

Development of microbial protective Kolliphor-based nanocomposite hydrogels

Kokkarachedu Varaprasad,¹ Rotimi Sadiku²

¹Center for Advanced Polymer Research, CIPA, Beltran Mathieu 224, Floor 2, Concepción, Chile

²Department of Polymer Technology, Tshwane University of Technology, Pretoria 0029, South Africa

Correspondence to: K. Varaprasad (E-mail: varmaindian@gmail.com or prasad@cipachile.cl)

ABSTRACT: In this scientific work, a novel class of antimicrobial nanocomposite hydrogels were designed and synthesized by chemical and environmentally bioprocess using Kolliphor, acrylamide, and mint leaves in order to achieve antiseptic property for wound applications. In the bioprocess approach, silver nitrate and gold chloride were nucleated with mint leaves in order to obtain effective free individual nano-inorganic compounds to provide superior antibacterial assets. The formations of dual inorganic nanoparticles were confirmed by transmission electron microscopy, which indicated the size of nanoparticles in the range of approximately 3 ± 2 nm and without agglomeration. The formations of biomaterials were characterized using Fourier transform infrared spectroscopy, thermogravimetric analysis, scanning electron microscopic–energy dispersive spectrometric studies and their swelling properties were determined. Furthermore, the pure hydrogel and the dual inorganic nanocomposite hydrogels developed were tested for antibacterial activities. When compared with the neat hydrogel, the nanocomposite hydrogels significantly improved their anti-bacterial activities on *Bacillus* bacterium. © 2015 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2015**, *132*, 42781.

KEYWORDS: gels; hydrophilic polymers; nanoparticles; swelling

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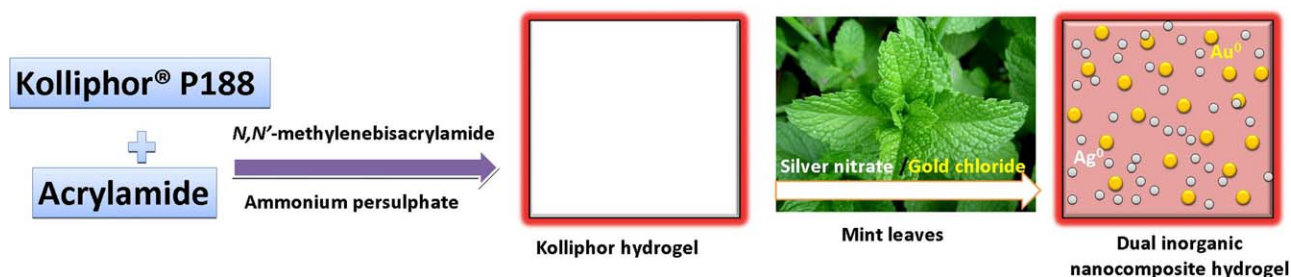
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INTRODUCTION

In recent decades, increasing significant time and resources (in research and development) have been devoted to the inorganic nanocomposite hydrogels with suitable polymer substrates. This is because of their unique biomedical usefulness, as they enhance their bioactivity in the biomedical fields, such as catalysts, biosensors, antibacterial, and anticancer agents.^{1–3} Advanced hydrogels developments specify that via the selection of polymers that are able to regulate the three-dimensional (3D) microstructures network of the hydrogels, the hydrogels can be stabilized by the organic and inorganic nanoparticles and such stability enhances the hydrogels applicability in biomedicine.^{4–9} Recently, polyoxypropylene–polyoxyethylene block copolymer (Kolliphor[®] P 188) has been highly used in sequence of gels due to their hydrophobic and hydrophilic blocks, which make them to be sensitive and intelligent materials in biomedical field.^{3,10,11} In general, Kolliphor[®] P 188 is used as a wetting agent, emulsifiers, and stabilizing agent of polymer nanoparticles.^{3,11} These significant characteristics of Kolliphor[®] P 188, make it suitable for the development of various hydrogel systems.¹² Especially, the 3D microstructures can serve as a temporary support for the accommodation of inorganic nanoparticles and they are excellent inorganic nanoparticles transporters.⁷

Principally, inorganic hydrogels are synthesized via chemical cross-linking and physical cross-linking methods. In the methods used for the development of inorganic nanocomposite hydrogels, there are few toxic nucleation agents, hence giving effect to the living systems. In order to solve the difficulty of toxicity and increase the use of nano-inorganic–hydrogels applicability in the wound-dressing application, Jayaramdu and his research group developed a green process.¹³ In the technique, they utilized leaves extract as a nucleation agent, which enhances the hydrogel's wound-healing capacity/capability. Generally, leaves are naturally available materials and limited leaves are non-toxic and good bioactive materials^{14–16} which cannot give harmful side effects to the human living system. Therefore, naturally available leaves which have antibacterial capacity/capability, in that limited leaves are useful for the nucleation of inorganic materials.

Previously, several studies have been undertaken in order to propose formulation, evaluate characteristics, as well as study possible applications of wound dressing hydrogels based on a combination of several polymers with other components. Among, dual inorganic nanomaterials are receiving increasing attention in biomedicine. Especially, silver, gold and their core/shell nanoparticles are have come into prominence since, they



Scheme 1. Schematic diagram for the formation of dual individual inorganic nanocomposites hydrogel. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

are nontoxic and biocompatible.¹⁷ Nevertheless, the difficulty occurs for the duration of the preparation of the two individual nanoparticles within the polymer material, since they interact with each other and exceedingly agglomerated.¹⁷ Owing to this phenomenon, most of the inorganic nanoparticles lose their individualities; which can reduce their applicability in the biomedical field.

In order to solve this drawback, we report the development of a free radical polymerization method for synthesizing dual individual inorganic nanoparticles within the hydrogels using Kolliphor® P 188 polymer as a stabilizing agent of dual individual inorganic nanoparticles without any functional interaction between inorganic nanoparticles and agglomeration. In this method, the mint leaf was used as a nucleation agent for the dual inorganic nanoparticles. Structural, thermal, and morphological studies of the dual inorganic nanocomposite hydrogels were carried out by using Fourier transform infrared spectroscopy (FTIR). The stabilization and distribution of nanoparticles in dual inorganic nanocomposite hydrogels were determined by thermogravimetric analysis (TGA), transmission electron microscopy (TEM), and scanning electron microscopic–energy dispersive spectrometric (SEM-EDS) studies.

EXPERIMENTAL

Materials

Kolliphor® P 188 [polyoxyethylene polyoxypropylene glycol] (Lubrizol, Germany) (188KP) was obtained as a gift sample from Lubrizol Advanced Materials Europe. Acrylamide (AM), N,N' -methylene-bis-acrylamide (MBA), ammonium persulphate (APS), silver nitrate, and gold (III) chloride trihydrate were purchased from SD Fine Chemicals (Mumbai, India). Di-distilled water was used for all the chemical cross-linking process, solution preparations, and swelling studies.

Synthesis of Dual Inorganic Nanocomposite Hydrogels

Microbial resistant dual inorganic nanocomposite hydrogels were prepared through green a process (Scheme 1). In this green method, mint leaf extracts were primarily prepared using the standard process described by Raghavendra *et al.*¹⁸ Mint leaves were collected and thoroughly washed with twice distilled water. Leaf broth was obtained by taking 7.5 g of mint leaves and finely cutting the leaves in a 500 mL Erlenmeyer flask with 150 mL of distilled water. The distilled water was heated (at 100°C for 5 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 mint leaf solutions were stored at 4°C.

Secondly, AM (14.06 mM) and different ratios (0.05–0.15 g) of 188KP polymer were dissolved in 3 mL of cold twice distilled water by stirring at 300 rpm for 2 h at room temperature. To this solution, 1 mL of MBA (10.64 mM) and 1 mL of APS (2.191 mM) solution were added. Following the addition of the reactants, the temperature of the hydrogel system was raised from 25°C to 45°C for 30 min. Furthermore, the chemical reaction was carried out at room temperature for 8 h. Following the completion of the reaction, the hydrogel was immersed in twice distilled water at room temperature for 48 h in order to remove the unreacted hydrogel materials present in their 3D network. Lastly, the [P(188KP-AM) $x=1-3$] hydrogels were dried at room temperature for further studies.

Finally, in order to prepare the dual inorganic nanoparticles onto the nanocomposite hydrogel, the required amounts of dried P(188KP-AM) hydrogels were immersed in a 50 mL of twice distilled water for 2 days in order to swell it to a full extent. The swollen hydrogel was transferred into a 250 mL beaker and immersed in 20 mL of silver nitrate (5 mM) and 10 mL of gold (III) chloride trihydrate (5 mM) aqueous solutions in order to allow for equilibration for 24 h. The P(188KP-AM) hydrogels were removed and transferred to a cold mint leaf solution for nucleation of the inorganic ions in the hydrogel network for 8 h. The dual nanoparticles composite [P(188KP-AM) $x + Ag^0/Au^0$, $x=0-3$] hydrogels developed were dried at room temperature for 5 days. Furthermore, it was powdered and used for different characterizations.

Characterizations

FTIR was used to study the transmission of the P(188KP-AM) hydrogel pattern, dual nanoparticles interactions (or lack of) in the polymer hydrogel matrices. The basic hydrogels and the dual nanoparticles-P(188KP-AM) hydrogels were completely dried in the oven at 50°C for 4 h before their FTIR experiments. Samples were examined between 500 and 4000 cm^{-1} on a Perkin Elmer Model Impact 410 (Wisconsin, MI) spectrophotometer. SEM–EDS analysis of P(188KP-AM) hydrogel and dual nanoparticles impregnated P(188KP-AM) hydrogels were performed using a JEOL JEM-7500F (Tokyo, Japan). SEM micrographs were recorded at acceleration voltages of 2 kV. TGA was conducted in a nitrogen atmosphere (100 mL/min) with a heating rate of 10°C/min using a SDT Q 600 thermal analyzer (T.A. Instruments-water LLC, Newcastle, DE). TEM micrographs were

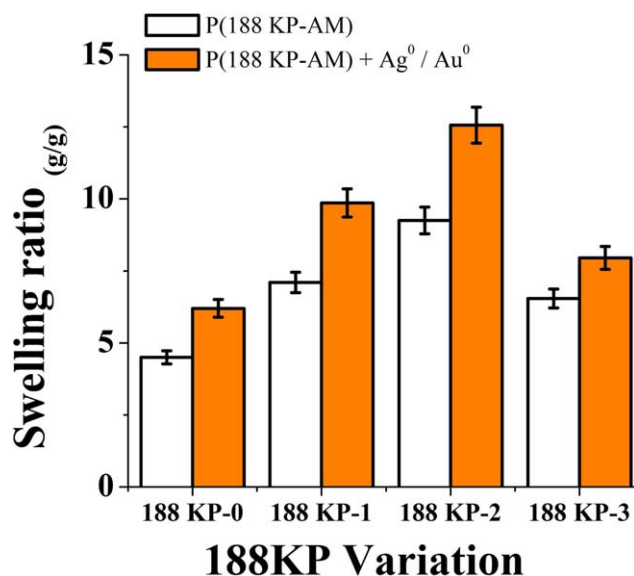


Figure 1. Swelling behavior of plain and dual individual inorganic hydrogels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

obtained using JEM-1200EX, JEOL Transmission electron microscope operating with an electron beam of energy of 200 kV. Samples were prepared by placing three to four drops of finely grinded P(188KP-AM) dual nanocomposite (1 mg/1 mL) solution on a 3-mm copper grid and allowing the liquid to evaporate in the air.

The swelling behavior of hydrophilic hydrogel samples were measured at room temperature, using a swelling method.^{19,20} The completely dried hydrogels were immersed in a 100 mL beaker containing twice distilled water until their weight became constant (24 h). The hydrogels were removed from the water and their surfaces were blotted with filter paper before being weighed. Furthermore, swollen hydrogels were treated with dual inorganic metallic aqueous solutions and subsequently with a mint solution via the bioprocess, as clarified in section "Synthesis of dual inorganic nanocomposite hydrogels." The

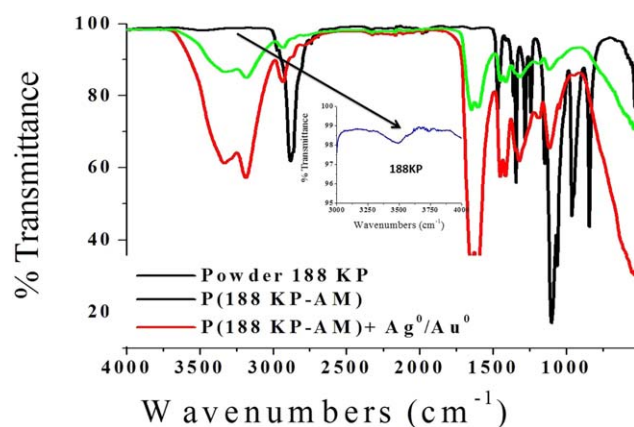


Figure 2. FTIR spectra of 188KP, plain hydrogels, and dual individual inorganic nanocomposite hydrogels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

swelling ratio ($S_{g/g}$) of the hydrogel developed and their dual nanocomposite hydrogels were calculated using eq. (1) below:²¹

$$\text{Swelling ratio, } S_{g/g} = \frac{H_s - H_d}{H_d} \quad (1)$$

where H_s and H_d denote the weight of the swollen hydrogel at equilibrium and the weight of the pure hydrogels, respectively.

Antibacterial Properties of Inorganic Nanoparticle Hydrogels

Bacterial inactivation of the dual-metallic nanocomposite P(188KP-AM) hydrogels, under study, was investigated using the disc diffusion technique, according to the standard procedure described elsewhere.^{22,23} Agar nutrient medium was developed by mixing 2.5 g of peptone, 1.5 g of beef extract, and 2.5 g of sodium chloride in 500 mL twice distilled water and the pH was adjusted to 7.0. Finally, 7 g of agar was added to the aqueous solution. The agar medium was sterilized in a conical flask at a pressure of 15 lbs in⁻² for 30 min. This agar medium was transferred into sterilized glass petri dishes in a laminar air flow chamber (Microfilt Laminar Flow Ultra Clean Air Unit, India, Mumbai) at 37°C. After solidification of the agar media, bacteria (*Bacillus*) (MTCC-1133; 50 μ L) were spread on the solid surface of the agar media for culture experiment. Over this inoculated glass petri dish, one drop of gel solution (10 mg of dual inorganic nanocomposite/5 mL of distilled water) was added using a 10 μ L tip and the plates were incubated for 24 h at 37°C, and bacterial inhibition zone was calculated by subtracting disc diameter from the total inhibition zone diameter.

RESULTS AND DISCUSSION

Individual silver and gold nanoparticles were prepared by nucleation of silver and gold salts on 188KP hydrogel (Scheme 1) network, using mint leaf extraction, which contains —OH associated with menthol, offer sufficient reduction capacity for the reduction of Ag⁺ and Au³⁺.²⁴ According to the literature, gold ions are reduced first then silver ions due to the difference in their reduction potentials.²⁵ The gold ions and silver ions were individually reduced with mint solution in the hydrogel (hydrophobic and hydrophilic) network. However, the nanoparticles

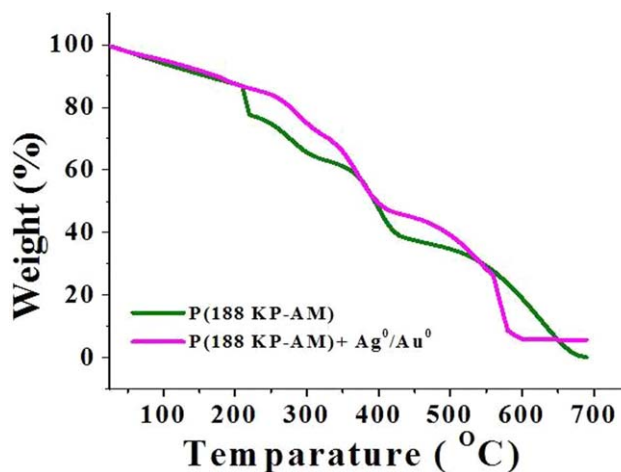


Figure 3. TGA cure of plain and dual individual inorganic nanocomposite hydrogel. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

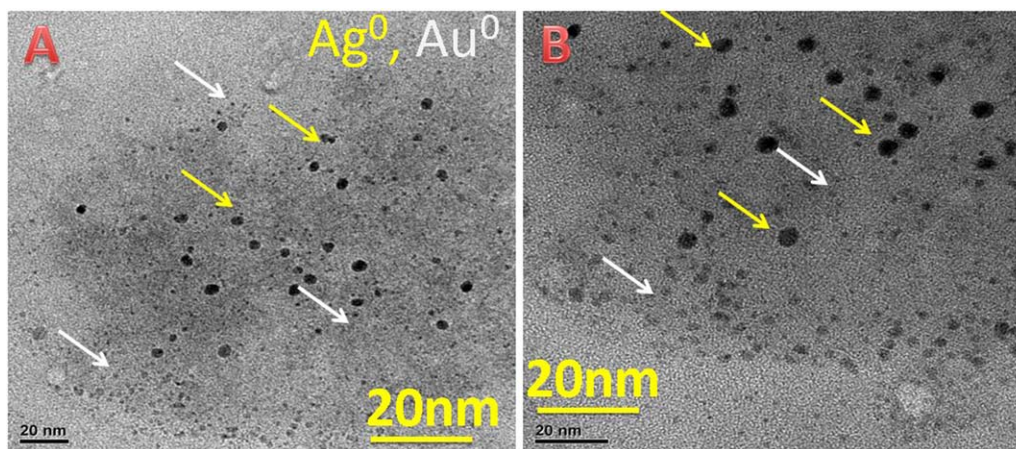


Figure 4. TEM image of dual individual inorganic nanoparticles obtained from P(188KP-AM)2. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

stabilization and agglomeration were highly controlled via the hydrophobic polyoxypropylene and hydrophilic polyoxyethylene blocks of Kolliphor[®] P 188 polymer.^{3,10} The formation of individual nanoparticles and their significance were determined from the following studies: Swelling, FTIR, TGA, TEM, SEM-EDS, and antibacterial activity and are reported in this segment.

Figure 1 illustrates the swelling capacity of plain [P(188KP-AM)] and dual nanocomposite [P(188KP-AM) + Ag⁰/Au⁰] hydrogels with different amounts of 188KP at room temperature, from which some variations (of the swelling) can be observed. The values of the hydrogels swelling ability were influenced by the 188KP concentration; with an increase of the 188KP content effecting, primarily, in an increased swelling capacity following which the swelling ability decreased. This behavior is attributed to the availability of more cross-linking sites for crosslink possibility at higher 188KP, concentration, which resulted in an overall hydrogels swelling decrease. However, the silver/gold nanocomposite hydrogels exhibited higher swelling ratio when compared with pure 188KP-based hydrogels. This result is attributed that a high concentration of 188KP which can significantly stabilize the inorganic nanoparticles within the hydrogels space and in nucleation time, the hydrogel network is expanded (the nanoparticles create holes) in the presence of the nanoparticles and water. The 188KP hydrogels

network highly stabilized the nano-inorganic particles. This stabilization is extremely helpful for the controlled release that (for a long time) of the nanoparticles in an aqueous medium and it can significantly improve the inactivation effect of bacteria in biomedical applications.

The formation of hydrogels and the interactions that occurred between the hydrogels and the nanoparticles were study via FTIR spectra. The FTIR spectra of clean powdered 188KP, pure hydrogel, and silver/gold nanocomposites are shown in Figure 2. The FTIR spectra of 188KP show characteristic peaks at 3483 (—OH), 2892 (—CH), which is due to the presence of —OH and —CH stretching bonds. The bonds at 1463 and 1353 indicate the presence of —CH₂ and —OH bending vibration peaks, respectively. The main C—O—C stretching peak was observed at 1107 cm⁻¹.^{23,26} FTIR studies of the pure hydrogel and nanoparticles hydrogel indicate interactions between 188KP, acrylamide and the interactions between hydrogel networks with the dual inorganic nanoparticles. The absorption peaks at 1107 cm⁻¹ for P(188KP-AM) hydrogel was due to the stretching vibration of C—O—C unit (188KP). The absorption peaks at 1107 cm⁻¹ shifted to 1121 cm⁻¹ in the case of P(188KP-AM) + Ag⁰/Au⁰. Similarly, the broad band at 3340 and 3191 cm⁻¹, corresponding to a stretching vibration of O—H/NH₂ groups in P(188KP-AM) hydrogel, shifted to 3330 and 3192 cm⁻¹ for the individual P(188KP-AM) + Ag⁰/Au⁰ nanocomposite

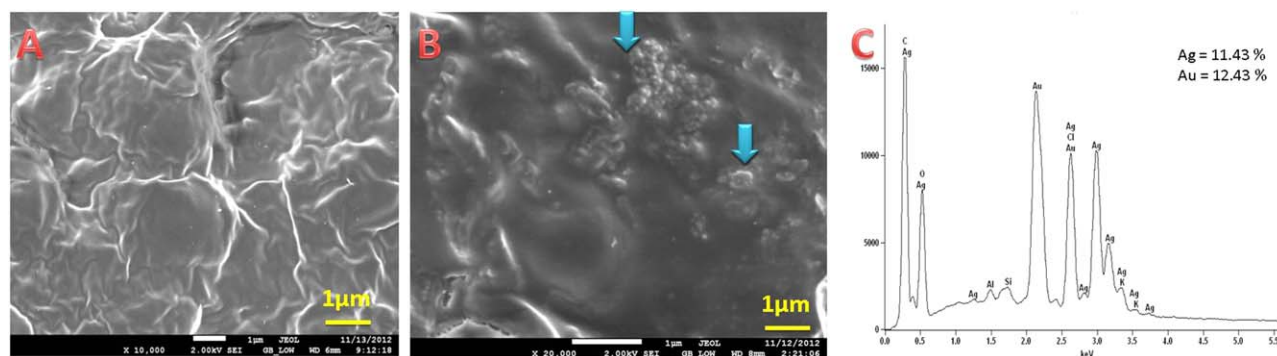


Figure 5. Scanning electron microscopy of plain hydrogel (A), dual individual inorganic nanocomposite hydrogels (B), and EDS images of dual individual inorganic nanocomposite hydrogel (C). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

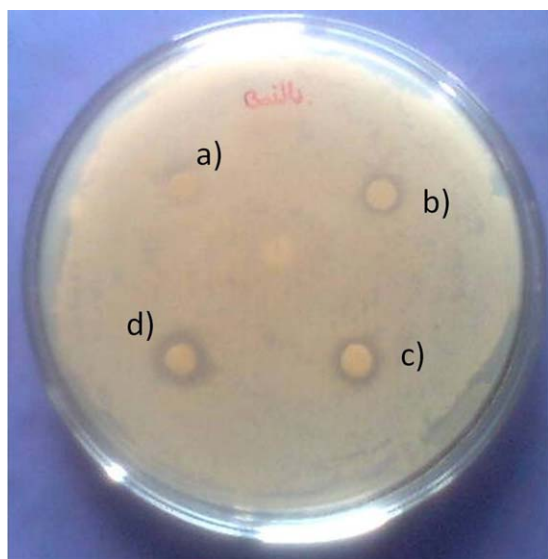


Figure 6. Antibacterial activity of plain and single and dual individual inorganic nanocomposite hydrogels (a) P(188KP-AM)₂, (b) P(188KP-AM)₂ + Ag⁰, (c) P(188KP-AM)₂ + Au⁰, and (d) P(188KP-AM)₂ + Ag⁰/Au⁰ nanocomposite hydrogels on *Bacillus*. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

hydrogels. Therefore, the 188KP-based hydrogels network has, no doubt, been highly involved in the synthesis and stabilization of the dual nanoparticles (silver/gold).

In order to analyze the thermal stability of the nanocomposite hydrogels and the formation of silver/gold nanoparticles in the hydrogel network, TGA experiments on the individual nanocomposite hydrogels were performed. As shown in Figure 3, the weight loss in P(188KP-AM), occurred at 677°C, with a significant mass loss of approximately 99.6%. For the P(188KP-AM) + Ag⁰/Au⁰ hydrogel, a comparatively less weight loss (~94.5%) was found at 677°C, which was due to the partial decomposition of the silver/gold nanoparticles. From the TGA results, the P(188KP-AM) + Ag⁰/Au⁰ hydrogels show a higher thermal stability when compared with the pure hydrogel.

TEM analysis [Figure 4(A,B)] shows that the dual inorganic [silver/gold] nanoparticles are independently well-dispersed in the hydrogel 3D network and they are spherical in shape with a size of around 3 ± 2 nm. This is due to the hydrophobic and hydrophilic blocks of Kolliphor[®] P 188 polymer, which independently stabilized the dual nanoparticles with mint leaves. Similarly, in order to ascertain the presence of silver/gold nanoparticles on the hydrogel surface, SEM examination [Figure 5(A,B)] was carried out. The distribution of silver/gold nanoparticles over the hydrogel surface can be visualized in Figure 5(B). Furthermore, EDS analysis was employed in order to confirm the presence of silver/gold nanoparticles in the P(188KP-AM) hydrogel, hence the EDS spectra of the dual inorganic nanocomposite hydrogel were investigated. The spectra of the dual inorganic nanocomposite hydrogels [Figure 5(C)], show clearly, the intensity peaks of silver/gold nanoparticles, which peaks are proportional to the metal concentration in the nanohydrogels.

In order to evaluate a realistic applicability of the nanohydrogel, it was tested against *Bacillus* bacteria. Figure 6 shows the antibacterial effect of nanohydrogels in terms of the zone of inhibition. The results indicate that the nanohydrogels containing dual inorganic nanoparticles show higher inhibition zone when compared with nanohydrogels containing single inorganic nanoparticles. However, inorganic nanoparticles are known to be reactive and thus will interact with cell-surface of the bacteria, eventually killing the bacteria.² The nanohydrogels containing dual individual inorganic nanoparticles formed have two different surface charges. Due to the dual inorganic nanoparticles, the nanohydrogels will, no doubt, have better interaction with bacteria in comparison to other nanohydrogels. In addition, it was found that the size of the inhibitory zone increased with an increase in the 188KP content in hydrogel, due to the higher stability of the inorganic nanoparticles. Therefore, these results are significant and will be useful and helpful in several scientifically related materials for several applications.

CONCLUSIONS

Nanohydrogel containing dual individual inorganic nanoparticles were successfully synthesized in 188KP hydrogel network via a green process. These composite hydrogels were developed and characterized by spectral, thermal, and electron microscopy. The nanocomposite hydrogels prepared have significant antibacterial activity against *Bacillus*. These results suggest that hydrogels containing individual dual nanoparticles would be useful for effective and convenient treatment of inactivation of bacteria. Therefore, by employing dual individual inorganic nanoparticles loaded in hydrogel networks could be helpful in developing effective pharmaceutical formulations for biomedical and biotechnological applications.

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