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Development of novel biodegradable Au nanocomposite hydrogels based on wheat: For inactivation of bacteria

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ABSTRACT

The design and fabrication of novel biodegradable gold nanocomposites hydrogels were developed as antibacterial agent. Biodegradable gold nanocomposite hydrogels were developed by using acrylamide (AM) and wheat protein isolate (WPI). The gold nanoparticles were prepared as a gold colloid by reducing $HAuCl_4 \cdot XH_2O$ with leaf extracts of *Azadirachta indica* (neem leaf) that formed hydrogel network. The characterization of developed biodegradable hydrogels were studied using fourier transforms infrared (FTIR) spectroscopy, ultraviolet–visible (UV–vis) spectroscopy, X-ray diffraction (XRD), thermo-gravimetric analysis (TGA), differential scanning calorimetry (DSC), scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS) and transmission electron microscopy (TEM). The biodegradable gold nanoparticle composite hydrogels developed were tested for antibacterial properties. The results indicate that these biodegradable gold nanocomposite hydrogels can be used as potential candidates for antibacterial applications.

1. Introduction

Biodegradable hydrogels containing metal nanoparticles have been actively studied for applications in the biomedical and biotechnological fields (Ramesh Babu, Kim, Kim, Ahn, & Yong-Ill, 2010; Sorrentino, Gorrasi, & Vittoria, 2007). In general, their origins are (in most cases) from natural materials. Wheat isolate (globular protein) is one of the biological proteins (Smithers, 2008) that is in great abundance in nature with low cost of amino acids source and has good biodegradability potential (Fitzsimons, Mulvihill, & Edwin, 2008; Jagadeesh, Jeevan Prasad Reddy, & Varadarajulu, 2011). Due to these properties, they are used as recombinants in DNA technology (Shanshan, Cao, & Hongbin, 2012) and for biomedical applications (Bounous, Batist, & Gold, 1991; Regester, Belford, West, & Goddard, 2003). Recently, Betz et al. (2012) reported the antioxidant capacity of bilberry extract microencapsulated in whey protein hydrogels. In their study, they provided new insights regarding protein-based microencapsulation of phenolics and studied their degradation property. Gunasekaran, Sanghoon, and Lan (2007) prepared whey protein hydrogels for encapsulation and control delivery applications. Doherty et al. (2012) fabricated whey protein micro beads 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 (Jonker, Lowik, & Van Hest, 2012; Van Vlierberghe, Dubruel, & Schacht, 2011). These biomaterials provide unique properties to address some of the challenges in biology, medicine and material science (Smithers, 2008). To enhance their bioactivity in wound care applications, a number of researchers introduced inorganic metals (Murali Mohan et al., 2010; Varaprasad, Murali Mohan, & et al., 2010; Varaprasad, Ravindra, Narayana Reddy, Vimala, & Mohana Raju, 2010; Ranga Reddy, Varaprasad, Narayana Reddy, Mohana Raju, & Subbarami Reddy, 2012). Because inorganic nanoparticles can easily functionalize with biomaterials, this characteristic makes them attractive in the biomedical and biotechnological fields (Vimala, Samba Sivudu, Murali Mohan, Sreedhar, & Mohana Raju, 2009). Recently, hydrogels with gold nanoparticles have attracted attention for their potential applications as antimicrobial materials (Ranga Reddy et al., 2012; Vimala et al., 2009; Luo, Xu, Zhang, Yang, & Chen, 2005). They can be prepared by chemical, photoinduced and microwave-assisted reduction methods, but the chemical reduction methods are the most common (Shiv Shankar, Akhilesh, Absar, & Murali, 2004). The reduction of gold nanoparticles procedures depends on toxic chemicals (Murali Mohan, Lee, Premkumar, & Geckeler, 2007; Zan, Kozlov, McCarthy, & Su, 2010). To overcome this

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

Hydrogel code	AM (mM)	WPI (g)	MBA (mM)	KPS (mM)	TMEDA (mM)
P(WPI-AM)0	14.084	–	0.648	1.851	0.8620
P(WPI-AM)1	14.084	0.10	0.648	1.851	0.8620
P(WPI-AM)2	14.084	0.15	0.648	1.851	0.8620
P(WPI-AM)3	14.084	0.25	0.648	1.851	0.8620
P(WPI-AM)4	14.084	0.35	0.648	1.851	0.8620

problem, many researchers have introduced the green process (Carlo et al., 2012). In the green process, few researchers used plant leaf extracts as reducing agents for metal nanoparticles, which are cost-effective and also utilize ambient condition for reduction reaction (Vivekanandhan, Christensen, Misra, & Amar Kumar, 2012). Therefore, the development of metal nanoparticles based on natural extracts is considered the most appropriate method for obvious environmental reasons (Iravani, 2011).

In view of the importance of the above work, the present investigation involves the development of gold nanoparticles in wheat protein isolate based polyacrylamide by reducing the hydrogel with 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 microscopy-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. Herein, a study of the design of P(WPI-AM) gold nanocomposites hydrogels for significant antibacterial applications is presented.

2. Materials

Wheat protein isolate (WPI) powder was obtained from Honeyville Food Products, Salt Lake City, Utah, and USA. WPI was reported, by the manufacturer, to contain 90% protein, 4% fat (acid hydrolysis), about 5% ash and 1% other minor constituents. Acrylamide (AM), *N*,*N*′-methylenebisacrylamide (MBA), potassium persulphate (KPS), *N*,*N*,*N*′,*N*′-tetramethylrthylenediamine (TMEDA), gold chloride ($HAuCl_4{\cdot}XH_2O$) and sodium hydroxide (SH) were purchased from S. D. Fine Chemicals (Mumbai, India). All chemicals were used without further purification.

2.1. Preparation of the leafs extract

Leaf extracts were prepared by a green process technique, using the standard procedure described by Ravindra, Murali Mohan, Narayana Reddy, and Mohana Raju (2010). Neem leafs (*Azadirachta indica*) were collected from neem tree and thoroughly washed with distilled water. Neem leaf broth was prepared by taking 25 g of thoroughly washed leafs and finely cut leaves in a 1000 ml Erlenmeyer flask with 500 ml of sterile distilled water. The solution was heated at 100 °C for 2 min in order to extract the contents of the leaves and filtered through 0.45 μm PVDF Millex Filter using a 50 ml syringe. The extracted leaves solutions were stored at 4 °C.

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

Different amount of wheat protein isolate powder were dissolved in (0.05 N) aqueous sodium hydroxide solution in a 50 ml beaker. To this solution, 1 g of AM, 1 ml of MBA and 1 ml of KPS/1 ml of TMEDA as an initiating system, were added. Each mixture was stirred for 30 min over a magnetic stirrer at 100 rpm. The gel matrix formed was carefully transferred into a 1 l beaker containing 500 ml distilled water and the distilled water was repeatedly changed (every 5 h) 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.

2.3. Preparation of gold nanocomposite hydrogels

To prepare WPI-based Au nanocomposite hydrogels 100 mg of dry P(WPI-AM) was equilibrated with distilled water for 48 h and then transferred to a beaker containing 30 ml of $HAuCl_4{\cdot}XH_2O$ (400 mg/150 ml) aqueous solution and then allowed to equilibrate for 24 h. 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 50 ml of cold neem leaf extracts (*A. indica* (AI)) solution. The beaker was left in the refrigerator (4 °C) for 8 h in order to reduce the Au^{3+} ions into Au° 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 method, the 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.

2.4. Swelling studies

Accurately weighed dry P(WPI-AM) based hydrogels were immersed in a 100 ml beaker containing twice distilled water, until the hydrogel reached equilibrium swelling at ambient temperature for 48 h. Swollen hydrogels were treated with $HAuCl_4$ and subsequently with *A. indica* (neem solution) via a green process as explained in the experimental section. The swelling ratio or swelling capacity ($S_{g/g}$) of the hydrogel developed and their nanocomposite was calculated using equation (1):

$$\text{Swelling ratio}\,(S_{g/g}) = \frac{[W_s - W_d]}{W_d} \quad (1)$$

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 is an average value of three individual sample readings.

3. Characterizations

3.1. Fourier transforms 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 h before their FTIR experiments. Samples were examined

between 500 and 4000 cm^{-1} on a Bruker IFS 66V FTIR spectrometer (Ettlingen, Germany), using the KBr disk method.

3.2. UV–visible

UV–vis spectra of P(WPI-AM) gold nanocomposites hydrogels were recorded on an ELICO SL 164 Model UV–vis spectrophotometer (The Elico co, Hyderabad, India) from 200 to 700 nm. For this study, 100 mg of P(WPI-AM) gold nanocomposite hydrogels were dispersed in 10 ml of distilled water and allowed to stand for 24 h 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.

3.3. 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).

3.4. 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_α radiation, $\lambda = 0.1546$ nm) at 40 kV and 50 mA.

3.5. SEM-EDS

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 samples were carbon-coated, prior to examination on a field emission scanning electron microscope.

3.6. TEM

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

3.7. Antibacterial activity

The antibacterial activity of the gold nanocomposite P(WPI-AM) hydrogels, under study, was investigated by disc method, using the standard procedure described elsewhere (Varaprasad, Murali Mohan, Vimala, & Mohana Raju, 2011; Varaprasad, Vimala, & et al., 2011). Nutrient agar medium was prepared by mixing peptone (5.0 g), beef extract (3.0 g) and sodium chloride (NaCl) (5.0 g) in a 1000 ml distilled water and the pH was adjusted to 7.0. Finally, agar (15.0 g) was added to the solution. The agar medium was sterilized in a conical flask at a pressure of 6.8 kg (15 lbs) for 30 min. 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 (*Streptococcus pyogenes* and *Escherichia coli*) (50 μl) culture was spread on the solid surface of the media. Over this inoculated Petri dish, one drop of gel solutions (20 mg/10 ml distilled water) was added using a 10 μl tip and the plates were incubated for 48 h at 37 °C.

3.8. Biodegradation characterizations

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

3.9. Method A

Nutrient agar medium was prepared by using the standard procedure described elsewhere (Varaprasad, Murali Mohan, & et al., 2011; Varaprasad, Vimala, & et al., 2011). The agar medium was sterilized by autoclaving at 121 °C for 30 min at a pressure of 6.8 kg (15 lbs). An *E. coli* bacterium was inoculated in this medium and the pure culture was maintained separately in the incubator. Then, to 10 ml of sterilized broth, 0.100 g each of the samples, i.e. both 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 *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 deionized water, oven-dried at 40 ± 1 °C for 24 h. Then, the samples were weighed to determine the weight loss.

3.10. 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, 0.01 M) with proteinese K, at a concentration of 0.2 mg/ml. The mixture was then incubated at 37 °C under constant shaking (60 rpm). 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:

$$W_r = \frac{W_d}{W_o} \quad (2)$$

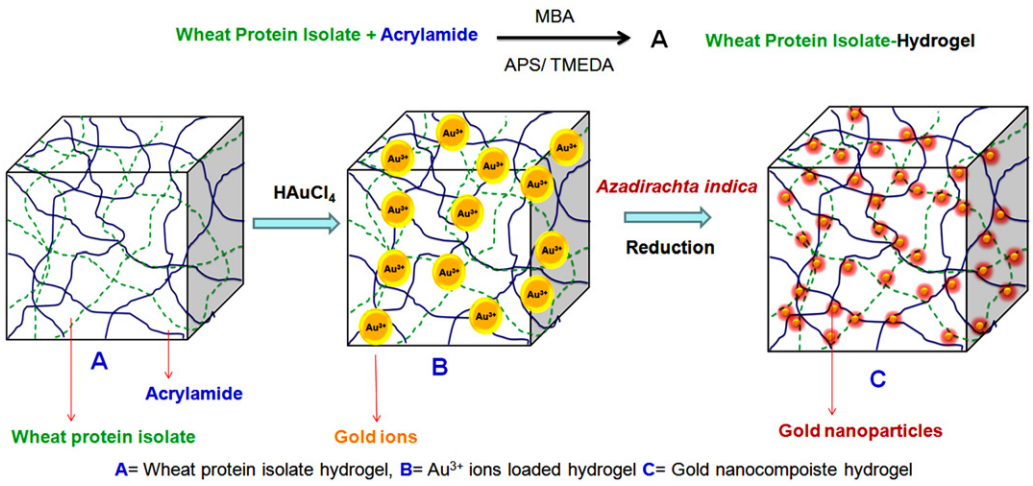
where W_o is the initial weight of the dried gel sample and W_d is the weight of the dried sample after degradation at a given time.

4. Results and discussion

During the past decade, inorganic biodegradable hydrogels have become an active field of research because of its tremendous potential for a variety of applications (Bardajee, Hooshyar, & Rezanezhad, 2012; Bojana, Jasmina, Zeljka, & Vesna, 2012). In this work, a 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. Scheme 1 illustrates the fabrication of gold nanocomposite hydrogels.

4.1. Swelling properties

The swelling capability of the hydrogels plays a significant role in their biomedical applications, particularly in antibacterial applications (Murali Mohan et al., 2010; Varaprasad, Murali Mohan, & et al., 2010; Varaprasad, Ravindra, & et al., 2010). Based on swelling properties, Varaprasad et al. (Varaprasad, Murali Mohan, & et al., 2010; Varaprasad, Ravindra, & et al., 2010; Varaprasad, Murali Mohan, & et al., 2011; Varaprasad, Vimala, & et al., 2011; Varaprasad et al., 2012) have prepared different type of hydrogels for drug delivery and antibacterial applications. However, in



Scheme 1. Schematic diagram for the formation of P(WPI-AM) gold nanocomposite hydrogels.

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. 1A. The values of the swelling ratio were influenced by the WPI concentration; with increase of the WPI ratio resulting in increases of the swelling ratio values. This is due to the hydrophilic nature of WPI. In addition, Fig. 1A indicates that the swelling capacity of the Au^{3+} ions loaded hydrogels and gold nanoparticles formed hydrogels, resulted in an increase of the WPI content in the hydrogel system. The swelling ratio depends on the Au^{3+} ions loading and the Au nanoparticle formed. However, the swelling capacity follows in this order: Au° hydrogel > Au^{3+} ions hydrogel > hydrogel. The reason being that when Au^{3+} ions loaded hydrogels are treated with *A. indica*, they turned into dark coffee colors, indicating the formation of nanoparticles throughout the hydrogel networks. During this step, the addition of many 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. (Varaprasad, Murali Mohan, & et al., 2010; Varaprasad, Ravindra, & et al., 2010; Varaprasad, Murali Mohan, & et al., 2011; Varaprasad, Vimala, & et al., 2011; Ranga Reddy et al., 2012; Varaprasad et al., 2012) also observed similar phenomena.

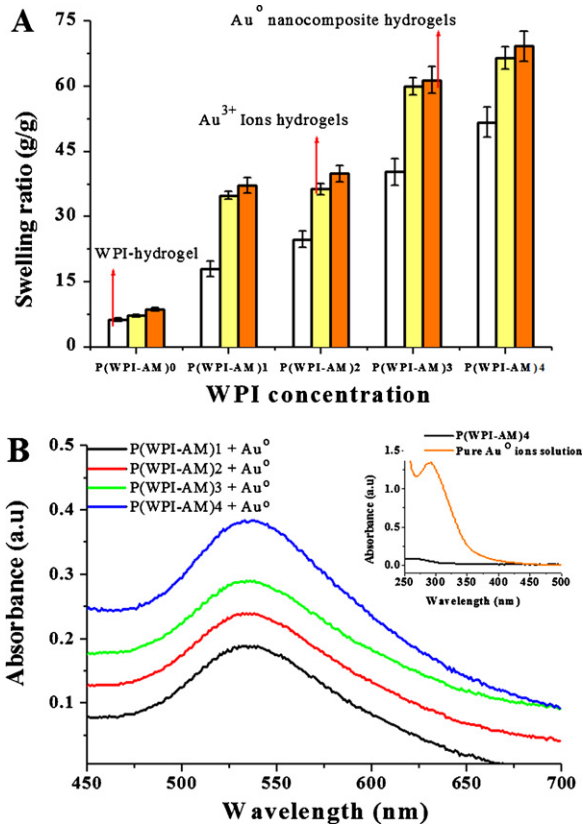


Fig. 1. Swelling behavior of A) WPI varied hydrogels, gold ion and gold nanocomposite hydrogels and B) UV–visible spectra of P(WPI-AM) gold nanocomposite (P(WPI-AM)1–P(WPI-AM)4) hydrogels (insert aqueous gold, Pure P(WPI-AM) hydrogel spectra).

4.2. UV–vis spectrophotometer

The formation of gold nanoparticles was also demonstrated by UV–vis spectra (Fig. 1B). The absorption spectra of the gold ions solution, pure P(WPI-AM) hydrogel, P(WPI-AM) gold nanocomposites are shown in Fig. 1B. In the figure, a strong absorption peak shows at λ_{max} 290 nm for Au^{3+} ion aqueous solution (Jiang, Wang, & Chen, 2007). A significant improvement in the absorption peak (λ_{max} = 530 nm) was observed for gold nanocomposite hydrogel (Kim, Boote, Pham, Hu, & Byun, 2012). 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 is no intensity peaks around 290 and 530 nm, as was observed in pure P(WPI-AM) hydrogel.

4.3. FTIR spectroscopy

The formation of gold nanoparticles inside the hydrogels network was also investigated by FTIR analysis (Fig. 2). The P(WPI-AM) hydrogel (Fig. 2A) shows absorption peaks at 1677.83 and 1452 cm^{-1} associated with to the C=O stretching vibrations of AM and WPI units. The peak observed at 3417.85 cm^{-1} is due to the stretching vibrations of NH_2 and COOH functional groups in the hydrogel networks. The gold nanocomposite (Fig. 2B) did show all the above characteristic peaks, with a slight shift in wavelengths (3440.24 cm^{-1} corresponding to NH_2 and COOH functional groups and 1659.44 and 1437 cm^{-1} relating to C=O stretching 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

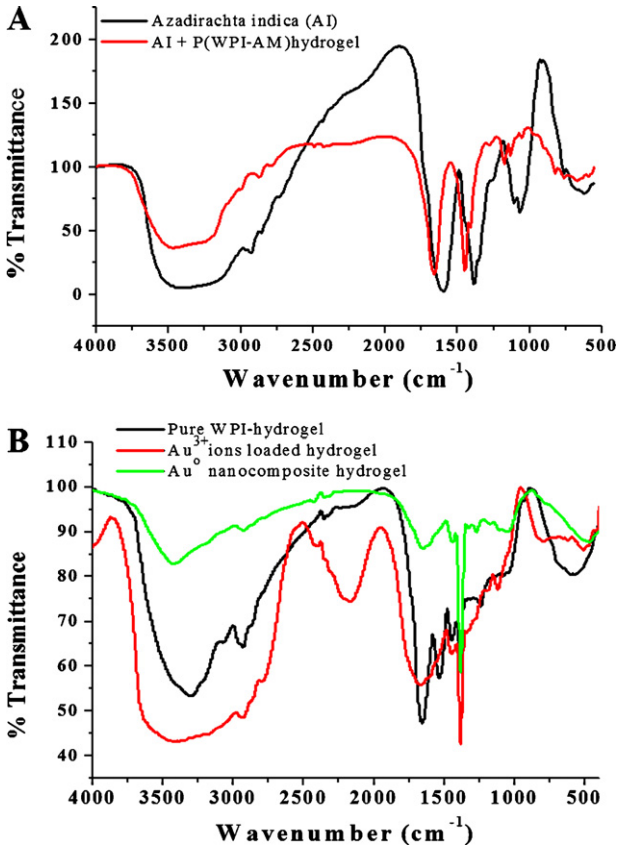


Fig. 2. FTIR spectra of: A) pure AI and AI + P(WPI-AM) hydrogel, B) pure WPI, pure P(WPI-AM) and P(WPI-AM) gold nanocomposite hydrogels.

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.

4.4. 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. 3 shows the SEM micrographs of the P(WPI-AM) and P(WPI-AM) gold nanocomposite hydrogels. Fig. 3a shows a clear surface feature for the pure P(WPI-AM) hydrogel, whereas gold nanoparticles loaded P(WPI-AM) hydrogel (Fig. 3b) 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 particles.

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. 3). The EDS spectrum for the neat hydrogel (Fig. 3a[1]) did not show the characteristic peak of gold, while the EDS spectrum of gold-loaded hydrogel (Fig. 3b[1]) showed clearly the peak of gold. The intensity of the gold peak is proportional to the metal concentration in the composites hydrogel. Hence, the existence of gold nanoparticles on the hydrogel is confirmed by EDS spectra. The use of WPI in the networks is to stabilize the gold nanoparticles formed in the hydrogel networks.

4.5. X-ray diffraction

Analysis of the X-ray diffraction patterns is a suitable technique to identify the crystallinity of the inorganic polymer materials. Fig. 4A shows the XRD pattern of P(WPI-AM) hydrogel stabilized gold nanoparticles, synthesized via a green process at ambient temperature. Fig. 4 XRD pattern shows clear peaks of gold nanoparticles in the faced-centered cubic (fcc) structure. The broad peak at 24.73° is characteristic of the crystal plane (1 1 0) of P(WPI-AM) hydrogel structure. The other five diffraction peaks at 2θ: 38.06, 44.10, 64.54, 77.42 and 81.81° correspond to the reflections of crystal planes (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2) respectively. On the other hand, the pure P(WPI-AM) hydrogel did not show any sharp intensity peaks in XRD diffractions pattern. Therefore, XRD

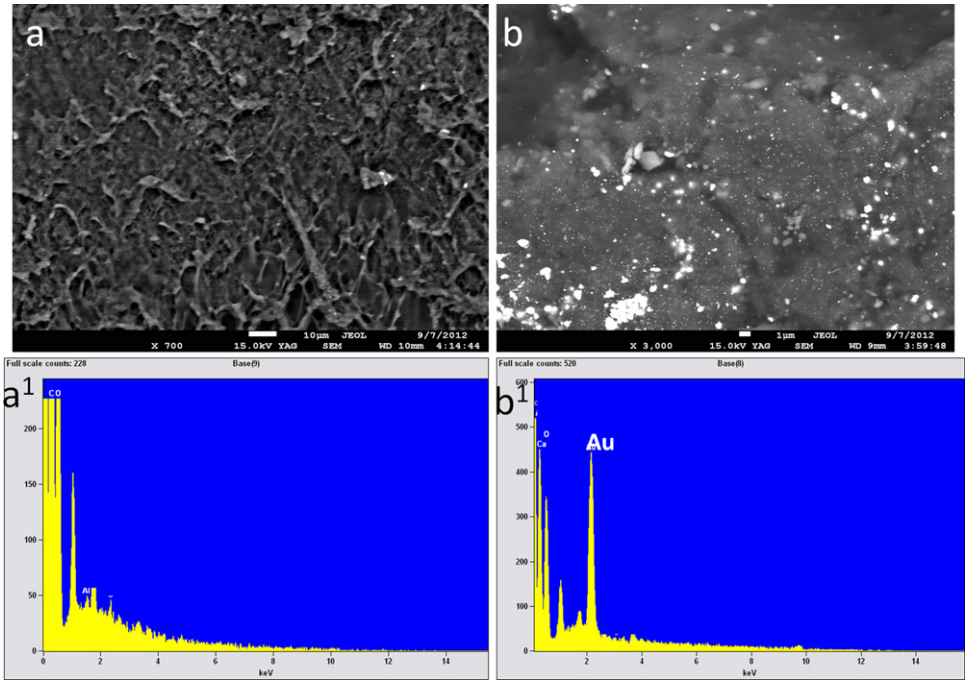


Fig. 3. SEM images of: a) P(WPI-AM), b) P(WPI-AM) gold nanocomposite hydrogels, EDS images of: a[1]) P(WPI-AM) and b[1]) P(WPI-AM) gold nanocomposite hydrogel.

spectrum confirms the presence of gold nanoparticles as revealed in the visible area of the spectrophotometer.

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

4.6. Thermal stability of the P(WPI-AM) gold nanocomposites

The thermal stability and the formation of the 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 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 thermogram, 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

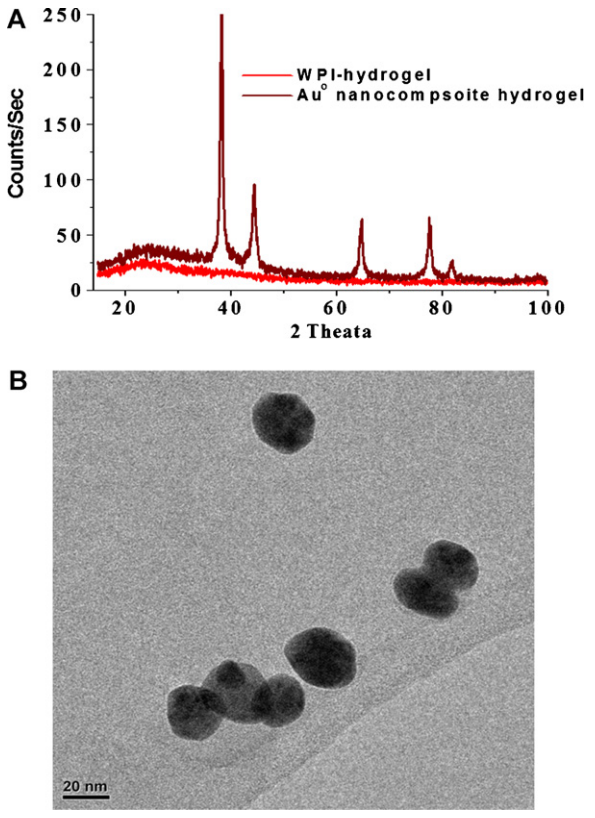


Fig. 4. XRD patterns of: A) pure P(WPI-AM)4 and P(WPI-AM)4 gold nanocomposites hydrogels, B) TEM images of gold nanoparticles hydrogel.

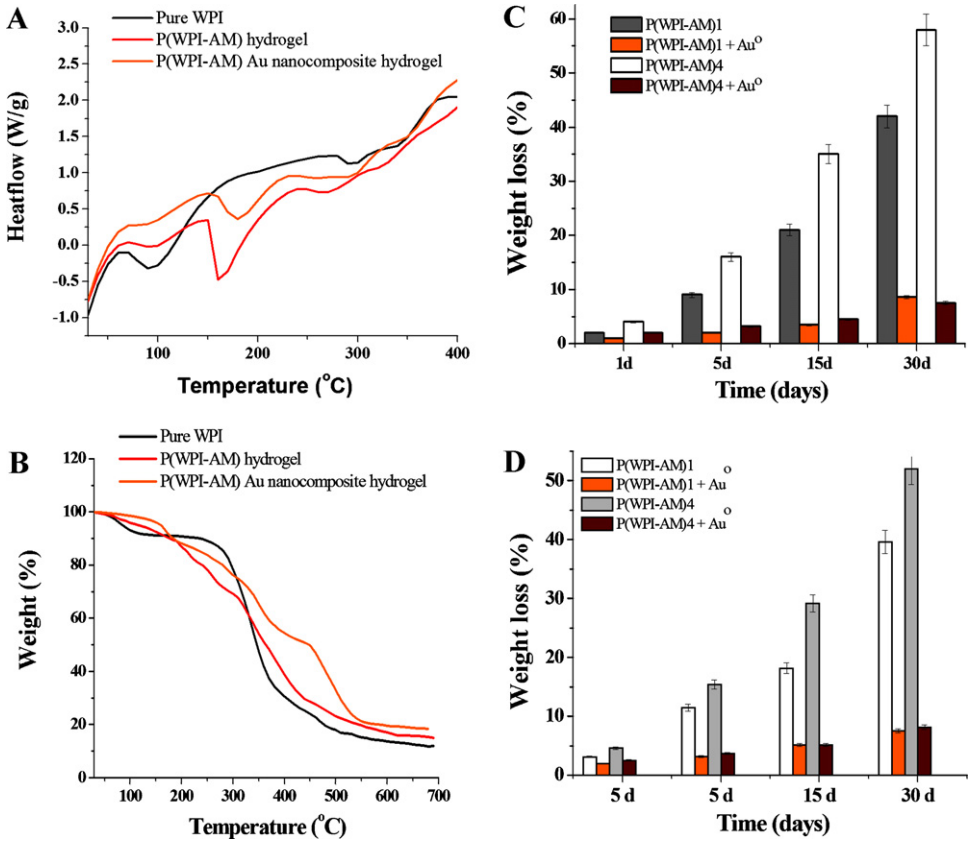


Fig. 5. DSC thermograms of: (A) pure WPI, P(WPI-AM)4 and P(WPI-AM)4 + gold nanocomposite hydrogels and TGA curves of: (B) pure WPI, P(WPI-AM)4 and P(WPI-AM)4 + gold nanocomposite hydrogels. Biodegradation of: C) WPI-hydrogels (P(WPI-AM)1, P(WPI-AM)4) and gold nanocomposite (P(WPI-AM)1 + Au° and P(WPI-AM)4 + Au°) hydrogels by *E. coil* and D) weight loss (%) of WPI-hydrogels (P(WPI-AM)1 and P(WPI-AM)4) and gold nanocomposite (P(WPI-AM)1 + Au° and P(WPI-AM)4 + Au°) hydrogels incubated in proteinese K for 30 days.

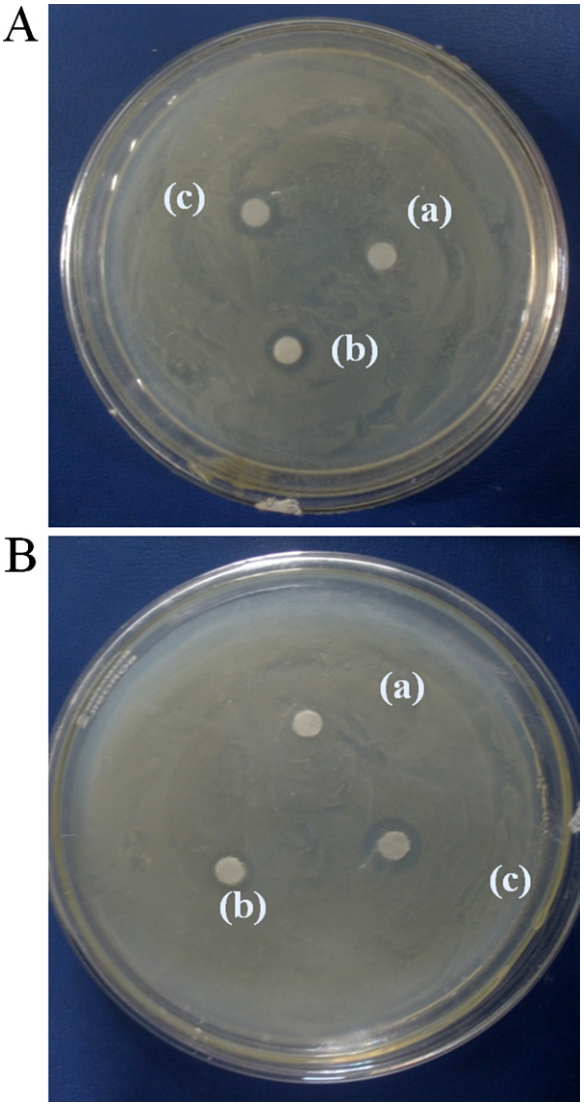


Fig. 6. Antibacterial activity of (A) a) plain P(WPI-AM)4 b) AI + P(WPI-AM)4 and c) P(WPI-AM) gold nanocomposite hydrogels on *S. pyogenes* and (B) a) plain P(WPI-AM)4, b) AI + P(WPI-AM)4 and c) P(WPI-AM) gold nanocomposite hydrogels on *E. coli*.

(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.

4.7. 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 (Bardajee et al., 2012). 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. 5C. From the figure, it is observed that pure P(WPI-AM) hydrogel shows high 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 nanocompoaites.

Similarly, these types of phenomena were observed in the enzymatic biodegradation of these hydrogels. The resulting degradation profiles of the bio-nanocomposite hydrogels are shown in Fig. 5D. However, the hydrogels show different degradations 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.

4.8. Antibacterial activity

Whey has been used in cancer therapy, wound care and repair applications (Bounous et al., 1991; Regester et al., 2003). Due to its unique properties, WPI was selected for the preparation of gold nanocomposite hydrogels. Similarly, *A. indica* is a highly biologically active compound (Schmutterer, 1995). It is a naturally occurring, non-toxic and bioactive agent in human life (Biswas, Chattopadhyay, Ranajit, & Uday, 2002).

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. 6. The diameter of the inhibition zone for the P(WPI-AM) gold nanocomposite hydrogel (Fig. 6Ac 0.9 cm and Fig. 6Bc 1 cm) is larger than that for AI with P(WPI-AM) hydrogel (Fig. 6Ab 0.7 cm and Fig. 6Bb 0.6 cm) samples, whereas the pure P(WPI-AM) hydrogels (Fig. 6Aa 0.0 cm and Fig 6Ba 0.0 cm) showed no inhibition ability. Therefore, WPI in combination with gold nanocomposites hydrogels exhibit excellent antibacterial activity.

5. 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 *A. 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 *S. pyogenes* and *E. coli*. These agents will easily find applications in wound/burn dressings.

Authors' contribution

KVP and KMR are responsible for design and execution of experiments and manuscript preparation. TJM, GMR and RS participated in experiments and manuscript preparation related to (swelling), (FTIR), DSC/TGA, (SEM-EDX) and (TEM). KVP is participated in the discussion of the results, providing expertise and extensive revise of the manuscript.

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