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Imparting Antimicrobial Properties to Natural Rubber Latex Foam via Green Synthesized Silver Nanoparticles

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ABSTRACT: The green synthesis of silver nanoparticles (AgNPs) in centrifuged natural rubber latex (NRL) by *in situ* reduction of silver nitrate by NRL is described. The synthesis of AgNP within NRL was successfully carried out without the addition of any reducing agent or stabilizers. The modified AgNP incorporated with centrifuged NRL (GAgNP_NRL) was used to make NRL foam (NRLF) by the Dunlop production method. An ultraviolet–visible (UV-Vis) spectrophotometer analysis, Zeta potential analysis data and transmission electron micrograph analysis proved that the modified centrifuged NRL consisted of stable nanometer-sized silver particles. A scanning electron microscopic (SEM)/energy-dispersive X-ray spectroscopy (EDX) analysis and UV-Vis analysis of a latex film made out of the modified GAgNP_NRL compound showed nano-sized silver particles inside the rubber matrix. The final product of the NRLF (GAgNP_NRLF) made out of the GAgNP_NRL compound was tested for antimicrobial properties against gram-negative *Escherichia coli*, gram-positive *Staphylococcus aureus* and *Staphylococcus epidermidis*. The resultant GAgNP_NRLF strongly inhibited the bacteria. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40155.

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INTRODUCTION

Research into the *in situ* reduction in silver nitrate to metallic silver nanoparticles (AgNPs) inside polymers,[1,2] hydro gells,[3,4] resins,[5,6] and layered silicate clays[7–9] is very important in modern nanotechnology. Silver nitrate is reduced by several types of reduction agents within those materials. The most common types of reducing agents used by researchers are trisodium citrate,[10–13] ascorbic acid,[14,15] polyvinylpyrrolidone (PVP),[16] sodium borohydride,[17,18] and different kinds of glucose.[18–20] The reduction in silver nitrate to metallic AgNPs using biologically originated materials is a more attractive and important research area among several researchers.[21,22]

Because of the concerns about toxic chemicals and environmental safety, the green synthesis of nano particles by biologically oriented materials such as bacteria,[23] fungus,[24,25] and plant extracts has become an interesting field in modern nanotechnology. Pandey et al.[26] found that a naturally occurring polysaccharide commonly known as guar gum (extracted from a plant called *Cyamopsis tetragonaloba*) can be used both as a reducing and a stabilizing agent for the preparation of AgNPs. Dubey et al.[27] reported that leaf extracts of the *Rosa regosa* can produce 12-nm-sized AgNPs and 11-nm-sized gold nanoparticles. They also have found that the stability of the synthesized nanoparticles appreciated greatly in a wide range of pH values. Several studies can be found in the literature on the formation of AgNPs using naturally occurring latex from different types of plants. Mondal et al.[28] found that AgNPs synthesized within naturally occurring latex from several plants, such as the *Alstonia scholaris, Hevea brasiliensis, Ficus religiosa, Calotropis gigantean, Musa paradisiacal,* and *Achras sapota,* can give a surface plasmon resonance (SPR) peak of between 401and 434 nm in ultraviolet–visible (UV-Vis) spectroscopy. But they have not reported on any further analysis of the resultant nanoparticles of silver in the so-called latex. In 2007, Abu Bakar et al.[21] published that AgNPs can be easily synthesized in natural rubber latex (NRL) by UV-irradiation of Ag^+ ions in NRL. In their investigation, the sizes of the AgNPs were in the range of 4–10 nm. Another study carried out by the same author and his coauthors[29] found that the preparation of PVP-grafted natural rubber having 4.1-nm-sized AgNPs can be easily synthesized by the UV irradiation method.

The investigation of the antimicrobial activities of AgNPs and AgNPs incorporated with polymeric materials has been a

popular area of research for many years.[30–33] The mechanism for the antimicrobial action of AgNPs is believed to be due to many reasons, such as the production of silver ions (Ag^+) that can interact with the thiol groups in proteins. It results in the inactivation of respiratory enzymes leading to the production of reactive oxygen species (ROS). Furthermore, excessive amounts of ROS can oxidize proteins in mitochondria, attack lipids and DNA leading to the breakdown of the cell membrane. Also ROS can inhibit the role of the mitochondria or prevent the reproduction of DNA.[34,35] Some researchers[36,37] have suggested that silver ions are also found to be photoactive in the presence of UV-A and UV-C irradiation, leading to enhance UV inactivation of bacteria and viruses.

In previous research studies,[38] it was found that the chemical reduction in silver nitrate in the presence of NRL foam (NRLF) materials can provide antimicrobial NRLF with very good antimicrobial properties. The silver nanocolloids produced had AgNPs with an average size of 29 nm, whereas the AgNPs adsorbed on the surface of the NRLF matrix had sizes within the range of 50–70 nm. It was also found that silver nanocolloids can be easily mixed with a NRL compound and the modified compound can be used to make antimicrobial NRLF. After mixing with NRL, the AgNPs were very stable inside the NRL compound as well.[39] Recently, another study[40] on AgNPs and NRLF also found that the novel method can be used to incorporate AgNPs into the NRLF matrix. It was reported that the chemical reduction in the silver ions within potassium oleate (KOL) soap by trisodium citrate can incorporate the AgNPs into the KOL soap. The synthesized SNP_KOL itself has antimicrobial activities against gram-negative *E.coli* and can be used as a convenient carrier of AgNPs to the NRLF, while acting as a foaming agent for making NRLF. The resultant end product of the NRLF also gave very good antimicrobial properties against gram-negative *E.coli*. Recently, we have found that incorporation of ZnO nanoparticles into NRLF can enhance antimicrobial activities to a greater extent. It was found that the antibacterial activities against for both gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus* for nano sized ZnO were much greater than that of micro sized ZnO. Also we reported that there can be several possibilities of killing microbes by ZnO nanoparticles.[41]

This work concerns the green synthesis of AgNPs inside the liquid dispersion centrifuged NRL having 60% dry rubber content (DRC) and its use for making antimicrobial NRLF materials. According to the literature findings, no research work has been published on the green synthesis of AgNPs inside centrifuged NRL. Also no published data can be found about the synthesis of antimicrobial NRLF materials using green synthesized AgNPs inside the liquid dispersion of NRL. This novel method can be used to make many products having AgNPs incorporated into NRL-based materials such as foam, gloves, catheters, condoms, and so on. In the synthesis of NRLF, 60% of DRC is a necessary requirement to make a fine cell-structured foam material.[42,43] So it is very important to incorporate the AgNPs into the NRL without affecting the DRC.

MATERIALS AND METHODS

The chemicals for the synthesis of AgNPs were purchased from Merck KGaA, Germany, and Sigma Aldrich Chemicals. The chemicals for making the SNP_NRL material, such as NRL (low Ammonia (LATZ) type), were purchased from Zarm Scientific and Supplies (M) Sdn. Bhd., Malaysia. Chemical additives for the preparation of NRLF, such as sulfur (S), phenolic type antioxidant (Naftocit ZMP), zinc 2-mercaptobenzhiozolate (ZMBT), zinc diethyldithiocarbamate (ZDEC), zinc oxide (ZnO), diphenylguanidine (DPG), and sodium silicofluoride (SSF), were also supplied by Zarm Scientific and Supplies (M) Sdn. Bhd., Malaysia. The chemicals for the antimicrobial susceptibility tests were obtained from Sigma Aldrich Chemicals and from Fisher Scientific. The *E. coli*, *S. aureus*, and *Staphylococcus epidermidis* strains were supplied by Ward's Science.

Table I. Formulation for Latex Compounds for Synthesis of AgNPs Incorporated NRLF Samples and Control Sample of NRLF

	Dry parts per hundred rubber (pphr)	
Ingredients	Control Sample	GAgNP_ NRLF
60% Pure NR Latex	100.00	0.00
60% GAgNP_NR Latex	0.00	100.00
20% KOL soap	3.00	3.00
50% Sulfur	2.50	2.50
50% Phenolic type antioxidant (vulkanox SKF)	1.00	1.00
50% ZMBT	1.00	1.00
50% ZDEC	1.00	1.00
40% ZnO	1.00	1.00
40% DPG	0.70	0.70
25% SSF	3.50	3.50

Synthesis of AgNPs Inside Centrifuged Latex and Synthesis of NRLF

Green Synthesis of AgNPs Inside Centrifuged NRL (GAgNP_NRL). Centrifuged NRL (DRC 60%) containing AgNPs was prepared as follows: 10 mL of 0.1*M* silver nitrate was added to 250 mL of centrifuged latex (60% DRC). The mixer was kept in a steam bath at 60°C while the mixture was mixed vigorously by means of a mechanical stir for 8 h. Next, the temperature of the reaction mixer was reduced to ambient temperature, while the mixture continued to be stirred at a low speed for 24 h. The synthesized NRL was left to cool down to room temperature and was stored in a closed amber glass container. The DRC of the control NRL and GAgNP_NRL was measured according to the ISO 126, and it was found that the initial and final DRC contents were 60.24 and 58.02, respectively.

Compounding and Production of AgNPs Incorporated NRLF (GAgNP_NRLF) Using GAgNP_NRL. The formulation used to make the GAgNP_NRLF and the control sample of NRLF is shown in Table I. First, the modified GAgNP_NRL was mixed with sulfur, antioxidants, and KOL soap, while stirring at 10 rpm. After 2 h, ZMBT and ZDEC were slowly added to the mixture. Then the compound was matured for 8 h at room temperature while stirring at 10 rpm.

After maturation, the GAgNP_NRLF compound was vigorously beaten using a stand mixer (KENWOOD, kMix) to make a fine foam until the volume was increased up to three times of the initial volume (beating time about 5 min). After that 3.00 pphr of ZnO together with 0.30 pphr of DPG were added as the primary gelling agents to the foam and the beating was continued for another 90 s. Then a secondary gelling agent, 1.00 pphr of SSF, was quickly added and the foam was beaten for another 90 s. Finally, the ungelled foam was immediately poured into an aluminium mould and allowed to gel for 2 min at ambient temperature. The gelled foam was then cured in a hot air oven at 100°C for 2 h. Then the cured foam was stripped from the mould and thoroughly washed with deionized water to remove any soap and nonreacted elements. After washing, the cured GAgNP_NRLF was dried in a hot air oven at 80°C for 8 h. The resultant foam was yellowish in color. The same procedure was used to prepare a control sample of NRLF, which was off-white in color.

CHARACTERIZATION

1. Pure NRL and modified GAgNP_NRL were evaluated for fundamental properties such as DRC, total solid content (TSC), volatile fatty acid (VFA) content, mechanical stability time (MST), alkalinity, and pH as per the ISO methods. The respective ISO methods and the results are given in Table II.
2. The GAgNP_NRL sample was tested using a VARIAN Cary 50 conc. UV/Vis Spectrophotometer. A liquid sample of pure silver nanocolloid synthesized in deionized water by reduction in silver nitrate by trisodium citrate as explained in a previous research work[44] was tested for UV-Vis analysis in reference to AgNPs. The pure NRL sample was also tested for UV-Vis analysis.
3. The Philips CM12 transmission electron microscope (TEM) was used to capture the TEM images. A small drop of a diluted sample of the modified GAgNP_NRL was put on the copper grid very carefully and allowed to dry for 1 h. Then the copper grid was placed carefully inside the instrument and tested to get the TEM images. The resultant TEM images were analyzed using the Docu version 3.2 image analysis.
4. A Malvern Zeta Sizer was used to analyze the zeta potential of a liquid sample of GAgNP_NRL.
5. The ZEISS Supra TM 35VP (Germany) scanning electron microscope coupled with EDAX Genesis operated at 10.00 kV was used to analyze the scanning electron microscopic (SEM) images and to carry out an EDX analysis. Before analyzing by the scanning electron microscope, all the foam rubber samples were coated with an alloy consisting of 80% gold and 20% palladium in a Bio-RAD Polaron Division SEM coating system.
6. The quantitative and qualitative determination of the antimicrobial testing was carried out as described in the following method.

Determination of Antibacterial Activities by Agar Diffusion Method

The agar diffusion method was used to evaluate the antimicrobial activity against *S. epidermidis*, methicillin resistant *S. aureus* (MRSA), and *E. coli* strains (HB101 and DH5d). Bacterial concentrations were determined by measuring the optical density (OD) at 600 nm. The OD value of 0.3 corresponds to a bacteria concentration of 1 × 108 CFU/mL.[45] An amount of 100 μL of the bacterial solutions was inoculated on to Muller Hinton agar (MHA) plates by evenly spreading procedure. AgNPs incorporated natural foam rubber (GAgNP_NRLF) samples and unmodified foam materials (NRLF) were placed in separate positions on agar plates under aseptic conditions. A thin layer of Tryptic Soya agar (TSA) was poured on to the top of the foam samples so that the foam materials were sandwiched between the TSA layers. For the lower layer, 15 mL of MHA was poured into the sterilized Petri dishes under aseptic conditions. For the upper thin layer, 10 mL of TSA was poured onto the two pieces of foam material. The inoculated agar plates were incubated at 37°C for 24 h. The antibacterial activities of modified GAgNP_NRLF samples were evaluated by measuring the zone of bacterial inhibition in comparison with the unmodified NRLF control samples.

Table II. Results of DRC, TSC, VFA, MST, Alkalinity and pH of the Modified and Pure NRL

Name of the property	ISO test method	Standard values[42]	Pure NRL	GAgNP_NRL
DRC	ISO 126	Min 60.3%	60.24%	58.02%
TSC	ISO 124	Min 61.5%	61.62%	56.60%
VFA	ISO-506	Max 0.2	0.0249	0.0235
MST	iso 2006	Min 540 s	1034	960
Alkalinity	ISO 125	0.22-0.25	0.27	0.198
pH		10-11	10.3	9.88

Determination of Antibacterial Activities by Measuring the OD

This method of evaluation involves testing bacterial suspensions directly inoculated into modified GAgNP_NRLF samples and unmodified foam materials (NRLF). The test was performed in accordance to ISO 20743:2007 standards (ISO, 2010). *S.aureus*, *S.epidermidis*, and *E.coli* bacterial suspensions were prepared by taking a single colony from the original bacterial culture plate with a loop and inoculating 20 mL of sterile tryptic soya broth in a 25-mL separate culture tube in aseptic conditions. The culture tubes were then incubated in a shaking incubator at 37°C at 110 rpm for 3 h and the OD values of the tubes were measured at 600 nm. After incubation, 0.100 μL of the bacteria suspension was transferred to separate sterile culture tubes containing 20 mL of tryptic soya broth with GAgNP_NRLF samples and unmodified foam materials (NRLF; 2 × 2 cm). The culture tubes were initially shaken for 5 min. Then the culture tubes were incubated in a shaking incubator at 37°C and 110 rpm. The OD of the culture tubes at 600 nm was measured in every 30 min to evaluate the rate of bacterial growth in the presence of GAgNP_NRLF samples and unmodified foam materials (NRLF).

RESULTS AND DISCUSSION

Fundamental Properties of the Raw Latex

To make good NRLF, it is very important to measure the fundamental properties of the raw latex. The values were measured

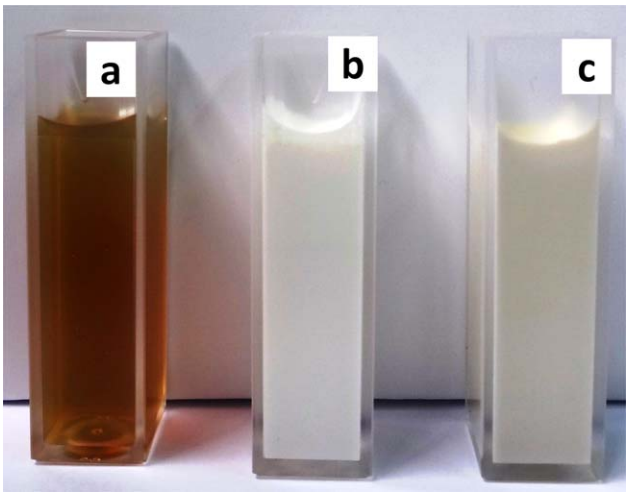

Figure 1. (a) Reference sample of silver nanocolloid, (b) pure NRL, (c) sample of GAgNP_NRL. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

for both pure NRL and modified GAgNP_NRL and compared against the standard values**.** According to Table II, after being modified by AgNPs, all the values of the DRC, TSC, alkalinity, and VFAs number were changed. However, the changed values of the DRC and TSC did not have a great effect on the lab scale foam rubber synthesis and the resultant foam had a very fine cell structure. The VFA content is also a very important parameter to be checked in raw latex. The formation of VFA is mainly due to the effect of bacteria on the organic materials present in the serum of NRL. If the VFA is high the stability of the foam is reduced, so the VFA content should be as low as possible. The VFA of the resultant GAgNP_NRL was reduced after the modification, probably due to the evaporation of the VFAs during the heat treatment of the green synthesis of AgNPs. Further investigation into the VFA of the stored GAgNP_NRL will be a very important factor to make high-quality preserved latex by AgNPs. The bactericidal action of AgNPs may suppress the growth of microorganisms in the serum fraction of NRL. Hence, the VFA of GAgNP_NRL will be reduced. Alkalinity was also reduced after the modification. The alkalinity of the raw latex is mainly due to the presence of ammonia in the raw centrifuged latex. During the preservation of latex, ammonia is added as a preservative agent together with small amounts of ZnO and tetramethylthiuram disulfide. The reduction in ammonia was due to the evaporation of ammonia during the high-speed stirring and heat treatment for the synthesis of GAgNP_NRL. The pH value was also decreased due to the low ammonia content. The MST was also decreased from 1034 s to 960 s due to the high-speed stirring and heat application to the latex. However, during the synthesis of natural rubber foam, KOL was added as the foaming agent. As KOL acts as an anionic stabilizing agent for the NRL, KOL could increase the MST values, alkalinity and pH values of the compounded latex. So the reduction in the MST, alkalinity, and pH values before adding the KOL soap will not affect the final quality of the NRLF.

Figure 1(a) shows the appearance of the referenced silver nanocolloid produced in this research work, Figure 1(b) shows the appearance of pure NRL (NRL) and Figure 1(c) shows the modified GAgNP_NRL. It can be observed that the GAgP_NRL is yellowish whereas the pure NRL is whitish in color. This was the fundamental observation with regard to the modified NRL and this observation was further proven by analyzing the maximum absorbance peak using UV-Vis spectroscopy.

UV-Vis Analysis of GAgNP_NRL, Pure Silver Nanocolloid, and Pure Natural Rubber

Metal nanoparticles such as silver exhibit an exceptional intense peak in UV-Vis spectroscopic analysis due to SPR.[46] The conduction band and the valance band of metal nanoparticles lie very close to each other where electrons can move very easily. These free electrons give rise to a SPR absorption band in UV-Vis spectroscopy analysis.[11,47] In the UV-Vis spectrophotometer analysis, the modified GAgNP_NRL showed a maximum absorbance peak of 0.34 at 429 nm wavelength and the reference sample of pure silver nanocolloid gave a maximum absorbance peak of 0.70 at 425 nm wavelength (Figure 2). However, the pure NRL did not give any peak for the UV-Vis spectrophotometer analysis. The characteristic absorption peak at 425 nm seen in the pure referenced sample of the silver nanocolloid was shifted to a higher wavelength in the modified GAgNP_NRL, the reason being that the green synthesized AgNPs in the centrifuged NRL produced larger particles than the pure silver nanocolloid. In previous research work it was reported that the pure silver nanocolloid had 22–35 nm sized AgNPs.[39] Abu Bakar et al.[21] reported that the UV-Vis data for the casting film of natural rubber–silver composite showed absorption peaks in the range of 425–484 nm range. Another research work published on green synthesis of AgNPs using green leaf extract also reported that the maximum absorption peak in UV-Vis spectrophotometer analysis for the obtained AgNPs was 440 nm. They have further reported that another peak at 370 nm also can be found due to the transverse plasmon vibration in the AgNPs.[48]

TEM Analysis of GAgNP_NRL

The TEM images taken for the modified GAgNP_NRL in Figure 3 clearly show that the bigger particles of natural rubber are covered with nano-sized silver particles. It can be seen that the sizes of the individual particles of silver are below 100 nm, whereas the sizes of the natural rubber particles (large circular shaped particles) are higher than 500 nm. As the TEM images

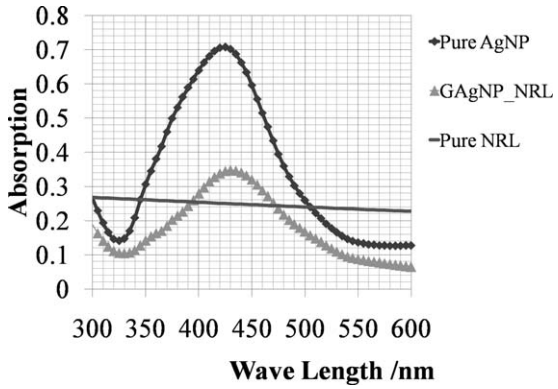

Figure 2. Absorption versus wavelength of referenced sample of silver nanocolloid, modified GAgNP_NRL, and pure NRL.

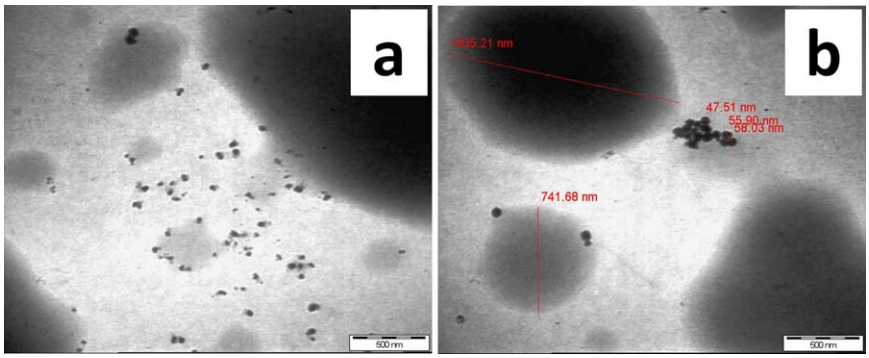

Figure 3. TEM micrograph of the liquid sample of Green AgNP incorporated NRL, (a) magnification at ×3K, (b) showing sizes of natural rubber particles and AgNPs magnification at ×3K. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

were taken after drying the latex samples on the copper grid, the agglomerated AgNPs in the TEM images could be seen. In our previous studies also we have found that the agglomerations of nanoparticles in TEM images were mainly due to the sample preparation technique carried out in TEM analysis.[39] The formation mechanism of the AgNPs inside the latex serum has yet to be investigated, however some research work conducted on the green synthesis of AgNPs by several kinds of plants and microorganisms suggest that the proteins in the living organisms can act as a reducing agent for the reduction in ionic silver to AgNPs. Abu Bakar et al.[21] have reported that the protein content present in NRL plays a very important role as a potential reducing agent and as well as a potential stabilizing agent. But in their experiment reduction in silver ions to silver nanopartciles was mainly carried out by using UV rays. Further investigation is going on about the green synthesis of AgNPs inside deproteinized NRL to prove above statements.

According to the Figure 4, it is suggested that the proteins inside latex serum (2–3%) are act as both reducing and stabilizing agents for AgNPs. According to the review carried out by Jacob et al.,[49] the polymeric part (cis-1,4-polyisoprene) of NRL is covered mainly with phospholipids and protein layers (α-globulins). These layers act as the stabilizing envelops to prevent agglomeration of rubber particles inside the NRL. Figure 4 shows that the silver ions are adsorbed on the protein layer and eventually reduce them to make AgNPs. The formed AgNPs are further stabilized by the same protein layers. This suggested mechanism also proved from the TEM image results (Figure 4) that shows most of the AgNPs are seen on the surface of the rubber particles.

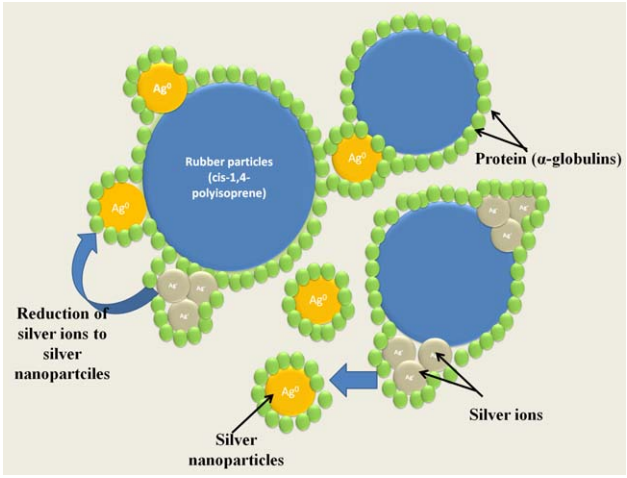

Figure 4. Suggested mechanism for the formation and stabilization of AgNPs inside the modified liquid NR latex. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Zeta Potential Analysis of GAgNP_NRL

Zeta potential measurement is a very important technique that can be used to measure the stability of the dispersion system. It can be also used to check the charge of the stabilizing system, whether the stabilizing system is anionically stable or catatonically stable. As shown in Figure 5, the value of the zeta potential for modified NRL by AgNPs (GAgNP_NRL) is −66.7 whereas zeta potential for pure NRL and pure silver nanocolloid in aqueous media are −60.6 and −31.8 respectively. The more stable the dispersion, the higher the value of the zeta potential. Any dispersion is extremely stable if it has an absolute zeta potential value of more than 60, and it is physically stable if the absolute value is more than 30.[50] According to the results of the zeta potential, it can be said that the stability of silver nanopartciles inside the serum fraction of NRL is very high. Also the absolute value of zeta potential of pure NRL did not change due to the presence of AgNPs inside the serum fraction. This happened as the zeta potential of pure nanocolloid was also

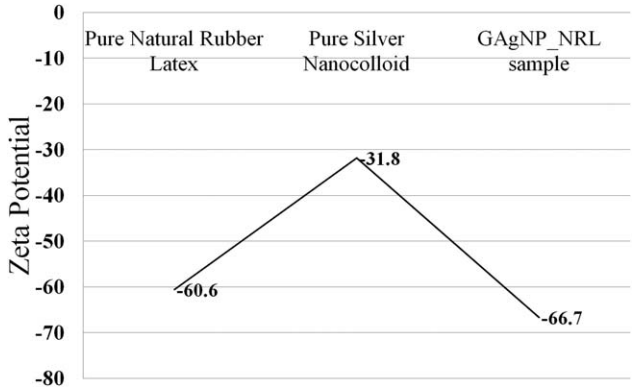

Figure 5. The graph of Zeta potential analysis of GAgNP_NRL, pure silver nanocolloid, and pure NRL.

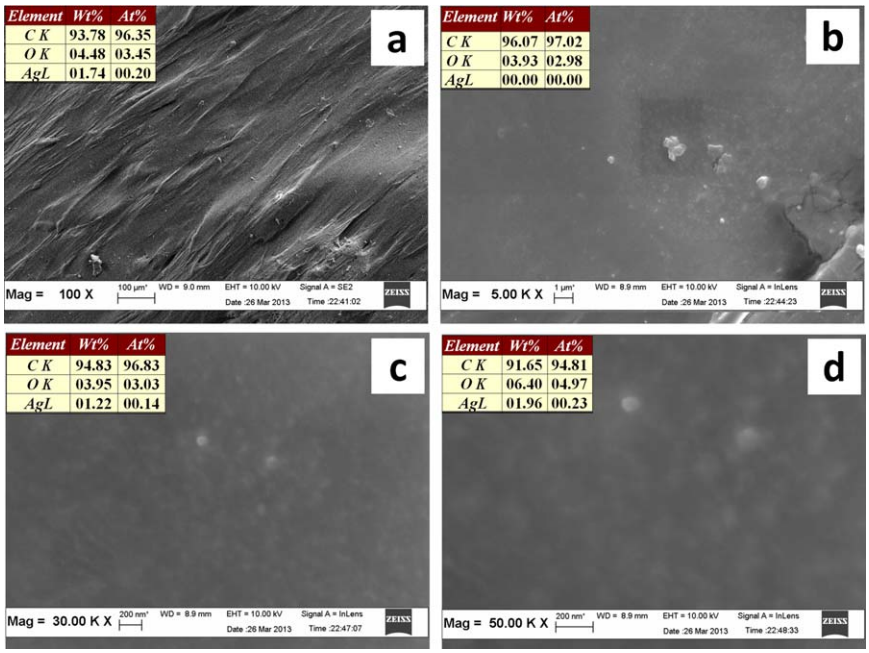

Figure 6. SEM micrograph and EDX analysis of GAgNP_NRL film; (a): magnification at ×100, (b) magnification at ×5.0K, (c) magnification at ×30.00K, and (d) magnification at ×50.00K. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

−31.8 (negatively stable). As both stabilizing systems were anionic stabilizing systems (surrounded by anions), their values were higher enough to give a good stable dispersion. It is obvious that the mixing or synthesizing of AgNPs inside NRL would create a good stable dispersion. This stable colloidal dispersion of modified GAgNP_NRL is a very important property for making the final material of NRLF.

SEM-EDX Analysis of NRL Film Made from GAgNP_NRL

A latex film was prepared using the same compound that was used to prepare the modified NRLF. It was evaluated using SEM-EDX analysis. The resultant SEM micrograph (Figure 6) showed nanometre-sized particles embedded in the latex film. The overall EDX analysis of the film is shown at the top left corner of each image. In Figure 6(d), the elemental silver percentage was 1.96% of the wt % and 00.23% of the atoms. The major element present in the latex film was C (91.65 wt %, 94.81 atom %). The reason for that was because the main polymer part was cis-1,4-polyisoperen (—C_5H_7—) units that mainly consisted of elemental carbon. Also, 6.40 wt % and 4.97 atom % of elemental O were also present in the film. The main source for this could be the nonrubber contents in the serum fraction of the NRL which consisted of carbonic materials.

SEM micrographs and EDX analysis of the GAgNP_NRLF further confirmed that the resultant foam material consisted of AgNPs. Figure 7 shows the SEM images of the GAgNP_NRLF at different magnifications. At the higher magnification, the AgNPs in the microsized cells of the modified foam sample can be clearly seen. EDX analysis results showed in Figure 7(e) confirmed that the area of the agglomerated nanosizedparticles is consisted with minor amount of silver element and other elements such as C, O, F, and Zn.

Figure 8 shows the overall EDX analysis of the GAgNP_NRLF. The luminous blue spots shown in Figure 8(c) are the elemental silver mapping results of the resultant foam. It is shown that 6% of weight and 0.72 atomic % of elemental silver are present in the resultant GAgNP_NRLF. The film made with the AgNP_NRL showed a wt % of 1.96 and atomic % of 0.23. The reason for the difference in values was based on the scanned area and the surface condition of the scanned area. As the foam surface consisted of microsized holes, the scanner could scan a larger surface area and give higher values for the elements in the EDX scanning. However, the EDX scanning results proved that the resultant foam sample consisted of silver elements and a higher amount consisted of carbon elements, the reason being that the main polymer part was cis-1,4-polyisoperen (—C_5H_7—) units that mainly consist of C elements. A small amount of sulfur was also present due to the use of sulfur in the NRLF-making process

Figure 9 shows the physical appearance of the treated and untreated NRLF. Figure 9 (a) is the control sample of NRLF where the color is white, whereas the treated sample (Figure 9(b)) is somewhat yellowish in color. The reason for the slight difference in color is due to the AgNP particles present in the NRLF sample. During the synthesis of the foam rubber, the end product was washed several times before the final drying of the foam material. However, the yellowish color of the modified foam samples did not change during the washing. This proved that the AgNPs adsorbed on the foam matrix were strongly bound and could not be washed off by water.

Results of the Antimicrobial Test

Determination of Antibacterial Activities by Agar Diffusion Method. As shown in Figure 10, the antimicrobial test results against gram-negative *E.coli* and gram-positive *S.aureus* and *S.epidermidis* showed that the treated NRLF sample by green synthesized AgNPs strongly enhanced the antibacterial activities.

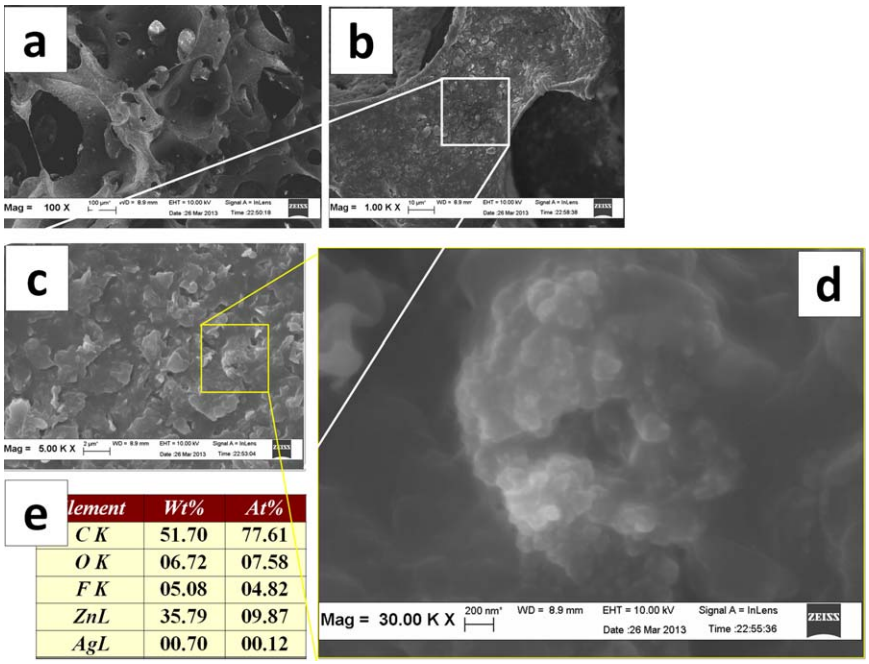

Figure 7. SEM micrograph and EDX analysis of modified natural rubber AgNP_NRLF; (a) magnification at ×100, (b) magnification at ×1K, (c) magnification at ×5K, (d) magnification at ×30K, (e) EDX analysis results of SEM image "d". [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The antimicrobial activities of silver ions and silver-based compounds against several types of microorganisms have been reported by several researchers.[51–54] The imparting of AgNPs into various kinds of materials to make them resistant to pathogenic microbes is well known among many researchers.[18,37,53,55,56] The cell envelopes of bacteria are complex,dynamic structures that play a veriety of protective and adaptive roles. The major concerved component of all bacteria cell envelopes is peptidoglycon, which is essential for stabilizing cell membranes against high internal osmetic pressures. An essential function of the peptidoglycan layer of the bacterial cell between gram-positive *S. aureus* and gram-negative *E. coli* is to

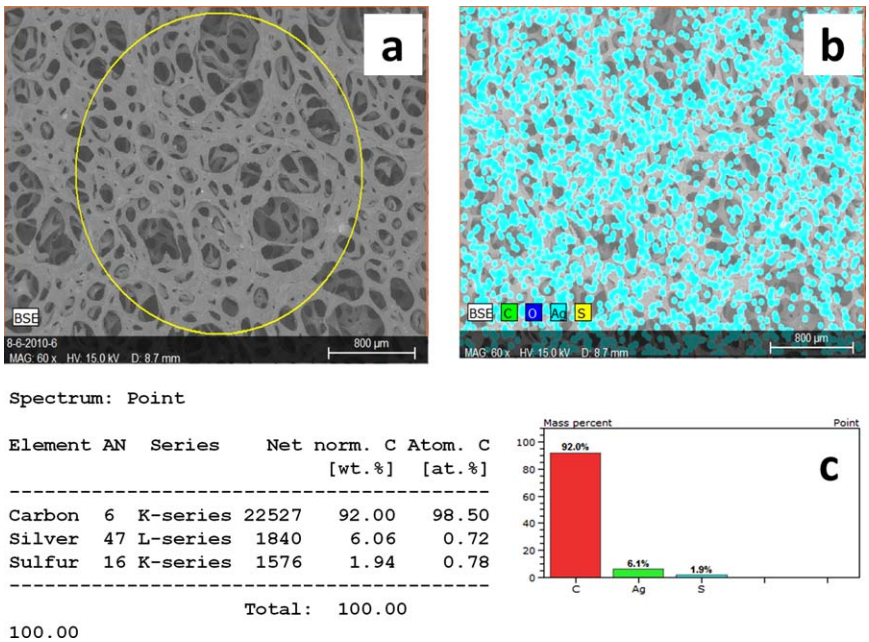

Figure 8. EDX mapping of overall area of modified natural rubber AgNP_NRLF showing EDX results magnification at ×60, (a) circular scanned area, (b) silver elemental mapping results, (c) mass percentages of the scanned area. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

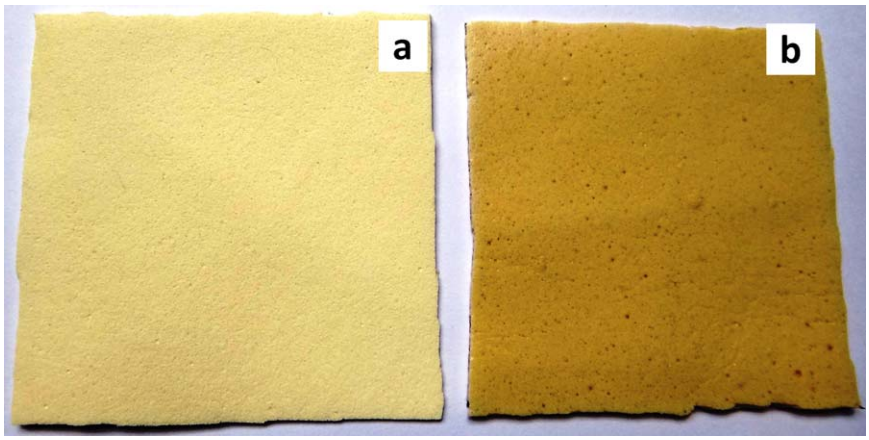

Figure 9. External appearance of the latex foam rubber samples (a) control sample of NRLF piece and (b) AgNPs incorporated NRLF piece. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

protect against antibacterial agents such as antibiotics, toxins, chemicals, and degradative enzymes. According to the bacterial killing mechanism explained by Thomas J., Silhavy, D. Kahne, et al.,[57] the gram-negative cell envelope consists of outer membrane, thin peptidoglycan layer, and cell membrane. Beside to this, gram-positive cell envelope consists of lipoteichoic acid containing thick peptidoglycan layer and cell membrane. Whereas gram-negative peptidoglycan is only a few nanometers thick, gram-positive peptidoglycan is 30 nm through 100 nm thick. However peptidoglycon alone is not sufficient to enable gram-positive bacteria to survive in their different environments due to the lack of their outer membrane. Accordingly the modified GAgNP-NRLF samples exhibited a larger inhibition zone of 10 mm for *S.aureus* (Figure 10 plate-1a) and *S.epidermidis* (Figure 10 plate-2a) compared with the inhibition zone of 9 mm for *E.coil* (Figure 10 plate-3a). The relatively larger inhibition zones (10 mm) observed for gram-positive *S.aureus* and *S.epidermidis* suggests that the thick peptidoglycan layer of gram-positive bacteria is not good enough to protect them from reactive oxygen species (ROS), which are formed by Ag-NPS.[58]

We have previously evaluated the antimicrobial activities of NRLF samples modified with AgNPs, which were synthesized using trisodium citrate as the reducing agent.[38,39] AgNP-incorporated NRFL samples using chemical reduction method produced an inhibition zone of 5 mm for gram-positive *S.aureus*. Our GAgNP-NRFL samples exhibit comparable or higher inhibition zones for the three bacteria species studied. We confirm that the AgNPs formed using the green synthesized method produced nanoparticles with antimicrobial activities comparable to those synthesized foam materials using chemical reduction methods.[38,39]

Determination of the Rate of Growth of Bacteria by Measuring the OD Values. OD of the culture samples were monitored over time to evaluate the bacterial inhibition quntitatively using modified GAgNP-NRLF samples. Figure 11 depicts the variation in the OD of modified GAgNP-NRLF and unmodified NRLF (control) samples against *S.aureus*, *S. epidermidis*, and *E. coli* bacteria species, respectively. The antibacterial activity of the modified GAgNP-NRLF samples was determined using shake flask method. Tryptic soya broth was used as the medium. According to Figure 11, a reduction in microbial population was evident with time after the bactera were incubated with the modified GAgNP-NRL samples. After 3 h, more than 95% of the OD of the bacteria culture media was reduced in the presence of the modified GAgNP-NRLF samples confirming strong antimicrobial acivities. Control NRLF samples did not

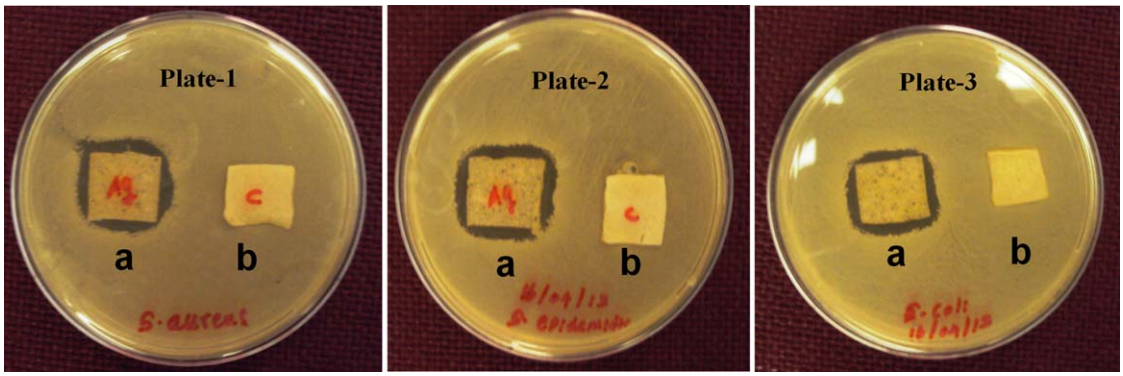

Figure 10. Qualitative testing of antibacterial properties of plate-1: (a) GAgNP_NRLF and (b) control NRLF against *S.aureus* bacteria; plate-2: (a) GAgNP_NRLF and (b) control NRLF, against *S.epidermidis*; and plate-3: (a) GAgNP_NRLF and (b) control NRLF against *E.coli*. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

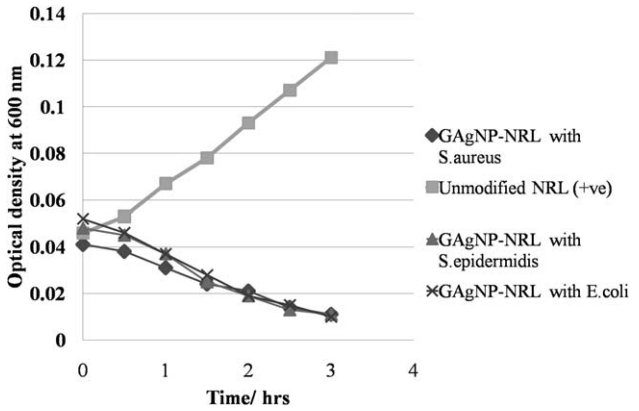

Figure 11. Variation in OD values of *S.aureus, S. epidermidis*, and *E. coli* bacteria cultures with time.

show any antimicrobial activity where the OD value was increased by 50% due to the increment in the bacterial populations. *In situ* production of GAgNP in NRLF would interact with the bacteria and destroy them in a strong manner. Perviously, we have evaluated the OD of chemically reduced AgNPs-incorperated NRLF samples using *S.aureus*.[38] In this research work, we have investigated the antimicrobial properties of the resultant foam rubber materials against two additional bacteria species and it was found that the resultant foam samples inhibited the bacterial growth in a strong manner. Chemically reduced nanoparticles exhibited antimicrobial activities in a similar assay platform used here in. Both chemically reduced AgNPs- (in our previous work) and GAgNP-NRLF samples (current work) showed a significant reduction in the OD after 2 h of incubation with bacteria. Therefore, the current green method produced silver nanoparticles incorperated NRLF materials with a very similar antimicrbial behavour to the nanoparticles produced using tri sodium citrate reduction method.

Antimicrobial activities of synthesized silver nanoaprticles using green routine using polysaccharides have been well-established in the literature.[59] These green synthesized procedures include an environmentally benign solvents such as water and polysaccharides such as glucose, galactose, and maltose, which serve as a capping ligand and as well as a reducing agent. The resulting nanoparticles exhibit antimicrobial activities against *S.aureus, E. coli*, and *S. epidermidis*. We believe our green approach method would provide a novel way to prepare AgNPs-incorperated NRLF samples for potential use in future clinical applications with impressive antimicrobial activities. Furthermore, incorparation of silver nanopartciles into the NRL via green approach will be a new technique that can be used to increase the service life of centrifuged NRL.

CONCLUSION

It can be concluded that the silver ions in silver nitrate can be reduced to metallic AgNPs by materials present in the serum of centrifuged NRL. The biological reducing agents inside the serum fraction of the centrifuged NRL which are responsible for this are yet to be investigated. The modified NRL can be used as the main raw material for the synthesis of NRLF by the Dunlop production method. The synthesized foam material also had nanosized silver particles inside the foam matrix. The resultant foam rubber samples strongly inhibited the growth of both gram-negative *E.coli* and gram-positive *S. aureus* and *S. epidermidis*. The quantitative analysis by OD measurements further confirmed the inhibition of bacterial performances by the treated foam rubber samples. The GAgNP-NRLF samples exhibited antimicrobial activities comparable to those prepared NRLF materials using trisodium citrate reduction method. Finally, it can be concluded that the novel green synthesized AgNPs incorporated NRLF samples can be used to make antibacterial foam materials.

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