CHAPTER 7

Discussion and Conclusions

In the previous 3 chapters, the results presented were discussed in sequence. In this concluding chapter, these results will be reviewed as a whole in an attempt to bring together and, where possible, interrelate the previous conclusions to form a coherent picture highlighting the main achievements of this project.

7.1 Choice of Main Monomer – Why Na-AMPS?

AMPS acid and its sodium salt, Na-AMPS, have come to the fore in recent years as new water-soluble monomers which, when polymerised in aqueous solution, yield flexible, elastic, highly absorbent hydrogels with a range of properties suitable for use in biomedical applications. They are prime examples of what are referred to, in the current terminology, as ***molecular engineering monomers*** in the sense that their structures have been specifically "engineered" (i.e., designed) for applications such as contact lenses, polymer electrode membranes, skin adhesives and wound dressings. As shown below in Figure 7.1, each part of the molecule has a specific purpose related to either monomer polymerisability or polymer properties.

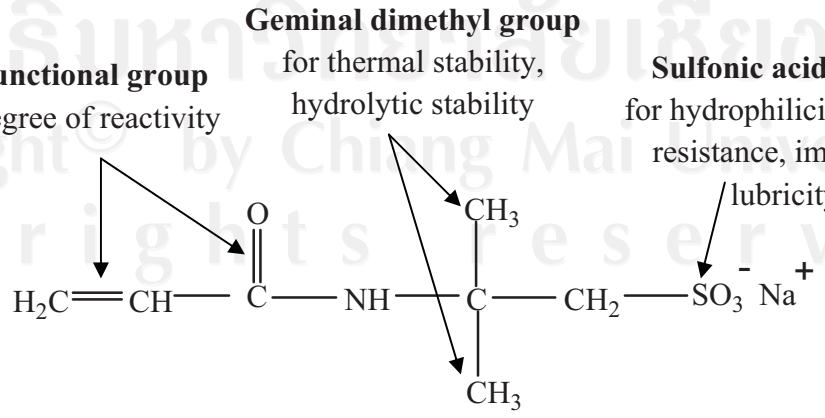


Figure 7.1 : The "molecularly engineered" chemical structure of Na-AMPS.

The fact that AMPS and Na-AMPS can be polymerised in aqueous solution is an important advantage in itself. Any residual monomer can be easily removed by water extraction plus the hydrogel network is formed in the presence of water so that the final product is a, non-collapsible, ready-hydrated hydrogel in the shape of the container. In this work, the container was a mould consisting of parallel glass plates so that the hydrogel could be obtained in the form of a thin sheet of the required thickness. Of the two monomers, Na-AMPS was preferred to AMPS acid so that the hydrogel sheet would be pH-neutral rather than acidic, bearing in mind its intended use as a wound dressing.

Another reason why Na-AMPS, was chosen was because poly(Na-AMPS) is starting to attract increasing attention as a result of suggestions that it may have ***biomimetic*** properties in wound healing. ***Biomimesis***, or the ability to mimic the function of the natural system, is currently one of the hottest topics in biomaterials research. What it implies is that, rather than just being specialist (or advanced) materials, polymers are now becoming "smart" (or "intelligent") in the sense that they are able to respond to the physiological environment that they find themselves in and, in some cases, actually play a part in the physiological process.

But how can polymers do this? In order to answer this question, it is first necessary to be able to understand the natural system. For a wound dressing, the natural system with which it is in contact is the wound itself. As mentioned earlier, one of the most important functions of a wound dressing is to help maintain a moist environment at the wound surface since this has been shown to accelerate the healing process. This moist environment is due to the wound exudate, which is mainly composed of the extracellular fluid. This, in turn, is part of the extracellular matrix, the biological structure of which is shown in Figure 7.2. It is now widely accepted that the dominant water-structuring feature of the extracellular fluid is the proteoglycan molecules. These proteoglycan molecules achieve this through their fixed charge density which osmotically maintains the hydration of natural tissue. This is vital to the function of, for example, intervertebral discs. As shown in Figure 7.3, the charged groups in question are carboxylate, COO^-, and sulfonate, SO_3^-.

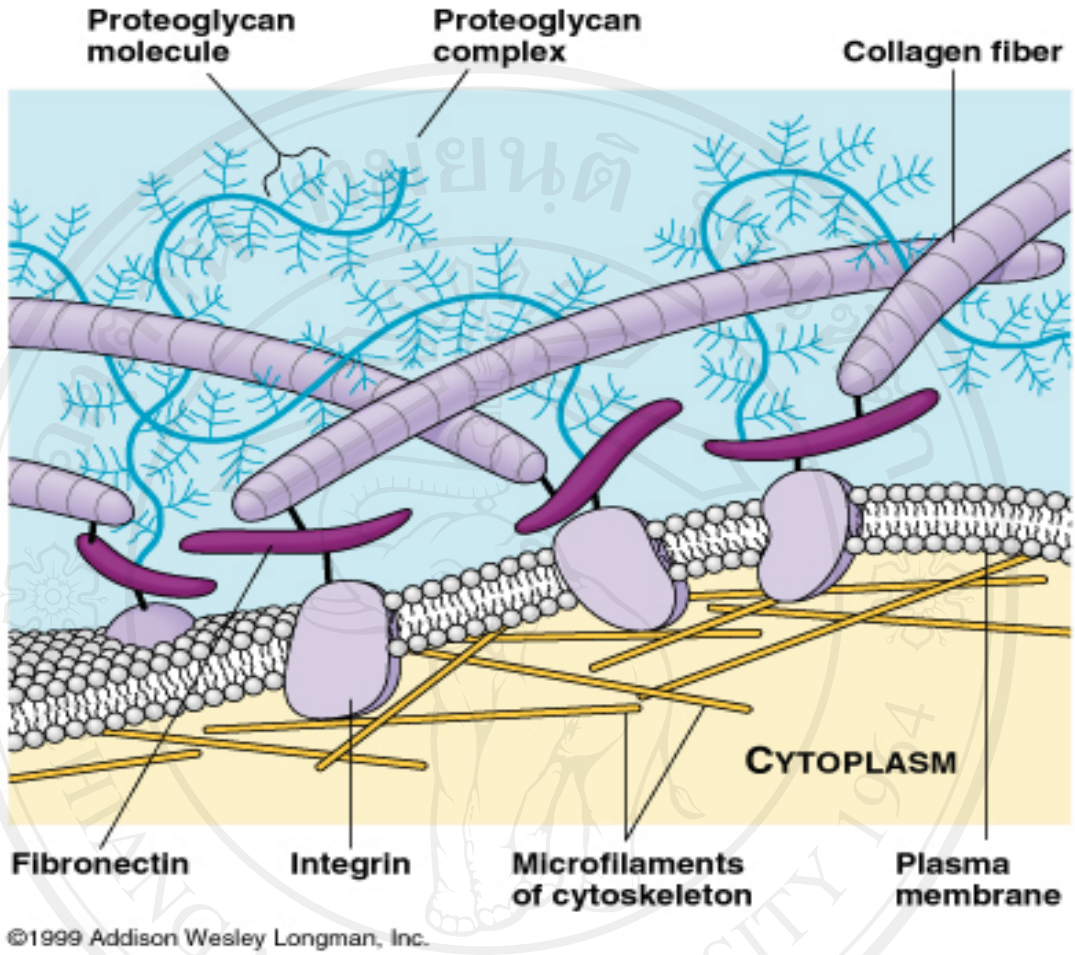


Figure 7.2 : Diagram showing the biological structure of the extracellular matrix.

Since proteoglycans play such an important role in water-structuring in the wound exudate, it is reasonable to assume that they also play a role in wound healing. This view has recently received support from the observation that wound dressings made from glycosaminoglycans enhanced the rate of epithelialisation, reduced pain and promoted bioactivity in some long-standing ulcerated wounds. Consequently, when similar observations were made with other sulfonate-containing hydrogels, including poly(Na-AMPS), it was concluded that we were perhaps seeing the first steps in the biomimetic design of wound dressings based on structural features of the extracellular matrix.

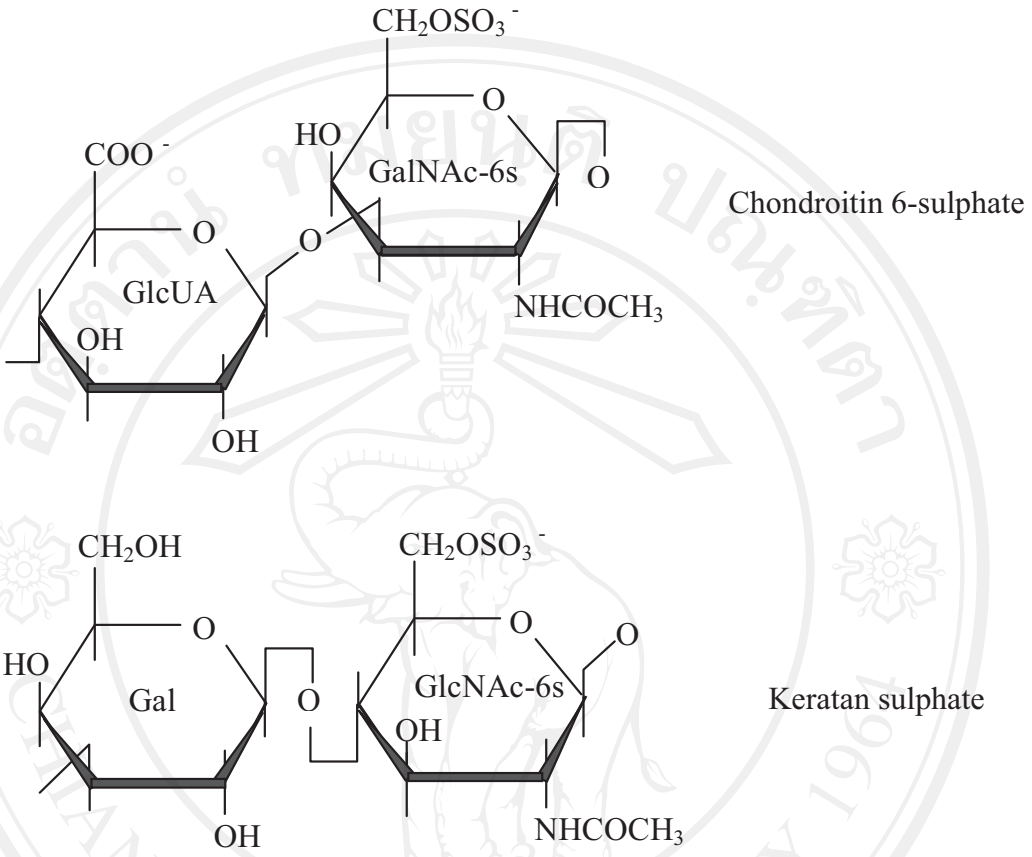


Figure 7.3 : Proteoglycan molecules which maintain the hydration of natural tissue.

Although the subject of biomimesis was always going to be beyond the scope of this project, it was nevertheless an influential factor in the choice of Na-AMPS as the main monomer of interest. The structural similarities between Na-AMPS in Figure 7.1, and hence poly(Na-AMPS), and the proteoglycan molecules in Figure 7.3 are obvious. In addition to the sulfonate group, which is the key feature, there is also the amido group. It was with this in mind that Na-AMPS was seen not only as a ***molecular engineering monomer*** but also as a ***proteoglycan-mimic monomer*** even though the scope of this work would not extend as far as being able to study this.

7.2 Hydrogel Preparation – Polymerisation Conditions

Provided that the monomer is water-soluble, it is common practice to synthesize hydrogels in aqueous solution. This has the advantage that, as the monomer polymerises, the resultant polymer automatically becomes hydrated to form an expanded hydrogel network. Any residual monomer remaining in solution within the hydrogel can be easily leached out by water extraction if necessary. This is much preferred to bulk polymerisation which, if highly exothermic, may be difficult to control and which, in the absence of water, would give a collapsed network structure which would make it more difficult to remove any entrapped residual monomer.

At the outset of this research, one of the initial aims was to design a suitable mould for thin sheets so that the polymerisation and fabrication steps could be combined into one. The glass mould described in Chapter 3 served this purpose well and hydrogel sheets of consistent thickness (1.20 ± 0.02 mm) could be produced throughout this project.

However, it was found that, in order to obtain sheets of suitable quality, both in terms of physical appearance and mechanical properties, both the initial monomer and crosslinker concentrations, [Na-AMPS] and [EGDM], needed to be balanced. Too low a monomer concentration and the sheet lacked cohesive strength due to insufficient polymer in the hydrogel network. Conversely, too high a monomer concentration and the sheet became less elastic and more brittle as the balance between the polymer-polymer interactions and solubilization/plasticization by water shifted in favour of the former. Similarly, for the EGDM crosslinker, while a certain amount of crosslinking was essential to give the sheet sufficient elasticity and tear strength, too much crosslinking made it less extensible and more brittle.

As for the method of initiation, all 3 methods used – thermal, redox and photoinitiation – gave similar results, the only noticeable difference being that photoinitiation gave colourless sheets whereas thermal and redox initiation gave sheets with a slight pale yellow/brown discoloration.

Thus, the polymerisation conditions that gave the best quality hydrogel sheets during the first part of this work were:

Monomer concentration	=	[Na-AMPS]	=	40 % w/v
Crosslinker concentration	=	[EGDM]	=	1.0 % by mol
Method of initiation	=	photoinitiation		

In addition to these results, there were also some other relevant observations regarding synthesis which are worth mentioning:

(1) Na-AMPS is a highly reactive monomer which, over a period of time, could slowly self-polymerise in aqueous solution at room temperature. Therefore, in this work, it was normally used freshly prepared. If it needed to be kept for any length of time, it was stored in a refrigerator at 0-4°C.

(2) Na-AMPS could be photopolymerised in aqueous solution even without the addition of a photoinitiator, albeit more slowly. This is a reflection of the fact that the amido moiety in Na-AMPS can activate the C=C double bond sufficiently to produce free radicals on UV-irradiation. However, the use of a purposely-added photoinitiator is always recommended since it greatly enhances the efficiency of photoinitiation (quantum yield) and, in doing so, ensures fast kinetics and a polymerisation reaction that proceeds to near-quantitative conversion.

(3) On removal from the mould, there was no apparent difference between the two sides of the hydrogel sheet in terms of tackiness which might have resulted from non-uniform polymerisation. It was therefore concluded that the combined thickness of the front glass plate and the cavity containing the monomer solution was less than the depth of penetration of the incident UV light.

7.3 Preferred Method of Initiation – Why Photoinitiation?

Initially, ***thermal*** and ***redox*** initiation were used for polymerisation. Of these two methods, redox initiation was preferred since it could be carried out more conveniently at room temperature. Consequently, the earliest results from this work, some of which were reported in a paper published in the ***Chiang Mai Journal of Science*** (see reference below), were for hydrogel sheets prepared by redox initiation. A copy of this paper is included in the Appendix of this thesis.

Design and Preparation of AMPS-Based Hydrogels for Biomedical Use as Wound Dressings

K. Nalampang, N. Suebsanit, C. Witthayaprapakorn and R. Molloy

Chiang Mai J. Sci., 34(2), 183-189 (2007)

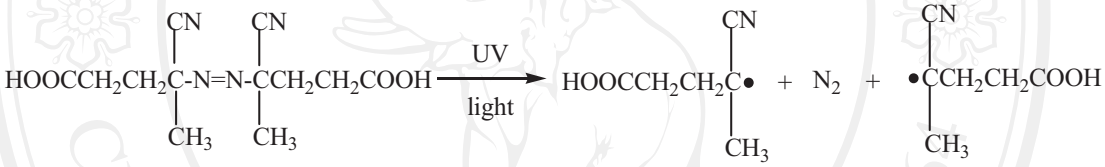
However, through an international collaboration with Professor Brian Tighe's Biomaterials Research Unit, Aston University, Birmingham, UK, a commercially available domestic ultraviolet (UV) lamp was acquired. This type of UV lamp had already been used by the Aston group and found to be suitable for photoinitiation of monomers in aqueous solution.

It was soon confirmed in this work too that UV-induced photoinitiation was not only more controllable than redox initiation, in that it could literally be switched "On" and "Off", but also gave better quality hydrogel sheets in terms of physical appearance. Photopolymerisation was also much faster and would be more easily convertible into a continuous process for industrial production.

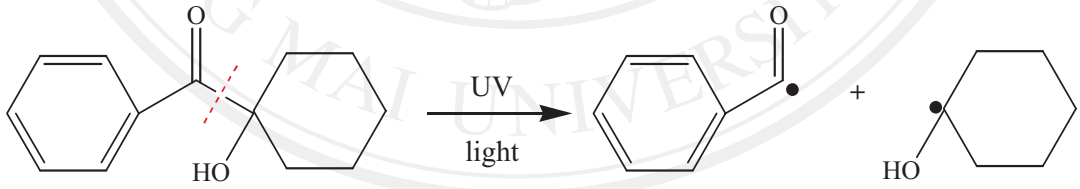
The main practical limitation of photoinitiation, as described previously in Chapter 5 (pages 62-63), is that, because the UV light source is invariably placed outside the reaction vessel, the polymerisation tends to become "frontal" as the light intensity becomes attenuated in passing through the vessel. However, this effect decreases as the thickness of the vessel decreases until it becomes negligible, as appeared to be the case for the sheet mould used in this work. Thus, photoinitiation

lends itself ideally to hydrogel sheet production and, as the ion chromatography results have shown, gives near-quantitative conversion with only trace amounts of residual monomer.

When comparing the two photoinitiators used in this research, both ACPA and Irgacure 184 were found to be highly efficient although it should be added that photoinitiation is as much to do with the UV light source as with the photoinitiator. It is therefore difficult to conclude from the results presented here that either one was better than the other; both were very effective. While ACPA was the photoinitiator of choice at the beginning of this research, Irgacure 184 was a commercial alternative suggested to us later by the Aston Biomaterials Group.



4,4′-Azo-bis(4-cyanopentanoic acid) (ACPA)



1-Hydroxycyclohexyl phenyl ketone (Irgacure 184)

Following the successful use of photoinitiation, a second paper was published in the journal ***Advanced Materials Research*** (see reference below), a copy of which is also included in the Appendix.

Design and Preparation of a Bioresponsive Hydrogel for Biomedical Application as a Wound Dressing

C. Witthayaprapakorn, R. Molloy, K. Nalampang and B.J. Tighe

Adv. Mater. Res., 55-57, 681-684 (2008)

7.4 Hydrogel Sheet Properties – A Myriad of Requirements

At the beginning of Chapter 2, in Table 2.1 on page 17, a list of properties was given for an "ideal" wound dressing. Most of these properties are obviously essential while the others, if not essential, are considered useful. To be able to achieve such a wide range of properties in a single material is an unlikely prospect and so, realistically, the aim of the research reduces to achieving all of the essential properties and as many of the useful ones as possible.

In the previous Chapters 5 and 6, the test results for a range of hydrogel sheet properties were presented and discussed in sequence. In this concluding chapter, the main aim is to combine and, wherever possible, correlate these results to form a bigger and more coherent picture, rather like piecing together a jigsaw. That said, it is evident that this work has not uncovered all of the pieces but, from those that it has, the main conclusions that can be drawn are as follows:

(1) All of the Na-AMPS hydrogel sheets, including both the homopolymers and the copolymers, attained ***equilibrium water contents***, EWC_{water}, of around 99% within 30 mins when immersed in distilled water at 37°C. This is very high and very fast water absorption by any standards. Even in ion-containing solutions such as physiological saline and synthetic body fluid (SBF), in which the osmotic effect is reduced, the EWC_{water} was still over 90% within the same time period of 30 mins. Thus, it is clear that these hydrogels fall into the category of "high water-absorbing polymers" (HWAPs), even more water-absorbing than they need to be for wound dressings.

(2) However, high water absorption is only advantageous if it is balanced by an appropriate rate of ***evaporative water loss*** (EWL). If it is not, then high water absorption can actually be disadvantageous because it only leads to high volume swell and loss of cohesive strength. Moreover, in its use as a wound dressing, if the hydrogel were to absorb a large amount of exudate quickly but only release it

slowly, it would lead to "pooling" of the exudate at the wound surface. The negative consequences of this would be seepage from underneath the dressing and, with the wound in a continuously wet state, a slowing down of the wound healing process. Conversely, if the rate of EWL is too fast, the wound may "dry out" which, besides causing soreness and pain to the patient, would also be detrimental to the healing process.

It has therefore been encouraging to see from the water retention and water vapour transmission–time profiles that the rate of EWL from the hydrogels in this work is sufficiently high to attain and maintain an appropriate balance between water absorption and release. The ***water vapour transmission rate*** (WVTR) is significantly lower (by about 30-40%) than the rate of EWL from a typical 2nd degree burn which satisfies one of the most essential property requirements. As explained previously in Chapter 5 (Figure 5.17, page 89), water transport through a hydrogel is a complex process involving absorption, diffusion and evaporation, all of which have their own individual yet interrelated rate constants. This would appear to present a rather complicated picture to the polymer scientist but, essentially, all that really matters to doctors and nurses is how efficiently the dressing can absorb the wound exudate and control the rate of loss of body fluid from a 2nd degree burn. In these respects, the results here are encouraging.

(3) The ***peel strength*** results provided useful, if somewhat subjective, measures of ***skin adhesion***. To be more specific, skin adhesion for a wound dressing really refers to its adhesion to the healthy skin surrounding the wound rather than to the wound itself. Adhesion to the wound surface is actually undesirable since it would tend to cause detachment ("stripping") of the new epidermal cells during removal (Chapter 5, page 92). Furthermore, skin adhesion to the healthy skin only needs to be sufficient to keep the dressing in place. It does not need to be as strong as, for example, a skin adhesive hydrogel pad for use as an ECG electrode. A hydrogel wound dressing will lose some of its adhesiveness as it becomes more hydrated. This is because the water in the hydrogel not only acts as a lubricant for reducing the coefficient of friction at the hydrogel-skin interface but also as a

surface energy “bridge”. However, this is not necessarily problematical since lowering the adhesive strength relative to its cohesive strength reduces the possibility that the hydrogel may tear during either re-positioning or removal.

The results here have shown that the hydrogels are all skin adhesive with sufficient cohesive strength, with or without mesh reinforcement, to resist tearing on application and removal. The only hydrogel sample that was noticeably less adhesive than the rest (by sensory perception) was the highest-NVP content copolymer, poly(Na-AMPS-*co*-NVP) 70:30. This was thought to be due to the higher concentration of lubricating free water at the hydrogel surface.

(4) Due as much to their high water contents as their chemical structures, all of the hydrogels exhibited high ***oxygen permeabilities*** (Dk) well in excess of, for example, a P(HEMA) hydrogel that would be used as a soft contact lens. Thus, high oxygen permeability is an advantage of Na-AMPS hydrogels that can even be further enhanced by copolymerisation with NVP. Oxygen exchange to and from the wound surface allows the wound to “breathe” and, in doing so, helps to accelerate the healing process through promoting cell growth.

(5) A common concern with biomedical polymers is the possible presence of toxic substances (antigens) which may be already present in a natural polymer or toxic impurities remaining after synthesis in a synthetic polymer. With the polymers here being synthetic, the main concern was ***residual monomer*** (Na-AMPS or NVP), even allowing for the fact that the hydrogels sheets would normally be sterilized by γ-irradiation before use which would further reduce its concentration. However, the ***cytotoxicity*** tests showed that whatever residual monomer was still remaining was non-cytotoxic to L929 mouse fibroblast cells. (L929 mouse fibroblast cells are generally recognized as being particularly sensitive to toxins in a direct contact cytotoxicity test.) These results can therefore also be taken as indicative that the photopolymerisation conditions used in aqueous solution were suitably efficient in keeping the amount of residual monomer remaining at the end of the reaction to an acceptably low level.

(6) In this research, two ***photoinitiator-crosslinker*** systems were compared, namely:

System I : 4,4′-azo-bis(4-cyanopentanoic acid) (ACPA) – ethylene glycol dimethacrylate (EGDM)

System II : 1-hydroxycyclohexyl phenyl ketone (Irgacure 184) – poly(ethylene glycol) diacrylate, PEGDA 600 (Ebecryl 11)

System I was chosen by the author at the outset of this research as a novel system not yet reported in the literature, while System II was suggested as an interesting alternative through our international collaboration with the Aston Biomaterials Group. The main differences between these two systems were seen to be:

(a) different efficiencies of the two photoinitiators
(b) different reactivities and lengths of the crosslinkers

The prospect that these two systems could lead to different hydrogel network structures at the molecular level (crosslink density, uniformity, length) resulting in different properties at the macroscopic level was considered to be one of the novel aspects of this study. In the event, the differences in properties between the System I and System II Na-AMPS hydrogels sheets were less than expected. Both Systems were similarly efficient. The only significant differences were that the System II hydrogel gave:

(i) an approximately 30% higher water vapour transmission rate (WVTR)
(ii) an approximately 10% lower oxygen permeability (Dk)
(iii) an order of magnitude lower residual monomer content

Of these 3 differences, the lower residual monomer is considered to be the most important and so, if a choice had to be made between these two Systems, it would be for System II on this basis. These differences are most likely due to the longer chain PEGDA 600 crosslinker giving a looser 3-D network but more work needs to be done, possibly with PEGDAs of different molecular weights, to confirm this.

(7) The main purpose of adding a ***humectant*** is to decrease the rate of dehydration of the hydrogel. Two humectants were compared here: glycerol and poly(ethylene glycol) (PEG). Neither had much effect on the absorption properties but they did have an effect on the WVTR, slowing it down quite significantly, PEG more so than glycerol. Thus, the humectant's main function is to bind some of the free water so that the hydrogel does not lose its flexibility and elasticity due to excessive evaporative water loss. However, this function also impacts on other properties, notably peel strength (and, hence, skin adhesion) which decreases quite dramatically (Figure 5.39, page 115). Its effect on oxygen permeability (Dk) was less clear (Figure 5.40, page 116) since it was masked by its effect on water content but the indication was that it did not seriously impair Dk.

Consequently, the use of a humectant depends on the type of hydrogel that is being used and its intended application. On the basis of the results obtained here, together with information gleaned from the literature, it appears that the use of a humectant is actually more concerned with extending the shelf-life of the hydrogel in storage rather than any effect which it may have on the hydrogel's performance in use as a wound dressing.

(8) Provided that water absorption and water loss are sufficiently well balanced for volume swell not to be excessive, the incorporation of a ***reinforcing mesh*** can improve the cohesive strength of the hydrogel without compromising its other properties. Moreover, this is an inexpensive and easy-to-perform modification. The only drawback is that it obviously impairs the transparency of the hydrogel sheet. Having said this, consultations with doctors have suggested that, although transparency is useful for allowing visual observation of the wound surface, it is not an essential requirement. Therefore, mesh reinforcement would certainly be a design construction refinement well worth considering in a prototype dressing for possible commercialization.

(9) Finally, ***copolymerisation*** with *N*-vinyl-2-pyrrolidone (NVP) showed how the hydrogel properties can be varied quite significantly with composition, thereby introducing an element of "property tailoring" into the molecular design process. Increasing the NVP content increased oxygen permeability but decreased peel strength. Water transport properties were largely unaffected. It is also worth noting that NVP is much cheaper in price than AMPS acid and so copolymerisation can be a way of lowering the unit cost of the final product – an important consideration in any research project which is aimed at developing a new commercial product.

7.5 Concluding Remarks

In conclusion of this thesis, it is appropriate now to look back and reflect on the main aims of this project and the extent to which they have been achieved. As the title of this thesis states, the overall aim has been the

Design and Preparation of Synthetic Hydrogels
for Biomedical Use as Wound Dressings

This could have been written more specifically as ***"… as Wound Dressings for Second-Degree Burns"*** but a certain degree of flexibility in the title was considered advisable.

To go back to the very beginning, the primary function of a wound dressing is to encourage the various stages of wound healing by creating a set of favourable conditions – in effect, a sort of "micro-environment" – around the wound that can aid and, if possible, accelerate recovery. While the results in this thesis have focused on properties such as water absorption, water vapour transmission and oxygen permeability, all of these properties are directed towards one objective – namely, promoting cell growth. Apart from confirming that the hydrogels are non-cytotoxic, this project has not gone as far as studying cell growth, viability and proliferation. However, the results presented here are encouraging enough for the next stage of this

research to proceed to biological testing. In support of this claim, Table 7.1 on the following page presents a "check list" comparing what has been achieved property-wise with the formidable array of properties that was given in Table 2.1 (page 17) in Chapter 2 for an "ideal" wound dressing. The checks (✓) may not yet be complete but, importantly, there are no items in the list that have crosses (✗) against them.

As with any research project such as this which is directed towards the development of a new product for commercial use, it has necessarily involved a wide range of activities starting from molecular design through to polymer synthesis, characterisation, fabrication and testing, as shown in the diagram below. With such stringent property requirements and the modern trend towards "smart" materials, biomedical polymer research is now justifiably being described as a branch of "molecular engineering". To what extent this project qualifies for this description is a matter of opinion but it certainly stretched the imagination as to what the molecules involved are doing. Hopefully, the results described in this thesis will provide a solid platform for this work to continue on towards its logical and successful conclusion.

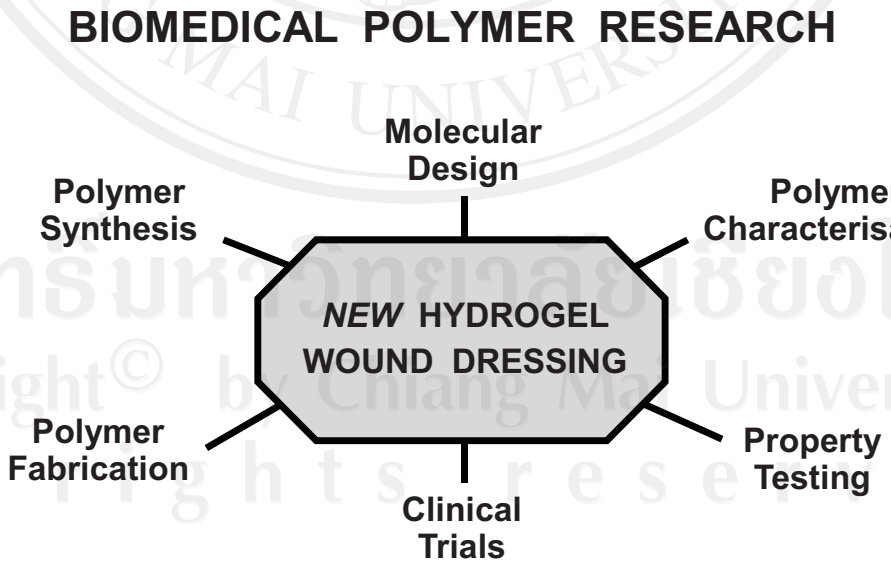


Table 7.1 : Check list of hydrogel sheet properties for suitability as a wound dressing (with reference to the list given in Table 2.1 on page 17).

HYDROGEL PROPERTIES	CHECK	COMMENTS
MEDICAL		
• Good adhesion to the wound bed	✓	Wound adhesion not tested but good adhesion to healthy skin
• Maintenance of moist environment	–	Not tested
• Occlusiveness to microorganisms	–	Not tested
• Control of water vapour loss	✓	WVTR in appropriate range for a second-degree burn
• Absorption of wound exudate	✓	Actual wound exudate not tested but good absorption of SBF
• Conducive to cell proliferation	–	Not tested
• Antisepticity	–	Not tested
• Ability to improve the healing process (biomimesis) and control pain	–	Not tested
• Durability	✓	Elastic with reasonable tear strength Could be reinforced with a mesh
• Ease of application and removal	✓	Easy to apply and remove
SAFETY		
• Sterility	✓	Sterilizable with no adverse effects
• Non-pyrogenicity	–	Not tested - but no reports of such
• Non-toxicity	✓	Cytotoxicity test results negative
• Non-antigenicity	–	Not tested - but no antigens expected
• Lack of release of toxic substances	✓	Residual monomer non-cytotoxic
GENERAL		
• Oxygen permeability	✓	High oxygen permeability due to high EWC
• Water vapour permeability	✓	Good water vapour permeability from WVTR
• Flexibility	✓	Highly flexible
• Transparency	✓	Transparent
• Good handling characteristics	✓	Easy to handle and manipulate
ECONOMIC		
• Availability	✓	All materials readily available
• Reasonable shelf-life	✓	Could be stored without change over a period of months
• Cost effectiveness	✓	Could certainly be made cheaper than current commercial materials
• Minimal storage requirements	✓	If sealed in sterilized packs, can be stored under normal conditions