Antibacterial properties of Au doped polycarbonate synthesized by gamma radiation assisted diffusion method

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HIGHLIGHTS
- Au–PC matrix has been synthesized by gamma assisted diffusion method.
- RBS study showed the diffusion depth of gold in PC as ~0.85 μm.
- This depth of diffusion of Au in PC is suspected for the study of anti-bacterial.
- The prepared Au–PC samples showed moderate anti-bacterial properties.

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ABSTRACT
Gold (Au)–Polycarbonate (PC) matrix was prepared by gamma radiation assisted diffusion of Au nanoparticles in PC matrix. UV–Visible spectroscopy showed the surface plasmon resonance around 550 nm which corresponds to Au and this peak shift towards lower wavelength i.e. blue shift indicating the decrease in particle size of Au. Rutherford Backscattering (RBS) experiment confirmed the diffusion of Au in PC and depth of diffusion is found to be around 0.85 μm. X-ray Diffractogram (XRD) results also revealed the diffusion of Au in PC where the peak observed at 2θ ∼ 38.29° which correspond to the FCC structure. Scanning Electron Microscope (SEM) images showed the hexagonal shaped Au nanoparticles and average particle size is found to be around 110 nm. These samples also showed anti-bacterial properties with both gram positive and gram negative bacteria’s and revealed the inhibition of the overall growth of the bacteria with gamma dose.

1. Introduction

In the last few decades, synthesis of metal particles/polymer has been widely studied interms of combining properties of metal nanoparticles and polymer matrices (Rozenberg and Tenne, 2008). The doping of suitable metal nanoparticles in polymer can be done by different techniques like sol–gel (Tachikawa et al., 2011), co-precipitation (Alam et al., 2013), plasma treatment (Mackova et al., 2006), ion implantation (Prakash et al., 2011) or diffusion by gamma radiation (Akhavan et al., 2014). When compared to other conventional chemical methods, gamma irradiation was a clean, simple and efficient method to prepare, nanosized metal particles (Li et al., 2007).

Among various metal nanoparticles, gold nanoparticles are multifunctional inorganic metal which has application in different fields such as nano-engineering (Li et al., 2003), nano-electronics (Barnes et al., 2003), biosensors (Elghanian et al., 1997), biological imaging (Jain et al., 2006) and other technologies. Even, it exhibits excellent anti-bacterial properties (Zhao et al., 2010; Demurtas and Perry, 2014). Polycarbonate (PC) is the most important polymer which is utilized in all fields of life, especially in optical (Blair, 1985), electronic (MacDonald et al., 2007), space (Iskanderova et al., 1998), medical (James et al., 1991) and for preparing nanotubes and nanowires (Piraux et al., 1997). There are a numerous reports available in the literature on the use of light and heavy ions for modifying physico-chemical changes in polycarbonate films (Hareesh et al., 2013a,b; Kumar et al., 2012) like chain scission, cross-linking, creation of free radicals, evolution of gases, creation
of defects and enhancement in fractional free volume. This enhancement in free volume will result in enhancement in the diffusion co-efficient of inorganic species in polymer (Shariff et al., 2002).

In literature, different research groups reported the synthesis of different type metal particles/polymer matrix viz. gold–polyvinyl pyrrolidone by gamma irradiation (Mistra et al., 2012), gold–polyvinyl alcohol (Deore et al., 2014) by low energy electron irradiation, gold–polyimide by high energy electron irradiation (Bogle et al., 2007), Gold-Poly(methyl methacrylate) (Alsawafta et al., 2011) by thermal treatment. In addition to this, gold in flower (Dhumale et al., 2010) and in cube (Dhumale et al., 2011) like structures have also been synthesized by high energy electron irradiation. Aleksandra et al. (2012) have characterized polyvinyl alcohol–gold samples synthesized by insitu gamma irradiation method and found uniform spherical Au nanoparticles homogeneously embedded in PVA matrix.

In this work, we have synthesized the gold–polycarbonate matrix by gamma radiation assisted diffusion method. The prepared samples are characterised by UV–Visible spectroscopy, Rutherford Backscattering Spectroscopy (RBS), X-ray diffractogram (XRD), Scanning Electron Microscopy (SEM), Energy Dispersive Spectroscopy (EDS) and Fourier Transform Infrared (FTIR) spectroscopy. The prepared samples have been checked for its antibacterial properties by Agar diffusion method against gram negative bacteria, Escherichia coli (E. coli) and gram positive bacteria, Staphylococcus aureus (S. aureus). These two bacteria’s will spread via water or meat and may cause serious food poisoning, anaemia and fever etc. Therefore, it is important to diffuse nanoparticles in polymer matrix that will inhibit these kinds of bacteria’s. Therefore, in the present study the synthesized Au–PC matrix is being effectively used for the inhibition of the overall growth of these kinds of bacteria.

2. Experimental details and characterization

2.1. Sample preparation

340 mg of pure HAuCl4 powder was dissolved in 100 ml of double distilled water and the mixture was stirred continuously for 30 min at room temperature. Seven glass bottles were taken and each bottle was filled with 10 ml of this solution. In every bottle Polycarbonate (Lexan, C10H14O3, thickness — 200 μm) film of size (1.5 × 1.5) cm2 was immersed. These bottles were exposed to Co-60 gamma source for the integrated doses of 50, 100, 200, 300, 400, 500, 600 kGy at a dose rate of 5.4 kGy/h. PC samples were removed from the bottle, dried and washed with double distilled water.

2.2. Sample characterization

The surface plasma resonance of gold was studied by UV–Visible spectrophotometer of model JASCO, V-670 by taking PC as a reference in the wavelength range 300–700 nm. The diffusion of Au in PC was estimated by Rutherford Backscattering Spectroscopy (RBS) technique using helium ions of energy 2 MeV. XRD diffractograms 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. The surface morphology and atomic percentage of gold for all the samples was studied using SEM of model JEOL JSM 6390LV attached with electron diffusion spectroscopy (EDS). FTIR spectroscopy measurement was carried out for all the samples using JASCO, 1600 FTIR spectrophotometer.

2.3. Anti-bacterial inhibition

The anti-bacterial activity of Au–PC samples against gram negative bacteria, E. coli and gram positive bacteria, S. aureus was studied by the agar well diffusion method. The samples were dissolved in dichloromethane (DCM) and used for anti-bacterial activity. The bacterial cultures of known inoculums size (0.2 ml, 10^3 CFU/ml) of test microorganisms were spread on nutrient agar plates. In aseptic condition, the sterile borer was used to prepare well of 5 mm diameter, in the nutrient agar medium of each Petri dish. The sample of 2 mg concentration was used 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.

3. Results and discussion

3.1. UV–Visible spectroscopy

UV–Visible absorption spectrum for Au–PC matrix irradiated by different gamma doses is as shown in Fig. 1. Inset figure shows UV–Visible spectrum for pristine PC. It can be observed from inset figure that the PC showed main absorption edge at 285 nm and a small absorption edge at 385 nm. After irradiating PC in gold solution by a dose of 50 kGy, it did not show any peak. At this dose, Au particles may form but they may not diffuse inside the polymer. But, a new significant peak appears around 550 nm which corresponds to surface plasmon resonance of gold (Dhumale et al., 2011) at a dose of 100 kGy. The intensity of this peak increases with increase in dose indicating the increase in concentration of gold particles inside PC. Also, with increase in dose this peak shift towards lower wavelength side i.e. the blue shift indicating the decrease in particle size of gold. Based on the mechanism reported in literature (Wu et al., 2005; Lee et al., 2007), the reaction occurred during irradiation can be summarized as follows.

\[ n \cdot H_2O \xrightarrow{\text{irradiation}} e_{aq}^- + H^+ + OOH, H_2O^+, H_2O_2, \ldots \text{etc.} \]  
\[ e_{aq}^- + M^{n+} \rightarrow M^{(n-1)+} \]  
\[ e_{aq}^- + M^{n+} \rightarrow M^{0} \]  

In an aqueous solution, high energy gamma rays generate free electrons through photoelectric absorption and Compton
scattering. They are also responsible for the production of species like hydrated electrons, $e_{aq}$ and hydroxide radicals arising from radiolysis of water (Zhou et al., 1999). As the generated electrons are strong reducing agents, they reduce gold ions to zerovalent state. In turn, electrons reduce gold ions into gold atoms. These gold atoms are transferred to the defects sites or voids created in the polycarbonate during gamma irradiation. The local heat created during irradiation and some momentum transfer to the atoms in solution through Compton electrons may be the driving forces for the atoms to diffuse into the polymer. These atoms finally coalesce to form aggregates of metal nanoparticles in polymer as depicted by following reaction. Simultaneously, the reduced gold atoms start coalescing to form metal nanoparticles in the solution (Li et al., 2007).

$$nM^0 \rightarrow (M)_2 \rightarrow \ldots (M)_h \ldots \rightarrow (M)_{agg} \quad (4)$$

It is interesting to note that, the nanoparticles size has a relation with the gamma dose, and it is well explained by the report of Naghavi et al. (2010). For gold ions, the electron capture is high thus producing large number of neutral gold atoms.

$$M^+ + e_{aq} \rightarrow M^0 \quad (5)$$

$$M^0 + M^0 \rightarrow M_2 \quad (6)$$

As reported by Naghavi et al. (2010) the bonding between atoms/clusters with unreduced ions is also strong and hence association becomes fast. The neutral gold atoms and unreduced gold ions combine results in the formation of gold nanoparticles.

$$M^0 + M^+ \rightarrow M^+_2 \quad (7)$$

$$M_{m+n} + M^+ \rightarrow M_{m+n+1}^+ \quad (8)$$

$$M_{m+1}^+ + e_{aq} \rightarrow M_{m+1} \quad (9)$$

The last two reaction of ion association with atoms/clusters are important in the cluster growth mechanism. There is simultaneous reduction of free gold ions and of absorbed ones and both are controlled by the rate of formation of reducing radical. The cluster formation by direct reduction followed by coalescence is dominant at higher gamma doses and the final aggregates are having smaller size as compared to that of lower doses. This can be clearly seen in case of UV-Visible spectroscopy results that the particle size decreases (i.e. blue shift of surface plasmon resonance) with increase in gamma dose. At higher doses, long irradiation time favors adsorption of ions which are not yet reduced onto neutral gold atoms or small clusters formed at earlier stage. This process is independent and cannot be controlled by the reduction in situ of the ions by electron transfer from reducing radicals. The electrons from smaller aggregates can be transferred to larger ones coated with adsorbed ions (Naghavi et al., 2010). In this manner, during gamma irradiation, the number of gold nanoparticles increases and gold particles of nanometric dimensions can be synthesized.

3.2. Rutherford backscattering

The content of Au particle and its depth profile were determined from the RBS spectra measured with the 2 MeV $^4$He beam (0° incidence angle, 165° laboratory scattering angle), and the back scattered particles were detected with a surface barrier detector. Depth profile of the incorporated Au 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 polymers using the data base from the SRIM code (Ziegler et al., 2010). The RBS spectrum of PC and Au–PC sample is as shown in Fig. 2. In the curve 2, the peak edge at the channel ~1837 corresponds to gold. This indicates the diffusion of gold nanoparticles inside PC and depth of distribution of gold nanoparticles in PC at a gamma dose of 600 kGy is found to be 0.85 μm.

3.3. X-ray diffractogram

XRD pattern for pristine PC and gold diffused PC is shown in Fig. 3. Fig. 3(a) showed a broad peak around 16.93° (2θ) indicating the amorphous nature of PC, where as Fig. 3(b) and (c) are for PC irradiated in gold solution at different gamma doses. At a dose of 50 kGy, a main sharp peak at 2θ ~38.29° with index (111) and small peaks at 2θ ~44.56°, 64.65°, 77.68° with index (200), (220), (311) respectively are observed which corresponds to face centered cubic structured gold nanoparticles (JCPDS file no. 04.0784). In addition Fig. 3(b) and (c) also showed the increase in intensity and broadening of XRD peaks indicating the diffusion of more gold particles and the average size of the particles decreases with increase in gamma dose. The lattice parameter of (111) peak found to be 4.07 Å which is in good agreement with standard value of 4.07 Å (JCPDS file no. 04.0784). The average crystallite size (L) value was calculated using the following equation (Kumar et al., 2012) and are listed in Table 1.

$$L = \frac{C \lambda}{\beta \cos(\theta)} \quad (10)$$

where C is constant (0.9), $\lambda$ is wavelength of the Cu-Kα X-ray radiation used (1.5406 Å), $\beta$ is full width at half maximum of the diffraction peak, $\theta$ is the Bragg angle. It can be seen from Table 1 that the average crystallite size value of the peak (111) decreases with increase in gamma dose.

3.4. Scanning electron microscopy

SEM images of pristine PC and gold diffused PC at different doses are shown in Fig. 4. It can be seen from Fig. 4(a) that the surface of PC is smooth and homogeneous. At a dose of 50 kGy, the gold particles start nucleating (Fig. 4(b)) and at a dose of 100 kGy, gold particles of hexagonal shape were formed (Fig. 4(c)). The process of synthesis of metal nanoparticles by radiolysis from aqueous solutions may take place in the following steps (Engelbrekt et al., 2013). First step, typically in a solution, gamma radiation may reduces HAuCl₄ to three reaction intermediates i.e., tetrachloroaurate(III) (AuCl₄)⁻, the monoqua-substituted...
complex (AuCl3OH2), and the deprotonated monohydroxo-analog (AuCl3OH)\(^{-}\) \cite{Sen1998}. \(\text{AuCl3OH}^{-}\) is hypothesized to be the dominating oxidizing Au(III) form which is reduced to Au(I) in the form of \(\text{AuCl2}\) by two electrons, and further to metallic gold (Au\(^{0}\)). As produced Au\(^{0}\) may occupy these defects (voids or free volume) inside the polymer produced by gamma irradiation. In the second step, freshly formed gold atoms in polymer start coalescing to form bigger particles (~nm) of several nuclei. Finally, over a period of gamma dose, remaining smaller particles diffuse and redeposit into larger particles. At a dose of 600 kGy, the average particles size is found to be around 110 nm. The atomic percentage of gold is also increased with increase in dose as studied from EDS method and are listed in Table 1.

### 3.5. FTIR spectroscopy

To investigate the origin of interaction between Au particles and PC matrix, FTIR spectroscopy of pristine PC and gamma irradiated PC in gold solution were carried out and is as shown in Fig. 5. All of them exhibit the characteristic signature of vibrations of benzene ring, \(-\text{CH}_2-\) and asymmetric vibrations of \(\text{CH}_3\) at 1505 cm\(^{-1}\), 1014 cm\(^{-1}\) and 2968 cm\(^{-1}\) respectively \cite{Sinha2004, Sunjie2010}. The peaks present between the wave numbers 1150 cm\(^{-1}\) and 1220 cm\(^{-1}\) are due to C–O–C vibrations (aromatic). It is clearly observed that, there is change in the intensities of all these bands. This clearly indicates the scissioning of the corresponding bonds on gamma exposure of PC. A peak at 1602 cm\(^{-1}\) is assigned to the stretching band of C–O and is observed shifting from 1602 cm\(^{-1}\) to 1648 cm\(^{-1}\). This shift identifies that the oxygen lone pair in –\(\text{C}–\text{O}–\) participates in the interaction and is the main interaction between surface of Au nanoparticles and PC chains \cite{Sunjie2010}. At 3260 cm\(^{-1}\), a new broad band appears in case of gamma irradiated samples, which is due to O–H bonds. Sinha et al. \cite{Sinha2004} reported that, phenolic groups (–OH groups) are formed in PC after gamma irradiation. Also, this may be due to the presence of water molecules adsorbed on the surface of Au nanoparticles synthesized on the surface region of PC after gamma irradiation \cite{Sunjie2010}.

### 3.6. Anti-bacterial properties

Fig. 6 shows the photograph (on dark background) of zone of inhibition of pristine PC and Au-PC samples with gram positive (\(S. \text{Aureus}\)) and gram negative (\(E. \text{Coli}\)) bacteria. Pristine PC did not show any anti-bacterial patterns, but Au–PC samples showed zone of inhibition pattern (some white patches) for both kind of bacteria’s. Many studies are devoted to understand the mechanism of anti-bacterial action of metallic nanoparticles, but it is still not well understood. There are two hypotheses which give some idea about the mechanism of gold bactericidal activity. In first mechanism, gold nanoparticles produce reactive oxygen species

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**Table 1**

Average crystallite size (L) Atomic percentage (A) and antibacterial property of Au–PC matrix.

<table>
<thead>
<tr>
<th>Dose (kGy)</th>
<th>L (nm)</th>
<th>A (%)</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(S. \text{Aureus})</td>
</tr>
<tr>
<td>50</td>
<td>126</td>
<td>0.05</td>
<td>1.1 ± 0.14</td>
</tr>
<tr>
<td>100</td>
<td>119</td>
<td>0.37</td>
<td>1.3 ± 0.14</td>
</tr>
<tr>
<td>200</td>
<td>108</td>
<td>0.48</td>
<td>1.5 ± 0.12</td>
</tr>
<tr>
<td>300</td>
<td>92</td>
<td>0.67</td>
<td>1.8 ± 0.09</td>
</tr>
<tr>
<td>400</td>
<td>80</td>
<td>1.03</td>
<td>2.0 ± 0.23</td>
</tr>
<tr>
<td>500</td>
<td>72</td>
<td>1.37</td>
<td>2.5 ± 0.31</td>
</tr>
<tr>
<td>600</td>
<td>66</td>
<td>2.58</td>
<td>2.9 ± 0.15</td>
</tr>
<tr>
<td>Control (Tetracycline)</td>
<td>–</td>
<td>–</td>
<td>12 ± 1.06</td>
</tr>
</tbody>
</table>
(ROS) in the vicinity of the bacterial cell membrane leads to the cell permeability and hence cell death (Ivan and Branka, 2004; Maness et al., 1999). The second hypothesis tells that, there is slow release of metal ions from the surface of metallic nanostructures. These released metal ions interact with the DNA and cellular enzymes which is done by coordinating to electron donating groups of microbes (Smetana et al., 2008; Feng et al., 2000). The damage to the cell membrane results into the disruption of respiratory chain reactions. In such a way metal ion–DNA interaction inhibits bacterial cell and finally kills the cells (Singh et al., 2009). Therefore in the present work, the Au–PC samples prepared in this work showed moderate anti-bacterial activity against both gram negative and gram positive bacteria's. In addition to this, it is also observed from Table 1 that the zone of inhibition is more in case of
gram negative bacteria compared to that of gram positive bacteria which may be due to the difference in their structure in the cell wall. 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 more rigid structure which leads to difficulty for Au nanoparticles to penetrate inside the cell, but the cell walls of gram positive bacteria possesses thinner layer of peptidoglycan (Jelena et al., 2014; Kaviya et al., 2011).

4. Conclusions

Au–PC samples were synthesized by gamma radiation assisted diffusion method. UV–Visible spectroscopic results showed decrease in wavelength of gold peak i.e. blue shift with increase in dose indicating the decrease in particles size. RBS results confirmed the diffusion of gold in PC and depth of diffusion was found to be 0.85 µm. XRD results also revealed the diffusion of gold in PC and decrease in average size of particle after irradiation. SEM results showed the formation of hexagonal shape gold nanoparticles and the size is around ~110 nm. The zone of inhibition of both gram positive and gram negative bacteria’s increases with increase in dose for the prepared Au–PC samples due to decrease in Au particle size with dose. The present approach provides easy and convenient method to prepare Au–PC samples with anti-bacterial properties which may be used in food industry and medical applications.

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References


