8th National and International Academic Conference
Huachiew Chalermprakiet University

Effect of prepared condition of polyurethane-silver nanocomposites on the growth inhibition of the opportunistic Gram-negative bacterial

Pornpen Atorngitjawat1* and Wisatre Kongcharoensuntorn[2]
Faculty of Science, Burapha University
*Corresponding Author's Email: pornpena@go.buu.ac.th Telephone: 038103054

Rationale

Recently, opportunistic Gram-negative bacteria play a big role on bacteria pathogens, especially for low-immune people. It has been reported that bacterial infection in hospitals was increased due to antibiotic resistance in bacteria . Metal nanoparticles have been widely employed and prepared in different ways for applying to the hospital equipment. Polyurethane ionomer is one of the functional polymers used as a template for preparing embedded silver nanoparticles and widely used in medical equipment. In this research, the anionic waterborne polyurethane was selected due to the coordination of anions and silver ions with spontaneous reduction of silver ions to silver nanoparticle.
Therefore, in this study, the anionic polyurethanes were used to neutralize with silver ions in four different concentrations, 100, 500, 1000 and 2000 ppm at three different temperatures (25, 60 and 80 ^{o}C). The antibacterial activity of samples was tested against opportunistic Gram-negative bacteria, which were *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and drug resistant *Pseudomonas aeruginosa*, compared to neat polyurethane.

Research Objectives

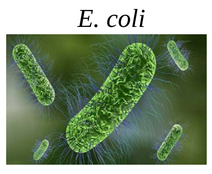
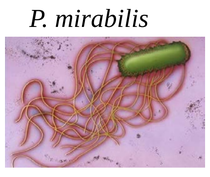
To study the effect of prepared condition of PU/Ag nanocomposites in order to use as an opportunistic Gram-negative antibacterial compound

Methodology

Bacterial strains:

Four bacteria were used in this study. *Pseudomonas aeruginosa* (*P. aeruginosa*) and drug resistant *P. aeruginosa* (1–375/04–2013) were collected from Chonburi hospital, Chonburi, Thailand. *Escherichia coli* (*E. coli* ATCC 25913) and *Proteus mirabilis* (*P. mirabilis*) were kindly provided from Department of Microbiology, Burapha University, Chonburi, Thailand.

P. aeruginosa *E. coli* *P. mirabilis*

Research Methods:

Polyurethane – silver nanocomposites (PU/Ag):

Anionic waterborne polyurethanes were purchased from sincere Group Company, Bangkok, Thailand. $AgNO_3$ was dissolved in deionized water with four concentrations (100, 500,1000 and 2000 ppm) and then added to PU solution with stirring for 15 min at 25 ^{o}C. The temperature was changed to 60 ^{o}C and 80 ^{o}C for the next batch. The PU/Ag films was prepared by solution casting. The PU containing 100, 500, 1000 and 2000 ppm of Ag is referred to as PU/100Ag, PU/500Ag, PU/1000Ag and PU/2000Ag, respectively.

Antibacterial activity test:

The 4 – 6 pure colonies of bacteria from Nutrient agar (NA) were sub-cultured in Mueller Hinton broth (MHB) and then incubated at 37 ^{o}C for three hours. The number of bacterial cultures was adjusted to $1.5x10^8$ CFU/mL by 0.5 McFarland standard. For making the test plate, 50 µL bacterial cultures were mixed with 1 ml of MHB. Then, PU and PU/Ag samples were put in the plates. All samples were incubated at 37 ^{o}C for three hours, and then diluted ten-fold serial dilution by 0.85 % phosphate buffer solution. Next, the diluted samples were poured into NA and incubated for 37 ^{o}C for 24 hours and the bacteria were counted by CFU/mL. In this study PU was used as a control. The effectiveness of antibacterial activities (EAA) of PU/Ag samples and PU were calculated followed by

$$EAA(\%) = \frac{N_0 - N_s}{N_0} \times 100$$

Where N_0 and N_s refer to the number of bacterial colonies (CFU/mL) of PU and PU/Ag samples, respectively.

Bacterial growth kinetic study :

The 5 mL of $1.5x10^8$ CFU/mL of bacterial cultures in MHB was incubated at 37 ^{o}C for 18 hours. The number of bacterial cultures was adjusted with 0.5 McFarland standard. Then pouring into 20 mL tubes which contained PU/Ag samples. The tube was incubated at 37 ^{o}C for 0, 2, 4, 6, 8, 10, and 24 hours, respectively. After incubated by time intervals, all bacteria were diluted by serial dilution, grown on NA for 24 hours, and counted for the survival colonies. The tests were conducted in triplicates. The number of colonies was estimated by $\log_{10}$ CFU/mL.

Results

Figure 1 presented the number of colony of bacteria in $\log_{10}$CFU/mL scale of neat PU, PU/100Ag, PU/500Ag, PU/1000Ag and PU/2000Ag prepared at 25 ^{o}C tested by Broth Dilution Susceptibility. The positive result against the growth of bacteria was found for all PU/Ag samples, compared to PU.

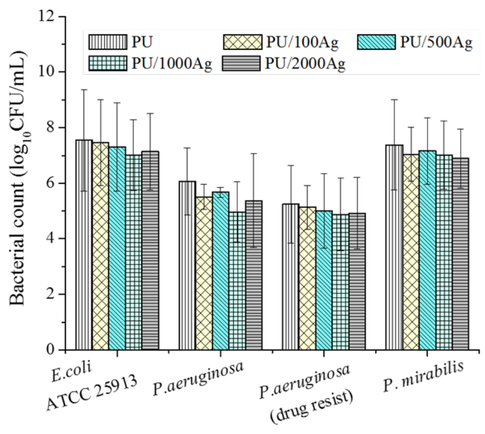


Figure 1 Number of bacterial colonies ($\log_{10}$ CFU/mL).

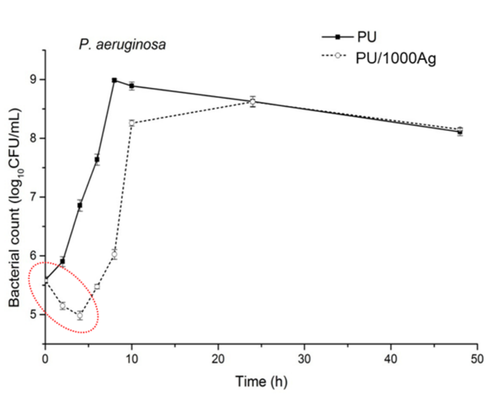


Figure 2 Dynamic growth curve.

From the dynamic growth kinetic study on *P. aeruginosa* alive shown in **Figure 2**, PU/1000 Ag initially inhibited the growth of *P. aeruginosa* at the 2nd hour, and mostly inhibit at the 4th hour and until at the 6th hour. After the number of *P. aeruginosa* decreased from 2 to 8 hours, the bacteria started growing up again at 8th hour, and maintained the growth as the control PU.

Table 1 %EAA of PU/Ag samples prepared at 25 ^{o}C

	Effectiveness of antibacterial activity (%)			
	PU/100Ag	PU/500Ag	PU/1000Ag	PU/2000Ag
E.coli ATCC 25913	16.50	41.55	60.97	59.90
P. aeruginosa	72.36	60.11	86.70	79.14
P. aeruginosa (drug resist)	21.69	41.65	56.43	51.72
P. mirabilis	54.08	39.70	57.40	67.64

As seen in **Table 1**, PU/1000Ag provided the best EAA against *E. coli* ATCC 25913, *P. aeruginosa*, and drug-resistant *P. aeruginosa*. PU/2000Ag was the best against *P. mirabilis*. For *E. coli* ATCC 25913 *and P. aeruginosa* (drug resist), the EAA is in the order of PU/1000Ag > PU/2000Ag> PU/500Ag > PU/100Ag. PU/500Ag shows the lowest EAA against *P. aeruginosa* and *P. mirabilis*.

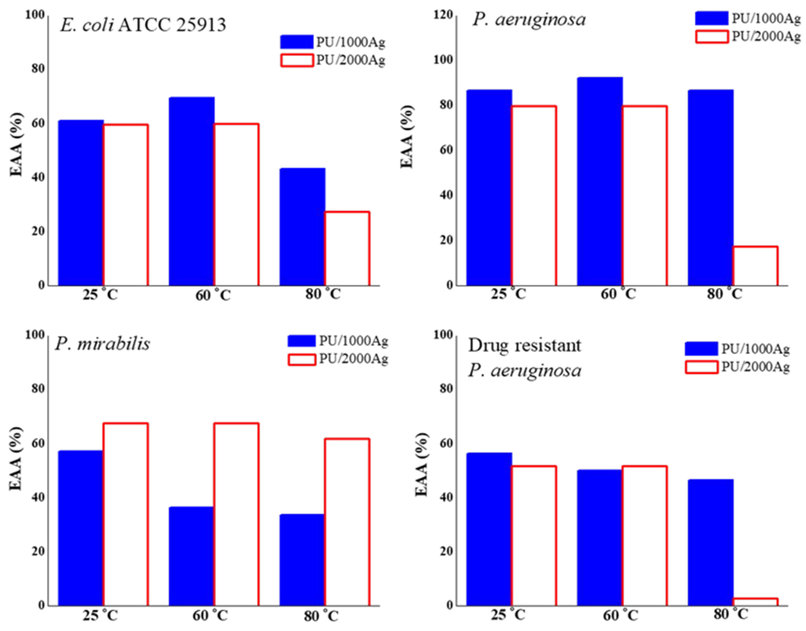


Figure 3 %EAA of PU/1000Ag and PU/2000Ag prepared at temperatures of 25, 60 and 80 ^{o}C.

The effect of different temperatures for preparing PU/Ag against bacterial growth was studied and shown in **Figure 3**. The result shows that PU/Ag prepared at 25 and 60 ^{o}C had no significant different of EAA but was better than the sample prepared at 80 ^{o}C for all opportunistic Gram-negative bacteria.

Discussion

Increasing silver concentrations mostly increased antibacterial activity. Silver nanoparticles can directly inhibit bacteria growth by ether interacting with the cell wall and gradually destroying the metabolic responses or releasing Ag^+ ions via oxidative dissolution and bind to thiol groups of proteins. Not only proteins in organelles and cytoplasm are denatured, but also the phosphate groups in DNA are destroyed and mutated. Therefore, the denaturation of the proteins and enzymes results in stopping DNA synthesis and leads to disruption of the bacterial cell. Therefore, the high increase of silver concentration, the more rate of dissipation of Ag^+ ions.

The different temperature, 25, 60 and 80 ^{o}C for preparing PU/Ag nanocomposites influenced on inhibition of bacterial growth. This is due to at low temperatures, polymer chains are not packed well, containing free volumes, which let Ag^+ ions easier to produce and release into aqueous solution.

This study revealed the efficacy of antibacterial activities against opportunistic Gram-negative bacteria of PU/Ag nanocomposites, which were prepared at different concentrations (100, 500, 1000 and 2000 ppm) and temperatures (25, 60 and 80 ^{o}C) PU/1000Ag exhibited the best antibacterial activity against *E. coli* ATCC 25913, *P. aeruginosa*, and drug-resistant *P. aeruginosa* , which gave the EAA about 61, 87 and 56 %, respectively. PU/2000Ag showed the best effectiveness of antibacterial activities against *P. mirabilis*, which was 68 %. PU/Ag samples prepared at 25 and 60 ^{o}C displayed the greater antibacterial activity against opportunistic Gram-negative bacteria than the sample prepared at 80 ^{o}C.

References

Asamoah, R.B., Yaya, A., Mensah, B., Nbalayim, P., Apalangya, V., Bensah, Y.D., Damoah, L.N.W., Agyei-Tuffour, B., Dodoo-Arhin, D., & Annan, E. (2020). Synthesis and characterization of zinc and copper oxide nanoparticles and their antibacteria activity. *Results in Materials, 7*, 1-11.

Clinical and standard Institute. (2010). *Performance standards for antimicrobial susceptibility testing*: Twentieth informational supplement. Document M100-S20. Wayne, PA: CLSI.

Francolini, I., D′llario, L., Guaglianone, E., Donelli, G., Martinelli, A., & Piozzi, A. (2010). Polyurethane anionomers containing metal ions with antimicrobial properties: thermal, mechanical and biological characterization. *Acta Biomater, 6*, 3482-3490.

Guo, R., Wen, J., Gao, Y., Li1, T., Yan, H., Wang, H., Niu, B., & Jiang, K. (2018). Effect of the adhesion of Ag coatings on the effectiveness and durability of antibacterial properties. *Journal of Material Science, 53*, 4759–4767.

Jia, Y., Sun, R., Pan, Y., Wang, X., Zhai, Z., Min, Z., Zheng, G., Liu, C., Shen, C., & Liu, X. (2021). Flexible and thin multifunctional waterborne polyurethane/Ag film for high-efficiency electromagnetic interference shielding, electro-thermal and strain sensing performances. *Composites Part B, 210*, 1-10.

Lokhande1, G.P., Chambhare1, S.U., & Jagtap, R.N. (2017). Anionic water-based polyurethane dispersions for antimicrobial coating application. *Polymer bulletin, 74*, 4781–4798.

Morones, J.R., Elechiguerra, J.L., Camacho, A., Holt, K., Kouri, J.B., Ramírez, J.T., & Yacama, M.J. (2005). The bactericidal effect of silver nanoparticles. *Nanotechnology, 16*, 2346-2353.

Nagvekar, V., Sawant, S., & Amey, S. (2020). Prevalence of multidrug-resistant Gram-negative bacteria cases atadmission in a multispeciality hospital. *Journal of Global Antimicrobial Resistance, 22*, 457-461.

Park, H., Kim, J.Y., Kim, J., Lee, J., Hahn, J., Gu, M.B., & Yoon, J. (2009). Silver-ion-mediated reactive oxygen species generation affecting bactericidal activity. *Water Research, 43*, 1027-1032.

Sanpui, P., Murugadoss, A., Prasad, P.V.D., Ghosh, S.S., & Chattopadhyay, A. (2008). The antibacterial properties of a novel chitosan-Ag-nanoparticle composite. *International Journal of Food Microbiology, 124*,142-146.

Sedlarik, V., Galya, T., Sedlarikova, J., Valasek, P., & Saha, P. (2010). The effect of preparation temperature on the mechanical and antibacterial properties of poly (vinyl alcohol)/silver nitrate films. *Polymer Degradation and Stability, 95*, 399-404.

Shrivastava, S., Bera, T., Roy, A., Singh, G., Ramachandrarao, P., & Dash, D. (2007). Characterization of enhanced antibacterial effects of novel silver nanoparticles. *Nanotechnology, 18*, 225103-225112.

Stamm, W.E. (1992). *Hospital Infection*. Boston: Little Brown.

Warren, J.W. (1997). *Prevention and control of nosocomial infection*. Baltimore: Williams & Wilkins.

Zapata, P.A., Tamayo, L., Páez, M., Cerda, E., Azócar, I., & Rabagliati, F.M. (2011). Nanocomposites based on polyethylene and nanosilver particles produced by metallocene ‘‘in situ’’ polymerization: synthesis, characterization, and antimicrobial behavior. *European Polymer Journal, 47*, 1541-1549.