# Biosynthesis of Siver Nanoparticles by Using Papaya Fruit Extract

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**Abstract:** Nanotechnology in the latest contest is acquiring its potential due to its capability of changing metals into their nanoparticles. There are few studies on the nanoparticles in plants. Our present research is to investigate on the novel synthesis of biodegradable silver nanoparticles using fruit extract of papaya. In this green synthesis we used papaya fruit extract which acts as natural reducing agent to synthesize biodegradable silver nanoparticles by reducing AgNO<sub>3</sub> with fruit extract from papaya.Hydrogel of fruit extract characteristic color is changed from pale yellow to dark brown by reduction. Characterization of newly synthesized biodegradable silver nanoparticles was analysed by using UV – visible spectroscopic technique,FTIR,DSC,TGA,XRD,SEM and TEM.In the present study 10 nm size nanoparticles are synthesized. These nanoparticles have antimicrobial applications especially against Bacillus and Escherichia coli.

**Keywords:** Silver Nanoparticles, Acrylamide (AM), Green Synthesis, Transmission electron microscopy (TEM)

# I. Introduction

The development of green processes for the synthesis of nanoparticles is evolving into a significant field. In the present scenario nanomaterials have gained as innovative antimicrobial agents due to their high surface area to volume ratio and the unique chemical and physical properties. Chemical, Physical and Biological methods these nanoparticles can be synthesized.

Biosynthesis of nanoparticles using microorganisms, enzymes, and extract of plant have been suggested as environmental friendly alternatives to conventional methods, (Ahmad et al., 2004).

Metal nanoparticles have potential applications in catalysis, photonics, antimicrobial activity and optics (Wang et al., 2004; Biswas et al., 2002; Shipway et al., 2001; Nie et al., 1997; Govindraju et al., 2008; Govindraju et al., 2009). The biosynthesis of nanoparticles performed using micro-bial strains, enzymes ,metabolites (Ali et al., 2011) and plant extracts (Harekrishna et al., 2009; Nagati et al., 2013), and Biodegradable polymeric nanoparticles based drug delivery systems (Avnesh et al., 2010).

Silver nanoparticles are of between 1 nm and 100 nm in size and have gained a lot of future research concern. Biological synthesis of nanoparticles were synthesized by some researchers (Bhyan et al., 2007; Calvo et al., 2006) and testing for antimicrobial activities (Saxena et al., 2010; Khandelwal et al., 2010; Thirumurgan et al., 2010). silver nanoparticles are capable of rendering high antifungal efficacy and hence has a great potential in the preparation of drugs used against fungal diseases. Nanosilver is highly toxic to several strains of bacteria including so called gram positive bacteria such as Staphylococcus aureus and streptococcus pneumonia and gram negative bacteria including E.coli and Pseudomonas aeruginosa.

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 (Jayaramudu et al., 2013). Wheat protein isolate-based inorganic biodegradable hydrogels are also used for inactivation of bacteria.

Papaya fruit extract for Ag-nanoparticals synthesis was reported first by (Devendra J et al., 2009) and that was the first Ag-nanoparticals synthesis from any plant fruit extract. Synthesis of Ag- nanoparticals from papaya was also reported by (Namrata et al., 2009) using Callus extract of papaya. The size of spherical Ag-nanoparticles of 60-80 nm was reported. Later, (Farooqui M et al., 2013) have demonstrated the synthesis of Ag- nanoparticles which are highly toxic against different multidrug resistant human pathogens using papaya fruit extracts. Enamala Narmadha et.al. reported 10 nm nanoparticle size when papaya fruit extract was used.

Natural polymer component of a biodegradable hydrogel can consist of three dimensional network structures which are biocompatible and biodegradable (Sun, Zhuo, & Liu, 2003). In general, biomaterial based hydrogels are developed by using natural and synthetic components (polymers) (Varaprasad, K., Murali Mohan, Y., Vimala et al., 2011), Murthy, P.S.K., Murali Mohan, et al., 2008). It consists of inorganic-based ones are

particularly promising for inactivation of bacterial applications in materials and engineering science, have generated a lot of interest (Jayaramudu et al., 2013).

Biomaterial based inorganic nanocomposite hydrogels are obtained by embedding various types of metallic nanoparticles: metals, clay, and ceramics in hydrogel matrix. However, greater attraction in biomedical field is due to the incorporation of natural component/compound in hydrogels because of their biocompatibility, biodegradability and their non-toxic nature.

The fabricated hydrogels and their nanocomposite hydrogels structural and morphological studies were carried out by using Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD) and the formation of the  $Ag^0$  nano particles was confirmed by using UV-vis spectra. The thermal stability, surface morphology, the amount and distribution of  $Ag^0$  nano particles in Poly (Papaya Fruit Extract-Acrylamide) P(PFE-AM) hydrogels were determined by thermo-gravimetric analysis (TGA), scanning electron microscopy-energy dispersive spectroscopy (SEM) and transmission electron microscopy (TEM).

In the present research the effect of  $Ag^0$  nano particles on the antibacterial activity of the P(PFE-AM) hydrogels was studied. In this investigation the fabrication of Poly (Papaya Fruit Extract-Acrylamide) P(PFE-AM)  $Ag^0$  nanocomposite hydrogels for significant antibacterial applications is presented. Hence, the present work deals with the simple, effective, low cost biological (green) synthesis of silver nanoparticles using papaya fruit extract. Further, the bioreduction process was monitored by the UV-visible spectroscopy.

#### **II.** Materials

Papaya fruit was used to make the aqueous extract. Unripe papaya fruit weighing 10g was thoroughly washed in distilled water, whipped slightly with tissue paper, cut into small pieces and crushed into 100 ml distilled water with the help of mortar and pestle. Then the mixture filtered through Watman No1 filter paper. The filter was further filtered through another Watman No1 filter paper. Papaya (Papain) Fruit extract (PFE) - 10% (w/w) in the Sri Krishnadevaraya University Campus, Acryl amide (AM), N, N<sup>1</sup>-methylenebisacrylamide (MBA), potassium persulfate (KPS) and N, N, N<sup>1</sup>, N<sup>1</sup>-tetramethylethylenediamine (TMEDA) was purchased from S.D Fine Chemicals (Mumbai, India). Silver nitrate AgNO<sub>3</sub> was supplied by Merck (Mumbai, India). All the chemicals were used without further purification. Throughout the experiments double distilled water was used. The department of Microbiology (Sri Krishnadevaraya University, Anantapur, India) has provided standard cultures of the micro organisms.

#### 2.1. Preparation of poly (Papaya Fruit Extract-Acrylamide P(PFE-AM) Hydrogels

Different amounts (1 ml, 2ml, 3ml, and 4ml) of papaya fruit extract were taken in a 50ml beaker. To this solution, 1g of AM, 1ml 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(PFE-AM) hydrogels obtained were allowed to dry at ambient temperature for 2 days. The feed Composition of the gels prepared is presented in Table 1.

#### 2.2 Fabrication of silver nanocomposite hydrogels

Briefly, 500 mg of dry hydrogels were equilibrated in double distilled water for 48 h and the swollen hydrogel species were transferred in to a beaker containing 50 ml of  $AgNO_3$  (100.07 mM (5.1 g/ 300 ml)) aqueous solution and then allowed to equilibrate for 24 h. During this equilibrium stage, the  $Ag^+$  ions are being exchanged from solution to the P(PFE-AM) hydrogel networks. The beaker was left in the refrigerator (4<sup>o</sup>C) for 8 h in order to reduce the  $Ag^+$  ions into  $Ag^0$  nanoparticles. In this process papaya fruit extract act as self reducing agent. The  $Ag^0$  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 papaya fruit extract -based hydrogels were prepared by varying the papaya fruit extract concentration.

# 2.3. Characterization

## Fourier transform infrared (FTIR) spectroscopy

FTIR spectrophotometer is used to study the transmission of the hydrogel pattern,  $Ag^+$  ions incorporation and  $Ag^0$  nanoparticles patterns in hydrogel networks. The hydrogels and the  $Ag^0$  nano particlesembedded P(PFE-AM) hydrogels were completely dried in the oven (Baheti Enterprises, Hyderabad, India) at  $60^{\circ}$ C for 6 h 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.

#### UV-vis spectrophotometer

UV–vis spectra of P(PFE-AM)  $Ag^0$  nanocomposites hydrogels were recorded on an ELICO SL 164 Model UV–vis spectrophotometer (The Elico co, Hyderabad, India) from 300 to 550 nm. For this study, 100 mg of P(PFE-AM)  $Ag^0$  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  $Ag^0$  nanoparticles into aqueous phase and these solutions were recorded for their UV-vis absorption spectra.

#### Thermal studies

Thermal analysis (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 10  $^{0}$ C/min under a constant nitrogen flow (100 ml/min).

### X-ray diffraction (XRD)

X-ray diffraction (XRD) method was used to identify the formation of  $Ag^0$  nano particles in the P(PFE-AM) hydrogels network. These measurements were carried out on dried and finely grounded samples on a Rikagu diffractometer (Cu, K $\alpha$  radiation,  $\lambda$ = 0.1546 nm) at 40 kV and 50 mA.

### Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) analysis of plain P (PFE-AM) hydrogel and Ag<sup>0</sup> nanoparticles impregnated P (PFE-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.

### Transmission electron microscopy

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(PFE-AM)  $Ag^0$  nanocomposite (1mg/1ml) solution on a 3 mm copper grid and dried at ambient temperature after removing the excess solution using filter paper.

### Swelling studies:

Sample Code	AM	PFE	MBA (mM)	KPS	TMEDA(mM)	Swelling ratio of
	(gm)	( <b>ml</b> )		( <b>mM</b> )		equilibrium(S <sub>eq</sub> )(S <sub>g/g</sub> )
P(PFE-AM) <sub>1</sub>	1	1	1	1	1	9.22275
P(PFE-AM) <sub>2</sub>	1	2	1	1	1	31.44673
P(PFE-AM) <sub>3</sub>	1	3	1	1	1	44.77300
P(PFE-AM) <sub>4</sub>	1	4	1	1	1	65.69686

Table 1: Preparation of biodegradable P(PFE-AM) hydrogels feed composition

The swelling ratios of hydrogel samples were measured at ambient temperature using a gravimetric method. The dried hydrogels were immersed in a 50 ml beaker containing double distilled water until their weight becomes constant. The hydrogels were then removed from water and their surfaces were blotted with filter paper before being weighed. Furthermore, the swollen hydrogels were treated with  $AgNO_3$  via a green process. The swelling ratio or swelling capacity (Sg/g) of the hydrogels developed and their nanocomposites were calculated using the equation 1:

Swelling ratio: 
$$(S_{g/g}) = \frac{(W_s - W_d)}{W_d}$$
 (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 4 individual sample readings. The swelling studies are presented in the form of graph in Fig.2 in the results & discussion section.

# 2.4. Antibacterial activity:

The antibacterial activity of the  $Ag^0$  nanocomposite P (PFE-AM) hydrogels, under study, was investigated by disc method, using the standard procedure described elsewhere (Vimala, Samba Sivudu, Murali Mohan, Sreedhar, & Mohana Raju, 2009; Varaprasad, Vimala, Ravindra, Narayana Reddy, Venkata Subba Reddy, et al., 2011; Varaprasad, Vimala, Ravindra, Narayana Reddy, & Mohana Raju, 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 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 using Autoclave 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 (*Bacillus* and *Escherichia coli*) (50

 $\mu$ l) 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  $\mu$ l tip and the plates were incubated for 48 h at 37  $^{0}$ C.

#### 2.5. Biodegradation characterizations:

Biodegradation study was performed by using the weight loss (%) method. In this method the gravimetric study was used by using AR0640 analytical balance (OHAUS Corp., Pine Brook; NJ, USA)

#### 2.6. Method

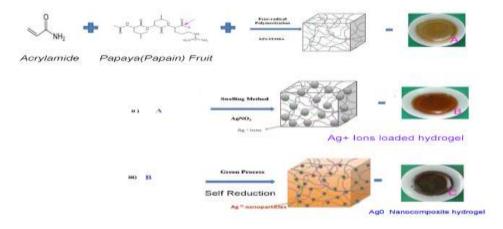
Nutrient agar medium was prepared by using the standard procedure described elsewhere (Varaprasad, Murali Mohan, et al., 2011;Varaprasad, Narayana Reddy, et al., 2011; Varaprasad, Vimala, Ravindra, Narayana Reddy, Venkata Subba Reddy, et al., 2011;Varaprasad, Vimala, Ravindra, Narayana Reddy, & Mohana Raju,2011)). The agar medium was sterilized by autoclaving at 121  $^{0}$ C for 30 min at a pressure of 6.8 kg (15 lbs). An *Escherichia 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(PFE-AM) hydrogel and their Ag<sup>0</sup> 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, 8, 15 and 30 days. After the required time period, samples were washed repeatedly with deionized water, oven-dried at  $40\pm1^{0}$ C for 24 h. Then, the samples were weighed to determine the weight loss. The ratio of weight remained (Wr) was calculated based on Eq.2:

$$W_r = \frac{W_d}{W_0} \tag{2}$$

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.

#### **III. Results and discussion**

The synthetic procedure of  $Ag^0$  nanocomposite P(PFE-AM) hydrogels consists of the following three steps, as shown, schematically, in Scheme 1: (i) the fabrication of P(PFE-AM) hydrogels via free-radical reaction; (ii) the addition of  $Ag^+$  ions-loaded hydrogels via swelling method and (iii) the formation of  $Ag^0$  nanocomposite P(PFE-AM) hydrogels via green process (The  $Ag^0$  nano particles were prepared by reducing AgNO<sub>3</sub> with self reduction in the P(PFE-AM) hydrogels network).

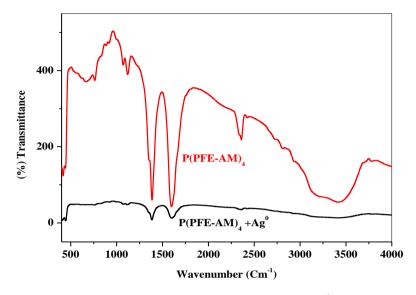


**Scheme1:** The synthetic procedure of  $Ag^0$  nanocomposite P(PFE-AM) hydrogels consists of the following three steps, as shown, schematically (i) the fabrication of P(PFE-AM) hydrogels via free-radical reaction; (ii) the addition of  $Ag^+$  ions-loaded hydrogels via swelling method and (iii) the formation of  $Ag^0$  nanocomposite P(PFE-AM) hydrogels via green process (The  $Ag^0$  nanoparticles were prepared by reducing  $AgNO_3$  with self reduction in the P(PFE-AM) hydrogels network

#### 3.1. Fourier transform infrared (FTIR) spectroscopy analysis:

The evidence for the successful preparation of  $Ag^0$  nanocomposite hydrogel was analyzed by FTIR spectral comparison, as shown in Fig. 1A. The spectrum of P(PFE-AM) sheets shows a broad absorption band at 3424 cm<sup>-1</sup> that is related to the NH asymmetric symmetric stretching vibrations groups, band at 2354 cm<sup>-1</sup> are

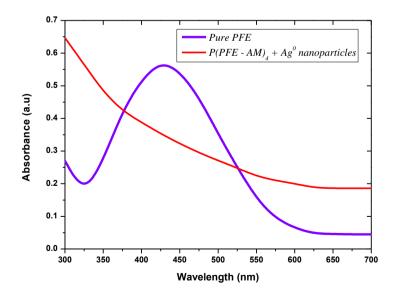
attributed to stretching vibrations of CH<sub>3</sub> units and an absorption band at 1615 cm<sup>-1</sup> from the carbonyl groups of P(PFE-AM) hydrogel (Soleimani, Sadeghi, & Shahsavari, 2012). These peaks have shifted to 3444, 2342 and 1604 cm<sup>-1</sup> in Ag<sup>0</sup> nanocomposite P(PFE-AM). As a result, it can be concluded that Ag<sup>0</sup> nanoparticles are present in P(PFE-AM) hydrogels.



**Fig.1** FTIR spectra of pure P(PFE-AM)<sub>4</sub> hydrogel and P(PFE-AM)<sub>4</sub>+Ag<sup>0</sup> nanocomposite biodegradable hydrogels

#### 3.2. UV–Visible Analysis:

To prove the formation of the  $Ag^0$  nanoparticles in the hydrogel networks the UV–Vis spectra was used. Fig.2 shows the absorption characteristics of the Pure PFE & their P(PFE-AM)  $Ag^0$  nanocomposite hydrogel. The characteristic plasmonic resonance peak of  $Ag^0$  nanoparticles was observed at  $\lambda_{max}$  430.50nm showing the absorption spectra of which measured after 5 hours and the absorbance peak observed at 430.50nm, broadening of peak indicated that the particles are polydispersed.However, no intense peak around 430.50nm was observed in P(PFE-AM)  $Ag^0$  hydrogel. This clearly indicates the formation of silver nano particles in P(PFE-AM) hydrogel and their dispersion.



**Fig.2** UV-vis of spectra of Pure PFE and P(PFE-AM)<sub>4</sub>+Ag<sup>0</sup>

<sup>3.3.</sup> Thermo gravimetric analysis (TGA):

Thermogravimetric analysis (TGA) was used to study the formation of  $Ag^0$  nano particles in the hydrogel network. The primary thermogram of the hydrogel and the nanocomposite hydrogel are shown in Fig.3. The thermal decomposition of P(PFE-AM) hydrogel occurred at  $500^{\circ}$ C with a significant weight loss (83%), but for the  $Ag^0$  nanocomposite hydrogel a comparatively very low weight loss (58.8%) was found at  $500^{\circ}$ C, which is due to the incomplete decomposition of the  $Ag^0$  nanoparticles. Moreover, according to the TGA results, the  $Ag^0$  nanocomposite hydrogels showed a higher thermal stability than the P(PFE-AM) hydrogel. The difference in be 24.2% and it confirms the presence of  $Ag^0$  nano particles (by weight loss) in the hydrogel.

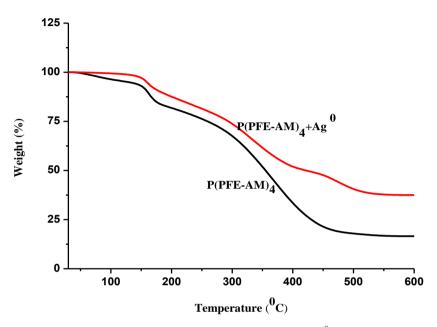
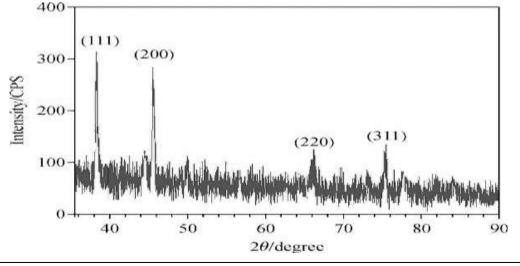


Fig.3 TGA curves of pure P(PFE-AM)<sub>4</sub> and P(PFE-AM)<sub>4</sub>+Ag<sup>0</sup> nanocomposite hydrogels

#### 3.4. X-ray diffraction (XRD) analysis:

The X-ray diffraction is a suitable technique to identify the crystallinity of the inorganic particles present in the hydrogels and hence the chemical composition of the material under investigation can be obtained. Fig.4 shows the XRD pattern of P(PFE-AM) hydrogel stabilized  $Ag^0$  nano particles, synthesized via a gas-solution process at room temperature. In Fig. 4 the insert figure shows the XRD pattern of pure P(PFF-AM) which exhibits strong and broad diffraction peaks at  $2\theta = 24.9$  and 41.30. The four other characteristic peaks showed in the case of  $Ag^0$  nanocomposite hydrogel indicates the face-centered cubic (fcc) structure. The diffraction peaks at  $2\theta = 38.9, 44.62, 64.9$  and 77.53 corresponding to the reflections of crystal planes (1 1 1), (2 0 0), (2 2 0) and (3 1 1) respectively. This face centered cubic (fcc) structure indicates that  $Ag^0$  nano particles were dispersed in the P(PFF-AM) hydrogels network



# Fig.4 XRD patterns of pure P(PFE-AM)<sub>4</sub> hydrogel and P(PFE-AM)<sub>4</sub> Ag<sup>0</sup> nanocomposite biodegradable

hydrogels

# 3.5. Scanning electron microscopy:

The surface morphology of P(PFE-AM) and  $Ag^0$  nanocomposite hydrogels were investigated with SEM. Fig.5(a) shows the SEM micrographs of the P(PFE-AM) and Fig.5(b) shows  $Ag^0$  nanocomposite hydrogels. It indicates a clear rough morphology for the pure P(PFE-AM) hydrogel, Fig.5(a) whereas  $Ag^0$  nano particles loaded P(PFE-AM) hydrogel as shown in Fig.5(b) exhibited smaller nano particles distributed throughout the gel matrix having porous in nature which is in confirmity with the UV-Vis spectra because PFE slightly escaped from the hydrogels as it can be seen in Fig.5(c). It is worth mentioning from the SEM analysis that no individual silver nano particles were observed outside the P(PFE-AM) hydrogels, indicating a strong interaction between the P(PFE-AM) and the  $Ag^0$  particles. In order to further confirm the presence of  $Ag^0$  nanoparticles in the P(PFE-AM) hydrogel and to determine the identity of the  $Ag^0$  nanoparticles.

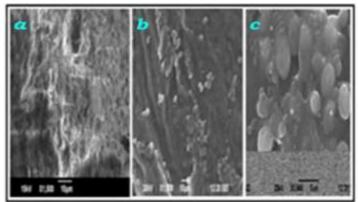


Fig.5 SEM images of: pure P(PFE-AM)<sub>4</sub>, P(PFE-AM)<sub>4</sub>+Ag<sup>+</sup> and P(PFE-AM)<sub>4</sub>+Ag<sup>0</sup> nanocomposite hydrogels

# 3.6. Transmission electron microscopy (TEM) analysis:

TEM analysis also confirmed the formation of spherical  $Ag^0$  nanoparticles in the P(PFE-AM) hydrogels network. The TEM image is shown in Fig.6 The average size of the nanoparticles was found to be about  $\Box 10 nm$ . It is evident that  $Ag^0$  nanoparticles were highly stabilized by using PFE in the hydrogel network. These results are mainly due to the strong interaction between the  $Ag^0$  nanoparticles and P(PFE-AM)<sub>4</sub>

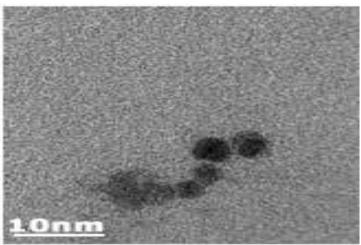


Fig.6 TEM images of P(PFE-AM)<sub>4</sub>+Ag<sup>0</sup> nanocomposite hydrogel

# 3.7. Swelling properties

The result present in Fig. 7 indicates that the  $Ag^0$  nanocomposite hydrogels have superior swelling ratio, when compared to the blank P(PFE-AM) hydrogels. The reason for this is that when  $Ag^+$  ions-loaded hydrogels were reduced by self reduction, the many  $Ag^+$  ions present in the hydrogels led to the formation of silver nanoparticles within the hydrogel, which expanded the gel networks and promoted higher water molecules uptake capacity. This interesting phenomenon can play significant role in biomedical applications, particularly in antibacterial applications. Based on this characteristic, (Varaprasad et al., 2011) have prepared different type of hydrogels for drug delivery and antibacterial applications.

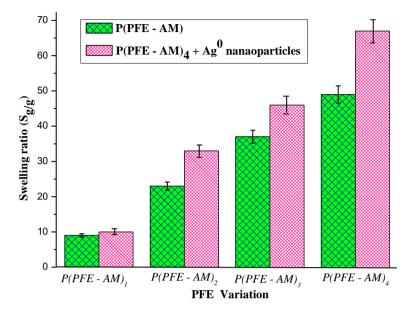
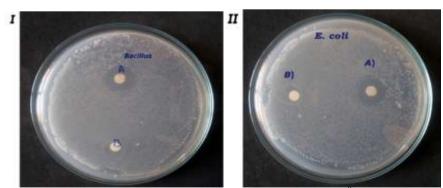


Fig.7 Swelling behaviour of P( PFE-AM) varied hydrogels and Ag<sup>0</sup> nanocomposite

# 3.8. Antibacterial activity:

 $Ag^0$  nanoparticles are well thought-out environmentally eco friendly and nontoxic antibacterial activity material, but the main disadvantage is their poor binding characteristic and stability, which restrict their utility. Therefore, polymerstabilized nanoparticles (Raghavendraa, G M., Jayaramudua et al.,) and nanoparticles embedded in hydrogel networks (Jayaramudua, T., Raghavendra, G. M et al.,2013) are outstanding approaches for antibacterial applications (Jayaramudua, T., Raghavendra, G. M et al.,2013). The antibacterial abilities of P(PFE-AM) and  $Ag^0$  nanoparticles contained hydrogels were investigated by calculating their capacity to inhibit the zone of bacillus and E. coli growth on agar culture dishes. After 48 h of incubation at 37 <sup>0</sup>C, there was the inactivation of bacterial zones and no bacterial colonies were clearly observed (Fig. 8) in the Petri dishes. The diameter of the inhibition zone for the  $Ag^0$  nanocomposite P(PFE-AM) hydrogel is as follows: (Fig. 8 I(A) (1.5 cm)and II(A) (1.2 cm)), whereas for the pure P(PFE-AM) hydrogels (Fig. 8 I(B) 0.0 cm andII(B) 0.0 cm), it showed no inhibition ability. Therefore, PFE in combination with  $Ag^0$  nanocomposites hydrogels exhibited excellent antibacterial activity.



**Fig.8.** Antibacterial activity of I(B) plain P(PFE-AM)<sub>4</sub>, I(A) P(PFE-AM)<sub>4</sub>+Ag<sup>0</sup> nanocomposite hydrogels on Bacillus and II(B) plain P(PFE-AM)<sub>4</sub>, II(A) P(PFE-AM)<sub>4</sub>+Ag<sup>0</sup> nanocomposite hydrogels on E.coli

# 3.9. Biodegradation studies

The biodegradation property of pure P(PFE-AM) hydrogel and Ag<sup>0</sup>nanocomposite hydrogel developed, were carried out by weight loss methods. The degradation behaviours of P(PFE-AM) hydrogel and Ag<sup>0</sup>nanocomposites hydrogel are shown in (Fig.9) From the figure, it is observed that pure P(PFE-AM)

hydrogel shows high weight loss (%) than P(PFE-AM) Ag<sup>0</sup> nanocomposite hydrogels. This is due to the fact that Ag<sup>0</sup> nano particles 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(PFE-AM) hydrogel which does not have inorganic Ag<sup>0</sup> nano particles. Therefore, it readily undergoes degradation when compared to Ag<sup>0</sup> nanocomposites.

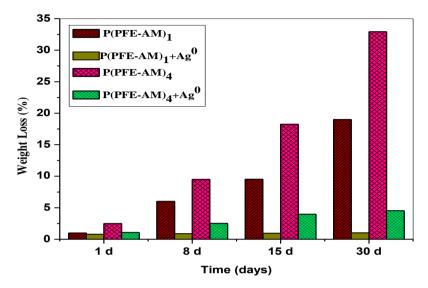


Fig.9 Biodegradation of PFE-hydrogels(P(PFE-AM)<sub>1</sub> and P(PFE-AM)<sub>4</sub>) and  $Ag^0$  nanocomposite (P(PFE-AM)<sub>1</sub>+Ag<sup>0</sup> and P(PFE-AM)<sub>4</sub>+Ag<sup>0</sup>) hydrogels by E.coli.

#### **IV.** Conclusion

An effective green process for the fabrication of a novel biodegradable silver nanocomposite hydrogel with papaya fruit extract as a stabilizing agent for the silver nanoparticles has been described. The  $Ag^0$  nanoparticles were prepared by reducing  $AgNO_3$  with papaya fruit extract. These composites were developed and characterized by spectral, thermal and electron microscopy. In our study it is found that nanoparticles obtained were sized  $\Box 10 nm$ . The synthesis of silver nanoparticles were confirmed by the change of colour papaya fruit extract. The  $Ag^0$  nanocomposite hydrogels prepared have significant antibacterial activity against Bacillus and E. coli. Toxicity studies of silver nanoparticles on human pathogen opens a door for a new range of antibacterial agents. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other nanomaterials.

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