SYNTHESIS OF POLYVINYL ALCOHOL-CHITOSAN HYDROGEL AND STUDY OF ITS SWELLING AND ANTIBACTERIAL PROPERTIES

Sintesis Hidrogel Polivinil Alkohol-Kitosan dan Studi Sifat Mengembang dan Antibakterinya

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ABSTRACT

The aim of this research was to synthesize a hydrogel for wound dressing by mixing of polyvinyl alcohol (PVA) and chitosan (CTS) and processed by combination technique of freezing-thawing and irradiation by gamma ray, and to study of its properties. PVA aqueous solution 10% (w/v) was mixed with 2% (w/v) chitosan (CTS) solution and homogenized. The PVA-CTS mixture was processed by freezing-thawing up to 3 cycles, and then irradiated by gamma rays at the dose ranged of 20-50 kGy (dose rate was 10 kGy/hour). Result showed that PVA-CTS hydrogel with the gel fraction of 83%, 87%, 90%, and 83% were obtained at the irradiation dose of 20 kGy, 30 kGy, 40 kGy, and 50 kGy, respectively. Increasing of irradiation dose caused increasing of water absorption of hydrogel, i.e. 1.700 %, 1.715 %, 1.913 %, and 2.036 %, respectively, and the hydrogel reached the equilibrium in 25 hours. The hydrogel showed very slow water evaporation rate (≈ 2%) at the initial time (1 hour) and then increased very fast (up ~50 %) at 24 h, i.e. 43%, 39.13%, 44%, and 53%, respectively. The elongation at break of hydrogels were obtained 245%, 322%, 322%, and 205% with the maximum value were obtained at irradiation dose ranged of 30-40 kGy. The presence of chitosan in the PVA hydrogel made it having higher antibacterial properties with the inhibition zone value of 8 mm at irradiation dose of 30-40 kGy compared to PVA hydrogel as a negative control (6 mm) and to chloramphenicol as a positive control (8 mm).

Keywords: hydrogel, polyvinyl alcohol (PVA), Chitosan (CTS), freezing-thawing, gamma irradiation

ABSTRAK

Tujuan dari penelitian ini adalah mensintesis hidrogel untuk pembalut luka dengan mencampurkan polivinil alkohol (PVA) dan kitosan (CTS) dan diproses dengan kombinasi teknik beku-leleh dan irradiasi sinar gamma, serta mempelajari sifat-sifatnya. Larutan PVA 10% (b/v) dicampur dengan 2% (b/v) larutan kitosan (CTS) dan dihomogenkan. Campuran PVA-CTS diproses dengan beku-leleh hingga 3 siklus, dan kemudian diirradiasi dengan sinar gamma pada kisaran dosis 20-50 kGy (laju dosis adalah 10 kGy/jam). Hasil penelitian menunjukkan bahwa hidrogel PVA-CTS dengan fraksi gel 83%, 87%, 90%, dan 83% didapatkan pada dosis irradiasi masing-masing 20 kGy, 30 kGy, 40 kGy, and 50 kGy. Meningkatnya dosis irradiasi mengakibatkan meningkatnya absorpsi air oleh hidrogel, yaitu masing-masing 1.700 %, 1.715 %, 1.913 %, dan 2.036 %, dan hidrogel mencapai kondisi keseimbangan dalam 25 jam. Hidrogel menunjukkan laju penguapan air sangat lambat (≈ 2%) pada awal waktu (1 jam) dan selanjutnya meningkat dengan sangat cepat (hingga ~50 %) pada 24 jam, yaitu masing-masing 43%, 39.13%, 44%, dan 53%. Nilai perpanjangan putus dari hidrogel didapatkan 245%, 322%, 322%, dan 205% dengan nilai maksimum didapatkan pada dosis irradiasi berkisar 30-40 kGy. Adanya kitosan dalam hidrogel PVA menjadikannya memiliki sifat antibakteri lebih tinggi dengan nilai zona inhibisi 8 mm pada dosis irradiasi 30-40 kGy dibandingkan dengan hidrogel PVA sebagai kontrol negatif (6 mm) dan dengan kloramfenikol sebagai kontrol positif (8 mm).

Kata Kunci: hidrogel, polivinil alkohol (PVA), kitosan (CTS), beku-leleh, irradiasi gamma

INTRODUCTION

Wound healing is an active area of research on accounts of its importance in the treatment of burns, prevention of post surgical adhesions and cosmetics surgery (Risbud *et al*., 2000). The purpose served by dressing includes protecting wounds, promoting healing, and providing, retaining or removing moisture

(Park & Barbul, 2004). Hydrogels are cross-linked hydrophilic polymer networks which absorb large amounts of water. Hydrogel wound dressing has humectants properties such that the wound is kept hydrated to prevent any scabbing or drying out so that the wound is allowed to heal from inside out. The absorption of secretion causes an expansion of the hydrogel making room for the inclusion of foreign bodies such as bacteria detritus and odor molecules that are irreversibly taken up along the liquid (Pai *et al*., 2006).

Based on their similar physical properties to human tissues and their excellent tissue compatibility, hydrogels have been studied extensively for biomedical applications. They can be used as soft contact lenses (Dillehay & Miller, 2007; Cheng *et al*., 2004), tissue engineering scaffold (Seng *et al*., 2012), controlled drug-release vehicles (Barndl *et al*., 2010) and wound dressings (Wang *et al*., 2012). Hydrogels have many advantages as wound dressings, for instance, absorb excess of wound exudates, protect the wound from secondary infection and effectively promote the healing process by providing a moisturized wound healing environment. They also can be removed without causing trauma to the wound (Thomas, 2007).

Polyvinyl alcohol (PVA) is a water-soluble polyhydroxy polymer. It has been used in practical applications because of its easy preparation, chemical resistance and physical properties, biodegradable, and cheap (Anon., 2006; Stasko *et al*., 2009). Chitosan (CTS), partially deacetylated of chitin, is a well known material in the wound dressing field. It has an excellent antibacterial activity, biodegradability, hemostatic, and biocompatibility (Goosen, 1997; Tombs & Harding, 1998; Liu *et al*., 2001; Panos *et al*., 2008). The use of chitosan as an additive in hydrogels can improve its performance for wound dressing. Chemical structure of PVA and CTS are presented in Fig. 1.

Hydrogels can be prepared by irradiation, freezing-thawing or chemical methods. Irradiation is considered to be a suitable tool for the formation of hydrogel. The main disadvantage of hydrogel prepared by irradiation is their poor mechanical strength. In addition, the technique is easy control of processing, no necessity to add any initiators or cross-linkers which may be harmful and difficult to remove, and possesses the possibility of combining hydrogels formation and sterilization in one technological step. Hydrogels of PVA in aqueous solutions prepared by freezing-thawing have shown many interesting properties (Stasko *et al*., 2009). The main disadvantages of this hydrogel are its opaque appearance and the limited swelling capacity and thermal stability. They have good mechanical strength, stable at room temperature, and with no initiators or cross-linkers.

Up to date, much work have been done on preparing hydrogels by irradiation (Erizal & Chosdu, 2009; Erizal *et al*., 2011) or by freezing-thawing (Lopergolo *et al*., 2002; Nugent *et al*., 2005; Zhao *et al*., 2003; Bajpai & Saini, 2005). However, there is very little information on preparation of hydrogels by combining the two processing techniques (Nho & Park, 2002; Erizal *et al*., 2011), especially by freezing-thawing followed by irradiation.

In this research, PVA hydrogels containing chitosan was prepared by combination technique of freezing-thawing and followed by γ-irradiation. The properties of the hydrogel including the gel fraction, the water absorption, the water evaporation rate, and the antibacterial properties were investigated.

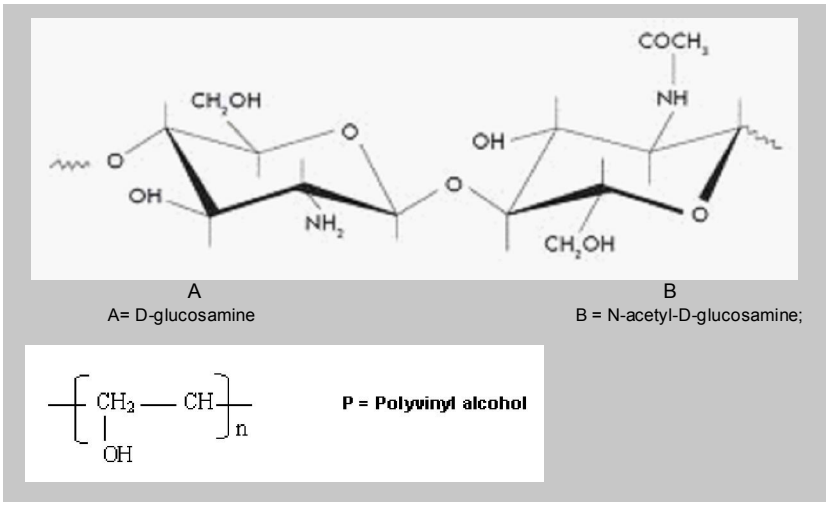

Figure 1. Repetitive units on the chemical structure of chitosan and polyvinyl alcohol. A= D-glucosamine; and B = N-acetyl-D-glucosamine; P = Polyvinyl alcohol.

MATERIAL AND METHOD

Materials

Polivinyl alcohol (PVA) with molecular weight of 72000 was purchased from Merck and used without any pretreatment. Chitosan (CTS) was purchased from Biotech Surindo, Indonesia with degree of deacetylation of 92 %. Acetic acid was purchased from Merck. All chemicals were of analytical grade and distilled water was obtained in our lab using aqua distilator.

The 7 (seven) species of bacteria indicator namely *Staphylococcus aureus, Salmonella typhimurium, Escherichia coli, Pseudomonas aerouginosa, Streptococcus* sp.*, Bacillus stearothermophillus, and Bacillus subtilis* were obtained from collection of bacteria culture laboratory at Research and Development Center for Marine and Fisheries Product Processing and Biotechnology.

Preparation of PVA-CTS Hydrogel

A 10% (w/v) of PVA solution was prepared by dissolving PVA in distilled water and autoclaving at 121°C for 20 minutes. A 2% (w/v) of chitosan solution was prepared by dissolving chitosan in 1% acetic acid solution. Both of solutions were mixed and homogenized by stirring at room temperature to get the final volume of 500 mL. The mixture was poured into polyethylene plastic bags, dimension of (20 cm x 10 cm x 0.5 cm), sealed and kept it in freezer at -80°C for 2 hours, then was thawed at room temperature for 1 h (this is called as 1 cycle). The process of freezing-thawing was repeated for 3 cycles. Finally, all the samples were irradiated by gamma rays from ^{60}Co source at the doses of 20 kGy, 30 kGy, 40 kGy, and 50 kGy (dose rate: 10 kGy/h) at room temperature. The hydrogels were washed directly for gel fraction determination and the rest were used for swelling studies. Each treatment was done in triplicate.

Gel Fraction

The hydrogel samples (2 cm x 2 cm x 0.5 cm) were put in the glass cup containing excess distilled water and were taken into shaker incubator at room temperature for 24 h, to remove the soluble fraction. The gels were dried under vacuum to constant weight. The insoluble fraction in the samples was determined gravimetrically and calculated as follow:

$$\text{Gel Fraction (\%)} = (W_2/W_1) \times 100\%$$

where W_1 is the initial weight of the gel and W_2 is the weight of dry gel after extraction.

Water Absorption

The water absorption of hydrogel was determined by gravimetric method. The gel samples (dried to constant weight) were immersed in a glass cup containing excess distilled water at room temperature. The hydrogel were periodically weighed after the water on the gel surface was removed with a filter paper. The water absorption was calculated as follow:

$$\text{Water absorption} = \{(Ws-Wd)/Wd\} \times 100\%$$

where Ws is the weight of the swollen gels at time t and Wd is the initial weight of dried gels.

Elongation at Break

Elongation at break is an important physical parameter of hydrogel representing its elasticity, and measured based on ASTM standard method by using Instron tester instrument. The hydrogels were moulded with dumbbell for the preparation of the standard size measurement. Both ends of the pieces were firmly clamped in the jaws of a testing machine. One jaw was fixed and the other was movable. The movable jaw moved at the rate of 30 mm/min at room temperature. The resultant data was showed at the recorder. This procedure was repeated for three times for each result. The elongation at break was calculated as follow:

$$\text{Elongation at break} = L_1/L_o \times 100\%$$

Where L_o is the initial length and L_1 is the final length.

Antibacterial Assay

Antibacterial activity of PVA-CTS hydrogels were tested using Kirby-Bauer method (Rollins & Joseph, 2000) against seven species bacteria, *Escherichia coli (E. coli), Staphylococcus aureus, Bacillus stearothermophilus, Salmonella typhimurium, Streptococcus* sp., *Pseudomonas aeroginosa, Bacillus subtilis.* As a negative control was disc blank (PVA hydrogel disc), a positive control was chloramphenicol (PVA hydrogel disc + 100 ppm chloramphenicol), and a treatment was PVA-CTS hydrogel disc. At amount of 15 mL agar Mueller Hinton was poured into 90 mm petridish until solidification. At about 10^5 CFU/mL microbial culture suspension was spread over the surface of a sterile agar plate evenly by a sterile swab, then added another 15 mL agar solution, homogenized and waited at room temperature until the plates cooled down. The hydrogels treatment and control were applied on the centre of plates and the mixture was kept at 37°C for 48 hour. The antibacterial inhibition of hydrogel was

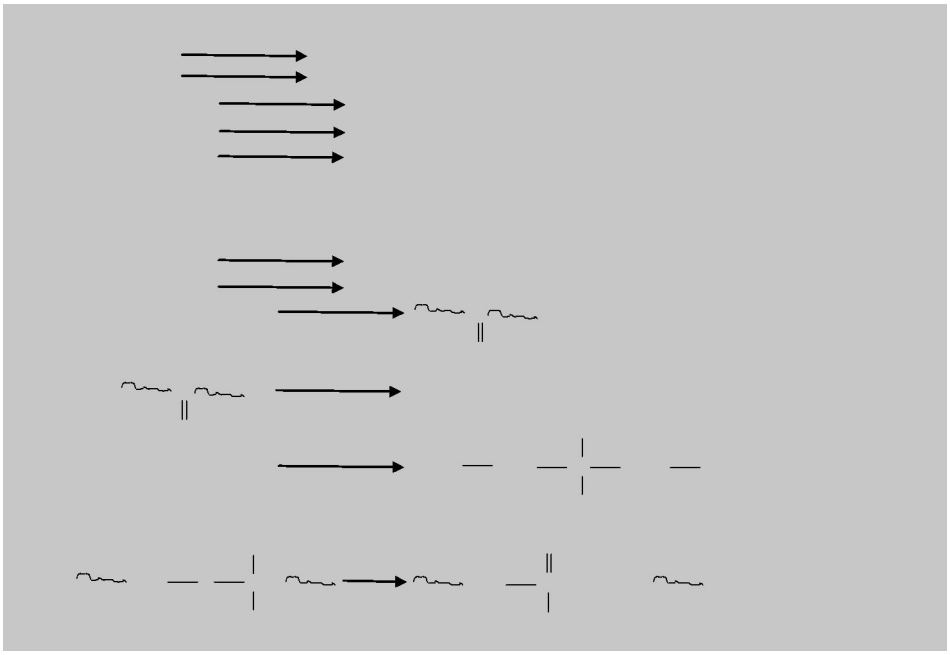

Figure 2. Effect of Gamma irradiation on polyvinyl alcohol polymer in aqueous solution, crosslinking and degradation reaction (Sakurada & Ikada, 1963).

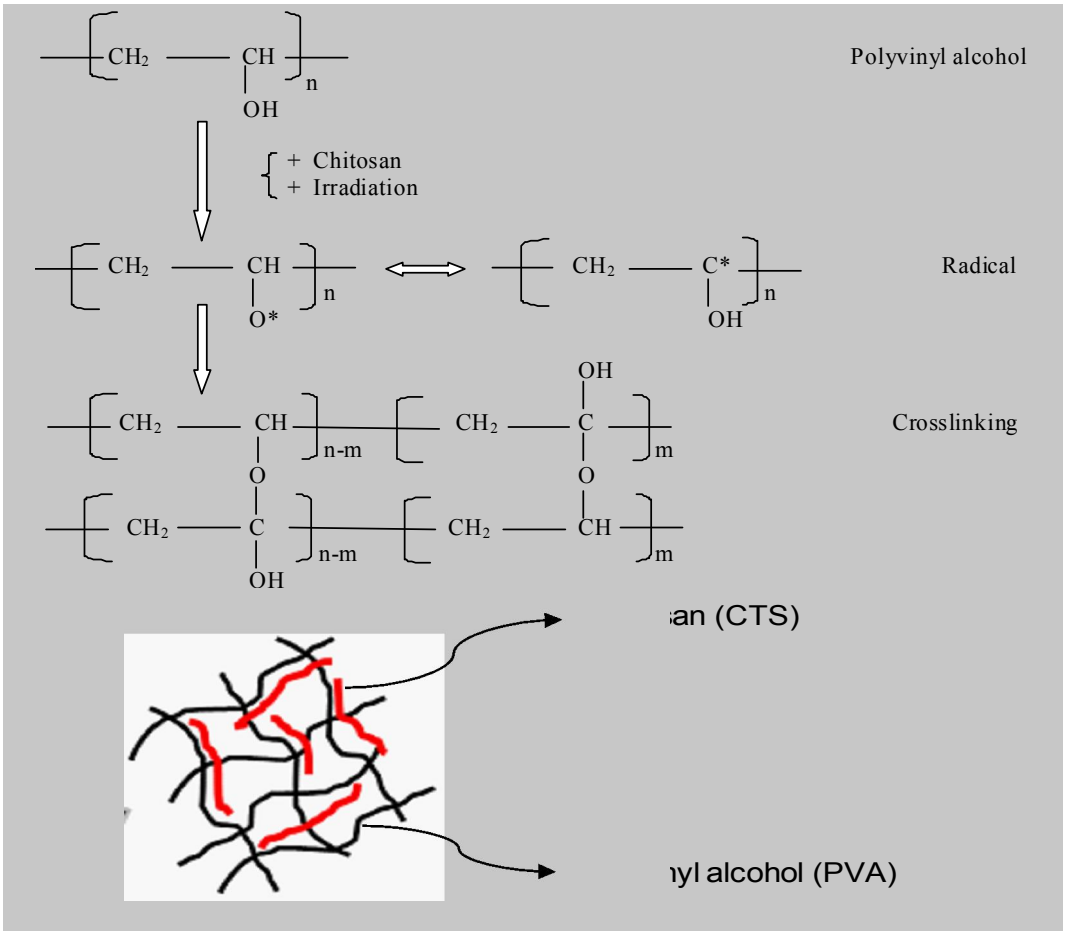

Figure 3. Effect of gamma irradiation on polyvinyl alcohol polymer in aqueous solution (Sakurada & Ikada, 1963). Interpenetrating polymer network (IPN) formed on PVA-CTS (Hoarea & Kohaneb, 2008).

determined by measuring the diameter of each clear zone in millimeter at around of gels using a ruler provided. All of the operation procedures were done under aseptic condition.

RESULT AND DISCUSSION

When irradiation from source interacts with a polymer material, the polymer absorbs its energy and active species such as radicals are produced, thereby, initiating various chemical reaction. Crosslinking and degradation are two competing process that always co-exist under radiation (Mishra *et al*., 2007).

In brief, there was a mechanism of crosslinking and degradation on this material when it is exposed to the irradiation. Let say, P is a symbol of PVA, and P* is a radical produced as a result of hydrogen abstraction from the main-chain, and the radiolysis of PVA aqueous solution can be described in Fig. 2 and Fig. 3 (Sakurada & Ikada, 1963). The amount of P* produced by reaction (2) is very small compared to that produced by reaction (3) and (4). The rate of reaction of (7) is generally consider to be much higher than that of (5).

Gel Fraction

The variation of gel fraction of PVA-CTS hydrogels versus irradiation dose on hydrogels is presented in Fig. 4. It shows that the increase of the irradiation dose, the gel fraction of hydrogels gradually increased from 80% to 85% at the irradiation dose of 20 kGy and 30 kGy, respectively, and reached the maximum value of gel fraction of 90 % at the irradiation dose of 40 kGy. Then, the gel fraction decreases until 80% at the irradiation dose of 50 kGy. Thus, there is a significant decrease in the gel fraction value as the irradiation dose increased in the range of this study. This result indicates that the PVA-CTS hydrogels with high gel fraction of 90% can be obtained in the presence of 2% chitosan at the irradiation dose of 40 kGy.

Rekso & Sunarni (2009) used 10% PVA and CTS solutions with the ratio of PVA-CTS as 80% : 20% and irradiated the polymer at the dose of 20 kGy, 30 kGy, and 40kGy. He obtained the gel fraction of the polymer as 76.4%, 86.3%, and 87.4%, respectively. This gel fraction result is lower compared with our result by using the ratio of PVA-CTS as 83.33% :17.67% and same irradiation dose, i.e. 83%, 87%, and 90%. Hydrogel of PVA-CTS blend has the higher gel fraction then PVA alone. Based on those data, the higher the CTS content in the hydrogel, the lower the gel fraction.

Chitosan is a natural polysaccharide, which is degraded on irradiation by breakdown of the main chains (Hien *et al*., 2012). However, in this study PVA is the main component that is known as a polymer and crosslinked in aqueous medium (Zheng *et al*., *2008)*. When the mixture of PVA-CTS is freezed-thawed and then it is irradiated, the interpenetrating polymer network (IPN) is formed (Fig. 3) with the chemical crosslink of PVA and physical crosslink of CTS (Herman *et al*., 2009). Soerens *et al*. (2005, USA Patent 6967261) and Yang *et.al* (2008, USA Patent 20090297587) reported that the hydrogels with the gel fraction of ~80 % are suitable to be applied as wound dressings. It means that based on gel fraction, all treatments used in this research are met the requirement for wound dressing, and the best one is resulted from irradiation dose of 40 kGy.

Water Absorption

The water absorption of PVA-CTS hydrogel with variation of the irradiation dose is presented in Fig.5. It shows that the absorption of water increases along

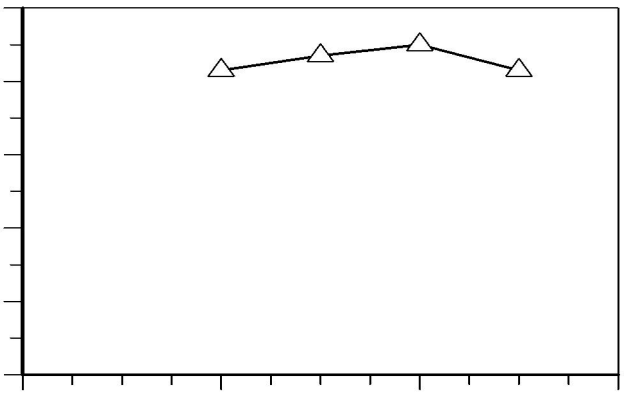

Figure 4. Relationship of the irradiation dose (kGy) and the gel fraction (%) of PVA-CTS hydrogels.

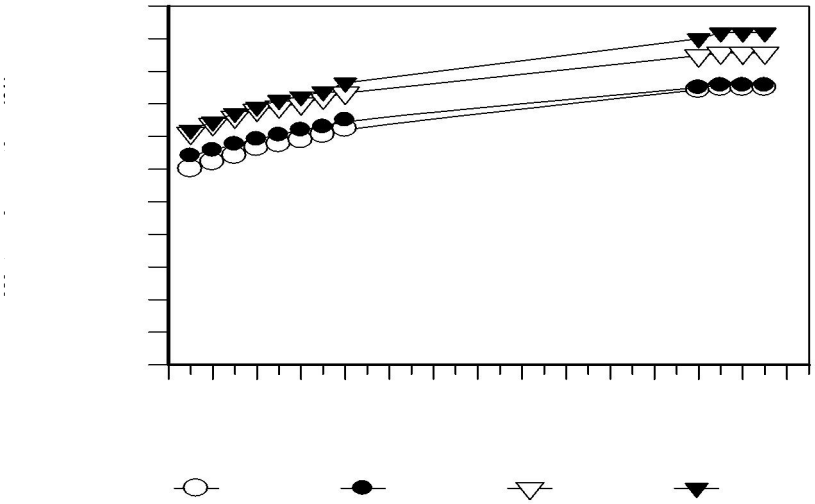

Figure 5. Relationship of time (hours) and water absorption (%) of PVA-CTS hydrogel that was irradiated with different dose.

with the times howevers after 26 hours, it reached a limiting value which is an equilibrium condition. It was found that the water absorption of hydrogels was increasing in accordance with the increasing of the irradiation dose. All hydrogels were reached the equilibrium condition in 25 hours, i.e. 1.700%, 1.715%, 1.913%, and 2.036%, respectively, with the highest water absorption of ≈ 2.000% at the irradiation of 40 kGy and 50 kGy.

The water absorption of hydrogels may be supported by hydroxyl (OH) groups of PVA and amino ($-NH_2$) groups of CTS which is interacted with water molecules through hydrogen bonding, and by the presence of porous network in the hydrogel. By increasing the irradiation dose, the crosslink density of PVA hydrogel increase, therefore the porosity of PVA hydrogel increase and the water diffusion into hydrogel increase. On the other hand, the increasing the irradiation dose the degradation of IPN CTS in the hydrogel increase, and the degradation product of its CTS was dissolved out of the hydrogel, and in turn due to the porosity of PVA hydrogel increase, the water absorption of hydrogel or water diffusion into hydrogel increase.

The PVA-CTS hydrogel prepared by gamma irradiation at the irradiation dose range of 40 kGy to 50 kGy has water absorption of ≈ 2.000%, it is equivalent to swelling ratio of 20 g/g. Thus, it indicates that this hydrogel absorb all the effusive wound exudates if it is used as wound dressing. Soeren *et al.* (2005) reported that the swelling ratio of hydrogel at value of 20 g/g is an ideal value to absorb an excess of wound exudates. According to Yang *et al.* (2008, USA Patent 20090297587) if the hydrogel has a water absorption value of 1/2 times lower of the ideal value, on the applications of hydrogel as a wound dressing it is needed to change wound dressing very often at about every 2-3 days. Based on those statements, the hydrogel prepared by irradiation dose of 50 kGy is the most ideal one, and that of by 40 kGy is also ideally. In contrast, Rekso & Sunarni using PVA-CTS ratio as 80% : 20% reported that the higher the irradiation dose, the lower the water absorption of hydrogel, i.e. at the irradiation dose of 20 kGy, 30 Gy, and 40 kGy, got the water absorption as 166.1%, 104.6%, and 90.9%, respectively. This water absorption result is lower compared with our result by using the ratio of PVA-CTS as 83.33% : 17.67% and same irradiation dose, i.e. 1700%, 1715%, and 1913 %, respectively. Rekso & Sunarni the found the water absorption of hydrogel with the irradiation dose of 20 kGy, 30 Gy, and 40 kGy were decreased, i.e. 92.2%, 57.8%, and 54.5% at 2 hours and 104%, 72.5%, and 70.3% at 4 hours dipping in aquadest, respectively. Whilst, we got much higher result and were increased on the water absorption of hydrogel with the irradiation dose of 20 kGy, 30 Gy, and 40 kGy, i.e. 1.244%, 1.316%, and 1.475% at 2 hours and 1.332%, 1.384%, and 1.563% at 4 hours dipping in aquadest, respectively. The difference result probably because of mainly they used a PVA with lower molecular weight and also the chitosan with a lower molecular weight and degree of deacetylation, that it was not specified.

Water Evaporation

To examine the possibility of the hydrogel to be used as wound dressing, we investigate the water evaporation rate from hydrogel at the temperature of 30°C with 40% humidity. Result is presented in Fig.

6. The water evaporation rate from hydrogel irradiated at the dose of 50 kGy at the initial 1 hour was very slow (~2%) and then increased very fast up to ~50 % at 24 hour. The water evaporation rate of hydrogel irradiated at the dose of 20 kGy, 30 kGy, 40 kGy, and 50 kGy were 43%, 39.13%, 44%, and 53%, respectively. The water evaporation rate of hydrogel irradiated by 20 kGy, 30 kGy, and 40 kGy are relatively

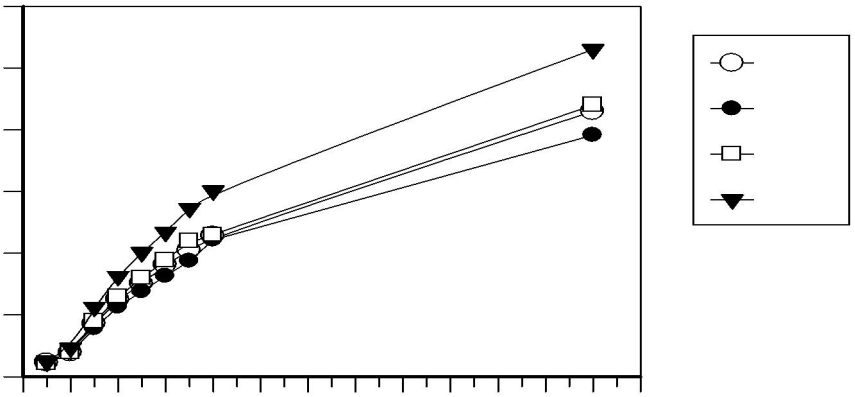

Figure 6. Relationship of time and water evaporation rate of PVA-CTS hydrogel that was irradiated with different dose.

have same value, at about 40%, except irradiated by 50kGy is much higher.

It is known that swelling capacity can prevent the accumulation of wound exudates, and a smaller evaporation rate can avoid very often changing the wound dressing. Therefore, hydrogel prepared by irradiation dose of 20 kGy, 30 kGy, 40 kGy are suitable for wound dressing, and among of those, hydrogel irradiated by the dose of 30 kGy is the most suitable one. Whilst, hydrogel prepared by 50 kGy is the worse. Yang *et al.* (2008) reported that irradiation dose of 30 kGy is suitable irradiation dose for preparation of PVA-CTS hydrogel. They obtained that water evaporation rate of the PVA-CTS hydrogel was very fast (~100 %) for 6 hour standing at room temperature.

Elongation at Break

Elongation at break is an important physical factor of hydrogel representing its flexibility when it is used for wound dressings. The aims of measurement on the elongation at break of hydrogel in this experiment was to get a supporting data for hydrogel product specification. Until now, there is no available reference related with the standard values of elongation at break for wound dressing. Generally, the more flexible of hydrogel the easier to follow the skin surface contour (Soerens & Malik, 2005; Yang *et al*., 2008). The relationship of elongation at break of PVA-CTS hydrogel with the irradiation dose is presented in Fig 7. The initial elongation at break of hydrogel with irradiation dose of 20 kGy is 245%. The elongation at break of hydrogel increased with increasing the irradiation dose from 20 kGy to 30 kGy, become 322%, and kept constant at 40 kGy (322%), and then decreased at 50 kGy, become 205%. It indicates that irradiation dose at range of 30-40 kGy is the optimum dose to get maximum value of the elongation at break of PVA-CTS hydrogel.

Decreasing of elongation at break of hydrogel at the irradiation dose more than of 40 kGy may be due to degradation of chitosan in the hydrogel matrix, distributed unhomogeneously and uncrosslinked with resulting a harder and less extensible hydrogel.

***In Vitro* Antibacterial Assay**

In the biomedical field, the product used must be free of bacteria or has an antibacterial activity. Hydrogel prepared for wound dressing or biomedical application has to be sterilized or free of any microorganisms. Here, the PVA hydrogel with and without chitosan have been made by irradiation and were tested against many bacteria to confirm whether chitosan addition on PVA can improve the antibacterial activity of the PVA hydrogel or the product meet the requirement for biomedical application. The clear zones diameter formed as representing the inhibition activity of PVA-CTS hydrogel tested against 7 bacteria is presented in Table 1. The results showed that all the hydrogel containing chitosan (treatment) inhibited the growth of all the bacteria tested with bigger inhibition zone (7-8 mm) than PVA hydrogel without chitosan or negative control (6-7 mm). Comparing to

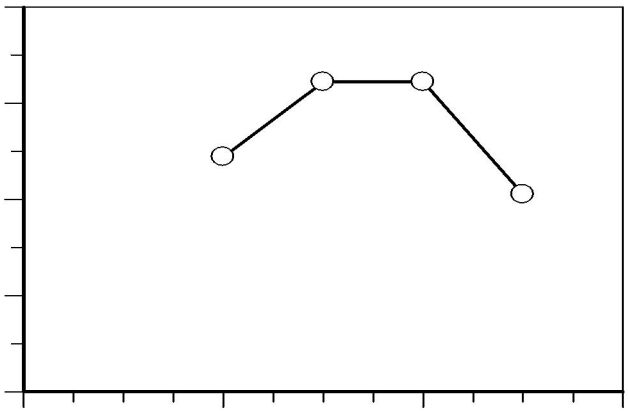

Figure 7. Relationship of irradiation dose (kGy) and elongation at break of PVA-CTS hydrogel.

Table 1. Antimicrobial activities of irradiated PVA-CTS hydrogel

Notes:

Negative control were 1). PVA (20 kGy); 2). PVA (30 kGy); 3). (40 kGy); 4). PVA (50kGy); Treatments were 5). PVA-CTS (20 kGy); 6). PVA-CTS (30 kGy); 7). PVA-CTS (40 kGy); 8). PVA-CTS (50 kGy); Positive control was 9). Chloroamphenicol;

Disc diameter was 5 mm; Bacteria tester : *Ec = E. coli; Sa = Staphylococcus aureus; Bst = Bacillus stearothermophilus, Sty = Salmonella typhimurium; Sh = Streptococcus* sp.*; Pa = Pseudomonas aeroginosa; Bs = Bacillus subtilis;*

chloramphenicol as a positive control (8 mm), the antibacterial activity of PVA-CTS hydrogel (30 kGy and 40 kGy) have same potency (8 mm) with chloramphenicol. The antibacterial properties of hydrogel containing chitosan is due to a positive charge of the amino-group in chitosan that and it has the ability to bind to a negative charge in bacteria (Tsai & Su, 1999).

CONCLUSION

A series of PVA hydrogel containing chitosan were synthesized by combination of freeze-thawing and irradiation technique and their properties were compared with PVA hydrogel without chitosan. Results showed that PVA-CTS hydrogels have high gel fraction (83-90%), high water absorption capacity (1.201-1.441% in 1 hour; 1.700-2.036% in 25 hours), low water evaporation rate (43-53%), high elongation at break (205-322%), good antibacterial activity, and translucent appearance. Increasing the irradiation dose of 20 kGy until 50 kGy, will result in increased the gel fraction, increased the water absorption, relatively low and constant of water evaporation rate except at the dose of 50 kGy, increased elongation at break except at the dose of 50 kGy, and increased the antibacterial activity except at the dose of 50 kGy.

All the PVA-CTS hydrogels showed an antibacterial activity against *E. coli*, *Staphylococcus aureus, Bacillus stearothermophilus, Salmonella typhimurium, Streptococcus sp., Pseudomonas aeroginosa, and Bacillus subtilis.* Based on all parameters measured, PVA-Chitosan hydrogels produced by irradiation dose of 40 kGy is the best one. In fact, the product of 30 kGy and 40 kGy has similar properties, hence from economical view point (time of irradiation, dose rate was 10 kGy/hour), the product of 30 kGy is more promising.

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