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Antimicrobial Activity of Bimetallic Cu/Pd Nanofluids

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Abstract

A series of copper/palladium bimetallic nanostructures based nanofluids has been prepared with the aim of investigating antimicrobial activity. Synthesized nanofluids were characterized by UV-visible spectroscopy, X-ray diffraction, scanning electron microscopy and acoustic spectroscopy to determine their optical absorbance, structure, surface morphology and particle size distribution, respectively. Prepared nanofluids were tested for their antimicrobial activity using the agar disc diffusion method and their minimum inhibitory concentration (MIC) values were calculated by micro-dilution method. Results of antimicrobial activity revealed that prepared nanofluids possessed a good antibacterial activity against microbial species. In summary, the application of these bimetallic nanofluids as antimicrobial agent will be very valuable for biomedical and industrial applications.

Keywords: Nanofluids; Bimetallic; Antibacterial activity; MIC

Introduction

Nanofluids (the dispersion of nano-sized particles in fluids) have been extensively used in a wide variety of applications [1-7]. In recent years, nanofluids have been found to possess excellent antimicrobial activity, largely due to the ability of small sized particles being able to penetrate the living microbe-cells and causing chemically induced internal damage [1,6,7]. In this regard, although both nano-sized organic and inorganic particles have been extensively investigated, inorganic nanomaterials are considered to be better antimicrobial agents [6] as organic materials become unstable at high temperatures or pressures. Therefore, the use of inorganic materials has emerged up as novel antimicrobial agents. Among inorganic metallic nanomaterials, antibacterial properties of silver and gold nanoparticles are comprehensively studied for their potential applications in food packaging, in disinfection of water and in the infection control in medicine [7].

Due to improved catalytic property, bimetallic nanoparticles are of greater interest in comparison of monometallic nanoparticles [8-14]. By virtue of their unique properties, they have been applied in sensors, catalysts, electronic devices and optics. Depending on preparation conditions, bimetallic nanoparticles can be in alloy form or core-shell structure. Among various bimetallic nanoparticles, considerable interest has been paid to the preparation of the bimetallic nanoparticles of Cu since Cu exhibits excellent features like electrical conductivity and chemical activity [10-12]. Few reports are available on synthesis of Cu/Pd nanoparticles using different techniques [13-15]. However, there is no literature on the bactericidal properties of Cu/Pd bimetallic nanofluids (BMNFs). Also among the different antimicrobial agents, silver and gold nanoparticles have been extensively studied and used since ancient times to fight infections and prevent spoilage. Cu/Pd nanofluids are cost effective in comparison to silver and gold. Hence, we prepared a series of Cu/Pd nanofluids by varying their molar ratios and the antimicrobial properties of the nanofluids (NFs) have been studied. Antimicrobial activities of the prepared nanofluids were evaluated using the agar disc diffusion method against standard strains viz. *E. coli*, *P. aeruginosa*, *E. faecalis*, *S. aureus* and against the yeasts *C. albicans*, *C. tropicalis*, *C. neoformans* and their MIC values were calculated by micro-dilution method. The antibacterial activity measurements for copper-palladium nanofluids are performed first time. Results of antibacterial activity were found to depend on the concentration of copper and palladium in nanofluids.

Experimental

A series of bimetallic Cu/Pd nanostructures based nanofluids were prepared via a facile method [10] by taking the three different molar ratios - 1/20, 1/1 and 20/1 of Cu/Pd bimetals at nanoscale. A flow chart for the synthesis is shown in Figure 1. Trisodium citrate (147 mg) was used as a complexing agent and polyvinyl pyrrolidone (PVP) (500 mg) as a protecting agent. Whole synthesis was carried on at room temperature. The synthesized Cu:Pd in ratio 1:20, 1:1 and 20:1, were labelled as S1, S2 and S3, respectively. The structural properties and crystallite size of the material was analyzed with X-ray diffractometer (X-Pert PRO PANalytical) using monochromatized Cu K_α radiation (λ=1.54059 Å). Thin film was used as target material in the XRD measurement. The morphology of the thin film was investigated with scanning electron microscope (SEM, LEO-0430). Particle size of Cu/Pd bimetallic nanoparticles and their distribution in the nanofluids were determined with a Matec Applied Sciences acoustic particle sizer (APS-100). APS-100 determines particles size and their distribution

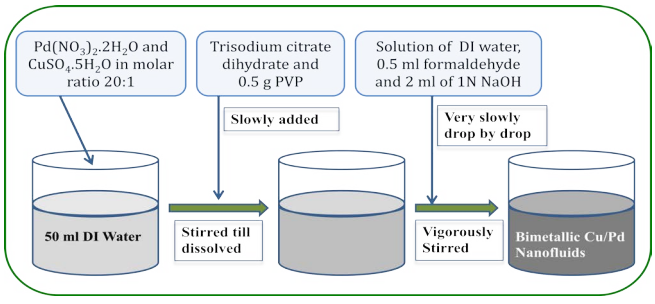

Figure 1: Flow chart of the synthesis of copper/palladium nanofluids.

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from the acoustic attenuation data using the software based on Epstein and Carhart theory [16]. The UV-Vis spectra of the NFs were recorded by Ultrospec 4000 UV/Visible Spectrophotometer.

Antimicrobial (antibacterial and antifungal) activities of NFs were evaluated by measuring the zone of inhibition in the agar disc as per standard protocol [17]. The disc diffusion method was used to screen the antibacterial activity and antifungal activity. In this study, different bacterial strains viz. *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (clinical isolate), *Staphylococcus aureus* (ATCC 25323) were used to test the antibacterial activity and different strains of candida viz. *Candida albicans* (ATCC 90028), *Candida tropicalis* (ATCC 750), *Cryptococcus neoformans* were tested for the antifungal properties of BMNFs. All cultures were preserved at Department of Microbiology, Institute of Medical Sciences, BHU, Varanasi, India. For antimicrobial Susceptibility Test: Muller Hinton agar (MHA) plates were prepared by pouring 15 ml of molten media into sterile petriplates. The fresh grown bacteria were suspended in sterile saline to achieve concentration of 10^7 cfu/ml. This suspension was spread on the surface of plates. The different concentrations of nanofluids were loaded on 6 mm sterile disc and were placed on the surface of medium. The plates were kept for incubation at 37°C for 24 hr for bacteria and 48 hr at 25°C for fungal agents. At the end of incubation, inhibition zones were examined around the discs, were measured with transparent ruler in millimetres.

Further, MIC values were also determined for the standard solution of ciprofloxacin (CPF) and nanofluid samples by micro-dilution method [18,19] using serially diluted (2 fold) test compounds according to guidelines of National Committee for Clinical Laboratory Standards (NCCLS) [20]. MIC was determined by dilution of nanofluids with different concentration. Standardized inoculums of bacteria/fungus were added in each well of microtiter plate. The nanofluids were serially diluted in specific well and were then incubated at 35°C for 24 hr for bacterial growth and at 25°C for fungal growth for 48 hr. The lowest concentration (highest dilution) of the nanofluid which inhibits the visible bacterial growth of microorganism was regarded as MIC.

Results and Discussion

Figure 2a displays the XRD pattern of the bimetallic nanoparticles which indicates that diffraction peaks at 2θ=39.6°, 45.7° and 67.8° correspond to the (111), (200) and (220) planes of Pd (JCPDS card no. 00-05-0681) of the fcc lattice, respectively. Other peaks located at 2θ=43.6° and 74.4° can be assigned to the reflections of corresponding (111) and (220) planes, respectively of Cu (JCPDS card no. 00-04-0836). The average crystallite size estimated from the Debye–Scherrer relation was 3 nm. The UV-vis absorption spectra of S1, S2 and S3 are presented in Figure 2b. It can be clearly seen that there is no absorption peak above the 300 nm in case of S1 which indicates the reduction of Pd(II) [21]. The peak observed at 650 nm for sample S3 shows the formation of copper nanoparticles due to more proportionality of Cu in comparison to Pd. Figure 2c shows the particle size distribution determined with the principle of ultrasonic spectroscopy using acoustic particle sizer (APS-100). The APS-100 measures the ultrasonic attenuation (dB/cm) at different frequencies of the ultrasonic waves (1-100 MHz) of nanofluids with high accuracy. These measurements are commonly referred to as ultrasonic attenuation spectroscopy. The APS-100 simultaneously measures the velocity of the waves at different frequencies. The measured ultrasonic attenuation spectra are converted into particle size distribution data using the software based on Epstein and Carhart theory [16]. This theory incorporates the intrinsic absorption, visco-internal dissipation losses, thermal dissipation losses and scattering losses of ultrasonic energy interacting with the nanofluids. It is obvious from the Figure 2c that the particle size distributions are not very much different for S1, S2 and S3. It is clear from figure that particle distributions of S1 and S2 are in the range of 10 to 20 nm, while particle distribution of S3 is in the range of 10 to 15 nm. For S1 and S2, distribution curve overlaps. However, it is slightly different for S3. It seems due to more concentration of copper in the ratio. Figure 2d-2f shows SEM micrographs of the S1, S2 and S3, respectively. Figure 2d shows at random distribution of the particles. Figure 2e shows hexagonal shaped nanostructures. Figure 2f shows the mixed shaped nanostructures. Thus, a varied composition of Cu/Pd bimetallic system was observed on their optical and surface morphological features. The surface morphology of S2 may be helpful for its application for the development of sensors as it possesses special surface structure [22].

Table 1 shows results of antimicrobial activity of nanofluids. It is obvious from the Table 1 that antibacterial activity of S3 is found to be higher against both Gram positive and negative bacteria than those of S2. The antibacterial activity of S1 and S3 is equal against the Gram negative bacteria - *P. aeruginosa.* Interestingly, S3 shows higher antibacterial activity against *S. aureus* and *E. coli* than S2; however, lesser activity is recorded *E. faecalis*. The response of antibacterial activity is depicted in Figure 3. Although the difference in antibacterial activity of S1 and S3 is insignificant, the bacterial inhibition potential was displayed significantly increased as against the standard solution of well-known antibiotic drug, CPF. The zone of bacterial growth

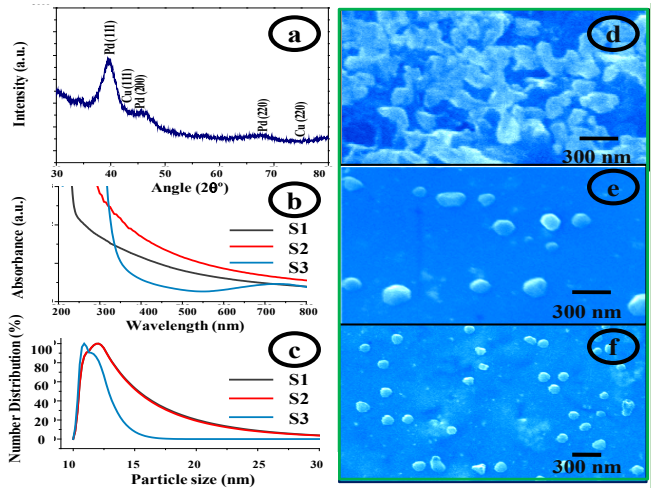

Figure 2: (a) XRD pattern of S1, (b) UV-Vis spectra, (c) particle size distribution curves determined with APS, and (d-f) SEM images of S1, S2 and S3 respectively.

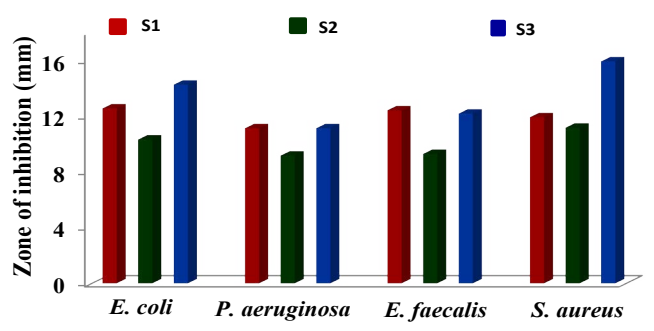

Figure 3: Antibacterial response in terms of inhibition zone (in mm).

(a) In terms of inhibition zone (in mm)							
Microbial species							
	Gram negative bacteria		Gram positive bacteria		Fungi		
Nanofluids	***E. coli***	***P. aeruginosa***	***E. faecalis***	***S. aureus***	***C. albicans***	***C. tropicalis***	***C. neoformans***
S1	12.54 ± 0.47	11.12 ± 0.33	12.41 ± 0.68	11.91 ± 0.43	-	-	-
S2	10.31 ± 0.19	9.16 ± 0.66	9.28 ± 0.11	11.16 ± 0.66	-	-	-
S3	14.24 ± 0.21	11.12 ± 0.33	12.17 ± 0.18	15.91 ± 0.43	-	-	-
Ciprofloxacin	28. 06 ± 1.30	24.76 ± 0.76	23.7 ± 1.05	29.93 ± 0.49	-	-	-
Amphotericin B	-	-	-	-	18.07 ± 0.28	16.22 ± 0.40	19.51 ± 0.52
(b) In terms of MIC values (µg/ml)							
Microbial species							
	Gram negative bacteria		Gram positive bacteria		Fungi		
Nanofluids	***E. coli***	***P.aeruginosa***	***E. faecalis***	***S. aureus***	***C. albicans***	***C. tropicalis***	***C. neoformans***
S1	46.98	93.97	46.98	93.97	-	-	-
S2	93.97	187.96	375.9	93.98	-	-	-
S3	46.98	93.97	93.98	46.98	-	-	-
Ciprofloxacin	6.25	6.25	3.12	6.25	-	-	-
Amphotericin B	-	-	-	-	0.5	0.5	0.5

Table 1: Antimicrobial activity of nanofluids (a) in terms of inhibition zone (in mm) and (b) in terms of MIC values (µg/ml) against various microbial species.

inhibition on culture dishes was ranged from 9-16 mm by application of S1, S2 and S3 NFs at very low concentration, i.e., 46.98, 93.97 and 46.98 µg/ml, respectively. It is revealed from these data that selected samples of BMNFs have mild antibacterial property. This experiment further exhibited substantially increased state of MIC values in S2 sample in comparison to S1 and S3 samples as well as CPF. Antifungal characteristics of these nanofluids were found completely absent as against the amphotericin B, a well-known antifungal drug.

Although a few antibacterial inducing mechanisms have been postulated by exposure of some mono and/or bimetallic nanoparticles [6,23], but none of the mechanism was established as central mechanism of mortality of different strains of bacteria. Xiu et al. [23] suggested that metal ions, released from metallic nanoparticles, strongly bound to cell wall of the bacteria and easily pass through membrane into the protoplasm, and thus damage the single stranded DNA which ultimately leads to cell death. We may also speculate the similar mechanism of action of metallic nanofluids at certain ratio and concentration. Their large surface area of nanoparticles provides better contact with the microorganisms and hence, provides better fusion with the bacterial cell membranes leading to membrane damage and cell death. Hence, this laboratory is pioneer to report antibacterial property of some selected bimetallic nanofluids at certain ratios. The smaller is the particle, the greater is its surface area to volume ratio. This enhances its biological and chemical activity by increasing the contact area of the bimetal with a microorganism. The use of nanoscale bimetals allows achieving hundred time decreased concentration and at the same time increase in antimicrobial properties.

In the present investigation in order to improve antibacterial properties, we have synthesized bimetallic nanoparticles based nanofluids. Previously, Wu et al. [24] tried to synthesize combined nanoparticles for this purpose with advanced antibacterial properties. On perusal of Table 1, it is obvious that S3 has Cu:Pd in the ratio 20:1. Since Cu nanoparticles have larger antibacterial activity in comparison to Pd nanoparticles, therefore S3 has greater antibacterial activity in comparison to S1 in which Cu:Pd ratio is 1:20. The lowest antibacterial activity of S2 was probably due to the equal proportion of Cu and Pd nanoparticles (1:1) present in bimetallic nanofluid. In S1, Pd nanoparticle proportion is greater and antibacterial activity is larger than that of S2. It shows that antibacterial activity due to Pd nanoparticles is significant but smaller in comparison to Cu addition. Antibacterial activity of Pd nanoparticles is not reported in the literature. Lowest value of antibacterial activity in S2 may be understood due to influence of shape of the particles and effective concentration of Cu/Pd bimetals. Further investigations are required for substantial increase of antibacterial characteristics of Cu/Pd nanofluids by modulating the molar ratios of nanoparticles considering their applied use in pharmaco-therapeutic industry and nanodrug delivery system. The different antimicrobial agents, such as Ag and Au nanoparticles extensively studied by the others, are very costly. Cu/Pd BMNFs are cost effective in comparison to these novel metal nanoparticles.

Conclusion

In the present study, a facile approach was used for one-pot synthesis of polyvinylpyrrolidone (PVP) stabilised copper/palladium bimetallic nanostructure based nanofluids with small range distribution (~10-20 nm) at room temperature. The results on the investigations of the nanofluids demonstrate that these nanofluids inhibited the growth of bacteria at very low concentrations. Also the influence of Pd nanoparticles and morphology of the Cu/Pd nanostructure is significant for the antimicrobial activity of nanofluids which is important for pharmaceutical industries. Thus, the application of these nanofluids as antibacterial agent will be very valuable for biomedical and industrial applications.

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