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Screening of most effective nano metal between AgNP, CuNP and Ag-Cu NP's synergistic by invitro antibacterial comparison

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ABSTRACT

The metal nanoparticles like silver, copper have attracted much attention as potential antimicrobial agents. In order to trace out very effective antimicrobial therapy needs invitro comparison of these nanoparticles and Synergistic activity by combining these two molecules together for their commercial application. The present work concluded that CuNP are most potent antimicrobial agents in comparison to AgNP and synergistic activity. © 2016 Trade Science Inc. - INDIA

KEYWORDS

Antibacterial properties;
Coppernanoparticles;
Silver nanoparticles;
Synergistic antimicrobial.

INTRODUCTION

The silver and copper nanoparticles are emerged as novel antimicrobial therapy to solve the problems like microbial resistance and has shown promising commercial applications[9]. For commercial application of these nanomaterial's it is essential to sort out most potent antimicrobial agent between them. For this invitro comparison of antimicrobial activity and also synergistic antimicrobial activity plays key role. While formulating commercial preparations it is necessary to use most potent antimicrobial agent in order to get ideal results. The invitro evaluation was carried out by maintaining constant evaluation parameters like bioburdan, temperature, cup diameter, volume of testing sample in cup for proper evaluation.

MATERIAL AND METHODS

Synthesis of NPs

1. Silver nitrate an equal mole amount of salt was used concentration as 0.02M, the reducing agent 0.5% and 1.5% trisodium citrate was added drop by drop during boiling the solution, the capping and stabilizing agent PVP was added in the concentration as 1% to the solutions. colour change was prominent with change from colorless to pale yellow[1].

2. The salt used was copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) A solution consisting of deionized water and the corresponding metal salt with concentration 0.1M and 0.2M was prepared with various concentrations of reducing agents 0.1M concentration of reducing agents (NaBH4) was added. Excess concentration 1% of ascorbic acid. After the addition of the reducing agent the solution was stirred and kept at room temperature. Finally, to store the nanoparticles and avoid unstability PVP was added in a sufficient amount to submerge them completely[2].

TABLE 1 : Synthesis of nanoparticles

Sr.no.	Nano Particle	Concentration (M)	Concentration of Reducing Agent	Capping Agent (1%)	Particle size (nm) average	U.V. Absorbance (400 nm)
1.	Silver nanoparticle	0.02	5ml 0.5% trisodium citrate	-	40-60 nm	0.371
2.	Silver nanoparticle	0.02	6ml 1.5% trisodium citrate	-	40-60 nm	0.270
3.	Silver nanoparticle	0.02	4ml 0.5% trisodium citrate	PVP	40-60nm	0.148
4.	Silver nanoparticle	0.02	5ml 1.5% trisodium citrate	PVP	40-60nm	0.202
5.	Copper nanoparticle	0.1	4ml 0.1(M) sodium borohydride	-	40-60nm	0.242
6.	Copper nanoparticle	0.1	7ml 0.1(M) sodium borohydride		40-60nm	0.091
7.	Copper nanoparticle	0.2	2ml 0.1(M) sodium borohydride	PVP	40-60nm	0.08
8.	Copper nanoparticle	0.2	10ml 0.1(M) sodium borohydride	PVP	40-60nm	0.170

CHARACTERIZATION

The synthesized Cu, Ag and bimetallic Cu–Ag NPs were characterized, UV–visible spectrophotometry at 420 nm. The particle size of corresponding nano particles was determined on Malvern particle size analyzer instrument NS 300 model[4].

ANTIBACTERIAL ACTIVITY

The antimicrobial evaluation was done by keeping all parameters constant bioburdan, temperature, cup diameter, volume of testing sample in cup for proper evaluation. The experiments on the antimicrobial activity were carried out. The parameters were kept constant by using four petri dishes as follow,

Temperature

The nanoparticles were poured in the four petridishes consisting of different concentrations and then this petridishes temperature were kept constant by placing it in the incubator which had the same and uniform temperature(37^0c) throughout the growth of the organisms as well as for the antimicrobial activity evaluation study.

Bioburden

E-Coli was used in the this evaluation by using the same strain in all the petri dishes and at the same time it was incubated for 24 hours so as to keep the uniformity in the growth environment and growth rate.

Diameter of the cup

The cup plate method was used in the study and the cups diameter was kept constant by using the cork borer, each petri dishe had two cups consisting of the silver and copper nanoparticle for the evaluation of the synergistic activity of the nanoparticles.

Volume

The volume of the NPs was constant for synergistic activity it was half the quantity of both NPs. For others the cups were filled to the maximum volume by using the syringe.

Antimicrobial activity of the synthesized NPs was tested against the human pathogenic bacteria *Escherichia coli* by determining the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) following the cup-plate method. Selective media were used to culture each strain. For culturing E *coli* the agars used were: The samples were initially incubated at 37 °C for 24 h for the bacterial culture. Each set was inoculated aseptically with 10 mL of the respective bacterial suspension (approximately 10^8 CFU/mL).. We used a positive control (only bacteria) and a negative control (only NPs). Tests were performed three times for each strain. The inoculated sets were incubated at 37 °C for 24 h. The zone of inhibition in each plate were observed and calculated, also in the present study we also invented novel technology of angle of inhibition for antimicrobial activity calculation was by using protractor III camera software for better evaluation of antibacterial activity.[1]

Synergistic antibacterial activity

To analyze synergistic antibacterial activity of CuNP and AgNP, they were mixed at equal volume, no spontaneous reaction, no separation of phases was observed after mixing.

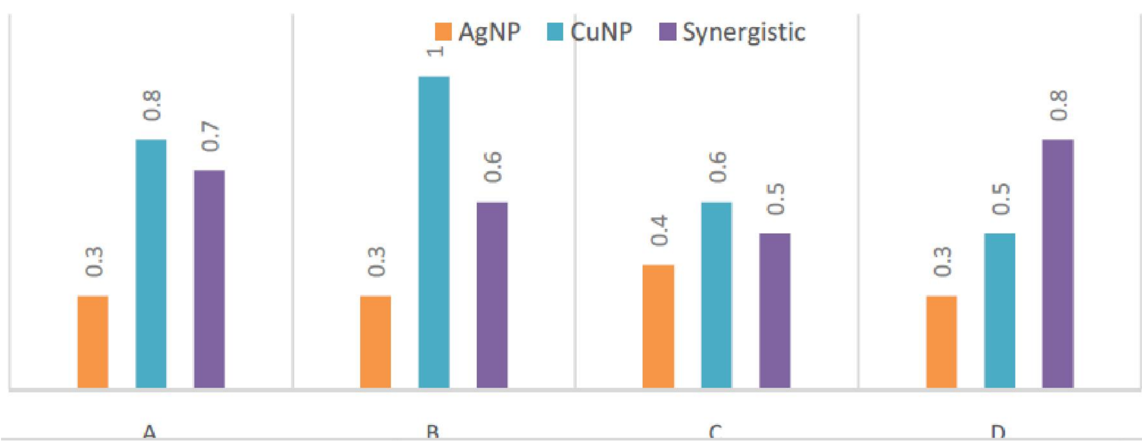
RESULTS

Figure 1 : Shows maximum angle of inhibition 20.8^{0}and zone of inhibition 1 cm for 0.1M CuSO4 NaBH4 (PVP)

TABLE 2

Sr.no.		Silver nanoparticle				Copper nanoparticle				Synergistic			
1.	Concentration	I	II	III	IV	I	II	III	IV	A	B	C	D
2.	Zone of Inhibition(cm)	0.3	0.3	0.4	0.3	0.8	1	0.6	0.5	0.7	0.6	0.5	0.8
3	Angle of inhibition (degree)	2.9	2.9	4.7	2.9	18	20.8	16	15	17	16	15	18

I-0.02M AgNO3 0.5% trisodium citrate, II-0.02M AgNO3 1.5% trisodium citrate, III-0.02M AgNO3 0.5% trisodium citrate 1%(PVP), IV-0.02M AgNO3 1.5% trisodium citrate 1% (PVP), I-0.1M CuSO4 NaBH4, II- 0.1M CuSO4 NaBH4 (PVP), III-0.2M CuSO4 NaBH4, IV-0.2M CuSO4 NaBH4(PVP)



A 0.5% trisodium citrate 0.02M AgNO3 +0.1M CuSO4 +Combine effect, B 1.5% trisodium citrate 0.02M AGNO3 +0.2MCuSO4 +combine effect, C 0.5% trisodium citrate 0.02M AgNO3 +0.1M CuSO4 +Combine effect with capping agent, D 1.5% trisodium citrate, 0.02M AgNO3 +0.2M CuSO4 +combine effect with capping agent

Figure 2 : Graph shows antibacterial activity

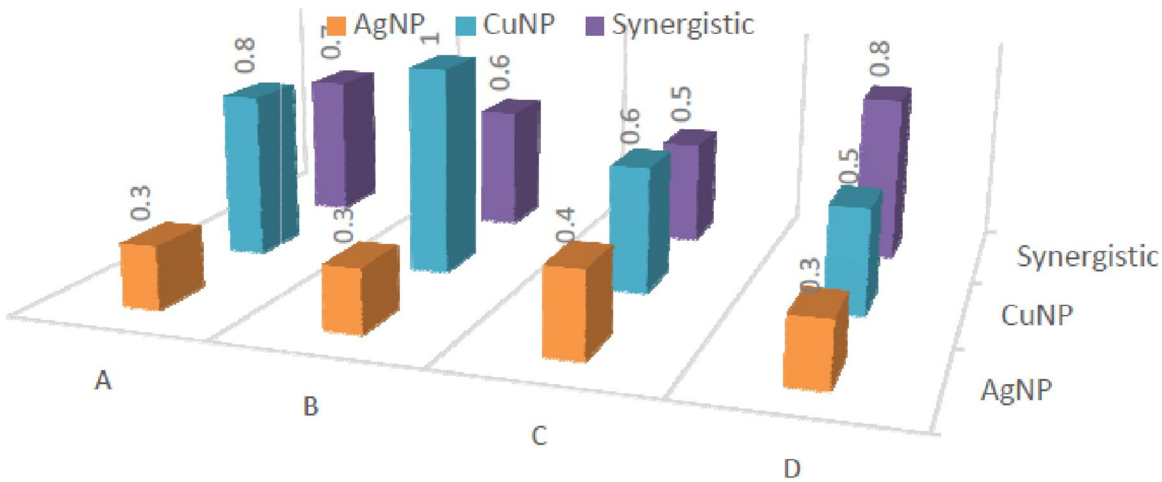


Figure 3

CONCLUSIONS

The copper nanoparticles are most effective antimicrobial agent than silver nanoparticles and copper-silver synergistic, out of various concentrations of copper nano particles 0.1M copper nanoparticles coated with PVP shows maximum zone of inhibition and angle of inhibition. The synergistic effect of these two nanoparticles also increases antibacterial activity. The novel angle of inhibition by protractor III camera for evaluation antimicrobial activity is successfully invented.

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