CHAPTER IV
RESULTS AND DISCUSSION

4.1 Composition of Hydrogels

In this work AMPS-Na^+ hydrogels were prepared by both UV-curing and Gamma radiation technique, the composition of AMPS-Na^+ hydrogels that produced by Ultraviolet radiation technique and Gamma radiation technique was showed in Table 4.1

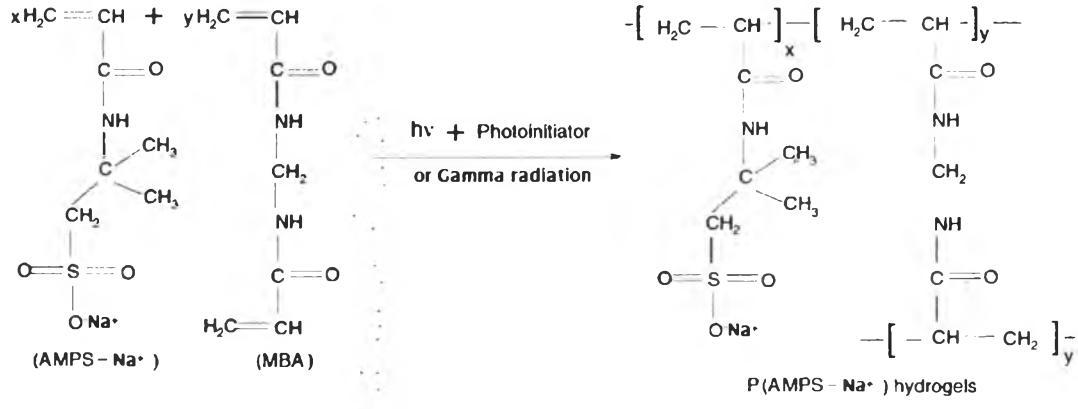
Table 4.1 Compositions of poly(AMPS-Na+) hydrogels produced by UV and Gamma radiation

Sample		Weight	
		MBA (g)	Photoinitiator (mg)
30% AMPS-Na^+	0.1 mol% MBA	0.02	3
	0.5 mol% MBA	0.11	3
	1 mol% MBA	0.22	3
40% AMPS-Na^+	0.1 mol% MBA	0.03	4
	0.5 mol% MBA	0.15	4
	1 mol% MBA	0.30	4
50% AMPS-Na^+	0.1 mol% MBA	0.04	5
	0.5 mol% MBA	0.18	5
	1 mol% MBA	0.37	5

Aqueous solutions of AMPS-Na^+ in the presenced of *N-N'*-methylenebisacrylamide (MBA) crosslinker and 1-Hydroxycycloxehyl phenyl ketone photoinitiator crosslink on irradiation and form gels. In case of UV radiation, when aqueous solution of AMPS-Na^+, MBA crosslinker and 1-Hydroxycycloxehyl phenyl ketone photoinitiator is expose to Ultraviolet radiation, photoinitiators decomposed on exposure to UV light to produce initiating free radicals and these free radicals reacted further with monomer and crosslinker to start the polymerization reaction. But in case of Gamma radiation when aqueous solution of AMPS-Na^+ and MBA crosslinker is exposed to radiation, hydroxy free radicals (•OH), hydrogen free radicals (•H) and hydrated electrons are produced. •OH radicals are mostly

responsible for crosslinking of monomer with crosslinker and form into three-dimensional crosslinking network or hydrogel structure.

Chemical structures of monomer and possible binding mechanism between AMPS-Na^+ and MBA crosslinker was showed in Scheme 4.1



Scheme 4.1 Synthesis of poly AMPS-Na^+ hydrogels.

4.2 Chemical Structure Analysis of Chitin Whiskers

The preparation of chitin whiskers by hydrolysis of chitin flakes with 3 N HCl resulted in colloidal solution. Chitin, which is a poly-N-acetyl-D-glucosamine as shown in Figure 4.1, is never fully acetylated, and amino groups (NH_2) are always present on the chitin crystallite surface, in acid media they are protonated to form NH_3^+. The protonation of these amino groups is resulting in electrostatic repulsive force in suspension. Therefore, chitin whisker displayed colloidal behavior; according to Equation 7.

$$Glu—NH_2 + H^+ \longrightarrow Glu—NH_3^+ \quad (7)$$

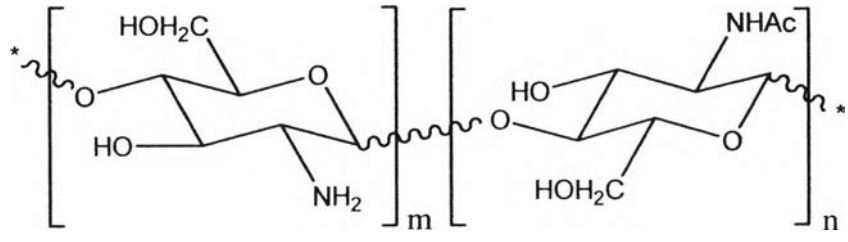


Figure 4.1 Chemical structure of chitin (n > 50%).

The FTIR spectrum of chitin whiskers (Fig. 4.2a) demonstrates a typical type of chitin. When compared to the FTIR spectrum of chitin flake (Fig 4.2b), chitin whiskers shows sharp characteristic peak in all ranges, as seen from the peaks at 3500-3300 cm^{-1} (-OH); 2900-2800 cm^{-1} (C-H stretching); and 1660,1621,1557 cm^{-1} (C=O stretching of amide I and II). This might be due to the highly crystalline chitin whiskers which can confirm the successful hydrolysis of chitin. The different wavenumbers of amide I and II could be due to the differences of interaction effects. Amide I was described to the effect of the intermolecular hydrogen bonding of C=O--$OHCH_2$ (Lavall, R.L. *et al.*, 2006). In case of amide II, the band at 1557 cm^{-1} arose from the deformation of the intermolecular hydrogen bonding, N-H bond in the plane of the CONH group (Nge, T.T. et al., 2003).

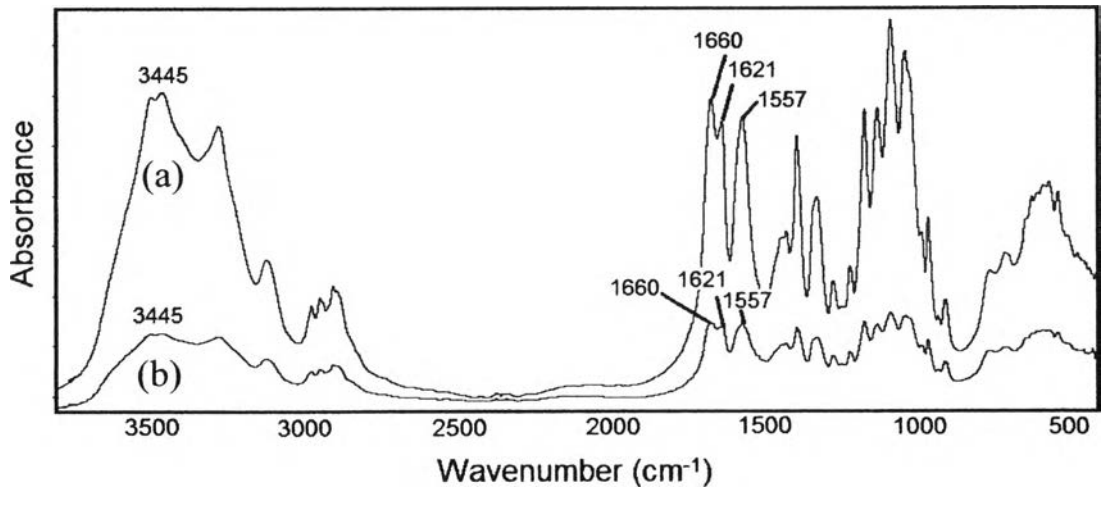


Figure 4.2 FTIR spectras of chitin whiskers (a), and chitin flakes (b).

The effect of UV and Gamma radiation to chemical structure of chitin whiskers can be investigated by irradiation chitin whiskers suspension with UV radiation and Gamma radiation and then characterized with FTIR. The obtain results showed that FTIR spectrum demonstrated similar peaks as in as- prepared chitin whiskers as shown in Figure 4.3. This can be concluded that chemical structure of chitin whiskers was not changed after irradiation.

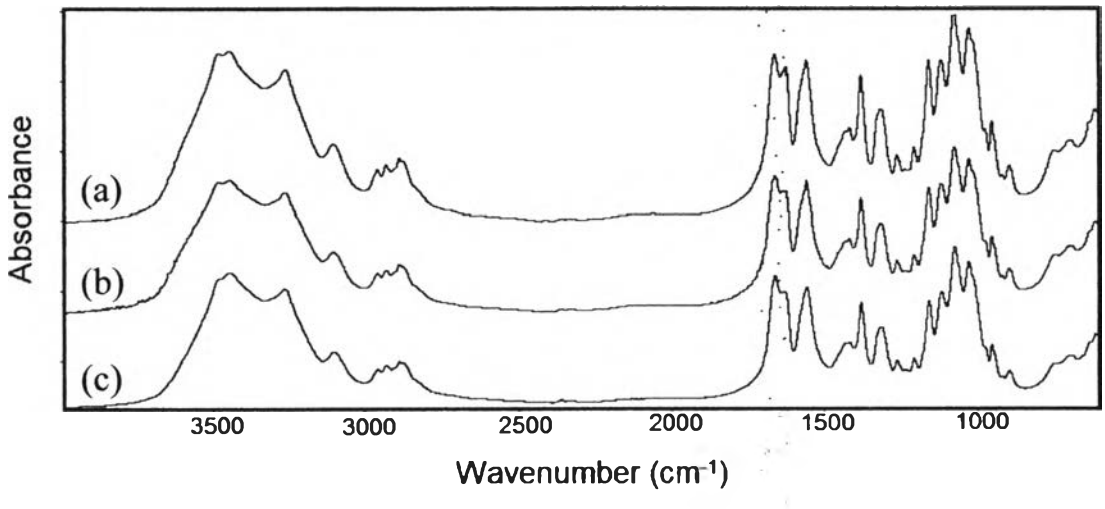


Figure 4.3 FTIR spectras of chitin whiskers (a), and chitin whiskers after UV irradiation (b), chitin whiskers after Gamma irradiation (c).

4.3 Morphological Appearance and Size of Chitin Whiskers

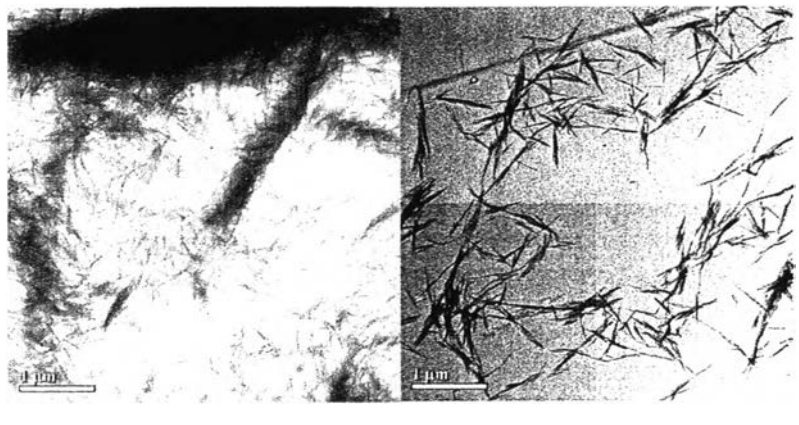


Figure 4.4 TEM images of chitin whiskers.

Transmission electron microscopy (TEM) gives the information to determine size of chitin whiskers. The TEM images of chitin whiskers from a dilute suspension of chitin whiskers are shown in Figure 4.4. The suspension contained chitin fragments consisting of both individual crystallites and crystal bundles. The size distribution of the chitin whiskers is shown in Figure 4.5 and 4.6. The width of these chitin fragments range from 12 to 66 nm ,while the length ranged from 120 to 990 nm. The average width and length of these whiskers were 31.19 nm and 499.9 nm, respectively. The aspect ratio (average length to width ratio *L/D*) of these whiskers was calculated to be about 16. The solid content of the as-prepared chitin whiskers suspension was about 6.17 %w/v. These dimensions are in the same range of the previously reported values for chitin whiskers obtained from the shrimp shell (L = 180 to 820 nm and d = 8–74 nm (P. Wongpanit *et al.*,2007).

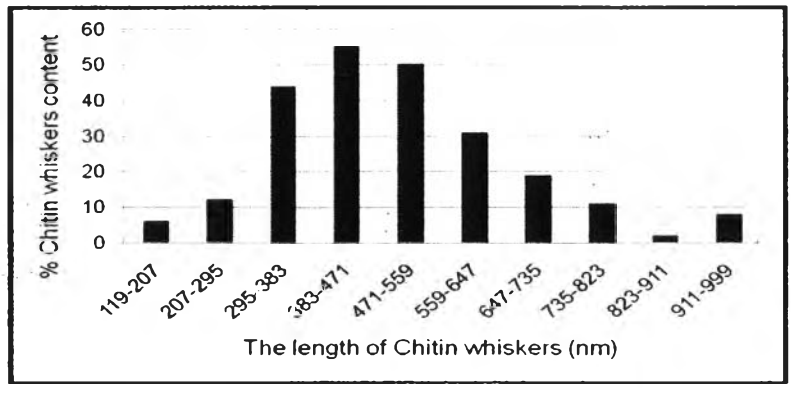


Figure 4.5 Histogram of the length distribution of chitin whiskers.

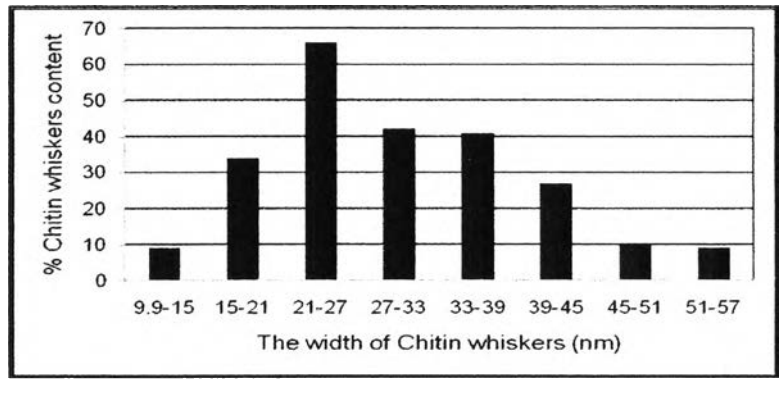


Figure 4.6 Histogram of the width distribution of chitin whiskers.

4.4 Composition of Chitin Whiskers-Reinforced Hydrogels

The composition of Chitin whiskers-reinforced hydrogels that produced by Ultraviolet radiation technique and Gamma radiation technique were showed in Table 4.2.

Table 4.2 Compositions of Chitin whiskers-reinforced Hydrogels produced by UV and Gamma radiation

Sample		MBA		Chitin whiskers		PI (mg)
		%	Weight (g)	Weight (g)	Volume	
30% AMPS-Na+	3 wt%	0.5	0.11	0.9	15	3
	5 wt%			1.5	25	
	8 wt%			2.4	40	
40% AMPS-Na+	3 wt%	0.1	0.03	1.2	20	4
	5 wt%			2	33	
	3 wt%			2.8	45	

Hydrogels produced from 30% (w/v) AMPS-Na^+ with 0.1%MBA was glutinous, soft, tacky and did not stay in the sheet form while hydrogels from 50% (w/v) AMPS-Na^+ had less flexibility and easy to lacerate. Also, from an economic perspective, the cost of the monomer needs to be taken into account since a higher monomer concentration will increase the production cost of the hydrogel, therefore only 2 conditions, which were 30%AMPS-Na^+ with 0.5 %MBA and 40% AMPS-Na^+ with 0.1% MBA, was suitable for improve dimensional stability by incorporated with chitin whiskers.

The chitin whiskers/AMPS-Na^+ solutions were prepared by neutralize AMPS solution with NaOH solution until pH = 7.00 and then mixed with homogenized chitin whisker suspension. Due to the solid content of as-prepared chitin whiskers was 6.17 wt%, which this suspension volume must be consider with the final volume after neutralize AMPS solution with NaOH solution. So, the maximum content of chitin whiskers in 30% and 40% AMPS-Na^+ solution was limited to be only 8 and 7 wt%, respectively.

4.5 Appearance of AMPS-Na^+ Hydrogels and Chitin Whiskers-Reinforced AMPS-Na^+ Hydrogels

AMPS-Na^+ hydrogels and chitin whiskers-reinforced AMPS-Na^+ hydrogels were formed by Ultraviolet radiation and Gamma radiation technique. The pure AMPS-NA^+ hydrogels were transparent which is the advantage for using AMPS-Na^+ hydrogels as a wound dressing because it allow easy inspection of wound, whereas chitin whiskers-reinforced hydrogels were more translucent as chitin whiskers content increase as showed in Figure 4.7

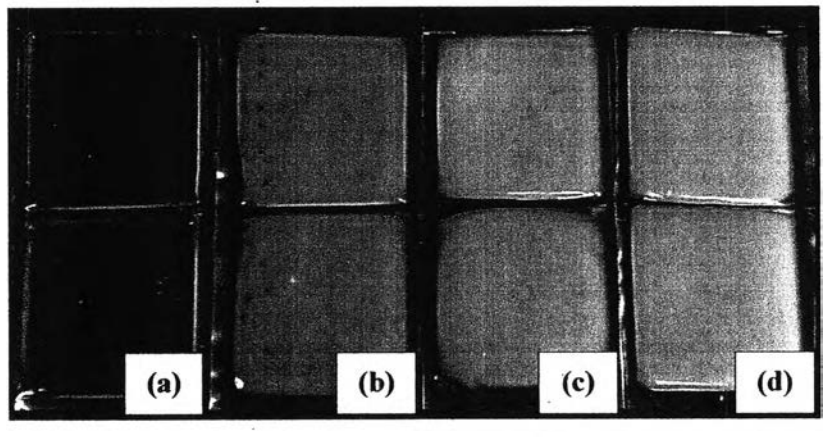


Figure 4.7 Appearance of neat AMPS-Na^+ hydrogel (a) AMPS-Na^+ hydrogel reinforced with 3 wt% chitin whiskers (b), 5 wt% chitin whiskers (c) and 8 wt% chitin whiskers (d).

4.6 Structural Characterization

FTIR spectra of neat AMPS-Na^+ hydrogel and hydrogel that contained chitin whiskers in various amount (3-8% w/w) are shown in Figure 4.8 FTIR spectrum of neat AMPS-NA^+ hydrogel reveal characteristic absorption bands of acrylamides. The main bands in the AMPS-Na^+ hydrogel spectrum were as follows: 3420-3090 cm^{-1} (NH- and OH- stretching vibrations), 1655 cm^{-1} (C=O stretching of Amide I), 1550 cm^{-1} (C=O stretching of Amide II) and the isopropyl methyl deformation bands at 1386 and 1366 cm^{-1} (C.Alarcon *et al.*,2005 and R.M.

Silverstein *et al.*, 2005) The bands due to the presence of sulfonic acid groups were in the range 1192-1040 cm^{-1} (asymmetric and symmetric O=S=O stretching) and 630 cm^{-1} (C-S stretching). (R.M. Silverstein *et al.*, 2005 and S.Durmaz and O.Okay, 2000)

The spectra of reinforced hydrogel are uneffect. All characteristic peaks of neat hydrogel were observed in all spectra of reinforced hydrogel. No peaks shift and no new peaks are observed. This investigation, may possibly explain that the interaction of chitin whiskers and AMPS-Na^+ matrix is only physical interaction via hydrogen bonding.

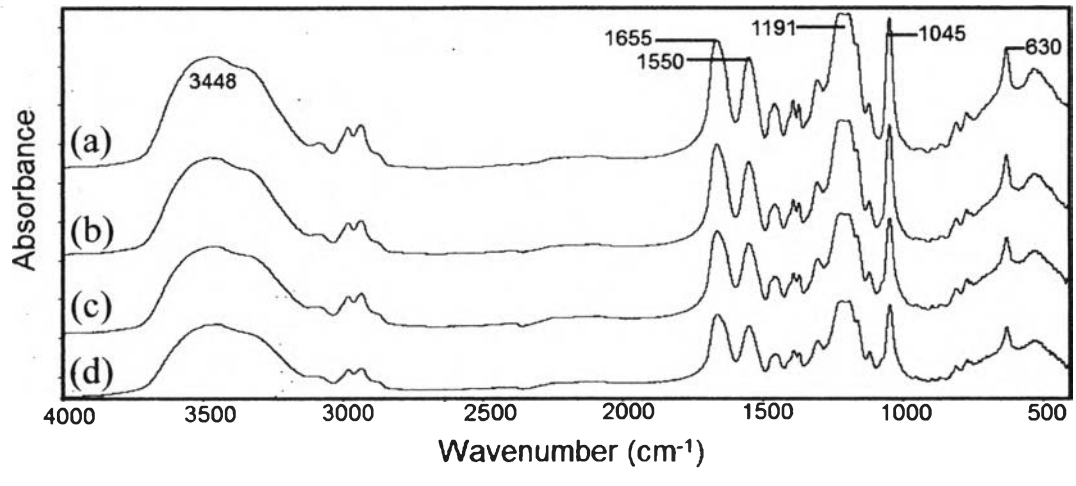
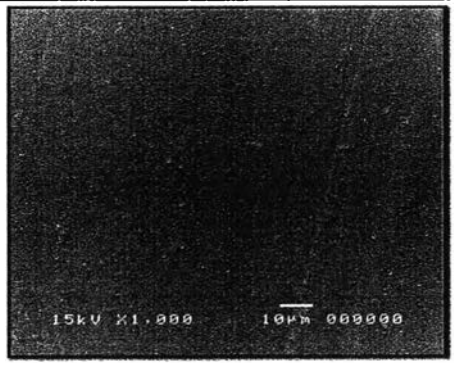
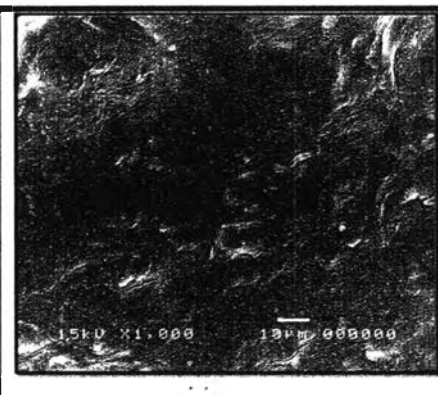
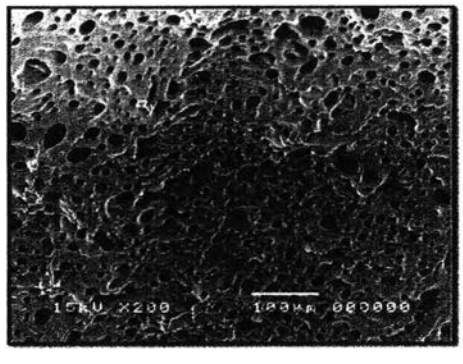
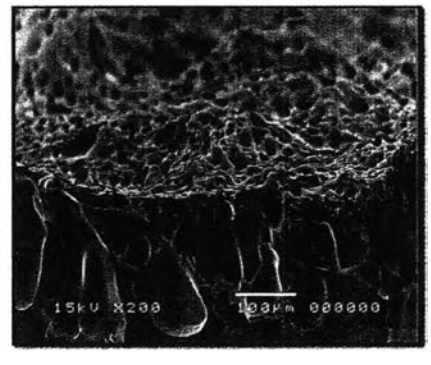


Figure 4.8 FTIR spectras of AMPS-Na^+ hydrogel (a) and AMPS-Na^+ hydrogel containing (b) 3%, (c) 5%, and (d) 8% w/w chitin whiskers.

4.7 Morphology of Neat Hydrogels and Chitin Whiskers-Reinforced Hydrogels

Table 4.3 Selected SEM Images of 30% AMPS-Na^+ neat hydrogel with 0.5 % MBA Crosslinker and hydrogel Containing 8% w/w Chitin Whiskers

 Neat hydrogel (30% AMPS-Na^+)	 8% CW reinforced hydrogel
 Top surface of freeze-dried hydrogel	 Transverse and top section of freeze-dried Hydrogel

Selected SEM images of neat AMPS-Na^+ hydrogels and chitin whiskers-reinforced hydrogels that contain 8% w/w of chitin whiskers are shown in Table 4.3 ; It further proved that all hydrogels formed homogeneous structure in irradiation process. The surface of neat hydrogel was smooth but for chitin whiskers-reinforced hydrogel the surface of hydrogel was quite rough, with the evidence of aggregates of the whiskers appearing on their surface. The SEM photographs of freeze-dried hydrogel showed highly porous structure as seen in top and transverse section. Well defined pore structure could be due to the evaporation of water from basic network structure of hydrogel after irradiation.

4.8 Gel Fraction

Irradiation of AMPS-Na^+ aqueous solution leads to the formation of insoluble polymer network (gel). A typical dependence of gel fraction on the monomer concentration and percentage of crosslinker after UV irradiation and Gamma irradiation (25 kGy) is shown in Figure 4.9 and 4.10, respectively. It can be seen that the gel fraction increases with increasing monomer concentration and MBA crosslinker and it seems never to reach 100% of gel. When a higher concentration of monomer and crosslinker are employed, the number of crosslink points in the polymer network increases, the gel fraction of hydrogel also increases. This is supported by the observation that the low crosslink density gives softer and more elastic hydrogel sheets. The obtained results shown that 50% AMPS-Na^+ hydrogels at every percentage of MBA crosslinker give the highest gel fraction values which was about 96% and 98% for UV radaition and Gamma radiation process, respectively. And the results also shown that hydrogel produced by Gamma radiation technique at radiation dose of 25 kGy can form more crosslinking structure than using UV radiation technique.

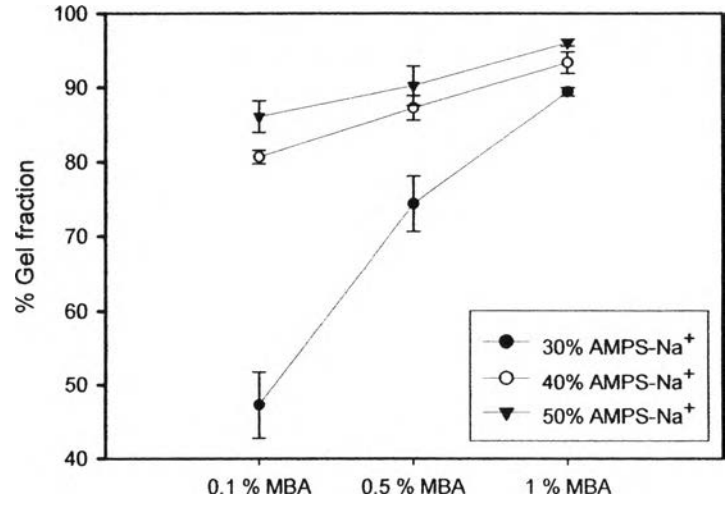


Figure 4.9 Gel fraction of 30% , 40% and 50% AMPS-Na^+ hydrogel at various percentage of MBA crosslinker produced by UV radiation technique.

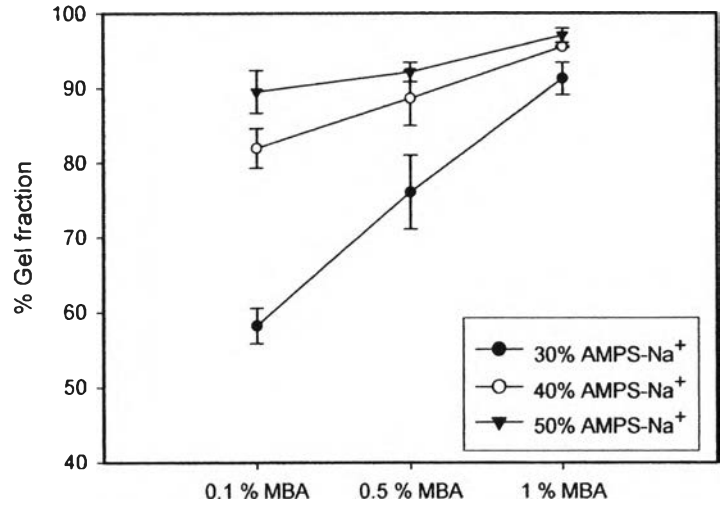


Figure 4.10 Gel fraction of 30% , 40% and 50% AMPS-Na^+ hydrogel at various percentage of MBA crosslinker produced by Gamma radiation technique.

4.9 Swelling Behavior of Hydrogels

The degree of swelling in stimulated body fluid (SBF, pH 7.40) of neat AMPS-Na^+ hydrogels which produced from both technique were plotted as a function of the immersion time, as shown in Figure 4.11 and Figure 4.12. The percentage of swelling gradually increased with time and reach equilibrium point after an immersion time of about 1 day. The results showed that equilibrium degree of swelling of AMPS-Na^+ hydrogel decreased with increasing monomer content and percentage of MBA crosslinker. As discussed in the gel fraction part that hydrogels with higher monomer concentration and MBA crosslinker have higher crosslinking density, resulting in more compact and denser structure. Therefore, it has less of free volume inside their structure and it is difficult for water molecules to diffuse inside the hydrogel structure. So, degree of swelling of hydrogels decreases.

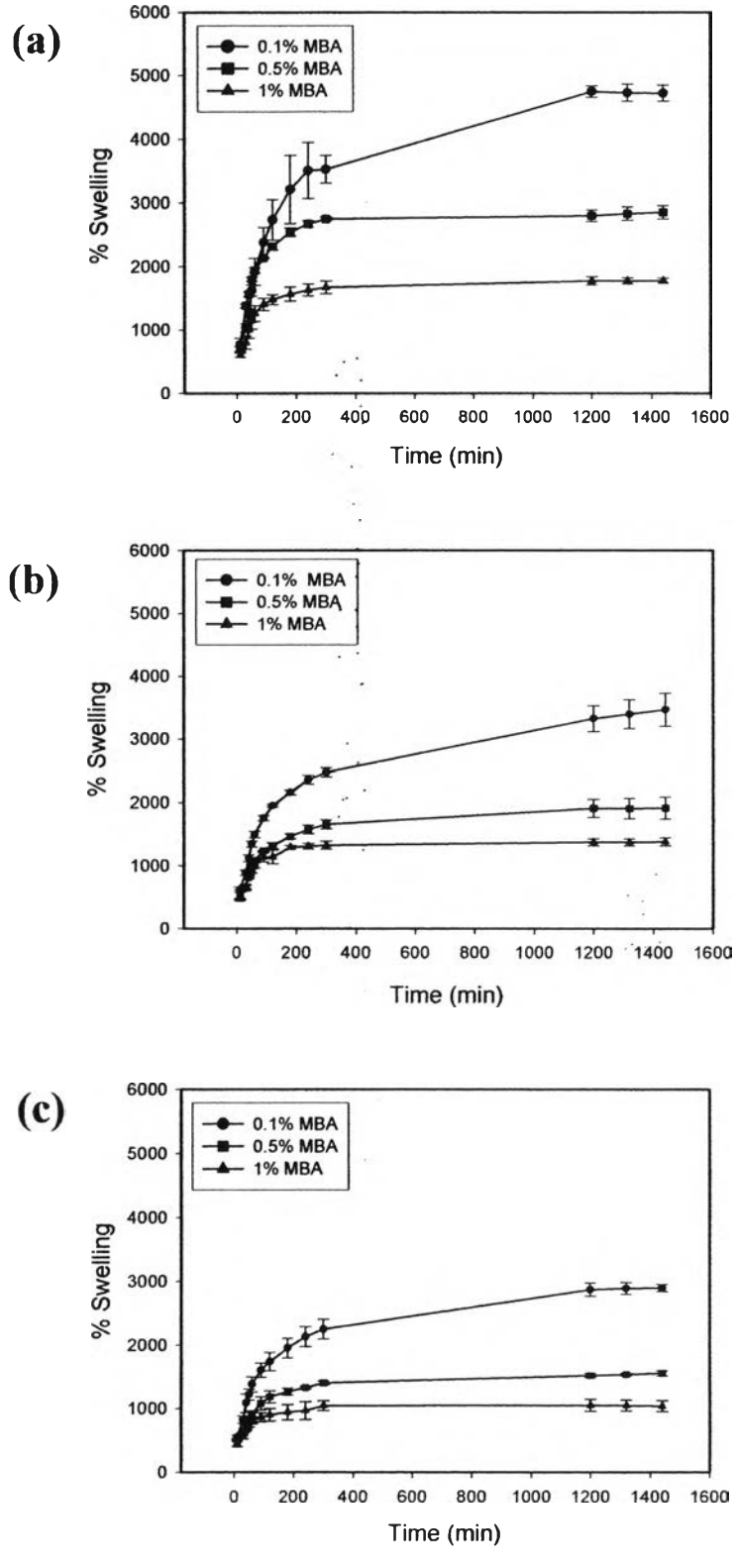


Figure 4.11 Percentage of swelling of 30% (a), 40% (b) and 50% AMPS-Na^+ from UV radiation technique.

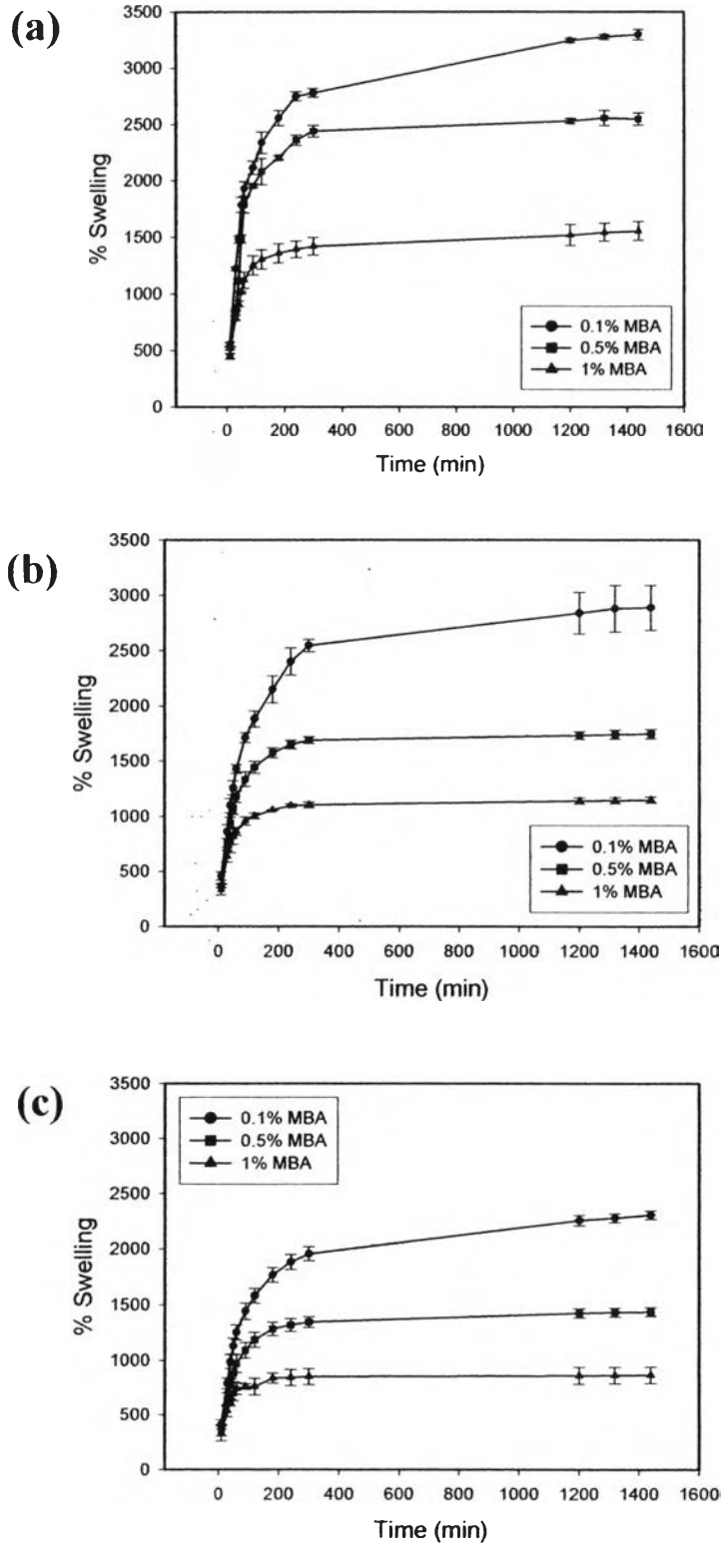


Figure 4.12 Percentage of swelling of 30% (a), 40% (b) and 50% AMPS-Na^+ from Gamma radiation technique.

Also, the hydrogel takes a shorter time to approach equilibrium as the % crosslinker is increased to 1 mol%. This is because a hydrogel with a higher crosslink density shows more resistance to expansion and absorbs less amounts of water. But for hydrogel with 0.1 mol%, water can diffuse inside the crosslinking structure easily and show the large expansion of hydrogel sheet.

However, it was observed that these synthetic hydrogels can absorb relatively large amounts of water. On immersion in SBF, the hydrogels expanded dramatically from approximately 5 x 5 x 0.1 cm^3 to 12 x 12.5 x 0.3 cm^3, as shown in Figure 4.13. Their hydrophilic property have a high affinity for water but their hydrophobic main chains and crosslinks prevent them from dissolving. Hence, they swell rather than dissolve when immersed in water or SBF.

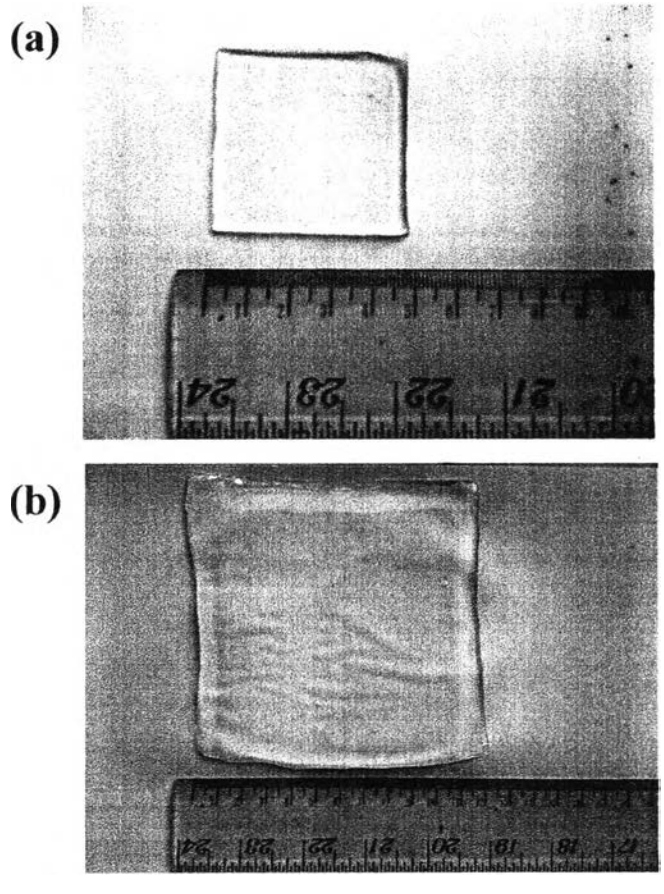


Figure 4.13 Hydrogel sheets synthesized from 30% AMPS-Na^+ crosslinked with 0.5 % MBA (a) hydrogel sheet before immersion in SBF (5 x 5 x 0.1 cm) and (b)swollen hydrogel sheet after immersion in SBF to an equilibrium state (12 x 12.5 x 0.3 cm).

And it was clearly seen that hydrogels produced from UV radiation technique reach higher equilium degree of swelling than hydrogels produced from Gamma radiation technique. This agree with the sesults in gel fraction of hydrogel that Gamma radiation technique at a dose rate of 25 kGy give hydrogel with higher degree of cresslinking than UV radiation. Swelling value reached the highest point with UV radiation production of neat 30% AMPS-Na^+, 0.1% MBA (4717%).

Figure 4.14 showed the swelling ratio of chitin whiskers-reinforced hydrogel produced by UV radiation technique. The nanocomposite hydrogels provided a lower percentage of swelling than the pure AMPS-Na^+ hydrogel. The equilibrium degree of swelling were continueously decrease with the increase in the chitin whisker content, including 2550%, 2399% and 2234% of 3%, 5% and 8% chitin whiskers-reinforced 30% AMPS-Na^+ and 2872%, 2611% of 3%, 5% and 7% chitin whisker-reinforced 40% AMPS-Na^+ hydrogels. A similar trend was observed in chitin whiskers-reinforced hydrogel produced by Gamma radiation technique (Figure 4.15).

The swelling beheavior of hydrogels in this study suggested that it is dependent on the presence of chitin whisker, which is inversely proportional to the degree of swelling. This might come from the fact that chitin whiskers acts as a water barrier for water diffusion into hydrogel structure.

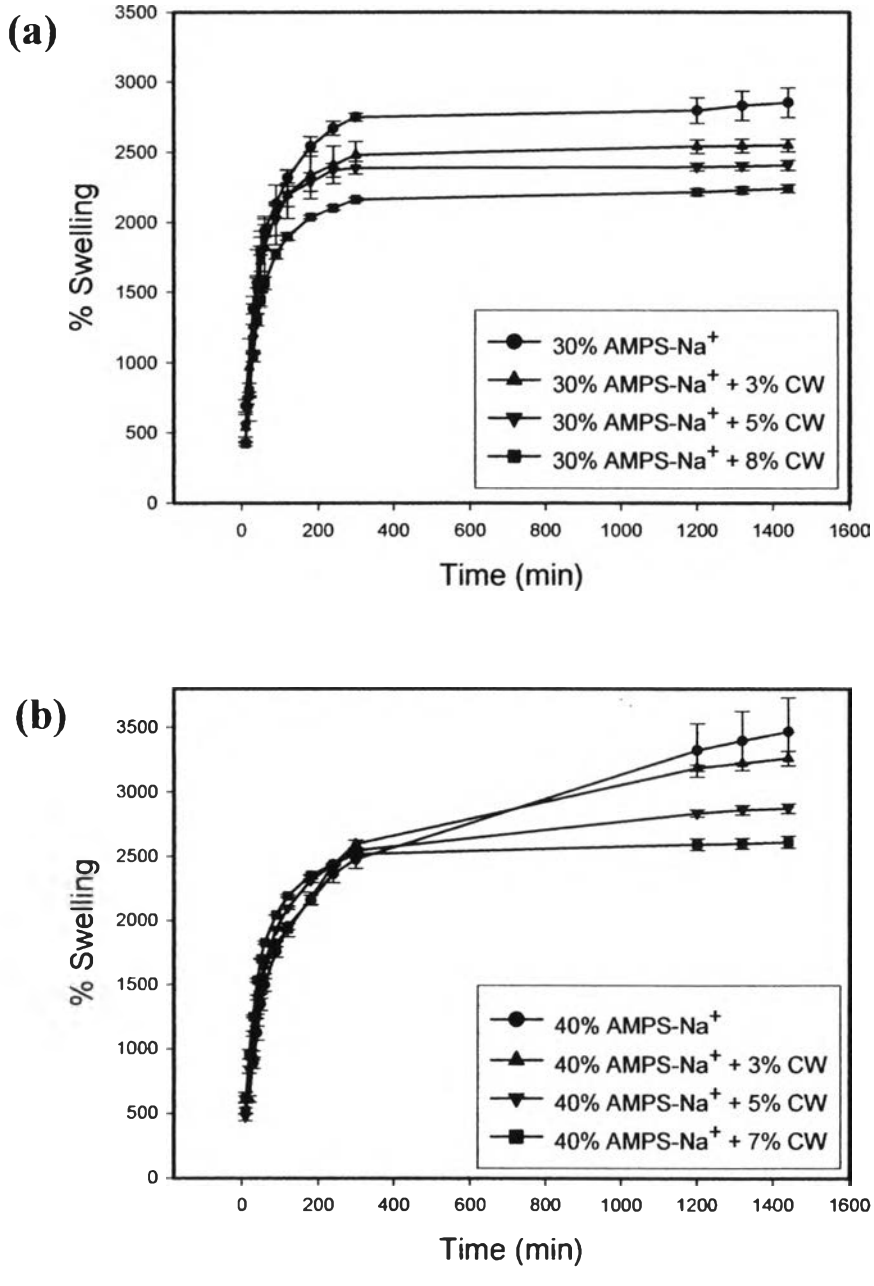


Figure 4.14 Percentage of swelling of chitin whisker-reinforced 30% (a), and 40% (b) AMPS-Na^+ from UV radiation technique.

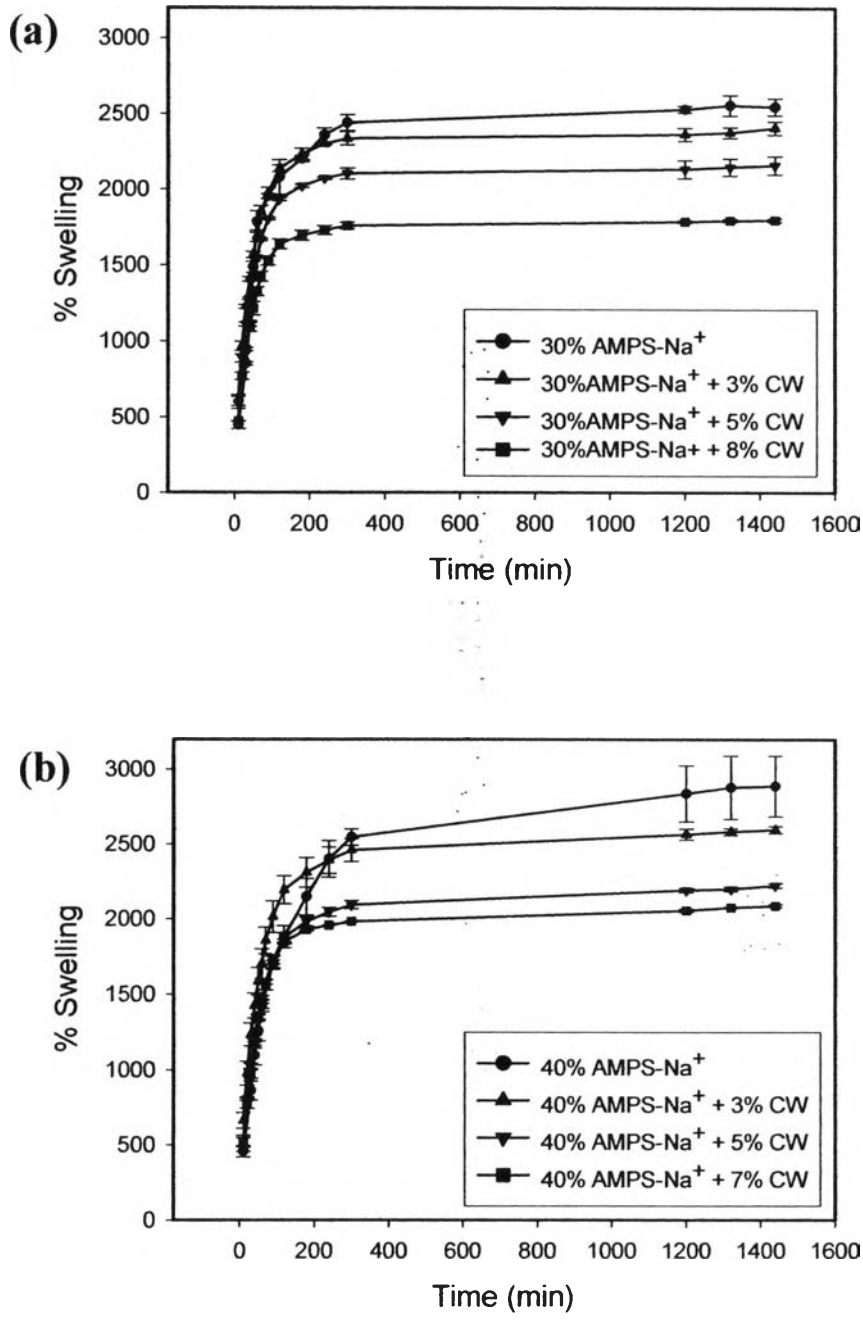


Figure 4.15 Percentage of swelling of chitin whisker-reinforced 30% (a), and 40% (b) AMPS-Na^+ from Gamma radiation technique.

4.10 Mechanical Properties of Hydrogels

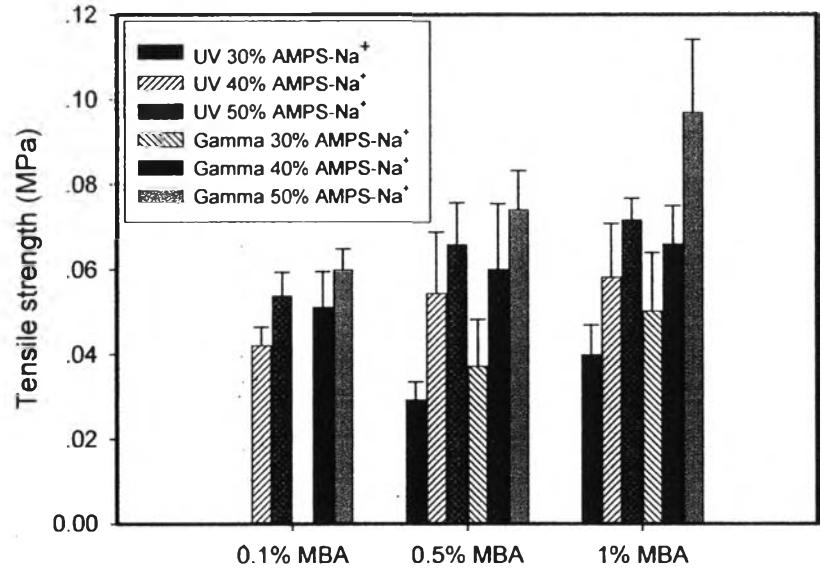


Figure 4.16 Tensile strength (MPa) of neat AMPS-Na^+ hydrogels produced from UV and Gamma radiation technique at various concentration of MBA crosslinker from 0.1-1 mol%.

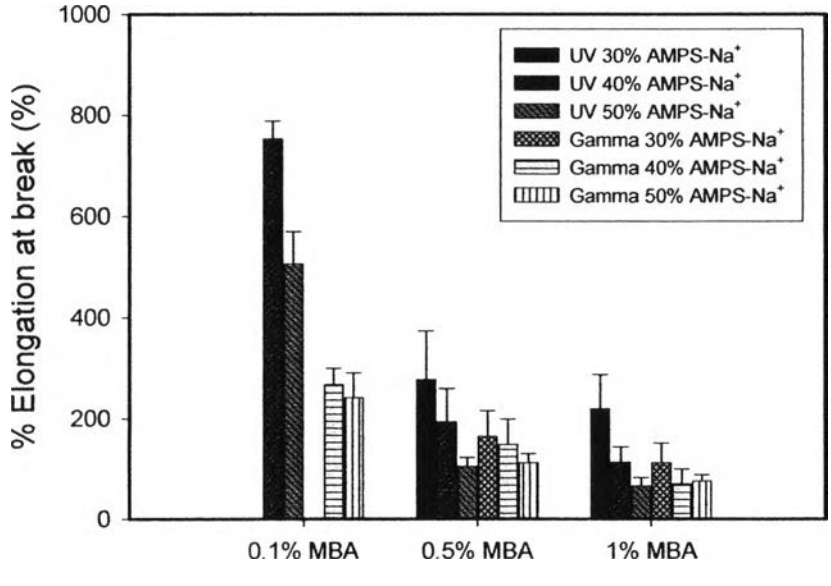


Figure 4.17 % Elongation of neat AMPS-Na^+ hydrogels produced from UV and Gamma radiation at various concentration of MBA crosslinker from 0.1-1 mol%.

Tensile strength and %Elongation of neat AMPs-Na^+ hydrogels at each concentration of monomer and MBA crosslinker from two techniques were investigated and showed in Figure 4.16 and 4.17. The data for 30% AMPS-Na^+ with 0.1% MBA from UV and Gamma techniques was not showed here because hydrogels from this condition were too soft and too sticky cannot cut into the test shape to measure their mechanical properties from testing machine.

However, The results of other conditions found that increasing of monomer concentration from 30% to 50% trend to higher tensile strength. In case of UV radiation process initially from 30% AMPS-Na^+ with 0.5% MBA tensile strength of hydrogel was 0.029±0.004 MPa. For 40% AMPS-Na^+ with 0.5% MBA tensile strength of hydrogel was 0.054±0.014 MPa and for 50% AMPS-Na^+ with 0.5% MBA tensile strength of hydrogel was 0.066±0.01 MPa. And percentage of MBA crosslinker from 0.1%, 0.5% to1 mol% also effect the tensile strength of hydrogels, which higher percentage of crosslinker hydrogels showed higher tensile strength. On the other of tensile strength, %Elongation decreased steadily as incrase monomer concentration and MBA crosslinker. The %Elongation of 40% AMPS-Na^+ with 0.5% MBA showed the highest value which was 753.1±35.3%.

The radiation that used to forming hydrogel also effects the mechanical properties of hydrogels. Hydrogels produced from Gamma radiation at dose rate of 25 KGy shown higher value of tensile strength and lower value of percentage of elongation (ie. 0.037±0.01 MPa and 164.13±51.8 % ,respectively for 30% AMPS-Na^+ with 0.1% MBA hydrogel) than hydrogels that produced from UV radiation technique (ie. 0.02914±0.004 MPa and 277.2±96.3% ,respectively for 30% AMPS-Na^+ with 0.1% MBA hydrogel) at the same condition, this is due to the crosslinking density of hydrogels as discussed before.

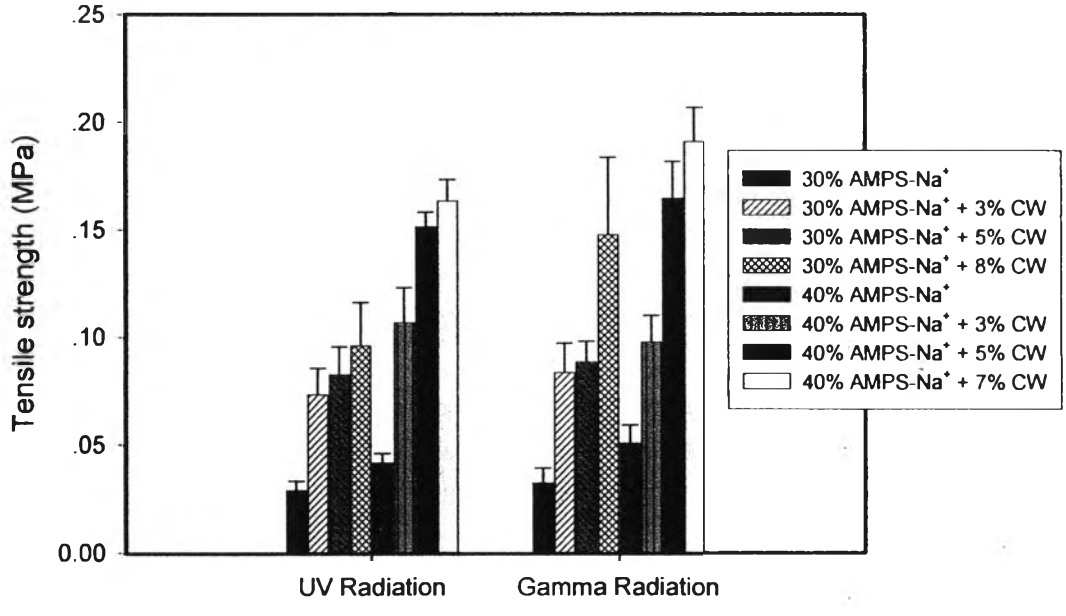


Figure 4.18 Tensile strength (MPa) of chitin whisker reinforced AMPS-Na^+ hydrogels produced from UV and Gamma radiation technique.

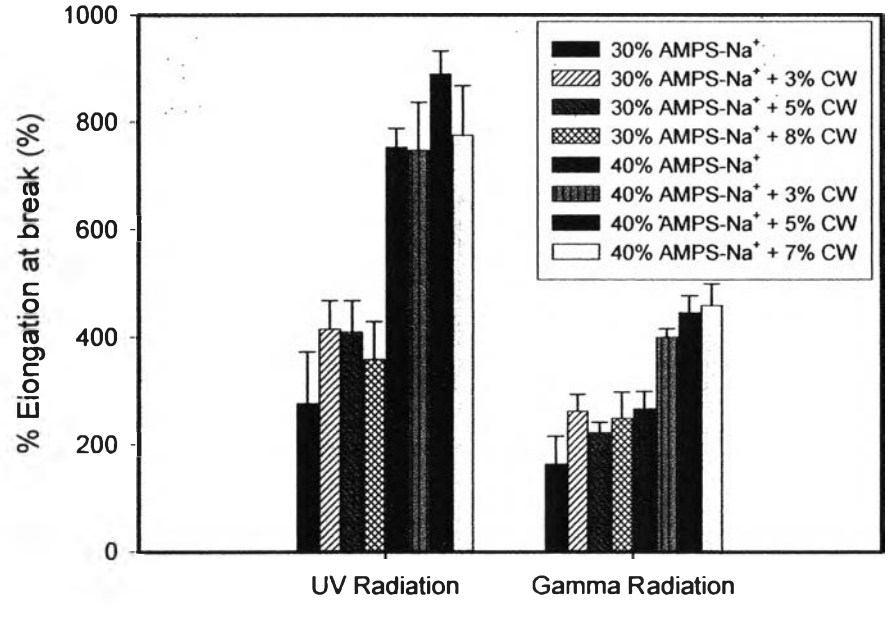


Figure 4.19 % Elongation of chitin whiskered reinforced AMPS-Na^+ hydrogels produced from UV and Gamma radiation technique.

Figure. 4.18 and 4.19 showed tensile strength and percentage of elongation at break of pure AMPS-Na^+ hydrogels and chitin whiskered reinforced AMPS-Na^+ hydrogels having whisker content in the range of 3-8 wt%. The tensile strength of reinforced hydrogels increased from that of pure hydrogel (ie. 0.0291±0.004 MPa for pure 30% AMPS-Na^+ hydrogel) with increasing chitin whisker content from 3, 5 to 8 wt% (ie. 0.0735±0.0122 MPa, 0.0828±0.0130 MPa and 0.0962±0.0201 MPa, respectively). The percentage of elongation trend to follow in the same way of tensile strength , the % elongation of chitin whiskers reinforced hydrogels (ie. 415.73±52.5 MPa for 30% AMPS-Na^+ hydrogel reinforced with 3wt% chitin whiskers) was higher than the pue one (ie. 277.2±96.3 MPa for pure 30% AMPS-Na^+ hydrogel). And the results also showed that hydrogel produced by Gamma radiation technique at radiation dose of 25 kGy can forms more crosslinking structure than using UV radiation technique as its showed higher tensile strength and lower percentage of elongation.

The increase in the tensile strength and %Elongation of chitin whiskered reinforced hydrogels might due to the hydrogen bonding between polymer and chitin whiskers reinforcing filler. This can be concluded that chitin whiskers fillers helps improved the mechanical properties of neat hydrogels.

4.11 Water Vapor Transmission Rate of Hydrogel (WVTR)

The hydrogel wound dressing must avoid or at least reduce the body liquid lost by controlling absorption and transmission as well as by maintaining the high humidity in the wound area. Table 4.4, 4.5, 4.6 and 4.7 showed the water vapor transmission rate of neat hydrogel and chitin whisker-reinforced hydrogels produced from UV and Gamma radiation technique.

Table 4.4 The value of WVTR of AMPS-Na^+ hydrogels from UV radiation

Sample	WVTR ($g/h.m^2$)		
	0.1% MBA	0.5% MBA	1% MBA
30% AMPS-Na^+	141.1 ±4.1	129.1 ±1.9	115.5 ±4.4
40% AMPS-Na^+	120.8 ±1.0	107.5 ±3.1	102.3 ±0.9
50% AMPS-Na^+	106.1 ±4.0	97.9 ±1.7	84.0 ±1.1

Table 4.5 The value of WVTR of AMPS-NA^+ hydrogels from Gamma radiation

Sample	WVTR ($g/h.m^2$)		
	0.1% MBA	0.5% MBA	1% MBA
30% AMPS-Na^+	125.9 ±2.8	121.2 ±0.6	111.4 ±2.9
40% AMPS-Na^+	112.9 ±0.8	104.7 ±4.2	93.9 ±3.1
50% AMPS-Na^+	80.8 ±2.4	75.76 ±3.2	69.4 ±2.3

Table 4.6 The value of WVTR of Chitin whiskers-reinforced AMPS-Na^+ hydrogels from UV radiation

Sample		WVTR ($g/h.m^2$)
30% AMPS-Na^+ (0.5% MBA)	0% CW	129.1 ±1.9
	3% CW	124.9 ±3.0
	5% CW	110.8 ±3.0
	8% CW	102.6 ±3.2
40% AMPS-Na^+ (0.1% MBA)	0% CW	120.8 ±1.0
	3% CW	100.4 ±7.4
	5% CW	99.6 ±5.7
	7% CW	85.8 ±3.7

Table 4.7 The value of WVTR of Chitin whiskers-reinforced AMPS-Na^+ hydrogels from Gamma radiation

Sample		WVTR ($g/h.m^2$)
30% AMPS-Na^+ (0.5% MBA)	0% CW	121.2 ±0.6
	3% CW	109.8 ±1.4
	5% CW	105 ±0.9
	8% CW	97.5 ±0.7
40% AMPS-Na^+ (0.1% MBA)	0% CW	112.8 ±0.8
	3% CW	95.3 ±1.4
	5% CW	90.3 ±1.7
	7% CW	88 ±1.5

It can be seen that the WVTR values of neat AMPS-Na^+ hydrogel are around 69 to 141 $g/h.m^2$, whereas the chitin whiskers-reinforced hydrogels show a lower WVTR value with an increase in chitin whisker content. This results sugget that the addition of chitin whiskers obstructs the permeability of water through the hydrogels. Though there is not an exact ideal value of WVTR for wound dressing, the value must not be so high because it will cause a dry condition in the wound area. On the other hand, if the WVTR value is so low, then it will make the accumulation of exudates which may cause the deceleration of healing process and opens up the risk of bacterial growth.

The WVTR values of AMPS-Na^+ hydrogels seem to be lower than evaporative water loss of second and third degree of burn skin (Table 4.8) which have value of 178.1 ± 5.5 $g/h.m^2$ and 143.2 ± 4.5 $g/h.m^2$, respectively. (Nilsson, G.E.,1997) So, these can be concluded that hydrogels from AMPS-Na^+ can control the loss of body fluid and can keep moist environment to wound.

Table 4.8 Evaporative water loss of normal skin and each degree of burn skin (Nilsson, G.E., 1997)

Skin types	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

4.12 Moisture Retention Capability and Equilibrium Degree of Swelling

Being an important factor of wound dressing, Moisture retention capability and Equilibrium degree of swelling (EDS) in stimulated body fluid (SBF) of hydrogels were investigated and shown in Table 4.9, 4.10, 4.11, 4.12. Moisture retention capability of all hydrogels tends to be linear with the slope (water losing rate) of about -3 to -5 $\times 10^{-4}$ g/min, and the ratio of water holding in the hydrogel slice (Rh) in 3 h was about 77% . AMPS-Na^+ hydrogels with lower EDS values are observed in higher percentage of crosslinker than the lower one due to higher crosslink density achievable under the experimental conditions.

Table 4.9 EDS and moisture retention capability of AMPS-Na^+ hydrogels by UV radiation

	30% AMPS-Na^+			40% AMPS-Na^+			50% AMPS-Na^+		
% MBA	0.1	0.5	1	0.1	0.5	1	0.1	0.5	1
EDS in SBF	986	801	632	865	639	482	820	567	431
Water losing rate (10^{-4} g/min)	-4.7	-3.7	-3.7	-4.4	-4.4	-4.1	-3.4	-3.2	-3.0
$R_{h,3h}$ (%)	73.98	71.91	71.62	78.19	78.00	78.91	82.57	82.61	81.66

Table 4.10 EDS and moisture retention capability of AMPS-Na^+ hydrogels by UV radiation

	30% AMPS-Na^+			40% AMPS-Na^+			50% AMPS-Na^+		
% MBA	0.1	0.5	1	0.1	0.5	1	0.1	0.5	1
EDS in SBF	1002	740	558	818	729	451	805	613	466
Water losing rate (10^{-4} g/min)	-5.3	-4.6	-4.1	-4.5	-3.7	-3.2	-4.3	-3.8	-3.1
$R_{h,3h}$ (%)	76.23	75.71	70.25	78.74	75.63	75.60	82.01	81.01	80.04

Table 4.11 EDS and moisture retention capability of chitin whiskers reinforced AMPS-Na^+ hydrogels by UV Radiation

	30% AMPS-Na^+ + 0.5 MBA				40% AMPS-Na^+ + 0.1 MBA			
% chitin whiskers	0	3	5	8	0	3	5	8
EDS in SBF	801	906	891	650	865	1026	980	954
Water losing rate (10^{-4} g/min)	-3.7	-3.7	-3.5	-3.4	-4.4	-3.7	-3.4	-3.4
$R_{h,3h}$ (%)	71.91	71.88	71.41	71.19	78.19	78.97	78.62	78.08

Table 4.12 EDS and moisture retention capability of chitin whiskers reinforced AMPS-Na^+ hydrogels by Gamma Radiation

	30% AMPS-Na^+ + 0.5 MBA				40% AMPS-Na^+ + 0.1 MBA			
% chitin whiskers	0	3	5	8	0	3	5	8
EDS in SBF	740	663	639	540	818	872	818	772
Water losing rate (10^{-4} g/min)	-4.6	-4.5	-4.1	-4.6	-4.5	-4.4	-4.8	-3.6
$R_{h,3h}$ (%)	75.71	75.8	75.8	74.9	78.74	78.4	78.6	78.6

4.13 Release of Chitin Oligomers from Hydrogels

Chitin is known to be a biopolymer that can be degraded in a suitable enzyme. Lysozyme from hen egg white could be used to degrade chitin to oligochitin and later to N-acetylglucosamine (CHO, Y.W. *et al.*, 1999). To evaluate the release of chitin whiskers from chitin whiskers-reinforced hydrogels, the hydrogel samples were incubated in PBS buffer and PBS buffer containing lysozyme at 37°C for 5 days and compare the weight after dry to a constant weight. Figure 4.20, 4.21, 4.22,and 4.23 shows the percentage of weight loss of hydrogel after enzymatic hydrolysis. The obtained results show that most of chitin whisker reinforced hydrogels could release chitin whisker by enzymatic hydrolysis within 5 days. The weight reduction of the gels probably due to the loss of chitin whiskers in hydrogels and weight loss of hydrogel increase with chitin-whisker content increased.

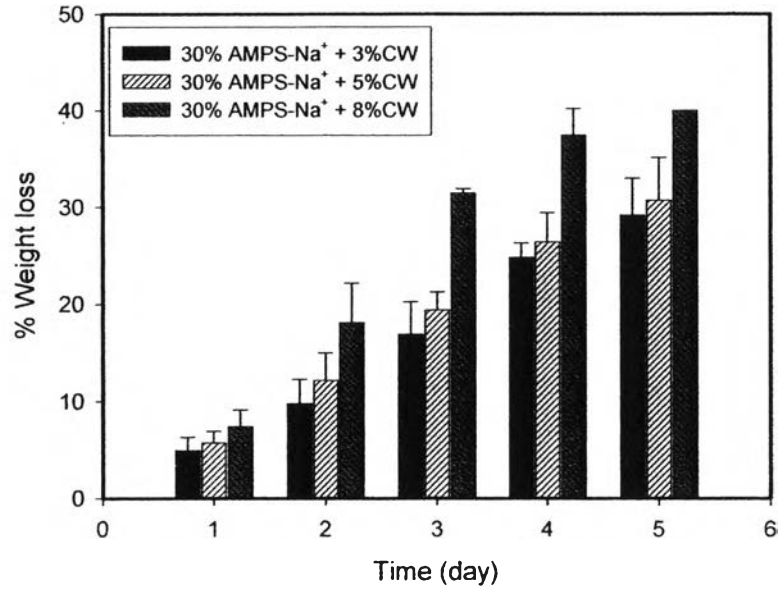


Figure 4.20 Percentage of weight loss after enzymatic hydrolysis in PBS buffer solution containing lysozyme of 30% AMPS-Na^+ hydrogels with 0.5% of MBA crosslinker containing 3,5,and 8% w/w chitin whiskers produced by UV radiation.

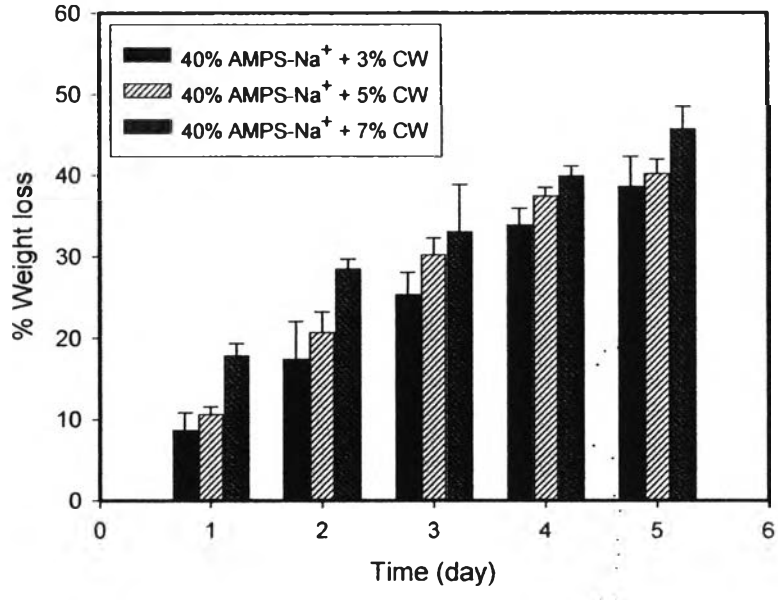


Figure 4.21 Percentage of weight loss after enzymatic hydrolysis in PBS buffer solution containing lysozyme of 40% AMPS-Na^+ hydrogels with 0.1% of MBA crosslinker containing 3,5,and 7% w/w chitin whiskers produced by UV radiation.

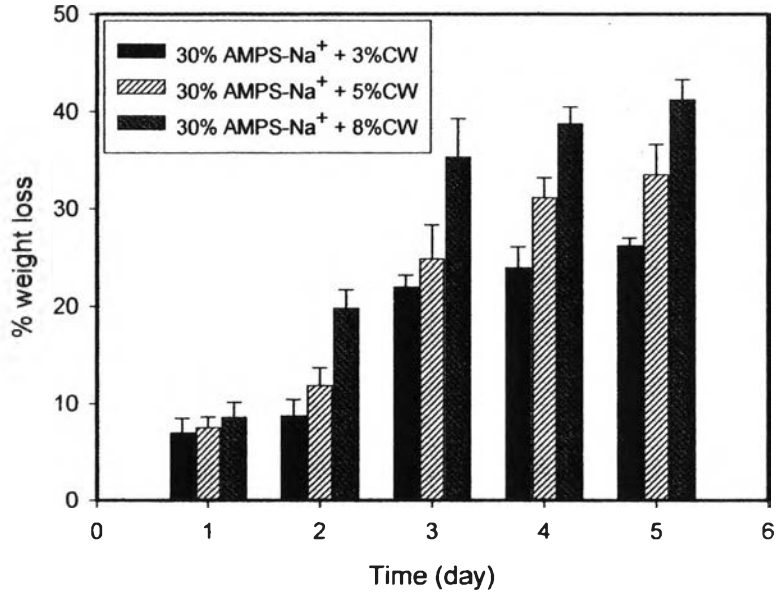


Figure 4.22 Percentage of weight loss after enzymatic hydrolysis in PBS buffer solution containing lysozyme of 30% AMPS-Na^+ hydrogels with 0.5% of MBA crosslinker containing 3,5,and 8% w/w chitin whiskers produced by Gamma.

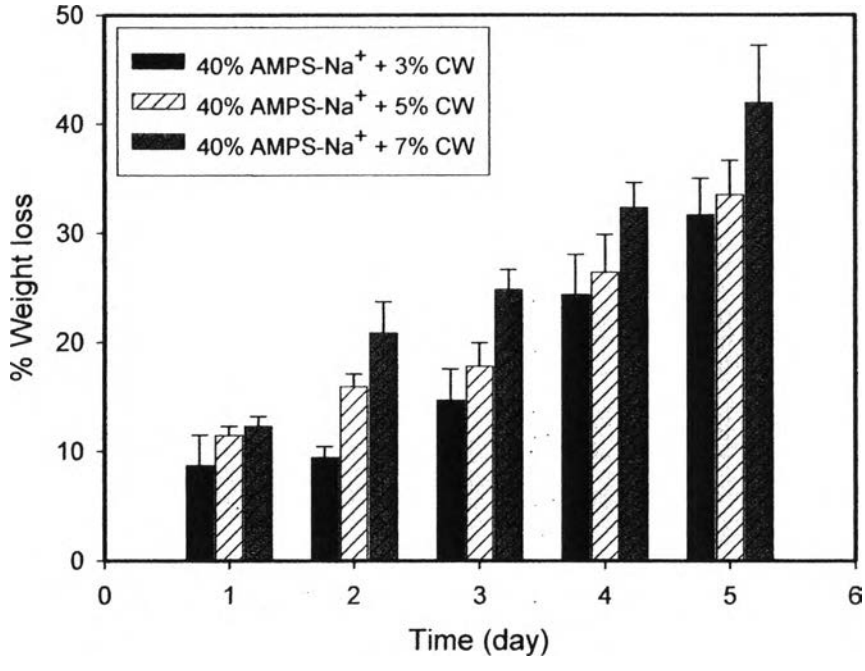
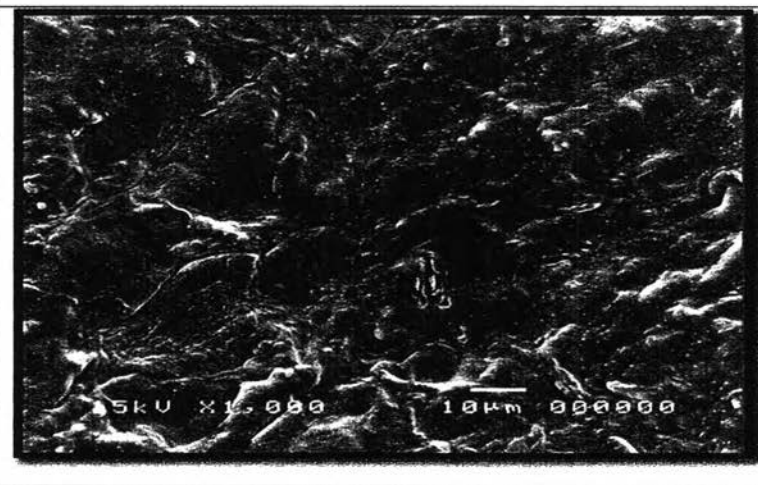
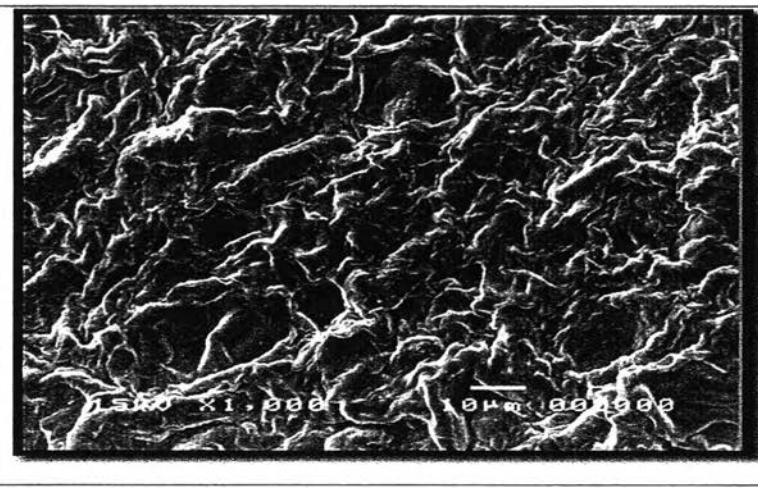


Figure 4.23 Percentage of weight loss after enzymatic hydrolysis in PBS buffer solution containing lysozyme of 40% AMPS-Na^+ hydrogels with 0.1% of MBA crosslinker containing 3,5,and 7% w/w chitin whiskers produced by Gamma.

To confirm the enzymatic hydrolysis, the morphology of hydrogels was analyzed by SEM. The results show that the surface morphology of hydrogels was smoother and the evidence of the aggregates of the whiskers was disappeared from the surface after 5 days of enzymatic hydrolysis as shown in Table 4.13.

Table 4.13 Selected SEM Images of 30% AMPS-Na^+ hydrogel with 0.5 % MBA Crosslinker Containing 8% w/w Chitin Whiskers After Submersion in PBS Buffer Solution Containing Lysozyme at 1,3,and 5 Days

Day 1	
Day 3	
Day 5	

4.14 Indirect Cytotoxicity Test (MTT assay)

Toxicity test is an important aspect of biomaterials. MTT assay was used to determine the viability of living L929 fibroblasts for detecting toxic products or adverse reactions, which could be evaluated through in vitro cytotoxic tests. MTT reagent is a yellow tetrazolium salt that produces a dark-blue formazan crystal when incubated with viable cells. Therefore, the level of the reduction of MTT into formazan can reflect the level of cell metabolism. Fig. 4.24 and 4.25 showed percent viability of cells obtained from MTT assay for L929 cells.

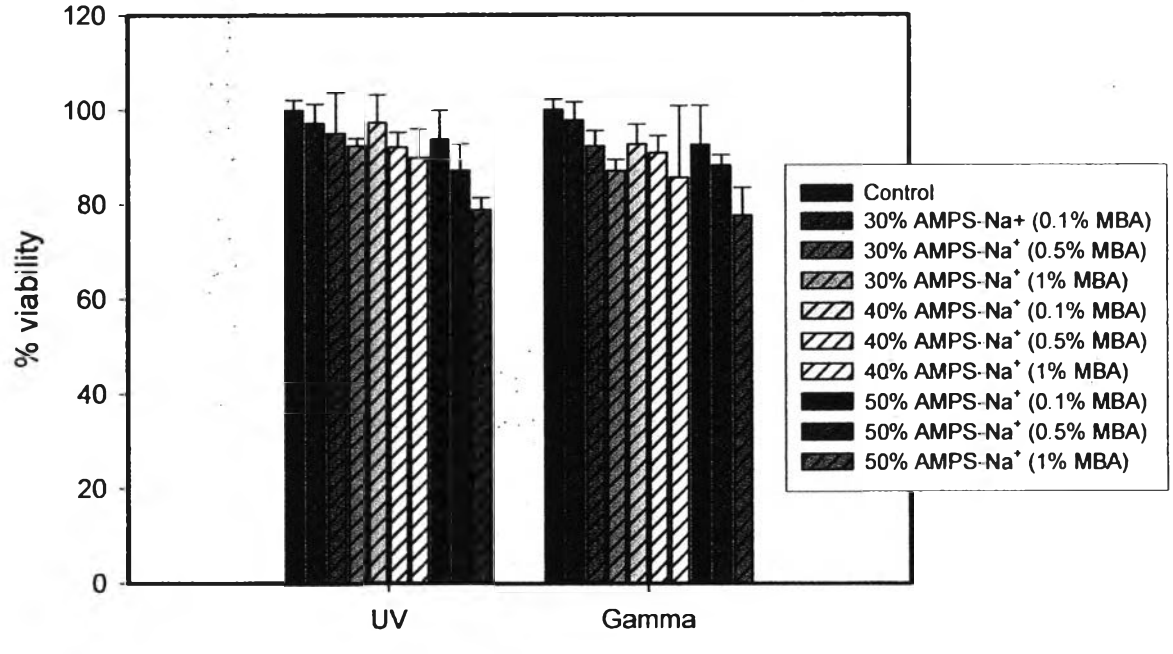


Figure 4.24 The percent viability of L929 fibroblast for control and extract solutions from AMPS-Na^+ hydrogels that produced by UV and Gamma radiation.

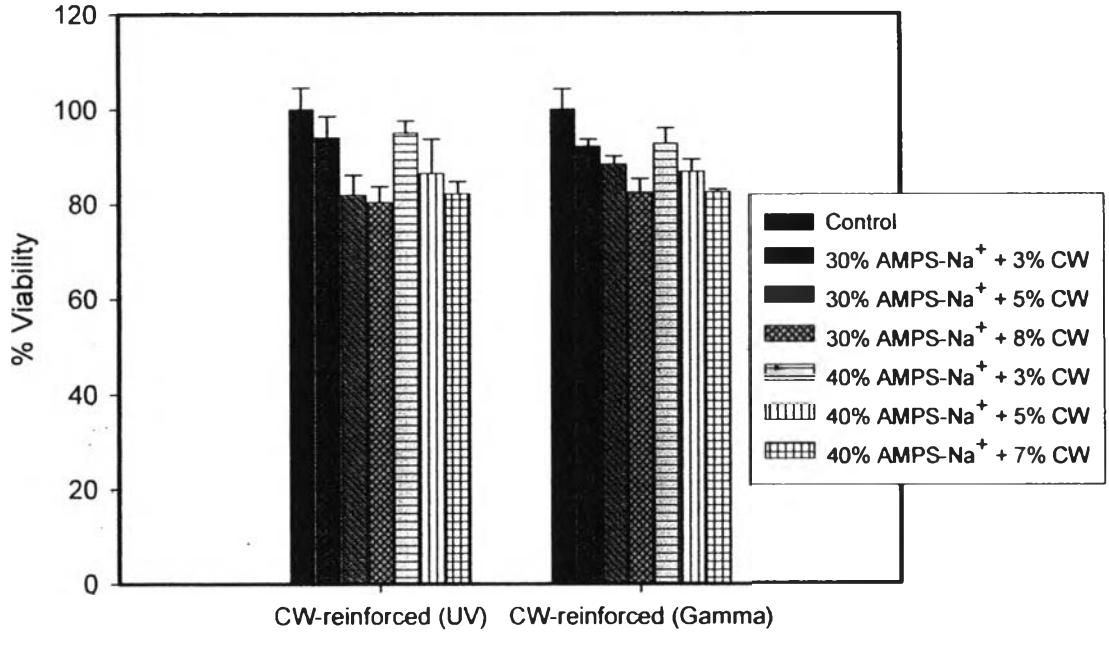


Figure 4.25 The percent viability of L929 fibroblast for control and extract solutions from Chitin Whisker-reinforced AMPS-Na^+ hydrogels that produced by UV and Gamma radiation.

All of the obtained results show that the viability of L929 fibroblasts cultured in all hydrogel extracted solution were lower than control but greater than 75% of viability. This was not significantly different from that of the control and its indicated that hydrogel samples were non-toxic to L929 fibroblast and AMPS-Na^+ hydrogels and chitin whisker reinforced hydrogel in all condition were a good candidate to be used as wound dressing.

4.15 Treatment of Open Wounds on Dog Using AMPS-Na^+ Hydrogel Dressing; Case Report*

* Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn Universiry

A seven-year-old female Schnauzer dog has gangrenous skin lesions on its left-side of body caused by moist gangrene with the size of 20×40 cm^2.

On day 0 surgical debridement was performed on gangrenous skin lesions followed by wound irrigation with 0.9% normal saline and covered with wet-to dry bandage and changed the dressing everyday.

On week 1, granulation tissues occurred on the top-half of wound, the bottom-half still had gangrenous skin lesions and the wound has been treated with the same procedure but begin to used IntraSite Gel™ (Smith & Nephew) with non-adherent dressing.

On week 2, all gangrenous skin lesions were disappeared. The wound has been treated with the same procedure but this time begin to used AMPS-Na^+ hydrogels on the top-half of wound and IntraSite Gel™ (Smith & Nephew) on the bottom–half of wound with non-adherent dressing and changed the dressing every other day.

On week 3, 4 and 5 Size wound reduced to 65%, 50%, 45% and 30%, respectively (about 7×17 cm^2.)

On week 6, no exudates from wound and wound size reduced continuously and fully healed on week 8.

This case report from Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn Universiry indicates an alternative use of AMPS-Na^+ hydrogels as a dressing to promote wound healing. The results show that AMPS-Na^+ hydrogels found to be comparable to the IntraSite Gel™ (Smith & Nephew) commercial dressings.

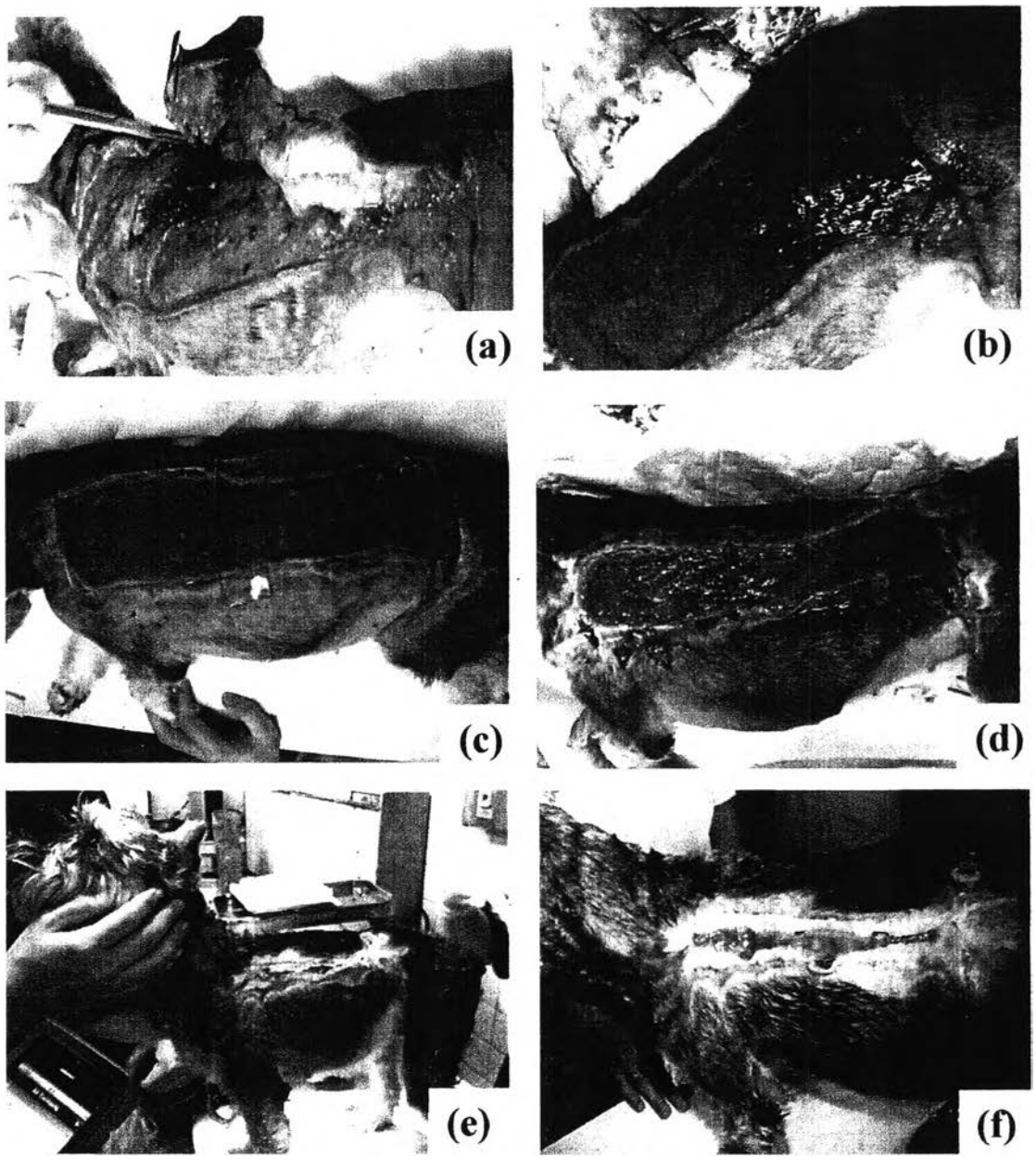


Figure 4.26 Wounds on skin. a: On day 0 before treatment with dressing; b, c, d, e and f: wound characteristics in weeks 1 through 8 of treatment, respectively.