JOURNAL OF PURE AND APPLIED MICROBIOLOGY, September 2013. Vol. 7(3), p. 2213-2218

Antibacterial Effects of Polymers Impregnated by Nano Silver Manufactured as Computer Parts

Fahimdokht Mokhtari[1], Esmaeil Salahi[2], Ramesh Mehrpour[1], Mehdi Asgari[2], Mojgan Heidarpour[1]* and Fataneh Shokrollahi[1]

[1]Faculty of Food Industry and Agriculture, Department of Biology, Standard Research Institute (SRI), Karaj P. O. Box 31745-139, Iran,
[2]Materials and Energy Research Center (MERC), Karaj, P.O. Box: 14155-4777, Iran,

(Received: 16 January 2013; accepted: 24 February 2013)

Some certain materials exhibit remarkably unusual physical, chemical and biological properties, including unique interactions with microorganisms at nanoscale, among them the silver nanoparticles have been used vastly in different materials. In this study, the antibacterial effects of acrylonitryl polymers containing 3 different concentrations of silver nano particles incorporated in zeolite as an organic carrier were assessed. These polymers are intended to be used in computer parts such as keyboards and mouse-buttons. Two standard test methods, modified agar diffusion and surface method were used to assess the antibacterial activity of the samples. The methods were evaluated according to the consistency of results obtained from samples compared to the negative controls. The results showed that the agar diffusion test method was not as reliable as surface method. By Comparing the number of viable bacteria survived in the samples impregnated by nanosilver to the negative controls (polymers without nanosilver), the antibacterial potential of silver nanoparticles in this form has been proved, and it has been demonstrated that the antibacterial efficacy of polymers containing nanosilver increases by enhancing the amount of silver to a certain amount.

Key words: Antibacterial, Nanosilver, Polymer, Surface method, Agar diffusion method.

Nanotechnology is the most developing field for generating new applications in different areas, including manufacturing products having antimicrobial effects. Among the various antimicrobials silver is one of the most versatile antimicrobial agents due to its intense antimicrobial properties such as high thermal stability and long-term activity against a broad spectrum of microbes as well as little toxicity to mammalian cells and tissues[1-3]. The silver nanoparticles are used in various products such as textiles, certain implants and some polymers either by coatings on the surface, or impregnated into their structure to offer them antimicrobial activity[4-6].

* To whom all correspondence should be addressed.
E-mail: m.heidarpour@standard.ac.ir

The silver filled materials release the silver ions to be effective on microorganisms. It was shown that silver ions interact with thiol groups in proteins, resulting in inactivation of respiratory enzymes and leading to the production of ROS and also prevent DNA replication and affect the structure and permeability of the cell membrane[7], whereas it has been reported that silver nanoparticles may damage some bacterial cells and viruses by mechanisms other than the release of silver ions[8].Some results indicated that the bactericidal properties of the nanoparticles are size dependent, and nanoparticles that present a direct interaction with the bacteria preferentially have a diameter of ~1–10 nm[9-11].

The process of silver ion release from silver filled material includes diffusion of water into the composite specimen, reaction between the

silver and water molecules leading to the formation of silver ions and the migration of silver ions through the composite specimen leading to the release from the composite specimen to the aqueous environment[12-13]. So selection of a hygrophill matrix for the production of silver filled polymers is essential. Polar polymers like polyamides are excellent candidates for silver releasing, while the polymers which are commercially interested, such as polypropylene, because of their nonpolar and crystalline nature show less compatibility and conformity with silver ions and the nanoparticles may be agglomerated easily due to their vast surface[13-14].

According to preceding studies, the carrier materials can be effective in enhancing the water uptake, accelerating the diffusion of water molecules and the migration of silver ions through the matrix polymer[14]. Evidences have shown that integrating of the metallic nano particles in a polymeric base without carrier results lower efficacy than incorporating in a suitable carrier. Some compounds such as zeolite, apatite, phosphates, titanium oxides and glass have been used as inorganic carriers for nanoparticles[15]. The zeolite provides channels for the water molecules to enter the polymer matrix, improves water diffusion and acts as an ion pump providing controlled time release of silver ions. In addition, its pores make a good space for creating stable silver clusters in nanoscale[16]. Silver exhibits a strong affinity for zeolite, a porous crystalline material of hydrated sodium aluminosilicate, and can electrostatically bind this ion up to ca. 40% (w/w) of its framework[17].

The computer parts such as keyboards and mouse-buttons are of the most contaminated devices which are used daily by different groups of people[18]. These parts could be sources of microbial contamination resulting consequently in indirect transmission of potential pathogens and nosocomial infections[19-21].

In the current study, we evaluated the antibacterial efficacy of the nano silver polymers which were produced for applying in the mentioned computer parts. In order to achieve the most reliable results, the antibacterial tests were done based on two standard methods, the best method was chosen and the antibacterial potential of the polymers impregnated by different amounts of nano silver zeolite were assessed.

The results showed that the antibacterial efficacy increases by enhancing the amount of nano silver concentration, but in high concentrations of nano silver (20%), the activity decreases. Furthermore the methods such as agar diffusion are not appropriate for non porous materials like polymers, instead of which the methods involving enumeration of viable bacteria, are more reliable, and can enable us to estimate the antibacterial activity in a quantification way.

MATERIALS AND METHODS

Preparation of polymers containing nanosilver zeolite

As an initial suspension, different concentrations of aqueous $AgNO_3$ (5%, 10% and 20%) solutions were prepared. An aliquot of 1 g of zeolite powder A (Merck) was added to each suspension. The suspended solutions were adjusted to pH 7 by HNO_3 then stirred at room temperature for 270 min in darkness (to prevent formation of Ag oxide) [Marcos Marques da Silva Paula *et al*, 2009 [22]. After completing the ion exchange, the suspensions were filtered and the remained powder on the filtration paper, was washed by distilled water twice, and left in the ambient temperature to be dried. In this way, zeolite incorporated by different amounts of Ag nitrate was obtained[14]. The aqueous phase was used to determine the exchanged Ag+ ion.

The Ag zeolite powders were mixed by polymer granules in concentrations of 5%, 10% and 20% in an internal blending apparatus supplied by Brabender inc. The granules were melted at 160 °C, then Ag- Z powders were added to the granules gradually in 3% w/w. After 10 min the composite was formed as a paste and cut into small pieces [C.Damma *et al*, 2008].

Test samples and controls

Treated and untreated polymers cleaned and disinfected by an alcoholic solution, were used as test samples and controls respectively.

Test strains

The following bacterial strains were used as testing microorganisms in both mentioned methods:

E. coli ATCC 8739 and *Staph. aureus* ATCC 6538p, obtained from certified purchaser.

The microbial suspensions were prepared

by inoculating of a loopful taken from working culture (a16-24h slant culture from stock cultures) into 1/500 Nutrient Broth incubated at 37°C for an overnight. The concentration of microorganisms in initial suspensions was calculated using spectroscopy at 625 nm, adjusted to 2.5×10^8 - 10×10^8 cfu/ml. The number of bacteria was determined by serial dilutions and pour plate method in each test.

Testing the antimicrobial activity

The antimicrobial activity of polymers containing different concentrations of silver was assessed by both modified disc diffusion[23-24] and surface method[25].

Comparing the test methods

Disc diffusion method recommended by previous studies and surface method based on ISO 22196 were compared in order to choose the most appropriate method. Both test methods were done on three replicates recommended by ISO standard 22196 on three different days by the same users.

Disc diffusion method

For each sample, each control and each bacterium, a TSA plate was used. Each plate was seeded by 10^6 cfu/ml bacterial suspension.

Testing for each strain was performed using three samples and three controls, along with an untreated textile as a porous sample. A seeded plate without any sample was also used as growth control.

The samples and controls with the same size were planted on seeded plates, incubated at 37°C for 24-48 h. After incubation time (24 h and then 48 h), the inhibitory zones around and beneath the samples were assessed and the diameters were recorded.

Surface method

For each test, 3 treated polymer as test samples and 6 untreated polymer as controls were used, 3 of them for determining the number of viable bacteria immediately and the remained 3 ones for counting bacteria after 24 h.

Test samples and controls, were put in separate Petri dishes, and inoculated by 0.2 ml of bacterial suspension containing 2.5×10^5-10×10^5 cfu/ml (the 10^{-3} of initial suspension). The surface of each sample and control was covered by a polyethylene film. Penetration of the suspension through the cover may lead to reducing the number of viable counts.

The inoculated samples and half of the controls were incubated at 37°C for enumeration after 24 h, and the remained controls were subjected to enumeration immediately after inoculation. Recovering of bacteria was done by washing the surface of the polymer and the polyethylene cover by 20 ml Soy Casein Digest Lecithin Polysorbate (SCDLP), a diluent containing neutralizers.

Counting of bacteria was done using serial dilution and pour plate method. The number of viable bacteria immediately and 24 h incubation was determined.

The antibacterial efficacy was calculated by the following formula:

$$R = \text{Log}\,(N_0 / N_t)$$

where:

R The antibacterial efficacy

N_0 Mean of recovered bacteria immediately after inoculation (cfu/cm^2)

N_t Mean of recovered bacteria 24 h after inoculation (cfu/cm^2)

Antimicrobial activity

After choosing the preferred method the results attained by it were considered as the antibacterial activity of the nanosilver polymer.

RESULTS

Disc diffusion method

After incubation, no zone was observed around the samples and controls (Fig. 1), but a transparent region due to lack of growth was observed beneath all of the treated and untreated

Table 1. Number of recovered *St. aureus* (ATCC 6538) immediately (0 h) and 24 h after inoculation

Counts Samples	N_0	N_t	R
5%	1.7×10^4	3×10^3	0.75
10%	4×10^4	2.2×10^2	2.26
20%	2.2×10^4	5×10^3	0.64
Control	2.5×10^4	4×10^7	-3.2
Inoculated suspension		5.5×10^5	

The results showed that *St. aureus* had a remarkable growth after 24 h in contact with untreated samples. A significant decrease of 0.64 to 2.26 log was detected after 24 h contact with polymers impregnated with nanosilver, while log of decrease was equal to -3.2 for controls.

polymers and its size was as same as the sample (figures 2-3), while no sign of reduction of growth was detected under the textiles (fig 4,5). Although the zones under some of the treated polymers were a little greater than the surface of the related sample, no significant differences between samples and controls were observed.

Table 2. Number of recovered *E. coli* (PTCC 1330) immediately (0 h) and 24 h after inoculation

Counts Samples	N_0	N_t	R
5%	2×10^4	2×10^4	0
10%	2.4×10^4	4×10^2	2.20
20%	1.7×10^4	1.5×10^3	1.05
Control	2.5×10^4	8.1×10^7	-3.52
Inoculated suspension		7×10^5	

The results showed that *E. coli* had a remarkable growth after 24 h in contact with untreated samples. A significant decrease of 0 to 2.20 log was detected after 24 h contact with polymers incorporated with nano silver, while log of decrease was equal to -3.52 for controls.

Surface method

The number of bacteria recovered from untreated and treated polymers with 5%, 10% and 20% nano silver were are shown in table 1 and 2.

DISCUSSION

Methods

According to the results, it is suggested that agar diffusion is not a suitable method for polymers. Because of non porous structure of polymers, bacteria are not able to grow under the sample due to lack of oxygen, and the formed transparent zone may mislead to antibacterial activity, while the mentioned method is appropriate for porous materials such as textiles. The mentioned method has been used in several studies for evaluating of antimicrobial efficacy of silver colloidal solutions[26-27] and even for copolymers impregnated with silver [Marcos Marques da Silva Paula et al, 2009] nanoparticles. Although the disk diffusion method can be suitable for solutions or porous materials, it can not be used for rigid material such as polymers, and the results may be ambiguous. Comparison between two methods showed that the surface method [ISO 22196] is the most appropriate test for assessing the antibacterial activity of surfaces. Some modifications in this method will improve it. Using a semi solid inoculation suspension can reduce loss of inoculums.

We made a correction in the formula for calculating the antibacterial activity[25], it means that we calculated the log of the ratio of counts obtained immediately and after 24 h (N_0 / N_t) for each sample, while in the original reference [25], the log reduction of counts recovered from controls (U_t) and test samples (A_t), both after 24 h had been recommended (U_t-A_t). It is suggested that comparison between number of recovered bacteria immediately and after a specific time can lead us to quantify the antibacterial activity of each sample. In some related studies, similar methods to the surface method have been used[13-15]. In these studies the number of survived bacteria in contact with nanomaterial after a specific time (usually 24 h) was calculated without comparison to the number counted at the beginning time or even to controls. Therefore ISO method is suggested for such evaluation as a reliable and comparable reference method.

Antibacterial efficacy of nano polymers

The polymers impregnated by different amounts of nano silver had shown different antibacterial activity compared to untreated polymers. The antibacterial activity rises by increasing the amount of silver, but in high concentrations of nano silver (20%), the activity decreases. Same results have been reported by Marcos Marques da Silva Paula *et al*, 2009 [22] that the inhibition zone increased with increasing concentration of Ag nanoparticles soaked in the polymeric matrix and was dependent to the silver content in the polymer composite. In our study the results show that in high concentrations of nano silver (20%), because of formation of OH^-, the ion exchange declines, and increasing the silver ions can not affect the antibacterial activity.

In our study both test microorganisms *E.coli* and *S. aureus* represented same sensitivity to the silver nanoparticles which is similar to results reported by Ki-Young Yoon *et al*, 2007[28], while Kim *et al*., 2007 [4] reported that gram positive *S. aureus* is more resistant to silver nanoparticles compared to gram negative bacteria[28].

Using zeolite as a carrier in polymer

background assisted to better distribution of silver and releasing it steadily. The best ratio of silver to zeolite was 7.8% (w/w), i.e. 78.1 mg silver in 1 g zeolite. As Douglas Roberto Monteiro, 2009 [17] stated that Silver ions can bind to zeolite up to ca. 40% (w/w). Obtaining high antibacterial activity after 24 h, can be due to boosting of silver ions released during given time. By performing the mentioned antibacterial test in certain intervals, a curve can be achieved for quantification.

ACKNOWLEDGEMENTS

The authors wish to thank the microbiology department of standard research institute, also Materials and Energy Research Center for their financial and technical supports.

REFERENCES

1. Volker Alta, Thorsten Bechertb, Peter Steinr. Uckeb, Michael Wagenerc, Peter Seideld, Elvira Dingeldeind, Eugen Domanne, Reinhard Schnettlera, An in vitro assessment of the antibacterial properties and cytotoxicity of nanoparticulate silver bone cement, *Biomaterials* 2004; **25**: 4383–4391.
2. Wen-Tso Liu, 2006, Review, Nanoparticles and Their Biological and Environmental Applications, *Journal of Bioscience and Bioengineering*, **102** (1): 1-7.
3. Mahendra Rai, Alka Yadav, Aniket Gade, Silver nanoparticles as a new generation of antimicrobials, *Biotechnology Advances,* 2009; **27**(1): 76-83.
4. Jun Sung Kim, Eunye Kuk, Kyeong Nam Yu, Jong-Ho Kim, Sung Jin Park, Hu Jang Lee, So Hyun Kim, Young Kyung Park, Yong Ho Park, Cheol-Yong Hwang, Yong-Kwon Kim, Yoon-Sik Lee, Dae Hong Jeong, Myung-Haing Cho, Antimicrobial effects of silver nanoparticles, Nanomedicine: *Nanotechnology, Biology, and Medicine,* 2007; **3**: 95-101.
5. Shuhua Wang, Wensheng Hou, Liqiao Wei, Husheng Jia, Xuguang Liu, Bingshe Xu, Antibacterial activity of nano-SiO_2 antibacterial agent grafted on wool surface, *Surface & Coatings Technology*, 2007; **202**: 460-465.
6. Volker Alt, Thorsten Bechert, Peter Steinr, Michael Wagener, Peter Seidel, Elvira Dingeldein, Eugen Domann, Reinhard Schnettler, An in vitro assessment of the antibacterial properties and cytotoxicity of nanoparticulate silver bone cement, *Biomaterials* 2004; **25**: 4383-4391.
7. Qilin Li, Shaily Mahendra, Delina Y. Lyon, Lena Brunet, Michael V. Liga, Dong Li, Pedro J.J. Alvarez, Review, Antimicrobial nanomaterials for water disinfection and microbial control: Potential applications and implications, *water research* 2008; **42**: 4591– 4602.
8. Katherine Zodrow, Le´na Brunet, Shaily Mahendra, Dong Li, Anna Zhang, Qilin Li, Pedro J.J. Alvarez, Polysulfone ultrafiltration membranes impregnated with silver nanoparticles show improved biofouling resistance and virus removal, *water research* 2009; **43**: 715–723.
9. Jose Ruben Morones, Jose Luis Elechiguerra, Alejandra Camacho, Katherine Holt, Juan B Kouri, Jose Tapia Ramírez and Miguel Jose Yacaman, The bactericidal effect of silver nanoparticles, *Nanotechnology* 2005; **16**: 2346.
10. G. A. Martínez-Castañón, N. Niño-Martínez, F. Martínez-Gutierrez, J. R. Martínez-Mendoza and Facundo Ruiz, Synthesis and antibacterial activity of silver nanoparticles with different sizes*, Journal of Nanoparticle Research*, 2008; **10**(8): 1343-1348.
11. Elechiguerra, J.L., *et al*., "Interaction of silver nanoparticles with HIV-1." *Journal, of Nanobiotechnology* 2005; **3**(6): 1-10.
12. Bernd Nowack, Thomas D. Bucheli, Review Occurrence, behavior and effects of nanoparticles in the environment*, Environmental Pollution* 2007; **150**: 5-22.
13. C. Damm, H. Munstedt, A. Rosch, The antimicrobial efficacy of polyamide 6/silver-nano- and microcomposites, *Materials Chemistry and Physics* 2008; **108**: 61-66.
14. C. Radheshkumar, H. Munstedt, Antimicrobial polymers from polypropylene/silver, composites—Ag+ release measured by anode stripping voltammetry, *Reactive & Functional Polymers* 2006; **66**: 780-788.
15. Jia Husheng,b, Hou Wensheng, Wei Liqiao, Xu Bingshea, Liu Xuguang, The structures and antibacterial properties of nano-SiO_2 supported silver/zinc–silver materials, dental materials 2008; **24**: 244-249.
16. H. Pehlivan, D. Balköse, S. Ülkü, F. TihminliogÇlu, Characterization of pure and silverexchanged natural zeolite filled polypropylene composite films, *Composites Science and Technology*, 2005; **65**(13): 2049-2058.
17. Douglas Roberto Monteiro, Luiz Fernando Gorup, Aline Satie Takamiya, Adhemar Colla Ruvollo-Filho, Emerson Rodrigues de Camargo, Debora Barros Barbosa, Review, The growing

importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver, *International Journal of Antimicrobial Agents*, 2009; **34**: 103–110.

18. A. K. Al-Ghamdi, S. M. A. Abdelmalek, A. M. Ashshi, H. Faidah, H. Shukri, A. A. Jiman-Fatani, Bacterial contamination of computer keyboards and mice, elevator buttons and shopping carts, *African Journal of Microbiology Research,* 2011; **5**(23): 3998-4003.
19. Schultz M, Gill J, Zubairi S, Huber R, Gordin F., Bacterial contamination of computer keyboards in a teaching hospital, *Infect Control Hosp Epidemiol*. 2003; **24**(4):302-3. ((Veterans Affairs Medical Center, Washington, DC 20422, USA.,))
20. Lu PL, Siu LK, Chen TC, Ma L, Chiang WG, Chen YH, Lin SF, Chen TP., Methicillin-resistant *Staphylococcus aureus* and *Acinetobacter baumannii* on computer interface surfaces of hospital wards and association with clinical isolates. *BMC Infect Dis*. 2009; **1**(9): 164.
21. DJ, Waghorn, WY Wan, C. Greaves, N. Whittome, HC Bosley, and S. Cantrill, Contamination of computer keyboards in clinical areas: potential reservoir for nosocomial spread of organisms, *British Journal of Infection Control* 2005; **6**(3): 22-24.
22. Marcos Marques da Silva Paula, César Vitório Franco, Mario César Baldin, Larissa Rodrigues, Tatiana Barichello, Geovana Dagostim Savi, Luiz Felipe Bellato, Márcio Antônio Fiori, Luciano da Silva, Synthesis, characterization and antibacterial activity studies of poly-{styrene-acrylic acid} with silver nanoparticles *Materials Science and Engineering* C 2009; **29**: 647–650.
23. A.W. Bauer, W.M.M. Kirby, J.C. Sherris, M. Turck, Antimicrobial susceptibility testing by a standardized single disk method, *American Journal of Clinical Pathology* 1966; **45**(4): 493-496.
24. ISO 20645, Textile fabrics -Determination of antibacterial activity- Agar diffusion test, 2004.
25. ISO 22196, Measurement of antibacterial activity on plastics and other non-porous surfaces, 2011.
26. A. Petica a,", S. Gavriliu a, M. Lungua, N. Burunteaa, C. Panzarub, Colloidal silver solutions with antimicrobial properties, *Materials Science and Engineering* B 2008; **152**: 22–27.
27. M.Z. Kassaee, A. Akhavan, N. Sheikh, R. Beteshobabrud, g-Ray synthesis of starch-stabilized silver nanoparticles, with antibacterial activities, *Radiation Physics and Chemistry*, 2008; **77**: 1074–1078.
28. Ki-Young Yoon, Jeong Hoon Byeon, Jae-Hong Park, Jungho Hwang, Susceptibility constants of Escherichia coli and Bacillus subtilis to silver and copper nanoparticles, *Science of the Total Environment* 2007; **373**: 572–575.