CHARACTERISATION AND MODIFICATION OF HYDROGEL POLYMERS

by

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SUMMARY

The work described in this thesis can be broadly divided into two sections. The first being the characterisation of hydrogel polymers in both their hydrated and dehydrated states and the second some aspects of the structural modification of polymers.

The characterisation of hydrogel polymers in their dehydrated state (xerogels) involves such techniques as elemental analysis, pyrolysis gas liquid chromatography, infra-red spectroscopy, density determination and surface characterisation by contact angle measurements. The characterisation of some commercially available hydrogel materials was undertaken using such techniques and the results obtained were compared to laboratory synthesised systems in an attempt to assess the value of the combination of techniques employed. In the characterisation of hydrated polymers the amount and nature of water present is the single most important factor. The most convenient method of characterising this water involves the use of differential scanning calorimetry (DSC), coupled with total equilibrium water content measurements. DSC distinguishes between non-freezing and freezing water but in addition provides some information on the continuum of states in the freezing water fraction.

Two aspects of the structural modification of hydrogel polymers were studied. The first involved the incorporation of acrylamide and substituted acrylamide monomers into a copolymer system and an examination of the effect of this on the amino acid interaction of the polymers. The second was the attempted synthesis of cell surface analogues by the attachment of sugar type molecules to the polymer using a variety of reaction methods.

KEY WORDS

Hydrogel
Glycoprotein Analogue
Poly(2-hydroxyethyl methacrylate)
Water Binding

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CHAPTER ONE

INTRODUCTION

Hydrogel polymers can be broadly defined as polymeric materials which exhibit the ability to swell in water and retain a significant amount of water absorbed within their structure. The amount of water absorbed can vary over a fairly wide range and will tend to affect the physical properties of the polymer without causing dissolution in water. This general definition of a hydrogel includes a wide range of materials both natural and synthetic in origin. The work described in this thesis is concerned with some aspects of the characterisation and modification of synthetic hydrogel materials in both hydrated and dehydrated forms.

Hydrogel materials have found applications in a wide variety of fields and are particularly useful in biomedical applications[1]. In all applications, the success with which a hydrogel can be employed is governed by the properties exhibited by the material in question. This is especially true in the case of biomedical applications in which the hydrogel is in contact with a physiological environment. This introductory chapter presents a brief overall view of the nature, properties and applications of synthetic hydrogels particularly in relation to their use in biomedicine. Properties of interest in these applications may be broadly classified as:

(i) surface properties: which govern the interfacial phenomena (such as blood clotting) when a hydrogel is in contact with a biological environment,

(ii) transport properties: which are important if the material is to be used as a semi-permeable membrane (eg in dialysis membranes, contact lenses and liver support systems), and

(iii) mechanical properties: such as rigidity, tear strength and tensile strength.

As can be readily appreciated, there is a significant requirement to match the properties and structure of a hydrogel material to a given application, this being of particular importance in the field of biomedicine. In order to fulfil this requirement the need to characterise materials with respect to their structure and properties becomes apparent. This in itself is not necessarily an easy process because the basis for hydrogel synthesis is vinyl polymerisation and hydrogels are usually copolymers of two or more monomers, hence, such factors as reactivity ratios and monomer residuals will affect the ultimate composition of any polymer produced. In discussing the characterisation of hydrogel polymers, it is convenient to divide the methods employed into two categories.

(a) Characterisation of structure

In this category, it is convenient to class such techniques as

the detection of any monomer residuals in the polymer and also the determination of polymer composition in relation to monomer feed ratio.

(b) Determination of properties

This category comprises both properties that are dependent on both the chemical composition (eg density) and also the broader spectrum of physical behaviour (eg mechanical properties). The measurement of the amount and nature of water in a hydrogel network is of considerable importance within this section as this is the single most important factor in governing hydrogel properties.

This thesis then is concerned with the characterisation in the broadest sense of hydrogel polymers and also some synthetic routes for the modification of materials, particularly modification of the surface behaviour.

1.1 Development and Application of Hydrogel Materials

The usage of synthetic polymeric materials in medical applications is a comparatively recent development and it is only within the last thirty years that some commercially available polymers have been found suitable, in some circumstances, for use as surgical implants and protheses. During the last ten to fifteen years, however, the use of synthetic polymers in medical applications has increased markedly. It is during this time that

hydrogel materials have emerged and gained importance in the biomedical field due to their enhanced compatibility with surrounding tissue and interesting transport properties.

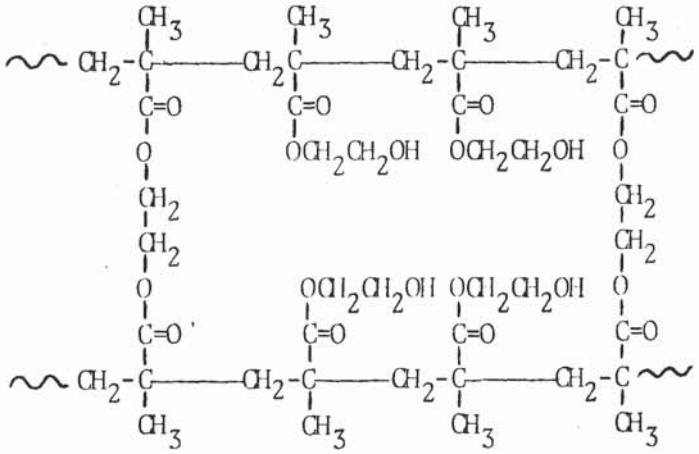
The first detailed work on synthetic hydrogel polymers was reported by Wichterle et al in 1960[2]. Wichterle claimed that covalently cross-linked water swollen glycol methacrylates such as poly(ethylene glycol methacrylate) (as shown below) were most suitable for use in biomedical applications becasue:

(i) the hydrogel hydrolyses only with difficulty, even under severe conditions,

(ii) it is relatively unaffected by biological systems,

(iii) it can withstand heat sterilisation with little damage, and

(iv) it's water content and mechanical properties can be adjusted.



Cross-linked poly(ethylene glycol methacrylate) gel shown in the absence of water (dehydrated form).

Hydrogel polymers resemble living tissue in their physical characteristics more so than do other types of material used in biomedical applications. The main reason for this enhanced resemblance to tissue is the inherent water content of the gel which influences its mechanical, bulk and surface behaviour. Although the presence of water within a hydrogel does not in itself guarantee biocompatibility, it is believed that the relatively large fraction of water present is related to their biocompatibility[3]. With reference to the water content of a hydrogel, it has been found that if the water content of a polymer is increased then the mechanical properties of the gel tend to be affected due to the plastisising effect of water within the network. The nature or states of water within the network are, however, of importance in both these aspects of hydrogel behaviour. This point is discussed in greater detail at a later stage.

As previously described, the initial work on cross-linked hydrogel polymers was reported by Wichterle et al and was based on cross-linked glycol methacrylate gels. Following this work, a wide variety of hydrogel polymers have been synthesised utilising a range of differing monomers such as those discussed in the following section.

Poly(hydroxy alkyl acrylates and methacrylates)

Included in this group of polymers are poly(2-hydroxyethyl

methacrylate) (PHEMA), poly(glyceryl methacrylate) and poly-(hydroxypropyl acrylate) (PHPA)[4].

Poly(2-hydroxyethyl methacrylate) hydrogels were first synthesised by Wichterle and Lim in an aqueous medium in the presence of a cross-linking agent. This polymer had been prepared as early as 1936 by Du Pont[5] but was not synthesised in the presence of a cross-linking agent. The synthesis and properties of polyHEMA have been described by Refojo and Yasuda[6]. If the monomer is polymerised in the presence of a good solvent for both monomer and polymer, then a homogeneous transparent polymer is formed. If the polymerisation is carried out in the presence of a poor solvent, then the resulting polymer will be heterogeneous and opaque. During polymerisation, if the water content of the HEMA/water mixture is 40% or less, then a homogeneous transparent polymer will be formed[7,8].

One of the major problems encountered in the preparation of polyHEMA hydrogels is that of impurities in the monomer. These impurities include methacrylic acid, ethylene glycol and ethylene glycol dimethacrylate. The monomer can be purified by a procedure involving extraction with hexane, distillation under vacuum and treatment with alumina[9,10]. Even after this lengthy purification, impurities still remain present at low levels in the monomer. It has been suggested that lack of reproducibility in the measurement of thrombogenicity of PHEMA materials in 'in vivo' tests may be due to as yet unidentified

impurities in HEMA monomer.

PHEMA hydrogels have been found to be particularly useful in biomedical applications due to their high degree of chemical stability. PHEMA gels are resistant to hydrolysis even under severe conditions and are relatively temperature stable[11,12]. Hydrogels made from HEMA can also be heat sterilised without apparent damage[13]. One of the principal uses for PHEMA gels has been in the manufacture of hydrophilic 'soft' contact lenses. Many studies have been published examining the usage of PHEMA gels as soft contact lens materials and indeed many patents exist on the manufacture of PHEMA contact lenses[14]. A number of drawbacks have, however, been observed in the uses of hydrophilic contact lenses. These include inadequate permeability of some materials, low visual acuity as compared to conventional hard lenses, susceptibility to mechanical damage and the need for frequent sterilisation[15,16].

Poly(hydroxypropyl methacrylate)[17] and poly(glyceryl methacrylate) hydrogels have not received as much attention as PHEMA gels. Both these materials exhibit different water binding characteristics as compared with PHEMA. Poly(hydroxypropyl methacrylate) showing a lower water content and poly(glyceryl methacrylate) exhibiting a considerably higher water content[18-20].

Poly(acrylamide) and Derivatives

Hydrogels of poly(acrylamide) and of some N-substituted derivatives of poly(acrylamide) can be prepared in an aqueous medium containing a small amount of cross-linking agent. The hydrogels are mechanically weak, optically transparent and have a water content in the order of 90%.

Studies have been carried out on the hydrolytic stability of acrylamide and methacrylamide hydrogels[21-23]. Some hydrolysis has been observed at elevated temperatures under acidic or basic conditions. However, within physiological conditions of temperature and pH acrylamide gels have been shown to be relatively stable. The tissue compatibility of poly(N-substituted acrylamides) has been studies, these materials being used in preference to poly(acrylamide) due to their improved hydrolytic stability[24]. These studies have shown that subcutaneously implanted materials of this type are well tolerated and do not provoke unfavourable reactions in test animals. The long term biological characteristics of implants of these hydrogels has been described as being similar to responses shown with PHEMA hydrogels. The thrombogenicity of poly(acrylamide) hydrogels has been investigated and has been found to be dependent on the purity of the monomer used, the purer the monomer, the less thrombogenic reaction observed[25].

Poly(N-vinyl-2-pyrrolidone)

Poly(N-vinyl-2-pyrrolidone), poly(NVP) exhibits a significant difference to the two previously discussed groups of polymers in that in an uncross-linked state it is extremely soluble in water and in many other solvents. Poly(NVP) hydrogels exhibit high water contents and hence high cross-link concentrations are required to produce hydrogels with useful mechanical properties.

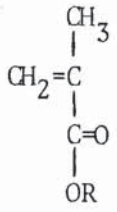
Poly(NVP) has found applications in the biomedical field most importantly as a plasma expander[26]. Poly(NVP) infused intravenously is non-toxic and non-thrombogenic and can usefully be used to maintain fluid volume within the body in cases of severe injury and trauma. At present, poly(NVP) is not used as a plasma expander as in humans as it is not metabolised and is not retained in circulation as well as other expanders available[27]. The thrombogenicity of poly(NVP) gels has been studied and residual NVP monomer has been found to have an adverse effect on blood clotting times as measured by the Lee-White test[25].

Other hydrogel materials have been synthesised from a range of monomers and examined in various applications. Table 1 illustrates some typical examples of the monomers from which hydrogel polymers have been synthesised.

Table 1 Typical monomers used in hydrogel synthesis

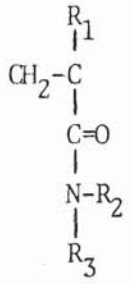
Hydrophilic monomers

Hydroxyalkyl methacrylates



$R = -CH_2CH_2OH$, $-CH_2-CH(CH_3)$, $-CH_2-CH(OH)-CH_2-OH$

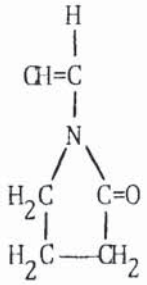
Acrylamide derivatives



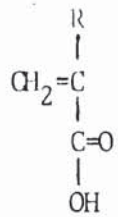
$R_1 = -H$, $-CH_3$

$R_2, R_3 = -H$, $-CH_3$, $-C_2H_5$, $CH_2CHOHCH_3$

N-vinyl pyrrolidone



Acrylic acid derivatives

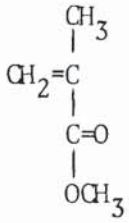


$= -H$, $-CH_3$

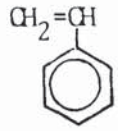
Continued ...

Hydrophobic monomers (used as comonomers)

Methyl methacrylate



Styrene



1.2 Biomedical Applications of Synthetic Hydrogels

Some typical biomedical applications of synthetic hydrogels are illustrated in Table 2. As can be appreciated from Table 2, there are a wide variety of applications in which hydrogel polymers are of value. Due to this wide spectrum of biomedical uses, it is of value to subdivide the field into applications, some specific requirements of which are discussed below.

Oesophagus prothesis: It has been found that for a successful oesophageal substitution, it is necessary for the implant to have a sufficient degree of rigidity to prevent stricture, combined with a degree of flexibility to allow movement and manipulation. Moulded cylinders of hydron (PHEMA) reinforced with Dacron fibre have been used for this purpose[28]. Such implants have only achieved limited success and failure was found to be due to the Dacron or Teflon reinforcement, it would therefore seem that hydron appears to be a good material for use in prostheses.

Ureter prothesis: A ureter prosthesis should conduct urine satisfactorily and be non-irritating and in such applications a tube of knitted polyester fibre encased in a layer of a glycol methacrylate gel has been used[29]. The polyester fibre was used to increase rigidity and to facilitate suture fixation. The hydrophilicity of the glycol methacrylate gel coupled with the use of a knitted polyester fabric enables water to be

Table 2 Applications of synthetic hydrogels

Coated Materials	Homogeneous Materials	Implanted Devices
Sutures	Contact lenses	Artifical organis
Catheters	Artificial corneas	Drug delivery systems
Vascular grafts	Soft tissue substitutes	
Blood detoxicants	Haemodialysis membranes	
Cell culture substrates	Burn dressings	
Electrophoresis cells	Vitreous Humor replacements	
	Electrophoresis gels	

transferred from the isotonic tissue fluids to the hypertonic urine and this slight flow of water into the prothesis helps to prevent incrustation.

Bile duct prothesis: Once again, a Hydron gel tube has been used for bile duct protheses in dogs and it has been shown that animals with such replacements have survived six months or more. Hydron gel was used because of its good permeability and hydrophilic nature[30].

Sutures: It has been found that bare polyamide fibres used as sutures have a toxic effect on cell multiplication and appearance, however if the same material is coated with hydron gel cell appearance is unaltered and cell multiplication is little affected[31]. Surgical sutures made of terylene caused blood clotting and tissue rejection in dogs, however if the sutures were coated in Hydron, nearly all such bodily reactions could be eliminated with no apparent effect on the mechanical properties of the fibre[32].

Plastic surgery: Transplants of fatty tissue used in breast surgery are often unsatisfactory due to resorption and scarring and for this reason Hydron has been used[33]. Hydron is permeable to fluids and is therefore preferable to other plastic materials such as poly(vinyl alcohol) and silicones (which are hydrophobic) and tend to act as impermeable barriers to physiological processes and fluid transfer. Hydron is also

inert, relatively biocompatible and has good mechanical resistance.

Drug delivery systems: Poly(glycol methacrylate) gels permit a controlled release of drugs into their surroundings and therefore are suitable where the required concentration cannot be delivered by other means[34,35]. For example, they may be used in the area of the respiratory tract and in the middle ear cavity[36]. In the simplest drug delivery system, the hydrogel can be saturated with a solution of the drug which will leach out into the surrounding tissue on subsequent implantation. With simple homogeneous hydrogels saturated with a drug solution, the rate of drug delivery generally decreases rapidly. By using a hydrogel membrane device filled with a drug in the form of a pure liquid, constant drug delivery rates and extended treatment times can be obtained[37].

Ocular surgery: Hydrogels have been used for vitreous implants due to their good biocompatiblity, permeability and optical properties. Hydrogels tend to be difficult to suture and attachment to the cornea has been found to be more successful when cyanoacrylate adhesives are used. The optical properties of hydrogels are very similar to those of the gel found within the eye and hydrogels have the advantage that they can be implanted in a dehydrated state[38]. On implantation, the polymer will absorb moisture and swell, filling the available cavity. Poly(2-hydroxyethyl methacrylate) has been used in

ocular experiments involving the implantation of glaucoma drainage strips in the eyes of rabbits[39]. The strips allow slow drainage of fluid by means of capillary channels and swell on hydration to plug the surgical incission.

Artificial kidney dialysis membranes: The aim of the artificial kidney machine is to remove waste toxins from the blood, thus carrying out the action which a diseased or deficient kidney is not able to perform. This is carried out by dialysis in which the unpurified blood is separated from the dialysing fluid by a semi-permeable membrane. The most commonly used membranes are based on cellulosic films, such as xanthate derivatives or cuprophane. These function by sieving the smaller species, such as water molecules, glucose, urea and other ionic particles. The process which largely depends on the pore size and thickness of the film, removes both impurities and also some biologically essential components which have to be replaced by back-diffusion from the dialysing fluid. Non-ionic hydrogels have been suggested as possible materials for use as kidney dialysis membranes (eg poly(glycol methacrylates)[40,41]). These materials have potential advantages such as chemical stability, good biocompatibility and relatively good mechanical stability. Despite the seemingly advantageous properties of these polymers, they do not give a significant improvement over the materials already in use.

Artificial liver support systems: The area of artificial liver support

systems is a more complex field than that of artificial kidney dialysis membranes. The artificial kidney dialysis membrane separates molecules according to their size by a simple sieving mechanism, while an artificial liver support system must remove specific toxins from the blood[42,43]. In many cases where the liver has been damaged, for example in a drug overdose, an artificial liver support system may be of value to remove specific blood toxins, whilst the liver recovers sufficiently to enable it to resume its normal biological functions. Anion exchange and uncharged resin columns have been used to remove protein bound cholephilic anions from plasma, these are normally excreted in bile. An example of a resin used for this purpose is Dowex 1X4, a polystyrene/divinyl benzene copolymer, substituted with quaternary ammonium groups with 4% cross-linking, which was converted to the bicarbonate form and then washed with distilled water to neutrality. Although resins of this type have produced some limited success in experiments with animals, they also produced some adverse effects. 'In vitro' studies have indicated that little haemolysis took place during the passage of canine or human blood through such resins and erythrocyte counts did not change. Leucocyte counts decreased and platelet counts were markedly lower in addition fragmentation of red blood cells has also been observed. The use of activated charcoal for liver support systems has also produced a loss of white blood cells and platelets. A coating of poly(HEMA) has been shown to reduce the loss of cells whilst not affecting the absorption properties of the charcoal[44].

1.3 Properties of Hydrogel Polymers

Although it is apparent that the presence of water within a hydrogel network will affect the mechanical properties[45] simply because of the role of water as a plasticiser, there are other factors which need to be taken into consideration.

The existence of water in differing states within a hydrogel is derived from different monomer systems in terms of the equilibrium water content and also the wide divergence in polymer structure which can be synthesised[46]. These effects combine to produce a polymer in which the mechanical properties may not be readily predicted from a knowledge of the water content and may vary widely depending on such factors as cross-link density and molecular structure.

The term mechanical property in the case of hydrogels encompasses a variety of types of strength which are not simply interrelated. Various conventional test methods exist for the determination of the mechanical behaviour of hydrogel polymers and these include:

Tensile test: A polymer specimen if subjected to tensile stress will undergo elongation. Conventionally, the tensile test is carried out with a tensometer and the resultant stress-strain relationship affords considerable information as to the mechanical properties of the material under test.

(i) the ultimate tensile strength of the material under test,

(ii) the elongation to break of the material,

(iii) the elastic modulus of the material as determined from the initial slope of the stress-strain curve, and

(iv) the toughness of the material as determined by the area under the stress-strain curve.

Tear strength: The tear strength of a material is defined as the force required to propagate a tear in a notched specimen of the material under test. Tear strength is expressed as the force per unit length of the resultant tear and depends on factors such as the depth of notch, mode of application of the tearing force, the thickness of the specimen and the temperature at which the test is carried out.

Hardness: Hardness can be expressed as the resistance of a test specimen to penetration. In hardness tests, an indentor of a hard material is pressed into the surface of the test specimen and the extent to which it penetrates as a function of time and under a given pressure is an inverse measure of hardness.

Flexural testing: In flexural testing the specimen is repeatedly flexed until it fractures. The number of flex cycles to break can be used to asses the flexural strength of the material under

test.

Creep test: Plastics often tend to deform if they are constantly loaded. A creep test records the deformation of a test specimen as a function of time for a constant applied stress.

The surface properties of a hydrogel material both in the dehydrated and hydrated states are important factors for differing reasons. In the case of dehydrated hydrogels, the surface properties are of interest in the fabrication process. In moulding the particles of a polymer are fused into a continuous phase which must then be made sufficiently fluid to flow into the mould shape. On cooling, the forces of cohesion between the polymer chains must be greater than the forces of adhesion between the solid and the mould in order to allow release from the mould. This release cannot be achieved by the use of processing aids and lubricants as they would subsequently come into contact with a biological environment. In the hydrated state, the surface properties are of interest as the relationship of the properties of the polymer to any interactions with the environment into which hydrogels are placed are important. It is therefore important to investigate the effect of chemical structure on surface properties and conversely whether it is possible to relate observed surface phenomena to the bulk structure of the polymer. In the case of hydrated hydrogels, the principle area of interest is that of biocompatibility and many workers have attemped to correlate the

surface properties of hydrogels with their biocompatibility. This aspect of the determination of surface properties is discussed later in this thesis as is the relationship of surface properties with the behaviour of hydrogels in a biological environment.

One property that is particularly dependent on the nature and quantity of water present in a hydrogel is that of its permeability or transport behaviour. There are in general two distinct types of requirement in the design of hydrogels which possess specific transport behaviour. The first and more obvious requirement is for hydrogels that are capable of transporting a permeant (such as oxygen or other dissolved metabolites[47]) across a membrane as quickly and efficiently as possible. An application in which this type of behaviour is typified is that of the extended wear contact lens[48,49]. Such lenses would be required to be worn for extended time periods including times when the eye is closed. The cornea is avascular and so relies on oxygen dissolved in the tear fluid in order to respire. In cases where a contact lens is fitted, oxygen would be required to diffuse through the polymer in order to maintain corneal metabolism and so a polymer with relatively high oxygen permeability would be required.

The second requirement is the phenomenon known as permselectivity, that is the preferential transport of one species with respect to another. The most common example of this type of behaviour is

illustrated by the reverse osmosis membrane. A typical application of such a membrane is in the desalination of sea water. Such membranes need to have properties which are predominantly governed by the nature of water within the hydrogel[50]. The nature of water contained in hydrogels can be determined using differential scanning calorimetry and this aspect will be discussed in greater detail in a later chapter.

1.4 Modification of Hydrogel Polymers

Hydrogels, in many respects, are well suited for use as starting materials in the making of biologically active biomedical materials. There are a number of advantages in using hydrogels in these types of system. Small molecules, eg drugs, can diffuse through hydrogels and the rate of diffusion can be controlled by copolymerising the hydrogel in varying ratios with other monomers. Hydrogels may interact less strongly than more hydrophobic materials with molecules which are immobilised on or within them, thus leaving a larger proportion of the molecules active[51]. Hydrogels can be left in contact with blood or tissue for extended periods of time without causing adverse reactions making them useful for devices to be used in long term treatment of various conditions. Hydrogels usually have a large number of polar reactive sites on which molecules can be immobilised by relatively simple chemistries. Biologically active molecules can be immobilised within hydrogels both on a temporary or permanent basis. If the hydrogel is designed to release the attached

active molecule at a predetermined rate then these materials are well suited to applications as drug delivery devices. In the simplest of such devices, the hydrogel can be swollen in solutions of various drugs which will leach out into the surrounding tissue on implantation. In such systems the rate of drug delivery generally decreases rapidly. By using a hydrogel membrane device, filled with a drug in the form of a pure liquid or solid, constant drug delivery rates and extended treatment times can be obtained. An even more sophisticated approach to this problem involves the catalytic release of drugs from specifically designed polymers[52].

Many biomolecules have been bound to supports such as Sephadex and Sepharose (modified polysaccharides) which allow large amounts of active biomolecules to be immobilised but which would not be expected to show significant biocompatibility. Devices designed for the immobilisation of enzymes to be used in contact with blood have been made from such materials as poly(methyl methacrylate), poly(vinyl chloride) and polycarbonates[53-55], all of which are considered to have rather thrombogenic surfaces. Hoffman et al have studied the immobilisation of albumin and heparin to hydrogels based on PHEMA and PNVP. Nguyen and Wilkes have also reported the grafting of enzymes onto a polymer utilising acrylic acid and N-acryloyl-para-phenylene diamine[53]. Another approach to preparing a blood compatible surface based on the immobilisation of biomolecules has been described by Lee et al[56]. They prepared a three

layered support material and onto the surface esterified first half cholesterol esters of dicarboxylic acids and next, the half sialic acid ester of a longer chain dicarboxylic acid. Finally the surface was treated with a tissue culture medium to condition it with salts and proteins found in the blood. Despite the complex construction of such materials, vena cava ring tests have indicated that these surfaces have generally poor thromoresistance. A number of chemical techniques have been developed for the coupling of biomolecules to hydrogels (Table 3) and some specific aspects of this type of reaction are discussed in a later chapter.

1.5 Scope of Present Work

Because no previous attempt has been made to collect together and examine techniques that might be applicable to the particular problem of hydrogel characterisation as outlined above, the first and major objective of the work described here was to do this. In particular, it was hoped to examine the extent to which the chemical structure of commercially available hydrogels could be determined by a collection of such techniques. In addition, it was hoped to characterise the surface properties and the water binding properties of hydrogels in order to provide a basis that would enable differences in behaviour (transport and biocompatibility) between apparently similar hydrogels to be understood. Finally, since the bulk properties of hydrogels are determined by the nature and quantity of water contained in

the gel network (and since changes in bulk and chemical structure affect this) attempts were made to modify the surface structure of hydrogels without necessarily altering the bulk properties.

Table 3 Coupling proteins on carboxyl containing surfaces

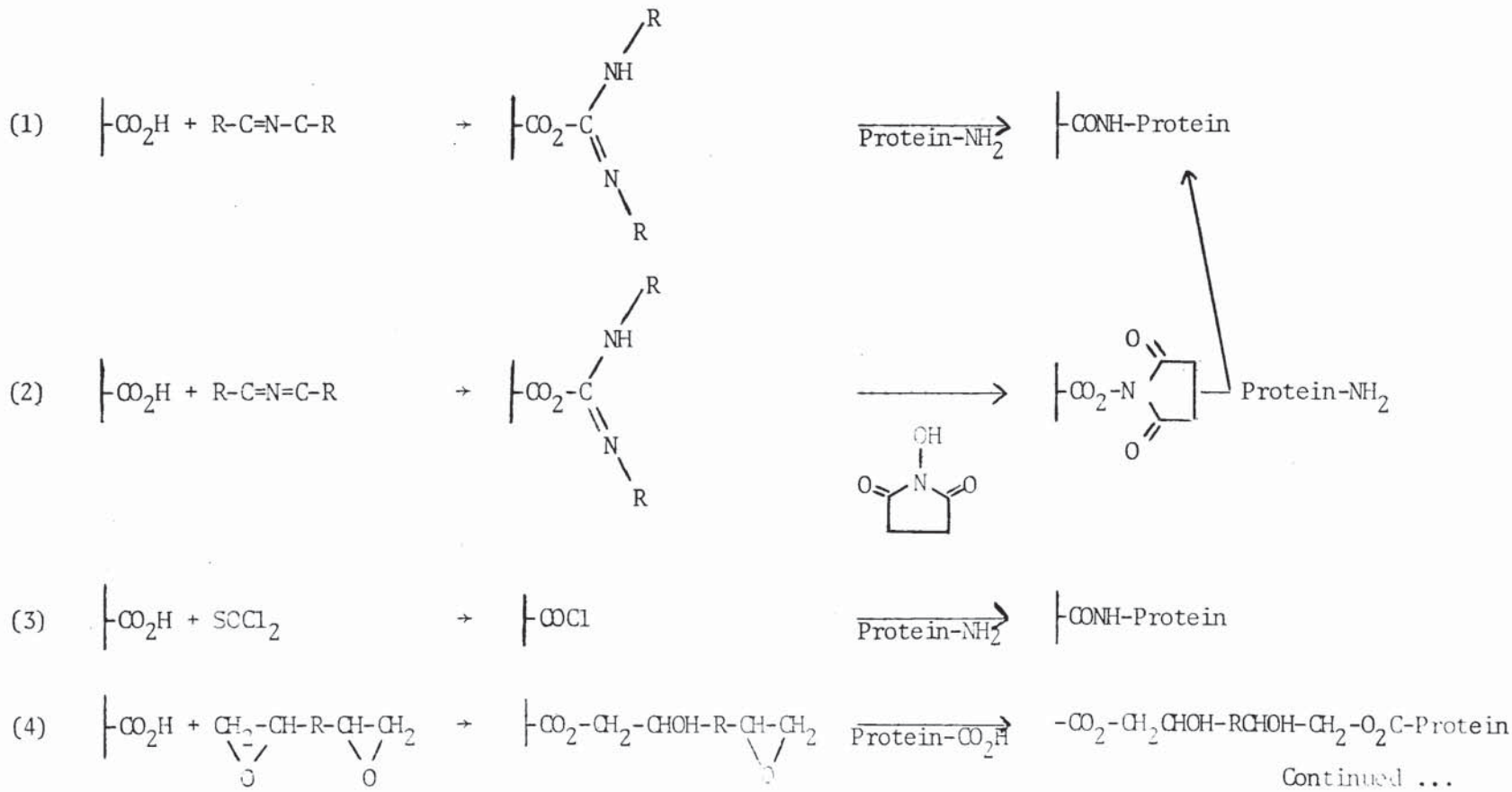


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Table 3 Continued

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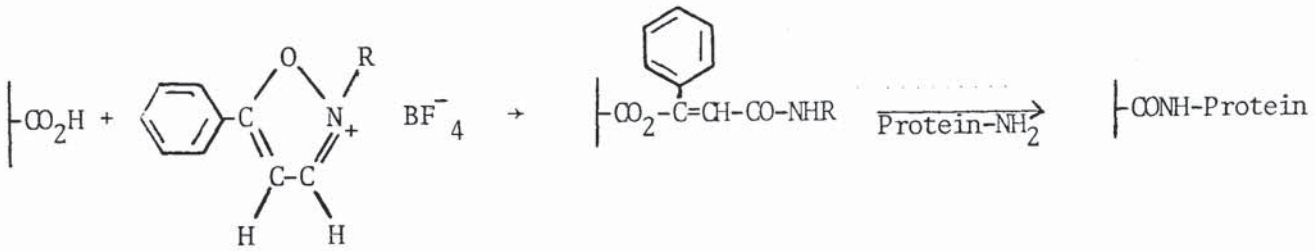
(6) 

CHAPTER TWO

EXPERIMENTAL METHODS

2.1 Source and Purification of Monomers and Reagents

Monomers

The monomers shown in Table 4 were purified by conventional methods. The purified monomers were stored in a refrigerator until required for polymer synthesis.

Catalysts, Initiators and Solvents

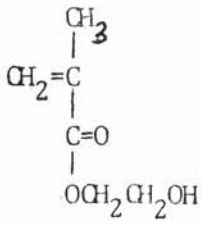
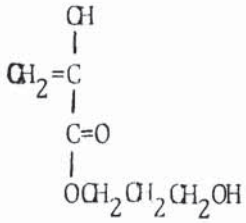
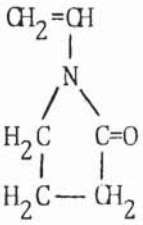
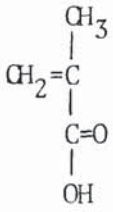
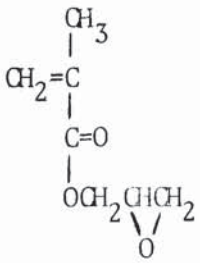
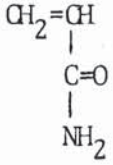
Uranyl nitrate, supplied by Hopkin and Williams was used as supplied.

Benzoyl peroxide, supplied by Fisons was purified by recrystallisation from chloroform.

α-Azo-isobutyronitrile, supplied by BDH was used without further purification.

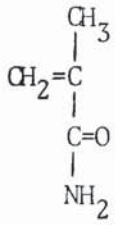
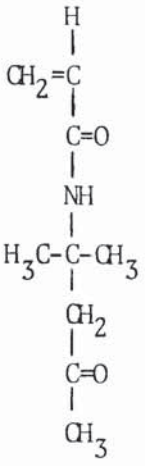
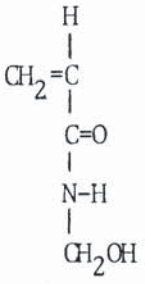
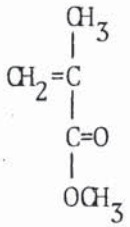
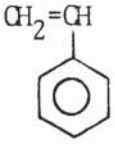
The following solvents were used as supplied by the manufacturers and without further purification:

Table 4 Monomers used in polymer synthesis

Monomer	Structure	Supplier
2-Hydroxyethyl methacrylate (HEMA)		BDH
Hydroxypropyl acrylate (HPA)		Wychem Ltd
N-vinyl pyrrolidone (NVP)		Koch Light Chemicals
Methacrylic acid (MAA)		BDH
Glycidyl methacrylate (GMA)		BDH
Acrylamide (ACM)		Koch Light Chemicals

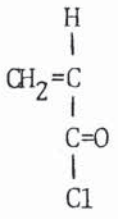
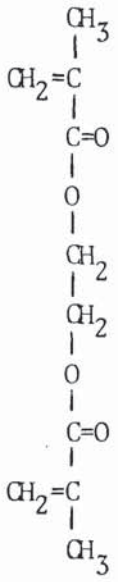
Continued ...

Table 4 Continued

Methacrylamide (MACM)		Koch Light Chemicals
Diacetone acrylamide (DAACM)		Koch Light Chemicals
N-methyl acrylamide (NMACM)		Koch Light Chemicals
Methyl methacrylate (MMA)		BDH
Styrene (STY)		BDH

Continued ...

Table 4 Continued

Acryloyl chloride		Cambrian Chemicals
Ethylene glycol dimethacrylate (EDMA)		BDH

Toluene	BDH
Chloroform	BDH
DMF	BDH
THF	BDH

1,4-Dioxan was purified using the standard technique described by Vogel[57].

2.2 Experimental Techniques

2.2.1 Analytical Techniques

Infra-red spectra: These were obtained using a Perkin Elmer infra-red spectrophotometer (Model 457). Sample details are given with individual spectra.

Microanalysis: Elemental analysis for quantitative determination of carbon, hydrogen and nitrogen was carried out on a Perkin Elmer Analyser (Model 240).

Gas liquid chromatography: Chromatograms were obtained using a Pye Series 104 Model 24/34 gas chromatograph. A general purpose silicone gum SE30 column was used in conjunction with a flame ionisation detector.

Pyrolysis gas chromatography: Chromatograms were obtained using a Perkin Elmer F11 gas chromatograph. A fluoro silicone oil

column was used with a flame ionisation detector.

Differential scanning calorimetry: Traces were obtained using a Perkin Elmer (Model DSC 2) calorimeter. Sample details and experimental conditions are given with individual results.

2.2.2 Polymer Synthesis

Polymers were prepared by both bulk and solution techniques. In the case of bulk polymerisation, polymers were synthesised in rod and membrane form.

Solution polymerisation

Free radical solution polymerisations were carried out on a 0.5 litre scale using normal techniques and precautions.

In a typical homopolymerisation, 13.0 g (0.1 M) 2-hydroxyethyl methacrylate and 1% by weight AZBN were dissolved in 250 ml of 1,4-dioxan contained in a 3-necked 500 ml flask which was equipped with a stirrer, condenser, thermometer and a nitrogen bleed. Polymerisation was carried out under a nitrogen blanket in a water bath at 60°C for 8 hours. The contents of the flask were then allowed to cool and added dropwise to 2.5 litres of stirred ether. The precipitate obtained was filtered, washed with ether and dried in a vacuum oven at 60°C.

Bulk polymerisation

As previously mentioned, both rods and membranes were produced by bulk polymerisation.

Rod form: Rods were prepared by polymerising a mixture of monomers in the presence of a free radical initiator and a cross-linking agent. Polyethylene tubes were used to carry out the polymerisations as it has been found that they facilitate the removal of polymerised rods.

In a typical polymerisation, a mixture of 2-hydroxyethyl methacrylate, methyl methacrylate and methacrylic acid in a molar ratio of 80 : 17 : 3 and AZBN (0.1% by weight) were weighed into a polyethylene tube sealed at one end. The mixture was flushed with nitrogen and the tube sealed with a rubber bung covered with a thin polyethylene film and secured with adhesive tape. Polymerisation was carried out in a water bath at $65^{o}C$ for 24 hours and finally in an oven at $90^{o}C$ to ensure complete polymerisation. The rod was easily removed by cutting away the polyethylene tube.

Membrane form: Hydrogel membranes were prepared by bulk polymerisation in a glass mould. Two glass plates (12.5 cm x 10 cm) each covered with a sheet of Melinex (polyethylene terephthalate) on one side were separated by a polyethylene gasket. The mould was held together by six spring clips (Fig 1).

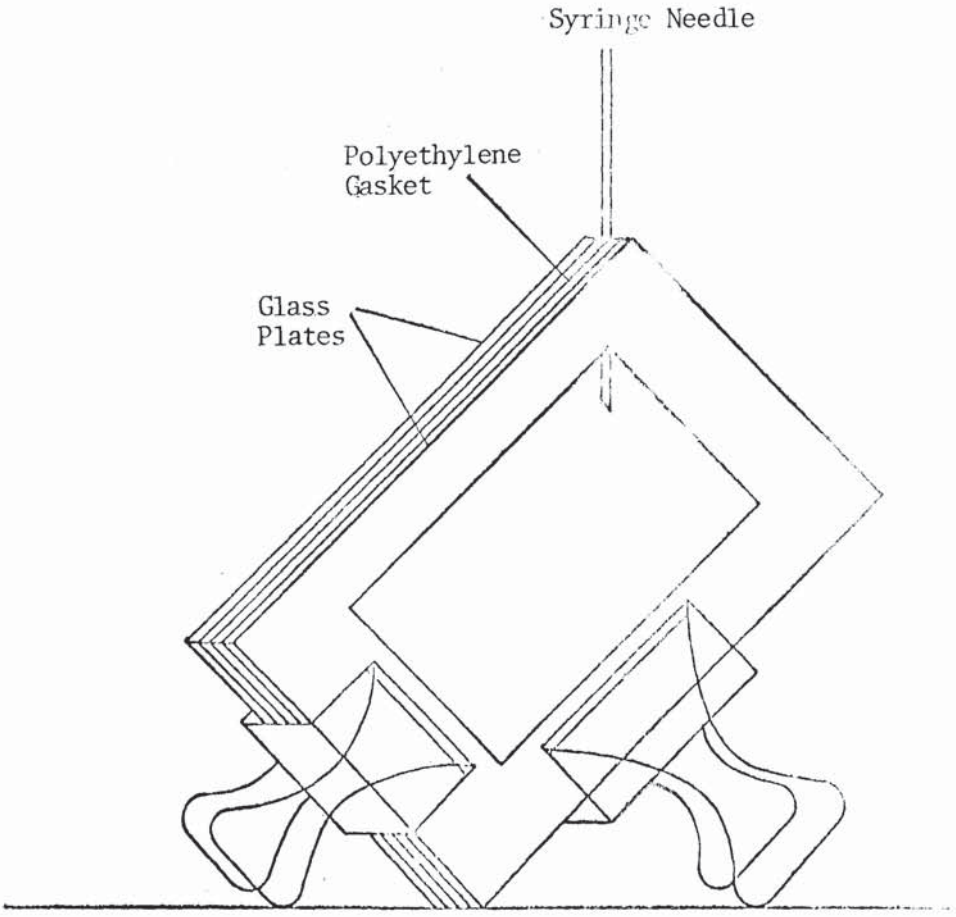


Fig 1 Polymerisation cell for synthesis of polymers in membrane form

In a typical synthesis, a monomer mixture of 2-hydroxyethyl methacrylate, methyl methacrylate and methacrylic acid in a molar ratio of 80 : 17 : 3 and AZBN (0.1% by weight) was purged with nitrogen and introduced into the mould by means of a syringe taking care to avoid the inclusion of air bubbles in the mould. The mould was placed in an oven at 65°C for 72 hours followed by 2 hours post-curing at 90°C. When the polymerisation was complete, the spring clips were removed and the mould halves separated leaving the polymer sheet adhering to only one of the halves. This was then placed in distilled water to hydrate. The hydrogel in its hydrated state could then be easily removed from the poly(ethylene terephthalate) covered glass plate.

2.3 Polymer Properties

2.3.1 Equilibrium Water Content

Hydrogels were conveniently characterised by their water contents when swollen to equilibrium in distilled water at room temperature.

The equilibrium water content (EWC) of a hydrogel membrane was measured after allowing the membrane to hydrate in distilled water for at least three weeks. Not less than three samples were then cut using a cork borer (1 cm in diameter) from the hydrated membrane. The surface water of each sample was carefully removed with a tissue and the sample transferred to

and weighed in a closed weighing bottle of known weight. The lid of the weighing bottle was then partially opened and the sample dehydrated to constant weight in a vacuum oven at 60°C. When a constant weight was reached, this was noted and the EWC calculated by the equation shown below:

$$EWC = \frac{\text{weight of hydrated sample - weight of dehydrated sample}}{\text{weight of hydrated sample}} \times 100\%$$

The EWC's of at least three samples were measured and an average EWC calculated.

2.3.2 Contact Angles

Contact angles were measured using the Owens and Wendt[58] technique for dehydrated polymers and the Hamilton technique[59] for hydrated samples.

Owens and Wendt technique: Prior to contact angle measurements, the polymer surfaces were cleaned by washing with a detergent solution followed by thorough rinsing in distilled water and subsequent drying in a vacuum desiccator.

A sessile drop of water was formed on the surface of the polymer using a G.25 hypodermic needle, the position of which could be accurately controlled using a Prior micromanipulator. The polymer sample was supported in a glass cell with optically flat sides in an atmosphere saturated with water vapour in order to

eliminate evaporation (Fig 2). The volume of the drop was controlled by an Agla micrometer syringe and was slowly increased to enable several measurements of the advancing contact angle to be made. The contact angle could be measured directly using a goniometer eyepiece fitted to a cathetometer or by photographing the image of the drop projected on a back projection screen (Plates 1 and 2). Having obtained a photograph the contact angle can be determined by drawing a tanget to the drop surface at the three-phase interface and measuring the contact angle with a protractor. In all contact angle determinations, at least six measurements were made on each polymer sample and each measurement was the average of the contact angles on either side of the sessile drop. In the Owens and Wendt technique contact angles are measured using distilled water and methylene iodide and the results inserted into the equation of Owens and Wendt. This procedure yields an estimate of the polar and dispersive components of surface energy of the hydrogel surface. In order to assist in the processing of this data a computer programme has been developed.

Hamiliton's technique: This technique involves the measurement of octane contact angles on surface whilst under water. It is therefore apparent that this would be a valuable method for studying hydrogel surfaces. The hydrogels remain in their hydrated state and the problems associated with the removal of surface water would be eliminated. Hamilton's technique has been adapted for use with hydrogel polymers using the following

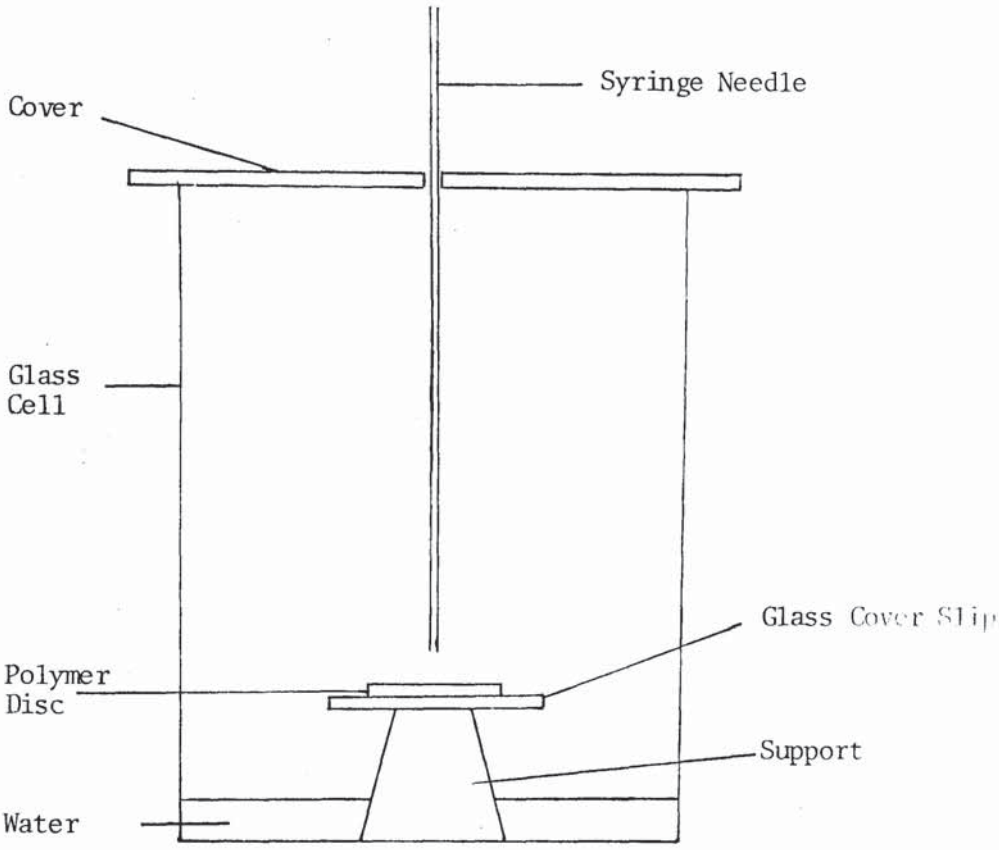


Fig 2 Layout of apparatus for the determination of contact angles for hydrogels in the dehydrated state

Plate 1 Layout of contact angle apparatus

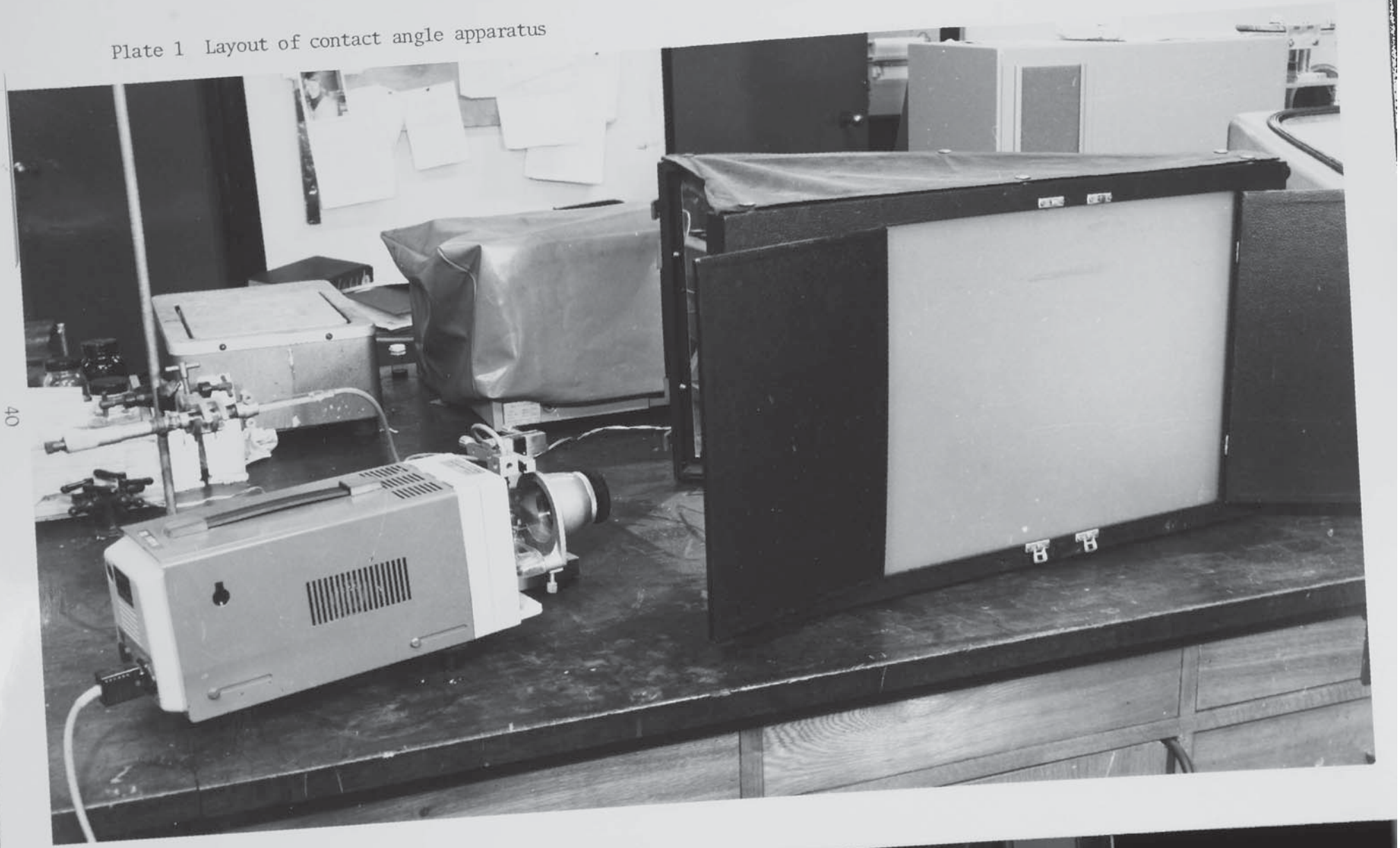
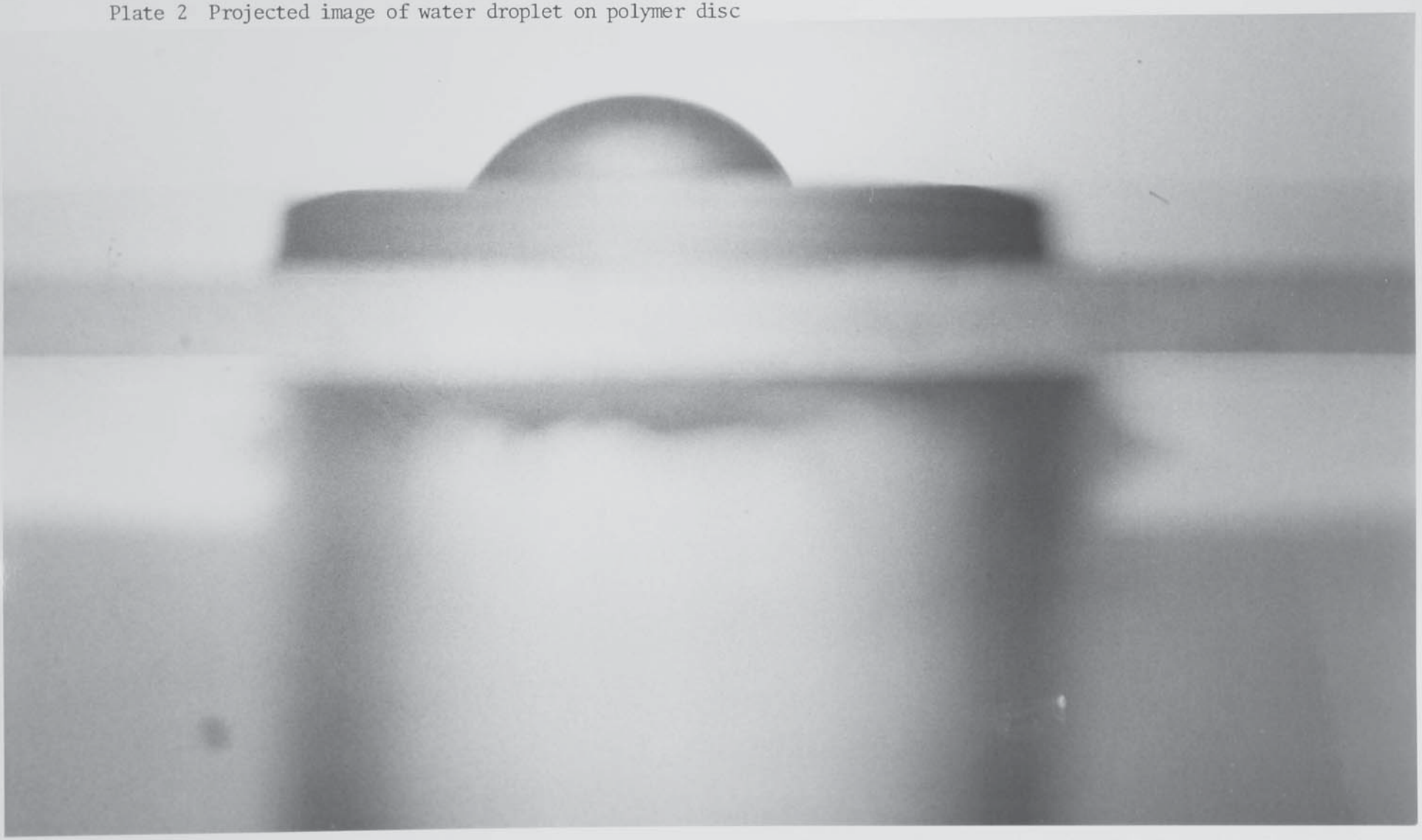


Plate 2 Projected image of water droplet on polymer disc



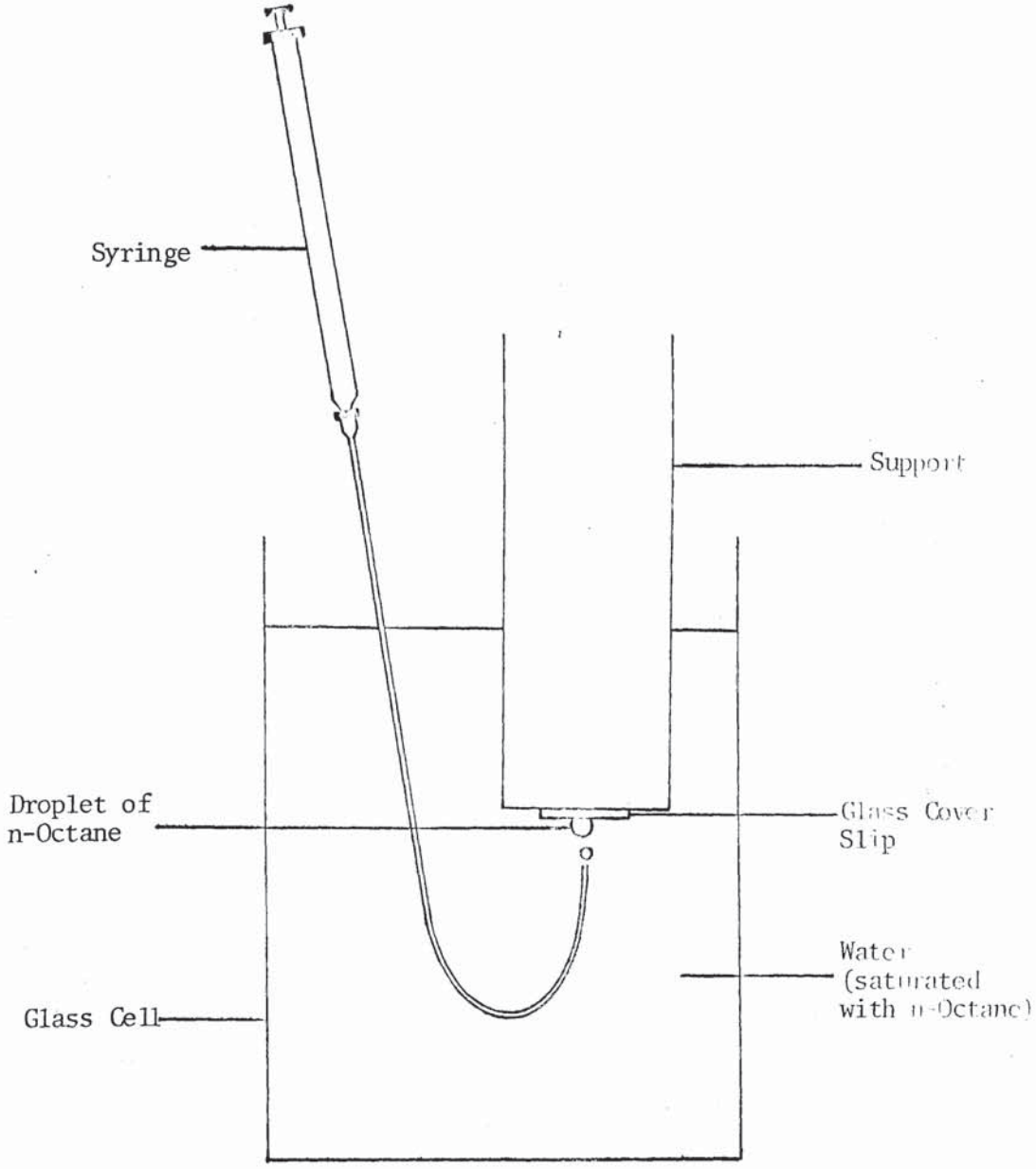


Fig 3 Layout of apparatus for the determination of contact angles for hydrogels in the hydrated state

procedure. Hydrogel samples were cut from a hydrated membrane and stuck to microscope cover slips using cyanoacrylate adhesive. The hydrogel surfaces were then cleaned using a detergent solution followed by a thorough rinsing with distilled water. The samples were then allowed to re-equilibrate in distilled water for several hours.

The cover slips with the samples attached were supported in a glass cell containing octane saturated water. Sessile drops of octane were formed on the hydrogel surface by releasing octane drops from a syringe through a bent hypodermic needle as shown in Fig 3. As octane has a lower density than that of water, the octane droplets float upwards to the polymer surface and form the interface. An enlarged image of the sessile drops formed can then be projected and the contact angles measured or the angle can be observed directly using a goniometer eyepiece in conjunction with a low power microscope.

CHAPTER THREE

CHARACTERISATION OF SOME COMMERCIALLY AVAILABLE HYDROGEL POLYMERS IN THE DEHYDRATED STATE

As mentioned in Chapter One, one of the main aspects of the work described in this thesis is the application of a number of techniques to the characterisation of hydrogel polymers. In this Chapter, some of the techniques applicable to dehydrated hydrogels will be discussed with reference to the use of these techniques in the characterisation of some commercially available hydrogel polymers. The techniques described are primarily for use with dehydrated polymers and although the equilibrium water content is used as a characterisation technique in this Chapter, the water binding properties of hydrogels are not discussed as this aspect is dealt with in more detail in the following Chapter. As a basis for investigation, some hydrogel polymers used for soft contact lens applications were selected and the techniques described were applied to each.

3.1 Experimental Techniques and Their Application to Hydrogel Systems

Density: The densities of the various lens materials in both the dehydrated and hydrated states were measured using a density gradient column calibrated using glass floats of known density. In the case of dehydrated samples, small samples of the material in question were placed in a vacuum oven at 60°C for one

week in order to dehydrate thoroughly. On removal from the oven, the sample was placed in the density gradient column. The progress of the sample down the column was observed and when an equilibrium position was reached, a reading of the sample position within the column was made, enabling the density of the sample to be readily calculated from the calibration curve. To measure the density of hydrated polymers, the samples were first placed in distilled water for two weeks to hydrate thoroughly. The samples were then removed from distilled water and any surface moisture removed using a tissue. The procedure used for dehydrated samples was then followed. In the measurement of polymer density using a density gradient column care has to be taken in the preparation and storage of the columns in order to ensure good results. It has been shown that the readings for dehydrated polymers can alter as a function of time in the column and, therefore, readings have to be taken as soon as equilibrium is reached[60]. Care is also necessary to ensure that samples are thoroughly dehydrated so that any discrepancies due to residual water are kept to a minimum.

Elemental analysis: Carbon, hydrogen and nitrogen were quantitively determined using the instrument described in Chapter Two. The accuracy with which CHN analysis can be applied to polymers is limited by the fact that the analysis requires complete combustion of the sample and in the case of polymers this is sometimes not achieved. In addition, the hygroscopic properties of hydrogels in the dehydrated state can lead to errors.

Infra-red spectroscopy: The polymer was ground into a fine powder using a fine grade of glass paper. The resulting powder was mixed with potassium bromide, a disc prepared and an infra-red spectrum recorded on the instrument described in Chapter Two. Two difficulties were encountered in obtaining infra-red spectra of the polymers. Firstly it was difficult to obtain a homogeneous dispersion of the polymer in potassium bromide and secondly it was difficult to remove all traces of water from the samples as indicated by absorbtion bands in the resulting infra-red spectra.

X-ray diffraction: Diffraction patterns for polymeric materials are generally poorer in quality than those obtained for other solid materials, tending to show broad patterns. The diffraction patterns shown by amorphous polymers tend to give rise to diffuse haloes and those of crystalline polymers sharper circles. The sample was ground to a fine powder and exposed to X-rays for six hours in a Debye Scherrer camera. For the reasons outlined above it was not possible to draw any firm conclusions from the patterns obtained as they were too diffuse to be of any value.

Equilibrium water content: The equlibrium water content of a hydrogel can be defined as:

$$EWC = \frac{\text{weight of hydrated sample - weight of dehydrated sample}}{\text{weight of hydrated sample}} \times 100\%$$

The polymer samples were hydrated in distilled water or saline solution for two weeks at room temperature. The samples were then removed from the hydrating medium, excess surface moisture removed and the sample placed in a closed weighing bottle and weighed. The sample was then placed in a vacuum oven at 60°C and dried to constant weight. The above procedure was repeated on at least three samples of each material.

Contact angle measurements: Contact angles were obtained using the Owens and Wendt technique as described in Chapter Two. The calculation of surface free energy from contact angle data is shown below:

$$\text{Cos } \theta + 1 = \frac{2}{\gamma LV} \quad (\gamma_1^d \gamma^d s)^{\frac{1}{2}} + (\gamma^p_1 \gamma^p s)^{\frac{1}{2}}$$

where θ = contact angle

γLV = surface tension of wetting liquid

γ_1^d = dispersive component of surface free energy of wetting liquid

$\gamma^d s$ = dispersive component of surface free energy of solid surfaces

γ^p_1 = polar component of surface free energy of wetting liquid

$\gamma^p s$ = polar component of surface free energy of solid surface

This method relies on having two wetting liquids both fully characterised in terms of polar and dispersive component and solving the resulting simultaneous equations for $\gamma^p s$ and $\gamma^d s$.

Prior to the measurement of contact angles, the polymer surfaces were polished using a fine grade of glass paper. The surfaces were then washed using a dilute detergent solution and thoroughly rinsed using distilled water. Finally the samples were dried in vacuo at 60°C and subsequently carefully handled to avoid any contamination of the surfaces. The primary difficulty encountered is that of obtaining a smooth surface on which to determine a contact angle. It has been shown[61] that the contact angle is related to surface roughness and in order to obtain consistent results, it is necessary to obtain a smooth surface.

Pyrolysis gas chromatography: Non-volatile substances may be studied by gas chromatography if they are first thermally degraded and the degradation products then flushed into the carrier gas flow of a chromatograph.

A Perkin Elmer F11 gas chromatograph fitted with a Fluorosilicone oil FS1265 column was used in conjunction with a flame ionisation detector and associated recorder. The inlet part of the chromatograph was suitably modified to allow a pyrolysis filament to be fitted. A small piece of the polymer sample was placed on a helical platinum filament which was inserted into the inlet part. The filament was then heated electrically to a temperature of 500°C. The pyrolysates were then swept into the column by the nitrogen carrier gas, the pyrolysis was continued for 10 seconds to afford complete pyrolysis of the sample. After pyrolysis, the column was heated at a rate of 10°C per minute from a temperature

of 50°C to 170°C. Prior to the investigation of unknown polymer samples, chromatograms of the polymers thought to be present in the commercial materials were obtained in order to obtain the basis for a 'finger printing' technique. In addition, preliminary work was often carried out with the column held at 80-100°C.

Whereas the application of these techniques (eg elemental analysis and infra-red spectroscopy) to hydrogel characterisation is so obvious as to require no further explanation, others present special problems and advantages. In particular, density measurement and pyrolysis gas liquid chromatography require preliminary comment. Some indication of the combined value and problems of these techniques may be obtained by examining the results for 2-hydroxyethyl methacrylate (HEMA) copolymers.

A series of such copolymers was prepared in membrane form (Chapter Two) by bulk copolymerisation of various proportions of the monomers, styrene and HEMA, in the presence of 0.5% azobisisobutyronitrile at 60°C. To each combination, a nominal cross-link density was introduced by incorporating 1% by weight of ethylene glycol dimethacrylate. The membranes were hydrated to equilibrium (changing the hydration medium several times to ensure complete removal of any residuals) after which the water content together with the density of the hydrated and dehydrated materials.

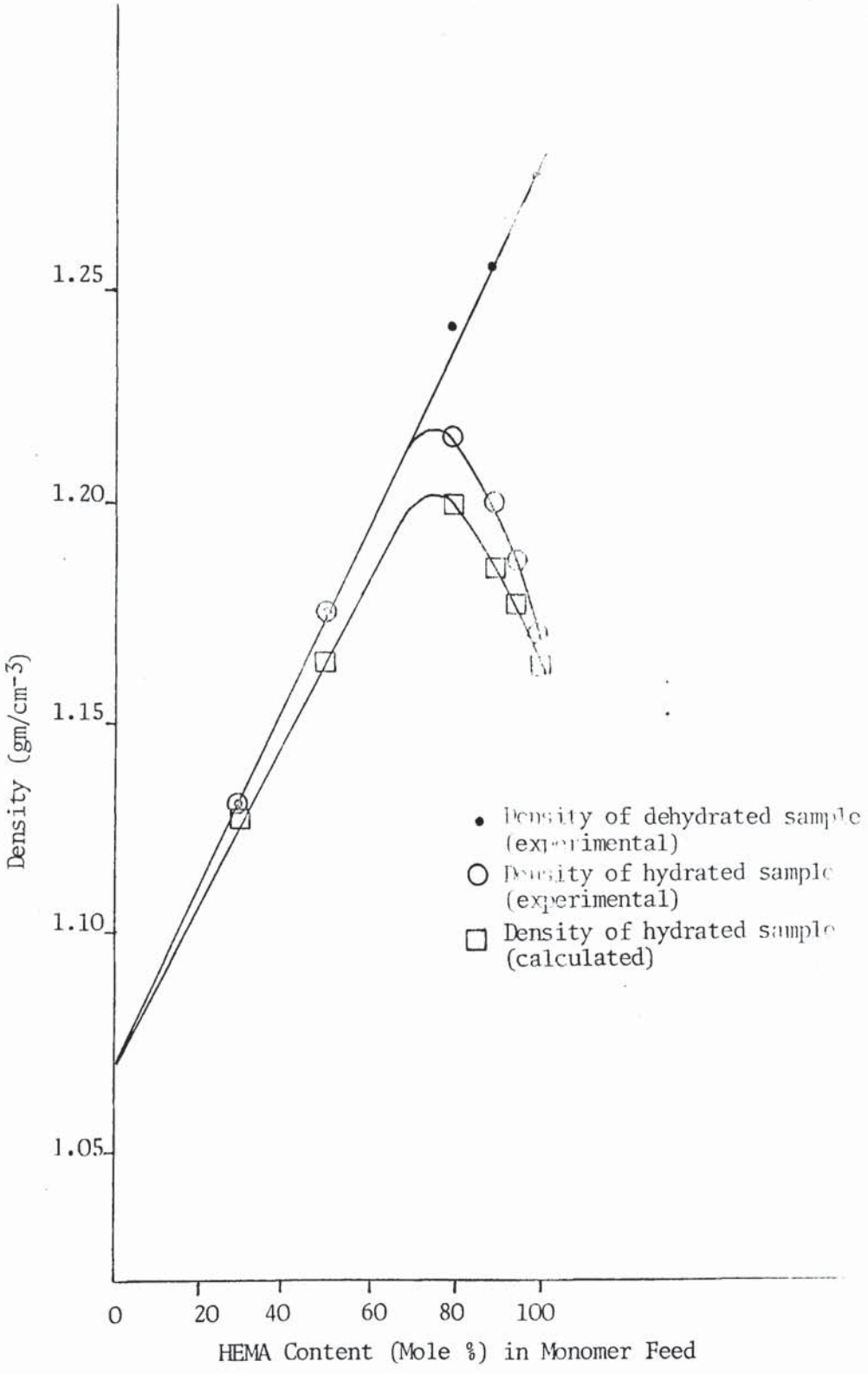
A suitable density gradient column was prepared from carbon

tetrachloride and xylene (mixed isomers) in the following manner. Eleven solutions were prepared covering a range of densities by mixing the two liquids in the volume ratios indicated

	1	2	3	4	5	6	7	8	9	10	11
CCl_4 (ml)	100	90	80	70	60	50	40	30	20	10	0
Xylene (ml)	0	10	20	30	40	50	60	70	80	90	100

The solutions were gently poured down a glass rod into a suitable cylinder starting with the most dense. The column was finally stirred with a rotating motion. The calibration was carried out using coloured floats of accurately known (four decimal places) density. The Davenport density gradient column has a facility for mixing and introducing the liquids automatically but requires similar calibration using standard floats. Once a height versus density calibration was made the samples were introduced. Fig 4 shows the results obtained for the styrene/HEMA copolymers described above. In addition to the experimental points calculated on the basis of the measured water contents, assuming that the density of water is 1.0. Two points are apparent. Firstly, the measurement of density of hydrated and dehydrated samples of these copolymers provides a valuable method of identifying a copolymer of unknown styrene : HEMA ratio. The second point applies to the difference between experimental and calculated values. There is no problem in the case of dehydrated materials which show no more than expected experimental variation from the line joining the density of the individual homopolymers. Since

Fig 4 Effect of HEMA content on dens· of HEMA/styrene copolymers



the materials are non-crystalline, this is in accordance with expectation. The difference between experimental and calculated values of the hydrated materials is quite marked, however, and appears to be related in some way to the water content. One possible explanation for this lies in the different states in which it is possible for water to exist in hydrogels (Chapter Four). If the so called 'bound' or 'non-freezing' water is considered to exhibit a somewhat higher apparent density than the free water, the problem disappears. This point will be elaborated in Chapter Four. Suffice it to say that if the quantity of non-freezing water in an unknown hydrogel is measured (eg by differential scanning calorimetry and total water content measurements) the differences between experimental and calculated density can apparently be resolved.

The problems arising with pyrolysis gas liquid chromatography arise firstly from the fact that the hydrophilic monomers used in hydrogels frequently complicate the pyrolysis pattern of the resultant polymers. Typical examples are found in Figs 5 and 6. These are pyrolyses carried out at 500°C in conjunction with isothermal chromatography. In the case of PHEMA (Fig 5) the explanation of the shape of the chromatogram is reasonably simple. Esters are well known to undergo a β-elimination reaction at pyrolysis temperatures, involving the hydrogen attached to the β-carbon of the alcohol residue.

Fig 5 Pyrolysis gas liquid chromatogram of PHEMA at 500°C (pyrolysis). Fluoro silicone oil column at 150°C.

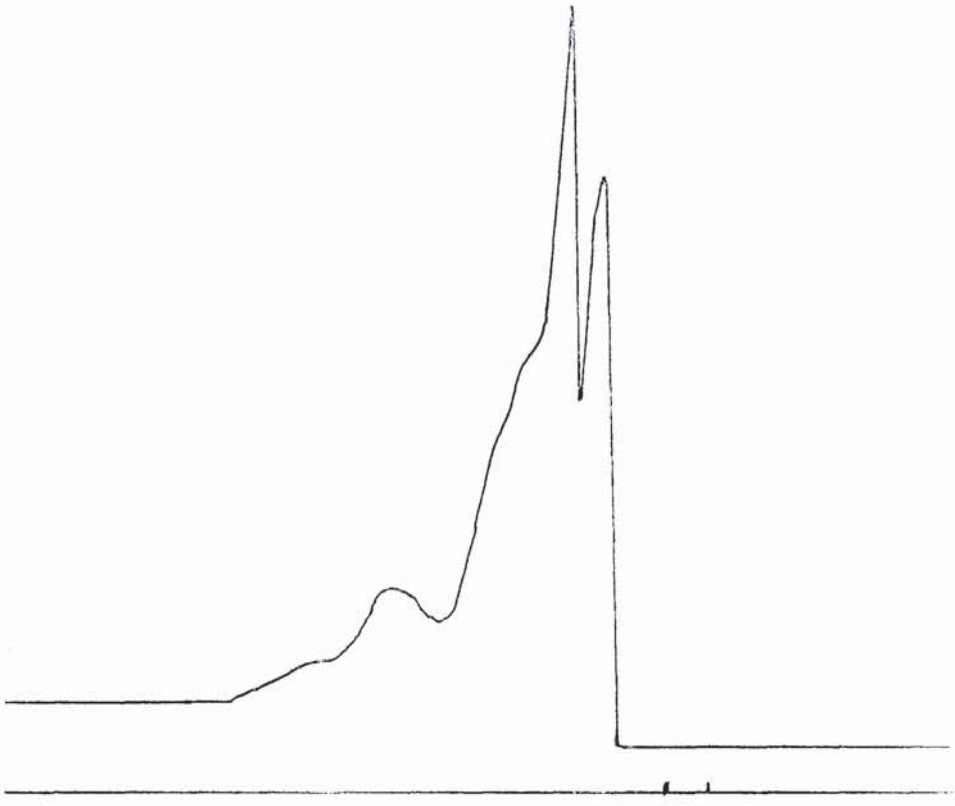
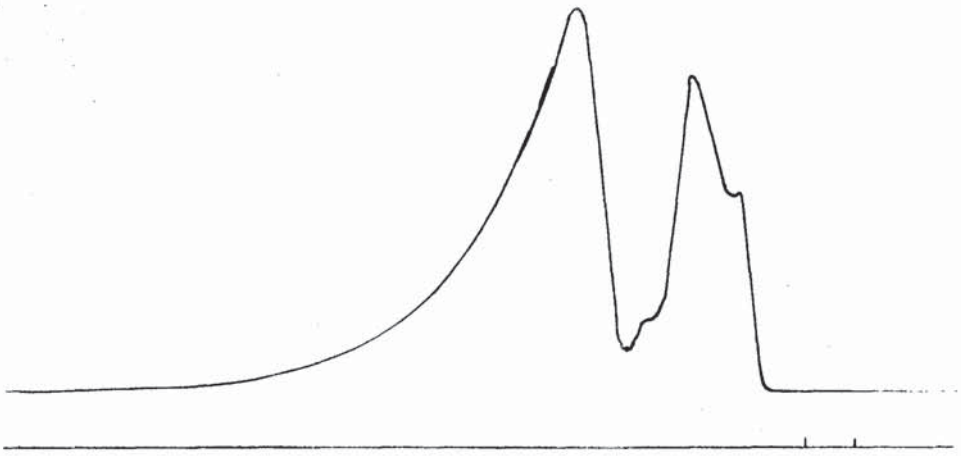
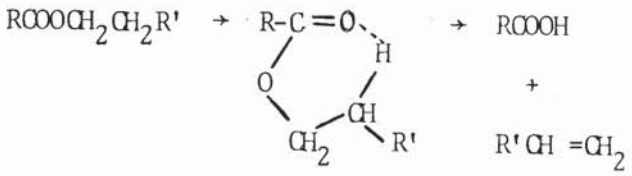


Fig 6 Pyrolysis gas liquid chromatogram of poly(N-vinyl pyrrolidone) at 500°C (pyrolysis). Fluoro silicone oil column at 150°C.



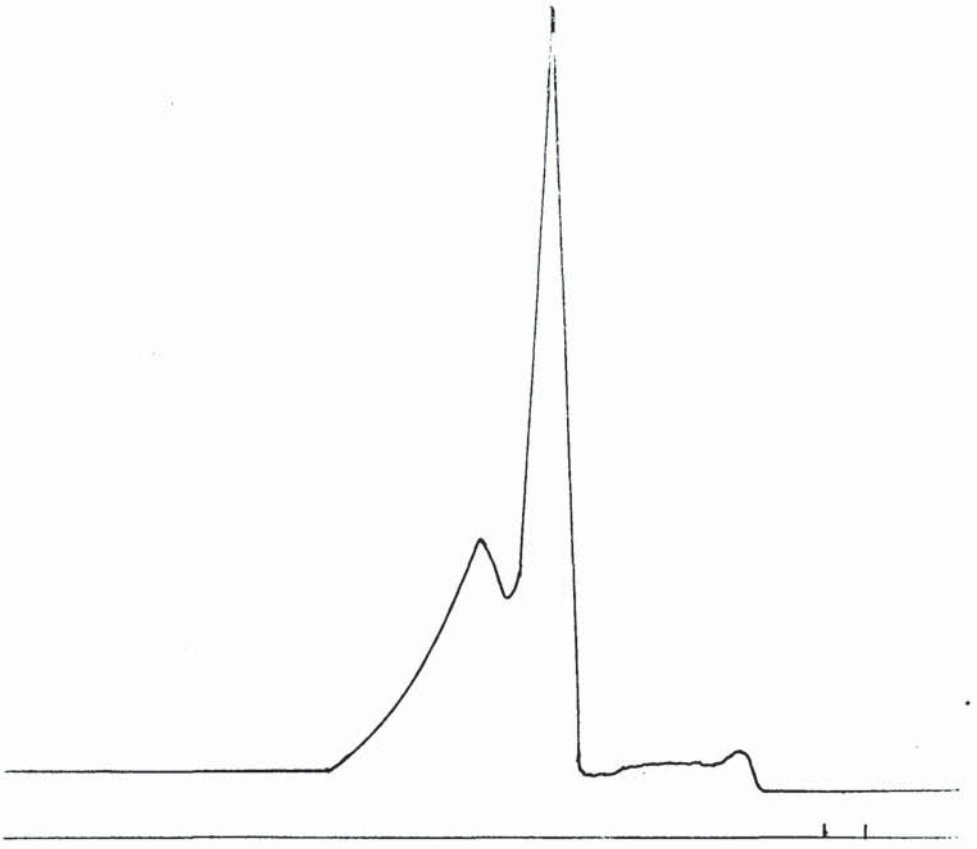
For example:



In the case of 2-hydroxyethyl methacrylate the products are methacrylic acid and vinyl alcohol which, of course, rearranges to acetaldehyde. This reaction competes with the depolymerisation and the major products are therefore monomer, methacrylic acid and acetaldehyde. Of these three, it is methacrylic acid that is longest retained showing a characteristic tailed peak. Poly(vinyl pyrrolidone) (Fig 6) again undergoes concurrent fragmentation and depolymerisation. The longest retained peak in this case corresponding to vinyl pyrrolidone monomer. In both cases the size of monomer peak produced is very much smaller than that obtained from a polymer that produces almost quantitative yields of monomer on depolymerisation, such as poly(methyl methacrylate) (Fig 7).

Although for convenience, isothermal chromatograms are shown, it will be appreciated that problems of peak overlap arise in this situation. By first carrying out an isothermal run and subsequently a temperature programmed run (which produces a much more extended chromatogram) the individual components of copolymers are resolvable. In some cases, variations of pyrolysis temperature, which modifies the finger print pattern,

Fig 7 Pyrolysis gas liquid chromatogram of poly(methyl methacrylate) at 500°C (pyrolysis). Fluoro silicone oil column at 150°C.



provides a useful additional method of resolving the copolymer composition.

In contrast to the precise analytical value of density and pyrolysis gas liquid chromatography measurements, allowing the provisos indicated above, surface characterisation does not generally give precise information about the bulk composition of unknown copolymers. The problem is two fold. In the first place the surface properties of the copolymer are not directly representative of bulk composition. Secondly the surface properties relating to a particular composition may often be modified by fabrication techniques and surface contamination. In the best instances, however, where a copolymer of widely different monomers that do not mutually interact is under consideration. The surface properties may give some indication of composition. Such a case is that of styrene and HEMA (Table 1 and even here the determined value of polar and dispersive components do not vary in a simple additive manner down the polymer series.

3.2 Application of Characterisation Techniques to Unknown Copolymers

A particularly severe test of the combined characterisation techniques discussed previously is found in the analysis of commercially available contact lens materials. This more than any other field is one in which a range of hydrogel compositions are employed. A selection of commercially available contact lens

Table 5 Effect of polymer composition on surface properties of HEMA/styrene copolymers

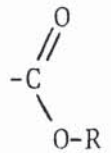
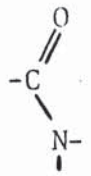
Polymer Composition, %		Contact Angle, ^{o}		Surface Free Energy, dynes cm^{-1}		
HEMA : Styrene		Water	Diiodomethane	Dispersive Component γ_d	Polar Component γ_p	Total γ
100 :	0	53	41	31.42	20.24	51.66
90 :	10	55	41	31.71	18.81	50.53
50 :	50	61	42	32.11	14.91	47.03
30 :	70	65	42	32.76	12.30	45.07
10 :	90	73	40	35.25	7.31	42.56
0 :	100	85	35	40.21	1.94	42.15

materials was collected and examined using the above mentioned techniques. The results are presented in Table 6.

Discussion

The application of the various characterisation techniques described is perhaps best discussed with reference to the results obtained from their application to commercially available hydrogel materials. Although the techniques give more accurate information when applied to purpose made polymers of known composition, a more realistic assessment of their value is to be gained from their application to polymers of a more complex nature.

The infra-red spectra of the commercially available materials show a characteristic carbonyl absorption, the position of which depends on the type of carbonyl group present.

		Absorbtion
	$R = CH_3, CH_2CH_2OH$	$\simeq 1720\ cm^{-1}$
		$\simeq 1680\ cm^{-1}$

The position of the carbonyl absorbtion in the infra-red spectrum

Table 6 Summary of data obtained from characterisation of commercially available soft contact lens materials (* by difference only)

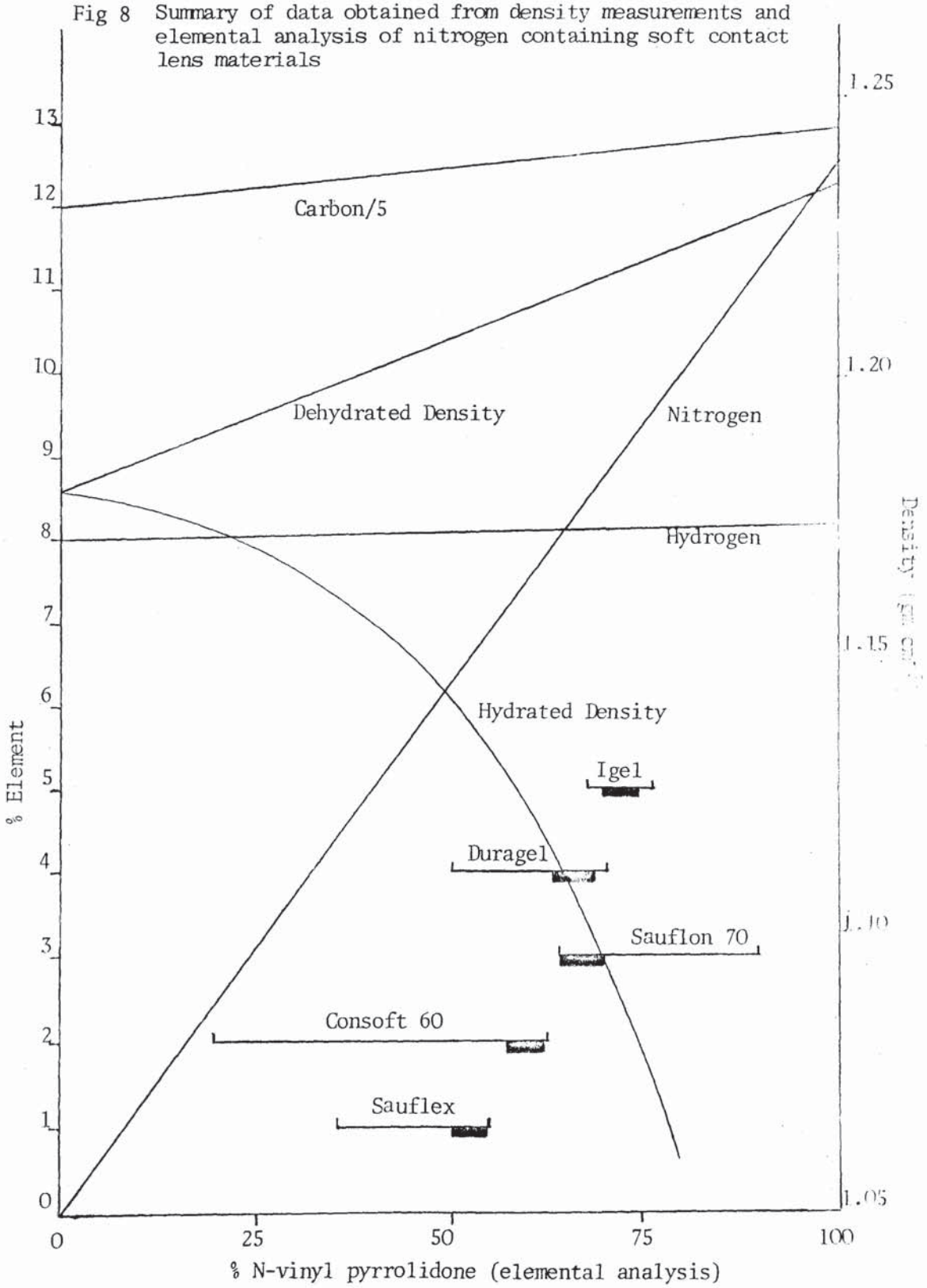
Sample	Density g cm^{-3}		Elemental Analysis %				Contact Angle°		Water Content %		Carbonyl Absorbtions IR cm^{-1}	Indicated from Pyrolysis Gas Chromatography
	Dehydrated	Hydrated	C	H	N	O*	H_2O	CH_2I_2	H_2O	Saline		
Hydrocurve II	1.26	1.16	57.78	8.52	≈1	33.7	76	36	42	40	1720	HEMA + 2 unknown
Flexol 35	1.26	1.19	53.9	8.27	-	37.83	75	32	36	35	1710	HEMA + 1 unknown
Flexol 72	1.25	1.20	53.9	7.86	-	38.34	73	32	29	28	1725	HEMA, MMA + 1 unknown
Eurocon	1.29	1.15	53.59	7.93	-	38.48	84	24	39	38	1720	HEMA + 1 unknown
Igel	1.22	1.07	59.0	8.26	9.22	23.52	85	11	78	79	1720 1680	NVP, HEMA, MMA
Duragel	1.22	1.14	59.68	8.62	8.17	23.36	79	39	63	64	1700 (broad)	NVP, MMA
Sauflon 70	1.23	1.10	59.65	8.06	7.94	24.35	78	23	71	72	1720 1680	NVP, MMA
Consoft 60	1.19	1.11	60.2	7.90	7.79	24.12	77	28	63	64	1700 (broad)	NVP, MMA
Sauflex	1.20	1.13	60.09	8.49	7.02	24.4	69	12	55	54	1710 1680	NVP, MMA

therefore affords information as to the possible presence of nitrogen in the sample. This carbonyl absorbtion is shown by materials such as Sauflon 70, Consoft 60 and Sauflex. Although infra-red spectroscopy gives an indication of the presence of nitrogen, it is not possible solely on the basis of infra-red spectroscopy to deduce the nature of the nitrogen containing monomer. In these three cases, the presence of N-vinyl pyrrolidone is indicated by pyrolysis gas liquid chromatography and also by the relatively high water contents of polymers containing vinyl pyrrolidone. An examination of the elemental analyses obtained, shows that there is some relationship between the amount of nitrogen present on elemental analysis and the proportion of vinyl pyrrolidone present in the polymer.

Errors can arise, however, particularly from incomplete combustion and the presence of residual monomer (usually vinyl pyrrolidone rather than methacrylates). A useful way of interpreting CHN and density results together in the construction of a graph of the form shown in Fig 8. This shows expected elemental analysis figures together with hydrated and dehydrated densities for the complete composition range of a pair of comonomers (in this case methyl methacrylate and vinyl pyrrolidone).

Also shown on the graph are the range of vinyl pyrrolidone contents indicated by all results for the five vinyl pyrrolidone-containing materials. The shaded region in each case shows the

Fig 8 Summary of data obtained from density measurements and elemental analysis of nitrogen containing soft contact lens materials



most reliable range of values. It is noticeable that the dehydrated density is the least reliable (and reproducible) property because of swelling of the polymer as it passes down the column together with the effect of residual monomer. The indicated vinyl pyrrolidone contents of the materials are:

Sauflex	(50-55%)
Consoft 60	(57-62%)
Sauflon 70	(64-69%)
Duragel	(63-68%)
Igel	(69-74%)

The last two materials listed are open to more doubt as they appear to be slightly more complicated compositions. The only other N-containing material is Hydrocurve which appears to be PHEMA with a small proportion of a nitrogeneous comonomer. The remaining materials are substantially PHEMA containing various amounts of methacrylic acid (incorporated deliberately or as impurity).

The detection of methacrylic acid in hydrogels is complicated by the fact that it is one of the degradation products of PHEMA at the temperatures at which pyrolysis gas liquid chromatography is carried out and so this technique cannot be relied upon to detect its presence as a deliberately added comonomer. Two of the commercial polymers examined are known to contain small quantities of methacrylic acid. This is used

to increase the water content by treating the polymers (Flexol 35 and Flexol 72) with sodium bicarbonate to form the sodium salt of methacrylic acid which is considerably more hydrophilic than the free acid. In the case of Flexol 72, the water content shown in Table 6 can be increased to 72% on treatment with sodium bicarbonate solution. The only reliable method for the estimation of methacrylic acid content is titration (eg with sodium hydroxide).

The detection of other alkyl methacrylates is complicated by peak overlap in the pyrolysis gas liquid chromatograms and in cases where another alkyl methacrylate in small quantity is present, it is often difficult to distinguish between it and unmodified PHEMA. In the Flexol materials, it is known that there is another alkyl methacrylate present, however, it is not possible to confirm it's nature or presence from the results obtained in this work.

As can be seen from Table 5 there is a tendency for the water contact angle to increase with increasing styrene content. With all polymers, the surface structure and the effects caused by the surface are dictated by interactions which occur in the bulk. The values observed for surface free energy are functions of the amount of freedom the polymer chains have in taking up any preferential orientation under the constraints of such factors as inter and intramolecular bonding, ease of packing of any side chains and the amount of any cross-linking which has occured. With hydrophilic monomers present, there is a possibility of large

amounts of both intra and inter molecular hydrogen bonding and this will tend to lead to the non random presentation of groups at the surface. With large amounts of hydrogen bonding, it is expected that the surface free energy will be reduced because the amount of polar groups which are available to form the surface will be reduced by the bonding. In the case of styrene/HEMA copolymers, there would appear to be more polar groups at the surface than would be expected by adding together the surface energies of the two homopolymers in the correct proportions. This increase in the polar component of surface energy is probably due to some kind of packing restraint imposed on the hydrophilic monomer by the styrene molecule. The difficulty of packing the phenyl group and the hydroxyl containing side chains which occurs on HEMA could then lead to the exclusion of the side chain which would then be relatively free to form a surface of polar hydroxyl groups. The application of contact angle measurements to elucidate surface properties is a useful technique in controlled systems such as the styrene/HEMA polymers described. With reference to the commercial polymers, however, the usefulness of the data obtained is much more limited. The contact angles measured on the commercially available materials seem markedly higher than would be expected for polymers with compositions similar to those shown. This could be due to surface contamination of some kind (eg residual monomers) and so the results obtained are not necessarily indicative of the surface properties of the polymers.

The techniques described when used in combination can provide useful information as to the composition of hydrogels. Naturally all the information obtained is of interest but it is important to realise the limitations of the methods used. The techniques used are at present of greater value in a controlled situation but as has been shown, can provide useful indications as to the composition of commercially available and more complex systems. With modification and refinement, there is no reason why more accurate characterisations of commercial materials cannot be undertaken.

CHAPTER FOUR

WATER BINDING PROPERTIES OF HYDROGEL POLYMERS

Although it has been suggested that the most important single property of a hydrogel is it's equilibrium water content, this statement requires further qualification. In addition to the total amount of water present within a hydrogel, as measured in terms of equilibrium water content, the nature or organisation of the water present is important, especially with respect to any specific interactions that this may induce. Water in the hydrogel network can exist in more than one state and it is this phenomenon of hydrogel structure/property relationships which governs many aspects of the behaviour of the material in a given environment. In this Chapter some aspects of the nature of water within synthetic hydrogels is discussed with reference to some of the techniques available to investigate water binding properties, the major technique discussed being the application of differential scanning calorimetry (DSC).

4.1 Nature and Determination of Water Binding Properties

It has long been recognised that water in biological systems can exist in more than one form. The relationship between water and biological macromolecules is one of the most important of all natural processes, connective tissue for example depends upon it's association with water for unique and specific mechanical

properties. Despite the obvious importance, many aspects of the structural protein-water interaction remain unresolved. This is largely due to the complex chemical nature of the protein molecule and the equally complex structure of connective tissue. It is because of the complex nature of the problem that the spectrum of interactions observed varies widely[62]. In the study of water binding properties in proteins, the water associated with the protein has been described as structural, bound and free water. X-ray diffraction data can provide information as to the spatial arrangement of water molecules located within a protein cyrstal and the structural water associated with the crystal can be described by a specific stoichiometry. The term bound water has been used to describe water in both the aqueous solution and in the hydrated condensed state. The best definition perhaps being considered as one in which bound water is considered as having properties that are measurably different from those of bulk (free) water as measured by the same technique. Water molecules that are in the vicinity and interact strongly with macromolecular surfaces have been found to exhibit properties that are measurably different from those shown in the bulk[63,64]. These water molecules exhibit a lower vapour pressure, lower mobility and greatly reduced freezing point. Such water has been referred to as bound water, however, this definition has limitations. Water molecules considered bound by some experimental methods may not be by others. Despite the obvious difficulties and limitations concerned with the definitions of water within biological systems, it is generally accepted that water molecules adjacent

to macromolecules exhibit different properties to those in the bulk.

In a synthetic system, the study of water binding properties is open to a much wider and more detailed examination. The problems of disturbing a biological environment as is the case with animal tissue, for instance, does not arise. The fact that synthetic systems are free of the constraints imposed by natural systems, although being an advantage in many ways, also has inherent disadvantages. In many natural systems, an active mechanism has been proposed to account for observed behaviour this being the case with the transport of metabolites in solution across some naturally occurring membranes. For this reason, the way in which water binding is related to polymer properties in purely synthetic systems is not necessarily concurrent with observations made in nature. The work described in this thesis is confined to the investigation of water binding properties in synthetic systems.

There is now considerable evidence to suggest that water molecules absorbed within synthetic hydrogel polymers may exist in two or more different forms[64 - 79]. Of these states, one probably consists of water which is bound to the polymer through hydrogen bonding. The rest of the water does not take part in hydrogen bonding (free water) and has a greater mobility. The relative amounts of bound and free are thought to have a considerable influence on many of the properties of hydrogels.

A number of terms have come into use when referring to the state of water within hydrogels and these are shown in Table 7. Although these terms are often used to describe the state of water within hydrogels, it is doubtful if any of the descriptions of the states of water are strictly correct. Perhaps the best way to describe the water present in hydrogels is to view the water as existing as a continuum consisting of highly bound water molecules interacting directly with the hydrophilic sites on the polymer, surrounded by successive hydration shells of less highly bound water. The concept of water existing in two states (bound and free) within a hydrogel is certainly an over-simplification, but is frequently used because of its simplicity and convenience. A number of techniques exist for the study of water binding properties in polymers and this can lead to differences as to the quantity of a particular type of water present dependent on the experimental technique used. This is because each technique may have a bearing on different aspects of water, eg thermodynamic, dynamic and structural.

The techniques used for the study of the states of water within hydrogels include differential scanning calorimetry (DSC)[81], nuclear magnetic resonance (NMR)[82], infra-red spectroscopy[83], dilatometry[74], specific conductivity[74] and dielectric studies[78]. Of these techniques, differential scanning calorimetry and nuclear magnetic resonance have been the most widely used.

Table 7 Terms used to describe the states of water in water swollen polymer systems

Bound Non-freezing Primary		Free Freezing Secondary	
Z W_3 Bound	Y W_2 Interfacial	X W_1 Bulk	
Primary Bond	Secondary Bond	Free	Bulk

NMR provides one of the simplest ways to observe the different states of water and has been used extensively to study the state of water in biological systems. Two kinds of NMR are used, pulse and broadline, the broadline technique being similar in principle to the pulse technique although in practice the samples, instrumentation and results, are quite different. The important difference being that the broadline technique can be used for the study of the solid state whereas the pulse method is better for the liquid state. Both techniques yield information mainly about the relative mobilities of the differing water states. Bound water being distinguished from free water because of its limited mobility as compared to free water. NMR also indicates that while bound water is relatively immobile compared to free water, it is still more mobile than pure ice and that there can be a rapid exchange between the bound and free water states.

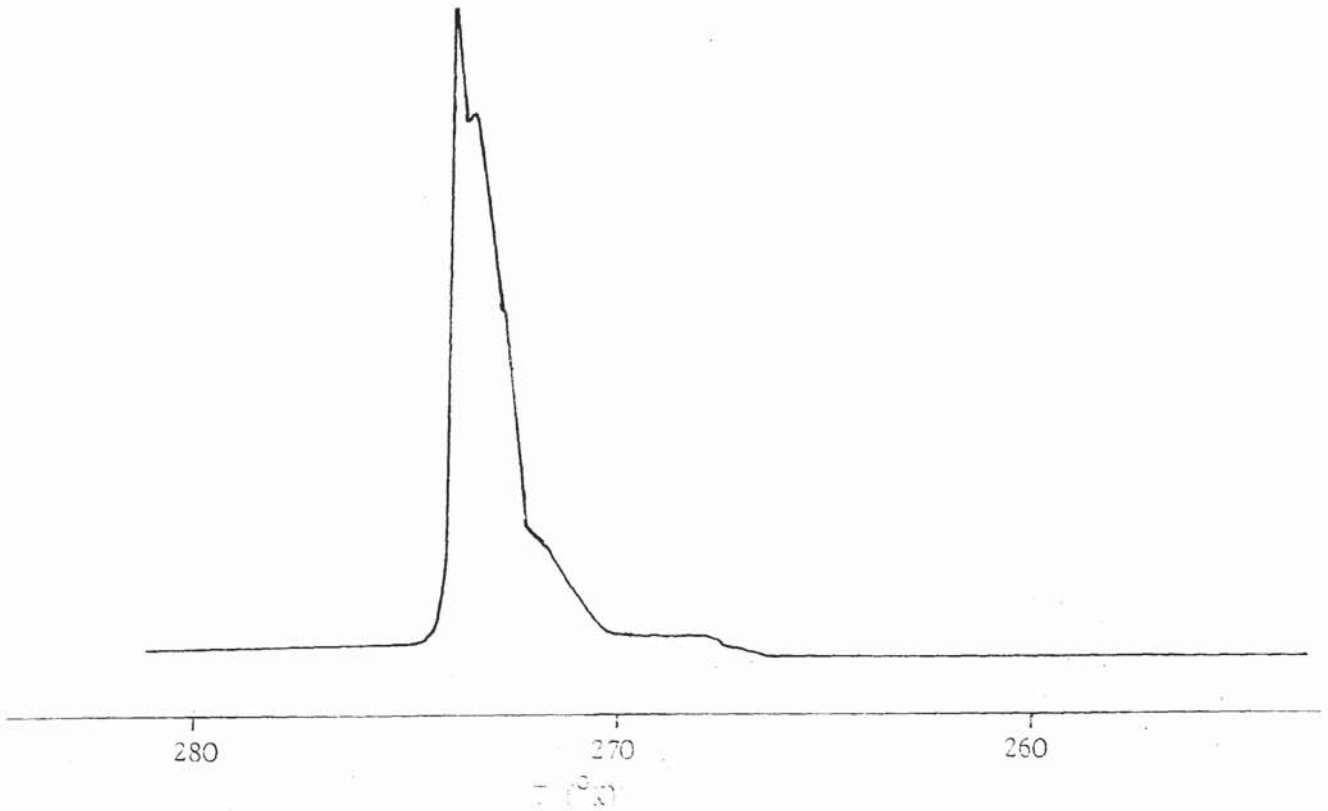
When a thermal transition takes place in a material, the reaction associated with the transition is either exothermic or endothermic. DSC measures the amount of heat evolved or absorbed when a thermal event such as melting or freezing occurs. During such a transition the instrument senses heat absorbed or evolved by the sample and alters the distribution of heating energy between the sample holder and reference material so as to maintain a thermal balance. In DSC the distance moved by the recorder pen from the base line is directly proportional to the rate of energy absorbtion or release and the area under the

thermogram measures the heat of transition. DSC when applied to the study of absorbed water in polymers can be used to quantitatively determine the amount of water which freezes near the freezing point of pure water. From a knowledge of the total water content of the system and the weight of the sample used the fraction of water that does not freeze at very low temperatures, typically -70°C, can be calculated. On this basis, non-freezing (bound) water can be taken to be water which is strongly associated with the polymer and freezing (free) water can be interpreted as that which is less strongly associated with the polymer and hence free to freeze at a temperature close to that shown by pure water.

4.2 Information Obtainable from Differential Scanning Calorimetry

The freezing and non-freezing water contents of hydrogel films (prepared by the method described in Chapter Two) were obtained using a Perkin-Elmer (DSC 2) differential scanning calorimeter in conjunction with a Servoscribe potentiometric recorder fitted with an integrator and event marker. The operating range of the instrument being -175°C to + 725°C. In a typical experiment, the samples to be studied by DSC were wiped with tissue to remove surface water and then hermetrically sealed in aluminium sample pans, the sample size being typically 3-7 mg. The sample pans were cooled to -50°C and then heated at 1.25°C/min or 5°C/min to 20°C, at least three samples of each material were used. Fig 9 shows a typical trace obtained

Fig 9 Typical melting endotherm obtained from DSC of a hydrogel material



from the above procedure. The area under the melting peak was measured and the amount of freezing water from a calibration graph obtained by measuring the peak areas produced by distilled water samples of known weight. It has been shown that the heat of fusion of freezing water contained in water swollen polymers is virtually identical to the heat of fusion of pure water[68, 79]. The freezing water content was expressed as a percentage of the weight of hydrated polymer. The amount of non-freezing water being taken as the difference between the total water content of the sample and the amount of freezing water.

Although the area under the melting endotherm is a measure of the total freezing water, it is interesting to examine in more detail the shape of these curves. Work carried out in these laboratories has shown a fine structure to the DSC curves obtained. Many of the results obtained do not show the simple melting behaviour exhibited by pure water but are more complicated which would tend to indicate that freezing water may exist in a number of states of water shown in Table 7. Two examples of this fine structure are illustrated in Figs 10 and 11. In the first example, a typical DSC trace is shown and on this trace it is apparent that there are two peaks at approximately 273 and 274 K in the melting endotherm. Temperature cycling between 223 and 293 K gives rise to a slight increase in the total area under the peaks but more importantly an increase in the size of the peak at 274 K is observed, combined with a corresponding decrease in the peak at 273 K. If

Fig 10 Melting endotherm after 3 melting cycles

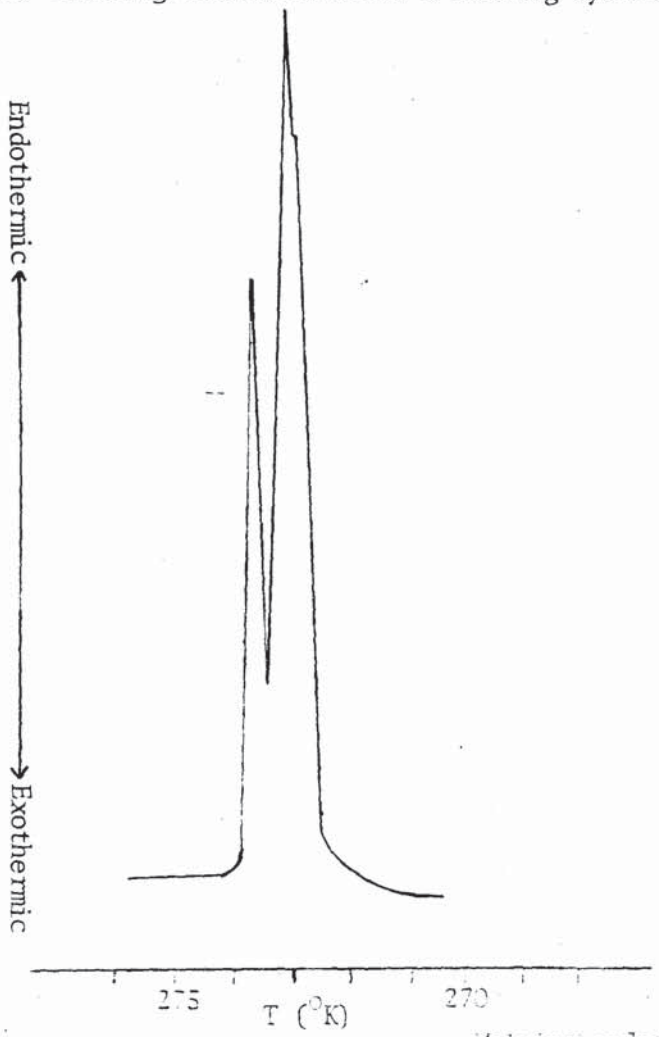
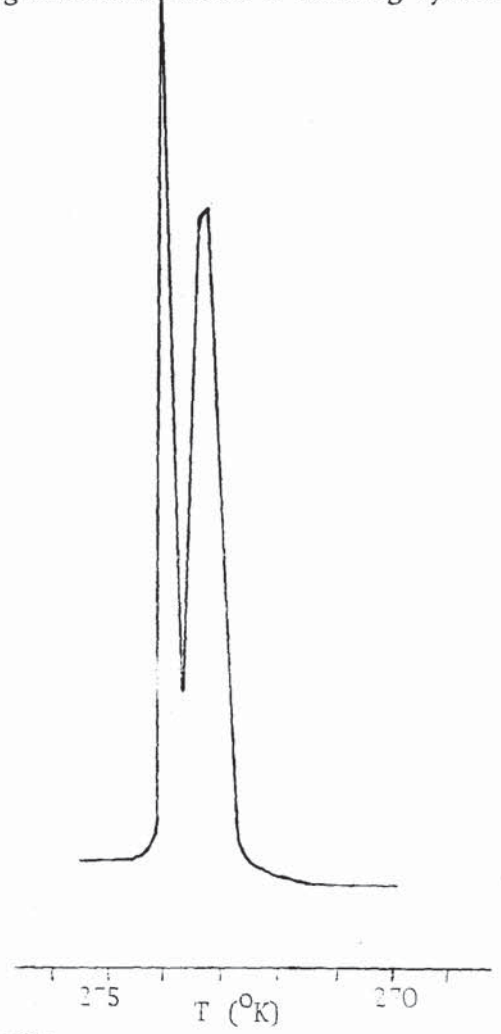


Fig 11 Melting endotherm after 5 melting cycles

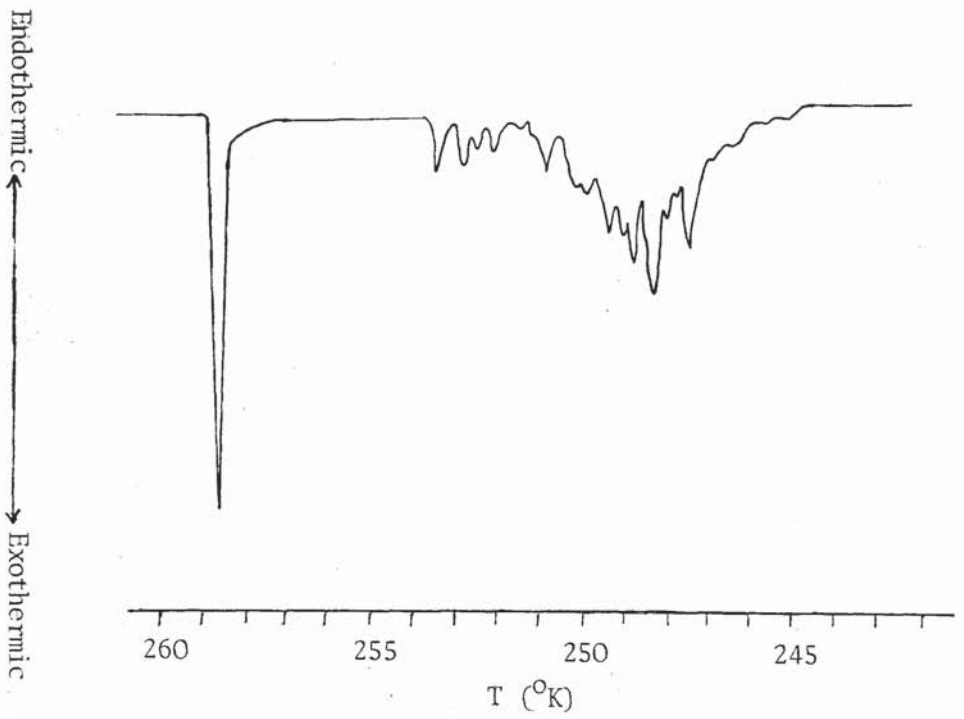


Melting endotherms of a hydrogel polymer

the area under the peak at 274 K is taken to be associated with the 'pure' water in the system, then temperature cycling can be seen to increase the size of this peak and would tend to indicate that the amount of pure water is increased on cycling. There is evidence to suggest the existence of water pores in hydrogels[84,85]. Given the presence of water pores in the gel the effect of temperature cycling is not difficult to visualise in terms of increased perfection of crystallisation of the water in the gel. In a typical freezing exotherm (Fig 12) there is a sharp peak and a broader more complex peak at a much lower temperature which may be indicative of a multiple nucleation process. From these observations, it is apparent that there is a difference between the descriptions of water states shown in Table 7 and the experimental results described above, because the descriptions in Table 7 are theoretical concepts which do not necessarily correlate with the results of any specific experimental technique. It is our belief that our results are consistent with the concept of a continuum of water states between water which is hydrogen bonded to functional groups in the polymer and water that is relatively unaffected by it's polymeric environment. The latter type of water crystallises at 274 K in the manner of pure water. There is then a continuum of water states whose behaviour is affected by the environment and in which crystallisation occurs more slowly.

The water binding process in hydrogels is a complex phenomenon, however, an understanding of this process is important because

Fig 12 Typical freezing endotherm of a hydrogel polymer



of the profound influence that this process has on the behaviour of the material itself and consequently the applications in which a given material may be utilised. Properties of hydrogel polymers which depend on the water binding process include: permeability (salt rejection), biocompatibility and mechanical properties.

The permeability of hydrogel polymers has been studied with particular reference to the use of hydrogel polymers as reverse osmosis membranes for desalination purposes and PHEMA has been the most widely studied polymer in this field. The structure of PHEMA suggests that it may have semipermeable characteristics similar to those shown by cellulose acetate. Like cellulose acetate, it has both ester and hydroxyl groups and has the considerable advantage of being relatively resistant to hydrolysis. Baddour et al[86] prepared copolymers of HEMA, ethyl methacrylate and ethylene glycol dimethacrylate (cross-linking agent), in the form of thin films. Using this system, the number of hydroxyl groups could be varied as could the cross-link density. The salt rejection of PHEMA was found to increase to a maximum of 80% as the membrane was more tightly cross-linked and then to decrease rapidly. The salt rejection of membranes containing ethyl methacrylate was found to be very low. The true permeabilities of these membranes could not be accurately determined because of the fact that filter paper was used to reinforce the membranes at the polymerisation stage. Subsequent work[66] on cross-linked PHEMA membranes has led to more

encouraging results. In reverse osmosis experiments, the salt rejections of these membranes was found to increase to a maximum of 94% as the amount of cross-linking agent was increased, although water fluxes decreased rapidly for even relatively small amounts of cross-linking agent. A variety of other systems have been studied and these include the preparation of thin solution cast membranes from uncross-linked PHEMA[87] using a suitable supporting material to form a composite material. Two disadvantages of uncross-linked PHEMA are apparent; firstly, the low mechanical strength of these materials and secondly, the fact that uncross-linked PHEMA is too hydrophilic to allow any marked salt rejection. To overcome this difficulty, HEMA has been copolymerised with a hydrophobic monomer[65]. In reverse osmosis tests with these hydrophilic/hydrophobic type membranes, some very high salt rejections have been observed (98%). Hoffman et al have suggested that improved performance in terms of salt rejection and water flux should be possible by the correct selection of type and concentration of the hydrophilic, hydrophobic monomers and the cross-linking agent. Although the performance of this type of membrane may be superior to that of say cellulose acetate, it still remains to prepare such membranes that will give a performance (in terms of water flux) approaching that of cellulose acetate membranes.

In addition to the physical construction of a membrane for permselective (eg reverse osmosis) applications, the nature of the water within the membrane is now accepted as the major

factor in controlling permselectivity. Because of its interaction with the hydrophilic sites on the polymer, bound water is unable to appreciably solvate any salts and consequently salt permeation is prevented. The bound water molecules can, however, still form hydrogen bonds with free water molecules and so permit the migration of free water from site to site within the membrane. The need, therefore, is for a membrane with good mechanical properties whilst maintaining a high permeability of water. This system therefore requires a relatively high water content with a large proportion of such water being in the bound state to allow transport of water but with a low free water content to discourage the passage of salts.

The biocompatibility is another property of a hydrogel material which is dependent not only on the amount of water present within a hydrogel but also on the nature of such imbibed water. The performance of hydrogel materials in a biological environment is now thought to be a function of the relative amounts of free and bound water in the gel network, although there has been no evidence of any hard relationship being established between the water binding properties of a hydrogel and its observed biocompatibility.

Previously it has been mentioned that the mechanical properties of a hydrogel tend to be adversely affected with increasing water content. This, however, is not strictly speaking true. Once again the mechanical properties of a hydrogel are dependent

not only on the amount of water present but also the nature of such water. A hydrogel can have a relatively high water content and still maintain good mechanical properties if the amount of bound water within the gel is sufficiently high, as is desirable in the reverse osmosis membrane. The ratio of bound to free water can be easily varied by altering the cross-link density of the material and this aspect is discussed in a later section with reference to PHEMA hydrogels with varying cross-link densities.

4.3 The Relationship between Water Binding Properties and Hydrogel Structure

In this section some aspects of the relationship between water binding properties and hydrogel structure will be discussed with reference to a variety of hydrogel systems of differing hydrophilic/hydrophobic characteristics. The effect of cross-link density on water binding will also be discussed with reference to PHEMA hydrogels. The commonest synthetic hydrogel systems are those based on PHEMA and a variety of studies have been carried out. Perhaps surprisingly there is no substantial agreement in the results obtained as regards the amount of free and bound water present in a given system. This lack of agreement is probably a function of both the large number of definitions that have come into use for describing water absorbed in hydrogels and also the number of techniques that have been used to investigate the water binding process. More important

than the precise state of the water present is the way in which the ratio of free to bound water in a given system, as defined by one particular experimental technique, varies as a function of hydrogel structure.

In the work described in this Chapter, measurements of freezing and non-freezing water were made using differential scanning calorimetry (DSC). The amount of freezing water expressed as a fraction of the total hydrated weight of the polymer is taken as a measure of the free water present. The amount of non-freezing (bound water) can then be calculated from a knowledge of the equilibrium water content of the material in question. All observations are therefore expressed in terms of freezing and non-freezing water as defined above. All the polymer membranes studied in this Chapter were synthesised using the standard methods as described in Chapter 2, the DSC thermograms being obtained as described in an earlier section of this Chapter.

4.3.1 Hydrophilic/Hydrophobic Systems

As an example of one of the above systems, a copolymer based on HEMA with the incorporation of varying amounts of styrene was studied. The addition of a hydrophobic monomer, in this case styrene, to reduce the hydrophilicity of a hydrogel has been a widely used technique in order to synthesise a hydrogel with specific properties (eg rigidity). The effect of the incorporation of styrene on the equilibrium water content of

HEMA/styrene hydrogels is shown in Fig 13 and as can be seen, as the amount of styrene incorporated increases, the equilibrium water content (EWC) of the copolymer falls from 40% (PHEMA) to 0% in the case of polystyrene homopolymer. Of greater importance than the reduction of the equilibrium water content due to styrene incorporation is the effect that this has on the amount of freezing water within the hydrogel. In the case of HEMA/styrene copolymers, PHEMA has an EWC of 40% of which previous work has indicated 23% is freezing water and 17% is non-freezing water[81,95]. The incorporation of 10 mole % of styrene in the copolymer reduces the EWC to 23% of which 4% is freezing water the remaining 19% being non-freezing water. With styrene contents of 20 mole % and above no freezing water is observed. The complete set of results with respect to the water binding properties observed in this type of system are presented in Figs 14 and 15 . Fig 14 shows the relationship between the HEMA content of the copolymers described and the equilibrium and freezing water contents observed. Fig 15 shows the total number and number of non-freezing water molecules associated with each HEMA unit in the copolymer system.

It is apparent that the incorporation of a bulky hydrophobic monomer even in relatively small amounts, has a marked influence on the amount and more importantly the nature of water present within the hydrogel. Non freezing water consists of water molecules which are hydrogen bonded to hydrophilic groups on the polymer chain. Freezing water molecules, however, are

Fig 13 Effect of the incorporation of styrene in HEMA/styrene copolymers on the equilibrium water content

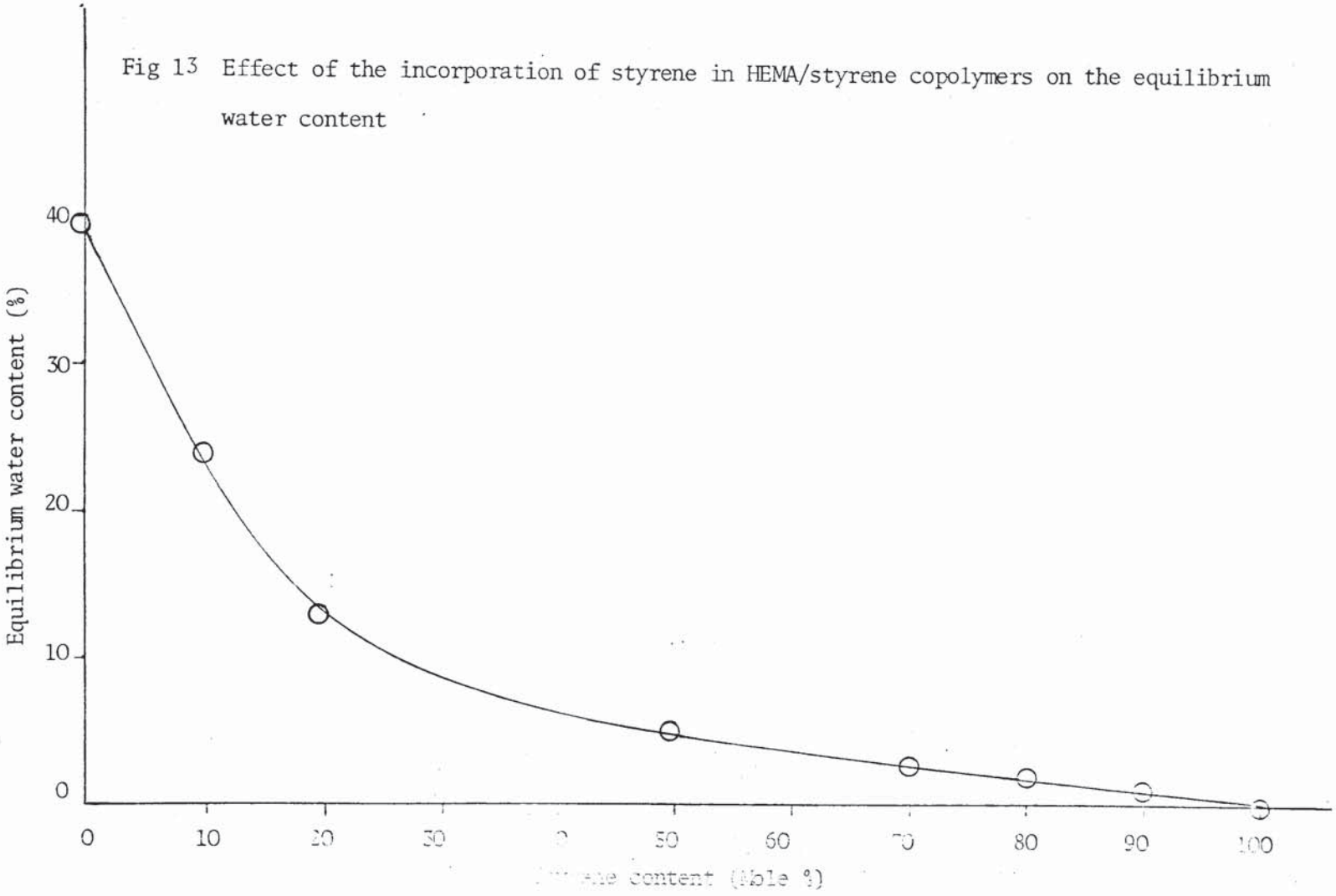


Fig 14 Equilibrium and freezing water contents of HEMA/styrene copolymers

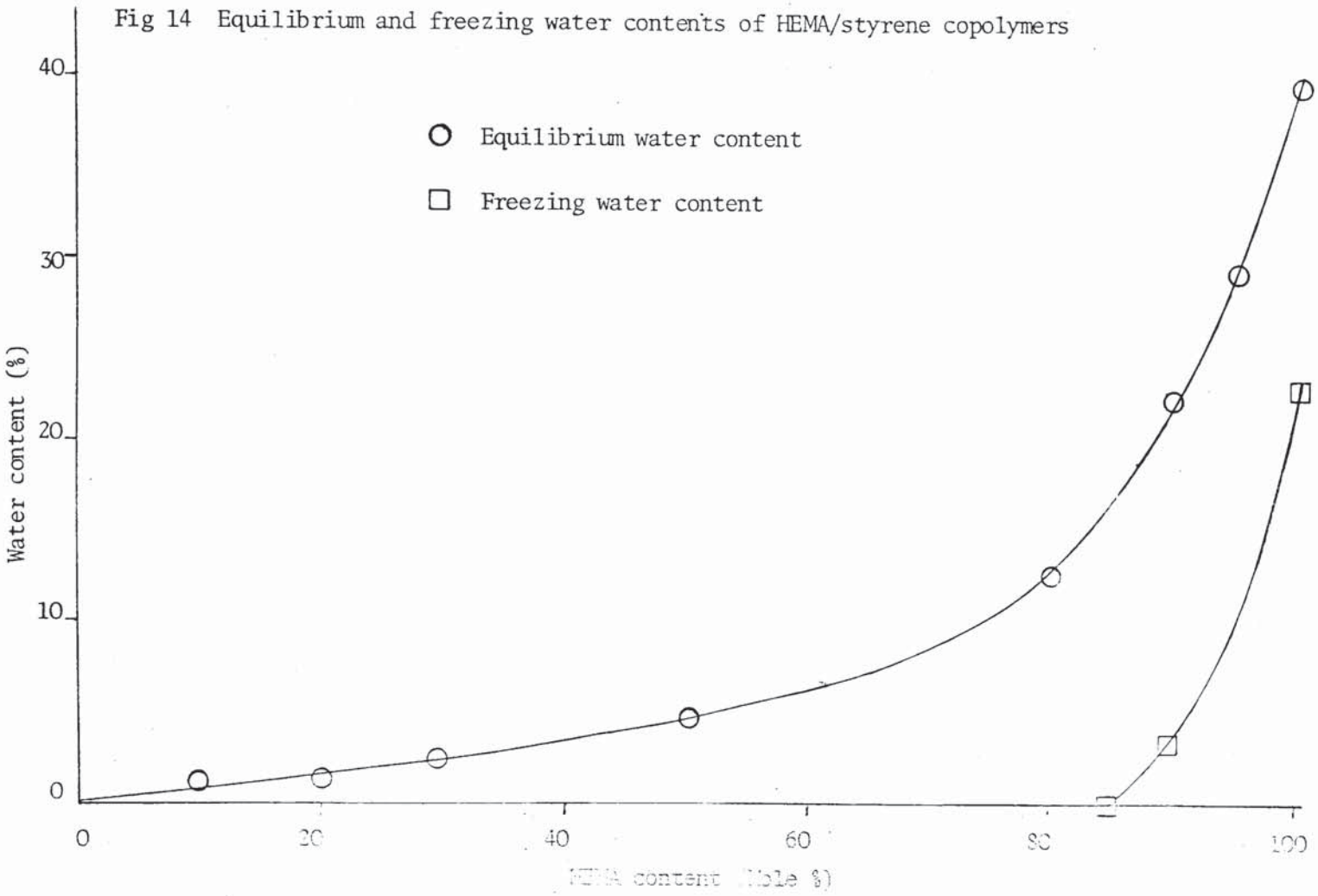
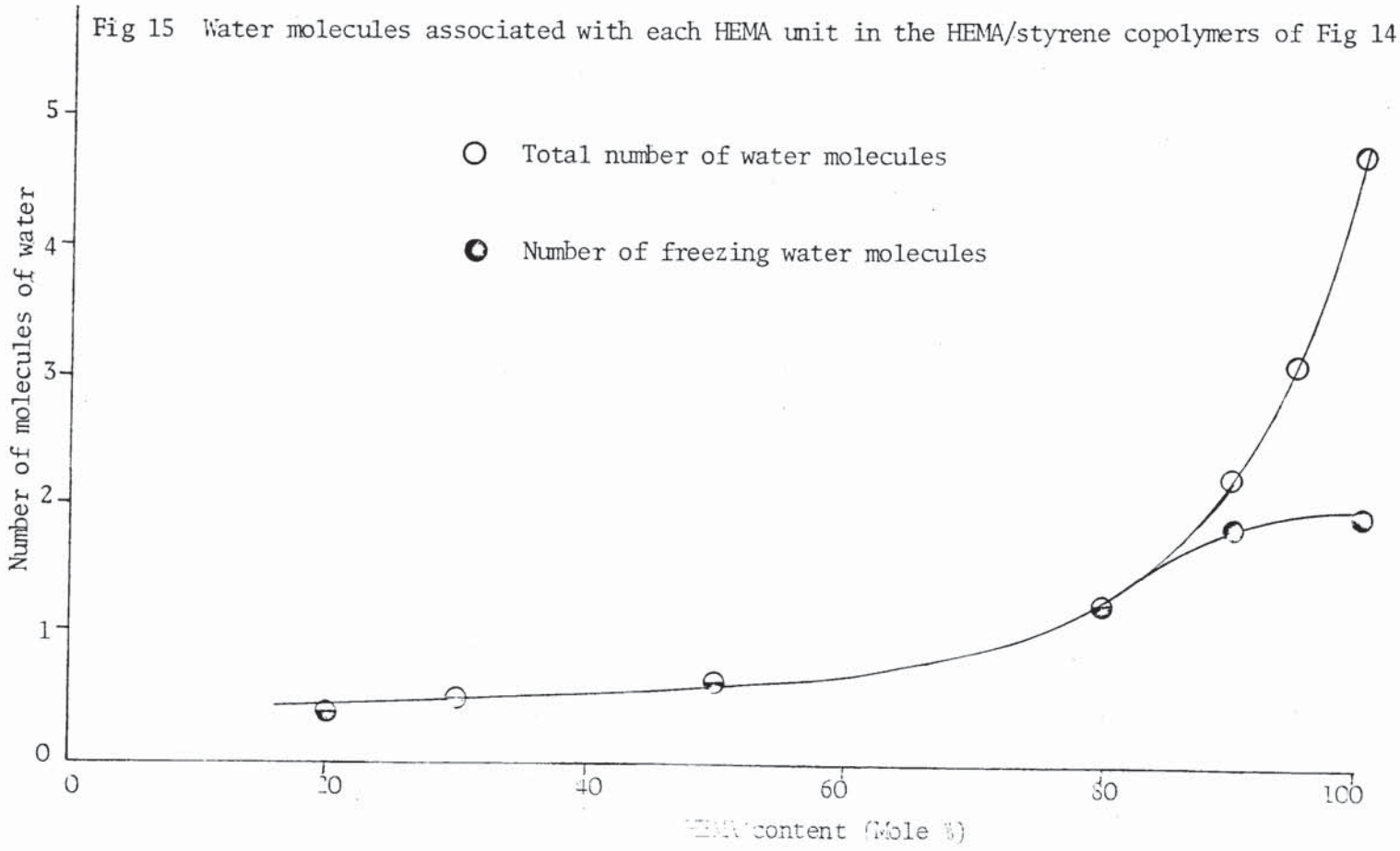


Fig 15 Water molecules associated with each HEMA unit in the HEMA/styrene copolymers of Fig 14



only hydrogen bonded to themselves or to non-freezing water molecules. The pendant hydrophilic groups on the polymer chain (hydroxyl groups in the case of PHEMA) are surrounded by a shell of non-freezing water molecules around which freezing water molecules exist. Using this concept of hydration shells it is possible to envisage the presence of many hydration shells around each hydrophilic site on the polymer chain. If a relatively bulky hydrohpobic group, such as the phenyl group in the case of styrene is incorporated, then its presence will tend to affect the freezing water molecules as these are the water molecules present in the outer hydration shells. The phenyl group although relatively bulky and comparatively large does not approach the hydrophilic groups close enough to have any significant effect on the non-freezing water present.

The effect of the incorporation of styrene in the HEMA/styrene series of copolymers is therefore two-fold. Firstly, the equilibrium water content of the system is decreased as the proportion of styrene present is increased because of the hydrophobic nature of styrene as a monomer. Secondly and perhaps more importantly the proportion of freezing water present is decreased with increasing styrene content as discussed earlier. Both these observed effects would seem to be due to the hydrophobic nature of the styrene molecule and not to any other more complex interactions.

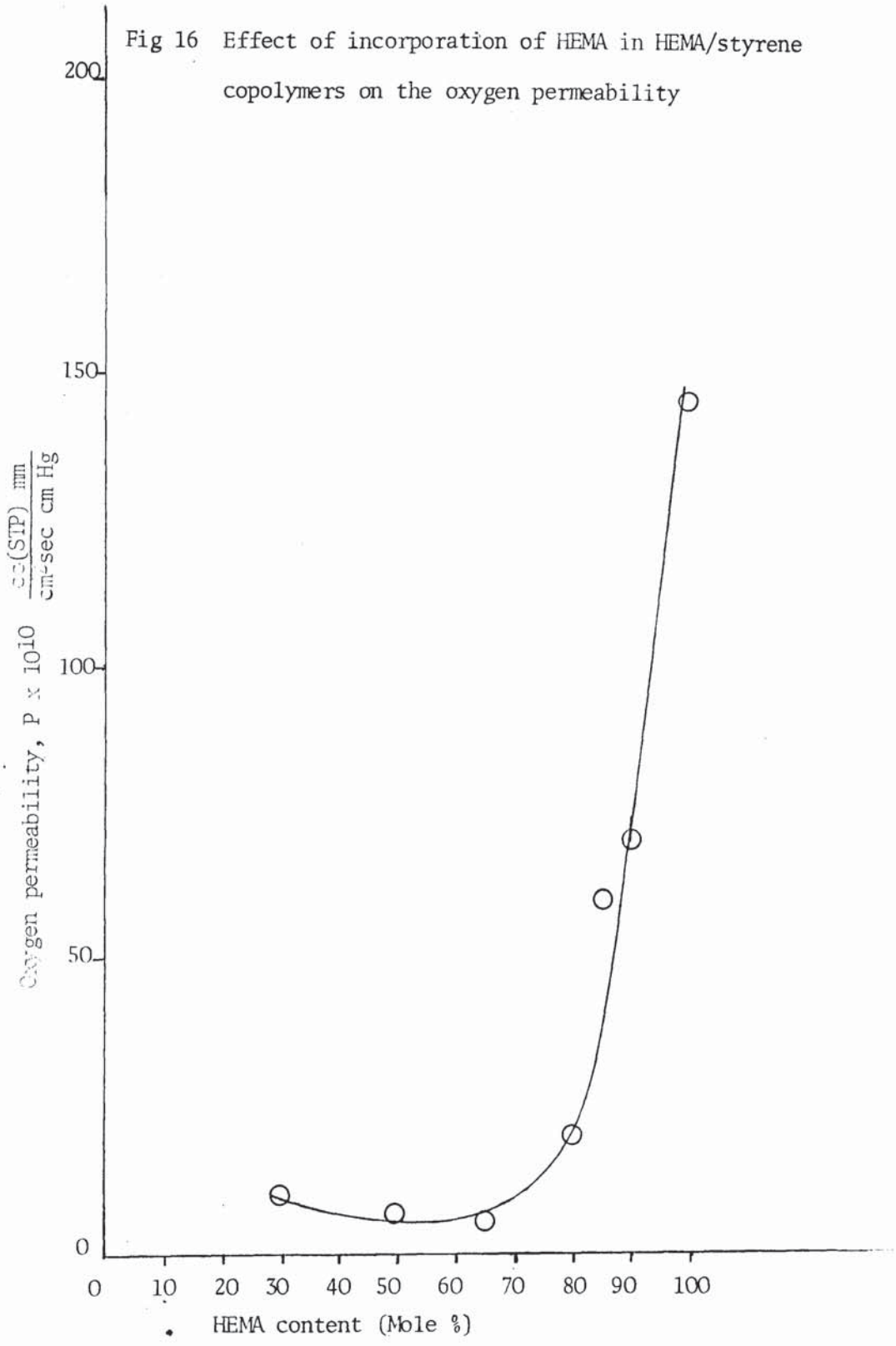
The consequences of this behaviour can be seen with reference

to the effect on a number of observed properties. Firstly, the difference between the calculated and measured densities of these copolymers suggests that the non-freezing (bound) water has a slightly different density than that of freezing (free) water. The results shown in Fig 4 (Chapter 3) suggests that non-freezing water has a density of approximately 1.1 g cm^{-3}. Although this is entirely speculative at this moment, it does provide a means of interpreting observed results. Secondly, the oxygen permeability of these materials, which has been reported elsewhere[96], shows a dramatic increase as the amount of freezing water in the hydrogel increases as shown in Fig 16. The oxygen molecule apparently has a hydration shell containing an average of 2.6 moles of water associated with it[97]. This hydration shell both increases the effective size of the diffusing species and also affects the water exchange interactions that provide a basis for understanding the rejection of hydrated species by hydrogel membranes. Finally the mechanical properties of the material change markedly at a molar composition of 90:10 (HEMA : styrene). This is illustrated by the relationship between flexibility and polymer composition which shows the same trends as those observed in the relationship between oxygen permeability and polymer composition

4.3.2 Hydrophilic/Hydrophilic Systems

In contrast to the previously mentioned system, both monomers in a hydrophilic/hydrophilic system are hydrophilic in their

Fig 16 Effect of incorporation of HEMA in HEMA/styrene copolymers on the oxygen permeability



own right. Therefore both monomers have the capability to hydrogen bond with themselves or with water molecules within the hydrogel.

A series of copolymers based on HEMA and acrylic acid (AA) were synthesised and the water binding characteristics studied by differential scanning calorimetry. The equilibrium water content (EWC) was also measured and the effect of increasing HEMA content in the copolymer is shown in Fig 17. As can be seen, the EWC of the copolymer decreases from 73% (polyacrylic acid) to 40% for PHEMA. Acrylic acid being a more hydrophilic monomer than HEMA. The amount of freezing water present in the gel was measured and the effect of the incorporation of HEMA is shown in Fig 18. The curve shown (Fig 18) follows the trend set by the EWC curve and shows no unusual features with respect to the water binding properties of the system.

In this system, both the monomers used are relatively weak in terms of their ability to form hydrogen bonds. There is therefore no strong competition between the monomers to form intermolecular hydrogen bonds with each other as opposed to the formation of hydrogen bonds with water molecules within the hydrogel network.

Fig 17 Effect of the incorporation of HEMA on HEMA/AA copolymers on equilibrium water content

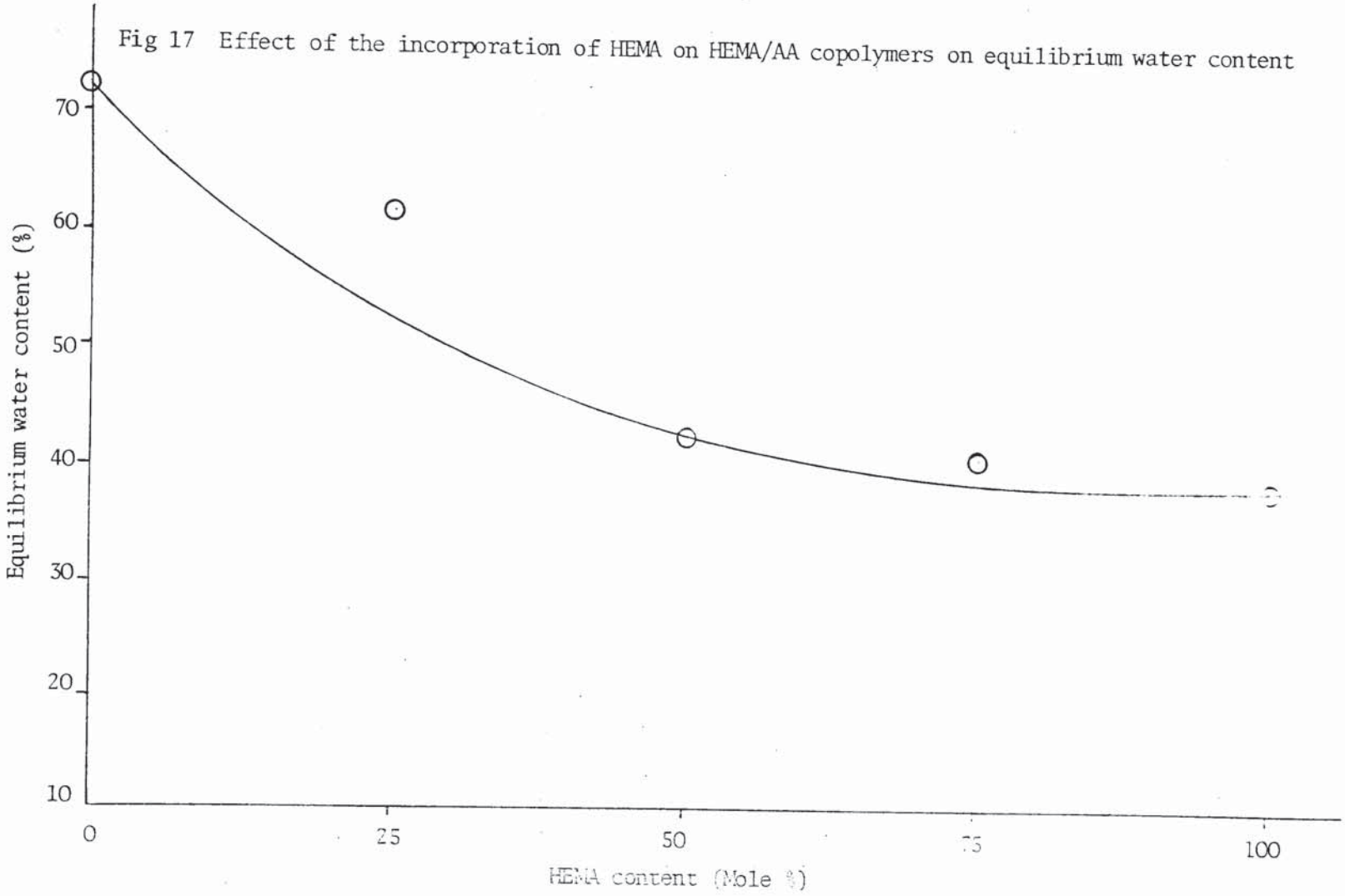
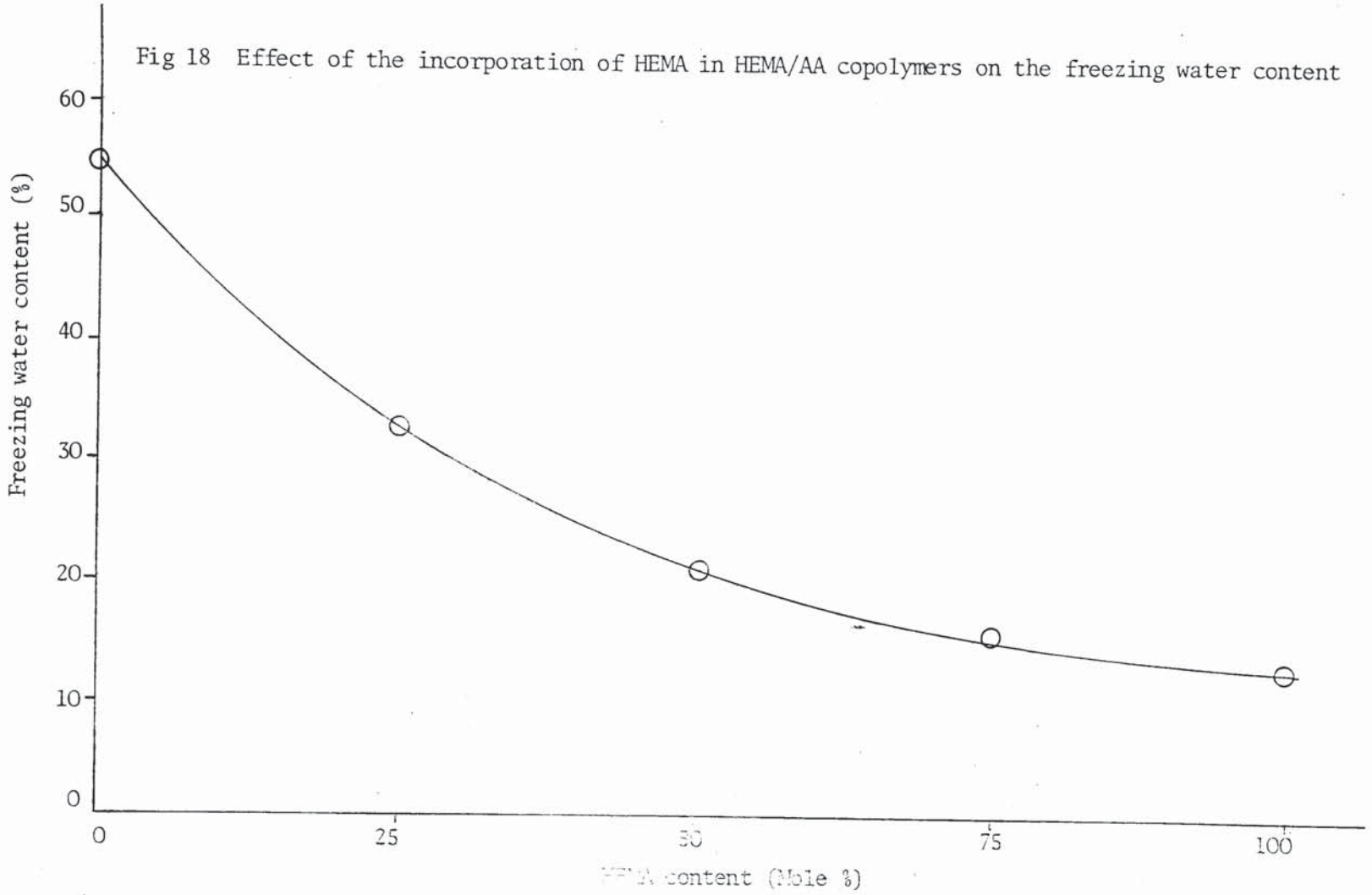


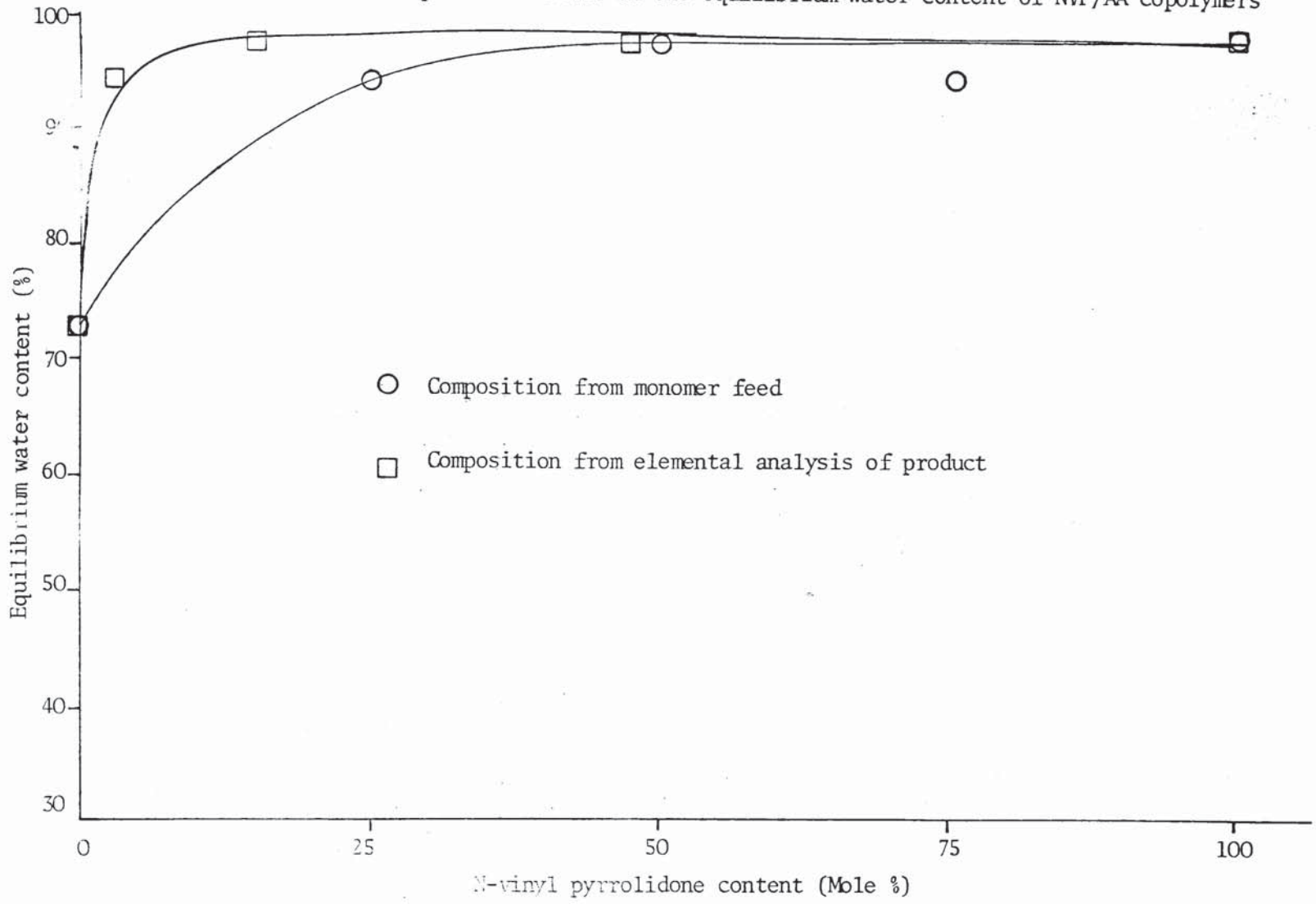
Fig 18 Effect of the incorporation of HEMA in HEMA/AA copolymers on the freezing water content



The hydrophilic/hydrophilic group of copolymers allows scope for systems involving stronger hydrogn bonding to be synthesised. These systems show a number of interesting properties and it is possible to make a polymer with a high equilibrium water content, a large fraction of which is present in the non-freezing (bound) state. Polymers with high non-freezing water content can be made to have good mechanical properties, (eg rigidity) and consequently can be used in applications such as reverse osmosis where a high non-freezing water content is desirable for salt rejection and mechanical strength is required because of the water pressures involved. During the course of this research, the effect of incorporating stronger hydrogen bonding monomers, in a copolymer system, on water binding properties has been examined. The effect of the incorporation of a hydrophobic monomer (styrene) into a hydrophilic/hydrophilic system has also been investigated with respect to the resultant water binding properties. Some interesting results have been obtained from such systems and these are discussed in the following section.

One type of hydrophilic/hydrophilic system based on monomers with stronger mutual hydrogen bonding capability is illustrated by the system acrylic acid (AA) -co- N-vinyl pyrrolidone (NVP). A series of copolymers using varying amounts of the above monomers with 1% cross-linking agent, was synthesised in membrane form using the standard method described in Chapter 2. The equilibrium water contents and freezing water contents of these copolymers were determined by the methods previously described. Fig 19

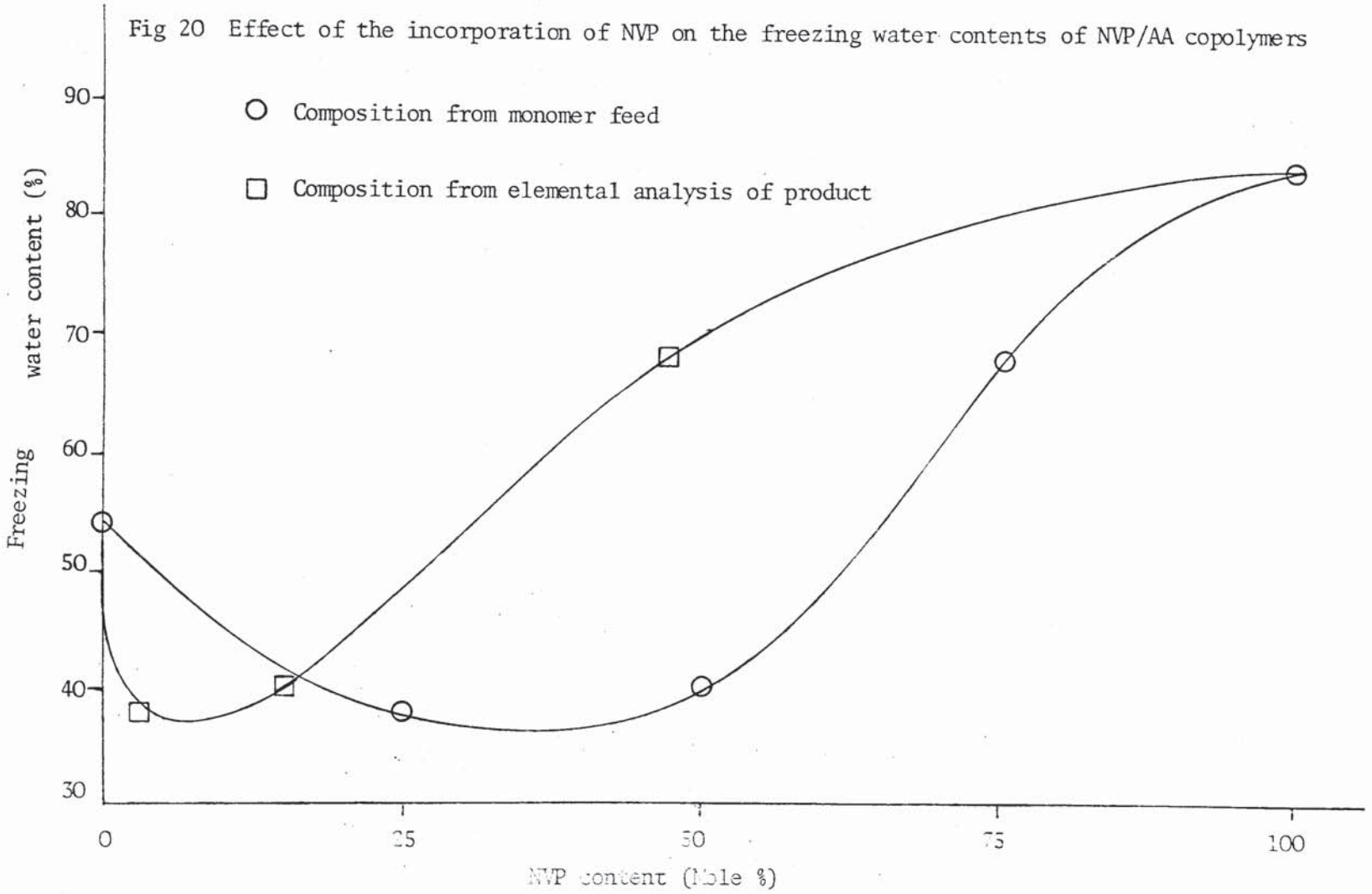
Fig 19 Effect of the incorporation of NVP on the equilibrium water content of NVP/AA copolymers



shows the relationship between the equilibrium water content of the copolymers as a function of the amount of NVP present. Two curves are shown (Fig 19) describing the equilibrium water content both in terms of the amount of NVP in the feed and also the amount of NVP as determined by elemental analysis, incorporated in the resultant polymer. Fig 20 shows the freezing water contents of the AA/NVP system with respect to the feed ratio and also the amount of NVP present in the polymer. The equilibrium water content curves (Fig 19) show an increase as the amount of NVP present is increased. This is to be expected due to the hydrophilic nature of NVP and although less NVP is incorporated than would be expected on the basis of the feed ratio (because of the low reactivity ratio of NVP) no unexpected results are observed in the curve for the polymer which was analysed.

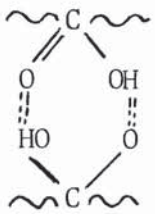
The freezing water content curves (Fig 20) however show interesting minima at small NVP concentrations. Although the equilibrium water content of the copolymers increases with increasing NVP content, the freezing water content decreases and then rises and hence the bound water content must increase correspondingly. One reason for this could be the competition between interchain hydrogen bonding and water binding, however, in view of the small amounts of NVP required to cause the decrease in freezing water content, this may not be the case. Another possibility is that when present in small amounts, NVP causes the water to be structured to a certain extent, perhaps incorporated in some interchain hydrogen bonding, causing the

Fig 20 Effect of the incorporation of NVP on the freezing water contents of NVP/AA copolymers



amount of freezing water present in the gel to fall. As the amount of NVP present in the system increases, this structuring effect disappears and the hydrophilic nature of NVP becomes the dominant effect, consequently the amount of free water present in the system increases following the trend set by the equilibrium water content.

An alternative way of expressing this would be to say that the incorporation of NVP into apolyacrylic acid network begins to disrupt the hydrogen bonding between carboxyl groups:



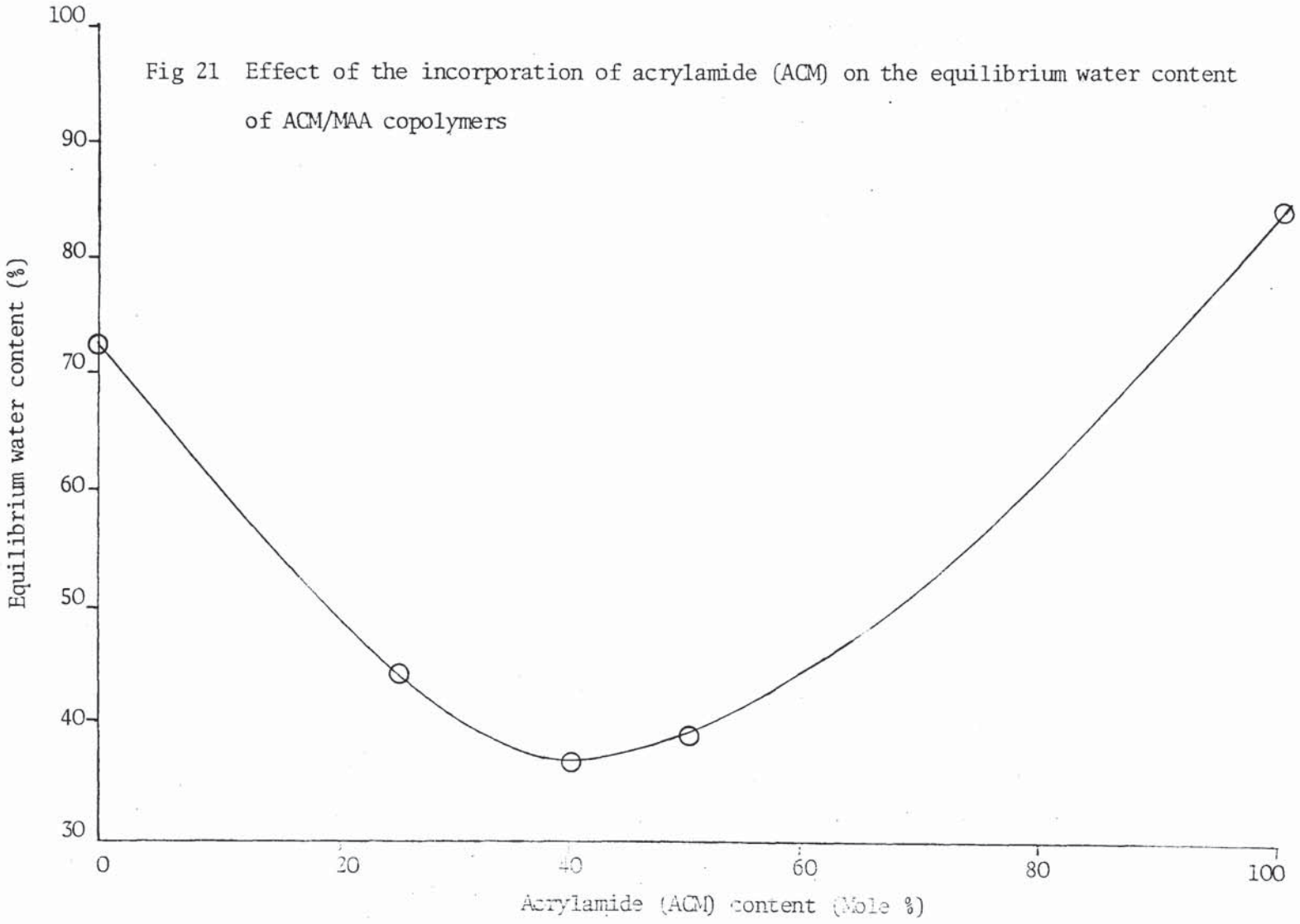
The net result in the initial stages of the incorporation is to make a greater proportion of carboxyl groups available for direct water binding. Thus, although the incorporation of the more hydrophilic NVP causes the total water content of the system to rise, it also causes an initial disproportionate increase in hydrogen bonded (non-freezing) water and, consequently, a decrease in freezing water. As the amount of NVP in the network is increased, the balance of water stages shows a smooth composiition-dependent variation.

This structuring effect would seem to be the best explanation

of the results obtained from this type system where the second monomer incorporated exhibits a somewhat stronger H-bonding (donor or acceptor) capability than the monomer forming the basis of the gel. There is a difference, for example, between NVP and HEMA in the strength of their hydrogen bonding capability with acrylic acid. Thus incorporation of HEMA into an acrylic acid system simply produces a smooth change in water content with no abnormal structuring effects. There is therefore a clear difference between the AA/NVP system and the AA/HEMA system, in the former the presence of a stronger hydrogen bonding monomer (NVP) causes the effects described above, whilst in the latter system, there is no evidence of the monomers causing any unusual effects on the water binding properties of the hydrogel.

The third type of system studied, within the hydrophilic/ hydrophilic groupings, was a system in which both monomers used show strong hydrogen bonding tendencies. Such a system is illustrated by copolymers of acrylamide (ACM) and methacrylic acid (MAA). A series of such copolymers with 1% cross-linking agent, was synthesised in membrane form according to the method previously described. Fig 21 shows the relationship between the equilibrium water content of these copolymers with respect to the acrylamide content[89]. Although monomers used in this system are very hydrophilic, cross-linked (1% EDMA) homopolymers of MAA and ACM showing equilibrium water contents of 73% and 84% respectively, there is an interesting minimum in the

Fig 21 Effect of the incorporation of acrylamide (ACM) on the equilibrium water content of ACM/MAA copolymers



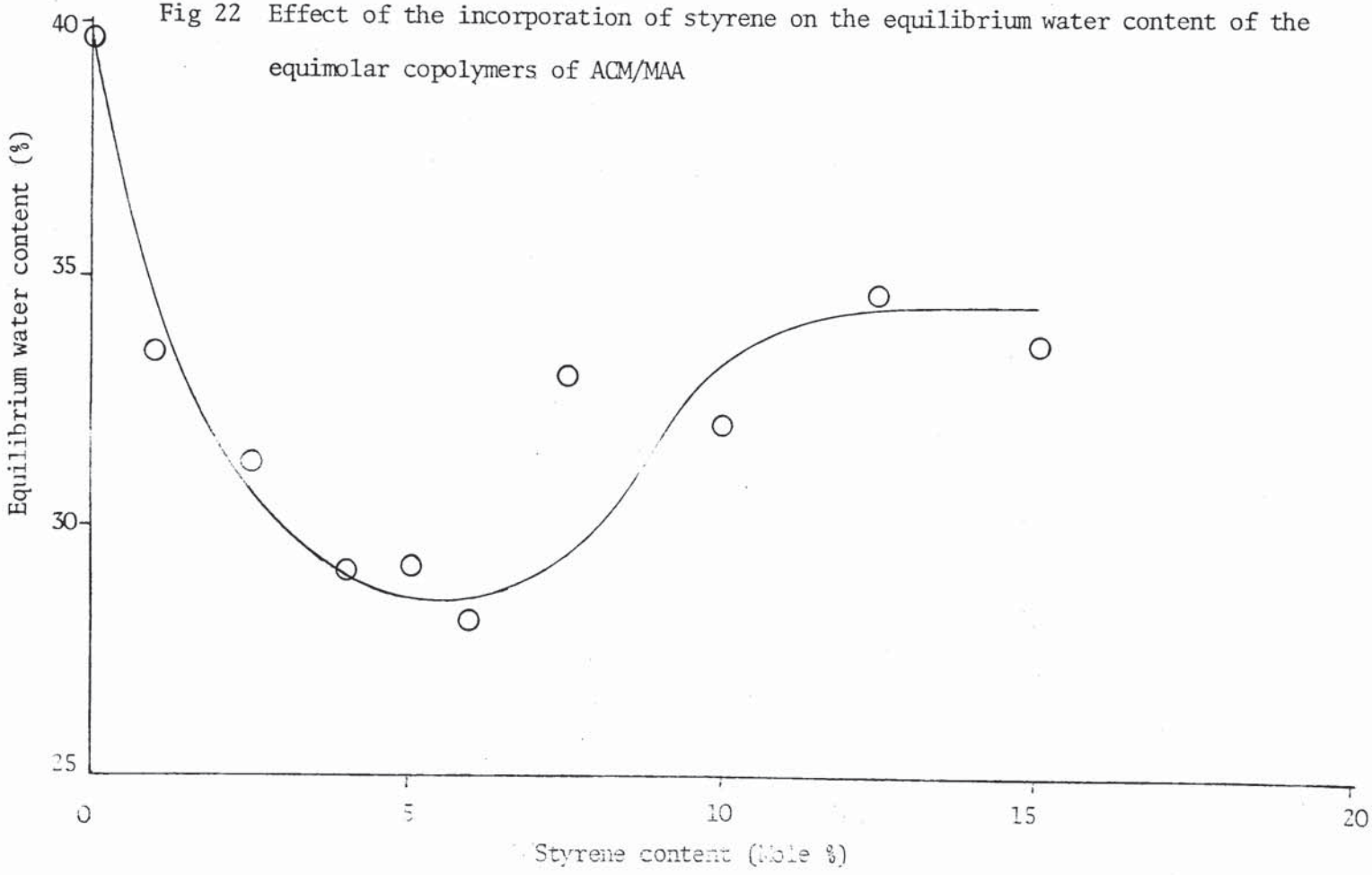
equilibrium water content when both monomers are present in approximately equimolar proportions. This effect is believed to be due to the competition between interchain hydrogen bonding and water binding. In this region, it would seem that strong hydrogen bonding occurs between the pendant carboxyl and amine groups resulting in a tightly bound network structure from which water is partially excluded. Also in this region, the polymers have similar equilibrium water contents to that of poly(2-hydroxyethyl methacrylate) (PHEMA) but show improved mechanical properties (eg tensile strength and rigidity). This is partially due to the interchain hydrogen bonding and is also related to the differing plasticising properties of freezing and non-freezing water.

Given this effect on equilibrium water content, ACM:MAA provide a useful system on which to base modifications which would give a relatively high equilibrium water content, coupled with a low freezing water content. Table 8 shows the equilibrium freezing and non-freezing water contents for an ACM/MAA series of copolymers in which the ratio of MAA to ACM is constant (equimolar) throughout, and in which the effect of adding increasing amounts of a hydrophobic monomer (styrene) to the system is investigated. Fig 22 shows the relationship between the equilibrium water content of the copolymer series (Table 8) with respect to the amount of styrene present in the system. With reference to this curve, it can be seen that there is a decrease in the equilibrium water content, falling to

Table 8 Water binding properties of methacrylic acid : acrylamide copolymers

Hydrogel	Molar Composition	Equilibrium Water Content,%	Freezing Water Content,%	Non-Freezing Water Content,%
MAA : ACM : St	49.5 : 49.5 : 1	33.5	6.8	26.7
MAA : ACM : St	48.75 : 48.75 : 2.5	31.3	4.4	26.9
MAA : ACM : St	48 : 48 : 4	29.1	2.1	27.0
MAA : ACM : St	47.5 : 47.5 : 5	29.2	1.1	28.1
MAA : ACM : St	47 : 47 : 6	28.1	0.7	27.4
MAA : ACM : St	46.25 : 46.25 : 7.5	33.1	0.7	32.4
MAA : ACM : St	45 : 45 : 10	35.1	0.1	35.0
MAA : ACM : St	43.75 : 43.75 : 12.5	34.7	0.4	34.3
MAA : ACM : St	42.5 : 42.5 : 15	33.8	0.3	33.5

Fig 22 Effect of the incorporation of styrene on the equilibrium water content of the equimolar copolymers of ACM/MAA



a minimum at a styrene content of approximately 5 mole %, this is to be expected due to the hydrophobic nature of the styrene molecule. The equilibrium water content then shows an unexpected rise as the styrene content is increased.

In the region 0 to 5 mole % of styrene, Fig 22 shows a similar trend to that seen in Fig 13 for HEMA/styrene copolymers. In HEMA/styrene copolymers, the incorporation of 5 mole % of styrene results in a decrease of equilibrium water content from around 40% to approximately 30%. In the case of the MAA/ACM series, 5 mole % of styrene reduces the equilibrium water content from 39.6% to about 29%. In this respect, it would appear that small amounts of styrene are acting in a similar manner to the behaviour observed for HEMA/styrene copolymers.

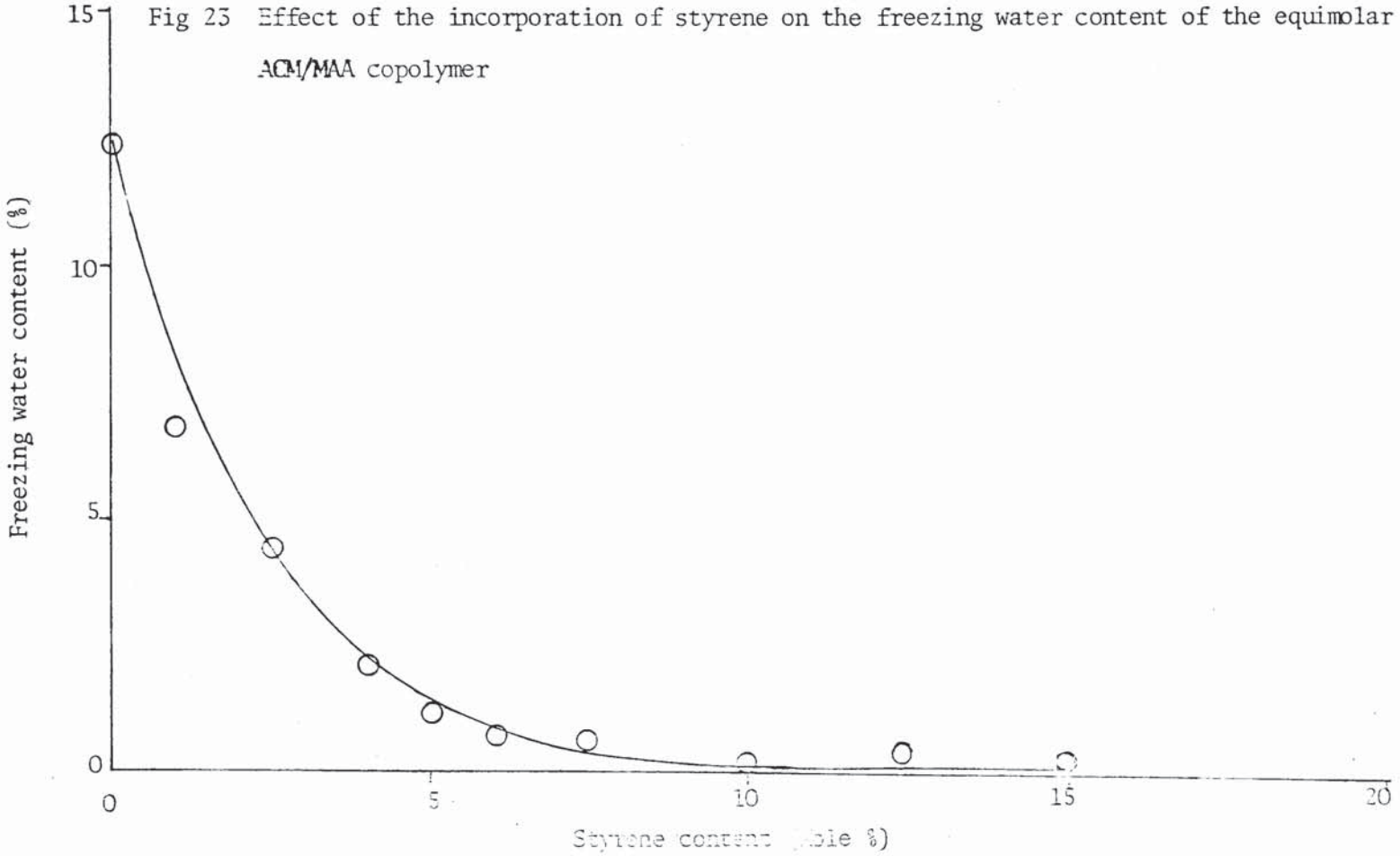
However, when the amount of styrene present is increased beyond 5 mole %, the equilibrium water contents of the MAA/ACM hydrogels show a marked difference from the behaviour of the HEMA based system. The key difference between the two systems being the presence of two monomers (ACM and MAA), both of which possess strong hydrogen bonding tendencies. The occurrence of a minimum in the equilibrium water content suggests that two different, competing effects are being exhibited. The initial decrease in the equilibrium water content (0 to 5 mole % styrene) can be explained by styrene, a hydrophobic monomer, causing a decrease in the hydrophilicity of the system. The rise in equilibrium water content for styrene contents above 5 mole %

is thought to be due to the disruptive effect of styrene on interchain hydrogen bonding. The presence of a greater number of styrene molecules in the system could lead to a reduction in the hydrogen bonding between the pendant groups in the polymer chains, thereby making more water binding sites available within the gel. Therefore, in the region of 5 to 10 mole % of styrene, the decrease in equilibrium water content caused by the hydrophobic effect of styrene would seem to be more than compensated for by the accompanying decrease in interchain hydrogen bonding resulting in a rise in equilibrium water content.

The observed rise in equilibrium water content is seen to reach a limiting value with styrene contents of between 10 and 15 mole %. This would seem to be due to the mechanism observed with small amounts of styrene where the hydrophobicity of this monomer is the controlling factor and the disruptive effect it has on interchain hydrogen bonding is not a determining influence.

The freezing water contents of the copolymers of MAA/ACM (Table 8) are shown in Fig 23 with respect to the amount of styrene present. Fig 23 shows an interesting effect in that the observed rise in equilibrium water content of these copolymers is not associated with a rise in the amount of freezing water present. The curve shows a steady decrease in the amount of freezing water present. As the equilibrium water

Fig 23 Effect of the incorporation of styrene on the freezing water content of the equimolar ACM/MAA copolymer



content rises over the range 5 to 10 mole % styrene and the freezing water content is decreasing over the same range, there must be an increase in the amount of non-freezing water present in the system. The decrease in the amount of interchain hydrogen bonding caused by incorporation of styrene, therefore, affects only the amount of bound water, to any appreciable degree.

To summarise it is perhaps useful to contrast the ACM/MAA system with styrene incorporated with the HEMA/styrene system. In both cases, styrene is present as a hydrophobic monomer, however, its influence on the behaviour of the system is profoundly different. In the simple hydrophilic/hydrophobic system as illustrated by HEMA/styrene, there are no strong interchain hydrogen bonding tendencies and the incorporation of styrene simply causes a decrease in both the equilibrium water content and freezing water content of the polymer series which is a result of the net decrease in hydrophilicity. In the MAA/ACM system, however, there are two competing effects in operation. These are the ability of the pendant groups on the polymer chain to form interchain hydrogen bonds and their ability to bind to water. It is because of this competition that styrene has the previously described interesting effects on this system and most importantly on the amount of non-freezing water present within such a system.

4.3.3 Macroporous Systems

In addition to the systems described earlier in this section, all of which are homogeneous, hydrogels that are heterogeneous and macroporous systems can be made. Macroporous gels are known to possess good mechanical and water flux properties and it is possible to make them with various chemical structures, thereby producing a thin homogeneous layer with varying salt retention properties.

Macroporous hydrogels have been prepared using a technique developed by Haldon and Lee[90]. A mixture of monomers, 2-hydroxethyl methacrylate (HEMA) and ethylene glycol dimethacrylate (EDMA) (cross-linking agent) were added to a solvent, aqueous ethylene glycol, containing uranyl nitrate as a photosensitiser. This mixture was injected into a glass mould and placed in contact with powdered solid carbon dioxide. When freezing had occurred, the mould was inverted onto a tray containing dry ice and the mixture was photopolymerised for 15 minutes using an ultra-violet lamp. The membrane was subsequently removed from the mould and hydrated in distilled water to equilibrium (at least two weeks). The water being changed frequently to ensure complete removal of the ethylene glycol. Table 9 shows the formulations used in the synthesis of macroporous hydrogels.

In all the above formulations, the monomer content refers to the total HEMA and EDMA, with EDMA present as 15.5 weight % of HEMA.

Table 9 Formulations of Macroporous Hydrogels

Monomer (weight %)	Solvent (weight %)
68	32
60	40
50	50
45	55

The solvent was a 4:1 mixture (by volume) of water : ethylene glycol. The amount of uranyl nitrate employed was 2% by weight of the total monomer content.

To date, no attempt has been made to compare the water binding properties of macroporous gels with those of a homogeneous system with comparable cross-link density. In order to investigate this, a series of poly(2-hydroxyethyl methacrylate) (PHEMA) hydrogels having cross-link densities between 1 and 20% were prepared in membrane form using the method previously described (Chapter 2). Fig 24 shows the relationship between the equilibrium water content of the macroporous gels and the percentage monomer in the monomer/solvent mixture. Figs 25 and 26 show the corresponding freezing and non-freezing water contents respectively. Fig 27 shows the relationship between equilibrium water content and cross-link density of the homogeneous gels and Figs 28 and 29 the corresponding amounts of freezing and non-freezing water respectively.

With reference to the homogeneous system, it can be seen from Fig 27 that the equilibrium water content of the system decreases steadily with increasing amounts of cross-linking as the system becomes less hydrophilic. The corresponding freezing and non-freezing water content curves (Figs 28 and 29) show evidence that the amount of cross-linking agent present is acting in two ways on the water binding properties of the system as a whole. Taking Fig 28 it can be seen that in the

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Fig 24 Effect of polymerisation diluent on equilibrium water content of macroporous HEMA gels

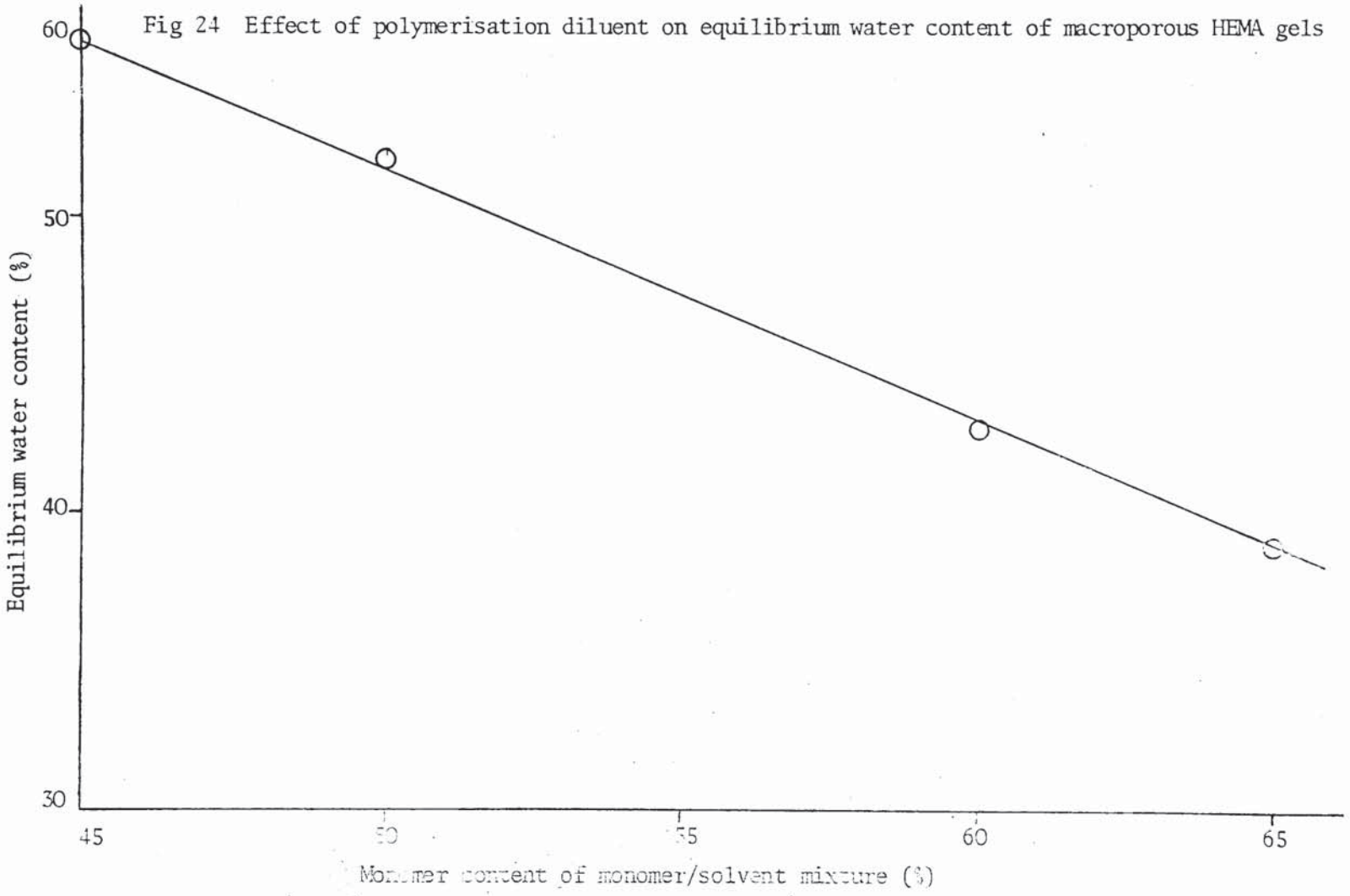


Fig 25 Effect of polymerisation diluent on the freezing water content of macroporous HEMA gels

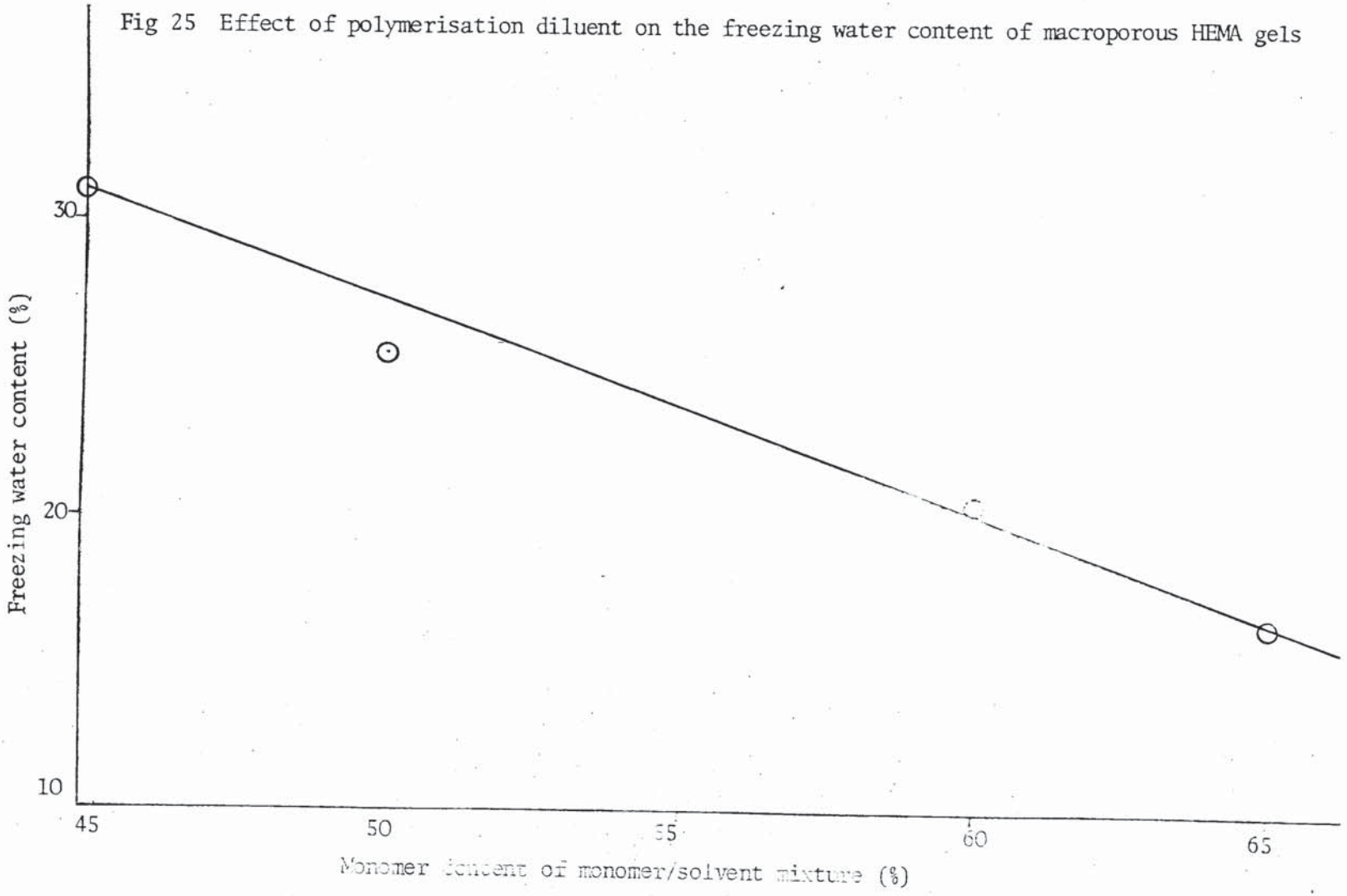


Fig 26 Effect of polymerisation diluent on the non-freezing water contents of macroporous HEMA gels

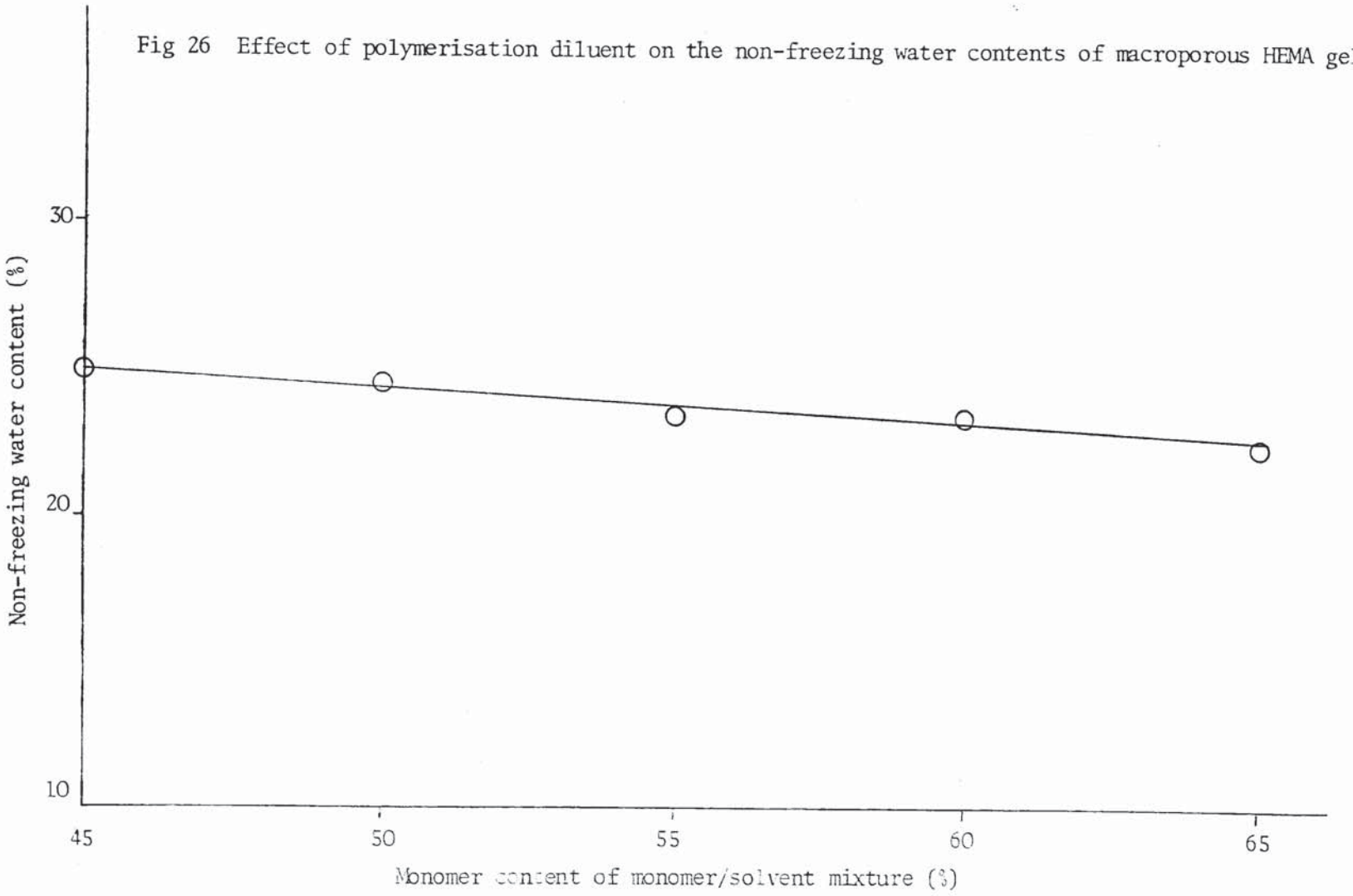


Fig 27 Effect of cross-linking on the equilibrium water content of homogeneous HEMA gels

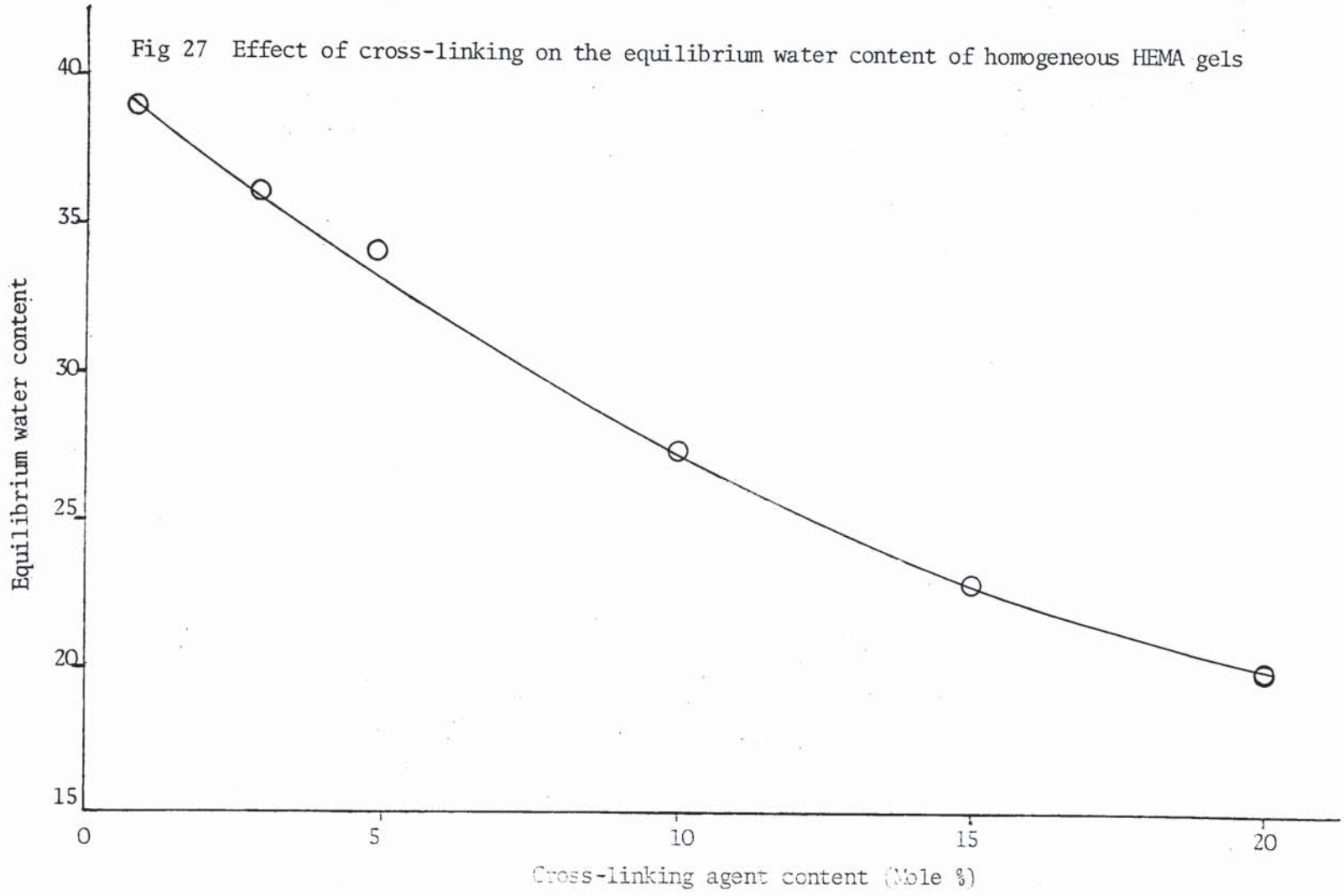


Fig 28 Effect of cross-linking on the freezing water contents of homogeneous HEMA gels

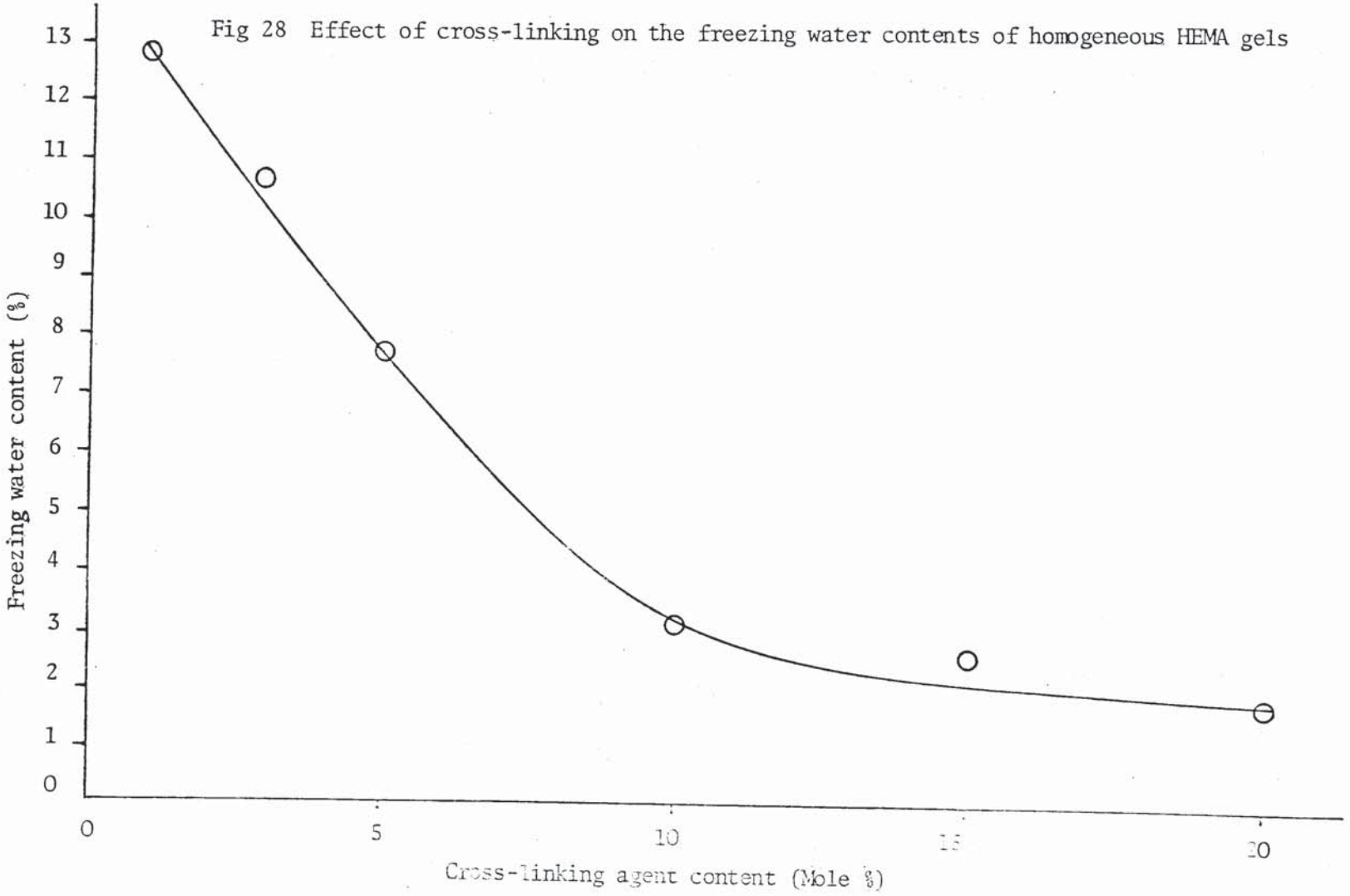
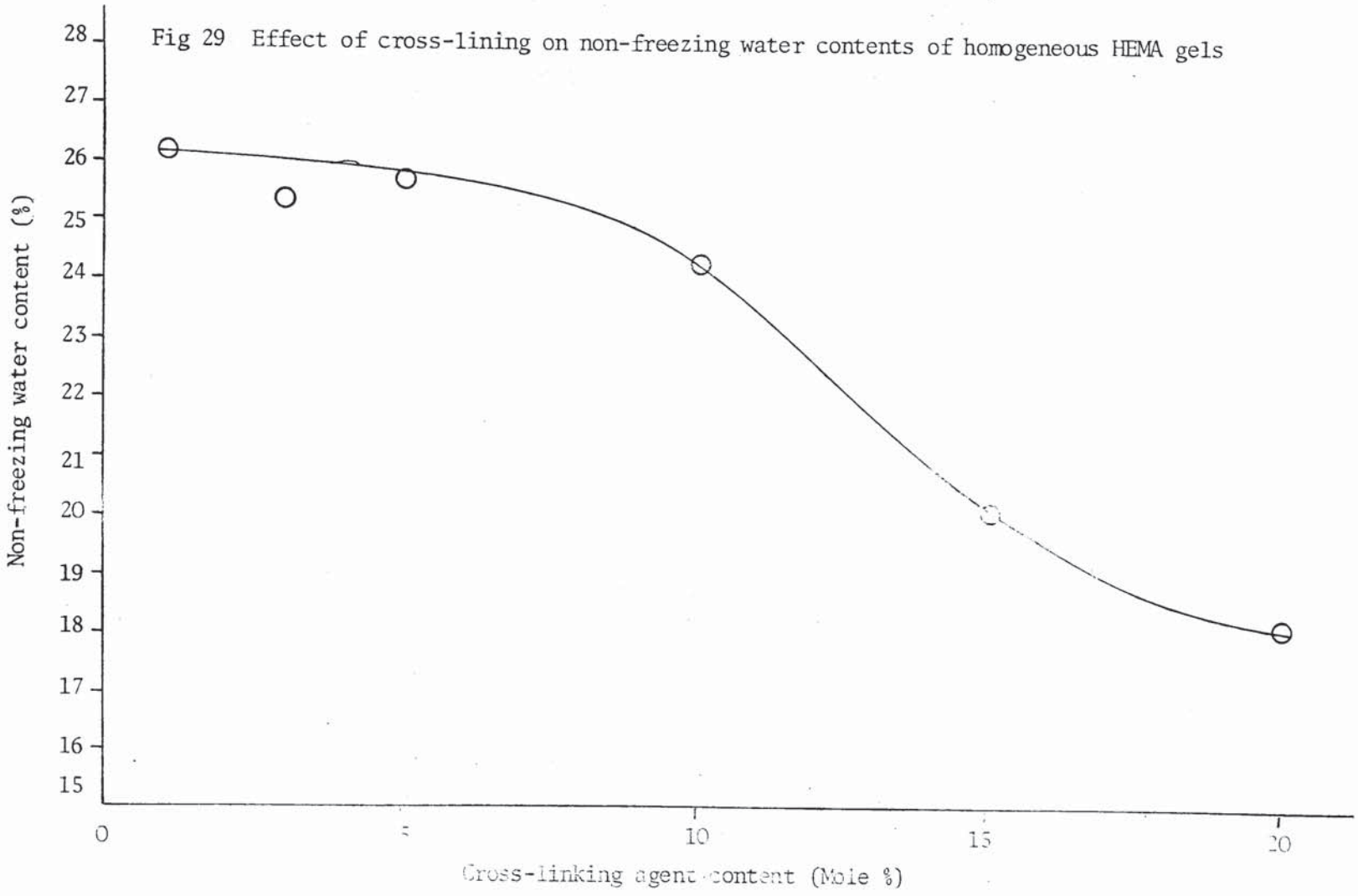


Fig 29 Effect of cross-lining on non-freezing water contents of homogeneous HEMA gels



initial stages (1 - 10% cross-linking) the amount of freezing water present is markedly affected by the cross-link density. This can be attributed to the fact that the cross-linking is tightening the network of the gel, thereby decreasing the space available for freezing water. As the cross-link density is increased, this network effect would seem to have less influence on the system and therefore the freezing water content decreases gradually following the equilibrium water content curve. It is interesting to note that at 20% cross-linking, there is only a small amount (1.9%) of freezing water present. The non-freezing water content (Fig 29) shows that in the initial stages the amount of cross-linking agent present has a less pronounced effect on the non-freezing water present than on the freezing water. This is believed to be due to the fact that in this region, the amount of cross-linking agent is not sufficient to disrupt the ability of the gel to bind water. At higher cross-link densities, the amount of cross-linking agent present is sufficient to affect the water binding of the gel and consequently the non-freezing water content is seen to decrease in a more pronounced manner. In this respect, the cross-linking agent is acting as a hydrophobic monomer to a certain degree (although not so efficiently) and so the general hydrophilicity of the system would be expected to decrease and this coupled with the increase in cross-link density would account for the observed decrease in non-freezing water present.

Comparing the homogeneous system with the macroporous system, there is one obvious difference. Whereas in the homogeneous system the non-freezing water content decreases with increasing cross-linking, in the macroporous system this is not the case. Fig 26 shows that the amount of non-freezing water in such a system remains fairly constant despite the decrease in both equilibrium and freezing water content (Figs 24 and 25) as the amount of monomer present in the monomer/solvent mixture increases. This would tend to indicate that the non-freezing water is present predominantly in the gel network as opposed to the pores in the system. This view is supported by the fact that at a given monomer/solvent ratio and comparable cross-link density the amounts of non-freezing water present in the homogeneous and macroporous gels are similar, approximately 20% and 22.5% respectively for a 65% monomer concentration and a cross-link density of 15.5%. It is interesting to note that the amount of non-freezing water present in the macroporous system, under these conditions, is slightly higher than that shown by homogeneous system. One explanation for this could be that although the majority of the non-freezing water in a macroporous system is present in the gel network, there could be a small amount of water which is bound to the inner surfaces of the pores. As this difference is so small, more detailed work will need to be undertaken in order to ascertain whether there is a larger amount of bound water in a macroporous gel by investigating gels of differing cross-link densities and comparing the results obtained with homogeneous gels of similar cross-link density.

Comparing the freezing water contents of the macroporous system (65% monomer in the monomer/solvent mixture), with the homogeneous system at 15.5% cross-linking it is observed that the macroporous system has more freezing water present. The freezing water in a macroporous system would therefore seem to be present mainly in the pores. As the cross-link density is constant, the observed decrease in freezing and equilibrium water content can be accounted for by the fact that as the monomer present in the monomer/solvent mixture increases, the structure of the system becomes less porous and so the freezing water content decreases. This effect can be likened to the network effect of cross-link density on a homogeneous system in the initial stages of cross-linking (1 - 10%).

At low cross-link densities, in the region of 1%, there is some doubt as to the precise amounts of freezing and non-freezing water present in a homogeneous PHEMA system. In this region, the purity of the monomer used is of importance as any impurities present (eg methacrylic acid or ethylene glycol dimethacrylate) can lead to discrepancies in the actual cross-link density and hence alter the freezing and non-freezing water contents. In this respect, further work is necessary on PHEMA gels at low cross-link density, using rigorously purified monomer in order to elucidate more fully the water binding properties of gels under these conditions.

4.4 Some Mechanical Properties of Poly(2-hydroxyethyl methacrylate) Hydrogels

At this stage in the work it was convenient to carry out a preliminary investigation into the mechanical properties of PHEMA gels of various cross-link densities with respect to their water binding properties. The deformation behaviour of the hydrogels was examined using a microindentation apparatus and the results are summarised in Fig 30. This shows the load applied to the specimen plotted against the depth of indentation on a logarithmic basis. For comparative purposes it is convenient to measure the load required to produce a given indentation, in this case, 2 micron. Fig 31 shows the relationship between cross-link density and load to 2 micron indentation and Fig 32 the relationship between equilibrium water content and load to 2 micron indentation. As would be expected, the load to 2 micron indentation increases with increasing cross-link density as the material becomes more rigid (Fig 31) and decreases with increasing equilibrium water content as the plasticising effect of the water present becomes more apparent (Fig 32). Fig 33 shows the effect of freezing water content (corresponding to the cross-link densities of the gels) on the load to 2 micron deformation. As can be seen from this curve, the effect of freezing water is more pronounced in the initial stages (up to 5%) and then the curve becomes less severe with increasing freezing water content. This can be accounted for by the fact that the freezing water has a marked effect as a plasticiser in

Fig 30 Effect of cross-lining on the deformational behaviour of various homogeneous HEMA gels (log/log)

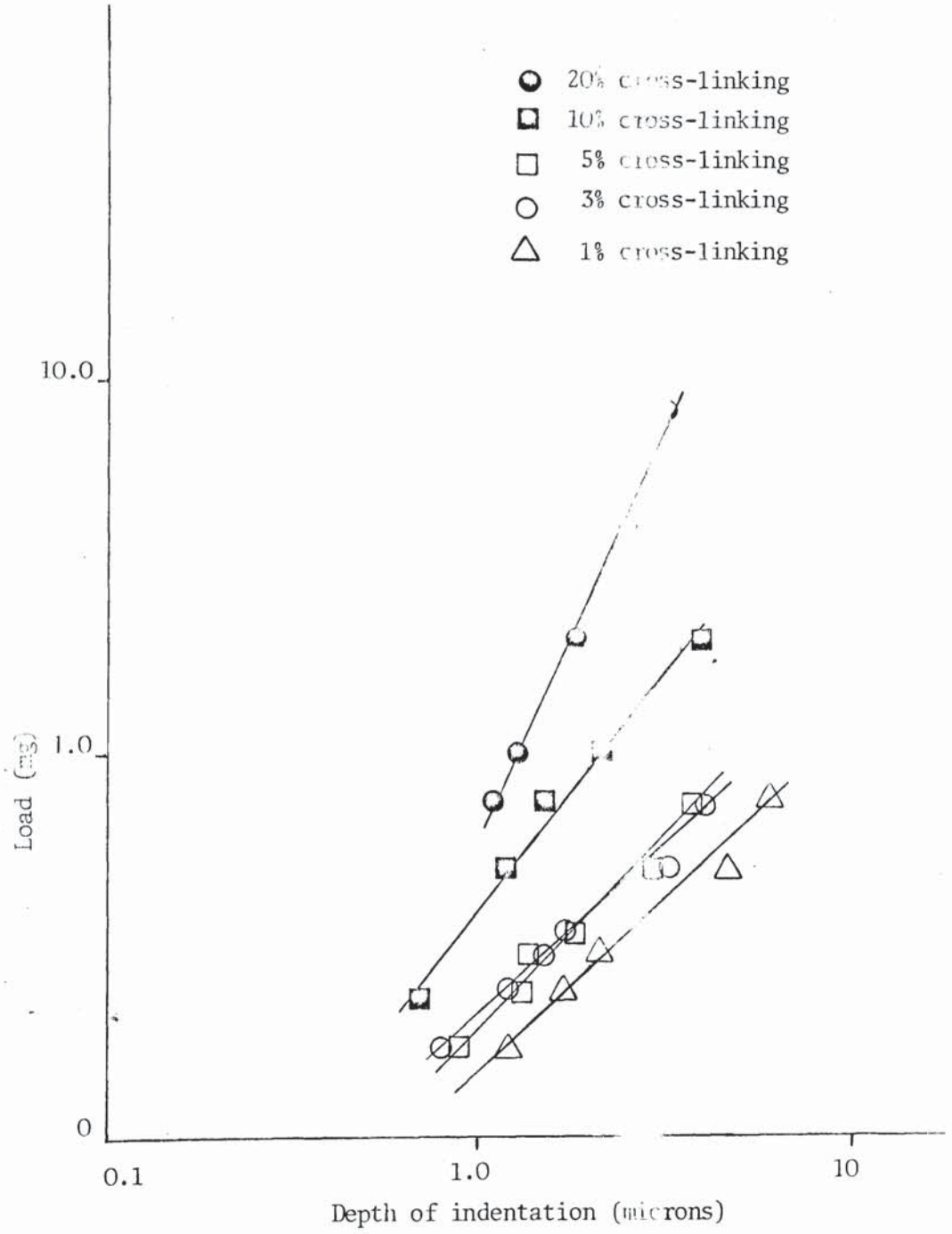


Fig 31 Effect of cross-linking on the load to 2 microns deformation of homogeneous HEMA gels

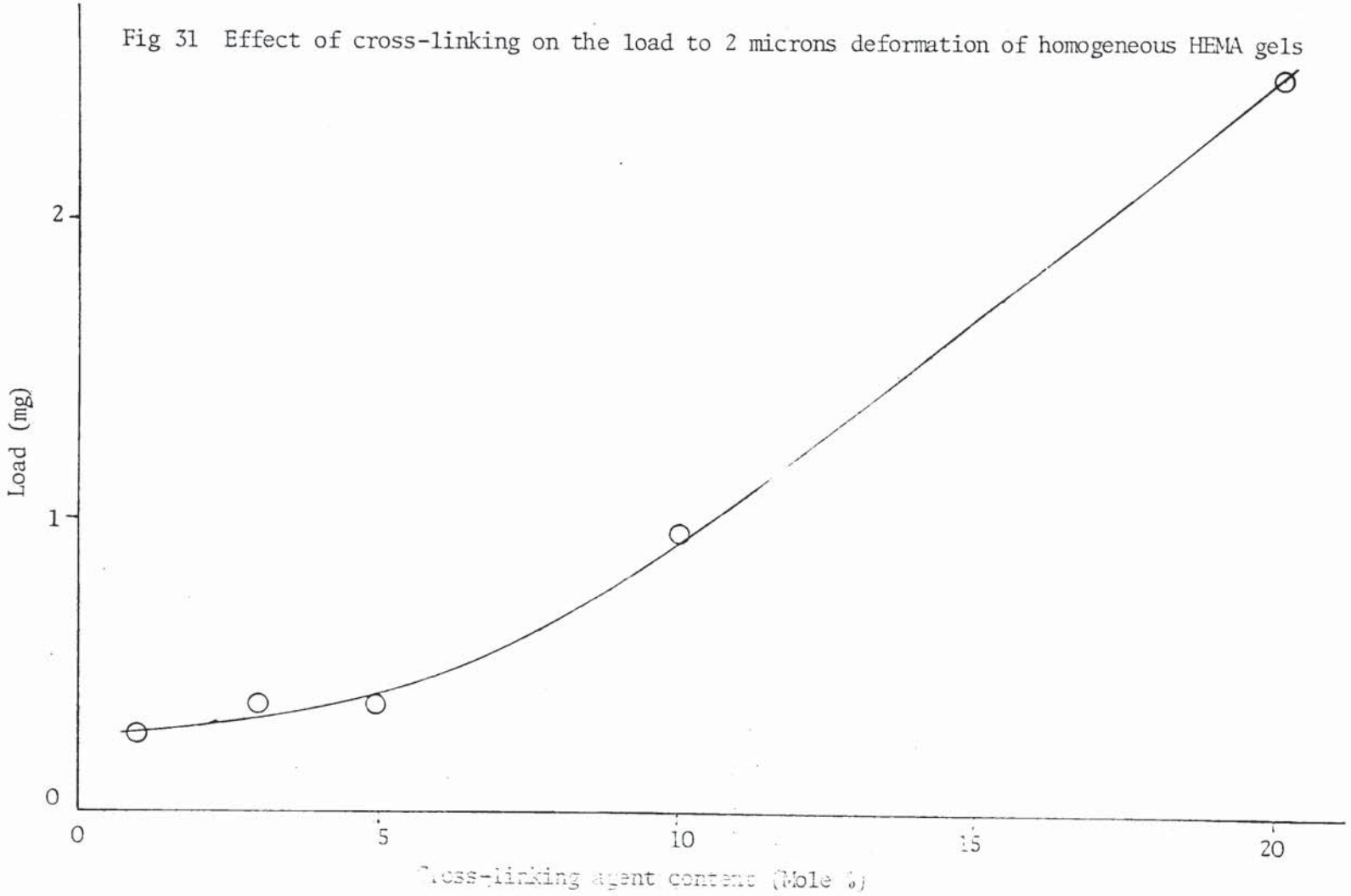


Fig 32 Effect of equilibrium water content on the load to 2 microns deformation of cross-linked homogeneous HEMA gels

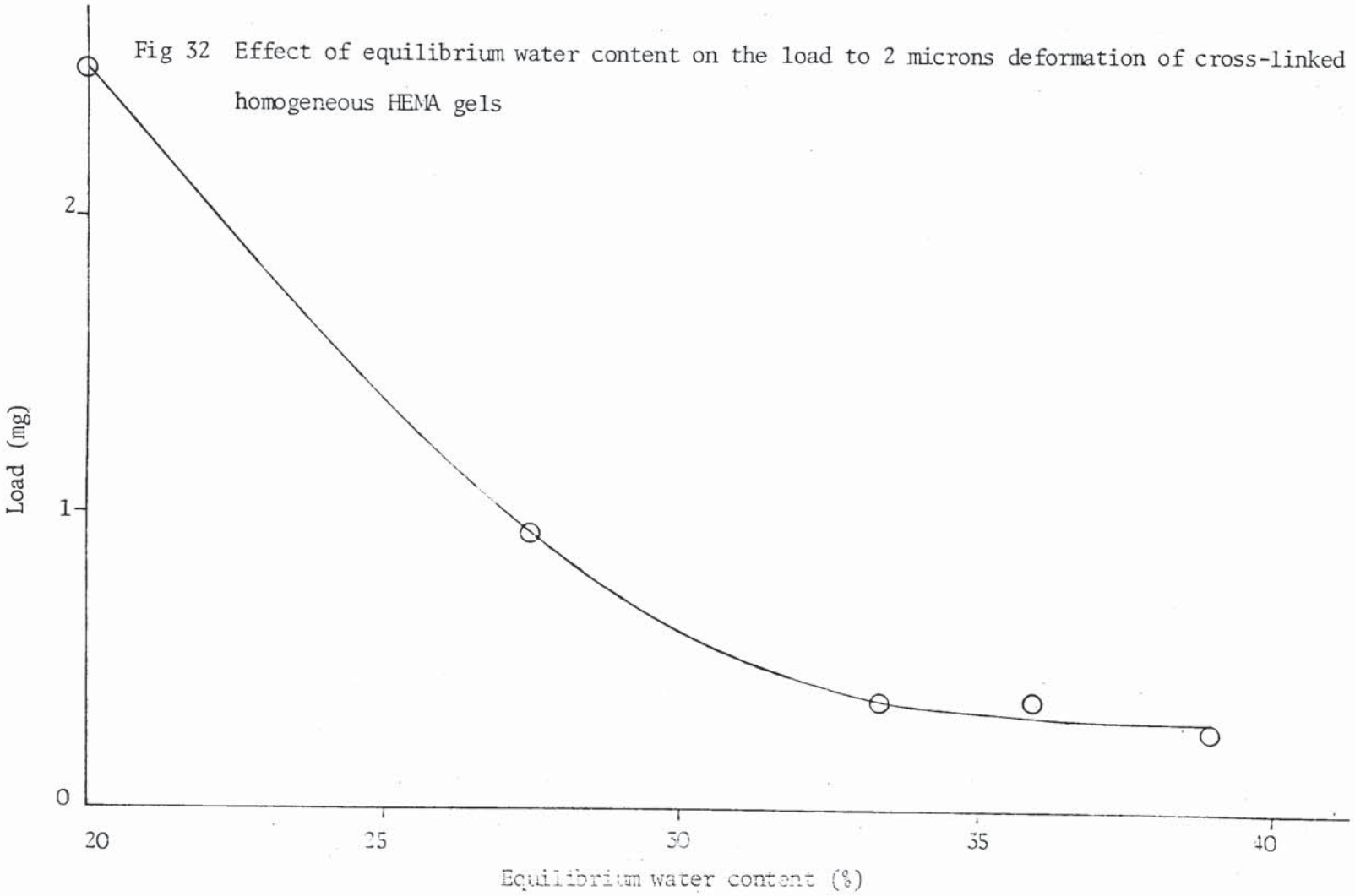
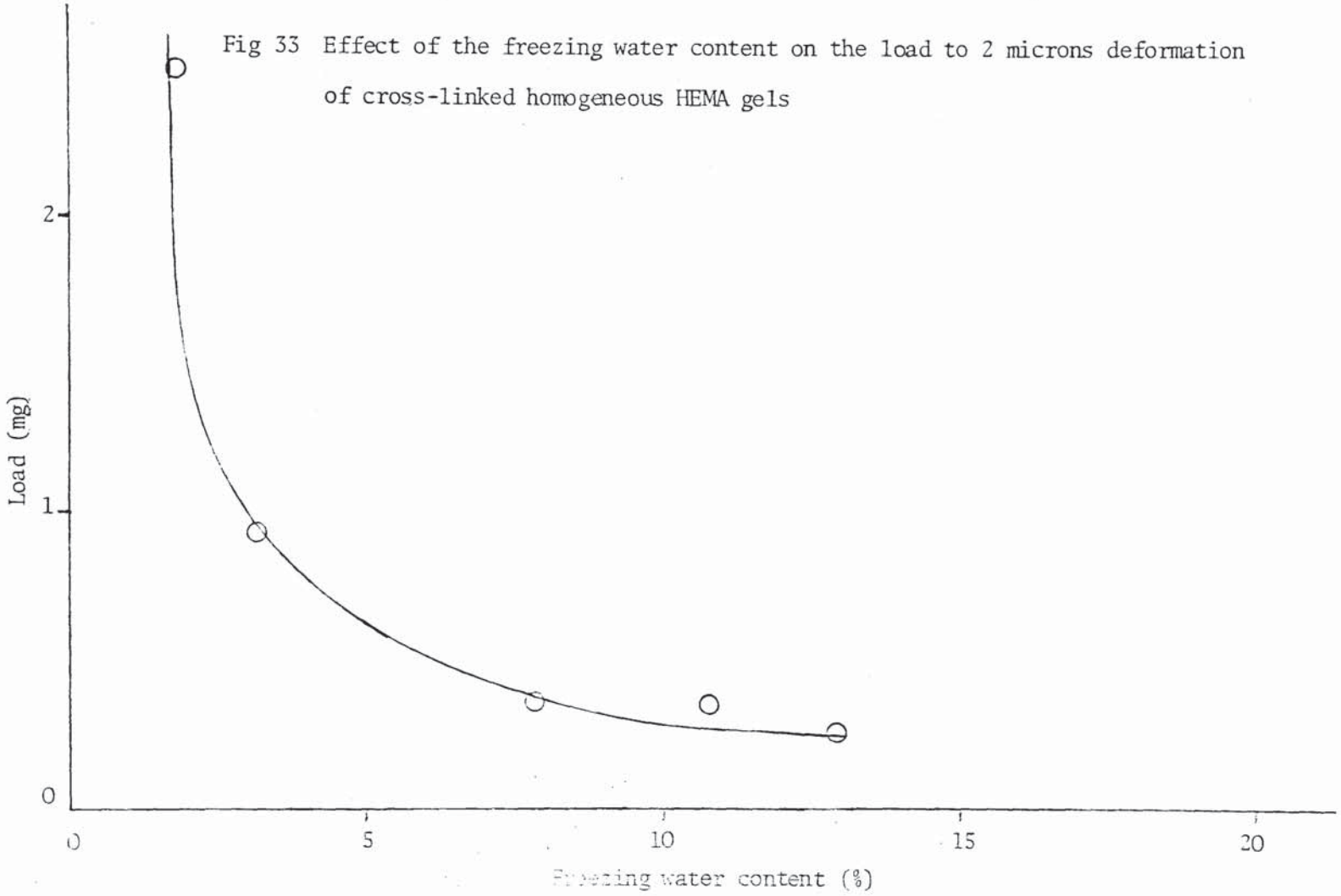


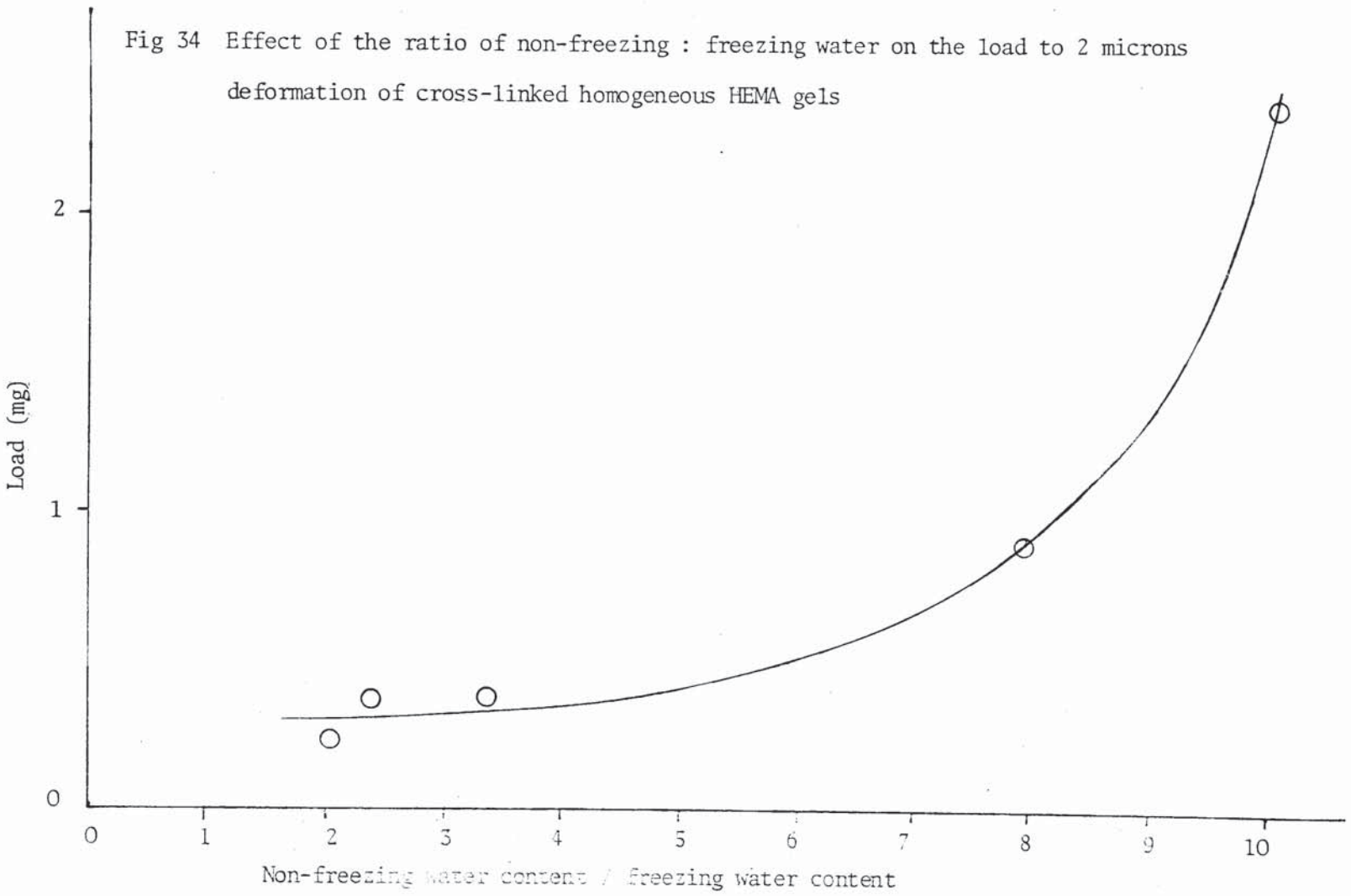
Fig 33 Effect of the freezing water content on the load to 2 microns deformation of cross-linked homogeneous HEMA gels



the initial stages causing the material to be more readily deformed. However, as the freezing water content increases, so does the non-freezing water content and therefore the overall water content of the system which would tend to compensate for the initial dramatic effect observed. Fig 34 shows the effect of the ratio of non-freezing to freezing water content on the load to 2 micron deformation. As this ratio increases, as a reuslt of increasing cross-link density, the load to 2 micron deformation increases, this can be attributed to the fact that as the amount of non-freezing water increases, there is less freezing water available to act as a plasticiser and the system becomes more rigid.

These results, although limited in their scope, provide an interesting basis for the understanding of the effect of cross-link density on the mechanical behaviour of PHEMA gels. The mechanical properties of the material can be related to the equilibrium water content in terms of the cross-link density. Also the water binding properties can be related to the mechanical behaviour of the system in terms of the amounts of freezing water and non-freezing water present. In order to determine the precise effect of cross-link density on the water binding properties and therefore the mechanical behaviour of these gels, it would be necessary to measure the cross-link density of the system after polymerisation as opposed to the amount of cross-linking agent added to the monomer feed this would be especially important at low cross-link densities where the purity of the monomer used would be an important factor.

Fig 34 Effect of the ratio of non-freezing : freezing water on the load to 2 microns deformation of cross-linked homogeneous HEMA gels



CHAPTER FIVE

MODIFICATION OF HYDROGEL POLYMERS: BACKBONE MODIFICATION AND AMINO ACID ADSORPTION

In this Chapter, the effect of backbone modification on the adsorption and release of amino acids is described. Backbone modification affords a direct and simple method of altering the water binding properties of hydrogels. Though this is a bulk property there is some feeling that such water binding may influence protein adsorption and therefore biocompatibility. The experiments described were designed to determine whether individual amino acids interact in different ways with hydrogel polymers having modified structures.

5.1 Adsorption and Interaction Processes

The development of hydrophilic polymeric materials for biomedical applications has brought not only the inherent advantages of this type of material over conventional hydrophobic polymers but also certain disadvantages. One such disadvantage of major importance associated with the prolonged contact of a synthetic material with biological systems is that of the formation of deposits on and within the expanded gel network. These deposits have been widely studied and have been found to be essentially proteinaceous in nature[91]. The problem of protein deposition on biomedical materials has been highlighted in the contact lens field although

the deposition of proteins is a well-known interfacial phenomenon which would seem to involve all situations where foreign bodies of both natural and synthetic origin are in contact with a proteinaceous environment.

The adsorption or deposition process of proteins has been shown to be similar to that of the adsorption of gas molecules onto a solid surface in which a mono layer of gaseous molecules is laid down in a manner described by Langmuir's adsorption isotherm. There are many examples in which materials come into contact with various biological solutions (eg blood, saliva, tears, sea water) and in so doing, demonstrate the phenomenon of protein adsorption. This primary adsorption process of proteins exerts a considerable influence over subsequent events in a given biological environment and gives rise to processes such as thrombus formation (in blood), the formation of dental plaque (in saliva) and marine fouling (in sea water). To date most studies of protein adsorption have been largely confined to the area of blood contact materials with particular reference to thromboresistance and biocompatibility.

It has been demonstrated that within a few seconds of first exposure to blood, all non-physiological materials acquire a rapidly thickening film of essentially pure protein (most likely fibrinogen), which on adsorption alters both the physical and chemical properties of the surface[92]. Studies carried out on this adsorption process lead to the theory that there are two types of adsorption, one that is hydrophilic, exothermic and

reversible and the other that is hydrophobic, endothermic and irreversible. The stability of proteins in solution depends upon them being able to retain their specific characteristic shapes, thus reversible adsorption takes place with no change in shape (conformation). Strong irreversible adsorption, however, involves some change in nature or conformation of the protein resulting in it being strongly bound to the surface. The precise conformational changes that occur on adsorption have not yet been fully resolved, although the configurational states and anchorage sites of adsorbed proteins have been shown to differ depending on the temperature, pH and concentration in solution. It appears that the principle of designing a surface to preferentially adsorb a particular protein from a given biological environment is a promising way of designing biocompatible surfaces for various applications.

In order to study one aspect of the adsorption process at hydrogel surfaces, amino acids were used as models for protein deposition. A series of hydrogel polymers were synthesised and the adsorption/desorption characteristics of the materials was studied with respect to the polymer composition and equilibrium water content.

5.2 Experimental Procedures

5.2.1 Polymer Synthesis

Polymer membranes were prepared using the method described in Chapter Two. In general the compositions were based on hydroxypropyl acrylate (HPA), methyl methacrylate and a third monomer. Details of copolymer compositions are given in Table 10 .

All the polymer compositions shown in Table 10 contained 1% by weight of ethylene glycol dimethacrylate (EDMA) as a cross-linking agent.

A further series of PHEMA hydrogels were synthesised using EDMA and trimethyl propane trimethacrylate as cross-linking agents in varying concentrations. All the membranes were hydrated in distilled water, which was frequently changed, for at least two weeks before any observations were undertaken.

5.2.2 Amino Acid Desorption Measurements

Hydrated samples of the hydrogels were soaked in a 0.5% solution of each of the amino acids shown below for at least a week.

Aspartic acid

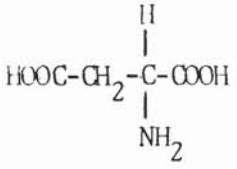
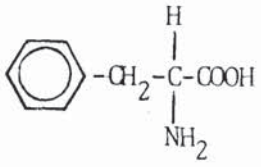


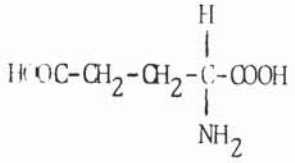
Table 10 Copolymer compositions

Monomer Mole %						
Hydroxypropyl acrylate	Methyl methacrylate	Acrylamide	Methacrylamide	N-Methylol acrylamide	Diacetone acrylamide	Styrene
80	10	10				
80	10		10			
80	10			10		
	10				10	
90	10					10

Phenyl alanine

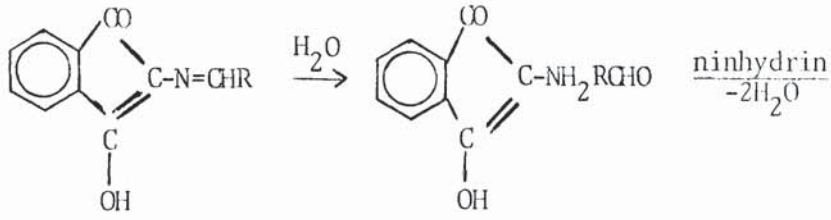


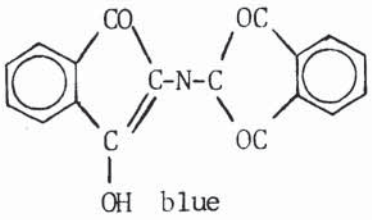
Glutamic acid



After allowing the samples to hydrate in the amino acid solution for at least one week, a rectangular sample of a size to fit the side of a spectrophotometer cell was cut from each material. The samples were then stained using a 0.5% solution of triketohydrindene hydrate (ninhydrin) which reacts on heating with α-amino acids to produce a blue coloration.

ninhydrin





blue

The initial optical density of the stained film was measured on a Higer Watts spectrophotometer at 579 nm, using the following procedure. The sample of material was blotted to remove excess moisture and placed on the inside face of a spectrometer cell, a reading of the optical density (arbitrary units) was then taken using distilled water as a standard. In order to follow the desorption of amino acid from the sample the following method was used. The polymer sample was placed in sufficient distilled water (5 ml) to fill a spectrometer cell and the desorption of amino acid was followed by measuring the increase in optical density of the solution as a function of time. Distilled water was again used as a standard. The equilibrium water contents of the polymers was determined using the method described in Chapter Two. All measurements were carried out at room temperature.

5.3 Results and Discussion

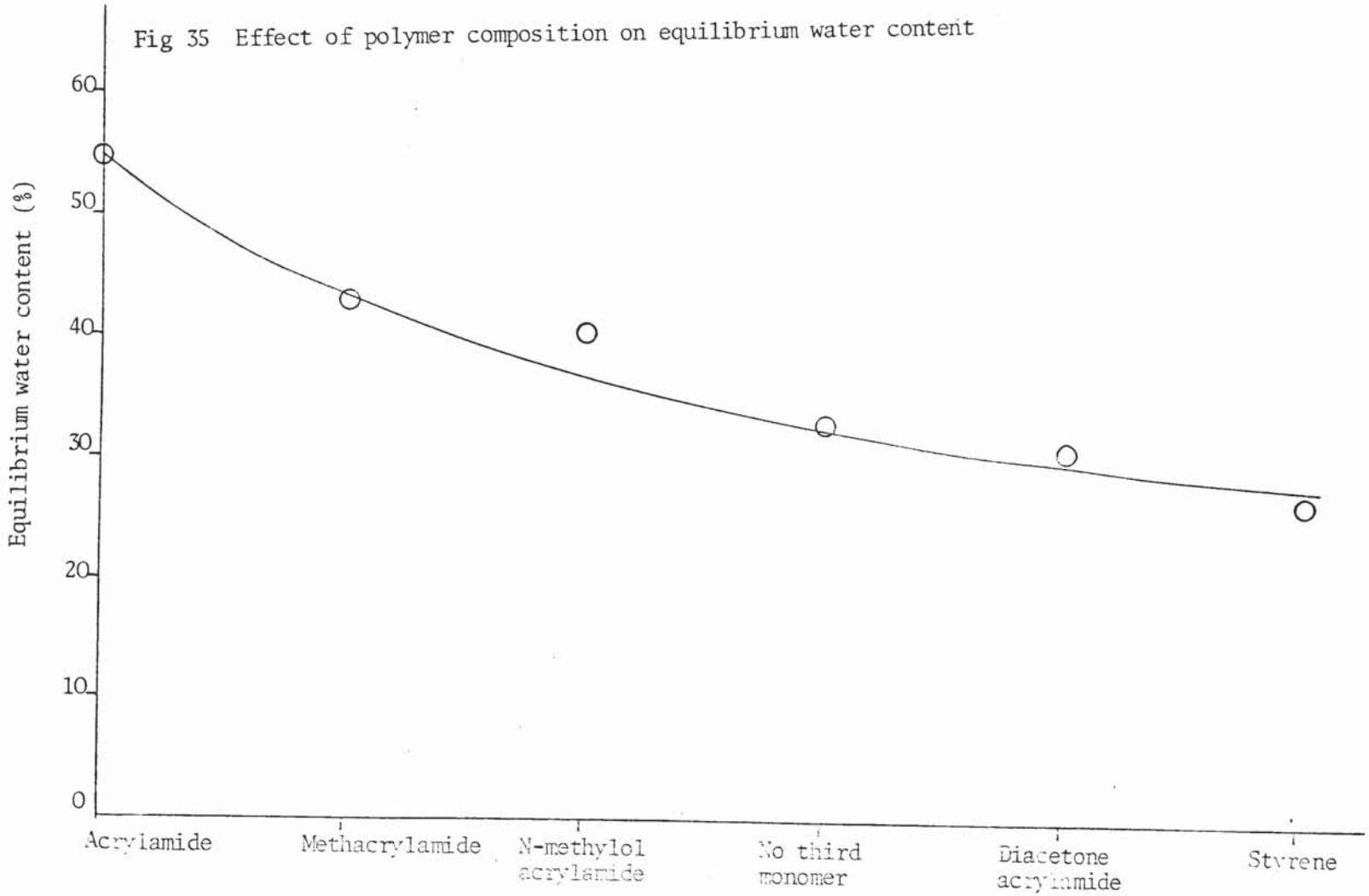
The effect of polymer composition on equilibrium water content is shown in Table 10 and Fig 35. As can be seen, the equilibrium water content of the copolymers decreases as follows:
No third monomer > acrylamide > methacrylamide > N-methylol acrylamide > diacetone acrylamide > styrene giving some indication as to the relative hydrophilicity of each monomer. It is the relationship between the water binding properties and the rate of amino acid desorption that is of primary interest.

The results of the optical density measurements are shown in

Table 11 Effect of polymer composition on equilibrium water content

Third Monomer	Equilibrium Water Content (%)
Acrylamide	55.0
Methacrylamide	43.0
N-methylol acrylamide	40.5
Diacetone acrylamide	31.0
No third monomer	33.0
Styrene	27.0

Fig 35 Effect of polymer composition on equilibrium water content



Tables 12 and 13. Figure 36 shows the way in which the optical density at a given time expressed as shown varies as a function of time. All the amino acids used show similar desorption characteristics. From the graph of optical density versus time, it is apparent that there are marked differences in the rate of desorption between the copolymers containing acrylamide type monomers and those with either no third monomer or styrene as the third monomer. All the polymers containing acrylamide type monomers show a desorption profile similar to that shown for the acrylamide containing copolymer whereas the styrene containing polymer and the polymer with no third monomer show markedly more rapid rates of desorption. Before considering the reasons for these differences, it is useful to note two factors concerning the styrene containing polymer. Firstly, the equilibrium water content of the styrene polymer is considerably less than that of the acrylamide type copolymers and secondly, as discussed in a prior Chapter, the amount of free water in the styrene polymer is very much less than that of the acrylamide type polymers.

The reasons for the differing rates of desorption would appear to be twofold. Firstly, the amino acid is probably absorbed to a certain degree by the hydrogel and this absorption would be more apparent in a hydrogel with a higher water content as in the case of acrylamide containing polymers. This however does not afford a total explanation as the difference in total water content between a copolymer containing diacetone acrylamide and a

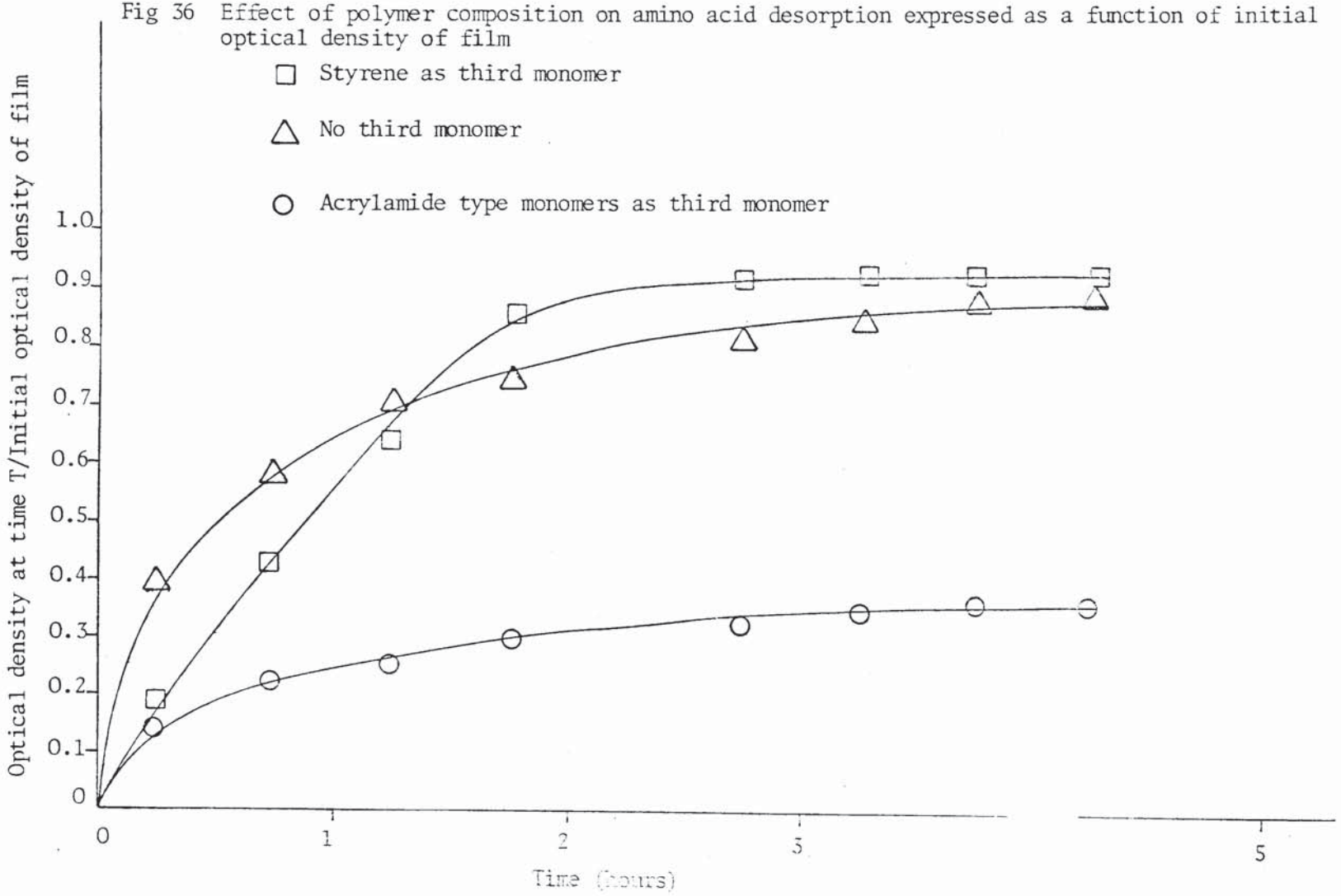
Table 12 Effect of polymer composition on amino acid desorption

Third Monomer	Initial Optical Density of Film	Optical Density of Solution After Time in Hours							
		0.25	0.75	1.25	1.75	2.75	3.25	3.75	4.25
Acrylamide	0.91	0.13	0.20	0.23	0.27	0.30	0.32	0.33	0.33
Methacrylamide									
N-methylol acrylamide	1.69	0.24	0.35	0.42	0.45	0.46	0.48	0.50	0.50
Diacetone acrylamide	1.23	0.16	0.25	0.31	[illegible]	[illegible]	0.43	0.49	0.49
None	0.57	0.22	0.33	[illegible]	[illegible]		0.48	0.51	0.51
Styrene	0.14	0.04	0.06	0.09	0.12	0.13	0.16	0.21	0.21

Table 13 Effect of polymer composition on amino acid desorption expressed as a function of initial optical density of film

Third Monomer	Initial Optical Density of Film	Optical Density at Time T/Initial Optical Density of Film							
		0.25	0.75	1.25	1.75	2.75	3.25	3.75	4.25
Acrylamide	0.91	0.14	0.22	0.25	0.30	0.33	0.35	0.36	0.36
Methacrylamide									
N-methylol acrylamide	1.69	0.14	0.21	0.25	0.27	0.27	0.28	0.30	0.30
Diacetone acrylamide	1.23	0.13	0.20	0.25	0.28	0.33	0.35	0.40	0.40
None	0.57	0.39	0.58	0.70	0.75	0.82	0.84	0.89	0.89
Styrene	0.14	0.29	0.43	0.64	0.86	0.93	0.94	0.94	0.94

Fig 36 Effect of polymer composition on amino acid desorption expressed as a function of initial optical density of film



copolymer containing styrene is only 4% being 31% as opposed to 27%. Secondly and more importantly, there is the question of interaction between the amino acid and the polymer resulting in hydrogen bonding of the amino acid to the acrylamide containing polymer. This interaction appears to be present even in cases such as the one indicated above in which the total water content of the gels is not appreciably different. The difference being not in the total water content but in the free water content of the gel. It would seem that the interactions of the amino acid are more apparent in polymers where although the equilibrium water contents may be similar the amount of free water is markedly different. Interactions appear to be strong in a polymer containing acrylamide for instance because the free (freezing) water content is appreciably higher than that of a polymer containing styrene. Relatively small amounts of styrene are required to alter the amount of freezing water present in a polymer and this phenomenon has been discussed in a previous Chapter. Although a series of copolymers using both EDMA and TMPTMA, in various concentrations, was prepared, there seemed to be no firm relationship between the amount and functionality of the cross-linking agent present and the rate of amino acid desorption. This would tend to suggest that the trends observed are predominantly a result of backbone modification of the polymer.

In conclusion, an examination of the results obtained would tend to indicate that the water binding properties of the hydrogels

exert an effect on the adsorption/desorption rates of the amino acids studied. The use of individual amino acids as models for more complex protein molecules would appear to be a useful approach in attempting to relate the chemical structure, by its effect on the water binding properties to the surface behaviour as observed by amino acid desorption. This relationship being of particular interest because of the effect of surface properties of a hydrogel on its biocompatibility.

CHAPTER SIX

MODIFICATION OF HYDROGEL POLYMERS: THE SYNTHESIS OF GLYCOPROTEIN ANALOGUES

Hydrogel polymers by virtue of their inherent water content tend to exhibit a certain degree of biocompatibility. There is, however, always a tissue response to a polymeric implant even in the case when the material is chemically inert. It is, therefore, important in the design of materials for implant applications that any tissue response is kept to a minimum.

A foreign body response can be defined as any response of the host tissue that results from the introduction of an alien material to the tissue environment[31]. Foreign body reactions can conveniently be divided into three categories:

(i) Reactions due to physical characteristics of the implanted material, for example epithelial encapsulation of the polymer and thickening of the connective tissue fibrous capsule.

(ii) Reactions due to the chemical properties of the implant for example epithelial hypertrophy and connective tissue inflammation.

(iii) Immune responses for example the invasion of the epithelium

by leucocytes and increased inflammatory tissue.

The effects of plastic implants in pigs, dogs and one human volunteer have been investigated by Ocumpaugh and Lee[31]. In this study, two types of implant were used, these were implants which penetrated through the skin, ie percutaneous and implants placed wholly below the skin, ie subcutaneous. Such implanted devices were made from graphite, Teflon, polyurethane and etched annealed and untreated Teflon. Percutaneous implants consisted of a disc-shaped flange inserted just below the skin with a projecting conduit through the skin. Subcutaneous implants were generally 'dogbone' shaped bars. The human volunteer received a subcutaneous flange shaped like a button.

Reactions of the type first described above, due to physical characteristics of the implanted material, were generally found to be associated with implants of Teflon, polyurethane and graphite. Epithelial encapsulation was found in implants which did not have steep angles were made of relatively inert materials and were free of infection. Excessive formation of keratin, usually found in response to pressure, was found at the angle between the conduit and the skirt. A fibrous capsule was formed around the implant and was found to be thicker at the conduit-skirt junctions. At sharp angled tips of the subcutaneous bars both glycoprotein and mucopolysaccharide substances were to be more prevalent than near other surfaces of the implant. Giant cells

were found to be formed at irregular surfaces of implants and at the convex surface of the human implant, the reason for this cell formation is not known. There appeared to be no inflammation, infection or necrosis, which would be indicative of a chemical reaction.

Adverse conditions due to the chemical properties of the implant were associated with polyelectrolyte coated plastics and certain epoxy resins which produced an inflammatory reaction. Signs of necrosis were also found to be present and fibrous capsules when present were outside the necrotic regions.

Reactions due to immune responses were found to be associated with implants which had projecting shafts through the skin. These implants produced chronic signs of infection at the skin-shaft interface. The reactions produced under these circumstances seem to have been due to the absence of sterile conditions and not due to the physical or chemical nature of the polymer.

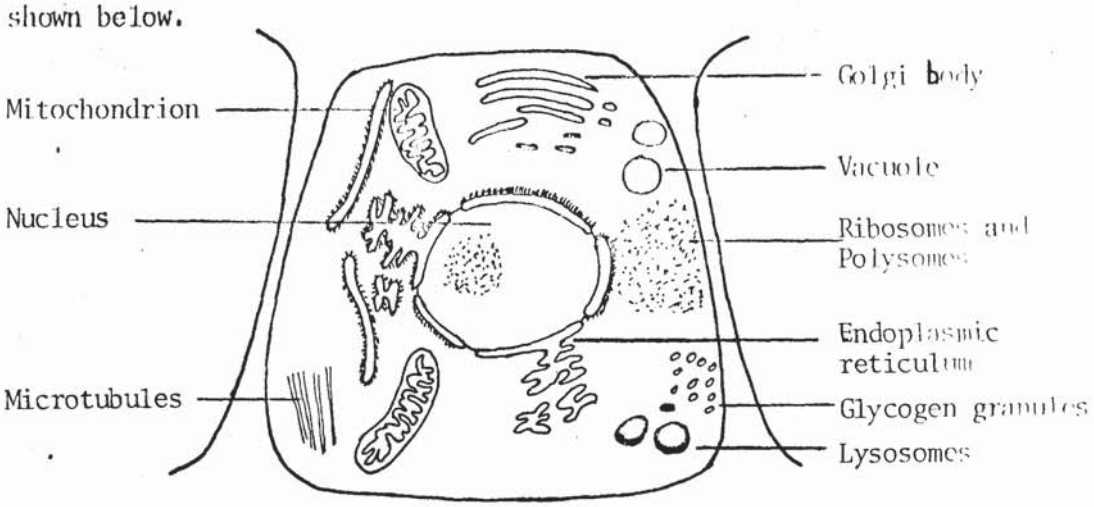
From the above account of some of the reactions invoked by the implantation of conventional polymers into a physiological environment, it can be appreciated that the situation is open to improvement. Hydrogel polymers exhibit biocompatibility which is a considerable improvement on results achieved with hydrophobic materials. Although hydrogel polymers exhibit a limited degree of biocompatibility, the situation is far from ideal, it is therefore desirable to modify hydrogel polymers to enhance their

inherent biocompatibility.

Polymers for use in biomedical applications require a variety of specific properties in order to be of value in a physiological environment. A useful approach to the problem of biocompatibility would be to appraise the reactions not only between a hydrogel and a surrounding environment but also between the cells in the environment which form the basis for any successful biomedical application. Such interactions include cell aggregation and differentiation and a polymer containing components capable of mimicking these processes would be useful in a biomedical situation.

6.1 Factors Involved in Intercellular Processes

Cells are those units of protoplasm which are controlled by a single nucleus and the boundaries of which are limited by a cytoplasmic membrane[93,94]. Most cells are in the order of 10 μ in diameter and the organisation of a typical cell is shown below.



Intercellular interactions would seem to involve only the membrane surface[95] and it is therefore apparent that this surface is of primary interest in cell-cell interactions. It has been observed that cells of different types when randomly mixed will move about, recognise each other and group selectively, but some completely different types of cells will aggregate together. This would tend to suggest that the adhesive mechanisms may be similar for different cell types with subtle differences within the cell membrane deciding as to whether or not different cells will aggregate.

Muscle proteins (actin and myosin) have been found in a variety of cells and are thought to be responsible for cell mobility and other movements within the cell. The large shape changes shown by platelets before aggregation are thought to depend on actin and myosin combining in solution to increase the amount of functional actomyosin. It has recently been confirmed that both actin and myosin are present in cell membranes and their presence would explain movements of the cell surface.

Three main lines of research have demonstrated the presence of glycosubstances at cell surfaces, these are the electrophoresis of intact cells, immunological studies and microscopical investigations using appropriate staining techniques. It has been proposed that the initial adhesion of cells could involve either a glycoprotein-enzyme interaction or a glycoprotein-glycoprotein interaction[95]. The glycoprotein-enzyme

interactions would involve glycosyltransferase (enzyme) on the surface of one cell attaching to a protruding carbohydrate on the surface of another cell. This glycoprotein-enzyme type of interaction would explain the specificity of many aggregation processes but due to the fact that such a process would involve transfer of a sugar unit to the glycoprotein, cleavage of the enzyme glycoprotein bond would follow on completion of the carbohydrate chain. Therefore such a process does not fully explain the formation of permanent adhesion on aggregation.

An alternative mechanism to that of the enzyme route is that adhesion proceeds through a glycoprotein glycoprotein interaction[95]. Glycoproteins on the surfaces of adjacent cells could form hydrogen bonds and the overall strength of interaction would depend on the number of hydrogen bonds. Similar glycoproteins would be expected to have a large number of intermolecular hydrogen bonds and would interact strongly while structurally dissimilar glycoproteins would have fewer hydrogen bonds and weaker interactions.

Available evidence, therefore, suggests that both specific and non-specific aggregation involve bonding between the branched glycoprotein residues found at the cell surface and that the arrangement of carbohydrates within the heterosaccharide chains governs the adhesive specificity on aggregation. The exact mechanism of aggregation is not known but could involve either a glycoprotein-enzyme interaction or glycoprotein-glycoprotein

interaction, in which case specificity of aggregation may depend on the ease of formation of hydrogen bonds between the polysaccharide chains.

6.1.1 Assessment of Biocompatibility

Due to the fact that a hydrogel in a biomedical application is in contact with a living system, the biological properties of the hydrogel are extremely important. Terms such as 'biocompatible' and 'non-thrombogenic' are often confused in the description of biological responses to a foreign material. Bruck has itemised a number of factors which can be detrimental to the long term performance of a biomedical material[3]. Based on his description, the ideal biomaterial could be defined as one which does not cause thrombosis, destruction of cells, alteration of plasma proteins, destruction of enzymes, depletion of electrolytes, adverse immune reactions, damage to adjacent tissue, cancer and toxic or allergic reactions. No synthetic material developed to date has been shown to fully satisfy all these conditions. One of the other problems encountered when discussing biological responses to materials is that there is no single test method available which is capable of evaluating all the above factors. Several methods can be used in the evaluation and some are discussed below.

'In vitro' coagulation assays: The Lee White test compares the coagulation time of whole blood in a test tube made or coated

with the material under test, with the coagulation time of blood in a control tube (usually made of glass). Several variables can affect the results obtained from this technique and these include changing the donor, storage time of the blood and in the experimental technique used to measure the clotting times.

Vena Cava ring test: This method involves the implantation of streamlined rings made of or coated with the material under test into the vana cava of dogs, usually for 2 hours or 2 week test periods. The materials are then examined for thrombus formation and can be described in terms of the time taken for thrombus formation. This test does not distinguish between materials that are truly non-thrombogenic and those which cause thrombus formation but are non-thromboadherent[96].

Renal embolus ring test: Rings of the material under test are implanted in the canine descenting aorta just above the renal arteries. A construction is made in the aorta below the renal arteries to force a large fraction of the blood flowing through the test ring into the kidneys. After a period of implantation, the rings are examined for adhering thrombi and the kidneys are dissected and examined for infarcts presumably caused by thrombi originating from the test ting surface. Thus this test should be able to distinguish between materials which are truly non-thrombogenic and those which are only non-thromboadherent. Almost all materials tested to date have shown some kidney damage to the test animal.

Soft tissue compatibility tests: These tests are used to evaluate response to a material implanted in soft tissue areas, ie not in direct contact with the bloodstream. Autian has suggested in a literature review[97] that intramuscular implantation may be the most sensitive site for evaluation of tissue response. In general, there is no standardised test procedure for soft tissue compatibility tests but a material can be said to be 'tissue compatible' if on implantation it shows a normal acute inflammation reaction and then rapidly 'heals in' to a passive state.

6.2 Synthetic Routes to Glycoprotein Analogues

In order to prepare a polymer with side chains containing thiol groups, which can then be reacted with sugar molecules, it is necessary to appraise two factors. Firstly, the available methods for preparing polymeric molecules containing thiol groups within the side chain and secondly the preparative routes to 1-thioglycosides which could be applied to this situation.

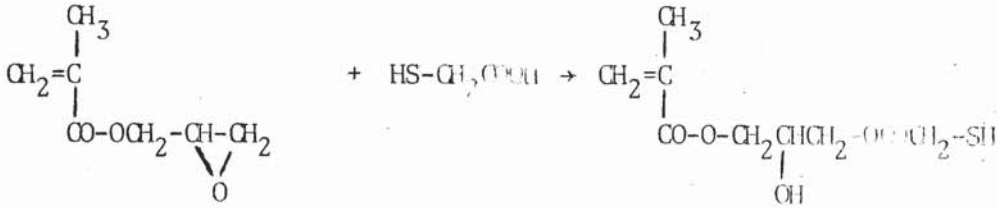
6.2.1 Preparation of Polymers Containing Thiol Groups on Side Chains

It is possible to produce a side chain containing a thiol group by carrying out reactions with active groups, such as -OH groups present either in the monomer or the polymer chain. In the case where the thiol group is attached to the monomer, the modified monomer must then be polymerised or copolymerised to form thiol

containing polymers. Two routes for the insertion of thiol groups have been examined.

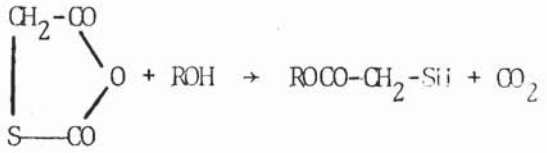
Reaction of glycidyl methacrylate with α-thio acids

Thioglycollic acid has been shown to react with glycidyl methacrylate as shown below[98].

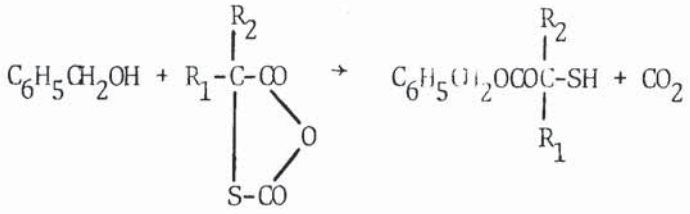


A similar reaction has been carried out using a copolymer of methyl methacrylate and glycidyl methacrylate. This copolymer was prepared in bulk at 80°C using benzoyl peroxide as a free radical initiator. Ten parts of redistilled thioglycollic acid were added to ten parts of polymer dissolved in 150 parts of chlorobenzene. The mixture was flushed with nitrogen and kept at 100°C for 30 hours. The polymer was then precipitated by slow addition to cold nitrogen flushed hexane. The spacing of the thiol groups on the polymer backbone could be varied by altering the proportions of α-thio acid, or by changing the composition of the copolymer.

6.2.2 Reaction of Sulphur Containing Heterocycles with Vinyl Monomers Containing -OH Groups



This type of reaction has been found to occur with a number of different sulphur containing heterocycles[99].

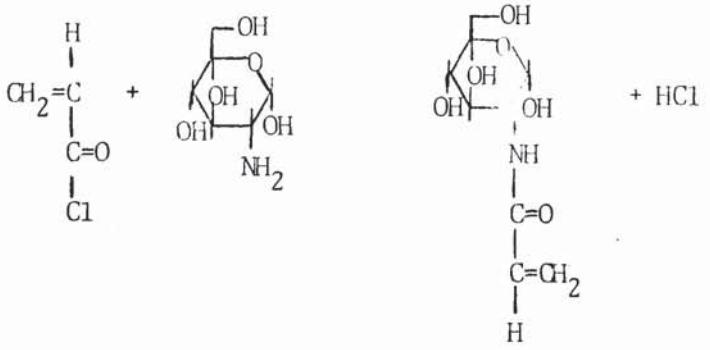


where: $R_1 = R_2 = H$

$R_1 = R_2 = CH_3$

$R_1 = H,\ R_2 = CH_3$

From the above two illustrations it is apparent that there are a number of available methods for attaching a thiol group either to a polymer chain or to a vinyl monomer. Another possible route which was considered was the use of acryloyl chloride, a vinyl monomer containing an acid chloride functional group. It was thought that the acid chloride group in acryloyl chloride could be reacted with the amino group on a sugar type molecule such as glucosamine.

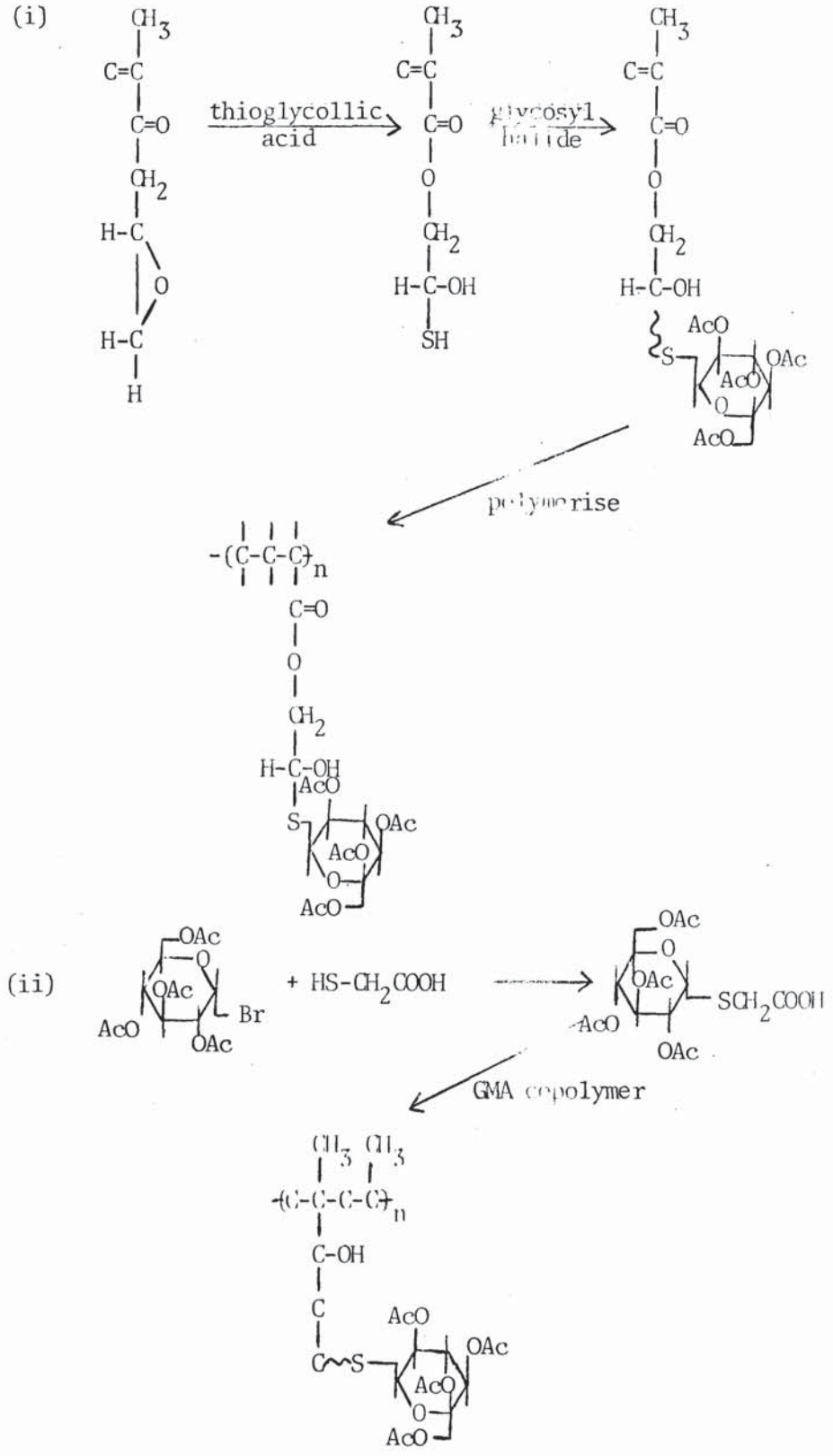


During the course of this work, two possible routes to the modification of hydrogels have been studied. Firstly reactions involving thioglycollic acid with glycidyl methacrylate to produce either a reactive monomer or a polymer with reactive sites. In both these cases, the product could be reacted with a sugar type molecule and in the case of the monomer it could subsequently be polymerised. Secondly, the reactions involving the use of acryloyl chloride which could be reacted with a molecule such as glucosamine to produce a monomer with a sugar residue attached.

6.3 Experimental Procedures and Results

6.3.1 Reactions Involving Thioglycollic Acid

In this section, reactions of the types shown below are described.


(i)
thioglycollic acid
glycosyl halide
polymerise
(ii)
+ HS-CH2COOH
GMA copolymer

Reaction of thioglycollic acid with glycidyl methacrylate

Glycidyl methacrylate 14.2 g (0.1 Moles) and thioglycollic acid 0.92 g (0.1 Moles) were mixed at room temperature and allowed to react for 24 hours. The clear solution became opaque forming a solid. This was washed and shaken with dry acetone to produce a white powdery solid.

Reaction of thioglycollic acid with pentacetylglucose

Pentaacetylglucose (2.50 g) was dissolved in 12.5 ml of thioglycollic acid containing anhydrous zinc chloride. The reaction was carried out in a refrigerator for 12 hours. After this time no solid had formed and ether was added to precipitate a sticky white solid.

Reaction of thioglycollic acid with acetobromoglucose

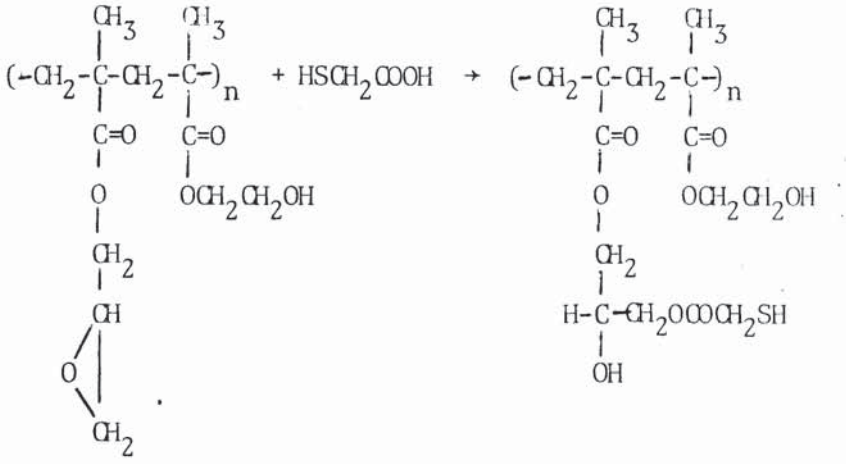
Acetobromoglucose (0.7882 g) and silver carbonate were added to 2 ml of thioglycollic acid and shaken for 1 hour. The mixture was warmed and filtered and washed with dry acetone. The acetone solution was concentrated and allowed to crystallise in a refrigerator.

The above reactions represent attempts to react thioglycollic acid with glycidyl methacrylate and with two glycosyl compounds. Another line of investigation would be to prepare a thiol

polymer and then to react this polymer with glycosyl compounds.

Reaction of a HEMA/GMA copolymer with thioglycollic acid

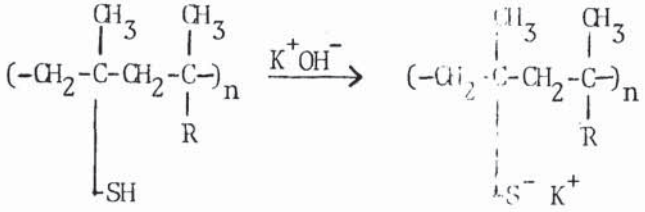
The reaction shown below was carried out with a 50 : 50 HEMA/GMA copolymer in membrane form which was described by the method in Chapter 2.



A piece of the copolymer membrane was placed in a small flask containing 15 ml of chlorobenzene. Thioglycollic acid was added and the mixture was heated under reflux conditions at 100°C for 30 hours. During the course of the reaction, the polymer was observed to swell. The solution was decanted and the polymer washed with acetone and then dried in a vacuum oven at 60°C.

Reaction of the thiol polymer with potassium hydroxide

It was apparent that the product from the above reaction contained thiol groups. In order to react these groups with monosaccharide molecules it is necessary to convert the thiol groups to sulphide groups.



A piece of the thiol polymer membrane was added to a 0.1 M solution of potassium hydroxide in methanol. On stirring the polymer broke up and both the polymer and the solution became yellow. The solution was decanted off and the polymer was washed with methanol and dried in a vacuum oven. It was apparent that decomposition of the polymer had occurred. The reaction was repeated, the polymer being added to a 0.1 M solution of potassium hydroxide in water. In this case the polymer did not break up and the solution remained clear. The polymer was removed and dried in a vacuum oven.

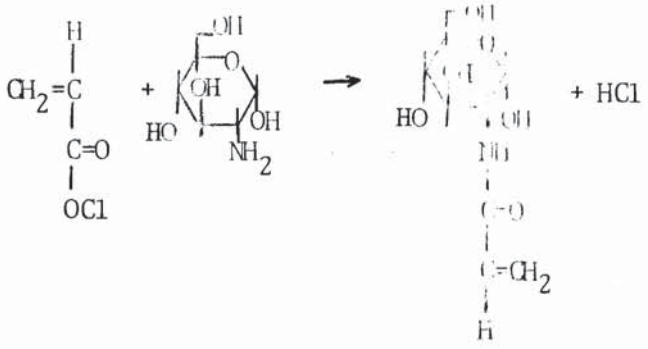
Reactions of the thiol polymer with various glycosyl compounds

The reaction of thioglycollic acid with the 50 : 50 GMA/HEMA copolymer has apparently produced a polymer containing thiol

groups. Table 14 summarises the reactions of the thiol polymers with some glycosyl compounds.

6.3.2 Reactions Involving Acryloyl Chloride

As mentioned previously, it is possible that acryloyl chloride could react in the manner shown below.



Preparation of glucosamine

Glucosamine hydrochloride was converted to glucosamine by reaction with diethylamine at room temperature in ethanol. The diethylamine hydrochloride formed was soluble in ethanol and so easily separable from the glucosamine which remains insoluble in ethanol. The product was washed thoroughly with ethanol and dried at room temperature in a vacuum oven. Glucosamine decomposes at 102°C and has a melting point of 104-105°C.

Table 14 Reactions of thiol polymers with various glycosyl compounds

Reaction	Weight of Polymer (g)		Comments
	Before Reaction	After Reaction	
Polymer + acetobromo glucose (ABG) in acetone for 30 h (with Ag_2CO_3)	0.0689	0.070	No change in thiol peak intensity in ir spectrum (2560 cm^{-1})
Polymer + ABG reflux in toluene for 5 days (with Ag_2CO_3)	0.0413	0.0441	No change in thiol peak intensity in ir
Polymer + ABG reflux in toluene for 30 h (with Ag_2CO_3)	0.0473	0.0466	No change in thiol peak intensity in ir
Polymer + ABG in acetone at 0°C for 5 days (with Ag_2CO_3)	0.0617	0.0641	No change in thiol peak intensity in ir

Table continued ...

Table 14 Continued

Polymer + ABG in pyridine for 30 h at room temperature (with Ag_2CO_3)	0.0476	0.0428	Slight decrease in thiol intensity in ir. Polymer broke up
Polymer + ABG in toluene for 5 days at room temperature (without Ag_2CO_3)	0.0646	0.0634	No change in thiol peak intensity in ir. Polymer broke up
Potassium salt of polymer + ABG in acetone for 30 h at room temperature	0.105	0.081	Thiol peak absent in ir of K + salt, present in ir of product
Polymer + pentaacetyl glucose + $ZnCl_2$ in acetone for 24 h at room temperature	0.0431	0.0492	No change in thiol peak intensity in ir
Polymer + pentaacetyl glucose + $ZnCl_2$ in acetone for 2 Weeks at room temperature	0.0375	0.0422	No change in thiol peak intensity in ir

Table Continued ...

Table 14 Continued

Polymer + pentaacetyl glucose + $ZnCl_2$ in THF for 24 h at room temperature	0.055	0.055	No change in thiol peak intensity in ir. Polymer broke up
Polymer + pentaacetyl glucose + $ZnCl_2$ in CCl_4 for 24 h at room temperature	0.062	0.060	No change in thiol peak intensity in ir. Polymer became discoloured and broke up
Polymer + pentaacetyl glucose + $ZnCl_2$ refluxed in DMF for 24 h	0.041	0.047	No change in thiol peak intensity in ir. Polymer broke up

Reactions of glucosamine and acryloyl chloride

Reactions between glucosamine and acryloyl chloride were carried our under various conditions. Some typical examples being shown in Table 15.

In all cases, the yield obtained was low and the products were sensitive to moisture and difficult to isolate.

6.4 Attempted Synthesis of Glycoprotein Analogues: Discussion

Reaction between thioglycollic acid and glycidyl methacrylate

This reaction was intented to produce a monomer containing a thiol group to which a sugar molecule could be attached and the monomer produced could then be copolymerised as required.

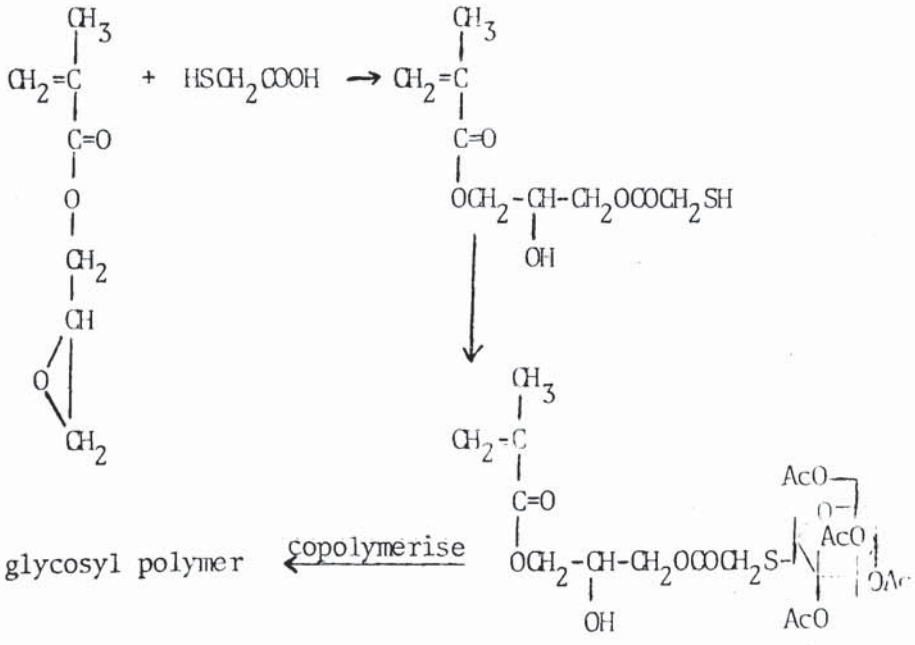


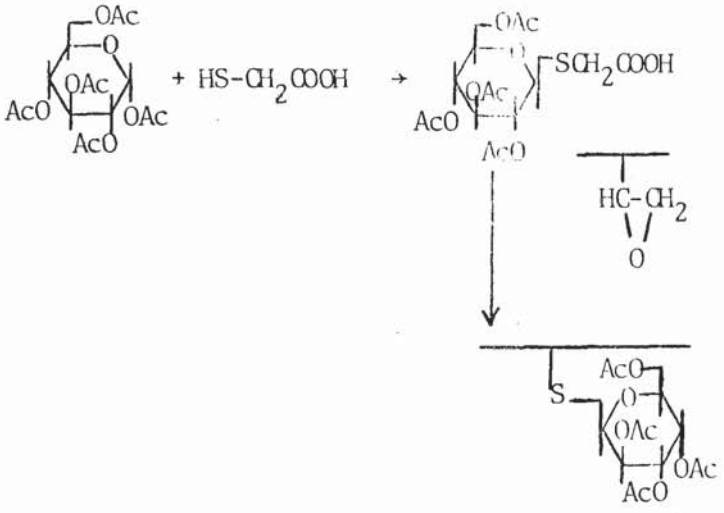
Table 15 Examples of reactions between acryloyl chloride and glucosamine

Reaction	Comments
Acryloyl chloride + glucosamine in DMF at room temperature	Glucosamine insoluble in DMF. On addition of acryloyl chloride the glucosamine appeared to dissolve. While precipitate appears on addition of mixture to acetone (non solvent). Product difficult to isolate.
Acryloyl chloride + glucosamine in DMF at 60°C	As above but with increased discolouration (brown) of reaction mixture
Acryloyl chloride + glucosamine in hot methanol	Gives a brown solution. Precipitate appears on addition to ether (non-solvent). Product difficult to isolate

The above reaction however did not take place, instead polymerisation occurred with thioglycollic acid being incorporated in the product. The product does not appear to contain thiol groups and it therefore seems probable that radical scission of the S-H bond has taken place producing the observed polymerisation. The polymer does not contain pendant thiol groups and hence could not be used for the preparation of thioglycoside polymers. The addition of an inhibitor such as p-benzoquinone may prevent polymerisation and enable the desired reaction to take place.

Reaction of thioglycollic acid with pentaacetylglucose

It was hoped that the reaction would produce a thioglycoside containing a pendant thiol group which could be reacted with a glydicyl containing polymer.

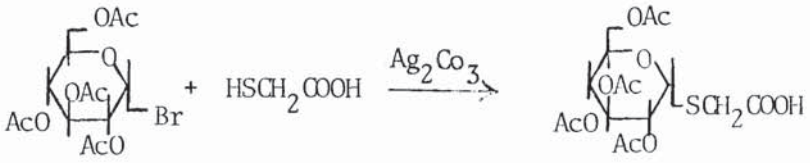


The only products observed were thioglycollic acid and pentaacetylglucose as well as a high melting point solid which was insoluble in organic solvents. The failure of this reaction

could be due to the presence of a carboxyl group in the thiol.

Reaction between acetobromoglucose and thioglycollic acid

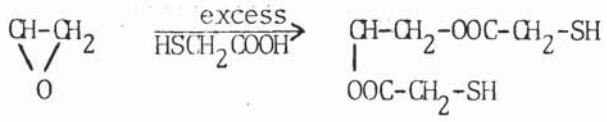
The object of the reaction was to produce a thioglycoside containing a pendant carboxylate group.



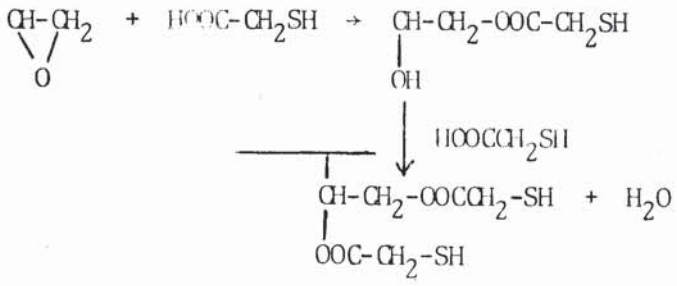
The above reactions however did not appear to take place. In fact methylation of thioglycollic acid takes place and there is no evidence of a sugar residue. It is possible that methylation of the thioglycollic acid could have involved either acetone, in which the reaction was carried out, or the acetyl groups of the acetobromoglucose.

Reaction between HEMA/GMA copolymer with thioglycollic acid

It was thought that one mole of thioglycollic acid would react with one mole of polymer. In fact the final weight of the polymer would tend to indicate that two moles of thioglycollic acid have reacted. A possible reaction is shown below.



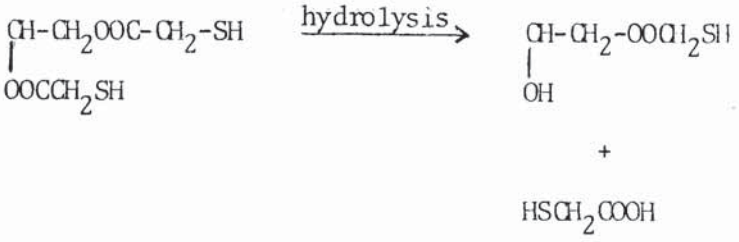
The reaction could proceed as shown below:



In this reaction, initially one mole of the thioacid adds to the epoxide followed by esterification of the resulting hydroxyl group with another mole of thioglycollic acid. It would seem that esterification of the hydroxyethyl methacrylate has not occurred as if this reaction had occurred three moles of thioglycollic acid would have been taken up by the polymer. Analytical results indicate that two moles of thioacid have reacted with the polymer but do not indicate whether the compound contains thiol or sulphide groups which could have been formed by oxidation of the thiol. The infra-red spectrum of thiol containing polymers contain a peak at 2560 cm^{-1} indicating S-H. The observation of this peak provides a qualitative indication as to whether oxidation or reaction of the thiol groups has taken place.

Reaction of thiopolymer with potassium hydroxide

It was intended that the postassium salt of the thiol polymer would be formed by the reaction between the polymer and potassium hydroxide and the product could then be reacted with a glycosyl halide. When the reaction was carried out in methanol, the solution became yellow and thioglycollic acid was apparent after the reaction. The product could have undergone base catalysed hydrolysis to produce thioglycollic acid.

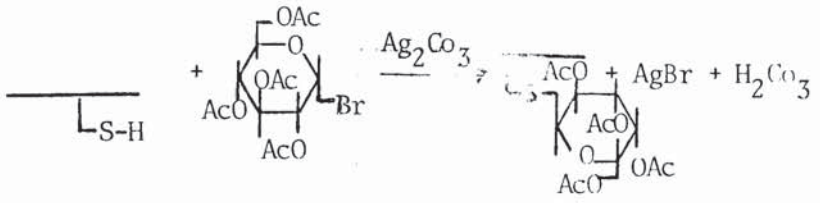


Hydrolysis could possibly remove a second molecule of thioglycollic acid to produce a diol or epoxide. Treatment with hydrochloric acid causes the thiol group to reappear would tend to indicate that the second molecule is not cleaved. When the reaction was carried out with aqueous potassium hydroxide, no free thioglycollic acid was formed and the formation of a potassium salt was indicated by the infra-red spectrum.

Reactions of thiopolymer with acetobromoglucose

The reactions shown in Table 14 all involve the attempted reaction of the pendant thiol group on the polymer with

acetobromoglucose, usually in the presence of silver carbonate which is expected to react with the hydrogen bromide produced as shown below.



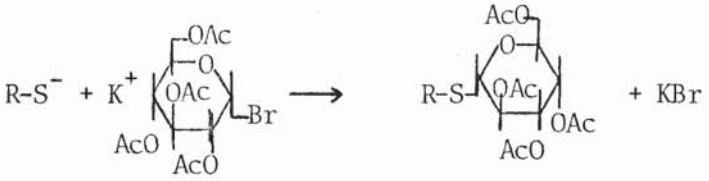
From the infra-red spectra and the weight of the polymer before and after reaction, it is apparent that no reaction has taken place in any of the reactions. The reaction of acetobromoglucose with the thiol polymer in acetone follows a literature method for the reaction of acetobromoglucose with water and it is possible that the insoluble nature of the polymer accounted for the failure of this reaction. No suitable solvent could be found for the polymer and although a number of solvents seemed to swell the polymer reaction did not take place. At higher reaction temperatures it can be assumed that decomposition of acetobromoglucose occurred. Also longer reaction periods either at low temperatures or at room temperature did not seem to be beneficial.

The failure of these reactions would seem to be due to the insoluble nature of the polymer or to a steric effect which might prevent reaction due to the large size of the acetobromoglucose molecule. The configuration of the polymer backbone and of the long side chain to which the thiol group is attached would be expected to have some effect on the ease of approach of the sugar molecule

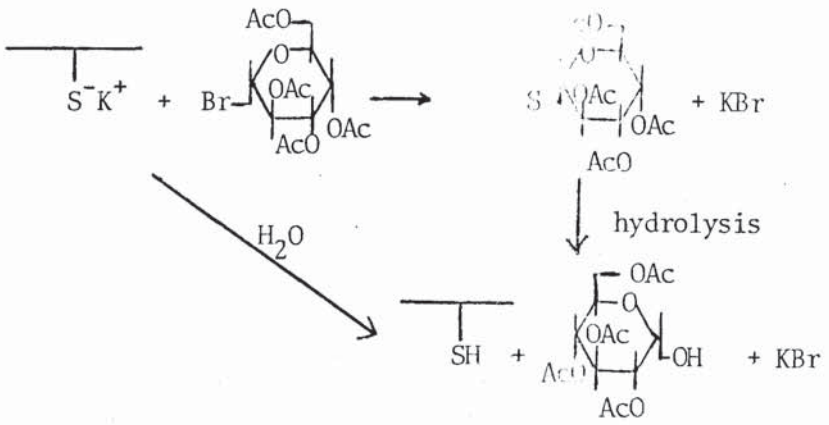
to the thiol group. An interaction could arise from hydrogen bonding between the hydroxyl groups of the HEMA and neighbouring carbonyl groups. A high degree of hydrogen bonding involving the thiol groups may explain the very low equilibrium water content found in the thiol polymer as such hydrogen bonding would constitute a form of cross-linking hence lowering the water content.

Reaction of the potassium salt of the thio polymer with acetobromoglucose

This reaction follows a literature reaction in which acetobromo-glucose is reacted with the potassium salt of a thiol.



It is possible that the thiosugar has been formed and subsequently decomposed to give the thiol, this however would not be expected as the C-S bond of the thiosugar should not be cleaved under the comparatively mild reaction conditions. It is more likely that the thiol has been formed by hydrolysis due to the presence of water.



Reaction of thiopolymer with pentaacetylglucose

This reaction follows a literature reaction for the preparation of thioglycosides which is known to give variable yields. Once again the failure of this reaction can be attributed to the insoluble nature of the polymer.

In conclusion it has been shown that copolymers of GMA and HEMA can be reacted via the epoxide groups in the polymer can be reacted with thioglycollic acid and this would seem to be a promising route to the attachment of sugar molecules to hydrogel polymers. Although the position of attachment of the thiol is not known, it has been shown that two moles of thioglycollic acid attach to each mole of epoxide thus it would be expected that two moles of a glycoside could be attached. The attempted preparations of thioglycosides in which the polymer was reacted with acetobromo glucose were unsuccessful under all conditions used. This is probably due to the insoluble nature of the polymer, coupled with steric effects which prevent the approach of the glycoside to the thiol groups in the polymer. Also the fact that acetobromoglucose is known to undergo hydrolysis is of importance as it is difficult

to remove all traces of water from hydrogel polymers. The reactions described above all involve the attachment of a spacer arm to the polymer followed by reaction of the end group with a glycosyl compound. Attempts at attaching the spacer arm to the glycoside before reaction with the polymer and at attaching the spacer arm to the monomer (GMA) before polymerisation all proved to be unsuccessful, the latter because dissociation of the spacer arm (thioglycollic acid) occurred producing polymerisation of the monomer. In the case of reactions of acryloyl chloride reactions the major difficulties encountered are two fold. Firstly the need to find a solvent system for the reaction and secondly the need to exclude water from the system and so prevent hydrolysis of the acid chloride group.

CHAPTER SEVEN

CONCLUDING DISCUSSION AND SUGGESTIONS FOR FURTHER WORK

7.1 Concluding Discussion

The work described in this thesis can be broadly divided into two categories. Firstly, the characterisation of some hydrogel materials (both commercially available and laboratory synthesised) using a combination of techniques in order to elucidate the structural features of the materials. Secondly, some aspects of the modification of hydrogels which may be of value in enhancing the properties required for their use in biomedical applications.

Although hydrogel polymers have been known for some time, their characterisation has not been studied in a coherent manner. The work described in this thesis has attempted to approach the problem of characterisation by investigating the properties of the gel as a whole and the equally important structural properties of the polymer backbone. The use of hydrophilic monomers creates problems in characterisation due to the varying radical reactivity ratios of the monomers used (free radical polymerisation being the most common method of synthesis). This is complicated by the fact that the polymer is swollen in water subsequent to synthesis and at this stage hydrophilic residues not bound into the network may be removed. One of the most important monomers in this respect is N-vinyl pyrrolidone which

is a widely used constituent of hydrogel materials. Attempts to incorporate more of this monomer into commercially available polymers (eg contact lens materials) often involves the use of a thermal postcure and the net result of this is the formation of low molecular weight poly(N-vinyl pyrrolidone) fragments which are removed along with N-vinyl pyrrolidone monomer when the gel is hydrated. There is frequently, therefore, no simple relationship between the structure of the hydrogel backbone and the monomer feed used in the synthesis. On the other hand, there are advantages in the characterisation of hydrogels and these are typified by the different techniques available for the characterisation of the material in both the hydrated and dehydrated state which provide two methods of approaching the problem.

The application of a variety of characterisation techniques to some commercially available hydrogel materials was described in Chapter 3. The results obtained indicate that the combination of techniques employed (eg pyrolysis gas liquid chromatography, elemental analysis and density) provide a simple but powerful means of assessing the compositions of the materials studied. The techniques used have been shown to be more accurate when applied to laboratory synthesised as opposed to commercially available materials and this is probably due to the wide variety of substances which may be incorporated into commercial systems. During part of the work, the equilibrium water content of the hydrogels was used as a characterisation technique. However, more important than the amount of water present in a gel is the nature of that water.

The characterisation of water in hydrogels presents problems and these have been investigated in previous studies on biological systems which lead to the expectation that water could exist in more than one state in synthetic gels. Several techniques have previously been applied to the study of water in hydrogels (eg NMR spectroscopy, dielectric and conductivity measurements). However, as is often the case, research groups that have been concerned with this work have specialised in the techniques available for studying water and have not involved themselves in synthetic chemistry. For this reason, most of the studies of water in synthetic hydrogels have been confined to poly(2-hydroxyethyl methacrylate) which is widely available commercially. The importance of the characterisation of water in hydrogels cannot be over-emphasised. The states of water present within a gel are known to exert a profound effect on the biocompatibility, permeability and permselectivity of hydrogels. These studies are as yet in their infancy and it is important to establish a set of reliable characterisation techniques to provide a basis for the interpretation of results as they become available. A single example of the importance of the states of water present in gels is typified by the fact that virtually nothing is known at present about the permeability and permselectivity of gels with respect to ammonia and amines. However, such effects are of great potential importance in the design of membranes for artifical liver support systems and for semi-conductor sensors in the field of water borne pollutants.

During the course of this work, differential scanning calorimetry (DSC) was used to identify water as being freezing or non-freezing depending upon its strength of association with the polymer network. A number of systems were investigated using differing combinations of hydrophilic and hydrophobic monomers. The water binding properties of these systems have been shown to be related to the structure of the monomers used and also to the relative amounts of monomer present in a copolymer system. The results obtained were interesting in that they did not always show simple relationships between polymer composition and the observed water binding properties.

This was the case when hydrophilic monomer pairs having strong hydrogen bonding tendencies were copolymerised. In this type of system, there was evidence of competing effects, ie hydrogen bonding between the monomers and their hydrogen bonding to the water present in the system. By using suitably chosen monomers it is possible to vary the relative amounts of each type of water present and so design a polymer having the water binding characteristics required to suit a particular application. An example of this is found in reverse osmosis where the salt rejection properties together with a degree of water flux associated exclusively with non-freezing water are required.

The effect of cross-link density on the water binding properties of some poly(2-hydroxyethyl methacrylate) (PHEMA) gels was investigated. The results from this work have shown two principal

features of this type of system. Firstly, at low cross-link densities (1-5%), it has been shown that increasing the cross-link density has a dramatic effect on the amount of freezing water present and after this stage as the cross-link density is increased the effect on the freezing water content is less pronounced. On the other hand, the non-freezing water content has been shown to decrease gradually over the region 1 to 10% cross-linking and then decrease more rapidly as the cross-link density is increased. Secondly, this work has shown that there is a need to investigate more fully the water binding behaviour of this system at cross-link densities (below 1%). This is necessary as there is some doubt as to the relative amounts of freezing and non-freezing water present in this region and this is thought to be due to impurities in the monomers used which could lead to inaccuracies in the cross-link density present and hence affect the observed water binding properties.

Macroporous hydrogels provide an interesting method of obtaining higher permeability to solutes that are too large to diffuse readily through the restricted pore network of conventional homogeneous hydrogels. Whereas pore sizes in the latter are controlled by water content but with diameters typically of the order of 10-20 A for a 60% water content gel, those in the former lie in the micron range.

The water binding properties of a typical macroporous system (which have not been previously studied in this respect) were

compared to those of a homogeneous PHEMA at similar cross-link densities. These two systems provided an interesting contrast in water binding properties because in a macroporous system the non-freezing water content is relatively constant despite changing the conditions (monomer in monomer solvent feed) under which the gel is made whilst the freezing water content increases if the structure is made more porous in this way. In a homogeneous system, however, both the freezing and non-freezing water contents of the material vary as a function of the cross-link density in the manner previously described. Fairly high (5-15%) cross-link densities are required for the preparation of macroporous gels and at similar cross-link densities, it is interesting to note that the non-freezing water contents of these two types of system are comparable with the macroporous system having a slightly higher non-freezing water content. This was thought to be due to the fact that although the majority of the non-freezing water would be associated with the polymer matrix, the free water existing predominantly in the pores of the system, there may be a small amount of water that is bound to the inner surfaces of the pores. In order to clarify this explanation, it would be necessary to carry out further comparisons of water binding behaviour between homogeneous and macroporous systems at a range of cross-link densities and perhaps using a different hydrogel composition.

The mechanical properties of PHEMA gels with various cross-link densities were investigated with respect to their deformation behaviour and water binding properties. The results from this

work indicate that freezing water acts as a plasticiser. Therefore, at low cross-link densities where the ratio of non-freezing to freezing water is low, the material is easily deformed and flexible, as the ratio of non-freezing to freezing water increases with increasing cross-link density, the material becomes more rigid and less deformable. Once again, there is a need to investigate the mechanical properties of PHEMA gels at low cross-link densities in order to discover the effects of water binding on the mechanical behaviour.

In broad terms, it has been shown that DSC can provide valuable information on existence of water in the freezing and non-freezing states. In addition, DSC shows the fine structure of the continuum of water states normally collected under the heading of 'freezing' water. It is important when interpreting the results of these studies to have a precise knowledge of the structure of the dehydrated polymer rather than simply the feed ratio of the monomers used. This illustrates one aspect of the importance of the characterisation techniques mentioned and how all the techniques available for characterisation can be interrelated.

The different states of water in hydrogel polymers is also important in relation to other surface and bulk properties. In addition to this, the chemical nature of the dehydrated polymer is capable of exerting a profound effect on these properties. Some preliminary studies of the effect of backbone structure on the behaviour of the hydrated and this was investigated with

respect to the absorption/adsorption and release of amino acids from various polymers (Chapter 5). The backbone modification of some hydrogel polymers was achieved by the incorporation of different acrylamide type monomers into the copolymer system described in Chapter 5 and studied by the relative release rates of these materials with polymers containing styrene as the third monomer in the system. The results showed a much slower rate of amino acid release than a polymer containing styrene. This is believed to be related to the interaction between the nitrogen containing monomers and the amino acid used and also possibly to the states of water present in the hydrated polymer. Amino acids were used in this work in order to model the behaviour of proteins on hydrogel surfaces as they provide results which are more readily interpreted than the more complex processes which are known to occur at protein/polymer interfaces.

An extremely important area of immense potential is the modification of hydrogel surfaces by the covalent attachment of certain biologically important species to the polymer matrix. The potential importance of this work is made clear by the increasing interest in the use of synthetic hydrogels as substrates for cell studies[100,101]. As yet, only poly(2-hydroxyethyl methacrylate) has been studied (because of its ready availability) and there is obviously considerable potential for the use of other hydrogel polymers. For this reason, an investigation into methods of attaching a sugar type molecule to some hydrogels was undertaken.

The attempted synthesis of glycoprotein analogues was described in Chapter 6 and it was in this area that problems were encountered. Two principal routes to the modification of hydrogels were investigated and both of these presented problems which were only partially resolved during the course of this work. The first route made use of a thiol intermediate in the modification process and was essentially a two-step reaction. Firstly, a suitable thiol intermediate has to be made and this is subsequently reacted with a sugar type molecule to produce the modified polymer. Although difficulties were encountered, it was finally found possible to attach thiol groups to the polymer backbone.

The reaction of the thiol polymer with a sugar type molecule, however, proved to be less successful. This is believed to be due to a number of factors and these include the insoluble nature of the polymer and possibly steric effects which prevent the reaction from occurring. Also the known susceptibility of sugars such as acetobromoglucose to hydrolysis is probably important because it is very difficult to remove all traces of water from hydrogel polymers. The problems associated with this type of modification route may be overcome by the use of a soluble polymer containing thiol groups which could then be reacted with a sugar type molecule under anhydrous conditions. This is itself may be difficult because the preparation of a soluble polymer containing glycidyl methacrylate is hindered by the cross-linking between epoxide groups.

The second route used in the attempted modification of hydrogels by the attachment of sugar-type molecules makes use of acryloyl chloride, a reactive vinyl monomer containing an acid chloride functional group. It was proposed to react this group with a nitrogen containing sugar molecule such as glucosamine thereby binding a sugar molecule to the hydrogel structure. The problems encountered with this system were two-fold. Firstly, the need to find a suitable solvent for the reaction and secondly overcoming the problems associated with keeping the reaction system free from moisture during the course of the reaction. The solvent problem arises because it was difficult to find a solvent in which glucosamine is soluble at low temperatures. This is necessary because at higher temperatures the sugar decomposes. This is coupled with the problem of excluding moisture from the system which causes hydrolysis of the acryolyl chloride in the system. Bearing in mind the problems associated with the use of acryloyl chloride, it was decided that future work if undertaken should make use of a material which is less unpleasant due to its lachrymatory effects and also less subject to hydrolysis. Although the results obtained from this section of the work were not entirely successful in terms of the attachment of a sugar molecule to a hydrogel, they have served to highlight the difficulties encountered in this type of synthesis and provide a basis for further work in this field.

Taking an overview of the work described in this thesis as a whole, the single most important factor in governing a wide

variety of the observed behaviour of hydrogels is the nature of the water contained within the gel. Typical examples in which the nature of water is important are illustrated by transport phenomena and biocompatibility which is also related to the work described on hydrogel modification. The oxygen permeability of hydrogels has been shown to be dependent on water content for high equilibrium water contents[60] but at lower water contents there is evidence that there is greater dependence on polymer structure and the nature of the water present. In the case of reverse osmosis membranes, the question of permselectivity is important. This phenomenon has been shown to be affected by the nature of the water present and in this application, a high water content is desirable but with a large fraction of the water being non-freezing (bound). This is necessary to promote salt rejection and increase the mechanical strength of the membrane. For hydrogels in biomedical applications the surface properties, are of importance and it was for this reason that the investigation into the modification of hydrogels was undertaken. In addition to the surface properties it has been suggested that the blood compatibility of the hydrogel material is related not only to the water content of the gel but also in some way to the nature of the water present.

7.2 Suggestions for Further Work

Arising from this work, several suggestions for further work are made as follows:

(1) The investigation of water binding properties should be continued examining new polymers as they become available. The temperature of hydration is known to affect the equilibrium water content of hydrogels. The effect of hydration temperature on the relative amounts of freezing and non-freezing water should be examined as should the effect of the incorporation of ionisable monomers (eg methacrylic acid).

(2) Possible new routes to the attachment of biologically important molecules to hydrogels should be studied in the light of the work described in this thesis. Additionally, it would be interesting to extend this study to investigate the effect of modified hydrogels on their interaction with amines, amino acids and proteins.

(3) In order to resolve the apparent anomalies in the observed water binding properties of PHEMA gels at low cross-link densities (in the order of 1%) further work should be carried out using carefully purified systems.

(4) The study of the water binding properties of homogeneous hydrogels in comparison with heterogeneous macroporous gels should be extended to include a variety of materials at different cross-link densities.

(5) The characterisation techniques described for use with dehydrated hydrogels should be refined and improved with special reference to pyrolysis gas liquid chromatography.

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