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НАУКА, НОВЫЕ ТЕХНОЛОГИИ И ИННОВАЦИИ КЫРГЫЗСТАНА, № 4, 2020

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СУЮКТУКТУН ИЧИНДЕГИ ПЛАЗМА МЕТОДУ МЕНЕН АЛЫНГАН Ag/Cu НАНОКОМПОЗИТТЕРИ ЖАНА АЛАРДЫН АНТИБАКТЕРИАЛДЫК КАСИЕТТЕРИ

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НАНОКОМПОЗИТЫ Ag/Cu ПОЛУЧЕННЫЕ МЕТОДОМ ПЛАЗМЫ В ЖИДКОСТИ И ИХ АНТИБАКТЕРИАЛЬНЫЕ СВОЙСТВА

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Ag/Cu NANOCOMPOSITES BY THE PLASMA IN LIQUID METHOD AND THEIR ANTIBACTERIAL PROPERTIES

УДК: 66.088

Бул изилдөөдө импульстук плазма ыкмасы менен күмүш жана жез таякчаларын анод жана катод катары колдонуп, синтездөө шарттарын өзгөртүү менен, тактап айтканда анод жана катоддун материалдарын өзгөртүү (Ag-Ag, Cu-Cu, Ag-Cu, CuAg, Ag-CuAg, CuAg-Ag, Cu-CuAg, CuAg-Cu) жана суюктуктун түрүн (суу, этил спирти, суу+беттик активдүү зат) өзгөртүү менен металл наноболүкчөлөр алынды. Синтезделген нанобөлүкчөлөрдү рентген-фазалык анализи менен жана ТЭМ (Трансмиссиондук Электрондук Микроскоп) микроскобу менен анализденип, андан соң антибактериалдык касиети аныкталды. Үлгүлөрдүн биологиялык антибактериалдык активдүүлүгү салмонелла бактериясы менен текшерилди. Тажрыйбанын натыйжасында антибактериалдык касиети боюнча синтезделген нанобөлүкчөлөрдүн арасынан Ag-Ag сууда алынган нанокомпозиттер эң жогорку антибактериалык касиетти көрсөттү.

Негизги сөздөр: *металлдык нанобөлүкчө, электрикалык разряд, импульстук плазма, кумуш, жез, күмүш-жез, антибактериалдык касиети, беттик активдүү заттар, эксперименталдык шарттар.*

В этом исследовании, метод импульсной плазмы использован для получения наночастиц серебра и меди с изменением экспериментальных условий таких как материалы анода и катода (Ag-Ag, Cu-Cu, Ag-Cu, CuAg Ag-CuAg, CuAg-Ag, Cu-CuAg, CuAg-Cu) и состав жидкой среды (вода, этиловый спирт, вода+ ПАВ). Синтезированные наночастицы были изучены с помощью рентгенофазового анализа, ПЭМ (просвечивающего электронного микроскопа), а затем были определены их антибактериальные свойства. Антибактериальная активность образцов была испытана с использованием бактерии сальмонеллы. Результаты опыта показали, что нанокомпозит Ag-Ag в воде имеет высокую антибактериальную активность.

Ключевые слова: *металлические наночастицы, электрический разряд, импульсная плазма, серебро, медь, серебро-медь, антибактериальная активность, поверхностно-активные вещества, экспериментальные условия.*

In this study, the pulsed plasma method was used to obtain silver and copper nanoparticles by changing experimental conditions such as anode and cathode material (Ag-Ag, Cu-Cu, Ag-Cu, CuAg Ag-CuAg, CuAg-Ag, Cu-CuAg, CuAg-Cu) and the composition of the liquid medium (water, ethanol, water + surfactant). The synthesized nanoparticles were studied using X-Ray Diffraction analysis, TEM (transmission electron microscope), and then the antibacterial properties were determined. The antibacterial activity of the samples was tested using Salmonella bacteria. The results of the experiment showed that the nanocomposite Ag-Ag in water has a high antibacterial activity.

Key words: *metal nanoparticles, electric discharge, pulsed plasma, silver, copper, silver-copper, antibacterial activity, surfactants, experimental conditions.*

Introduction. Nanotechnology deals with processes that take place on the nanometer scale, that is from approximately 1 to 100 nm. Development of simple methods for the preparation of nanosized metal particles has attracted significant attention because of their future applications due to unusual size-dependent optical and electronic properties [1-5]. In the recent past, considerable interest has been paid to the preparation of bimetallic nanoparticles. Bimetallic nanoparticles, either as alloys or as core-shell structures, exhibit unique electronic, optical and catalytic properties compared to pure metallic nanoparticles [6].

The antimicrobial properties of silver ions were known since ancient times and silver ions are widely used as bactericide in catheters, burn wounds and dental work. Metallic copper nanoparticles are striking materials primarily because of their unique properties and low cost compared to other metallic nanoparticles. Silver (Ag) and copper (Cu) nanoparticles have shown great potential in variety applications due to their excellent electrical and thermal properties resulting high demand in the market

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[7,8].

A number of methods for producing Ag and Cu nanoparticles have been developed using both physical and chemical approaches. Such as green synthesis by mixed-valence polyoxometallates, chemical synthesuisin solution, laser ablation, gamma and electron irradiations, photochemical methods, microwave processing, and biological synthetic methods were applied for synthesis of silver and copper nanoparticles possessing biological actibvity against diverse bacteria [9-12].

Experimental. An electric discharge synthesis method for copper and silver nanostructures using a pulsed plasma in liquid [13]. The method combines physical (spark discharge) and chemical (surrounding liquid) processes that provide a number of advantages (simple, one-step, low-energy, versatile) for the synthesis of various kinds of metastable nanomaterials. The possibility of achieving a high-temperature stable phase at very low temperature not only provides an economically viable route for applications but also opens up a new route for studying the structural kinetics and chemistry of various nanomaterials.

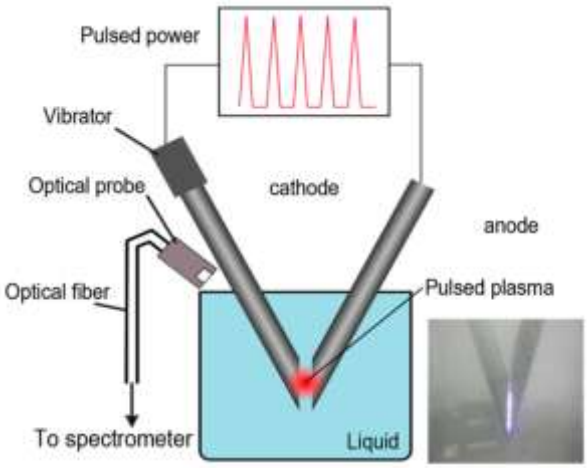

Figure 1. Schematic of pulsed-plasma-in-liquid method.

We have synthesized several metals' nanoparticles using different electrodes. Ag-Ag, Cu-Cu, Ag-Cu, Cu-Ag, Ag-CuAg, CuAg-Ag, Cu-CuAg, CuAg-Cu (with various liquid solutions: water, ethanol and water with active surface agents as PVP) metal electrodes were used and obtained nanoparticles of these metals.

Table 1

List of synthesized in various conditions Ag and Cu nanoparticles

1. **Cu – Cu** (water)	13. **CuAg (+) – Cu** (water)
2. **Cu – Cu** (Etanol 95%)	14. **CuAg (+) – Cu** (Etanol 95%)
3. **Cu – Cu** (water + PVP)	15. **CuAg (+) – Cu** (water + PVP)
4. **Ag – Ag** (water)	16. **Cu (+) – CuAg** (water)
5. **Ag – Ag** (Etanol 95%)	17. **Cu (+) – CuAg** (Etanol 95%)
6. **Ag – Ag** (water + PVP)	18. **Cu (+) – CuAg** (water + PVP)
7. **Cu (+) – Ag** (water)	19. **Ag (+) – CuAg** (water)
8. **Cu (+) – Ag** (Etanol 95%)	20. **Ag (+) – CuAg** (Etanol 95%)
9. **Cu (+) – Ag** (water + PVP)	21. **Ag (+) – CuAg** (water + PVP)
10. **Ag (+) – Cu** (water)	22. **CuAg (+) – Ag** (water)
11. **Ag (+) – Cu** (Etanol 95%)	23. **CuAg (+) – Ag** (Etanol 95%)
12. **Ag (+) – Cu** (water + PVP)	24. **CuAg (+) – Ag** (water + PVP)

After synthesizing nanoparticles with different conditions we obtained 24 samples as shown in figure 5.

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Figure 2. Photo of Ag and Cu nanoparticles obtained with different conditions.

Antibacterials activity test. Biological activity of silver and copper nanocomposites was tested by *Salmonella* the gram-negative bacteria. *Salmonella* species are intracellular pathogens [14] certain serotypes causing illness. Nontyphoidal serotypes can be transferred from animal-to-human and from human-to-human. They usually invade only the gastrointestinal tract and cause salmonellosis, the symptoms of which can be resolved without antibiotics. The antibacterial effect of the nanocomposites was evaluated by using BIO-RAD Model 680 Microplate Reader, showed in the Figure 3 .

Figure 3. BIO-RAD Model 680 Microplate Reader.

The Model 680 Microplate Reader is an eight-channel, vertical pathlength photometer that measures the absorbance of the contents in the wells of a 96-well microtitration plates were used reliably and easily to measure turbidity of bacterial samples in antibiotic susceptibility studies.

For the evaluation of the nanocomposites' antibacterial effect 2 ml of LB medium + Salmonella bacteria were incubated at 37ºC for 12 hours. 50 µl of this solution was added to 4950 µl of LB Medium and incubated for another 4 hours. Then it was 1000 times diluted and put in 96 wells. After incubation for 6 hours we measured our samples by Microplate Reader. The growth curve measurements were performed on a 96-well microplate, which showed in figure 4.

Results and Discussions. Analysis of x-ray difraction patterns of the products obtained by the pulsed plasma in water, ethanole and water+PVP are shown in Fig.4. When the combination of Cu-Cu (anode-cathode) was used, metallic Cu, CuO and Cu_2O phases were identified. Cu_2O phases was present in Cu-Cu in water+PVP and Cu-Cu in water samples in a small amount according to the intensity of the Cu_2O peak at 34 degrees. While Cu-Cu in ethanol sample showed mostly Cu metallic peaks.

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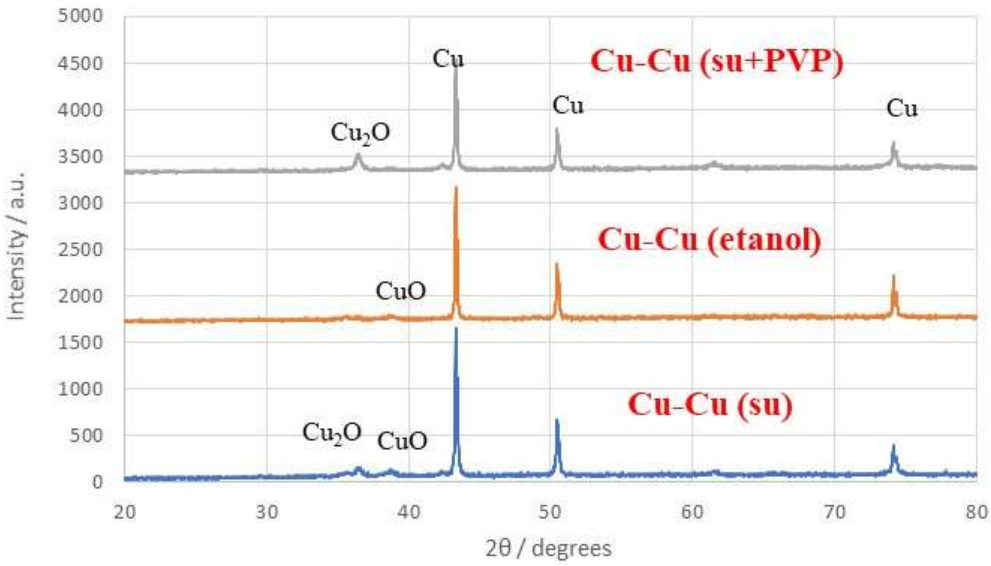

Figure 4. XRD patterns of samples, produced by Cu anode and Cu cathode in presence of water, ethanol and water+PVP.

In the Figure 5, we can see XRD pattern for the Ag-Ag samples. Similarly, Ag-Ag in ethanol sample was much higher content (almost 100%) of pure metallic particles of Ag, while the water and water+PVP contained AgO and Ag_2O phases.

When we used the combination of Cu-Ag (anode-cathode) electrodes, as shown in Fig. 6, erosion of anode electrode (Cu) was much higher than the cathode. XRD pattern shows the peak of mostly Cu. This is because the electrical breakdown was directed from cathode to anode, so the high density current hit the surface of anode electrode and pulverized it.

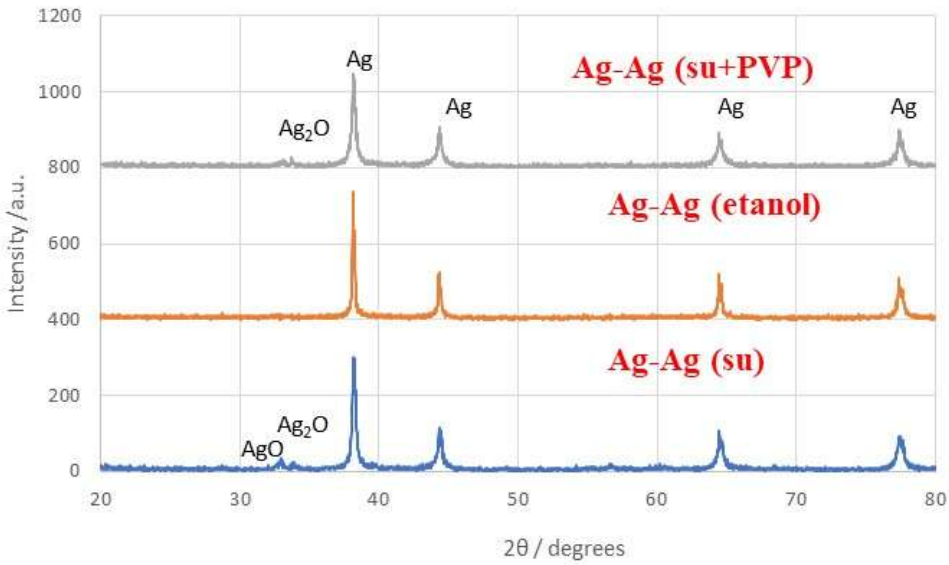

Figure 5. XRD patterns of samples, produced by Ag anode and Ag cathode in presence of water, ethanol and water+PVP.

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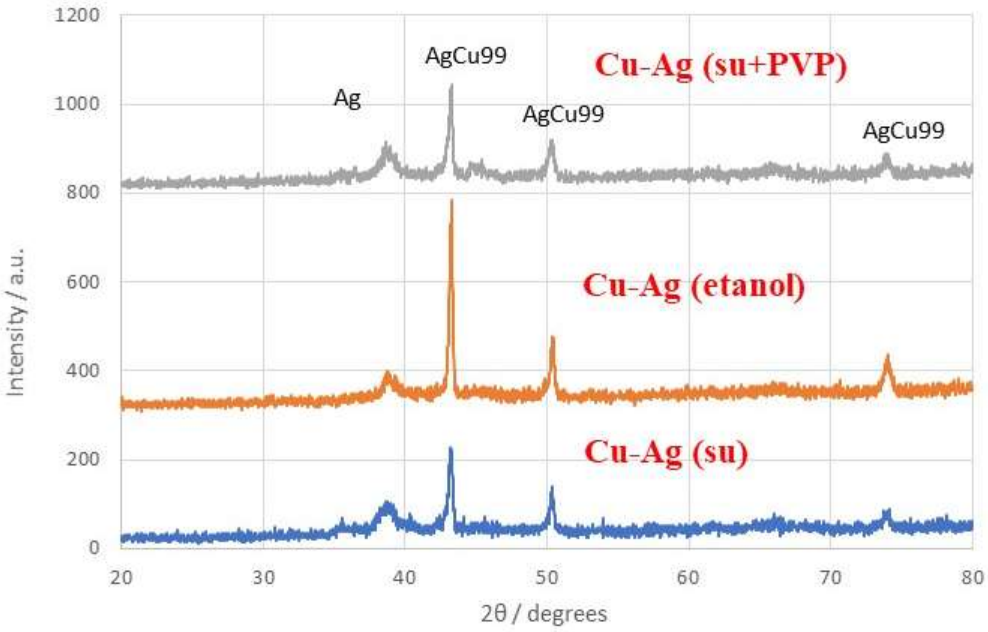

Figure 6. XRD patterns of samples, produced by Cu anode and Ag cathode in presence of water, ethanol and water+PVP.

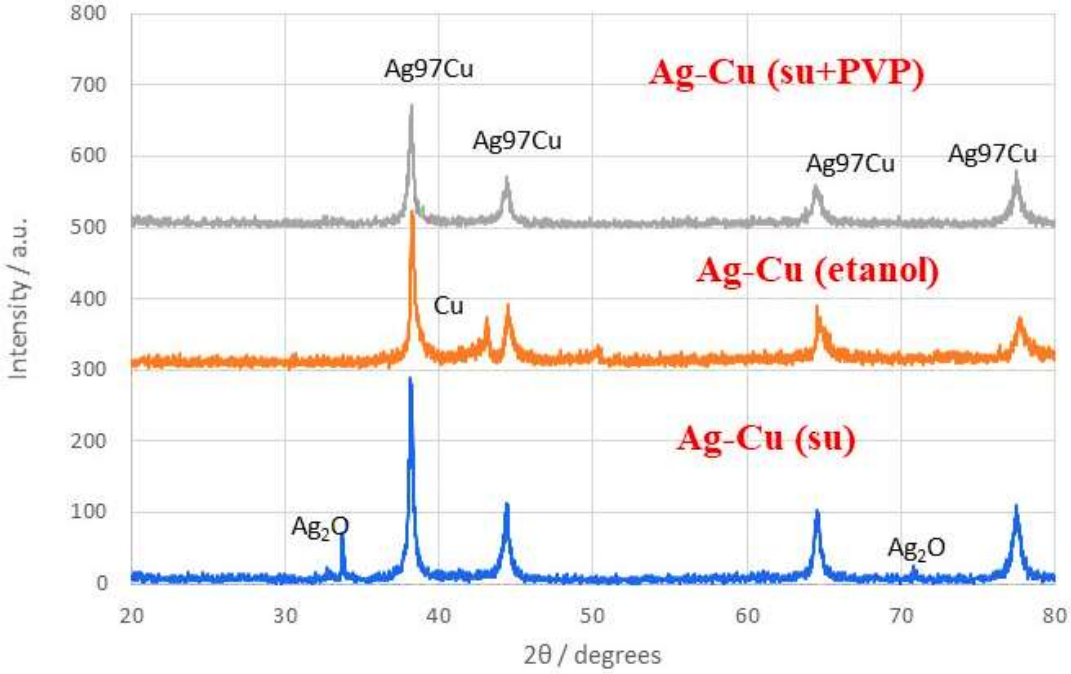

Figure 7. XRD patterns of samples, produced by Ag anode and Cu cathode in presence of water, ethanol and water+PVP.

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Figure 7 shows the XRD pattern of the sample produced when a combination of Ag-Cu (anode-cathode) wasused. This is opposite to Fig.6, where we used Cu-Ag combination in the cathode to anode discharge. Similarly, the anode material (Ag) was eroded much more in amount comparing to Cu. We can see formation of alloy of Ag and Cu with much more content of Ag (97%). Formation of oxides in this case was confirmed only in Ag-Cu in water condition, where we can see peaks of Ag_2O.

With the help of the transmission electron micrographs, the morphology and dimensions of the prepared samples were determined. Figure 8 exhibits the TEM photograph of pure metallic Ag nanoparticles, which were prepared by pulsed plasma method. We can see that the particles are under the 100 nm in size, about 5 to 30 nm. There can be seen particles with a rhombic shape (due to Ag_2O nanocrystals, which crystallizes in tetrahedral structure or to AgO which crystallizes in monoclinic structure) as well as hexagonal and spherical shapes.

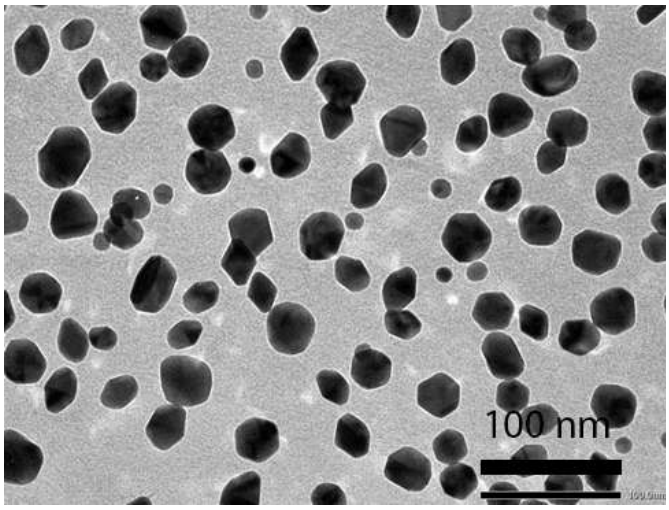

Figure 8. TEM image of the sample, produced by Ag anode and Ag cathode in presence of water.

Figure 9 and 10 show TEM images of Ag-Cu in water and Ag-CuAg in water accordingly. We can see similar picture with Ag-Ag in water conbination. The sizes of the nanocrystals are from few nanometers (3nm) to about 50 nm. Mostly hexagonally shaped nanocrystals was observed along with some spherically shaped ones.

In the case of Ag-AgCu in water sample, the size of the nanosrystals were smaller compared to above 2 samples. There are nanoparticles with size of 1-2 nm up to the crystals of 20 nm.

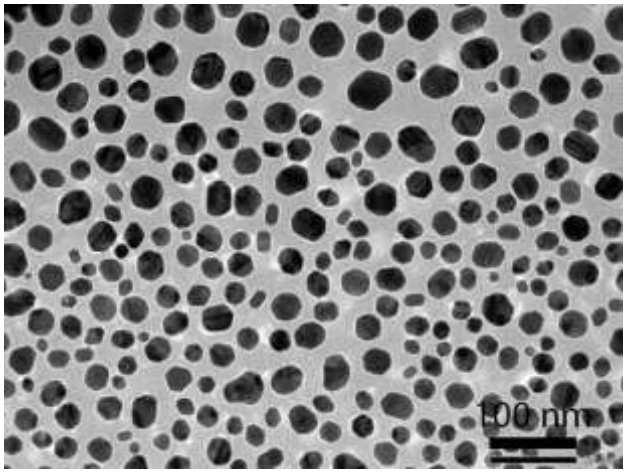

Figure 9. TEM image of, the sample produced by Ag anode and Cu cathode in presence of water.

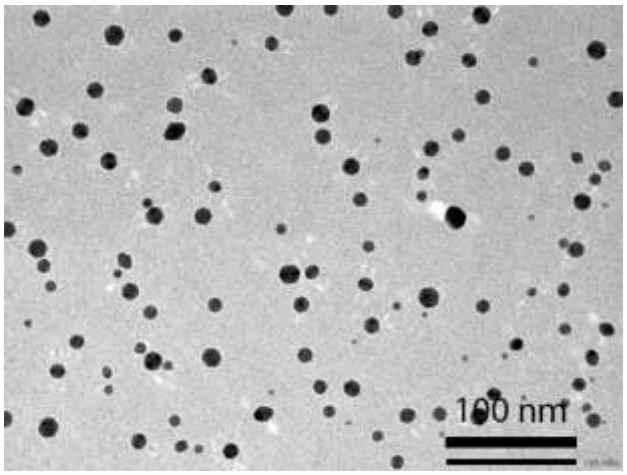

Figure 10. TEM image of the sample, produced by Ag anode and CuAg cathode in presence of water.

Figure 11 displays the graph of average particle sizes determined by using the taken TEM images for several samples. As we can see Ag-Ag in water + PVP and Ag-Cu-Ag in water+PVP showed the smallest particle sizes.

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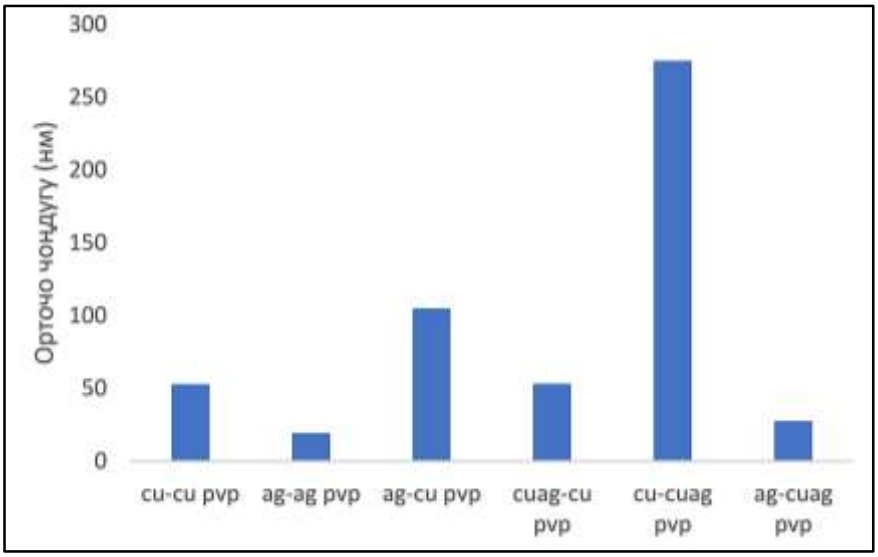

Figure 11. Average size of nanoparticles measured by usinf TEM images for the different samples.

The antibacterial activity of the samples were first measured by using 10 pm concentration of nanoparticles. Among the 24 samples tested, samples that showed antibacterial activity were selected for the next test, which was to lower their concentration to 5 ppm. And after that again the samples that showed antibacterial activity against salmonella bacteria were selected for the next test, which was to lower their concentration to 3 ppm. The antibacterial activity test results are given in Figure 12. Finally samples that showed antibacterial activity in 3 ppm were further selected for final test, which is 1 ppm. For the final test, where the nanoparticles with 1 ppm concentration were tested, the following samples remained: Ag-Ag , Ag-Ag (PVP), Ag-Cu, Ag-Cu (PVP), Ag-CuAg, Ag-CuAg (PVP).

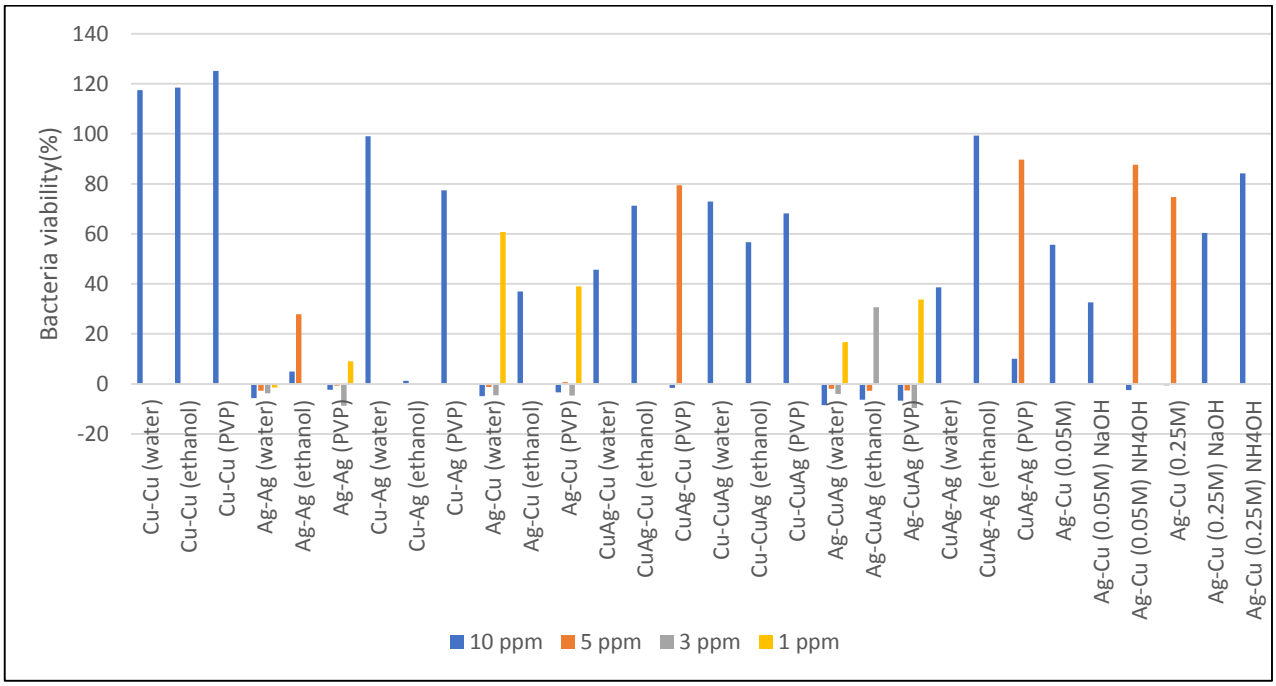

Figure 12. Antibacterial activity of synthesized silver and copper nanoparticles.

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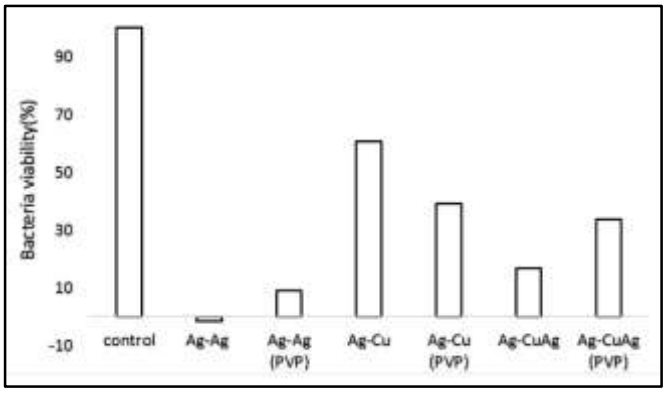

Figure 13. Results of antibacterial activity of synthesized silver and copper nanoparticles **(**1 ppm).

Figure 13 shows the antibacterial activity test results for the samples with 1 ppm concentration. As we can see, Ag-Ag in water sample showed the highest antibacterial activity against salmonella bacteria. This was followed by Ag-Ag in water solution of PVP surfactant sample and then Ag-CuAg in water sample. Antibacterial activity of the samples depended on concentration of solution with nanoparticles; also depends on place of Ag electrode (anode or cathode), when we placed Ag electrode to anode results were better than Cu or CuAg.

Conclusions. In summary, we have synthesized Ag and Cu NPs by pulsed plasma in liquid method using different experimental conditions. XRD analyses showed that ethanol as a medium for the pulsed plasma can serve as a good candidate for the production of pure Ag or Cu metallic phases nanoparticles, while the water and water + PVP surfactant induced formation of oxides of Ag_2O, Cuo, Cu_2O.

TEM analyses showed that sizes of the nanocrystals of Ag-Ag in water sample are from few nanometers (3nm) to about 50 nm. Mostly hexagonally shaped nanocrystals was observed along with some spherically shaped ones. Similarly, Ag-AgCu in water sample's size were smaller (2 – 20 nm) compared to other samples. Other samples had particle sizes under 100 nm, which can confirm that they were in nanometer scale.

Samples with highest antibacterial activity were selected by step by step decrease of their concentration starting from 10 ppm and ending with 1 ppm. The samples that showed antibacterials activity for the salmonella bacteria in 1 ppm concentration were (from highest activity to lower): Ag-Ag , Ag-Ag (PVP), Ag-Cu, Ag-Cu (PVP), Ag-CuAg, Ag-CuAg (PVP).

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