CHAPTER – 7.0

PVA-Ag AND PVA-Au NANOFIBRE MATS FOR SUSTAINED RELEASE OF NANOPARTICLES AND THEIR ANTIBACTERIAL AND ANTICANCER APPLICATIONS
7.1. Synthesis and characterization of PVA-Ag and PVA-Au nanofibre mats

Silver and gold nanoparticles were synthesized by reduction of AgNO$_3$ and HAuCl$_4$·3H$_2$O with leaves extract of *Bauhinia tomentosa* respectively. Twelve percent (12 % w/v) of PVA was dissolved in aqueous suspension of silver and gold nanoparticles respectively. The green synthesized nanoparticles were composited with electrospun PVA nanofibres.

UV-Visible spectrum showed characteristic absorbance for AgNP at 406 nm and AuNP at 525 nm. [Figure 7.1 and Figure 7.2] The spectrum for PVA-Ag and PVA-Au suspension also exhibited absorbance at 406 nm and 525 nm respectively [83].

![Figure 7.1: UV-Visible spectrum of Ag, PVA-Ag](image1)

![Figure 7.2: UV-Visible spectrum of Au, PVA-Au](image2)

7.2. Sustained release of nanoparticles

The electrospun nanofibres were further analyzed for release mechanism of Ag and Au nanoparticles in water and the presence of nanoparticles released with time were studied using UV-Visible spectrophotometer. The UV-Visible spectra [Figure 7.3 and 7.4] shows the continuous release of Ag and Au NPs with time.
FTIR analysis confirmed the presence of stretching and bending vibrational groups in PVA, PVA-Ag and PVA-Au nanofibre mats and solution respectively [Figure 7.5 and 7.6] [83].

Figure 7.7 shows the HR-SEM image of PVA nanofibre. HR-SEM images confirmed the formation of PVA nanofibres with silver and gold [Figure 7.8 and 7.9]. The average size of the Ag and Au nanoparticles was found to be 18.9 nm and 17.3 nm and the diameter of nanofibre was in the range of 54.4 nm. EDAX analysis confirmed the presence of elemental silver and gold at approximately at 3 keV and 2 keV respectively [Figure 7.8 (inset) and 7.9 (inset)] [81].
XRD confirmed the presence of nanocrystalline silver and gold with size 12.3 nm and 9.9 nm respectively [Figure 7.10 and 7.11] [81].

Figure 7.7: HR-SEM - PVA nanofibre

Figure 7.8: Release of Ag from PVA
Figure 7.9: Release of Au from PVA

Figure 7.10: XRD Spectrum of PVA-Ag

Figure 7.11: XRD Spectrum of PVA-Au
7.3. Antibacterial and anticancer applications of nanofibre mats

The antibacterial activity of PVA-Ag and PVA-Au nanofibre was confirmed by Resazurin assay on the cells of *E. coli*, *B. subtilis*, *S. aureus*, *S. typhi* and *P. aeruginosa*.

Figure 7.12 represents the antibacterial activity of PVA-Ag against the five bacterial cells. From the figure, wells in violet colour confirmed bacterial cell death while the wells in pink confirmed that the cells were alive. The pink colour appeared due to reduction of rezazurin to rezarufin. With decrease in concentration, the presence of more number of violet colour wells indicates high antibacterial activity of PVA-Ag.

![Figure 7.12: Antibacterial activity of PVA-Ag](image)

The minimum inhibitory concentration (MIC) of PVA-Ag against different bacteria is shown in Figure 7.13. The figure shows that the AgNP’s released from the PVA nanofibres exhibited high activity against *B. subtilis* while *S. typhi* showed least activity. *E. coli* and *S. aureus* exhibited higher activity than *P. aeruginosa* and *S. typhi*.[84]
Figure 7.13: MIC of PVA-Ag on microorganisms

Figure 7.14: UV-Visible spectrum showing sustained release of PVA-Ag on E. coli

Figure 7.15: UV-Visible spectrum showing sustained release of PVA-Ag on B. subtilis
Figure 7.16: UV-Visible spectrum showing sustained release of PVA-Ag on *S. aureus*

Figure 7.17: UV-Visible spectrum showing sustained release of PVA-Ag on *S. typhi*

Figure 7.18: UV-Visible spectrum showing sustained release of PVA-Ag on *P. aeruginosa*

Figure (7.14-7.18) depicts the UV-Visible spectrum showing sustained release of PVA-Ag against *E. coli, B. subtilis, S. aureus, S. typhi* and *P. aeruginosa*. The absorbance at 600 nm is characteristic of the presence of resazurin. This confirms that PVA-Ag nanofibre exhibits antibacterial activity while the absorbance spectrum at 570 nm is characteristic of resarufin which is pink in colour [314].
Figure 7.19 represents the antibacterial activity of PVA-Au against the five bacterial cells. The figure showed violet coloured wells confirming bacterial cell death while the pink coloured wells confirmed that the cells were alive. The pink colour appeared due to reduction of rezazurin to rezarufin. As the concentration of PVA-Au decreases, the presence of only one or two violet colour wells indicates low antibacterial activity.[84]

The minimum inhibitory concentration (MIC) of PVA-Au against different bacteria is shown in Figure 7.20. The figure shows that the AuNP’s released from the PVA nanofibres exhibited high activity against *S. typhi* while *E. coli*, *B. subtilis*, *S. aureus* and *P. aeruginosa* exhibited least activity.

**Figure 7.19:** Antibacterial activity of PVA-Au
Figure 7.20: MIC of PVA-Au on microorganisms

Figure 7.21: UV-Visible spectrum showing sustained release of PVA-Au on E. coli

Figure 7.22: UV-Visible spectrum showing sustained release of PVA-Au on B. subtilis
Figure 7.23: UV-Visible spectrum showing sustained release of PVA-Au on *S. aureus*

Figure 7.24: UV-Visible spectrum showing sustained release of PVA-Au on *S. typhi*

Figure 7.25: UV-Visible spectrum showing sustained release of PVA-Au on *P. aeruginosa*

Figure (7.21-7.25) depicts the UV-Visible spectrum showing sustained release of PVA-Au against *E. coli*, *B. subtilis*, *S. aureus*, *S. typhi* and *P. aeruginosa*. The absorbance peak at 600 nm is characteristic of the presence of resazurin. This confirms that PVA-Au nanofibre exhibits antibacterial activity while the absorbance spectrum exhibiting a peak at 570 nm is characteristic of resarufin which is pink in colour [314].

Figure 7.26 (a, b, c) depict the control A-549 cells, cells treated with PVA-Ag and cells treated with PVA-Au respectively. Microscopic images of A-
549 cells treated with PVA-Ag and PVA-Au nanofibres showed spherical cells, cell shrinkage, blebbing and cell surface protuberances. Anticancer activity of PVA-Ag and PVA-Au nanofibres against A-549 cells confirmed by MTT assay exhibited IC$_{50}$ values 62.5 µg mL$^{-1}$ and 56.25 µg mL$^{-1}$ respectively.[83].

The normal cell cycle of A-549 cells, cell cycle arrest after treatment with PVA-Ag and cell cycle arrest after treatment with PVA-Au are shown in Figures [7.27, 7.28, 7.29] respectively. The figures showed that cells treated with PVA-Ag and PVA-Au exhibited apoptosis or programmed cell death while control A-549 cells exhibited normal activity in all phases.

**Figure 7.26** Microscopic images of a) Control A-549 cells b) PVA-Ag c) PVA-Au

**Figure 7.27:** Control-A-549 Cells **Figure 7.28:** Cells treated with PVA-Ag **Figure 7.29:** Cells treated with PVA-Au
Table 7.1 Flow Cytometry - A-549 - Anticancer efficacy of PVA-Ag and PVA-Au

<table>
<thead>
<tr>
<th>Phase</th>
<th>Control (%)</th>
<th>With PVA-Ag (%)</th>
<th>With PVA-Au (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P2 - Apoptosis</td>
<td>5.80</td>
<td>71.73</td>
<td>63.78</td>
</tr>
<tr>
<td>P3 - G0/G1</td>
<td>44.30</td>
<td>4.19</td>
<td>3.57</td>
</tr>
<tr>
<td>P4 – S</td>
<td>14.70</td>
<td>2.62</td>
<td>4.08</td>
</tr>
<tr>
<td>P5 – G2/M</td>
<td>28.10</td>
<td>21.47</td>
<td>28.57</td>
</tr>
</tbody>
</table>

Table 7.1 shows the different phases of cell cycle namely Apoptosis, G0/G1, S, G2 and M. Table shows that A-549 cells treated for 24 hours with PVA-Ag and PVA-Au nanofibres significantly reduced the DNA content, indicating apoptosis, with consequent loss of cells. Cells treated with PVA-Ag showed 71.73% apoptosis while cells treated with PVA-Au showed 63.78% apoptosis. Control cells showed only 5.8% apoptosis. Flow cytometry confirmed apoptosis of A-549 cells and the efficiency of PVA-Ag and PVA-Au after 24 hours was found to be 65.93 % and 57.98 % respectively. PVA-Ag exhibited better activity compared to PVA-Au.