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Evaluation of Antimicrobial Activity of Ionic Liquid-Assisted Synthesis of Monometallic Silver and Bimetallic Copper-silver Nanoparticles

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Abstract: The formation of monometallic silver and bimetallic copper-silver nanoparticles in 1-butyl-3-methylimidazolium methanesulfonate ionic liquid, through chemical reduction is reported. The synthesized particles were characterized using SEM/EDX, UV-vis, and FTIR spectroscopy. UV-vis and FTIR revealed the formation of nanoparticles with active components being adsorbed on the surface of the particles, as stabilizers. SEM revealed uniformed microspheres and microcubes for AgNPs and AgCuNPs, respectively. On the bactericidal and fungicidal activity of AgNPs and AgCuNPs against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebseilla pneumonia* (bacteria) and *Candidaalbicans* (fungus), we observed that AgNPs inhibited *Pseudomonas aeruginosa (23 mm)* and *Candida albicans* (29 mm) higher than the bimetallic particles and the antibiotics used as control. It is interesting to note that AgCuNPs inhibited *Staphylococcus aureus* (21 mm) better than AgNPs (15 mm) indicative of the synergistic effect of two metals.

Introduction

The interest in metal / or bimetallic nanoparticles stems from their applications in diverse areas such as catalysis, chemical sensors, biocatalysts and nanomaterial technology (Yu *et al*., 2012; Esteban *et al*., 2015; Marcos *et al*., 2015; Hatakeyama *et al*., 2016; Janiak , 2014). In particular, silver nanoparticles (Ag-NPs) are potential building blocks for the creation of new materials with tailored properties for optical and medical applications (Graf *et al.*, 2009; Mantion *et al*., 2008; 2011). Silver nanoparticles have proven useful in sports wears due to their anti-bacterial tendency and their compatibility with the human system at low concentration which have made them useful as disinfectants. Also, their unique optical property has made them useful in decorative arts and human make up (Ayi *et al.*, 2014; Pacioni *et al.*, 2015).

It has been demonstrated by different research groups that ionic liquids (ILs) offer outstanding possibilities as a green solvent in the synthesis of metal nanoparticles (Wegner and Janiak 2017; Zhu and Hou, 2012, Ayi *et al*., 2011; 2015). This is not only as a result of achievable high reaction and nucleation rates with the formation of small particles, but also for their electronic and steric stabilization that prevents particle aggregation [Richter *et al*., 2013]. Imidazolium-based ILs have been extensively utilized in the formation and stabilization of M-NPs (Khare *et al*., 2010; Ayi *et al*., 2010; Ayi *et al*., 2015; Zhang *et al.*, 2015). Imidazolium ILs are air, water and electrochemically stable with a wide liquidus range. 1-Alkyl-3-alkyl'-imidazolium ILs simultaneously act as reaction media, hydrogen sources, catalysts, templating agents and stabilizers for the synthesis of metal nanoparticles. According to Werner and Janiak (2017), there are ILs which have a strong influence on particle formation, good nucleation aids, but poor stabilizers, good nucleation aids and good stabilizers, and those which are none of this. ILs with the 1-n-butyl-3-methylimidazolium [BMIM] cation and the relatively weakly coordinating anions such as tetrafluoroborate, hexafluorophosphate and trifluoromethanesulfonate, are liquids over a large range of temperatures, possess high thermal and chemical stability, a large electrochemical window, high ion density, relatively low viscosity, and negligible vapor pressure [Dupont *et al*., 2002].

The present study reports on the antimicrobial activities of silver monometallic and copper-silver bimetallic nanoparticles synthesized in 1-n-butyl-3-methylimidazolium methane sulfonate [BMIM][MS] ionic liquid.

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Materials and Methods

Materials

All chemicals (1-Butyl-3-methyl-imidazolium methane sulfonate ($C_9H_{10}N_2O_3S$) ionic liquid, silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$)) were of analytical grade and were used as purchased from Sigma-Aldrich.

Synthesis of AgNPs (monometallic)

The procedure adopted for the synthesis of silver nanoparticles (AgNP) used 1-butyl-3-methyl-imidazolium methane sulfonate ionic liquid as a solvent and sodium borohydride as reducing agent. In a typical synthesis, $AgNO_3$ (1.0g, 5.83×10^{-06} mM)was dispersed in 1-butyl-3-methyl-imidazolium methane sulfonate ionic liquid (1.4g, 5.97×10^{-06} mM)followed by the addition of$NaBH_4$(0.74g, 1.938×10^{-05} mM) under continuous stirring. The resultant black mixture was heated at 120 °C under reflux. Six portions of $1cm^3$ of the mixture was taken out from the reaction vessel after every 1 h interval, the reaction was stopped after 6 hours, a colour change from black to ash with a dirty white supernatant was observed. The products were centrifuged at 3000 rpm for 15 min, filtered and wash with distilled water, dried at room temperature and stored in airtight container for further analysis.

Synthesis of CuAgNPs bimetallic nanoparticles

The procedure adopted for the synthesis of Cu-Ag bimetallic nanoparticles (Cu-AgNP) used Copper (II) chloride, silver nitrate, 1-butyl-3-methyl-imidazolium methane sulfonate, sodium borohydride. In a typical synthesis, $AgNO_3$ (2.0g, 1.17×10^{-05} mM) was dispersed in BMIMMS ionic liquid (4.0g, 1.74×10^{-05} mM) followed by $CuCl_2$ (2.0g, 2.02×10^{-05} mM). The reducing agent, $NaBH_4$ (0.3g, 7.930×10^{-06}mM) was then added and the reaction mixture was stirredcontinuously for 30 mins. The resultant homogenous black colloidalmixture was heated under reflux at 120°C for 6 h. Six portions of $1cm^3$ of the mixture were taken out from the reaction vessel after every 1h for UV-vis measurements. A colour change from black to light green was observed at the end of the reaction time. The products were centrifuged at 3000 rpm for 15 min, washed with distilled water, dried at room temperature and stored in an airtight container for further analysis.

Characterization

The reductions were monitored using UV-visible spectrophotometer (Evolution 201 spectrophotometer) at regular interval with samples dissolved in ethanol using quartz cuvette operated with a resolution of $1cm^3$.

The FTIR Spectrophotometer (Shimadzu IR Affinity-1S) in the spectral range 4000–500 cm^{-1} KBr pellets (sample: KBr = 20 : 1). The scanning electron microscopy (SEM) was performed on a Hitachi S-4800 microscope attached with EDX at a voltage of 15Kv.

Antimicrobial studies

Antimicrobial susceptibility test was done in the bacteriology lab of the General Hospital Calabar, Cross River State, Nigeria. The agar diffusion test (disc diffusion method) was adopted [Prescott et al., 2005]. Muller-Hilton agar was prepared from a dehydrated base according to the manufacturer's instruction. The medium was allowed to cool to 47 °C and poured into petri-dishes that were arranged and labeled according to their microbial isolates, and allowed to set on a level surface to a depth of approximately 4 mm. When the agar had solidified, the plates were dried for 20 minutes at 35°C by placing them in an upright position in hot air oven with the lids tilted. A discrete colony of each of the isolate was picked with a sterile wire loop and streaked on the Muller-Hilton agar according to their names as labeled: staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebseilla pneumonia (bacteria) and Candidaalbicans (fungus). Filter paper discs carrying 2000 µg/ml AgNPs, CuAgNPs and antibiotics; Ciprofloxacin (10 µg) Ofloxacin (30 µg) Augmentin (25 µg), Ceftriaxone (25 µg), Meropenem (30 µg) and Racinef (30 µg) were transferred to the appropriate locations on the agar plates with the help of sterile forceps. The plates were then incubated at 37 ^oC for 18 h. At the end of the incubation, the zones of inhibition or no inhibition (for resistant strains) were measured and recorded in millimeters.

Results and Discussions

Nanoparticles of silver and copper-silver alloy were synthesized in 1-butyl-3-methylimidazolium methanesulfonate ionic liquid via chemical reduction by$NaBH_4$. The reduction reactions were performed under reflux at a temperature of 120 ^oC. The colour of AgNPs changed from dark to grey, while that of AgCuNPs changed from dark to green, indicating the possible oxidation of metallic copper to copper chloride. The effect of heating time on the reaction was monitored with UV-vis spectroscopic analysis. Figure 1 shows representative UV/vis spectra of AgNPs (Fig. 1a) and AgCuNPs (Fig. 1b) synthesized in IL (BMIMMS). The UV-vis spectra shows signals in the range 300 – 350 nm attributed to molecules of the ionic liquid adsorbed on the surface of the particles similar to earlier reports [Ayi *et al*., 2010; 2015; Khare *et al*., 2010]. The surface plasmon absorbance for the AgNPs is observed in the range 400 - 450 nm for 1 -5 h of heating and disappears after 6 h heating period. The bimetallic nanoparticles

showed absorbance in the range 400-480 nm throughout the 6 h heating period.

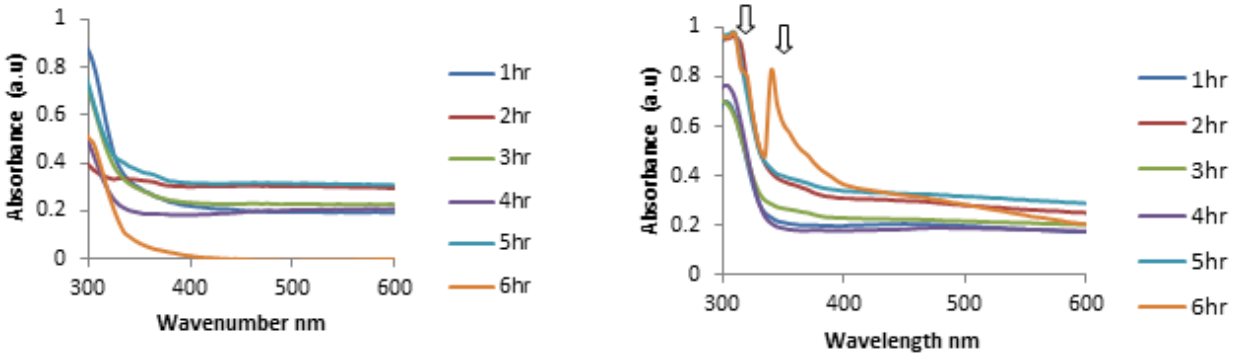


Figure1: UV-Vis of particles synthesised in 1-butyl-3-methyl-imidazolium methane sulfonate (a) AgNPs (b) AgCuNPs.

SEM analysis was used to study the surface morphology of synthesized nanoparticles. The SEM micrographs of the AgNPs and AgCuNPs are shown in Fig. 2. The SEM images demonstrated that AgNPs are spherical, while the AgCuNPs are cubic in shape. The energy dispersive analysis by x-ray was employed to quantify the composition of the synthesized nanoparticles. The EDAX spectra of the particles presented in Fig. 3 confirmed the presence of Ag (79.78 %), C (8.84 %), N (6.59 %), O (3.59 %) and S (1.21) for AgNPs (Fig.3a) and the presence of Cu (73.20 %), Ag (0.94 %), C (18.64 %), O (3.93 %), Cl (3.29 %) for AgCuNPs (Fig.3b). The C and N belong to the cationic part while S and O belong to the anionic part of the ionic liquid providing electrostatic stabilization for synthesized particles.

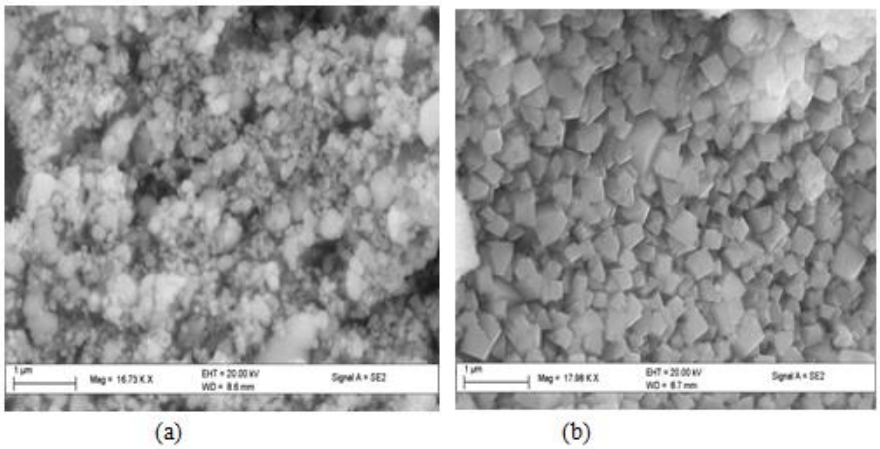


Fig.2 SEM images of particles synthesized in ionic liquid [BMIM][MS] (a) AgNPs (b) AgCuNPs

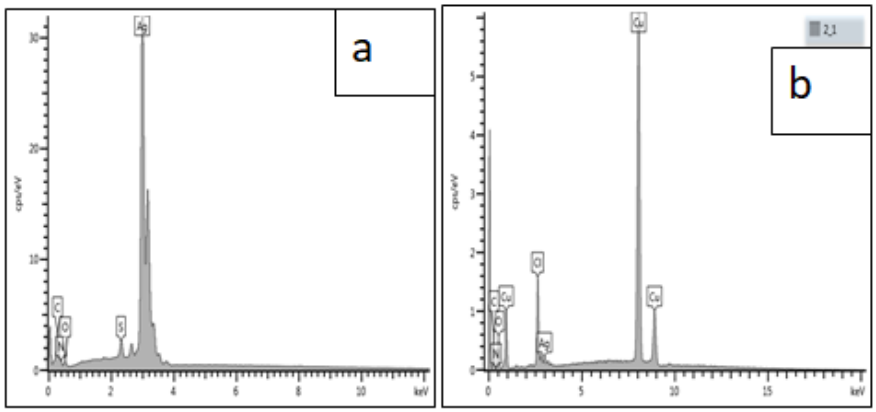


Fig. 3 EDAX spectra of nanoparticles synthesized in ionic liquid (a) AgNPs (b) AgCuNPs

According to Derjaguin-Landau-Verwey-Overbeek theory (DLVO theory), the anion is the primary source of stabilization for the metal nanoparticles (Verwey and Overbeek 1999; Finke, 2002). The metal nanoparticles and their anion layer on the surface form an overall negatively charged particle and are subject to Coulomb repulsion within the DLVO theory [Vollmer and Janiak, 2011]. Thus the ionic liquid cations are attracted to the surface of a negatively charged nanoparticle to form a positive ion layer, and then counter anions form a second layer on the nanoparticle surface by electrostatic attraction [Obliosca *et al*., 2010; Rubim *et al*., 2008; Schrekker *et al*., 2007].To determine the possible functional groups involved in the stabilization of both AgNPs and the bimetallic AgCuNPs, FTIR spectroscopy was employed (Fig. 4). The spectra showed broad absorption band at 3458 cm^{-1} for AgNPs, which can be assigned to stretching vibration of v(N-H) group of the imidazolium cation involved in hydrogen bonding. This band is split into 3346 and 3439 cm^{-1}in the case of AgCuNPs. The bands between the region 3005 - 2925 cm^{-1} alongside the bands 1645 - 1615 cm^{-1}(for both AgNPs and AgCuNPs) are attributed to C–H(*in-plane*) and C=C stretching vibrations of the imidazolium moiety. The band at 1382 cm^{-1} is ascribed to the v(C-N) stretching vibration of the tertiary amine. Also the bands in the region 1197 -1051 cm^{-1} indicates the presence of v(C-S) stretching vibration of the methanesulfonates, while the absorption band at 987 – 780 cm^{-1} region is a clear indication of the presence of sulfonates bending vibration and absorption bands at 597 -412 cm^{-1} indicates the presence of M-O bending vibration. The various assignments agree with related compounds reported in literature (Roldan *et al*., 2012; Markova-Deneva. 2010).

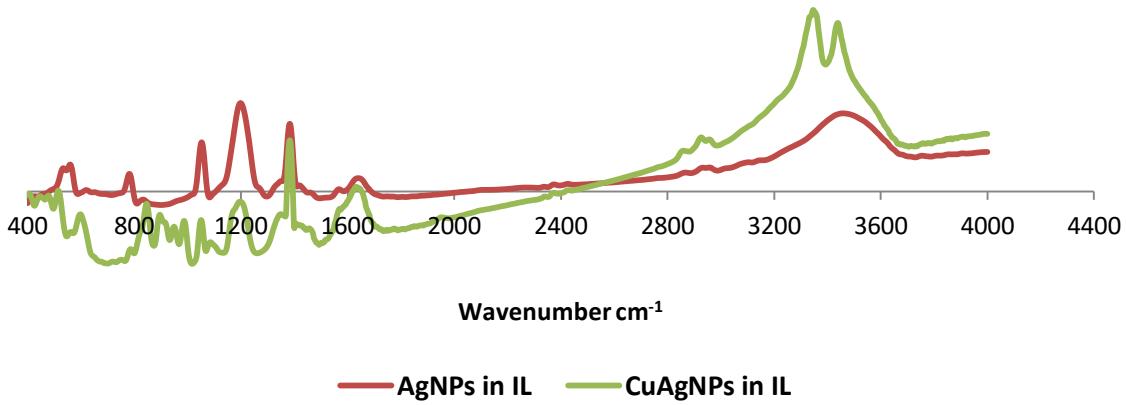


Fig.4 FTIR spectra AgNPs (red) and AgCuNPs (green) in ionic liquid [BMIM] MS

Antimicrobial activities of the synthesized AgNPs and AgCuNPs were investigated using standard agar diffusion assay. The nanoparticles showed inhibition zone against all the studied bacteria and fungus (*Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Pseudomonas and Candida albicans* sp) as presented in Figure 5 and Figure 6. The zone of inhibition of AgNPs against *Pseudomonas* and *Candida albicans* are 23 and 29 mm, respectively which are higher than the inhibition zones of the bimetallic particles (AgCuNPs) and also the antibiotics used as control. Monometallic silver nanoparticles inhibited other organisms except *staphylococcus aureus* better than the bimetallic copper-silver nanoparticles. While AgCuNPs inhibited *Staphylococcus aureus* (21 mm) better than AgNPs (15 mm) this indicates that the synergistic effect of two metals plays a vital role in the inhibition of staphylococcus (Ashishie *et al*., 2018; Nazeruddin *et al*., 2014; Nalawade *et al*., 2014; Jaiswal*et al*., 2016). On a general scale, it is worthy of note that; nanobiotics (nanoparticles) synthesized from 1-butyl-3-methyl imidazolium methane sulfonate showed prominent inhibition against the microbes under study than antibiotics most especially, racinef.

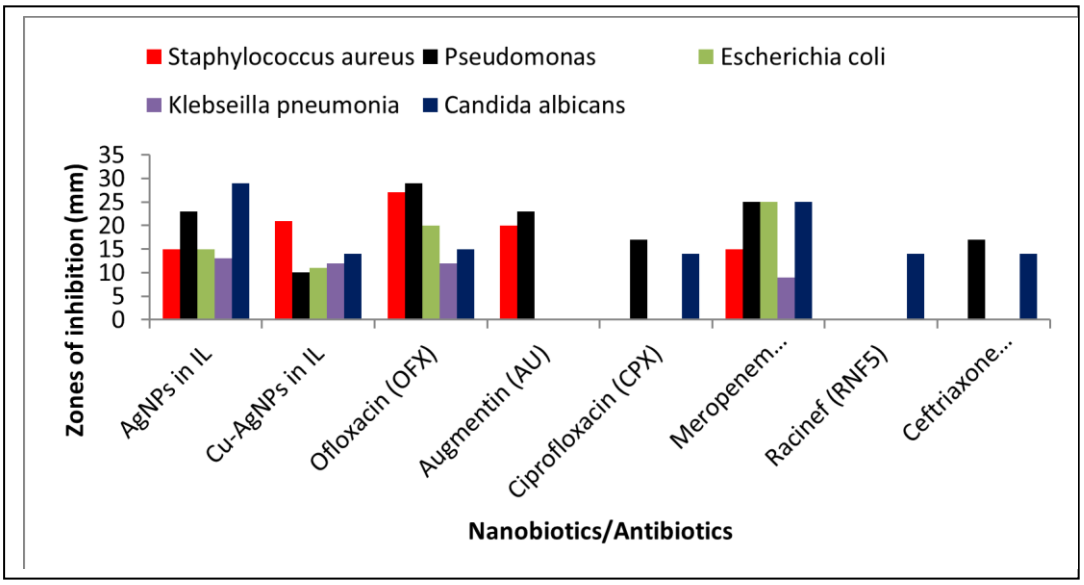
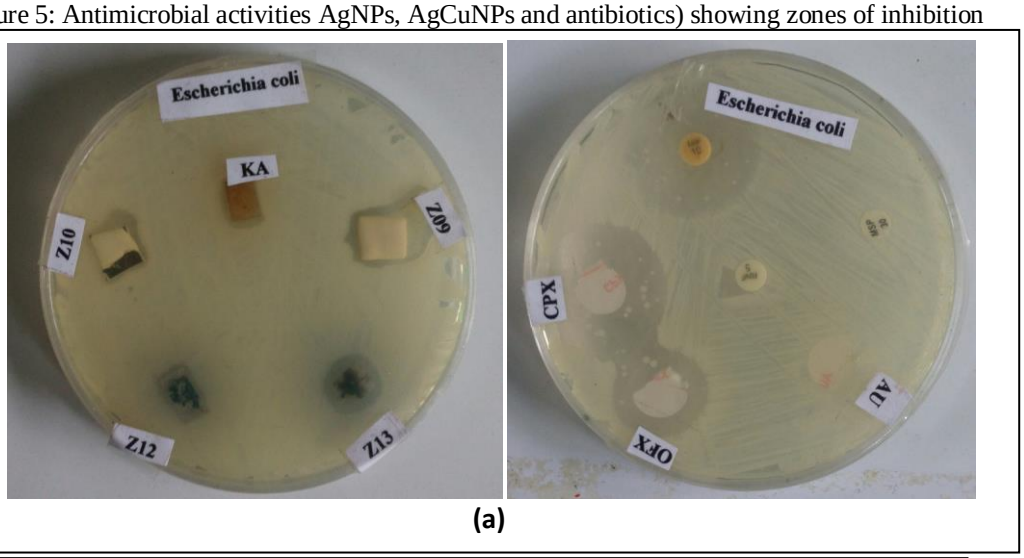
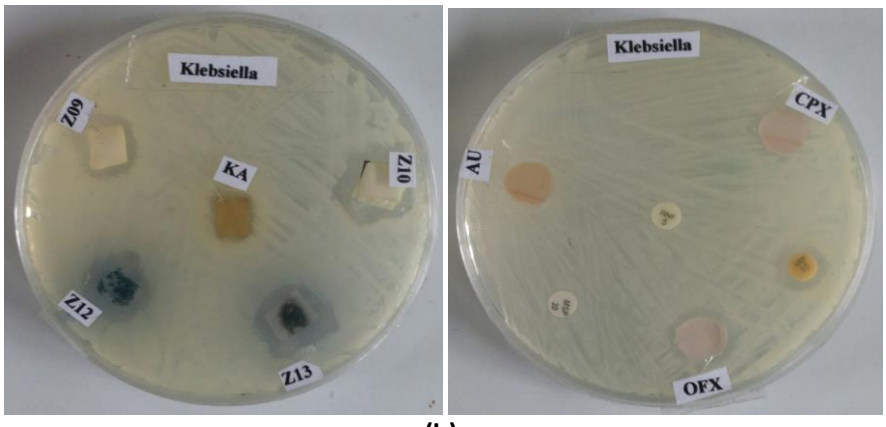
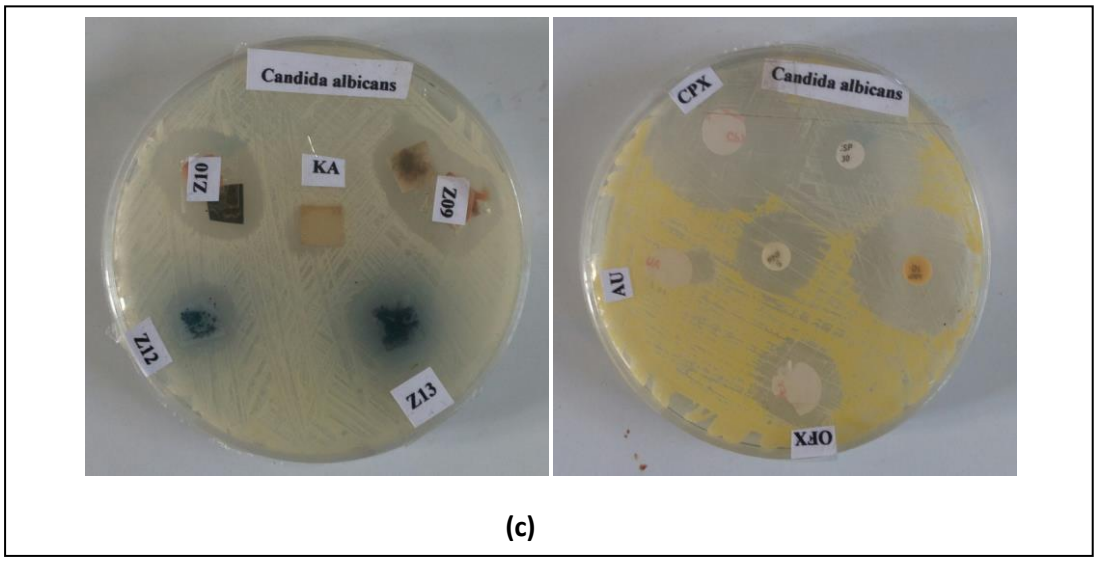


Figure 5: Antimicrobial activities AgNPs, AgCuNPs and antibiotics) showing zones of inhibition







(c)

Figure 6: Representativeplates (a-e) showing zones of inhibition: Z 10= AgNPs in IL, ,Z 12 =Cu-AgNPs, ,OFX=ofloxacine, CPX=ciprofloxacin,AU= augmentin

Conclusion

Using ionic liquid as a solvent and stabilizer, silver monometallic and silver-copper bimetallic nanoparticles have been successfully synthesized via chemical reduction with $NaBH_4$. The UV-vis and IR measurements showed that nanoparticles were formed and that molecules of the ionic liquids were adsorbed on the surface of the particles. An EDAX measurement also lends credence to this fact. The silver monometallic nanoparticles were found to be spherical while the bimetallic particles are cubic in shape. The larger zone of inhibition obtained against microbes proves that the synthesized nanoparticles in ionic liquids have good potentials in drug delivery applications.

REFERENCES

1. Ashishie, P.B, Anyama, C.A, Ayi, A.A, Oseghale, C.O, Adesuji, E.T, Labulo, A.H. (2018). Green synthesis of silver monometallic and copper-silver bimetallic nanoparticles using Kigelia Africana fruit extract and evaluation of their antimicrobial activities. International *Journal of Physical Sciences*, 13(3): 24-32.
2. Ayi, A.A, Anyama, C.A, Khare, V. (2015). On the synthesis of molybdenum nanoparticles under reducing conditions in ionic liquids. *Journals of Materials*, 2015.
3. Ayi, A.A, Khare, V, Strauch, P, Girard, J, Fromm, K.M, Taubert, A. (2010). On the chemical synthesis of titanium nanoparticles from ionic liquids. *MontashChem*, 14: 1273-1278.
4. Ayi, A.A., Aniefiok, J.U, Anyama, C. A, Samuel, S. E, Ogar, J.O. (2014). Synthesis, Spectral Characterization of Anti-Microbial Activity of Silver Nanoparticles in 1-Alkyl-3-Methylimidazolium Methanesulfonate Ionic Liquids.International *Journal of Innovative Research in Science & Engineering*, 3(5): 2347 – 3207.
5. Dupont, J, de Souza, R.F, Suarez, P.A.Z. (2002). Ionic Liquid (Molten salt) Phase Organometallic Catalysis. *Chemical Reviews*, 102 (10): 3667–369.
6. Esteban, M. R, Meyer, H, Kim, J, Gemel, C, Fischer, R. A, Janiak, C. (2015). Comparative synthesis of Cu and Cu_2O Nanoparticles from different copper precursors in ionic liquid or propylene carbonate, *European Journal of Inorganic Chemistry*, DOI: 10.1002/ejic.201500969.
7. Finke, R. G. (2002). Metal nanoparticle: synthesis, characterization and applications. Marcel Dekker: New York.
8. Graf, P, Mantion, A, Foelske, A, Shkilnyy, A, Masic, A, Thünemann, A. F, Taubert, A. (2009). Peptide-coated silver nanoparticles: synthesis, surface chemistry, and pH-triggered, reversible assembly into particles assemblies. *Chemistry-A European Journal*, 15 (23): 5831–5844.
9. Hatakeyama, Y., Judai, K., Onishi, K., Takahashi, S., Kimura, S., Nishikawa, K., (2016). Anion and cation effects on the size control of Au nanoparticles prepared by sputter deposition in imidazolium-based ionic liquids. *Physical Chemistry Chemical Physics*, 18 (4): 2339-2349.
10. Jaiswal, A.K, Gangwar, M, Nath, G, Yadav, R.R. (2016). Antimicrobial activity of bimetallic Cu/Pd nanofluids. *Journal of Chemical Engineering*. 6 (2): 151.
11. Janiak., C. (2014). Metal Nanoparticle Synthesis in Ionic Liquids, in Catalysis in Ionic Liquids: From Catalyst Synthesis to Application, ed. C. Hardacre and V. Parvulescu, RSC Publishing: Cambridge, ch. 11: p. 537–577.
12. Khare, V, Li, Z, Mantion, A, Ayi, A. A, Sonkaria, S, Voelkl, A, Thunemann, A. F, Taubert, A. (2010). Strong anion effects on gold nanoparticles formation in ionic liquids. *Journal of Materials Chemistry*, 20 (7): 1332–1339.
13. Mantion, A. (2011). Biomimetic synthesis of chiral erbium-doped silver/pepetide/silica core-shell nanoparticles (ESPN). *Nanoscale*, 2011(3): 5168-5179.
14. Mantion, A, Guex, A. G, Foelske, A, Mirolo, L, Fromm, K. M, Painsi, M, Taubert, A. (2008). Silver nanoparticles engineering via oligovalineorganogels. *Soft Matter*, 4 (3): 606–617.
15. Marcos Esteban, R, Schütte, K, Marquardt, D, Barthel, J, Beckert, F, Mülhaupt, R, Janiak, C. (2015). Synthesis of ruthenium@graphene nanomaterials in propylene carbonates as reusable catalyst for the solvent-free hydrogenation of benzene. *Nano-Structures &Nano-Objects*, 2: 28 –34.
16. Markova-Deneva, I. (2010). Infrared Spectroscopy investigation of metallic nanoparticles based on copper, cobalt and nickel synthesis through borohydride reduction method (review). *Journal of the University of Chemical Technology and Metallurgy*, 45 (4): 351-378.

17. Nalawade, P, Mukherjee, P, Kapoor, S. (2014). Triethylamine induced synthesis of silver and bimetallic (Ag/Au) nanoparticles in glycerol and their antibacterial study. *Journal of Nanostructure Chemistry*, 4: 113.
18. Nazeruddin, G.M, Prasad, R.N, Shaikh, Y.I, Shaikh, A.A.(2014). Synergetic effect of Ag-Cu bimetallic nanoparticles on antimicrobial activity. *Der Pharmacia Letter*, 6 (3): 129-136.
19. Obliosca, J.M, Arellano, I.H.J, Huang, M.H, Arco, S.D. (2010). Double layer micellar stabilization of gold nanocrystals by greener ionic liquid 1-butyl-3-methylimidazolium lauryl sulfate. *Materials Letters*, 64: 1109–1112.
20. Pacioni, N.L, Borsarelli, C.D, Rey, V, Veglia, A.V. (2015). Synthetic Routes for the preparation of silver nanoparticles; A mechanistic perspective. Springer international Publishing, Switzerland.
21. Prescott, L. M, Harley, J. P, Klein, D. A. (2005). Microbiology (6th edition).McGraw-Hill: Boston.
22. Richter, K, Campbell, P. S, Baecker, T, Schimitzek, A, Yaprak, D, Mudring, A.-V. (2013). Ionic liquids for the synthesis of metal nanoparticles. *Physica Status Solidi B*, 250: 1152 – 1164.
23. Roldan, M.V, Pellegri, N.S, de Sanctis, O.A. (2012). Optical response of silver nanoparticles stabilized by amines to LSPR based sensors. *Procedia Materials Science*, 1:594-600.
24. Rubim, J.C, Trindade, F.A, Gelesky, M. A, Aroca, R.F, Dupont, J. (2008). Surface-Enhanced vibrational spectroscopy of tetrafluoroborate 1-n-Butyl-3-methylimidazolium (BMIBF4) Ionic liquid on silver surfaces. *Journal of Physical Chemistry C*, 112: 19670–19675.
25. Schrekker, H.S, Gelesky, M.A, Stracke, M.P, Schrekker, C.M.L, Machado, G, Teixeira, S.R, Rubim, J.C, Dupont, J. (2007). Disclosure of the imidazolium cation coordination and stabilization mode in ionic liquid stabilized gold (0) nanoparticles. *Journal of Colloidal Interface Science*, 316, 189–195.
26. Verwey, E.J.W, Overbeek, J.T.G. (1999). Theory of the stability of lyophobic colloids. Dover Publications Mineola, New York 101.
27. Vollmer, C, Janiak, C. (2011). Naked metal nanoparticles from metal carbonyls in ionic liquids easy synthesis and stabilization. *Coordination Chemistry Review*, 255: 2039–2057.
28. Wegner, S, Janiak, C. (2017). Metal Nanoparticles in Ionic Liquids. *Topics in Current Chemistry*, 375 (65).
29. Yu, S. H, MacGillivray, L. R, Janiak, C. 2012. Nanocrystals. CrystalEngComm, 14: 7531–7534.
30. Zhang, B, Yuan, Y., Philippot, K, Yan, N. (2015). Ag-Pd and CuO-Pd nanoparticles in a hydroxyl-group functionalized ionic liquid: Synthesis, characterization and catalytic performance. *Catalysis Science Technology*, 5: 1683–1692.
31. Zhu, S, Tong, Y, Lv, Y, Wu, Y. (2012). Use of Ionic liquids to improve the production of hydroxymethylfurfural from renewable biomass. *Bioresources*, 7(1): 3-4.