Journal of Biotechnology **14**(4): 699-704, 2016

PREPARATION OF ANTIBACTERIAL POLYPROPYLENE GRAFTED ACRYLIC ACID AND IMMOBILIZED SILVER NANOPARTICLES BY γ-IRRADIATION METHOD

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Received: 04.5.2016
Accepted: 20.12.2016

SUMMARY

Silver nanoparticles (AgNPs) are now being widely used as antibacterial agents due to their strong bactericidal properties and low toxicity on mammalian cells. In this study, γ-rays irradiation method was used to synthesize AgNPs from silver nitrate ($AgNO_3$) solution and to graft acrylic acid (AAc) onto porous polypropylene (PP). Porous PP grafted with AAc (PP-g-AAc) was then immobilized with AgNPs for preparing the antimicrobial materials (PP-g-AAc/AgNPs). The results demonstrated that the grafting yield of AAc onto PP increased by the increasing of irradiation dose as well as AAc concentration. The PP-g-AAc samples with grafting degrees from 1.2 to 29.8% were immobilized with AgNPs (d ~ 10 nm, 500 ppm) to obtain antimicrobial properties. The immobilized Ag contents were from 132 to 392 ppm and corresponded to the PP-g-AAc samples at grafting degrees from 1.2 to 29.8%. The *in vitro* antibacterial properties of PP-g-AAc/AgNPs materials on *E. coli* were evaluated and the results indicated that the bactericidal efficiency (η) increased by the increase of Ag contents in the tested materials. The germicidal activities against *E. coli* of PP-g-AAc/AgNPs containing 363 ppm Ag were found to be nearly 100% after treating in 30 min. In addition, the inhibition zone of this PP-g-AAc/AgNPs on *E. coli* was also found up to 28 mm in diameter. Thus, γ-rays radiation demonstrated a strong capability in grafting functional groups (AAc) onto porous PP. Furthermore, the porous PP grafted with AAc and immobilized with AgNPs might potentially be used for elimination of bacteria in water filtering.

Keywords: *Bactericidal, γ-irradiation, polypropylene, silver nanoparticles, water treatment*

INTRODUCTION

Waterborne diseases caused by microorganisms especially bacteria are now leading to millions of deaths per year. According to WHO GLAAS reports (2014), an estimated of 748 millions of people are now lacking access to an improved source of water, billions are lacking access to safe water that is reliably and continuously delivered in sufficient. Moreover, hundreds of millions of people do not have soap and clean water to wash their hands, a simple practice that prevents the spread of diarrhea and respiratory illness. Therefore, development of an innovative water treatment technology is now highly important and utmost necessary.

PP filter cartridges are now being widely used in many water filtration systems as raw filters. Due to their pore size varied from 0.1 to 70 µm, they play an important role in sediment and small particles separation. However, one of the biggest disadvantages is the lack of antibacterial properties of raw PP, which would eventually make them become microorganism niches in the water filtration systems. Therefore, research works on modification of PP to create bactericidal PP materials for water treatment have been carried out. Due to the bactericidal properties of silver have been widely known, Sung *et al.,* (2005) has already created antibacterial polymer fiber which could killed over 99% of *S. aureus* at the silver concentration from 5%. Moreover, Phu *et al.,* (2014) reported that over 99% of *E. coli* was inhibited after only 2 min contacted with hand wash containing AgNPs and the best particle size for antibacterial activities was about 7.6 nm. This author's other study also reported that ~100% of *E. coli* was inhibited after 30 min treated with polyethylene film containing AgNPs (Phu *et al.*, 2013). Earlier studies of Kornackar *et al.,* (2014) on the grafting of AAc on PP for metal ion absorption indicated that γ irradiation could induce a grafting yield up to 20%. The fixing of silver onto PP filter cartridge using in water treatment was tested by Heidapur *et al.,* (2010) by using a spraying machine to spray silver onto the cartridge. Other study of the research group also indicated that the silver-coated filter cartridge had eliminated 100% of *E. coli* at 10^3 cfu/ml with a flow rate of 3 l/h. However, the production of these silver-coated PP filters required complicated machines and a relatively large amount of silver, which would consequently increase the cost and reduce the potential of large-scale production. In this study, porous PP samples were immersed into AAc solution and irradiated with γ-Co-60 radiation to induce AAc grafting. The grafted PP was then immobilized with AgNPs and the antibacterial properties of the materials were investigated on *E. coli* using growth test and inhibition zone test. The germicidal effectiveness of polypropylene immobilized with AgNPs against *E. coli* was also estimated.

MATERIALS AND METHODS

Materials

Polypropylene filter cartridges with pore size 5 μm were purchased from Nishimen, Malaysia. Acrylic acid, silver nitrate, PVP k90 and Mohr's salt were obtained from Merck, Germany. Luria-Bertani (LB) broth and agar were purchased from Himedia, India and *E. coli* was provided by the University of Agriculture and Forestry in Ho Chi Minh City, Vietnam.

Preparation of PP grafted with AAc

Porous PP filter cartridges were cut into smaller pieces (d = 70 mm, 10 mm thickness) and immersed into AAc solution with concentrations of 5, 10, 15 and 20%. Mohr's salt was added into the AAc solutions at 0.05% (w/v) for preventing the homo-polymerization of AAc (Said *et al.,* 2009). Porous PP pieces were then removed before irradiating by γ-rays from a Co-60 source at doses of 8, 12, 16 and 20 kGy to induce grafting. Afterwards, the irradiated samples were rinsed thoroughly with deionized water to remove un-grafted AAc. The PP samples grafted with AAc (PP-g-AAc) were then dried at 50ºC in 24 h and weighed for W_g. The degree of grafting was determined using the following equation (Said *et al.*, 2009):

$$\text{Degree of grafting (\%)} = (W_g - W_0)/W_0 \times 100\%;$$

Where W_0 and W_g represent the weights of initial and grafted samples, respectively.

Immobilization of AgNPs into PP-g-AAc

AgNPs with d ~ 10 nm were prepared by irradiation with the solution of 5 mM Ag^+ and 1% polyvinyl pyrrolidone (PVP) at 16 kGy. The UV-Vis spectrum and TEM image of AgNPs were taken using a Shimadzu UV-vis mini 1240 spectrophotometer (Japan) and Transmission Electron Microscope (JEM 1400, Japan). For AgNPs immobilization, grafted PP samples were immersed into AgNPs solution (500 ppm). The immobilized PP samples were then washed with deionized water to remove the excess AgNPs. The Ag contents in PP-g-AAc/AgNPs were analyzed by an inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Optima 5300 DV Perkin- Elmer, USA).

FTIR (Fourier-Transform IR) spectrometry

The PP-g-AAc and PP-g-AAc/AgNPs samples were crush into powder and prepared in KBr pellet formed by well-dried mixtures of 3 mg sample in 100 mg KBr. The spectra were obtained on a Shimadzu FTIR-8400s spectrophotometer (Japan).

Antibacterial activities

Luria-Bertani broth and agar were used as cultivation media. To investigate the antibacterial activities, PP-g-AAc/AgNPs sample with the size of 5x5x5 mm were immersed into cultural media contained 10^4 cfu/ml *E. coli*. The cultures were soaked thoroughly for 30 min at room temperature and 0.1 ml of these cultural media were then spread on LB agar plates. These media were then incubated at 37ºC for 24 h, the surviving *E. coli* colonies were counted and the bactericidal efficiency was calculated using the following equation: $\eta(\%) = (N_0 - N)\times100/N_0$, where N_0 and N are the survival numbers of bacteria in the control and treated water samples, respectively (Zhang *et al.*, 2009).

For inhibition zone test, 0.1 ml of *E. coli* suspension (10^7 cfu/ml) was spread on LB agar plates and PP-g-AAc/AgNPs samples were placed on the surface before incubating at 37ºC for 24 h.

RESULTS AND DISCUSSION

Graft of AAc on porous PP

Fig. 1 indicated the grafting degree onto porous PP at different irradiation doses and AAc concentration. The irradiation dose was showed as a function for grafting of AAc onto PP and grafting degree increased by the increase of radiation dose. The results also indicated that the higher AAc content induced the higher grafting degree. The highest grafting yield with 41.5% was found by the treatment of 20% AAc at 20 kGy, while the lowest grafting yield with 1.2% was obtained by the treatment with 5% AAc at 5 kGy. The reasons that grafting yield increases relatively with the increase of radiation dose and AAc concentration can be explained based on the interaction of AAc with the radical species (Said *et al.*, 2009).

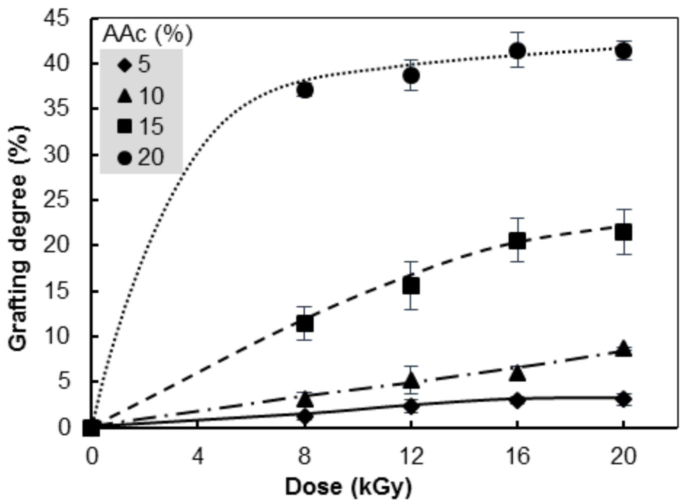

Figure 1. Grafting degree of polypropylene at different doses of radiation and acrylic acid concentration

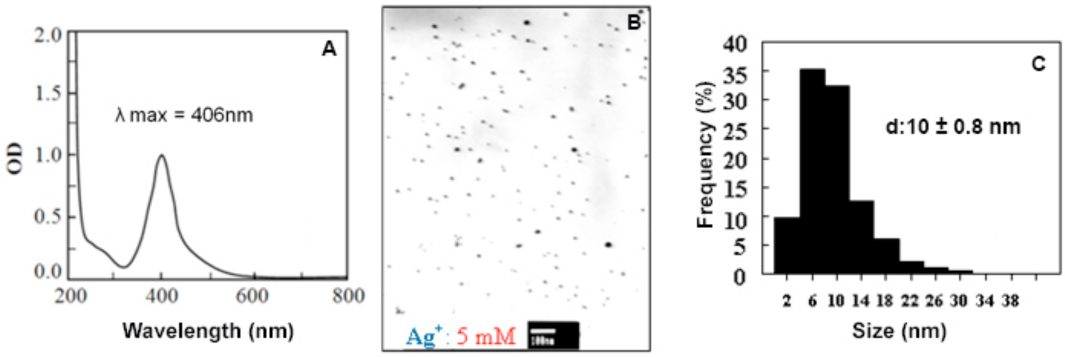

Figure 2. UV-vis spectrum (A), TEM image (B) and size distribution (c) of AgNPs prepared by γ-irradiation.

Immobilization of AgNPs onto PP-g-AAc

Fig. 2 showed the UV-vis spectrum of AgNPs in PVP with the maximum absorption at 406 nm. The AgNPs size was ~10 nm determined by using TEM image.

The content of AgNPs immobilized onto PP-g-AAc was displayed in Fig. 3 and the results showed that the immobilized Ag content in PP-g-AAc strongly increased from 132 to 363 ppm by the increase of AAc grafting degree in samples from 1.2 to 10.8%, respectively. Then the immobilized Ag content slightly increased with grafting degree to 392 ppm at 21.5% before dropped back to 380 ppm at 29.8% AAc and the highest Ag content (392 ppm) in PP-g-AAc was obtained by the sample with 21.5% AAc grafting degree. The samples with grafting degree from 1.2 to 10.8% and the immobilized Ag content from 132 to 363 ppm were selected to investigate the antibacterial activities.

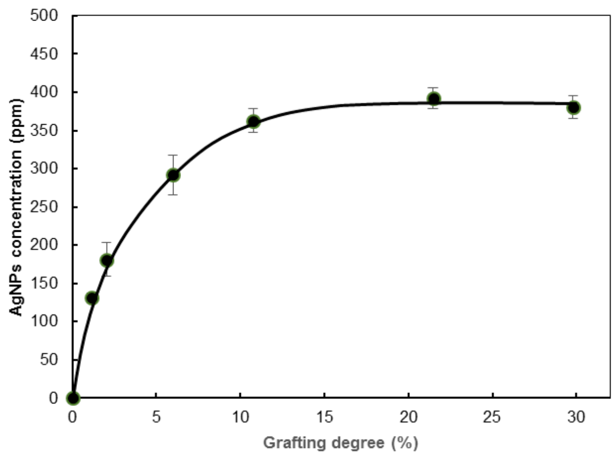

Figure 3. Content of Ag immobilized onto PP-g-AAc at different grafting degree.

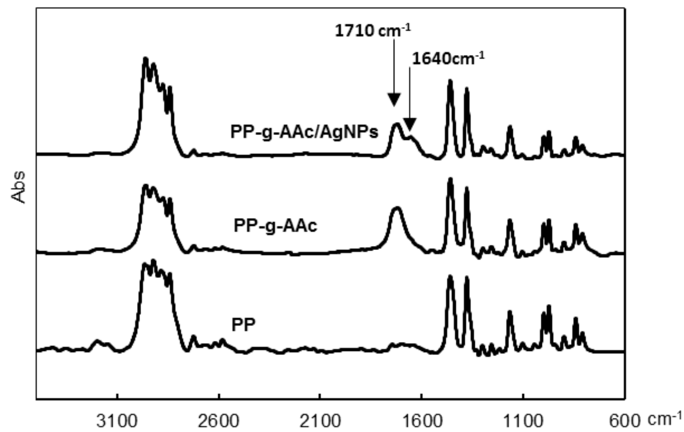

Figure 4. FTIR spectra of PP-g-AAc and PP-g-AAc/AgNPs samples.

FTIR characterization

Fig. 4 showed the FTIR spectra of untreated PP, PP-g-AAc and PP-g-AAc/AgNPs. The specific peaks for PP such as 2952 cm^{-1}, 2910 cm^{-1} and 2850 cm^{-1} for -C-H alkane; 1453 cm^{-1}, 1464 cm^{-1} and 1373 cm^{-1} for -C-C- alkane were clearly visible. However, the FTIR spectra of PP-g-AAc and PP-g-AAc/AgNPs had additional peak at 1710 cm^{-1} assigned to carboxylic groups. The FTIR spectrum of PP-g-AAc/AgNPs had another peak at and 1640 cm^{-1}, which is related to the interaction between the carboxylic groups of AAc with AgNPs/PVP. These results are in agreement with those observed in polyethylene grafted with AAc and immobilized with AgNPs (Zhang *et al.*, 2009; Phu *et al.*, 2013).

Bactericidal activities

Although the antimicrobial mechanism of AgNPs still remains unclear, several recent studies suggested that the antimicrobial activities of AgNPs are related to toxicity mechanism the same as Ag^+ (Zodrow *et al.*, 2009; Klemenčič *et al.*, 2010; Dankovich and Gray, 2011). Other studies also indicated that the antibacterial properties of AgNPs is related to the capability of AgNPs in interacting with bacteria cell surface which leads to structural changes and make the cell membrane become more permeable (Lazar, 2011). Furthermore, due to larger interactive surface and stable release of Ag^+, AgNPs have high bactericidal ability at low concentration compared to other constituents of silver (Klemenčič *et al.*, 2010).

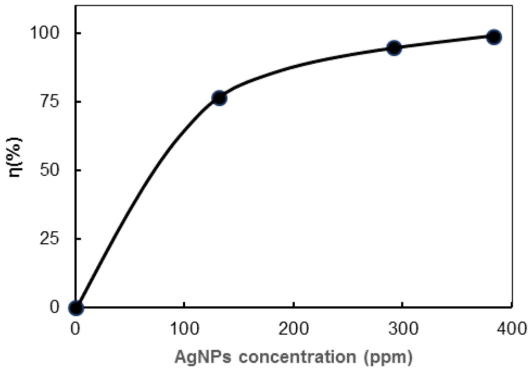

Fig. 5. Bactericidal efficiency of porous PP-g-AAc/AgNPs contained different Ag concentration on *E. coli.*

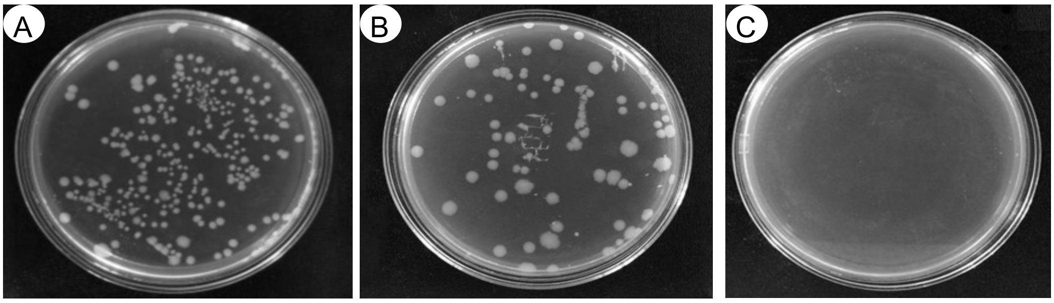

Figure 6. Bactericidal efficiency of PP-g-AAc/ AgNPs on *E. coli*; A: Control, B: Untreated PP, C: Porous PP-g-AAc/AgNPs contained 363 ppm Ag.

The results in Fig. 5 and 6 indicated the bactericidal efficiency of PP-g-AAc/AgNPs increased to nearly 100% at the AgNPs concentration of 363 ppm. The amount of *E. coli* in suspension dropped from ~10^4 cfu/ml to below 10^2 cfu/ml after 30 minutes treated with porous PP-g-AAc/AgNPs material in 5 ml tested suspension.

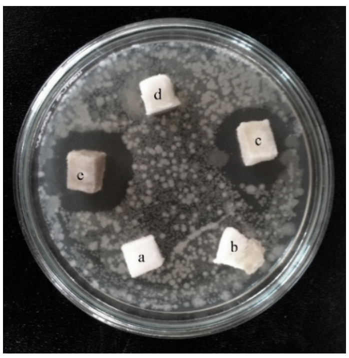

Figure 7. Inhibition zone test of untreated PP (a), PP-g-AAc (b), PP-g-AAc/AgNPs contained 132 ppm Ag (c) and 363 ppm Ag (e) and washed untreated PP immobilized with AgNPs (d).

The inhibition zone test of Ag decorated materials was also conducted on *E. coli.* The PP-g-AAc/AgNPs showed a strong antibacterial activity with the inhibition zone up to 25 and 28 mm in diameter corresponded to the PP-g-AAc/AgNPs materials contained 132 and 363 ppm Ag. The untreated PP, PP-g-AAc and untreated PP immobilized with AgNPs then washed with deionized water did not show any bactericidal effect (Fig. 7).

CONCLUSION

Irradiation using γ rays was a useful method for grafting AAc onto PP as well as synthesizing AgNPs. The PP-g-AAc materials showed a great capability for AgNPs immobilization and materials with the grafting degree from 1.2 to 21.5% were suitable for immobilization of AgNPs. The PP-g-Ac/AgNPs displayed a strong bactericidal activity against *E. coli in vitro*. Therefore, this method can have great potential for the application of preparing antibacterial PP filter cartridges, which can be applied for point-of-use drinking water treatment.

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CHẾ TẠO VẬT LIỆU KHÁNG KHUẨN POLYPROPYLENE GHÉP MẠCH VỚI ACRYLIC ACID VÀ CỐ ĐỊNH NANO BẠC BẰNG PHƯƠNG PHÁP CHIẾU XẠ TIA GAMMA

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TÓM TẮT

Nano bạc (AgNPs) hiện nay đang được ứng dụng rất rộng rãi để làm chất kháng khuẩn do đặc tính kháng khuẩn cao và độc tính thấp đối với tế bào động vật. Trong nghiên cứu này, phương pháp chiếu xạ tia gamma được sử dụng để tổng hợp nano bạc từ dung dịch bạc nitrate ($AgNO_3$) và ghép acrylic acid (AAc) lên vật liệu xốp polypropylene (PP). Vật liệu xốp PP sau khi ghép mạch với AAc (PP-g-AAc) đã được cố định AgNPs để chế tạo vật liệu kháng khuẩn (PP-g-AAc/AgNPs). Các kết quả nghiên cứu cho thấy hàm lượng ghép của AAc lên PP tăng lên khi gia tăng liều xạ cũng như nồng độ AAc. Vật liệu PP-g-AAc với hàm lượng ghép từ 1,2 đến 29,8% đã được lựa chọn để cố định AgNPs (d ~ 10 nm, 500 ppm) nhằm tạo ra vật liệu có đặc tính kháng khuẩn. Hàm lượng bạc cố định đạt được tương ứng từ 132 đến 392 ppm khi cố định AgNPs lên vật liệu PP-g-AAc có hàm lượng ghép từ 1,2 đến 29,8%. Hoạt tính kháng khuẩn của vật liệu PP-g-AAc/AgNPs đối với *E. coli* đã được đánh giá trong điều kiện *in vitro* và kết quả cho thấy hiệu lực kháng khuẩn (η) gia tăng tỉ lệ thuận với hàm lượng bạc được cố định trong vật liệu. Hiệu lực kháng *E. coli* của vật liệu PP-g-AAc/AgNPs có hàm lượng bạc cố định 363 ppm đạt được ~100% sau 30 phút xử lí. Thêm vào đó, đường kính vòng kháng khuẩn của vật liệu này trên *E. coli* cũng lên đến 28 mm. Như vậy, có thể thấy chiếu xạ tia gamma là một phương pháp hiệu quả để ghép các monomer chứa nhóm chức năng (như AAc) lên vật liệu xốp PP. Hơn nữa, vật liệu PP-g-AAc/AgNPs đã cho thấy có tiềm năng to lớn trong việc xử lí các vi sinh vật trong công nghệ lọc nước.

Từ khóa: *Chiếu xạ gamma, kháng khuẩn, nano bạc, polypropylene, xử lí nước*