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Antibacterial silver nanoparticles in polyvinyl alcohol/sodium alginate blend produced by gamma irradiation

Naeimeh Eghbalifam [a], Masoud Frounchi [a,*], Susan Dadbin [b]

[a] Sharif University of Technology, Department of Chemical and Petroleum Engineering, Tehran, Iran
[b] Radiation Applications Research School, Nuclear Science and Technology Research Institute, North Kargar Street, Tehran, Iran

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ABSTRACT

Polyvinyl alcohol/sodium alginate/nano silver (PVA/SA/Ag) composite films were made by solution casting method. Gamma irradiation was used to synthesize silver nanoparticles in situ via reduction of silver nitrate without using harmful chemical agents for biomedical applications. UV–vis and XRD results demonstrated that spherical silver nanoparticles were produced even at low irradiation dose of 5 kGy. By increasing irradiation dose, more nanoparticles were synthesized while no PVA hydrogel was formed up to 15 kGy. Also the size of nanoparticles was reduced with increasing gamma dose evidenced by higher release rate of silver nanoparticles in lukewarm water and SEM images. Comparing SEM images with DLS results indicated good performance of PVA/SA as an efficient stabilizer in preventing agglomeration of the silver nanoparticles. Good miscibility of polyvinyl alcohol and sodium alginate observed on the SEM images was supported with FTIR spectroscopy. Upon addition of sodium alginate to polyvinyl alcohol and increasing silver nanoparticles, the melting peak shifted to lower temperature and crystallinity percent was decreased. Addition of sodium alginate led to remarkable increase in rigidity of PVA. The composites exhibited strong antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* even at very low level of silver nanoparticles.

1. Introduction

Silver-filled polymeric materials have the potential to provide antimicrobial devices such as catheters, wound dressings, heart valve sewing cuffs and bone cement. Indeed elemental silver particles in contact with water and dissolved oxygen can release small amounts of silver ions which exhibit high antimicrobial efficacy in combination with a fairly low toxicity against human tissue. Polymers containing elemental silver nanoparticles release silver ions much more effectively than materials filled with silver microparticles [1–5]. Various methods have been used for synthesis of silver nanoparticles. Among these methods gamma irradiation possesses important advantages over chemical methods in terms of no need for use of harmful reducing agents or other chemical and also easy control on synthesis process. Indeed upon irradiation hydrated electrons are generated through water radiolysis acting as strong reducing agents [6]. However, the silver nanoparticles agglomerate rapidly in aqueous solutions [7] and they should be stabilized by polymers. Polyvinyl alcohol (PVA) is a semi-crystalline biocompatible polymer with high transparency, good flexibility, highly hydrophilic and soluble in hot water with high chemical stability [8]. It is used in biomedical applications such as artificial blood vessels, artificial intestines, contact lenses and drug-delivery system due to its biocompatibility with body organs [9]. Blending of natural polymers with PVA is also of interest for achieving desirable physical properties along with biocompatibility [10–12]. Sodium alginate is a natural polymer possessing unique biological, chemical and physical properties that has application in wound dressing [13] and forms miscible blend with PVA [14]. Incorporation of antibacterial properties into biocompatible polymers is desirable for many applications where silver nanoparticles stand out for their strong antibacterial activities [15–17].

In this work, we have prepared polyvinyl alcohol/sodium alginate/nano silver (PVA/SA/Ag) composite films by in situ gamma irradiation and investigated the effect of various doses of gamma irradiation on the size and concentration of in situ synthesized silver nanoparticles within the blend of sodium alginate and polyvinyl alcohol with emphasis on the antibacterial properties.

* Corresponding author. Tel.: +98 2166165422.
E-mail address: frounchi@sharif.edu (M. Frounchi).

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2. Experimental

2.1. Materials

Polyvinyl alcohol (M_w = 72,000 g/mol) and sodium alginate were purchased from Fluka and Sigma–Aldrich, respectively. Silver nitrate, $AgNO_3$ (99.9%) and isopropanol were obtained from Merck Co. All chemicals were of analytical grade and used without purification. All aqueous solutions were made using deionized water.

2.2. Sample preparation

1.125 g PVA was dissolved in 70 ml deionized water at 90 °C with magnetic stirring until clear solution was obtained. Silver nitrate solutions at three different concentrations of 5.24×10^{-3}, 1.04×10^{-2} and 1.57×10^{-2} mol/lit of $AgNO_3$ were prepared. Then 10 ml of the prepared silver nitrate solution and 10 ml isopropanol, as hydroxyl radical scavenger [7], were added to the prepared PVA solution with continuous stirring for about 1 h to ensure homogeneity of the solution. As the silver nitrate is light sensitive, it was kept inside an opaque container. The mixture was purged by N_2 to remove the oxygen, sealed and irradiated at different doses of 5, 10 and 15 kGy using ^{60}CO γ-rays at a dose rate of 3.19 Gy/s at room temperature. Then 0.125 g sodium alginate dissolved in 10 ml deionized water and was added to the gamma irradiated PVA/Ag solutions so that the concentration of sodium alginate in the blend of PVA/sodium alginate was 10 wt.%. At higher concentrations the PVA/SA blends were less flexible and tending to be brittle as it will be shown later in the mechanical properties. It should be noted that sodium alginate was added after irradiation as it is susceptible to degradation upon exposing to gamma irradiation. The calculated concentration of Ag^+ ion in PVA/SA blends was 0.45, 0.89 and 1.33 wt.%. The composite with 0.45 wt.% Ag^+ and irradiated at 15 kGy was denoted as PVA/SA/0.45Ag^+/15kGy. The amount of Ag^+ ion converted to Ag depends on the dose of irradiation and initial Ag^+ ion concentration. Then the prepared solution of PVA/SA/Ag was cast on a petri dish and dried in a vacuum oven at 50 °C through evaporation of the solvent. The formed films were stripped off the petri dish. After gamma irradiation, the color of PVA/Ag solution was light brown to dark gray depending on the concentration of Ag and the given gamma dose. The change of color of the primary clear solution of PVA and silver nitrate was indicative of formation of silver nanoparticles that was confirmed by UV–Visible Spectrophotometer. It was interesting to find that no PVA hydrogel formed upon irradiation even at 15 kGy. This was while neat PVA solution turned into hydrogel after gamma irradiated even at a low dose of 5 kGy manifesting the fact that silver nitrate solution absorbed gamma radiation faster than PVA inhibiting cross-linking of PVA at the dose levels that were applied.

2.3. Characterization

2.3.1. UV–vis spectrophotometry

In order to confirm the formation of Ag nanoparticles, the UV–vis absorption spectra were measured in the wavelength range of 200–1100 nm using Perkin-Elmer Spectrophotometer.

2.3.2. X-ray analysis

X-ray diffraction (XRD) scans were obtained using STOE, D-64295 diffractometer using Cu K_α radiation (where λ = 1.54 Å and the Bragg's angel, 2θ, in the range of 5–85°).

2.3.3. Dynamic light scattering

Particle diameter and the particle size distribution of silver nanoparticles in aqueous solution were determined by dynamic light scattering (DLS) using Zetasizer Nano series of Malvern Instruments.

2.3.4. Scanning electron microscopy

Scanning electron microscopy, SEM, was done by Hitachi, F4160 in order to study morphology, shape and size of silver nanoparticles.

2.3.5. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was used to study thermal behavior of the composites by a Perkin-Elmer Pyris 1 DSC. It was done at a heating rate of 10 °C/min under N_2 gas blanket. Crystallinity percent is calculated by the following relation:

$$X_c(\%) = \frac{\Delta H_m/\phi}{\Delta H_m^*} \times 100$$

where ΔH_m^* is the heat of fusion for the 100% crystalline polymer, which is $\Delta H_m^* = 152$ J/g for PVA and ϕ is the weight fraction of PVA in the composite.

2.3.6. Tensile measurements

The mechanical properties (tensile modulus, strength and strain at break) of the samples were measured at crosshead speed of 50 mm per min using a universal tensile machine. The samples had initial length of 40 mm, width of 10 mm and thickness of 100–150 μm.

2.3.7. Silver release measurements

A piece of film (~50 mg) was placed in a flask containing 10 ml of water at 37 °C and then the flask was oscillated at a frequency of 120 rpm in a rotary shaker. Then the amounts of released silver nanoparticles from films were determined by measuring optical density (O.D.) of the solutions at the wavelength of maximum absorption, λ_{max}, at different lengths of time.

2.3.8. Antibacterial properties

The antibacterial properties of composite films against *Staphylococcus aureus* (PTCC 1113) and *Escherichia coli* (ATCC 11105) were studied by using the agar diffusion test. A piece of sample (1.2 cm × 1.2 cm) was cut and placed on the surface of a nutrient agar plate, and then bacterial solutions of 1.2×10^7 CFU *S. aureus* per ml and 3.9×10^7 CFU *E. coli* per ml were spread uniformly. After 24 h incubation at 37 °C the inhibition zones around the samples (the radius of a circle inscribed in the square inhibition zone) was measured in five directions and the average was determined.

3. Results and discussion

3.1. UV–vis spectra analysis

The UV–vis absorption spectra of PVA/SA/Ag composites are shown in Figs. 1 and 2 at different concentrations of silver ion and irradiation doses, respectively. As shown in Fig. 1 there are no peaks of absorption on the absorption spectrum of the PVA/SA transparent film over the wavelength range of 300–1000 nm; however, a new peak appeared at λ_{max} = 420–460 nm in absorption spectra of the gamma irradiated silver nitrate containing samples whose intensity increased with increase in silver nitrate concentration. Upon exposing PVA/silver nitrate solution to gamma irradiation silver nanoparticles were produced. As shown in Fig. 2, at a fixed concentration of Ag^+ ion of 1.33 wt.%, the height of the peak at λ_{max} increased with increase in gamma irradiation dose from 5 to 15 kGy indicating increase in concentration of produced silver nanoparticles with irradiation dose.