CHAPTER 6

Na-AMPS Hydrogel Property Modification by Copolymerisation with *N*-Vinyl Pyrrolidone

6.1 Copolymerisation Definitions and Classification

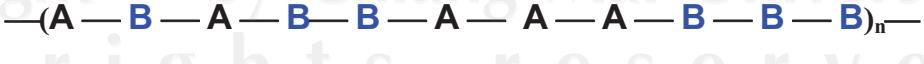
A copolymer (or heteropolymer) is a polymer derived from two (or more) monomers, as opposed to a homopolymer where only one monomer is used [62].

Since a copolymer consists of at least two types of constituent units (also referred to as structural units), copolymers can be classified based on how these units are arranged along the chain [121-124]. These include:

(1) Alternating Copolymers with regularly alternating A and B units

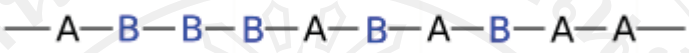
poly(A-*alt*-B)

(2) Periodic Copolymers with A and B units arranged in a repeating sequence



poly(A-*per*-B)

(3) Statistical Copolymers are copolymers in which the sequence of monomer units follows a statistical rule and are the most common type of copolymer. If the probability of finding a given type of monomer unit at a particular point in the chain is equal to the mole fraction of that monomer unit in the chain, then the polymer may be referred to as a truly **Random Copolymer** [125].



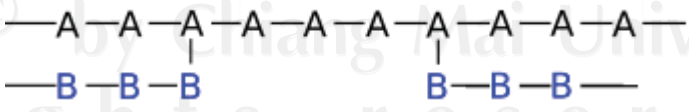
poly(A-*stat*-B) or poly(A-*ran*-B)

commonly written as **poly(A-*co*-B)**

(4) Block Copolymers comprise two or more homopolymer sub-units linked by covalent bonds. The union of the homopolymer sub-units may require an intermediate non-repeating sub-unit known as a junction block. Block copolymers with two or three distinct blocks are called diblock copolymers and triblock copolymers respectively.

poly(A-*b*-B)

(5) Graft Copolymers are a special type of branched copolymer in which the side chains are structurally distinct from the main chain.



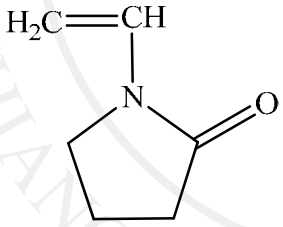
poly(A-*g*-B)

Copolymers may also be described in terms of the existence of or arrangement of branches in the polymer structure. Linear copolymers consist of a single main chain

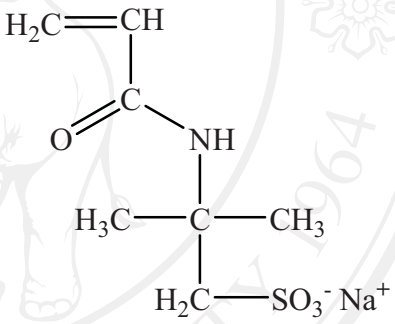
whereas branched copolymers consist of a single main chain with one or more polymeric side chains. Other special types of branched copolymers include star copolymers, brush copolymers, and comb copolymers.

6.2 *N*-Vinyl Pyrrolidone

In this work, *N*-vinyl pyrrolidone (NVP) was chosen as the comonomer with Na-AMPS for hydrogel property modification. Unlike Na-AMPS, NVP is a non-ionic monomer but, like Na-AMPS, its polymer is water-soluble. The chemical structures of NVP and Na-AMPS are compared below.



N-Vinyl pyrrolidone (NVP)



Na-AMPS

Although the 2-pyrrolidone group in NVP is a bulky group, it is still smaller in terms of the space that it occupies than the amido-2-methylpropane sodium sulfonate group in Na-AMPS. Both groups bind strongly with water but, because it is non-ionic, crosslinked poly(NVP) as a hydrogel is less strong osmotically in its water absorption characteristics than poly(Na-AMPS). Nevertheless, poly(NVP), or simply PNVP, is strongly hydrophilic and has found widespread biomedical use in its own right in numerous applications in medicine and pharmaceuticals because of its ability to bind reversibly to various molecules in solution while maintaining excellent biocompatibility within the human body [126-130]. PNVP hydrogels, formed either by physical or chemical cross-linking, have also been widely investigated [131-136].

In previous work, Declan and Clement [132] studied crosslinked copolymers of NVP and acrylic acid P(NVP–*co*–AA) that were prepared from a mixture of NVP, AA and EGDM crosslinker. The rheometry results indicated that there was a significant difference in the comparative gel strength at different pHs due to the increased water uptake. It was also found that by varying the molecular weight of the crosslinking agent, an increase in comparative gel strength could be achieved. Fechine and co-workers [133-134] studied PNVP hydrogels produced by direct ultraviolet irradiation compared with other methods of hydrogel production. They concluded that physical properties such as crosslink density and pore size affect not only the swelling properties of the hydrogel but also the storage and loss moduli obtained from rheological tests.

However, to date, very little appears to have been reported in the literature about copolymers of Na-AMPS and NVP. When copolymerised together, Na-AMPS and NVP should produce statistical copolymers based on their monomer reactivity ratios of 0.66 and 0.13 respectively [137]. Thus, copolymers of Na-AMPS and NVP can be expected to show a fairly random monomer sequencing in which the NVP units in the copolymer chain can act as “spacers” between the Na-AMPS units. In this way, the incorporation of NVP units into the P(Na-AMPS) chain should bring about measurable changes in hydrogel properties, not necessarily in terms of water affinity but certainly in some other structure-dependent properties.

6.3 Copolymer Synthesis

Copolymers of Na-AMPS and NVP were prepared in the form of hydrated sheets as described previously for Na-AMPS alone. The System I photoinitiator/crosslinker (ACPA/EGDM) combination was used. As shown in Table 6.1, 3 different copolymer compositions were compared, prepared from comonomer feed ratios of:

40% Na-AMPS solution : NVP = 90:10, 80:20, 70:30 (vol %)

These volume ratios corresponded to actual comonomer mole ratios of:

Na-AMPS : NVP = 62:38, 42:58, 30:70 (mol %)

Table 6.1 : Comonomer feed ratios used in the synthesis of the Na-AMPS-NVP hydrogel copolymers.

Comonomer Volume Ratio		**Volume of Each Monomer in 20 ml**		**Comonomer Mole Ratio**		**Total Moles of Monomers (mol)**
Na-AMPS * (%)	**NVP (%)**	**Na-AMPS * (ml)**	**NVP (ml)**	**Na-AMPS (%)**	**NVP (%)**	
100	0	20	0	100	0	0.0350
90	10	18	2	62	38	0.0509
80	20	16	4	42	58	0.0668
70	30	14	6	30	70	0.0827

* Used as a 40% w/v aqueous solution of Na-AMPS

6.4 Hydrogel Sheet Properties

6.4.1 Water Absorption, Retention and Vapour Transmission

From the results in Figures 6.1-6.3 and Table 6.2, the following conclusions can be drawn.

(1) From Figure 6.1, increasing the % NVP appeared to have relatively little effect on the water absorption properties of the hydrogel. The EWCs (98.6-99.6%) were all within 1% of each other and the approach to equilibrium was similarly fast in each case.

(2) From Figure 6.2, increasing the % NVP did not affect the rate of evaporative water loss in air but did slightly decrease the final EWC from 22 to 18%.

(3) The most noticeable effect of the NVP is on the water vapour transmission rate (WVTR). In Figure 6.3, increasing the % NVP decreased the WVTR by up to 20% (from 101.9 to 80.7 $g.m^{-2}.hr^{-1}$). This is a sign that, despite the very similar absorption and retention properties, when diffusion through the sample is also taken into consideration, as in water vapour transmission, there is some other effect(s) involved through which the increasing NVP content slows down the rate of water transport. It is by no means conclusive but one possible explanation is the apparent increase in sheet thickness at EWC with NVP content, as shown in Table 6.2. In other words, increasing the amount of NVP increases the amount of volume swell for the same level of hydration. This would increase the length of the diffusion pathway through the thickness of the hydrogel, thereby slowing down the WVTR.

Thus, it can concluded that incorporating up to 30% NVP by volume (= 70% by mole) into a crosslinked Na-AMPS hydrogel network has relatively little effect on water absorption/retention/vapour transmission properties. Despite the fact Na-AMPS is ionic while NVP is non-ionic, both repeating units are extremely hydrophilic (both

uncrosslinked homopolymers are readily water-soluble) and so copolymerisation offers little scope for property modification in terms of water transport.

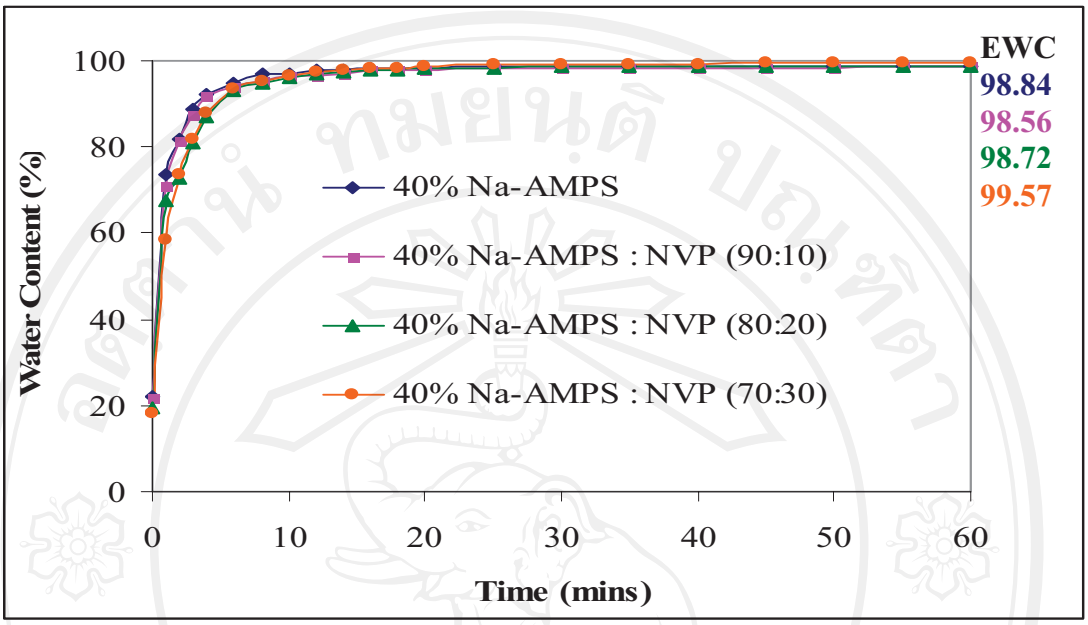


Figure 6.1 : Water absorption - time profiles for the 40% w/v Na-AMPS and 40% w/v Na-AMPS : NVP System I hydrogels when immersed in distilled water at 37°C.

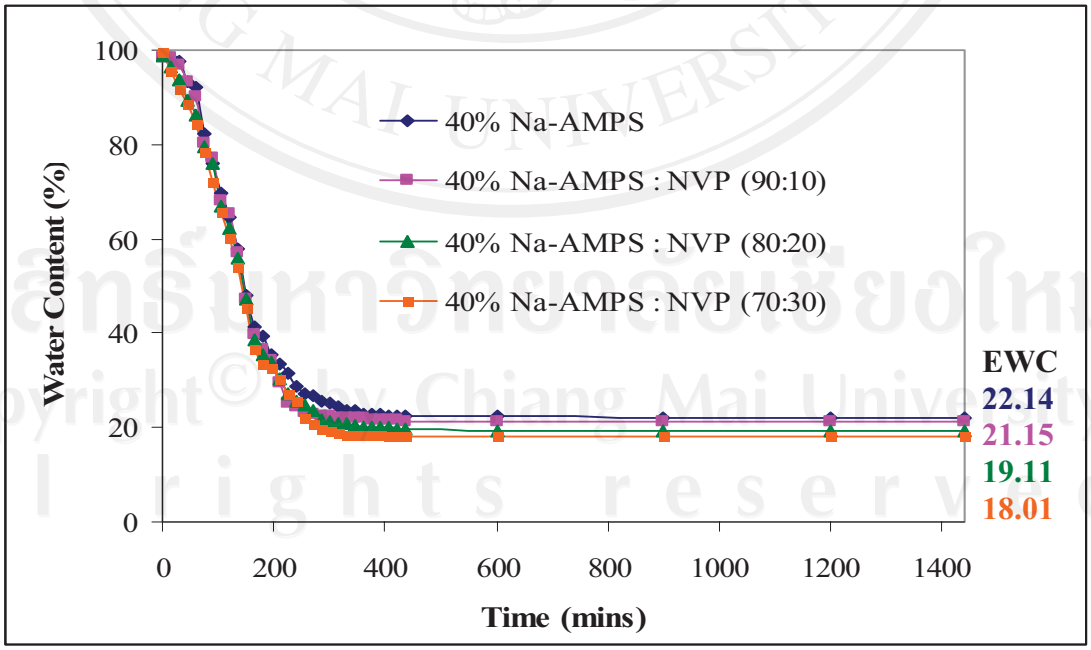


Figure 6.2 : Water retention - time profiles for the 40% w/v Na-AMPS and 40% w/v Na-AMPS : NVP System I hydrogels in air at room temperature.

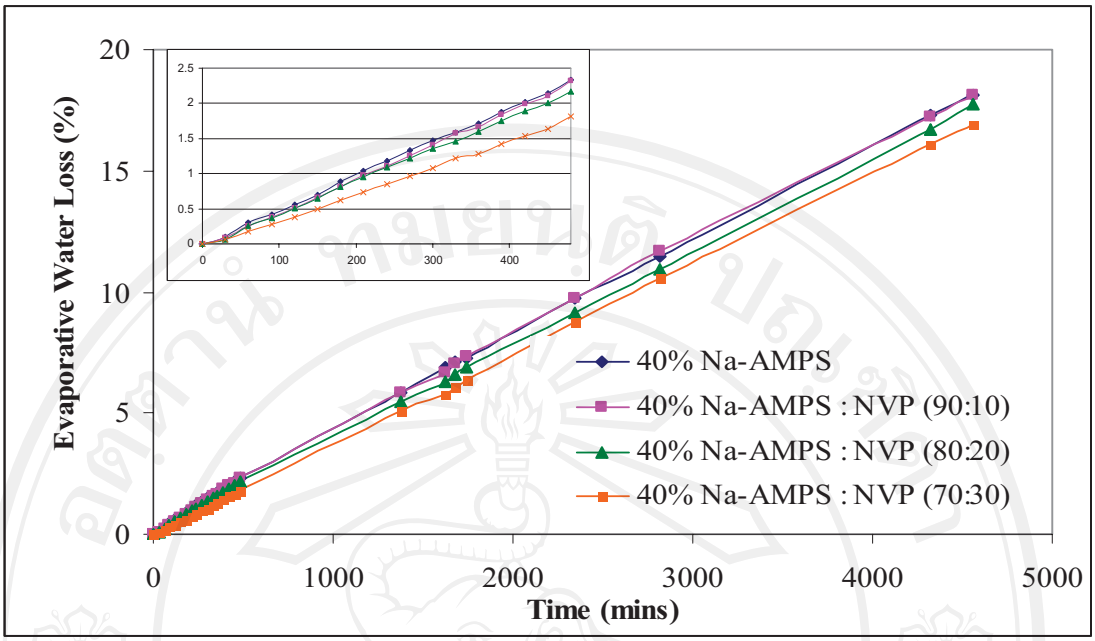


Figure 6.3 : Water vapour transmission - time profiles for the 40% w/v Na-AMPS and 40% w/v Na-AMPS : NVP System I hydrogel sheets at 37°C and 55-60% relative humidity over a 76 hrs time period.

Table 6.2 : Comparison of the water absorption, retention and vapour transmission rates of the 40% w/v Na-AMPS and 40% w/v Na-AMPS : NVP System I hydrogel sheets.

Comonomer Ratio (% v/v)	**Thickness (mm)**	**Water Absorption EWC (%)**	**Water Retention EWC (%)**	**WVTR * initial 8 hrs. ($g.m^2.hr^{-1}$)**
40% Na-AMPS : NVP (100:0)	1.20	98.84	22.14	101.87
40% Na-AMPS : NVP (90:10)	1.22	98.56	21.15	99.75
40% Na-AMPS : NVP (80:20)	1.47	98.72	19.11	97.63
40% Na-AMPS : NVP (70:30)	1.49	99.57	18.01	80.65

* WVTR = water vapour transmission rate

6.4.2 Peel Strength

In contrast to the relatively small effects on water transport properties, copolymerisation with NVP had a much more noticeable effect on peel strength. As shown in Figure 6.4, as the NVP content of the hydrogel increased, the peel strength from human skin decreased. As described in the previous chapter, these peel strength tests were carried out using the modified Hounsfield Tensometer with the sample (150 x 25 x 1.20 mm) adhered to the author's forearm as the substrate. Prior to testing, the sample was equilibrated to its EWC in air in the instrument testing room.

The decrease in peel strength with NVP content may be explained in terms of a charge dilution effect at the surface of the hydrogel. Increasing the proportion of NVP units decreases the ionic charge density of the Na-AMPS units at the hydrogel surface. This, in turn, decreases the level of ionic interaction and, hence, the strength of adhesion at the hydrogel-skin interface. Another possibility is that there could be a higher concentration of water molecules at the hydrogel surface as the NVP content increases which also reduces the hydrogel-skin interaction. Whatever the reason is it is clear that NVP copolymerisation, like humectant addition previously, provides a means of varying the peel strength of the hydrogel.

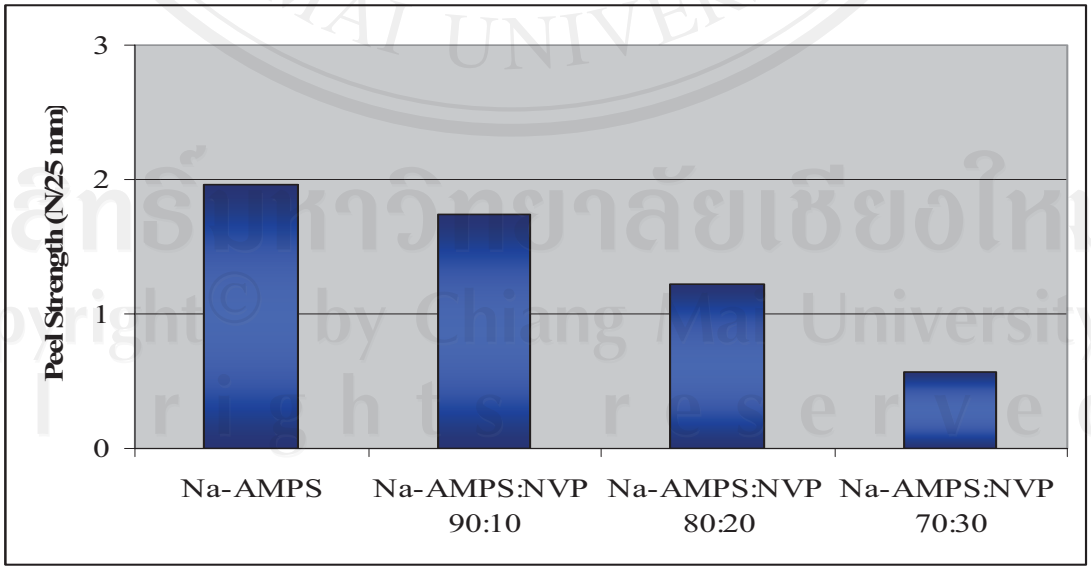


Figure 6.4 : Peel strengths of Na-AMPS and Na-AMPS-*co*-NVP hydrogels sheets from human skin, each tested at their EWC in air.

It is interesting to compare the instrumentally measured peel strength results in Figure 6.4 with purely subjective assessments based on the physical sensation as the sample was being removed from the skin. Skin adhesion is, after all, as much a human perception as a material property. In the subjective opinion of the author, all of the 4 samples in Figure 6.4 were what could be considered to be “skin adhesive”. However, the 70:30 sample was noticeably less adhesive than the other 3 compositions. Thus, it can be said that, subjective though they are, patient assessments of skin adhesion can be correlated to some extent at least with instrumental measurements.

6.4.3 Oxygen Permeability

It was mentioned in the previous chapter that oxygen permeability (Dk) in hydrogels tends to follow the same trend as the equilibrium water content (EWC) at EWC values of about 30% and above. It is therefore interesting to see in Figure 6.5 that, even though the EWCs of the Na-AMPS and Na-AMPS-*co*-NVP hydrogels are approximately equal (95±1%), their Dk values increase significantly with NVP content. The obvious conclusion to be drawn from this is that the Dk-EWC correspondence only holds for the same chemical structure. As the chemical structure changes with NVP incorporation, especially at NVP ≥ 20% v/v (≥ 58% by mol), the Dk value deviates from the approximately constant EWC.

The most likely explanation for the increase in Dk with NVP content comes from the balance between free and bound water. Dk is actually more specifically related to the free water since it is the free water molecules, with their greater freedom of molecular mobility, that are responsible for transporting the oxygen molecules through the hydrogel. It is therefore reasonable to conclude that the NVP units, even though they absorb just as much water as the Na-AMPS units, bind proportionately less. This is an interesting finding made possible by the structural variation affecting Dk more than EWC. Since it is well known that the presence of oxygen at the wound surface is beneficial to the wound healing process, this effect of NVP in increasing Dk

could provide a convenient means of tailor-making high oxygen permeability hydrogels for specific purposes.

Figure 6.5 below also compares the Dk values with that of a poly(2-hydroxyethyl methacrylate), P(HEMA), hydrogel sample at its EWC of 37%. P(HEMA) is commonly used in soft contact lenses, an application in which oxygen permeability is also an essential requirement so that the eye can "breathe" sufficiently. Although the much lower Dk value of P(HEMA) is as much to do with its lower EWC as with its chemical structure, what its comparison in Figure 6.5 serves to show is that, with their much higher Dk values, the hydrogels in this work should be able to allow more than enough oxygen to pass through in order to support the wound healing process.

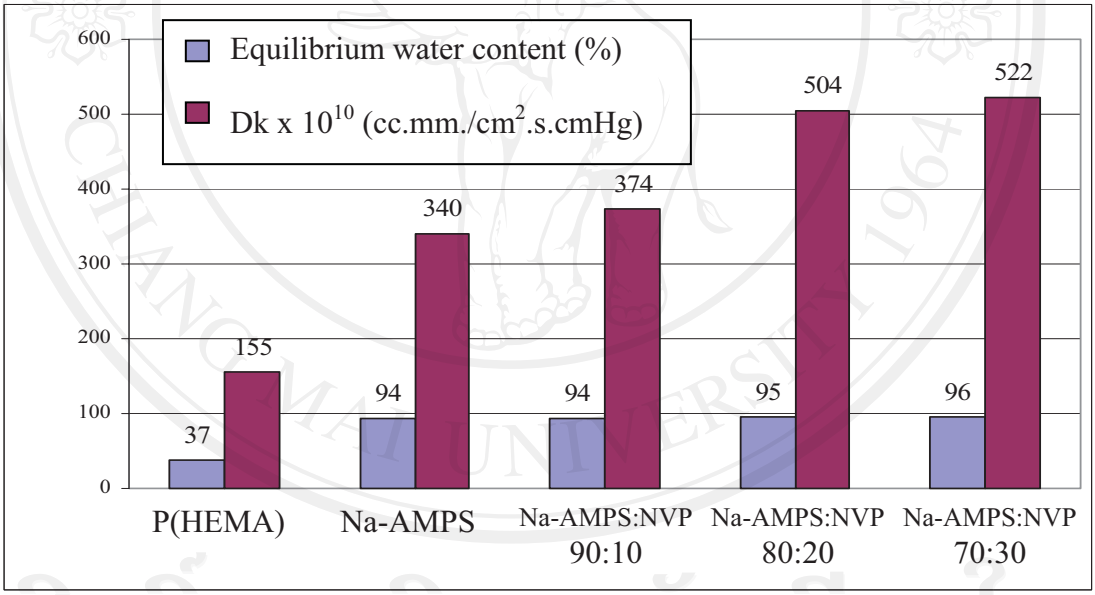
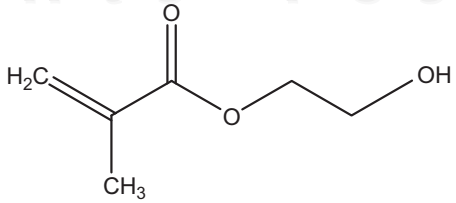


Figure 6.5 : Oxygen permeabilities (Dk) of the Na-AMPS and Na-AMPS-*co*-NVP hydrogel sheets compared with P(HEMA).
Sheet thickness = 1.35 ± 0.15 mm



P(HEMA) = poly(2-hydroxyethyl methacrylate)

6.4.4 Cytotoxicity Testing

Since PNVP homopolymer is already widely used in biomedical applications, it was not expected that copolymerisation with NVP would have an adverse effect on the non-cytotoxicity of Na-AMPS hydrogels. However, cytotoxicity testing was nevertheless necessary, not only for regulatory reasons but also to check that the photopolymerisation procedure did not leave behind toxic levels of residual NVP monomer. This possibility had to be considered since NVP has a lower reactivity ratio (0.13) than Na-AMPS (0.66) in their copolymerisation and so there was the likelihood that most of the residual monomer, whatever the amount, would be NVP.

Consequently, the Na-AMPS-*co*-NVP hydrogel sheet that was chosen for cytotoxicity testing was that which had the highest NVP content (i.e., 70:30 v/v = 70 mol % NVP). As the SEM results in Figure 6.6 show, this Na-AMPS-*co*-NVP 70:30 hydrogel, like the Na-AMPS alone (100:0) hydrogel, is clearly non-toxic since it exhibited similar cell responses to the HDPE negative control. This similarity extended to both cell viability (number) and morphology (size and shape). This confirms that NVP can be copolymerised with Na-AMPS to give non-cytotoxic hydrogel sheets which could be safely used as wound dressings in contact with living tissue.

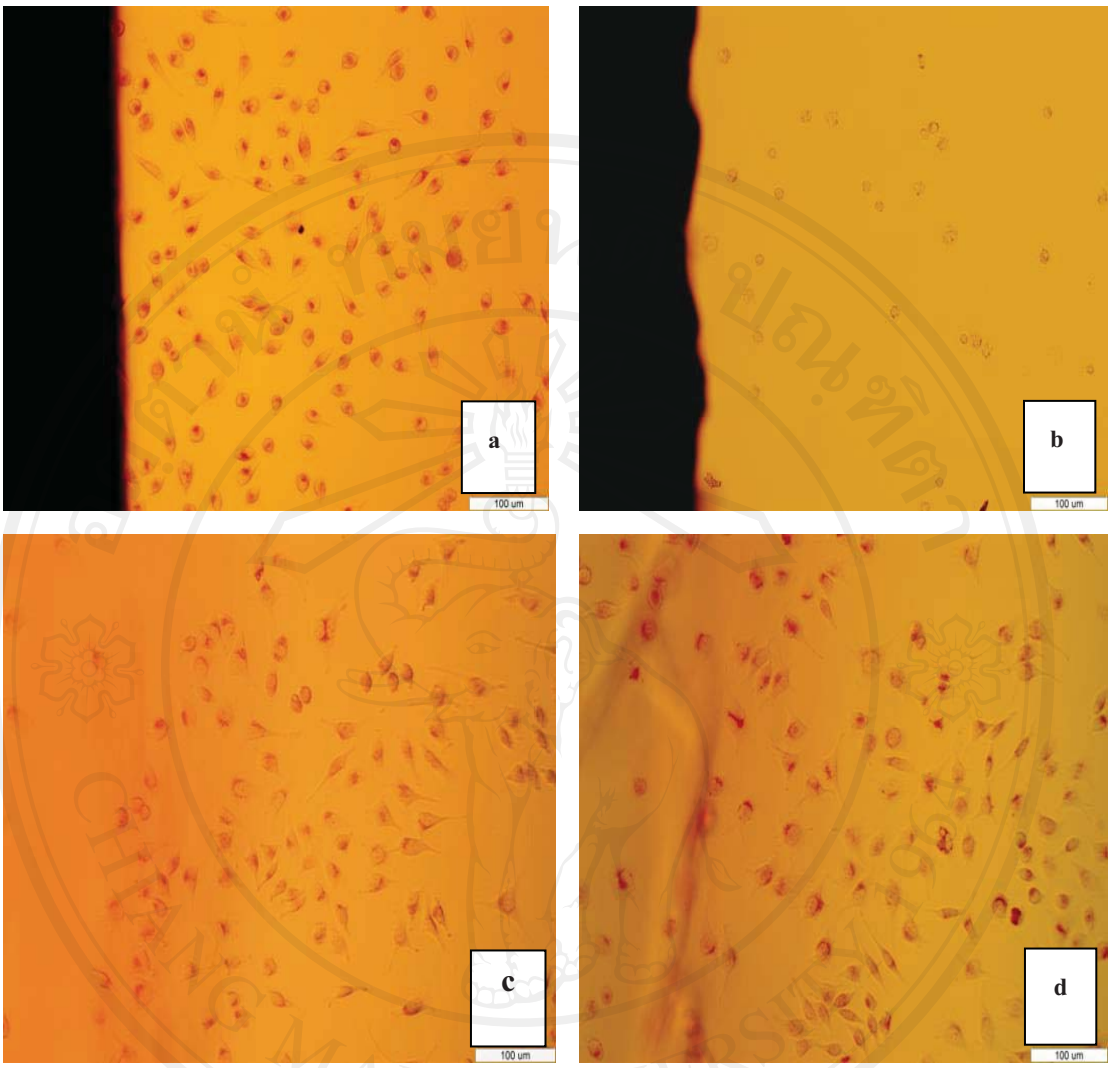


Figure 6.6 : Scanning electron micrographs (magnification x 200) showing the L929 mouse fibroblast cells after cytotoxicity testing for 48 hrs at 37°C on the following substrates :

(a) HDPE (negative control)

(b) natural rubber containing carbon black (positive control)

(c) Na-AMPS System I hydrogel

(d) Na-AMPS-*co*-NVP (70:30) System I hydrogel

6.4.5 Dynamic Vapour Sorption Analysis [138-140]

Finally, to conclude this chapter, an additional specialist technique is described which was used during a short overseas training visit made by the author to the Biomaterials Research Unit, Aston University, Birmingham, UK. This technique is ***dynamic vapour sorption*** (DVS) analysis in which measurements were carried out using a Scientific and Medical DVS Analyzer, as shown in Figure 6.7. DVS is a gravimetric technique that measures how quickly and how much of a solvent (usually water) is absorbed or desorbed by a sample. Thus, the main usefulness of this technique to this project was seen to be to determine the rate at which water vapour sorption or desorption occurs in a hydrogel sample on varying the surrounding humidity. In this way, it can provide useful information as to how the hydrogel behaves under dynamically changing conditions.

The relative humidity was generated by bubbling N_2 gas through a water reservoir so that it became saturated with moisture. In the mixing chamber, the wet N_2 gas was mixed with dry N_2 at a fixed ratio to give the required relative humidity. As illustrated in Figure 6.8, the DVS apparatus contains two sample chambers. The reference chamber contains only an empty pan while the other contains the material being monitored. Both sample pans were connected to a highly sensitive microbalance located in the temperature-controlled chamber which was set to 37°C. Before any experiments were conducted, the relative humidity was initially set to 40% and the balance zeroed with no sample present. Each sample was cut using a size 2 cork borer and weighed less than 100 mg. The sample was carefully placed onto the pan at which point its mass was registered in the DVS software. Samples were subjected to preset relative humidity conditions. The sample would either take up moisture, causing an increase in mass, or it would lose moisture causing a decrease in mass. Stabilisation of the mass indicated that equilibrium had been reached.

Figure 6.7 : Photograph showing the DVS instrument used in this work.

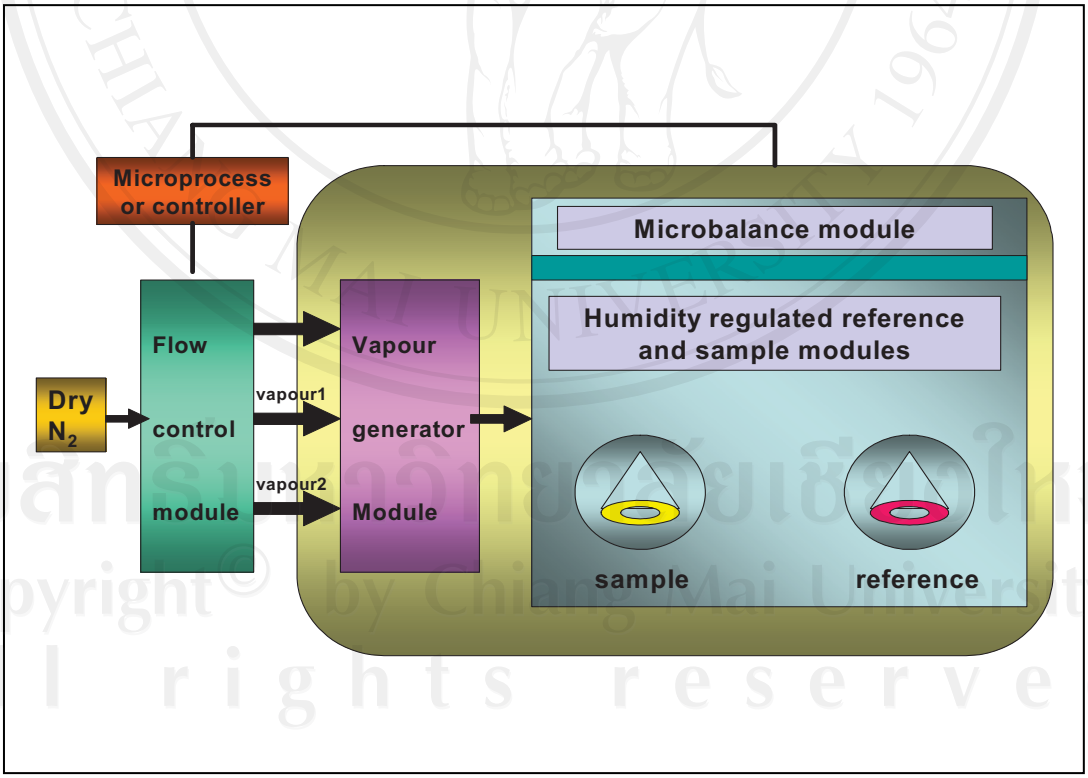


Figure 6.8 : Schematic diagram of the DVS instrument showing the flow stream of controlled humidity.

The experimental conditions which were used to determine the rate at which vapour sorption or desorption occurred in the Na-AMPS and Na-AMPS-*co*-NVP hydrogels on varying the humidity were

- Temperature = 37°C
- Humidity range = 40 – 70%
- N_2 flow-rate = 200 ml/min

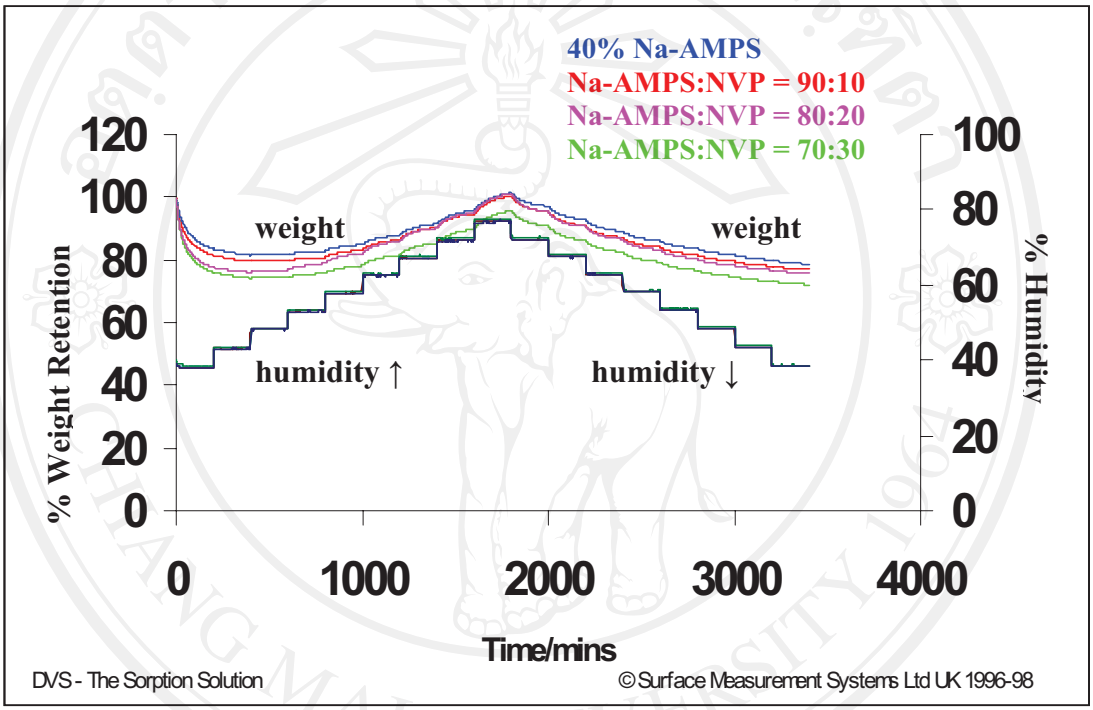


Figure 6.9 : DVS results showing the relation between weight retention (%) and % humidity with time for the 40% Na-AMPS and Na-AMPS-*co*-NVP hydrogel sheets.

As the DVS isotherms in Figure 6.9 show, starting at 40% humidity, weight loss (desorbed moisture) occurred up to about 50% humidity. Thereafter, as the % humidity continued to rise up to 70%, the hydrogels gained weight (absorbed moisture) back up to approximately their starting values. The weight loss was greater and the weight recovery slower as the NVP content in the hydrogel increased. This observation is consistent with the previous conclusion (from the oxygen permeability results) that increasing the NVP content increases the proportion of free water relative

to bound water. In these DVS experiments, the weight loss due to desorbed moisture would be loss of free water.

Interesting though this DVS technique is, it does not really add significantly to the previous water absorption / retention / vapour transmission results. It appears that DVS analysis is more applicable to the study of dry samples rather than samples which are already considerably hydrated. Hence, it is more commonly used in, for example, studying the moisture absorption properties of pharmaceutical materials such as excipients, drug formulations and drug packaging films. Another example of its application is to study the dehydration of soft contact lenses, which is what the DVS analyzer was mainly being used for in the Aston Biomaterials Research Unit.