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Natural Synthesis of Silver Nanoparticles by Banana Peel Extract and as an Antibacterial Agent

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Abstract: ***Natural of silver nanoparticles has now become a novel method to physical and chemical approaches. In the present study, silver nanoparticles (AgNPs) were synthesized from banana peel extract (BPE). Our present research is to investigate on the synthesis eco-friendly silver nanoparticles using banana peel extract. In this natural synthesis we used banana peel extract which act as natural reducing agent to synthesize biodegradable silver nanoparticles. Natural silver nanocomposite hydrogels were prepared by a green process using Acrylamide (AM) with banana peel extract. The silver nanoparticles were prepared by reducing $AgNO_3$ with banana peel extract. Banana peel extract was found to reduce the silver ions (Ag^+ to Ag^0) and capping agent. The characteristic color of hydrogel is changed to reddish brown in the reaction due to reduction. The UV-Vis spectrum of silver nanoparticles of our results revealed a characteristic surface plasmon- resonance (SPR) peak at 460 nm. X-ray diffraction (XRD) revealed their crystalline nature. Scanning electron microscope showed Monodispersed spherical shaped nanoparticles. The average size of nanoparticles was 10nm as confirmed by Transmission electron microscope. Fourier transform infrared spectroscopy (FT-IR) affirmed the role of BPE as a reducing and capping agent of silver ions. The antibacterial activity of these nanoparticles was also studied against Gram-positive and Gram-negative bacteria. The characteristic color of hydrogel is changed to reddish brown in the reaction due to reduction.***

Keywords: Silver nanoparticles, Banana peel extract, Antibacterial, Free radical, Polymerisation

1. Introduction

Nanoparticle research is inevitable because of its application and synthesis (Gopinath et al., 2012).Nanotechnology is a fast developing field with its application in science and technology. Nanoparticles are of great interest due catalytic, optical, magnetic, and electrical properties.

Nanoparticles have unique electrical, optical as well as biological properties and are thus applied in catalysis, biosensing, imaging, drug delivery, nanodevice fabrication and in medicine (Jain et al., 2008 and Nair and Laurencin, 2007).Synthesis of silver nanoparticles was extensively studied employing chemical and physical methods, but the development of reliable natural technology to produce nanoparticles is an important aspect of nanotechnology (Natarajan et al., 2010). Using microorganisms and enzymes, are suggested as possible natural alternatives (Mohanpuria, Rana, & Yadav, 2008).

The plants or plants extract, which act as reducing and capping agents for nanoparticles synthesis, are more advantageous over other biological processes (Valli & Vaseeharan, 2012), because they avoid the elaborated process of culturing and maintaining of the cell (Saxena, Tripathi, Zafar, & Singh, 2012). Moreover, nanoparticles from plant is preferred because it is cost-effective, ecofriendly, a single-step method for biosynthesis process and human friendly (Kumar & Yadav, 2009).

Different parts of plant materials such as extracts (MubarakAli, Thajuddin, Jeganathan, & Gunasekaran, 2011), fruit (Prathna, Chandrasekaran, Raichur, & Mukherjee, 2011), bark (Satishkumar et al., 2009), fruit peels (Bankar, Joshi, Kumar, & Zinjarde, 2010), root (Ahmad et al., 2010) and callus (Nabikhan, Kandasamy, Raj, & Alikunhi, 2010) have been studied so far for the synthesis of silver, gold, platinum and titanium nanoparticles in different sizes and shapes (Gopinath et al., 2012).

Natural nanoparticle synthesis is achieved by using plant extracts. Bananas are consumed all over the world, after consumption of the pulp, the peels are generally discarded (Bankar et al., 2010). A few applications of banana peels discussed in the literature include (i) exploitation for their medicinal properties (Parmar & Kar, 2008); (ii) in ethanol fermentation (Tewari, Marwaha, & Rupal, 1986); (iii) application as a substrate for generating fungal biomass (Essien, Akpan, & Essien, 2005); (iv) use in the production of laccase (Osma, Toca, Rodriguez Couto, 2007); and (v) utilization as a biosorbent for heavy metal removal (Annadurai, Juang, & Lee, 2003). In addition, banana peels that are inherently rich in polymers such as lignin, cellulose, hemicellulose and pectins (Emaga, Andrianaivo, Wathelet, Tchango, & Paquot, 2007) could be used in the synthesis of silver nanoparticles.

Banana peels are rich in polymers such as lignin, cellulose, hemicellulose and pectins could be used in the synthesis of silver nanoparticles. Pectin is a complex polysaccharide mainly consisting of D-galacturonic acid polymers form the backbone of pectin materials which link together through α-1,4-glycosidic linkage. Different pectins may have different side chains of arabinose, galactan, arabinogalactan, glucose, mannose and xylose (Lee *et al*., 2010). The extraction of pectins are reported in apple pomace, sugar beet, citrus peels, peach promace, and pumpkin (Qiu *et al*., 2010)and in banana skin Emaga *et al*., (2008).
The present study aims to synthesize silver nanoparticles by a green biological route, using an extract derived from banana peel waste, and characterization of the synthesized nanoparticles utilizing UV–visible spectroscopy, scanning

electron microscope (SEM), transmission electron microscope (TEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FT-IR) analysis.

There is an increasing interest in silver nanoparticles on account of the antimicrobial properties (Choi et al., 2008). Silver is a nontoxic, safe inorganic antibacterial agent that is capable of killing about 650 types of diseases causing microorganisms (Jeong, Yeo, & Yi, 2005).They are even being projected as future generation antimicrobial agents (Rai, Yadav, & Gade, 2009).

2. Materials

Fresh banana was obtained from (Sri Krishnadevaraya University, Campus, Anantapur), Acrylamide (AM), *N,N*^{l}-methylenebisacrylamide (MBA), Ammonium persulphate (APS), *N,N,N*^{l},*N*^{l}- tetramethylrthylenediamine (TMEDA), Silver nitrate (AgNO3) were supplied by Merck (Mumbai, India). All chemicals were used without further purification. Throughout the experiments double distilled water was used. The department of Botany (Sri Krishnadevaraya University, Anantapur, India) has provided standard cultures of the micro organisms.

2.1. Preparation of Banana Peel Extract (BPE)

Banana peels were cut it into small pieces (< 5 mm), washed three times with tap water and three times with distilled water to remove external dirt impurities present in it. Then, the peels were removed and dried on paper towelling. About 25 g of peel was taken in a 100 ml beaker containing 50 ml double distilled water and then the peel was boiled at 80^{0}C for 10 min and filtered through Whatman No. 1 filter paper twice to remove insoluble fractions and macromolecules. The resultant filtrate was stored at 4^{0} C. In this Banana peel extract act as a self reducing agent.

2.2. Preparation of Poly (Banana Peel Extract-Acrylamide P(AM-BPE) Hydrogels

To prepare the P(AM-BPE) hydrogels, the initial solution consisting of monomer AM (1g), double distilled water (3 ml) and various ratios (1ml to 2.5ml) of BPE was stirred at 300 rpm for 2 h at ambient temperature. Then the activator TMEDA (1.0 mM) was added with stirring. Finally, an aqueous solution of the initiator APS (1.0mM) and MBA as crosslinker (0.5mM) were added to the solution. After the addition of the reaction mixture by stirring at 100 rpm on a magnetic stirrer then the free-radical addition polymerization was carried out at ambient temperature. After the reaction has been completed, the hydrogels were immersed in distilled water separately at ambient temperature for 24 h to remove the un-reacted materials present in the hydrogel network. Finally, the hydrogel was dried at ambient temperature for 48 h. Similarly, other hydrogels were prepared by the above procedure.

2.3. 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(AM-BPE) hydrogel networks. The Ag^{+} ions loaded P(AM-BPE) hydrogels were wiped off using tissue paper. The beaker was left in the refrigerator (4^{0}C) for 5 h in order to reduce the Ag^{+} ions into Ag^{0} nano particles. The Ag^{0} nano particles in the hydrogel obtained were allowed to dry at ambient temperature and the product was used for further studies. In a similar method, the BPE-based hydrogels were prepared by varying the BPE concentration. It was powdered and used for characterization. Table 1 illustrates the various components used in the preparation of P(BPE-AM) hydrogels.

2.4. 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 particles-embedded P(AM-BPE) hydrogels were completely dried in the oven (Baheti Enterprises, Hyderabad, India) at 60^{0}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.

2.5 UV-vis spectrophotometer

UV–vis spectra of P(AM-BPE)+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(AM-BPE)+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.

2.6 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).

2.7 X-ray diffraction (XRD)

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

2.8 Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) analysis of plain P(AM-BPE) hydrogel and Ag^{0} nanoparticles impregnated P(AM-BPE) 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.

2.9 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(AM-BPE)+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.

2.10 Swelling studies

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

Sample Code	AM (gm)	BPE (ml)	MBA (1%)(mL)	APS (1%)(mL)	TMEDA(1%)(mL)	Swelling ratio of equilibrium(S_{eq})($S_{g/g}$)
$P(AM\text{-}BPE)_1$	1	1	0.5	1	1	6.314
$P(AM\text{-}BPE)_2$	1	1.5	0.5	1	1	10.587
$P(AM\text{-}BPE)_3$	1	2.0	0.5	1	1	25.900
$P(AM\text{-}BPE)_4$	1	2.5	0.5	1	1	36.258

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:

$$Swelling ratio : (S_{g/g}) = \frac{(W_s - W_d)}{W_d} \quad (1)$$

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 4 individual sample readings. The swelling studies are presented in the form of graph in Fig.2 in the results & discussion section.

2.11 Antibacterial activity

The antibacterial activity of the Ag^0 nanocomposite P(AM-BPE) 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 culture *(*Staphylococcus aureus and *Escherichia coli)* (50 µl) 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 ^{0}C. After the incubation period, the zone of inhibition (in mm diameter) was observed and tabulated.

3. Results & Discussion

3.1 Characterization

FTIR measurements were carried out to identify the major functional groups on the $P(AM\text{-}BPE)_4$ surface and their involvement in the synthesis and stabilization of silver nanoparticles. The spectra of $P(AM\text{-}BPE)_4$ before and after reaction with silver nitrate are represented in Fig. 1. Control spectrum ($P(AM\text{-}BPE)_4$ untreated with $AgNO_3$) showed several peaks indicating the complex nature of the biological material. The bands appearing at 2798.4, 2428.5, 1595, 1384, 1108.7, 915.6,762.6 and 616.6 cm_1 were assigned to stretching vibration of O-H of alcohol or N-H of amines, C-H of alkanes, C=O of carboxylic acid,or ester, N-C=O of amide I bond of proteins , CH_2 of alkanes, C-O of carboxylic acid , ester, or ether ,C-N of aliphatic amines or alcohol/phenol, N-H deformation of amines, and C-C bending, respectively (Socrates, 1980). After reaction with $AgNO_3$ there was a shift in the following peaks: 2798.4 to 2926.9, 2428.5 to 2363.9, 1595 to 1598.6, 1384 to 1414.1, 1108.7 to 1150.1, 915.6 to 1021and 616.6 to 557.6cm^{-1} indicating that carboxyl, hydroxyl and amide groups on the surface of $P(AM\text{-}BPE)_4$ +Ag^0 nanocomposites may be participating in the process of nanoparticle synthesis (Bankar et al., 2010). Banana peel extract are mainly composed of pectin, cellulose and hemicelluloses (Emaga et al., 2007) and the functional groups associated with biological components are known to interact with metal salts and mediate their reduction to nanoparticles (Bar et al., 2009).

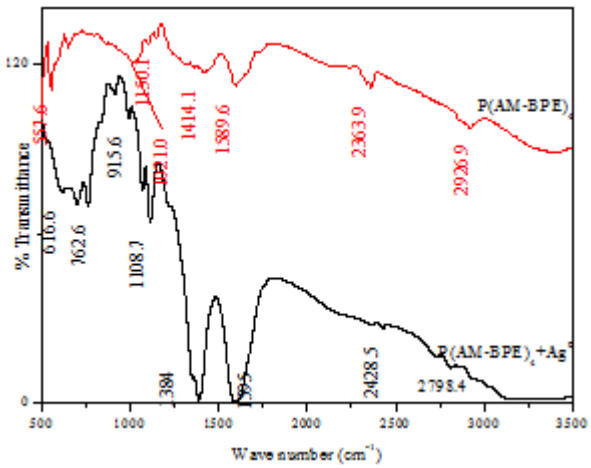

Figure 1: FTIR spectra of pure $P(AM\text{-}BPE)_4$ hydrogel and $P(AM\text{-}BPE)_4$+Ag^0 nanocomposite hydrogel

3.2 Visual observation and UV–visible spectroscopy

Noble metals exhibit unique optical properties due to surface plasmon-resonance (SPR) (Bindhu & Umadevi, 2013). Formation of silver nanoparticles was predicted by *the color change from yellowish brown to reddish brown.* The UV intense absorbance peaks at, 454.07, 455.42, 456, 456.10 and 460 nm are observed i.e., in between 450-460 excitation vibrations of the silver nanoparticles. It is due to the reduction of silver ions (Ag^{+})into silver nanoparticles(Ag°) in the BPE (Ahmad et al., 2003).Red shift indicates either an increase in the size or aggregation of AgNPs in BPE. The same was confirmed by TEM analysis. These results are in agreement with (Mulvaney, 1996) who obtained peak at λ 460 nm

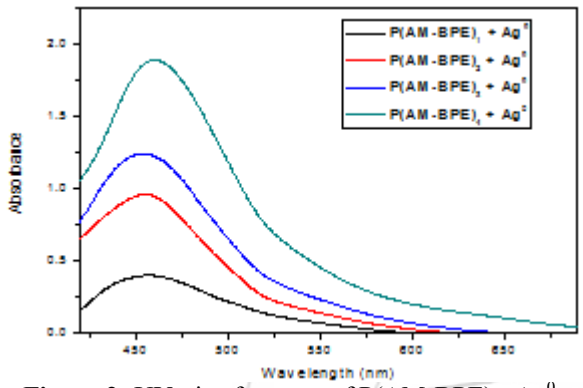

Figure 2: UV-vis of spectra of P(AM-BPE) $+Ag^{0}$ nanocomposite hydrogels

3.3 Thermogravimetric Analysis

The initial weight loss (around 100^{0}C) observed is due to loss of moisture present in the hydrogels. The weight loss observed in the case of P $(AM\text{-}BPE)_4$ hydrogels is 78.17% at 487^{0}C where as the weight loss observed for $P(AM\text{-}BPE)_4$ $+Ag^{0}$ at this temperature is 76.29%. The weight loss difference between the $P(AM\text{-}BPE)_4$ and $P(AM\text{-}BPE)_4$ $+Ag^{0}$ represents the presence of silver nanoparticles in $P(AM\text{-}BPE)_4$ $+Ag^{0}$. The percentage amount of silver nanoparticles present in the $P(AM\text{-}BPE)_4$ $+Ag^{0}$ hydrogels can be calculated from the difference in the weight loss between the $P(AM\text{-}BPE)_4$ and $P(AM\text{-}BPE)_4$ $+Ag^{0}$ hydrogels at 800^{0}C (~8%), which is ~ 7.89%.

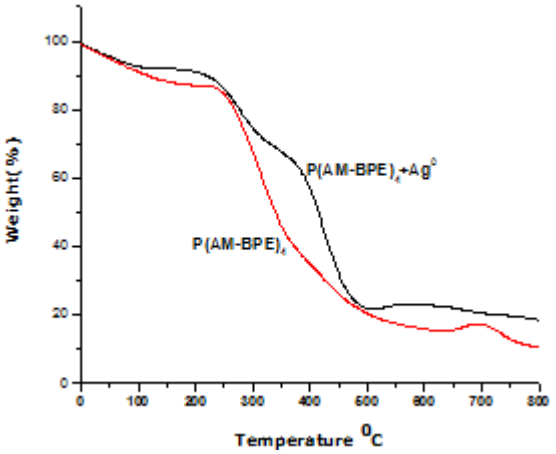

Figure 3: Thermogravimetric analysis of $P(AM\text{-}BPE)_4$ hydrogel and $P(AM\text{-}BPE)_4$ $+Ag^{0}$ nanocomposite hydrogel

3.4 X-ray diffraction (XRD) analysis:

The crystalline nature of AgNPs is confirmed by X-ray diffraction (XRD) analysis. It is used to characterize crystallographic structure, grain size, and orientation. Silver nanoparticles showed Bragg Reflection peaks at 38.06^{0}, 44.26^{0}, 64.5^{0} and 78^{0} in the 2θ range between 10-80^{0} (111), (200), (220) and (311) planes of face centered cubic (fcc) crystal, respectively. They have a good match with the standard diffraction corresponding to the reflections of crystal planes. Synthesized silver nanoparticles are composed of pure crystalline silver and the particle size is approximately 10 nm. The peak corresponding to (111) plane is more intense than the other planes, suggesting that the (111) plane is in the predominant orientation.

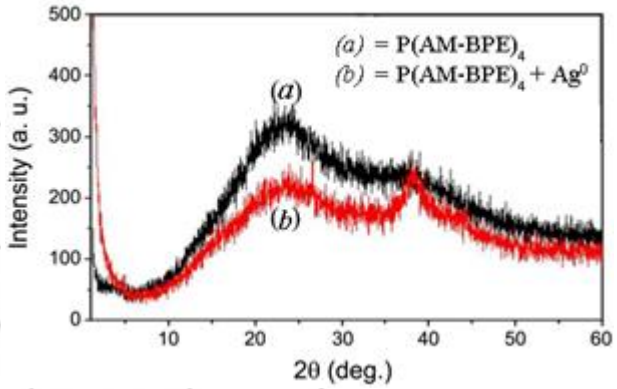

Figure 4: XRD patterns of pure $P(AM\text{-}BPE)_4$ hydrogel and $P(AM\text{-}BPE)_4$ $+Ag^{0}$ nanocomposite hydrogel

3.5 Scanning electron microscopy

Fig.5 (a) shows the SEM micrographs of the P(AM-BPE) .It shows a clear rough morphology for the pure P(AM-BPE) hydrogel, whereas Ag^{0} nanoparticles loaded P(AM-BPE) hydrogel as shown in Fig.5(b) exhibited smaller nanoparticles distributed throughout the porous gel matrix which is in agreement with the UV-Vis spectra because BPE slightly escaped from the hydrogels as it can be seen in Fig.5(c). No individual silver nanoparticles were observed outside the P(AM-BPE) hydrogels, indicating a strong interaction between the P(AM-BPE) and the Ag^{0} particles.

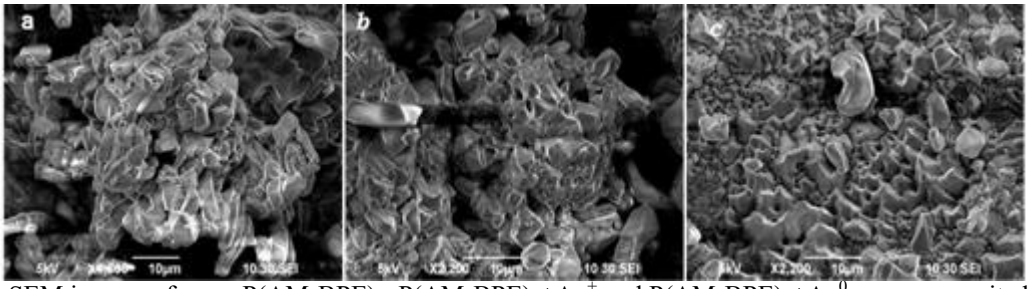

Figure 5: SEM images of: pure P(AM-BPE)$_4$, P(AM-BPE)$_4$+Ag$^+$ and P(AM-BPE)$_4$+Ag^0 nanocomposite hydrogels

3.6 Transmission electron microscopy (TEM) analysis:

TEM analysis also confirmed the formation of spherical Ag^0 nanoparticles in the P(AM-BPE) hydrogels network. It conforms the results obtained by SEM. The TEM image is shown in Fig.6 The average size of the nanoparticles was found to be about 10 *nm*. Nanopartical crystalline nature is evidenced by selected area electron diffraction patrons with bright light spots. It is evident that Ag^0 nanoparticles were highly stabilized by using BPE in the hydrogel network.

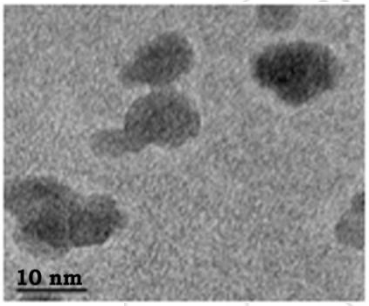

Figure 6: TEM images of P(AM-BPE)$_4$+Ag^0 nanocomposite hydrogel

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(AM-BPE) 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

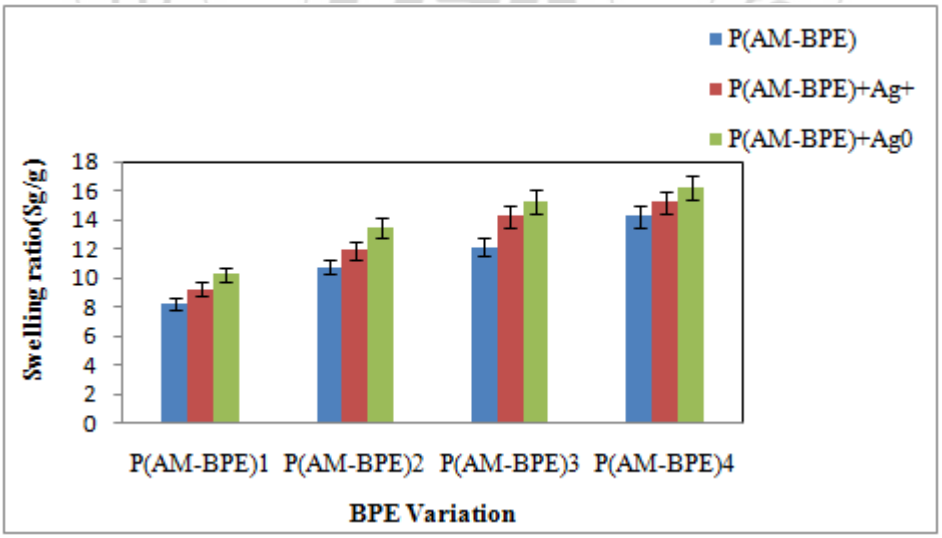

Figure 7: Swelling behaviour of P(AM-BPE), P(AM-BPE)+Ag$^+$ varied hydrogels and Ag^0 nanocomposite

3.8 Antibacterial activity of silver nanoparticles

The natural synthesized silver nanoparticles **showed** excellent antimicrobial activity against clinically isolated Multidrug-resistant human pathogens such as Gram-positive bacteria Staphylococcus aureus, and Gram-negative bacteria E.coli .The antibacterial abilities of P(AM-BPE) 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 ^{0}C, there was the inactivation of bacterial zones and no of bacterial colonies were clearly observed (Fig. 8) in the Petri dishes. The diameter of the inhibition zone for the Ag^0 nanocomposite P(AM-BPE) hydrogel is as follows: (Fig. 8 I(b) (1.7 cm)and II(b) (1.6 cm)), whereas

for the pure P(AM-BPE) hydrogels (Fig. 8 I(a) 0.0 cm and II(a) 0.0 cm), it showed no inhibition ability.

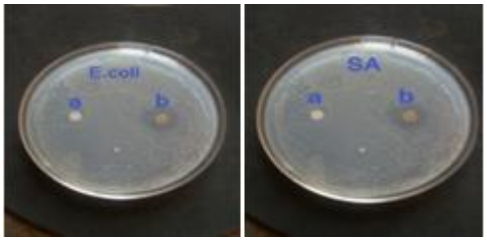

Figure 8: Antibacterial activity of I(a) plain $P(AM\text{-}BPE)_4$, I(b) $P(AM\text{-}BPE)_4+Ag^0$ nanocomposite hydrogels on Staphylococcus aureus, and II(a) plain $P(AM\text{-}BPE)_4$, II(b) $P(AM\text{-}BPE)_4+Ag^0$ nanocomposite hydrogels on E.coli

4. Conclusion

An effective natural process for the fabrication of a novel silver nanocomposite hydrogel with banana peel extract as a stabilizing agent for the silver nanoparticles has been investigated. The Ag^0 nanoparticles were prepared by reducing $AgNO_3$ with banana peel extract. These were analysed by spectral, thermal and electron microscopy. In our study it is found that nanoparticles obtained were sized 10 *nm* The synthesis of silver nanoparticles were confirmed by the change of color banana peel extract. The Ag^0 nanocomposite hydrogels have significant antibacterial activity against Staphylococcus aureus and E. coli. Toxicity studies of silver nanoparticles on human pathogen opens a new door for a new range of antibacterial agents.

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