Nanomed. J. 5(2): 90-95, Spring 2018

RESEARCH PAPER

Optimization of copper nanoparticles synthesis using E. coli and the study of its antifungal activity

Mehrdad Haghighi [1], Hamid Reza Ghorbani [2*]

[1]Department of Chemistry, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran
[2]Department of Chemical Engineering, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran

ABSTRACT

Objective (s): The green synthesis of copper nanoparticles using metabolites of microorganisms has gained much interest in recent years. In this work, it was studied optimization of copper nanoparticles synthesis using E. coli and its antifungal activity.

Materials and Methods: The copper nanoparticles were synthesized by Escherichia coli. Effect of copper nitrate concentration and temperature was studied on size and production efficiency. In addition, copper nanoparticles were analyzed by UV – VIS spectroscopy, transmission electron microscopy (TEM), and dynamic light scattering (DLS). Finally, the antifungal properties of synthesized nanoparticles were tested against Penicillium by disc diffusion method in different concentrations of nanoparticles.

Results: It was found that initial concentration of copper nitrate plays a key role in formation of nanoparticles. Also, it was indicated that in lower temperatures, the size of copper nanoparticles is smaller and their distribution is narrower. It was determined the concentration of 15% w/v of copper nanoparticles in distilled water is optimum concentration for the maximum of antifungal activity.

Conclusion: The biosynthesized copper nanoparticles displayed antifungal activity against Penicillium. The experiments showed the usability of these nanoparticles in water purification, air purification and antifungal packaging.

Keywords: *Antifungal Activity, Biosynthesis, Copper Nanoparticles, Optimum*

How to cite this article

Haghighi M, Ghorbani H R. Optimization of copper nanoparticles synthesis using E. coli and the study of its antifungal activity. Nanomed J. 2018; 5(2): 90-95. DOI: 10.22038/nmj.2018.005.005

INTRODUCTION

The past decade has seen significant interest directed towards the synthesis of metal nanoparticles due to their unique physicochemical properties and their potential applications in various fields, which include biological labelling, catalysis, diagnostics, electronics, and sensor technology [1, 2]. The techniques used to produce nanoparticles can be classified as physicochemical or biological. Many physicochemical synthetic methods have been devised, but they are often expensive and involve the use of toxic or hazardous chemicals that pose potential environmental and biological risks [3, 4]. Therefore, biological processes based on the use of microorganisms or plants have been considered as possible means for synthesizing nanoparticles, especially "green" synthetic processes [5, 6]. Furthermore, the nanoparticles formed by biological methods are stable for a long time, whereas those produced using chemical methods are unstable and tend to aggregate [7, 8]. Accordingly, biological entities, such as, microorganisms (algae, bacteria, fungi, and yeast) and plants have been investigated for the synthesis of copper nanoparticles [9, 10]. Of the many possible biological sources, microorganisms are preferred for biosynthesis due to their processing speeds, ease of culture, and downstream processing and manipulation considerations [11, 12, 13]. In addition, the synthesis of copper nanoparticles using microorganisms does not require the laborious extraction processes needed for processing plant biomasses. The microbial synthesis of copper nanoparticles can take place either intracellularly or extracellularly. Copper nanoparticles have wide applications in heat transfer fluids, sensors [14,

* *Corresponding Author Email: hamidghorbani6@gmail.com*
Note. This manuscript was submitted on January 5, 2018; approved on February 15, 2018

15, 16], catalysis, solar energy, antibacterial and antifungal materials and batteries [17, 18, 19].

In the present study, E. coli was used for the extracellular synthesis of copper nanoparticles. Environmental parameters (concentration and temperature) were optimized to synthesize of copper nanoparticles, and their antifungal activities were determined.

MATERIALS AND METHODS

Tryptic Soy Broth (TSB) was prepared, sterilized and inoculated with a fresh growth of E. coli. The cultured flask was incubated at 37ºC for 24 h. After incubation the culture was centrifuged at 4500 rpm for 25 min and the supernatant was used for subsequent experiments.

The effect of copper nitrate (II) concentration

100 ml of 0.01M copper nitrate(II) solution, 100 ml of 0.005 M copper nitrate(II) solution, and 100 ml of 0.001 M copper nitrate(II) solution were mixed with 1 ml Polyvinylpyrrolidone (PVP) in three separate flasks. 10 ml supernatant was added to each flask and after 2 hr., the color of the solution turned yellow to ocher. The prepared copper nanoparticles were characterized by uv-vis spectroscopy, transmission electron microscopy (TEM) and DLS analysis.

The effect of temperature

Three flasks containing 100 ml of 0.001M copper nitrate (II) solution with 1 ml pvp were kept at three different temperatures. For this aim, water bath was used to maintain the flasks at 5°C, 25 °C and 50°C. Then, 10 ml of supernatant was added to each flask and it was detected a color change in the solution after 2 hr. The produced samples were characterized by UV-Vis spectroscopy, transmission electron microscopy (TEM), and DLS analysis.

Anti-fungal properties of copper nanoparticles

The anti-fungal activity was studied by agar well diffusion method. *Penicillium* fungus was used in this study. At first, the copper nanoparticles suspensions (optimal conditions) were centrifuged at 5000 rpm for 20 minutes. The precipitated copper nanoparticles were washed with distilled water and then prepared in four concentrations of 5%, 10%, 15% and 20% w / v in distilled water. A fresh fungal culture was spreader on agar plates with glass spreader. A well was punched off in pertiplates with sterile cup borer and then copper nanoparticle (5%, 10%, 15% and 20%), control sample and two antibiotics tetracycline and cefalexin were loaded. Plates were incubated at 37˚C for 2 days, until appearance of zone of inhibition. The zone of inhibition was measured as a property of anti-fungal activity.

RESULTS AND DISCUSSIONS

The study of copper nitrate (II) concentration

The color of the solution in the flask containing mixture of copper nitrate (II) and supernatant changed to ocher after 2 hrs.

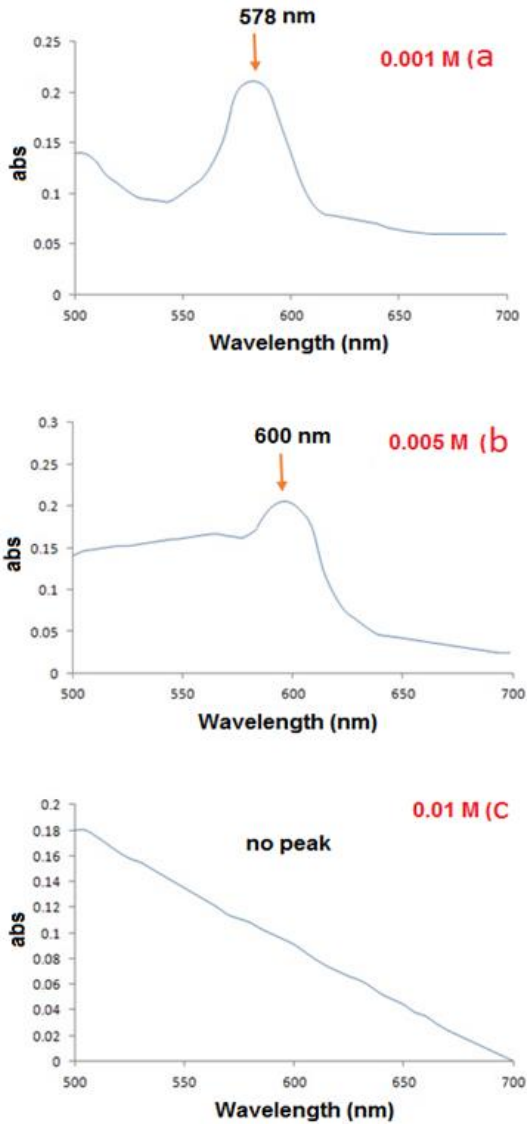

Fig 1. UV–Vis spectra of copper nanoparticles suspension a) 0.001 M, b) 0.005 M, and c) 0.01 M of copper nitrate solution

This color change indicated the possible of copper nanoparticles formation. The nanoparticles were primarily characterized by UV–Vis spectroscopy, which has proved to be a very useful technique for the analysis of nanoparticles [9, 10, 20]. As illustrated in Fig 1 (a), a strong surface plasmon resonance was centered at ~578 nm. Observation of this strong but broad surface plasmon peak has been well documented for various metal nanoparticles, with sizes ranging widely from 2 to100 nm [11, 21, 22].

By increasing concentration from 0.001 M to 0.005 M, the peak was shifted from 578 nm to 600 nm (Fig 1b); while at concentration of 0.01 M, no peak was observed (Fig 1c). It is apparent that nanoparticles were not formed at concentration of 0.01 M. It was suggested that microparticles were formed at high concentration. Dynamic light scattering (DLS) analysis was used to obtain the distribution of nanoparticles size. As shown in Fig 2, the size of copper nanoparticles increased with increasing the concentration of copper nitrate from 0.001 M to 0.005 M.

This confirmed the results of UV-Vis spectroscopy analysis. In addition, the average size of copper nanoparticles was 64 nm at concentration of 0.001 M (Fig 2a). Also, two peaks were observed at 49 nm and 135 nm for concentration of 0.005 M that indicated particles size were larger than 100 nm (Fig 2b).

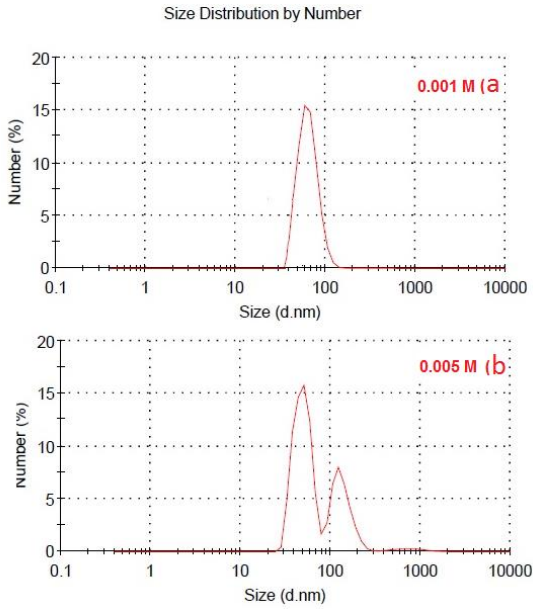

Fig 2. The curve of size distribution by number, a) 0.001 M, b) 0.005 M of copper nitrate solution

This expressed an inappropriate distribution of particles in this state. Thus, the best the size distribution of nanoparticles was in concentration of 0.001 M.

The study of temperature

In this work, three different temperatures were used to study the temperature effect on the copper nanoparticles synthesis. The color changes were started at 5ºC quicker than other temperatures.

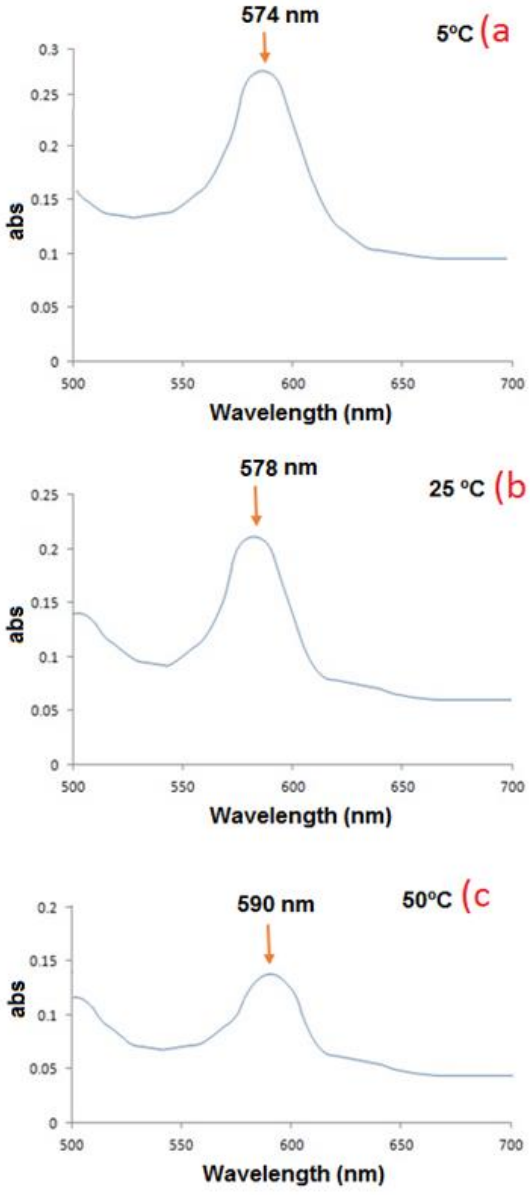

Fig 3. UV–Vis spectra of copper nanoparticles suspension a) T=5°C, b) T=25°C, and c) 50°C

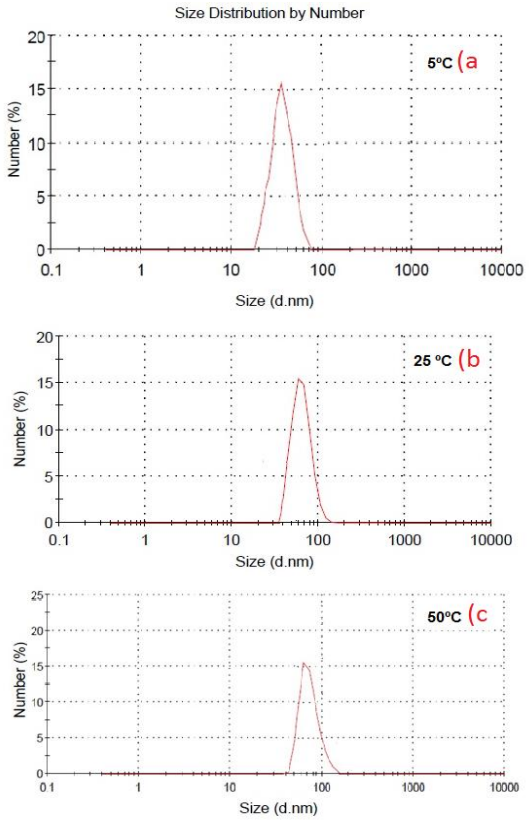

Fig 4. The curve of size distribution by number a) T=5°C, b) T=25°C, and c) 50°C

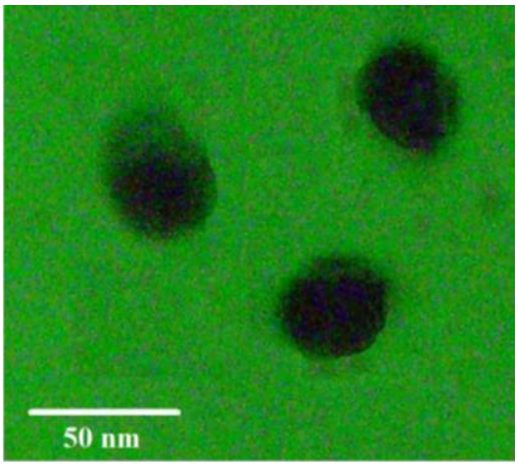

Fig 5. TEM micrograph of copper nanoparticles

To prove the existence of nanoparticles, three samples were monitored by measuring UV-visible absorption within wave lengths of 500-700nm. As seen in Fig 3, peaks are at 572 nm, 578 nm and 590 nm for temperatures at 5°C, 25 °C and 50°C, respectively. This means that the smallest of the nanoparticles were formed at 5 °C and also, the absorption amount is more than two other samples that indicates the more reduction of copper ions. Dynamic light scattering (DLS) analysis was used to investigate the temperature effect on the nanoparticles size. As illustrated in Fig 4, the nanoparticles size was increased with increasing temperature. At 5 ºC, the average size of copper nanoparticles is about 35 nm, while at 25ºC and 50ºC are about 64 nm and 88 nm, respectively. These results were completely confirmed with the results of UV-Vis spectroscopy. Since the enzyme nitrate reductase (NR) released by E. coli is the major factor in copper ions reduction, it seems the enzyme was deactivated with increasing temperature, and thus the reduction amount was decreased.

Therefore, the nanoparticles size was decreased and the amount of production was increased with decreasing temperature.

The above results suggest that the best of synthesis conditions is 0.001 M and 5 °C for the copper nitrate concentration and the reaction temperature, respectively.

As shown in the table, the antifungal activity of nanoparticles is related to the concentration of nanoparticles. The zone of inhibition increased with increasing the concentration of nanoparticles to 15% but the inhibition zone is approximately constant with increasing the concentration to 20%. This means that the minimum concentration, which has the maximum antifungal activity, is 15%. In fact, a suspension containing copper nanoparticles with a concentration of 15% w/v in distilled water is an optimal concentration for the *penicillium* antifungal activity. Fig 6 shows the inhibition zone curve of *Penicillium* fungus according to copper nanoparticles percentage.

Table 1. Antifungal activity of the nanoparticles on fungus Penicillium(mm)

zone of inhibition(mm)	sample
0	control
4.5	Copper nanoparticles 5%
8.5	Copper nanoparticles 10%
13	Copper nanoparticles 15%
13.5	Copper nanoparticles 20%
15	tetracycline
0	cefalexin

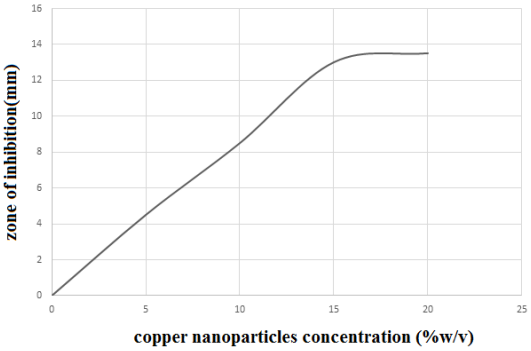

Fig 6. The inhibition zone curve of Penicillium fungus according to copper nanoparticles percentage

CONCLUSION

In this research, the copper nanoparticles were synthesized by E. coli and it was investigated the effect of copper nitrate concentration and temperature on nanoparticles size. The results showed in low concentrations of copper ions in solution, it is possible to form more nanoparticles. In this study, copper nitrate solution with a concentration of 0.001 M and a temperature of 5°C has the best performance in the formation of nanoparticles and their small size. All the results were analyzed using UV-Vis spectroscopy, dynamic light scattering (DLS), and transmission electron microscopy (TEM). These nanoparticles were used to determine the antifungal activity. The copper nanoparticles were prepared at four concentrations of 5%, 10%, 15% and 20% w/v in distilled water. It was studied its antifungal activity against *Penicillium* fungus by disk diffusion method. It was determined the concentration of 15% w/v of copper nanoparticles in distilled water is optimum concentration for the maximum of antifungal activity. The experiments showed the usability of these nanoparticles in water purification, air purification and antifungal packaging.

ACKNOWLEDGMENTS

This study was supported by Islamic Azad University, Qaemshahr Branch, Qaemshahr, Iran.

References

1.Wei L, Lu J, Xu H, Patel A, Chen ZS, Chen G. Silver nanoparticles: synthesis, properties, and therapeutic applications, Drug Discov Today. 2015; 20: 595-601.

2.Ruparelia JP, Chatterjee AK, Duttagupta SP, Mukherji S. Strain specificity in antimicrobial activity of silver and copper nanoparticles, Acta Biomater. 2008; 4: 707–716.

3.Ghorbani HR. Biological and non-biological methods for Fabrication of Copper Nnanoparticles. Chem Eng Commun 2015; 202: 1463–1467.

4.Raffi M, Mehrwan S, Bhatti TM, Akhter JI, Hameed A, Yawar W, Hasan MN. Investigations into the antibacterial behavior of copper nanoparticles against Escherichia coli. Ann Microbiol. 2010; 60: 75–80.

5.Chatterjee AK, Sarkar RK, Chattopadhyay AP, Aich P, Chakraborty R, Basu T. A simple robust method for synthesis of metallic copper nanoparticles of high antibacterial potency against E. coli, Nanotechnol. 2012; 23: 1–11.

6.Wei Y, Chen S, Kowalczyk B, Huda S, Gray TP, Grzybowski BA. Synthesis of stable, low-dispersity copper nanoparticles and nanorods and their antifungal and catalytic properties, J Phys Chem C. 2010; 114: 15612–15616.

7.Shao W, Wang S, Wu J, Huang M, Liu H, Min H. Synthesis and antimicrobial activity of copper nanoparticle loaded regenerated bacterial cellulose membranes, RSC Adv. 2016; 6: 65879-65884.

8.Ramyadevi J, Jeyasubramanian K, Marikani A, Rajakumar G, Rahuman AA. Synthesis and antimicrobial activity of copper nanoparticles, Mater Lett. 2012; 71: 114–116.

9.Deryabin DG, Aleshina ES, Vasilchenko AS, Deryabina TD, Efremova LV, Karimov IF, Korolevskaya LB. Investigation of copper nanoparticles antibacterial mechanisms tested by luminescent Escherichia coli strains, Nanotechnol Russia. 2013; 8: 402-408.

10.DeAlba-Montero I, Guajardo-Pacheco J, Morales-Sánchez E, Araujo-Martínez R, Loredo-Becerra GM, Martínez-Castañón GA, Ruiz F, Compeán Jasso ME. Antimicrobial Properties of Copper Nanoparticles and Amino Acid Chelated Copper Nanoparticles Produced by Using a Soya Extract, Bioinorg. Chem Appl. 2017; 2017: 1064918.

11.Kaszuba M, McKnight D, Connah MT, McNeil-Watson FK, Nobbmann U. Measuring sub nanometre sizes using dynamic light scattering, J Nanopart Res. 2008; 10: 823-829.

12.Chandra S, Kumar A, Tomar PK. Synthesis and characterization of copper nanoparticles by reducing agent, J Saudi Chem Soc. 2014; 18: 149–153.

13.Doodi M, Naghsh N, Heidarpour A. Effect of silver nanoparticles on pathogenic Gram-negative bacilli resistant to extended spectrum beta-lactamase (ESBLs) antibiotics. Lab J. 2011; 5(2): 44-51.

14.Monsef Khosh-hesab Z. Synthesis of ZnO nanoparticles using chemical deposition method. Int Nano Lett 2011; 1(4): 39-49.

15.Naghsh N, Safari M, Haj Mehrabi P. Effect of silver nanoparticles on E. coli growth. J Qom Univ Med Sci. 2011; 6(2): 65-68.

16.Veissi Malekshahi Z, Afshar D, Ranjbar R, Shirazi MH, Rezaei F, Mahboobi R, and colleagues. Antimicrobial properties of ZnO nanoparticles. J MDPI Tropical Med. 2012; 17(59): 1-4.

17.Soo-Hwan K, Lee HS, Ryo DS, Choi SJ, Lee DS. Antibacterial Activity of Silver-nanoparticles Against Staphylococcus aureus and Escherichia coli. Korean J Microbiol Biotechnol. 2011; 39(1): 77-85.

18.Zhang YC, Tang JY, Wang GL, Zhang M, Hu XY. Facile synthesis of submicron Cu_2O and CuO crystallites from a solid metallorganic molecular precursor. J Crys Growth. 2006; 294(2): 278-282.

19.Yoon K, Hoon Byeon J, Park JH, Hwang J. Susceptibility constants of *Escherichia coli* and *Bacillus subtilis* to silver and copper nanoparticles. Sci Total Environ. 2007; 373(2-

3): 572-575.
20.Ruparelia JP, Chatterjee AK, Duttagupta SP, Mukherji S. Strain specificity in antimicrobial activity of silver and copper nanoparticles. Acta Biomater. 2008; 4(3): 707-716.
21.Singh R, Shedbalkar UU, Wadhwani SA, Chopade BA. Bacteriagenic silver nanoparticles: synthesis, mechanism, and applications. Appl Microbiol Biotechnol. 2015; 99(11): 4579–4593.
22.Hosseini-Abari A, Emtiazi G, Lee SH, Kim BG, Kim JH. Biosynthesis of silver nanoparticles by *Bacillus stratosphericus* spores and the role of dipicolinic acid in this process. Appl Biochem Biotechno. 2014; 174(1)**:** 270-282.