



Antimicrobial Evaluation of Silver Nanoparticle-Polymer Composites Prepared by Gamma Radiation

Rita Singh*, Durgeshwer Singh, Antaryami Singh

Radiation Dosimetry and Processing Group, Defence Laboratory, Defence Research and Development Organization, Jodhpur, India

Email address:

rita.singh@dl.drdo.in (R. Singh)

*Corresponding author

To cite this article:

Rita Singh, Durgeshwer Singh, Antaryami Singh. Antimicrobial Evaluation of Silver Nanoparticle-Polymer Composites Prepared by Gamma Radiation. *American Journal of Polymer Science and Technology*. Vol. 2, No. 2, 2016, pp. 40-46. doi: 10.11648/j.ajpst.20160202.14

Received: October 17, 2016; **Accepted:** December 22, 2016; **Published:** January 12, 2017

Abstract: The aim of the present study was to compare the antimicrobial activity of silver nanoparticle-polymer composites prepared by *in situ* synthesis of the silver nanoparticles within the polyvinyl pyrrolidone (PVP) hydrogel and by direct addition of the silver nanoparticles into the polymer matrix prepared using gamma radiation technology. The antimicrobial activity of the PVP-nanosilver hydrogels prepared with different concentrations of 30, 50, 70 and 100 ppm silver was tested against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Hydrogels with 100 ppm nanosilver prepared by *in situ* reduction of silver resulted in about 3 to 5 log reduction in microbial counts after 3 hours as compared to about 2-log reduction with hydrogels prepared by addition of nanosilver. Comparison of the microbial reduction rates in the presence of two types of hydrogels have shown higher antimicrobial effects of nanosilver prepared by *in situ* reduction of silver by gamma radiation in the polymer matrix.

Keywords: Silver Nanoparticles, Polyvinyl Pyrrolidone, Gamma Radiation, Antimicrobial Activity

1. Introduction

Polymeric materials containing metallic nanoparticles have attracted tremendous scientific and technological interest due to their potential applications in the field of optical, electrical, chemical, biological and medical devices [1, 2]. Nanoparticle-polymer composites are functional materials made through the combination of nanoparticles and polymer. Three dimensional network hydrogels are more suitable as templates for the production of nanoparticles than conventional non-aqueous or polymeric systems especially for biomedical applications, owing to their exceptional compatibility with biological molecules, cells and tissues. The combination of nanoparticles and hydrogels create synergistic, unique and potentially useful properties that are not found in the individual components. Properties imparted to the composites depend on the type of nanoparticles incorporated [3].

Polymers with silver nanoparticles have been identified as materials commonly used in biological and medical applications [4, 5]. Nanosilver particles are known for their antimicrobial properties and have been applied to wide range

of healthcare products such as burn dressings, scaffold, skin donor and recipient sites, and medical devices [6]. Nanosilver is an effective killing agent against a broad spectrum of Gram-negative and Gram-positive bacteria [7, 8], including antibiotic-resistant strains [9, 10]. The combination of nanoparticles with polymers forming composites offers easier utilization of the antimicrobial activity of these nanoparticles.

Two general approaches can be employed for the preparation of polymer-metal nanocomposites depending on where the nanoparticles are synthesized- *in situ* by using the polymer matrix as the reaction medium and *ex situ*, meaning that the particle is synthesized before their incorporation into the polymer and in this way the matrix is just the dispersion medium. The present study was focused on the preparation of antimicrobial polymer-metal nanocomposites by two different routes using gamma radiation technology. Polymer polyvinyl pyrrolidone (PVP) as reaction medium for *in-situ* synthesis of silver nanoparticles and polymer PVP as a dispersion medium of pre-synthesized silver nanoparticles was used and the antimicrobial activity of PVP-nanosilver hydrogel composites prepared by the two methods was evaluated.

2. Materials and Methods

2.1. Synthesis of Hydrogels Containing Silver Nanoparticles

Hydrogel was prepared using aqueous solutions of 15% Polyvinyl pyrrolidone (PVP) of molecular weight 1,300,000. PVP hydrogels were prepared with 0, 30, 50, 70 and 100 ppm silver. Two methods for nanosilver incorporation were studied – *in situ* reduction of silver by gamma radiation and addition of nanosilver prepared by gamma radiation. Silver nitrate (AgNO_3) at concentrations of 30, 50, 70 and 100 ppm silver was added to the PVP mixture. Radiolytic synthesis of silver nanoparticles within the PVP hydrogel was attempted. The hydrogel matrix was obtained by gamma irradiation induced crosslinking, while the *in situ* reduction of Ag^+ ions was performed by strong reducing species formed under water radiolysis. The second method involved addition of nanosilver prepared with 0.3% alginate and gamma irradiation dose of 50 kGy to the PVP before irradiation. PVP nanosilver composite hydrogels were prepared by gamma irradiation at 25 kGy.

2.2. Characterization of PVP Hydrogels for *in situ* Synthesis of Silver Nanoparticles

UV-Vis Spectroscopy and Scanning Electron Microscopy with EDX Imaging was used to confirm *in situ* synthesis of nanosilver in polymer matrix by gamma radiation. The UV-Vis absorption spectra of PVP hydrogels with and without nanosilver were recorded using Dynamica Halo DB-30 UV-Vis Spectrophotometer (Dynamica Pty. Ltd. Prahran East Victoria, Australia) in the range of 300-700 nm. Scanning Electron Microscope Carl Zeiss EVO MA 15 (Carl Zeiss NTS GmbH, Germany) was used to observe the morphologies of hydrogels. Samples were coated with gold before analysis. The morphological structure of the hydrogels was examined using SEM at an accelerating voltage of 20 KV. Oxford INCA Energy 250 (135eV INCA X-act Peltier cooled SDD detector) Energy Dispersive X-ray Analysis (EDX) coupled to SEM was used to identify the elemental composition of hydrogels with nanosilver.

2.3. Evaluation of Antimicrobial Activity by Log Reduction Assay

The antimicrobial efficacy of PVP hydrogels containing different concentrations of silver nanoparticles was tested quantitatively by log reduction assay. Antimicrobial activity of nanosilver hydrogels prepared by *in situ* reduction of silver by gamma irradiation and nanosilver hydrogels prepared by addition of silver nanoparticles prepared by gamma irradiation to polymer before radiation polymerization was compared. Three bacteria *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* were grown in nutrient broth medium and incubated aerobically for 24 h at $35^\circ\pm 2^\circ\text{C}$. Fungal culture *Candida albicans* was grown in soybean casein digest broth and incubated at $22^\circ\pm 2^\circ\text{C}$ for 5 days. The inoculation was

prepared as suspension representing 10^4 to 10^5 CFU/ml. The silver nanoparticles incorporated hydrogels were immersed in soyabean casein digest broth inoculated with the test organisms. Control broths with inoculation and without hydrogels were also included. The broths were incubated with agitation and samples were withdrawn at specific time intervals. The solutions were serially diluted and plated for viable counts using soyabean casein digest agar medium. The plates were then incubated at $35^\circ\pm 2^\circ\text{C}$ for bacteria and $22^\circ\pm 2^\circ\text{C}$ for fungus, and colonies were counted. The number of surviving organisms was determined in the presence of PVP-nanosilver hydrogels prepared by the two methods with different concentration of 0, 30, 50, 70 and 100 ppm silver. Plates counts were measured in triplicate, and each experiment was repeated three times to obtain mean value of CFU counts.

2.4. Zone of Inhibition Test

The antimicrobial property of the hydrogels containing silver nanoparticles synthesized by *in situ* reduction of silver by gamma radiation and by addition of silver nanoparticles prepared by gamma radiation to polymer matrix before radiation polymerization was evaluated against various microbial strains using the zone of inhibition test. Test was performed using three clinically relevant organisms- *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*. Agar plates were evenly inoculated with the test organism. Pieces of hydrogels were placed on the inoculated agar plates. The zone of inhibition around the hydrogels was measured.

3. Results

Synthesis of polyvinyl pyrrolidone (PVP) hydrogels was carried out by gamma irradiation. The presence of embedded silver nanoparticles within the gel macromolecular network was confirmed by UV-Vis spectral studies, using hydrogels of PVP with and without nanosilver. Fig. 1 depicts the absorption spectra of PVP-nanosilver hydrogel. The absorption spectrum of PVP-nanosilver composite hydrogel has surface plasmon absorption band due to the plasmon resonance effect originating from the quantum size of the silver nanoparticles with peak at 402 nm.

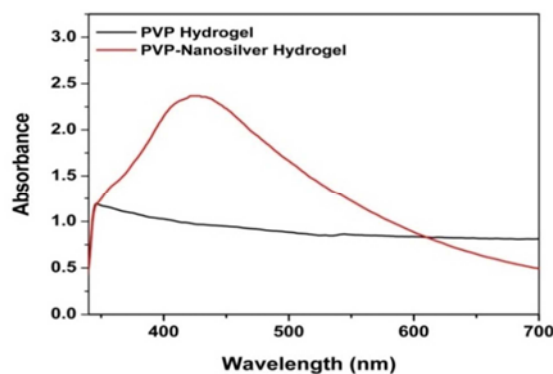


Figure 1. UV-Vis absorption spectra of PVP hydrogels.

The surface morphology of PVP hydrogels and PVP-nanosilver hydrogels was characterized by Scanning Electron Microscopy (SEM). SEM images of the PVP-nanosilver hydrogels are illustrated in Fig. 2. Roughness in the surface of polymer impregnated with silver nanoparticles was

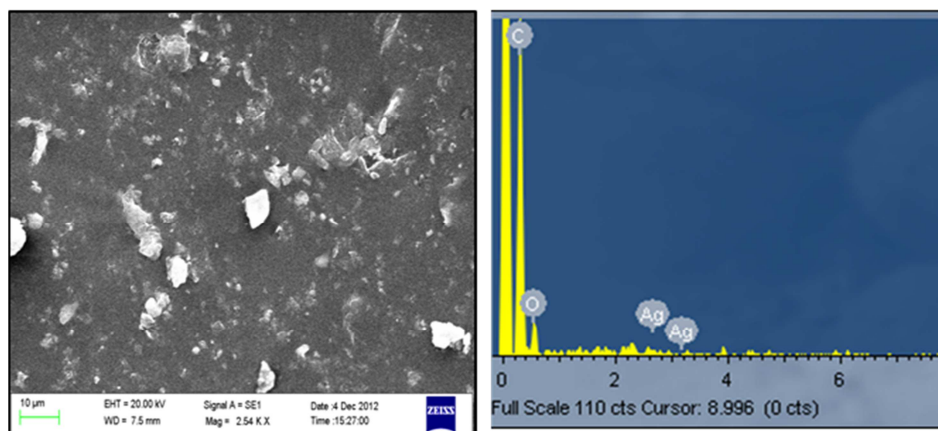


Figure 2. SEM and EDX image of PVP hydrogel containing nanosilver.

Antimicrobial activity of nanosilver hydrogels prepared by two different methods- by *in situ* reduction of silver by gamma radiation and by addition of nanosilver prepared *ex situ* by gamma radiation was evaluated. The antimicrobial efficacy of the hydrogels containing nanosilver synthesized by *in situ* reduction of silver by gamma radiation against Gram-negative bacteria, *Pseudomonas aeruginosa* is presented in Fig. 3a.

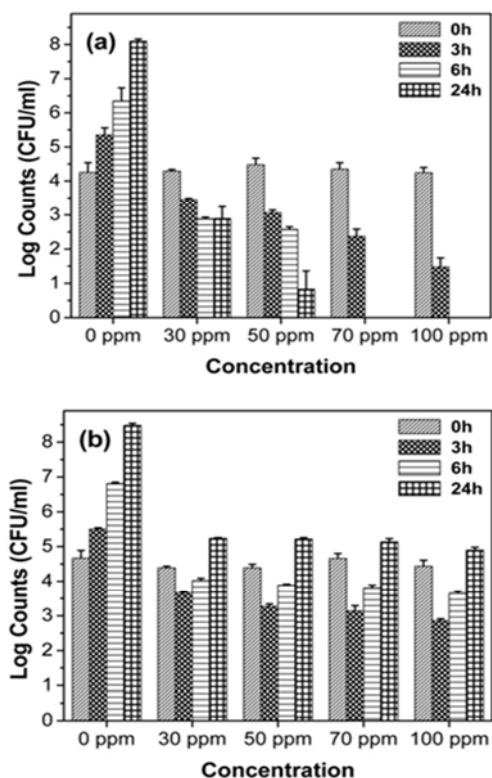


Figure 3. Effect of PVP hydrogels containing nanosilver on *Pseudomonas aeruginosa* (a) *in situ* reduction of silver by gamma radiation (b) addition of nanosilver prepared by gamma radiation. Data are mean \pm SD, $N = 4$.

observed. In order to confirm the presence of silver nanoparticles in the hydrogel samples, the elemental analysis of the hydrogels was done through energy dispersive X-ray spectroscopy (EDX) coupled to SEM. The EDX spectrum showed the presence of silver (Ag) in the PVP hydrogels.

The average initial counts of *Pseudomonas aeruginosa* in the broth was $1.10 - 4.24 \times 10^4$ CFU/ml. About 4-log reduction in cell counts was observed with 30 ppm and 50 ppm nanosilver dressing after 6 hours as compared to counts in the absence of nanosilver hydrogels. About 2-log reduction in the counts were observed with 70 ppm nanosilver dressing after 3 hours and complete killing was observed at 6 hours. No viable counts for *Pseudomonas aeruginosa* were detected with 100 ppm nanosilver hydrogels after 6 hours of exposure.

Antimicrobial effect of PVP-nanosilver hydrogels prepared by incorporation of nanosilver synthesized by gamma radiation is presented in Fig. 3b. Reduction of *Pseudomonas aeruginosa* in the presence of nanosilver hydrogels was observed ($p < 0.01$). However, hydrogels with 30, 50, 70 and 100 ppm nanosilver showed increase in viable counts after 3 hours. The hydrogels prepared by addition of nanosilver did not demonstrate significant bactericidal activity against *Pseudomonas aeruginosa* as compared to hydrogels with *in situ* reduction of silver. However, in the presence of hydrogel-nanosilver composite membranes about 3 log reduction in the viable counts ($p < 0.05$) was observed after 24 hours as compared to hydrogels with no nanosilver.

The effect of hydrogels containing nanosilver on Gram-positive bacteria *Staphylococcus aureus* is presented in Fig. 4a. Bactericidal effect was observed in the presence of both the types of nanosilver hydrogels, whereas the counts progressively increased in the absence of nanosilver hydrogels. With PVP-nanosilver hydrogels prepared by *in situ* reduction of silver about 1-log reduction in the counts was observed in the presence of 50 ppm nanosilver hydrogel after 3 hours of exposure. The reduction was observed with time and the cell counts were found to decrease after 6 hours in the presence of hydrogel containing 30 and 50 ppm nanosilver. With increasing exposure time, no viable counts were detected in the presence of 30 and 50 ppm. Complete killing was observed after 6 hours with 70 ppm and after 3

hours with hydrogels containing 100 ppm nanosilver. Maximum bactericidal effect was observed with 100 ppm nanosilver dressing.

With hydrogels prepared by incorporation of nanosilver (Fig. 4b), no viable counts were detected after 6 hours in the presence of 50, 70 and 100 ppm nanosilver hydrogels. No viable counts were observed after 24 hours in the presence of nanosilver hydrogels. Comparison of the microbial reduction rates in the presence of two types of hydrogels indicate higher antimicrobial effects of nanosilver prepared by *in situ* reduction of silver by gamma radiation in the polymer matrix.

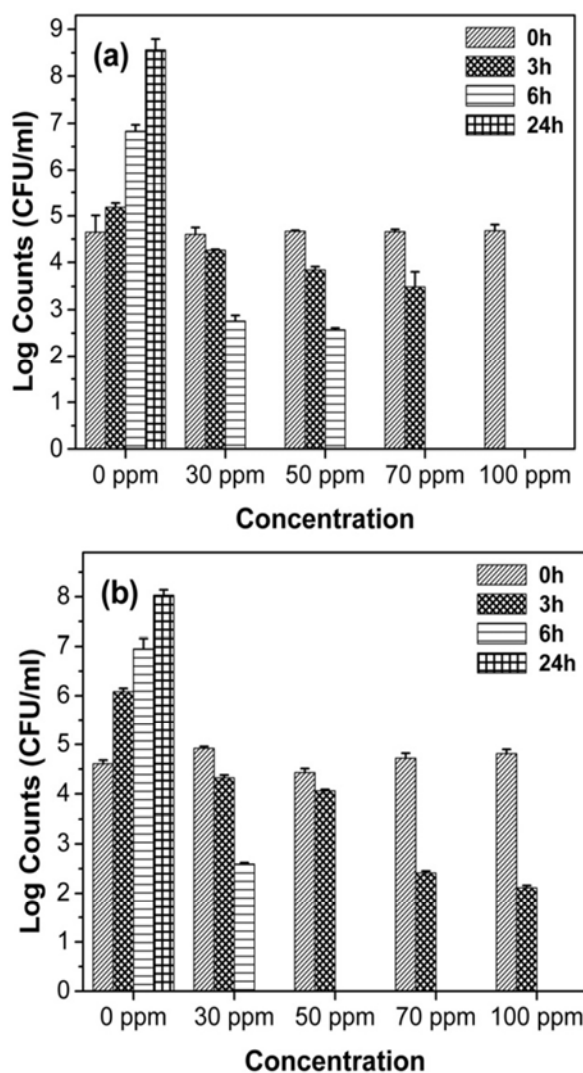


Figure 4. Effect of PVP hydrogels containing nanosilver on *Staphylococcus aureus* (a) *in situ* reduction of silver by gamma radiation (b) addition of nanosilver prepared by gamma radiation. Data are mean \pm SD, $N = 4$.

Fig. 5a presents the effect of PVP hydrogels containing 30, 50, 70 and 100 ppm nanosilver on *E. coli*. Strong microbicidal effect of the hydrogels prepared by *in situ* reduction of silver by gamma radiation was observed (Fig. 5a). No viable counts were detected after 6 hours of exposure with 50 to 100 ppm nanosilver.

Lower antimicrobial activity against the Gram-negative

bacteria *E. coli* was observed with hydrogels prepared by addition of nanosilver as compared to hydrogels with *in situ* reduction of silver to nanosilver (Fig. 5b). Reduction in cell number was in the range of 50-71% after 6 hours of exposure. No viable counts were detected with 70 ppm and 100 ppm nanosilver after 24 hours of treatment.

Antimicrobial activity of PVP hydrogels containing different concentrations of nanosilver (0, 30, 50, 70 and 100 ppm) against *Candida albicans* is presented in Fig. 6. Initial counts of *Candida* log 4.28 ± 0.09 to 4.49 ± 0.08 CFU/ml were found to increase to log 6.31 ± 0.32 to 6.45 ± 0.09 CFU/ml after 6 hours of incubation.

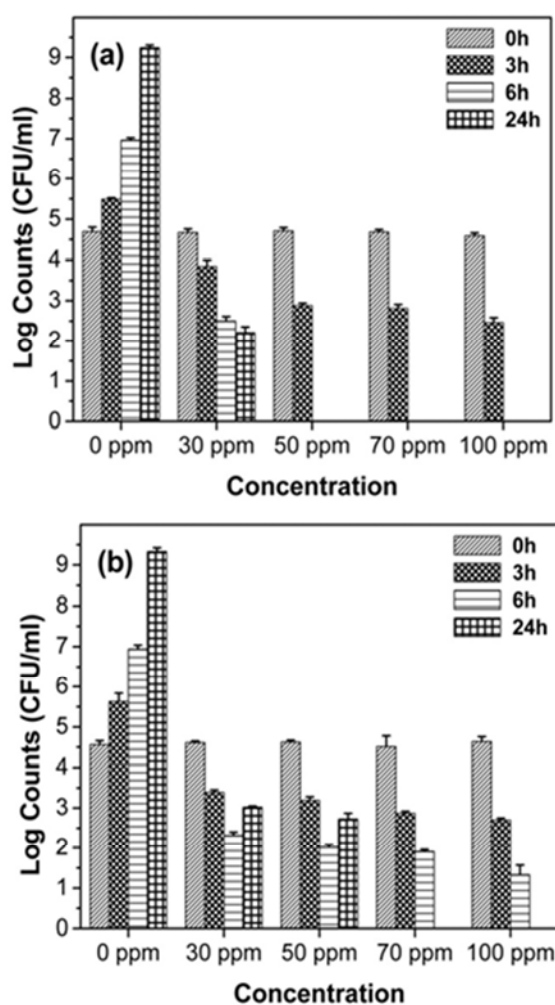


Figure 5. Effect of PVP hydrogels containing nanosilver on *E. coli* (a) *in situ* reduction of silver by gamma radiation (b) addition of nanosilver prepared by gamma radiation. Data presented are mean \pm SD, $N = 4$.

Significant reduction was observed in the presence of nanosilver hydrogels. About 3-log reduction in counts was observed with 30 and 50 ppm nanosilver hydrogels after 6 hours of treatment. Progressive decrease in counts were observed with time and no viable counts were detected in presence of 50 ppm after 24 hours. With 70 and 100 ppm nanosilver hydrogel about 2-log reduction was observed after 3 hours and no viable counts were detected after 6 hours of treatment.

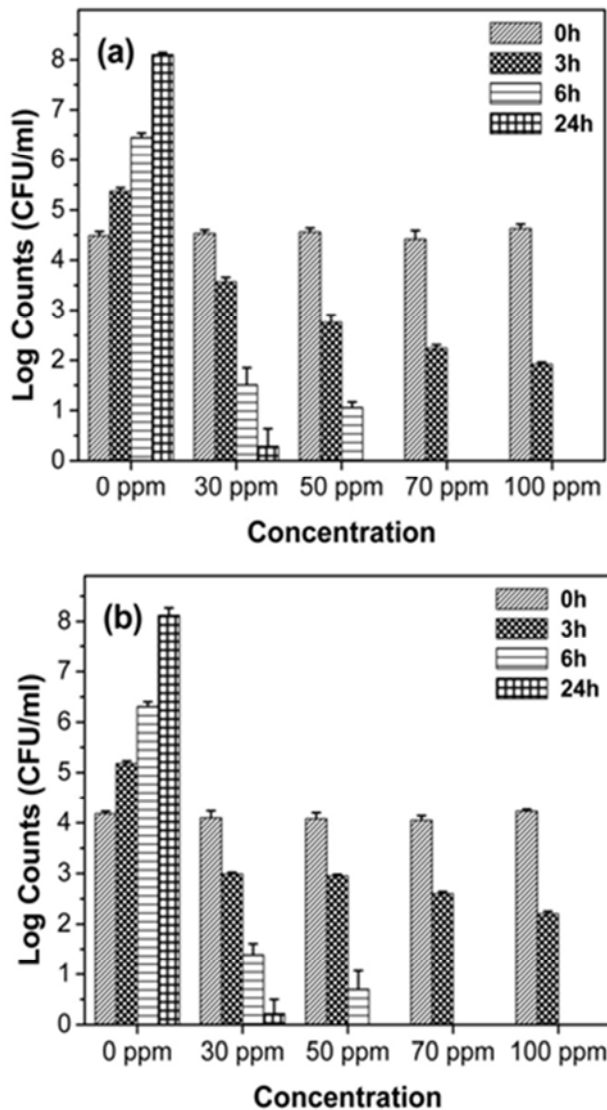


Figure 6. Effect of PVP hydrogels containing nanosilver on *Candida albicans* (a) *in situ* reduction of silver by gamma radiation (b) addition of nanosilver prepared by gamma radiation. Data are mean \pm SD, N = 4.

Similar antimicrobial effect was observed with hydrogels prepared by addition of nanosilver (Fig. 6b). Hydrogels with 100 ppm nanosilver resulted in complete killing after 5 hours. No viable counts were detected with 70 ppm nanosilver hydrogel after 6 hours. No proliferation or growth was observed after 24 hours of incubation in presence of hydrogels containing 50-100 ppm nanosilver.

Table 1. Zone of Inhibition against different microbes.

Test organisms	Silver nanoparticle-polymer composites	
	In situ synthesis	Ex situ synthesis
<i>Pseudomonas aeruginosa</i>	4.88 \pm 0.60 mm	3.00 \pm 0.71 mm
<i>Staphylococcus aureus</i>	5.50 \pm 1.00 mm	2.75 \pm 0.66 mm
<i>Escherichia coli</i>	3.50 \pm 1.41 mm	1.25 \pm 0.66 mm

Values are mean \pm standard error (N=8)

PVP hydrogels containing 100 ppm nanosilver were evaluated for antimicrobial activity by zone of inhibition test

(Table 1). Zone of inhibition were determined for three clinically relevant microorganisms- *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *E. coli*. Zone of inhibition was higher (3.50 \pm 1.41 mm to 5.50 \pm 1.00 mm) in the presence of hydrogels prepared by *in situ* reduction of nanosilver as compared to hydrogels prepared with addition of radiation synthesized nanosilver. For hydrogels prepared by addition of nanosilver, the zone of inhibition was 1.25 \pm 0.66mm to 3.0 \pm 0.71mm. Zone of inhibition test demonstrated higher antibacterial activity of hydrogels containing nanosilver prepared by *in situ* reduction of silver by gamma radiation

4. Discussion

Hydrogels, which are a crosslinked network of hydrophilic polymers, have the ability to absorb large amounts of water and swell, while maintaining their three-dimensional structure. Hydrogels have received increasing attention in biomedical and biochemical application because of their permeability, biocompatibility and biodegradability. Polyvinyl pyrrolidone (PVP) is a synthetic polymer with good biocompatibility [11]. PVP is a water-soluble polymer and has been successfully used as hydrogel for biomedical application. Hydrogels using PVP were synthesized by gamma radiation technology in the present study and silver nanoparticles were incorporated in hydrogel network.

Irradiation is a useful tool to make hydrogels for biomedical applications [12]. Radiation reactions utilize gamma ray to excite a polymer and produce a crosslinked structure. The radiation crosslinking can be easily adjusted by controlling the radiation dose and is reproducible. There are no initiators or crosslinkers which may be harmful and difficult to remove. The hydrogel formation and sterilization can be achieved in one step. Rosiak and coworkers have reported hydrogel wound dressing by radiation crosslinking of polymers in aqueous solution [13]. Usually a dose of 25 kGy is applied in order to ensure sterility of the product [14]. Under ionizing radiation, PVP undergoes crosslinking to form transparent hydrogels with good biocompatibility [15]. Aqueous PVP solutions are permanently gelled when crosslinked by irradiation with gamma rays [16, 17]. PVP hydrogel has excellent transparency, biocompatibility, swelling capacity, ability to disperse different active compounds and have been applied as dressings for wound treatment such as burns and skin ulceration [18], drug delivery system [14] and in protein release [19].

Hydrogels have been extensively studied for dermal wound healing applications. Their advantages compared to other wound dressings are good mechanical properties, permeability to oxygen, fluid absorption, hydration of the wound bed, shape stability and softness similar to that of the soft surrounding tissue and cooling of the wound surface [20]. Depending on the state of hydration of the tissue, hydrogels can absorb or donate water to the wound environment. Hydrogels leave no residue, are malleable and improve reepithelialization of wounds [21]. The use of

hydrogels to replace damaged tissues was the main incentive for their synthesis and investigation of antimicrobial activity. Hydrogels containing nanosilver can provide an excellent environment to wound healing; it can absorb exudates generated during the healing process, while protecting the wound from secondary infection [22, 23]. Several chemical and physical methods exist to prepare antimicrobial polymer-silver nanocomposites. The main fabrication approach is to disperse previously prepared silver nanoparticles in the polymer matrix. However, this often leads to the inhomogeneous distribution of the particles in the polymer. Another approach is to prepare a system in which the silver ions are reduced to a zero valent state by a reducing agent.

In the present study, two methods for fabrication of PVP hydrogels with silver nanoparticles were used and the antimicrobial activity was evaluated. Silver nanoparticles synthesized by gamma radiation were dispersed in PVP matrix before radiation polymerization. Secondly, radiolytic synthesis of silver nanoparticles within the PVP hydrogel was attempted. The hydrogel matrix was obtained by gamma irradiation induced crosslinking, while the *in situ* reduction of Ag^+ ions was performed using strong reducing species formed under water radiolysis. The radiolytic method is very suitable for generating metal nanoparticles in the solution. Radiolytically generated species, solvated electrons and secondary radicals exhibit strong reduction potentials, and consequently metal ions are reduced. The control of particle size is achieved by the use of capping agents such as polymers, which are present during the formation of metal clusters. Polymer molecules interact with the growing metal particles, inhibiting the aggregation process [24]. UV-Vis and EDX spectra confirmed the formation of silver nanoparticles in the hydrogel network.

Antimicrobial activity of polymer networks containing silver nanoparticles prepared by two different methods was evaluated against the common wound contaminants. The killing curves were determined for the four clinically relevant microorganisms- *P. aeruginosa*, *S. aureus*, *E. coli* and *C. albicans*. According to the results obtained with PVP hydrogels, it is evident that the antimicrobial activity of the hydrogels varied with the method of nanosilver incorporation. Several mechanisms have been postulated for the antimicrobial properties of silver nanoparticles. Nanosilver may release silver ions and generate reactive oxygen species (ROS); interact with membrane proteins affecting their function; accumulate in the cell membrane affecting membrane permeability; and enter into the cell where it can generate ROS, release silver ions, and affect DNA [25]. Several factors have been reported to influence activity of silver nanoparticles. The bactericidal effect of silver nanoparticles is size dependent [26]. Change in reactivity and properties of nanoparticles are attributable to their small size, compared with bulk matter. The smaller the size, the larger the surface-area to volume ratio; hence, the antimicrobial activity of silver nanoparticles is affected by the size of the nanoparticles. Depending on the size of the nanoparticles, large surface area comes in contact with the bacterial cells to provide a higher percentage of interaction

than bigger particles. The bactericidal potential of nanoparticles is also influenced by their shapes, which is shown by studying the bacterial growth inhibition by differentially shaped nanoparticles [26]. Stability of silver nanoparticles also influences activity since the formation of aggregates tends to decrease biocidal activity. Different surfactants and polymers have been used to stabilize silver nanoparticle dispersions and enhance biocidal activity [27, 28]. The results of the present study indicated remarkably strong antimicrobial activity of silver nanoparticles-polymer composite prepared by *in situ* reduction of silver as compared to ex-situ addition of silver nanoparticles to the polymer matrix.

5. Conclusion

Two methods for fabrication of PVP hydrogels with silver nanoparticles were used. Silver nanoparticles synthesized by gamma radiation were dispersed in PVP matrix before radiation polymerization. Secondly, radiolytic synthesis of silver nanoparticles within the PVP hydrogel was carried out. Higher antimicrobial activity was observed for silver nanoparticles-polymer composite prepared by *in situ* reduction of silver in polymer matrix.

References

- [1] Lu Y, Spyra P, Mei Y, Ballauff M, Pich A (2007) Composite Hydrogels: Robust Carriers for Catalytic Nanoparticles. *Macromol Chem Phys* 208 (3): 254-261.
- [2] Murali Mohan Y, Lee K, Premkumar T, Geckeler KE (2007) Hydrogel networks as nanoreactors: A novel approach to silver nanoparticles for antibacterial applications. *Polymer* 48: 158-164.
- [3] Palza H (2015) Antimicrobial polymers with metal nanoparticles. *Int J Mol Sci* 16: 2099-2116.
- [4] Yu H, Xu X, Chen X, Lu T, Zhang P, Jing X (2007) Preparation and antibacterial effects of PVA-PVP hydrogels containing silver nanoparticles. *J Appl Polym Sci* 103 (1): 125-133.
- [5] Kong H, Jang J (2008) Antibacterial properties of novel poly(methyl methacrylate) nanofiber containing silver nanoparticles. *Langmuir* 24: 2051-2156.
- [6] Boonkaew B, Barber PM, Rengpipat S, Supaphol P, Kempf M, He J, John VT and Cuttle L (2014) Development and characterization of a novel, antimicrobial, sterile hydrogel dressing for burn wounds: Single-step production with gamma irradiation creates silver nanoparticles and radical polymerization. *J Pharm Sci* 103 (10): 3244-3253.
- [7] Guzman MG, Dille J, Godet S (2012) Synthesis and antibacterial activity of silver nanoparticles against Gram-positive and Gram-negative bacteria. *Nanomedicine* 8: 37-45.
- [8] Radzig MA, Nadtochenko VA, Koksharova OA, Kiwi J, Lipasova VA, Khmel IA (2013) Antibacterial effects of silver nanoparticles on gram-negative bacteria: Influence on the growth and biofilms formation, mechanisms of action. *Colloid Surface B* 102: 300-306.

- [9] Percival SL, Thomas J, Linton S, Okel T, Corum L, Slone W (2012) The antimicrobial efficacy of silver on antibiotic-resistant bacteria isolated from burn wounds. *Int Wound J* 9 (5): 488-493.
- [10] Rai MK, Desmukh SD, Ingle AP, Gade AK (2012) Silver nanoparticles: The powerful nanoweapon against multi-resistant bacteria. *J Applied Microbiol* 112: 841-852.
- [11] Lamponi S, Leone G, Consumi M, Greco G, Magnani A (2012) In vitro biocompatibility of new PVA-based hydrogels as vitreous body substitutes. *J Biomater Sci Polym Ed* 23: 555-557.
- [12] Soler DM, Rodríguez Y, Correa H, Moreno A, Carrizales L (2012) Pilot scale-up and shelf stability of hydrogel wound dressings obtained by gamma radiation. *Radiat Phys Chem* 81 (8): 1249-1253.
- [13] Rosiak JM, Janik I, Kadlubowski S, Kozicki M, Kujawa P, Stasica P, Ulanski P (2002) Radiation formation of hydrogels for biomedical applications. *IAEA-Tech Doc* 1324: 5-47.
- [14] Ajji Z, Othman I, Rosiak JM (2005) Production of hydrogel wound dressings using gamma radiation. *Nucl Instrum Methods Phys Res B* 229: 75-380.
- [15] Rosiak JM, Ulanski P, Pajewski LA, Yoshii F, Makuuchi K (1995) Radiation formation of hydrogels for biomedical purposes. Some remarks and comments. *Radiat Phys Chem* 46: 161-168.
- [16] Kaplan H, Guner A (2000) Characterization and determination of swelling and diffusion characteristics of poly(n-vinyl-2-pyrrolidone) hydrogels in water. *J Appl Polym Sci* 78:994-1000.
- [17] Singh R, Singh D (2012) Radiation synthesis of PVP/Alginate hydrogel containing nanosilver as wound dressing. *J Mater Sci Mater Med* 23: 2649-2658.
- [18] Benamer S, Mahlous M, Boukrif A, Mansouri B, Larbi Youcef S (2006) Synthesis and characterisation of hydrogels based on poly(vinyl pyrrolidone). *Nucl Instrum Methods Phys Res B* 248: 284-290.
- [19] Wang D, Hill DJT, Rasoul F, Whittaker AK (2011) A study of the swelling and model protein release behaviours of radiation-formed poly(n-vinyl-2-pyrrolidone-co-acrylicacid) hydrogels. *Radiat Phys Chem* 80: 207-212.
- [20] Yu T, Ober CK (2003) Methods for the topographical patterning and patterned surface modification of hydrogels based on hydroxyethyl methacrylate. *Biomacromolecules* 4: 1126-1131.
- [21] Amadeu TP, Seabra AB, deOliveira MG, Costa AM (2007) S-nitrosoglutathione containing hydrogel accelerates rat cutaneous wound repair. *J Eur Acad Dermatol Venereol* 21: 629-637.
- [22] Gwon HJ, Lim YM, Nho YC, Baik SH (2010) Humectants effect on aqueous fluids absorption of γ -irradiated PVA hydrogel followed by freeze thawing. *Radiat Phys Chem* 79: 650-653.
- [23] Singh D, Singh A, Singh R (2015) Polyvinyl pyrrolidone/carrageenan blend hydrogels with nanosilver prepared by gamma radiation for use as an antimicrobial wound dressing. *J Biomater Sci Polym Ed* 26: 1269-1285.
- [24] Krkljes AN, Marinovic-Cincovic MT, Kacarevic-Popovic ZM, Nedeljkovic JM (2007) Radiolytic synthesis and characterization of Ag-PVA nanocomposites. *Eur Polym J* 43: 2171-2176.
- [25] Marambio-Jones C, Hoek EMV (2010) A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. *J Nanopart Res* 12: 1531-1551.
- [26] Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT, Yacaman M (2005) The bactericidal effect of silver nanoparticles. *Nanotechnology* 16: 2346-2353.
- [27] 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.
- [28] Kvitek L, Panacek A, Soukupova J, Kolar M, Vecerova R, Prucek R, Holecova M, Zboril R (2008) Effect of surfactants and polymers on stability and antibacterial activity of silver nanoparticles (NPs). *J Phys Chem C* 112: 5825-5834.