Some Thermo-, Photo- and Electro- Responsive Hydrogels

A thesis presented by

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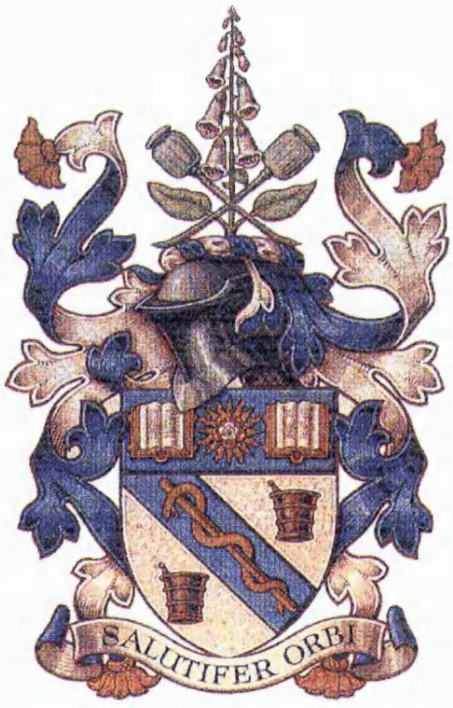

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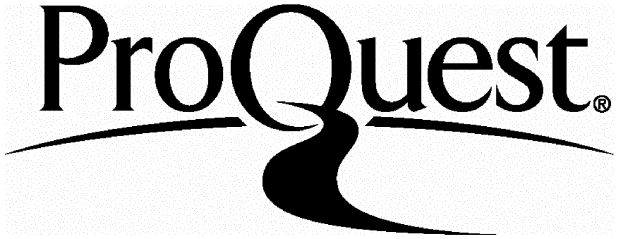

To my parents, Shoshana and Zvulun,
my wife, Esti, and my daughter, Amit,
without whose consistent support, encouragement and love
it would have been impossible.

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Abstract

This work has focused on externally stimulated hydrogels which are thermo-responsive, photo-responsive and electro-responsive as matrices for drug delivery.

Thermo-responsive hydrogels were fabricated from N-isopropylacrylamide (NiPAAm) and exhibited behaviour analogous to a lower critical solution temperature(LCST) phenomenon- being highly swollen at room temperature but deswelling when the temperature was raised and collapsing fully above the LCST. Copolymerization of different monomers and changing the crosslinking concentration controlled the swelling profiles of the gels, and the release of water soluble model drugs and solutes was investigated. On-off release of flurbiprofen was achieved by temperature modulation.

Photo-responsive hydrogels were prepared by incorporating an azobenzene chromophore into the NiPAAm network. Azobenzene naturally occurring as the *trans* isomer undergoes reversible isomerisation to *cis* under irradiation. Two different azobenzene derivatives were used- methacryloylaminoazobenzene and di(methacryloylamino)azobenzene, the first used as a pendant group inside the network, and the second as crosslinking unit. When using azobenzene as a pendant group, solute release from the network increases upon UV irradiation, while as a crosslinking unit, isomerization decreases the release of the model solute, although the maximum difference was not greater than 10%.

Hyaluronic acid (HA) was used to synthesize an electro-responsive release hydrogel. HA is a naturally occurring polysaccharide and due to its carboxylic groups enables the formation of highly swollen polyelectrolyte gels.Unloaded HA gels deswell dramatically under an electric field due to electro-osmosis that causes partial protonation of the network. The responsive swelling behaviour of loaded HA gels was dependent upon the initial swelling state of those gels. Release of two negatively charged macromolecules was investigated and on-off switchable release was demonstrated.

Possible future responsive release systems based on the knowledge acquired in this work are envisioned in the end of this thesis.

Abbreviations:

P.... - Poly(....)

AAc - Acrylic acid

AIBN - N,N'-Azoisobutyronitrile

AMPS - 2-Acrylamido-2-methyl-1-propanesulfonic acid

APS - Ammonium persulfate

BIS - Methylenebis(acrylamide)

DMAAB - Di(methacryloylamino) azobenzene

DMSO - Methyl sulfoxide

EGDGE - Ethyleneglycol diglycidylether

HA - Hyaluronic acid

HEMA - Hydroxyethyl methacrylate

HEMC - Hydroxyethylmethyl cellulose

HPC - Hydroxypropyl cellulose

HPMC - Hydroxypropylmethyl cellulose

LCST - Lower Critical Solution Temperature

Leuco-CN - Bis(4-(dimethylaminophenyl))(4-vinylphenyl)methyl leucocyanide

Leuco-OH - Bis(4-(dimethylaminophenyl))(4-vinylphenyl)methyl leucohydroxide

MAA - Methacrylic acid

MAAB - Methacryloylamino azobenzene

MMA - Methyl methacrylate

NiPAAm - N-Isopropylacrylamide

ONPG - o-Nitrophenol-β-D-galactosidase

PEO - Poly(ethylene oxide)

PGT - Poly(glutamic acid,tyrosin) 4:1

PSSA - Poly(styrene sulfonic acid)

PVA - Poly(vinyl alcohol)

PVP - Poly(N-vinyl pyrrolidone)

TEMED - N,N,N',N'-Tetramethylene diamine

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Chapter One - Introduction

1.1 Hydrogels

1.1.1. Hydrogels for biological and pharmaceutical application

" Plastics to-day enjoy wide use in many fields, and it is natural that the possibility of their employment in permanent contact with living tissues has been seriously considered.... The demand to be placed upon suitable plastics are thus: (1) a structure permitting the desired water content; (2) inertness to normal biological processes; (3) permeability for metabolites. Materials with these properties must have hydrophilic groups. Further they must have a three dimensional structure with at least enough cross-linkage to prevent absorption. Of a large number of plastics we found co-polymers of glycomonomethacrylate with several tenths of glycodimethacrylate to be most suitable. They hydrolyse only with difficulty, even under severe conditions, and are indifferent to biological materials.... The water content, and thus the shape of the object, is, after attaining equilibrium at given temperature, constant and dependent on the structure and number of cross-linkage.... The desired shapes are prepared by polymerization of aqueous solution in suitable moulds.... These materials have now passed through the stage of application such as filling after enucleation of the eye. Promising results have also been obtained in experiments in other cases, for example, in manufacturing contact lenses, arteries, etc."

(O. Wichterle and D. Lim, Hydrophilic gels for biological use, Nature 1960)

This paper published in 1960 is regarded as the first milestone in the use of hydrogels in the medical field. Webster's New Twentieth Century Dictionary (McKechnie J.L., (ed.)) gives the following definitions:

Gel A jelly-like substance formed by a colloidal solution in its solid phase: opposed to sol.

Jelly A soft, resistant, partially transparent, semisolid, gelatinous food resulting from the cooling of fruit juice boiled with sugar or of meat juice cooked down.

Scientifically, hydrogels can be defined as hydrophilic, polymeric, three dimensional, usually cross-linked networks which are insoluble in water but exhibit the ability to be hydrated and retain water within their structure. The polymers used for fabrication of such hydrogels can vary from synthetic to natural materials, and include synthetically modified natural structures.

As indicated in their paper, Wichterle and Lim found hydrogels to be suitable for biological use because they, more than any other synthetic biomaterials, resemble living tissues in their physical properties, especially due to their higher water content and their rubbery soft consistency. Another of their advantages is that metabolites and ions can easily diffuse through the matrix. Their physical properties can be easily adjusted to suit many requirements. However, due to their high water content they are usually mechanically weak.

Over the last three decades the research in the field of hydrogels has widely spread. Table 1.1 indicates some of the great variety of the published data and commercially available products containing hydrogels for biological use. Some data on the use of hydrogels as drug carriers for different administration routes is shown in table 1.2 . Their biocompatibility allows them to be administered in any possible route from oral to subcutaneous implants and buccal devices. Hydrogels have many other applications, some of them are trivial as in the food industry, but many other in more sophisticated fields such as chromatography, electrophoresis, crystallography, photography, adhesives, tyres (elastomers) and many more.

Table 1.1

Some hydrogels for biological use

Monomer/Polymer	Description	Reference
PHEMA/PVP	Contact lenses	Tighe 1987
Hyaluronan	Viscosurgical implant in the eye	Balazs *et al.* 1989
Hyaluronan	Viscosurgical implant in the ear	Laurent *et al.* 1986
Cellulose acetate	Membranes for hemodialysis	Klein 1977
	(Cuprophan®, Celanese®)	Kerr *et al.* 1974
PVA	Plasma separation membrane (Kuraray®)	Randerson *et al.* 1983
PHEMA/ silicone	Reconstruction of sexual organs	Peppas 1987a
PVA	Reconstruction of vocal cords	Peppas *et al.* 1980
PHEMA	Mammaplasty (Hydron®)	Calnan *et al.* 1971
Acrylate/ lactate/ PEG	Agioplasty- support arteries open after surgery	Franscinella 1994
PVA	Artificial skin	Chardack *et al.* 1962
Collagen/glycosaminoglycans	Artificial skin	Yannas *et al.* 1981
Polylactide/ polyurethane	Artificial skin	Gogolewski *et al.* 1983
Hyaluronan	Joint viscosupplemation	Balazs *et al.* 1989
MAA/ polydimethacrylate	Denture basis, crown basis and	Ruyter *et al.* 1988
	artificial teeth	Sheela *et al.* 1991
PVP	Artificial liver (carrier of metabolic enzymes)	Jauegui *et al.* 1983
PVA	Carriers of β cells as an artificial pancreas	Sun *et al.* 1983

Table 1.2

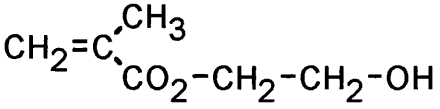
Hydrogels as drug delivery systems

Monomer/Polymer	Drug	Route of administration	Reference
PHEMA	Pilocarpine	Soft contact lens	Maddox *et al.* 1972
PVA	Chloramphenicol	Soft contact lens	Praus *et al.* 1972
PHEMA/ MAA	Fluoride	Dental implant	Cowsar *et al.* 1976
HPC/ HPMC/ Karaya gum	Benzydamine HCl	Buccal	Saito *et al.* 1990
HPC/ Carbapol	Lidocaine	Buccal	Ishida *et al.* 1982
HPC/ PVP/ PVA	Protirelin (TRH)	Buccal	Andres *et al.* 1989
HPMC	Misoprostol	Oral (stomach)	Oth *et al.* 1992
PVP	Flavin mononucleotide	Oral (Stomach)	Shalaby *et al.* 1992
Sod. alginate/ Xanthan gum	Theophyline	Oral (enteric)	Fu Lu *et al.* 1991
Ca. alginate	Nucleic acids	Oral (enteric)	Smith 1994
PHEMA	Progesterone	Transdermal film	Song *et al.* 1981
PHEMA	Insulin/ Calcitonin	Transdermal (iontophoresis)	Banga *et al.* 1993
PAA	Insulin	Vaginal	Morimoto *et al.* 1982
HEMC	Prostaglandin E2	Vaginal	Rayburn *et al.* 1992
PEO/ PVA	cp-53,607	Ruminal	Tombre *et al.* 1992

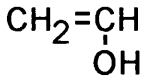
1.1.2 Common monomers and polymers for hydrogel preparation

The wide range of monomers and polymers available has resulted in a wide spectrum of hydrogels, some of them consist of homopolymer and other of two or more copolymers with different properties.

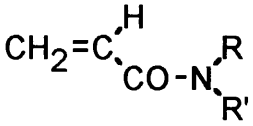
Methacrylate derivatives are the most studied as synthetic hydrogels. In that group polyHEMA (PHEMA) which was first reported by Wichterle and Lim (1960) is the most widely used. Drug delivery systems, diffusion control membranes, cell separation and protein absorption devices, and matrices for enzyme immobilization were fabricated from PHEMA and its related copolymers (Mack *et al.* 1987, Yashuda *et al.* 1968, Yoshida *et al.* 1980, Pywell *et al.* 1987, Rao *et al.* 1994). The related acrylate derivatives which include acrylic acid (Gracia-Gonzales *et al.* 1993) and acrylamide and their n-substituted polymers (Okano *et al.* 1990) are also widely used in drug delivery and related fields. Poly(vinyl alcohol) (PVA) is a product of hydrolysis of poly(vinyl acetate). Because of its crystallization tendency, the polymer can form a gel without cross-linking using the freeze-thaw method (Ficek and peppas 1993) or heat treatment (Wan and Lim 1992). Gel forming by chemical crosslinking of PVA can be easily achieved utilizing its free hydroxyl groups (Peppas 1987b). Poly(ethylene oxide) (PEO) is a high molecular weight polymer (range between 100,000 to 1,000,000), it can crystallize or be cross-linked to form a hydrogel. Graham (1986) demonstrated differences in release patterns between different states of PEO gels.



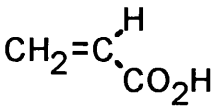
Hydroxyethylmethacrylate (HEMA)



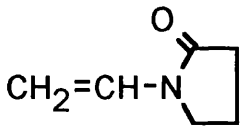
Vinyl alcohol



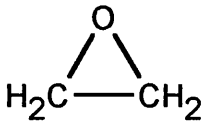
N-alkyl acrylamide



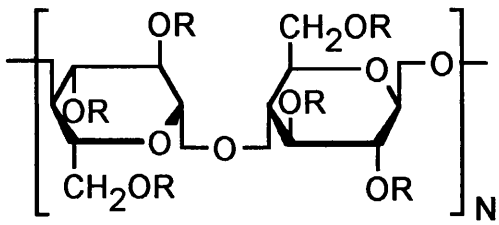
Acrylic acid



N-vinyl pyrrolidone



Ethylene oxide



Substituted cellulose

Figure 1.1 Some common monomers for synthesis of hydrogels.

Poly(vinyl pyrrolidone) (PVP) is a highly hydrophilic polymer that can form highly swollen gel matrices. Shalaby *et al.* (1992) used this property to increase gastric retention time of such a gel. PVP can be polymerized with PHEMA in order to increase the swelling of PHEMA gels (Yoshida *et al.* 1980).

Polysaccharides are natural polymers which are widely used as a source for the synthesis of hydrogels. They are produced by bacterial fermentation or isolated from animals or plants. Due to their high molecular weight some form a gel without cross-linking, but if necessary they can be easily cross-linked. Most of them have an acidic group like sulphonate (heparin, carragenans) or carboxylic group (alginic acid, hyaluronic acid, xanthan) (Atkins 1985). Chitosan is singular in this group since it has a basic group obtained by acetylation of chitin, the main component in crab shell (Miyazaki *et al.* 1981). In the pharmaceutical field the most used polysaccharides are the cellulose derivatives.

1.1.3 Preparation and drug loading of hydrogels

Hydrogels can be synthesised by covalent cross-linking (chemical hydrogels) (Peppas and Khare 1993) or with other crosslinking methods (physical hydrogels) (Ross-Murphy 1994).

Chemical hydrogels can be synthesized in two different ways. In the more common technique the monomers are mixed with the cross-linking agent and polymerization and cross-linking take place simultaneously, while in the second technique the cross-linking step is carried out after obtaining the

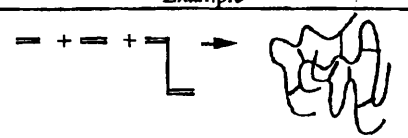
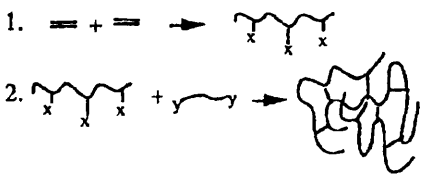
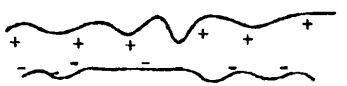
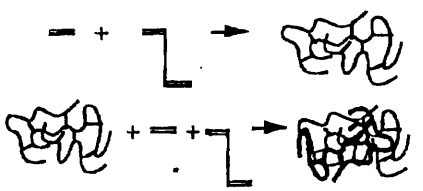
Method	Example	Comments
Crosslinking copolymerization		An extensive amount of different monomers are available. Hydrogels with different properties may be obtained by varying the monomers.
Crosslinking of polymeric precursors		The gel point is reached very fast. Good control of molecular weight of polymer backbone.
Polyelectrolyte complexes		Physical crosslinks (ion-ion interaction). Swells drastically in solutions of high ionic strength.
Interpenetrating networks		Can be formed in one step using two different polymerization mechanisms, or in two steps polymerizing one network at a time.

Figure 1.2 Some methods for preparation of crosslinked hydrogels (from: Brondsted and Kopecek 1992a)

polymer. The cross-linking agent is usually a multi-functional monomer that reacts with different polymer chains. The polymerization reaction is generally initiated by free radicals which are generated by thermal, light irradiation, chemical radiation or redox means. A wide variety of initiators can be used, such as peroxides, azo-compounds and redox agents. Gamma-radiation (from cobalt 60, cesium 137 or electron beam) can be used as well to initiate the polymerization of the network. Crosslinking by irradiation removes the necessity for addition of another chemical agent results in a more pure and homogenous product. Wood *et al.* (1981) have showed that chemically polymerized PHEMA swell to higher extent than irradiation polymerized gels with the same initial water content. This is due to the ionized chemical initiator that is entrapped in the network. This residual initiator slowly diffuses out of the hydrogel, causing a moderate deswelling, during few days of incubation of the hydrogel in water, before it reaches the equilibrium swelling. On the other hand, swelling of irradiation polymerized hydrogels rapidly reaches a steady state.

The polymerization process can be carried out in bulk, solution or suspension. Bulk polymerization is the reaction of the neat monomers. A monolithic block of hard material is prepared in a mould and then cut out to the desirable shape and size. Solution polymerization is also used to obtain directly the swollen hydrogel and is carried out in an excess of water. Suspension polymerization is the preferred method for preparation of hydrogel beads.

Physical hydrogels are formed due to association of polymeric chains in some "junction zones". Many biological large scale molecular structures are held together by interactions between their polymers. Physical gels are formed from polymeric blends. Methods for physical cross-linking include ionic interactions, hydrophobic interactions and hydrogen bonding.

Interpenetrating polymer networks (IPN) are a mesh of two polymers which are produced by synthesizing one linear polymer, swelling it in the second monomer and polymerizing the latter. These systems are mainly use to modify the surface characteristics of the network in order to produce a biocompatible and thrombogenic hydrogel (Bae and Kim 1993).

Drug loading in hydrogels can be achieved by adding it before polymerization or incorporation after the gel has been formed by immersion of the gel inside the drug solution in the appropriate solvent. The drug diffuses inside the hydrogel often by the same process that it will diffuse out in a controlled release application. The first loading method is more simple but requires an inert drug, and poses a problem in purification of the hydrogel after its preparation without loosing its content. In the second method the gel can be washed before the loading phase.

1.1.4 Swelling and the nature of water inside hydrogels

The ability of dry hydrogels to take up water and swell is probably their most distinctive feature. Since the polymer segments and water are attracted to each other, the average chain dimension tends to increase in order to

increase the number of interactions between it and the water. The dimensions increase until the polymeric network has stretched so much that the resulting elastic force counteracts the inflow of water molecules. The extent of elasticity is dependent on the degree of cross-linking of the network (cross-linking density). Increasing the cross-linking density is known to effect the diffusion rate from hydrogels, through its influence on the hydration, which can be explained by its effect on the pore size, and the mobility of the polymeric chains and the solvent. PHEMA hydrogels were reported to exhibit bi-phasic linear correlation between diffusivity and hydration (it exhibits a high slope at low hydration levels and more moderated slope for high hydration) probably due to different classes of water that are present inside the hydrogel (Wood *et al.* 1982). When a poor solvent is introduced to the network the polymeric chains tend to avoid that solvent and the network shrinks (Hirokawa and Tanaka 1984, Mukae *et al.* 1993).

Wood *et al.* (1981) reported on the effect of the solute structure in aqueous solution on the swelling of PHEMA hydrogels. Sugars and inorganic electrolytes caused partial dehydration of the hydrogel, due to the elevation of the solution osmotic pressure. The opposite effect was observed due to organic solutes with hydrogen bonding polar groups (such as ethylurea and acetamide), that usually induce swelling. Those solutes break hydrogen bonds within the network and interfere with the internal hydrophobic forces. These phenomena have a major influence on the effect of loaded solutes on the matrix swelling, and therefore on their release characteristics.

An interesting phenomenon exhibited by some hydrogels is the phase transition which occurs between a swollen and a collapsed phase of the hydrogel in response to chemical or physical stimuli (Tanaka 1992). This phenomenon is analogous to the behaviour of 'critical fluid' which can convert from a liquid to a vapour phase and back to liquid due to small temperature or pressure changes (Osada and Ross-Murphy 1993). This stimuli responsive phase transition phenomenon can be a basis for preparation of responsive drug delivery systems as will be discussed in this thesis.

Andrade and co-workers (Andrade *et al.* 1973, Lee *et al.* 1975) have suggested that hydrogels contain three classes of water : bound water, bulk (or free) water, and interfacial water. More than 20 years after their publication the condition of water inside hydrogel is still subject to controversy (Roorda 1994), with many contradicting results (mainly of DSC and NMR studies) concerning the number of classes of water in hydrogel, though the crude classification which is widely used describes the two main classes (bound and free water). The free water molecules are more significant for drug delivery because, unlike the bound water, they provide a good environment for solute transport.

1.1.5 Polyelectrolyte hydrogels

Polyelectrolyte hydrogels are in common use and can be found in pharmaceutical products (bioadhesive and enteric-coated formulation) as well as in personal care products (feminine care products, nappies and other

incontinence products) and industrial processes (sewage treatment, membranes and resins processes, and protein and biological synthesis products) (Harland and Prudhomme 1992). They can form the basis for pH, electro- or other responsive hydrogels (Brondsted and Kopecek 1992, Sawahata *et al.* 1990).

Polyelectrolyte hydrogels contain ionizable groups along the polymer chains. In pure water the chains tend to expand in order to minimize the reaction between them. The high osmotic pressure inside the hydrogels supports the high uptake of water. Many factors are known to effect the swelling of these hydrogels, such as the pKa of the ionizable groups and the solution pH which determines the degree of ionization, and the ionic strength of the solution (Brondsted and Kopecek 1992). The counter-ions have a vast influence upon the swelling of the polyelectrolyte hydrogels due to the high charge density inside the gels. Small changes in their concentration affect the hydrogel water content. In solution a charge separation is developed between the network and an oppositely charged layer from the fixed charge of the hydrogel. This layer is known as a 'double layer' which is defined as a very thin diffuse region in the pore of the hydrogel that contains enough counter ions to maintain gel neutrality (Atkins 1990).

Increasing the ionic strength of the medium reduces the swelling of polyelectrolyte hydrogels because of the shielding effect of those ions which reduces the electrostatic repulsion between the fixed charges and the reduction in the osmotic pressure. Multi-valent ions play an additional role in the swelling of such hydrogels since they can form, in some conditions, ionic bridges between the polymeric chains restricting the swelling, and at higher

concentrations they cause a phase transition in these gels (Ohmine and Tanaka 1982).

1.1.6 Immobilization of enzymes in hydrogels

It has been known for many years that the catalytic activity of enzymes can be better exploited if the enzyme could be insolublized (Goldman *et al.* 1971). Enzymes are usually immobilized in order to increase their stability and enable a long term operation. Since enzymes usually require an aqueous environment, hydrogels are suitable candidates for an immobilization matrix. The various techniques known for immobilization are entrapment (which is based on the occlusion of the biomaterial within the constraining hydrogel, this is a simple process that usually does not effect the enzyme structure though some leakage of it usually occurs), adsorption (not preferable because of its reversibility, but useful for cellular immobilization), and covalent binding (more complicated but prevents desorption). Combination of these techniques has also been reported (Pollak *et al.* 1980).

Gombotz and Hoffman (1987) reviewed the many applications of immobilized enzymes which include analytical and diagnostic applications, therapeutic bioreactors, drug delivery systems and other industrial applications.

1.2 Responsive polymers and hydrogels

The sea cucumber is essentially a water swollen gel which contains some primitive organs, but it exhibits an interesting capability for self-protection against attacks. In a response to any touch, the sea cucumber stiffens its usually flexible body and if the attack continues it can turn a part of his body wall into a viscous fluid mass which protects it from being grasped firmly (Osada and Ross-Murphy 1993).

Since the early 1950's scientists tried to mimic such biological response behaviour with synthetic responsive polymers. At that time most of the attention was focused on ways of converting chemical energy into mechanical work. Kuhn and Katchalsky pioneered this field, and their first published work described a dilation and contraction of polymeric networks by changing their ionization state (Kuhn *et al.* 1950). In recent years research into the field of responsive systems has been carried out in various scientific areas concerning sensing devices, robotics, artificial organs and drug delivery.

Responsive delivery of chemical substances takes place regularly in living animals. Membranes change their permeability due to environmental conditions and some substances are secreted by various organs in a pulsatile manner. Mast cells, for example, are secretory granules which in response to an electrical signal swell dramatically and release their histamine content within milliseconds (Navati and Fernandez 1993).

In the past delivery of drugs meant a rapid increase in the drug concentration after administration reaching a peak and then declining. This

caused some problems with some medicines, which have been overcome by the development of some polymeric release systems which allow better control of the drug release (sustained release, delayed release etc.). Nowadays more sophisticated drugs are being developed which require even better control and special patterns of release. In addition, some clinical situations require a more flexible administration regime. In these cases a better controlled and regulated delivery system is desirable. Delivery of insulin to diabetic patients, acid inhibitors for ulcer control, hormone replacement therapy, contraception, immunization and cancer chemotherapy are some of the areas where responsive and pulsatile drug delivery systems can be beneficial.

Responsive drug delivery systems can be divided into two types- as self regulated and externally responsive systems. Self regulated systems can detect a specific environmental condition and respond accordingly, adjusting the release to the physiological needs. Such systems includes pH sensitive polymers, enzymatically erodible polymers, competitive binding and metal concentration-promoted-hydrolysis. Externally responsive systems respond to thermal, photo-irradiation, electrical, ultrasonic and magnetic stimuli.

1.2.1 Self regulated polymeric systems

1.2.1.a pH-sensitive polymeric systems

pH-sensitive coating of pharmaceutical formulations represents an old form of responsive release. Formulations of acid-labile drugs were coated with

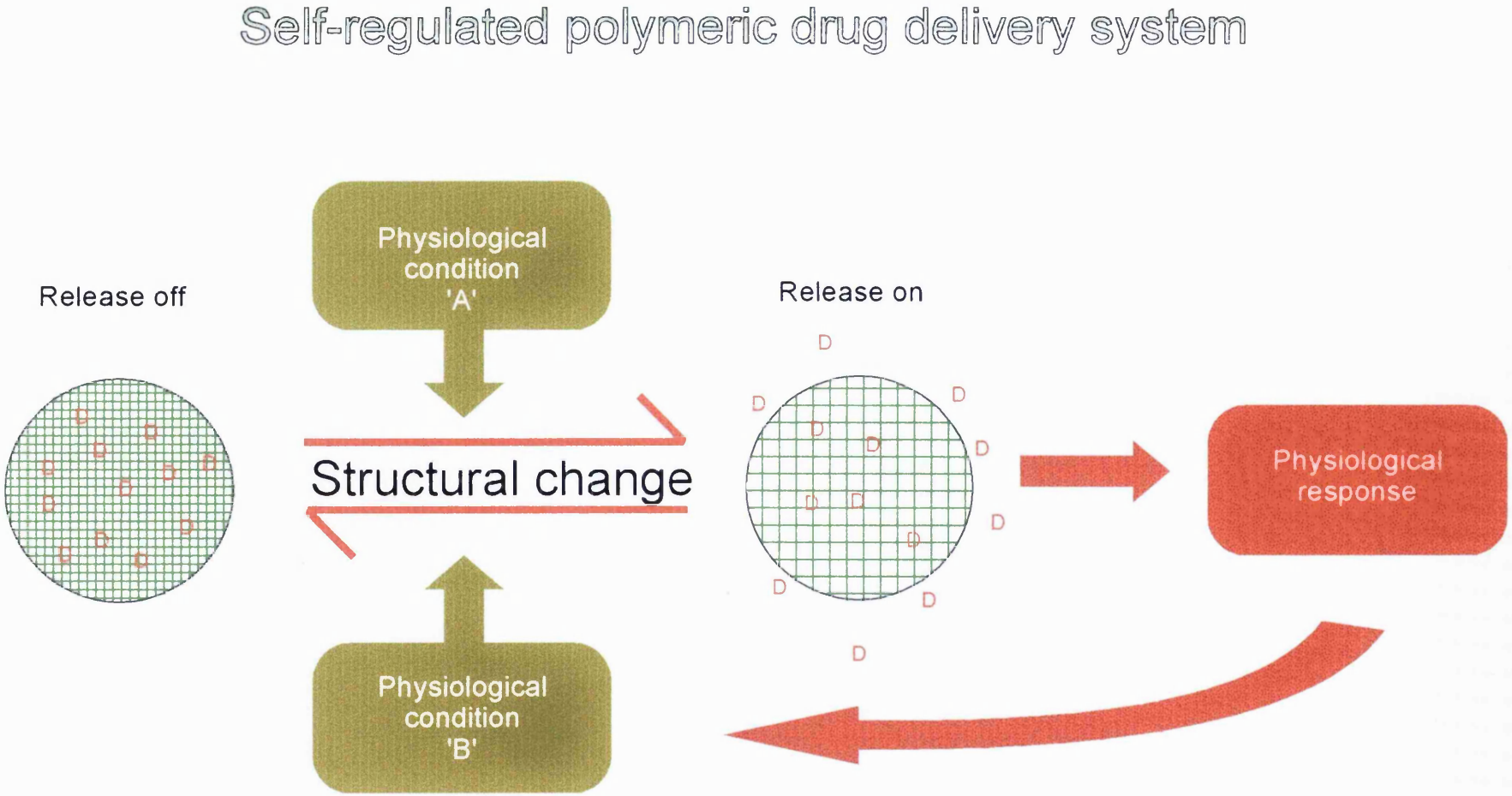


Figure 1.3 Self-regulated polymeric drug delivery system

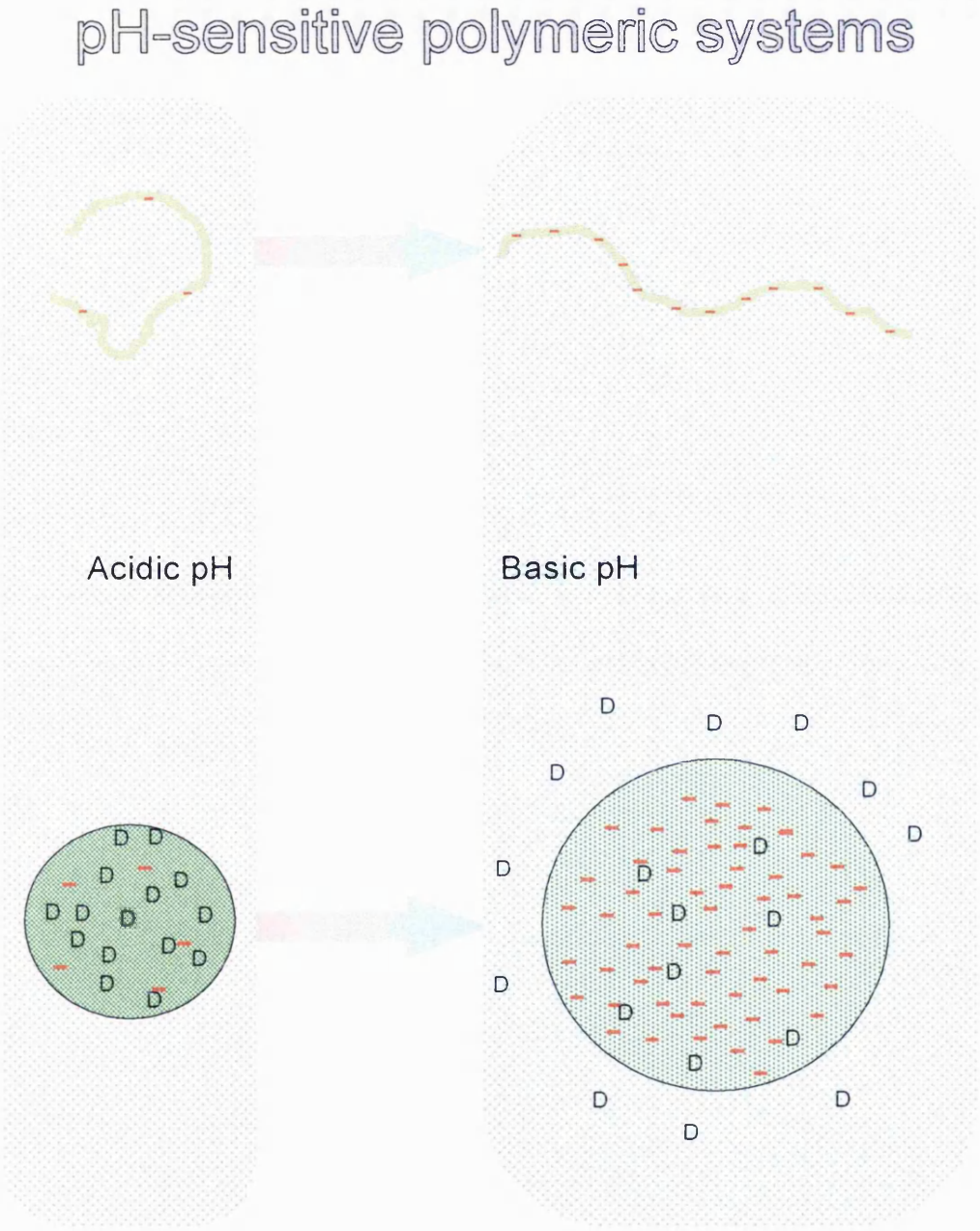


Figure 1.4 pH-sensitive polyacidic polymer and hydrogel. [−] negative charge

polymers which were only dissolved in the high pH of the intestine and therefore protected the drug from decomposition in the stomach (Jones 1970). In recent years pH-sensitive hydrogels have been developed, in which the whole network responds to changes in pH, and swells or deswells accordingly. These networks contain in their backbones acidic or basic groups. Polybasic polymers (usually containing amine groups) will swell extensively in an acidic pH environment due to the protonation of the amine groups, but increasing the pH causes deionization of this functional group resulting in deswelling of the network. Polyacidic polymers (usually containing carboxylate or sulphonate groups) will demonstrate the opposite behaviour. They are moderately swollen at lower pH, but highly swollen at high pH. Both types of hydrogels generally exhibit phase transition (reversible discontinuous shrinking) at certain critical pH values (Tanaka 1992). Factors affecting the equilibrium degree of swelling of pH sensitive hydrogels are the pH and the ionic strength of the solution, the charge concentration and pKa of the polymer, and the crosslinking density and hydrophilicity of the network (Brondsted and Kopecek 1992a).

Polybasic hydrogels composed of methylmethacrylate and dimethylaminoethyl methacrylate were investigated by Siegel and co-workers (1988). Those gels exhibit a sharp phase transition upon increasing the pH and deswell dramatically at pH 6.5. Caffeine was released rapidly at a lower pH (3 and 5) where the gel is highly swollen, but at pH 7 the solute was retained in the collapsed network. In a different study, the dependence of the swelling of such a hydrogel upon the ionic strength and ionic valence of the salts in the buffer medium was demonstrated (Firestone and Siegel 1994). Polyacidic

hydrogels which contain poly(acrylic acid) and poly(methacrylic acid) showed the opposite effect (Khare and Peppas 1993). They retain the model solute at low pH, and release it at higher pH. These hydrogels are suitable for targeting drugs to the intestine and colon.

pH modulation of drug permeability that involves different conformational and structural changes (but not swelling changes) have also been reported. Polyvinyl-polypeptide membranes can undergo conformational change from a rigid α-helix structure at low pH to a random coil at high pH. Sugar permeation through such membranes increases at high pH due to this conformational change (Chung *et al.* 1986). In a similar manner a poly maleic acid/ methylvinyl ether membrane changes its permeability in response to pH changes (Higuchi *et al.* 1986). pH responsive liposomes (Yatvin *et al.* 1980, Kitano *et al.* 1990, Wheatly *et al.* 1994) and microspheres (Bala and Vasudevan 1982, Kokufuta *et al.* 1988) have been reported as means of effecting responsive drug delivery.

1.2.1.b Glucose-sensitive polymers

Our bodies regulate glucose levels in the blood by an auto biofeedback process regulated by the pancreas, which releases insulin upon demand. Two different approaches, both aiming to fabricate a system that is able to sense the glucose levels and release insulin accordingly, were investigated.

Systems based on competitive binding:

Lectins are known to have high binding affinity to glucose. Synthetic glycosylated-insulin which maintains the biological activity of insulin can be easily bound to lectin (Brownlee and Cerami 1979). Kim and co-workers (Sato *et al.* 1984, Kim *et al.* 1990) prepared a complex of glycosylated-insulin within a PHEMA matrix. When the concentration of glucose increases it displaces the glycosylated-insulin which is associated with the lectin concanavalin A (con A) within the matrix causing it to diffuse out. *In vivo* studies of this implanted system in diabetic rats demonstrated its capability to maintain the blood glucose concentration in its normal range (Jeong *et al.* 1985). A different approach utilizing the same mechanism was described recently by Lee and Park (1994). They synthesized a water soluble polymer of PVP containing glucose pendant groups. In the presence of con A a hydrogel was formed. Each con A molecule can bind up to four glucose molecules allowing it to act as a crosslinking agent between the glucose containing polymeric chains. When exposed to high level of glucose, the polymer-con A complex dissociates causing degradation of the hydrogel which results in release of the gel content (i.e. insulin). The main problem posed by such competitive binding systems is the lag time (up to few hours) of response.

Systems based on immobilization of glucose oxidase in the matrix:

Glucose oxidase is an enzyme which oxidizes glucose in the presence of oxygen to gluconic acid and hydrogen peroxide. Formation of gluconic acid lowers the local pH, and this effect has been utilized to produce dimensional

changes in pH-sensitive hydrogels that can regulate the release pattern of insulin from it. The enzyme was immobilized in different networks that contain the basic monomer dimethylaminoethyl methacrylate which includes a *tert*-amine group. When exposed to high concentrations of glucose, the local pH was reduced due to the enzymatic reaction and the amides were ionized, resulting in increased network swelling, allowing the release of insulin. When glucose levels decreased, the production of gluconic acid stopped and the local pH increased, the hydrogel shrunk and the release of insulin was halted (Horbett *et al.* 1984, Ishihara 1988, Luz and Kost 1994).

Delvin and Tirrell (1986) used the same mechanism for synthesis of glucose-sensitive vesicles. Phosphatidylcholine vesicles were immersed in a solution of polyethylene acrylic acid and glucose oxidase and were shown to release their insulin content in response to elevation of glucose concentration. A different approach was reported by Ishihara *et al.* (1983). They immobilized glucose oxidase within a polyacrylamide network which contains a redox group (nicotinamide). In the presence of hydrogen peroxidase (that is produced by the enzyme in the presence of glucose) nicotinamide was oxidized and generated a positively charged group in the membrane, that increased the network water uptake and the permeation of insulin from the network by factor of 2.

1.2.1.c Other self-regulated polymeric systems

Enzyme digestible hydrogels were prepared to enable targeting

especially for specific parts of the gastro-intestinal tract. The common approach is to use an enzyme digestible crosslinking unit, which causes erosion of the gel when exposed to the digestive enzyme. Park (1988) used albumin as a bioerodible crosslinker. Brondsted and Kopecek (1992b) prepared hydrogels which were crosslinked by azobenzene-containing crosslinker which was degraded by an azoreductase for colon specific drug delivery. Another reported approach was to coat a polymeric matrix loaded with hydrocortisone with a bioerodible hydrogel composed of methylvinyl ether/ maleic anhydride copolymer which undergoes surface erosion at high pH. An enzyme such as urease is immobilized in these hydrogels. In the presence of urea, urease produces NH_4HCO_3 and NH_4OH which increase the local pH and stimulate the degradation of the hydrogel and the release of hydrocortisone from the internal polymer (Heller *et al.* 1987). These systems can be altered to be sensitive to different chemical substances by incorporating different enzymes in the hydrogel.

Responsive systems based on hapten-antibody association were reported by Pitt *et al.* (1985). Such systems have an enzyme which is covalently bound to a trigger molecule (which is an hapten). This is connected to an antibody within the polymeric network. The enzyme-heptane-antibody complex deactivates the enzyme due to steric hindrance of the antibody. When a free hapten is introduced, the antibody dissociates from the complex and the enzyme regains its activity which serves as a trigger for drug delivery.

1.2.2 Externally stimulated polymeric systems

1.2.2.a Thermo-responsive polymeric systems

Temperature change is a convenient way to induce responses in polymeric matrices since it is easy to apply and control with good precision. Thermally responsive polymers can be used to form different systems in different fields such as extracting and concentrating biosynthetic products (Trank *et al.* 1989, Park and Orozco-Avila 1993), bio-conjugates (Park and Hoffman 1993, Takei *et al.* 1993a,b), surface grafting (Okahata *et al.* 1986), immobilization of enzymes and biological cells (Dong and Hoffman 1986) and drug delivery systems (Affrassiabi *et al.* 1987, Okano *et al.* 1990). For drug delivery purposes temperature control can be easily applied externally using uninvasive techniques. physiological temperature changes within the body can be used as well for self-regulation and targeting of drugs. For example, hyperthermic treatment is known to potentiate the cytotoxic activity of doxorubicin in chemotherapy (Hahn *et al.* 1975). Using a thermo-responsive delivery system for doxorubicin can enhance this synergistic effect (Merlin 1991b).

Thermo-responsive polymers are known to exhibit the lower critical solution temperature (LCST) or upper critical solution temperature (UCST) phenomenon. Polymers exhibiting a LCST are soluble at temperatures below the LCST, but aggregate and precipitate when the temperature exceeds the LCST. Polymers exhibiting a UCST have the opposite behaviour.

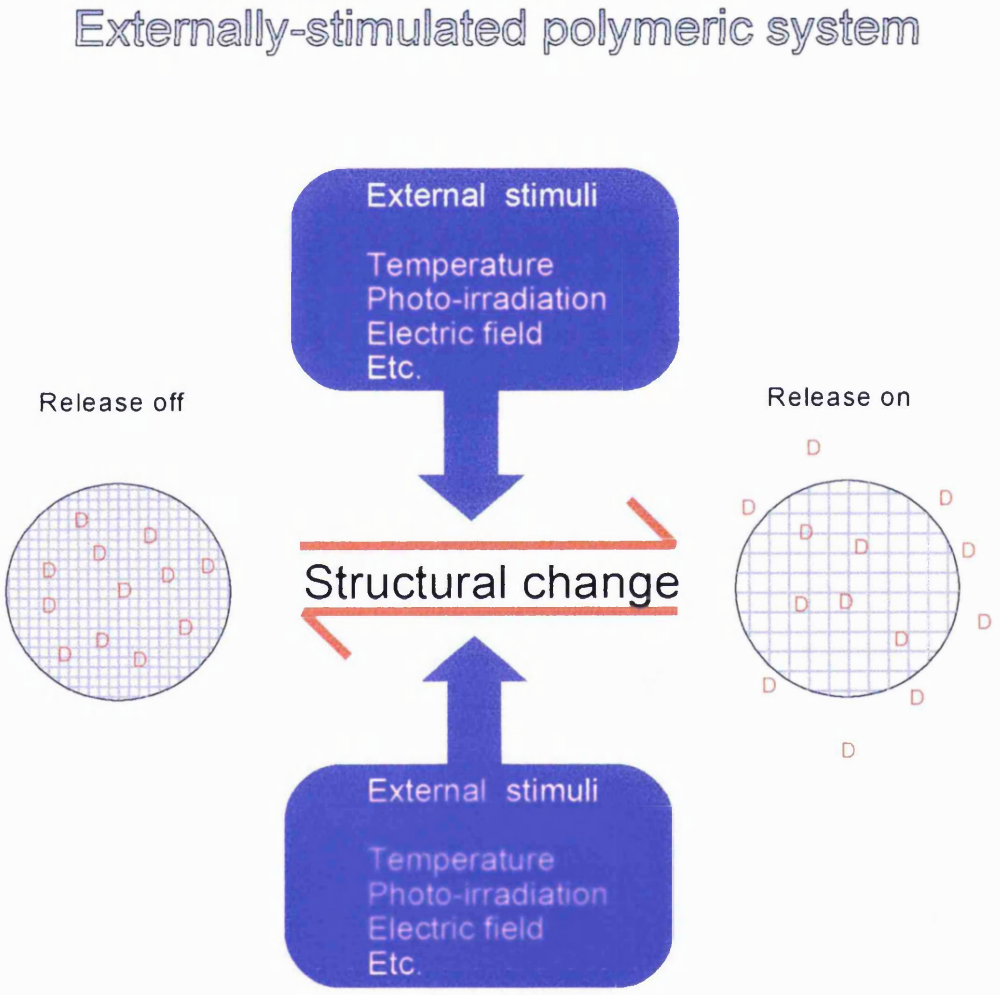


Figure 1.5 Externally-stimulated drug delivery system. An external stimulus can trigger and/or stop the release from the matrix.

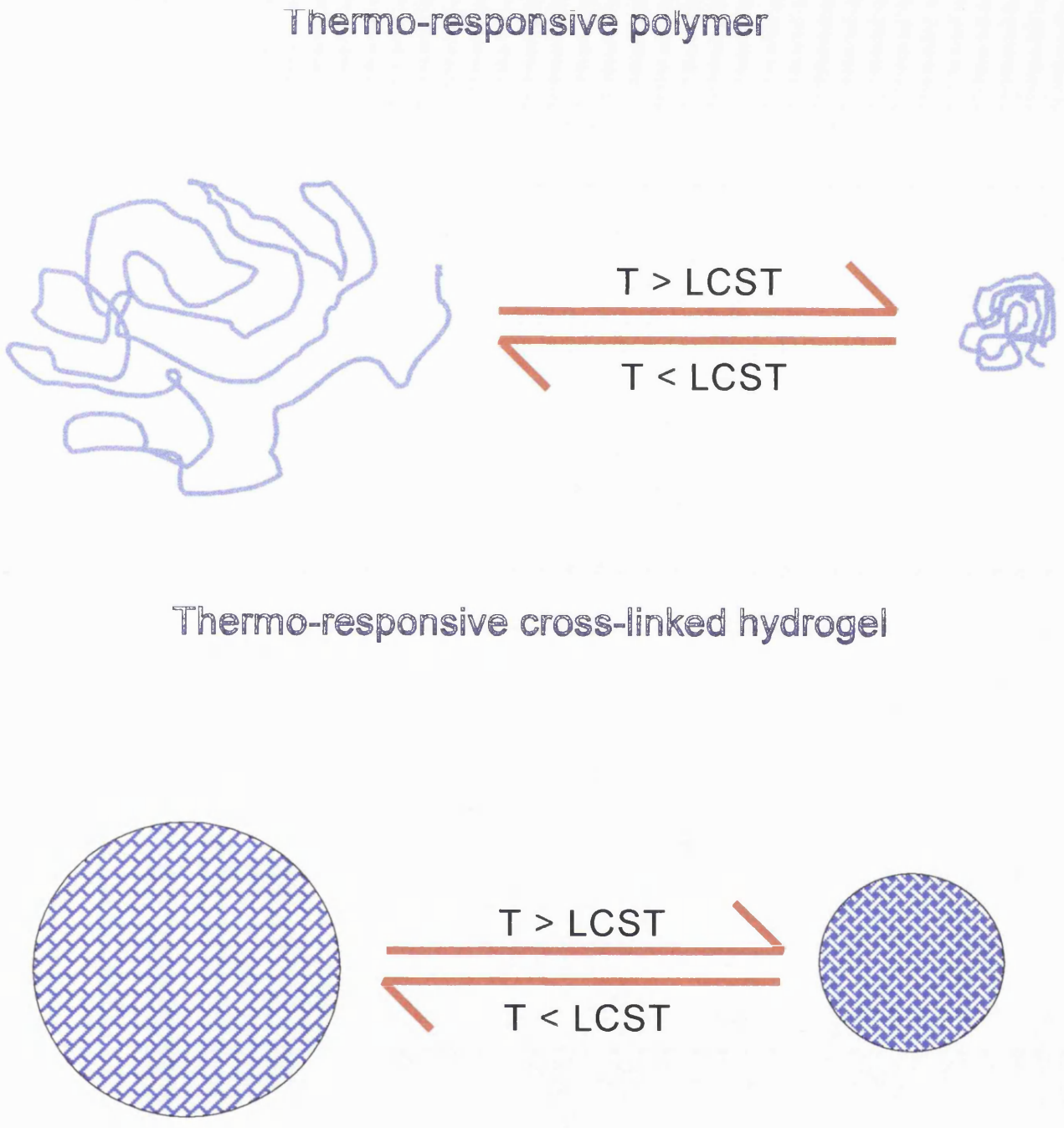


Figure 1.6 Structural changes in a thermo-responsive polymer and a cross-linked hydrogel

Typical thermo-responsive polymers which have been reported are: poly(N-alkylacrylamide) (Okano *et al.* 1990), poly(ethyleneoxide) (Baily and Callard 1959), poly(vinylethyl ether) (Horne *et al.* 1971), and poly(hydroxypropylacrylate) (Taylor and Cerankowsski 1975). Different systems that were reported for controlled drug delivery include hydrogels, lipid bilayers and membranes containing liquid crystal phases.

Thermo-sensitive hydrogels can be divided into two groups based on the origin of their thermo-sensitivity. The first is based on polymer-water interactions, especially the specific hydrophobic/hydrophilic balance of the polymer pendant group which affects its conformation, and the second is based on polymer-polymer interactions.

Poly(alkylacrylamide) hydrogels belong to the first group. Okano *et al.* (1990) investigated different derivatives with different alkyl groups and found that poly(N-isopropylacrylamide) (NiPAAm) showed the most temperature dependence profile, with significant deswelling upon increasing temperature and collapsing when the temperature exceeds its LCST (~32.0°C). Poly(diethylacrylamide) collapsed as well at temperatures above 32.0°C, but this process starts at a lower temperature and is more moderate than with NiPAAm. Poly(acryloylpyrrolidine) (poly(APy)) and poly(ethylacryl)amide also deswell upon heating but do not totally collapse. NiPAAm hydrogels exhibit the highest thermo-sensitivity due to their highly hydrophobic isopropyl group, whose mobility increases in response to temperature elevation, resulting in a reduction of its hydration capability (Bae *et al.* 1990). Several techniques were used to investigate the LCST of NiPAAm in aqueous solution, such as viscosity

(Fujishige 1987), fluorescence (Winnik 1990), differential scanning calorimetry (Schild and Tirrell 1990), and light scattering (Fujishige *et al.* 1989).

Opposite data on the effect of temperature on solute release from NiPAAm-containing hydrogels were reported. Hoffman and co-workers (Hoffman *et al.* 1986, Afrassiabi *et al.* 1987, Hoffman 1987) found that the shrinking of the hydrogel matrix at high temperature increases the release of vitamin B12 and myoglobin due to the 'squeezing effect' (where the solute is squeezed out with the water when the hydrogel collapses). Okano and co-workers (1990, 1991, Yoshida *et al.* 1994) demonstrated different release patterns from hydrogel matrices in response to different temperatures. Release of glucose, insulin and indomethacin was reduced or stopped upon deswelling of the matrix at high temperatures. There findings were explained as being due to the rapid formation of a 'shrinking layer' on the surface of the hydrogel as the first phase of the shrinking process. Such a layer restricts the diffusion of the solute, but allows the small water molecules to migrate out of the hydrogel. Okano and co-workers also utilized this phenomenon to achieve an on-off switchable release pattern from a poly(NiPAAm/ butylmethacrylate) hydrogel which stopped solute diffusion at a temperature above its LCST and regenerated it when the temperature was lowered. Hydrogel shrinking due to polymer-polymer interactions have been reported by Bae *et al.* (1988). They described an IPN that consist of Poly(APy) and PEO, a network which showed continuous deswelling upon increasing of temperature, and lost most of its water content above 50°C. This phenomenon can be explained by the involvement of the two polymers in an endothermic mixing process (i.e.

increasing of miscibility of two polymers upon elevation of temperature). At high temperature the interactions between the two polymers increase and the network contracts, whilst at lower temperatures the repulsion between the polymers increase, as well as an increase of the polymer-water interaction, causing expansion of the network.

Thermo-responsive liposomes have an advantage due to their ability of instantaneously targeting and controlling the release of drugs, though their response is not reversible (Weinstein *et al.* 1980). Different liposomes were reported to undergo phase transition of their membranes at 37°-45°C, which results in release of their hyper-osmotic content. Such systems include the release of doxorubicin from dipalmitoylphosphtidylcholine (DPPC)/ distearyolphosphatidylcholine (DSPC)/ cholesterol liposomes at 43°C (Merlin 1991a), calcein from DPPC/ DSPC liposomes at 40° and 45°C (Ono *et al.* 1994), dextran from DPPC/ DSPC/ dicetylphosphate at 42°C (Oku *et al.* 1994) and many more. A different approach was taken in preparing thermo-sensitive liposomes bearing NiPAAm on the outer membrane. Calcein or carboxy fluracein were loaded in DPPC or egg yolk phosphatidylcholine bearing a co-polymer of NiPAAm/ octadecylacrylate (LCST~27°C). These liposomes showed negligible release at 20°C, and rapid release above 25°C, probably due to the increase of hydrophobicity of the co-polymer (at higher temperatures) which induces strong interactions with the lipid bilayer resulting in destabilization of the vesicle (Kono *et al.* 1994).

On-off control of permeability of alkali metal ions through liquid crystal (LC) phases in a composite membrane due to thermal changes has been

suggested by Shinkai *et al.* (1987). The membrane was composed from polycarbonate / N-(4-ethoxybenzylidene)-4'-butylaniline (a liquid crystal) / amphiphilic crown ethers. Potassium ions (in a complex with the crown ether) diffused through that membrane at 40°C (which is above the LC phase transition temperature). The diffusion was in site-to-site mechanism through the fluid crystal membrane phase (formed by the LC organisation at that temperature). At 10°C the thermal motion of the LC was frozen causing a negligible diffusion of the potassium-crown ether complexes through the crystalline membrane.

Some other thermo-responsive drug delivery systems that were reported include nylon capsules grafted with NiPAAm and other poly(alkylacrylamide) which demonstrated reduced permeability at temperatures above the LCST due to a compact arrangement of the NiPAAm polymers (Okahata 1986), and surface grafting of NiPAAm polymers that entrapped heparin in their aqueous content, and released it at high temperature where they were losing their water content (Kim 1994).

Thermo-sensitive polymeric systems have other applications outside the drug delivery field. They can be utilized for isolation and concentration of large bio-synthetic products. NiPAAm hydrogels were used to isolate soy protein (Trank *et al.* 1989) and to concentrate bacterially produced cellulose (Park and Orozco-Avila 1993). The procedure of such process is to swell a dry hydrogel within the aqueous solution of the extracted protein or polysaccharide at low temperature (~5°C) (it takes up water and small molecules from the solution but excludes the high molecular weight protein/polysaccharide), then the hydrogel

is separated from the unabsorbed solution (retentate) by filtration or centrifugation and immersed in water at a high temperature (50°C), where it collapses and releases its content. Then it is returned to the retentate for another cycle until the desired product is purified and/or concentrated.

Conjugating a thermo-sensitive polymer to a bioactive substance can be useful especially for responsive separation and biological assays. The bio-conjugate is soluble and active at temperatures below the LCST of the polymer, but deactivated and precipitated when the temperature is increased to exceed the LCST. NiPAAm polymer was coupled to a carboxylate containing polymer and then conjugated to a bioactive molecule and chemical substances such as an enzyme (alkaline phosphate) (Park and Hoffman 1993), collagen (Takei *et al.* 1993a), BSA and bovine serum fibrinogen (Takai *et al.* 1993b). An example of purification using thermo-sensitive bioconjugates is the conjugation of NiPAAm to aminophenylphosphorylcholine which has high affinity to rabbit c-reactive protein (Mori *et al.* 1994).

Thermally reversible hydrogels were used as an immobilization matrix for enzymes. As previously mentioned hydrogels are a preferable matrix for immobilization of enzymes, but pose a problem due to their mass transfer resistance to the substrate, co-factors, or oxygen which can affect the enzyme activity. Increasing the mixing of the solution gives only partial answer to the problem. Immobilization of enzymes in thermo-responsive matrices allows feedback control and could help to overcome the mass transport problem, by inducing shrinking-swelling cycles of the hydrogel that act as a 'hydraulic pump' which enhance the transport and reduce the problem. Dong and Hoffman

(1986) first reported the thermal regulation of asparaginase immobilized in NiPAAm co-polymeric hydrogel, and later reported on an immobilized *Arthtobacter simplex* (which converts hydrocortisone to prednisolone) and increased its conversion rate due to temperature cycles that induce mass transfer (Park and Hoffman 1990).

1.2.2.b. Photo-responsive polymeric systems

Photo-responsive polymers are defined as polymers that change their properties reversibly under photo-irradiation. These polymers are synthesised by incorporation of a chromophore inside the backbone of the polymeric chains or as a pendant group. Such chromophores include the derivatives of azobenzene, triphenylmethyl leucohydroxide (or leucocyanide) and spirobenzopyran (Fig. 1.7 and 1.8).

Azobenzene neutrally exists as the *trans* isomer, but is isomerized to the *cis* isomer when irradiated by ultra-violet (UV) light. This conformational change alters the dimensions of the chromophore from 9.0Å to 5.5Å. The isomerization is slowly reversible in the dark and can be enhanced under visual light (Hartlet 1938, Hampson and Robertson 1941). Incorporating azobenzene pendant groups in the polymeric chain can result in two - opposite-effects as was previously reported (Lovrien, 1967; Matejka and Dusek, 1981) from viscosity measurements. In some structures the *trans* isomer increases the internal hydrophobic forces supporting a compact arrangement, whilst the formation of the *cis* isomer reduces those forces and allows the polymer chains

to expand. In other structures the isomers showed the opposite effect. Therefore, in some cases, UV irradiation can expand the polymer (Lovrien, 1967) and in others the opposite effect can be observed (Matejka and Dusek, 1981). A more simple mechanism occurs when an azobenzene chromophore is incorporated in the backbone of the polymer. Isomerization from the *trans* to *cis* form twists the polymer causing a more compact arrangement. This returns to the original dimensions when exposed to visual light. Viscosity measurements of polyamides confirmed this theory (Irie and Hayashi 1979, Irie *et al.* 1981). Kungwatchakum and Irie (1988) reported on photo-stimulated phase separation of polymer solutions. NiPAAm is known to undergo phase separation in solution at temperatures above its LCST. A 1% aqueous solution of a co-polymer of NiPAAm including 2.7% azobenzene pendant groups exhibits phase separation in the dark at 19.4°C. Upon UV irradiation the phase separation temperature (i.e. LCST) increases to 26.0°C. This can be explained by the change in the intramolecular hydrophobic forces which are known to effect the LCST. Increasing or reducing the azobenzene concentration was found to reduce the difference in the LCST between the different isomers containing polymers. The LCST of polymers containing above 3% or below 2% of azobenzene pendant groups were not affected by the photo-irradiation induced isomerization. At concentrations above 3% the polymer exhibits many hydrophobic interactions and the change induced by the isomerization does not affect the balance while at 2.7% the balance is sensitive to the photo-induced change. In different report Irie and Kungwatchakum (1992) demonstrated that such a system can undergo a reversible phase transition upon UV or visual radiation at temperature between 19.4 to 26.0°C.

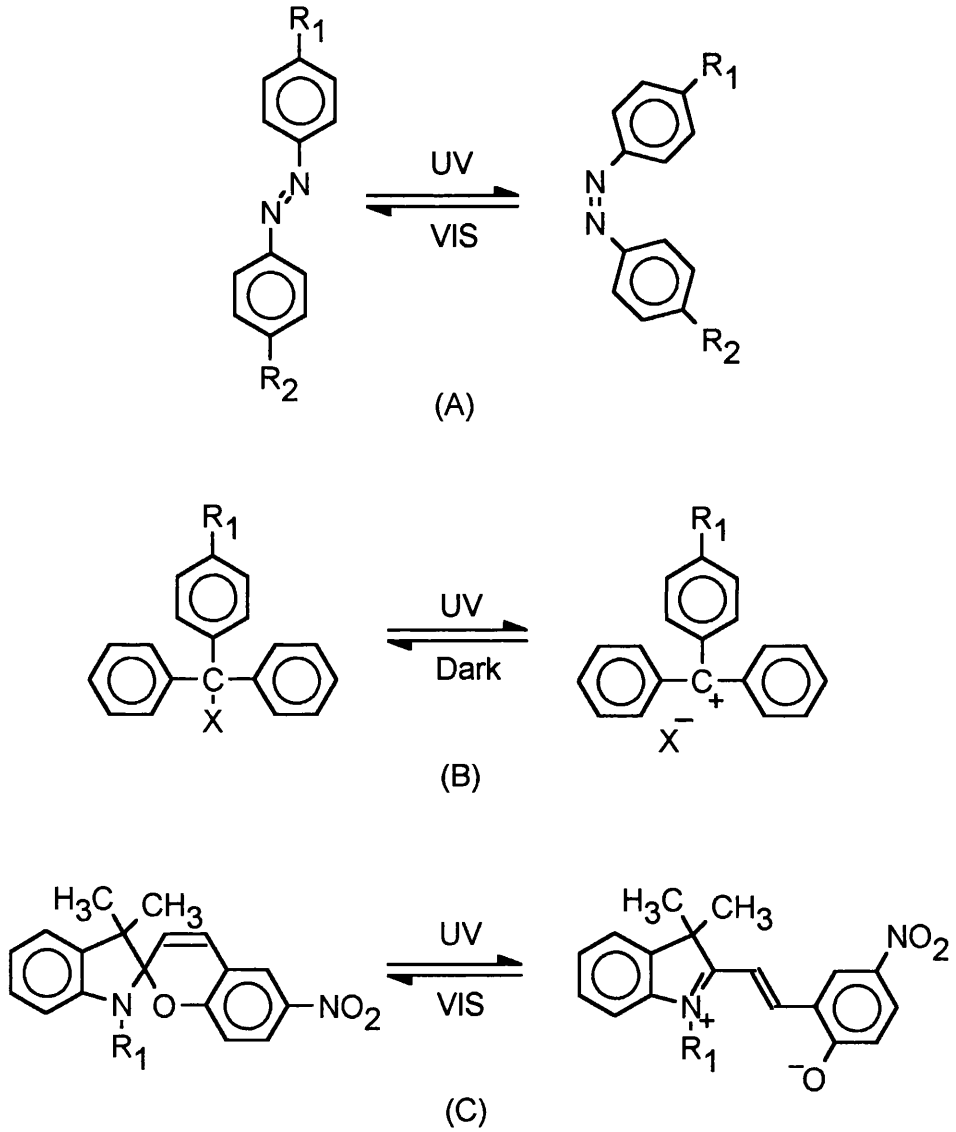


Figure 1.7 Three chromophore used in photo-responsive polymeric systems. A - azobenzene; B - Triphenylmethane leucohydroxide/ cyanide; C- Spirobenzopyran.

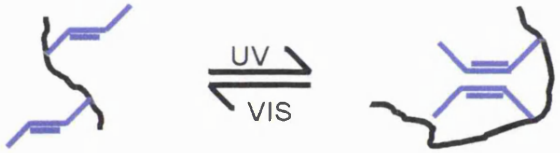
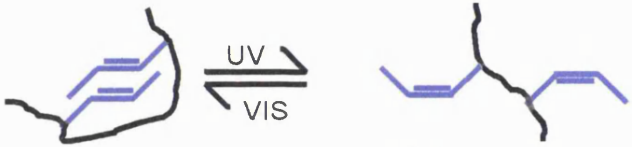
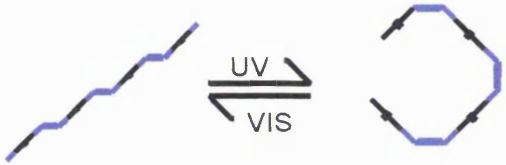
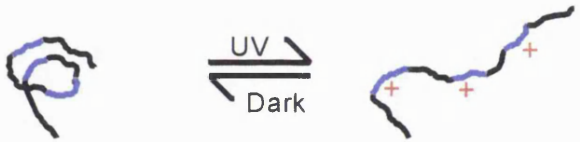
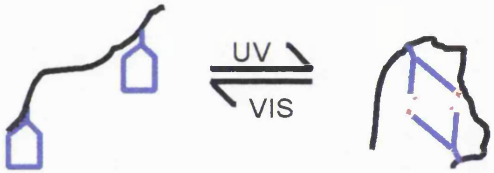
Chromophore	Photochemical reaction	Reference
Azobenzene (pendant group)		Matejka and Dusek 1981
Azobenzene (pendant group)		Lovrien et al. 1967
Azobenzene (backbone)		Irie et al. 1981
Triphenylmethane leucohydroxide		Irie and Kungwatchakum 1986
Spirobenzopyran		Irie et al. 1979

Figure 1.8 Chromophore used for the preparation of photo-responsive polymers and their photochemical reactions. Polymer -black; chromphore - blue.

Triphenylmethane leuco derivatives can be used as photo-receptor molecules. This chromophore dissociates into an ion pair under UV irradiation, generating an intensely green-coloured triphenylmethyl cation. This process is slowly reversible in the dark and can be induced by heating. Triphenylmethane leucohydroxide groups were incorporated inside an acrylamide hydrogel disc. The gel swelled by factor of 3 upon UV irradiation for 1 hour, due to the generation of positive charges on the polymeric network, and deswelled to its original size after 18 hours in the dark. Similar results were achieved using thin gel rods (10-180μm) (Irie and Kungwatchakum 1986). Incorporating triphenylmethane leucocyanide inside very thin poly(NiPAAm) hydrogels caused elevation of the LCST and a discontinuous phase transition when irradiated by UV light . When the temperature was fixed at 32.0°C (which is between the LCST of the hydrogel in the dark and its LCST under UV irradiation) the gel underwent a discontinuous swelling-shrinking switching upon exposure to and removal of UV radiation (Mamada *et al.* 1990).

Spirobenzopyran derivatives showed another response upon exposure to UV radiation. It undergoes a ring opening reaction leading to the formation of a zwitterionic merocyamine molecule which has a strong dipole. That reaction is reversible under visual light or heating. Viscosity changes of a solution of poly(MMA) with spirobenzopyran pendant groups upon UV irradiation have been reported (Irie *et al.* 1979).

Susiki and Tanaka (1990) utilized a different concept to achieve photo-stimulated phase transition in hydrogels. The trisodium salt of copper chlorophylline is a chromophore which can absorb light and dissipate heat.

They incorporated that chromophore inside a NiPAAm hydrogel and illuminated it with visual light from a laser source. The thermal dissipation of light energy caused a thermal-induced phase transition.

Only a handful of reports on photo-responsive drug delivery systems can be found. Mathiowitz *et al.* (1981 and 1989) reported on photochemical release from polyamide microcapsules. Azobisisobutyronitrile (AIBN) was encapsulated in these microcapsules. Under UV irradiation the AIBN releases nitrogen gas. A burst of release of a model solute in response to exposure to UV light was observed, due to photo-rupture and force diffusion caused by the gas produced.

Photo-degradation was reported as well as a basis for photo-responsive drug release. Photo-degradable microspheres, prepared by a coupling reaction of disuccinimo-4,4'-azobis(4-cyanovalerate) with L-lysine, demonstrated light-responsive release of BSA by irradiation from a UV source (Captain *et al.* 1991). Photo-degradation of hyaluronic acid (HA) hydrogels using visual light was described by Yui *et al.* (1993). A complex of HA with methylene blue was formed. Methylene blue is known to undergo photo-chemical auto-oxidation under visual light. The consequent production of free radicals or radical anions, causes degradation of the HA, resulting in release of the gel contents.

Willner and co-workers (Willner *et al.* 1993) described the regulation of immobilized α-chymotrypsin activity by light irradiation. The enzyme was immobilized inside three different matrices of acrylamide hydrogels with either azobenzene, triphenylmethane leucohydroxide or spirobenzopyran pendant groups. In all three hydrogel matrices, the enzyme was not originally active but

gained its activity under UV irradiation. The activity was lost when the chromophores were reversibly isomerized under visual light. This mechanism proved to be due to changes in the substrate permeation through the network, which was induced by UV radiation.

1.2.2.c Electro-responsive polymeric systems

The combination of applied electric field and drug delivery was first reported in the early years of this century (Leduc 1908). This was the first report on a method, which later came to be known as iontophoresis, of drug delivery through the skin. The permeation of the drug is induced by application of an electric field. The electric influence is on the drug, while the release matrix is inert (Burnette 1989).

In the last decade different electro-responsive systems were described, in which the matrix responds to the stimuli. Matrices which change their dimensions or degrade upon the application of an electric-stimuli were used as diffusion control membranes or drug carriers, as well as matrices that transform their electro-chemical energy to mechanical energy. In all such electro-responsive systems the matrix is composed of polyelectrolyte polymers. Reversible alteration of the electrostatic swelling forces, arising from the polyelectrolyte matrix fixed charged groups, can significantly change the matrix microstructure, dimensions and its diffusion properties. A great similarity can be found between the response to an electric field and the pH-induced change of such matrices.

The first hydrogel matrices which changed their dimensions under electric field were reported by Tanaka *et al.* (1982). A polyacrylamide was hydrolysed to obtain a poly(acrylamide/acrylic acid) (80:20) hydrogel. This hydrogel totally collapsed under electric field of 5V in water/acetone solution. The authors explain this response by two mechanisms: a stationary current which passes through the hydrogel and shields the fixed charges on it, and a stress gradient which deforms the matrix. The stress is caused by the fixed negative polymer that is unable to migrate towards the anode. That stress inside the hydrogel is higher near the positive electrode than near the negative one and causes the collapsing process to start near the anode. Osada and Hasebe (1985) described the same phenomenon in poly(2-acrylamido-2-methyl-1-propanesulfonic acid) (AMPS) hydrogels which lose 70% of their water content under an electric field of 6.3 V cm^{-1}.

Grodzisky and co-workers investigated the diffusion of solutes through electro-responsive hydrogel poly(methacrylic acid) (MAA) membrane, which its swelling exhibits sensitivity to the charge of the polymer. First, they demonstrated the pH dependence of the membrane. The membrane hydration increased by factor of 4 when the pH was elevated from 3 to 6, due to the ionization of the carboxylic groups of the polymer (Wiess *et al.* 1986). Applying an electric field across the MAA membrane also increased its hydration and swelling (Eisenberg and Grodzisky 1984, Grimshaw *et al.* 1990). Application of an electric field across the hydrated MAA membrane gives rise to a net force on the charge in the fluid phase, this is transferred to the solvent, resulting in electro-osmotic fluid flow through the membrane (Grimshaw *et al.* 1989).

Permeation of low and high (M.W. >6000) molecular weight solutes through the membrane was augmented under the influence if the electric field (Weiss *et al.* 1986, Grimshaw *et al.* 1989). The diffusion through the electro-responsive MAA membrane was compared to the diffusion of the same solute through a non-ionic (non electro-responsive) membrane. The results indicated that the influence of the electric field on the augmentation of the permeation is mainly due to the swelling of the membrane, while the effect of solute electrophoresis is much smaller. The electro-induced permeability enhancement of high molecular weight solutes was larger by at least one magnitude than the permeability enhancement of the low molecular weight solutes (up to factor of 54 for large solutes, compared with factor of 5 for small solutes). This indicates that for macromolecules the main factor influencing their permeability is the restricted diffusion due to steric hindrance, and electro-induced swelling reduces that hindrance.

Electro-responsive hydrogels have also been used as drug carriers. Electro-responsive release of hydrocortisone from poly (AMPS/BMA) hydrogel was demonstrated (Kwon *et al.* 1991b). The release was enhanced when an electric field was applied causing the shrinkage of the hydrogel and the squeezing of the solute from it. Better control of release was achieved when a positively charged solute was incorporated inside the hydrogel. A rapid release under electric field was observed, but the release rate was drastically reduced when the field was turned off. Electrostatic force and electro-osmosis, as well as the 'squeezing effect', were proposed as mechanisms. Sawahata *et al.* (1990) reported on switchable release of pilocarpine and raffinose from

MAA hydrogel due to on-off switching of a 12V cm^{-1} electric field.

A different electro-responsive delivery system was reported by Kwon and co-workers (1991a). A hydrogel was fabricated from a complex of PMAA and poly(ethyloxazoline) (POEx). The complex was formed, at a pH below 5.4, due to intramolecular hydrogen bonds between the carboxylic and oxazoline groups. This polymeric complex swelled and formed a hydrogel. The gel was placed on the cathode inside an acidic medium (pH<5.4). When a current was applied, hydroxyl ions were formed on the cathode causing the elevation of the local pH, this resulted in disruption of the hydrogen bonds and disintegration of the hydrogel into two water soluble polymers. Insulin was loaded in such a system, and was released in response to the electric current. This system demonstrates a well-defined pulsatile release, but it needs an acidic environment (pH<5.4). In a similar manner a complex hydrogel of heparin/poly(allyl amine) was synthesised which is stable at neutral pH. Heparin was dissolved and released when the current was switched on. In this system the drug is one of the polymers which forms the hydrogel (Kwon *et al.* 1994).

Different 'intelligent' hydrogels, which are able to transform electrochemical energy to mechanical energy, hve been reported. A gel pendulum was prepared from AMPS hydrogel (Okuzaki and Osada 1994). The hydrogel was fixed in one edge and suspended inside a surfactant solution between two electrodes. Different positively charged N-alkylpyridinium chloride surfactants were used. When an electric field was applied, the gel bends towards the anode, and when the polarity was periodically reversed, the gel

showed a repeated swinging movement like a pendulum. This bending was caused by an electro-stimulated complexation of the negatively charged network and the positively charged surfactant. The electric field induces the migration of the surfactant molecules toward the cathode. The migrating molecules are stopped by the suspended hydrogel rod and react to form a complex on the side facing the anode. This reduces the amount of charged groups on this side and consequently the electrostatic repulsion, causing an anisotropic contraction and bending of the hydrogel toward the anode. A similar gel network was used to create a 'worm-like' movement of a hydrogel rod which was suspended with two hooks from a long plastic ratchet bar in a surfactant solution (Osada *et al.* 1992). The polarity was reversed every 2 seconds, causing the bending and stretching of the gel, which exhibit a 'worm-like' motion in velocity of 25cm/min.

Many other interesting intelligent devices were reported such as 'golf club' that can strike a ball (Osada and Ross-Murphy 1993), gel 'fingers' which act in the open air and can hold a piece of paper, a gel beetle that can crawl up an incline, a flagging gel wing and many more (Kajiwara and Ross-Murphy 1992). Conceptual design of a swimming robotic structure was described using AAm/ PVA/ AAc polymeric hydrogel as a swinging tail which attaches to the head that contains the electrical device (Shahinpoor 1992). Applications of such electro-mechanical hydrogels can eventually include the contraction of gels which could possibly be inserted into a muscle to emulate the nervous system.

1.2.2.d Other externally stimulated polymeric systems

Other perturbations that were reported for external regulation of drug delivery systems include ultrasound, magnetic field and microwave irradiation.

Kost and co-workers described the use of ultrasound for responsive drug release from polymeric matrices. Bioerodible polymers (Kost et al 1988 and 1989) and non-erodible polymers (Kost *et al.* 1989, Lavon and Kost 1994) were used. Rapid erosion of the bio-erodible polymer matrices were observed. In both type of matrices the release was enhanced by exposure to ultrasound. Cavitation and acoustic streaming were suggested as the mechanism for the release enhancement. Increase of temperature and ultrasound induced mixing could have an influence as well. An *in vivo* experiment using an ethylene/vinyl alcohol copolymer implant for insulin release in diabetic rats was preformed. A sharp drop in blood glucose levels was observed, after the ultrasound irradiation, due to the fast release of insulin from the implant (Miyazaki *et al.* 1985). Another application of ultrasound is as an enhancer of drug permeation through the skin (defined as 'phonophoresis'). Levy *et al.* (1987) showed an ultrasound induced permeation (by factor of 5 to 20) of mannitol and insulin through the skin in rats and guinea pigs.

Oscillating magnetic field can be used as well to induce drug release. Magnetic beads were incorporated within polymeric matrices and their micromovements under the oscillating field enhanced the release. Kost *et al.* (1987) used an ethylene/vinyl acetate implant that entraps the magnetic beads and was loaded with insulin. *in vivo* studies in diabetic rats showed reduced

glucose levels upon demand due to the applied magnetic field. The release enhancement was reported to increase as the magnetic amplitude was risen (Edelman *et al.* 1985). The extent of release enhancement is dependent on the elasticity of the matrix. The enhancement of release due to the magnetic field increases as the matrix elasticity decreases (Kost *et al.* 1986).

Enhancement of release of 5-fluorouracil from ethylene/ vinylalcohol matrix upon microwave irradiation was reported, due to the heating of the system (Miyazaki *et al.* 1989).

1.3 Outline of work

The objective of the work described in this thesis is to demonstrate the probability of modulating drug release from hydrogels by external stimuli. Three different perturbations will be used: temperature, photo-irradiation, and electric field. The effect of the external stimuli on the swelling and release of different model drugs and solutes will be investigated with the aim of achieving an on-off switchable release systems.

Chapter 2 -Thermo-responsive hydrogels

2.1 Introduction

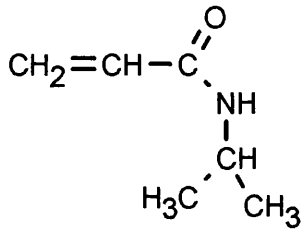
This chapter presents data from studies which investigate the potential of thermal control in drug delivery from hydrogels. All the matrices were based on the NiPAAm monomer because of its ability to display thermo-sensitive behaviour at convenient temperatures (20-50°C). The swelling profile of such hydrogels was studied, in order to understand the influence of temperature changes on the swelling state and to find some parameters which allow the modification of that profile. The release of water soluble and slightly water soluble solutes is examined as a function of different temperatures, with the objective of achieving an on-off switchable control of solute release. Lastly, a different aspect of thermo-control is investigated, namely the control on the activity of an enzyme which is immobilized within a thermo-sensitive hydrogel, and the mechanism of that effect will be examined.

2.2 Materials

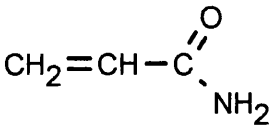
The materials used in the various experiments discussed in this chapter are listed in table 2.1. Methacryloylaminoazobenzene(MAAB) was synthesized in a procedure described in chapter 3. All proprietary materials were used as received without further purification. The water source was from an ultra high quality reverse osmosis water purifier (Elgastat UHQ PS - Elga U.K.). The equipment sources are stated within the relevant portions of the text.

Table 2.1 Materials used in the study of thermo-responsive hydrogels

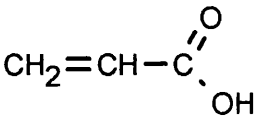
Material	Source
Acrylamide (AAm)	Aldrich Chemical Company (UK)Ltd.
Acrylic Acid (AAc)	Aldrich Chemical Company (UK)Ltd.
Ammonium persulfate (APS)	Fluka Chemika (Germany) Ltd.
Azoisobutyronitrile (AIBN)	Fluka Chemika (Germany) Ltd.
Caffeine	Aldrich Chemical Company (UK)Ltd.
Chlorhexidine diacetate	Sigma Chemical Company (UK)Ltd.
Flurbiprofen	Sigma Chemical Company (UK)Ltd.
β-Galactosidase (from *Escherichia coli*)	Sigma Chemical Company (UK)Ltd.
N- Isopropylacrylamide (NiPAAm)	Eastman Kodak (USA) Ltd.
Magnesium chloride ($MgCl_2$)	Sigma Chemical Company (UK)Ltd.
β-Mercaptoethanol	Sigma Chemical Company (UK)Ltd.
Methyl sulfoxide (DMSO)	Aldrich Chemical Company (UK)Ltd.
Methylenebis(acrylamide) (BIS)	Aldrich Chemical Company (UK)Ltd.
Myoglobin	Sigma Chemical Company (UK)Ltd.
o-Nitrophenol-β-D-galactopyranoside (ONPG)	Sigma Chemical Company (UK)Ltd.
Poly(styrenesulfonic acid) sod. salt (MW. 70000)	Polysciences (USA) Ltd.
Sodium chloride (NaCl)	BDH Laboratory Supplies (UK)
Sodium phosphate, monobasic (NaH_2HPO_4)	BDH Laboratory Supplies (UK)
Sodium phosphate, dibasic (Na_2HPO_4)	BDH Laboratory Supplies (UK)
N,N,N',N'-Tetramethylene diamine (TEMED)	Aldrich Chemical Company (UK)Ltd.



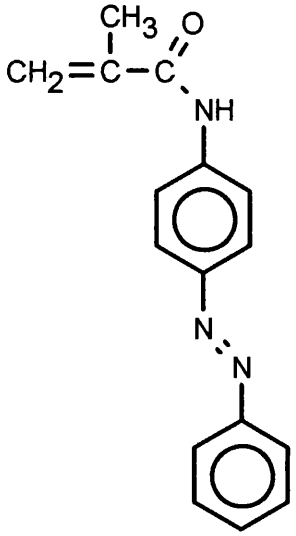
N-isopropylacrylamide (NiPAAm)



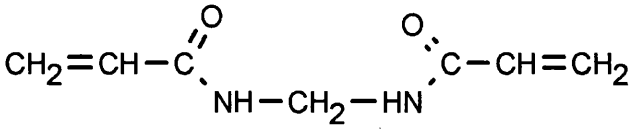
Acrylamide (AAm)



Acrylic acid (AAc)



Methacryloylaminoazobenzene (MAAB)



Methylenbis(acrylamide) (BIS)

Figure 2.1 Monomers used for the preparation of thermo-sensitive hydrogels.

2.3 Methods

2.3.1 Preparation of various NiPAAm hydrogels

All hydrogels discussed in this chapter were prepared by one phase synthesis (i.e. instantaneous polymerization and cross-linking) using chemical initiation in DMSO or an aqueous solution. Table 2.2 presents the amount and ratio of each chemical used in the different formulations. The bi-functional methylenebis(acrylamide) (BIS) was used as a cross-linking agent and AIBN, which is known to undergo a cleavage of its azo bond upon heating resulting in two free radical fragments, was used as the chemical initiator (at concentration of 7% w/v). The solution of the monomers, cross-linker and initiator was degassed by bubbling nitrogen through it (to remove dissolved oxygen) and then was injected into 1.5mm bore tubing. The polymerization was carried out at 60°C for 4 hours. Cylindrical gels (2-3mm in diameter) were cut and removed from the tubing, then washed with DMSO and water. They were then immersed in water for at least 7 days at 5°C, in which period the water was changed at least once.

Table 2.2 Gel compositions used in the preparation of thermo-responsive hydrogels

NiPAAm mg (%)	Co-monomer mg (%)	Cross-linker mg (%)	DMSO ml
Poly(NiPAAm) hydrogels			
1000 (100%)	--	0.3 (0.023%)	1
1000 (100%)	--	0.5 (0.038%)	1
1000 (100%)	--	1.0 (0.076%)	1
1000 (100%)	--	5.0 (0.378%)	1
1000 (100%)	--	10.0 (0.755%)	1
1000 (100%)	--	20.0 (1.510%)	1
1000 (100%)	--	40.0 (3.020%)	1
Poly(NiPAAm/MAAB) hydrogels			
977 (99.1%)	23 (0.9%)	1.0 (0.076%)	1
960 (98.2%)	40 (1.8%)	1.0 (0.076%)	1
928 (96.8%)	72 (3.2%)	1.0 (0.076%)	1
935 (97.1%)	65 (2.9%)	10.0 (0.755%)	1
Poly(NiPAAm/AAc) hydrogel			
977 (97.6%)	15 (2.4%)	1.0 (0.076%)	1

The figures in brackets refer to the molar percentage ratio of the total monomers.

2.3.2 Swelling measurements

Swelling studies were preformed on swollen hydrogels incubated at different temperatures between 22° to 50°C. The hydrogels were incubated for at least 4hr before the swelling ratios were recorded. The temperature was controlled using a water bath (SS40-D5, Grant U.K.) and a cooling system (CS-25, Grant U.K.) with the accuracy of ±0.1°C. The swelling ratio was calculated by using either weight or volume measurements.

Weight measurements: The swollen gels were taken out of the water and blotted on filter paper in order to remove water from the surface, before immediately weighing. The swelling ratio was calculated thus:

$$Swelling\ ratio = (W_{polymer} + W_{water}) / W_{polymer} = W_{swollen\ state} / W_{dry\ state}$$

Volume measurements: The radius (r) and the height (h) of the cylindrical hydrogel were measured using an image analyzer system and the object volume was calculated as $V=\prod r^2 h$. The swelling ratio was defined as the volume ratio of the swollen hydrogel to the non-swollen hydrogel (i.e. the hydrogel which was taken out from the tubing after the polymerization), and was calculated as:

$$Swelling\ ratio = V_{swollen\ state} / V_{non\text{-}swollen\ state}$$

Deswelling kinetics studies were preformed by pre-equilibrating the hydrogels at 22°C for 24 hours, and then transferring them to water at various temperatures. Their swelling ratios were recorded at different time intervals.

2.3.3 Drug/solute loading in the hydrogels

The water soluble solutes and drugs which were used in this chapter are caffeine, chlorhexidine, myoglobin and PSSA. Water swollen hydrogels were incubated at 50°C for 15min., then the collapsed hydrogels were transferred to 15mg/ml aqueous loading solutions of each of the solutes at 5°C and were stored at 5°C. The percentage of loading was calculated as the ratio of the maximum amount released from the hydrogels to the theoretical amount of solute (calculated as: $A_{theoretical}$= water content in the gel (ml)• concentration of the loading solution). The percentage of loading was calculated thus:

$$\% \text{ loading} = A_{experimental} / A_{theoretical}$$

The slightly water soluble drug, flurbiprofen, was loaded on the hydrogel via a different procedure. A 15mg/ml solution of flurbiprofen in ethanol/water (80:20) was used. The hydrogels were loaded using the same procedure described above, and then were frozen at -45°C and freeze-dried for 48hr. The freeze-drying method was used in order to eliminate the possible migration of the drug towards the surface which can occur when drying at room temperature. The dried hydrogels were stored at 5°C (in the presence of silica gel).

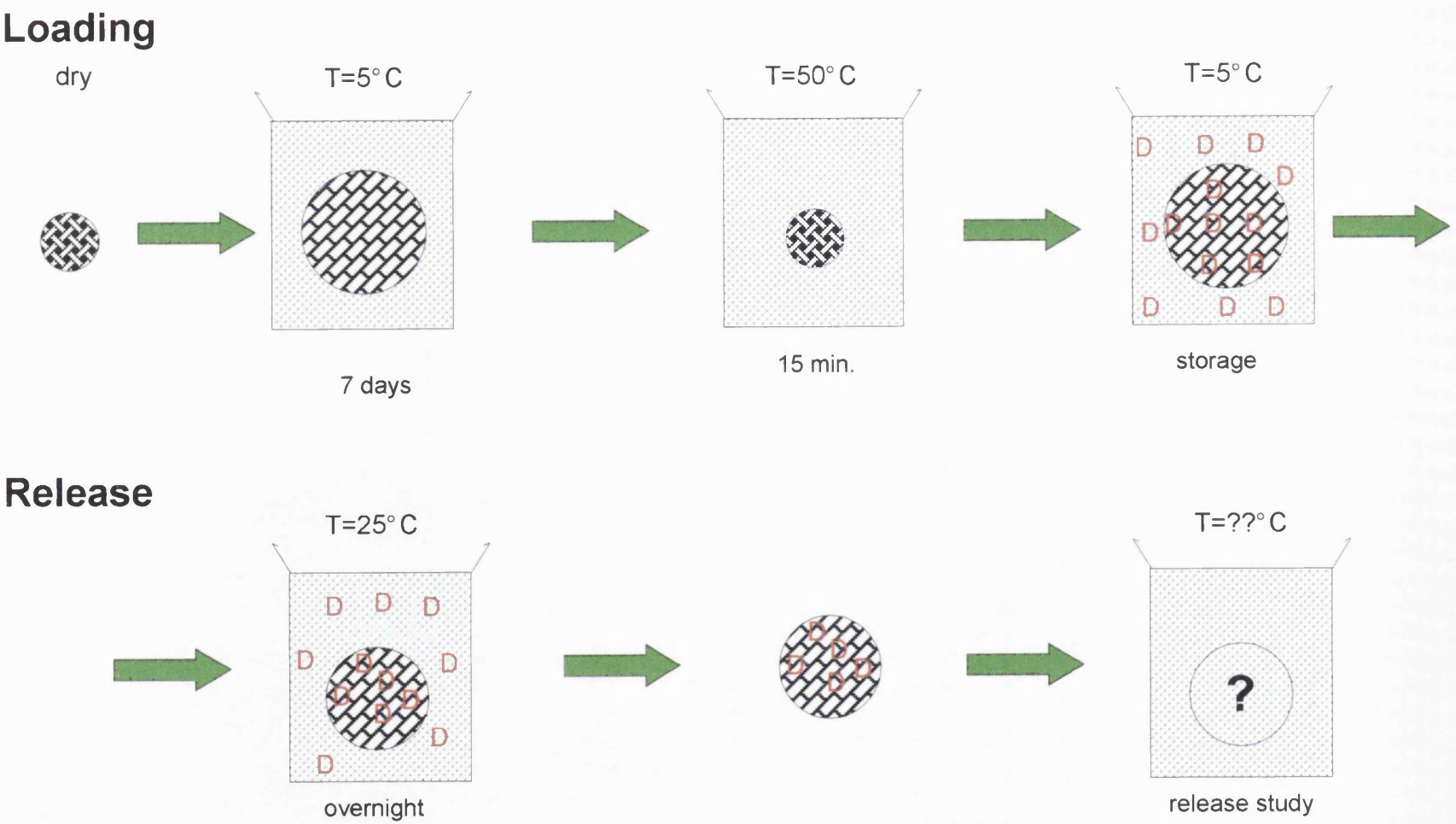


Figure 2.2 Procedure for the loading and release of solutes on/from thermo-sensitive hydrogels

2.3.4 Drug/solute release from the hydrogels

All hydrogels were equilibrated overnight in the loading solution at 25°C before any release study. The hydrogel samples were immersed in 5-25ml of fresh water at various temperatures in a shaking water bath. 0.25-1ml aliquots of the release media were taken at specific intervals and the volume made up with fresh water. Solute concentrations were determined using a Shimadzu spectrophotometer (MPS-2000) at the following wavelengths: caffeine (273nm), chlorhexidine (254nm), myoglobin (279nm), PSSA (224,261nm) and flurbiprofen (247nm). Percent release was calculated as the percent ratio of the amount of solute released at a specific time to the maximum drug released (measured after a week at 5°C). The release of flurbiprofen from dry hydrogels was presented as the amount released which was normalized to the dry hydrogel weight. All measurements were performed in triplicate.

2.3.5 Immobilization of an enzyme within thermo-sensitive hydrogels

β-Galactosidase was immobilized within NiPAAm/AAm hydrogels of different monomer ratios as described in table 2.3.The monomers and 3 mole% of cross-linker (BIS) were dissolved in 11ml of 0.1M sodium phosphate buffer and then degassed by bubbling nitrogen through. 17ml of initiator (APS) and 1000 units of β-galactosidase were dissolved in 1ml buffer, mixed with the monomer solution and degassed again. 175μl of TEMED (an accelerator) was added and the solution was immediately injected between two glass plates

separated with a 0.5mm Teflon spacer and cooled to 5°C. The polymerization was performed at low temperature to avoid reaching the heat of polymerization which could denature the enzyme. The hydrogel sheet was removed from the glass plates after 24hr and the discs were punctured out of the sheet with a cork borer. The hydrogels were stored in buffer solution at 5°C.

The hydrogels were tested for leakage of enzyme by immersing a hydrogel disc in a buffer for 3 days and performing an activity assay on an aliquot of the buffer medium. No activity was detected in any hydrogel batch which might indicate a negligible leakage of enzyme.

2.3.6 Assay of enzyme activity

o-Nitrophenol-β-D-galactopyranosidase (ONPG) was used as the enzyme substrate. The product of the enzymatic reaction, o-nitrophenol, was measured spectrophotometrically at 410nm, after 10min. of incubation of the immobilized or free enzyme within the assay solution (2mM ONPG, 10mM β-mercaptoethanol, 10mM magnesium chloride).

The specific activity was defined as:

$$\textit{Specific activity} = W_{product}(\mu g) \,/\, [\textit{assay time (min)} \bullet W_{(\textit{dry gel or free enzyme})}(mg)]$$

2.3.7 Diffusion study of ONPG through NiPAAm/AAm hydrogel membrane

Hydrogels membranes were equilibrated at the appropriate temperature and then placed in a diffusion cell as a membrane separating the donor and the acceptor compartments. A buffer solution was circulated through the donor compartment at the desired temperature and an aliquot of it was analyzed spectrophotomerically (262nm) over 10min. The contents of the donor compartment were continuously stirred using a magnetic stirrer.

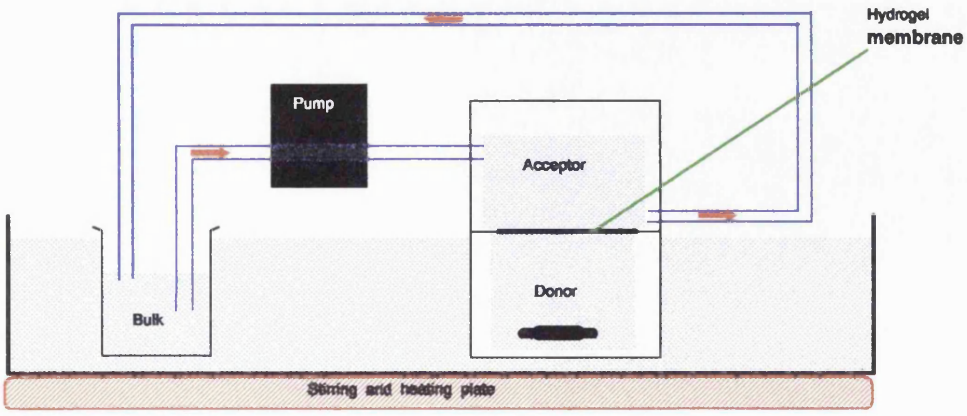


Table 2.3 Gel compositions used for the immobilization of an enzyme within thermo-responsive hydrogels

NiPAAm	AAM	Cross-linker	Buffer Solution
mg (%)	mg (%)	mg (%)	ml
--	1750 (100%)	110 (3.0%)	12
2250 (80%)	350 (20%)	110 (3.0%)	12
2425 (90%)	175 (10%)	110 (3.0%)	12
2500 (94%)	100 (6%)	110 (3.0%)	12
2550 (97%)	50 (3%)	110 (3.0%)	12
2600 (100%)	--	110 (3.0%)	12

The figures in brackets refer to the molar percentage ratio of the total monomer.

2.4 Swelling behaviour of thermo-responsive hydrogels

The amount of water taken up by hydrogel matrices is a very significant factor which affects the controlled release of solutes. Generally, greater water sorption increases the rate of release of solutes from the matrix, since it generates a more open and less tortuous pore structure. Investigating the effect of different factors, such as the degree of cross-linking and the incorporation of different co-monomers into the network, on the swelling behaviour of the thermo-sensitive hydrogels, is useful in order to understand some of the processes which occur in the hydrogels as a response to temperature changes, and can influence the release profile from such hydrogels, as will be demonstrated later in this chapter.

A typical swelling profile of NiPAAm hydrogel is shown in fig. 2.3. The hydrogel is highly swollen at 22°C, but deswells moderately when the temperature increases. As the temperature nears the LCST of the polymer, the rate of deswelling becomes rapid, and above the LCST the hydrogel totally collapses, exhibiting a swelling ratio of 1 (which means that the hydrogel has lost all of its water content, its weight being equal to the dry hydrogel weight). Hydrogels which were prepared with various cross-linker concentrations show different swelling ratios at temperatures below the LCST (fig. 2.4). The degree of cross-linking affects the elasticity of the network. A high degree of cross-linking reduces the elasticity and restricts the ability of the hydrogel to stretch and uptake more water. This explains the difference in swelling of the three NiPAAm hydrogels at temperatures below the LCST. The difference is mainly

in the content of the free water, whose portion increases in the less cross-linked network. As the temperature is elevated the hydrogels start losing their water content (mainly the free water fraction). The less cross-linked hydrogels, which contain more free water, deswells more rapidly than the most cross-linked hydrogel (which has less free water). The three hydrogels collapse when the temperature reaches the LCST losing all their free and bound water. The fact that the LCST is independent of the cross-linking density shows that this phenomenon is solely due to the polymer characteristics and is independent of the three dimensional structure of the the network. The LCST occurs because of the increase in hydrophobicity of the polymer which reduces its ability to become hydrated. This phenomenon can be explained thermodynamically. Increasing the temperature results in a decrease of the entropy of the network because of the collapse process that results with a more compact arrangement. The unfavourable entropy decrease and increase of enthalpy are compensated for by a large entropy gain due to the water which is released from the bound water state to the free water outside the hydrogel. The total entropy gain supports thermodynamically the collapsed form of the gel (Prange et al. 1989).

Co-polymerization of another monomer within the network influences its swelling profile. Fig. 2.5 shows the effect of incorporating a hydrophobic monomer (MAAB) on the thermo-sensitivity of the network. 0.9mole% of MAAB reduces the LCST by 2°C, as well as reducing the swelling ratio of the matrix below the LCST. MAAB increases the hydrophobic interactions between the polymeric chains and reduces the ability of the network to become hydrated. Increasing the proportion of MAAB reduces further the LCST and the swelling

below that temperature. At high concentration of 3.2mole%, the deswelling process becomes linear upon elevation of temperature, and the collapse of the gel is more moderate at the LCST. This can be explained due to the initial high hydrophobicity of the network, which is therefore less sensitive to hydrophobicity changes when the temperature is increased. Incorporation of a highly hydrophillic co-monomer, such as acrylic acid (AAc), results in the opposite effect (fig. 2.6). The LCST is elevated to 44°C and the swelling ratio below that temperature increases extensively (to a swelling ratio of up to 80), and is insensitive to temperature changes below 37°C. AAc introduces a negative charge into the network which increases its hydrophilicity and therefore reduces the effect of the hydrophobicity changes.

Fig. 2.7 shows the effect of acrylamide (AAm) which was copolymerized with NiPAAm. These hydrogels were prepared using a different procedure described in section 2.3.5, which affects the extent of initial swelling of the hydrogels but the thermo-sensitive behaviour which is described below exhibited in all circumstances. Homogeneous AAm hydrogels are not sensitive to changes of temperature, because AAm does not have any pendant group. Copolymer hydrogels with equal concentration of each monomer show a moderate linear thermo-sensitivity but do not exhibit the LCST phenomenon. The thermo-sensitivity increases when the proportion of NiPAAm is increased in the network. Hydrogels with a molar ratio of 90:10 (NiPAAm/AAm) regain the typical thermosensitive swelling profile of NiPAAm hydrogels and exhibit the LCST phenomenon (at 37°C).

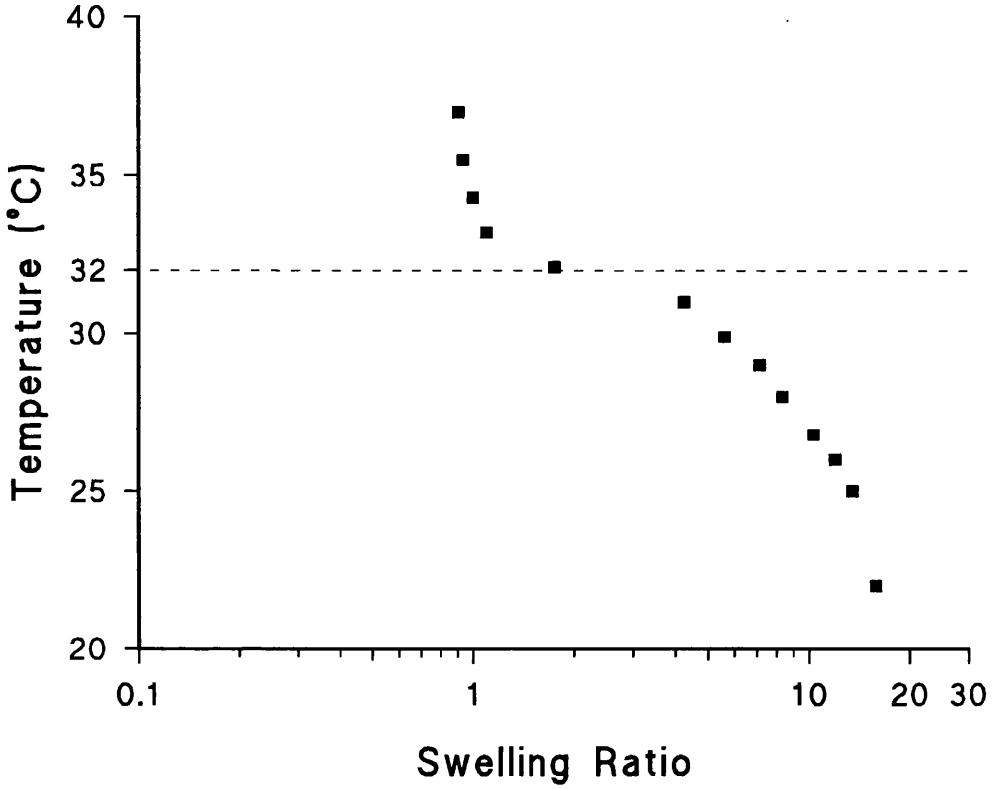


Figure 2.3 Swelling profile of NiPAAm hydrogels with 0.023 mole% cross-linker in water, as a function of temperature.

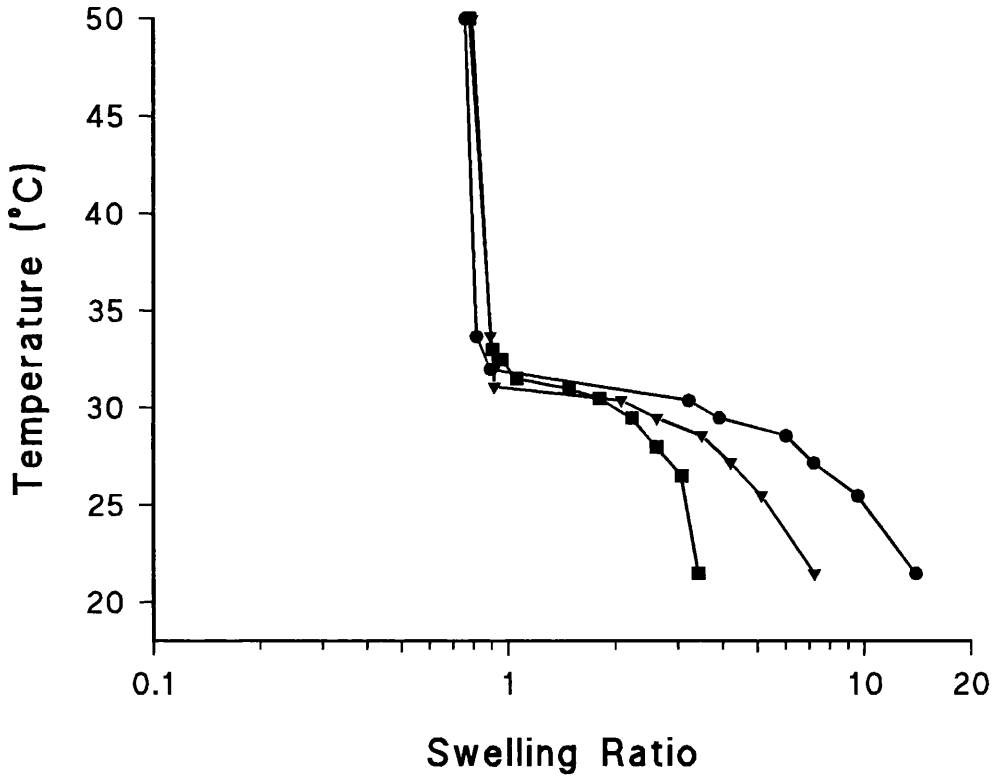


Figure 2.4 Swelling profile of NiPAAm hydrogels with 0.076 mole% (●), 0.378mole% (▼) and 3.02 mole% (■) in water, as a function of temperature.

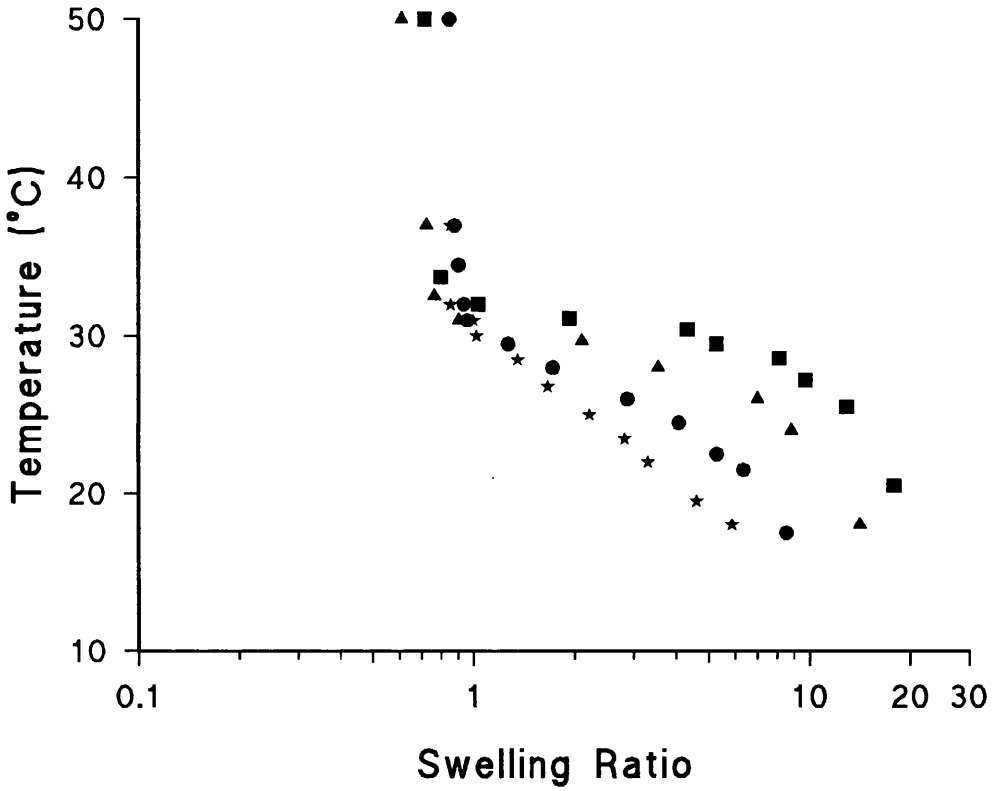


Figure 2.5 Swelling profile of co-polymerized hydrogels of NiPAAm with 0 mole% (■), 0.9 mole% (▲), 1.8 mole% (●) and 3.2 mole% (★) of MAAB in water.

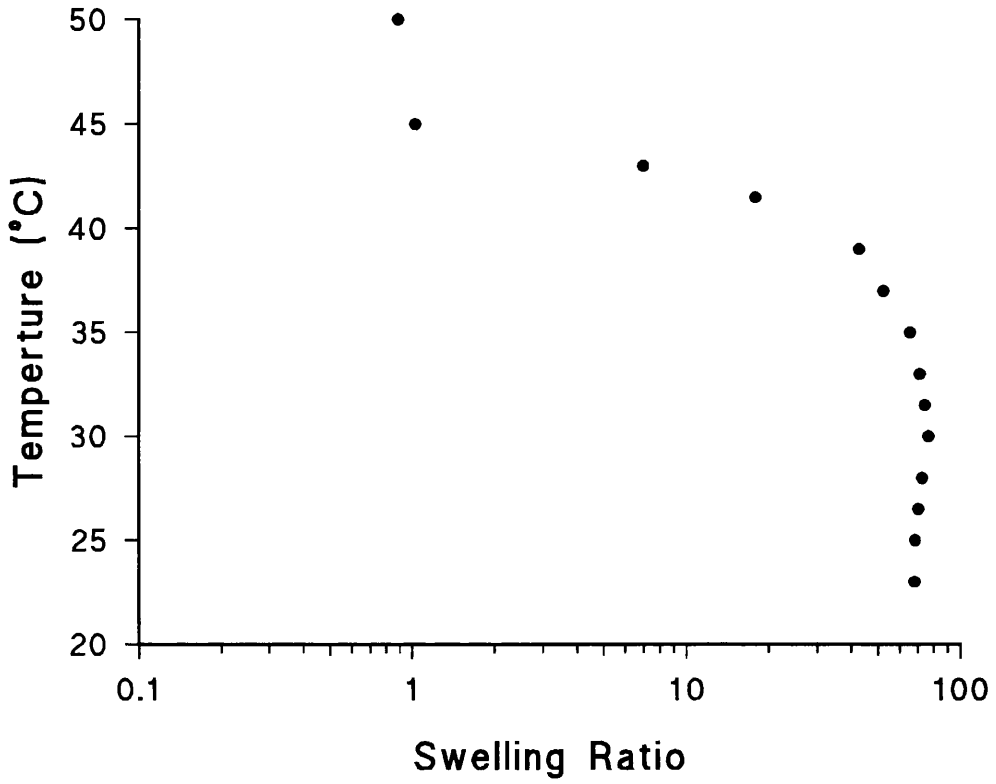


Figure 2.6 Swelling profile of NiPAAm/acrylic acid (97.6/2.4 mole%) hydrogels with 0.076mole% cross-linker in water, as a function of temperature.

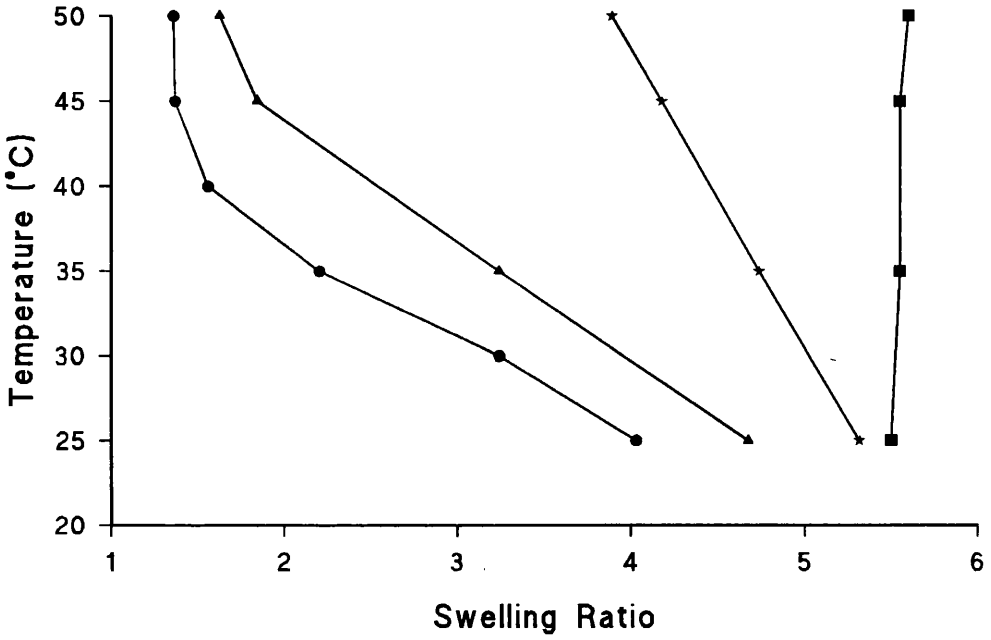


Figure 2.7 Swelling profiles of hydrogels consisting of NiPAAm/ AAm in different ratios (0/100mole%[■], 50/50mole%[★], 80/20mole%[▲] and 90/10mole%[●]).

The examples discussed above (figs. 2.3, 2.4, 2.5, 2.6) show the main principal which can be used in the preparation of hydrogels which are 'tailored' to exhibit a required swelling profile by using various monomers and cross-linker in different concentrations.

Deswelling kinetic studies were performed in order to add the time dimension which is important for understanding the release profiles from NiPAAm hydrogels. The hydrogels were equilibrated at 22°C and then the temperature was increased and the deswelling process was recorded. As seen in fig. 2.8, hydrogels which were transferred to higher temperatures deswelled more rapidly then those transferred to lower temperatures. All the temperatures examined in this study were above the LCST. At 33°C (just above the LCST) the deswelling was slow and the collapsing process took more than two hours, while at a higher temperature of 50°C, the hydrogels collapsed faster within 30 min.

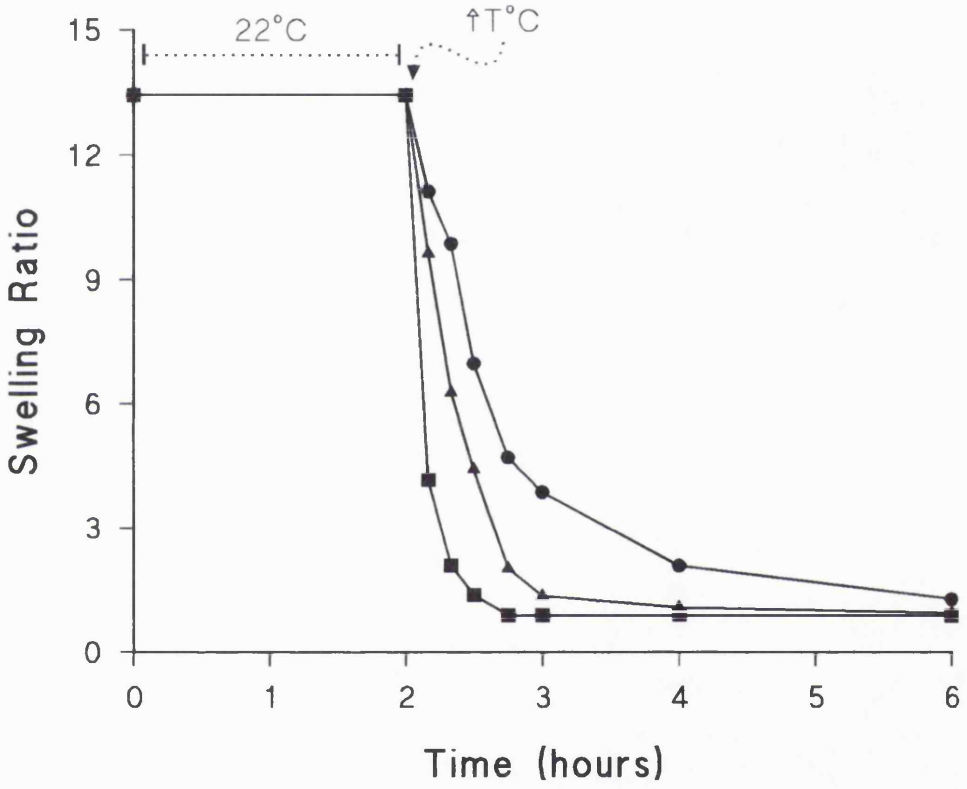


Figure 2.8 Deswelling kinetics of NiPAAm hydrogels with 0.076mole% cross-linker, upon increase of temperature to 33°C (●), 37°C (▲) and 50°C (■).

2.5 Loading of solutes on NiPAAm hydrogels

Fig. 2.9 shows the percent loading of solute of various molecular weights on NiPAAm hydrogels at 5°C. Solutes of low molecular weight such as caffeine and chlorhexidine (which is not shown in this figure) were quantitatively loaded, but solutes of high molecular weight (myoglobin and PSSA) were only partially loaded. The NiPAAm hydrogels were prepared in a random copolymerization which resulted in some areas exhibiting a higher cross-linking density than others, and which consequently would not be accessible to the solute molecules due to steric hindrance. The effect of cross-linking density on the loading can be seen in fig. 2.10 . Hydrogels with a low degree of cross-linking (0.076mole%) showed 60% loading, whilst that figure reduced by a third (to ~20%) when the cross-linker concentration was increased by ten fold.

2.6 Release of water-soluble solute from thermo-responsive hydrogels

The release of caffeine from NiPAAm hydrogels at different temperatures is described in fig. 2.11. At 25°C, caffeine diffuses rapidly from the matrix reaching 100% release before the first half hour. This is due to the highly swollen state of the hydrogel which does not restrict the diffusion of the small highly soluble solute from the matrix. The release profile at 33°C shows the same pattern, despite the fact that this temperature is above the LCST of the polymer. The reason for this similarity can be found in the deswelling profile of the hydrogel at that temperature, as seen at fig. 2.8. The NiPAAm hydrogel

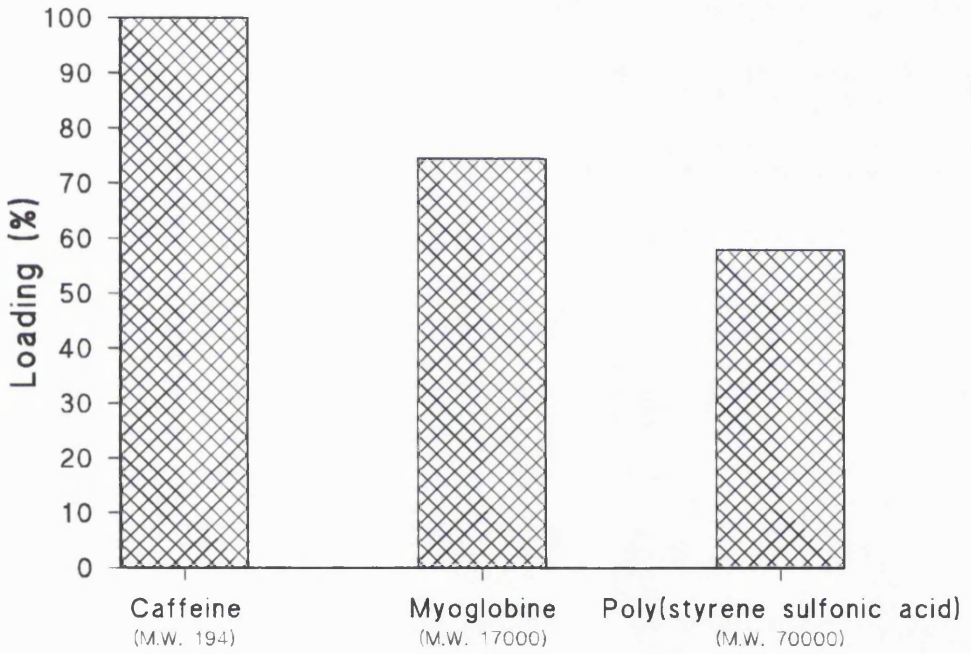


Figure 2.9 Percentage of loading of caffeine, myoglobin and PSSA in NiPAAm hydrogels with 0.076mole% of crosslinker.

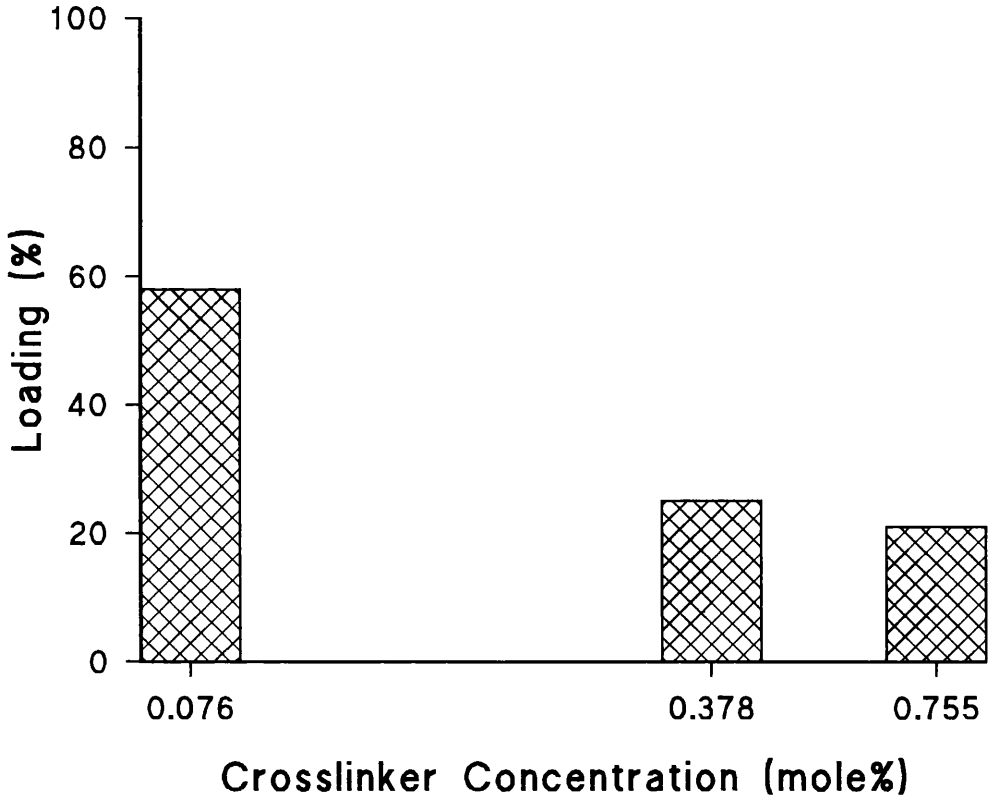


Figure 2.10 Percentage of loading of PSSA on NiPAAm hydrogels as a function of the crosslinker concentration.

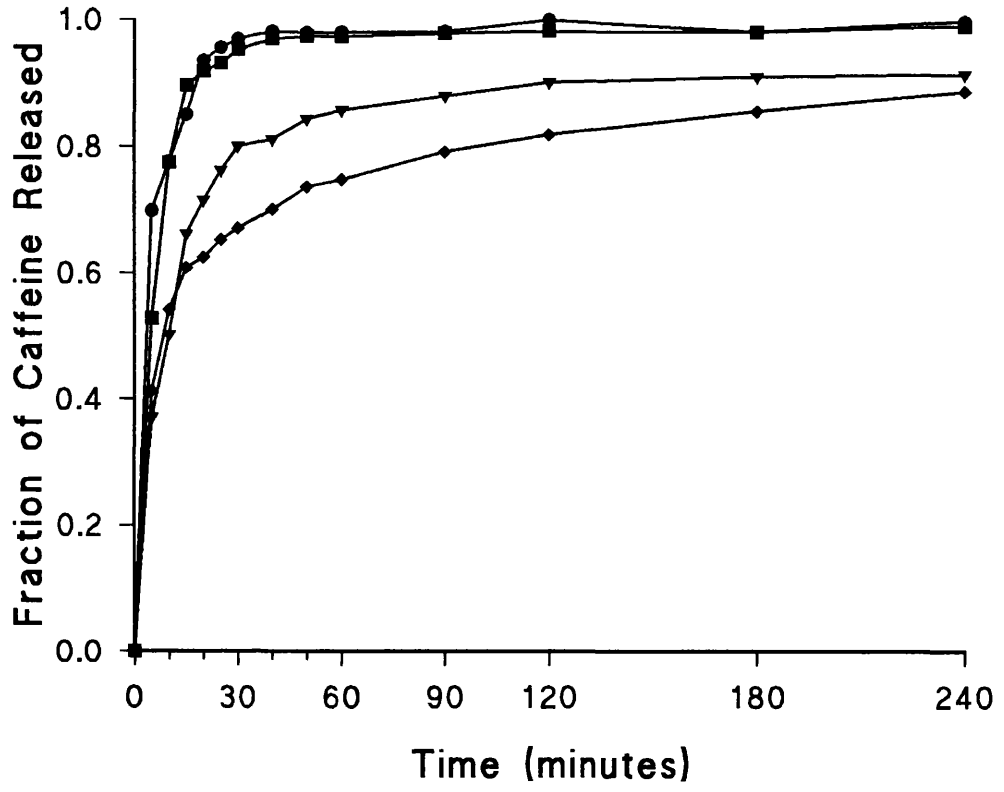


Figure 2.11 Release of caffeine from NiPAAm hydrogels with 3.02mole% crosslinker at 25°C (●), 33°C (■), 37°C (▼) and 50°C (♦).

deswells slowly at 33°C and totally collapses after more then 2hr. Since all the caffeine is released within the first 30 min., the release is not affected by the slow volume change.

The influence of the deswelling network on the release profile is notably observed at 37°C, the release is slower (just 85% is released within the first hour) and only 90% of the loaded dose is released whilst the rest is trapped inside the collapsed hydrogel. The NiPAAm hydrogel collapses faster at 37°C and reaches a swelling ratio of 1 after approximately an hour, though the effect of the collapsing process can be seen earlier in the first 10 minutes. This could be due to the formation of a thin and dense 'shrinking layer' on the hydrogel surface as described by Okano and co-workers (Okano et al. 1990, Yoshida et al. 1994). Since the surface is the first to 'sense' the high temperature of the release medium, it is the first to collapse and forms the 'shrinking layer' which restricts the release of the small solute from the matrix but allows the diffusion of the small water molecules out of the hydrogel (fig. 2.12). The diffusion of the solute is thus restricted before the hydrogel is totally collapsed. At 50°C the deswelling of the hydrogel (and probably the formation of the surface 'shrinking layer') is even faster and the release of caffeine is more restricted (~72% is released within the first hour).

Figures 2.13, 2.14, 2.15 and 2.16 describe the effect of different cross-linking concentrations on the release of caffeine at various temperatures. At 25° and 33°C the release is very fast and is not affected by the degree of cross-linking of the hydrogels. At higher temperatures the influence of the cross-linker concentration on the release becomes apparent, increasing the

cross-linker concentration reducing the rate and the extent of the release. Fig. 2.17 summarizes the effect of various temperatures and cross-linker concentrations on the percent of caffeine release.

The release of a macromolecular solute, PSSA, was more affected by the degree of cross-linking. Differences in release can be observed even at 25°C (fig. 2.18) due to steric hindrance which affects the diffusion of the large PSSA molecule. At 37°C (fig 2.19) the difference is greater since the effective pore dimensions are further reduced by the collapsing process. Figs. 2.20 and 2.21 compare the release of three water soluble solutes from the NiPAAm hydrogels. As expected the diffusion of the larger solute is slower from the same hydrogel at both temperatures.

The main obstacle in using water soluble solutes at all molecular weights is that they diffuse very rapidly from the hydrophilic swollen network, therefore the temperature-induced structural changes have only a partial or negligible effect on the release characteristics. The approach which was chosen to overcome this limitation was to load a slightly water-soluble drug into the hydrogel. Such a drug would be released over a prolonged period in comparison to that of the water soluble solutes (a few days instead of several hours).

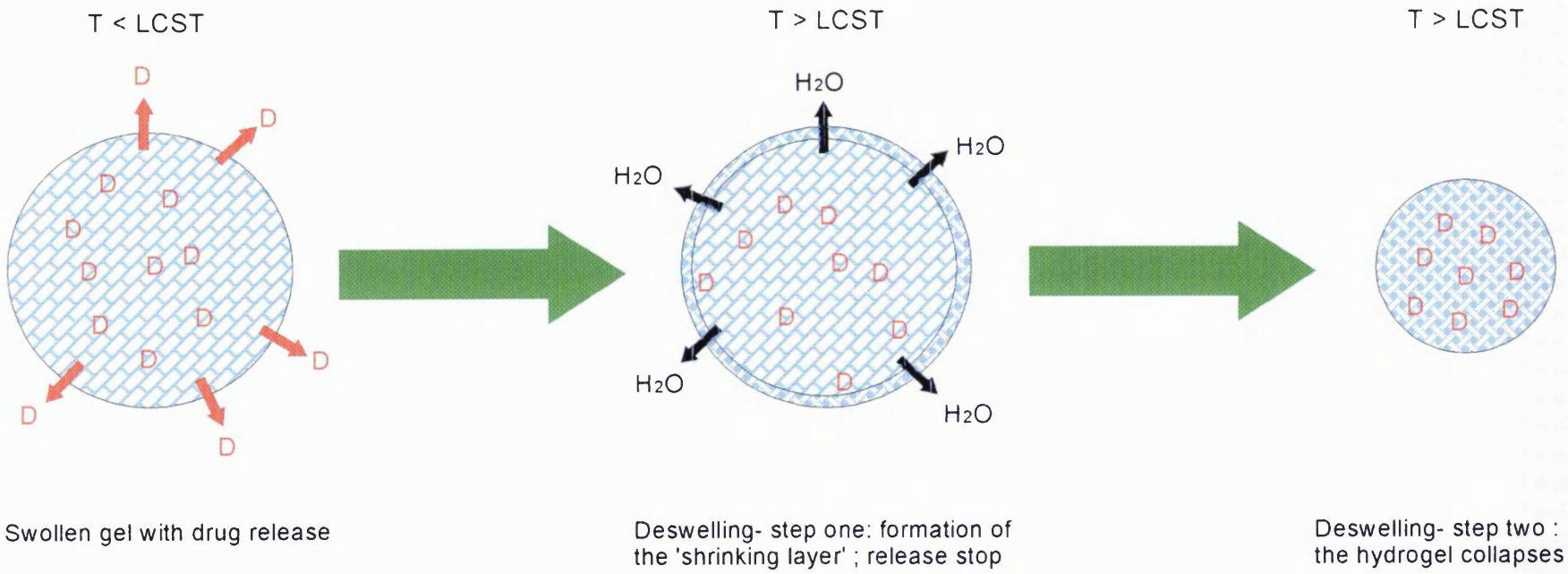


Figure 2.12 The deswelling process of NiPAAm hydrogel upon elevation of the temperature above the LCST.

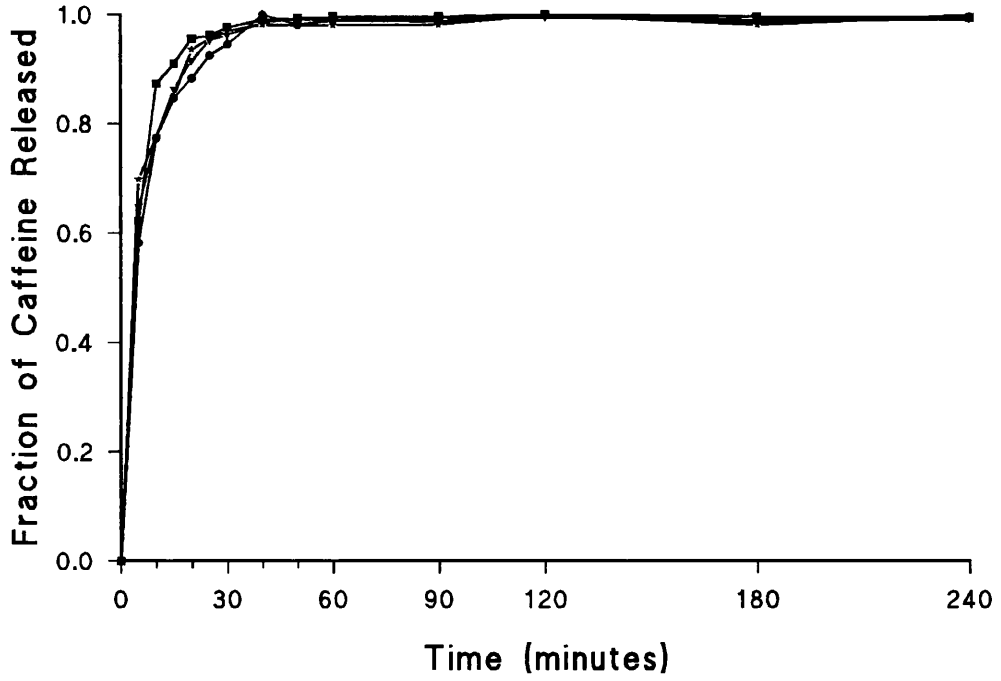


Figure 2.13 Release of caffeine from NiPAAm hydrogels with 0.076mole% (●), 0.755mole% (■), 1.51mole% (▼) and 3.02mole% (★) cross-linker **at 25°C**.

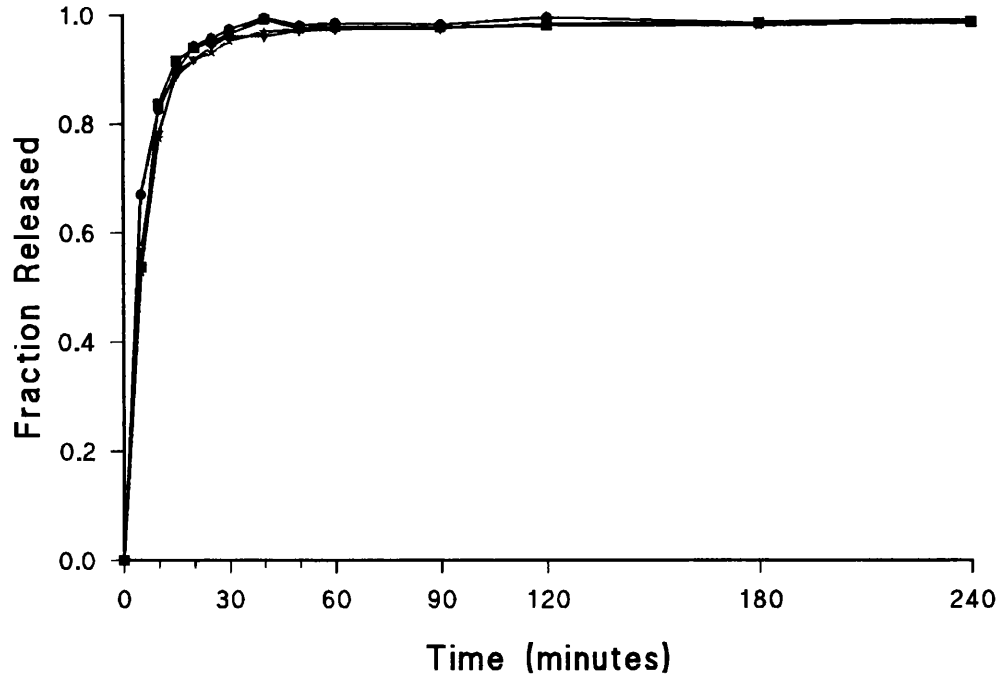


Figure 2.14 Release of caffeine from NiPAAm hydrogels with 0.076mole% (●), 0.755mole% (■), 1.51mole% (▼) and 3.02mole% (★) cross-linker **at 33°C**.

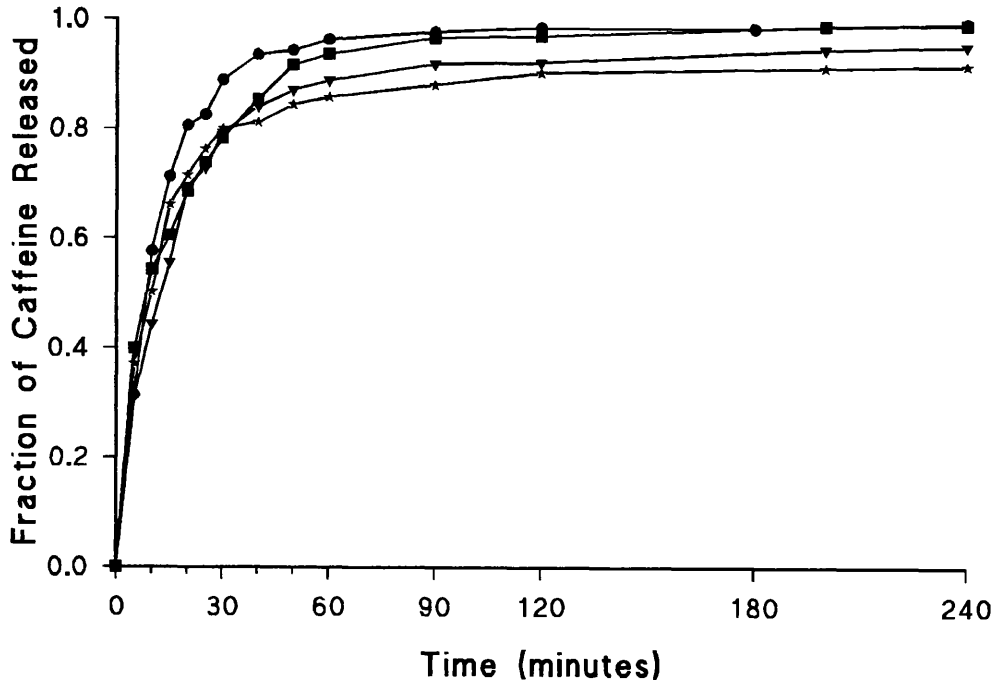


Figure 2.15 Release of caffeine from NiPAAm hydrogels with 0.076mole% (●), 0.755mole% (■), 1.51mole% (▼) and 3.02mole% (★) cross-linker **at 37°C**.

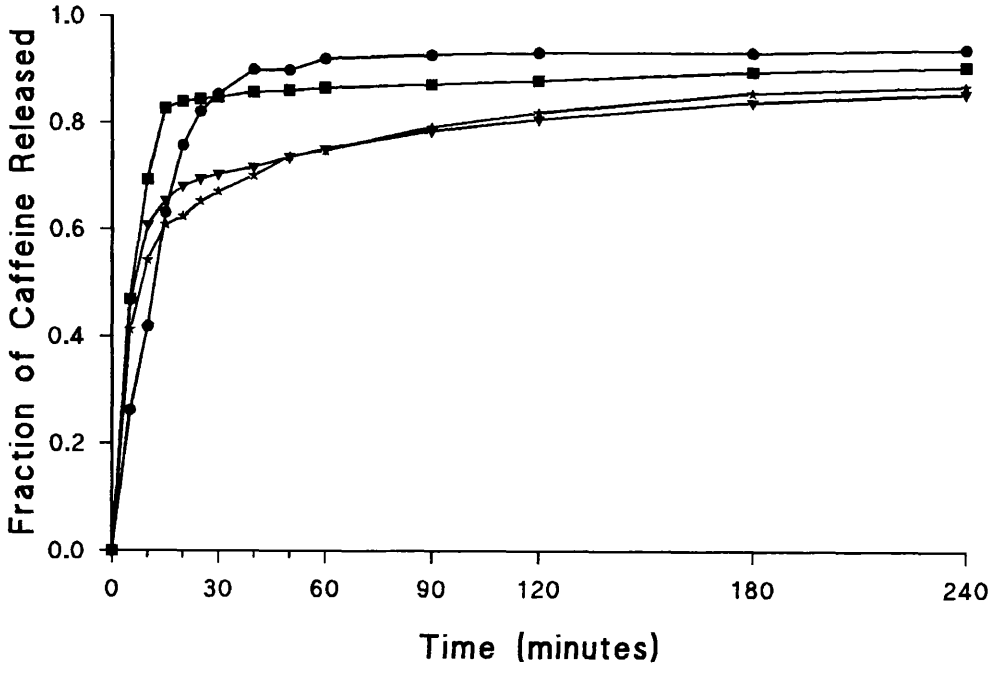


Figure 2.16 Release of caffeine from NiPAAm hydrogels with 0.076mole% (●), 0.755mole% (■), 1.51mole% (▼) and 3.02mole% (★) cross-linker **at 50°C**.

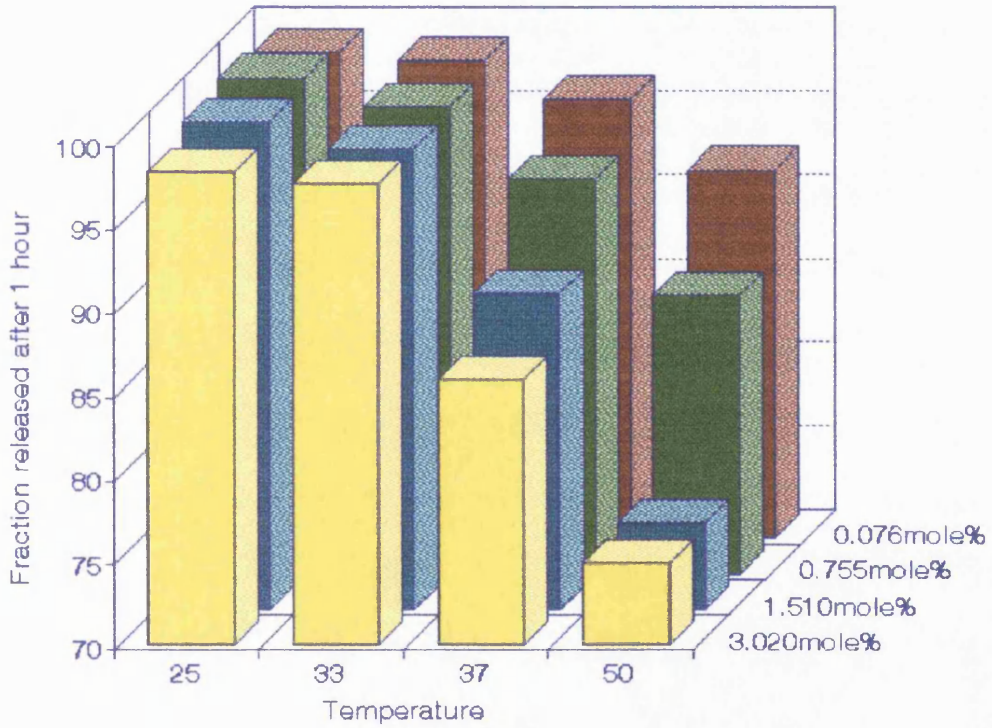


Figure 2.17 Fraction of caffeine released from NiPAAm hydrogels after 1 hour as a function of temperature and the concentration of the cross-linker.

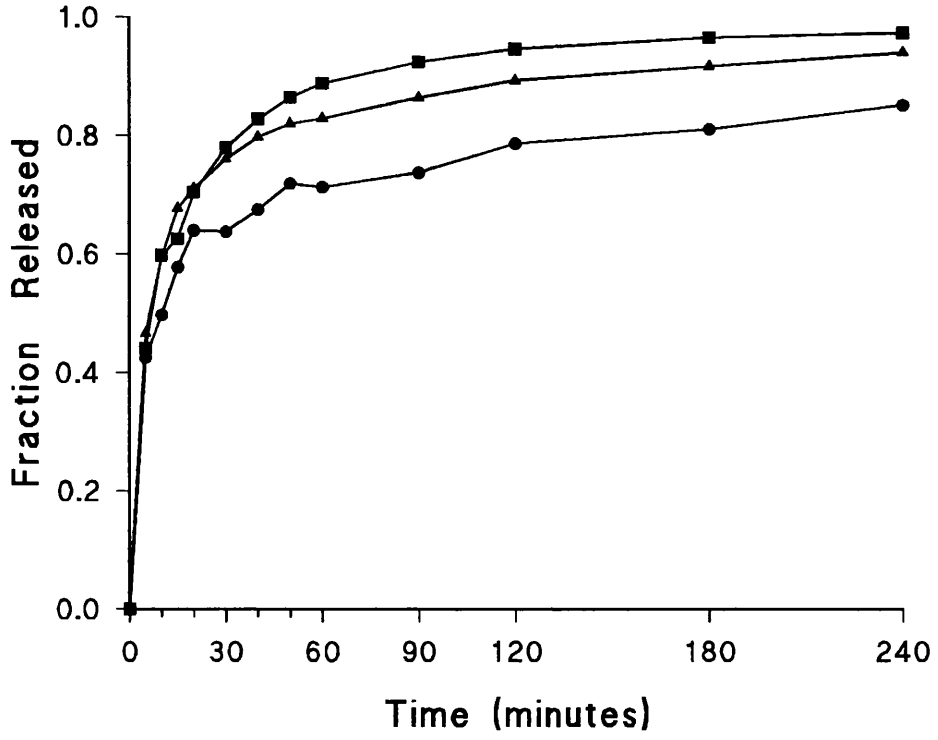


Figure 2.18 Release of PSSA from NiPAAm hydrogels with 0.076mole% (■), 0.378mole% (▲) and 0.755mole% (●) cross-linker **at 25°C.**

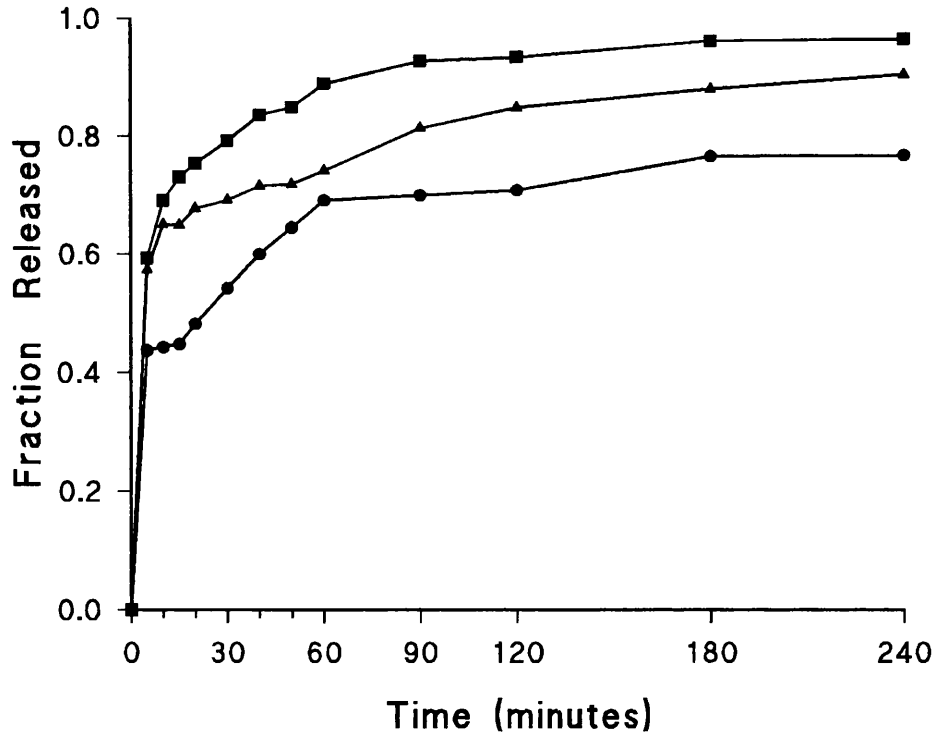


Figure 2.19 Release of PSSA from NiPAAm hydrogels with 0.076mole% (■), 0.378mole% (▲) and 0.755mole% (●) cross-linker **at 37°C.**

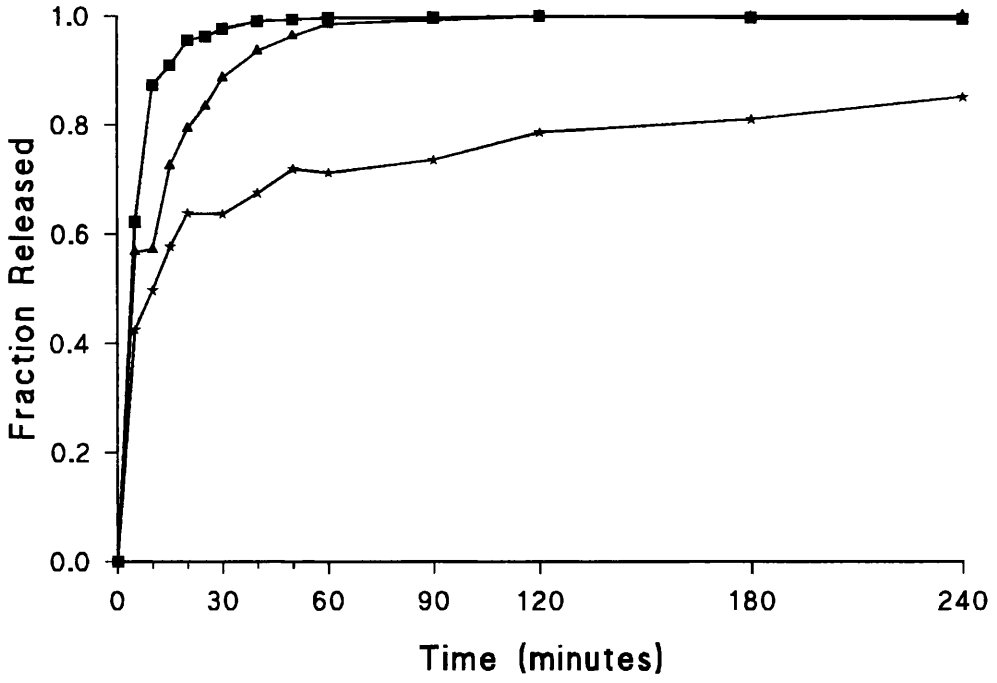


Figure 2.20 Release of caffeine (MW 194,■), chlorhexidine (MW 505,▲) and PSSA (MW 70000,★) from NiPAAm hydrogels with 0.755mole% crosslinker **at 25°c.**

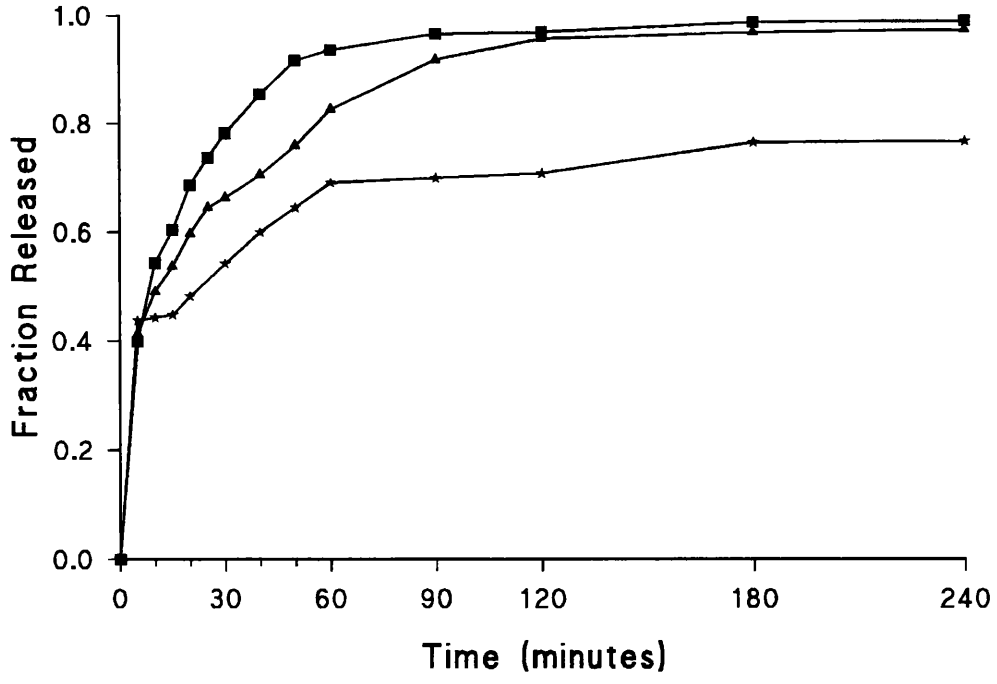


Figure 2.21 Release of caffeine (MW 194,■), chlorhexidine (MW 505,▲) and PSSA (MW 70000,★) from NiPAAm hydrogels with 0.755mole% crosslinker **at 37°c.**

2.7 Release of a slightly water-soluble drug from thermo-responsive hydrogels

Flurbiprofen was chosen as the model drug and was loaded using an ethanol/water solution onto the hydrogels which were later dried. The release study was carried out with initially dry hydrogels. Fig. 2.22 describes the release of flurbiprofen at 26°C and 37°C, from co-polymerized hydrogels of NiPAAm/MAAB (97.1/2.9). This polymer exhibits a LCST of ~27°C. The LCST was reduced upon the addition of the highly hydrophobic monomer (MAAB), due to the reasons described in section 2.4 . A hydrogel exhibiting a lower LCST behaviour was preferable, since it could work over a more moderate range of temperatures. The release duration of flurbiprofen is prolonged and the lower temperature range also prevents the possibility of evaporation of a fraction of the release medium at that long period. Flurbiprofen was continuously released over 3 days from the hydrogels at 26°C, whilst at 37°C a fraction was released in the first few hours, but then the release ceased. The release in the early period at 37°C could be attributed to the portion of the drug that was adsorbed onto the surface of the dry hydrogel (the initial faster release rate observed at 26°C that slows after few hours could be explained in the same way). Two explanations can be suggested as the mechanism which reduces the release at 37°C. Firstly, at this temperature (above the LCST) the water content of the hydrogel is minimal, and since flurbiprofen is poorly soluble in water the absence of free water inside the hydrogel prevents even small quantities from being continuously dissolved and released. Restriction in diffusion is the second reason that prevents the drug release at 37°C. Due to

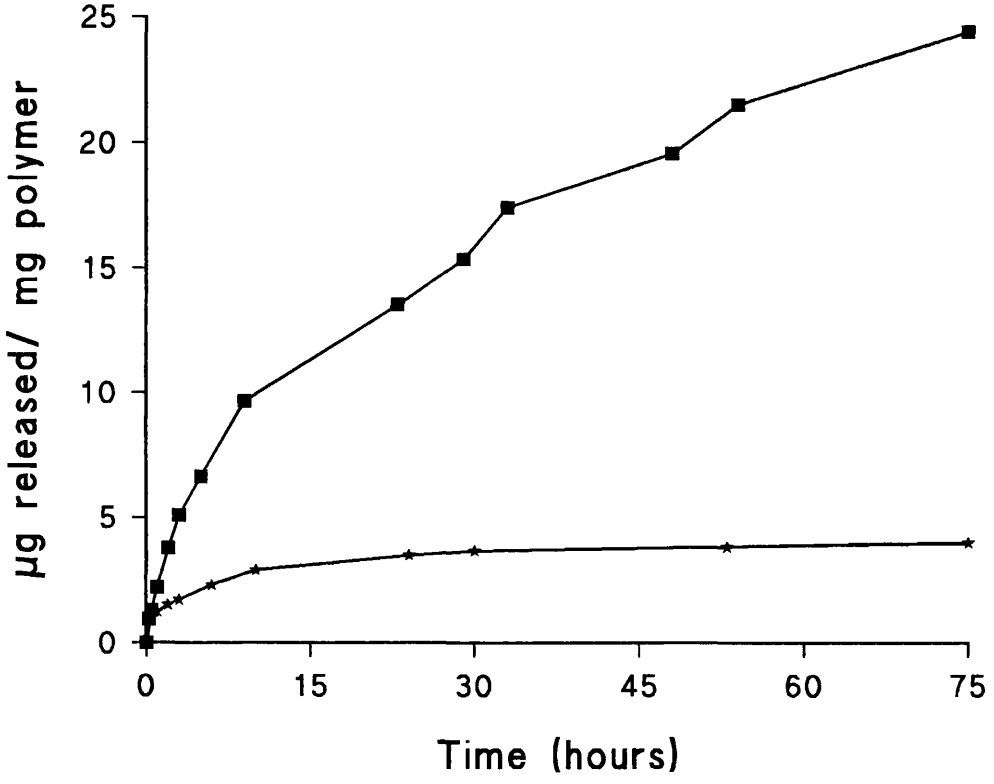


Figure 2.22 Release of flurbiprofen from NiPAAm/MAAB (97.1/2.9mole%) hydrogels with 0.755mole% crosslinker at 26°C (■) and 37°C (★).

hydrophobic interactions that increase substantially above the LCST a more compact network is formed which restricts the diffusion (in the same manner as described in section 2.6). The incorporation of the hydrophobic MAAB monomer into the matrix increases substantially those interactions and produces a more compact network.

The large difference in the release profiles above and below the LCST allows us to achieve an on-off switchable release pattern of flurbiprofen as seen in fig. 2.23 . The temperature was cycled between 22° and 37°C. At the lower temperature the hydrogel swells and releases its content, whilst elevation of the temperature to 37°C stops the release. The rate of release in the first cycle was much higher than in the following cycles, due to the release of the adsorbed drug as well as the loaded one. When the temperature was raised to 37°C the release rapidly stopped without the initial phase of release (that was observed at fig. 2.22), since all the adsorbed drug was released previously at 22°C. This study emphasises the ability to prepare a slow and pulsatile release system for slightly water-soluble drugs using temperature regulation. The range of temperatures used can be modified by using different compositions and concentrations of co-monomers to suit any specific requirements.

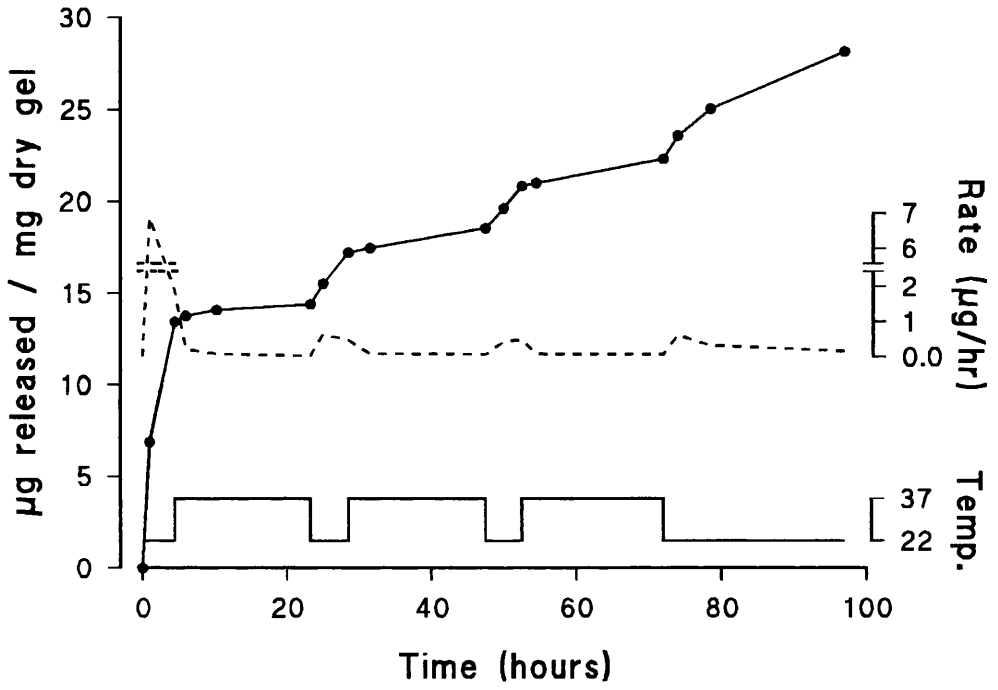


Figure 2.23 On-off switchable release of flurbiprofen from NiPAAm\MAAB (97.1/2.9mole%) hydrogels (amount released- solid line, rate of release- dashed line).

2.8 Immobilization of β-galactosidase in thermo-responsive hydrogels

β-Galactosidase was immobilized within a thermo-sensetive network, and its activity at different temperautres and different swelling level was investigated. The following three different NiPAAm/AAm hydrogels were chosen as the immobilization matrices:

GE1 - 90mole% NiPAAm / 10mole% AAm

GE2 - 94mole% NiPAAm / 6mole% AAm

GE3 - 97mole% NiPAAm / 3mole% AAm

Fig. 2.24 shows the swelling profile of such hydrogels. These heterogeneous hydrogels were chosen since the homogenous NiPAAm hydrogels collapse at 32°C, and above this temperature exhibits the same swelling ratio (~1), whilst most enzymes and biological cells require higher temperatures for efficient activity. The NiPAAm/AAm co-polymeric hydrogels exhibit a LCST at around 40°C and therefore are more suitable. Increasing the proportion of AAm over 10mole% results in the gradual loss of thermo-sensitivity in such hydrogels (Fig. 2.7).

Fig. 2.25 presents the specific activity of the immobilized β-galactosidase in the different hydrogels as a function of temperature. When the temperature was elevated the hydrogels deswelled causing a reduction in enzyme activity. This could be attributed to the mechanical pressure of the shrunken network on the enzyme or due to the reduction of the substrate and the products diffusion through the deswollen hydrogel (as discussed in part 2.6) (fig. 2.26). Thus the temperature dependence of the activity of the immobilized enzyme is opposed to that of the free enzyme as seen in fig. 2.27.

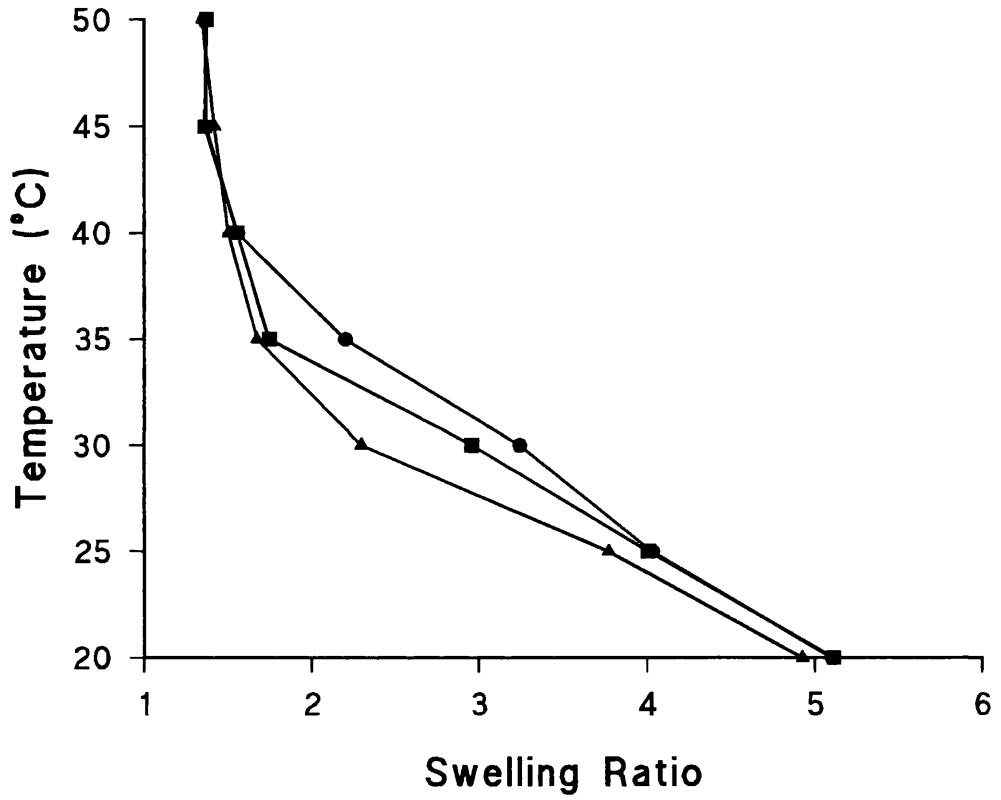


Figure 2.24 Swelling profiles of three different NiPAAm/AAm hydrogels in water, as a function of temperature. ● - GE1 ; ■ - GE2 ; ▲ - GE3.

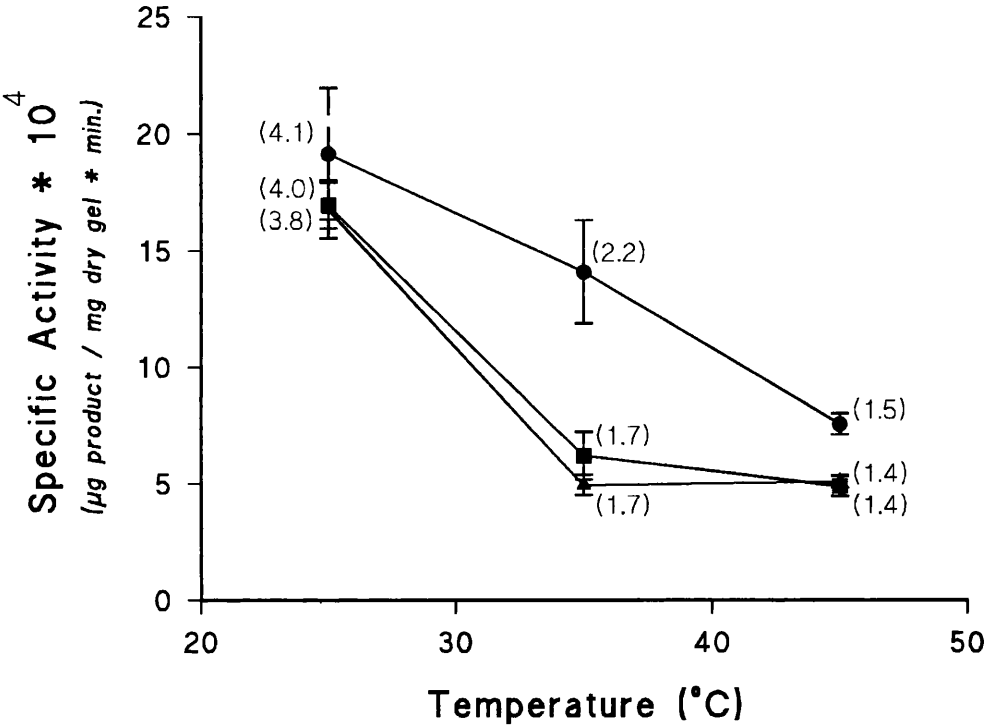


Figure 2.25 Specific activity of immobilized β-galactosidase in GE1(●), GE2 (■) and GE3 (▲) hydrogels at different temperatures.

(The figures in brackets represent the swelling ratio of each gel at the specific temperature)

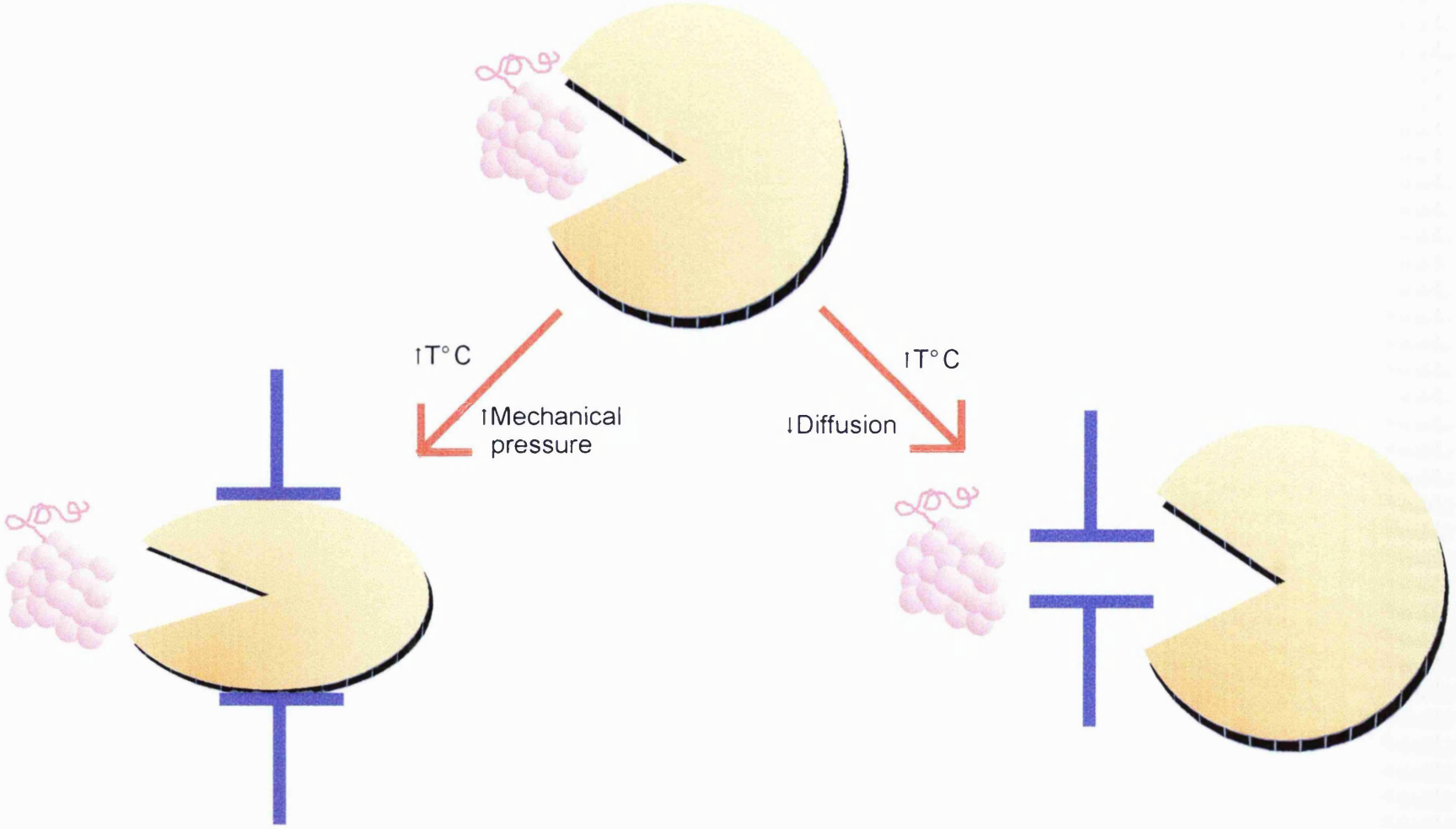


Figure 2.26 Two possible mechanisms for regulation of immobilized enzyme activity.

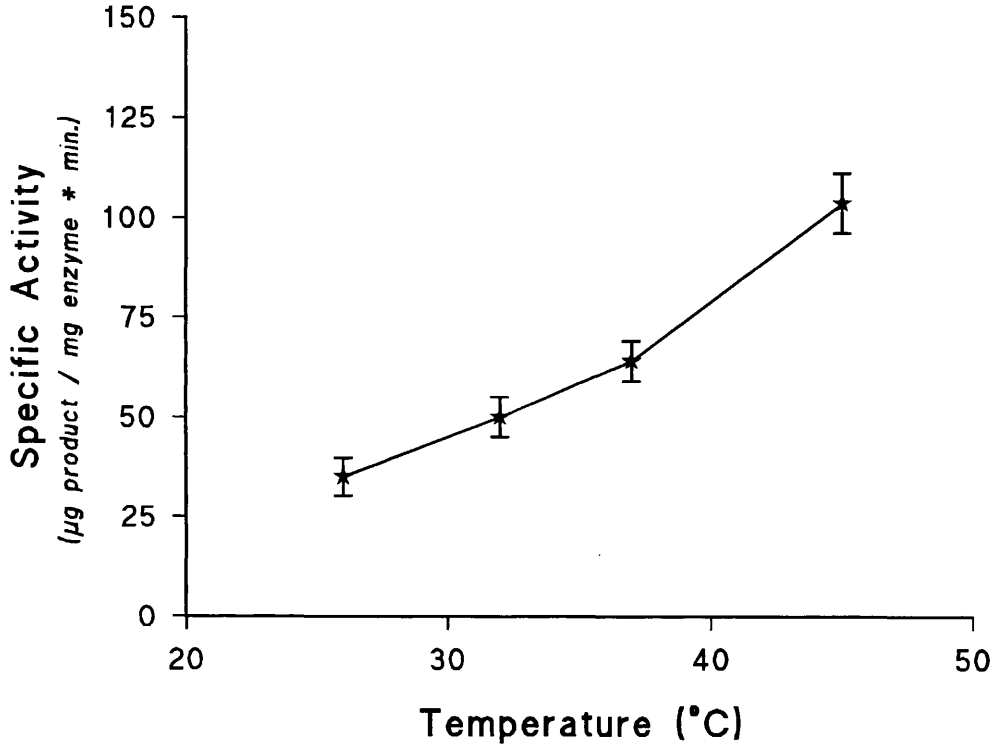


Figure 2.27 Specific activity of free β-galactosidase at different temperatures.

The free β-galactosidase activity increases as the temperature is elevated, and the results of fig. 2.25 should be examined in the light of this fact. For example, the activity of the enzyme immobilized in GE1 is reduced by factor of 2 when the temperature is elevated from 25°C to 45°C, but at 45°C the intrinsic activity of β-galactosidase increases by a factor of 4 (of the activity at 25°C), and this indicates that the shrunken gel restricts the enzyme activity by factor of 8 approximately. To allow clarification of the relationship between the enzyme activity and the swelling, the activity values were normalized to the proportional increase in the intrinsic activity at a certain temperature, compensating for the effect of the temperature on the intrinsic activity.

$$\text{Normalized specific activity (T°C)} = \frac{\text{Specific activity T°C (immobilized enzyme)} \cdot \text{Specific activity 25°C (free enzyme)}}{\text{Specific activity T°C (free enzyme)}}$$

Fig. 2.28 shows the resulting linear correlation between the hydrogel swelling ratio and the enzyme activity. It can be seen that there is no difference between the three hydrogels, from which it can be concluded that achieving a certain activity of an immobilized enzyme at a specific temperature is possible by synthesizing a copolymer hydrogel which exhibits the appropriate swelling ratio at the indicated temperature to produce the desired activity.

The effect of swelling on the activity of the enzyme can be due to two reasons: the shrunken matrix reduces the availability of the substrate or reduces the intrinsic activity of the enzyme due to mechanical pressure. A diffusion study of substrate through a gel disc (for gel GE1) at different temperatures (Fig. 2.29) shows that increasing the temperature reduces the

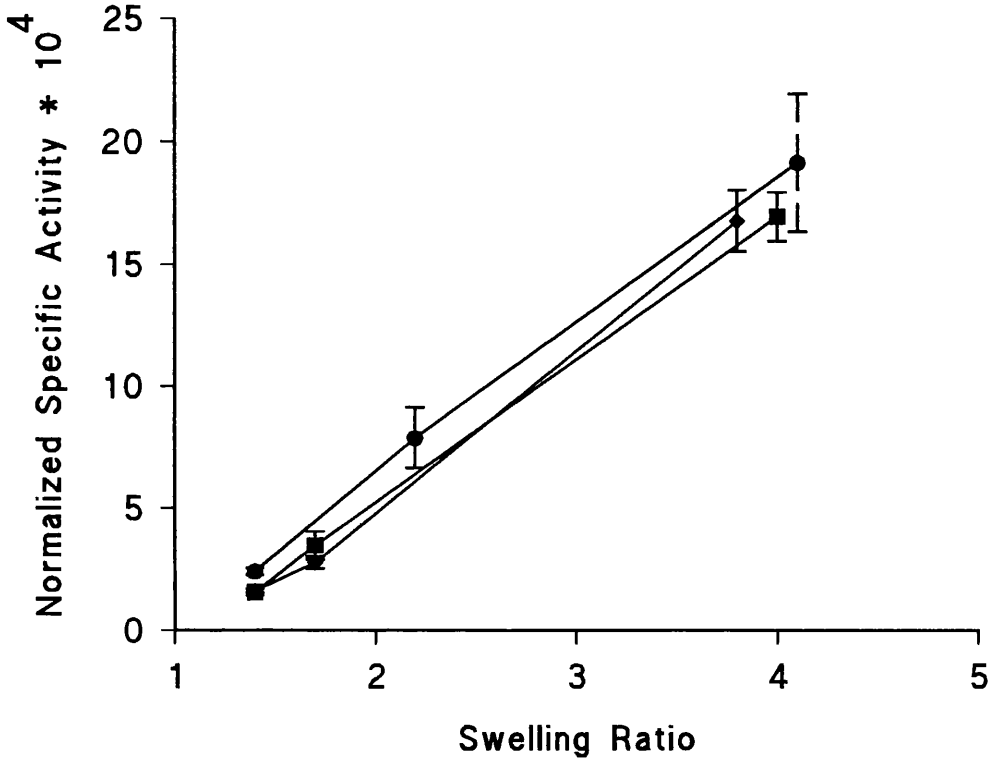


Figure 2.28 Normalized specific activity of immobilized β-galactosidase in GE1 (●), GE2 (■) and GE3 (▲) hydrogels at different temperatures.

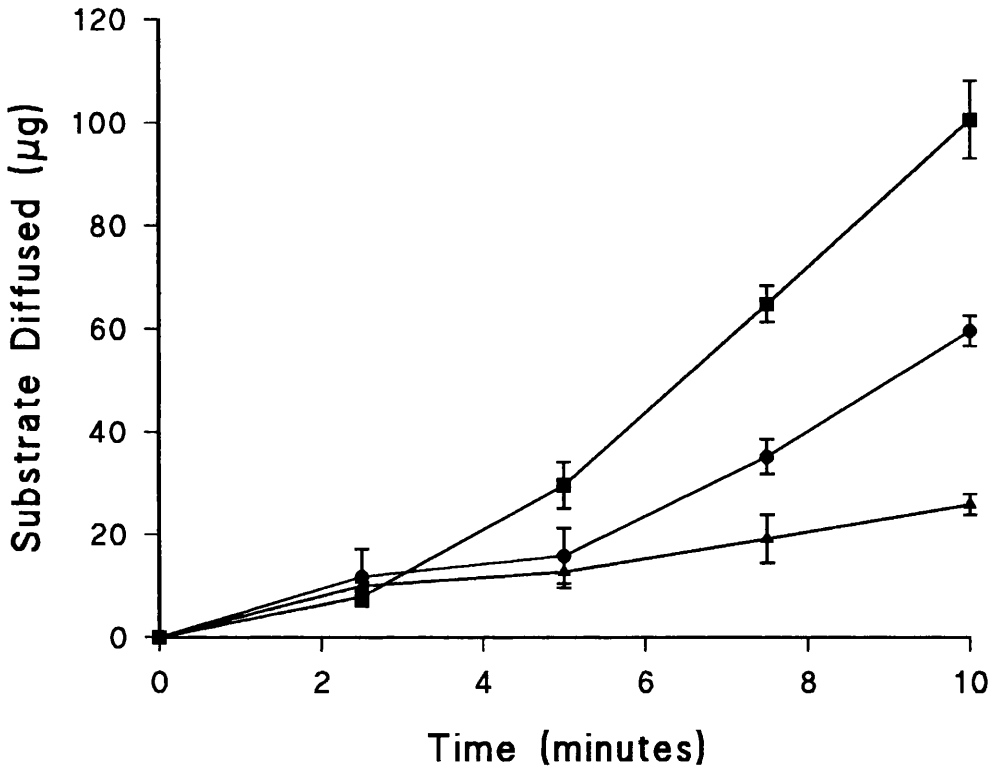


Figure 2.29 Diffusion of ONPG through NiPAAm/AAm (90/10mole%) hydrogel (GE1) membrane at 25°C (■), 35°C (●) and 45°C (▲).

diffusion rate of the substrate. The reduction of diffusion plays a key role in the reduction of enzyme activity at high temperatures.

In order to find whether this is the only factor affecting the activity, we normalized the specific activity of the enzyme in GE1 gel to the diffusion rate at the same temperature (calculated from the slopes of the diffusion profiles in Fig. 2.29). By this normalization the effect of the different availabilities of the substrate to the enzyme at a range of temperatures is accounted for. Table 2.4 shows the relative diffusion normalized activity of the immobilized gel to the relative activity of the free enzyme. Relative activity was calculated as the ratio of activity at a specific time to the activity at 25°C. If the reduction of diffusion is the only factor affecting the enzyme activity, we would expect to find the two data sets of relative activities similar. However the relative activity of the immobilized enzyme is lower than that of the free enzyme at 35°C and 45°C indicating that the mechanical pressure of the shrunken network on the immobilized enzyme plays an important part in the reduction of enzyme activity.

Table 2.4 Diffusion normalized and relative activity values for free and immobilized β-galactosidase.

Temp. (T°C)	Diffusion Normalized Specific Activity	Relative Diffusion Normalized Activity	Relative Activity of Free Enzyme
25°	1.85	1.00	1.00
35°	2.47	1.33	2.33
45°	3.13	1.69	3.67

$$\text{Diffusion normalized activity} = \frac{\text{Specific activity of immobilized enzyme at T°C}}{\text{Diffusion rate at T°C}}$$

$$\text{Relative diffusion normalized activity} = \frac{\text{Diffusion normalized activity at T°C}}{\text{Diffusion normalized activity at 25°C}}$$

$$\text{Relative activity of free enzyme} = \frac{\text{Specific activity of free enzyme at T°C}}{\text{Specific activity of free enzyme at 25°C}}$$

Chapter 3- Photo-responsive hydrogels

3.1 Introduction

Some chromophores are known to exhibit a reversible structural response when exposed to radiation at different wavelengths. Some experiments which describe the photo-response of polymers which contain such chromophore have been reported, but only a handful are dealing with polymeric matrices and none with drug delivery from such matrices. This chapter describes the synthesis of monomers which contain either one of the two chromophores, azobenzene or triphenylmethane, and the effect of radiation at different wavelengths on the release from hydrogels containing such monomers.

3.2 Materials

The materials used in the various experiments discussed in this chapter are described in the previous chapter. Additional materials are described in table 3.1 .

Table 3.1 Additional materials used in the study of photo-responsive hydrogels

Material	Source
Azobenzene	Aldrich chemical company (UK) Ltd.
4,4'-Azodianiline	Pfaltz & Bauer (USA)
4-Bromostyrene	Lancaster (UK) Ltd.
Glacial acetic acid	BDH Laboratory Supplies (UK)
Hydrochloric Acid	BDH Laboratory Supplies (UK)
Methacryloyl chloride	Aldrich Chemical Company (UK) Ltd.
Methanol (HPLC grade)	Rathburn Chemicals (UK)
Michler's ketone	Aldrich Chemical Company (UK) Ltd.
4-Phenylazoaniline	Aldrich Chemical Company (UK) Ltd.
Potassium cyanide	BDH Laboratory Supplies (UK)
Pyridine	Aldrich Chemical Company (UK) Ltd.
Sodium	Aldrich Chemical Company (UK) Ltd.
Sodium bicarbonate	BDH Laboratory Supplies (UK)
Toluene	BDH Laboratory Supplies (UK)

3.3 Methods

3.3.1 Synthesis of methacryloylaminoazobenzene (MAAB)

MAAB was synthesized by the method described by Eisenbach (1978) (fig. 3.1). Methacryloyl Chloride (1.57mg) was slowly added to a solution of phenylazoaniline (1.97gr) in pyridine(20ml) at 30°C. The mixture was stirred at 60°C for 1 hour. The contents were poured into 10ml ice water and acidified with concentrated hydrochloric acid. The product was collected by vacuum filtration, washed with a 10% sodium bicarbonate solution and purified water. The product was purified by recrystallisation from ethanol and water. Yield 1.95gr (73.5%). -MS(EI): m/z(%) = 265(46)$[M]^+$, 228(3), 196(3), 188(25)$[M-C_6H_5]^+$, 160(100),$[M-(N=N-C_6H_5)]^+$, 145(4), 130(3), 117(10), 105(11), 91(13), 77(77), 69(50). -^{1}H NMR ($CDCl_3$): δ= 2.20(s,3H,-CH_3), 5.52, 5.84 (2d,2H,CH_2=), 7.44 (d,1H,aromatic H), 7.49, 7.88 (2d ab,4H,aromatic H), 7.79, 7.95 (2d ab,4H,aromatic H).

Anal. $C_{16}H_{15}N_3O$ (265.31)

Calcd. C 72.45 H 5.67 N 15.89

Found C 70.15 H 5.79 N 15.13

3.3.2 Synthesis of di(methacryloylamino)azobenzene (DMAAB)

Synthesis was carried out using the same procedure as in 3.3.1 exchanging azodianiline (1.0gr), instead of Phenylazoaniline. Yield

0.42gr(25.6%). -MS(EI): m/z(%) = 348(32)$[M]^+$, 279(2), 188(11), 160(88), 145(4), 132(8), 117(12), 107(7), 91(8), 79(8), 69(80), 41(100). -H^1 NMR ($CDCl_3$): δ= 1.59 (s,6H,-CH_3), 5.51, 5.86 (d,2H,CH_3=), 7.75, 7.94 (2d ab,8H,aromatic H).

Anal. $C_{20}H_{20}N_4O_2$ (348.40)

Calcd. C 69.94 H 5.79 N 16.08

Found C 62.71 H 6.14 N 14.33

3.3.3 Synthesis of Bis(4-(dimethylamino)phenyl)(4-vinylphenyl)methyl leucocyanide (leuco-CN)

This is a two phase synthesis. In the first phase the leucohydroxide derivative was synthesized and purified and in the second phase it was converted to the leucocyanide derivative (fig. 3.2).

Bis(4-(dimethylaminophenyl))(4-vinylphenyl)methyl leucohydroxide (leuco-OH)

A mixture of o-bromostyrene (4.2 g), Michler's ketone (6.2 g), finely cut sodium (1.3 g) and dry toluene (170 ml) were refluxed at 140°c under nitrogen and stirred for 9 hours. The product was purified by flash chromatography. Yield 3.44 g(40%). -MS(FAB): m/z(%) = 373(4) $[M+H]]^+$,355(100) $[M-OH]^+$, 339(18), 269(49), 253(17), 237(7), 208(6), 185(13), 165(10), 148(63), 120(15), 105(11). -^{1}H NMR ($CDCl_3$): δ= 3.43 (s,12H,N-CH_3), 5.50, 5.99 (2d,2H,CH_2=), 6.84 (m,1H,CH=), 7.03, 7.42 (2d ab,8H,N-aromatic H), 7.32, 7.56 (2d ab,4H,vinyl aromatic H).

Anal. $C_{25}H_{28}N_2O$ (372.49),

Calcd. C 80.60 H 7.58 N 7.52

Found C 80.51 H 7.52 N 7.30

Bis(4-(dimethylamino)phenyl)(4-vinylphenyl)methyl leucocyanide (leuco-CN)

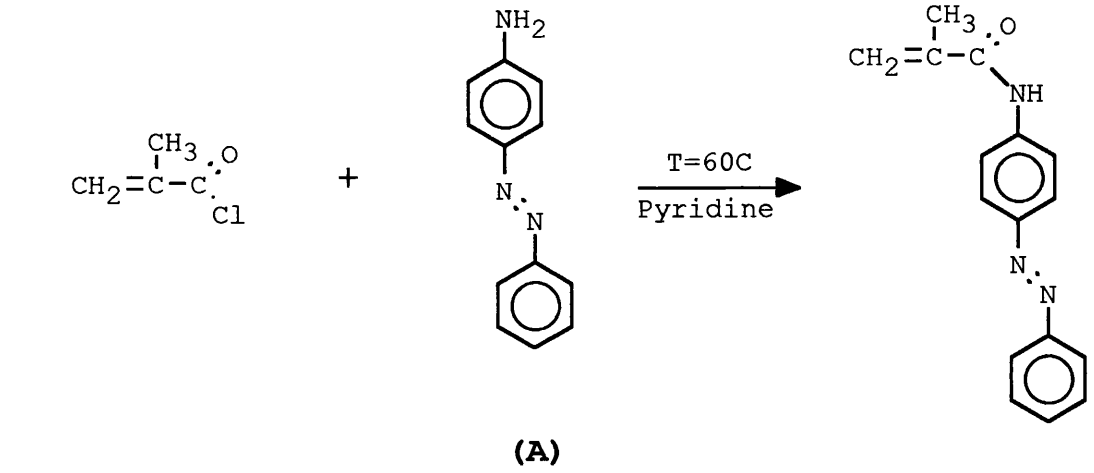
Bis(4-(dimethylamino)phenyl)(4-vinylphenyl)methylleucohydroxide(1.34 g) was dissolved in DMSO (35 ml) and glacial acetic acid (0.6 ml) was added. A deep coloured solution was produced. The solution was heated to 100°c and potassium cyanide (0.92 g) was added to the solution which immediately decolourized. Ethanol (15ml) was added and stirred followed by a slow addition of hot water (15 ml). The solution became turbid. The mixture was cooled slowly and filtered, to yield yellow crystals of the product. Yield 0.681 g(49%).

-MS(EI): m/z(%) = 381(91) $[M]^+$, 356(27)$[M\text{-}CN]^+$, 355(18),342(11), 278(100), 261(19), 253(9), 208(2), 191(7), 148(20), 120(5), 105(3). -^{1}H NMR ($CDCl_3$): δ= 2.95 (s,12H,N-CH_3), 5.30, 5.75 (2d,2H,CH_2=), 6.66 (m,1H,CH=), 6.65, 7.08 (2d ab,8H,N-aromatic H), 7.23, 7.38 (2d ab,4H,vinyl aromatic H). -^{13}C NMR ($CDCl_3$) δ= 114 (s,1C,-CN)

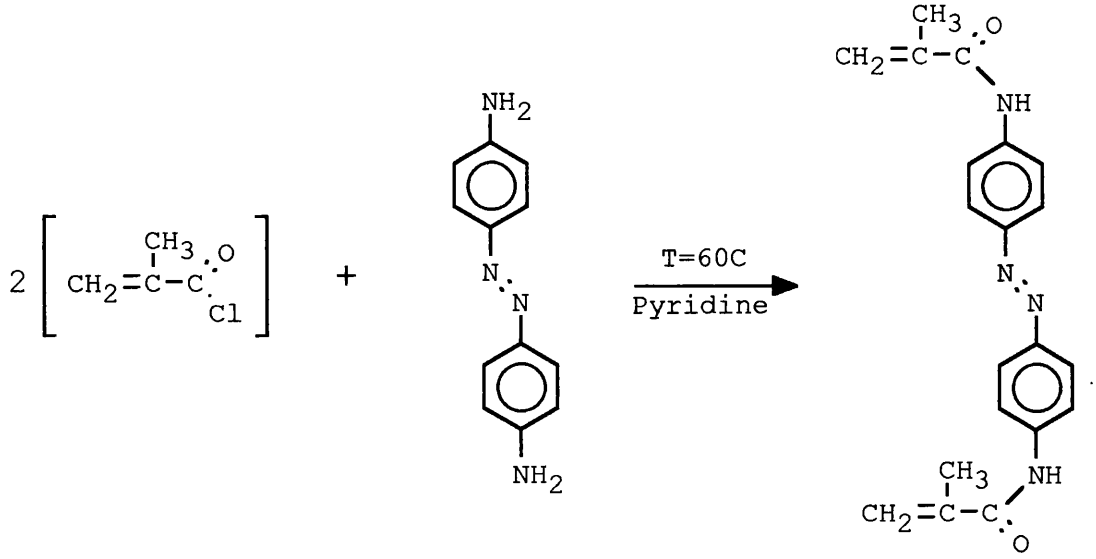
Anal. $C_{26}H_{27}N_3$ (381.51)

Calcd. C 81.85 H 7.14 N 11.02

Found C 81.58 H 7.10 N 10.82



(A)



(B)

Figure 3.1 Synthesis reactions of MAAB (A) and DMMAB (B).

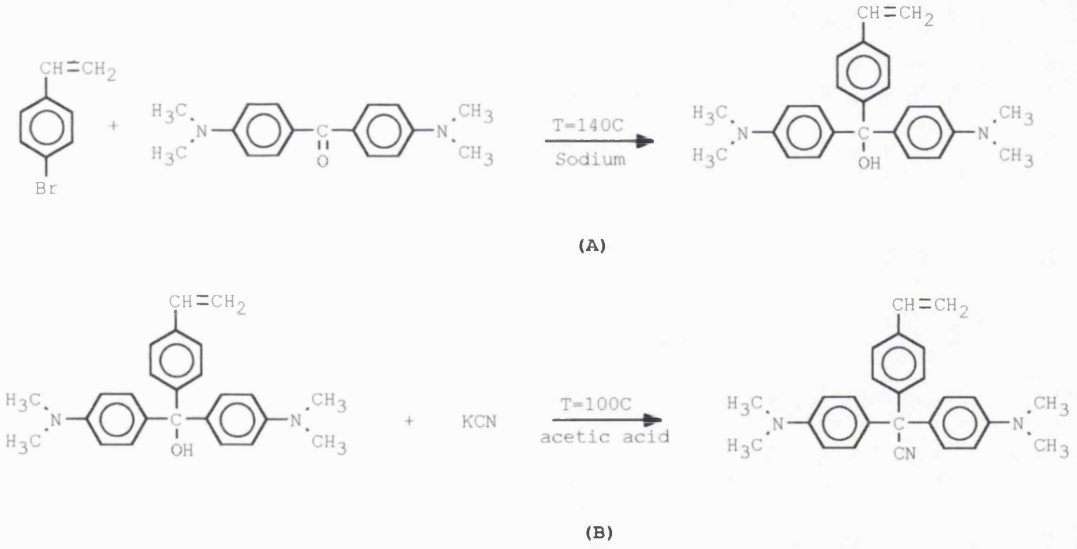


Figure 3.2 Synthesis reactions of leuco-OH (A) and leuco-CN (B).

3.3.4 Preparation of chromophore containing hydrogels

All hydrogels discussed in this chapter were prepared using the method described at part 2.3.1 . Table 3.2 presents the amount and ratio of each chemical used in the different formulations.

3.3.5 Loading and release of solutes from hydrogels

Caffeine and PSSA were loaded on the hydrogels in the same methods described in part 2.3.3 .

The release studies were performed in a similar manner to that described in part 2.3.4, inside a silica cuvette which was part of the setup that allows simultaneous illumination and control the temperature of the sample (fig. 3.3). An arc lamp housing(model no. 66007, Oriel Corporation USA) with a power supplier (68805, Oriel Corporation USA) were used with different lamps. The light source was equipped with a fused silica condenser, a water filter (which absorbed the IR radiation and reduced the consequent heating), and a filter holder. The light beam was focused on the cuvette which was placed at a distance of 10cm from the source inside a special holder on a heating unit, placed on a stirring plate. A temperature probe was immersed inside the cuvette and the temperature was maintained to ±0.2°C by a temperature controller.

A 200W mercury-xenon lamp was used for all experiments involving the azobenzene chromophore. For UV radiation a filter (59154, Oriel Corporation

USA), which transmitted over 90% of light in the range of 300-360nm was used, and for visible light a filter which blocks light below 420nm (59484, Oriel Corporation USA) was chosen.

A 150W (UV version) xenon lamp, which is made with silica glass and produces high irradiation at the far-UV range, was used in the experiment involving the leuco-CN chromophore, which requires UV radiation below 300nm. A special mirror (Oriel Corporation USA) which reflects UV light in the required 260-320nm range was used.

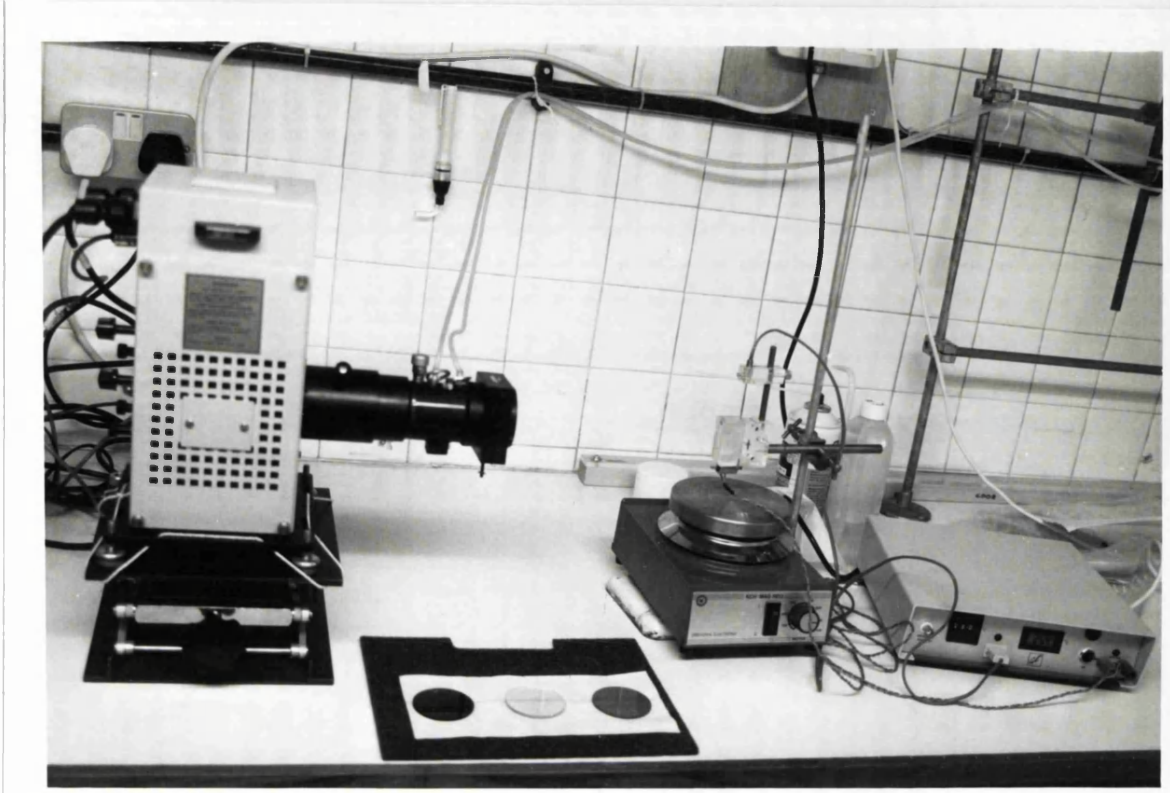
3.3.6 HPLC analysis of the azobenzene chromophore

The analysis was preformed using a Spherisorb ODS2 column (Phase Sep, U.K.) on a Gilson HPLC system. The mobile phase was methanol/water (80/20%). Fig. 3.4 shows the effect of the methanol/water ratio on the retention time of both isomers. The polymer was dissolved in the mobile phase and 20µl of the 175µg/ml polymer solution were injected. A Gilson UV detector was used, and the absorbence at 254nm was measured (since this wavelength is not affected by the absorption changes caused by the isomerization as can be seen in fig. 3.7).

Table 3.2 Gel compositions used in the preparation of photo-responsive hydrogels

NiPAAm mg (%)	Co-monomer mg (%)	Cross-linker mg (%)	DMSO ml
Poly(NiPAAm/MAAB) hydrogels cross-linked with BIS			
1000 (100%)	--	10.0 (0.755%)	1
977 (99.1%)	23 (0.9%)	10.0 (0.755%)	1
960 (98.2%)	40 (1.8%)	10.0 (0.755%)	1
935 (97.1%)	65 (2.9%)	10.0 (0.755%)	1
928 (96.8%)	72 (3.2%)	10.0 (0.755%)	1
Poly(NiPAAm) hydrogels cross-linked with DMAAB			
1000 (100%)	--	11.0 (0.36%)	1
Poly(AAm) hydrogels cross-linked with DMAAB			
--	630 (100%)	11.0 (0.36%)	1
Poly(NiPAAm/leuco-CN) hydrogels cross-linked with BIS			
970 (98.8%)	40 (1.2%)	0.3 (0.023%)	1

The figures in brackets refer to the molar percentage ratio of the total monomers.



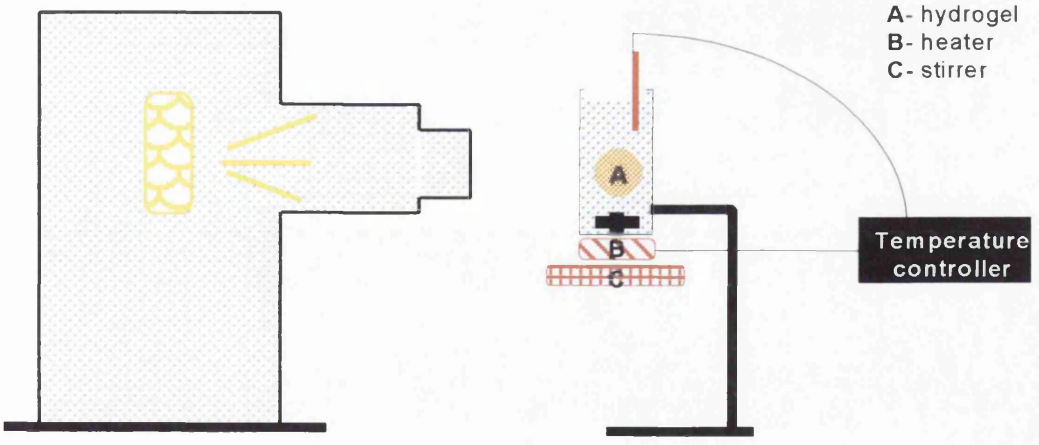


Figure 3.3 Apparatus used for illumination of the hydrogel samples at specific temperatures.

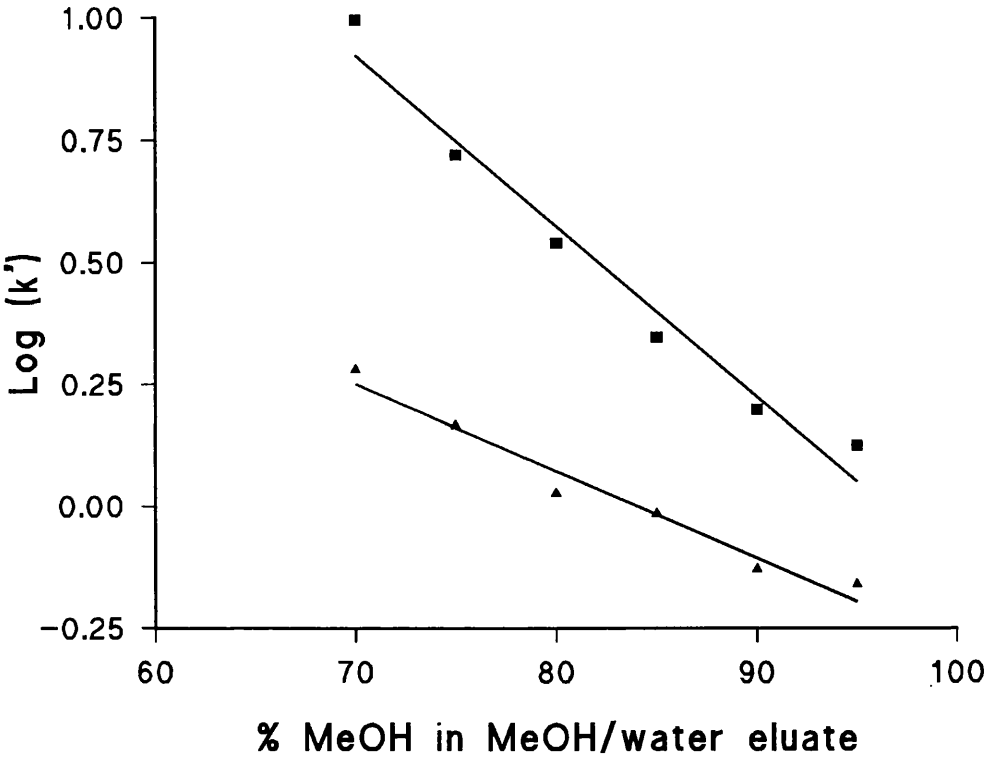


Figure 3.4 The separation of the peaks of trans(■) and cis(▲) isomers as a function of the mobile phase. Log (k')=Log (Tr-To/To), k'- mass distribution ratio; Tr- retention time; To-dead time.

3.4 The azobenzene chromophore

Azobenzene derivatives generally occur in the yellow to red colour range. The main feature of their absorption spectra is a relatively weak long wavelength band well separated from the intense shorter wavelength band system. Fig. 3.5 shows a typical absorption spectrum for azobenzene. The intense absorption at 320nm is due to the Π-Π^* transition of the *trans* isomer, whilst the less intense absorption at around 430nm is due to the n-Π^* absorption of the *cis* isomer (Kumar and Neckers 1989).

This chromophore is characterized by a reversible conversion from the dominant *trans* form to the *cis* isomer on exposure to UV radiation. This isomerization causes a major dimensional change of the molecule as seen in fig. 1.7(A). The isomerization changes the absorption spectrum of the azobenzene. The high intensity absorption of the *trans* isomer is reduced, whilst the low intensity absorbence of the *cis* isomer is slightly increased. Fig. 3.6 describes the photo-isomerization of a NiPAAm/MAAB co-polymer (97.1/2.9mole%). The hydrogels discussed in part 3.5.1 were synthesized from this co-polymer. After 1min of UV radiation the maximal change was observed. 1min of irradiation with visible light of the pre-UV exposed solution reversed a portion of the *cis* isomer back to *trans*, and after 5min a full recovery was achieved. This indicated that the UV-induced isomerization is faster than the visible light-induced recovery. Fig. 3.7 illustrates the slow recovery process in the dark after exposure to UV radiation; only after 8 days the system totally recover.

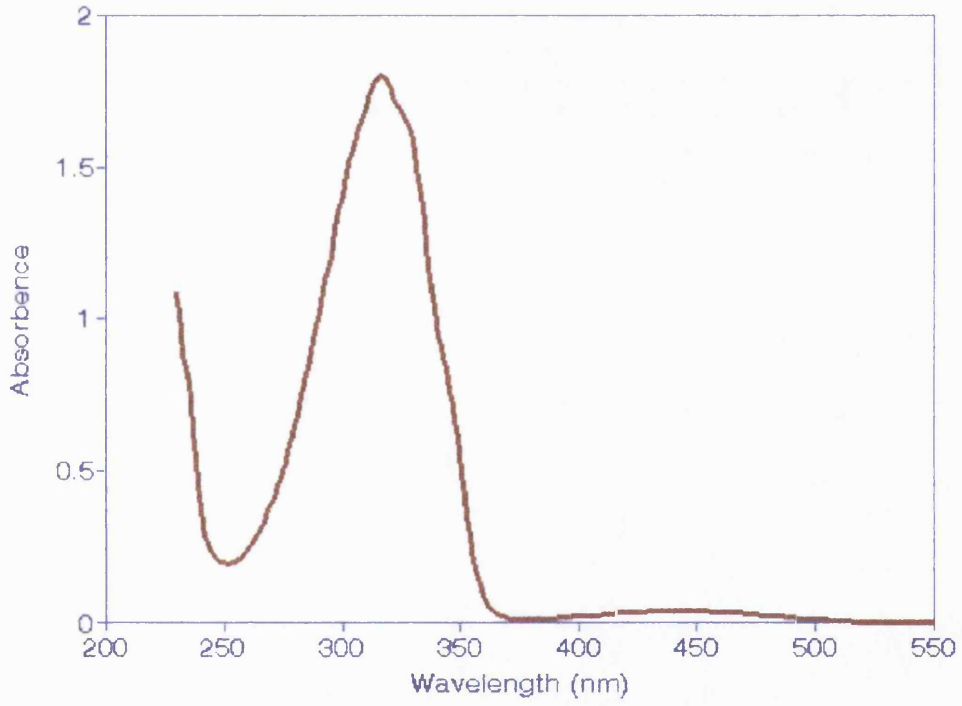


Figure 3.5 Absorption spectrum of 30µg/ml azobenzene solution in ethanol.

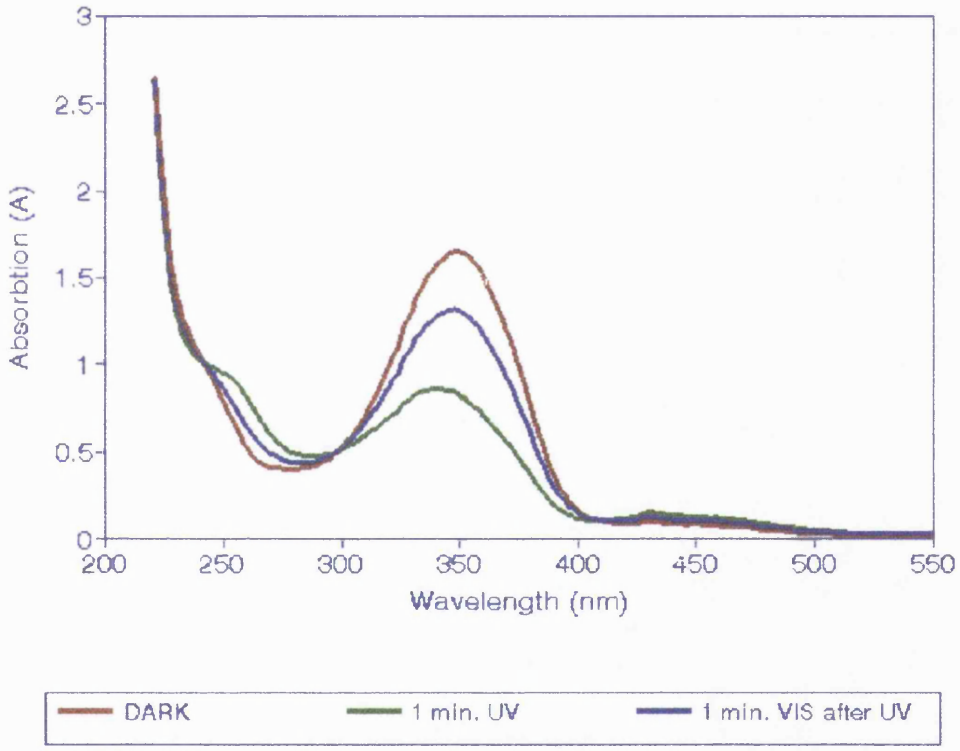


Figure 3.6 Absorption spectrum of 175µg/ml NiPAAm/MAAB (97.1/2.9mole%) co-polymer in ethanol before and after light irradiations.

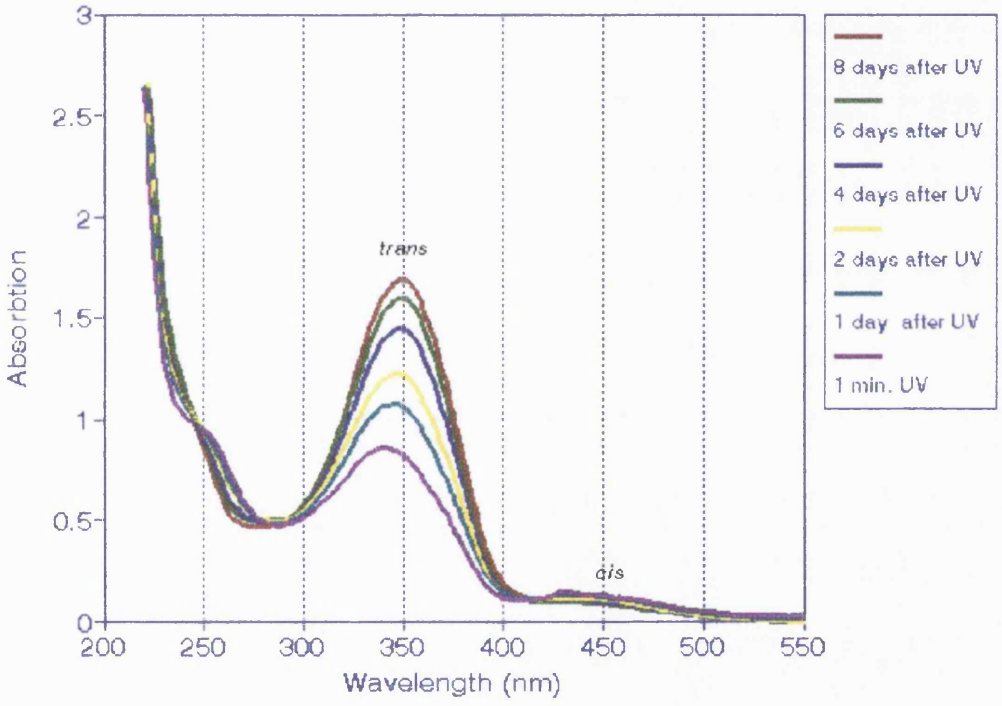


Figure 3.7 Absorption spectrum of 175 µg/ml NiPAAm/MAAB (97.1/2.9mole%) solution in ethanol after 1 min UV irradiation and during 8 days recovery in the dark.

3.5 Solute release from NiPAAm hydrogels containing the azobenzene chromophore

3.5.1 Hydrogeis containing an azobenzene pendant group

Co-polymerized NiPAAm/MAAB hydrogels were chosen as the release matrix. NiPAAm based hydrogels were preferred after examination of the data found in the literature (mainly on solutions of such polymers) since NiPAAm polymers demonstrated the greatest change in response to light, especially at temperatures near the LCST. A change in the collapsing pattern of such hydrogels affects the release profile from the matrix as discussed in chapter 2. Fig. 3.8 compares the release of caffeine from NiPAAm/MAAB (97.1/ 2.9mole%) at 30°C when exposed to UV and visible light. While the UV radiation causes the isomerization of azobenzene, the exposure to visible radiation was used as control since it supports the existing *trans* isomer. The UV radiation increased the release of caffeine from the matrix by 10.4%. Isomerization of the azobenzene-containing polymer can result in two opposing effects, as described in section 1.2.2.b . From the release results it seems that the isomerization of azobenzene to the *cis* form reduces the hydrophobic forces between the azobenzene groups. The release study was performed at 30°C, which is above the LCST of the gel. At such a temperature any change in the hydrophobic interactions within the hydrogel can have an effect on the release of solute from it since the hydrophobic forces play an important role in the collapsing process. Apparently, the reduction in the hydrophobic interactions

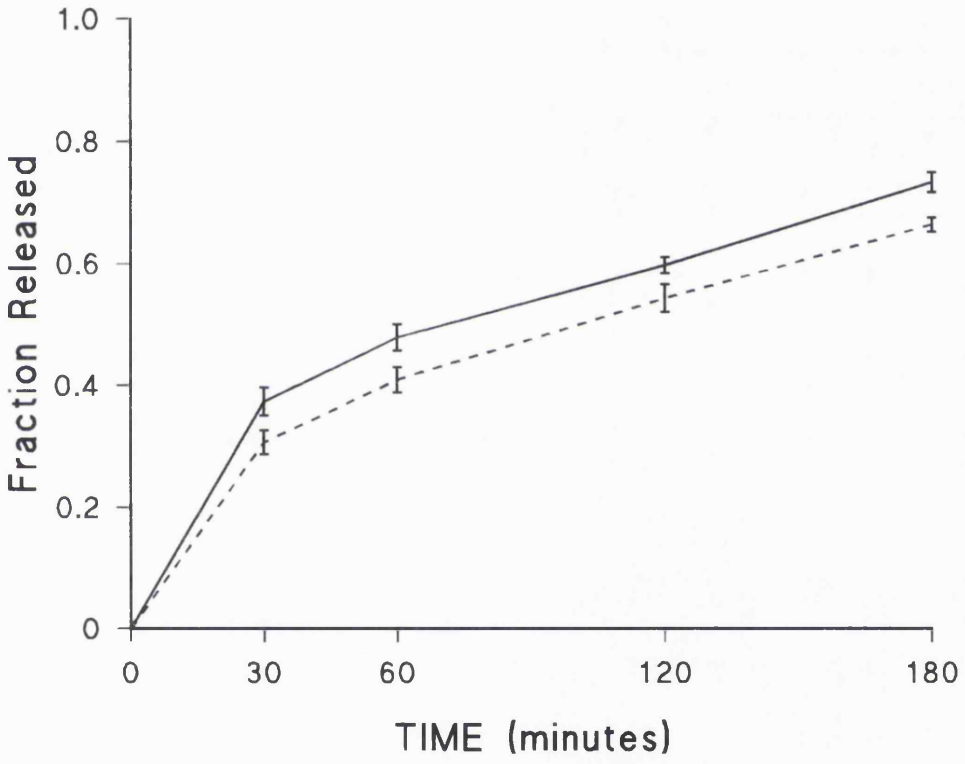


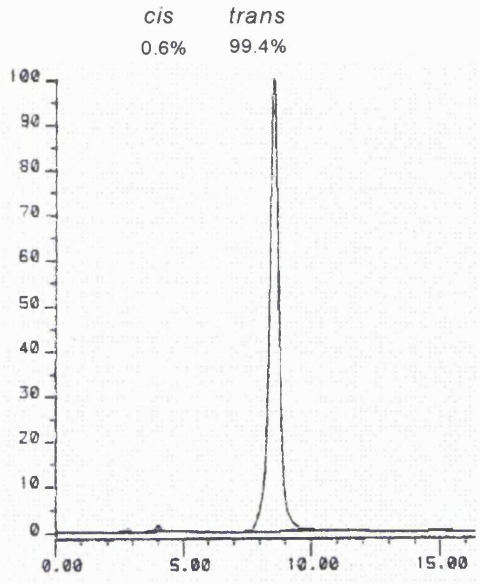
Figure 3.8 Release of caffeine from NiPAAm/MAAB (97.1/2.9mole%) hydrogels under UV radiation (—) and visible light (- -) at 30°C.

upon exposure to UV radiation causes the hydrogel to collapse differently and to exhibit a less compact arrangement during that process which allows the release of caffeine to a higher extent, but at the end of the collapsing process the hydrogel is not affected any longer by the UV irradiation, as can be seen in fig. 3.8 . The difference in release was observed within the first 30min; after that period the release rate is almost identical. That effect of UV irradiation on the thermo-sensitive hydrogel matrix is the same as was described by Kungwatchakum and Irie (1988, section 1.2.2.b). A 5°C increase in the LCST of the NiPAAm/MAAB polymer solution was observed on exposure to UV radiation probably due to the reduction in the hydrophobic forces caused by the isomerization to the *cis* isomer. Table 3.3 illustrates the difference in fraction of release of caffeine from hydrogels with different concentrations of MAAB. The maximum concentration of MAAB which was examined was 3.2mole% since above this concentration the texture of the dry hydrogel was very soft and consequently impossible to handle. At 3.2mole% the difference in release between samples irradiated with UV and visible light was reduced to 6.9%, whilst at 1.8mole% it was only 2.6mole%. A 100mole% NiPAAm hydrogel was used as a control and showed no difference in release under UV or visible light at 37°C (above its LCST).

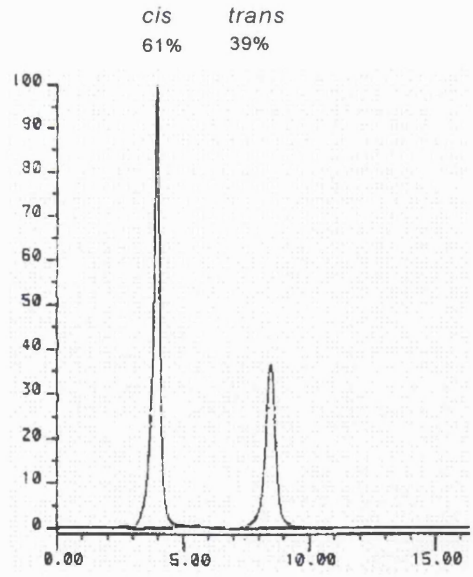
An HPLC analysis was performed to investigate the extent of UV-induced isomerization. Since the matrix is insoluble, the analysis was performed with a NiPAAm/MAAB (97.1/2.9mole%) polymer. Fig. 3.9(A) confirms that the neutral state of azobenzene is the *trans* isomer, the peak at ~8.5min belongs to the *trans* isomer(99.4%) and the peak at ~4.0min is due to the *cis* isomer (0.6%).

Table 3.3 Percentage of increase in the fraction of caffeine released from NiPAAm/MAAB hydrogels, after 3hr of UV irradiation.

NiPAAm mole%	MAAB mole%	temperature (°C)	increase in release under UV radiation
96.8	3.2	30	6.9%
97.1	2.9	30	10.4%
98.2	1.8	30	2.6%
100	---	37	-0.4%



(A)



(B)

Figure 3.9 HPLC analysis of the azobenzene isomers before (A) and after (B) 10min of UV irradiation.

After exposure to UV radiation for 10min 60% of the azobenzene group were isomerized to the *cis* form (fig. 3.9(B)), being the maximum isomerization rate achieved. It can be assumed that inside a three dimensional network a smaller amount of azobenzene groups will be isomerized due to steric hindrance, which could be one of the reasons for the small change in release which was observed under UV radiation.

5.3.2 Hydrogels containing azobenzene cross-linking units

Azobenzene was introduced as part of the cross-linking units inside the network by using the bi-functional DMAAB cross-linker. A NiPAAm hydrogel with 0.36mole% of DMAAB was used in this experiment. Its swelling profile is similar to NiPAAm hydrogels cross-linked with BIS and its LCST is lowered to 31°C. Fig. 3.10 illustrates the effect of isomerization (under UV radiation) on the release of PSSA from such hydrogels. Caffeine which was used in section 3.5.1. as a model solute was not suitable for these hydrogels which are more swollen resulting in a very rapid release of the drug. It can be seen that UV radiation reduced the release from the matrix. Upon isomerization the length of the cross-linking unit is shortened due to the dimensional change of the azobenzene. The formation of the *cis* isomer brings the two NiPAAm chains closer and consequently the pore size is reduced causing a decrease in the release due to steric hindrance. Figure 3.11(A,B) shows a model of the behaviour of such isomerization.

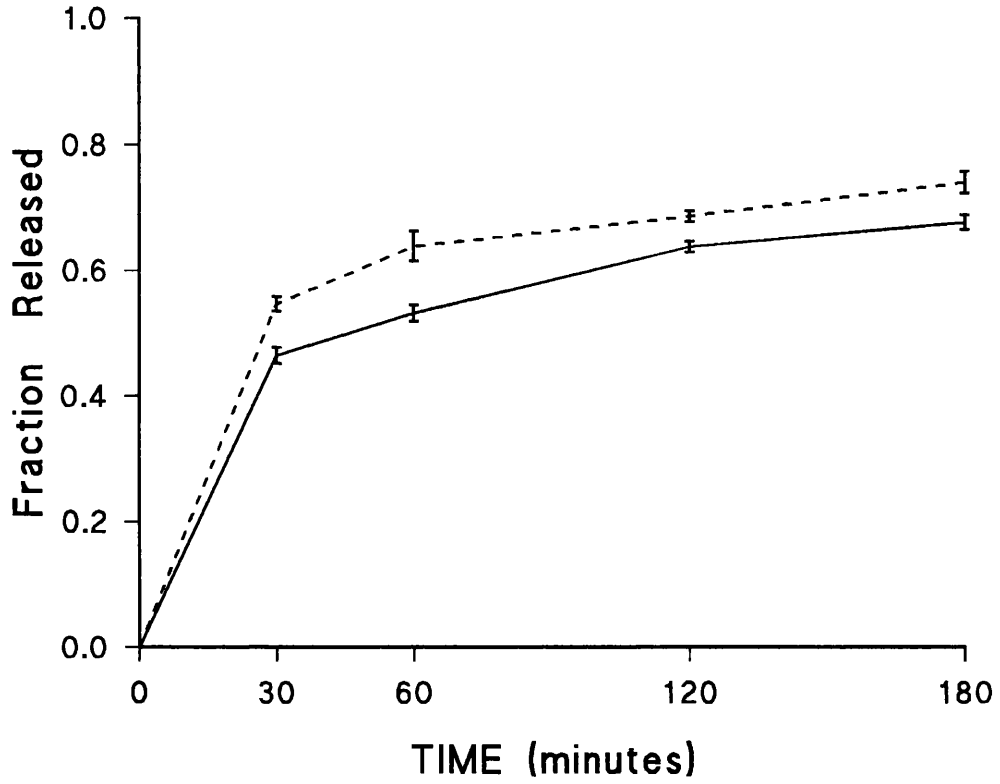


Figure 3.10 Release of PSSA from NiPAAm hydrogels cross-linked with 0.378mole% of DMAAB under UV radiation (—) and visible radiation (- -) at 30°C.

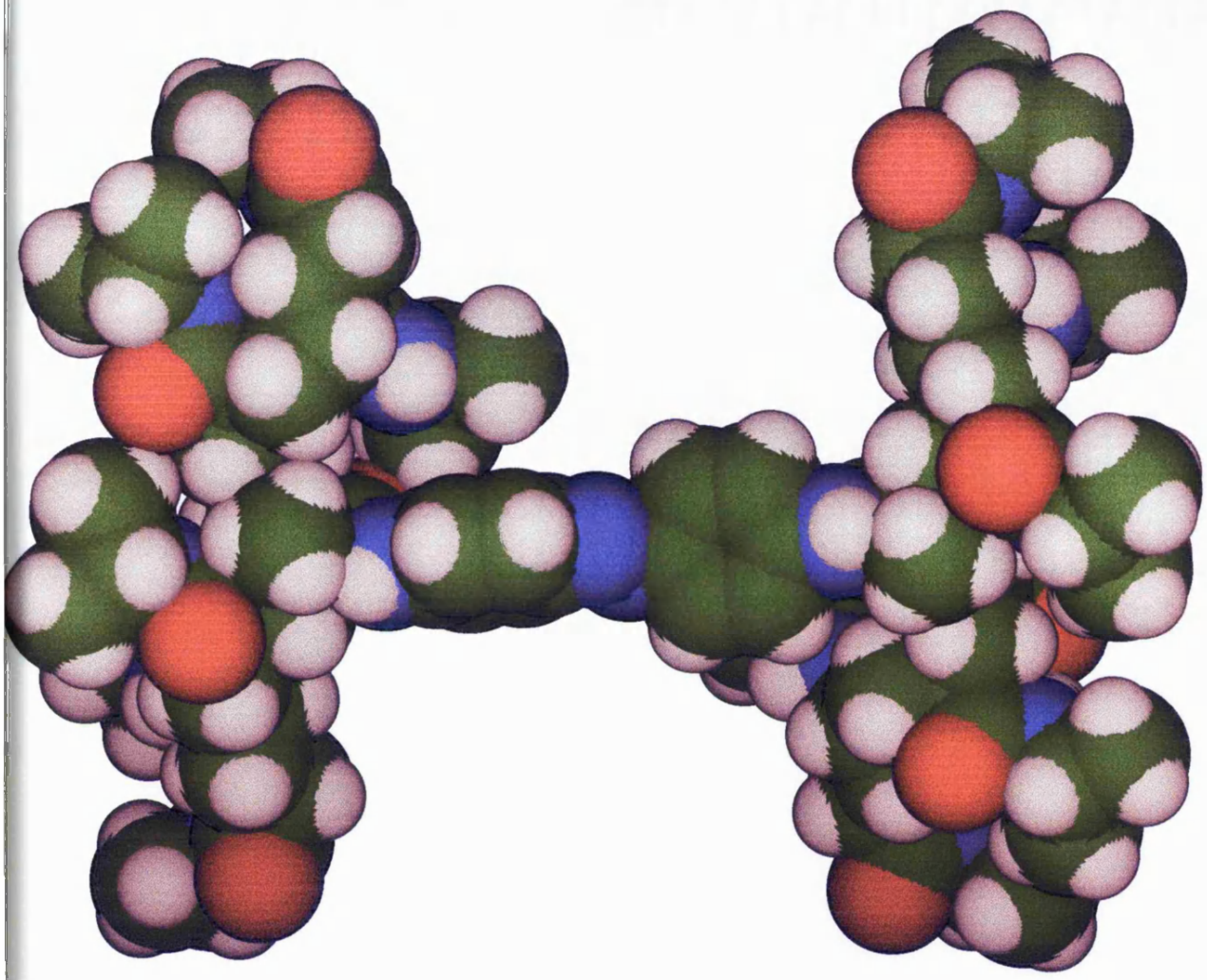


Figure 3.11(A) Azobenzene cross-linking unit (***trans* isomer**) between two NiPAAm chains.

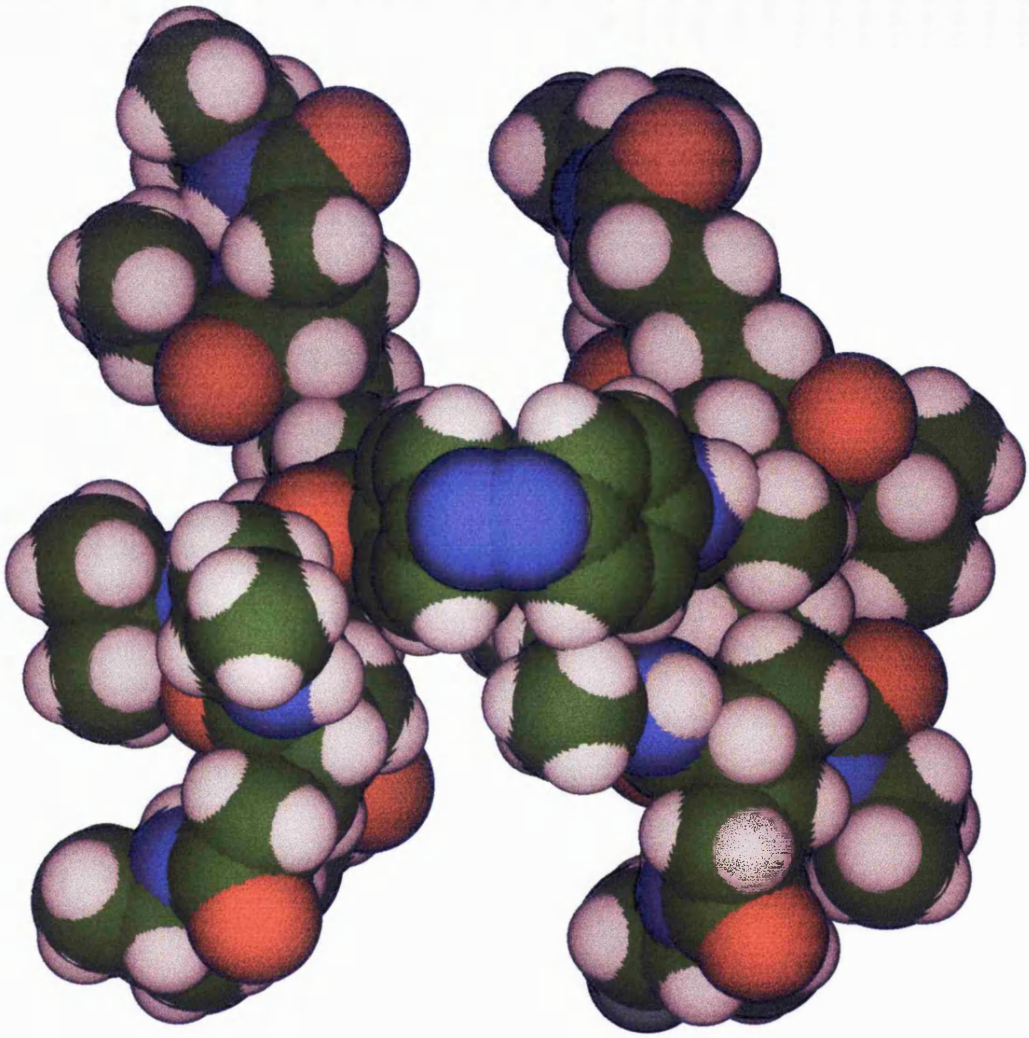


Figure 3.11(B) Azobenzene cross-linking unit (***cis* isomer**) between two NiPAAm chains.

It should be noted that the extent of isomerization is restricted as was described earlier (section 5.3.1). The polymer containing azobenzene pendant groups showed only 60% isomerization. The cross-linking azobenzene groups are expected to be even less isomerized, because of the high energy required for moving the long polymeric chains attached on both sides of the chromophore. A non-thermosensitive acrylamide hydrogel with 0.36mole% DMAAB was prepared in order to investigate whether some swelling changes occur solely due to the photo-induced isomerization. No significant change was observed under UV radiation, which indicates that the isomerization is limited (due to the high energy barrier) and could only affect the microstructure of the hydrogel and not its entire swelling. This obstacle can be overcome by using a light source, such as a laser, which produces much higher energy. Restriction in the penetration of light (especially in the UV range) through the hydrogel can pose another problem. The entire surface of the cylindrical hydrogel was illuminated but the extent of light penetration was not clear. The use of microspheres and beads can possibly solve this problem, although the disadvantage in working with such systems is the inability to focus the light on a specific part of the network.

3.6 Hydrogels containing a triphenylmethane leucocyanide chromophore

The triphenylmethane leuco-derivatives are known to be ionized upon exposure to UV radiation below 300nm (fig. 1.7(B)). A derivative of such a chromophore (leuco-CN) which contains a vinyl group on one of its aromatic rings, was

polymerized within the backbone of the polymer. Ionization of such a chromophore which is incorporated within a hydrogel matrix would be expected to increase the swelling of such gel (in the same manner that pH-induced ionization affects the swelling of pH-sensitive hydrogels). A hydrogel containing 1.2mole% of leuco-CN was exposed to UV radiation at different temperatures and its swelling ratio was recorded. No significant swelling difference was observed between such a hydrogel and a non-irradiated one(fig. 3.12), but a change in colour from pale to dark green was observed (fig 3.13). The colour change is an indication that some leuco-CN molecules were ionized, although this change was not reversible over a long period as can be expected. These hydrogels were not transparent even before irradiation and possibly the irradiated light could not penetrate through the hydrogel and effect only some of the chromophore. A formation of ionic complex between the carbo-cation and the cyanide ion which shield the charge could be another explanation.

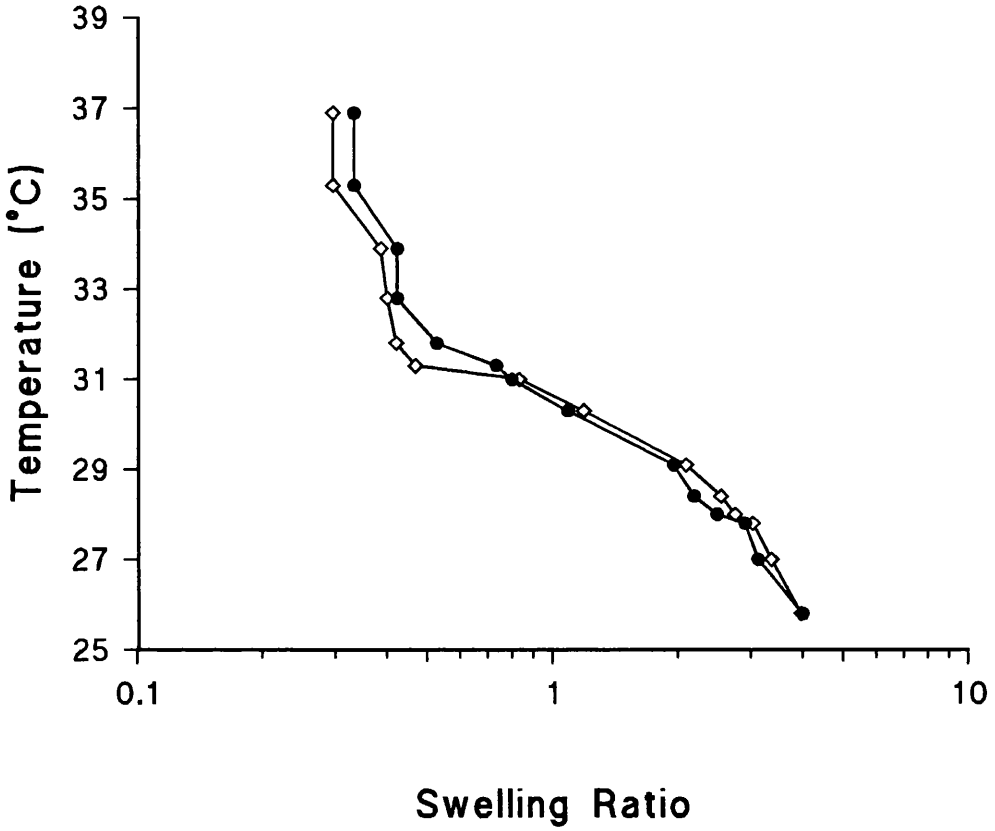


Figure 3.12 Swelling profile of NiPAAm/Leuco-CN hydrogels without (□) and on exposure (■) to UV radiation.

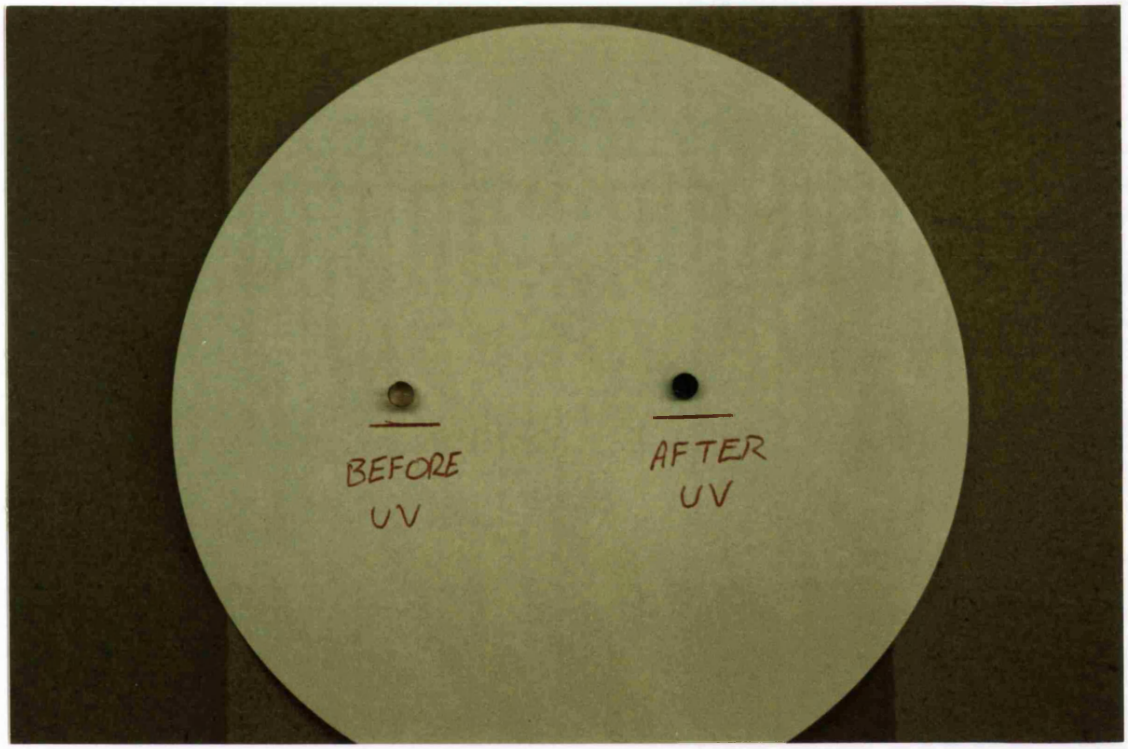


Figure 3.13 Leuco-CN containing hydrogels before and after 1Hr of irradiation with UV.

Chapter 4- Electro-responsive hydrogels

4.1 Introduction

In this chapter the application of an electric field has been investigated as a mean of controlling the dimensions of hydrogel networks. Electrical stimuli can be easily applied to any network merely with a small battery and electrodes.

Hyaluronic acid (HA) hydrogels were chosen to form a suitable polyelectrolyte network. HA is a naturally occurring polysaccharide, and is an important intercellular material in the space between skin, cartilage and muscle cells. It is a linear polymer of repeating units of D-glucuronic acid and N-acetylglucosamine linked at β1-3 and β1-4. It is extensively used as a viscosurgical tool in ophthalmology (Balazs and Band 1984) and has other clinical uses (Balazs and Delinger 1989). A crosslinked derivative of HA which provides highly viscoelastic solutions in water known as Hylan (Balazs and Leshchiner 1989) has been used to study the release of growth hormone and gentamicin (Larsen *et al.* 1990). In this chapter the effect of the application of an electric field both on the swelling state of such hydrogels and on the release profile from the matrix in order to prepare a pulsatile release system which responds to an on-off switching of an electrical stimulus is examined. The different effects which the application of an electric field has on methacrylic acid hydrogels (a polyelectrolyte network with different characteristic) is also demonstrated.

4.2 Materials

The materials used in the various experiments discussed in this chapter are given in the previous chapters. Additional materials are given in table 4.1

.

4.3 Methods

4.3.1 Preparation of hyaluronic acid hydrogels

HA hydrogels were prepared by cross-linking the sodium hyaluronate polymer with ethyleneglycol diglycidylether (EGDGE) in basic conditions, using the following procedure: Sodium hyaluronate (200mg) was dissolved in 0.9ml of 1N NaOH solution and degassed, while EGDGE (82µl) was dissolved in 0.1ml of ethanol. Both solutions were vigorously mixed and degassed, then cast into a mould, washed with distilled water and ethanol and placed in distilled water for 3 days to remove any unreacted materials.

4.3.2 Preparation of methacrylic acid hydrogels

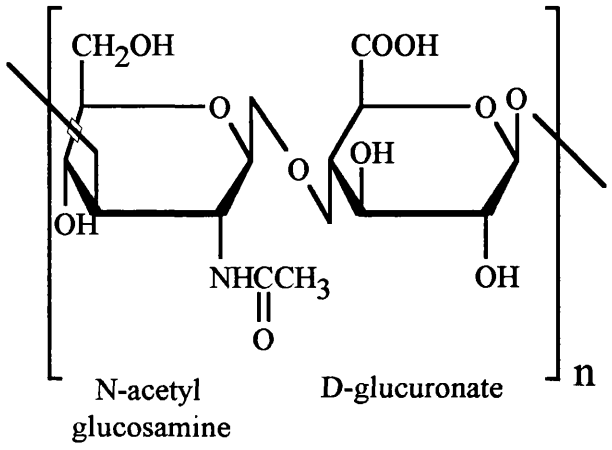
MAA Hydrogels were prepared using the same method described in section 2.3.1, using 2g of MAA and 100mg of the cross-linker BIS.

4.3.3 Swelling measurements of HA hydrogels

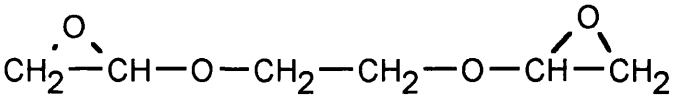
The swelling ratio of HA hydrogels was determined as being the ratio

Table 4.1 Additional materials used in the study of electro-responsive hydrogels

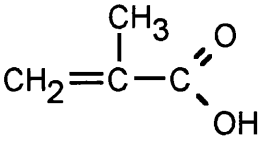
Materials	Source
Bovine serum albumin (BSA)	Sigma Chemical Company (UK) Ltd.
Ethyleneglycol diglycidylether (Technical grade) (EGDGE)	Aldrich Chemical Company (UK) Ltd.
Hyaluronic acid, sod. salt(from *Streptococcus zooepidemicus*)	Sigma Chemical Company (UK) Ltd.
Methacrylic acid (MAA)	Fluka Chemika (Germany) Ltd.
Poly(glutamic acid,tyrosin) 4:1, sodium salt (PGT)	Sigma Chemical Company (UK) Ltd.
Ranitidine HCl	Sigma Chemical Company (UK) Ltd.
Sodium hydroxide	BDH Laboratory Supplies (UK) Ltd.
Sodiume sulfite	Aldrich Chemical Company (UK) Ltd.



Hyaluronic acid



Ethyleneglycol diglycidylether (EGDGE)



Methacrylic acid (MAA)

Figure 4.1 Monomers used for the preparation of electro-responsive hydrogels.

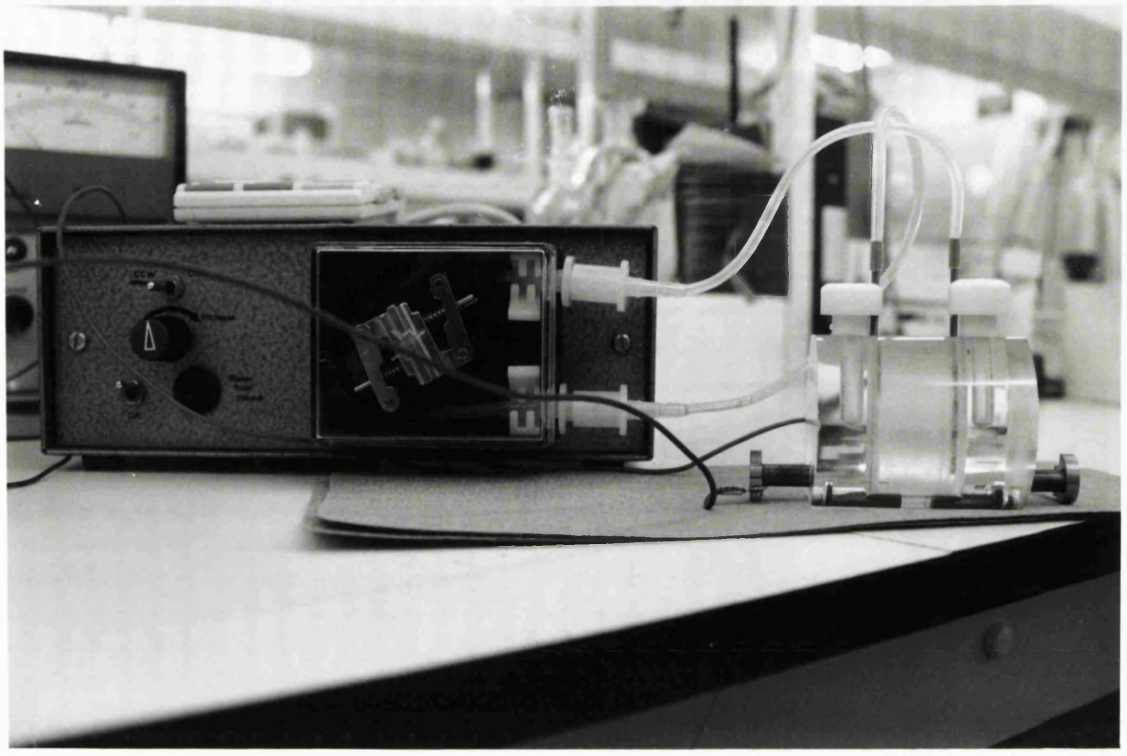
between the swollen and dry states of the hydrogels. The hydrogels were freeze-dryied for at least 36h. Since these hydrogels were highly swollen their dry weight was only in the range of 10-250μg. Determination of the true weight of the light lyophilised network was rather inaccurate, and hence the swelling state of those hydrogels is described in this chapter as relative swelling. Relative swelling is the ratio of the swelling of the hydrogel at T=t to its initial swelling ($T=t_0$) (or the ratio between the weight of the hydrogel to its initial weight) calculated as a percentage.

4.3.4 Application of an electric field to hydrogel samples

An electric field was applied to the hydrogel samples using the apparatus described in fig. 4.2 . The hydrogels were placed in the circulating water bath between two carbon electrodes (5mm diameter, 1cm apart) and constant voltage was applied from a D.C. source.

4.3.5 Loading and release from HA and MAA hydrogels

The hydrogels were immersed in the loading solutions of 50mg/ml of the model solutes (BSA,PSSA,PGT) or 15mg/ml of caffeine, and were incubated at 5°C for at least 3 days. The solute release measurements were performed using the same apparatus described in section 4.3.3 . Aliquots of the release media were taken at specific intervals and analysed spectrophotometrically at 261nm (PSSA), 273nm (caffeine), 275nm (PGT) and 280nm (BSA).



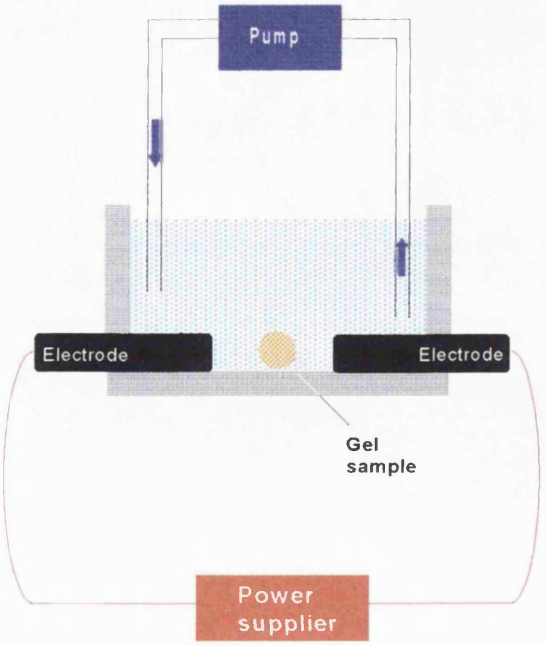


Figure 4.2 Appartus used for the application of an electric field to hydrogel samples.

4.4 Electro-responsive swelling of hyaluronic acid (HA) hydrogels

HA hydrogels are highly swollen in water due to the ionization of their carboxylate groups (pKa 3.2) at neutral pH. The swelling ratio of such hydrogels was around 500, indicating that 99.8% of the matrix is water and only 0.2% is polymer. The existence of fixed negative charges in the network increases its hydrophilicity and causes repulsion between the polymeric chains, consequently stretching the network and increasing its ability to become further hydrated. As with other polyelectrolyte hydrogels, HA gels are sensitive to the pH and ionic strength of their medium (Shah and Barnett 1992), the former changing the ionization state of the network, whilst the latter affects the repulsion between the polymeric chains and the osmotic pressure between the hydrogel and in its surrounding medium. Each polyelectrolyte polymeric chain is surrounded by an electrical double layer which contains counter-ions in an equal number to the fixed charges. The radius of the double layer is defined as the Debye length. The electric field established due to the fixed charges decays significantly after one Debye length, and consequently the network stretches in order to keep the polymeric chains at a distance above this Debye length in order to reduce the repulsion forces. Increasing the ionic strength of the medium leads to smaller Debye lengths due to the increase of concentration of the counter-ions (Martin *et al.* 1983, Atkins 1990). Therefore increasing the ionic concentration to a certain level causes the shielding of the fixed charges and allows a more compact arrangement of the network. It also increases the osmotic pressure of the solution outside the hydrogel, hence

limiting the hydration of the network. Figure 4.3 shows the effect of the ionic strength on the swelling of the hydrogels. Upon increasing the salt concentration to 1mM the gel deswells and further elevation of the ionic strength reduces the swelling of the hydrogel. The uni-divalent salt (sodium sulfite) has a greater effect on the swelling then the uni-univalent salt (sodium chloride) at the same salt concentration as expected.

When exposed to an electric field ($5V \bullet cm^{-1}$), the hydrogels shrink and lose almost two thirds of their water content after 30min (fig. 4.4). A small movement of the hydrogels toward the anode was observed. Two mechanisms are involved in the deswelling process (fig. 4.5). Firstly the electric field causes the migration of the Na^+ counter ions in the hydrogel toward the cathode, which produces a stationary current in the hydrogel, resulting in partial shielding of the carboxylate groups. The dimensions of the double layer are also reduced (reducing the Debye length) which results in reduction of the extent of hydration of the hydrogel. The second mechanism involves an uniaxial stress gradient which is caused by the electrostatic force on the fixed carboxylate groups by the anode, which is greatest at the positive electrode and least near the negative electrode. This gradient plays an important role in the deformation of the hydrogel (Tanaka *et al.* 1982) and can explain the initial anisotropic deswelling of the network (the process starts near the anode) as seen in fig. 4.6 . Figure 4.7 describes the effect of different electric fields on the deswelling process. Under an electric field of $5V \bullet cm^{-1}$ upwards the process was rapid causing the loss of over 40% of the hydrogel water content within the first 10min.

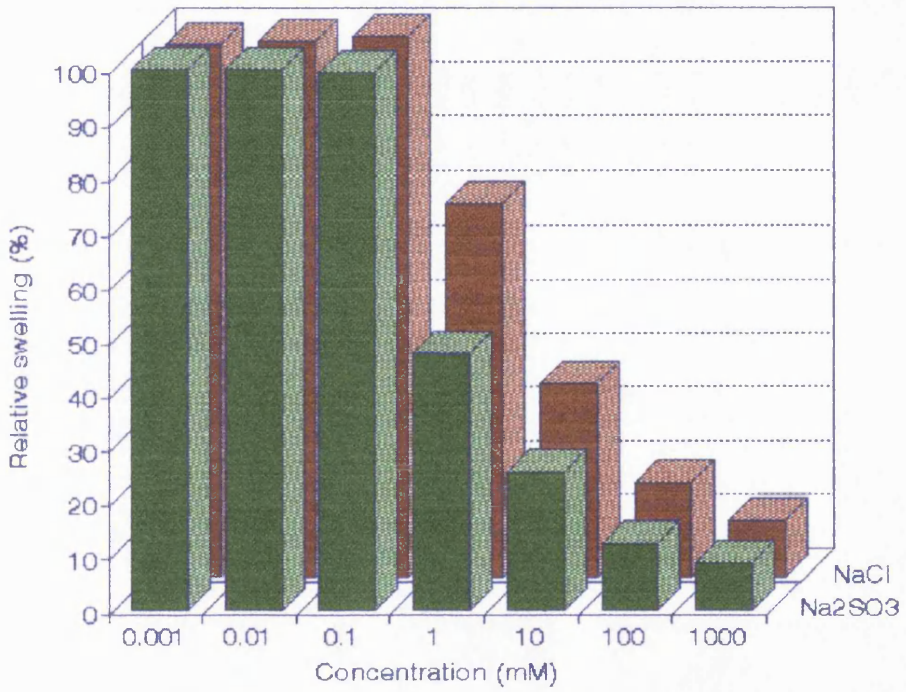


Figure 4.3 Relative swelling of HA hydrogels in solutions of sodium chloride and sodium sulfite, as a function of their concentration.

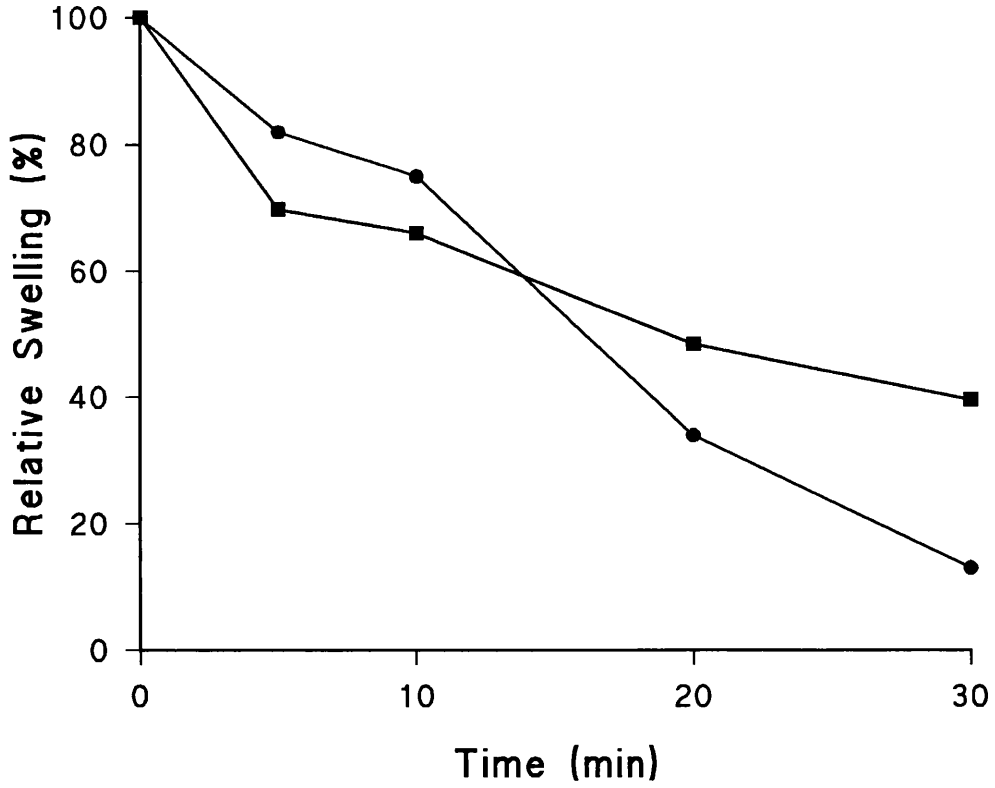


Figure 4.4 Deswelling process of unloaded (■) and BSA-loaded (●) HA hydrogels under an electric field of $5V \cdot cm^{-1}$.

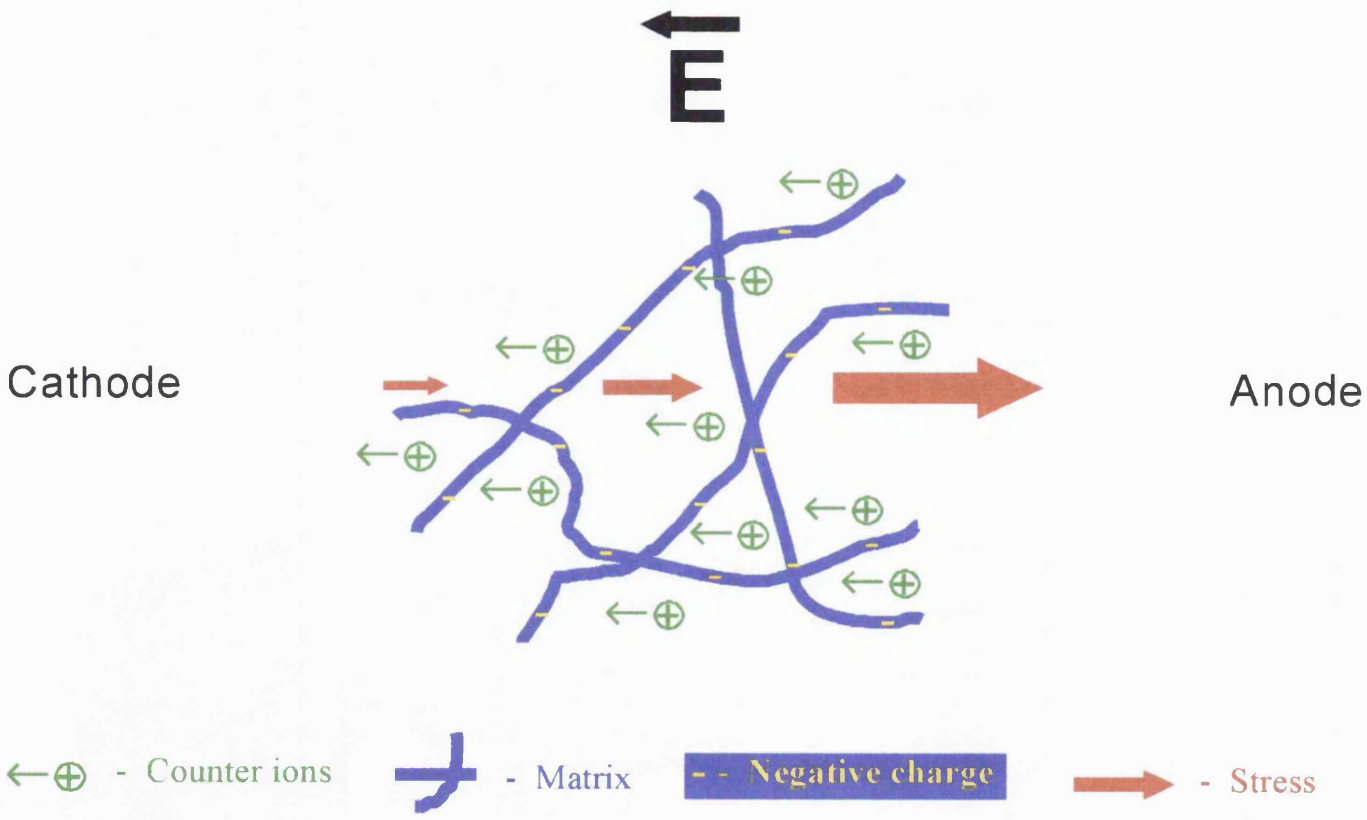


Figure 4.5 The effect of application of an electric field to a polyelectrolyte network.

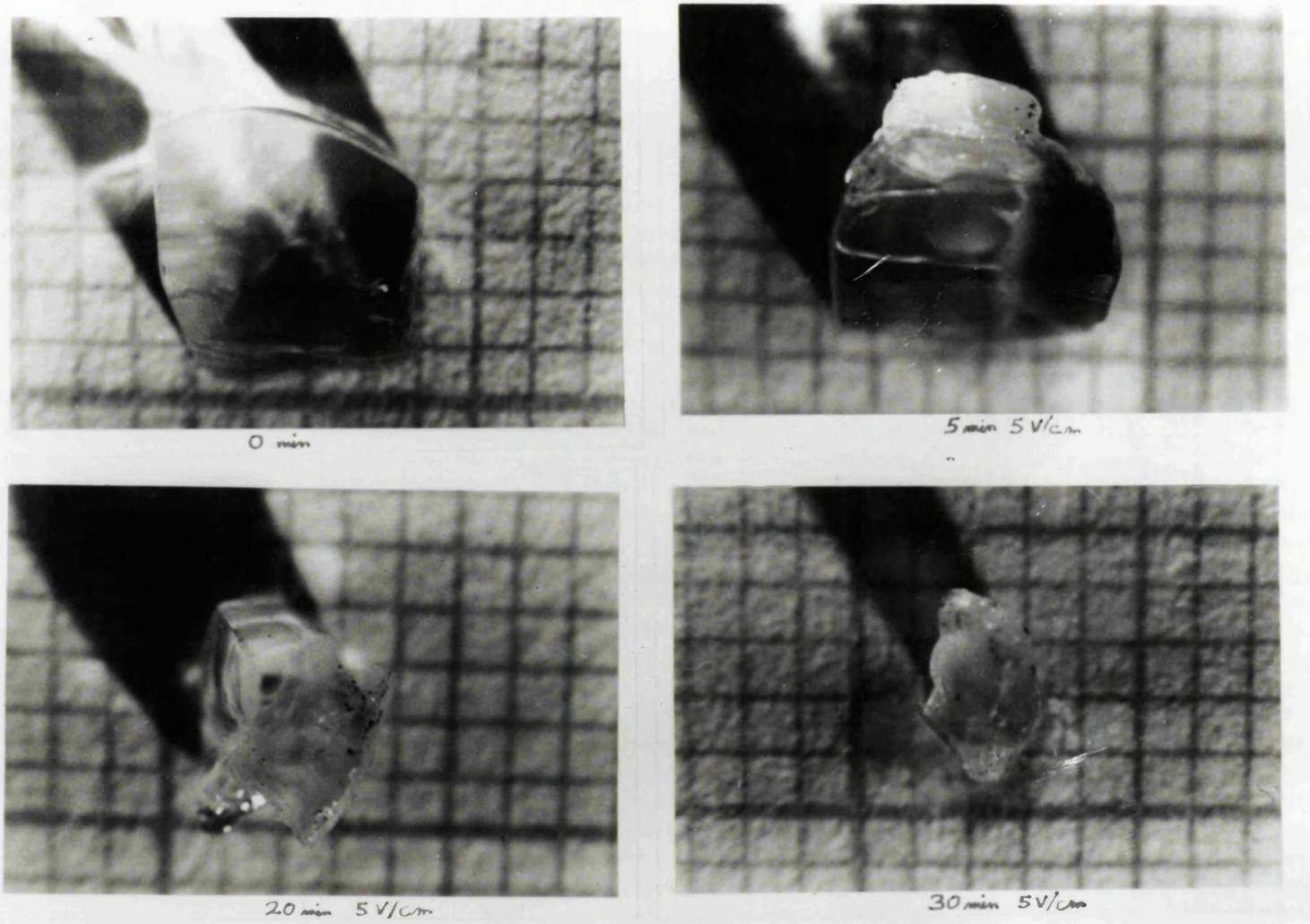


Figure 4.6 BSA loaded HA hydrogels before and after 5,20 and 30 min application of an electric field of $5V \bullet cm^{-1}$.

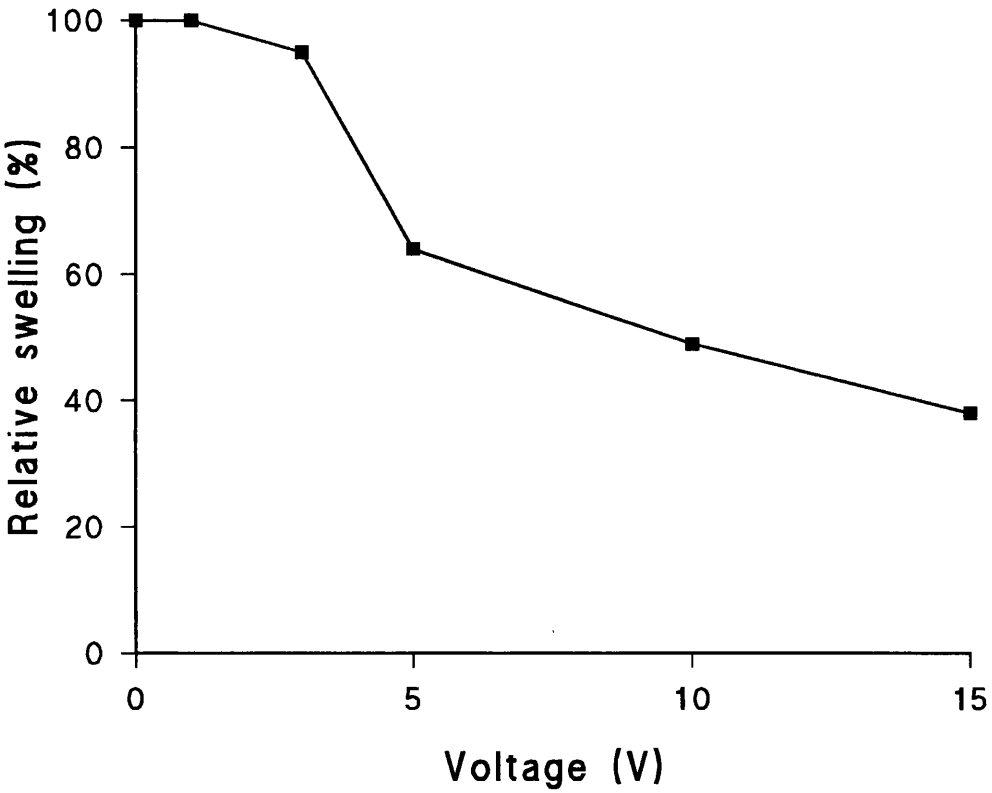


Figure 4.7 Relative swelling of HA hydrogels after 10 min under different electric fields.

HA gels loaded with BSA exhibit the same swelling ratio at neutral pH as the unloaded gels (~500). BSA is a zwitterionic protein, with an overall negative charge of 18 at pH 7.4, and is known to have the ability to bind anions (Swaney and Klotz 1970). BSA loaded hydrogels significantly deswell on application of an electric field, losing about 85% of their water content (figs. 4.4 and 4.6). While the contraction of the unloaded gels was mostly reversible, the collapsed BSA loaded gels did not reswell (fig. 4.8) and became turbid. Complex formation between the anionic gel and the BSA is a possible explanation.

HA gels loaded in 50mg ml^{-1} solutions of PSSA showed a different swelling pattern. They swell marginally to 6% of the swelling ratio of the unloaded gels similar to the effect displayed in fig. 4.3. While unloaded gels and gels loaded with BSA showed maximum swelling, the gels loaded with the highly ionized PSSA solution behaved like those in high ionic strength solutions of NaCl and Na_2SO_3. After transferring the PSSA loaded gels to water, swelling occurs rapidly and the gel swelling ratio increases by 16 fold over 30min, as shown in figs. 4.9 and 4.10. The release of PSSA into the solution reduces the internal osmotic pressure as the concentration of PSSA around the gel decreases. Applying an electric field restricts the swelling of the loaded hydrogels (figs. 4.9 and 4.10). The moderate swelling is due to the same mechanism which forces the deswelling of the unloaded hydrogels. Upon application of an electric field of $10V \bullet cm^{-1}$ the gels swell moderately and

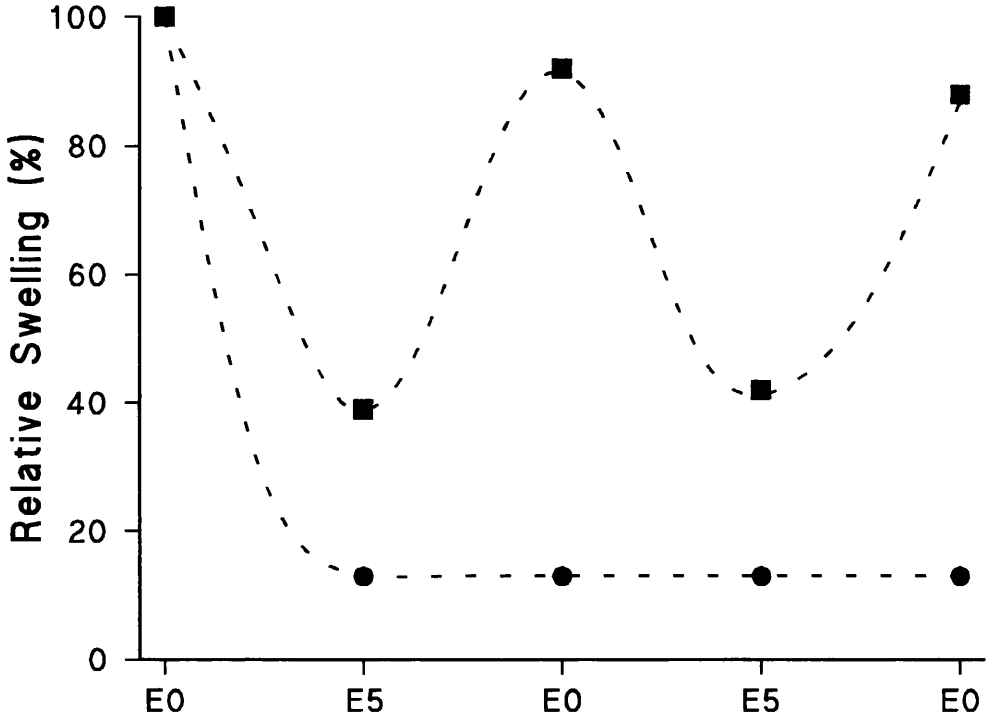


Figure 4.8 Relative swelling of unloaded (■) and BSA-loaded (●) HA hydrogels under electric field of $5V \cdot cm^{-1}$. E0- 48hr, no electric field; E5- 30min, electric field of $5V \cdot cm^{-1}$

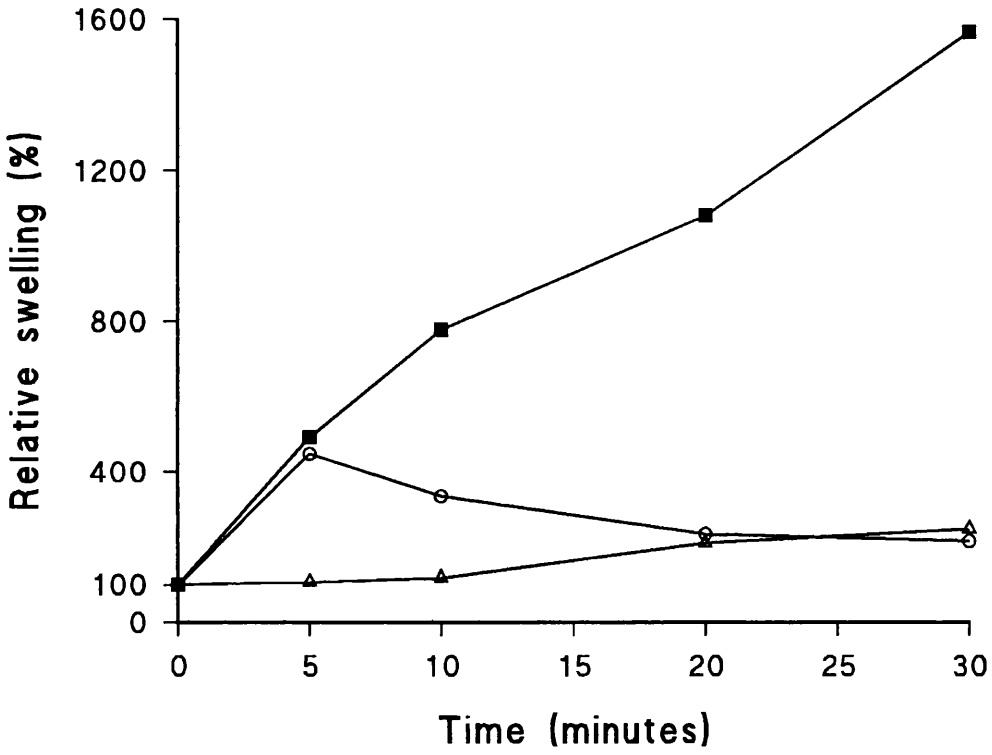


Figure 4.9 Relative swelling of PSSA-loaded HA hydrogels without (■) and under an electric field of 5V•cm^{-1} (O) and 10V•cm^{-1} (Δ).

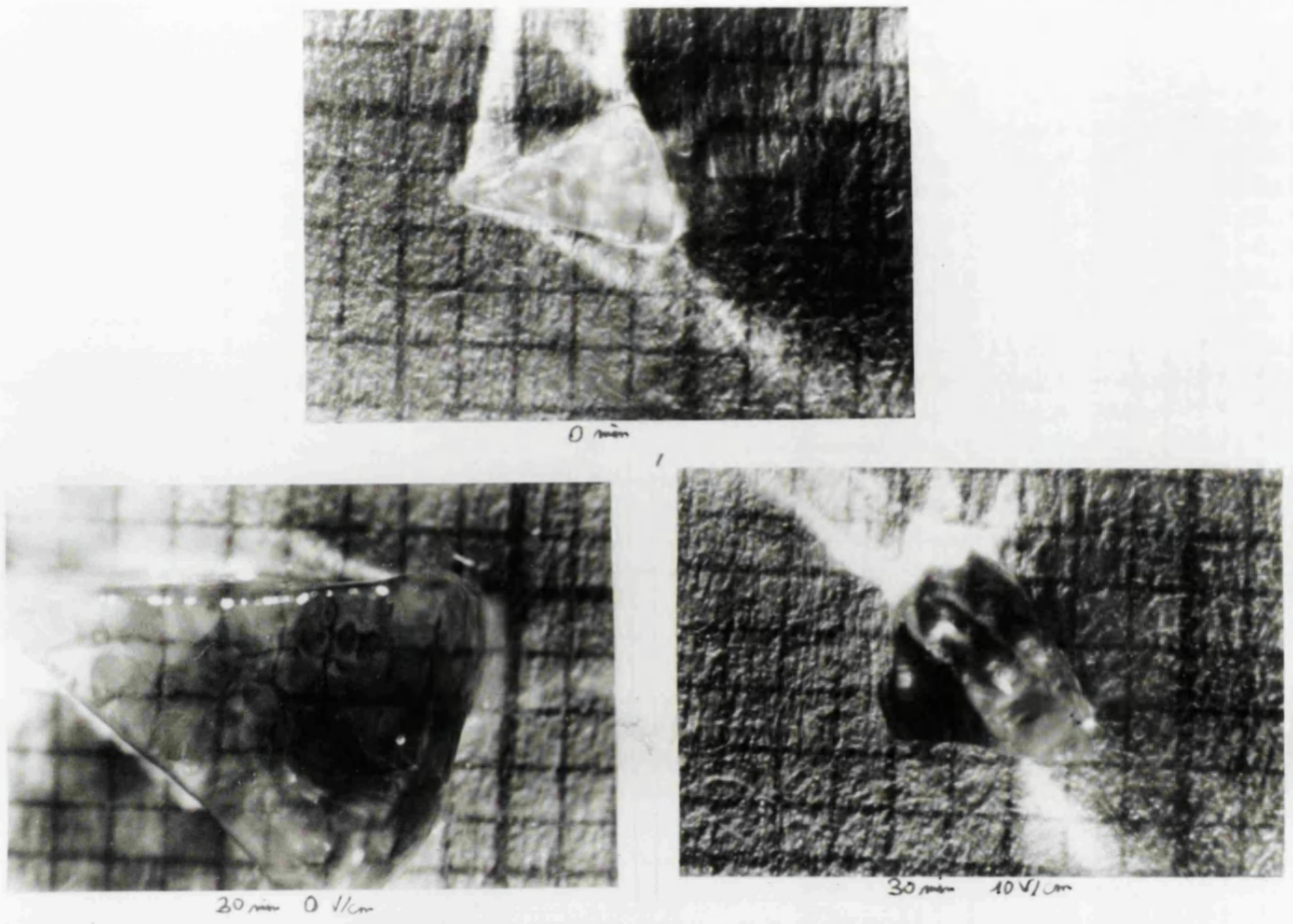


Figure 4.10 Swelling of PSSA-loaded HA hydrogels in the loading solution (t=0min) and after 30min in water with and without exposure to an electric field of $10V \bullet cm^{-1}$.

doubled their weight after 30min. Under an electric field of $5V \bullet cm^{-1}$ a phase of swelling was first observed, followed by the deswelling of the network. The rate of response to the electric field is dependent on its potency (as seen in fig. 4.7). The change in swelling pattern after 5min under $5V \bullet cm^{-1}$ can be explained by a shift in balance of two elements: the swelling caused by changes in the osmotic pressure and the electric field-induced deswelling.

A different swelling experiment is shown in fig. 4.11 . HA hydrogels were loaded with 5mM NaCl (in order to improve conductivity), before being taken out of the bathing solution and attached to two platinum electrodes. A 10V electrical stimulus was applied to the hydrogel. The gel swelled at the cathode and deswelled at the anode, a clear separation between the two regions of the hydrogel could be seen easily after 5min. The mechanism which affects the swelling behaviour of this hydrogel is different from the previous case. The electrically induced hydrolysis causes local pH changes around the electrodes:

$$\text{Anode:} \quad H_2O \rightarrow \tfrac{1}{2}O_2 + 2H^+ + 2e^-$$

$$\text{Cathode:} \quad 2H_2O + 2e^- \rightarrow H_2 + 2OH^-$$

The pH values were lowered near the anode and increased near the cathode resulting in differing pH values in various regions of the hydrogel. Such hydrolysis induced pH changes have been reported by Kwon *et al.* (1991b). The decrease in pH near the anode causes the protonation of some carboxylate groups on the hydrogel in this region and the opposite effect occurs near the cathode, resulting in the shifting of water toward the cathode. During the experiment a small quantity of water is expelled from the hydrogel and

accumulated on the surface near the cathode, a phenomenon described by Osada and Hasebe (1985), which indicates that an electro-syneresis process is occurring within the hydrogel as a result of the electric stimuli.

Hydrolysis can also occur when an electric field is applied to the hydrogel in solution (in the setting described in fig. 4.2), but in this case the effect is limited for two reasons: firstly, under these circumstances the solutions have a significantly lower conductivity which restricts the extent of hydrolysis, and secondly the water is circulated from one electrode to the other causing mixing which minimises any local pH differences. The difference in the swelling pattern (in response to the electric stimuli) between the different experimental conditions emphasises the different mechanisms affecting the swelling in these different cases.

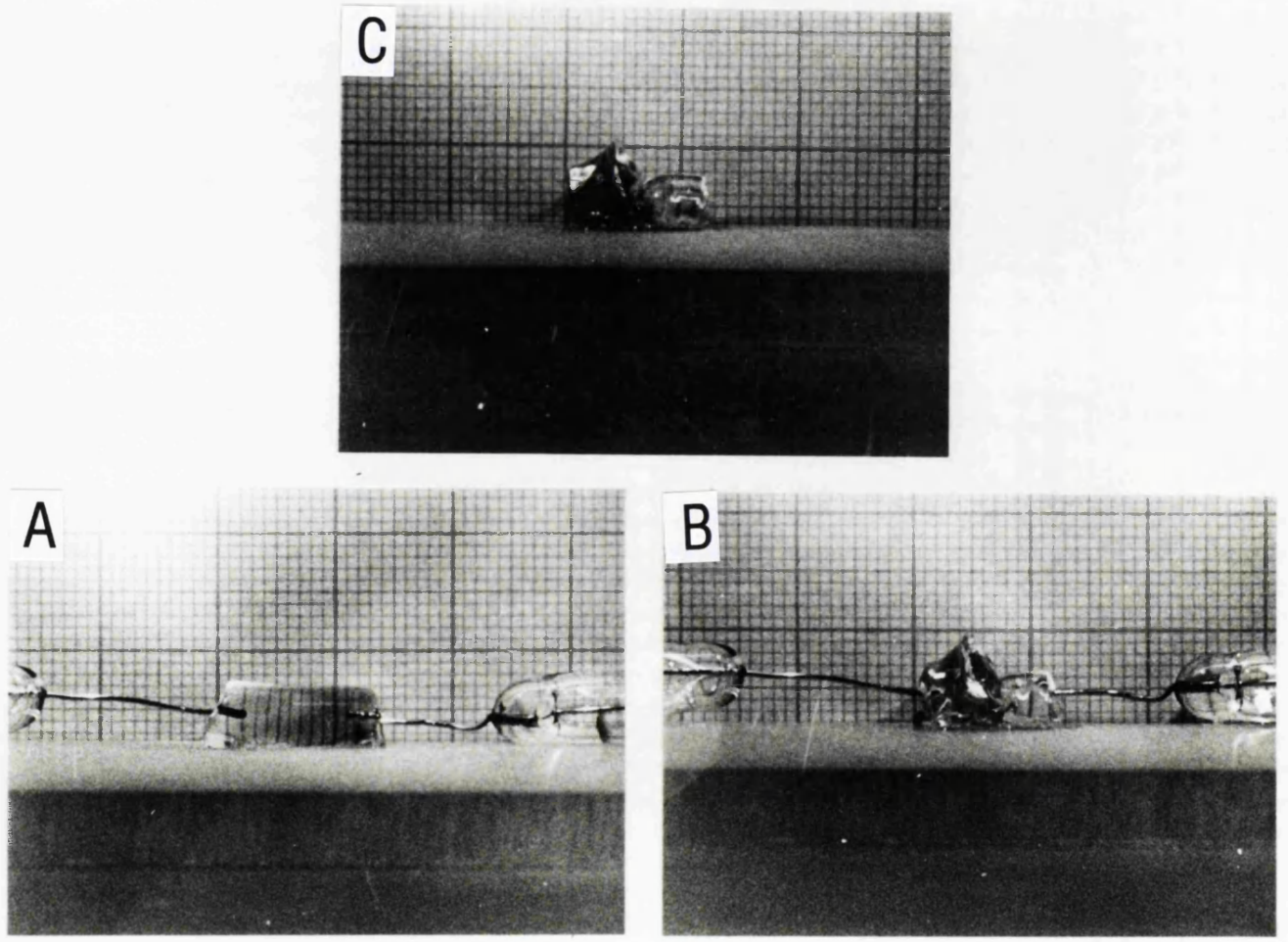


Figure 4.11 HA hydrogels loaded with 5mM NaCl before (A) and after (B,C) 5min under a 10V electrical stimulus. Anode- right; Cathode- left.

4.5 Electro-responsive release of macromolecules from hyaluronic acid (HA) hydrogels

The release of a small solute, caffeine, from HA hydrogels is described in fig. 4.12 . A similar release profile regardless of exposure to an electric field was observed, which can be attributed to the fast release of the small molecule from the highly swollen network. Most of the solute was released within the first 10min before any dimensional changes of the matrix could affect the diffusion. It should be noted that the initial extent of release (within the first 5 min) is higher under the electric field, possibly due to the 'squeezing effect' and electro-osmosis of water. The result of this study indicates that small solutes are not suitable candidates for release from the HA matrix, therefore the release of macromolecules was subsequently investigated.

Macromolecules diffuse from and through hydrogels by the two mechanisms of 'pore' and 'partition' permeation (Sato and Kim 1984). In swollen HA hydrogels permeation occurs through the aqueous channels of the hydrogel. Partition permeation is negligible due to the repulsion between the negatively charged network and the negatively charged solute. Yashuda *et al.* (1969,1971) developed the free volume theory for the diffusion of solute through a polymer membrane which was based on Eyring's concept of diffusion (Eyring 1936), whereby a solute diffuses by jumping from 'hole' to 'hole'. In the Eyring model the diffusion coefficient is derived as:

$$D = v \exp(-F/kt) = v \exp(S/k) \exp(-E/kt) \quad (1)$$

where v is the transitional oscillating frequency of the diffusion species and F,S

and E are the free energy, entropy and energy of activation for the diffusion, respectively. The energy of activation describes the temperature dependence of the diffusion, and the entropy term consist of two contributions: the conformational probability of formation of a hole sufficiently large for the passage of the diffusing molecule (exp [$-Br^2/V_p$]), and the probability of finding space in the membrane for such a hole with cross-section equal to or larger than r ($\varphi(r^2)$).

$$S/k = -Br^2/V_p + \ln(\varphi(r^2)) \quad (2)$$

where V_p is the total free volume in the polymeric matrix, and Br^2 is a characteristic volume parameter describing the diffusion of the permeant molecule (where r^2 is the effective cross-sectional area of the solute and B is a proportionality factor). From equations (1) and (2) the diffusion coefficient of solutes in the polymeric matrix can be written as

$$D_p = v\varphi(r^2) \exp(-Br^2/V_p) \exp(-E/kt) \quad (3)$$

and the diffusion coefficient of the solute in water is

$$D_w = v \exp(-Br^2/V_w) \exp(-E/kt) \quad (4)$$

assuming that the activation energy of diffusion is the same as in the matrix and φ=1. V_w is the free volume of H_2O. The free volume in the matrix for the solute diffusion can be described as the free volume of water in the swollen matrix (V_{wp}), that is:

$$V_p = V_{wp} = HV_w \quad (5)$$

where H is the degree of hydration of the matrix. Dividing equation 3 by 4 and substituting V_p with equation 5 results with

$$D_p/D_w = \varphi(r^2) \exp[-Br^2/V_w (1/H -1)] \quad (6)$$

From these model Yashuda and co-workers predict that the diffusivity of a solute through a polymeric network increases with the network hydration and decreases with the solute size. Wiess *et al.* (1986) estimated the dependence of solute diffusion on the hydration of the network. They found that the small changes of flux of small solutes can be predicted solely from the hydration changes, whilst the larger flux changes for macromolecular solutes were due mainly to steric hindrance.

The substantial difference in the swelling behaviour of PSSA-loaded HA hydrogels with or without exposure to an electric field affects the release profile from the hydrogels, as seen in fig. 4.13. Under an electric field of $10V \bullet cm^{-1}$ the release is restricted (due to the low swollen state) and levels off after 10min. The release in the first period can be attributed mainly to solute that was adsorbed onto the surface of the hydrogel. In the absence of an electric field the rapid swelling allows continuous release of PSSA. Under $5V \bullet cm^{-1}$ the release was only partially restricted due to the different swelling response to the application of an electric field. Fig. 4.14 describes the responsive swelling and release from HA hydrogels due to the switching off of the electric field after one hour. The hydrogel which swells moderately under an electric field responds by rapid swelling after the field is switched off, which causes a burst of release from the hydrogel. On-off switching of the electric field results in a pulsatile release pattern from the hydrogel, which releases some of its PSSA content when the field is switched off.

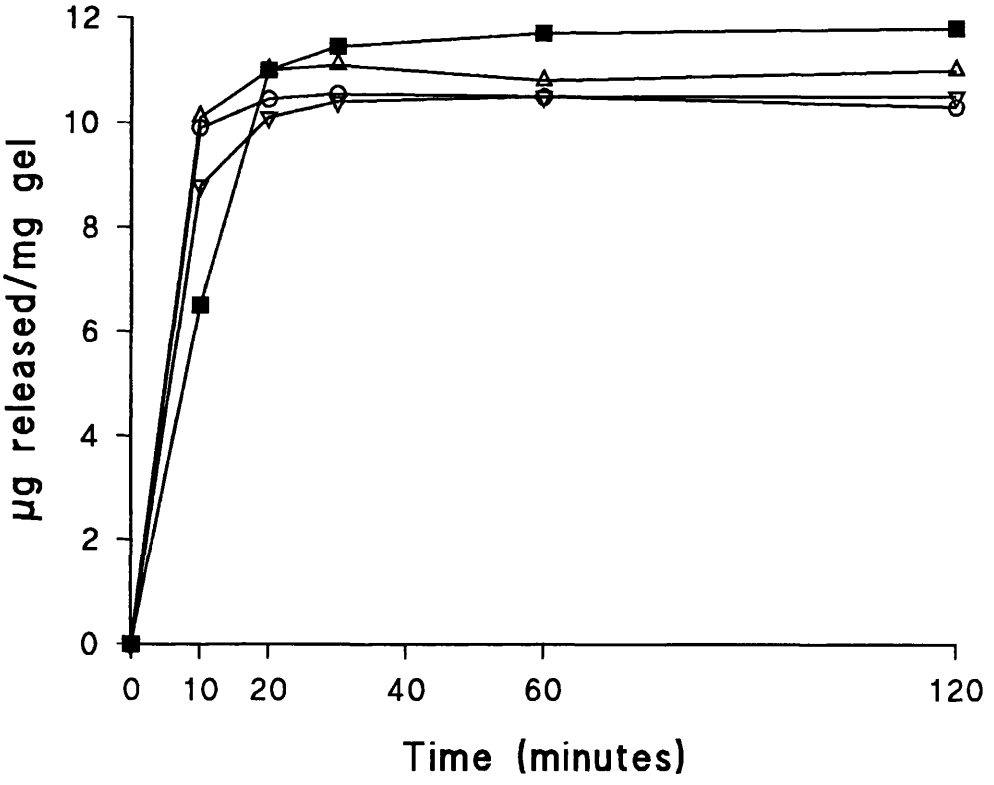


Figure 4.12 Caffeine release from HA hydrogels under electric fields of 0V•cm^{-1} (■), 3V•cm^{-1} (▽), 5V•cm^{-1} (○) and 10V•cm^{-1} (△).

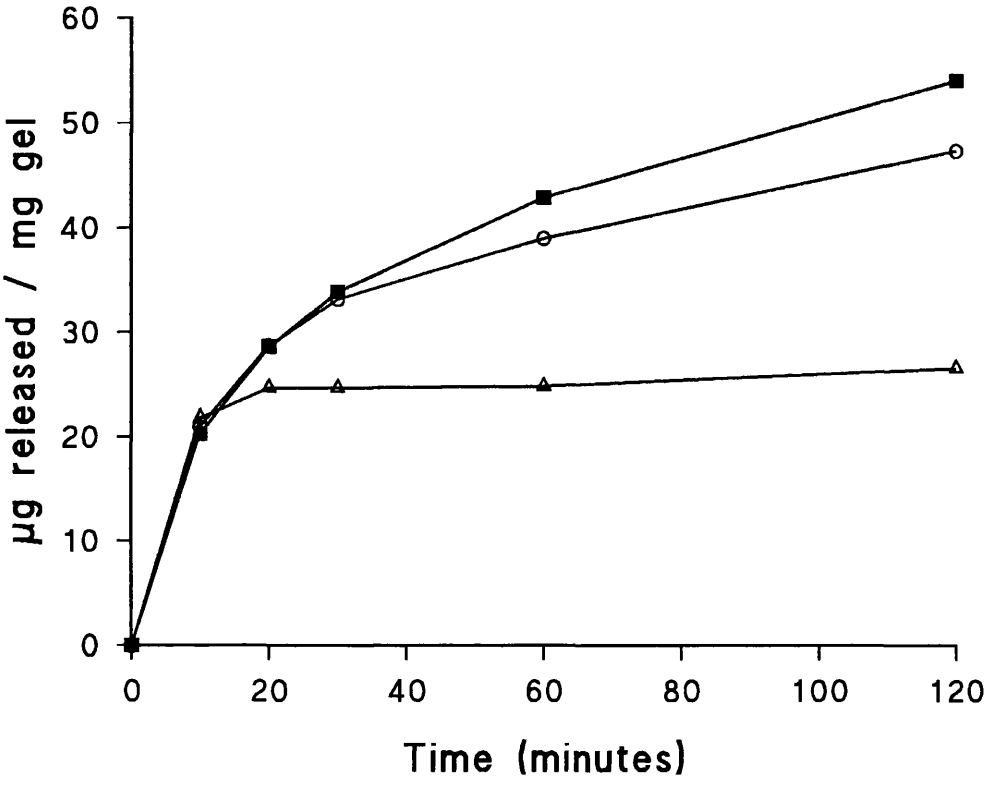


Figure 4.13 Release of PSSA from HA hydrogels without (■) and under electric fields of 5V•cm^{-1} (O) and 10V•cm^{-1} (Δ).

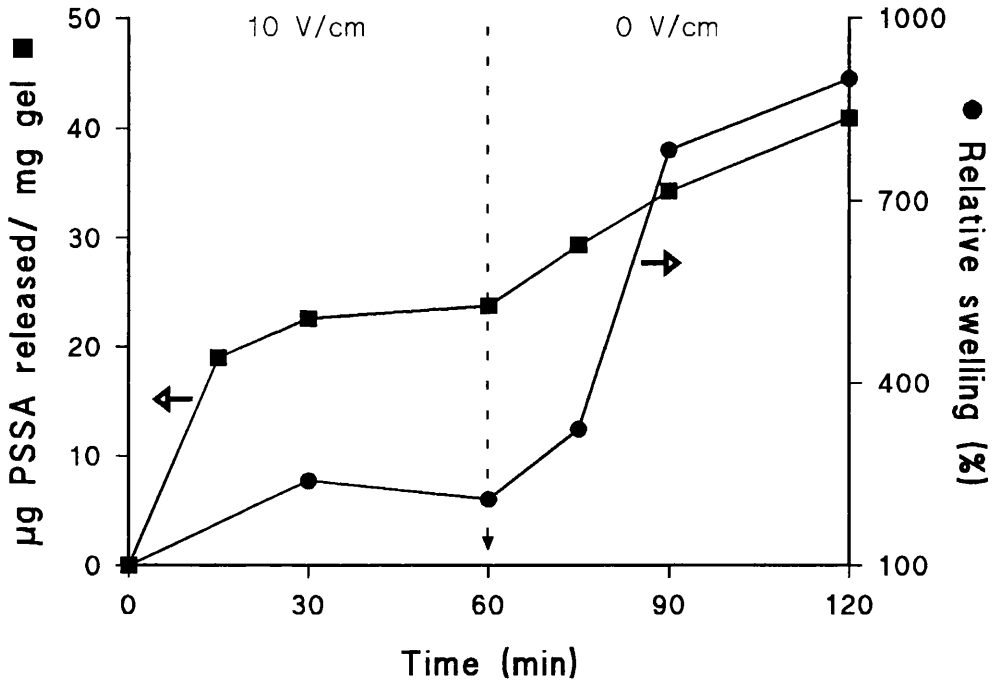


Figure 4.14 Responsive swelling (●) and release (■) of PSSA when an electric field is switched off.

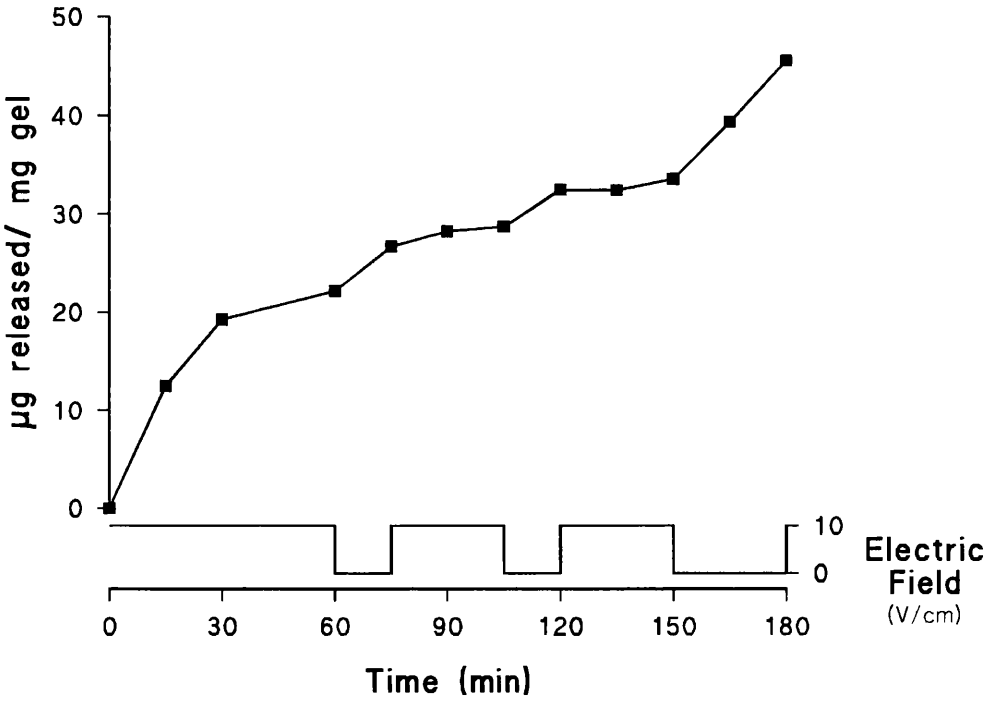


Figure 4.15 Pulsatile release of PSSA from HA hydrogels upon on-off switching of an electric field of $10V \cdot cm^{-1}$.

In a similar manner, release of the negatively charged polypeptide PGT was studied. The results in fig. 4.16 shows the difference in release with and without exposure to an electric field, a difference which enabled the preparation of a simple pulsatile release system for PGT (fig. 4.17).

The release characteristics of this pulsatile system (shown at figs. 4.15 and 4.17) are different from those described elsewhere (Osada and Hasebe 1985, Sawahata *et al.* 1990, Kwon *et al.* 1991), which are synthetic polyelectrolyte matrices that are less swollen and are used for pulsatile release of small solutes. These other hydrogels release their content when an electric field is applied due to the effect of the deswelling hydrogels. The release of macromolecules from HA hydrogels described here shows the reverse response to the electrical stimuli. The solute is released when the electric field is switched off and stopped when the field is turned on again. HA hydrogels are not suitable for the release of positively charged macromolecules because of their interaction with the negatively charged network under an electric field. Positively charged solutes require a positively charged polyelectrolyte network to achieve a similar result.

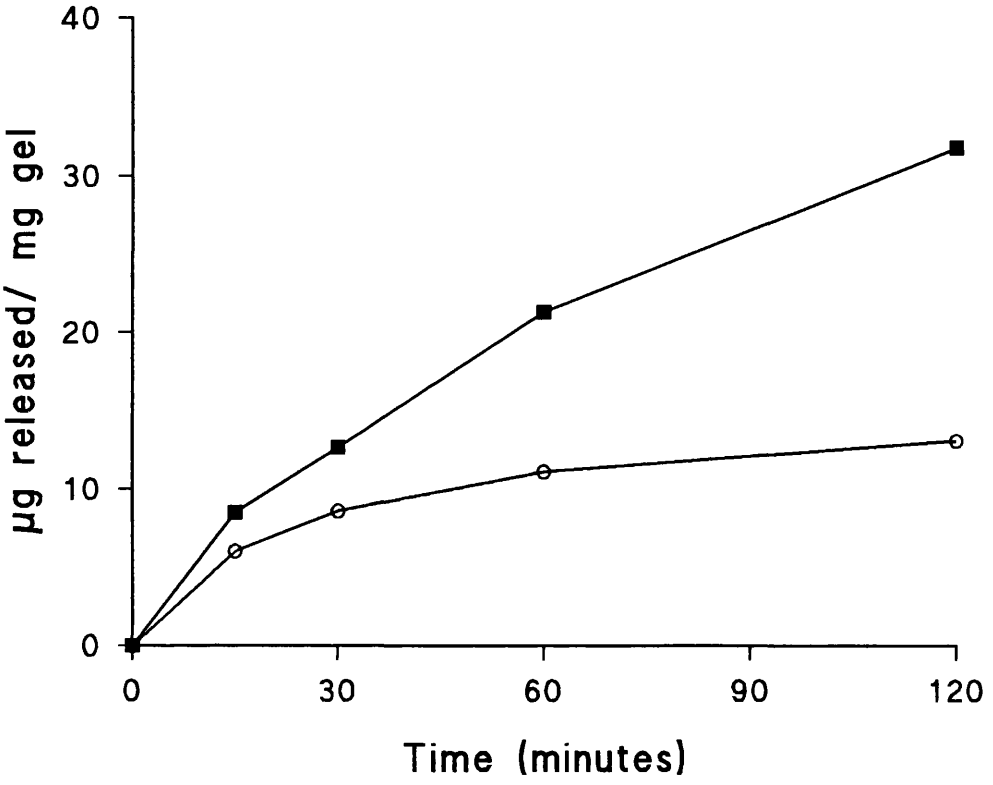


Figure 4.16 Release of PGT from HA hydrogels without (■) and under an electric field of $10V \cdot cm^{-1}$(O).

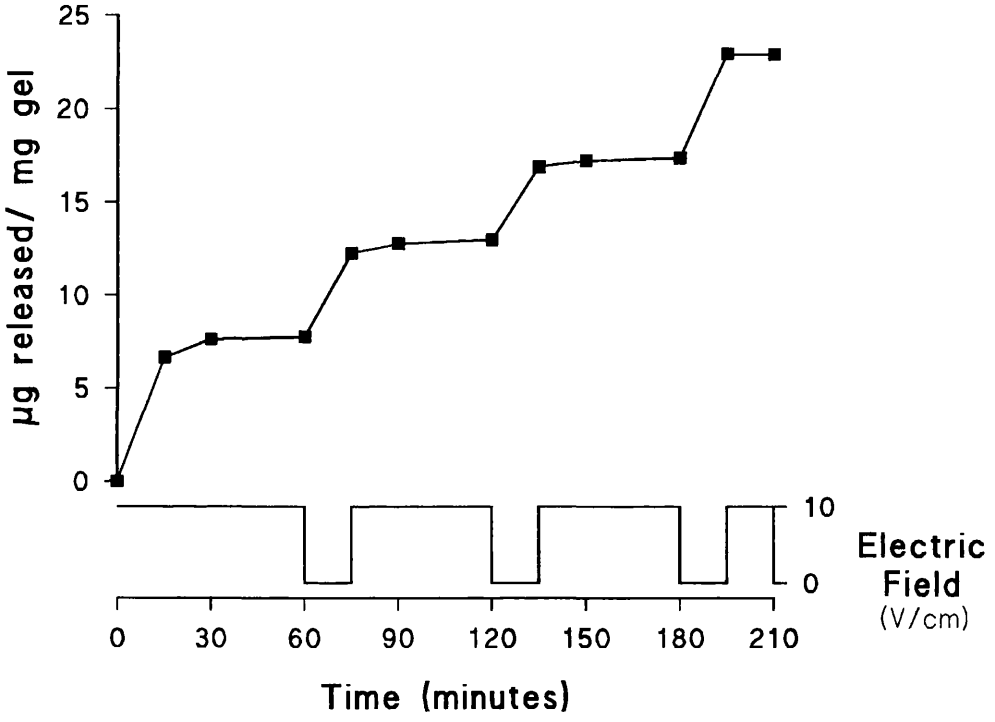


Figure 4.17 Pulsatile release of PGT from HA hydrogels upon on-off switching of an electric field of $10V \cdot cm^{-1}$.

4.6 Electro-responsive behaviour of methacrylic acid (MAA) hydrogels

The electro-responsive behaviour of MAA hydrogels was studied in order to demonstrate that an electric field can induce an opposite swelling effect on different matrices from the effect that was described earlier with HA hydrogels. MAA is a weak acid which is only slightly ionized at neutral pH and therefore is only moderately swollen in water. Application of an electric field was reported to induce swelling of the MAA hydrogel matrices (Sawahata *et al.* 1990) and membranes (Grimshaw *et al.* 1989,1990). Sawahata *et al.* explained this phenomenon by the electro-diffusion effect which forces the hydrogen ions out of the matrix and induces the ionization of the carboxylic groups. The structural change results in an increase of solute permeation and release from the matrix. Fig. 4.18 presents the change in the swollen state of MAA after 2h under an electric field of $5V \cdot cm^{-1}$. The gels swell in an anisotropic pattern, the extent of swelling being greater near the cathode due to the stress gradient caused by the electrostatic force applied by the anode. The amount of caffeine released from the matrix after 2h almost doubled upon application of the electric field due to the induced swelling change (fig. 4.19).

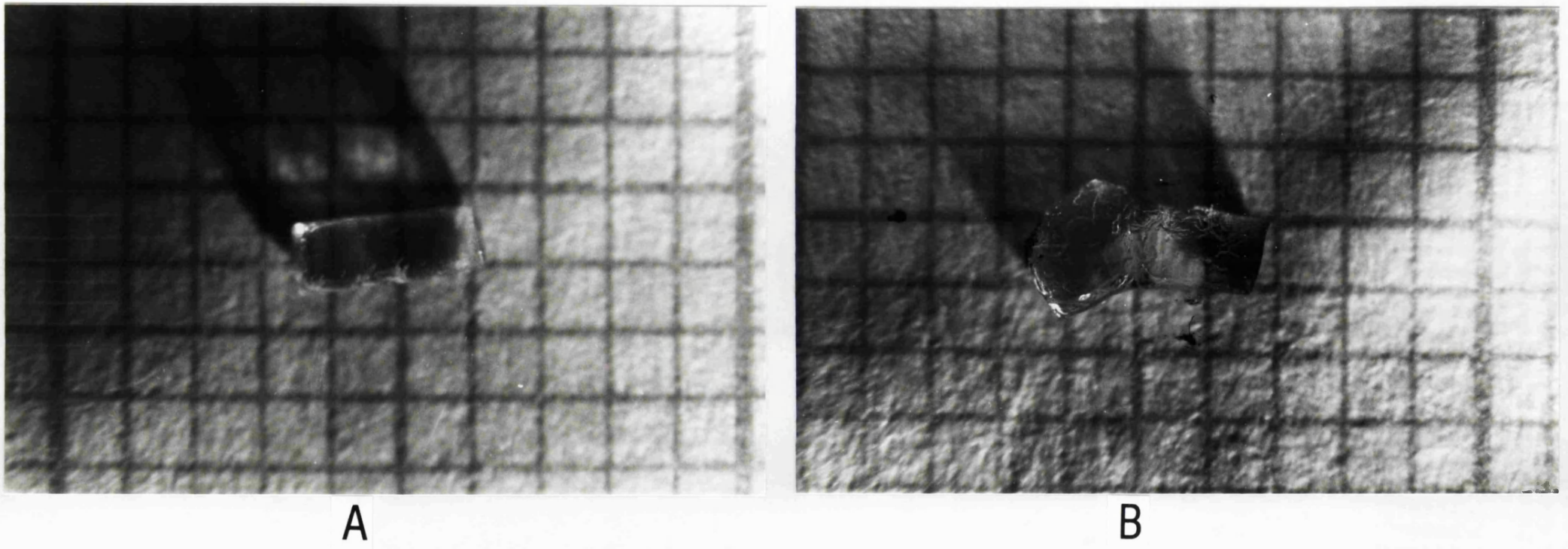


Figure 4.18 MAA hydrogels before (A) and after (B) exposure for 2hr to an electric field ($5V \bullet cm^{-1}$)

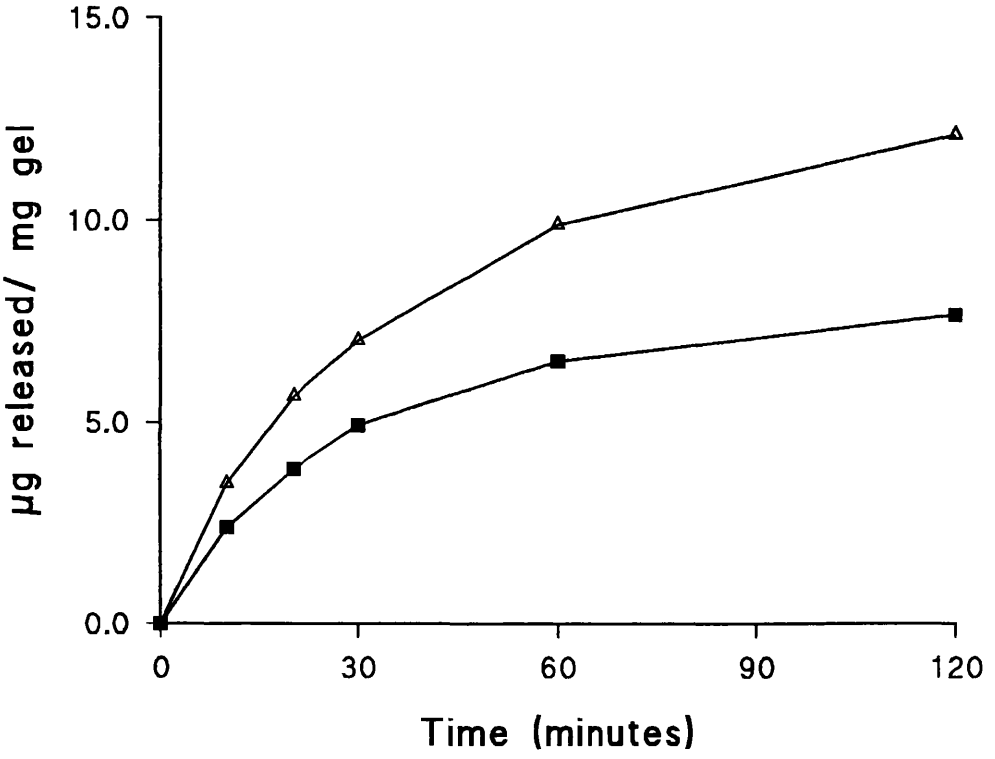


Figure 4.19 Release of caffeine from MAA hydrogels without (■) and under (▵) electric field of $5V \bullet cm^{-1}$.

Chapter 5- conclusions and future work

5. Conclusions and future work

This study has laid some milestones in the long journey to prepare responsive pulsatile release system. The potential for the use of an external stimulus, either thermal, photo-irradiation and electrical, for modulation of drug release from hydrogel matrices has been demonstrated.

Temperature responsive hydrogels were synthesized using poly(N-isopropylacrylamide) and some of its co-polymers. These hydrogels showed negative thermo-sensitivity, highly swollen at low temperatures and deswelled on temperature elevation. Release of water soluble solutes was modulated by this thermo-induced change, although the extent of modulation was limited due to the fast solute diffusion from the highly soluble matrix. Generally, release from the matrix is reduced upon elevation of the temperature because of the formation of a thin and dense 'surface shrinking layer'. Better release control was achieved using a slightly water soluble drug, and a pulsatile release pattern due to temperature cycles was demonstrated for such drug.

Photo-responsive hydrogels were prepared by incorporation of two chromophores into a hydrogel matrix. Photo-irradiation was observed to have only limited effect on the release profile from such hydrogels. Release form hydrogels containing the azobenzene pendant group was moderately increased upon exposure to UV radiation, whilst the same radiation slightly reduced the release from hydrogels containing azobenzene in their cross-linking unit. The effect of irradiation on the first group of hydrogels can be attributed to the change in hydrophobic interactions and on the latter gels to geometrical

changes in the length of the cross-linking unit. The limited response to the photo stimuli can be attributed to two factors: firstly, the chromophores are an integral part of the polymeric network and possibly require a very high energy to force the structural change; secondly, light penetration was restricted due to the hydrogel's bulky structure. These problems could be overcome by using a laser source, which can supply high energy in a very short pulses, with a thinner structure which will transmit light.

Electrical stimuli were used to control the dimensions of polyelectrolyte hydrogels and modulate release from them. A pulsatile release system for macromolecules was prepared using hyaluronic acid hydrogels. Such a system showed a reverse response to the stimulus, the hydrogels swelled and released their content when the electric field was switched off, and deswelled stopping the release when the stimulus was reapplied.

This work explored only the first step in the challenge to produce an applicable responsive drug delivery system. The next steps should include the optimization of such a system and an attempt to reduce the size of the whole setup.

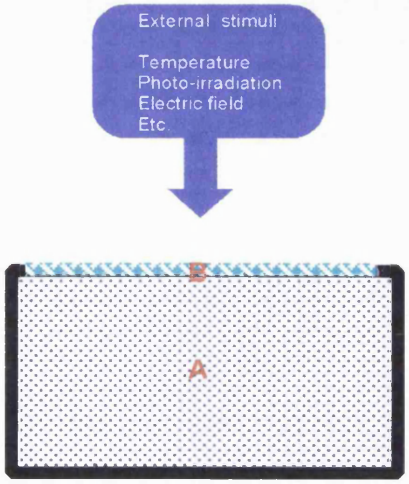
Other matrices should be examined, for instance vesicular and particular systems having the same functional groups and monomers described in this thesis. Reduction of the dimensions of the matrix can improve the response time to the stimuli. Mast cells were earlier mentioned as such an example. These are granular structures which respond within milliseconds to a change in the electrical potential of their membrane and rapidly release their content (Nanavati and Fernandez 1993). Those granules consist of the bipolymer

heparin sulphate proteoglycan. In order to mimic such responsive behaviour, heparin (or similar polysaccharide) microspheres could be prepared which may demonstrate a similarly rapid response to electrical stimuli. For photo responsive systems microstructures may be favourable since they will not prevent the penetration of light as the bulky hydrogels. Vesicular and particular systems can be utilized in combined systems of responsive release and targeting. They can be administrated intravenously and circulate in the blood. The stimuli could be applied near the target organ and induce local release of the drug.

Application of responsive hydrogels will require implantation subcutaneously or near the target organ. The external stimuli could be applied by external local heating, photo-irradiation via fibre optics and implantable miniature batteries and electrodes or an external electric field. Responsive hydrogels have a big disadvantage when used as implanted drug carriers because of their restricted loading capacity and their short period of usage. The alternative is to use a hydrogel membrane or similar thin film to control drug release from a reservoir. Such a system could have a drug capacity for a long operation life, and would require a hydrogel which can be reliably operated for the whole period during many cycles. A different approach is to utilize the great dimensional changes in some hydrogels (as was demonstrated with electro-responsive hydrogels) in order to mimic a pump action. Such a theoretical system is described in fig. 5.1. This system contains two compartments: one will include the hydrogel, miniature batteries and the electrodes and is permeable to water, and the other contains the drug solution.

The two compartments are separated by an agar piston. When a stimulus is applied the gel will expand forcing some of the drug solution out of the capsule through a small hole in the drug compartment. Release will cease when the stimulus is stopped, and will start again when another stimuli will cause further expansion of the hydrogel. In this manner small quantities of the drug can be released in a pulsatile pattern.

Responsive hydrogels can be used in other applications. Chromatography could be a suitable field for such systems which change their pore size as well as other microstructural characteristics under external stimuli. Selective absorbtion gels can used for humidification which waste no energy on cooling due to their ability to suck water from the air. Other hydrogels can be designed for purification and isolation of products. For example, it would be useful for the production of baby food, where they could sop up worthless whey (small molecules) and leave behind the valuable curds (large protein molecules) and then deswell to release their load of whey and reused again. The ability to regulate the activity of enzymes which are immobilized within a responsive network could be very beneficial for industrial purposes. The work in this thesis has shown the ability to affect enzyme activity by thermal-induced swelling changes of a hydrogel network. Electrical stimulus should be investigated as other mean for regulation of immobilized enzyme activity because of the large scale volume changes it induces. It seem that such industrial aspects could be achieved earlier than responsive drug delivery systems since they pose less technological difficulties in the production of an applicable apparatus.



A- Drug resevoir

B- Hydrogel membrane

(A)

A- water permeable wall

B- Battery

C- Electrodes

D- Hydrogel

E- Piston

F- One way valve

G- Drug reservoir

(B)

Figure 5.1 Possible responsive drug delivery systems based on : A- hydrogel membrane; B- hydrogel 'pump'.

Systems which respond to more then one stimulus could also be prepared to allow multi-phasial control of the hydrogel properties. Such a system could combine the photo-stimuli on triphenylmethane leucoderivatives, which induce its ionization, with electrical stimuli that reacts on the ionized group. By different periods of light irradiation , we can control the quantity of ionized group which will exist on the network and therefore control the effect to the electric stimuli.

During the 3 years in which this subject has been researched the amount of publications concerning responsive systems have more than tripled and many research groups have joined the effort to prepare similar networks. In 1994 the first company ("Gel Med Inc.") was established for the purpose of developing pharmaceutical and other applications of Engineered Response™ hydrogels, and it is already reporting on some promising financial contracts. In this thesis only the first steps have been made and there is still a long way ahead before the goal of an effective responsive drug delivery system will be achieved. Although at present such a system could be seen as science-fiction, sometime in the near future such an applicable system will be commercially available. After all, 30 years ago 'key hole' surgery was regarded as science-fiction and gene therapy as just another 'wild' idea.

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