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Synthesis, morphological, characterization and evaluation of antibacterial effects of Silver-Polyaniline nanocomposites against *Escherichia coli*

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ABSTRACT

Silver-Polyaniline (Ag-PANI) nanocomposites were prepared by in-situ oxidative polymerization of aniline monomer in sodium bis(2-ethylhexyl) sulfosuccinate (AOT) solution as an emulsifier. The synthesis of Silver-Polyaniline nanocomposites was investigated as a function of several parameters such as aniline concentration, concentration of emulsifier (AOT), concentration of oxidation agent and concentration of silver nitrate. The formation of Ag-PANI nanocomposite was characterized by scanning electron microscopy (SEM) in each stage of controlled parameters. The particle size of PANI was increased from 43 nm to 56 nm with decrease in aniline concentration. Moreover the particle size of silver nanocomposites decreased with increasing oxidation agent concentration. In addition, the nanoparticle size was raised with decrease of AOT concentration. Also, when the concentration of silver nitrate was decreased, the particle size of nanocomposites was decreased too. In vitro antibacterial tests were performed using *Escherichia coli (E. coli)* to determine the antibacterial capability of the Ag-Polyaniline nanocomposites.

Keywords: *Nanocomposites; Polyaniline; Silver nanoparticles; Micro emulsion; Antibacterial properties.*

INTRODUCTION

Conducting polymer–metal nanocomposites provide exciting systems to investigate the possibility of designing device functionality [1] and exhibit enhanced sensing and catalytic capabilities compared to the pure conducting polymer [2]. In the synthesis of PANI–metal nanocomposites, metal ions are often reduced in the presence of preformed PANI and metal nanoparticles deposited on the surface of the polymer where the metal ions and PANI contact [3]. Polymerization of aniline in the presence of preformed metal nanoparticles also leads to the formation of PANI–metal nanocomposites [4].

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Although the simplest method for the synthesis of bulk PANI is chemical oxidative precipitation polymerization of aniline (ANI) using a chemical oxidant such as an ammonium persulfate (APS), the resulting PANI is highly aggregated and therefore unsatisfactory for most applications. Industrial demand has led to the development of several strategies to overcome such problems. The synthesis of PANI in colloidal form is one of the attractive alternatives to overcome its poor process ability due to its insolubility in common organic solvents and water [5]. In many studies on the synthesis of PANI–metal composites by chemical oxidative polymerization using metal salts, almost all of the composites were obtained in the form of aggregates. In terms of improving the process ability and performance of PANI–metal composites, it is crucial to synthesize Ag– PANI composites in the form of colloidal particles. Polyaniline (PANI) has been extensively studied because of its facile synthesis, environmental stability, electrical, electrochemical and optical properties, and has found applications in antistatic and anticorrosion coatings, biological and chemical sensors, electrodes for light-emitting diodes and batteries [6]. Polyaniline exists in three oxidation states, such as leucoemeraldine (reduced form), emeraldine (the stable intermediate form), and pernigraniline (oxidized form).It has been observed that emeraldine can reduce noble-metal compounds to corresponding metals. This fact has been illustrated especially for silver, gold, palladium, platinum, and rhodium. The process can be used for the noble-metal recovery, the deposition of noble metals in catalysts, and in preparation of nanostructure materials. The conducting composites are the obvious target. Silver is a metal of the choice in the last application due to its relatively low cost and easy availability. Silver and its compounds have been studied for many years not only for their antibacterial activity, but also for their low toxicity [7]. Silver is also the most conducting among metals, its conductivity being 6.3×10^5 S cm^{-1} at 20˚C [8]. Polyaniline has been used as a reductant for the generation of metallic silver from the corresponding silver salts [9]. The present study reports the polymerization of aniline in an AOT solution yielding PANI. In addition with reduction of Ag ions in surface of PANI are produced silver-polyaniline nanocomposites. We compared different concentrations of different parameters such as concentrations of aniline, emulsifier (AOT), oxidation agent ($FeCl_3$) and silver nitrate in synthesis of silver-polyaniline nanocomposite (Ag-PANI) and studied the effects of stirring method on the distribution and particle size in such systems. The study was stimulated by several reasons: (1) composite materials containing silver particles embedded in a matrix of conducting polymer may exhibit good electrical and thermal conductivities [10]. (2) Silver makes a good model for the deposition of more expensive noble metals, such as gold, platinum, palladium, and rhodium that are used in catalytic and electro catalytic applications. (3) The antibacterial activity of silver is well known and a similar ability has recently been reported for PANI [11]. The combination of these two materials might have synergetic effects worthy of consideration. Scanning Electron Microscopy (SEM) was used to characterize the Ag-PANI nanocomposites.

EXPERIMENTAL

Materials

Aniline (99%, monomer), sodium borohydride (NaBH4) (95%, Riedel-de Haen), AOT (96%, Acros Organics), and Silver nitrate crystal (99.8%, Merck) were purchased as analytical grade.

Synthesis of PANI in micro emulsion medium of sodium bis (2- ethylhexyl) sulfosuccinate (AOT)

Polyaniline were prepared as follows: aniline monomer solution (0.025 M) was added to 100 mL micro emulsion medium solution of AOT (0.01 M) under stirring. AOT is a micellar structure which may be considered as a set of nanoscale chemical ‘reactors’ formed by surfactant molecules. The aqueous solution of $FeCl_3$ (0.05 M) was slowly added to the above aniline and AOT solution under constant stirring and the polymerization reaction continued for 5 hr at room temperature. The formed dark-green precipitation was removed by filtration and washed repeatedly with ethanol and distilled water and was dried in oven for 12 h at 40˚C.

Incorporation of silver nanoparticles into PANI

In a typical synthesis process, 0.15 g as prepared PANI was added to the $AgNO_3$ solution (30 mL, 0.003 M), followed by addition of the aqueous solution of $NaBH_4$ (50 mL, 0.006 M). The mixture was settled in oven for 24 h until the Ag^+ ions had reduced to Ag nanoparticles. The product was centrifuged and then dried in oven for overnight. The SEM image of the Ag-Polyaniline nanocomposites with the particle size of 38 nm is shown in Figure 1.

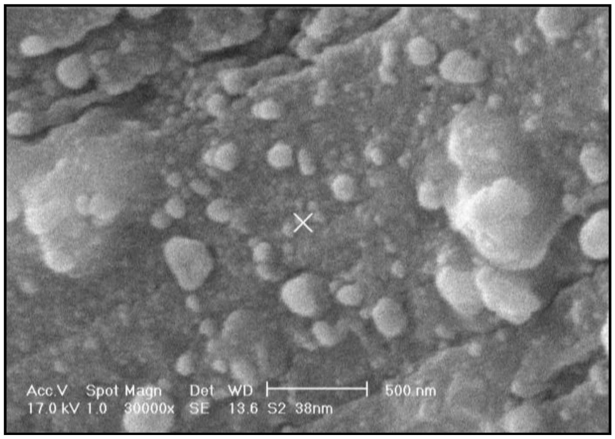

Fig. 1. Scanning Electron Microscopy(SEM) of Ag-PANI nanocomposite

Antibacterial assay

The antibacterial activities of Ag-Polyaniline nanocomposites against *E. coli* were carefully evaluated by turbidimetric method [19]. *E. coli* was grown in nutrient broth (0.5% peptone, 0.5% meat extract and 0.5% NaCl, and pH 7.5) incubated overnight at 37°C. The cultures obtained were diluted with autoclaved distilled water to obtain cell suspensions with optical absorbance at 620 nm of 0.4±0.02. One milliliter of the cell suspension was added to 50ml nutrient broth (NB) mixed with Ag-Polyaniline nanocomposites that had been autoclaved at 121°C for 20 min, then cultivated at 110 rpm and 37°C for 10 h. The number of bacteria was measured spectrophotometrically at 620nm (*A*620nm). Ag-Polyaniline nanocomposites with serial concentrations of each sample were added to NB before sterilization to investigate the antibacterial activity of Ag-Polyaniline nanocomposites with different surface structure.

RESULTS AND DISCUSSION

Effect of aniline concentration on the morphology of polyaniline

The influence of aniline concentration on morphology of polyaniline was investigated. The effect of aniline concentration on the morphology of PANI was studied by carrying out a series of experiments at different concentrations ranging from 0.0125 M to 0.05 M. The particle size of PANI was increased from 43 nm to 56 nm with decrease in aniline concentration. It would be illustrated that increasing of aniline monomers is affected on particle size of PANI. The result is shown in Figure 2.

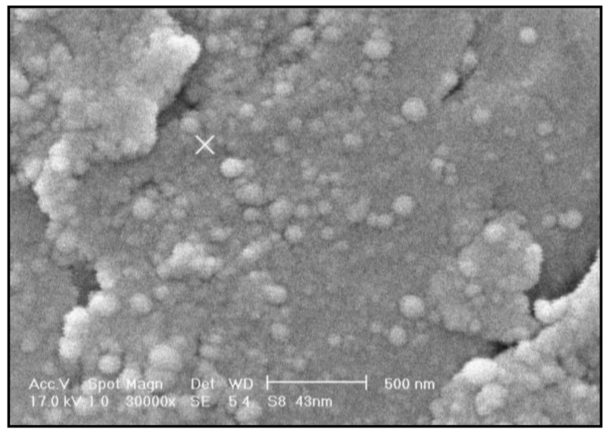

Fig. 2. Scanning Electron Microscopy (SEM) of Ag-PANI nanocomposite in concentration of 0.0125 M aniline

Effect of concentration of oxidation agent in PANI morphology

Effect of $FeCl_3$ concentration was studied at different concentrations in the range of 0.025 to 0.1 M. The particle size of silver nanocomposites decreased with increasing oxidation agent concentration. The lowest particle size of silver nanocomposites 18 nm was obtained with the concentration of 0.1 M of $FeCl_3$. This can be attributed to the fact that higher $FeCl_3$ concentrations (0.1 M) provide the higher oxidation reaction rate for synthesis of PANI to improved morphology of PANI. In addition, it should be mentioned that when the concentration of $FeCl_3$ is decreased to 0.025 M the synthesis of polyaniline is not occurred. Figure 3 is shown the results of the effect of $FeCl_3$ concentration in the morphology of PANI.

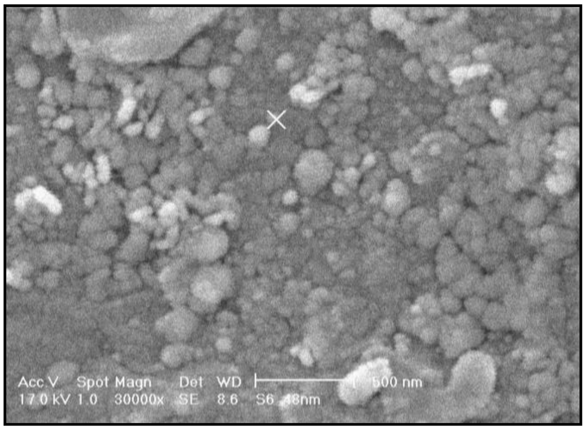

Fig. 3. Scanning Electron Microscopy (SEM) of Ag-PANI nanocomposite in concentration of 0.1 M $FeCl_3$

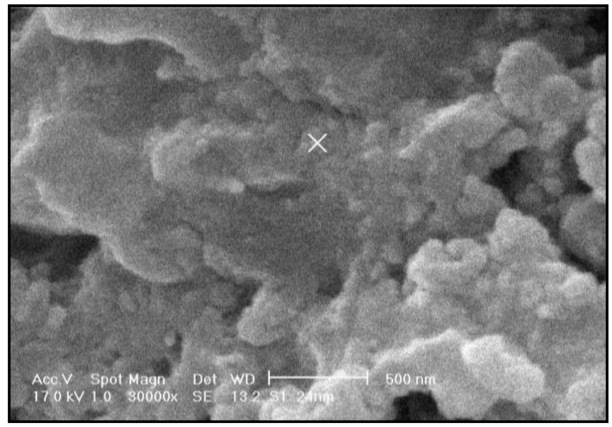

Fig. 4. Scanning Electron Microscopy (SEM) of Ag-PANI nanocomposite in concentration of 0.001 M silver nitrate

Effect of concentration of emulsifier in nanoparticle size

The results of experiments to determine the effects of emulsifier concentration on nanoparticle size is shown in Figure3 and reveal that the size of the silver nanocomposite increases with decrease in concentration of sodium bis(2-ethylhexyl) sulfosuccinate (AOT). It rose from 38 nm to 43 nm with the decrease of AOT concentration ranging from 0.01 M to 0.005 M. This suggests that there is a decrease in formation of microemultion to stabilize of nanoparticles during the decrease in AOT concentration. Increasing AOT concentrations above 0.2 M causes solutions to agglomerate.

Effect of concentration of Silver nitrate in nanoparticle size

The concentration of the silver nitrate is an important controlling parameter in the particle size of silver nanocomposites. Figure 4 represents the effect of silver nitrate concentration on the synthesis of silver nanocomposites. The lowest particle size of nanocomposites 24 nm was obtained with the concentration of 0.001 M of silver nitrate. This suggests that Increases in silver ions concentration is caused to agglomerate; therefore the nanoparticle size is increased with association of particles, as a result when the concentration of silver nitrate decrease, the particle size of nanocomposites decrease.

Antibacterial Test

Antibacterial properties of metallic silver and its compounds are known for scientists. In small concentrations, silver is safe for human cells but lethal for the majority of bacteria and viruses and hence widely used in disinfection of water and food in everyday life and in the infection control in medicine [12].Bactericidal properties of metallic silver are associated with its slow oxidation and releases of Ag^+ ions to the environment; hence, it seems promising to use nanosilver drugs as an especial class of biocidal agents.

The mechanism of nanoparticle penetration into a cell is not yet entirely clear. Strong changes in the membrane structure of the bacterium E. coli, an increase in its permeability and its ultimate death upon the interaction with nanoparticles were reported. Observations of the membrane-bound and intracellular nanoparticles are important for understanding the bactericidal action of nanosilver. A bacterium wall contains a large amount of sulfur and phosphorus containing molecules that interact with nanoparticle and lose their activity. Inside a bacterium, nanoparticle can interact with DNA; the latter thus loses its ability to replication, which may lead to the cell death [13].

As supplementary data, the antibacterial property of the silver nanocomposite was exhibited. *Escherichia coli (E.coli)* were used for this test as the common microorganisms involved in hospital-acquired infections. As the result suggested, Ag-Polyaniline nanocomposites showed strong antibacterial properties. These results show that the Ag-Polyaniline nanocomposites are more effective in killing the bacterium in contrast with pure Ag and polyaniline, which this is for the synergic effect of PANI with silver nanoparticles. This effect also can be through improving the interfacial contacting inhibitory effect which is occurred on the surface of the microspheres. The killing of the microbial organism might be the result of cytoplasmic membrane disruption or permeability. Figure 5 is shown the result of antibacterial test.

Fig. 5. Antibacterial test image of Ag-polyaniline nanocomposites.

CONCLUSIONS

The aim of present study was the synthesis of Ag-Polyaniline nanocomposite in the sodium bis(2-ethylhexyl) sulfosuccinate (AOT) solution as an emulsifier. Several parameters were investigated in the synthesis of silver-polyaniline nanocomposites. In the investigation of effect of aniline concentration on the morphology of polyaniline it was obvious that increasing of aniline monomers was affected on particle size of PANI. The particle size of PANI was increased from 43 nm to 56 nm with decrease in aniline concentration. Moreover the particle size of silver nanocomposites decreased with increasing oxidation agent concentration. In addition, the nanoparticle size was raised with decrease of AOT concentration. Also, when the concentration of silver nitrate was decreased, the particle size of nanocomposites was decreased too. For the antibacterial test of silver-Polyaniline nanocomposites, *Escherichia coli (E.coli)* were used and as the results, Ag-Polyaniline nanocomposites showed strong antibacterial properties.

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