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SYNTHESIS AND CHARACTERIZATION OF COPOLYMERIC AND TERPOLYMERIC HYDROGEL-SILVER NANOCOMPOSITES BASED ON ACRYLIC ACID, ACRYLAMIDE AND ITACONIC ACID: INVESTIGATION OF THEIR ANTIBACTERIAL ACTIVITY AGAINST GRAM-NEGATIVE BACTERIA

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Abstract - In this study, copolymeric and terpolymeric hydrogel-silver nanocomposites based on poly(acrylamide-co-itaconic acid), poly(acrylic acid-co-itaconic acid) and poly(acrylic acid-co-acrylamide-co-itaconic acid) were synthesized by free-radical polymerization. These nanocomposites were characterized by Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), UV-Visible Spectrophotometry (UV-Vis) and X-Ray Diffraction (XRD) analysis, as well as their swelling behaviors. In addition, antibacterial properties of these hydrogel-silver nanocomposites were investigated against *Pseudomonas aeruginosa*. Acrylic-based hydrogel-silver nanocomposites demonstrated antibacterial activity against Gram-negative bacteria. These hydrogel-silver nanocomposites can be used as antibacterial material in the medical field.
Keywords: Antibacterial; Gram-negative bacteria; Hydrogel; Nanocomposite; Silver nanoparticle.

INTRODUCTION

Hydrogels are three-dimensionally crosslinked polymer networks composed of hydrophilic homo or hetero copolymers, and they have the ability to absorb significant amounts of water (Byrne *et al*., 2002). Hydrogels have been used in agricultural applications, the food industry, water treatment processes, and biotechnological and medical fields (Karadağ *et al*., 1997; Karadağ and Üzüm, 2012; Ju *et al*., 2009; Özkahraman *et al*., 2011). Today, hydrogel-silver nanocomposites have also been widely used in biomedical applications (Kim *et al*., 2004; Varaprasad *et al*., 2010; Yiamsawas *et al*., 2008; Gils *et al*., 2010; Guzman *et al*., 2012).

Nano-sized metal particles such as silver, gold, and copper are highly toxic to microorganisms, and they exhibits strong biocidal effects (Thomas *et al*.,

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2007). In recent years, silver nanoparticles have been preferred because of their antimicrobial effect to fight against infection and diseases (Ravindra *et al*., 2012). Silver exhibits strong cytotoxicity for various microorganisms and it has been extensively used to control infections since ancient times (Travan *et al*., 2009; Pinto *et al*., 2009; Zhou *et al*., 2012). Metallic and ionic forms of silver have a bacteriostatic (growth inhibition) or a bactericidal (antibacterial) impact (Pinto *et al*., 2009). In addition, low concentrations of silver nanoparticles are non-toxic to human cells and silver is known to be an environmentally friendly antibacterial material (Murthy *et al*., 2008; Thatiparti *et al*., 2009). Therefore, silver has been extensively preferred for synthesis of medical products. Silver-based medical products can be used as ointments and bandages which have retarding and preventing properties for bacterial infections (Travan *et al*., 2009). Hydrogels play an important role in the stabilization of silver nanoparticles (Khan *et al*., 2011). Because of this, silver nanoparticles are embedded in hydrogel networks for preparation of antibacterial hydrogel-silver nanocomposites (Murthy *et al*., 2008). Today, there has been growing interest in the development of antibacterial materials combining the antibacterial properties of silver with the original performance of the polymer matrix (Travan *et al*., 2009) and there are numerous research studies on hydrogel/silver nanocomposites.

Poly(acrylic acid-co-acrylamide) (Yiamsawas *et al*., 2008; Thomas *et al*., 2007; Buikliskii *et al*., 2012; Mohan *et al*., 2010; Aggor *et al*., 2010), poly(acrylamide)/poly(vinyl pyrrolidone) (Murthy *et al*., 2008), poly(acrylamide)/poly(vinyl alcohol) (Varaprasad *et al*., 2010), poly(2-hydroxyethyl methacrylate-co-acrylic acid) (Gils *et al*., 2010), poly(ethylene glycol dimethacrylate-co-acrylonitrile) (Kim *et al*., 2004), poly(N-isopropylacrylamide)-co-sodium acrylate) (Mohan *et al*., 2007; Mohan *et al*., 2006); poly(N-isopropylacrylamide-co-acrylic acid-co-butylmethacrylate) (Thatiparti *et al*., 2009), poly (acrylamide-co-2-acrylamido-2-methylpropanesulfonic acid) (Ravindra *et al*., 2012), poly(2-hydroxyethyl methacrylate-(poly(ethylene glycol) methyl ether methacrylate-methacrylic acid) (Xiang and Chen, 2007), poly(N,N-dimethylacrylamide)-g-poly(vinyl alcohol) (Luo *et al*., 2009) and poly(2-hydroxylethyl acrylate)epoly(ethylenimine) (Ho *et al*., 2004) based hydrogel-silver nanocomposites have been previously synthesized.

In the above mentioned studies, the antibacterial activities of hydrogel-silver nanocomposites were investigated and the use of these nanocomposite hydrogels in pharmaceutical and biomedical fields foreseen. In addition, there are various studies on the use of hydrogel-silver nanocomposites as wound dressings (Hong, 2007; Rujitanaroj *et al.,* 2008), for biologic labeling (Xu *et al.,* 2012) and drug delivery (Xiang and Chen, 2007), and as biosensors (Endo *et al.,* 2008) and photonic crystals (Xu *et al.,* 2003). There are also different articles about polymeric membranes containing silver salts (Pollo *et al.,* 2012) and, MgO nanoparticles as antibacterial agent (Tang *et al.,* 2012; Tang *et al.,* 2014.)

A literature survey has not yielded any research about terpolymeric hydrogel-silver nanocomposites based on poly(acrylic acid-co-acrylamide-co-itaconic acid). There were no detailed data about the investigation of antibacterial activity against gram-negative bacteria of this terpolymer in the literature.

In this study, poly(acrylamide-co-itaconic acid) [p(AAm-co-IA)], poly(acrylic acid-co- itaconic acid) [p(AA-co-IA)] and poly(acrylic acid-co-acrylamide-co-itaconic acid) [p(AA-co-AAm-co-IA)] based hydrogel-silver nanocomposites were synthesized by free-radical polymerization. These nanocomposites were characterized by Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), UV-Visible Spectrophotometry (UV-Vis) and X-Ray Diffraction (XRD) analysis. Their swelling behaviors were also investigated in detail. In addition, antibacterial properties of these hydrogel-silver nanocomposites were investigated against the Gram-negative bacterium, *Pseudomonas aeruginosa*.

EXPERIMENTAL

Materials

Acrylamide (AAm), acrylic acid (AA), polyethylene glycol (PEG) with an average molecular weight of 10.000, silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$), were purchased from Merck (Germany). Ammonium persulphate (APS), itaconic acid (IA) and N,N-methylenebisacrylamide (NMBA) were obtained from ABCR (Germany), Fluka (USA) and Aldrich (USA), respectively. Other reagents were chemically pure grade, and all solutions and standards were prepared with distilled water.

Synthesis of Hydrogels

Hydrogels abbreviated as "p(AAm-co-IA)", "p(AA-co-IA)", "p(AA-co-AAm-co-IA)" were synthesized by free radical polymerization using crosslinker (NMBA) and initiator (APS). PEG was also used as pore maker in order to form a porous structure. Syntheses of

hydrogels were performed as follows.

Firstly, monomers were dissolved in deionized water at the desired mole ratios in cylindrical glass tubes and PEG (5% w/w of total monomer weight) was added to this aqueous monomer solution. Then, initiator (1% w/w of total monomer weight) and crosslinking agent (5% w/w of total monomer weight) were also directly added. After sealing the mouth of these tubes with rubber caps, the solution was purged with nitrogen gas for 30 min and the polymerization reaction was performed at 80 °C for 3 h. At the end of the reaction, the glass tubes were carefully broken and hydrogels were cut into discs 10 mm in length. These hydrogel discs were immersed in deionized water at room temperature for 72 h. During this time, the water was replaced once a day with fresh distilled water in order to remove residual monomer. Afterwards, hydrogels were dried in an oven at 50 °C. Dried pure hydrogels were used for preparation of hydrogel-silver nanocomposites. Feed compositions and symbols of the pure hydrogels are given in Table 1.

Table 1: Feed compositions of the hydrogels.

Sample	AAm	AA	IA	APS	NMBA	PEG
	(molar ratio)			(weight %)*		
p(AAm-co-IA)	2	-	1	1	5	5
p(AA-co-IA)	-	2	1	1	5	5
p(AA-co-AAm-co-IA)	1	1	1	1	5	5

* based on total monomer amount

Preparation of Hydrogel-Silver Nanocomposites

Hydrogel-silver nanocomposites (HSNC) were prepared according to a procedure given in the literature (Mohan *et al*., 2010). This procedure was realized as follows.

Firstly, dry pure hydrogel (H) discs (50 mg) were completely swollen in distilled water for 2 days and then the freshly swollen hydrogels were equilibrated in 30 mL of aqueous $AgNO_3$ solution (2g/L, 0.012 mol/L) for 24 hours. After removing the excess of $AgNO_3$ solution from the surface of the swollen hydrogels with filter paper, the silver salt loaded hydrogels (HS) were immersed in 50 mL of $NaBH_4$ solution (2g/L, 0.053 mol/L) for 24 hours to reduce the absorbed silver ion (Ag^+) in the hydrogel structure to metallic silver nanoparticles (Ag^o). The formation of the silver nanoparticles in the hydrogel structure was observed by the appearance of a brown color. Symbols for the pure hydrogels, silver loaded hydrogels and hydrogel-silver nanocomposites are given in Table 2.

Table 2: Symbols of the hydrogels, silver-loaded hydrogels and hydrogel-silver nanocomposites.

Composition	Symbols		
	Hydrogels	Silver-loaded hydrogels	Hydrogel-silver nanocomposites
p(AAm-co-IA)	H1	HS1	HSNC1
p(AA-co-IA)	H2	HS2	HSNC2
p(AA-co-AAm-co-IA)	H3	HS3	HSNC3

Swelling Studies

The swelling properties of each one of the H, HS, and HSNC samples were investigated gravimetrically in distilled water at room temperature according to the well-known tea-bag method (Yazdani-Pedram *et al.,* 2000; Al *et al.,* 2008; Dalaran *et al.,* 2009; Dalaran *et al.,* 2011; Çöle *et al.,* 2013). To apply this method, the tea-bag made of 200 mesh nylon screen containing dried sample was immersed in water until the equilibrium swelling ratio (Q_e) was reached. Then the tea-bag was taken out and the excess water removed with filter paper. The tea-bag containing the swollen hydrogel was weighed. The equilibrium swelling ratio (swelling capacity) of samples was calculated using to following equation:

$$Q_e = \frac{W_{wet} - W_{dry}}{W_{dry}}$$

Q_e is the equilibrium swelling ratio in grams of water per gram of sample. W_{wet} and W_{dry} are the weights of the swollen sample and the dry sample in grams, respectively.

FTIR Analyses

The infrared spectra of the H and HS samples were obtained with a "Perkin-Elmer Spectrum One" FTIR Spectrophotometer in the range 400-4000 cm^{-1}. In order to prepare the samples in pellet form for FTIR analysis, dried samples were diluted with IR grade Merck potassium bromide powder (KBr) (samples/ KBr: 1/200 (w/w)) and compressed into a disc under a pressure. Then these discs were subjected to FTIR analysis.

XRD Analyses

X-ray diffraction (XRD) patterns of the HSNC samples were obtained using a "Philips Panalytical X'Pert Pro X-ray Diffractometer" with CuK α radiation (45 kV, 40 mA and λ=1.5406). Powder samples were pressed into a tablet shaped disc before XRD

analysis, and these samples were placed in the measurement chamber of diffractometer and subjected to XRD analysis.

UV-Vis Spectroscopic Analyses

UV-Vis spectroscopic analyses of the HSNC samples were carried out with a "Shimadzu UV-160A UV-Visible Spectrophotometer" with a scan range of 200-600 nm. For UV analysis, hydrogel-silver nanocomposites were allowed to swell with deionized water, and then UV-Vis spectra were recorded using deionized water for background correction. At this stage, silver nanoparticles were extracted from the swollen hydrogel-silver nanocomposite samples into the deionized water sample. Then a certain amount of sample was taken from this medium to measure of absorption spectra.

UV -Vis. analyses were applied to selected samples. Sample selection for UV-Vis. analysis was determined based on the swelling experiment results. In the swelling studies, the equilibrium swelling ratio values of HSNC2 and HSNC3 were very close to each other. Therefore, two samples (HSNC1 and HSNC3) that had different equilibrium swelling ratio values were selected.

SEM Analyses

The scanning electron microscopy (SEM) images of the H and HSNC samples were obtained using a "Quanta FEG 450 Scanning Electron Microscope". In these analyses, the samples were coated with gold before SEM measurements. This coating is required to obtain a clear image of an insulating material, but is so thin (200 A°) that it does not hinder the identification of specific minerals (Welton, 2003). At the end of the SEM analysis, EDAX spectra and SEM micrographs were obtained.

The SEM micrographs of H samples were very similar to each other. Similarly, the SEM micrographs of HSNC samples were also very similar to each other. Since the surfaces of all H and HSNC samples were almost the same, only the SEM micrograph of H3 and HSNC3 samples are given in the manuscript.

Antibacterial Activity

The antibacterial activities of the HSNC samples were determined by the modified disc diffusion method (Bauer *et al*., 1966) against *Pseudomonas aeruginosa* ATCC 9027 (Gram negative bacterium). The overnight bacterial culture was diluted with tryptic soy broth (TSB) medium up to 0.5 McFarland standards to obtain a bacterial cell density around 108 CFU/mL and 100 mL of this culture was spread homogenously on the tryptic soy agar (TSA) plate. The HSNC samples (5mg/mL) were deposited on the plate after drying and incubated for 24 h at 37 °C. Geneticin (10 µg, Oxoid) and the hydrogels without Ag were used as positive and negative controls, respectively. The tests were carried out in triplicate. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organism. These values were calculated with Graph Pad Prism software and reported as mean ±SD.

Antibacterial activity tests were applied to selected samples. Sample selection for the antibacterial tests was determined based on the swelling experiment results. Because the equilibrium swelling ratio values of HSNC2 and HSNC3 were very close to each other, two samples (HSNC1 and HSNC3) with different equilibrium swelling ratio values were selected.

RESULTS AND DISCUSSION

This study deals with both the synthesis of antibacterial hydrogel-silver nanocomposites based on acrylic acid, acrylamide, itaconic acid and the investigation of the antibacterial activities of these hydrogel-silver nanaocomposites against the Gram-negative bacterium, *Pseudomonas aeruginosa*. For this purpose, three types of hydrogels were synthesized and hydrogel-silver nanocomposites were prepared using $AgNO_3$-$NaBH_4$. These HSNC samples were characterized by FTIR, UV-Vis. spectroscopy, XRD and SEM techniques. In addition, the swelling properties and antibacterial activities of HSNC samples were also investigated.

Structural analysis of the samples by FTIR indicated that the copolymeric and terpolymeric hydrogels were successfully synthesized. FTIR analysis also indicated that the interaction between silver and the polymer network occurred. This interaction is evidence for the presence of silver ions in the silver-loaded hydrogel structure. The results of XRD and UV-Vis. analyses confirmed the presence of silver nanoparticles in the hydrogel structure. Morphological analysis of samples by SEM clearly indicated the formation of silver nanoparticles in the hydrogel network. The EDAX spectrum also confirmed this result. In swelling studies, the order of the swelling capacity of the gels was found to be in the following order:

$$\text{pure hydrogel} > \text{hydrogel} - \text{silver nanocomposite} > \text{silver} - \text{loaded hydrogel.}$$

In the antibacterial activity studies, the hydrogel-silver nanocomposites exhibited antibacterial activity against the Gram-negative bacterium, *Pseudomonas aeruginosa*. All analyses are examined in detail in the separate headings below.

FTIR Analyses

The FTIR spectra of H1, H2, H3, HS1, HS2 and HS3 are given in Figures 1-3. The characteristic peaks of acrylic-based copolymers were found at about 1400 cm^{-1} (C-O symmetric stretching) and 1450 cm^{-1} attributed to the -CH_2 group (Silverstein and Bassler, 1966). As expected, these peaks were observed in all spectra.

In the case of the AAm-IA based copolymers H1, the double peaks observed at about 1668 and 1720 cm^{-1} correspond to the amide and carboxyl groups (Silverstein and Bassler, 1966), respectively. In the case of the AA-IA based copolymer H2, a single peak was observed at 1721 cm^{-1} corresponding to the carboxyl groups of acids. In parallel, in the case of the AA-AAm-IA based terpolymer H3, peaks were observed at 1660 and 1727 cm^{-1} for the amide (shoulder) and carboxyl groups (sharp peak), respectively.

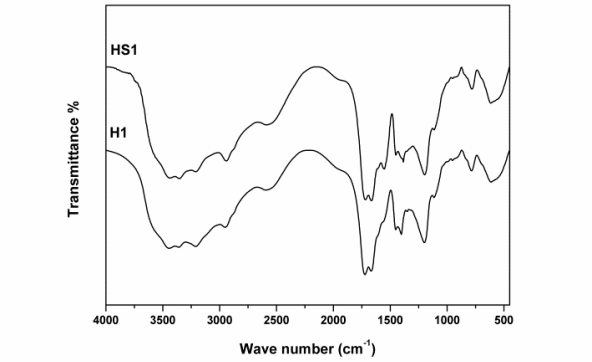


Figure 1: The FTIR spectra of H1 and HS1.

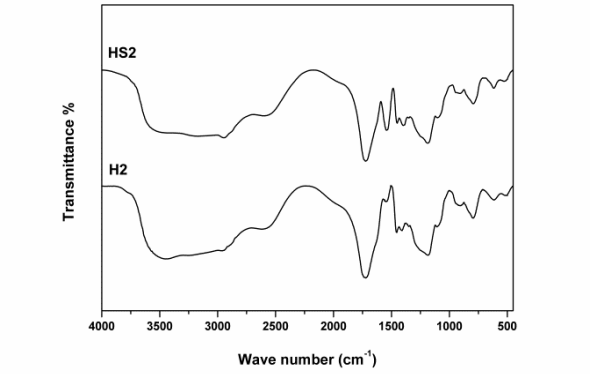


Figure 2: The FTIR spectra of H2 and HS2.

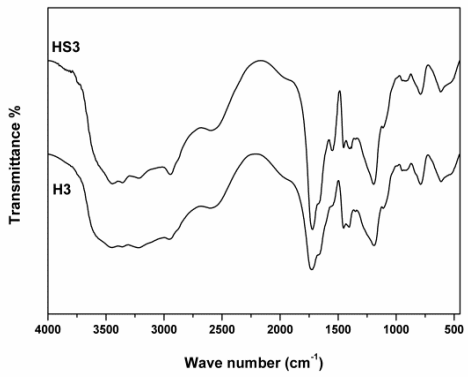


Figure 3: The FTIR spectra of H3 and HS3.

For the silver loaded hydrogels (H-Ag^{+}) HS1, HS2, HS3 a new peak was observed at about 1550 cm^{-1} attributed to the ionized carboxylate ion (Gils *et al*., 2010; Silverstein and Bassler, 1966). This indicates ionization of carboxyl groups and complexation between the COO^{-} groups and Ag+ ions (Xiang and Chen, 2007; Khan *et al.,* 2011). This provides evidence of the presence of silver ions in the hydrogel structure and of interaction between silver nanoparticles and the polymer backbone.

XRD Analyses

The XRD pattern of H and HSNC samples was used to determine the nanoparticle formation in the gel networks. XRD patterns of H1, H2, H3, HSCN1, HSCN2 and HSCN3 are demonstrated in Figures 4-6. Pure hydrogel samples did not exhibit any sharp peaks in XRD patterns, with only a broad peak at ~20 θ attributed to the polymer networks (Varapsad *et al*., 2009). In the case of HSNC, four sharp peaks were observed. These four diffraction peaks in the XRD patterns of HSCN samples, at angles $2\theta=38.06^{o}$, 44.09^{o}, 64.27^{o}, 77.45^{o} are ascribed to Bragg reflections through (111), (200), (220) and (311) planes of the face-centered cubic (fcc) packing of silver nanoparticles, respectively (Gils *et al*., 2010; Murthy *et al*., 2008). However, these peaks due to the formation of metallic silver nanoparticles in the gel networks (Varapsad *et al.,* 2009) were not observed in XRD patterns of H1, H2 and H3 hydrogels, as expected. These results confirm that all HSCN samples contain silver nanoparticles.

UV-Vis Spectroscopic Analyses

To confirm the presence of silver nanoparticles in the hydrogel structure, UV-Vis. Spectroscopy was used. UV-Vis. spectra of HSNC1 and HSNC3 samples

are given in Figure 7. For this study, 150 mg of HSNC was dispersed in 15 mL of deionized water for 2 days to extract all silver nanoparticles into the aqueous phase and absorption spectra recorded with a scan range of 200–600 nm for these solutions. As shown in Figure 7, the spectra of HSNC samples have a characteristic absorption peak at around 405-410 nm due to the surface-plasmon resonance band of the silver nanoparticles (Yiamsawas *et al*., 2008; Mohan *et al*., 2007), indicating the presence of silver nanoparticles (Varapsad *et al.,* 2010) in the hydrogel structure.

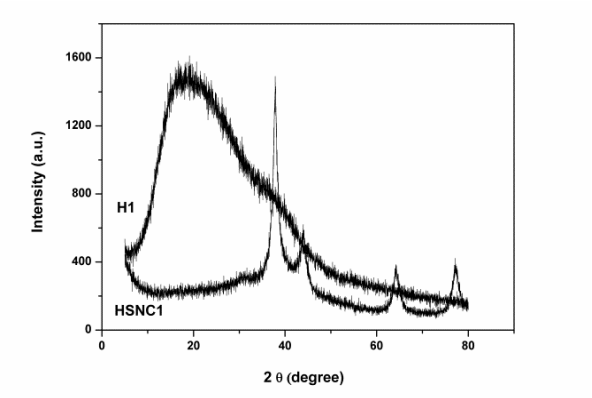


Figure 4: XRD patterns of H1 and HSCN1.

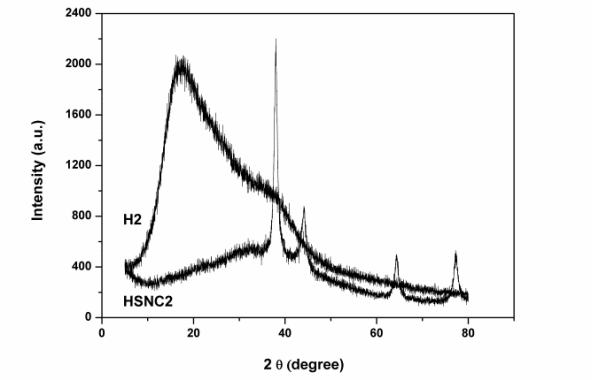


Figure 5: XRD patterns of H2 and HSCN2.

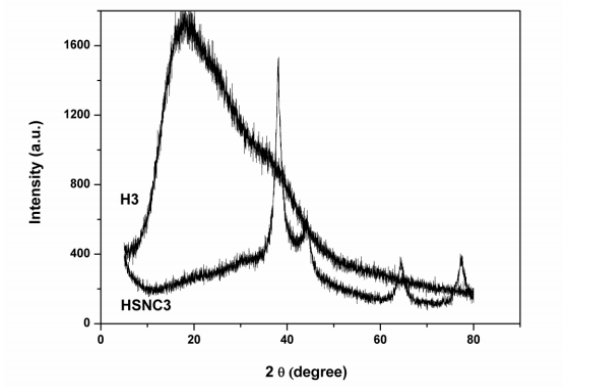


Figure 6: XRD patterns of H3 and HSCN3.

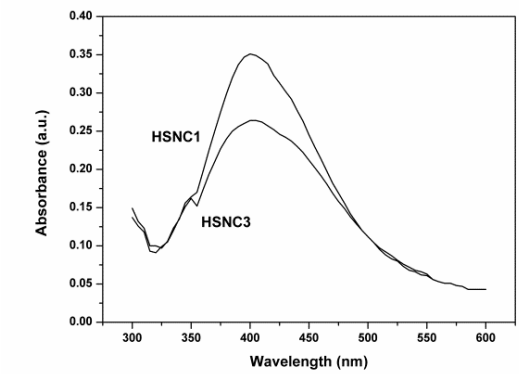


Figure 7: UV-Vis. spectra of HSNC1 and HSNC3.

SEM Analyses

Surface morphologies of the hydrogels and hydrogel-silver nanocomposites were investigated by SEM and EDAX analysis. SEM micrographs of H3, HSNC3 samples and the EDAX spectrum of the HSNC3 sample are illustrated in Figures 8, 9 and 10, respectively. The SEM micrographs were taken at 80.000 magnifications.

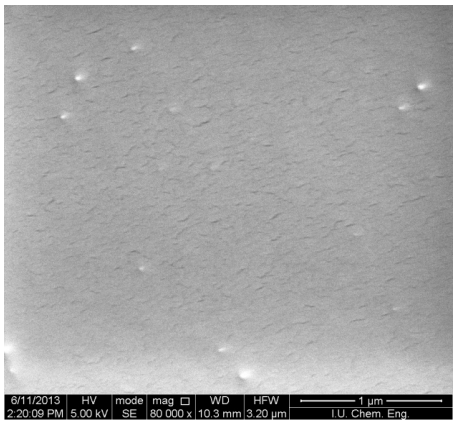


Figure 8: SEM micrograph of H3.

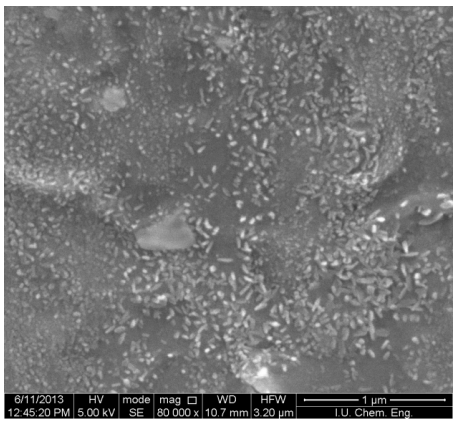


Figure 9: SEM micrograph of HSNC3.

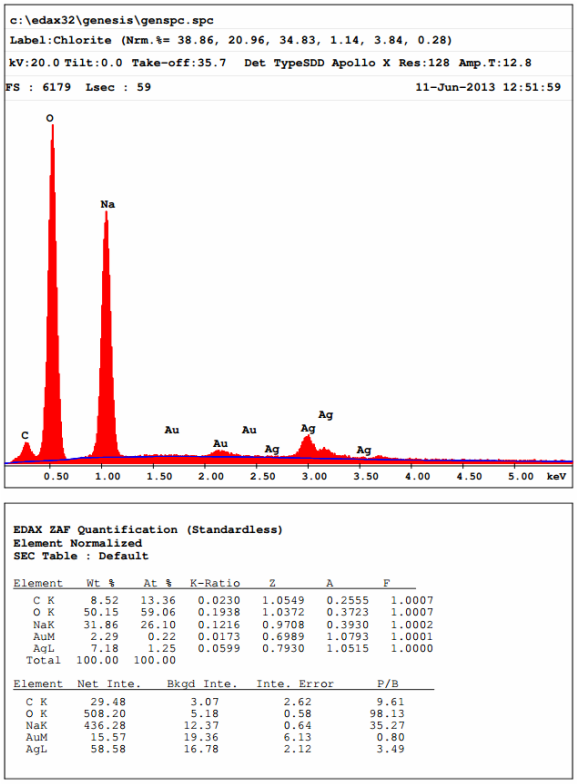


Figure 10: EDAX spectrum of HSNC3

The SEM micrograph of pure hydrogel (H, without nanoparticles) showed a clear network and smooth surface throughout the structure, while the SEM micrograph of HSNC clearly showed the formation of silver nanoparticles in the network. The SEM micrographs of HSNC showed that the silver nanoparticles were dispersed and embedded in the polymer matrix in all micrographs. The EDAX spectrum of the HSNC sample in Figure 10 also confirmed this result.

Swelling Studies

The results of swelling studies are presented together in Figure 11. As seen from the figure, the swelling capacities of pure hydrogels are higher than those of hydrogel-silver nanocomposites and silver-loaded hydrogels.

After the hydrogels were treated with $AgNO_3$ solution and silver ions loaded into the hydrogel network, the presence of silver ions in the hydrogel network caused a decrease of the swelling degree of silver-loaded hydrogels. This diminution originates from complexation of the Ag+ ion with carboxyl groups of the polymer backbone. As seen from the figure, after the addition of Ag+, a significant amount of shrinkage was observed in the hydrogel network. When the silver-loaded hydrogels were treated with $NaBH_4$ solution, silver nanoparticles were found in the hydrogel network. Thus, the swelling ratio of hydrogel-silver nanocomposites improved upon formation of Ag^o nanoparticles by the reduction reaction. The improvement of the swelling capacity in the hydrogel-silver nanocomposites can be attributed to the presence of silver nanoparticles of different sizes and different surface charges in the hydrogel network (Gils *et al*., 2010; Murthy *et al*., 2008; Vimala *et al*., 2009, Yiamsawas *et al*., 2008). As a result, the order of the swelling capacity of the gels is the following: H > HSNC > HS.

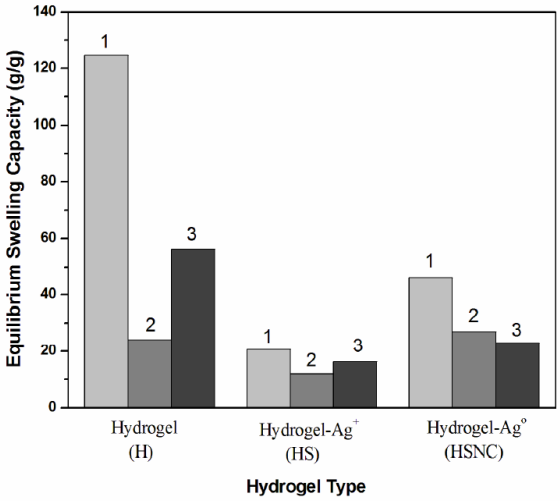
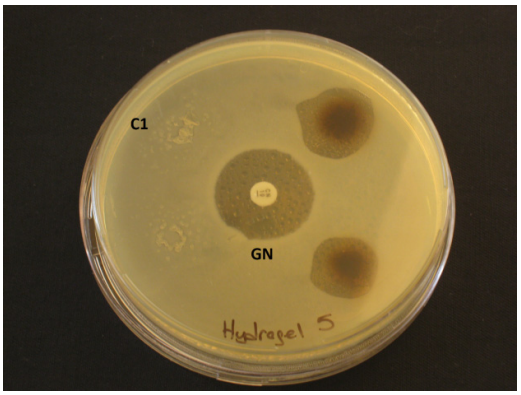


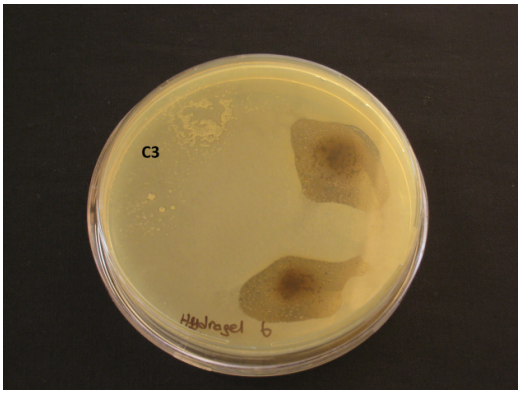
Figure 11: Equilibrium swelling ratio values of hydrogels (H), silver-loaded hydrogels (HS) and hydrogel-silver nanocomposites (HSNC).

Antibacterial Activity

The assay results show that two of these hydrogels (H1 and H3) exhibited antibacterial activity against *Pseudomonas aeruginosa* (ATCC 9027) (Figure 12a and 12b). Clear inhibition zones occurred for HSNC1 and HSNC3 samples compared with control hydrogels without silver (C1 and C3) (Figure 12a and 12b). Hydrogel-silver nanocomposites exhibited antibacterial activity against *Pseudomonas aeruginosa.* As shown in Table 3, inhibition zone diameters of hydrogel-silver nanocomposites were close to geneticin as positive control. As a result, hydrogel-silver nanocomposites can be used as antibacterial agent against gram-negative bacteria.



(a)



(b)

Figure 12: Antibacterial activity of (a) HSNC1 and (b) HSNC3 against *Pseudomonas aeruginosa* (ATCC 9027), C1: Hydrogel 1 without silver and C3: Hydrogel 3 without silver as negative control, GN: Geneticin as positive control.

Table 3: Antibacterial activity of HSNC samples.

Gram-negative bacteria	Samples		GN (Control)
	HSNC1	HSNC3	
Pseudomonas aeruginosa (ATCC 9027)	1.8±0.17	2.27±0.24	2.4±0

Inhibition zone diameters were expressed as mean ±SD (mm).
GN: Geneticin (10 µg)

CONCLUSION

In this study, copolymeric and terpolymeric hydrogel-silver nanocomposites based on acrylic acid, acrylamide and itaconic acid were synthesized for investigation of antibacterial activities against *Pseudomonas aeruginosa*. These hydrogel-silver nanocomposites were characterized by FTIR, UV-Vis. spectroscopy, XRD and SEM techniques as well as their swelling properties. The following conclusions can be drawn from the results obtained:

- FTIR spectra of HS samples showed the presence of silver ions in the hydrogel structure, and interaction between silver nanoparticles and the polymer backbone.
- XRD patterns of HSNC samples confirmed the presence of silver nanoparticles in hydrogel structure.
- UV-Vis. spectra of HSNC indicated the formation of silver nanoparticles.
- SEM micrographs of HSNC showed the distribution of silver nanoparticles in the polymer network. In addition, silver nanoparticles embedded in the polymer matrix were also observed in the EDAX spectrum.
- In swelling studies, a significant amount of shrinkage of the gel network was observed after the addition of Ag+ ion. Nevertheless, when Ag^{o} nanoparticles were formed by reduction, the swelling capacity of hydrogel-silver nanocomposites improved. This situation reflects the surface charge of the nanoparticles and an expansion in hydrogel network. The order of the swelling capacity of the gels was the following: Hydrogel > Hydrogel-Ag^{o} > Hydrogel-Ag^{+}.
- Acrylic-based hydrogel-silver nanocomposites exhibited antibacterial activity against *Pseudomonas aeruginosa* according to antibacterial test results.

In conclusion, the silver nanoparticles were successfully produced within hydrogel networks. Synthesized acrylic-based hydrogel-silver nanocomposites demonstrated antibacterial activity against the Gram-negative bacterium, *Pseudomonas aeruginosa*. The hydrogel-silver nanocomposites obtained in this study are suitable for antibacterial applications in the medical field.

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