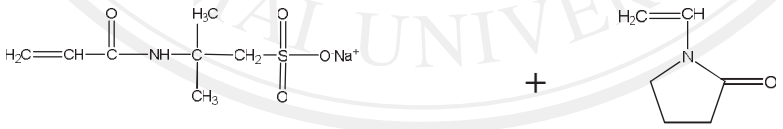
CHAPTER 4
CONCLUSIONS

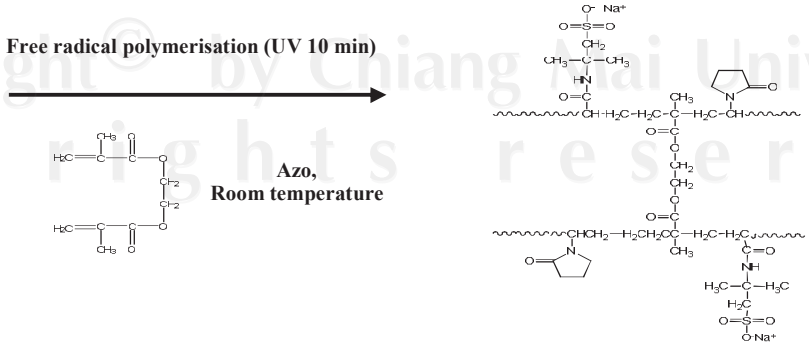
In this final Chapter 4, the following conclusions can be drawn from the experimental results obtained and presented in the previous Chapter 3. The main point of this research has been achieved in this initial study of synthetic hydrogels as wound dressings. This work investigated the development of hydrated hydrogels. This focus was placed upon the synthesis of three dimensional polymer networks possessing good water absorption and mechanical properties. Understanding the basic principles of photopolymerization and mechanical properties testing has enabled modifications to be implemented for specific biomedical applications.

4.1. Hydrogel Synthesis

The primary objective of this thesis is to synthesize hydrogels based on monomer AMPS-Na^+, NVP and MAA, by UV-photopolymerization. Both EGDM and NMBA, were used as crosslinking agents to improve mechanical strength and also as a convenient means of controlling water absorption properties via crosslink density of the network.



AMPS-Na^+ NVP



Scheme 1. Synthesis of the crosslinked poly(AMPS-Na^+-co-NVP).

The three dimensional network structure of crosslinked represents above showing how the functional of crosslink units serve to join the monomer chains together at points throughout the matrix. The structural similarity between crosslinking and the monomer ensures that the crosslinking does not seriously affect the chemical nature of the polymer.

4.2. Photopolymerization

Partially hydrated hydrogels composed of an unsaturated ionic monomer AMPS-Na^+, NVP or MAA, were converted into a three dimensional polymer matrix by photopolymerization. This technique using UV light exposed to monomer solution and converted to hydrogel. It was found to be the most efficient and the most convenient due to the shorter time spent to polymerize hydrogel sheets to complete conversion within 10 mins at ambient temperature.

4.2.1. Effects of Crosslinking

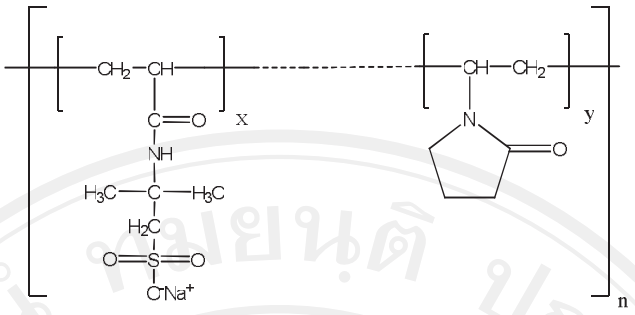
In this study, the effects of crosslinker, EGDM and NMBA, in the water uptake and equilibrium water content, EWC, of copolymer were studied. It is indicated that the EWC of all hydrogels was reached after immersion in distilled water at 35.0±1.0 °C within 25 mins in which the synthesized hydrogels with more crosslinker concentrations approach equilibrium level quicker than hydrogels with less amount of crosslinker. This is as would be expected since the crosslinks give the effect of tying the polymer chains together in a three dimentional network, thereby stiffening the matrix and decreasing its capacity to expand or swell. This, in turn, resulting in a denser and more compact structure which porosity decreases towards the water uptake molecules. Thus, the crosslink density, which simply depends on the amount of crosslinking agent added, provides an easy and convenient means of controlling the EWC of the hydrogel polymer.

In addition to its effect on water absorption properties, crosslinking also has an important effect on mechanical properties. The incorporation of EGDM and NMBA crosslinks does indeed increases the mechanical properties. In this work, the hydrogel

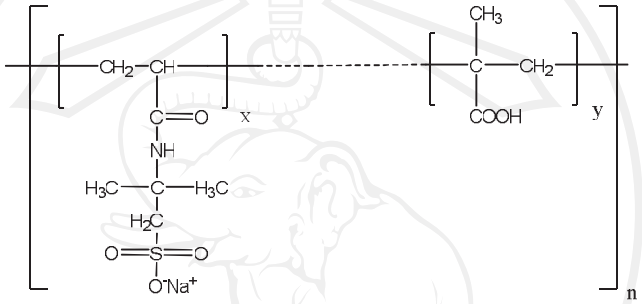
sheets did not examined for their the tensile strength, but their mechanical strength and coherency were judged manually by simply stretching and pressing the sheets. The amount of crosslinking agent employed has a profound influence on water absorption properties.

4.2.2. Effects of Copolymerization

The water binding behavior of hydrogels can be varied to a certain extent by copolymerization. In this work, NVP and MAA, were chosen as the modifying comonomers. The incorporation of NVP and MAA units into the poly(AMPS-Na^+) chain lead to an increase in hydrophilicity and, therefore, increasing water absorption. The effects of copolymer composition on the water uptake and EWC of crosslinked poly(AMPS-Na^+-co-NVP) and poly(AMPS-Na^+-co-MAA) hydrogels were studied. The EWC of crosslinked poly(AMPS-Na^+) was approximately 90-95% while the EWCs of the crosslinked poly(AMPS-Na^+-co-NVP) and poly(AMPS-Na^+-co-MAA) copolymer were a little bit higher in the range of 95-98% since the NVP and MAA content in the comonomer feed increased. It was also observed that the copolymers became more pliable. This latter effect of NVP is probably due to the steric effect of the bulky NVP groups decreasing the intermolecular interaction between the polymer chains. In case of MAA, the hydrophilic segments of the poly(AMPS-Na^+-co-MAA) hydrogels such as carboxylic acid groups could be able to form hydrogen bonds with water molecules. These bonds act cooperatively to form a stable environment of hydration around the hydrophilic segments, resulting in a greater water uptake and producing a larger swelling ratio. Also, of course, an increase in EWC effectively means an increase in the amount of free water between the chains. Since this free water acts like a plasticizer, it is reasonable to expect that a higher EWC will result in a more pliable material.



(a)



(b)

Scheme 2. The chemical structure of copolymers (a) poly(AMPS-Na^+-co-NVP) and (b) poly(AMPS-Na^+-co-MAA).

The EWC of a poly(AMPS-Na^+-co-NVP) and poly(AMPS-Na^+-co-MAA) copolymer at a given composition can be considered to depend upon the balance of contributing steric and polar effects. The polar effect arises predominantly from the amide group in the AMPS units and, to a lesser extent, from the carbonyl groups in AMPS-Na^+, NVP and MAA whereas the steric effect arises from the combined contributions of the bulky pyrrolidone group in NVP together with the methyl group (alkyl side chain) in AMPS-Na^+. Thus, in the poly(AMPS-Na^+-co-NVP) and poly(AMPS-Na^+-co-MAA) copolymers, in which AMPS-Na^+ is by far the major component, are polar effects predominate. However, as the proportion of NVP units increases, the contribution from steric effects increases which leads to an increase in EWC.

4.3. Balance of Hydrophilicity and Mechanical Properties

In designing a polymer for use as wound dressings, many property requirements need to be taken into consideration. They need to be biocompatible and non-toxic, perhaps the most important properties which determine the performance characteristics are the hydrophilicity and mechanical properties. Expressed more specifically, the polymer must be able to absorb water, diffusion and allow water vapor to be transmitted, and at the same time be strong and flexible.

Increasing comonomer NVP and MAA contents in the copolymers gave more effect on the EWC than mechanical properties. Since the EWC increases consistently with NVP and MAA contents, the mechanical properties show no definite trend. It is indicated that at the proportion of 25:75 poly(AMPS-Na^+-co-NVP) and poly(AMPS-Na^+-co-MAA) exhibited the highest water content as compared with other compositions. This may be due to the hydrophilic of pendant group in their structure which content of NVP and MAA comonomers produced the high water content. However, the hydrogel sheets at the proportions of 50:50 poly(AMPS-Na^+-co-NVP) and 25:75 poly(AMPS-Na^+-co-MAA) were very flexible and exhibits coherent sheet which gave the optimum synthesis condition to achieve the physical properties.

4.4. Water Absorption in Deionized Water, Saline and SBF solutions

Poly(AMPS-Na^+) superabsorbents are ionized hydrogels; their swelling capacity depends on the properties of the physical and chemical structure and the medium to be absorbed such as saline and SBF solutions.

In this research, the water absorption of poly(AMPS-Na^+) in deionized water, saline and SBF solutions were 99.15, 94.01 and 92.30%, respectively. It is clearly shown that the water absorption in deionized water was the highest while those in saline and SBF were not statistically significant. This well-known phenomenon, commonly observed in the swelling of ionic hydrogels, often results from a “charge screening effect” of the additional cations causing a non-perfect anion-anion electrostatic repulsion. This leads to a decreased osmotic pressure (ionic pressure) difference between the polymer network and the external solution.

4.5. Effect of Cationic Charges

Crosslinking polymerization of AMPS-Na^+ monomer using EGDM as crosslinker was performed in an aqueous medium. Variations in the reaction parameters affecting the ultimate swelling capacity of the final product optimized the synthesis of superabsorbent hydrogel.

The swelling capacity decreased when hydrogels were immersed in the solution contains higher charge of the cation (multivalent < univalent). This swelling behavior may be explained by the complexing ability arising from the coordination of the multivalent cations with the carboxylate and sulfonate groups on the superabsorbent polymer chains.

4.6. Water Vapor Transmission Rate (WVTR)

Hydrogels which are used as wound dressing materials would avoid or at least reduce the body liquid loss through controlling absorption and transmission as well as keeping the optimum humidity in the wound area in order to accelerate the healing process. WVTR is a distinct factor that shows the potential of hydrogel in transmission of body liquid or wound exudates.

The WVTR of hydrogels in the present investigation showed a range of 56-147 g/hr.m^2 which seem to be intermediate level between the WVTR for first and second degree burns. It was found to be optimal for maintaining a moist environment conducive for wound healing. The highest value of WVTR of hydrogel from AMPS-Na^+ 30% w/v is 146 g/hr.m^2 which gave the WVTR higher than copolymer of poly(AMPS-Na^+-co-NVP) and poly(AMPS-Na^+-co-MAA) for all compositions. The WVTR decreases with increasing monomer and crosslinker concentrations, leading to more crosslinked density and rigid structure with less free volume inside the crosslinked network. On the other hand, the WVTR increases with increasing NVP and MAA monomer content in the poly(AMPS-Na^+-co-NVP) and poly(AMPS-Na^+-co-MAA) because NVP and MAA comprises of the –NH and COO^- groups respectively which increase hydrophilicity.

Clearly, the hydrogel samples prepared in this work were able to control water vapor transmission within reasonable limits. Since this ability is vital function of a wound covering, the samples would appear to have sufficient potential in this area of biomedical application to warrant future study.

4.7. Diffusion Kinetics

In the presence of higher crosslinking agent concentrations (NMBA), more crosslinked network could be constructed to give rigid chains and polymer network that reduce the swelling of the gel. The water absorbency of the crosslinked copolymer was followed by extent of swelling with time in distilled water. In this study, the superabsorbent polymers of poly(AMPS-Na^+) exhibited the Fickian type diffusion, n, in the range of 0.15-0.44, leading to the rate of water diffusion much slower than the expansion of the network, with diffusion coefficients in the range of 0.03-3.29 x 10^{-9} $cm^2.s^{-1}$ which indicates the characteristic of the macromolecular network and penetrant system.

4.8. Peel Strength

The peel strength is the force required to remove the adhesive at a peel angle of 90^o, for the anionic adhesive hydrogel sheets. It was observed that the proportion of 75:25 poly(AMPS-Na^+-co-NVP) showed the highest increase in peel strength, while keeping the concentration of AMPS-Na^+ monomer constant at 40% w/v. As increasing the concentration of NVP from 50% wt to 75% wt results in lower peel strength. The hydrogel sheets containing of NVP 75% wt exhibited the lowest peel strength because of the strong charge of sulfonate and amide group of AMPS-Na^+ were decreased leading to a decrease in intermolecular interaction.

In addition, the hydrogel sheet of 75:25 poly(AMPS-Na^+-co-MAA) showed the lowest peel strength, while the 25:75 poly(AMPS-Na^+-co-MAA) showed the highest peel strength. Addition of MAA content to polymer compositions increased the strength of the adhesive bond. These results indicate that the MAA improved cohesive properties of hydrogel sheet as copolymerized with AMPS-Na^+. This is

possible since MAA increases water binding and removes the interfacial layer of moisture.

4.9. Effect of pH

Effect of pH of solution medium on the swelling behavior of hydrogels were investigated. The experimental results have demonstrated that the gels did not swell greatly at low pH environments whereas they can swell sufficiently at higher pH solutions, exhibiting smart pH sensitivity. The swelling of poly(AMPS-Na^+-co-MAA) hydrogels was also influenced by ionic strength and copolymer compositional ratio variables.

As pH increased from 5.8 to 7.4, the swelling ratio values of all samples increased. The highest swelling ratio value at pH 7.4 was 42 g H_2O/g polymer received from 25:75 of poly(AMPS-Na^+-co-MAA). This is because the carboxylic groups became ionized, meanwhile, electrostatic repulsion caused the network to expand. The content of MAA in the hydrogel network and the pH value of the medium solution had great effect on the swelling ratio of the hydrogel.

However, swelling of hydrogels depended not only on the pH media, ionization ability and the matrix swelling effects, but the copolymer composition also played an important role on swelling ratio. The pH response, as expected, can be controlled or modulated by varying or adjusting pH values, ionic strength as well as the proportion of hydrophilic domains.

4.10. Oxygen Permeability

The permeability of "dissolved" oxygen through hydrogel sheet is a roughly exponential function of equilibrium water content over a wide range of water contents. It also depends on the intrinsic nature of the chemical constituents.

Under normal circumstances, purified water has a dissolved oxygen value in the range of 357 to 694 cc.cm/cm^2.s.cmHg at 25^oC by using the thickness of hydrogel sheets ranging of 1.49 to 1.94 mm. The hydrogel sheet with copolymer composition 75:25 of poly(AMPS-Na^+-co-MAA) showed the highest oxygen permeability. It

should be note that the highest of oxygen permeability value was 694 cc.cm/cm^2.s.cmHg. The same progressive decrease in equilibrium water content was shown with more complex of comonomers.

Thus, 75:25 of poly(AMPS-Na$^+$-co-MAA) is more flexible, slightly more hydrophilic and in addition has a somewhat higher oxygen permeability than the others compositions.

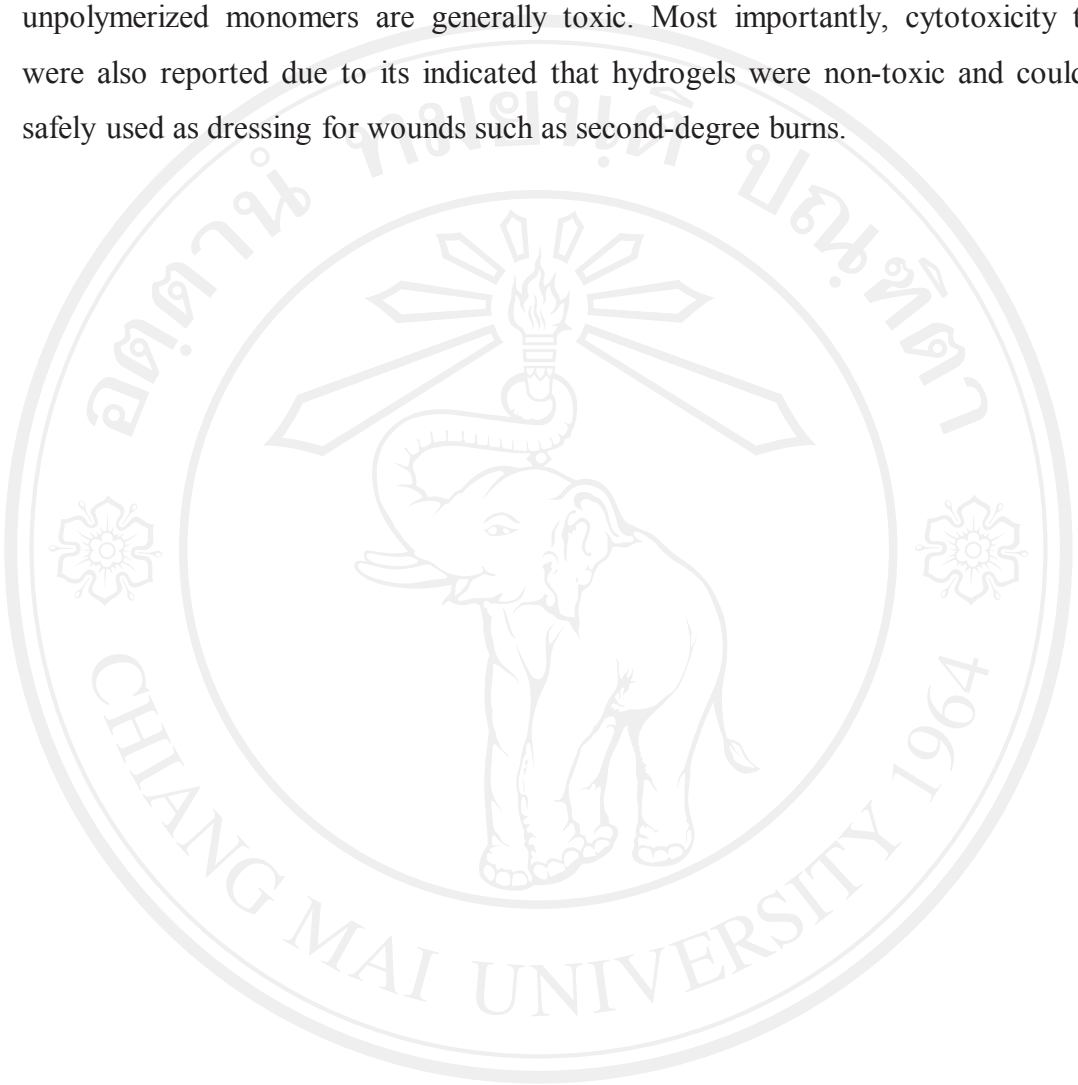
4.11. Monomer Residual and Cytotoxicity

Ion chromatography was used to detect the residual of AMPS-Na$^+$ anionic monomer in polymerized hydrogels. The AMPS-Na$^+$ monomer concentration was expressed as a percent area by ion chromatogram. It was found that the percent area of AMPS-Na$^+$ showed 12.17% and approximately 0.15% w/v of AMPS-Na$^+$ monomer concentration was found.

Finally, to examine the possibility of using these synthesized hydrogel as wound dressing application, determination of cell viability is an assay to evaluate the *in vitro* cytotoxicity of hydrogel sheets. The predictive value of *in vitro* cytotoxicity test is based on the concept that toxic chemicals affect the basic functions of cells. The cell line L929 is constituted by a highly proliferative population allowing rapid screening assays, mainly regarding acute toxicity testing towards cellular vitality and proliferation established from normal tissues. The results of L929 cell possessed normal morphology after 48 hrs incubation and were well stained with neutral red, indicating that synthesized hydrogels were non-toxic.

In overall conclusions, the hydrogels which have been synthesized and characterized in this research work, in accordance with its objectives, have been shown to possess hydration properties suitable for use as wound dressings. Furthermore, there is plenty of scope in their design for these properties to be controlled through variables, such as monomer and crosslink concentrations, copolymer composition, water swelling properties, effects of pH, diffusion kinetics, oxygen permeability and the peel strength of their mechanical properties. In addition,

to detect and quantify monomers would provide a method for determining the amount of residual monomer by ion chromatography method. This is desired as residual unpolymerized monomers are generally toxic. Most importantly, cytotoxicity tests were also reported due to its indicated that hydrogels were non-toxic and could be safely used as dressing for wounds such as second-degree burns.



Suggestions for the Futher Work

In continuation of the research initiated in this project, the following suggestions for further work are now made:

1. In this work, hydrogel sheets of uniform thickness 1.0±0.1 mm were studied. Since sample thickness can be expected to have an effect on both water absorption / vapour transmission and mechanical properties (e.g., flexibility, skin adhesive), thus sheets of different thickness should be prepared and examined for their absorption and mechanical properties.

2. It would be useful at this initial stage of the research to compare the properties of samples prepared with those of commercial materials, in hydrogel-type wound dressings. This would indicate the level of performance that needs to be achieved.

3. It has been suggested that a small amount of a volatile solvent added to the polymerization mixture can increase the matrix porosity of the hydrogel as it forms which, in turn, increases its capacity for subsequent water absorption. This is an interesting possibility which is worth investigating.

4. Methacrylic acid is thought to improve the cohesiveness of skin adhesives; thus the effect of adding methacrylic acid on cohesiveness of neutral hydrogels could be evaluated. Furthermore, the potential to produce cohesive neutral hydrogels from novel neutral macromers would be another interesting area of study. In addition, the lack of cohesiveness may have been due to the inefficacy of the polymerization process; hence varying the crosslinker-photoinitiator ratios as well as amounts and also the use of other crosslinkers and photoinitiators could be investigated to optimize the polymerization process. This could also be extended to exploring other methods of initiation that would allow hydrogels to be used in other applications such as in-situ polymerization for use as a "smart" drug delivery agent to deliver the right amount of drug when the appropriate signal is given.