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Antibacterial Properties of Silver-polycarbonate Nanocomposite Synthesized by Gamma Radiation-assisted Diffusion Method

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ABSTRACT

Radiolytic preparations of silver-polycarbonate (Ag-PC) composites have been characterized for their structural and antibacterial properties. Rutherford backscattering, X-ray diffractogram, and transmission electron microscopy measurements verified the presence of face-centered cubic structured Ag nanoparticles. The microstructural changes of pristine PC and Ag doped PC were monitored using positron annihilation lifetime spectroscopy (PALS). Experiments indicated a decrease in free volume hole size and fractional free volume of Ag-PC composites. PALS results were consistent with Doppler broadening spectroscopy measurements. The antibacterial activity of the Ag-PC composites was evaluated by agar diffusion method against Gram-negative bacteria and Gram-positive bacteria and showed the prepared composite's ability to inhibit bacterial growth.

Key words: *Polymer composite, Antibacterial property, Radiolytic synthesis.*

1. INTRODUCTION

The antimicrobial properties of silver, known for centuries, can be attributed to its ability to interrupt the bacteria cell's capability to form the chemical bonds essential to its survival [1]. Bandages with silver ions prevent bacterial growth and speed healing time. Similarly, the inclusion of silver into polymers in food packaging may reduce, inhibit, or retard the growth of microorganisms that may be present in packed food or the packaging material.

The present work focuses on structural and antibacterial properties of silver-polycarbonate (Ag-PC) nanocomposite synthesized by a gamma radiation-assisted diffusion method, which is simple, clean, and efficient [2]. The structural properties of Ag-PC nanocomposites have been characterized using Rutherford backscattering (RBS) technique, X-ray diffractogram (XRD), transmission electron microscopy (TEM), and by positron annihilation lifetime spectroscopy (PALS).

2. EXPERIMENT

2.1. Preparation of Ag-PC Matrix

PC film of thickness 200 μm (Lexan, $C_{16}H_{14}O_3$) was immersed in a glass bottle containing 10 ml of 1 mM $AgNO_3$ solution and exposed to ^{60}Co gamma radiation chamber with a dose rate of 5.4 kGy/h. The sample was then removed from the bottle, washed with double distilled water, dried and used for further characterization [2].

2.2. RBS Technique

RBS technique was employed to study the diffusion of Ag into PC using helium ions of energy 2 MeV.

2.3. XRD

XRDs were recorded in the 2θ range (10-80°) using a Bruker AXS D8 Advance X-ray diffractometer with CuKα radiation at a wavelength of 1.5406 Å.

2.4. TEM

TEM images were acquired using of model Tecnai G^2 U-thin 200 kV model with LaB_6 filament.

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2.5. PALS

PALS measurements were performed at a room temperature using a fast-fast coincidence system with BaF_2 scintillators coupled to XP2020Q photomultiplier tubes and a system time resolution of 220 ps. A 30 μCi ^{22}Na positron source, deposited on Kapton foil, is sandwiched between two identical pieces of the sample, 1 mm thick. About 10^6 counts were accumulated for each lifetime spectrum and analyzed using the PALSfit program [3].

2.6. Doppler Broadening Spectroscopy (DBS)

The DBS were performed at room temperature with a high purity germanium detector, with an energy resolution of 1.1 keV at 662 keV γ-line of ^{137}Cs radioactive source. Around 10^6-10^7 counts were collected under each Doppler broadened 511 keV γ ray spectrum. The DB spectra were analyzed using computer program SP-I to evaluate the line shape parameters S and W [4].

2.7. Antibacterial Properties of Ag-PC Samples

The antibacterial activity of Ag-PC composites against Gram-negative bacteria, *Escherichia coli* and Gram-positive bacteria, *Staphylococcus aureus* were examined applying the "agar well" diffusion method. The bacterial cultures of known inoculums size (0.2 ml, 105 CFU/ml) of test microorganisms were spread on nutrient agar plates. A well of 5 mm diameter was made in the plates sample (dissolved in dichloromethane) of 2 mg concentration to each well, and the plates were further incubated for 18-24 h at 37°C. The antibacterial activity was evaluated by measuring the zone of inhibition [2].

3. RESULTS AND DISCUSSION

3.1. RBS Results

Figure 1 shows RBS spectrum for pristine PC and Ag-PC sample prepared at 600 kGy. Depth profiles of the incorporated Ag atoms were determined by a simple channel-by-channel method taking into account the energy dependence of the alpha particle stopping power, calculated for pristine polymer using the database from the SRIM code [5]. As can be seen from Figure 1, the curve 2 showed a peak edge around ~1725 which corresponds to Ag. The appearance of this peak edge confirms the diffusion of Ag in PC and the depth of diffusion of Ag in PC found to be 1.02 μm.

3.2. XRD

The AgNPs present in PC is also confirmed by XRD as shown in Figure 2. After irradiation, a peak appears at 37.45° corresponding to (111) crystal planes of Ag with a face-centered cubic (FCC) crystal structure. The d-spacing corresponding to this angle is found to be 2.405 Å. The intensity of this peak increases with increase in gamma dose which may be due to diffusion of more Ag in PC.

3.3. TEM

TEM image showed Figure 3 the interplanar spacing as 2.41 Å, which is in agreement with XRD results and corresponds to (111) crystal planes of Ag. The average particle sizes of AgNPs are found to be ~10 nm as studied from TEM analysis. These all results confirm the diffusion of AgNPs in PC.

3.4. Positron Annihilation Spectroscopy (PAS)

The positron lifetime spectra are resolved into three-lifetime components τ_1, τ_2, and τ_3 using PALSfit program with variances χ^2 of fits ≈ 1 and with respective intensities I_1, I_2, and I_3. The long life

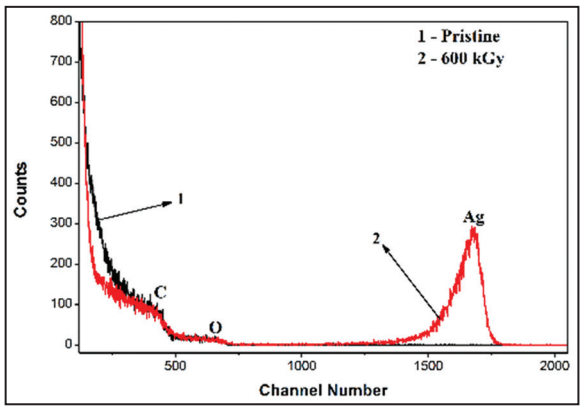

Figure 1: Rutherford backscattering spectrum for (1) pristine polycarbonate (PC) and (2) silver-PC matrix.

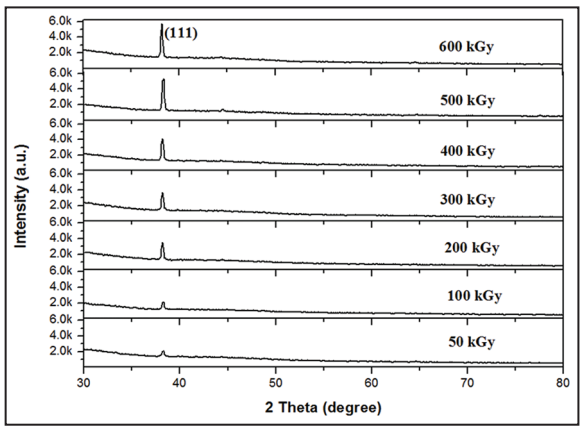

Figure 2: X-ray diffractogram of silver-polycarbonate matrix.

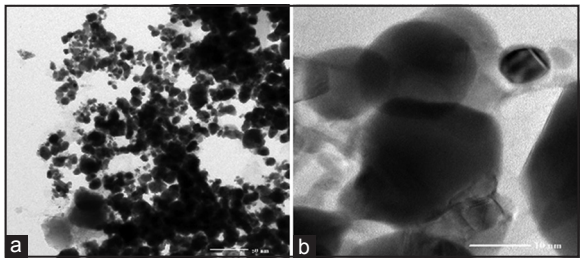

Figure 3: Transmission electron microscopy images of silver-polycarbonate matrix prepared at 600 kGy.

component (τ_3) of o-Ps responds sensitively to the size and the distribution of the free volumes as Ps atoms are preferentially trapped in holes of atomic dimensions.

Relation connecting the o-Ps lifetime (τ_3) and free volume hole radius (R) is given by [6]:

$$\frac{1}{\lambda_3} = \tau_3 = 0.5\left[1 - \frac{R}{R+\Delta R} + \frac{1}{2\pi}\sin\left(\frac{2\pi R}{R+\Delta R}\right)\right]^{-1} \text{ ns}$$

Where, R is a free volume hole radius and ΔR = 1.656 Å [6]. The average free volume hole size V_f is then evaluated as $V_f = (4/3)\pi R^3$, and the fractional free volume or the free volume content is given by $F_V = CI_3V_f$; where C = 0.0018 $Å^{-3}$.

The diffusion of Ag nanoparticles influences the free volume of the polymer. Further diffusion of dopants in polymers is controlled by the free volume and its content. Hence, the o-Ps lifetime τ_3 and its intensity I_3 are the most suitable parameters in understanding the free volume modifications due to diffusion of Ag nanoparticles into PC.

When the PC samples were irradiated in silver solution, the τ_3, I_3 (Table 1), and F_V (Figure 4) values decreased gradually. The decrease in values of τ_3, I_3, and F_V for this set of samples can be attributed to:

i. Cross linking of the polymer chains resulting in close packing of polymer chains thereby decreasing the average free volume size and number density of free volume sites.
ii. Diffusion of Ag nanoparticles into the free volume holes.

3.5. DBS

The ratio of the area of the central part of the DB spectrum (low momentum) to the total area under the DB spectrum gives the S-parameter. The W-parameter is taken in the high momentum regions far from the center. It is the ratio of the area under the wings in a fixed window divided by the total area [4].

The shape of the DB spectrum is determined by the summed momentum distribution of electrons with which the positron interacts, i.e., valence or core and the type of atom donating the electron. If the annihilation occurs in a large open volume site such as a defect in a crystalline material or free volume in amorphous material (polymer), the fraction of low momentum conduction and valence electrons (represented by the S-parameter) participating in the process increases relative to the fraction of high momentum core electrons (represented by the W-parameter).

Thus, a defect-rich/higher free volume material will have a narrower electron momentum distribution than would a defect-free material.

As can be seen from the Figure 5, the line shape parameter S decreases, whereas W increases with increasing dose. As the diffusion of Ag particles into PC matrix increases, the overall crystalline region of the sample increases (due to increase in crystalline Ag particles). Thus, with an increase in dosage the crystallinity of the sample increases, which is supported by the decrease in I_3 values and the RBS and XRD results which show an increase in the diffusion of Ag particles into the polymer matrix as the irradiation dose increased.

3.6. Antibacterial Properties

There were no antibacterial effects or zone of inhibition observed for pristine PC, but the Ag-PC sample shows the zone of inhibition pattern for both kind of bacteria. The zones of inhibition by the prepared Ag-PC sample are given in Table 2, and they show moderate antibacterial activity against both Gram-negative and Gram-positive bacteria.

The zone of inhibition by Ag-PC sample may be due to two reasons. (i) Ag nanoparticles produce reactive oxygen species (ROS) in the vicinity of the bacterial cell membrane leading to the cell permeability and thereby cell death [7] and (ii) the metal ions released from the surface of metallic nanostructures may interact with the DNA and cellular enzymes. This will be done by coordinating to electron donating groups of microbes. The damage to the cell membrane results in the disruption of respiratory chain reactions. In such a way metal ion - DNA interaction inhibits bacterial cell and finally kills the cells [8]. Furthermore, it can be

Table 1: PALS results of Ag-PC samples.

Dose (kGy)	τ_3±0.01 (in ns)	I_3±0.23 (in %)	V_f±0.8 (in $Å^3$)
0	2.128	32.14	109.8
200	2.069	30.71	104.17
400	2.025	29.55	99.95
600	2.038	28.31	101.21

PALS=Positron annihilation lifetime spectroscopy, Ag-PC=Silver-polycarbonate

Table 2: Antibacterial property of Ag-PC matrix.

Dose (kGy)	Zone of inhibition (mm)	
	Staphylococcus aureus	*Escherichia coli*
200	1.8±0.46	1.3±0.22
400	2.3±0.58	1.8±0.31
600	3.2±0.48	2.4±0.15

Ag-PC=Silver-polycarbonate

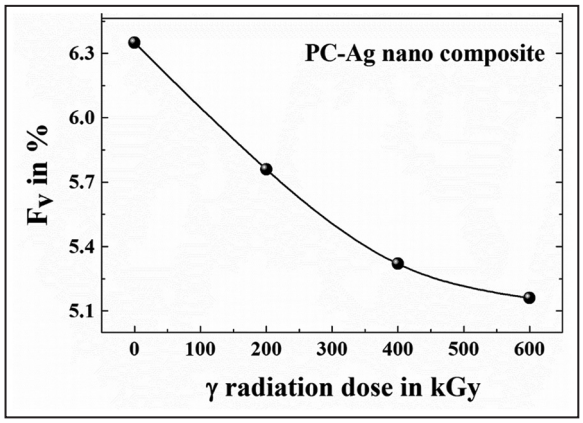

Figure 4: Variation of fractional free volume with gamma dose.

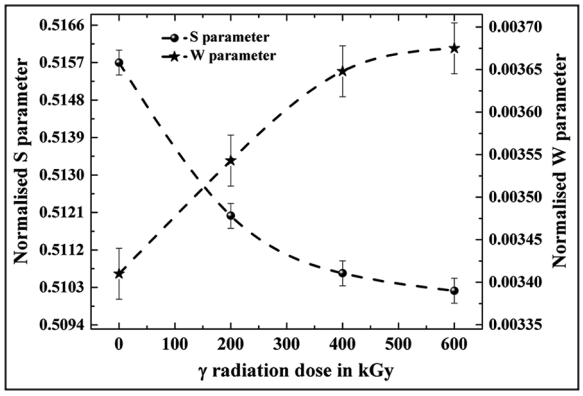

Figure 5: Plot of S and W parameters as a function of gamma dose for the silver doped polycarbonate.

seen from Table 2 that the zone of inhibition is more in the case of Gram-negative bacteria compared to that of Gram-positive bacteria. This can be attributed to the difference in their cell wall structure. The cell wall of the Gram-positive bacteria consists of a thick layer of peptidoglycan, (linear polysaccharide chains cross linked by short peptides), thus forming a more rigid structure, which leads to difficulty for Ag nanoparticles to penetrate inside the cell, but the cell walls of Gram-positive bacteria possess thinner layer of peptidoglycan [2].

4. CONCLUSION

Ag-PC polymer composites were synthesized by g-irradiation method, an easy, cost effective, and environmental friendly approach. Effect of g-radiation with varying dosage on Ag-PC sample has been studied through RBS, XRD, TEM, and PAS. The outcome of the measurements confirmed the diffusion of Ag in PC matrix. XRD and TEM results revealed that the diffused AgNPs have FCC structure. The observed reductions in positron lifetime parameters were due to cross linking of polymer chains and diffusion of Ag nanoparticles into the amorphous region of the polymer matrix. These results were further supported by the line shape parameters S and W. The zone of inhibition of both Gram-positive and Gram-negative bacteria increased with increase in dose for the prepared sample owing to the decrease in Ag particle size with gamma dose. The results indicated the efficacy of the synthesized Ag-PC matrix in inhibition of the overall growth of these types of bacteria. The obtained results support the prospective use of the Ag-PC nanocomposite in the food packaging industry.

5. ACKNOWLEDGMENTS

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***Bibliographical Sketch**

Dr. P. Ramya obtained her PhD degree in Physics, under the supervision of Professor. C. Ranganathaih, from the University of Mysore, India. She was awarded an Australian Government Endeavour Post-doctoral Research Fellowship in 2014 with Professor. Jim Williams at the ARC Centre for Antimatter and Matter Studies, School of Physics, University of Western Australia, Australia. Subsequently, she was appointed as a Research Assistant Professor, and later in 2015 joined the Antimatter and Nuclear Engineering Group lead by Professor Filip Tuomisto, as a Post-doctoral Researcher at the Department of Applied Physics, Aalto University School of Science, Finland. She also holds a concurrent Honorary Research Fellowship that the University of Western Australia, Australia. Her research interests include Positron Annihilation Spectroscopy, Polymers, composites/hybrid materials and their applications.