Carbohydrate Polymers 95 (2013) 188–194

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

# Iota-Carrageenan-based biodegradable $Ag^0$ nanocomposite hydrogels for the inactivation of bacteria

Tippabattini Jayaramudu[a], Gownolla Malegowd Raghavendra[a], Kokkarachedu Varaprasad[b,c,*], Rotimi Sadiku[b], Koduri Ramam[c], Konduru Mohana Raju[a,**]

[a] Synthetic Polymer Laboratory, Department of Polymer Science & Technology, Sri Krishnadevaraya University, Anantapur 515003, India
[b] Department of Polymer Technology, Tshwane University of Technology, CSIR Campus, Building 14D, Private Bag X025, Lynwood Ridge 0040, Pretoria, South Africa
[c] Departamento de Ingeniería de Materiales – DIMAT, Facultad de Ingeniería Universidad de Concepción, Concepción, Chile

ARTICLE INFO

*Article history:*
Received 2 February 2013
Received in revised form 20 February 2013
Accepted 28 February 2013
Available online 7 March 2013

*Keywords:*
I-Carrageenan
Hydrogels
Green process
Biodegradable
*Azadirachta indica*

ABSTRACT

In this paper, we report the synthesis and characterization of Iota-Carrageenan based on a novel biodegradable silver nanocomposite hydrogels. The aim of study was to investigate whether these hydrogels have the potential to be used in bacterial inactivation applications. Biodegradable silver nanocomposite hydrogels were prepared by a green process using acrylamide (AM) with I-Carrageenan (IC). The silver nanoparticles were prepared as silver colloid by reducing $AgNO_3$ with leaf extracts of *Azadirachta indica* (neem leaf) that ($Ag^0$) formed the hydrogel network. The formation of biodegradable silver nanoparticles in the hydrogels was characterized using UV–vis spectroscopy, thermo gravimetrical analysis, X-ray diffractometry studies, scanning electron microscopy and transmission electron microscopy studies. In addition, swelling behavior and degradation properties were systematically investigated. Furthermore, the biodegradable silver nanoparticle composite hydrogels developed were tested for antibacterial activities. The antibacterial activity of the biodegradable silver nanocomposite hydrogels was studied by inhibition zone method against *Bacillus* and *Escherichia coli*, which suggested that the silver nanocomposite hydrogels developed were effective as potential candidates for antimicrobial applications. Therefore, the inorganic biodegradable hydrogels developed can be used effectively for biomedical application.



## 1. Introduction

In the past years, of polymer nanotechnology, attention in biodegradable hydrogels has developed significantly. These materials are widely used in the biomedical field such as: drug delivery, tissue engineering and especially in anti-bacterial applications (Tiwari, Grailer, Pilla, Steeber, & Gong, 2009; Varaprasad et al., 2012; Woo, Mittelman, & Santerre, 2000). Natural polymer component of a biodegradable hydrogel can consist of three dimensional network structures which are biocompatible and biodegradable (He et al., 2010; Sun, Zhuo, & Liu, 2003). Biodegradation is extremely interesting as far as hydrogels are concerned. Therefore, natural polymers have been widely used as biodegradable components of hydrogels. This is due to the biocompatibility of their degradative products (Bhattarai, Gunn, & Zhang, 2010; Paulino et al., 2012; Varaprasad, Murali Mohan, Vimala, & Mohana Raju, 2011; Varaprasad, Narayana Reddy, Ravindra, Vimala, & Mohana Raju, 2011; Varaprasad, Vimala, Ravindra, Narayana Reddy, Venkata Subba Reddy, et al., 2011; Varaprasad, Vimala, Ravindra, Narayana Reddy, & Mohana Raju, 2011). Due to their biodegradability, various natural polymers have been explored for the synthesis of biodegradable hydrogels. Lately, "biodegradability" is the main and essential concept which is required for the hydrogels applied in the tissue engineering, since they will allow complete replacement by the regenerated tissue and their biological interaction with the body components; hence these biodegradable hydrogels are used in biomedical applications (Jayaramudu, Raghavendra, Varaprasad, Sadiku, & Raju, 2013). I-Carrageenan is one of the natural carbohydrates (polysaccharide) (Bonferoni, Rossi, Ferrari, Bettinetti, & Caramella, 2000; Soleimani, Sadeghi, & Shahsavari, 2012) that is in great abundance in nature

* Corresponding author at: Departamento de Ingeniería de Materiales – DIMAT, Facultad de Ingeniería Universidad de Concepción, Concepción, Chile.
** Corresponding author.
*E-mail addresses:* varmaindian@gmail.com, prasadadc@gmail.com, kvaraprasad@udec.cl (K. Varaprasad).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.02.075

with low cost and has good biodegradability (hydrolyzed with acid) potential. Lately, Hezaveh and Muhamad (2012) reported the use of carrageenan-based hydrogels for control release application. Salgueiro, Daniel-da-Silva, Fateixa, and Trindade (2013) prepared carrageen hydrogel nanocomposites for drug carriers.

Among biodegradable hydrogels, inorganic-based ones are particularly promising for inactivation of bacterial applications in materials and engineering science, have generated a lot of interest lately (Jayaramudu et al., 2013). Because of they are easily functionalized with inorganic materials and biocompatible, this characteristic makes them attractive in the biomedical and biotechnological fields. The synthesis of inorganic based hydrogels can proceed in various methods. In this process toxic chemical were used for reduction of inorganic particles (Shiv Shankar, Akhilesh, Absar, & Murali, 2004; Varaprasad, Murali Mohan, et al., 2010; Varaprasad, Ravindra, Narayana Reddy, Vimala, & Mohana Raju, 2010b; 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). To solve this problem, we have introduced the green process (Jayaramudu et al., 2013; Raghavendra et al., 2013). 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). Therefore, the development of metal nanoparticles based on natural extracts is considered as the most appropriate method for obvious environmental reasons.

In a recent work, we described the use of wheat protein isolate-based inorganic biodegradable hydrogels for inactivation of bacteria. In their study, they reported the green process, hydrogels degradation and the antibacterial activity of biodegradable hydrogels (Jayaramudu et al., 2013). The present work explores the use of another inorganic biodegradable hydrogels which are prepared form I-Carrageenan and acrylamide by green process. Carrageenan was chosen on the basis of its significant characteristics in biomedical fields. Carrageenan and its derivative products (gels) have been used against to HSV 1 and 2 (Herpes simplex virus 1 and 2) (Buck et al., 2006) transmission, extremely potent inhibitors of human papillomavirus (HPV) (Roberts et al., 2007), human immunodeficiency virus (HIV) infection (Turville, Aravantinou, Miller, Kenney, & Teitelbaum, 2008) and cancer therapy (Tobacman, 2001) and various other biomedical applications (Eccles et al., 2010). Due to its unique properties, IC was selected for the preparation of $Ag^0$ nanocomposite hydrogels. Similarly, we have taken *Azadirachta indica* as a natural reducing agent which is a highly bio-logically active compound (Schmutterer, 1995). It is a naturally occurring, non-toxic and bioactive agent in human life (Biswas, Chattopadhyay, Ranajit, & Uday, 2002). Structural, thermal and morphological studies of the hydrogels and their corresponding $Ag^0$ nanocomposite hydrogels were carried out by Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD). The content and distribution of $Ag^0$ nanoparticles in P(IC-AM) hydrogels were determined by thermogravimetric analysis (TGA), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The effect of silver nanoparticles on the antibacterial activity of the P(IC-AM) hydrogels was studied. Herein, a study of the design of P(IC-AM) $Ag^0$ nanocomposites hydrogels for significant antibacterial applications is presented.

## 2. Materials

I-Carrageenan (IC) was purchased from Himedia Laboratories Pvt. Ltd. (Product code: *RM 1576*-Mumbai, India) Acrylamide (AM), N,$N^1$-methylenebisacrylamide (MBA), potassium pursulfate (KPS) and N,N,$N^1$,$N^1$-tetramethylethylenediamine (TMEDA) were purchased from S.D Fine Chemicals (Mumbai, India). Silver nitrate ($AgNO_3$) was supplied by Merck (Mumbai, India). Neem leaves were used as a reduction agent and obtained from neem tree in the Sri Krishnadevaraya University campus, Anantapur, Andhra Pradesh, 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) provided standard cultures of the organisms.

### 2.1. Preparation of the leaf extract

Leaf extracts were prepared by a green process technique, using the standard procedure described in our earlier study (Jayaramudu et al., 2013). Neem leaves (*A. indica*) were collected from neem tree and thoroughly washed with distilled water. Neem leaf broth was prepared by taking 25 g of thoroughly washed leaves 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. Fabrication of P(IC-AM) hydrogels

To prepare the P(IC-AM) hydrogels, the initial solution consisting of monomer AM (14.06 mM), double distilled water (3 ml) and various ratios (0.15–2) of IC (linear sulfated polysaccharides) was stirred at 300 rpm for 2 h at ambient temperature. Then the activator TMEDA (0.86 mM) was added with stirring. Finally, an aqueous solution of the initiator KPS (1.89 mM) was added to the solution. After the addition of the reactants the temperature of the system was raised to 50 °C for 15 min. The free-radical polymerization was carried out at ambient temperature for 8 h. After the reaction has been completed, the hydrogel was immersed in distilled water 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 distilled water for 48 h and the swollen hydrogel species were transferred 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(IC-AM) hydrogel networks.

The $Ag^+$ ions loaded P(IC-AM) hydrogels were wiped off using tissue paper and transferred to a beaker containing 50 ml of cold neem leaf extracts *A. indica* solution. The beaker was left in the refrigerator (4 °C) for 8 h in order to reduce the $Ag^+$ ions into $Ag^0$ nanoparticles. 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 IC-based hydrogels were prepared by varying the IC concentration. The feed compositions of the P(IC-AM) hydrogels are presented in Table 1.

### 2.4. Characterization

FTIR spectrophotometer is used to study the transmission of the hydrogel pattern, and $Ag^0$ nanoparticles patterns in hydrogel networks. The hydrogels and the $Ag^0$ nanoparticles-embedded P(IC-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

**Table 1**
Preparation of biodegradable P(IC-AM) hydrogels feed composition.

| Hydrogel code | IC (g) | AM (mM) | MBA (mM) | KPS (mM) | TMEDA (mM) | Swelling ratio at equilibrium ($S_{eq}$) ($S_{g/g}$) |
|---|---|---|---|---|---|---|
| P(IC-AM)0 | – | 14.068 | 0.648 | 1.849 | 0.8605 | 7.728 |
| P(IC-AM)1 | 0.15 | 14.068 | 0.648 | 1.849 | 0.8605 | 23.80 |
| P(IC-AM)2 | 0.2 | 14.068 | 0.648 | 1.849 | 0.8605 | 36.45 |
| P(IC-AM)3 | 0.25 | 14.068 | 0.648 | 1.849 | 0.8605 | 49.10 |

between 500 and 4000 $cm^{-1}$ on a Bruker IFS 66V FTIR spectrometer (Ettlingen, Germany), using the KBr disk method. UV–vis spectra of P(IC-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(IC-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 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 °C/min under a constant nitrogen flow (100 ml/min). Wide X-ray diffraction (XRD) method was used to identify the formation of $Ag^0$ nanoparticles in the P(IC-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) analysis of plain P(IC-AM) hydrogel and $Ag^0$ nanoparticles impregnated P(IC-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. 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(IC-AM) $Ag^0$ nanocomposite (1 mg/1 ml) solution on a 3 mm copper grid and dried at ambient temperature after removing excess solution using filter paper.

The swelling ratios of hydrogel samples were measured in the ambient temperature using a gravimetric method (Varaprasad, Murali Mohan, et al., 2010; Varaprasad, Ravindra, et al., 2010; 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 dried hydrogels were immersed in a 50 ml beaker containing double distilled water until their weight became constant. The hydrogels were then removed from the water and their surfaces were blotted with filter paper before being weighed. Furthermore, swollen hydrogels were treated with $AgNO_3$ 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 Eq. (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 3 individual sample readings.

### 2.5. Antibacterial activity

The antibacterial activity of the $Ag^0$ nanocomposite P(IC-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 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, Mumbai, India). After solidification of the media, bacteria (*Bacillus* 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.

### 2.6. Biodegradation characterizations

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

### 2.7. Method

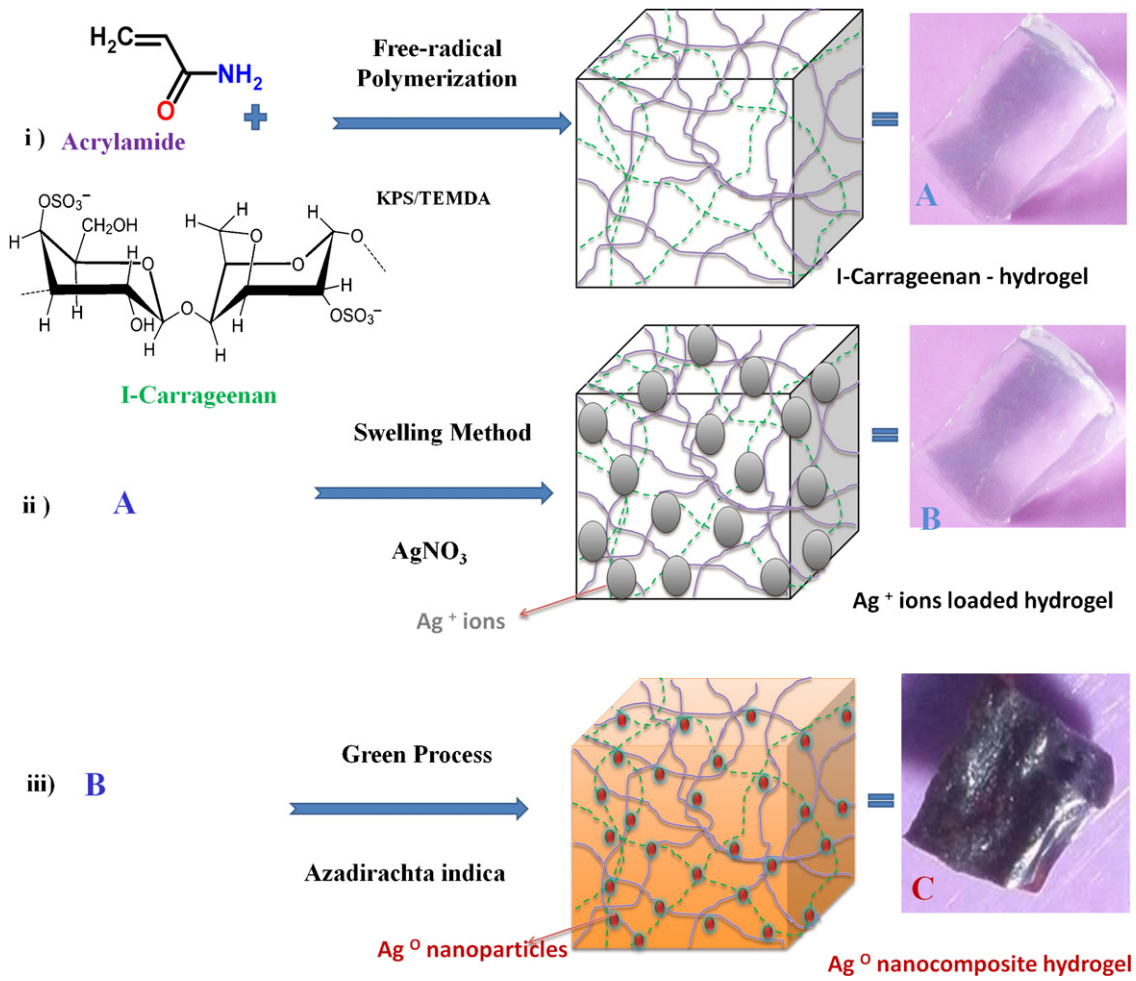
Nutrient agar medium was prepared using the standard procedure described elsewhere (Varaprasad, Murali Mohan, et al., 2011; Varaprasad, Narayana Reddy, et al., 2011; Varaprasad, Vimala, Ravindra, Narayana Reddy, Venkata Subba Reddy, et al., 2011; Varaprasad, Vimala, Ravindra, Narayana Reddy, & Mohana Raju, 2011). The agar medium was sterilized by autoclaving at 121 °C for 30 min at a pressure of 6.8 kg (15 lbs). An *E. coli* and bacterium was inoculated in this medium and the pure culture was maintained separately in the incubator. Then, to the 10 ml of sterilized broth, 0.100 g each of the samples, i.e. both P(IC-AM) hydrogel and their $Ag^0$ 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 ± 1 °C for 24 h. Then, the samples were weighed to determine the weight loss. The ratio of weight remained ($W_r$) was calculated based on Eq. (2):

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

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.

## 3. Results and discussion

The synthesis procedure for the $Ag^0$ nanocomposite P(IC-AM) hydrogels consists of the following three steps, as shown, schematically, in Scheme 1: (i) the fabrication of P(IC-AM) hydrogels via free-radical reaction; (ii) the preparation of $Ag^+$ ions-loaded hydrogels via swelling method and (iii) the synthesis of $Ag^0$ nanocomposite P(IC-AM) hydrogels via green process (the $Ag^0$ nanoparticles were prepared by reducing $AgNO_3$ with *A. indica* in the P(IC-AM) hydrogels network).




**Scheme 1.** Biodegradable P(IC-AM) silver nanocomposite hydrogel preparative schematic illustration in three steps. (i) The fabrication of P(IC-AM) hydrogels via free-radical reaction; (ii) the preparation of $Ag^+$ ions-loaded hydrogels via swelling method and (iii) the synthesis of $Ag^0$ nanocomposite P(IC-AM) hydrogels via green process (The $Ag^0$ nanoparticles were prepared by reducing $AgNO_3$ with *A. indica* in the P(IC-AM) hydrogels network).

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(IC-AM) sheets shows a broad absorption band at 3398 $cm^{-1}$ that is related to the —NH asymmetric and —OH symmetric stretching vibrations groups, band at 2923 $cm^{-1}$ are attributed to stretching vibrations of —$CH_3$ units and an absorption band at 1659 $cm^{-1}$ from the carbonyl groups of P(IC-AM) hydrogel (Soleimani, Sadeghi, & Shahsavari, 2012). These peaks have shifted to 3391, 2938 and 1655 $cm^{-1}$ in $Ag^0$ nanocomposite P(IC-AM). As a result, it can be concluded that $Ag^0$ nanoparticles are present in P(IC-AM) hydrogels.

Thermogravimetric analysis was used to study the formation of $Ag^0$ and the thermal stability of the hydrogels. As seen in Fig. 1B, the thermal decomposition of P(IC-AM) occurred at 425 °C with a significant mass loss (79%). For $Ag^0$ nanocomposite P(IC-AM) hydrogel (Fig. 1B), a comparatively very low mass loss (55%) was found at 425 °C, which was due to the partial decomposition of the $Ag^0$ nanoparticles. Moreover, according to the TGA results, the $Ag^0$ nanocomposite P(IC-AM) hydrogels showed a higher thermal stability when compared with the P(IC-AM) hydrogel.

Another piece of evidence for the formation $Ag^0$ nanocomposite P(IC-AM) hydrogels came from UV spectra. Fig. 1C shows the

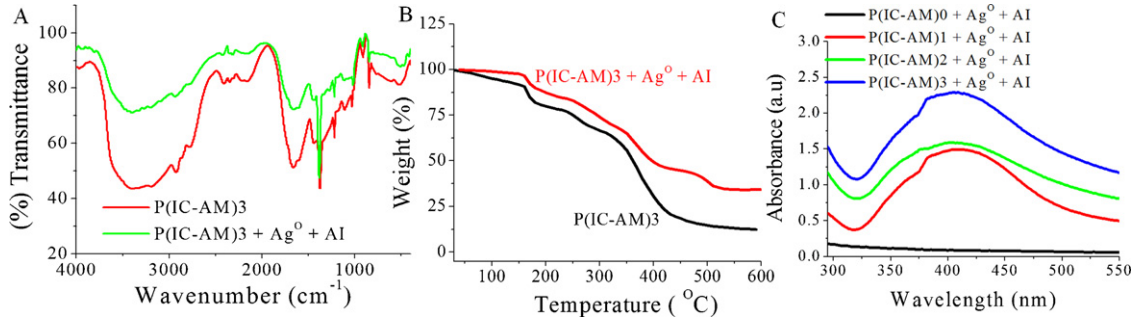



**Fig. 1.** (A) FTIR spectra of pure P(IC-AM)3 hydrogel and P(IC-AM)3 $Ag^0$ + AI nanocomposite biodegradable hydrogels. (B) TGA curves of pure P(IC-AM)3 and P(IC-AM)3 + $Ag^0$ + AI nanocomposite hydrogels and (C) UV–vis spectra of P(IC-AM) $Ag^0$ nanocomposite (P(IC-AM)0–P(IC-AM)3 + $Ag^0$ + AI) hydrogels.

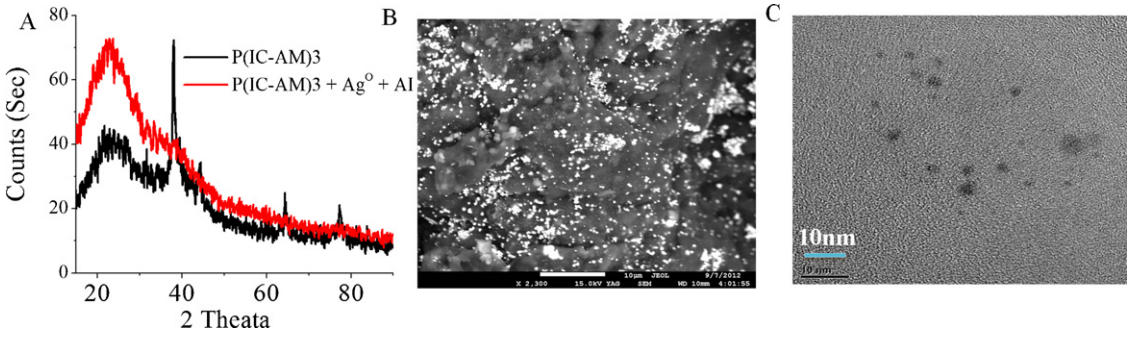



**Fig. 2.** (A) XRD patterns of pure P(IC-AM)3 and P(IC-AM)3 + $Ag^0$ + AI hydrogels, (B) SEM images of: (a) P(IC-AM) and (b) P(IC-AM) $Ag^0$ nanocomposite hydrogels and (C) TEM images of P(IC-AM)3 + $Ag^0$ + AI nanocomposite hydrogel.

absorption characteristics of the $Ag^0$ nanoparticles and the characteristic plasmonic resonance peak of silver nanoparticles at $\lambda_{max}$ 408 nm. These results imply that the $Ag^0$ nanoparticle content increased with increased IC concentration in the hydrogel and these peaks reveal the presence of stable nanoparticles in the diluted sample. However, there are no intense peaks around 408 nm, as was observed in pure P(IC-AM) hydrogel.

From Fig. 2A, it can be clearly seen that no diffraction peak was observed for the pure P(IC-AM) hydrogels, while the $Ag^0$ nanocomposite P(IC-AM) hydrogel showed a diffraction peak at $2\theta = 38.16$, 44.26, 64.39 and 77.40, corresponding to the reflections of crystal planes (1 1 1), (2 0 0), (2 2 0) and (3 1 1), respectively, of the face-centered cubic structure of $Ag^0$. This faced-centered cubic ($f_{cc}$) structure indicates that $Ag^0$ nanoparticles were dispersed in the P(IC-AM) hydrogels, which was confirmed with the scanning electron micrograph image shown in Fig. 2B. TEM was employed to further confirm the formation of $Ag^0$ nanoparticles (Fig. 2C), which are spherical in shape and its average size is $\sim 3 \pm 2$ nm. It is evident that $Ag^0$ nanoparticles are highly stabilized using IC in the hydrogel network. These results are mainly due to the strong interaction between the $Ag^0$ nanoparticles and P(IC-AM) hydrogels.

The results in Fig. 3A show that $Ag^0$ nanocomposite hydrogels formed have superior swelling ratio, when compared to the conventional P(IC-AM) hydrogels. The reason being that when $Ag^+$ ions-loaded hydrogels were treated with *A. indica*, with the addition of many $Ag^+$ ions led to the formation of the nanoparticles within the hydrogel, 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 defining characteristic, Varaprasad et al. have prepared different type of hydrogels for drug delivery and antibacterial applications (Varaprasad, Murali Mohan, et al., 2010; Varaprasad, Ravindra, et al., 2010; 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).

However, the values of the swelling ratio were influenced by the IC concentration; with increase of the IC ratio resulting in increase of the swelling ratio values. This is due to the hydrophilic nature of IC. Similarly, with increase in IC concentration, hydrogels degradation increases. The biodegradation property of pure and $Ag^0$ nanocomposite P(IC-AM) hydrogel developed were carried out by weight loss methods. The degradation behaviors of pure hydrogel and $Ag^0$ nanocomposites P(IC-AM) hydrogel are shown in Fig. 3B. From the Figure, it is observed that pure P(IC-AM) hydrogels show high weight loss (%) than $Ag^0$ nanocomposite P(IC-AM) hydrogels. This is due to the fact that $Ag^0$ nanoparticles that escaped 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 reduced) and also strong nitration between $Ag^0$ and IC hydrogels networks. But this is not the case for pure P(IC-AM) hydrogel which does not have $Ag^0$ nanoparticles. Therefore, it readily undergoes degradation when compared to $Ag^0$ nanocomposites. The antibacterial properties of the biodegradable hydogels were investigated by calculating their ability to inhibit *Bacillus* and *E. coli* growth on agar culture dishes. After 48 h of incubation, there was the inactivation of bacterial zones and no bacterial colonies were clearly observed (Fig. 4) in the Petri dishes. The diameter of the inhibition zone for the $Ag^0$ nanocomposite P(IC-AM) hydrogel is as follows: [Fig. 4A(b and c) (0.8 cm and 1.2 cm) and B(b and c) (0.6 cm and 0. 9 cm)], whereas for the pure P(IC-AM) hydrogels (Fig. 4A(a) 0.0 cm and B(a) (0.0 cm)), it showed no inhibition ability. Therefore, IC in

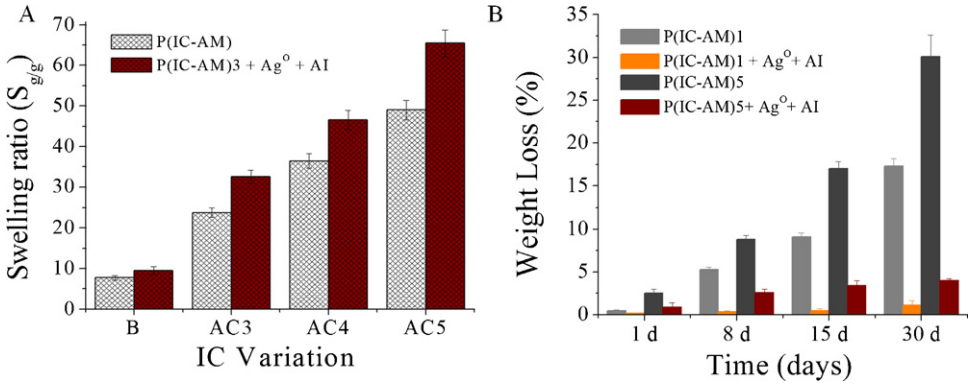



**Fig. 3.** (A) Swelling behavior of IC varied hydrogels and $Ag^0$ nanocomposite hydrogels and (B) biodegradation of IC-hydrogels (P(IC-AM)1–P(IC-AM)3) and $Ag^0$ nanocomposite (P(IC-AM)1 + $Ag^0$ + AI and P(IC-AM)3 + $Ag^0$) hydrogels by *E. coil*.

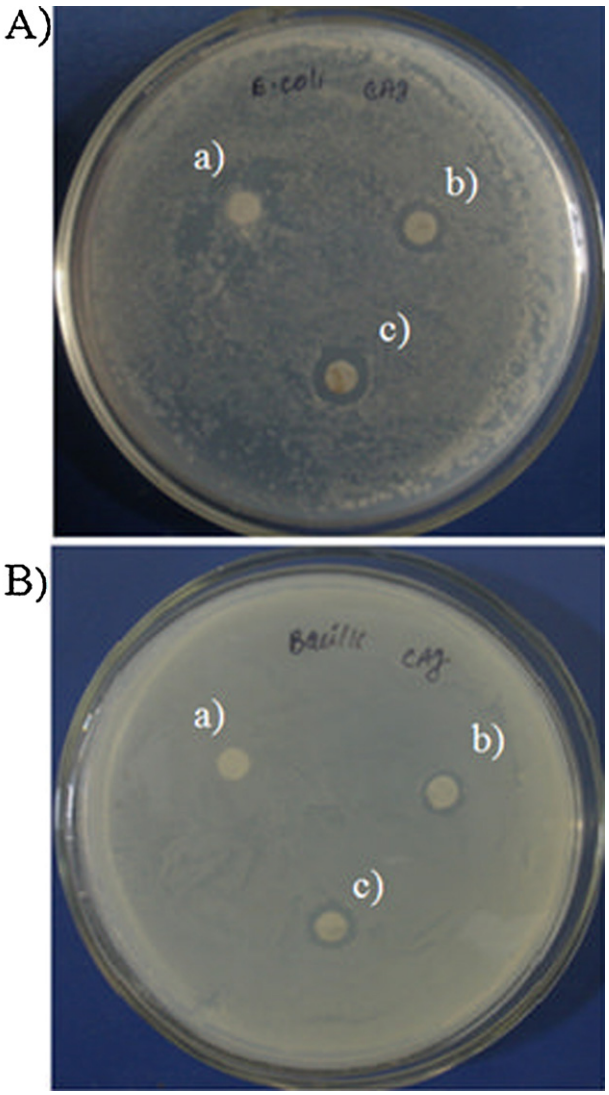



**Fig. 4.** Antibacterial activity of (A) (a) plain P(IC-AM)3 (b) AI + P(IC-AM)3 and (c) P(IC-AM) + $Ag^0$ nanocomposite hydrogels on *E. coli* and (B) (a) plain P(IC-AM)3, (b) AI + P(IC-AM)3 and (c) P(IC-AM) $Ag^0$ nanocomposite hydrogels on *Bacillus*.

combination with $Ag^0$ nanocomposites hydrogels exhibits excellent antibacterial activity.

## 4. Conclusion

An effective green process for the fabrication of a novel biodegradable silver nanocomposite hydrogel with I-Carrageenan as a stabilizing agent for the silver nanoparticles, has been described. The $Ag^0$ nanoparticles were prepared by reducing $AgNO_3$ with *A. indica* in the hydrogels network. These composites were developed and characterized by spectral, thermal and electron microscopy. The $Ag^0$ nanocomposite hydrogels prepared have significant antibacterial activity against *Bacillus* and *E. coli*. Application of $Ag^0$ nanocomposites hydrogels based on these findings is expected to lead to valuable discoveries in various fields; such as medical devices and antimicrobial agents.

## Acknowledgements

One of the author (TJ) thanks the UGC, New Delhi, India for the award of JRF under UGC-BSR Meritorious Fellowship Scheme. Two of the authors (KVP & KRM) thank the Fondecyt Proyecto No.: 3130748 & 1110583 Chile (South America).

## References

Bhattarai, N., Gunn, J., & Zhang, M. (2010). Chitosan-based hydrogels for controlled, localized drug delivery. *Advanced Drug Delivery Reviews*, *62*, 83–99.
Biswas, K., Chattopadhyay, I., Ranajit, K. B., & Uday, B. (2002). Biological activities and medicinal properties of neem (*Azadirachta indica*). *Current Science*, *82*, 1336–1345.
Bonferoni, M. C., Rossi, S., Ferrari, F., Bettinetti, G. P., & Caramella, V. (2000). Characterization of a diltiazem–lambda carrageenan complex. *International Journal of Pharmaceutics*, *200*, 207–216.
Buck, C. B., Thompson, C. D., Roberts, J. N., Muller, M., Lowy, D. R., & Schiller, J. T. (2006). Carrageenan is a potent inhibitor of papillomavirus infection. *PLoS Pathogens*, *2*, 671–680.
Eccles, R., Meier, C., Jawad, M., Weinmüllner, R., Grassauer, A., & Prieschl-Grassauer, E. (2010). Efficacy and safety of an antiviral Iota-Carrageenan nasal spray: A randomized, double-blind, placebo-controlled exploratory study in volunteers with early symptoms of the common cold. *Respiratory Research*, *11*(108), 1–10.
He, J., Ni, P., Wang, S., Shao, H., Zhang, M., & Zhu, X. (2010). Synthesis and physicochemical characterization of biodegradable and pH-responsive hydrogels based on polyphosphoester for protein delivery. *Journal of Polymer Science Part A: Polymer Chemistry*, *48*, 1919–1930.
Hezaveh, H., & Muhamad, I. I. (2012). Modification and swelling kinetic study of kappa-carrageenan-based hydrogel for controlled release study. *Journal of the Taiwan Institute of Chemical Engineers*, *44*, 182–191.
Jayaramudu, T., Raghavendra, G. M., Varaprasad, K., Sadiku, R., & Raju, K. M. (2013). Development of novel biodegradable Au nanocomposite hydrogels based on wheat: For inactivation of bacteria. *Carbohydrate Polymers*, *92*, 2193–2200.
Paulino, A. T., Pereirad, A. G. B., Fajardod, A. R., Ericksonb, K., Kipperb, M. J., Munizd, E. C., et al. (2012). Natural polymer-based magnetic hydrogels: Potential vectors for remote-controlled drug release. *Carbohydrate Polymers*, *90*, 1216–1225.
Raghavendra, G. M., Jayaramudu, T., Varaprasad, K., Sadiku, R., Ray, S. S., & Raju, K. M. (2013). Cellulose–polymer–Ag nanocomposite fibres for antibacterial fabrics/skin scaffolds. *Carbohydrate Polymers*, *93*, 553–560.
Roberts, J., Buck, C., Thompson, C., Kines, R., Bernardo, M., Choyke, P., et al. (2007). Genital transmission of HPV in a mouse model is potentiated by nonoxynol-9 and inhibited by carrageenan. *Nature Medicine*, *13*(7), 857–861.
Salgueiro, A. M., Daniel-da-Silva, A. L., Fateixa, S., & Trindade, T. (2013). Carrageenan hydrogel nanocomposites with release behavior mediated by morphological distinct Au nanofillers. *Carbohydrate Polymers*, *91*, 100–109.
Schmutterer, H. (Ed.). (1995). *The neem tree: Source of unique natural products for integrated pest management, medicine, industry and other purposes*. (pp. 1–696). Weinheim, Germany: VCH.
Shiv Shankar, S., Akhilesh, R., Absar, A., & Murali, S. (2004). Rapid synthesis of Au, Ag and bimetallic Au core–Ag shell nanoparticles using neem (*Azadirachta indica*) leaf broth. *Journal of Colloid and Interface Science*, *275*, 496–502.
Soleimani, F., Sadeghi, M., & Shahsavari, H. (2012). Preparation and swelling behavior of carrageenan-graft-polymethacrylamide superabsorbent hydrogel as a releasing drug system. *Indian Journal of Science and Technology*, *5*, 2143–2147.
Sun, L. F., Zhuo, R. X., & Liu, Z. L. (2003). Studies on the synthesis and properties of temperature responsive and biodegradable hydrogels. *Macromolecular Bioscience*, *3*, 725–728.
Tiwari, A., Grailer, J. J., Pilla, S., Steeber, D. A., & Gong, S. (2009). Biodegradable hydrogels based on novel photopolymerizable guar gum–methacrylate macromonomers for in situ fabrication of tissue engineering scaffolds. *Acta Biomaterialia*, *5*, 3441–3452.
Tobacman, J. K. (2001). Review of harmful gastrointestinal effects of carrageenan animal experiments. *Environmental Health Perspectives*, *109*(10), 983.
Turville, S. G., Aravantinou, M., Miller, T., Kenney, J., & Teitelbaum, A. (2008). Efficacy of Carraguard-Based microbicides in vivo despite variable in vitro activity. *PLoS ONE*, *3*(9), e3162.
Varaprasad, K., Murali Mohan, Y., Ravindra, S., Narayana Reddy, N., Vimala, K., Monika, K., et al. (2010). Hydrogel–silver nanoparticle composites: A new generation of antimicrobials. *Journal of Applied Polymer Science*, *115*, 119–1207.
Varaprasad, K., Murali Mohan, Y., Vimala, K., & Mohana Raju, K. (2011). Synthesis and characterization of hydrogel–silver nanoparticle–curcumin composites for wound dressing and antibacterial application. *Journal of Applied Polymer Science*, *121*, 784–796.
Varaprasad, K., Narayana Reddy, N., Ravindra, S., Vimala, K., & Mohana Raju, K. (2011). Synthesis and characterizations of macroporous poly(acrylamide-2-acrylamido-2-methyl-1-propanesulfonic acid) hydrogels for in vitro drug release of ranitidine hydrochloride. *International Journal of Polymeric Materials*, *60*, 490–503.
Varaprasad, K., Ravindra, S., Narayana Reddy, N., Vimala, K., & Mohana Raju, K. (2010). Design and development of temperature sensitive porous poly(NIPAAm-AMPS) hydrogels for drug release of doxorubicin—A cancer chemotherapy drug. *Journal of Applied Polymer Science*, *116*, 3593–3602.
Varaprasad, K., Vimala, K., Ravindra, S., Narayana Reddy, N., & Mohana Raju, K. (2011). Development of sodium carboxymethyl cellulose-based poly (acrylamide-co-2acrylamido-2-methyl-1-propane sulfonic acid) hydrogels for in vitro drug release studies of ranitidine hydrochloride an anti-ulcer drug. *Polymer – Plastics Technology and Engineering*, *50*(12), 1199–1207.

Varaprasad, K., Vimala, K., Ravindra, S., Narayana Reddy, N., Siva Mohana Reddy, G., & Mohana Raju, K. (2012). Biodegradable chitosan hydrogels for in vitro drug release studies of 5-flurouracil an anticancer drug. *Journal of Polymers and the Environment*, *20*, 573–582.

Varaprasad, K., Vimala, K., Ravindra, S., Narayana Reddy, N., Venkata Subba Reddy, G., & Mohana Raju, K. (2011). Fabrication of silver nanocomposite films impregnated with curcumin for superior antibacterial applications. *Journal of Materials Science: Materials in Medicine*, *22*, 1863–1872.

Vimala, K., Samba Sivudu, K., Murali Mohan, Y., Sreedhar, B., & Mohana Raju, K. (2009). Controlled silver nanoparticles synthesis in semi-hydrogel networks of poly(acrylamide) and carbohydrates: A rational methodology for antibacterial application. *Carbohydrate Polymers*, *75*, 463–471.

Woo, G. L. Y., Mittelman, M. W., & Santerre, J. P. (2000). Synthesis and characterization of a novel biodegradable antimicrobial polymer. *Biomaterials*, *21*, 1235–1246.