Fabrication of Au and Ag Bi-Metallic Nanocomposite for Antimicrobial Applications

P. Ranga Reddy, K. Varaprasad, N. Narayana Reddy, K. Mohana Raju, N. Subbarami Reddy

Synthetic Polymer Laboratory, Department of Polymer Science and Technology, Sri Krishnadevaraya University, Anantapur, Andhra Pradesh-515055, India

Received 3 February 2011; accepted 5 July 2011
DOI 10.1002/app.35192
Published online 13 January 2012 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Hydrogel silver nanocomposites have shown immense potential in many biomedical applications, specifically wound healing. The combination of bi-metallic (Ag, Au) hydrogel nanocomposites are developed to enhance their antimicrobial activity. This paper presents the fabrication of bi-metallic nanocomposites obtained from the synthesis of acrylamide (AM) and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) based hydrogels for antimicrobial applications. The nanocomposite formation was confirmed by scanning electron microscopy (SEM), thermal analysis (TGA/DSC), as well as X-ray diffraction (XRD) methods. The bi-metallic nanocomposite hydrogel has shown significant antibacterial activity on bacillus. Therefore, these bi-metallic antibacterial materials are promising candidates for a wide range of biomedical applications. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 125: 1357–1362, 2012

Key words: biomedical applications; antimicrobial activity; bi-metallic; hydrogels

INTRODUCTION

The development of metal nanoparticles (NPs) and nanostructured materials are attracting attention in recent research because of their extensive properties which enhance the biomedical properties[1] such as drug delivery[2] and wound dressing properties.[3] Generally, bulk metallic fabrics such as Au and Ag are the most noble of metals and are considered inert.[4] This property is useful in macroscopic level but in nanoscales level they are useful in plasmonics-related fields,[5] drug delivery,[2] as well as wound dressing.[6,7] Nowadays polymer templates are highly used for the formation of nanoparticles due to their biodegradability and nonhazard nature, and they also provide long-term stability to the particles without aggregation/agglomeration. These nanocomposite polymers are considered to be novel functional materials with a wide range of potential applications. The formation principle of polymer-based metal nanocomposites is thought as interaction between polymer and nanometal particles,[8] such as electrostatic, hydrogen bond, charge-transfers, and so on. However, polymeric materials can play key roles in controlling the physical, chemical, optical, and electronic properties of these nanoscopic materials.[9,10]

Recently, Varaprasad et al.[6,11,12] have reported a hydrogel-silver nanocomposites consisting of acrylamide and various polymers (natural or synthetic), which are prepared using a various crosslinker. In their studies they were optioned small size nanoparticles within the hydrogel network which exhibited superior antibacterial properties. Yan Hong Gao et al.[13] also reported the preparation of gold nanoparticles using polymer composites. The composite showed good antibacterial activities because gold deposited on the surface of carbon spheres without aggregation. The development of nanocomposites and nanostructural materials have opened a new era for constructing well designed nanostructures that have been considered as a novel class of materials for catalytic, optical,[14,15] electronic,[15,16] and biomedical applications.[16–19] It is widely renowned that nano-sized metal particles such as silver, gold, and copper are highly toxic to microorganisms exhibiting strong biocide effects on bacteria.

2-Acrylamido-2-methyl-1-propanesulfonic acid is one of the strong acid[20] and it can copolymerize with other monomers to synthesize water-soluble and nonwater-soluble copolymers due to its good reactivity and acidity.[8] AMPS have been found as a good host for many guests of inorganic nanoparticles[21] such as Ag and Au. Therefore, they can be used in various fields[8,22] such as biomedical applications.

The objective of this study was to improve the swelling properties as well as improved wound

Correspondence to: N. S. Reddy (nsramireddy@gmail.com).

Contract grant sponsors: University Grants Commission, New Delhi, India.

dressing properties of hydrogels by generating bi-metallic nanoparticles. For this purpose, the combination of poly(acrylamide) hydrogel and acrylamide-2-acrylamido-2-methyl-1-propanesulfonic acid systems are selected because of their relevance for pharmaceutical and biomedical applications.

EXPERIMENTAL

Material

Acrylamide (AM), 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS), *N,N*[1]-methylenebisacrylamide (MBA), ammonium persulphate (APS) and *N,N,N*[1]*,N*[1]-teramethylethylenediamine (TMEDA), silver nitrate($AgNO_3$), gold chloride ($H.AuCl_4.XH_2O$), and sodium borohydride ($NaBH_4$) were purchased from SD Fine Chemicals (Mumbai, India). Double distilled water was used throughout the investigations for the preparation of all solutions.

Preparation of P(AM-AMPS) hydrogels

Highly swollen AM-AMPS hydrogels were prepared by free radical polymerization of AM monomer with the addition of hydrophilic vinyl monomer (co monomer) such as AMPS and a hydrophilic crosslinker such as *N,N*[1]-methylenebisacrylamide (MBA). In the preparation of hydrogel, 14.08 m*M* of AM was dissolved in 2 mL of distilled water taken in a 100-mL beaker and 0.2412 m*M* of AMPS was added as a comonomer; 0.648 m*M* of MBA as a hydrophilic crosslinker and 2.191 m*M* of APS, 0.172 m*M* of TMEDA as an initiating pair system were also added to the mixture of the monomers by stirring at 100 rpm on a magnetic stir plate. The temperature of the system was raised to 50°C for (30 min). After the reaction was completed, the hydrogel formed was immersed in distilled water at room temperature for 24 h to remove the unreacted materials (monomers, crosslinker, as well as initiator) present in the hydrogel network. Finally, the hydrogel was dried at room temperature for 2 days. Similarly, other hydrogels were prepared by the above procedure. The feed compositions of the hydrogels are presented in Table I.

Preparation of Au-Ag Bi-metallic P(AM-AMPS) nanocomposite

Required amounts of dried hydrogel were immersed for 24 h in a large amount of distilled water for 2 days to swell to full extent. The swollen hydrogel was transferred into a 250-mL beaker and immersed in 20 mL of 5 m*M* $AgNO_3$ and 10 mL of gold chloride (5 m*M*) aqueous solutions to permit equilibrating for 1 day. The hydrogel was removed and transferred to $NaBH_4$ (10 m*M*/20 mL) solution for reducing the metal ions for 4 h. The hydrogel nanocomposite was dried at ambient temperature. It was powdered and used for characterization.

TABLE I
Feed Compositions of APMS Based Hydrogels Preparation Conditions

Hydrogels code	AAm (mM)	AMPS (mM)
B	14.08	–
A-AS1	14.08	0.2412
A-AS2	14.08	0.4825
A-AS3	14.08	0.9650
A-AS4	14.08	1.4475

MBA, 0.648 m*M*; APS, 2.191 m*M*; TEMDA, 1.721 m*M*, at room temperature for 24 h.

Swelling studies

The equilibrium swelling ratio of hydrogels was measured by gravimetric method. The swelling characteristic of the hydrogels provides the information about the hydrogel network integrity after loading gold and silver salts and formation of bimetallic nanocomposites inside the hydrogel networks. To study this phenomenon, the same weights of dried hydrogels were equilibrated in distilled water at ambient temperature for 2 days. The swollen hydrogels were treated first with $AgNO_3 + H.AuCl_4.XH_2O$ and then with $NaBH_4$ solutions. The swelling ratio (Q) of the gels was calculated from the following equation:

$$\text{Swelling ratio } (Q) = \text{the weight of the swollen hydrogel} / \text{the weight of the dry hydrogel}$$

Characterization

The UV-vis spectra are recorded on an ELICO SL 210 Model UV-vis spectrophotometer (Elico Co., Hyderabad, India). The morphological variations are observed (coated with a thin layer of palladium gold alloy) by using a JOEL JSM 840A (Tokyo, Japan) scanning electron microscope (SEM). Differential scanning calorimetry (DSC) of nanocomposites were studied by using a SDT Q 600 DSC instrument (TA Instruments-water LLC, Newcastle, DE 19720, USA) at a heating ramp 20°C/min under a constant nitrogen flow (100 mL/min). The samples were run from 30 to 600°C. Thermogravimetric analyses (TGA) of nanocomposites were evaluated on a SDT Q 600 TGA instrument (TA Instruments-water LLC, Newcastle, DE 19720, USA) at a heating rate of 10°C/min under a constant nitrogen flow (100 mL/min). The samples were run from 30 to 600°C. X-ray diffraction analysis was

carried out using a Model D/Max-2500Pc X-ray diffractometer (Rigagu, Tokyo, Japan) with Cu Kα radiation.

Antibacterial activity

Dish method

Nutrient agar medium was prepared by mixing peptone (5.0 g), beef extract (3.0 g), and sodium chloride (NaCl) (5.0 g) in 1000 mL of distilled water and the pH was adjusted to 7.0. Finally, agar (15.0 g) was added to the solution. The agar medium was sterilized in a conical flask at a pressure of 15 lbs for 30 min. This mixture was transferred into sterilized Petri dishes in a laminar air flow chamber. After solidification of the media, bacillus culture (50 μL) was spread on the solid surface of the media. To the inoculated Petri dish, one drop of nanoparticles solution (20 mg/10 mL distilled water with alkali treatment) was added using 50-μL tip and incubated for 2 days at 37°C in the incubation chamber

Absorbance count method

The effect of bacterial growth of bacillus in mineral salts medium (MSM) was studied in the presence of nanoparticles (Ag, Au, Au-Ag nanoparticles). This medium was prepared by the following composition: NH_4NO_3 (1.5 g), KH_2PO_4 (2.5 g), K_2HPO_4 (0.5 g), NaCl (1.0 g), $MgSO_4$ (1.5 g), $MnSO_4$ (0.01 g), $FeSO_4$ (0.05 g), and $CaCl_2$ (0.05 g) were added to 1000 mL of distilled water and the pH was adjusted to 7.0. Then, yeast extract (0.01%) was added for bacterial growth. After that the MSM medium was sterilized, and 50 mL of solution was transferred into a sterilized 250-mL conical flask. Afterward, 100 μL *bacillus bacterium* was added into the media. Finally, 100 μL of nanoparticles solution (10 mg/5 mL distilled water) or its equivalent nanoparticles suspension was added, and the optical density of the bacterial medium was measured using a UV-vis spectrophotometer at 600 nm.

RESULTS AND DISCUSSION

Nanoparticles have an extremely large relative surface area, thus increasing their contact with bacteria or fungi, and improving their bactericidal and fungicidal effectiveness. Nanocomposite hydrogels have many diverse applications in the chemical, physical, and biological fields.[6] In this study the PAM(poly acylamide) hydrogel networks have not only regulated the gel networks but also influenced the control of metal salts, alloy formation, and embedding the nanoparticles into the gel networks.

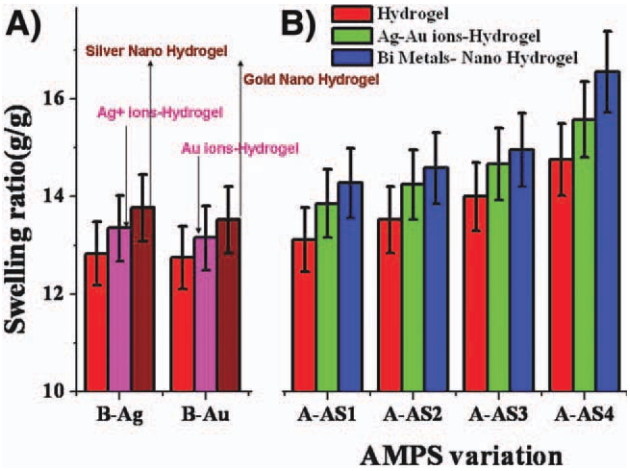

Figure 1 (A) Swelling behavior of pure hydrogel, ions loaded hydrogel, and nano-hydrogels. (B) Influence of AMPS content on swelling behavior of hydrogels (pure hydrogels, Ag-Au ions-hydrogel, and Bi-metals-nano-hydrogel). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Swelling studies

Swelling properties is mainly dependent on hydrogels composition by varying their parameters (such as monomers, polymer, crosslinker, and initiator/activator) which can improve and control the on-off swelling characteristics of hydrogel.[23–29] Depending on absorption and desorption properties, the inorganic ions are loaded from the metal nanoparticle by reduction reaction, and the hydrogel network controls the particle size, shape, and its release from their networks, which are important for antibacterial activity or wound healing processes.[6]

Effect of Amps concentration on swelling behavior

The experiment results of swelling ratio of the hydrogels were evaluated at ambient temperature. The relationship between the AMPS concentration and water absorbency values was studied by varying the AMPS concentration from 0.2412 to 1.4475 m*M*. The results are presented in Figure 1 indicating that the swelling ratio increases, when the AMPS concentration increases. The increase in water absorbency with an increase in the amount of AMPS concentration is due to the hydrophilic nature (acid groups) of the AMPS monomer. Thereby more number of water molecules will be bound to the AMPS chains which in turn increases the swelling capacity.[30]

Bi-metallic nanoparticle composite hydrogel

Ag ions and Au ions were absorbed from its silver nitrate and gold chloride solution into hydrogels by utilizing the ion exchange ability of the amide groups of acrylamide units as well as the coordination

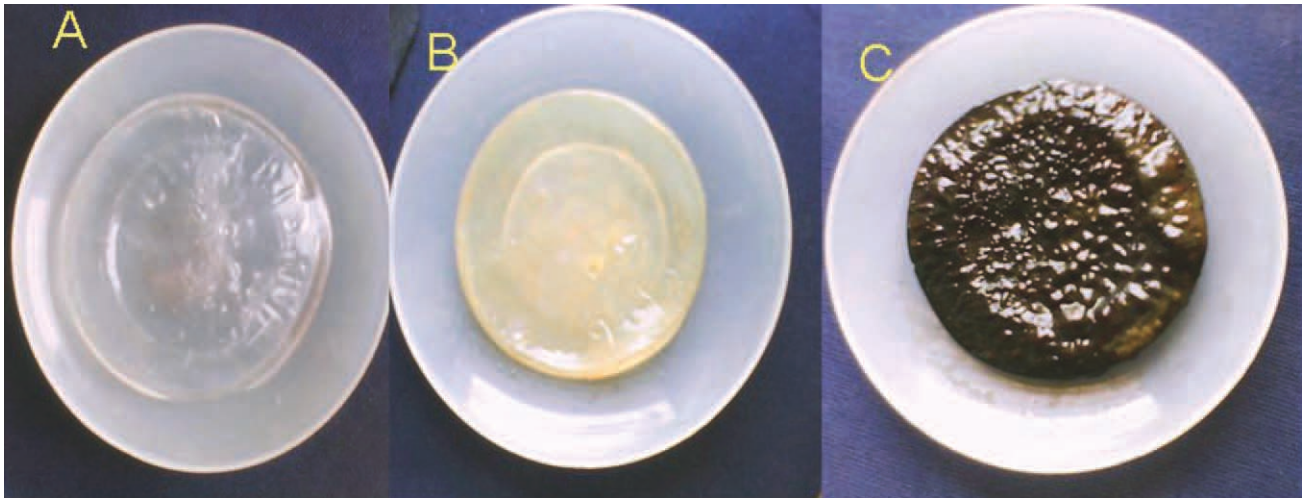

Figure 2 (A) Plain hydrogel (A-AS2). (B) Au-Ag ions loaded hydrogel (A-AS2). (C) Ag and Au nano composite (A-AS2) hydrogels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

capability of nitrogen atom in the AM of the hydrogel. The Ag ions and Au ions loaded hydrogels treated with $NaBH_4$ have turned into a dark brown color, indicating the formation of bi-metallic nanoparticles throughout the hydrogel networks. During this step, the addition of many silver as well as gold atoms to form the nanoparticles within the hydrogel networks expand the gel networks and promote higher water uptake capacity (Fig. 1). The increase in their swelling capacities is found to be directly proportional to their original hydrogel networks. The order of swelling capacity follows in this order: bi-metallic nanocomposite hydrogel > ions bonded hydrogel > hydrogel (Fig. 2).

UV-vis spectra of Au-Ag nanocomposite

The formation of Au-Ag nanoparticle in the hydrogel network can be analyzed by comparing the UV-spectra of metallic and bimetallic nanocomposite solutions (Fig. 3). Hydrogels containing silver nanoparticles show a characteristic peak at 417.55 nm which indicates the formation of silver nanoparticles.[6] Similarly, UV abortion peak of Au nanoparticles was obtained at 549.47 nm.[31] However, the mixture of pure Au and pure Ag nanoparticles (spectrum) shows a red shift at 445.59 nm which indicates the formation of both silver as well as gold nanoparticles in the gel network.[32] This spectral study once again suggests that simultaneous reduction of $HAuCl_4$ and $AgNO_3$ in the presence of hydrogel network produces homogeneous alloy nanoparticles and not a mixture of Au and Ag particles.[33]

Scanning electron microscopy

The morphology of P(AM-AMPS) and P(AM-AMPS) nanocomposite hydrogels were examined by SEM images and presented in Figure 4. It was observed that P(AM-AMPS) hydrogel has a smooth channel network structure [Fig. 4(A). In Figure 4(B,C), clear and flat surfaces of hydrogel can be observed, and it clearly explains the distribution of Ag and Au particles inside the hydrogels network. But nanocomposite hydrogels [Fig. 4(D)] show particles distributed throughout the hydrogel network, which clearly appear on the surface of the hydrogel network.

X-ray diffraction studies

The X-ray diffraction study gives spotting information of nanoparticles formed in the hydrogels networks which shows crystalline peaks[6,34] in Figure 6. When compared to the bimetallic nanocomposite, blank hydrogels does not exposed such type of peaks [Fig. 5(A)]. However in the case of Ag with Au nanocomposite, hydrogels can exhibit (2θ) highly sharp peaks at 24.24, 38.28, 44.52, and 64.83 when

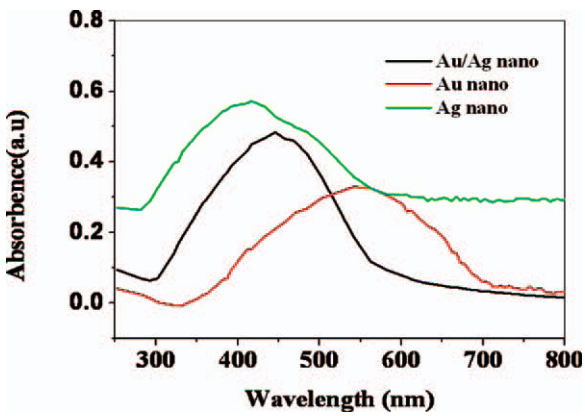

Figure 3 Uv spectrum of nanocomposites (Ag, Au, and Ag-Au nanocomposites). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

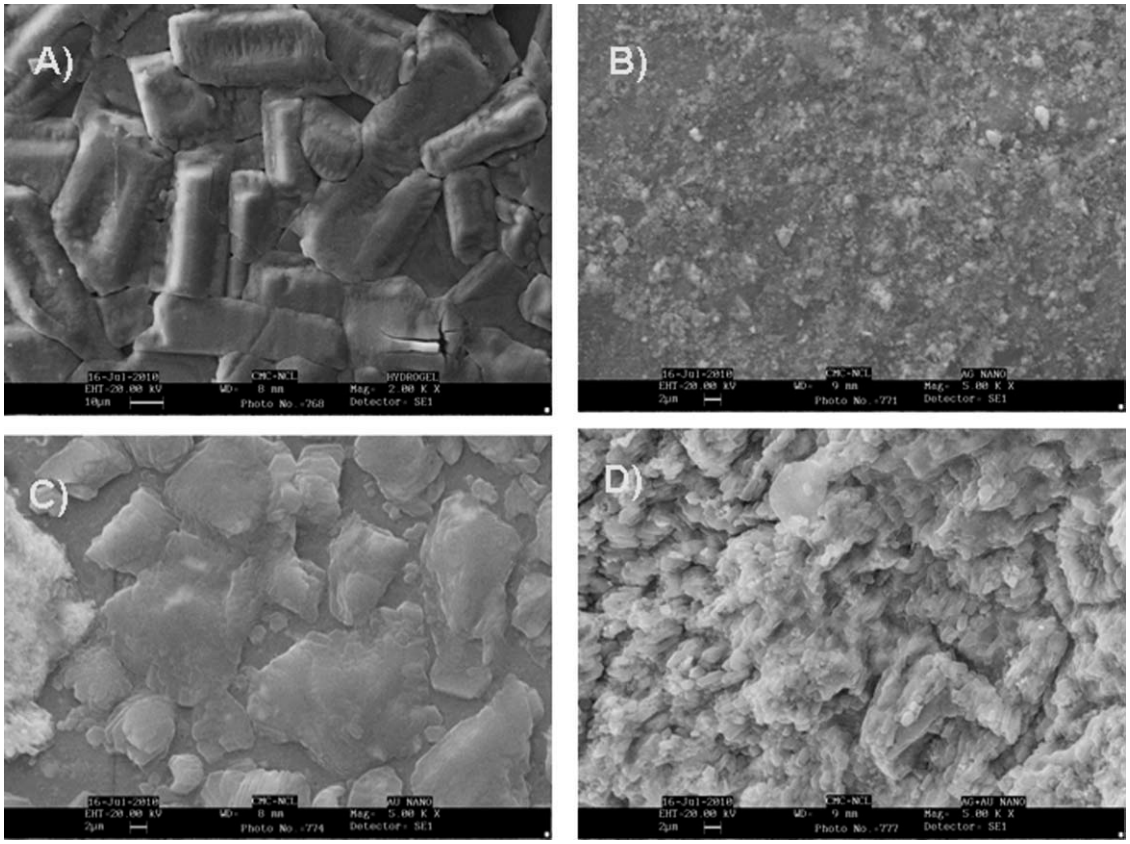

Figure 4 Scanning electron microscopy of (A) pure hydrogel (A-AS3), (B) Ag (A-AS3 Ag NCH), (C) Au (A-AS3 Au NCH), and (D) Ag and Au nanocomposite hydrogels (A-AS4 Au + Ag NCH).

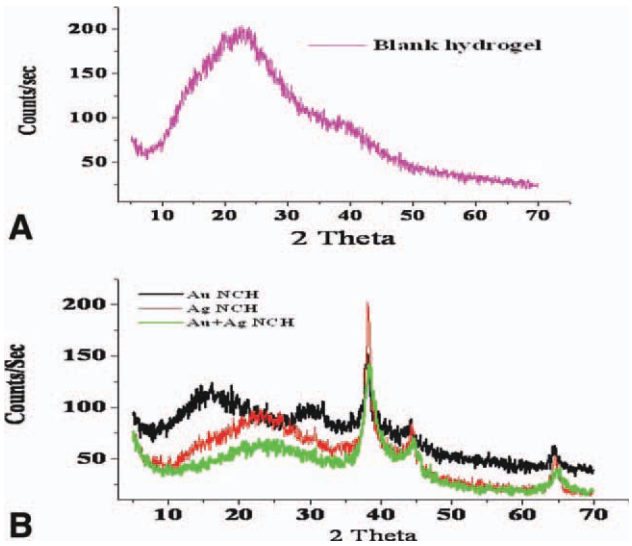

Figure 5 X-ray diffraction patterns of (A) blank hydrogel (A-AS4), (B) Au (A-AS4 Au NCH), Ag (A-AS4 Ag NCH), and Au with Ag nancomposite hydrogel (A-AS4 Au+Ag NCH) [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.].

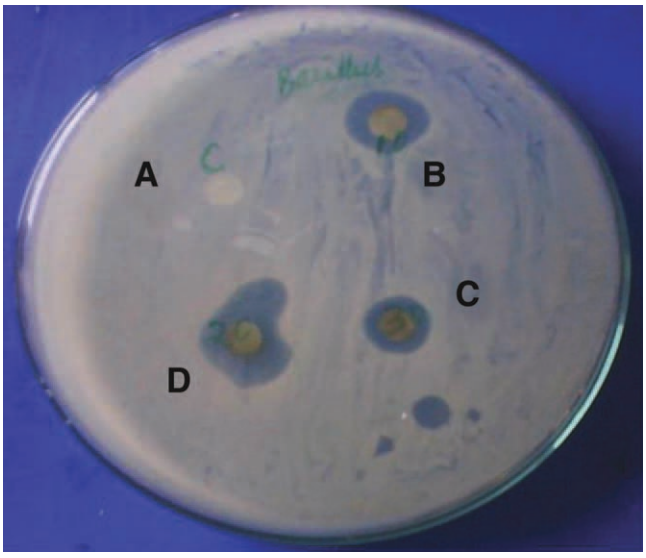

Figure 6 Antibacterial activity of (A) blank hydrogel (A-AS3), (B) Ag nanocomposite hydrogel (Ag NCH), (C) Au nanocomposite (A-AS3 Au NCH), and (D) Au with Ag nanocomposite hydrogel (A-AS3 Au + Ag NCH). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

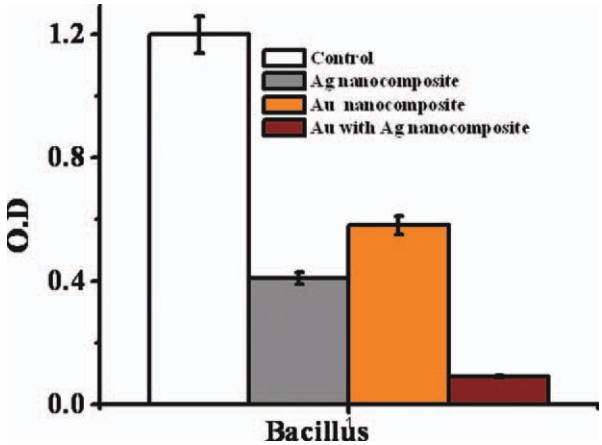

Figure 7 Antibacterial activity with silver nanoparticles, Au nanoparticles, and gold-silver nanoparticles containing hydrogels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

compared to other hydrogels, which can be corroborated to (111), (200), (220), and (222) reflections, due to the formation of bi-metallic Au with Ag nanocomposite hydrogel [Fig. 5(B)].

Evaluation of antibacterial activity

The main aim of this study was to develop a new antimicrobial/wound dressing agent. By addition of bimetallic compounds, the bacterial inhibition is increased. The antimicrobial activities of the nanocomposite (blank, Au, Ag, and Au+Ag nanocomposite) hydrogels were tested with nutrient agar media. Figure 6(D) shows the higher inhibition capacity than others. The hydrogel containing bi-metallic nanoparticles highly inhibited bacillus growth compared to blank < Au < Ag hydrogels [Fig. 6(A–C)]. Similarly, in the absorbance count method, we have found that bi-metallic nanoparticles exhibit higher activity on *E. coli* compared to Ag and Au nanoparticles (Fig. 7). The results of this study clearly demonstrated that the colloidal bi-metal nanoparticles 97% inhibited *bacillus* bacterial growth. It can be inferred from the study of the bi-metals nanocomposite hydrogels could be used for antimicrobial and biomedical applications.

CONCLUSIONS

In summary, we have synthesized Au-Ag bimetallic nanocomposites by varying the crosslinker, initiator, and activator concentrations. It is clearly illustrated that the Au-Ag nanoparticles are being formed not only on the surface of hydrogels but also throughout the networks. The nanocomposites are confirmed by using spectral, thermal, and electron microscopy methods. They are confirmed as excellent antibacterial materials.

References

1. Pal, K.; Banitha, K.; Majundar, D. K. Design Mono Polym 2009, 12, 197.
2. Qiu, Y.; Park, K. Adv Drug Deli Ver Rev 2001, 53, 321.
3. Kokabi, M.; Sirousazar, M.; Hassan, Z. M. Eur Polym Mater 2007, 43, 773.
4. Brown, C.; Whitehouse, M.; Tiekink, E.; Bushell, G. 2008, 6, 133.
5. Marsich, E.; Travan, A.; Donati, I.; Di Luca, A.; Benincasa, M. Biointerfaces 2011, 83, 331.
6. Varaprasad, K.; Murali Mohan, Y.; Ravindra, S.; Narayana Reddy, N.; Vimala, K.; Monika, K.; Sreedhar, B.; Mohana Raju, K. J Appl Polym Sci 2010, 115, 1199.
7. Sharma, V. K.; Yngard, R. A.; Lin, Y. Adv Coll Inter Sci 2009, 145, 83.
8. Xu, G. C.; Shi, J. J.; Li, D. J.; Xing, H. L. J Polym Res 2009, 16, 295.
9. Kamat, P. V. J Phys Chem B 2002, 106, 7729.
10. EI-Sayed, M. A. Acc Chem 2001, 34, 257.
11. Murali Mohan, Y.; Vimala, K.; Thomas, V.; Varaprasad, K.; Sreedhar, B.; Bajpai, S. K.; Mohana Raju, K. J Coll Inter Sci 2010, 342, 73.
12. Varaprasad, K.; Murali Mohan, Y.; Vimala, K.; Mohana Raju, K. J Appl Polym Sci 2011, 121, 784.
13. Gao, Y.-H.; Zhang, N.-C.; Zhong, Y.-W.; Cai, H.-H.; Liu, Y.-L. Appl Surf Sci 2010, 256, 6580.
14. Alqudami, A.; Annapoorni, S.; Govind, S.; Shivaprasad, M.; J Nanopart Res 2008, 10, 1027.
15. Hayward, R. C.; Saville, D. A.; Aksay, I. A. Nature 2000, 404, 56.
16. Ispasoiu, R. G.; Balogh, L.; Varnavski, O. P.; Tomalia, D. A.; Goodson, T. J Am Chem Soc 2000, 122, 11005.
17. Kiesow, A.; Morris, J. E.; Radehaus, C.; Heilmann, A. J Appl Phys 2003, 94, 6988.
18. Poizot, P.; Laruelle, S.; Grugeon, S.; Dupont, L.; Tarascon, J. M. Nature 2000, 407, 496.
19. Geckeler, K. E.; Rosenberg, E., Eds. Functional Nanomaterials; American Scientific: Valencia, USA, 2006.
20. Varaprasad, K.; Ravindra, S.; Narayana Reddy, N.; Vimala, K.; Mohana Raju, K. J Appl Polym Sci 2010, 116, 3593.
21. Kabiri, K.; Mirzadeh, H.; Zohuriaan-Mehr, M. J.; Daliri, M. Polym Int 2009, 58, 1252.
22. Kabiri, K.; Mirzadeh, H.; Zohuriaan-Mehr, M. J. J Appl Polym Sci 2010, 116, 2548.
23. Daniel, M. C.; Astruc, D. Chem Rev 2004, 104, 293.
24. Varaprasad, K.; Ravindra, S.; Narayana Reddy, N.; Vimala, K.; Mohana Raju, K. Int J Polym Mater 2010, 59, 981.
25. Lee, S. M.; Ju, S. S.; Chung, H. Y.; Ha, C. S.; Cho, W. J. Polym Bull 2001, 46, 241.
26. Baba, M.; Schols, D.; Pauwels, R.; Nakashima, H.; De-Clercq, E. J. Acq Immune Defcien Syndr 1990, 3, 493.
27. Mbemba, E.; Chams, V.; Gluckman, J. C.; Klatzmann, D.; Gattegno, L. Biochim Biophys Acta 1992, 1138, 62.
28. Neyts, J.; Reymen, D.; Letourneur, D.; Jozefonvicz, J.; Schols,D.; Este, J.; Andrei, G.; McKenna, P.; Witvrouw, M.; Ikeda, S.; Clement, J.; De Clercq, E. Biochem Pharm 1995, 50, 743.
29. Muniz, E. C.; Geuskens, G. J Mater Sci Mater Med 2004, 14, 879.
30. Ekici, S.; Saraydin, D. Polym Int 2007, 56, 1371.
31. Rodríguez-González, B.; Sánchez-Iglesias, A.; Giersig, M.; Luis, M.; Marzán, L. Faraday Discuss 2004, 125, 133.
32. Valodkar, M.; Modi, S.; Pal, A.; Thakore, S. Mater Res Bull 2011, 46, 384.
33. Sinzig, J.; Radtke, U.; Quinten, M.; Kreibig, U. Z. Physica D. 1993, 26, 242.
34. Murthy, P. S. K.; Murali Mohan, Y.; Varaprasad, K.; Sreedhar, B.; Mohana Raju, K. J Coll Inter Sci 2008, 318, 217.