CHAPTER V

NOVEL COPPER (II) STEARATE CONTAINING PVP HYDROGEL AND THEIR POTENTIAL FOR USE AS ANTIBACTERIAL WOUND DRESSING

5.1 Abstract

The antibacterial wound dressing successfully prepared using gamma radiation. The gamma ray induced the reduction of copper (II) stearate within hydrogel pad to copper nanoparticles. The materials exhibited the good antibacterial activity against *E.coli*, *S. aurous*, *MRSA*, *S. epidermidis*, *S.pyogenes*. The addition of copper (II) stearate was affected to the swelling of the hydrogels. The swelling decreased when concentration of additional copper (II) stearate increased. All of the hydrogel samples had ability to allow water vapor to pass through (in the tenn of WVTR) which can be used for burn wound. For the indirect cytotoxicity, 0.5 % copper (II) stearate containing PVP hydrogel showed the least toxicity (more than 80% cell viability).

5.2 Introduction

It is well known that there is certain requirement to accelerate would healing is providing moist environment which allows the wound fluids remaining in contact with wound. Owing to moist wound, an environment rich in white blood cells, enzymes, cytokines and growth factors can be easily generated [1-5].Many research works have been interested to develop such a materials which provide moisture since 1960s [6-8] .One of the most important candidate is hydrogel. The first hydrogel was developed by radiation and further study which was surprised in its characteristics as pain reliever and enhanced healings properties [7-11]

Hydrogel chains are hold cross-linked between polymer chains which are synthesized from either synthetic or natural polymers along with other chemical ingredients. Since hydrophilic polymer like poly (vinyl alcohol), poly (vinyl pyrrolidone), poly (acrylic acid), etc. play a major role using to synthesize hydrogel which directly depends on the desirable properties of the hydrogel. The invasive wound infection is influenced by the type and quantity of microorganisms that colonize the burn wound. The well-known pathogens, that infect the wound and resist to broad antimicrobial agents, are gram-positive metthicilin-resistant *Staphylococus*

aureus (MRSA) and gram-negative *Peudomonas aeuginosa*. Due to the resistant, antibiotics are become less effective.

Among the metals with antimicrobial properties, copper has the most effective antibacterial inhibition and the least toxicity to animal cells[12]. The antibacterial activity of copper and its derivatives were not significantly different from silver and its complex. However, there was not frequently studied because of instability of copper itself and can be easily oxidized from air[13]. In order to overcome this problem, the long chain of copper (II) stearate was interested which expected less reactivity to oxidize in air.

In this study, copper (II) stearate was synthesized and then added to PVP solution in order to prepare hydrogel pad using gamma radiation. The hydrogel samples were tested for hydrogel properties and the potential for use as anti bacterial wound dressing.

5.3 Experimental

5.3.1 Materials

Stearic acid, NaOH and HNO_3 were purchased from Carlo Erba (Italy). Copper (II) sulfate ($CuSO_4.5H_2O$) was purchased from Fisher Scientific. Poly(vinylpyrrolidone) , PVP was purchased from Sigma-Aldrich. All chemicals were used without further purification.

5.3.2 Synthesis of Copper (II) Stearate

Stearic acid (48.5 g) was dissolved in 1 liter of distill water at 80 °C, followed by addition of 120 mL of 1.5 M NaOH and 10 mL of 1.3 M HNO_3 consequently. Afterward, Copper (II) sulfate 43.5 g were added to the mixed solution and then stirred for 30 min. The blue precipitate of copper (II) stearate was obtained. The solid was filtered and rinsed in ethanol and distill water to get rid of un-reacted copper (II) ions and vacuum dried at room temperature.

5.3.3 Characterization of Copper (II) Stearate

The phase structure of copper (II) stearate was characterized by an X-ray diffractometer (XRD) (Rigaku Corp., D/max-2400) equipped with graphite

monochromatized Cu K radiation. Chemical interaction between the copper (II) ions and certain chemical functional groups of stearate was examined using a Nicolet Fourier transform infrared (FT-IR) 360 spectrophotometer (the KBr pellet method).

5.3.4 Preparation of PVP Hydrogel Using Gamma Radiation

First step is the preparation of polymer solution. PVP was dissolved in distilled water at room temperature. PVP in the final solution was 10 %w/v (hereafter: Neat). Polymer solutions were poured into a 5x10 cm^2 nylon bag (10 mL each bag) and the solutions were sealed. The contents were then exposed to gamma radiation at 35 kGy to form the hydrogel pads.

5.3.5 Preparation of PVP Hydrogel Pad Containing Copper (II) Stearate

10 g of PVP was dissolved in 100 mL of distilled water at room temperature. Copper (II) stearate (0.50, 1.00 and 2.00 g $Cu(C_{17}H_{35}COO)$) was dissolved in PVP solutions to obtain the desired concentrations (0.5, 1.0 and 2.0 %w/v) following by stirring at room temperature for 30 min to obtain homogeneous solutions. 10 ml of each mixture was loaded into a 5x10 cm^2 nylon bag and the contents were sealed. The contents were then exposed to gamma radiation at 35 kGy to form the hydrogel pads.

5.3.6 Characterizations of Hydrogels

The characterizations included the verification of the formation of copper nanoparticles using surface plasmon bands and TEM. The physical and mechanical properties of the hydrogels containing copper (II) stearate were carried out in parallel with a neat hydrogel.

5.3.7 Characterization of Nanocopper Formation in The PVP Hydrogels Containing Copper (II) Stearate.

5.3.7.1 Surface Plasmon Band

The neat hydrogel and PVP containing copper nanoparticles hydrogel pads that were exposed to gamma radiation were cut to 3 x 4 cm^2 and the

formation of copper nanoparticles was monitored by surface plasmon bands using UV-vis spectrophotometer on a Shumadzu UV-2550 UV-vis spectrophotometer.

5.3.7.2 TEM Images

The nanocopper infused PVP hydrogels were cut to discs (15 mm in diameter) and immersed in DI water (40 ml) at 37°C under shaking (60 rpm) for 24 h to obtain the nanocopper releasing solutions. The morphology and size of copper nanoparticles in the solutions were identified by transmission electron microcopy (TEM) using a JEOL JEM-2100 transmission electron microscope.

5.3.8 Physical Properties of Hydrogels

5.3.8.1 Measurement of water vapor transmission rate (WVTR)

The water vapor transmission rate (WVTR) was measured following the monograph of the European Pharmacopeia. The experiment measured the rate of water transport from a glass bottle containing 25 mL of water, which used a disc of hydrogel (14 mm in diameter) as the cap for the bottle. The glass bottle with the hydrogel cap was kept in an oven at 35 °C for 24 h. WVTR was calculated by using following equation

$$\text{WVTR} = \frac{(W_i - W_t)}{A \times 24} \times 10^6 \quad \text{g/(m}^2\text{.h)} \qquad (1)$$

where WVTR is expressed in g/(m^2.h), A is the area of the diameter of the bottle (mm^2), W_i and W_t are the weight of bottle before and after placed in an oven, respectively.

5.3.8.3 Gel Fraction

The hydrogels were cut to 0.5x0.5 cm^2 and dried in an oven at 60°C for 24 h to obtain the original weight (W_0). Each dried specimen was placed in a tea bag which was submersed in DI water at 121°C for 4 h in an autoclave. The hydrogels were then dried again at 60 °C for 72 h to obtain weight after extraction (W_E). The gel fraction was determined by the percentage of gelation using the equation below.

$$\text{Gelation (\%)} = \frac{WE}{Wo} \times 100 \ \% \qquad (2)$$

5.3.8.4 Swelling Behavior

Studies were carried out based on the kinetics of water sorption dynamics method utilized by Balakrishnan and Lee [14, 15]. Briefly, the films were weighed for initial dry weight before being immersed in distilled water at 37 °C. After a specified immersion time, the films were taken out and the water droplets on the surface of the films were removed with filter paper sheets prior to being weighed. The swelling ratio (Q_m) was calculated by following expression:

$$Q_m = \frac{W_s - W_i}{W_i}, \quad (3)$$

where W_s and W_i are the weights of the film hydrogels in their swollen state and initial dry state, respectively.

5.3.9 Mechanical Properties of Hydrogels

5.3.9.1 Tensile Strength and Percentage of Elongation

The mechanical properties of the neat hydrogel and nanocopper infused hydrogels were evaluated in terms of tensile strength and percent elongation at break. The hydrogels were cut to 5.0 cm x 1.0 cm (thickness of 0.214 cm), and the mechanical properties were measured using a 500 N load and cross-head speed of 50 mm/min on a Lloyd Universal testing machine model LRX.

5.3.10 Evaluation of The Applicability as Antibacterial Wound Dressing

5.3.10.1Cumulative Copper Release

The hydrogels containing copper nanoparticles were cut to discs (15 mm in diameter, 2.38 ± 0.10 mm in thickness, 320.0 ± 12.0 mg) and each individual disc was immersed in a bottle containing 50 ml of SBF solution at 37°C under shaking at 60 rpm. At various time intervals, the immersion solution was collected for investigation of the cumulative release of copper (either in the form of Cu^{2+}, Cu^{+} cation or the as-formed nCu). The replacement of 50 ml of SBF solution into the bottles was performed at each time interval. The concentration of copper was measured using Varian SpectrAA300.

The actual amounts of copper (either in the form of Cu^{2+}, Cu^{+} cation or the as-formed nCu) released from the nanocopper infused hydrogels were determined. The hydrogels were cut into discs (15 mm in diameter) and each individual disc was immersed in a bottle containing 50 ml of nitrate solution (HNO_3) at 37°C under shaking at 60 rpm for 24 h. The solutions were collected and the actual concentration of silver was measured using Varian SpectrAA300.

5.3.10.2 Indirect Cytotoxicity

The ISO 10993-5 standard test method was used to evaluate toxicity of the PVP hydrogel pads containning copper (II) stearate, which was used NHDF (Normal human dermal fibroblast). The cells were cultured in Dulbecco's modified Eagle's medium, DMEM [composition of 10% fetal bovine serum (FBS; Invitrogen Corp., USA), 1% l-glutamine (Invitrogen Corp., USA) and 1% antibiotic and antimycotic formulation which was contained penicillin G sodium, streptomycin sulfate, and amphotericin B (Invitrogen Corp., USA)]. The disc specimens (14 mm in diameter) were sterilized with 70%v/v ethanol for 30 min. The extraction medium was prepared by immersing each disc specimen (weighed in mg of hydrogel) in serum free media (SFM; DMEM containing 1% l-glutamine, 1% lactalbumin and 1% antibiotic and antimycotic formulation) for 1, 2 and 3 days with extraction ratio of 25 mg/mL, respectively. The cells were then separately cultured using SFM in 24-well tissue-culture polystyrene plates (TCPS; NunclonTM, Denmark) which were incubated at 37 °C for 24 h. (10,000 cells per well for NHDF). The obtained extraction media was used as the media for the cells to grow. The relative cell viability was determined using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay.

5.3.11 Antibacterial Evaluation

Specimens of hydrogel were cut into discs (9 mm in diameter), which were further studied via the disc diffusion method [The US clinical and laboratory standards institute (CLSI) disc diffusion method]. Both gram-negative bacteria, *E. coli*, ATCC 25922 and gram-positive bacteria, *S. aureus*, ATCC 25923 were used to test for antibacterial activity of copper (II) alginate hydrogels. Methicillin resistant *S.*

aureus (MRSA), DMST 20654, *S. epidermidis,* ATCC 12228 and *S. pyogenes,* DMST 17020 which caused dermal infection, were also selected to evaluate their antibacterial activity. All of the bacteria was diluted until the colonies equal to 10^8 CFU (colony forming unit)/ mL and then 200 μL bacteria solution were transferred on Difco™ Mueller Hinton agar dishes. The hydrogel specimens were placed on the agar culture dishes and incubated at 37 °C for 24 h. An inhibition zone was clearly seen around each specimen whenever there was antibacterial activity.

The hydrogel pads that were kept at room temperature for 7 days, 30 days and 90 days, were evaluated for their antibacterial activity using same procedure as described. In order to ensure the stability antibacterial activity of the material.

5.4. Results and Discussion

5.4.1 Characterization of Copper (II) Stearate

FTIR spectra was demonstrated the chemical composition of copper (II) stearate. The FTIR spectrum of stearic acid showed the carbonyl stretching peak around 1702 cm^{-1} which disappeared in FTIR spectrum of copper (II) stearate (fig.5.1). From FTIR spectrum of copper (II) stearate, the symmetric stretching of carboxylate peak and asymmetric stretching carboxylate peak showed at 1550 and 1462 cm^{-1}, respectively. The results clearly confirm the formation of stearate[16].

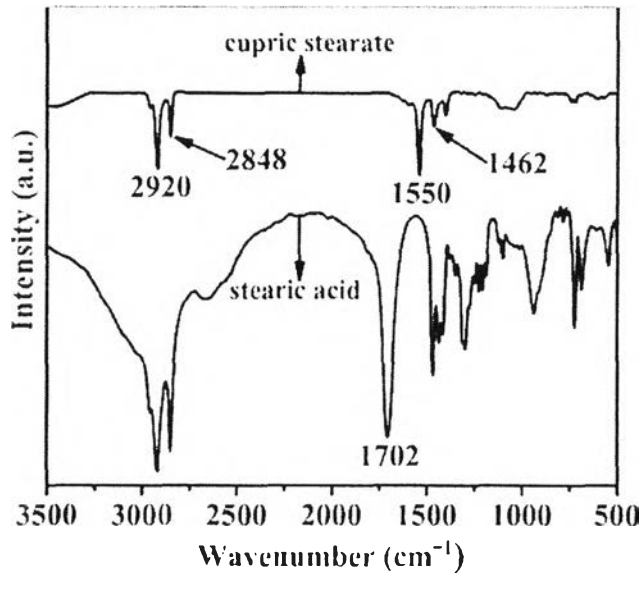

Figure 5.1 FTIR spectra of copper (II) stearate (cupric stearate) and Stearic acid

The XRD pattern was confirmed crystallization of as-prepared copper (II) stearate (fig.5.2). The XRD spectrum showed four peaks which indicated to copper (II) stearate[17].

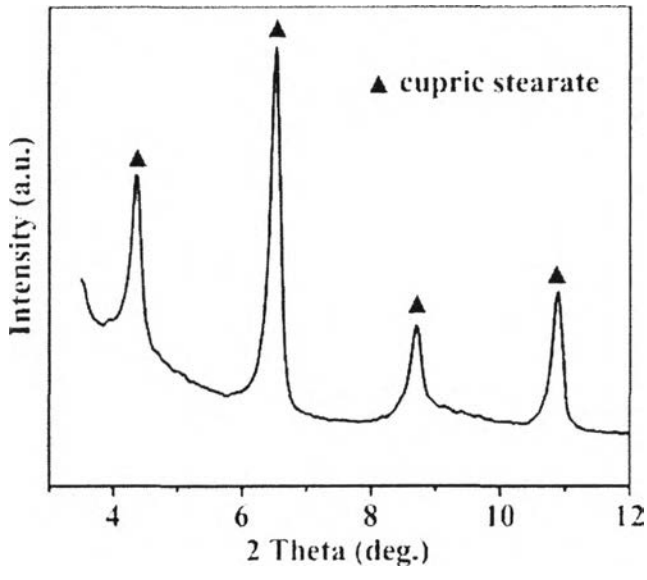

Figure 5.2 The XRD diffraction pattern of copper (II) stearate powder.

5.4.2 Characterization of Hydrogels

5.4.2.1 Characterization of Nanocopper Formation in the PVP Hydrogel Pad

- *Surface Plasmon Resonance*

The dark red particles can be observed from the copper (II) stearate containing PVP hydrogel pad after exposing to the gamma ray. Surface plasmon resonance absorption peaks around 577 nm can be used to identify the formation of copper nanoparticles[1] Figure 5.3 showed surface plasmon resonance of the hydrogels. The neat hydrogel was used as a control, and absorption peaks in the association with the copper nanoparticles were not observed. No nanocopper formation was expected to be observed for the neat hydrogel. On the other hand, a peak at 580 nm was observed for hydrogel containing 0.5% copper (II) stearate. This observation can be used to confirm that copper nanoparticles were formed. In the case

of 1.0% and 2.0% copper (II) stearate hydrogels, the absorption bands also showed peaks in this position in the higher absorbance.

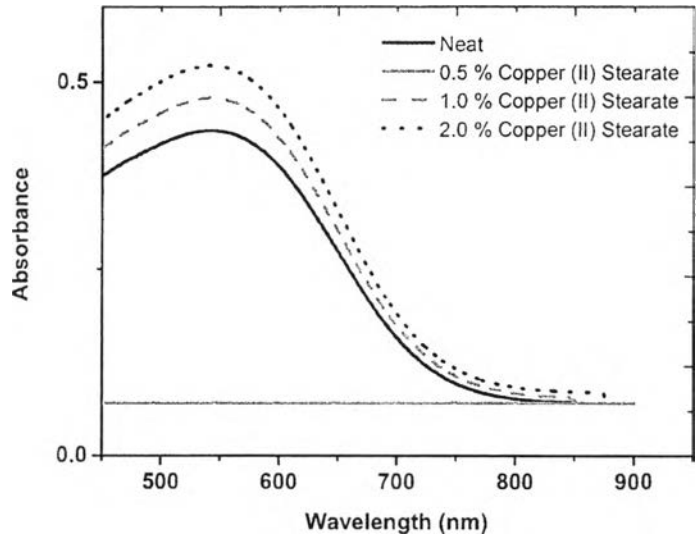

Figure 5.3 Representative of surface plasmon of 0.5%, 1.0% and 2.0% copper (II) stearate

- *TEM Images*

Figure 5.4 displays the transmission electron microscopy (TEM) images of 2.0% copper (II) stearate hydrogel. It was observed that the size of nanocopper particles released from the submerged solution of 2.0% copper (II) stearate hydrogel was in the range of 10.4-20.6 nm.

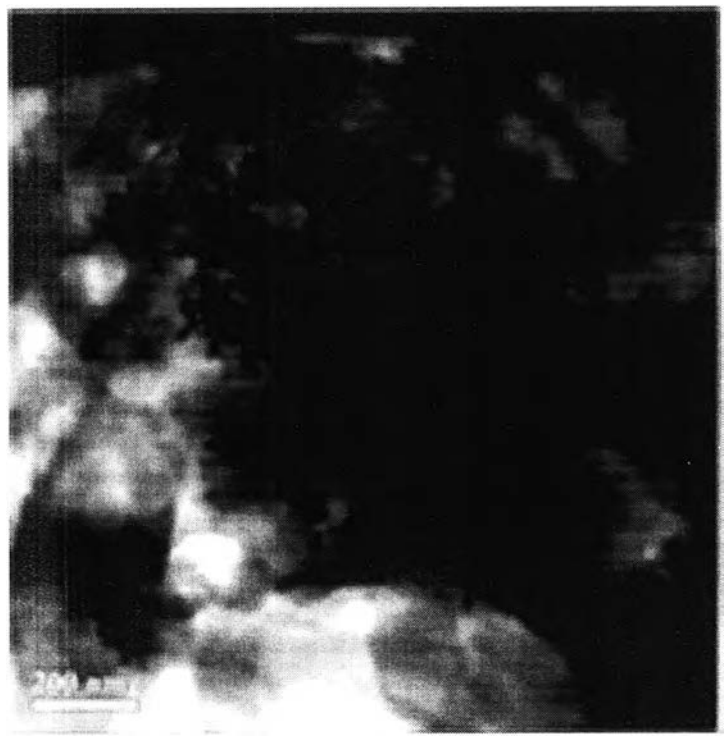

Figure 5.4 TEM image of 2.0% copper (II) stearate from 24 h released solution

5.4.3 Physical Property Characterizations

5.4.3.1 Gelation

Gel fraction for Neat, 0.5%, 1.0% and 2.0% copper (II) stearate hydrogels was measured at 80.83%, 79.31%, 81.71% and 82.63%, respectively. It was clearly seen that copper (II) stearate contents affects percent gelation of PVP hydrogels. Percent gelation inversely relates to the free volume inside the gel networks 0.5% copper (II) stearate hydrogels had the lowest percent gelation. The results for 1.0% and 2.0% copper (II) stearate hydrogels indicate that increasing the copper (II) concentration increases the percent gelation.

5.4.3.1 Water Vapor Transmission Rate

Wound dressing materials are expected to reduce body liquid loss and be able to control absorption and transmission rates of exudate by maintaining the humidity of the wound environment. The results found that the WVTR values of all hydrogels (range between 92.17-116.22 g/h.m^2) were lower than

the evaporative water loss of second and third degree burned skin (shown in Table 5.2 which are 178 ± 5.5 g/h.m^2 and 143.2 ± 4.5 g/h.m^2, respectively). This indicates that PVP hydrogels can be used to control body fluid loss and keep a moist environment for burn wounds. It is one of their advantageous properties for use as potential wound dressings.

Table 5.1 Representative of gel fraction (%) and WVTR of Neat and 0.5%, 1.0% and 2.0% copper (II) stearate containing PVP hydogel pads

Sample	Gel Fraction (%)	WVTR (g/m^2/h)
Neat	80.83 ± 3.52	116.22 ± 8.64
0.5 % copper (II) stearate	79.31 ± 5.72	110.10 ± 9.88
1.0 % copper (II) stearate	81.71 ± 5.97	98.17 ± 6.23
2.0% copper (II) stearate	82.63 ± 8.34	92.17 ± 9.34

Table 5.2 Evaporative water loss of normal skin and each degree of burn skin (Nilsson, G.E., 1997)

	Evaporative water loss (g/h.m^2)
Healthy skin	8.5 ± 0.5
First degree burn	11.6 ± 1.1
Second degree burn	178.1 ± 5.5
Third degree burn	143.2 ± 4.5

5.4.3.3 Swelling Behavior

The swelling behavior in simulated body fluid (SBF, pH 7.40) of the neat and various copper (II) stearate hydrogels (0.5%, 1.0% and 2.0%) is shown in Figure 5.5. The plots of percent swelling as a function of immersion time were required. The experiments were carried out until the hydrogels reached their equilibrium. At the initial state, all hydrogels had great slopes in the percent swelling graphs. After 12 h, the percent swelling rate slowed, and they reached equilibrium at 16 h. It was observed that the concentration of copper (II) stearate affected the

swelling behaviors of the hydrogels. Neat had the highest swelling capacity at each time interval, followed by 0.5%, 1.0% and 2.0%, respectively. As described in percent gelation, the concentration of copper (II) stearate affects the free volume inside the hydrogels. The greater the concentration of copper (II) stearate, the less the free volume of the hydrogel network. It can be predicted that, the copper nanoparticles filled up the free volume of the hydrogels. However, in the case of neat hydrogel, the swelling behavior was the highest. It can be hypothesized that the hydrophobic property of stearate chain may limited the absorption ability of the hydrogel.

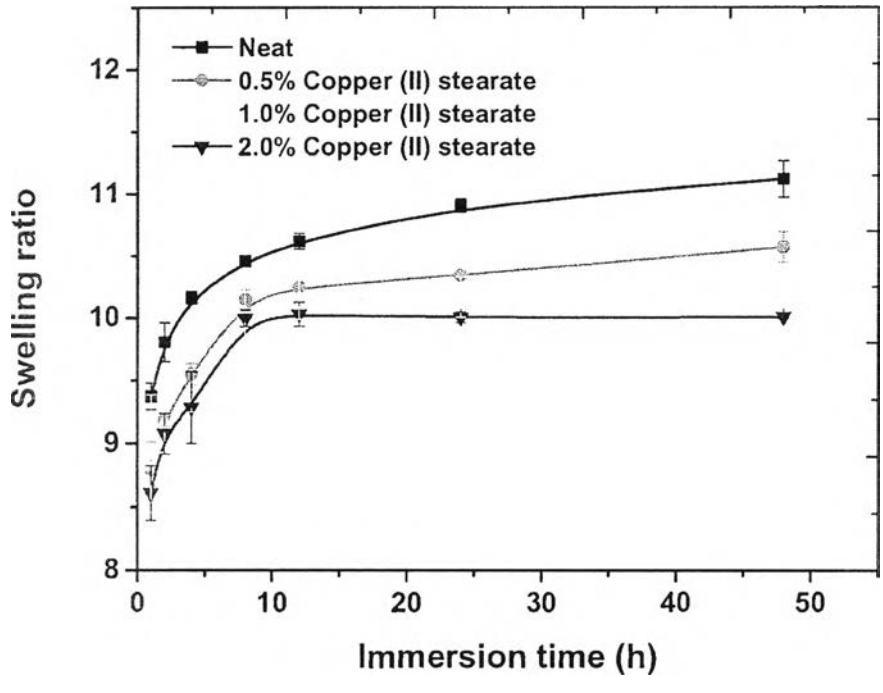

Figure 5.5 Swelling behavior of neat, 0.5%, 1.0% and 2.0% copper (II) stearate hydrogel

5.4.4 Mechanical properties of hydrogels

The investigation of the mechanical properties of hydrogel wound dressings is important to determine their actual applicability. Tensile strength and percentage of elongation were used to evaluate the mechanical properties of the neat hydrogel, copper (II) stearate loaded (by 0.5%, 1.0% and 2.0%) PVP hydrogels.

From Table 5.3, it was seen that all CuSt loaded hydrogels have tensile strength values (0.0281-0.0296 MPa) similar to the neat hydrogel (0.0268 MPa). Percent elongation at break values for all copper (II) stearate loaded hydrogels (197.3 %-222.6 %) was slightly higher than percent elongation at break of the neat hydrogel (187.5%).

Table 5.3 Representative of Tensile strength (MPa) and Elongation at break (%) of Neat and 0.5%, 1.0% and 2.0% copper (II) stearate containing PVP hydogel pads

Sample	Tensile strength (MPa)	Elongation at break (%)
Neat	0.0268 ± 0.0066	187.5 ± 53.8
0.5 % copper (II) stearate	0.0281 ± 0.0015	197.3 ± 62.2
1.0 % copper (II) stearate	0.0291 ± 0.0051	210.4 ± 55.5
2.0% copper (II) stearate	0.0296 ± 0.0059	222.6 ± 43.1

5.4.5 Cumulative Copper Release

Figure 5.6 showed the actual amount of copper (either in the form of the free Cu^{+},Cu^{2+} cation or the as-formed nAgs) as determined by the amount released from the copper (II) stearate hydrogels when submerged in nitric acid solution. The cumulative release of nanosilver infused hydrogels immersed in SBF solution was also determined. After a 72 h immersion in SBF, the released contents of copper within the hydrogels were 3.94 ± 0.23, 8.84 ± 0.10 and 15.56± 0.30 mg for alginate hydrogel containing 0.5%, 1.0% and 2.0% w/v copper (II) stearate, respectively. These values corresponded to 86 ± 2, 89 ± 3 and 91 ± 2 cumulative release (%) based on the initial amount of copper loaded in the polymer solutions.

The cumulative release of copper from all hydrogels in a function of the submersion time was shown in Figure 6. The cumulative amount of the released copper (II) ions increased rapidly during the first 8 h of the submersion time. A slight but continuously released up to 24 h of the submersion time was observed. An initial fast release is possibly because the copper particles dispersing close to the surface of

polymer hydrogels and absorbing near the surface could diffuse to the solution rapidly in initial time. The slow release rate might be described by the assumption that the ions were encapsulated in the inner core of the hydrogel network and a long distance to diffuse through the network leaded to a longer releasing time.

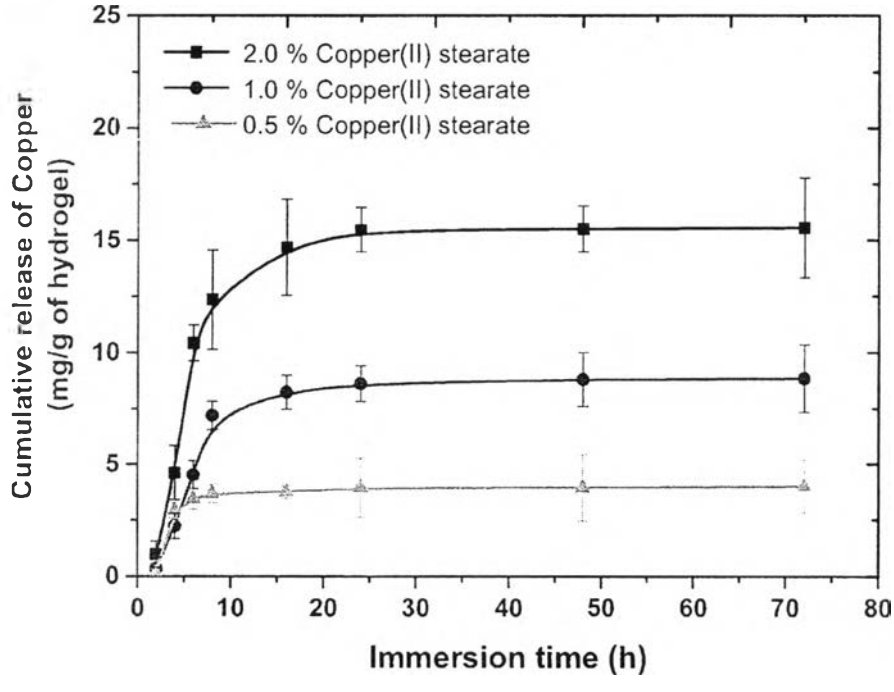

Figure 5.6 Cumulative copper release of hydrogel containing copper (II) stearate (0.5%, 1.0% and 2.0%)

5.4.6 Indirect Cytotoxicity Evaluation

The copper (II) stearate containing PVP hydrogels were tested for their toxicity using NHDF cells (normal human dermal fibroblast). As shown in Figure 5.7 the relative cell viability is acceptable when concentration of copper (II) stearate was 0.5 % w/v, but tended to slightly reduce to 70 % cell viability when corresponding copper (II) stearate concentration increased to 2.0 % w/v.

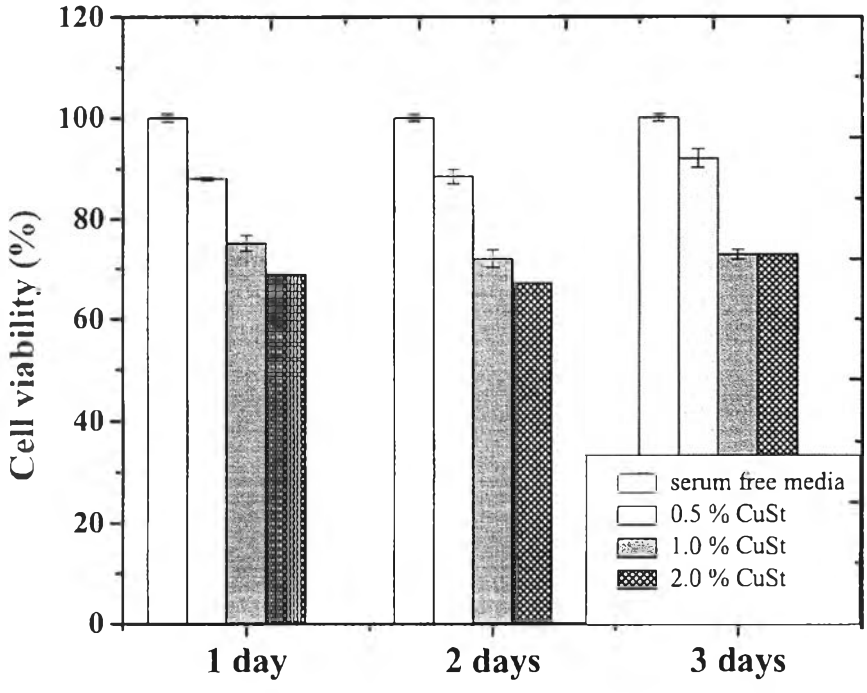

Figure 8.7 Representative of NHDF cell viability after seeded with extraction media of 0.5%, 1.0% and 2.0% copper (II) stearate, respectively

5.4.6 Antibacterial evaluation

Disc specimens of copper (II) stearate containing PVP were tested via the disc diffusion method against *E. coli*, *S. aureus*, Methicillin resistant *S. aureus* (MRSA), *S. epidermidis* and *S. pyogenes*. The activity increased with increasing in copper (II) stearate concentration which can be observed through the zone of inhibition. Table 5.4 shows the activities of hydrogels using different concentrations of copper (II) stearate solution. The activity was determined by the circular area around each sample which correlated with ability to release antibacterial substances to inhibit the bacterial growth. The hydrogels using 0.5 to 2.0 % w/v of copper (II) sulfate solution are active toward *S.aureus, E.coli,* Methicillin resistant *S. aureus* (MRSA), *S. epidermidis* and *S. pyogenes*.

Table 5.4 Inhibition zone length (mm) of bacteria treated with the hydrogels via disc diffusion method. The size of each swelled hydrogel was 9 mm in diameter and the inhibition zone length was measured in terms of the diameter of the inhibition zone (n=3).

Sample	Inhibition zone length (mm)				
	S.aureus	MRSA	*S.Epidermidis*	*E. coli*	*S.pyogenes*
Neat	0	0	0	0	0
0.5 % copper (II) stearate	11.7 ± 0.6	11.9 ± 0.2	12.6 ± 0.2	10.9 ± 0.2	11.4 ± 0.4
1.0 % copper (II) stearate	12.4 ± 0.4	12.3 ± 0.3	14.3 ± 0.6	11.8 ± 0.4	12.8 ± 0.6
2.0% copper (II) stearate	14.3 ± 0.6	13.1 ± 0.4	15.2 ± 0.3	13.0 ± 0.7	13.6± 0.2

From the antibacterial results were corresponding to release characteristic of copper from hydrogel. The higher amount copper release showed the higher antibacterial activity. The released copper from each copper hydrogel specimen can be estimated to a 0.39-1.56 mg of copper (9 mm in diameter, 1 mm in thickness, 102.02 ± 14.0 mg which were satisfied amount to be an inhibitor of the microbial activity at relatively low copper (II) concentration [17]. The ability to inhibit the growth of bacteria of the copper hydrogel was higher withgram positive bacteria (i.e. *S. aureus,* Methicillin resistant *S. aureus* (MRSA), *S. epidermidis* and *S. pyogenes*) than gram negative bacteria (*E. coli*). This can be explained in term of the antisense targeting of the genes, as the gene encoding bacterial is highly similar in sequence among Staphyloccus species (85-100% gene similarity in S. aureus, Methicillin resistant *S. aureus* (MRSA), *S. epidermidis* and *S. pyogenes*) and different sequences in *E.coli* species[18]. Therefore, Copper nanoparticles possibly preferred to be bound with the antisense target of gram positive more than gram negative bacteria.

Antibacterial testing of samples after store at room temperature for 7 days, 30 days and 90 days was similar trend as short term activity. This can be expected that the copper (II) stearate hydrogels showed the stable activity which can be applied for prolong use.

5.5 Conclusion

The antibacterial wound dressing successfully prepared using gamma radiation. The gamma ray induced the reduction of copper (II) stearate within hydrogel pad to copper nanoparticles. The materials exhibited the good antibacterial activity against *E.coli*, *S. aurous*, *MRSA*, *S. epidermidis*, *S.pyogenes*. The addition of copper (II) stearate was affected to the swelling of the hydrogels. The swelling decreased when concentration of additional copper (II) stearate increased. All of the hydrogel samples had ability to allow water vapor to pass through (in the term of WVTR) which can be used for burn wound. For the indirect cytotoxicity, 0.5 % copper (II) stearate containing PVP hydrogel showed the least toxicity (more than 80%). From all of the results, the author suggested that the 0.5% copper (II) stearate containing PVP hydrogel was the optimum condition to be an antibacterial wound dressing due to their swelling and their satisfied concentration to disinfect bacteria but low-toxicity to the skin cell.

5.6 Ackhowledgements

The authors acknowledge partial support received from the Thailand research fund (TRF, grant no.DBG5280015 and a doctoral scholarship from Golden Jubilee Ph.D. Program), "the Integrated Innovation Academic Center IIAC (RES_01_54_63)" Chulalongkorn University Centenary Academic Development Project, Chulalongkorn University, the Center of Excellence on Petrochemical and Materials Technology, Chulalongkorn University, and The Petroleum and Petrochemical College (PPC), Chulalongkorn University.

5.7 References

[1] Boateng, J.S., Matthews, K.H. and Stevens, H.N. (2008) Wound healing dressings and drug delivery systems: A review. Journal of Pharmaceutical Science, 97, 2892–2923.

[2] Kokabi, M., Sirousazar, M. and Hassan, Z.M. (2007) PVA–clay nanocomposite hydrogels for wound dressing. European Polymer Journal, 43, 773–781.

[3] Lin, S.Y., Chen, K.O. and Run-Chu, L. (2001) Design and evaluation of drug-loaded wound dressing having thermoresponsive, adhesive, absorptive and easy peeling properties. Journal Biomatter, 22, 2999–3004.

[4] Purna, S.K. and Babu, M. (2000) Collagen based dressings: A review. Burns, 26, 54–62.

[5] Singh, B. and Pal, L. (2008) Development of sterculia gum-based wound dressings for use in drug delivery. European Polymer Journal, 44, 3222–3230.

[6] Rosiak, J.M., Rusika-rybus, A. and Pekal, A.W. (1989) Method of manufacturing of hydrogel dressings. U.S. Patent 4 871 490.

[7] Winter, G.D. (1962) Formation of scab and the rate of epithelialization of superficial wounds in the skin of the domestic pig. Nature, 193, 293-294.

[8] Rosiak, J.M., Ulanski, P., Pajenski, L.A. and Yoshii, K. (1995) Radiation formation of hydrogels for biomedical purpose. Radiation Physical Chemistry, 46, 161-168.

[9] Gilchrist, T. and Martin, A.M. (1983) Wound treatment with sorbsan-an alginate fibre dressing. Journal Biomatter, 4(4), 317-320.

[10] Julian, T.N., Radebaugh, G.W. and Wisniewski, S.J. (1983) Permeability characteristics of calcium alginate films. Journal of Control Release, 7, 165–169.

[11] Jones, V and Harding, K (2001) Moist wound healing. Chronic Wound Care: A Clinical Source Book for Healthcare Professionals (pp. 245-252). Wayne, PA.

[12] Wang, S., Feng, L. and Jiang, L. (2006) One-step solution-immersion process for the fabrication of stable bionic superhydrophobic surfaces. Advance Mater. 18, 767–770.

[13] Lia, J., Liua, X., Yea, Y., Zhoua, H. and Chen, J. (2011) A simple solution-immersion process for the fabrication of superhydrophobic cupric stearate surface with easy repairable property, Applied Surface Science, 258 , 1772–1775.

[14] Balakrishnan, B. and Jayakrishnan, A. (2005) Self-cross-linking biopolymers as injectable in- situ forming biodegradable scaffolds. Journal Biomatter, 26, 3941–3951.

[15] Bagchi, B. (2012) Antimicrobial efficacy and biocompatibility study of copper nanoparticle adsorbed mullite aggregates. Materials Science and Engineering C, 32, 1897–1905.

[16] Hermann, L. (1879) Allgemeine nervenphysiologie, Handbuch der Physiologie. Lster Theil (pp.1-196), Vogel, Leipzig.

[17] Koechlin, B. A. (1955) On the chemical composition of the axoplasm of squid giant nerve fibers with particular reference to its ion pattern. Biophysical Biochemistry Cytology, 1, 511-529.

[18] Steck, T. L., Fox, C. F. (1972) Membrane proteins. Membrane Molecular Biology (pp. 27-75), Sinauer Associates, Inc., Stamford.