Antibacterial ethylene propylene rubber impregnated with silver nanopowder: AgNP@EPR

M. Miranzadeh[a], M.Z. Kassaee[a,*], L. Sadeghi, M. Sadroddini[b], M. Razzaghi Kashani[b] and N. Khoramabadi[c]

[a]Department of Chemistry, Tarbiat Modares University, Tehran, Iran

[b]Department of Polymer Engineering, Faculty of Chemical Engineering, Tarbiat Modares University, Tehran, Iran

[c]Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

ABSTRACT: Following our interest in reaching for a molded rubber article with possible detergent contact applications, durable silver nanopowder (AgNP) is synthesized by arc discharge, then mixed with varying ratios of ethylene propylene rubber (EPR), affording novel AgNP@EPR nanocomposites. X-ray diffraction (XRD) patterns of AgNP as well as AgNP@EPR show no trace of impurity, while scanning electron microscopy (SEM) indicates an average diameter of 50 nm for the former. Transmission electron microscopy (TEM) images while confirm the SEM results, show quite a few 5 nm AgNP particles lying beside some micro crumbs. Our DC arc discharge technique involves explosion of movable silver anode and static cathode by a current pulse between 5 to 10 A cm^{-2}. A solution blending method is employed for preparation of AgNP@EPR nanocomposites. The AgNP is first dispersed in toluene using an ultrasonic homogenizer, and then thoroughly mixed with EPR in the same solvent whose removal gives nanocomposites of 2, 4, 6 and 8 vol% AgNP in EPR, showing strong antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus*.

INTRODUCTION

The emergence and increase in the number of multiple antibiotic-resistant microorganisms have caused scientists develop new effective antimicrobial agents that overcome such resistance. In particular, silver ions have long been known to exert strong inhibitory effects as well as to possess a broad spectrum of antimicrobial activities [1-6]. Hence nowadays, nanosilver is drawing increasing attention for potential prevention of bacterial/fungal and viral infections [1,2].

The effects of silver nanoparticles on bacterial cells are complicated [2]. Yet compared to organic antimicrobial agents, they are generally safer and more stable [7-10]. Silver nanoparticles are known to produce low concentrations of Ag$^+$ ions along with Ag atoms [11,12].

These, as well as other Ag-based compounds, have strong antimicrobial effects [13-16]. Evidently, they have a distinct advantage over conventional chemical antimicrobial agents. Ag$^+$ ions and Ag salts have been used for decades as antimicrobial agents in various fields because of their growth-inhibitory capacity against microorganisms. Also, many other researchers have tried to measure the activity of metal ions against microorganisms [17].

Gram positive *Staphylococcus aureus* and Gram negative *Escherichia coli* are widely used in bacterial experiments. They live on the body surface of mammals while sometimes cause infection [2]. Therefore *S. aureus* and *E. coli* strains are selected for this antibacterial study. The generation of stable and efficient AgNP offers an advanced perspective in the field of environmental hygiene and sterilization.

Nonetheless, fabrication and characterization of nanosilver has attracted considerable attention as a result of

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*Corresponding author. E-mail: kassaeem@modares.ac.ir

its significant applications in the fundamental sciences and nanotechnology [9]. Specifically, arc discharge technique is an economical and powerful method for preparation of nanoparticles [16]. Pulsed wire evaporation (PWE) technique, a physical process, allows for better production rate and particle size control with higher efficiency [18-21].

Associating metal nanoparticles to polymer matrix extend new applications of bio-films with antimicrobial effects [22,23]. The properties of nanocomposites can be modified by changing particle size, size distribution or shape of particles [9]. EPR is one of the most widely used and fastest growing synthetic rubbers having both specialty and general-purpose applications [24,25].

Following our interest in reaching for a molded rubber article with possible detergent contact applications, here we have utilized arc discharge to synthesize very durable AgNP, and used it in different quantities for formulation of novel nanocomposites involving EPR. Nanocomposites of AgNP@EPR are evaluated for their antibacterial activities against *E. coli* and *S. aureus.*

EXPERIMENTAL

Preparation of AgNP

Silver electrodes, with an 80° angle, are exposed to pulses of 5-10 A cm^{-2} in 10% glycerin/water. The resulting nanopowder is separated upon centrifuging and drying at 70 °C for 24 h.

Nanostructures are characterized using a Holland Philips Xpert X-ray powder diffractometer (CuK = 0.9, radiation, λ = 0.154056 nm), at a scanning speed of 2° min^{-1} from 20° to 80° (2θ). To estimate the average grain size and their quality with the (111) diffraction peak, Scherrer's equation is used [26]. In addition the particle shape and morphology are determined by SEM (KYKY EM3200-25 KV) and TEM (Zeiss EM10C 80 KV). Size distribution is obtained by Malvern Zetasizer ZEN3600.

Preparation of AgNP/EPR Nanocomposites

The desired amount of EPR is dissolved in enough toluene by stirring at 40 °C. At the same time, specific volume fraction of AgNP is dispersed in toluene under sonication. Then, the latter is poured into the EPR solution. The obtained mixture is stirred at 40 °C for 3 h and then at 120 °C for about 4 h to evaporate the solvent. The resulting composite is heated at 80 °C in a vacuum oven to remove traces of the solvent. Finally, disk-shaped samples of 15 mm diameter and 1.5 mm thick are compression-molded at 160 °C for 10 min, under a pressure of about 10 MPa. SEM images show the size and distribution of nanoparticles in the polymer matrix.

Antibacterial Assays

Antibacterial activity of AgNP@EPR composites with 2, 4, 6, 8 vol% AgNP are probed against *E. coli* (Gram negative) and *S. aureus* (Gram positive). Samples are cut with the same size (3 mm*5 mm) and placed in the test tube. After 15 min autoclaving at 121°C/15 lb, they are removed from autoclave and 100 µl bacterial suspension containing 6×10^7 is added to them in a laminar hood under aseptic conditions. Suspension of bacteria is prepared by culturing bacteria on a nutrient medium (such as BHI agar) in saline for 18 h. The tubes are kept for 16 to 20 h at 37 °C. After that 10 ml of sterile saline is added to each tube under aseptic conditions, and vortexed for 2 min until bacteria are completely transported in saline from the samples. Then, 1:10 dilutions of each tube are done using 2 ml of sterile saline in the micro tubes (100 µl sample and 900 µl normal saline). From each dilution 100 µl is cultured on the surface of Mueller Hinton agar medium. The plates are kept at 37 °C for 18 h. After incubation the number of colonies per samples is counted.

RESULTS AND DISCUSSION

Size, morphology, and durability of nanosilver highly depend on method of synthesis. Following our quest for stable nanomaterials [27-30], and our recent interest in reaching for a molded rubber article with possible detergent contact applications, here we take up fabrication of durable AgNP, through arc discharge in 10% glycerin/water. The AgNP is mixed with varying ratios of ethylene propylene rubber (EPR), affording novel antibacterial AgNP@EPR nanocomposites.

Fabrication of Durable AgNP through Arc Discharge
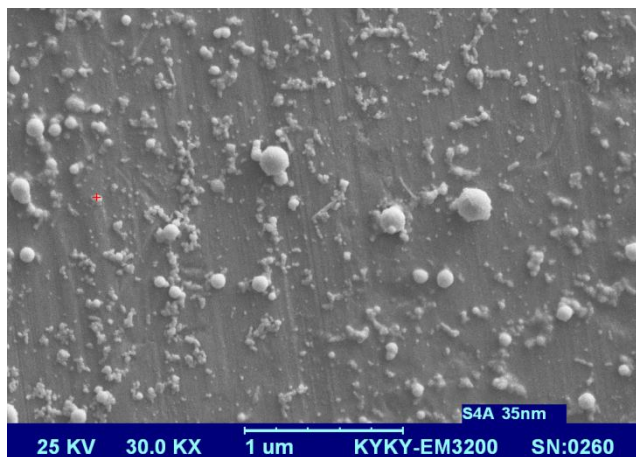
Our DC arc discharge technique involves explosion of

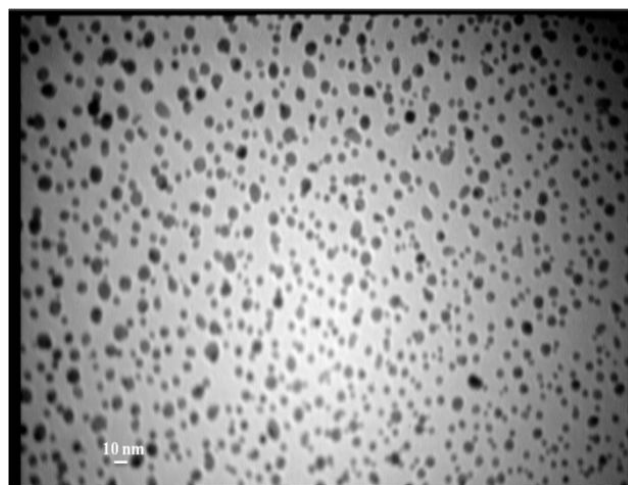**Fig. 1.** SEM image of arc fabricated silver nanopowder (AgNP).

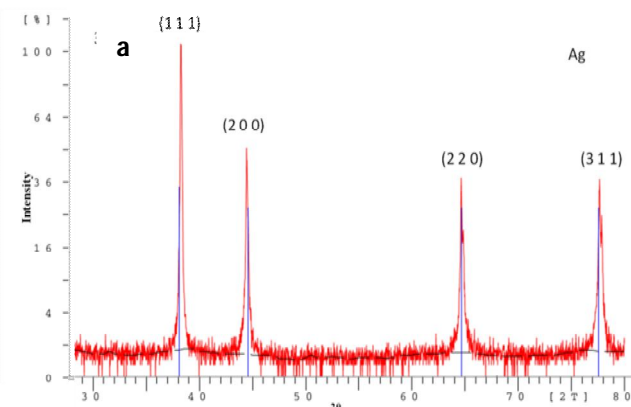**Fig. 3.** TEM image of arc fabricated silver nanopowder (AgNP).

Fig. 2. The XRD pattern of arc fabricated silver nanopowder (AgNP).

silver rods by a current pulse between 5 to 10 A cm^{-2}. Fairly pure silver electrodes (99%) with diameters of 2 mm and lengths of 40 mm are used as anode and cathode. Arc experiment is initiated by slowly detaching the moveable anode from the static cathode. In order to maintain a stable discharge current between 5 to 10 A cm^{-2}, the cathode-anode gap is controlled at approximately 1 mm. To sustain the arc inside the medium, the angle between the two electrodes is maximized to 80°. Gas bubbles are formed in

the aqueous media during the arc process, due to the plasma vaporization/decomposition of the anode material and boiling plus decomposition of the medium. The vaporized metal can be condensed more efficiently in the polar liquids than the gas phase [31]. These media pose rather low explosion risk. Again, they play a role in quenching and capping the atomized Ag vapor into dispersed solid nanostructures.

The SEM image of AgNP shows reasonable morphological uniformity and size distribution (Fig. 1). Due to capping potential of glycerin, AgNP turns out very pure (Fig. 2). Particle size for AgNP appears about 41.0 nm according to Debye-Scherrer equation, $D_{h,k,l} = k\lambda/\beta cos\theta$, where k is a constant (generally considered as 0.89), λ is the wavelength of Co Ka (1.78897 Å), β is the corrected diffraction line full-width at half-maximum (FWHM), and θ is Bragg's angle. Our TEM images show the presence of AgNP as small as 8 nm (Fig. 3). However for economic reasons, we deliberately employ the as-prepared unfiltered AgNP, which is naturally accompanied by agglomerations that give an excessive size distribution manifested by DLS through Zetasizer analyses (Fig. 4).

Preparation of AgNP@EPR Nanocomposites

The AgNP is first dispersed in toluene, using ultrasonic homogenizer for 30 min, and then thoroughly mixed with
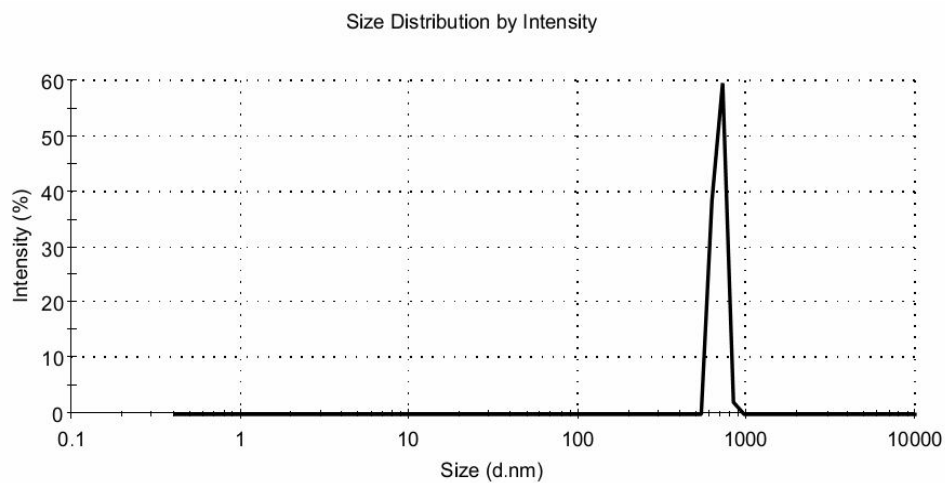
Fig. 4. Size distribution manifested by DLS through Zetasizer analysis where arc fabricated silver nanopowder (AgNP) show an intrinsically wider size distribution in water.
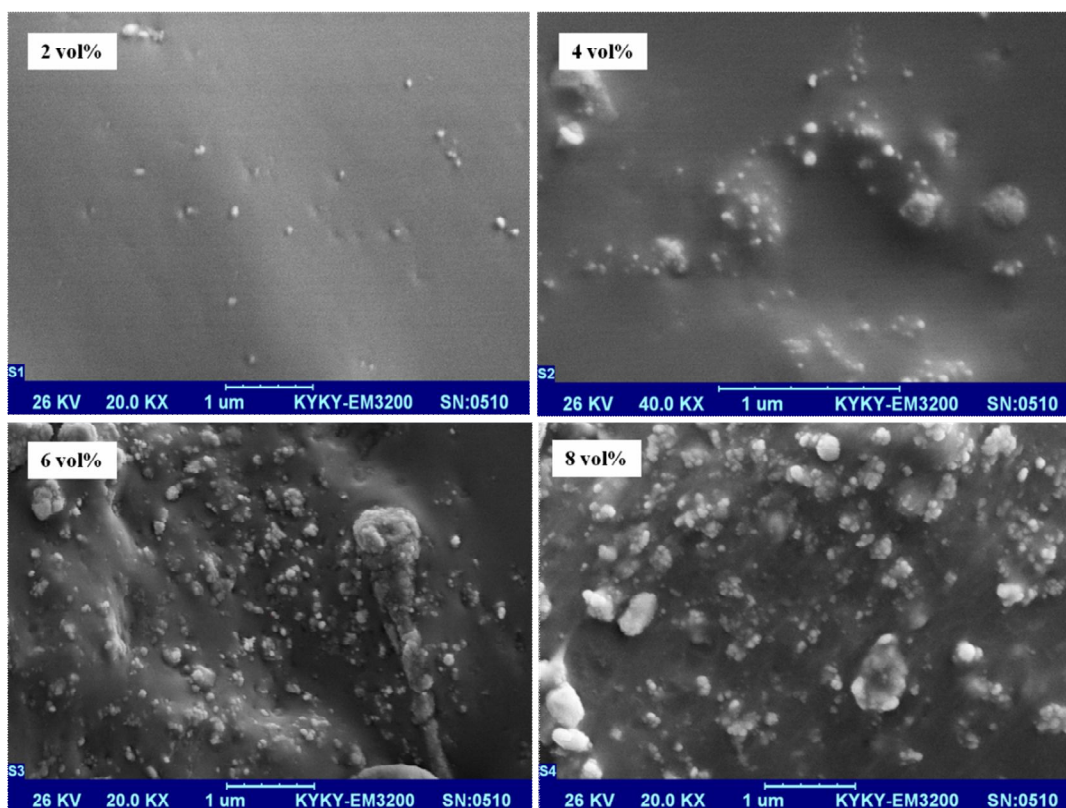

Fig. 5. The SEM images of AgNP@EPR composites with 2, 4, 6 and 8 vol% of AgNP.

EPR in the same solvent. Its evaporation under reduced pressure gives nanocomposites of 2, 4, 6 and 8 vol% AgNP in EPR. The SEM images of these nanocomposites show acceptable distribution of AgNP in EPR matrix along with some agglomeration due to differing polarities of AgNP and EPR (Fig. 5). The XRD pattern of AgNP@EPR shows peaks of silver and a broad band at $2\theta = 20$ attributed to EPR (Fig. 6).

Antibacterial Studies

Antibacterial tests of these nanocomposites show total eradication of both *E. coli* and *S. aureus* due to Ag^+ and $Ag°$ seemingly released by AgNP@EPR (Figs. 7-8) [11,12].

The mechanism of the inhibitory effects of Ag^+ ions on microorganisms is partially known. Some studies have reported that the positive charge on the Ag^+ ion is crucial for its antimicrobial activity through the electrostatic attractions between the negatively charged cell membrane of microorganisms and the positively charged nanoparticles [16,32-33]. In contrast, Sondi and Salopek-Sondi (2004) report that the antimicrobial activity of AgNPs on Gram-negative bacteria is dependent on the concentration of the Ag nanoparticles used, and is closely associated with the formation of pits in the cell wall of bacteria [34]. Following this, Ag nanoparticles are accumulated in the bacterial membrane and cause permeability, resulting in the cell death. Amro *et al*. suggested that metal depletion may cause the formation of irregularly shaped pits in the outer membrane and change membrane permeability, which is caused by the progressive release of lipopolysaccharide molecules and membrane proteins [35]. Also, Sondi and Salopek-Sondi speculate that a similar mechanism may cause the degradation of the membrane structure of *E. coli* during treatment with AgNPs [34]. Although their inference involves some sort of binding mechanism, the mechanism of the interaction between AgNPs and components of the outer membrane is still unclear. From another perspective, silver ions may cause the release of K^+ ions from bacteria; thus, the bacterial plasma or cytoplasmic membrane, which is associated with many important enzymes and DNA, is an important target site of silver ions [36-39]. When bacterial growth is inhibited, silver ions are deposited into the vacuole and cell walls as granules [40]. So, they may inhibit cell division and damage the cell envelope and cellular contents of the bacteria [41]. The sizes of the bacterial cells increase and the cytoplasmic membrane, cytoplasmic contents, and outer cell layers exhibit structural abnormalities. In addition, silver ions can interact with nucleic acids [40]. They preferentially interact with the bases in the DNA rather than with the phosphate groups, although the importance of this mechanism in terms of their lethal action remains unclear [2,43-45].
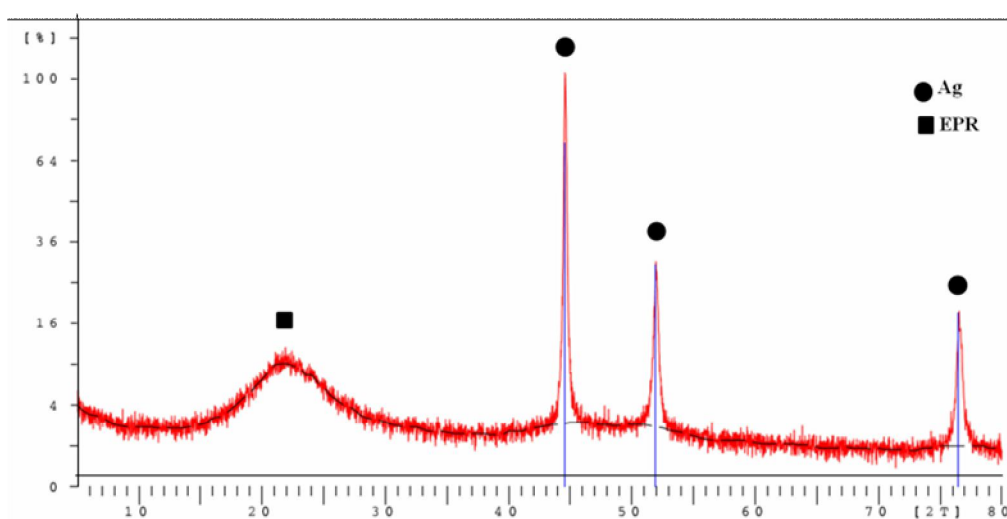

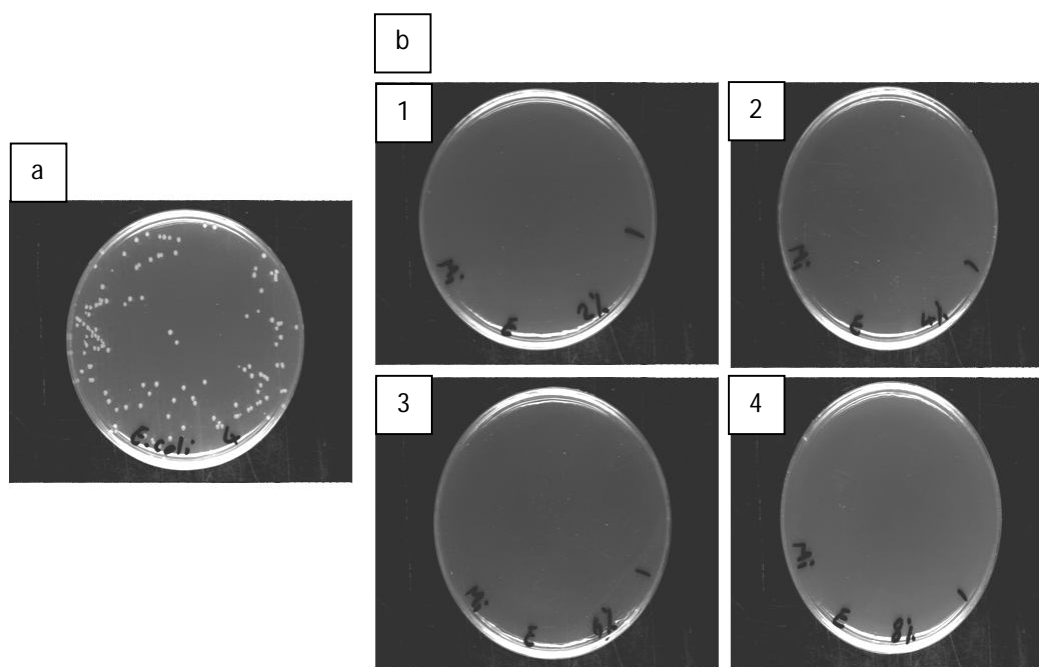**Fig. 6.** The XRD pattern of AgNP@EPR composites.

Fig. 7. Antibacterial activity of AgNP@EPR composites against *E. coli* (a) blank without composite (b) in present of composites (1) 2 vol% (2) 4 vol% (3) 6 vol% (4) 8 vol% AgNP.
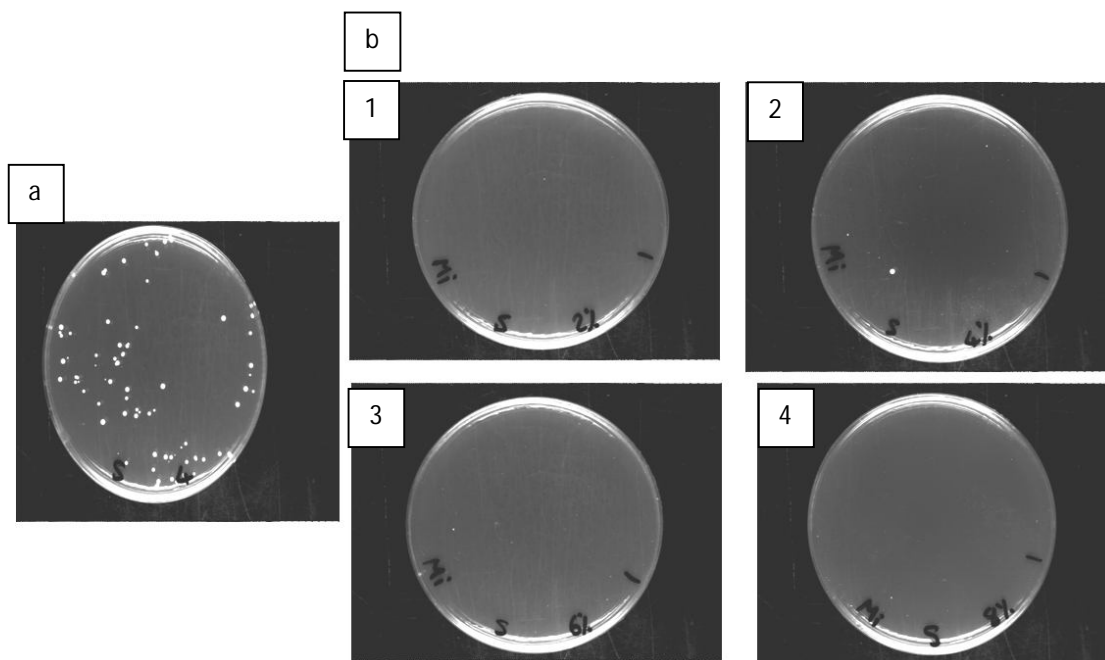

Fig. 8. Antibacterial activity of AgNP@EPR composites against *S. aureus* (a) blank without composite (b) in present of composites (1) 2 vol% (2) 4 vol% (3) 6 vol% (4) 8 vol% AgNP.

The possibility of free-radical involvement in the antibacterial activity of silver nanoparticles (Ag-NPs) has been previously reported [46], but the underlying mechanism and characteristics remain unclear. The interaction between reactive oxygen species (ROS) and bacterial cell death is revealed in a previous study [47]. Accordingly, bacterial DNA or mitochondria can be affected by ROS. Thus, for instance, some of them show good antibacterial and antiviral effects, producing ROS such as superoxide anion (O^{2-}), hydroxyl radical ($OH^{.}$) and singlet oxygen ($^1O^2$) with subsequent oxidative damage [2]. The results show that although AgNP blend with EPR, its antibacterial activity remains intact. Indeed, AgNP in EPR matrix can release both Ag nanoparticles and Ag^+ in aqua which play the role of antibacterial agents.

CONCLUSIONS

Durable silver nanopowder (AgNP) is synthesized through arc discharge, then curved in ethylene propylene rubber (EPR), resulting in novel AgNP@EPR nanocomposites, which show antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus*. Such an effect upgrades EPR and may expand its numerous applications in the green sanitization industry. Purity, size, and structural make-ups of AgNP as well AgNP@EPR are authenticated by XRD, SEM and TEM.

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