THE DESIGN OF HYDROGEL POLYMERS FOR ARTIFICIAL LIVER SUPPORT SYSTEMS

by

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SUMMARY

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This thesis examines various approaches to the design of hydrogel polymers for use in artificial liver support systems. It contains an extensive literature survey which in addition to providing information for the present project was designed to facilitate future research in this field. The experimental investigations fall into three main areas. Firstly, homogeneous hydrogel systems which provide information on many of the relationships between molecular composition and eg the water content and surface properties of hydrogels. These membranes were used for blood compatibility tests, thereby establishing relationships between the physicochemical properties of a hydrogel and its gross interaction with blood. Secondly macroporous hydrogel membranes were examined. The pores of these are discrete physical entities created by freezing a monomer-solvent mixture prior to polymerization thereby creating a polymer matrix with large macropores running through it. These materials are interesting because of their greater permeability to large molecules, a property of interest if the dialytic method of removing blood toxins is considered. These materials did indeed show good permeability to large molecules but other properties limit their immediate use in dialytic applications. The knowledge gained making macroporous membranes was however applied in the third area, namely the design of a biocompatible adsorbent. A novel method of preparation was devised, various compositions were synthesised and their adsorbtion properties and biocompatibility examined. This work was extended to include the more conventional technique of suspension polymerization. In both cases however it proved difficult to prepare beads meeting all the requirements for practical use in liver support systems. Nevertheless much useful information was gained which it is thought may lead to a solution to this problem by using adsorbents such as activated carbon or ion exchange resins which need a permeable biocompatible coating for in vivo use. Hydrogels remain the most promising polymers for this application.

KEYWORDS

Hydrogels
Liver support
Macroporous adsorbents

L I S T O F C O N T E N T S

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LIST OF ABBREVIATIONS USED IN THE TEXT

AA	Acrylic Acid
ACR	Acrylamide
ADP	Adenosine Diphosphate
AN	Acrylonitrile
APTES	Aminopropyltriethoxysilane
BCT	Blood Clotting Time
BSP	Bromosulpho phthalein
CA	Crotonic Acid
CLD	Crosslink Density
DAA	Diacetone Acrylamide
DADACℓ	Diallyl dimethyl ammonium chloride
DMAEMA	Dimethyl ethyl amino ethyl methacrylate
DMAPN	Dimethyl amino propionitrile
DMS	Poly (Dimethyl siloxane)
EA	Ethyl acrylate
EAA	Ethylene/acrylic acid polymers
EDM	Ethylene dimethacrylate
EG	Ethylene glycol
EVS	Ethylene/vinyl sulphonate polymers
EWC	Equilibrium Water Content
FM	Fluorinated Monomers
FS	Poly (fluoro siloxanes)
GBH	Graphite Benzalkonium Heparin
HEMA	Hydroxy ethyl methacrylate
HFIPA	Hexafluoro isopropyl acrylate
HFIPMA	Hexafluoro isopropyl meth

HPA	Hydroxy propyl acrylate
LTI	Low Temperature Isotropic
MAA	Methacrylic Acid
MACR	Methacrylamide
NaSS	Sodium styrene suophonate
NNMBA	NN Methylene bis acrylamide
NVP	N-vinyl pyrrolidone
PAA	Poly (acrylic acid)
PACR	Poly (acrylamide)
PAN	Poly (acrylonitrile)
PETMA	Pentaerythrital tetramethacrylate
pDAA	poly (diacetone acrylamide)
pHEMA	poly (hydroxy ethyl methacrylate)
pHPA	poly (hydroxy propyl acrylate)
PHPMA	Poly (hydroxy propyl methacrylate)
pGMA	poly (glyacryl methacrylate)
pVAℓ	poly (vinyl alcohol)
PVP	Poly (vinyl pyrrolidone)
SEM	Scanning Electron Microscopy
ST	Styrene
TDMAC	Tridecyl methyl ammonium chloride
TFEM	Tri fluoro ethyl methacrylate
TPT	Trimethylol propane tri methacrylate
VA	Vinyl acetate
VBTAC	Venyl benzyl trimethyl ammonium chloride
Wf	Weight fraction

CHAPTER 1 HYDROGELS

CHAPTER 2 BIOCOMPATIBILITY

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CHAPTER 4 EXPERIMENTAL TECHNIQUES.

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CHAPTER I

HYDROGELS

1.1 INTRODUCTION

Hydrogels are polymeric gels which are hydrophilic in character. Three main types of hydrogel have been defined[1]

(1) Non-ionic hydrogels - in which the network is formed by primary chemical bonds between chains of macromolecules, for example, the hydrogels of poly (hydroxyethyl methacrylate) crosslinked by ethylene dimethacrylate.

(2) Thermally reversible hydrogels - in which the 3-dimensional structure is held together by weak secondary forces, for example the hydrogels of polyelectrolyte complexes.

(3) Novel microcrystal hydrogels - which contain discrete colloidal particles or microcrystals. Examples are gels of cellulose and collagen.

All three types of hydrogels have been used in biomedical applications.

The range of each type of hydrogel will now be dealt with: special emphasis is put on the non-ionic hydrogels, which are the type with which this thesis is mainly concerned.

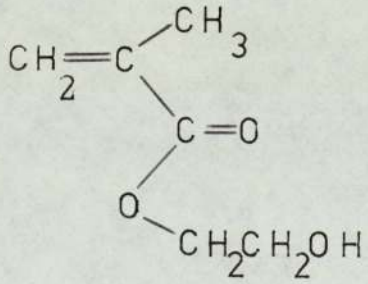
1.1 (a) SYNTHETIC NON IONIC HYDROGELS

There are two main ways of making synthetic non ionic hydrogels: - (1) by polymerization of hydrophilic monomers and (2) by derivatization of hydrophobic polymers. These are now dealt with.

(1) Monomers used to manufacture synthetic non ionic hydrogels (by polymerization)

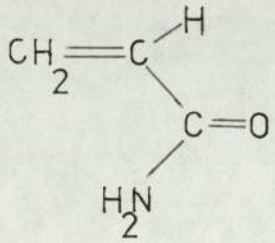
A wide variety of monomers have been used in making (non ionic) hydrogels. Some examples are given below.

(i) The hydroxyalkyl acrylates and methacrylates, one of the most important being hydroxyethyl methacrylate whose structure is:-



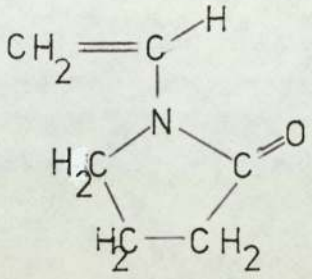
(H E M A)

(ii) The acrylamides and methacrylamides, substituted and unsubstituted. The structure of acrylamide is given below:-



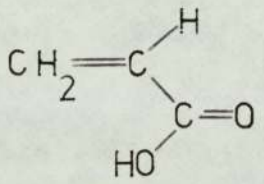
(A C R)

(iii) The N-vinyl lactams, of which N-vinyl pyrrolidone is the most important:-

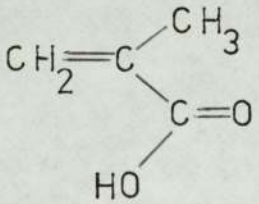


(N V P)

In addition, acidic, cation-exchanging ability can be conferred on a hydrogel by copolymerization with acidic monomers such as acrylic and methacrylic acid:-

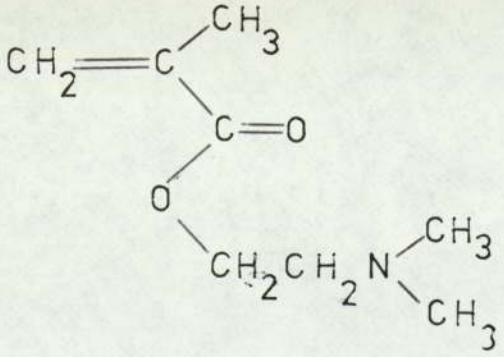


(A A) - acrylic acid



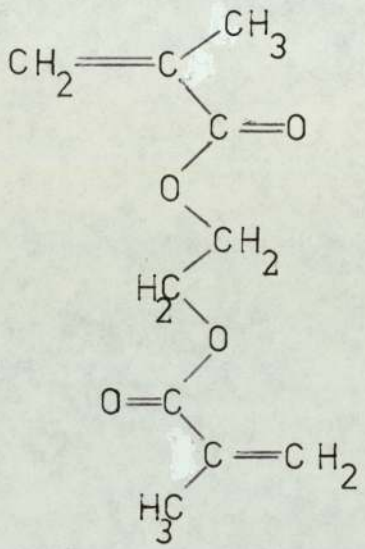
(M A A) - methacrylic acid

Also, basic, anion-exchanging ability can be conferred by copolymerization with basic monomers such as N-substituted amino methacrylates; an example of which is dimethyl amino ethyl methacrylate:-

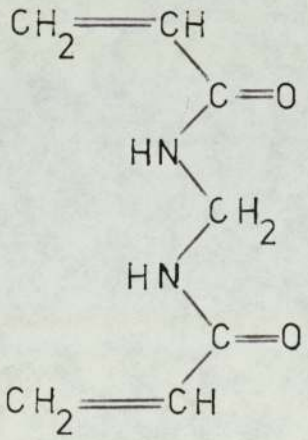


(D M A E M A)

Also needed are crosslinking agents to hold the gel structure together. There are a wide variety of these having different reactivities, hydrophilicities and other properties. Two of the most important are ethylene dimethacrylate and NN' methylene bisacrylamide whose structures are shown below:-



E D M - ethylene dimethacrylate



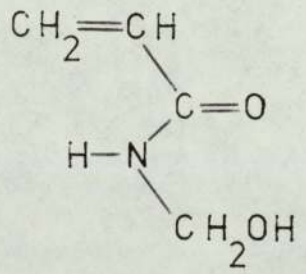
N N 'M B A - NN' methylene bisacrylamide

As can be seen these monomers have two vinyl groups of identical reactivity, thus crosslinked structures are produced on polymerization.

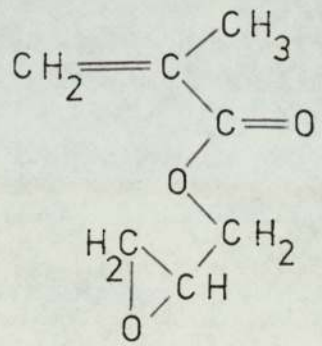
Alternatively other crosslinking agents, which have two different functional groups of different reactivity can be used. Typically one of these is a vinyl group activated during the polymerization process to copolymerize with the other monomers to create a linear soluble polymer. The other functional group of lower reactivity can be activated by higher temperature or other means after the polymer has been processed e.g. perhaps by moulding into a desired shape.

Some monomers used for this purpose are the following:-

(a) N-hydroxy methacrylamide



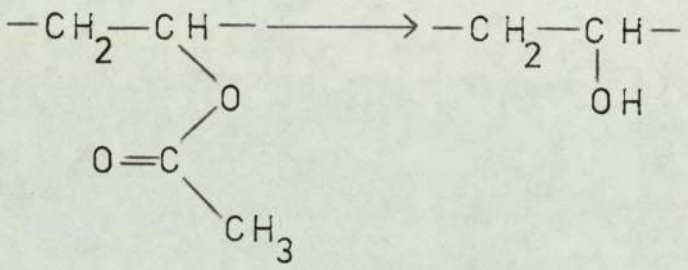
(b) Glycidyl methacrylate



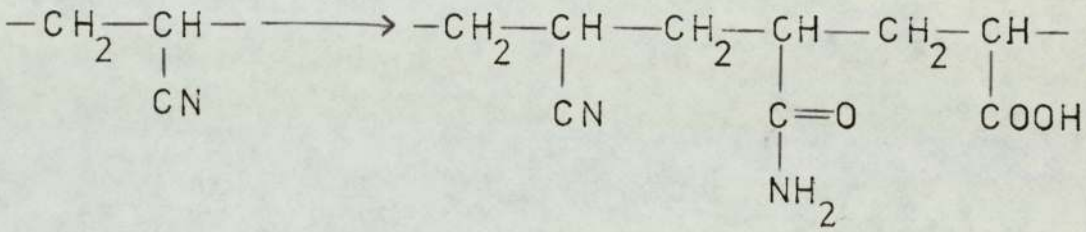
Even methacrylic acid can be considered in this light as the ester reaction between the carboxyl group of the acid and the hydroxyl groups of other monomers takes place at high temperature to produce a crosslinked structure.

(2) Derivatization

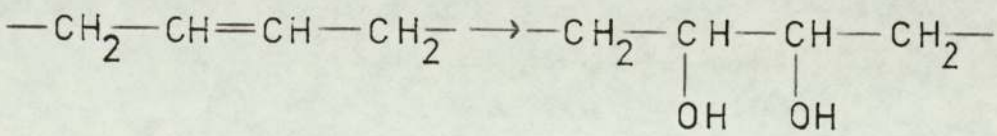
In addition to creating non-ionic hydrogels by copolymerization of hydrophilic monomers, non-ionic hydrogels can also be created by derivatizing hydrophobic polymer networks[2], for example, hydrolysis of poly (vinyl acetate) to yield poly (vinyl alcohol):-



or of acrylonitrile



or by addition to double bonds



1.1 (b) THE POLYELECTROLYTE COMPLEXES (PEC's)

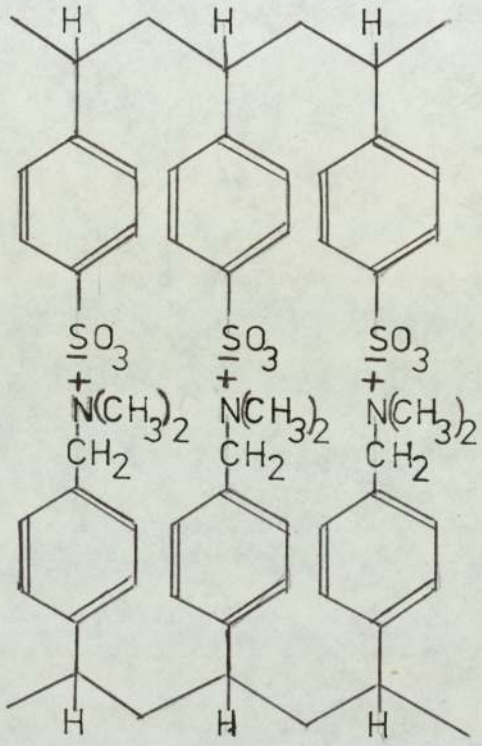
The PEC's are ionically bonded hydrogels; they are synthesized by coreacting linear, water soluble ionic polymers of opposite electrical charge under carefully controlled conditions. The resulting material is insoluble in water, electrolytes, organic or common solvents but soluble in special ternary solvents.(3)

Two examples of polyelectrolyte complexes are Ioplex 101 and Ioplex 103. Ioplex 101 is prepared from sodium poly (styrene sulphonate), [Na S S] , and poly (vinylbenzyl trimethyl ammonium chloride) [V B T A Cℓ] and Ioplex 103 from Na S S and poly (diallyldimethyl ammonium chloride) D A D A Cℓ. The structures of Ioplex 101 and 103 are given to illustrate this type of hydrogel in Figure 1.(3)

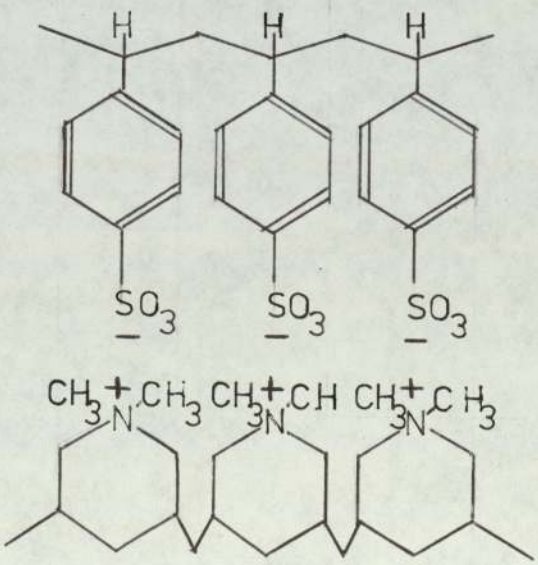
FIGURE 1

Structures of Ioplex 101 and 103

IOPLEX 101



IOPLEX 103



1.1 (c) NOVEL MICROCRYSTAL HYDROGELS

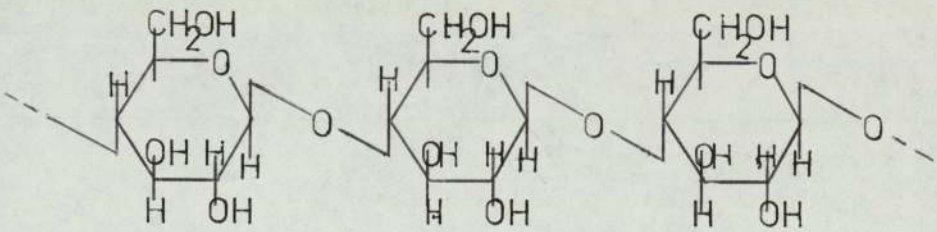
The most important hydrogels in this class from the point of view of biomedical applications are the gels of cellulose and collagen, which are the main structural biopolymers in the animal and plant kingdoms, respectively.

(a) Cellulose

This is a straight-chain polysaccharide consisting of β-1,4 linked glucose units. Its molecular weight is about 500,000 and its structure is shown in Figure 2.[4]

FIGURE 2[4]

Structure of Cellulose



From basic cellulose many important materials of biomedical application can be synthesised such as cuprophane, cellophane and collodion.

(b) Collagen

The collagens are, unlike cellulose, made up of polypeptides and structurally consist of three coiled polypeptide chains

which are helically wound round each other.[5]

Like cellulose it too can be converted into other interesting materials of biomedical interest e.g. gelatin.

Gelatin - when collagen is boiled with water, it is converted into the familiar water soluble protein gelatin, which when the solution is cooled does not revert to collagen but sets to a gel. Gelatin has a molecular weight one-third that of collagen. Evidently the treatment separates the strands of the helix, breaking inter-chain hydrogen bonds and replacing them with hydrogen bonds to water.[6]

Gelatin and collagen have been used to make biomaterials for various uses.

1.2 PROPERTIES OF HYDROGELS

(a) Hydrophilicity

This property is one of the most important of a hydrogel since it is integrally related to the other important properties such as permeability, mechanical strength and biocompatibility.

The hydrophilicity of a polymer is the result of the presence of hydrophilic groups on the polymeric chains; it enables swelling of crosslinked polymers when they are equilibrated with water. The equilibrium water content, EWC, is controllable because it depends on the hydrophilicity of the polymer structure and the experimental conditions of the preparation of the hydrogel, (obviously the EWC increases with the increasing hydrophilicity of the hydrogel).

The swelling of non-ionic hydrogels was found to be dependent on the following factors:-

1) the interaction parameter between the polymer and solvent
2) the extent of crosslinking or the molecular weight of the polymer chain between crosslinks
3) the relative swelling of the polymer at the time of crosslinking.

These factors and others which concern hydrogels with ionic groups will be considered on the following pages.

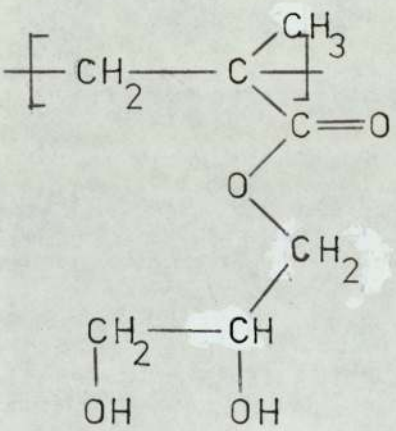
1) The Interaction Parameter Between the Polymer and Solvent

The interaction parameter is a most important factor in determining the EWC of a hydrogel. If it is low then water is a bad solvent for the polymer and the hydrogel does not swell to a great degree,

and if it is very high the polymer will dissolve unless factors such as crosslinks prevent it. An example of the first case is polyHEMA and of the second, PVP.

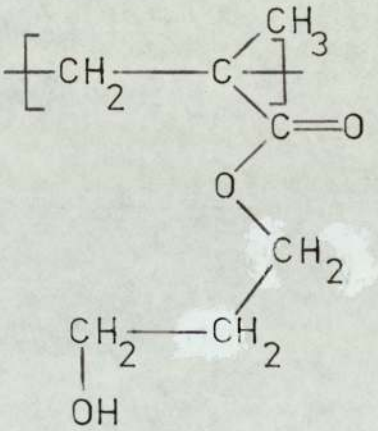
The reason for the differences in interaction between polymers is, of course, the number and nature of the hydrophilic groups on the polymer backbone. These separate effects are illustrated by the following examples.

The increase in hydrophilicity caused by the increase in the number of hydrophilic groups is best shown by the differences in EWC for poly(glyceryl methacrylate) (PGMA), and poly(hydroxypropyl methacrylate) (PHPMA), as shown below:



P.G.M.A.

E.W.C.= 75%.(7).



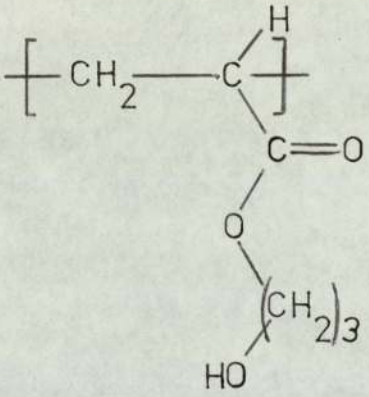
P.H.M.A.

E.W.C.= 22.5%.(8).

The more polar the groups the more hydrophilic the polymer is and therefore the more it is solvated, Thus poly(acrylamide), which has the highly polar amide group is water soluble in its uncrosslinked

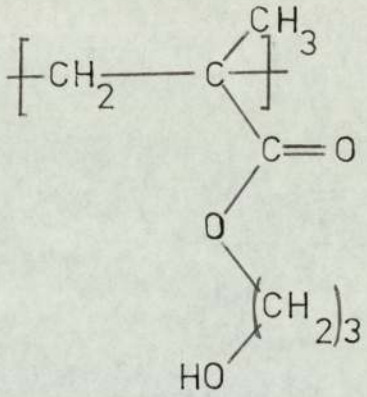
state; whereas poly(HEMA) with its less hydrophilic hydroxyl group absorbs only up to 40% its own weight in water. The effect of ionic groups on EWC is illustrated later.

Also important in determining the EWC is whether the polymer is an acrylate or a methacrylate, large differences between the EWC's of comparable polymers can be seen as shown below in the EWC's of poly hydroxy propyl acrylate and methacrylate (PHPA and PHPMA):-



P.H.P.A.

E.W.C.= 50%. (8).



P.H.P.M.A.

E.W.C.= 22.5%. (8).

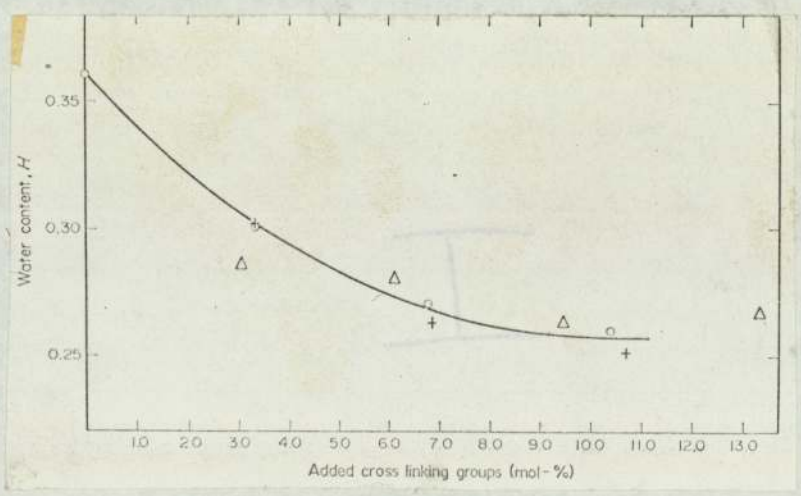
In these cases it is not only the fact that a hydrophobic group has been removed that causes the differences in EWC; it is also due to the increases in chain rotation about the polymer backbone caused by the disappearance of the methyl group. This allows the hydrophilic groups more freedom and consequently they can be solvated more easily.

2) The Extent of Crosslinking

The extent of crosslinking can be an important factor in determining the water content of hydrogels especially high water content hydrogels such as poly(vinyl pyrrolidone) and poly(acrylamide). However, for polymers such as polyHEMA, which have a low interaction with water, the extent of crosslinking does not have a marked influence on the EWC until very high levels of crosslinking agent are attained. This is illustrated in Figure 3.

FIGURE 3[9]

The EWC of polyHEMA crosslinked with EDMA, TPT, PETMA in water:ethylene glycol 4:1



Nor are covalent crosslinks the only type of crosslinks which exist in the hydrogel systems, ionic and hydrogen bonds can limit the EWC which is obtained.

3) The Effect of Ionic Groups

The presence of ionic groups in the hydrogel can profoundly influence the equilibrium water content of the hydrogels, one study which investigated this has been carried out by Gregonis et al[10] on copolymers and terpolymers of HEMA with MAA (acidic) and DMAEMA (basic). The results are shown graphically below in Figures 4a, 4b and 4c.

FIGURE 4a[10]

Equilibrium water weight fraction, w_f, of HEMA-MAA copolymers

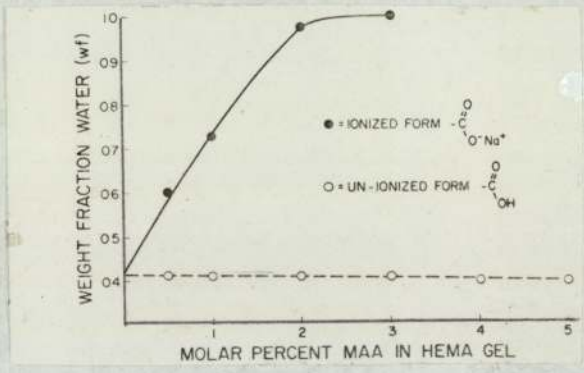


FIGURE 4b[10]

Equilibrium water weight fraction, w_f, of HEMA-DMAEMA copolymers

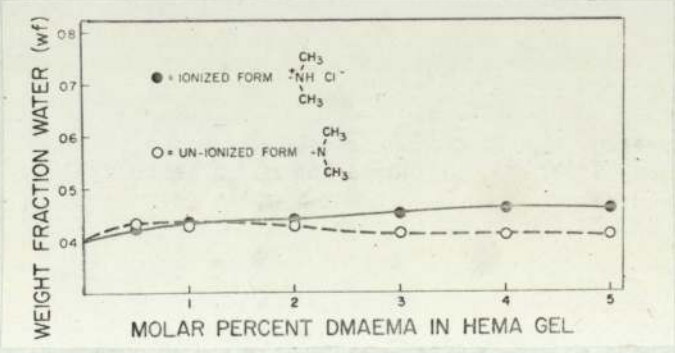
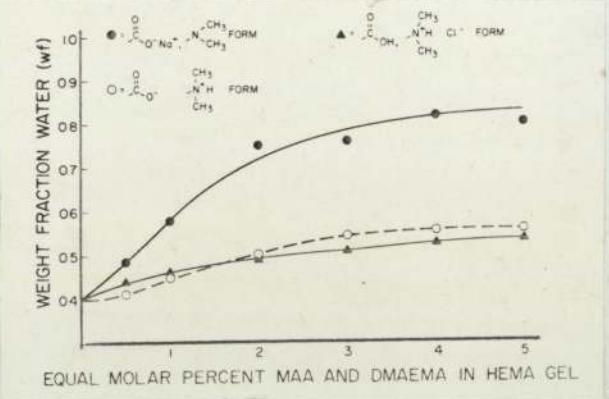


FIGURE 4c[10]

Equilibrium water weight fraction, w_f, of HEMA-MAA-DMAEMA terpolymers. MAA and DMAEMA are polymerized at equal molar concentrations.



It can be seen from Figure 4a, that the conversion of MAA to its carboxylate salt in the gels dramatically increases the EWC of the gel, eg a HEMA uncrosslinked gel containing only 2 molar % MAA equilibrates with a water fraction of greater than 90%.[10]

Surprisingly converting the HEMA-DMAEMA gel to its hydrochloride salt did not show the same dramatic swelling effect. This is believed to be due to the tertiary amines acting as chain transfer agents with the methacrylate system; this could lead to a higher crosslink density, which could account for the low degree of swelling.[10] Thus other effects may confuse our understanding of the effect of ionic changes on EWC.

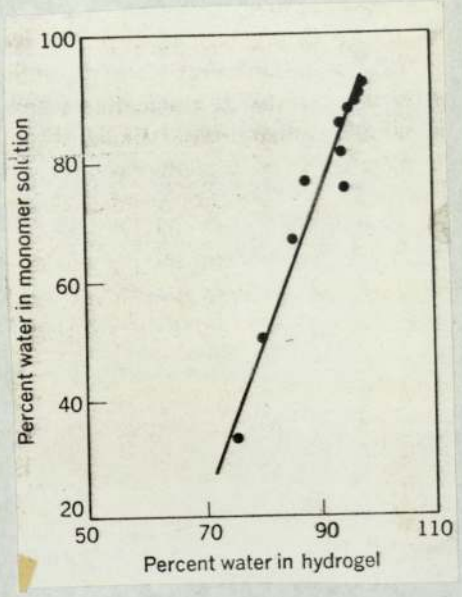
4) Pre-Swelling

Additionally other factors such as the relative swelling of the polymer at the time of crosslinking can be important in cases where

solution rather than bulk polymerization is used. An example of this is shown below in Figure 4d, which shows the effects of the amount of water in the monomer solution upon the amount of water in glyceryl methacrylate hydrogels.

FIGURE 4d[11]

The Effect of the Amount of Water in the Monomer Solution upon Final Water Content



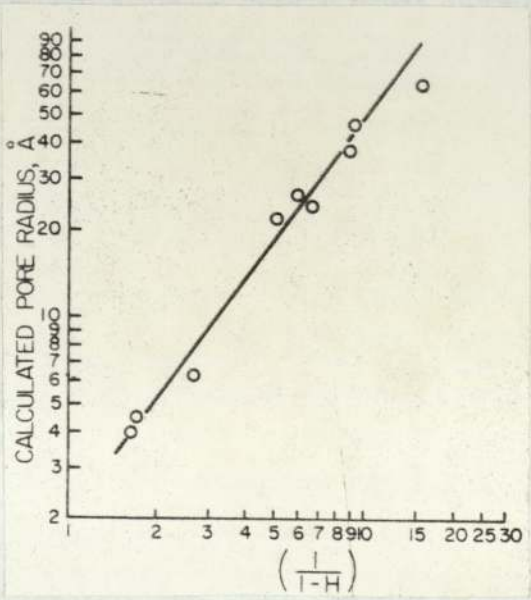
(b) Permeability

Permeability is a very important property from the point of view of the biomedical applications of hydrogels such as dialysis, ultrafiltration or adsorption of toxins; it is determined for any one particular solute by many factors including the physical and chemical nature of the water, (whether it is bound or free).

Hydrogels can be of different sorts, ie they can be homogeneous in which case the water is dispersed evenly throughout the hydrogel and there are therefore no true discrete pores only a randomly fluctuating system of hydrated polymer chains. Therefore for homogeneous hydrogels pore size is only a theoretical concept, it is nevertheless a useful one in so far as it allows us to predict the size of solute molecule, which can travel through a gel. Figure 5 shows the calculated pore radius of methacrylate hydrogels set against their swelling ratios. H is the equilibrium water content divided by ten.

FIGURE 5[12]

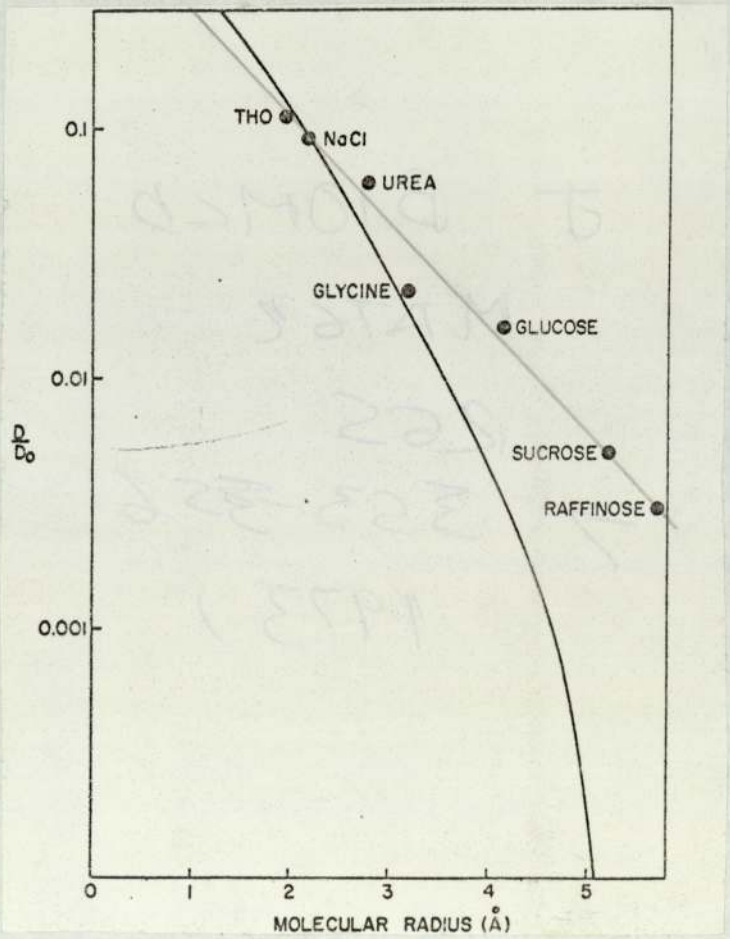
Dependence of calculated pore radius on swelling ratio of methacrylate hydrogels,



From the graph the average pore radius for hydrogels of different water contents can be calculated, eg polyHEMA (EWC = 40%) will have theoretical pores of average radius 3 Å and thus would only be expected to be able to let through molecules whose molecular diameters are just over 6 Å. This is borne out by experiment as can be seen from Figure 6, which shows the ratio of the diffusion coefficient of the molecular species through the hydrogel and that found in free solution, set against the molecular radius of various solute molecules.

FIGURE 6[13]

Restriction to diffusion of various solutes through poly(HEMA)



There is quite a fast molecular size cut off and as the molecular radius approaches 6 Å the diffusion rate becomes extremely low but the rate does not fall off as quickly as might be expected from theory as there is a pore size distribution which extends upwards to allow, (slowly), molecules of large size to penetrate and diffuse through the network. Also shown on Figure 6 is the theoretical line of diffusion constants predicted from the Ferry-Faxen equation:-

$$D/D_o = (1 - a/r)^2 \left[1 - 2.104(a/r) + 2.09\ (a/r)^3 - 0.95\ (a/r)^5 + \ldots\right]$$

This model is a sieve pore flow one in which two factors reduce the diffusion rate; firstly there is a geometrical restraint, the molecule of radius, a, must pass into a cylindrical pore of radius, r, without hitting the pore edge. Secondly there is friction between the molecule and the pore wall. The remaining terms of the equation are D_o, the self diffusion constant in free solution, and D the actual diffusion constant in the membrane.[13]

To create hydrogels of greater permeability either the water content of the gel can be increased to high levels to give large pores, but this can result in a drastic fall off in, for example, mechanical properties. Other methods can be used to give discrete pores in which the water is not homogeneously dispersed throughout the gel but inhomogeneously between a polymer network of low water content and a porous water network in which water molecules overwhelmingly predominate.

There are several ways of creating such a discrete porous network in hydrogels. One is by the conventional way used in the manufacture of macroporous ion exchange resins, ie by using a high

concentration of crosslinking agent and a diluent in which the monomers are not highly soluble one can create a porous structure of polymer nuclei (interconnected with polymer chains), between which are discrete pores. The degree of porosity, of course, depends on factors such as the monomer to solvent (diluent) ratio and the percentage of crosslinking monomer.

Another way of creating a highly porous hydrogel structure has been investigated by Haldon and Lee; this method involves the initial freezing of a monomer solvent mixture to produce a network of solvent crystals between which, in the interstices is the monomer. If the monomer has had a UV photocatalyst added to it prior to freezing then it is possible by using UV light to create a polymer network. Then on thawing and hydration a highly porous hydrogel structure will result.(9)

One further technique that is known is to directly freeze the hydrogel which results in ice crystals being formed and the polymer structure changed into a more porous mode.(14)

A further factor that is important in determining the permeability of membranes to solute molecules is the presence of ionic charges. This dependence has been examined by Kopecek and Lim(15) using membranes of 2-hydroxy ethyl methacrylate (HEMA) with methacrylic acid (MAA) and 2-diethyl amino ethyl methacrylate (DMAEMA). The ionization of the later two monomers enables the membrane to acquire a positive or negative charge.

It was found that membranes with permanent positive or negative charges exhibited low permeability presumably due to Donnan's

excluding effect; the transport of the coion will be slowed down, so that, with respect to the necessity of the conservation of electroneutrality, the diffusion of the salt as a whole will be slowed down.(15)

Ampholytic membranes on the other hand exhibit very high permeability as in this case the electric charge leads to an acceleration of the diffusion of both ions. pH is of critical importance as this determines the degree of ionization of the functional groups in the membrane.(15)

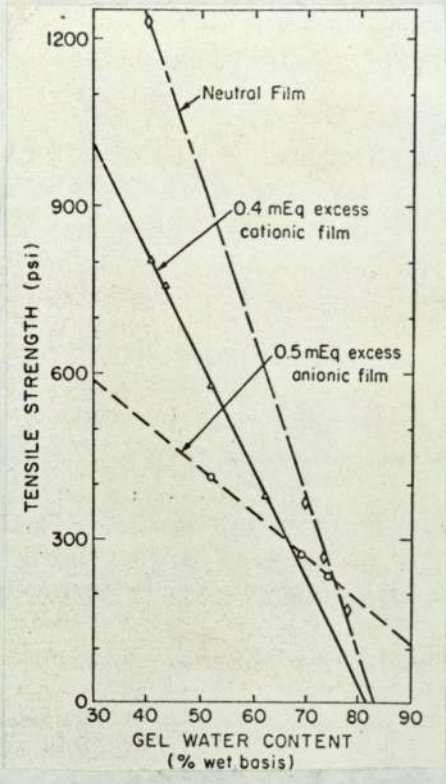
(c) Mechanical Strength

There have been some studies of the mechanical strengths of hydrogels in relation to their other properties such as equilibrium water content, chemical composition and physical make-up of the gel. But most studies, however, have been on the dry dehydrated hydrogel, the xerogel,and are not of interest in this review of properties as they tell us nothing about the probable mechanical strength of the water swollen hydrogel.

The best known relationship between mechanical strength and another property is that between strength and water content. This relationship is well illustrated in Figure 7, which shows the tensile strength of several polyelectrolyte complexes of different water content. As can be seen there is a drastic fall-off in strength as water content increases; this inverse relationship is known for all hydrogels.

FIGURE 7[15]

Tensile strength vs water content for Ioplex 101 film



(d) Biocompatibility

The biocompatibility of hydrogels is, of course, the most important property of hydrogels from the point of view of biomedical application and for this reason is discussed in detail in a separate chapter, Chapter 2. Briefly, however, the biocompatibility of hydrogels in comparison to other materials is believed to be due to the fact that they present a less abnormal surface for blood and tissue to interact with than other hydrophobic substances. The lack of such an abnormal interface does not cause the blood or tissue to be disturbed into unusual physiological reactions, such as blood clotting.

The presence of water in the hydrogel is a most important factor in lessening the possibility of undesirable interactions but it is not the only, or necessarily most important, one.

(e) Optical Properties

A further property, which is obviously important in some of the biomedical applications of hydrogels, such as contact lenses and vitreous implants, is the clarity, or otherwise of the hydrogel. The prime determinant in whether a hydrogel is clear or not is the distribution of water molecules in the hydrated polymer. If the water is dispersed homogeneously throughout the hydrogel then the hydrogel is clear. If however the water is dispersed into two phases, one of which is the hydrated polymer with the water molecules intimately associated with the polymer molecules, and the other bulk water within the hydrated polymer matrix; then if the bulk water region is significant compared to the wavelength of light in size, there will be regions within the polymer of different refractive indices, which will cause light scattering and hence translucency and opacity.

1.3 THE BIOMEDICAL APPLICATIONS OF HYDROGELS

1.3 (a) INTRODUCTION

Hydrogels are one of the main groups of materials, which have been considered for biomedical applications. In the following pages a review of the uses and attempted uses of these materials in the biomedical field is given. The division of their applications into separate areas within this field is to a certain extent rather arbitrary as applications can overlap; however, the division of the field decided upon is that shown in Table 1. In addition, for completeness sake, the biomedical uses of the linear uncrosslinked hydrophilic polymers manufactured from the same monomers as the hydrogels are included because, once again, the applications of these two sets of materials overlap. Also certain of the linear, synthetic non-ionic polymers yield water-insoluble polymers as their hydrophilicity is so low and therefore it is difficult to know which category they should be put in.

Finally it should be noted that the main emphasis of the review will be on the applications of hydrogels and linear hydrophilic polymers manufactured from synthetic monomers, rather than hydrogels made from natural products such as the cellulosics (and other polysaccharides), and collagen. This emphasis is necessary to confine the scope of the review.

TABLE 1

Biomedical Uses of Hydrogels and Linear Hydrophilic Polymers

(1) Reconstructive Surgery	Plastic surgery
	Replacement of ducts and canals
	Replacement of hard tissues
(2) Ophthalmic Applications	Soft contact lenses
	Scleral buckling agents
	Glaucoma drainage devices
	Artificial vitreous implants
(3) Artificial Kidney Membranes	Dialysis
	Ultrafiltration
(4) Coatings for Miscellaneous Biomedical Devices	Sutures
	IUD's
	Urinary catheters
	Artificial heart components
	Blood detoxicants
(5) Vehicles for the Release of Physiologically Active Compounds	Antibiotics
	Contraceptives
	Anticoagulants
	Anti-bacteria agents
	Anti-cancer drugs
	Antibodies
	Drug Antagonists
	Enzymes
(6) Pharmaceutical and other uses of linear synthetic hydrophilic polymers	Plasma substitutes
	Pharmaceutical applications
	Miscellaneous applications

1.3 (b) RECONSTRUCTIVE SURGERY

The first application of hydrogels which is considered is their use in replacing the various tissues in the body:- reconstructive surgery. This field can be further subdivided into plastic surgery, the replacement of ducts and canals in the body and the substitution for miscellaneous other tissues, including the tissues of the eye, (which for simplicity's sake is considered in the next section which deals with all ophthalmic applications of hydrogels).

(1) Plastic Surgery

One of the practical ways in which hydrogels have been used to help people is in the field of plastic surgery, so far mainly for breast and nose prostheses; the principal leaders in this field have been the Czechoslovaks who first suggested their use as biomaterials in 1960.[16]

PolyHema was reported as having been used for augmentation of the breasts in 1968. It was developed for this use because of the substantial decrease in volume or complete resorption, which has been found when autogeneous fatty tissues are used for transplants. Also, the operations necessary for obtaining the autogeneous tissues leave many scars on the patient's body.[17]

The workers involved felt that in contrast to the hydrophobic materials which have been used such as poly (methyl methacrylate), polyethylene and the silicones; hydrogels of poly (HEMA) would have the advantage of being permeable to the transfer of fluids

and would thus, not present an impermeable barrier to all physiological processes. Other advantages are the chemical stability, biocompatibility and ease of modelling. In addition, the gels can be prepared with a wide variety of mechanical properties and hence their consistency can be matched to the original tissues.[17]

The same gel, poly (HEMA), has also been used in plastic surgery operations on the nose. Between 1964 and 1970, this gel was implanted into 62 patients with nose malformations in Czechoslovakia. In a check up, (1973), most were found to be satisfied with the results of the operation with no complaints about the long term application of the gel.[18]

(2) Replacement of Ducts and Canals

Hydrogels have also been considered as coatings for ureter[19] bile duct[20] and esophagus[21] prostheses; the use of these prostheses has so far only been attempted in experimental animals and not in human beings, unlike the previous application.

(a) Ureter Prosthesis - experiments have been carried out in dogs in which the ureter was replaced by a new type of ureteral gel prosthesis, consisting of a tube of knitted polyester fibre encased in a layer of poly HEMA gel. The prosthesis was found to be non-irritating and conducted urine satisfactorily; other materials have been unsatisfactory.[19]

The poly HEMA gel, (~50% water), was used for the construction

of the prosthesis, but since this is a very flexible and rather weak material, to facilitate suture fixation and prevent bending strictures, the prosthesis was reinforced with polyester fibres. The success of this prosthesis over other materials is believed to be due to the slight transfer of water from the isotonic tissue fluid on the outside to the hypertonic urine in the inside through the hydrophilic gel; this permeation is believed to preserve a clear inner surface on the prosthesis, preventing incrustation.(19)

(b) Bile duct prosthesis There has also been interest in the possibility of using a poly HEMA gel tube as a bile duct prosthesis, this stems from the lack of a suitable artificial conduit to replace a diseased or damaged common duct segment. A high success rate was reported for the animal experiments, 13 of the 14 dogs whose common duct was replaced survived for six or more months. The workers concluded that poly HEMA's hydrophilicity and permeability may provide a compatible bile-plastic interface suitable for use in this application.(20)

(c) Esophagus Prosthesis Artificial conduits to replace resected segments of thoraic esophagus have been extensively investigated and a variety of plastic materials have been proposed including woven Teflon, nylon mesh, silicone rubber and others, but experimental and clinical failures in the application of these synthetic channels have been found. On the basis of reported experience the requirements for a successful permanent esophageal substitution have been decided upon,

and to fulfill these criteria poly HEMA was selected in the form of moulded supple cylinders reinforced with a Dacron fibre matrix. Unfortunately the prosthesis was found to be only successful in about half the experimental animals it was tried on, but the failure has been attributed to the other materials, Dacron and Teflon felt, used in the prosthesis and it is believed by the workers in this field that poly HEMA's distinctive properties permit its modification towards the development of an important esophageal substitute.(21)

(3) Hard Tissue

More recently poly HEMA with suitable fillers have been investigated as replacements for hard tissues, such as bones and teeth, but at the moment it seems that the materials are still very much inferior to bone in their mechanical properties. There is interest in hydrogels in this application over other materials such as polyethylene and PMMA because of the better tissue biocompatibility exhibited by hydrogels.(22)

Their present inferior properties need not however rule these composites out for applications in which mechanical strength is not so important and crosslinked pHEMA with $BaSO_4$ and silica has been suggested as a tooth root canal filling composition.(23)

There are in addition to the previously mentioned applications others applying to the replacement of tissues by linear synthetic polymers and these are discussed in the final section on linear hydrophilic polymers.

1.3 (c) OPHTHALMIC APPLICATIONS

The most important commercial ophthalmic application of hydrogels has, of course, been that of the development of soft contact lenses, but there are others and these will be the main ones discussed in this review as soft contact lenses have been reviewed in detail by others.[24][25]

(1) Soft Contact Lenses

Briefly, the properties of hydrogels which make them suitable for this application are their transparency, their high water contents which allow the passage of oxygen into the cornea, (thereby helping to maintain a normal corneal metabolism), and their softness, which prevents the damage to the eye, which can occur with hard contact lenses. Many hydrogels are being used in this application but the most important ones at the moment are poly HEMA and copolymers of hydroxethyl methacrylate with monomers such as N-vinyl pyrrolidone.

Other important uses of hydrogels in ophthalmological applications are as glaucoma drainage devices, scleral buckling agents and as replacements for tissues of the eye, and these uses are dealt with on the following pages.

(2) Scleral Buckling Agents

When the retina separates from the wall of the eye, because of traction exerted by vitreous structures, trauma, or disease, it loses contact with its source of nutrition, the

blood vessels of the choroid. Retinal detachment, if left untreated causes blindness; however, the traction on the retina can be counteracted by indenting the wall of the eye from the outside in the area of the retinal break.[26]

Several absorbable and non-absorbable scleral implants have been used in retinal detachment to produce a buckling effect. Implants made of materials that swell postoperatively such as absorbable gelatin[26], or a non-absorbable material such as poly (glyceryl methacrylate) hydrogel[27], can be used to produce scleral buckles. However, full indentation is somewhat delayed by the gradual swelling process.

(3) Glaucoma Drainage Device

Another area of ocular surgery for which hydrogels have been tried experimentally is for glaucoma drainage devices. Glaucoma is a serious eye disease causing tension, hardening of the eyeball and a rise in pressure within the eye which destroys the visual nerve fibres. One method of treatment which has been attempted experimentally on rabbits is to partially insert a strip of poly HEMA into the anterior chamber; the strip then swells and softens on hydration to plug the surgical incision. The strip permits the slow drainage of fluid with a consequent lowering of intraocular pressure. This device was found to significantly mitigate elevation of intraocular pressure following subconjuctival injection of concentrated sodium chloride solution into the eyes of rabbits.[28]

(4) Artificial Vitreous Implants

In some complex cases of retinal detachment, it is useful to supplement expand or replace the vitreous humour of the eye. Many materials have been tried, among them have been bio-degradable collagen gels[29] and acrylic gels[30], these have been used on animals and a limited number of patients. The most successful one appears to have been poly (glyceryl methacrylate) hydrogel, with about 98% water by weight in physiological saline. The dehydrated gel is placed in the vitreous cavity through a small incision and then swells by absorbing injected saline and available intraocular fluids. The final volume of the gel being known prior to implantation.[31][32] The softness of these hydrogels approximates that of the vitreous body and they are well tolerated by ocular tissues.[26]

It has even been suggested that the total intraocular content of the eye might be replaced by a PGMA hydrogel ball in desperate cases of glaucoma, phthisis bulbi, retinal detachment and vitreous opacities.[27]

1.3 (d) ARTIFICIAL KIDNEY MEMBRANES

Hydrogel membranes of the three main types have been suggested for the two main different types of artificial kidneys, examples of hydrogels for these are given on the following pages.

(1) Dialysis

In dialysis we are attempting to remove blood toxins from the blood stream of patients whose kidneys (which normally remove toxins), have failed. Membranes currently used in haemodialysis consist of cellulose film regenerated from ammoniacal copper hydroxide solution, e.g. Cuprophane or from a xanthate derivate, such as Cellophane and Nephrophane[31], but it is felt that better membranes possessing improved permeability and higher selectivity towards blood components, in addition to better biocompatibility could be made with hydrogels.

A variety of membranes manufactured from hydrogels have been suggested for haemodialysis, a list of some of them is given in Table 2.

TABLE 2

Hydrogel membranes suggested for haemodialysis

Non-ionic	Poly vinyl alcohol[33]
	Poly (2-hydroxy ethyl methacrylate)[34]
	NVP-EA and NVP-ST[35]
	AA-Butyl methacrylate and AN-DMAEMA[36]
Others	Poly electrolyte complexes[37]
	Collagen[38]

In addition to those membranes mentioned already, a wide variety of dialysis membranes have been manufactured from solvent cast polymer blends, one of the components usually being PVP. Those manufactured include:-

a) PAN-PVP[39][40]

b) Cellulose nitrate - PVP[41]

c) Cellulose acetate - PVP[42]

d) Polyurethane - PVP[43]

(2) Ultrafiltration

Ultrafiltration has long been considered a potentially attractive means of separating molecules in solution on the basis of size and shape but the process has never been widely used because of the unavailability of membranes that combine high permeability with high permselectivity. However, the permeability of P.E.C's has been shown to be high and their permselectivity can be controlled within fairly narrow margins; these factors have led to their development as practical ultrafiltration membranes.[37]

Ultrafiltration offers the advantage of a more complete detoxification of uremic patients. This is because conventional extracorporeal dialysis relies on diffusion and the removal of a solute depends on its size, which gives rise to the observation that small solutes such as urea are removed by dialysis more readily than larger solutes such as creatinine and uric acid and it is widely believed that large molecular weight solutes,

the so-called "middle molecules" are very important in uremic symptomatology. In addition the rate of solute removal falls exponentially with time following a first order curve, as a result dialysis efficiency is low at low plasma solute concentration.(44)

Ultrafiltration has the advantage of removing solutes small enough to pass through the ultrafilter in proportion to their plasma concentration rather than their concentration gradient as with diffusion. With the driving force being a pressure gradient rather than a concentration gradient there would not be as sharp a reduction in solute removal at low plasma concentrations as seen with diffusion. In addition, the rate of solute removal would be proportional to the applied pressure and so this could be adjusted to meet the needs of the clinical situation.(44)

As mentioned before it is P.E.C's such as those formed by Na S.S. and V.B.T.A.C have been examined and have been found to give a variety of molecular weight cut offs so membranes can be made which remove all solutes molecules of lower size than the plasma proteins. Since ultrafiltration removes blood solutes which are essential such as glucose it is necessary to reconstitute the plasma continuously with a reconstituting fluid composed of physiological quantities of the major non-protein components in aqueous solution.(44)

In addition, other types of poly electrolyte complexes have been examined as possible ultrafiltration membranes, these

include copolymers of acrylonitrile, such as those prepared from AN-1,6-dimethyl-3-vinyl pyridinum methyl sulphate and AN-Na methallyl sulphone copolymers[45]
ACN-Na M.A.S. + ACR-3 vinyl-6-methyl pyridine[46]

(3) Hollow Fibres

These are in a sense a subclass of the ultrafiltration membranes; their structure is that of an inner skin of low porosity overlaid by a much more porous sponge region, which is a rigid and highly-voided domain containing straight through channels with diameters in the 1-5 micron range.[47]

It is believed that they have a wide application in the biomedical field in blood oxygenation, treatment of enzymatic and cellular deficiencies (e.g. hepatic necrosis, diabetes).[47]

To date, it is mainly hydrophobic materials which have been investigated for the manufacture of these materials but linear hydrophilic polymers are being used in some cases to manufacture these fibres.[48][49][50]

In addition to their potential in haemodialysis there has also been a recognition of the potential as an artificial capillary bed in which cultured cells could be grown in a compact structure resembling an artificial organ. Among the cells which have cultured in this artificial capillary system are choriocarcinoma cells, (to synthesize chorionic gonadotrophin), pancreatic beta cells, (to synthesize insulin) and in work described later, in the chapter on Artificial Liver Support,

cells of hepatic origin have been used to perform a complex hepatic function.[51]

The hollow fibre artificial capillary bed is well suited to cell growth because it is dimensionally stable with strong membrane surfaces which grow to a minimum volume.[51]

1.3 (e) BIOMEDICAL USES OF HYDROGEL COATINGS

Hydrogels, because of their biocompatibility, have been suggested as coatings for various devices and materials. These are listed in Table 3.

TABLE 3

Biomedical Uses of Hydrogel Coatings

(1) Suture coatings
(2) I.U.D. coatings
(3) Urinary catheter coatings
(4) Artificial heart component coatings
(5) Blood detoxicant coatings

(1) Sutures

Toxicity tests in vitro using cell cultures have shown that the protective effect of coating a suture with poly HEMA is impressive. The multiplication of cells found in the presence of a polyamide fibre represent about 20% of the control cell population. When, however, the fibre coated with poly HEMA gel was used, the growth action was 80% of the growth action of the control cell population. Also, the cells cultivated in the presence of a fibre coated with poly HEMA gel show almost no difference in appearance from control cells. On the other hand, cells in cell cultures in the presence of bare fibres became distorted and impaired.(52)

It has also been found, on trials, on dogs, that, whereas surgical sutures made of terylene commonly provoke a foreign

body reaction in the tissue and the formation of blood clots in trials in which poly HEMA has been used to coat the terylene sutures, these unfavourable reactions are almost totally eliminated. The experiments suggested that the use of similarly coated sutures might enable wounds to heal and small blood vessels to be sutured without the risk of them becoming blocked.(53)

The differences between the effects of the coated and uncoated sutures have been again attributed to the hydrophilicity of poly HEMA, which reduces the fluid to solid interface. The authors conclude that, while the fact that poly HEMA coated sutures appear not to promote the formation of blood clots is of great importance, the absence of the violent forming body giant cell reaction to the coated sutures may have wide applications. It may help in lowering the incidence of dehiscence of sutured wounds, gut anastomoses and have uses in inter-cardiac repairs and artificial or grafted heart valves.(53)

In addition, appropriate biologically active species, e.g. antibiotics, prevented either the development or spread of an infection along the fissure. The antibiotics may be incorporated by diffusion into the gel layer. Neither the knotting ability nor other mechanical properties of the fibre are influenced by the gel coating.(52)

(2) Coatings for I.U.D.'s

Polyethylene I.U.D.'s wrapped with copper wire have good contraceptive effectiveness, but some anxiety still exists about

fragmentation and systemic toxicity. A copper bearing I.U.D. coated with hydrogel appears to lend itself to the minimization of expulsions, composition changes, bleeding and pain according to studies carried out on rabbits. This improved tolerance and contraceptive effect leads the authors to believe that these devices can be used by women.(54)

(3) Latex Urinary Catheters

A problem with untreated latex catheters is the accumulation of calculus on their surfaces; this is usually encountered in patients with urinary tract infections owing to bacteria which provide urease to hydrolyse urea to ammonia. The formation of this ammonia leads to a rise in pH and effects the solubility product relationships of the dissolved compounds, resulting in precipitation of various salt crystals, some of which form on the walls of the catheter, which provides sites for nucleation. This accumulation is a clinical annoyance and it results in obstruction of the catheter which necessitates its replacement.(55)

It has been found that if poly HEMA (which bonds well with latex and adds a soft semipermeable membrane to the rubber), is coated on catheters, the deposition is significantly less. Poly HEMA is believed to act in this way because it modifies the interface between the latex and the solution which is contained so inhibiting crystal formation. The modified latex catheter has so far only been tried in vitro.(55)

(4) Components of Artificial Hearts

Ioplex 101 and 103 have been investigated as coatings for light weight fabrics, including those made from glass, Dacron, polypropylene and others, for making thromboresistant materials for use in artificial heart valve components. Methods for the adhesion of Ioplex 101 to both flexible and rigid substrates have been developed and several of these reinforced Ioplexes have been evaluated in in vivo tests.(56)

(5) Blood Detoxicants

One of the most important applications of hydrogels as coatings has been in the field of blood detoxicants; hydrogels, such as poly HEMA, have been used to coat materials like charcoal granules to make them more biocompatible, but this application of hydrogels is more properly discussed in the chapter dealing with artificial liver support systems.

1.3 (f) VEHICLES FOR THE RELEASE OF PHYSIOLOGICALLY ACTIVE SUBSTANCES

Hydrogels have been considered as vehicles for the release of many physiologically active substances, in addition they have also been used to encapsulate or immobilize materials for various purposes; their uses in this field and the reasons for their application to it are described on the following pages.

The reasons for interest in hydrogels as vehicles to release active substances are manifold, First of all, by placing the gel vehicle in the region of the target organ or tissue, the effect of the drug or other substance is localised in one area and the rest of the body will not suffer toxic side effects from the substance. Secondly, if a zero-order release rate can be obtained then the concentration of the drug in the blood will remain constant for a long time and the wide variation in blood levels of drugs encountered with other methods of delivery, i.e. oral or injection, will not be seen. Thus, for these and other reasons, there is a great deal of interest in hydrogels in this general application. The substances which have been entrapped or immobilized in hydrogels are shown in Table 4.

TABLE 4

Substances Entrapped, Diffused into or Immobilized with Hydrogels[2]

Antibiotics
Anticoagulants
Anticancer drugs
Antibodies
Drug antagonists
Enzymes
Contraceptives
Antibacterial agents

Examples of the proposed uses of these materials with hydrogels are shown in the following pages.

(1) Antibiotics

These are the materials, which probably have the most general application as agents released by hydrogel vehicles, as has already been mentioned they have been infused into prosthetic implants in the general area near the implant.

It is believed that hydrogel vehicles for the release of antibiotics would be especially suitable for application into tissues and spaces attacked with primary or secondary infection as they permit a protracted release of the drug into the immediate environment and thus attain the optimum concentration. This is especially advantageous in media where the required concentration cannot be attained by other application methods. Therefore they can be used with advantage in areas of the middle ear, the pneumatic system of the mastoid process and the area of the respiratory tract.(57)

Another major area in which they can be used is as ocular insert devices to deliver medication directly to the eye. One method of doing this is to use drug-impregnated hydrogels, which will render the drug into the tear film at a variable rate dependent upon the concentration difference; another is to make a vehicle of the drugs thoroughly mixed with a soluble or biodegradable polymer, such as gelatin which will deliver the drug at the rate of degradation or dissolution of the polymer in the tear film.(26)

(2) Anti-narcotic Agents

As has been previously mentioned among the advantages of implantable, sustained release drug delivery devices over oral ingestion or injection are delivery at a constant therapeutic rate, thus avoiding intermittent and massive dose effects, as well as reliance on patients taking their prescribed dosages. These advantages offer the solution to the problem of drug antagonists.(58)

Drug antagonists are anti-narcotic agents which are given to addicts to help in their cure; they have the effect of blocking the action of the narcotic on the central nervous system by crossing the blood-brain barrier and occupying opiate receptor sites on the nerve tissue that narcotics normally occupy. However, a major drawback to their use is that their potency is short lived so that the patient has to return for an oral dose of the antagonist on a daily basis; this results in a high failure rate by addicts taking this treatment.(58)

Poly HEMA was chosen as a suitable gel vehicle to overcome the problems of sustained release over a long period and also of tissue compatibility; it was found that by altering the rate of diffusion of the drug through the barrier membrane, a zero order diffusion rate could be obtained, and this approach seems to be a highly promising one for the treatment of drug addicts.(58)

(3) Anti-tumour Agents

A further use for which hydrogels are being considered is

as release devices for drugs is that of the local chemotherapy of inoperable malignancies. Here again the aim is to create a high concentration of the drug in the affected region, (in this case a tumour), without flooding the rest of the body with chemotherapeutics. Wichterle has described a pouch shaped poly HEMA device which can be applied on the surface of the tumour and which delivers the drug at the optimal rate by diffusion.(59)

The most important part of the device is the membrane through which the diffusion occurs; the rate of diffusion is controlled by the chemical and physical properties of the membrane and Wichterle has looked at the HEMA/butyl methacrylate system in this connection.(59)

Also investigated, has been the use of a hydrogel formed by polymerizing L-asparaginase, acrylamide and NN'MBA, which gives an ACR-NNMBA copolymer matrix containing embedded L-asparaginase, which retains its enzymatic activity longer than free L-asparaginase and is more effective for the treatment of leukemia and lymphona.(60)

(4) Fertility Control

Another group of physiologically active chemicals that hydrogels are being used to release are the prostaglandins; this work is being done with a view to fertility control in humans, but at present it has only been tested on animals. The hydrogels used have been PVP, PVP with another polymer such as silicone

rubber and poly (acrylamide).[60] The purpose of the PVP is described as being to stabilize the chemical potency of the prostaglandin.[61]

It was found that PVP-silicone rubber implants containing prostaglandin E2 could suppress fertility in female hamsters without apparent side effects.[62] A similar device was also found to be capable of inducing abortion in rats, hamsters and rabbits.[63][64] The PACR prostaglandin device also terminated pregnancy when implanted into hamsters.[61]

(5) Miscellaneous Applications

In addition, many other applications have been suggested for hydrogels as release vehicles or supports for immobilized materials, these include.

(a) Controlled release of fluoride (for dental applications) from ribbon-shaped HEMA/MMA copolymer devices.[65]

(b) Enzyme replacement therapy using spherical polymer beads containing entrapped active enzyme.[66][67][68]

1.3 (g) LINEAR SYNTHETIC HYDROPHILIC POLYMERS

Lastly, the biomedical uses of the linear hydrophilic polymers whose uses in many cases overlap and interrelate to those of the hydrogels, will be considered. These materials in the main have pharmaceutical applications; their various biomedical uses are illustrated in Table 5.

TABLE 5

Biomedical uses of linear hydrophilic polymers

(1) Blood plasma substitutes

(2) Pharmaceutical applications:

- (a) Tablet disintegrators
- (b) Tablet binders
- (c) Increasing solubility of drugs
- (d) Increasing drug stability
- (e) Germicidal and bactericidal compositions
- (f) Wound dressings
- (g) Miscellaneous uses

(1) Blood Plasma Substitutes

One of the most publicized early uses for poly (N-vinyl-2-pyrrolidone) (PVP), was as a blood-plasma substitute. Research conducted by Reppe and Weese[69] in 1940 showed that this use was possible because of the low intravenous toxicity and ability of the homopolymers to absorb and eliminate toxins, viruses and drugs in the bloodstream.[70] It was later found that the PVP collected in the bone marrow and organs of those treated.

Since then a variety of other hydrophilic materials have been examined as possible blood plasma substitutes and infusion solutions, such as the gelatin and dextran products[71], polymers of N-ethylmethacrylate, NN'-dimethylmethacrylamide, glycerol monomethacrylate and others[72].

(2) Pharmaceutical Applications

(a) Tablet disintegrators

The high water absorbability of polymers such as lightly crosslinked PAA and PMAA, crosslinked PVP and cellulose derivatives are being evaluated for increasing the dissolution efficiency of tablets, e.g. PAA and PMAA have been evaluated for the disintegration of materials such as valnoctamide, (which are difficult to disintegrate in water), tablets containing these polymers were found to cause more rapid disintegration if added than controls.[73]

(b) Tablet binders

Tablet binders that have been tried to improve tablet strength, physical standards and dissolution characteristics, include PVP, gelatin, starch, methyl hydroxyethyl cellulose and others. PVP and gelatin have been judged the most suitable for certain preparations.[74]

(c) Increasing the solubility of drugs

Due to their high hydrophilicity, the linear hydrophilic polymers can be used to make hydrophobic drugs much more water soluble by coupling them. Among the antibiotics and hormones which have been coupled with NVP copolymers are the penicillins,[75] tetracycline,[76] ampicillin,[77] insulin[78][79] and others.

By carrying out this process and increasing their water solubility it has been found to be possible to increase their concentration in body fluids and also to increase their stability and hence prolong their effective life, e.g. in the case of tetracycline derivatives reacted with NVP copolymers, these were approximately 700 times more soluble than tetracycline base, and on intra-muscular injection into animals the tetracycline levels in the lymph and blood were increased[76] and in the case of insulin, preparations bound to NVP-crotonic acid copolymers have been shown to have a stronger and more pronounced hypoglycemic activity than pure insulin or to act for longer periods, depending on the nature of the linkage to the polymer.[78]

(d) Increasing drug stability

PVP has been found to complex with certain drugs such as resorcinol, salicylic acid, sulphathiozole, aminophenazone and others. This complexing interaction has been used to make sublingual nitroglycerine tablets, which show vastly improved stability over existing brands. The solid phase interaction lowers the fugacity of nitroglycerine in the dosage form.

However, in an aqueous environment the complex is not stable, because of preferential solvation of PVP by water so that the nitroglycerine is released.[81][82]

(e) Germicidal and bactericidal compositions

The complex formed by PVP and iodine has been used for a wide variety of applications including drugs, dermatological salves, gargles, tablets, aerosols[83] abrasive cleansing compositions.[84] PVP reacts with iodine to form a water soluble compound which retains the germicidal and chemical nature of iodine but is more stable, less irritating and less toxic.[85]

Other complexes with PVP, which have been tried in the above-mentioned applications and others, are those with di- and polyiodides,[86] the interhalogens (ICℓ, IBr), (for deodorants and disinfectants).[87]

(f) Wound and burn dressings

The basic idea in most cases for this application is that the dissolved hydrophilic polymer, (in a volatile solvent such as ethanol), is applied by an aerosol spray onto the wound. The solvent dries off leaving a hydrophilic film over the effected area. Films of this sort exhibit good skin adhesion and elasticity; they also have the advantage that they can be easily removed by washing with water as they are water soluble.[88][89]

(g) Miscellaneous applications

In addition to those applications already mentioned, a wider range of uses exist for these materials in the cosmetic field and in other miscellaneous applications, these include:

(i) Synthetic physiological mucus - a vaginal and surgical lubricant having characteristics similar to natural mucus based on a solution of high molecular weight polyacrylamide.(90)

(ii) Artificial lubricants for the joints in arthritis and osteoarthritis and in artificial joints - aqueous solution of PVP and PVP-hyaluronic acid complex.(91)

(iii) Stabilization of liquid smallpox vaccine by PVP, PVAc and dextran.(92)

(iv) Antidiorrhea pharmaceutical composition containing PVP.(93)

CHAPTER 2

BIOCOMPATIBILITY

2.1 INTRODUCTION

In this chapter biocompatibility will be dealt with, but since this is a very large field it has been necessary to confine the subject matter a great deal, and so most of the material describes work on hydrogels and related areas of research, in addition to giving a basic review of the nature of blood and biocompatibility. Other areas are mentioned briefly.

2.2 BIOCOMPATIBILITY

There are two main types of biocompatibility - tissue and blood compatibility. As has been shown in the last chapter, hydrogels of various sorts are thought to possess these properties or, at least, if not the property of compatibility then the property of tolerability. This chapter will be concerned exclusively with blood compatibility, which is a property necessary for a viable artificial liver support system.

Bruck has listed the effects that materials that are to be useful for blood-contacting biomedical applications should not cause as being: (1) thrombosis, (2) destruction of the cellular elements of the blood, (3) alteration of the plasma proteins, (4) destruction of enzymes, (5) depletion of electrolytes, (6) adverse immune reactions, (7) toxic and allergic reactions.[94]

Before discussing blood compatibility and the factors that influence it we must first consider the nature of blood and its component parts.

2.3 (a) BLOOD

Blood is a heterogeneous system composed of cellular elements (red blood cells, white blood cells and platelets), which are suspended in the plasma. The plasma constitutes the fluid portion of the blood and represents 5% of the body weight. Blood is about five times as viscous as water, the viscosity depends largely on the cellular elements and to a lesser degree on the concentration of plasma proteins.[95]

The blood cells are chiefly erythrocytes and together with the others they make up 40-45% of the blood volume[96] because of their importance in blood compatibility they will be discussed individually on the following pages, followed by a brief outline of the plasma proteins.

2.3 (b) ERYTHROCYTES (RED CELLS)

These are derived from primitive nucleated cells in the bone marrow and go through several stages of development[97] the last of which is to the mature erythrocyte, (the main erythrocyte type in blood).[98] These mature erythrocytes are nonnucleated biconcave discs about 7μ in diameter and their main function is the transport of oxygen from the lungs to the tissues.[97]

2.3 (c) LEUKOCYTES (WHITE CELLS)

These cells differ structurally from erythrocytes in many particulars, such as being larger in size and possessing ameboid movement.(99) There are several different types of white cells, but collectively they are few in number compared to the red cells.(99) The most important function of these cells is to combat foreign substances that enter the body, for this the most important of the white cells are the neutrophils, (which make up 60-70% of the white cells); they act by moving towards the substance attracted by chemicals liberated from the tissue damaged by the invading material and then engulf it, (phagocytosis). Then cell intracellular enzymes go to work and effectively destroy or neutralize the substance.(100)

The other types of white cells, such as monocytes, basophils and eosinophils function in a similar way to neutrophils, but it is thought that they may have other specific functions in combating invasion. In addition, basophils release heparin into circulating blood, a function which may be essential in preventing intravascular clotting.(100)

Another type, the lymphocytes are something of a mystery with many roles being assigned to them.(100)

2.3 (d) THROMBOCYTES (PLATELETS)

Platelets are small colourless, usually spherical or oval shaped and vary from 2 - 4 μ in diameter. Their morphology changes with the nature of the contacting surface, presence and

concentration of an anticoagulant and other factors.

The known functions of platelets in the body are three; they participate in haemostasis, serve as vehicles for pharmacologically active agents and contribute in endothelial integrity.

(1) Haemostasis The arrest of haemorrhage in small blood vessels is largely due to platelet activity. Platelets perform a mechanical function by adherence to the cut end of vessels, additional platelets forming a platelet plug, that grows by accretion until it presents an effective barrier to further bleeding. In addition changes in the platelet membrane lead to the development of clotting promoting activity (platelet factor 3), which accelerates plasma coagulation.

(2) Pharmacological effects The platelet membrane contains enzyme systems for active transport and concentration of 5-hydroxy tryptamine, catecholamines and probably other pharmacologically active agents. Essentially all the serotonin in the blood is contained in the platelets. These compounds can be secreted by the platelets under the influence of thrombin and other specific stimuli in a "release reaction" with important physiological consequences; their action may account for significant side effects of thrombotic disorders.(101)

(3) Endothelial support Fragility of blood vessels has been described in thrombocytopenia and in qualitative disorders of

platelet function. There is evidence that maintenance of vascular integrity during extracorporeal perfusion is enhanced by the use of platelet rich perfusion media. Although these effects may simply reflect the haemostatic function of platelets, provision of vital nutrients or other activities are possible.(101)

2.3 (e) THE PLASMA PROTEINS

The plasma is the fluid in which the cells are suspended, the total proteins content of the blood is about 7-7.5 gm/100 ml and the plasma proteins comprise the major part of the solids of the plasma. The proteins of the plasma are actually a very complex mixture which includes not only simple proteins but also mixed or conjugated proteins such as glycoproteins and various types of lipoproteins.(102) It is customary to separate the proteins of the plasma into three major groups (albumin, globulin and fibrinogen).

(1) Albumin This fraction accounts for over 50% of the total body pool of plasma proteins. Albumin is the most important plasma protein in the respect of the maintenance of colloid osmotic pressure. Its second major function is one of transport and many substances are thought to be transported by albumin.

(2) Globulins The globulin fraction of the serum proteins is a very complex mixture. The main components are:-

(a) Mucoproteins and glycoproteins - These are combinations of carbohydrate moieties with globulin.

(b) Lipoproteins - These are combinations of lipid and protein, they are believed to function as major carriers of the lipids of the plasma since most of the plasma fat is associated with them.

(c) Metal binding proteins - These are important in transporting metals in the plasma, for example, transferrin which binds iron.

(d) Gamma globulins - These are the principal site of circulating antibodies, the so-called 'immunoglobulins', which constitute a family of closely related proteins possessing all of the known antibody activity of the serum.

(3) Fibrinogen Fibrinogen is the precursor of fibrin, the substance of the blood clot. It normally constitutes 4-6% of the total protein of the plasma. This protein is manufactured in the liver. In any situation where excessive destruction of liver tissue has occurred, a sharp fall in blood fibrinogen results.(102)

2.4 UNDESIRABLE EFFECTS FOR BLOOD COMPATIBILITY

2.4 (a) INTRODUCTION

The undesirable effects which mitigate against good blood compatibility have already been listed. The three most important and interrelated effects of thrombosis, haemolysis and denaturation will now be discussed.

2.4 (b) THROMBOSIS (BLOOD COAGULATION)

This is an extremely complicated, and as yet not completely understood process; in this section a brief outline of the processes involved will be given. The following figures 8 and 9 illustrate current theories.

The most important process in clotting is the conversion of the coagulable protein,fibrinogen, into the polymer fibrin, which gives the clot its strength. This final process is proceeded by a series of reactions involving several blood proteins (factors), which act alternately as substrates in the inactive form and as enzymes in the active form. This is illustrated in Figure 8; it can be seen that coagulation involves a complex chain reaction, the "waterfall sequence".[103]

As is shown in Figure 8, there are two routes to the initiation of blood coagulation; the intrinsic and extrinsic systems. The extrinsic system is important when a person sustains a tissue injury; extracts from the damaged tissue gain access to the blood and clotting is promoted. The other system, the

FIGURE 8

The Intrinsic and Extrinsic Systems for Initiation of Clotting[102]

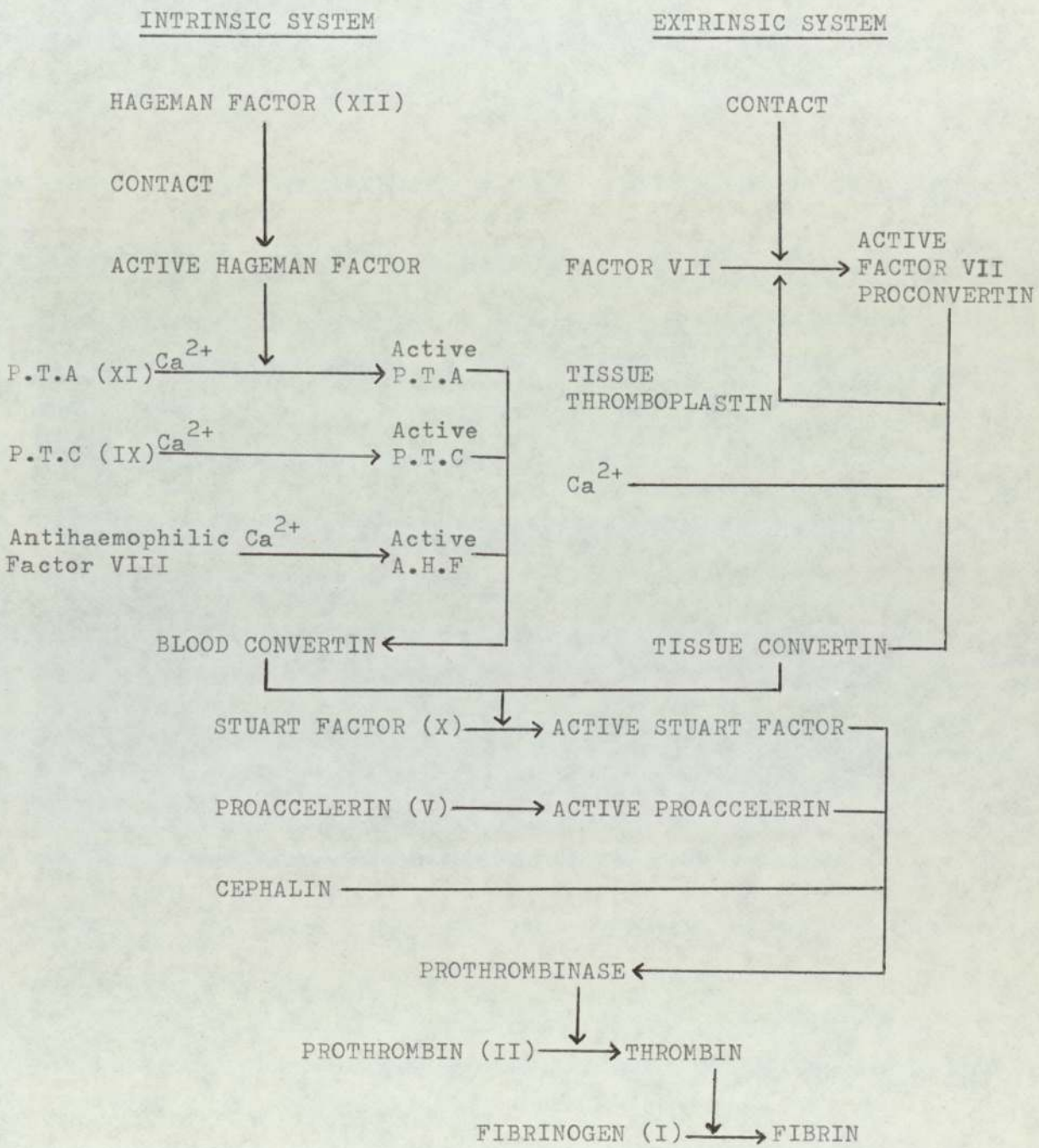
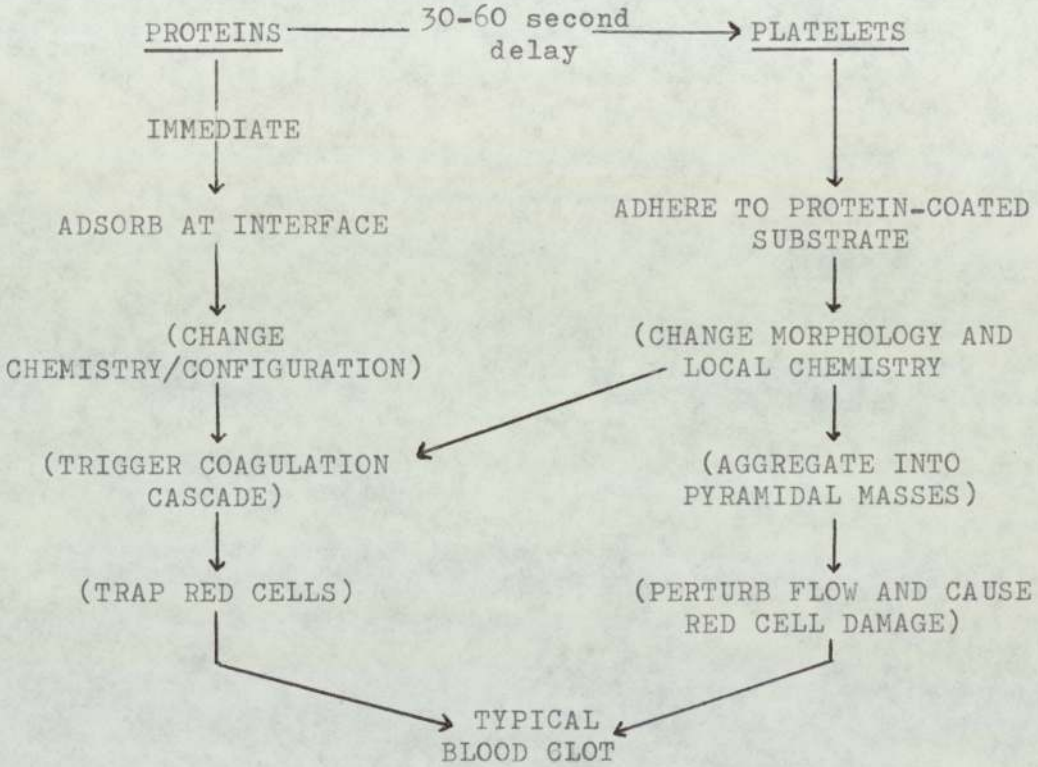


FIGURE 9

Usual Sequency of Interactions When Blood Contacts Strange Interfaces[104]



intrinsic system, which is the important one from the point of view of blood compatible biomaterials, since it is activated by contact with foreign surfaces. It is less well understood. It is believed, however, that Factor XII, (Hageman factor), is activated in some way by surfaces and that it is this factor which is the initial one in the intrinsic sequence.(103)

However, as can be seen from Figure 9,which is a generalised view of what happens when blood contacts foreign interfaces, other blood components are involved in forming a blood clot most notably the platelets whose role in haemostasis has already been briefly described. As shown in Figure 9, platelets adhere to the protein coat which forms on a strange surface, they change shape and thrombin, generated in the course of plasma coagulation, as shown in Figure 9, helps platelet aggregation, probably by causing platelet release of ADP and other platelet clumping agents.(104)

The platelets then aggregate into pyramidal masses which cause the blood flow over the surface to be perturbed, resulting in red cell damage. The red cells are then entrapped in the protein-platelet matrix and the typical red blood clot forms. In addition, platelets also participate by providing a phospholipid surface which functions as an accelerator at several points in the intrinsic coagulation system.(104)

Another important aspect of the clotting mechanism, which should be briefly mentioned is autocatalysis; clotting is a fast process because certain products formed in the coagulation process actually catalyse the reactions by which they themselves were formed.(102)

Thrombosis is a very undesirable effect in haemoperfusion systems etc, since not only does it cause the loss of cellular elements and plasma proteins with subsequent undesirable effects, but non-adherent thrombi if introduced into the bloodstream of the patient can lead to blood clots forming in the patient's body with potentially very serious consequences.

2.4 (c) HAEMOLYSIS

Haemolysis is the liberation of free haemoglobin into the bloodstream. This threatens the kidneys, whose task it then becomes to clear the blood. Curiously a healthy body can tolerate very high levels of haemolysis - up in 1000 or more milligrams of haemoglobin per 100 millilitres of blood. Unfortunately diseased bodies have no such tolerance and therefore haemolysis is a major problem in the perfusion of whole blood. It is avoidable by use of the celltrifuge, (which is described later), but this is only a partial solution.

The causes of haemolysis can be physical or chemical. The physical causes which could be important in perfusion have been investigated by Blackshear.(105) He looked at the effects of pressure, shear stress, prolonged shear stress, turbulence, occlusion and other factors and concluded that the application of shear stress to red blood cells, which had adhered to the foreign surfaces was the cause of lysis. On the basis of a theoretical model, backed by experimental data, he calculated that shear stresses of the order of 1000 dynes/cm^2 were sufficient to cause the tethered red cells to undergo lysis,

although away from foreign surfaces they could withstand shear stress in excess of 40,000 dynes/cm^2.[105] Thus it is the interaction of red cells with surfaces which is the important factor in physically created haemolysis.

2.4 (d) DENATURATION

A further undesirable effect that perfusion can have on the blood is the denaturation of the plasma proteins. A denatured protein is one that has lost its 3-dimensional native structure, following interference with those bonds responsible for secondary, tertiary and quaternary structure. Denaturation can be brought about by a wide range of physical and chemical agents, e.g. heating, extremes of pH, shaking, urea, alcohol, detergents, etc. In general it is the hydrogen bonds and disulphide bridges between cysteine amino acids, that are the most vulnerable to such treatment.

A general model for the process is believed to be the following. The first stage is reversible and consists mainly of rupturing the hydrogen bonds which stabilize the tertiary structure. This leads to random coiling of the polypeptide chain with an increase in viscosity and decrease in solubility. With many globular proteins these early stages of denaturation are characterized by either molecular association or dissociation. If the process continues beyond the first stage, the disulphide bridges may be ruptured and new reactive-thiol groups formed. This stage is usually irreversible and results in complete denaturation of the molecule. At this point the protein is in

an amorphous insoluble form with the polypeptide chains probably in the configuration of fibrous proteins (β-extended) and the protein biologically inactive.[98]

2.5 INTERACTIONS OF MATERIALS AND BLOOD

2.5 (a) INTRODUCTION

In this section the interactions between blood and materials will be dealt with, and the various means of measuring these interactions. The attempts to interrelate surface properties and blood compatibility will be discussed, with the results of the studies on the effects of haemoperfusion and haemodialysis systems on blood. Lastly, the various means which have been attempted to make materials more blood compatible will be given with particular reference to the use of hydrogels.

Since this area is potentially a very large one; it has been necessary to set limitations as to its scope, thus this section deals mainly with hydrogels and areas related to the study of their interactions with blood and the tests, which have been used to measure this.

2.5 (b) MEANS OF MEASUREMENT OF BLOOD COMPATIBILITY

Introduction

Many methods of determining the blood compatibility of materials from direct biological experiments exist; these can be divided into three areas:- in vivo, ex vivo and in vitro.

(1) In vivo tests involve the implantation of the biomaterial into an experimental animal followed by its removal at a later time to find the amount of thrombus, which has formed on it.

(2) Ex vivo tests are similar, but involve passing the blood from the experimental animal through a test device outside the organism, and then back into the animal.

(3) Lastly, in vitro tests involve the removal of blood from the organism, which is then placed in a test device and the time for clotting or some other physiological reaction to occur is measured or observed.

The order of importance of the tests is the same as that in which they have been defined; the tests become less physiologically normal as we move from in vivo to in vitro, although it could be argued that some ex vivo tests resemble haemoperfusion systems, (so knowledge gained from these may be especially applicable to the artificial liver support system devices).

In addition to these tests many other means of measurement have been devised to look at the interactions of individual blood components, such as the plasma proteins and platelets, with surfaces. These tests, in so far as they apply to hydrogels and the general understanding of the field, will be discussed, but it must be borne in mind that the individual interactions of blood components with surfaces are not a true indication of that surface's biocompatibility, as blood clotting is, (as has been shown, Section 2.4(b)) a complex system involving several interacting components.

On the following pages are some of the important tests and methods, which have been applied to hydrogels, (and the results of which will be described later).

(1) INVIVO TESTS

The two most important 'in vivo' tests are the vena cava and renal embolus tests.

The vena cava test involves the insertion of representative polymers as small cylindrical rings into the canine inferior vena cava for periods of two hours in an acute test or two weeks in a chronic test.(104) The purpose of the test is to find whether thrombi form on the surface of the ring. The chief objection to this test is however that it does not indicate whether the material is truly thromboresistant or merely that the surface is non-thromboadherent and thrombi which form are merely swept into the bloodstream to do damage elsewhere. To study this effect the renal embolus test was devised.(106)

The renal embolus test utilizes rings fabricated from the materials to be evaluated, which are implanted in the canine descending aorta just above the renal arteries. A constriction 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 adherent thrombi, and the kidneys are dissected and examined for infarcts, presumably caused by thrombi shed from the ring surface. Thus this test should be able to distinguish between those materials which are truly non-thrombogenic and those which are only non-thromboadherent.(106) For this reason it is the more important test for hydrogels, to which thrombi do not usually adhere strongly because of the physical nature of the hydrogel.

(2) EX VIVO TESTS

The most important of these are the arteriovenous shunt chamber and the stagnation point flow tests,[107] but as these tests do not appear to have been applied to hydrogels they will not be discussed in this review.

(3) IN VITRO TESTS

There are a wide number of these but, as has been mentioned, the validity of the results, particularly as they apply to situations involving contact with flowing blood, have frequently been questioned.[106] The major tests are the Lee White Test and the Lindholm Test.

The Lee White Test compares the coagulation of recalcified whole blood in a test tube made of, or coated with, the material to be evaluated with the coagulation time of blood in a standard control tube (usually glass). There are a large number of variables, which can affect the results from this test, including changing the donor, storage time of the blood, changes in the diet or medication of the donor, venipuncture technique, and variations in the experimental technique used to measure the clotting times.[106] The test is also criticized because of the large blood-air interface which is exposed,[106] since the blood-air interface has been reported to activate the intrinsic clotting system.[107]

There are several variations of this test, for example, the

one described by Courtney et al (Strathcylde)[108] and for the reasons given the results cannot be too reliable. Several of the variants of the technique describe massive changes in the volume of the blood sample leading to dilution of the blood elements, the use of platelet rich plasma and massive amounts of calcium chloride solution; this must make the results less reliable from the point of view of relating them to the normal physiological environment.

The Lindholm Test is also a modified whole blood clotting time test, it is said to be well suited for biomaterials in the form of sheets or films. Fresh blood from a human volunteer is taken and used to cover the bottom of a test cell which has the material of interest on it, and the time for a clot to form is measured.[107]

Workers who have used the Lindholm test on hydrogels have reported that it is difficult to determine the endpoint clot on the slick hydrogel surface and that the movement of small amounts of water from the gel to the blood also make the determination difficult.[107]

ADDITIONAL INVESTIGATION TECHNIQUES

Besides tests on materials using whole blood, there are additionally, various techniques of investigating the interactions of specific blood components with surfaces. These tests, of course, can be applied to the whole blood experiments but are more properly dealt with apart from the previous tests as they are more of an investigative nature, rather than the previously

described series, which seek to give a definitive conclusive answer as to whether materials are blood compatible or not.

The other techniques of studying the interactions of blood components with foreign surfaces are briefly described on the following pages; these are listed in Table 6.

TABLE 6

Some Additional Techniques used in Studying the Interactions of Blood Components with Foreign Surfaces

MAIR Spectroscopy

Ellipsometry

Contact angle measurements

Electron microscopy

Platelet adhesiveness tests

Miscellaneous techniques

MAIR Spectroscopy (Multiple Attenuated Internal Reflection Spectroscopy)

This technique involves the internal reflection of infra-red radiation between germanium prisms. If these prisms have specimen films (plasma protein coated) on them and the infra-red spectrum is taken; it is possible to deduce the nature of the chemical groups present and the conformations of the protein molecules. This makes it possible to determine whether or not the protein film is denatured.(109)

MAIR spectroscopy has also been used to determine the nature of the natural surfaces in the body which are in contact with blood, such as the jugular veins.(110) It was hoped that

by use of this method knowledge would be gained which would enable similar synthetic surfaces to be produced, which would be expected to be blood compatible. However, it is believed that the process of preparation of these materials so change their surface constitution as to make the results misleading.(111)

Ellipsometry

This technique enables film thicknesses to be determined with a fair degree of accuracy. It involves again the use of germanium prisms. From the positions of rotation of an analyzer and quarter wave plate necessary to produce minimum intensity of a polarized mercury lamp beam reflected from the prism faces, it is possible by plotting these values against known film thicknesses of a series of long chain carboxylic acids to calculate an approximate value for the thickness of the plasma protein film. It is necessary to assume that the refractive index of the specimen film is close to that of fatty acid and this is believed to be a valid assumption.(112)

Besides this, a major difficulty in applying this technique to polymer surfaces is that proteins and polymers have similar refractive indices.

Contact Angle Measurements

Various contact angle measurements have been used in the analysis of surfaces with regard to the interrelationship between blood compatibility and surface properties; (this is dealt with

in the next section). In this sub-section their use in determining the effect of protein deposition on surface properties is discussed.

It has been found by means of contact angle measurements that when blood contacts a foreign surface in most cases protein deposition occurs causing the surface properties to be modified. An example of this are the L.T.I carbons, these exhibit critical surface tensions* of 50 dynes/cm before implantation which drops to about 28 dynes/cm after implantation,[109] and it has in fact been found that almost all synthetic materials and metals, (with the possible exception of hydrogels), become coated with proteins exhibiting critical surface tension values in the range 25-28 dynes/cm.[109]

Thus contact angle measurements provide a means of examining the effect of protein deposition on surfaces, and the effect of initial surface properties on thromboresistance and thrombogenicity. The investigations of the latter relationship is discussed in the next section.

Electron Microscopy

This technique can be used to look at the structure of the clotting film, which forms on a foreign surface, for example, the thickness and structure of the film can be determined,[112] and as very high resolution is possible small individual features can be identified. An example of this is that electron microscopy has been used to directly count individual fibrinogen

* This quantity is defined in the next section.

molecules adsorbed onto mica surfaces.[113]

However, as polymer surfaces are rougher and the materials, (polymer and protein), are not so easily distinguishable, electron microscopy is best used to look at the macroscopic effects of clotting, such as the strands and masses of fibrin, the density of cells and their distribution, as well as the actual structure of the cells in the film.[104]

Platelet Adhesiveness Tests

There are several techniques available for measuring platelet adhesiveness; these tests yield valuable information but cannot be regarded as a measure of overall blood compatibility, as platelets are only one part, (although a crucial one), of the blood clotting mechanism.

Many of the tests devised make use of a standard surface such as glass beads and do not lend themselves to evaluation of the thrombogenic character of various foreign surfaces.[114]

Those that do, however, will now be dealt with.

(a) The Rocking Test Cell (Lindsay et al)

This system, designed by Lindsay, comprises two identical test cells and compares the retention of blood platelets by films following contact with human blood. Platelet counts are measured prior to contact with the polymer and after a ten minute rocking cycle. The drop in platelet count expressed as a percentage

of the initial count gives what is termed the platelet adhesiveness. By comparing the results on different films in the two test cells, which have been treated exactly the same way, direct comparisons can be made.(115)

(b) Static Platelet-Polymer Surface Studies (Lyman)

Two techniques have been devised by Lyman.

(i) Blood from a vein is drawn into a siliconized glass cell containing acid citrate. The polymer samples, either as self-supporting films or films coated on glass slides, were held in a clamp and immersed in the blood for one minute then removed and immersed in a stirred rinse solution for about one minute or until all red cells appeared to have been removed. The slides are then fixed and stained; then the average number of platelets on a given surface area determined by standard microscope techniques.(116)

(ii) In the second procedure, a special cell is used to eliminate any undesirable effect resulting from the blood-air interface. The blood enters at the bottom of the vertically clamped cell, displacing a phosphate-buffered saline solution. There is little or no visible mixing of blood and saline and the blood-air interface is eliminated. The flow of blood is continued for one minute after the cell has been completely filled. The surfaces are rinsed, fixed, stained and counted as in the first procedure.(116)

(c) Platelet-Polymer Surface Studies under Continuous Flow Conditions

The previous tests, while valid for comparing two surfaces under the conditions of the test, did not compare membranes under conditions similar to those of haemodialysis, where blood is continuously flowing in response to a pulsatile pressure driving force.

A small-scale in vitro test to compare the adhesion of platelets to various surfaces under conditions of continuous blood flow for various time periods has been devised and tested by Muzykewicz et al.[114]

The device consists of two sheets of membrane placed on plexiglass blocks separated by a gasket. The space between the membranes is filled with distilled water followed by saline solution and then heparinized saline. Then, (after the system has been thoroughly flushed with the final solution), fresh human heparinized blood is first used to flush the system out and then a second aliquot is used for the actual experiment. The tubing is filled with blood and pumped through the system at a constant rate; samples are taken at various times and platelet counts done by phase microscopy. The degree of platelet adhesion is determined by the drop in platelet count observed for each blood sample.[114][116]

The % adhesion at time t was determined as follows:-

$$\% \text{ adhesion} = \frac{\text{Initial Platelet-Platelet at time t}}{\text{Initial Platelet Count}} \qquad (114)$$

It would seem however, difficult to analyse the results of this test as heparinized blood is used and heparin, (which is discussed later in this chapter), is a powerful anti-coagulant. Therefore the blood is not in a normal physiological state.

(d) Human Platelets and Polymer Microspheres (Rembaum and Kronick)

A recent technique, which has been devised to look at the interactions of platelets and microspheres of polymer, (particularly hydrogel microspheres), is that described by Rembaum and Kronick.[117] This technique is used to follow the aggregation of platelets by monitoring the light transmission of a platelet suspension while it is being stirred at 37°C. If the platelets aggregate their contribution to turbidity decreases.[117]

However, once again, the results cannot be considered of much use in assessing the true blood compatibility of polymers as a suspension of platelets without plasma or other cells being used.

The adherence of erythrocytes and leukocytes has also been examined.

(e) Miscellaneous Techniques

In addition to the techniques described previously there are various other miscellaneous techniques which have been used to investigate the interactions of blood components and surfaces but which are of peripheral interest. These techniques include:-

(i) Immunoelectrophoresis

This technique is employed for the estimation of qualitative changes in the plasma proteins.[109] It involves electrophoresis with specific antigen-antibody precipitation reactions. Migration of the protein fractions is carried out in a gel medium and the immunologic identification of the electrophoretically separated proteins accomplished by the addition of immune serum to a trough in the gel block adjacent to the separated protein fractions. The specific precipitation antibodies in the immune serum reacting with the protein fractions (acting as antigens) cause visible lines of precipitation. Quantitation of the separated fractions is related to the extent and intensity of the observed precipitin reaction.[102]

The techniques of immunoassay and immunodiffusion rely on the same basic principle.

(ii) The Use of Radiolabelled Proteins

Using radiolabelled proteins it is possible to study the competitive adsorption of plasma proteins onto surfaces.[118]

(iii) Other Chemical Techniques

There are many chemical techniques of studying the nature of blood components, too many to describe in this review, e.g. many plasma enzyme activities can be measured colourimetrically[119] and there are many methods of determining the degree of protein denaturation.

(f) Cell Culture Evaluation

Finally, in addition to the tests involving the blood cells, tests involving cultured tissue cells of human origin have been devised. It is believed that if cell growth is unaffected by the presence of a foreign surface, that this is a favourable indication of that surface's blood compatibility, but it must be realized that the results obtained using tissue cells must be inferior, (at least for blood compatibility), compared to those obtained using blood cells.

2.5 (c) SURFACE PROPERTIES AND BLOOD COMPATIBILITY

One of the most interesting fields of research is the attempt to relate the physical properties of surfaces to their blood compatibility. In the following section, the various surface properties of materials and their relationship to blood compatibility will be briefly dealt with. The various factors to be considered are shown in Table 7.

TABLE 7

Surface Properties which may Correlate with Blood Compatibility

(1) Surface charge characteristics - type of charge
- density

(2) Surface smoothness

(3) Wettability - Basic wettability
- Critical surface tension
- Surface Hydrophilicity
- Interfacial Free Energy

It is obviously to be hoped that from a better understanding of the relationship between surface properties and blood compatibility that more thromboresistant surfaces can be made using the knowledge gained.

(1) Surface Charge Characteristics

The effect of surface charge on the biocompatibility of surfaces is an area of controversy as there is conflicting evidence as to its importance. The electrical charge on the surface of a polymer was originally thought to be of importance because of the naturally occurring negative potentional on the vascular surface; which is present due to its composition, (that of an anionic mucopolysaccharide.[119] Also many blood components such as platelets are known to be negatively charged. It therefore seemed reasonable to investigate whether the presence of negative charge creates thromboresistant surfaces.

There are many examples of experiments of this nature in the literature and the weight of evidence does indeed appear to favour such a correlation. Among the examples which can be given are the following:-

(a) Electrets - The antithrombogenic properties of electrets have been considered. An electret is formed by solidifying molten polymer in either a high-intensity electric field or a magnetic field thereby inducing a permanent charge on the polymer surface. The experimenters found, in general, that surfaces with a negative charge resist thrombosis, whereas those with a positive charge promote thrombosis.[103][120]

(b) Modified Cellulose Surfaces - It has been found that anionic modified celluloses (phosphate, sulphonate, carboxymethyl) showed thromboresistance, but cationic celluloses (diethylaminoethyl, quaternary ammonium) did not.(103)

(c) Polyelectrolyte Complexes - PEC's of the type poly-vinyl benzyltrimethylammonium chloride and polystyrene sulphonate has been examined; the ionic charge being varied by altering the ratio of the two polyelectrolytes. A possible correlation between the anionic density and thromboresistance was found.(103)

PEC's of this type are illustrated in Figure I of the first chapter.

(d) Sulphonated polystyrenes - Closely related to the previous example are sulphonated polystyrenes, (polyelectrolytes); these are considered to be potentional blood compatible surface coatings. It has been found that optimum blood thromboresistance (based on vena cava and renal embolus tests), is exhibited by styrene-sulphonate copolymers having sulphonate contents in the range 8.3 - 14.5 per cent.(107)

Further evidence for the influence of surface charge is given later when dealing with anionically charged hydrogels, in Section 2.5.

It should be noted that there is conflicting evidence to suggest that some cationic surfaces may be thromboresistant and it is also believed that it may be that it is the distribution

of charge on the surface, (the surface charge density), rather than the total charge, which is an important factor in determining whether a surface is thromboresistant or thrombogenic.[106]

(2) Surface Smoothness

The smoothness of a surface has been held to be important in the prevention of clotting, but even very smooth surfaces, such as glass, are thrombogenic. Less smooth surfaces have increased thrombogenicity probably through the creation of turbulent flow, which can culminate in mechanical damage to the blood. The haemodynamics of a flowing blood system are of importance because either excessive turbulence or excessive stasis can lead to coagulation.[103] Some workers have shown that glass tubes carrying blood will remain open if sufficient attention is paid to streamlining the flow. It appears quite possible that even thrombogenic surfaces may not initiate coagulation if there is sufficient flow of blood past the surface.[103]

The results of Bernstein and Blackshear indicate that haemolysis is due primarily to destructive interaction of the red cells with the wall of the vessel or pump and appears to be proportional to the rate of red cell-wall collision; factors such as turbulence, occlusion etc would tend to effect this collision rate.[103]

(3) Wettability

Of the surface characteristics of importance, wettability received the earliest attention. Lampert (1930) determined that the coagulation time of blood on a variety of surfaces (glass, metal, paraffin, phenolformaldehyde polymer, etc) is inversely proportional to the wettability of the surface by water or other liquids, i.e. a measure of the attractive forces existing between the blood and the surface. With a glass surface, which is quite wettable, coagulation time is faster with a surface coated with the relatively non-wettable paraffin. Subsequent workers also obtained data which were interpreted as following "Lampert's Rule".[103] However, subsequent work by other experimentalists has shown that this simple rule does not hold, and other relationships have been examined between other surface measurements and blood compatibility. Some of these are dealt with on the following pages.

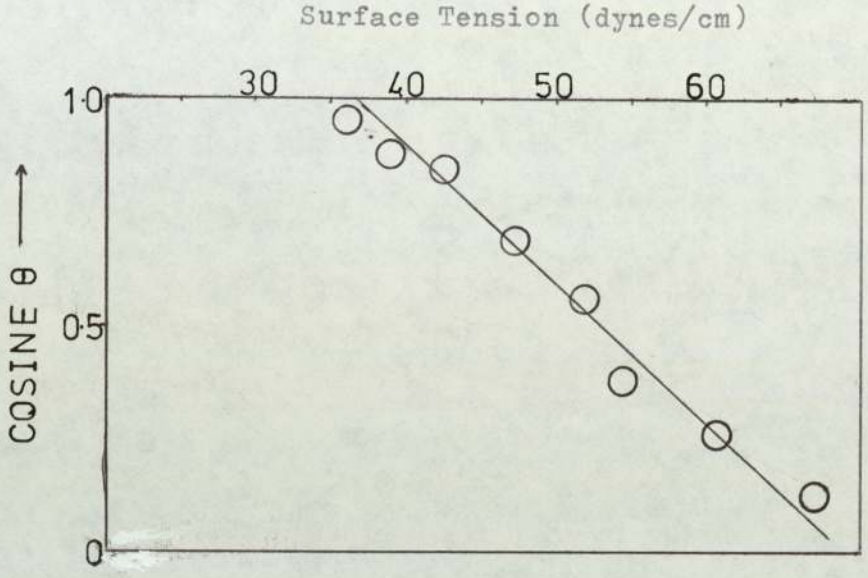
Critical Surface Tension

A physical measurement of surfaces, which has been related to the interactions of blood with surfaces is the critical surface tension. It is found by plotting the cosine of the contact angle various liquids make with the surface against those various liquids surface tensions, as shown in Figure 10.

A rough straight line relationship is observed, and by extrapolating the line to the point where $\cos \theta = 1$ the critical surface tension is found. It is the theoretical surface tension of a liquid which will just wet the surface, [$\cos \theta = 1$, $\theta = 0^{\circ}$].

FIGURE 10

Method of Determining Critical Surface Tension



The critical surface tensions derived from plots as shown correlate very well with the chemical nature of the polymer surface[120] and some critical surface tensions of various polymeric solids are shown in Table 8.

TABLE 8

Critical Surface Tensions of Various Polymeric Solids[120]

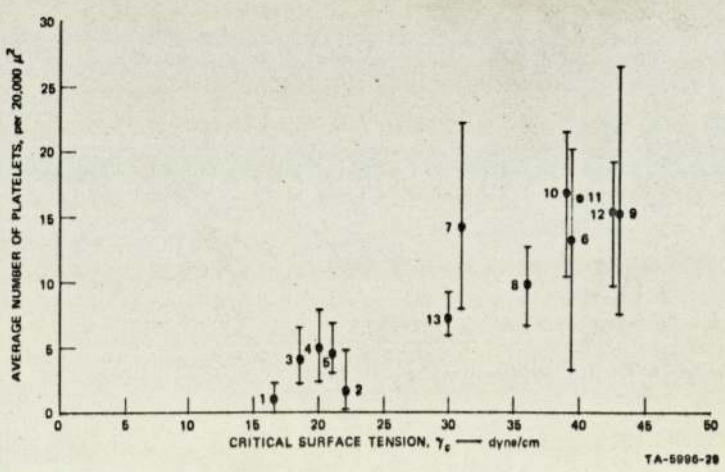
Poly hexafluoropropylene	16.2 dynes/cm
Poly tetrafluoro ethylene	18.5 "
Poly trifluoroethylene	22 "
Polyethylene	31 "
Polystyrene	33 "
Poly (vinyl alcohol)	37 "
Poly (methyl methacrylate)	39 "
Poly (vinyl chloride)	39 "
Nylon 6/6	46 "

It can be seen that polymeric materials have a wide range of values, the more polar substances having higher values.

Several relationships have been established between interactions of blood and the critical surface tensions of surfaces. The interpretation of these is made difficult however because of the modification of the surface due to the deposition of plasma proteins soon after the blood contacts the foreign surface.(112)

Lyman has shown on experiments with neutral hydrophobic polymer surfaces that there was a definite trend of increasing platelet adhesion with increasing critical surface tension as shown in Figure 11.(121)

FIGURE 11(121)



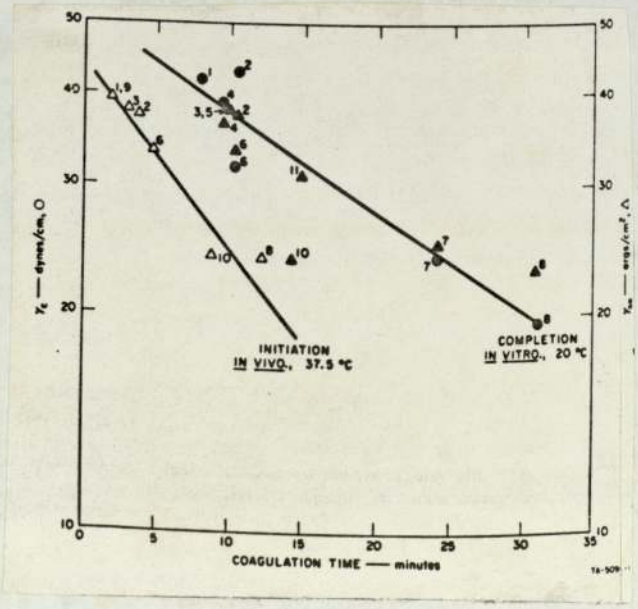
Platelet adhesion versus critical surface tension for hydrophobic polymer surfaces.

1. Poly(1,1-dihydroperfluorobutyl)acrylate
2. Polybutyl acrylate
3. Polytetrafluoroethylene
4. Fluorinated ethylene/propylene copolymer
5. Polydimethyl siloxane
6. $[NH\bigcirc CH_2\bigcirc NHCOCH_2CH_2OC]_n$
7. Polyethylene
8. Polystyrene
9. Polyethylene terephthalate
10. Polyvinyl chloride
11. Polynomamethylene adipamide
12. Lexan polycarbonate
13. Polypropylene

Also, on experiments with dog blood it has been found the coagulation time increases with the decreasing critical surface tension of the materials, (hydrophobic polymers), as shown on Figure 12.(121)

FIGURE 12(121)

The Relationship of Critical Surface Tension γ_c(O) and Surface Free Energy γ_{so} (Δ) of Various Polymer Surfaces to the Coagulation Time of Dog Blood. Polymers are: 1) Poly(caprolactam); 2) Poly(ethylene terephthalate); 3) Polystyrene; 4) Poly(methyl methacrylate); 5) Poly(trifluorochloroethylene); 6) Polyethylene; 7) Paraffin; 8) Silicone Rubber; 9) Poly(hexamethylene adipamide); 10) Polytetrafluoroethylene; 11) Poly (isopropylidenediphenylene carbonate). (▲ ●) Coagulation completion times, in vitro, 20°C (Δ O) Coagulation initiation times, in vivo, 37.5°C.



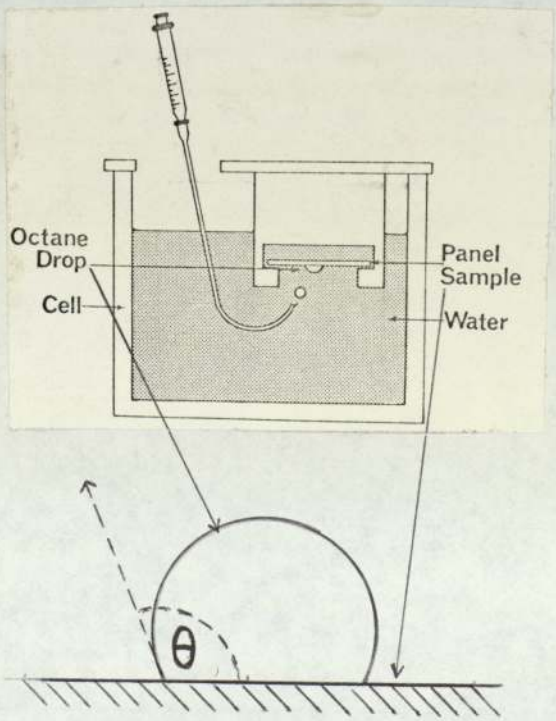
However, the critical surface tension obtained by organic liquids is a rather useless quantity as a criterion of hydrophilicity. This fact is illustrated by the results of the critical surface tension experiments on polyethylene and poly (acrylamide), whose critical surface tensions are very similar, (31 and 33 dynes/cm respectively). Thus other surface measurements have been made to try to correlate blood compatibility and surface characteristics.[122]

The Hamilton Contact Angle

Another method of measuring the physical properties of surfaces is the Hamilton contact angle test, in which the polar interfacial energy of hydrated surfaces is measured by finding the contact angle a drop of octane makes on an inverted surface submerged underwater, as shown in the following diagram: Figure 13

FIGURE 13

Apparatus to Measure Hamilton Contact Angle



The reason that octane and water have been chosen as the two liquids is that the dispersive components of surface free energy of water and octane are identical (21.8 dynes/cm), but water alone also has a polar component to its surface free energy (50.2 dynes/cm). Thus since the dispersive components cancel and underwater contact angles of octane on surfaces are a direct measurement of the polar forces at the surface of the sample. Therefore, the stronger the polar force, that is, the more hydrophilic the surface, the larger the contact angle is. Octane/water contact angles ranging from 50° to nearly 180° are possible depending on the magnitude of the surface's polar forces.[123] The Hamilton contact angles of some common polymers illustrate this point: Table 9.

TABLE 9

Octane/Water/Solid Contact Angles of Some Common Polymers[120][123]

Polymer	Octane/Water Contact Angle	Critical Surface Tension
Polytetrafluoroethylene	50°	18 (dynes/cm)
Polyethylene	50°	33 "
Poly propylene	50°	$\sim$30 "
Polystyrene	55°	29 "
Poly (methyl acrylate)	85°	41 "
Cellulose acetate	120°	36 "
Nylon 6,6	140°	46 "
Poly HEMA	150°	47 "

Also shown in Table 9 are the critical surface tensions of these surfaces. As can be seen the critical surface tension

values do not reflect the changes in surface hydrophilicity,[123] as it is a measure of the dispersive component of surface free energy.

The Hamilton test has been applied to the endothelium and the polar interfacial energy was found to be at a minimum (the octane drops forming nearly perfect spheres). The same test applied to a poly (HEMA) gel gave a contact angle of about 150° indicating a small but detectable interfacial energy.[111] Therefore, this hydrogel is much closer in its surface properties, (as measured by this test), to the endothelium than any other of the polymers shown in Table 9.

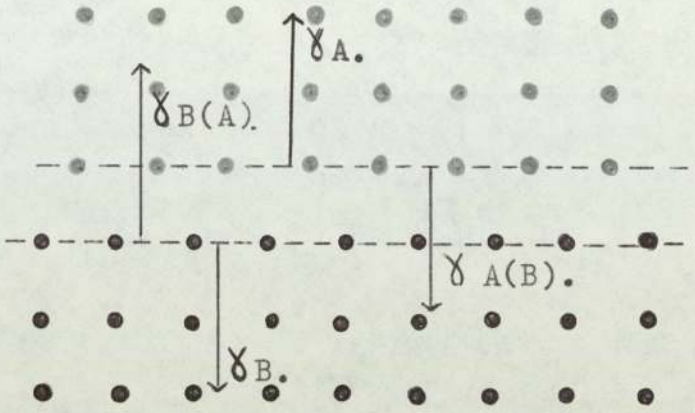
Interfacial Free Energy (Interfacial Tension)

This property is believed by Andrade and others to be a more important factor in determining the blood compatibility of surfaces. The theory on which he bases this idea is that to make a non-reactive interface means to make one, which will not be involved in adsorption or adhesion phenomena, double layer formation or other undesirable interactions. He points out that in natural systems the interface between cells and the extracellular environment has a very low interfacial tension, in the region of 1-3 dynes/cm but in some cases as low as 0.1 dyne/cm for certain cell types.[111] Therefore the best blood compatible surface is likely to be one which results in a very low interfacial free energy.

The theory behind the concept of interfacial free energy is illustrated in Figure 14, and the following equations and explanations.

FIGURE 14 (120)

Hypothetical Interface between two phases, A and B



In Figure 14, which represents an interface between two phases, γ_A represents the surface free energy of Phase A and γ_B, the surface free energy of Phase B. The terms $\gamma_{A(B)}$, (the effect on A due to the presence of B) and $\gamma_{B(A)}$, (the effect on B due to the presence of A), serve to reduce the free energy at the interface. Thus we can say that the interfacial free energy, (γ_{AB}) is given by the following equation:-

$$\gamma_{AB} = \gamma_A - \gamma_{A(B)} + \gamma_B - \gamma_{B(A)} \quad (120)$$

The presence of B partially satisfies the unsatisfied bonding of A and vice versa. The unsatisfied bonding capacity at the interface is known as the interfacial energy, and γ_{AB} is known as the interfacial tension or interfacial free energy.(120)

However, various forces contribute to the interfacial free energy, e.g. hydrogen bonding, dipole-dipole interactions and dispersion forces. The methods of measurement of these forces usually depends on a solid - liquid - vapour contact angles, which

are not very applicable to physiological conditions,[111] i.e. the results are less meaningful than they appear. Taking this factor into consideration with the additional fact that the slight differences in the measured contact angle have a marked effect on the measured values of the components of interfacial free energy, it is not surprising that the values obtained have been shown to be sensitive to experimental variation.[123] Therefore, although the concept of interfacial free energy is probably a very important one in relating blood compatibility to surface/interface properties, experimental difficulties make it too difficult to apply.

The Hamilton contact angle, which has already been dealt with, allows the interfacial polar binding component to be measured in situ by measurement of the n-octane/water/solid contact angle,[111] it is not subject to the same difficulties as measuring the total interfacial free energy and so has become of great interest.

2.5 (d) THE EFFECTS OF DIALYSIS AND PERFUSION ON BLOOD

It is important to understand these effects from several points of view; it is another source of information on the effect of a foreign interface on blood and also these techniques are the main means of removal of toxins from the body in kidney and liver failure so undesirable effects on patients who are already very ill are extremely important. As regards the effects of perfusion on blood the effects of specific types of perfusion such as charcoal, ion-exchange and neutral resins are discussed more fully in the chapter on Artificial Liver Support; here we will be looking at general effects.

Of the two types of system the effects of perfusion are considered to be more drastic as we are dealing with a larger foreign surface area, pressure effects and turbulence; it is considered first.

(1) Perfusion

The effects of perfusion are greatest on the cellular elements of blood which are fragile; these effects on the individual blood cell types are considered on the following pages, with the effect on plasma proteins.

(a) Red Blood Cells

The vulnerability of red cells has been well established, prosthetic circulatory devices can inflict two magnitudes of injury upon the erythrocytic elements of blood. A certain

fraction of the total number may suffer outright or prompt destruction. Others may be injured to a degree that falls short of outright destruction, (sublethal injury). Such injury, however, may nonetheless cause important alterations with respect to red cell morphology, metabolism, cell life span and cellular functional capacity.(124)

(b) White Cells

Less is known about the perfusion induced injury of leukocytes but the following effects have been demonstrated:-

(1) alterations in total and differential white cell populations
(2) leukocyte morphological aberrations
(3) metabolic changes
(4) impairment of leukocyte phagocytic capability.

Since white cells play a crucial role in combating infection and are fundamentally important participants in tissue inflammatory response, it is clear that if prolonged in vivo perfusion renders a certain fraction of circulating leukocytes functionally incompetent, the ability to combat infection generally may be compromised. The degree of leukocyte destruction and injury required to bring about intolerable impairment is yet unknown.(124)

(c) Thrombocytes

These cells suffer most in haemoperfusion systems and thrombo-embolic phenomena are encountered in all perfusion systems. The main effects noted are the destruction of

platelets and a change of platelet adhesiveness; it is believed that these effects may be due to phospholipid adsorption or protein denaturation or to actual cellular breakdown caused by surface contact in the external circuit. Clotting, as has been mentioned before, can be promoted by the release of thromboplastic substances from damaged platelets.(125)

(d) Plasma Proteins

Denaturation of plasma proteins can often occur quite readily at material-blood interfaces. Denatured proteins might contribute to long-range allergy or hypersensitivity reactions. Intravascular sludging has also resulted from extracorporeal circulation, and this change of viscosity appears to result from protein denaturation.(125)

(2) Effects of Dialysis

These are less severe than for haemoperfusion for the reasons already mentioned, nonetheless for long term haemodialysis they can be quite severe and the effects noted previously, that are due to extracorporeal circulation, of course, also apply to haemodialysis. Among the effects found with haemodialysis have been: accelerated aging of red cells and increased haemolysis.(125)

2.5 (e) ATTEMPTED MEANS OF INCREASING BLOOD COMPATIBILITY

(1) Introduction

Many different techniques have been tried to make materials more blood compatible; the principal ones are shown in Table 10.

TABLE 10

Techniques to increase the blood compatibility of materials

(1) The use of the anti-coagulant heparin
(2) The attachment of fibrinolytic enzymes and analogous materials
(3) Coating with the plasma protein albumin
(4) Grafting on hydrogels.

These techniques are individually discussed on the following pages.

(2) The Use of Heparin

Action of heparin

Heparin is a natural component of blood and one of the important compounds involved in natural processes of coagulation and anticoagulation,[126] it is extremely potent, as little as 1 mg will prevent the clotting of 100 ml or more of blood.[102] Its action as an anti-coagulant is caused in part, by its direct and immediate combination with various coagulation factors, most notably it decreases the rate of interaction of thrombin and fibrinogen, (the last stage of the plasma protein clotting mechanism). It is also an antiprothrombin and prevents platelet agglutination, so preventing thrombus formation. Additionally

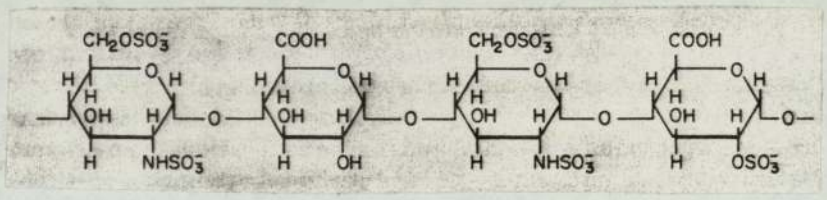
it enhances the activity of "clearing factors", (lipoprotein lipases).[102]

Heparin's structure is that of a polysaccharide having a molecular weight of about 12,000 and is characterized by a high negative fixed charge due to sulphonate and sulphate groups attached to a mixed copolymer of glucosamine and glucuronic acid. The chain also contains a high concentration of fixed carboxylic groups. An average of one sulphonate or sulphate group is found for each chain unit along with two carboxylic groups for every three chain units.[126]

A proposed structure for heparin is shown in Figure 15.

FIGURE 15

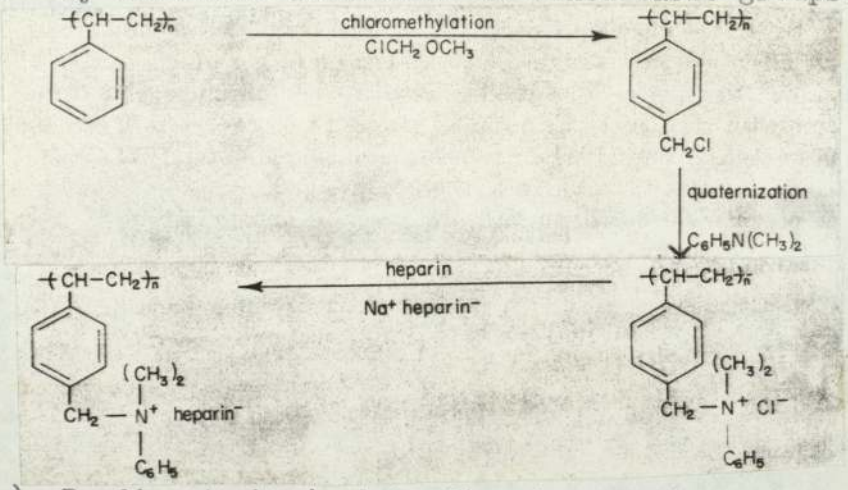
Proposed Structure of Heparin[103]



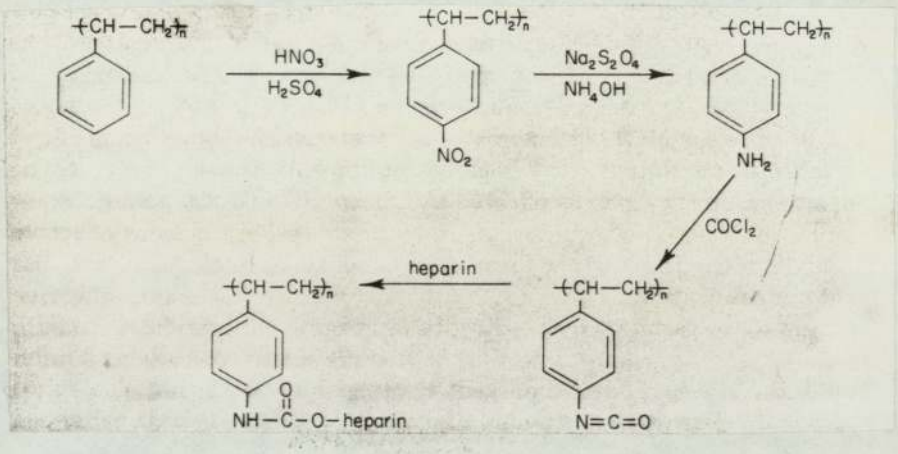
Means of Attachment

Heparin has been employed rather extensively for the purpose of imparting non-thrombogenicity to the surface of plastics by several methods:-

(a) By coulombic attachment to fixed basic groups, for example[103]



(b) By direct chemical bonding to reactive groups on the surface of the plastic, for example[103]



(c) Or, by occlusion into a microporous plastic material from which it is diffused slowly.[126]

Examples of Use

There are several examples of heparin being used to make thrombogenic materials more thromboresistant, for example:-

(a) GBH treatment (graphite-benzalkonium-heparin)

It has been found that a polymer or metal surface coated with graphite can be used to absorb a cation (usually the

benzalkonium group); this in turn then can be used to adsorb heparin, binding it ionically. Polymers treated in this manner are nonthrombogenic, for example a high percentage of rings with the GBH treatment placed in the vena cava of dogs have remained clot free for more than a year.(103)

(b) Poly ether polyurethanes

Polyether polyurethanes containing positive charges in the backbone or in the side chains were synthesized from commercially available diisocyanates. These cationic polyurethanes were reacted with sodium heparin to yield polyurethane heparin complexes; solutions of these have been used to produce thromboresistant coatings on commercial tubing.(127)

(c) Heparinized silicone rubbers

Several techniques exist for making heparinized silicone rubbers, (these can be used too for certain other polymers.) Firstly by the use of coupling agents, such as the quaternary ammonium salt tridecylmethammonium chloride, (TDMAC), and γ-aminopropyltriethoxysilane (APTES). In each case the heparin molecule is ionically linked to the coupling agent, for TDMAC the process involves the formation of an ionic complex between the coupling agent and heparin which is then applied to the polymer and, in the case of APTES, the coupling agent is covalently bonded to the hydroxyl groups of the silica filler present in the elastomer, and the resulting structure is ionically complexed to heparin.(109)

Materials produced by both techniques show good thromboresistance in the vena cava ring tests. However, for arterial-venous shunt tests the TDMAC-heparinized silicone rubber showed complete occlusion by thrombus after only a few days. This phenomenon is due to the elution of heparin by the increased blood velocity, pressure and turbulence and so this test underlies the limitation of these sorts of material in this type of blood flow region, despite excellent performance in other areas.(109)

There are many other examples of heparin being used to make blood thromboresistant materials and some of these applying to more hydrophilic materials, (hydrogels), will be mentioned later in the introduction.

Additionally there is an extensive literature on synthetic heparinoids. A variety of polymers ranging from poly(styrene sulphonic) acid to a number of sulphonated or sulphated natural products, (including sulphated chitin), have been examined. Invariably, these materials have demonstrated limited effectiveness, and often a high toxicity. Similarly, surfaces to which polystyrene sulphonic acid has been attached have not shown promise for non-thrombogenicity and there are reports of red cell damage by their use.(126)

(3) <u>The Attachment of Fibrinolytic Enzymes</u>

This technique of preventing thrombosis on prosthetic surfaces (by the use of fibrinolytic enzymes) is very new, so far two systems have been tried:-

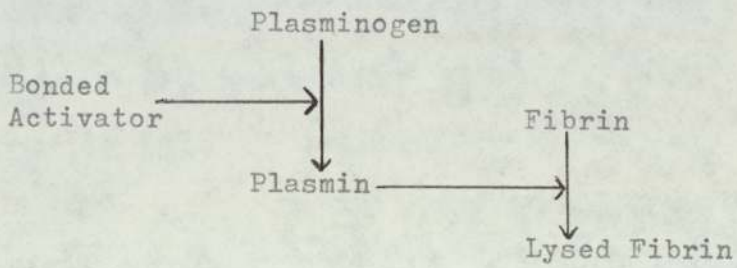
(a) The urokinase-heparin bonded synthetic surface.[128]

(b) Brinolase on a solid support.[129]

In the case of the first technique the rationale as to its use was that a great improvement in blood compatibility might be achieved if a surface had on it both a surface factor which would inhibit thrombus formation (heparin) and a second surface factor (a plasminogen activator, such as urokinase), which would initiate the lysis of any minute mural thrombotic nuclei resulting from the failure of heparin activity. The action of the first factor, heparin, has already been discussed; urokinase is relatively stable and has a powerful indirect clot lysing ability, which acts by activating plasminogen, a substance known to be intimately associated with fibrin, the network of which is important for the cohesion and propagation of the initial thrombus. The mechanism of this lysis is shown schematically in Figure 16.

FIGURE 16

Mechanism of Lysis



From results on whole blood and plasma the authors felt that there was evidence that adsorptively bonded urokinase was exhibiting an effective fibrinolytic activity on the surface and so it appears that the presence of this surface activator may

continuously destroy small thrombi and thereby exert a continuous cleansing action at the prosthetic surface.(128)

A further approach involves the use of brinolase; this involved, unlike the previous example, the permanent coupling of the enzyme to the support. The enzyme chosen for the study was the fungal enzyme, brinolase, which is a proteolytic enzyme, which is very effective in dissolving emboli in vivo. This enzyme was successfully chemically bound to derivatized poly (acrylamide) and evidence found that the material retained fibrinolytic activity after being used for repeated fibrinolysis.(129)

In addition to these fibrinolytic enzymes other materials of a similar nature have been suggested as possibilities in increasing the biocompatibility of surfaces, e.g. the attachment covalently of platelet protective agents to polymeric surfaces including prostaglandin EI and others.(130)

(4) Coating with Albumin

This technique is widely believed to be effective in increasing the blood compatibility of materials. The theory behind this technique is that if the surface of a material is coated with the main plasma protein of blood it will prevent the clotting mechanism being activated as the surface presented to the blood will be less physiologically abnormal.

If the surface is allowed to acquire a layer of adsorbed albumin by equilibrating it with an albumin solution, the surface, when initially exposed to blood, cannot participate to the

clotting mechanism. However, as time goes on this pre-adsorbed albumin layer will equilibrate with other plasma proteins such as fibrinogen and γ-globulin, (which do not create a thromboresistant surface), and the clotting mechanism will be initiated. In order to prevent this process of desorption taking place it is necessary to immobilize the albumin on the surface; (several techniques of achieving this are known). Alternatively, the albumin can be cross-linked chemically to encapsulate the material. It has been found that crosslinked albumin exhibits thromboresistance for a longer time than adsorbed albumin,[131] and that albuminated surfaces appear to be less haemolytic than the original polymer surface.[132]

(5) Grafting on Hydrogels

This is a further way of making surfaces more biocompatible; there are several techniques available for use: - Table 11.

TABLE 11[106]

Techniques for Depositing Hydrogel Coatings

(a) Dip-coat in pre-polymer and solvent.

(b) Dip in monomer(s) with or without solvent and polymer, then polymerize using a catalyst with or without heat.

(c) Pre-activate surface ("active vapour", ionizing radiation in air) then contact with monomer(s) with or without heat to polymerize.

(d) Irradiate with ionizing radiation while in contact with vapour or liquid solution of monomer(s).

The final section of this chapter deals with the interaction of hydrogels and blood.

2.5 (f) HYDROGELS AND BLOOD

(1) Introduction

In this section the results of the various blood compatibility tests and other miscellaneous tests, (described in the first section of this chapter), which have been applied to hydrogels will be discussed. For simplicity, the various types of hydrogel are dealt with individually; the division of the field, which has been chosen is shown in Table 12.

TABLE 12

Types of hydrogel considered in Blood Compatibility Studies

(a) Poly (acrylamides and methacrylamides)

(b) Poly (hydroxy alkyl methacrylates and acrylates) and their copolymers

(c) Poly (N-vinyl lactams)

(d) Poly (electrolyte complexes) and Poly electrolytes

(e) Modified Hydrogels

On the following pages the results for these hydrogels in their homogeneous form or as coatings are given. From the table it can be seen that once again the main emphasis will be on synthetic hydrogels, and it should also be noted that there is some degree of overlap between the various divisions, for example with regard to copolymers.

(2) Poly (acrylamides and methacrylamides) and their copolymers

The main polymeric acrylamide which has been investigated is that of the simplest monomer - acrylamide. There has been great interest in coatings of polyacrylamide to make blood compatible coatings because of good experimental evidence that it does produce such coatings.

The results of in vivo and in vitro tests using poly (acrylamide) are shown in Table 13.

TABLE 13[133]

Biological Properties of Bulk Poly (acrylamide*)
(EWC = 65-75%)

(a) In vivo (canine)

Vena cava (2 hours)	thrombus on ring	- none
(2 weeks)	thrombus on ring	- very small on few rings
Renal embolus	thrombus on ring	- none
	renal infarcts	- few

(b) In vitro

Effect on 18 plasma proteins	- none
Effect on 8 plasma enzymes	- none
Calcium replacement clotting time	- not prolonged
Adherence of cells	
- erythrocytes (whole blood)	- 70,000/cm^2
- leucocytes (whole blood)	- 0
- platelets (platelet rich plasma)	- 256,000/cm^2
Platelet aggregation and	- none

* Crosslinked with NN methylene bis acrylamide

As can be seen the evidence is that there is little thrombus formation in the vena cava and renal embolus test, and from the

renal embolus test it can also be seen that the observation that little thrombus formed on the ring was not due to any appreicable extent by the thrombi being non-adherent as few renal infarcts were found on autopsy of the kidney. It is also observed that no effect on the plasma proteins or enzymes investigated could be discerned in in vitro tests, and that platelets did not aggregate, although there is some adherence to the surface by the various cell types.

Additionally, the thrombogenicity of a number of polyacrylamide, (PACR) gels has been investigated in vitro, using the Lee-White coagulation time test. It was found that the coagulation time of fresh blood samples in PACR tubes prepared using singly recrystalized acrylamide monomer showed a clotting time of ~45 minutes, against 12 minutes for glass tubes. However the clotting times obtained were much improved if the gels were prepared from triply recrystallized monomer; times in excess of 24 hours were recorded. Thus the deleterious effects of incomplete removal of impurities is shown by this study, as were the bad effects of the incomplete removal of initiator by-products. It was also observed that varying the crosslinking agent concentration in the PACR gel did not significantly alter the Lee-White clotting times.(106)

The effects of polyacrylamide grafting on the blood compatibility of materials has been examined; it has been found that polyacrylamide coated materials have good blood compatibility, e.g. uncoated polyurethane causes a large number of platelets to adhere to its surface, whereas a polyurethane surface with

grafted polyacrylamide causes a negligible number to adhere.[133]

It has also been found by the use of radio labelled proteins that the chemical grafting of polyacrylamide onto polyurethanes causes less plasma protein adsorption, especially of fibrinogen and γ-globulin, which are known to make surfaces thrombogenic. This result is illustrated in Table 14.

TABLE 14[133]

Uptake of single labelled proteins by polyacrylamides covalently grafted onto polyurethanes in static experiments

	Proteins $\mu g/cm^2$		
	Albumin	Fibrinogen	γ-Globulin
(a) Nongrafted polyurethane	0.58	0.92	0.93
(b) Polyurethane chemically grafted with PACR with cross-linker	0.19	0.18	0.29
(c) Polyurethane chemically grafted with PACR without cross-linker	0.97	0.11	0.09

A large number of copolymer gels of acrylamide and another monomer have had their thrombogenicity investigated by Halpern et al using the Lee-White techniques. The comonomers used with acrylamide in these studies are listed in Table 15.[106]

Only DMAEMA-acrylamide hydrogel showed a significant extension in clotting time over the control, (poly acrylamide) - 24 hours as against 38-40 minutes. The amount of copolymerized DMAEMA in the copolymer was very small 1/20th the amount of ACR.[134]

TABLE 15[106]

Comonomers used with acrylamide to make hydrogels, and investigated for thrombogenicity using the Lee-White Technique

(a) Dimethyl amino ethyl methacrylate (DMAEMA)

(b) t-butyl amino ethyl methacrylate

(c) 2-sulphoethyl methacrylate sodium salt

(d) 2-hydroxy-3-methacryloloxypropyltrimethylammonium chloride

(e) acrylic acid (AA)

(f) methacrylic acid (MAA)

(g) 2-vinyl pyridine

(h) 4-vinyl pyridine

(i) 2-methyl 5-vinyl pyridine

This result is surprising as DMAEMA-ACR hydrogels will be positively charged at physiological pH and positively charged materials are usually thought to be thrombogenic.

Polymers of other acrylamides, poly (N-substituted acrylamides), have been investigated from the point of view of tissue rather than blood compatibility because of their superior hydrolytic stability compared to poly (acrylamide), and do not appear to have been studied for blood compatibility.[135]

(3) Poly (hydroxy alkyl methacrylates/acrylates) and their copolymers

In this series of polymers the one of most interest has been, of course, poly (hydroxyethyl methacrylate) (poly HEMA), which

has been used in many biomedical applications. However, due to its poor mechanical properties there have always been experimental difficulties in evaluating the blood compatibility of these materials by accepted in vivo tests, but there are a few studies of in vitro blood compatibility, and using less conventional techniques some in vivo studies.[106]

One of the most interesting in vivo studies was done by Levowitz et al[106] using hydron cylinders in the vena cava of dogs; it was found that poly HEMA was a relatively "non thrombogenic" (or at least non-thromboadherent) material,[106] and mural thrombus only formed on the cylinder after a long period of implantation, over a week at least.[136] Unfortunately it does not appear that any renal embolus tests have been done using this polymer and the observation that clots do not adhere well to hydrogel surfaces[107] must give rise to anxiety that poly HEMA may only be a non-thromboadherent surface.

Of the in vitro tests, the standard Lee-White clotting time test has been carried out on poly HEMA coated test tubes and times of the order of one hour found,[136] which is less than that found for polymers made from thoroughly recrystallized poly (acrylamide).

There is, however, more evidence as to the better blood compatibility of poly HEMA as against other materials from the work done with grafted HEMA hydrogels on other materials, e.g. Figure 17 shows the decrease in platelet adhesion to radiation grafted HEMA hydrogels on cellulose acetate with increasing HEMA

concentration, and Figure 18 a corresponding decrease in fibrinogen adsorption with increasing HEMA grafting on silicone rubber.

FIGURE 17(137)

Platelet adhesion to radiation grafted HEMA hydrogels on cellulose acetate

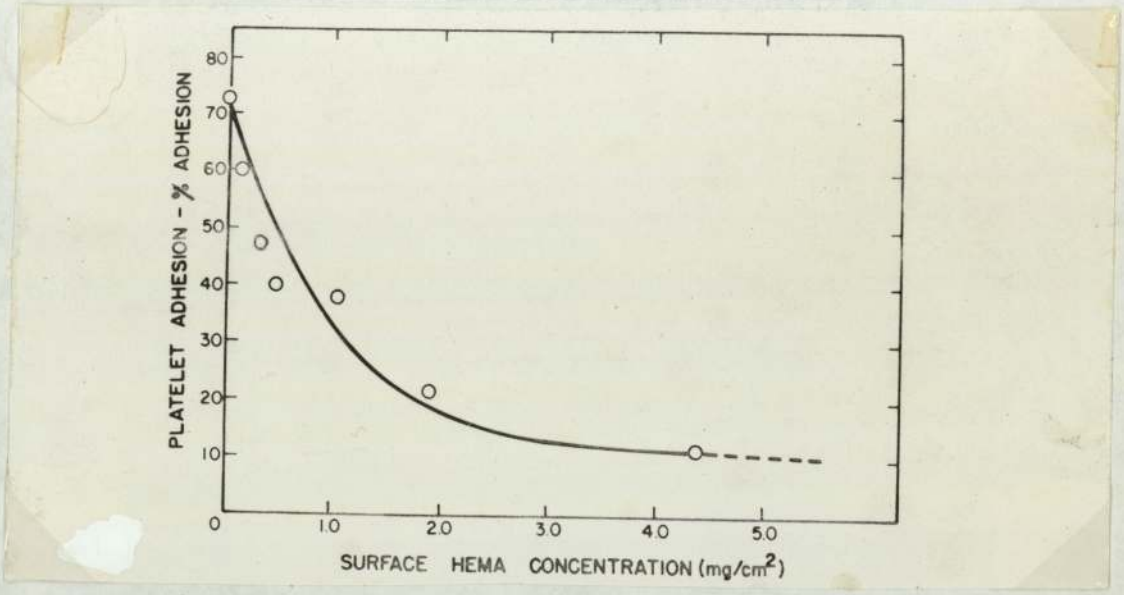
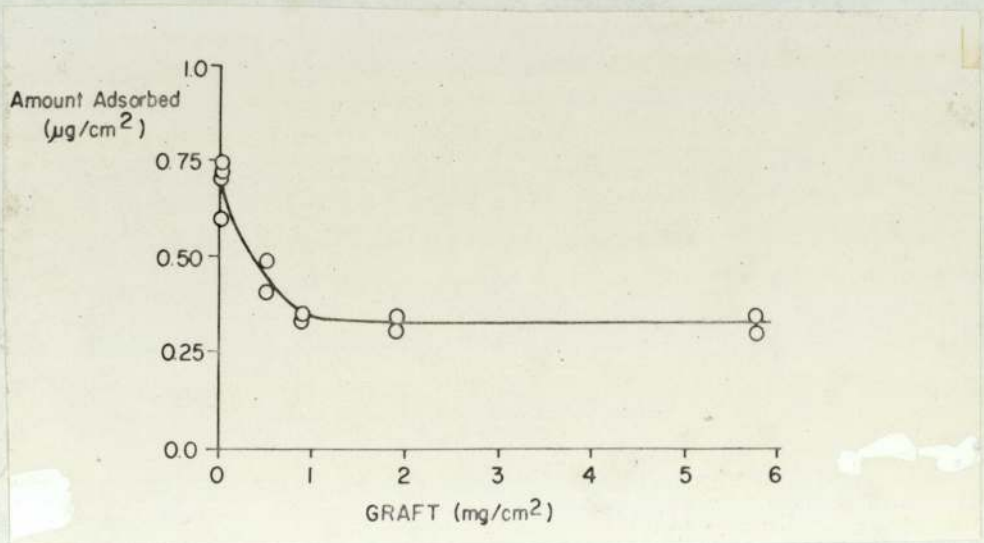


FIGURE 18(138)

Fibrinogen adsorption to radiation grafted HEMA hydrogels on silicone rubber

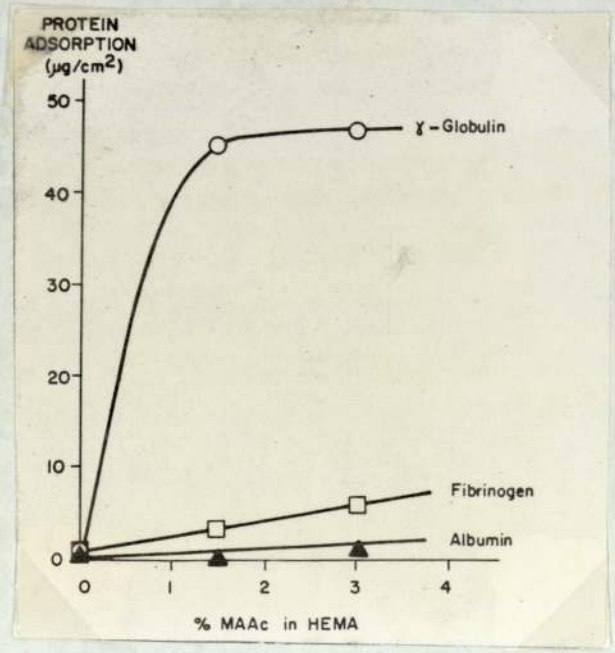


These results show that the presence of poly HEMA on the surface of a material decreases the concentrations of two of the most important components involved in blood clotting, mainly the platelets and fibrinogen.

Another interesting study of protein adsorption onto poly HEMA has been done by Hoffmann et al; he showed that the presence of methacrylic acid in the polymer has the effect of increasing plasma protein adsorption on the grafted hydrogel, especially of undesirable plasma proteins such as γ-globulin and fibrinogen, which create thrombogenic surfaces. His results for the adsorption of plasma proteins onto HEMA:MAA copolymer grafts on silastic supports are shown in Figure 19.(139)

FIGURE 19

Adsorption of plasma proteins onto methacrylic acid/HEMA copolymer grafts on Silastic supports. Dose = 0.25 Mrad. Protein concentrations = 0.5 mg/ml, pH = 7.4, no added salt, T = 37°C



Since methacrylic acid is a common impurity in HEMA and as we can see from Figure 19 it has such a pronounced effect on the character of plasma protein deposition, even in quite small concentrations this result again stresses the importance of purity, as did the results on polyacrylamide.

On the other hand evidence that poly HEMA is not a good biomaterial compared to poly ACR comes from the platelet-hydrogel microsphere tests of Rembaum. He found that poly HEMA microspheres were potent in stimulating platelet aggregation unlike poly ACR microspheres, and also, surprisingly, that HEMA beads containing 20 per cent methacrylic acid, (MAA), were inactive in stimulating platelets to aggregate. Thus he concluded that "MAA is important in reducing the thrombogenicity of poly HEMA at the level of the reaction with platelets."[117] The inherent caution in his statement is present because, as has been mentioned before, the blood clotting mechanism has many components and platelet adhesion to surfaces is only one aspect of blood clotting, although an important one. Also investigated was the HEMA:ACR copolymer series, containing 20 to 80 per cent acrylamide; these copolymers within the range indicated did not cause platelet aggregation.[117]

(4) Poly (N-vinyl lactams)

The most investigated polymer of this series is crosslinked or grafted poly (vinyl pyrrolidone) (PVP), although the testing of it as a material for blood compatible coatings has not been as thorough as for PACR or negatively charged Ioplex 101. The tests

carried out have mainly been in vitro rather than in vivo for the same reasons as were given for poly HEMA, mainly that high concentrations of crosslinking agent (20 per cent) are needed to produce a material with useful mechanical properties.(106)

The in vitro test carried out was again the Lee-White clotting time test, it was found the PVP gels and P-(NVP-ACR) copolymer gels showed some extension of clotting times, although there is the complicating factor of the low reactivity of NVP, which results in high amounts of residual monomer in the gels.(106)

Like other hydrogels it has been grafted onto other surfaces in an attempt to increase blood compatibility and it has been found that PVP hydrogels grafted onto silicone rubber will greatly reduce the thromogenicity of the silicone rubber as judged by the vena cava ring test.(106)

(5) Poly electrolyte complexes and poly electrolytes (crosslinked)

Both these types of polymeric material have been examined for blood compatibility and are dealt with separately on the following pages.

(a) Poly electrolyte complexes

The nature and attempted biomedical uses to which these materials have been put has already been discussed in the first chapter; it has also been mentioned, in this chapter, that results obtained from experiments with these complexes provide evidence that the presence of excess negative charge increases

the blood compatibility of the materials.

A net charge (anionic cationic) can be incorporated into polyelectrolyte complexes with great ease, simply by adding stoichiometrically greater or lesser amounts of the two polymeric components during formulation. It was determined, using the in vivo vena cava ring test that Ioplex 101, (whose structure is shown in Figure 1), containing 0.5 meq excess anionic component showed the greatest thromboresistance.(106)

A complete table of the biological tests carried out on Ioplex 101 with 0.5 meq excess anionic charge is shown in Table 16. It can be seen that it appears to be quite a good material, although not as good as poly (acrylamide), as there are many more renal infarcts in the renal embolus test, some effects on plasma proteins and enzymes, and, also a greatly increased adherence of cells to its surface.

TABLE 16

Biological Properties of Ioplex 101
(0.5 meq excess anionic) EWC = 55%+

(a) In vivo (canine)		
Vena cava (2 hours)	thrombus on ring	- mostly free
(2 weeks)	thrombus on ring	- very small on few rings
Renal embolus	thrombus on ring	- mostly free
	renal infarcts	- moderate to severe
(b) In vitro		
Effect on 18 plasma proteins		- more, except fibrinogen
Effect on 8 plasma enzymes		- some, alkaline and acid phosphase
Calcium replacement clotting time		- not prolonged
Adherence of cells		
- erythrocytes (whole blood)		- 510,000/cm^2
- leucocytes (whole blood)		- 0
- platelets (platelet rich plasma)		- 320,000/cm^2

Neutral Ioplex 101 with an EWC of approximately 50-55% exhibited only moderately less blood compatibility than the sample which contained 0.5 meq excess anionic charge. Gels with greater anionic charge than this, and those that were cationically charged showed much reduced blood compatibility.

(b) Polyelectrolytes

Anionic and cationic hydrogels are polyelectrolytes, they are usually formed by copolymerizing small amounts of anionic or cationic monomers with neutral hydrophilic monomer, but can also be prepared by derivatization or, in the case of polyelectrolyte complexes by adding an excess of the polyanion or polycation component.(106)

Several examples of the blood compatibility of anionic and cationic hydrogels of various sorts have already been given, especially for acrylamide copolymers, From that study no particular relationship could be drawn between type and quantity of charge and blood compatibility. Additionally only one copolymer, that of ACR-DMAEMA, showed any increased thromboresistance over p-ACR.

A more rigorous study of the importance and interrelated effects between the concentration of ionizable groups, degree of neutralization, hydrophilicity and blood compatibility for four series of negatively charged polyelectrolytes has been carried out by Lundell et al. The four series of copolymers investigated are shown in Table 17, a variety of in vitro and in vivo blood compatibility tests were performed on them.

TABLE 17

Series of hydrogels investigated for blood compatibility

(a) Ethylene/acrylic acid (EAA)

(b) Ethylene/vinyl sulphonate (EVS)

(c) N-vinyl pyrrolidone/acrylic acid (NVP/AA)

(d) Vinyl acetate/crotonic acid (VA/CA)

The results for these copolymers are considered separately:-

(a) Ethylene/acrylic acid polymers (EAA)

The two polymers in this series that exhibited a moderate level of thromboresistance were the 19% AA copolymers, and its highly neutralized sodium ionomer, whereas copolymers at lower neutralization or lower acrylic acid content, as well as calcium monomers were thrombogenic.

(b) Ethylene/vinyl sulphonate polymers (EVS)

This series was investigated because the sulphonic acid moieties ($-SO_3H$) are present in naturally occurring polyelectrolytes (most notably heparin), and also, because sulphonic acids are stronger than their carboxylic acid analogue, so their chemical reactions with plasma would be expected to differ. This series was found to have poor blood compatibility, however.

(c) N-vinyl pyrrolidone/acrylic acid polymers (NVP/AA)

These polymers are much more hydrophilic than the previous series and were investigated to try to determine the influence of hydrophilicity of blood compatibility by comparison with the previous series. The results for this series were discouraging and all the polymers and ionomers were found to be thrombogenic.

(d) Vinyl acetate/crotonic acid polymers (VA/CA)

As the previous series of highly hydrophilic polymers had proved to be thrombogenic, a series with an intermediate hydrophilicity between the NVP/AA and the EAA/EVS series was examined.

Some members of the VA/CA series of polymers were found to be thromboresistant; the material which consistently demonstrated the most encouraging thromboresistance is the 60% sodium ionomer of vinyl acetate/2% crotonic acid copolymer.

From these investigations blood compatibility could be seen to depend on the following factors:-

i) the chemical nature of the ionic moiety bound to the polymer backbone and its counterion.

ii) the surface charge density

iii) the hydrophilicity of the polyelectrolyte.

It was also shown within each series that there is a thrombogenic dependence on surface charge density, and the results, taken together, imply that the antithrombogenic character of a

surface does not increase indefinitely as the hydrophilicity and surface charge density increases. Rather, an optimum hydrophilicity and charge density is reached beyond which the surface again becomes thrombogenic.(140)

(6) Modified Hydrogels

In this subsection modifications to hydrogels to improve blood compatibility are briefly dealt with; this includes the use of techniques discussed in the previous section, which were considered in their application to materials in general. In that previous section, some of the modifications to hydrogels to make them more blood compatible were dealt with, specifically the use of fibrinolytic enzymes; this will not be dealt with again, only the remaining fields; the use of heparin and albumin.

(a) Heparin and hydrogels

One of the most mentioned examples of the use of heparin and hydrogels is a hydrogel consisting of poly vinyl alcohol and heparin crosslinked together with glutaraldehyde/formaldehyde mixtures. This polymer has been shown to have low thrombogenicity in in vitro tests and such hydrogels have been evaluated for haemodialysis membranes.(106)

Other hydrogels make use of the ability of heparin to form ionic bonds with positively charged species; an example of this is the improvement in mean recalcification times of heparinised coatings of dimethyl aminoethyl methacrylate-methyl methacrylate copolymers compared to the unheparinized coatings, as shown in

Table 18.

TABLE 18[108]

Mean Recalcification Times of Heparinized and Unheparinized DMAEMA-MMA copolymer coatings

DMAEMA in Copolymer (%)	Mean Recalcification Time (minutes)	
	Unheparinized	Heparin
17.8%	3.8	30
24.4%	7.8	60
27.0%	5.6	60

(b) Albumin and hydrogels

The techniques described earlier to coat materials with albumin can be applied to hydrogels and in the literature there are examples of the coupling of albumin to derivatives of polyacrylamide[141] and other gels[142][143] but these have been mainly to make adsorbants for protein bound metabolites and many have not proved to be very biocompatible.

(7) The Role of Water in Blood Compatibility

As hydrogels consist mainly or partly of water, the role of water in the blood compatibility of hydrophilic and hydrophobic surfaces should be considered, especially as the structure of water is of known importance in a wide range of biological phenomena. It is reasonable to hypothesize that this factor may be important in blood interface phenomena.[111]

Water in and at the surface of hydrogels is believed to be of several types and various authorities speak of "bound" and "free" water, and X, Y and Z water. The nature of the bulk water in hydrogels is being studied by a variety of techniques:- NMR, differential scanning calorimetry, dielectric work, infra-red spectroscopy, thermal expansion data and ion-conduction studies,[106] but there must be doubt as to how far the structure of water in the bulk hydrogel is related to the structure on or at the surface. So far no correlations have yet been drawn between blood compatibility and water structure, but some workers believe that high water content hydrogels approximate more closely to the outer cell coat of the endothelium than most materials. The outer cell coat being most likely hydrophilic, gelatinous and of very high water content.[111]

The following scheme has been advanced for the importance of structured (ordered) water at the surface of hydrophilic and hydrophobic materials.

"If a polar, wettable surface is placed in an aqueous environment, there should be dipole-dipole interactions occurring between the polar sites on the surface and nearby water molecules, orientating these molecules so as to maximize the dipolar interactions. If there are ionic sites on either surface, (due to adsorption of ions from the medium or ionization of the foreign surface in the medium), then there will be regions in the interface characterized by the type of structured water existing in the hydration layers of such ions. A diffuse double layer of counterions will also be set up to neutralize the field of the

interfacial ions and interaction of protein ionic charge with foreign surface ionic charges will release the counter ions of each and discharge the surface double layer (resulting in an entropy gain due to the increased freedom of these counterions).[144]. Thus, the process is thermodynamically favoured.

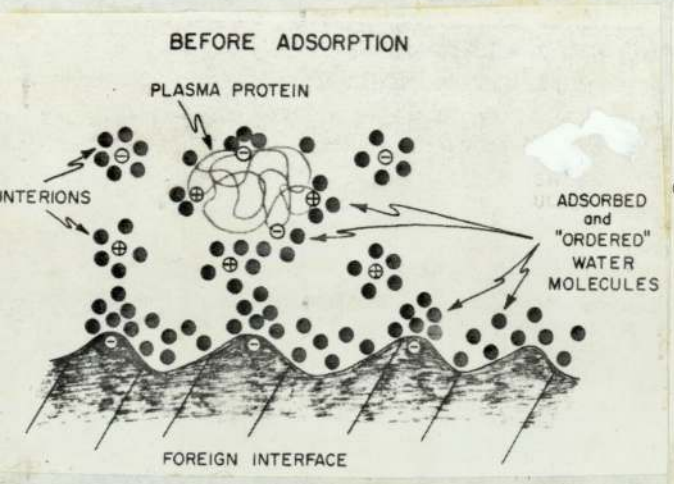
In the case of nonpolar foreign surfaces, the case is different especially in the final stages. Nonpolar surfaces cause structuring of the aqueous medium to quite a large extent, and 'ice-like' structured water overlies the entire surface, to a greater extent than is the case for a neutral hydrogel. Adsorption of a plasma protein on a non-polar surface should be dominated by nonpolar or dispersion force interactions, releasing the ice-like water, which is structured in the vicinity of the interface. The plasma protein may undergo some conformational changes upon adsorbing in order to expose its own internal nonpolar groups to the foreign surface,[144] as hydrophobic bonding could then occur between the internal hydrophobic groups of the plasma protein and the hydrophobic polymer surface. Such an "unfolding" could result in an irrevsible denaturation of the protein.[144]

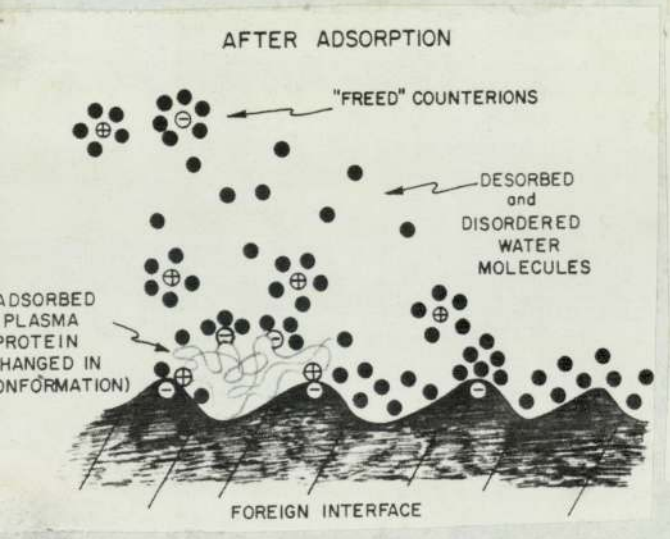
Since, as has already been shown, plasma protein adsorption and denaturation is of great importance in thrombosis, it should be expected that the nonpolar surfaces are more likely to cause irrevsible denaturation (with the exposing of reactive groups, causing further reactions) than polar hydrogel surfaces. The processes are depicted schematically in Figure 20, for a nonpolar polymeric surface and for such a surface coated with a hydrogel.

FIGURE 20 (144)

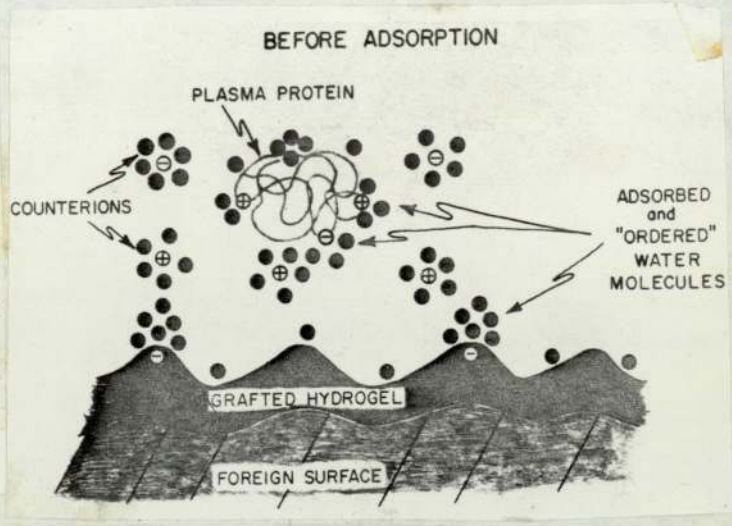
Expected interactions of nonpolar and hydrogel surfaces in an aqueous medium with plasma proteins

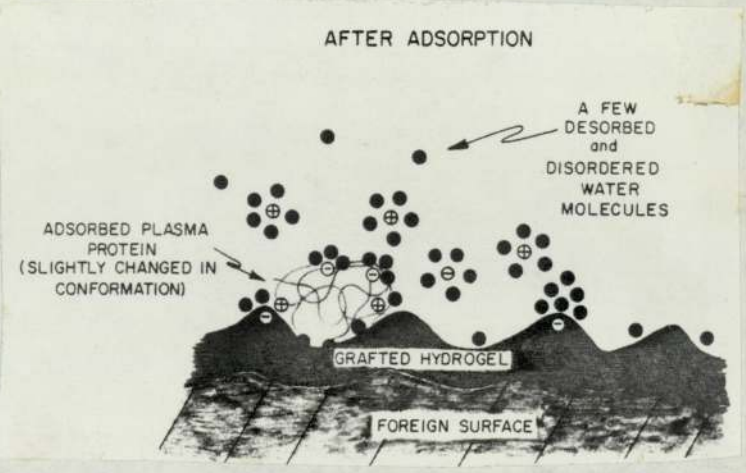
Non-polar surface





Hydrogel-coated surface





Support for the hypothesis of Hoffman comes from several adsorption studies. e.g. it has been found that fibrinogen adsorbs to a greater extent on silastic surfaces at 22°C than at 37°C, while there is little difference in the amount of its adsorption to radiation-grafted hydrogel/Silastic surfaces at these temperatures. This suggests the importance of nonpolar ("hydrophobic") bonding between fibrinogen and Silastic surfaces in contrast to the hydrogel coated surfaces,[144] as hydrophobic bonding is very temperature dependent and its importance decreases as temperature increases. It is also found that when adsorbed proteins, such as fibrinogen or albumin are exposed to desorbing solutions, only a small fraction of the protein on the hydrophobic Silastic can be desorbed, while a large fraction of that or the hydrogel coated surface comes off easily.[144] This effect is illustrated in Figure 21.[139] This observation also tends to support the hypothesis as plasma proteins, which are denatured and highly bonded to the surface are less likely to be desorbed.

As was mentioned earlier a wide variety of techniques are being used to investigate the organization of water within hydrogels, but only a few preliminary results are available of the interrelationship of the physical and chemical nature of the hydrogel, and the organization of gel water.

The gel water can be of several types:[106]

(a) polarized around charged ionic groups

(b) orientated around hydrogen bonding groups or other dipoles

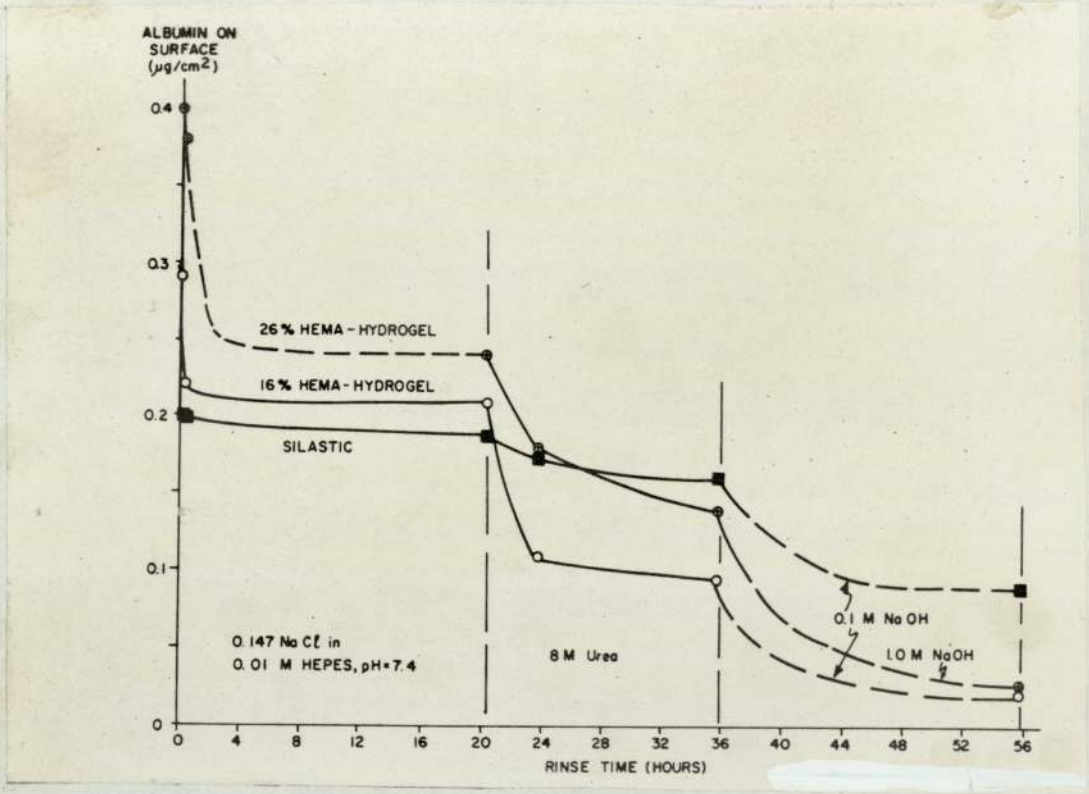
(c) structured in "ice-like" configurations around hydrophobic groups

(d) imbibed in large pores as "normal", "free" bulk water.

FIGURE 21[139]

Desorption of albumin from two different polyHEMA/Silastic hydrogels and from Silastic

Three different elution solvents were used in sequence, each at room temperature.



The organization and content of gel water will therefore vary significantly with hydrogel composition, and some expected effects have been seen, for example, that gels with higher water contents will have lower fractions of bound and interfacial water.[145]

It is believed that surfaces, which have a water structure similar to that of the plasma, will probably be more blood compatible than those that do not;[111] however, experimental work on such an inter-relationship is just beginning.

(8) Comparison of Hydrogels to other Materials

Hydrogels are not the only materials which have been considered for biomedical applications; it is therefore interesting to compare their performance to other materials in comparable blood compatibility experiments. The following tables give compilations of such results, they are divided into the various in vivo and in vitro tests mentioned and referred to earlier.

The materials, which are dealt with, are the following:-

(a) hydrogels, which have been discussed previously, and against which the others are compared.

(b) two kinds of polyurethanes, the first is a polyether block copolyurethane and the second, "Estane", a polyester-type block copolyurethane. They have been considered for cardiac assist devices of various sorts.

(c) polyfluorosiloxanes and blends of these with polydimethyl siloxane elastomers in the role percentages given. Polyfluoro siloxanes have been developed to improve the generally poor blood compatibility of polydimethylsiloxane elastomers. The presence of fluorine groups, it is believed, would tend to increase the negative polarization of the surfaces and such negative polarization is considered by some to be one of the parameters involved in achieving blood compatibility.

(d) heparinized silicone rubbers, which have already been discussed as examples of the use of heparin to increase blood compatibility.

(e) a low-temperature-isotropic (LTI) carbon, and carbons of this type are being successfully used in applications such as heart valves.

(f) a polycarbonate/polydimethylsiloxane copolymer, and such copolymers are undergoing test and evaluation in blood oxygenators (hollow fibre type).

The tests on the materials are divided up into the following groups:-

Table 19 In vivo vena cava tests

Table 20 In vivo renal embolus tests

Table 21 In vitro, plasma proteins and enzyme test results and calcium replacement clotting time tests

Table 22 In vitro tests on the adherence of blood cells

Table 23 Platelet aggregation and activation with cell growth results.

It can be seen by comparing the results obtained in the blood compatibility tests that the hydrogels studied compare very favourably with the other materials, and so must be thought of as representing a very promising approach to the problem of increasing the blood compatibility of surfaces.

TABLE 19

Vena cava test results on Hydrogels and other Materials

Material	Vena cava (2 hours)	Vena cava (2 weeks)
(a) Hydrogels		
Polyacrylamide	Excellent	Very good
Ioplex 101 (0.5 meq anionic)	Excellent	Very good
(b) Polyurethanes		
Copolyether urethane	Excellent	Moderate
Estane	Good	Poor
(c) Fluorosilicone Elastomers		
100% FS	Moderate	Moderate
65/35 mole % FS/DMS	Good	Good
38/62 mole % FS/DMS	Good-Moderate	Good-Moderate
(d) Heparinized silicones		
TDMAC	Excellent	Excellent
APTES	Very good	Very good
(e) LTI Carbon	Excellent	Excellent
(f) Polycarbonate/Silicone copolymer	Moderate-Poor	-

TABLE 20

Renal Embolus test results on Hydrogels and other Materials

Material	Thrombus on ring	Deposit below ring	Renal infarcts
(a) Hydrogels			
Polyacrylamide	None	—	Few
Ioplex 101 (0.5 meq anionic)	Mostly free	—	Moderate-severe
(b) Polyurethanes			
Copolyether urethane	Moderate	Partial blockage	Numerous
Estane	—	—	—
(c) Fluorosilicone elastomer			
100% FS	Moderate	Moderate	Moderate
65/35 mole % FS/DMS	Moderate	None	Good-moderate
38/62 mole % FS/DMS	Moderate	None	Moderate-none
(d) Heparinized silicones			
TDMAC	—	—	—
APTES	—	—	—
(e) LTI Carbon	V E R Y G O O D		
(f) Polycarbonate/ Silicone Copolymer	—	—	—

TABLE 21

Summary of in vitro tests dealing with plasma proteins and enzymes, and the calcium replacement clotting times

Material	Effect on plasma proteins 17	Effect on plasma enzymes	Calcium replacement clotting times
(a) Hydrogels			
Polyacrylamide	None	None	Not prolonged
Ioplex 101 (0.5 meq anionic)	None (except fibrinogen)	Some	Not prolonged
(b) Polyurethanes			
Copolyether urethane	None	None	Not prolonged
Estane	None	Some	Prolonged
(c) Fluorosilicone elastomer			
100% FS	None	None	Not prolonged
65/35 mole % FS/DMS	None	None	Not prolonged
38/62 mole % FS/DMS	None	None	Not prolonged
(d) Heparinized silicones			
TDMAC	None	None	Not prolonged
APTES	None	None	Not prolonged
(e) LTI Carbon	None	None-slight	Not prolonged
(f) Polycarbonate/ Silicone Copolymer	None	None	Not prolonged

TABLE 22

Adherence of blood cells to hydrogels and other materials

Material	Adherence of blood cells		
	Erythrocytes (Whole Blood)	Leucocytes (Whole Blood)	Platelets (Platelet rich plasma)
(a) Hydrogels			
Polyacrylamide	70,000/cm^2	0	256,000/cm^2
Ioplex 101 (0.5 meq anionic)	510,000/cm^2	0	320,000/cm^2
(b) Polyurethanes			
Copolyether urethane	20,000/cm^2	60,000/cm^2	2,320,000/cm^2
Estane	30,000/cm^2	60,000/cm^2	2,080,000/cm^2
(c) Fluorosilicone elastomer			
100% FS	320,000/cm^2	250,000/cm^2	3,680,000/cm^2
65/35 mole % FS/DMS	100,000/cm^2	200,000/cm^2	2,560,000/cm^2
38/32 mole % FS/DMS	150,000/cm^2	80,000/cm^2	2,240,000/cm^2
(d) Heparinized silicones			
TDMAC	10,000/cm^2	0	150,000/cm^2
APTES	180,000/cm^2	0	90,000/cm^2
(e) LTI Carbon	Light*	None*	Moderate*
(f) Polycarbonate/ Silicone Copolymer	30,000/cm^2	60,000/cm^2	1,550,000/cm^2

* Quantitive figures are not available.

TABLE 23

Platelet aggregation and activation and cell growth results for Hydrogels and other materials

Material	Platelet Aggregation and Activation	Cell Growth (amnion)
(a) Hydrogels		
Polyacrylamide	None	Slightly less than control
Ioplex 101 (0.5 meq. anionic)	—	—
(b) Polyurethanes		
Copolyether urethane	Very low-None	Slightly less than control
Estane	Low	50% of control
(c) Fluorosilicone Elastomer		
100% FS	None	Slight-None
65/35 mole % FS/DMS	None	Slightly less than control
38/62 mole % FS/DMS	None	Slightly less than control
(d) Heparinized silicones		
TDMAC	Very low	None
APTES	None	Little
(e) LTI Carbons	Slight	Near 100%
(f) Polycarbonate/ Silicone Copolymer	Some	Slightly less than control

(9) Conclusion

In this chapter it has been shown that homogeneous or grafted hydrogels represent one of the most promising approaches to the problem of increasing the blood compatibility of materials. It has also been shown that there are many problems remaining as to the relationship between surface properties and blood compatibility; a complete elucidation of these should result in the preparation of more promising materials, such as novel or modified hydrogels.

CHAPTER 3

ARTIFICIAL LIVER SUPPORT

3.1 THE LIVER

The liver is the largest and, from a metabolic standpoint, the most complex internal organ in the body;[102] its functions being numerous and complex. These have been classified in five major groups.[146]

(A) Circulatory functions - the transfer of blood from the portal to systemic circulation; the activity of its reticuloendothelial system (Kupffer cells) in immune mechanisms and blood storage, (the regulation of blood volume).

(B) Excretory functions - bile formation and excretion of bile into the intestine, secretion in the bile of products emanating from the liver parenchymal cells, eg. bilirubin conjugates, cholesterol, cholic acid as bile salts, and excretion of substances withdrawn from the blood by hepatic activity, eg heavy metals.

(C) Metabolic functions - carbohydrate, protein, lipid, mineral and vitamin metabolism, and heat production.

(D) Protective functions and detoxification - Kupffer cell activity in removing foreign bodies from the blood (phagocytosis); detoxification of various chemicals and drugs by conjugation, methylation, oxidation and reduction. Particularly important is the removal of ammonia from blood, especially that absorbed from the intestine by way of the portal vein.

(E) Haematologic functions - (haematopoiesis and coagulation) Blood formation in the embryo and, in some abnormal states, in the adult, production of albumin and blood clotting factors, eg fibrinogen, prothrombin, heparin, and erythrocyte destruction.

When a patient's liver fails it is appropriate to think of replacing the various aspects of the liver's functions, for example, intravenous infusion of clotting factors and albumin can, in part, replace its synthetic function and the avoidance of certain drugs obviates the need for a part of the liver's detoxification function,[147] but one function is very difficult to replace:- the excretory aspect, the replacement of which is the main subject of this chapter.

These difficulties in replacing the functions of the liver are to some extent counterbalanced by the ability of the liver, given the correct conditions, to regenerate rapidly. Thus if the functions of the liver can be supplemented by temporary liver support during an acute crisis, total recovery is possible in many cases.[147]

On the other hand the central metabolic role of the liver results in disturbances in other organs, notably the brain, when liver function is impaired.[147]

3.2 CONDITIONS NEEDING TREATMENT

Williams et al have identified three main groups of patients who could benefit from a temporary liver support system.[147]

The first consists of those in acute liver failure due to viral hepatitis, exposure to hepatotoxins or drug reactions. When the patient has lapsed into deep coma the mortality rate is around 80 per cent. This accounts for about 400 deaths per annum in England and Wales and includes a number of deaths from an overdose of paracetamol. This drug, an effective and safe analgesic in normal quantities, can result in severe liver damage if taken in excessive

quantities in suicidal attempts, and now constitutes the largest single group of patients seen in the liver unit in hepatic coma.[147] Another major cause is halothane - induced hepatic necrosis which with a few exceptions has followed the use of multiple anaesthetics.[148] Table 24 shows the causes of fulminant hepatic failure (Grade III and IV) as seen in the Liver Unit of King's College Hospital over a 7-year period.

TABLE 24

Causes of Fulminant Hepatic Failure seen in the Liver Unit over 7 years

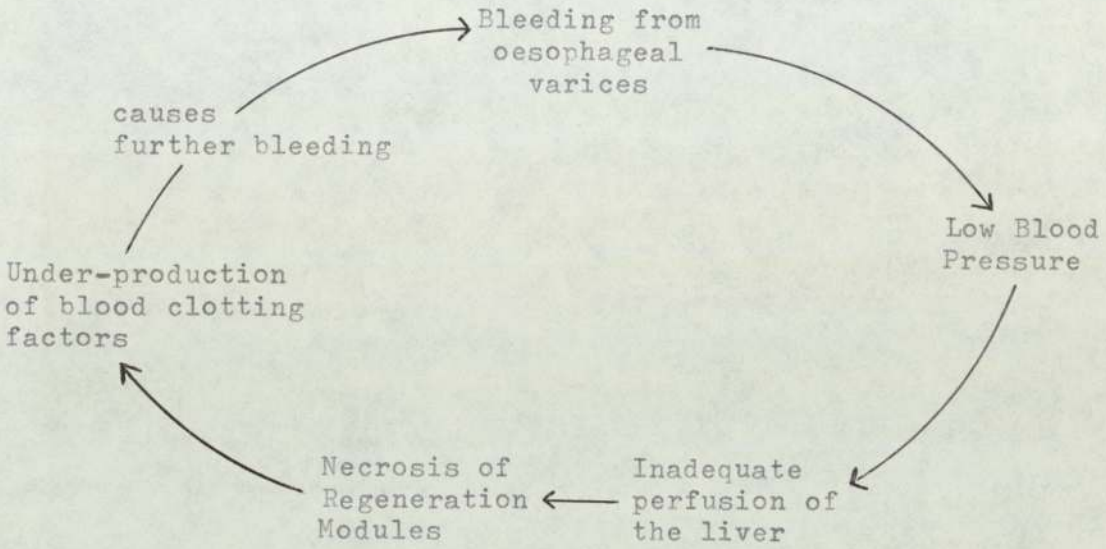
Acute Viral Hepatitis	59
Paracetamol Overdose	46
Halothane Associated	20
Other drug toxicity	4
Amanita Phalloides	1
Fatty Liver of Pregnancy	2
	132

The second group is formed by patients with chronic underlying liver disturbances, such as cirrhosis, subsequently exacerbated by, for example, bleeding from oesophageal varices. The vicious circle illustrated in Figure 22 then results.

A means of breaking this circle would result in improvement and stabilization of the patient's condition and allow the underlying liver disease to be treated by other means.[147]

FIGURE 22

Effect of Chronic Underlying Liver Disturbances



The third group consists of those with advanced cirrhosis and irreversible liver failure for whom the only possible treatment is transplantation. This is potentially the largest group of patients as cirrhosis alone accounts for about 1,400 deaths per annum in England and Wales. Kidney transplantation would not be undertaken without dialysis support, yet no equivalent procedure can be carried out before the technically more difficult liver transplant. Likewise, at present, there are no means whereby a patient with liver failure can be kept alive until such time as a donor becomes available.(147)

3.3 BASIC SUPPORTIVE THERAPY

3.3 (a) INTRODUCTION

We shall now consider the basic therapeutic measures which are necessary for the survival and recovery of the patient; the four major areas in which this is necessary are shown in Table 25.[148]

TABLE 25

Areas in which therapy is necessary

A	Haemorrhagic diathesis	- Coagulation factors - Thrombocytopenia	
B	Infections	- Local, eg pneumonia - Septicaemia	
C	Metabolic derangements	- Hypoglycaemia - Hypokalaemia	Early
		- Fluid overload - Renal failure - Hypernatraemia	Latter
D	Encephalopathy	- Secondary factors - Primary factors	

The factors shown in Table 25 will be individually discussed in the following pages.

3.3 (b) HAEMORRHAGIC DIATHESIS

The haemorrhagic diathesis of fulminant hepatic failure is considered to be only second in importance to encephalopathy. Major bleeding from the gastrointestinal tract is often the immediate cause of death, and earlier episodes of bleeding because of ensuing hypotension must have a deleterious effect on cerebral and renal function as well as causing further ischaemic damage to the hepatocytes.[149]

Study of the coagulation tests found on admission of patients with fulminant hepatic failure showed that, the prothrombin time was usually considerably prolonged and the fibrinogen concentration was often reduced. These changes could be solely due to failure of synthesis by the liver but in addition platelet count was frequently reduced, despite apparently normal production in the marrow. This and other observations lead to the suggestion that intravascular coagulation was occurring in these patients, with an increased consumption of platelets and coagulation factors.(149)

The development of intravascular coagulation is presumably due to the release of tissue thromboplastins into the circulation from necrotic hepatocytes. It is also believed that in addition to intensifying the coagulation factor deficiency of these patients, intravascular coagulation could also theoretically lead, as a result of thrombotic occlusion of the microcirculation, to secondary organ damage. Because of continuing fibrinolysis, microthrombi may not be found in histological sections but in some cases they have been seen in the hepatic sinusoids. There is also evidence from experiments on rats that the kidney, heart, liver and spleen were affected by intravascular coagulation.(149)

This effect of liver failure has been treated by a combination of heparin for the intravascular coagulation and fresh frozen plasma for the synthetic defect. This therapy, even in patients with severe encephalopathy in whom the coagulation disturbance was most marked, has been followed by rapid falls in prothrombin times and rises in plasma fibrinogen levels indicating that the degree of coagulation within the liver must have lessened.(149)

3.3 (c) INFECTIONS

These are quite a severe risk since the unconscious state and inadequate ventilation favour respiratory infection and the need for intravenous infusion and a urinary catheter predispose the likelyhood of infections by these routes.(149)

To prevent infection from indwelling catheters, these should be charged every three days and the inguinal area avoided.(149)

3.3 (d) METABOLIC DERANGEMENTS

Several different types of metabolic derangements can arise in liver failure, these can be sub-divided into two sets:- those that arise early in treatment and those which arise later.

Two which can arise early are hypoglycaemia and hypokalaemia. Profound hypoglycaemia is often missed but is important because of the brain damage it can cause.(81) The depressed level of consciousness resulting from it can be reversed, (at least temporarily) by giving glucose. Often massive quantities are needed to correct the defect, 2.5 kg per day has been reported.(149) Similarly, the serum electolytes also need frequent estimation, hypokaleamia is present early and complicated changes in the acid-base balance develop. Supplementary potassium is nearly always needed and the exact therapy given depends on the changes found.(149)

The two main metabolic derangements which occur late in treatment are renal failure and fluid overload.

Renal failure with severe oliguria is a frequent happening, it

has been found that a high percentage of patients who enter deep coma have evidence of renal impairment. It is best treated by peritoneal dialysis.(149)

Fluid overload is a problem, partly because of the effects of plasma and other substances given in treatment, particularly when renal failure develops.(148)

Hypernatraemia can also be of potential importance late in treatment as it alone can cause coma. It can be caused by osmotic diuresis precipitated by hypertonic dextrose given intravenously for nutritional purposes. An additional factor is the content of sodium in fresh frozen plasma used to correct the coagulation disturbance, when renal excretion of this ion is inappropriately low.(149) These last two effects can again be relieved by dialysis.

3.3 (e) ENCEPHALOPATHY

This is the main subject of the following section, which deals with the toxins that arise in liver failure and their means of removal. In this short section an overall look at the subject is taken leaving out the previously mentioned areas, which are the subject of the next sections.

The occurrence of encephalopathy is of central importance in the syndrome of hepatic failure. The severity of encephalopathy has been divided into four stages:-

Stage I - Mild confusion and irrational behaviour

Stage II - Patients become confused but do not lose consciousness

Stage III - Coma with some brain activity

Stage IV - Deep coma, little brain activity.

It has been found, as might be expected that the survival rates decrease with increasing grade of coma, eg in one study a survival rate of 66 per cent was found for Stage II, and 18 per cent for Stage IV.(149)

The development of encephalopathy is usually attributed to the effect of toxins, whose synthesis and removal are discussed in the next section. There are, however, various secondary factors which are also of importance, these too can damage the brain. These factors include anoxia, which is a result of cardio-respiratory failure and is very common in the later stages of hepatic failure, hypoglycaemia, (which has already been mentioned), and also from the low p CO_2, resulting from the hyperpnoea, which is often a striking feature of the early stages. This last factor causes a reduction in cerebral blood flow, possibly to critical levels. Mechanical ventilation should be instituted as soon as the respiratory rate begins to drop, but it has been observed that recovery is unusual once ventilation becomes necessary.(149)

3.4 POTENTIAL TOXINS TO BE REMOVED

3.4 (a) INTRODUCTION

There are many different types of toxins present in the body during liver failure whose removal should result in a clinical improvement for the patient. Some of these toxins are toxic only because they are "free" rather than plasma bound, or because they are present in unusually high concentrations, and if their levels were reduced too far deleterious effects would result. An example of this latter class of toxins are the amino acids, some of which

are coma producing yet necessary for liver regeneration.

A list of known potential toxins is given in Table 26. Besides those shown, drugs, which can cause liver failure, eg paracetamol, must be considered. In addition to the toxic effects of the individual toxins it is known that synergistic effects also occur.

TABLE 26

Potential Toxins in Liver Failure

1 Ammonia
2 Amino acids
3 Fatty acids
4 Mercaptans
5 False neurotransmitters
6 Bile acids) Minor toxins
7 Bilirubin)

The production of and effects of these are considered in the following pages.

3.4 (b) AMMONIA

Ammonia is produced by the deamination of amino-acids and other compounds. Normally, it is detoxified rapidly in the liver by conversion to urea; however, when liver failure occurs this process is interrupted and the concentration of ammonia in the body rises.(4)

There is a great deal of evidence to show that pure ammonia intoxication produces a state which results in convulsions and

coma;[150] however, it is also apparent that although disturbed ammonia metabolism is in some way basic to the syndrome of hepatic coma it is not the only factor.[150] In general the correlation between blood ammonia and the degree of hepatic encephalopathy is poor, though positive.

The primary disposal route of ammonia in the brain is by forming glutamine,[150] and a good correlation has been found between the spinal fluid glutamine concentration and the degree of encephalopathy.[151] No other parameter has correlated as well with clinical severity,[150] with the possible exception of the false neurotransmitter octopamine.[89]

3.4 (c) AMINO-ACIDS

Amino-acids are normally utilised by the body for protein synthesis with the excess quantities being deaminated to urea. It is believed that their accumulation in liver failure may have a role in the pathogenesis of coma,[147] since it has been found that large intra-peritoneal doses of essential amino acids have been uniformly fatal to rats, with individual essential amino acids comprising a lethal dose creating high levels of blood ammonia.[150]

It is now known that hepatic failure is characterized by an increase in the plasma of straight chain amino acids, and a decrease in branched chain amino acid, (which are the least toxic). The predominating amino acids that increase in the plasma are methionine, phenylalanine, aspartate, glutamate, tyrosine and histidine.[150]

Only a few studies have been carried out on amino acid

alterations in the brain or spinal fluid, they showed that glutamine, tyrosine, tryptophan, phenylalanine, and histidine, all increased several fold.(150)

Some amino acids - aspartate, glutamate and arginine, have particular theoretical, if not practical significance because they have arousal properties in patients with hepatic encephalopathy; they are coma-preventing.(150)

3.4 (d) FATTY ACIDS

It has been found that fatty acids injected into animals cause coma which is rapid in onset but reversible. The longer the fatty acid chain the more potent the effect. Biochemically, free fatty acids have widespread metabolic effects at very low concentrations such as the inhibition of enzymes and uncoupling oxidative phosphorylation.

4-5 fold rises in serum short-chain fatty acids, particularly hexanoic acid, have been observed in hepatic coma and as much as a $2\frac{1}{2}$ fold increase has been seen in long-chain fatty acids, though this was highly variable and could not be correlated with the degree of encephalopathy.(150)

In addition to their intrinsic toxicity, the free fatty acids have potential significance in relation to the possible toxicity of tryptophan and as augmenters of the toxic effect of ammonia and mercaptans (synergism).(150) Normally at low concentrations fatty acids in the blood are not toxic because they are bound to the plasma protein, albumin.

3.4 (e) MERCAPTANS

An association has been established between mercaptans and hepatic coma; from breath analyses, it has been found that mercaptans increase approximately four-fold in hepatic coma. Animal experiments have shown that reversible coma can be induced by them and that they enhance the toxicity of ammonia and fatty acids.(150)

3.4 (f) NEUROTRANSMITTERS AND FALSE NEUROTRANSMITTERS

Intimately connected with the amino acids and their chemistry are the neurotransmitters and false neurotransmitters. The neurotransmitters are substances such as norepinephrine and serotonin, histamine and possibly even several highly neuroactive amino acids such as glycine, alanine, taurine, γ -amino butyrate, glutamate and aspartate, which in addition to acetyl choline may play important roles in normal synaptic transmission in the brain and spinal cord. In the peripheral sympathetic nervous system nonepinephrine is the putative neurotransmitter, however, other β -hydroxylated phenylethylamines can replace norepinephrine and act as false or relatively inactive neurochemical transmitters. (The β -hydroxylation on the side chain has been shown to be a basic structural requirement for false transmitters in the peripheral sympathetic nervous system.(152)

The hypothesis set out by Fischer(152) to explain the production of hepatic coma and its complications is as follows. Precursors such as phenylalanine, tyrosine and their respective amines, which are produced from food proteins in the gut, by the action of bacteria are, (when hepatic function is impaired and blood is shunted around the liver), not catabolised by monoamine oxidase(MAO)

(which acts mainly in the liver) but flood the peripheral and central nervous system and are taken up, β-hydroxylated by a relatively non-specific enzyme, and replace the normal endogenous neurotransmitters at presynaptic storage sites. It is then possible to explain many of the physiological effects seen in hepatic failure, such as cardiovascular changes, asterixis (a sign of disorded extrapyramidal control of movement) and many others.(154)

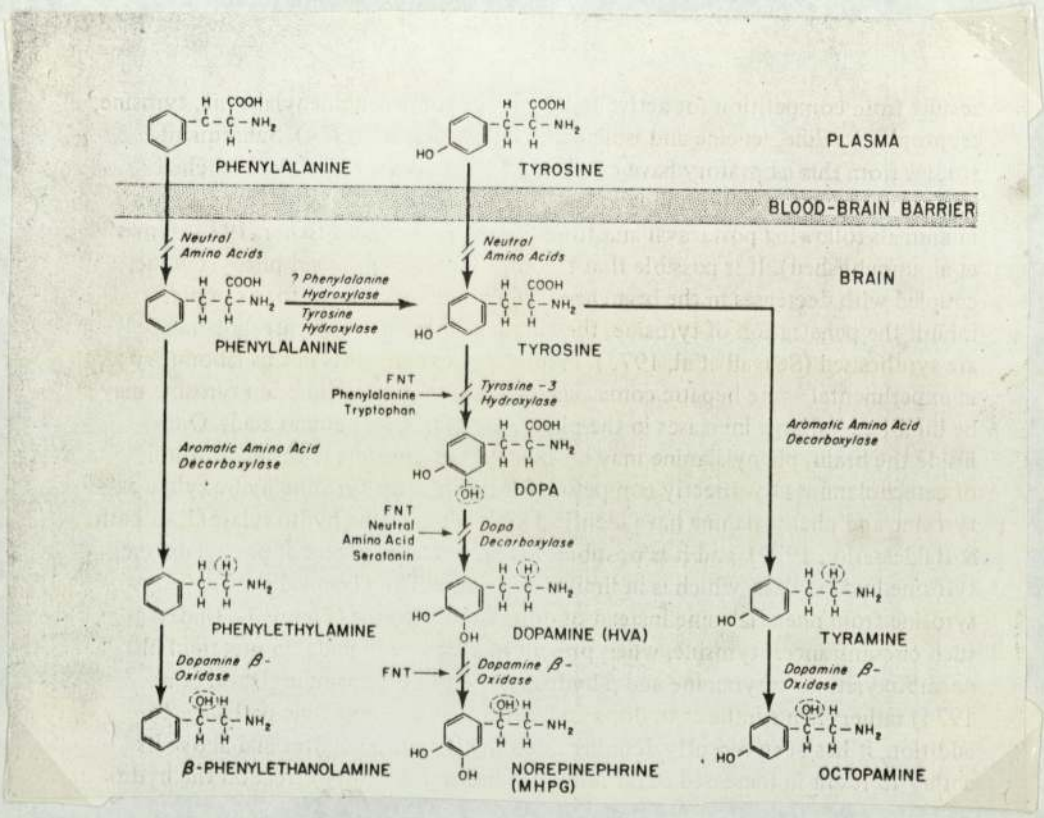
One of the most important false neurotransmitters is believed to be octopamine, since the concentration of octopamine in the blood has correlated better to the state of consciousness of the patient in liver failure than any other biochemical measurement. Although octopamine in the brain, (the important locus of its action), is not caused by accumulation of octopamine in the blood, as it could not traverse the blood brain barrier, it is believed the rise in the blood is paralleled by the rise in the brain due to increased synthesis in both systems.

A scheme of synthesis of catecholamines and phenylethylamines has been suggested and is shown in Figure 23. Blocks in the synthetic function are indicated by lines and suggest that there are numerous sites at which catecholamine synthesis can be blocked. If tyrosine, for example, cannot go to DOPA it is primarly decarboxylated to tyramine and then to octopamine, thus perhaps explaining the increase in brain octopamine while brain norepinephrine is decreased on the basis of diminished synthesis.(152)

However, the complete picture of the synthesis and action of these materials has still to be determined.

FIGURE 23

Synthesis of Some False and True Neurotransmitters (152)



3.4 (g) BILE ACIDS

The bile acids are a group of cholesterol derivatives synthesized by the liver and excreted into the bile to aid the digestion of fat, after which they are reabsorbed into the bloodstream, thus establishing a cyclic path (the "enterohepatic circulation").(147)

Disorders of bile excretion are sometimes accompanied by severe itching (pruritis), which is related to the accumulation of bile acids in the skin.(147) Other toxic effects believed to be caused by the bile acids are:-

1. inactivation of cholinesterase, which is thought to account for the bradycardia, increased sweating and increased neuromuscular activity. Very high doses of bile acids may lead to muscular paralysis, probably owing to suppression at the myoneural junction.
2. a haemolytic effect on red cells
3. an anticoagulant effect by binding calcium
4. a metabolic effect: the depletion of the sulphur amino acids
5. production of gastric ulcers, if bile acid is brought into contact with the gastric mucosa.(153)

The bile acids are often used in the assessment of artificial liver support systems.

3.4 (h) BILIRUBIN

Another minor toxin in liver disease but an important one in neo-natal jaundice is bilirubin, a breakdown product of haemoglobin which, when present in high concentration is responsible for the yellow colour of jaundice. It is water insoluble and tightly bound

to protein, primarily albumin, in the blood. The liver normally conjugates bilirubin to the diglucuronide which makes it water soluble, less strongly bound to plasma protein, and less toxic. It is in this form that it is excreted into the bile.[147] In liver failure, however, this process does not occur and the concentration of the unconjugated form rises.

The toxicity of bilirubin is likely to be low, although in the newborn infant in whom the blood brain barrier is immature, unconjugated bilirubin may damage the brain (kernicterus) if levels exceed 20 mg/100 ml.[147] It may also play a synergistic effect with other toxins by displacing them from sites on the albumin molecule.

3.4 (i) SYNERGISM

In addition to the toxic effects of the individual toxins it has been found that the coma-producing potential of some of these toxic substances can be multiplied several fold when they are present together, as a result of their interdependent metabolism or interrelated effects. This synergism has been demonstrated experimentally with ammonia, fatty acids and mercaptans,[150] and some examples of known cases have been given on the previous pages.

3.5 METHODS OF REMOVING TOXINS

3.5 (a) INTRODUCTION

Many different methods of removing the toxins described in the previous section have been tried; these are described individually

on the following pages, and in addition to the removal of endotoxins such as ammonia, the removal of exotoxins such as paracetamol and other drugs will be discussed as this can be an integral part of artificial liver support in cases of drug overdose.

The means of treatment tried in the past and being tried presently both clinically and experimentally, are given in Table 27.

TABLE 27

Potential means of removing Exo and Endotoxins

1	(1	Extracorporeal liver perfusion
	(2	Cross circulation
2	(3	Conventional dialysis
	(4	Polyacrylonitrile membrane haemodialysis
	(5	Liquid - liquid interfaces
3	(6	Resin perfusion
	(7	Activated carbons
	(8	Affinity chromatography
4	(9	Gell-entrapped liver cell microsomes
	(10	Microsomal enzymes bound to artificial carriers
	(11	Haemoperfusion through liver cells in tissue cultures

It will be seen that four basic approaches have been made to the problem of the removal of toxins. Firstly, techniques such as extracorporeal liver perfusion and crosscirculation, which involve in some way the use of an animal or human liver to replace the excretory function of the damaged liver. Secondly, dialytic techniques similar

to those used on people suffering from kidney failure. Thirdly, haemoperfusion which involves passing blood or plasma over an adsorbent of some sort to remove the toxic substances. Fourthly, the use of elements of the liver:- cells, microsomes, or enzymes to remove and or detoxify the blood (and even in some cases to replace the liver's synthetic function).

The different methods will be discussed under these headings and the main emphasis will be on the second and third groups, as these are at the moment the most important in practical application and the experimental work carried out has relevance to this area. There is, of course, some overlap between the various sections and the division is to some extent arbitrary.

3.5 (b) TECHNIQUES INVOLVING A HUMAN OR ANIMAL LIVER

These techniques were tried early on in the therapeutic field for detoxifying the blood of the patient suffering from liver failure. They were the most logical approaches as the liver itself is, of course, the best "device" to replace a damaged liver. The two main approaches were cross-circulation and extracorporeal liver perfusion.

(1) Extracorporeal Liver Perfusion

Connection of the patient to an isolated but functional animal liver in an extracorporeal perfusion circuit has been a frequently tried technique. In Britain the liver is usually obtained from a pig, but calves, baboons and monkeys have also been used in other parts of the world. A human liver obtained from a cadaver very

shortly after death is another possibility and has the advantage that, as with the liver of a baboon, perfusion can be continued for longer periods of time - up to about forty eight hours, as compared with a maximum of six to eight with a pig's liver.(149)

Although a perfused liver can be shown to have excellent biochemical function, this gives no indication of its haematological function. Indeed such a liver can deplete the patient's blood of platelets, worsen the prothrombin time and produce severe bleeding. In a study Williams et al detected a significant loss of fibrinogen in the fluid that weeps from the surface of the liver during perfusion. There was also a loss of fibrinogen into the perfused liver itself in most cases, with a parallel drop in the platelet count of the perfused blood. The most likely cause appears to be intravascular coagulation within the perfused liver, probably because of damage to the sinusoidal endothelium occasioned by ischaemia during hepatectomy.(149) Since there is no certain method of predicting whether such intravascular coagulation will be slight or severe this makes this technique unreliable.

It has been found that although the patient may be brought out of coma by extracorporeal perfusion the total figures for patient survival have been disappointing; but there have been individual cases of long term survival and a few cases of complete recovery.(149)

(2) Cross circulation

A further method of bringing the patient's blood into prolonged contact with a normal liver is by cross-circulation with a volunteer having compatible blood.(149) The technique is limited to

non-infectious cases[147] but even then it is not without risk to the partner; fever, falls in platelet counts and abnormal liver function test results being observed.[154]

The baboon may also be used as a partner in cross-circulation. If its blood is washed out and replaced by human blood, the animal will live normally for five to seven days before the human red cells undergo haemolysis.[149] Some of the patients thus treated have improved.[155][156] The use of baboons, however, carries a potential virological hazard to patients and staff which necessitates special facilities.

Some success has been reported in the few cases in which this method has been tried, eg Saunders et al reported the case of one patient who before cross-circulation with a baboon was decerebrate regained normal consciousness afterwards,[149] but the overall lack of success with this and the previous technique has lead to the investigation of other approaches.

3.5 (c) DIALYSIS

Another obvious approach to the problem of liver failure is dialysis, the technique which has been found to be so efficacious in the treatment of patients suffering from kidney failure. However, while there are evident similarities between the roles and function of the liver and kidneys, there are also fundamental differences. In particular the excretory functions of the liver deal with a much wider spectrum of compounds, in terms of molecular weights, physico-chemical properties, and protein binding, than do those of the kidney and although a relatively detailed understanding of renal

excretory physiology was an essential foundation for the development of haemodialysis, no such comparable foundation exists for the liver.[158] Thus conventional dialysis using materials developed for the artificial kidney were unlikely to be as useful in the treatment of liver failure.

On the following pages the use of conventional dialysis and the use of new more porous membranes is described. In addition, a type of haemoperfusion, which developed from certain ideas in the dialytic field will be discussed.

(1) Conventional Haemodialysis

The first use of conventional haemodialysis in patients with hepatic failure was reported in 1950, and small clinical trials were reported in the 1950's and 1960's.[158] Haemodialysis was found to be effective in lowering blood ammonia levels,[158] and in experiments on the dialysis of blood toxins and model compounds Willson et al found that compounds not bound to albumin, (methionine, glutamine, butyrate, ammonia and urea) were readily dialysed; whereas dialysis of protein-bound anions, (unconjugated bilirubin, chenodexoxycholate, BSP and methyl orange) was negligible and enhanced little by addition of a competitive anion, (eg salicylate) or an acceptor, (plasma or a water-soluble polycation) to the dialysate. The major rate-limiting factor was the low concentration of the unbound species, rather than the membrane.[159]

It was this failure of the haemodialysis system to remove protein bound material and higher molecular weight materials that lead to the development of haemoperfusion systems of various sorts;

those with high surface areas and adsorptive capabilities did not have the same limitations. However, it is now felt by various workers on a re-examination of the data of the early clinical trials, that the rejection of the haemodialysis system as a means of treatment was premature,[159] and there has been renewed interest in it as a means of treatment. The greatest advance in this area has been the work of Opolon using a new improved haemodialysis membrane.[160]

(2) Polyacrylonitrile membrane haemodialysis

The new membrane consists basically of polyacrylonitrile and is strong, very thin and permeable to substances up to a molecular weight of 15,000 (but not to the plasma proteins). Experiments with comatose pigs for false and true neurotransmitters and their precursors in different parts of the brain before dialysis, after dialysis, (with polyacrylonitrile and cuprophane membranes) and on controls, showed that the concentrations of false and true neurotransmitters after haemodialysis with the polyacrylonitrile membrane approached closely those of the controls; whereas, surprisingly, the cuprophane membrane showed deleterious effects, amplifying the difference between false and true neurotransmitters.[160]

The new membrane has also been clinically tried on 24 patients with fulminant viral hepatitis and coma, (grades III and IV). Total recovery of consciousness was achieved in 13 out of 22, and four patients recovered (22 per cent), death in the others being due to a variety of causes. This is a high success rate by present standards, and this membrane is now under investigation and is being used for treatment at King's College Hospital's Liver Unit in

preference to other techniques.[161]

It is thought that the new membrane is more successful than cuprophane because unlike cuprophane it is better at dialysing higher molecular weight substances, (such as polypeptides, whose role in the origin of false neuro-transmitters or directly on neurotransmission is still hypothetical) compared to cuprophane haemodialysis which appears to amplify the imbalance between the normal and false neurotransmitters by being highly permeable to only low molecular weight substances, (such as the neurotransmitter precursors).[160]

It is also believed that the hollow fibre artificial kidneys capable of efficiently clearing substances of high molecular weight may be of great importance in the treatment of hepatic failure and a use of these materials and liver cells in a specialised artificial liver device is described later in this review.

(3) Liquid - liquid interfaces

When the conventional type of dialysis was found to be less successful than desired in the removal of certain drugs, the use of lipid-containing solutions for peritoneal dialysis was examined and it was found that glutethimide extraction was increased five-fold as compared to conventional dialysis. Subsequently cottonseed oil on the outside of the cellophane membrane was tried and found to be preferable to conventional dialysis for the removal of certain drugs. This technique lead to the idea of using oil-haemoperfusion, in which direct liquid - liquid extraction is used to remove toxic or unwanted substances from whole blood. By choosing

the proper oil or modification of an oleaginous liquid, total bilirubin and lipid soluble drugs such as glutethimide can be selectively removed or adjusted to the desired concentration with a single liquid - liquid extraction.(162)

The oils investigated were vegetable oils, (cottonseed, linseed, soyabean, corn), mineral oil (liquid petrolatum), ion exchange oil and silicone and fluorocarbon liquids.

Experiments showed that, for the present, liquid ion-exchange materials are unsuitable because erythrocytes in whole blood are grossly haemolysed when put in contact with them. The toxicity, safety and other potential side effects of silicone and fluorocarbon liquids are not yet known. Consequently, vegetable oils, particularly corn oil have been the most studied.(162)

The researchers found that in in vitro experiments with blood that they could remove 80 per cent of the glutethimide present in blood in a single extraction with corn oil without any unwanted losses or adverse effects upon blood being detected, such as, increased osmotic fragility, haemolysis, or alterations in the lipid concentration of the serum, the leukocyte, erythrocyte, platelet or differential cell count.

These experiments were backed up by further in vivo experiments with rhesus monkeys. No loss of platelets or other cellular components were observed during or after the procedure.(162)

Difficulties with the technique are the danger of forming an emulsion of blood and oil or even small droplets within the liquids. Pumping oil into the veins could produce life-endangering fat emboli.

Another undesirable factor is that some essential substance might also be extracted from blood, such as, lipid-soluble vitamins. It is however, believed that rapid re-equilibration with tissue stores would occur when the oil-haemoperfusion procedure is discontinued. On the other hand, such depletion of tissue stores is the goal in patients intoxicated with a drug such as glutethimide. Previously, the rate of extraction of the drug has probably been the rate-limiting step rather than the equilibration between blood and tissue concentrations of the drug.(162)

This field of research is in its infancy and needs much more development before application to human patients.(147)

3.5 (d) HAEMOPERFUSION

The next method for the removal of blood toxins, which will be considered is the use of adsorbents of various sorts; the main ones investigated have been neutral and ion-exchange resins of different types and activated charcoal. As will be shown the technique of haemoperfusion as well as having certain advantages:- selectivity, efficiency (due to the high surface area of the adsorbents) also has certain disadvantages, mainly the low blood compatibility of the systems. Measures to improve the latter, in which hydrogels have played an important role, will be discussed.

(1) Resin Perfusion

Resins of all three types (anion exchange, cation exchange and neutral), have been tried in the treatment of liver failure and the often associated problem of drug overdose; their uses are

illustrated on the following pages.

(a) Cation-exchange resins

Resins of this type have been mainly looked at from the point of view of removing ammonia from the bloodstream; this compound, as has been shown, is probably the most important coma producing substance in hepatic failure. The main difficulty with them is their adverse ion-exchange reactions, resins such as Amberlite IR-120, (a polystyrene sulphonic acid, strong cation exchange resin), has been found to be efficient at extracting ammonia but to sequester other cations from the blood resulting in the development of a dangerous hypokalaemia, hypocalcaemia and hypomagnesaemia. Hypokalaemia, besides its other harmful effects, is known to increase ammonia toxicity.(163)

The way round this problem is to modify the resin and use the sodium, potassium, calcium and magnesium forms of the resins in the right proportions so that ion-exchange will not adversely affect the ionic balance of the blood.(163)

Modified resins of this type have been used by Juggi in the treatment of a few cases of hyperammonaemia due to hepatic failure. He found no significant changes in end-perfusion levels of plasma sodium, potassium, calcium and magnesium and that the resin was a selective and efficient method of remaining ammonia from the circulation, which brought down the elevated blood ammonium levels to within normal limits.(163)

(b) Uncharged hydrophobic resins

Several types of hydrophobic resins have been looked at mainly for the removal of blood exotoxins such as drug molecules, but also some potential blood endotoxins. The two main types of resins examined have been:-

1) macroreticular styrene-divinyl benzene copolymers (XAD2 and 4)
2) macroreticular acrylic ester resins (XAD 7).

The properties of these materials and their adsorption properties will be discussed invidually.

XAD 2 and 4

The physical properties of these highly hydrophobic resins are shown in Table 28.

TABLE 28

Physical characteristics of XAD-2 and XAD-4[164]

	XAD-2	XAD-4
Porosity volume	42 per cent	51 per cent
Surface area	330 metres per cc	750 metres per cc
Average pore diameter	90 Å	50 Å

As can be seen they both have very high surface areas and pores, which are large in comparison to the blood toxins and drug molecules they are intended to remove.

The drugs which XAD-2 has been found to have a specific adsorptive capacity are barbiturates, glutethimide, ethchlorvinyl, methaqualone, thyroxin, digitalis and cephaloridine.[165] However, it

has been found that for more water-soluble drugs such as the salicylates saturation of the columns was rapid and clearance ineffective.[166] Willson has done a study of the effectiveness of XAD-2 at removing bile acids, bilirubin and test molecules such as BSP from saline and plasma in comparison to the anion-exchange resin (Dowex I-X4). He found that XAD-2 was inferior as regards to BSP adsorption from plasma and saline solutions, but that the bile acids were more readily bound to XAD-2.[167] It therefore appears that for hydrophobic molecules, such as the bile acids and certain drugs, XAD-2 is a good adsorbent, but is not a good adsorbent for more water soluble materials such as BSP and the salicylates, as should be expected.

The chemically identical XAD-4 has been found to have an increased adsorptive capacity for lipid soluble molecules due to its much higher surface area. In some cases, however, pore size can be an important factor and for larger molecules, eg alkylbenzene sulphonate XAD-2 has a higher capacity than XAD-4.[165]

Haemoperfusions were found to have no effect on the leukocytes or red blood cells of the circulating blood, but caused reductions in platelet concentrations. XAD-2 has also been found to have effects on the plasma proteins; prealbumin, haptoglobin and ceruloplasmin showed decreases in concentration. Ceruloplasmin appears to be denatured by the haemoperfusion.[168] XAD-4 must be expected to show similar effects.

XAD-2 haemoperfusion has been used on patients with life-threatening acute drug intoxication. The haemoperfusions were associated with dramatic clinical responses and a high success rate

obtained. However, platelet levels dropped by 40 per cent during the procedure, although this and other possible toxic effects were not believed to have lead to the few deaths that occurred.(165)

Rosenbaum, one of the principal workers in this field, has expressed the view that before these resins can be used on a consistent or repeat basis, as with prolonged hepatic failure, effective means of preventing significant embolisation of particulate matter into the circulation must be developed.(165)

XAD-7

A further uncharged resin which has been investigated for toxin removal is XAD-7, it has been found to be capable of adsorbing a wide range of compounds. Its physical, chemical and structural nature are shown in Table 29 and Figure 24.

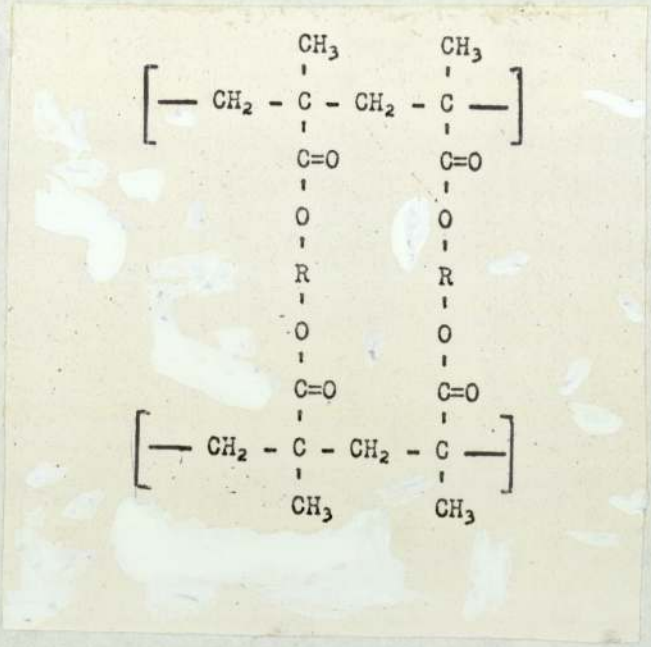
TABLE 29

Physical and chemical characteristics of XAD-7

Porosity of beads	0.50-0.55 millilitre of pore/millilitre of bead
Surface area	450 square metres/gram
Average Pore diameter	80 Ångstrom units
Skeletal density	1.24 grams/cc
Chemical Nature	Acrylic ester

FIGURE 24

Molecular Structure of XAD-7



As can be seen from Figure 24 the precise nature of the material has not been given and there is great reluctance on the part of the manufacturers to completely describe it and reveal the nature of the group R, which they simply describe as a "polyfunctional aliphatic residue".(171)

The adsorption of compounds by this material has been well described.(147)(169)(170)(171) It has been found that due to their hydrophobicity they can be used to adsorb through hydrophobic interactions when used in polar solvents, eg water.(171) One group of compounds whose adsorption has been studied are the fatty acids. It has been found that the adsorption from aqueous solution increases as the chain length of the fatty acid increases(171) and this must be due to greater hydrophobic interactions. It is also believed that dipole-dipole interactions also occur and aid the adsorption process.(169)

In the biomedical field at the Liver Unit, King's College Hospital, it has been found that this resin is more efficient at removing bilirubin than XAD-2 and has a similar capacity with regard to the bile acids.(147)

There are in addition to XAD-7 other resins of a similar composition XAD-6 and 8, these do not seem to have been tried biomedically, but their ability to adsorb fatty acids compared to XAD-7 has been investigated.(171) The structures of these resins is better described than that of XAD-7, they are crosslinked poly (methyl methacrylates).(170)(171)

(c) Anion-exchange resins

As with the cation-exchange resins there is with anion-exchange resins the problem of their effect on the ionic balance of the blood or plasma which is passed over them. Again the problem is solved by modifying the resin so that ion exchange between the blood or plasma and the resin does not alter the concentration of ions in the blood. Thus these resins adsorb toxic anions such as barbiturate, salicylate or bile acid ions in exchange for chloride and bicarbonate ions with no appreciable effect on systemic electrolyte balance.(172)

The use of these materials started with cholestyramine, a small size anion exchange resin, which was ingested orally to remove cholephilic ions such as the bile acids from the digestive tract, thus mitigating the toxic effects they cause when present in high concentrations in certain diseases.(36)(39) Now larger sized resins such as Dowex 1X4 and Amberlite IRA900 are being examined for haemoperfusion. Dowex IX4 is a polystyrene-divinylbenzene copolymer

substituted with quaternary ammonium ions[36] and Amberlite IRA900 an anion exchange resin derived from trimethylamine.[173]

These resins have been found capable of removing protein bound anions; they are especially effective with the more water soluble ones, the hydrophobic ones being more efficiently removed by hydrophobic resins as has already been mentioned.

As regards to biocompatibility in vitro perfusion studies using Dowex I showed large decreases in platelets and leukocytes resulting in leukopenia and thrombocytopenia, as well as occasional schistocytes and burr cells, but no measurable adsorption of any protein.[168] Similar results have been observed on haemoperfusion with dogs, the animals, however, did recover following treatment and showed no other clinical evidence of toxicity from the haemoperfusion.[165]

As has been mentioned resin haemoperfusion has been employed clinically for the treatment of drug overdose. However, the striking losses of white blood cells (60 - 80 per cent) and platelets (60 - 98 per cent) noted in both animal and clinical trials has not encouraged their widespread use in hepatic failure, where additional haemostatic defects are likely to be particularly poorly tolerated.[158] Thus adsorbents which are biocompatible are being sought, these would be an ideal solution but for the present one has not been found.[147] There is, however, an alternative approach which will now be described.

(d) Resin biocompatibility - an alternative approach

Alternative methods of achieving better biocompatibility are

by physical separation of the adsorbent with a biocompatible polymer, which is permeable to the desired molecules; or by perfusing the adsorbent with plasma only, the formed elements having been removed by continuous centrifugation. This second alternative is not as impractible as it seems since the technology has already been developed for treatment of leukaemia, in which white cells are selectively removed or irradiated on a continuous basis with the continuous flow celltrifuge.(147)

The use of the celltrifuge has been attempted in this application as perfusions of whole blood through columns of resins is frequently accompanied by mechanical difficulties due to a build-up of pressure across the resin bed, in addition to losses of white blood cells and platelets (platelet losses in patients with liver disease being particularly important).(174)

A solution to these difficulties would be to perfuse the resin with plasma from which white cells and platelets have been removed. The use of the celltrifuge which separates blood continuously into plasma and red cell phases by centrifugation is one way in which this could be achieved. If blood is spun at great enough centrifugal force, the plasma becomes free of platelets and white and red blood cells. The cell free plasma may then be perfused through a column of resin without build-up of pressure. In practice, however, there are still unavoidable losses of blood cells in this technique but it does confer advantages such as plasma being mechanically easier to handle and, since, the extraction of metabolites may be limited by the slow moving fluid (boundary layer) surrounding particles in a fluid stream and the boundary layer is thicker in whole blood than

plasma, metabolite extraction may be more efficient from plasma.[174]

The other alternative of physical separation of the blood and adsorbent by a biocompatible polymer has been attempted for charcoal perfusion which is now described.

(2) Charcoal Perfusion

(a) Introduction

With the resins the other major group of materials which have been used as adsorbents for blood toxins present in liver failure are the activated charcoals.

Activated charcoal has been used in the chemical industry as a highly effective adsorbent material for many years.[147] They are highly porous materials prepared by carbonizing and activating organic substances mainly of biological origin. The product of simple carbonization is an inactive material with a specific surface area of several square meters per gram. A sorbent with a highly developed porosity and a correspondingly large surface area is obtained by reaction with an activating agent. Activated carbon is prepared either by physical activation (reaction with steam or CO_2) or by chemical activation (reaction with zinc chloride).[175]

Their high internal surface area (up to 1500 m^2/gm) gives a large adsorption capacity. It has also been used medically since at least 1912 as a "universal antidote" which is instilled into the stomach following the ingestion of toxic substances.[147] In the last decade investigations have started on the use of activated carbon to remove toxins in uremia, hepatic failure and acute

poisoning.[175]

There are, however, two major problems associated with direct haemoperfusion over activated carbon: carbon microparticles (micro-emboli), and blood compatibility.[131] Attempts have been made to overcome these difficulties by applying a coating to the surface of the carbon which allows permeation of toxins to the activated charcoal but minimizes unfavourable interactions between the blood elements and the charcoal. Also care in selection of the specific activated charcoal is important.

(b) Selection of Activated Carbon

Great care has to be exercised in choosing the activated carbon as there are many different sorts which possess diverse properties.

One factor of major importance is the pore structure of the carbon, since the blood elements range in size from 2-10 μ in diameter it is clearly desirable that the macropores in the carbon be less than the dimensions of the blood cells so that there is no penetration into the particle and therefore no cell loss.[115]

Other important factors are the hardness, size and shape of the particles[115] as these affect the flow properties of the column.

Granular activated carbons can be divided into two distinct classes according to their physical shape, which is related to the method of manufacture. The first is granular carbon produced by breaking and sieving activated carbon from the natural raw materials, such as, coal, lignite, peat, wood, coconut shell and petroleum coke. In this case the particle shape is irregular and the macropore

structure is a function of the original raw material because during carbonisation the volatiles are removed and the macropore structure is formed.(115)

The second is extruded granular carbon and this type is produced by extruding previously carbonised powdered material, eg peat coke with a binder followed by the carbonisation of the binder and steam activation. In this case, any large macropores present in the original carbonised material have been destroyed by grinding and so the largest pores in an extruded carbon are the interparticle interstices between the carbon particles. This means that it is possible to control the upper limit of the macropore structure.(115)

It has been found that the extruded granular carbon types are better because blood cell loss is less due to the smaller pores which do not allow diffusion into the carbon. They are also more regular in shape and size; and their relatively smoother surface is believed to be a contributory factor in reducing blood damage.(115)

(c) Problems in use

The main problems with the use of untreated activated carbon are micro-emboli and blood compatibility.

The microparticles are in part due to the highly porous nature of activated carbon, which makes it inherently fragile. Scanning electromicroscopy shows these microparticles and rough surfaces, which can be seen to be very susceptible to abrasion and further fracturing.(131)

In addition, haemoperfusion of columns of uncoated activated

charcoal generally results in acute thrombo- and leucocytopenia. The columns are also susceptible to sludging and channeling which leads to pressure gradients and often reduced clearances. High heparin doses are generally used for haemoperfusion of uncoated activated carbon. Such blood incompatibility is a common characteristic of most high surface area particulate systems, due to high surface area to blood volume ratios, relatively long blood residence times, and generally poor local haemodynamics.

Other additional problems are the ash content and ion-exchange capacity of some carbons, which can lead to electrolyte changes in perfused blood.

These various problems have been dealt with in the following ways:[131]

1) The microparticle problem can be greatly reduced by proper selection, washing of the carbon and by coating.
2) The soluble ash and electrolytes can be extracted out of the carbon by the proper design of the washing procedure.
3) Blood incompatibility can be reduced by coating or encapsulation.

(d) Coatings

The characteristics of an 'ideal' coating system have been defined by Bruck[131] as being:-

1) The coating must be strong enough to eliminate all fragmentation and generation of microemboli.
2) The coating must be freely permeable to the toxins of interest.

3) The coating must be blood tolerable, particularly with respect to the adhesion of blood cellular elements.

4) The coating must permit good flow and low pressure drops in the column, ie coated particles must not adhere or aggregate in the column.

5) The coating must be readily sterilisable, non-toxic and non-pyrogenic.

In addition to these properties the coating might also be used to impart a degree of selectivity to the charcoal.

The coatings in Table 30 have been used to coat activated carbons for medical applications. As can be seen from the table the various methods of increasing the biocompatibility of materials described in the previous chapter have been applied in the case of activated charcoals. The ones that will be considered in this review are the use of synthetic hydrogels particularly poly HEMA which has been investigated by several groups and shown much promise.

Several studies have been done on the properties of poly HEMA coatings of various sorts in improving the biocompatibility of activated charcoal; the main group interested in Britain is Smith and Nephew. Working in conjunction with King's College Hospital Liver Unit, they have produced and tested a poly HEMA coated activated charcoal for use in the treatment of hepatic failure. In America the use of poly HEMA has been investigated by Andrade's group at the University of Utah.

The techniques used to apply poly HEMA coatings in this field have been based on dip-coating. Andrade for example has used coating

TABLE 30

Biomedical coatings used for activated charcoal

(1) Albumin (adsorbed)[176][177]

(2) Albumin (adsorbed on cellulose nitrate)[178]

(3) Albumin (crosslinked)[179][176]

(4) Cellulose acetate[180][181]

(5) Cellulose triacetate (deacetylated)[182]

(6) Cellulose nitrate (collodion)[183][184]

(7) Dextan (adsorbed)[185]

(8) Haemoglobin (adsorbed)[186]

(9) Heparin complexed cellulose nitrate[187]

(10) Hydroxyethyl cellulose[188]

(11) Methacrylate copolymers[189]

(12) Nylon[190]

(13) Polyhydroxyethyl methacrylate[191][192]

(14) Poly (acrylic acid and stryrene copolymer)[193]

(15) Poly (acrylonitrile) and Acrylonitrile methyl acrylate copolymer [194][195]

(16) Poly (acrylic acid Butyl methacrylate)[196]

solutions such as HEMA in 95 per cent ethanol containing 0.4 per cent t-butyl peroctoate initiator.[197] After coating the granules, polymerization in vacuo at 80°C for 2 hours is carried out[197], this results in a poly HEMA coated charcoal adsorbent.

The other commonly used technique is to encapsulate the charcoal by adding it to a solution of polymer and then removing the solvent in a rotary evaporator.[200] In this way a polymer coating is obtained.

A substantial improvement in the blood compatibility of the charcoals using coatings of poly HEMA has been found, eg Table 31 shows the per cent removal of platelets and white cells from blood using impregnating and encapsulating coatings.

TABLE 31

Removal of platelets and white cells (expressed as a percentage of the initial count) during perfusion with fresh blood in vitro

Each value represents the mean of six separate measurements

Coating (% by weight)	Removal of formed elements (%)	
	Platelets	White cells
Uncoated	50	15
2% Impregnated	29	12
4% Impregnated	27	10
2% Encapsulated	44	6
4% Encapsulated	20	6
10% Combined	10	0

It can be seen that both types of coating, the impregnating (which covers the external surface and the interior macropores) and the encapsulating (which gives a total covering of the external granules surface), gives a great improvement in the blood compatibility of the activated carbons. It is interesting to note that the impregnation technique produced a less complete coating and that the lower percentage (by weight) coatings were the least complete; the efficiency of coating is determined by dye-adsorption studies using materials such as Lissamine scarlet which can be adsorbed by uncoated charcoal, but which cannot penetrate or bind to

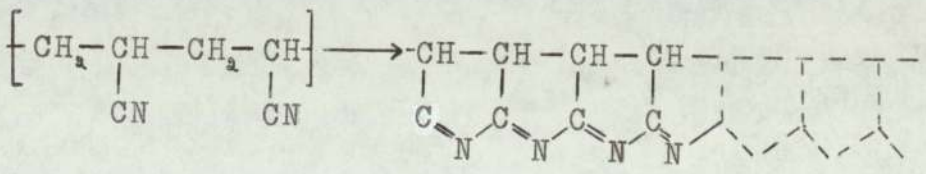
poly HEMA. Adsorption continues until a dye monolayer is complete, then the concentration in the dye solution can be found spectrophotometrically and so the amount of surface uncovered deduced.[198]

Besides the poly (hydroxy ethyl methacrylate) type of coating another hydrogel type of coating is being investigated by Gilchrist et al at Strathclyde University;[115] this is a copolymer of acrylonitrile and dimethylaminoethylmethacrylate (DMAEMA). On polymerization the acrylonitrile gives the highly stable polyacrylonitrile and DMAEMA a cationic water soluble material. By blending these two monomers together in a copolymer system one obtains water sensitive cationic films which can be prepared by solvent casting; these membranes have been used in their own right as potentional haemodialysis membranes.

It is possible to change the properties of these films by several procedures, for example, acrylonitrile (AN) polymers and copolymers undergo structural changes on the application of heat to give rise to insoluble ladder polymers at temperatures of 100-200°C[115] as shown below in Figure 25.

FIGURE 25

Ladder Polymer Formation in Poly (acrylonitrile)



In addition a degree of hydrophilicity can be retained by reacting the AN/DMAEMA copolymer with ethylene oxide gas.(115)

However the blood compatibility of this system has proved not to be high, clotting occurring within a matter of minutes. This undesirable effect has, however, been countered by ionically attaching heparin; the blood clotting times of charcoal coated in this way have improved dramatically.(115)

A further type of membrane system under investigation by the Strathclyde group is based on a copolymer of acrylic acid and n-butyl methacrylate. In this case the methacrylate species determines the mechanical characteristics of the membrane film whereas the acrylic acid gives rise to a water sensitive charged membrane species. This copolymer can also undergo chemical modification with ethylene oxide to give rise to a highly hydrophilic hydroxyethylacrylate polymer.(115)

Lastly the blood compatibility of some of the other types of hydrogel coatings using cellulose and collodion should be mentioned. Chang has reported a post-haemoperfusion value of 91.8 $\pm$ 11.8 per cent in platelet levels using 300 g of albumin-coated collodion-microencapsulated activated carbon at a flow rate of 150-200 ml/min; similarly Odaka et al reported a 20 to 30 per cent platelet drop and no charge in white blood cell level using 90 g of albumin/collodion-coated spherical carbon clinically.(175)

In the use of cellulosics, Denti obtained a 54 per cent platelet drop after one hour using 125 g of cellulose-coated carbon at a flow rate of 50 ml/min in dogs.(175)

So it can be seen that these other types of hydrogel coating are important too for creating potentional blood compatible adsorbents.

The importance of the coatings in imparting selectivity to the adsorbent is given in the next section, which deals with the adsorbability of activated carbons.

(e) Adsorbability of Activated Carbons

The adsorbability of activated carbons to various compounds has been investigated. It has been found that the type of substances adsorbed by charcoal depends on several factors including the size of the internal pores which allow access to the binding sites. The criterion for good adsorption appears to be the polarisability of the molecule as distinct from the possession of a permanent dipole.(147)

The adsorption of many different types of substances such as the amino acids, bilirubin, urea, uric acid, creatinine and drug molecules such as paracetamol have been studied. It has been found that large pore charcoal is capable of adsorbing as much as 100 mgs of bilirubin per gram from chloroform solution but the high affinity of bilirubin for albumin is such that the same charcoal only adsorbs 5 mgs of bilirubin from plasma. On the other hand the same is not true of other compounds and charcoal is capable of adsorbing fairly large quantities of amino acids and the drug paracetamol from plasma.(147) In addition to paracetamol, other drugs such as salicylate, pentobarbitone, phenobarbitone,(199) glutethimide(147) and other drugs have been shown to be removed by coated charcoals.(199) This shows their potential in the treatment of drug overdoses, an

application for which coated charcoals are now being used in Western Europe.

They have also been shown to be efficient in removing some of the markers for uraemia, creatinine and uric acid being strongly adsorbed; however urea is adsorbed poorly.(199)

This wide range of toxic substances which activated carbons are capable of adsorbing makes them one of the most interesting adsorbents for artificial liver and kidney haemoperfusion system.

(f) The Effect of Polymer Coating on Adsorbability

One of the main factors which can influence the adsorption of drugs and metabolites by the coated charcoal is the physical and chemical nature of the coating, these factors will now be individually discussed.

Physical Nature

The main physical factor which effects the rate of permeation of the metabolite through the membrane is the thickness of the coating. The thicker the coating the better the coverage of the surface is likely to be, and hence the biocompatibility is improved. However the thicker the coating the lower the rate of permeation will be, causing each haemoperfusion to be less effective.

A further factor of importance is the technique by which the carbon is coated. This effect of the coating on adsorption has been studied as regards to paracetamol removal by Gazzard et al.(198) It was found that charcoals which had been "impregnated", (that is

an attempt made to give a coating to both the surface and internal adsorptive area) were able to adsorb paracetamol faster than those which had been "encapsulated", ie those which had a thin external coating alone.(198)

This effect is presumably due to the greater surface area available as the macropores into the carbon's structure are not covered over by "impregnation".

It should be noted that these factors do not affect the total adsorption of paracetamol at equilibrium, only the speed of adsorption.(198)

Chemical Nature

The chemical nature of the coating plays an important part in determining the rate of adsorption of toxins from blood or plasma; thus the chemical nature of the coating can be used to influence what chemical type of toxins are taken up. Examples of this effect are that anionic, acrylic acid-butylmethacrylate copolymer membranes show rapid diffusion of (basic) creatinine and cationic, acrylonitrile-dimethylaminoethyl methacrylate copolymer membranes shows increased rates of clearance of acidic solutes such as uric acid and phosphate.(36) Both types of membrane have been suggested and tried as coatings for activated charcoal; their selectivity is, of course, due to ionic attraction for polymers and solutes, which are oppositely charged and repulsion for those which are similarly charged.

(g) Trials on Patients

There are several series of clinical trials of coated activated charcoals described in the literature, one of the biggest has been that carried out by King's College Hospital's Liver Unit on patients suffering from fulminant hepatic failure, despite high initial success rates this means of treatment had to be discontinued as the initial success was not maintained.(201) The coated charcoal used, which is coated with pHEMA is now used throughout Western Europe for cases of drug overdose. For this group of patients in which the organs of the body are not badly damaged, the treatment is not too traumatic to be considered for use.(202) It therefore appears that further work is necessary on the coating of charcoal with hydrogel before an acceptable haemoperfusion is made for the treatment of patients suffering from hepatic failure.

(3) Competition Chromatography

Lastly, a technique has been developed for removing protein bound toxins by a type of competition chromatography involving agarose beads, which have been coupled via cyanogen bromide to human serum albumin. This technique was developed for a specific case, an adolescent girl with the Crigler-Najjar syndrome, type I, who abruptly developed neurologic signs suggesting kernicterus after a minor illness. The agarose-albumin gels removed bilirubin efficiently from plasma or whole blood and the beads could be regenerated easily using aqueous ethanol.(142)

The immobilized albumin was found to behave qualitatively much like serum albumin. Digitoxin was removed, digoxin not,

chenodeoxycholate was, taurocholate was only retarded, a portion of thyroxine was removed, and BSP and indocyanine green were removed. However, the removal of the fatty acids was much less than expected and it is thought that although the hydrophobic pockets responsible for binding bilirubin are not significantly affected by the coupling process, the charged anion-binding sites, which are likely to be on the surface of the protein may be damaged. Ammonia, too, was not removed.(142)

The biocompatibility of the beads was examined by in vitro tests and the results found were that the blood cells, clotting factors, and the proteins detectable by immunoelectrophoresis were virtually unchanged by passage over such albumin-agarose gels.(142) In experiments on rats carried out later only minimal changes were observed in either formed elements or other fluid phase components of blood as a result of haemoperfusion.(143) Unfortunately more recent experiments on higher animals (monkeys), have been less successful and the favourable results on rats have been explained as being due to the high resistance of rat blood to trauma.(202)

Another drawback to this system is that the weight of albumin-agarose needed to remove significant quantities of toxin from man would probably be too large in terms of expense and volume of blood needed to prime the column of adsorbent. Albumin as an adsorbent would be attractive if a less bulky support system were devised.(147)

3.5 (e) THE USE OF LIVER ELEMENTS

These techniques represent something of a return to the earlier approach of using the liver of another organism for support, but in this case only elements of the liver are used, in this way the effects obtained can be more specific and greater safety achieved as there should be less doubt what is happening in the device and less individual variation between results with them, than livers themselves.

(1) Gel-entrapped liver cell microsomes

An approach to an artificial liver support system using hydrogels is the work done on entrapping microsomes from the endoplasmic reticulum of liver cells. These microsomes play an important part in the metabolism of endogenous and exogenous compounds; they are obtained from liver cell homogenate and are relatively unstable, which is a serious obstacle to the utilization of the recovered enzymatic activities. However, the stability of microsomal enzymes is often improved by their inclusion in cross-linked polymeric matrices. Denti succeeded in entrapping animal liver microsomes in gamma-ray crosslinked polyvinyl pyrrolidone and found stabilization of some enzymatic activities.(203)

The recovery values, (after irradiation and entrapment), of biochemical activity and their storage characteristics lead the authors to believe that these preparations could be successfully utilized in an artificial liver support system.(203)

(2) Microsomal Enzymes Bound to Artificial Carriers

This is a similar approach to the previous one in that it is attempting to eliminate unwanted substances alone from the blood, extracorporeally, by a route which is close to the physiological pathway. Again the technique utilizes hydrogels.(204)

UDP-glucuronyltransferase from rabbit liver was detached from microsomal membranes, purified and then the free enzyme covalently bound to artificial carriers on an acrylamide or sepharose base. After binding the enzyme to artificial carriers it was found that certain phenolic substances were not glucuronidated by the carrier bound enzyme, but this problem was overcome by using different carriers for binding the enzyme. This effect could be explained by the existence of more than one UDP-glucuronyltransferase or steric effects of the shape and size of the substrate molecule. Paracetamol was one of the investigated phenols.(204)

It was found that enzyme carriers with a smooth surface such as membranes or beads were more biocompatible than granules. The carrier bound UDP-glucuronyltransferose was found to remove free phenols in vitro from the blood of a patient in liver failure and also eliminated phenols in vivo from the blood of paracetamol intoxicated rabbits. Brunner hopes in possible future clinical applications of this method to use hydroxylases, sulphatases and other detoxifying enzymes in multienzyme complexes or in succession for treatment.(204)

(3) Haemoperfusion Through Liver Cells in Tissue Cultures

Lastly, improvements in the ability to grow liver cells in tissue culture have lead to attempts to incorporate such cultures into extracorporeal "artificial livers", in which both the synthetic and excretory capabilities of the cultured cells could be harnessed.(158)

Two such attempts have been so far described, one involving semi-permeable membranes, the other hollow fibres.

(a) In one device monolayers of liver cells are held between two semi-permeable membranes. Blood flows over one membrane, and a dialysate designed both to remove the waste products produced by the cells and to provide them with nutrients flows over the other. This permits access by protein-bound metabolites to the liver cells.(158) The actual device consists of multiple sets of monolayer cell cultures and associated membranes arranged in parallel. Haemoperfusion of jaundiced rats with chemically induced hepatic insufficiency through these prototype devices has resulted in improved BSP clearance, a fall in serum bilirubin and blood ammonia concentrations, and a slight increase in serum albumin,(158)(205) showing some replacement of the synthetic function of the liver.

The materials for use as membranes so far suggested are cellulosics of various sorts and the author of the patent suggests that a similar system could be used as an artificial endocrine pancreas-kidney for use in cases of diabetic electrolyte imbalance.(205)

(b) The second device consists of liver cells grown in culture on bundles of semipermeable hollow fibres of cellulose acetate and acrylic copolymer with high nominal retention limits in the region of 30,000 and 50,000 molecular weight. These devices have shown in preliminary in vivo studies on rats that it is technically feasible to haemoperfuse these capillaries with blood (fibre occlusion was not pronounced). Additionally in vitro studies have shown both bilirubin removal and conjugation by these cell cultures. The results are, however, preliminary and limitations in capacity and scale may be significant obstacles to their clinical application,[51][158] but they are extremely interesting and further refinements have been suggested and are being explored.[51]

CHAPTER 4

EXPERIMENTAL TECHNIQUES

4.1 INTRODUCTION

In this chapter the main methods of making the membranes used in the course of this project will be outlined. Additionally, the tests these materials have been examined by are described, and information given on the source, and means of purification of the monomers and other chemicals used in the course of the project.

The main techniques used to make beads - suspension polymerization and a novel type of frozen droplet polymerization are described in Chapter 7 and 8, as they are more properly dealt with there. The methods of testing them are however discussed, ie the tests for the adsorption of bilirubin and the model compound BSP.

4.2 PURIFICATION OF MONOMERS

All the monomers used were purified by conventional methods, using GLC to monitor purity, and are listed with the suppliers, below in Table 32.

TABLE 32

Monomers and Suppliers

Hydroxyethyl methacrylate	BP Ltd
N-vinyl pyrrolidone	Koch-Light Ltd
Acrylic acid	Koch-Light Ltd
Methacrylic acid	Koch-Light Ltd
Acrylamide	Koch-Light Ltd
Dimethylaminoethyl methacrylate	ICI
Ethylene dimethacrylate	BDH Ltd
NN'-Methylene bis acrylamide	Koch-Light Laboratories

4.3 INITIATORS AND CATALYSTS (SUPPLIERS)

A list of the suppliers of initiators and catalysts is given below in Table 33.

TABLE 33

Suppliers of Initiators and Catalysts

∝∝'Azobisisobutyronitrile (AZBN)	- BDH Ltd
Uranyl Nitrate (UN)	- Hopkin & Williams Ltd
Methylene blue	- BDH Ltd
Bezoin	- BDH Ltd
Toluene p-sulphinic acid (Sodium salt)	- BDH Ltd
Ammonium persulphate	- Hopkin & Williams Ltd

4.4 MISCELLANEOUS CHEMICALS (SUPPLIERS)

A list of the miscellaneous chemicals used in the project is given in Table 34.

TABLE 34

Miscellaneous Chemicals Used in Project

Benzalkonium chloride (50% aqueous solution)	- Koch-Light Laboratories Ltd
1, 3, 5 - Trioxane	- Koch-Light Laboratories Ltd
Ethanediol (Ethylene glycol)	- Fisons Ltd
Tween 21*) Span 60+) Span 20‡) Suspension Stabilizers	- Honeywell-Atlas Ltd
Sodium chloride AR	- Fisons Ltd
Silicone fluid DC 200/5 cs^{x} Silicone fluid DC 200/20 cs^{x}	- Hopkin & Williams Ltd

* Poly oxyethylene (4) sorbitan monolaurate
\+ Sorbitan monostearate
‡ Sorbitan monolaurate
x centi stokes

4.5 PREPARATION OF POLYMER FILMS

(a) Thermal free-radical polymerization

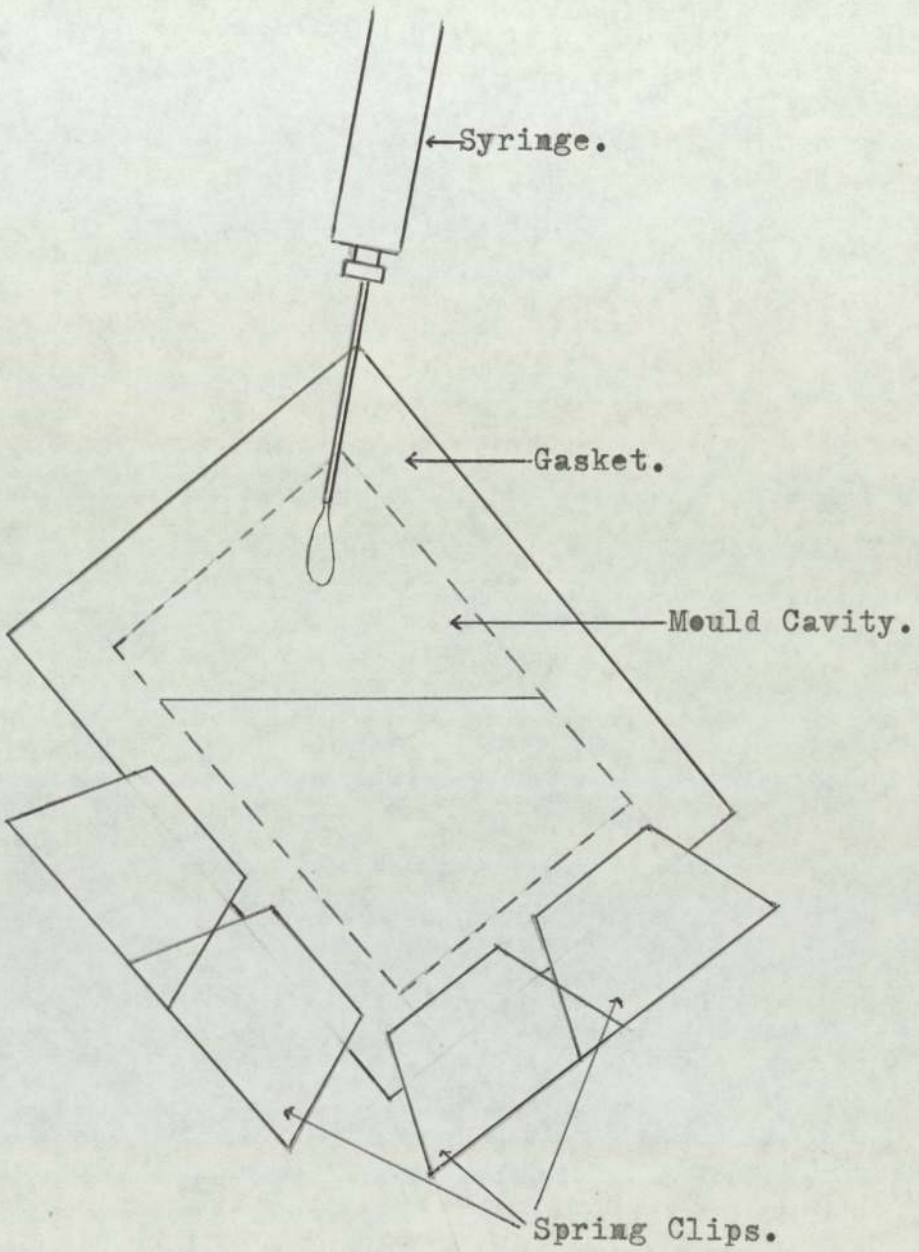
The technique of making hydrogel films by thermal polymerization has already been described by Barnes[206], Ng[207] and Pedley[208]; the basic method is as follows.

The hydrogel film was made by thermal polymerization in bulk of a mixture of monomers and a free radical initiator in a suitable mould. The glass mould consisted of two glass plates, (about 4" x 4"), each coated with a layer of melinex, (polyethylene terephthalate film), bonded to the glass plates by a spray-on adhesive; this film was applied to the glass as it had been found previously that if the film was left out the adhesive bond formed between the polymer and the glass made it very difficult to separate the cell without breaking up the hydrogel film. The covered glass sheets were separated by a polyethylene gasket, and the whole assemblage clamped tight together with spring clips on two sides. A syringe needle was inserted at the apex of the glass plates, the tip lying well inside the cavity, ready to allow the solution to be passed in, as shown in Figure 26.

A monomer mixture containing several monomers and a free radical initiator, usually AZBN, was made ensuring a homogeneous solution was created. After being flushed with a stream of nitrogen gas the monomer mixture was introduced into the glass mould through the syringe needle until the level of liquid inside the cavity almost reached the apex of the gasket, the syringe needle was removed and the remaining sides of the mould were clamped together with spring clips.

FIGURE 26

Preparation of a Polymer Film



The mould containing the polymerization mixture was then placed in an oven thermostated at 65°C for three days, and then post-cured at 90°C for three hours in an attempt to ensure complete polymerization.

After polymerization had occurred, the spring clips and polyethene gasket were removed and the glass plates separated by inserting a knife between them. The hydrogel membrane was then swollen off the melinex sheet in a water bath, unless it could be easily removed without cracking.

(b) UV Polymerization

This technique does not differ substantially from the previously described procedure (Thermal free radical polymerization). The major differences being that instead of the monomers being polymerized thermally, a UV photo-catalyst, photo-sensitizer or initiator was added to the monomers and the polymerization initiated by UV radiation from a lamp.

In many cases for the making of membranes the above technique was used as it stands, however in the case of macroporous membranes a different procedure was adopted after the initial filling of the cavity with a monomer solution. The assemblage of glass plates and cavity with the solution was placed horizontally, and a very fine dry ice powder poured over one of the glass plates. This addition results in the formation of solvent crystals with the monomer at the interstices of the crystals; if this frozen film is now irradiated by a UV lamp a polymer matrix is formed which after polymerization and melting of the solvent results in a highly porous polymer film: a macroporous hydrogel.

Alternatively a macroporous film may be made by pouring the monomer-solvent mixture over a cold plate and then irradiating the surface as before with UV light once the solution has frozen.

(c) Measurement of Equilibrium Water Contents

The technique of measuring the EWC of a hydrogel polymer has already been described by Ng and others[207, 206, 208].

Several disc samples were cut with a cork-borer (about 1 cm in diameter) from each hydrated polymer sheet which had been allowed to hydrate in distilled water at room temperature (22°C) for at least two weeks, by which time the samples were thought to reach equilibrium hydration under that experimental condition. The surface water of each hydrated sample was removed with a soft tissue paper and the sample was quickly transferred to and weighed in a closed weighing bottle. The hydrated sample was then dried in a vacuum oven at 60°C for about 8 hours, the weight taken and the procedure repeated again until a constant weight was obtained. The EWC of the hydrogel was then calculated using the equation shown below:

$$EWC = \frac{\text{Initial Weight of Hydrogel - Weight of Dehydrated Sample}}{\text{Initial Weight of Hydrogel}} \times 100\%$$

$$= \frac{\text{Weight of water in hydrogel}}{\text{Weight of Hydrogel}} \times 100\%$$

The EWC's of four or more samples were determined in this way for each material, and an average EWC was taken. No more than four samples had their EWC's taken if at least three of the samples had their EWC's within 1.5%, but if this was not the case, more were determined and an average value obtained.

(d) Measurement of Hamilton Contact Angles

Hamilton contact angles were measured using the apparatus shown in Figure 27, by the technique previously described by Barnes[206].

"The sample was glued to a glass cover slip, which was held in contact with a hollow tube, by a suction bulb contained in the tube. This apparatus was inverted and placed in the optical cell. Sufficient water, previously saturated with n-octane was added to the cell to cover the sample."

"The wetting liquid which was delivered by the bent syringe needle was n-octane. The needle was connected to an 'Agla' micro-syringe which was attached to a micrometer, so that small volumes of liquid could be placed accurately on the sample surface. A needle with a flat rather than a pointed tip was used to ensure the drop was formed symmetrically."

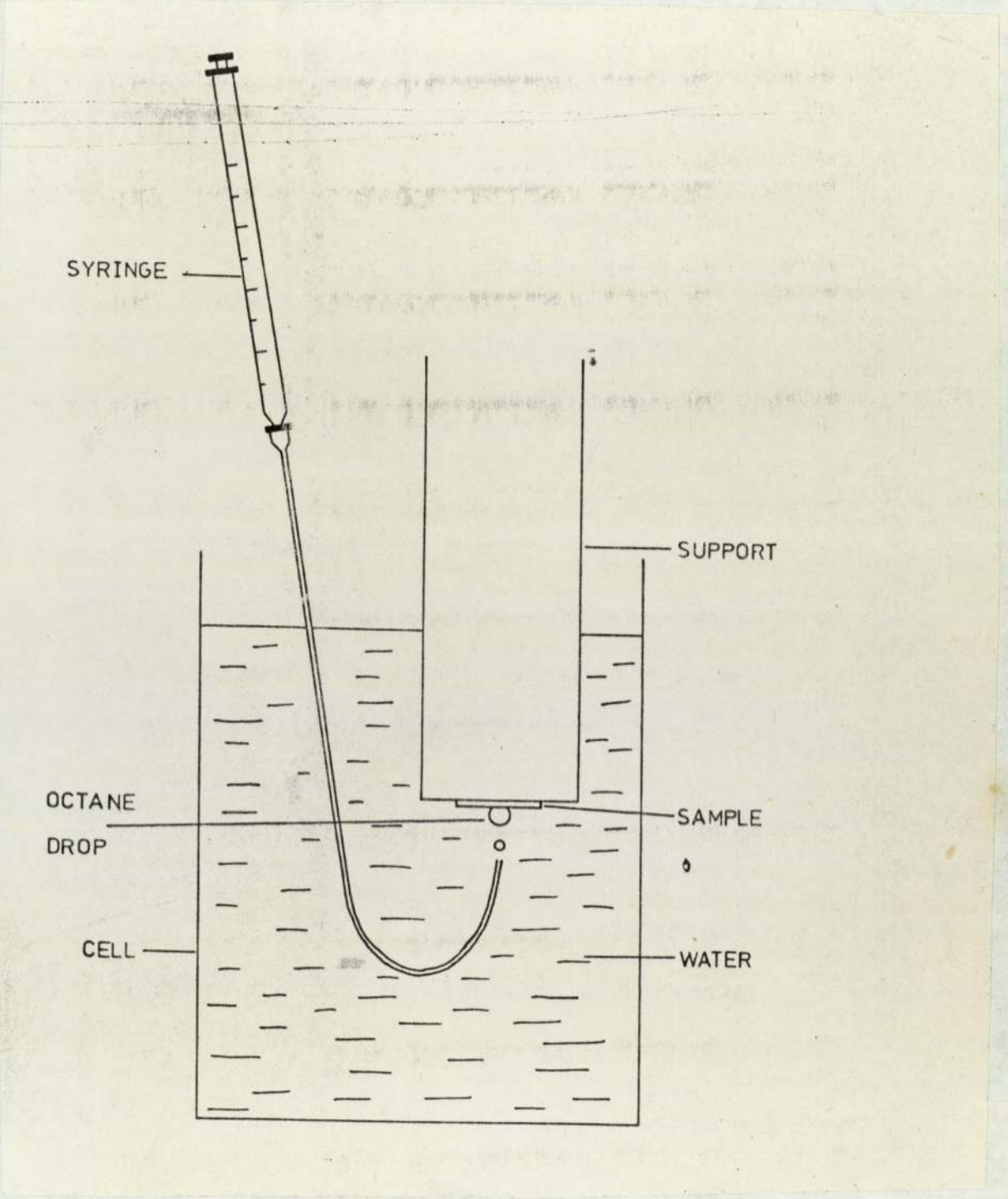
"The cell was placed in the light path of a Rank Aldis Tutor 2 slide projector which had been fitted with a short focal length (5 cm) lens and an image of the drop was thrown onto a back projection screen." From this image the Hamilton contact angle could be found by drawing the tangent to the drop at the surface of the sample and measuring the included angle with a protractor. Alternatively, if a permanent record was desired the image could be photographed and by placing the developed photographic film in an enlarger a large image of the drop was produced and from this the contact angle could be determined.

4.6 THE DIFFUSION CELL AND THE MEASUREMENT OF BSP CONCENTRATIONS

In order to assess the permeability of high molecular weight

FIGURE 27

Measurement of Hamilton Contact Angle



solutes through hydrogel membranes, a diffusion cell and the high molecular weight compound - bromosulphalein, (BSP), was used.

(a) The Diffusion Cell

The diffusion cell used for the study of BSP permeability through hydrogel membranes was the same one that has been used in a previous project[209].

It consists basically of two cell compartments made of perspex with glass sides. The two cells have a circular hole in each of one of their perspex sides, in this hole a rubber ring was placed. The two cell compartments were clamped together with the two rubber rings in contact with one another; between these rings the hydrogel membrane was placed.

In order to prevent stagnation of the solutions and to prevent boundary layer effects agitation of the solutions was provided by a nitrogen bubbler system. In use, a small sheet of cut melinex is put between the two cells to prevent small drops of the BSP solution splashing into the solution in the other cell, as a result of the bubbles bursting from the nitrogen agitation system. Additionally sheets of melinex were put over the test cells to prevent spatter and thus an excessive loss of solution. This latter problem was a significant one over the length of time the experiment was carried out, ie 5-20 hours.

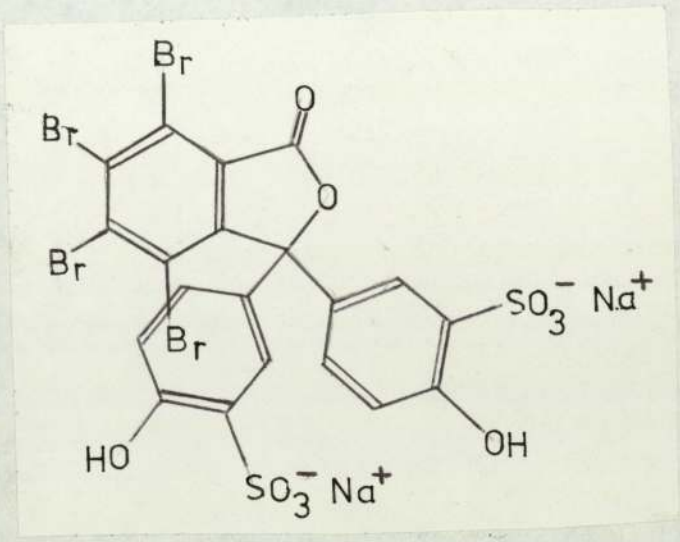
(b) BSP Concentrations

The permeant used in these studies was BSP (disodium phenol tetrabromopthalein disulphonate, $C_{20}H_8Br_4Na_2O_{10}S_2$), which is a high

molecular weight compound, 837.84. Its structure is shown below in Figure 28.

FIGURE 28

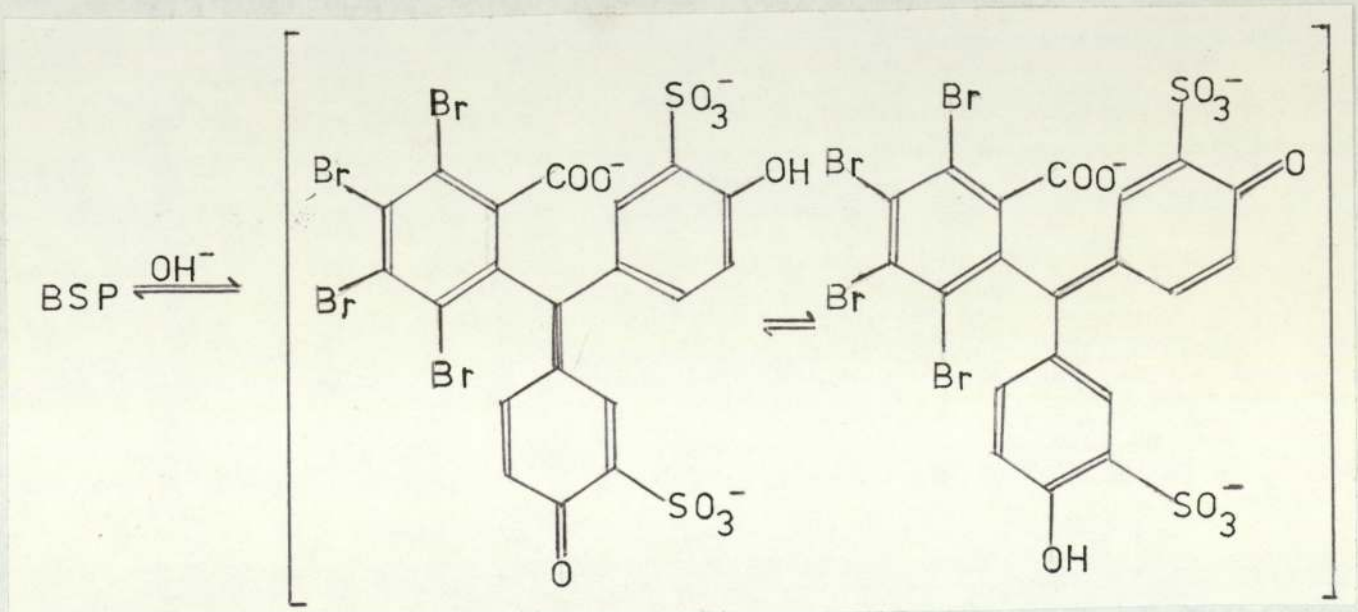
The Structure of BSP



BSP with this structure is a white water soluble powder, however if a solution of BSP is added to a 0.1 m sodium hydroxide solution, a chemical reaction occurs to create a highly conjugated molecule:- Figure 29. The maximum absorbance of this species occurs at 580 nm.

FIGURE 29

Result of Chemical Reaction Between BSP and the Hydroxide ion



This highly conjugated system gives BSP in sodium hydroxide solution its characteristic purple colour, and hence enables its easy colourimetric assay. Since the ionic species is highly conjugated it is very highly coloured and its concentration can be detected down to very low values; as is demonstrated in Figure 30, which shows the graph of optical absorbance against (BSP) for several solutions. It can be seen that BSP can be detected colourimetrically down to levels of 1×10^{-6} moles $litre^{-1}$.

(b) Experimental Procedure

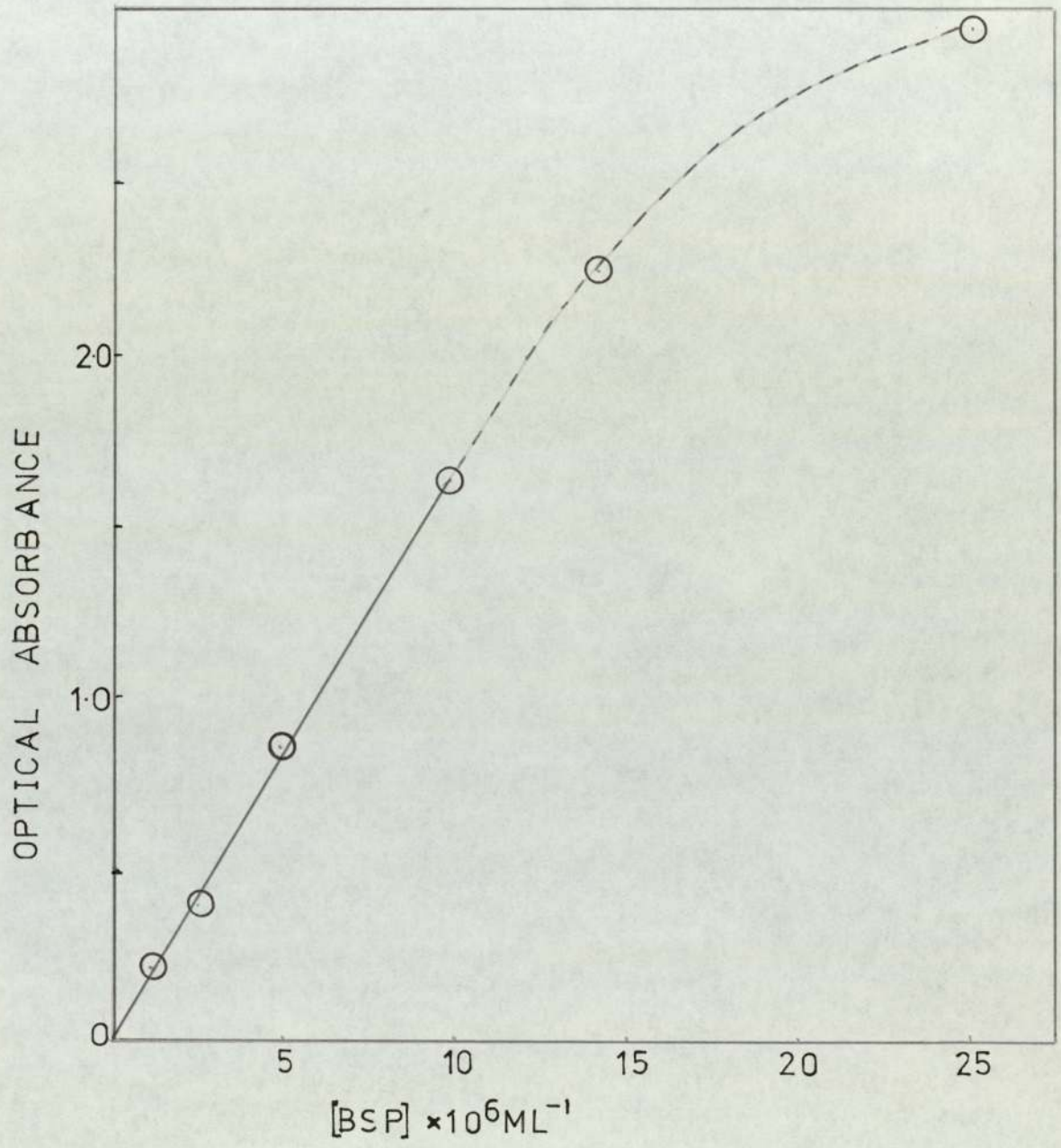
The actual experimental procedure adopted to find the diffusion rate of BSP across the membranes was the following:-

First, a circular piece of hydrogel membrane was cut using a cork borer and this disc then had its thickness measured using a spring loaded micrometer, (which exerts little pressure of the hydrogel and so does not squeeze out water and reduce its thickness). The thickness at various points on the disc was measured and the results averaged to give an average value for the thickness of the hydrogel membrane.

The disc was then inserted between the rubber rings of the test cells and clamped in place. Then in order to test the membrane's integrity, a Dextran blue solution was added to one side of the test cell and distilled water to the other cell; the cell was left overnight and if the next morning there was a blue colour in what had been the cell which had been pure water then the membrane was discarded and the procedure carried out using a new piece of membrane. Dextran blue was chosen as the test of membrane integrity as it had

FIGURE 30

[BSP] Against Optical Absorbance



been used in another study[209]; it has a molecular weight of 2×10^6, thus if it is found to have penetrated the membrane it indicates a fault of some sort such as a pinhole in the membrane, as it could not have diffused through the pores of the membrane.

If, however, the membrane was shown not to be defective then the test cell with Dextran blue was emptied and then refilled by a BSP solution; this solution was of the concentration of 1×10^{-3} moles litre^{-1} for all the tests done.

After a period of several hours, 5-20, the experiment was stopped and the concentration of BSP in the cell to which the BSP was diffusing, was measured by taking a volume of this liquid and diluting ten times with 0.1 m NaOH solution to create the blue conjugated form of BSP which then had its optical absorbance measured and hence the concentration of BSP in the test cell could be determined. Additionally, the volume of liquid in the cell was measured so that the number of moles of BSP which had crossed the membrane could be determined, as evaporation (aided by the nitrogen bubbler system) was severe enough to change the initial volume of the solution to a lower value. The experiment was carried out in duplicate.

The diffusion rate of BSP across the membrane could be determined as the number of moles of BSP which had crossed the membrane, the time over which the experiment had been carried out, the area of membrane and its thickness through which the BSP had diffused, had been determined. Thus the diffusion rate could be calculated in the units:- moles hr^{-1} cm^{-2} mm^{-1}.

4.7 THE MEASUREMENT OF BSP ADSORPTION BY HYDROGEL BEADS

The measurement of BSP concentrations was carried out in a similar manner to that used in the case of BSP travelling through hydrogel membranes. However in these cases the procedure adopted was the following:- a known volume of BSP solution, usually 1×10^{-3} Moles litre^{-1} or 1×10^{-4} Moles litre^{-1} was added to a known weight of hydrogel or other beads. Then, as with the samples, in the case of the BSP diffusion experiments, the samples of BSP solution removed after long time intervals were diluted with 10 volumes of 0.1 M NaOH solution and the optical transmittances of the solutions determined. From these, of course, the concentrations of BSP in the solutions could be calculated and therefore the amount of BSP absorbed by the test material determined.

In the case of the hydrogel beads the dilution effect had to be taken into account, this is due to high water content of the beads which effectively dilute the BSP concentration of the system as a whole. If not taken into consideration it would lead to the observation that the test compound was being adsorbed when in fact it was not.

4.8 THE MEASUREMENT OF BILIRUBIN ADSORPTION BY HYDROGEL BEADS

A similar technique to that described in the previous experiments was adopted, but the experiments had to be modified in order to allow for the greater instability of the bilirubin molecule, which decomposes under the action of light and heat. The following technique was used for the preparation of bilirubin solutions.

(a) Preparation of Bilirubin Solution

Bilirubin is not water soluble, thus for the adsorption experiments it has to be used in another form of solution; it was decided to use it in the form of a plasma solution, (the normal state it is found in the blood).

Firstly, a small quantity of bilirubin was weighed out in a piece of aluminium foil using a very accurate balance, an Oertling micro-analytical balance. Only very small quantities can be dissolved up to just over 20 mgs per 100 mls of plasma.

The aluminium foil boat with the bilirubin was then added to a graduated flask containing a small volume of 0.1 m NaOH solution; the flask is wrapped in aluminium foil to prevent decomposition of the bilirubin by light. When the bilirubin was seen to have dissolved in the sodium hydroxide solution, a partially reconstituted plasma solution was added to the flask to form the bilirubin-plasma solution. Then an equal volume of 0.1 m HCl (ie equal to the 0.1 m NaOH solution the bilirubin had been dissolved in initially) was added to neutralize the solution. The solution was then made up to the correct volume recommended for the reconstituted plasma with distilled water and the pH of the solution checked to see if it was at the correct value:- 7.3. The solution is then ready for use, if not used initially, it was stored in a refrigerator in the dark, as bilirubin decomposes at temperatures above $4^{o}C$. It should be noted too that reconstituted plasma has a limited life even when stored under refrigerated conditions. It is also desirable to purge the bilirubin-plasma solution with nitrogen after it is made up to remove dissolved oxygen and hence slow down the oxidative

degradation reactions bilirubin is subject to.

(b) Experimental Procedure used to Measure the Adsorption of Bilirubin

A known volume of prepared bilirubin solution, usually 20 mgs/100 mls or a lower value of concentration was added by pipette to previously weighed samples of hydrogel or other beads in sample bottles, (covered with aluminium foil to prevent photo-oxidation). The sample bottles were then put on a mechanical shaker and agitated at a slow speed to give good mixing between the beads and bilirubin solution. Excessively high speeds of agitation are not desirable as this can lead to denaturation and polymerization of albumin, the main plasma protein.

The bilirubin concentrations in the sample bottles are then determined at various time intervals using a Boehringer bilirubin test kit, which enables the Van der Bergh test to be carried out. It is possible to determine bilirubin concentrations simply from colourimetric assay of the plasma-bilirubin solution as the bilirubin gives the solution a bright yellow colour. However, it has been found that such an assay is not particularly accurate, mainly because of denatured plasma etc which interferes with the optical transmittance readings.

(c) The Van der Bergh test

The basis of the Van der Bergh test is the coupling of diazotized sulphanilic acid and bilirubin to produce a reddish-purple azo compound which can be easily assayed. The maximum absorbance of this compound occurs at about 590 nm and it is at

this wavelength that optical absorbance readings were taken. It should be noted that the addition of alcohol is necessary too, as a solvent in which both the unconjugated bilirubin and the reagent are both soluble, so that the reaction can take place.

In the case of the actual physical measurement of bilirubin concentration it is necessary when each set of readings are taken to standardize the solution against a blank which contains fresh plasma instead of a bilirubin plasma solution.

It is also desirable to have a standard bilirubin solution of the same volume, as that added to the individual test bottles containing the hydrogel beads or other materials, in an empty sample bottle. If this is then treated under exactly the same conditions as the other bottles and the solution tested at the same time intervals as the other solution, then some measure of the importance of degradative mechanisms such as oxidation or deposition on the glass walls of the sample bottle, (which also leads to a reduction in the solution concentration), can be gained.

Additionally, the solution can also be examined for the presence of biliverdin, one of the main products of the oxidative degradation of bilirubin. Biliverdin can be detected spectrophotometrically as it has an absorption maxima at 680 nm[210].

CHAPTER 5

HOMOGENEOUS MEMBRANES

5.1 INTRODUCTION

Homogeneous membranes were synthesized and characterized, as materials of this type have been suggested as coatings for blood detoxicants and as haemodialysis membranes. Producing these materials in their bulk form enables their physical properties to be easily determined, facilitates the investigation of their reactions with blood in a suitable test cell and their permeability to solutes.

It should be noted that in addition to those materials prepared and characterized by the author, other polymers investigated were prepared by other students in the course of their work. The origin of these materials is clearly stated in the text.

The following monomers shown in Table 35 were used in the preparation of the homogeneous hydrogel membranes by the technique described in the previous chapter.

TABLE 35

Monomers used to synthesize Hydrogel Membranes

1) Acrylamide (ACR)
2) N-Vinyl Pyrrolidone (NVP)
3) 2-Hydroxyethyl Methacrylate (HEMA)
4) Acrylic acid (AA)
5) Methacrylic acid (MAA)
6) Ethylene dimethacrylate (EDM)
7) Diacetone acrylamide (DAA)

A complete list of the homogeneous hydrogel membranes prepared in the course of this project is given in Table 36.

TABLE 36

"Homogeneous" Hydrogel Membranes Prepared by Thermal Bulk Polymerization and UV Polymerization

	Composition	Initial Molar Ratio	EWC
(A)	Thermally Polymerized		
A1	HEMA : MAA	75 : 25	30.0 ± 1.0%
A2	" : "	50 : 50	28.5 ± 1.0%
A3	" : "	25 : 75	40.5 ± 0.5%
A4	- : "	0 : 100	73.5 ± 1.0%
A5	HEMA : AA	75 : 25	37.5 ± 1.0%
A6	" : "	50 : 50	38.0 ± 1.0%
A7	" : "	25 : 75	43.5 ± 1.0%
A8	- : "	0 : 100	73.0 ± 1.0%
A9	NVP : -	100 : 0	97.0 %
A10	NVP : MAA	75 : 25	56.5 ± 1.0%
A11	" : "	50 : 50	48.0 ± 1.0%
A12	" : "	25 : 75	58.5 ± 0.5%
A13	NVP : AA	75 : 25	75.0 ± 1.0%
A14	" : "	50 : 50	62.0 ± 1.0%
A15	" : "	25 : 75	———
A16	ACR : MAA	50 : 50	40.0 ± 1.5%
A17	" : "	25 : 75	44.5 ± 0.5%
A18	ACR : AA	50 : 50	50.5 ± 1.0%
A19	" : "	25 : 75	49.5 ± 0.5%
A20	HEMA : ACR	50 : 50	65.5 ± 0.5%
A21	" : "	75 : 25	56.0 ± 0.5%
(B)	Polymerized by UV Radiation of Bulk Polymers		
B1	AA : EDM	100 : 8.5	40 ± 0.5%
B2	" : "	100 : 4	55 ± 1.5%

As can be seen a great deal of attention was devoted to copolymers of methacrylic and acrylic acid, since these have proved to be of interest biomedically[140].

The interaction of these polymers with blood is dealt with in Chapter 9, in this chapter other important properties of these materials are examined such as their equilibrium water contents, (EWC's) and their surface hydrophilicities. Firstly, however, their composition will be dealt with.

5.2 ANALYSIS OF HYDROGELS BY C, H, N ELEMENTAL ANALYSIS

(a) Introduction

The hydrogels were examined by C, H, N elemental analysis to determine the true monomer ratios present in the copolymers that were synthesized; this was done to check the assumptions made in previous work, mainly that for the monomers used that after post-curing, the initial monomer feed ratios gave the same monomer ratios in the polymer. This method was used rather than the determination of residual monomer as water soluble homopolymer may be formed in the polymerization, the amount of crosslinking agent in the polymerization being small. The presence of homopolymer and the potential accuracy of C, H, N analysis led to its application in the determination.

It was hoped that by knowing the stiochiometric ratios of monomers more exactly a better understanding of the nature of complexing between functional groups would result, and also of the relationship between blood clotting times and true composition.

Additionally, the study also gave information on the relative reactivities of the various monomers used.

The theoretical C, H, N and O weight percentages for the various monomers used in these studies are shown in Table 37.

TABLE 37

Theoretical elemental weight percentages in monomers examined

	% C	% H	% O	% N
Styrene	92.26	7.74	-	-
N-vinyl pyrrolidone	64.84	8.16	14.40	12.60
Ethylene dimethacrylate	60.59	7.12	32.29	-
Methacrylic acid	55.81	7.02	37.17	-
Hydroxyethyl methacrylate	55.37	7.75	36.88	-
NN'Methylene bisacrylamide	54.54	6.54	20.76	18.17
Acrylamide	50.69	7.09	22.51	19.71
Acrylic acid	50.00	5.59	44.40	-

It can be seen from the table that there are some monomers whose percentages of elements are very close for example HEMA and MAA, and this makes analysis of copolymers of these monomers, and others, difficult by this method.

The results obtained for various copolymers and terpolymers are shown on the following pages; the monomer ratios were obtained by the simple use of simultaneous equations. The results have been split up into the following groups:- Table 38.

TABLE 38

The Division of C, H, N results for the Various Hydrogels

The NVP:MAA and the NVP:AA series

Miscellaneous NVP copolymers and terpolymers

Copolymers of HEMA and the fluorinated monomers

Copolymers of ACR and AA/MAA

Miscellaneous Copolymers and Terpolymers of HEMA

It should be noted that the HEMA/fluorinated monomer composition were prepared previously by the author as part of his MSc Project[209].

(b) NVP:MAA and NVP:AA Series

1) NVP:MAA series

		NVP	MAA	Membrane
Initial molar monomer feed ratios	(1)	75	25	(A10)
	(2)	50	50	(A11)
	(3)	25	75	(A12)

True molar ratios from C, H, N analysis

(1) 47 ± 6% : 53 ± 6%

(2) 17.5 ± 1% : 82.5 ± 1%

(3) 2.6 ± 0.2%, 97.4 ± 0.2%

2) NVP:AA series

		NVP	AA	Membrane
Initial molar ratios	(1)	75	25	(A13)
	(2)	50	50	(A14)
	(3)	25	75	(A15)

True molar ratios

(1) 47 ± 2% : 53 ± 2%

(2) 15 ± 1%, 85 ± 1%

(3) 3.0 ± 0.5%, 97.0 ± 0.5%

These results are shown graphically in Figures 31 and 32. It can

FIGURE 31

The Composition of NVP:MAA copolymers as a function of Initial Monomer Feed Composition

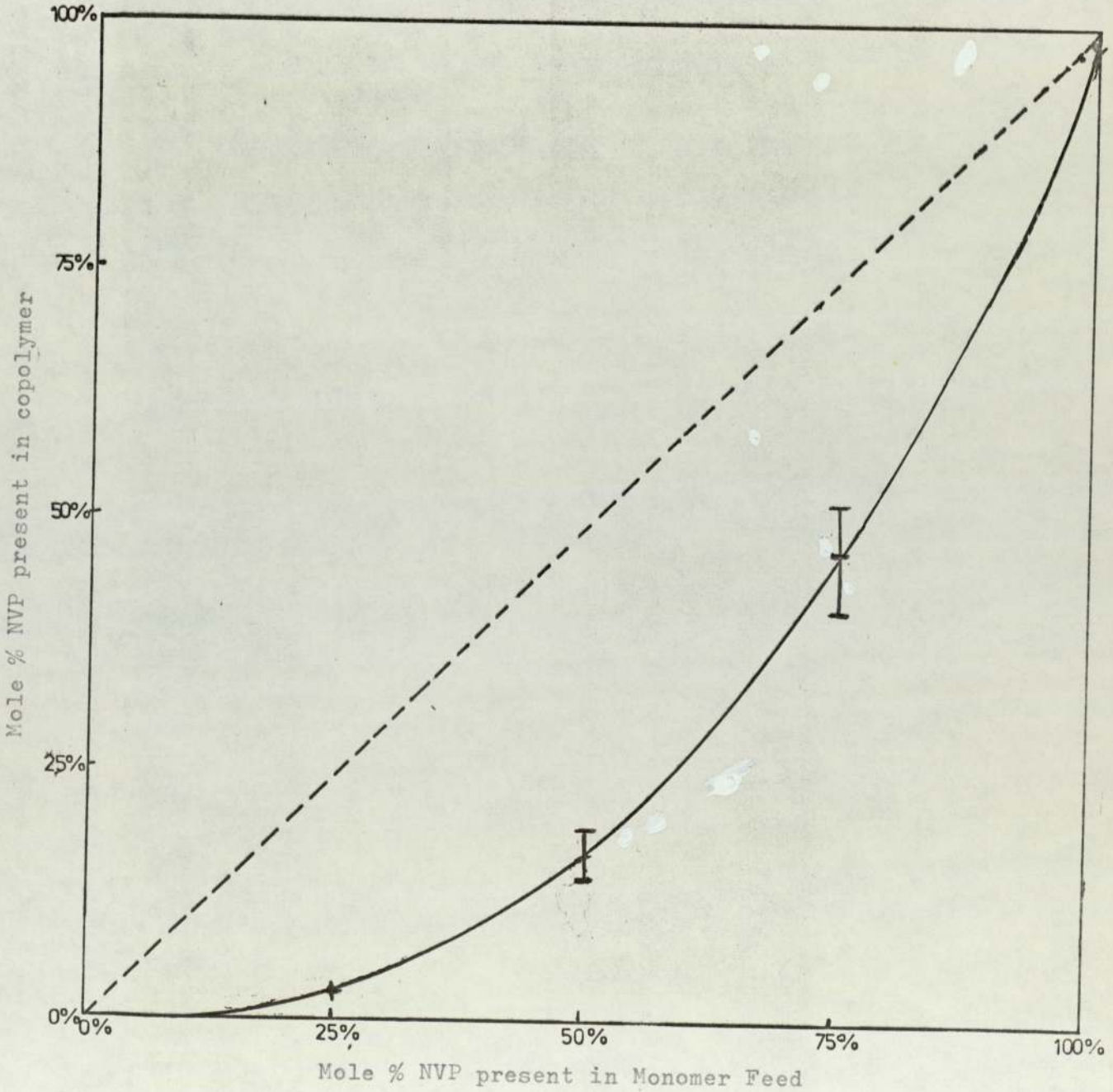
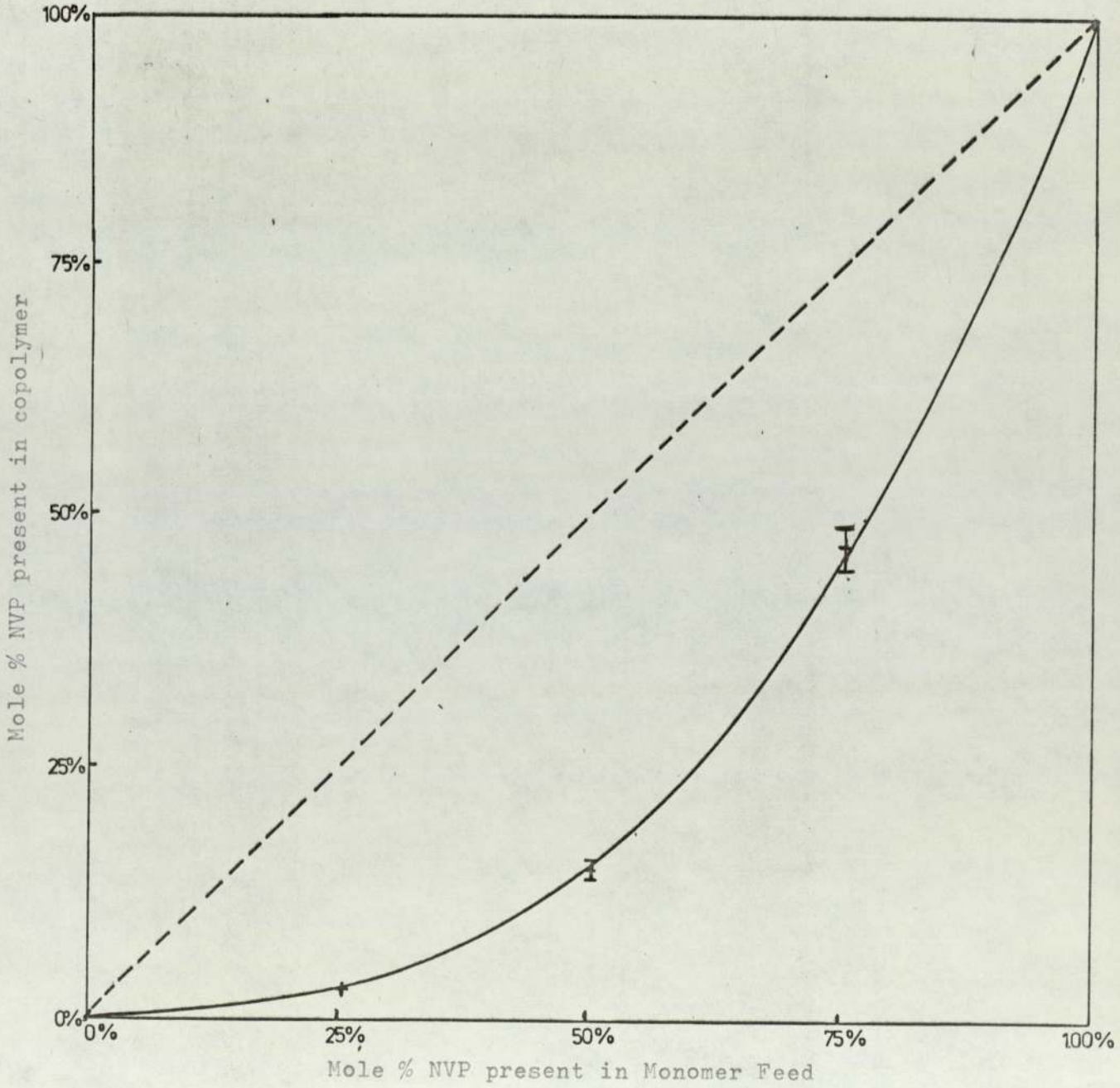


FIGURE 32

The Composition of NVP:AA copolymers as a function of Initial Monomer Feed Composition



be seen that NVP is quite unreactive in these polymerization systems and little of it is incorporated in the polymers unless a high initial amount is present.

(c) Miscellaneous NVP Copolymers and Terpolymers

In these too the low reactivity of NVP showed itself in its low per cent in the polymer.

1) NVP:HPMA

Initial feed ratio - 50 : 50

C, H, N analysis - 39 ± 1% : 61 ± 1%

2) NVP:HEMA

Initial feed ratio - 30 : 70

C, H, N analysis - 15 ± 0.5% : 85 ± 0.5%

3) HPA:NVP:ST

Initial feed ratio - 45 : 45 : 10

C, H, N analysis - ~67.5 ~23.5, ~8.5

These polymers were made by other students.

(d) HEMA and Fluorinated Monomers

It was found in these polymers that their final compositions were close to their initial monomer feed ratio.

1) HEMA:Hexafluoro isopropyl acrylate

Initial - 50 : 50

C, H analysis - 50 ± 1% : 50 ± 1%

2) HEMA:Hexafluoro isopropyl methacrylate

Initial - 50 : 50

C, H analysis - 54.5 ± 2.5% : 45.5 ± 2.5%

3) HEMA:2,22-trifluoroethyl methacrylate

Initial - 50 : 50

C, H analysis - 50 ± 5% : 50 ± 5%

4) HEMA:ACR:2,2,2-trifluoroethyl methacrylate

Initial - 50 : 25 : 25

C, H, N analysis - ~47, ~22 ~31

(e) ACR and AA/MAA Copolymers

For these copolymers it was observed that ACR and AA must be very similar in reactivity, as the molar ratios in the copolymer are almost the same as those in the initial formulation. For the MAA/ACR system it was found that an excess of MAA was present in the copolymer suggesting that it is more reactive than ACR. The results of these tests are shown below:

1) ACR:MAA

Membrane

Initial feed ratio - 50 : 50

(A16)

C, H, N analysis - 40-41.5% : 60-58.5%

2) ACR:AA

Initial feed ratio - 50 : 50

(A18)

C, H, N analysis - 49.5% : 50.5%

(f) Miscellaneous Copolymers and Terpolymers of HEMA

Various miscellaneous copolymers have had their molar ratios of monomers in the final composition measured, it has been found that the deviations of initial from final compositions were not great.

1) HEMA:ST(209)

Initial ratio - 70 : 30

C, H, N analysis - 77 ± 4 : 23 ± 4

2) In addition several HEMA co- and terpolymers were examined but unfortunately due to the similarity of the C, H, N % of the monomers, analysis was impossible.

(g) Conclusion

It can be seen from the results obtained that the basic assumption made in previous work, that post-curing ensures that the final monomer ratio is basically true for most copolymer and terpolymer systems except those containing the unreactive monomer N-vinyl pyrrolidone, which is not present in high concentrations in the copolymers and terpolymers synthesized, unless very high concentrations are present in the initial feed.

5.3 FACTORS AFFECTING THE EQUILIBRIUM WATER CONTENT OF HYDROGELS

In the introductory chapters of this thesis the importance of EWC with respect to strength, permeability and biocompatibility were dealt with; since it has been shown to be such an important factor, it was measured for this series of materials. The three most important factors in determining it were the hydrophilicity of the backbone polymer, the crosslink density, and complexing between functional groups. The latter two factors are the main ones dealt with in this section as the importance and nature of the hydrophilicity of the backbone polymer of the polymers used in this study is well known and self-evident from the results in the Table for the EWC's of crosslinked homopolymers.

(a) Crosslink Density and Equilibrium Water Content

The relationship between EWC and crosslink density has been investigated for crosslinked polyacrylamide and poly(acrylic acid). In both cases the crosslinking agent was ethylene dimethacrylate EDM, the former series involved UV polymerization of an ACR-EDM solution in a gasket between glass plates, and the latter by the normal bulk polymerization, (both techniques described in Chapter 4).

A further series of polymer membranes consisting of poly acrylamide and poly (hydroxyethyl methacrylate) crosslinked with NN-methylene bisacrylamide (NNMBA) have been investigated, but the results for these membranes are dealt with in the later chapter dealing with suspension polymerization, as they were prepared in connection with that work.

FIGURE 33
The Effect of the EDM concentration in poly(acrylamide) hydrogel membranes (prepared by UV polymerization) on EWC

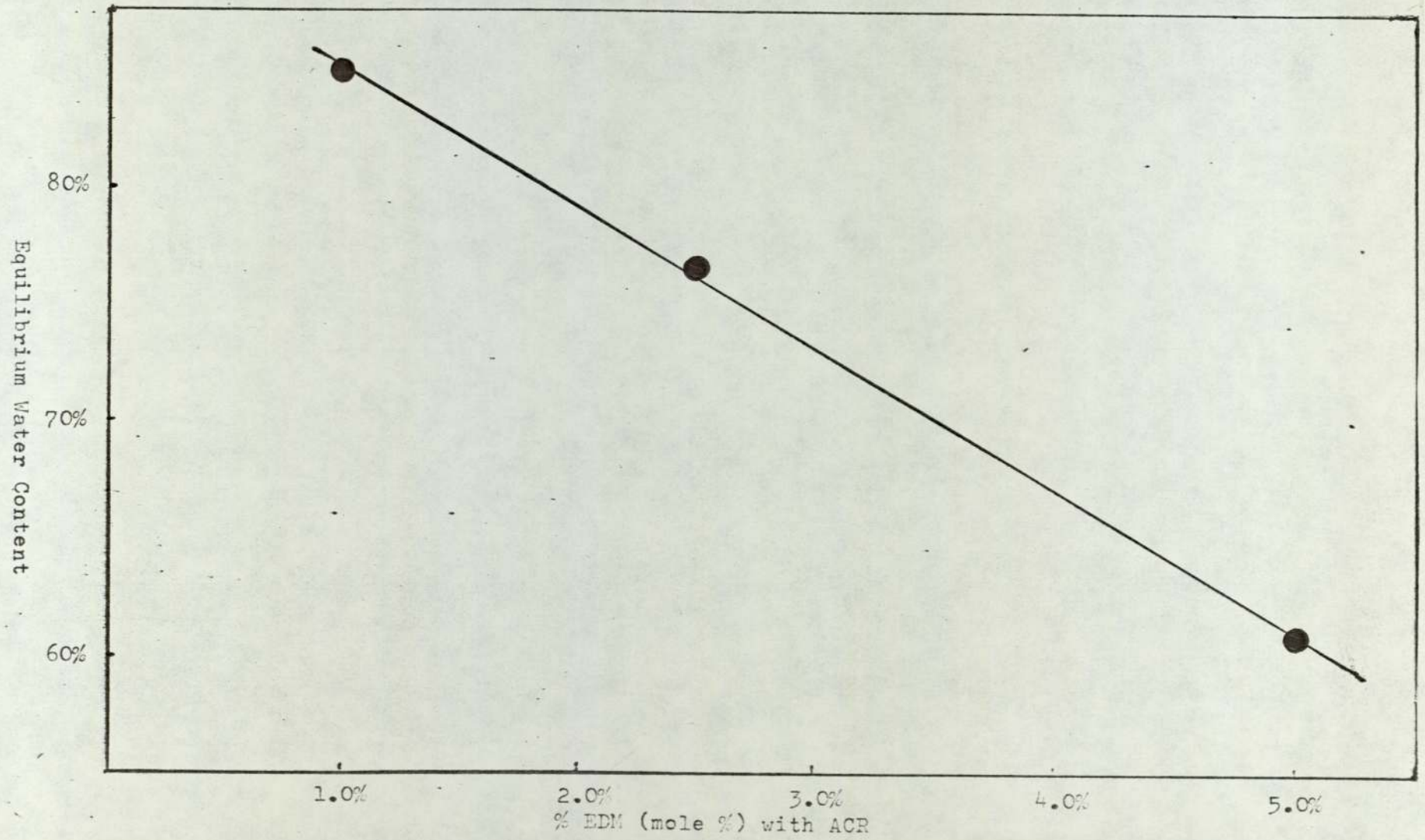
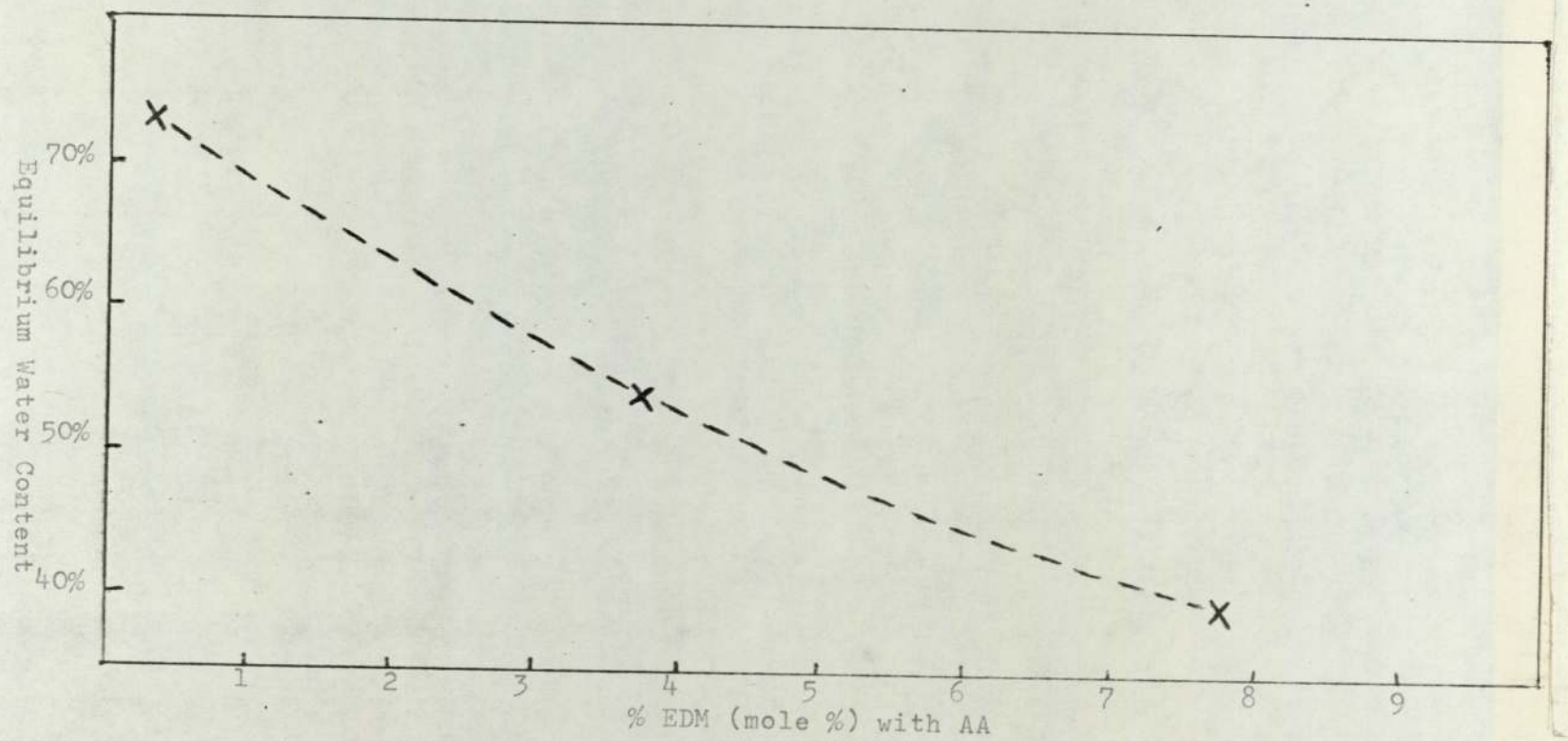


FIGURE 34

The Effect of EDM concentration in poly(acrylic acid) membranes on EWC



The results of the EWC experiments for the series dealt with here are shown in Figures 33 and 34, as well as in Table 36.

It can be seen that, as expected, and found previously by other workers for pHEMA[162], pMAA[211], pHPA:NVP[207] and pHPMA:NVP[207], homopolymers and copolymers, mainly that increasing the amount of crosslinking agent decreases the EWC of the hydrogel.

(b) Complexing and Equilibrium Water Content

More interesting than the relationship between crosslink density and EWC is the effect of complexing between functional groups in hydrogels, on the EWC. This has been examined for copolymers of methacrylic and acrylic acids with acrylamide, N-vinyl pyrrolidone and 2-hydroxy ethyl methacrylate. The EWC's of members of these series are given in Table 36 and shown graphically against the composition of the hydrogel in Figures 35, 36 and 37. All the membranes made were synthesised by the thin film method and contained 1 per cent by weight of EDM. It should be noted that those series with acrylamide as a co-monomer do not have the same range of molar ratios in the copolymer series as the other monomers due to acrylamide's limited solubility in its comonomers even at the temperature of polymerization, 65°C, (the figure for polyacrylamide shown was obtained from the EWC of membranes C, made by UV polymerization of an acrylamide solution.

The results for the individual series are discussed with the figures on the following pages.

1) ACR:AA and ACR:MAA Hydrogels (Figure 35)

As can be seen from the figure, the EWC's of these hydrogels drops to a minimum as the composition of the comonomers approaches a one to one equimolar ratio. This must be due to hydrogen bonding between the carboxylic acid and amide functional groups in the hydrogel which will reach a maximum as an equimolar composition is achieved.

The minimum of the copolymer system with MAA as one of the comonomers is lower in this case and the further examples dealt with. This probably has two causes:- the lower hydrophilicity of MAA due to its hydrophobic methyl group and the possibility of hydrophobic bonding in the case of methacrylic acid copolymers, which will lead to a stabilizing of the hydrogen bond and hence a lower water content, as the hydrogel is effectively more crosslinked.

2) HEMA:AA and HEMA:MAA Hydrogels (Figure 36)

These series of hydrogels show the same basic trends as the ACR/(M)AA and NVP/(M)AA series, however the effect is less marked. This is almost certainly an indication of the lower hydrogen bonding interaction which will occur between the hydroxyl group of HEMA and the acid group of MAA; the effect is bound to be lower as in the other cases there is more electrophilic nitrogen atom as part of the hydrogen bond.

3) NVP:AA and NVP:MAA Hydrogels (Figure 37)

Similarly to the two previous cases, interaction between the

FIGURE 35

The EWC's of the ACR:MAA and ACR:AA Copolymer Hydrogel Membranes

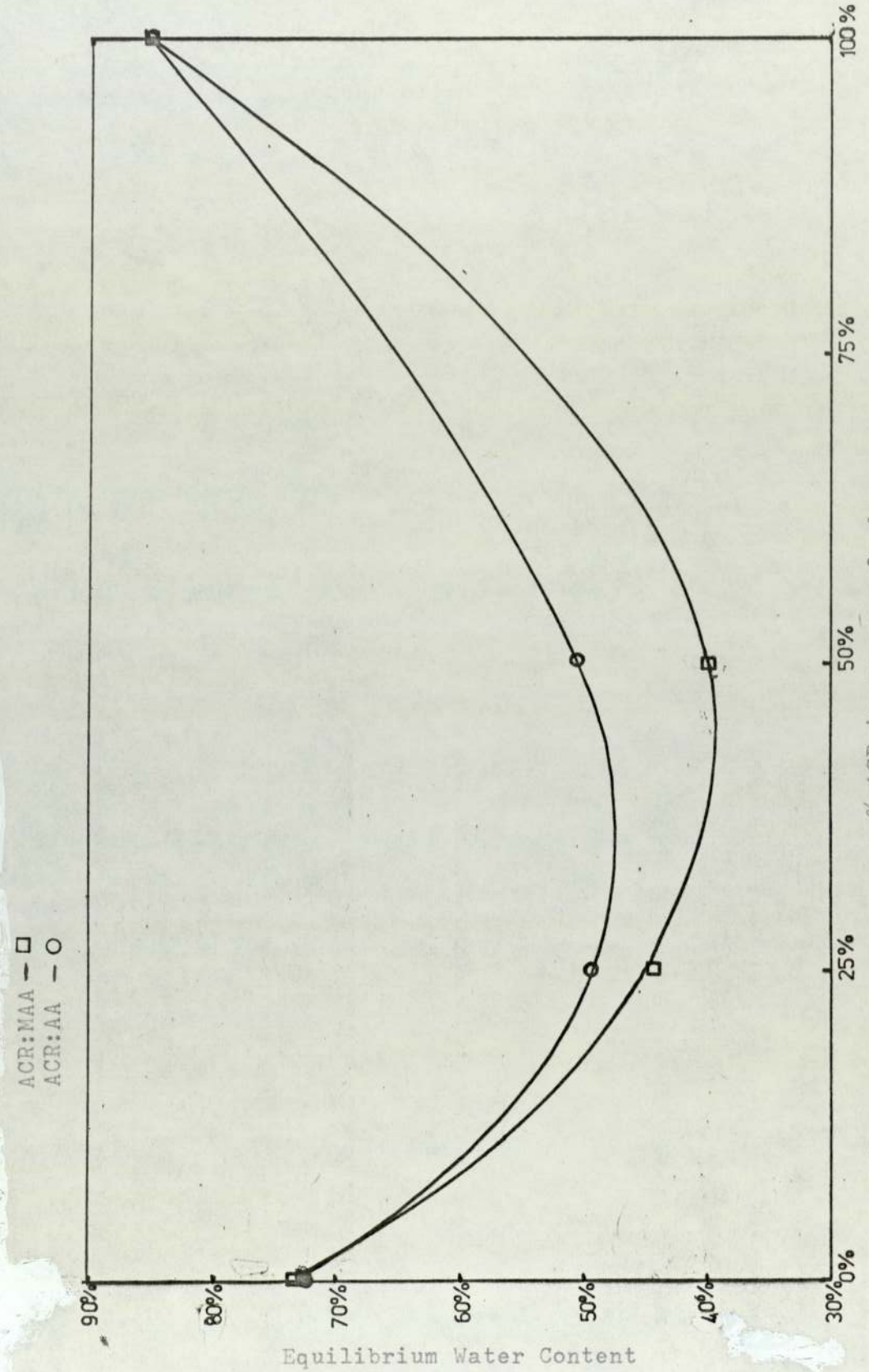


FIGURE 36

The EWC's of the HEMA:MAA and HEMA:AA Copolymer Hydrogel Membranes

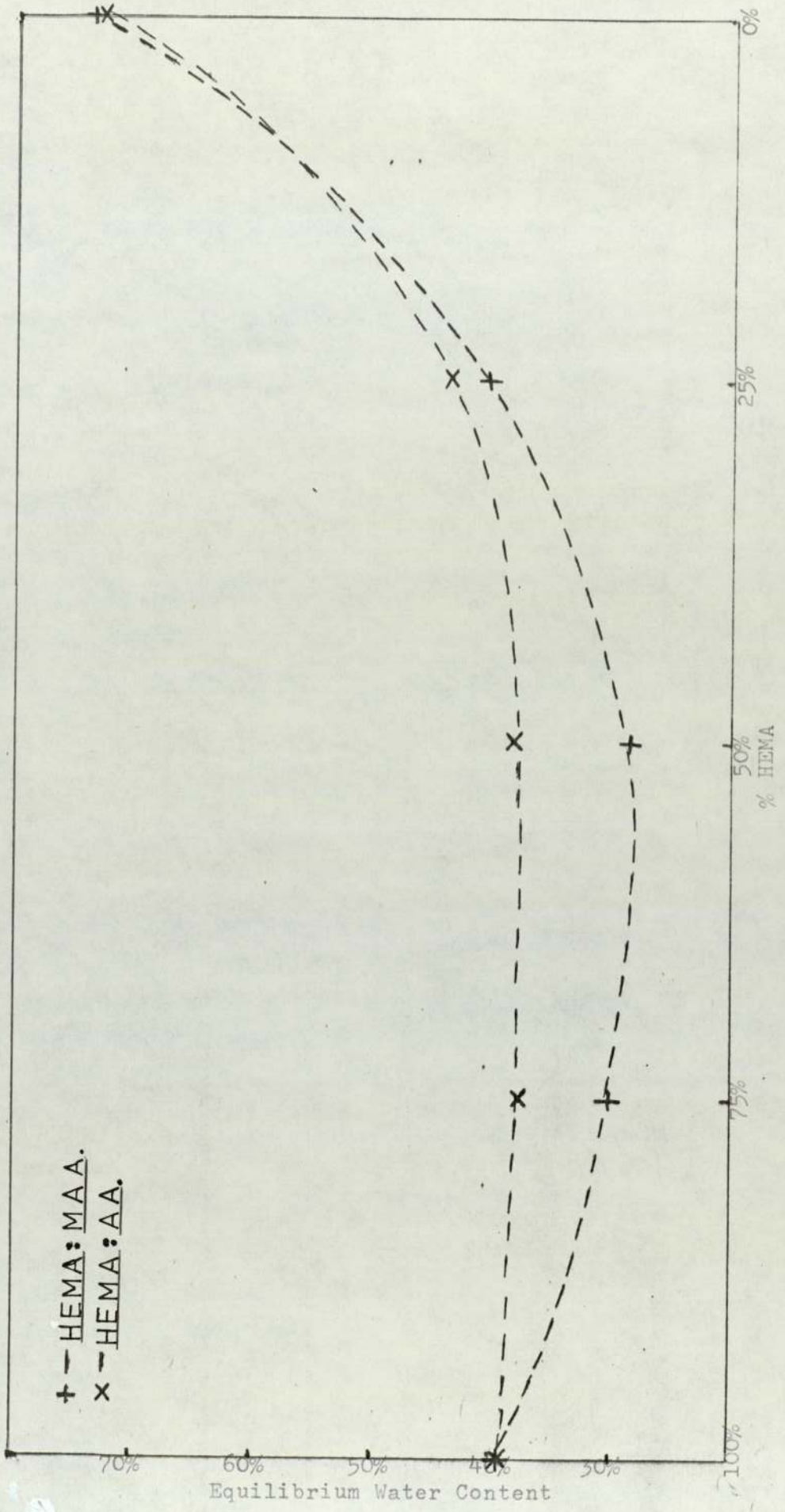
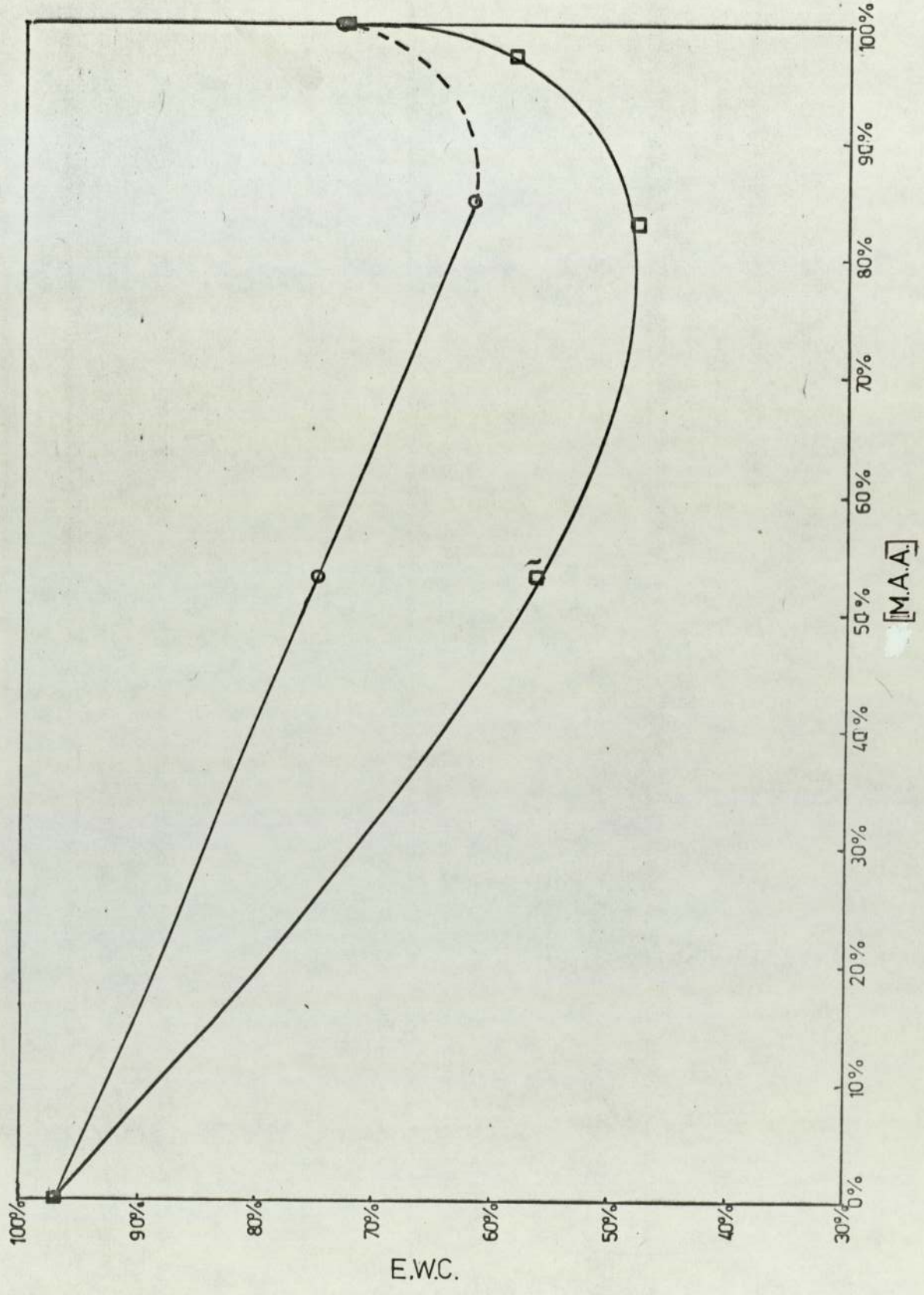


FIGURE 37



The EWC's of the NVP:MAA and NVP:AA Copolymers Hydrogel Membranes set against their true composition

○- NVP:AA
□- NVP:MAA

functional groups in these hydrogels cause the EWC's of these copolymers to decrease from those found for the crosslinked homopolymers. However, as can be seen from the graph these series of copolymers do not show a minima in EWC around the equi molar points but considerably shifted to the high methacrylic and acrylic acid containing copolymers. This can be explained by the importance of other factors than complexing influencing the EWC; one of these factors is the high chain transfer rate of NVP[212] which will lead to a low effective crosslink density in the polymers, (with a high amount of this monomer), and hence will allow the polymers to swell to a greater extent.

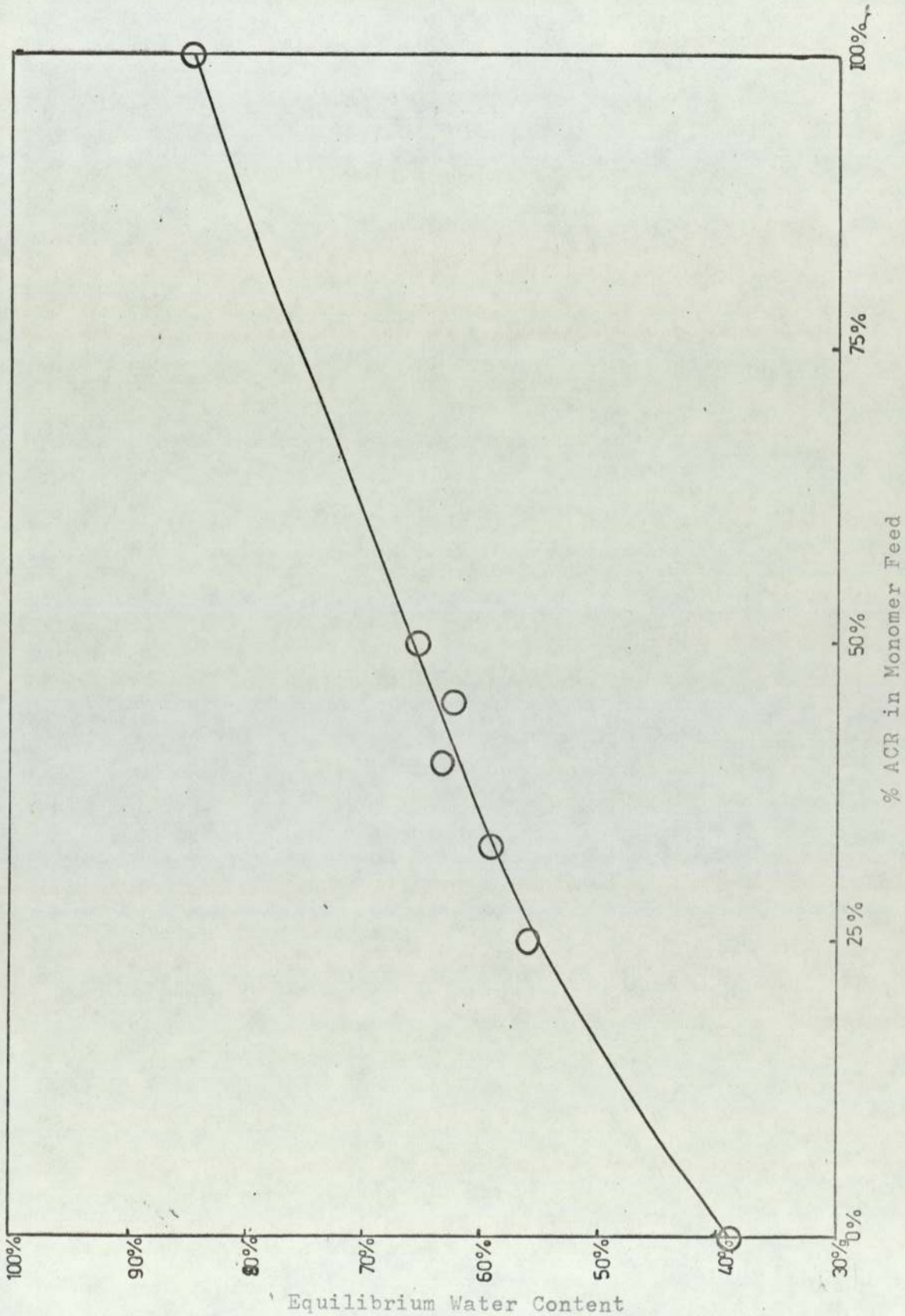
(c) Non-Interacting Polymers

In addition to the previously described series, which were synthesised to provide membranes for blood compatibility testing and whose EWC's were measured and then were found to show the effect of complexing and crosslink density on EWC; other series were manufactured which showed situations in which there was little or no interaction between diverse groups and hence no effect on EWC. An example of this type of copolymer series is the HEMA:ACR series, whose EWC's are given in Table 36 and Figure 38.

As can be seen from the figure, (which contains results obtained by previous worker[213], in addition to those of the author), there is an almost linear increase in EWC as the concentration of the more hydrophilic component, ACR, is increased.

FIGURE 38

The EWC's of the HEMA:ACR Copolymer Hydrogel Series



5.4 SURFACE HYDROPHILICITY AND POLYMER STRUCTURE

The surface hydrophilicities of the homogeneous hydrogel membranes were measured by the Hamilton contact angle technique described in the previous chapter. This was done in order to try to establish relationships between structure, water content and general composition with the surface hydrophilicity. These relationships have been investigated as there appears to be a relationship between the time blood takes to clot on a hydrogel surface and the surface hydrophilicity it exhibits; this relationship is examined in the chapter on Blood Compatibility, Chapter 9.

Since this relationship was found it was decided to carry out a more complete survey of the relationships already mentioned. Many of the results were obtained by Barnes in the course of his work on hydrogel membranes, many of which were prepared and characterized by the author; his results are clearly indicated.

On the following pages a similar structure of presentation is adopted to that made in the previous section dealing with EWC, ie the relationship between surface hydrophilicity and the other properties of the hydrogel are examined. These individual effects were not considered by Barnes in the course of his work.

The separate sub-headings into which this work is divided is given in Table 39.

TABLE 39

Division of Results (Hamilton Contact Angles)

(a) The Effect of Water Content on Hamilton Contact Angle

(b) The Effect of Polymer Composition on Hamilton Contact Angle

1) The Effect of NVP (N-vinyl pyrrolidone)

2) The Effect of DAA (diacetone acrylamide)

3) The Effect of Introducing Fluorinated Monomers

4) The Effect of Complexing Between Functional Groups

(a) The Effect of Water Content on Hamilton Contact Angle

The general trend of the relationship between the Hamilton Contact angles and the EWC's of hydrogels has been elucidated by Barnes[206]. He found that with increasing EWC the contact angles, and thus surface polarity increased; this is shown in Figure 39, which is a compilation of results for many hydrogels of different EWC's.

It was observed by him that there was an initial very sharp increase in the Hamilton contact angle for hydrogels with small amounts of water; an effect which he attributed to the plasticizing effect of water on the hydrogel, which should allow the more polar groups at the water-hydrogel interface to rotate and orientate themselves into the water layer. This effect is best illustrated however by a series of Barnes's results for the HEMA:ST series:- Table 40.

TABLE 40

Hamilton Contact Angle Results for the HEMA:ST Series

HEMA:ST Initial Molar Feed Ratio	EWC	Hamilton Contact Angle
100 : 0	40.5%	148 ± 2°
90 : 10	23.0%	136 ± 2°
80 : 20	13.0%	126 ± 2°
50 : 50	5.0%	108 ± 2°
10 : 90	1.5%	93 ± 2°

This table shows the dramatic change in surface polarity even small amounts of additional water can make.

The main subject however of this section is the relationship between polymer composition and surface hydrophilicity for hydrogels within that broad band of values found for higher water content hydrogels, illustrated in Figure 39.

(b) The Effect of Polymer Composition on Hamilton Contact Angle

Polymer composition is obviously a very important factor in determining the surface polarity of a hydrogel; many effects have been observed and these are dealt with on the following pages. Among the most interesting are the effects due to hydrophilic polymers with large hydrophobic side chains and these are considered first.

1) The Effect of N-Vinyl Pyrrolidone (NVP)

The effect of NVP on surface polarity is most clearly illustrated

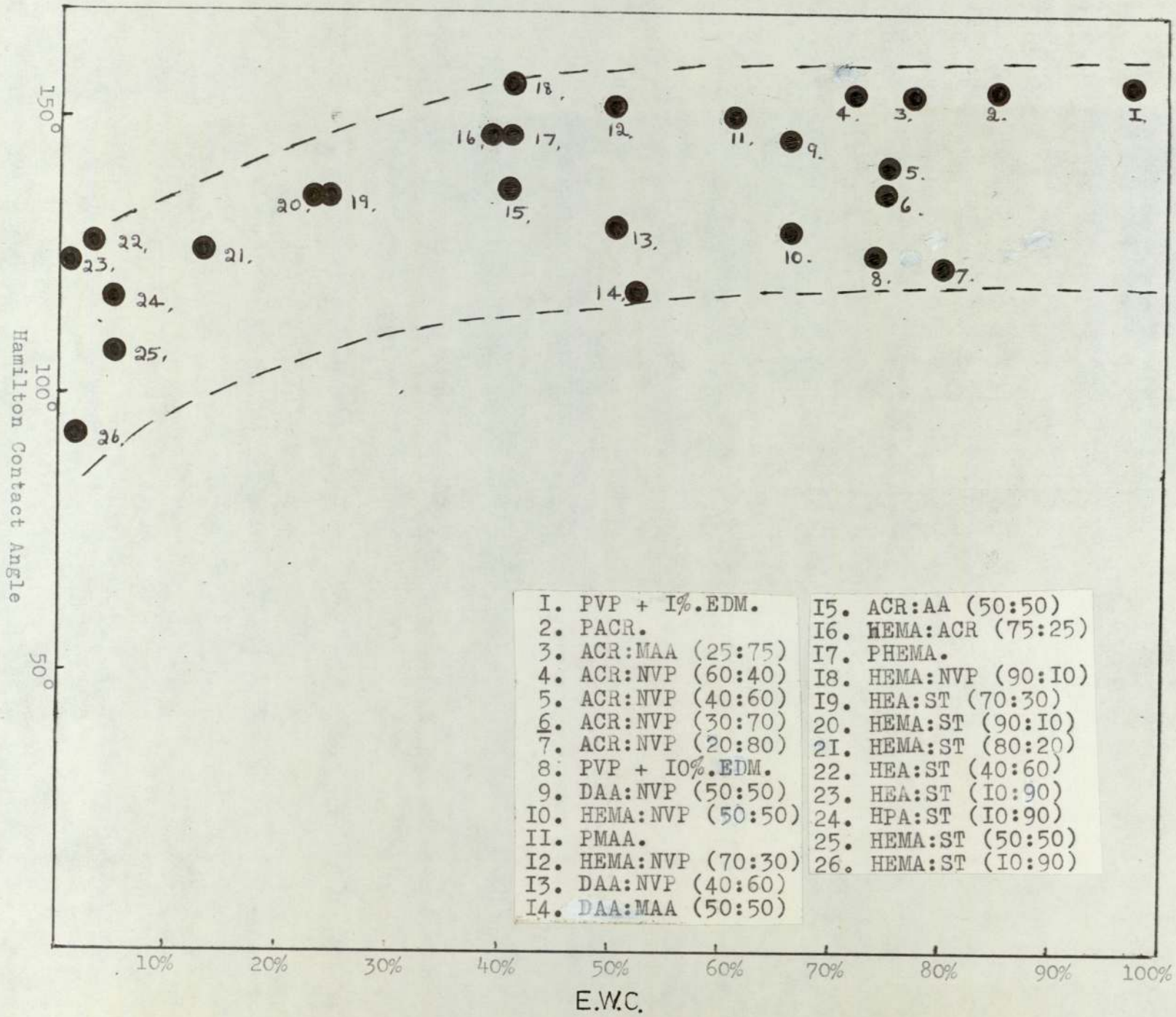


FIGURE 39

The Relationship between Hamilton Contact Angle and Equilibrium Water Content for Various Hydrogels

for the copolymer series ACR:NVP and HEMA:NVP, the results for which are shown in Table 41, and plotted in Figure 40.

TABLE 41

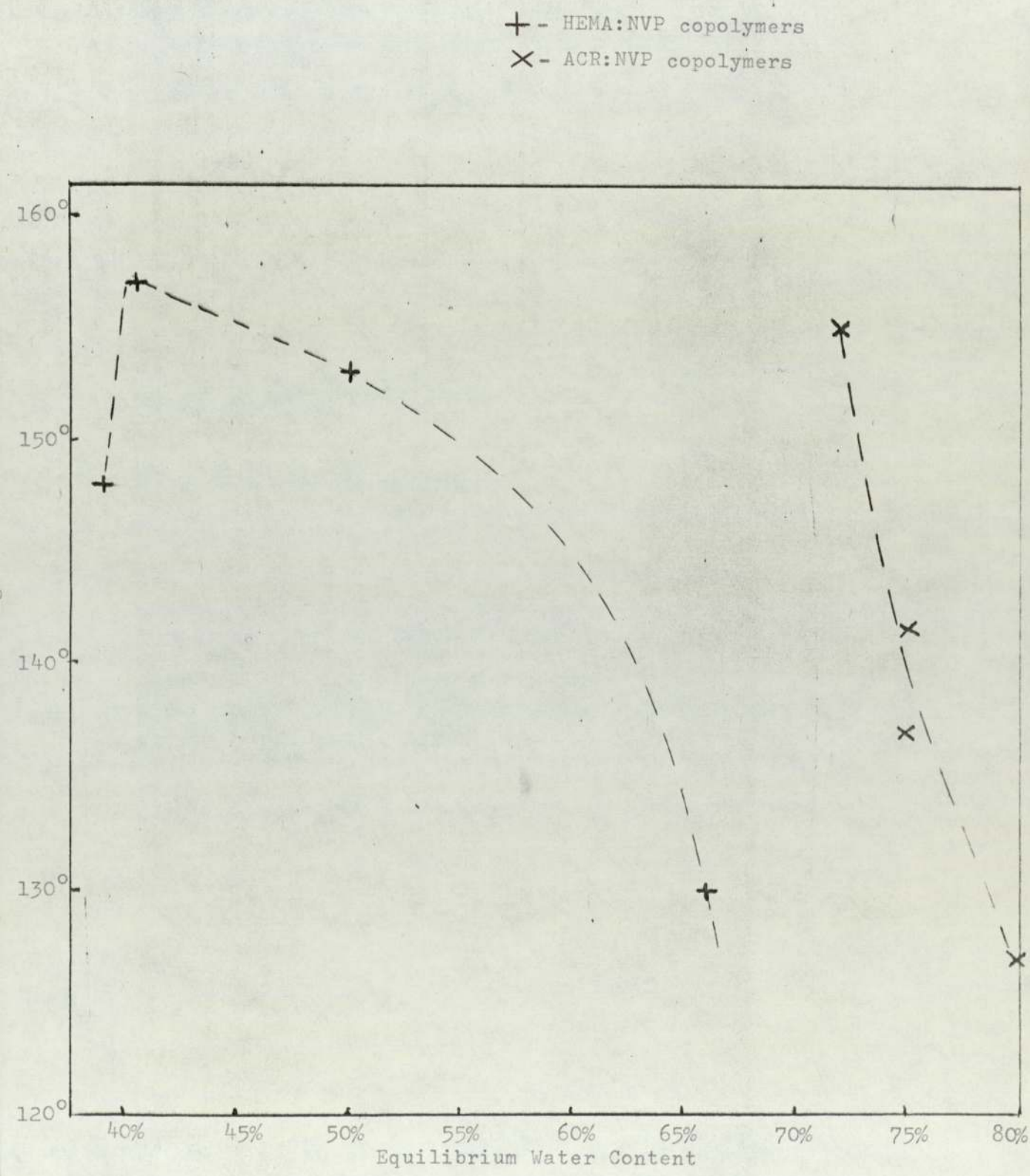
Hamilton Contact Angle and Composition/EWC of NVP containing hydrogel films

Composition	Initial Molar Feed Ratio	EWC	Average Hamilton Contact Angle
ACR : NVP	60 : 40	72.0%	155°
" : "	40 : 60	75.0%	141.5°
" : "	30 : 70	75.0%	137°
" : "	20 : 80	80.0%	124.5°
HEMA : NVP	100 : 0	39.0%	148 ± 2°
" : "	90 : 10	40.5%	157 ± 1°
" : "	70 : 30	50.0%	153 ± 1°
" : "	50 : 50	66.0%	130°

In the case of the ACR:NVP series 5% EDM was added as cross-linking agent and for the HEMA:NVP series 1% EDM was added. The initial molar feed ratio does not represent the final composition of the polymer as C, H, N analysis has shown that the unreactivity of this monomer leads to a low amount of it being incorporated in the polymerization. Nevertheless, in both cases it can be seen that as the amount of NVP increases the surface polarity of the hydrogel decreases. This effect is accompanied by an increase in EWC with increasing NVP content, it is therefore going against the general trend of increasing Hamilton Contact Angle with increasing EWC, and it is believed to be due to the presence of the hydrophobic methylene groups on the NVP ring shielding the polar hydrophilic amide group, thus leading to this unusual effect, mainly, increasing

FIGURE 40

The Effect of N-vinyl pyrrolidone (NVP) on the Hamilton Contact Angle of two hydrogel compositions



bulk hydrophilicity with decreasing surface polarity.

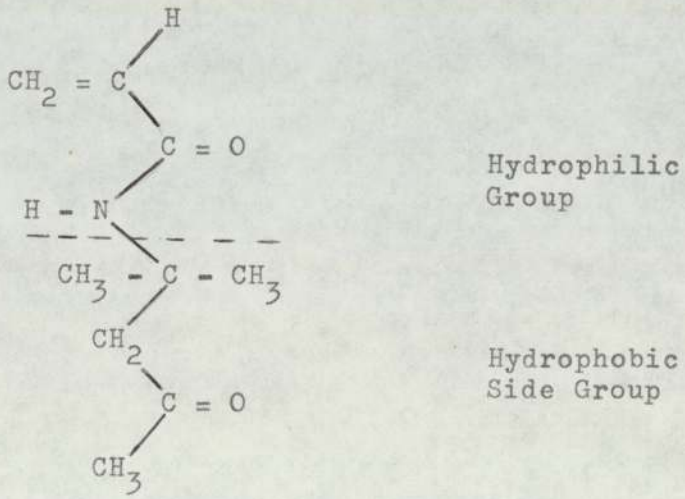
Another unusual effect, which can be seen in Table 41 and Figure 40, is an unusual jump in Hamilton contact angle on going from p-HEMA to HEMA-NVP (90:10) before reduction of the Hamilton Contact Angles start; this effect can be attributed, possibly, to the "structure breaking" effect of a small concentration of diluent, leading to the initial exposure of a higher concentration of polar groups.

2) The Effect of Diacetone Acrylamide

Another monomer which appears to act in a similar way to NVP is diacetone acrylamide, whose structure is shown in Figure 41.

FIGURE 41

Structure of Diacetone Acrylamide (DAA)



As can be seen from the figure showing the structure, DAA has a large hydrophobic side chain. In the polymer, therefore, there will be a polymer backbone with large hydrophobic side groups.

Between the non-polar backbone and the non-polar side group there is the highly hydrophilic amide group, which is shielded by the hydrophobic group and backbone. Thus, hopefully, polymers and copolymers of DAA will have the property of possessing high bulk hydrophilicity due to the amide entity and low surface hydrophilicity (non-polarity) due to the existence of the shielding thus increasing the range of properties that a hydrogel can have. It should be possible to make materials with high water contents but low surface polarities.

Several polymers and copolymers of DAA have been produced in this and other studies; the presence of the above mentioned property is discussed and exemplified. The polymers and copolymers of DAA which have been investigated are shown in Table 42.

TABLE 42

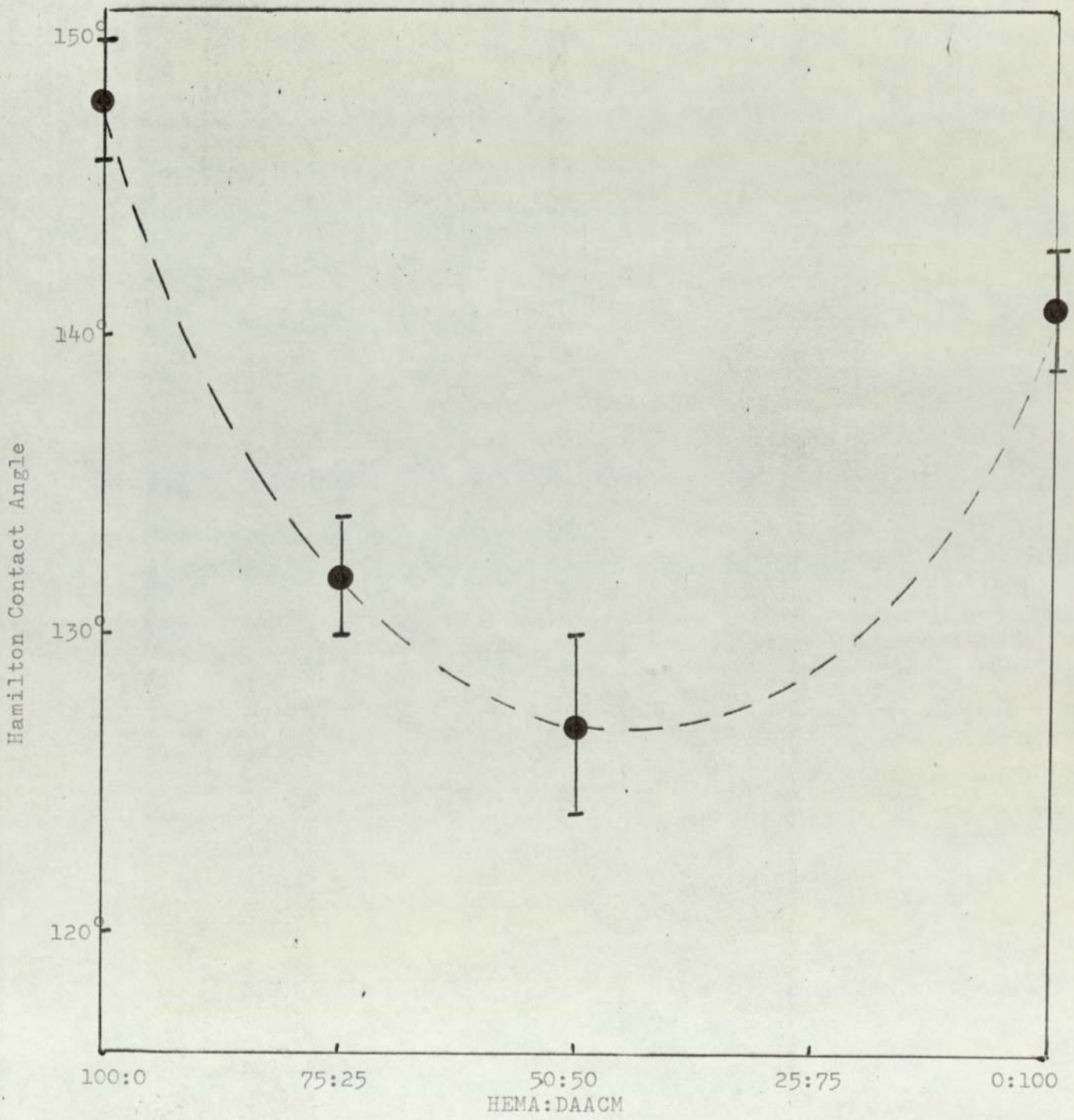
Hamilton Contact Angles on Various DAA Polymers

Composition	Initial Molar Ratio*	EWC	Hamilton Contact Angle
(1) pDAA	-	-	$141 \pm 2^{\circ}$
(2) DAA : HEMA	(75 : 25)	-	$132 \pm 2^{\circ}$
(3) DAA : HEMA	(50 : 50)	23.7%	$127 \pm 3^{\circ}$
(4) DAA : MAA	(50 : 50)	51.0%	119.5°
(5) DAA : AA	(50 : 50)	28.3%	$130 \pm 3.5^{\circ}$
(6) DAA : NVP	(40 : 60)	50%	132°
(7) DAA : NVP	(50 : 50)	66%	147°

* (EDM) is 1% by weight with the exception of (6) which has 5%

FIGURE 42

The Relationship Between Hamilton Contact Angle and the Composition of HEMA:DAA Copolymers



These materials were all manufactured by the normal thermally catalysed bulk polymerized method using AZBN, with the exception of the poly (diacetone acrylamide), which was made by polymerizing a solution of DAA in water.

As can be seen from the table the main series of DAA copolymers which have been investigated are the HEMA:DAA series; the relationship between composition and Hamilton contact angle for these hydrogels is shown graphically in Figure 42.

From the figure it can be seen that the surface polarity of the copolymers decreases as more DAA is added to the initial composition, but that the value for Hamilton contact angle reaches a minimum at about a 50:50 molar ratio.

Also given in Table 42 are the Hamilton contact angle results obtained on a variety of other hydrogels. It can be seen that in comparison with other hydrogels several exhibit abnormally low Hamilton contact angle values for such hydrophilic, high water content materials. As can be observed 50:50 molar ratios of DAA with a comonomer were the main compositions studied; these compositions were used to make hydrogels in the hope of making materials which could be compared easily.

However, many peculiar anomalies have appeared, for example, it might have been expected that copolymers of DAA with NVP would have exceptionally low surface hydrophilicities as one would expect the hydrophobic contributions from the hydrophobic side groups to add and create an especially low polarity surface. Unfortunately this does not appear to occur.

With the copolymers of DAA and MAA/AA the effect demonstrated with the HEMA:DAA series is reproduced; mainly, there is a reduction in the Hamilton contact angle as the molar ratios approach unity. Although in this case we cannot be sure that the minima are in this region due to shortage of results. Figure 43 is a graphic representation of this data, it is observed that the reduction in surface polarity is much greater in the case of the copolymers, which contain methacrylic acid, than acrylic acid, an effect which can be attributed to the hydrophobic effect of the methyl group.

3) The Effect of Introducing Fluorinated Monomers

It might be expected that the introduction of fluorinated side chains into hydrogel copolymers might have a similar effect to that described previously for NVP and DAA, mainly that the surface hydrophilicity of the materials would be substantially lower. Two sets of hydrogel copolymers have been examined for this effect, one manufactured by the author in the course of an MSc project and another by Hussein Berry (MSc); the results of these studies are given in Table 43.

Examining the table and comparing the Hamilton contact angle values with those obtained on poly HEMA and poly HPA, 148° and 145°, respectively, it can be seen that the addition of fluorine groups to the hydrogel do not appear in most cases, to appreciably effect the surface hydrophilicity.

FIGURE 43

The Relationship Between Hamilton Contact Angle and Composition for Diacetone acrylamide:(Meth) acrylic acid Hydrogels

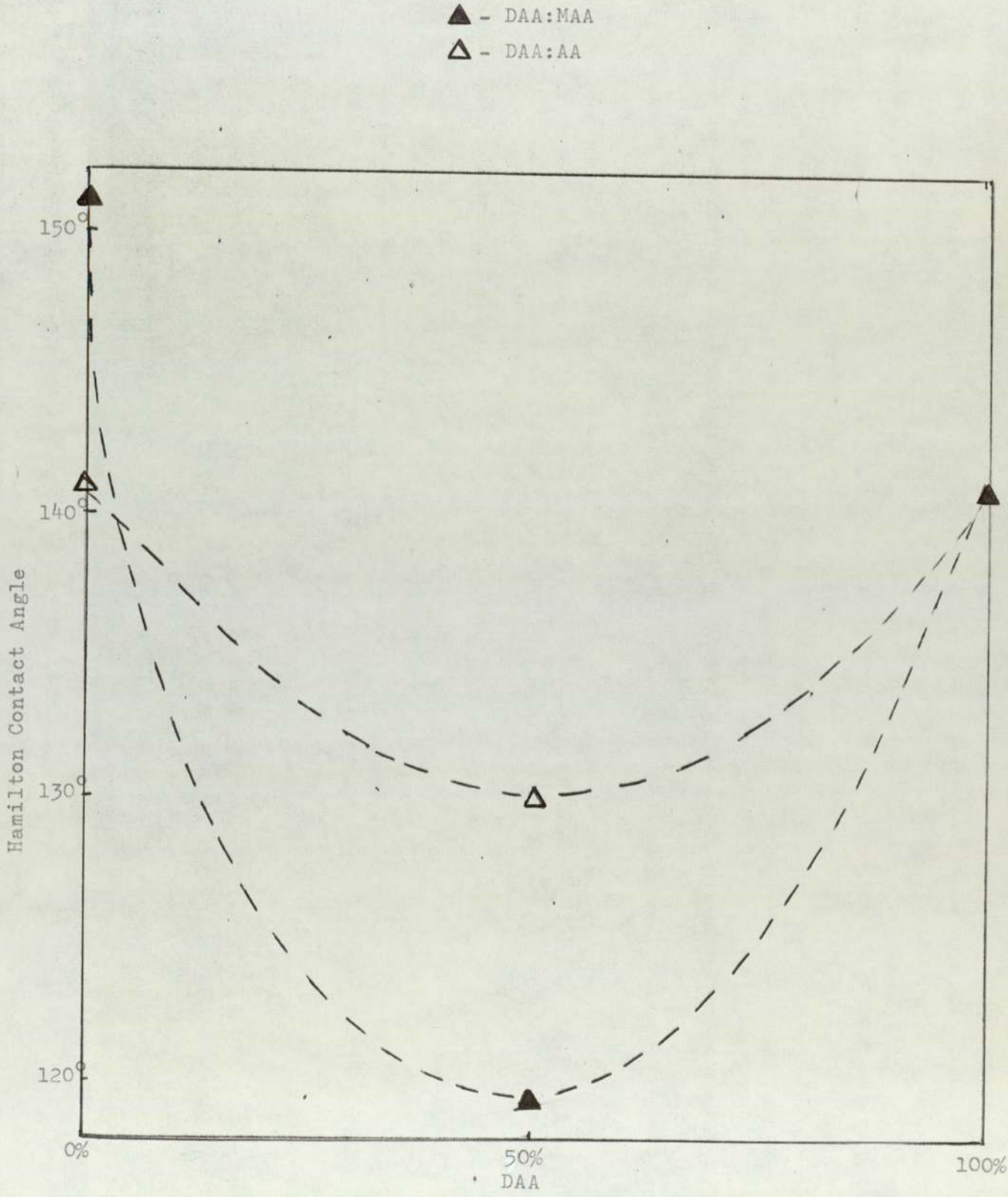


TABLE 43

Hamilton Contact Angles of Fluorinated Hydrogels

Composition	Initial Molar Ratio	EWC	Hamilton Contact Angle
HEMA:HFIPA	(50:50)	12.0%	147 ± 2.5°
HEMA:HFIPMA	(50:50)	11.5%	137 ± 1.5°
HEMA:222TFEM	(50:50)	8.0%	141 ± 1.5°
HEMA:ACR:222TFEM	(50:25:25)	22.0%	119 ± 3.5°
HPA:222TFEMA	90:10	30%	142 ± 4° (214)
HPA:1,1,5THPFPMA	90:10	22%	135 ± 2° "
HPA:1,1,7THPFHMA	90:10	20.5%	133 ± 3° "
HPA:1,1DHHFBMA	90:10	31%	137 ± 3° "
HPA:1,1,3THPFPMA	90:10	31.5%	136 ± 3° "
HPA:HFIPA	90:10	28%	141 ± 4° "
HPA:HFIPMA	90:10	21%	137 ± 3° "

4) The Effect of Complexing Between Functional Groups

A further factor, which can have a great influence on the Hamilton contact angle is complexing between functional groups; here again, as with the effect on increasing CLD there are several effects occurring which are difficult to disentangle, since complexing effects the EWC, (as has been previously shown), and hence effects Hamilton contact angle. The effect has again been investigated by Barnes and the author on membranes prepared and characterized by the author.

The copolymer compositions investigated are shown in Table 44

TABLE 44

The Effects of Complexing on Hamilton Contact Angle for Various Hydrogel Series

		Initial Molar Monomer Feed Ratio	EWC	Hamilton Contact Angle
(1)	HEMA:MAA	100:0	39.0%	148 ± 2°
		75:25	30.0%	140°
		50:50	28.5%	134.5 ± 1.5°
		25:75	40.5%	146 ± 0.5°
		0:100	73.5%	151 ± 1°
(2)	HEMA:AA	100:0	39.0%	148 ± 2°
		75:25	37.5%	140°
		50:50	38.0%	131 ± 1.5°
		25:75	43.5%	136 ± 1.5°
		0:100	73.0%	141 ± 1.5°
(3)	NVP:MAA	100:0	97.0%	157 ± 3°
		75:25	56.5%	139.5 ± 1.5°
		50:50	48.0%	143.5 ± 1.5°
		25:75	58.5%	150.5 ± 0.5°
		0:100	73.5%	151 ± 1°
(4)	NVP:AA	100:0	97.0%	157 ± 3°
		75:25	75.0%	145 ± 1.5°
		50:50	62.0%	136 ± 1°
		25:75	—	148 ± 3°
		0:100	73.0%	141 ± 1.5°
(5)	ACR:MAA	50:50	40.0%	117 ± 2°
		25:75	44.5%	153.5 ± 2°
		0:100	73.5%	151 ± 1°
(6)	ACR:AA	50:50	50.5%	136 ± 2°
		25:75	49.5%	146 ± 2.5°
		0:100	73.0%	141 ± 1.5°

and the results shown graphically in Figures 19 and 20. The individual series are discussed on the following pages.

HEMA:MAA and HEMA:AA copolymers (Figure 44)

In both these series there is a pronounced drop in the Hamilton contact angle as the composition approaches equimolar amounts of acrylic/methacrylic acid and HEMA in the copolymer. This suggests that a hydrogen bonding complex exists and that by fixing polar groups, thus inhibiting their orientation towards the water-polymer interface, the complexing reduces surface polarity.

NVP:MAA and NVP:AA series (Figure 44)

These series too show a similar effect and since complexing between poly (meth)acrylic acid and poly-vinyl pyrrolidone is well known and, also, the effects of complexing on the polymerization of the monomers, (the linear polymer of one monomer providing a template for the polymerization of the other) , it is clear that complexing is effecting the surface polarity of the polymers.

This effect is shown along with the one for the previously described series in Figure 44, which shows the Hamilton contact angles plotted against the EWC's of the polymers.

ACR:MAA and ACR:AA series (Figure 45)

In these series too there is a pronounced drop in the surface polarity (as shown by Hamilton contact angle) as we approach the equimolar compositions in the two series (Figure 45). However,

FIGURE 44

The Effect on Hamilton Contact Angles of Polymer Composition for HEMA:MAA, HEMA:AA, NVP:MAA and NVP:AA series, as set against EWC

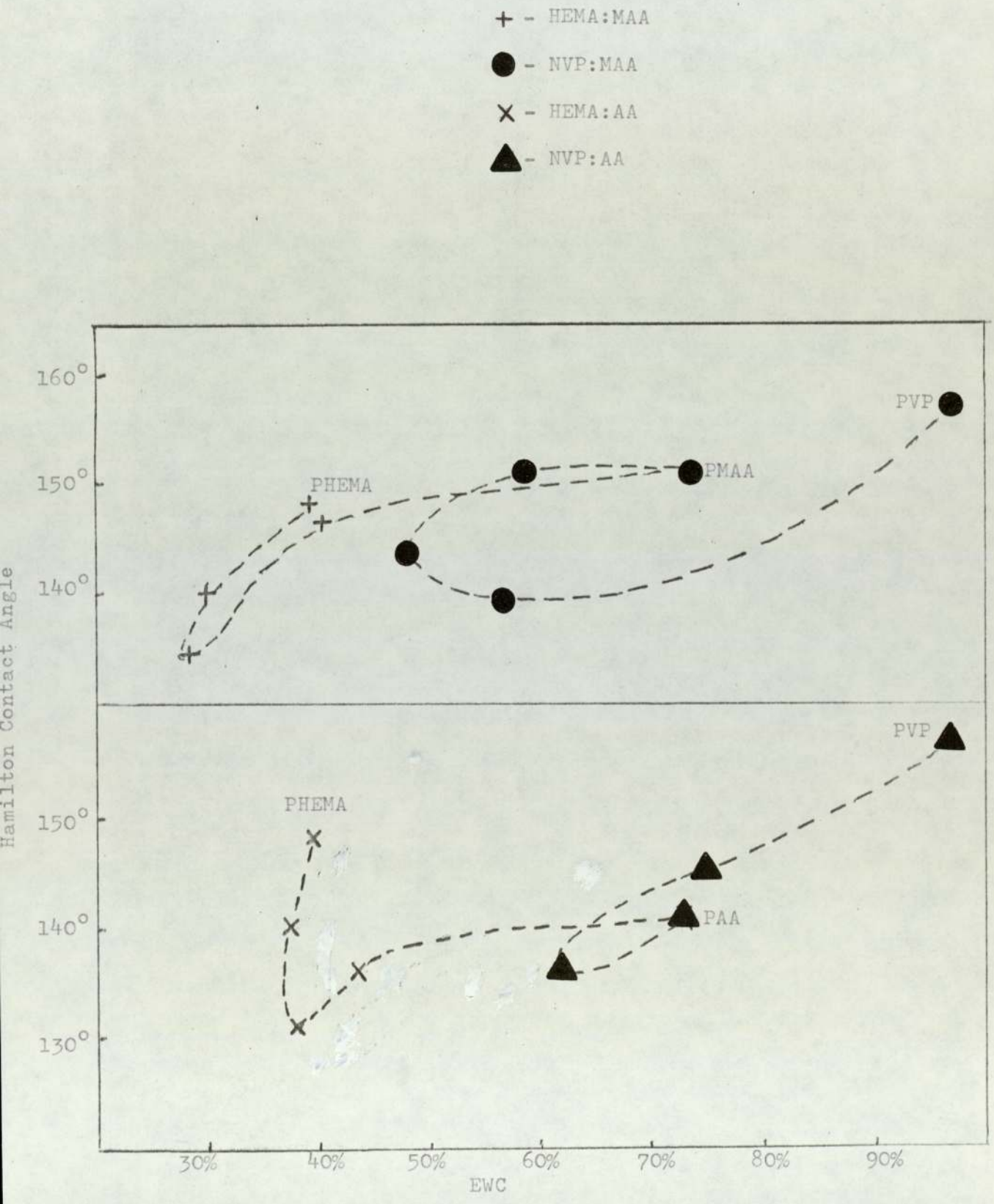
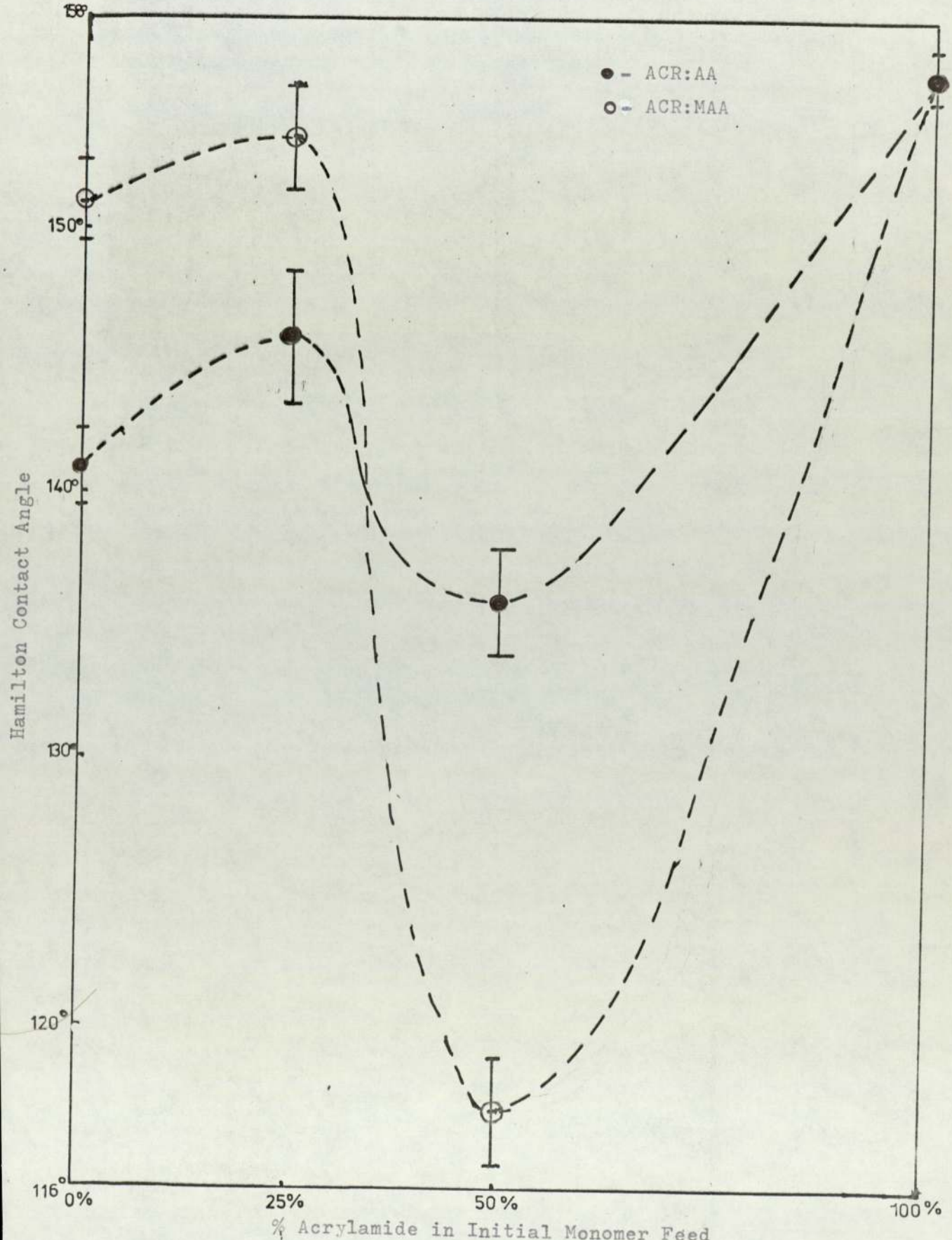


FIGURE 45

The Relationship between Hamilton Contact Angle and the Composition of Acrylamide:Acrylic Acid and Acrylamide:Methacrylic Acid Copolymers



it is noticeable in this case that there is a very pronounced difference in the magnitudes of the drop; it is much greater for the ACR:MAA series. This can be explained by hydrophobic bonding. Hydrophobic bonding between methyl groups is known to stabilize complexes between carboxylic acid groups ; it is therefore possible to speculate that this accounts for the differences between the two series. To test this theory the Hamilton contact angle measurements were applied to the surfaces of membranes of methacrylamide:(meth)acrylic acid. If the theory was correct then we should expect MACR:MAA (50:50) to have an even lower contact angle than ACR:MAA (50:50), since there will be even more hydrophobic bonding present, as there are more methyl groups. Also, the MACR:AA (50:50) copolymer should show a drop in Hamilton contact angle against ACR:AA (50:50) for the same reason. The results on these membranes, prepared by Ian Middleton, are shown in Table 45.

TABLE 45

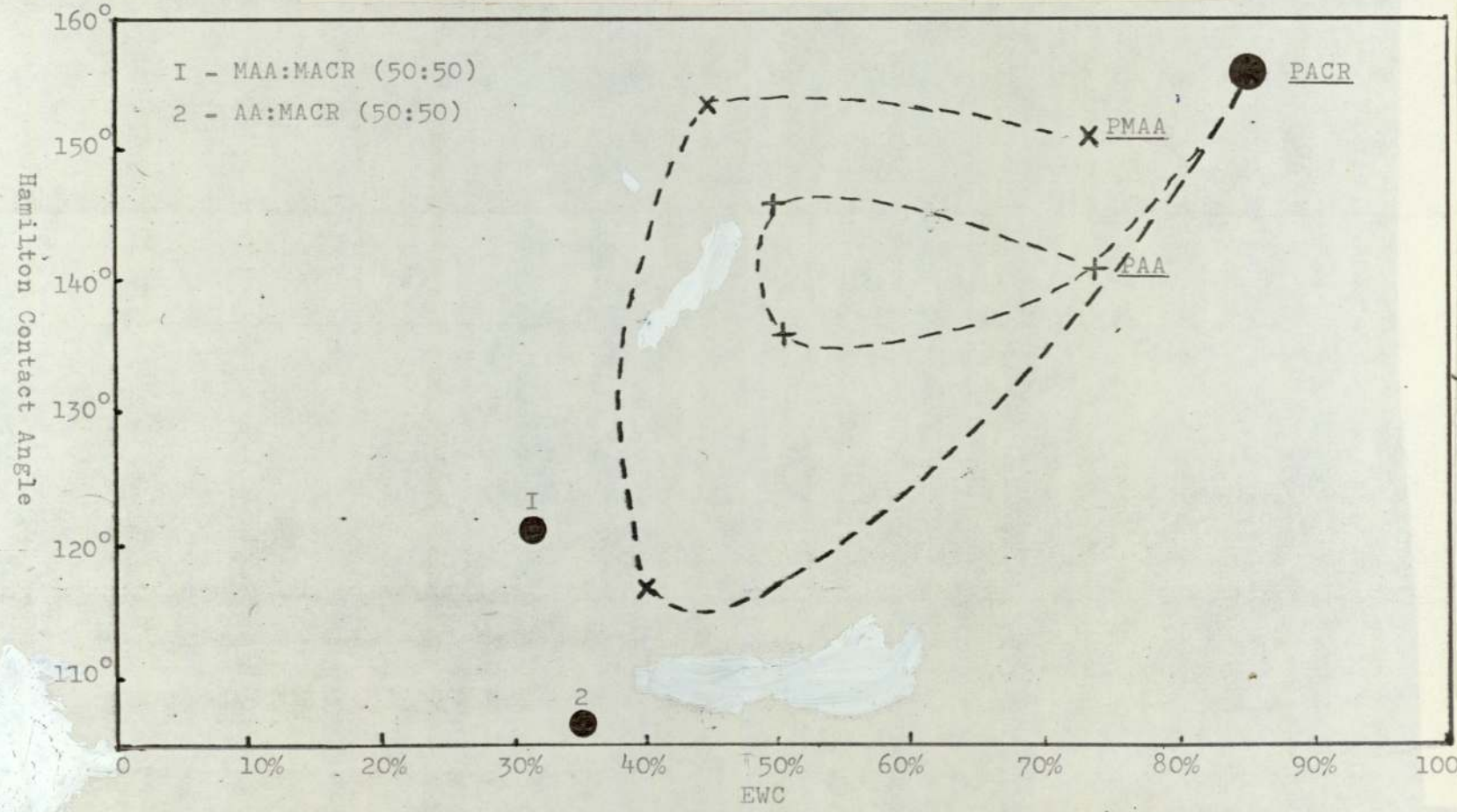
Hamilton Contact Angles of MACR/ACR:MAA/AA Copolymers

Composition	Initial Molar Ratio	EWC	Hamilton Contact Angle
MACR:MAA	(50:50)	31.5%	$111.5 \pm 3^{\circ}$
ACR:MAA	(50:50)	40%	$117 \pm 2^{\circ}$
MACR:AA	(50:50)	35%	$121 \pm 1.5^{\circ}$
ACR:AA	(50:50)	50.5%	$136 \pm 2^{\circ}$

As can be seen from the table the results are as predicted. Also, it is noticeable that there is a difference between the ACR:MAA and MACR:AA results, this is probably due to the locus of action of the hydrophobic bond being different in each case, in

FIGURE 46

The Effect on Surface Hydrophilicity, (Hamilton Contact Angle) of the Interactions between the Amide and Carboxylic Acid group in Acrylamide/Methacrylamide and Acrylic/Methacrylic acid copolymers set against their EWC's



the methacrylamide copolymers it is further away from the interacting carboxylic acid groups and thus has less stabilizing effects. Another possibility is that the bonds have a self-enhancing effect as they are created, ie that the stability of one type of bond helps to create the other, this effect would be greater if the bonds were in close proximity with each other.

Lastly, it is noticeable that both series in common with other series shown, such as the NVP:HEMA series, exhibit an initial rise in surface polarity as the weakly crosslinked homopolymers of acrylic and methacrylic acid are diluted with acrylamide. This effect occurs despite the fact that complexing must be quite considerable when the compositions reach 75% AA/MAA:25% ACR, and it is probably due to the structure breaking effect of the diluent co-monomer, acrylamide, on the complexed helical coils of pMAA and pAA, which by breaking up the structure cause more free polar groups to be present.

HEMA:ACR series

As certain membranes containing HEMA and ACR (whose Hamilton contact angles had been determined in connection with the blood clotting time results), appeared to be very low compared to other membranes of similar EWC, it was decided that the HEMA:ACR series would be manufactured and their Hamilton contact angles determined. The results of the experiments are shown in Table 46. These results are also shown graphically in Figure 47.

From Table 46 and Figure 47 we can see that there is indeed a slight drop in Hamilton contact angle, indicating perhaps some

FIGURE 47

The Relationship between Hamilton Contact Angle and the Composition of the Hydroxyethylmethacrylate:Acrylamide Copolymer Series

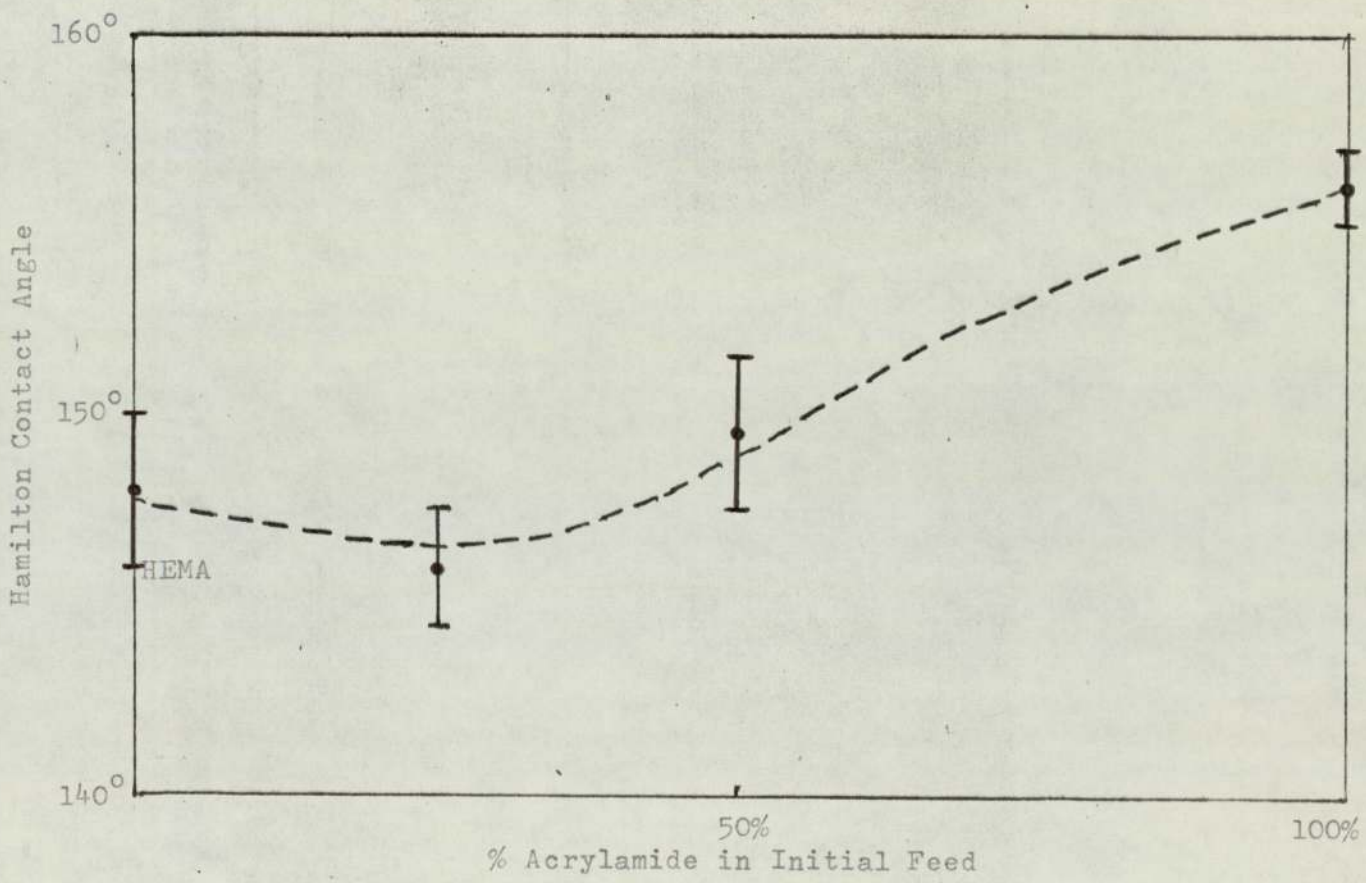


TABLE 46

Hamilton Contact Angles of the HEMA:ACR series

Composition	Initial Molar Ratio	EWC	Hamilton Contact Angle
HEMA:ACR	100:0	39.0%	148 ± 2°
" "	75:25	56.0%	146 ± 1.5°
" "	50:50	65.5%	149.5 ± 2°
" "	0:100	85.0%	156 ± 1°

degree of hydrogen bonding between the amide and hydroxyl groups in the copolymer. However, the effect is of a very low order and cannot be very significant in producing the low values observed in the terpolymers mentioned.

5.5 MISCELLANEOUS PROPERTIES OF THE HOMOGENEOUS HYDROGELS

The properties dealt with earlier in this chapter are not, of course, the only properties which are important in the final application of these materials. Other properties, such as strength have been dealt with on a more superficial level.

(a) Strength

The strength of the materials synthesized in this study was not rigorously investigated, since the polymers could not be used in the form produced for biomedical applications and if it was desired to use them in such applications many other factors would influence the strength of the materials, eg if hydrogels are being

used as coatings for blood detoxicants, the grafting technique, post-polymerization crosslinking reactions and other factors would be the prime determinants in the strength of the hydrogel. Nevertheless, some observations have been made as to the variations in strength of the homogeneous hydrogels, which may be useful in further work, and these are given in Appendix B.

(b) Permeability and Blood Compatibility

Lastly, the clotting times of blood on many of the homogeneous hydrogels were found; the results of these tests are discussed with those on other materials, ie non-homogeneous membranes and beads, in Chapter 9. The permeability of the membranes to the large solute molecule, BSP, was examined and these results are dealt with in a section in the next chapter, in which they are compared with the results found for the macroporous membranes and reference materials.

(c) Transparency and Opacity

The transparency, translucency or opacity of the films are obviously of little importance to the present work, except perhaps as a minor indication of porosity. However, as the optical properties of hydrogels are important in other applications such as contact lenses, the optical properties of the materials made in the present study have been noted and are given in Appendix C.

5.6 CONCLUSIONS

From the work done on the homogeneous membranes the following conclusions have been reached.

(1) C, H, N analysis is an effective technique for measuring the final molar ratios of many copolymer systems.

(2) NVP is a highly unreactive monomer, which is not incorporated to a large extent by a thermally catalysed polymerization system.

(3) There are many interesting interrelationships between the structure of the hydrogel polymers and their surface properties.

CHAPTER 6

MACROPOROUS MEMBRANES

6.1 INTRODUCTION

Macroporous hydrogel membranes, ie hydrated polymer films with pores of the order of microns in size, were synthesized and characterized, as it was considered that they would have better permeability to higher molecular weight molecules of the type which are more interesting from the point of view of artificial liver support, the so-called "middle molecules".

The means of creating such membranes was first discovered by Haldon and Lee[9], and the technique described in a previous chapter. It basically consists of freezing a monomer solution on a cold plate so as to create a system which has monomer molecules between solvent crystals. This monomer matrix is then polymerized using UV radiation and utilizing a UV sensitive photo active catalyst, such as uranyl nitrate. After polymerization the solvent is removed by thawing and a macroporous film results. The technique has the additional advantage over the previously described free radical polymerization method in that it could conceivably be used to produce large areas of membrane suitable for haemodialysis, unlike the previously described method.

In this chapter the synthesis and characterization of the films to determine their water contents, permeability to solute molecules, surface properties and other characteristics are described. In a later chapter, 9, their interaction with blood is dealt with.

6.2 RANGE OF MONOMERS USED AND POLYMERS MADE

(a) Monomers

A wide range of formulations were used to make macroporous hydrogel membranes of different compositions and physical characteristics. The monomers used in this study are listed in Table 47, and the general results found with each one are discussed on the following pages. A complete list of membranes is given in Table 48.

TABLE 47

Monomers used in making Macroporous Hydrogel Membranes

(1) 2-Hydroxy ethyl methacrylate (HEMA)
(2) Methacrylic acid (MAA)
(3) Acrylic acid (AA)
(4) Ethylene dimethacrylate (EDM)
(5) Acrylamide (ACR)
(6) NN Methylene bis acrylamide (NNMBA)

The monomers given in Table 47 were chosen as several of them are well known for the manufacture of biomaterials. Additionally, several of them have also been used successfully by Haldon and Lee.[9]

(b) Macroporous Polymers Synthesized

A wide range of macroporous hydrogel membranes were synthesized: Table 48. The materials were made to investigate the effects listed below.

1) The effect of the percentage of crosslinking agent in the initial composition on polymer structure, M1-3, as Haldon and Lee

observe that it is the hydrophobicity of the ethylene dimethacrylate (EDM) which leads to the separation of monomer and solvent phases on freezing and hence the macroporous structure.

2) In line with (1) the effect of using a more hydrophilic cross-linking agent, NN' methylene bis acrylamide, (NN'MBA), on final polymer structure was examined:- M6 against M7, membranes which differ only in crosslinker composition.

3) The range of monomers which can be used for making hydrogel macroporous membranes, extending the number used by Haldon and Lee to acrylamide and acrylic acid. Also to examine the structures of these materials and find what differences are caused by differences in composition.

4) The examination of the differences that the technique of preparation of macroporous hydrogels causes ie the thin film method as opposed to the method of pouring the solution onto a very cold surface and polymerizing the cast sheet.

5) The examination of the permeability and biocompatibility of these materials in comparison with the homogeneous hydrogels and known biomaterials such as cuprophane, to evaluate the possible use of macroporous hydrogels as biomaterials.

TABLE 48

Hydrogel Membranes prepared by "Freezing Solvent" Technique

	Composition	Initial Molar Ratio	Monomer Solvent* Ratio	Solvent* Ratio	EWC
M1	HEMA:EDM	100:4	50:50	1/4	51.0%
M2	" "	100:2	50:50	1/4	53.5%
M3	" "	100:1	50:50	1/4	51.5%
M4	AA:EDM	100:8.5	70:30	1/4	52.5%
M5	" "	100:8.5	60:40	1/4	58.5%
M6	" "	100:8.5	50:50	1/4	62.0%
M7	AA:NNMBA	100:8.7	50:50	1/4	65.0%
M8	ACR:EDM	100:8.6	50:50	1/4	77.0%
M9	" "	100:5	60:40	1/2	67.5%
M10	" "	95:5	60:40	1/4	61.0%
M11	ACR:AA:EDM	50:50:8.5	60:40	1/4	46.5%
M12	HEMA:MAA:EDM	75:25:8.5	50:50	1/2	42.0%
M13	AA:HEMA:EDM	75:25:8.5	50:50	1/4	56.0%

* Solvent ethylene glycol:H_2O (Ratio by volume)

6.3 PHASE DIAGRAMS

(a) Introduction

Phase diagrams of the various systems investigated in this study were used for the reasons listed below:

1) To find the concentrations of EDM, which can be tolerated by a solution without phase separation occurring. The level of EDM which can be attained is important as it is the presence of EDM that determines the porosity and strength of the material.
2) To determine the effect of the solvent ethylene glycol on the solubility of the crosslinking agent, EDM, in the monomer/solvent system, as the presence of ethylene glycol allows more EDM to be dissolved, ie it is a better solvent than water for this monomer.

It should also be noted that temperature has an important effect on phase separation in the monomer solvent systems. This effect has not been investigated in this study however as although the solubility of the systems can be increased by raising the temperature, the time taken to freeze will also increase. As a consequence of this last effect a hot solution would undergo phase separation before freezing and photopolymerization which would result in an inferior film being produced, ie an inhomogeous one.

The phase diagrams made are listed in Table 49, the figures are shown in the form of three co-ordinate graphs but as most possible compositions are of no interest, (as phase separation occurs) the figures are shown in a truncated form with the area of interest highlighted.

TABLE 49

Key to Phase Diagram Figures

Figure 49 (a) HEMA:EDM:H_2O

HEMA:EDM:H_2O:EG(4:1)*

(b) AA:EDM:H_2O

Figure 49 (a) HEMA/AA(50:50):EDM:H_2O

(b) ACR:EDM:(H_2O)

ACR:EDM:(H_2O:EG)(4:1)*

Figure 50 MAA/EDM:EG:H_2O

Solvents used ethylene glycol (EG) and water

* The ratios of H_2O:EG are by volume

(b) Hydroxy ethyl methacrylate solutions

(HEMA:EDM:H_2O and HEMA:EDM:(H_2O:EG)) Figure 48A

From the phase diagram of this system it can be seen that the concentration of EDM that can be tolerated by the system increases as the concentration of HEMA increases, as might be expected. It can also be seen that the addition of ethylene glycol allows the concentration of EDM that can be tolerated, without phase separation, to increase.

These effects are shown quantitatively in Table 50.

FIGURES 48A and B

Phase Diagrams of the HEMA/EDM/(H_2O:EG) System and the AA/EDM/H_2O System

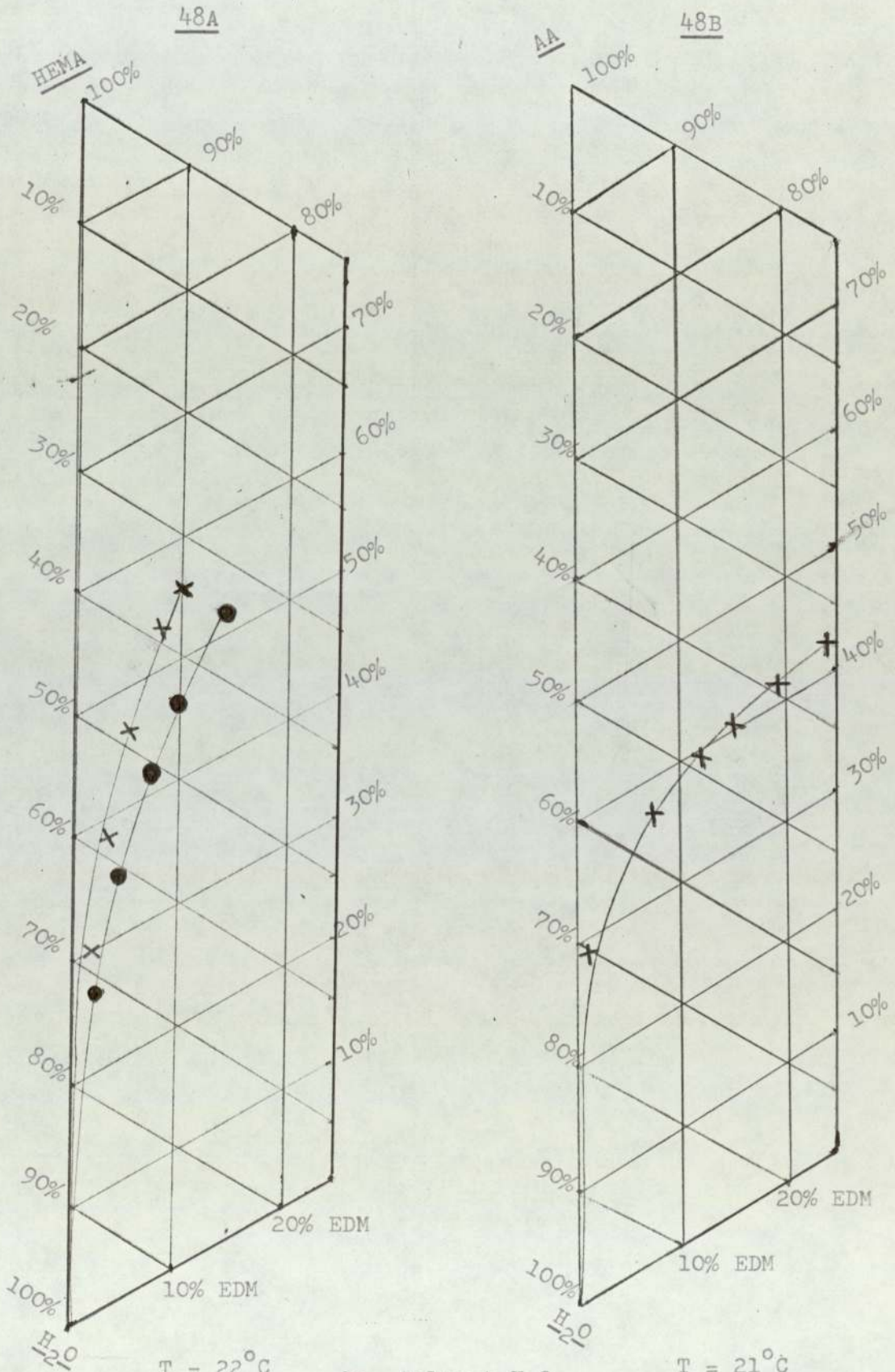


TABLE 50

Maximum concentrations of EDM which can be tolerated by the system, without phase separation occurring, at various monomer:solvent ratios (HEMA:EDM)

Monomer:Solvent Ratio	Solvent Ratio H_2O:EG	Lowest Molar Ratio obtainable HEMA:EDM	Temperature
30:70	100:0	96.6:3.4	22°C
40:60	100:0	95.0:5.0	"
50:50	100:0	93.6:6.4	"
60:40	100:0	92.0:8.0	"
30:70	80:20	95.5:4.5	"
40:60	80:20	94.1:5.9	"
50:50	80:20	90.0:10.0	"
60:40	80:20	86.9:13.1	"

(c) Acrylic Acid Solutions (AA:EDM:H_2O (Figure 48B))

In the case of this system it was found that the concentration of EDM, which could be tolerated by the system increased dramatically as the concentration of AA rose. From the phase diagram the following table of results for maximum concentrations of EDM in the system can be made, Table 51.

TABLE 51

Maximum concentration of ethylene dimethacrylate which can be added to the system without phase separation occurring at different monomer:solvent ratios

Monomer:Solvent Ratio	Solvent	Lowest Molar Ratio obtainable AA:EDM	Temperature
30:70	H_2O	98.1:1.9	21°C
40:60	"	95.1:4.9	"
50:50	"	91.4:8.6	"
60:40	"	85.8:14.2	"

(d) Mixed Hydroxy ethyl methacrylate:acrylic acid systems

(HEMA/AA(50/50):EDM:H_2O) (Figure 49A)

Since the systems HEMA:EDM:H_2O and AA:EDM:H_2O have been investigated it was decided to look at an intermediate system, that of (HEMA/AA):EDM:H_2O, in which HEMA and AA were equimolar. The results from the phase diagram are shown in Table 52.

TABLE 52

Maximum concentrations of EDM which can be tolerated by the system, without phase separation occurring, at various monomer:solvent ratios

Monomer:Solvent Ratio	Solvent	Lowest Molar Ratio Obtained Monomers:EDM
70:30	H_2O	96.5:3.5
60:40	"	93.3:6.7
50:50	"	90.0:10.0

Thus it would appear, comparing the results with those of Tables 50 and 51, that mixed monomers can give the EDM a higher solubility.

(e) Acrylamide Solutions

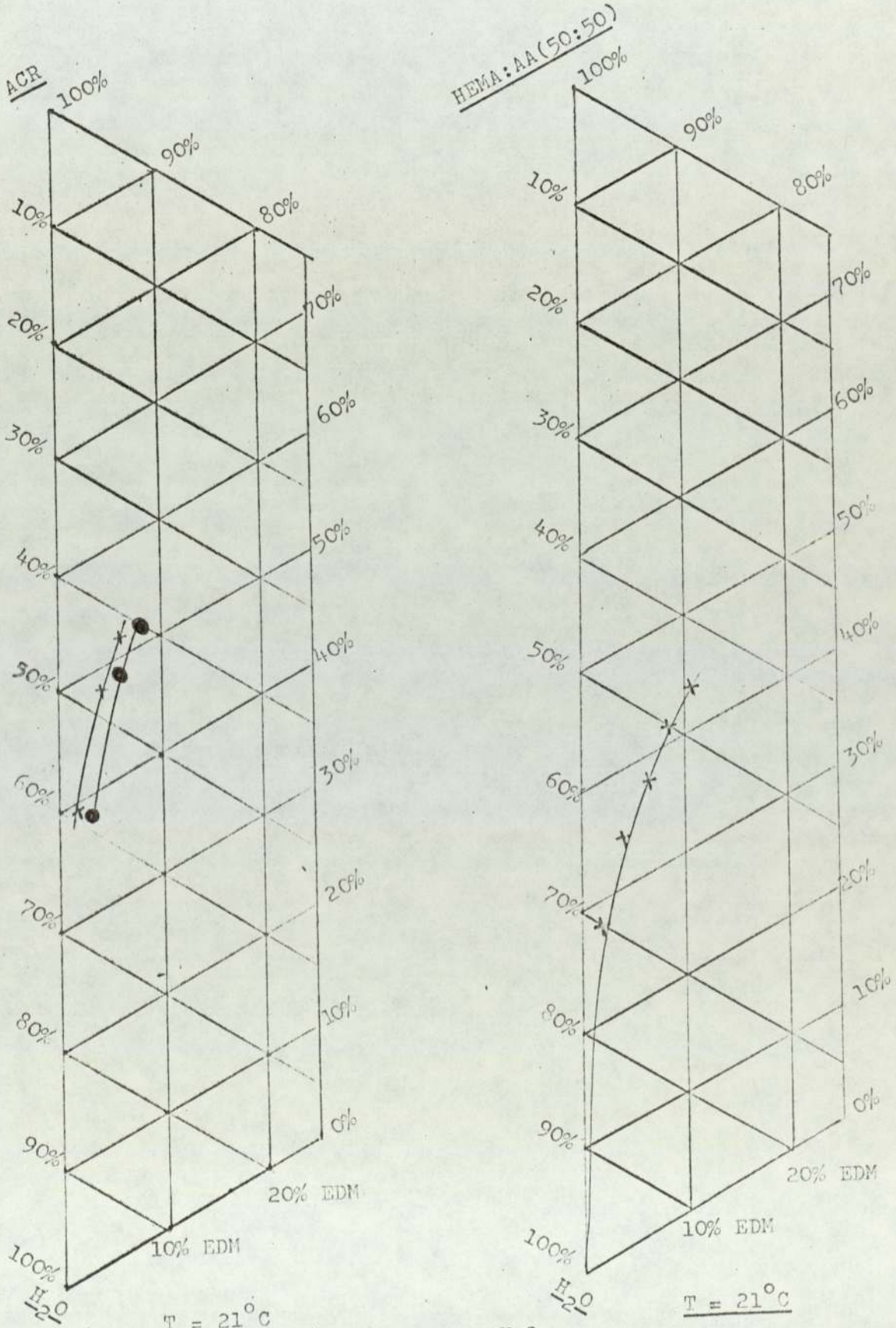
(ACR:EDM:H_2O, ACR:EDM:(H_2O:EG)) (Figure 49B)

This phase diagram which is rather limited as acrylamide is not very soluble in water or the ethylene glycol water mixture, at room temperature.

The diagram shows that acrylamide:water solution cannot dissolve a high concentration of EDM without phase separation taking

FIGURE 49(A)(B)

Phase Diagrams of the ACR/EDM/(H_2O:EG) System and the HEMA:AA(50:50)/EDM/H_2O System



place. It also shows that the addition of ethylene glycol as co-solvent does not help matters much only increasing EDM's solubility by a small amount. This low solubility is to be expected as more hydrophilic compounds will not have a good effect in altering the solubility of hydrophobic EDM.

Thus if it is desired to make strong macroporous membranes or beads containing acrylamide it may be necessary to add a comonomer so that sufficient EDM can be added.

(f) Methacrylic Acid Solutions

(MAA/EDM:H_2O:EG) (Figure 50)

This system is different to the others in that it shows not how much EDM can be tolerated in a system without phase separation occurring, but how bad MAA is as comonomer in these systems as it requires large amounts of ethylene glycol as a co-solvent with water to form a homogeneous solution. From the phase diagram, (Figure 50), the following table, (53), of results to illustrate this effect is shown.

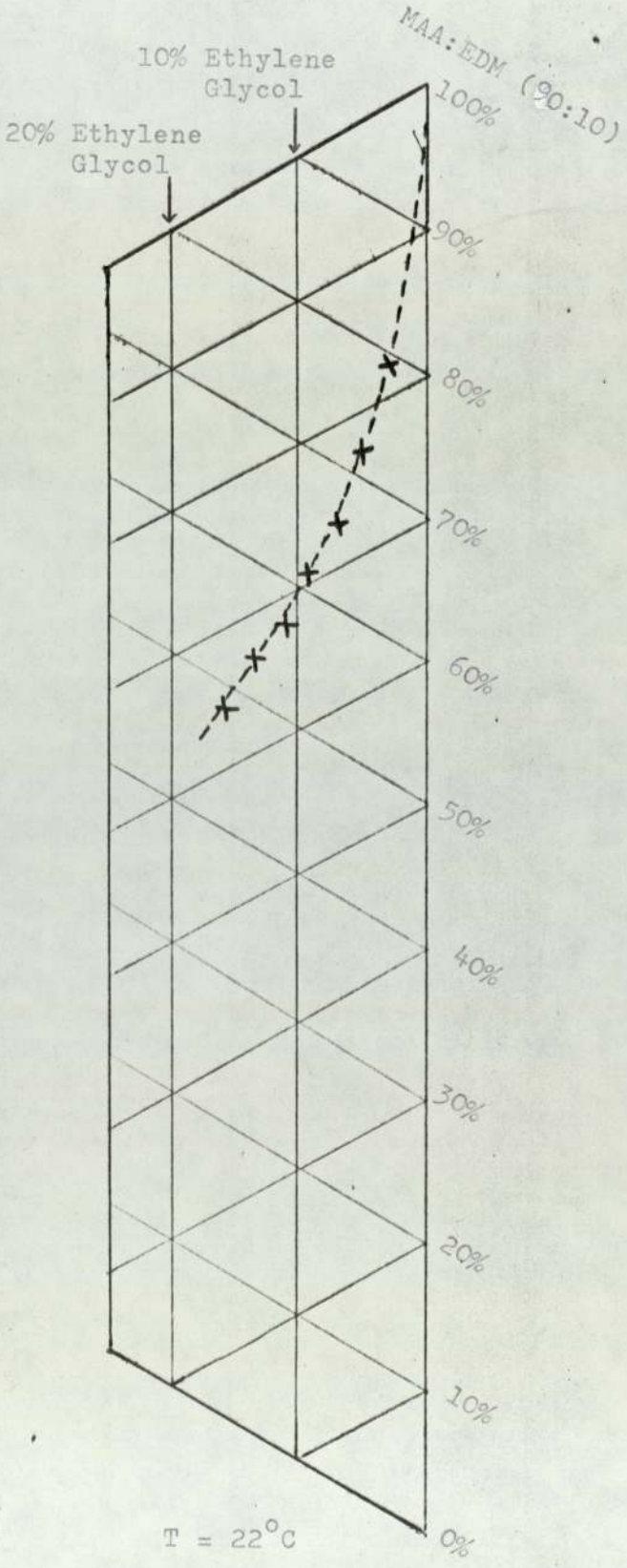
TABLE 53

Solvent Ratio of Ethylene glycol to water needed to make a homogeneous solution at various monomer to solvent ratios

Monomer:Solvent Ratio	Molar Ratio of Monomers MAA:EDM	Lowest weight ratio of EG:H_2O obtainable	Temperature
70:30	90:10	19:81	21°C
60:40	90:10	24:76	"
50:50	90:10	30:70	"

FIGURE 50

Phase diagram of the (MAA/EDM):H_2O:EG System



Thus to get a solution with large amounts of EDM present it is necessary to have a solvent mixture with a large amount of ethylene glycol.

Since it is undesirable to have a formulation with a great deal of ethylene glycol present, (as the solution's freezing point is decreased), hence an increased danger of macrophase separation leading to an inhomogeneous membrane results. For polymers in which acidic groups were to be added, acrylic acid instead of methacrylic acid was used. Acrylic acid as has been shown is more water soluble.

6.4 THE EQUILIBRIUM WATER CONTENTS OF MACROPOROUS HYDROGELS

(a) Introduction

As with homogeneous hydrogels the EWC's of macroporous hydrogels are important in determining the strength and permeability of the material together with other characteristics. For this reason they have been measured and the results are given in Table 48.

The main factors which have been found to determine the EWC of macroporous hydrogels are the following: (1) the initial monomer:solvent ratio, (2) the crosslinking density, and (3) the hydrophilicity of the polymer backbone. These factors are discussed individually on the following pages.

(b) The Initial Monomer:Solvent Ratio and EWC

The effect of the initial monomer:solvent ratio on final EWC is illustrated for two copolymer series and several individual hydrogel copolymers in Figure 51. The results on which the figure is based are shown in Table 48. It should be noted that the HEMA:EDM series were prepared by the author during a previous project.(209)

It can be seen from the table, (54), and Figure 51 that as the initial monomer:solvent ratio is decreased the EWC of the resultant macroporous hydrogel increased, as would be expected.

More importantly, it shows that as the hydrophilicity of the polymer component is increased the initial monomer solvent ratio becomes a less important factor in determining the EWC. This can

FIGURE 51

The Effect of the Initial Monomer:Solvent Ratio on the Final EWC of the Macroporous Hydrogels

Solvent - Ethylene glycol:H_2O, (1:4) by volume

(EDM) - 8.5% (mole %) of all membranes but HEMA series)

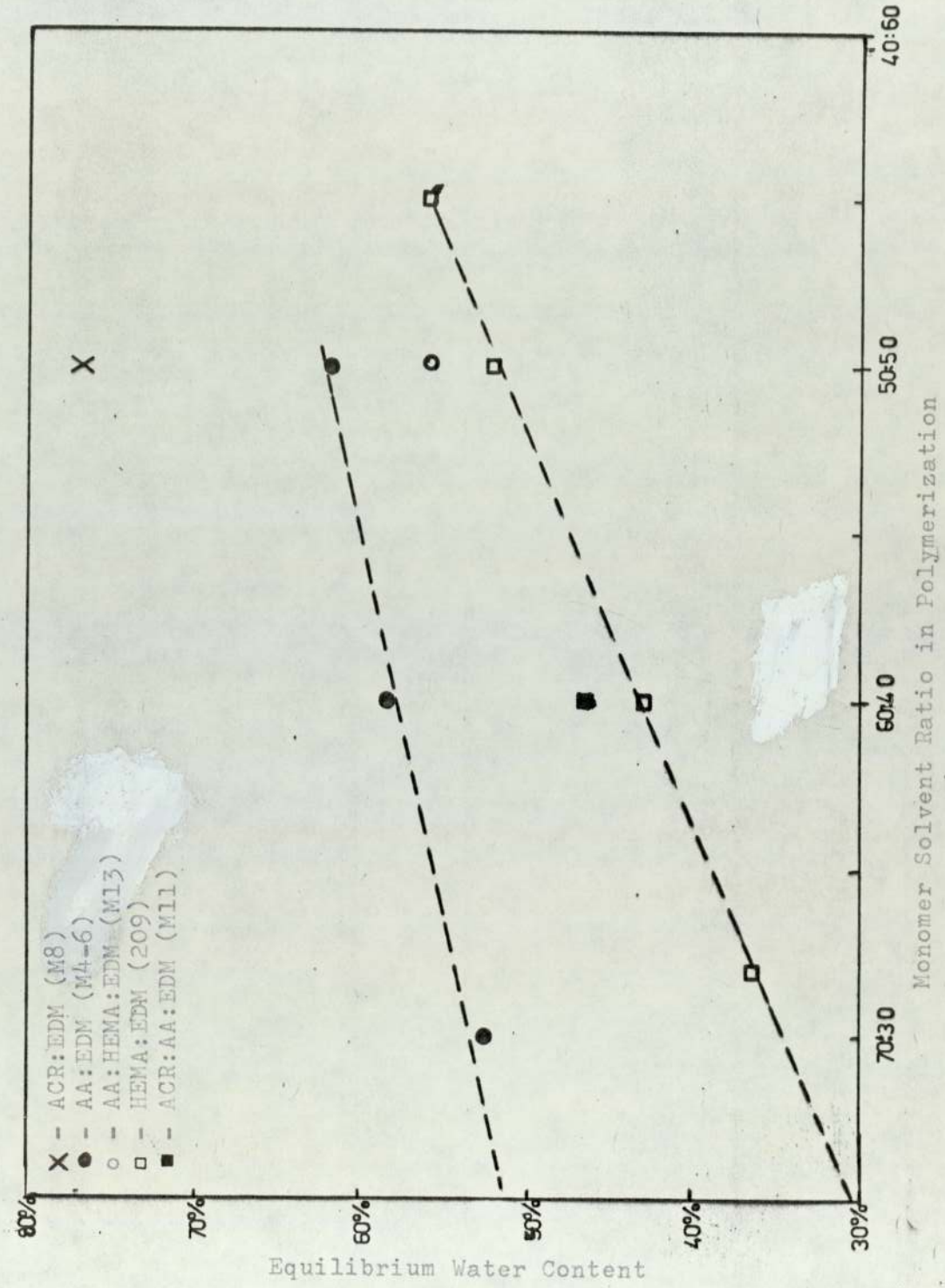


TABLE 54

The Macroporous Membranes and the Effect of Monomer:Solvent Ratio on EWC
(Solvent-Ethylene glycol:H_2O, (1:4 by volume)

Membrane	Composition	Molar Ratio	Monomer:Solvent	EWC
-	HEMA:EDM	90:10	68:32	36.5%
-	" "	90:10	60:40	43%
-	" "	90:10	50:50	52%
-	" "	90:10	45:55	56%
M6	AA:EDM	92:8	50:50	62%
M5	" "	92:8	60:40	58.5%
M4	" "	92:8	70:30	52.5%
M13	HEMA:AA:EDM	23:69:8	50:50	56%
M11	ACR:AA:EDM	46:46:8	60:40	46.5%
M8	ACR:EDM	92:8	50:50	77%

be observed by comparing the results for the HEMA and AA copolymers; the gradient on the line drawn through values for the EWC's of the various macroporous AA:EDM polymers, is much less steep than the line through the values for the less hydrophilic HEMA:EDM macroporous hydrogels.

(c) Crosslink Density and EWC

The effect of crosslink density on the EWC of bulk polymerized hydrogel polymers has been illustrated in the previous chapter for various monomers; the best illustration of this was given in Chapter 5, Figure 34, which showed the effect of increasing (EDM)

on the EWC of a series of AA:EDM polymers. This shows the importance of the amount of crosslinking agent in the polymer on EWC for such highly hydrophilic polymers such as poly (acrylic acid).

However, as can be seen from the EWC values for the HEMA:EDM Macroporous hydrogels M1-3, for less hydrophilic polymers, ie crosslinked polyHEMA the amount of crosslinking agent present does not appreciably effect its EWC.

(d) The Hydrophilicity of the Polymer Backbone

Also important as a factor in determining the EWC of the macroporous hydrogels, as it was in the homogeneous hydrogels, is the hydrophilicity of the polymer backbone. The results illustrating this are given graphically on Figure 51.

It can be observed that the more hydrophilic polymers, ie ACR:EDM have the higher water contents. More interestingly the effect of complexing between functional groups is again shown to have an effect of EWC, as can be seen for the ACR:AA macroporous hydrogel which has a much lower EWC than the hydrogels of the AA:EDM series or the ACR:EDM polymer.

6.5 SCANNING ELECTRON MICROSCOPY OF MACROPOROUS MEMBRANES

(a) Introduction

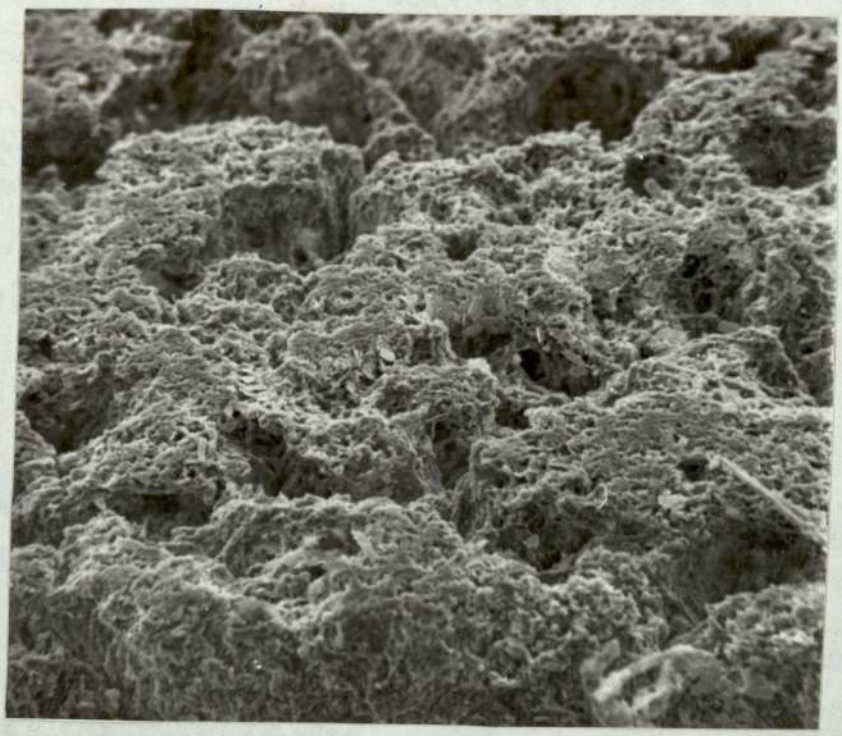
Scanning electron microscopy was found to be of great value in examining the surfaces of the dehydrated membranes, since the photographs yield information on pore sizes and surface rugosity. On the following pages selected photographs are shown to illustrate the various effects seen using this technique. Table 55 gives a key to the various photographs selected.

TABLE 55

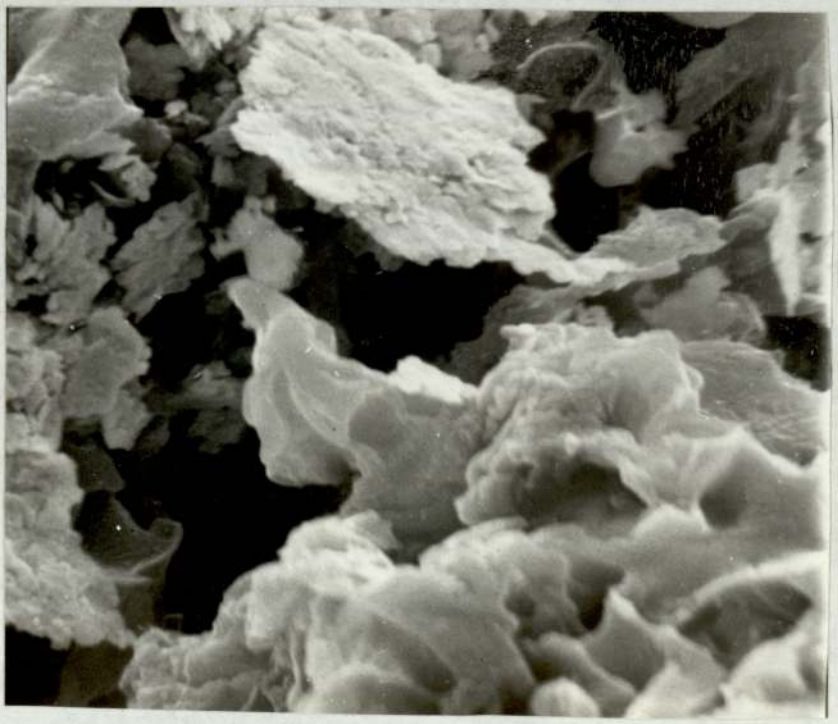
Key to Photographs of Macroporous Hydrogel Membranes

Plate 1	Photograph 1	- Top surface of M5 (x 100)
	Photograph 2	- Top surface of M5 (x 2000)
Plate 2	Photograph 3	- Freezing surface of M5 (x 2000)
	Photograph 4	- Freezing surface of M5 (x 1000)
Plate 3	Photograph 5	- Surface of M1 (x 1000)
	Photograph 6	- Surface of M3 (x 5000)
Plate 4	Photograph 7	- Surface of M7 (x 1000)
	Photograph 8	- Surface of M7 (x 5000)
Plate 5	Photograph 9	- Surface of M6 (x 1000)
	Photograph 10	- Surface of M6 (x 5000)
Plate 6	Photograph 11	- Surface of M8 (x 1000)
	Photograph 12	- Surface of M8 (x 5000)
Plate 7	Photograph 13	- Surface of M12 (x 1000)
	Photograph 14	- Surface of M12 (x 5000)

PLATE I

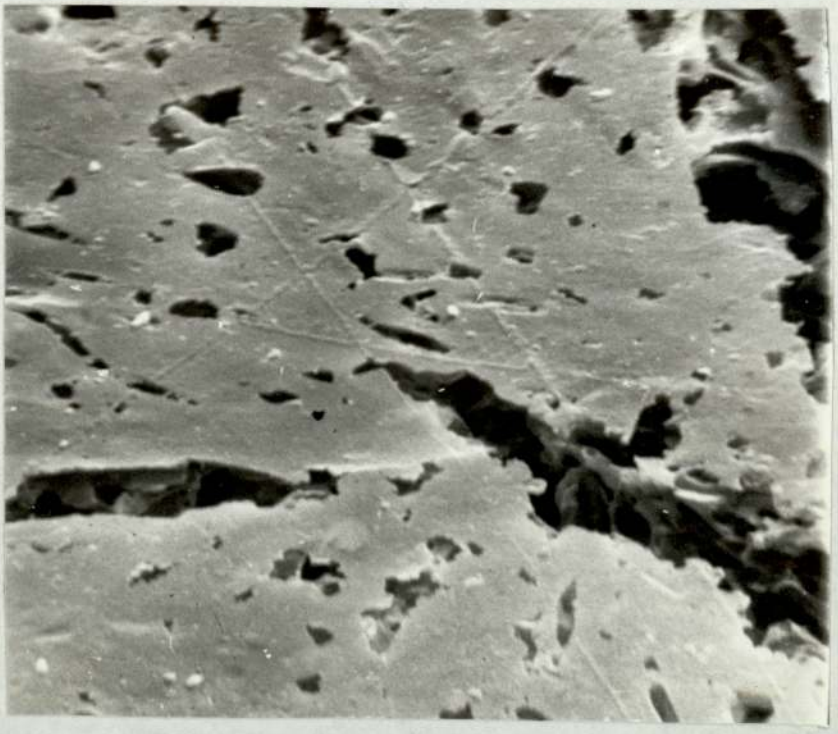


PHOTOGRAPH I

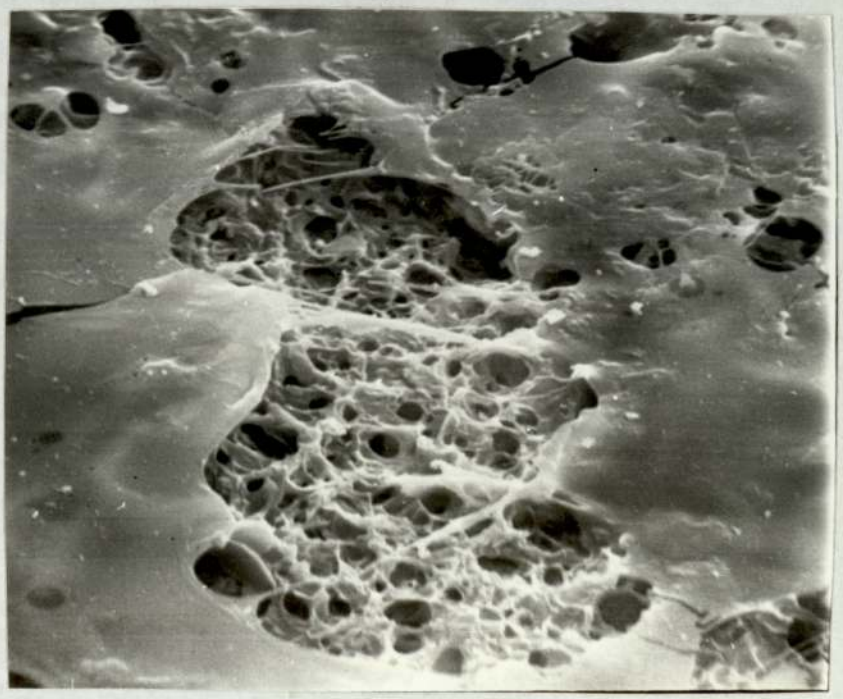


PHOTOGRAPH 2

PLATE 2

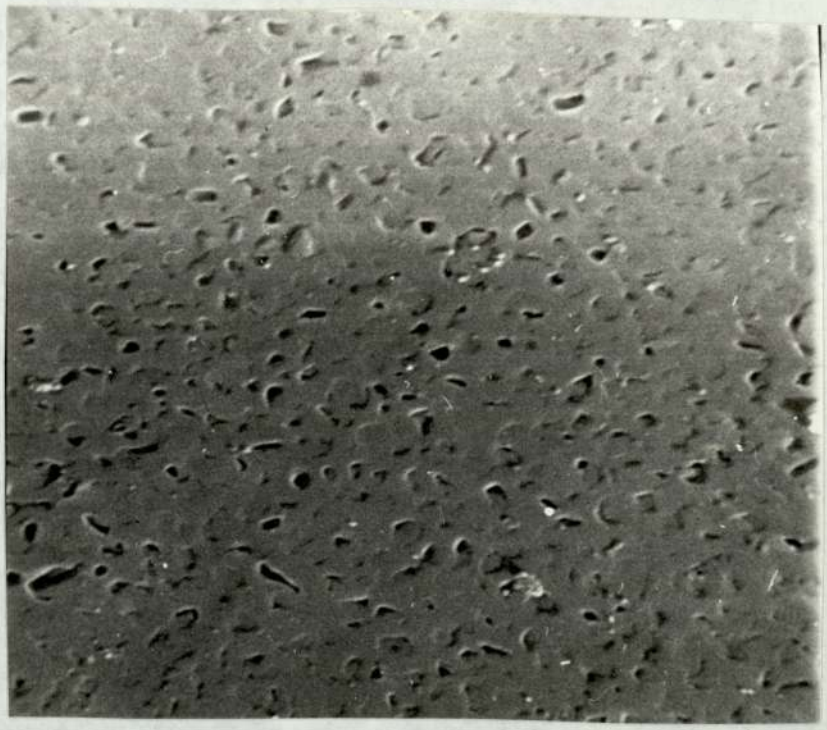


PHOTOGRAPH 3



PHOTOGRAPH 4

PLATE 3



PHOTOGRAPH 5

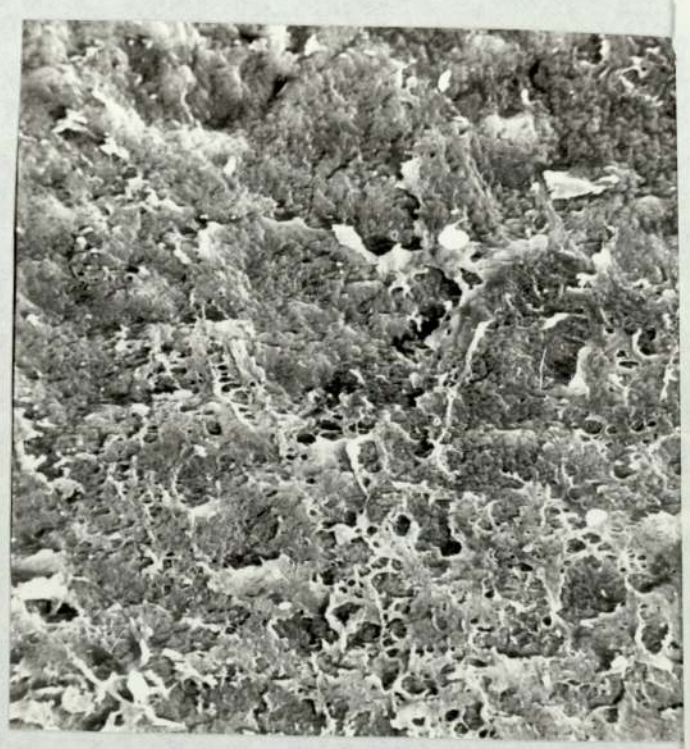
PHOTOGRAPH 6

PLATE 4

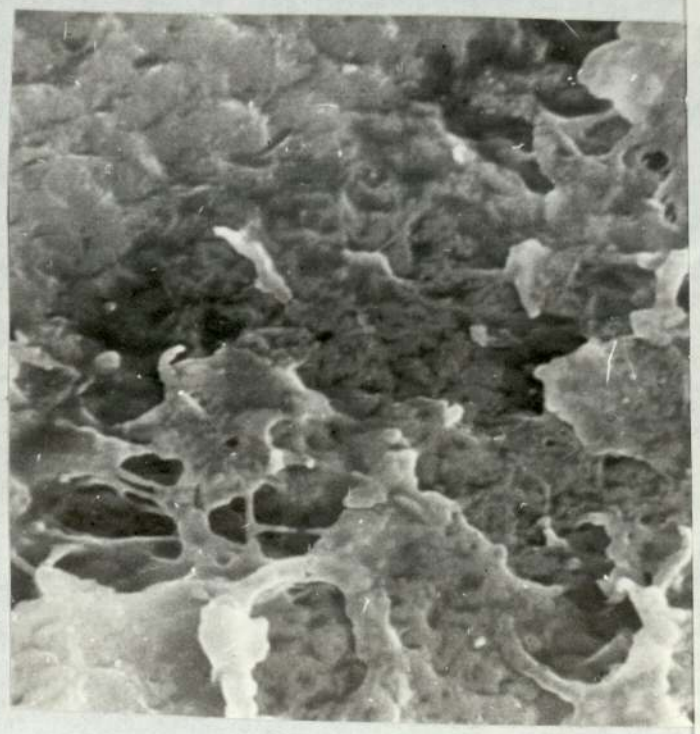
PHOTOGRAPH 7

PHOTOGRAPH 8

PLATE 6

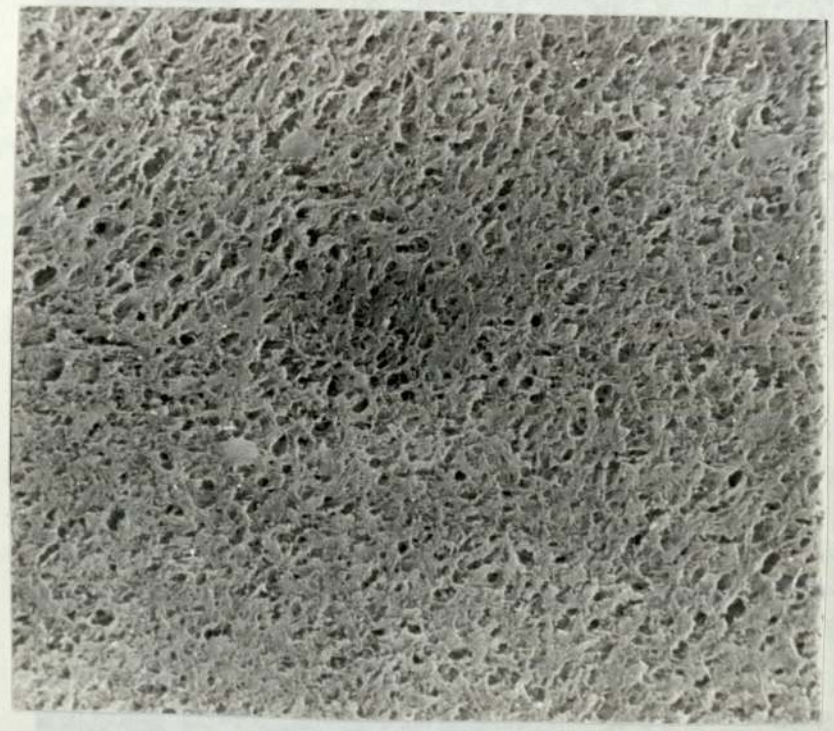


PHOTOGRAPH 11

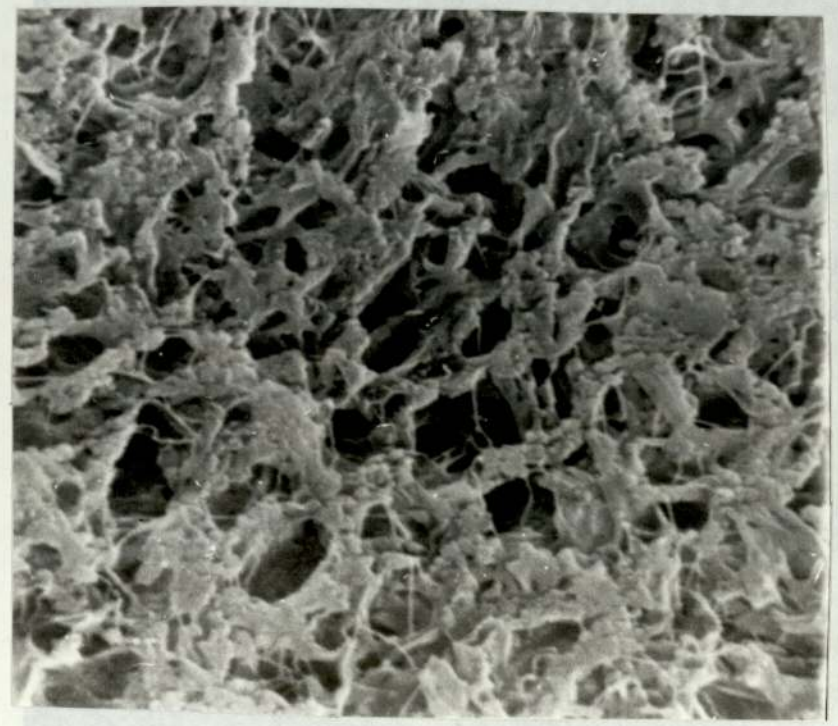


PHOTOGRAPH 12

PLATE 5

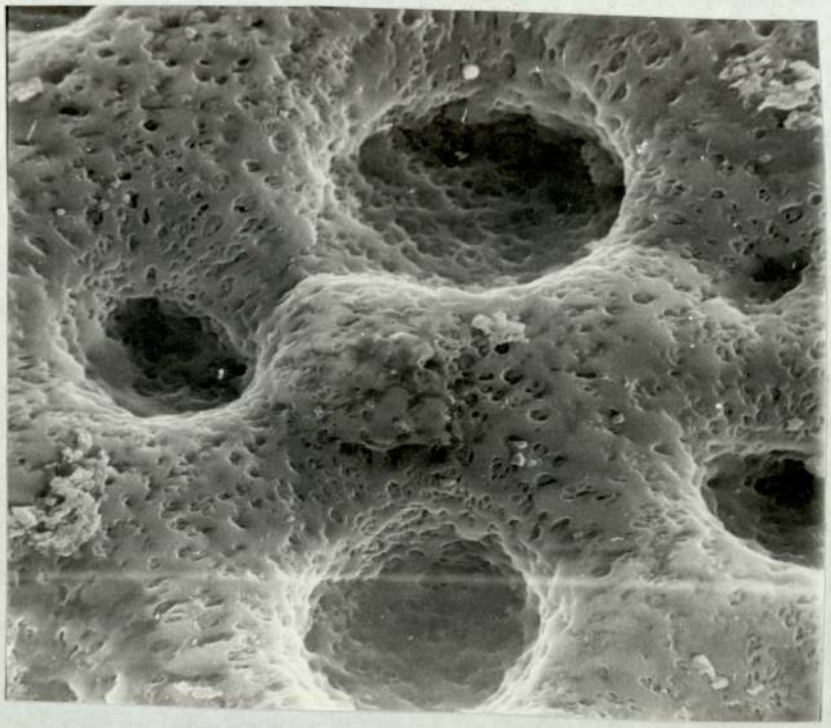


PHOTOGRAPH 9

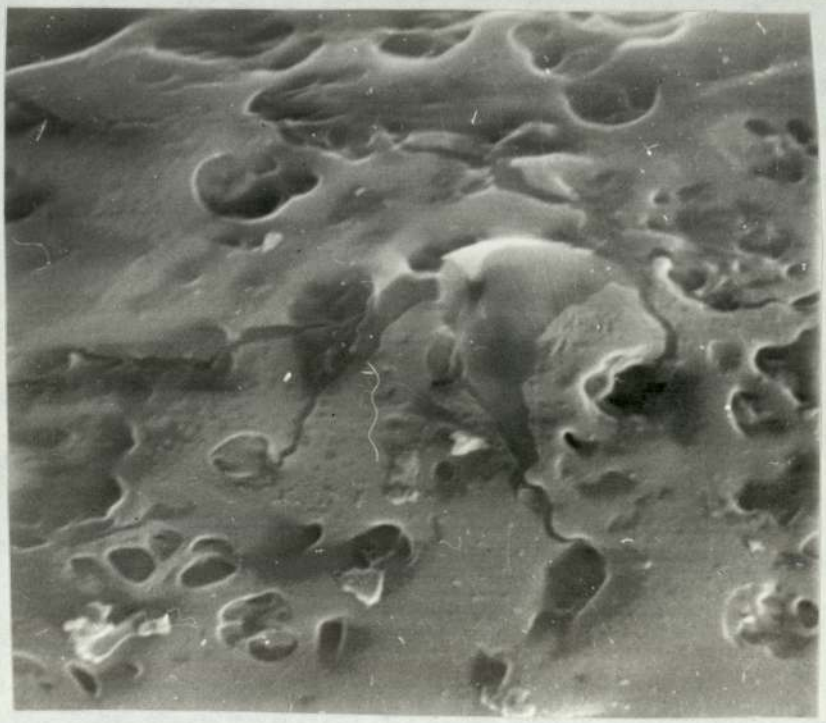


PHOTOGRAPH 10

PLATE 7



PHOTOGRAPH 13



PHOTOGRAPH 14

(b) Discussion of the Results of the Scanning Electron Microscopy of Dehydrated Samples of the Macroporous Hydrogel Membranes

The scanning electron micrograph photographs shown on the previous pages have been chosen to illustrate the various effects of membrane composition and means of manufacture of the film on the final physical nature of the film.

1) The differences between the "freezing" and "top" surfaces of a Macroporous Membrane:-

This difference for the macroporous membrane made by pouring the monomer/solvent solution over a glass plate cooled to dry ice temperatures and then polymerized, (M5) is shown in photographs 1, 2, 3 and 4.

It is found that the membrane surface which was in contact with the freezing glass plate, photographs 3 and 4, was relatively smooth, as would be expected, but that there was often a thin, non-porous, film lying on top of a much more porous interior structure which is visible through gaps in the thin film's structure. The origin of this thin homogeneous film could perhaps be attributed to extremely fast freezing of the composition, without phase separation, occurring initially on the cold glass plate. Then, the remaining solution might freeze at a slower rate with phase separation of the monomers and solvent occurring, to give the porous underlying structure, on polymerization. The patchy nature of the thin film is almost certainly due to its partial adherence to the glass plate it is polymerized on.

The top surfaces of the macroporous membranes are shown in

photographs 1 and 2, (these are the surfaces in contact with the air not the glass plate). It can be seen that the structure of the surface is rough and highly porous.

2) The effect of (EDM) in the formulation on polymer structure

This is best illustrated by comparing photographs 4, 5 and 6, which show respectively polymers containing 8.5%, 4.0% and 1.0% of EDM. It can be seen that there is a decrease in the porosity until at 1% no porosity at all is observable. This result confirms the observation of Haldon and Lee.(9)

3) The effect of using a more hydrophilic crosslinking agent

Photographs 7 and 8 illustrate the effect on structure due to changing from a formulation with a hydrophobic crosslinking agent to one with a hydrophilic one, NN'MBA, Membrane M7, illustrated in the photographs. These pictures should be compared with the photographs on the following page, 9 and 10, which show a similar polymer but one in which EDM is the crosslinking agent. It can be seen that this one is much more porous.

Thus the observation that Haldon and Lee make that a hydrophobic crosslinking agent is necessary for good phase separation on freezing and hence macroporosity on polymerizing is borne out; as membranes with a hydrophilic crosslinking agent are not very porous.

4) Other effects of changing polymer structure by changing the Monomer formulation

The last photographs show some unusual effects, eg the acrylic acid/ethylene dimethacrylate membrane has a very porous structure but presents a very fibrous structure at the surface:- photographs 9 and 10. It is difficult to see how such a surface, on surface rugosity lines alone, could be compatible with blood.

Photographs 11 and 12 show an acrylamide/ethylene dimethacrylate membrane's surface. Again a very friable surface is uncovered.

Finally photographs 13 and 14 show the porous structure of a HEMA:MAA:EDM which has a very unusual surface structure with large depressions as well as macropores. The depressions are about 50 microns across.

Clearly a wealth of structures can be created by changing the composition of the formulation used to make the membrane, or the technique used to make it.

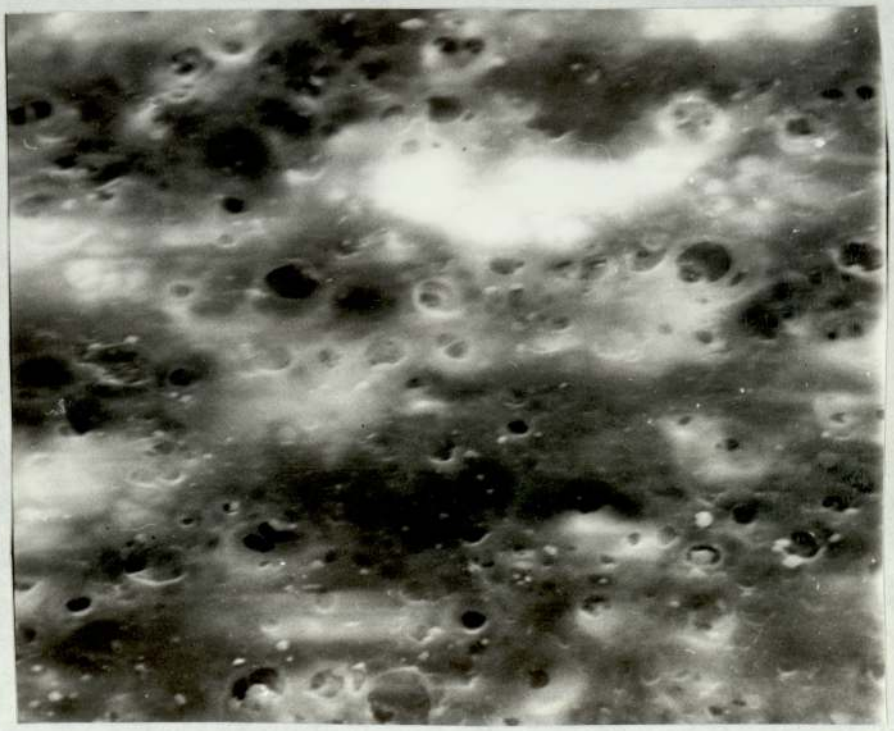
5) Comparison membranes

In addition to the macroporous hydrogel membranes examined, certain membranes of comparison materials used in biomedical applications and more specifically in tests described in this chapter such as permeability tests, were examined by scanning electron microscopy.

The photographs on the following page show firstly the Cuprophane membrane at magnification x1000, and a porous polyurethane at x1000. The Rhone-Poulenc membrane was also examined at up to

PLATE 8

PHOTOGRAPH 15
Surface of Cuprophane (X1000)



PHOTOGRAPH 16
Surface of Porous Polyurethane (X1000)
(Porvair)

x5000 magnification, but as it was featureless it is not shown.

The photographs on Plate 8 show that the Cuprophane membrane has a very smooth surface and in the case of the Porvair polyurethane membrane, the pores formed by the micronised salt particles that were present in the polymer before being dissolved away can clearly be seen.

6.6 ELEMENTAL ANALYSIS

This technique could not be used to determine the monomer ratios in the polyners for most of the materials made, as the differences in the ratios of carbon, hydrogen and oxygen in their constituent monomers are not great. It was however applied, with value, to the two polymers made, which had been prepared with nitrogen containing monomers, thus giving some information on the reactivity of these monomers in this system. The results are given below:-

(a) HEMA:NVP:EDM

A terpolymer of this composition had been prepared during an earlier study[206] and was found to be extremely weak; it was analysed by C,H,N analysis in order to determine how much NVP had polymerized. Unfortunately it proved impossible to calculate accurately on a mole % basis how much NVP had copolymerized, but by comparing the per cent of nitrogen in the C,H,N result that was found and what would be expected on the basis of the initial molar ratios of the monomers. The results are shown in the following table, 56.

From the table it can be seen that very little of the NVP monomer has been incorporated into the terpolymer; so NVP is an unreactive monomer in this polymerization system.

TABLE 56

C,H,N Results for a HEMA:NVP:EDM Terpolymer

	% Expected on basis of initial molar ratios	% Found	
		Result 1	Result 2
C	59.9	54.5	54.9
H	7.8	7.5	7.4
N	5.0	0.5	0.8

(b) Acrylamide:Ethylene Dimethacrylate (M11)

The C,H,N analysis of this material gives percentages of C,H,N almost exactly the same as those that would be expected on the basis of the initial molar ratios of the monomers in the formulation, as shown below:-

Monomer %'s on basis of C,H,N results	ACR = 91.5 $\pm$ 0.5 mole % EDM = 8.5 $\pm$ 0.5 mole %
Initial monomer molar ratios	ACR = 92.1 mole % EDM = 7.9 mole %

6.7 SURFACE HYDROPHILICITIES OF MACROPOROUS HYDROGELS

Since, in the case of homogeneous hydrogels, relationships have been found between surface hydrophilicity and composition, it was decided to measure the Hamilton contact angle in the case of some macroporous hydrogels. The results of these experiments are shown in Table 57.

TABLE 57

Hamilton Contact Angles found for Macroporous Membranes

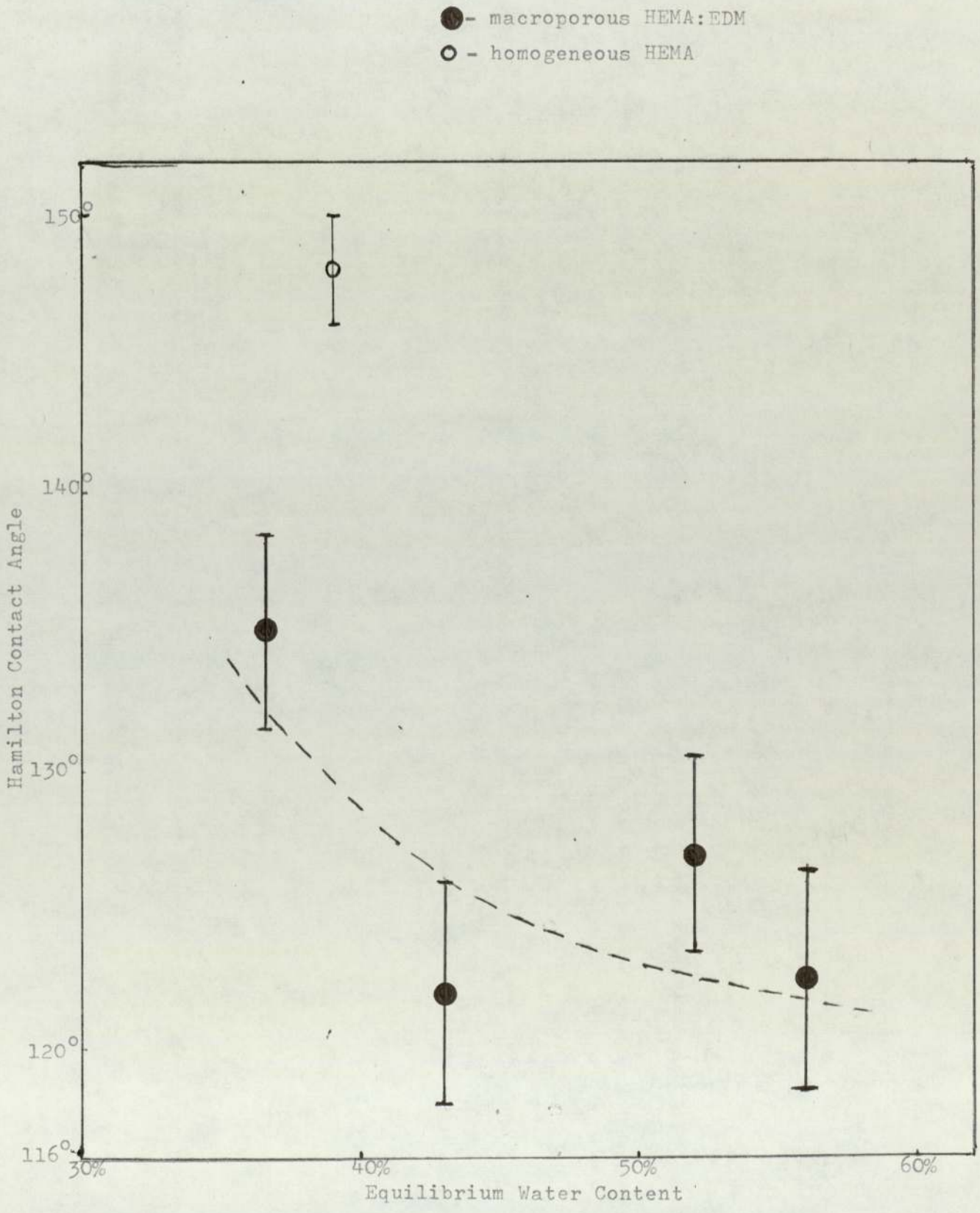
Composition	EWC	Hamilton Contact Angle
HEMA:EDM	56.0%	$122.5 \pm 4^{\circ}$
" "	52.0%	$127.0 \pm 3.5^{\circ}$
" "	43.0%	$122 \pm 4^{\circ}$
" "	36.5%	$135 \pm 3.5^{\circ}$
ACR:EDM	77.0%	$153.5 \pm 3^{\circ}$

The Hamilton contact angles for the HEMA:EDM series have been plotted against their EWC's and the result of this is shown in Figure 52. For comparison the result for a homogeneous polyHEMA membrane is included.

It can be seen from the figure that the surface hydrophilicities of the macroporous materials are very much lower than that of the homogeneous parent material; this effect can be attributed to much of the surface being composed of areas of water, (the macropores), which will not contribute much to the overall polarity of the surface, hence reducing the Hamilton contact angle. However,

FIGURE 52

The Hamilton Contact Angle of Macroporous HEMA Hydrogel Membranes set against that of a PolyHEMA Membrane (Homogeneous)



caution is required when making this interpretation as the surfaces of the macroporous membranes are very rough compared to the homogeneous materials, and it is known that surface roughness effects contact angle measurements.

Due to doubt about the validity of this technique with regard to its application on these materials lead to it only being applied to those macroporous hydrogels for which blood clotting times were available. This was done to provide further information as to the possible relationship between blood clotting time and surface hydrophilicity. The relevant results in this connection are discussed in Chapter 9

6.8 PERMEABILITY OF MACROPOROUS HYDROGELS TO A LARGE SOLUTE MOLECULE

The permeability of the macroporous hydrogel membranes has been investigated using the permeability cell and general technique given in Chapter 4. As described in Chapter 4 the permeant chosen was bromosulphaphalein BSP. Two macroporous hydrogels made as part of a previous study[209] were compared with various other materials, some of which have a biomedical application already in the artificial kidney field.

The membranes, that the macroporous hydrogel membranes were compared with, were Cuprophane and the new Rhone Poulenc membrane, (mentioned in Chapter 3), the former being an important artificial kidney membrane, and the latter a more experimental membrane for clearing blood toxins. Other membranes than the macroporous hydrogels were compared with were two high water content homogeneous hydrogel membranes prepared previously by other workers, and a porous polyurethane membrane, (the physical structure and means of manufacture of which has already been described in this chapter).

The physical characteristics of the membranes, both the comparison and macroporous hydrogel are shown in Table 58.

The method described in the experimental techniques section was used, and at least two experiments were tried on each membrane; the worst materials from the point of view of reproducibility were the homogeneous high water content hydrogels, which were weak and of variable thickness.

TABLE 58

Physical Characteristics of the Test Membranes

	Material	EWC	Average Hydrated Thickness
(1)	Cuprophane	50.5 ± 0.5%	1.8×10^{-2} mm
(2)	Rhone-Poulenc Membrane	59.5 ± 0.5%	2.5×10^{-2} mm
(3)	Porous polyurethane	71.0 ± 0.5%	3.8×10^{-1} mm
(4)	HPA-NVP-ST(47.5:47.5:5)	74.5%	1.6×10^{-2} mm
(5)	HPA-NVP-ST(15:75:10)	78.5%	1.2×10^{-2} mm
(6)	HEMA:EDM (Macroporous)	52%	2.0×10^{-1} mm
(7)	HEMA:EDM (Macroporous)	56%	1.9×10^{-1} mm

4 and 5 are the high water content homogeneous hydrogels

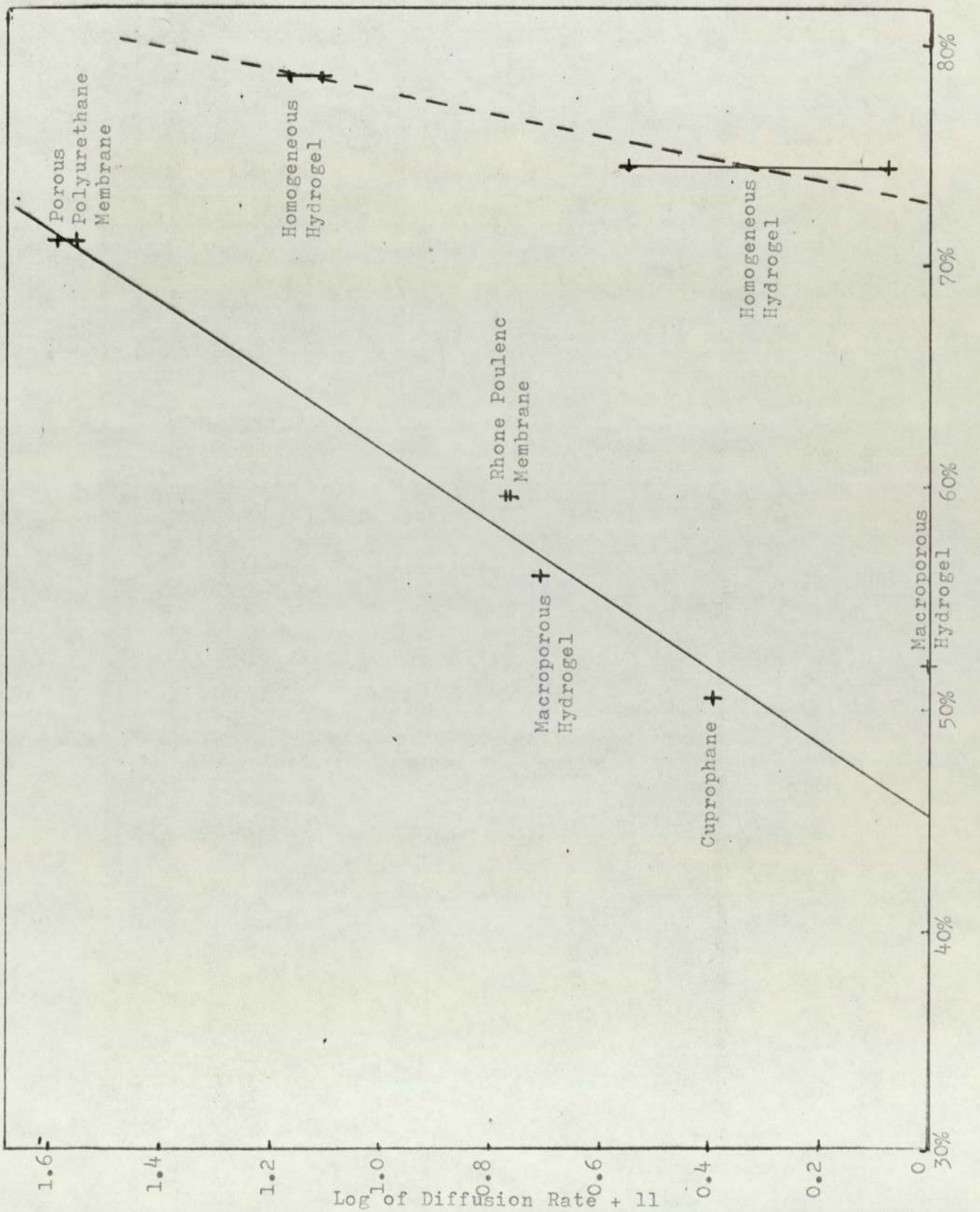
6 and 7 are the macroporous hydrogels

The results of the experiments are shown graphically in Figure 28, which plots the logarithm of the diffusion rate of BSP through the membranes, (corrected for thickness), against the membrane's equilibrium water content.

As can be seen from the graph the materials split into two distinct groups. Firstly, there are those materials which have pores in their structure, which are much larger than the BSP molecule:- macroporous hydrogels, cuprophane, the Rhone Poulenc membrane and the porous polyurethane membrane. In the case of these materials there is a straight line relationship between the log of their diffusion rates and their EWC's. However, for the homogeneous hydrogels, which do not have discrete pores, a high rate of diffusion of BSP comparable to that found for the macroporous materials is only observed at very high equilibrium water contents. Consequently

FIGURE 53

The Log of the Diffusion Rate of BSP versus EWC for Macroporous hydrogels, Homogeneous hydrogels and Comparison Materials



it can be said with regard to biomedical applications in which a high permeability to large solute/toxin molecules is required that heterogeneous/macroporous hydrogel materials are required, if hydrogels are needed.

6.9 ACRYLAMIDE-TRIOXANE FORMULATIONS

Additionally to the use of monomer solution in water/ethylene glycol solvents attempts were made to use another system, described by Haldon and Lee,[9] to make macroporous hydrogel membranes. This system uses a substance as a solvent which is a solid at room temperature but melts at 64°C, (1,3,5 trioxane).

Various monomers, such as acrylamide and NN' methylene bis acrylamide are quite soluble in 1,3,5 trioxane at high temperatures, thus by adding photocatalysts or UV catalysts to the system, the same basic procedure that was adopted for the monomer solutions in water/ethylene glycol can be used, ie freezing of the monomer solution to form a frozen film with crystals containing monomer at their interstices, followed by irradiation and polymerization.

A large variety of photocatalysts and UV catalysts were used in an attempt to make membranes using acrylamide/trioxane systems:- Table 59.

TABLE 59

Photocatalysts and UV Catalysts used with Acrylamide:Trioxane

1) Photocatalysts Methylene Blue/Sodium p-toluene sulphonate
2) UV catalysts (a) Uranyl nitrate
   (b) Benzoin
   (c) Benzil

In the case of UV catalysts, the standard UV lamp was used and in the case of the photocatalysed systems a 500 watt photoflood bulb was used in a standard lamp fitting.

The polymer formulations had crosslinking agents such as NN Methylene bis acrylamide and ethylene dimethacrylate added to them to create crosslinked systems.

Unfortunately the membranes produced were without exception weak and unusable.

6.10 CONCLUSIONS

The following conslusions were drawn from the work on macroporous membranes:-

(1) That the technique for making macroporous membranes can be applied to a wide variety of monomers.

(2) That macroporous hydrogel membranes are superior in permeability to large molecules compared with homogeneous hydrogel membranes, and are also physically stronger.

(3) That the macroporous hydrogel membranes were superior in strength to cuprophane but inferior compared to the new Rhone-Poulenc polyacrylonitrile membranes.

(4) That the method of making macroporous membranes could not easily be applied to make large amounts of membrane, or membranes of similar thickness to the new experimental haemodialysis membranes such as those manufactured by Rhone-Poulenc.

CHAPTER 7

MACROPOROUS BEADS

7.1 INTRODUCTION

Hydrogel macroporous beads were synthesised and characterized as they have a possible application in haemoperfusion systems as biocompatible adsorbents for various water-soluble substances and as supports for albumin in the removal of protein-bound toxins. The method used to make the beads is essentially a modification of the process of producing the macroporous membranes, and similar formulations to those used to produce the films were utilized.

A complete list of the various types of macroporous hydrogel beads made is given in Table 60.

TABLE 60

Samples of Hydrogel Beads prepared by the Freezing Technique

Composition	Initial Molar Ratio	Monomer Solvent Ratio	Macroporous Membrane (same composition)
S1 HEMA:EDM	100:8.5	50:50	—
S2 HEMA:EDM	100:4	50:50	M1
S3 HEMA:EDM	100:2	50:50	M2
S4 HEMA:EDM	100:1	50:50	M3
S5 AA:EDM	100:8.5	50:50	M6
S6 AA:EDM	100:8.5	60:40	M5
S7 HEMA:MAA:EDM	75:25:8.5	50:50	M12
S8 AA:HEMA:EDM	25:75:8.5	50:50	M13

It can be seen from the Table that much attention has been given again to acrylic acid systems. This was primarily due to the success with formulations containing this monomer.

In this chapter the techniques used to make different sizes

of beads are dealt with first, then the tests used to examine the beads such as scanning electron microscopy and surface area measurements. The most important tests related to the 'end stage' use of materials of this sort, ie adsorption of toxins and blood compatibility are not dealt with here but in Chapter 9 and 10, where these properties are discussed in relation to other materials, mainly other resins such as XAD-2 and XAD-7.

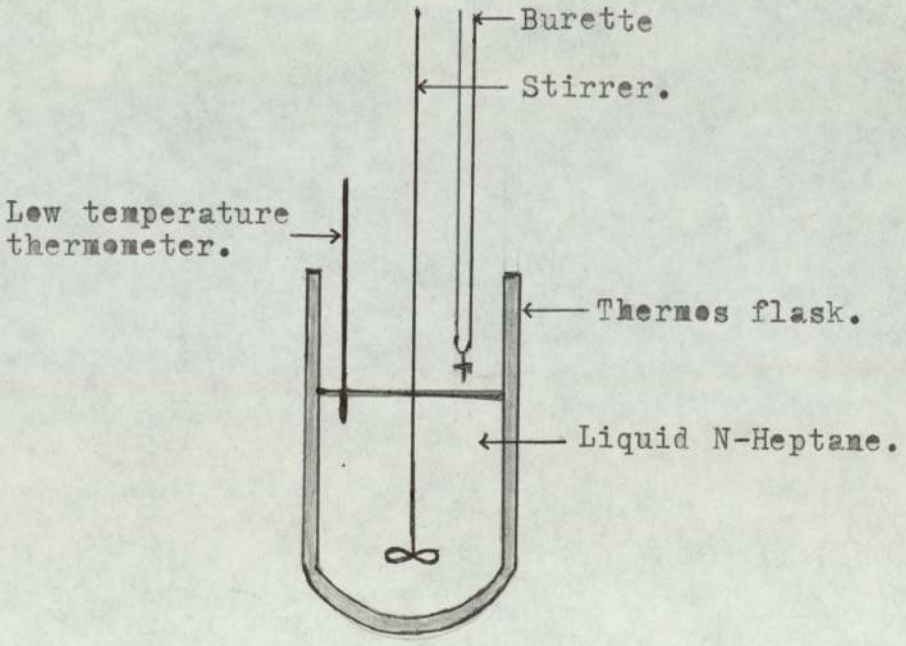
The polymers made were in most cases similar to the formulations used for making membranes, difficulties were encountered using acrylamide however, the droplets froze very slowly and so coalesced. This behaviour also observed making membranes of acrylamide can be attributed to the high solvation energy of acrylamide. Acrylamide on dissolving in water cools the solution a great deal due to this, so on freezing the acrylamide solution this heat is put back into the system causing a reduction in the rate of cooling.

7.2 TECHNIQUE OF MAKING LARGE MACROPOROUS BEADS

The technique used for making large macroporous beads was relatively simple, basically it consisted of introducing droplets of a monomer/solvent formulation into a very cold bath of n-heptane cooled by dry ice. The droplets, (fed from a burette), froze within a second or two and dropped through the n-heptane to the bottom of the vessel; it was found to be advantageous to have the n-heptane stirred as the droplets were added as this largely prevented coalescence of the droplets which could lead to deformed beads and clumps of partially united beads. The basic apparatus used is shown in Figure 54.

FIGURE 54

Apparatus Used to make Large Macroporous Beads



After the last droplets had been added to the n-heptane bath, the frozen beads were allowed to settle and the bulk of the n-heptane was removed from the thermos flask. Then the frozen beads with a small quantity of n-heptane were transferred to a large beaker and the beaker put on top of a magnetic stirrer. To maintain the beads in a frozen state the n-heptane was kept cool by adding powdered dry ice; and a magnetic follower was added so that the mixture could be stirred.

Above the beaker the UV lamp was placed and the stirred beads were irradiated while still frozen. Due to heat from the lamp it was occasionally necessary to add powdered dry ice to the n-heptane to prevent the melting of the beads. The irradiation continued until the beads were polymerized, then the n-heptane was removed and finally added to distilled water to enable hydration to take place.

Some of the points which should be noted about the technique are that the non-solvent in which the beads are frozen must remain a liquid to very low temperatures (approximately -70^{o}C). It should also be less dense than water so that the beads will sink and it should also be a non-solvent for the monomers in the beads. The most suitable liquids with these properties are the hydrocarbon liquids such as n-hexane and n-heptane.

It must be pointed out however that the method of producing these beads has several drawbacks, including phase separation and freezing of the monomer/solvent solution in the burette above the cold n-heptane bath. This particular difficulty was overcome by electrically heating the tip of the burette to keep the solution warm.

A further undesirable feature of the technique which could not be so easily overcome was the creation of many irregularly shaped beads. These were observed to be caused by a variety of mechanisms, such as partial freezing of the droplet as it forms on the burette end prior to being dropped into the n-heptane. Other possible causes were the droplet falling through the cold n-heptane before fully freezing on the surface, which results in frozen "tails" forming, and distortion of the droplet generally, and distortion of the droplets while in the process of freezing by stirrer action or bubbles from the dry ice used to cool the cold non-solvent. As has been mentioned previously coalescence of beads was also a problem.

7.3 INCREASING THE SIZE DISTRIBUTION OF THE HYDROGEL BEADS

(a) Introduction

It was considered desirable to look at modifications to the procedure of making the hydrogel beads for three main reasons:-

1) the technique of making the large hydrogel beads is slow and unpredictable;
2) many irregularly shaped beads were produced;
3) the size of bead produced, (approximately 3 mm in diameter) is large and this is undesirable from several points of view, mainly because a column of beads with a much higher external surface area should be better from the point of view of the materials adsorption properties. Also such large beads will take longer to polymerize fully and there will be the danger of possible residual monomer at their cores.

Two methods were examined as means of producing better and smaller beads:-

1) the addition of surfactants;
2) injection and fast freezing of monomer/solvent droplets.

(b) Reduction in Size of Beads by Use of Surfactants

The first method investigated as a means of creating smaller bead sizes was the addition of surfactants. The surfactant chosen was benzalkonium chloride because it is particularly effective in reducing the surface tension of water, (the weight a droplet can attain before it drops is governed by the surface tension of the liquid).

As an initial trial this effect was measured by simply using water solutions of benzalkonium chloride and calculating the experimental radii of the water droplets from the mass of a certain counted number of drops and knowing the density of the benzalkonium chloride solutions. It was found that change in the theoretical radius of the water droplet did not differ much as the benzalkonium chloride concentration was increased and the surface tension of the liquid fell.

From the value for the radius of the droplet it is of course possible to calculate the surface area a certain mass of droplets would have. When this calculation was carried out the maximum increase in surface area for the same mass of beads that could be expected was in the region of 20 per cent. Thus it was decided that this technique had no practical value for increasing the external surface area of hydrogel beads and its use was not attempted in the manufacture of hydrogel beads.

(c) Reduction in Size of Beads by Injection and Stirring

Finding that the size of the beads would not be significantly changed by the addition of surfactants, and in any case would still be a slow technique, the possibilities of high speed injection and stirring were looked at.

A monomer solution (acrylic acid/ethylene dimethacrylate in water) was injected at varying speeds into a bath of n-heptane (-70°C) using a motorised syringe. The beads formed were polymerized and thawed in the same way as the larger beads had been and after

FIGURE 55(A)

The Effect of Injection Rate on Bead Size

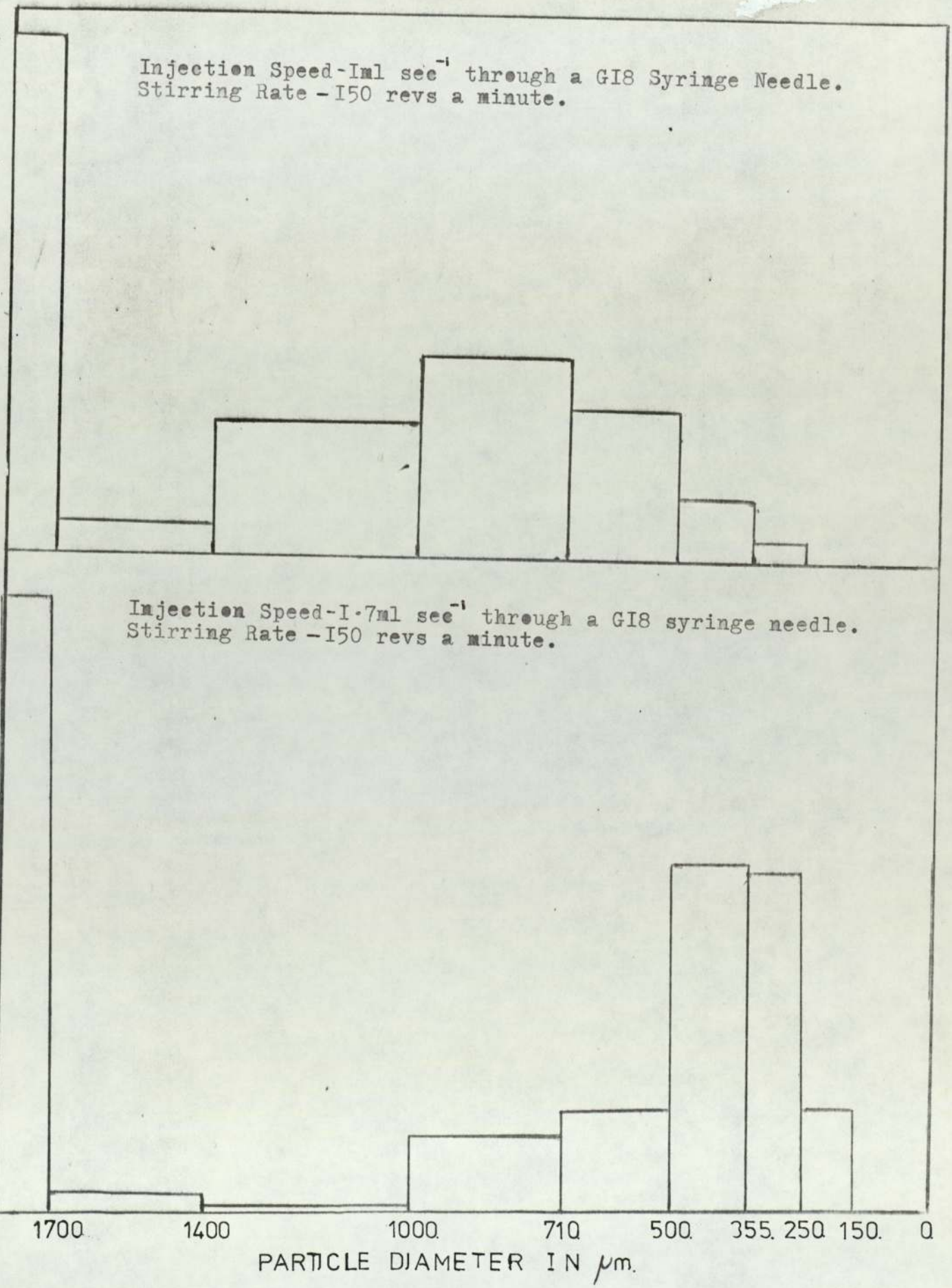
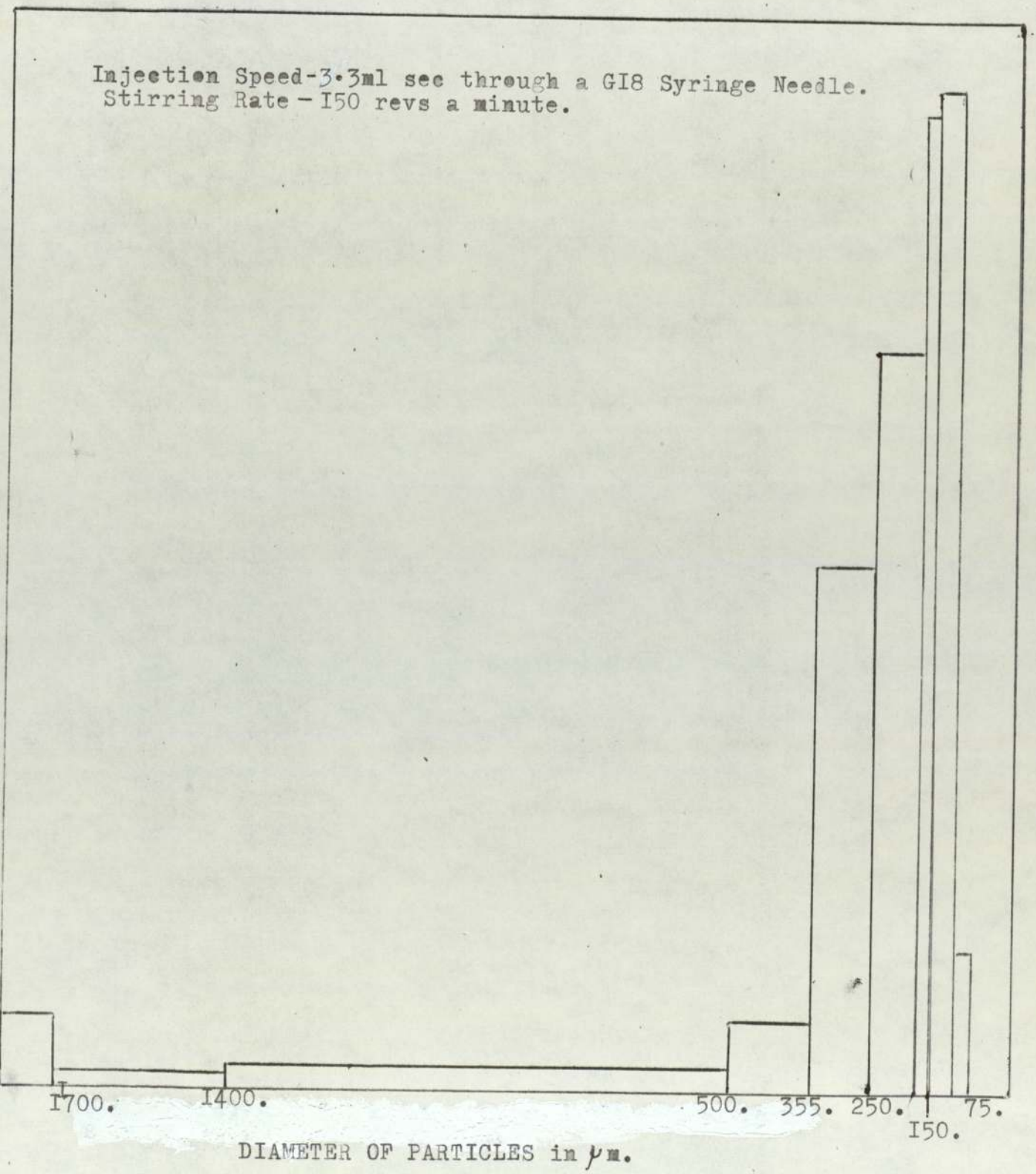


FIGURE 55(B)

The Effect of Injection Rate on Bead Size



hydration they were sieved into specific size ranges, then the beads of the different sizes were dehydrated and weighed.

The weight distributions of the beads with size are shown in the form of column charts in Figures 55 A, B and C. It can be seen that for a bath being stirred at the same rate, as the injection speed is increased the size distribution of the beads is moved to lower values.

This technique was not however investigated in greater detail as it was found that it was only applicable to solutions which had freezing points close to room temperature so that injection into the n-heptane resulted in the same droplets that were formed freezing instantaneously. However for HEMA solutions for example as the freezing point of the solutions is very low, small beads were not formed. So the technique can unfortunately only be used for solutions of monomers with a high freezing point, eg acrylic acid.

(d) Acrylamide/Trioxane Beads

As in the case of the synthesis of the macroporous hydrogel membranes the use of 1,3,5 trioxane as the solvent for the monomers in the preparation of films was attempted; this approach was also tried for the macroporous beads.

As with the membrane work on these or similar formulations however, the results were disappointing. In the experiments carried out droplets of a hot formulation were dropped into liquid paraffin at room temperature; the droplets froze but did so to produce very

irregularly shaped particles. This must be due to shrinkage of the droplet on freezing, due to the different densities of the solid and liquid phases. It does occur with the water monomer solution freezing experiments as ice has a lower density than water so the droplet is not deformed by this effect at least.

The fast injection system to produce small beads was also used, but the results too were discouraging; the beads after polymerization and hydration were extremely weak and fragile.

7.4 SCANNING ELECTRON MICROSCOPY OF MACROPOROUS BEADS

(a) Introduction

Scanning electron microscopy was carried out on the surfaces of the macroporous beads and their interiors. The latter being done by cutting the hydrated beads in half with a sharp knife. In addition XAD-2 and XAD-7 were examined for comparison.

The chief reasons for this study are listed below:-

1) To find out whether a similar macroporous structure to that created in the membranes has been produced in the beads.
2) To observe if the general surface rugosity of the beads is the same or higher than those of the membranes which are made with the surfaces in contact with smooth glass plate.
3) To find out if the macroporous nature of the bead is uniform throughout the bead or is simply a surface feature.
4) To see if differences in the formulations used to make the beads made appreciable differences to their structures as has been observed for the macroporous membranes.

In order to illustrate the answers to these questions and others, the photographs given on the following pages have been chosen, they are itemised in Table 61, which is a key to the selection of photographs.

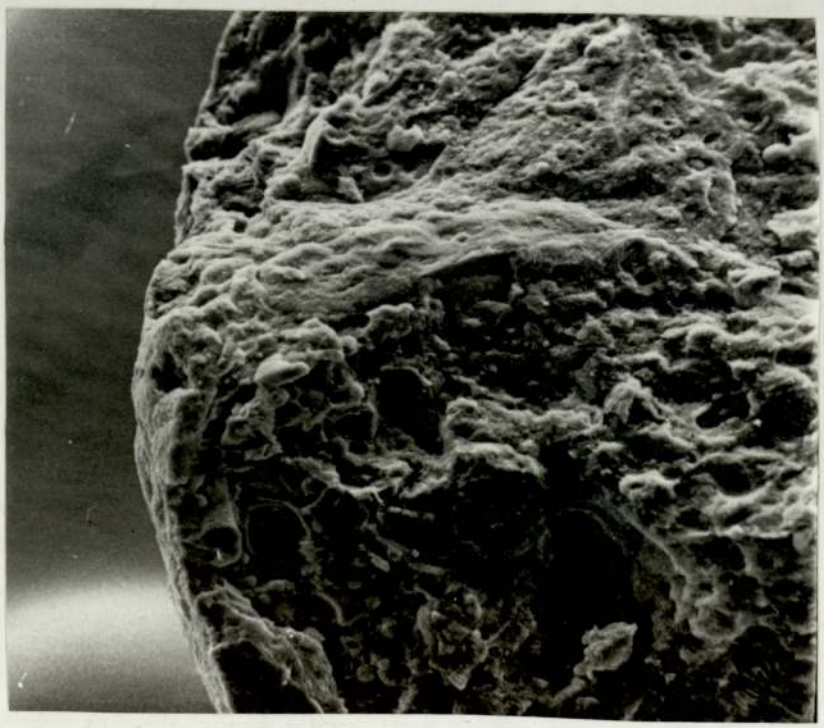
TABLE 61

Key to the Scanning Electron Micrograph Photographs of the Macroporous Hydrogel Beads (Dehydrated)

Plate 9	Photograph 17	Surface of S1 (x240)
	Photograph 18	Interior of S1 (x220)
Plate 10	Photograph 19	Surface of S1 (x1200)
	Photograph 20	Interior of S1 (x1100)
Plate 11	Photograph 21	Surface of S1 (x6000)
	Photograph 22	Interior of S1 (x5500)
Plate 12	Photograph 23	Surface of S5 (x2600)
	Photograph 24	Surface of S5 (x6000)
Plate 13	Photograph 25	Surface of S2 (x1100)
	Photograph 26	Surface of S2 (x5500)
Plate 14	Photograph 27	Surface of S4 (x1200)
	Photograph 28	Interior of S4 (x5500)
Plate 15	Photograph 29	Cut edge of S3 (x260)
	Photograph 30	Cut edge of S3 (x1300)
Plate 16	Photograph 31	Cut edge of S8 (x260)
	Photograph 32	Cut edge of S8 (x1300)
Plate 17	Photograph 33	Surface of S8 (x1200)
	Photograph 34	Surface of S8 (x200)
Plate 18	Photograph 35	Surface of XAD-7 (x1000)
	Photograph 36	Surface of XAD-2 (x1000)

PLATE 9

PHOTOGRAPH 17



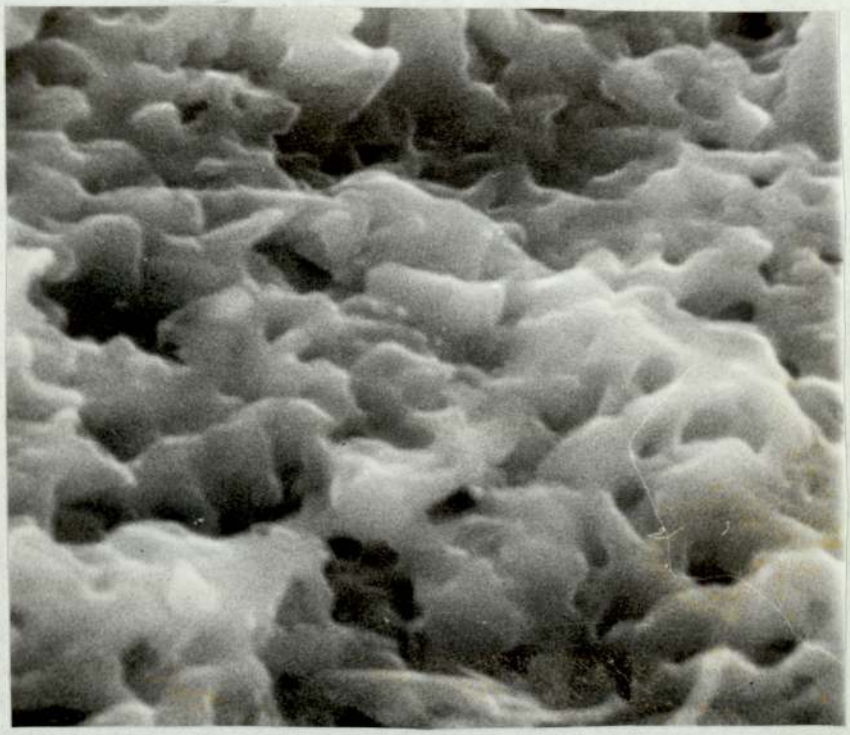
PHOTOGRAPH 18

PLATE 10

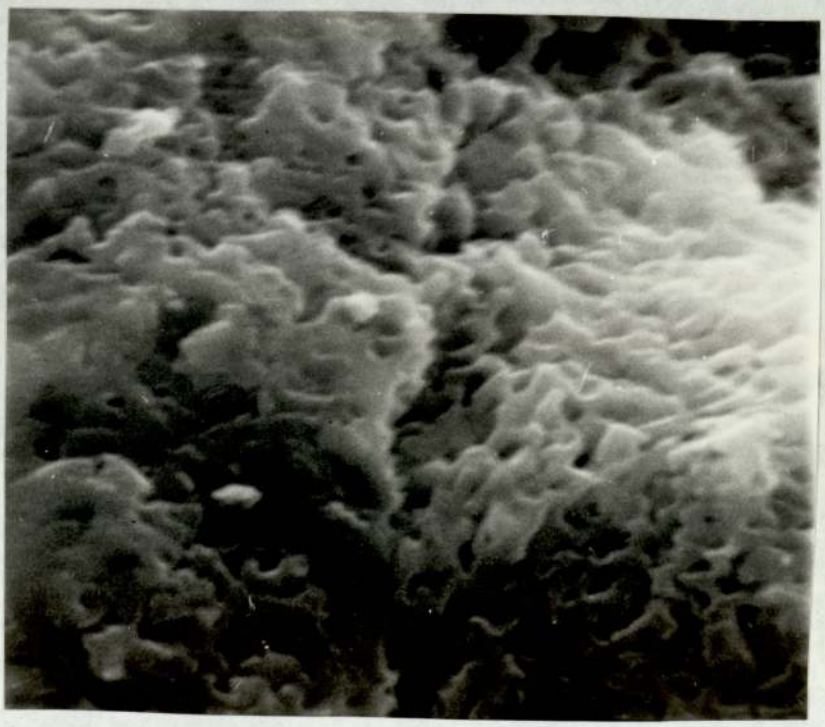
PHOTOGRAPH 19

PHOTOGRAPH 20

PLATE 11

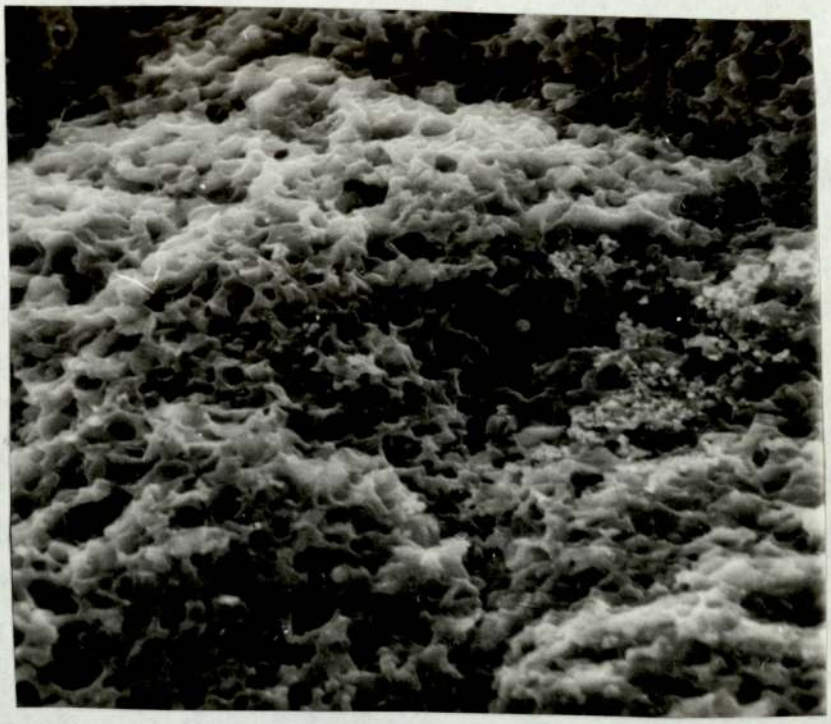


PHOTOGRAPH 21



PHOTOGRAPH 22

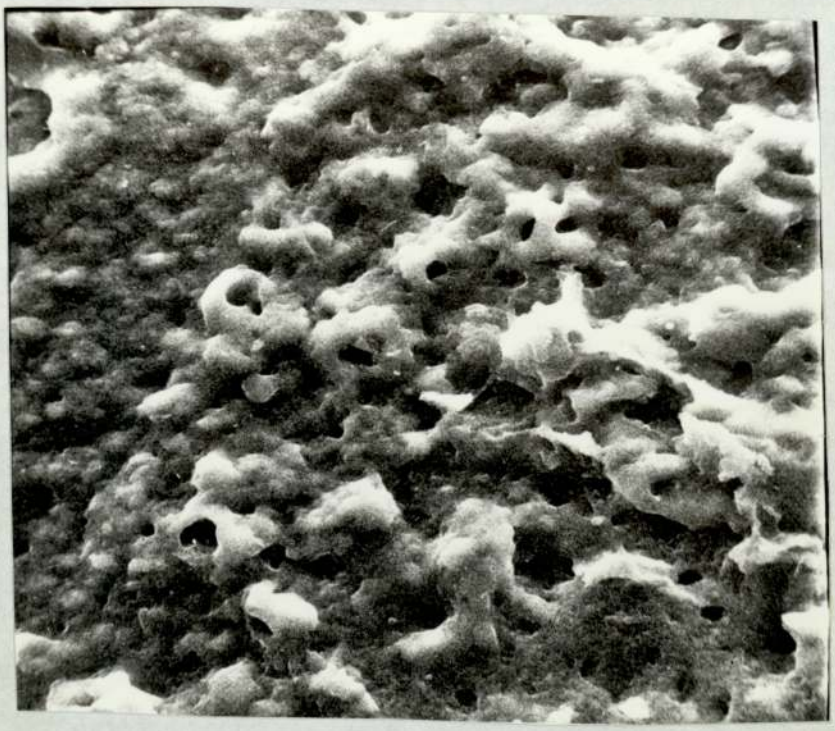
PLATE 12



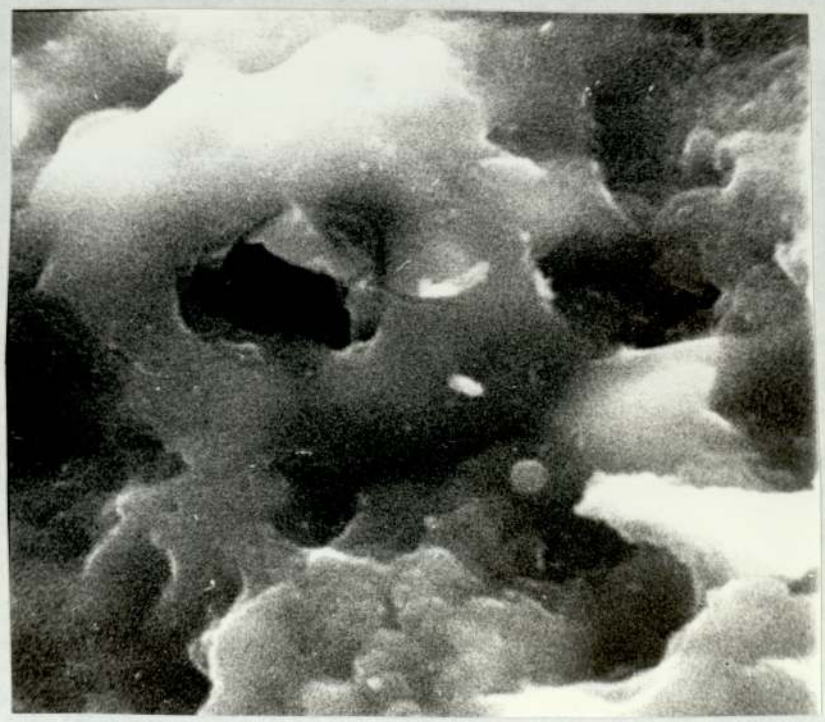
PHOTOGRAPH 23

PHOTOGRAPH 24

PLATE 13

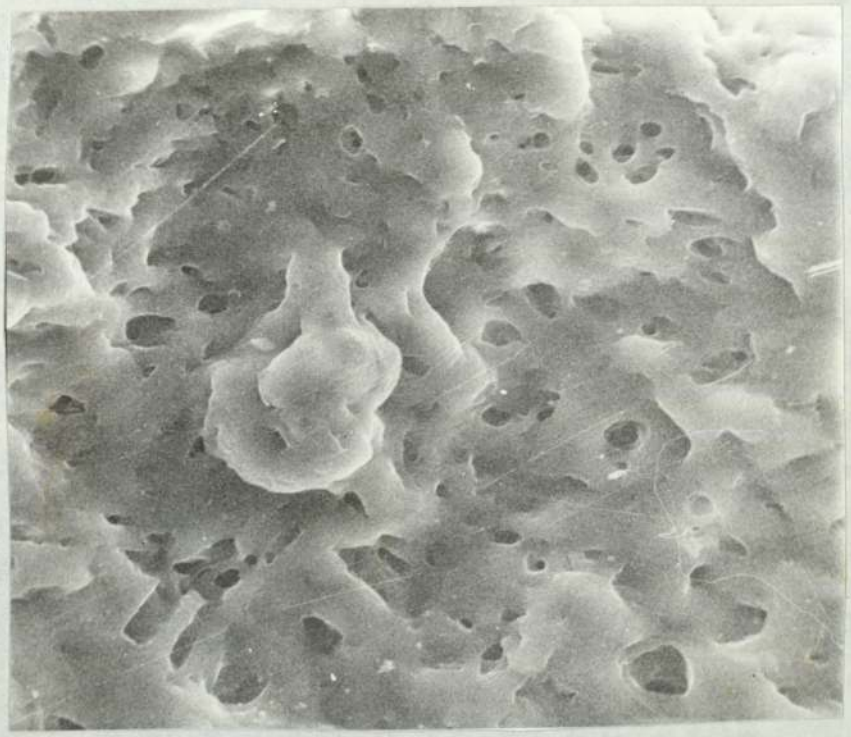


PHOTOGRAPH 25

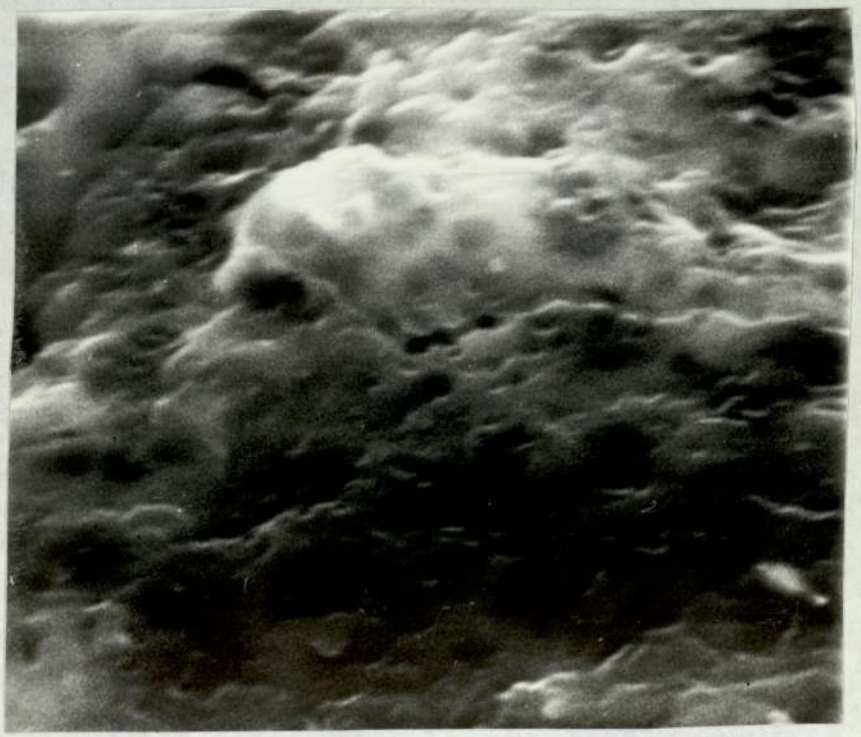


PHOTOGRAPH 26

PLATE 14

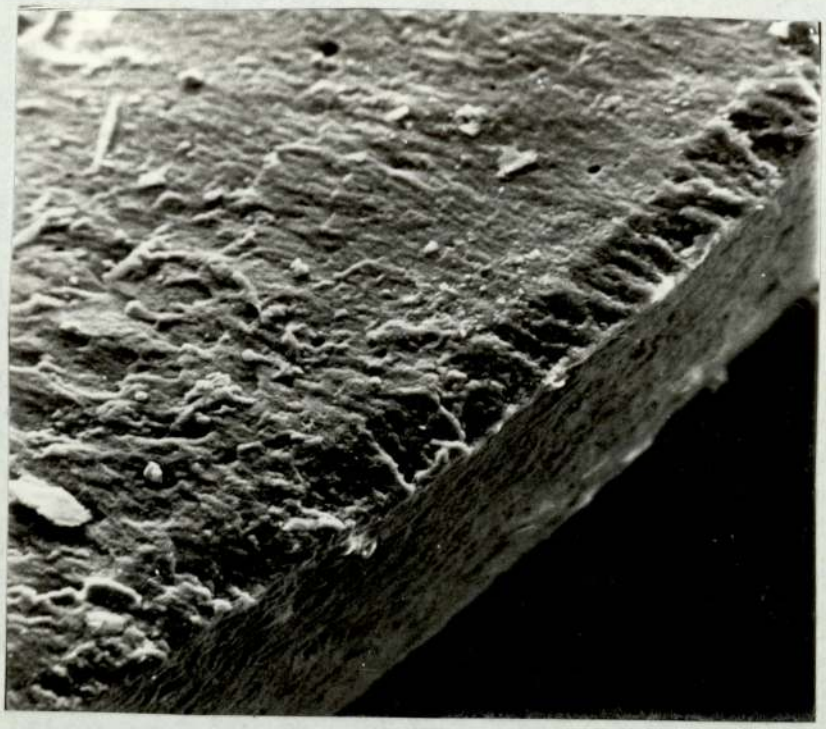


PHOTOGRAPH 27



PHOTOGRAPH 28

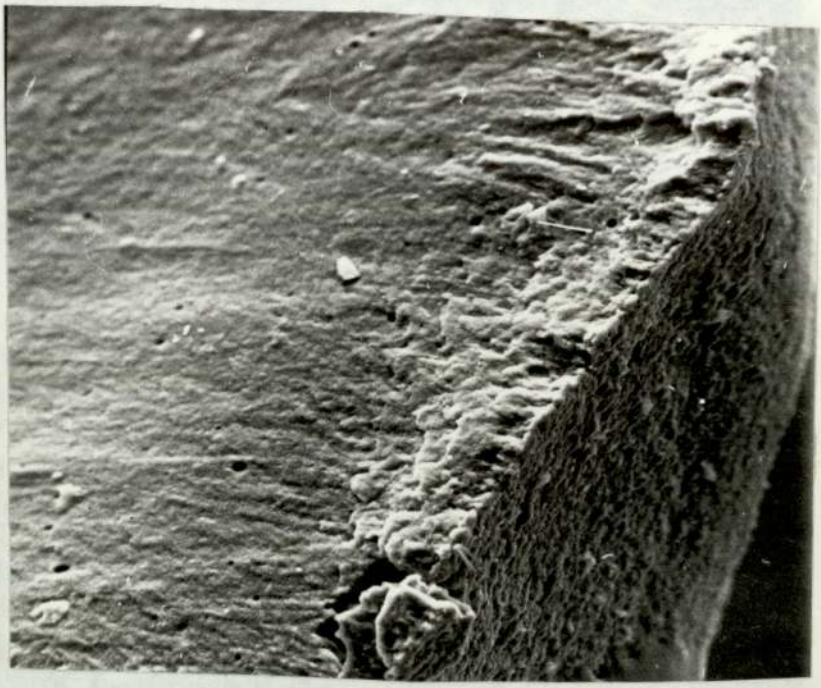
PLATE 15



PHOTOGRAPH 29

PHOTOGRAPH 30

PLATE 16



PHOTOGRAPH 31

PHOTOGRAPH 32

PLATE 18

PHOTOGRAPH 35

PHOTOGRAPH 36

(b) Discussion of the Scanning Electron Micrograph Photographs

1) The Macroporous Nature of the Surface of the Beads

This is best illustrated in photographs 19, 21, 23, 24, 25, 26, 27, 33 and 34. It can be seen that well-defined macropores are not usually present on the surface of the bead, which presents a generally porous, broken up appearance. However, on several photographs distinct macropores can be seen, eg 9 and 11, and the sizes of pore has been determined as being of several microns in diameter, ($\sim 2\ \mu$).

2) The Macroporous Nature of the Interiors of the Beads

The macroporous nature of the interiors of the beads is well illustrated by photographs 20, 22, 28, 30 and 32. It can be observed in these photographs that the macroporous nature of the surface extends into the interior of the bead. It is, however, noticeable on some of the photographs (29-32), that there is an apparent discontinuity between the edge of the bead and the interior. It appears that the pore structure is much more unidirectional at the immediate edge of the beads with the pores pointing towards the centre of the bead. This might be expected as the solution will freeze much faster at the outside of the monomer-solvent droplet producing an organized structure, whereas in the interior of the droplet slower freezing will produce a disorganized porous structure. This is, in effect, the observation made by Haldon and Lee for the structure created in membranes under different freezing conditions.(9)

In other cases, eg S2, photographs 18 and 20, a very confused

structure appears to have been produced and it could be speculated that strong convection currents, during the freezing process, could result in frozen material at the surface being swept into the centre and thereby creating the disorderly structure seen in some of the photographs.

3) Surface Rugosity and the Presence of Debris on the Surface

In several cases, photographs 17 and 33 especially, it is possible to see the presence of debris on the surface of the beads. In some cases the pieces of material appear to be attached to the surface, photograph 33, and will therefore only add to surface rugosity. In other cases, photograph 17, the particles appear to be completely detachable and thus present the danger of creating microemboli if beads of this material were used in a haemoperfusion system.

It is also noticeable that surface roughness of the beads in general is quite high and much higher than that shown by the comparison materials: XAD-2 and XAD-7, photographs 35 and 36.

4) Composition and Macroporous Structure

Lastly, it was noticed that the composition of the formulation particularly with regard to ethylene dimethacrylate content did not appear to have the same overriding importance it did in the case of membranes. Even compositions with EDM concentrations of 1 per cent did produce macroporous structures: photographs 27 and 28. Precisely why this should be the case for beads but not membranes is not clear, but it is still advantageous to have a high concentration of EDM in the formulation from the point of view of other considerations such as strength.

7.5 SURFACE AREAS OF THE BEADS

The macroporous internal surface areas of the hydrogel beads were measured using a BET surface area apparatus available in the department; this apparatus was built by D Cooper[215] and modified by A Moir and works by measuring the absorption of nitrogen gas onto surfaces which have been caused to desorb all gas molecules by heating to high temperatures under high vacuum.

The surface areas of the dehydrated beads measured by this technique were in the region of 8-11 square metres per gram, which is low in comparison to commercially available resins and carbons. However, it should be noted that this value only represents the surface area of the macropores and does not take into account the microporous nature of the polymer matrix, which is destroyed by dehydration and therefore not measured by this technique. (Also, regrettably, the surface area apparatus has been designed to measure very high surface areas in the region of at least 100 metres square/per gram and the results obtained for lower surface area materials are less reliable.)

7.6 BLOOD COMPATIBILITY AND ADSORBABILITY

The blood compatibility of the beads and their adsorption of the blood toxin, bilirubin, from plasma are discussed in Chapter 9 and 10, respectively, as it is best to deal with these properties in relation to the results obtained for other materials synthesized during this project.

7.7 CONCLUSIONS

The main conclusions drawn from the work on macroporous beads were that:-

(1) The technique for making large macroporous beads is too slow and cumbersome to be of practical value in making materials for artificial liver support systems.

(2) That the technique of making small macroporous beads is very limited and can be practically applied only to monomers which have high freezing points $\sim 0^{\circ}C$ and allow freezing to take place quickly, ie are not highly solvated, for example, acrylamide.

(3) That the internal surface area, size of pores and other cardinal factors of beads cannot be altered easily to more favourable values without destroying other important features such as physical strength or increased aggregation in the pre-polymerization process.

CHAPTER 8

SUSPENSION POLYMERIZATION

8.1 INTRODUCTION

Also investigated as a means of creating hydrogel beads was the technique of inverse suspension polymerization. This technique was tried in addition to the freezing technique described previously as the latter technique had severe limitations as to the range of compositions, which could be made and the variety of physical properties that could be developed in the materials eg pore size and the size distribution of the particles cannot easily be controlled.

However, as the beads proved difficult to make by the suspension technique much of the work described in this chapter has been done in investigating the basic properties of monomer-solvent polymerized membranes by the thin film method. This has been done to provide much of the basic information on the systems needed to develop beads; since many of the physical properties of membranes should be identical to beads of the same composition and produced under much the same conditions.

The main system investigated was the acrylamide-NN'methylene bis acrylamide system, (ACR-NN'MBA). This system was chosen for several reasons:-

(1) There is a great deal of experimental evidence that polyacrylamide is one of the most promising biocompatible materials.[133]

(2) Polyacrylamide can be readily derivatized into a large variety of products including many which might be useful for toxin removal by ion exchange.[141] It can also be used as a template for the albumin molecule and hence, for the removal of toxins which bind to albumin.[141]

(3) The monomers are quite water soluble thus water can be used as the solvent rather than other liquids which might contaminate the product.

(4) Some work has already been carried out on this system to prepare beads by suspension polymerization.[134][68]

(5) As the crosslinking agent, NN'MBA is quite soluble to an acrylamide/water solution high amounts can be incorporated into the polymer leading to a highly porous structure by the same method as is used for conventional ion exchange resins, ie high (crosslinking agent) plus high solvent to monomer ratio leads to high porosity.[216]

In this chapter, the first important factor dealt with is the solubility of the crosslinking agent in the monomer/solvent solution, especially how this varies with monomer/solvent ratios, temperature and the ratio of acrylamide to NN'methylene bis acrylamide. Then the properties of the polymerized membranes made from these solutions are discussed, ie their strength, porosity, equilibrium water contents. Tests on the membranes, not of direct relevance to the manufacture of hydrogel beads, were also carried out, ie measurements of surface hydrophilicity to further elucidate the relationship between surface properties and composition.

Next the properties of another system, HEMA-NN'MBA, are discussed and finally the experiments to produce hydrogel beads and the tests carried out on the materials produced.

8.2 SOLUBILITY OF ACRYLAMIDE/NN'METHYLENE BIS ACRYLAMIDE FORMULATIONS IN WATER

The solubility of the systems were investigated by filtering the solutions of ACR-NNMBA in water through a (previously weighed) heated sinter filter prior to mixing the solution with the initiator and then using it to make membranes. The sinter filters were then heated, under vacuum, until constant weight was obtained.

(a) The Effect of Increasing (NNMBA) and reducing (ACR) on Solubility

The effect of increasing the concentration of NNMBA on the solubility of various systems with different initial monomer:solvent ratios is shown in Table 62, and graphically in Figure 56. It should be noted that the value for pure NNMBA was obtained from the literature.(217)

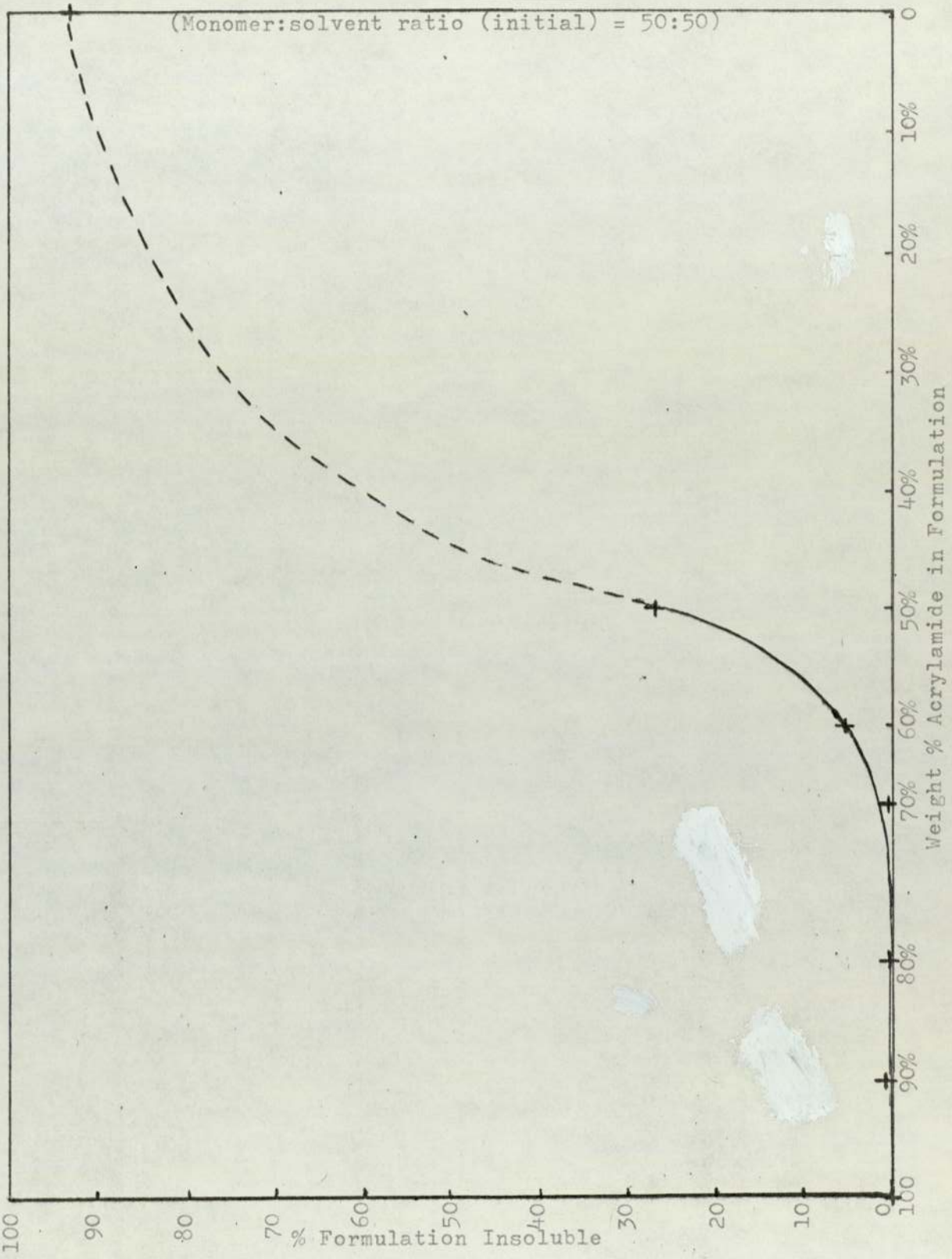
TABLE 62

The Effect of ACR:NNMBA weight ratio on the solubility of the monomers in water at 50°C

Initial Weight Ratio of ACR:NNMBA	Initial Monomer Solvent Ratio	% Material Insoluble	Membrane made from formulation
4.5:0.5	50:50	0.3	D1
4:1	50:50	0.2	D2
2:1	50:50	0.5	D3
3:2	50:50	5.4	D4
1:1	50:50	27.0	D5
2:1	40:60	0.8	D6
3:2	40:60	2.6	D7
2:1	30:70	0.5	D8
3:2	30:70	1.4	D9
2:1	20:80	0.7	D10

FIGURE 56

The Solubility of the ACR/NN'MBA formulations in water with differing amounts of acrylamide and NN'methylene bisacrylamide at 50°C



It can be seen from the table and figure that the amount of material remaining insoluble of the formulation starts to increase dramatically when the weight % of NNMBA is about 35-50%, (for solutions at 50°C). Therefore it is possible to add quite high levels of this fairly hydrophobic crosslinking agent before the formulation starts to become insoluble.

(b) Effect of Monomer:Solvent Ratio on Solubility

This effect can also be seen from the data on Table 62 but for ease of interpretation the relevant results are shown rearranged in Table 63.

TABLE 63

The Effect of Monomer:Solvent Ratio on the Solubility of ACR/NNMBA Formulations at 50°C

Initial Monomer Solvent Ratio	Initial Weight Ratio of ACR:NNMBA	% Material Insoluble	Membrane made from formulation
50:50	2:1	0.5	D3
40:60	2:1	0.8	D6
30:70	2:1	0.5	D8
20:80	2:1	0.7	D10
50:50	3:2	5.4	D4
40:60	3:2	2.6	D7
30:70	3:2	1.4	D9

As can be seen from the Table there is no discernable effect for the first series, those with an initial weight ratio of ACR:NNMBA of 2:1, but for the second series with a weight ratio of 3:2 it appears that the solubility of the formulation is increasing with the amount of solvent present.

(c) Variation of Solubility of the Formulation with Temperature

The variation of solubility of the ACR/NNMBA systems with the temperature of the solution has also been investigated. The temperatures chosen were 30°C, 40°C and 50°C, higher temperatures were not used as the solutions become unstable and tend to polymerize thermally and spontaneously.

The results of the experiments are shown in Table 64, and graphically in Figure 57.

TABLE 64

Solubility of ACR/NNMBA Solutions with Different Temperatures

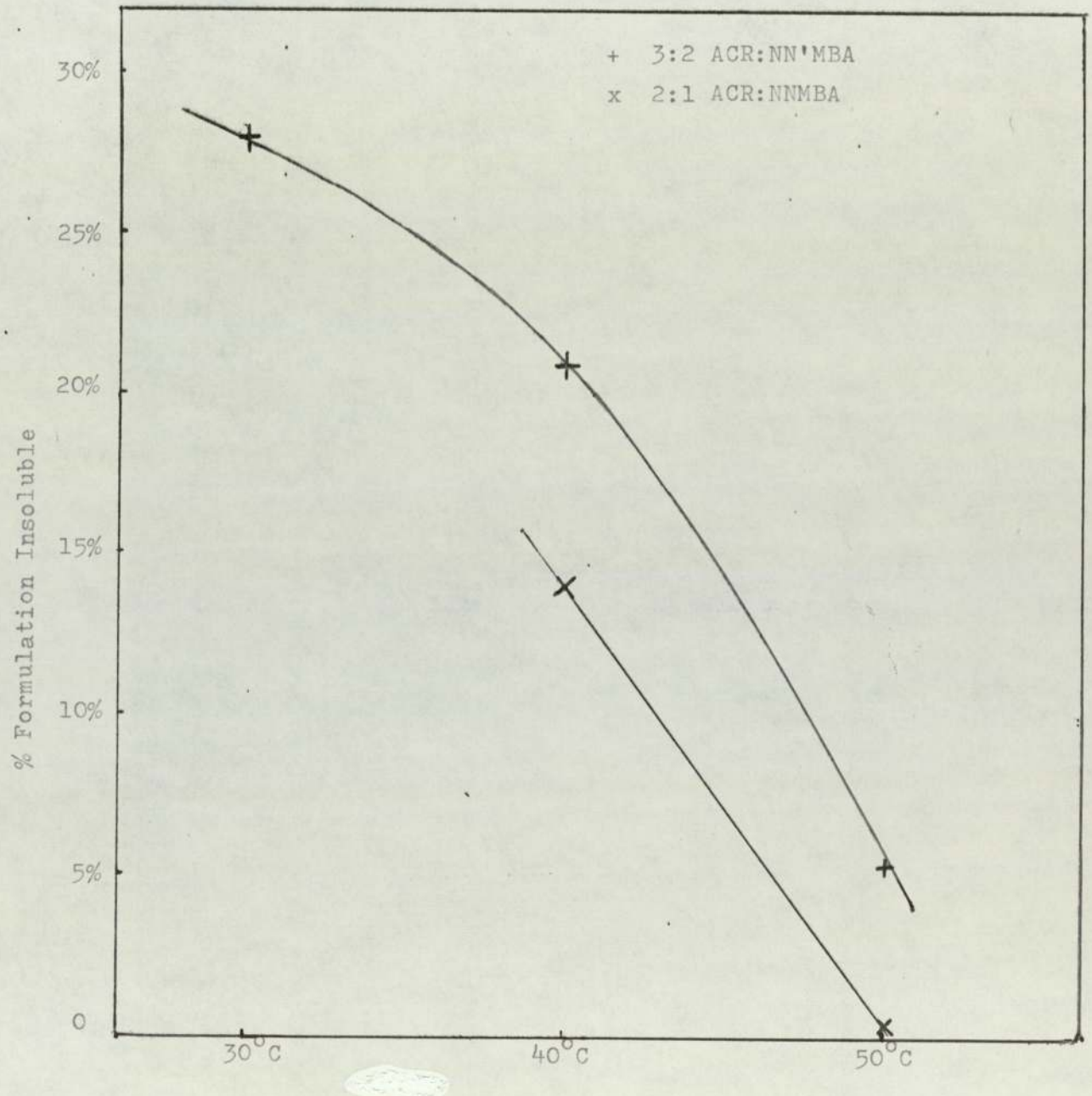
Temperature of Solution	Initial Monomer Solvent Ratio	Initial Weight Ratio ACR:NNMBA	% Insoluble Material	Membrane made from Formulation
50°C	50:50	3:2	5.4	D4
40°C	50:50	3:2	21.0	-
30°C	50:50	3:2	28.0	-
50°C	50:50	2:1	0.5	D3
40°C	50:50	2:1	14.0	-

The results of the experiments show clearly that the amounts of the monomers, which remain insoluble, are reduced dramatically as the temperature is increased to 50°C.

FIGURE 57

The Solubility of the ACR/NN'MBA formulation in water at Different Temperatures

(Initial Monomer Solvent Ratio = 50:50)



8.3 PROPERTIES OF ACRYLAMIDE-NN'METHYLENE BIS ACRYLAMIDE MEMBRANES

A series of the above mentioned membranes were made to provide basic information about this system, such as the insolubility of the formulations in water, the relationships between EWC and the amount of crosslinking agent, the effect of the monomer/solvent ratio on EWC and the relationship between porosity and the various properties of the polymer. These properties are discussed on the following pages.

(a) The EWC's of the Acrylamide NN'Methylene bis acrylamide membranes

The Equilibrium Water Contents of these hydrogel membranes have been measured by the technique previously described and the results are given in Appendix D, but they are also shown graphically on the following pages to illustrate the following relationships.

(1) EWC and initial concentration of NN'MBA - The effect of the initial concentration of NN'MBA on EWC is shown in Figure 58; it can be seen that as the concentration of NN'MBA rises the EWC of the membranes initially drops very steeply and then levels out at a roughly constant value for membranes with very high amounts of the crosslinking agent. The slight upturn of EWC values at very high initial concentrations of NN'MBA is, of course, due to the insolubility of the formulation which causes a change in the monomer: solvent ratio and leads to a higher EWC value.

(2) EWC and Monomer:Solvent ratio - The interrelation between these two factors is shown in Figure 59; it can be seen that for the formulations chosen, (which all have high concentrations of NN'MBA),

FIGURE 58

The Effect on the EWC of crosslinked poly(acrylamide) hydrogels of the concentration of crosslinking agent (NN'MBA) (Monomer:Solvent Weight Ratio is 50:50)

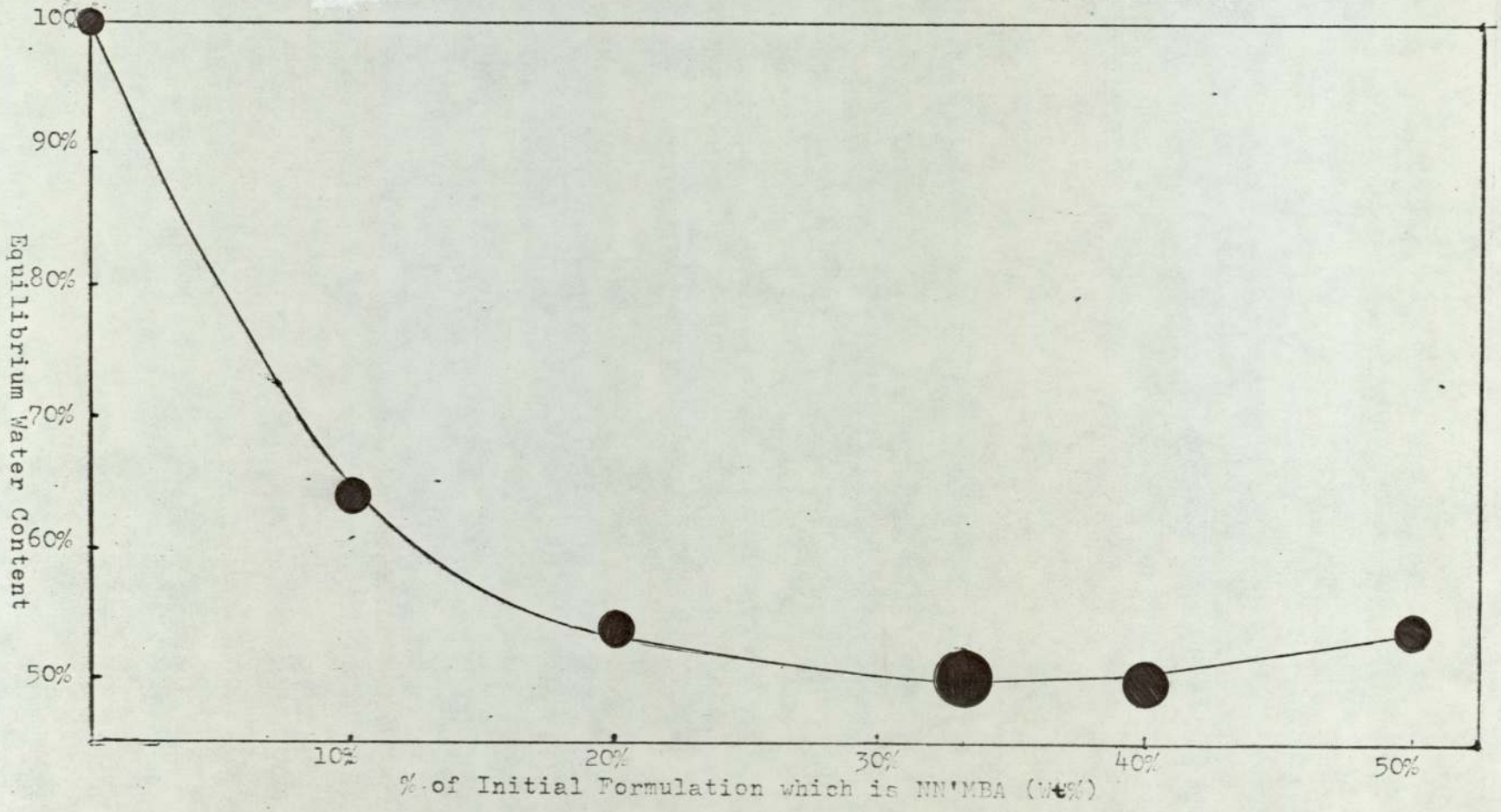
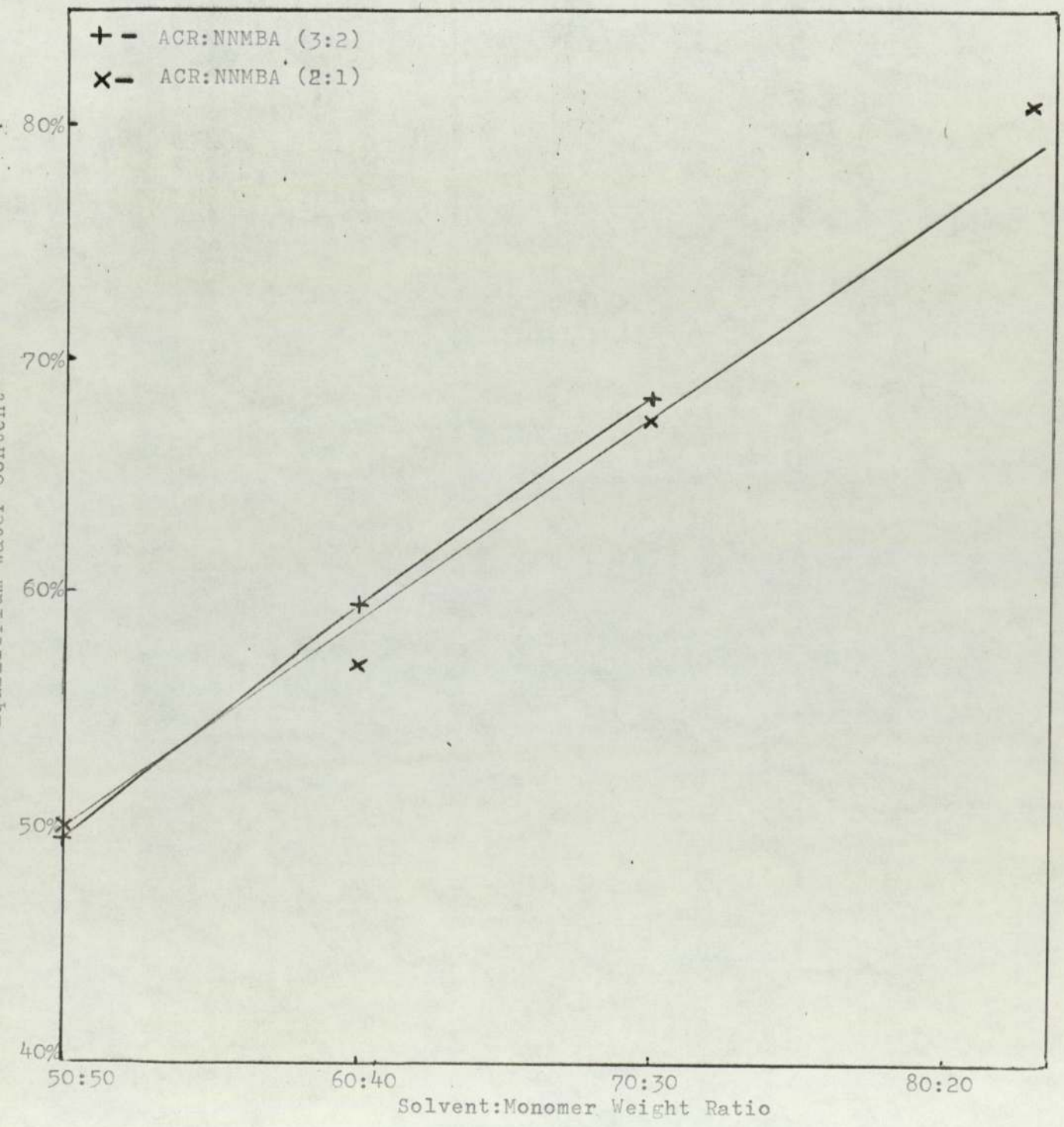


FIGURE 59

The Effect of the Initial Monomer:Solvent Ratio on the EWC of Two Series of Crosslinked Polyacrylamide Hydrogels



the EWC is very close to the percentage of solvent in the initial formulation.

Thus taking the results as shown in Figures 58 and 59, together it is obvious that the concentration of cross linking agent is the main determinant of EWC, when it is at a low concentration and the monomer:solvent ratio is the main factor when the amount of cross linking agent is high.

(b) The Porosity of Acrylamide-NN'Methylene Bis Acrylamide Membranes

Attempts were made to look at the porous nature of these membranes by several methods.

(1) Scanning Electron Microscopy - The available scanning electron microscope in the University did not have the resolution to detect porosity in these membranes.

(2) Hydraulic Permeability - Several attempts were made to measure the rate of passage of water across these membranes under pressure, unfortunately they proved too weak and failed even under slight pressures. So this method of assessing porosity could not be used.

(3) Porosity to High Molecular Weight Solutes - As the two previous methods had not yielded results the technique previously used of measuring porosity by the diffusion of BSP across the membrane was tried. Unfortunately the membranes proved in many cases to be too weak to use, so too few meaningful results were obtained to yield much information on the interrelationship between structure and rates of diffusion of solutes.

(4) Transparency - Some indication as to the porosity of the hydrogels can be gained by examining their optical properties, given in Appendix D. In these hydrogels translucency and opacity cannot be due to block copolymer formation as the reactivity of the two monomers used is very similar; it is therefore the case for these hydrated copolymers that if a material is translucent or opaque it is an indication that we do not have a homogeneous network of hydrated polymer chains, but have nuclei of polymer with water filling the gaps between them, ie porosity.

The process of making porous materials such as macroporous resins has been described previously in Chapter 1; it was shown that to make porous resins a high density of crosslinking agent was required plus a diluent. The situation described applies in the present set of synthesized materials.

Two main effects can be seen from the list of optical properties of the films, both expected. Firstly, as the amount of crosslinking agent increases in the formulation the membrane produced becomes translucent and then opaque; this effect is most clearly seen for the membranes made with an initial monomer to solvent ratio of 1:1. Secondly, that as the initial solvent to monomer ratio is increased from 50:50 to 60:40, the membranes produced go from transparent-translucent to opaque; this happens in every case for the formulations used.

It therefore appears that the theory is borne out for hydrogels, and also that varying the monomer solvent ratio has a more potent effect than altering the ratio of ACR to NNMBA in creating a porous network.

However, a more detailed investigation is necessary to verify these tentative results using a permeability cell to measure the diffusion of high molecular weight solutes through these materials.

(c) Surface Hydrophilicities of the Acrylamide-NN'Methylene Bis Acrylamide Series of Membranes

The surface hydrophilicities of the membranes have again been examined by the Hamilton contact angle technique since the membrane made presented an ideal opportunity to look at more basic relationships between surface polarity and the structure of the hydrogel, eg the effect of increasing the amount of crosslinking agent on surface polarity while keeping the EWC constant. Although, admittedly, the effects shown are not clear cut since several factors are operating at the same time.

The Hamilton contact angles of the materials have been measured and are given in Appendix D. On the following pages some of the basic relationships which have been found are illustrated graphically and are discussed.

(1) The relationship between the amount of crosslinking agent and Hamilton contact angle

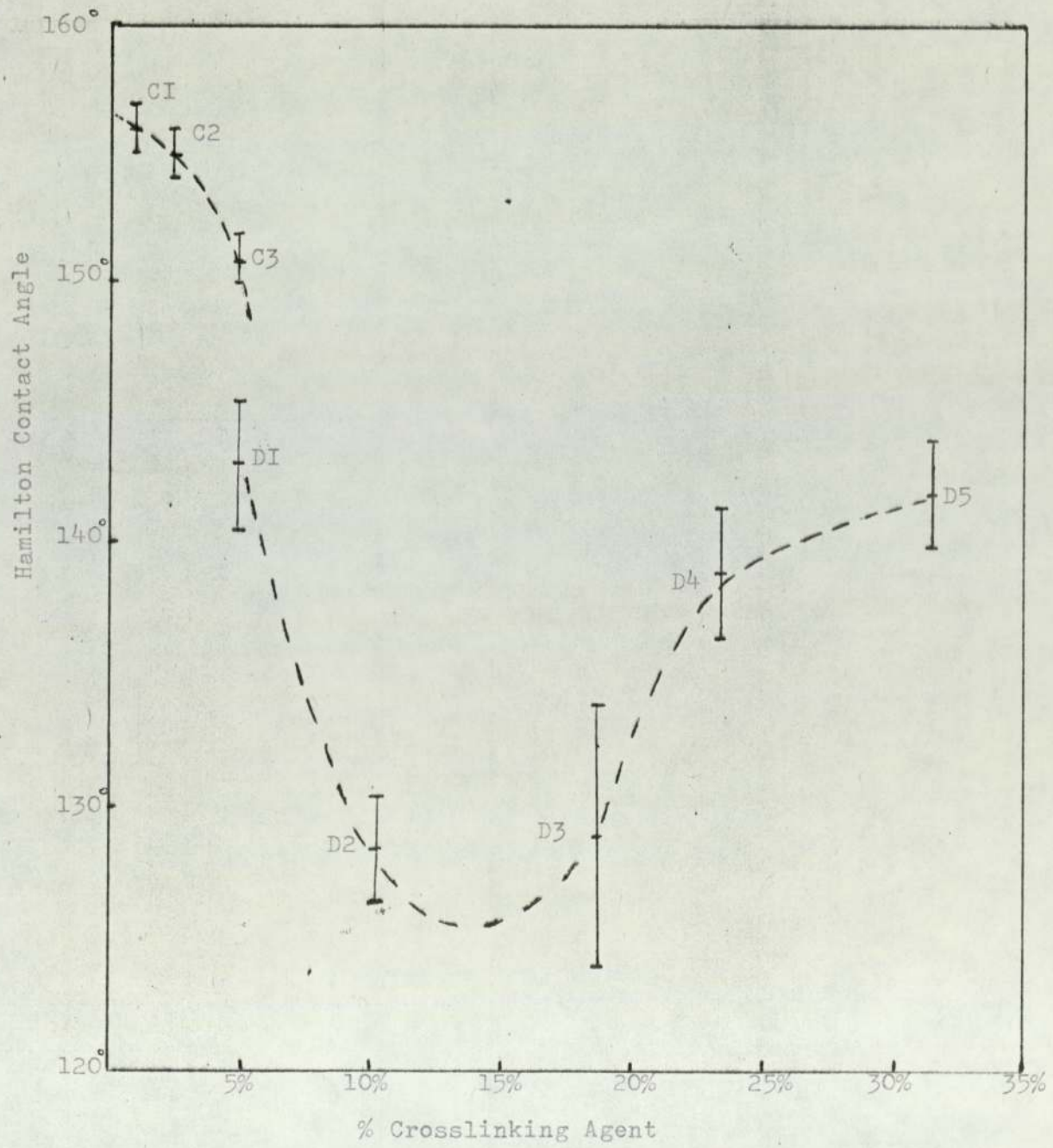
This relationship is well illustrated in Figure 61, which shows the combined results obtained by Hamilton contact angle measurements on two series of crosslinked polyacrylamide hydrogel membranes: firstly a series of ACR:EDM membranes made by UV polymerization of an acrylamide solution, (Appendix E) and secondly the series of ACR:NN'MBA membranes, whose synthesis and properties have been one of the main subjects of this chapter.

FIGURE 60

The Effect on the Surface Hydrophilicity (Hamilton Contact Angle) of Crosslinked Poly(acrylamide) of increasing the amount of crosslinking agent

CI-3 - ACR:EDM See Appendix E

DI-5 - ACR:NNMBA See Appendix D



As can be seen from the Figure, the surface hydrophilicity of the materials decreases as the amount of the crosslinking agent increases in the membranes; this effect can be attributed to two factors, firstly the decrease in the EWC's of the films caused by the increase in CLD, and secondly because of the increase in hydrophobicity of the polymer matrix as the concentration of the more hydrophobic NN'MBA rises relative to that of the more hydrophilic ACR.

However, as can be seen, this is not the complete picture as with higher amounts of crosslinking agent the gels surfaces become apparently more hydrophilic as the measured Hamilton contact angle increases. This increase is difficult to explain but may be due to 'freezing' of the polymer structure by the high levels of crosslinking; this might cause polar hydrophilic groups to be held in certain orientations and prevent them from interacting with each other, causing free non-interacting polar hydrophilic groups to be present at the surface of the polymer.

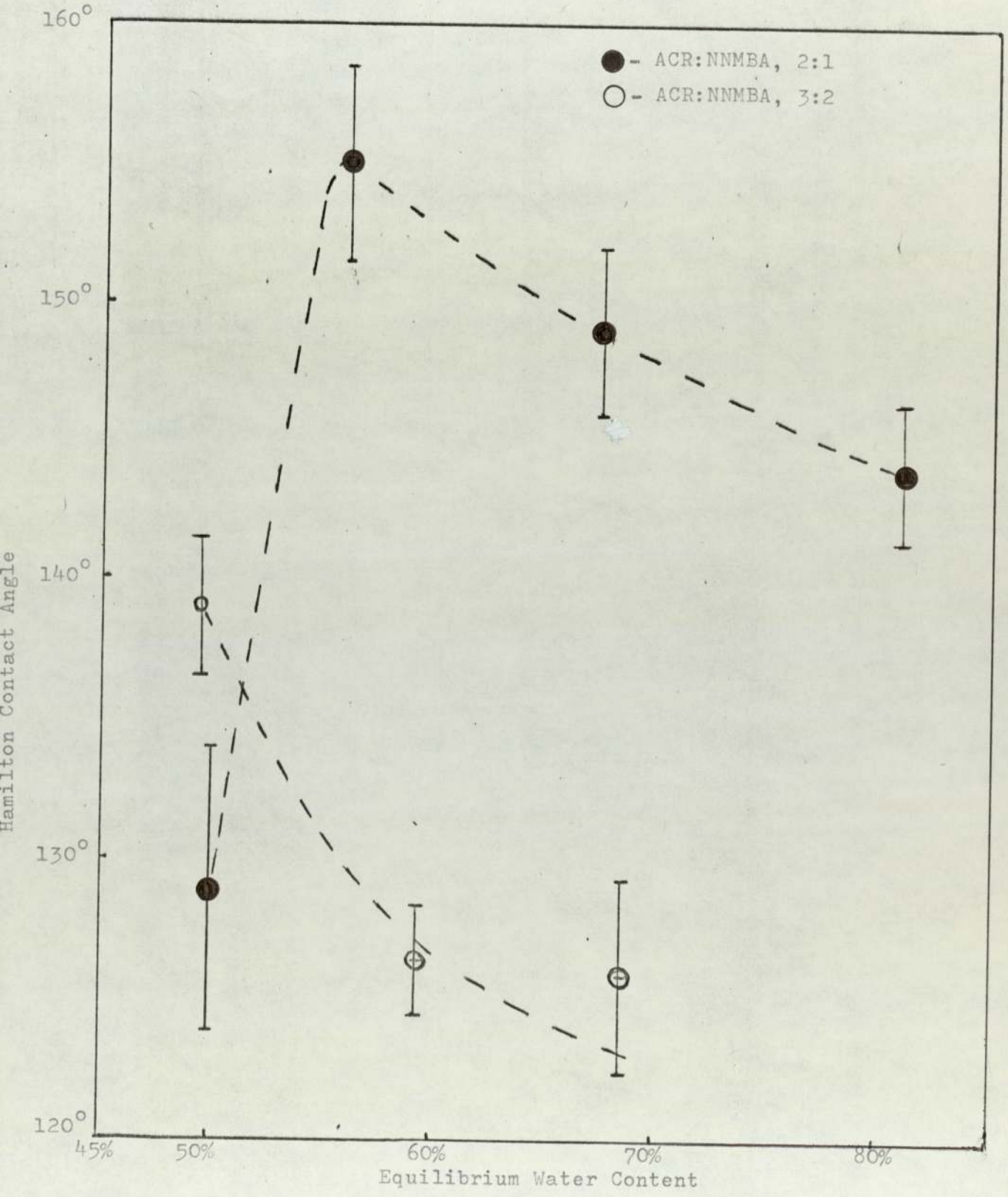
(2) The relationship between Hamilton contact angle and EWC

This relationship is illustrated in Figure 61 which shows the Hamilton contact angles of two series of crosslinked polyacrylamide membranes which both had the same ratio of ACR to NNMBA in the initial formulation, but different monomer to solvent ratios and hence different EWC's.

It seems that for these materials, as the EWC increases the surface hydrophilicity decreases within certain limits; this effect probably has the same cause as that attributed to the results

FIGURE 61

The Effect of Polymer Structure on Hamilton Contact Angle as set against EWC for Poly(ACR-NNMBA) Copolymers



obtained for the macroporous membranes, mainly that the contribution to the surface hydrophilicity of the water regions (pores) is less than that from the surrounding polymer matrix; therefore as porosity increases surface polarity will decrease. In the case of these materials the increase in porosity is not due simply to the increase of the EWC, but also because with increasing dilution in the initial formulation the size of the pores is increasing.

Two further important points should be noted:-

(1) Substantial changes in the surface hydrophobicity appear to occur as we go from similar formulations whose optical properties change from transparency to opacity (eg D3-D6); these cannot be entirely explained at present.

(2) The surface rugosity of these materials is not likely to be as critical in effecting the contact angle results as it was in the case of the macroporous membranes, since the surface features are not of the same magnitude, ie they cannot be detected by SEM as could the features of the macroporous films.

8.4 THE 2-HYDROXY ETHYL METHACRYLATE-NN'METHYLENE BIS ACRYLAMIDE SYSTEM

In addition to the ACR-NN'MBA system the HEMA-NN'MBA system was examined but in less depth. This system was examined because it was considered possible that with the high reactivity of the acrylamides as against that of HEMA it might be possible using a crosslinking acrylamide NN'MBA to make nuclei of NNMBA interlocked by strands of polyHEMA - one of the classic techniques of making macroporous membranes and beads.

(a) Solubility of NN'MBA in the HEMA:NN'MBA:H_2O System

The solubility experiments were conducted in the same way as those involving ACR:NN'MBA:H_2O as previously described. The new system had however, one advantage over the previous one, in that both monomers were not solids so the insoluble material remaining must be NN'MBA. The results are illustrated in Table 65.

TABLE 65

% NN'MBA Remaining Insoluble in HEMA:NN'MBA:H_2O Systems at various temperatures

Temperature of Solution	Initial Molar Ratio HEMA:NN'MBA	Initial Monomer:Solvent Ratio	% NN'MBA Insoluble	Membrane made from Formulation
50°C	75:25	50:50	7%	D11
40°C	75:25	50:50	8%	D12
30°C	75:25	50:50	36%	D13

(b) The EWC's of the HEMA:NN'MBA Membranes

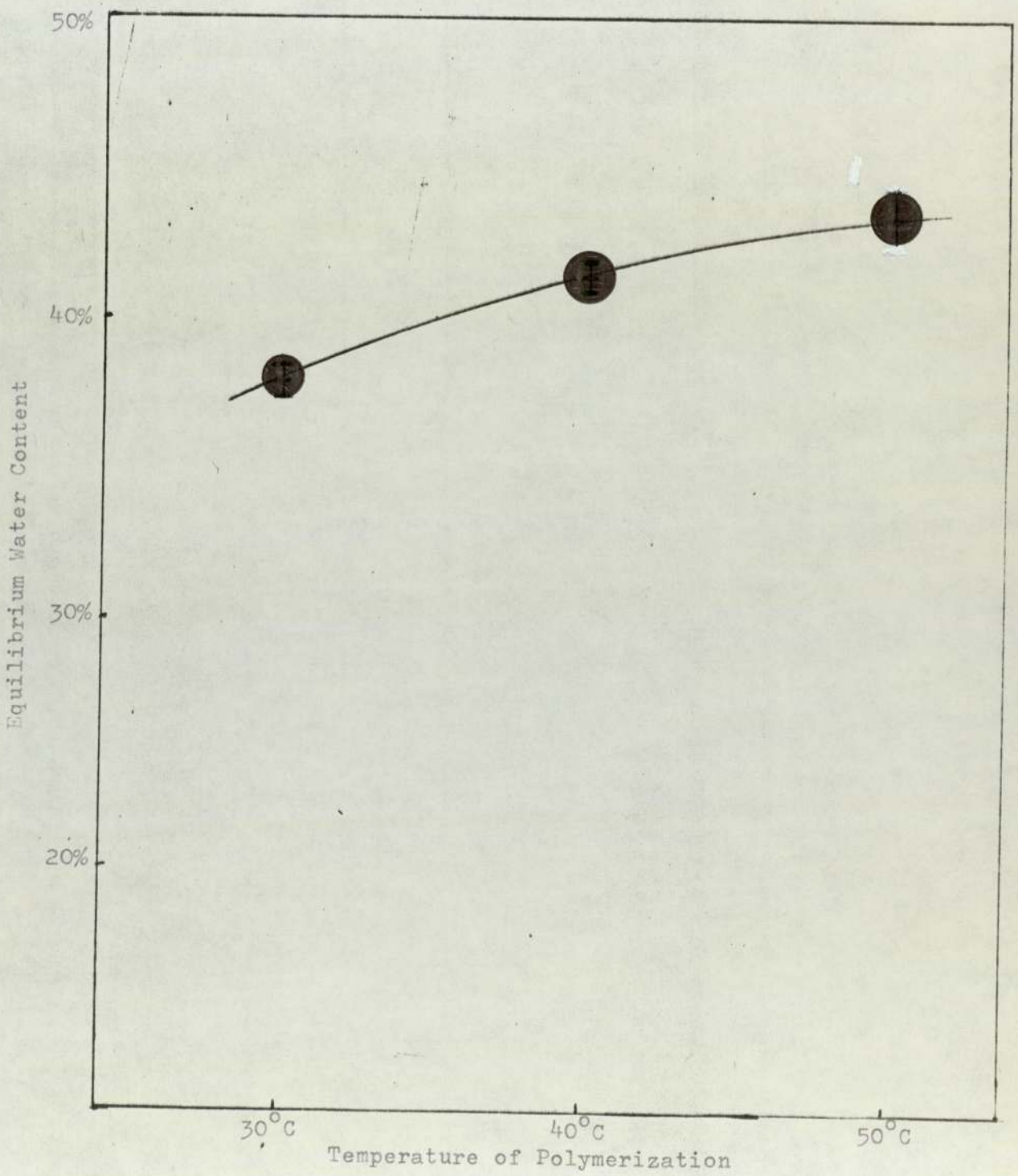
The EWC's of these membranes have been measured as described previously; the results are given in Appendix F, and also graphically in Figure 62.

From Figure 62 it can be seen that the EWC's of the membranes are all less than would be expected from their monomer:solvent ratios, (50:50) so it appears that desorption of water from the membranes has occurred. The increase in water content as the temperature of polymerization is increased is not easily explained as more crosslinking agent will be present in those membranes with higher EWC's.

This system although interesting was not investigated as the membranes formed were not very porous, a fact which is shown by the transparency of D11, and their low water contents.

FIGURE 62

The EWC's of the HEMA/NN'MBA hydrated Copolymers versus the Temperature of Polymerization



8.5 SUSPENSION POLYMERIZATION OF THE ACRYLAMIDE-NN'METHYLENE BIS ACRYLAMIDE-H_2O SYSTEM

(a) Introduction

This system has been investigated in the Department of Chemistry by I Pasha, an MSc student, and the author. It was found, despite references in the literature, to be difficult to produce a workable system. In chronological order the work done was as follows: initial work by the author, the MSc project, and then further work by the author. The experimental work is described under these headings.

(b) Initial Experiments

The initial experiments involved basically a repeat of the work of Halpern and Greenburg.[134] A resin flask was set up in a water bath, 65°C, and a litre of paraffin oil added to it. As in Halpern's previous work a very high solvent to monomer ratio was used, (95:5), and the same catalyst system and stabilizers used, dimethyl amino propionitrile sodium persulphate and Tween 21/Span 20. The basic formulation used in the experiments is shown below:

Solution	- ACR, NN'MBA, DMApN, $N_aS_2O_8$ (catalyst system)		5%
Non-Solvent	- Paraffin		95%

+ Suspension stabilizer

The initial experiments were not very successful. This was probably due to the differences in procedure adopted as a different

product is desirable, ie one that is highly porous. This results in higher temperatures having to be used to dissolve the NN'MBA but this also results in a very fast polymerization even when the catalyst for the persulphate, dimethyl amino propionitrile is left out.

The materials made by the process described were tested and examined. The dehydrated particles were studied by scanning electron microscopy and the results are shown in photographs 37-39. It can be seen that the particles appear in actuality to be agglomerated masses of smaller beads, these are about 5 μ in diameter. The magnification of the photographs on Plate 19 are x55, x220 and x1100.

(c) The MSc Project of I Pasha[218]

In this work the same system as was used in the previously described work was used, ie an ACR/NN'MBA solution in liquid parrafin with stabilizers and initiators. Although a variety of stabilizers were tried and other factors such as stirring speed altered, no success was achieved and in most cases a large amount of gel was created. It was evident that basic changes in experimental set-up were necessary.

(d) Subsequent Experiments

The major modification introduced after the completion of Pasha's report[218] was the use of a silicone oil as the suspending medium in order to reduce the amount of agitation necessary to keep the acrylamide solution droplets in suspension as they are polymerizing. This is effected by the higher density of the medium which is

Plate I9. Photographs 37,38 and 39.

much closer to that of the acrylamide-water solution. The silicone oil used was DC 200/5 cs and this had a density of 0.9 gms/cm^3 which being close to that of water meant that less agitation was necessary.

Another modification that was found to give a more acceptable product was the use of glass anchor type stirrers. As this sweeps across the glass sides of the resin flask it prevents the build-up of gel on the glass which was observed in the previous work (polymerizing poly(acrylamide) has a tendency to stick to glass). Also a glass stirrer has an advantage over the metal stirrers used initially, as their high energy surfaces cause even more polymer to stick to them.

The material quantities and conditions used for the most successful experiment are shown in Table 66.

TABLE 66

Conditions and Quantities of Materials Used in Best Suspension Polymerization Experiment

Non Solvent - 250 mls of DC 200/5cs Silicone Fluid

Water Soluble System - Acrylamide - 5.0 gms
NN'MBA - 1.25 gms
H_2O - 6.25 gms

Initiator - Ammonium persulphate - 0.06 gms

Suspension Stabilizer - Span 20 (Sorbitan Monolaurate)

Ratio of Water Soluble System to Non Solvent - 5:95

Temperature of Polymerization - 50°C

Reaction time - 4 hours

Type of Stirrer - Anchor type (width 13.5 cms)

Stirring speed - 240 revs/min

The experimental procedure adopted for the experiment was the following; firstly the water soluble system, ie ACR and NN'MBA was dissolved in the water by heating at 50°C for one hour. This solution was then filtered and the initiator added and allowed to dissolve. The solution was then added to a purged stirred volume of silicone oil plus Span 20 and stirred for four hours at 50°C. Polymerization could be observed to have taken place after this time as white specks rather than clear solution droplets could be seen in the reaction flask. The polymer particles were then separated off, allowed to hydrate and examined.

The polymer or hydration was sieved and the fractions dehydrated and weighed. From these results Figure 63 was constructed and it can be seen that many large polymer particles are formed. Unfortunately, examining the particles produced shows that they are granular rather than spherical in shape; it appears that stirrer action is deforming the particles as they polymerize, hence creating granulated particles rather than the desired beads.

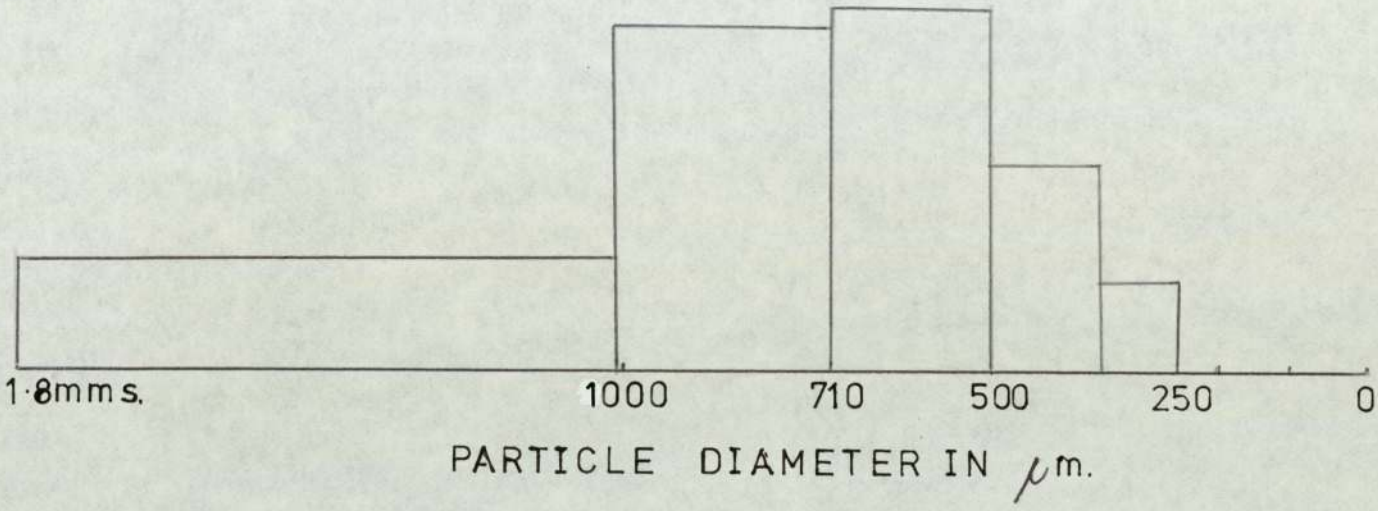
Further experiments with the same formulation but more agitation resulted in massive aggregation of the droplets to form a cloudy gel. It had been hoped to create smaller more stable droplets which would be less subject to deformation.

Similarly attempts with lower agitation, ie stirrer speeds in the region of 150 revs per minute, result in insufficient agitation leading again to aggregation.

Changes in the suspension stabilizer were considered but Span 20 proved to be best as it is soluble in silicone oil.

FIGURE 63

Size Distribution for Suspension-Polymerized Granules



8.6 CONCLUSION

The following conclusions have been drawn from the work on suspension polymerization and work related to it.

(1) It appears difficult to make large regular spherical beads of crosslinked polyacrylamide as the gel particles as they polymerize are very susceptible to deformation.

(2) The use of higher density non-solvents in the suspension polymerization is essential for the production of large beads. Therefore silicone oil rather than paraffin must be used.

(3) Further work with non-solvents of higher density and other suspension stabilizers will be necessary before the possibility of making hydrogel beads by this technique is dismissed.

CHAPTER 9

BLOOD COMPATIBILITY

9.1 INTRODUCTION

An attempt was made to assess the blood compatibility of the hydrogel membranes and beads by measuring the blood clotting times, which were found in an in vitro test. The tests were carried out at the Liver Unit, King's College Hospital by Dr R Hughes and Dr E H Dunlop; the technique basically consisted of passing fresh human blood into a cell containing a hydrogel membrane and measuring the time taken for the blood to clot. It was not an ideal method of measuring blood compatibility however, as the technique does not eliminate the blood-air interface, and, as has been mentioned before, plasma proteins denature at this interface causing an abnormal physiological condition to arise.

As well as assessing their blood compatibilities, possible interrelationships between such characteristics as surface rugosity, surface hydrophilicity, the water content of the gel and the blood clotting times were investigated.

The results of the blood clotting time tests for the different types of material;- homogeneous hydrogels, macroporous hydrogels and macroporous beads are given on the following pages.

9.2 RESULTS AND DISCUSSION

(a) Blood Clotting Time Experiments on Homogeneous Hydrogel Membranes

The results of the tests on homogeneous hydrogels are given in the following table, Table 67, which splits the hydrogels into separate series according to their composition. It will be seen that some of the results are repeated in separate series as they are copolymers of the main different monomers used to differentiate the large number of hydrogels tested.

The figures given in brackets after the hydrogel's composition are the initial monomer feed ratios of the monomers in the polymerization. It should also be noted that the values for the blood clotting times of the same hydrogels are often not very close and that in some cases the time could not be accurately determined and was said to lie between certain times, eg 30-35 minutes. This is more evidence of the difficulty of determining clot formation on hydrogels. The EWC's of the hydrogels are also given in the table.

It is interesting when considering the blood clotting times, to know what blood clotting times are obtained using the same technique on reference materials; this has been done and the results obtained are shown in Table 68, for thrombogenic glass and the widely used biomaterial, Cuprophane.

TABLE 67

Blood Clotting Times of Blood on Homogeneous Hydrogels

	Initial Molar Ratio	EWC %	Blood Clotting Time (mins)
1 Fluorine Containing Hydrogels			
(a) 2,2,2TFEM:HEMA	50:50	8.0	19, 25
(b) IPTFA:HEMA	50:50	11.0	22, 24
(c) IPTFMA:HEMA	50:50	12.0	28, 24
(d) 2,2,2TFEM:HEMA:ACR	25:50:25	22.0	30-35, 35, 22
2 Acrylamide Containing Hydrogels			
(a) ACR:AA	50:50	50.5	74, 95
(b) ACR:MAA	50:50	40.0	10, 12
(c) ACR:HEMA:MAA	25:50:25	38.5	27, 40
(d) ACR:HEMA:2,2,2TFEM	25:50:25	22.0	30-35, 35, 22
(e) ACR:HPA:EA	25:50:25	59.0	21, > 40
3 N-Vinyl Pyrrolidone Containing Hydrogels			
(a) NVP:MAA	75:25	58.5	27
(b) NVP:MAA	50:50	40.0	60, 90
(c) NVP:HPA:ST	45:45:10	61.5	15, 27, 40
(d) NVP:HEMA:MAA	25:50:25	54.5	30, 32
4 Methacrylic and Acrylic Acid Containing Hydrogels			
(a) MAA	-	73.5	18
(b) AA	-	73.0	90, 150
(c) MAA:HEMA	50:50	28.5	> 150
(d) MAA:NVP	50:50	40.0	60, 90
(e) MAA:ACR	50:50	40.0	10, 12
(f) MAA:HEMA:EDM			80
5 Hydroxyethyl Methacrylate Containing Hydrogels			
(a) HEMA	-	39.0	45-63, 34
(b) HEMA:FM*	50:50	8-12	19-28
(c) HEMA:ACR:MAA	50:25:25	38.5	27, 40
(d) HEMA:ACR:2,2,2TFEM	50:25:25	22.0	30-35, 35, 22
(e) HEMA:NVP:MAA	50:25:25	54.5	30, 32
(f) HEMA:MAA	50:50	28.5	> 150
(g) HEMA:MAA:EDM	50:50:8.5	25.0	80

* FM - Fluorinated Monomers in general

TABLE 68

Blood Clotting Times Using Same Technique on Other Materials

Material	EWC	BCT
Glass	0	22-26 minutes
Cuprophane	50.5%	47-70 minutes

Looking at the results for the hydrogels it can be seen that no individual group shows outstanding performance over any others, except that those containing the acidic monomers do in some cases show exceptionally high values, eg 150 minutes. However, it should be noted that this enhancement may not represent a desirable feature since it may be that the acidic hydrogel surface is adsorbing clotting factors from the blood and thus inactivating the clotting mechanism.(139)

(b) Blood Clotting Time Experiments on Macroporous Hydrogels

The same technique as that which was used on the homogeneous membranes was applied to the macroporous membranes; the results obtained are shown in Table 69.

TABLE 69

Blood Clotting Times Obtained on Macroporous Membranes

Macroporous Membranes Composition	EWC	BCT (mins)
(a) HEMA:EDM (92:8)	36.5%	32, 40
(b) HEMA:EDM (92:8)	43.0%	36, 30
(c) HEMA:EDM (92:8)	52.0%	28, 40
(d) HEMA:EDM (92:8)	56.0%	16, 40
(e) HEMA:NVP:EDM (50:50:8.5)	45.0%	28, 40
(f) HEMA:MAA:EDM (75:25:8.5)	42.0%	150
(g) ACR:EDM (100:8.5)	77.0%	16

It can be seen that (with the exception of the single result for the HEMA:MAA:EDM macroporous membrane), the blood clotting time results obtained are slightly lower than the overall results for the homogeneous membranes. Particularly interesting in this respect are the results for the crosslinked macroporous HEMA membranes, which are significantly lower than the results for the homogeneous poly HEMA membrane. It seems likely that the increase in surface rugosity and the macroporous nature of the gel are causing this loss in biocompatibility.

(c) Blood Clotting Time Studies on Macroporous Beads and Comparable Materials

A very limited amount of work on the blood clotting times exhibited by the macroporous beads has been carried out; the results obtained by Dr Dunlop and Dr Hughes are shown below in Table 70, with their results on comparable materials.

TABLE 70

Blood Clotting Times Obtained for Macroporous Beads and Other Materials

Material	Composition	Initial Molar Feed Ratio	BCT (mins)
a) Macroporous Beads	(S2) HEMA:EDM	(100:4)	21
	(S3) HEMA:EDM	(100:2)	27
	(S4) HEMA:EDM	(100:1)	27
	(S5) AA:EDM	(100:8.5)	18
	(S8) HEMA:MAA:EDM	(75:25:8.5)	21
b) Comparison Materials			
XAD-7			12
XAD-7 (albumin coated)			17
XAD-7 (albumin coated and autoclaved)			18
			18
Charcoal granules coated with 4% poly HEMA			12

It should be noted that the technique used in these experiments was a variant of the one described previously, for these beads were wrapped in cuprophane and the blood then passed over them. This was done to prevent the large thrombogenic surface of the test cell contacting the blood and swamping any thrombogenic effect from the beads themselves; however, it does mean that the results obtained are not directly comparable to those obtained for the membranes since the blood clotting time of the cuprophane imposes an upper limit on the observed blood clotting time.

From Table 70 it can be seen that the macroporous beads appear to be marginally better than the comparison materials as regards to the length of blood clotting times. It is interesting to compare the results for the beads with those obtained by the membranes (the membranes caused the blood to clot much later); since the composition of the materials is very similar it appears that the higher surface rugosity of the beads is responsible for the difference, but it must also be noted that the test procedure is slightly different.

A further interesting point to be noted is that the coated charcoal granules (Smith and Newphew's), have a much lower blood clotting time than the material which coats them, poly HEMA. This suggests that the coating may not cover the entire surface of the granules or is ineffective for other reasons.

It can also be seen that the resin XAD-7 even after various treatments such as coating with albumin and stabilizing the coating on the surface by autoclaving which denatures and polymerizes it, does not lead to great enhancement of blood compatibility vis a vis the hydrogel materials.

9.3 THE RELATION OF BLOOD CLOTTING TIMES TO OTHER FACTORS

Following the determination of the blood clotting times for the various hydrogels, an attempt was made to relate these measurements to other characteristics of the hydrogels such as their equilibrium water contents, compositions, surface properties and types of water present in the gels.

(a) Equilibrium Water Content

The blood clotting times of the hydrogels were plotted against their equilibrium water contents; the graph showed no observable relationship between EWC and blood clotting time either for the hydrogels studied in general or specific groups of similar composition.

(b) Surface Free Energy and Critical Surface Tension

The surface free energies and critical surface tensions of many of the hydrogels tested for blood clotting times were measured by Andrew Barnes as part of his research project. The measurements were carried out on dehydrated samples of hydrogel and it is therefore not surprising that no correlations were found between the blood clotting times of the hydrated materials and the surface properties of the materials in the dehydrated state, as the surface properties are greatly effected by hydration.

(c) Surface Hydrophilicity (Hamilton Contact Angle)

Following the failure to relate the clotting times to the surface properties of dehydrated hydrogels, (Xerogels), the hydrogel surfaces were examined by the Hamilton technique. Again most of the

work was carried out by Andrew Barnes on materials prepared and characterized by the author. Table 71 shows the compositions, blood clotting times and Hamilton contact angles of various hydrogels.

From the table no clear picture of a relationship of Hamilton contact angle and blood clotting time emerges; however, if the results are graphed a distinct relationship does emerge: Figure 64.

It can be seen from the graph that there appear to be two distinct classes of hydrogels showing a different relationship between blood clotting times and Hamilton contact angle. Firstly there are the non-ionic hydrogels that show the expected increase in blood clotting times with increasing Hamilton contact angle, although it must be stressed that the relationship is not well defined. The two high peaks in this particular series are poly HEMA and Cuprophane, both well known biomaterials; they both lie at the high end of the Hamilton contact angle spectrum.

Secondly, there are the acrylic acid and methacrylic acid containing hydrogels, many of these appear to lie in a band showing an inverse relationship between Hamilton contact angle and blood clotting times. However, there are exceptions in the acrylic acid and methacrylic acid series especially those which have exceptionally low contact angles; these show lower blood clotting times. It may therefore possibly be that this relationship only occurs for the more hydrophilic hydrogel surfaces.

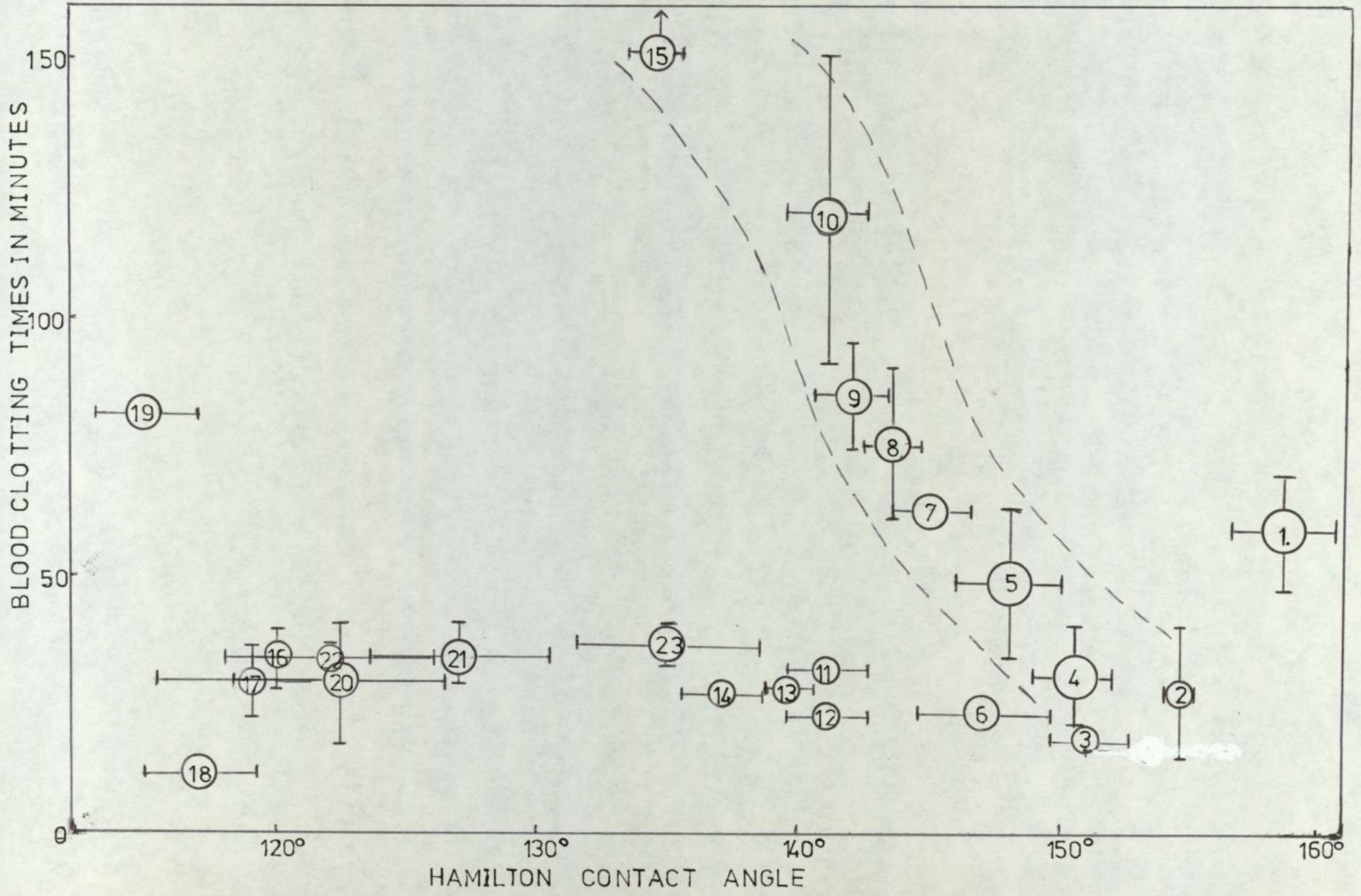
TABLE 71

The Relationship between Hamilton Contact Angle and the Blood Clotting Times of Hydrogels

Material	Hamilton Contact Angle	BCT (mins) Blood Clotting Time
(a) Homogeneous Hydrogels		
(1) Cuprophane	$158.5 \pm 2^{\circ}$	47-70
(2) HPA:NVP:ST (45:45:10)	$154.5 \pm 0.5^{\circ *}$	15, 27, 40
(3) MAA	$151 \pm 1.5^{\circ *}$	~18
(4) ACR:HPA:EA (25:50:25)	$150.5 \pm 1.5^{\circ *}$	21, >40
(5) HEMA	$148 \pm 2^{\circ *}$	45-63, 34
(6) HEMA:HFIA (50:50)	$147 \pm 2.5^{\circ *}$	22, 24
(7) NVP:AA (75:25)	$145 \pm 1.5^{\circ *}$	~62
(8) NVP:MAA (50:50)	$143.5 \pm 1^{\circ *}$	60, 90
(9) ACR:AA (50:50)	$142 \pm 1.5^{\circ *}$	74, 95
(10) AA	$141 \pm 1.5^{\circ *}$	90, 150
(11) HEMA:NVP:MAA (50:25:25)	$141 \pm 1.5^{\circ *}$	30, 32
(12) HEMA:2,2,2TFEM (50:50)	$141 \pm 1.5^{\circ *}$	19, 25
(13) NVP:MAA (75:25)	$139.5 \pm 1^{\circ *}$	~62
(14) HEMA:HFIMA (50:50)	$137 \pm 1.5^{\circ *}$	28, 24
(15) HEMA:MAA (50:50)	$134.5 \pm 1^{\circ *}$	>150
(16) HEMA:ACR:MAA (50:25:25)	$120^{\circ *}$	27, 40
(17) HEMA:ACR:2,2,2TFEM (50:25:25)	$119 \pm 3.5^{\circ *}$	30-35, 35, 22
(18) ACR:MAA (50:50)	$117 \pm 2^{\circ}$	10, 12
(19) HEMA:MAA:EDM	$115 \pm 4^{\circ}$	80
(b) Macroporous Hydrogels		
(20) HEMA:EDM (100:8.5)	$122.5 \pm 4^{\circ}$	16, >40
(21) " " " "	$127 \pm 3.5^{\circ}$	28, >40
(22) " " " "	$122 \pm 4^{\circ}$	36, 30
(23) " " " "	$135 \pm 3.5^{\circ}$	32, >40

FIGURE 64.

BLOOD CLOTTING TIMES OF HYDROGEL MEMBRANES AGAINST THEIR SURFACE HYDROPHILICITIES AS MEASURED BY HAMILTON CONTACT ANGLE TECHNIQUE.



9.4 CONCLUSIONS

Although the work done has been fairly limited the following general conclusions have been arrived at.

(1) The EWC of a hydrogel does not appear to be an important factor in determining the degree of blood compatibility of a hydrogel.

(2) Surface rugosity and surface hydrophilicity do appear to be important factors in determining a hydrogel's degree of compatibility with blood.

CHAPTER 10

ADSORPTION STUDIES

10.1 INTRODUCTION

Adsorption studies have been carried out on the materials synthesized in order to determine how effective as adsorbents they are in comparison with other materials such as XAD-2, XAD-7 and Dowex I-X4, which have been used in clinical and experimental toxin removal.

The substances chosen for this study were BSP and unconjugated bilirubin, both commonly used in studies of this kind. Both these substances have been previously mentioned, the reasons for their choice in these experiments was that they can be easily colourimetrically assayed. Also, as regards to bilirubin, this is known minor toxin, which is strongly bound to albumin; thus its removal demonstrates good adsorptive power for the material under study.(219)

The means of assay for these materials has been already described in Chapter 4; in this chapter the results of the adsorption experiments will be dealt with. The experimental results have been divided into the following sub-sections:-

10.2 The Adsorption of Bilirubin from Plasma (Kinetic study)

10.3 The Adsorption of Bilirubin from Plasma (Equilibrium adsorption study)

10.4 The Adsorption of BSP from aqueous solution (Equilibrium adsorption study)

10.5 Miscellaneous Experiments

10.2 THE ADSORPTION OF BILIRUBIN FROM PLASMA (KINETIC STUDY)

This experimental work was carried out early in the project and was simply done to observe if any adsorption of bilirubin by hydrogel beads occurred, and if so over what sort of time scale this could be observed. The hydrogel bead types used are shown in Table 72.

TABLE 72

Hydrogel Beads Used in Kinetic Study

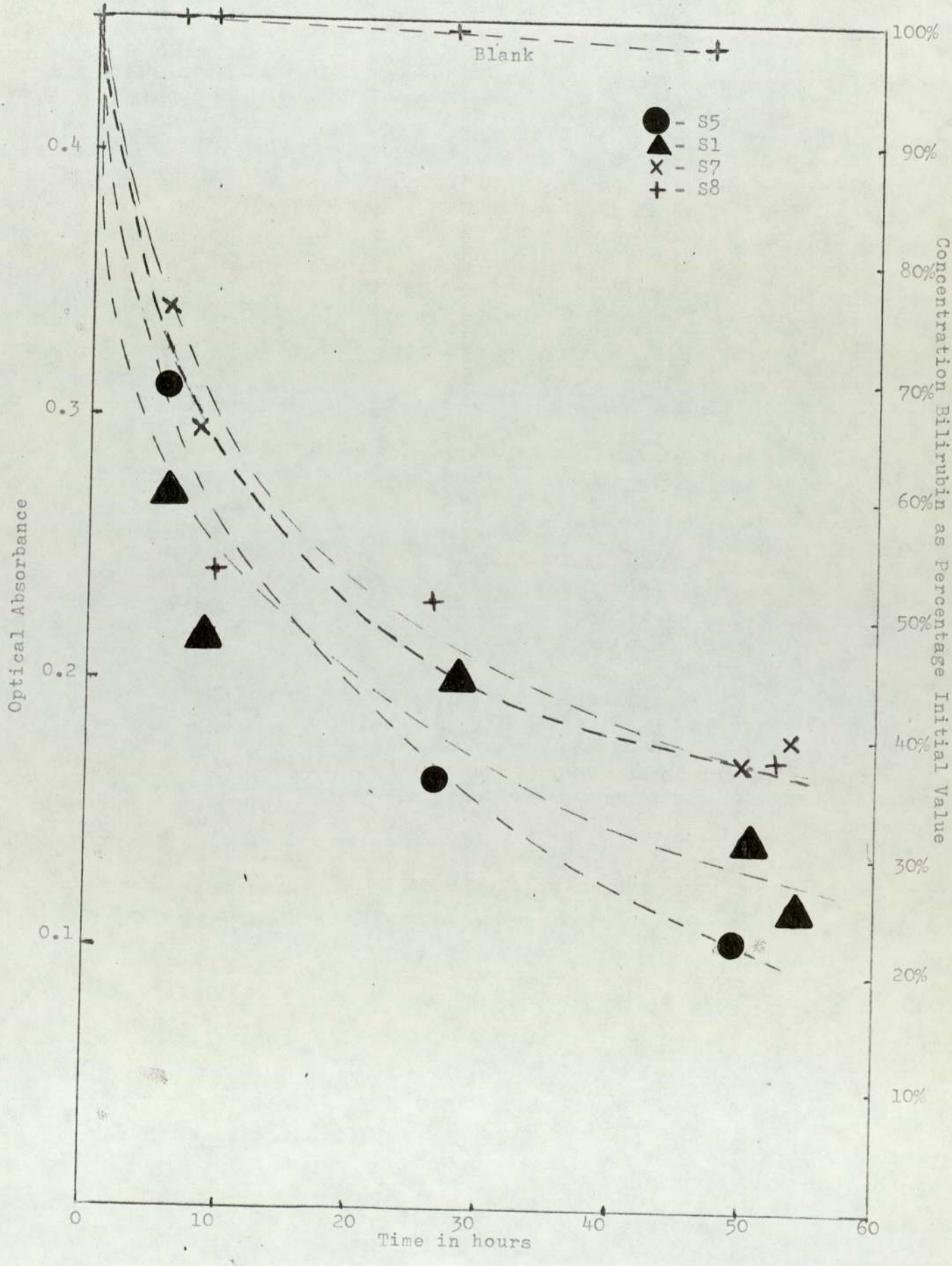
1) S1 - HEMA/EDM (100:8.5)
2) S5 - AA/EDM (100:8.5)
3) S7 - HEMA/MAA/EDM (75:25:8.5)
4) S8 - AA/HEMA/EDM (25:75:8.5)

These initial experiments were not and could not be very accurate as the volume of solution used for the test is quite large compared to the original volume of bilirubin solution, (5%), and as the tests carried out at later stages involve even greater volumes of liquid on a percentage basis, it can be seen that the results obtained only give a rough indication of adsorption. As the test too is not particularly accurate, it is obvious that no true value can be calculated for the amount of bilirubin adsorbed.

The results of the experiments are shown in Figure 64, which is a graph of the absorbance of the tested bilirubin solution, (and hence the bilirubin concentration) against time in hours. Also shown are the results for a blank solution of bilirubin, and it can be seen that in the deoxygenated solution kept in the dark there is

FIGURE 64

Adsorption of Bilirubin from Plasma by Macroporous Hydrogel Beads with Time



little degradation at room temperature.

The figure shows that all the hydrogel beads examined showed adsorption over the time period taken, 55 hours, and the concentration of bilirubin was still dropping at the end of this period. The process appeared to be that of an initially very fast adsorption followed by a more gradual effect which levels off and appeared to be reaching equilibrium. The experiment had, however, to be terminated as plasma solutions do not last for long periods of time at room temperature.

The initial fast drop in bilirubin concentration may however be caused simply by the dilution effect which is due to the exchange of solutions between the macropores in the hydrogel beads and the bilirubin solution that surrounds them; this leads to effective reduction in the concentration of bilirubin in solution. However in the present case this effect cannot explain the total reduction of the bilirubin concentration as the maximum effect could only be in the region of $\sim$25%, ie if all the water in the hydrogel is available to dilute the bilirubin concentration.

Therefore this experiment demonstrated that there was a real adsorption of bilirubin by hydrogel beads; it was however necessary to carry out an equilibrium adsorption study to determine the precise amounts adsorbed.

10.3 THE ADSORPTION OF BILIRUBIN FROM PLASMA (EQUILIBRIUM ADSORPTION STUDY)

In these experiments, unlike the much earlier kinetic study the adsorption of bilirubin from plasma was examined against various comparison materials, eg XAD-7, XAD-2, activated charcoal and Dowex I-X4. In this way some idea of the effectiveness of hydrogel beads as adsorbents as against other materials was gained. The acrylamide granules made by suspension polymerization were also examined.

For this study two solutions of bilirubin in plasma were prepared, the concentrations used were 20 mg/100 ml and 8.5 mg/100 ml (the first of these being close to the maximum value of bilirubin in plasma that can be found, ie at the limit of its solubility). The materials used to study the adsorption are shown in Table 73.

TABLE 73

Adsorbents used in Study of Bilirubin Adsorption and their Compositions and Properties

		EWC	Composition
(a) Hydrogel Beads	S9	44.5%	HEMA(90)EDM(10)
	S10	57.0%	HEMA(50)AA(50)EDM(10)
	S11	49.0%	HEMA(75)AA(25)EDM(10)
(b) Comparison Materials	XAD-2	-	Polystyrene
	XAD-7	-	Poly Methacrylate
	Dowex I-X4	-	Ion exchange resin
	Activated Carbon	-	
(c) p(Acrylamide) Granules	-	54.0%	ACR/NN'MBA

Three special types of hydrogel beads were prepared for these tests, their compositions are given in Table 73 with their code number. They were extensions of the large macroporous bead series prepared by the freezing of monomer solution droplets and subsequent polymerization.

The procedure adopted was that given in Chapter 4 the mass of beads or resin particles used was such that the dry weight of resin or beads was half a gram. The volume of bilirubin solution was 5 ml in each case. The time allowed for adsorption to occur was 50 hours as the kinetic study had shown that equilibrium adsorption had almost been achieved by this time.

The following tables, 74 and 75, show the concentrations of bilirubin in plasma after the period of adsorption by the hydrogel beads and the comparison materials has taken place.

TABLE 74

Adsorption of Bilirubin by Various Materials

Series I	Concentration mgs/100 ml	Initial Concentration in Sample Bottle Allowing for Dilution effect
Initial Concentration (Blank)	20	20 mgs/100 ml
Activated charcoal	8.9 ± 0.7	"
XAD-7	6.3 ± 0.3	"
XAD-2	17.8 ± 0.5	"
Dowex I-X4	4.4 ± 0.5	"
Hydrogel Beads S9	15.0 ± 0.5	18.5 mgs/100 ml
S10	5.3 ± 0.1	17.9 "
S11	9.7 ± 0.5	18.2 "
Acrylamide Granules	18.8 ± 0.3	19.0 "

TABLE 75

Adsorption of Bilirubin by Various Materials

Series 2	Concentration mgs/100 ml	Initial Concentration in Sample Bottle Allowing for Dilution effect
Initial Concentration (Blank)	8.5	8.5 mgs/100 ml
Activated Charcoal	4.8 ± 0.4	"
XAD-7	4.1 ± 0.3	"
XAD-2	8.0 ± 0.3	"
Dowex I-X4	4.2 ± 0.6	"
Hydrogel Beads S9	6.5 ± 0.4	7.8 mgs/100 ml
S10	3.3 ± 0.4	7.6 "
S11	4.7 ± 0.1	8.1 "
Acrylamide Granules	7.8 ± 0.3	8.1 "

As can be seen from the Tables, allowance has been made for the dilution effect in the case of the hydrogels as the water content of these materials effectively reduces the bilirubin concentration in the system.

From the results given in Tables 74 and 75, and knowing the weights of beads used it is possible to calculate the amount of bilirubin adsorbed per gram of dry resin, and these results are plotted for the two series in Figure 65.

As can be seen from the graph of these results, Figure 65, the hydrogel beads compare favourably with the comparison materials such as Dowex I-X4, XAD-7 and activated carbon as regards adsorption. The hydrogel results are shown in the form of a band as it is not possible to calculate the exact contribution from the dilution

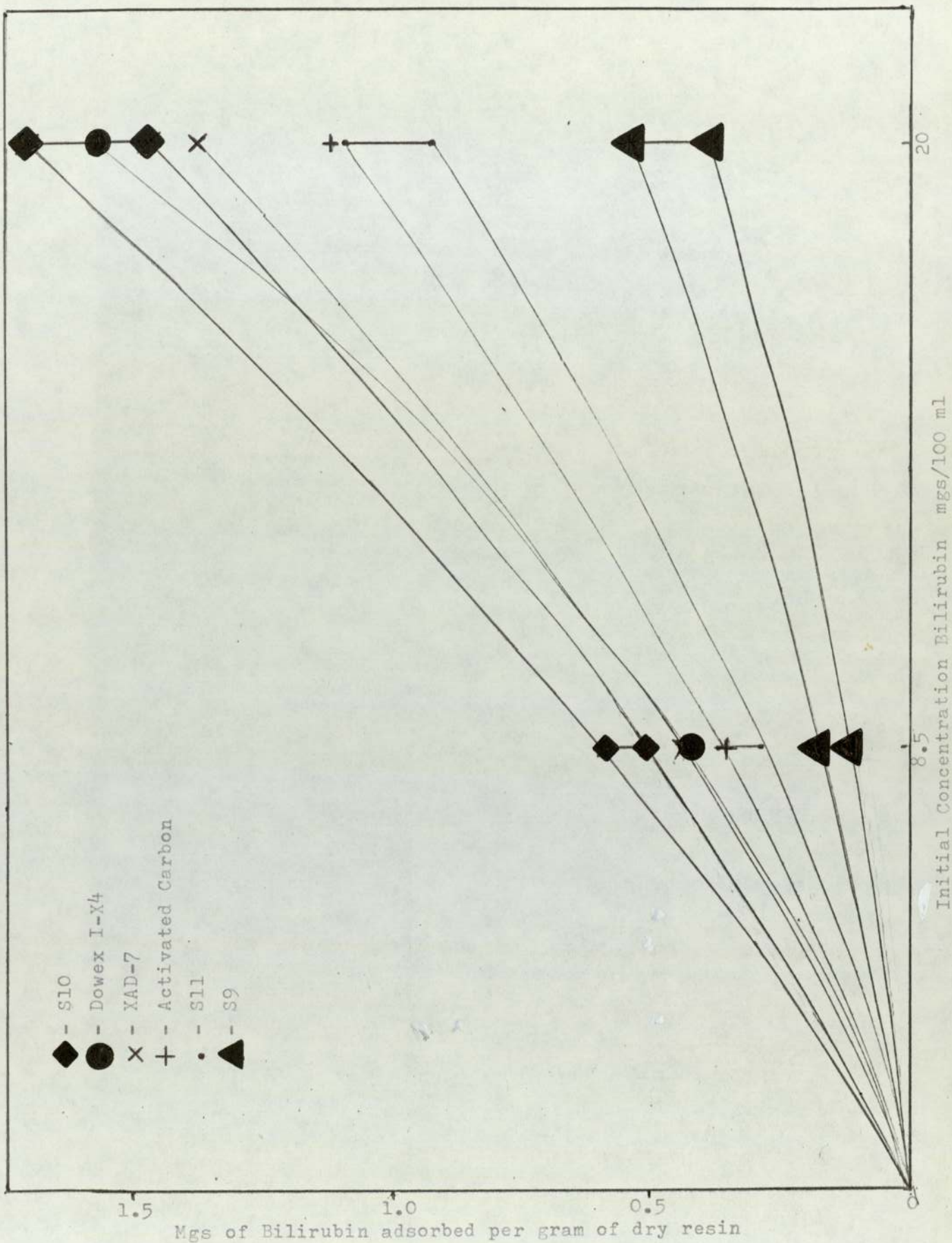


FIGURE 65

Amount of Bilirubin adsorbed per gram of dry resin for Hydrogel Beads and Other Materials

effect as not all the water in the beads will be available to contribute to the dilution of the bilirubin concentration. It is likely that only the water in the macropores is available to dilute the surrounding bilirubin solution, but as the relative amounts of the two types of water in the macropores and polymer matrix are not known, the true value for adsorption will lie within the band which gives the values that will be found if all the water in the beads takes part in the dilution effect or if more of it is involved, ie, the top value is made assuming that there is no dilution effect and the bottom value is made assuming the dilution effect is total.

The most noticeable feature of the hydrogel bead experiments was the increase in adsorption as the acrylic acid concentration in the beads increased, this can be attributed to increased hydrogen bonding occurring between the bilirubin molecule and the polymer, due to interaction between the carboxylic acid groups of acrylic acid and bilirubin.

XAD-2, the polystyrene resin, did not adsorb bilirubin at all, therefore it appears that ion exchange or some sort of polar interaction is necessary for the adsorption of bilirubin.

10.4 THE ADSORPTION OF BSP FROM SALINE SOLUTION (EQUILIBRIUM ADSORPTION STUDY)

The adsorption of BSP from saline solution was also investigated as it was thought that it would be interesting to find out if an ionic species such as BSP would be adsorbed by a hydrogel, in comparison with activated charcoal, ion exchange resins and other

sorts of adsorbents. The basic experimental set-up is the same as that described for the adsorption of bilirubin. The same weights of beads and volume of solution were used; the concentrations of BSP used were 1×10^{-3} ML^{-1} and 1×10^{-4} ML^{-1}. The results are shown in Table 76.

TABLE 76

Adsorption of BSP by Adsorbents

Adsorbent	Initial (BSP)	Final (BSP)
Activated Carbon	1×10^{-3} ML^{-1}	0.18×10^{-3} ML^{-1}
	1×10^{-4} ML^{-1}	0.23×10^{-4} ML^{-1}
XAD-7	1×10^{-3} ML^{-1}	0.12×10^{-3} ML^{-1}
	1×10^{-4} ML^{-1}	0.18×10^{-6} ML^{-1}
Dowex I-X4	1×10^{-3} ML^{-1}	0
	1×10^{-4} ML^{-1}	0

As can be seen from Table 76, the three adsorbents caused a great adsorption of BSP especially, the ion exchange resin, Dowex I-X4, which completely adsorbed the BSP from solution.

However in the case of the hydrogel beads, of whatever sort these apparently showed a minimal adsorption, but when the dilution effect was taken into consideration the adsorption effect disappeared and it is concluded that no adsorption had taken place. A similar effect was seen for XAD-2.

10.5 MISCELLANEOUS EXPERIMENTS

In addition to the previously described experiments certain others were carried out involving primarily pieces of hydrogel membrane which had been cut up into small sized discs with a cork borer. These were then placed in sample bottles with the plasma-bilirubin solutions.

It was intended that as there was much greater variation in the composition of the homogeneous hydrogel membranes than those of the beads, that more information could be gained as to the effect of polymer composition on adsorption. However, reproducable results were not obtained from these experiments; the principal cause of this failure is believed to be the stickiness of the hydrogel surfaces in plasma solution, which results in different surface areas of polymer being available for adsorption in different experiments. Such an effect is not seen in the case of the hydrogel polymer beads as the surface areas of beads which can be in contact with each other is much less.

10.6 CONCLUSIONS

1 Hydrogel beads are capable of the adsorption of bilirubin from plasma solution, and are capable of adsorbing amounts comparable to materials used by other researchers, ie XAD-2, XAD-7 and Dowex I-X4.

2 The amount of bilirubin adsorbed in the case of the large hydrogel beads is directly related to the concentration of acrylic acid in the polymer matrix.

3 Hydrogel beads will not be good adsorbents for ionically changed toxins, unless ion exchange groups are incorporated in their structure.

4 Given the difference in adsorption capability of hydrogel beads towards organic molecules (bilirubin), and ionic species, (BSP), it may be hydrogel beads could be developed as a selective adsorbent. This is important as a range of substances with different adsorptive capabilities is desired by many workers in the field of liver support (220) to enable a more selective adsorption to take place in individual cases of liver failure. Thus enabling the adsorption of toxins to be more closely tailored to suit the individual patient.

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CHAPTER 6

A P P E N D I X B

STRENGTH OF THE HOMOGENEOUS HYDROGELS

The following table, Table 77, shows the relative strengths of the homogeneous hydrogels made.

TABLE 77

Relative Strengths of the Homogeneous Hydrogels

Membrane	Composition	Initial Molar Ratio	Strength
A1	HEMA:MAA	75:25	Strong
A2	"	50:50	Strong
A3	"	25:75	Strong
A4	MAA	0:100	Weak
A5	HEMA:AA	75:25	Weak
A6	"	50:50	Weak
A7	"	25:75	Weak
A8	AA	0:100	Strong
A9	NVP	100:0	Very weak
A10	NVP:MAA	75:25	Strong
A11	"	50:50	Tough
A12	"	25:75	Tough
A13	NVP:AA	75:25	Weak
A14	"	50:50	Quite Strong
A15	"	25:75	Weak
A16	ACR:MAA	50:50	Strong
A17	ACR:MAA	25:75	Weak
A18	ACR:AA	50:50	Strong
A19	"	25:75	Weak
A20	HEMA:ACR	50:50	Weak
A21	"	75:25	Weak
B1	AA:EDM	100:8.5	Weak
B2	"	100:4	Weak

As can be seen from some of the series in the table such as NVP:MAA and NVP:AA, the strength of many of these compositions reaches a maximum at the equimolar point of the copolymer compositions.

A P P E N D I X C

OPTICAL PROPERTIES OF HOMOGENEOUS HYDROGELS

The Optical Properties of the homogeneous hydrogels are shown below in Table 78.

TABLE 78

Optical Properties of the Homogeneous Hydrogels

Membrane	Composition	Initial Molar Ratio	Transparency
A1	HEMA:MAA	75:25	Transparent
A2	"	50:50	Transparent
A3	"	25:75	Translucent
A4	MAA	0:100	Translucent
A5	HEMA:AA	75:25	Transparent
A6	"	50:50	Transparent
A7	"	25:75	Transparent
A8	AA	0:100	Translucent
A9	NVP	100;0	Translucent
A10	NVP:MAA	75:25	Opaque
A11	"	50:50	Translucent
A12	"	25:75	Translucent
A13	NVP:AA	75:25	Transparent
A14	"	50:50	Transparent
A15	"	25:75	Transparent
A16	ACR:MAA	50:50	Translucent
A17	"	25:75	Translucent
A18	ACR:AA	50:50	Translucent
A19	"	25:75	Opaque
A20	HEMA:ACR	50:50	Transparent
A21	"	75:25	Transparent
B1	AA:EDM	100:8.5	Translucent
B2	"	100:4	Translucent

A P P E N D I X D

PROPERTIES OF THE ACR/NNMBA MEMBRANES

The following table shows the relevant properties that were examined:

TABLE 79

Properties of the ACR/NNMBA Membranes

Membrane	Initial Weight Ratio of ACR:NNMBA	Initial Monomer Solvent Ratio	EWC
D1	4.5:0.5	50:50	64 ± 1%
D2	4:1	50:50	54.0 ± 0.5%
D3	2:1	50:50	50.0 ± 2.5%
D4	3:2	50:50	49.5 ± 1.5%
D5	1:1	50:50	53.5 ± 1.0%
D6	2:1	40:60	57.0 ± 0.5%
D7	3:2	40:60	59.5 ± 0.5%
D8	2:1	30:70	67.5 ± 1.0%
D9	3:2	30:70	68.5 ± 0.5%
D10	2:1	20:80	81.0 ± 1.0%

Membrane	Strength	Transparency	Hamilton Contact Angle
D1	Strong	Transparent	143 ± 2.5°
D2	Tough	Transparent	128.5 ± 2°
D3	Weak	Translucent	129 ± 5°
D4	Fairly Strong	Translucent	139 ± 2.5°
D5	Weak	Translucent	142 ± 2°
D6	Weak	Opaque	155 ± 3.5°
D7	Quite Strong	Opaque	126.5 ± 2°
D8	Weak	Opaque	149 ± 3°
D9	Weak	Opaque	126 ± 3.5°
D10	Very Weak	Opaque	144 ± 2.5°

A P P E N D I X E

THE ACR:EDM MEMBRANES MADE BY UV POLYMERIZATION

Some acrylamide membranes were made with ethylene dimethacrylate in solution by UV polymerization using uranyl nitrate as the photo-initiator. They are designated C1-C3 and their properties are shown below in Table 80.

TABLE 80

Properties of ACR:EDM Membranes and Conditions of Polymerization

Membrane	Initial Monomer Ratio, ACR:EDM	Monomer:Solvent*	EWC
C1	99:1	60:40	85%
C2	97.5:2.5	60:40	76.5%
C3	95:5	60:40	61.0%

Membrane	Strength	Transparency
C1	Fairly Strong	Transparent
C2	Fairly Strong	Transparent
C3	Fairly Strong	Translucent

* Solvent - Ethylene glycol:Water (1:4)

The EWC values are given also in Figure 33

A P P E N D I X F

PROPERTIES OF THE HEMA:NNMBA MEMBRANES

The properties of the few HEMA:NNMBA membranes made are shown below in Table 81.

TABLE 81

Properties of the HEMA:NNMBA Membranes

Membrane	Initial Molar Ratio of HEMA:NNMBA	Initial Monomer: Solvent Ratio	Temperature of Polymerization
D11	75:25	50:50	50°C
D12	75:25	50:50	40°C
D13	75:25	50:50	30°C

Membrane	EWC	Optical Properties	Strength
D11	43.5 ± 1.0%	Transparent	Strong
D12	41.5 ± 0.5%	Translucent	Strong
D13	38.0 ± 0.5%	Opaque	Weak

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