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FABRICATION OF SILVER-NANOPARTICLES-EMBEDDED POLYMER MASTERBATCHS WITH EXCELLENT ANTIBACTERIAL PERFORMANCE

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ABSTRACT

In the present work, a versatile and effective synthesis method of the silver-nanoparticles-embedded polyethylene (PE)-based polymer masterbatchs was demonstrated. Antibacterial investigations revealed that the nano-silver masterbatchs consisting of oleate capped silver nanoparticles dispersed in PE polymer matrix exhibited excellent antibacterial performance against Gram-negative Escherichia Coli (E.coli) and Staphylococcus aureus (S. aureus) bacteria. A complete inhibition in bacteria growth was found at a silver nanoparticles concentration as low as 600 ppm. The origin of bactericidal effect and interaction mechanism of the stabilized silver nanoparticles with the Gram-negative E. coli and Gram-positive S. aureus bacteria can be understood in the light of electron microscopic observation. These advances make the synthesized nano-silver masterbatchs ideal for mass production of effectively antibacterial green products in medical, biological and industrial sectors. The type of polymer resin and silver concentration can be adjusted depending on the application area.

Keywords: *Silver nanoparticles; nanosilver masterbatch; anti-microbial effect, green synthesis, thermal decomposition.*

I - INTRODUCTION

During the last decade, due to the emergence of a new generation of high technology materials, the number of groups involved in nanomaterials has increased rapidly. Nanomaterials are implicated in several domains such as chemistry, electronics, high density magnetic recording media, sensors and biotechnology. This is, in part, due to their outstanding properties, that differ from both the isolated atoms and the bulk phase [1 - 3].

The recent development of functional polymer nanocomposites is rapidly emerging as a multidisciplinary research activity, resulting in new applications of polymers for the great benefit of various industries [4]. Polymer compounds containing functional inorganic nanoparticles have demonstrated significant improvements in mechanical, thermal, and electrical properties [5]. The homogeneous dispersion of nanoparticles within a polymer matrix is an important issue, because the high performance of nanocomposite materials can be achieved only by controlling their phase structure in nanosized dimensions [6]. However, the fine dispersion of nanoparticles in a polymer using conventional compounding techniques is a

very difficult task, because of the strong tendency of nanoparticles to agglomerate and aggregate. Therefore, the homogeneous deposition of silver nanoparticles on ready-made solid polymers is still a challenging area of investigation.

Polymer compounds containing silver-based nanoadditives are of special interest because of their antibacterial activity. Poly Ethylene (PE) is one of the most widely used polymer materials [7]. There is a high demand for PE with antimicrobial properties for use in a variety of applications, for example, in appliances, as filters, in packaging, and in the textile industry in various forms such as nonwoven films and fibers, and so forth. Significant efforts have been made in the development of silver-based antimicrobial additives for PE [8]. Most of the commercial silver-based antimicrobial additives for PE are available as an active agent in powder form, or as already precompounded master-batches.

In this work, our efforts have been devoted to the fabrication with uniform dispersion of silver nanoparticles within PE materials for making highly bactericidal PE-based pellets and films- type polymer masterbatchs. These nanosilver-embedded masterbatchs also exhibited an extra high antibacterial activity against tested bacteria including both Gram-negative *Escherichia Coli (E. coli)* bacteria and highly multiresistant bacteria such as *Staphylococcus aureus (S. aureus)*. The origin of bactericidal effect and interaction mechanism of the stabilized silver NPs with the Gram-negative *E.coli* and Gram-positive *S. aureus* bacteria was demonstrated by adapting the electron microscopic technique.

II - EXPERIMENTAL PROCEDURES

1. Synthesis of silver nanoparticles (NPs) powder

Silver nitrate ($AgNO_3$, 99%) and sodium oleate (99%) were purchased from Korean Chemical Co. and used without further purification. A silver-oleate precursor was firstly made. In a typical experiment, 1.7 g of $AgNO_3$ (10 mmol) was dissolved in deoxygenated water (100 ml, 18 MΩ, nitrogen gas bubbling for 30 minutes), then added the resulting solution into 3.05 g of sodium oleate (10 mmol) under vigorous stirring for two hours. The obtained solution was separated the precipitate by filtration and washed with three times deionized water to free it of sodium and nitrate ions. The resulting complex powder was dried at room temperature.

After drying, the Ag^{+1}-oleate complex of white powder was transferred into a pyrex tube to perform a thermal decomposition reaction. The complex was then flushed with nitrogen, and the tube sealed at 0.3 Torr. The sample was slowly heated from room temperature to 300ºC with a heating rate of 2ºC/min, and annealed at 300ºC for 1 hour, and then was cooled to room temperature. Finally, silver NPs powders were obtained, which can be easily redispersed in octane and toluene [9].

2. Measurements

The crystalline structure of silver NPs was analyzed by X-ray diffraction (XRD, Bruker D5005) using CuKα radiation (λ = 0.154 nm) at a step of 0.02□ (2θ) at room temperature. Transmission electron microscope (TEM, JEOL-JEM 1010) was conducted to determine the morphology and distribution of silver nanoparticles. Thermal gravimetric analysis (TGA, SDT 2960 TA Instruments) was employed to examine the decomposition of silver-oleate complex. The composition of silver NPs was characterized by Energy-dispersive X-ray (EDX, 5410 LV JEOL).

3. Preparation of nanosilver-embedded masterbatch

A typical fabrication process including three-steps was described in Fig. 1. First, the synthesized silver NPs and Disperplast 1010 (BYK), Chimassorb 81 (Ciba), Irganox 1010 (Ciba) were mixed by a super mixer (KAWATA) at high speed in 1 minute to get A compound. Second, B compound mixing of LDPE resins and Canation oil was formed by a Tumble at speed rate of 20 rpm in 5 minutes. Then, A and B compounds were mixed in 10

minutes to obtain C labeled compound. Finally, C compound was added to a push machine (CTE 35) to produce serially nanosilver-pellets. All experimental compositions for production of nanosilver-embedded masterbatch products were summarized in table 1.

Table 1

No	Materials	C (%)	Note
1	LDPE (LF20184, VALENE)	98.98	Base resin LDPE: Low Density Poly Ethylene
2	Manufactured silver nanoparticles	0.01	
3	Irganox 1010 (Ciba)	0.3	
4	Chimassorb 81 (Ciba)	0.5	
5	Canation oil	0.01	
6	Disperplast 1010 (BYK)	0.2	
7	**Total**	**100**	

4. Antimicrobial tests

The antibacterial activity was tested against both Gram-negative *E. coli* and Gram-positive *S. aureus* bacteria. The studied bacteria were cultured into a Luria-Bertani (LB) liquid nutrient broth medium with pH = 7. The culture medium was incubated at 37^0 C after 24 hours, the bacteria concentration reached to 10^8 colony-forming units (CFU ml^{-1}).

Bactericidal activity of silver-nanoparticles-embedded masterbatchs on *E. coli* and *S. aureus* was studied using diffusion method. The nanosilver masterbatchs were cut in rectangular-shape pieces of 5 mm in length and 3 mm in width. 100 □l of bacterial suspension containing 10^8 CFU was pipetted and spread plating on the surface of polymer masterbatchs containing silver NPs. Masterbatch samples without loading silver nanoparticles were used as control sample. After 24 h incubation at 37□C, these samples were observed for bacterial colony formation. All these experiments were performed under sterile conditions and in triplicate.

The percentage reduction ratio of the bacteria for quantitative antibacterial evaluation has been expressed as [10]:

$$R = \frac{A - B}{A} \times 100\% \quad (1)$$

where R is the percentage reduction ratio, A-the number of bacterial colonies in the masterbatchs without loading silver NPs and B-the number of colonies in the masterbatchs containing silver NPs.

III - RESULTS AND DISCUSSION

Firstly, the XRD technique was used to determine the crystal structure of silver nanoparticles. The XRD pattern of synthesized silver nanoparticles is shown in Fig. 2. There were three well-defined diffraction peaks at 38.2□, 44.5□ and 64.4□ respectively, corresponding to (111), (200) and (220) planes of face centered cubic (fcc) crystal structure of metallic silver (JCPDS PDF 04-0783). Thus XRD pattern clearly demonstrates that nanoparticles formed by thermal decomposition technique, were crystalline in nature. Also, the broadening of the diffraction peaks was observed due to the effect of nano-sized particle [11]. The inset shows a photograph of a jar containing 20 g of silver nanoparticles powder prepared by the thermal decomposition of silver-oleate complex at 300ºC for 1 hour.

Next, in order to determine the morphology and distribution of the obtained silver NPs, the TEM analyses were conducted. As one can see clearly from Fig. 3, the nano-sized silver particles were formed and well-dispersed. Almost no aggregates of silver NPs were observed through TEM investigation. The particle size histogram was obtained by

measuring the size of about 100 nanoparticles and their diameter distribution. Size of the particles ranged from 5 - 20 nm and the average particle size is about 10□0.8 nm.

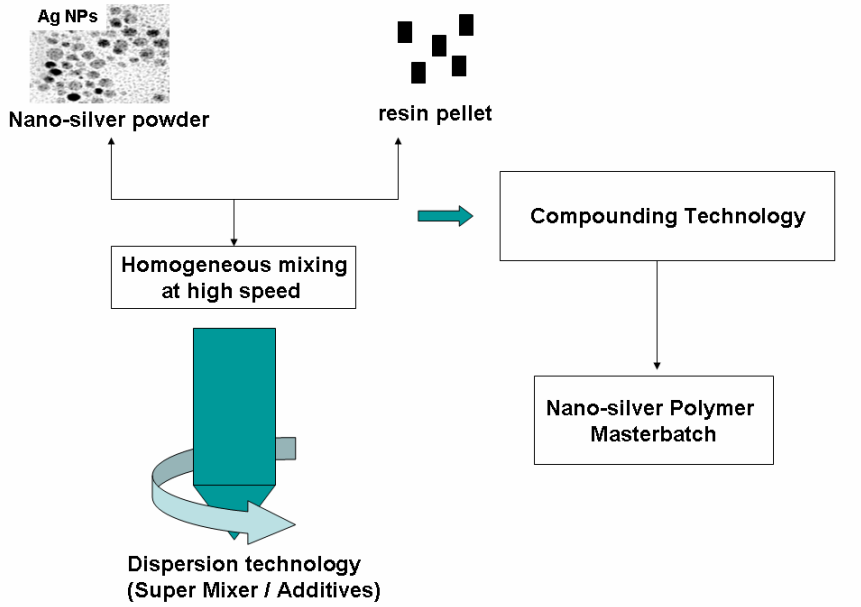

Figure 1: A typical fabrication process of nanosilver-added polymer masterbatch including three-steps: (i) Homogeneous mixing of nano-silver powder and resin pellet at high speed; (ii) Making dispersions with additives by super mixer; and (iii) Producing silver-nanoparticles-embedded compound pellets

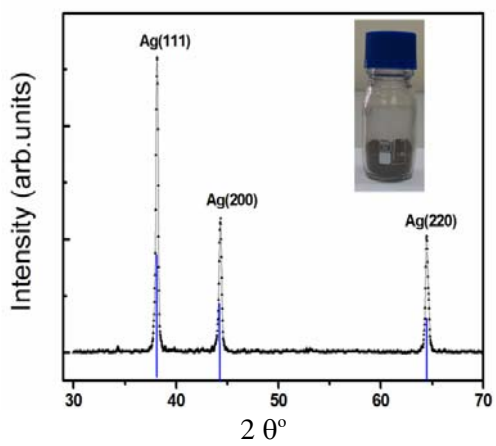

Figure 2: The X-ray diffraction pattern of the silver nanoparticles powder. The inset displays a photograph showing a jar containing 20 g of prepared silver NPs powder

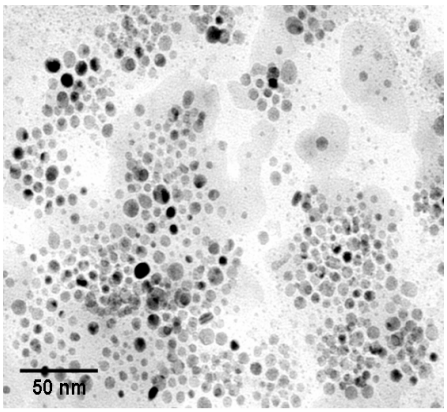

Figure 3: The TEM image of morphology and distribution of the as-prepared silver nanoparticles

In order to apply these synthesized silver NPs as an effective antibacterial medium, a production of nanosilver-embedded masterbatch was performed. This work has been closely

collaborated with a European Plastic Joint-Stock Company partner for commercial products [12]. Silver concentration / PE polymer ratios were varied from 0.01-0.1 % (100 ppm - 1.000 ppm) as shown in Figs. 4 and 5. These obtained results have revealed that the optimal conditions for complete inhibition in bacteria growth were of a silver concentration at 600 ppm for both Gram-negative *E. coli* and Gram-positive *S. aureus* bacteria. It is necessary to emphasize that the developed nanosilver masterbatchs have bactericidal effects resulting not only in inhibition of bacterial growth but also in killing bacteria. Importantly, the excellent antibacterial effect of nano-silver masterbatch over *E. coli* and *S. aureus* bacteria obtained without deterioration of color and mechanical properties of final products.

To gain deeper insights into interaction of silver-nanoparticles-embedded masterbatch with the bacteria, the electron microscopic observation was also conducted. It was evidently observed from Fig. 6, for an example of *E. coli*, that in addition to being fixed to the cell membrane, synthesized silver nanoparticles are capable of penetrating through it to be distributed inside a bacterium. The silver nanoparticles after interaction with *E. coli* bacteria changed the cell wall of bacteria and penetrated though the cell membrane resulting into the inhibition of bacterial cell growth and multiplication.

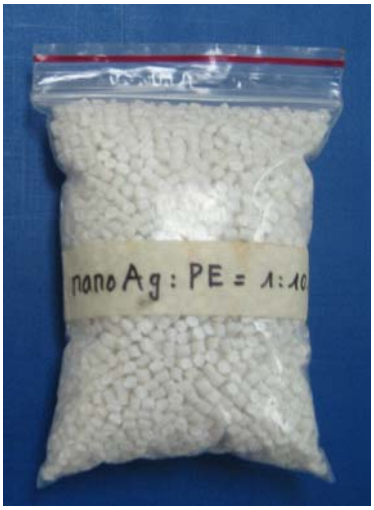

Figure 4: The photograph of silver-nanoparticles-embedded pellets masterbach with 1000 ppm of silver concentration in

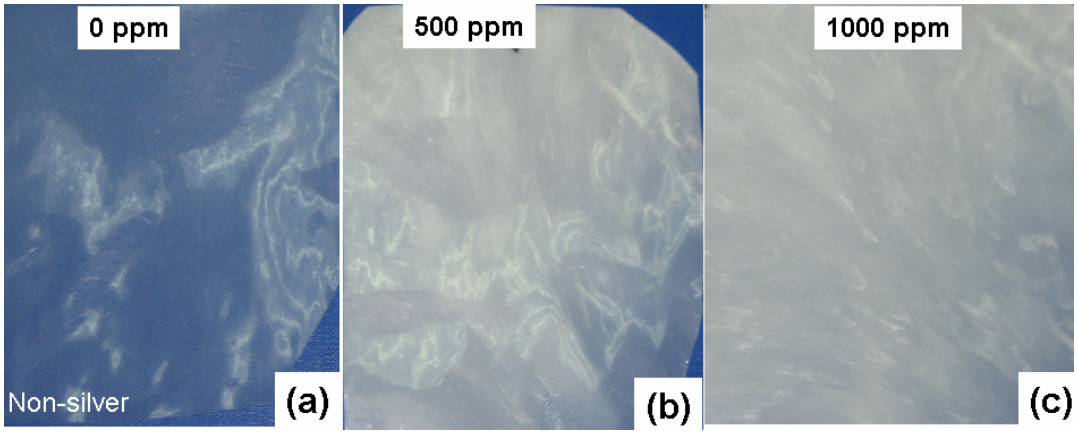

Figure 5: The photographs of film-type masterbachs in which (a) an original plastic film without adding nanosilver (0 ppm), and nanosilver-embedded masterbatchs with (b) 500 ppm of silver concentration in, and (c) 1000 ppm of silver concentration in

It demonstrated the high bactericidal activity of the silver nanoparticles to oleate capping which can insert easily into lipid bilayer of gram negative bacterial cell wall there by allowing the silver nanoparticles to exhibit its bactericidal activity in an efficient way. Thus silver nanoparticles capped with oleate bilayer were more potent in terms of concentration (lower)

and magnitude of cells killed (much higher) as compared to nanoparticles stabilized by other capping agents [13]. This is attributed to formation of the oleate surfactant ions as the capping layer on the surface of synthesized silver particles. The surfactant molecules render inhibition to this aggregation association through capping/template effect and thus act as particle stabilizer [14]. It is worthy to note that that the hydroxymethyl functionalities of the surfactant molecules anchor the molecule at the cluster surface while the hydrophobic chain protects the cluster from aggregation with the next neighbor due to electrostatic repulsion and steric hindrance and thus inhibit coalescence [15].

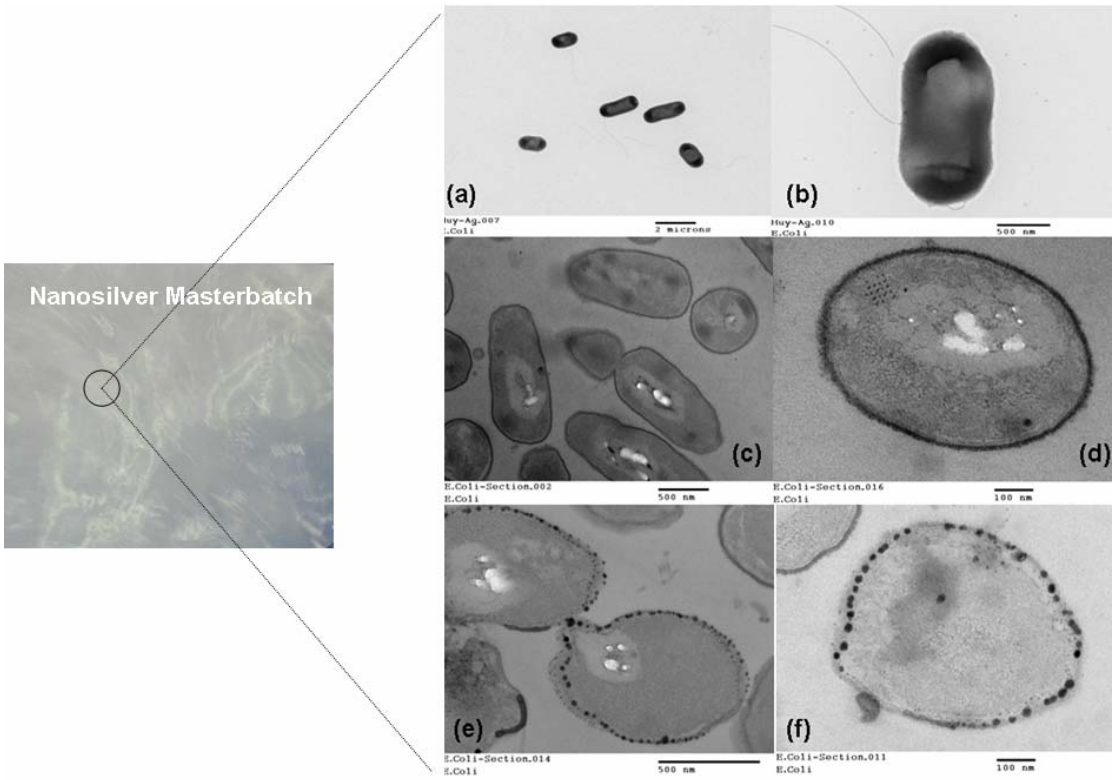

Figure 6: The interaction mechanism of nanosilver masterbatch with tested *E. coli* bacteria observed by the transmission electron microscope technique

At the present, the exact mechanism of action of silver on the microbes is still not known but the possible mechanism of action of metallic silver, silver ions and silver nanoparticles have been suggested according to the morphological and structural changes found in the bacterial cells. For the case of silver metal, the mechanism of action of silver is linked with its interaction with thiol group compounds found in the respiratory enzymes of bacterial cells [16]. Silver binds to the bacterial cell wall and cell membrane and inhibits the respiration process. For an example of *E. coli*, silver acts by inhibiting the uptake of phosphate and releasing phosphate, mannitol, succinate, proline and glutamine from *E. coli* cells. In the case of silver nanoparticle [17], the silver nanoparticles show efficient antimicrobial property compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. The nanoparticles get attached to the cell membrane and also penetrate inside the bacteria. The bacterial membrane contains sulfur-containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus containing

compounds like DNA. When silver nanoparticles enter the bacterial cell it forms a low molecular weight region in the center of the bacteria to which the bacteria conglomerates thus, protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity. The silver nanoparticles with their unique chemical and physical properties are proving as an alternative for the development of high effective antibacterial agents.

IV - CONCLUSIONS

The silver-nanoparticles-embedded PE-based polymer masterbatchs with excellent antibacterial performance were successfully synthesized. A fine dispersion of silver nanoparticles in PE polymer matrix was obtained with use of appropriate dispersion supporting reagents of Disperplast 1010 (BYK), Chimassorb 81 (Ciba), Irganox 1010 (Ciba). At a silver nanoparticles concentration of 600 ppm, a complete inhibition in *E. coli* bacteria growth was achieved. These nanosilver masterbatchs can be effectively used for various industrial applications such as food container, packing film, breathable film, etc. This opens new opportunities to fabricate other different kinds of nanosilver polymer masterbatch upon request of the customer in terms of polymer type and silver concentration.

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REFERENCES

1. A. M. Spasic. Finely dispersed particles: micro-, nano-, atto-engineering, CRC Taylor and Francis Group (2006).
2. G. Cao. Nanostructures and nanomaterials: synthesis, properties and applications, Imperial College Press, 2004.
3. K. Ohno, M. Tanaka, J. Takeda, Y. Kawazoe. Nano- and micromaterials, Springer Publisher, 2008.
4. N. Perkas et al. Journal of Polymer Science: Part A: Polymer Chemistry, 46, 1719 (2008).
5. http://www.nanoine.com
6. M. Z. Kassaee et al. Journal of Applied Polymer Science, 110, 1699 (2008).
7. W. Zhang et al. Chemical Physics, 330, 495 (2006).
8. W. Zhang et al. J. Colloids Interface. Sci., 302, 370 (2006).
9. J. Park, J. Joo, S. G. Kwon, Y. Jang, T. Hyeon. Angew. Chem. Int. Ed., 46, 4630 (2007).
10. J. R. Morones, J. L. Elechiguerra, A. Camacho, K. Holt, J.B. Kouri, J. T. Ramirez, M. J. Yacaman. Nanotechnology, 16, 2346 (2005).
11. Y. Sun, Y. Xia. Science, 298, 2176 (2002).
12. http://www.europlast.com.vn
13. Y. A. Krutyakov, A. A. Kudrinskiy, A. Y. Olenin, G. V. Lisichkin. Russ. Chem. Rev., 77, 233 (2008).
14. I. Sondi, B. Salopek-Sondi. J. Colloids. Interface. Sci., 275, 177 (2004).
15. M. Raffi, F. Hussain, T. M. Bhatti, J. I. Akhter, A. Hameed, M. M. Hasan, J. Mater. Sci. Technol., 24, 192 (2008).
16. W. Yang, C. Shen, Q. Ji, H. An, J. Wang, Q. Liu, Z. Zhang. Nanotechnology, 20, 085102 (2009).
17. S. Shrivastava, T. Bera, A. Roy, G. Singh, P. Ramachandrarao, D. Dash. Nanotechnology, 18, 225103 (2007).

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