Chapter 2

Development of novel biodegradable Au\(^0\) nanocomposite hydrogels based on wheat protein: for inactivation of bacteria

$\text{Wheat Protein Isolate} + \text{Acrylamide} \xrightarrow{\text{MBA KPS/TMEDA}} \text{A} \xrightarrow{\text{Wheat Protein Isolate-Hydrogel}}$

\[ \text{HAuCl}_4 \xrightarrow{\text{Azadirachta indica}} \text{Reduction} \]

A: Wheat protein isolate hydrogel, B: Au\(^{3+}\) ions loaded hydrogel, C: Gold nanocomposite hydrogel

Formation of $P(WPI-AM)$ gold nanocomposite hydrogels
2. 1. INTRODUCTION

Biodegradable hydrogels containing metal nanoparticles have received great importance for many applications in the biomedical and biotechnological fields (1-2). In general, their origin is (in most cases) from natural materials. Wheat isolate (globular protein) is one of the biological proteins (3) that is in great abundance in nature rich in amino acids and has good biodegradability potential available at a low cost (4-5). Due to superior properties, they are used as recombinants in DNA technology (6) and for biomedical applications (7-8). Recently, Betz et al., (9) reported the antioxidant capacity of bilberry extract microencapsulated in wheat (or whey) protein hydrogels. In their study, they brought new insights regarding protein-based microencapsulation of phenolics and studied their degradation property. Gunasekaran et al., (10) prepared wheat protein hydrogels for encapsulation and control delivery applications. Doherty et al., (11) fabricated wheat protein microbeads for biomedical applications (gastro-intestinal transit).

However, various natural biopolymers that are protein-based (e.g., elastin, collagen, gelatin, fibrin and globular proteins), are interesting materials for hydrogel developments. This is due to their biodegradability, biocompatibility and non-toxicity (12-13). These biomaterials provide unique properties to address some of the challenges in biology, medicine and material science (3). To enhance their bioactivity in wound care applications, a number of researchers introduced inorganic metals (14-16). Because inorganic nanoparticles can easily functionalize with biomaterials, this characteristic makes them attractive in the biomedical and biotechnological fields (17). Recently, hydrogels with gold nanoparticles have attracted attention for their potential applications as antimicrobial materials (16-18). They are prepared by
chemical, photoinduced and microwave-assisted reduction methods, but the chemical reduction methods are the most common (19). The reduction of nanoparticles procedures depends on toxic chemicals (20-21). To overcome this problem, many researchers have introduced the green process (22). In the green process, few researchers have used plant leaf extracts as reducing agents for metal nanoparticles, which are cost-effective and also utilize ambient condition for reduction reaction (23). Therefore, the development of metal nanoparticles based on natural extracts is considered the most appropriate method for obvious environmental reasons (24).

In view of the importance of the above work, the present investigation involves the development of gold nanoparticles in wheat protein isolate based polyacrylamide hydrogels by reducing the gold chloride, using neem leaf’s extracts. Structural and morphological studies of the hydrogels and their corresponding gold nanocomposite hydrogels were carried out by using fourier transforms infrared (FTIR) spectroscopy and X-ray diffraction (XRD). The content and distribution of gold nanoparticles in $P(WPI-AM)$ hydrogels were determined by thermo-gravimetric analysis (TGA), scanning electron microscop-energy dispersive spectroscopy (SEM-EDS) and transmission electron microscopy (TEM). The effect of gold nanoparticles on the antibacterial activity of the $P(WPI-AM)$ hydrogels was studied. Therefore this study involves the design of $P(WPI-AM)$ gold nanocomposites hydrogels for significant antibacterial applications and presented in this chapter.

2.2. MATERIALS AND METHODS

2.2.1. Materials

Wheat protein isolate (WPI) powder was obtained from Honeyville Food Products, Salt Lake City, Utah, and USA. WPI as reported, by the manufacturer, contains 90% protein, 4% fat (acid hydrolysis), about 5% ash and 1% other minor
constituents.

Acrylamide (AM), \(N,N'\text{-methylenebisacrylamide (MBA), potassium persulphate (KP S), }\ N,N,N',N'\text{-tetramethylrthylenediamine (TMEDA), gold chloride (}HAuCl_4 \ X \ H_2O\text{) and sodium hydroxide (SH) were purchased from S. D. Fine Chemicals (Mumbai, India). All chemicals were used without further purification.}

2.2.2. Preparation of the neem leafs extract

Leaf extracts were prepared by a green process technique, using the standard procedure described by Ravindra et al (25). Neem leafs (\textit{Azadirachta Indica or A.Indica}) were collected from neem tree in the Sri Krishnadevaraya University (Anantapur, India) and thoroughly washed with distilled water. Neem leaf broth was prepared by taking 25g of thoroughly washed leafs and finely cut them in a 1000ml Erlenmeyer flask with 500ml of sterile distilled water. The solution was heated at 100\(^\circ\)C for 2min in order to extract the contents of the leaves and filtered through 0.45\(\mu\)m PVDF Millex Filter using a 50ml syringe. The extracted leaves solutions were stored at 4\(\circ\)C.

2.2.3. Preparation of poly (wheat protein isolate-acrylamide) \(P(WPI-AM)\) hydrogels

Different amounts of wheat protein isolate powder were dissolved in (0.05N) aqueous sodium hydroxide solution in a 50ml beaker. To this solution, 1g of AM, 1 ml of MBA as crosslinker and 1ml of KPS/1ml of TMEDA as redox initiating system, were added. Each mixture was stirred for 30 min over a magnetic stirrer at 100rpm. The gel matrix formed was carefully transferred into a 1liter beaker containing 500ml distilled water and the distilled water was repeatedly changed (for every 5hrs) for 2 days in order to remove unreacted products such as: monomer, cross-linker, initiator and soluble polymers etc. The \(P(WPI-AM)\) hydrogels obtained was allowed to dry at
ambient temperature for 2 days. The feed Composition of the gels prepared is presented in Table 1.

<table>
<thead>
<tr>
<th>Hydrogel code</th>
<th>AM (mM)</th>
<th>WPI (g)</th>
<th>MBA (mM)</th>
<th>KPS (mM)</th>
<th>TMEDA (mM)</th>
</tr>
</thead>
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<tr>
<td>P(WPI-AM)0</td>
<td>14.084</td>
<td>-</td>
<td>0.648</td>
<td>1.851</td>
<td>0.8620</td>
</tr>
<tr>
<td>P(WPI-AM)1</td>
<td>14.084</td>
<td>0.10</td>
<td>0.648</td>
<td>1.851</td>
<td>0.8620</td>
</tr>
<tr>
<td>P(WPI-AM)2</td>
<td>14.084</td>
<td>0.15</td>
<td>0.648</td>
<td>1.851</td>
<td>0.8620</td>
</tr>
<tr>
<td>P(WPI-AM)3</td>
<td>14.084</td>
<td>0.25</td>
<td>0.648</td>
<td>1.851</td>
<td>0.8620</td>
</tr>
<tr>
<td>P(WPI-AM)4</td>
<td>14.084</td>
<td>0.35</td>
<td>0.648</td>
<td>1.851</td>
<td>0.8620</td>
</tr>
</tbody>
</table>

Table 1: Feed composition of $P(WPI-AM)$ hydrogels

2.2.4. Preparation of gold nanocomposite hydrogels

To prepare WPI-based Au nanocomposite hydrogels 100mg of dry $P(WPI-AM)$ was equilibrated with distilled water for 48hrs and then transferred in to a beaker containing 30ml of $HAuCl_4 \times H_2O$ (400mg/150 ml) aqueous solution and then allowed to equilibrate for 24hrs. During this stage, the $Au^{3+}$ ions are being exchanged from solution to the $P(WPI-AM)$ hydrogel networks.

The Au salts loaded $P(WPI-AM)$ hydrogels were wiped off using tissue paper and transferred to a beaker containing 50ml of cold neem leaf extracts ($Azadirachta Indica$ (AI) solution. The beaker was left in the refrigerator (4°C) for 8hrs in order to reduce the $Au^{3+}$ ions into $Au^0$ nanoparticles. The Au nanoparticles in the hydrogel obtained were allowed to dry at ambient temperature and the product was used for further studies. In a similar manner, the other WPI-based hydrogels were prepared by varying the WPI concentration. Table 1 illustrates the various components used in the preparation of $P(WPI-AM)$ hydrogels.
2.2.5. Swelling studies

Accurately weighed dry P(WPI-AM) based hydrogels were immersed in a 100ml beaker containing twice distilled water, until the hydrogel reached the equilibrium swelling at ambient temperature for 48 hrs. The swollen hydrogels were treated with HAuCl₄ and subsequently with Azadirachta Indica (Neem solution) via a green process as explained in the experimental section. The swelling ratio or swelling capacity \( S_{g/g} \) of the hydrogels developed and their nanocomposite was calculated using the equation 1:

\[
S_{g/g} = \frac{W_s - W_d}{W_d} \quad \text{equation 1}
\]

Where \( W_s \) and \( W_d \) denote the weight of the swollen hydrogel at equilibrium and the weight of the dry hydrogel, respectively. The data provided in the form of Fig.2 in Results & Discussion is an average value of 3 individual sample readings.

2.2.6. Fourier transform infrared (FTIR) spectroscopy

FTIR spectrophotometer is used to study the transmission of the hydrogel pattern, gold salt incorporation and gold nanoparticles patterns in hydrogel networks. The hydrogels and the gold nanoparticles-embedded P(WPI-AM) hydrogels were completely dried in the oven (Baheti Enterprises, Hyderabad, India) at 60°C for 6 hrs before their FTIR experiments. Samples were examined between 500 and 4000cm\(^{-1}\) on a Bruker IFS 66V FTIR spectrometer (Ettlingen, Germany), using the KBr disk method.

2.2.7. UV-Vis spectrophotometer

UV–Vis spectra of P(WPI-AM) gold nanocomposites hydrogels were recorded using ELICO SL 164 Model UV–Vis spectrophotometer (The Elico co, Hyderabad, India) from 200 to 700nm. For this study, 100mg of P(WPI-AM) gold nanocomposite hydrogels were dispersed in 10ml of distilled water and allowed to stand for 24hrs in
order to extract, as much as possible, the gold nanoparticles into aqueous phase and these solutions were recorded for their UV-Vis absorption spectra.

2.2.8. Thermal studies

Thermal analyses (DSC and TGA) of the samples were carried out using SDT Q 600 DSC instrument (T.A. Instruments-water LLC, Newcastle, DE 19720, USA), at a heating rate of 20°C/min under a constant nitrogen flow (100 ml/min).

2.2.9. X-ray diffraction (XRD)

Wide X-ray diffraction (XRD) method was used to identify the formation of gold nanoparticles in the $P(WPI-AM)$ hydrogels network. These measurements were carried out for dried and finely grounded samples on a Rikagu diffractometer ($Cu k_{\alpha}$ radiation, $\lambda = 0.1546$ nm) at 40 kV and 50 mA.

2.2.10. Scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS) analysis

Scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS) analysis of plain $P(WPI-AM)$ hydrogel and gold nanoparticles impregnated $P(WPI-AM)$ hydrogels were performed using a JEOL JEM-7500F (Tokyo, Japan) operated at an accelerating voltage of 2 kV. All the samples were carbon-coated, prior to examination on a field emission scanning electron microscope.

2.2.11. Transmission electron microscopy

Transmission electron microscope (TEM) (JEM-1200EX, JEOL, Tokyo, Japan) was used for nanoparticles formation. TEM sample was prepared by dispersing two to three drops of finely grinded $P(WPI-AM)$ gold nanocomposite (1mg/1ml) solution on a 3mm copper grid and dried at ambient temperature after removing excess solution using filter paper.
2.2.12. Antibacterial activity

The antibacterial activity of the gold nanocomposite \textit{P(WPI-AM)} hydrogels, under study, was investigated by disc method, using the standard procedure described elsewhere (26). Nutrient agar medium was prepared by mixing peptone (5.0g), beef extract (3.0g) and sodium chloride (NaCl) (5.0g) in a1000 ml distilled water and the pH was adjusted to 7.0. Finally, agar (15.0g) was added to the solution. The agar medium was sterilized in an Autoclave at a pressure of 6.8kg (15 lbs) for 30min. This medium was transferred into sterilized petri dishes in a laminar air flow chamber (Microfilt Laminar Flow Ultra Clean Air Unit, India, Mumbai). After solidification of the media, bacteria (\textit{Streptococcus pyogenes} and \textit{Escherichia coli}) (50µl) culture was spread on the solid surface of the media. Over this inoculated petri dish, one drop of gel solutions (20mg/10ml distilled water) was added using a 10µl tip and the plates were incubated for 48 hrs at 37°C.

2.2.13. Biodegradation characterizations

Biodegradation study was performed by using the weight loss (\%) methods A and B.

Method A

Nutrient agar medium was prepared by using the standard procedure described elsewhere (26). The agar medium was sterilized in an autoclave at 121°C for 30min at a pressure of 6.8kg (15 lbs). An \textit{Escherichia coli} bacterium was inoculated in this medium and the pure culture was maintained separately in the incubator. Then, to 10ml of sterilized broth, 0.100g each of the samples, i.e. both \textit{P(WPI-AM)} hydrogel and their gold nanocomposites samples were added aseptically in separate test tubes and each tube of samples was supplemented with inoculums of the bacterial strains separately. The degradation of samples by \textit{E. coli} was monitored at time intervals of 1, 5, 10, 15 and 30 days. After the required time period, samples were washed
repeatedly with de-ionized water, oven-dried at 40±1°C for 24 hrs. Then, the samples were weighed to determine the weight loss.

**Method B**

Enzymatic biodegradation of the hydrogels and their gold nanocomposites were carried out in a small vial containing a small piece of dry hydrogel sample and phosphate buffer solution (pH=7.4, 0.01M) with proteinase K, at a concentration of 0.2mg/ml. The mixture was then incubated at 37°C under constant shaking (60rpm). At regular intervals (1, 5, 15 and 30 days), the hydrogels were taken out and rinsed thoroughly with deionized water and blotted on filter paper in order to remove surface solution; they were then lyophilized in order to determine the dry weights of the hydrogels. The ratio of weight remained ($W_r$) was calculated based on the following equation:

$$W_r = \frac{W_d}{W_o}$$  \textit{equation 2}

Where $W_0$ is the initial weight of the dried gel sample and $W_d$ is the weight of the dried sample after degradation at a given time.

**2.3. RESULTS AND DISCUSSION**

Wheat protein isolate (WPI) obtained from wheat seeds of an annual plant, primarily contains 90% protein, higher than other wheat protein products: wheat gluten (76.5% protein) and defatted wheat flour. WPI primarily consist of β-lactoglobulin (β- Lg) and α-lactalbumin (α-La) are the essential main compounds but WPI having β-lactoglobulin is (~75%) higher than α-lactalbumin (27) and these are generally biological active, performing catalytic and regulating functions (28).

In ancient time Neem tree (Azadirachta indica) has been a part of Ayurvedic and East Indian folk medicine. The backgrounds of chemical investigations of neem were undertaken by Indian pharmaceutical chemists in 1919. However, real chemical
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Research originated in 1942 with isolation of three active compounds: nimbin, nimbidin, and nimbinene. Nimbin is the first bitter compound isolated from neem oil, and more than 135 compounds have been found in neem tree; the chemical structure of nimbin shown in Fig.1 with this it may be one of the reasons for the reduction of nanoparticles (29). Among the various bioreductants, Neem leaves extract was chosen for the present study, since (i) Neem is a quite commonly available plant (our university campus itself and also in all over Andhra Pradesh, India), (ii) it excludes addition of external stabilizing agent during synthesis and (iii) antimicrobial properties of the synthesized gold nanoparticles (one of the major end uses) might be enhanced due to synergistic effects of Neem leaves. Some of research works have been reported on synthesis of gold nanoparticles by using neem leaf (*Azadirachta Indica*) (30-31). In the present study we have investigated synthesis of gold nanocomposite hydrogels by neem leaf extract (*Azadirachta Indica*).

![Fig.1 A) Neem leaves (*Azadirachta Indica*) B) Nimbin Chemical Structure](image)

During the past decade, inorganic biodegradable hydrogels have become an active area of research because of their tremendous potential for a variety of applications (32-33). In this work, novel biodegradable P(WPI-AM) gold nanocomposite hydrogels were synthesized by free radical polymerization of AM in the presence of WPI. This combinational approach will enhance their antibacterial
efficacy and will open a new era in antimicrobial materials. **Scheme1** illustrates the fabrication of gold nanocomposite hydrogels.

![Scheme1: Schematic diagram for the formation of P(WPI-AM) gold nanocomposite hydrogels]

**2.3.1. Swelling properties**

The swelling capability of the hydrogels plays a significant role in their biomedical applications, particularly in antibacterial applications (14-15). Basing on the swelling properties, Varaprasad et al (34-35) have prepared different type of hydrogels for drug delivery and antibacterial applications. However, in this investigation, biodegradable $P(WPI-AM)$ template hydrogels were prepared for superior antibacterial applications. The swelling behaviors of $P(WPI-AM)$ hydrogels, Au$^{3+}$ ions embedded hydrogels and gold nanocomposites hydrogels are shown in Fig. 2. The values of the swelling ratio were influenced by the WPI concentration; with increase of WPI ratio in the hydrogels increases the swelling ratio values. This is due to the hydrophilic nature of WPI. In addition, Fig. 2 indicates that the swelling capacity of the Au$^{3+}$ ions loaded hydrogels and gold nanoparticles formed hydrogels,
resulted in further increase of swelling ratio of the hydrogel system. The swelling ratio depends on the $\text{Au}^{3+}$ ions loading and the Au nano particle formed. As per the results the swelling capacity of the hydrogels follows in this order: $\text{Au}^0$ hydrogel $>$ $\text{Au}^{3+}$ ions hydrogel $>$ Pure hydrogel. The reason being that when $\text{Au}^{3+}$ ions loaded hydrogels are treated with *Azadiractha Indica*, they turned into dark coffee colors, indicating that the formation of nanoparticles throughout the hydrogel networks. During this step, the addition of many $\text{Au}^{3+}$ ions leading to the formation of the nanoparticles within the hydrogel, expands the gel networks and promotes higher water uptake capacity. Varaprasad et al., [16, 34-36] also observed similar phenomena.

![Swelling behaviour of WPI varied hydrogels, gold ion and gold nanocomposite hydrogels](image)

**Fig 2:** Swelling behaviour of WPI varied hydrogels, gold ion and gold nanocomposite hydrogels

### 2.3.2. Fourier transforms infrared (FTIR) spectroscopy

The formation of gold nanoparticles inside the hydrogels network was also investigated by FTIR analysis (Fig.3). The $P(\text{WPI-AM})$ hydrogel (Fig. 3A) showed the absorption peaks at 1677.83cm$^{-1}$ and 1452cm$^{-1}$ associated with to the C = O stretching and NH bending vibrations of AM and WPI units. The broad peak observed at 3417.85cm$^{-1}$ is due to the overlapping stretching vibrations of NH$_2$ and
COOH functional groups in the hydrogel networks. The gold nanocomposite (Fig. 3B) did show all the above characteristic peaks, with a slight shift in wavelengths (3440.24 cm\(^{-1}\) corresponding to overlapping vibrations of NH\(_2\) and COOH functional groups and 1659.44 cm\(^{-1}\) and 1437 cm\(^{-1}\) relating to C = O stretching and –NH bending vibrations of AM and WPI, respectively). This is due to the coordination bond between the nano-gold and electron rich groups present in the hydrogel network.

Similarly, this type of difference was observed in the case of the AI treated \(P(WPI-AM)\) hydrogel. This indicates that the intermolecular hydrogen bonding between amide moieties remains intact and holds the hydrogel network. Therefore, \(P(WPI-AM)\) hydrogels and gold nanocomposites hydrogels can improve the inactivation effect of bacteria in biomedical applications.

![FTIR spectra](image)

Fig 3: FTIR spectra of: A) pure AI and AI+ \(P(WPI-AM)\) hydrogel, B) pure WPI hydrogel, gold ions loaded \(P(WPI-AM)\) hydrogel and \(P(WPI-AM)\) gold nanocomposite hydrogel.
2.3.4. UV-Vis spectrophotometer

The formation of gold nanoparticles was also demonstrated by UV-Vis spectra (Fig 4B). The absorption spectra of the gold ions solution, pure $P(WPI-AM)$ hydrogel, $P(WPI-AM)$ gold nanocomposites are shown in Fig. 4A. In the figure, a strong absorption peak shows at $\lambda_{\text{max}}$ 290nm for Au$^{3+}$ ion aqueous solution (37). A significant improvement in the absorption peak ($\lambda_{\text{max}}$= 530nm) was observed for gold nanocomposite hydrogel (38). This is due to the formation of gold nanoparticles in the $P(WPI-AM)$ hydrogels, as observed in the FTIR spectra, SEM micrographs and X-ray diffraction. However, there are no intensity peaks around 290 and 530 nm, in the case of pure $P(WPI-AM)$ hydrogel.

![UV-Visible spectra of A) Aqueous gold, Pure $P(WPI-AM)$4 hydrogel spectra B) $P(WPI-AM)$ gold nanocomposite $P(WPI-AM)$1to $P(WPI-AM)$4 hydrogels.](image)

Fig 4: UV-Visible spectra of A) Aqueous gold, Pure $P(WPI-AM)$4 hydrogel spectra B) $P(WPI-AM)$ gold nanocomposite $P(WPI-AM)$1to $P(WPI-AM)$4 hydrogels.
2.3.5. Thermal stability of the $P(WPI-AM)$ gold nanocomposites

The thermal stability and the formation of different hydrogels is measured with TGA and DSC. Fig. 5 shows the TG and DSC curves of the pure WPI, $P(WPI-AM)$ hydrogel, $P(WPI-AM)$ gold nanocomposite, obtained by testing in air, at a heating rate of 10°C/min. WPI, $P(WPI-AM)$ hydrogel and $P(WPI-AM)$ gold nanocomposite hydrogel exhibit a small endothermic peak at 90°C due to the presence of moisture in the samples (Fig. 5A). $P(WPI-AM)$ hydrogel shows a new peak at 160.23°C, this is due to the formation of hydrogels. But gold nanoparticle loaded hydrogel displays a stronger peak at 180.38°C when compared to $P(WPI-AM)$ hydrogels. This is due to the incorporation of gold nanoparticles in the said hydrogel.

The gold nanocomposite hydrogels were characterized by thermogravimetric analysis in order to determine the percentage weight loss of WPI, $P(WPI-AM)$ hydrogel and $P(WPI-AM)$ gold nanocomposite hydrogels. Fig. 5B shows the percentage decomposition of hydrogel and nanocomposite hydrogel. From the TGA thermograms the weight loss observed in the case of WPI-hydrogel is 87.54% at 646°C (Fig. 5B), whereas the weight loss in the $P(WPI-AM)$ gold nanocomposite hydrogel is (83.95%) at this temperature (646°C) (Fig. 5B). The difference in decomposition between the hydrogel and gold nanocomposite hydrogel is found to be 3.6% and it confirms the presence of gold nanoparticles (weight loss) in the hydrogel.
2.3.6. X-ray diffraction

Analysis of the X-ray diffraction patterns is a suitable technique to identify the crystallinity of the inorganic polymer materials. Fig.6 shows the XRD pattern of $P(WPI-AM)$ hydrogel stabilized gold nanoparticles, synthesized via green process at ambient temperature. Fig.6. XRD pattern shows clear peaks of gold nanoparticles in the faced-centered cubic (fcc) structure. The broad peak at $2\theta = 24.73^\circ$ is characteristic of the crystal plane (110) of $P(WPI-AM)$ hydrogel structure. The other five diffraction peaks at $2\theta$: 38.06, 44.10, 64.54, 77.42 and 81.81° correspond to the reflections of crystal planes (111), (200), (220), (311) and (222) respectively. On the other hand, the pure $P(WPI-AM)$ hydrogel did not show any sharp intensity peaks in XRD diffractions pattern. Therefore, XRD spectrum confirms the presence of gold nanoparticles as revealed in the visible area of the spectrophotometer.
2.3.7. Scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS) analysis

The morphology of \( P(WPI-AM) \) and \( P(WPI-AM) \) gold nanocomposite hydrogels were investigated with SEM. Fig. 7 shows the SEM micrographs of the Pure \( P(WPI-AM) \) and \( P(WPI-AM) \) gold nanocomposite hydrogels. Fig. 7a shows a clear surface feature for the pure \( P(WPI-AM) \) hydrogel, whereas gold nanoparticles loaded \( P(WPI-AM) \) hydrogel (Fig. 7b) exhibits smaller nanoparticles distributed throughout the hydrogel networks. It is worth mentioning that no individual gold particles were observed outside the \( P(WPI-AM) \) hydrogels, indicating a strong interaction between the \( P(WPI-AM) \) and the gold nanoparticles.

In order to confirm the presence of gold nanoparticles in the \( P(WPI-AM) \) hydrogel, EDS spectra of the unloaded and the gold-loaded hydrogel were investigated (Fig. 7). The EDS spectrum for the neat hydrogel (Fig. 7a) did not show the characteristic peak of gold, while the EDS spectrum of gold-loaded hydrogel (Fig. 7b) showed clearly the peak of the gold. The intensity of the gold peak is proportional to the metal concentration in the composites hydrogel. Hence, the
existence of gold nano particles in the hydrogel is confirmed by EDS spectra. The use of WPI in the networks is to stabilize the gold nano particles formed in the hydrogel networks.

Fig 7: SEM images of: a) $P(WPI-AM)4$, b) $P(WPI-AM)4$ gold nanocomposite hydrogels, EDS images of: a$^1$) $P(WPI-AM)4$ and b$^1$) $P(WPI-AM)4$ gold nanocomposite hydrogel

2.3.8. Transmission Electron Microscopy (TEM) Analysis

TEM analysis also demonstrated the formation of spherical gold nanoparticles in the $P(WPI-AM)$ hydrogels network. Their TEM image is shown in Fig 8. The average size of the gold nano particles is about $\sim 10 \pm 2$nm. It is evident that gold nanoparticles are highly stabilized by using WPI in the hydrogel network.
Fig 8: TEM images of \( P(WPI-AM)4 \) gold nanoparticles hydrogel.

2.3.9. Antibacterial activity

Wheat Protein Isolate (WPI) has been used in cancer therapy, wound care and repair applications (7-8). Due to its unique properties, WPI was selected for the preparation of gold nanocomposite hydrogels. Similarly, \textit{Azadirachta Indica} is a highly biologically active tree (39). It is a naturally occurring tree, non-toxic and bioactive agent in human life (40). The bactericidal effects of (plain \( P(WPI-AM) \) hydrogel, AI with \( P(WPI-AM) \) hydrogel and \( P(WPI-AM) \) gold nanocomposite) biodegradable hydrogels are shown in Fig 9. The diameter of the inhibition zone for the \( P(WPI-AM) \) gold nanocomposite hydrogel (Fig 9Ac 0.9cm and Fig 9Bc 1cm) is larger than that for AI with \( P(WPI-AM) \) hydrogel (Fig 9Ab 0.7cm and Fig 9Bb 0.6cm) samples, whereas the pure \( P(WPI-AM) \) hydrogels (Fig 9Aa 0.0cm and Fig 9Ba 0.0cm) showed no inhibition ability. Therefore, WPI in combination with gold nanocomposites hydrogels exhibit excellent antibacterial activity.
2.3.10. Biodegradation studies

Currently, many researchers have focused on biodegradable hydrogels, because of their activity and their biological interaction with body components; hence they are used in biomedical applications (32). The biodegradation property of pure \( P(WPI-AM) \) hydrogel and gold nanocomposite hydrogel developed, were carried out by weight loss methods. The degradation behaviors of \( P(WPI-AM) \) hydrogel and gold nanocomposites hydrogel are shown in Fig.10A. From the figure, it is observed that pure \( P(WPI-AM) \) hydrogel shows higher weight loss (%) than \( P(WPI-AM) \) gold nanocomposite hydrogels. This is due to the fact that gold nanoparticles that escape from the hydrogel in aqueous medium got attached to the negatively charged bacterial cell wall, which causes cell death to the bacteria. Therefore, cells metabolic activity is reduced (degradation also less). But this is not the case for pure \( P(WPI-AM) \) hydrogel which does not have inorganic gold nanoparticles. Therefore, it readily undergoes degradation when compared to gold nanocomposites.
Similarly, this type of phenomena was observed in the enzymatic biodegradation of these hydrogels. The resulting degradation profiles of the bio-nanocomposite hydrogels are shown in Fig. 10B. However, the hydrogels show different degradation rates, depending on the WPI content in the hydrogels. It was found that a higher content of WPI in the \((P(WPI-AM)\) hydrogel and gold nanocomposite hydrogel), resulted in a faster degradation rate. Moreover, the gold nanoparticle contained hydrogel, shows less degradation than the \(P(WPI-AM)\) hydrogels.

**Fig 10:** Biodegradation of: A) WPI-hydrogels \((P(WPI-AM)1, P(WPI-AM)4)\) and gold nanocomposite \((P(WPI-AM)1 + Au^0 \) and \(P(WPI-AM)4 + Au^0\) hydrogels by *E. coli* and B) Weight loss (%) of WPI-hydrogels \((P(WPI-AM)1\) and \(P(WPI-AM)4\) and gold nanocomposite \((P(WPI-AM)1 + Au^0 \) and \(P(WPI-AM)4 + Au^0\) hydrogels incubated in proteinease K for 30 days.
2.4. Conclusion

Biodegradable \( P(WPI-AM) \) gold nanocomposite hydrogels with excellent antimicrobial properties have been successfully synthesized through a green process (the gold nanoparticles were prepared by reducing \( HAuCl_4 \) with \( Azadirachta Indica \) in the \( P(WPI-AM) \) hydrogels network). These composites were developed and characterized by spectral, thermal and electron microscopy studies. These results showed that gold nanoparticles were dispersed in the \( P(WPI-AM) \) hydrogel and that strong interaction was formed between the WPI and gold particles. The \( P(WPI-AM) \) hydrogel gold nanocomposites exhibited a strong antibacterial activity against \( Streptococcus pyogenes \) and \( Escherichia coli \). These agents will easily find applications in wound/burn dressings.

This work is an important contribution in the field of Bio-degradable metal nanocomposites prepared via green process for bio-medical applications.

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2.5. References


