SYNTHESIS AND CHARACTERISATION OF NOVEL N-VINYL PYRROLIDINONE/ACRYLIC ACID COPOLYMERS FOR USE IN BIOMEDICAL APPLICATIONS

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Declaration

I hereby declare that this thesis submitted to the Higher Education and Training Awards Council for the degree of Doctor of Philosophy, is a result of my own work and has not in the same or altered form, been presented to this institute or any other institute in support for any degree other than for which I am now a candidate.

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Declan Devine

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Abstract

The aim of this study was to develop a novel hydrophilic polymer for use in biomedical applications. The polymers synthesised in this work were based on the monomers N-vinyl pyrrolidinone (NVP) and acrylic acid (AA) and were UV polymerised using a suitable photoinitiator depending upon the application. These polymers were analysed both with and without the incorporation of chemical crosslinking agents using a variety of test methods. From these tests it was found that the incorporation of AA imparted pH sensitivity to the hydrogel, which had a critical pH range of between 4.07 and 4.49. Above a pH of 4.49 there is a progressive break up of the polymer chain due to a reduction in the amount of intermolecular hydrogen bonding. There is also a significant increase in the polymer solubility and swelling of the crosslinked polymers at higher pHs. The Ftir spectra of PVP-PAA copolymer complexes indicates the presence of hydrogen bonding between the carbonyl group in the PVP and the carboxylic acid group in the PAA moiety, and the formation of an AA dimer. The incorporation of crosslinking agents caused a reduction in hydrogen bonding, signifying that crosslinking agents acted as a spacer between molecular chains.

These polymers were physically characterised using parallel plate rheometry. The rheometry results indicated that there was a significant difference in the oscillating torque at break and the comparative strength of the hydrogels at different pHs, due to the increased water uptake. It was also found that by varying the molecular weight of the crosslinking agent, oscillating torque at break and the comparative strength of the hydrogels could be altered. After the initial characterisation of the hydrogels had taken place, suitable hydrogels were chosen for further analysis for specific applications. A chemically crosslinked monomeric mixture of 70-30 wt% NVP-AA was tested as a drug eluting lubricious hydrophilic coating that could be cured directly onto a substrate (Pebax ® 3533) without the aid of a solvent, using a dip coating/UV curing procedure. Ftir analysis illustrated that no characteristic monomeric peaks were found, and that curing the coating directly onto a substrate did not affect the chemical structure of the crosslinked polymer when compared to the Ftir spectrum of the polymer cured in bulk. Optical microscopy suggested that 2 coating cycles yielded the most consistent coating throughout the entire length of the substrate. Frictional analysis confirmed the lubricious nature of the coating, and showed that a dramatic reduction in the instantaneous force and kinetic force required to move a sample of Pebax ® 3533 was observed with the incorporation of the hydrated coating. Drug release analysis showed that the release of an active agent could be controlled by varying the molecular weight of the crosslinking agent used.

The extractable content of the crosslinked hydrogels was analysed in relation to increasing crosslinking agent content. It has been shown that the extractable content of these hydrogels is relatively low, allowing these hydrogels to be used in applications where this is advantageous. We have demonstrated that the dissolution profile of active agents from these polymers vary, depending upon the dissolution media used. Finally, favourable cytotoxicity and genotoxicity results have been obtained proving that these novel polymers have potential in a variety of biomedical applications.

Abbreviations

AA	Acrylic acid
COX	Cyclo-oxygenase
CS	Chondroitin sulphate
DMEM	Dulbecco's Modified Eagles Medium
DSC	Differential scanning calorimetry
ECM	Extracellular matrix
EG	Ethylene glycol
EGDMA	Ethylene glycol dimethacrylate
EGF	Epidermal growth factor
EWC	Equilibrium water content
FCS	Foetal Calf Serum
FDA	Food and drugs authority
FGF	Fibroblast growth factor
Ftir	Fourier transform infrared spectroscopy
G′	Storage modulus
G″	Loss modulus
GAGs	Glycosaminoglycans
GI	Gastro intestinal
GPC	Gel permeation chromatography
HA	Hyaluronan
HDEEMA	Hydroxydiethoxyethyl methacrylate
HEEMA	Hydroxyethoxyethyl methacrylate
HEMA	Hydroxyethyl methacrylate
HPMA	N-(2-hydroxypropyl) methacrylamide
Irgacure ® 184	1-hydroxycyclohexyl phenol ketone
IL	Interleukin
IPM	Isopropyl myristrate
LMP	Low melting point
MAA	Methacrylic acid
MDA	Methylene diacrylamide

MDEEMA	Methoxydiethoxyethyl methacrylate
MEEMA	Methoxyethoxyethyl methacrylate
MEMA	Methoxyethyl methacrylate
MMA	Methyl methacrylate
Mn	Number average molecular weight
Mp	Peak average molecular weight
Mw	Weight average molecular weight
MTT	3-(4,5 Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide
NIPAAm	N-isopropyl acrylamide
η_{rel}	Relative viscosities
NSAID	Non-steroidal anti-inflammatory drug
NVP	N-vinyl pyrrolidinone
O.S. cal	Outside calibration
OVA	Ovalbumin
PA	Polyamide
PAA	Poly acrylic acid
PBT	Poly butylene terephthalate
PCL	Poly ε-caprolactone
PDGF	Platelet-derived growth factor
PE	Polyethylene
PEG	Poly ethylene glycol
PEGA	Poly ethylene glycol acrylate
PEGDA	Poly ethylene glycol diacrylate
PEGDMA	Poly ethylene glycol dimethacrylate
PEGMA	Poly ethylene glycol methacrylate
PEO	Polyethyleneoxide
PGNA	Poly(α, L-Glutamate)
PHEMA	Poly(hydroxy ethyl methacrylate)
PLGA	Poly(lactic-co-glycolic acid)
PMMA	Poly methyl methacrylate
PS	Polystyrene
PTFE	Polytetrafluoroethylene

PVOH	Polyvinyl alcohol
PVP	Polyvinyl pyrrolidinone
SCGE	Single cell gel electrophoresis
SRC	Self reinforced composite
t_0	Flow time for the solvent
TGF-b	Transforming growth factor-beta
t_{sol}	Flow time for the coating solution
Vac	Vinyl acetate
W_0	Weight of hydrogel before swelling
W_c	Water content
W_t	Weight of hydrogel at time t
W_u	Water uptake

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Chapter 1

Literature Review

1.1 Biomaterials

In the treatment of disease or injury it has been found that a variety of non-living materials are of use. Common examples include sutures, bone fixation plates, synthetic cement used in hip replacement and tooth fillings. A biomaterial is a material employed in, or used as a medical device, which is intended to interact with biological systems (Egan and Waterman 1998) other than food or drugs.

Biomaterial applications are not a modern phenomenon. The Romans, Chinese and Aztec used gold in dentistry more than 2000 years ago. Also throughout recorded history, glass eyes and wooden teeth have being in common use (Ratner 1996). The use of biomaterials did not become practical until the advent of aseptic surgical techniques as developed by Lister (1865 cited Das 2000). Problems of infection tend to be exacerbated in the presence of biomaterials, since the implant can provide a region inaccessible to the body's immunlogically competent cells. Work to counteract this response has been done by Modak and Sampath (1996), and has led to a patent that enables medical devices to have anti-infective agents to be impregnated onto their surfaces.

The earliest successful implants were in the skeletal system and this is still a large fraction of modern biomaterial implants. Bone plates were introduced in the early 1900s to aid the fixation of fractures. Many of these early plates broke as a result of unsophisticated mechanical design. It was also discovered that materials such as vanadium steel, which were chosen for good mechanical properties, corroded rapidly in the body. Following the introduction of stainless steels and cobalt chromium alloys in the 1930s, greater success was achieved in fracture fixation, and the first joint replacement surgeries were performed. As for polymers, it was found that warplane pilots in World War II who had been injured by fragments of plastic [poly methyl methacrylate (PMMA)] aircraft canopy did not suffer adverse chronic reactions from the presence of the fragments in the body. PMMA became widely used after that time for corneal replacement and for replacements of sections of damaged skull bones. PMMA is still in use and Wright et al. (1997) has investigated the use of self-reinforced composite PMMA (SRC-PMMA) for use in orthopaedic prosthesis.

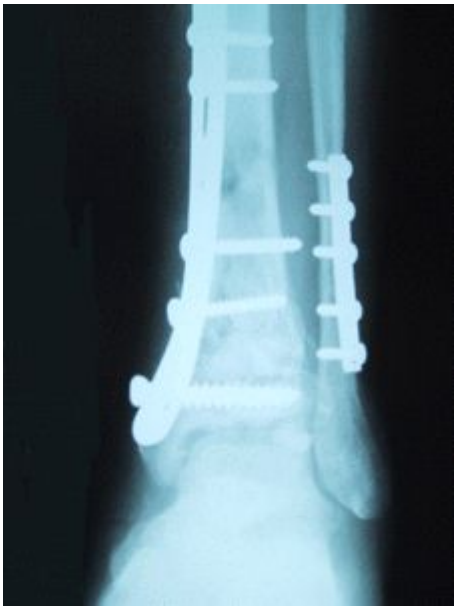


Figure 1.1 *X-rays depicting internal fixation of the tibia and fibula with plates and screws*

Following further advances in materials and in surgical technique, blood vessel replacements were tried in the 1950s, and heart valve replacements and cemented joint replacements in the 1960s. It was also at this time that researchers formed a new field called tissue engineering in order to combine the fields of materials science and cell biology. The term tissue engineering was introduced into medical research for the development of biological substitutes to maintain, restore and improve functions of lost organs and tissues (Nomi et al. 2002). Thomas et al. (2003) describes some the advances in relation to vascular tissue engineering, but states that synthetic materials are suitable for large bore arteries but often thrombose when used in smaller arteries. Rémy-Zolghadri et al. (2004) also recognises this problem and presents work carried out on a tissue engineered blood vessel produced *in vitro* by the self assembly method for the replacement of small-diameter blood vessels.

Applications using biomaterials include the replacement of body parts that have lost functionality due to disease or trauma (Figure 1.2), to assist in healing, to improve function and to correct abnormalities. For example, the human eye is a complex organ, which is a major target for a range of implants and accessory

biomedical devices. A wide range of biomaterials e.g. hydrogels are used to fabricate ocular devices to correct functional deficiencies caused by disease, age and ocular trauma. Hydrogels are also used to assist wound healing as they provide a moist healing environment. This allows wounds to heal up to 40% faster than wounds that are exposed to air (Park and Nho 2003). Advances in many areas of medicine have influenced the role of biomaterials considerably. For example, with the advent of antibiotics, infectious disease is less of a threat than in former times, so that degenerative disease assumes a greater importance. Moreover, advances in surgical technique have permitted materials to be used in ways that were not previously possible (Ratner 1996).

Figure 1.2 *A compression plate used as an internal fixation device to restore functionality in a deteriorated hip joint.*

Although biomaterials are primarily used for medical applications, they are also used to grow cells in culture. These cell cultures are of vast importance in their own right. Wilke et al. (1993) describes a cell culture system for biocompatibility testing, using bone marrow cells, while Lombello et al. (2000) describes the use of poly(hydroxyethyl methacrylate) (PHEMA) to culture cells not dependent on

anchorage to name but two. Biomaterials can also be used in apparatus for handling proteins, in the aquaculture of oysters and possibly in the near future they will be used in a cell-silicon biochip that would be integrated into computers. Bienias (2002) discusses the changing role of the medical device manufacturer. In a bid to increase market share, suppliers are being led into supplementing their traditional services or products with new value-added services that enhance their products and bring new value to the customer.

Biomaterials must always be considered in the context of their final fabricated, sterilised form. For example when a polyurethane elastomer is cast from a solvent onto a mould to form a heart assist device, it will elicit different blood-material interaction than when injection moulding is used to form the same device. The world biomedical device market in 1995 was estimated at being worth €89 billion. Biomaterials based medical devices accounted for around 12% of this market that amounts to €10.5 billion in sales value. With annual growth of around 15% (Egan and Waterman 1998) the 2005 global biomaterials based market was expected to be around €43 billion and should rise to €49.5 billion in 2006.

1.2 Polymers as biomaterials

Polymers have been used in biomedical applications, ranging from naturally occurring materials such as natural rubber and cellulose to synthetic elastomer, polyurethanes and hydrogels. Polymers are selected so that they can serve both the purposes of tissue engineering (degradable) and biosensors (stable) as well as implants (degradable/stable). Polymers have found applications in such diverse bio-medical fields as tissue engineering, implantation of medical devices and artificial organs, dentistry, bone repair, coatings for medical devices. Nathan et al. (1995) describes tissue engineering perivascular endothelial cell implants. These endothelial cells were engrafted onto gelfoam bio polymeric matrices. Gelfoam is a natural polymer and is isolated from porcine dermal gelatine. These extravascular implants were then used to control vasculoproliferative disease. Hong et al. (1996a, 1996b, 1998) describes polyvinyl pyrrolidinone (PVP) as a possible vitreous substitute and describes experiments based on implantation of crosslinked PVP into the rabbit eye. Nurdin et al. (1996) and Conroy (1998) both describe the use of hydrophilic polymers as coatings for medical devices, and publish results to prove that there is a

significant reduction in friction when using hydrophilic-coated medical devices compared to non-coated medical devices.

The main requirements of polymers used in the body are that they be biocompatible, at least on their surface, not eliciting an excessive or chronic inflammatory response upon implantation, and for those that degrade, that they breakdown into non-toxic products (Mickiewicz 2001). Functional groups properly located on a polymer, as well as in its structure are usually responsible for its biocompatibility and / or biodegradability and may impart on it either therapeutic and / or toxic characteristics. For example carboxylic groups induce therapeutic activity of many drugs.

Jagur-Grodzinski (1998) notes that many polymer systems used for implantation of medical devices are considered to be biocompatible, however after implantation they become isolated from the tissues of the body by collagenous encapsulation, therefore the body rejects the polymer. However as a bio-film is generated on the entire surface, they do not produce any harmful effects.

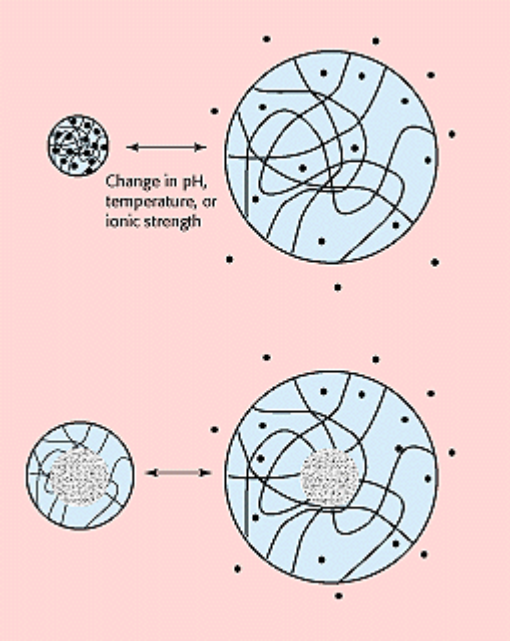


Figure 1.3 *Drug release and drug entrapment from polymeric hydrogels.*

Biodegradable polymers may be either synthetic or of natural origin. Piskin (1995) undertook a review of biodegradable polymers used as biomaterials and considered them with reference to natural polymers (albumin, collagen and gelatin, chitin and chitosan) and synthetic polymers (poly α-hydroxy acids, poly α-amino

acids, poly ε-caprolactone, poly ortho-esters, polyanhydrides, etc.). Some of the mechanisms of the degradation of these polymers are firstly hydrolysis *in vivo* to give off non-toxic alcohols, acids and other low molecular weight products which are easily eliminated from the body, secondly hydrolytic erosion of unstable crosslinks in high molecular weight polymers, and thirdly water insoluble polymers may be converted into water-soluble polymers as a result of ionisation, protonation, or hydrolysis of side chains. Such conversions do not significantly affect molecular weight, but may be responsible for bio-erosion in typical applications.

1.3 Polyvinyl pyrrolidinone

Polyvinyl pyrrolidinone was first synthesised in the 1930s by Walter Reppe and was patented in 1939, since then there has been extensive research and use of PVP in the biomedical field. The first use of PVP in medicine was during World War II when a 3.5% w/v solution of PVP was infused into patients as a synthetic blood plasma volume extender. This application showed PVP's solubility in water and demonstrated that it appeared to be biologically inert. As well as the properties of PVP shown by this application, it has been found that PVP also exhibits a unique combination of properties, including solubility in water and in organic solvents, very low toxicity, high complexing ability, good film forming characteristics, and the ability to adhere to a number of substrates. As a result of these properties, PVP finds use in a wide variety of industries. Its film-forming and adhesive properties are utilised in hair sprays, adhesives, and photographic and lithographic coatings. Its complexing ability is utilised in the textile industry for improving dye-ability and dye stripping, in the pharmaceutical industry and agriculture for drug dissolution and sustained release of drugs and chemicals, and in the food industry for the clarification of beer and wine (Kroschwitz 1985a).

Ravichandran et al. (1997) states that PVP undergoes very slow hydrolytic degradation in the physiological environment to yield non-toxic normal metabolites. This is desirable in many controlled release systems, as it is not necessary to remove the polymer after diffusion controlled release has taken place. The toxicity of PVP has been extensively studied in a variety of species, including humans and other primates and has been proved to be of a very low order (Robinson et al. 1990). However, Pemawansa and Khan (2001) notes that intravenous applications of PVP

are now restricted because of the accumulation of high molecular weight fraction in the body since it is not metabolised or degraded contrary to what Ravichandran et al. (1997) had stated. Ravichandran did however note that this process of degradation is very slow and may take from several weeks to months depending on the molecular weight. PVP is still widely used in food and pharmaceutical formulations since it is physiologically inactive. Therefore, the dose of LD50 at peroral and intravenous applications is 100g/Kg and 10-15g/Kg respectively (Kirsh 1998). Hong et al. (1998, 1996a, 1996b) investigated the use of PVP as a vitreous substitute. In one research article (Hong et al. 1996a) over 300 copolymers of PVP were examined as vitreous substitutes. After preliminary selection based on mechanical and optical properties only 13 hydrogels were felt suitable for further study. In other work (Hong et al. 1996b) it was stated that the PVP hydrogel in question showed no *in vitro* cytocidal effects and no adverse reactions in the retinas of experimental animals. However histology experiments showed the existence of polymer particles in some retinal layers. This indicated possible biodegradation of the polymer. This biodegradation was further studied (Hong et al. 1998) both *in vitro* (using two proteolytic enzymes) and *in vivo* in the rabbit vitreous over four weeks. While no biodegradation occurred *in vitro*, half the PVP hydrogel disappeared from the rabbit vitreous.

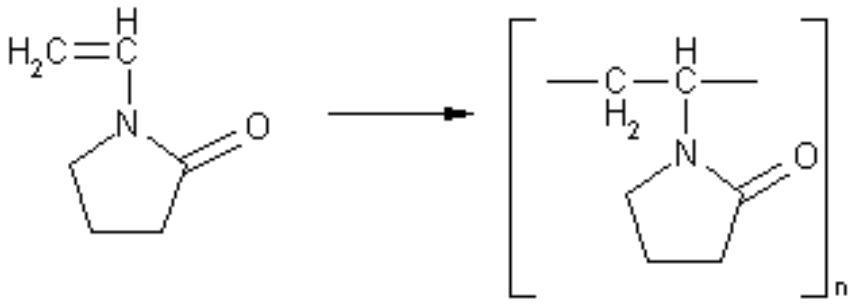


Figure 1.4 *Structures of N-vinyl pyrrolidinone, and polyvinyl pyrrolidinone*

Both Coombes et al. (1998) and Bezemer et al. (2000) compared the use of PVP and polyvinyl alcohol (PVOH) as stabilisers in the external phase in the water-in-oil-in-water method of polymerisation. The resultant polymers were then used in protein release. The former used poly (DL-lactide co-glycolide), while the latter used copolymers of poly (ethylene glycol) (PEG) and poly(butylene terephthalate) (PBT). Coombes found that PVP caused a reduction in the initial burst release of ovalbumin

(OVA) together with sustained protein release over 28 days and an increase in the protein delivery capacity, and suggested that diffusion of PVP from the external phase into the polymer solution droplets caused an increase in viscosity which was attributed to the changes in protein loading and delivery characteristics. However Bezemer found that PVP was less effective in stabilising the emulsion droplets than PVOH.

PVP is a white or slightly yellow hydroscopic powder, which forms hard, clear films. The physical properties of PVP are generally determined on either films or powder. The polymer interacts strongly through dipole attraction, and so the melt viscosity is too high for typical thermoplastic forming operations. However these interactions do have their advantages. Pemawansa and Khan (2001) notes that PVP interacts with a range of guest molecules, for example iodine, β-carotene, tolbutamide and resepine by accepting hydrogen bonds as well as non-polar groups and with this in mind non-covalent adducts of PVP and poly(α, L-Glutamate) (PGNA) were examined as part of an ongoing program involving the delivery of peptide drugs from polymer-peptide complexes. Preliminary data suggests that PVP and PGNA molecules are well dispersed in the non-covalent adduct.

PVP can be prepared in a variety of molecular weights, depending on the conditions of polymerisation. The hydrogen peroxide-ammonia process gives a molecular weight range of 2500 to about 1.1×10^6. It has being suggested that the amount of water absorbed by PVP is virtually independent of the molecular weight (Kroschwitz 1985a). The equilibrium water content varies with the ambient relative humidity and is approximately one-third of the latter value (Figure 1.5). Approximately 0.5 mol of water is associated with one monomer unit, which corresponds to the values given for the hydration of proteins.

In accordance with the characteristics of pyrrolidinone, PVP is chemically inert. The dry polymer can be stored under normal conditions without decomposition, degradation, or structural change. The heat sensitivity of PVP is quite low and it is stable at 130°C for short intervals. However, at 150°C in air, solubility is reduced and colour increased: after an extended period of time, the polymer becomes insoluble. Heating with ammonium persulfate at 90°C for 30 minutes renders PVP insoluble. Diazo compounds and oxidative agents in the presence of light results in gelation. Gels retain their structure on drying and can be

swollen with the absorption of water. When heated with strong bases, PVP precipitates irreversibly from aqueous solution because of an opening in the pyrrolidinone ring and subsequent crosslinking (Kroschwitz 1985a).

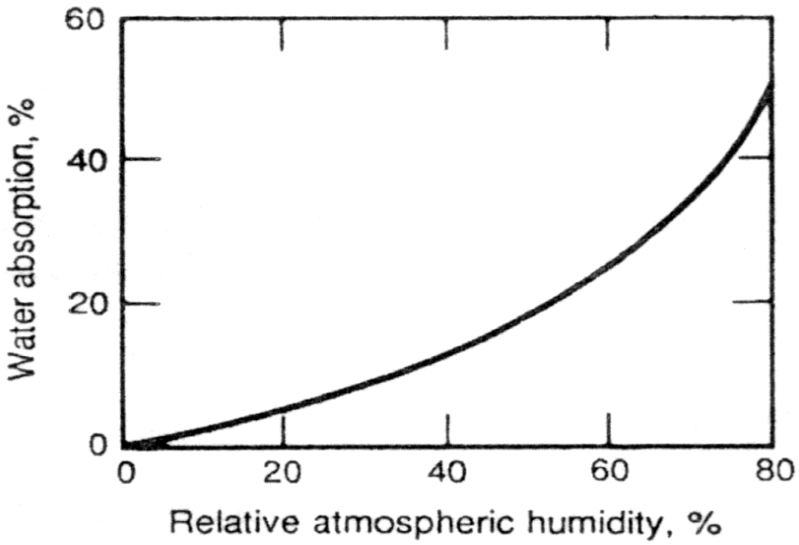


Figure 1.5 *Hydroscopicity of PVP (Kroschwitz 1985a)*

Lightly crosslinking PVP can be prepared by treatment of linear PVP with persulfates, hydrazine, and hydroperoxide, with diolefins in the presence of peroxides or with γ-irradiation. The products gel when treated with water but do not dissolve. Copolymerisation with multifunctional, unsaturated compounds gives densely crosslinked polymers swellable in water and organic solvents. Nurtin et al. (1996) describes the use of a PVP polymer crosslinked with an isocyanate (Hydromer®) coating for a polyurethane catheter. It was found that the PVP coating induced a hydrophilic surface after immersion of the catheters in water for several hours. It was concluded that the PVP coating constituted an effective treatment for improving wettability and frictional properties of polyurethane catheters at a micro-scale. However, it was also suggested that prehydration may be necessary as the surface was highly hydrophobic in air, whereas after several hours in water, the surface was totally wetted by water.

1.4 Acrylic acid

Acrylic acid (AA) Figure 1.6, was first prepared in 1843 by air oxidation of acrolein obtained from the high temperature cracking of glycerine. However, despite considerable research on the synthesis, chemistry, and polymerisation of AA, it was

not until 1930 that the technical obstacles for the manufacture and handling of these reactive monomers were overcome. AA and its esters are primarily used to prepare polymers and copolymers. The polymeric products can be made to vary widely in physical properties through control of the nature and ratio of monomers employed in the preparation, and control of the degree of crosslinking and molecular weight. AA is used widely to impart pH sensitivity (Am Ende and Peppas 1996; Ravichandran et al. 1997; Yaung and Kwei 1997; Peppas and Wright 1998; Jones 1999; Bures and Peppas 2000). Am Ende and Peppas (1996) polymerised two polymers, poly (acrylic acid) (PAA) and PAA-co-2-hydroxyethyl methacrylate (PAA-co-HEMA), and studied their potential as bioadhesive controlled release dosage forms. Among other results the effect of pH and crosslinking ratio on water up-take were illustrated, as well as the ionisation of PAA hydrogels.

PAA has being widely studied for its pH dependent swelling characteristics. Peppas and Wright (1998) investigated the use of PVOH and PAA copolymers for use in controlling drug and protein diffusion. The content of PAA was varied in order to examine the effect of ionisation on drug diffusion at pH 3 and pH 6. It was found that drug release differed from pH 3 to pH 6 due to expansion of the mesh size at pH 6, as at pH 6 PAA is readily ionised, and therefore there is a reduction in intermolecular hydrogen bonding. Ravichandran et al. (1997) studied the development of a pH sensitive, biocompatible and biodegradable hydrogel for stomach specific drug delivery. The effect of pH on the swelling of the hydrogels was studied and it was found that at pH 6.8 the gels swelled to a higher degree than at pH 1.2, for example with initiator concentration at 2.75% the gels had swollen to 93% in pH 1.2 and 222% in pH 6.8. Yaung and Kwei (1997) photopolymerised a copolymer of PVP-PAA and again studied the swelling properties in various pH media. It was found that the release of caffeine was higher above a critical pH value of 3.8.

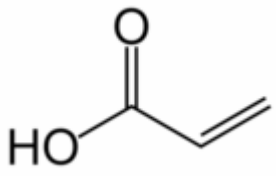


Figure 1.6 *Structure of acrylic acid*

AA is commercially available in better than 98% purity and usually contains 10-200 ppm of hydroquinone, monomethyl ether, or phenothiazine as polymerisation inhibitor. Because of their relatively high freezing points and potentially violent polymerisation, AA requires special handling conditions to avoid freezing and thawing. Thawing of bulk amounts of AA can be dangerous because the inhibitors may not be uniformly distributed between solid and liquid phases.

Linear polymers of acrylic acid can be prepared using the general free-radical initiation systems used for other vinyl monomers. Polymerisation in the aqueous solution at less than 20-30% concentration is readily controlled. However, the high heat of polymerisation makes it difficult to control polymerisation of highly concentrated solutions. In this instance, an uncontrolled exotherm may be produced, which may result in the production of a crosslinked polymer. AA is also readily copolymerised with many other monomers. Its versatility arises from the combination of their highly reactive double bonds and their miscibility with both water-soluble and oil soluble monomers.

1.5 Photo polymerisation

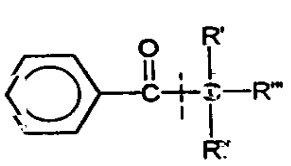
A photoinduced polymerisation reaction is usually considered as a reaction where the initiation step is produced by a photochemical event. Thus, on exposure to UV or visible light the liquid monomer formulation is converted into a solid polymer. However, if the monomer contains more than one reactive function, a crosslinked polymer network is readily produced. Photo polymerisation is different from photocrosslinking where every chain propagation step requires the absorption of a photon of light.

In the biomedical industry UV curing has evolved from technologies used in less demanding areas, such as the painting industry. Salthammer et al. (2002) investigated some of these properties in relation to the UV curing of surface coatings of furniture. It was found that although some problems did exist, UV coating technology had a number of benefits and these problems could be avoided by optimisation of the process. Kim et al. (1999) also stated that UV curing offers several advantages over conventional processes in the coating industry. These benefits included high speed of processing and high-energy efficiency since the polymerisation was carried out at room temperature. These properties are relevant to all coating industries. Most monomers, oligomers or pre-polymers commonly

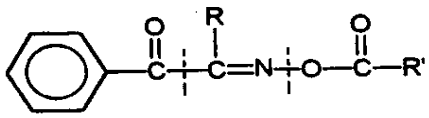
employed in photopolymerisation do not readily produce sufficient initiating species upon light exposure. Therefore, it is usually necessary to incorporate a photoinitiator that will start the polymerisation (Fouassier 1998).

A photoinitiator is an organic molecule employed alone or in a chemical system involving two or more molecules, which absorbs light and forms reactive initiating species e.g., radicals. This process is referred to as photoinitiation and results in both photophysical and photochemical processes occurring. An ideal commercial photoinitiator is expected to exhibit properties such as, easy synthesis, low price, absence of toxicity and odour and excellent shelf stability and pot life when dissolved in reactive monomers. A photoinitiator should also have a high absorption of incident light; this high absorption can be achieved either by matching the absorption spectrum of the photoinitiator with the emission spectrum of the light source as closely as possible, or designing molecules having high molar extinction co-efficient. When a photoinitiator is used in a chemical system, it is usually incorporated with a co-initiator and/or a photosensitiser. A co-initiator is the part of the chemical system that does not absorb light, but instead participates in the production of reactive species. A photosensitiser is a molecule that is able to absorb light and transfer the excitation to another molecule that will be used as a photoinitiator. The excitation transfer should basically be depicted by an energy transfer mechanism (Fouassier 1995; Angiolini and Carlini 1997).

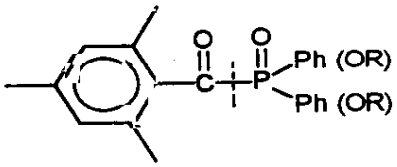
It is understood that photoinitiators react mainly through a triplet-state mechanism, which results in the initiation of species from the triplet state or to a lesser degree the singlet state. Therefore, the initiator should also have excellent reactivity of the initiating species towards the monomer compared to a number of competing processes such as reactions with oxygen, the solvent used (if any), impurities or additives present. There should also be no evidence of yellowing while in the presence of the crosslinked network.



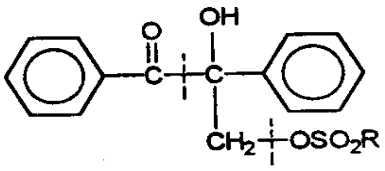
benzoin (R' = H, R'' = OH, R''' = Ph)
benzoin alkylethers (R' = H, R'' = OR, R''' = Ph)
benzil ketals (R' = R'' = OR, R''' = Ph)
dialkoxy acetophenones (R' = R'' = OR, R''' = Ph)
hydroxyalkylphenones (R' = R'' = R, R''' = OH)



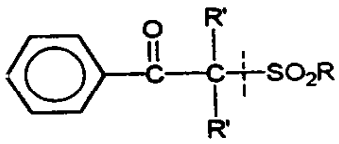
benzoyloxime esters



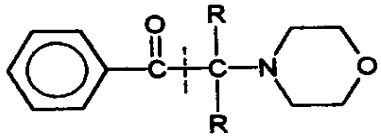
benzoyl phosphinoxides (phosphonates)



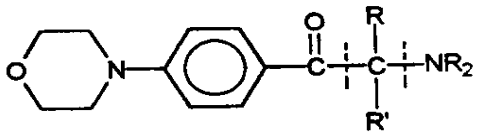
α–hydroxymethyl benzoin sulphonic esters



sulphonyl ketones



α–morpholino acetophenones



p-morpholino dialkylamino acetophenones

Figure 1.7 *Type I photoinitiators (dotted lines represent the most usual site of bond cleavage (Angiolini and Carlini 1997)*

There are two classes of photoinitiators that meet the required criteria for free radical polymerisation and these are derived from arylketones. The first type gives α or β cleavage upon UV irradiation with the formation of two radical species and the

second type generate bimolecular reactions between the excited state of a sensitizer and a synergist to give one initiating radical species. The type I photoinitiators (Figure 1.7) are most commonly used in the area of photocurable coatings due to their availability, high quantum yields of radical generation and monomer radical formations. In addition to these benefits, the excited triplet state of those molecules is not damped by the presence of oxygen, thus the photoinitiation is unaffected by the presence of air during the curing process.

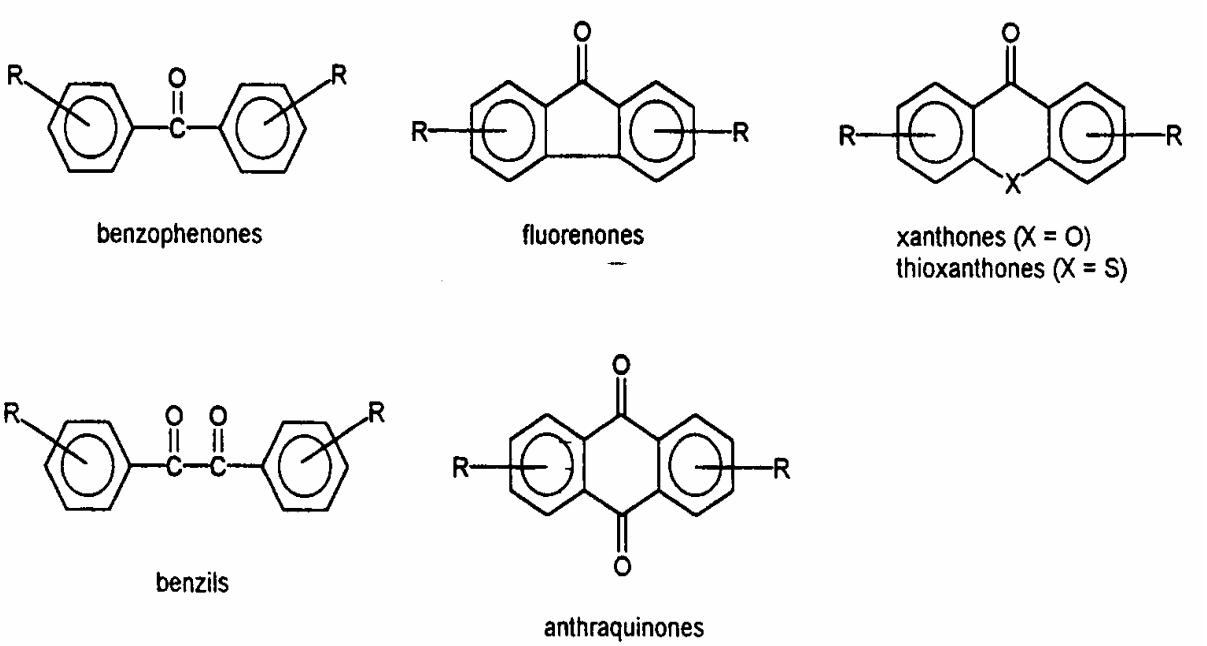


Figure 1.8 *Type II photoinitiator (Angiolini and Carlini 1997)*

Type II photoinitiators as illustrated in Figure 1.8 react through the hydrogen abstraction mechanism, in which the sensitiser chromophore (mainly a diarylketone Ar_2 C=O), is excited by the UV light to the triplet state which is similar to the type I photoinitiators, which undergoes photoreduction of the carbonyl group by reacting with a hydrogen donor, to give a ketyl radical and a primary radical species (Angiolini and Carlini 1997; Kroschwitz 1985b).

It has also been established that in the photoinduced vinyl polymerisation promoted by benzophenone, the ketyl (semipinacol) radical is mainly involved in termination reactions of the growing polymer chains, whereas the radical derived from the hydrogen donor is the actual initiating species. Photoreduction of type II photoinitiators can also be achieved by a reaction with electron donor molecules, such as amines, with formation of the ketyl radical and of an initiating amine-

derived primary radical species. Scherzer et al. (2002) examined the process of free radical formation from benzophenone. It was stated that the excitation of the carbonyl group in the benzophenone by UV light leads to the lowest triplet state that can be described as a biradical. By abstraction of a hydrogen atom from the polymer backbone, a ketyl and carbon-centered radical are formed. Recombination of these species leads to crosslinks. Allmér (1995) and Tierney et al. (2004) both employed benzophenone to abstract a hydrogen atom from the backbone of the polymer to develop a surface bound free radical. This allowed the grafting of an unsaturated monomer onto the surface and thus introduced a hydrophilic layer onto the surface of a hydrophobic polymer.

Photopolymerisation is currently being used for an increased number of biomedical applications due to its ability to rapidly convert liquid monomer into a crosslinked network and also because the use of organic solvents during the polymerisation process is not always necessary (Lopérgolo et al. 2003). Nguyen and West (2000) presented a review on UV curable hydrogels that may be used as biomaterials in medical applications including tissue engineering. The use of photopolymerisation of these hydrogels in situ is also discussed and the possibility of their use in a minimally invasive manner where the liquid monomer can be injected and polymerised in situ.

1.6 Hydrogels

The term hydrogel is used to describe materials that are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water or biological fluids. These hydrogels exhibit a thermodynamic compatibility with water, which allows them to swell in aqueous media. Hydrogels are best considered as polymeric materials, which are able to swell in water and retain a significant fraction (typically 30%) of water within their structure, but do not completely dissolve in water, (LaPorte 1997) although some do undergo hydrolytic degradation (Ravichandran et al. 1997; Kunioka and Choi 1998). Hydrogels are prepared by synthetic chemical reactions from a range of specialty vinyl and acrylic monomers. A list of monomers most commonly used in the preparation of polymeric hydrogels is presented in Table 1.1.

Table 1.1 *Monomers most often used in the synthesis of synthetic hydrogels for pharmaceutical applications*

Monomer abbreviation	***Monomer***
HEMA	Hydroxyethyl methacrylate
HEEMA	Hydroxyethoxyethyl methacrylate
HDEEMA	Hydroxydiethoxyethyl methacrylate
MEMA	Methoxyethyl methacrylate
MEEMA	Methoxyethoxyethyl methacrylate
MDEEMA	Methoxydiethoxyethyl methacrylate
EGDMA	Ethylene glycol dimethacrylate
NVP	N-vinyl-2-pyrrolidinone
NIPAAm	N-isopropyl acrylamide
Vac	Vinyl acetate
AA	Acrylic acid
MAA	Methacrylic acid
HPMA	N- (2-hydroxypropyl) methacrylamide
EG	Ethylene glycol
PEGA	PEG acrylate
PEGMA	PEG methacrylate
PEGDA	PEG diacrylate
PEGDMA	PEG dimethacrylate

Hydrogels are becoming increasingly important materials for pharmaceutical applications. They are used in a variety of applications including diagnostic, therapeutic and implantable devices for example controlled release drug delivery systems which have been studied extensively (Graham 1990; Florence 1993; Brazel and Peppas 1996; Am Ende and Peppas 1996; McNair 1996; LaPorte 1997; Ravichandran et al. 1997; Aikawa et al. 1998a; Aikawa 1998b; Grass et al. 2000; Murata et al. 2000; Risbud et al. 2000; Ruel-Gariépy et al. 2000; Kishida and Ikada 2002; Varshosaz and Koopaie 2002;), contact lenses (Perera and Shanks 1996; LaPorte 1997; Pöhlmann et al. 1997; Shoji et al. 1997; Kishida and Ikada 2002), wound dressings (Corkhill et al. 1989; Chen et al. 1993; Hilmy et al. 1993; Weibin et al. 1993; Higa et al.1999; Jandera et al. 2000; ; Razzak et al. 2001; Ishihara et al. 2002; Kirker et al. 2002; Lugáo et al. 2002; Scherzer et al. 2002; Park and Nho 2003;

Tanodekaew et al. 2004) and tissue engineering (Nguyen and West 2000). Hydrogels have been widely used in such applications because of their biocompatibility with the human body and also because they resemble natural living tissue more than any other class of synthetic biomaterials. This is due to their high water content and soft consistency that is similar to natural tissue, (Anseth et al. 1996; Ravichandran et al. 1997; Nguyen and West 2000; Peppas and Bures 2000; Risbud et al. 2000; Kishida and Ikada 2002), which has a equilibrium water content (EWC) in excess of 99% (Lovell and Bowman 2003).

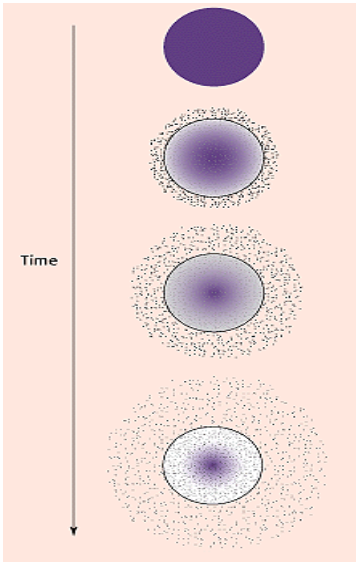


Figure 1.9 *Swelling and drug release from a hydrogel*

Hydrogels are prepared by synthetic chemical reactions from a range of speciality vinyl and acrylic monomers. Hydrogels may be composed of homopolymers or copolymers and are insoluble due to the presence of chemical crosslinks, or physical crosslinks such as entanglements or crystallites (Peppas et al. 2000). Anseth et al. (1996) explored methods for analysing mechanical properties of hydrogels and their dependence on polymer structure, especially the crosslinking density and the degree of swelling. Methods for measuring the elastic and viscoelastic properties of hydrogels were examined along with mechanisms for controlling these properties. Suggested examples for controlling these properties are: variations in the polymer composition; the crosslinking density and the polymerisation conditions. Perera and Shanks (1996) co-polymerised hydrogels of HEMA/NVP and methyl methacrylate (MMA)/NVP in the presence of ethylene glycol dimethacrylate and methylene diacrylamide (MDA) as chemical crosslinking

agents, using photo-polymerisation. It was found that the use of MDA crosslinked networks appeared to be softer (lower elastic modulus) than EGDMA.

Chemical gels are three-dimensional molecular networks formed by the introduction of primary covalent crosslinks. Unless the covalent bonds can be broken, these types of gels do not dissolve in water or other organic solvents even upon heating: rather, they are observed to swell. As the properties of hydrogels are highly dependent on the components used to produce the polymer, and these components can be interchanged easily, a limitless number of hydrogels can be produced. Hong et al. (1996b) prepared over 300 chemically crosslinked hydrogels for initial evaluation, however it was found that only 4% of the hydrogels tested warranted further assessment.

Two common techniques used to produce chemically cross-linked hydrogels are the polymerisation of a water-soluble monomer in the presence of an appropriate crosslinking agent and crosslinking of an existing hydrophilic polymer, again with the use of a crosslinking agent. Both general methods obey common reaction mechanisms and can be implemented in a variety of ways. It is essential that monomeric species have functionality greater than two in order to form the characteristic three-dimensional structure of a gel (LaPorte 1997). A commonly used chemical crosslinking agent is ethylene glycol dimethacrylate, (Davis et al. 1988; Hong et al. 1996b; Hong et al. 1998; Peppas and Wright 1998) as well as polyethylene glycol dimethacrylates with various molecular weights (Hong et al. 1996b).

Physical gels are hydrophilic networks comprised of an amorphous hydrophilic polymer phase held together by highly ordered aggregates of polymers chain segments arising from secondary molecular forces (Van der Waals forces) in conjunction with other types of molecular interaction. Unlike chemical gels, these types of hydrogel systems eventually will dissolve in water or solvents and can be melted by applying heat to the systems. Stauffer and Peppas (1992) described a method of preparing a strong polyvinyl alcohol hydrogel without utilisation of chemical crosslinking or other reinforcing agents. This was achieved by freezing/thawing cycles of an aqueous solution containing 10-15wt% PVOH. The process of reinforcement was a densification of the macromolecular structure. It was noted that the strength, stability and swelling ratio of the gels were a function of the solution concentration, freezing time and number of freezing/thawing cycles, and

that freezing 15 wt% PVOH solution at -20°C for 24hrs followed by thawing at 23°C for 24 hrs produced the strongest gels. Investigation of the swelling ratios indicated that denser structures were observed after five freezing/thawing cycles. Nugent and Higginbotham (2005) prepared novel PVOH hydrogels also utilizing the freeze/thaw method. However it was found that the incorporation of NaOH into the solution prior to freeze/thawing produced a strong gel, thus the need for repeated freeze/thawing cycles was alleviated. Hassan and Peppas (2000) further discussed the use of the freeze/thaw method for physically crosslinking PVOH hydrogels. This method of preparing hydrogels addresses the toxicity issues because it does not require the presence of a crosslinking agent. It was also noted that such physically cross-linked materials also exhibit improved properties, most notably higher mechanical strength than PVOH gels cross-linked by chemical or irradiative techniques because the mechanical load can be distributed along the crystallites of the 3-dimensional structures. However the long-term stability of such gels still remains an important issue. Jianqi and Lixia (2002) also discussed how physically crosslinked hydrogels could be formed: however, in this instance a thermo-crosslinking method was employed.

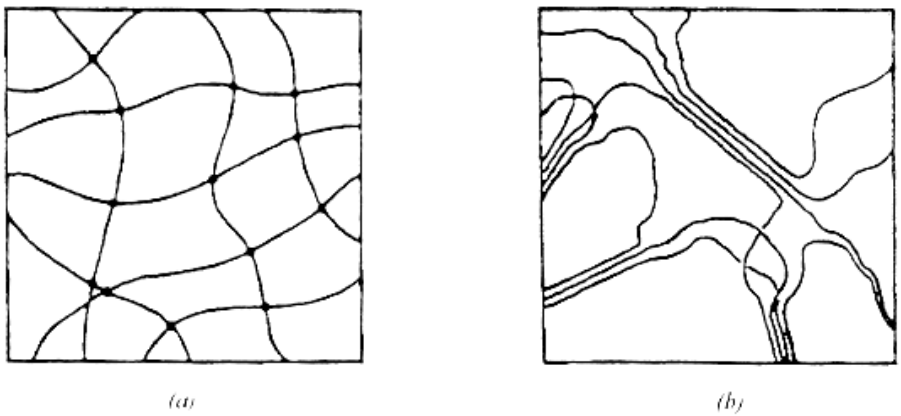


Figure 1.10 *Schematic representation of general structure of (a) chemical and (b) physical gels (LaPorte 1997)*

The configuration of the ordered domains in physically crosslinked hydrogels varies with the type of secondary forces involved in their formation, as well as the general molecular structure and configuration of the hydrophilic polymers that comprise them. Some of the intermolecular forces responsible for giving rise to these physical bonds include London dispersion forces, permanent dipoles, hydrogen

bonding, and ionic attraction. These forces are cumulative in nature and contribute greatly to the ordered structure exhibited by the physical bonds. The so-called "bonds" that hold physical gels together are actually sections of the polymer chain that are in the crystalline state. Segments of the molecule arrange themselves into a tightly packed repeating structure. To achieve this, the polymer must exhibit a certain degree of structural regularity along the main chain (LaPorte 1997).

1.7 Hydrogels in wound healing applications

A dressing often covers the wound to accelerate its healing. There are two types of dressings, dry and wet. Yoshii et al. (1999) notes that Winter, (1965) reported that healing in a wet environment is faster than a dry environment. Wound dressings before the 1960s were considered only to be passive products having a minimal role in the healing process. The pioneering research of Winter initiated the concept of an active involvement of a wound dressing in establishing and maintaining an optimal environment for wound repair. This awareness resulted in the development of wound dressings from being traditionally passive materials to functional active dressings, which through the interaction with the wounds they cover, create and maintain a moist and healing environment. This is due to the fact that renewed skin, without the formation of eschar, takes place during healing in a wet environment. The concepts put forward by Winter have continually been developed, and recently Kirker et al. (2002) reported the development of a hydrogel film as bio-interactive dressings for wound healing. The hydrogel films described are glycosaminoglycans (GAGs) based hydrogels. GAGs including hyaluronan (HA) and chondroitin sulphate (CS) are aminosugar-containing polysaccharides in the extracellular matrix (ECM) of all vertebrates. From *in vivo* analysis of the GAG film plus Tegaderm ™ and Tegaderm ™ only, it was found that a significant increase in re-epithelialisation was observed with the incorporation of the GAG hydrogel films, and that no significant difference in inflammatory response was observed.

Maintaining a moist wound environment facilitates the wound healing process. The beneficial effects of a moist versus a dry wound environment include: prevention of tissue degradation and cell death, accelerated angiogenesis, increased breakdown of dead tissue and fibrin, i.e., pericapillary fibrin cuffs, and potentiating the interaction of growth factors with their target cells. In addition, pain is significantly reduced when wounds are covered with an occlusive dressing.

Concerns that moisture in wounds may increase the risk of clinical infection over traditional therapies are unfounded. The use of hydrocolloid occlusive dressings in maintaining a moist environment has proved to be useful adjunct in facilitating wound healing (Field and Kerstein 1994). Hydrocolloid-type dressings that give better conditions for healing have been developed consisting of gelatin, pectin, water and hydrophilic polymer which promote healing. However, some of the dressing material can stick to the wound because of its low strength, and the wound often needs rinsing with physiological saline solution to remove the remaining material of the dressing. Such treatment can cause damage to the renewed skin. Hence, a hydrogel having good strength is expected to be better as a dressing material (Yoshii et al. 1999).

The development of synthetic occlusive wound dressings, used for the treatment of burns, granulation tissue, dermatitis, ulcerations, blisters, fissures, herpes and several other skin conditions is a subject of great commercial interest (Corkhill et al. 1989). An ideal wound dressing should have several key attributes. The dressing should protect the wound from bacterial infection, control permeability of oxygen and carbon dioxide, absorb wound extrudate, provide a moist healing environment and be permeable to water vapour and metabolites. Additionally, it should be composed of materials that are biocompatible, non-toxic, non-immunogenic, non-antigenic, flexible, strong, durable, and comfortable when worn (Corkhill et al. 1989; Ishihara et al. 2002; Kirker et al. 2002).

From a review by Field and Kerstein (1994) it can be seen that there has been contradictory reports on the role of oxygen in the wound healing process. In the past it was believed that increased rates of epithelialisation and collagen production (two integral components of wound healing) was dependent on increased concentration of ambient oxygen in the wound. However Field states that more recent evidence supports the exact opposite hypothesis; i.e., wound healing is stimulated and facilitated by a relatively hypoxic wound environment. *In vitro* trials have demonstrated that optimal growth of fibroblasts in tissue culture occurs at low partial pressures of oxygen (5-10 mm Hg), and epidermal cell growth is inhibited at oxygen levels higher than that in the surrounding air.

Hydrogels possess many of the above properties and because of this they have been used extensively as wound dressing materials, sometimes alone but frequently in the form of composites, primarily to enhance their mechanical strength.

Synthetic materials such as polyurethane (PU), PVOH, PHEMA and copolymers of these materials, as well as biological materials like bovine collagen, chitin and alginate, have been investigated, in the search for an ideal wound dressing (Kirker et al. 2002).

Figure 1.11 *(Left) Characteristics of moist wound healing are rapid movement of epithelial cells across the surface and decreased surface inflammation. (Right) Characteristics of dry wound dressings are slower epithelial movement as enzyme debridement through eschar is required and increased wound inflammation (extrudate)*

The healing of full thickness skin defects, such as in chronic wounds (leg ulcers) or deep burns comprise complex processes leading to the formation of new tissue. Wound contraction and scar formation are still unavoidable components of the healing process. In chronic wounds the imbalance between synthesis and degradation of extracellular matrix is one of the characteristics leading to non-healing (Middelkoop et al. 2004). The healing of a wound proceeds in three overlapping phases, namely (i) inflammation, (ii) granulation tissue formation, and (iii) matrix formation and remodelling. This sequential process requires the interaction of cells in the dermis and epidermis, as well as the activity of chemical mediators released from inflammatory cells, fibroblasts and keratinocytes. The proliferation of mesenchymal cells and capillaries, as well as the influx of macrophages composing granulation tissue serve to replace the lost dermis and to provide substrates and inducers for re-epithelialisation. It is hypothesised that the application of growth factors which induce fibroblast and/or endothelial cell proliferation to healing-impaired wounds might increase the rate and degree of granulation tissue formation and thus stimulate wound repair (Obara et al. 2003). Following wounding, platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF-β) are considered to be among the first growth factors to be

released, for the purpose of stimulating fibroblast proliferation and as chemoattractants for macrophages, neutrophils, and smooth muscle cells. Macrophages, specialised white blood cells present in the wound bed during the inflammatory and repair process, also secrete growth factors (including epidermal growth factor (EGF), fibroblast growth factor (FGF), interleukin-1 (IL-1) PDGF, and TGF-β. Among others, these growth factors act as epidermal cells or keratinocytes as they differentiate to form the outer stratum corneum and help the process of angiogenesis by providing a stable collagen matrix. In essence, during the healing cycle, numerous growth factors are released, depending on the needs of the wound. However until such time as which combinations of growth factors are needed and, when an environment that has been shown to retain those provided by nature should be provided (Field and Kerstein 1994). Work on delivering such growth factors has taken place, Obara et al. (2003) evaluated the use of a chitosan hydrogel as a carrier material for the controlled release of FGF-2 for use in wound occlusion and healing acceleration in the healing impaired, for example in diabetics. It was found that accelerated healing occurred *in vivo* in the healing impaired model, due to the addition of FGF-2 and that FGF-2 remained bioactive and was released upon biodegradation of the hydrogel. It is important to note that until the epidermal layer of skin has regenerated and the protective barrier is restored, a patient will remain at risk of infection and will continue to suffer loss of water, heat, and nutrients from the wound surface (Kirker et al. 2002).

Cooling the burn wound has been used empirically for centuries in an attempt to reduce pain and decrease mortality. Ordinary tap water is recommended by the British Burn Association as the treatment of choice for the first aid management of burns and scalds. However, the best method and the ideal temperature required to achieve cooling are unknown, as the published data regarding cooling is both confusing and sometimes even contradictory. This is largely because different models have been used in experimentation and different criteria have been used to assess outcome (Jandera et al. 2000). Jandera et al. (2000) investigated the cooling effect of different modalities on the burn wound and to assess their effect on wound healing, using a new commercial dressing designed for the acute treatment of burns. The hydrogel by composition contains 96% water, melaleuca alternifolia oil and emulsifiers at a pH 5.5 – pH 7. The hydrogel was

impregnated within a thin layer of foam and presented sterile in different size sheets within sealed aluminium packets. It had a turpentine-like odour. The melaleuca oil was compared with tap water in a controlled deep partial thickness burn created on the back of a porcine model. It was found that a single application of melaleuca hydrogel was as effective as repeated cold water compresses, which is advantageous as it is unlikely that a melaleuca hydrogel application would lead to significant hyperthermia when applied on a large burn. However, it was also found that exposed melaleuca hydrogel showed signs of drying out after one hour and an additional application may be required, if cooling were to be continued for an extended period.

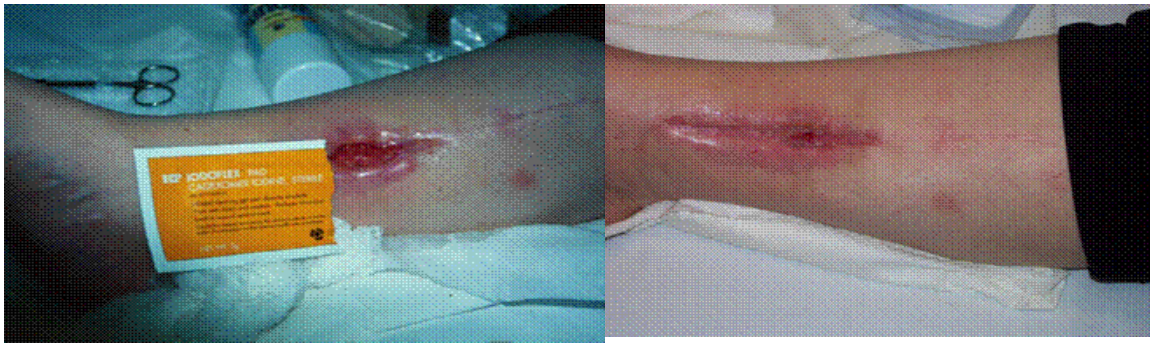


Figure 1.12 *Single wound treated on one side with a hydrogel type dressing. As can clearly be seen the wound portion that was covered with the hydrogel type dressing has healed considerably more than the control portion of the wound.*

Wound dressings manufactured by the simultaneous radio-induced crosslinking and sterilisation of hydrophilic polymers were invented by Rosiak et al. (1989). These dressings showed good biocompatibility and are widely used, not only as wound dressings, but also as drug delivery systems and in many others applications (Lugáo et al. 1998). These dressings were made by simultaneous sterilisation and radiation crosslinking and of polyvinyl pyrrolidone, polyethyleneglycol and agar. The resultant hydrogels had many interesting properties that include: immediate pain control; easy replacement; transparency to allow healing follow up; absorption and prevention of loss of body fluids; acting as a barrier against bacteria; good adhesion; good handling; oxygen permeability; control of drug dosage and so on (Higa et al.1999). Since the original patent several papers have been published using radiation induced curing of PVP copolymers. Hilmy et al. (1993) found that the addition of agar and PEG could be

used to improve the mechanical properties of a PVP hydrogel. It was reported that the hydrogels produced acted as a barrier against bacteria, absorbed a significant amount of water having a EWC of over 90%, and that they were elastic, transparent and soft, but strong enough to be used as a dressing. Even in a tropical environment (temperature 29 ± 2°C and humidity 80 ± 10%) the hydrogels were not penetrable for microorganisms. Lugáo et al. (1998) further investigated these hydrogels and found that the hydrogel network formed was composed of pure PVP and that agar and PEG acted only as a plasticiser and were not grafted or crosslinked to the network. The lubriciousness of the hydrogels was connected mainly to the amount of water and plasticiser present in the PVP network. Higa et al. (1999) investigated the *in vitro* biocompatibility of the wound dressing. The results showed that the mechanical properties were in an acceptable range of values and that the membranes could be considered non-toxic and non-hemolytic to cells. Lugáo et al. (2002) also studied the rheological behaviour of these hydrogels. The effects of different chemical substances used as additives in the PVP network were analysed in order to change the gel characteristics. It was found that glycerol increased the fluidity of the gel. The influence of PEG was dependent on the amount of PEG, and its molecular weight. As the amount and molecular weight of PEG increased, an increase in the crosslink density was observed. Finally it was found that the incorporation of high molecular weight polyethyleneoxide (PEO) yielded gels with higher elasticity. In somewhat of a review, Rosiak et al. (2003) described several applications that these hydrogels can and have been used. Some of these applications are: drug delivery systems; encapsulation of living cells towards hybrid organs; an approach to develop polymeric materials for intervertebral disc implant; temperature sensitive membranes; gel dosimeter for radiotherapy; degradation resistant nanoporous hydrogels and microporous hydrogels for biomedical purposes, hydrogel based dietary products and biopolymers of controlled molecular weight.

Razzak et al. (2001) also investigated a PVP copolymer prepared by high-energy irradiation for biomedical applications. However, PVOH was used as a second component instead of agar. The PVOH-PVP blended hydrogel showed some of the requirements of an ideal wound dressing: it effectively absorbed extrudate from the wound; it was pleasant to touch and painless to remove; it

exhibited high elasticity and good mechanical strength; it had good transparency and could act as a barrier against microbes. It was found that the hydrogel wound dressing had potential for use in a tropical environment. However, the EWC of 60-80% did not compare favourably to that achieved by the Rosiak method, which was over 90% (Hilmy et al. 1993; Lugáo et al. 1998).

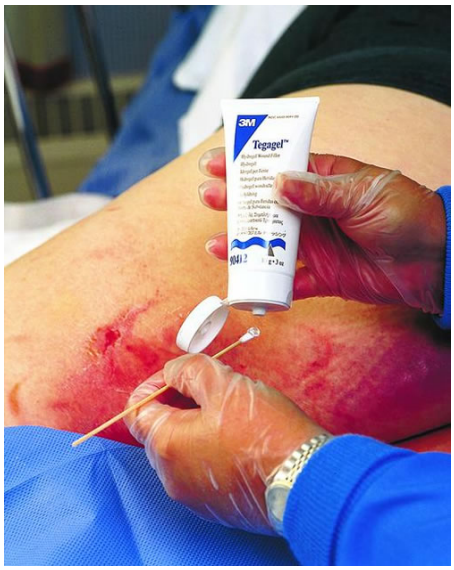


Figure 1.13 *A commercially available hydrogel for use in wound healing applications. The hydrogel is a clear hydrating gel for management of non-draining, minimally-draining and necrotic wounds.*

As illustrated, much of the work carried out on hydrogels for biomedical purposes, has utilised a high-energy irradiative source for the production of the hydrogel. An alternative method is the use of UV crosslinking in conjunction with monomers, and/or photoinitiators. Lopérgolo et al. (2003) describes the UV photocrosslinking of a hydrogel similar to that patented by Rosiak et al. (1989). It was found that the hydrogel produced had similar microscopic and macroscopic properties to hydrogels produced by high energy radiation and that the hydrogel presented no cytotoxicity. It was stated that these results demonstrated the viability of using this method as a versatile alternative to high energy hydrogel production, broadening the possibilities where high energy radiation facilities are not available. Ishihara et al. (2002) and Obara et al. (2003) also used UV irradiation of polymers for the production of hydrogels for wound dressings. However, in these cases the base polymer was chitosan. Ishihara produced a hydrogel for both wound occlusion

and wound healing that was found *in vivo* to stop bleeding within 30 seconds, and assisted in the accelerated healing of a wound.

The dissolution of fibrin (fibrinolysis) occurs naturally in wounds through the action of local plasminogen-activator or other enzymes. Dead or inflamed tissue contains fibrin that needs to be removed before healing can occur. Field and Kerstein (1994) states that both *in vitro* and *in vivo* research showed that wound fluid from occluded wounds contained a protease that begins the hydrolytic breakdown of proteins usually by splitting them into polypeptide chains. In addition, it was observed *in vitro* that one hydrocolloid dressing was able to lyse fibrin i.e. cause destruction of the cell.

One of the earliest and most widely studied hydrogel based wound dressing is the Hydron Burn Bandage (Hydro Med. Sciences Inc., New Brunswick, NJ, USA). The dressing is formed directly on the burn wound from a two-component system, PHEMA and PEG the solvent. Alternate layers of PEG and PHEMA are applied to the wound, either by spraying the components from a compressor or by direct application of PEG and powdered PHEMA, until three or four layers have been built up. The PEG dissolves the PHEMA forming a saturated solution, which solidifies after some 30 minutes. Corkhill et al. (1989) reports that some conflicting reports from clinical studies of Hydrons suitability as a wound dressing material exist. Some of these reports suggest that Hydron is difficult to apply, does not adhere well to the wound, especially if it is moist, and cracking of the film is a problem in a significant number of cases. Additionally, as the dressing is translucent it is not possible to monitor visually the wound healing and the dressing must be cut away to check the wound for infection. Other clinical studies have however, reported promising results using Hydron. These reports suggested that Hydron is easy and painless to apply, reduces the pain for the patient after application, is fairly flexible and although the tensile strength is relatively low, the translucent dressing can remain in place for up to a week between changes. The rate of infection was found to be higher in wounds dressed with Hydron, than in the control wounds, but the healing time was shorter. The permeability of Hydron enabled the problem of infection in the wound to be countered by loading the dressing with an antibacterial agent.

1.8 Administration and elimination of drugs

The possible routes of administration of drugs into the body can be divided into two classes enteral and parenteral. In enteral administration, the drug is placed directly into the gastro intestinal (GI) tract either by placing under the tongue, by swallowing it, or by rectal administration. In parenteral administration the gastrointestinal tract is bypassed. There are many parenteral routes. The most common of these routes are subcutaneous, intramuscular, and intravascular, but the drugs may also by injected intradermally or applied to the skin (transdermally). For local effect or to be absorbed transcutaneously, they may be introduced intranasally, or may be inhaled for direct action on the bronchial tree or to be absorbed into the blood at the alveoli. They may also be injected into or near the spinal column, introduced intra-vaginally or directly into other body cavities, or they may be injected directly into pathologic cavities such as abscesses and tumour cavities (Pratt and Taylor 1990). Drugs administered in any of the ways outlined above distribute themselves to some extent throughout the entire body after absorption into the blood stream, thus, medicating the whole body, rather then the specific area in which the drug has its primary pharmacological effect (Florence 1993).

A drug leaves the circulatory system by distribution into tissues, where it may be metabolised or excreted. Some drugs are metabolised in the plasma by various esterases, which are a variety of enzymes that catalyze the hydrolysis of an ester. The rate of each of these processes that contribute to initiation of drug action is determined by the chemical and physical properties of the drug and its interaction with the specialised tissues responsible for the pharmacological effects or elimination process. The kidneys play a major role in drug excretion. However, other excretory routes may also be of prime, e.g. the excretion of volatile agents via the lungs. The liver is of chief importance in drug metabolism, but drugs are also biotransformed in other tissues. Often metabolic alteration of structure is a prerequisite to rectal excretion e.g. converting a lipid-soluble drug into a more water soluble one (Pratt and Taylor 1990).

1.9 Polymeric controlled drug delivery

Controlled drug delivery occurs when a polymer, whether natural or synthetic, is judiciously combined with a drug or other active agent in such a way that the active agent is released from the material in a pre-designed manner. Griffith

(2000) describes the classic drug delivery system as the implantable contractive Norplant ™, which comprises a silicone rubber (poly dimethylsiloxane) tube filled with a steroid dispersion. Drug release is controlled by the permeability of the steroid in the tube wall and remains fairly constant over several years after an initial transient.

Using polymers as drug carriers can enable the release of the active agent to be constant over a long time period, it may be cyclic over a long time period, or it may be triggered by the environmental or other external events. Regardless, the purpose behind controlling the drug delivery is to achieve more effective therapies, while eliminating the potential for both underdosing and overdosing. Conventionally drugs were administered to the human body directly via oral, rectal and intravenous means, these substances were then released immediately, which lead to the concentration of drug rising rapidly to a therapeutic level. However, depending on the plasma half-life of the drug, the concentration would fall below the minimum effective concentration and another dose of the drug is required in order for it to be effective. This cycle of drug taking and relatively short retention times can lead to sharp differences in drug concentration in the body and thus the drug is not being effective on a continual basis. This cycle is shown in Figure 1.14 and can in fact lead to harmful side effects (Nitsch and Banakar 1994)

New oral drug delivery approaches were outlined in review form by Florence (1993). Oral controlled release systems were discussed, that could be used to control the rate of drug release in specific regions of the GI tract. Methods for site specific drug release in the GI tract are outlined, such as the use of floating tablets and adhesive preparations to maintain residence in the stomach; disintegrating pelletised systems that unload the active agent in the stomach or intestine. Formulations for delivery to the colon can be based on pH dependent dissolution of a polymer coating or on breakdown of the azo-polymer barrier membrane by azo-reductases from the colonic bacterial flora. Murata et al. (2000) describes work carried out using floating alginate gel beads that can be used for stomach-specific drug delivery. These gel beads were capable of floating in the gastric cavity, which allowed the site specific release of the active agent.

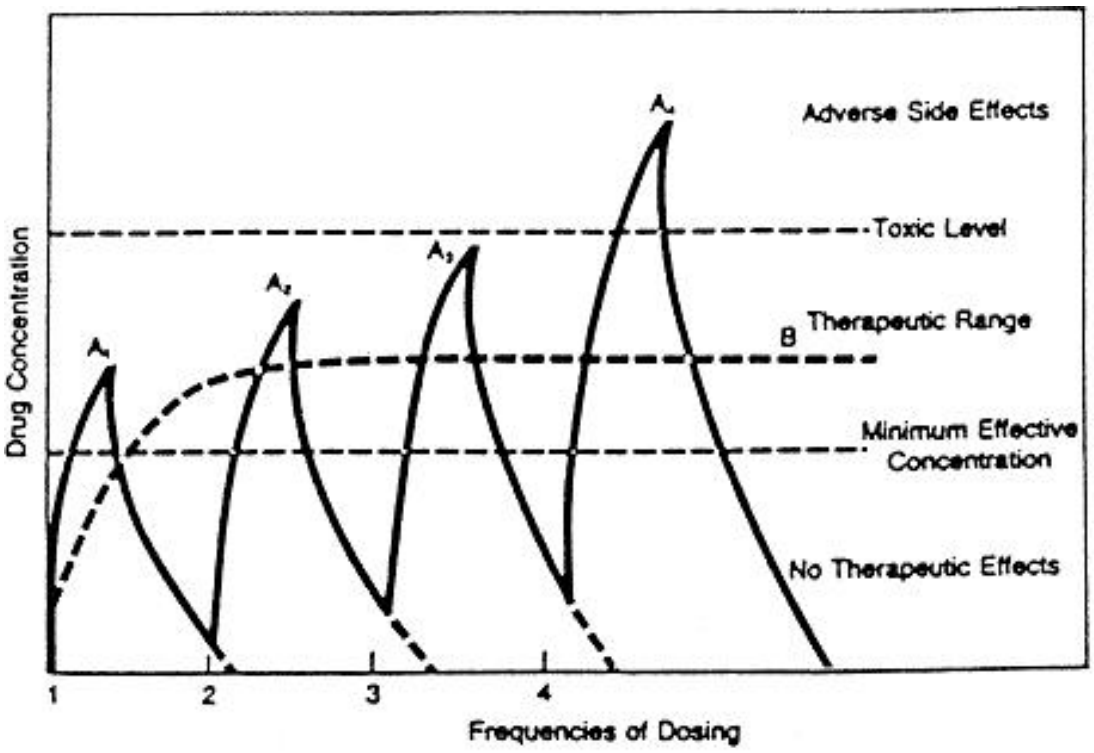


Figure 1.14 *Schematic of conventional drug delivery compared to ideal delivery*

The need to maintain a desired release rate for a wide range of drugs has led to the development of polymeric drug delivery systems that can swell in biological fluids and allow drugs to diffuse out, release drugs depending on the surrounding pH, change release rates depending on body temperature, or biodegrade to release the drug. Another approach for optimisation of drug delivery and improvement of drug efficiency is the use of pro-drugs, a concept first suggested by Albert (1958) (Dumitriu 1994) to temporarily alter the properties of drugs, increasing their usefulness and reducing their toxicity. A pro-drug may be considered as a pharmacologically inactive chemical derivative of a parent drug requiring chemical and or enzymatic transformation within the body to release the parent drug. Sartore et al. (1997) describes the synthesis and pharmacokinetic behaviour of four pro-drug derivatives of Ibuprofen. These pro-drugs were made to counteract the main disadvantages of Ibuprofen, which are a relatively short plasma half-life and significant gut toxicity.

Using polymers as drug carriers has several advantages over conventional drug administration methods. One of these advantages is the ability to give a much higher concentration of drug in a single dose keeping the concentration within the therapeutic range while not causing adverse side effects. This can be utilised in the

administration of anticancer drugs whose nature is to be toxic. Beck et al. (1993) investigated the use of poly butylcyanoacrylate nanoparticles and liposomes loaded with mitoxantrone, a highly effective anticancer drug. It was found that both nanoparticles and liposomes prolonged survival time in mice models with P388 leukaemia, whereas nanoparticles led to a significant tumour volume reduction at the B16 melanoma mice models. However neither nanoparticles nor liposomes were able to reduce the toxic side effects caused by mitoxantrone. Dordunoo et al. (1997) described the development of a taxol-loaded polymeric surgical paste based on poly (ε-caprolactone) (PCL) for application at tumor resection sites. This paste could be applied in the molten state from a syringe to these sites. It was found that taxol released *in vivo* using a subcutaneous tumor in mice caused a mean tumor regression of approximately 63%. Wang et al. (1997) also described the use of a taxol-loaded polymer poly(lactic-co-glycolic acid) (PLGA) microspheres containing isopropyl myristate (IPM). It was found that drug diffusion in the matrix was the important factor that dominated the release rate, while polymer degradation rate was only a moderate factor. Both *in vitro* and *in vivo* results suggested that the taxol concentration in the lung after intravenous injection of the taxol-IPM-PLGA microspheres was 2-4 times higher over one week, as compared with those after injection of the microspheres without IPM.

Reduction in the frequency at which the drug must be administered is advantageous and can also be achieved via polymeric drug release. An example of this is the use of heparin post percutaneous transluminal coronary angioplasty (PTCA) operations to prevent acute occlusions. Traditionally heparin is used for days by means of continuous infusion. Both Edelman et al. (2000) and Yang et al. (1999) investigated heparin delivery from PLGA. Edelman encapsulated heparin within PLGA microspheres sequestered in an alginate gel, while Yang encapsulated heparin into PLGA by using a spray-drying technique. Both systems were examined for use as inhibitors for undesirable vascular response to injury caused during surgery for example restenosis and venous bypass graft disease. Other advantages are optimal use of the drug in question, and increased patient compliance. An example of the latter where patient compliance can be a major factor is the administration of a narcotic antagonist. Maa and Heller (1990) describe the use of linear poly (orthoesters) for the controlled release of naltrexone pamoate.

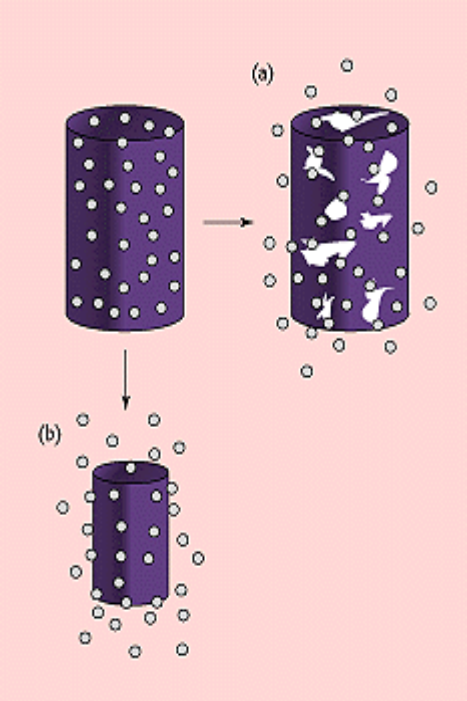


Figure 1.15 *Release of active agent from a bulk polymer through biodegradation*

While the advantages to polymeric controlled delivery can be significant, the potential disadvantages cannot be ignored. Some of these disadvantages include the possibility of toxic or non-biocompatibility of the material used: undesirable by products of degradation; any surgery required for the implantation or removal of the delivery system; the chance of patient discomfort from the delivery device. Controlled release systems also have a higher cost in comparison to traditional dosing methods.

Providing control over the drug delivery can be the most important factor at times when traditional methods of drug delivery cannot be used. These include situations requiring the slow release of water soluble drugs, the fast release of low solubility drugs, drug delivery of two or more agents with the same formulation and systems based on carriers that can dissolve or degrade and be readily eliminated. Both drug loading and molecular weight can affect drug release properties. Tamilvanan and Sa (2000) found while studying the *in vitro* release characteristics of Ibuprofen loaded polystyrene (PS) microparticles, that an increase in drug loading increases drug release from the microparticles and that a biphasic linear relationship is observed between the time required for 50% drug release and the drug loading.

However, Meilander et al. (2001) found that the molecular weight of proteins inversely affected the release rate from lipid-based microtubular drug delivery vehicles. It was found that samples loaded with a higher protein concentration increased the mass but not the percentage of initially loaded protein released daily. Park et al. (1998) analysed the release rate of three drugs with varying physiochemical properties, cefazolin sodium, bupivacaine and taxol from a biodegradable polyanhydride device. It was found that the release rate of each drug was very different even though identical delivery devices composed of the same polymer was used. This would indicate that any controlled delivery device would have to be evaluated for the release of each drug.

The ideal drug delivery system should be inert, biocompatible, mechanically strong, comfortable for the patient, capable of achieving high drug loading, safe from accidental release, simple to administer and remove and easy to fabricate and sterilise. If the polymer matrix does not degrade inside the body, then it has to be surgically removed after it is depleted of the drug. Hence to avoid the extra costs as well as risks associated with multiple surgeries, the polymer used should be biologically degradable (Brannon-Peppas 1997).

1.10 Aspirin

Aspirin (Figure 1.16) is a class of drug that belongs to the salicylates family of drugs. Salicylic acid containing compounds derived from willow bark, have been in use for hundreds of years as antipyretic (fever-reducing), analgesic (pain killing), and anti-inflammatory agents. It was eventually discovered that salicylic acid is the portion of these molecules responsible for the therapeutic effect. Two ester derivatives of this acid, synthesized as shown in Figure 1.17 are currently in common medicinal use: methylsalicylate (oil of wintergreen) for external applications and acetylsalicylic acid (aspirin) for internal consumption. The particular derivatives are chosen for their effectiveness in penetrating the physiological environment in the respective applications i.e. the skin or the intestines. Once within the body tissue, both compounds are hydrolyzed to a salicylic acid, the active agent.

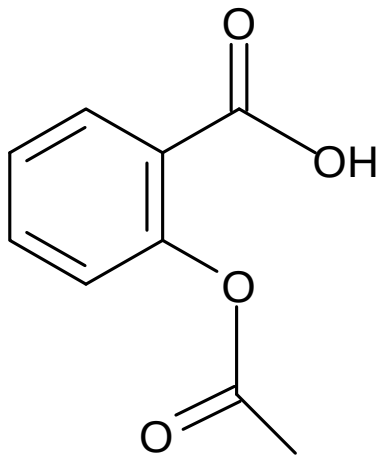


Figure 1.16 *The aspirin molecule*

Aspirin is superior to salicylic acid for oral dosage in that the latter is very bitter and causes stomach upset. Aspirin possesses these properties to a markedly lesser degree and passes through the stomach largely unchanged, undergoing hydrolysis only in the small intestine and after absorption in the blood stream. Ancient Asian records indicate that aspirin or one of its derivatives was being used more than 2400 years ago (Osborne 1997). Hipprocrates also wrote of a bitter powder extracted from willow bark that could ease aches and pains and reduce fevers as long ago as the fifth century B.C. Aspirin is a non-steroidal anti-inflammatory drug (NSAID) and is widely used to reduce pain, fever, inflammation, and rheumatism (Osborne 1997). Aspirin is also used in the treatment of unstable angina as it slows the blood clotting process.

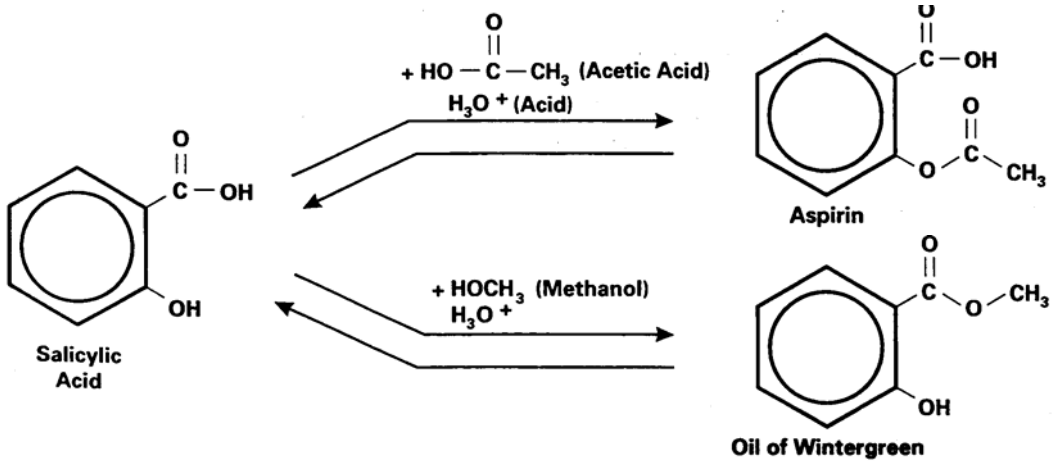


Figure 1.17 *Synthesis routes of aspirin and oil of wintergreen*

The principle of pain and inflammation relief properties of aspirin appears to be that aspirin bonds to an enzyme called cyclo-oxygenase 2 (COX 2). When tissue is damaged the body produces several chemicals, some of which are prostaglandins that are made using the enzyme COX 2. These chemicals appear to magnify the pain signal sent to the brain and also cause inflammation of the region. As aspirin bonds to COX 2 it prevents the production of prostaglandins and therefore cause a reduction in the magnitude of the pain signals that the brain receives and also in the inflammation of the region. However aspirin also reacts with other enzymes in the body, one of which is COX 1. COX 1 produces a prostaglandin that appears to help keep a thick lining on the stomach walls. As aspirin also bonds to COX 1 the stomach lining can become thin over time and thus allows the digestive juices to irritate it: this in turn can lead to stomach ulcers (Osborne 1997).

1.11 Paracetamol

The painkilling properties of paracetamol were discovered by accident when a similar molecule (acetanilide) was added to a patient's prescription about 100 years ago. But since acetanilide is toxic in moderate doses, chemists modified its structure to try and find a compound that was less harmful but which still retained the analgesic properties. One of these compounds is N-acetyl-para-aminophenol (Figure 1.18), which is also known as acetaminophen in the US and paracetamol in the UK.

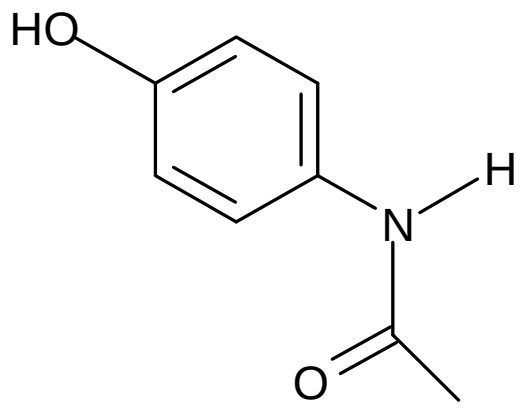


Figure 1.18 *The paracetamol molecule*

Paracetamol is one of the most common drugs used in the world, and is manufactured in huge quantities. The starting material for the commercial

manufacture of paracetamol is phenol, which is nitrated to give a mixture of the ortho and para-nitrotoluene. The o-isomer is removed by steam distillation, and the p-nitro group reduced to a p-amino group. This is then acetylated to give paracetamol as shown in Figure 1.19.

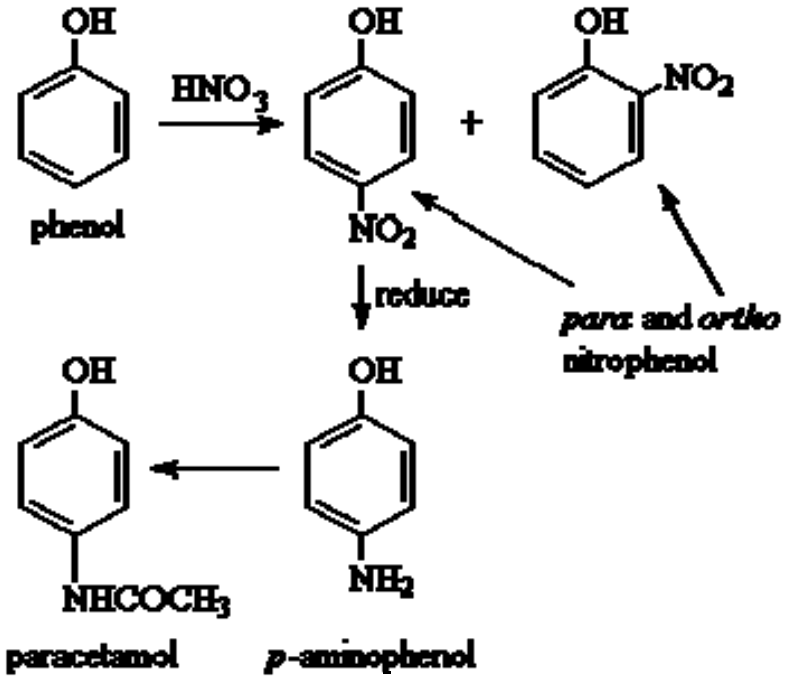


Figure 1.19 *Synthesis route for paracetamol*

As with many medicines, the effectiveness of paracetamol was discovered without understanding the mechanism that makes it effective. Its mode of action was known to be different to other pain relievers, but although it produces pain relief throughout the body the exact mechanism was not clear. The production of prostaglandins is part of the body's inflammatory response to injury, and inhibition of prostaglandin production around the body by blocking the COX 1 and COX 2 enzymes has long been known to be the mechanism of action of aspirin and other NSAIDs such as ibuprofen. However, their action in blocking COX 1 is known to be responsible for also causing the unwanted gastrointestinal side effects associated with these drugs. Paracetamol has no significant action on COX 1 and COX 2, which left its mode of action a mystery but did explain its lack of anti-inflammatory action and also, more importantly, its freedom from gastrointestinal side effects typical of NSAIDs. Early work had suggested that the fever reducing action of paracetamol was due to activity in the brain while its lack of any clinically useful anti-inflammatory action was consistent with a lack of prostaglandin inhibition

peripherally in the body. However, research has shown the presence of a new, previously unknown enzyme COX 3, found in the brain and spinal cord, which is selectively inhibited by paracetamol, and is distinct from the two already known COX 1 and COX 2 enzymes. It is now believed that this selective inhibition of the enzyme COX 3 in the brain and spinal cord explains the effectiveness of paracetamol in relieving pain and reducing fever without having unwanted gastrointestinal side effects (Chandrasekharan et al. 2002).

Aim of current study

The aim of this work is to prepare novel hydrogels for use in biomedical applications. The materials used in this work are N-vinylpyrrolidinone which has been proven to be biocompatible (Robinson et al. 1990), copolymerised with acrylic acid in order to increase the level of hydrogen bonding in the system (Kaczmarek et al. 2001) and also impart pH sensitivity, which is beneficial for site specific drug delivery (Yaung and Kwei 1997). A random copolymer formed from a mixture of the monomers was chosen, as this system has not been published in the literature. This random copolymer complex was expected to dissolve in appropriate solvents, therefore a chemical crosslinking agent was used to form a covalently crosslinked structure, which was not expected to dissolve. Ethyleneglycol dimethacrylate was used to crosslink the random copolymer, as it had been previously used for the production of covalently crosslinked hydrogels and also as it was available in various molecular weights which enabled characterisation of the effect of length of crosslink chain on the properties of the hydrogel (Hong et al. 1996b).

UV irradiation was the method of choice to initiate the curing reaction and therefore Irgacure ® 184 was incorporated into the solution to initiate the photocuring process. Where grafting of the copolymer onto a substrate was desired, Benzophenone was incorporated as the photoinitiator as it has been shown to induce grafting (Allmér 1995; Tierney et al. 2004). A low energy UV light source was used for the bulk of this work, as low levels of heat were generated by the lamps during the curing process and therefore active agents incorporated into the solution should not be affected by the curing process. UVA 340 UV lamps were chosen as the light produced by these lamps closely resemble UV light generated by the sun. For coating applications in situ onto a polymer substrate, these lamps were not practical as curing was slow and the viscous solution would easily drip from the substrate.

Therefore high intensity UV lamps were used, however as the curing time was short (120 seconds) it is believed that active agents would not be affected.

The active agents used in this work were aspirin and paracetamol. As one potential application of the copolymer produced in this work was a lubricious hydrophilic coating that could be used during coronary angioplasty procedures, controlled release of aspirin from the coating was felt to be beneficial. Aspirin is known to reduce swelling due to trauma; therefore it should reduce inflammation of blood vessels injured during the procedure. Aspirin is also administered post operatively to thin the blood and thus prevent clotting (Osborne 1997). Aspirin has a pKa of approximately 3.49 and paracetamol has a pKa of 9.35. Both active agents are widely used and have similar molecular weights. As the polymeric system developed in this work was pH sensitive, drug release of these active agents was analysed to determine the effect of pKa on the release of active agent from the system.

Chapter 2

Experimental Details

2.1 Preparation of samples

The hydrogels investigated in this work were prepared by free-radical polymerisation. The monomers used were N-vinylpyrrolidinone (NVP, Lancaster synthesis) and acrylic acid (AA, Merck-Schuchardt, Germany). The monomeric feed ratios analysed were 100wt% NVP (100-0), 90wt% NVP 10wt% AA (90-10), 80wt% 20wt% AA (80-20) and 70wt% NVP 30wt%AA (70-30). The crosslinking agents used to form 3-dimensional structures within these polymers were ethylene glycol dimethacrylate (Figure 2.1) and poly (ethylene glycol) dimethacrylate with molecular weights of 200, 400 and 600 (EGDMA, PEG200DMA, PEG400DMA, and PEG600DMA respectively, all Sigma Aldrich) at 0.1%wt of the total monomer content for the bulk of this work. Values of 0.1wt%, 0.25wt% 0.5wt% and 1wt% crosslinking agent were used for soxhlet extractions. Both monomers and crosslinking agents were used as received. To initiate the reactions, 1-hydroxycyclohexylphenylketone (Irgacure ® 184, Ciba speciality chemicals) was used as a UV-light sensitive initiator at 3wt% of the total monomer weight. This was added to the NVP/AA monomeric mixture and stirred continuously until completely dissolved. This solution was pipetted into a silicone mould (W.P. Notcutt, Middlesex) that contained disk impressions and rectangular impressions for use in Ftir. The mould was positioned horizontally to the gravity direction under two UVA 340 UV lamps (Q-panel products) and the solution was cured for 1-2 hours in an enclosed environment. The cured samples were then dried in a vacuum oven at 40°C, 260 mmHg for 24 hours prior to use.

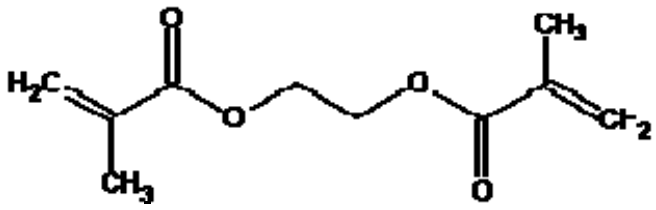


Figure 2.1 *Structure of ethylene glycol dimethacrylate*

The hydrogels investigated for their suitability as coatings for medical devices had monomeric feed ratios of 70-30, crosslinked with 0.1%wt EGDMA and PEG600DMA. To this monomeric concentration a photoinitiator that enabled

grafting to occur (benzophenone, Sigma Aldrich) was added at 3wt% of the total monomer content. The substrate used was a polyether block amide Pebax® 3533, that had been extruded through a tape head die using a Prism T20 U-Tron Soder twin-screw extruder and the extrudate fed through a Thermo Prism haul-off mechanism. This tape was stored in airtight bags until required for coating applications. For drug release characterisation aspirin (Sigma Aldrich) was added to the monomeric concentration at 25wt% of the total monomer content, prior to the coating been cured onto the Pebax ® 3533 substrate.

For drug dissolution characterisation, aspirin and paracetamol (both Sigma Aldrich) were added to the monomeric concentration at 25wt% of the total monomer content. The active agents were added to the liquid monomers and stirred continuously until dissolved, or for 1 hour whichever occurred first. The photoinitiator was added to the solution as before and the samples UV cured as described previously.

2.2 Differential scanning calorimetry

Differential scanning calorimetry (DSC) was carried out using a Perkin Elmer, Pyris 6 DSC. A sample size of between 10-12.5 mg was used for DSC experiments. The samples were weighed using a Sartorius microbalance capable of being read to 5 decimal places, and crimped using pierced lid pans. Prior to testing, the thermal history of each sample was removed by heating the samples from 20°C to 200°C at 30°C per minute, holding the sample isothermally at this temperature for 10 minutes, cooling the sample back to 20°C at 30°C per minute and again holding the sample isothermally for 10 minutes. After the removal of the samples thermal history, DSC experiments were performed from 20°C to 200°C at a ramp rate of 10°C per minute, under a 20mL per minute flow of nitrogen to prevent oxidation.

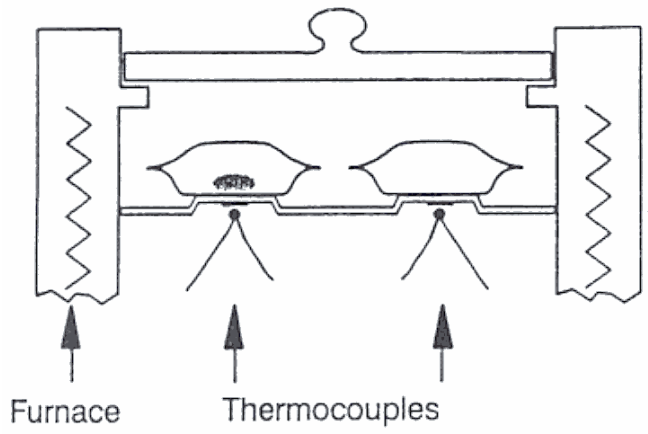


Figure 2.2 *Diagram that illustrates the positioning of the sample (left) and reference (right) in a DSC (Shah 1998)*

2.3 Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (Ftir) was carried out on UV polymerised rectangular samples with and without 25wt% active agent. The samples were exposed to atmospheric conditions for a minimum of seven days prior to Ftir testing. Ftir was also carried out on coated Pebax® 3533. This test was carried out by firstly performing a background on uncoated Pebax® 3533, and thus the resultant scan was of the coating and not of the coating and the Pebax® 3533. Ftir experiments were carried out using a Nicolet Avator 360 Ftir, with a 32 scan per sample cycle.

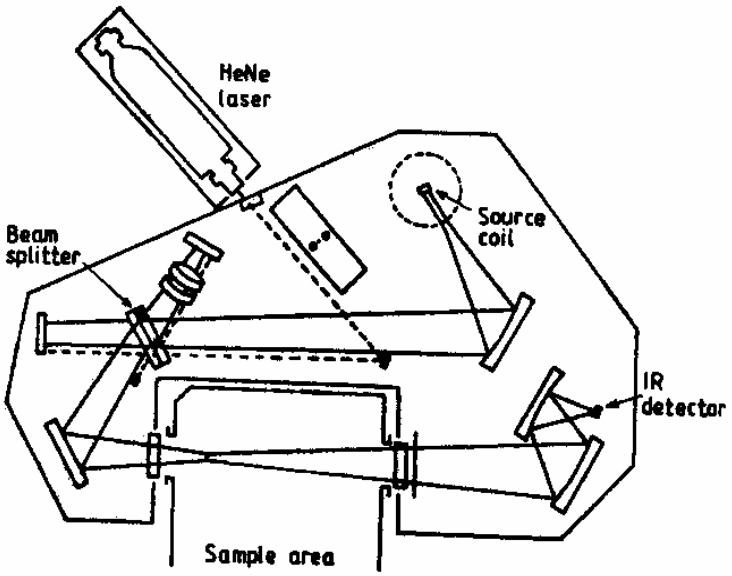


Figure 2.3 Schematic of the general arrangement of optics of an IR interferometer (Campbell and White 1989)

2.4 Potentiometric titration

The potentiometric titrations of the UV cured PVP/PAA copolymers without crosslinking agent, and crosslinked with PEG200DMA, were executed by firstly grinding the sample that had being vacuum dried as described previously. The sample was placed into a 200mL bottle, into which 25mL of 0.05M HCl containing 0.1M NaCl was added. A lid was placed on the bottle to prevent evaporation. The sample was stirred continuously for 24 hours prior to use. A burette that could be read to ±0.1 mL was used to titrate 0.05M NaOH containing 0.05M NaCl with the HCl solution. An Orion 420A+ pH meter, capable of reading pH changes of 0.01, was used to monitor the progress of the titration.

2.5 Polymer solubility testing

Polymer solubility testing was preformed in buffered solutions (buffered tablets, BDH Ltd. Poole, England) in triplicate at pH values of pH 4.0, pH 7.0 and pH 9.2 at ambient temperature. Samples of the cured polymer with a mass of 1.38g ± 0.42g were placed into a petri dish; the petri dish was then filled with the appropriate buffered solution. Periodically, excess buffered solution was removed by pouring the solution through a Buchner funnel, the samples were then pat-dried with filter paper and weighed. The samples were re-submerged in fresh-buffered solution. The percentage that the hydrogels swelled was calculated using the formula: -

$$\% \text{ Swelling} = W_t / W_0 \times 100$$

Where W_t is the weight of the hydrogel at a predetermined time, and W_0 is the weight of the hydrogel before swelling experiments took place. Pictures of the swollen samples were taken before the removal of the buffered solution for comparative reasons. This process was continued until the sample appeared to have dissolved, or for approximately 120 hours, whichever occurred first.

2.6 Gel Permeation Chromatography

Gel permeation chromatography (GPC) was carried out using a Polymer Laboratories aquagel-OH 8μm mixed column, with a suitable guard column. The mobile phase used consisted of water/methanol mixture at a ratio of 10:1. The mixture was filtered and degassed using a Millipore filtration system under vacuum

using 50μm polytetrafluoroethylene (PTFE) filter paper. The mixture was then further degassed by sparging helium through it.

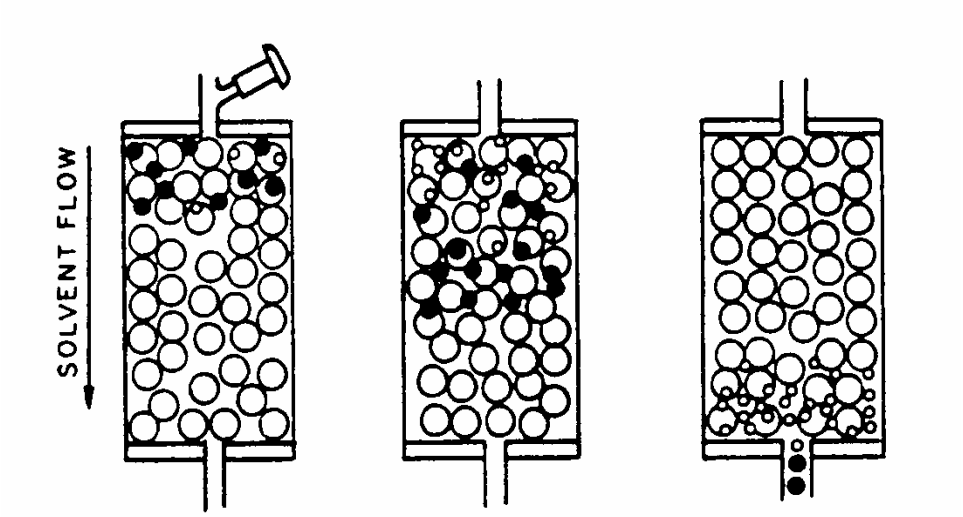


Figure 2.4 *Schematic diagram of gel permeation chromatography (Brown 1973)*

The detector used for these tests was a Polymer Laboratories evaporative light scattering (PL ELS 1000) detector, with an evaporator temperature set at 100°C, nebuliser temperature set at 85°C and a nitrogen flow rate set at 1.5mL/min. Polyethylene glycol calibration standards were used as reference standards. However, results dictated that a wider range was necessary, therefore high molecular weight polyethylene oxides (up to 8 million) were also used. These standards were prepared by weighing 25-25.5 mg of PEG into 28mL bottles; 25mL of high purity water was then pipetted into the bottle. The samples were allowed to dissolve for 24 hours prior to use. All tests were carried out using a flow rate of 1mL per minute.

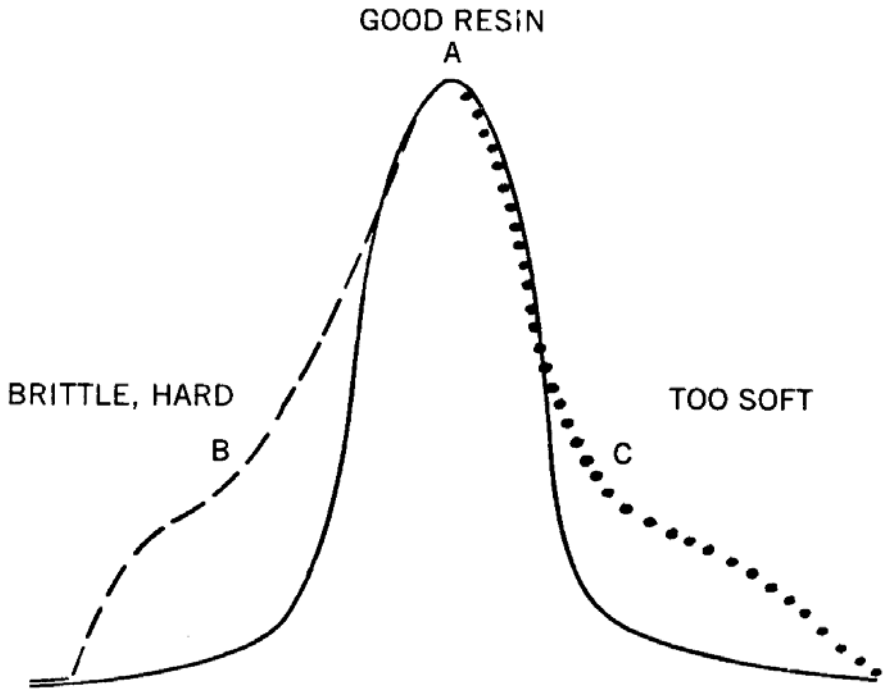


Figure 2.5 *An example of differing molecular weight curves achievable using GPC. A good resin; B resin with excessively high molecular weight is hard and brittle; C resin with excessively low molecular weight is soft and sticky (Shah 1998)*

The samples tested were collected by swelling the hydrogels in deionised water for defined periods of time, removing a sample of water, and then replacing the water with fresh water to maintain a constant volume of media.

2.7 Swelling studies

Swelling experiments were preformed on crosslinked samples in buffered solutions (buffered tablets, BDH Ltd. Poole, England) in triplicate at pH values of pH 4.0, pH 7.0 and pH 9.2 at ambient temperature. Samples of the cured polymer with an average mass of 1.44g, were placed into a petri dish, and the petri dish was filled with the appropriate buffered solution. Periodically excess buffered solution was removed by pouring the solution through a Buchner funnel, the samples were then pat-dried with filter paper and weighed. The samples were re-submerged in fresh-buffered solution. The percentage that the hydrogels swelled was calculated using the formula: -

$$\% \text{ Swelling} = \frac{W_t}{W_0} \times 100$$

where W_t is the weight of the swollen hydrogel and W_0 is the weight of the hydrogel before swelling experiments took place. Both the water content (W_c) and water uptake (W_u) of the fully hydrated hydrogels was also calculated using the formulas: -

$$W_c = 100\frac{(W_t - W_0)}{W_t}$$

&

$$W_u = 100\frac{(W_t - W_0)}{W_o}$$

respectively. This process was continued for approximately 120hrs at which point the sample was removed from the buffered solution, weighed and dried in an oven at 80°C for 24 hours.

2.8 Parallel plate rheometry

Parallel plate rheometry was carried out on samples designated 80-20 and 70-30 using an Advanced Rheometer AR1000 (TA instruments, Figure 2.6) fitted with Peltier temperature control. These samples had previously been swelled in a buffered solution for at least 120hrs. The samples were tested using a 6 cm steel plate, as shown in Figure 2.7. The swollen hydrogel was placed on the Peltier plate, and tests carried out using a strain sweep from 1 x E^{-4} to 1 at a frequency of 1Hz. The test was performed at 37°C with a constant normal force of 5 ±0.5 N exerted on the samples. This procedure was used to determine the point at which the samples interactions were increasingly stretched until they are broken, and thus the oscillating torque at break could be observed. This resulted in a drop in the storage modulus G', and thus there was a crossing of G' and the loss modulus, G". The comparative strength of the hydrogels was taken from the linear G' region and is a measure of comparative strength. For some samples it was necessary to increase the normal force to 10N depending on the strength of the hydrogel.

Figure 2.6 *TA Instruments, Advanced Rheometer AR 1000*

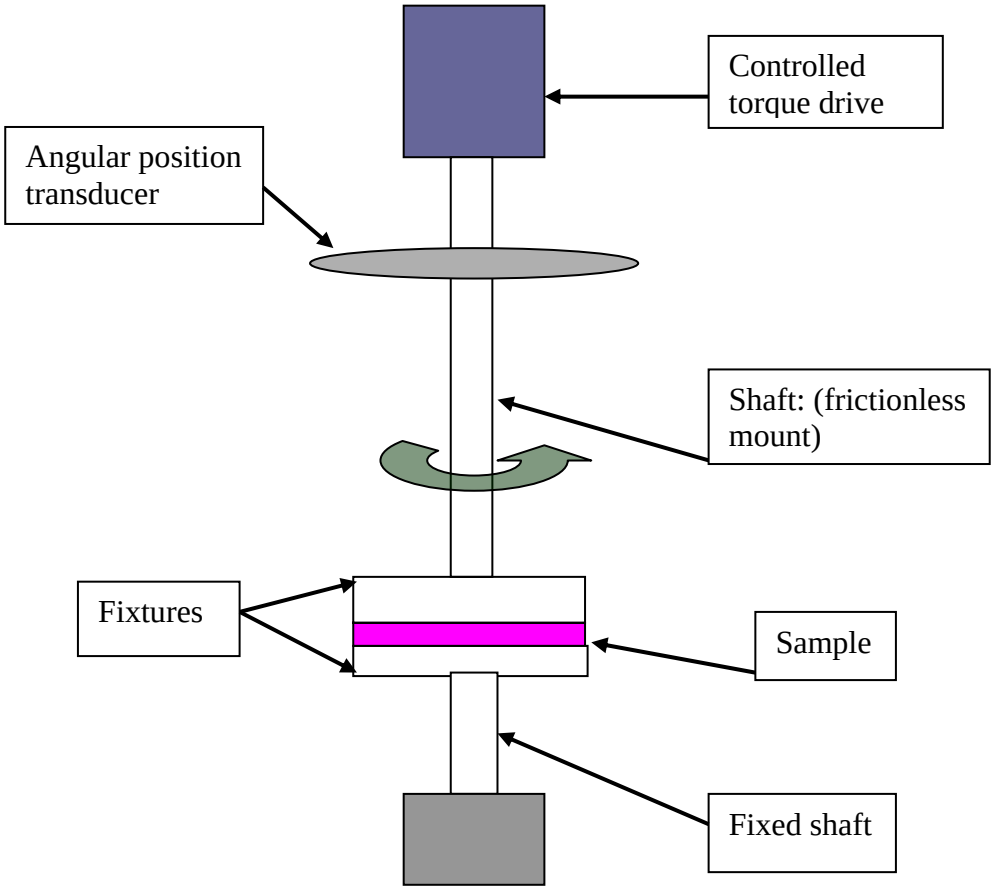


Figure 2.7 *Set-up of sample for parallel plate rheometry*

2.9 Dip coating and UV curing

Before dip coating/UV curing was performed the Pebax ® 3533 substrate was hand-dipped into propan-1-ol to remove surface impurities, and then oven dried at 50°C for at least 30 minutes to evaporate the solvent. Dip coating was carried out

by immersing the substrate in a 100mL graduated cylinder that had been filled with the appropriate coating solution. The dipping speeds used were 22.1 ± 0.5 mm/sec while dipping the sample, and 5.2 ±0.035 mm/sec while withdrawing the sample. Immediately after dipping, the substrate was placed in the curing chamber (Figure 2.9), which consisted of two, high intensity Dymax ® 2000EC UV light sources, and cured for 120 seconds. These lamps were used as it would be impractical to attempt to cure a low viscosity coating on a substrate over a long period of time using low intensity UV lamps, as were used previously. When samples were to undergo more than one coating cycle, they were suspended from a retort stand for at least 5 minutes before a subsequent coating was applied. The samples were then oven dried at 50°C to remove any volatiles that may have remained after curing.

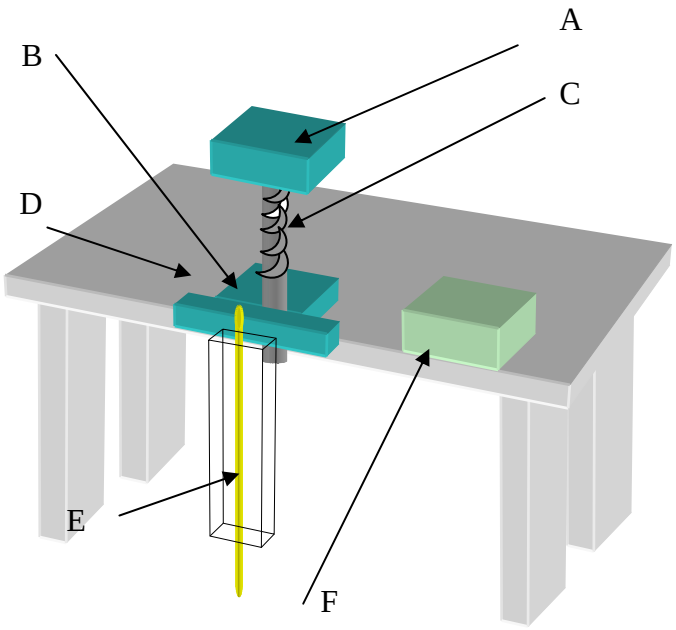


Figure 2.8 *Dip-coating apparatus. Where: - A = Rig motor, B = Moving clamp, C = Reciprocal screw, D = Dipping bench, E = Coating solution container & F = Control unit.*

In order to keep the substrate straight during coating cycles it was necessary to suspend a 20g weight from the substrate. This was achieved by placing a bull-clip on the sample after dipping, and attaching the weight to the bull-clip. This was done to prevent the sample distorting during UV curing.

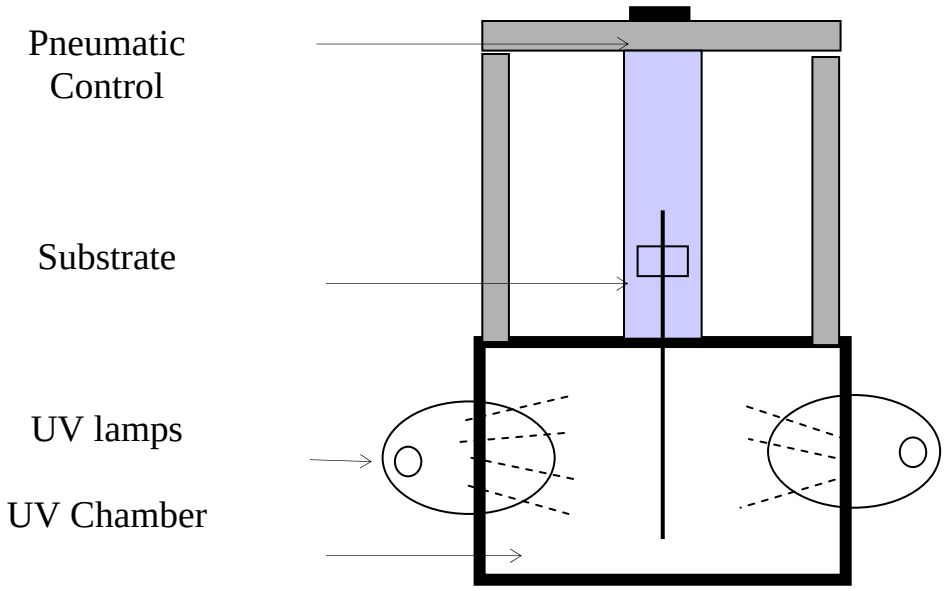


Figure 2.9 *UV curing apparatus*

2.10 Optical analysis

Optical analysis was carried out on samples that underwent a predetermined number of dipping/ UV curing cycles to determine the optimal coverage over the entire length of the substrate. The test was carried out by swelling the pre-coated substrate in a pH 7 buffered solution that contained Congo red indicator, for twenty minutes. The Congo red indicator was used to aid the visualisation of the coating in situ. Pictures of the samples were taken with a Sony cyber-shot digital camera. Microscopic analysis of the coating was preformed using an Olympus BX60 microscope with a magnification of 10 X.

Figure 2.10 *An Olympus BX60 microscope*

2.11 Coating characterisation

Coating characterisation was carried out on samples that had undergone 2 dip coating/UV curing cycles. The thickness of the coating was measured using a micrometer, capable of being read to 3 decimal places. The samples, whose thickness was to be measured, were dried for a period of 24hrs at 50°C prior to been measured. The measurement was performed at three points along the length of 10 different samples, and the average thickness is illustrated in this work. This procedure was then repeated after the samples had been coated using the coating procedure described previously, and the coating thickness calculated. The contact angle made between the coating solutions and the substrate was measured in triplicate using a goniometer. The relative viscosities (η_{rel}) of the coatings were determined by placing 5mL of a coating into a 50mL volumetric flask, and then filling the volumetric flask to the mark with acetone. Using an Oswald viscometer the flow times for acetone and the coating solutions was measured in triplicate. From these results the relative viscosity could be calculated using the formula: -

$$\eta_{rel} = t_{sol}/t_0$$

where t_{sol} is the flow time for the coating solution, and t_0 is the flow time for the solvent i.e. acetone.

The densities of the coating solutions were determined by placing 5mL of the coating solutions into a pre-weighed beaker and determining the mass of the known volume of liquid. The density could then be calculated using the simple formula: -

Density = mass/volume

2.12 Friction analysis

Samples of Pebax ® 3533 that had undergone the optimum number of coating cycles were suspended in a beaker containing pH 7 buffered solution. At five minute time intervals a sample was removed and frictional analysis carried out. Frictional analysis was performed on the coating/glass interface, using a Lloyd LRX tensile testing machine with a 2.5 KN load cell. The test was carried out by placing the tape under a sled that was attached to the load cell as shown in Figure 2.11. A force of 7.36 N was then placed on top of the sled. The sample and sled were then aligned with the crosshead of the test apparatus, and the sample was pulled at an angle of 30° for a distance of 100mm at a speed of 100mm/min. From this test the optimal swelling time for the coating was established. As the samples been tested were lubricious in nature, it was necessary to bend the test specimen over the top of the sled, and tape it in position.

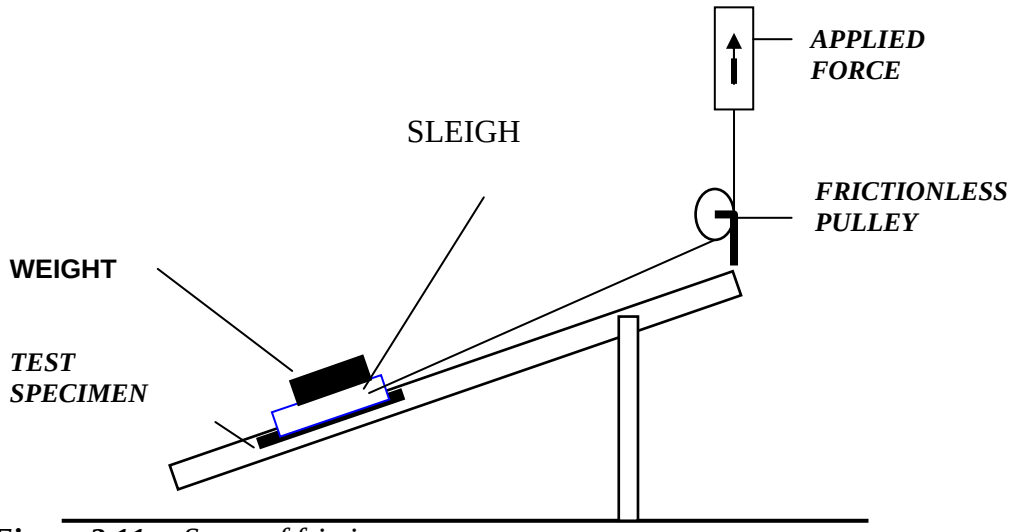


Figure 2.11 *Setup of friction apparatus*

2.13 Aspirin release from PVP/PAA coating

For aspirin release testing from the PVP/PAA copolymer coating, 7 x 100mm coated samples with aspirin incorporated were placed into a 100mL bottle that contained 100mL of pre-heated (37°C) pH 7 buffered solution. The test specimens were placed in an oven at 37°C. Aspirin release was determined by performing UV spectroscopy on the swelling media, at predetermined time intervals

using coated samples without entrapped drug as a reference. The UV spectroscopy was performed using a Shimadzu UV 160 UV spectrometer.

2.14 Soxhlet extractions

Soxhlet extractions were carried out on UV cured samples designated 100-0, 90-10, 80-20, and 70-30, crosslinked with varying amounts of either EGDMA or PEG600DMA. The test was carried out by placing a pre-weighed circular disk with an average weight of 0.99g into a 25mm x 80mm Soxhlet thimble. The thimble was placed into a 100mL capacity Soxhlet extraction apparatus and the test was carried out in distilled water at 100°C for 72hrs. When the test had ended the samples were removed from the thimbles and dried in an oven at 80°C for a minimum of 48hrs. The samples were then re-weighed so as to calculate the extractable content of the hydrogel.

2.15 Dissolution of active agents

The media used for drug dissolution experiments were 0.2M buffered solutions with pH values of 2, 6.8 and 9. Potassium chloride, monobasic potassium phosphate, and boric acid/ potassium chloride were used to prepare pH buffers 2, pH 6.8, and pH 9 respectively. The buffered solutions were prepared in the laboratory and filtered under vacuum using Millipore filtration apparatus and 50μm (polytetrafluoroethylene) PTFE filter paper prior to use. The pH of the buffered solution was checked using an Orion 420A+ pH meter capable of reading pH changes of 0.01. Drug dissolution studies were conducted on polymers using a Sotax ® on-line dissolution system as shown in Figure 2.12. The test was carried out in triplicate using the Basket method (USP XXV) at 37ºC ± 0.5ºC at 100 rpm. At predetermined time intervals, samples were withdrawn automatically, filtered, passed through a Perkin Elmer Lambda 20 UV/Vis spectrometer, the UV of the sample at the preset wavelength analysed, and finally the sample was returned to the vessel. The wavelength and absorption of a 100% drug concentration for each drug and pH value was determined in triplicate using a Perkin Elmer Lambda 40 UV/Vis spectrometer. The average values were entered into software calculations prior to commencement of testing to form a reference standard. The active agents used in this experiment were aspirin and paracetamol.

Figure 2.12 *Sotax ® on-line dissolution apparatus and associated equipment*

2.16 Cytotoxicity testing

MTT assay is a laboratory test and a standard colorimetric assay for measuring cellular proliferation. The amount of yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduced to purple formazan is measured spectrophotometrically. This reduction takes place only when mitochondrial reductase enzymes are active, and thus conversion is directly related to the number of viable cells. The production of purple formazan in cells treated with an agent is measured relative to the production in control cells, and a dose-response curve can be generated.

Prior to biocompatibility testing individual hydrogel discs were weighed and dissolved in an appropriate volume of complete culture medium (Table 2.1) to give a final concentration of 25 mg/ml. Following incubation overnight at 37ºC, and brief vortexing, hydrogel suspensions were filter sterilised (0.2 µm pore size) and subjected to serial dilutions in complete culture medium to give the concentration range 25 – 0.025 mg/ml.

HepG2 cells were seeded at 1 x 10^4 cells per 100 µl per well and microtitre plates incubated overnight at 37ºC. Following subsequent exposure for 3 h or 24 h, hydrogel containing culture medium was replaced with fresh culture medium supplemented with MTT at a final concentration of 0.05 mg/ml for 4 h at 37°C. After careful removal of MTT medium, blue formazan crystals were solubilised in 100 µl per well acidified isopropanol and microtitre plates agitated for 10 seconds at medium intensity prior to recording optical densities at 560 nm using an Anthos (htIII) microplate reader (Mosmann, 1983; Vistica, 1991).

Table 2.1 *Typical HepG2 Cell Complete Medium Composition*

DMEM (Dulbecco's Modified Eagles Medium) 44 ml
Hams F-12 44 ml
Foetal Calf Serum (FCS) 10 ml (10%)
L-glutamine 1 ml (1%)
Penicillin-streptomycin (0.5 ml)
Amphotericin-B 'Fungizone' (0.5 ml)

All Sigma.

2.17 Genotoxicity testing

The Comet assay is a means of measuring DNA damage, particularly DNA strand breaks. A cell is embedded in agar and exposed to the test sample. The cell is then permeabilised by adding detergent and an electric field is applied. If the cell's genomic DNA has been broken into small fragments, these fragments move out of the cell by electrophoresis and form a streak or "tail" leading away of the cell. The appearance is that of a comet, hence the name of the assay.

Duplicate wells of a 24 well culture plate were seeded at 2 x 10^4 cells per ml in 2 ml aliquots. Following overnight incubation at 37ºC culture medium was replaced with various concentrations of hydrogel containing culture medium for a further 3 h or 24 h. Subsequently, the Comet assay procedure of Klaude et al. (1996) was employed, with slight modifications, to detect DNA strand breakage in individual cells. Cells were embedded in 1% LMP agarose on gel bond electrophoresis film and subject to lysis, alkaline unwinding and electrophoresis at high pH. DNA damage was expressed as a function of the measured 'tail moment' parameter, a product of the fluorescence intensity of the tail and the extent of migration.

Chapter 3

The synthesis and characterisation of physically crosslinked N-vinyl pyrrolidinone – acrylic acid hydrogels

3.1 Introduction

Hydrogels may be composed of homopolymers or copolymers and are insoluble due to the presence of chemical crosslinks or physical crosslinks (Peppas et al. 2000). If the polymer is crosslinked covalently, it is called a chemical gel (Hughes et al. 2001). Chemical gels can be prepared by the crosslinking of linear polymers, which involves organic reactions between pendant functional groups of the linear polymers, or by simultaneous polymerisation using multifunctional monomers.

Physical gels maintain their ordered structure by non-covalent cohesion interactions, such as hydrophobic interaction, stereocomplex formation, ionic complexation and crystallinity. The resultant physical associations act as physical junction domains that are not crosslinking points (Bae et al. 2000). Although non-covalent associations are reversible and weaker than chemical crosslinking, they allow solvent casting and thermal processing, and the resulting polymers often show elastic or viscoelastic properties. Most known physical gels lack controlled degradability, which limits their use in the body, especially for delivery applications. However, biodegradable polymers such as polylactide, polyglycolide, and their copolymers are often employed as delivery carriers for various drugs, but do not fulfil all properties required for a wide range of delivery purposes. Biodegradable physical hydrogels may offer an alternative material of choice in designing drug delivery systems as well as other biomedical applications (Bae et al. 2000).

Before a hydrogel can be used as a drug delivery device that utilises a diffusion delivery process, it is essential that the swelling properties and the soluble fractions of the hydrogel are characterised. In order to facilitate this, complexes of polyvinyl pyrrolidinone-polyacrylic acid were prepared by photopolymerisation from a mixture of the monomers NVP and AA. The complexes were characterised by means of DSC, Ftir, potentiometric titration, swelling studies and GPC. As it was desirable to remove as many variables as possible, the photoinitiator content was kept constant and no crosslinking agent was used. Therefore, the hydrogel produced would have no chemical crosslinks, thus any crosslinking that may occur would be

of a physical nature, occurring due to the presence of hydrogen bonding, entanglements or crystalline segments. Depending on the nature and quantity of crosslinks present, these hydrogels would likely be weaker and have lower gel integrity than ones produced using a chemical crosslinking agent in the polymerisation reaction.

However, these hydrogels would be useful where the polymer was expected to dissolve, as a hydrogel of this type would undergo hydrolytic degradation in the physiological environment to yield non-toxic normal metabolites (Ravichandran et al. 1997). This may be desirable if for example, an expensive active agent was incorporated into the polymer matrix. The entire active agent content would be released as the polymer dissolved, and localized drug delivery could be achieved by ingestion, implantation, etc. Physical hydrogels also have the advantage in that they do not incorporate a chemical crosslinking agent that may itself be toxic, and impart a toxic effect on the entire hydrogel.

3.2 Results and Discussion

3.2.1 Preparation of samples

Samples of both NVP and NVP/AA were photopolymerised using Irgacure® 184 as a photoinitiator. Irgacure® 184 is a benzophenone type photoinitiator. The structure of Irgacure® 184 is shown in Figure 3.1.

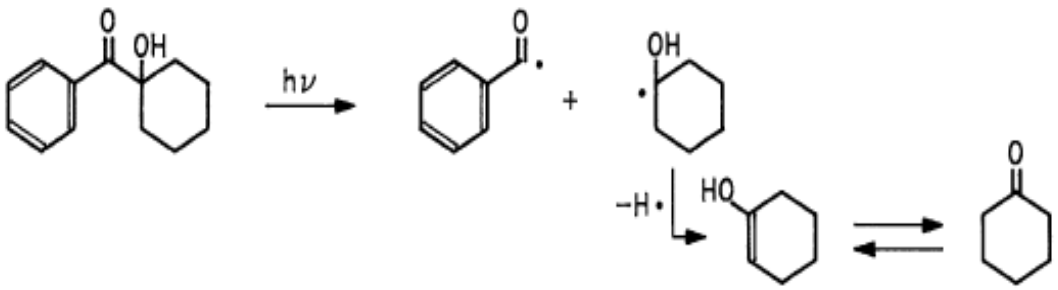
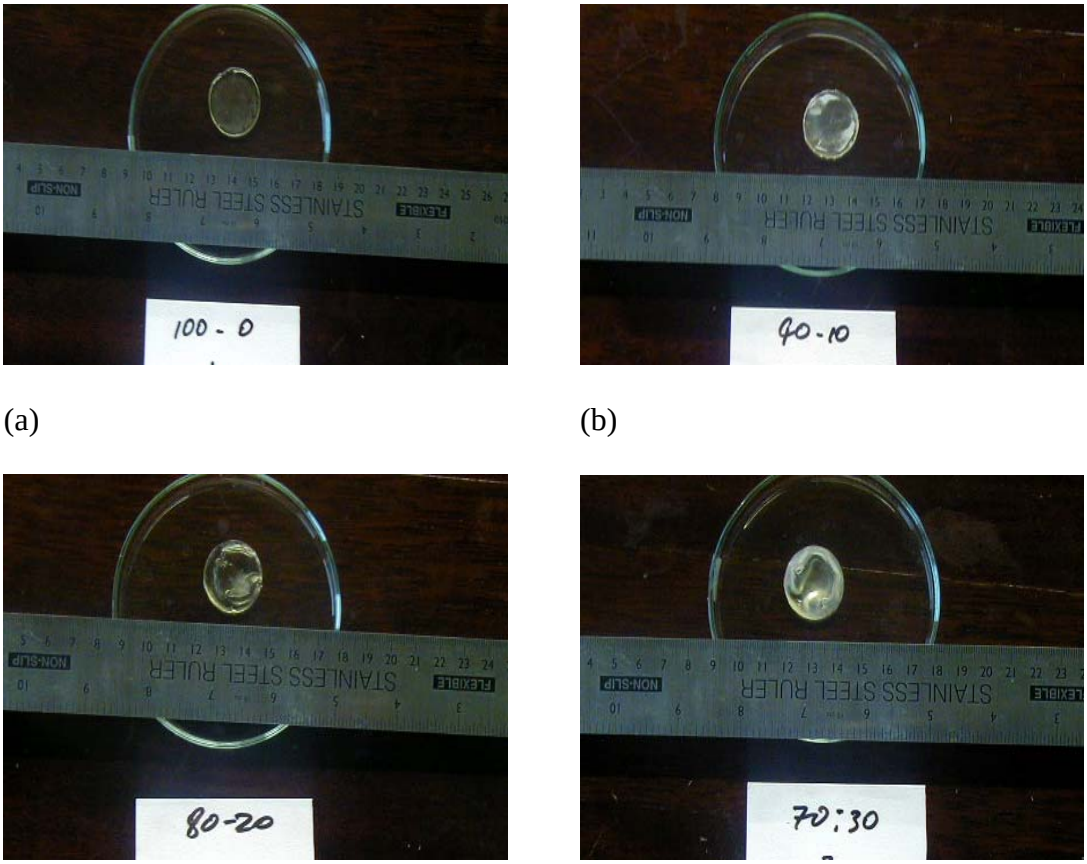


Figure 3.1 *The Irgacure® 184 molecule, when exposed to UV light Irgacure® 184 structure breaks down to form a radical (Satlhammer et al. 1999)*

Irgacure® 184 differs from benzophenone in that it consists of a cyclohexane group and an OH group instead of the phenyl group that exists in benzophenone. The advantage of using Irgacure® 184 instead of benzophenone is that, Irgacure® 184

minimises yellowing in photocuring (Kroschwitz 1985b) and can form a radical without abstraction of a hydrogen atom.

These samples were cured on a silicone moulding, and prior to use dried for 24hrs in a vacuum oven. Cured samples are shown in Figure 3.2. The 100-0 samples cured with a uniform smooth surface, which would indicate that the material could have potential in coating applications. With the addition of AA the uniformity of the surface of the samples deteriorated. This deterioration increased with an increase in AA concentration as is shown in Figure 3.2. The 100-0 samples were transparent in appearance and yellowish in colour after photopolymerisation. This suggested that no phase separation occurred in the polymer on the scale exceeding the wavelength of visible light. However, the addition of AA caused the formation of some white solids. Lau and Mi (2002) also noted the formation of white solids in a PVP/PAA copolymer and suggested that they were formed by phase separation of PAA in the cured co-polymer.



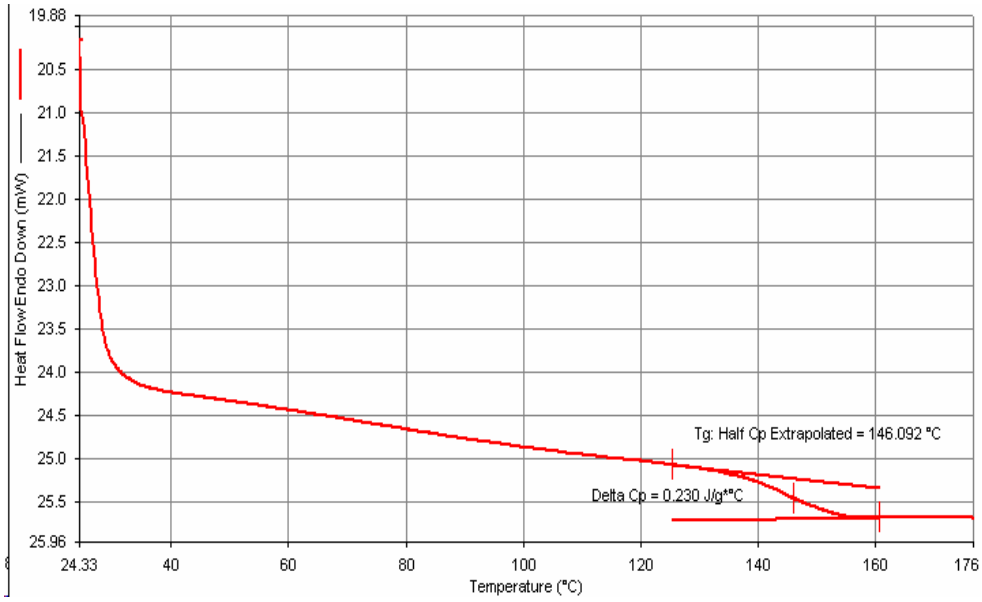
(a) (b)

(c) (d)

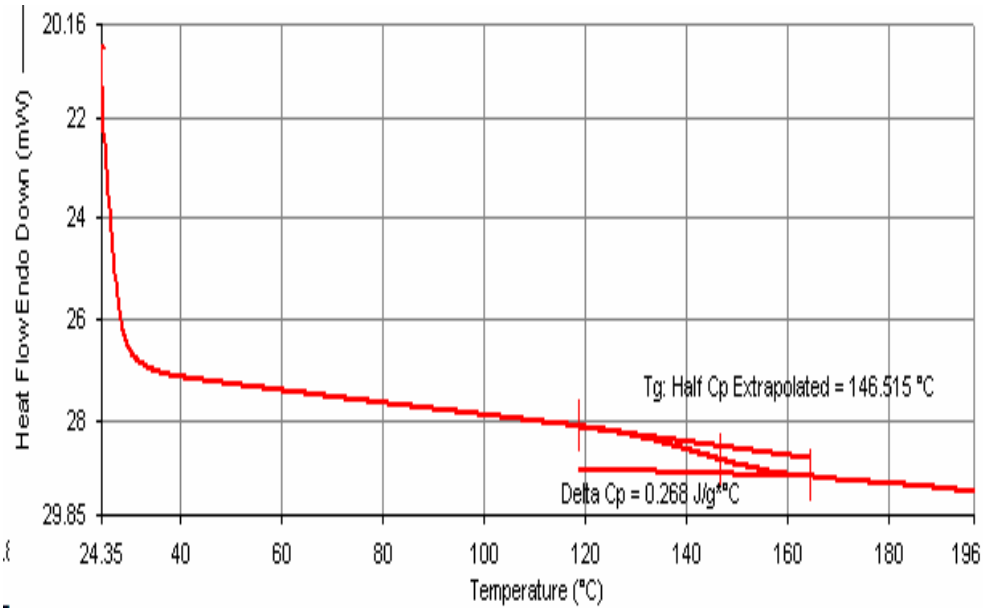
Figure 3.2 *Cured samples of PVP/PAA complexes. The initial composition of these were (a) 100-0, (b) 90-10, (c) 80-20, and (d) 70-30*

3.2.2 Differential Scanning Calorimetry

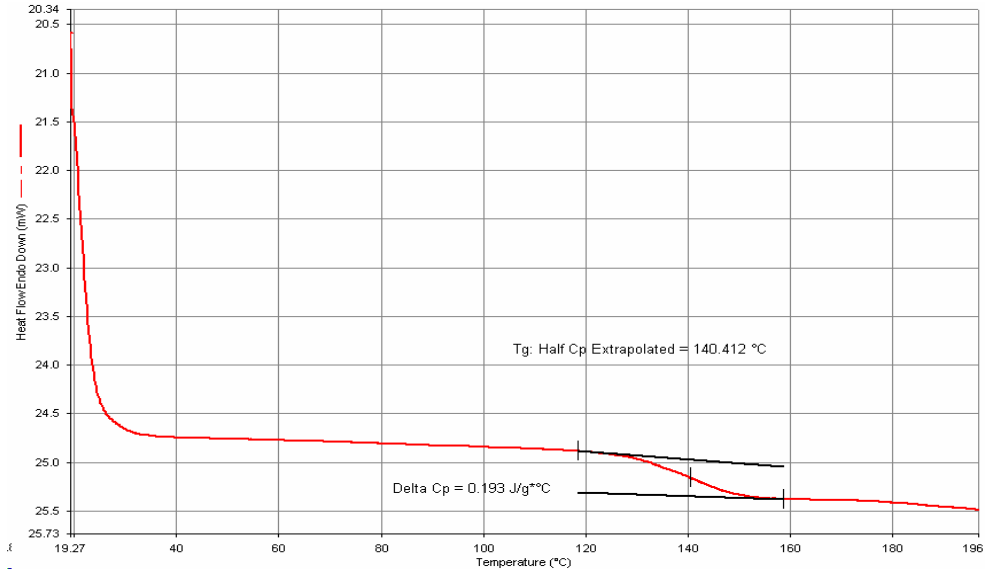
DSC was carried out by firstly removing the thermal history of each sample by heating the sample to 200ºC and cooling the sample back to ambient temperature at 30°C/minute. The samples were then heated back to 200°C at 10°C per minute and the DSC thermograms analysed for primary and secondary transitions. The resultant DSC thermograms are shown in Figure 3.3.



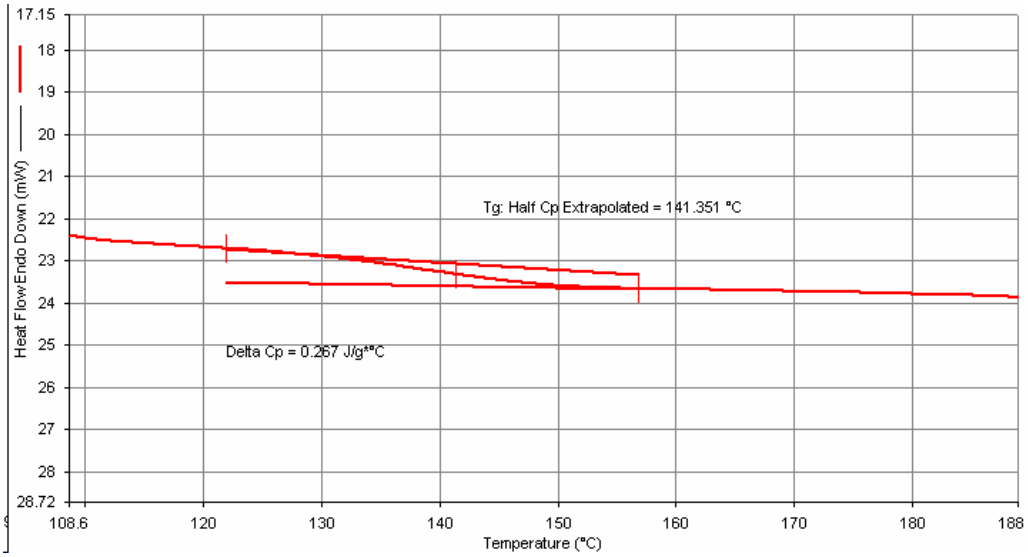
(a)



(b)



(c)



(d)

Figure 3.3 *DSC thermograms performed on cured polymers. The monomeric feed ratios of these samples were designated (a) 100-0, (b) 90-10, (c) 80-20 & (d) 70-30*

DSC scans were performed in order to determine the effect that the addition of PAA had on the properties of PVP. Yaung and Kwei (1997) also performed DSC scans on PVP-PAA complexes and found, that for the three formulations tested the glass transition temperature (T_g) of the samples were 134°C, 142°C, and 146°C. In this work the T_g of the samples tested were found to be 146.1°C, 146.5°C, 140.4°C and 141.3°C for samples 100-0, 90-10, 80-20 and 70-30 respectively, and therefore correlate to those presented by Yaung and Kwei. It can however be seen that there appears to be a decrease in T_g with increased amounts of AA. It is believed that low

molecular weight, phase separated AA oligomers acted as a plasticiser, thus increasing chain mobility and therefore reducing T_g.

It can be seen from the DSC thermograms obtained that the T_g of each of the samples was above 140°C, which would have no impact on the behaviour of the gel at body temperature, but does however give an indication of the stiffness of the dry polymer. It can also be seen that there appears to be no secondary transitions in any of these polymers in the region tested i.e. 20°C - 200°C.

The T_g is the temperature below which molecules have little relative mobility. T_g is usually applicable to wholly or partially amorphous phases, such as in glasses and plastics. Thermoplastic polymers have a T_g below which they become rigid and brittle, and can crack and shatter. Above T_g, the rigidity of the polymer molecules is reduced as the secondary, non-covalent bonds between the polymer chains become weak in comparison to thermal motion, and the polymer becomes rubbery and capable of elastic or plastic deformation without fracture. The T_g of some common polymers are 100ºC for polystyrene and values of between -20ºC and -80ºC have been quoted for polyethylene (PE) (Stehling and Mandelkern 1970). When the properties of these polymers are compared at ambient temperature, the effect of T_g becomes apparent. At ambient temperature PE is above its T_g and therefore the polymer is tough and flexible. PS however is below its T_g and is strong but brittle. Therefore, the results obtained in this work indicate that the polymer is a strong brittle material in the dry state.

3.2.3 Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy was carried out on UV polymerised rectangular samples designated 100-0, 90-10, 80-20, and 70-30. The samples were exposed to atmospheric conditions for a minimum of seven days prior to Ftir testing. Ftir experiments were carried out using a Nicolet Avator 360 Ftir, with a 32 scan per sample cycle.

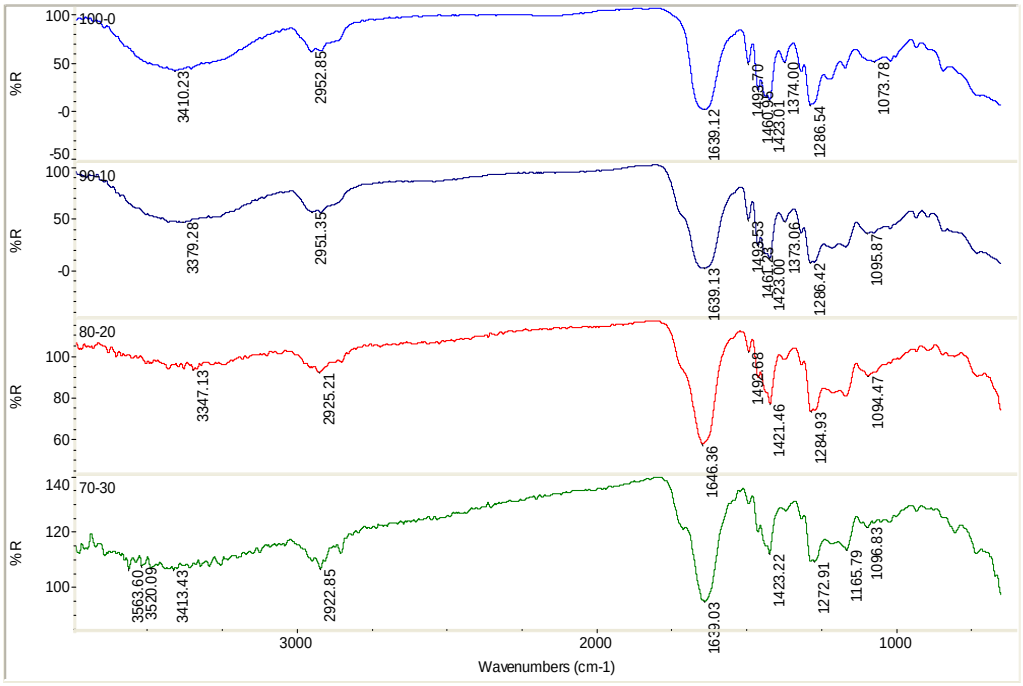


Figure 3.4 *A comparison of FTIR scans preformed on UV cured polymers.*

Mixing of polymeric hydrogen donors with polymers containing hydrogen acceptors (i.e. polyacid with polybase) causes the formation of complexes with strong hydrogen bonds. Such behaviour is shown by mixtures of PAA and PVP. The structures of these copolymers are complicated because of the numerous possibilities of interactions between the polymer molecules (Kaczmarek et al. 2001). The interactions between PVP and PAA were examined by Ftir. The range of OH stretching vibration (3000 – 3700 cm^{-1}) was not considered because of the difficulty of removing water residue completely. Kaczmarek et al. (2001) also found this and stated that long drying in vacuum at elevated temperatures can be dangerous for the polyacid structure because cyclic anhydride can be formed. However, a broad peak existed in all samples that are characteristic of the stretching vibration associated with hydroxyl groups as shown in Figure 3.4, (additional Ftir spectrum are illustrated in appendix A).

The formation of hydrogen bonding is exhibited on the IR spectrum as a negative shift of the stretching vibration of the functional group involved in the hydrogen bond, which is typically a carbonyl group. According to Bures and Peppas (2000) the carboxylic acid group in AA can exist in both free and dimeric form (Figure 3.5), depending upon their environment. Lee et al. (1988a, 1988b) describes how the stretching frequency of the carbonyl moiety in the carboxylic acid group is

defined by absorption at 1750cm^{-1}, whereas the dimer stretching frequency was reported as being 1700cm^{-1} by both Lee and Bures and Peppas (2000) and as 1717cm^{-1} by Yaung and Kwei (1997). In this work the dimer was identified by the clear formation of a shoulder on the PVP carboxyl group absorption peak. This shoulder is visible in both samples 90-10 and 80-20. This shoulder develops into a small peak in the 70-30 sample. This peak appears at 1719cm^{-1} and corresponds to values reported in the literature.

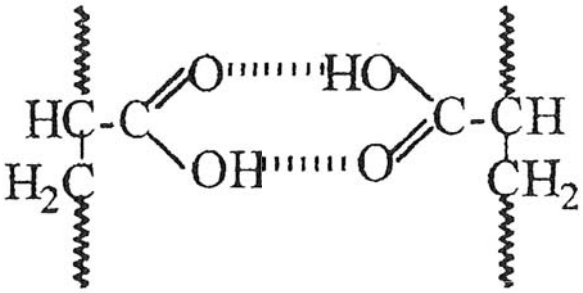


Figure 3.5 *Illustration of AA molecules forming a dimer through hydrogen bonding*

Lau and Mi (2002) states that for pure PVP, the absorption band at 1660 cm^{-1} is called amide-I and is a combined mode with the contribution of >C=O and C-N stretch. Owing to this combination mode, this band occurs at a lower wave number than expected for pure ester carbonyl band (1750 – 1700 cm^{-1}). This peak is commonly known as the PVP carbonyl peak. Yaung and Kwei (1997) describes how the frequency of the PVP carbonyl group shifts from 1670 - 1680 cm^{-1} to 1630 - 1640 cm^{-1} when it forms hydrogen bonds to the carboxyl group of the AA. In this work the PVP carbonyl group was found to exhibit a peak at 1650cm$^{-1.}$ This could indicate a low level of hydrogen bonding in the PVP homopolymer. This peak shifted to 1639cm^{-1} with the inclusion of AA thus giving evidence of a higher level of hydrogen bonding in the copolymer complex. It was also found that the peaks attributed to C=C and CH_2= (1617 cm^{-1} and 1409 cm^{-1} respectively (Ju et al. 2002)) had disappeared. This signified that a very high conversion rate from the monomers to polymer chains had being achieved.

3.2.4 Potentiometric titrations

Potentiometric titrations were carried out on pre-dried samples that were firstly ground and then dissolved in a 0.05M HCl solution. Into this solution a 0.05M solution of NaOH was titrated using a burette capable of being read to ±0.1 mL. All titrations were performed in duplicate to confirm reproducibility. Initially a titration was carried out (Figure 3.6) on HCl and NaOH in order to determine the volume of NaOH required to neutralise 40mL of HCl. As both HCl and NaOH used were volumetric standards, as expected the neutralisation point was approximately 40mL.

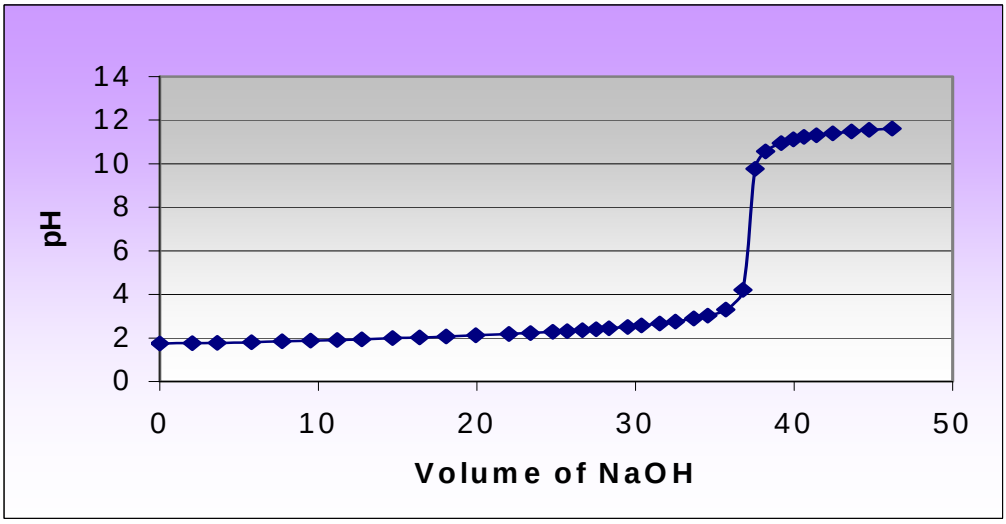


Figure 3.6 *Equilibrium pH for HCl/NaOH*

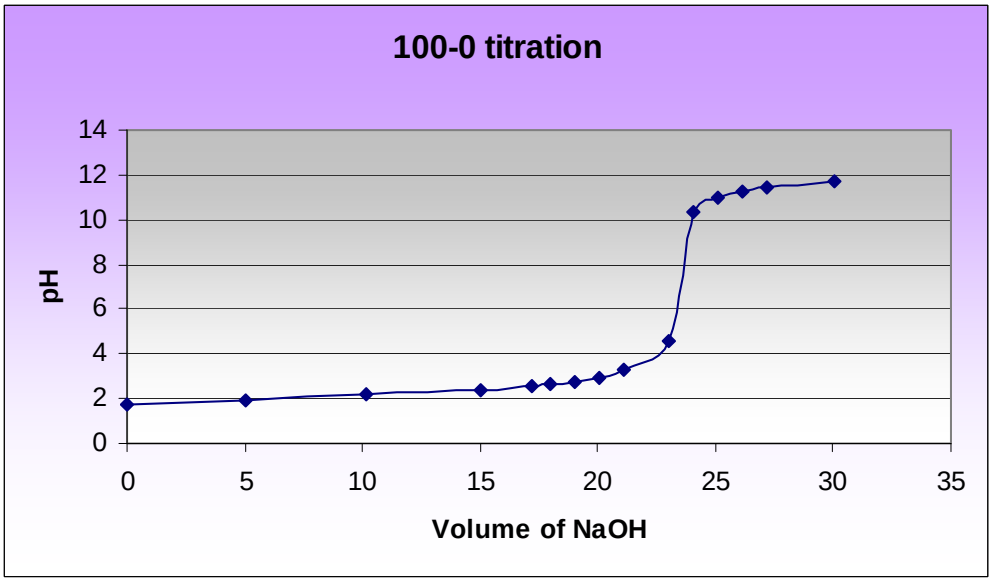


Figure 3.7 *Titration curve obtained from the titration of 100-0 photopolymerised polymer*

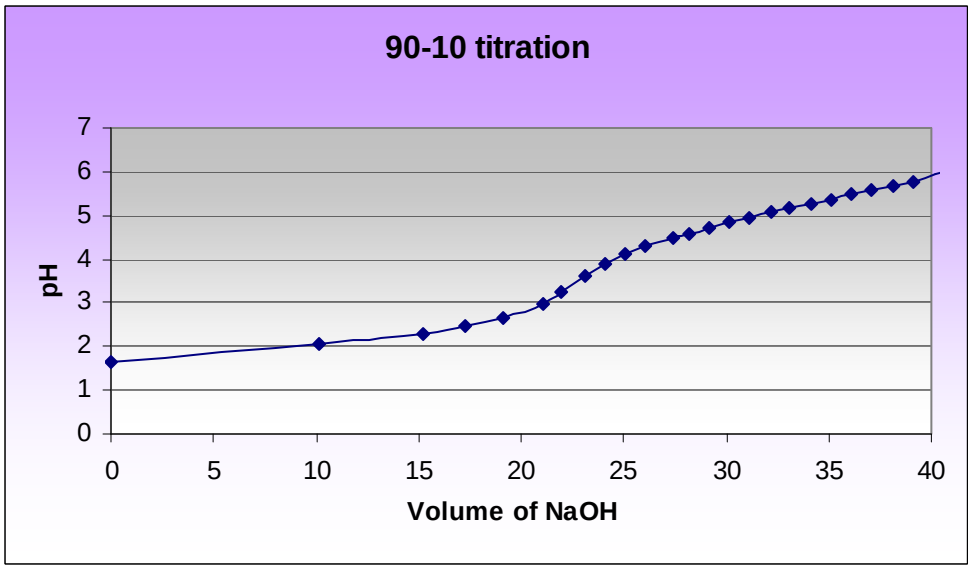


Figure 3.8 *Titration curve obtained from the titration of 90-10 photopolymerised copolymer*

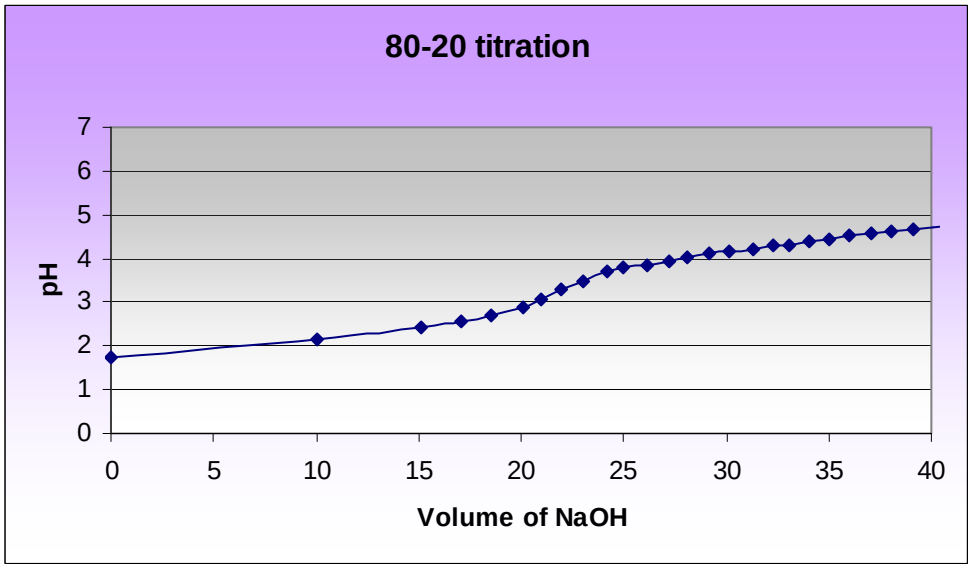


Figure 3.9 *Titration curve obtained from the titration of 80-20 photopolymerised copolymer*

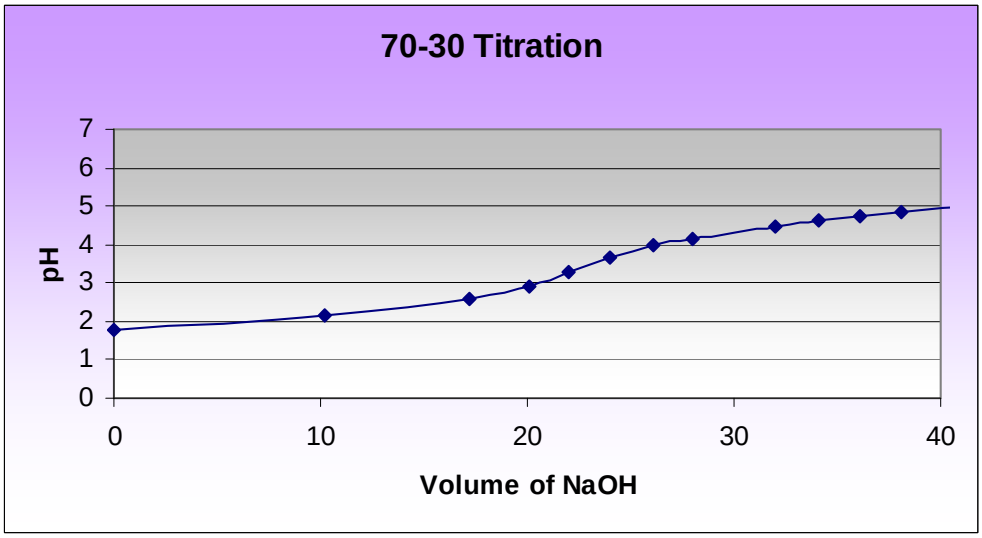


Figure 3.10 *Titration curve obtained from the titration of 70-30 photopolymerised copolymer*

The resultant potentiometric titration curves obtained for photopolymerised copolymers that contained PVP-PAA (3.7 – 3.10) displayed evidence of displaying two neutralisation transitions. The latter transition is merely the neutralisation of HCl. The first neutralisation transition can be attributed to the neutralisation of the carboxylic acid group associated with PAA/AA. From this neutralisation transition the $pK_{initial}$ can be defined. Yaung and Kwei (1997) described the $pK_{initial}$ for a weak polyelectrolyte such as PAA as the first point of pH drop from equilibrium. When the pH value of the solution is above $pK_{initial}$, which is the lowest pH at which the carboxylic acid can be neutralised, a fraction of the carboxylic acid groups dissociate to form carboxylate ions. The number of ionic charges on the backbone depends upon the pH of the solution.

In this work the $pK_{initial}$ was taken as the first point of pH drop from equilibrium. The range of $pK_{initial}$ values obtained was 4.07 to 4.49. These values compare favourably to values obtained by Yaung who recorded a $pK_{initial}$ range 4.22 of to 4.39. Therefore under pH 4 swelling conditions the carboxylic acid groups will not be fully dissociated. Therefore the bonds between PVP and PAA are expected to remain intact.

3.2.5 Polymer solubility testing

Polymer solubility testing was performed in buffered solutions at various pH values. The solubility experiments were carried out by placing a circular disc of

photopolymerised polymer into a petri dish. The polymer was then immersed in the appropriate buffered solution, and allowed to swell. Periodically removing the buffered solution and weighing the sample, obtained the amount that the hydrogel swelled. Pictures were taken of each of these samples to correlate the weight changes recorded.

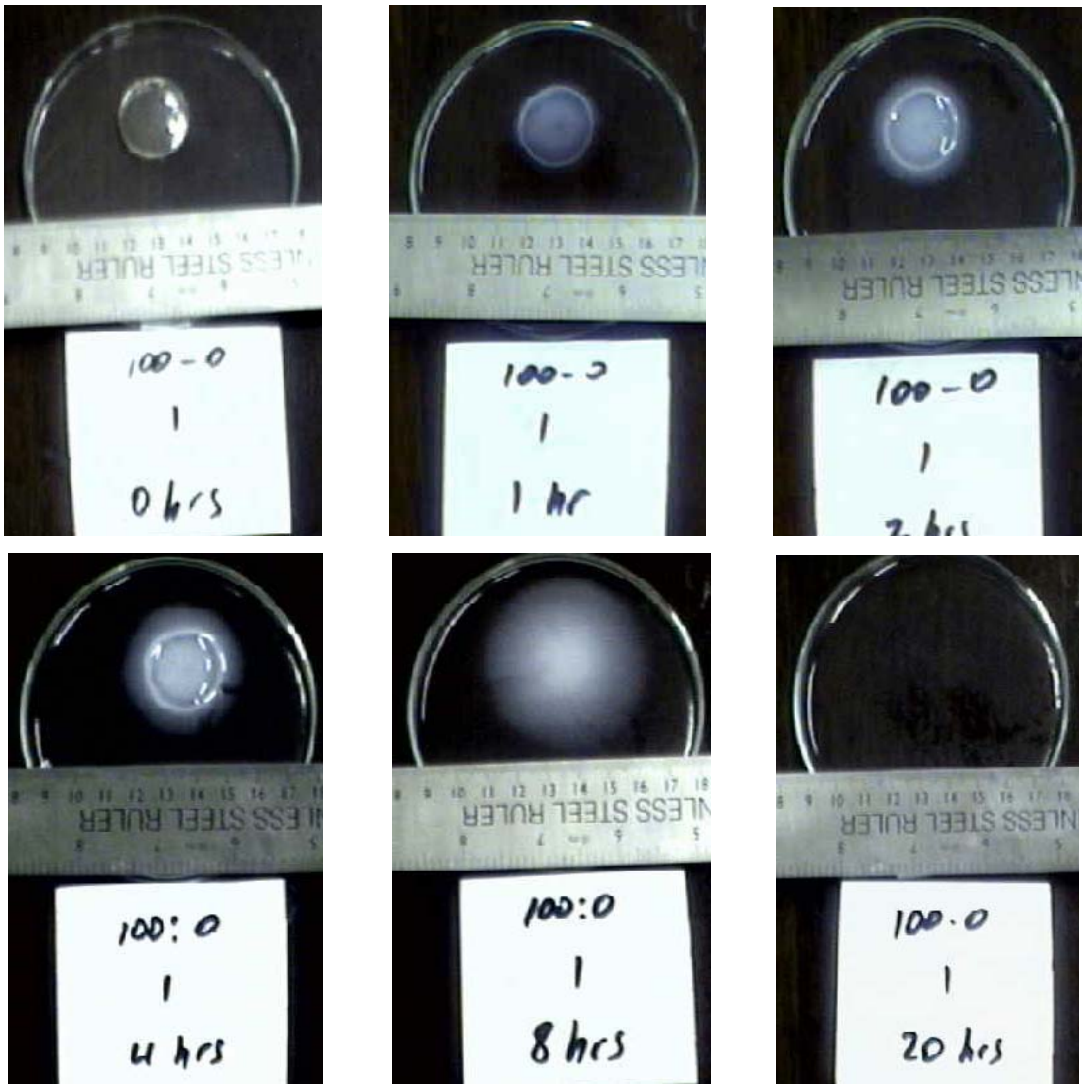
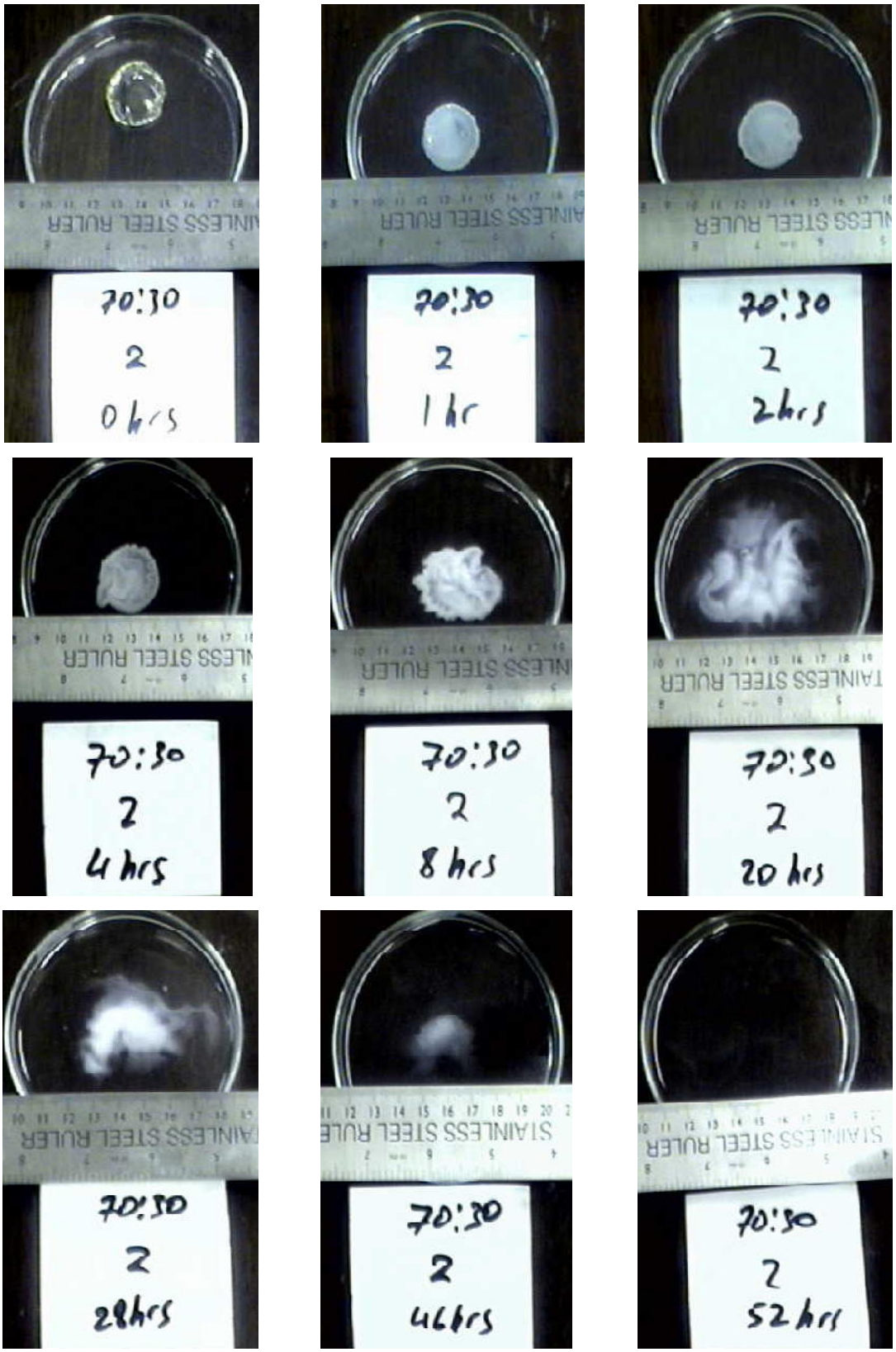


Figure 3.11 *Typical swelling characteristics of hydrogel designated 100-0. This sample was swollen in pH7 buffered solution*


70:30
2
0 hrs
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1 hr
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2
20 hrs
70:30
2
28 hrs
70:30
2
46 hrs
70:30
2
52 hrs

Figure 3.12 *Typical swelling characteristics of hydrogel designated 70-30. This sample was swollen in pH7 buffered solution. It should be noted that this sample became transparent after 52hours but did not dissolve until after 143hours.*

At a glance it would appear the samples whose original composition was 100% NVP appeared to swell less as the pH increased. However on re-evaluation of these samples with reference to the pictures taken it is clear that the samples swelled more as the pH increased. As no chemical crosslinking could occur and little evidence of intermolecular bonding was observed in Ftir, the rapid swelling of this polymer led to rapid dissolution of the samples in all pHs used (within 24 hours). All samples reached their maximum swollen weight from 0-2hrs, which would also indicate rapid dissolution (swelling data observed is shown in appendix B).

When the pH value of the solution is above $pK_{initial}$, which is the lowest pH at which the carboxylic acid can be neutralised, a fraction of the carboxylic acid groups dissociate to form carboxylate ions (Yaung and Kwei 1997). From examination of the 90-10 samples, it can be seen that the samples swollen in pH 4 buffered solution tended to swell less than samples swollen in pH 7 and pH 9. However if all three swelling media are compared at 30 hours it can be seen that samples swelled in pH 7 and pH 9 had dissolved while the samples swollen at pH 4 were still approximately 20% of original weight. However, samples that were swollen in pH 9 did not appear to swell as much as samples swollen in pH 7. In pH 7 and pH 9 buffered solutions the $pK_{initial}$ values (between 4.07 and 4.49) are exceeded, and the progressive break up of the polymer chains liberates more and more free polymer. The number of carboxylate ions also increases in the process. This leads to an increase in solubility

of the complex as the pH increases and this may give rise to an apparent decrease in swelling at pH 9. Similar trends were also seen for samples 80-20 and 70-30. As the AA content was increased the hydrogels were capable of swelling more and did not dissolve as quickly. This is due to the increase in intermolecular bonding caused by the addition of AA. Figures 3.11 and 3.12 show typical dissolution processes observed.

Solubility experiments were also carried out using distilled water as the dissolution media. On examination of the samples swollen in distilled water it was found that the sample prepared from 100% NVP dissolved rapidly. The samples that contained AA appeared to dissolve slower than would be expected, as the pH of water is approximately 7. However as there was no buffer present to maintain the pH level, the AA content tended to reduce the pH of the distilled water to approximately 4. Thus the intermolecular hydrogen bonding was not affected and so the dissolution process was slowed down.

3.2.6 Gel permeation chromatography

GPC experiments were carried out using a mobile – phase of water and methanol in the ratio of 10:1 at a flow rate of 1mL/minute, using an aquagel-OH 30 8μm mixed column. The calibration used in this experiment had a range of between 400 and 8,000,000. It was possible to calculate molecular weights outside this range, however as these results would be estimates at best they will not be presented in this work. These results are denoted as O.S. cal or outside calibration. The calibration curve used was a polynomial of the 4^{th} order. This curve was used as each calibration point had a fit ratio within ± 0.029 (Figure 3.13). The molecular weight values quoted are Mp or peak average molecular weight. Mp is a function of the weight average molecular weight (Mw), and the number average molecular weight (Mn) of the polymer, where: -

$$Mp \sim (Mw \times Mn)^{0.5}$$ (Coombes et al. 1998)

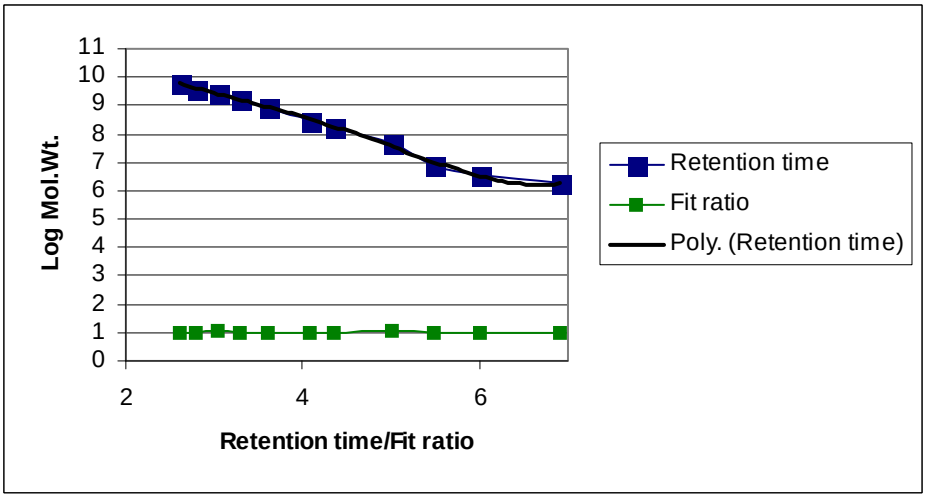


Figure 3.13 *GPC Calibration curve used in this work*

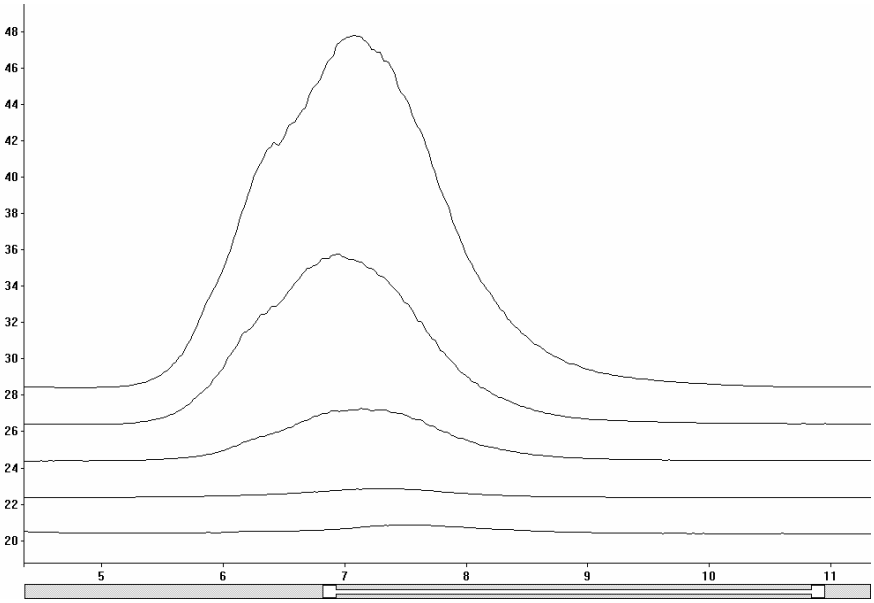


Figure 3.14 *GPC curves obtained for 100-0 samples. From bottom to top, sample taken after 1hr, 2hrs, 4hrs, 8hrs & 24hrs respectively.*

Table 3.1 *Mp values of 100-0 samples*

Time sample was taken (hours)	Retention time (minutes)	Calculated Mp
1	7.489	98845
2	7.315	132060
4	7.194	165073
8	6.943	291797
24	7.087	205993

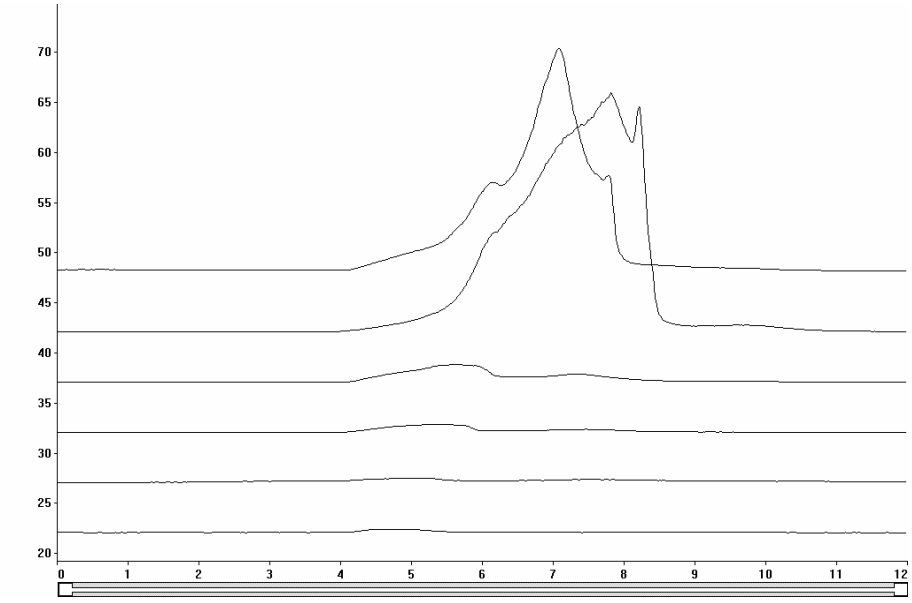


Figure 3.15 *GPC curves obtained for 90-10. From bottom to top, sample taken after 1hr, 2hrs, 4hrs, 8hrs, 24hr &, 48hrs respectively*

Table 3.2 *Mp of 90-10 samples*

Time sample was taken (hours)	Retention time (minutes)	Calculated Mp
1	4.799	O.S. cal
2	4.855	O.S. cal
4	5.456 + 7.443	O.S. cal 106467
8	5.583 + 7.364	O.S. cal + 121374
24	7.816 + 8.211	58468 + 278823
48	6.161 + 7.079 + 7.784	O.S. cal + 209670 + 61660

From Table 3.1 Mp of 100-0 samples, it can be seen that as the sample dissolved an increase of Mp was recorded up to a time 8 hours. The Mp value of the polymer eluted after 24 hours showed a reduction in molecular weight. However, as can be seen from Figure 3.14 the molecular weight distribution was broader after 24 hours as the sample had completely dissolved. This could cause a reduction in the Mp value as up to this point only surface polymer was released, but at 24 hours the bulk material had completely dissolved, reducing the Mp value. However, the possibility that hydrolytic degradation had taken place cannot be ignored (Ravichandran et al. 1997), although this is unlikely over such a short time period. In GPC the peak height is not normally taken into consideration, as the retention time is the significant value and is related to the molecular weight of the polymer been

tested. However, the peak height is an indication of the concentration of polymer been tested. It can clearly be seen from Figure 3.14 that the polymer concentration been tested increased steadily until complete dissolution of the polymer had taken place.

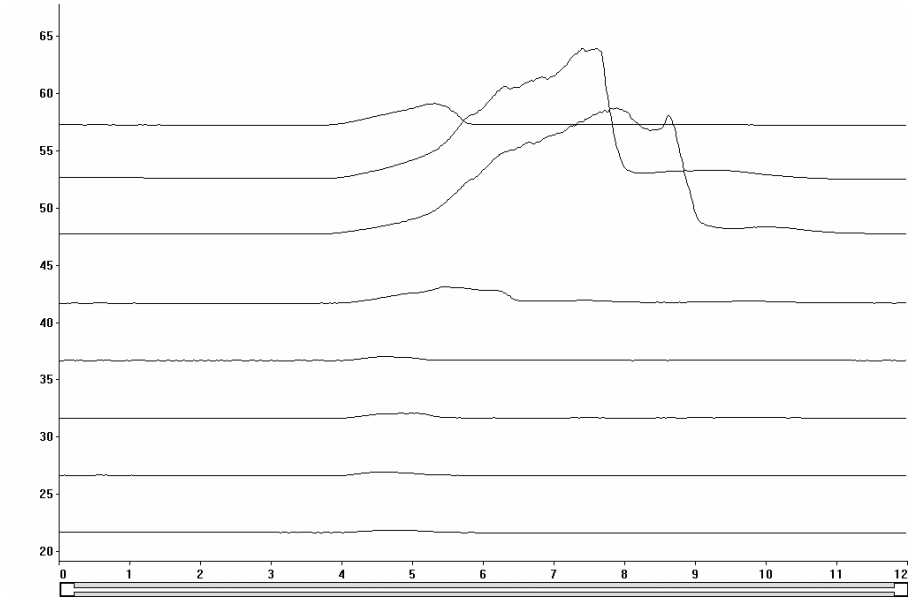


Figure 3.16 *GPC curves obtained for 80-20 samples. From bottom to top, sample taken after 1hr, 2hrs, 4hrs, 8hrs, 24hrs, 48hrs, 72hrs, & 98hrs respectively.*

Table 3.3 *Mp of 80-20 samples*

Time sample was taken (hours)	Retention time (minutes)	Calculated Mp
1	4.775	O.S. cal
2	4.65	O.S. cal
4	4.938	O.S. cal
8	4.688	O.S. cal
24	5.598	O.S. cal
48	7.913 + 8.637	49515 + 9860
72	7.4 + 7.587	114264 + 84588
98	5.288	O.S. cal

As can be seen from Table 3.2, 3.3, and 3.4 Mp values of 90-10, 80-20, and 70-30 samples respectively, there is a large number of molecular weight points outside of calibration. The Mp values eluted prior to 6.25 minutes are all in excess of

a molecular weight of 8 million. As the polymerisation process was identical to that which provided a molecular weight in the region of 290,000 for polymerised NVP it is reasonable to assume that a polymer of this molecular weight should not be possible using this polymerisation method. Thus, it must be assumed that these ultra high molecular weights achieved were caused by a physically crosslinked polymer rapidly passing through the GPC column.

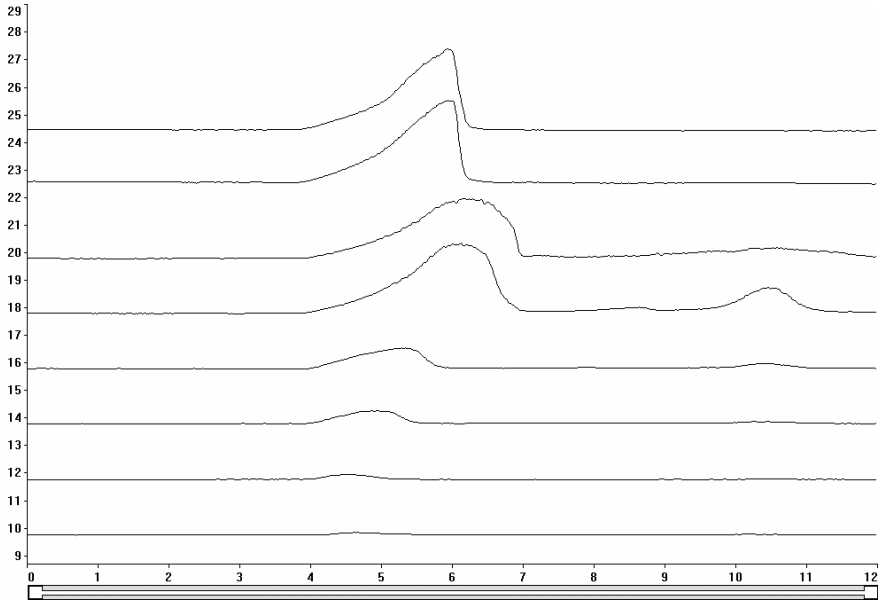


Figure 3.17 *GPC curves obtained for 70-30 samples. From bottom to top, sample taken after 1hr, 2hrs, 4hrs, 8hrs, 24hrs, 48hrs, 72hrs, & 98hrs respectively*

Table 3.4 *Mp of 70-30 samples*

Time sample was taken (hours)	Retention time (minutes)	Calculated Mp
1	4.638	O.S. cal
2	4.563	O.S. cal
4	4.888	O.S. cal
8	5.325 + 10.425	O.S. cal + O.S. cal
24	6.1 + 8.650 + 10.5	O.S. cal + 9516 + O.S. cal
48	6.238 + 10.575	O.S. cal + O.S cal
72	5.938	O.S. cal
98	5.95	O.S. cal

Conversely polymers eluted after 9.78 minutes had a molecular weight of less than 400. In Figure 3.15, the GPC chromatogram of 90-10 samples, there is evidence of a peak in this region after 24 hours. In Figure 3.16, the GPC chromatogram of 80-20 samples, there is even more evidence of peaks in this region after 24 hours, 48 hours and 72 hours. However in Figure 3.17, the GPC chromatogram of 70-30 samples, there is a clear peak attained at time intervals post 24 hours with further evidence of peaks in samples analysed after 24 hours. As the evidence for a peak in this region increased with increasing AA content, coupled with the visual inspection performed after sample preparation that showed phase separation due to the AA content, it can be assumed that this peak is caused by phase separated AA oligomers, the molecular weight of which was below 400.

From Tables 3.2, 3.3 and 3.4 it can also be seen that a molecular weight was also recorded in the region that was achieved by polymerising 100% NVP. This would signify that a portion of the copolymer complex was a PVP homopolymer. The concentration of this homopolymer reduced as expected with the increase of AA, giving way to a combination of physically crosslinked copolymer and AA oligomers.

3.3 Conclusion

A series of random copolymers, containing different monomeric concentrations of NVP and AA were synthesised by photopolymerisation. From visual inspection of the samples, it was found that a solid polymer was produced, which was slightly yellow in colour. Samples produced from 100wt% NVP had a smooth uniform surface. However samples which had AA incorporated did not have a uniform surface, and white solids on the surface indicated phase separation of some AA segments had taken place. DSC thermograms showed that the T_g of the samples tested was approximately 140ºC, with higher levels of AA the copolymers did show a reduction in T_g due to the plasticising effect of low molecular weight AA. DSC tests showed no secondary transitions in the region tested. The Ftir spectra of PVP-PAA copolymer complexes indicate hydrogen bonding between the carbonyl group in the PVP and the carboxylic acid group in the PAA moiety. There is also evidence that the AA molecules formed cyclic rings through hydrogen bonding of the carboxylic acid side groups. As the percentage of AA increased in the copolymer there is an increase in intermolecular hydrogen bonding between the carboxylic acid

groups of the AA segments. Polymer solubility analysis of the PVP-PAA complex in a higher pH medium is significantly different from results in low pH solutions. The critical pH range was found by potentiometric titrations to be between 4.07 and 4.49. Above a pH of 4.49 there is a progressive break up of the polymer chain due to a reduction in the amount of intermolecular hydrogen bonding, and thus the polymer can dissolve much quicker than in lower pHs where a high level of hydrogen bonding is possible. This is caused by an increase in the amount of carboxylate ions present, as the pH increases. The low solubility of the copolymer at low pH may make the complex suitable for gastric drug delivery systems.

As the polymers analysed in this chapter dissolved, it was deemed necessary to incorporate a chemical crosslinking agent into the monomeric solution to increase the usefulness of the hydrogel. Ethyleneglycol dimethacrylate's of various molecular weights were chosen as the chemical crosslinking agents so that the effect of molecular weight of the crosslinking agent, on the properties of the hydrogel could be determined.

Chapter 4

Synthesis and characterisation of chemically crosslinked N-vinyl pyrrolidinone -acrylic acid hydrogels

4.1 Introduction

The mechanical properties of hydrogels are very important for pharmaceutical applications. For example, the integrity of the drug device during the lifetime of the application is very important to obtain food and drugs authority (FDA) approval, unless the device is designed as a biodegradable system. A drug delivery system designed to protect a sensitive therapeutic agent, such as a protein, must maintain its integrity to be able to protect the protein until it is released out of the system.

Changing the degree of crosslinking has being utilised to achieve the desired mechanical properties of the hydrogel. Increasing the degree of crosslinking of the system will result in a stronger gel (Anseth et al. 1996). However, a higher degree of crosslinking creates a more brittle structure. Hence, there is an optimum degree of crosslinking at which a relatively strong and yet elastic hydrogel is produced. Copolymerisation has also being utilised to achieve the desired mechanical properties of hydrogels. Incorporating a co-monomer that will contribute to hydrogen bonding can increase the strength of the hydrogel (Peppas et al. 2000).

The swelling of hydrogels, following penetration of water into a glassy matrix, follows three steps that occur in succession. These steps are: diffusion of water molecules into the polymer network; relaxation of polymer chains with hydration; and finally expansion of the polymer network into the surrounding bulk water medium upon relaxation. Swelling behaviour differs depending on which step becomes dominant in determining the rate. Depending on the dominant factor, the mechanism of transport for solvent penetration into the polymers can be classified as either Fickian (Case-I) diffusion or anomalous (non-Fickian, Case-II) transport. When no structural changes of the polymer network occur throughout the whole process or when the rate of diffusion or penetration is much less than that of the polymer hydration relaxation process, the penetration of solvent into the polymer is governed by solvent molecule diffusion through the polymer network (Figure 4.1, step 1). In the extreme case that the solvent diffusion rate is much faster than the relaxation rate, the relaxation process becomes the rate-determining step (step 2).

When the gel expansion process dominates, the swelling kinetics is governed by collective diffusion (step 3) (Okano 1998).

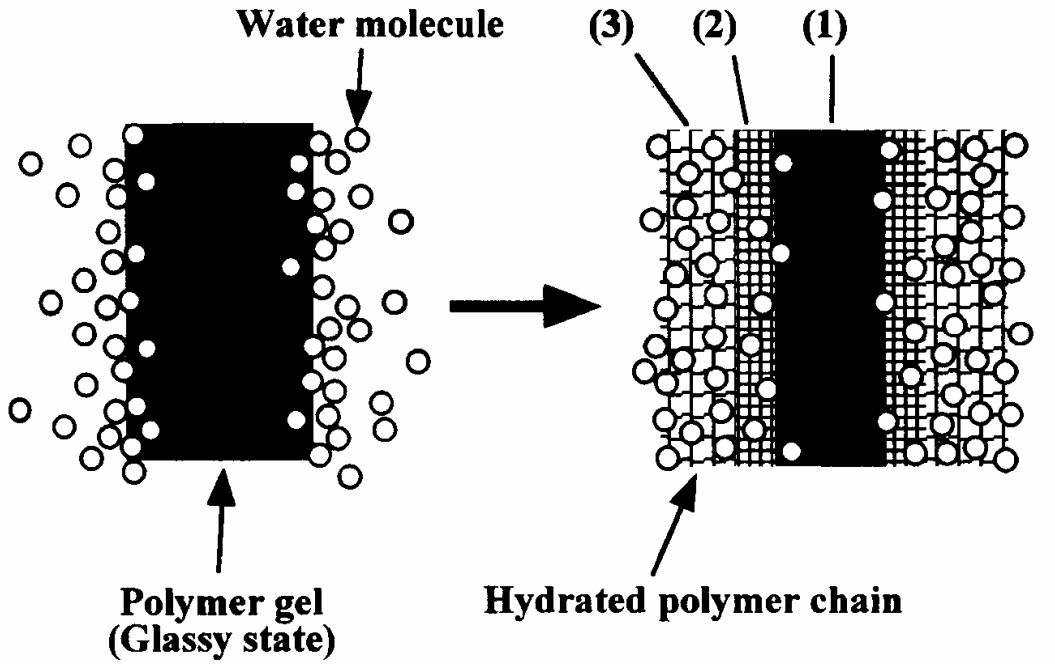


Figure 4.1 *Water uptake process for polymer gels from initially glassy dry state (Okano 1998)*

In chapter 3, it was shown that random copolymers of NVP and AA tended to dissolve in aqueous solutions at various pHs. Therefore crosslinking agents were incorporated into the monomeric feed concentration to further improve the usefulness of these gels. In this chapter polyethylene glycol dimethacrylate crosslinking agents of various molecular weights were copolymerised with NVP/AA by UV polymerisation and characterised by means of Ftir and Potentiometric titrations. Swelling studies were performed to determine the uptake of water in various pHs medias. Parallel plate rheometry was carried out at 37°C to investigate the comparative strength of these hydrogels at body temperature.

4.2 Results and Discussion

4.2.1 Preparation of samples

Crosslinked samples of both NVP and NVP/AA were photopolymerised using Irgacure® 184 as a photoinitiator. These samples were cured on a silicone moulding, and prior to testing dried for 24hrs in a vacuum oven.

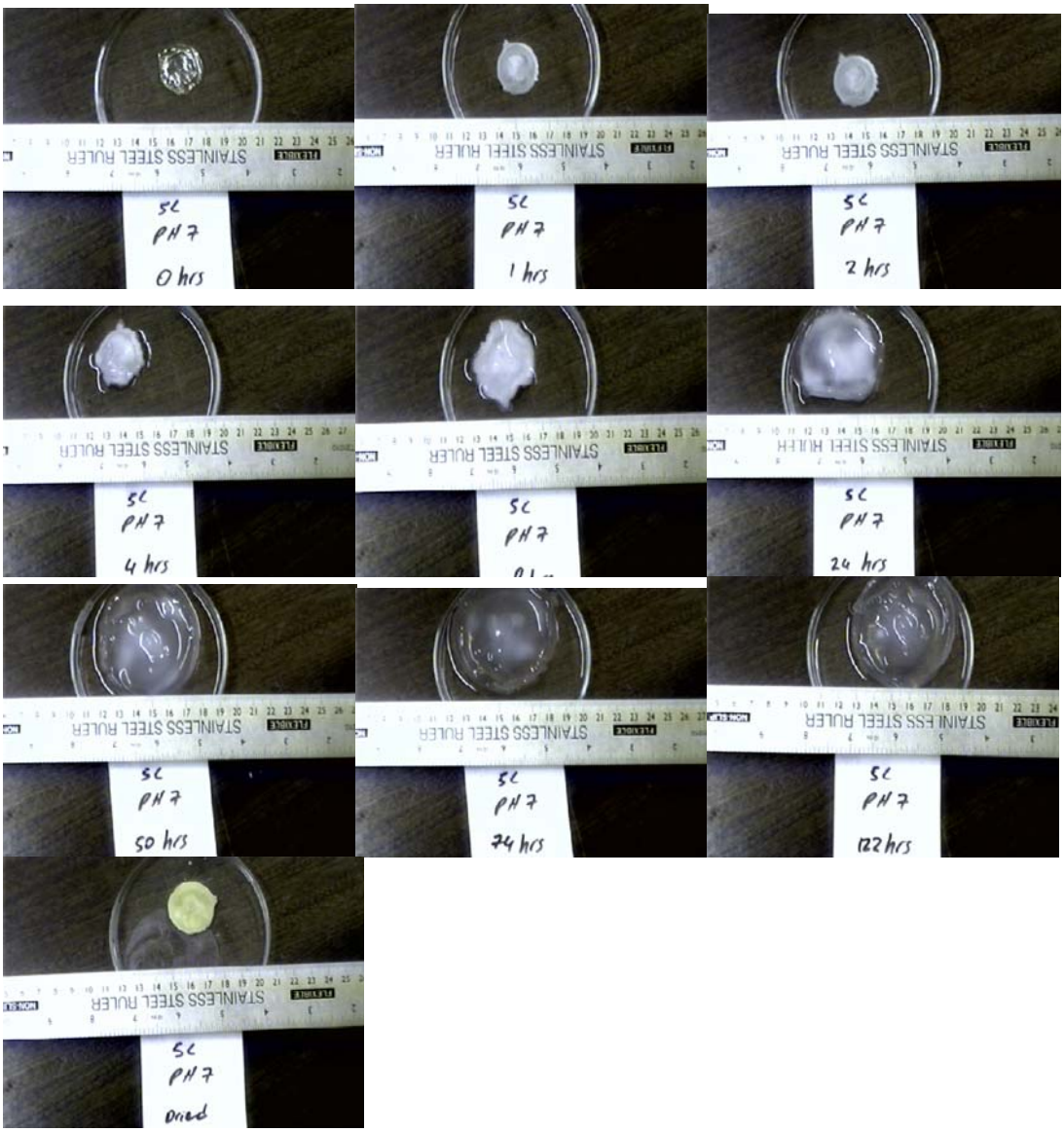


Figure 4.2 *Swelling characteristics of 70-30 sample crosslinked with 0.1wt% PEG600DMA; swelling occurred from 0 to 122 hours, and the sample oven dried at the end of test.*

On visual inspection of the UV cured samples, it was found that the addition of a crosslinking agent had no effect on the samples as the same observations were made for both crosslinked samples and uncrosslinked samples. These observations were that the 100-0 samples cured with a uniform smooth surface. With the addition of AA the uniformity of the surface of the samples deteriorated. This deterioration increased with an increase in AA concentration: in the samples whose initial composition was 100 wt% NVP all the samples were transparent in appearance and yellowish in colour after photopolymerisation. However the addition of AA caused

the formation of some white solids. These white solids would suggest a degree of phase separation in the cured co-polymer (Lau and Mi 2002).

Ramazani-Harandi et al. (2006) notes that visual observations of hydrogels are often the preferred method to quantify the strength of a hydrogel. It is suggested that a sample with good gel strength may be identified using simple qualitative methods such as ensuring the sample has a geometrically stable shape with sharp edges and corners, and feeling its strength by pressuring the sample between the fingers. In this work the samples retained a good shape as described by Ramazani-Harandi et al. and felt strong. Figure 4.2 shows the swelling profile of one of the hydrogels tested, and illustrates the stability of the geometrically shape.

4.2.2 Fourier transform infrared spectroscopy

Ftir experiments were carried out to determine the effect that the addition of a crosslinking agent had in comparison to the physically crosslinked hydrogel. This hydrogel consisted of a monomeric mixture of NVP, AA and a crosslinking agent, which underwent free-radical photopolymerisation.

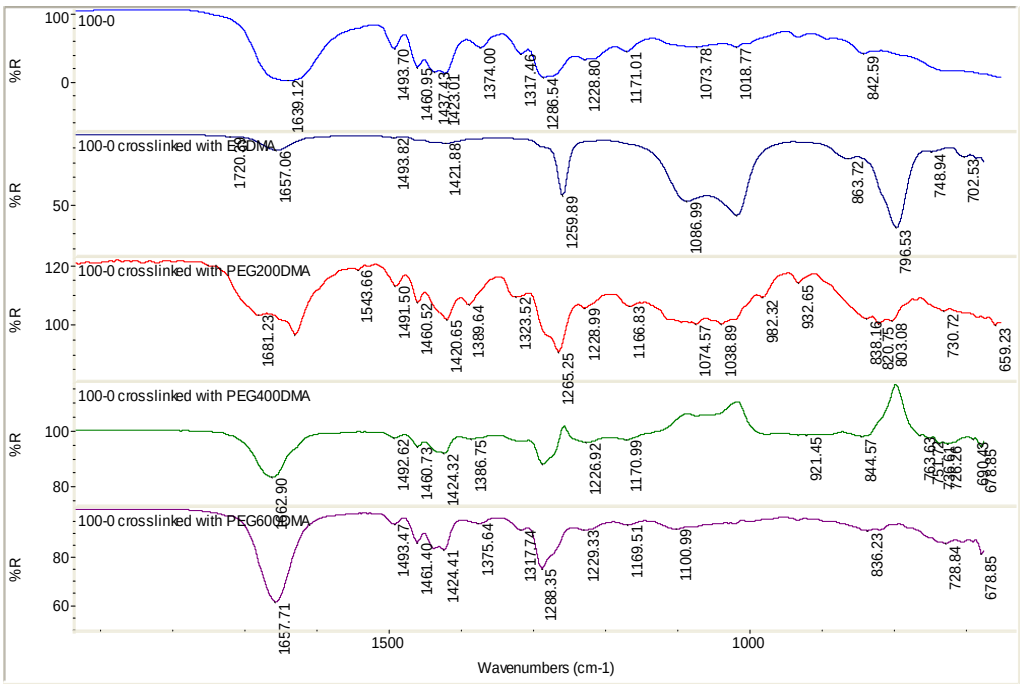


Figure 4.3 *Ftir spectrum of all 100-0 polymers tested*

From the literature the carbonyl group of PVP exhibits a peak between 1670-1680 cm^{-1} (Yaung and Kwei 1997). When the carbonyl group forms intermolecular

bonding there is a negative shift exhibited in the IR spectrum. In chapter 3, it was found that the carbonyl group exhibited a peak at approximately 1650cm^{-1}, which may indicate a low level of hydrogen bonding. The Ftir spectrum for crosslinked samples (Figure 4.3) exhibited a peak at approximately 1660 cm^{-1} associated with the carbonyl group of PVP, higher than that observed for the uncrosslinked homopolymer. This value is in the region where the PVP carbonyl peak should be if it is not involved in hydrogen bonding, and therefore indicates that the crosslinking agent acted as a spacer that did not allow a high level of intermolecular bonding to occur in the carbonyl group of PVP. However it is also evident that a small shoulder was formed on the PVP carbonyl peak. This would indicate that some intermolecular bonding did still occur, but the bulk of the polymer was not involved in intermolecular hydrogen bonding.

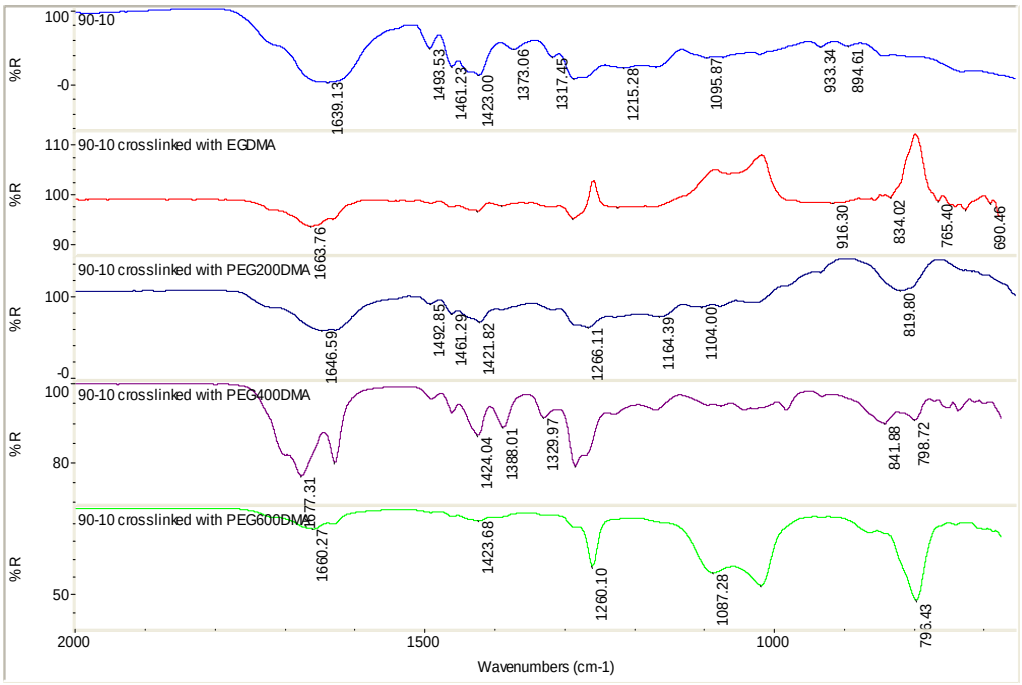


Figure 4.4 *Ftir spectrum of all 90-10 polymers tested*

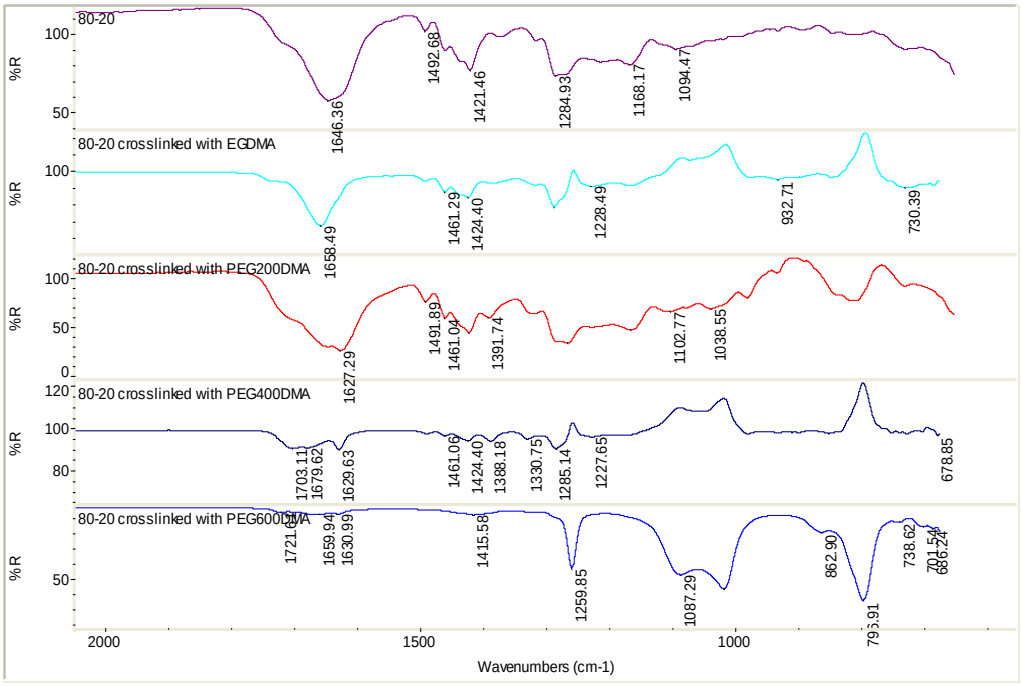


Figure 4.5 *Ftir spectrum of all 80-20 polymers tested*

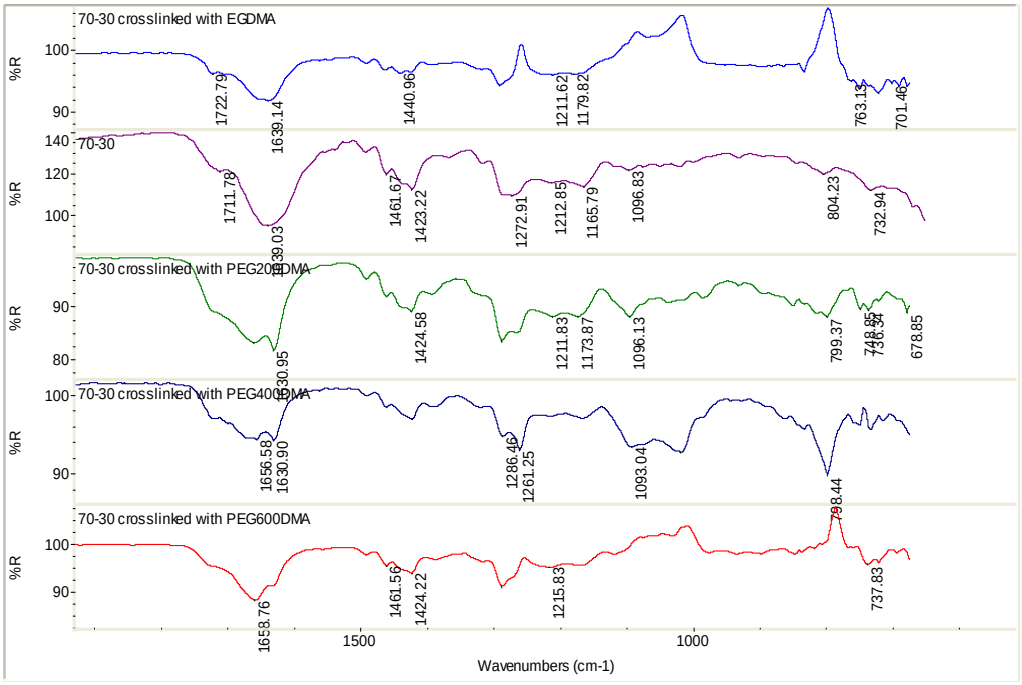


Figure 4.6 *Ftir spectrum of all 70-30 polymers tested*

As outlined in the chapter 3, Bures and Peppas (2000) states that the carboxylic acid groups can exist in both free and dimeric form depending upon their environment. Lee et al. (1988a, 1988b) describes how the stretching frequency of the carbonyl moiety in the carboxylic acid group, as is defined by absorption at 1750 cm^{-1}, whereas the dimer stretching frequency has been reported as being between

1700-1720 cm^{-1} (Yaung and Kwei 1997; Bures and Peppas 2000; Lee et al. 1988a; Lee et al. 1988b). In chapter 3 it was shown that the incorporation of AA caused the PVP carbonyl group to shift to 1639 cm^{-1} and also caused the formation of a shoulder in the region 1700-1720 cm^{-1} on this peak, which indicated the presence of the AA dimer. When a crosslinking agent was incorporated into the PVP/PAA copolymer (Figures 4.4 – 4.6, and additional spectrum illustrated in appendix C), two carbonyl peaks were formed indicating the presence of both hydrogen bonded PVP and non-hydrogen bonded PVP, with the hydrogen bonded PVP exhibiting a much smaller peak. There is also evidence of the formation of shoulders on the non-hydrogen bonded PVP carbonyl group in the region of 1721 and 1736 cm^{-1} that indicates the presence of both the AA dimer and hydrogen bond bonding between the carbonyl peak of PVP and the carboxylic acid group of PAA (Yaung and Kwei 1997).

As the AA content was increased the hydrogen bonded PVP carbonyl peak increased in size indicating further hydrogen bonding. The shoulders in the region of 1721 cm^{-1} and 1736 cm^{-1} also increased in size to form small peaks as expected.

4.2.3 Potentiometric titration

Potentiometric titrations were carried out on pre-dried samples that were firstly ground and then dispersed in a 0.05M HCl solution. Into this solution a 0.05M solution of NaOH was titrated using a burette capable of being read to ±0.1 mL. Experiments were performed using samples crosslinked with PEG200DMA to analyse the effect that crosslinking the polymer had on the $pK_{initial}$ value. PEG200DMA was the only crosslinking agent analysed as the structure of all the crosslinking agents were similar, with the only difference being the molecular weight. All titrations were repeated to confirm reproducibility.

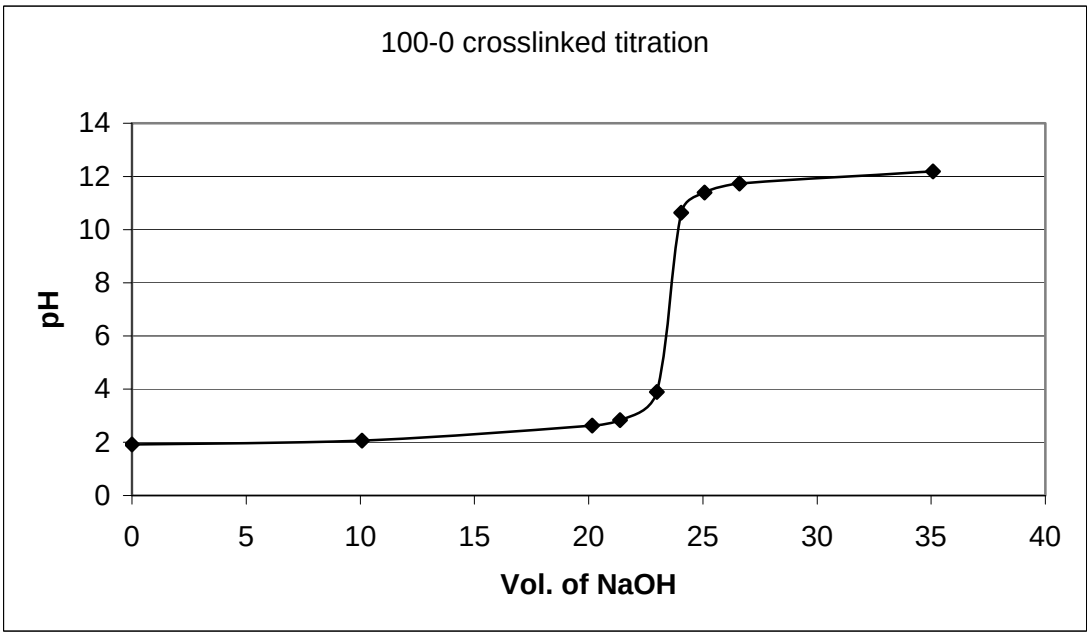


Figure 4.7 *Titration curve obtained from the titration of 100-0 photopolymerised polymer, crosslinked with PEG200DMA*

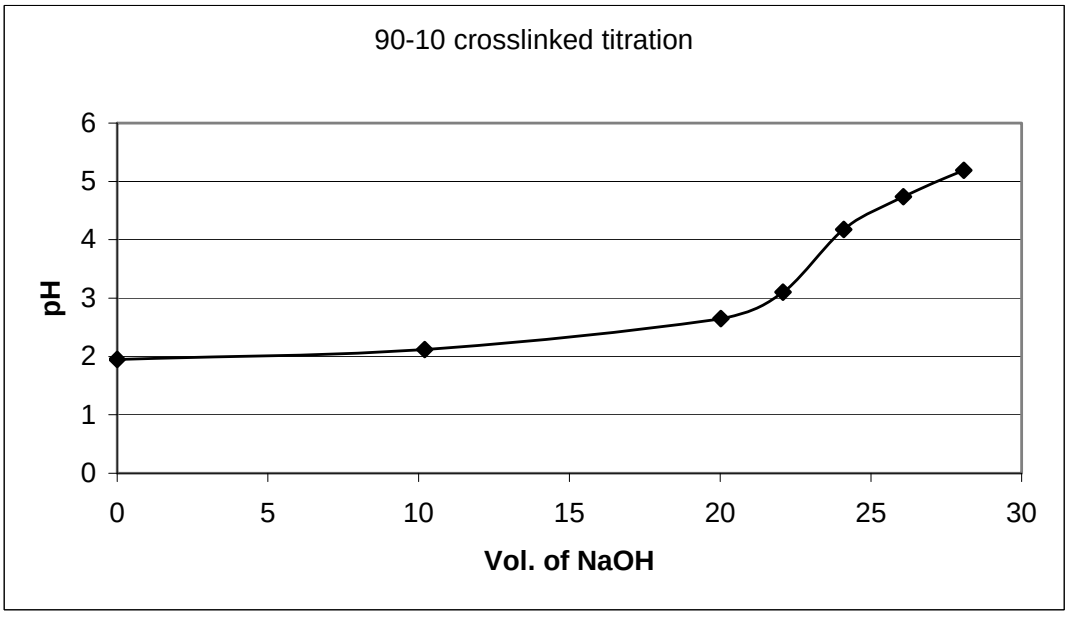


Figure 4.8 *Titration curve obtained from the titration of 90-10 photopolymerised copolymer,* crosslinked *with PEG200DMA*

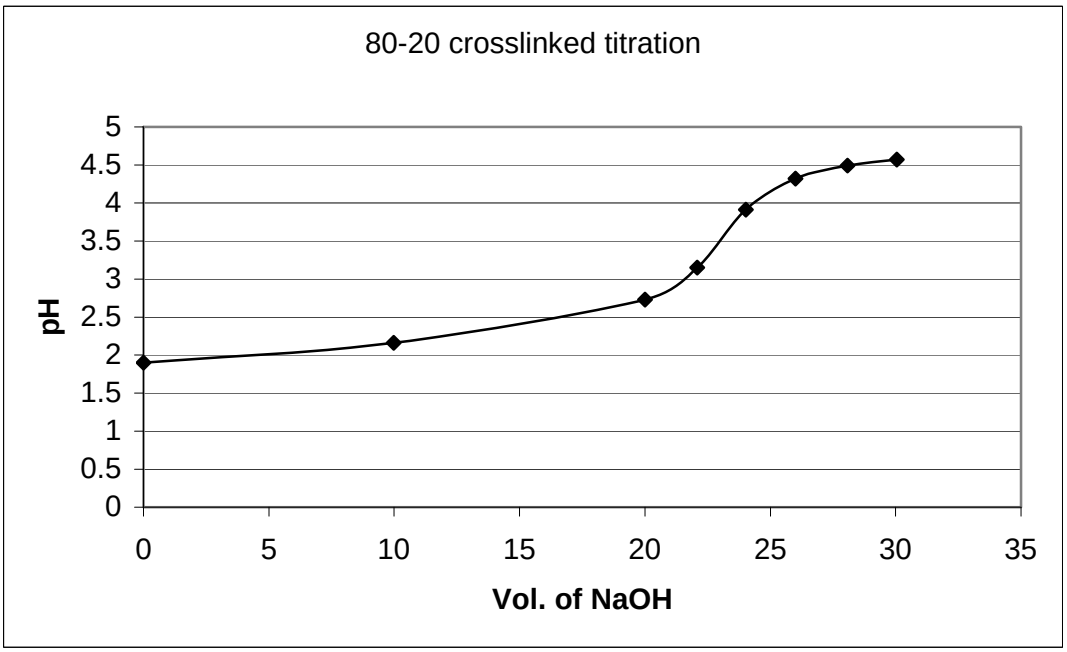


Figure 4.9 *Titration curve obtained from the titration of 80-20 photopolymerised copolymer, crosslinked with PEG200DMA*

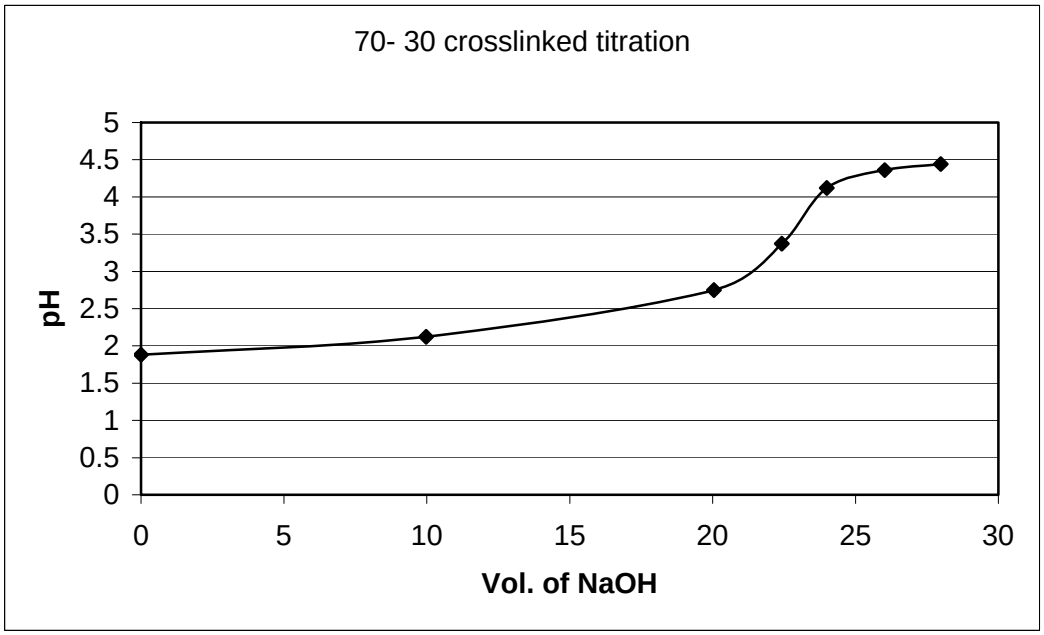


Figure 4.10 *Titration curve obtained from the titration of 70-30 photopolymerised copolymer,* crosslinked *with PEG200DMA*

From the resultant graphs two neutralisation transitions could clearly be seen. As was the case in chapter 3, the latter transition is merely the neutralisation of the HCl, and therefore has been omitted from this work. The first neutralisation transition can be attributed to the neutralisation of the carboxylic acid group associated with PAA/AA. From this neutralisation transition the $pK_{initial}$ can be

determined. The $pK_{initial}$ was taken as the first point of pH drop from equilibrium. The range of $pK_{initial}$ values obtained in this work was 4.41 to 4.48, and these values are within the range determined in chapter 3. It can therefore be concluded that the incorporation of the crosslinking agent had no effect on the $pK_{initial}$ values. These values compare favourably to values obtained by Yaung and Kwei (1997) who recorded a $pK_{initial}$ range of 4.22 to 4.39.

4.2.4 Swelling studies

Swelling experiments were performed in solutions of various pH values. The swelling experiments were carried out by placing a circular disc of photopolymerised polymer into a petri dish. The polymer was then immersed in the appropriate buffered solution and allowed to swell. The amount that the hydrogel swelled was determined by periodically removing the buffered solution, pat-drying and weighing the sample.

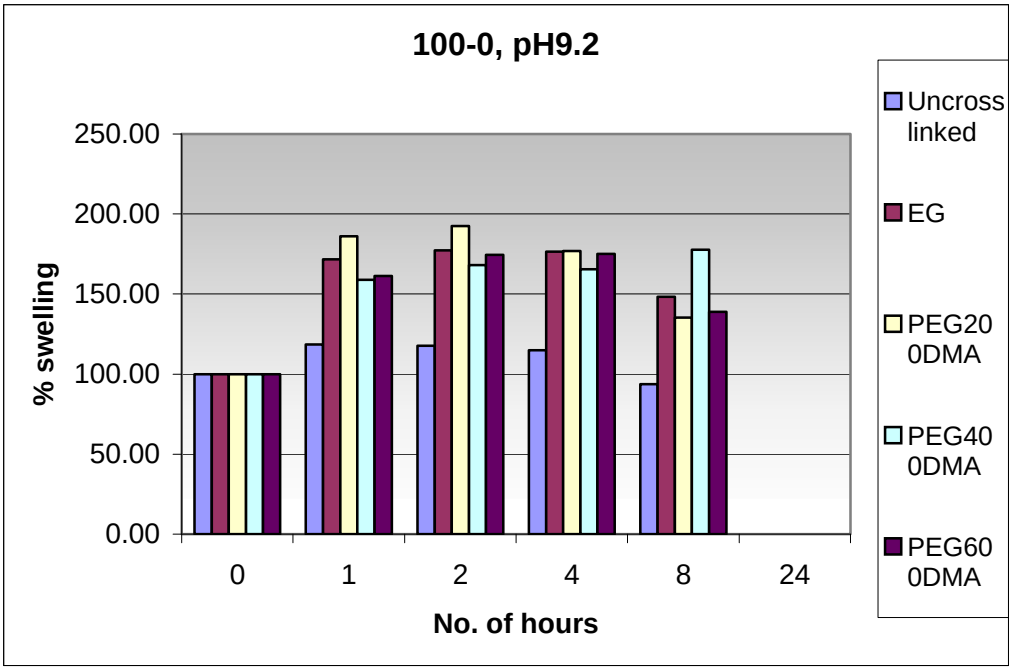


Figure 4.11 *Swelling of 100-0 crosslinked samples in pH9.2 buffered solution*

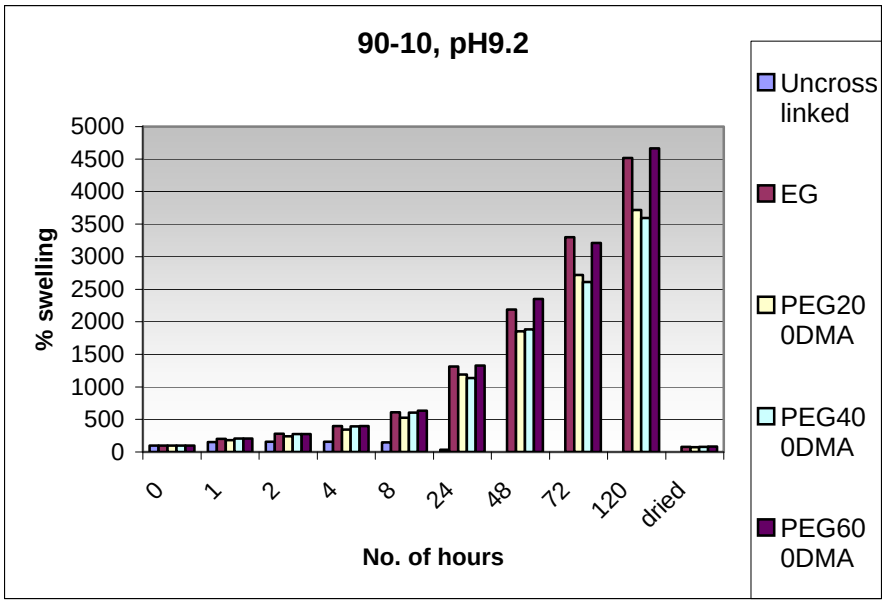


Figure 4.12 *Swelling of 90-10 crosslinked samples in pH9.2 buffered solution*

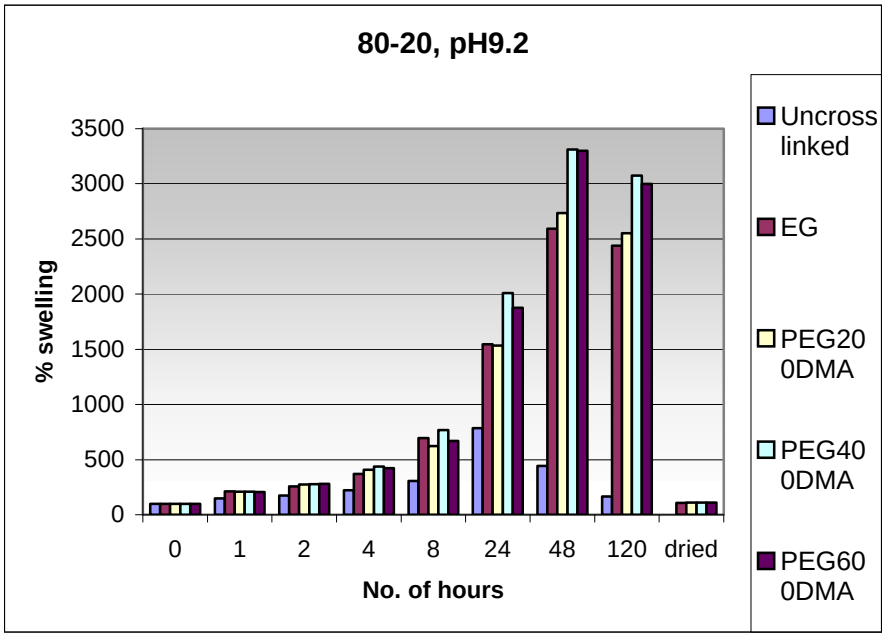


Figure 4.13 *Swelling of 80-20 crosslinked samples in pH9.2 buffered solution*

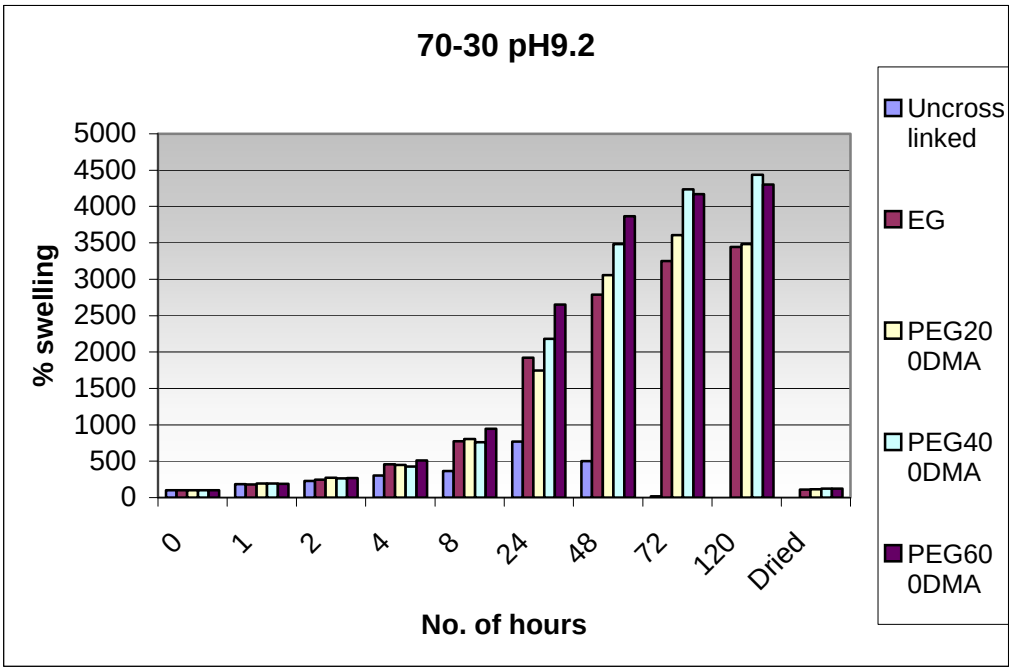


Figure 4.14 *Swelling of 70-30 crosslinked samples in pH9.2 buffered solution*

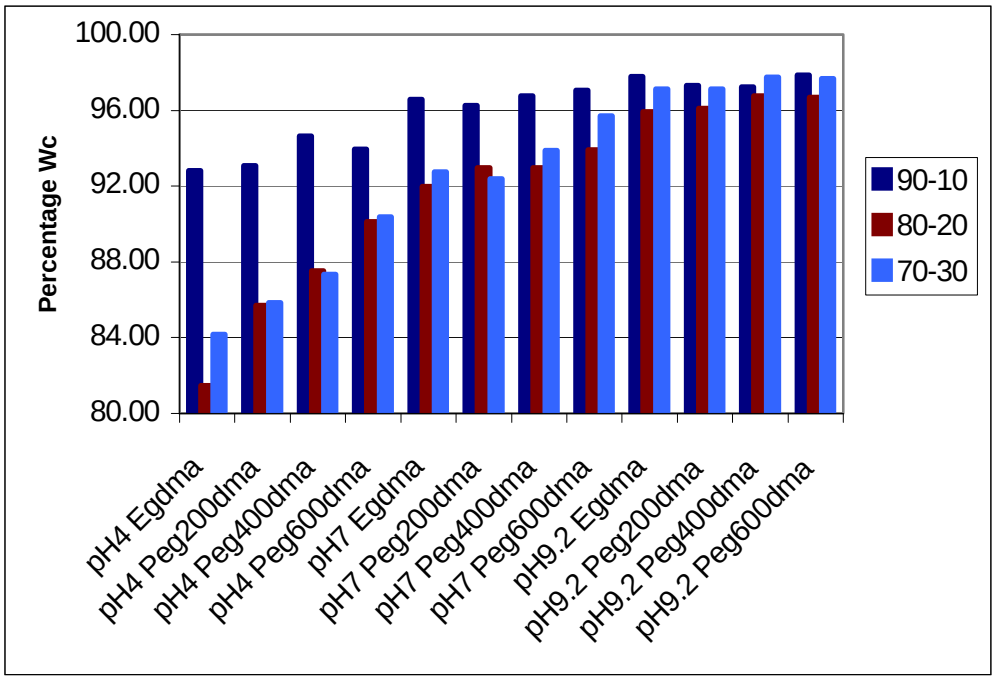


Figure 4.15 *The percentage water content of the hydrogels analysed*

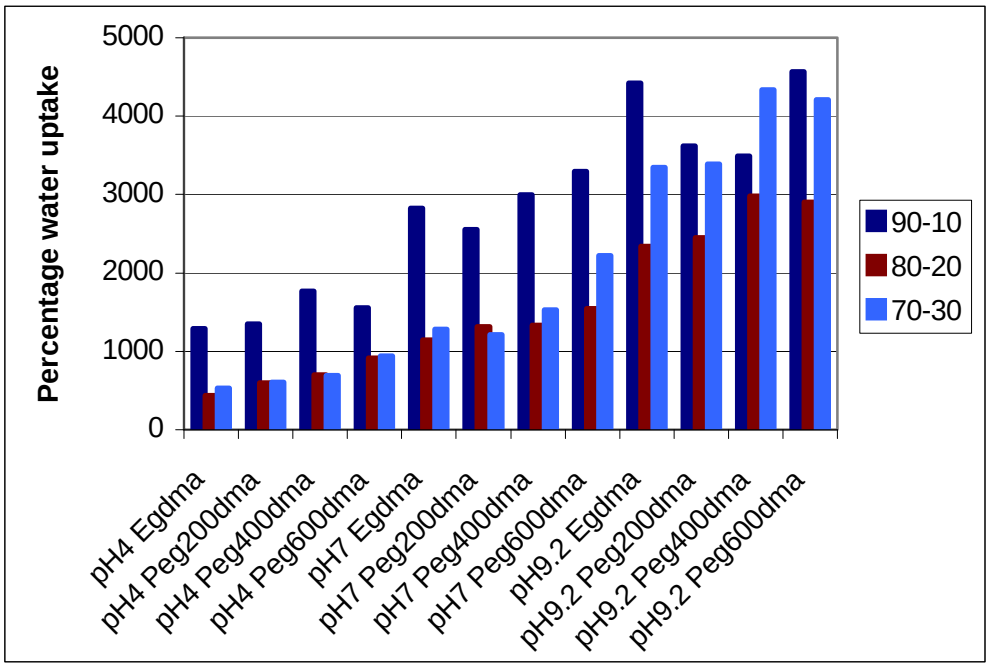


Figure 4.16 *The percentage water uptake of the hydrogels tested*

As the crosslinking agent incorporated in all instances was 0.1wt% of the total monomer content, the number of crosslinking chains per mass of liquid decreases as the molecular weight of the crosslinking agent increases. This, combined with the low degree of physical crosslinking in the 100-0 samples, led to relatively high swelling values for the lower molecular weight EGDMA and PEG200DMA. For example when swollen in pH 9.2 for 4hrs (Figure 4.11), the uncrosslinked polymer had a swollen percentage of 114%, while the crosslinked samples that contained EGDMA, PEG200DMA, PEG400DMA, and PEG600DMA yielded values of 176%, 177%, 166% and 175% respectively. When all 100% NVP crosslinked samples are compared to the uncrosslinked polymer it was found that a higher degree of swelling was achieved in the crosslinked samples. This was due to the crosslinks decreasing the polymer solubility, and hence an apparent higher degree of swelling was observed. However none of the crosslinked samples tested yielded a significant swollen polymer after 24hrs as dissolution had taken place. Therefore complete crosslinking did not occur, and thus it was not possible to calculate W_c and W_u. Hong et al. (1996b) also found that the swelling of NVP based polymers crosslinked with low levels of crosslinking agents, yielded a homogeneous solution or a viscous fluid on hydration rather than producing swollen polymers.

The $pK_{initial}$ of AA is in the range of 4.07 to 4.49. When the pH is less than $pK_{initial,}$ the H^+ ionic strength is very high and this effectively suppresses the ionisation of the carboxylic acid groups. The gel is neutral and the flexibility of the polymeric chains is rather low. Carboxylic acid groups within the network ionise as the pH of the environmental solution rises above its $pK_{initial}$. This effectively raises the concentration of free ions inside the gel causing the swelling to increase. Additionally, the gel tends to expand to minimise the repulsive forces between the ionised carboxylic acid groups (Jianqi and Lixia 2002).

Initially the 90-10 samples followed the same trend as the 100-0 samples, with the lower molecular weight crosslinking agents swelling more than the higher molecular weight crosslinking agents. However, as the test samples approached complete hydration, the samples containing higher molecular weight crosslinking agents did swell to a higher extent by virtue of their longer crosslinked chain lengths. Representative results are shown in Figure 4.12 and additional swelling results are illustrated in appendix D. These crosslinked samples, while they did not dissolve for the duration of the test, did however exhibit very poor gel integrity. This poor gel integrity may be caused by the fact that the polymer had absorbed more water than its structure was capable of holding. The corresponding uncrosslinked polymer had almost completely dissolved within 24hrs. When a swollen polymer is separable from the water fraction, but was too fluid to handle, however displaying an increased viscosity as compared to solutions, it is classified as a viscous fluid (Hong et al. 1996b). This was found to be the case with the 90-10 samples. The percentage swelling, water content and water uptake for all 90-10 crosslinked samples increased as the pH of the swelling media and the molecular weight of the crosslinking agents increased. In fact the W_c (Figure 4.15) and W_u (Figure 4.16) values achieved were higher than the equivalent 80-20 and most of the 70-30 samples. However these values were achieved at the expense of the gel integrity.

The crosslinked 80-20 and 70-30 samples (representative swelling results are shown in Figures 4.13 and 4.14, additional swelling results are shown in appendix D) had good gel integrity at the end of the swelling experiment. As expected the W_c and W_u values obtained increased as both the pH of the swelling media increased and the molecular weight of the crosslinking agents increased. The values obtained by both sets of gels were comparable in pH 4, however as the pH of the swelling media was increased there was a notable difference in the swelling characteristics of

each set of samples, with the 70-30 monomeric concentrations yielding a W_u of up to 1300% more then 80-20 samples. This was due to the increased internal repulsion of the gel caused by the increased number of free ions liberated from the carboxylic acid groups of AA. The hydrogels were completely swollen, transparent, and had a smooth, continuous surface. They were also relatively firm but flexible. These observations are characteristic of a homogeneous crosslinked hydrogel, which does not lose material by disentanglement of the polymer chains (Rodríguez et al. 2003).

4.2.5 Parallel plate rheometry

Parallel plate rheometry was carried out at 37°C on crosslinked 80-20 and 70-30 hydrogels swollen in various pH buffers, to investigate the comparative strength and the oscillating torque at break of these gels at body temperature. A strain sweep from 1 x E^{-4} to 1 at a frequency of 1Hz, with a constant normal force of 5 ±0.5 N exerted on the samples, was preformed to determine the point at which the hydrogel breaks down. This was the point at which the samples' interactions are increasingly stretched until they were broken and this resulted in a drop in the elastic component G', and thus there was a crossing of G' and G", this point was taken as the oscillating torque at break. The comparative strength of the hydrogels was taken from the linear region of the G' curve.

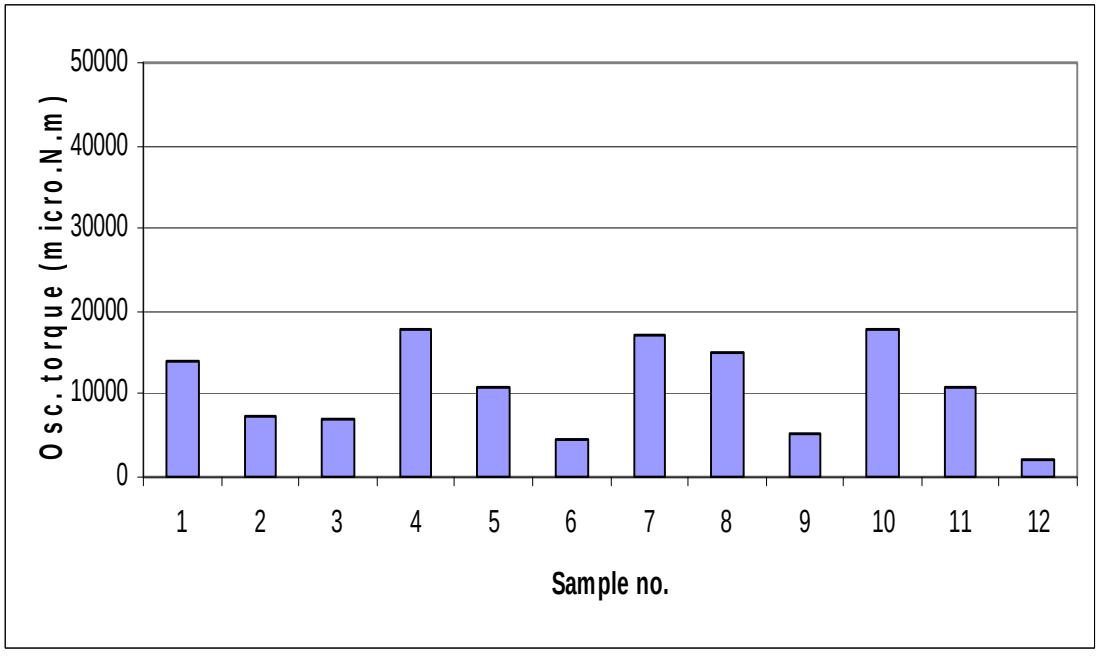


Figure 4.17 *Oscillating torque at break of the samples whose composition is shown in Table 4.1*

Table 4.1 *Composition of samples tested for oscillating torque at break and comparative strength on parallel plate rheometer*

Sample number	NVP (%wt)	AA (%wt)	Crosslinking agent	Buffer pH
1	80	20	EGDMA	4
2	80	20	EGDMA	7
3	80	20	EGDMA	9.2
4	80	20	PEG200DMA	4
5	80	20	PEG200DMA	7
6	80	20	PEG200DMA	9.2
7	80	20	PEG400DMA	4
8	80	20	PEG400DMA	7
9	80	20	PEG400DMA	9.2
10	80	20	PEG600DMA	4
11	80	20	PEG600DMA	7
12	80	20	PEG600DMA	9.2
13	70	30	EGDMA	4
14	70	30	EGDMA	7
15	70	30	EGDMA	9.2
16	70	30	PEG200DMA	4
17	70	30	PEG200DMA	7
18	70	30	PEG200DMA	9.2
19	70	30	PEG400DMA	4
20	70	30	PEG400DMA	7
21	70	30	PEG400DMA	9.2
22	70	30	PEG600DMA	4
23	70	30	PEG600DMA	7
24	70	30	PEG600DMA	9.2

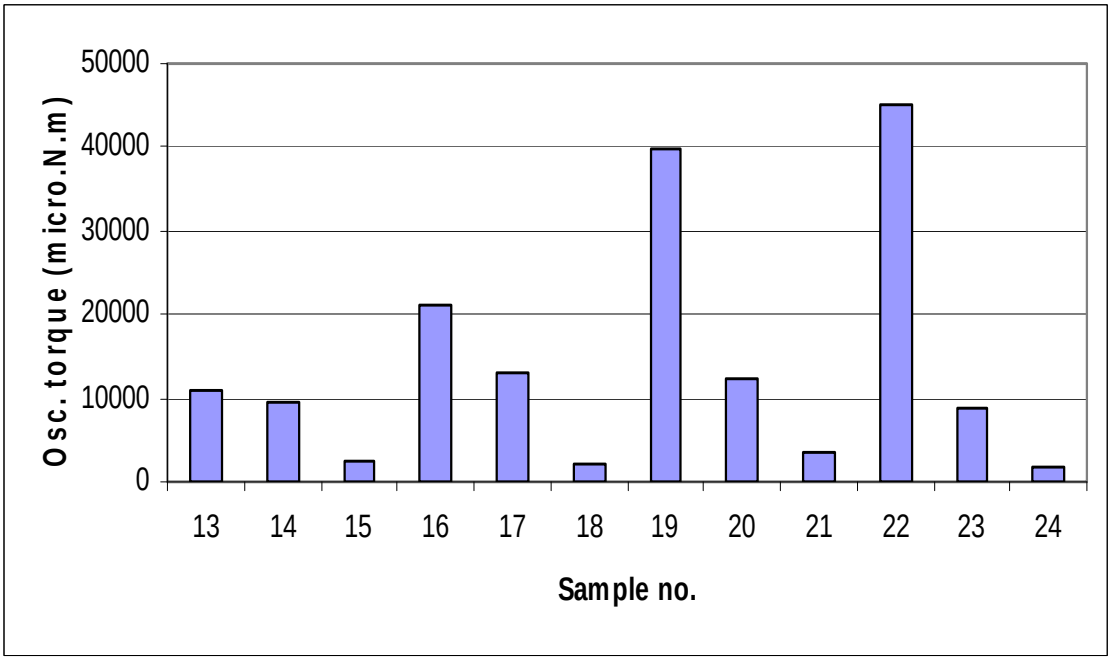


Figure 4.18 *Oscillating torque at break of the samples whose composition is shown in Table 4.1*

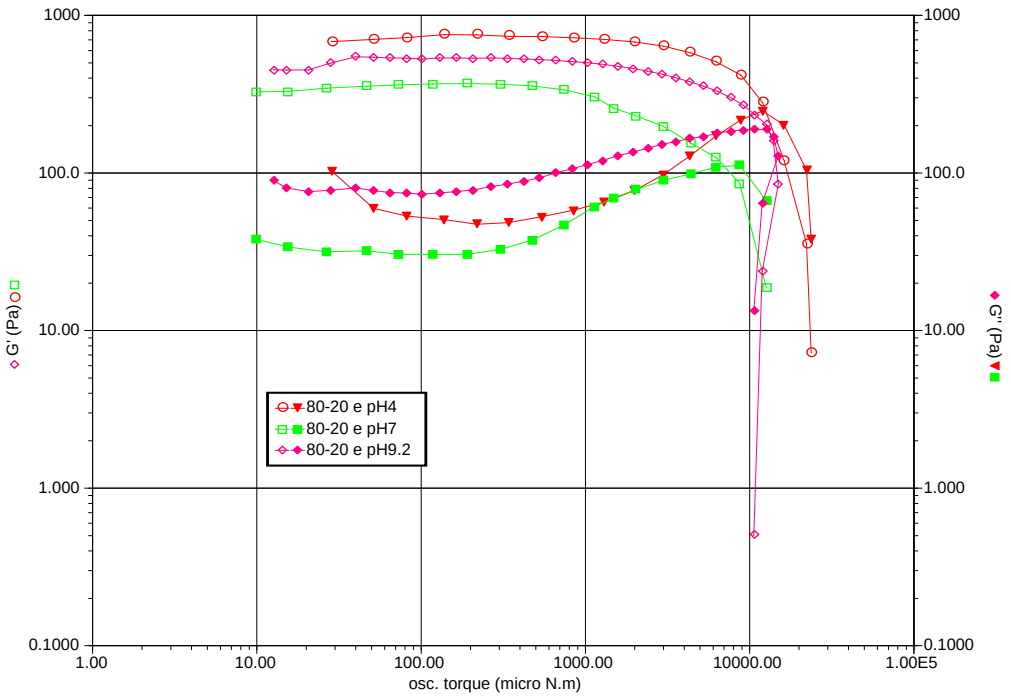


Figure 4.19 *Rheometric data for the 80-20 hydrogel crosslinked with EGDMA.*

From the results presented in Figures 4.17 and 4.18 (Table 4.1) it was found that at pH 4, the crosslinked 80-20 monomeric concentration produced a weaker gel as the oscillating torque at break at the crossing point of G′ and G′′ was lower than for the equivalent 70-30 monomeric concentration. This was caused by reduced

hydrogen bonding in the 80-20 polymers due to a smaller quantity of carboxylic acid groups present.

At pH 9.2 the crosslinked 80-20 monomeric concentration exhibits an oscillating torque at break slightly higher than their equivalent 70-30 samples. It is believed that the crosslinked 70-30 polymer becoming brittle at pH 9.2 due to higher internal forces caused by the swelling pressure due to free ions inside the hydrogel (Jianqi and Lixia 2002).

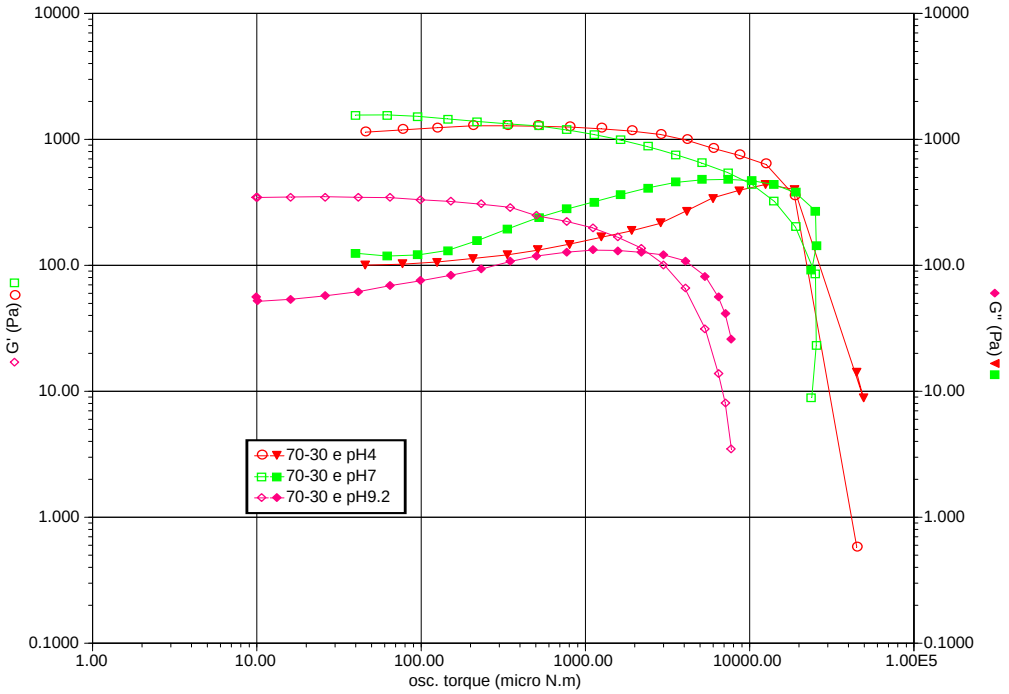


Figure 4.20 *Rheometric data for the 70-30 hydrogel crosslinked with EGDMA.*

At pH 7 the results obtained show only slight differences between the monomeric concentrations tested. As pH 7 is above the $pK_{initial}$ of AA, ionisation of the carboxylic acid can take place: however, the internal repulsion in the polymer would not be as high as is observed in pH 9.2 buffered solution. Therefore the brittle nature of the 70-30 samples is not as evident at pH 7. It was found that as the pH of the swelling media increased, the oscillating torque at break of the crosslinked hydrogels fell. This was expected as the gels exhibited an increase in W_c and W_u as the pH increased. Therefore per volume, the polymer content in a fixed volume of hydrogel decreased as the pH of the swelling media increased.

When the different molecular weight crosslinking agents were compared it was found that the oscillating torque at break achieved by the polymers increased as the molecular weight of the crosslinking agent increased. Hong et al. (1996b) stated

that, at low concentrations of crosslinking agents, branched polymers are mainly formed. Even though low concentrations of crosslinking agents were used in this work, the polymer retained its shape and did not dissolve, thus indicating that crosslinking did occur. However, it is probable that some branching did occur. The molecular length of these branches would increase with increasing molecular weight of the crosslinking agent, and therefore these longer branches could act as a tie between molecular chains, and distribute any load more evenly, and also allow for slippage over molecular chains. This yielded higher values for oscillating torque at break, as the longer molecular chains could also impart greater flexibility and therefore a greater oscillatory force would be required to break the polymer chains.

Ramazani-Harandi et al. (2006) notes that it is practically preferred in many cases to qualitatively assess the strength of hydrogels by observation and feeling the swollen gels. However this is method is highly dependent on the experience of the test operator. Therefore in this work the elastic component (G′) portion of rheology curves were used to evaluate the comparative strength of the hydrogels. Figures 4.19 – 4.20 illustrates representative results observed, for EGDMA crosslinked 80-20 and 70-30 copolymers at pH 4, 7, and 9.2, additional results are illustrated in appendix E. These results illustrate that in general, the comparative strength of the hydrogels decrease as the molecular weight of the chemical crosslinking agent increases. As the crosslinking chains are longer, more water can penetrate the hydrogel allowing the sample to swell to a higher degree. Thus, as the Wc in the hydrogel is increased, there are less polymer molecules available to retain the structure of the hydrogel and therefore a decrease in comparative strength is observed. Jones and Vaughan (2005) quotes elasticity values for five hydrogel wound healing dressings at pH values ranging from pH 5.9 to pH 6.8. The G′ or elastic component of the hydrogels tested in this work yielded a maximum value of 1050Pa for the 70-30 sample crosslinked with EGDMA at pH 7. This value is in excess of the values quoted for Granugel, 340-370 Pa, and NuGel, 580-590 Pa. However, it is less then the values quoted for Intrasite, Aquaform and Purilon which had values ranging from 1140 Pa to 2490 Pa.

4.3 Conclusion

In this chapter, a series of crosslinked random copolymers of NVP and AA have been synthesised by varying the molecular weight of the crosslinking agent used. The Ftir spectra of PVP-PAA copolymer complexes indicates both hydrogen bonded and non-hydrogen bonded PVP is formed. As the percentage of AA increases in the copolymer there is evidence of increased intermolecular hydrogen bonding between the carboxylic acid groups of the AA segments. Potentiometric titrations on the crosslinked samples indicates that the crosslinking agent did not affect the pH sensitivity of the polymers containing AA. Swelling of the PVP-PAA complex in a higher pH medium is significantly different from results in low pH solutions. Above a critical pH the carboxylic acid group of AA dissociates and is ionized, therefore there is a reduction in the intermolecular bonding and so the hydrogels tend to swell to a greater degree yielding higher water content and water uptake values. In-fact, water uptake values of over 4200% and water content values of over 97% were observed in the swollen samples. The water content values observed are similar to those found in the human body and would therefore indicate biocompatibility of the hydrogels. The rheometry results showed that there was a significant difference in the oscillating torque at break and the comparative gel strength at different pHs due to the increased water uptake as the pH of the swelling medium was increased. It was also found that by varying the molecular weight of the crosslinking agent an increase in oscillating torque at break could be achieved. However this increase in oscillating torque at break was offset by a decrease in the comparative gel strength as the water content in the hydrogel increased, with an increase in the molecular weight of the crosslinking agent. Despite this the comparative gel strength observed in this work at pH 7 did compare favourably to values quoted in the literature for hydrogel wound dressings. In conclusion, as the water uptake, oscillating torque at break and the gel strength can be controlled; these gels have potential applications in a variety of biomedical applications. These could include drug eluting polymeric coatings or wound dressing applications.

From the initial physical and chemical analysis of the hydrogels, various monomeric ratios were selected for particular applications. For instance a crosslinked 70-30 hydrogel was chosen as a coating because of its mechanical strength and its ability to swell to relatively high EWC values at plasma pH. By

varying the molecular weight of the crosslinking agent, the EWC should allow for the controlled release of an active agent, for example aspirin which reduces inflammation and is administered to patients post coronary angioplasty to thin the blood and therefore reduce the incidence of blood clot formation.

Various soluble polymers and a number of chemically crosslinked hydrogels were analysed for their potential in controlled delivery applications. Two active agents of similar molecular weight, but varying pKa, (aspirin and paracetamol) were incorporated into the monomeric solution prior to UV curing. These active agents were chosen to determine the effect of pKa of the active agent and pH of the dissolution media have on the release profile of the active agent.

Extractable content analysis, cytotoxicity and genotoxicity testing was carried out to determine if these polymers indeed had potential in biomedical applications.

Chapter 5

Development of a UV polymerised drug eluting lubricious hydrophilic coating

5.1 Introduction

Biomaterials to be used for catheterisation in urinary, tracheal, and cardiovascular tracts should have a surface that has good handling characteristics when dry, but which preferably becomes slippery upon contact with aqueous body fluids. Such a low-friction surface would enable easy insertion and removal from a patient. It would further prevent mechanical injury to the mucous membranes and would minimise discomfort to the patient (Ikada and Uyama 1993). Minimising friction between a medical device and the surrounding tissue is a major design consideration. The extent to which hydrophilic coatings can reduce the coefficient of friction on the surface of a device is so dramatic that the term frictionless coating is commonly used to describe them. For example, LaPorte (1997) described samples of high-density polyethylene (HDPE), which had a static coefficient of friction of approximately 0.74. This value was reduced to 0.38 by applying a conventional silicone oil-based coating to the sample. However, the application of a hydrophilic coating reduced the static coefficient of friction of the samples to 0.05, a reduction of more than an order of magnitude. Additional evaluations on a variety of other polymer substrates, including silicone elastomers, polyurethane, PMMA and PVC have demonstrated similar reductions in the coefficient of friction of 60 to 90% (LaPorte 1997). Conroy (1998) also notes that the use of polytetrafluoroethylene (PTFE), glycerine or silicone fluid can be used to render the surface slippery. However, these coating have the disadvantage that they can make the device difficult for the physician to manipulate, as silicone fluids and glycerine are greasy and sticky, and PTFE is slippery even when dry. It is for these reasons that hydrophilic coatings are finding increasing application in reducing an apparent surface friction of contact lenses and devices used in the field of minimally invasive surgery.

Hydrophilic coatings are applied to medical devices to impart functional properties to the surface of a given device. Many medical devices are fabricated from conventional polymeric materials. Most polymeric materials exhibit poor wettability due to their low surface tensions. Even though some polymers exhibit relatively lubricious surfaces, e.g. PTFE, PE, and various polyamides (PAs), when in

dynamic contact with the biological environment, they tend to irritate or damage surrounding tissue. By applying a hydrophilic coating, wettability or dynamic lubricity can be imparted to the existing device, thus minimising the trauma to the biological environment.

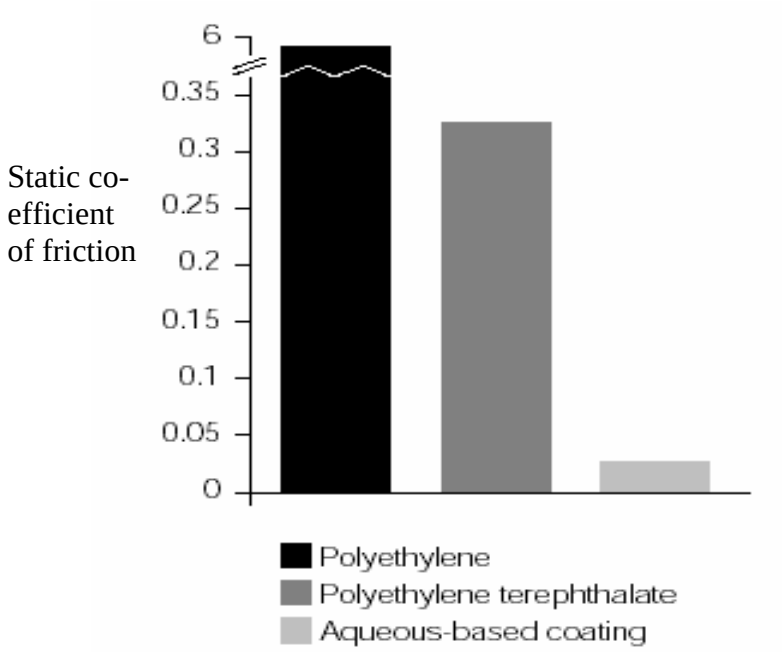


Figure 5.1 *Comparison of co-efficient of friction of an aqueous-based coating, with polyethylene and polyethylene terephthalate (Conroy 1998)*

Other devices fabricated from conventional polymers can come into contact with biological fluids or surfaces for extended periods of time in the form of short-term, long-term, or permanent implants. Frequently, the biological response is to exude the device from the body or encapsulate it in tissue. There are many factors contributing to this response, one of which is the interfacial tension existing between the implant and biological fluids or surfaces, and another is the attachment of certain biological factors to the surface of the implant. Hydrophilic coatings, because of their diffuse structure, exhibit extremely low interfacial tensions with surrounding biological fluids and surfaces. Additionally, because of the dynamic nature of their surfaces, hydrophilic coatings inhibit the attachment of factors that induce rejection or encapsulation of the implant. Hence, hydrophilic coatings are applied to impart biocompatability and antithrombogenicity to the implant (LaPorte 1997).

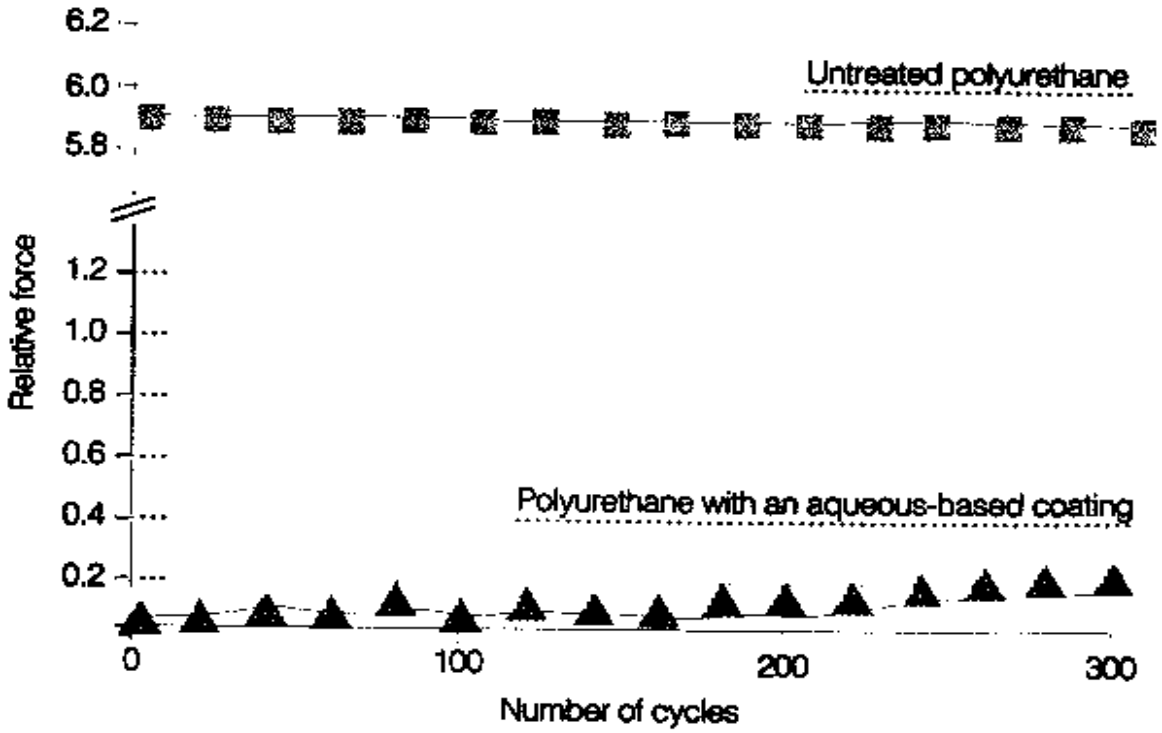


Figure 5.2 *Durability of aqueous-based coating in push/pull tests (Conroy 1998)*

Hydrogels are often used to improve or impart properties such as biocompatibility, wettability, and lubricity to systems deficient in these attributes. The extent to which the hydrogel can exhibit these properties is directly dependent on the amount of water that the hydrogel can imbibe into its molecular matrix, which is referred to as the degree of swelling. Several factors in the service environment have a direct bearing on the hydrogels intended function and, may actually be used to control the response of the hydrogel in dynamic environments such as the human body. Typical factors that can induce such influence include temperature, pH, chemical agents, mechanical stress, electric potential, and magnetic fields (LaPorte 1997).

5.2 Results and Discussion

5.2.1 Preparation of samples

Pebax® 3533 tape was extruded through a tape head die using a Prism T20 U-Tron Soder twin-screw extruder, and the extrudate fed through a Thermo Prism haul-off mechanism. Rolls of tape were then stored in airtight bags until required. The tape was wiped with propan-1-ol soaked filter paper to remove any dust or other impurities that may have been on the surface, prior to the application of a lubricious

coating. The dimensions of the extruded tape were 16.15mm ± 0.85mm x 0.615mm ± 0.015mm. The tape was cut into lengths of 150mm ±1mm for dip coating. After the curing process the samples were cut to lengths of 100mm ±1 mm to remove any inconsistencies in the coating from the top and bottom of the tape caused by the method used to suspend the samples for dipping, or droplets that may have formed on the bottom of the tape during the curing process.

It was noteworthy that the stiffness of the tape increased significantly after coating, and as the coating was built up over a number of dipping/curing cycles the stiffness appeared to increase. This led to the coating cracking when the tape was bent, however the coating did not chip off, which indicated that grafting had taken place.

5.2.2 Dip coating and UV curing

Dip coating was carried out by immersing the substrate into a 100mL-graduated cylinder that had been filled with the appropriate coating solution. Immediately after dipping, the substrate was placed on the curing apparatus and cured for 120 seconds.

The coating achieved on the Pebax ® 3533 tape was tacky after curing. However after air drying for approximately 15 minutes the tacky nature of the coating was less apparent. It was therefore deemed necessary to dry the samples in an oven at 50°C for 24hrs, prior to further testing, to remove any volatiles.

5.2.3 Optical analysis

Optical analysis was carried out on samples that underwent a predetermined number of dipping/curing cycles to determine the optimal coverage possible using the described coating/curing procedure. The test was carried out by firstly swelling the pre-coated tubing in a pH 7 buffered solution that contained congo red indicator, for twenty minutes to fully hydrate the coating. Pictures of the samples were taken with a Sony cyber-shot digital camera. An Olympus BX60 microscope with a magnification of 10X was used to characterise the coating at a microscopic level.

Figure 5.3 *Samples coated with 70-30 copolymer crosslinked with 0.1wt% EGDMA. From left to right: no coating; 1 coating cycle; 2 coating cycles; 3 coating cycles; and 4 coating cycles.*

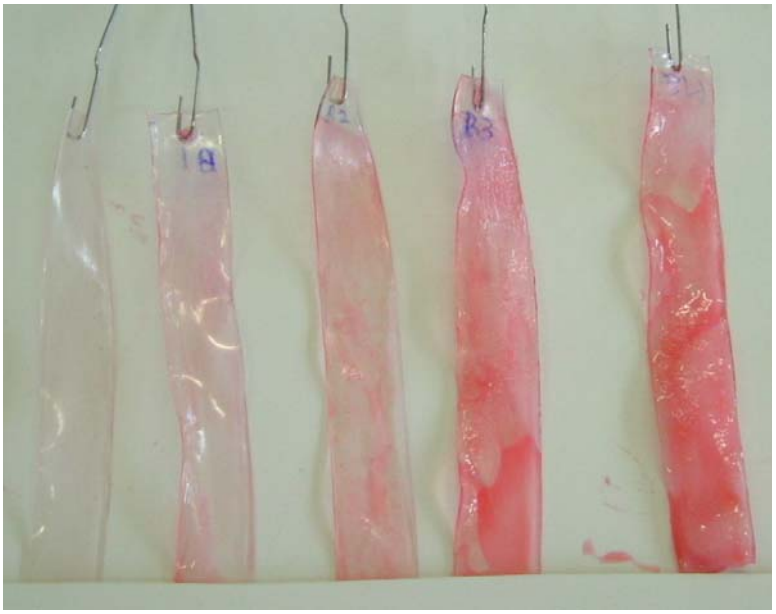


Figure 5.4 *Samples coated with 70-30 copolymer crosslinked with 0.1wt% PEG600DMA. From left to right: no coating; 1 coating cycle; 2 coating cycles; 3 coating cycles; and 4 coating cycles.*

Figure 5.5 *Pebax® 3533 uncoated*

In this work, uncoated Pebax ® 3533 was used as a reference for the coated samples. From Figures 5.3 and 5.4 it can be seen that all samples tested absorbed buffered solution containing Congo red indicator, with the exception of the uncoated Pebax ® 3533. This illustrates that a hydrophilic coating was in situ on the surface of the Pebax ® 3533. When the number of dipping cycles was compared visually it can be seen that as the number of coating cycles increased the uptake of Condo red indicating that a thicker coatings were achieved by repeating the coating procedure. Indeed it was found that clumps of coating were found on samples that had undergone 3 and 4 dipping cycles. When the coated samples that contained PEG600DMA crosslinking agent, had undergone 4 dipping cycles it was found that on removal of the sample from the buffered solution, some of the coating dripped off. This was due to large amounts of buffered solution being absorbed into the relatively thick coating. This caused molecular bonds to break under the strain, which resulted in the coating dripping off. This was not observed when the coating that contained EGDMA was removed from the buffered solution, as the space into which the buffered solution could diffuse was not as large due to the lower molecular weight of the crosslinking agent. Thus, the weight of water imbibed into the coating was not sufficient to cause bond cleavage. These observations correlate with swelling results presented in chapter 4, where it was found that an increase in the molecular weight of the crosslinking agent enabled the polymer to swell to a higher degree.

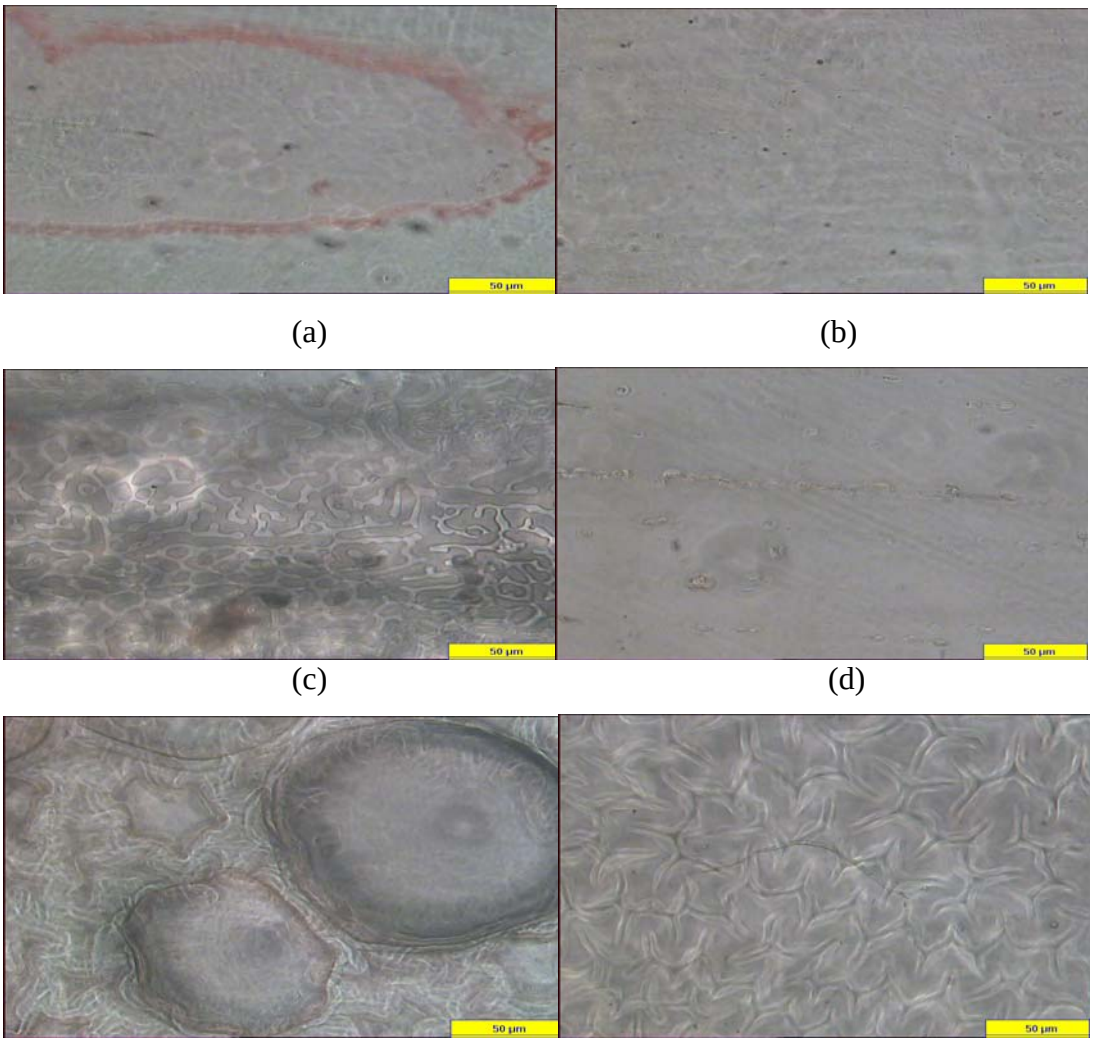


Figure 5.6 *Pebax ® 3533 coated with 70-30 copolymer crosslinked with EGDMA after various coating cycles.*
1 coating cycle (a) Top of the substrate, where coated and uncoated sections were observed. (b) Bottom of the substrate, where a consistent coating was observed.
2 coating cycles (c) Top of the substrate, where a slightly inconsistent coating were observed. (d) Bottom of the substrate where a consistent coating was observed.
3 coating cycles (e) Top of the substrate where lumps of inconsistent coating were observed. (f) Bottom of the substrate where a relatively consistent coating was observed.

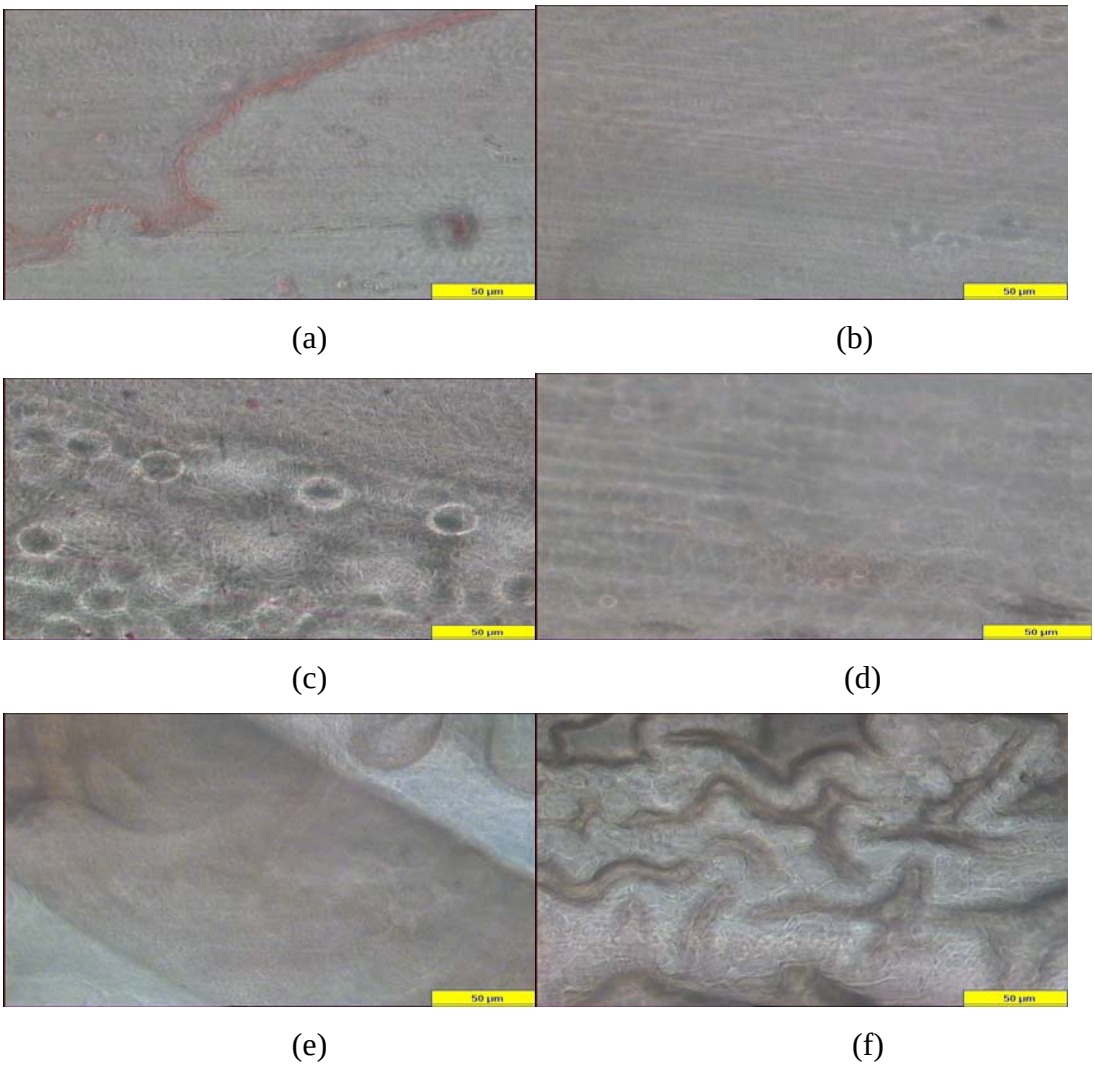


Figure 5.7 *Pebax ® 3533 coated with 70-30 copolymer crosslinked with PEG600DMA after various coating cycles.*
1 coating cycle (a) Top of the substrate, where coated and uncoated sections were observed, (b) Bottom of substrate where a consistent coating was observed.
2 coating cycles (c) Top of the substrate, where a slightly inconsistent coating were observed, (d) Bottom of the substrate where a consistent coating was observed.
3 coating cycles (e) Top of the substrate, where clumps of inconsistent coating were observed, (f) Bottom of the substrate where smaller clumps of inconsistent coating was observed.

When the coated samples were examined at a microscopic level it was found that after 1 coating cycle, the coating obtained was inconsistent over the length of the sample. Figures 5.6 and 5.7 illustrate this, as only partial coverage of the substrate was achieved by the coating, at the top of the samples. However, the coating obtained at the bottom of the substrate appeared smooth and consistent. This was a result of the method used for coating the sample, as the bottom of the substrate

was in contact with the coating solution for a longer period of time than the top of the substrate. Therefore, the coating solution had more time to diffuse into the substrate and thus achieved better coverage of the substrate. It was for this reason that a faster dipping speed than withdrawal speed was used when dip coating the substrate, in an attempt to minimise this effect.

When the samples were dip coated a second time, it was found that the build-up of coating at the top of the samples greatly reduced the coverage problems observed after 1 coating cycle. It was also noticeable that the coating at the bottom of the substrate was at least equal to the coating achieved after 1 coating cycle. After 3 coating cycles, it was found that both small clumps and large clumps of coating were formed. This led to a poor surface finish being obtained, and a large difference in the coating along the length of the substrate. When the samples that had undergone 4 coating cycles were analysed, it was found that as well as both small and large clumps present as occurred with 3 coating cycles, there was also some very thick coating present. This also led to a poor surface finish being obtained, and a large difference in the coating along the length of the substrate. It was also noticeable that some sections appeared to have little or no coating present. This was due to sections of coating that had been built up during previous dipping cycles, being effectively washed off by the final dipping cycle. These results illustrate that the best coverage by either coating tested, was achieved when the substrate underwent 2 coating cycles.

5.3.4 Coating characterisation

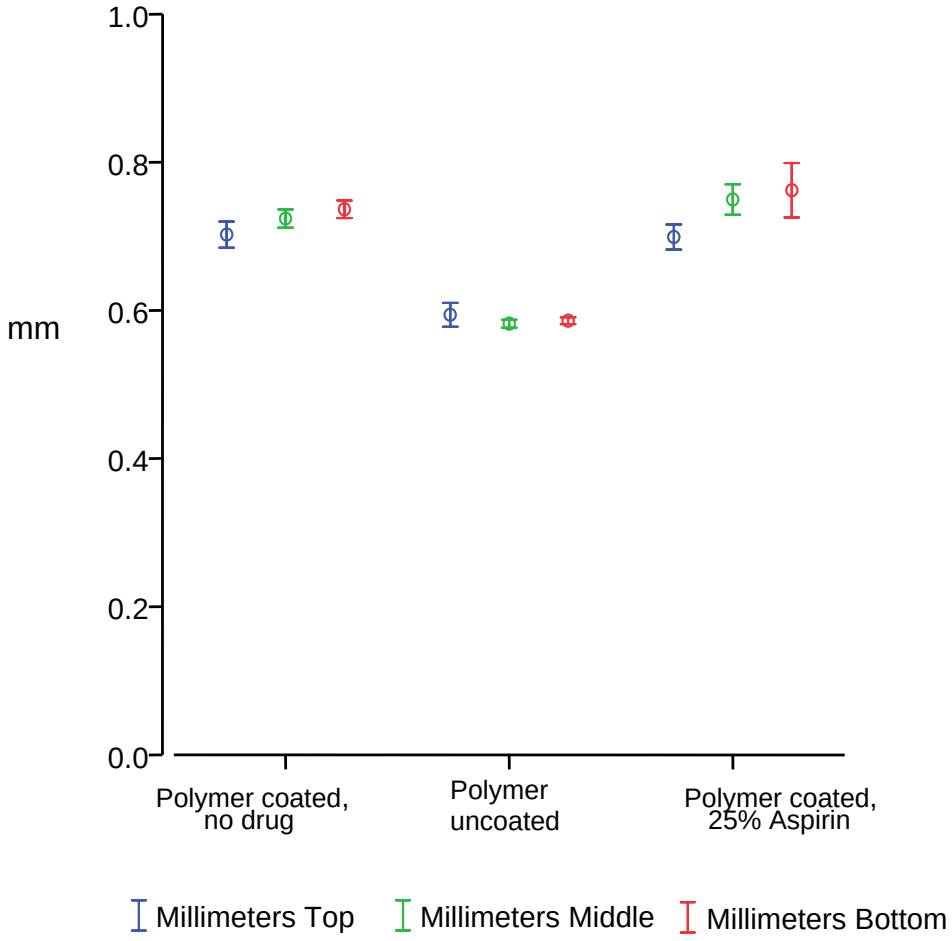
The thickness of the coating was measured using a micrometer, capable of being read to 3 decimal places. The coating thickness was measured at three points along its length, and the average taken as the thickness of the coating. The thickness of the coating grafted to the substrate was 67 μm for the EGDMA crosslinked coating and 56 μm for the PEG600DMA crosslinked coating. This coating is thicker than the coating developed by Tierney et al. (2004), which had a thickness of approximately 10 μm. The thickness of both coatings increased to approximately 75 μm with the incorporation of aspirin as shown in Table 5.1. This could be caused by lower polymerisation induced shrinkage of the coating material, due to the incorporation of aspirin. This is justified in that there was an increase observed in the standard deviation with the incorporation of aspirin, which suggested that the drug

was not evenly distributed throughout the coating and may have phase separated during polymerisation. However, a thicker coating is advantageous in this instance as with a drug loading of 25wt%, approximately 80 mg and 63 mg of aspirin was incorporated onto the surface of Pebax® 3533 substrates, using the EGDMA crosslinked coating and PEG600DMA crosslinked coating respectively. Thus, a coating of one-seventh the thickness would only be capable of incorporating 9 to 11.4 mg of active agent at 25wt% of the polymer/monomer weight in the coating. The coating described in this work also has the added advantage over that described by Tierney in that no solvent is necessary within the system and therefore any possible harmful effects from residual solvent are eliminated.

Table 5.1. *Coating Characterisation*

Crosslinking agent used				
	EGDMA		**PEG600DMA**	
Aspirin content (%)	0	25	0	25
Thickness (μm)	67.1	75.4	55.8	74.8
Std Dev	0.0396	0.0495	0.0336	0.0681
Contact angle (degrees)	36.167	41.33	38.83	42.67
Relative viscosity	1.212	1.235	1.212	1.242
Density	1.042	1.081	1.049	1.066

It was also found that there was an increase in the contact angle that the coating solution made with the substrate with the incorporation of aspirin. There was also a slight increase in both the viscosity and the density of the coatings with the incorporation of aspirin, which may also have contributed to a thicker coating being achieved when aspirin was incorporated into coating solution.



Statistical analysis of uncoated Pebax ® 3533, Pebax ® 3533 coated with PEG600DMA crosslinked coating and PEG600DMA crosslinked coating with 25wt% aspirin was carried out using SPSS (SPSS Inc., USA) analytical software, the average result from 7 different samples was used for analysis. Repeated measures of ANOVA (analysis of variance) with Bonferroni's pot-hoc test were used to determine statistically significant differences between the groups of samples tested. Further analysis was carried out between the coating thickness at the top, middle and bottom of the sample, using Huynh-Feldt correction factor where Sphericity was not assumed. Factors with p values below 0.05 were considered significant.

The uncoated Pebax ® 3533 showed a statistically significant difference to both the coated without drug and coated with 25% aspirin samples. However, there did not appear to be a significant difference between the coated sample without aspirin and that of Pebax ® 3533 coated with 25% aspirin. Furthermore, it was

found that the location of the taken measurement is an important factor since a significant difference (Huynh-Feldt p=0.03) was found between location of the measurements taken at the top, middle and bottom of the samples for different groups but no significant difference was determined within the same group.

5.2.5 Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy was carried out on coated Pebax® 3533, so as to analyse the composition of the coating. The test was carried out by firstly performing a background scan on uncoated Pebax® 3533, and thus the resultant scan was of the coating only.

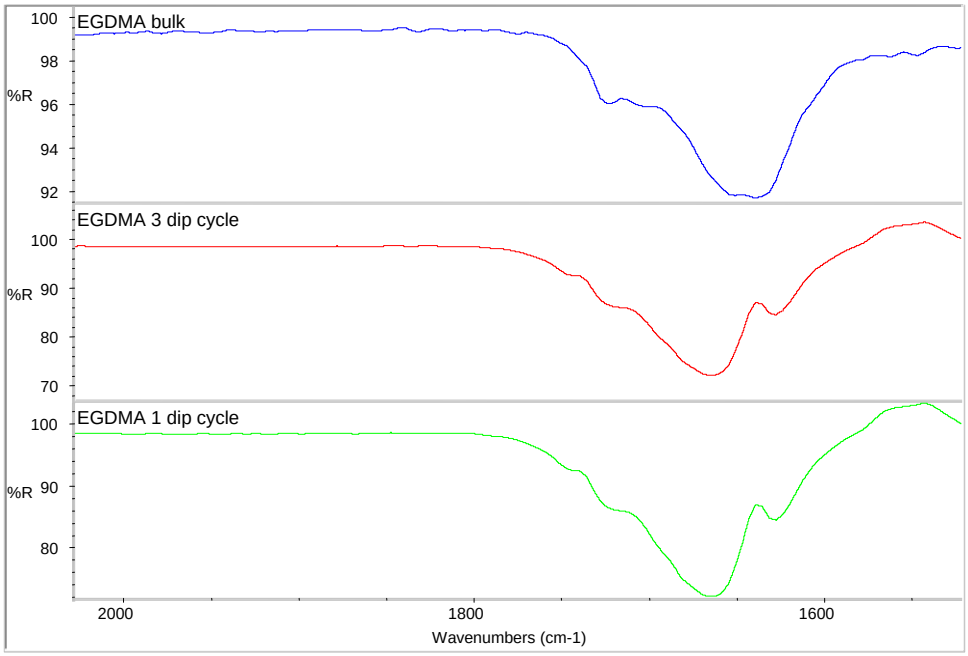


Figure 5.8 *Comparison of the PVP carbonyl spectrum for EGDMA crosslinked polymer as described in chapter 4, and the Ftir spectrum for EGDMA crosslinked coating after 1 and 3 coating cycles*

In chapter 4 the characteristic peaks for a random crosslinked copolymer of PVP and PAA has been discussed. On analysis of the IR spectrum of the PVP/PAA coating it was found that these characteristic peaks were also present (Figures 5.8 and 5.9). Briefly, the PVP carbonyl group exhibited peaks in the region of 1660 to 1680 cm^{-1}, and at approximately 1630 cm^{-1}. This indicated that the crosslinking agent acted as a spacer, which did not allow a high degree of intermolecular bonding to occur. It was also found that two shoulders were visible on the peak associated

with the PVP carbonyl group. The first shoulder is in the region 1720 cm^{-1}, which indicated the presence of the AA dimer. The second shoulder was in the region of 1740 – 1750 cm^{-1}, which indicated non-hydrogen bonded AA.

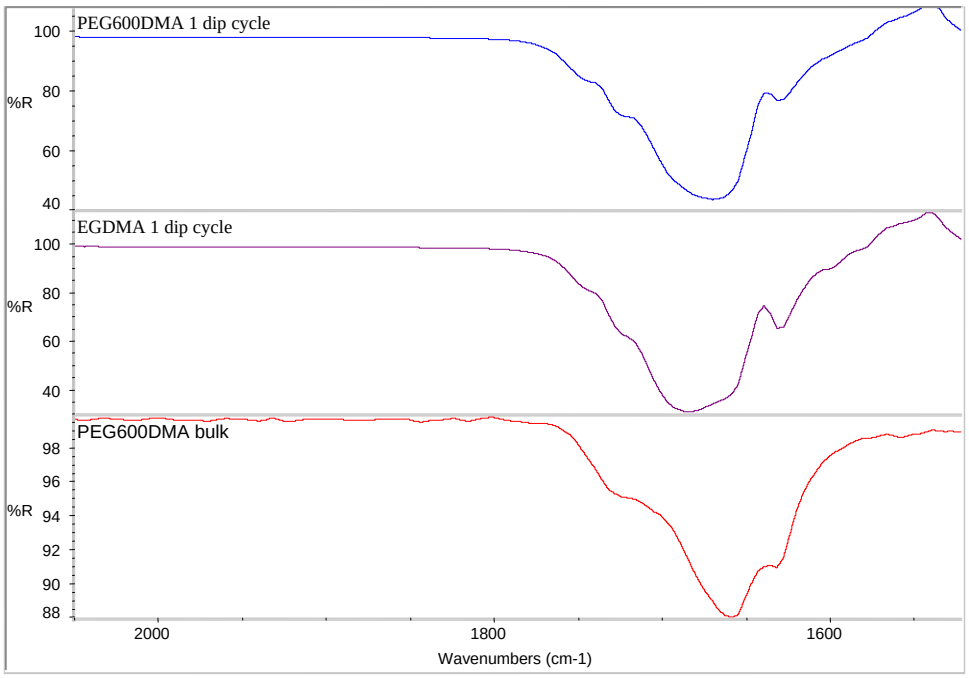


Figure 5.9 *Comparison of the PVP carbonyl spectrum for PEG600DMA crosslinked polymer as described in chapter 4, and the Ftir spectrum for PEG600DMA crosslinked coating after 1 and 3 coating cycles*

It was also found that the peaks associated with C=C and CH_2= at 1617 cm^{-1} and 1409 cm^{-1} respectively were not present, (Ju et al. 2002) thus proving that a high degree of polymerisation had taken place. These results show that the application of this copolymer to a substrate for coating applications does not affect its chemical structure. Further Ftir spectra of PVP/PAA copolymer lubricious coatings are shown in appendix F.

5.2.6 Friction analysis

Friction analysis was carried out on samples to characterise the lubricious nature of the hydrogels that contained either EGDMA, or PEG600DMA as a crosslinking agent. Frictional analysis was performed on uncoated Pebax ® 3533 and on samples that had undergone two coating cycles, as two coating cycles gave the optimum coverage possible, using the methods utilized. The dry, coated reference samples were dried in an oven at 50°C for 24 hours prior to testing to

remove any moisture that may have being absorbed. Frictional analysis of the hydrated coated samples was performed on samples swollen for predetermined time intervals in pH 7 buffered solution. Representative friction curved obtained for the hydrated coatings are shown in Figures 5.12 and 5.14, and the remaining frictional curves are illustrated in appendix G.

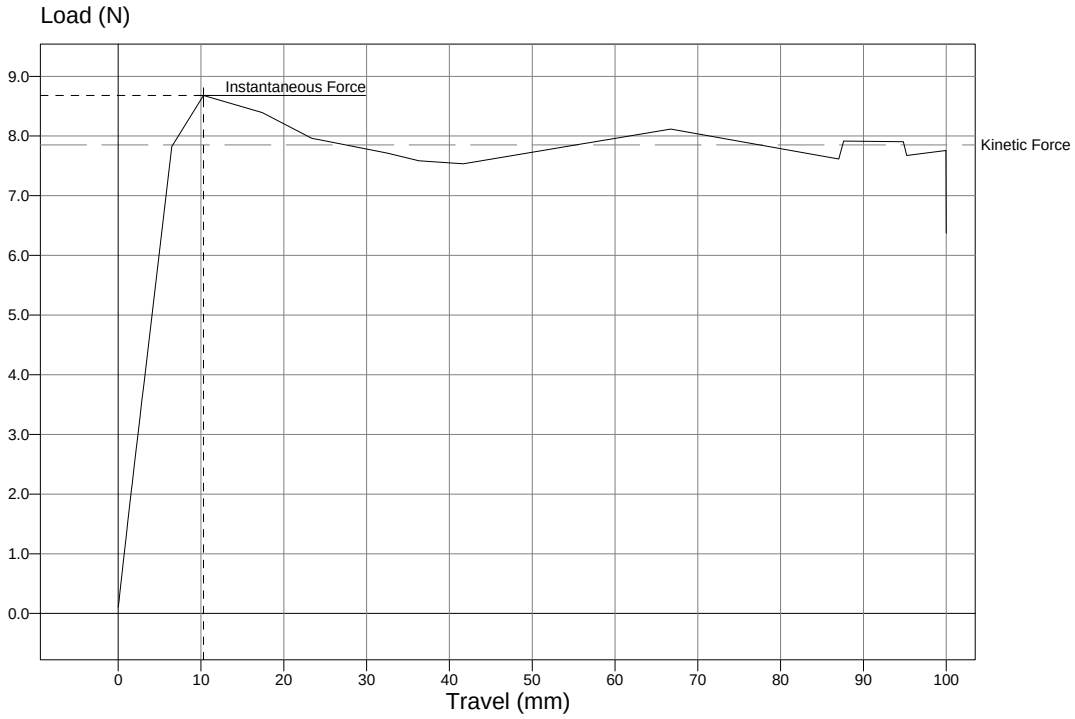


Figure 5.10 *Frictional analysis of Pebax ® 3533.*

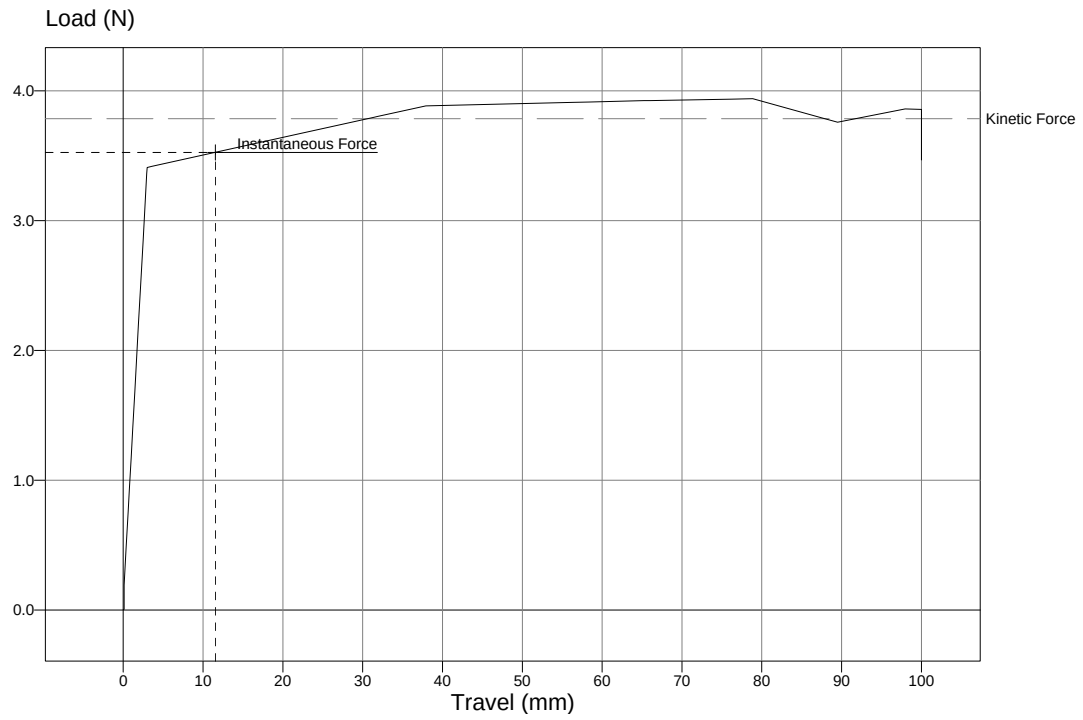


Figure 5.11 *Frictional analysis of coated Pebax ® 3533 after drying at 50ºC for 24 hours, using EGDMA as crosslinking agent.*

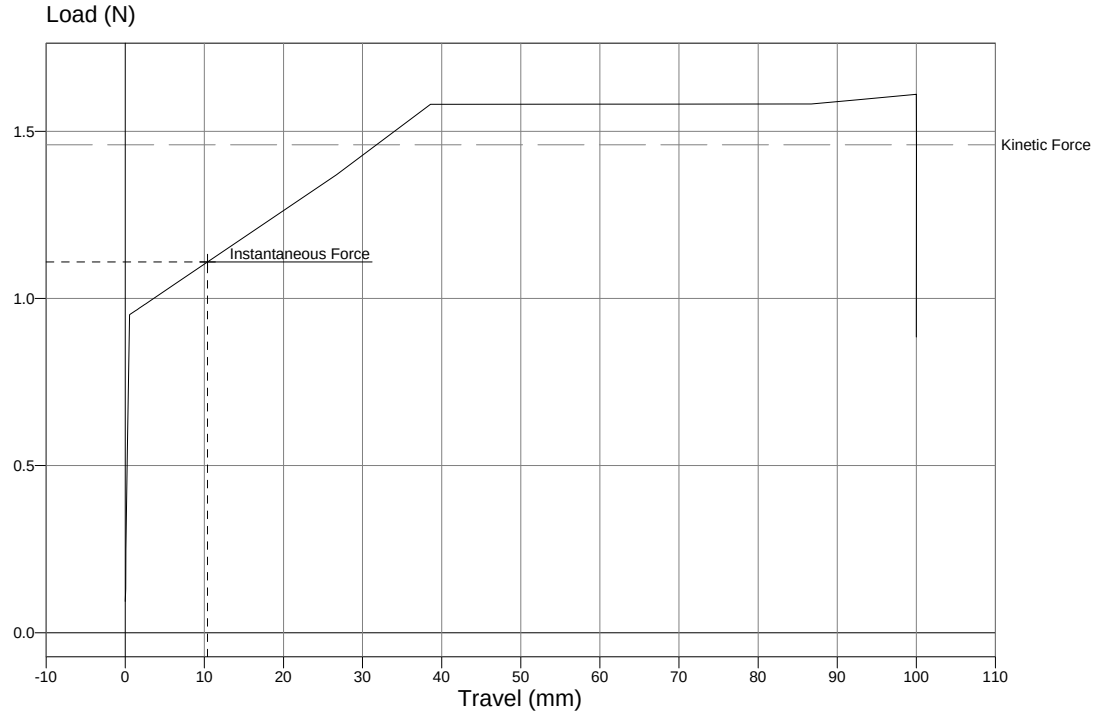


Figure 5.12 *Frictional analysis of coated Pebax ® 3533, using EGDMA as crosslinking agent, after* 20 *minutes submerged in pH7 buffered solution.*

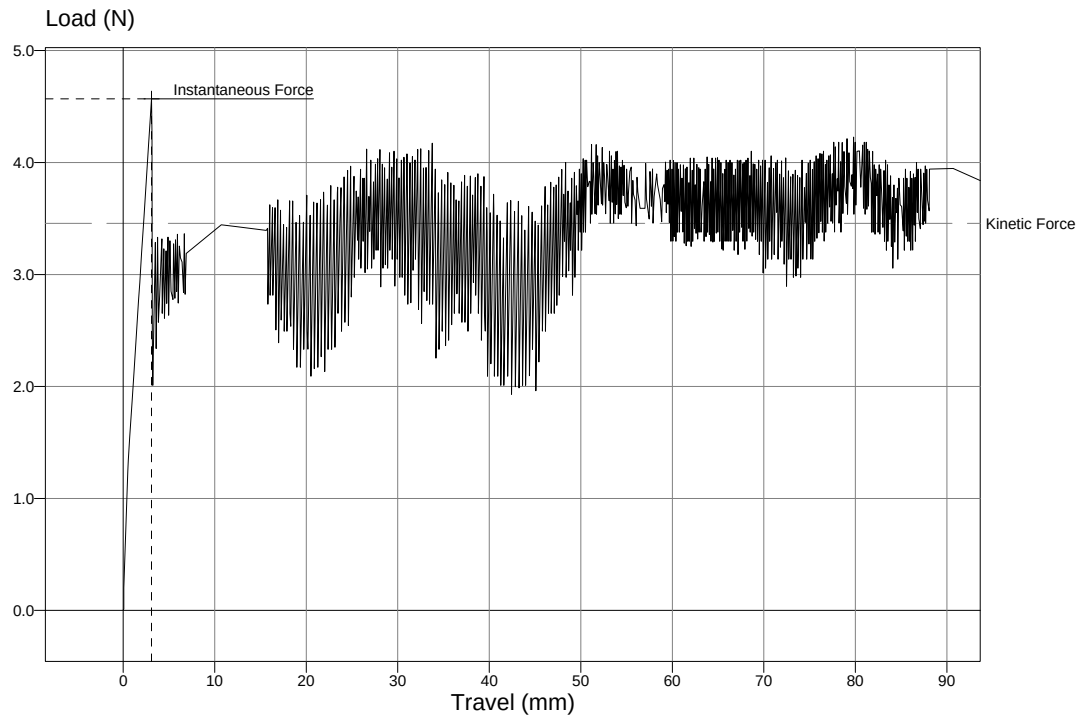


Figure 5.13 *Frictional analysis of coated Pebax ® 3533 after drying at 50ºC for 24 hours, using PEG600DMA as crosslinking agent.*

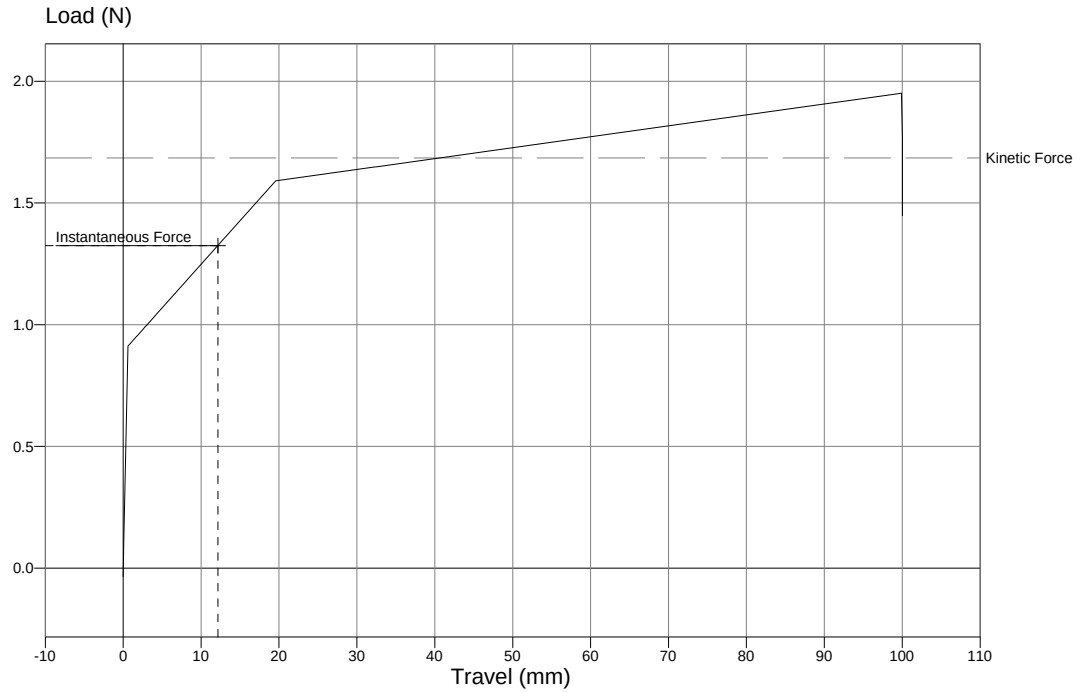


Figure 5.14 *Frictional analysis of coated Pebax ® 3533, using PEG600DMA as crosslinking agent, after 20 minutes submerged in pH7 buffered solution.*

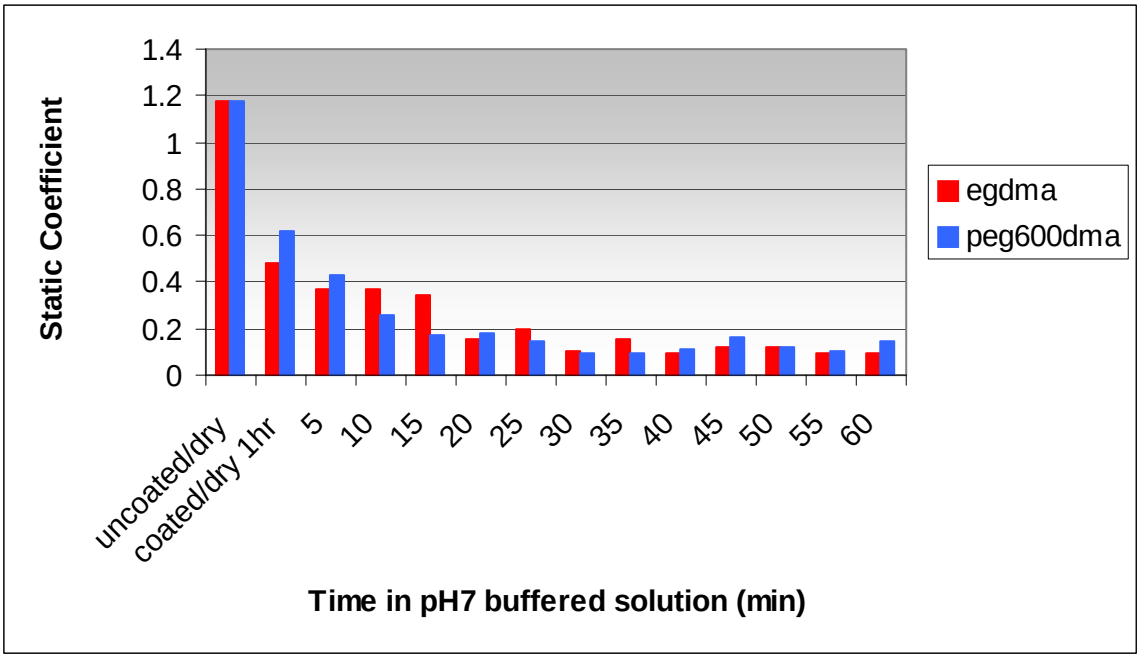


Figure 5.15 *Coefficient of friction results for samples crosslinked with EGDMA and PEG600DMA.*

From the frictional analysis of uncoated Pebax ® 3533 (Figure 5.10) it can be seen that the force required to start the sample moving (instantaneous force) was approximately 8.7N, and the force required to maintain the constant movement of the sample (kinetic force) was approximately 7.9N. These force readings were reduced to approximately half the instantaneous force and kinetic force observed for the uncoated Pebax ® 3533, with the incorporation of the PVP/PAA coating in the dry state, as shown in Figures 5.11 and 5.13. On hydration of the coating, the instantaneous force and kinetic force readings further reduced, and after 20 minutes in pH 7 buffered solution, instantaneous force readings of approximately 1.1N and 1.3N, and kinetic force readings of approximately 1.4N and 1.7N for the EGDMA and PEG600DMA crosslinked coatings respectively, were obtained. It is noteworthy that the kinetic force should in general be lower than the instantaneous force as it should take less force to maintain a sample moving then to start the movement. However, in this work the kinetic force values obtained for the coatings were higher than the instantaneous force values obtained for some samples tested. This was caused by the buffered solution absorbed into the coating, being forced out of the hydrogel during the test, reducing the coatings lubricity and therefore a higher force was required to maintain the movement of the sample. It is also noteworthy that in Figure 5.13, a typical slip-stick friction curve is produced characteristic of a dry

surface. As the sample tested had been dried for 24hrs prior to testing, this result was not unexpected.

From the static coefficient of friction values illustrated in Figure 5.15 for the coatings that contained EGDMA and PEG600DMA as they absorbed water, it can clearly be seen that the static coefficient of friction, for the coated samples whose coating was fully hydrated was below 0.2. This value was significantly less than the value achieved for the uncoated substrate (1.12), thus proving the lubricious nature of the coating. LaPorte (1997) quoted values of 0.74 for the static coefficient of friction of HDPE from the sales literature of Boston Scientific Corporation. This value was reduced to 0.38 when a silicone oil-based coating was used on the substrate and 0.05 when a hydrophilic coating was used. The values found in this work were half that of the values quoted for the silicone oil-based coating and would not have the handling problems associated with this type of coating (Conroy 1998). However the values observed were higher then the values quoted for the hydrophilic coating. Nevertheless it is noteworthy that in this work, the static coefficient of friction values quoted are frictional results between the coating being tested and a glass fixed plane at an angle of 30°. As friction is a characteristic of the surfaces tested, the values observed cannot be compared directly to those quoted by LaPorte as the test conditions for the quoted results were not clear. It should also be said that the values achieved in this work are below 0.2 and in many cases in the region of 0.1.

It was found that complete hydration of the coatings tested differed with the molecular weight of the crosslinking used. In essence when EGDMA was the crosslinking agent used, complete hydration did not occur until the coating was tested after 20 minutes. When PEG600DMA was used to crosslink the copolymer complete hydration had almost been achieved after 10 minutes. This correlates with results from swelling studies in chapter 4, where it was found that the Wc and the Wu values increased as the molecular weight of the crosslinking agent used increased. This was due to an increase in free space in the coating, into which water could diffuse, thus the polymer could swell more and as a coating swell faster. Even though the coating did not swell instantly, it does compare favourably with swelling rates found by Nurdin et al. (1996). Their work involved the analysis of a commercially available catheter coating, Hydromer ®. It was found that after several

hours in water, the surface was totally wetted by water, and suggested that pre-hydration may be necessary.

5.2.7 Aspirin release

Aspirin release was determined by performing UV spectroscopy using a Shimadzu UV 160 UV spectrometer. A sample of the swelling media was periodically tested to determine the Aspirin release profile.

From the release profile shown in Figure 5.16 it can be see that the release profile observed can be broken down into three almost linear regions. The first of these regions was caused by the release of aspirin close to or on the surface of the coating. This release took place within the first 15 minutes of the test, where 60% of the total drug content was released. This release was replaced by a slower release rate that consisted of the release of aspirin that was entrapped within the bulk of the coating, which continued for approximately 60 minutes. Finally the third and slowest release rate observed was the release of any remaining drug.

The release profile achieved for the co-polymer crosslinked with PEG600DMA (Figure 5.17) yielded a similar release profile. However, as the crosslinks in the polymer were longer, the polymer swelled quicker. This resulted in a prolonged initial period of release which lasted 45 minutes, in which 80% of the total drug content had been released. The second release phase was also extended in relation to the EGDMA crosslinked coating, and lasted up to 2 hours. However most of the drug had already been released prior to this release phase. This correlates with friction results in that full hydration of the PEG600DMA crosslinked copolymer occurred more rapidly then the EGDMA crosslinked copolymer, and therefore the buffered solution could penetrate the hydrogel structure quicker and allow the aspirin to diffuse out faster.

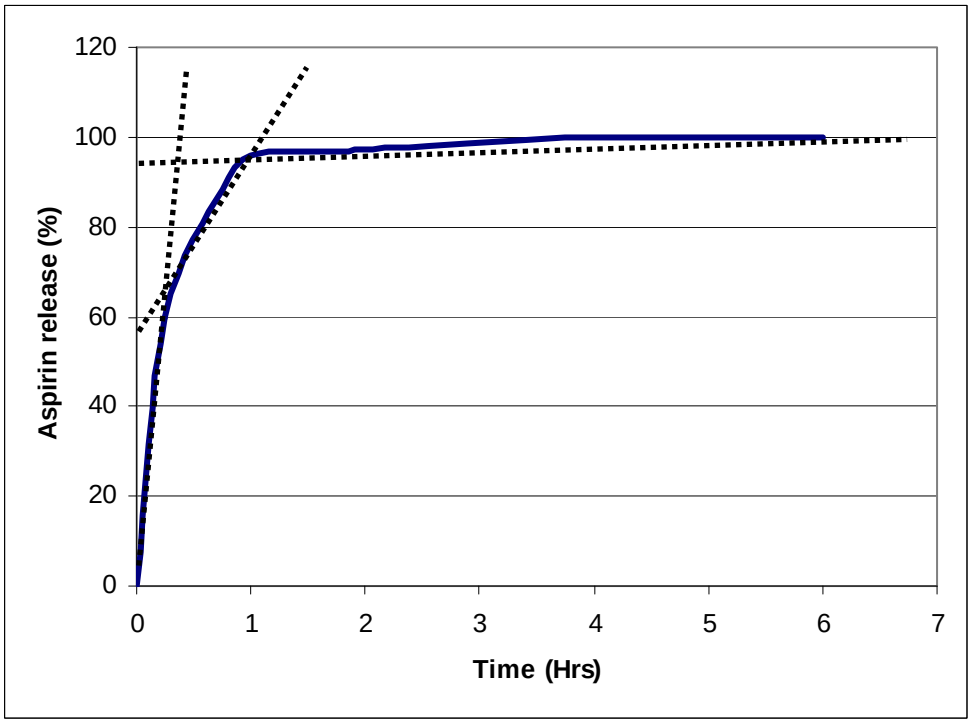


Figure 5.16 *Release profile for 70-30 coating, crosslinked with EGDMA crosslinking agent.*

It is noteworthy that the drug content was slightly higher in the EGDMA crosslinked copolymer (80mg) in comparison to the PEG600DMA (63mg) crosslinked copolymer. The drug content was calculated using the dimensions of the tape prior to coating, the coating thickness, total mass of the samples after coating, as well as the density of Pebax ® 3533 [msds sheet].

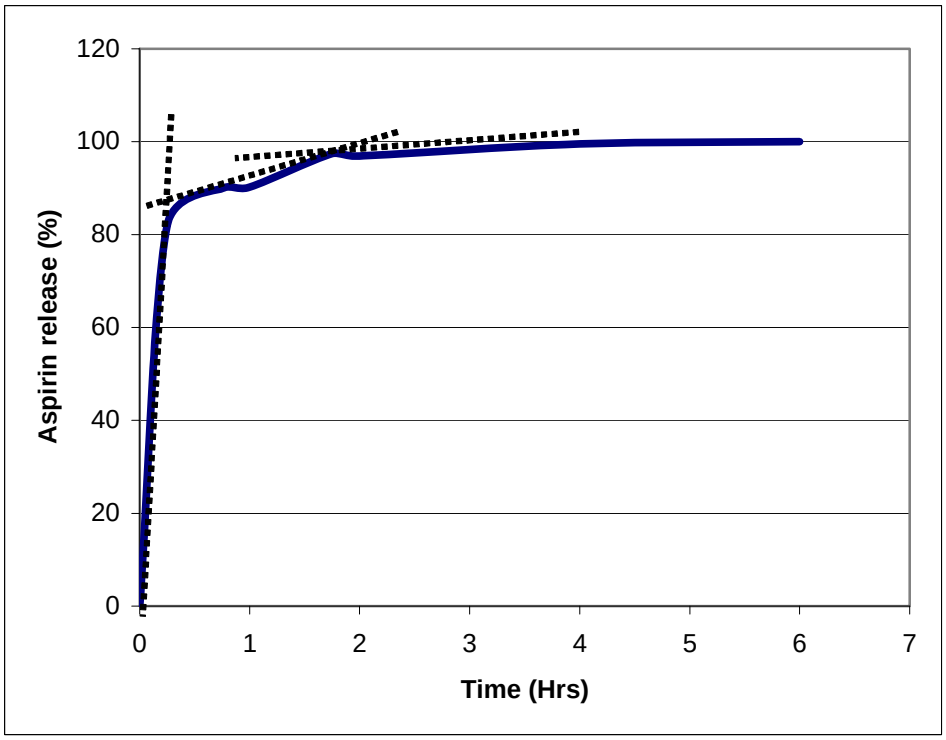


Figure 5.17 *Release profile for 70-30 coating, crosslinked with PEG600DMA crosslinking agent.*

5.3 Conclusions

In this work a lubricious polymer with potential for use as a medical device coating that can be cured directly onto the surface of the substrate without the need for solvents has been developed. It was shown that increasing the thickness of a traditional solvent based hydrophilic coating by the polymerisation of monomers directly onto the surface of the substrate is both an effective and simple method of encapsulating a drug within a coating and eliminates the use of potentially hazardous solvents. Even though the samples were slightly tacky after curing, any remaining volitiles were removed by oven drying the samples. This was proven using Ftir analysis where no characteristic monomeric peaks were found. Ftir also proved that curing the coating directly onto a substrate did not affect the chemical structure of the crosslinked polymer when compared to the Ftir spectrum of the polymer cured in bulk in chapter 4. Optical microscopy suggested that 2 coating cycles yielded the most consistent coating throughout the entire length of the substrate, as excessive coating lead to inconsistencies in the coating and even to parts of the coating been washed off by additional coating cycles. Frictional analysis proved the lubricious nature of the coating. It has been shown that the instantaneous force and kinetic

force required to move a sample of Pebax ® 3533 reduced significantly with the incorporation of the hydrated coating. Frictional analysis also illustrated the time taken for the coating to hydrate and become lubricious. Finally, drug release analysis showed that the release of an active agent could be controlled by varying the molecular weight of the crosslinking agent utilised.

In conclusion this coating has potential to coat several different substrates. The coating also has the potential to release several active agents, due to the method of incorporation and release. As the coating significantly reduces the force required to move a substrate in a frictional environment, the coating would reduce friction on the surface of a medical device such as a catheter, and therefore reduce trauma for the patient.

Chapter 6

Development of NVP based hydrogels for the controlled release of active agents

6.1 Introduction

Hydrogels are becoming increasingly important materials for pharmaceutical applications. They are used in a variety of applications including diagnostic, therapeutic, and implantable devices (e.g. catheters (Whitbourne 1994), biosensors, artificial skin, controlled release drug delivery systems (Graham 1990; Ravichandran et al. 1997; Risbud et al. 2000; Varshosaz and Koopaie 2002; Akhgari et al. 2005) and contact lenses (Shoji et al. 1997). Hydrogels have being widely used in such applications because of their biocompatibility with the human body and also because hydrogels resemble natural living tissue more than any other class of synthetic biomaterial. This is due to their high water content and soft consistency that is similar to natural tissue (Rathner and Hoffman 1976).

Selection of hydrogels used in pharmaceutical processes depends on the characteristics of the hydrogel and on the application of the drug or protein. Hydrogels have several important characteristics that play an important role in drug diffusion including ionisation of the gel, swelling ratio, and specific mesh or pore size. Functional groups along the polymer chain can also react to the external environment for example temperature (Kapnistos et al.1996; Aikawa et al. 1998a; Kono et al. 1999a; Kono et al. 1999b; Dobashi et al. 2000; Hendri et al. 2001; Roos et al. 2003; Geever et al. 2006), ionic strength (Peppas and Wright 1998; Bales et al. 2000; Rodríguez et al. 2003), pH of the swelling agent (Am Ende and Peppas 1996; Yaung and Kwei 1997; Bures and Peppas 2000) or a combination of two or more factors (Jones 1999; Aikawa et al. 1998a; Alvarez-Lorenzo and Concheiron 2002). Aikawa et al. (1998a) investigated the effect of both pH and temperature on hydrogel formations of polyvinylacetal diethylaminoacetate (AEA) and drug release from these polymers. Scanning electron microscope (SEM) observations suggested that AEA in solution at pH 4 becomes a hydrogel on change in pH to pH 7.4 and that temperature change accelerates network formation in the hydrogel.

The swelling ratio is also a very important parameter because it describes the amount of water that is contained within the hydrogel at equilibrium and is a function of the network structure, crosslinking ratio, hydrophilicity, and ionisation of

the functional groups. It is calculated from swelling studies and can be used to determine the molecular weight between crosslinks and the mesh size of the hydrogel. The mesh or pore size is the space available for drug transport (Peppas and Wright 1998).

Sustained release dosage forms are designed to prolong drug dissolution and hence absorption. These formulations move down the GI tract at rates dependent on their location. As drug is released from the formulation as it passes down the gut, it is absorbed at a rate depending on the drug's permeability properties and other factors. Unabsorbed drug or drug not released from the formulation is excreted in the faeces (Friend 2005). Many pharmaceutical systems are essentially made up of a polymeric carrier hosting the active agent inside a three dimensional network. In the case of oral administration, these drug delivery systems are often utilised since these forms present remarkable advantages over the single unit devices. Easy dispersion inside the stomach reflects in an appreciable reduction of local drug concentration, which is usually responsible for gastric irritation (Grass et al. 2000). The use of hydrogels as carriers for these active agents has been studied, as well as methods for controlling their release (Ravichandran et al. 1997; Varshosaz and Koopaie 2002; Akhgari et al. 2005; Friend 2005; Lyons et al. 2006).

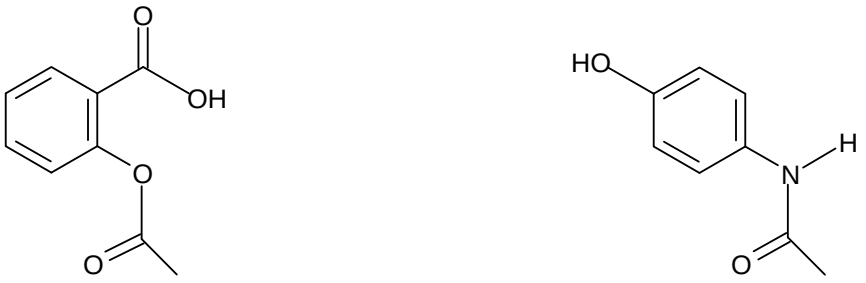
Ravichandran et al. (1997) studied a polyvinyl pyrrolidinone-acrylic acid-polyethylene glycol copolymer and performed drug release experiments in simulated gastric fluids. It was found that the drug was released in an ordered fashion, and that modification of the crosslink density of the polymeric matrix could be used to achieve desirable drug release profiles. Varshosaz and Koopaie (2002) analysed the release of an active agent from a crosslinked PVOH polymer and found that the crosslink density of the hydrogel affected the release of the drug used, i.e. there was a significant decrease in drug release as the percentage of crosslinking agent was increased. Friend (2005) puts forward a review of several methods of controlling the release of active agents in the GI tract. Some of these methods include time-based delivery systems, pH based systems using enteric coatings and combinations of both, among others. Akhgari et al. (2005) discusses the use of polymer coatings over pellets that contained active agents. It was found that these coatings delayed the release of the active agent, thus allowing site specific drug release. Lyons et al. (2006) compared the use of fillers within the polymer matrix to slow drug dissolution and reduce the cost of the overall drug delivery system. It was found that

agar significantly reduced the release rate of the active agent, and as agar is biocompatible and relatively cheap it would have potential in commercial products. In this work a series of PVP-PAA copolymers were analysed for their potential as carriers of two different active agents. The extractable content of the crosslinked hydrogels and cytotoxicity tests were carried out to determine the potential of the various hydrogels for use in pharmaceutical applications.

6.2 Results and Discussion

6.2.1 Preparation of samples

Crosslinked and uncrosslinked samples of both NVP and NVP/AA were photopolymerised using Irgacure 184 ® as a photoinitiator. These samples were cured on a silicone moulding, and prior to use dried for 24 hours in a vacuum oven. From visual inspection of these samples there was no significant difference in the polymers prepared in this work and those prepared in previous chapters. However, although the addition of aspirin did not affect the curing rate of the polymer, it was found that paracetamol slowed the curing process. Therefore longer curing times and longer drying periods in the vacuum oven were used for samples containing paracetamol. It was also observed that in samples containing 30wt% acrylic acid, paracetamol did not appear to fully dissolve prior to curing, but was instead dispersed within the monomeric solution.



(a) *(b)*

Figure 6.1 *The structure of (a) aspirin and (b) paracetamol*

6.2.2 Soxhlet extractions

Soxhlet extractions were carried out using distilled water as the solvent for 72hrs to determine the effect that crosslinking agent content had on the extractable content of the copolymers.

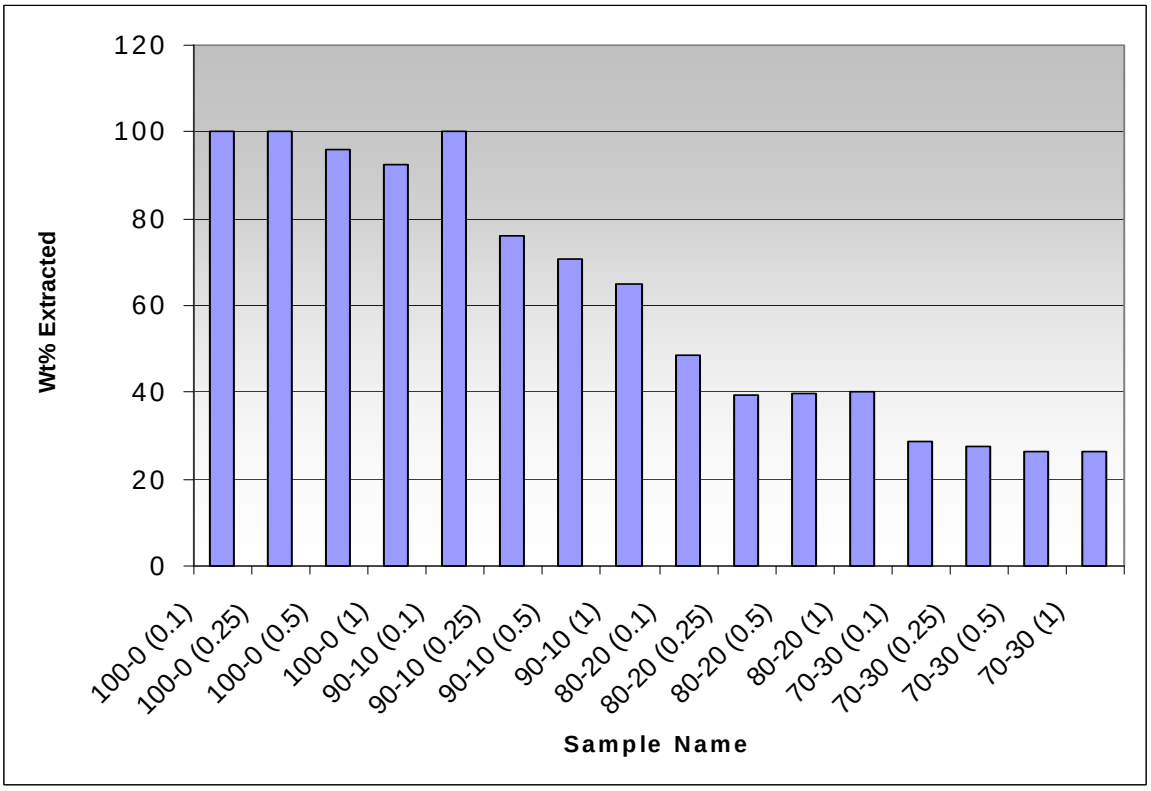


Figure 6.2 *Extractable content of hydrogels crosslinked with varying concentration of EGDMA. Where 100-0 (0.1) signifies that 0.1wt% EGDMA crosslinking agent was used to crosslink the sample designated 100-0*

From the results illustrated in Figures 6.2 and 6.3 it can clearly be seen that the samples designated 100-0 lost at least 80wt% of their total weight during extractions, irrespective of the crosslinking agent content. Similarly, the 90-10 samples lost at least 60wt% of their total weight during extraction. This indicated that these samples were not fully crosslinked and therefore did not warrant further study, as their extractable content was too high. These high extractable contents were due to a lack of intermolecular bonding, as the AA content in the samples was 0wt% and 10wt% respectively. However, it can be seen from the data obtained that as the crosslinking agent content increased there was a corresponding decrease in the extractable content of the polymer. Hong et al. (1996b) analysed a similar polymer system, and found that as the crosslinking agent content increased, the polymer went from being a homogeneous solution at 0.1wt% to a transparent material, with minimal fragmentation at 1wt% crosslinking content.

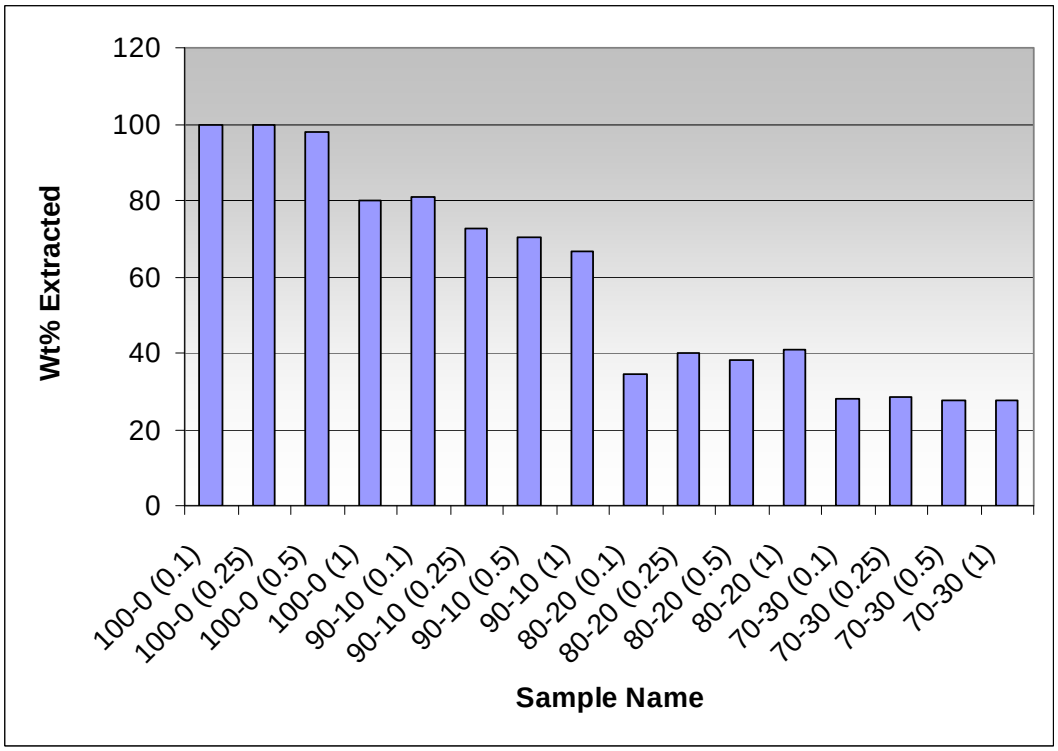


Figure 6.3 *Extractable content of hydrogels crosslinked with varying concentration of PEG600DMA. Where 100-0 (0.1) signifies that 0.1wt% PEG600DMA crosslinking agent was used to crosslink the sample designated 100-0.*

From analysis of the crosslinked samples designated 80-20 and 70-30 it was found that only a slight difference in solubility was observed with comparison to the crosslinking agent content. It was also found that the 70-30 polymers exhibited greater stability over the duration of the test as the recorded weight loss was approximately 28wt%, compared to approximately 40wt% for the 80-20 samples. These results correlate with results described in chapters 3 and 4, where it was found that an increase in AA content yielded an increase in intermolecular bonding, which in turn decreased solubility of the polymer, even without the incorporation of chemical crosslinking agents. The results observed in this work exceed the values illustrated by Nho and Park (2002), who performed a similar test at 70°C for approximately 48 hours on PVOH/PVP – chitosan copolymers. From results stated it was found that the polymers tested had weight losses in excess of 40%.

When the hydrogels are compared with reference to the molecular weight of the crosslinking agent it was found that the samples crosslinked with EGDMA exhibited a slightly higher solubility profile in comparison to samples crosslinked with PEG600DMA. This also correlates with results described in chapter 4, where it

was found that the oscillatory torque at break and therefore the flexibility of the copolymers increased with an increase in the molecular weight of the crosslinking agent. Therefore it is believed that this increased flexibility, decreased the number of main chain breakages during the test. In chapter 4 it was also found that at a pH above the $pK_{initial}$ of AA that the hydrogel that was designated 70-30 began to exhibit slight brittle behaviour at 0.1wt% crosslinking agent. It was therefore decided to carry out further analysis on the 70-30 copolymers crosslinked with 0.1wt% crosslinking agent only, as additional crosslinking agent would increase the brittleness of the copolymer.

6.2.3 Fourier transform infrared spectroscopy

Ftir experiments were carried out in order to determine the effect that the addition of an active agent had on the UV cured polymers. The polymers tested consisted of a monomeric mixture of NVP, AA and in some cases a crosslinking agent.

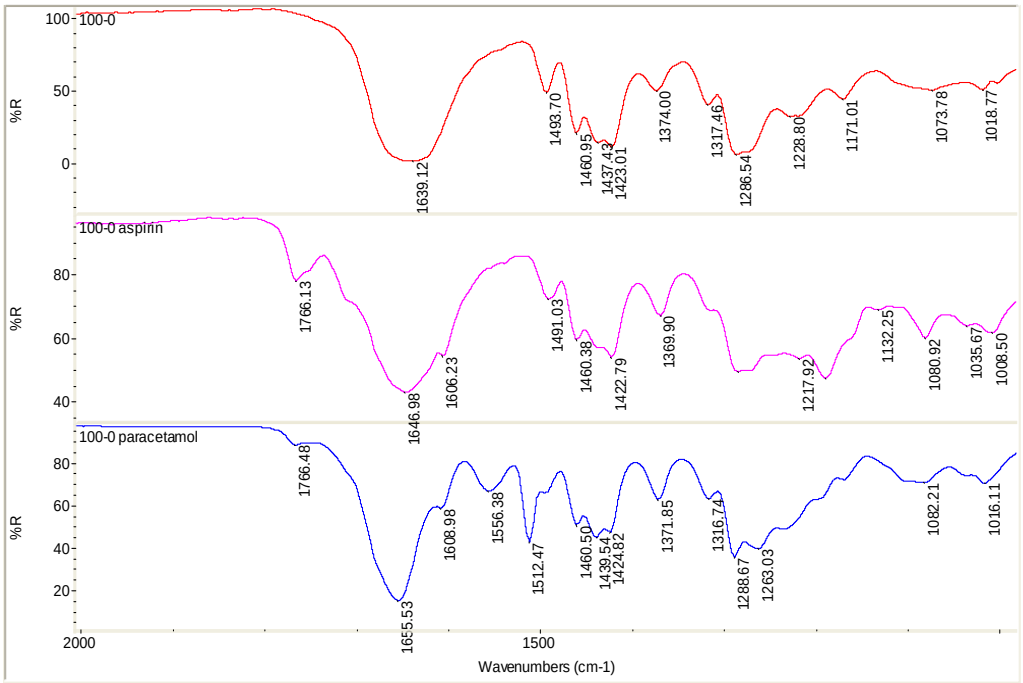


Figure 6.4 *Comparison of Ftir spectra for 100-0 sample, and 100-0 samples containing actives agents*

The Ftir spectra of the polymers tested have been discussed extensively in chapters 3 and 4 and therefore will not be discussed in this chapter. However, from analysis of the Ftir spectra of the polymers, and of the polymers containing an active

agent (Figures 6.4 and 6.5 illustrate representative results, remaining results are illustrated in appendix H), it was found that the presence of paracetamol within the polymer structure was confirmed by the presence of characteristic peaks in the region of 1512 cm^{-1} attributed to C-H symmetric bends. More importantly a peak attributed to N-H in plane deformation was observed in the region of 1556 cm^{-1}. The significance of this peak is attributed to the fact that no other compound used in this work, contains an N-H bond (Moynihan and O'Hare 2002).

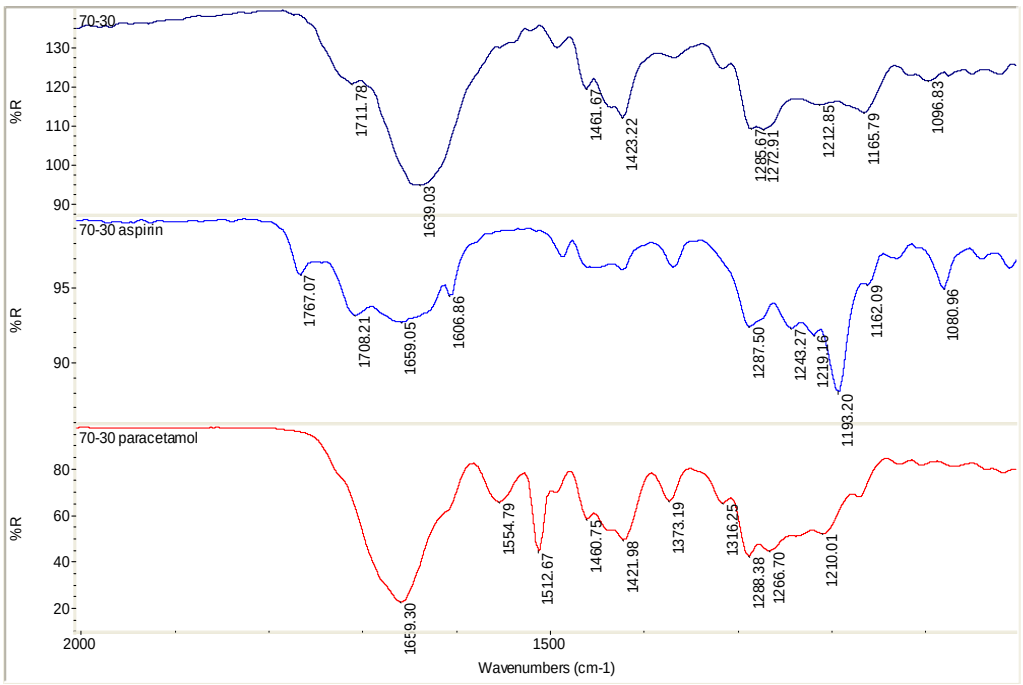
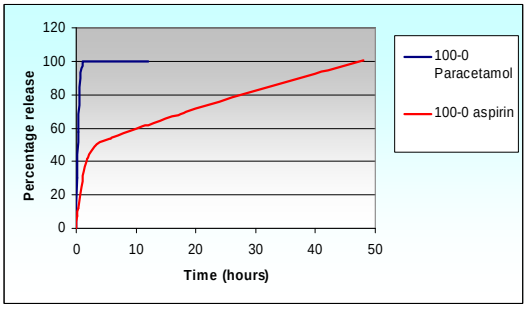


Figure 6.5 *Comparison of Ftir spectra for 70-30 sample, and 70-30 samples containing actives agents*

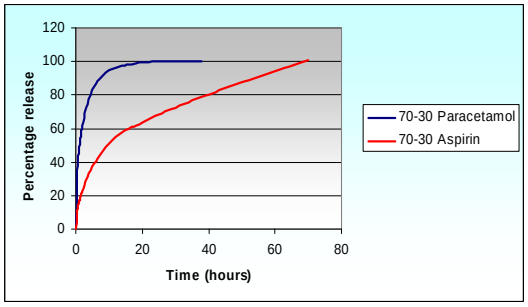
The Ftir spectra of the samples that contains aspirin, gave evidence that the carboxylic acid group of aspirin formed a dimer, similar to what was observed in previous chapters where the carboxylic acid group of acrylic acid formed a dimer by hydrogen bonding to an adjacent carboxylic acid group. This is observed as a shoulder/peak on the main PVP carbonyl peak in the region of 1708cm^{-1}. This peak was not evident in 100-0 samples or 100-0 samples with paracetamol incorporated. With the addition of acrylic acid a shoulder did appear in the spectra of the polymer without aspirin as was found previously. However the magnitude of this peak increased with the addition of aspirin, signifying that hydrogen bonding occurred between the carboxylic groups of both aspirin and acrylic acid.

6.2.4 Drug dissolution

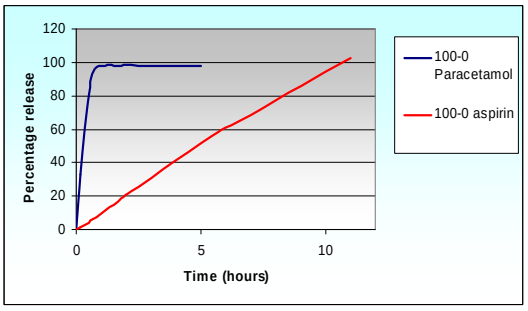
Dissolution studies were performed in triplicate using the Basket method (USP XXV) at 37ºC ± 0.5ºC at 100 rpm. The media used for drug dissolution testing were pH 2, pH 6.8 and pH 9 buffered solutions, using a Sotax ® on-line dissolution system. At predetermined time intervals, samples were withdrawn automatically, filtered, passed through a UV/Vis spectrometer, the UV of the sample at the preset wavelength analysed, and finally the sample was returned to the bowl. The wavelength and absorption of a 100% drug concentration for each drug and pH, were determined using a Perkin Elmer Lambda 40 UV/Vis spectrometer. These values were entered into software calculations prior to commencement of testing, and an E11 value was calculated. The values determined in this work are shown in Table 6.1. The drugs used in this experiment were aspirin and paracetamol.



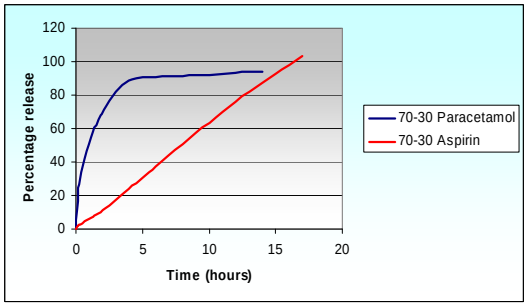
(a) 100wt% PVP, pH2



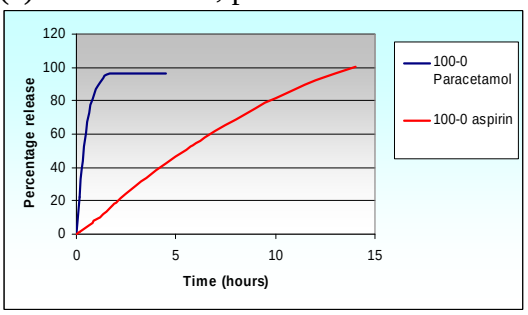
(b) 70wt% PVP – 30wt% PAA, pH2



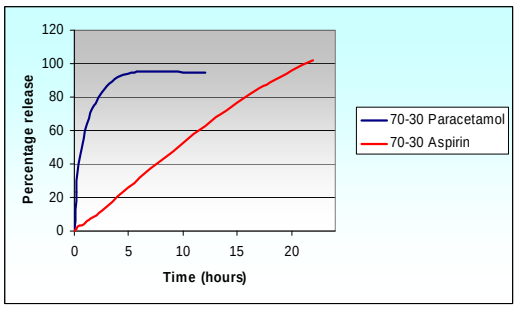
(c) 100wt% PVP, pH6.8



(d) 70wt% PVP – 30wt% PAA, pH6.8

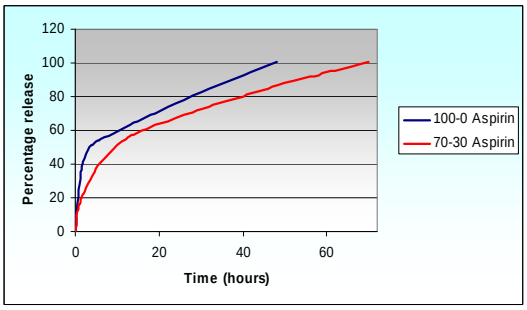


(e) 100wt% PVP, pH9

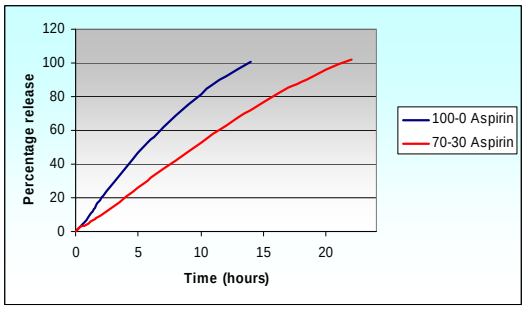


(f) 70wt% PVP – 30wt% PAA, pH9

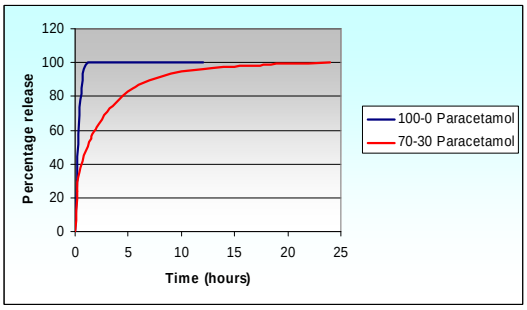
Figure 6.7 *Comparison of the release rate of aspirin and paracetamol at each pH value tested*



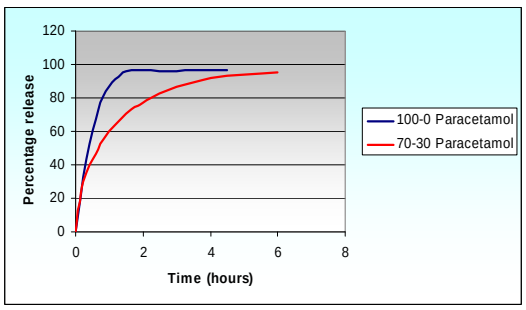
Aspirin release pH 2.0



Aspirin release pH 9.0



Paracetamol release pH 2.0

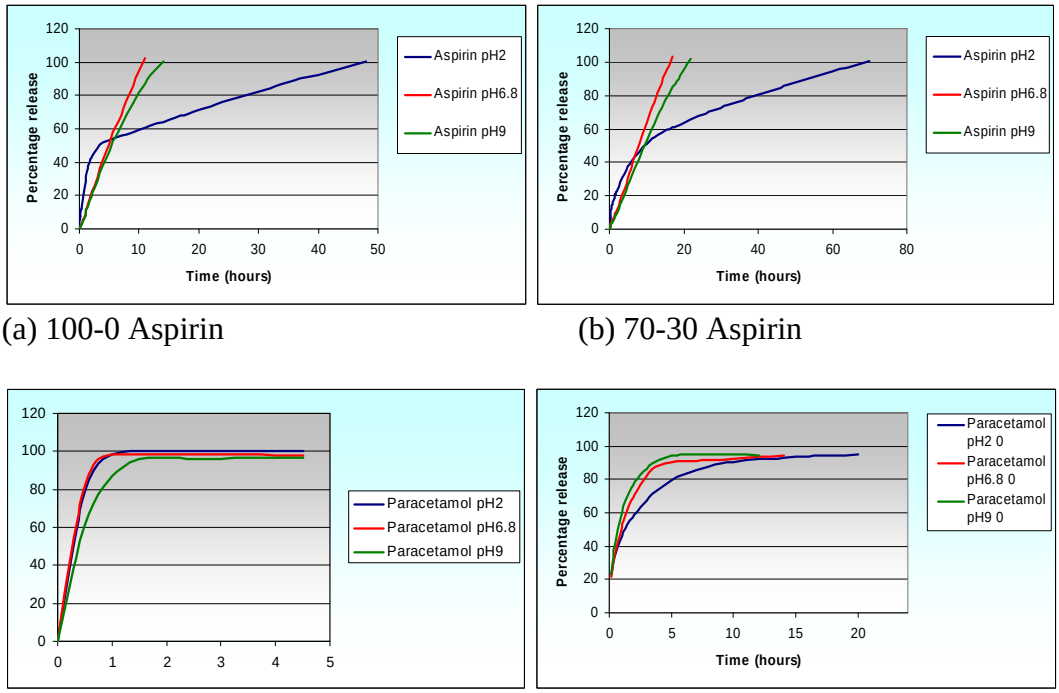


Paracetamol release pH 9.0

Figure 6.8 *Dissolution curves illustrating the effect of acrylic acid on the release rate of both active agents*

From the dissolution results obtained, it was found that for all polymers analysed at each pH value aspirin was released slower than paracetamol. In this work (Figure 6.7), it was found that at pH 2, the 100-0 samples had released its paracetamol content after approximately 1 hour 20 minutes. However, total aspirin release was not observed until approximately 48 hours. With the incorporation of 30wt% AA, the release of both active agents had slowed with paracetamol release reaching a maximum after approximately 24 hours; however the release of aspirin was also prolonged up to 70 hours. The release of the active agents at pH 6.8 and pH 9 followed a similar trend to the release of the active agents at pH 2. The 100-0 samples had released their paracetamol content after approximately 1 hour 10 minutes and 1 hour 40 minutes, and total aspirin release was observed after approximately 11 hours and 14 hours for pH 6.8 and pH 9 respectively. The addition of 30wt% AA again prolonged the controlled release of both active agents. In this instance paracetamol was released after approximately 5 hours at both pH 6.8 and pH 9, and aspirin was released after approximately 17 hours and 22 hours at pH 6.8 and pH 9, respectively. Hydrogen bonding between the carboxylic acid group of aspirin and the carboxylic acid group of acrylic acid in the copolymer is believed to

be the primary cause of the retardation of the aspirin release in comparison to paracetamol.



(a) 100-0 Aspirin (b) 70-30 Aspirin

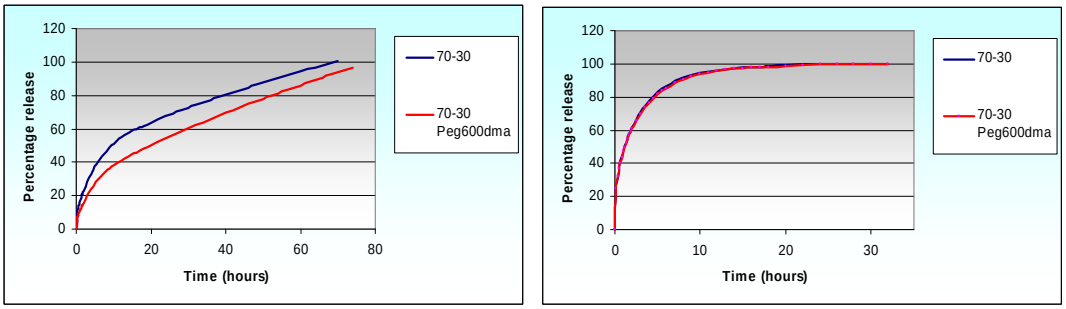
(c) 100-0 Paracetamol (d) 70-30 Paracetamol

Figure 6.9 *The release profile of aspirin and paracetamol at various pH values*

Figure 6.8 illustrates the release profile obtained by both aspirin and paracetamol, above and below the $pK_{initial}$ of acrylic acid (4.07 – 4.49) as described in chapter 3. It can be seen that at pH 2, samples containing aspirin, which has a pKa of 3.49, released the active agent in a slow and sustained manner after an initial burst release of the drug. The incorporation of acrylic acid further slows the release of the active agent, as a higher degree of physical crosslinking is achievable via the carboxylic acid groups of both acrylic acid and aspirin. At pH 9, it was found that the release of aspirin was faster then that obtained at pH 2. This was expected as above the $pK_{initial}$, the carboxylic acid groups are ionized, and therefore hydrogen bonding is not readily achievable.

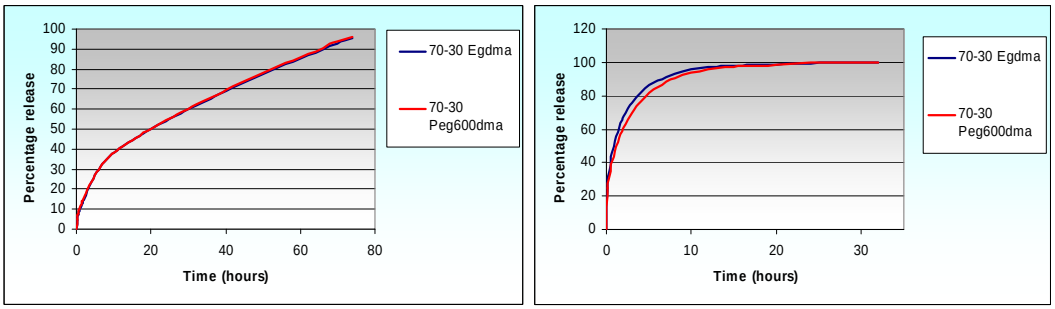
The release profile observed for samples containing paracetamol, which has a pKa of 9.35, shows that without the incorporation of acrylic acid, the active agent is released relatively quickly at both pH values. With the incorporation of acrylic acid, paracetamol release is retarded at both pH values; however at pH 2 the difference in release rate is far more pronounced. These release profiles can be explained by virtue of the fact that paracetamol is not as likely to form hydrogen bonds as aspirin,

either to itself or to the monomers used. Therefore without acrylic acid the release of the active agent is dependent on the dissolution rate of the polymer and is not affected by the pH of the dissolution media. With the incorporation of acrylic acid a more physically crosslinked structure is obtained below the $pK_{initial}$ and therefore a slower release rate is observed. The release profiles obtained for paracetamol also showed a reduction in the release rate at all pH values as the acrylic acid content was increased: however, in this case the slowed release rate was due to increased bonding in the polymer only and not polymer/drug intermolecular bonding.



(a) 70-30 Aspirin pH2 (b) 70-30 Paracetamol pH2

Figure 6.10 *The effect of crosslinking agent on the release rate of the active agents*



(a) 70-30 Aspirin pH2 crosslinked (b) 70-30 Paracetamol pH2 crosslinked

Figure 6.11 *The effect of varying the molecular weight of the crosslinking agent on the release rate of the active agents*

Figure 6.9 illustrates the release profile of both active agents in relation to change in the pH value of the dissolution media. On examination of the release profiles observed for aspirin (Figure 6.9 a and b), it was found that below the $pK_{initial}$ of acrylic acid and the pKa of aspirin, i.e. pH 2, where a relatively high level of hydrogen bonding is obtainable, the release rate of aspirin is relatively slow. However samples containing acrylic acid yielded a slower release profile due to increased hydrogen bonding. It was found that the release profile at both pH 6.8 and

9 were similar. Above the $pK_{initial}$ of AA and the pKa of aspirin, the carboxylic acid groups may be ionized and therefore hydrogen bonding between these groups is not readily possible. Therefore, the polymers tested would not have a high level of physical crosslinks at either pH value, thus the release profiles achieved would be similar.

Table 6.1 *Wavelength and absorbtion values used in dissolution studies*

	Aspirin			**Paracetamol**		
pH	Wavelength	Absorbance	E 11	Wavelength	Absorbance	E11
2	290	1.0863	58.56065	290	1.1048	59.55795
6.8	295	1.123233	60.54987	290	1.21457	65.47547
9	290	1.6566	89.30458	290	1.0863	58.56065

Figure 6.9 (c and d) also illustrates the effect that the pH of the dissolution media has on the release profile of paracetamol. It was found that for the 100-0 polymers, the release profile observed at pH 2 and 6.8 are similar, however the profile obtained at pH 9 is slower. The pKa of paracetamol is 9.35, therefore ionisation occurs at pH values higher then 9.35 and ionic bonds can form, thus slowing the release of the active agent. It is believed that at pH 9.0 some intermolecular bonding did take place which slowed the release of paracetamol. With the incorporation of acrylic acid the overall pH of the system would be acidic. Therefore above the $pK_{initial}$ of AA the release profile observed was similar, while below the $pK_{initial}$ of AA the release rate of the active agent slowed down due to the physical crosslinks within the polymer.

Chemical crosslinking agents were incorporated into the monomeric feed ratio to determine their effect on the release rate of the active agent. Samples designated 70-30 were used in these experiments. Drug release from these crosslinked samples were carried out in pH 2 media only as these samples could swell to over 1000% in a pH above the $pK_{initial}$ of acrylic acid, as shown in chapter 4. From Figure 6.10 it can be seen that with the incorporation of PEG600DMA, the release rate of aspirin is slowed down. This may be due to the crosslinks holding the molecular chains more tightly together. The release of paracetamol was similar to that obtained without the incorporation of crosslinking agent.

Figure 6.11 illustrates the release profiles obtained when the molecular weight of the crosslinking agent is altered. It was found that the molecular weight of the crosslinking agent did not have an effect on the release profile of the active agents at pH 2. It is believed that at pH 2 the molecular chains of the hydrogel were held tightly together with physical crosslinks, therefore the molecular weight of the crosslinks had little overall effect.

Additional drug dissolution data is shown in appendix I.

6.2.5 Cytotoxicity and genotoxicity testing

Prior to biocompatibility testing individual hydrogel discs were weighed and dissolved in an appropriate volume of complete culture medium to give a final concentration of 25 mg ml^{-1}. Following incubation overnight at 37°C, and brief vortexing, hydrogel suspensions were filter sterilised (0.2 µm pore-size) and subject to serial dilution in complete culture medium to give the concentration range 25–0.025 mg ml^{-1}.

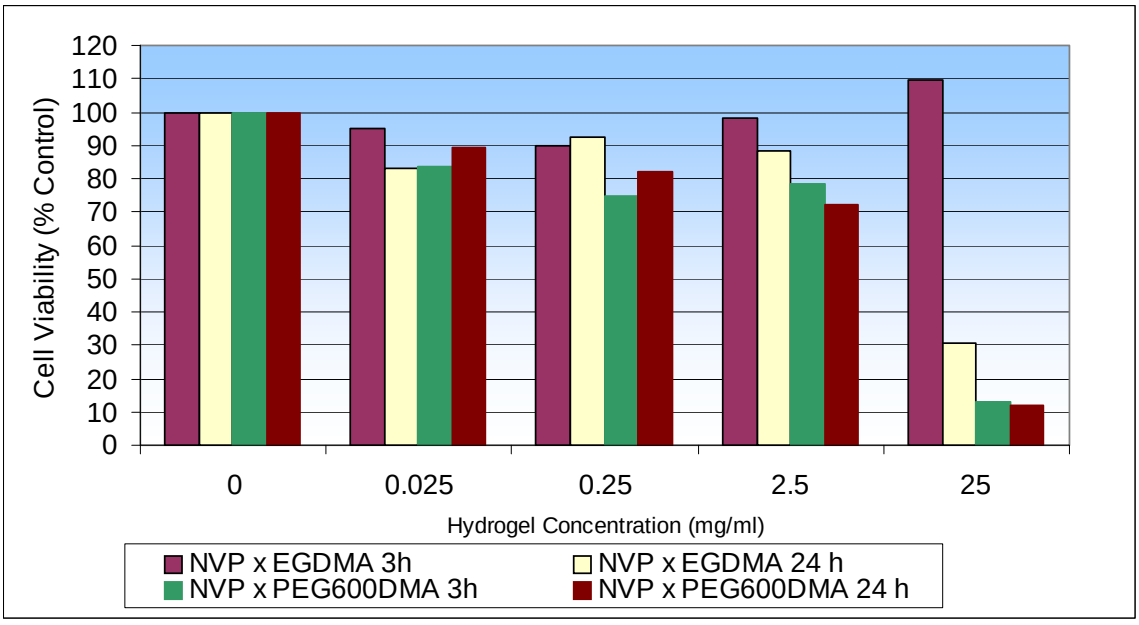


Figure 6.17 *Effect of NVP-based hydrogels crosslinked with EGDMA or PEG600DMA on HepG2 cell viability in the MTT assay after 3 h and 24 h exposures at 37°C. Each data point represents the mean of three separate experiments (n=18)*

Human hepatoma-derived cell line HepG2, were used in this work as the liver is the major site for the biotransformation /detoxification of toxicants (i.e.

major target organ). The human hepatoma-derived cell line HepG2 (Aden et al. 1979) was chosen as it retains many of the anatomical and functional characteristics of primary human hepatocytes (Devery and Tomkins, (1998; 1999) (i.e. best approximates hepatocyte physiology and biochemistry without the difficulties associated with primary cell isolation and culture.

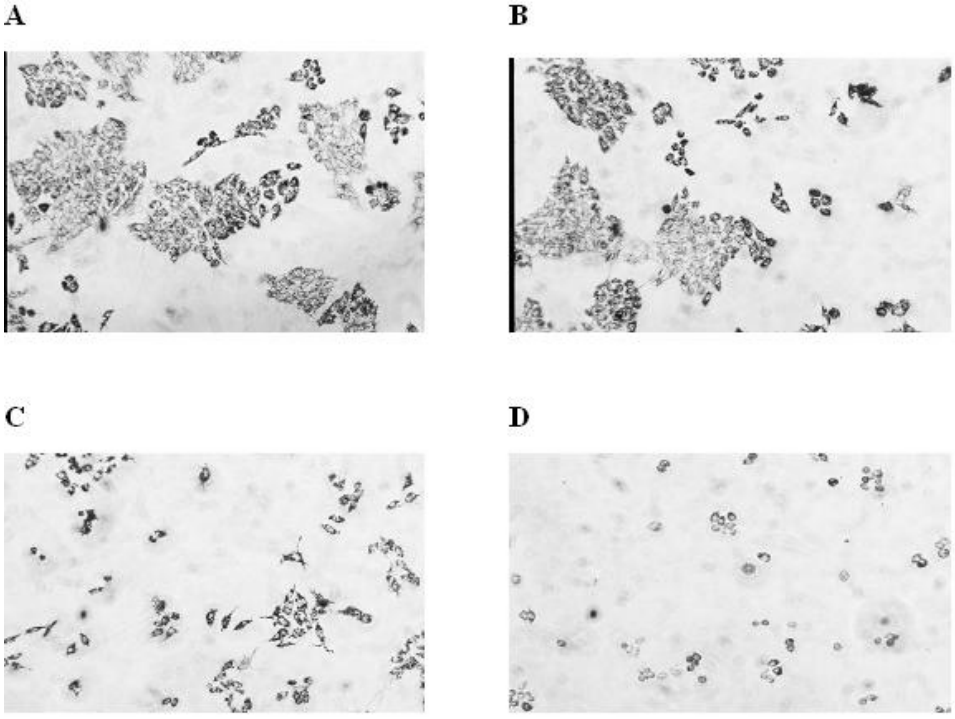


Figure 6.13 *HepG2 cell reduction of MTT tetrazolium salt following culture for: (A) 24 h in Hepes modified DMEM + Hams F-12 (1:1) complete culture medium (negative control); (B) 3 h in the presence of 0.025 mg ml^{-1} 100-0 sample crosslinked with EGDMA; (C) 24 h in the presence of 25 mg ml^{-1} 100-0 sample crosslinked with EGDMA; (D) 24 h in the presence of 25 mg ml^{-1} 100-0 sample crosslinked with PEG600DMA (magnification 200x)*

A dose-dependent decrease in HepG2 cell viability was observed in the MTT assay for samples designated 100-0, crosslinked with PEG600DMA (100-0 x PEG600DMA). This effect was most pronounced at concentrations > 2.5 mg ml^{-1} with a 100-0 x PEG600DMA concentration of 25 mg ml^{-1} reducing cell viability to approximately 10 % of untreated control cells, independent of exposure time as shown in Figure 6.17. While 100-0 x PEG600DMA hydrogel concentrations in the range 0.025-2.5 mg ml^{-1} also decreased cell viability, such reductions in viability were no greater than 70% of untreated cell values after either 3 hour or 24 hour

exposure (Figure 6.12). Conversely 100-0 samples crosslinked with EGDMA (100-0 x EGDMA) produced rather conflicting results at concentrations $\geq$ 2.5 mg ml^{-1}, also Figure 6.12. Essentially, following 24 h exposure a reduction in cell viability was observed at all 100-0 x EGDMA hydrogel concentrations in the range 0.025-25 mg ml^{-1} and again in keeping with the results obtained for 100-0 x PEG600DMA. This decrease was most pronounced at 25 mg ml^{-1}, representing a reduction in cell viability to 30 % of untreated cell values. In contrast, short-term 3 hour exposure seemed to exert a slight proliferative effect on HepG2 growth at 100-0 x EGDMA hydrogel concentrations $\geq$ 2.5 mg ml^{-1} (Figure 6.12).

Table 6.2 *Sample comet assay data for HepG2 cells exposed to 100-0 samples crosslinked with EGDMA for 3h at 37°C*

100-0 samples crosslinked with EGDMA (mg/ml)	Mean Tail Moment# (± SEM)	
0	0.768 ± 0.165	(1.0)
0.025	1.106 ± 0.268	(1.4)
0.250	3.270 ± 0.815	(4.3)
2.500	3.570 ± 0.678	(4.7)
25.000	4.610 ± 1.762	(6.0)

 # Values are the mean ± the standard error of the mean from a single experiment in which 50 single cells were analysed per concentration. Values in parentheses represent tail moment values expressed as a multiple of the untreated control.

Overall such effects on HepG2 cell viability, in response to direct contact or incubation with 100-0 containing media, did not correspond to alterations in HepG2 cell epithelial morphology as observed by light microscopy (Figure 6.13). Interestingly such results are somewhat similar to those obtained by Smith et al. (2006), whereby PVP hydrogels crosslinked with EGDMA reduced the viability of human dermal fibroblasts in direct contact studies while promoting the growth of such cells when there is indirect contact. Indeed the growth promoting effects of 100-0 x EGDMA has also been reported by others, albeit during indirect contact but in a polymer concentration-dependent manner independent of serum (Hong et al. 1997).

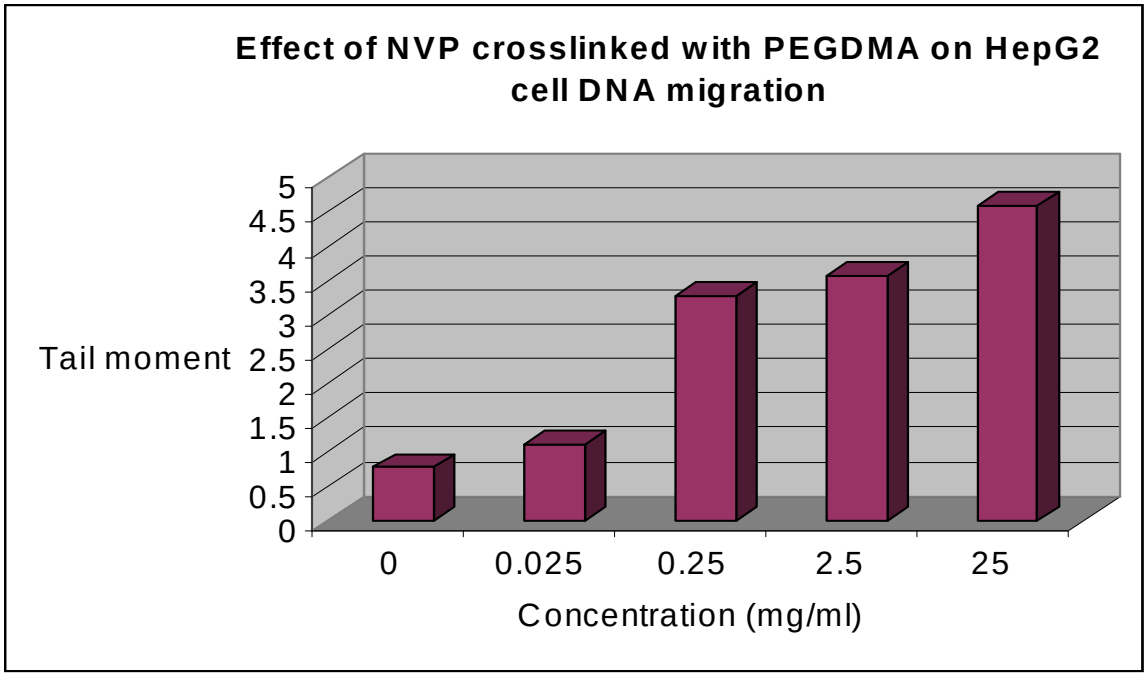


Figure 6.14 *DNA strand breakage expressed as tail moment in HepG2 cells following exposure for 3h at 37°C to 100-0 samples crosslinked with EGDMA. Results represent the mean of a single experiment, with 50 comets scored per concentration*

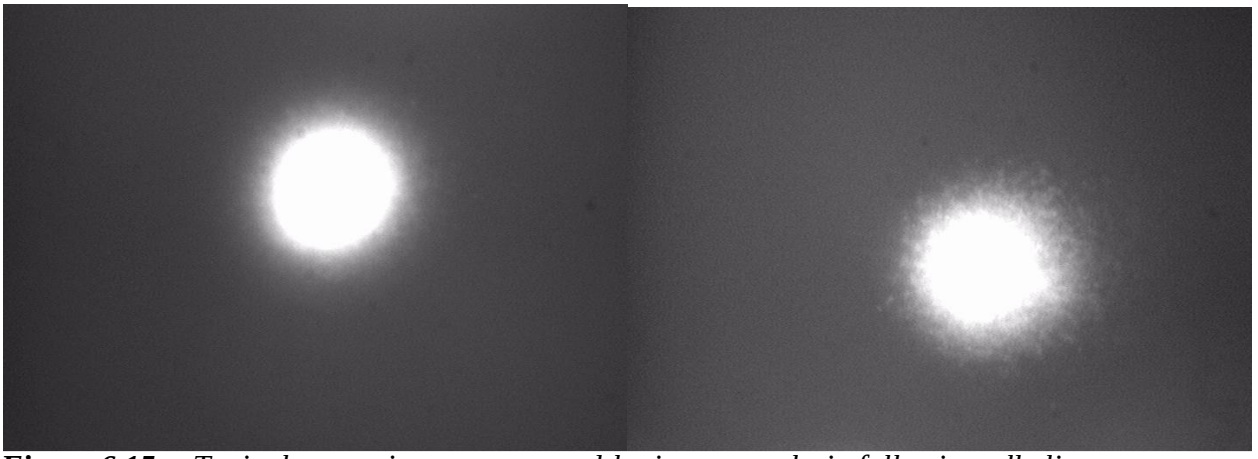


Figure 6.15 *Typical comet images captured by image analysis following alkaline SCGE and ethidium bromide staining.* ***Panel A:*** *Untreated HepG2 control cell.* ***Panel B:*** *HepG2 cell exposed for 3 h at 37°C to 25 mg ml^{-1} PVP crosslinked with EGDMA (magnification 1000x)*

Despite the fact that hydrogels, including those synthesised from NVP, are considered biocompatible it is possible that decreased viability observed for HepG2 cells may be the result of high concentrations of unreacted monomer, oligomers, initiator or indeed methacrylates (Peppas et al. 2000). Long-term animal toxicity studies have previously shown the liver and upper respiratory tract as the main targets for NVP toxicity (Klimisch et al. 1997). Furthermore, methacrylates tend to

be less cytotoxic than acrylates, although this appears to be structure-related and dependent upon the length of oxyethylene chains. The mutagenic effects of acrylate esters have also been documented (Dearfield et al. 1989; Yoshii 1997; Schweikl and Schmalz 1999). However it is inappropriate at such an early stage in the biocompatibility assessment of NVP-based hydrogels crosslinked with either PEG600DMA or EGDMA to comment on the exact mechanisms underlying the decreased cell viability and proliferative effects observed.

Preliminary results for 100-0 x EGDMA in the alkaline comet assay suggest a four to six fold increase in HepG2 cell DNA single strand breaks in the range 0.25-25 mg ml^{-1} compared to untreated cells following 3 hour exposure, as outlined in Table 6.2 and illustrated in Figures 6.14 and 6.15. Indeed such increases in DNA migration correlate with the cytotoxic and proliferative effects observed for 100-0 x EGDMA samples following short-term exposure in the MTT assay. In effect, the lowest levels of DNA migration correspond with the slight decreases in cell viability observed over a similar concentration range of 0.025-0.25 mg ml^{-1} (Figures 6.14 and 6.12 respectively). Moreover the slightly higher levels of DNA migration at concentrations $\geq$ 2.5 mg ml^{-1}, are compatible with the proliferative effects also observed at such concentrations in the MTT assay. This may be attributed to the fact that sites of active DNA replication can also lead to increased DNA migration and tail moment values in the alkaline Comet assay (Salagovic et al. 1997).

Conclusions

In this work the potential of polyvinylpyrrolidinone-polyacrylic acid copolymers developed for use as multifunctional hydrogels in biomedical applications has been evaluated. The extractable content of the crosslinked hydrogels was analysed in relation to increasing crosslinking agent content. It was found that the solubility of the polymers designated 100-0 decreased with high crosslinking agent loadings. This reduction in solubility was also observed with polymers designated 90-10, where a sharp decrease in solubility was observed with increasing crosslinking agent content. However, the polymers designated 80-20 and 70-30 were relatively insoluble, and therefore only a slight reduction in solubility was observed with increased crosslinking agent content. It has been shown that the extractable content of the 80-30 and 70-30 hydrogels is relatively low, allowing these hydrogels to be used in applications where this is advantageous. Drug dissolution was carried

out on some crosslinked and a range of uncrosslinked polymers. It was found that the dissolution profile of active agents from these polymers vary, depending upon the dissolution media used and on the chemical structure of the active agent incorporated. Finally preliminary *in vitro* biocompatibility results obtained for polyvinylpyrrolidinone crosslinked with either EGDMA or PEG600DMA are encouraging; with only slight increases in cytotoxicity and genotoxicity observed for metabolically competent hepatoma HepG2 cells when in direct contact with low concentrations of hydrogel-containing media.

In conclusion these hydrogels are capable of releasing various active agents in a predictable and controlled fashion depending on the pH of the dissolution media and therefore have potential for use in oral drug delivery applications. Due to their relatively low extractable content and the favourable cytotoxicity and genotoxicity results, they also have potential for use in other biomedical applications such as medical device coatings and wound healing dressings.

Chapter 7

Conclusions

The aim of this study was to develop a novel hydrophilic polymer for use in biomedical applications. The polymers synthesised in this work were based on the monomers N-vinyl pyrrolidinone and acrylic acid, which were UV polymerised using a suitable photoinitiator depending upon the application. Visual inspection of the samples showed that a solid polymer was produced after UV curing, which was slightly yellow in colour. Samples produced from 100wt% NVP had a smooth uniform surface. However samples that had AA incorporated did not have a uniform surface, and white solids on the surface indicated phase separation of some AA segments had taken place.

These polymers were chemically characterised using differential scanning calorimetry, Fourier transform infrared spectroscopy, potentiometric titrations, polymer solubility tests and gel permeation chromatography. DSC thermograms showed that the T_g of the samples tested was approximately 140ºC regardless of the AA content and proved that the stiffness of the polymer was not affected by the AA content, as low molecular weight PAA caused a plasticising effect and that no secondary transitions were present in the region tested. The Ftir spectra of PVP-PAA copolymer complexes indicate hydrogen bonding between the carbonyl group in the PVP and the carboxylic acid group in the PAA moiety. There is also evidence that the AA molecules formed cyclic rings through hydrogen bonding of the carboxylic acid side groups. As the percentage of AA increased in the copolymer there is an increase in intermolecular hydrogen bonding between the carboxylic acid groups of the AA segments. Polymer solubility analysis of the PVP-PAA complex in a higher pH medium is significantly different from results in low pH solutions. The critical pH range or $pK_{initial}$ was found by potentiometric titrations to be between 4.07 and 4.49. Above a pH of 4.49 there is a progressive break up of the polymer chain due to a reduction in the amount of intermolecular hydrogen bonding and thus the polymer can dissolve much quicker than in lower pHs where a high level of hydrogen bonding is possible. This is caused by an increase in the amount of carboxylate ions present, as the pH increases.

Ethylene glycol dimethacrylate with various molecular weights were added to the copolymer as chemically crosslinking agents and the polymers again chemically analysed by potentiometric titrations and Ftir. It was found that the

incorporation of crosslinking agents had no effect on the $pK_{initial}$, however the Ftir spectra of PVP-PAA copolymer complexes indicates both hydrogen bonded and non-hydrogen bonded PVP, signifying the crosslinking agents acted as a spacer and thus caused a reduction in hydrogen bonding.

The crosslinked copolymers were physically characterised using swelling studies and parallel plate rheometry. Swelling of the PVP-PAA complex follows similar trends to those observed in polymer solubility testing. Above the $pK_{initial}$ AA dissociates and is ionized, therefore there is a reduction in the intermolecular bonding and so the hydrogels tend to swell to a greater degree yielding higher water content and water uptake values. In fact water uptake values of over 4200% and water content values of over 97% were observed in the swollen samples. The water content values observed are similar to those found in the human body and would therefore indicate biocompatibility of the hydrogels. The rheometry results showed that there was a significant difference in the oscillating torque at break and the comparative gel strength of the hydrogels in different pH media, due to the increased water uptake as the pH of the swelling medium was increased. It was also found that by varying the molecular weight of the crosslinking agent an increase in oscillating torque at break could be achieved. However this increase in oscillating torque at break was offset by a decrease in the comparative gel strength as the water content in the hydrogel increased, with an increase in the molecular weight of the crosslinking agent. Even so the comparative gel strength observed in this work at pH 7 did compare favourably to values quoted in the literature for hydrogel wound dressings.

After the initial characterisation of the hydrogels had taken place, hydrogels were chosen for further analysis for specific applications. A monomeric mixture of 70-30 wt% NVP-AA was crosslinked with 0.1 wt% crosslinking agent for testing as a lubricious hydrophilic coating that could be cured directly onto a substrate without the aid of a solvent and potentially control the release of an active agent. The coating was applied to the Pebax ® 3533 substrate using a dip coating/UV curing procedure. It was shown that increasing the thickness of a traditional solvent based hydrophilic coating, by the polymerisation of monomers directly onto the surface of the substrate is both an effective and simple method of encapsulating a drug within a coating and eliminates the use of potentially hazardous solvents. Even though the samples were slightly tacky after curing, any remaining monomers were removed by oven drying the samples. This was proven using Ftir analysis where no characteristic monomeric

peaks were found. Ftir also proved that curing the coating directly onto a substrate did not affect the chemical structure of the crosslinked polymer when compared to the Ftir spectrum of the polymer cured in bulk. Optical microscopy suggested that 2 coating cycles yielded the most consistent coating throughout the entire length of the substrate, as excessive coating lead to inconsistencies in the coating and even to parts of the coating being washed off by additional coating cycles. Frictional analysis proved the lubricious nature of the coating. It has been shown that the instantaneous force and kinetic force required to move a sample of Pebax ® 3533 reduced significantly with the incorporation of the hydrated coating. Frictional analysis also illustrated the time taken for the coating to hydrate and become lubricious. Finally, drug release analysis showed that the release of an active agent could be controlled by varying the molecular weight of the crosslinking agent utilised.

The extractable content of the crosslinked hydrogels was analysed in relation to increasing crosslinking agent content. It was found that the solubility of the polymers designated 100-0 decreased with high crosslinking agent loadings. This reduction in solubility was also observed with polymers designated 90-10, where a sharp decrease in solubility was observed with increasing crosslinking agent content. However, the polymers designated 80-20 and 70-30 were relatively insoluble and therefore only a slight reduction in solubility was observed with increased crosslinking agent content. Drug dissolution was carried out on some crosslinked and a range of uncrosslinked polymers. It was found that the dissolution profile of active agents from these polymers vary, depending upon the dissolution media used and on the chemical structure of the active agent incorporated.

Preliminary *in vitro* biocompatibility results obtained for PVP crosslinked with either EGDMA or PEG600DMA are encouraging: with only slight increases in cytotoxicity and genotoxicity observed for metabolically competent hepatoma HepG2 cells when in direct contact with low concentrations of hydrogel containing media.

In conclusion, the water uptake, water content, oscillating torque at break and the gel strength can be controlled and the extractable content for the 80-20 and 70-30 hydrogels was found to be relatively low. Therefore these hydrogels have potential to be used in biomedical applications such as moist wound healing. When the polymers described in this work are utilised as lubricious hydrophilic drug eluting

polymeric coatings, it was found that the coating has potential to coat several different substrates. The coating also had the potential to release several active agents, due to the method of incorporation and release. As the coating significantly reduces the force required to move a substrate in a frictional environment, the coating would reduce friction on the surface of a medical device such as a catheter, and therefore reduce trauma for the patient. Drug dissolution testing confirmed that these hydrogels are capable of releasing various active agents in a predictable and controlled fashion depending on the pH of the dissolution media and therefore have potential for use in oral drug delivery applications.

Finally, as favourable cytotoxicity and genotoxicity results have been obtained, these polymers have potential for use in a variety of biomedical applications including but not limited to those outlined here.

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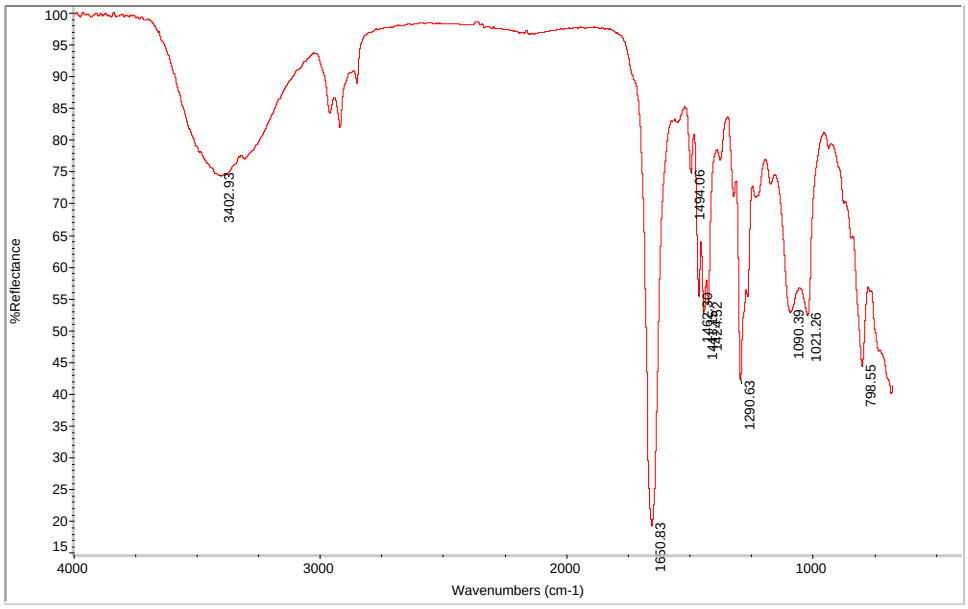
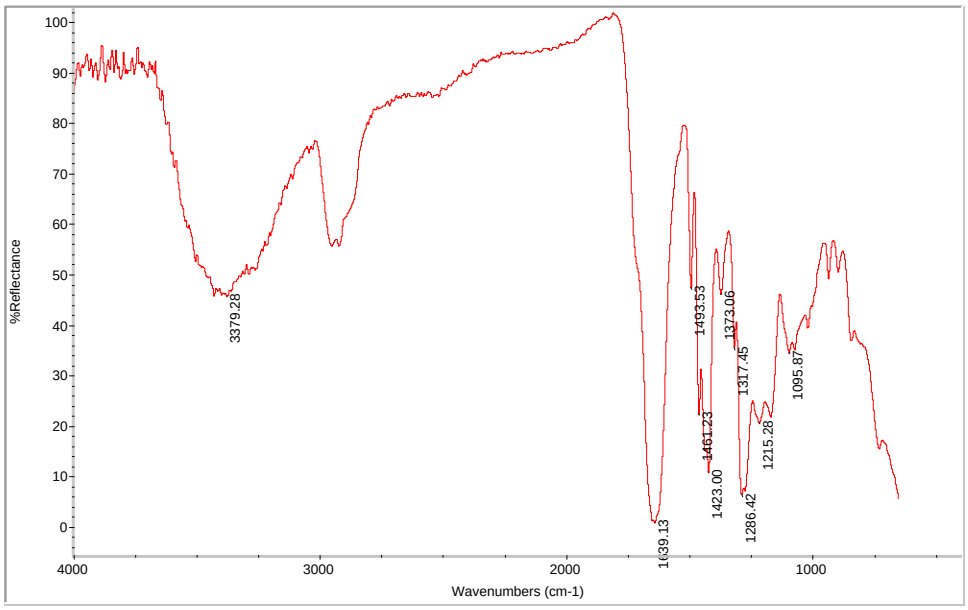
Appendices

Appendix A

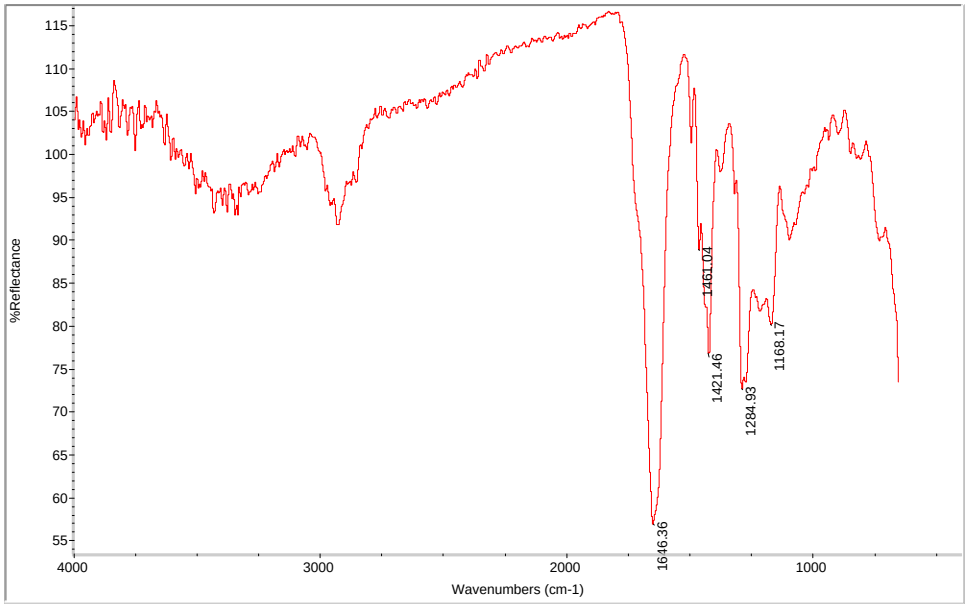
Ftir of physically crosslinked samples



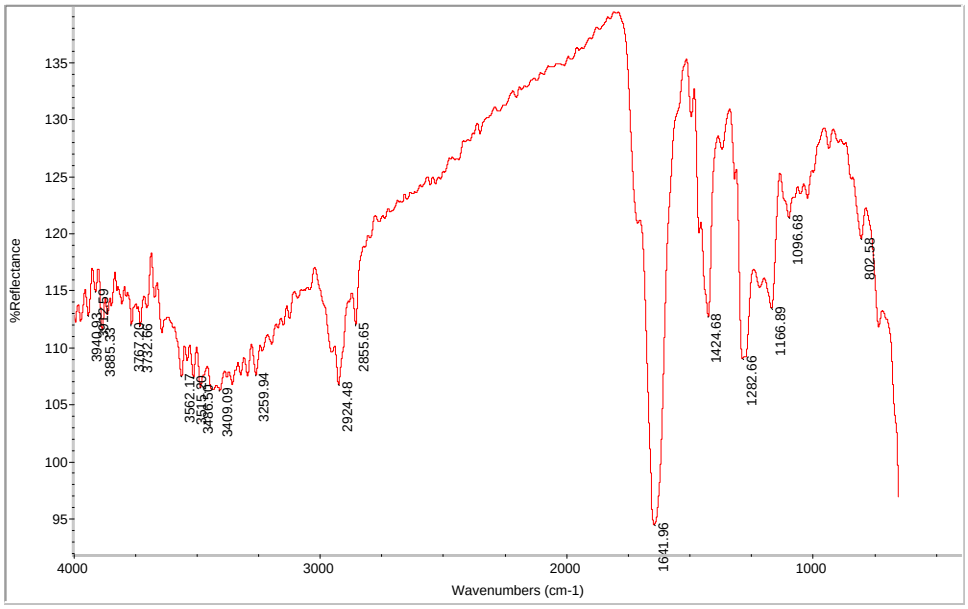
FTIR spectra of 100-0 UV cured polymer



FTIR spectra of 90-10 UV cured copolymer



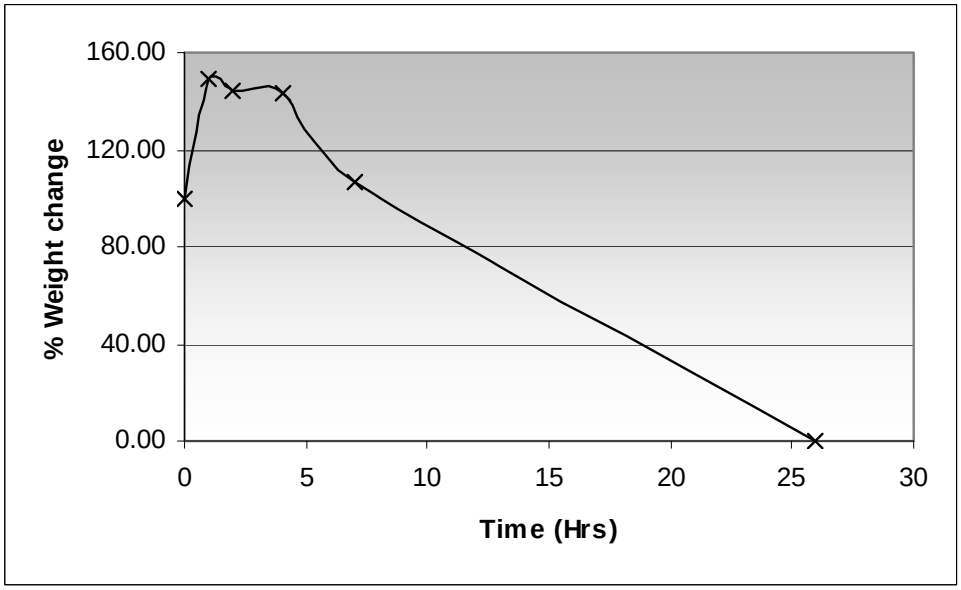
FTIR spectra of 80-20 UV cured copolymer



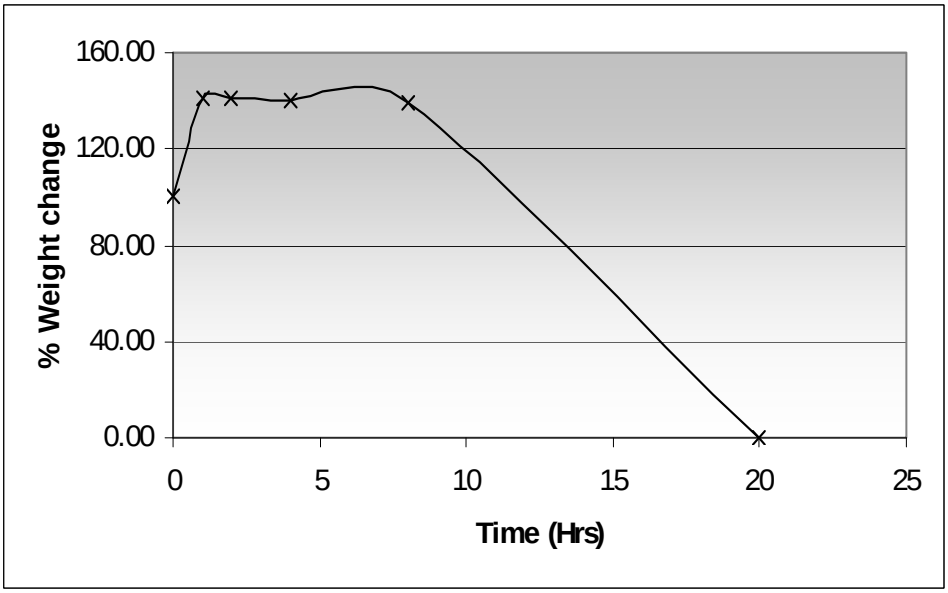
FTIR spectra of 70-30 UV cured copolymer

Appendix B

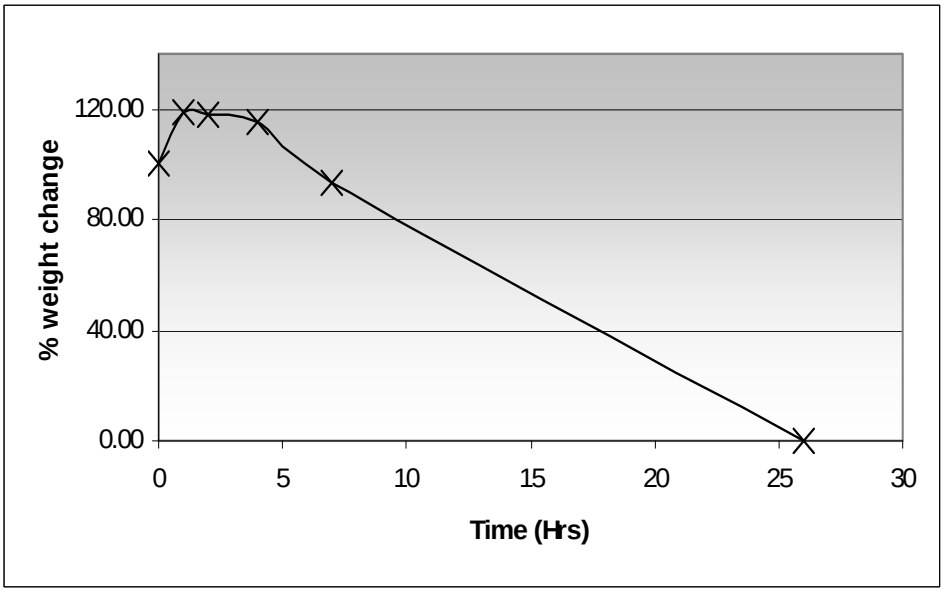
Polymer solubility tests



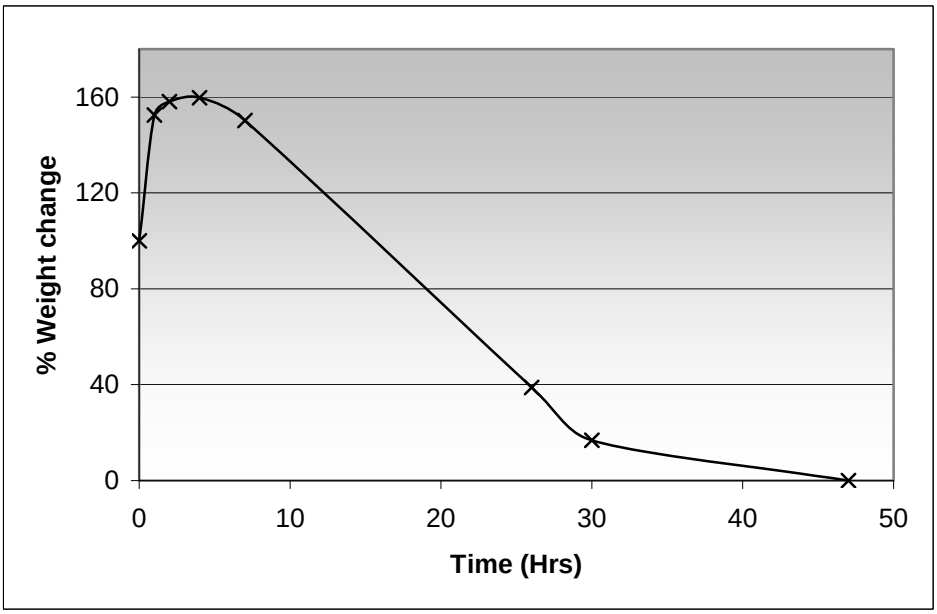
Solubility of sample 100-0 in pH 4 buffered solution



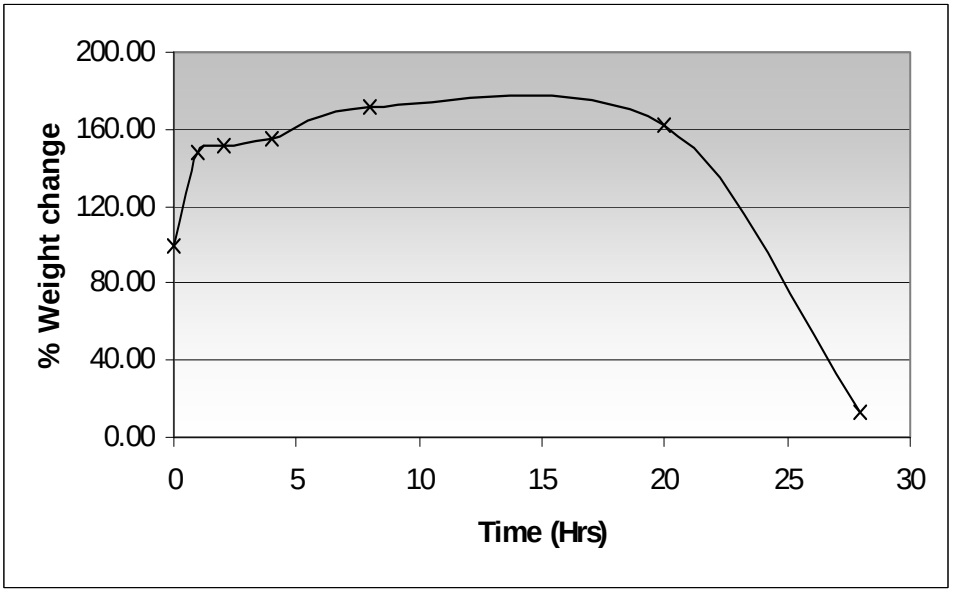
Solubility of sample 100-0 in pH 7 buffered solution



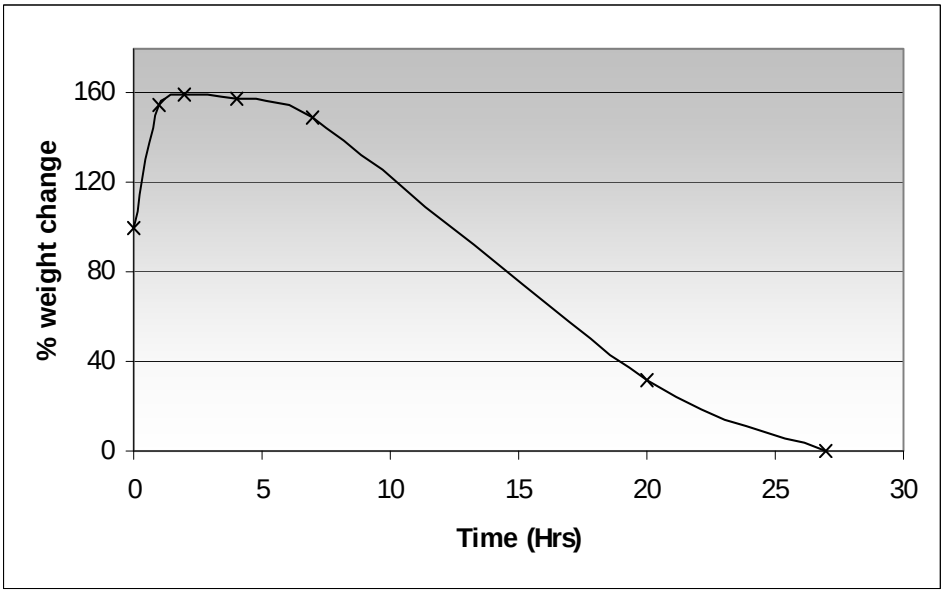
Solubility of sample 100-0 in pH 9 buffered solution



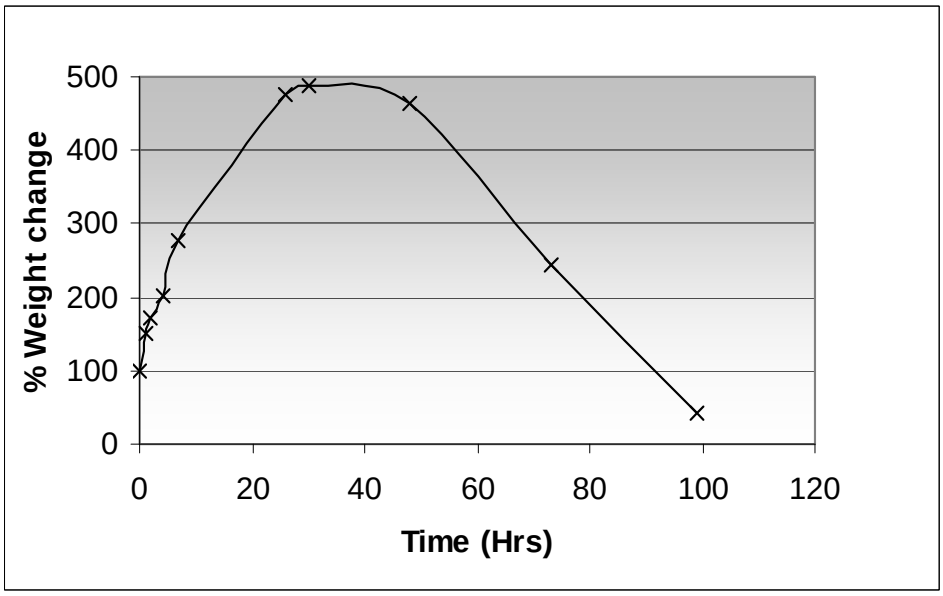
Solubility of sample 90-10 in pH 4 buffered solution



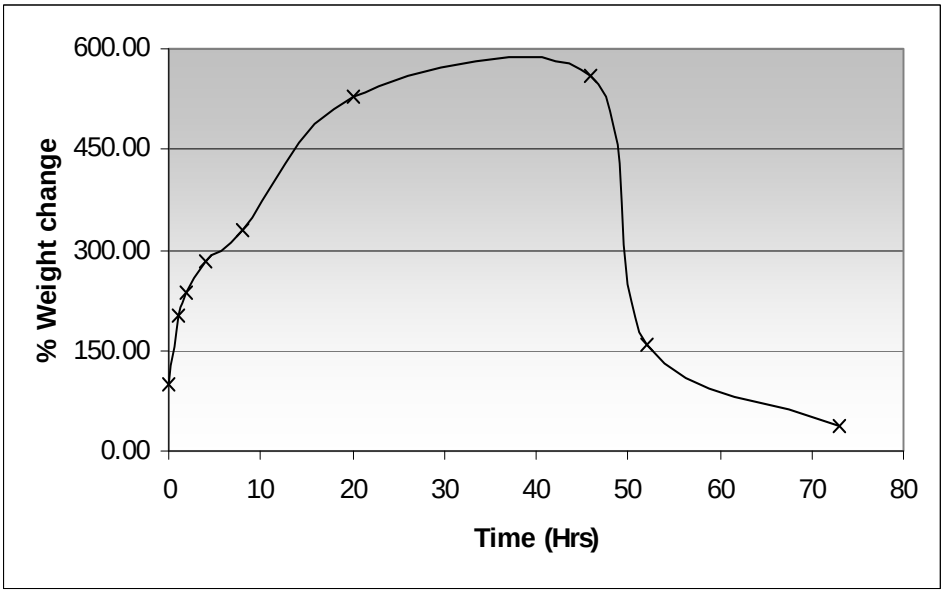
Solubility of sample 90-10 in pH 7 buffered solution



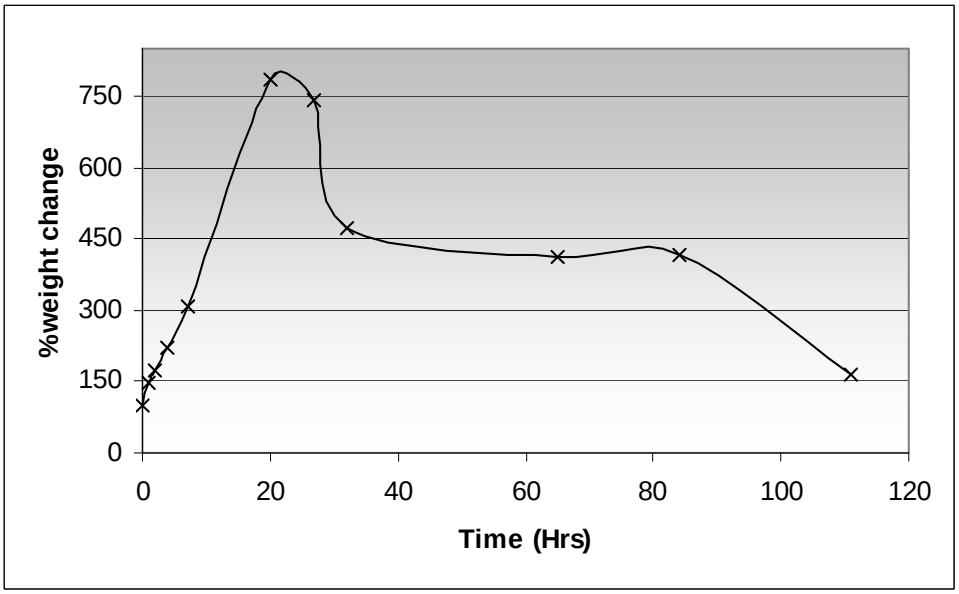
Solubility of sample 90-10 in pH 9 buffered solution



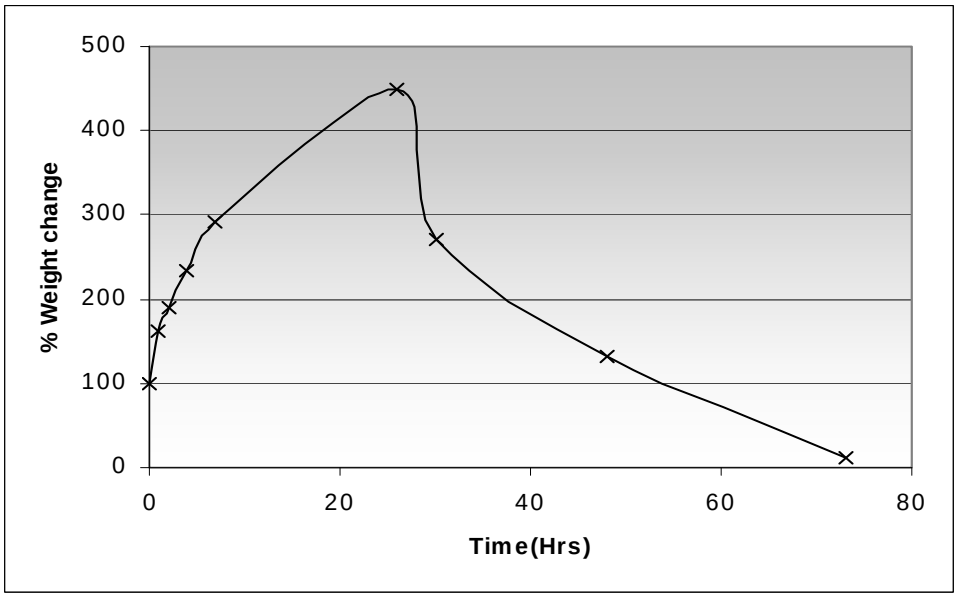
Solubility of sample 80-20 in pH 4 buffered solution



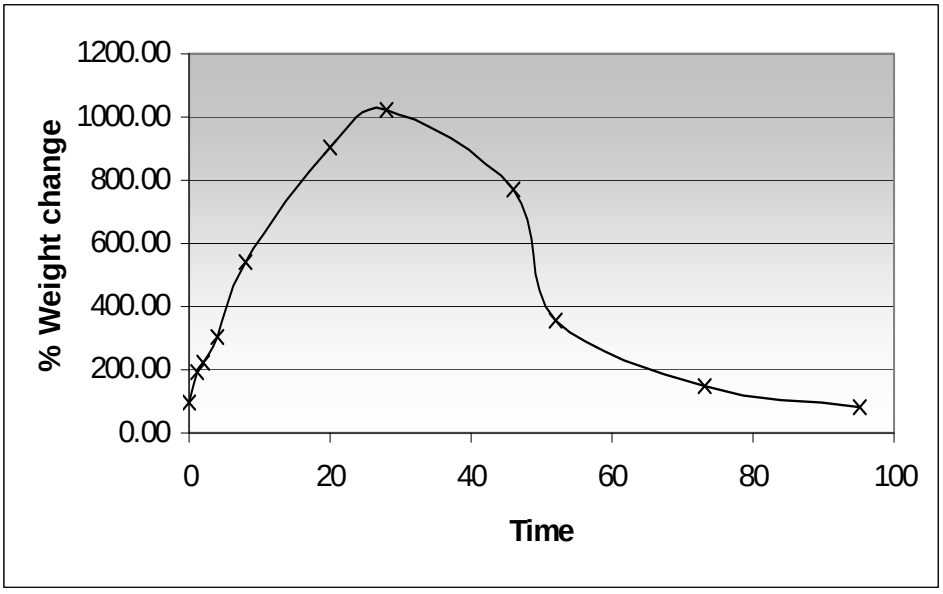
Solubility of sample 80-20 in pH 7 buffered solution



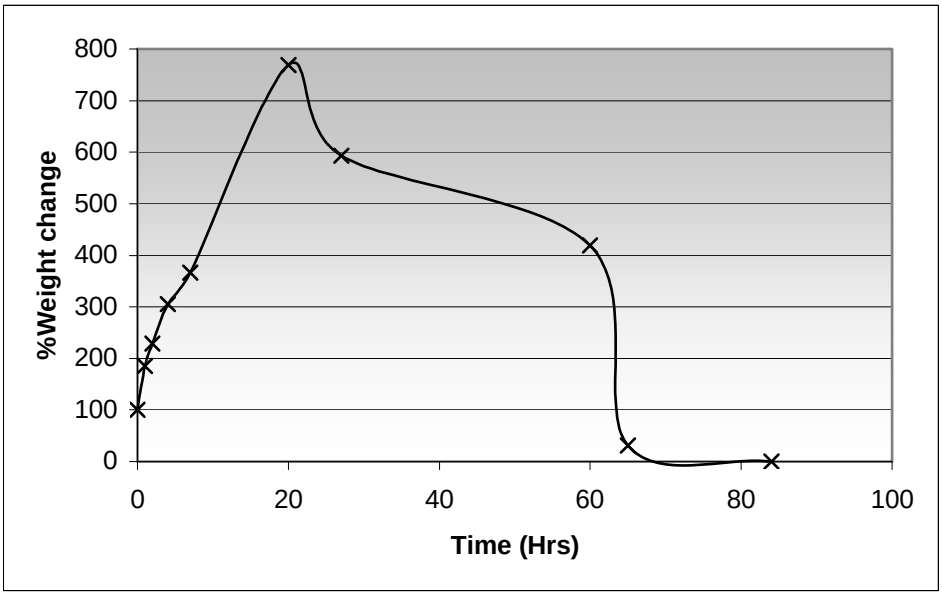
Solubility of sample 80-20 in pH 9 buffered solution



Solubility of sample 70-30 in pH 4 buffered solution



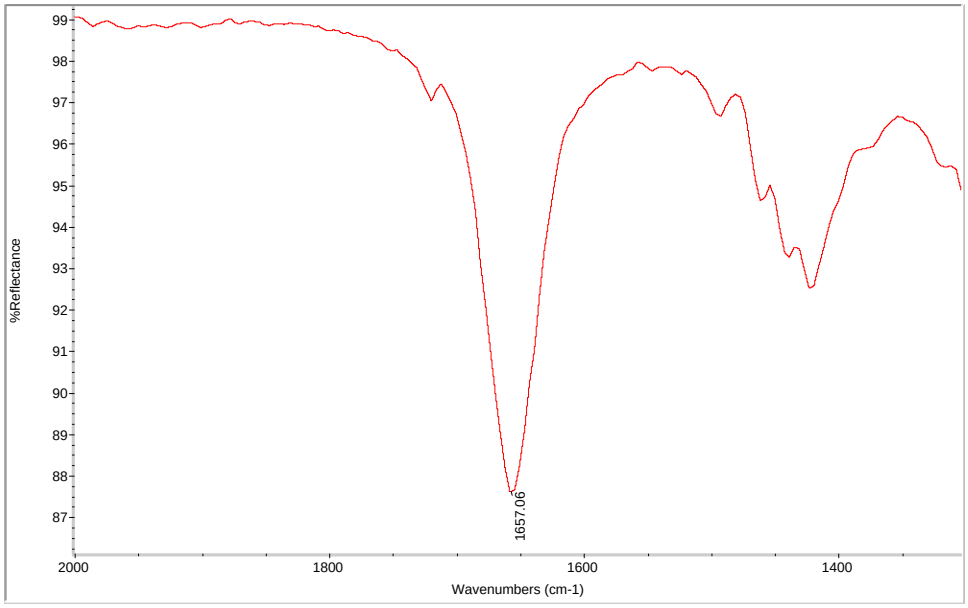
Solubility of sample 70-30 in pH 7 buffered solution



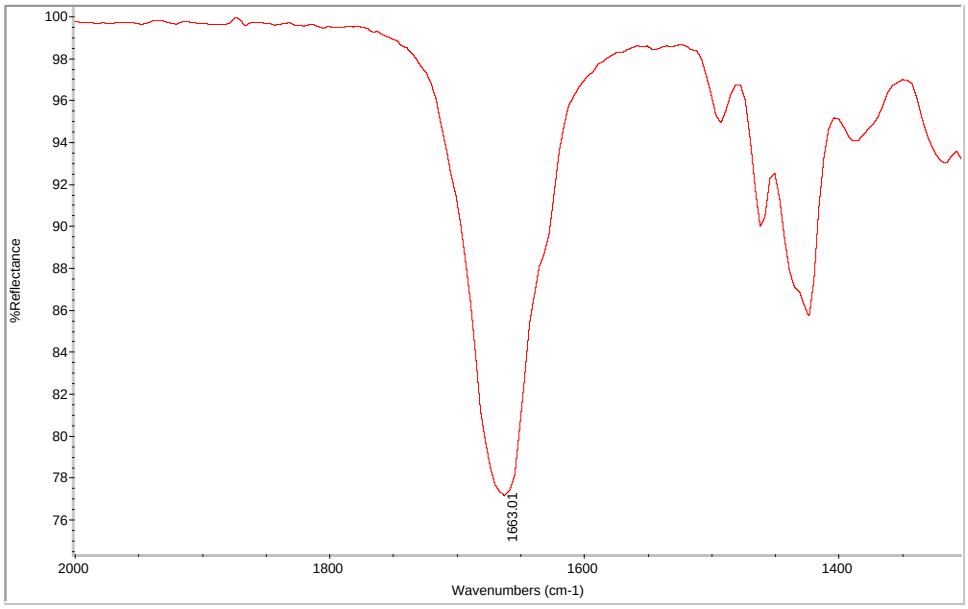
Solubility of sample 70-30 in pH 9 buffered solution

Appendix C

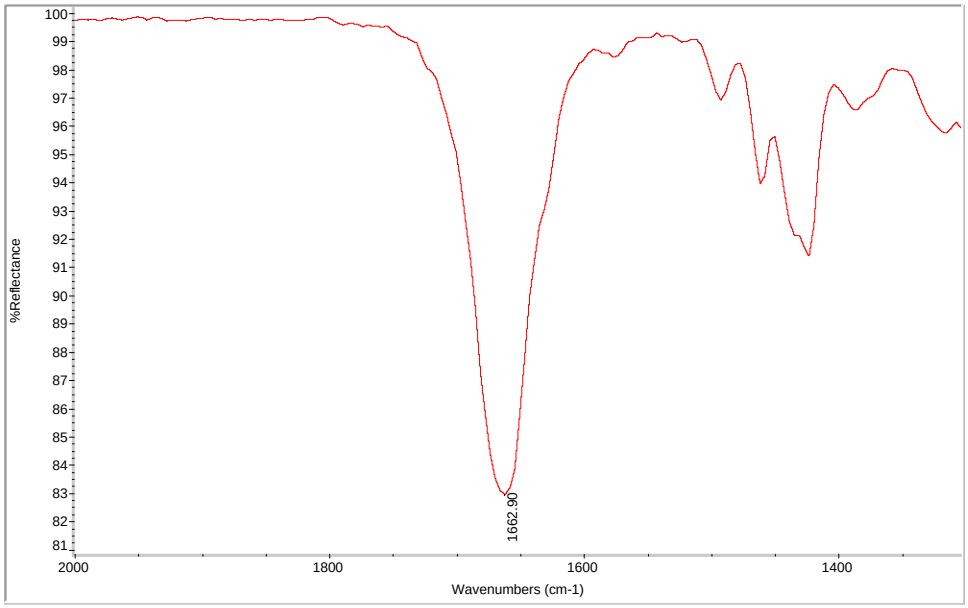
Ftir of chemically crosslinked samples



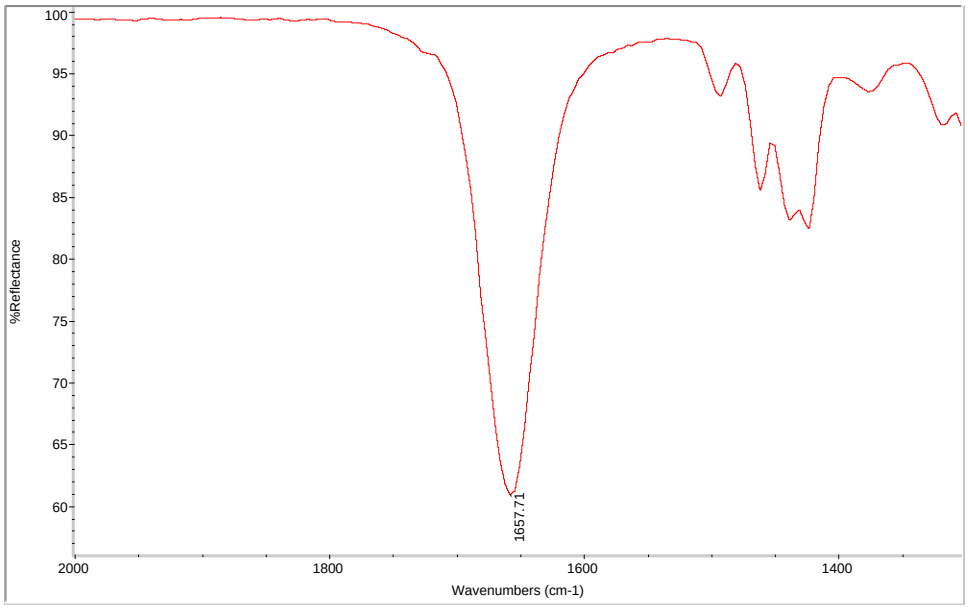
Ftir spectrum of the polymer 100-0, crosslinked with EGDMA.



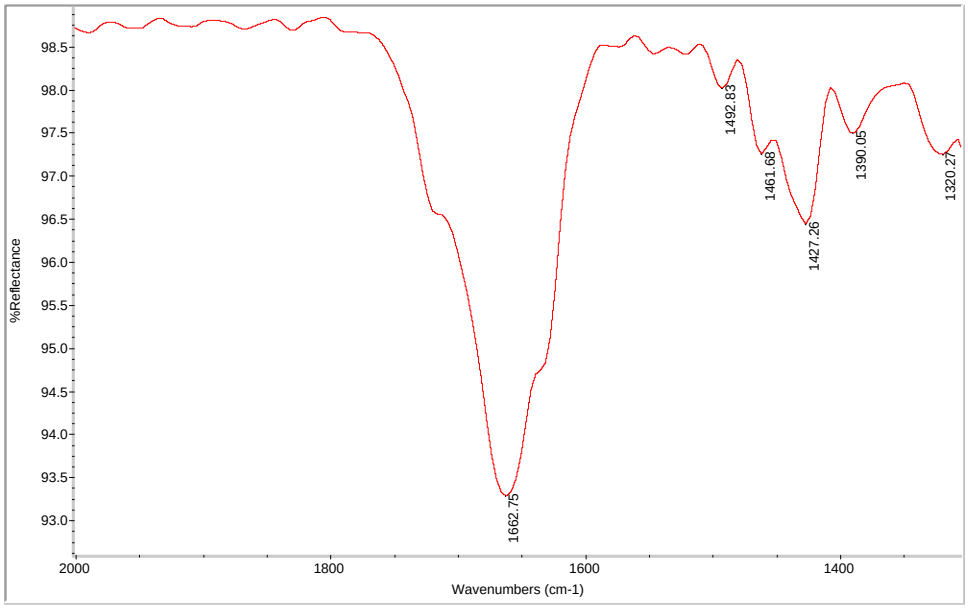
Ftir spectrum of the polymer 100-0, crosslinked with PEG200DMA.



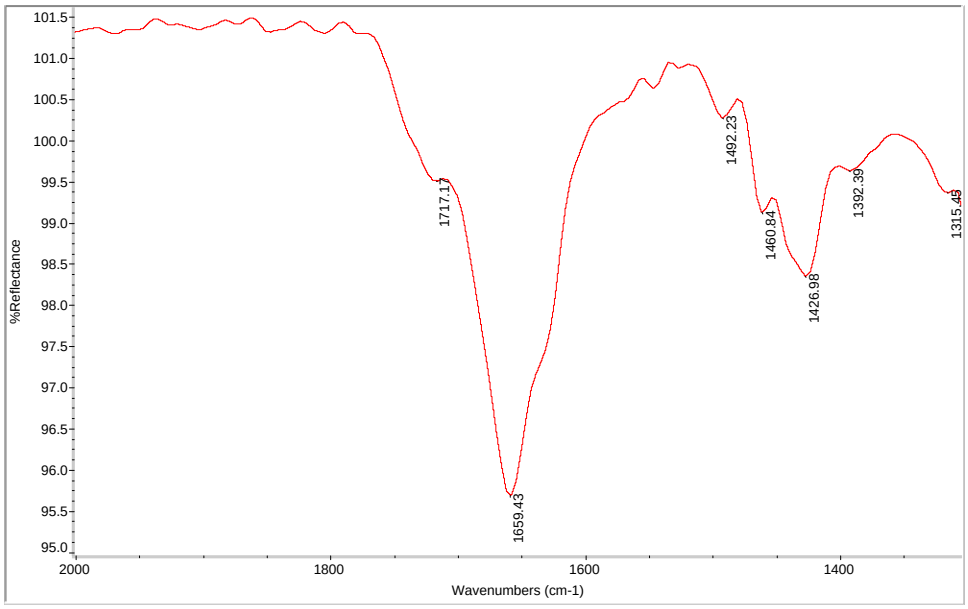
Ftir spectrum of the polymer 100-0, crosslinked with PEG400DMA.



Ftir spectrum of the polymer 100-0, crosslinked with PEG600DMA.



Ftir spectrum of the polymer 90-10, crosslinked with EGDMA.



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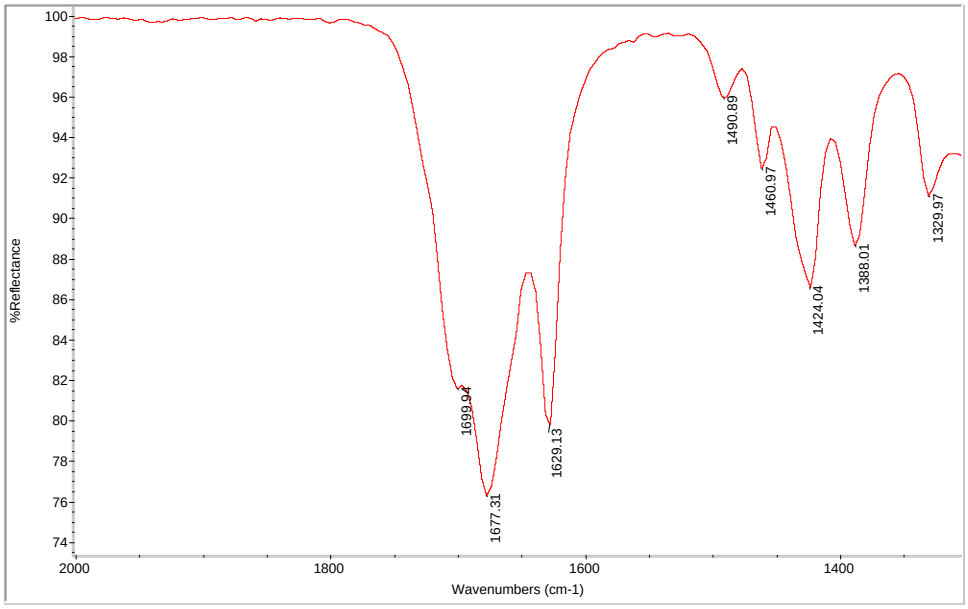
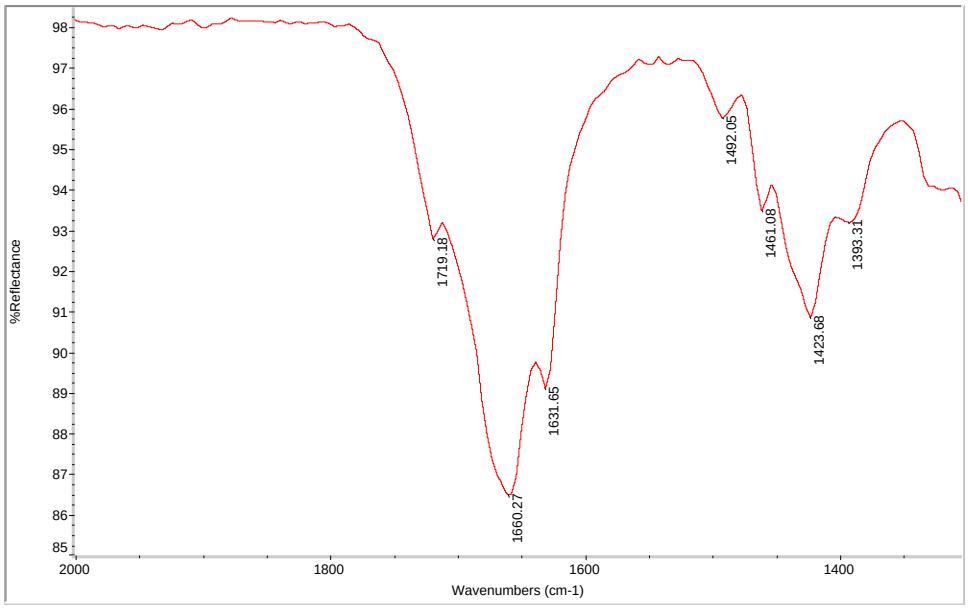
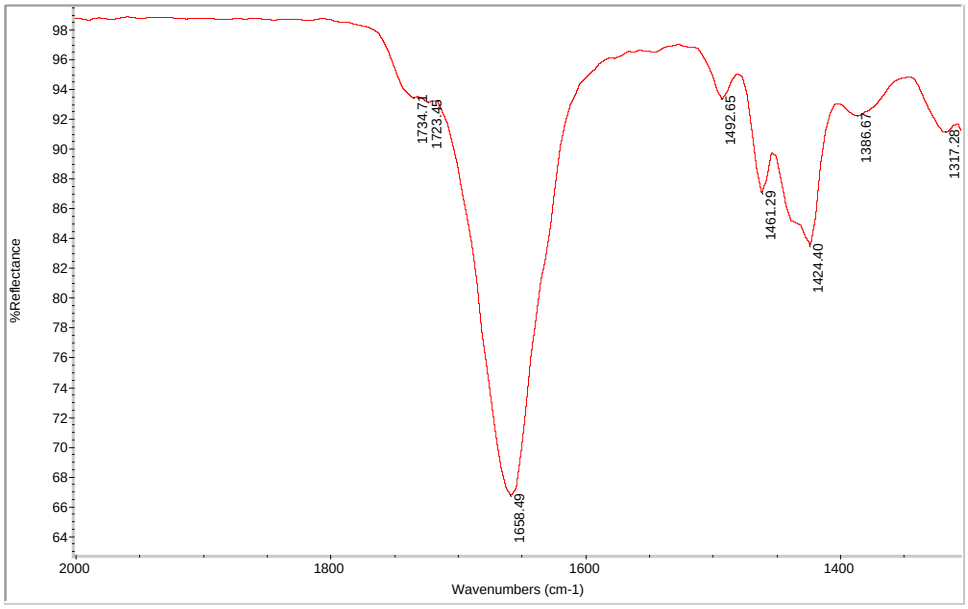


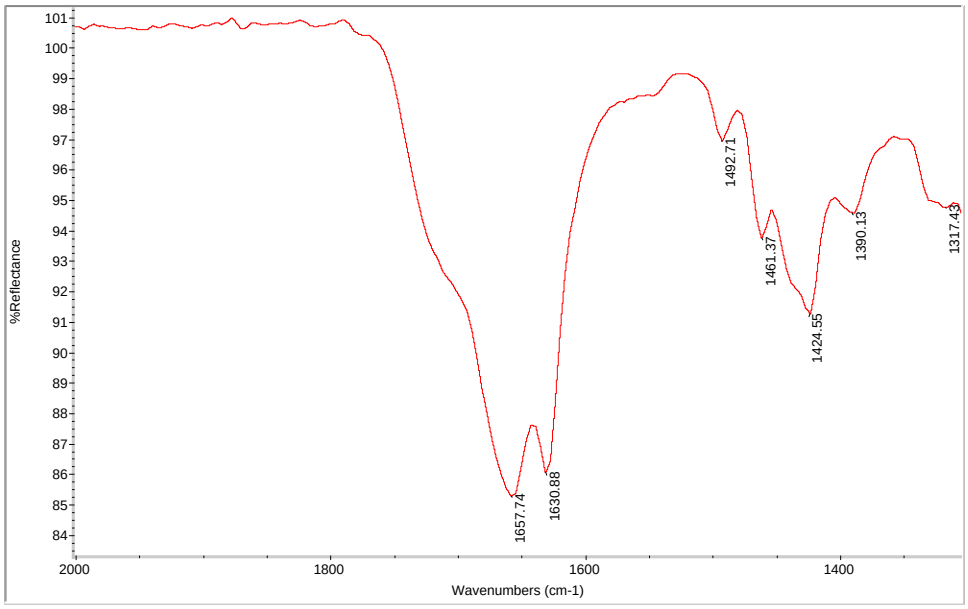
Ftir spectrum of the polymer 90-10, crosslinked with PEG400DMA.



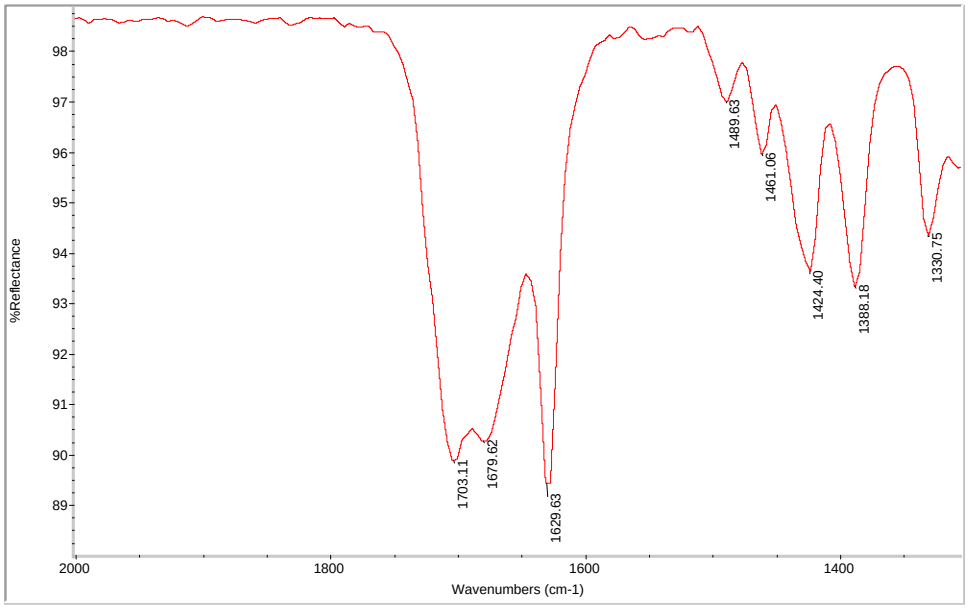
Ftir spectrum of the polymer 90-10, crosslinked with PEG600DMA.



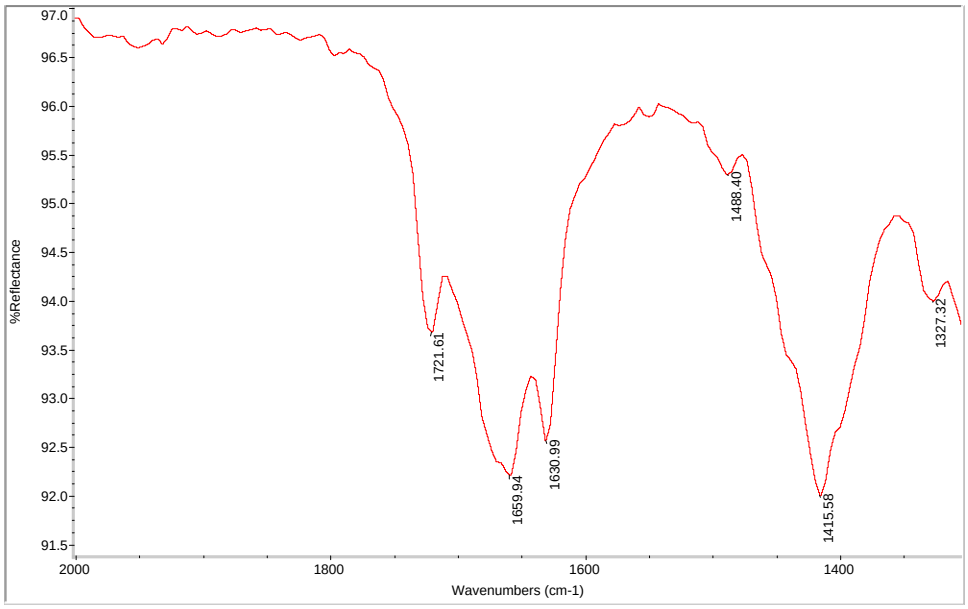
Ftir spectrum of the polymer 80-20, crosslinked with EGDMA.



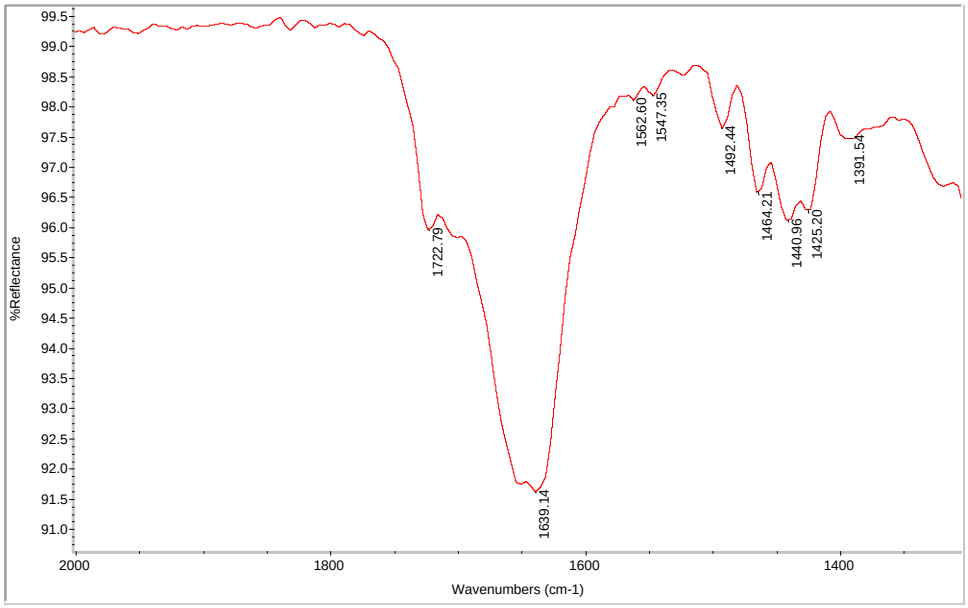
Ftir spectrum of the polymer 80-20, crosslinked with PEG200DMA.



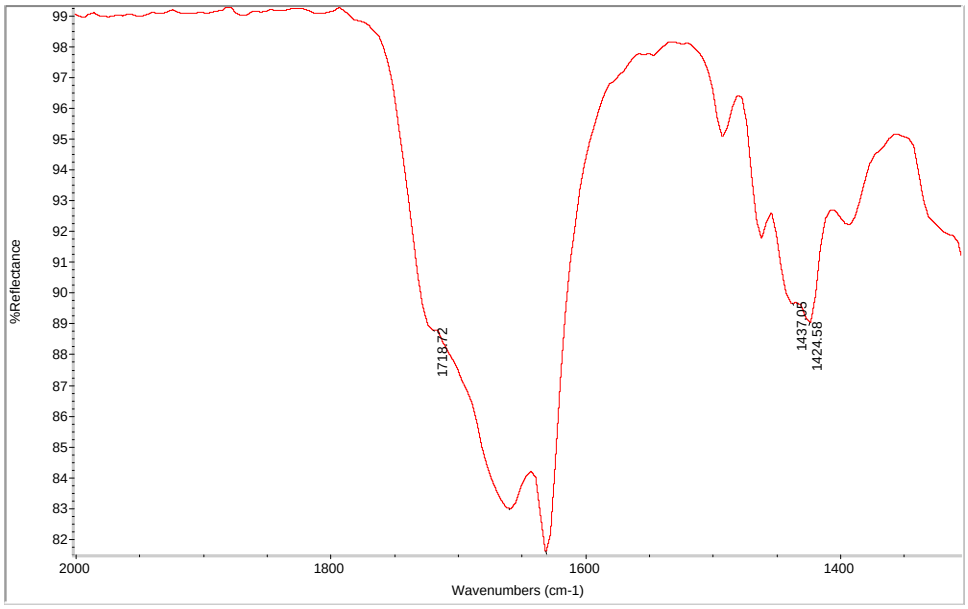
Ftir spectrum of the polymer 80-20, crosslinked with PEG400DMA.



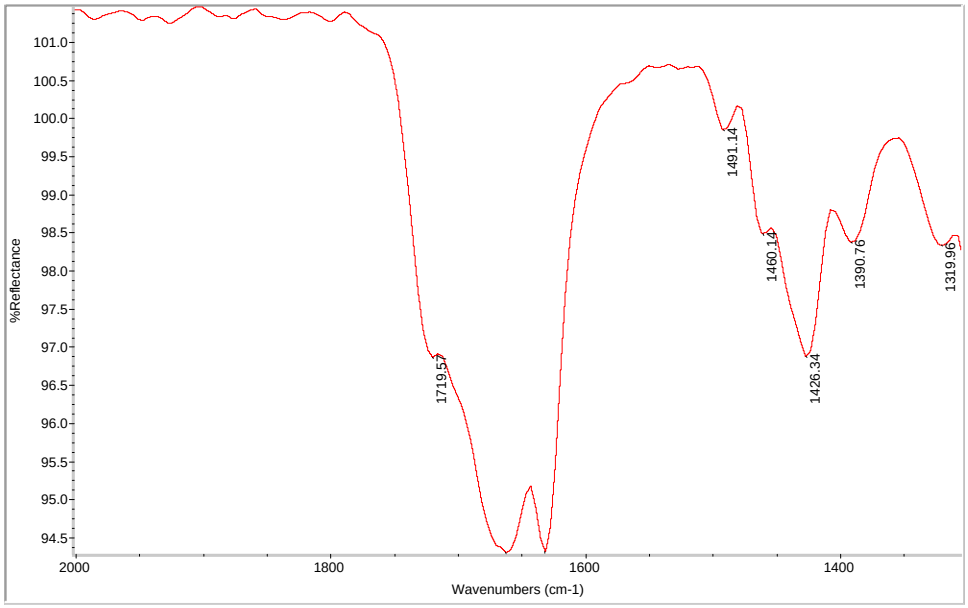
Ftir spectrum of the polymer 80-20, crosslinked with PEG600DMA.



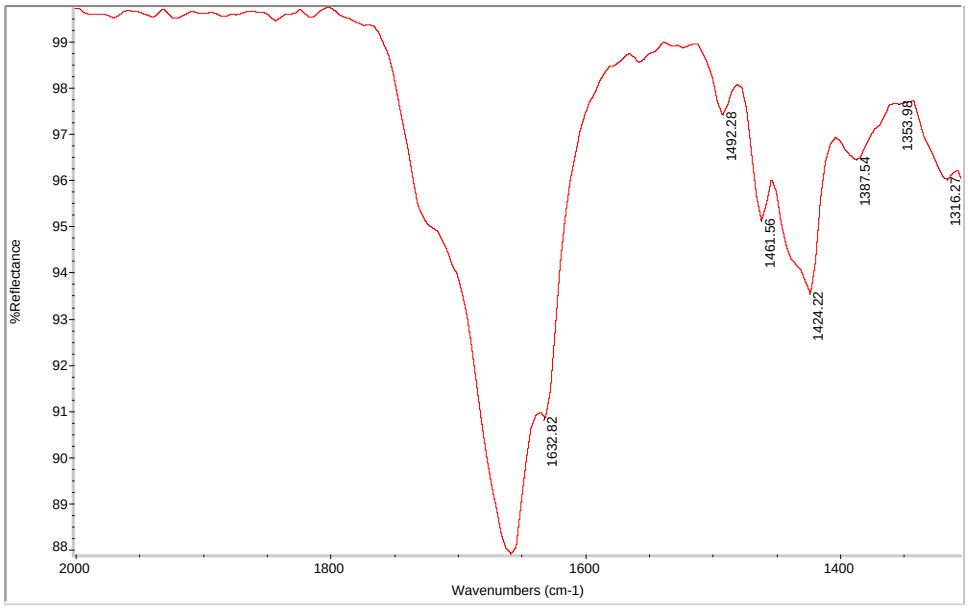
Ftir spectrum of the polymer 70-30, crosslinked with EGDMA.



Ftir spectrum of the polymer 70-30, crosslinked with PEG200DMA.



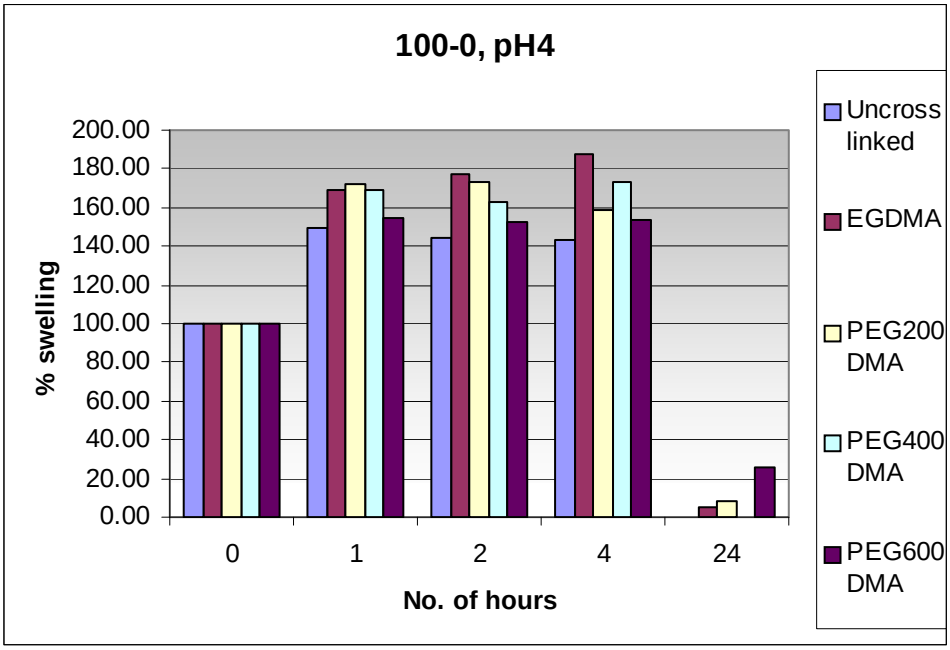
Ftir spectrum of the polymer 70-30, crosslinked with PEG400DMA.



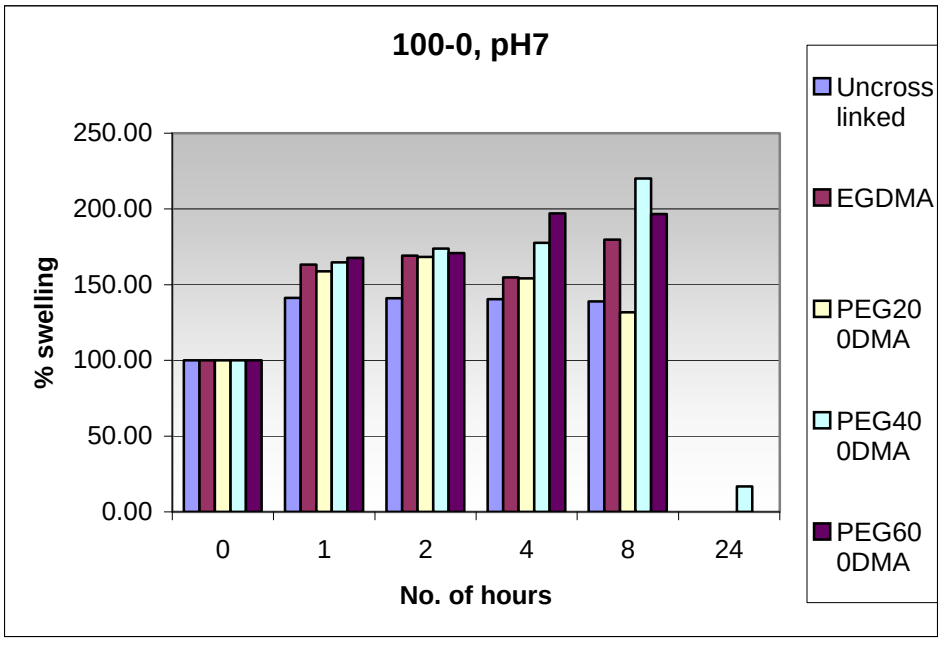
Ftir spectrum of the polymer 70-30, crosslinked with PEG600DMA.

Appendix D

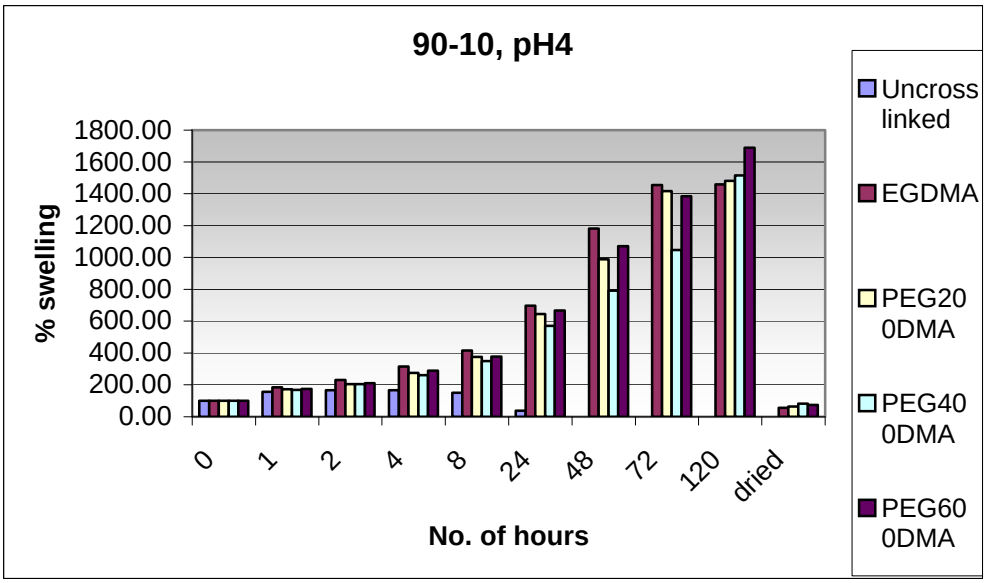
Swelling of chemically crosslinked samples



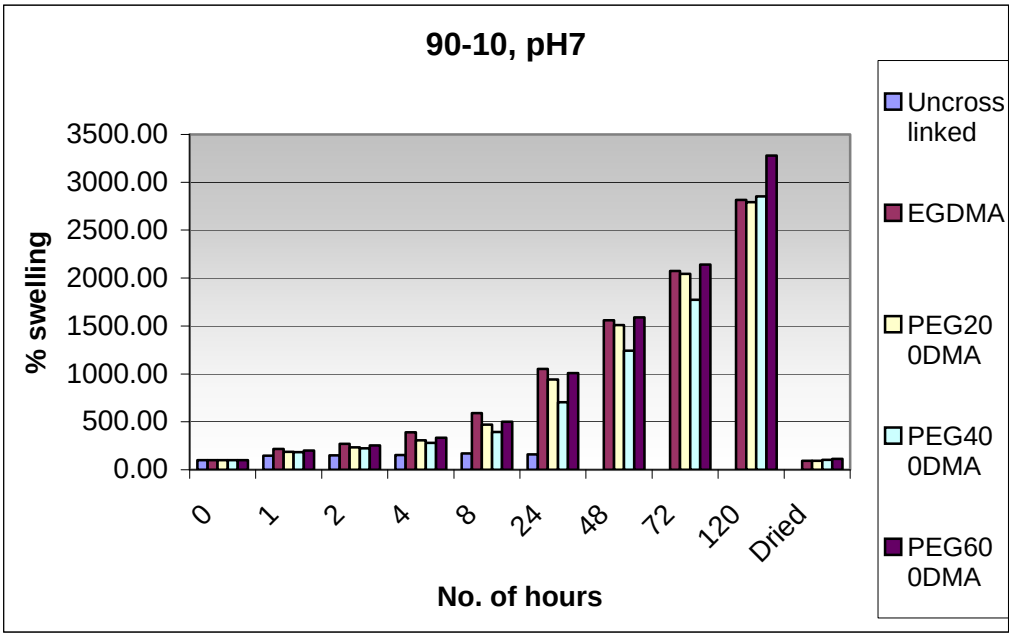
Swelling of 100-0 crosslinked samples in pH4 buffered solution



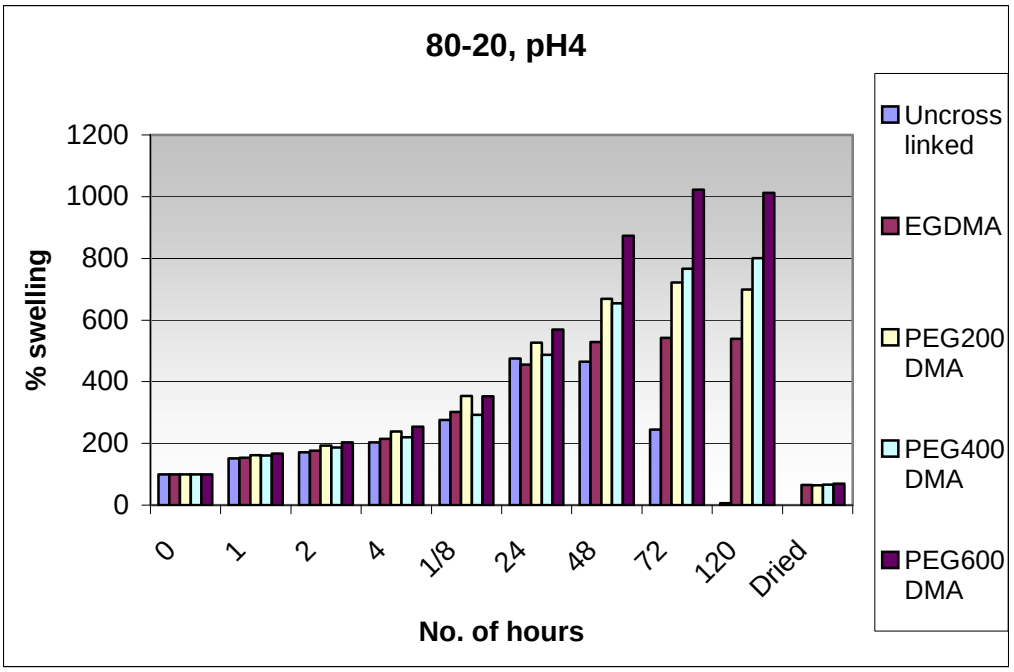
Swelling of 100-0 crosslinked samples in pH7 buffered solution



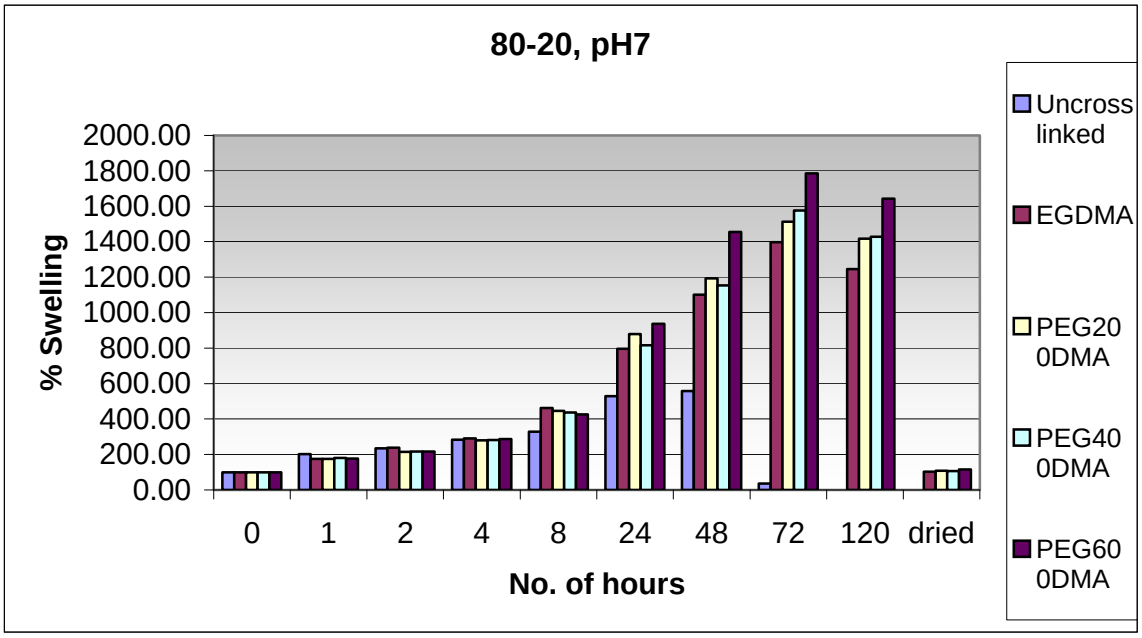
Swelling of 90-10 crosslinked samples in pH4 buffered solution



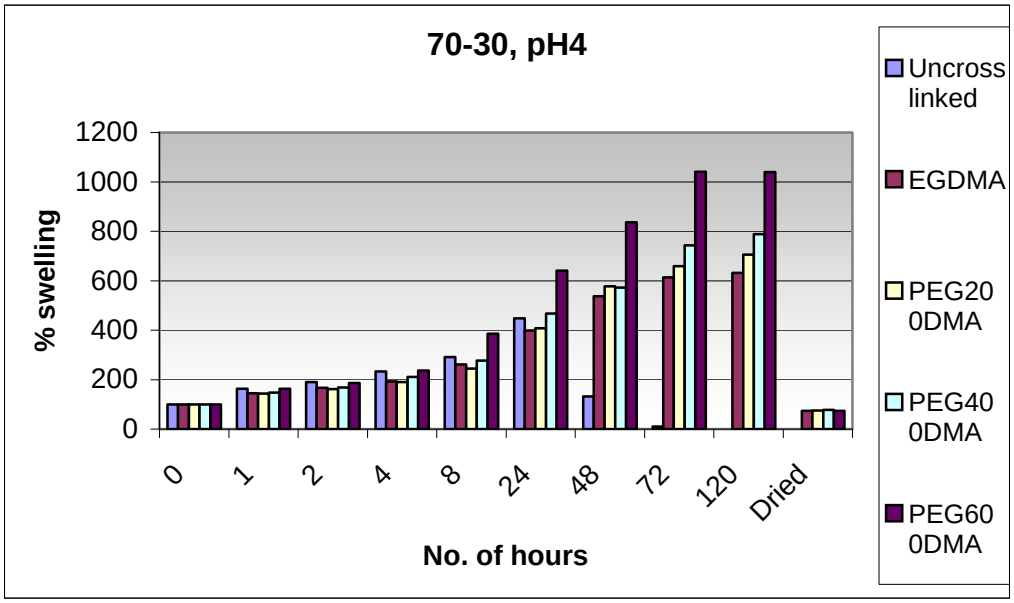
Swelling of 90-10 crosslinked samples in pH7 buffered solution



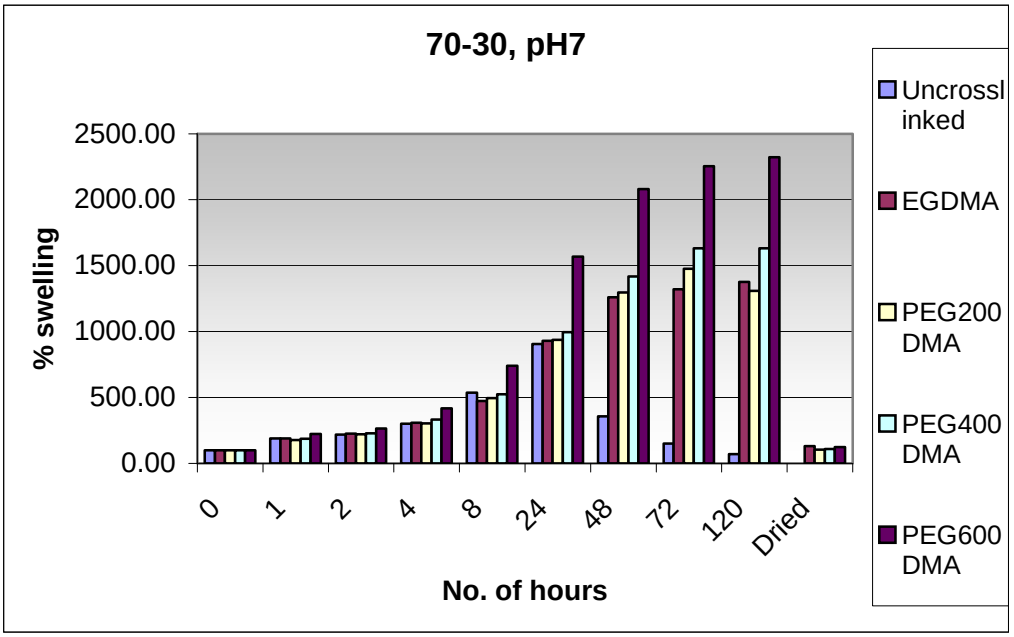
Swelling of 80-20 crosslinked samples in pH4 buffered solution



Swelling of 80-20 crosslinked samples in pH7 buffered solution



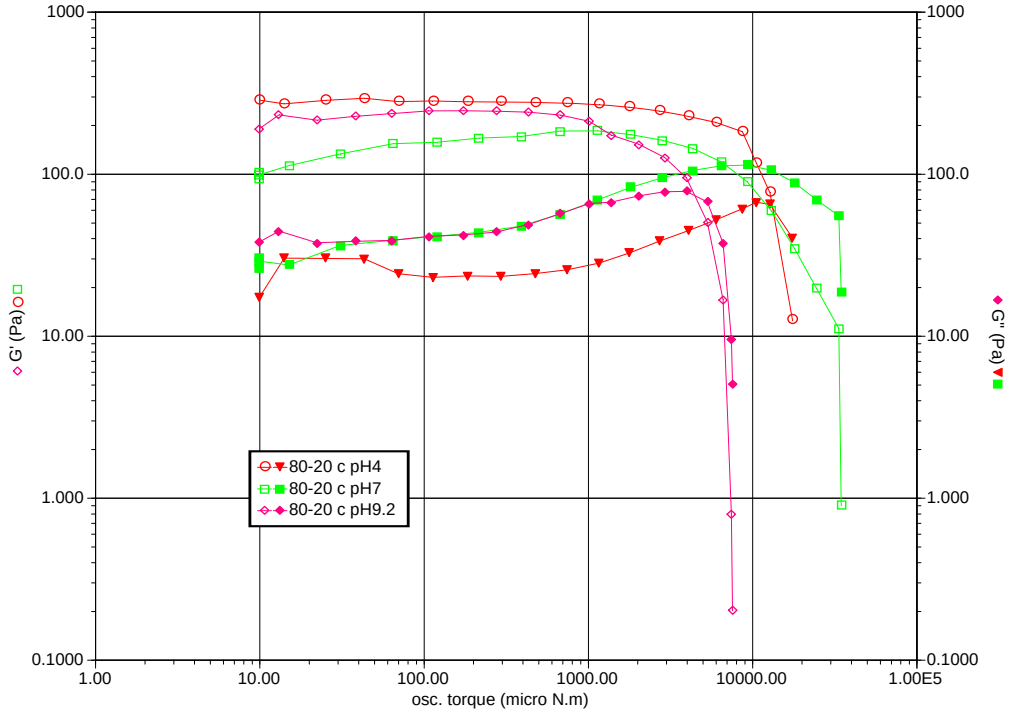
Swelling of 70-30 crosslinked samples in pH4 buffered solution



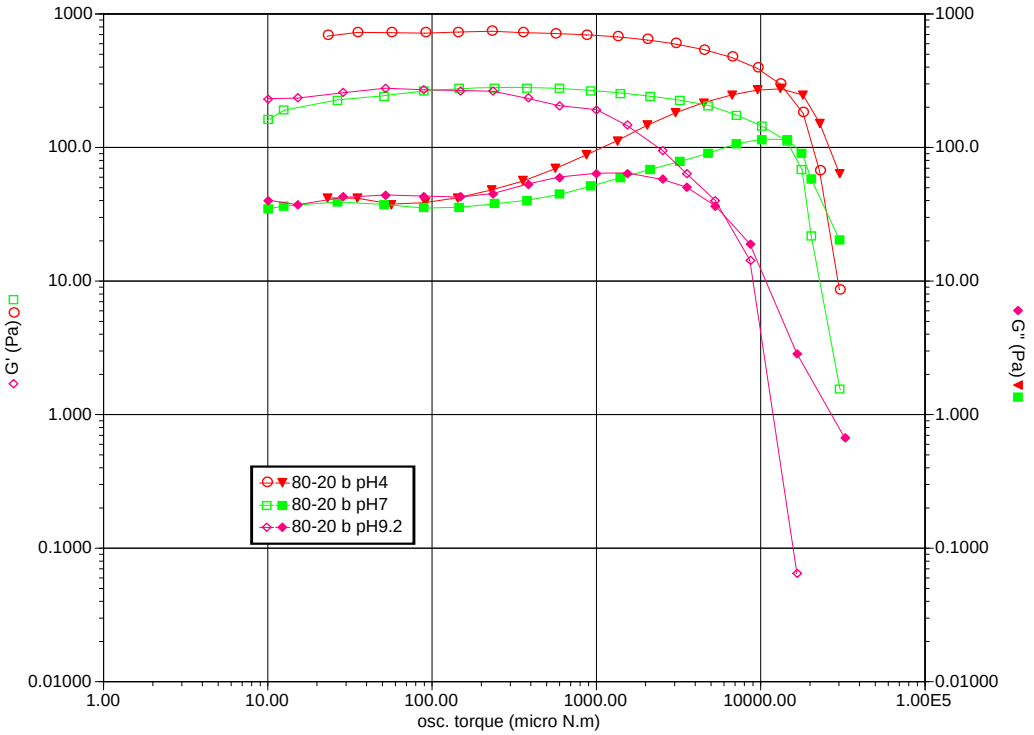
Swelling of 70-30 crosslinked samples in pH7 buffered solution

Appendix E

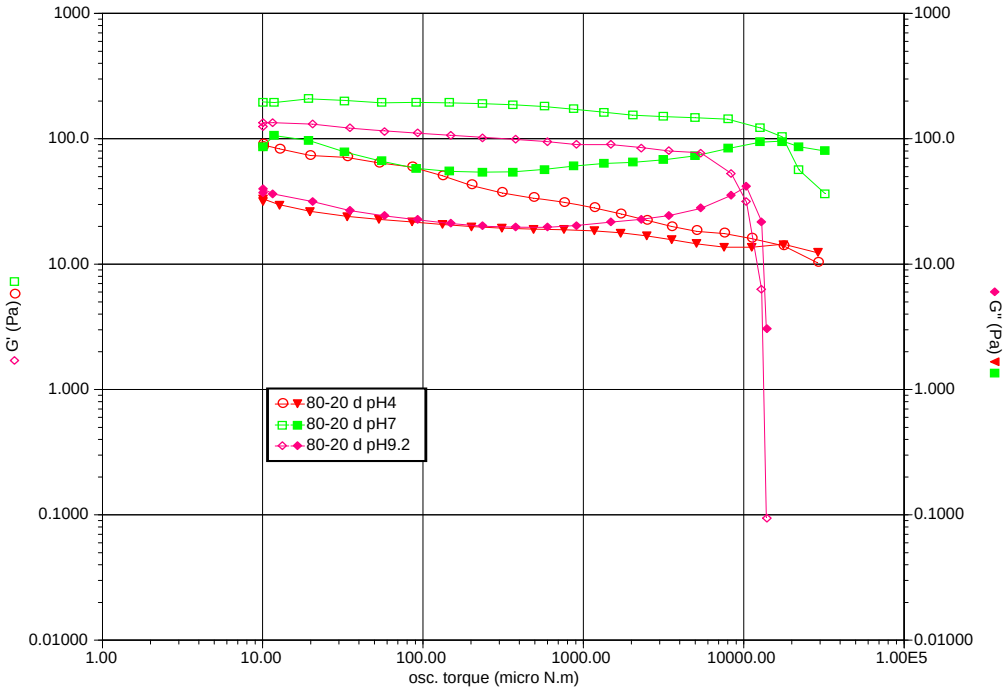
Parallel plate rheometry results



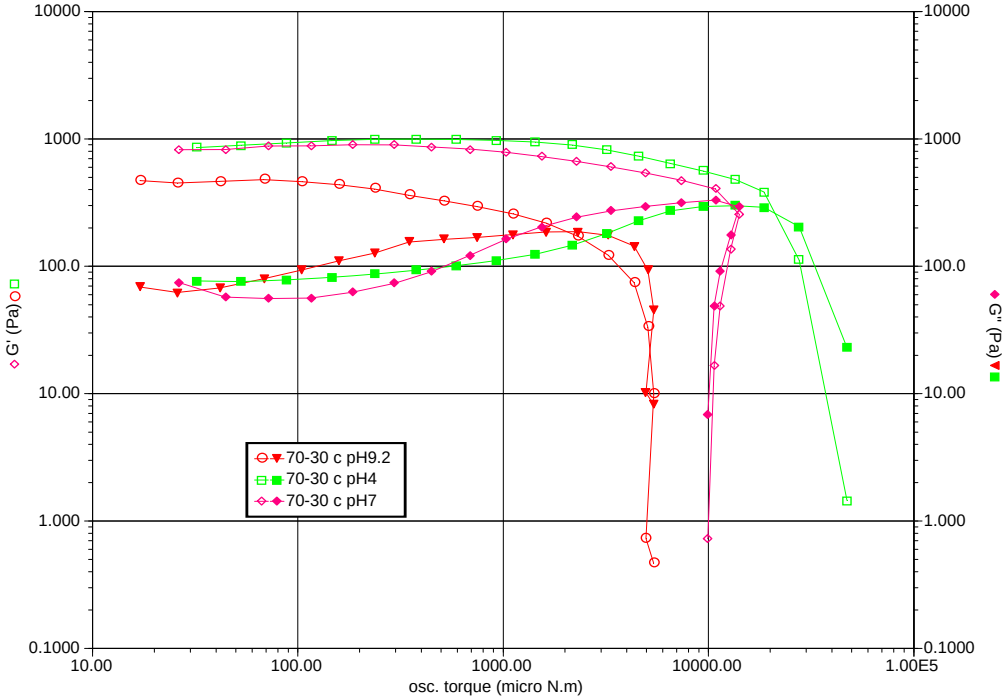
Rheometric data for the 80-20 hydrogel crosslinked with PEG200DMA.



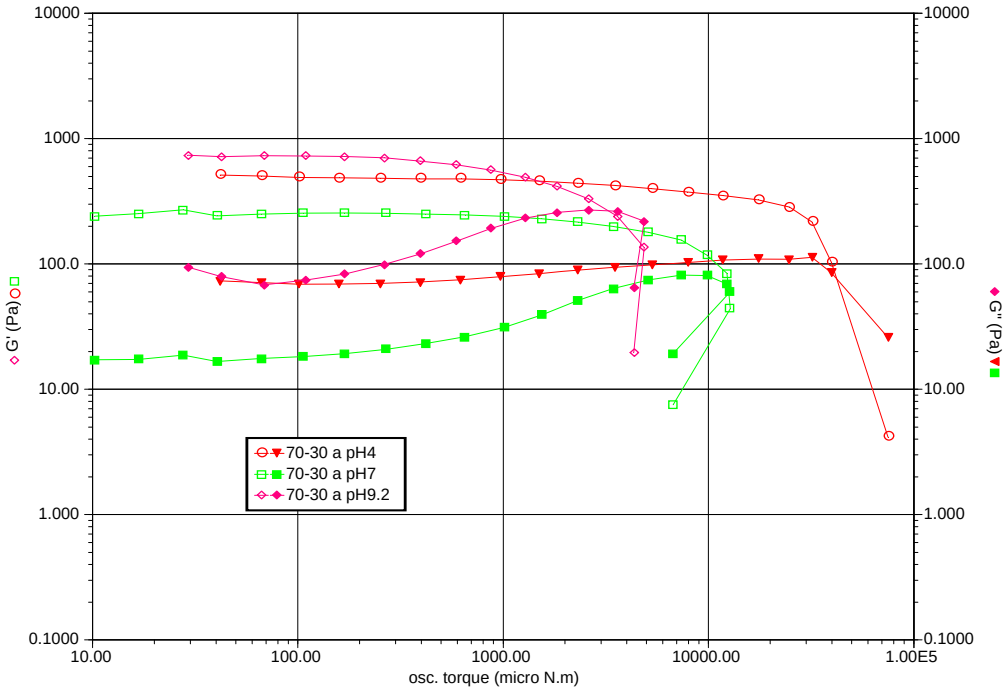
Rheometric data for the 80-20 hydrogel crosslinked with PEG400DMA.



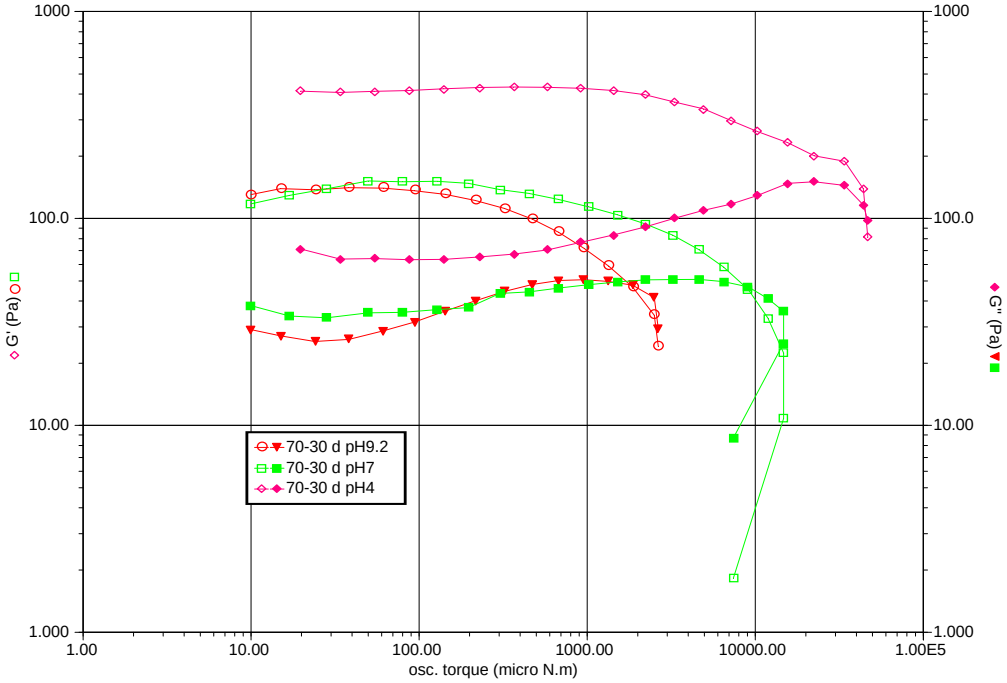
Rheometric data for the 80-20 hydrogel crosslinked with PEG600DMA.



Rheometric data for the 70-30 hydrogel crosslinked with PEG200DMA.



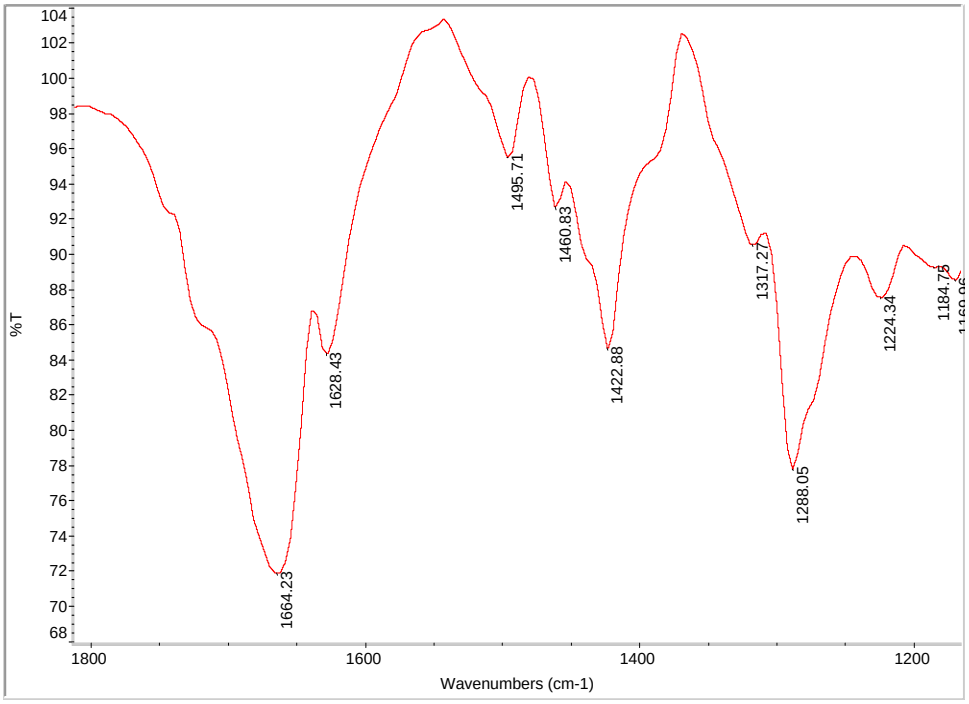
Rheometric data for the 70-30 hydrogel crosslinked with PEG400DMA.



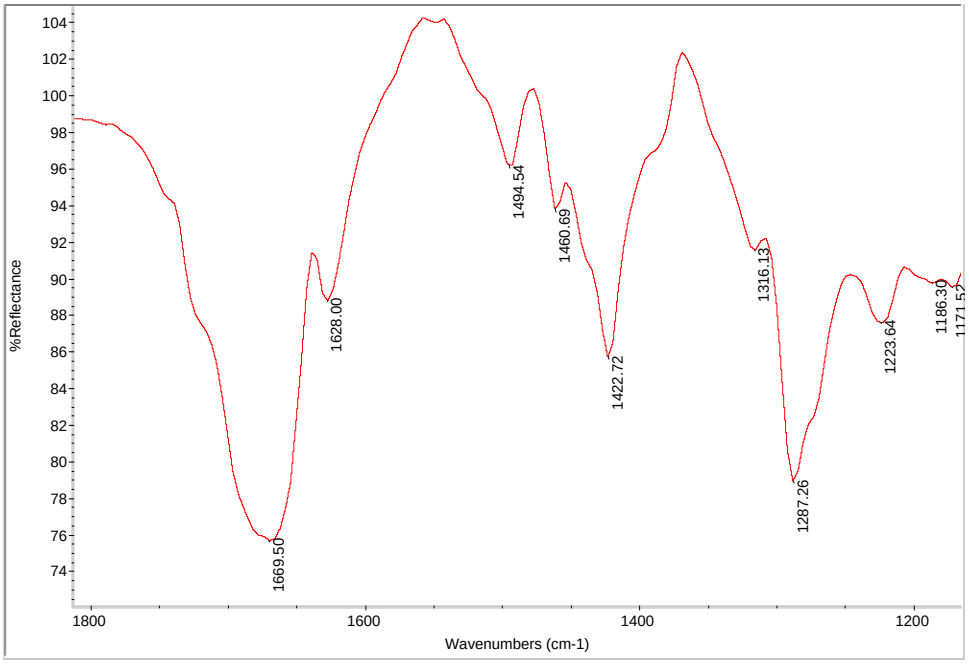
Rheometric data for the 70-30 hydrogel crosslinked with PEG600DMA

Appendix F

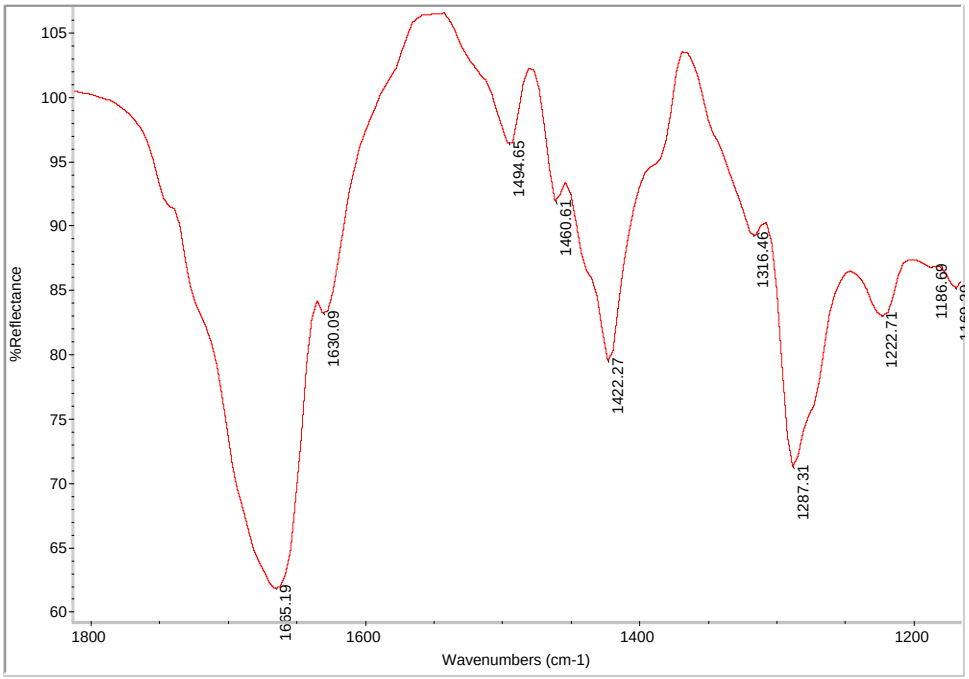
Ftir spectra of PVP/PAA copolymer coating



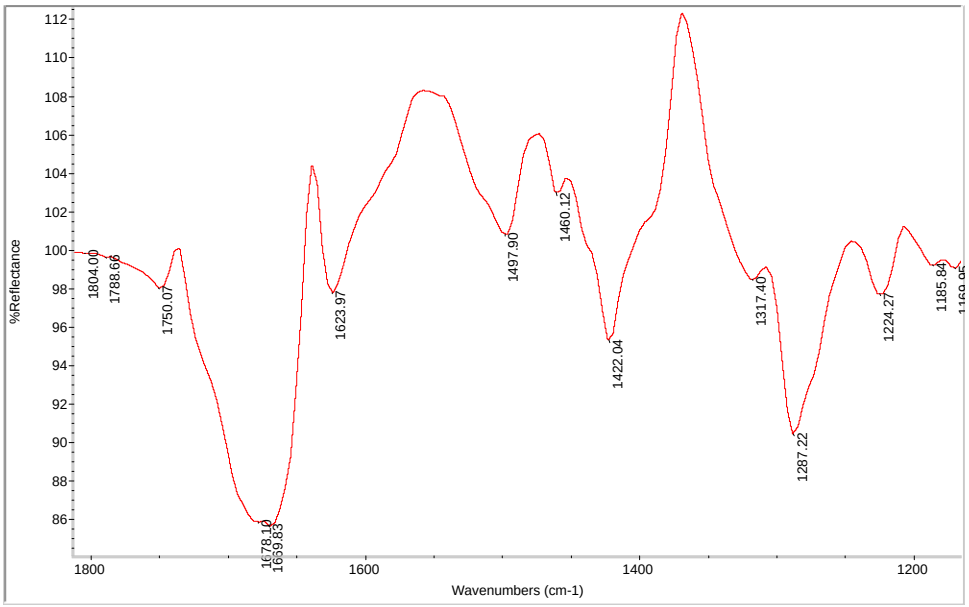
Coating containing EGDMA crosslinking agent, 1 coating cycle



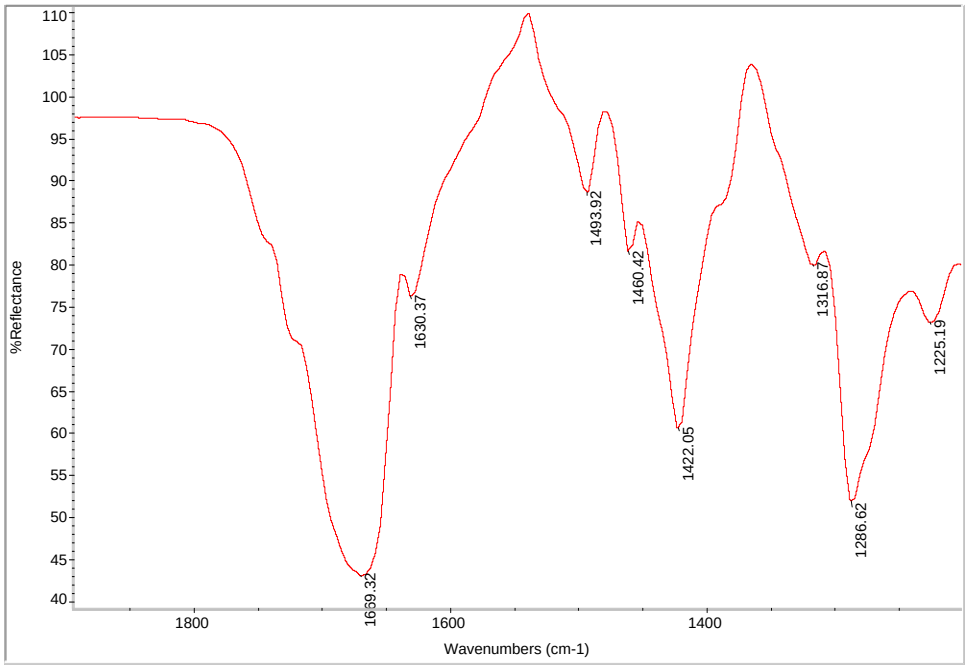
Coating containing EGDMA crosslinking agent, 2 coating cycles



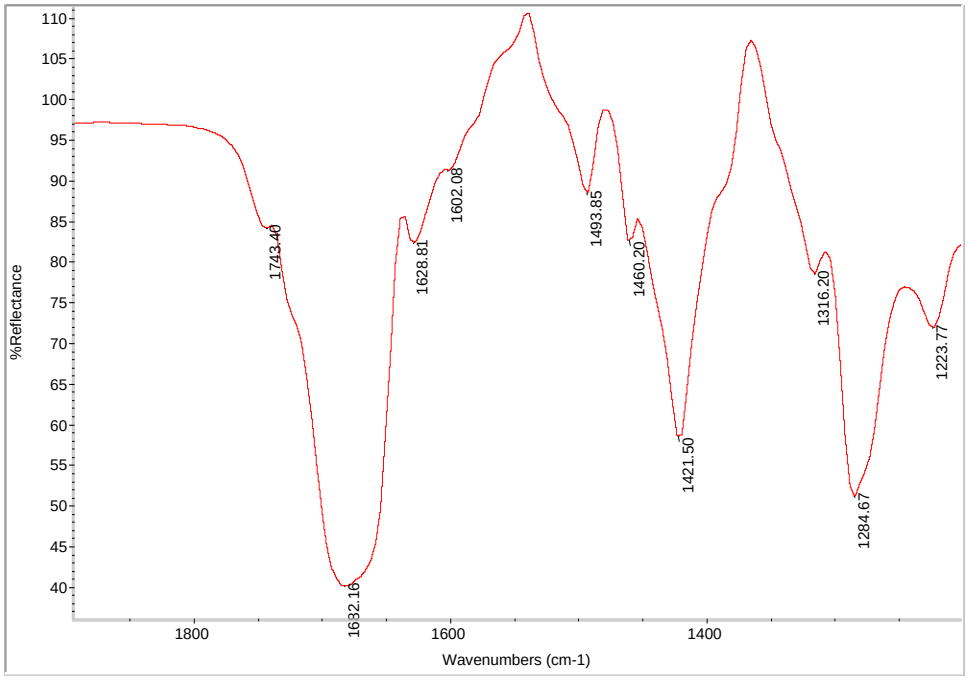
Coating containing EGDMA crosslinking agent, 3 coating cycles



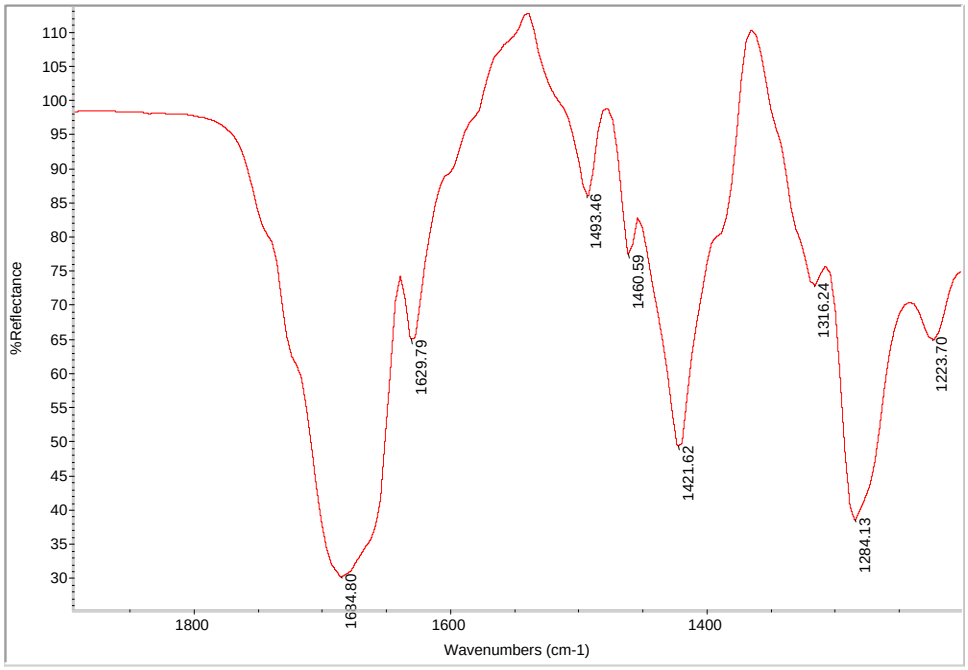
Coating containing EGDMA crosslinking agent, 4 coating cycles



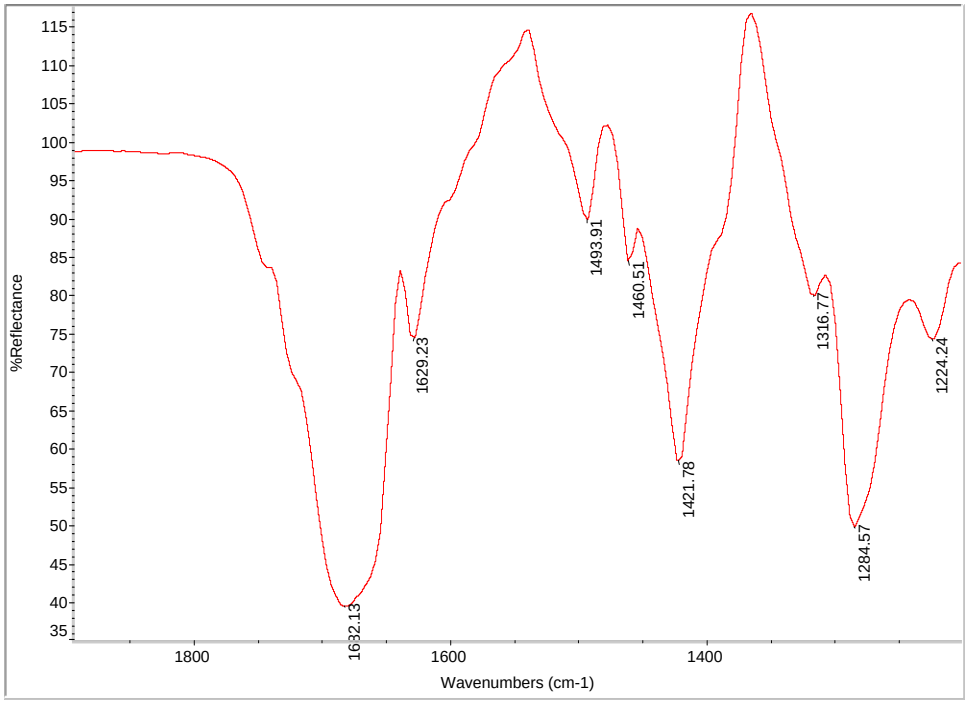
Coating containing PEG600DMA crosslinking agent, 1 coating cycle



Coating containing PEG600DMA crosslinking agent, 2 coating cycles



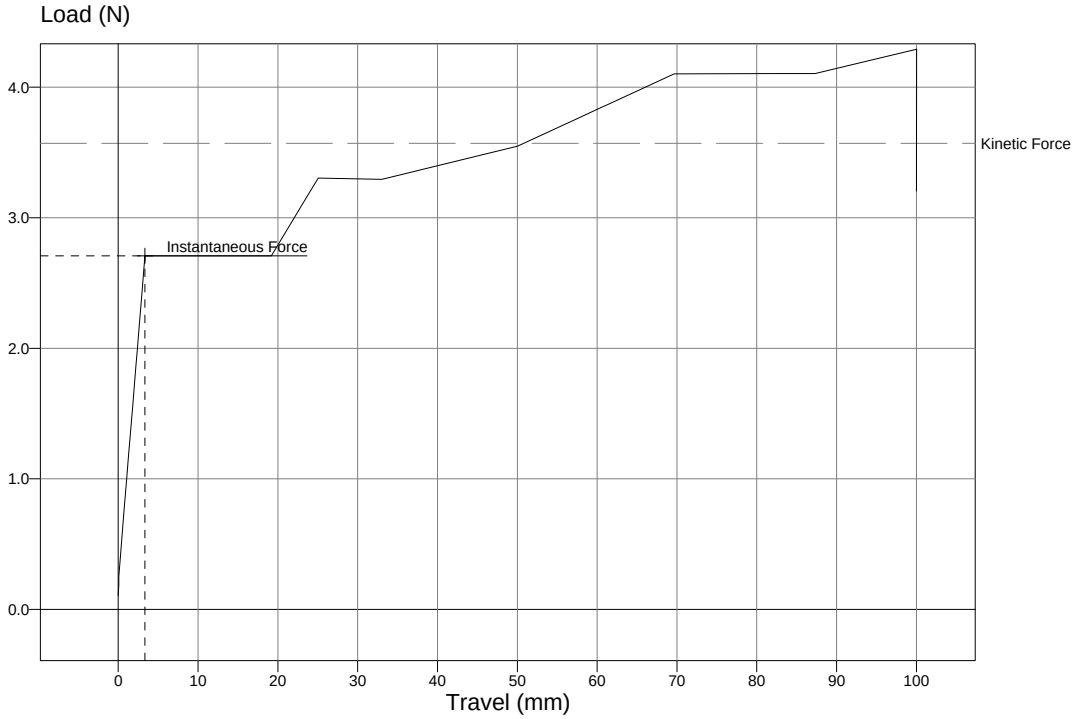
Coating containing PEG600DMA crosslinking agent, 3 coating cycles



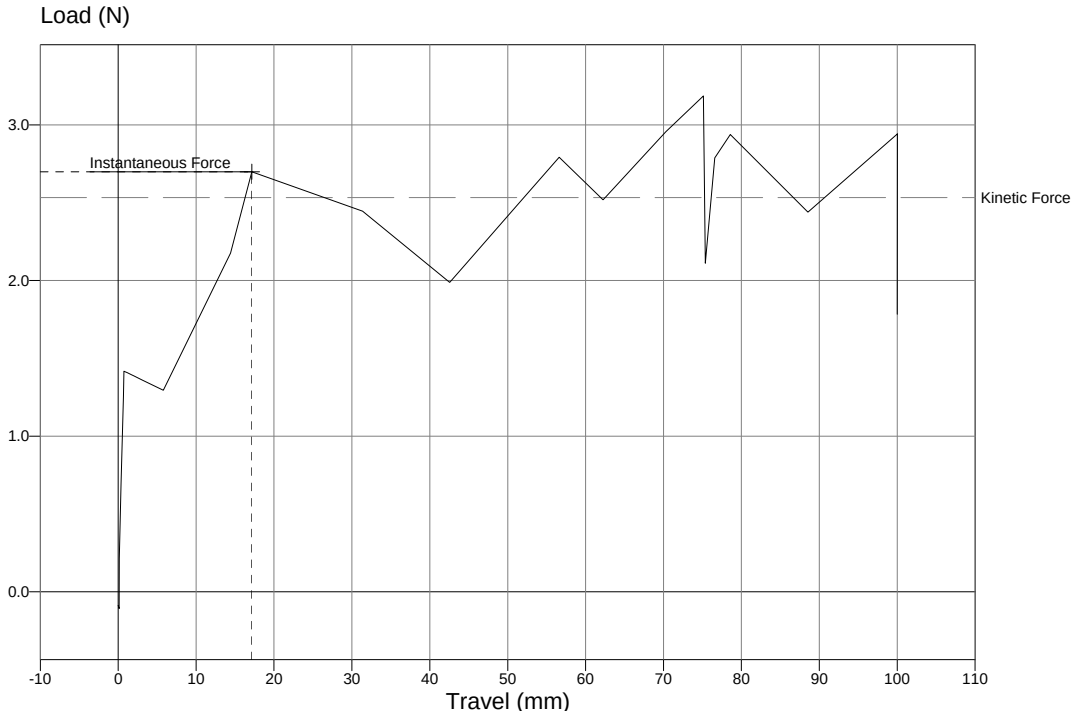
Coating containing PEG600DMA crosslinking agent, 4 coating cycles

Appendix G

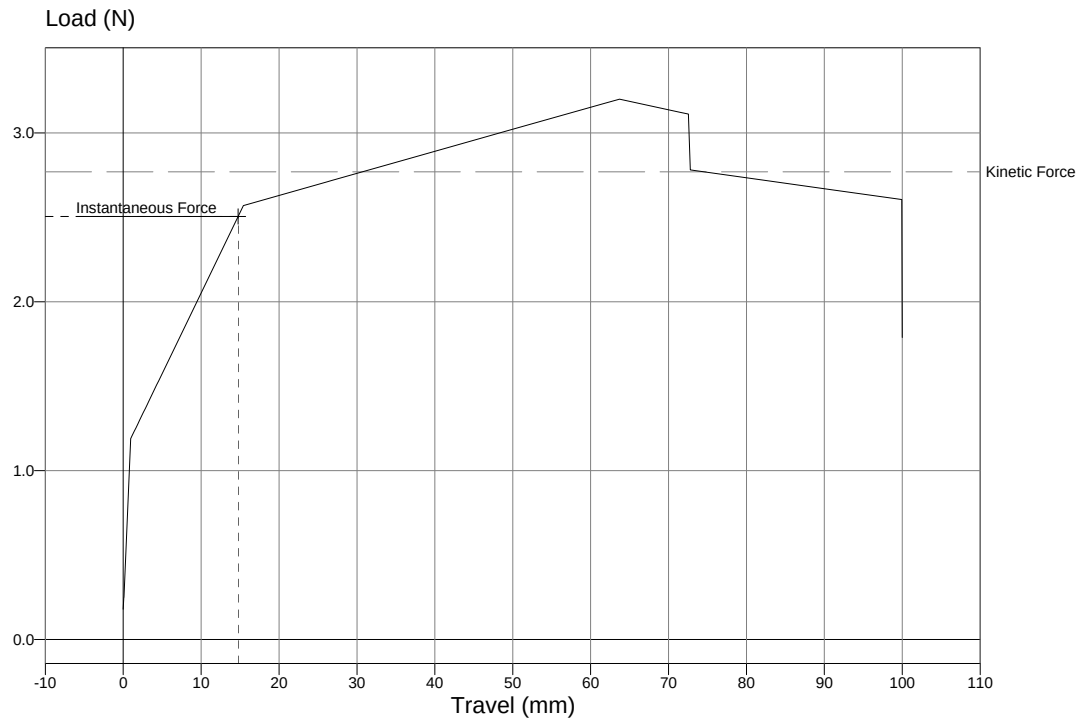
Friction results



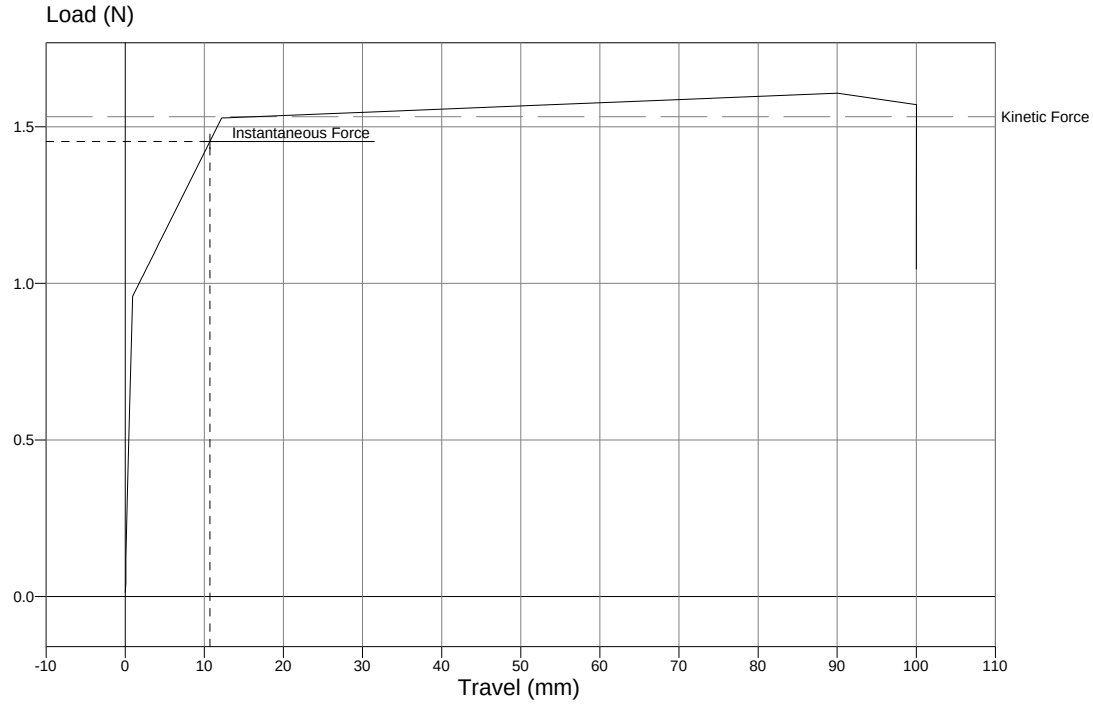
Frictional analysis of coated Pebax ® 3533, using EGDMA as crosslinking agent, after 5 minutes submerged in pH7 buffered solution.



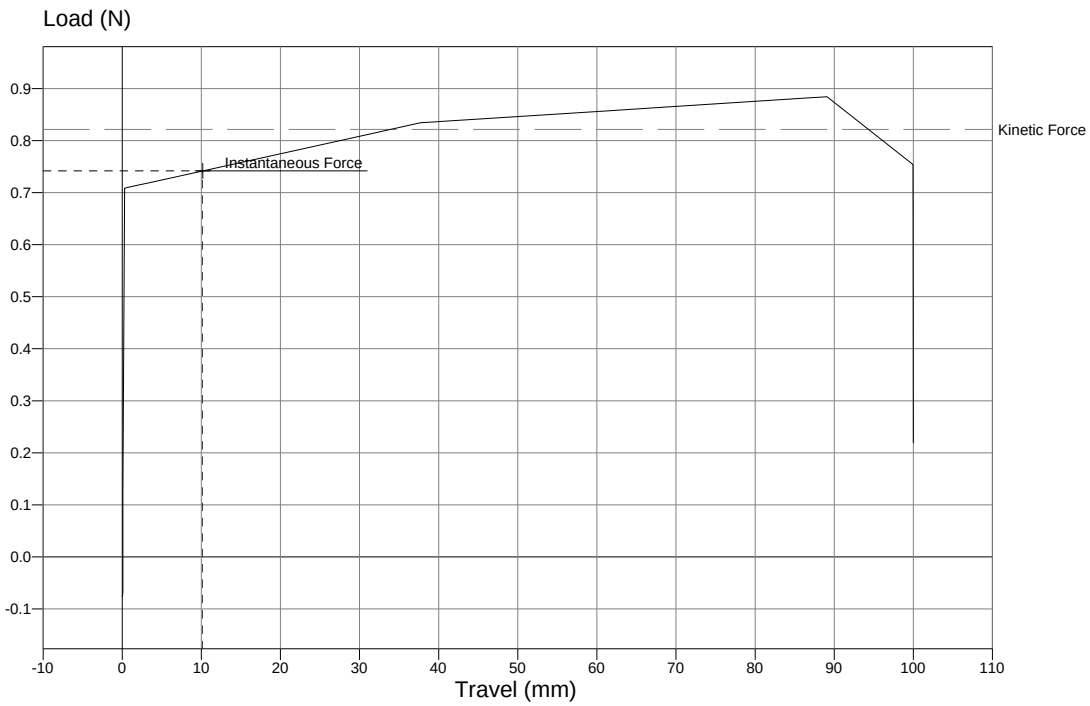
Frictional analysis of coated Pebax ® 3533, using EGDMA as crosslinking agent, after 10 minutes submerged in pH7 buffered solution.



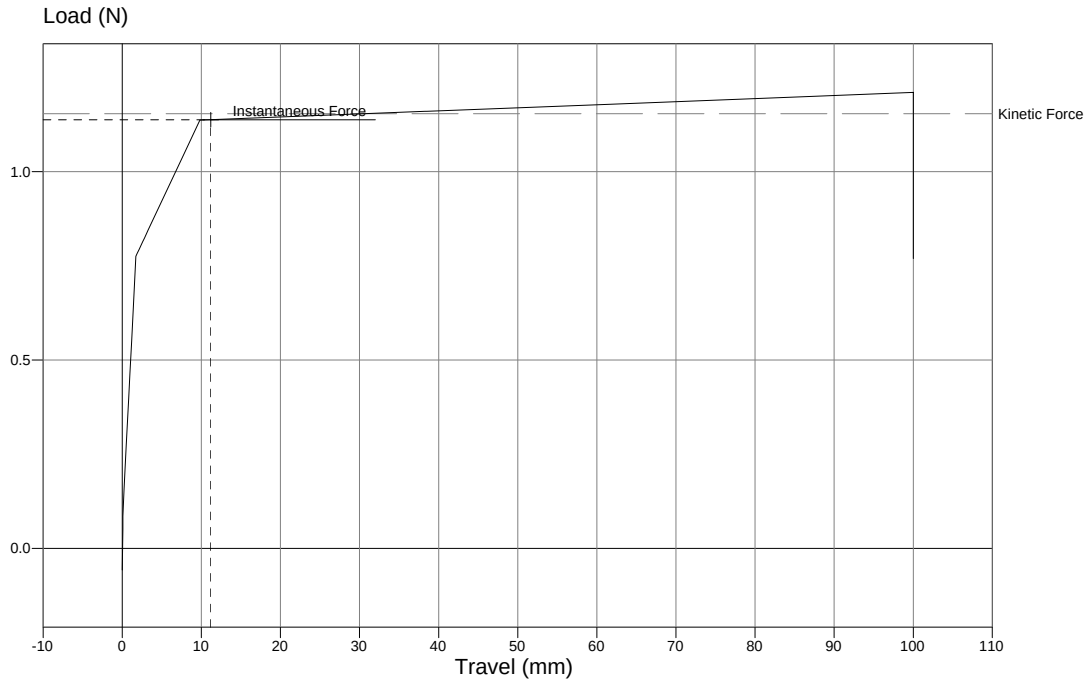
Frictional analysis of coated Pebax ® 3533, using EGDMA as crosslinking agent, after 15 minutes submerged in pH7 buffered solution.



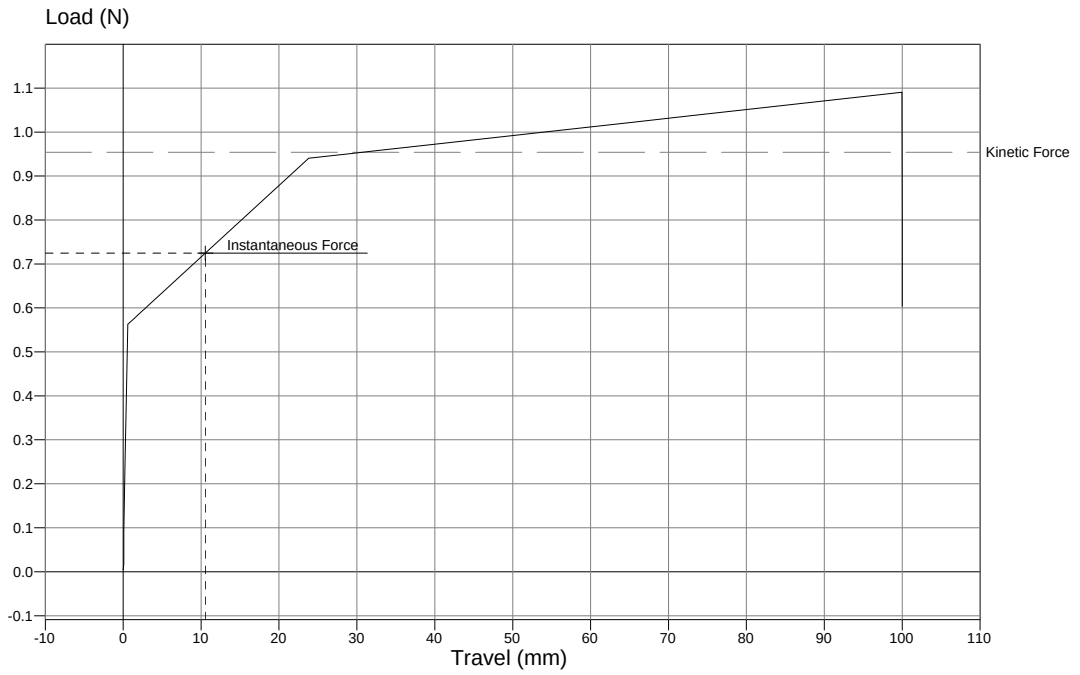
Frictional analysis of coated Pebax ® 3533, using EGDMA as crosslinking agent, after 25 *minutes submerged in pH7 buffered solution.*



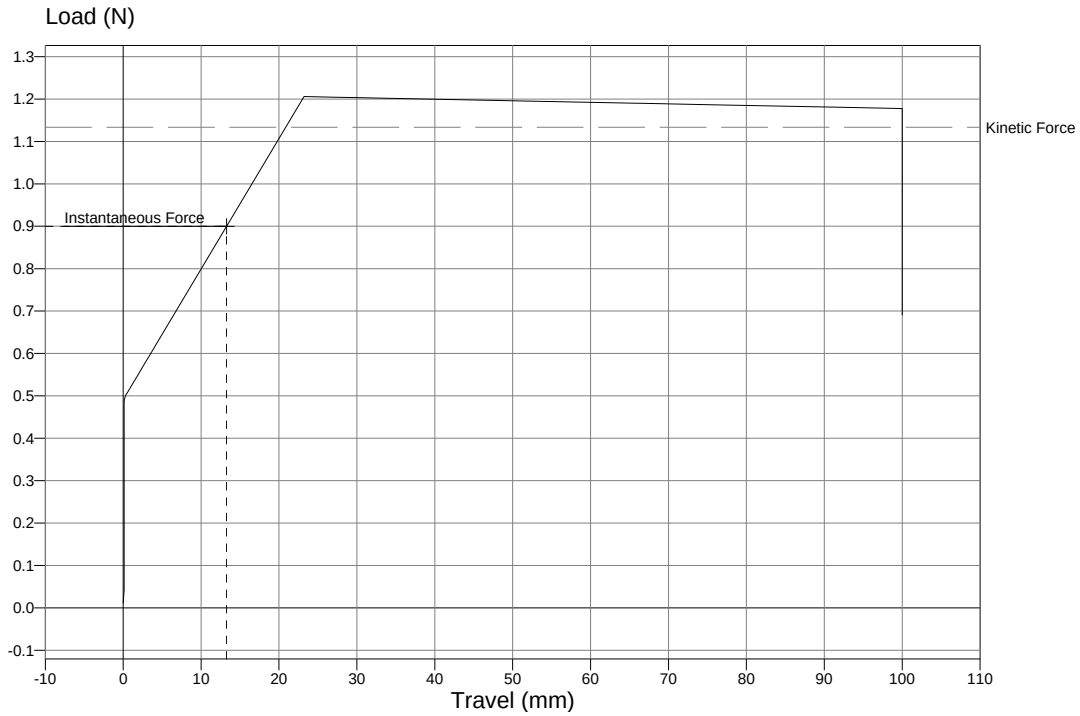
Frictional analysis of coated Pebax ® 3533, using EGDMA as crosslinking agent, after 30 *minutes submerged in pH7 buffered solution.*



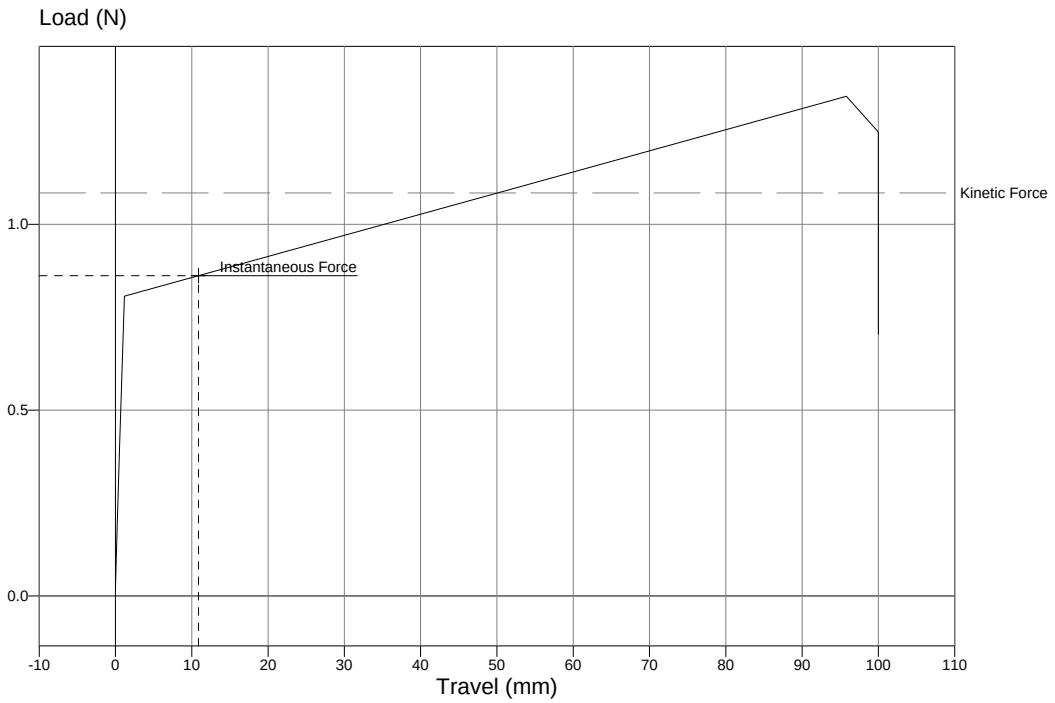
Frictional analysis of coated Pebax ® 3533, using EGDMA as crosslinking agent, after 35 *minutes submerged in pH7 buffered solution.*



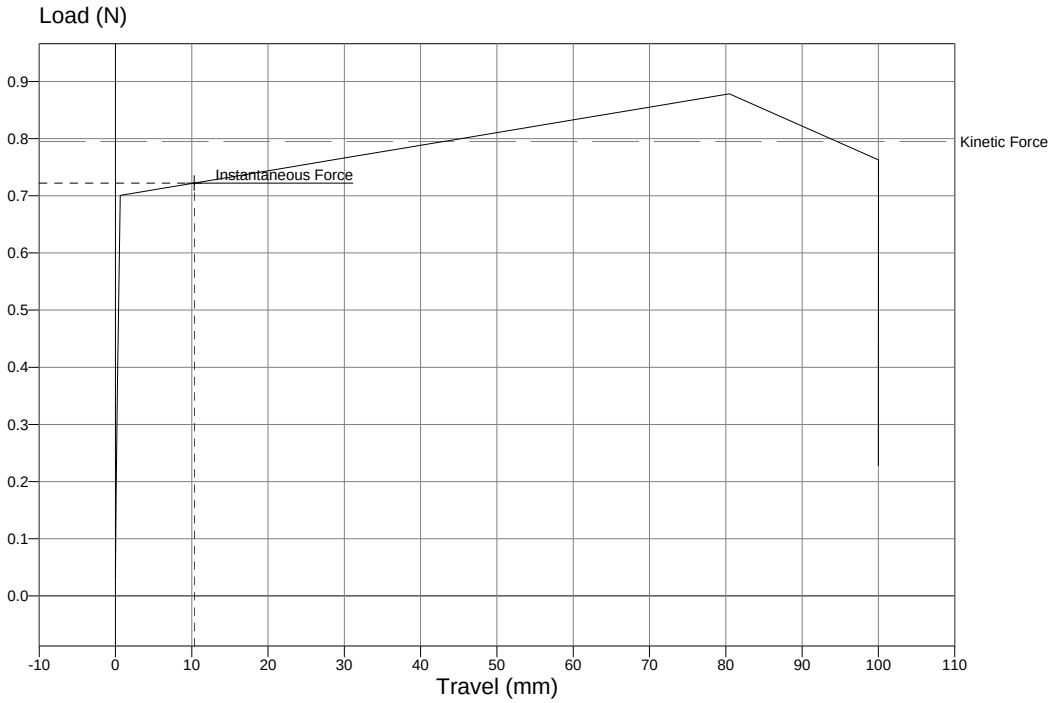
Frictional analysis of coated Pebax ® 3533, using EGDMA as crosslinking agent, after 40 *minutes submerged in pH7 buffered solution.*



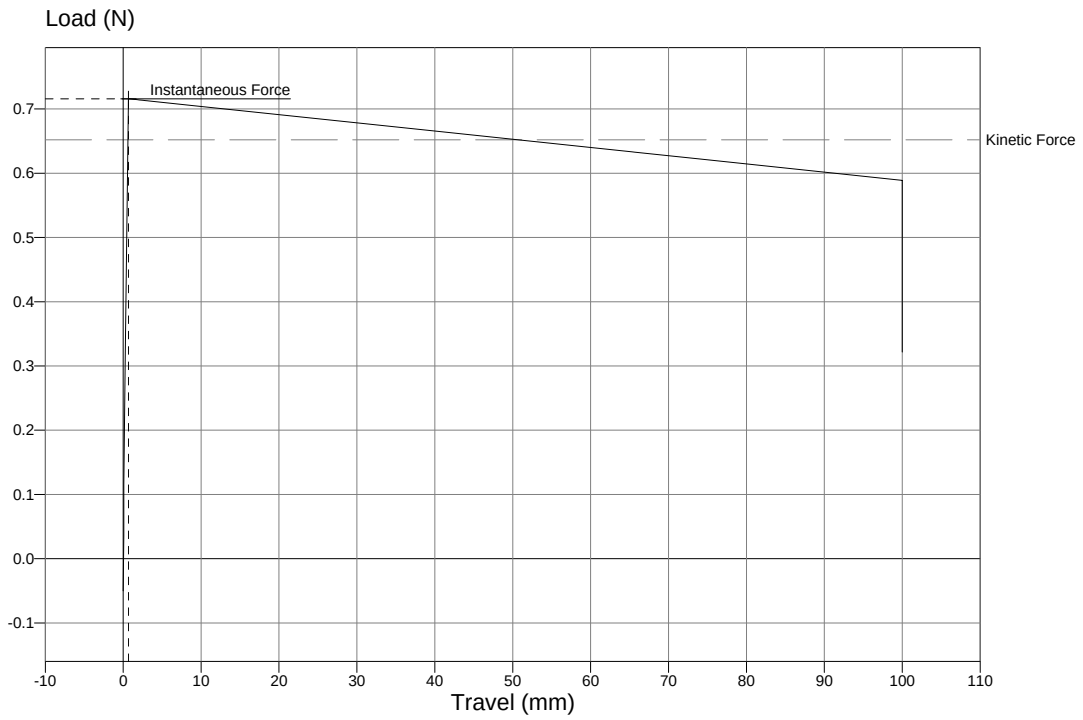
Frictional analysis of coated Pebax ® 3533, using EGDMA as crosslinking agent, after 45 *minutes submerged in pH7 buffered solution.*



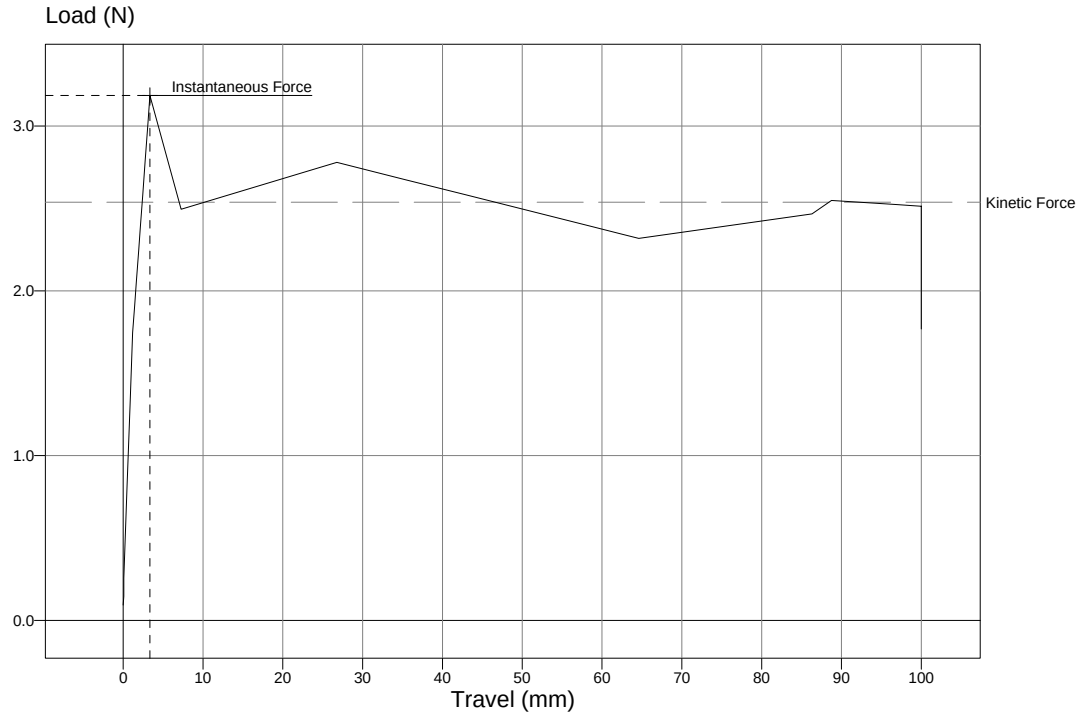
Frictional analysis of coated Pebax ® 3533, using EGDMA as crosslinking agent, after 50 *minutes submerged in pH7 buffered solution.*



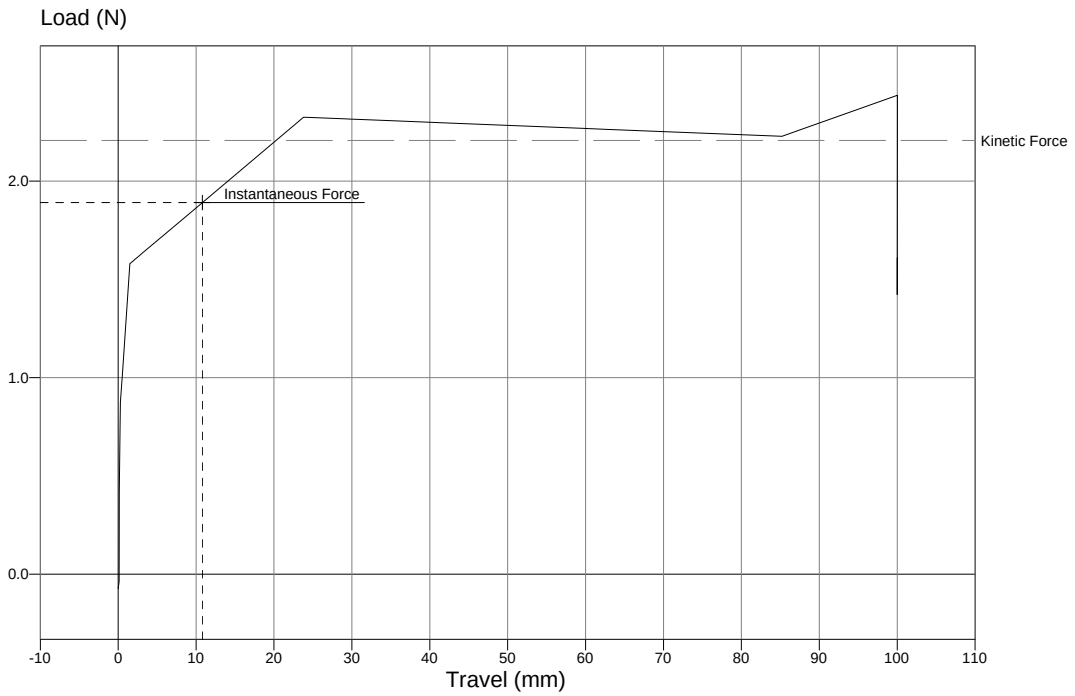
Frictional analysis of coated Pebax ® 3533, using EGDMA as crosslinking agent, after 55 *minutes submerged in pH7 buffered solution.*



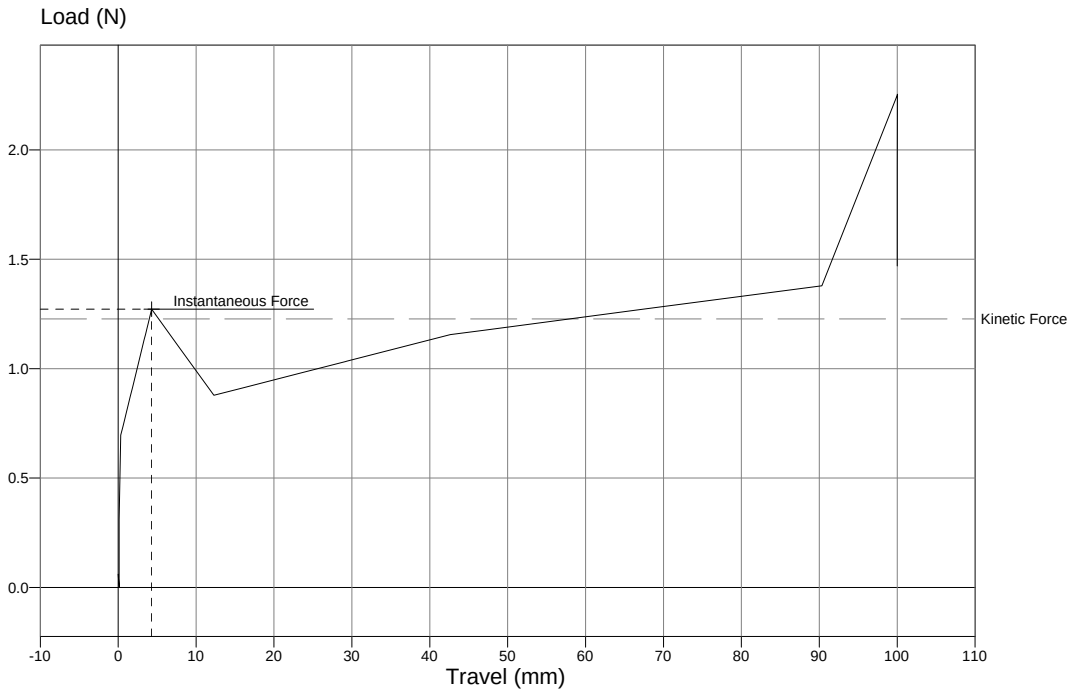
Frictional analysis of coated Pebax ® 3533, using EGDMA as crosslinking agent, after 60 minutes submerged in pH7 buffered solution.



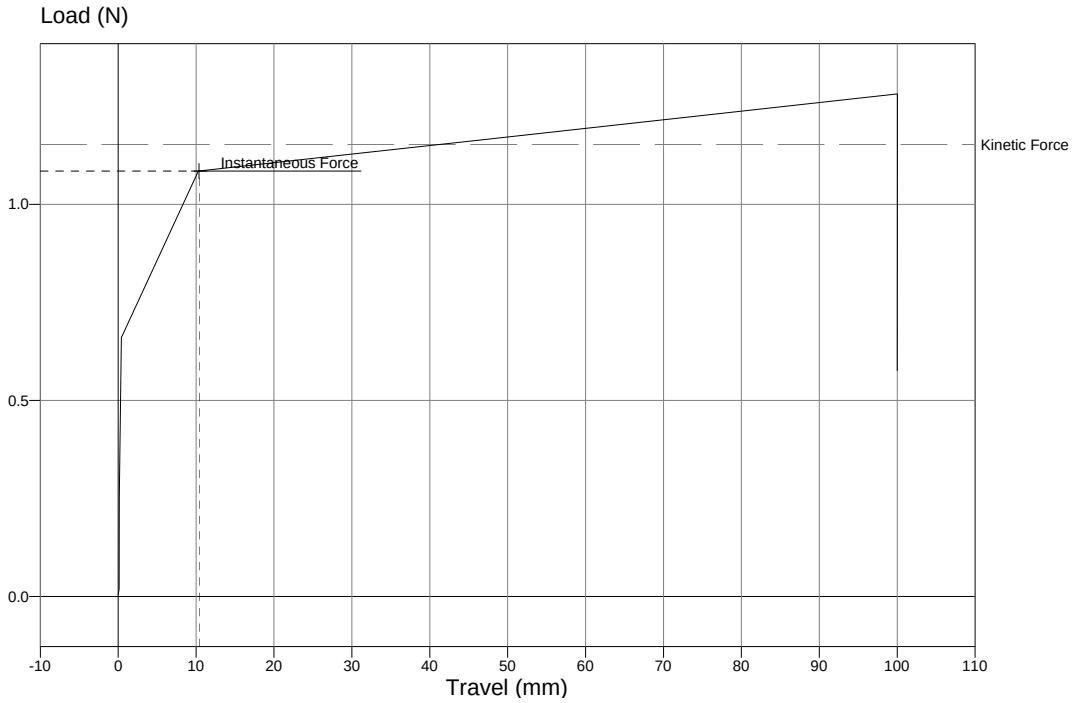
Frictional analysis of coated Pebax ® 3533, using PEG600DMA as crosslinking agent, after 5 minutes submerged in pH7 buffered solution.



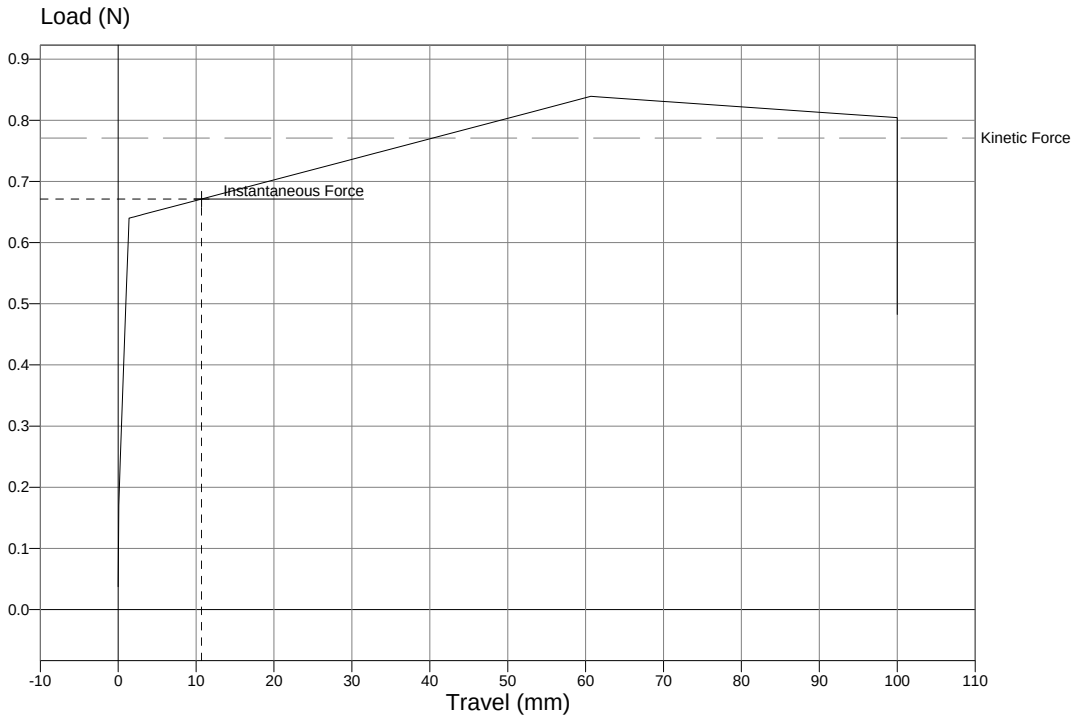
Frictional analysis of coated Pebax ® 3533, using PEG600DMA as crosslinking agent, after 10 minutes submerged in pH7 buffered solution.



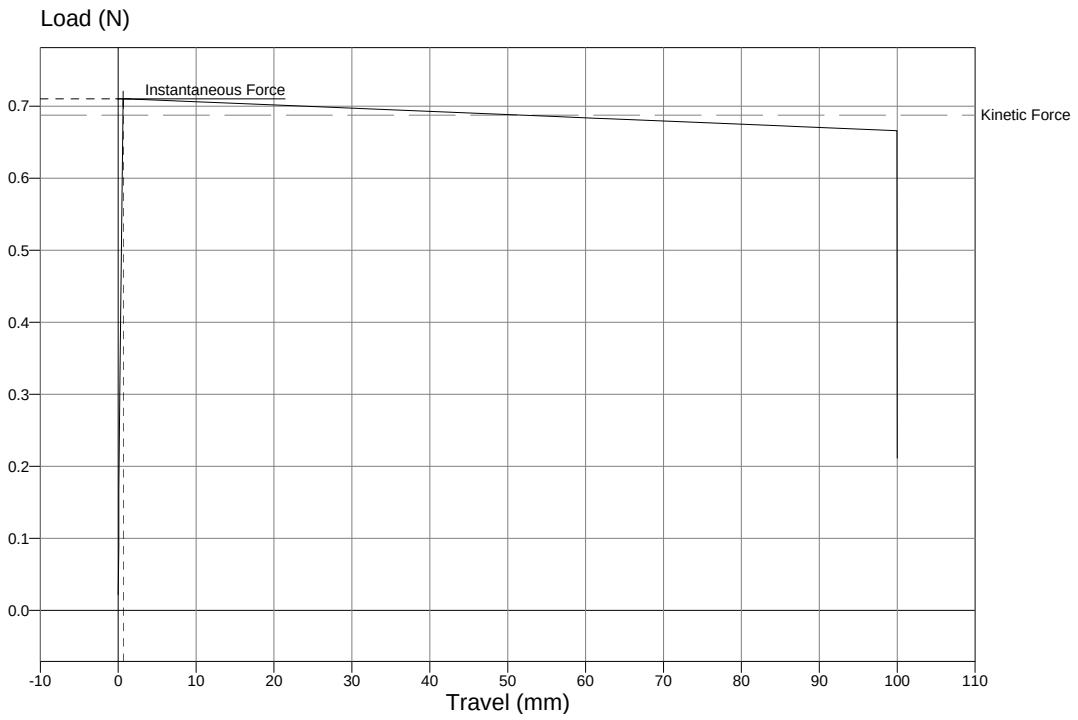
Frictional analysis of coated Pebax ® 3533, using PEG600DMA as crosslinking agent, after 15 minutes submerged in pH7 buffered solution.



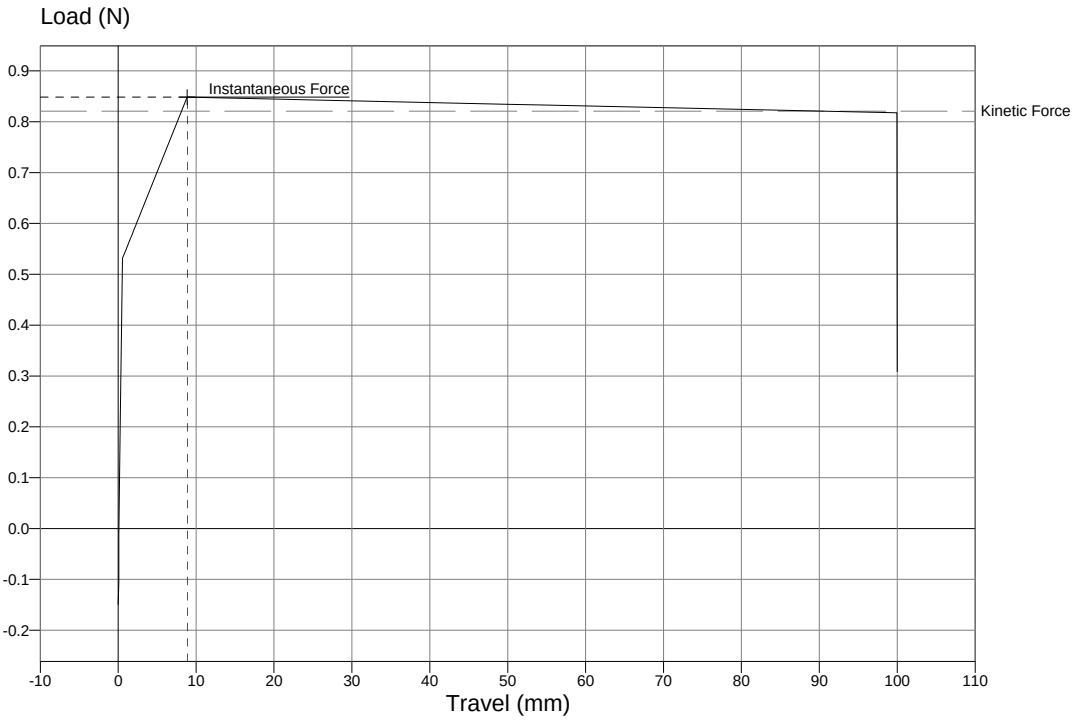
Frictional analysis of coated Pebax ® 3533, using PEG600DMA as crosslinking agent, after 25 minutes submerged in pH7 buffered solution.



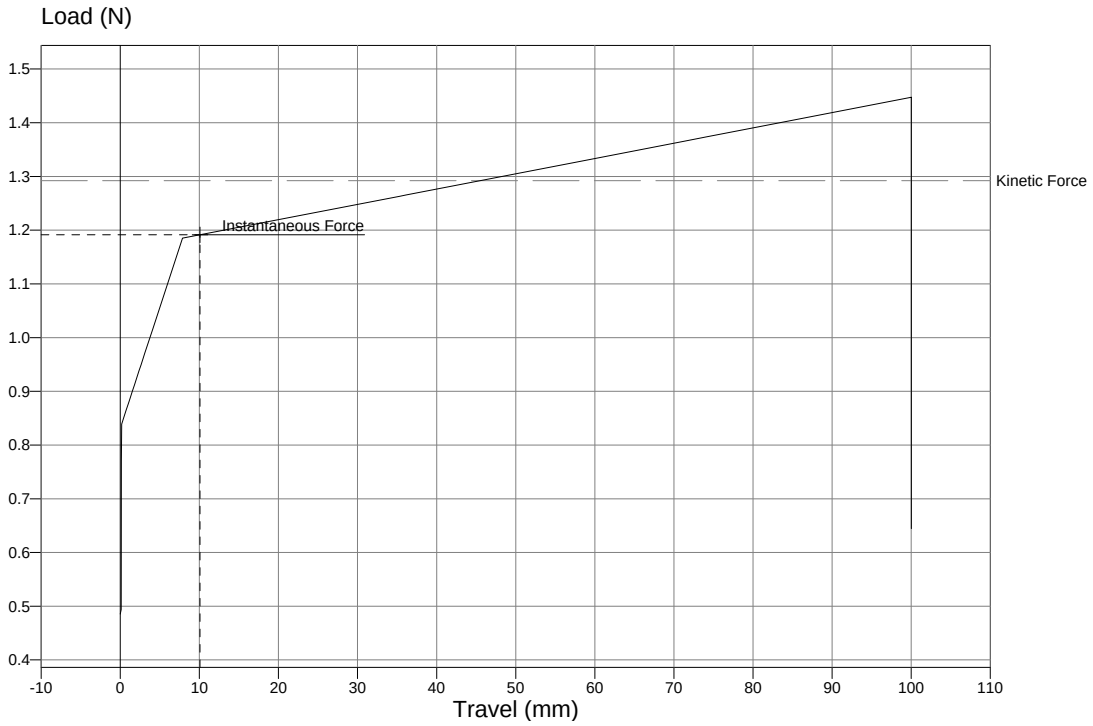
Frictional analysis of coated Pebax ® 3533, using PEG600DMA as crosslinking agent, after 30 minutes submerged in pH7 buffered solution.



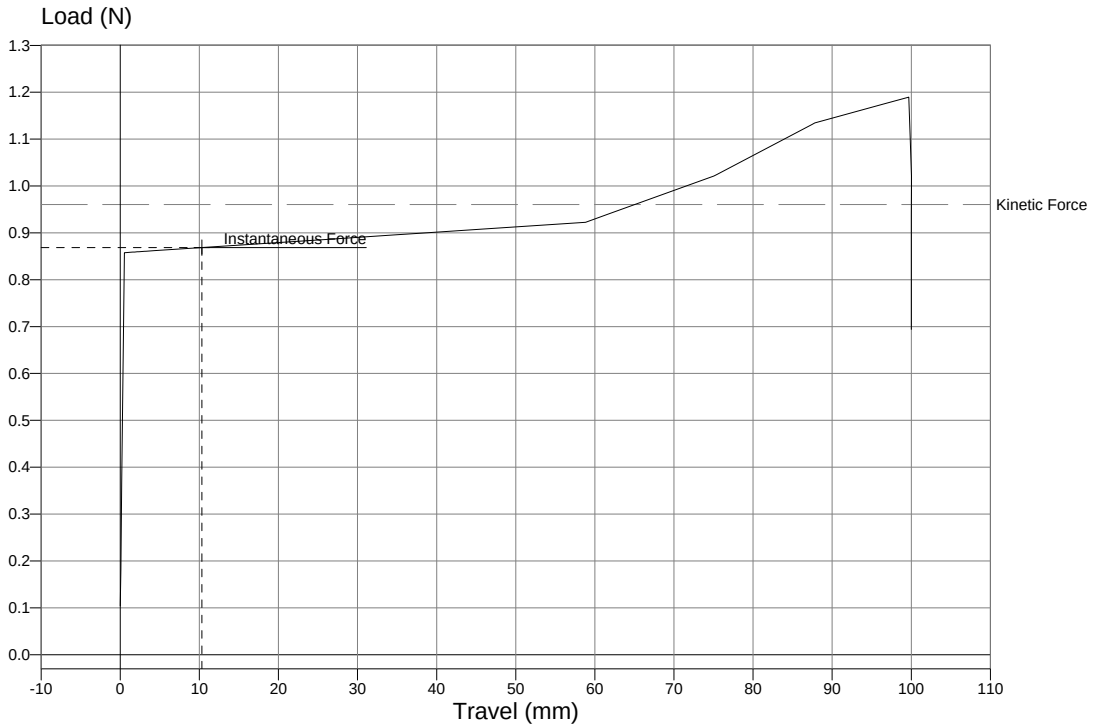
Frictional analysis of coated Pebax ® 3533, using PEG600DMA as crosslinking agent, after 35 minutes submerged in pH7 buffered solution.



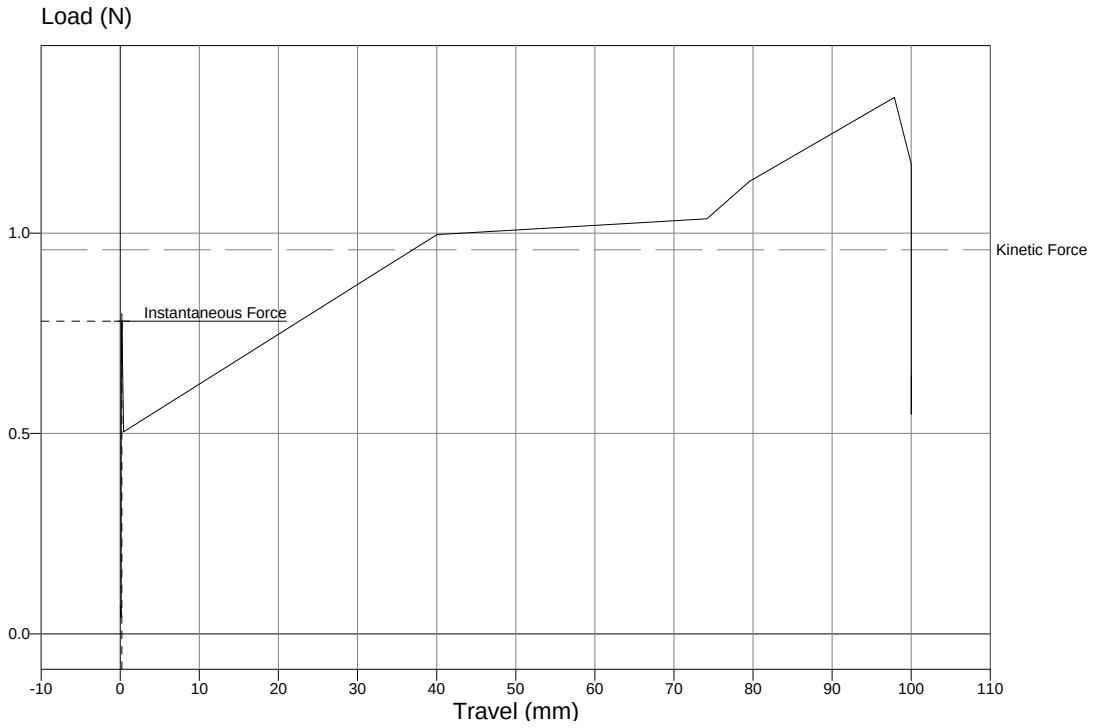
Frictional analysis of coated Pebax ® 3533, using PEG600DMA as crosslinking agent, after 40 minutes submerged in pH7 buffered solution.



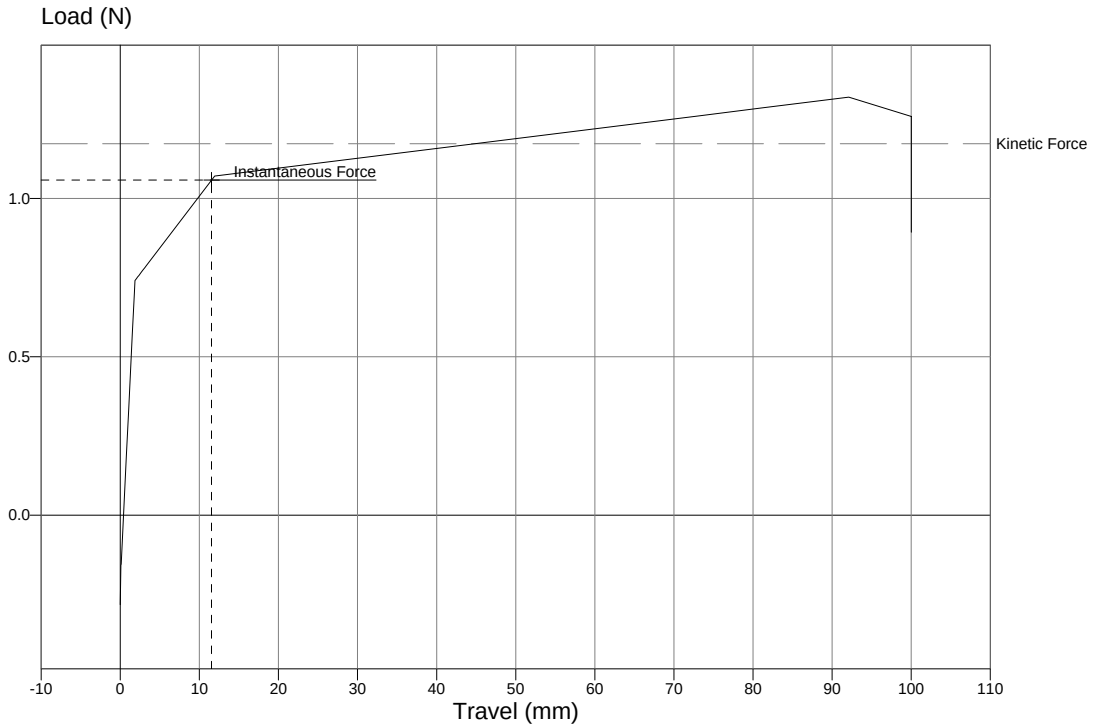
Frictional analysis of coated Pebax ® 3533, using PEG600DMA as crosslinking agent, after 45 minutes submerged in pH7 buffered solution.



Frictional analysis of coated Pebax ® 3533, using PEG600DMA as crosslinking agent, after 50 minutes submerged in pH7 buffered solution.



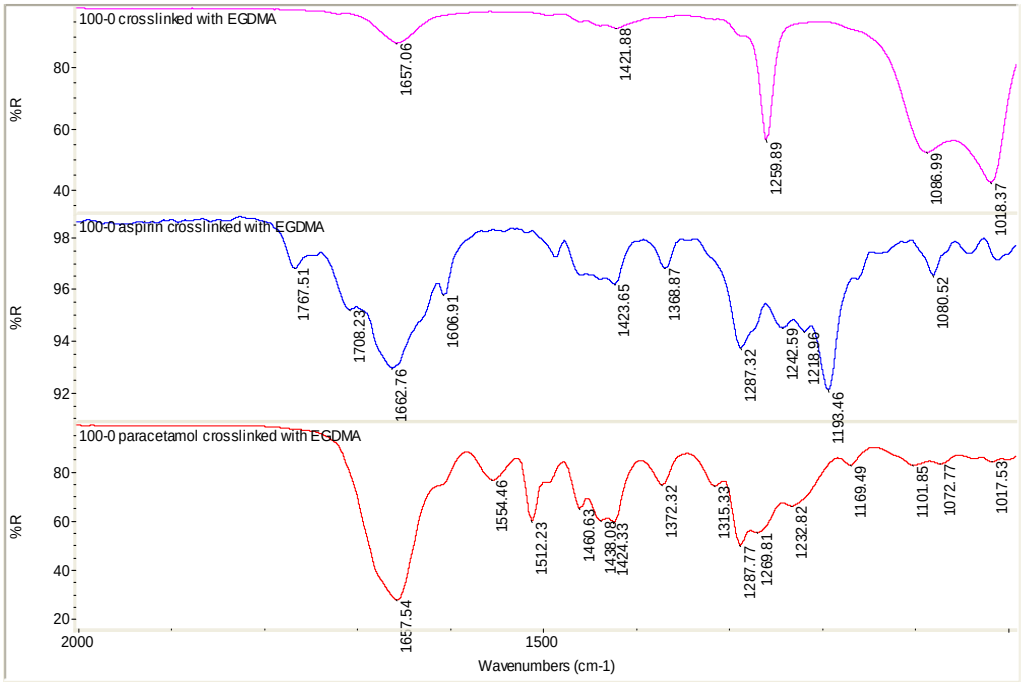
Frictional analysis of coated Pebax ® 3533, using PEG600DMA as crosslinking agent, after 55 minutes submerged in pH7 buffered solution.



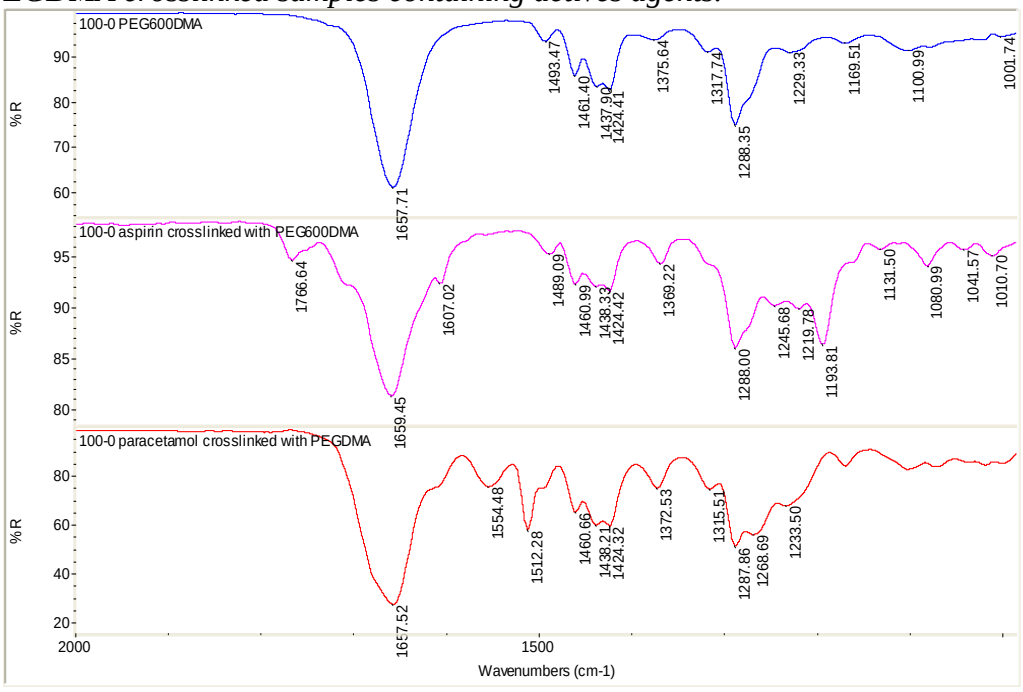
Frictional analysis of coated Pebax ® 3533, using PEG600DMA as crosslinking agent, after 60 minutes submerged in pH7 buffered solution.

Appendix H

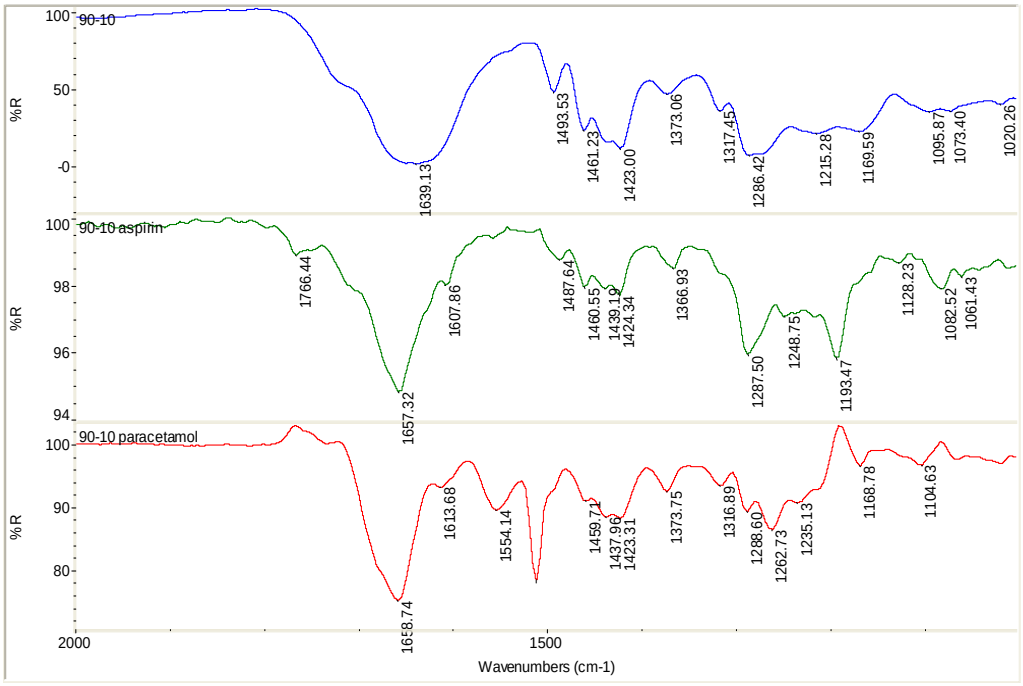
Ftir of polymers containing active agent



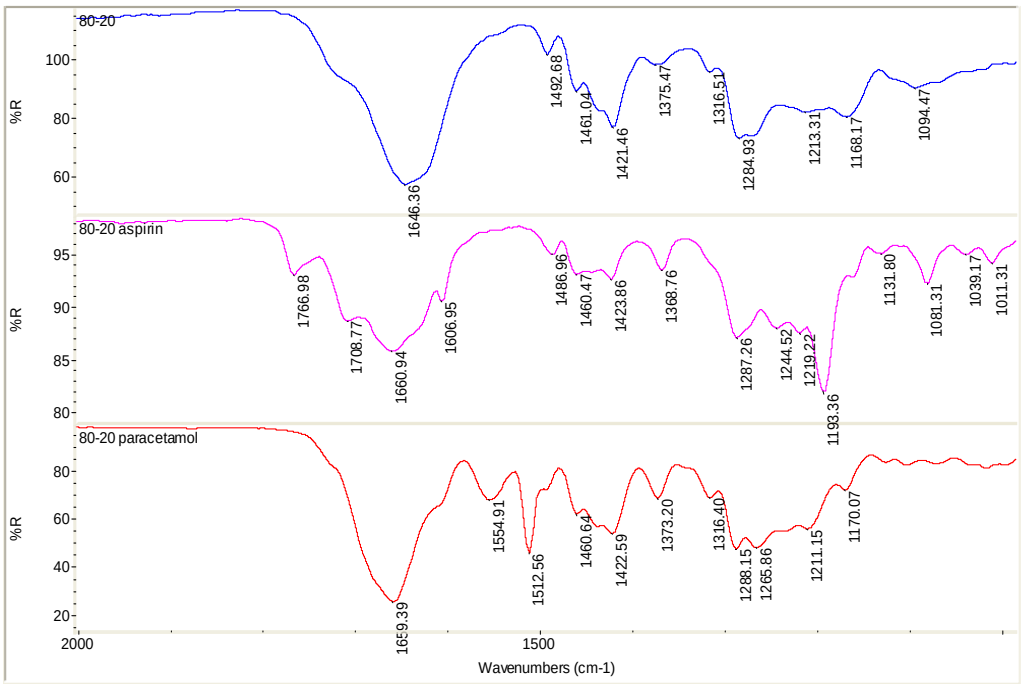
Comparison of Ftir spectra for 100-0 EGDMA crosslinked sample, and 100-0 EGDMA crosslinked samples containing actives agents.



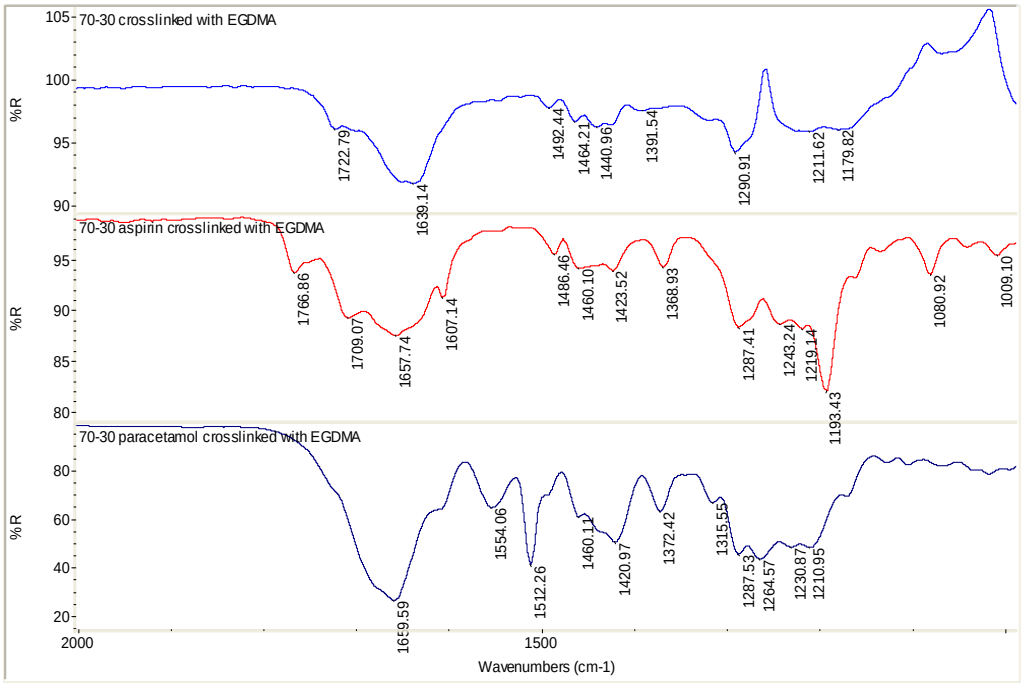
Comparison of Ftir spectra for 100-0 PEG600DMA crosslinked sample, and 100-0 PEG600DMA crosslinked samples containing actives agents.



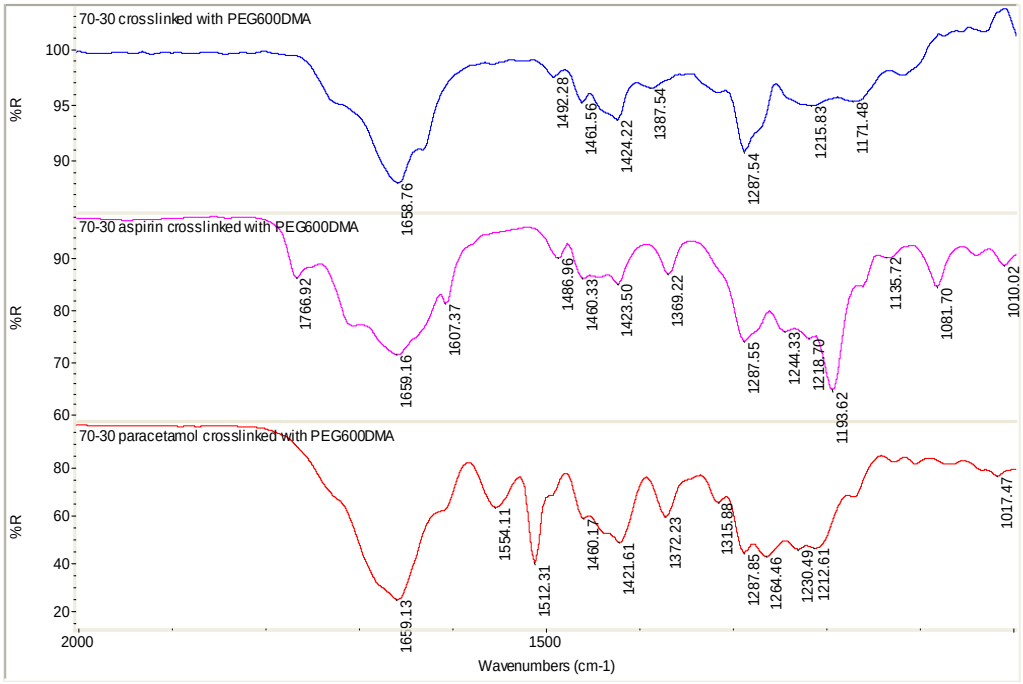
Comparison of Ftir spectra for 90-10 sample, and 90-10 samples containing actives agents.



Comparison of Ftir spectra for 80-20 sample, and 80-20 samples containing actives agents.



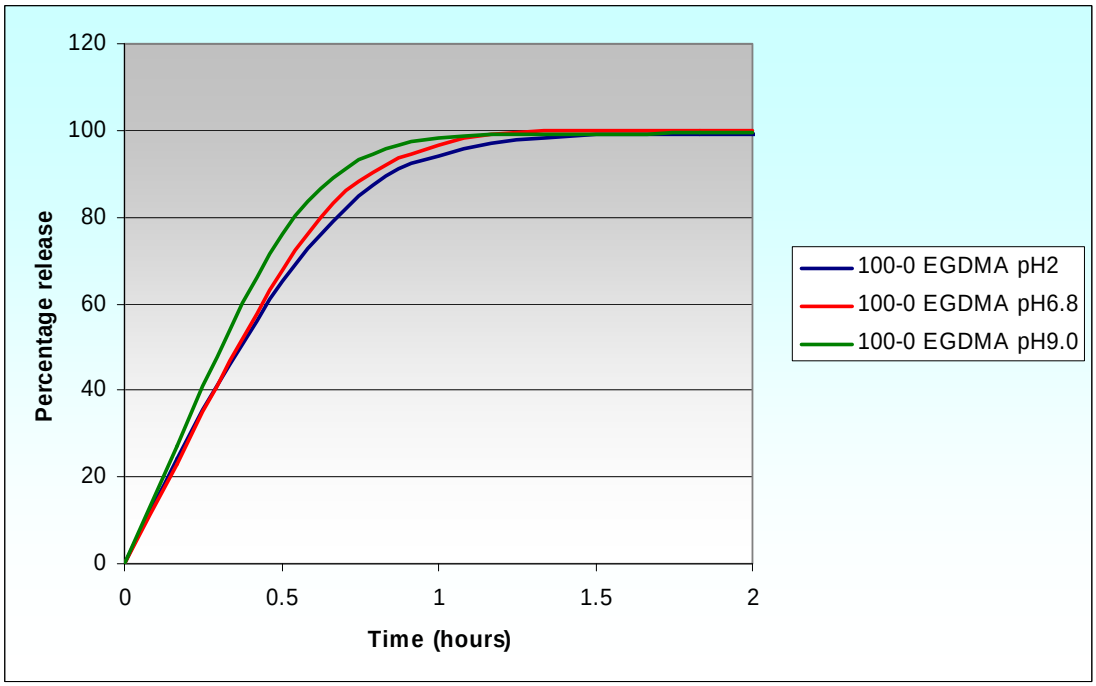
Comparison of Ftir spectra for 70-30 EGDMA crosslinked sample, and 70-30 EGDMA crosslinked samples containing actives agents.



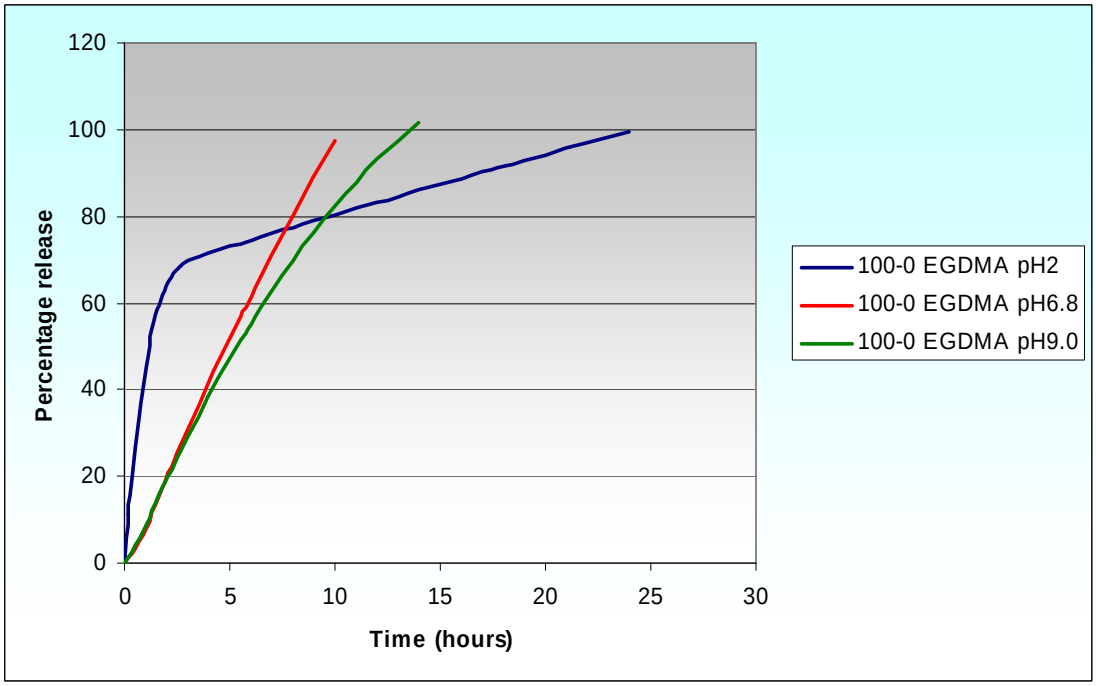
Comparison of Ftir spectra for 70-30 PEG600DMA crosslinked sample, and 70-30 PEG600DMA crosslinked samples containing actives agents.

Appendix I

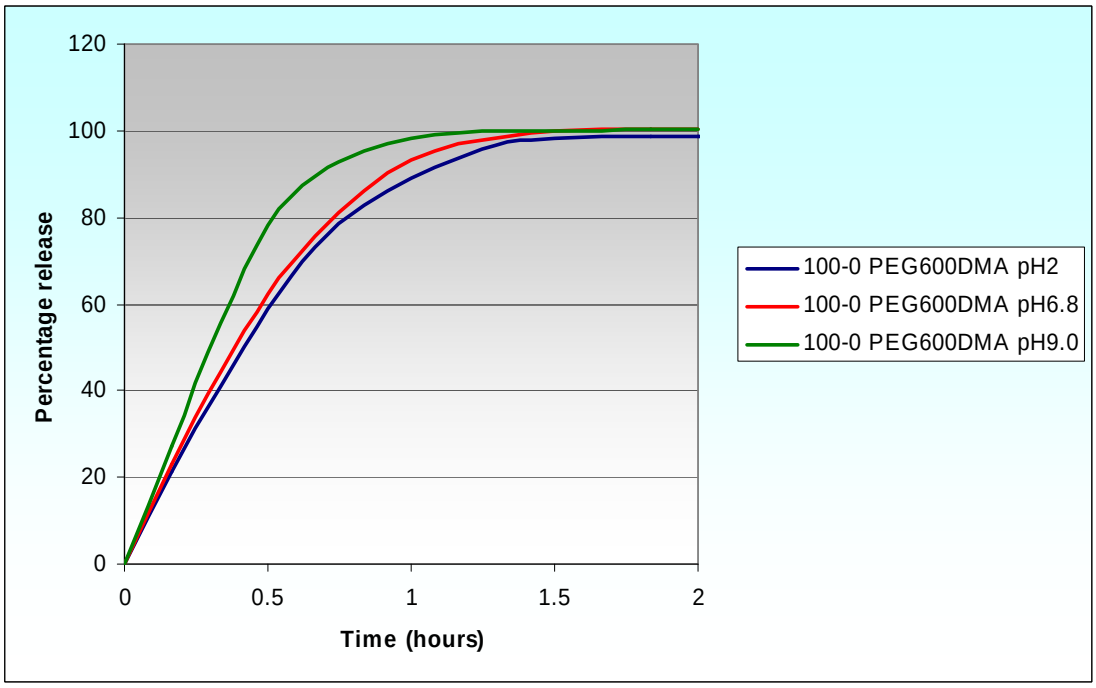
Drug dissolution data



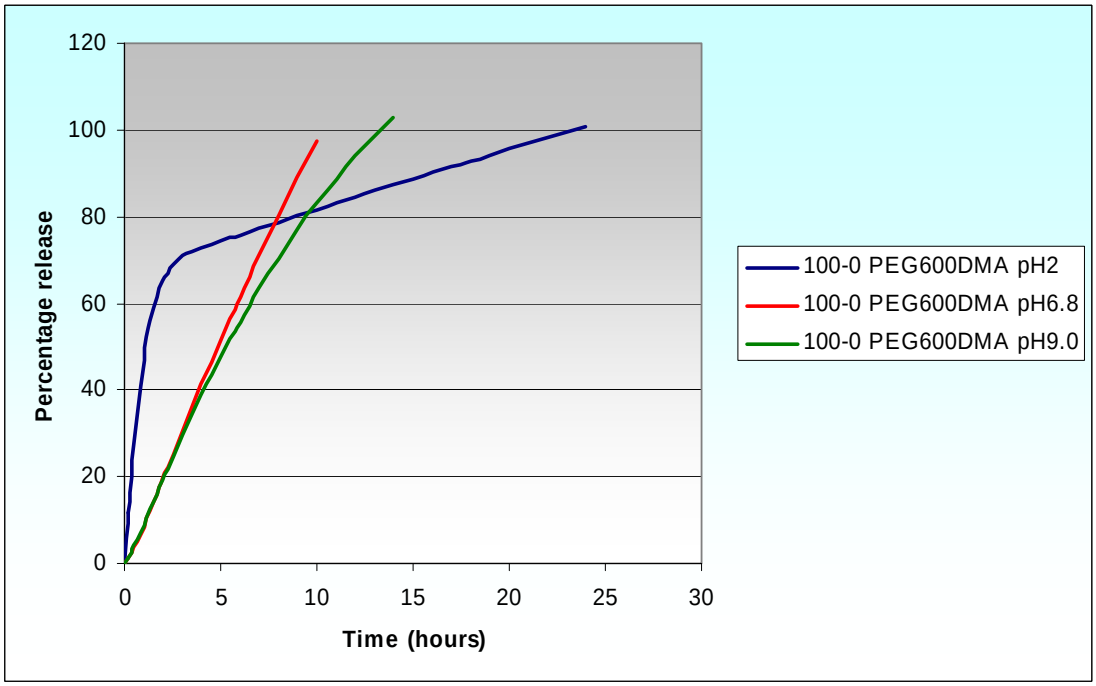
Paracetamol dissolution profiles for the polymers designated 100-0 crosslinked with EGDMA, at various pH values



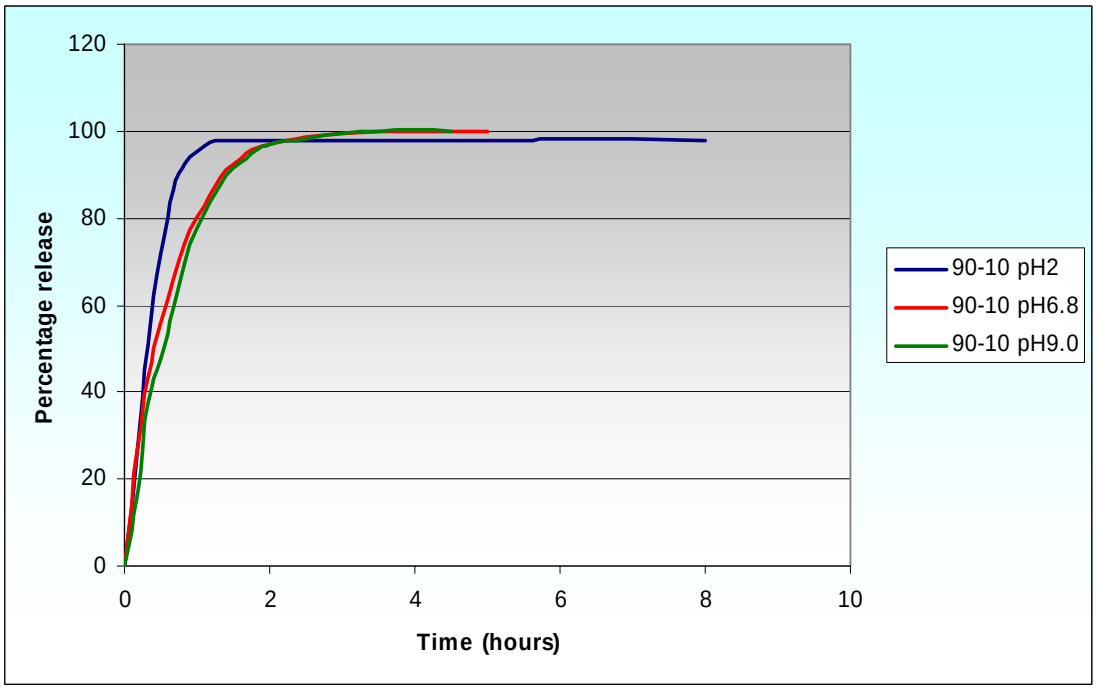
Aspirin dissolution profiles for the polymers designated 100-0 crosslinked with EGDMA, at various pH values



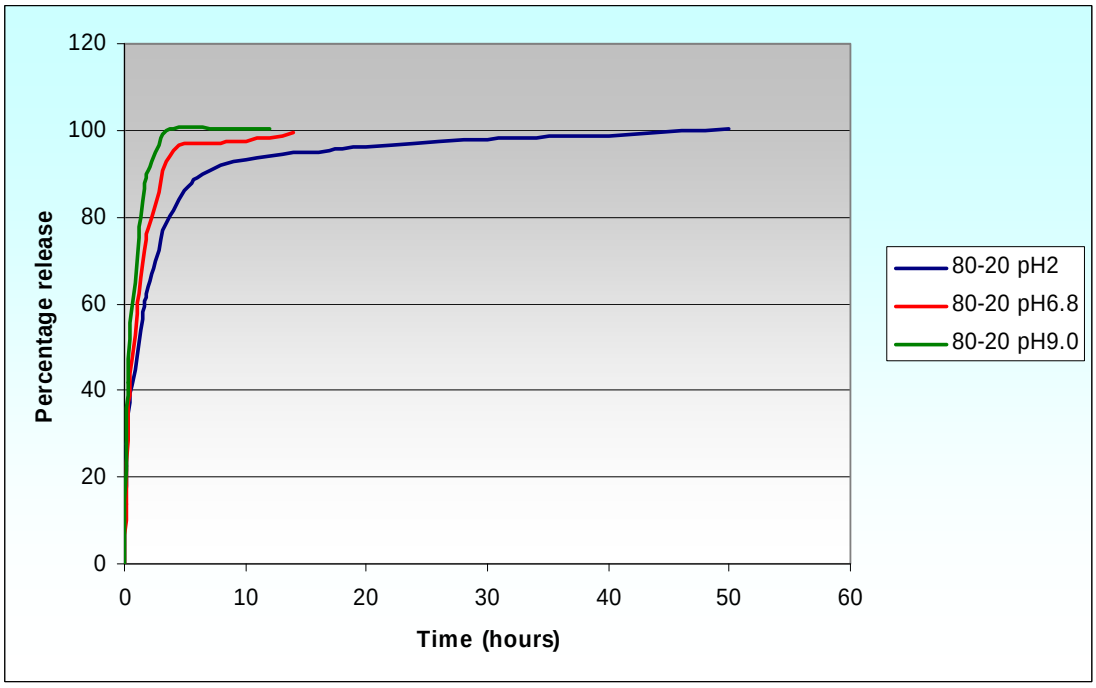
Paracetamol dissolution profiles for the polymers designated 100-0 crosslinked with PEG600DMA, at various pH values



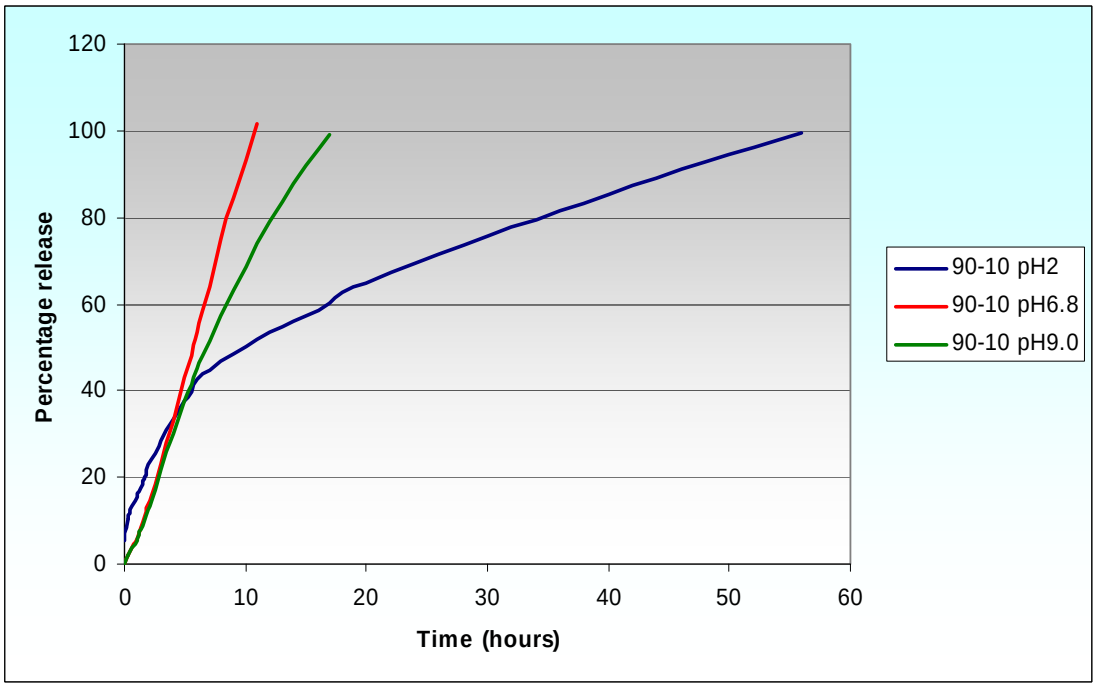
Aspirin dissolution profiles for the polymers designated 100-0 crosslinked with PEG600DMA, at various pH values



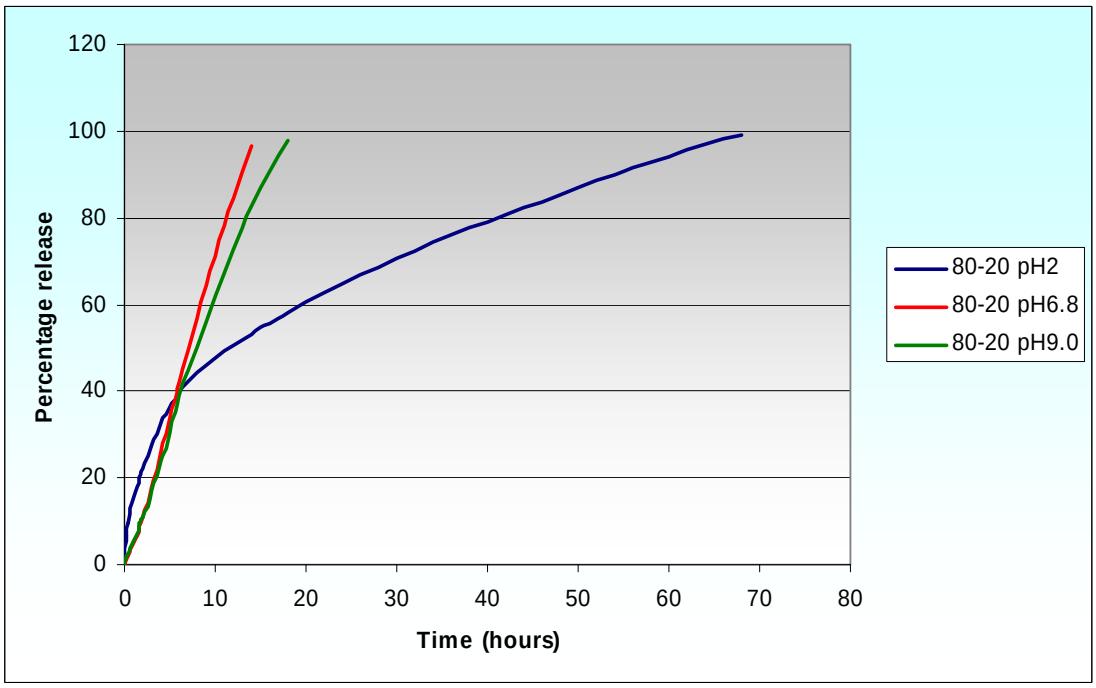
Paracetamol dissolution profiles for the polymers designated 90-10 at various pH values



Paracetamol dissolution profiles for the polymers designated 80-20 at various pH values



Aspirin dissolution profiles for the polymers designated 90-10 at various pH values



Aspirin dissolution profiles for the polymers designated 80-20 at various pH values