

# Synthesis and characterization of novel bactericidal Cu/HPMC BNCs using chemical reduction method for food packaging

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**Abstract** In this research copper nanoparticles (Cu NPs) were incorporated in the biodegradable hydroxypropyl methylcellulose (HPMC) matrix using the simple and low cost chemical reduction method for application as food packaging material. The properties of Cu/HPMC bionanocomposites (BNCs) were studied as a function of the  $\text{CuSO}_4$  concentration. Surface morphology of the film was investigated by scanning electron microscopy. Mechanical analysis and water vapor barrier properties of HPMC/Cu nanocomposites were analyzed. It was observed that mechanical and water vapor barrier properties of the films were improved by the concentration of  $\text{CuSO}_4$ . The antibacterial activity of HPMC/Cu thin films were evaluated based on the diameter of inhibition zone in a disk diffusion test against Gram positive bacteria, ie, *Streptococcus A.*, *S. epidermidis*, *S.aureus*, *B.cereus* and Gram negative bacteria, ie, *E. coli*, *E. faecalis*, *Salmonella*, *P. aeruginosa* using Mueller Hinton agar at different concentration of  $\text{CuSO}_4$ . The results revealed a greater bactericidal effectiveness for nanocomposite films containing 5 % of  $\text{CuSO}_4$ . Packages prepared from HPMC/Cu nanocomposite films were used for meat packaging. The films were filled with meat and then stored at 4 °C. Microbial stability of the meat was evaluated after 3, 7, 10 and 15 days of storage. The results showed that microbial growth rate significantly reduced as a result of using this nanocomposite packaging material.

**Keywords** Nanocomposite · Antibacterial · Active packaging · Biodegradable · Cu NPs

## Introduction

In recent global competitive markets, active packaging is an important concept to inhibit or retard the growth of microorganisms that may be present in the packed food or packaging material itself. The researches in antimicrobial packaging have been expanded to extend of the shelf life or enhance quality and safety of the food products. The incorporation of antimicrobials into packaging brought in a new generation of nanocomposites comprising antibacterial nanoparticles blended in the polymer matrix.

Among the cellulose-based materials, Hydroxypropyl methylcellulose (HPMC) is a semisynthetic, inert, viscoelastic biopolymer. HPMC has been examined for many different applications due to its biocompatibility, non-toxicity to mammals, barrier properties, low cost, antimicrobial activity and solubility in aqueous medium (Imran et al. 2010). Moreover, HPMC films are water-soluble, odorless and tasteless, and present moderate resistance to moisture and oxygen permeability (Krochta and Mulder-Johnston 1997). Consequently, they have a variety of current and potential applications in deep frying food products (Balasubramaniam et al. 1997; Kester and Fennema 1986) drug delivery and ophthalmology (De Silva and Olver 2005; Williams et al. 2001) and food processing (Moura et al. 2012; Hiroyasu 2003).

Nanomaterials are being widely used as they offer antimicrobial properties due to their high surface area/volume ratio. Gold and silver nanoparticles are the most widely studied particles. Different inorganic

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antibacterial materials, including Au and Ag nanoparticles have been expanded and some are in commercial use (Kawashita et al. 2000). In recent years, copper nanoparticles (Cu NPs) have attracted great interest due to their potential applications, such as, conductive films, lubrication, nanofluids, ink, metallic coatings and catalysis (Tilaki et al. 2007; Zhu et al. 2005; Larsen and Noriega 2004; Patel et al. 2005; Wang et al. 2004). The antimicrobial activity of copper nanoparticles (Cu NPs) is a new features issued against different microorganism (SCI Finder Scholar™, American Chemical Society). Since the copper has been known as an antibacterial agent (Faundez et al. 2004; Mary et al. 2009; Young et al. 2012), higher disinfecting effects are expected in nano scales. However, no study has been conducted on application of the Cu NPs for antibacterial food packaging.

In this work, Cu NPs have been embedded in a biodegradable polymer matrix, as a means to combine the antimicrobial properties of copper with the biocompatible and antimicrobial features of HPMC for active food packaging.

## Materials and methods

### Chemicals

Hydroxypropyl methylcellulose (Methocel E15) was obtained from Dow Chemical Co. (Midland, MI, USA.).  $\text{CuSO}_4$  (99.98) used as the copper precursor, was obtained from Merck (Darmstadt, Germany) and used without purification. Deionized water was used to prepare all the solutions employed.  $\text{NaBH}_4$  (98.5 %), used as a reduction agent was obtained from Sigma-Aldrich (St Louis, MO).

Gram positive: *Streptococcus A.* (ATCC 19615), *S. epidermidis* (ATCC 12228), *S. aureus* (ATCC 25922) *B. cereus* (ATCC 11788), and Gram negative: *E. coli* (ATCC 25923), *E. faecalis* (ATCC 29212), *Salmonella* (ATCC 14028), *P. aeruginosa* (ATCC 27853) bacteria, used for the antibacterial assay were obtained from the Bahar Afshan Ltd, Iran.

### Synthesis of (Cu NPs)/HPMC film

Net HPMC films (control films) were obtained by casting from a solution containing 3.0 g of HPMC in 100 ml of distilled water kept under magnetic stirring for 12 h. The HPMC: water ratio was kept at 3:97 (w: w) in all film-forming solutions. Following the usual preparation method for Cu NPs,  $\text{CuSO}_4$  solutions were added to each soluble HPMC sample under constant stirring for synthesis of the  $\text{CuSO}_4$ / HPMC solutions.

The Cu content of the samples was 0.5, 1.0, 2.0, and 5.0 g Cu/100 g HPMC. Freshly prepared  $\text{NaBH}_4$  ( $4 \times 10^{-2}$  M) solution was then added to the suspensions under continuous stirring to reach a constant  $\text{CuSO}_4$  /  $\text{NaBH}_4$  molar ratio (1:4). After the addition of the reducing agent, stirring was continued for another hour. The suspensions of Cu/HPMC BNCs were kept closed during 4 h to prevent micro bubble formation. The solutions were poured into a glass plate (30×30 cm) for films formation. Films were obtained at a wet thickness of 0.5 mm using casting bars and the plates were placed on a leveled surface at room temperature and let dry for 25 h. After drying, the films were removed and conditioned in sealed plastic bags, stored at room temperature.

### Evaluation of antibacterial activity

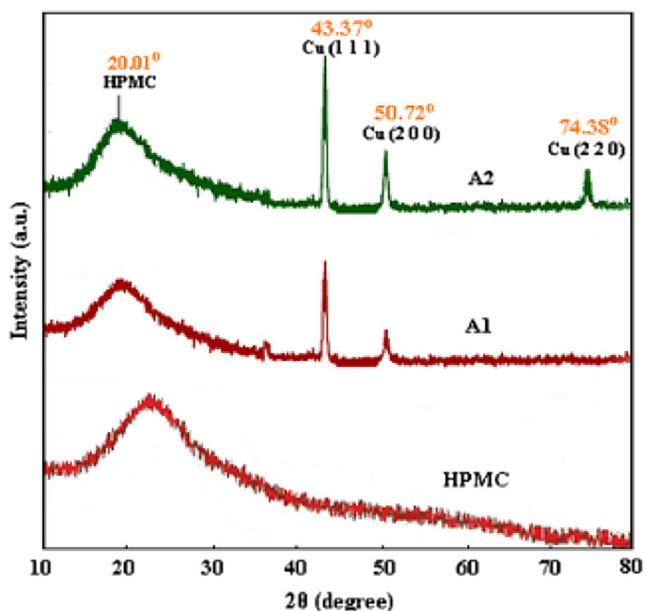
The in vitro antibacterial activity of the samples was evaluated by the well diffusion method using Mueller Hinton agar with determination of diameter of the inhibition zone formed around the well, which conformed to the recommended standards of the National Committee for Clinical Laboratory Standards (NCCLS, 2000). Petriplates containing 20 ml Muller Hinton medium were seeded with 24 h culture of bacterial strains. Wells were cut and 20  $\mu\text{l}$  of the BNC solution were added. The plates were then incubated at 37 °C for 24 h. Streptomycin antibiotic was used as a positive control and Dimethyl sulfoxide was used as a negative control.

### Characterization methods and instrument

The structures of the Cu/HPMC BNCs produced were examined by powder X-ray diffraction using the Siemens-D5000-1. SEM was performed using the Jeol JXA-840 instrument to study the morphology of Cu/HPMC BNCs. The water vapor permeability (WVP) was determined using a Labthink WVP System to determine the relative humidity (RH) at the films underside, according to Angles (Angles and Dufresne 2001). Three samples from each formulation were chosen and after drying for 24 h cut onto 23×23×0.1 mm diameter. After initial weighting, samples moved to desicator at 25 °C with 75 % relative humidity. Weights were taken periodically after steady state was achieved and used to calculate the percentage of RH at the films underside using following relation:

$$RH(\%) = \frac{W_t - W_0}{W_0} \times 100 \quad (1)$$

Where  $W_0$  is initial weight of the sample and  $W_t$  is the weights after t seconds. The mechanical properties of the composites were evaluated in rectangular pieces of the film

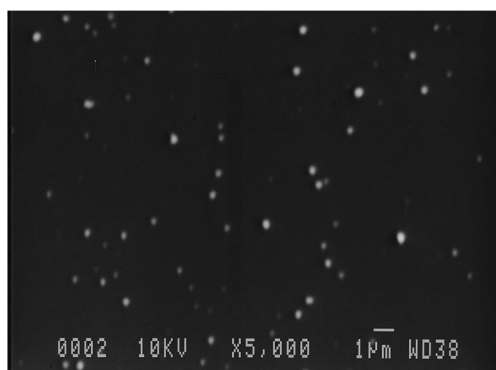


**Fig. 1** Powder X-ray diffraction patterns of hydroxypropyl methyl cellulose and copper/HPMC bionanocomposites for determination of copper crystals at different  $\text{CuSO}_4$  concentrations: (A1) 0.5 %, (A2) 5.0 %

with dimensions chosen in accordance to ASTM (1997) and conditioned at 24 °C for 48 h before measurements. An INSTRON instruments was used to determine the maximum tensile strength (TS) and elongation at break. Films were stretched up using a speed of 5 mm min<sup>-1</sup>. Tensile properties were calculated from the plot of stress (tensile force/initial cross-sectional area) versus strain (extension as a fraction of the original length). The mechanical properties were analyzed as a function of nanoparticles ratio by weight in the films.

#### Preparation and storage of meat in packages

To prepare minced, 300 g of fresh fatty beef meat were purchased from the local market in Tabriz, Iran. They were grinded using a semi-industrial meat grinder



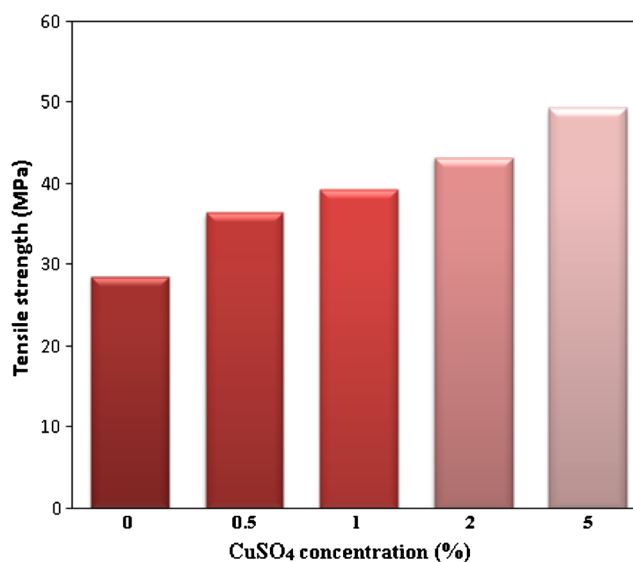
**Fig. 2** Scanning electron microscopy micrographs for prepared CuNPs/HPMC BNCs film

(Buffalo CB943 Meat Grinder, China), thoroughly washed with detergent and hot water. For perfect mixing of fat and meat the grind was repeated two times. The minced immediately transferred into a sterile glass container under sanitized conditions, sterilized in an autoclave at 121 °C for 15 min and refrigerated at 4 °C until processed. Packages were prepared by a hand heat sealer using Cu/HPMC BNC films and pure HPMC films 15×10 cm in size. The packages were immediately wrapped in aluminum foil and sanitized at 95 °C for 2 min. After cooling and under a sterile laboratory hood, 10 g of mince was poured into each package and sealed by the heat sealer. Packages containing mince were stored in dark and cool conditions (4 °C) and microbial counts of the samples were evaluated in duplicate by pour plate method using MRS agar and incubation for 48 h at 37 °C under CO<sub>2</sub> atmosphere (5 %), immediately after packaging and after 3, 7, 10 and 15 days of storing.

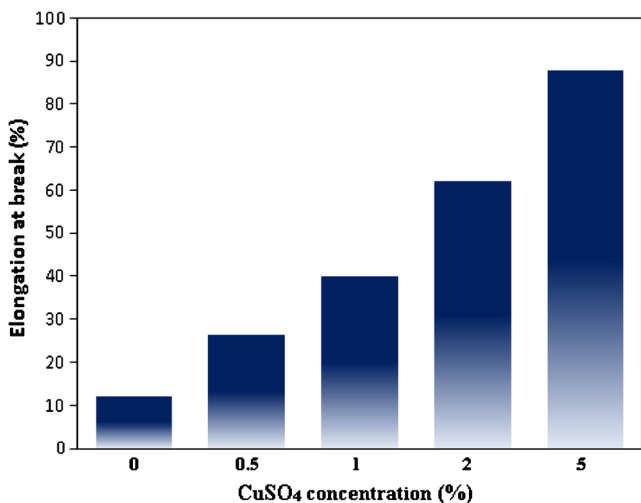
## Results and discussion

### X-ray diffraction analysis

Figure 1 showed the X-ray diffraction pattern of net HPMC and Cu/HPMC BNCs film with different concentration of  $\text{CuSO}_4$  (0.5 and 5.0 %). The X-ray diffraction pattern of HPMC film showed a broad scattering peak which indicated its highly amorphous structure. The Miller indices are shown above the diffractions.



**Fig. 3** Tensile strength for net HPMC films, and HPMC films containing  $\text{CuSO}_4$  with different concentration



**Fig. 4** Elongation at break for net HPMC films, and HPMC films containing CuSO<sub>4</sub> with different concentration

The diffraction patterns for Cu/HPMC BNCs showed peaks of Cu NPs (JCPDS file 04–0836) phase corresponding to (111), (200) and (220) crystallographic planes of the face-centered cubic copper crystals. The intensities of (111), (200) and (220) reflections due to the Cu NP phase were also found to increase along with the increased Cu NPs in the solid support matrix. For all samples, the main crystalline phase was copper, and no other obvious phases were found as impurities in the XRD patterns. The crystallite size of Cu NPs incorporated in the Cu/HPMC BNCs film was calculated by using Scherrer’s formula for the (111) peak for 2θ of 43.37° and was found to be 30 nm.

**Morphology**

The surface morphology of the synthesized Cu/HPMC BNC film is evaluated by SEM. Figure 2 shows the

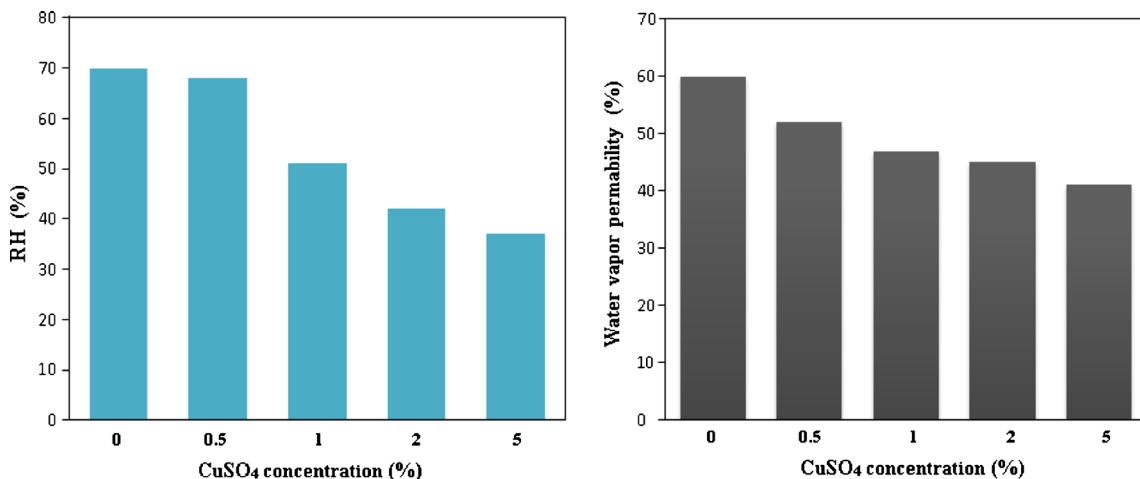
SEM image of the Cu/HPMC BNC, where the bright particles correspond to the immobilized copper nanoparticles. It is observed that the films are uniform and the nanoparticles are homogeneously distributed in the polymer matrix. The mean diameters of the Cu NPs were about 53.28±15.54 nm.

**Mechanical analysis**

With regard to the application of these films in packaging, mechanical stability is important to maintain integrity during processing and flexibility during shipping or handling. Tensile strength, elastic modulus, and elongation analyses describe how the mechanical properties of such film materials relate to their chemical structures (Ninnemann 1968). Figures 3 and 4 show the tensile strength and elongation at break of the Cu /HPMC BNCs in different concentration of CuSO<sub>4</sub>. As it is observed, the incorporation of Cu NPs in polymer matrix increased the mechanical resistance and elongation at break of the film for all concentrations, which leads to film of high toughness. This can be related to increase interaction of the nanoparticles and polymer chain and increase intermolecular bonding accordingly. The maximum tensile strength and elongation was observed for the film with 5 % CuSO<sub>4</sub> at 49.3±1.0 (MPa) and 87.6±2.0 (%), respectively.

**Water vapor permeability**

Water vapor barricading is critical in the packaging of moisture sensitive foods and pharmaceuticals to achieve the required quality, safety, and shelf life. Most edible films are characterized by a high water vapor permeation, which makes them inappropriate for several applications (Torres 1994). The hydrophilic natures of



**Fig. 5** Relative humidity at film underside and water vapor permeability of HPMC/CuNPs BNCs film in different concentration of CuSO<sub>4</sub>



**Table 1** Average inhibition zone for Cu/HPMC BNCs at different concentration of CuSO<sub>4</sub>

Bacteria	Inhibition zone (mm)		Control positive (mm) <i>SM</i>
	HPMC/Cu NPs (0.5 %)	HPMC/Cu NPs (5.0 %)	
<i>S.aureus</i>	NA	20.3	21.2
<i>B.cereus</i>	NA	22.1	21.2
<i>S.epidermidis</i>	NA	25.2	29.1
<i>Strept A</i>	13	27.9	29.1
<i>E.coli</i>	NA	NA	21.2
<i>E.Faecalis</i>	NA	NA	29.1
<i>Salmonella</i>	NA	NA	29.1
<i>P.aeruginosa</i>	NA	NA	21.2

Abriviations: *NA* not appearing;  
*SM* Streptomycin

polysaccharide films make them poor water barriers. The copper nanoparticles effect on the relative humidity at film underside is shown in Fig. 5 for pure HPMC film and Cu/HPMC films with different CuSO<sub>4</sub> concentration.

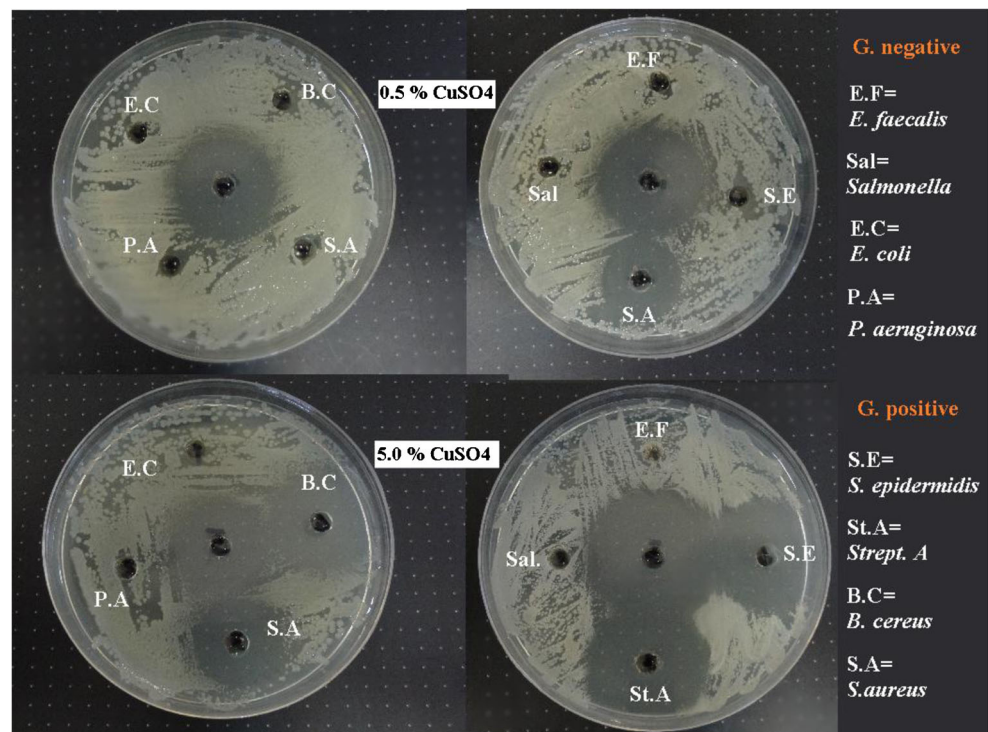
Upon addition of nanoparticles in HPMC matrix, a decrease in the WVP values and relative humidity at film underside was observed. The RH at film underside value was 70 % for the native HPMC film. Addition of nanoparticles induced a decrease in WVP values and RH at film underside. The RH values and WVP varied from 68±0.9 and 60±0.1 % for HPMC films containing 0.5 % CuSO<sub>4</sub> to 37±0.9 and 41±0.5 % for films

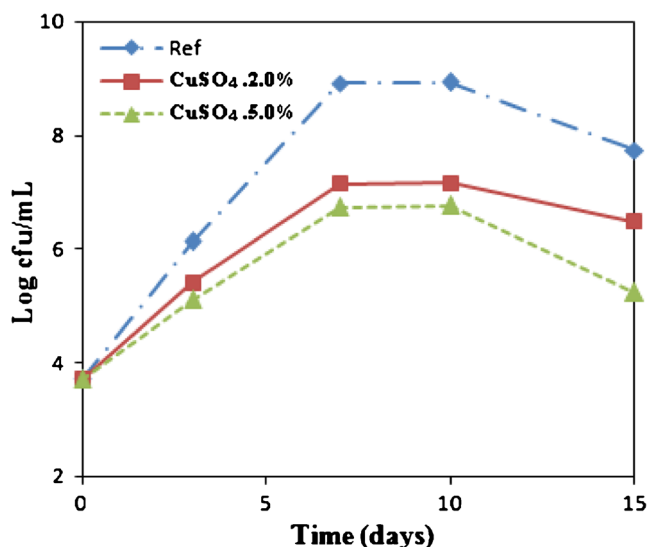
containing 5.0 % CuSO<sub>4</sub> respectively. The presence of nanoparticles reduced the intermolecular spacing within the films and increase in cohesion structures, thus reducing the water vapor permeability through of film. In the other words, small size nanoparticles have more ability in occupying the empty spaces of the porous HPMC film matrix, and prevent the diffusion of water into the film.

#### Antibacterial activity

Inhibition zone values were obtained for the synthesized Cu/ HPMC BNCs tested against Gram positive and

**Fig. 6** The antibacterial activity of nanobiocomposite at different concentration of CuSO<sub>4</sub> evaluated by well diffusion method





**Fig. 7** Effect of packaging containing Cu NPs on the microbial population during 15 days of storage at 4 °C

Gram negative bacteria listed in Table 1. The tests were repeated three times for each treated samples, are presented as average values in Table 1 and Fig. 6, respectively. The result shows that the Cu NPs in HPMC suspension had high antibacterial activity against Gram-positive bacteria and did not show any antibacterial activity against Gram negative bacteria. Inhibition effect of Cu NPs in lower concentrations did not observe except for *Strept A*, with 13 mm inhibition zone. Maximum inhibition was observed for *Streptococcus A* and *S.epidermis*.

The rigid cell walls of Gram negative bacteria limit the entry of hydrophobic and large molecules to reach the nuclear content of bacteria. Several mechanisms argue for the antibacterial activity of nanoparticles, including generation of oxygen species for degradation of cell structure or release of ions from the surface of nanoparticles to binding cell membrane (Morones et al. 2005; Lee et al. 2005; Lok et al. 2006; Sawai 2003). The result reinforces the idea that the lipopolysaccharide proteins in cell wall structures of Gram negative bacteria protect them against Cu NPs attack (Yoon et al. 2007). As well inclination of Cu NPs to amin and carboxyle groups on the cell walls of Gram positive bacteria is the reason for inhibition effect of Cu NPs against these bacteria (Ruparelia et al. 2009). With the regard to the excessive antibacterial effect of HPMC/Cu NPs film against Gram positive bacteria which is responsible to deterioration of foods and clinical infections, it can be used as substitute for commercial chemical antibiotics.

### Mean microbial population during refrigerated storage

Beef packaged in a high oxygen modified atmosphere typically retains a shelf-life of ten to 14 days for ground beef and 5 to 7 days for minced beef (Cornforth and Hunt 2008; Belcher 2006; Márquez et al. 2012). The variations in the microbial population during storage are shown in Fig. 7 after 3, 7 and 10 days of storage. Mean initial microbial population immediately after packaging was determined to be 3.7 log cfu/mL in minced. In all of packages, the mean population increased after 3, 7 and 10 days of storage. According to Fig. 7, the level of microbial population increased to 8.91 log cfu/mL after 7 days of storage in HPMC pure packages which is higher than Cu/HPMC BNC 2.0 % and 5.0 %. In all samples (2.0, and 5.0 % Cu SO<sub>4</sub>), significance decreases were observed over 10 days of storage. However, microbial growth in Cu/HPMC BNC 2.0 %, and Cu/HPMC BNC 5.0 % compared with pure HPMC packages, showed a higher reduction up to 15 days of storage at 4 °C. The microbial population decrease with the increasing in Cu SO<sub>4</sub> ratio from 2.0 to 5.0 %. Copper ions released from the surface of these nanoparticles can interact with thiol groups in protein to induce bacterial inactivation, condensation of DNA molecules, and loss of their replication ability (Feng et al. 2000). Based on electron spin resonance (ESR) measurements, the antimicrobial mechanism of nanoparticles, are related to the formation of free radicals and the subsequent free radical-induced membrane damage (Kim et al. 2007).

### Conclusion

Cu NPs were successfully prepared from CuSO<sub>4</sub>/HPMC suspension at different CuSO<sub>4</sub> concentrations by using NaBH<sub>4</sub> as a chemical reduction agent without any heat treatment or reducing agent. The XRD analysis confirmed that the crystallographic planes of the copper crystals were of the face-centered cubic type. SEM images show that the external morphology of Cu/HPMC BNCs is uniform with shiny points due to the presence of Cu NPs. Tensile strength (mechanical stability) and elongation at break (elasticity) of the synthesized Cu/HPMC BNCs increase by the presence of Cu NPs to 49.3 ± 1.0 MPa and 87.6 ± 2.0 % respectively. The decrease observed in the WVP values for the HPMC/Cu NPs system, in comparison to net HPMC systems. The antibacterial activities of Cu/HPMC BNCs at the different Cu NPs ratio showed strong antibacterial activity against Gram-positive bacteria. These results indicate that Cu/HPMC BNCs can be used in food packaging for certain bacterial inactivation and control. The prepared Cu/HPMC BNCs packages showed a significant

antimicrobial activity compared with pure HPMC in minced packaging.

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