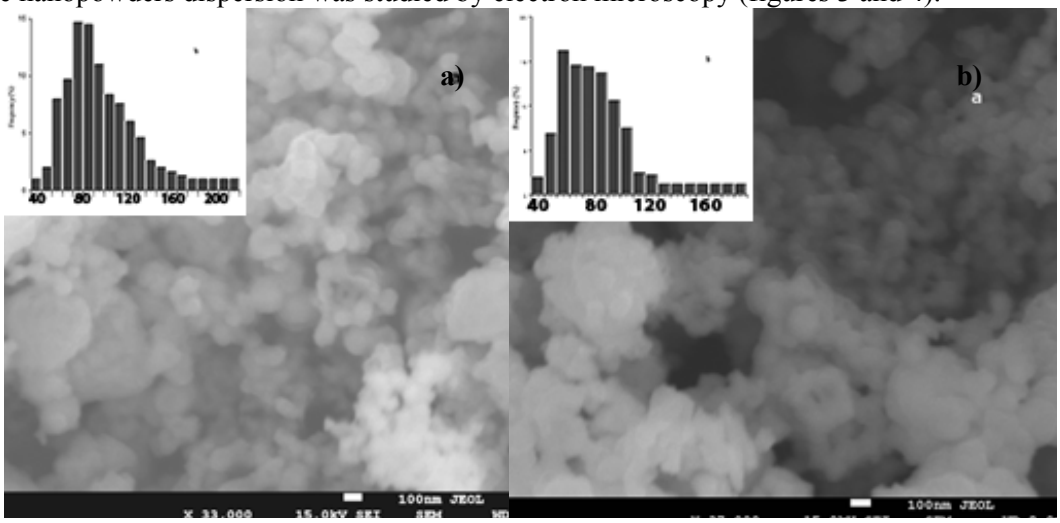

Figure 3. SEM images of Cu nanopowders produced in an alkaline medium (a) and Cu nanopowders obtained in an alkaline medium in the presence of gelatin (b) with their particle size histograms in their insets.

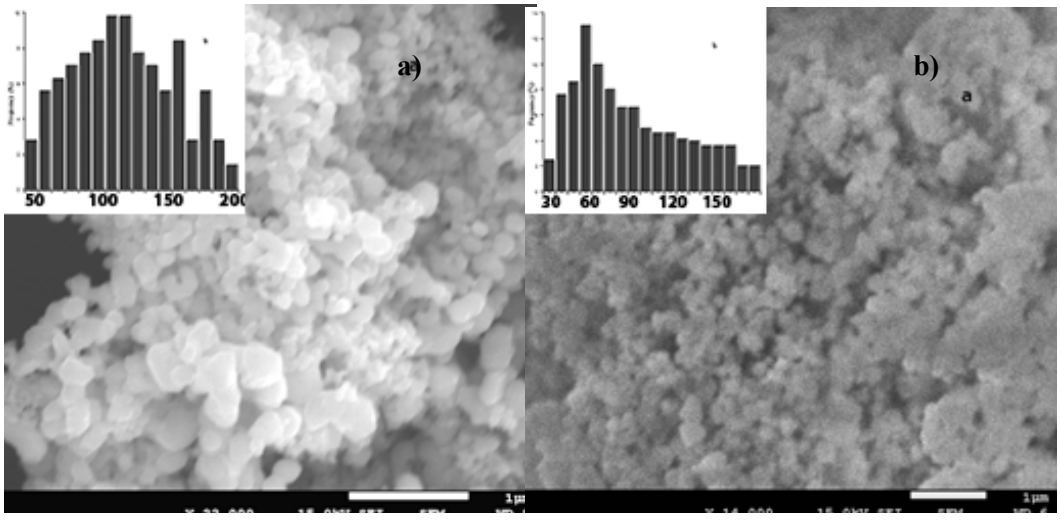

Figure 4. SEM images of Cu nanopowders obtained in an ammonia medium (a) and Cu nanoparticles obtained in the presence of gelatin in an ammonia medium (b) with their particle size histograms in their insets.

FESEM images of Cu nanoparticles synthesized in alkaline and ammonia media with and without gelatin show that in all four samples the particles are spherical in shape. Insets of the figures 3a and 4a

show histograms of Cu nanoparticles synthesized in alkaline and ammonium media, respectively. As shown from the figures, Cu nanoparticles synthesized in an alkali medium have an average size of 70-100 nm, and in an ammonia medium 100-140 nm. When comparing the photomicrographs of Cu nanoparticles obtained in an alkaline medium without a stabilizer and with the addition of gelatin (insets of the figures 3a and 3b), one can see that when gelatin stabilizes, more fine particles are formed and the particle size distribution interval is somewhat narrower than in the case of a sample obtained without a stabilizer in an alkaline medium.

The average size of Cu nanoparticles obtained in an ammonia medium in the presence of gelatin (figure 4a) lies in the range of 50-70 nm. Such a small diameter and narrow size distribution of Cu nanoparticles obtained in the presence of a stabilizer may be associated with an increase of nanoparticles stability in the presence of gelatin [5].

A comparative analysis showed that the average sizes of the nanoparticles determined by the FESEM are substantially larger than the dimensions of the CSR. This is because the FESEM captures the total particle size, including the polymer stabilizer shell. In addition, Cu nanoparticles are prone to some aggregation, even in the presence of a stabilizer. These facts allow us to assume that peaks that appear in the region of 160-200 nm in all four histograms belong to agglomerates consisting of smaller nanoparticles.

3.2. Biological activity of the Cu nanoparticles

The biological activity of Cu nanopowders synthesized during hydrazine reduction in alkali and ammonia media in the absence and presence of gelatin was tested against the causative agent of *Erwinia amylovora* crop diseases (the causative agent of bacterial apple and pear burn). Figure 5 shows the zones of inhibition of the growth of *Erwinia amylovora* (red circle marked) under the Cu nanoparticles action synthesized by chemical reduction.

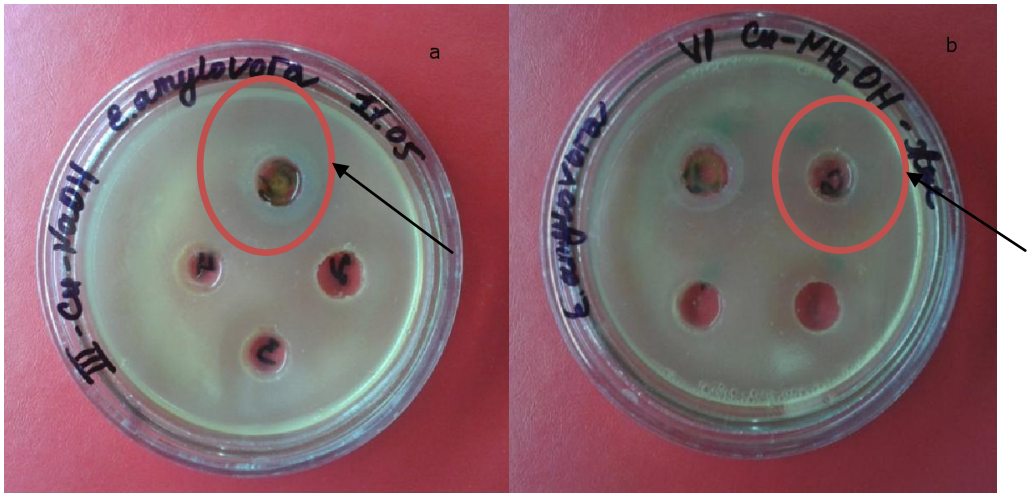

Figure 5. The zone of suppression of the growth of the *Erwinia amylovora* culture under the action of Cu nanoparticles obtained by chemical reduction with hydrazine in alkaline medium (a) and in ammonia medium (b).

The results of the study of the antibacterial activity of Cu nanopowders synthesized by the chemical reduction method show that all Cu nanopowders have a sufficiently high activity for the *Erwinia amylovora* [6] bacterial burn causative agent. The area of pathogen growth inhibition depends on the Cu nanoparticles concentration (table 1).

Table 1. Zone inhibition growth (mm) dependence of the bacterium *Erwinia amylovora* bacterium burner on the Cu nanoparticles concentration after 72 hours.

№	Copper nanopowders	Cu nanoparticle concentration			
		5 mg/ml	1 mg/ml	0,5 mg/ml	0,1 mg/ml
1	Cu-1	20,0	16,0	10,0	4,0
2	Cu-2	18,0	4,0	4,0	3,0
3	Cu-3	18,0	7,0	4,0	1,0
4	Cu-4	17,0	12,0	8,0	1,0

Cu-1 -copper nanopowder synthesized in alkaline medium.
Cu-2 copper nanopowder synthesized in ammonia medium;
Cu-3- copper nanopowder synthesized in alkaline medium in the presence of gelatin;
Cu-4 copper nanopowder synthesized in an ammonia medium in the presence of gelatin.

From the table 1 one can see that the Cu nanoparticles synthesized by the chemical reduction in an alkaline medium show the best results with respect to the causative agent of *Erwinia amylovora* bacterial burn. At a concentration of 5 mg / ml of Cu nanoparticles, the exciter growth inhibition zone reaches a maximum value within 72 hours and the lysis zone is 20 mm, and at a concentration of 1 mg / ml this value is 16 mm, which also indicates the significant antibacterial activity of this sample. Cu nanoparticles synthesized in alkaline medium at concentrations of 0.5 mg / ml and 0.1 mg / ml also show activity against this bacterium and the suppression zone is 10 and 4 mm, respectively. Biological activity of the Cu nanopowders obtained in alkaline medium in the presence of gelatin at concentrations of 1.0 mg / ml, 0.5 mg / ml and 0.1 mg / ml with respect to the causative agent of *Erwinia amylovora* bacterial burn is two or more times less than that of Cu nanopowders obtained in an alkaline medium in the absence of gelatin.

Comparison of the antibacterial activity of Cu nanopowders obtained in an ammonia medium in the absence and presence of gelatin shows that at a nanoparticle concentration of 5.0 mg /ml, the suppression zone of both samples practically coincides. At 1.0 mg / ml and 0.5 mg / ml concentrations, biological activity of Cu nanoparticles synthesized in the presence of gelatin is several times higher.

4. Conclusions

Copper nanoparticles with sizes less than 10 nm are formed during the chemical reduction, which form aggregates mainly with spherical shape and various sizes. The particle size distribution differs slightly depending the media and ranged from 20 to 40 nm. Presence of gelatin during the chemical reduction of copper can lead to smaller size distribution, which can be related to formation of protective layer.

Synthesized Cu nano-powders have a sufficiently high activity against the *Erwinia amylovora* bacterium, and the bacterial growth inhibition depends on the Cu nanoparticles concentration. Cu nanoparticles synthesized by the chemical reduction in an alkaline medium show the best results with respect to the causative agent of *Erwinia amylovora* bacterial burn. At a concentration of 5 mg / ml of Cu nanoparticles, the exciter growth inhibition zone reaches a maximum value within 72 hours and the lysis zone is 20 mm, and at a concentration of 1 mg / ml this value is 16 mm, which also indicates the significant antibacterial activity of this sample.

Acknowledgements

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