Protein Deposition at Polymer Surfaces: The Bulk and Surface Structure-Property Effects of Hydrogels.

By

David Baker.

A thesis submitted for the degree of:-

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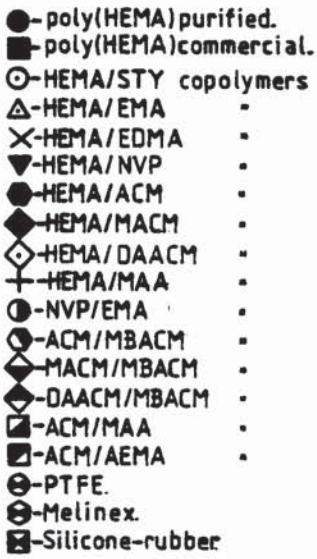
June, 1982.

General Key for the Scatter-graphs:



* N.B. Numerical suffix to the symbols denotes the second monomer content (Mole %)

THE UNIVERSITY OF ASTON IN BIRMINGHAM

PROTEIN DEPOSITION AT POLYMER SURFACES.

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SUMMARY

The primary objective of this research has been to investigate the interfacial phenomenon of protein adsorption in relation to the bulk and surface structure-property effects of hydrogel polymers. In order to achieve this it was first necessary to characterise the bulk and surface properties of the hydrogels, with regard to the structural chemistry of their component monomers. The bulk properties of the hydrogels were established using equilibrium water content measurements, together with water-binding studies by differential scanning calorimetry (D.S.C.). Hamilton and captive air bubble-contact angle techniques were employed to characterise the hydrogel-water interface and from which by a mathematical derivation, the interfacial free energy (γ_{sw}) and the surface free energy components (γ^{p}_{sv}, γ^{d}_{sv}, γ_{sv}) were obtained.

From the adsorption studies using the radio labelled iodinated (^{125}I) proteins of human serum albumin (H.S.A.) and human fibrinogen (H.Fb.), it was found that multilayered adsorption was occurring and that the rate and type of this adsorption was dependent on the physico-chemical behaviour of the adsorbing protein (and its bulk concentration in solution), together with the surface energetics of the adsorbent polymer. A potential method for the invitro evaluation of a material's 'biocompatibility' was also investigated, based on an empirically observed relationship between the adsorption of albumin and fibrinogen and the 'biocompatibility' of polymeric materials. Furthermore, some consideration was also given to the biocompatibility problem of proteinaceous deposit formation on hydrophilic 'soft' contact lenses and in addition a number of potential continual wear contact lens formulations now undergoing clinical trials, were characterised by the above techniques.

KEY WORDS

Hydrogel
Water-Interactions
Interfacial free energy
Biocompatibility
Multi-layered protein adsorption

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- - - -

This thesis is dedicated to Anna.

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GLOSSARY OF ABBREVIATIONS

HEMA	-	2-Hydroxy ethyl methacrylate
HPA	-	2-Hydroxy propyl acrylate
MMA	-	Methyl methacrylate
EMA	-	Ethyl methacrylate
STY	-	Styrene
NVP	-	N-vinyl pyrrolidone
ACM	-	Acrylamide
MACM	-	Methacrylamide
DAACM	-	Diacetone acrylamide
MBACM	-	N,N', Methylene-bis-acrylamide
EDMA	-	Ethylene glycol dimethacrylate
MAA	-	Methacrylic acid
AA	-	Acrylic acid
AEMA	-	Amino ethyl methacrylate
EWC	-	Equilibrium water content
HSA	-	Human serum albumin
HFb	-	Human fibrinogen
γ^{d}_{sv}	-	Surface free energy dispersive component
γ^{p}_{sv}	-	Surface free energy polar component
γ_{sv}	-	Total surface free energy
γ_{sw}	-	Interfacial free energy (tension)

N.B. The units of surface/interfacial free energy given in this work are in accordance with the traditional surface chemistry (C.G.S.) nomenclature of dynes. $cm.^{-1}$ however, with respect to the S.I. nomenclature please note that dynes. $cm.^{-1}$ = mN . $m.^{-1}$.

CHAPTER ONE

INTRODUCTION

1.1 General Introduction

The current widespread use of polymeric materials in many biomedical applications can be generally attributed to their wide range of material properties, their ready fabrication into many different forms, and their ease of chemical modification, (especially on the surface). Unfortunately, up until 10 - 15 years ago, the majority of polymeric materials used in biomedical applications were chosen somewhat empirically from commercially available materials, with sometimes disasterous consequences. In general there are two major problems associated with the bulk properties of such polymers:

(i) leaching of unreacted bulk fabrication products, which may cause local or systemic body reactions.

(ii) susceptibility to hydrolysis and/or enzymic attack in the body, deteriorating their physical properties, as well as releasing their degradation products into the body.

Even with the introduction of 'medical grade' polymeric materials, (with high biostability and very low leachable impurities), many deliterious effects resulted, simply due to the interface induced reactions when exposed to body fluids. When the more 'traditional' hydrophobic polymers come into contact with body fluids, intense interactions can occur at the interface which may ultimately lead to rejection reactions such as

excessive inflammation, extensive encapsulation, thrombosis and carcinogenesis. The need for more 'biocompatible' materials was soon evident however, the somewhat intangible complexities involved in actually defining and measuring the composite functional properties of the term 'biocompatibility', have made progress very difficult.

With the advent of hydrophilic polymeric materials (hydrogels), for biomedical applications, the inherent advantages of this type of material (such as soft tissue compatibility and high gas permeability), overcame many of the above disadvantages of the hydrophobic materials. Some background knowledge of biomedically useful hydrogels and their applications will be covered in the latter part of this chapter. Further details with regards the bulk and surface properties of hydrogels are covered more fully in Chapters 4 and 5, respectively.

A well-known phenomenon which is common to both types of material, and also to any foreign materials of natural or synthetic origin, is the interfacial phenomenon of protein adsorption. As early as 1925 research workers[1,2] showed that various materials when immersed in a protein containing solution adsorbed these proteins onto their surfaces. There are many examples, in the normal course of day to day events, in which materials come into contact with various biological solutions (e.g., blood, saliva, tears, sea-water) and in so doing demonstrate this

phenomenon of protein adsorption. This primary adsorption process then exerts a profound influence over subsequent events in the relevant biological environment and gives rise to such well recognised processes as thrombus formation (in blood)[3], the formation of dental plaque (in saliva)[4] and marine fouling (in sea-water)[5]. In addition, it appears that there is now good evidence to show that the phenomenon plays a role in fertility control by intrauterine devices (I.U.D.'s)[6]. To this list must now be added the progressive formation of deposits on contact lenses[7]. This latter problem has only been highlighted during recent years in the contact lens field, following the increased use of hydrophilic 'soft' contact lenses. A more complete discussion of this problem together with specific considerations relating solely to hydrophilic 'soft' contact lens wear, is given in Chapter 7.

To date most studies of these manifestations of the interfacial phenomenon of protein adsorption, have been in the field of blood contact materials, in particular with relation to the thromboresistance or 'biocompatibility' of the materials. Because of the apparent similarity of these various types of interactions of materials with biological environments, it is obvious that an understanding of one area will facilitate progress in another. In particular the following overview of the 'problem of biocompatibility' will aim to relate the above known blood contact behaviour to the associated problems of biocompatibility in 'soft' contact lens wear.

1.2 The Problem of Biocompatibility

1.2.1 Biocompatibility

The terms biocompatibility and thromboresistance are not synonymous with each other, however, they are often mistakenly interchanged; biocompatibility is a very inprecise term which is used to ascribe apparent biological inertness of a specific material, relative to a particular biological environment; whereas thromboresistance refers specifically to the compatibility (i.e., does not evoke thrombus formation) of a material in blood contact. For example, a material with the requisite apparent biocompatibility for a soft tissue implant, may not be sufficiently biocompatible for use as a blood contact material. Biocompatibility for a blood contact material (i.e., thromboresistance) is not in itself a definitive entity but is variable depending on the prevailing haemodynamics in which the material must function. These prevailing haemodynamics are in the main a composite function of:

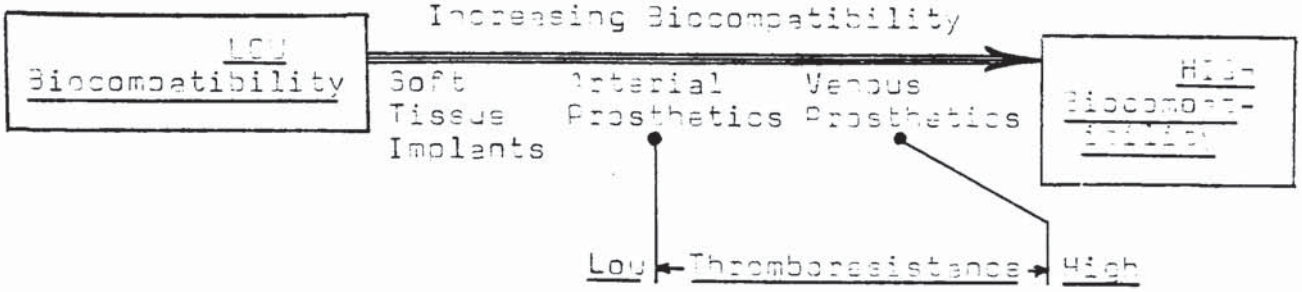
(i) blood vessel calibre (ii) blood velocity (iii) blood density and (iv) blood viscosity.

The variability of biocompatibility is perhaps more adequately illustrated by the schematic representation given in Fig 1.1

(See overleaf for Fig. 1.1)

Figure 1.1

BIOCOMPATIBILITY OF MATERIALS



The higher the relative biocompatibility the greater the number of criteria that the material must fulfill. The 'ideal' biomaterial may be thought of as exhibiting the highest biocompatibility or thromboresistance in a static or slow moving venous environment. Alternatively Bruck,[8] defined the 'ideal' biomaterial as being a material that does not produce the following adverse effects:

1. Thrombosis
2. Destruction of cellular elements
3. Alteration of plasma proteins
4. Destruction of enzymes
5. Depletion of electrolytes
6. Adverse immune responses
7. Damage to adjacent tissue
8. Carcinogenesis
9. Toxic or allergic reactions

To date no one synthetic material fully satisfies these criteria. In addition, no single test method for evaluating biomaterials is capable of measuring this wide range of factors relevant to the biocompatibility of a material. Although the majority of testing procedures for the evaluation of biological performance of potential biomaterials are far from ideal, some knowledge of the

more common test methods should enable a better understanding of the problems involved in the assessment of a material for biomedical applications. The tests employed can be divided into the three categories (i) Invivo (ii) Exvivo (iii) Invitro:

(i) Invivo

(a) Vena Cava Ring Test[9]: Rings made of, or coated by, the test material are implanted in the inferior vena cava (canine) for test periods of 2 hours, and 2 weeks. After this time the animals are sacrificed and the rings are evaluated for the degree of thrombus formation. The test does not distinguish between materials which are truely non-thrombogenic and those which cause thrombus formation but are non-thromboadherent.

(b) Renal Embolus Ring Test[10]: Rings of test material are implanted in the descending aorta (canine) just above the renal arteries. A constriction of the aorta below the renal arteries is made and this ensures that a large fraction of the blood flow through the test ring is directed into the kidneys. After 3 to 6 days the animals are sacraficed and the rings are examined for adherent thrombi and the kidneys are dissected and examined for embolic infarcts. Almost all materials to date have shown some renal infarction.

(c) Soft Tissue Compatibility Tests[11,12,13]: Test materials are implanted in soft tissue areas (not in direct contact with blood). Intramuscular implantation is cited as being the most sensitive site for evaluation of tissue responses. The majority of these types of tests do not

follow any standardised test procedures, and are very subjective in nature.

(ii) Exvivo

(a) Stagnation Point Flow Test[14,15]: In this test venous blood directly from animal or human subjects is shunted into a buffer-filled test-chamber (with the test material in situ) and is then disconnected from the venous supply, and sealed. An index of thromboresistance is derived from the time taken for thrombus formation to occur.

(iii) Invitro

(a) Lee White Test[16]: Coagulation times of recalcified whole blood in test-tubes made of or coated with, the test material are contrasted against the coagulation time of blood in a standard control tube (usually glass). From this an index of thromboresistance is derived. The test is apt to wide experimental variations. Criticism is also often drawn to the large blood-air interface which is present and which is believed to potentiate the clotting process.

(b) Lindholm Test[17]: This test is but a variant of the above Lee White test, however, in this test the material is in sheet form and is held in a test chamber. No substantial benefits are derived, over and above those of the Lee White test and again the same wide experimental variations apply.

Of the above tests, the invivo animal-testing of materials is by far the best technique for evaluating a material's potential, unfortunately the tests are expensive and time consuming. By far the most common investigative methods are the invitro tests however,

these tests are unfortunately the least representative of the normal physiological environment in which the materials must ultimately function. In addition to whole blood tests on materials, a large number of adjuvant invitro investigative techniques have been employed to characterise materials. A number of which are given below:

1. Cell culture evaluation[18]
2. Platelet Adhesiveness Tests[19-26]
3. Protein Adsorption Studies (see text)
4. Multiple Attenuated Internal Reflection (MAIR)[3,27] infra-red spectroscopy
5. Ellipsometry[3,28,29]
6. Electron Microscopy[30-32]
7. Contact Angle Measurements[3,23,33-35]

Some of the problems associated with the characterisation of surfaces by the above latter invitro physico-chemical surface techniques, are discussed in the following section.

1.2.2 The Characterisation of Natural and Synthetic Surfaces

The characterisation of solid surfaces presents several problems. Biological surfaces are, of course, only part of the total system and disturbance or removal of these surfaces in order to make measurements will inevitably cause some change to take place. This is particularly important in the case of the internal surface (intima) of blood vessels, for example. In addition, the nature of different types of surface varies so widely that complete characterisation is an extremely complex process. There are therefore a range

of chemical, physical and mechanical techniques which must be taken together in order to provide a complete characterisation. These include the chemical constitution of the surface, its rugosity or roughness, surface morphology, elasticity and the presence of ionic charges.

Surface science parameters such as, surface charge and density,[8,36-40] critical surface tension and surface free energy,[3,19,41-47] together with surface rugosity,[8,48] have all been assessed for their contributions towards the biocompatibility problem, and although some crude degree of correlation can be achieved with certain subsets of materials, to date no general correlations have been agreed upon.

One concept that has proved fairly useful however, is that of surface free energy. The most convenient way to describe this is in terms of the critical surface tension of the solid surface. A more detailed discussion of surface free energy and critical surface tension is given in Chapter 5, but for convenience a simplified definition of critical surface tension is as follows:

The critical surface tension of a solid surface is defined as the surface tension of a liquid that will just wet the surface and spread, instead of forming droplets on the surface. When an interface is formed between any liquid and a solid, the difference between the critical surface tension of the solid surface and the surface tension of the liquid is known as the interfacial tension, (or interfacial free energy). The inter-

facial tension is often used by workers to describe the relationship between a surface and a biological fluid.

1.2.2 (i). Cell Surface Models

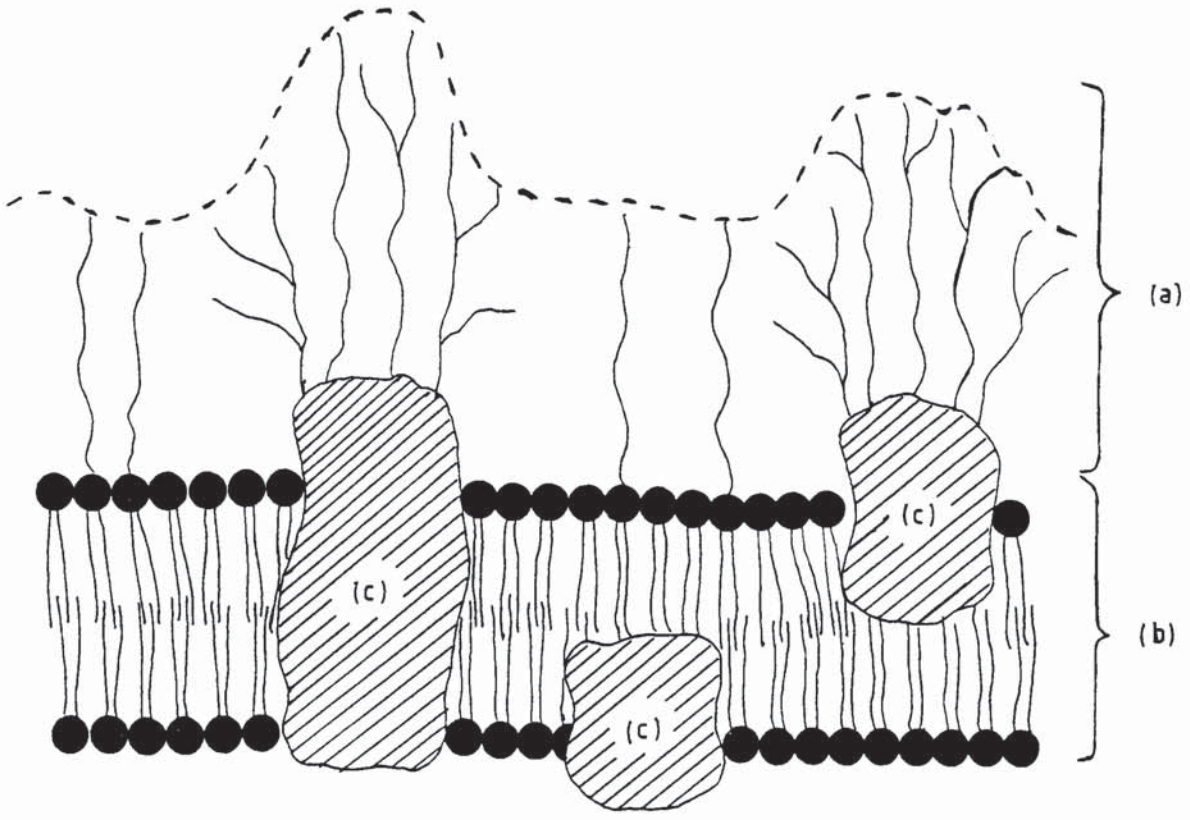
The cell surface is a highly complex organised surface, with many chemical groups present. Although the chemical nature of some of the major cell surface components are known[49] and illustrated in Figure 1.2, little detailed information exists about the invivo organisation of these components on the outer cell surface. Electron microscopy of endothelial cells often reveals a filamentous coat outside the cell membrane, composed of the oligosaccharide side chains of membrane glycoproteins together with the oligosaccharide head groups of the neutral glycosphingolipids. This cell coat is often referred to as the glycocalyx. These carbohydrate moieties, extending outwards into the extra-cellular fluid, have numerous hydroxyl groupings which are highly hydrated and it is believed that the water structuring around these groups determines the cellular recognition and antigenic responses of the cells.[50] Much work has yet to be carried out to elucidate the problems of the oligosaccharide structure/response relationships of cell surfaces, before any real attempts can be made to synthesise a model-cell surface with known antigenic responses.

1.2.2 (ii). Surface Science Criteria for Biocompatibility

Considerable conflict exists however, as to what criteria are necessary for defining a biocompatible material. It would seem reasonable to assume that to

Figure 1.2

CELL SURFACE MODEL



(a) Outermost layer (100-300 Å) containing hydrated oligosaccharide chains of glycoproteins and glycolipids together with mucopolysaccharides

(b) Lipid Bilayer

(c) Globular Proteins

obtain the ideal interfacial or surface caramaters, these should be modelled on the natural substrate which they are trying to emulate, (i.e., the endothelial cell surface). Several groups have reached a conclusion[51-54] which may be expressed as the 'Minimum Interfacial Energy'- hypothesis. This suggests that it is necessary for a material to exhibit a low interfacial tension (of the order of 5 dynes. cm^{-1} or less) with the biological system in order to achieve maximum biocompatibility. Hoffman[55] believes that this low interfacial tension between a hydrogel and an aqueous phase should reduce the tendency of plasma proteins to adsorb and also to reduce conformational changes in the secondary and tertiary structure of the globular proteins, when they adsorb.

An alternative approach to this 'Minimal Interfacial Energy'- hypothesis, which rests on theoretical considerations, is that of Baier et al.[3,43] This is based on the study and physico-chemical surface science measurements of materials that had successfully undergone long term inplantation, in areas of high thrombogenicity. From their studies, using multiple attenuated internal reflection (MAIR) infra-red spectroscopy and contact angle measurements, they found that materials exhibiting thromboresistance were all coated with a thin film of glycoprotein and this film had a critical surface tension of between 20 and 30 dynes. cm^{-1}. Similar studies were carried

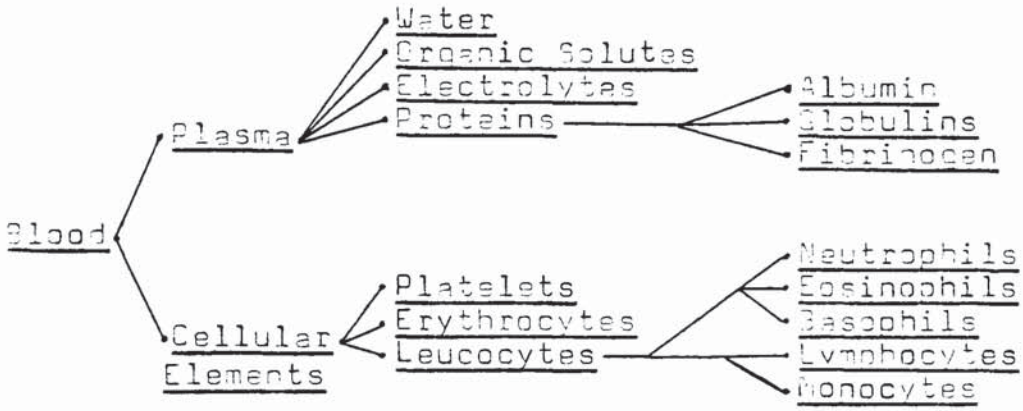
out by the same group, on the internal surface of blood vessels (endothelium), whose critical surface tensions also fell within the range of 20-30 dynes. cm^{-1}. They observed also that the intima surface chemistry was dominated by methyl groupings. From this they deduced that for a material to exhibit thrombo-resistance (blood biocompatibility), it must have a critical surface tension in the above range. This is known as the 'Moderate Surface Energy'- hypothesis.

The difficulties of this type of experimental work have been mentioned previously. Characterisation of the intima of blood vessels (or any other naturally hydrated material), by such surface science measurements in which the material under study must be in a dehydrated state, and thus far removed from the normal hydrated physiological state, provides information that must be interpreted with caution. It can be assumed, for example, that on dehydration a certain amount of chain rotation will have taken place, presenting the hydrophobic, methyl groups to the air/solid interface. At the same time the more hydrophilic (polar) groups (i.e., hydroxyl groupings normally associated with the carbohydrate glycocalyx) will turn into the matrix thus giving rise to a more stable thermodynamic conformation. When hydration takes place the reverse conformational changes would occur, presenting exterior hydrophilic groups with the hydrophobic groupings now hidden in the interior. Thus the invivo surface is more wettable (hydrophilic) than surface science measurements would predict.

1.2.3 The Nature of Blood

To understand blood interfacial phenomena, it is first necessary to consider the essential nature of blood or plasma. Blood is in the main a complex solution of electrolytes, globular proteins, lipids and carbohydrates together with an assortment of cellular elements in suspension, all of which contribute by complex interactions to its biochemical and physiological integrity. (Figure 1.3)

Figure 1.3 THE COMPOSITION OF BLOOD



Water, which accounts for approximately 90% of this system, is generally assumed to be a passive medium in which biochemical and physiological processes occur. Only in fairly recent years has it become clear however, that the fundamental H_2O unit is capable of existing in a range of structured forms which depend, for example, on the presence of dissolved species and its environment. These forms play a vital role in many biological processes, especially at interfaces.[51-53,56] Some appreciation of the complexity of this apparently simple molecule may be gained from the fact that a five volume work entitled 'A Comprehensive Treatise on Water' (edited by Felix Franks) was published in the 1970's.[57]

It is apparent then that much of the water in

blood (together with other extracellular fluids, i.e., tears) bears little or no resemblance to normal 'bulk' water. Water structured in, and around, proteins (and also in hydrogels) can conveniently be considered as being comprised of the three types listed below:

(i) 'Bulk' or 'free' water which is indistinguishable in properties from normal water and exists, for example, in large pores in biological and synthetic systems.

(ii) 'Bound' water which is electrostatically attracted to and structured around ionic and polar groupings. It behaves more as an extension of these groupings than as water per se.

(iii) 'Interfacial' water which consists of a continuum of water molecules intermediate in behaviour between 'bound' and 'free'. An example is water structured into ice-like configurations by hydrophobic (apolar) groups.

Further aspects concerning hydrogels and water structure may be found in Chapter 4.

In relation to the problems associated with the contact lens field, the composition of tears may be thought more simple than that of blood plasma. In fact each has its own elements of complexity together with certain similarities. Whereas the general composition of tears has been established for several years, more recent research has indicated a greater complexity of minor protein components than was previously appreciated. Table 1.1, taken from Alder's Treatise,[58] gives a good general picture of the similarities and differences in the overall composition of blood plasma and tears. A more detailed discussion of the physico-chemistry of tears and the related problem of biocompatibility in the contact lens field is given in Chapter 7.

Table 1.1

CHEMICAL COMPOSITION OF HUMAN TEARS AND PLASMA (58)

	TEARS	PLASMA
ELECTROLYTES		
Bicarbonate	26 mEq/litre	24.3 mEq/litre
Chloride	135 mEq/litre	102 mEq/litre
Potassium	15 to 29 mEq/litre	5 mEq/litre
Sodium	142 mEq/litre	137 to 142 mEq/litre
NITROGENOUS SUBSTANCES		
Total protein	0.669 to 0.800 gm/%	6.7 gm/%
Albumin	0.394 gm/%	4.0 to 4.8 gm/%
Globulins (α, β, & γ)	0.275 gm/%	2.3 gm/%
Fibrinogen	-	0.4 gm/%
Ammonia	0.005 gm/%	0.047 mg/%
Uric acid	-	3 to 5 mg/%
Urea	0.04 mg/%	26.3 mg/%
Nitrogen		
Total nitrogen	158 mg/%	1140 mg/%
Nonprotein nitrogen	51 mg/%	27 (15 to 42) mg/%
CARBOHYDRATES		
Glucose	2.5 (0 to 5.0) mg/%	80 to 90 mg/%
MISCELLANEOUS ORGANIC ACIDS, VITAMINS, ENZYMES		
Citric Acid	0.6 mg/%	2.0 to 2.6 mg/%
Ascorbic acid	0.14 mg/%	0.1 to 0.7 mg/%
Lysozyme	$\simeq$ 0.1 gm/%	-
Water	98.2 gm/%	94 gm/%

1.2.4 The Blood Clotting Process

The clotting of blood at interfaces is an extremely complex process which, superficially, bears no resemblance to the deposition processes occurring at the surface of contact lenses. There are several analogies between the two processes however, and despite the fact that blood clotting is a much more complex process, its sequential stages, beginning with proteinaceous adsorption, have certain analogies with deposition from tear fluid.

This necessarily brief description of the clotting process (thrombus formation) centres on two processes, namely, protein adsorption and platelet aggregation.

1.2.4 (i). Protein Adsorption

It has been demonstrated that within a few seconds of first exposure to blood, all non-physiological materials acquire a rapidly thickening film of essentially pure protein (normally fibrinogen), which on adsorption alters the surface texture, charge, and chemistry of these materials.[21,28,59-63] Once a peak film thickness of some 200 Å[21,32] has been established, Vroman et al,[21,28,60-63] and others,[32,64,65] have shown that it does not remain static, but is constantly changing, being altered, or replaced by other plasma proteins, for example, Hageman's factor (Factor XII). The part that this factor and fibrinogen play in the classical extrinsic and intrinsic (cascade) clotting systems, are illustrated in Figure 1.4.[66,67] Thermodynamic studies carried out by Dillman et al,[68] characterised this adsorption as being of two forms: one that is hydro-

Figure 1.4

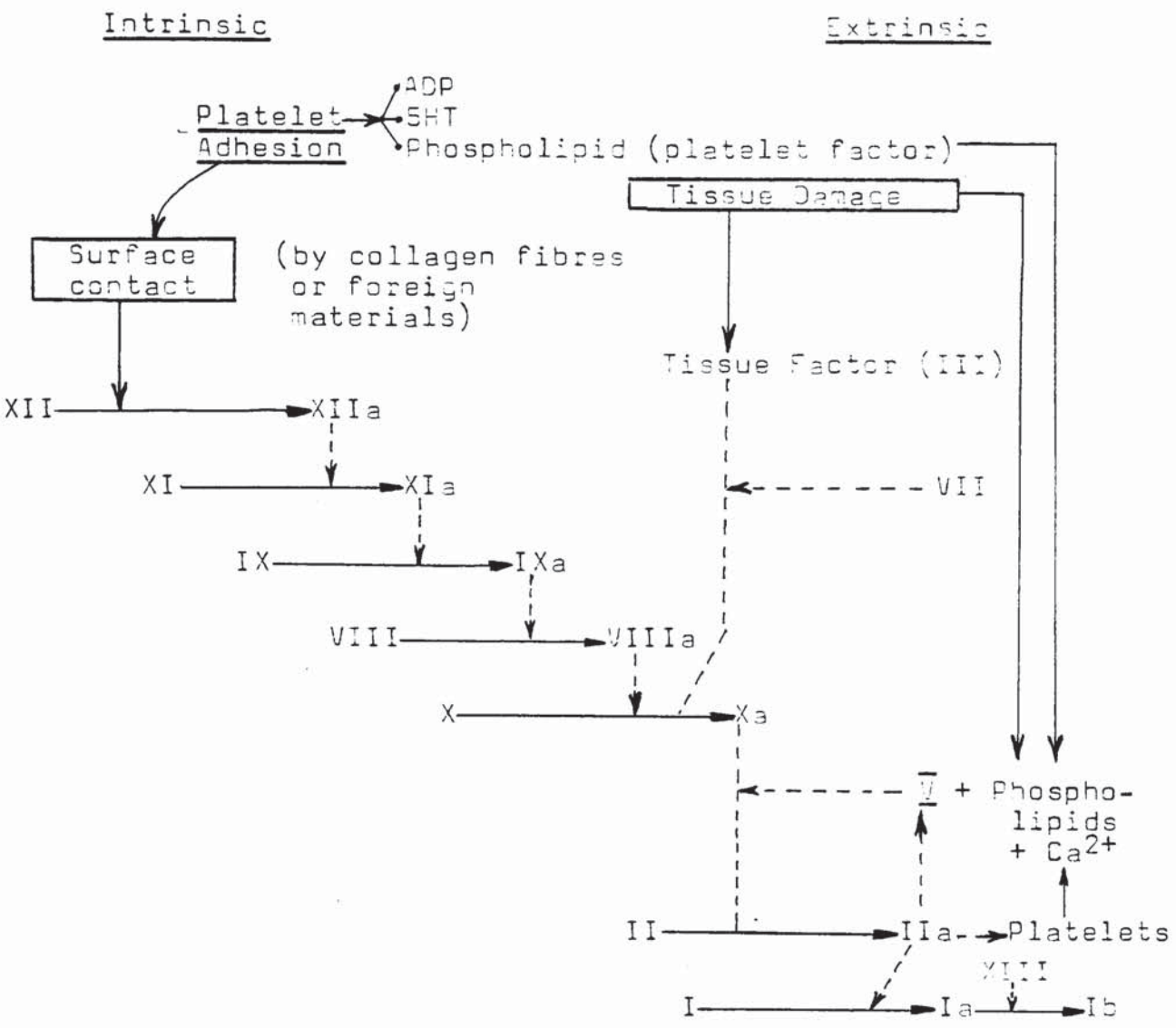


Figure 1.4: Scheme showing the sequence of changes which lead to Blood clotting by Activation of the Intrinsic and Extrinsic Systems[66,67]

XII = Hageman's Factor; II = Prothrombin; IIa = Thrombin.

I = Fibrinogen; Ia = Fibrin; Ib = Stabilized fibrin.

——→ Transformation

- - - - - - - → Action

philic, exothermic, and reversible and the other hydrophobic, endothermic, and strongly bound (irreversible). The 'reversibility' of protein adsorption is debatable and is very much dependent on the physico-chemistry of the adsorbing protein, together with the surface chemistry of the adsorbent material. The stability of proteins in solution depends upon them being able to retain their specific characteristic shapes, thus 'reversible' adsorption takes place with no change in shape (i.e., native conformation). Strong irreversible adsorption however involves some change in the nature, shape or conformation of the protein. When a protein adsorbs onto a surface the strength of its adhesion to the surface is due to the cumulative effects of the multiple points of contact.

The conformational changes in structure that proteins undergo in response to their environment has been the subject of many studies.[55,69-76] Conflict still exists as to the precise conformational changes that occur on adsorption, although the configurational states and anchorage sites of adsorbed proteins have been shown to differ depending on temperature, pH and concentration in solution. Morrissey et al,[69,71] who used infra-red measurements on bound fractions to determine the carbonyl exchange sites, found that the internal bonding of globular proteins, albumin and prothrombin were sufficient to hold them in their natural native or bulk conformation, but for fibrinogen this was not evident.

This correlates with the view that primary albumin adsorption leads to greater compatibility than fibrinogen adsorption.[19-26] It appears that the principle of designing a surface to preferentially adsorb a particular protein from a given biological environment is a promising way of designing biocompatible surfaces for various applications. A more detailed examination of the above interfacial phenomenon of protein adsorption is covered in Chapter 6.

1.2.4 (ii). Platelet Aggregation and Thrombus Formation

The majority of experimental work points to there being a mandatory conditioning film of protein which when established allows platelets to adhere and react specifically and individually with the surface of a material.[3,19-26,28,59-63]

The platelet population density in the first incompletely packed layer undergoes a morphological series of changes (or lack of) which then triggers a thrombogenic (or thromboresistant) response. This latter trigger mechanism is part of both the intrinsic and extrinsic clotting mechanisms, and the part that it plays together with that played by the surface activation of the plasma proteins (the 'intrinsic' clotting mechanism) is complex, involving several intermediate steps,[66,67] (Figure 1.4).

On adhesion, platelets flatten, degranulate and become sticky towards subsequent arriving platelets thus forming an aggregate. These aggregates grow in pyramidal

shapes out into the blood flow and are modelled according to the prevailing haemodynamics at that location. Approximately four minutes after initial contact of the material with the blood, white blood cells drawn by chemotaxis, adhere to the growing platelet aggregate, giving rise to a 'white' thrombus. Perturbation of the lamellar blood flow ensues, and this together with chemical modification of degranulating platelets and surface activation of the clotting factors, accelerates the coagulation process, forming fibrin strands, that enmesh red blood cells (erythrocytes) giving rise to a 'pale' thrombus (arterial) or 'red' thrombus (venous).[59,67,77] The proportion of fibrin deposited in a 'pale' thrombus is dependent on the rate of blood flow, to which it has an inverse relationship.[67]

The induction of platelet aggregation has been attributed to various factors, such as changes in water structuring[78] and high negative charge densities.[79,80] In some agreement with the above the present day view is that platelet aggregation (and to some extent adhesion) is due to an unstable prostaglandin-like substance, called thromboxane A_2, synthesized by the platelets. By contrast the endothelial cells lining the blood vessels protect themselves against platelets by forming prostacyclin, another unstable prostaglandin-like substance which inhibits platelet adhesion and aggregation.[67]

With regard to the mechanism of platelet adhesion, it is postulated by some research workers that for

adhesion to occur, the mandatory conditioning film of protein must be composed of specific plasma glycoproteins (such as fibrinogen and γ-globulins), so that membrane associated platelet glycosyl transferases may interact with their carbohydrate moieties and so bring about platelet surface fixation.[81,82] To some extent the above hypothesis is supported by the well recognised contrasting thrombogenicity exhibited by adsorbed fibrinogen and γ-globulins (with carbohydrate moieties), and the thromboresistance of adsorbed albumin (with no carbohydrate moieties).

1.2.5 Surface Modifications to Improve Blood Biocompatibility

It can be appreciated from the previous references to the role that carbohydrate moieties play in cell/cell recognition and antigenic responses, together with the belief that units of similar type affect platelet adhesion, that the order, or coding, of the carbohydrate units in a polysaccharide is of vital importance in determining the specificity and functional ability of molecules containing carbohydrate chains. This has recently been shown in the anti-coagulant action of the blood factor 'heparin', which is a strongly acidic polysaccharide distributed throughout the body in mast cells. Traditionally it is believed to be a polymer composed of tetrasaccharidic repeating units, however, the latest findings seem to indicate that the part of the molecule responsible for its anti-coagulant action is somewhat more complex.[83] Inhibition by heparin of invivo and invitro blood

coagulation is believed to be due to its interaction with a plasma protein called antithrombin, which subsequently inhibits thrombin. It is also believed that much of the anti-coagulant action is due to the strong electro-negative charge[30] that heparin possesses. Neutralisation of the negative charge by electropositive substances completely inhibits its anti-coagulant action.

When heparin is adsorbed or incorporated onto a substrate, it produces a high negatively charged surface, which in accordance with the Sawyer cell-surface model,[39,40] should mimic the surface charge of the blood vessel intima. Doubts have been cast on the use of surface-charge as the sole parameter for thromboresistance, however, and it may be that once again the order of specificity of the highly hydrated carbohydrate segments of the molecule determine its interactions. Recognition of the anti-coagulant actions of heparin has led to it being incorporated onto the surfaces of materials in an attempt to induce biocompatibility.[84-87] Success to date has, however, been rather limited. In the short term, incorporation of heparin works quite well but with time it will gradually leach out and thus the technique will not be suitable for long term implantation until a more permanent fixation process is developed.

This latter problem is also evident in a similar technique for rendering surfaces thrombo-

resistant, that of enzyme immobilisation.[88,89] In principle immobilising a fibrinolytic enzyme (i.e., plasminogen) on to a surface should ensure that fibrinogen does not adsorb and polymerise to form fibrin strands and thus no thrombus should develop. This is however rather an oversimplification of the clotting process.

It is apparent from numerous papers on both platelet adhesion and protein adsorption studies[19-26,33,81,82,90] than an index of thromboresistance or blood biocompatibility can be obtained from the premise that preferential adsorption of albumin over that of fibrinogen is indicative of a thromboresistant surface (or vice versa). As previously stated albumin has no carbohydrate moiety and therefore it cannot undergo reaction with platelet transferases which may account for its observed biocompatibility.[91,92] Accordingly, if a surface can be coated with albumin it should exhibit a degree of thromboresistance. Once again however the problem is that of achieving permanent fixation of albumin to a surface without loss of biological integrity, although some progress has been made in this field.

To date when an implanted material (biomedical prosthesis), exhibits thromboresistance, it would seem that it does so by undergoing one of two favourable outcomes:[59]

(i) the original layer of adherent platelets do not become adherent to arriving siblings. Through a poorly understood secondary adhesion of white blood

cells (predominantly neutrophils), the original platelets are removed to leave a residual protein film in dynamic equilibrium with the blood stream, and on which no further cellular deposition is noted.

(ii) implants of sufficiently large diameter, or in a region of sufficiently high flow rate, on which the original layer of platelet thrombus (with or without fibrin strands and enmeshed erythrocytes), remodels to form a smooth fibrin layer, or to support cellular ingrowth (normally by endothelial cells of the blood vessel intima), to provide a passive pseudo-intima.

Somewhat paradoxically the production of the above pseudo-intima can be achieved by the use of thrombogenic hydrophobic polymers such as poly (ethylene terephthalate), polypropylene and poly-urethanes, fabricated into woven mesh tubing (conduits)[8,93-97]. Of these polymer types, to date poly (ethylene terephthalate), in the form of 'Dacron' (U.S.A.) or 'Terylene' (U.K.) conduits, is often used successfully, in vascular surgery (such as arterial by-pass grafts and aortic repair grafts). Successful 'endothelialisation' of these conduit is largely governed by the mesh size and also whether it is possible to pre-clot the conduit in the patients own blood prior to implantation. Unfortunately the use of these blood vessel prosthetics is limited to large arterial replacement (i.e., aorta, femoral etc.), simply because of the haemodynamic considerations which apply.

Widespread research is presently in progress to overcome the above limitations and produce a successful synthetic small arterial prosthesis. Furthermore, it is believed that the inherent characteristics of hydrogels will illicit the necessary surface parameters to produce a thromboresistant biomaterial. Although the presence of imbibed water within a polymeric system is not a guarantee of blood biocompatibility (thromboresistance), it is believed that the relatively large fraction of water within certain hydrogel materials (and in particular the way in which it is structured) is intrinsically related to their high biocompatibility.[54,98] However, these highly hydrated and water-plasticised polymer networks are usually mechanically weak. Furthermore, the higher the water content of the gel, the poorer the mechanical properties of the gel become. Fortunately, several techniques are available to minimise this latter problem. With regard to the above, the general background of hydrogels and their biomedical applications are given further consideration in the following section.

1.3 Synthetic Hydrogels and their Biomedical Applications

1.3.1 Hydrogels: Some General Considerations

Hydrogels can be defined as polymeric materials, which have the ability to swell in water and retain a significant fraction within its three dimensional network. They can be natural or modified natural biopolymers as well as synthetic polymers. In this study it is the latter synthetic hydrogels which will

be under consideration and which were originally developed as potential biomaterials, due to their similarity to living soft tissue.

Due to the uniqueness of the hydrogel structure, they have a real advantage over other polymeric materials, in that, because of their expanded gel network, they have an inherent permeability to small molecules. This allows artefacts of polymerisation (i.e., excess initiator and products, solvent and excess monomer etc.) to be eliminated prior to the hydrogels coming into contact with living systems.[98] Leaching of fabrication additives, has been cited as a cause of inflammation and ultimate rejection in the production of materials for biomedical applications.[99] An example of this being the phthalate plasticiser used in the fabrication of poly (vinyl chloride),[100] for medical tubing and blood-bags. The phthalate was shown to produce a decrease in albumin adsorption and an increase in glycoprotein (fibrinogen and γ -globulin) adsorption, which is indicative of a thrombogenic agent.

Another distinct advantage of hydrogels, due to their above structure, is that it is possible to incorporate drugs into their gel-network, which when implanted (or administered), will give a controlled leaching or release of that drug, at a particular desired site of administration.[101] This technique is of particular advantage for anti-tumour chemotherapy, where the maximum dose is delivered directly to the region of tumour growth by implantation, thereby

minimising the normal systemic action of cytotoxic drugs used in chemotherapy.[102]

The most intriguing of the potential advantages for hydrogels is the low interfacial tension which may be exhibited between a hydrogel surface and an aqueous solution. As previously mentioned, this low interfacial tension should reduce the tendency of the proteins in body fluids to adsorb, or to unfold upon adsorption.[55] Surface adsorption and denaturation of proteins has been shown to ellicit a thrombogenic response and thus the use of hydrogels should minimise this problem. A comprehensive review of hydrogels as biomaterials is given by Ratner and Hoffman[54] and from this review a list of hydrogel biomedical applications is given in Table 1.2

1.3.2 Biomedically Useful Hydrogels

It is possible to synthesise innumerable permutations of hydrogel compositions, suitable for biomedical applications, from either monomers or conversion of preformed polymers, each of which will possess its own individual characteristic properties. It is not feasible however, to consider these in their entirety and for this reason only the more important commonly utilised classes of hydrogel will be discussed, and in particular those of interest to this project.

1.3.2 (i). Poly (hydroxy alkyl methacrylates and acrylates)

Of this class poly (2-hydroxy ethyl methacrylate), (poly (HEMA)), is probably the most widely used and studied of all synthetic hydrogels. Although originally

Table 1.2

BIOMEDICAL APPLICATIONS OF SYNTHETIC HYDROGELS[54]

Coatings	"Homogeneous" Materials	Devices
Sutures	Electrophoresis gels	Enzyme Therapeutic systems
Catheters	Contact lenses	Drug Delivery Systems
I.U.D.'s	Artificial corneas	Artificial organs
Blood Detoxicants	Vitreous Humour replacement	(Haemodialysis/Haemo-
Sensors (electrodes)	Breast or other soft tissue	perfusion)
Vascular grafts	substitutes	
Electrophoresis cells	Burn dressings	
Cell culture substrates	Bone Ingrowth sponges	
	Dentures	
	Ear-drum plugs	
	Synthetic cartilage	

prepared in 1936, by Du Pont scientists,[103] it was not until the early 1960's that Wichterle and Lim,[104] polymerised the monomer in an aqueous media with a cross-linking agent, and from this beginning came the advent of hydrogels for biomedical applications. If polymerised in a good solvent for the monomer and polymer, an optically transparent, homogeneous hydrogel is obtained. If however, the solvent system is poor, an opaque, spongy white (heterogeneous) hydrogel results. This latter problem can be negated by simply performing a bulk-polymerisation reaction (with cross-linking agent), in which the monomer acts as its own solvent, and produces a dehydrated hydrophilic polymer (Xerogel) which on hydration becomes an optically transparent, homogeneous hydrogel.

One of the problems encountered in the synthesis of poly (HEMA) for biomedical applications, is the presence of impurities in the monomer, such as methacrylic acid (MAA), ethylene glycol dimethacrylate (EDMA), and ethylene glycol (EG),[54,105] where the intrinsic biocompatibility, can be overshadowed by these low levels of monomeric impurity. This problem was encountered to some extent, by our own research group whilst trying to prepare linear (uncross-linked) poly (HEMA), which because of the above impurities, tended to undergo cross-linking to a slight degree. This has now been rectified by subjecting the monomer (HEMA) to a complex purification procedure, prior to polymerisation, (as outlined in Chapter 2).

Although poly (HEMA), (as with all synthetic hydrogels) is mechanically weak, it does possess properties

that are necessary for a biomedical material, in that it is chemically stable at extremes of pH and temperature (i.e., resistant to acid hydrolysis and reaction with amines). Indeed, one of the main priorities for a biomedical prosthetic device is that it can be steam sterilized (autoclaved).[106-108]

Of this same class as poly (HEMA) is poly (2-hydroxy propyl acrylate), poly (HPA), which is the isomer of poly (HEMA). It possesses slightly weaker mechanical properties than poly (HEMA) and also has a maximum permissible water content of ~ 50% (homogeneous composition), whereas poly (HEMA) has a maximum water content of ~43%. Due to the low glass transition temperature (Tg) of poly (HPA) its use is somewhat limited.

Poly (2-hydroxy propyl methacrylate) and poly (glyceryl methacrylate) hydrogels have not received as much attention as poly (HEMA) gels.[109] Both these materials exhibit different water binding characteristics as compared with poly (HEMA). Poly (2-hydroxy propyl methacrylate) showing a lower water content and poly (glyceryl methacrylate) exhibiting a considerably higher water content.[110-112]

1.3.2 (ii). Poly (Acrylamide) and Derivatives

The monomers of this class of hydrogel are crystalline solids at room temperature, and they are also of low solubility in other monomers. The solubility poses something of a problem, but it can be partially overcome by the addition of a small quantity of water and by a rise in temperature of the solvent monomers.

Alternatively the solution polymerisation of aqueous based acrylamide can be used to produce very high water content gels (> 95%), which also unfortunately possess low mechanical strength. For this reason, the former technique utilises both acrylamide and its derivatives (methacrylamide and diacetone acrylamide) as an adjuvant in hydrogel compositions, to boost the water content of the gels. Water content with this class is dependent on the cross-linking agent content in the system, unlike that of homogeneous poly (HEMA) gel systems.

A problem associated with this class is that although they are stable under physiological conditions of pH and temperature, they tend to hydrolyse at extremes of pH and temperature and therefore problems would be encountered with steam sterilization.[113-115] Better hydrolytic stability is exhibited by the N-substituted acrylamides.[116] The thrombogenicity of poly (acrylamide) hydrogels has been investigated and has been found to be dependent on the purity of the monomer.[117] Bruck,[98] cites that multiple recrystallization of acrylamide(s), markedly increases their thromboresistance.

1.3.2 (iii). Poly (N-Vinyl Pyrrolidone)

Poly (N-vinyl pyrrolidone), poly (NVP), in an uncross-linked, linear form is highly soluble in both polar solvents (such as water) and also many non-polar solvents. Because of its strong interaction with water it can be used to prepare gels of high water content. However, to produce a homogeneous poly (NVP) gel, it is necessary to incorporate some 5 - 20% (w/w) cross-linking agent to produce a mechanically stable hydrogel,

unfortunately however, monomer excess is always a problem.

Generally therefore, NVP is used as an adjuvant or modifying group to both increase water content and to some extent the strength of the resultant gel, due to its hydrogen bonding capacity.

In the past poly (NVP) has been used as a plasma expander,[118] at present however, dextrans have replaced it because they are retained better in the circulation. It was found that linear poly (NVP) plasma expanders tended to leak out of the circulation and to accumulate within organs (due to the fact that they are extremely stable and are not metabolised).[119] Other uses of poly (NVP) include, blood preservatives, tablet binder and coatings for solubilisation and stabilisation of drugs.[54]

The thrombogenicity of poly (NVP) gels has been studied and residual NVP monomer has been found to have an adverse effect on blood clotting times as measured by the Lee White test.[117]

1.3.2 (iv). Anionic and Cationic Hydrogels

These hydrogels are generally added to a composition in very low concentrations to alter the charge characteristics of a material. In the case of the anionic methacrylic acid (MAA), (which is found as an impurity in HEMA), it is capable of hydrogen bonding and thus will both increase the strength of a hydrogel composition as well as producing a negatively charged surface.

Based on the Sawyer model[39,40] of the blood vessel intimal charge, it is believed that negatively charged surfaces should be less thrombogenic than positively charged ones,[109] and experiments have been performed

which support this view. However, results indicating positively charged surfaces, (using the cation dimethyl amino ethyl methacrylate) have been suggested as an important factor with respect to the thrombogenicity of a surface.[120] At present the importance of surface charge in blood-hydrogel interactions is not at all clear.[54]

1.3.3 Methods to Graft Hydrogels

By grafting a hydrogel to the surface of another polymer a new composite material is formed whose mechanical properties more closely resemble those of the base polymer than the thin grafted hydrogel layer. This technique has the advantage of both minimising the problem of the inherent low mechanical strength of hydrogels whilst endowing the base polymer with the desirable surface properties of the hydrogels. Some techniques to coat surfaces with hydrogels are given below:[121]

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

(ii) Dip in monomer(s) (± solvent, polymer) then polymerise using catalyst ± heat.

(iii) Pre-activate surface ('active vapor', ionising radiation in gas) then contact with monomer(s) ± heat to polymerise.

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

By far the most permanent mode of fixation of hydrogels to a surface is by covalently bonding (grafting).

The most efficacious technique for preparing such materials involves the generation of free radicals on a plastic surface, followed by the polymerisation of monomer(s) directly onto that surface.

Detailed descriptions of methods which have been used to vary the surface character of grafted hydrogels for biomedical uses have been described in a number of papers.[122-124]

1.4 Scope of Thesis

Although the inherent characteristics of hydrophilic polymeric materials (hydrogels) do go some way towards a thromboresistant or 'biocompatible' material (the 'ideal' biomaterial), the previously outlined problems associated with contact of biomaterials with body fluids, is still evident and requires much further study. Moreover it is the intention of this project to primarily examine and characterise the bulk and surface structure-property effects of selected hydrogel formulations in particular, with respect to their adsorption of specific plasma proteins, based on the previously discussed relationship of certain plasma proteins (albumin and fibrinogen) adsorption, to biocompatibility. In so doing, it is hoped that this will help to generate a general understanding of the many complexities associated with biological solid/solution interfaces, especially with regards to the specific problems associated with the contact lens field.

CHAPTER 2

EXPERIMENTAL I - HYDROGEL SYNTHESIS AND CHARACTERISATION TECHNIQUES

2.1 Reagents:

All the monomers and catalysts used in the synthesis of the hydrogel polymers in this project are listed, together with their suppliers, below:

Monomers	Supplier(s)
Hydroxyethyl Methacrylate	BDH Ltd/Vidor Woolfe
Hydroxypropyl Acrylate	B. P. Chemicals
Ethyl Methacrylate	I.C.I.
Ethylene Glycol Dimethacrylate	B.D.H. Ltd
Methacrylic Acid	Koch-Light Ltd.
2-Amino Ethyl Methacrylate	Polysciences Inc.
N-Vinyl Pyrrolidone	Koch-Light Ltd.
Acrylamide	Koch-Light Ltd.
Methacrylamide	Koch-Light Ltd.
Diacetone Acrylamide	Koch-Light Ltd.
Styrene	B.D.H. Ltd.
N,N' Methylene-bis-Acrylamide	Koch-Light Ltd/ Sigma Ltd.
113,Trihydroperfluoropropyl Methacrylate	Polysciences Inc.
Hexafluoroisopropyl Methacrylate	Polysciences Inc.
Catalysts	**Supplier(s)**
α, α' Azobis-isobutyronitrile	B.D.H. Ltd.
Ammonium Persulphate ($(NH_4)_2S_2O8$)	Fisons Ltd.

2.2 Purification of Monomers

All monomers were purified by the conventional vacuum distillation or recrystallisation techniques,

where applicable. Additional purification procedures were carried out for Hydroxy ethyl Methacrylate and Acrylamide, as follows:

2.2.1 Purification of Hydroxy ethyl Methacrylate (H.E.M.A.)

Conventional purification procedures previously adopted for H.E.M.A., were found to be inadequate in the removal of the more polar impurities, (especially Methacrylic acid (M.A.A.)). Neutralisation of the M.A.A. in the H.E.M.A., was brought about by the titration of Sodium hydroxide solution until a pH of at least 7 was attained. Then the H.E.M.A. solution is passed through an anionic exchange resin (Dowex X-4 previously washed and regenerated). The total process is schematically illustrated in Fig. 2.1. The yield of purified H.E.M.A. by this technique (developed in our laboratories), was found to be approximately 50%. Analysis was determined by G.L.C., and the product was then stored refrigerated in a darkened bottle.

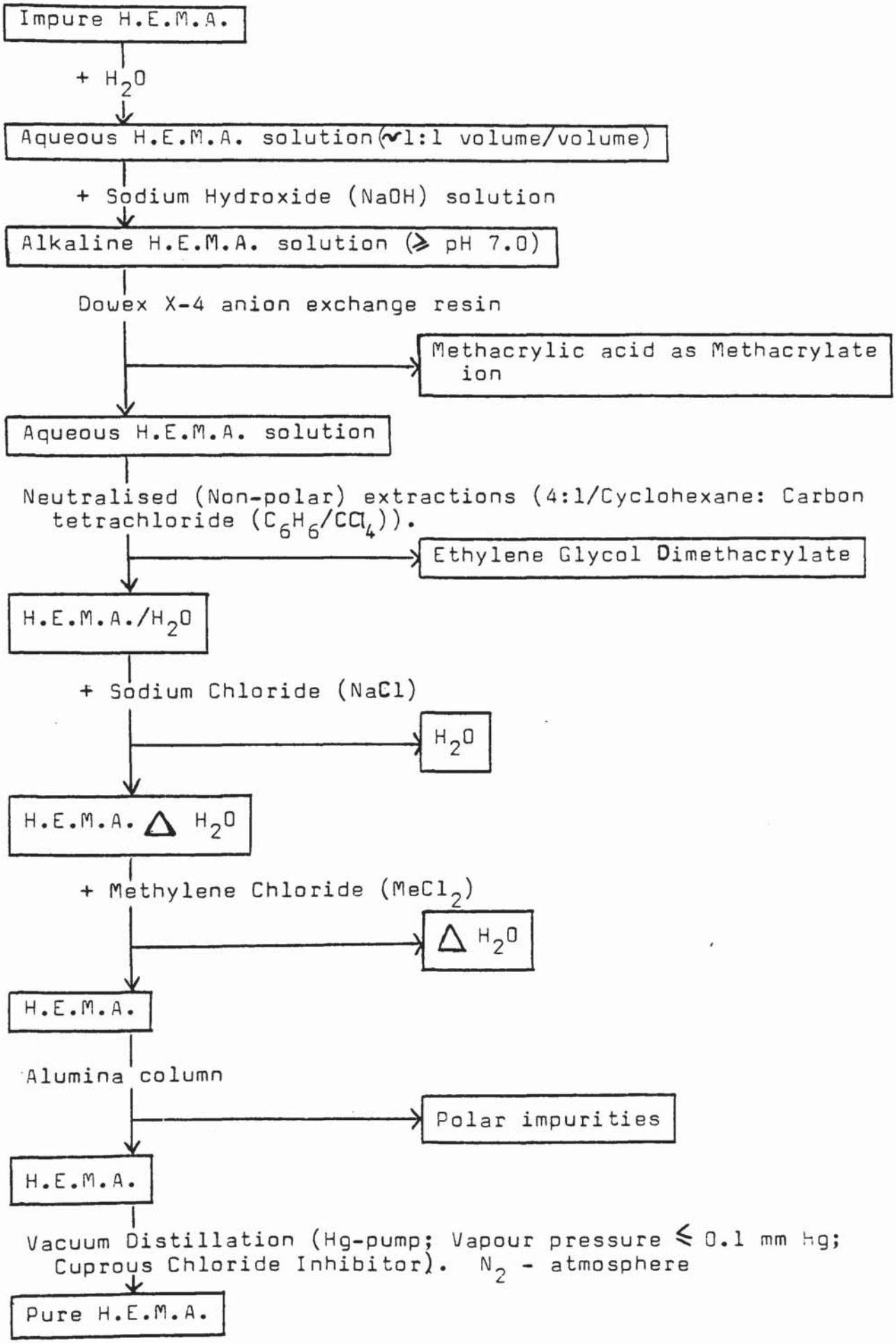
2.2.2 Purification of Acrylamide (A.C.M.)

Using ethyl acetate (A.R.) as the solvent, multiple-recrystallisation was carried out, as opposed to the standard single-recrystallisation purification method. Vacuum-sublimation was also undertaken, as a comparative purification procedure. This technique is often used in the pharmaceutics industry for drug purification. A larger than normal vacuum-sublimation apparatus was constructed (using pyrex glass). The purified monomer was analysed using both G.L.C. and infra red spectroscopy and subsequently stored in the dark in a vacuum-dessicator.

N.B. Caution must be exercised in the handling of all the

Figure 2.1

FLOW DIAGRAM OF H.E.M.A. PURIFICATION



monomers due to their deleterious biological effects. Special care must be taken with the crystalline acrylamide(s) which is a known neurotoxin.[125]

2.3 Analysis

The purity of the purified liquid monomers were determined by Gas-Liquid Chromatography (G.L.C.) and the purified crystalline monomers by infra-red spectroscopy.

Previous methodology for the quantitative analysis of M.A.A., relied on standardised alkali titration, which was both inaccurate and time consuming. Good reproducible quantitative and qualitative G.L.C. analysis was developed for M.A.A., during this project, using the G.L.C. operating conditions as laid down in Appendix 1. These operating conditions also gave much improved G.L.C. resolution for other acrylic/vinyl monomers.

The conventional analysis of acrylamide by infra-red spectroscopy can often give ambiguous results because of water interference (due to the inherent hygroscopic nature of acrylamide), and which makes for difficult interpretation of the results. Good qualitative and quantitative analysis of acrylamide was obtained by injection of acrylamide/methanol solutions onto the G.L.C. column, using the same operating conditions as above.

2.4 Preparation of Hydrogel Films

The crosslinked polymer films were synthesised by thermal free-radical addition polymerisation using either the technique of bulk-polymerisation or solution-polymerisation. Both techniques employed the use of

prepared polymerisation cells, one of which is illustrated in Fig. 2.2. The cell is composed of a sandwich of glass-plates, G, with polyethylene terephthalate (Melinex), M, sheets, to improve mould release, and a central G2000 - polyethylene gasket, P, all held together by spring-clips.

2.4.1 Bulk-Polymerisation

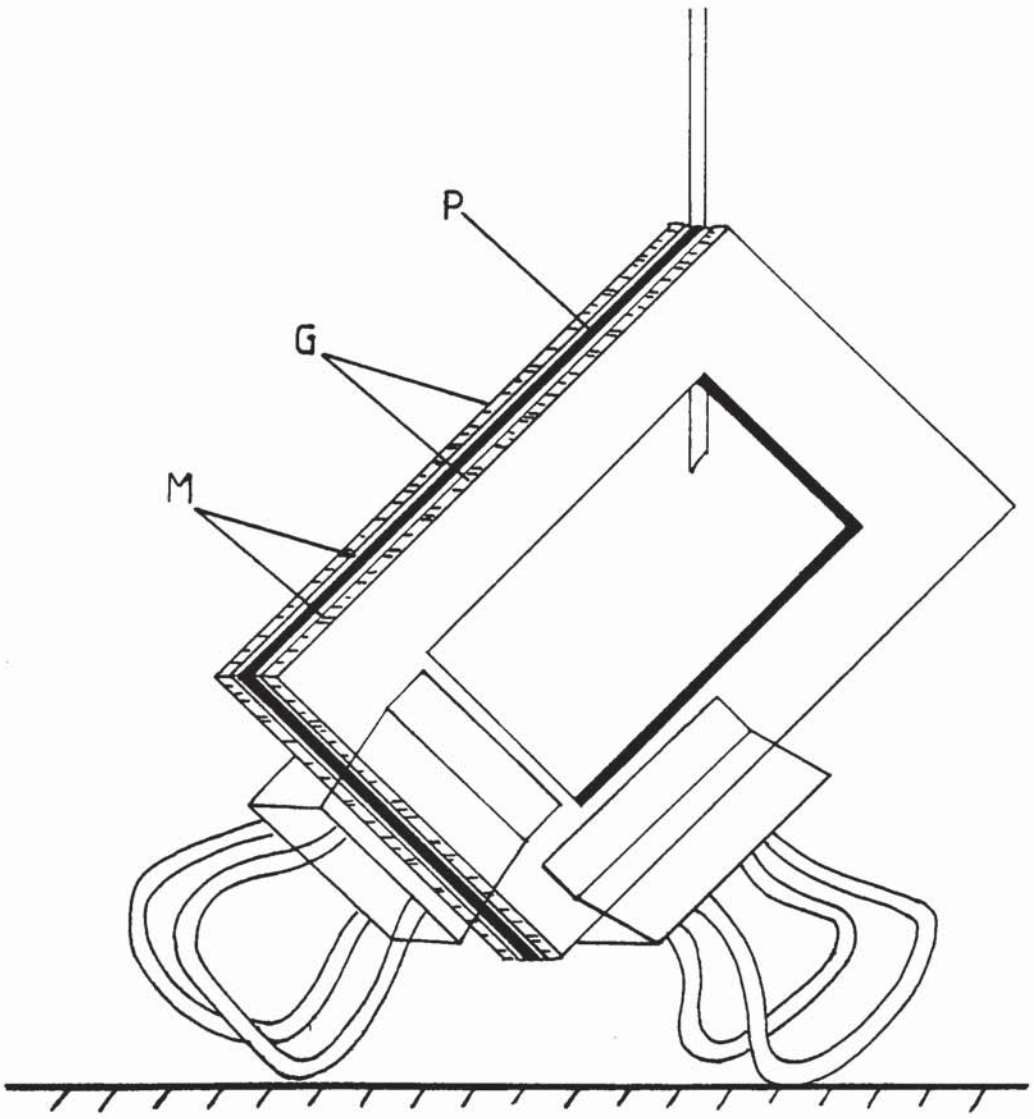
The monomeric compositions which were made-up typically included 1 molar % of E.D.M.A., as a cross-linking agent and 0.1% by weight of AZBN. See Appendix 3 for the compositions. These solutions were subsequently degassed, by bubbling nitrogen through them, to minimise oxygen inhibition of the polymerisation. Deoxygenated monomers with added catalyst and cross-linker were then injected through G22 syringe needles into the prepared polymerisation cells, which were then placed in an oven at 60°C for three days, followed by a post-cure of two hours at 90°C.

2.4.2 Solution-Polymerisation

Aqueous solutions of crystalline monomers, together with catalyst and cross-linker were degassed and injected into the polymerisation cells. Typically a 30 : 70 ACM/H_2O solution was made up and 1 molar % of the cross-linking agent methylene-bis-acrylamide was added together with 0.5% by weight of the catalyst $(NH4)_2S_2O_8$. See Appendix 3 for details of the compositions.

The solutions were subjected to centrifugation (for 15 mins. 15,000 rev/min), and then degassed (with N_2), and injected into previously warmed polymerisation

Figure 2.2 Schematic Illustration of Polymerisation Cell



cells, which were then placed in an oven at 60°C for one hour to enable polymerisation to occur.

2.4.3 Hydration of the Prepared Dehydrated Polymer Films

Following both the bulk and solution polymerisation, the formed polymer films were carefully removed from between the melinex sheets and then placed in distilled water to hydrate. If difficulty was encountered in releasing the films from the melinex sheets, soaking in distilled water allowed the hydrogel film to swell off the melinex. Hydration of the hydrogel films was allowed to take place for approximately four weeks, during which time frequent changes of water facilitated the elimination of small molecular weight artefacts of polymerisation, as well as ensuring the complete hydration of the gel(s). Subsequent to this the hydrogels were either stored ready for use in distilled water or were allowed to further re-equilibrate in a physiological-Kreb's solution for two weeks, with frequent changes of the solution, and then stored in sterile sealed bottles.

2.4. 4 Preparation of samples

All procedures were carried out using disposable sterile rubber gloves to minimise biological surface contamination. At no time were any of the polymer films, dehydrated or otherwise, allowed to come into contact with ungloved hands. For the characterisation of the hydrogels together with the static protein adsorption studies, discs were cut using a 12 mm dia Cork-borer (No. 7). For the dynamic adsorption system

studies rectangular films (5 x 2 cms) were cut using a scalpel. The handling and storage procedures minimised any contamination of the samples or their solutions, and therefore obviated the need for cleaning with detergent solutions, which in themselves would have induced chemical contamination of the samples.

2.5 Hydrogel Characterisation Techniques

Several techniques were used to investigate both the surface and bulk properties of the prepared hydrated hydrogel samples.

2.5.1 Contact Angle Measurements

Contact angle measurements were carried out by various techniques to characterise the hydrated hydrogel surface.

2.5.1 (i) Hamilton's Method

The apparatus used for this technique is illustrated in Fig. 2.3. Surface water was wiped from one side of the sample, S, and the sample was then glued using an α-cyanoacrylate glue, (See Appendix 1 for the method of adhesion), to a glass cover-slip, G, which was then suspended inverted in the optical cell, C. The cell was filled with either distilled water or Kreb's solution, both having been previously saturated with n-octane.

The G25 syringe needle, N, was controlled by a "Prior"-micromanipulator and the wetting liquid, n-octane, was delivered from an 'Agla' microsyringe and micrometer, which enabled small volumes of liquid to be accurately placed on the sample surface. The syringe needle point was removed by grinding, in order that the drop was formed symmetrically on the surface of

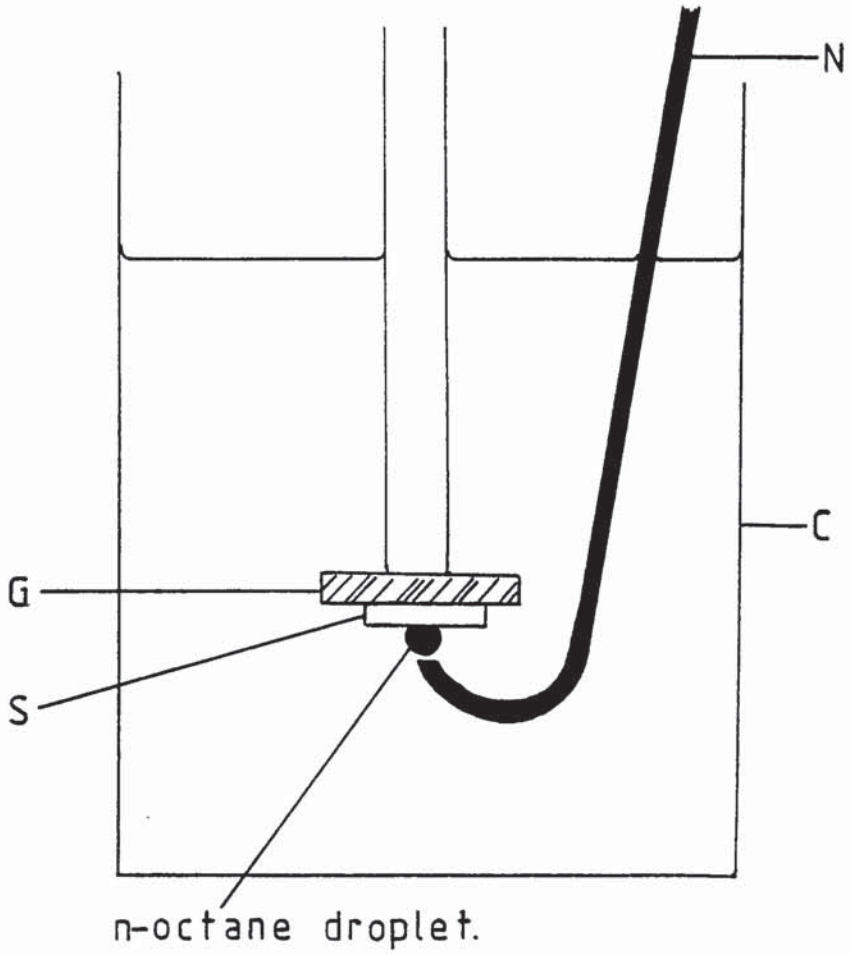


Figure 2.3 Hamilton's Method

the sample.

Angular measurements were taken for both the left and right contacting drop interface and then averaged. This was then repeated for at least three different n-octane droplets and the overall average value was taken. Contact angle measurements were made optically using a travelling telescope with a goniometer eyepiece equipped with a cross-wire and angular scale.

2.5.1 (ii) Captive Air Bubble Technique

In this technique a hydrogel sample is set up underwater exactly the same as in the Hamilton method, however, instead of n-octane droplets, air bubbles are released onto the sample surface. It is essential that the hydrogel surface is maintained in a horizontal plane for this technique. The smallest commercially available syringe needle was found to be too large to meet the experimental needs of this technique and it was therefore necessary to obtain a specially made curved G35 needle (2.032×10^{-4} m. dia) from a local manufacturing company (Aston Needles Ltd).

Using this needle in conjunction with the "Prior"-micromanipulator enables an air bubble to be delivered onto the sample surface, from a microsyringe, in such a way that the needle can be inserted into an air bubble so that its volume can be precisely controlled. The air bubble is enlarged to a diameter of approximately 5 - 6 m.m. and then reduced down to a size of 2 m.m., and the needle retracted. The contact angle measurements are

then taken in a similar manner to that used in the Hamilton method, and an average value taken.

2.5.2 Protein Adsorption Studies

This technique constitutes the main theme of the project, and it is made mention of here because it represents a somewhat novel approach to characterising the surface properties of a material in an aqueous environment. The theory and experimental procedures of this technique are fully discussed in later chapters.

2.5.3 Equilibrium Water Content (E.W.C.) - measurements

The E.W.C. of each hydrogel polymer prepared by bulk and solution polymerisation was measured after allowing sufficient time for complete hydration to occur, (see 2.4.3), either in distilled water and/or Kreb's solution.

Three samples were taken from each of the prepared hydrogel compositions, and surface water on each sample was carefully wiped off using filter paper and the sample quickly transferred to a preweighed glass vial for weighing. The samples were then dehydrated at 65°C in a vacuum oven until a constant weight was obtained and noted. This procedure generally took from 4 - 5 days.

From the known hydrated sample weight, W_2, and the dehydrated sample weight, W_1, the % E.W.C. can be derived from the equation:

$$\text{E.W.C.} \quad (\%) = \frac{W_2 - W_1}{W_2} \times 100$$

The E.W.C.'s of the samples were determined by this method and an average value taken.

2.5.4 Freezing and Non-Freezing Water - Content Measurements.

The freezing (unbound) and non-freezing (bound) water content measurements were determined by differential scanning calorimetry (D.S.C.), using a Perkin-Elmer D.S.C. - model DSC-2, fitted with a liquid nitrogen subambient accessory. For the operating conditions see Appendix 1. Areas under the melting peak were obtained from an integrater trace. The freezing component was computed from a calibration curve obtained by measuring melting peak areas from weighed samples of distilled water. The amount of the non-freezing component of the hydrogel sample was calculated from the difference between the freezing water content and the total E.W.C.

CHAPTER 3

EXPERIMENTAL II - PROTEIN ADSORPTION TECHNIQUES

3.1 Reagents

All main reagents used in the adsorption studies are listed, together with their supplier, below.

Reagents	Supplier
Human Serum Albumin (fraction V; 96 - 99% pure, remainder 1 - 4% mostly globulins)	Sigma Ltd.
Human Fibrinogen (fraction I; > 90% of protein is clottable)	Sigma Ltd..
Iodinated (^{125}I) Human Albumin Injection (Specific Activity = 2.5μ Ci/mg Albumin)	The Radiochemical Centre, Amersham.
Iodinated (^{125}I) Human Fibrinogen Injection. (specific activity > 100μ Ci/mg Fibrinogen)	The Radiochemical Centre, Amersham.
"Sigma 7 - 9^{R}". (Tris (hydroxy-methyl) aminomethane)	Sigma Ltd.

All protein solutions were made up in 'Physiological'-Kreb's solution, pH 7.4, as necessary, and its composition together with further technical information pertaining to the radiolabelled protein preparations, can be found in Appendix 2.

3.2 Preparation of Protein Solutions

3.2.1 Preparation of Human Serum Albumin (H.S.A.) Solutions

This product was supplied in a crystalline, freeze-dried preparation. Conventional procedures were followed to make up the H.S.A. in Kreb's solution, using a magnetic stirrer to fully dissolve the albumin.

3.2.2 Preparation of Human Fibrinogen (H.Fb) solutions

The commercially prepared H.Fb preparations are obtained by 'salting-out' of solution and then freeze-drying. Due to the low solubility of the commercially prepared H.Fb, some difficulty was experienced in preparing a H.Fb solution. The low solubility was due in part to the high salt concentration in the preparation, from the 'salting-out' process, and also the inherent chemical nature of the fibrinogen molecule.

This problem was resolved by the following procedure: the powder is scraped out of the bottle(s) as completely as possible and pulverised with a mortar and pestle. The remainder is rinsed out with 37^{o}C Tris-NaCl buffer, pH 7.4, and the total amount is suspended in the latter using about 50 ml per gram of fibrinogen. The suspension is stirred on a magnetic stirrer for about 30 minutes and then transferred to 'visking' - tubing, for dialysis against a Tris-NaCl buffer, pH 7.4, using 2 litres of buffer per 100 mls of fibrinogen suspension/ solution. (There is a considerable amount of undissolved material at this time). Dialysis is carried out at room temperature on a large magnetic stirrer, the heat from this produces some advantageous warming of the dialysis medium.

The dialysis 'visking'-tubing (tied at both ends) was frequently inverted in the initial period of dialysis, to aid in dispersal of the suspension. After some four hours a fresh buffer was substituted and further dialysis continued for 3 - 4 hours, after which the concentrated H.Fb solution was filtered

and stored frozen. The % clottability of these human preparations is believed to be above 95%.[126]

3.2.3 Preparation of Iodinated (^{125}I) - H.S.A. solutions

Each vial of ^{125}I-H.S.A. from the supplier, contained 200 mg of albumin and at the specified reference date this had an overall activity of 500μ Ci/ 10 ml, giving an effective specific activity of 2.5μ Ci/ mg albumin. It was found from experimentation that an optimum cost-effective specific activity of approximately 1.67μ Ci/mg albumin was necessary for good reproducible results. Typically the standard radiolabelled H.S.A. solution was made up from 200 mg of ^{125}I-H.S.A., together with 100 mg H.S.A., in 200 mls of Kreb's solution, to give an overall 150 mg% solution.

3.2.4 Preparation of Iodinated (^{125}I)-H.F - solutions

Each vial of ^{125}I-H.Fb from the supplier, contained on average 0.8 mg of freeze-dried fibrinogen. This was very easily solubilised in Kreb's solution and was added to a 200 ml volumetric flask together with subsequent rinsings from the vial. H.Fb solution of known concentration and volume was added to the flask, with a pipette, and a 20 mg% solution was made up. The total activity at the specified reference date was 110μCi and a specific activity of 137μ Ci/mg fibrinogen. It was found by experimentation that the optimum specific activity necessary for these studies was 2.75μ Ci/mg fibrinogen. The concentration of the concentrated frozen stock fibrinogen solution was determined following thawing and centrifuging, by ultraviolet spectroscopy (at 280 nm. and assuming $\varepsilon^{1\%}_{1\ cm} = 15.8$), using a Unican SP 1700 Ultraviolet

spectrometer.

3.3 Static Adsorption System

For each hydrogel composition under investigation, samples were taken and a quantitative measure of protein adsorption with time, was made.

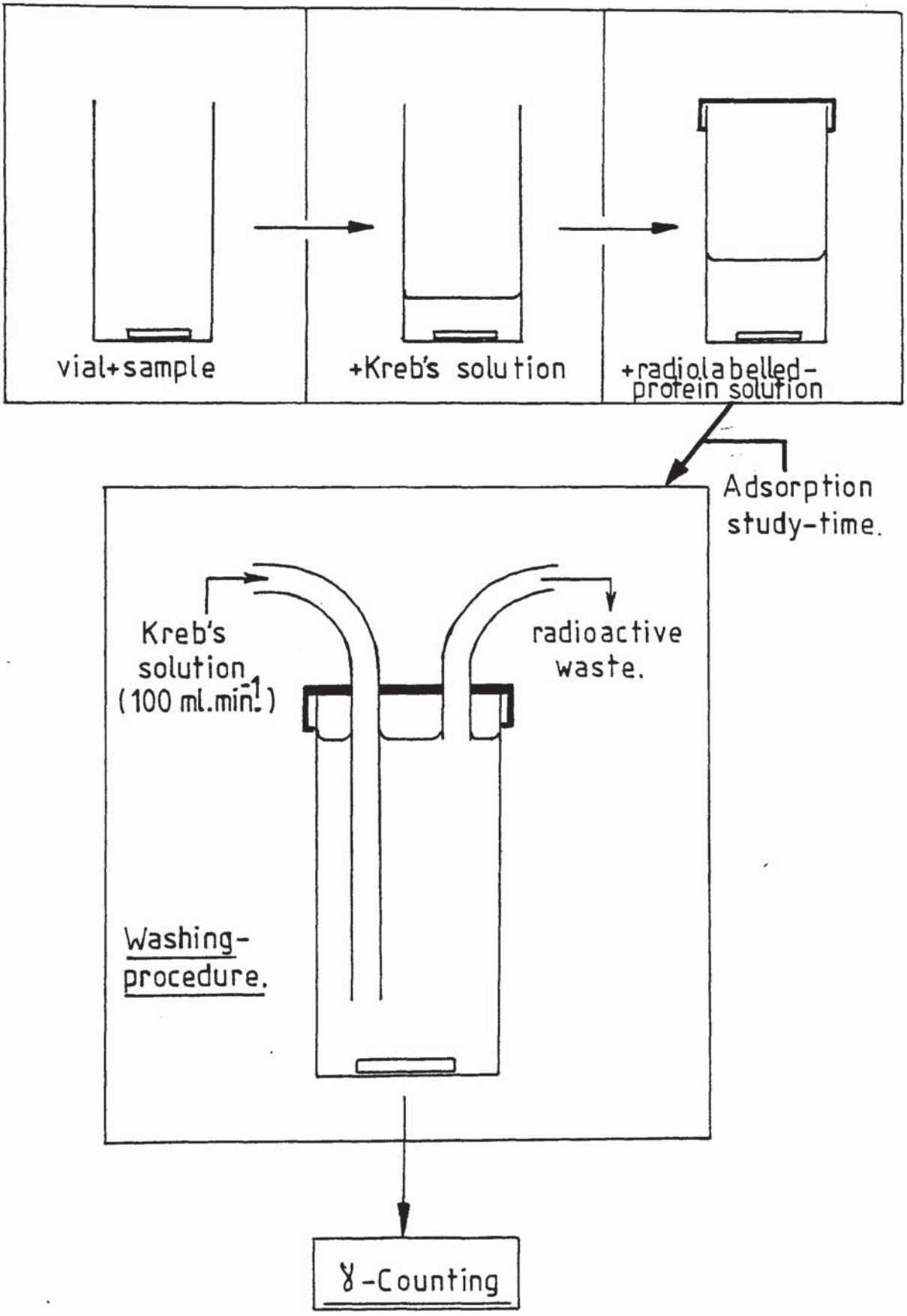
To obtain the optimum cost-effective use of the expensive radioisotopes, for each 200 ml batch of the radiolabelled protein solution, the maximum permissible number of 12 experimental study times, with staggered starts, were carried out. The apparatus used in these studies was of a simple design and construction, as illustrated in Fig. 3.1.

Each sample was placed in a vial (20 ml), to which was added 2 mls of Kreb's solution from an automatic "zipette"-dispenser, followed by 2 mls of radiolabelled protein solution, using a Gilson variable automatic pipette ($\leq$ 1000 μl), and the times were noted. At this point in time 3 x 100μl aliquots were taken from the radiolabelled protein 'stock' solution to be used later as internal counting standards.

Prior to the addition of the radiolabelled protein solution the sample should be checked in order to confirm that it is totally submerged beneath the Kreb's solution.

The vials were sealed and stored in the dark at room temperature ($\sim$ 20^{o}C), until the prescribed time(s) had elapsed, where upon the radiolabelled solution in each vial (4 mls) was displaced from the samples by a volume displacement method using 500 mls

Figure 3.1 Static Adsorption System



of Kreb's solution, at room temperature, and at a flow rate of 100 mls/min. The prescribed flow rate was attained using a Watson-Marlow multi-channelled "Delta" - Peristaltic pump, and had been ascertained by experimentation to be effective in eliminating all the radiolabelled solution(s) from the vial(s), leaving the sample(s) suspended in Kreb's solution. Aliquots, of 1.0 ml, were taken from this latter suspending Kreb's solution, to constantly monitor the efficiency of the 'washing' procedure.

The samples were then very carefully transferred to counting vials and sealed, in preparation for γ-counting.

The above experimental procedure was developed only after many preliminary experimental studies were carried out, for both ^{125}I-H.S.A. and ^{125}I-H.Fb, in order to establish the various criteria necessary to characterise hydrogels by this technique, such as protein concentrations, specific activities of solution and specific activities of adsorption etc.

3.4 Dynamic "Flowing" Adsorption System

As an adjunct to the previous 'cruder' static system, an improved more quantitatively accurate system, capable of both static and dynamic adsorption studies was designed and constructed during the course of this project. The construction and experimental methods of this system are dealt with below.

3.4.1 Construction

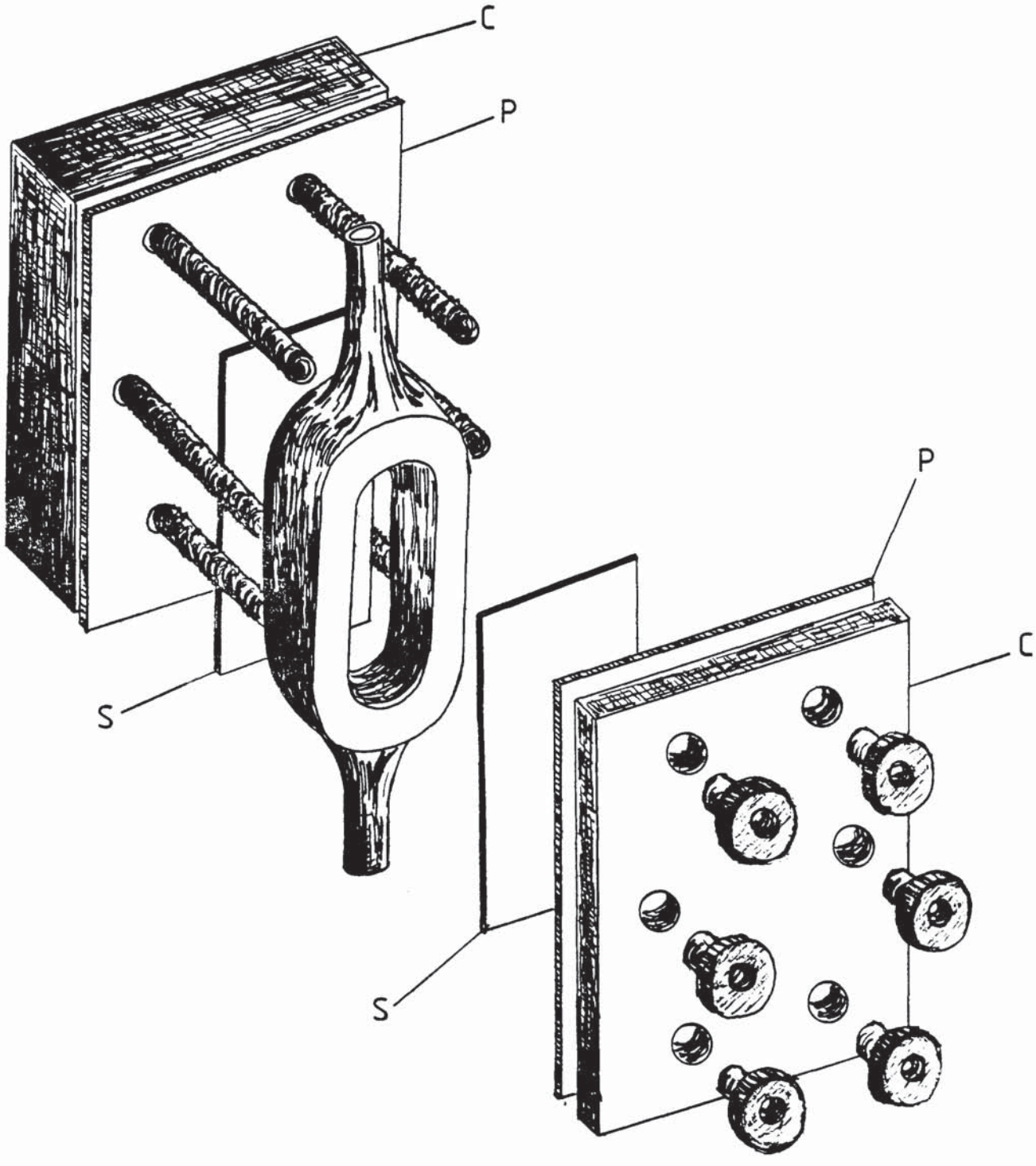
Initially one flow-cell was designed and constructed

to meet predetermined design criteria. After experimental testing five subsequent modified flow-cells, of improved design, were constructed. Each cell was constructed of pyrex-glass tubing (2.5 cm O.D. and 1.5 cm I.D.), drawn out at each end to form the inlet (0.5 cm I.D.) and the outlet(0.3 cm I.D.) of the cell. Two parallel diametrically opposing flat plane surfaces were then produced on each side of the main body of the cell, by grinding, and which effectively produced 'windows' in each side of the cell. The innermost edges of these 'windows' were shaped to prevent subsequent tearing of the hydrogel films under test, (which had occurred with the earlier cell). The surface area for each cell window was determined and is given in Appendix 2.

The cells were cleansed by conventional techniques using chromic acid and subsequently subjected to a siliconising process, by immersion in a bath of "Repelcote ^{R} ", air dried, and then washed with distilled water.

The materials under test in film form(s), are clamped between the cell windows and G2000-polyethylene gasket, G, by means of a purpose built perspex clamp, C, as illustrated in Fig. 3.2 By means of a system of medical grade polypropylene 3-way taps and medical grade 'Portex' silicone rubber tubing, an effective closed loop could be formed in which each cell could be alternatively perfused with Kreb's solution and then radiolabelled protein solution and finally Kreb's solution

Figure 3.2 Exploded view of Flow-cell



once again, without the test material ever coming into contact with the air/protein solution interface, and the implications thereof.

A perspex tank was also constructed to hold and contain the five flow-cells, filled with Kreb's solution. It's purpose was to maintain hydration of the samples on long time exposures, and also to act as a temperature bath. Each cell was labelled from A through to E, and each window I or II, to avoid any confusion. Perfusion was again by way of the Watson-Marlow multichannelled peristaltic pump.

3.4.2 Experimental method

The hydrogel films under test are cut into approximately 5 x 2 cms rectangular samples, S, and then each sample plus its gasket, G, backing, is positioned across the cell windows and firmly held in position by the clamp, C. Care must be taken to ensure that the clamping pressure is equally distributed across the rather fragile samples, otherwise leaks may develop.

The overall experimental procedure is best illustrated schematically in Fig. 3.3.

When the prescribed time(s) had elapsed, the experiment was ended by 'washing' through with Kreb's solution ($\sim$ 500 mls) at a flow rate of 100 mls/min.

Finally the Kreb's solution is displaced by air and the samples carefully transferred to counting vials and sealed in preparation for γ -counting.

After each experiment the whole apparatus is washed through with a 1% solution of Triton X-100 in borax pH 9.2

(0.2m H_3BO_3 titrated with 10% NaOH), and allowed to stand for a period of approximately 24 hours, where upon it is thoroughly rinsed out with hot water, followed by distilled water in readiness for the next experiment.

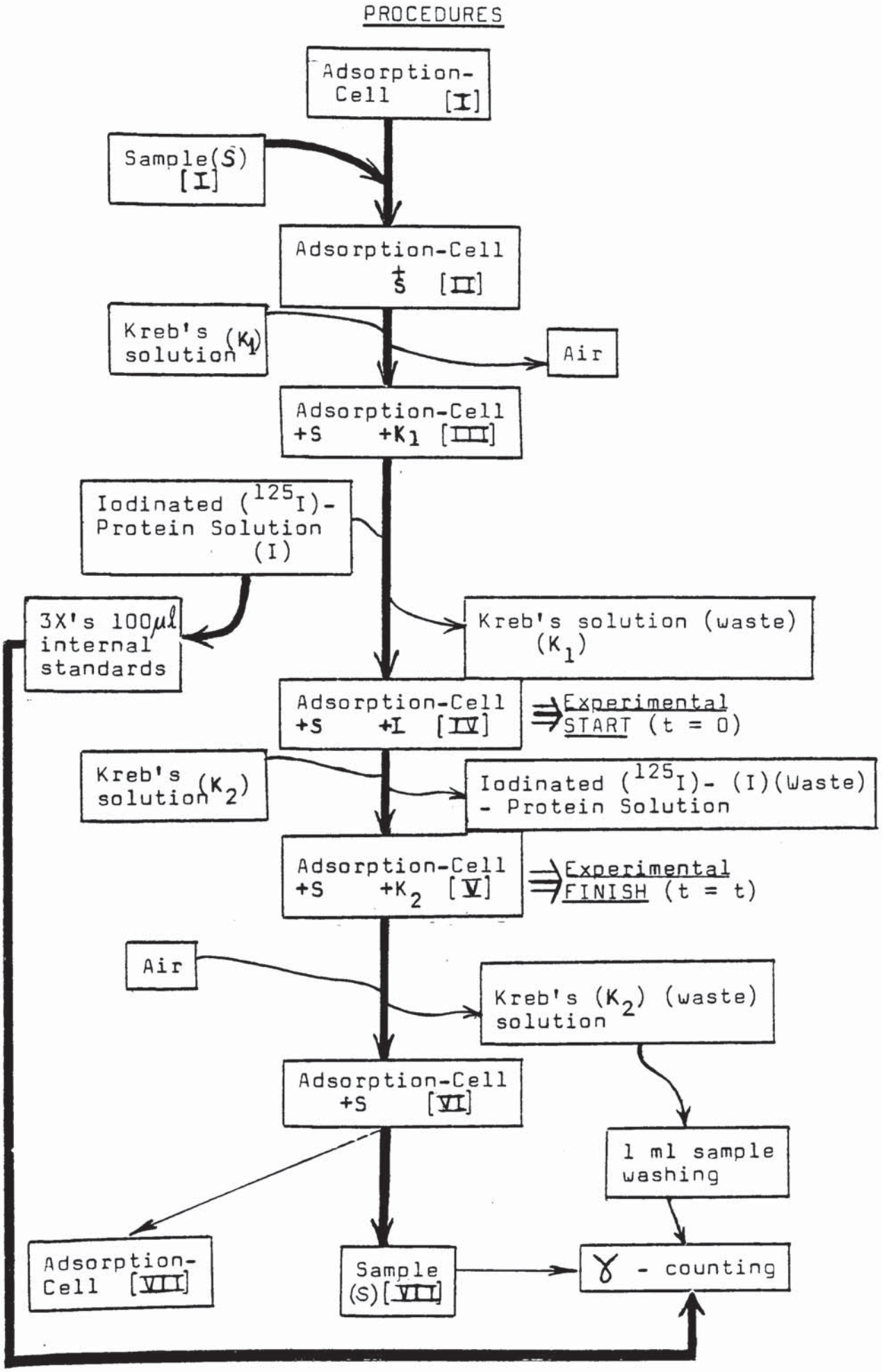
3.5 Radioisotope (^{125}I) - Counting Techniques

All samples ready for counting were loaded into the γ-counter, a Nuclear Enterprises Type: 8312 γ/β -counter, together with internal standards and a requisite number of blanks, for the estimation of background radiation and to act as experimental controls. The 8312 γ/β -counter is a solid crystal NaI (Tl)-scintillation counter with a total sample capacity of 400 samples (10 trays). Machine counting efficiency was determined for the ^{125}I-isotope by the conventional procedures and from which the optimum operating conditions were obtained, See Appendix 2 for details. Each sample was automatically counted for up to 2×10^5 counts or 600 seconds, which ever elapsed first, and the counts and time were printed out by teletype.

The standard radiochemical technique for the quantitative estimation of a material is by correcting the experimentally observed sample counts (using the machine counting efficiency), to give the theoretical counts (disintegrations per second), and then the quantity can be determined from the known theoretical specific activity of the material in μCi/mg (disintegrations per second/mg). This technique also requires correction for the decay of the radioisotope with time. The big advantage of using internal counting standards, as used

Figure 3.3

SCHEMATIC FLOW DIAGRAM FOR DYNAMIC ADSORPTION EXPERIMENTAL



in this project, is the simplicity of calculations, in that neither machine efficiency nor decay need be considered in the calculations. ^{125}I has a half-life of 60 days, and therefore, for the typical counting time of approximately six hours, for 60 samples, the decay during this period is insignificant, and it will therefore make no difference to the results, whether the internal standards are counted first or last in the counting run.

The calculated method is illustrated below:

Average internal standard (counts per 600 seconds) = $X_{c/600}$

Correction for Background radiation ($B_{c/600}$) = $X-B = X^{B}_{c/600}$

$X^{B}_{c/600}$ = P mg of protein

and therefore $1_{c/600} = P/X^{B}$ mg protein

Mean Sample Count, $\bar{x} = S_{c/600} \pm \sigma^{n-1} = d_{c/600}$

where n = 8 $\bar{x}$ = mean

σ^{n-1} = standard deviation for a small population

therefore the mean Adsorbed protein, $S_A = S \times P/X^{B}$ mg

and the standard deviation, $d_A = d \times P/X^{B}$ mg

therefore Adsorbed protein = $S_A \pm d_A$

CHAPTER 4

HYDROGEL-WATER: STRUCTURE-PROPERTY RELATIONSHIPS

4.1 Introduction

The intention of this chapter is to give some insight into those properties exhibited by hydrogels, as a direct consequence of their chemical structure and interactions with water. In the main this discussion will be centred on the hydrophilic properties of hydrogels, as determined by equilibrium water content and differential scanning calorimetry, water binding measurements. However, as an adjuct and to give a more complete scenario, other hydrogel properties, such as permeability, mechanical strength, surface/interfacial chemistry, optical and biocompatibility, will also be given some consideration.

Also included in Section 4.3 of this chapter are the results of the monomer purification procedures, outlined in Chapter 2. The purification of 2-hydroxy ethyl methacrylate is of particular interest, because it forms the basis for the majority of copolymer compositions in this project, and as such this data will allow true structure-property effects to be determined.

There is good evidence to suggest that many of the above hydrogel properties are directly related to the way in which the water is structured within the hydrogel-network, and therefore it would seem of value to give some consideration as to what is implied by the term 'water structuring'.

4.2 Water Structuring

The term structured water is used to describe a three dimensional network of aggregated water molecules held together with different degrees of rigidity by different degrees of hydrogen-bonding, (a special type of dipole-dipole interaction). A single molecule can be attracted to adjacent molecules by zero, one, two, three or four hydrogen-bonds. The greater the number of mutually shared bonds the greater the degree of structuring. The use of nuclear magnetic resonance (N.M.R.), has clearly indicated that water in biological systems is far more structured than water in the bulk (i.e., normal liquid water).[127] The term bulk water is used to refer to such relatively unstructured water, however this term does not imply that there is no structuring present in the bulk water, since all water has some degree of structuring.

Normally in bulk water hydrogen-bonds have an extremely short half-life (i.e., 10^{-11} seconds), however in structured water considerably longer half-lives would be expected.

The structure of water in any system can be influenced and changed by a number of factors, some of which are presented in Table 4.1.[128] (See Over).

Table 4.1

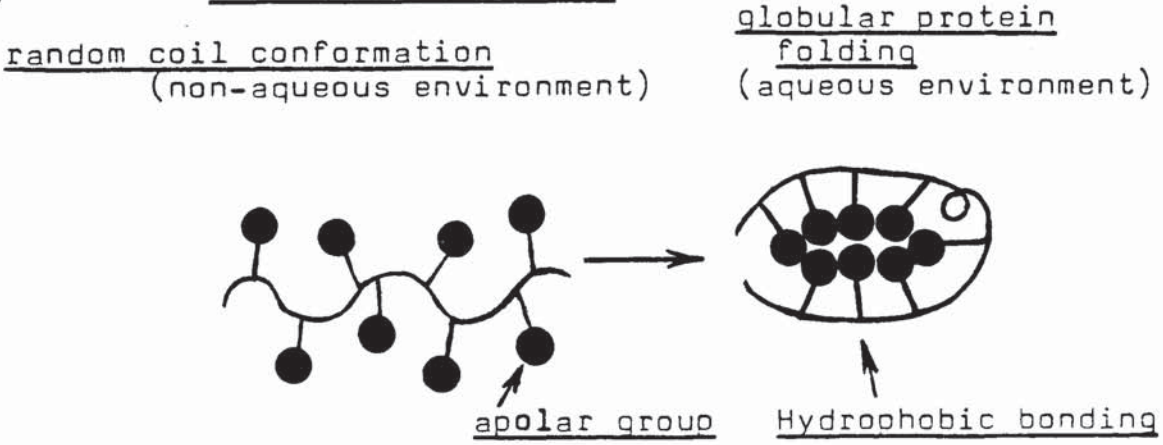
Factors Which Influence Water Structuring in Biological Systems[128]
A) <u>Factors which increase the degree of Structuring</u>: 1. Non-polar molecules 2. Non-polar segments of macromolecules 3. Polar ions such as H_3O^+, Na^+, Ca^{2+} 4. Conformational changes of protein towards a random coil conformation 5. Decreasing distance between adjacent surfaces 6. Decreasing temperature 7. Decreasing pH
B) <u>Factors which decrease the degree of Structuring</u>: 1. Polar molecules such as K^+ and NH_4^+ 2. Small non-polar ions which can break hydrophobic bonds i.e., urea and vitamin C. 3. Polar segments of macromolecules 4. Conformational changes of protein towards a globular conformation 5. Increasing distance between adjacent surfaces 6. Increasing temperature 7. Increasing pH

These factors can strengthen, weaken or completely break water structure. Depending on the effect of a solute on water structure, the solute is referred to as either a structure maker or a structure breaker.

Transfer of apolar molecules into an aqueous

environment is accompanied by a net negative entropy of hydration. This observation led to the suggestion that inert solutes, promote the structuring of neighbouring water molecules into clathrates (i.e., 'iceberg' effect)[129], so that hydrophobic, Van der Waals interactions of apolar molecules could be regarded as a partial reversal of the thermodynamically unfavourable process of solution. The water within these structures has a very low dielectric constant and in most ways it behaves more like a non-polar or lipid-phase, than a polar-phase. In this way complex aggregates, such as micelles, bimolecular layers and lamellar structures can exist, depending on the nature of the aggregating species. On a more complex level, water structuring due to apolar residues attached to a common polymeric backbone, can promote conformational changes of which the folding of globular proteins is the best known example[130], as illustrated below in Fig. 4.1.

Figure 4.1 Protein Conformation



Due to the above conformational changes, the hydrophilic (polar) groups of the globular proteins, will tend to be exposed to the aqueous environment and will become hydrated to a depth of only a few molecules.

In contrast to this fibrous proteins in the random-coil conformation, tend to become hydrated to a depth of a few thousand molecules,[131] in other words conformational changes of proteins will create changes in water structure.

Molecules with centres of high charge densities or inorganic ions cause the formation of so-called 'soft-ice' structures in their hydration shells. The well known binding of water molecules to potassium ions is a simple example of such a structure. Again the water in such hydration shells shows more lipoidal properties than bulk water properties.[128] Solid surfaces have been shown to influence water structuring and water molecules adsorb onto these surfaces in multiple layers in accordance with the B.E.T. multiple adsorption equation.[132]

Water molecules which interact strongly with macromolecular surfaces have been found to exhibit properties that are measurably different from those in the bulk.[133,134] These water molecules exhibit a lower vapour pressure, lower mobility and greatly reduced freezing point.

Considerable evidence now suggests that water molecules within hydrogel polymers, may exist in two or more different states.[52,135-149] Of these, one probably consists of water bound to the polymer through hydrogen-bonding, sometimes referred to as 'bound' or non-freezing water. All other water which does not take part in direct hydrogen-bonding with the polymer has a high

degree of mobility and is known as 'free' or freezing water. From nuclear magnetic resonance data (n.m.r.) of Andrade et al,[145] it was deduced that structured water in hydrogels takes the form of 'bulk', 'interfacial' and 'bound' water. In which the term 'bulk', refers to that water imbibed in large pores as normal water, 'interfacial' describes that water structured around hydrophobic groups in clathrate cages, and 'bound' defines the water structured around charged ionic groups or orientated around hydrogen-bonding groups or other dipoles. It has been suggested that the nature and relative amounts of the types of water have a considerable bearing on many of the properties of hydrogels. For hydrophilic polymers, water plays an important role in their interactions with blood and it is considered that there may be a correlation between the nature of water in a hydrogel and its biocompatibility.[51,54,98] Perhaps the best way to describe water present in hydrogels is to view the water as existing in a continuum, with highly bound water molecules interacting directly with polar (hydrophilic) sites on the polymer and surrounded by successive hydration shells of less highly bound water, interfacing with which are hydrophobic clathrate structured water, producing short and long range stabilising effects.

A variety of techniques exist for the study of water binding in polymers and this can lead to confusion as the quantity of a particular state of water is dependent on the experimental technique used. This is because each technique may have a bearing on different aspects

of the properties of water, i.e., thermodynamic, dynamic and structural.[150] The freezing behaviour of water-swollen polymers has been shown by a number of workers to be anomalous in the sense that only part of the available water shows a phase transition even down to very low temperatures. In partially dehydrated material often no phase transition is observed at all. Moreover, a considerable hysteresis of freeze-thaw behaviour is common.[150]

The use of differential scanning calorimetry (D.S.C.) thus enables a quantitative determination of the relative amounts of 'freezing' and 'non-freezing' water, to be made.

4.3 Monomer Purification Analysis

One of the main drawbacks in the use of 2-hydroxy ethyl methacrylate (HEMA) is the difficulty in attaining the monomer in a suitably purified state. Commercial preparations have been found inadequate for biomedical applications, due to their unpredictable characteristics. Two major impurities, methacrylic acid (MAA) and ethylene glycol dimethacrylate (EDMA) have been cited,[54] to account for the unpredictability of poly (HEMA) biomedical devices.

Commercial HEMA preparations were investigated for these impurities during this project, using the G.L.C. operating conditions as laid down in Appendix 1.

From these measurements it was determined that the commercial preparations contained impurity levels as high as 5.3% by weight of EDMA and 4.7% by weight of

MAA. Following the purification procedure, given in Chapter 2, subsequent G.L.C. analysis determined that these levels had been reduced to $\leqslant$ 0.5% (by weight) of EDMA and $\leqslant$ 0.1% by weight of MAA, and these levels of contamination were deemed to be acceptable for experimental purposes.

It was found that the two purification techniques for acrylamide (ACM), were of comparable efficiency, producing acrylic acid levels of $\leqslant$ 0.05% by weight. However due to the difficulties involved with large scale production by vacuum sublimation, the multiple recrystallisation technique was the adopted purification procedure used in this project.

Hydrogel-Water Interactions and their Relationship to Hydrogel Structure and Properties:

4.4 Introduction

Hydrogels have been defined as polymeric materials which have the ability to swell in water, generally in an isotropic manner, to form an expanded three dimensional gel-network. The way in which hydrogels swell, or hydrate, is generally accountable, to their inherent capability of hydrogen-bonding with water. Such a capability, infers that the polymer-solvent interactions are comparable to the polymer-polymer interactions. Thus a study of the bulk polymer-solvent interactions, by means of equilibrium water content determinations, can effectively be thought of as a means of characterising these polymers. Towards this end a number of different hydrogel copolymer series were synthesised by methods described in Chapter 2.

In addition to the total amount of water present within a hydrogel, as measured by equilibrium water content, the nature or organisation of the water is of great importance in relation to those properties exhibited by the hydrogel. Water within the hydrogel network has been shown to exist in more than one state, and it is this phenomenon of hydrogel-water/structure-property relationships which governs many of the interactions of hydrogels in a given environment.

The majority of past work covering the subject of hydrogel-water interactions, has been based on those properties exhibited by the hydrogels in a distilled-water environment, making data analysis a much simpler task. The validity of these results, when predicting the properties of such materials when in a physiological environment, is somewhat suspect, and this is indeed of particular merit when considering ionic hydrogels. To enable a better understanding of the behaviour of hydrogels in a physiological 'like' environment, characterisation of hydrogels by water content both in physiological Kreb's solution and in distilled-water were undertaken. To complement these studies differential scanning calorimetry measurements were carried out on a limited number of the polymer compositions, in order to determine the water structuring within their networks. To present a more complete discussion, the relevant findings of previous studies, concerning equilibrium water contents and differential scanning calorimetry measurements will also be included with those results obtained during the course of this research.

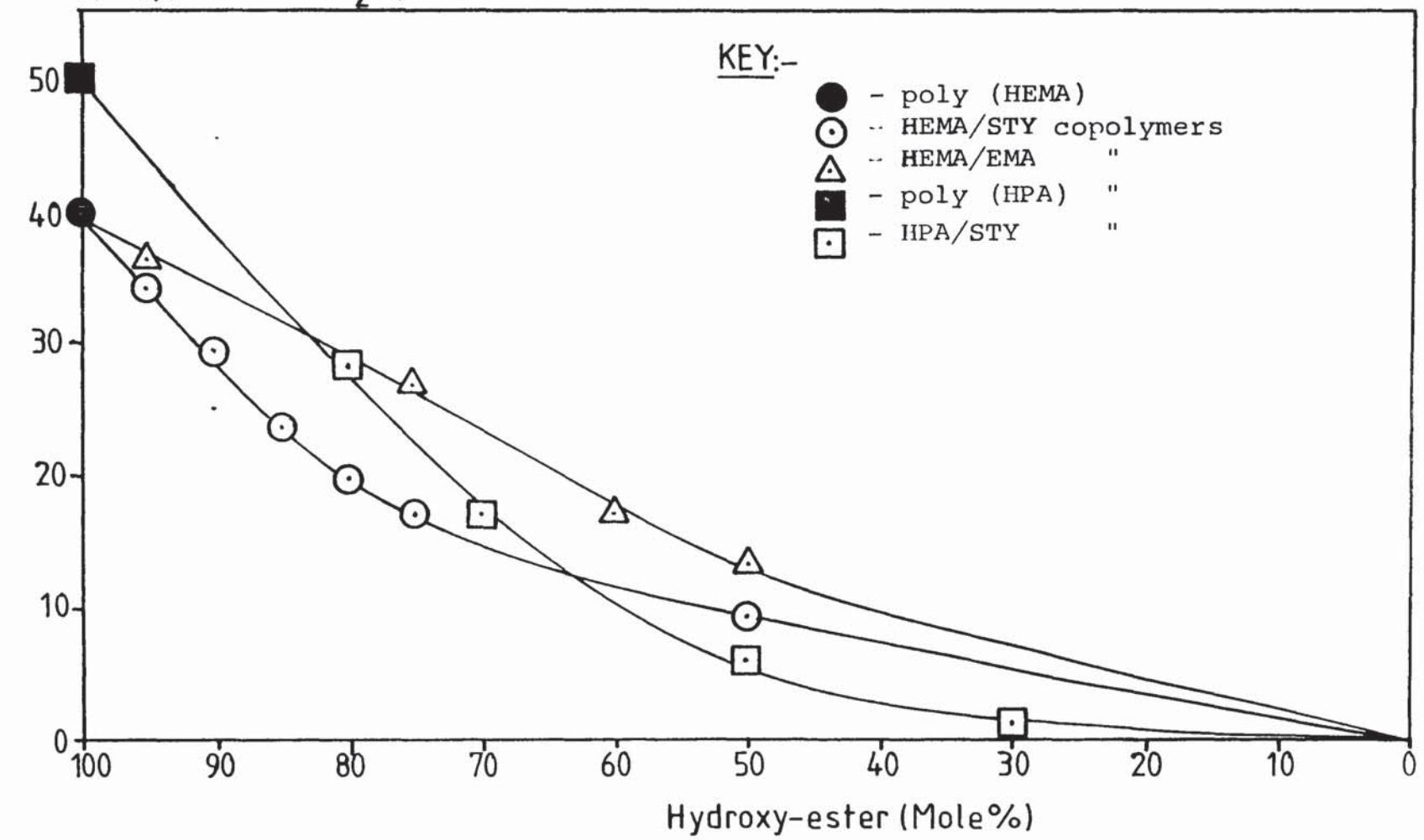
4.4.1 Hydrophilic/Hydrophobic Copolymer Interactions

All the bulk polymerised compositions in this series were based on purified 2-hydroxy ethyl methacrylate (HEMA) as the hydrophilic component, with the exception of those copolymers where N-vinyl pyrrolidone (NVP) was used in place of HEMA.

The equilibrium water contents of hydrogels prepared by copolymerising a hydroxyl substituted unsaturated aliphatic ester with monomers of varying hydrophilic and/or hydrophobic characters, has previously been reported.[151] Although simple 2-hydroxy propyl acrylate (HPA) copolymers have not been utilized in this present project, (due to low glass transition temperature (Tg) and poor mold release characteristics), they have been included in this discussion, because they enable structure/property comparisons with their isomeric counterparts, plus they represent one of the components of the complex hydrogel formulation for continual wear soft contact lenses, as discussed in Chapter 7.

It can be seen from Graph 4.1, that 2-hydroxy propyl acrylate (HPA) is more hydrophilic than its structural isomer 2-hydroxy ethyl methacrylate (HEMA). However the results also indicate a comparable dramatic depression of water content for both isomers when progressive amounts of the hydrophobic monomer, styrene, are added to the compositions. This progressive depression would appear to be more marked for the HPA copolymers until at approximately 65:35 (moles %) of hydroxyester to styrene, the HPA-copolymers are of lower hydrophilicity than their HEMA-cooolymer

Graph.4.1: Influence of Hydrophobic Monomers on Hydrogel Water Content



counterparts. These observations may be perhaps explained by a consideration of the monomer structures given in Figure 4.2 and Figure 4.3. Due to the α-methyl substituent on the backbone of HEMA, these copolymers experience greater steric hindrance to water absorption than the case of their structural isomer HPA. HPA-copolymers would appear to possess a higher water content due to the lower steric hindrance induced by its pendent side chain methyl group.

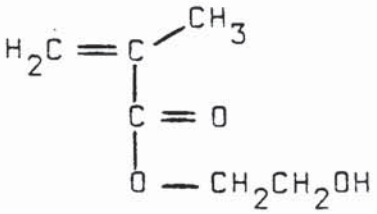
Inclusion of the hydrophobic monomers, styrene or ethyl methacrylate can be seen to produce a progressive decrease in the equilibrium water contents, but of the two, styrene produces by far the more dramatic effects.

This can be attributed to the bulky phenyl group of styrene, attached to the polymer backbone, limiting the accumulation of water within the gel-network by steric hindrance. The less dramatic decrease in water content observed with ethyl methacrylate is attributed to the smaller size of the pendant acrylate side chain, producing a smaller degree of steric hindrance together with its greater polarity (hydrophilicity), due to its ester and carbonyl groups.

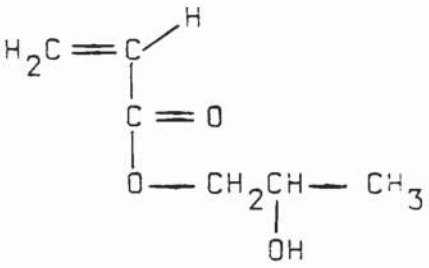
The greater efficiency in lowering water content observed in the HPA/STY copolymers, may also be attributed to the side chain methyl group of HPA (in the 2-position) being able to more easily undergo hydrophobic, Van der Waals interactions (bonding), with the phenyl group of styrene. These interactions are made easier by the greater freedom of movement of the HPA side chain methyl group as opposed to the restricted movement of the α-methyl group of HEMA.

Figure 4.2

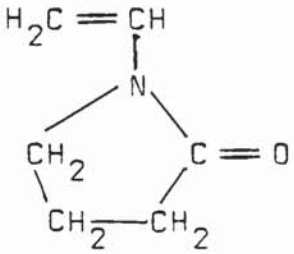
HYDROPHILIC MONOMER STRUCTURES



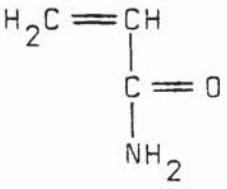
2-hydroxy ethyl methacrylate
(HEMA)



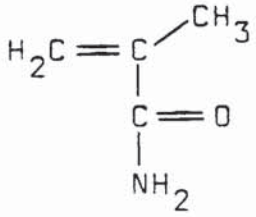
2-hydroxy propyl acrylate
(HPA)



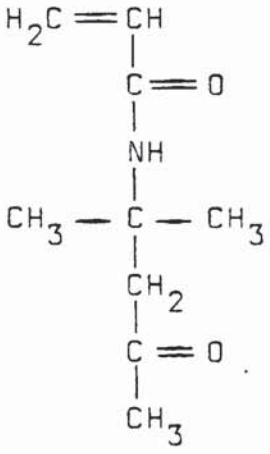
N-Vinyl Pyrrolidone
(NVP)



Acrylamide
(ACM)



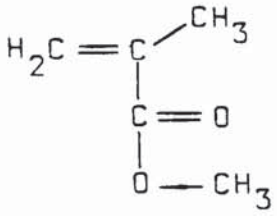
Methacrylamide
(MACM)



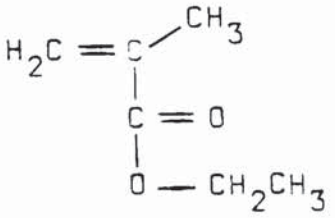
Diacetone Acrylamide
(DAACM)

Figure 4.3

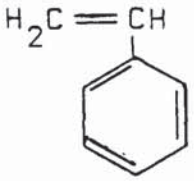
HYDROPHOBIC MONOMER STRUCTURES



Methyl methacrylate
(MMA)



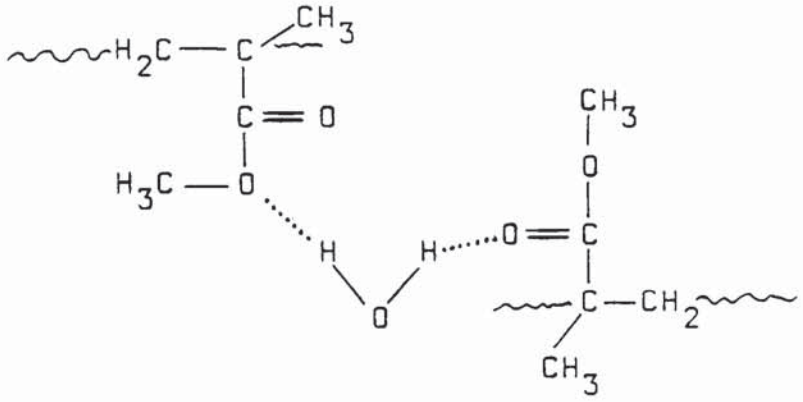
Ethyl methacrylate
(EMA)



Styrene
(STY)

It is perhaps of interest to consider the water-hydrogen bonding capacity of the so called 'hydrophobic' methacrylate and acrylate structures. Figure 4.4 illustrates a water ester-carbonyl hydrogen bonded bridge, as seen in poly methymethacrylate.

Figure 4.4 Poly Methyl Methacrylate Ester-Carbonyl Hydrogen-bonded Water Bridge



As the alkyl chain length is increased however, so the molecule loses its water binding properties, due to the ester group becoming progressively more sterically hindered, and thus a commensurate increase in its hydrophobic characteristics. This being so, it does indicate that even 'hydrophobic' polymers may bind water, and that the term hydrophobic is not an absolute. Even highly hydrophobic polymers, such as polyethylene, polypropylene and even poly (tetra-fluoroethylene) have been observed to bind water in small amounts (~ 6 mg per 100 g of polymer), at normal temperatures.[152]

Substitution of the highly hydrophilic N-vinyl pyrrolidone (NVP) for 2-hydroxy ethyl methacrylate (HEMA), in the HEMA/EMA compositions, produced similar trends in water content. The choice of this cooolymer composition was based on the need for a hydrogel of high equilibrium water content and with different

expected interfacial parameters to those of the high water content acrylamide copolymers. No observable variations were encountered for the NVP/EMA copolymer equilibrium water contents, as measured in either physiological 'Krebs' solution or distilled water. One very interesting observation was noticed however, regarding the unexpected strength of the NVP/EMA (75:25 moles %) composition (~71% EWC). This aspect will be dealt with in the relevant section concerning mechanical properties, however it would seem likely that strong interchain interactions are at work in this material, inspite of its high water content. Unfortunately no data yet exists as to the water structuring present in this copolymer, however it would seem very probable that a high non-freezing content would be measurable.

Having discussed the pronounced modifying effects of hydrophobic monomers on the general hydration patterns of hydrophilic polymers, to complete the picture we need to know whether these effects will affect the bound and free-water in a similar manner. A study has been carried out in our laboratories, using differential scanning calorimetry to determine the freezing (free) and the non-freezing (bound) water components of a series of HEMA/STY copolymers. Poly (2-hydroxy ethyl methacrylate) with a water content of approximately 40% has previously been shown to be comprised of 23% freezing and 17% non-freezing water.[153] The incorporation of 10 moles % of styrene in the copolymer was shown to reduce the water content to ~23% of which 4% is freezing and 19% is non-freezing water. At

approximately HEMA/STY (85:15 moles %) and above, it can be observed from the Table 4.2, that the water in the hydrogel is now totally bound. It is also of interest to note that at approximately this same monomer ratio, marked changes in the bulk properties of permeability and mechanical strength are ellicited. Such bulk effects would be expected to also exert an influence on the surface properties of hydrogels, and this will be one of the main areas of interest featured in the subsequent chapter concerning surface measurement.

From the concept of freezing water surrounding the bound (non-freezing) water in hydration shells, it can be envisaged that when the bulky hydrophobic phenyl group of styrene is introduced, its presence will tend to affect those freezing water molecules, near at hand in the outer hydration shells. The phenyl group due to its hydrophobic and steric characteristics appears to be decreasing the freezing water content simply by steric exclusion.

(See overleaf for Table 4.2)

Table 4.2

THE INFLUENCE OF THE HYDROPHOBIC STYRENE MOIETY

ON BOUND AND FREE WATER CONTENT

Hydrogel Copolymer	Monomer Ratio (Mole %)	EWC (%) in 'Krebs' at ~20°C	% Change *	Freezing water (%) (Free)	% Change *	Non-freezing water (%) (Bound)	% Change *	Bound/ free ratio
HEMA/STY	100:0	40	-	23	-	17	-	0.74
HEMA/STY	95:5	29	72.5	9.3	40.4	19.7	115.9	2.12
HEMA/STY	90:10	24	60.0	3.3	14.3	20.7	121.8	6.27
HEMA/STY	85:15	16.6	41.5	0.0	0.0	16.6	97.7	∞

* % Change for EWC, Bound and Free Water Contents refers to their percentage change with respect to purified poly (HEMA), (41.6% EWC), for the bulk polymerised compositions and to the poly (ACM), (93.3% EWC), for the solution polymerised compositions.

As one would expect the freezing water % change is decreasing with the % change in equilibrium water content (EWC), but in a more pronounced manner. Similarly the % change in the non-freezing water rises to a peak at the 90:10 ratio, before falling off to a fairly constant value (See Table 4.2). This would therefore seem to indicate that in fact the styrene molecule is structuring the water to some extent, (up to ~ 90:10 moles %), probably into the previously mentioned clathrate cages, which then tend to play a part in stabilising the hydration shells of the hydrogen-bonding polar groups, and thus raise the non-freezing water content. This is not evident simply from examining the bound/free ratio. Further increase in the styrene content above 10 moles % would seem to destabilise this previous structure, leading to a reduction in the non-freezing (bound) content.

A study of the measured water contents for neutral copolymers (See Appendix 3), hydrated in physiological solution or distilled water, would seem to indicate no significant changes in hydration interactions between the polymer and its two different aqueous environments. It would therefore seem safe to assume that the ionic moieties present in the physiological solution, do not markedly effect the neutral-polymer-aqueous hydration phenomena. However what cannot be determined from this data is whether the component ions influence the other bulk properties, by modifying the water structuring properties of the hydrogel.

All water-binding data on the physiologically hydrated hydrogels were corrected for the effects of the electrolytes, and thus should be comparable to measurements taken in distilled water. Therefore, no bound water should be directly attributable to the electrolytes present, however, there is the possibility that they will enhance or detract from the hydrogels own structuring effects indirectly. It should be emphasised that it is those hydrogel bulk properties exhibited in a physiological 'like' environment that this study is concerned with, and therefore the comparative differences between hydrogels hydrated in distilled water or physiological 'Krebs' solution, are of secondary importance.

From differential scanning calorimetry measurements obtained during the course of this research, large, as yet unexplained differences in the water binding properties are seen to have occurred, when compared to previous data, on supposed similar formulations. Non-freezing (bound) water in poly (HEMA) was found to be very high, 0.887 of the total EWC, compared to a previous figure of 0.425 bound fraction. Two alternative theories are available to explain this phenomena, first that the electrolytes present in the hydrating medium has influenced the structuring of the water by themselves or in combination with the inherent structuring of the hydrogel they have enhanced the bound content. The second alternative may be attributed to the much improved purity of the poly (HEMA), the

disruptive influence of the impurities, particularly methacrylic acid, has been eliminated, permitting a greater stability of the non-freezing (bound) hydration shells, and thus increasing the bound fraction. Concerning this alternative it should also be noted that the bound-free measurements of the physiologically hydrated commercial poly (HEMA) preparations gave a very large increase in the freezing water content, with a decrease in the bound content, which would seem to indicate the truth of the second alternative, and these together with the freezing water contents, which were obtained for the copolymers of HEMA/EMA, are given in Table 4.3.

(See overleaf for Table 4.3)

Table 4.3

THE INFLUENCE OF THE COMMERCIAL HEMA IMPURITIES, TOGETHER WITH THE INFLUENCE OF THE HYDROPHOBIC ETHYL METHACRYLATE MOIETY ON BOUND AND FREE-WATER CONTENT

Hydrogel Composition	Monomer Ratio (Mole %)	EWC (%) in 'Krebs' at ~20°C	% Change *	Freezing water (%) (Free)	% Change *	Non-Freezing water (%) (Bound)	% Change *	Bound/ Free Ratio
HEMA (Commercial)	100:0	57.2	137.5	28.9	614.9	28.3	76.7	0.979
HEMA (Pure)	100:0	41.6	-	4.7	-	36.9	-	7.85
HEMA/EMA	95:5	35.9	86.3	3.2	68.1	32.7	88.6	10.22
HEMA/EMA	75:25	29.0	69.7	0.7	14.9	28.3	76.7	40.4

* See Footnote for Table 4.2.

It would appear that the impurities present in the commercial poly (HEMA), (i.e., MAA and EDMA), produce a vast disproportionate rise in the freezing water compared to the overall rise in water content, as determined from the % change values. This can be attributed to the interaction between the carboxylate anion of MAA and the Na^+ cation in solution, producing a commensurate hydration in accordance with the hydrophilic cation.

With the HEMA/EMA copolymers, the previously reported action of the hydrophobic ethyl methacrylate on water content, can be seen to be reducing the water content of the gels, mainly by its effects on the free-water. Thus with increasing ethyl methacrylate content, the increasing hydrophobic content of the copolymers would appear to be a major factor in excluding the free (freezing) water. From the previously discussed considerations of the structural difference between ethyl methacrylate and styrene, this would appear anomalous with the structure enhancing properties attributed to styrene, and maybe these latter results were a consequence of interactions between the styrene and impurities present in the HEMA, and not as a direct result of the styrene molecule.

The incorporation of ethyl methacrylate into copolymers of HEMA would therefore seem to enable the much desired properties of highbound/low free-water content, necessary for a reverse osmosis membrane as used in desalination treatment of sea-water, to be achieved.[136]

Even poly (HEMA), which has previously been cited as having low potential in the above technique, may be shown to be of value, subsequent to more efficient purification of its monomer. Perhaps with a more hydrophilic monomer then HEMA, addition of ethyl methacrylate may provide even more desirable properties. To a certain extent this has been observed with even a high equilibrium water content of ~ 71% for the NVP/EMA 75:25 (moles %) copolymer, which exhibited greater strength than the ~ 40% EWC poly (HEMA) counterpart, and this may well be attributable to a very high bound water content, unfortunately this cannot be confirmed at present because to date no water binding data is available on this composition.

4.4.2 Hydrophilic/Hydrophilic Copolymer Interactions

In contrast to the previous section, this section will be concerned with the hydrogel-water interactions of copolymers, where both monomer components are of high polarity (hydrophilicity), and hence can exhibit high levels of both polymer-polymer and polymer-water interactions. A series of copolymers were synthesised, again using purified HEMA as the basic hydrophilic component and to it were added monomers with a variety of hydrophilicities. A study of the variance of equilibrium water content with structure for copolymers of 2-hydroxy ethyl methacrylate, with acrylamide and its derivatives, was undertaken. No experimentally significant variations in water content were observed for these copolymers, in either hydrating medium (See Appendix 3 for data), and so Graph 4.2 is representative of trends set in both

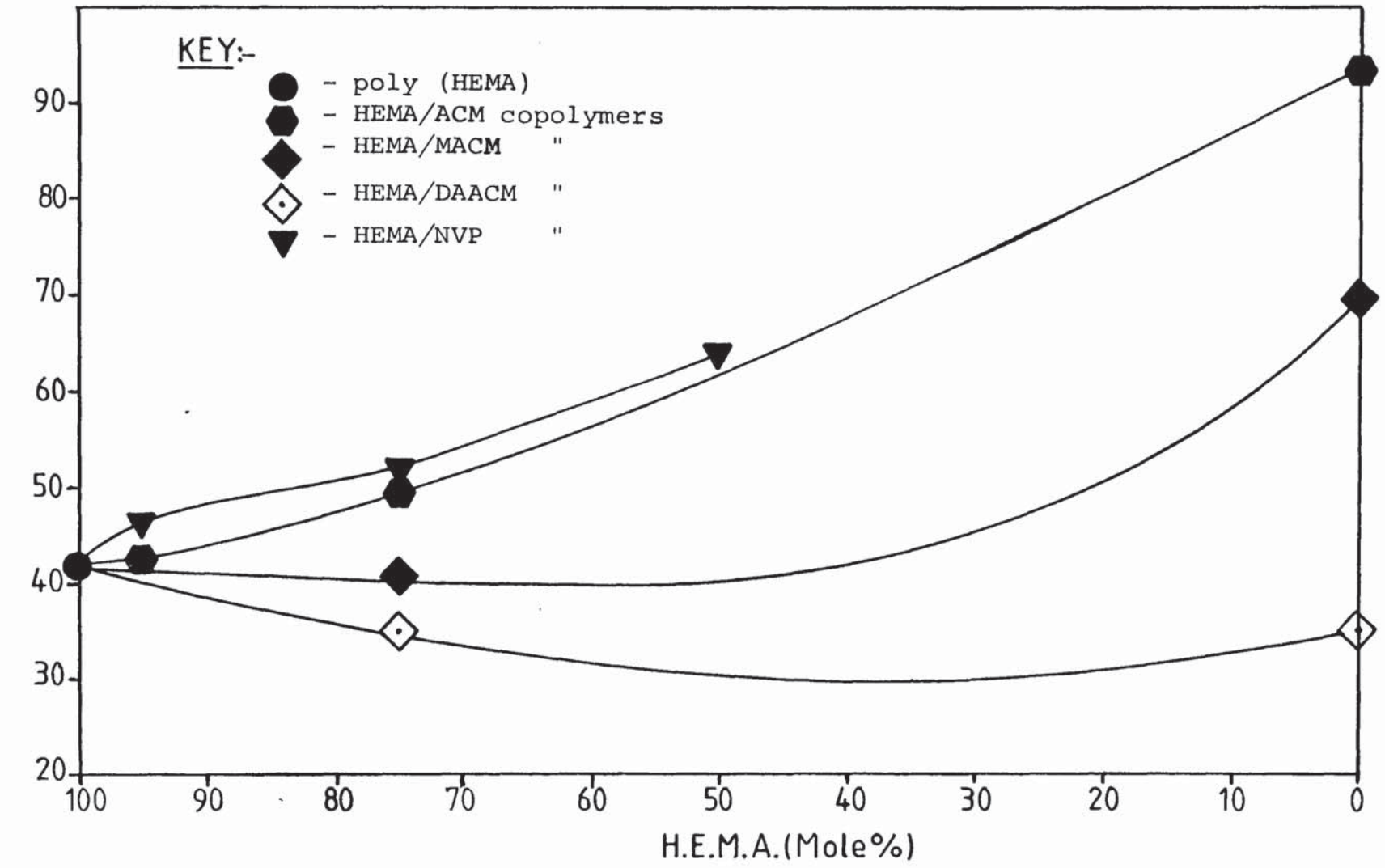
distilled water (for this work and others[154,155]) and physiological Krebs solution. The strong hydrogen bonding capacity of amides is also a characteristic feature of acrylamide, (ACM), due to the polar nature of its carbonyl and amine groups. Strong interactions take place between these groups and water, giving rise to very high water content gels. Accordingly the polymer-polymer interactions tend to be of lesser importance, but they do contribute significantly to the strength of these high water content gels.

Copolymers of HEMA/ACM tend not to undergo significant interchain hydrogen bonding, between their pendant polar side groups, due to the shielding of their carbonyl groups, and therefore a progressive increase in the acrylamide content gives a virtual linear rise in the equilibrium water content. The trends for the water contents of these copolymers are shown in Graph 4.2, and the differences in behaviour of the ACM copolymers and those of its derivatives are clearly illustrated. In the graph extrapolation of these copolymers towards the solution polymerised poly acrylamide, poly methacrylamide, and poly (diacetone acrylamide) has been made, to show the expected trend, based on the reported findings of other workers.[154,155] Bulk polymerised copolymers of HEMA with acrylamide and its derivatives has limitations due to the low solubility of the acrylamide derivatives in the HEMA.

Substitution of acrylamide by methacrylamide in these HEMA copolymers can be seen to cause a small initial depression in the water content trend, before a

Graph. 4.2: Influence of Hydrophilic Monomers on Hydrogel Water Content

E.W.C.(%),(Kreb's solution)



near linear rise is indicated. Similarly this trend is also observed when diacetone acrylamide is substituted for acrylamide, as previously reported by I. Middleton.[155] From an examination of the chemical structures of the acrylamide molecule and its derivatives in Fig. 4.2 a possible interpretation of these results is made possible.

Inclusion of methacrylamide in place of acrylamide, as previously stated, produces an initial depression of water content until at a certain concentration of amide groups, the inherent greater polarity (hydrophilicity) of methacrylamide as compared to HEMA, causes a near linear increase in equilibrium water content. The initial depression is quite probably due to hydrophobic, Van der Waals interchain interactions between the α-methyl substituents on the methacrylamide and 2-hydroxy ethyl methacrylate backbones, together with dipole-dipole interactions of the pendant side chain groups. With increasing methacrylamide so these constraining interactions are overcome by the high polarity of the amine group, and thus the equilibrium water content is increased accordingly.

The pronounced depression seen with the diacetone acrylamide copolymers can be attributed to the replacement of a hydrogen in the amine group by the 1,1 dimethyl 3-oxy-butyl group. This group can not only undergo hydrophobic, Van der Waals interactions with α-methyl substituent of HEMA units, but also it tends to shield the hydrophilic amine group. It is therefore not surprising that substitution of a hydrophobic group at the polar amine group site is more effective in causing depression of the water content

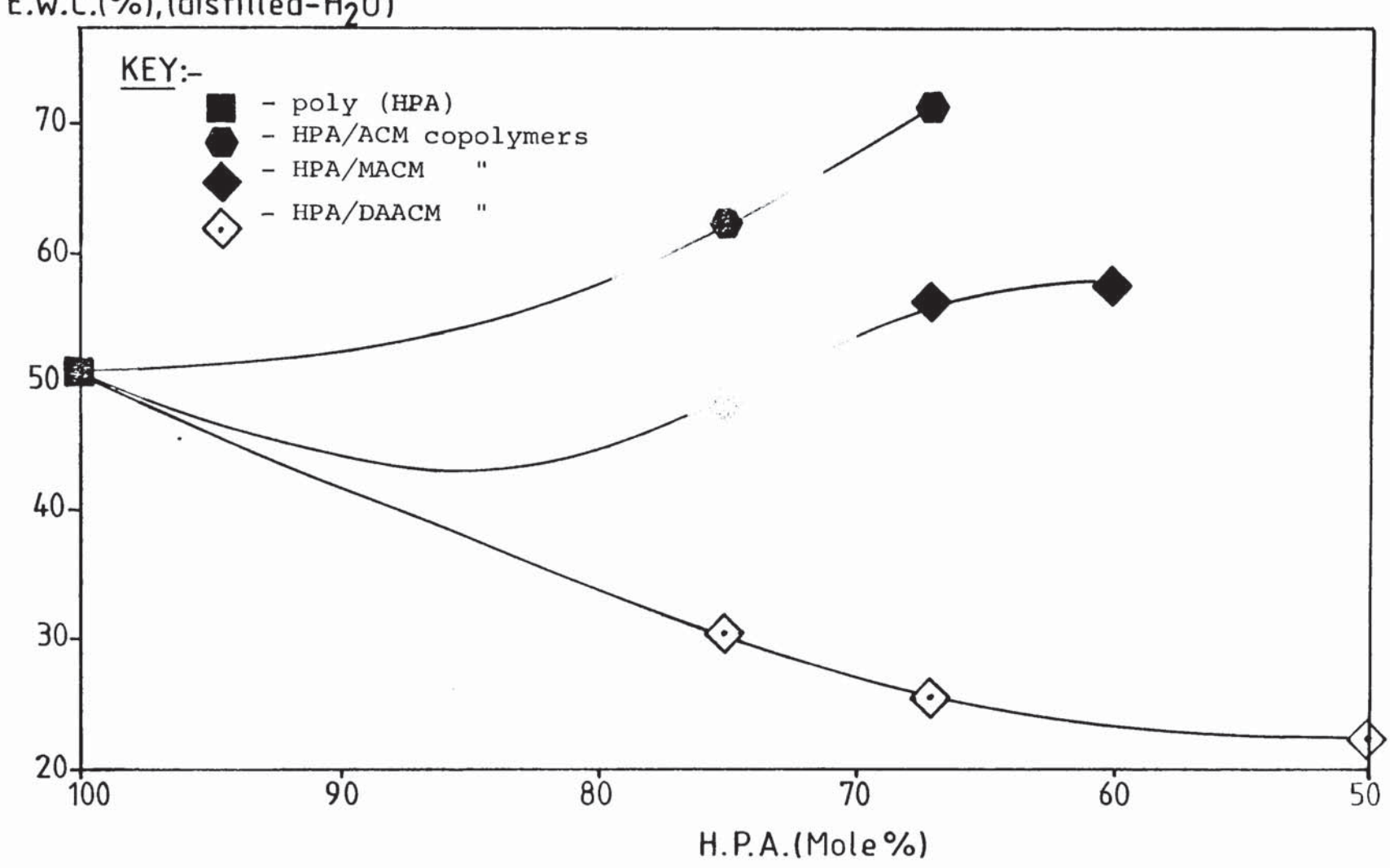
than the α-methyl substituent of methacrylamide.

As the diacetone acrylamide is increased above the 50:50 moles % a gradual increase in water content occurs until it reaches the water content of poly (DAACM) ($\sim$ 35%). Simply on the evidence of the equilibrium water contents for the solution polymerised poly (ACM), poly (MACM) and poly (DAACM), the effectiveness of these hydrophobic substitutions are demonstrated.

Similar studies have been carried out using the structural isomer of HEMA, hydroxy propyl acrylate,[155] and these results are reproduced in Graph 4.3. For all intents and purpose the trends for these copolymers, seem to be identical for each of its HEMA-copolymer counterparts, except that with the HPA/DAACM composition, no rise in water content at 50:50 (Moles %) is observed. This is somewhat surprising, in that previously HPA had been shown to be of greater hydrophilicity than its structural isomer. However, as with the trend seen with the HPA/STY and HEMA/STY copolymers, this can perhaps be attributed to the greater freedom of movement of the pendant 2-methyl group of HPA being able to more easily undergo hydrophobic, Van der Waals interactions with the hydrophobic 1,1, dimethyl 3-oxy-butyl group, together with possible dipole-dipole interchain interactions, which effectively exclude water uptake more efficiently than the HEMA/DAACM copolymer structure.

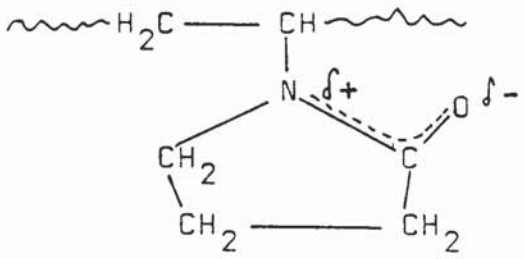
To investigate further the effects of hydrophilic/ hydrophilic interactions, a similar hydrophilic monomer to substitute for acrylamide was chosen, this being N-vinyl pyrrolidone, (NVP). The structure of N-vinyl pyrrolidone is given in Fig. 4.2. Due to the imide group

Graph.4.3: Influence of Acrylamide (and Derivatives) on Hydrogel Water Content



present in the pyrrolidone ring, a large dipole moment exists, producing a very polar, hydrophilic molecule, as indicated below in Fig. 4.5.

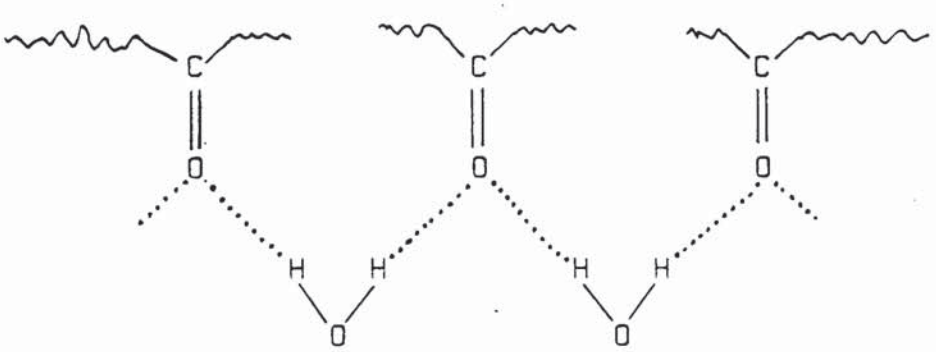
Figure 4.5



Dipole-moment on the N-VP imide group

Water uptake in cross-linked poly N-vinyl pyrrolidone has been shown,[152] to be endothermic up to a 50:50 moles % of poly (NVP):H_2O, and then subsequently becomes exothermic as a dilute solution is produced. High water solubility of this polymer is indicated by its use in a linear form as a completely soluble plasma expander.[118] Water molecules would be expected to bridge the carbonyl groups of adjacent pyrrolidone rings, (Fig. 4.6).

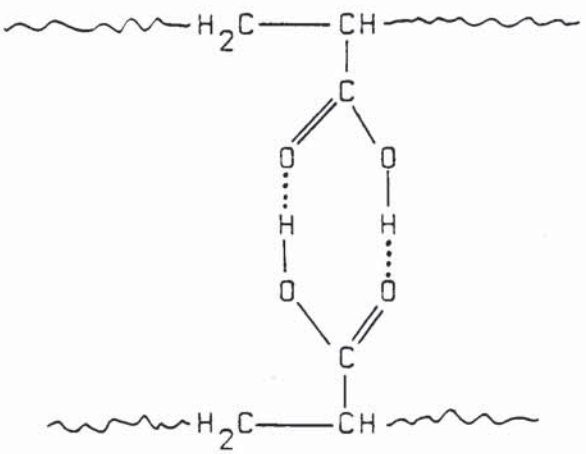
Figure 4.6 Poly (NVP)-Hydrogen-bonded water molecules



Poly N-vinyl pyrrolidone has supposed 'structure making' characteristics on water, because of the partial anionic centre (oxygen atom) and the partial cationic centre (Nitrogen atom), together with the hydrophobic alkyl groups of the pyrrolidone ring.

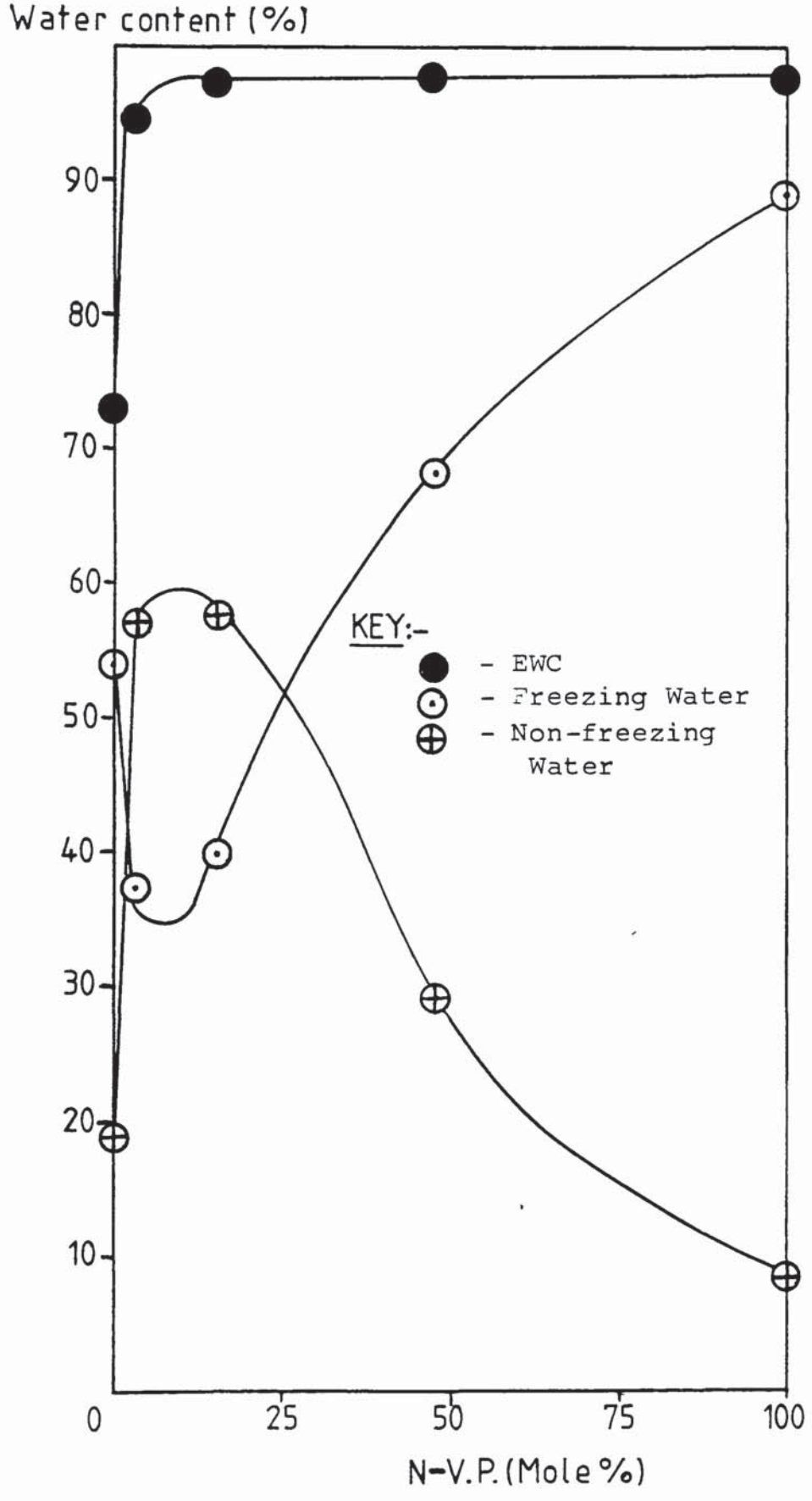
From the Graph 4.2, an initial small sharp rise in the equilibrium water content at the HEMA/NVP; 95:5(moles %) composition, can be observed. It would seem likely that a small amount of NVP molecules has a disruotive structure breaking effect on the poly (HEMA) composition, so that the stabilising hydrophobic, Van der Waals and dipole-dipole interchain interactions are disrupted, giving initially a higher water binding capacity than the NVP content would justify. Subsequent increase in the NVP content produces a near linear rise in water content as seen with the HEMA/ACM copolymers. Although no data is at present available on the bound and free water contents of these NVP/HEMA compositions, a DSC study, using NVP/acrylic acid copolymers, found similar disruption of poly acrylic acid interchain interactions by very small amounts of NVP.[156] (See Graph 4.4). Strong interchain Hydrogen-bonding in the poly acrylic acid network is known to occur between pendant carboxylic acid groups, as illustrated below in Fig. 4.7

Figure 4.7 Poly acrylic acid Interchain Hydrogen-bonding



Disruption of these bonds was seen to be accompanied by an increase in the bound water fraction with increasing

Graph.4.4: Water-binding Data for NVP/AA Copolymers



equilibrium water content, but with further increase in the NVP content, the water structuring effects decreased. Similarly for the HEMA/NVP copolymers it is expected that the initial increase in water content would be as a direct consequence of the interchain disruption giving rise to an increased water binding/structure making effect.

Based on the previous discussion concerning the influence of structural modifications of the acrylamide molecule on water content, similar predictable differences are observed for the water-binding characteristics of these copolymers, as illustrated in Table 4.4.

(See overleaf for Table 4.4)

Table 4.4

THE INFLUENCE OF ACRYLAMIDE AND ITS DERIVATIVES

ON BOUND AND FREE-WATER CONTENT

Hydrogel Copolymer	Monomer Ratio (Mole %)	EWC (%) in 'Krebs' at ~20°C	% Change *	Freezing Content (%) (Free)	% Change *	Non-Freezing Content (%) (Bound)	% Change *	Bound/ free ratio
HEMA	100:0	41.6	-	4.7	-	36.9	-	7.85
HEMA/ACM	75:25	49.7	119.5	16.1	342.6	33.6	91.1	2.09
HEMA/MACM	75:25	40.9	98.3	6.9	146.8	34.0	92.1	4.93
HEMA/DAACM	75:25	35.4	85.1	1.5	31.9	33.9	91.9	22.6
ACM/MBACM	99:1	93.3	-	71.3	-	22.0	-	0.31
ACM/MBACM	95:5	82.3	88.2	57.7	80.9	24.6	111.9	0.43
ACM/MBACM	90:10	75.7	81.1	45.0	63.1	30.7	139.6	0.68
MACM/MBACM	99:1	69.5	74.5	27.6	38.7	41.9	190.5	1.52
DAACM/MBACM	99:1	35.1	37.6	12.7	17.8	22.4	101.8	1.76

* See Footnote for Table 4.2.

addition of acrylamide to poly (HEMA) is observed to increase the equilibrium water content from 41.6% to 49.7%, a change of 119.5% but similar changes are not seen for the freezing (free) water content, which is observed to change by 342.6%. This data would seem to indicate that although the acrylamide molecule is very hydrophilic (polar), and is capable of giving much higher equilibrium water contents than HEMA, it would seem that it does not possess the necessary structure (hydrophobic groups ?), to stabilise the large hydration shells that it possesses, in order to give a higher non-freezing water content. Thus it would appear that in this example the acrylamide unit has had a net 'structure breaking' effect (i.e., decreased bound/free ratio), on water, similar to the 'soft-ice' structuring reported for the cation, potassium.[128] It should be noted that these terms are only of a relative nature however.

Addition of the α-methyl group to the acrylamide backbone to produce methacrylamide would seem to limit the above structuring effects of acrylamide. It too, however, is unable to match the water-binding properties of the HEMA-unit, as indicated by the lower bound/free ratio for approximately the same equilibrium water contents. A somewhat different trend is apparent with the HEMA/DAACM (75:25) copolymer, as a relatively small % change in EWC, is accompanied by a large reduction in the freezing water content, together with a very small decrease in bound content. This is quite obviously attributable to the substitution of the 1,1 dimethyl 3-oxy butyl group, which has the ability to exclude the freezing water by steric hindrance and due to its hydrophobic interactions, in a similar

manner to the hydrophobic monomer ethyl methacrylate. Similar trends are observed for the solution polymerised poly acrylamide, poly methacrylamide and poly diacetone acrylamide, where increasing bound/free ratio is observed, respectively.

4.4.3 Effects of Ionic Groups

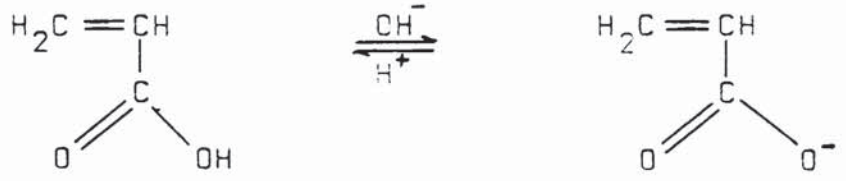
Amionic and cationic monomers are generally present as impurities in other neutral monomer preparations, however they may be used in low concentrations to alter the charge characteristics of a material. One very commonly used anionic monomer is methacrylic acid (MAA) previously cited in this chapter as a major contaminant of 2-hydroxy ethyl methacrylate and other methacrylates. Similarly the parent acid of acrylamide, acrylic acid (AA), also presents contamination problems, and its use is generally avoided, due to its tendency to form copolymers which behave as polyelectrolytes, giving water content instability with variation in pH.[157,158] Whilst acrylic acid is therefore unsatisfactory for use in potential biomedical applications, its use in research to give structure/property comparisons with methacrylic acid, is necessary, and accordingly some reference will be made to it. The structures of these acids together with the basic, 2-amino ethyl methacrylate is given in Fig. 4.8

The trends of equilibrium water content with structure for copolymers of HEMA/MAA, HEMA/AA, ACM/MAA, and ACM/AA have been previously reported,[154] and these results are given in Graph 4.5.

Figure 4.8

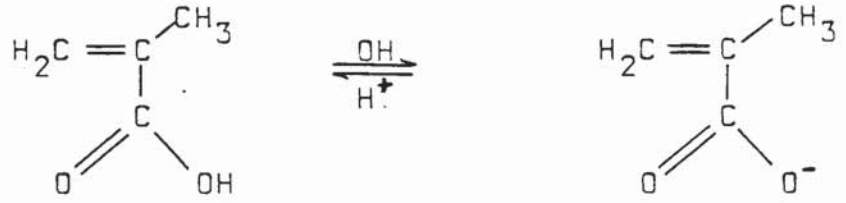
STRUCTURES OF THE ACIDIC AND BASIC MONOMERS

Acid Monomers



neutral ionized

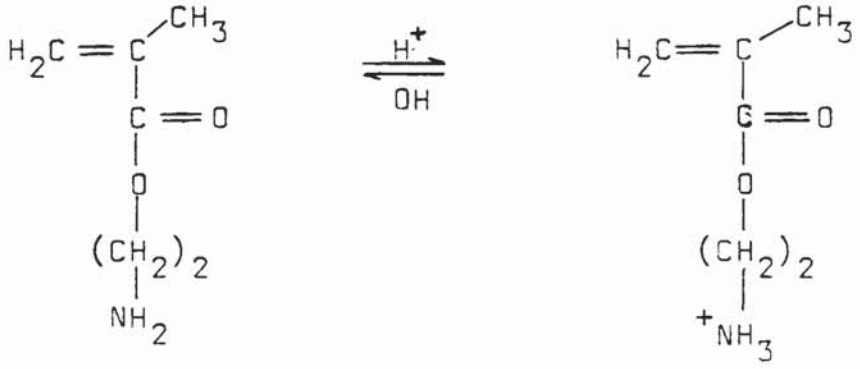
Acrylic Acid (AA)



neutral ionized

Methacrylic Acid (MAA)

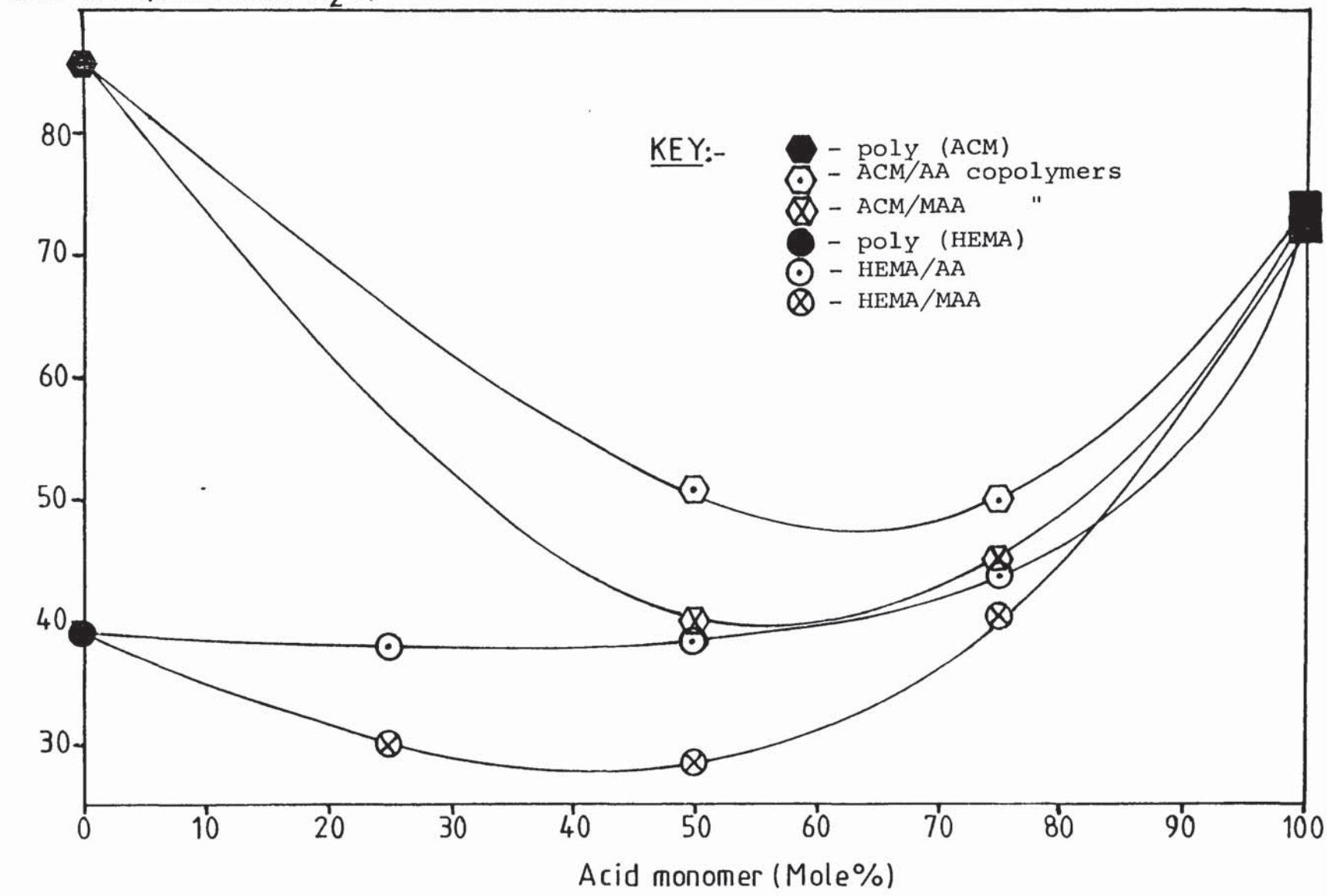
Basic Monomer



neutral ionized

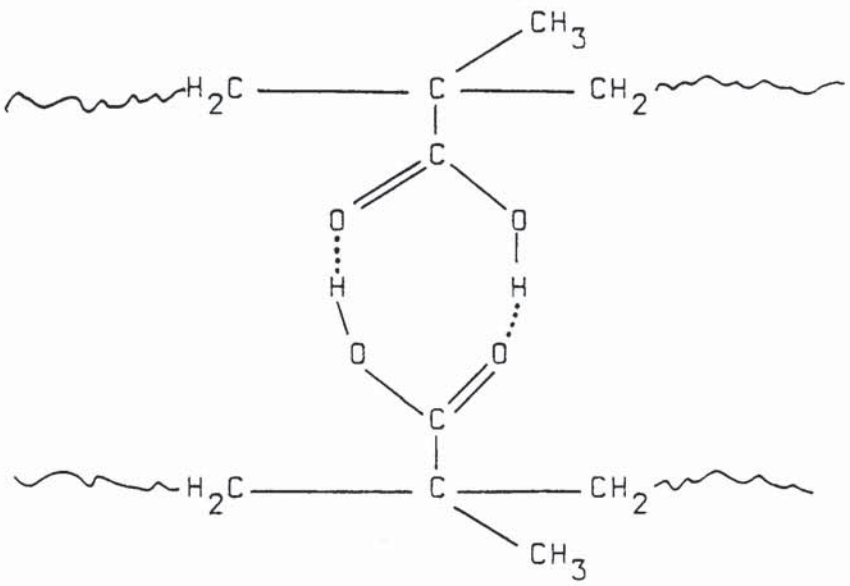
2-Amino ethyl Methacrylate (AEMA)

Graph.4.5: Effect of Hydrogen-bonding Interactions on Water Content



One of the main uses which has previously distinguished methacrylic acid for use in contact lens applications, is its ability to undergo strong hydrogen bonding, as seen in poly methacrylic acid, and which will invariable increase the strength of the hydrogel. See Fig. 4.9.

Figure 4.9 Interchain Hydrogen-bonding Interactions Exhibited by Poly methacrylic acid

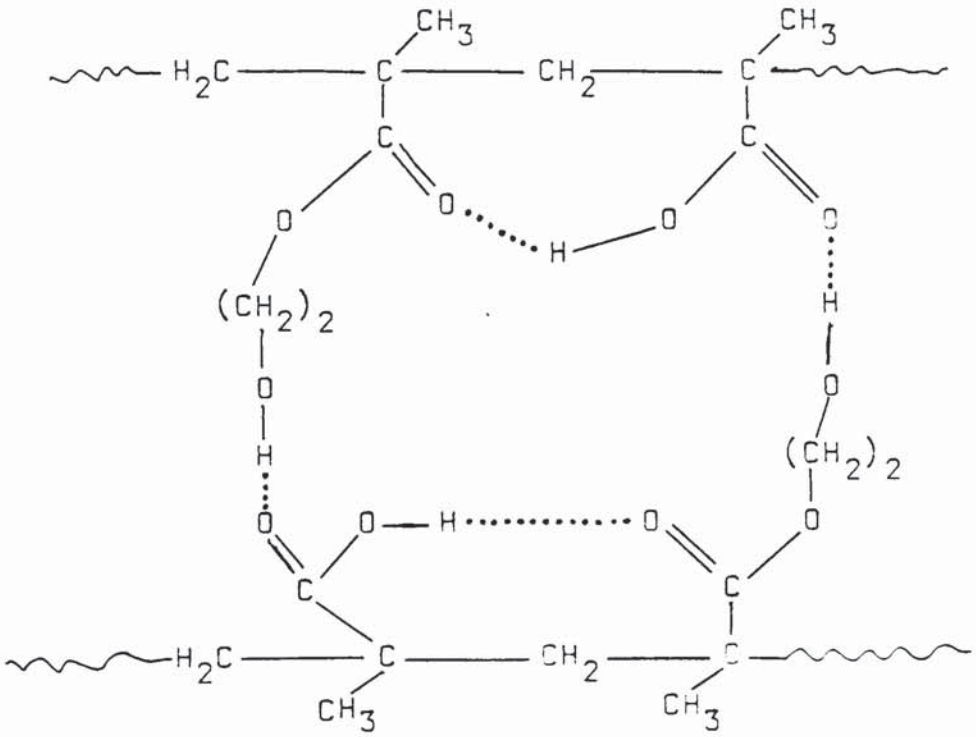


Both the HEMA and the ACM copolymer results given in Graph 4.5, can be explained by an increase in inter-chain polymer hydrogen bonding with increasing acid monomer content. The observable differences between the mathacrylic acid and acrylic acid copolymers can be attributed to the α-methyl group in the methacrylic acid molecule. This group would appear to be able to induce a greater depression of equilibrium water content, due to its hydrophobic Van der Waals interactions, together with its ability to sterically hinder chain rotation about the polymer backbone, which would allow

preferential arrangement for water absorption, as seen with acrylic acid. The results for the HEMA/MAA copolymers in this project, have been found to substantiate the previous workers findings, with a minimum equilibrium water content in distilled-water at approximately HEMA/MAA, 60:40 (Moles %), respectively. This is illustrated in Graph 4.6.

The depression in water content can, as previously stated, be attributed to the hydrogen-bonding between the pendant side chain hydroxyl and carbonyl groups of HEMA and MAA, as illustrated below in Fig. 4.10.

Figure 4.10 Idealised Structure in HEMA/MAA (60:40) for Preferential (Max) Hydrogen-bonding

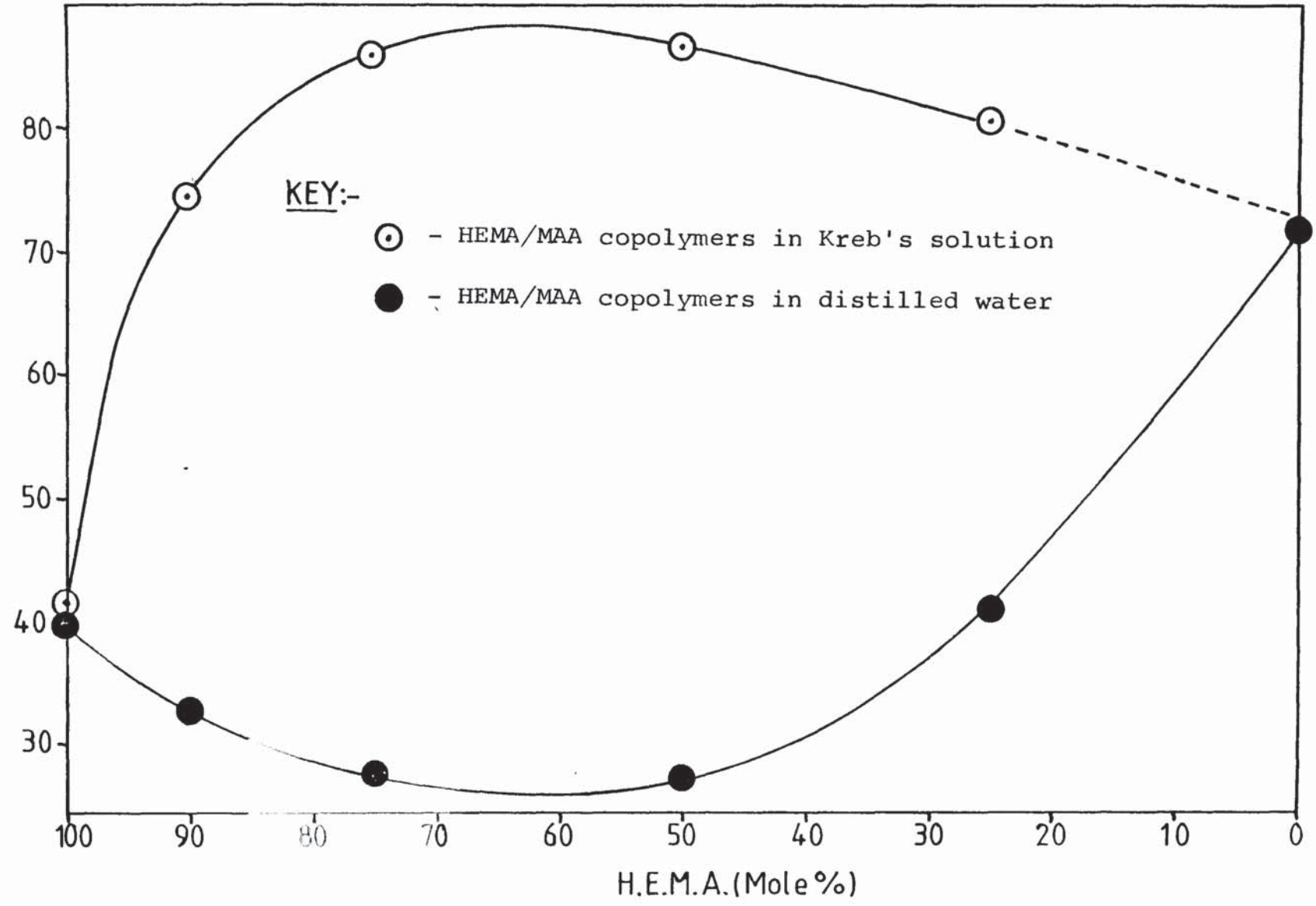


With increasing methacrylic acid the above structure is disrupted allowing greater water interactions with the increasing carboxylic units present.

An alternative theory to account for the depression in water content, that should also be considered, is that as the MAA units are increased, so a preferential spatial arrangement is attained at the HEMA/MAA 60:40 (Moles %), where one molecule of water is competitively shared between two or three, possible polymer hydrogen-bonding sites, thus reducing the water binding capacity of the system. This type of water-binding has been reported for the molecule of papain,[150] and may also apply to the results given earlier for acrylamide and its derivatives.

When the same HEMA/MAA copolymer series was hydrated in physiological Kreb's solution, very significant changes in equilibrium water content took place, as illustrated in Graph 4.6. A reversal of the above equilibrium water content depression phenomena is seen, with a massive rise in water content, reaching a peak of 87.5% at the HEMA/MAA 60:40 (Moles %) composition. Due to the high buffering capacity of the double-buffered Krebs solution, a pH7.4 is maintained and produces ionisation of the methacrylic acid to form the carboxylate anion. The double-buffer system is composed of the conjugate acid-base pair $H_2PO_4^-$ - HPO_4^{2-} ($pk' = 7.2$) and the bicarbonate buffer system H_2CO_3 - HCO_3^- ($pk' = 3.8$), and it is by this double-buffer system that normal blood maintains a pH 7.4. The ionisation effect will by itself produce an increase in water content, due to disruption of the polymer-polymer hydrogen-bonding and the hydrophilic nature of the carboxylate anion. However the major

Graph. 4.6: Influence of Hydration Medium on the Water content of HEMA/MAA Copolymers



rise in water content is mainly attributed to the interaction between the carboxylate anion and the predominate cationic specie present in solution, the sodium cation, which because of its high charge density, is very hydrophilic.

Water structuring by the solvated sodium-carboxylate groups will tend to limit the number of Na^+ ions that can enter into the gel matrix.[128] This would appear to be occurring at the peak water content HEMA/MAA 60:40 (Moles %) composition, where maximum hydrogen-bond disruption, by the above sodium-carboxylate system, would be indicated. With further increase in the methacrylic acid content, so the carboxylate groups would seem to be undergoing reformation of their interchain hydrogen-bonds as seen for poly methacrylic acid in Fig. 4.9, and accordingly a drop in water content is observed. The increased hydrogen-bonding capacity of the system may be thought of as being due to the electrochemical constraints imposed on ionisation within the gel-matrix by the nature of the water structuring and ionic species already present.

Divalent cations Mg^{2+} and Ca^{2+} can cause bridging between adjacent carboxylate anions, leading to a decrease in water content, however studies by a number of research groups, has indicated that divalent ions of Mg^{2+} and Ca^{2+} are unlikely to displace the monovalent sodium ions which are associated with the carboxylate groups,[154,155] and therefore this problem should not be of significance.

From the water binding data in Table 4.5 and illustrated in Graph 4.7, it is of interest to note

that where the maximum equilibrium water peak occurs, so the non-freezing (bound) water also attains its peak. The reduction in water content following this peak equilibrium water content to 80.6%, can be seen to coincide with a fairly substantial decrease in non-freezing (bound) water, together with a rise in freezing (free) water. Therefore it would seem that as the equilibrium water content decreases, interchain hydrogen-bonding is re-established and accordingly the potential water binding sites are diminished and non-freezing water content is correspondingly diminished. This being so, the interchain hydrogen-bonded carbonyl groups do still represent very weak binding or attractive sites, which will tend to polarise the water molecules around them, without binding, and so they give rise to the increased freezing (free) content with increasing methacrylic acid content.

The above trends, observed with increasing methacrylic acid content, are not so clearly defined in the solution polymerised acrylamide (ACM) : methacrylic acid (MAA) copolymers (as illustrated in Graph 4.8). Before discussing these results, it should be perhaps pointed out that small % change in equilibrium water content, at very high water content is a more significant indicator of structure/property effects than similar % change at low equilibrium water content, due simply to the method of derivation of equilibrium water content. Inherent experimental variations may however offset the significance of such fine variations in equilibrium water content, and therefore these results

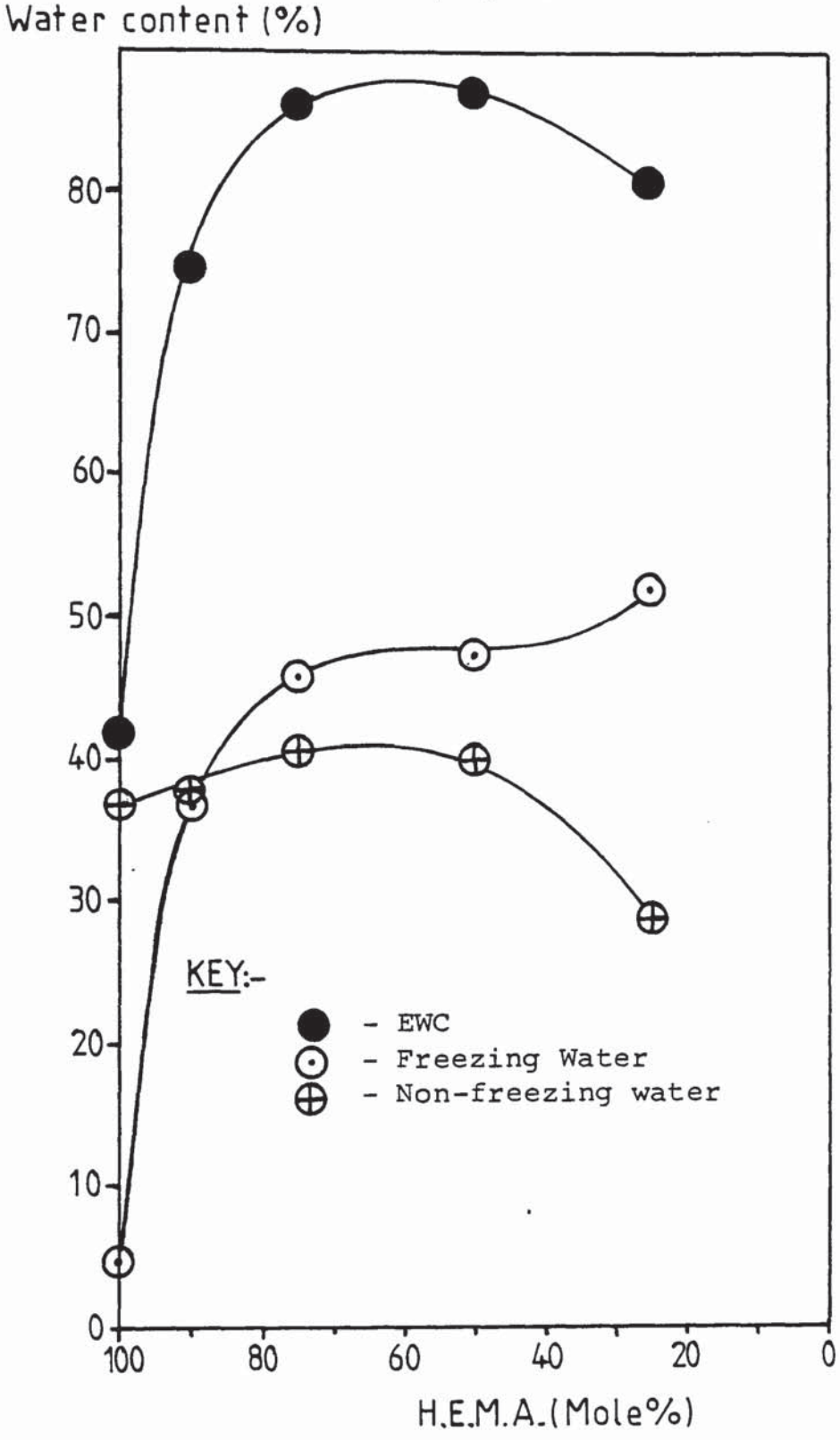
Table 4.5

THE INFLUENCE OF METHACRYLIC ACID ON BOUND AND FREE-WATER CONTENT

Hydrogel Composition	Monomer Ratio (Mole %)	EWC (%) in 'Krebs' at ~ 20°C	% Change *	Freezing water (%) (Free)	% Change *	Non-Freezing water (%) (Bound)	% Change *	Bound/ Free Ratio
HEMA	100:0	41.6	-	4.7	-	36.9	-	7.85
HEMA/MAA	90:10	74.5	179.1	36.9	785.1	37.6	101.9	1.02
HEMA/MAA	75:25	86.3	207.5	45.8	974.5	40.5	109.8	0.88
HEMA/MAA	50:50	86.9	208.9	47.2	1004.3	39.7	107.6	0.84
HEMA/MAA	25:75	80.6	193.8	51.9	1104.3	28.7	77.8	0.55

* See Footnote for Table 4.2

Graph. 4.7: Water-binding Data for HEMA/MAA Copolymers



can really only give a somewhat tentative prediction of structure/property effects.

With the initial introduction of Methacrylic acid (one mole %), the depression of EUC (distilled -H_2O), may be attributed to an increased polymer-polymer hydrogen-bonding, as previously cited for bulk polymerised HEMA/MAA snd ACM/MAA, however, as this trend does not continue, another possible alternative is that the methacrylic acid in low concentration, produces steric-disruption of the acrylamide water binding capacity. Subsequent increases in methacrylic acid content would be expected to ellicit the hydrogen-bonding-depressed equilibrium water content phenomena, as previously seen for the bulk polymerised ACM/MAA 40:60 (Moles %) copolymer (Graph 4.5). Due to the inherent very high hydrophilicity of the neutral poly acrylamide, further hydration by the sodium carboxylate group in physiological Krebs solution, will be limited by the hydro-dynamic, constraints imposed by the covalent, cross-link density, and therefore no great rise in physiological equilibrium water content would be expected. Physiologically hydrated ACM/MAA copolymers of the above type, do however seem to exhibit reduced mechanical strength, compared to the neutral poly acrylamid gels, and this can perhaps be attributed to the disruption of amine-carbonyl hydrogen-bonds, (as illustrated in Fig. 4.11), a number of these can normally be expected to exist in the poly acrylamide gel.

Graph. 4.8: Influence of Hydration Medium on the Water Content of Acidic and Basic Acrylamide Copolymers

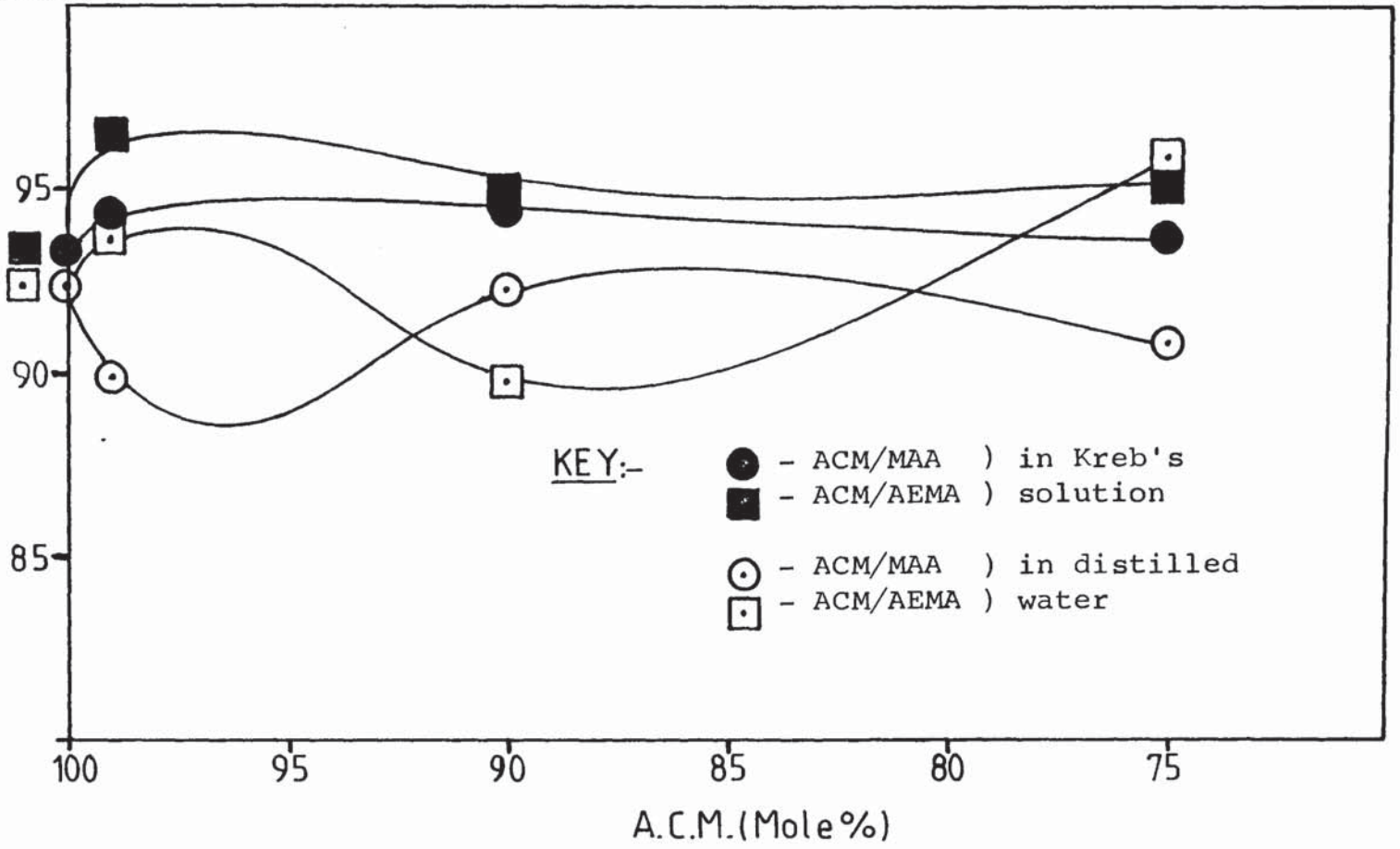
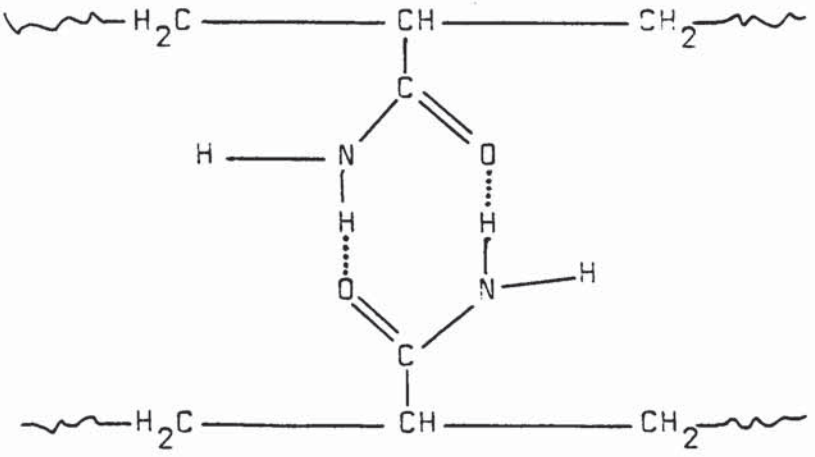


Figure 4.11 Poly-acrylamide amide-carbonyl hydrogen-Bonding



Similar studies on the behaviour of the basic monomer 2-amino ethyl methacrylate (AEMA), have been carried out. The structure of this monomer is given in Fig. 4.8, and it perhaps should be noted that this molecule will be ionized both in distilled-water (pH 6.5), and at physiological pH 7.4, (due to its high pK' value), and therefore it will be positively charged (i.e., cationic). A very small initial rise in water content both in distilled water and physiological solution may be seen as disruption of the hydrogen-bonding of poly acrylamide, following this at the ACM/ AEMA (90:10 Moles %) composition a fall in the water content may be possibly due to an ionic-dipole interaction between the carbonyl group of acrylamide and the quaternary ammonium ion of 2-amino-ethyl methacrylate. Possible dipole interactions with the carbonyl group of 2-amino ethyl methacrylate will tend to be limited due to the shielding effect of the ethyl group. This data analysis however is somewhat of a speculative

nature, there being no previous comparable work known, to date.

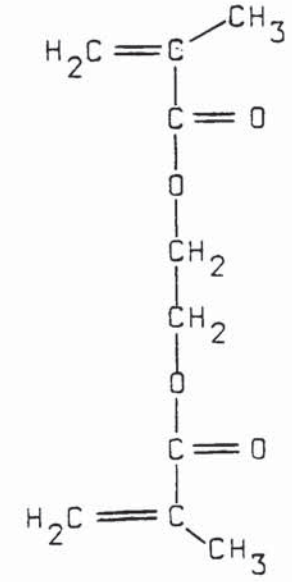
It is perhaps of interest to note a generally disregarded fact that methacrylic acid will be significantly ionized even in distilled water (pH 6.5), due to its low pK' value, and water behaving as conjugate acid-base-pair.

The obvious complexity of ionic monomers structure/property relationship require much greater study in order to elucidate some of the above reported findings.

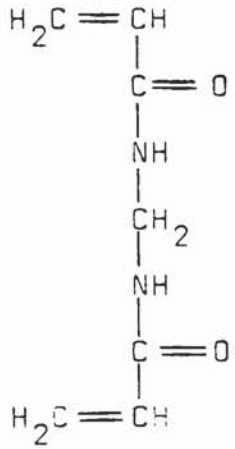
4.4.4 Extent of Cross-linking on Water Content and Structuring

From a consideration of the structural formulae of the cross-linking agents ethylene glycol dimethacrylate and N,N' methylene-bis-acrylamide, it should be possible to predict that the ethylene glycol dimethacrylate would decrease equilibrium water content more efficiently, due to its lower hydrophilicity (polarity), See Fig. 4.12.

Figure 4.12 Structures of the Cross-linking Agents



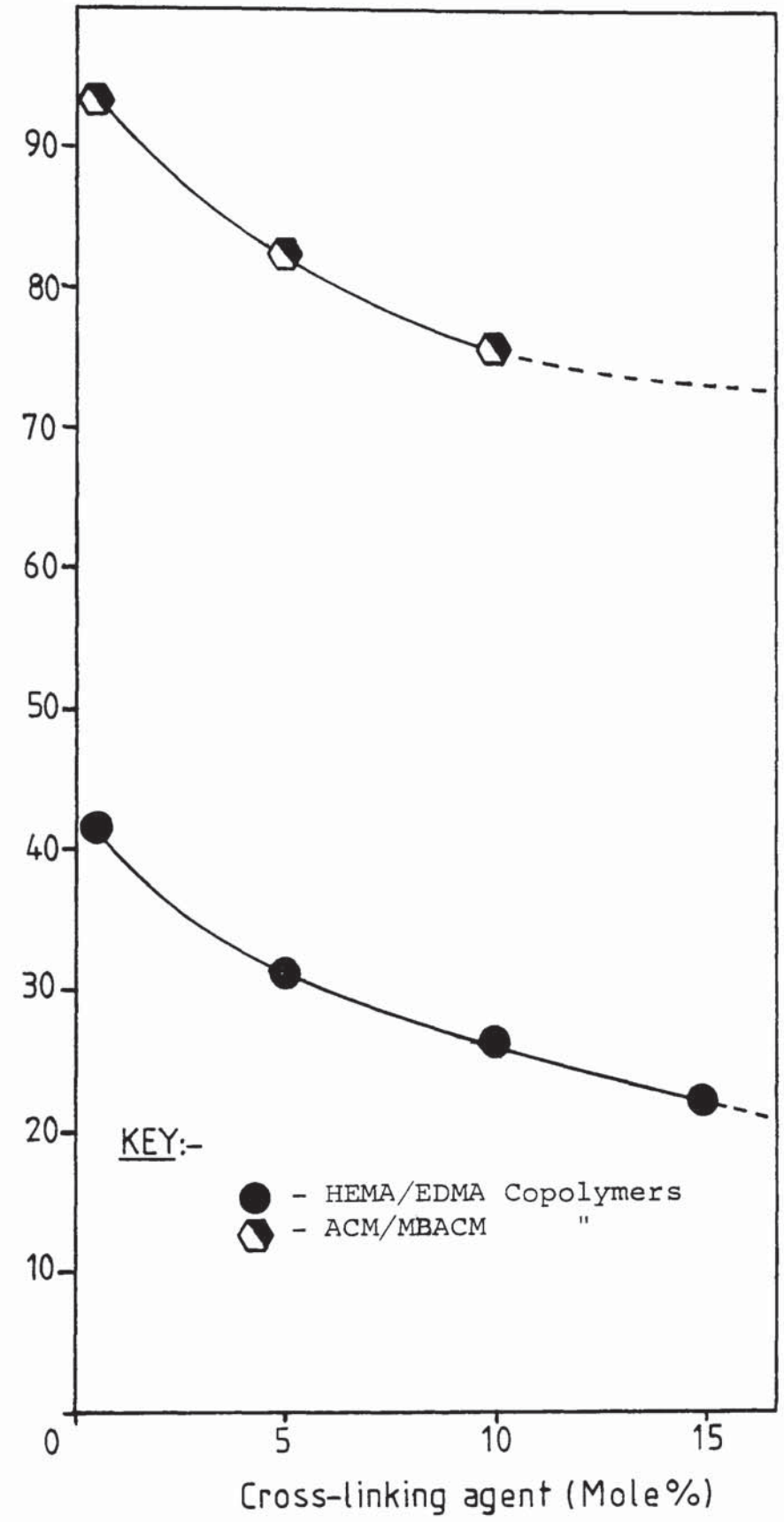
Ethylene glycol dimethacrylate (EDMA)



N'N-Methylene-bis-acrylamide (MBACM)

This however is not observed for the copolymers under consideration in this study, (See Graph 4.9). Initially an increase from 1 to 5 moles % of the cross-linking agents concentration, produces approximately the same rate of change of water content with respect to the concentration of both cross-linking agents. Subsequent to the initial sharp decrease in equilibrium water contents; both cross-linking agents are seen to be less efficient in further reducing the equilibrium water contents over the concentration range of 5 - 10 Moles % of cross-linking agent. This decrease in the efficiency of formation of cross-links with high concentrations of the cross-linking agents, would seem to confirm the idea that, the internal structure of hydrogels does not necessarily conform to the simple concept that each cross-link molecule incorporated into the network structure, produces one independent cross-link. Many such cross-links are wasted simply producing larger chains. Others occur too close to each other, and may only serve to extend the region which will mechanically act as a single cross-link, all covalent cross-links in that region acting as one. Hence many hydrogels react as though they had far fewer effective cross-links than apparently incorporated and as though they had much wider interchain openings ('pores') in them, than would be suspected from the amount of hydrogel substance present. Such effects would also be expected to modify the surface characteristics of a gel accordingly. Some confirmation of these ideas has come from lasar scattering results and some recent permeability studies from gels.[159]

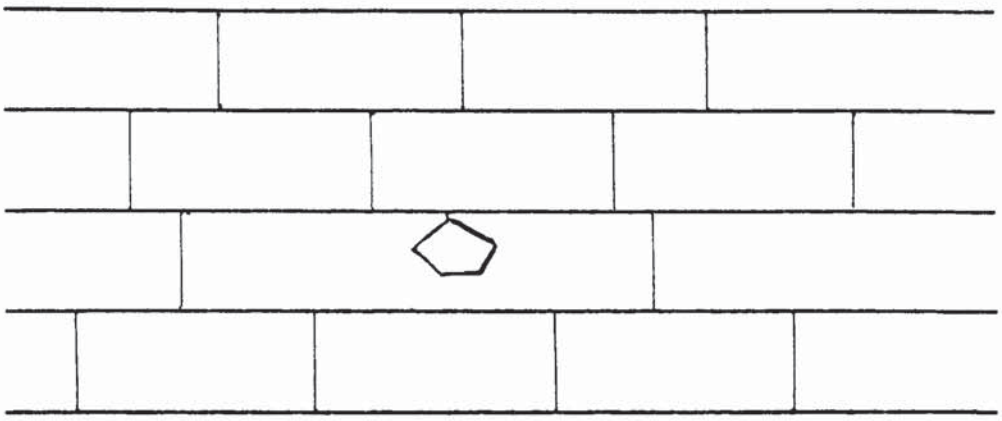
Graph. 4.9: Influence of Cross-linking Agents on Hydrogel Water Content



In the concentration range of 5 - 10 moles % of cross-linking agents, in fact N,N'-methylene bis-acrylamide shows a marginally greater efficiency in depressing the equilibrium water content of the gels. This may well be attributed to the high free/low bound water present in the high water content N,N' methylene-bis-acrylamide gels and the low free/high bound water present in the low water content ethylene glycol dimethacrylate gels. Therefore increasing the cross-link density would be expected to sterically hinder water uptake by the N,N' methylene-bis-acrylamide gels, more so than the ethylene glycol dimethacrylate gels. However an alternative reason in line with the earlier ideas in cross-link efficiency, concerns the possibility that ethylene glycol dimethacrylate in high concentrations, will have a greater tendency, than the other cross-linking agent, to undergo intra-molecular cross-link formation (cyclisation). A schematic representation of this is illustrated in Fig. 4.13. This will inevitably lead to imperfections in the gel-network and the apparent homogeneous gel, will contain large micropores, which do not give rise to phase separation, possibly because of the low equilibrium water content and the highly bound nature of the water.[155,160] This has to some extent been confirmed by the reported increased 'pore' size of poly (2-hydroxy ethyl methacrylate), when cross-linked by high concentrations of ethylene glycol dimethacrylate (EDMA).[161] These polymers were ascertained to have average 'pore' radii of up to 18A^{o} for gels of less than 30% water content, whereas normal poly (2-hydroxy ethyl methacylate) gels with 1 mole % of

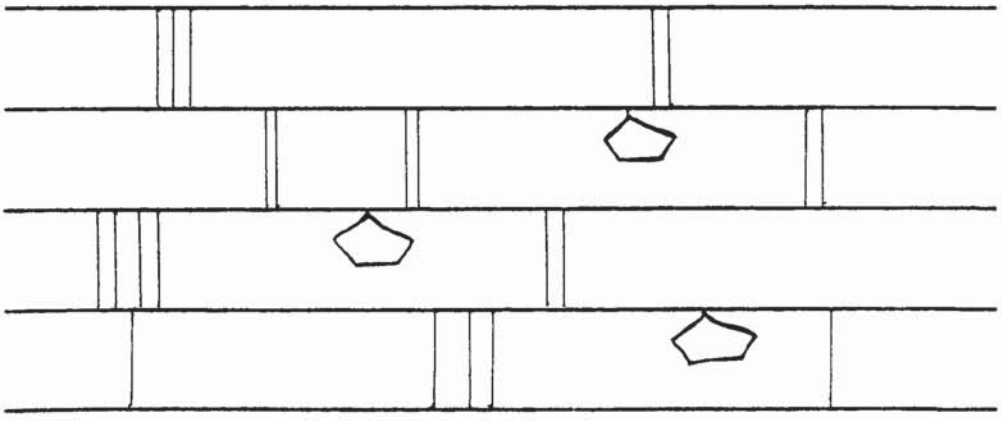
Figure 4.13

SCHEMATIC REPRESENTATION OF CROSS-LINK DENSITY ANOMALIES ON HYDROGEL 'POROSITY' AS A CONSEQUENCE OF CROSS-LINKING AGENT CONCENTRATION



HEMA:EDMA 99:1 (Moles %) (poly (HEMA)).

Isotropic distribution of cross-links, limits average pore size distribution to ~ 8Å.[162] Intra-molecular cross-links produce insignificant effects on net pore-size distribution.



HEMA:EDMA 85:15 (Moles %)

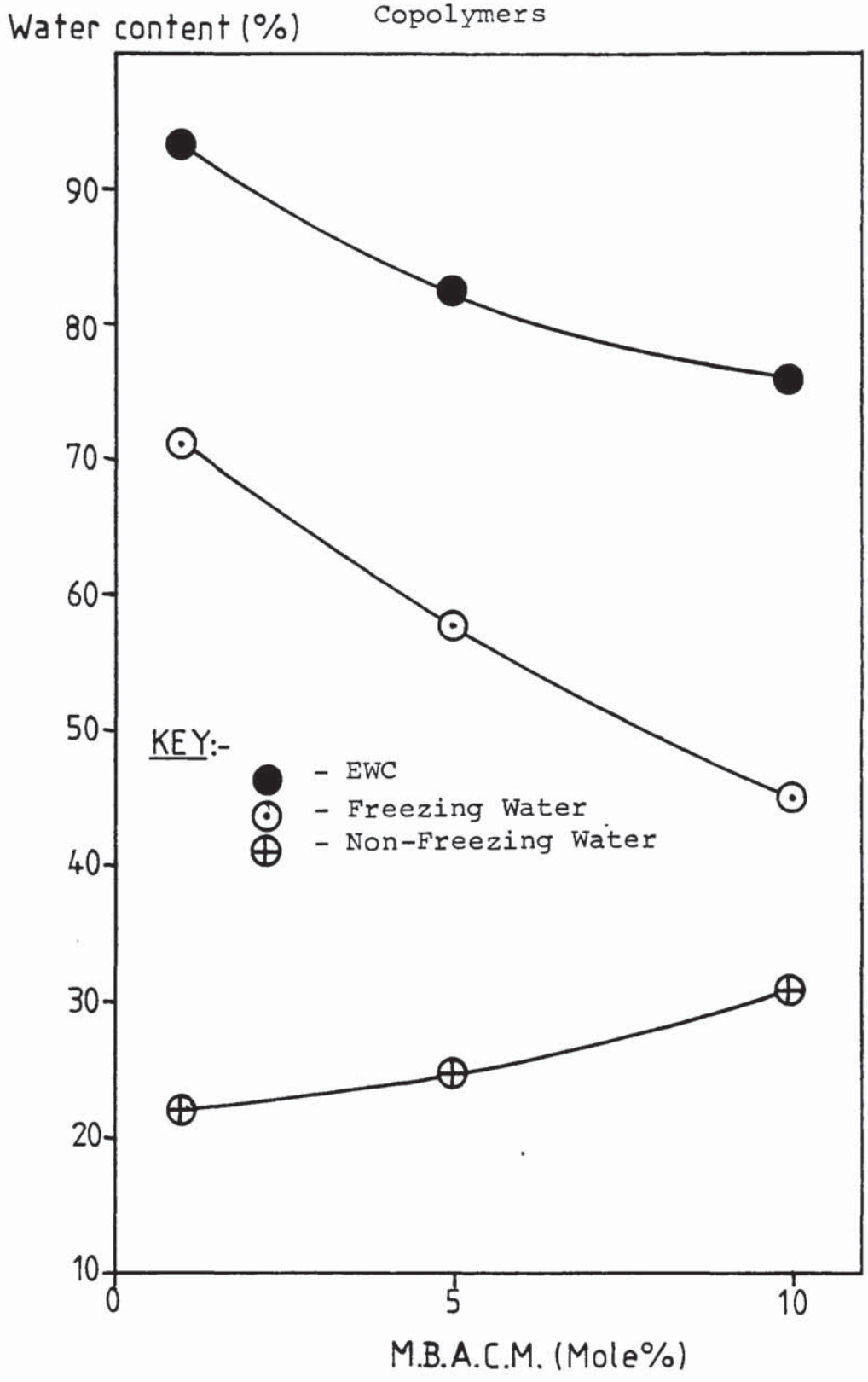
Non-isotropic cross-link distribution creates average pore-size distributions of ~ 18Å.[161] Intra-molecular cross-links (cyclisation) produce significant effects, due to increased numbers per unit volume and uneven cross-link distribution.

cross-linking agent (EWC ~ 40%) have average 'pore' radii of approximately 40^{o}.

From the water-binding data available on the acrylamide: N,N' methylene-bis-acrylamide copolymers (Graph 4.10 & Table 4.4), increasing the cross-linking agent is seen to not only effectively decrease the water content, but to also decrease the freezing water content whilst at the same time increase the non-freezing (bound) content. This may simply be attributed to the steric exclusion of free water by the increasing cross-link density however, as the efficiency of the cross-linking agent decreases with increasing concentration, so a more marked effect on the bound water content should be apparent. This does appear to be occurring, since the equilibrium water content for the 90:10 (moles %) ACM/MBACM copolymer has only decreased by 81.1%, however the free water has decreased by a much larger degree to 63.1%, and the bound (non-freezing) content has risen to 139.6% from the original ACM/MBACM 99:1 (Moles %) copolymer. This is quite obviously indicative of an increased structuring of the water into the non-freezing type by the N,N' methylene-bis-acrylamide molecule, and not simply steric hindrance to the free water uptake. These studies were carried out on physiologically hydrated hydrogels, corrected for the salt content.

Water-binding studies have been carried out on a series of 2-hydroxy ethyl methacrylate: ethylene glycol dimethacrylate copolymers, hydrated in distilled water in our laboratories,[156] and these results are

Graph. 4.10: Water-binding Data for ACM/MBACM Copolymers

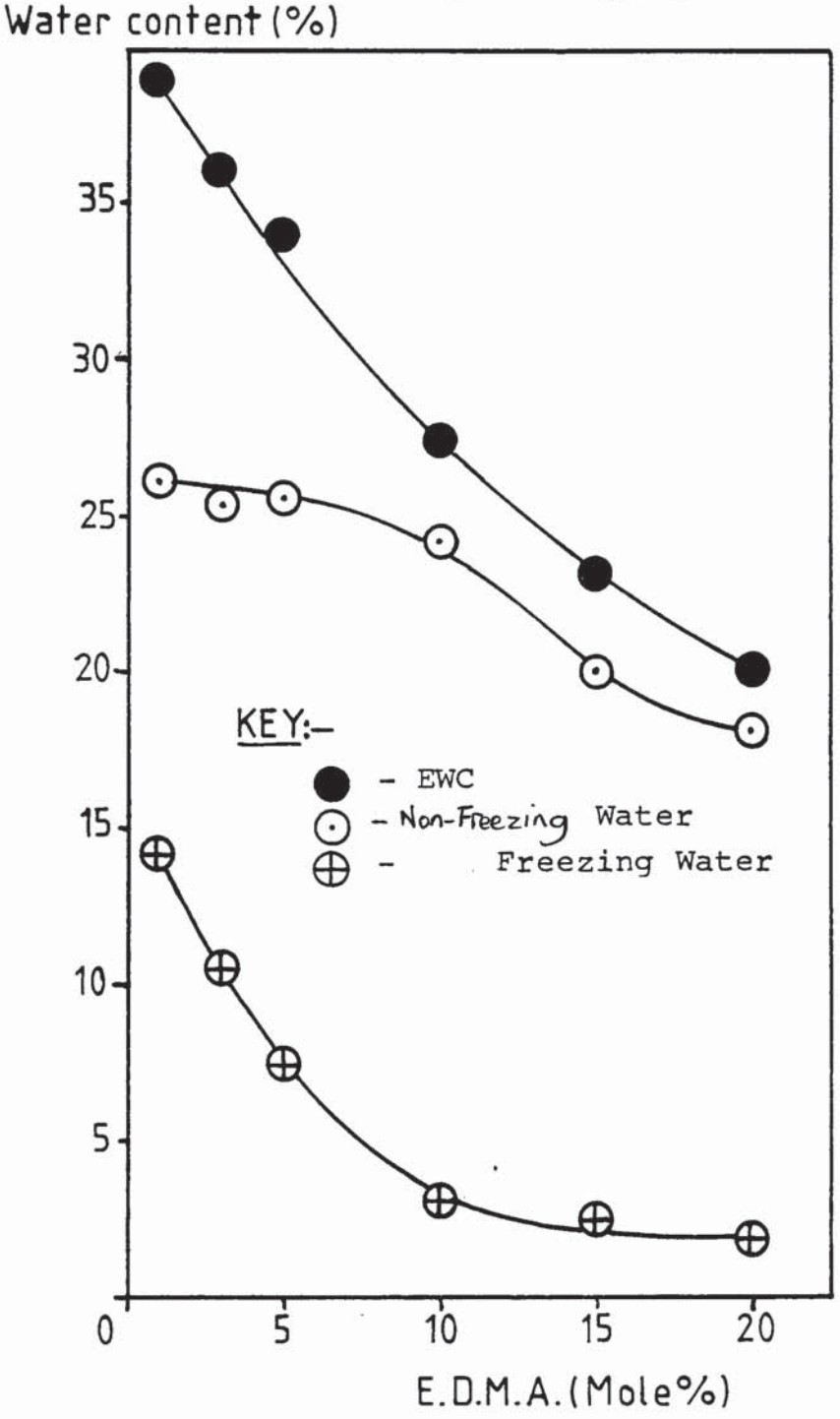


given in Graph 4.11. In these studies attention was drawn to the fact that the presence of the impurities methacrylic acid and ethylene glycol dimethacrylate in 2-hydroxy ethyl methacrylate, may effect the bound/ free water content measurements that were obtained. This problem has previously been elucidated in this chapter, and to some extent would bear out these convictions.

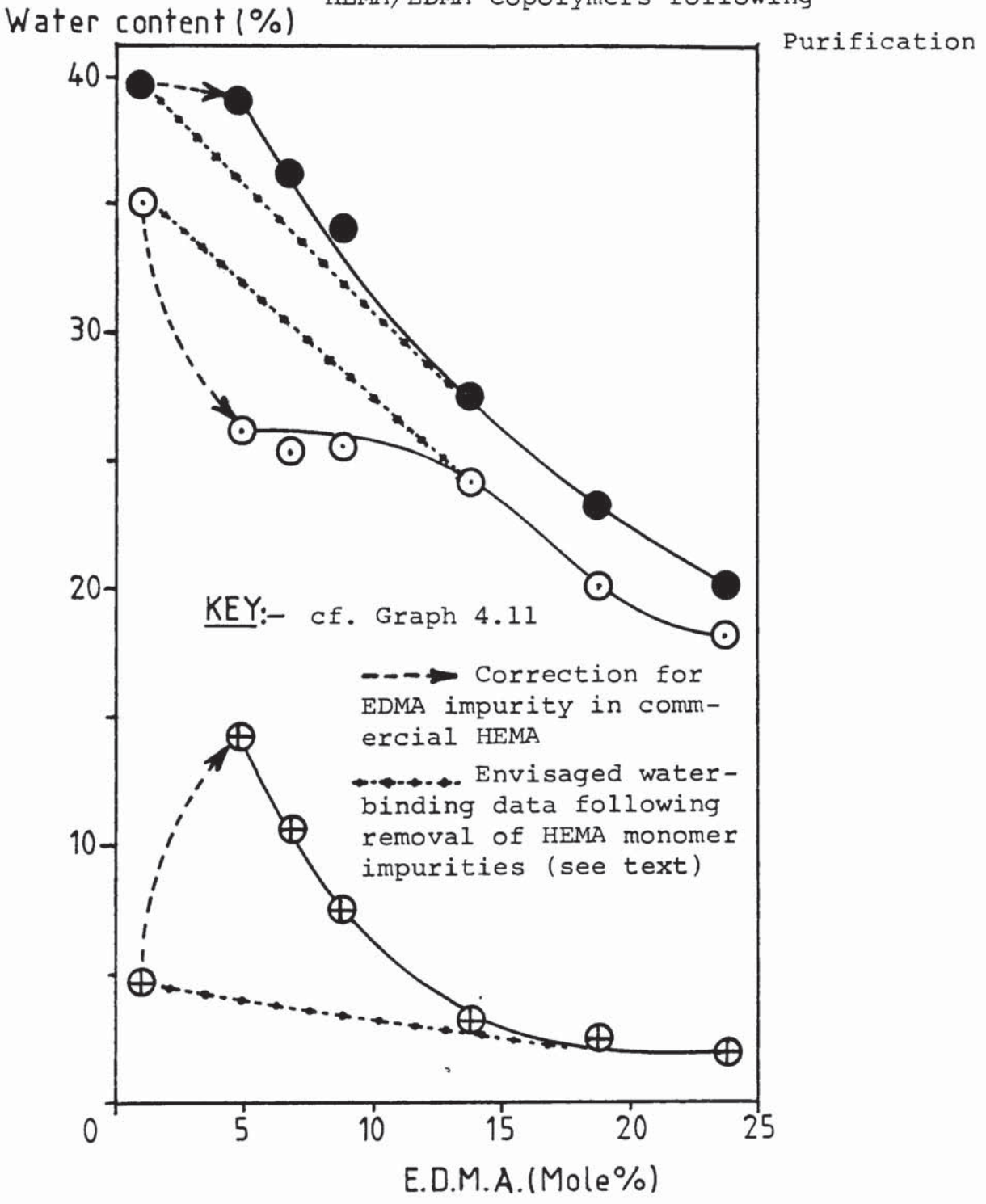
From these studies, it was deduced that over the range between 1 - 10 moles % of the cross-linking agent, ethylene glycol dimethacrylate, the amount of freezing water present, is markedly affected. This was attributed to the increasing cross-link density, sterically excluding the free water, conversely it was shown that for the same range no real change in non-freezing (bound) water was occurring, but that at higher concentrations of ethylene glycol dimethacrylate, reduction in non-freezing water did occur.

These observations do show a marked variation from the bound/free water contents, as determined for HEMA/EDMA, 99:1 (Mole %) copolymer in this project. The raised freezing content is presumably a direct consequence of the hydrogen-bonding interactions, due to the methacrylic acid impurities, as previously observed for the HEMA/MAA copolymers in distilled water. It should not be forgotten however that ethylene glycol dimethacrylate is also an impurity ($\leqslant$ 5.3 Mole %) and accordingly an envisaged 'true' or corrected, bound and free water-binding data is given in Graph 4.12, where Graph 4.11 is transposed to the right by the ethylene glycol dimethacrylate impurity level (taken as $\sim$ 4 Moles %), and corrected

Graph. 4.11: Water-binding Data for HEMA/EDMA Copolymers



Graph. 4.12: 'Envisaged' Water-binding Data for HEMA/EDMA Copolymers following Purification



for the envisaged modifying influence of the methacrylic acid content. Differential scanning calorimetry measurements on the purified HEMA/EDMA copolymers has yet to be undertaken, and until then the above would seem a reasonable working-hypothesis. From this envisaged data, the rate of decrease of the bound (non-freezing) water is directly parallel with the rate of decrease of equilibrium water content. This effect occurs for two reasons, one is the increase in cross-link density and the other is due to the low hydrophilic nature of the ethylene glycol dimethacrylate molecule. Eventually this trend disappears at 15 - 20 Moles %, to give rise to structuring effects due to imperfections in cross-links, as observed with high cross-linking agent concentration.[155,160,161]

In concluding this section it should be stressed that, covalent cross-linking effects are not the only type of cross-linking, previously mentioned interchain hydrogen-bonding, ionic, dipole-dipole and hydrophobic Van der Waals interactions are all valid cross-links and in as much, they will influence the hydrogel properties in a similar, but more transient manner to their covalent counterparts.

4.5 Permeability

Due to uniqueness of their structure, hydrogels have a real advantage over other polymeric materials, in that, because of their expanded gel network, they have an inherent permeability to small molecules. From the point of view of their biomedical applications, such as dialysis, ultrafiltration, haemoperfusion absorbent

for toxins, drug delivery systems, and low tissue toxicity (due to prior elimination of artefacts of polymerisation), their permeability characteristics are invaluable.

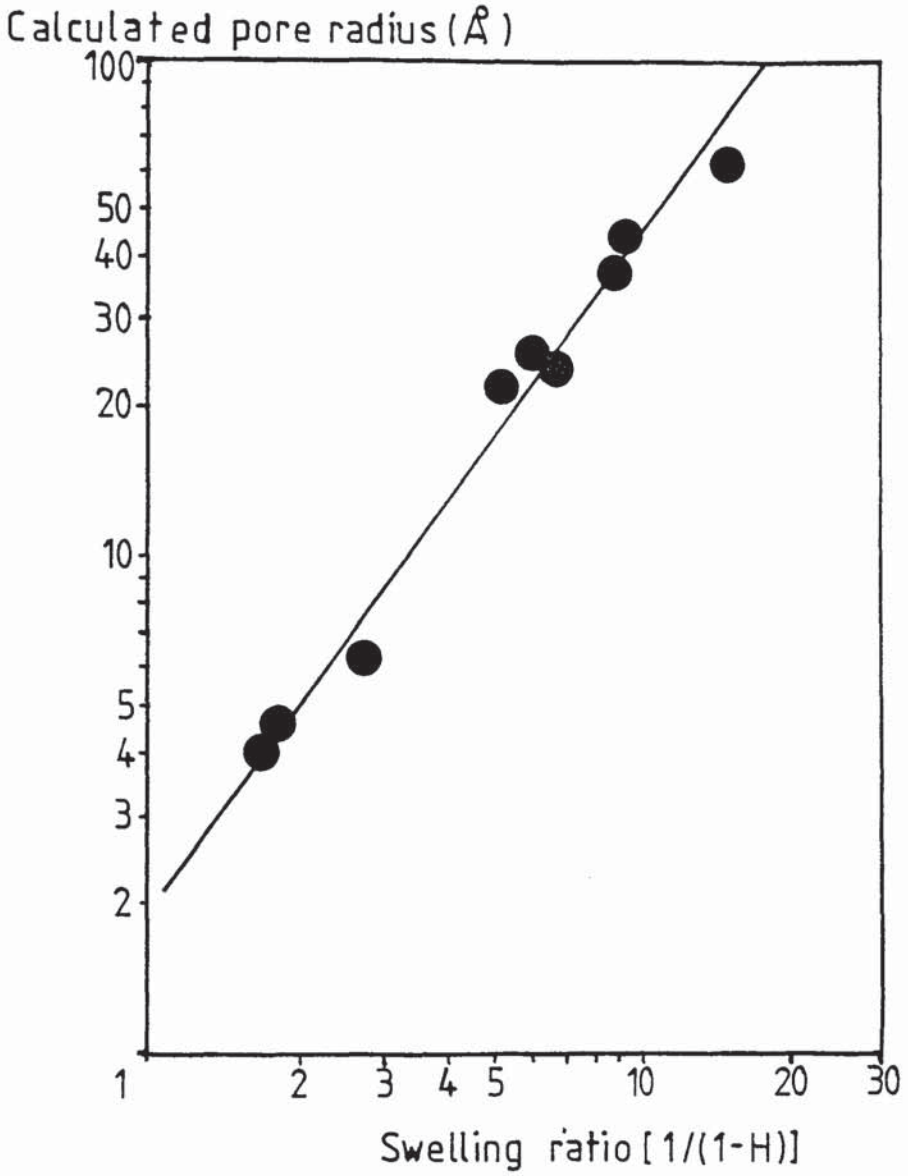
A theory has been proposed, for the permeation of solutes through a water-swollen membrane.[163] It was suggested that the diffusion of solutes through a water-swollen membrane can be related to the product of a pore size distribution factor, the free volume fraction of water in the polymer and the diffusion coefficient for the solute in pure water. Thus the diffusion coefficients in the hydrogel membrane (Dh), and in pure water (Dw), would be related in the following manner:

$$\frac{Dh}{Dw} = \emptyset\, q_2 \exp. \left[-B \left(q_2/V_{f,1}\right) \left(\frac{1}{H} - 1\right) \right]$$

where the term $\emptyset\, q_2$ describes the sieve mechanism by which molecules of greater cross-section than that of available pores (q_2) are rejected. $V_{f,1}$ is the free volume of pure water, B, a proportionality factor and H is the equilibrium water content fraction.

Homogeneous hydrogels in which the water is dispersed isotropically throughout the gel-network do not therefore behave as porous membranes, but as a randomly fluctuating system of hydrated polymer chains. The flow behaviour of methacrylate hydrogels has been shown to justify a linear correlation between swelling ratio (1/1-H) and the average pore radius,[162] and this relationship is illustrated in Graph 4.13. Pore size in hydrogels is a theoretical concept, never the less it does enable the prediction of permeability of a solute molecule, based

Graph. 4.13: Dependence of Calculated Radius on Swelling Ratio of Methacrylate Hydrogels



* N.B. H = EWC Fraction at~20°C.

on its size. Towards this end the copolymer series produced in this project have been characterised, from their equilibrium water contents (physiological) for their average pore radius by the above graphical relationship. (See Appendix 3).

It should be pointed out that the above derivation of average pore radius is based on the relationship by Ferry[164]

$r = \sqrt{8K/S}$, (where the term r, defines the average pore radius, K is the permeability coefficient and S is the specific water content),

and that the pore size may be too small, due to the following assumptions:

1. The water flow is determined by Poiseuille's law.
2. All pores are open to the surface.
3. There is no significant amounts of immobilised or bound water lining the walls of the pores.

Obviously not all these assumptions are valid for hydrogels.[98]

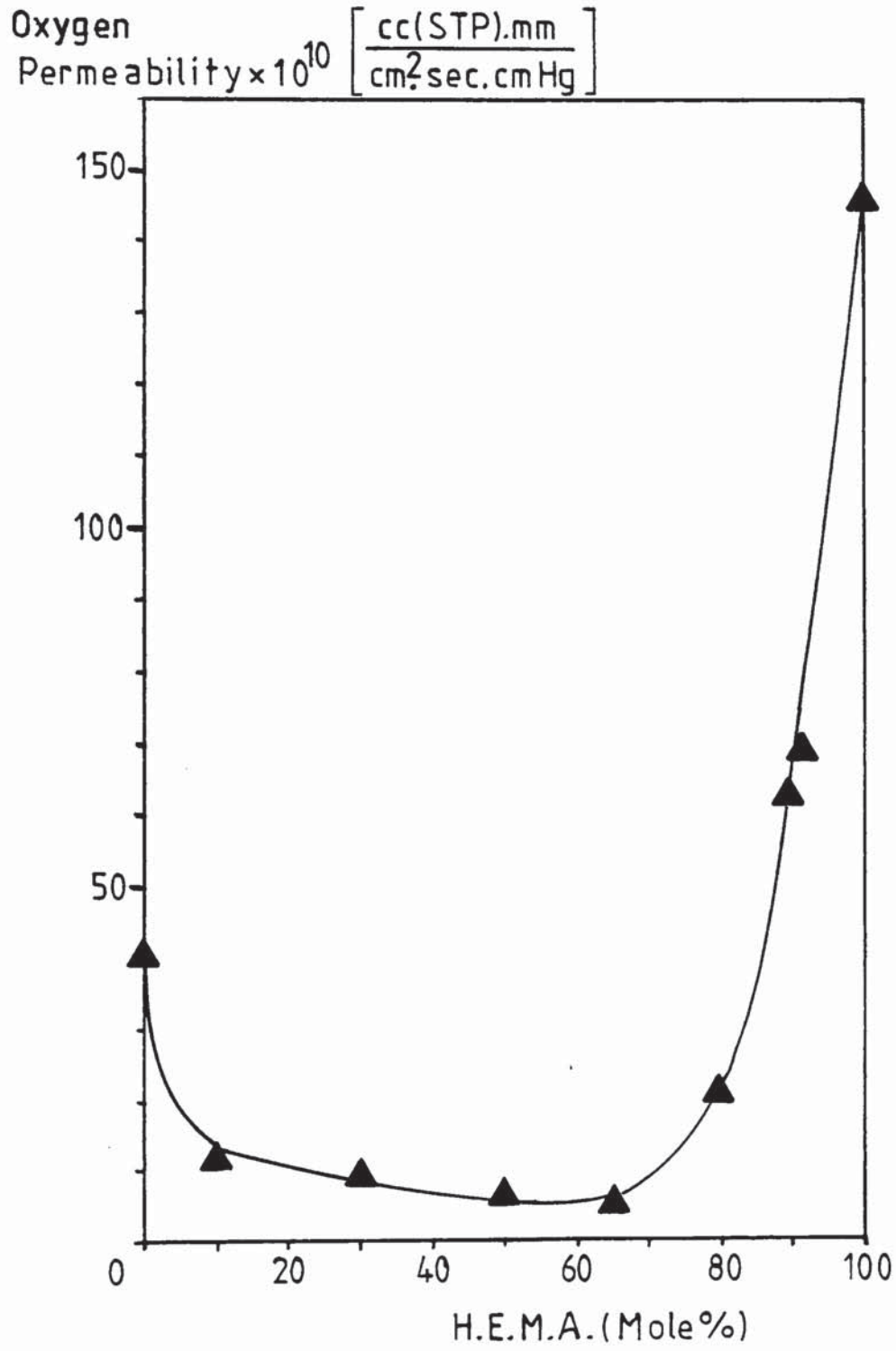
Using the Ferry equation, Refojo,[162] ascertained that for a homogeneous poly (2-hydroxy ethyl methacrylate) of approximately 39% water content, an average pore radius of 3 - 5 A^{o} was evident. However, somewhat anomalously, Haldon and Lee,[161] found that with increasing cross-linking agent instead of decreasing the pore size, it actually increased the average pore radius up to 18 A^{o} for a poly (HEMA) gel of less than 30% equilibrium water content. In a more recent report by Refojo,[165] he reports on the penetration of large biopolymers (poly-

saccharides and protein molecules), into hydrogel networks and also attributes poly (HEMA) as having pore size of at least 30 A°. The implications of this on the protein adsorption studies carried out during this project will be considered in the relevant Chapter 6.

The effects of equilibrium water content and temperature on dissolved oxygen permeability through hydrogels has been reported,[166] and as expected permeability was observed to increase with increasing water content and temperature. High oxygen permeability is an essential criterion for a continuous wear contact lens. An interesting phenomenon was observed by the same research group,[153] when considering the effects of bound (non-freezing)/free (freezing) water contents on dissolved oxygen permeability through HEMA/STY copolymers. As previously discussed these copolymers exhibit an equilibrium water content which is completely bound at approximately 85:15 (Moles %) HEMA/STY respectively, and in some agreement with this, it was observed that a rise in the free (freezing) water content, corresponded well with an observed vast increase in permeability (See Graph 4.14).

Other permeability studies on hydrogels have attempted to ascertain their applicability in the field of reverse osmosis membranes. In this application the hydrogel must function as a semipermeable membrane allowing only the transport of water, while rejecting any dissolved species, which in the case of desalination, the best known example, is sodium chloride. This can be achieved by the correct combination of hydrophilic and hydrophobic monomers, to attain a near totally bound water content, with high equilibrium water content. The most successful

Graph. 4.14: Oxygen Permeability of HEMA/STY Copolymers



material to date, for both reverse osmosis and kidney dialysis, has been cellulose acetate, with an equilibrium water content of 15% and a freezing water content of less than 1%.[153]

Copolymers of HEMA/EMA have shown very good salt rejection as the ethyl methacrylate content is increased, giving greater than 99% salt rejection, but with low water flux.[167] However on the basis of the results obtained in this project for the HEMA/EMA copolymers, (Table 4.3), very low freezing water contents and relatively high equilibrium water contents, as compared to cellulose acetate, were obtained. This is undoubtedly due to the improved purity of the 2-hydroxy ethyl methacrylate preparation.

Although water is a necessary criterion for hydrogels, it would appear that it is the way in which the water is structured, that is to say the ratio of bound to free water, which will ultimately determine the permeability, for a given pore size.

4.6 Mechanical Properties

For most biomedical applications of hydrogels, their inherent mechanical weakness, makes then an inappropriate choice of material. This disadvantage may to some extent be overcome by (radiation) grafting the hydrogels onto a strong supporting material. It has been suggested however that the low elasticity/rigidity moduli exhibited by hydrogels is a prerequisite for good blood compatibility, and also it is a necessary criteria for their application in soft tissue implants.[168]

One particular biomedical application in which grafting is unfeasible, is in the field of soft contact lenses, and it is in this field that the mechanical properties of hydrogels are most important. In relation to soft contact lenses, although tensile strength is relevant to the general durability and resistance to handling of a hydrogel, the shear and compressive forces due to eyelid motion, produce a quite different type of deformation. Indentation work under eyelid load conditions (2.6×10^4 dynes. cm^{-2}).[169] has shown a correlation with clinical studies. Although the ideal elastic behaviour exhibited by synthetic elastomers, under these low loads is preferable, appreciable deviations from this situation still produce acceptable clinical behaviour. Thus materials whose deformation characteristics are more time-dependent, exhibiting 'damped' curves, produce acceptable visual stability levels in lenses, provided that the overall deformation is not larger than 2μm deformation under eyelid load, as seen with poly (HEMA) lenses. Hydrogels whose deformation behaviour is characterised by large ($\sim$5μm) and rapid initial deformation give rise to lenses whose visual stability is unacceptable. Such behaviour is typically associated with hydrogels of fairly high equilibrium water content ($>$ 50%) and low 'effective' cross-link density. This 'effective' cross-link density is governed not only by the conventional covalent cross-links, but also ionic, hydrogen-bonding, dipole-dipole and hydrophobic-Van der Waals interchain-interactions.

The general inverse relationship between decreasing

mechanical strength and increasing equilibrium water content, can therefore to some extent be offset by the above interchain interactions. Strong hydrogen-bonding characteristics of the neutral acrylamide and its derivatives thereof, together with N-vinyl pyrrolidone, makes high water content gels with comparable strength to poly (HEMA), a distinct possibility. Previous work by Dusek and Janacek[170], found that by increasing the amide content of copolymers of 2-hydroxy ethyl methacrylate, with acrylamide and methacrylamide, high water content gels with comparable tensile strength to that of poly (HEMA), were formed and this they attributed to hydrogen-bonding. In more recent studies concerning the development of continual wear lenses in our own laboratories by I. Middleton and in conjunction with Kelvin Lenses Ltd. (See Chapter 7), much improved deformational characteristics over and above those shown by poly (HEMA) have been attained as illustrated in Table 4.6

(See overleaf for Table 4.6)

Table 4.6 THE INFLUENCE OF HYDROGEN-BONDING ON DEFORMATION CHARACTERISTICS[155]

Hydrogel Composition	Monomer Ratio	EWC %	Mass for 2μm deformation (g)	Force (dynes.cm^{-2}) for 2μm indentation
HEMA	100:0	39	0.108	106
HEMA/DAACM	50:50	27.5	0.278	273
HEMA/DAACM	67:33	26.4	0.284	279
HEMA/MACM	67:33	39.6	0.339	333
HEMA/MACM	75:25	36.2	0.531	521
HPA/ACM	67:33	71.3	0.679	666

These much improved mechanical properties observed with the strong hydrogen-bonding non-ionic copolymers has allowed the formulation of hydrogels with the requisite high water content, high oxygen permeability and mechanical stability, necessary for a continual wear soft contact lens, (for further details see Chapter 7).

Differential scanning calorimetry studies in our laboratories, on the water-binding properties of hydrogels have also observed marked changes in mechanical properties (tensile strength, rigidity modulus, tear strength), with changes in the bound and free water contents. Increased deformational behaviour is seen with high freezing water content gels, due to the very good plasticizing properties of free water. Generally the higher the bound water content for a particular equilibrium water content, then the greater the mechanical properties exhibited. This is a consequence of the fact that those polymers exhibiting good water binding properties also possess good polymer-polymer interactions and thereby

increase their strength. There is also a very real possibility that bound water, fixes the polymer chain and prevents chain rotation and thus reduces the strain on any polymer-polymer interchain-interactions, and thereby stabilises and effectively increases the mechanical strength of the polymer.

The hydrogel compositions used in this project have been assigned comparative strength values, based simply on physical examination, and these are given in Appendix 3. What is apparent from this classification is that the anionic methacrylic acid, far from increasing the strength, (when physiologically hydrated), due to its strong hydrogen-bonding capacity, has infact decreased the strength. It should of course be noted that this decrease in strength is accompanied by a vast increase in water content. Dimensional instability has also been attributed to methacrylic acid when subject to changes in temperature, due to autoclaving.[155] It would therefore seem that the biomedical use of compositions containing methacrylic acid or all anionic monomers, should be severely limited, until full knowledge of their behaviour is known.

The unexpected high strength exhibited by the N-vinyl pyrrolidone : ethyl methacrylate 75:25 (Moles %) copolymer has been attributed to strong interchain interactions and a very high bound water content.

This is perhaps not too surprising when one considers the structure of the N-vinyl pyrrolidone, Fig. 4.5, comprised of a hydrophobic alkyl ring and very strong dipole moments on the oxygen and nitrogen atoms.

Very powerful hydrophobic-Van der Waals, dipole-dipole and hydrogen-bonding interactions, would be expected between these groups and the ethyl methacrylate molecule, and the commensurate increased mechanical strength would then be expected. However, one other possibly somewhat speculative alternative exists and this is based on the low reactivity ratio of N-vinyl pyrrolidone. If during the polymerisation process only a small % of the N-vinyl pyrrolidone enters into reaction with the ethyl methacrylate, block copolymers of ethyl methacrylate will occur, interspersed with a few N-vinyl pyrrolidone molecules. During the later stages of the polymerisation and the post-cure procedures (as given in Chapter 2), this primary polymer network will be effectively swollen in the excess N-vinyl pyrrolidone, and this may then allow a secondary N-vinyl pyrrolidone polymer network to be set-up within the primary network. Thus a low hydrophilic polymer network with its merits of good mechanical properties will have its network interspersed by a very hydrophilic polymeric network, held together by simple physical chain entanglement and/or chemical interactions.

This is known as an interpenetrating polymer network, and several workers have demonstrated enhanced mechanical properties,[171] together with high water contents.[155,172]

Obviously compositions of the above type require a far greater in-depth study, which is outside the scope of this project.

Lastly with regards the copolymers of HEMA/EDMA, it was previously postulated that imperfections in gel-network occur with increasing cross-linking agent, due to non-isotropic distribution of the cross-links (by a mechanism proposed in Chapter 5.8.3). Micro-indentation studies were carried out in our laboratories which established that with increasing cross-linking agent concentration, so a proportional rise in strength was exhibited.[156] This would seem to indicate that although these cross-links are not evenly distributed throughout the bulk, they can still effectively raise the total bulk mechanical properties. Alternatively the increase in strength may be attributable to the rise in the bound water to free water ratio, exhibited by these gels with increasing concentration of cross-linking agent.

4.7 Hydrogel Surface/Interfacial Properties

Traditionally the surface of a material is considered as a geometrically abrupt change from the interior substance to the external environment, however in reality a hydrogel interfacial zone would be no more sharply defined than the mean distance between cross-links in the bulk. The region between these cross-links, at the gel 'surface' is composed of freely coiling chain segments, which terminate at the gel surface/interface, effectively smoothing out the coarse grain of the cross-link density distribution. In general it is these mobile chain segments coupled with the constraints placed on their mobility to rotate, by the cross-link density distribution, that will ultimately govern their surface/

interfacial properties. Dependent on the environment with which they interface, these mobile chain segments, will when possible, attempt to rotate to minimise the interfacial free energy of the system. Their ability to do just that will be as a direct consequence of the constraints imposed on these interfacial chain segments by the bulk properties, as characterised by water content and water-binding-studies. In turn their ability to attain this minimal interfacial free energy, will determine just to what extent the interfacial water will be structured. The implications of these bulk properties to the measured hydrated surface/interfacial properties will be the subject of discussion in the subsequent chapter.

4.8 Optical Properties of Hydrogels

In brief the necessary optical criteria that a potential soft contact lens must fulfill are:

1. It should swell in water, isotropically, to form a homogeneous gel-network, in order that no microphase dispersion occurs, which would lead to diffraction and translucency.

2. It should possess a similar refractive index to that of the cornea (i.e., 1.37).

3. It should possess sufficient mechanical properties (that mimic the cornea), and be able to resist the deforming forces of the eyelid during the blink cycle and yet be elastically deformable and rapidly recoverable in order to minimise mechanical pressure on the eye and avoid visual instability.[173] Visual acuity of such lens systems will generally always be slightly inferior to those achieved for hard lenses and spectacles.

Loss of visual acuity and increasing wearer discomfort has been reported with soft lenses, due to the build-up of proteinaceous deposits.[174] This latter problem will be looked at in more detail in Chapter 7.

All compositions synthesised for this project were transparent, except for the solution polymerised poly (diacetone acrylamide), which appeared opaque due to microphase separation.

4.9 Biocompatibility Properties

Various methods for the attempted determination of this parameter have been given in the introductory chapter. It is really a very indistinct property, but to some extent it may be thought of as a composite function of the afore-mentioned hydrogel properties, hydrophilicity, permeability, mechanical strength and probably the most important aspect, the surface/ interfacial properties. However to date, a physical measure of biocompatibility is possible only by comparative tests, which leave much to be desired, and therefore it remains a somewhat intangible property to evaluate.

CHAPTER 5

CHARACTERISATION OF HYDRATED SURFACES

5.1 Introduction

Important differences have been observed between the surface characteristics of hydrated and dehydrated polymers.[175] Since both surface and interfacial tensions are free energy properties, they will tend to minimise through molecular orientation and this is particularly evident when considering hydrogels with mixed polar (hydrophilic) and apolar (hydrophobic) surfaces. In the dehydrated state the surface free energy is minimised by the orientation of low energy methyl groups towards the vapour phase, and the high energy polar groups towards the hydrogel interior, due to bulk interactions. Increasing the alkyl side chain length will tend to raise the surface free energy due to the surface presence of slightly higher energy methylene groups. It can be appreciated that these orientations will be markedly altered when considering hydrogels in an aqueous environment. As previously mentioned in Chapter 4.2, hydration of apolar (hydrophobic) groups is thermodynamically unfavourable and in order to compensate the groups tend to undergo hydrophobic - Van der Waals interactions, to minimise their interface with the polar aqueous environment. Accordingly when hydrogels are in an aqueous environment, the majority of apolar groups will be located within the bulk and similarly polar groups will tend to orientate away from the bulk.

It should be remembered however, that the above will be reduced by a factor dependent on the amounts of bound and free water present within the hydrogel bulk.

The surface/interfacial free energy of a hydrogel will be determined by the orientation of the polar/apolar composition of the mobile chain segments near to the surface. The ability of these chain segments to undergo orientation to minimise interfacial free energy (γ_{sL}), will be directly related to the constraints imposed by covalent cross-link density and other bulk interactions (i.e., hydrogen-bonding, dipole-dipole, ionic and Van der Waals interactions).

A number of workers have attempted to correlate critical surface tension (γ_c), and surface polar (γ_s^p) and dispersive (γ_s^d) components with biological interactions of dehydrated polymers,[176,177] but as seen from the afore-mentioned section, the little success achieved in these studies is understandable. Therefore contact angle methods which take into account the presence of an aqueous interface in a biological environment, may be of more relevance to biomaterial surface energy evaluation, and especially when considering hydrogels. Two such techniques, the 'Hamilton'[178,179] and 'Captive Air Bubble',[180-182] have been developed, and normally these two techniques will enable the polar (γ_s^p) suface component and surface wettability, respectively, to be determined. Surface wettability is a composite function of both dispersive and polar surface components and therefore by a theoretical consideration,[183] to be given later, the two techniques may be combined to determine the total

surface free energy (γ_s), dispersive component (γ_s^d) and the interfacial free energy (γ_{SL}) for the hydrated surface.

The proposed characterisation of hydrogels by the protein adsorption studies, outlined in Chapter 3, necessitated the use of a physiological 'like' environment to maintain the proteins in as near a native conformation as possible. Therefore in order to establish any possible relationship that may exist between protein adsorption and hydrogel surface/interfacial chemistry, all hydrogel surface measurements were taken in physiological Krebs solution. From previous published results by Hoffman et al[184] and Van der Scheer et al,[185] it was determined that insignificant changes in contact angle data occurs, when measurements are taken in either distilled water or a physiological 'like' salt solution and that no significant change in surface free energy (γ_{sv}) with increasing salt content, would be expected for a neutral polymer. While no attempt has been made to verify these findings, (and they have been assumed true) this study has been solely concerned with those hydrogel properties exhibited in a physiological solution and confirmation of their validity is therefore inconsequential, as regards this study.

Although traditional surface chemistry measurement techniques, have been found inappropriate for the evaluation of hydrated surface characteristics, the fundamental theoretical derivations of surface chemistry from contact angle measurements, remains valid for hydrated surfaces. Therefore it is apparent that some knowledge of these theoretical derivations is necessary.

5.2 Derivation of Solid Surface Free Energies from Contact Angle Measurements

When a drop of liquid is placed on a flat solid surface, it may spread completely over the surface, or more likely it may remain as a drop having a definite angle of contact with the solid surface, as illustrated in Fig. 5.1. Assuming that the various surface forces can be represented by surface tensions acting in the direction of the surfaces, then, equating the horizontal components of these tensions yields the Young equation[186]:

$$\gamma_{sv} - \gamma_{sL} = \gamma_{Lv}.\cos\theta \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Dupré[187], demonstrated that the work of adhesion (W_A) could be defined in terms of Young's equation:

$$W_A = \gamma_{sv} + \gamma_{Lv} - \gamma_{sL} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

Combining equations (1) and (2), in terms of the interfacial tension, yields the Young-Dupré equation:

$$W_A - \gamma_{Lv} = \gamma_{Lv}\cos\theta = \gamma_{sv} - \gamma_{sL} \quad \ldots\ldots\ldots \quad (3)$$

This well known formula is based on a two dimensional horizontal force balance at the point of contact, assuming the system is in equilibrium. There are two experimental systems for measuring contact angles, liquid/air/solid systems and liquid/liquid/solid systems. Estimation of solid surface free energy (γ_s) from contact angle measurements presents problems, because this can only be achieved for a solid in a high vacuum. The difference between solid surface free energy (γ_s) and the solid-vapour interfacial free energy (γ_{sv}) is known as the equilibrium spreading pressure (π_e):

$$\pi_e = \gamma_s - \gamma_{sv} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

and

$$\gamma_s = \gamma_{sv} + \pi_e = \gamma_{Lv}.\cos\theta + \gamma_{LS} + \pi_e \quad \ldots\ldots\ldots \quad (5)$$

With low energy solids, such as polymeric materials it has been shown that the equilibrium spreading pressure (π_e), tends towards zero, when the liquid forms a finite contact angle on the solid surface.[188]

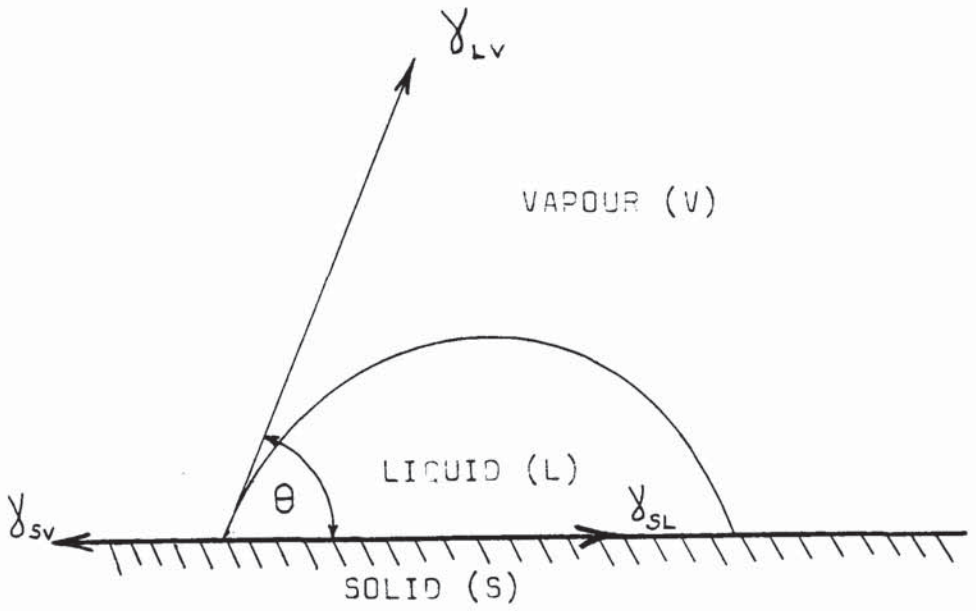
Since the solid-liquid interfacial free energy (γ_{SL}) is generally not measurable, the best expression is given by:

$$\gamma_s \approx \gamma_{sv} = \gamma_{LV} \cos\theta + \gamma_{SL} \quad \ldots\ldots\ldots\ldots \quad (6)$$

(See overleaf for Figure 5.1)

Figure 5.1

VECTOR COMPONENTS OF SURFACE FREE ENERGY



γ_{LV} = Surface tension of the liquid in equilibrium with its own vapour.

γ_{SL} = The interfacial tension of the solid-liquid interface.

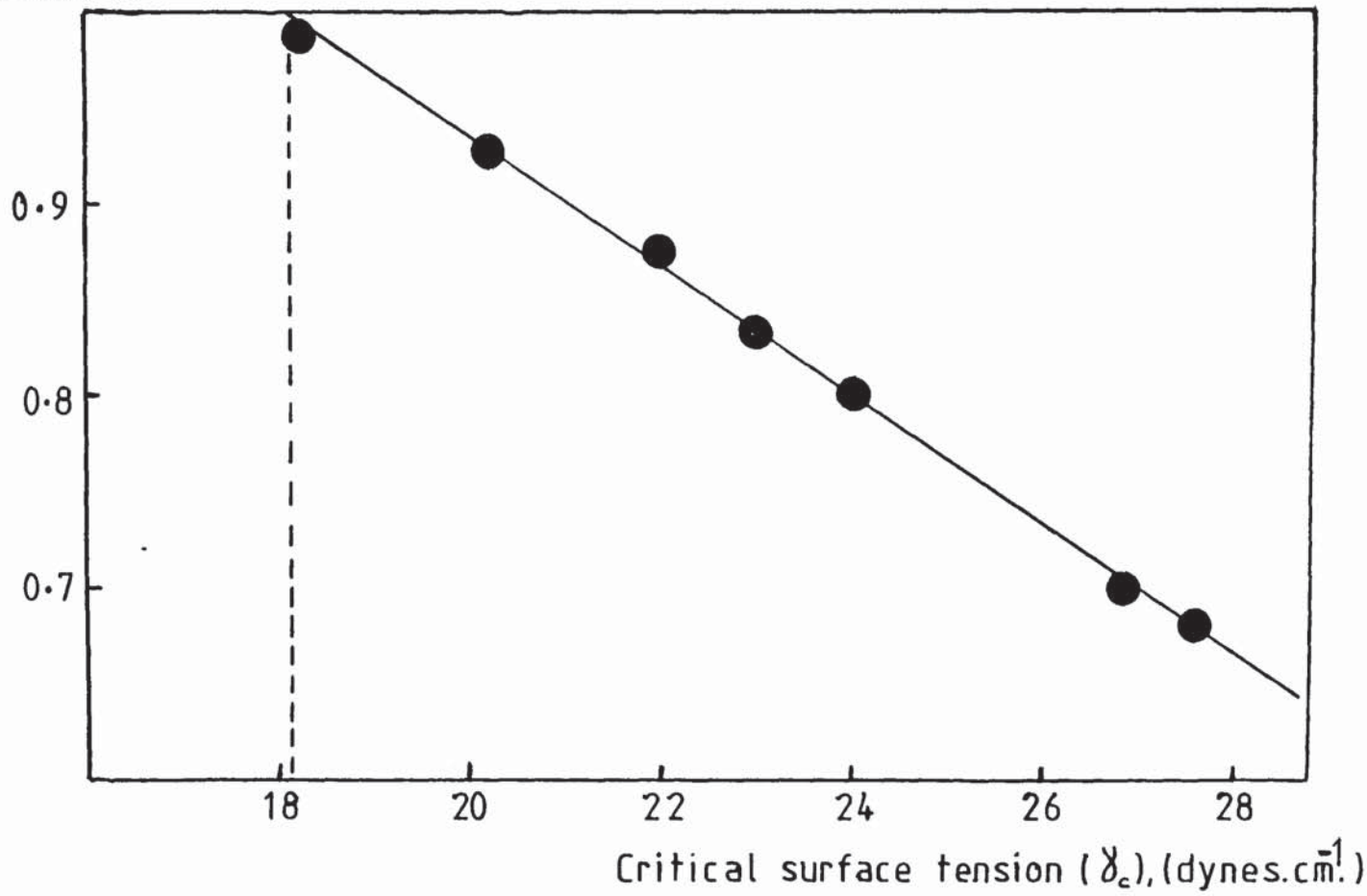
γ_{SV} = The solid "surface-tension" (free energy per unit area), in equilibrium with its own vapour, plus the vapour of the liquid.

5.3 Critical Surface Tension

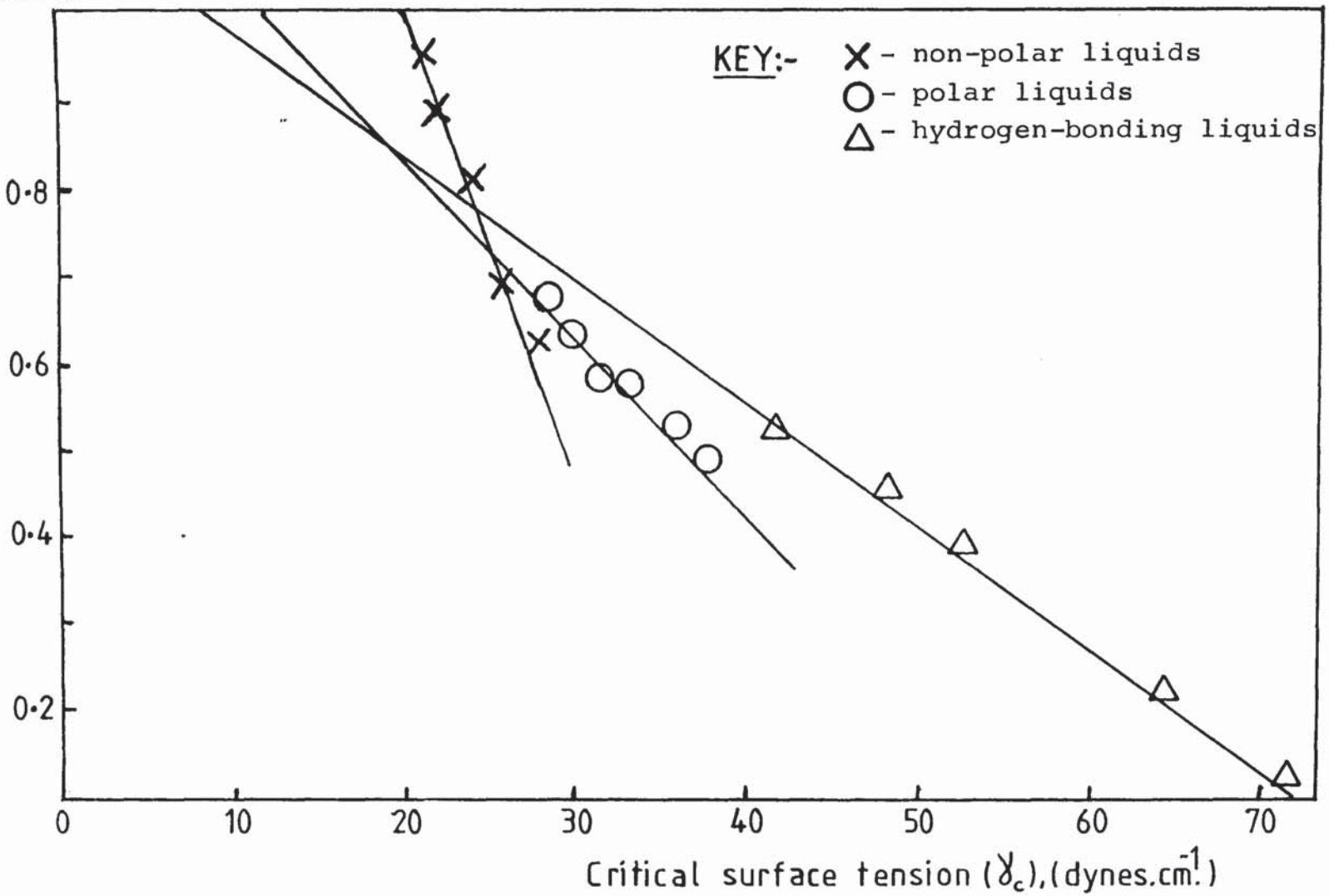
Zisman et al,[189-192] proposed the "critical surface tension of wetting", (γ_c), as a measure of solid surface free energies. In this technique the contact angles of a homologous series of liquids are measured on a solid polymeric surface, and a plot of the surface tensions (γ_{LV}) of the series of liquids against the cosine of the contact angles is then made. Extrapolation of the graph to $\cos\theta = 1$ (i.e., $\theta = 0^{\circ}$) is made, and at this intersect point a value for the critical surface tension (γ_c) is obtained, as illustrated in Graph 5.1. The critical surface tension of wetting (γ_c) is the theoretical surface tension (surface free energy), of a hypothetical liquid which will just spread on that particular solid surface. Variations in the values of critical surface tension will be obtained if different series of liquids (i.e., polar or apolar), are used, and it is therefore reasonable to assume that the liquid series most "like" the substrate polymer surface, will yield the most accurate and realistic estimation of critical surface tension (γ_c) (See Graph 5.2).

This technique represents the best documented approach to characterisation of solid surfaces, however it is applicable only to dehydrated solids. Therefore, although this technique may be applied to Xerogels (dehydrated hydrogels), it will not give a true indication of the hydrated-surface characteristics exhibited by hydrogels.

Graph. 5.1: Derivation of Critical Surface Tension of a Solid



Graph.5.2: Variability of 'Critical' Surface Tension of a Solid as a Function of the Wetting Solution Characteristics



5.4 Components of Solid Surface Free Energy

Girifalco and Good[193-195], developed a molecular theory for work of adhesion (W_A) (free energy of adhesion) based on the geometric mean of the free energies of cohesion of two separate phases:

$$W_A = \gamma_{LV} + \gamma_S - \gamma_{SL} = 2(\gamma_{LV} \cdot \gamma_S)^{\frac{1}{2}} \quad \ldots\ldots\ldots (7)$$

This equation was found to be inadequate for polar force interactions across the interface and so was improved accordingly:

$$W_A = \gamma_{LV} + \gamma_S - \gamma_{SL} = 2\,\phi\,(\gamma_{LV} \cdot \gamma_S)^{\frac{1}{2}} \quad \ldots\ldots\ldots (8)$$

where the bonding efficiency factor (ϕ) is equal to the product of $\phi_A \cdot \phi_V$ (where A = Attractive intermolecular interaction parameter and V = Molar volume of the two phases)

The term ϕ_A may be expressed as the geometric means of the polar (p) and dispersive (d) fractions of the energy density of the adjacent phases[196]:

$$\phi_A = (d_1 \cdot d_2)^{\frac{1}{2}} + (p_1 \cdot p_2)^{\frac{1}{2}} \quad \ldots\ldots\ldots (9)$$

Generally it is assumed that the molar volumes of the two phases are roughly equal and therefore ϕ_V tends towards unity. Thus equation (8) may be rewritten as:

$$W_A = 2(\gamma_{LV} \cdot \gamma_S)^{\frac{1}{2}} ((d_1 \cdot d_2)^{\frac{1}{2}} + (p_1 \cdot p_2)^{\frac{1}{2}}) \quad \ldots\ldots (10)$$

Independently to this Fowkes[197], suggested that for liquid-liquid or liquid-solid interfaces, across which only dispersive forces were significant, the work of adhesion (W_A^d) was equal to:

$$W_A^d = 2(\gamma_1^d \cdot \gamma_2^d)^{\frac{1}{2}} \quad \ldots\ldots\ldots\ldots\ldots (11)$$

which for a liquid-liquid interface:

$$W_A^d = (\gamma_{LV})_1 + (\gamma_{LV})_2 - \gamma_{L1/L2} = 2((\gamma_{LV}^d)_1 \cdot (\gamma_{LV}^d)_2)^{\frac{1}{2}} \quad \ldots\ldots (12)$$

and for a liquid-solid interface:

$$W_A^d = \gamma_{LV} + \gamma_S - \gamma_{SL} = 2(\gamma_{LV}^d \cdot \gamma_S^d)^{\frac{1}{2}} \quad \ldots\ldots (13)$$

Using this knowledge a number of liquids have been characterised into their disoersive and polar components where:

$$\gamma_{LV} = \gamma^{d}_{LV} + \gamma^{p}_{LV} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (14)$$

Some examples of which are given in Table 5.1.

Table 5.1

Dispersion and Polar Contributions to some Liquid Surface Tensions ($T = 20^{o}C$)[198,199]

Liquid	γ_{LV} dynes. $cm.^{-1}$	γ^{d}_{LV} dynes. $cm.^{-1}$	γ^{p}_{LV} dynes. $cm.^{-1}$
Water	72.8	21.8	51.0
Glycerol	63.4	37.0	26.4
Formamide	58.2	39.5	18.7
Methylene Iodide	50.8	48.5	2.3
N-Hexadecane	27.6	27.6	0.0
N-Octane	21.8	21.8	0.0
Carbon Tetrachloride	27.0	27.0	0.0

The work of adhesion between a liquid and a polymer will often have both polar and dispersive energy components, to account for this Owens and Wendt, Kaelble and others,[196,199,200] have evolved an equation:

$$W_A = \gamma_{LV} + \gamma_S - \gamma_{SL} = 2(\gamma^{d}_{LV}.\gamma^{d}_{S})^{\frac{1}{2}} + 2(\gamma^{p}_{LV}.\gamma^{p}_{S})^{\frac{1}{2}} \quad \ldots\ldots \quad (15)$$

This equation can then be applied to the Young-Dupré equation (3), to yield:

$$\cos\theta.\gamma_{LV} = 2(\gamma^{d}_{LV}.\gamma^{d}_{S})^{\frac{1}{2}} + 2(\gamma^{p}_{LV}.\gamma^{p}_{S})^{\frac{1}{2}} \quad \ldots\ldots\ldots\ldots\ldots \quad (16)$$

If contact angle measurements are now taken for a series of liquids (of known γ^{d}_{LV} and γ^{p}_{LV}), on a solid surface, the dispersive and polar components of surface free energy may be deduced. For simplicity, two liquids

are generally chosen for this task, and often these will be purified water and methylene iodide.[201] Again, as with the Zisman derivation of critical surface tension, the above technique is limited to dehydrated surfaces.

Total surface free energy (γ_S) of a polymer can be further sub-divided up into ionic (γ_S^i), hydrogen-bonding (γ_S^H), dipole-dipole and dipole-induced dipole (γ_S^P) giving:

$$\gamma_S = \gamma_S^H + \gamma_S^P + \gamma_S^d + \gamma_S^i \quad \ldots\ldots\ldots\ldots \quad (17)$$

Hydrogen bonding and polar interactions are basically similar and are generally combined in γ_S^P to give:

$$\gamma_S = \gamma_S^P + \gamma_S^d + \gamma_S^i \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (18)$$

The work of adhesion in such a situation is given by:

$$W_A = 2(\gamma_{LV}^d . \gamma_S^d)^{\frac{1}{2}} + 2(\gamma_{LV}^P . \gamma_S^P)^{\frac{1}{2}} + I_{SL}^i \ldots\ldots \quad (19)$$

It can be seen that this last term I_{SL}^i complicates the separation of surface free energy terms when the ionic interactions contribute significantly. Generally the ionic forces (I_{SL}^i) are ignored in the literature, since most polymeric solids exhibit neutral characteristics, and their small ionic content, due to impurities, will not contribute significantly.[202]

5.5 Hamilton's Technique[178,179]

The experimental procedure for this technique has been outlined in Chapter 2.

Hamilton proposed the contact angle measurements of small droplets* of n-octane at a solid-water interface, as illustrated in Figure 5.2.

---

* Assumption that gravity is negligible for small droplets.[183]

To which he applied the following theory:

Fowkes equation for work of adhesion at a solid-liquid interface Eq (13), was modified by Tamai et al,[203] to include stabilisation at the interface by non-dispersion forces:

$$\gamma_{SL} = \gamma_s + \gamma_{LV} - 2(\gamma_s^d . \gamma_{LV}^d)^{\frac{1}{2}} - I_{SL} \ldots\ldots (20)$$

Stabilisation by polar forces

$$I_{SL} = 2(\gamma_S^P . \gamma_{LV}^P)^{\frac{1}{2}} \ldots\ldots\ldots\ldots\ldots (21)$$

The γ_{LV}^d for n-octane and water (i.e., 21.8 dynes. cm.$^{-1}$) are fortuitously the same, and these effectively cancel the square-root terms in Eq.(20). Hamilton combined Eq.(20) with Young's Eq.(1), to derive an expression for the polar stabilisation parameter ($I_{s\omega}$)

$$I_{s\omega} = \gamma_{\omega v}' - \gamma_{ov} - \gamma_{o\omega} . \cos\theta \ldots\ldots\ldots (22)$$

By solving the above Eq.(22) for $I_{s\omega}$, the polar surface component (γ_S^P), may be determined from Eq.(21).

Hamilton attributed a value of 51.6 dynes. cm.$^{-1}$ to $\gamma_{\omega v}'$ (the surface tension of n-octane saturated water) however this has been disputed by El-Shimi and Goddard[204] and others,[183,205] who stated that the value for $\gamma_{\omega v}'$, is very close to the value for pure water, 72.8 dynes. cm.$^{-1}$. Solubility of n-octane in water is less than 1 ppm and accordingly it is assumed that $\gamma_{\omega v}' \approx \gamma_{\omega v}$ (72.8 dynes. cm.$^{-1}$).

A calibration curve for the Hamilton technique was constructed, using the value of 72.8 dynes. cm.$^{-1}$ for $\gamma_{\omega v}$ (See Graph 5.3). It should perhaps be pointed out, that often even the contact angle value is utilised in ranking surfaces in order of increasing polar component (i.e., the nearer θ is to 180^o the more polar will be the surface).

Calibration Curve for Hamilton Method

Graph. 5.3:

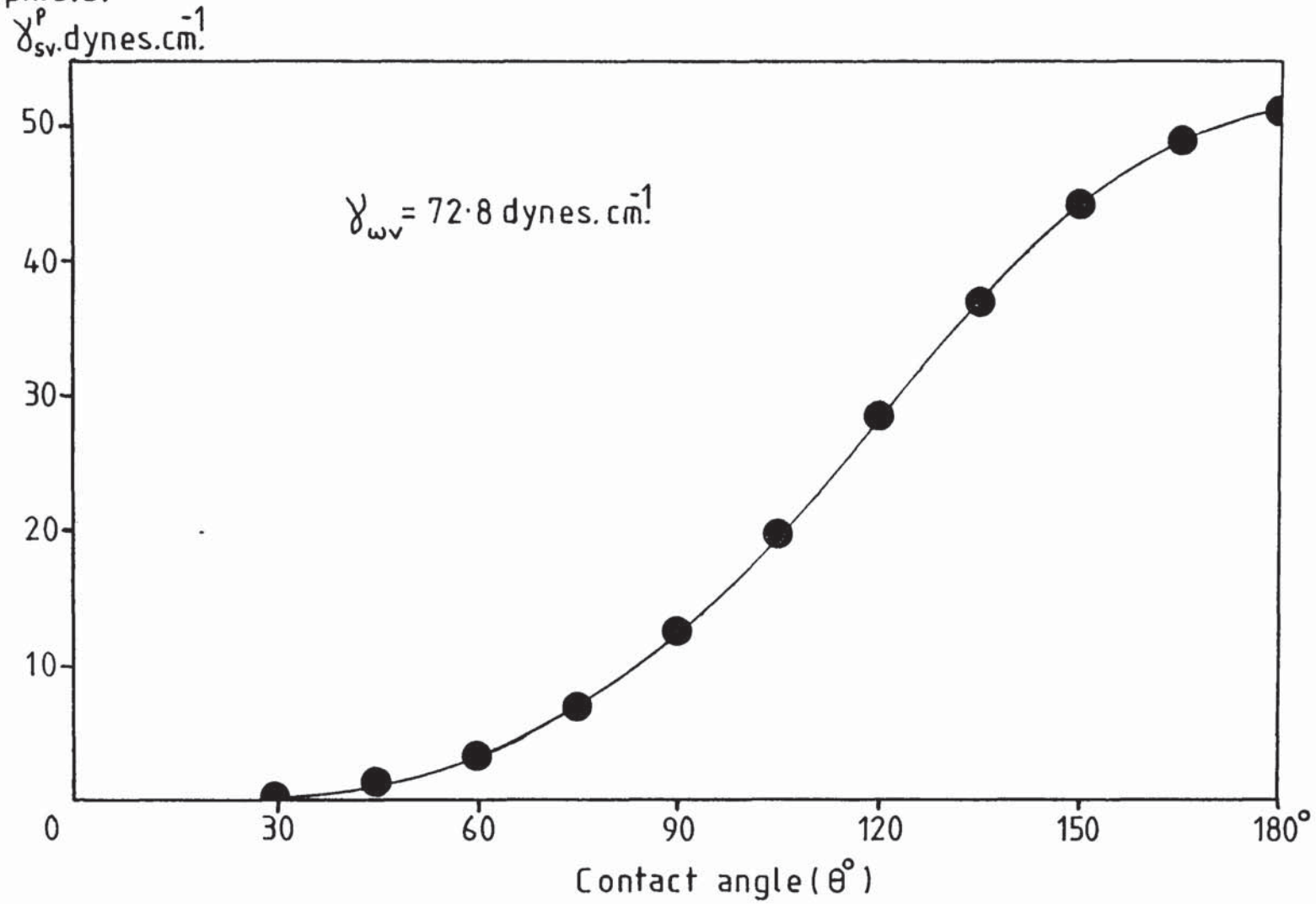


Figure 5.2

HAMILTON TECHNIQUE: SURFACE FREE ENERGY VECTORS

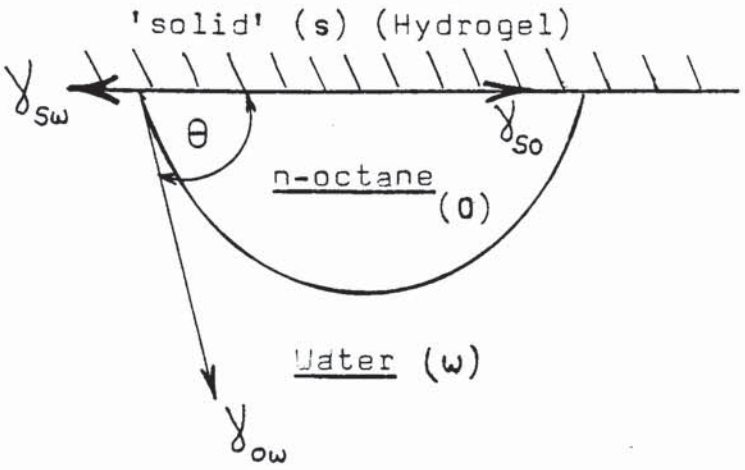
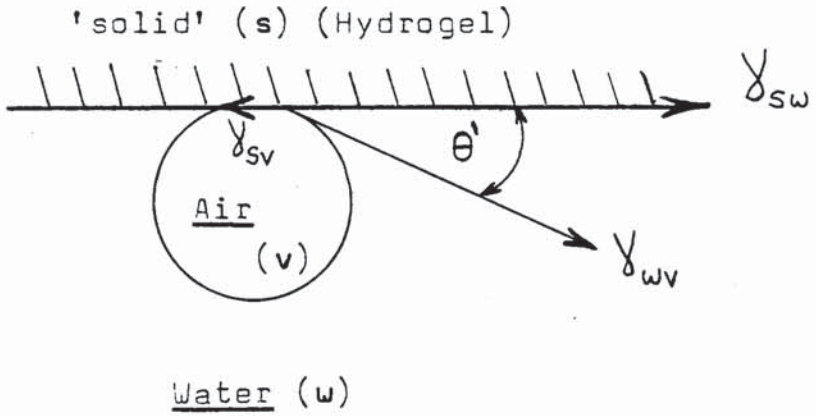


Figure 5.3

CAPTIVE AIR BUBBLE TECHNIQUE: SURFACE FREE ENERGY VECTORS



KEY:

$\gamma_{s\omega}$ = solid-water interfacial free energy

γ_{so} = solid-oil interfacial free energy

$\gamma_{o\omega}$ = oil-water interfacial free energy

$\gamma_s \approx \gamma_{sv}$ = solid surface free energy

γ_{wv} = surface tension of water (free energy)

5.6 Captive Air Bubble Technique[180-182]

The standard experimental procedure for this technique was outlined in Chapter 2.

When making a contact angle measurement with hydrated hydrogels or biopolymers in air, problems are encountered. One of the main problems is ensuring that the surface does not dehydrate, whilst at the same time reproducible efficient removal of surface films of water must be obtained.

The phenomena of hysteresis has been encountered in measurements made by the above mode,[206] which have been attributed to the ability of hydrogels to change their free energy by reorientation of the polymer side chains and chain segments depending on the adjacent phase. This phenomena may in some part be due to the problems of dehydration, however it is also possible that the kinetics of gel-deformation.[183] may also play a part. This later problem is seen to occur with low rigidity moduli hydrogels and high surface tension liquids.

The advantages of the captive air bubble and Hamilton's techniques over the above traditional contact angle methods is that the gel surface is fully hydrated and may be placed in either distilled-water or a physiological solution to perform the measurements. Also in the conventional method the composition of the vapour phase and its interaction with the solid, presents problems, however, these problems are eliminated using the captive air bubble technique.

The contact angles measurements from this technique

give a valid comparative measure of surface wettability which is a composite function of the surface dispersion and polar forces. The surface tension forces at play are illustrated in Fig. 5.3.

5.7 Theoretical Derivation for Hydrogel Surface/Interfacial free Energy Components, γ_{sv}, γ_{sv}^{p}, γ_{sv}^{d}, γ_{sw}.

Hydrogels are of considerable interest as blood and tissue interfacing materials and for many other biomedical applications.[54] They also are of interest as models for the study of cell surfaces.[51] Study of hydrogel systems should enable the 'minimum interfacial free energy' hypothesis[51,53] for protein adsorption and biocompatibility to be tested. This hypothesis, however is dependent on being able to measure the interfacial free energy and on the ability to prepare hydrogels with differing gel-water interfacial free energies (γ_{sw}). The hydrogel-aqueous interface is a very difficult subject to study, and the conventional classical surface chemistry assumptions do not apply. However, captive air bubble and Hamilton contact angle data can be used to estimate γ_{sv}, γ_{sv}^{p}, γ_{sv}^{d} and γ_{sw}, for the hydrogel-aqueous interface,[183] by the following theoretical considerations. Before embarking on this it should be remembered from Eq. (4) that $\gamma_s \approx \gamma_{sv}$ when π_e is tending towards zero, however it would seem more appropriate for a hydrogel, to consider γ_{sv}, because γ_s has no physical significance for the experimental conditions. Accordingly, γ_{sv} has been substituted in the equations previously written as γ_s.

From Young's Eq. (1) we have

$$\gamma_{sv} - \gamma_{sw} = \gamma_{wv} \cdot \cos\theta \quad \text{(where } \gamma_{sL} = \gamma_{sw}\text{)}$$

Taking the value of γ_{wv} to be 72.8 dynes. cm.$^{-1}$ at 20°C and θ' is the captive bubble contact angle, application to Eq. (1) yields ($\gamma_{sv} - \gamma_{sw}$), known as the adhesion tension. Application of the treatments used in the derivation of the Hamilton Eq. (22), gives the polar stabilisation parameter

$$I_{sw} = \gamma_{wv} - \gamma_{ov} - \gamma_{ow} . \cos\theta \quad \ldots\ldots\ldots\ldots \quad (22)$$

Using the data supplied in Table 5.1[198,199] and elsewhere[183] we have

γ_{wv} = 72.8 dynes. cm^{-1}., γ_{ov} = 21.8 dynes. cm.$^{-1}$ and γ_{ow} = 51 dynes. cm.$^{-1}$

Eq. (22) then becomes

$$I_{sw} = 51.0\ (1 - \cos\theta) \quad \ldots\ldots\ldots\ldots\ldots \quad (23)$$

Thus the polar stabilisation parameter (I_{sw}), also known as the polar work of adhesion, may be more easily derived.

From Eq. (15) for the work of adhesion, an expression for the adhesion tension ($\gamma_{sv} - \gamma_{sw}$), derived from Young's Eq. (1) using the captive air bubble technique can now be given:

$$(\gamma_{sv} - \gamma_{sw}) = 2(\gamma^d_{sv} . \gamma^d_{wv})^{\frac{1}{2}} + 2(\gamma^p_{sv} . \gamma^p_{wv})^{\frac{1}{2}} - \gamma_{wv} \quad \ldots\ldots \quad (24)$$

The above Eq. (24) may be simplified somewhat by the substitution of I_{sw} for the polar geometric mean expression, as previously seen in Eq. (21), to give

$$(\gamma_{sv} - \gamma_{sw}) = 2(\gamma^d_{sv} . \gamma^d_{wv}) + I_{sw} - \gamma_{wv} \quad \ldots\ldots\ldots \quad (25)$$

To derive the dispersive component (γ^d_{sv}) of the hydrogel, Eq. (25) may be transposed to give

$$\gamma^d_{sv} = \left[\frac{(\gamma_{sv} - \gamma_{sw}) - I_{sw} + \gamma_{wv}}{2(\gamma^d_{wv})^{\frac{1}{2}}} \right]^2 \quad \ldots\ldots\ldots \quad (26)$$

The polar component (γ^p_{sv}) of the hydrogel surface free energy (γ_{sv}) can now be simply derived by transposition of Eq. (21) to give

$$\gamma^p_{sv} = I^2_{sw}/(4\,\gamma^p_{wv}) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (27)$$

Computed values for ($\gamma_{sv} - \gamma_{sw}$), I_{sw}, γ^d_{sv}, γ^p_{sv} γ_{sv} and γ_{sw} are given in Appendix 4.

5.8 Results and Discussion

5.8.1 Surface/Interfacial free energy comparisons for the Hydrophilic/Hydrophobic Copolymer Systems

All contact angle data so derived from the captive air bubble and Hamilton techniques, together with the computed values for surface free energy components (γ_{sv}, γ^p_{sv}, γ^d_{sv}) and interfacial free energy (γ_{sw}), are given in tabular form in Appendix 4. Where necessary this data will be reproduced in the text, however, generally the discussion will be aided by reference to graphical representations of this said data.

It should also be noted that unless otherwise stated, the results and discussion will refer to those measurements derived in physiological 'Krebs' solution.

The contact angle data derived purely from the captive air bubble technique, by itself gives only an indication of the comparative wettability (hydrophilicity) of the various hydrogel surfaces. It is a measure of both the dispersive and the polar components of surface free energy, of these hydrogel copolymer systems. What it does not do however, is to give a value to these components or to differentiate between them.

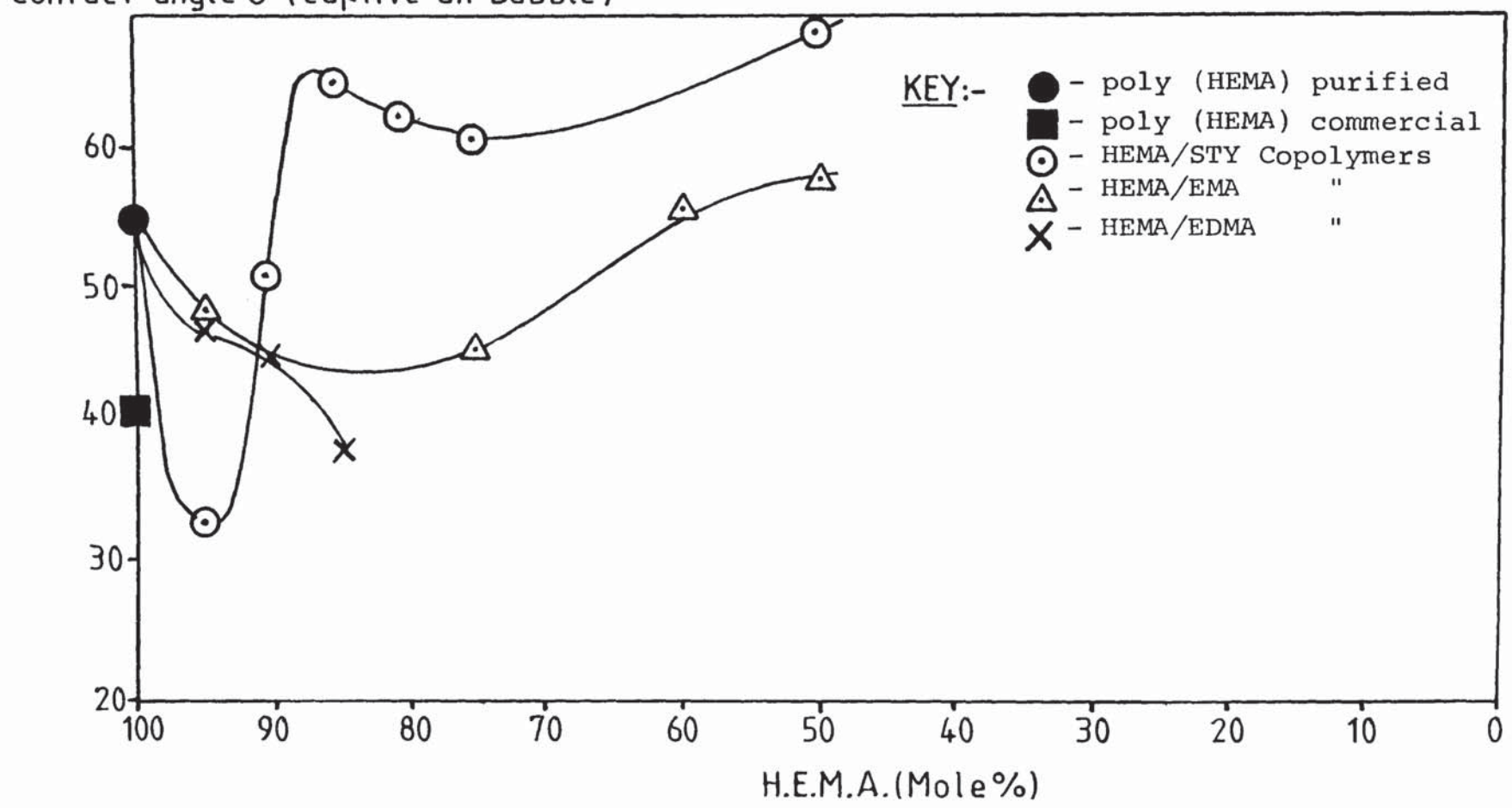
In order to study the effects of monomer structure and composition on the wettability of the 2-hydroxy ethyl methacrylate copolymer series, a plot of captive air bubble contact angles against monomer composition was made, as depicted in Graph 5.4.

As can be observed from the graph, additions of the various adjuvant monomers to 2-hydroxy ethyl methacrylate, leads to some very complex permutations of wettability. Considering the hydrophilic/hydrophobic compositions, it is perhaps not unreasonable to assume that additions of a hydrophobic type constituent to a hydrophilic one, would lead to a progressively diminishing wettability, with observable increasing contact angle. It can be seen however, that for the HEMA/STY copolymer compositions, initially the addition of a small % of the very hydrophobic styrene molecule, tends to sharply increase wettability, reaching a maximum at HEMA/STY 95:5 (Moles %). A further small increase in styrene content, ellicits an equally sharp decrease in wettability, up to the HEMA/STY 85:15 (Moles %) composition, where after a small rise in wettability, is followed by the expected trend of decreasing wettability with increasing hydrophobic content.

This pattern of behaviour is to some extent repeated in the Hamilton data (See Graph 5.5) and although for the HEMA/STY copolymers, the pattern does seem more complex, generally the trends are commensurate to those observed for wettability.

Surface/interfacial free energy data for these hydrophilic/hydrophobic compositions are reproduced in Table 5.2, from Appendix 4.

Graph. 5.4: Surface Wettability of the Hydrophilic/Hydrophobic Compositions (in Kreb's Solution)



Graph. 5.5: Surface Polarity of the Hydrophilic/Hydrophobic Compositions

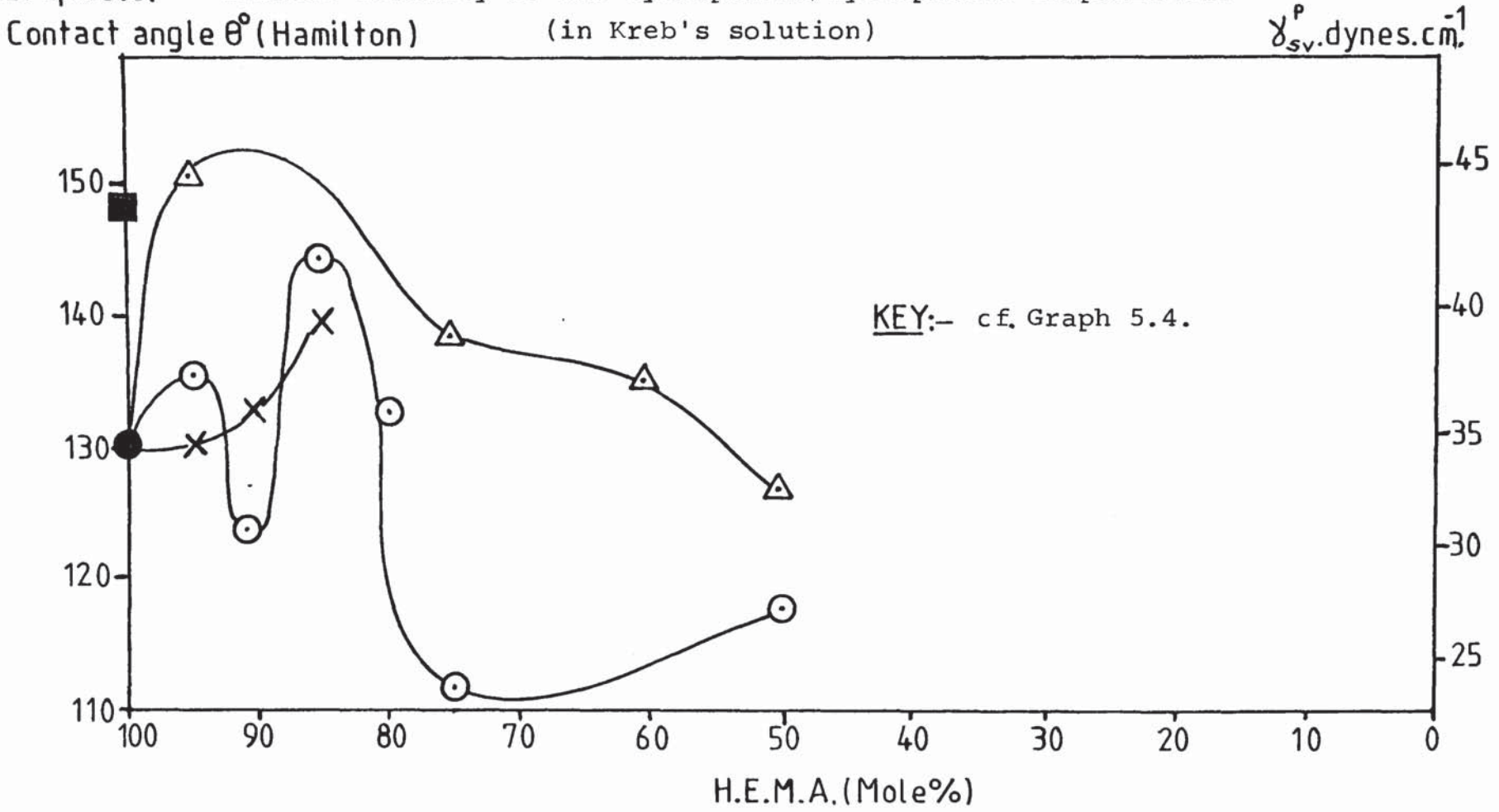


Table 5.2

SURFACE/INTERFACIAL-FREE ENERGY DATA FOR

THE HYDROPHILIC/HYDROPHOBIC COPOLYMERS

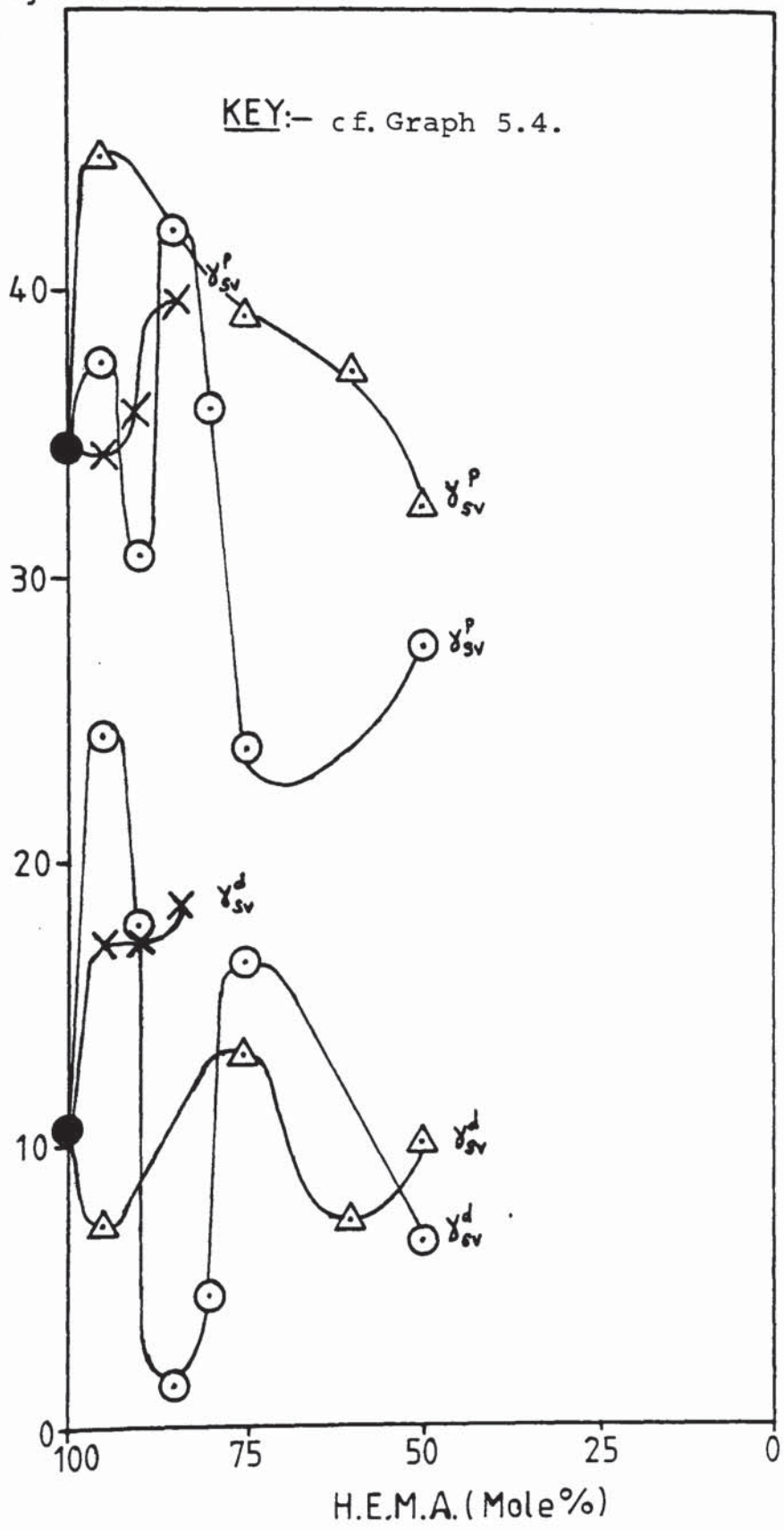
Hydrogel Composition	Monomer Ratio (Mole %)	EWC (%) 'Krebs'	γ_{sv}^{d} (dynes. cm.$^{-1}$)	γ_{Sv}^{P} (dynes. cm.$^{-1}$)	γ_{sv} (dynes. cm.$^{-1}$)	$\gamma_{s\omega}$ (dynes. cm.$^{-1}$)
HEMA	100:0	41.6	10.7	34.6	45.3	3.5
HEMA/STY	95:5	33.7	24.7	37.7	62.4	1.1
HEMA/STY	90:10	30.0	18.0	30.8	48.8	2.7
HEMA/STY	85:15	23.2	1.5	42.1	43.6	12.2
HEMA/STY	80:20	18.9	4.7	36.0	40.6	7.6
HEMA/STY	75:25	15.7	16.6	24.1	40.7	5.4
HEMA/STY	50:50	9.7	6.6	27.7	34.3	8.0
HEMA/EMA	95:5	35.9	7.2	44.9	52.2	4.1
HEMA/EMA	75:25	29.0	13.3	39.2	52.4	1.8
HEMA/EMA	60:40	17.9	7.4	37.4	44.8	4.8
HEMA/EMA	50:50	12.7	10.0	32.6	42.6	4.3
NVP/EMA	95:5	90.1	21.6	46.7	68.3	0.1
NVP/EMA	75:25	71.6	12.7	47.6	60.3	1.3

From this data it can be seen that the sharp increase in wettability observable with the HEMA/STY 95:5 (Moles %) is attributable to γ^d_{sv} = 24.7 dynes. $cm.^{-1}$ and γ^p_{sv}= 37.7 dynes. $cm.^{-1}$ giving a raised total surface free energy (γ_{sv}) of 62.4 dynes. $cm.^{-1}$. Similarly the subsequent drop in wettability, seen with HEMA/STY 85:15 (Moles %), is attributable to a very low dispersive component (γ^d_{sv}) of 1.5 dynes. $cm.^{-1}$ and a high polar component (γ^p_{sv}) of 42.1 dynes. $cm.^{-1}$, giving a total surface free energy of 42.1 dynes. $cm.^{-1}$. As would be expected it would seem that wettability is directly related to the surface free energy of the hydrogel (γ_{sv}), however it is perhaps somewhat anomalous that very high polar components (γ^p_{sv}) coupled to a very low dispersive component (γ^d_{sv}), do not raise the wettability.

When polar (γ^p_{sv}) and dispersive (γ^d_{sv}) components are plotted against the 2-hydroxy ethyl methacrylate copolymer compositions, as depicted in Graph 5.6, a large disparity between these components, does seem to correspond well with decreased wettability. Conversely, when the components γ^p_{sv} and γ^d_{sv} are tending towards each other, increasing wettability is generally observed for most copolymers.

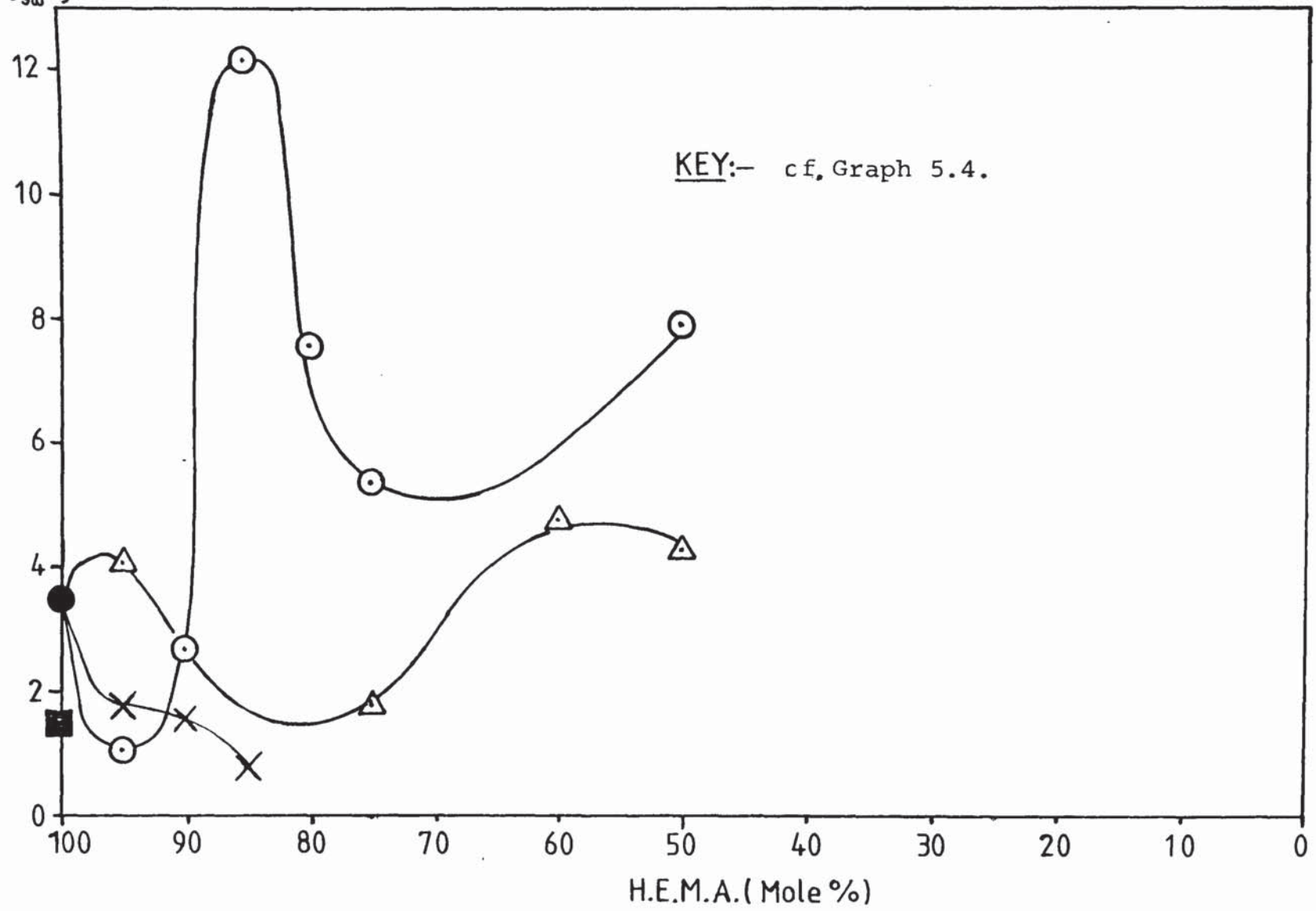
What is perhaps even more interesting is that where the above trends for the polar and dispersive components exist, corresponding maxima and minima for interfacial tension (free energy), are also observed (See Graph 5.7). This is not really very surprising when one considers that water wettability is a measure of the interfacial tension (γ_{sw}).

Graph. 5.6: Components of Surface Free Energy (γ^{p}_{sv}, γ^{d}_{sv}) dynes.cm.$^{-1}$ (Hydrophilic/Hydrophobic Compositions)



Graph.5.7: Interfacial Free Energy (γ_{sw})

γ_{sw} dynes.cm^{-1} (Hydrophilic/Hydrophobic Compositions in Kreb's solution)



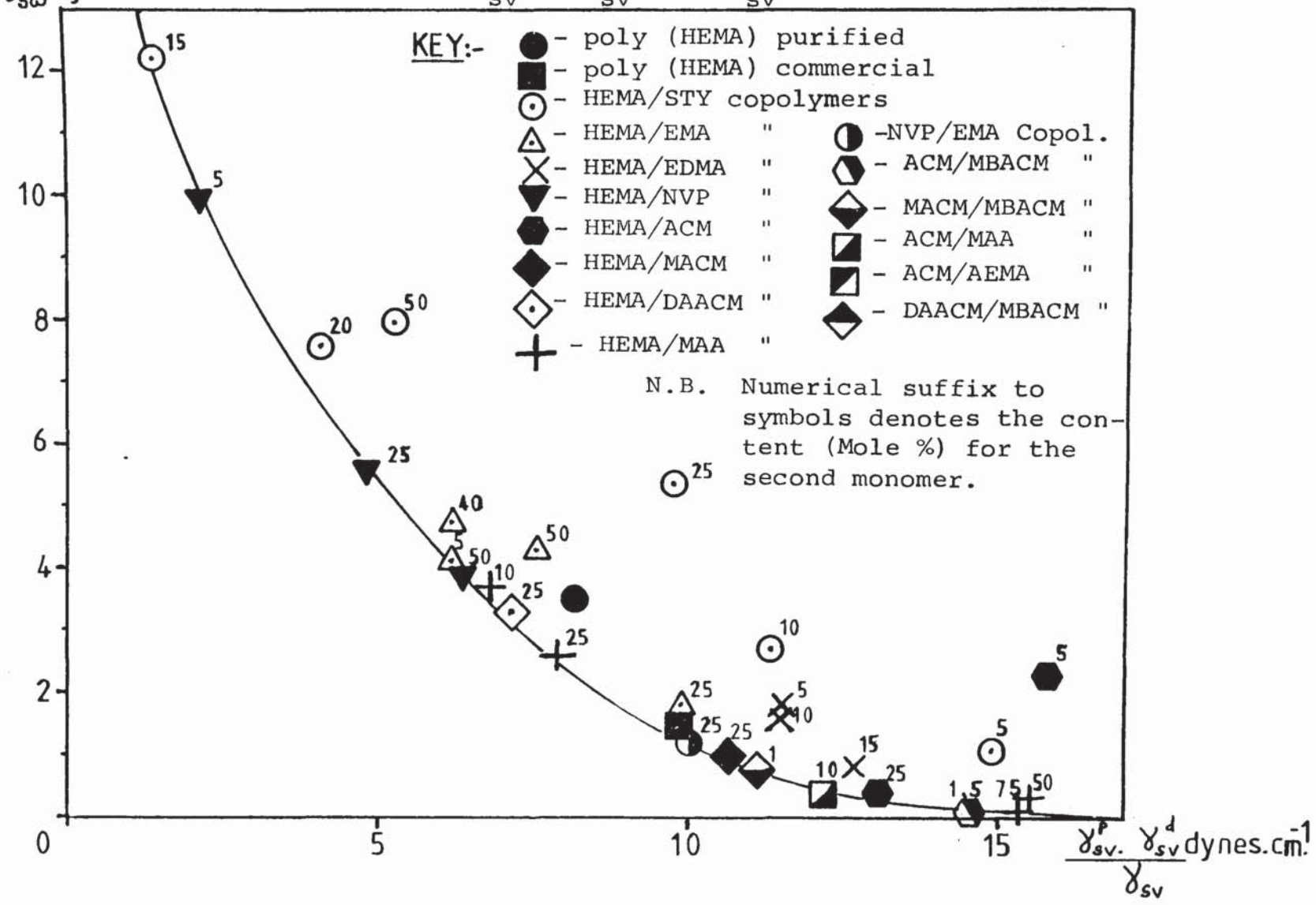
The relationship between surface free energy and interfacial tension (free energy) can be described as a composite function of the ratio between the products of the surface polar (γ^p_{sv}) and dispersive (γ^d_{sv}) free energy components and the total surface free energy (γ_{sv}), (i.e., γ^p_{sv}. γ^d_{sv}/ γ_{sv}). It can be seen from Graph 5.8, that although some scatter is observed for the HEMA/STY compositions, generally the hydrogels exhibited interfacial tensions in fairly close agreement with the above relationship.

The interfacial free energy changes observed in the 2-hydroxy ethyl methacrylate:styrene copolymers with increasing styrene content can be explained, based on the bulk constraints imposed on their surface chain segment reorientation.

With the HEMA/STY 95:5 (Moles %) composition, it would seem that the addition of the styrene has disrupted the bulk hydrophobic - Van der Waals and dipole-dipole interactions of poly (HEMA), and in so doing has reduced the bulk constraints to chain rotation thereby allowing a reduction in the interfacial free energy. With further increase in the styrene content of these copolymers a large rise in interfacial free energy is observed. It has previously been discussed in Chapter 4, that increases in the hydrophobic content of a hydrogel, will tend to raise the level of bulk hydrophobic - Van der Waals interactions.

Effectively therefore, all hydrophobic groups (i.e., methyl, methylene,phenyl) will be held within the bulk, whereas the polar, hydroxyl and carbonyl groups will be excluded where possible, thereby raising the polar and lowering the dispersive surface free energy components.

Graph.5.8: Interfacial Free Energy (γ_{sw}) Relationship to Surface Free Energy Components (γ^{p}_{sv}, γ^{d}_{sv} and γ_{sv})



This being so it is then understandable why a low surface dispersive component and high surface polar component is observed for the HEMA/STY 85:15 (Moles %) composition.

From the water-binding data given in Chapter 4 for the above HEMA/STY copolymers, it can be seen that for the HEMA/STY 95:5 (Moles %) composition, chain rotation to minimise interfacial free energy, will be possible, due to the plasticising effects of the free water. The water in the HEMA/STY 85:15 (Moles %) composition has been shown to be completely bound, therefore chain rotation will be severely limited and the above observed high interfacial tension due to bulk constraints, would seem to be confirmed.

A somewhat different trend is observed for the surface/interfacial free energies of the HEMA/EMA copolymers. With the HEMA/EMA 95:5 (Moles %) composition, the small content of ethyl methacrylate is seen to cause a large increase in the surface polar component (γ^p_{sv}) and a decrease in dispersive component (γ^d_{sv}). This pattern, when seen in the HEMA/STY copolymers, resulted in an increased interfacial tension (γ_{sw}), and as can be seen in Graph 5.7 a small increase in interfacial tension is observed for this composition. Probably because of the small size and polarity of the ethyl methacrylate side chain group, in comparison to styrene, and due to the similarities in size and structure to 2-hydroxy ethyl methacrylate, ethyl methacrylate does not disrupt the poly (HEMA) bulk interactions, to the same extent as the styrene molecule. Accordingly, increasing numbers of hydrophobic - Van der Waals interactions will occur in the

bulk, and as seen with the HEMA/STY compositions, raised polar and lowered dispersive surface free energy components result, (See Graph 5.6). With further increase in the ethyl methacrylate content a greater disruption of the poly (HEMA) bulk interactions would appear to be occurring, as evident from the lowered interfacial tension with the HEMA/EMA 75:25 (Moles %) composition. Further increase in the ethyl methacrylate again raises the interfacial tension as would be expected by a hydrophobic composition. It should be noted that simply by examination of the wetting characteristics and surface polarity given in Graphs 5.4 and 5.5 respectively, many but not all of the above trends may be deduced.

A number of research workers[18,207,208] have observed that HEMA/EMA copolymer compositions of approximately 75:25 (Moles %), or thereabouts, exhibited good thromboresistance and decreased cell adhesion in tissue culture, superior to both poly (HEMA) and poly (EMA). This perhaps may be directly attributable to the lowered interfacial tension (γ_{sw}).

Water binding data for the above HEMA/EMA copolymers, shows that they possess a high bound fraction; however, free water is still available to act as a plasticiser and allow chain rotation to minimise the interfacial free energy. From this it would seem that the surface chemistry of HEMA/EMA 75:25 (Moles %) is either less dependent on bulk interactions than its HEMA/STY counterpart, or that it inherently possesses the correct polar and dispersive surface free energy components, to minimise inter-

facial tension without chain rotation. Some degree of overlap between these alternatives, would be expected.

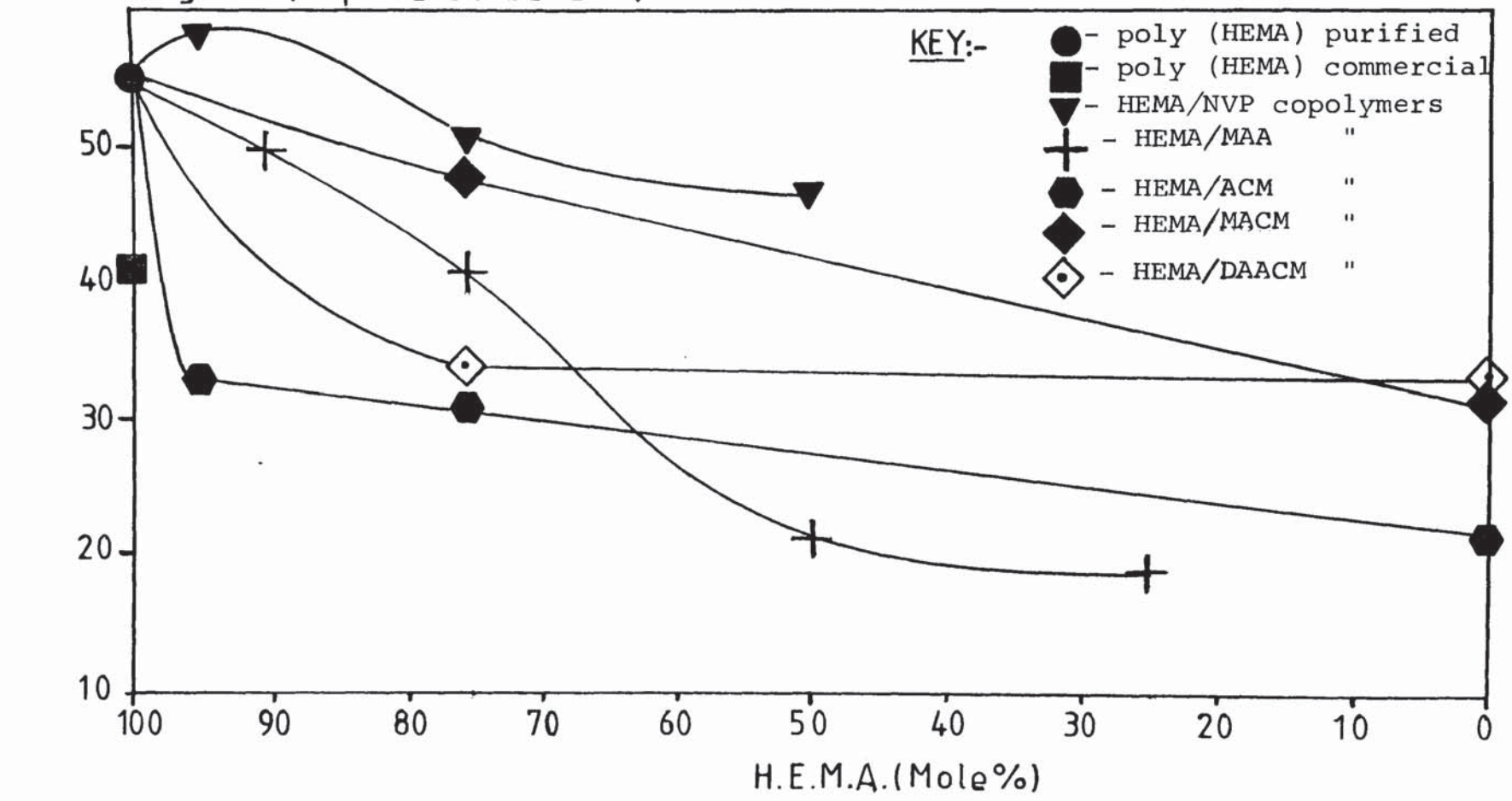
With the NVP/EMA copolymers, substitution of the hydrophilic 2-hydroxy ethyl methacrylate by the very hydrophilic N-vinyl pyrrolidone can be observed to markedly elevate the total surface free energy (γ_{sv}) of the hydrogel composition, and not unexpectedly lower the interfacial tension (γ_{sw}). (See Table 5.2). Direct comparisons of the effect of ethyl methacrylate on poly (NVP) as was undertaken for the poly (HEMA), is not possible because poly (NVP) cannot be synthesised at a controlled cross-link density.

Increasing the ethyl methacrylate content from the NVP/EMA 95:5 (Moles %) to the NVP/EMA 75:25 (Moles %) composition can be observed to markedly reduce the dispersive (i.e., hydrophobic) surface free energy component (γ^d_{sv}) with very little change in the polar surface free energy component (γ^p_{sv}). This may be attributed to the increased formation of hydrophobic - Van der Waals interactions between the pyrrolidone ring and the ethyl methacrylate, (methyl and ethyl groups), within the bulk, thereby reducing the surface dispersive groups. The high equilibrium water content ($\sim$71%) exhibited by the NVP/EMA 75:25 (Moles %), composition is somewhat anomalous to its high mechanical strength and low plasticity; this would therefore seem to be indicative of a very high degree of bulk polymer-polymer and polymer-water interactions, which would to some extent confirm the surface data analysis.

5.8.2 Hydrophilic/Hydrophilic Copolymer Systems

From Graphs 5.9 & 5.10, together with the data in Table 5.3, it can be observed that the addition of the hydrophilic monomer, acrylamide, to the poly (HEMA) structure, results in a predictable increase in wettability, but no significant change in surface polarity, for the HEMA/ACM 95:5 (Moles %) composition. The increased surface free energy (γ_{sv} = 63.4 dynes. cm.$^{-1}$), for this composition is due to a very high dispersive component (γ^{d}_{sv} = 29.4 dynes. cm.$^{-1}$), which can itself be attributed to the acrylamide molecule having a structure-breaking effect on the hydrophobic - Van der Waals interactions within the poly (HEMA) bulk, together with a structure-enhancing effect on bulk interchain dipole-dipole interactions. This will effectively exclude from the bulk, those α-methyl groups of 2-hydroxy ethyl methacrylate, residing near to the gel-surface and thereby raise the dispersive surface free energy component (γ^{d}_{sv}). Increasing the acrylamide content to the HEMA/ACM 75:25 (Moles %) composition, can be seen to raise surface polarity and lower surface dispersion forces, which can be seen as an isotropic increase in polar-groups and a corresponding isotropic diminution in hydrophobic groups, in both the bulk and surface, which is an expected trend. In accordance with these trends interfacial tension ($\gamma_{s\omega}$) is seen to be progressively reduced.

Graph. 5.9: Surface Wettability of the Hydrophilic/Hydrophilic Compositions (in Kreb's solution)



Graph. 5.10: Surface Polarity of the Hydrophilic/Hydrophilic Compositions

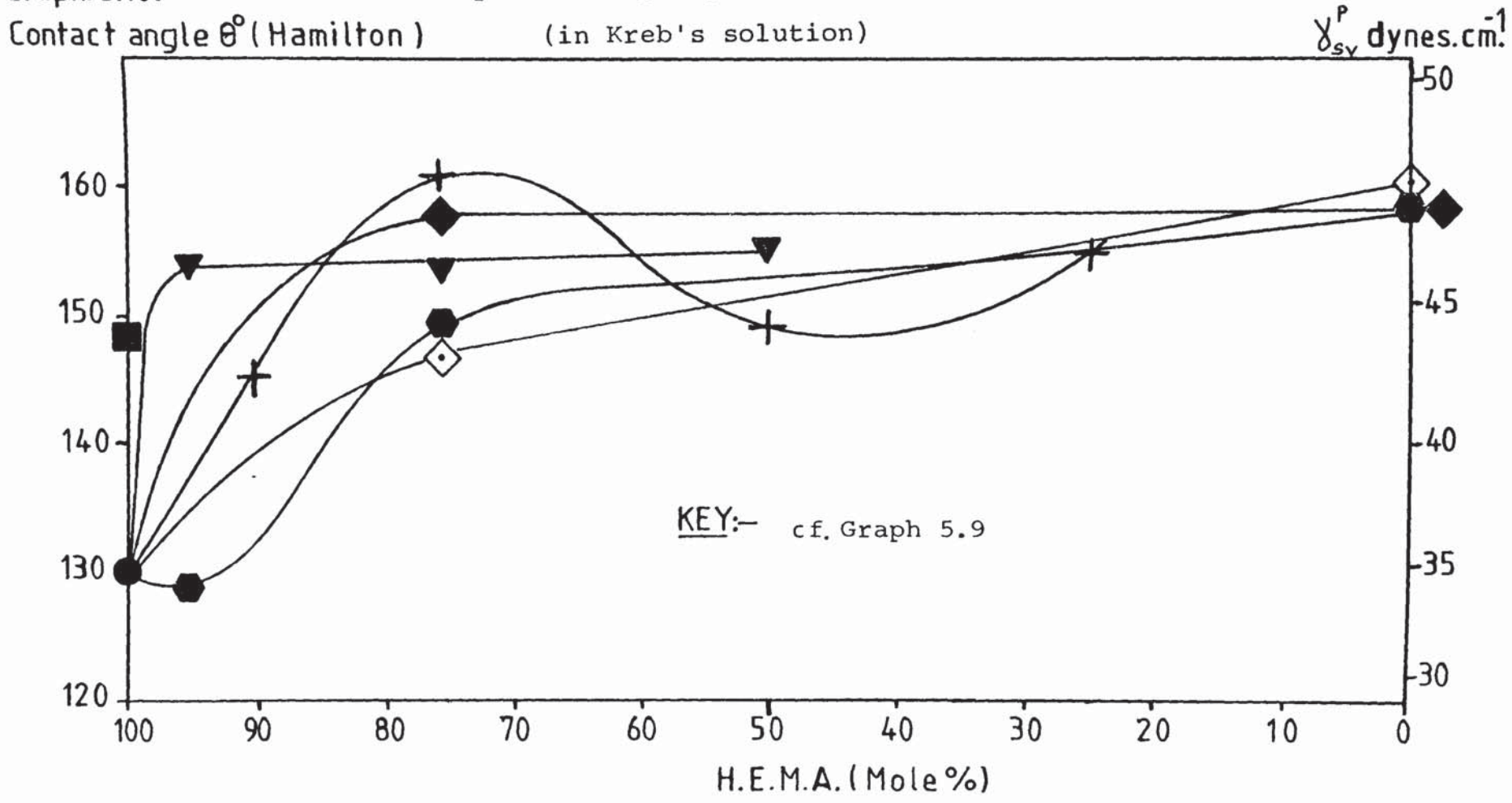


Table 5.3

SURFACE/INTERFACIAL-FREE ENERGY DATA FOR

THE HYDROPHILIC/HYDROPHILIC COPOLYMERS

Hydrogel Composition	Monomer Ratio (Mole %)	EWC (%) 'Krebs'	γ^{d}_{sv} (dynes. cm.$^{-1}$)	γ^{p}_{sv} (dynes. cm.$^{-1}$)	γ_{sv} (dynes. cm.$^{-1}$)	γ_{sw} (dynes. cm.$^{-1}$)
HEMA	100:0	41.6	10.7	34.6	45.3	3.5
HEMA/ACM	95:5	42.4	29.4	34.0	63.4	2.3
HEMA/ACM	75:25	49.7	18.5	44.4	62.9	0.4
HEMA/MACM	75:25	40.9	13.9	47.4	61.3	1.0
HEMA/DAACM	75:25	35.4	8.7	43.1	51.8	3.3
ACM/MBACM	99:1	93.3	20.4	47.8	68.1	0.1
MACM/MBACM	99:1	69.5	14.5	47.5	62.1	0.8
DAACM/MBACM	99:1	35.1	15.0	48.1	63.1	0.7
HEMA/NVP	95:5	46.0	2.3	45.9	48.2	10.0
HEMA/NVP	75:25	52.0	5.4	45.8	51.2	5.6
HEMA/NVP	50:50	64.2	7.4	46.5	53.9	3.9
HEMA/MAA	90:10	74.5	8.1	42.4	50.5	3.7
HEMA/MAA	75:25	86.3	9.4	48.3	57.8	2.6
HEMA/MAA	50:50	86.9	23.9	44.2	68.1	0.3
HEMA/MAA	25:75	80.6	22.8	46.3	69.1	0.1

Substitution of acrylamide by methacrylamide into the HEMA/ACM 75:25 (Moles %) composition, can be seen to reduce the total surface free energy (γ_{sv}), as would be expected. This it achieves by increasing the surface polar component and decreasing the surface dispersive component; this would not be expected based on the known lower hydrophilicity of methacrylamide compared to acrylamide. However, it is reasonable to assume that the α-methyl group of the substituted methacrylamide, will be capable of hydrophobic - Van der Waals interactions with the α-methyl groups of 2-hydroxy ethyl methacrylate, thereby reducing the surface methyl groups and increasing surface hydroxyl and amide groups accordingly.

Similarly with substitution of acrylamide by diacetone acrylamide to give a HEMA/DAACM 75:25 (Moles %) composition, the observed fall in both the polar and dispersive surface free energy components can be directly attributed to the structure and positioning of its 1,1, dimethyl 3-oxy-butyl substituent amide groups. In the above HEMA/MACM 75:25 (Moles %) composition it was proposed that when the methacrylamide molecules take part in hydrophobic bulk interaction, the side chain amide groups are then free to orientate towards the gel-aqueous interface, thereby increasing surface polarity. This however, is not the case for diacetone acrylamide, because when its large hydrophobic 1,1 dimethyl 3-oxy-butyl group undergoes similar hydrophobic - Van der Waals interaction, it very effectively

shields and fixes the polar amide group within the bulk, thereby giving the above stated surface effects.

Surface free energy data on the solution polymerised acrylamide derivatives are included in Table 5.3, and these would tend to confirm the surface effects observed with structural modifications to the acrylamide molecule, in the HEMA/ACM copolymers.

The degree of bulk interactions as determined from water-binding data (See Chapter 4), would seem to correlate very well with the computed interfacial tension data (γ_{sw}) for the acrylamide derivatives in both the bulk and solution polymerised copolymer preparations, in that the lower the bound water content (i.e., reduced bulk interactions) the lower the interfacial free energy, (See Tables 4.4 and 5.3).

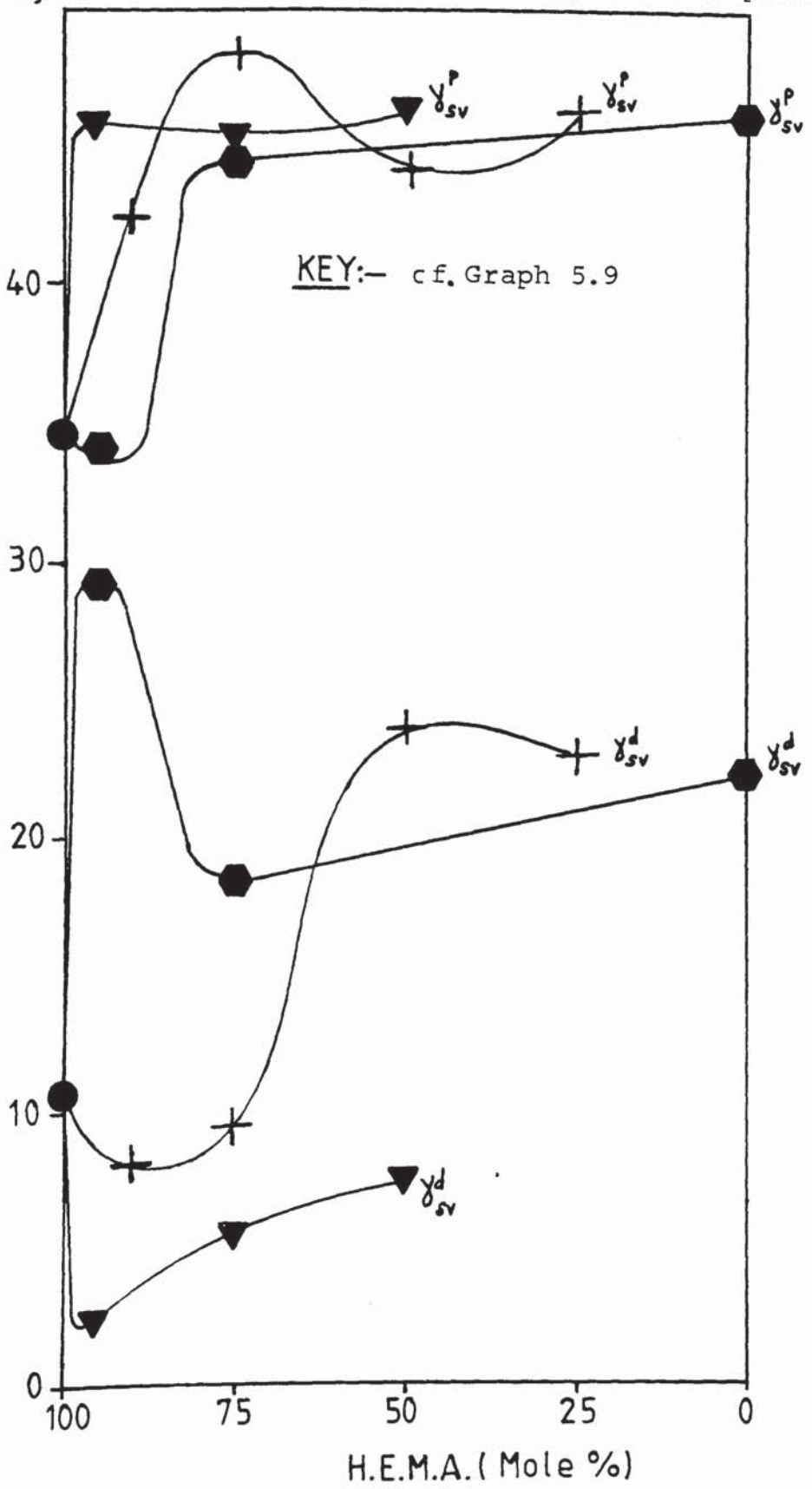
This would appear to further re-emphasise the importance of bulk interactions on the surface chemistry of hydrogels, and to make one more aware of the direct relationship that exists between them.

To further investigate the effects of monomer structure on surface properties and bulk-surface interactions, the hydrophilic monomer N-vinyl pyrrolidone was substituted for acrylamide in the 2-hydroxy ethyl methacrylate copolymers. Although N-vinyl pyrrolidone is of similar hydrophilicity (polarity) to acrylamide, it would not appear to exhibit similar surface effects when incorporated into poly (HEMA) copolymers. The surface wettability and polarity measurements for the HEMA/NVP copolymers exhibit very different trends to those obtained for their HEMA/ACM counterparts. With the HEMA/NVP 95:5 (Moles %) it can be seen that a small

N-vinyl pyrrolidone content, lowers the wettability and dramatically increases the surface polarity (See Graphs 5.9 and 5.10). From the data given in Table 5.3 and Graph 5.11, it can be observed that the above, produces a large disparity between the surface polar and dispersive components, which seem to generate a large interfacial tension ($\gamma_{s\omega}$). This effect was earlier observed with the HEMA/STY and HEMA/EMA copolymers. With reference to Graph 5.11, it can be observed, that the change in the surface free energy components, produced by the addition of hydrophilic N-vinyl pyrrolidone, to give the HEMA/NVP 95:5 (Moles %) composition, gave rise to very similar surface effects, as exhibited by its hydrophobic ethyl methacrylate, HEMA/EMA 95:5 (Moles %) copolymer counterpart. Similarly the surface effects observed for the HEMA/NVP 95:5 (Moles %) composition, can be attributed to enhanced hydrophobic - Van der Waals interactions within the bulk, as previously proposed for the HEMA/EMA 95:5 (Moles %), surface effects.

With increasing N-vinyl pyrrolidone content these hydrophobic bulk-interactions would appear to be reduced, as indicated by the rise in the surface dispersive component, and as a direct consequence of the decrease in the number of α-methyl groups available for interaction. From the previous hydration data in Chapter 4, the above same composition was shown to exhibit a larger than expected interaction with water, (indicative of a high bound water content), and because of these bulk interactions, constraints would be imposed, which would tend to limit the ability of the surface chain segments to

Graph.5.11: Components of Surface Free Energy (γ^{p}_{sv}, γ^{d}_{sv})



orientate to minimise the interfacial tension. This would appear to be confirmed by the large interfacial tension ($\gamma_{s\omega}$ = 10.0 dynes. cm.$^{-1}$), exhibited by this composition, as illustrated in Graph 5.12.

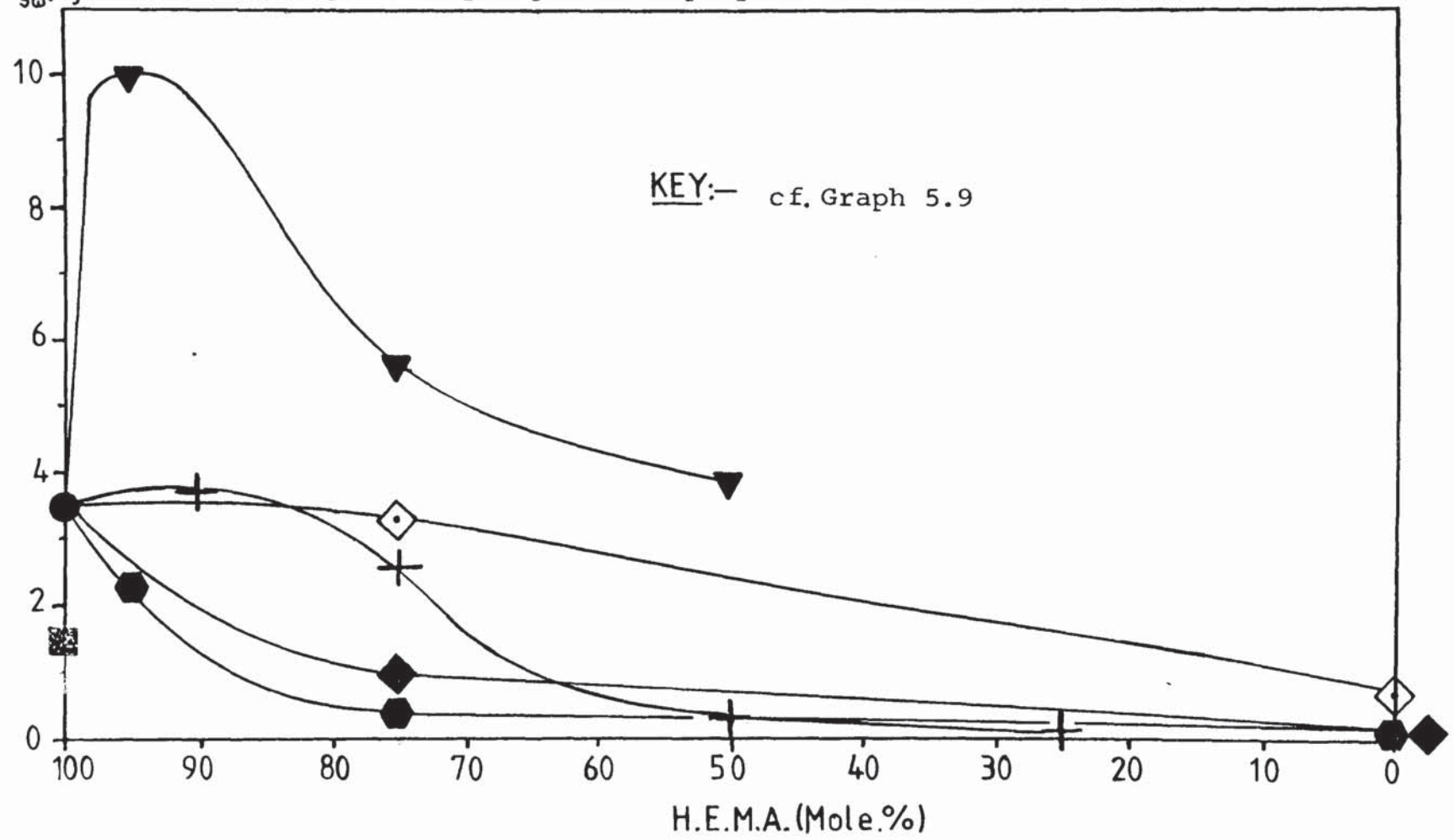
The last hydrophilic/hydrophilic copolymer compositions to be considered in this section are the HEMA/MAA copolymers. With increasing methacrylic acid content, progressive increases in surface wettability and surface polarity can be observed, which can be correlated with the increasing bulk hydrophilicity observed in the previous hydration studies (Chapter 4), and as a consequence of the formation of the methacrylic-sodium carboxylate groups. Although these HEMA/MAA copolymers do exhibit greater bulk hydrophilicity than comparable HEMA/ACM copolymers, the initial increase in surface wettability is less pronounced. This would seem to indicate that the sodium-carboxylate groups have a much greater effect within the bulk, rather than at the surface.

With regards to Graph 5.11, it can be observed that the surface free energy components for these compositions initially exhibit very similar trends to those of the HEMA/NVP and HEMA/EMA copolymers. It is probable that similar but less pronounced hydrophobic bulk interactions occur between the α-methyl groups of 2-hydroxy ethyl methacrylate and methacrylic acid.

Bulk interactions of this type, together with the increased bound water interactions, would account for the very small increase in interfacial energy

Graph. 5.12: Interfacial Free Energy (γ_{sw})

γ_{sw}.dynes.cm^{-1} (Hydrophilic/Hydrophilic Copolymers in Kreb's solution)



observed for HEMA/MAA 90:10 (Moles %). A 780% increase in the freezing (free) water content is observed for this methacrylic acid content (See Table 4.5), due to the hydrophilic sodium carboxylate group, but even so, the plasticising effects of this free water, cannot overcome the bulk interactions, which effectively raise the interfacial tension. Only when the methacrylic acid content is raised to HEMA/MAA 50:50 (Moles %), is a substantial drop in interfacial tension observed, (Graph 5.12), presumably due to further increases in free water content and a net reduction in bulk interactions.

5.8.3 Surface Chemistry Effects of the Divalent Ethylene Glycol Dimethacrylate and N,N'-Methylene-bis-Acrylamide Cross-linking agents

It would be expected that an increase in the cross-linking agent content of a gel would effectively limit the ability of the surface chain segments to orientate to minimise interfacial tension, in much the same way as other types of bulk interactions. Therefore with increasing content one would expect a rise in the interfacial tension.

From Table 5.4 it can be observed that this would seem to be true for the ACM/MBACM compositions, but not for the HEMA/EDMA copolymers. Previously in the hydration studies of Chapter 4, both cross-linking agents were shown to be of comparable efficiency in lowering equilibrium water content, and that with increasing content, so this efficiency was observed to decline. This decline was attributed to imperfections

in the cross-link density, and to some extent, these bulk defects can explain some of the observed surface effects, especially concerning the HEMA/EDMA copolymers.

The surface data for the N,N' Methylene-bis-acrylamide copolymers would appear to follow a predictable pattern. Total surface free energy (γ_{SV}) is seen to decrease, due to a net reduction in both the polar and dispersive surface free energy components. Previously it was shown that as the N,N' Methylene-bis-acrylamide content increases so the equilibrium water content decreases, as a direct result of steric hindrance to the freezing (free) water. Over the same range of ACM/MBACM copolymers the bound water content was observed to increase, and such structuring would be expected to perhaps raise the interfacial tension. This can be seen to be true, but the increase is only very small and not comparable to the increase in the bound water content. It would therefore seem likely that, either the large free water content, still present in the ACM/MBACM 90:10 (Moles %) composition, can still help plasticise the polymer chains, or that with the increase in cross-link density imperfections, competition exists between its effects and those of the bound water and effectively a net reduction in the bulk constraints on chain segment orientation, results.

(See overleaf for Table 5.4)

Table 5.4

THE INFLUENCE OF CROSS-LINKING AGENTS

ON SURFACE/INTERFACIAL-FREE ENERGY

Hydrogel Composition	Monomer Ratio (Mole %)	EWC (%) 'Krebs'	γ^d_{sv} (dynes. cm.$^{-1}$)	γ^p_{sv} (dynes. cm.$^{-1}$)	γ_{sv} (dynes. cm.$^{-1}$)	γ_{sw} (dynes. cm.$^{-1}$)
ACM/MBACM	99:1	93.3	20.4	47.8	68.2	0.1
ACM/MBACM	95:5	82.3	21.5	46.1	67.5	0.1
ACM/MBACM	90:10	75.7	18.0	44.1	62.1	0.4
HEMA/EDMA	99:1	41.6	10.7	34.6	45.3	3.5
HEMA/EDMA	95:5	31.1	17.1	34.6	51.7	1.8
HEMA/EDMA	90:10	26.2	16.8	36.1	52.9	1.6
HEMA/EDMA	85:15	22.0	18.6	39.8	58.4	0.8

An examination of the surface data in Table 5.4, indicates quite clearly that the Ethylene Glycol Dimethacrylate cross-linked-copolymers, exhibit quite different surface characteristics, to those observed with the N,N'-Methylene-bis-acrylamide copolymers. The trends would appear to be completely anomalous with the predicted actions of a cross-linking agent. Surface wettability and surface polarity are shown to increase in Graphs 5.4 and 5.5 respectively, which if based on the hydrophobic structure of ethylene glycol dimethacrylate, would have been expected to decrease. However we can see that from Table 5.4 and as illustrated in Graph 5.6, both the dispersive as well as the polar surface free energy component, show marked increase, giving rise to a progressive increase in the total surface free energy (γ_{sv}), a complete reversal of the trends set by NN' Methylene-bis-acrylamide.

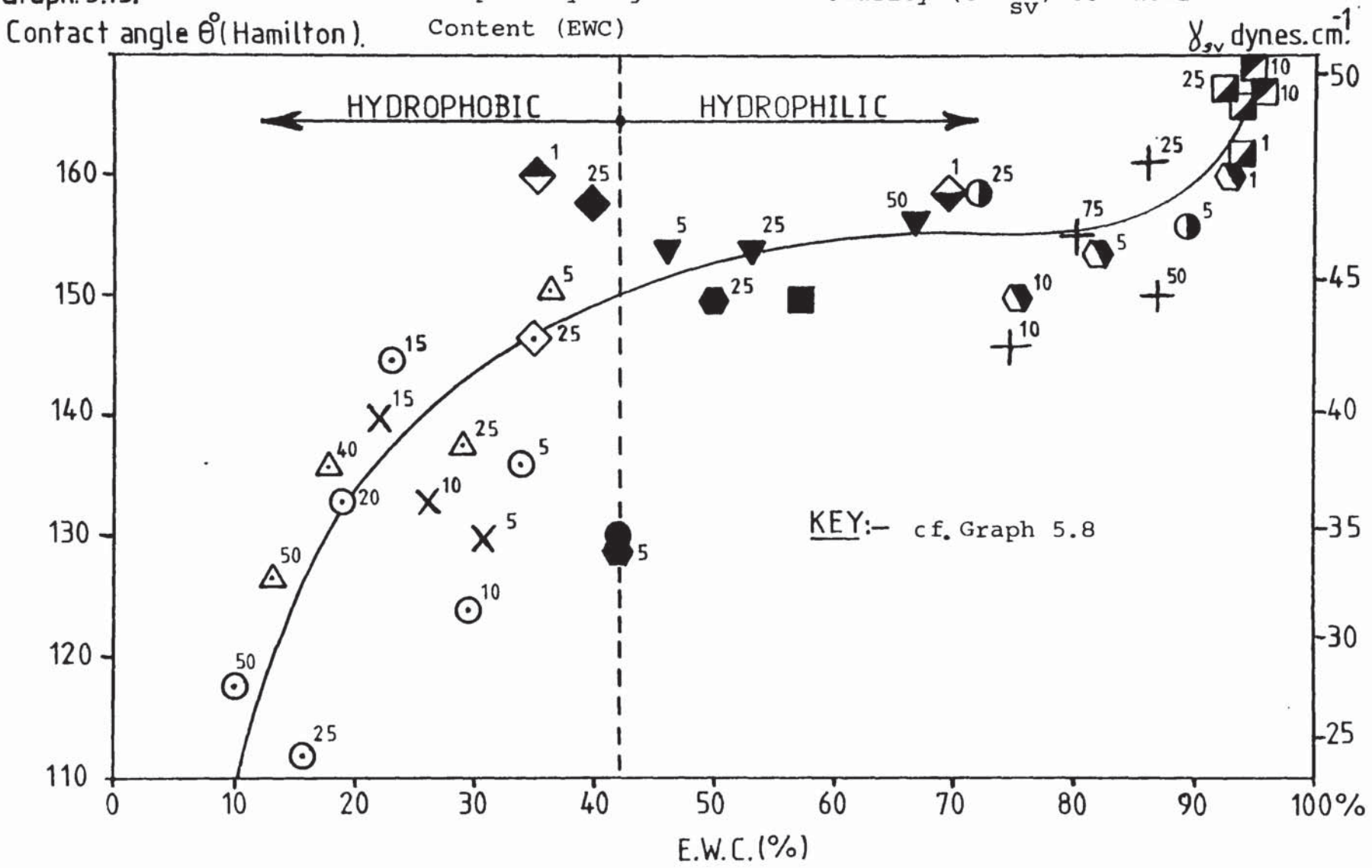
Further to this, it can be observed in Graph 5.7 that with increasing ethylene glycol dimethacrylate content in the HEMA/EDMA copolymers, a substantial reduction in interfacial tension occurs, which exceeds the reduction exhibited by the HEMA/NCM copolymers, for the same range. A continuation of this trend, would be expected to lead to a hydrogel which would exhibit a very low interfacial tension ($\sim$ 0.1 dynes. $cm.^{-1}$), even with low equilibrium water content ($\sim$ 20%). Imperfections in the gel-network of the above copolymers were previously cited in Chapter 4, as the reason for increased 'pore' size, observed with increasing cross-

linking agent concentration. These imperfections were attributed to cross-link density anomalies, which gave rise to non-isotropic distribution of cross-links, together with possible intramolecular (cyclisation) cross-links formation. The effects of this on 'pore' size was illustrated schematically in Fig. 4.13. The non-isotropic cross-link distribution may be attributable to increasing ethylene glycol dimethacrylate bulk interactions during the polymerisation, forming non-random block polymerisation, which increase with increasing concentration. Such effects, understandably would lead to an increased chain length and accordingly greater freedom of mobility, which would allow the surface chain segments to orientate to minimise interfacial tension, as is observed from the surface data for the HEMA/EDMA copolymers.

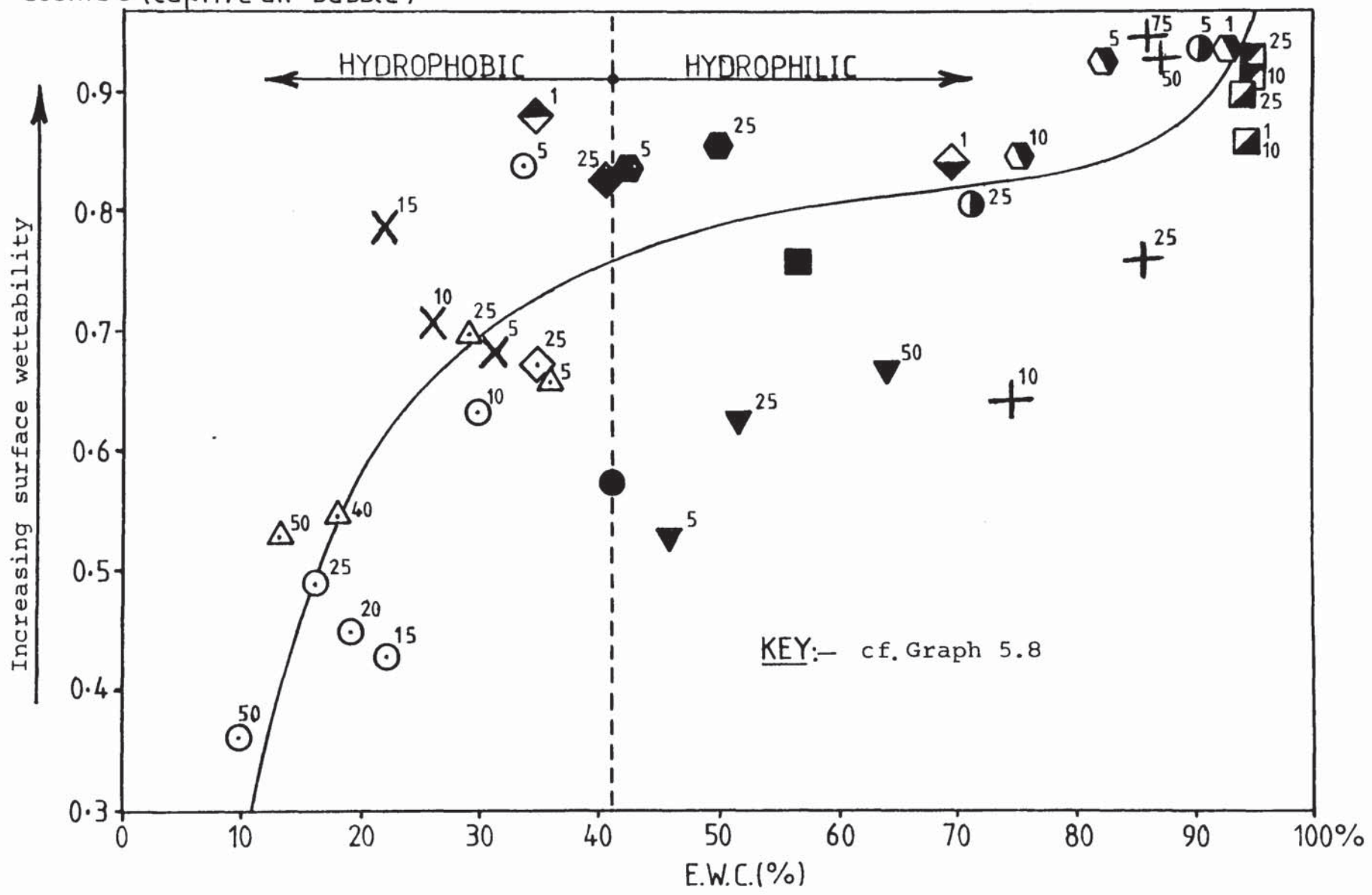
5.8.4 Some General Considerations of Surface-Chemistry and Bulk-Water Content Relationships

A plot of both Hamilton contact angles (θ) and the cosine of the captive air contact angles (θ') against equilibrium water content, as depicted in Graphs 5.13 and 5.14 respectively, indicates a general relationship between the measured surface and bulk properties of the polymers under study. Although scatter is apparent in each, there are definite gross surface property trends apparent across the spectrum of copolymer compositions, with increasing equilibrium water content. As one would expect both surface wettability and surface polarity increase with increasing bulk water content.

Graph. 5.13: The Relationship of Hydrogel Surface Polarity (γ^{p}_{sv}) to Water Content (EWC)



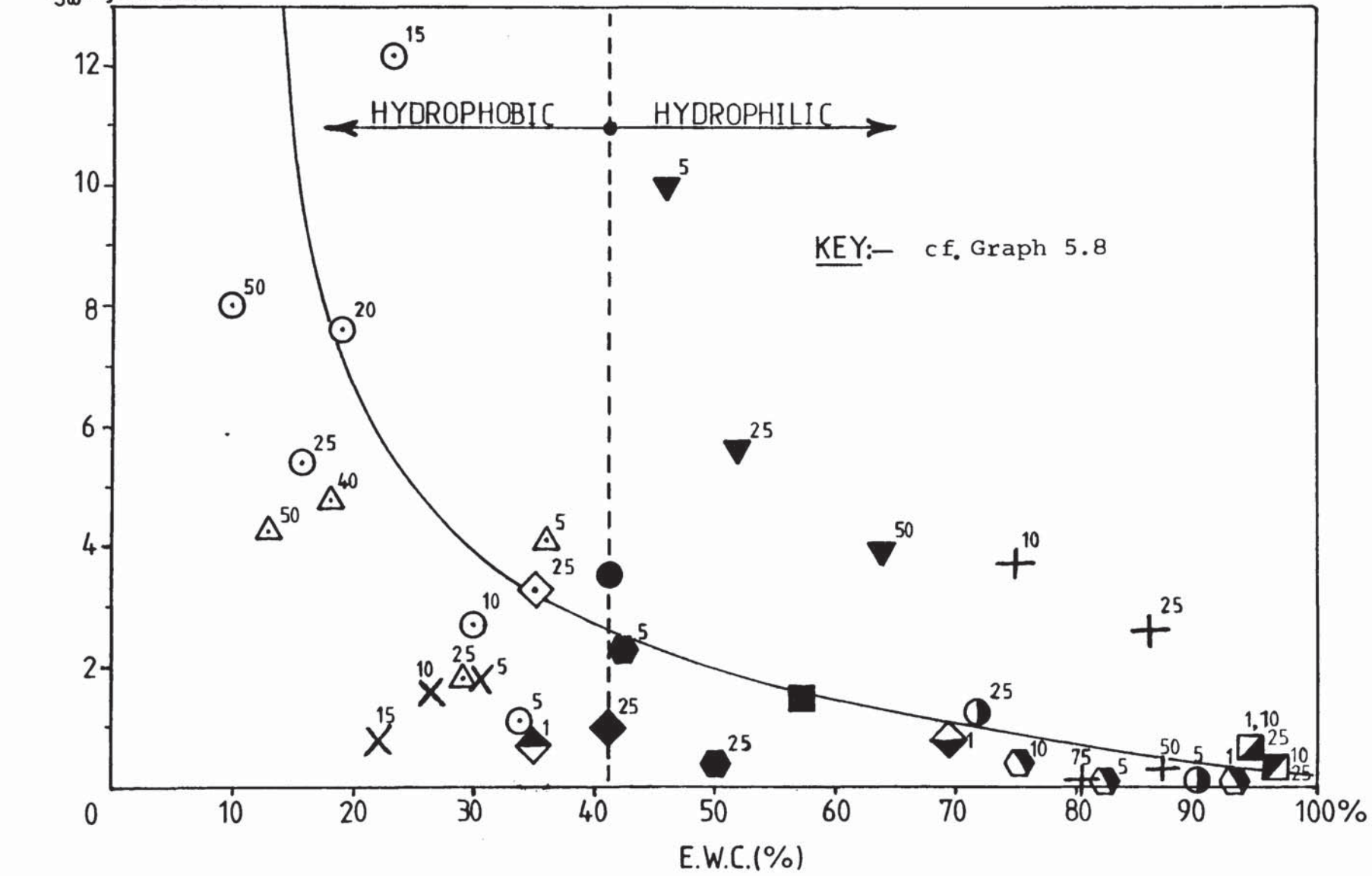
Graph. 5.14: The Relationship of Hydrogel Surface Wettability to Water Content (EWC)



It was generally observed in the previous chapter that the higher the equilibrium water content, the more freezing (free) to non-freezing (bound) water is present in the bulk. Therefore with an increase in the free to bound water content, a presumable increase in chain segment mobility would occur due to both the plasticising action of the free water and the reduction in bulk constraints due to the reduction in the bound water. Thus high water content gels as a direct consequence of their high degree of chain mobility, would be expected to exhibit low interfacial tensions, and as can be observed from Graph 5.15, this generally holds true. What is perhaps somewhat surprising is the ability of some of the low water content gels to also reduce their interfacial tension.

Previously it was noted that when the polar and dispersive surface free energy components, tended towards each other, the interfacial tension was observed to decrease, and accordingly when these components exhibited increased disparity the interfacial tension was raised. These gross observations do not give a clear indication of what is actually occurring at the hydrogel interface. If we consider the hypothetical case of a surface chain segment, with no bulk constraining forces acting on it, it will be absolutely free to orientate to minimise the interfacial free energy (tension), and in order to do this it must present the optimum ratio of polar to dispersive components towards the aqueous exterior. No better polar

Graph. 5.15: The Relationship of Hydrogel Interfacial Free Energy (γ_{sw}) to Water-Content (EWC)



to dispersive ratio exists than those exhibited by the surface free energy components of water (γ^d_{wv} = 21.8 dynes. $cm.^{-1}$ and γ^p_{wv} = 51.0 dynes. $cm.^{-1}$), and if we reconsider Graphs 5.6 & 5.7 and 5.11 & 5.12, it can be seen that when these values are approached, so the interfacial tension is reduced. It is therefore understandable that as the hydrogel water content increases, so it approximates more to the above polar/dispersive criteria, and we see measured decreases in surface wettability and polarity. However, we have noted that the low water content gels also exhibit lower than expected interfacial tensions, even with low free water contents. Therefore it would seem possible that the above optimum surface free energy components are attainable based purely on the polar/dispersive structural chemistry of the hydrogels.

Only the bulk constraints of polymer-polymer and polymer-water interactions will limit this ability, and it would appear that this factor is of prime importance throughout the complete range of equilibrium water contents, in determining the interfacial tension of the gels. Thus although hydrogels possess a hypothetical ability to attain the optimum surface free energy components, it is their chemical structure which determines the bulk interactions and ultimately their resultant interfacial tension.

5.9 Conclusions

The determination of the polar and dispersive surface free energy components, by the Hamilton and

captive air bubble techniques, can be directly related to the hydrogel monomer structure and composition ration. It was also found that the ratio of bound to free water in the hydrogel bulk, can influence the hydrogel interfacial free energy. Bound water tends to increase the interfacial tension by restricting the mobility of surface chain segments, whereas free water will tend to decrease the interfacial tension by way of its plasticising effects. Thus the relationships between surface/interfacial effects and bulk interactions can be seen as a composite function of polymer-polymer and polymer-water interactions, both of which are determined by the hydrogel monomer structure and composition ratio. A general gross correlation was observed between surface free energy (γ_{sv}) and interfacial free energy (γ_{sw}), however a more precise relationship is exhibited by the expression:

$$\gamma_{sw} \propto (\gamma^{p}_{sv} \cdot \gamma^{d}_{sv}) / \gamma_{sv}.$$

Generally it was observed that high water content hydrogels exhibited both high surface polarity and high surface wettability, together with a low interfacial tension, as would be expected. However, perhaps somewhat surprisingly, certain low water content hydrogel compositions, also exhibited the ability to reduce their interfacial tensions. This was found to be particularly true for the copolymers of 2-hydroxy ethyl methacrylate and ethylene glycol dimethacrylate, and which was attributed to the increasing non-isotropic distribution of cross-links, with increasing ethylene glycol dimethacrylate concentration.

CHAPTER 6

PROTEIN ADSORPTION STUDIES:

Hydrogel Structure-Property Effects and Their Relationship to the Interfacial Phenomenon of Protein Adsorption

6.1 Introduction

The biocompatibility of materials is a pre-requisite for their biomedical applications and this is especially true when considering materials for blood-contact applications such as haemodialysis and haemoperfusion. To date no invitro techniques including classical surface chemistry measurements have fully characterised the biocompatibility of a material. Invivo or exvivo animal techniques are perhaps the next best experimental model to actual clinical testing of materials however, both from a practical point of view and also perhaps on humanitarian grounds, large scale analysis of materials by these techniques are not feasible.

From many early invivo and invitro experiments there emerged the somewhat empirical relationship that fibrinogen adsorption was indicative of low biocompatibility (high thrombogenicity), whereas preferential albumin adsorption seemed to indicate a high biocompatibility (low thrombogenicity), (See Chapter 1): as previously outlined in Chapter 1 the surface activated blood coagulation processes are believed to be mediated by adsorbed proteins, such as fibrinogen, which because of their carbohydrate moieties, enables platelet adhesion and subsequent thrombus formation to occur.

A somewhat novel approach was envisaged to characterise hydrogel surface properties based on the adsorption of

proteins of known physico-chemistry. It was hoped that this technique together with other surface-chemistry measurements would give a viable and reproduceable index of surface properties relating to the structure-property effects of the constituent monomers. By inference from the above biocompatibility relationships between fibrinogen and albumin, it was believed that their respective adsorption levels would also give some indications as to the potential future use of these materials in biomedical applications.

It will therefore be the intention of this Chapter to attempt to relate human fibrinogen and human serum albumin adsorption levels both to their respective individual physico-chemical properties and the bulk and surface structure-property effects as discussed previously in Chapters 4 and 5.

6.2 General Principles of Adsorption

The unbalanced attractive forces which exist at solid surfaces give rise to their characteristic property of adsorption. When a gas or vapour is brought into contact with a clean solid surface some of it will become attached to the surface in the form of an adsorbed layer(s). The solid is generally referred to as the adsorbent, and the gas or vapour as the adsorbate. It is possible that uniform absorption into the bulk of the solid might also take place, and, since adsorption and absorption cannot always be distinguished experimentally, the generic term sorption, is sometimes used to describe the general phenomenon of gas uptake by solids.

Adsorption tends to reduce the imbalance of attractive forces (polar and dispersive), at a surface, and hence

reduces the interfacial/surface free energies of the system.

The forces involved in adsorption by solids have traditionally been classified as non-specific (Van der Waals) forces, similar to the forces involved in liquefaction; or stronger more specific forces, such as those which are operative in the formation of chemical bonds. The former are responsible for physical adsorption and the latter for chemisorption. When adsorption takes place, the adsorbed molecules are restricted to two-dimensional motion and accordingly a decrease in entropy is observed. Since adsorption also involves a decrease in free energy, then from the thermodynamic relationship for change in Gibb's free energy: (ΔG):

$$\Delta G = \Delta H - T.\Delta S \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (6.1)$$

the heat of adsorption, enthalpy (ΔH_{ads}), will be negative and thus an exothermic process. This argument does not necessarily hold for solute adsorption from solution, since a certain amount of destructuring may occur giving rise to a positive net entropy change.[209,210] This is often seen with conformational changes in protein molecules.[211]

Multilayer adsorption has been observed for a number of adsorbates, attributable to physical adsorption. Only monomolecular chemisorbed layers are feasible for simple gas molecules, but this may not be so for complex adsorbed macromolecules such as proteins.

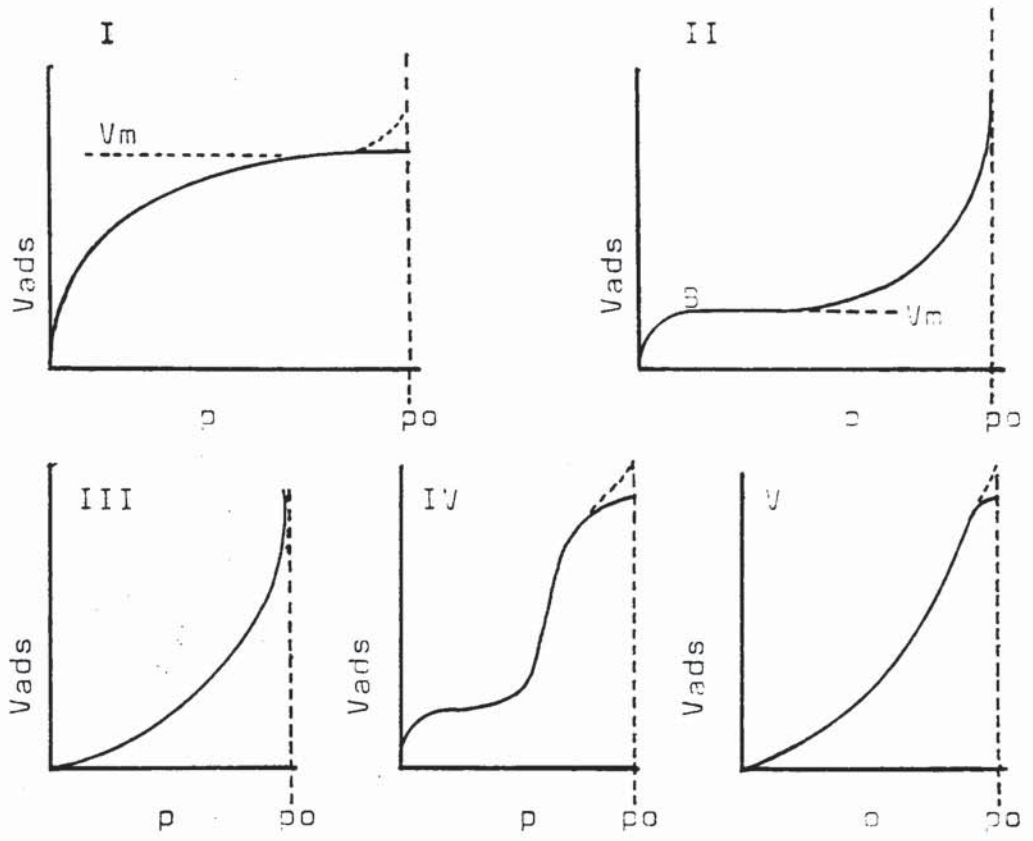
The heats of physical adsorption are very much smaller than those for chemisorbed molecules. Chemisorption is a specific process, which unlike physical adsorption, may require an activation energy and will therefore be relatively slow and not readily reversible. It is very likely that

physical adsorption is a prerequisite of chemisorption, by producing a reduction in the activation energy, which then enables the transition to chemisorption to occur.[209,210]

Brunauer[212] classified adsorption isotherms into the five characteristic types depicted in Fig. 6.1.

Figure 6.1

Adsorption Isotherms



Key: Vads = Volume of gas adsorbed at equilibrium.

Vm = Volume of gas to produce a complete monolayer.

p = concentration or pressure of gas.

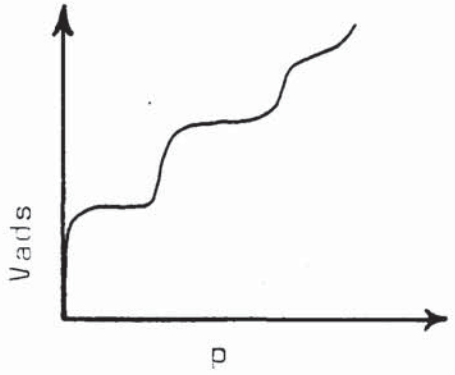
po = saturation vapour pressure.

Type I isotherms are referred to as Langmuir-type isotherms and are obtained when adsorption is restricted to a monolayer. Chemisorption isotherms, therefore

approximate to this shape. Type I isotherms have also been found for physical adsorption on solids containing a very fine pore structure. Type II isotherms are frequently encountered, and represent multi-layer physical adsorption on non-porous solids and are often referred to as sigmoid isotherms. Point B on this graph represents the formation of an adsorbed monolayer.

Frequently there is overlapping of two or more of these adsorption isotherms which makes interpretation very difficult. In addition there are some isotherms which do not fit into Brunauer's classification, the most notable being the step-wise isotherms[213] as depicted in Fig. 6.2

Figure 6.2



Each step is indicative of a successive monolayer on top of the first.

Langmuir[214] developed a mathematical expression from postulated adsorption mechanisms to fit the experimental isotherm curves of type I. He was of the opinion that, because intermolecular forces fall off very rapidly with distance, adsorbed layers are not likely to be more than one molecular layer in thickness.

This view is generally accepted for chemisorption and for physical adsorption at low pressures and moderately high temperatures.[209,210] The Langmuir adsorption isotherm is based on the assumptions that (a) only monomolecular

adsorption takes place, (b) adsorption is localised and (c) the heat of adsorption is independent of surface coverage. Langmuir's expression is derived by equating the velocities of adsorption and desorption together. The velocity of adsorption depends on (a) rate of collision of molecules with the solid surface, which is proportional to pressure (concentration), (b) the probability of striking a vacant site (1 - V/Vm) and (c) adsorption activation energy term (exp. (-E/RT). The velocity of desorption is dependent on (a) fraction of the surface which is covered, V/Vm, and (b) a desorption activation energy term exp. ($-E^1/RT$). From the above the following expression was derived:

$$ap = \frac{V/Vm}{(1 - V/Vm)} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (6.2)$$

or $$V = \frac{Vm \cdot ap}{(1 + ap)} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (6.3)$$

where V equals the equilibrium volume of gas adsorbed per unit mass of adsorbent at a pressure, p, and the term a, is a constant dependent on temperature, but independent of surface coverage.

An extension of Langmuir's treatment was proposed by Brunauer, Emmett and Teller[132] to allow for multilayer adsorption on non-porous solid surfaces. The BET equation, as it is commonly known, is derived by equating the rates of evaporation and condensation for the various adsorbed molecular layers. It is based on the assumption that a characteristic heat of adsorption, ΔH, applies to the first monolayer, whilst the heat of lique-

faction ΔH_L of the vapour under study, applies to adsorption in the subsequent successive monolayers. The expression is given by:

$$\frac{p}{V\,(p_o - p)} = \frac{1}{V_m C} + \frac{(C - 1)\,p}{V_m C\,p_o} \quad \ldots\ldots\ldots\ldots \quad (6.4)$$

where $C \approx \exp((\Delta H_L - \Delta H_1)/RT)$. Generally this equation is used to describe the type II isotherms.

The most notable criticism of both Langmuir's and the BET adsorption equations, concerns the simplifying assumption that the heat of adsorption is independent of surface coverage.

The heats of both monolayer physical adsorption and chemisorption would be expected to become significantly less exothermic as the surface coverage increases, due to depletion of the more active sites of a heterogeneous solid surface. The initial adsorption isotherm slope would be steeper than predicted by either of the above equations, but as the adsorption proceeds monolayer coverage becomes increasingly more difficult. To some extent this trend will be offset against the increasing lateral molecular interactions with increase in the monolayer coverage, and which would be expected to raise the exothermic heat of adsorption.

Generally there is a greater tendency for the initial monolayer to be completed, if it is significantly more exothermic than for further successive layers, but little tendency for the second monolayer to be comoleted prior to adsorption into the third and subsequent layers.

The theoretical treatment of adsorption from solution is generally more complicated than that of gas adsorption,

since adsorption from solution will always involve some degree of competition between solute(s) and solvent for the available adsorption sites. The amount of adsorption of each will be directly dependent on their ability to concentrate at the solid-solution interface and the solute concentration.[209,210]

Adsorption from solution can often be predicted in terms of the polar-nonpolar nature of the solid and of the solution components. A polar solute will be adsorbed strongly by a polar solid and a non-polar solute weakly, and vice versa. In addition, polar solutes will tend to be adsorbed strongly from non-polar solvents and weakly from polar solvents, and vice versa. Solute adsorption is often restricted to a monomolecular layer, however multilayer has been observed in a number of cases, being evident from the shape of the adsorption isotherms and from the calculated quantitative maximum value for a monolayer per unit area, based on the area per adsorbed molecule. Both the Langmuir and BET expressions are normally applied to solution adsorption.

The general theoretical treatments for adsorption of small solute molecules may not be applicable when considering large amphiphilic macromolecules, such as proteins, which when in solution can undergo conformational changes to mimic the polar/non-polar characteristics of the solvent and so increase solubility and reduce the interfacial free energy. In addition, they have the tendency to concentrate at an interface by conformational changes to expose the appropriate polar/non-polar groups, and thereby effectively reduce the interfacial/surface free energies of the system. Such materials are termed surface active and are known as surfactants.

6.3 The Phenomenon of Protein Adsorption at Interfaces

6.3.1 Introduction

The subject of proteins at interfaces is an extremely broad field of study, encompassing very many seemingly diverse fields of research, exhibiting apparently unrelated phenomena. Many events such as maritime fouling, thrombus formation, and dental plaque attachment are all very much dependent on cellular adhesion. It has been observed in general that adhesion in a biological environment of blood, saliva, sea-water, is directly attributable to a preconditioning film of protein, which mediates the adhesive interactions with arriving cells. It is the amphiphilic characteristics of proteins, resulting from their mixture of polar and non-polar side chains, which causes them to adsorb at interfaces, and so influence the above events. In order that some understanding of the fundamental relationship between these biological events and protein adsorption can be achieved, this section will endeavour to present the phenomenon of interfacial protein adsorption from a physico-chemical approach.

6.3.2 Mechanism of Adsorption

In order that a protein molecule can adsorb and exert its influence at a phase boundary or take part in an interfacial reaction, it must first arrive at the interface by a diffusion process. If it is assumed that there are no constraints to adsorption other than diffusion, simple diffusion theory may be applied to predict the rate of adsorption. Using this treatment it follows that all the protein molecules in the immediate

vicinity of an interface will be rapidly adsorbed and accordingly the protein concentration in a juxtapositioned interfacial sublayer, of several molecular diameters in thickness, will be reduced to zero. A diffusion process will then occur from the bulk solution phase into the sublayer. The rate of adsorption, dn/dt, will then be given by the classical diffusion theory[215] expression

$$dn/dt = Co\ (D/\pi)^{\frac{1}{2}} t^{-\frac{1}{2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (6.5)$$

where the term Co is the bulk concentration, D is the diffusion coefficient, t the time and n is the number of molecules adsorbed. (π = 3.142).

By integrating Eq. (6.5), an expression for the number of molecules adsorbed with respect to time (t), can be obtained.

$$n = 2\ Co\ (Dt/\pi)^{\frac{1}{2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (6.6)$$

This equation was applied to adsorption of bovine serum albumin (BSA) from solutions of different concentrations at an air/water interface.[216] For the higher concentrations close agreement with the Eq. (6.6) was found, but for the lower concentrations, higher adsorption rates than expected were observed and these were attributed to convection.

The Eq. (6.6) will be valid when there is an undisturbed fluid layer adjacent to the interface where mass transport occurs only by diffusion, this layer is often referred to as the stationary or boundary layer for mass transfer. Its width depends on the nature of the fluid and on conditions such as temperature fluid-dynamics, and the properties of the interfacial film.[217,218]

Application of Eq. (6.6) to adsorption at the solid/water interface, was found to exhibit agreement between the rates of adsorption and the predicted rates of diffusion.[219]

Results for the air/water interface[216] show that once a close-packed monolayer is present at the interface, the rate of adsorption progressively falls below the rate of diffusion, indicating the presence of an energy barrier to adsorption. This barrier is known as the interfacial pressure barrier, and is the energy necessary to compress molecules already adsorbed against the interfacial pressure π, to create an area of interface, $\triangle A$, equal to that required for the molecule to move into.[220] Several studies[216,221,222] have determined that these interfacial areas ($\triangle A$), are considerably smaller than those expected from the areas of molecules in either their native solution conformation or their air-water interfacial conformation. They fall within relatively narrow limits of 50 - 200 Å^2, which bear no relation to the molecular sizes of the proteins under study. For example the interfacial area ($\triangle A$), for human serum albumin (HSA), was found to be 100 Å^2, however from its native conformational dimensions in solution,[223] it has a minimum cross-sectional area of approximately 1100 Å^2, It was concluded from these studies that once a small portion of a protein molecule of sufficient energy to penetrate the interface has cleared the requisite interfacial area ($\triangle A$), its area grows spontaneously until the whole molecule has unfolded into its interfacial conformation.

The requisite interfacial area, $\triangle A$, necessary for adsorption to proceed was also shown to be directly dependent on bulk concentration, temperature and electrical charge.

When a protein adsorbs from a solution in which the pH is close to its isoelectric point, the rate of adsorption is controlled by the rate of diffusion to the interface and the interfacial pressure barrier. However when the protein molecule takes on a net electrical charge, an additional barrier to adsorption is observed as a consequence of the electrical potential set up at the interface, by the adsorbed protein.[224,225]

The rates of adsorption as measured in the fluid/fluid interface studies outlined above, can to some extent be correlated with the isoelectric points of proteins. However, the effects of counter ions in solution and changes in the isoelectric point on adsorption will in certain cases cloud the issue, and must therefore also be a consideration.

6.3.3 Protein Conformation

In its native state each type of protein molecule has a characteristic three-dimensional shape, referred to as its conformation. Depending on their conformation proteins can be placed in two major classes, fibrous and globular. The globular proteins with which this study is concerned, have their polypeptide chains tightly folded into compact spherical or globular shapes. This native conformation has four main types of weak interactions or bonds which stabilise the structure; (i) hydrogen bonding between peptide groups; (ii) hydrogen bonding between substituent groups; (iii) hydrophobic interactions; (iv) ionic bonds.

From studies on the relative contributions of each of these four types of interaction to the total conformational

stability of native protein molecules, it is now clear that hydrophobic interactions between the non-polar substituent groups, are by far the most important. Hydration of these non-polar groups induces the conformational folding of the protein in order to achieve the minimum free energy state of the molecule in solution, as previously outlined in Chapter 4. The stability of a native globular protein is thus the result of a balance between the entropically opposing forces of:

(i) the tendency of the polypeptide chain to unfold into a random arrangement and

(ii) the tendency of the surrounding water molecules to seek their most random state.

An excellent review by Privalov[226] gives a comprehensive insight into the conformational stability of globular proteins, as known to date.

The changes in conformation observed with adsorption of proteins at interfaces, has been the subject of many studies.[55,69-76] It is well established that proteins lose their native conformation (tertiary structure), at fluid/fluid interfaces, for example bovine serum albumin (BSA) molecules at an air/water interface were shown to exhibit a surface area per molecule of approximately 10,000 A^2, indicative of complete unfolding.[227] However, there are some indications that protein molecules at solid/liquid interfaces do not always undergo the drastic conformational changes, that occur at fluid/fluid interfaces. Electrophoretic studies by Chattoraj and Bull[70], found that adsorbed proteins exist in different conformational states with different numbers of anchorage sites, dependant on pH and protein concentration. In a study by Kochwa et

al,[72] he cites that adsorbed proteins were of a different conformation to those in the bulk solution, and thus cannot be considered to be in equilibrium with them. Somewhat in contrast to these studies, Morrissey and Stromberg,[69] using infrared differential spectroscopy, found that the internal bonding of globular proteins prothrombin and albumin was sufficient to retain a near native 'like' conformation on adsorption onto silica, however for fibrinogen the converse was true. As a consequence of the diversity of both protein structure and solid surfaces, it would seem that no one theory can account for protein conformational changes that occur on adsorption, for all situations.

Studies of synthetic polymer adsorption have shown that an adsorbed flexible polymer molecule may be divided into three parts: (i) the trains, which are segments adsorbed at the interface; (ii) the loops, which are segments of the chain that extend into the adjacent bulk phase(s); and (iii) the tails, the two segments at the two ends of each polymer chain; these tend to have lower free energies of adsorption than other segments and thus tend to extend into the bulk phase. The importance of tail segments decreases with increasing molecular weight. The free energies of adsorption of the trains, loops and tail segments, together with their chain flexibility, will determine their distribution at an interface. The majority of these studies have been carried out at the solid/liquid interface, and theoretical treatments have been developed.[228-230]

The high free energy of adsorption of proteins at fluid/fluid interfaces, results in practically all segments

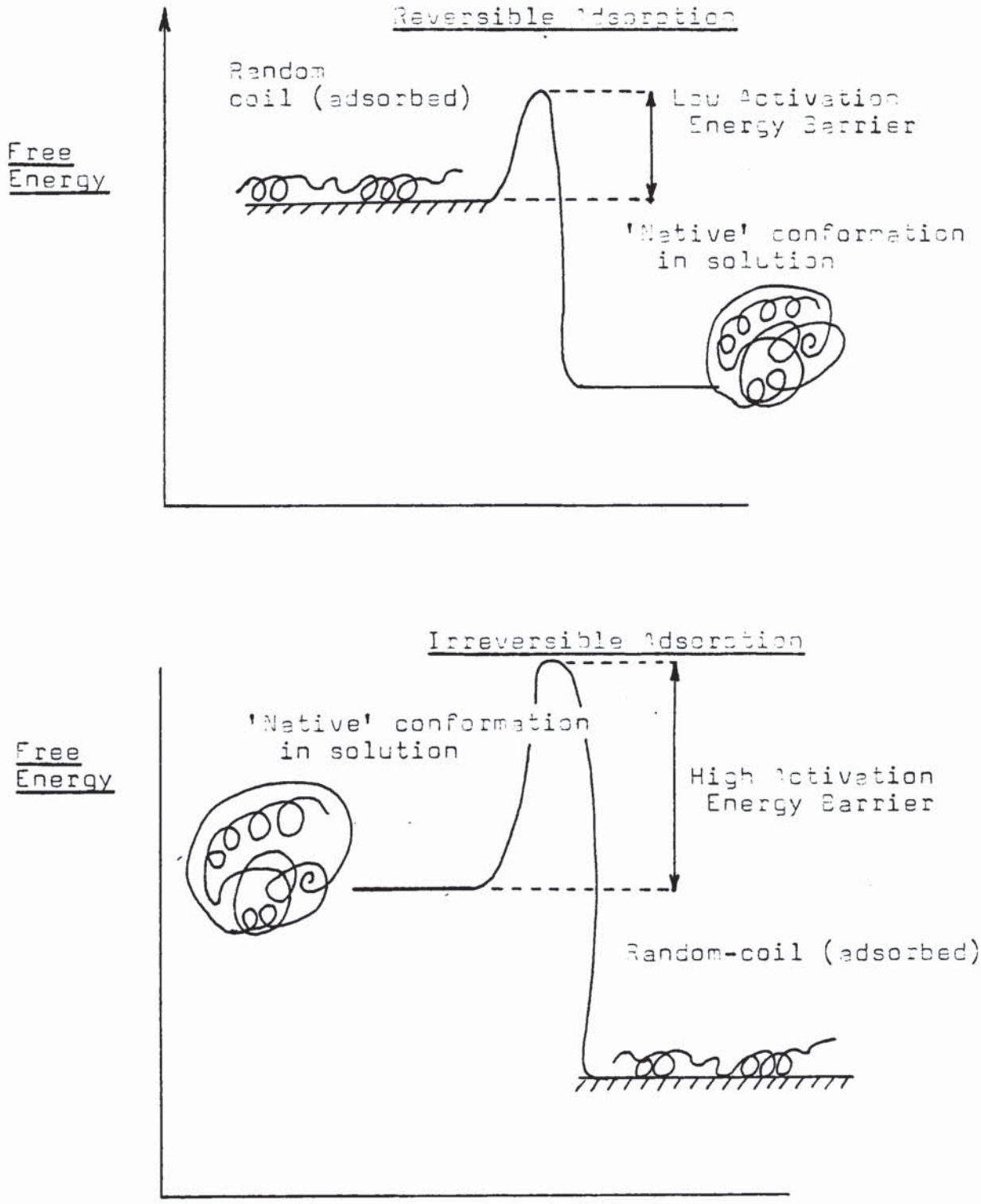
being at the interface at low interfacial pressures.[231] However compression of a monolayer causes displacement of segments, which is made evident by a decrease of interfacial pressure with time at a constant area or alternatively a decrease in area with time at a fixed interfacial pressure. It was found in the above study that a train of area 63 $\AA^2$ ($\triangle A$), for albumin, was equivalent to the area occupied at the interface by 3-4 amino acid units, and that this area is equivalent to the area of one turn of an α-helix. It is well known that certain segments of globular proteins contain α - helices in their secondary structure, however whether the adsorbed protein molecules retain this structure on adsorption has to date, yet to be determined.

Much evidence suggests that the folded, native conformation of globular proteins is only slightly more stable than the unfolded, or denatured conformation.[232] Previously it had been assumed that the native conformation was of a lower free energy than the unfolded random-coil form, under biological conditions, and that therefore it should exhibit higher stability. This assumption however, may not be true for all proteins and it is the subject of much debate and study.

Proteins that spontaneously refold into their native form, may indeed be more stable than their denatured forms under specific conditions of pH, ionic strength and temperature. On the other hand, the random coil form of some proteins may have less free energy than the native form (See Fig. 6.3). In such cases the unfolded form will not readily revert to the native conformation (i.e., irreversible). Much conflicting data exists as to the reversibility of protein adsorption and it again would appear that no one theory can account for all situations.

Figure 6.3

FREE-ENERGY CONCEPTS OF ADSORPTION



6.3.4 Protein Adsorption Studies at the Solid/Liquid Interface

Traditionally the study of adsorption at the solid/liquid interface has been experimentally more difficult than at the fluid/fluid interface. In recent years however, there has been a great impetus to the study of protein adsorption at the solid/liquid interface, largely motivated by biomedical problems. Many diversified techniques have been applied which yield a wider range of information and allow measurements of adsorption isotherms at these interfaces (See next Section).

A great deal of conflict exists in the literature to date as to the exact type of adsorption isotherm. Early workers[233,234] observed that the concentration dependence of adsorption followed a type I, that is a Langmuir type isotherm of a single monomolecular layer. Relatively few recent studies however have confirmed this.[68] A series of radiotracer studies employed by Kim and Lee et al deduced that adsorption isotherms was of the Langmuir type.[27,90,235-237] However based on the calculated weight per unit area as derived in this study (See Appendix 5 for the method of calculation), and from other workers[64,68,105,238,239] for a monolayer coverage for various plasma proteins, it is obvious from their results (See Table 6.1), that in fact multiple layering is occurring and all that is similar between their isotherm results and Langmuir adsorption isotherms is the shape of the isotherm curve. Application of the BET isotherm[132] model would perhaps therefore seem more appropriate however, with this as with the Langmuir isotherm model, there has been a tendency to doubt the applicability of these models

Table 6.1

ADSORPTION DATA OF KIM & LEE[235]

Proteins (60mg % solutions)	Polymer	Adsorption times (mins.)	Adsorbed Level x 10^{-8} g.cm^{-2}	Theoretical monolayer coverage. x 10^{-8} g.cm^{-2}	Ref.
Albumin	PEUU SR FEP	≤70 mins.	450 100 75	25 20	68 *
γ-Globulin	PEUU SR FEP	≤70 mins.	475 125 75	25 20	240 105
Pro-thrombin	PEUU SR FEP	≤70 mins.	475 200 75	-	-

KEY:

PEUU = segmented polyether-urethane-urea

SR = silicone rubber (polydimethyl siloxane)

FEP = fluorinated ethylene/propylene copolymer

N.B. The above values of Kim, S.W. & Lee, R.C. would seem to be evidence for multiple layer formation based on monomolecular coverage values. Therefore protein adsorption cannot follow the Langmuir isotherm type which relates solely to monolayer coverage. (See Text).

* Adsorbed HSA monolayer as derived in this project (See Appendix 5).

of adsorption, because of the apparent irreversibility.[72,238,240]

Desorption from a solid/liquid interface is generally very difficult, however certain studies,[64,65,75,105,209,241,242] have demonstrated some degree of desorption. Extensive **hysteresis** was evident for the adsorption/desorption process, and often chemical surfactants and even ultrasound[242] agitation, were used in an attempt to desorb the proteins. In the series of studies by Vroman et al,[21,28,60-63] using Ellipsometry measurements, and Brash and Samak[243], using a dual-labelled radiotracer technique, they show a dynamic exchange process is occuring at the interface under steady state conditions. The latter groups results indicated however that only a fraction of the surface shows reversibility and the remainder exhibits irreversible binding, this fraction being dependent on the conditions. The authors suggest that this behaviour may reflect heterogeneity in the binding energies of different sites. Similar conclusions have been reached by Dillman and Miller,[68] who deduced that the adsorption of bovine serum albumin, γ-globulin and fibrinogen onto various polymersurfaces, takes place in two separate and distinct ways, evidently a result of two types of adsorption sites. One, called type 1, was characterised by being easily 'reversible', hydrophilic, and exothermic with ΔH_{ads} value of approximately -40 $kJmol^{-1}$. The other type 2 adsorption, was characterised as being tightly bound (irreversible), of hydrophobic, and endothermic with a ΔH_{ads} value varying between 20 - 80 $kJmol^{-1}$. Their experimental determination of this data was not by direct microcalorimetry measurements and is somewhat suspect.

The free energy of adsorption can be expressed by the Gibb's expression:

$$\Delta G_{ads} = \Delta H_{ads} - T.\Delta S_{ads}, \quad \ldots\ldots\ldots (6.7)$$

where ΔH_{ads} & ΔS_{ads} are respectively, the net changes of enthalpy and entropy in the adsorption process. Each of the three components, interface, protein, and solvent make their own contributions to both ΔH_{ads} and ΔS_{ads}. For adsorption to occur a net negative value for ΔG_{ads} must be evident, and the larger the negative value, the greater the driving force for adsorption.

Thermodynamic studies by Chui and Nyilas,[238,240] using microcalorimetry deduced that adsorption was not of a Langmuir isotherm type and was in fact of the multilayer type and irreversible. They found adsorption was highly exothermic (ΔH_{ads} 10 - 15 x 10^6kcal/Mole), for fibrinogen on a hydrophilic glass surface, and less exothermic on LTI carbon (ΔH_{ads} 3 - 4 x 10^6kcal/Mole) for fibrinogen indicative of less conformational change. Similar results were also obtained for γ-globulin.

Various other workers have also attempted theoretical treatments for protein adsorption thermodynamics.[45,55,73] Multilayer adsorption has been shown to occur for both hydrophilic and hydrophobic surfaces.[4,5,30,64,72,105,238-240,244]

A greater adsorption rate is seen to occur at hydrophobic interfaces than at hydrophilic ones over the first monolayer.[64,245-247] However as the multilayers develop the hydrophobic surface would appear to reach a steady state (equilibrium) for a particular protein concentration very quickly, whereas, the hydrophilic surfaces keep on

slowly adsorbing with time until they greatly exceed the hydrophobic steady state level.[64] Brash, Uniyal and Samak[64] found that higher adsorption occurred with hydrophilic oxyethylene-urethane copolymers than hydrophobic oxypropylene-urethane copolymers with multilayers, but for a study just covering a time for a monolayer to appear the converse was true.[247] Similar results were found for the hydrophilic hydrogels of poly (HEMA) and copolymers thereof and poly (NVP) by Horbett,[105] however neither of these two groups commented on the above phenomena.

Apart from the above adsorption studies, at the solid/liquid interface, adsorption has been shown to increase at the isoelectric point of the proteins.[68,70] Scanning electron microscopy (S.E.M.) studies on fibrinogen have also tended to confirm multilayer formation,[30,32] together with large adsorbed films seen with immersion in sea-water[5] (800Å) after 1500 mins. and dental pellicles[4] (1000Å) due to adsorbed salivary proteins and glycoproteins.

Many of these studies show wide variation in their adsorption study times (and bulk protein concentrations) and many show great reluctance to admit to multilayer formation, and deliberately concentrate on the low protein concentrations and very short adsorption times in order that the monolayer criteria is not exceeded. Brash and Uniyal,[64] reported that adsorption on to the hydrophobic polymer polyethylene was not seen to produce multilayers at 10mg % of albumin, but that above this concentration, with time, multilayers did develop.

Chui and Nyilas[238,240] proposed that the reduction in enthalpy with the second and subsequent multilayers

was evidence for a change in the binding mechanism for the adsorbed proteins, and this would seem to be due to a time dependent change in the adsorbed initial monolayer.

Other workers have also noticed a time dependent change in adsorbed protein surface free energy (tension) at interfaces, as well as changes in adsorption due to bulk concentration.[34] [70,239]

6.4 Some Considerations of the Parameters Governing the Experimental Design of the Adsorption Systems.

Due to certain interfacial phenomena it was necessary for both the static and dynamic adsorption systems to employ experimental procedures which eliminated the air/solution interface from the test material.

Early experiments[248-250] had determined that the passage of a solid through an air/solution interface produced an adsorption of the solute onto the solid: this phenomenon is often referred to as Langmuir-Blodgett transfer, after the workers. Their early work was mainly concerned with long chain fatty acids, but subsequently their results and others confirmed that this phenomenon was also occurring with protein solutions.[34,251-253]

Due to their surface active amphoteric nature, proteins have the tendency to concentrate at an interface and in so doing reduce the interfacial tension. Depending on the hydrophilic/hydrophobic characteristics of the materials, the above researchers observed both transfer and stripping of adsorbed protein films on immersion and withdrawal from these protein solutions. Both Bull[34] and Vroman[254] found that adsorbed albumin and fibrinogen films due to the Langmuir-Blodgett transfer, remain adherent to hydro-

phobic poly (methyl methacrylate), even after extensive washing with solutions of trisodium phosphate and 8m urea. Quantitatively many of these results were variable and depended on the speed of immersion and withdrawal of the test material through the air/solution interface, and therefore because of the unpredictable nature of this phenomenon, both the static and dynamic adsorption systems were designed to eliminate this problem (as outlined in Chapter 3). The washing procedure to displace the protein solution by a physiological buffer, both eliminated the above problem and also the problem of contamination associated with the radiotracer techniques of protein adsorption measurements.[255] Generally speaking the most convenient and sensitive method of quantifying protein adsorption is by radiolabelling of the proteins. However as with any tagging of molecules either by a radio isotope or a fluorescent dye, questions are raised as to whether the labelling techniques modify the physico-chemical behaviour of the protein molecule. Studies on the antigenic specificity of proteins labelled with I^{131} or I^{125} isotopes seem to indicate that no significant change has occurred and that they can be assumed to be biologically identical to the untagged molecule.[256-259]

Radiotracer techniques have been used for many years as a technique to study polymer adsorption. They have been especially valuable in that the adsorbing surface can be studied directly, eliminating the need for high surface area substrates required for most solution depletion techniques. Such techniques as fluorescent tracer,[260,261] immunoelectrophoresis,[262] and isotachophoresis[263] all depend

on quantitative and qualitative analysis of the described washings, but because of the problem regarding the 'reversibility' of protein adsorption their use is questionable. Direct measurement by ellipsometry[264] cannot be used for hydrogel materials, because of the similarity between the refractive indices of hydrogels and proteins. Similarly multiple attenuated internal reflection (MAIR), infra-red spectroscopy[265] is feasible only for dehydrated specimens and is only semi-quantitative. One very promising technique for direct measurement of protein adsorption is by multiple internal reflection fluorescence spectroscopy.[266,267] This technique however is still in its infancy and as yet no known commercial equipment is available.

6.5 Human Serum Albumin (HSA)[268-272]

The normal physiological level of HSA is within the range 35 - 50g.l.$^{-1}$, (with a mean value of 42g.l.$^{-1}$). It comprises some 60% of all plasma proteins and accounts for almost all of the colloidal osmotic pressure of the plasma, which is about 25mm Hg. HSA is synthesized in the liver from the amino-acid pool which is maintained by dietary proteins. During protein starvation, HSA is formed at the expense of tissue proteins. In disease, the HSA level often falls whilst that of globulins increase. Only about 40% of the total albumin produced in the liver is contained within the circulation at any one time. Albumin is found in nearly every fluid or secretion of the body to some extent. An estimated 5% of the circulating HSA leaves the blood each hour to be returned (via the lymphatics), during the next 24 - 28 hours.

Physicochemical Properties:

Albumin has been a model protein for physical chemists, who have probed its behaviour by every conceivable technique. Selected physico-chemical data so obtained are listed in Table 6.2.[223,273-279]

Compared with other proteins, albumin is characterized by smaller size but greater solubility, total charge, net negative charge, stability, flexibility and diversity of ligand-binding affinities. A proposed multiple loop-like pattern illustrated in Fig. 6.4, gives the amino-acid sequence and primary structure for HSA and to some extent explains the flexibility or conformational adaptability.[273] From recent amino-acid composition and sequence analysis, as given in Fig. 6.4, a precise molecular weight determination of 66,248 daltons was obtained for HSA. Hydrodynamic studies have ascertained that at pH 5 to 8 HSA is a prolate ellipsoid with major and minor axes of 150 and 38Å.[223] From spectral analysis of albumin it was determined that the secondary structure contains 50 - 55% α-helix, about 15% β-pleated sheet and the remainder random coil.[279] It has been proposed that the numerous disulphide bridges (17 in HSA) impose considerable restraints which stabilize this secondary structure.[280] The tertiary structure of HSA has not been fully elucidated by X-ray crystallography, but it has been assumed that strong interactions between adjacent loops are responsible for the compact globular molecule observed at neutral pH. Associations of these loops into globular parts or domains at neutral pH have been postulated, as given in Fig. 6.5

Table 6.2

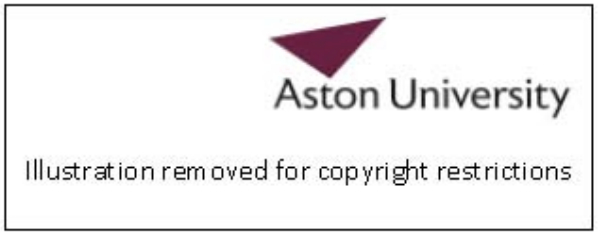
PHYSICO-CHEMICAL PARAMETERS OF HUMAN SERUM ALBUMIN (HSA)

Property	Value	Reference
Molecular weight (from amino-acid composition)	66,248	273
Sedimentation constant $S_{20,w} \times 10^{13}$		275
Monomer	4.6	"
Dimer	6.8	"
Diffusion constant. $D_{20,w} \times 10^{7}$	6.1	"
Partial specific volume. V_{20}	0.733	"
Intrinsic viscosity η	0.042	"
Frictional ratio f/fo	1.28	"
Overall dimensions A^{o}	38 x 150	223
Isoelectric point	4.7	"
Isionic point	5.2	"
Electrophoretic mobility pH 8.6	-5.9	274
Refractive index increment (578nm) $\times 10^{-3}$	1.89	276
Optical absorbance $A^{1\ gm/l}_{279\ nm}$	0.531	277,278
Estimated α-helix %	48	279
β-form %	15	"

Figure 6.4

AMINO-ACID SEQUENCE OF HSA[273]

Figure 6.5
Proposed Globular domains of HSA at pH 7.4[272]



Over 200 positive and negative charges distributed over the HSA molecule give a hydrophilic character and contribute to its high solubility in aqueous media. Titration curves of albumins indicate that it is a strong buffer at pH 4 or 10 but a weak one at pH 7. Nevertheless HSA does help to a small degree in buffering blood plasma to a near constant pH 7.4.

The ability of HSA to interact with, and bind a myriad of smaller molecules, is of both physiological and pharmacological important. The bindings of cations, Ca^{2+}, Cu^{2+}, Ni^{2+}, Ag^{+}, Hg^{2+}, Zn^{2+} etc., does occur but is far less dramatic than its binding of anions. The anionic long chain fatty acids are very insoluble at pH 7.4, and their presence in plasma is largely due to their binding to albumin. The association of fatty acids with albumin also causes conformational changes that help to increase the stability of the protein molecule and this is probably true for most tightly bound ligands.[272] Defatted albumins are notably unstable on storage.[281] Table 6.3 illustrates some of the many physiological anions which bind to HSA.[272]

Bilirubin molecules are less soluble than fatty acids at neutral pH, and in addition, are highly unstable. The affinity of HSA for bilirubin is very high, even so fatty acids compete for binding sites with bilirubin under physiological conditions. HSA binds thyroxine and numerous steroid hormones, but to a lesser extent than do the specific binding proteins such as thyroxine-binding globulin and cortisol-binding globulin. The HSA acts as an overflow reservoir when the primary binding protein becomes saturated or nearly so.

Table 6.3

BINDING OF SOME PHYSIOLOGICAL ANIONS TO HUMAN ALBUMIN (272)

Anion	Conditions				Est. % bound to albumin in normal plasma
	K (M^{-1})	n[a]	pH	Temp (°C)	
Oleate	2.6×10^8	1	7.4	37	99.9
Palmitate	6.2×10^7	1	7.4	37	99.9
Bilirubin	1×10^8	1	7.4	38	99.8
	7×10^7	1	7.4	25	
Hematin	5×10^7	1	7.5	23	
L-Thyroxine	1.6×10^6	1	7.4	24	10
L-Tryptophan	1.6×10^4	2	7.4	2	75
Estradiol	1×10^5	(1)	7.4	5	
Progesterone	3.7×10^4	(1)	7.4	5	
Cortisol	5×10^3	2	7.4	37	30
Corticosterone	1.3×10^4	(1)	7.4	5	30
Aldosterone	$< 5 \times 10^3$	(1)	7.4	5	60
Testosterone	4.2×10^4	(1)	7.4	25	6
Prostaglandin	7×10^4	2	7.5	37	
Urate	3×10^2	(1)	7.4	37	15

Table 6.4

BINDING OF SOME DRUGS TO HUMAN ALBUMIN (272)

Compound	K (M^{-1})	n	Temp (°C)	% bound to albumin
Salicylate	4×10^5	1	22	40
Sulfisoxazole	1.6×10^4	1	25	85
Oxacillin	4.7×10^3	1	37	94
Penicillin G	1.1×10^3	1	37	65
Ampicillin	5×10^2	1	37	23
Carbenicillin	2×10^3	1	37	50
Warfarin	2.2×10^5	2	37	97
Phenylbutazone	2.7×10^5	2	37	99
Acetrizoate	4×10^4	1	25	
Diphenylhydantoin	6×10^3	6	37	77
Chlorothiazide	1.5×10^4	2	37	89
Clofibrate	1.3×10^5	1	37	98

Examples of drug-binding to HSA are listed in Table 6.4. It is believed that hydrophobic aliphatic chains are inserted into hydrophobic clefts in the HSA molecule without any significant loss in the hydrophilicity of the HSA.

6.6 Human Fibrinogen: (HFb)[269,270,282]

The essential reaction in coagulation of blood is the conversion of the soluble protein fibrinogen into the insoluble scleroprotein, fibrin, by means of an enzyme, thrombin. This process may be activated by way of either the intrinsic clotting system due to surface activation by foreign material, or via the extrinsic clotting system arising from tissue factors released following cellular injury, as outlined in the introductory chapter. As like many other plasma proteins, fibrinogen is **synthesised** by the liver.

Physico-chemistry:

Many experimental techniques have been brought to bear, in an effort to elucidate the size and shape of the fibrinogen molecule. In the main these studies have been aimed at human and bovine fibrinogen and there is general agreement that the native molecular weight is 340,000 $\pm$ 20,000 daltons, based on sedimentation-diffusion data and on light scattering measurements, and that the native molecule is a dimer.[282]

The physico-chemical parameters as determined for human and bovine fibrinogen are listed in Table 6.5.

To date no clear agreement exists as to the general shape or size of the HFb molecule. At one extreme, if the

Table 6.5

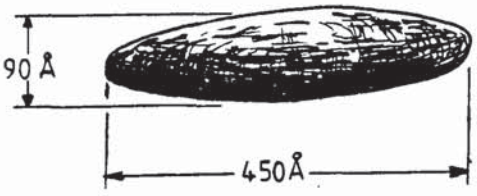
PHYSICOCHEMICAL PARAMETERS OF HUMAN AND BOVINE FIBRINOGEN (282)

Molecular weight (MW)	340,000±20,000
Sedimentation coefficient ($S_{20,w}$)	7.9 S
Translational diffusion coefficient ($D_{20,w}$)	2.0×10^{-7} cm^2. sec^{-1}
Rotary diffusion coefficient ($\theta_{20,w}$)	40,000 sec^{-1}
Intrinsic viscosity (η)	0.35dl/gm
Partial specific volume ($\bar{V}$)	0.71-0.72
Frictional ratio (f/f_o)	2.34
Molecular volume (calculated, unhydrated)	3.9×10^5 Å^3
Extinction coefficient ($E^1_{1cm\ 280}$)	15 - 16
Isoelectric point (IEP)	5.5
Percent α-helix	33

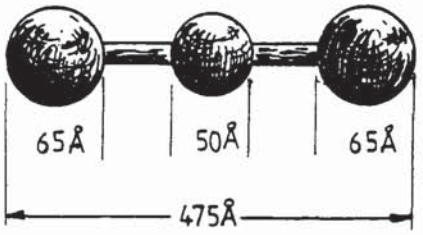
Figure 6.6

POSTULATED MOLECULAR SHAPES OF HFb

a) Prolate ellipsoid (from hydrodynamic studies) (282)



b) Triad of Hall & Slayter (283) (from E/M studies)



molecule is not in the least hydrated, then the hydrodynamic data are most consistent with a prolate ellipsoid of 900 x 30Å. At the other extreme a highly hydrated molecule behaves as a sphere of about 200Å diameter. Ancillary data however would seem to indicate a compromise structure resembling a prolate ellipsoid of 450 x 90Å. (See Figure 6.6a). Electron microscopy studies[283] have shown that the molecule is composed of three linearly attached globules, as illustrated in Fig. 6.6b. Fibrinogen is an unusually unstable, easily denatured molecule, and it obviously exhibits different conformational structures under different conditions of pH, ionic strength and temperature etc. Observations made under many conditions may have no bearing on the true native conformation, and the only ones which have any significance are those determined under near physiological conditions.

HFb molecules contain about 4% carbohydrate moieties, consisting of 19 galactose residues, 22 mannoses, 19 glucosamines and 6 sialic acid groups for every 340,000 molecular weight.[284,285] Various studies have indicated that all sulfhydryl groups of cysteine enter into disulphide bridge formation, but even here estimates range from 21 to 34 disulphide bridges per molecule.[282] Amino acid terminal analysis has shown that the native molecule is a dimer, each particle of 340,000 molecular weight having 3 pairs of non-identical polypeptide chains.[286] Two of the three different chains were found to undergo a change in amino terminal as a result of thrombin-catalyzed fibrin formation, corresponding to the release

of the fibrinopeptides A and B. By convention, the chains which lose the fibrinopeptides A are the α-chains and those which lose the fibrinopeptides B are the β-chains The chain whose amino terminal residue remains unchanged is termed the γ-chain.

Because of the unstable nature of the molecule, full elucidation of its primary, secondary and tertiary structures, has yet to be realised.

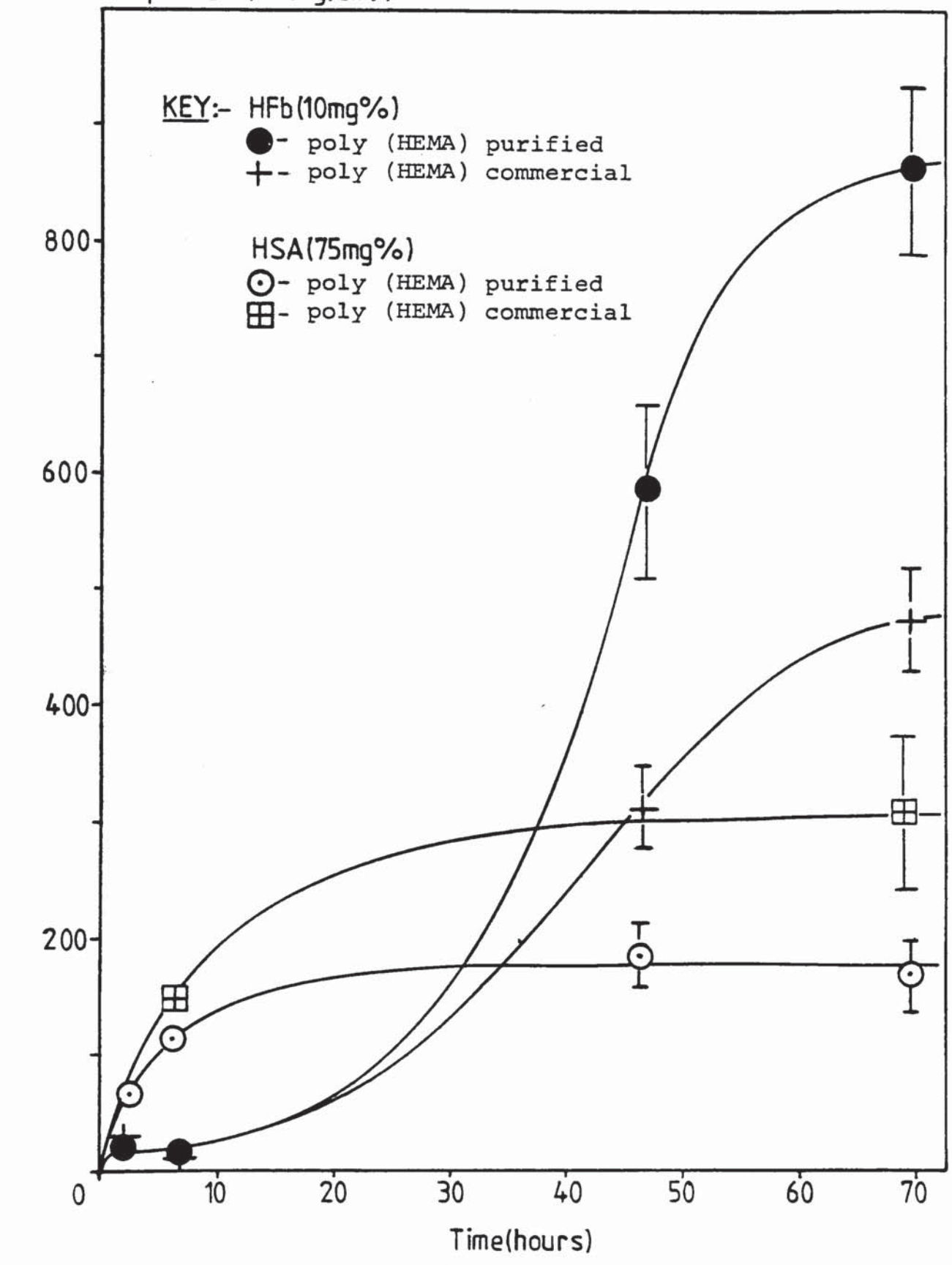
6.7 Results and Discussion

All adsorption study data has been tabulated in Appendix 5, and in order to facilitate their analysis and interpretation, the majority of the said data has been transcribed into a graphical format within the text. The hydrogel poly (2-hydroxy ethyl methacrylate), is by far the most commonly utilised hydrogel material, both for commercial and research purposes, and it was therefore chosen as the basic starting material for these studies. The structure-property effects of additions of adjuvant monomers, with a spectrum of chemical structures, to form 2-hydroxy-ethyl methacrylate (HEMA) copolymers, have been investigated. Of particular interest, especially with regards the viability of these materials in biomedical applications, is their structure-property effects on the interfacial phenomenon of protein adsorption. In order that comparative adsorption studies for these copolymers may be undertaken, some general principles of human fibrinogen (HFb) and human serum albumin (HSA) adsorption onto poly (2-hydroxy ethyl methacrylate), must first be considered.

6.7.1 Poly (2-hydroxy ethyl methacrylate)

It can be observed from Graph 6.1 (i) that the way in which HFb and HSA adsorb onto the purified and commercial poly (HEMA) preparations, differ markedly. The initial rate of adsorption, dA/dt, as determined from the slope, for HFb onto both types of poly (HEMA) is fairly rapid over the first hour or so, but this very quickly slows, to give a step or shoulder in the

Graph 6.1(i): Adsorption data for commercial and purified poly (HEMA)



curve, which persists for approximately 5 hours. The curve then proceeds to increase for a considerable time, before once again slowing to a steady state or 'near' steady state plateau at approximately 70 hours. From the previously mentioned calculated weight per unit area for a complete monolayer coverage of HFb molecules of native or near native conformation, ($\sim$ within the range (13-21) $\times 10^{-8}$ g.cm.$^{-2}$), it can be seen that multiple layering is occurring, and that the initial small step in the curve, would appear to correspond to an initial monomolecular coverage of HFb molecules. It would appear that the completion of the monolayer coverage is a time dependent or rate limiting step, after which each successive adsorbed layer becomes progressively easier. This time dependent step can be attributed to the progressive increase in the interfacial pressure Π, which will accompany the developing HFb monolayer coverage. This energy barrier to adsorption will attain a maximum value at or near complete monolayer coverage, and its value will be a function of its own physico-chemistry and that of the adsorbent material, and the bulk protein concentration.[34] Reduction in the interfacial pressure, Π, of adsorbed proteins at fluid-fluid interfaces with time have been reported,[220] and this has been attributed to the displacement of segments. It may well be that this interpretation can be applied to the above HFb monolayer coverage, in that before further successive layering can occur a time-dependent modification or restructuring must take place in the adsorbed protein film, before new adsorption sites become available. Alternatively,

if we consider that as monolayer coverage is attained so the more active hydrophilic adsorption sites are depleted and the enthalpy of adsorption will become significantly less exothermic ($\triangle G \longrightarrow +ve$). It is known that monolayers of HFb molecules at fluid-fluid interfaces undergo lateral molecular interactions, which are time-dependent forming a pseudo-fibrin polymer like film.[287] If this is occurring at the solid-solution interface in the afore-mentioned adsorption study, it would be expected to then raise the exothermic enthalpy (net $\triangle G = -ve$), for adsorption, enabling the second monolayer to adsorb onto the first monolayer. As previously stated in Section 6.2 there will be a greater tendency for the initial monolayer to be completed, if it is significantly more exothermic, ($\triangle G = -ve$), than for further successive layers, but little tendency for the second monolayer to be completed prior to adsorption into the third and subsequent layers. From the sigmoidal adsorption curves for HFb onto both types of poly (HEMA) preparations, the above would seem to be true, and if so, monolayer coverage for these materials would seem to be enthalpically driven, rather than entropically. Thus a low entropic component would seem to indicate little conformational change in the adsorbing HFb molecule onto hydrophilic poly (HEMA).

If the values for HFb adsorption onto the commercial poly (HEMA), ($\sim 475 \times 10^{-8}$ g.cm.$^{-2}$), and the purified poly (HEMA) preparation, ($\sim 865 \times 10^{-8}$ g.cm.$^{-2}$), are considered as being at or near a steady state value, ($\sim$ 70 hours), the relatively greater hydrophobic nature of the purified preparation can be observed to adsorb far higher levels of HFb.

This is perhaps indicative of the preference of HFb molecules to undergo hydrophobic interactions, as well as the more exothermic hydrophilic interactions of hydrogen-bonding and dipole-dipole, common to most proteins. The influence of bulk concentration on the multilayer formation can be ascertained from the data in Appendix 5. When the concentration is reduced, a much reduced multilayer level is evident.[64]

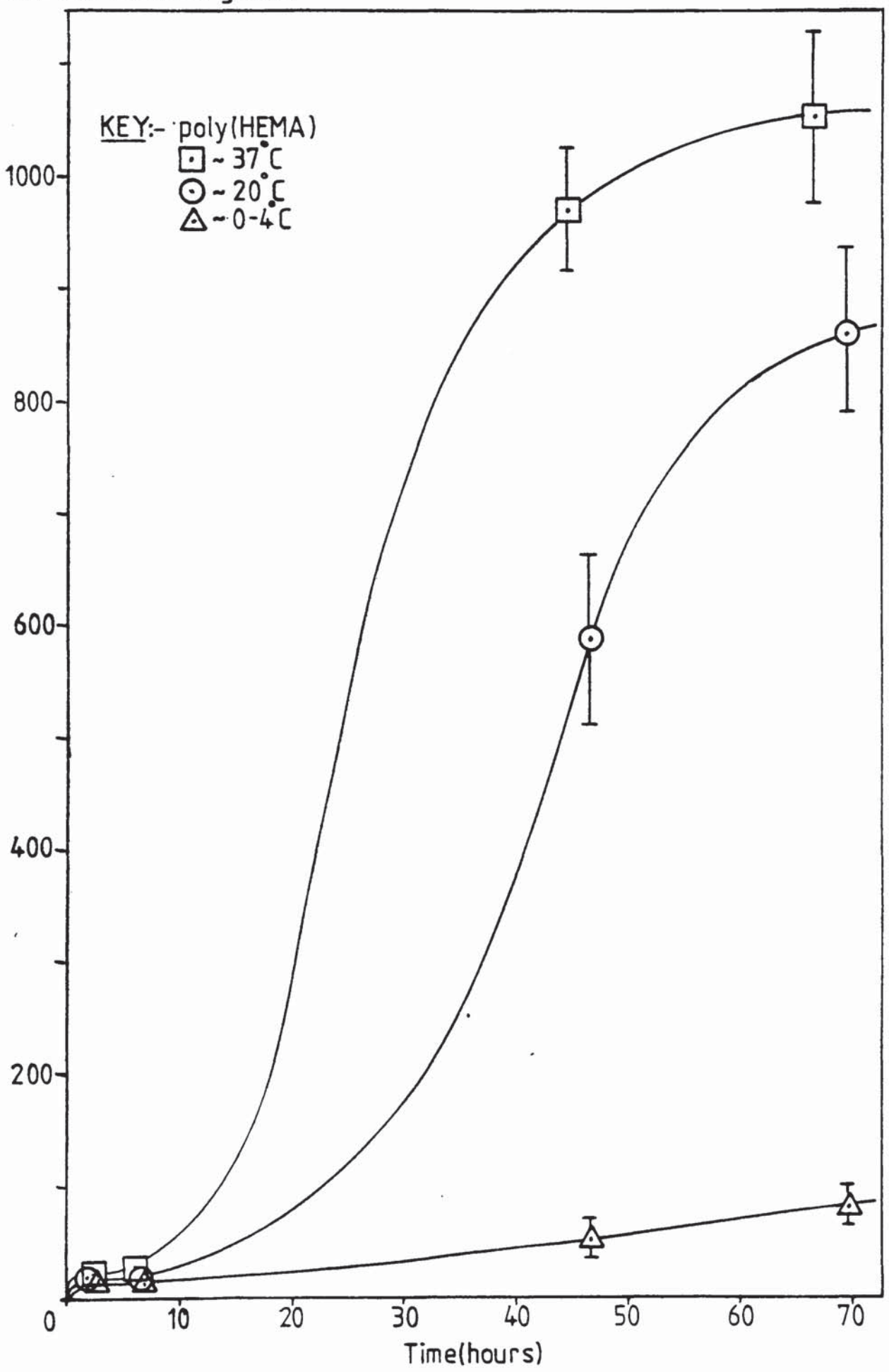
The effects of temperature on the adsorption of HFb molecules onto purified poly (HEMA) was investigated. The results illustrated in Graph 6.1 (ii), indicate a significant rise in the multilayer adsorption level with increasing temperature. However, over the same temperature range, no appreciable change in the rate of formation of the initial time dependent monolayer coverage is evident. This would seem to reaffirm that the monolayer formation is of an exothermic nature.

The rate of adsorption will of course also be governed by the temperature dependent diffusion constant for the molecular specie concerned, and this is indicated in Graph 6.1 (ii) by the increase slope with increasing temperature.

It can be seen in Graph 6.1 (i), that HSA adsorption onto the two types of poly (HEMA), preparation, are of a similar parabolic pattern to each other, in contrast to the sigmoidal pattern exhibited with the HFb adsorption.

The difference may be a function of the higher diffusion constant of HSA compared to HFb, which would mean that a higher adsorption rate is possible. This coupled with the higher bulk concentration of HSA to HFb,

Graph 6.1(ii): Influence of Temperature on Adsorption



(both are $\sim 1/50^{th}$ physiological concentrations of plasma), makes identification of a rate-limiting step for the monolayer coverage impossible, without further detailed study. Alternatively, however, this difference between HSA and HFb adsorption, may be attributed to the chemical differences between the two molecules. Substantial differences in surface/interfacial chemistry for these two proteins, have been reported for adsorption at fluid-fluid interfaces. HFb molecules in contrast to HSA molecules, tend to undergo time-dependent lateral interactions which increases the cohesive strength of the adsorbed film.[287] However all protein solution interfaces are known to undergo some form of time-dependent changes in the interfacial pressure of the adsorbed film.[34] It is perhaps therefore simpler to ascribe the observable differences in the adsorption curves for HFb and HSA as being directly attributable to their own particular physico-chemistry.

Again from the previously mentioned calculated weight per unit area for a complete monolayer coverage of HSA molecules of native or 'near' native conformation, (i.e., $\sim 20 \times 10^{-8}$ g.cm.$^{-2}$), would indicate that multilayer adsorption is occurring. It is of interest to note that the adsorbed levels of HSA on the commercial poly (HEMA) preparation, ($\sim 300 \times 10^{-8}$ g.cm.$^{-2}$) and purified poly (HEMA) preparation ($\sim 175 \times 10^{-8}$ g.cm.$^{-2}$), are of a much lower value and are the reverse of those found with HFb adsorption. Conformational studies[69] have shown that the albumin molecule possesses a far greater inherent conformational stability compared to fibrinogen molecules. With this information at hand, it is not hard to understand

why HFb molecules will tend to undergo greater conformational changes on adsorption, than HSA, and in so doing will tend to raise their multilayer adsorption due to entropic factors. Because of the greater molecular size of HFb it will have a greater number of interactions per molecule than the smaller HSA molecule and accordingly the increase in intramolecular strain due to these interactive forces will be greatest for HFb, and consequently results in its low stability. Of course the above adsorption process is also a surface-dependent phenomenon, and it will be the surface/interfacial chemistry of the adsorbent, which will determine the degree of interactions with a specific molecular specie. The greater HSA adsorption with the relatively more hydrophilic commercial poly (HEMA) (containing carboxyl group impurities) is precisely what would have been expected, based on the known highly hydrophilic, ligand-binding characteristics of the albumin molecule. Accordingly, from its lower solubility the HFb molecule will tend to show a greater preference for hydrophobic interactions than would the HSA molecule, and thus accounts for the higher level of adsorption of HFb on the purified poly (HEMA) with a lowered hydrophilicity.

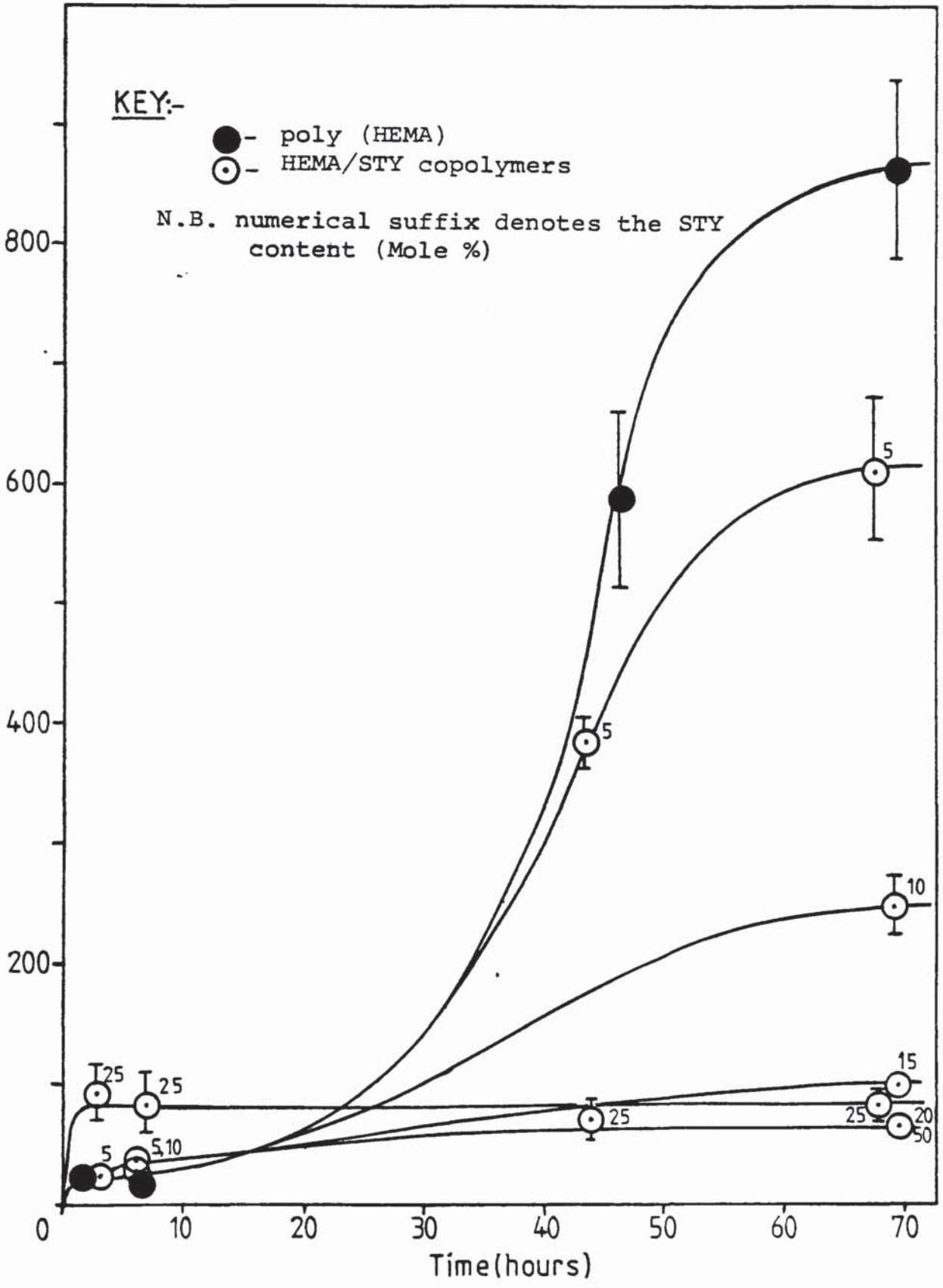
It should be pointed out that in no way can the adsorption characteristics of either fibrinogen or albumin molecules, be simply classified under the respective general headings of hydrophobic or hydrophilic interactions. Both molecules possess many polar and non-polar groups along their polypeptide chains, fibrinogen for example also contain very hydrophilic carbohydrate moieties (4% by weight). Therefore the adsorption phenomena for each individual molecule is an extremely complex array of interactions which

can consist of hydrophobic (entropic), Van der Waals, hydrogen-bonding, dipole-dipole, dipole-induced dipole, ionic, and in some circumstances possible covalent disulphide interactions will occur.

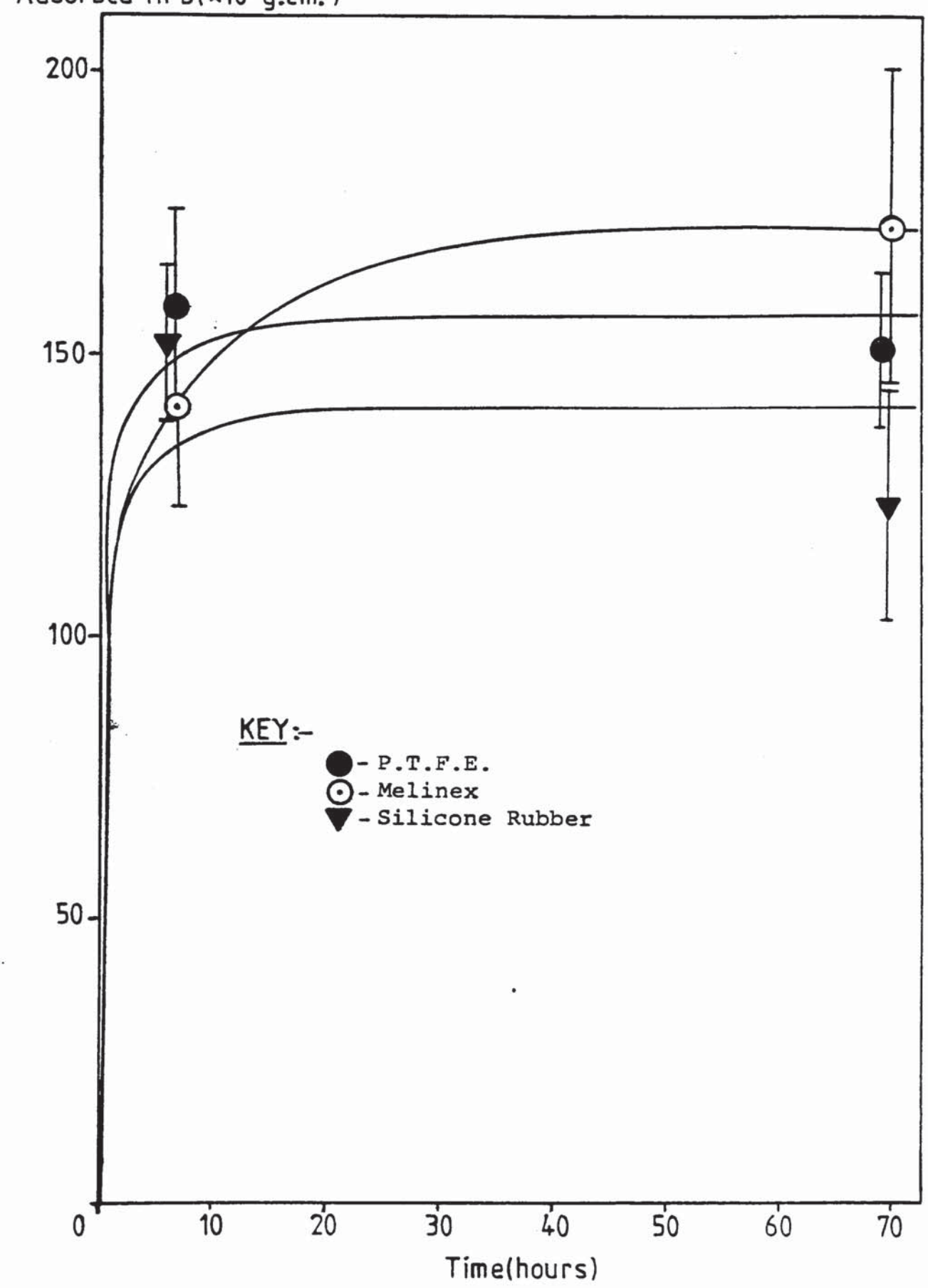
6.7.2 Hydrophilic-Hydrophobic Copolymers

Incorporation of an increasing percentage of the very hydrophobic monomer, styrene, to form HEMA/STY copolymers, somewhat in contrast to the findings for the commercial and purified poly (HEMA) preparations, produces a progressive decrease in the adsorption of HFb (Graph 6.2(i)). On closer examination of the HFb adsorption on these HEMA/STY copolymers, it is evident that not only is the HFb adsorption level decreasing as the hydrophobic content increases, but that the sigmoidal shape associated with the hydrophilic material(s), becomes modified to give a more parabolic curve. This latter aspect is reinforced by the HFb adsorption exhibited by the hydrophobic polymers of poly tetra-fluoroethylene (PTFE), polyethylene-terepthalate (melinex) and poly dimethyl-siloxane-rubber (silicone rubber), as depicted in Graph 6.2(ii). These hydrophobic materials exhibit a low level steady state (of between (140-175) $\times 10^{-8}$ g.cm.$^{-2}$), which is attained very rapidly in contrast to the slow build-up observed with the hydrophilic materials so far examined. The theoretical monomolecular adsorption level for HFb in its native conformation, lies somewhere in the range of (12-21) $\times 10^{-8}$ g.cm.$^{-2}$ (See Appendix 5), and it would therefore appear that far less multilayer formation is occurring on the more hydrophobic polymers/copolymers. This may simply be attributed to the low enthalpy-endothermic interactions which occur between hydrophobic groupings, however,

Graph 6.2(i): Adsorption data for the HEMA/STY copolymers



Graph 6.2(ii): Adsorption data for hydrophobic copolymers

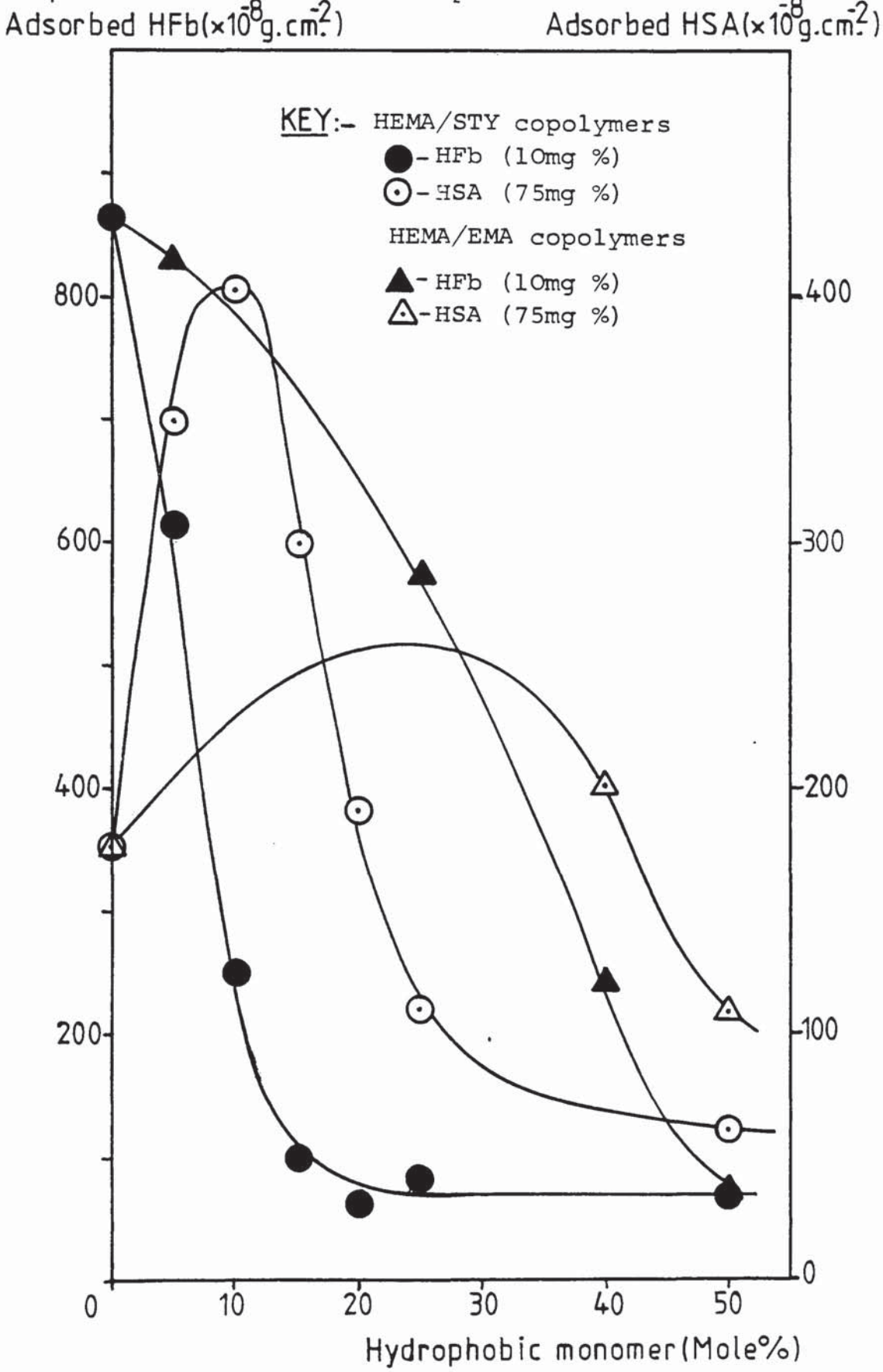


alternatively it is very likely that large conformational changes take place in the adsorbing HFb molecule, due to mainly entropic interactions (factors), which have the tendency to spread the molecule, and in so doing a theoretical monolayer coverage of 1.0×10^{-8} g.cm.$^{-2}$ or less, is feasible. Thus hydrophobic HFb multilayer formation may well be comparable to that observed with hydrophilic materials, however, direct comparisons of this nature are perhaps inadvisable, when considering the above somewhat obscure complexities of the adsorption phenomenon.

In Graph 6.2(iii), the values for adsorption at approximately 70 hours (which within the limits of experimental error can be taken as the steady-state value), for HFb and HSA adsorption onto HEMA/STY and HEMA/EMA copolymers, were plotted against the content of hydrophobic monomer (Moles %). It is interesting to note that certain of the trends in adsorption for the hydrophilic/hydrophobic copolymers do show correlation with the previously determined trends in bulk and surface structure-property effects. HFb adsorption decreases progressively with the progressive increase in styrene, until a 15-20 moles % content is achieved, after which further increase in the styrene content produces no apparent change in adsorption. As perhaps would be expected from the bulk and surface structure-property effects, the progressive increase of the less hydrophobic monomer ethyl methacrylate produces very similar effects on HFb adsorption, if less dramatic in nature than styrene.

Similarly, gross correlations can be observed for these hydrophilic/hydrophobic copolymers, in the rather

Graph 6.2(iii): Influence of Hydrophobic Monomer on Adsorption

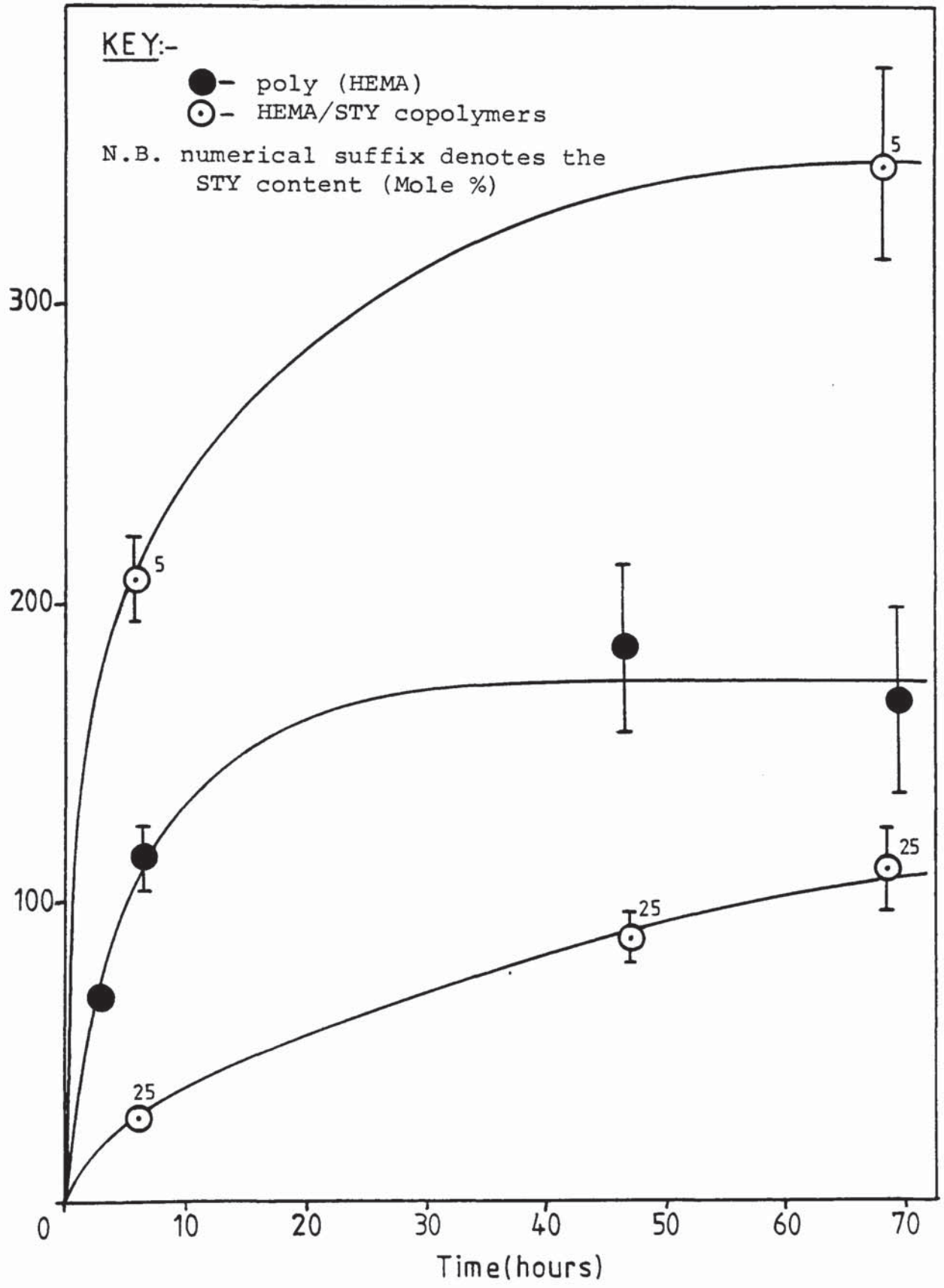


complex series of scatter diagrams, Graphs 6.6 to 6.10, relating their adsorption of HFb and HSA to their bulk and surface properties. The observed reduction in adsorbed HFb with increasing hydrophobic content can be correlated with a progressive decrease in water content (EWC%) a gross decrease in total surface free energy (γ_{sv}), and gross overall increases in the bound-water fraction and interfacial free energy (γ_{sw}).

The adsorption of HSA onto the HEMA/STY copolymers, as depicted in Graph 6.2(iv), exhibits quite substantial differences to those of HFb adsorption. The adsorption mechanism for HSA is believed to be a very different process from the adsorption of HFb molecules, in that albumin possesses sufficient internal bonding to stabilise and hold its native globular conformation, even on hydrophobic surfaces.[69] This parameter of HSA's physico-chemistry should render a far simpler interpretation of the adsorption data, in contrast to the problems encountered as a direct result of the dimensional instability of the HFb molecule.[282]

Previously in Chapter 5, it was found that an initial small percentage addition of styrene to poly (HEMA), instead of reducing surface wettability and surface free energy (γ_{sv}), actually increased these parameters to above that observed for poly (HEMA). With still further increase in the styrene content, the expected reduction in wettability and free energy were elicited. From Graph 6.2(iii), very similar gross effects can be seen for the HSA adsorption onto these HEMA/STY copolymers, and it would therefore seem very likely that the raised level of HSA adsorption over the range 5-20 (Moles %) STY content, was due in the

Graph 6.2(iv): Adsorption data for HEMA/STY copolymers



main to hydrophilic, exothermic interactions. Since adsorption of HSA attains a maximum on HEMA/STY 90:10 (Moles %), and based on the surface chemistry analysis (Chapter 5), it would seem that although hydrophilic interactions predominate, hydrophobic interactions cannot be discounted, for it would appear that maximum adsorption occurs at the most preferential ratio of hydrophilic (polar) - hydrophobic (non-polar) interactions feasible, and not solely at the maximum surface concentration of hydrophilic groupings, (i.e., HEMA/STY 90:10 (Mole %) γ^{d}_{sv} = 18.0 dynes. cm.$^{-1}$ and γ^{p}_{sv} = 30.8 dynes. cm.$^{-1}$).

With further increase in the hydrophobic content of the HEMA/STY copolymers, the observed decrease in the HSA adsorption onto the highly hydrophobic material, involves no entropic conformational changes and is simply due to a net decrease in interactions, as the adsorption process tends towards being exclusively endothermic-hydrophobic interactions. Again, similar, if less pronounced changes in the adsorption phenomena are seen to occur, when the smaller ethyl methacrylate molecule is substituted for the bulky highly hydrophobic phenyl group of styrene.

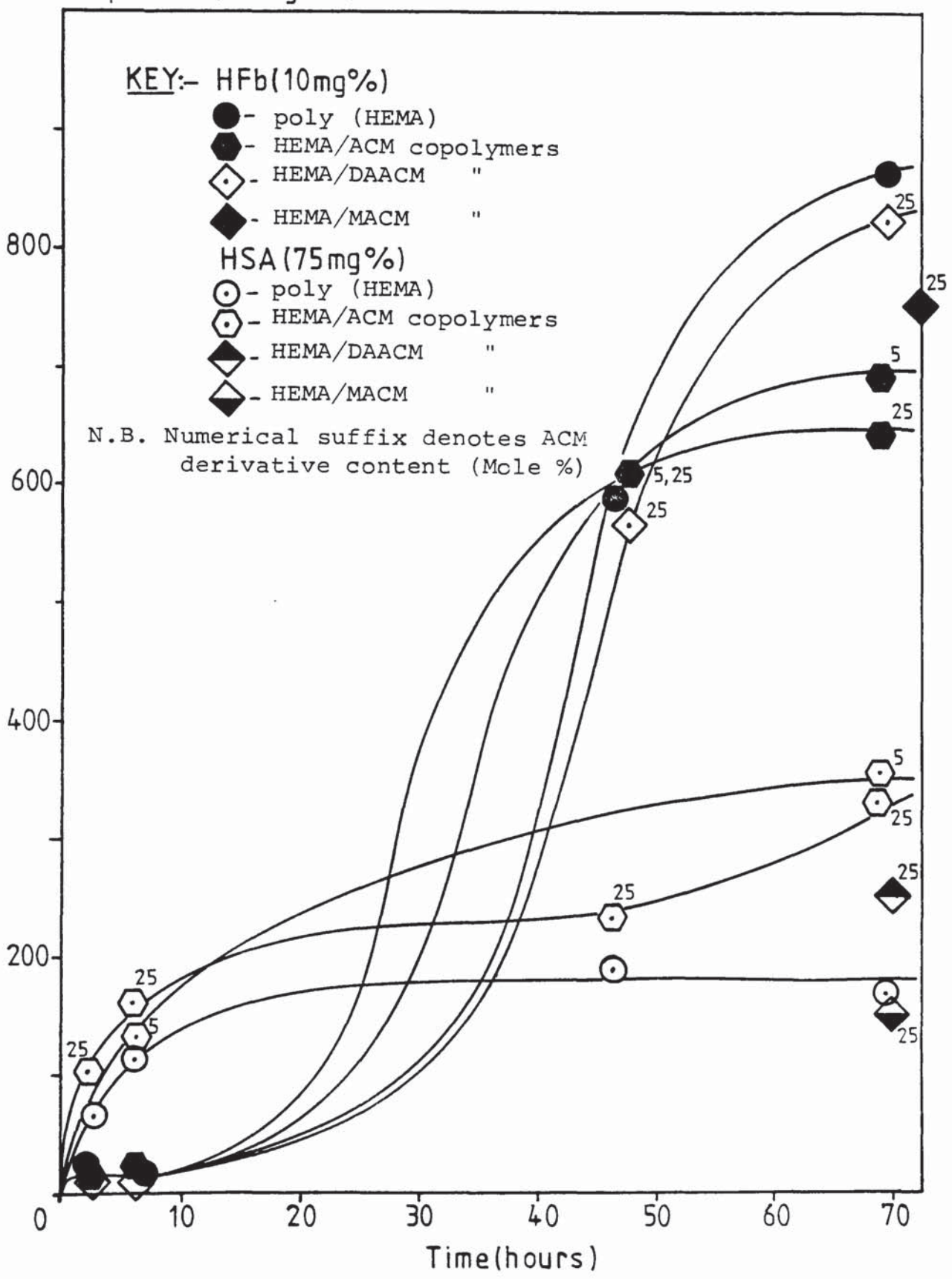
In contrast to the adsorption of HFb molecules, HSA adsorption does appear to follow a more precise correlation with the surface chemistry parameters of, total surface free energy (γ_{sv}) and interfacial free energy (γ_{sw}), and the surface active bulk chemistry parameters of water content (EWC %) and bound water-fraction measurements, (Graphs 6.6 to 6.10), for the hydrophilic/hydrophobic copolymers of HEMA/STY and HEMA/EMA.

With the substitution of 2-hydroxy ethyl methacrylate by the very much more hydrophilic N-vinyl pyrrolidone molecule, to form NVP/EMA copolymers, the unusual chemical characteristics of this monomer (discussed earlier in Chapters 4 and 5) do seem to greatly influence the adsorption of both HFb and HSA (See Appendix 5 for values), and results in far greater adsorption levels than its HEMA counterparts. These very high water content copolymer compositions of 95:5 and 75:25 (Moles %) NVP/EMA, although fitting into the criteria for the hydrogel material subset of hydrophilic/hydrophobic copolymers, they show quite different adsorption patterns, as compared to the other members of this subset so far discussed. Addition of the hydrophobic EMA dramatically increases the protein binding potential of this class of hydrogels, both for HFb and HSA. Again a maximum binding potential would be expected at the most preferential hydrophilic/hydrophobic ratio. Further increase in the EMA content, would be expected to produce a reduction in the adsorption level for both HFb and HSA, in a similar fashion as to that exhibited by the copolymers of HEMA/STY and HEMA/EMA.

6.7.3 Hydrophilic-Hydrophilic Copolymers

The previously described sigmoidal HFb adsorption pattern evident with hydrophilic poly (HEMA), is again observed for the HEMA/ACM (and ACM derivatives), and is indicative of multiple layering of the adsorbed HFb molecules, (as depicted in Graph 6.3(i)). Also depicted on this graph is the adsorption with time of HSA onto the above copolymers, and as previously observed with poly (HEMA),

Graph 6.3(i): Adsorption data for the ACM derivatives

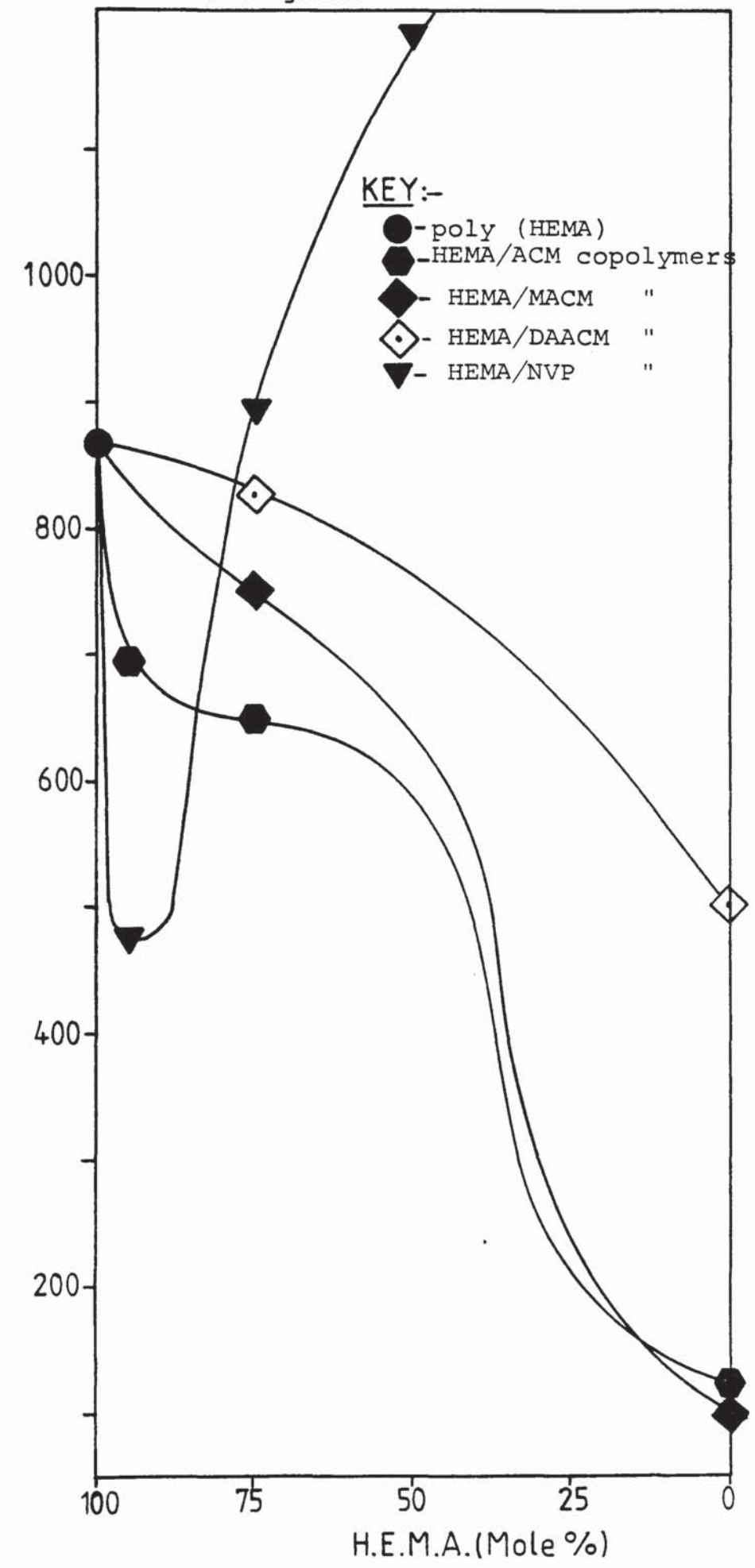


these show a more parabolic pattern, and multiple layering is self-evident, based on the adsorbed quantities (HSA monolayer coverage as previously derived $\sim 20 \times 10^{-8}$ g.cm.$^{-2}$)

Using the adsorption values at approximately 70 hours as the steady state values, these have been plotted against the HEMA content (Mole %) in the various copolymers of this hydrogel subset for both HFb and HSA, in Graphs 6.3(ii) and 6.3(iii) respectively.

From Graphs 6.3(i) and (ii) the addition of the monomer ACM, can be seen to reduce the adsorbed level of HFb quite drastically at first, but with further increase to a HEMA/ACM 75:25 (Moles %) composition the additional ACM molecules would seem to exhibit less surface activity, perhaps due to preferential bulk interactions. Extrapolation of the adsorption of HFb onto these HEMA/ACM copolymers is made possible by using the adsorption data for the solution polymerised poly (ACM). This seems to indicate that above the 50:50 Moles ratio, further additions of ACM once again are very effective in lowering the adsorbed level of HFb. It would therefore seem likely that, as previously mooted, HFb molecules tend to prefer hydrophobic interactions and that with the progressive increase in the surface hydrophilicity, due to ACM, an overall gross reduction in surface binding potential takes place. The reduction in surface binding potential for the HFb molecule, can also be attributed to the decrease in chain segment density per unit volume that occurs with high water content gels, such as poly (ACM). Effectively the exponential increase in pore size (See Chapter 4) with increasing water content will reduce the surface concentration of hydrophilic

Graph 6.3(ii): Influence of Hydrophilic Monomer(s) on Adsorption



and hydrophobic groupings on the chain segment backbone and side-chains, and the resultant spatial distribution of these hydrophilic/hydrophobic groups, tends towards a reduction in the total number of surface interactions possible, per unit area. Following on from this concept, it would appear that for maximum adsorption of a particular protein, not only must a preferential ration of hydrophilic/ hydrophobic groupings be present, but that they must co-exist in the "correct" preferential spatial arrangement(s) both on the "surface" of the adsorbent polymeric material and the adsorbing protein molecule. The adsorption level is of course also dependent on the strength or degree of intermolecular interactions, which are themselves dependent on the types of hydrophilic/hydrophobic chemical structures present; and this aspect is clearly evident, when the ACM derivatives, MACM and DAACM are substituted for ACM, in the HEMA/ACM copolymers depicted in Graph 6.3(ii).

The greater the hydrophobic character of these monomers, the stronger their interactions, and the greater their resultant net HFb adsorption level. Addition of the hydrophobic methyl group to the ACM backbone, to form MACM, shows in the HEMA/MACM 75:25 (Moles %) copolymer, a raised adsorption level in comparison to its HEMA/ACM counterpart. Similarly when the bulky hydrophobic 1,1 dimethyl-3-oxy-butyl substituent of DAACM is introduced, a still greater adsorption level is seen, as compared to either of its HEMA/ACM, HEMA/MACM counterparts. Extrapolation of the adsorption levels of these copolymers to that of their own respective solution polymerised poly (MACM) and poly (DAACM), indicates a net overall reduction in adsorbed HFb,

as previously seen with the ACM copolymers.

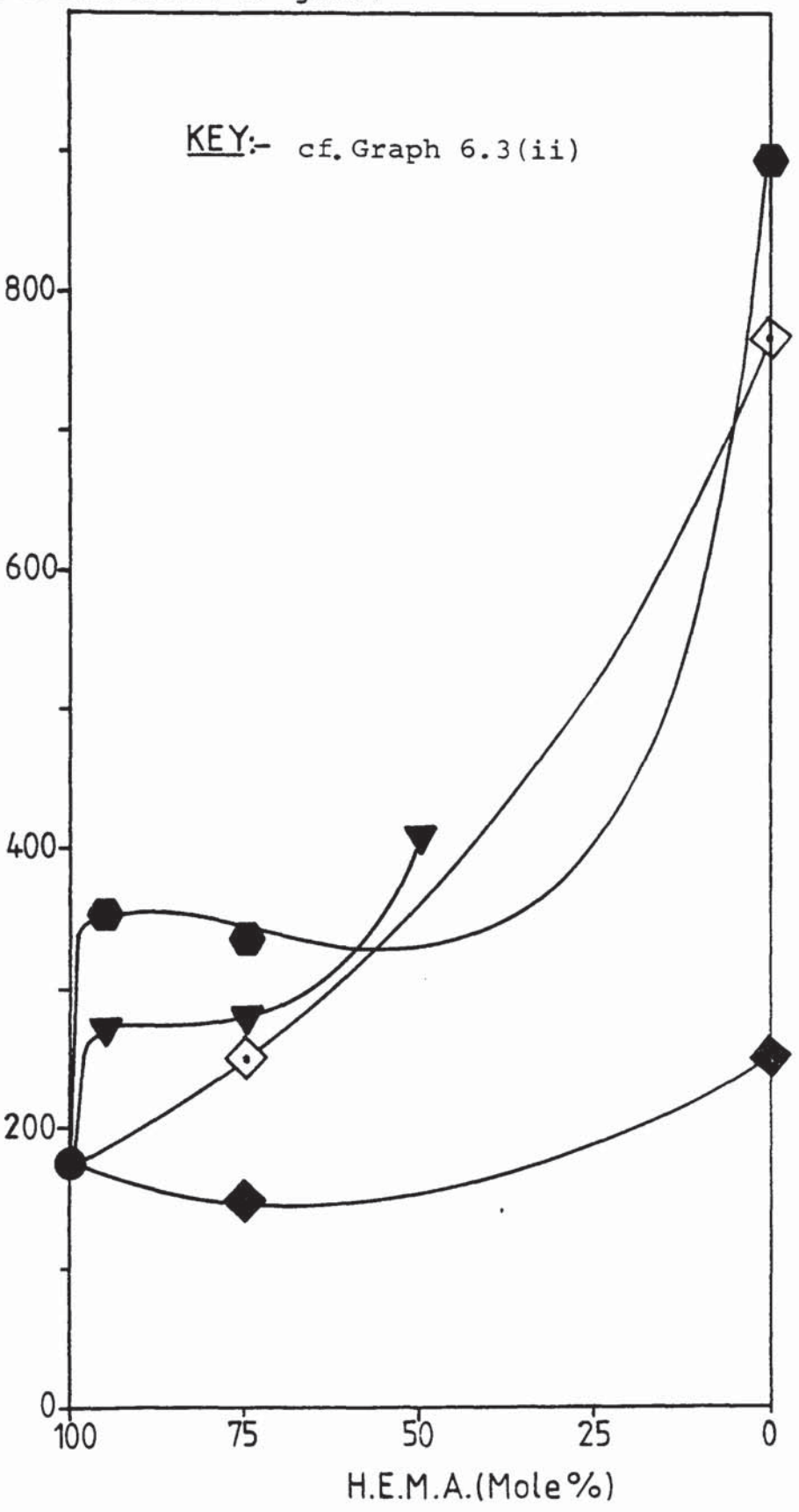
As would perhaps be expected, the decrease in HFb adsorption, resulting from the progressive increase of ACM, MACM and DAACM, can be seen to be accompanied by a rise in surface wettability and a commensurate fall in interfacial tension (γ_{sw}). (See Appendix 4 for data).

The opaque nature of the poly (DAACM), is indicative of phase-separation of the bulk-water and heterogeneity of the gel, and a possible anomalously large pore size, which would be expected to raise the adsorption level above that of its homogeneous counterpart. This would not be expected to greatly influence the adsorption of the large HFb molecule, but its effects on the smaller HSA molecule may well be significant.

In marked contrast to the adsorption of HFb, the adsorption of HSA onto the copolymers of HEMA/ACM, shows a significant rise in adsorption with a small 5 (Mole %) increase in the ACM content of the copolymers, as depicted in Graph 6.3(iii).

Further increase in the ACM content, is less effective in raising the HSA adsorption level, somewhat similar in nature to the reduced surface activity seen with the HFb adsorption, and again perhaps this may be attributed to the bulk interactions of the ACM molecule. Extrapolation of the adsorption data to that for poly (ACM), would seem to indicate that a fairly massive rise in HSA adsorption occurs with still further increases in ACM content. Although the rise in HSA adsorption with increase in ACM content is predictable, based on the preference of HSA to undergo hydrophilic-exothermic interactions, the "adsorption" of HSA onto the very high water content poly

Graph 6.3(iii): Influence of Hydrophilic Monomer(s) on Adsorption



(ACM) (~93% EWC), is somewhat suspect.

With such high water content gels, a fall in adsorption was to be expected, based on the previously discussed reduction in chain segment density and the accompanying reduction in surface interactions per unit area. However, this fall in adsorption is not observed and it may be that the very hydrophilic 1 mole % cross-linked poly (ACM) has the most preferential chemical arrangement for HSA adsorption. At such high water contents, an exponential increase in pore size is affected, and a theoretical pore size is produced large enough to enable the HSA molecule to penetrate or absorb into the gel matrix. The actual penetration or absorption of the HSA molecule into such high water content gels is therefore a distinct possibility, and will therefore be considered in the more appropriate section of this chapter concerning the cross-linking agents.

The introduction of the methyl group of MACM and the 1,1 dimethyl-3-oxy-butyl group of DAACM, as would be expected, produces less HSA adsorption than their ACM counterparts, as depicted in Graph 6.3(iii). Somewhat unexpectedly however, increasing the DAACM content would seem to progressively raise the adsorption level, whereas an increase in the MACM content, not unlike its bulk effects on water content (See Chapter 4), causes an initial depression of adsorption level prior to producing a net rise in adsorption, as determined by extrapolation to poly (MACM). It would appear that the hydrophobic 1,1 dimethyl-3-oxy-butyl and hydrophilic-amide substituent groups forming the long flexible side-chain of DAACM, confer a much greater potential for interaction with the amphoteric

HSA molecule, than the rather fixed hydrophobic backbone methyl group and side-chain amide group of MACM.

Substitution of ACM by the equally hydrophilic NVP molecule, to give HEMA/NVP copolymers, produces quite a different pattern of HFb adsorption, from that observed for ACM and its derivatives. From Graph 6.3(ii) it can be observed that a small content of 5 Mole % of NVP produces a significant fall in adsorbed HFb, but that further increase in NVP content effects an equally dramatic rise in the HFb adsorption level. This rather anomalous adsorption behaviour, does seem to bear out the equally anomalous surface chemistry structure-property effects discussed in Chapter 5.

From these surface chemistry measurements it was observed by comparison with poly (HEMA) that for the HEMA/NVP 95:5 (Mole %) copolymer, a fall in wettability and surface dispersive component (γ^{d}_{sv}) had occurred, together with a large rise in interfacial tension (γ_{sw}).

With further increase in the NVP content, a reverse in these trends was noted. It is very likely that if this copolymer series was extended, higher concentrations of NVP would inevitably raise surface wettability and lower interfacial tension, as a direct consequence of its very hydrophilic nature.

In conjunction with this, the accompanying rise in water content and resultant reduction in the chain segment densities, coupled with the change in hydrophilic/hydrophobic spatial distribution, a net reduction in surface binding potential takes place and thus the adsorption level falls.

This is born out to some extent by the copolymers of NVP/EMA, which show a fall in both HFb and HSA adsorption with an increase in the NVP content to give the high water content NVP/EMA 95:5 (Mole %) copolymer composition, (~ 90% EWC).

The adsorption of HSA onto the HEMA/NVP copolymers, as evident from Graph 6.3(iii), follows a very similar trend to that observed with the HEMA/ACM copolymers.

The addition of the monomer NVP can be seen to increase the adsorbed level of HSA, quite drastically at first, but with increase to give a HEMA/NVP, 75:25 (Moles %) composition, the additional NVP molecules would appear to exhibit less surface activity. This latter effect may well be attributable to bulk interactions, as previously proposed for the similar trend observed with ACM copolymers. Further increase in the NVP content to 50 Mole %, produces a substantial increase in adsorption and it is reasonable to assume that after reaching a peak adsorption level, even higher NVP content will subsequently produce a decrease in adsorption, in a similar manner to the previously described NVP/EMA copolymers. Extension of the HEMA/NVP copolymer series is necessary to confirm or disprove this hypothesis however, limitations will be imposed on this series by the problem of differentiating between actual adsorption or absorption of HSA, and both of which may occur with the high water content gels of 90% EWC and above.

6.7.4 The Influence of Cross-Link Density on the Protein Adsorption of Poly (HEMA) and Poly (ACM) Hydrogels

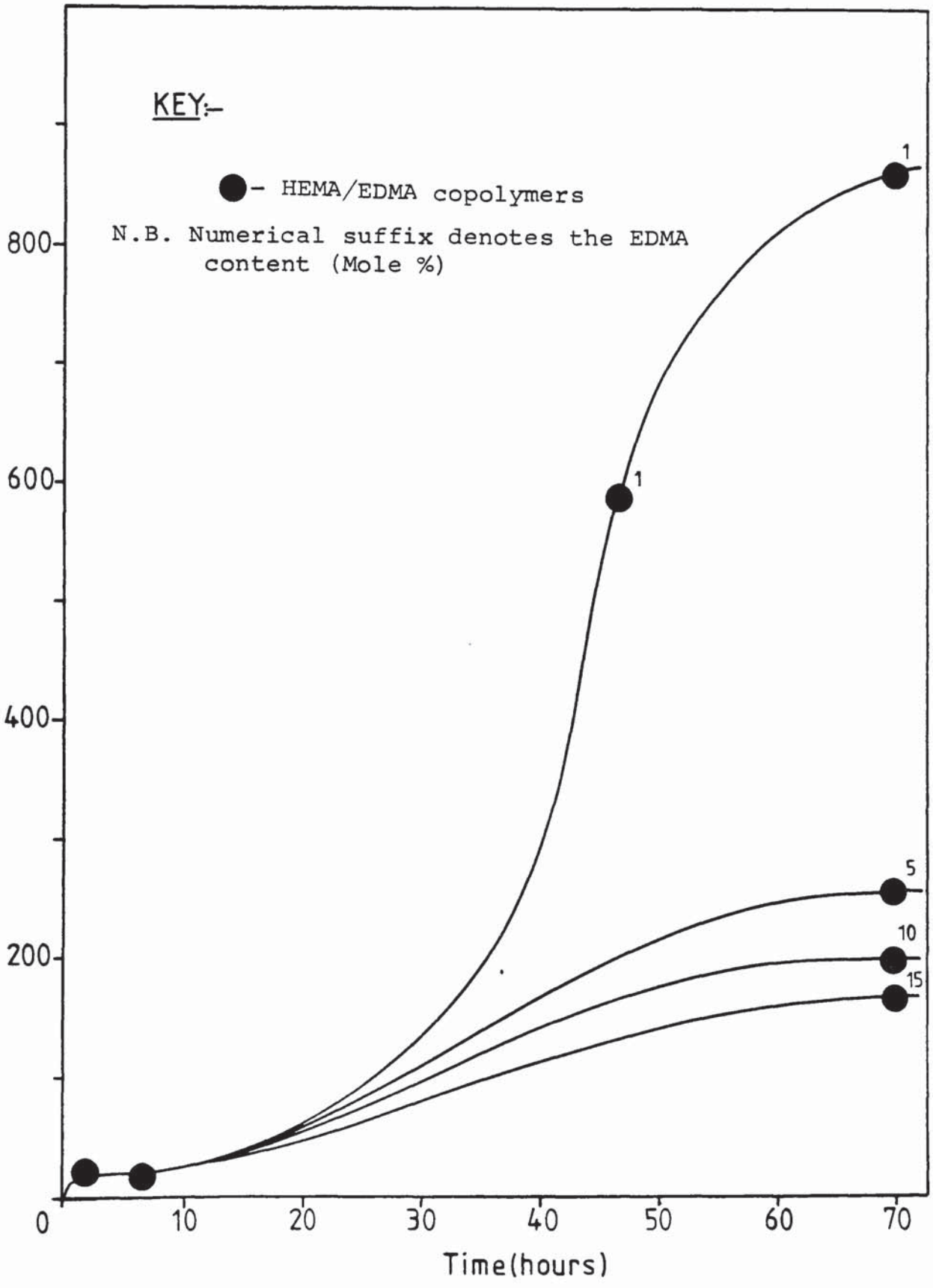
6.7.4(i): Poly (HEMA)

Graph 6.4(i) shows the adsorption of HFb with time,

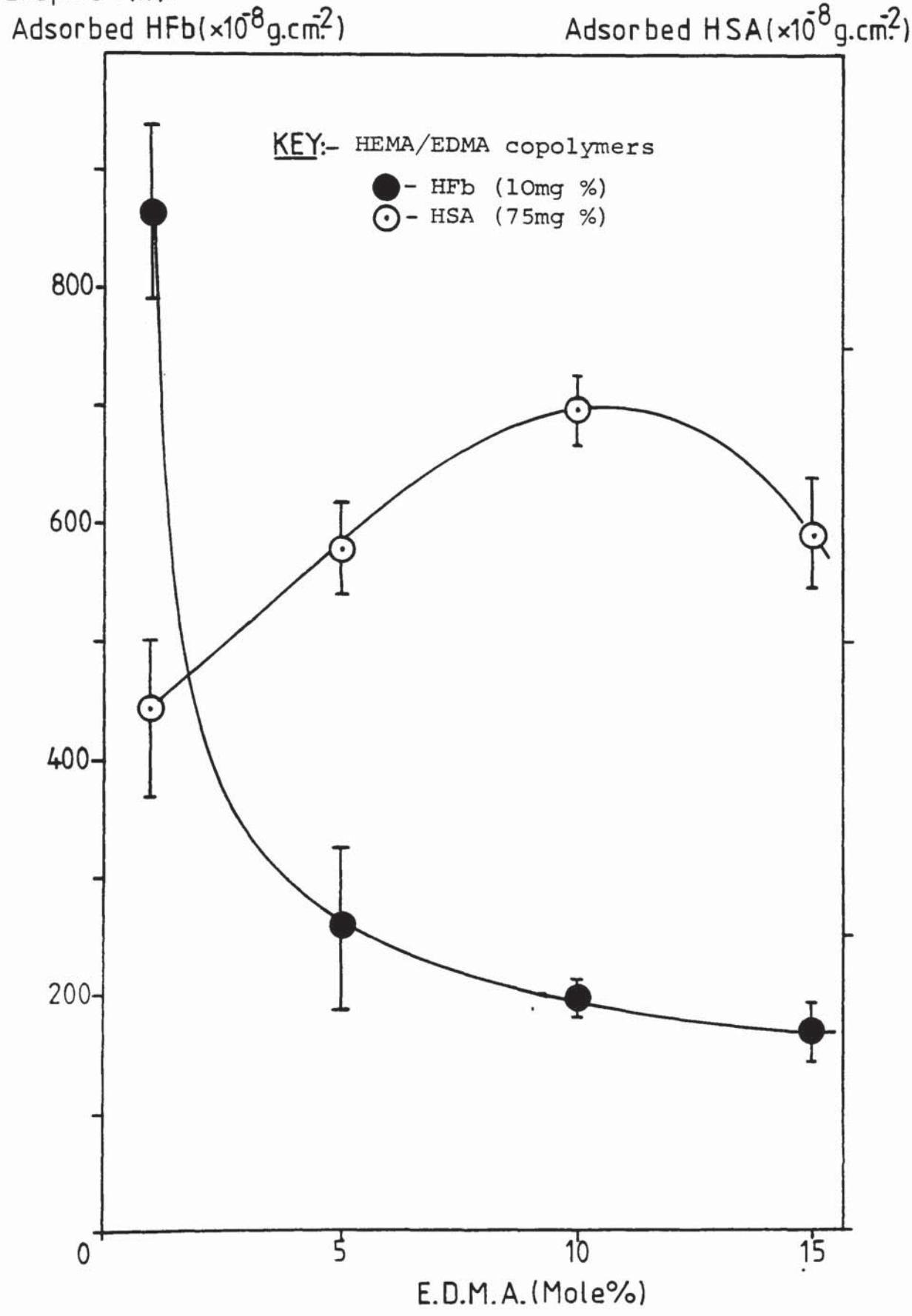
onto the copolymers of HEMA/EDMA, and that an increase in the EDMA content effectively reduces the HFb adsorption. This trend is perhaps more clearly illustrated by Graph 6.4(ii), which also depicts the HSA adsorption for these copolymers. It can be observed that initially a very marked reduction in HFb adsorption occurs when the EDMA content is raised to 5 Mole %, however, on further increase in the EDMA content its effectiveness in depressing the adsorption level, rapidly declines. Further increase in content above that for the HEMA/EDMA 85:15 (Mole %) copolymer, would not be expected to appreciably lower the HFb adsorption level. It is perhaps not unreasonable to assume that at a high enough EDMA content the "hydrogel" copolymers would exhibit similar adsorption profiles to those of the hydrophobic polymers discussed earlier. However, over the copolymer range covered in this project, the reduction in HFb adsorption levels is likely to be due to a very different mechanism from that postulated for the hydrophobic monomers of STY and EMA. This is to some extent reinforced by the rather remarkable surface data outlined in Chapter 5, in which it was discovered that progressive increase in the EDMA content produced a somewhat paradoxical increase in surface wettability, surface polarity (γ^{p}_{sv}) and total surface free energy (γ_{sv}), together with a reduction in interfacial free energy (γ_{sw}).

These surface effects were previously postulated as being attributable to the progressively non-random cross-link formation with increase in the EDMA content. This is believed to lead to anomalously large pore sizes, ($\leq 36\text{Å}$ dia),

Graph 6.4(i): Influence of Cross-linking on Adsorption



Graph 6.4(ii): Influence of EDMA on Adsorption



and long flexible chain segments, which allow re-orientation of the hydroxy ethyl side chains to present a higher than expected surface wettability.

This explanation can then fully justify both the fall in the HFb adsorption levels and also the rise in the HSA adsorption levels for these copolymers.

The peak HSA adsorption level observed with the HEMA/EDMA 90:10 (Moles %) copolymer, can be attributed in the main to hydrophilic-exothermic interactions. Subsequent increase in the EDMA content however, produces an increase in surface polarity/wettability, but a reduction in HSA adsorption and this would seem to reinforce the view that certain hydrophobic-low enthalpy interactions are also necessary for maximum HSA adsorption to occur.

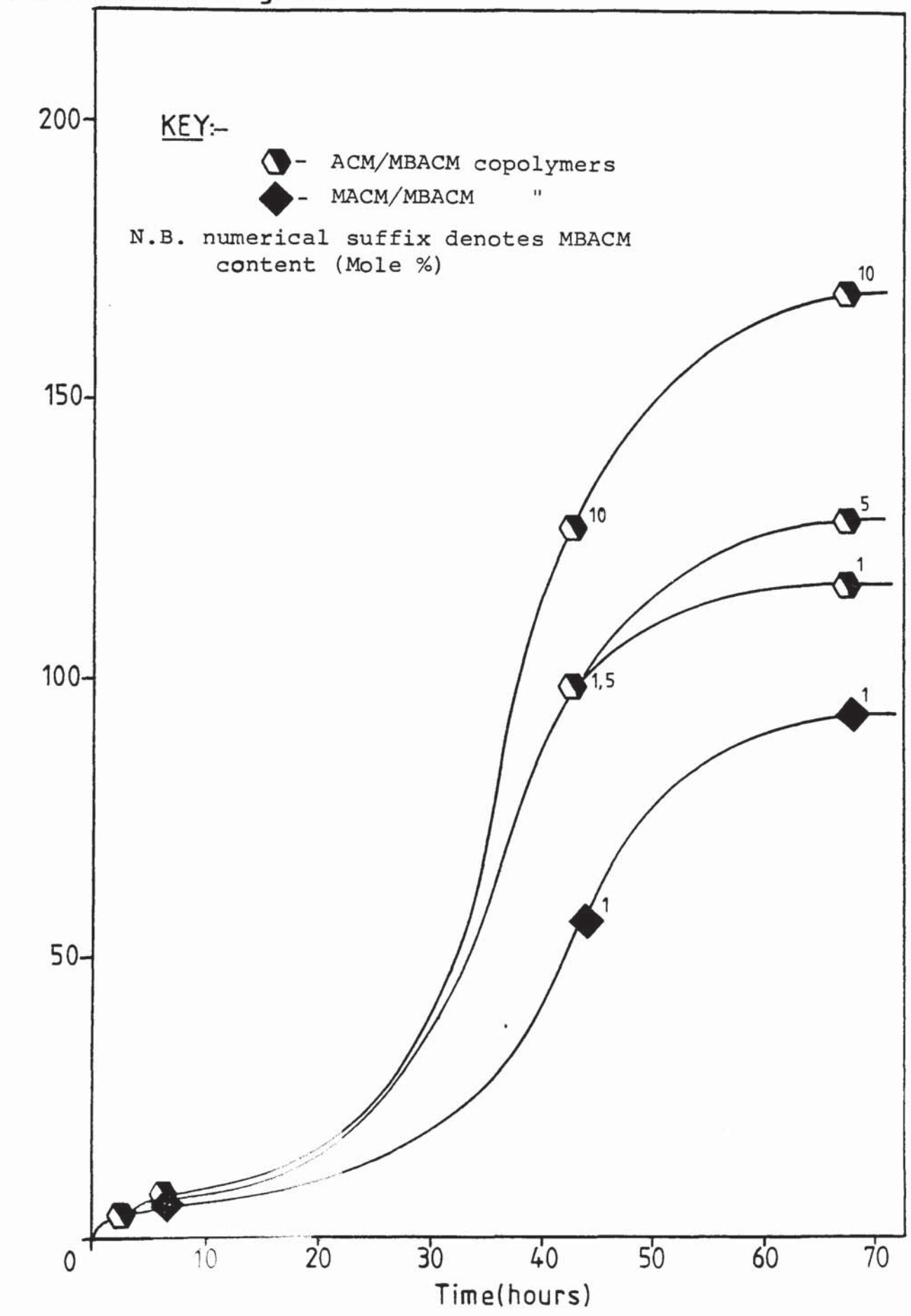
6.7.4(ii) Poly (ACM)

The adsorption of HFb onto the copolymers of ACM/MBACM, as evident from Graph 6.4(iii), follows the similar sigmoidal multiple-layering trend as observed for the other hydrophilic copolymers so far discussed.

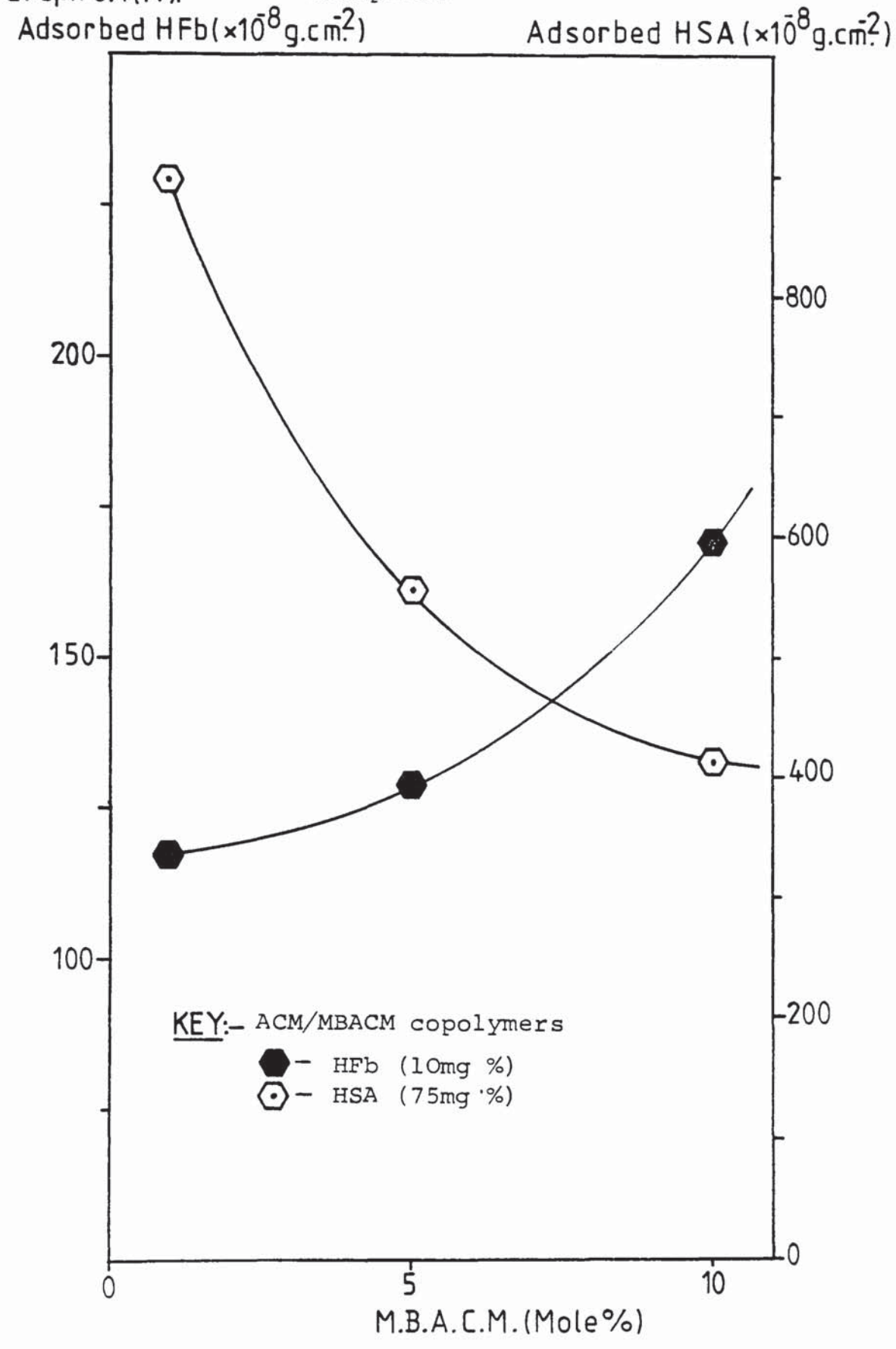
In contrast to other hydrophilic copolymers, the ACM/MBACM copolymers show very low HFb adsorption, as too does the ACM derivative, MACM/MBACM copolymer.

Addition of the MBACM cross-linking agent to poly (ACM), can be seen to produce only a relatively small increment in HFb adsorption, see Graph 6.4(iv). This rise may be attributable to the reduction in the surface polarity/wettability and/or, as a result of the increase in chain-segment density accompanying a reduction in water content, both of which will afford a greater degree of interaction with HFb molecules. In

Graph 6.4(iii): Influence of Cross-linking on Adsorption



Graph 6.4(iv): Influence of MBACM on Adsorption



contrast to the mechanism outlined for EDMA, the behaviour of MBACM would appear to be simply like that of a hydrophobic monomer. This is perhaps not too surprising when considering the high unbound 'bulk' water content of poly (ACM) as compared to that of poly (HEMA).

As previously mentioned, penetration or absorption of HSA molecules into the gel-matrix of the 93% EWC, poly (ACM) is a distinct possibility and therefore perhaps sorption may be a more appropriate term for such high water content gels. Based on the relationship between pore size and water content, as discussed in Chapter 4, then certainly the reduction in water content with increase in MBACM, should effectively exclude the penetration of HSA molecules and eliminate the problem of differentiating between adsorption and absorption. Some indication of this is given by the low HSA sorption evident with the 90% EWC NVP/EMA copolymer and by analogy, any absorption by the 82% EWC poly (ACM) will be insignificant. If this is then the case, the reduction in HSA sorption as depicted in Graph 6.4(iv), will be for the most part a reduction in adsorption, with reduction in absorption being prominent only over the high water content gel range above 90% EWC.

This reduction in HSA adsorption may therefore be ascribed to the increase in the hydrophobic nature of the gels and the commensurate decrease in protein-polymer interactions.

The very large standard deviations observed with HSA adsorption from a 75mg % solution, onto these copolymers, was not a feature of the HSA adsorption from a lower 50mg %

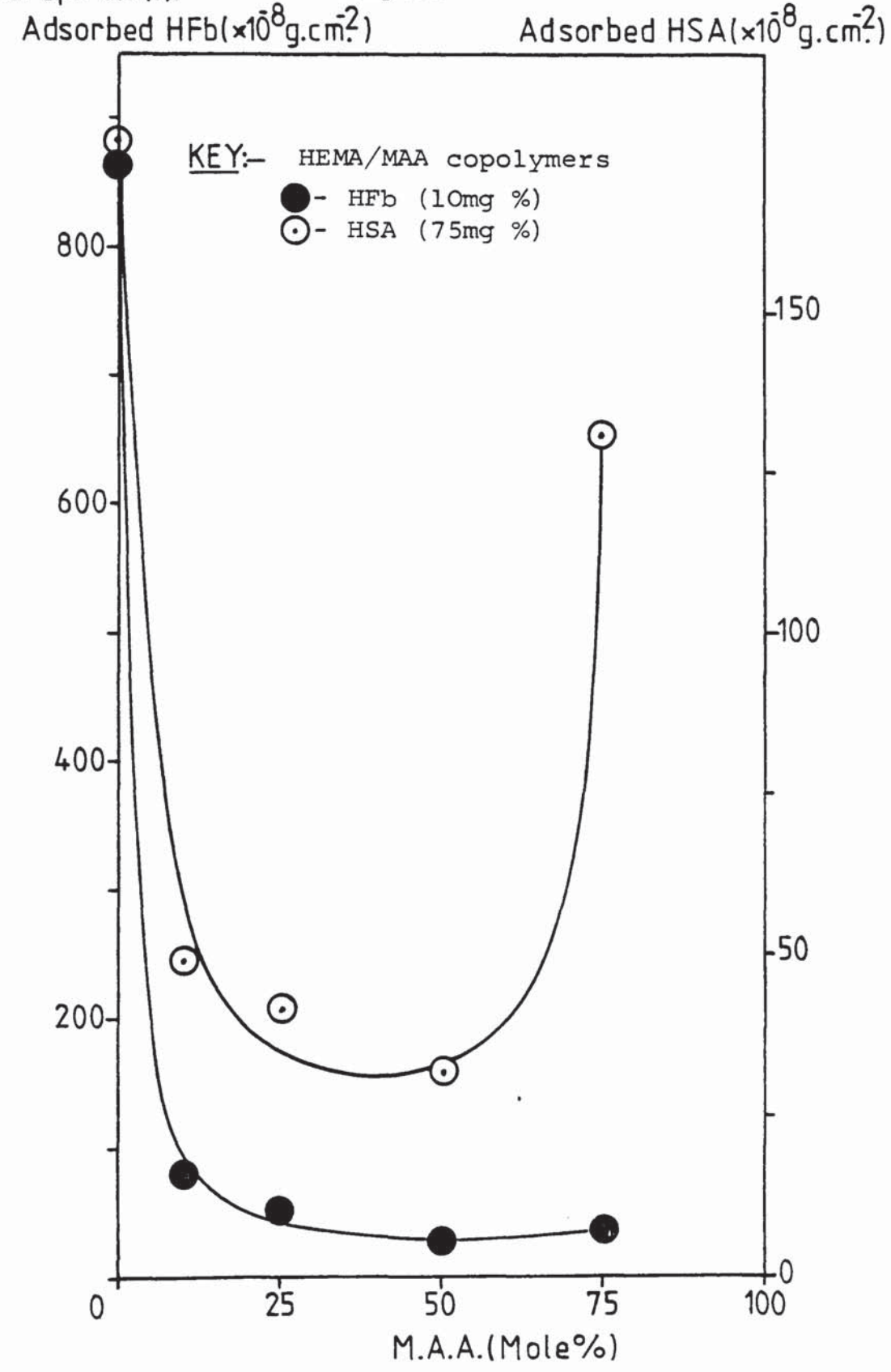
solution (See Appendix 5 for data), and this would seem to indicate that very high adsorbed multiple layers of HSA are unstable and perhaps therefore more prone to experimental error.

6.7.5 The Ionic Hydrophilic Copolymers

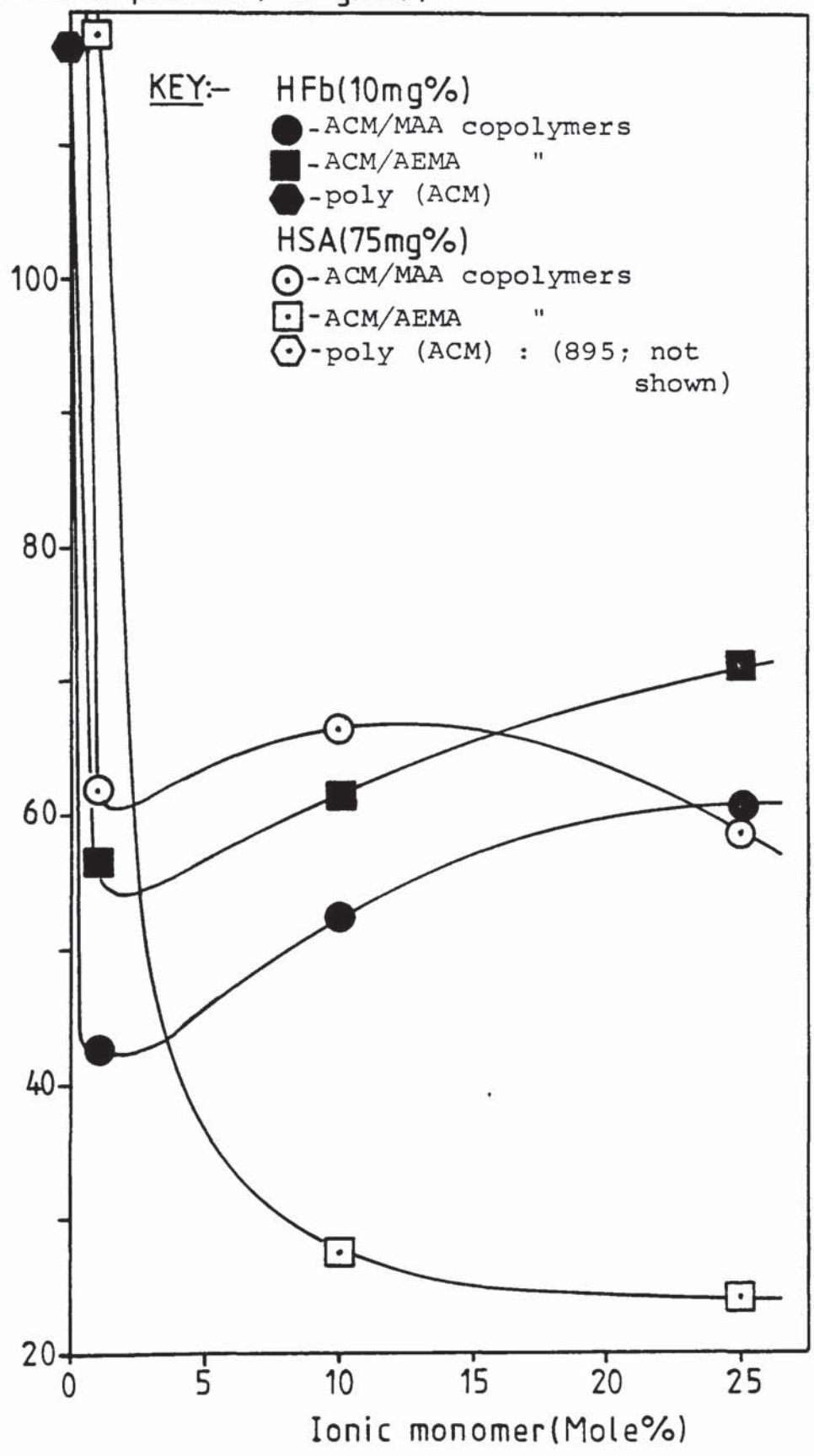
In contrast to the adsorption mechanisms so far postulated for the neutral hydrogels, the adsorption data for the ionic hydrogels as depicted in Graphs 6.5(i) and (ii), shows that for an increase in the ionic components both very low and HFb and HSA adsorption levels are attained, and these trends would seem to indicate that a substantially different adsorption mechanism is predominant for ionic hydrogels. From the known net negative charges possessed by both HSA and HFb at a physiological pH 7.4, it is believed that this adsorption mechanism is directly attributable to electrical double-layer effects which in turn produce a unifying electrostatic barrier to the phenomenon of multiple layer adsorption.

When the ionic copolymers are introduced into the physiological buffered Kreb's solution, they acquire a surface electric charge; possible charging mechanisms being ionisation, ion adsorption and ion dissolution. The surface charge influences the distribution of nearby ions in the aqueous medium. Ions of opposite charge (counter-ions), are attracted towards the surface and ions of like charge (co-ions) are repelled away from the surface. This, together with the mixing tendency of thermal motion leads to the formation of an electric

Graph 6.5(i): Influence of MAA on Adsorption



Graph 6.5(ii): Influence of Ionic Monomer(s) on Adsorption



double layer, made up of the charged hydrogel 'surface' and a neutralising excess of counter-ions over co-ions distributed in a diffuse manner in the aqueous medium.[209] The electric double layer can be regarded generally as consisting of two regions: an inner region which may include specifically adsorbed ions attached to the surface by electrostatic and/or Van der Waals forces strongly enough to overcome thermal agitation, and an outer region in which ions are distributed according to the influence of electrical forces and random thermal motion.

Surfaces in contact with aqueous media are more often negatively charged than positively charged. This is a consequence of the fact that cations are generally more hydrated than anions and so have the greater tendency to reside in the bulk aqueous medium, whereas the smaller, less hydrated and more polarising anions have the greater tendency to be specifically adsorbed.[209] Surfaces which are already charged by ionisation, usually show a preferential tendency to adsorb counter-ions, and it is possible that counter-ion adsorption can cause a reversal of surface charge.

If the negatively charged HSA and HFb molecules are considered as co-ions, then accordingly electrostatic repulsions would be expected to considerably reduce their adsorption onto a anionic hydrogel. This electrical double layer effect would seem to fit the adsorption data given for the HEMA/MAA copolymers in Graph 6.5(i). Why the HSA adsorption should increase with the increase in MAA content

to form the HEMA/MAA 25:75 (Mole %) copolymer is uncertain however, it is of interest to note that the HSA adsorption trends for these copolymers do seem to correlate with the decreases observed in EWC and bound fraction and perhaps indicates a reduction in ionisation.

In theory the ionisation of the HEMA/MAA copolymers would lead to a negative charge together with a commensurate rise in wettability and EWC plus fall in interfacial tension, all directly attributable to specifically sorbed highly hydrated sodium ions (the predominant counter-ions in solution).

The experimentally determined trends in surface and bulk characteristics of the HEMA/MAA copolymer series do seem to show some interesting, if somewhat complex correlation with the above postulated HFb and HSA adsorption mechanisms, as is illustrated in the series of scatter diagrams Graphs 6.6 to 6.10.

Inclusion of MAA (1 Mole %) and AEMA (1 Mole %) into the 93% EWC poly (ACM) composition produces similar dramatic reduction in both HFb and HSA adsorbed levels, as indicated in Graphs 6.5(i) and (ii). In fact because these ACM/MAA and ACM/AEMA copolymers have greater water contents, and by inference greater pore sizes than poly (ACM), it would seem that inclusion of these ionic moieties not only decreases adsorption but effectively decreases or eliminates any HSA absorption into the highly porous gel-matrix. It is likely that the above trends are again attributable to the electric double layer mechanism outlined

for the HEMA/MAA copolymers, however, by analogy the ACM/AEMA copolymers should show a raised adsorption. These somewhat anomalous findings may be explained by the argument that anions specifically-adsorb by way of electrostatic and/or Van der Waals forces to convert the surface to a negative charge.

As a direct consequence of their small hydration shells and higher polarity, anions have a greater tendency than cations to adsorb and produce this conversion of surface charge.

The ordering of ions by the electric double layer effect, together with their specific hydration shells, is not incompatible with the view that it is the water structuring effects of hydrogels that determines their adsorption. Similarly it is not incompatible with the above, that the changes in adsorption observed with these ionic hydrogels, is a feature of electrostatically induced intramolecular-strain in the conformation of the adsorbing protein molecules.

6.7.6 Bulk and Surface Effects

In an attempt to illustrate some of the previously mentioned correlations between bulk and surface characteristics and adsorbed HSA and HFb, a series of scatter-diagrams have been constructed, Graphs 6.6 to 6.10. Both the bulk and surface data and the adsorption data, are each of a somewhat diverse complex nature in their own right, and any attempt at their correlation will only be successful in all feasibility when limited to general trends or principles.

1. Bulk Effects

1.1 Equilibrium Water Content (Graphs 6.6(i) and (ii)

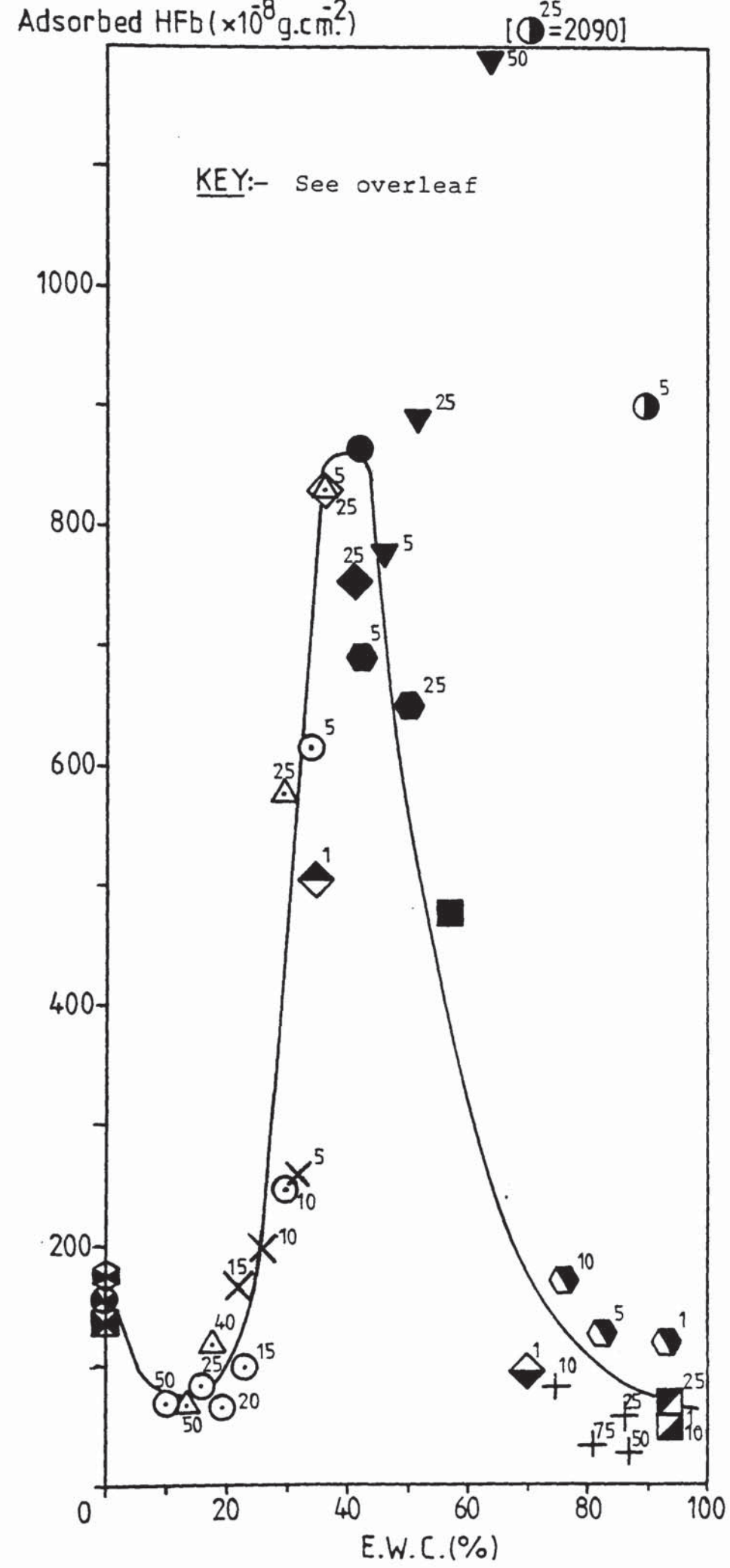
The relationship between HFb adsorption and EWC is illustrated in Graph 6.6(i). A somewhat empirical division of this data into three distinct types of adsorption is given below:

Region 1 - This consists of the low water content hydrogels (< 30% EWC) which have very small 'pore' size, high chain segment density and high surface concentration of hydrophobic groups. Very low HFb adsorption is evident and this may be attributed to mainly low enthalpy hydrophobic Van der Waals interactions, which are entropically driven by the conformational instability of the relatively hydrophobic HFb molecule.

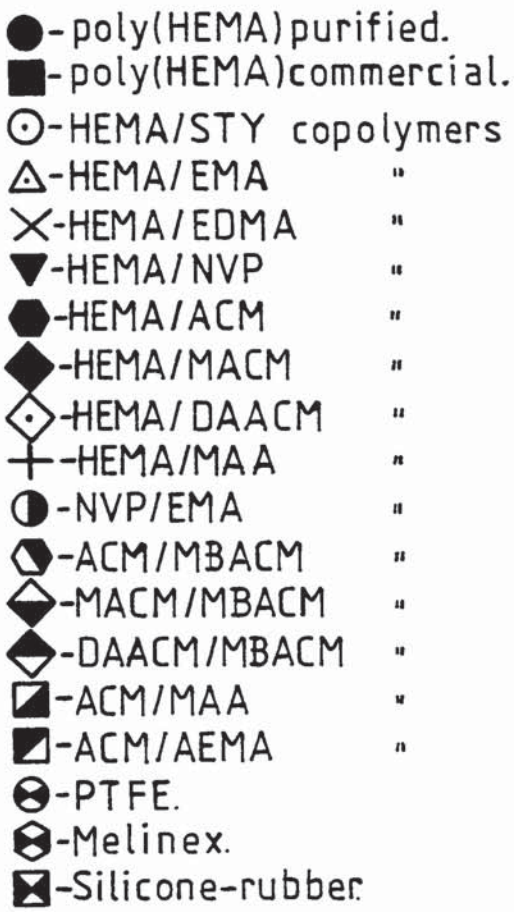
Region 2 - These gels have moderately high hydrophilicities within the $\sim$ range 30-60% EWC and accordingly small 'pore' size ($\sim \leq 10\text{Å}$ dia), high chain segment density and high surface concentration of both polar and non-polar groupings. Maximum adsorption is attained for these hydrogels due to their high surface binding potentials. Adsorption is driven by a combination of exothermic (high enthalpy) hydrophilic (polar) interactions together with the more low enthalpy hydrophobic interactions. Some entropic drive may also be present.

Region 3 - This region consists of very high water content gels (> 70% EWC), (moderate to large 'pore' sizes, $\sim$ 20-200Å dia), chain segment density and surface concentration of predominant hydrophilic groups are a variable feature, dependent on the pore dimensions. Low HFb adsorption is the feature of this region and results from both low enthalpy hydrophilic interactions and low entropy resulting from apparent conformational stability of adsorbing HFb molecules.

Graph 6.6(i): Relationship of Bulk Water to Adsorption



General Key for the Scatter-graphs:



* N.B. Numerical suffix to the symbols denotes the second monomer content (Mole %)

These three regions are not in complete isolation from each other and in fact a degree of continuity between the regions is indeed indicated, as would be expected.

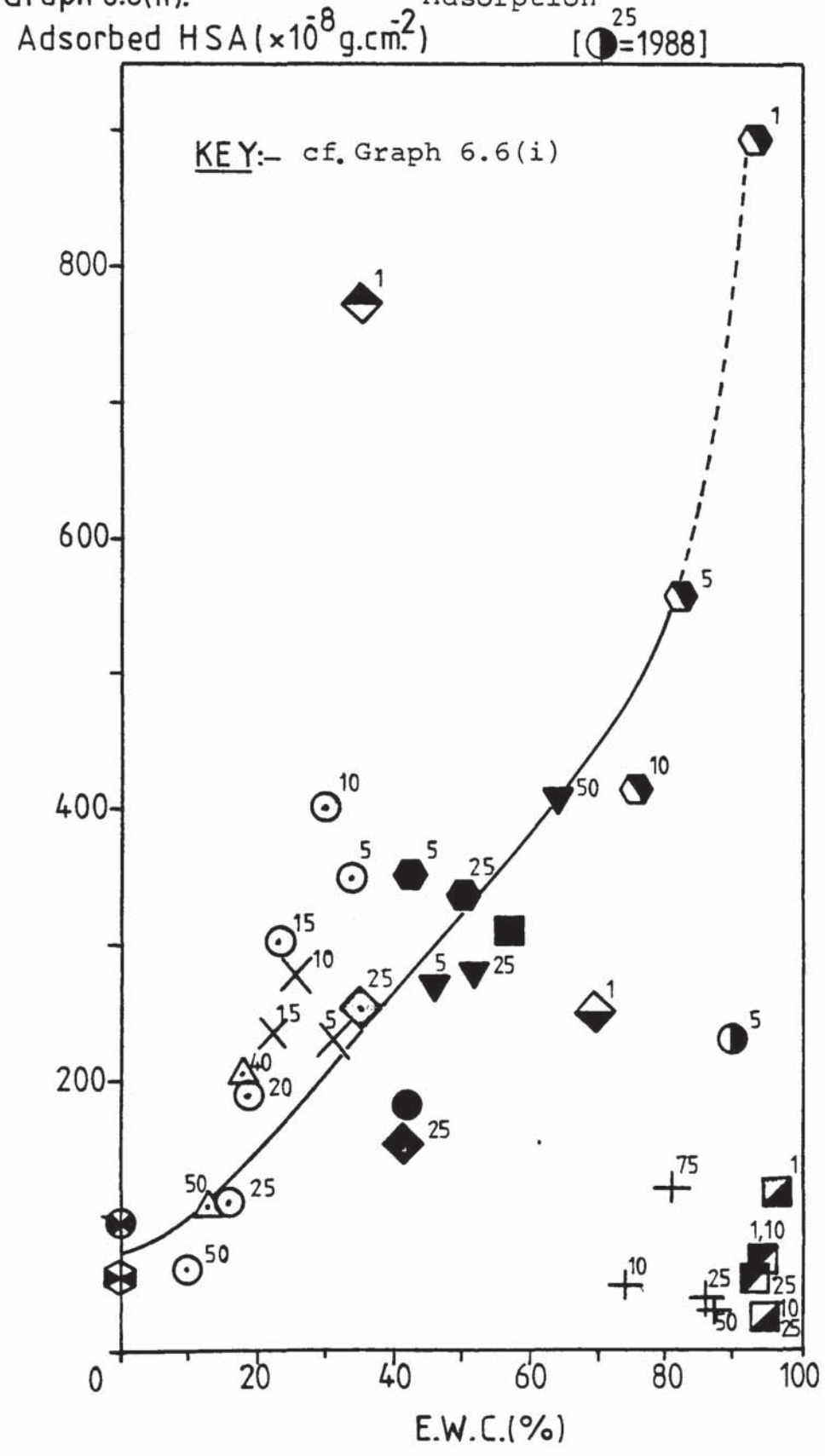
The somewhat anomalous results observed with the NVP copolymers can be related to the substantially different structure property effects of the pyrrolidone ring. By extrapolation of the data from these NVP copolymer compositions it would seem that a very high adsorption peak similar to the type 2 region of the HEMA copolymers, will occur, but in the 65-80% EWC range.

The HSA adsorption data given in Graph 6.6(ii), would seem to indicate that with the exception of the ionic hydrogels, the majority of hydrogels show an overall rise in adsorption with increase in hydrophilicity over the range 0-90% EWC. Above this value the problem in differentiating between true adsorption and absorption arises and further complicates data analysis. Individual variations are evident particularly for the hydrophilic/ hydrophobic copolymer compositions and HEMA/EDMA copolymers in the 20-35% EWC range.

Many past studies have reached the conclusion that in contrast to HFb, HSA has sufficient conformational stability to resist significant changes in its native globular state. Low HSA adsorption levels may therefore be regarded as being due to both low enthalpy and low entropy driven surface interactions between the hydrophobic (non-polar) 'gel'-surfaces and the few hydrophobic and predominant hydrophilic exterior groups of the HSA molecule.

As bulk hydrophilicity (i.e., EWC) increases so generally does the surface hydrophilicity/wettability, and

Graph 6.6(ii): Relationship of Bulk Water to Adsorption



because of this a progressive increase in exothermic-hydrophilic interactions between the gel-surface and the HSA molecules results in substantial increase in the adsorption levels.

The low adsorption observed with the ionic hydrogels do not fit into the above trends because as postulated in the text, electrostatic forces due to electric double layer effects produce a barrier to adsorption.

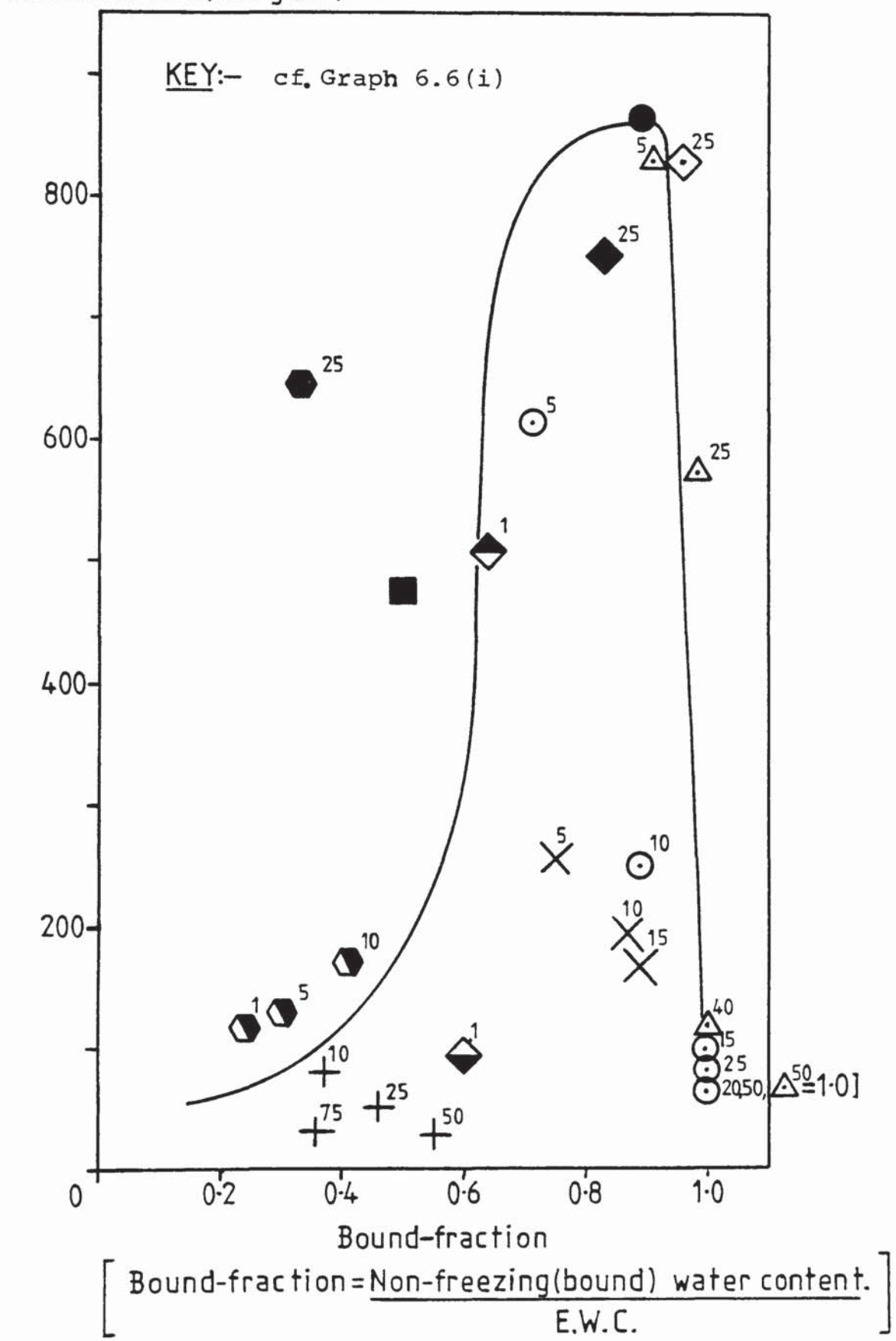
Fall in the adsorption levels for the NVP/EMA copolymers with increase in EWC, may be indicative of the reductions in surface interactions due to increased pore size and reduction in chain segment density, similar in nature to the considerations for the low HFb adsorption onto high water content gels.

1.2 Bound Fraction

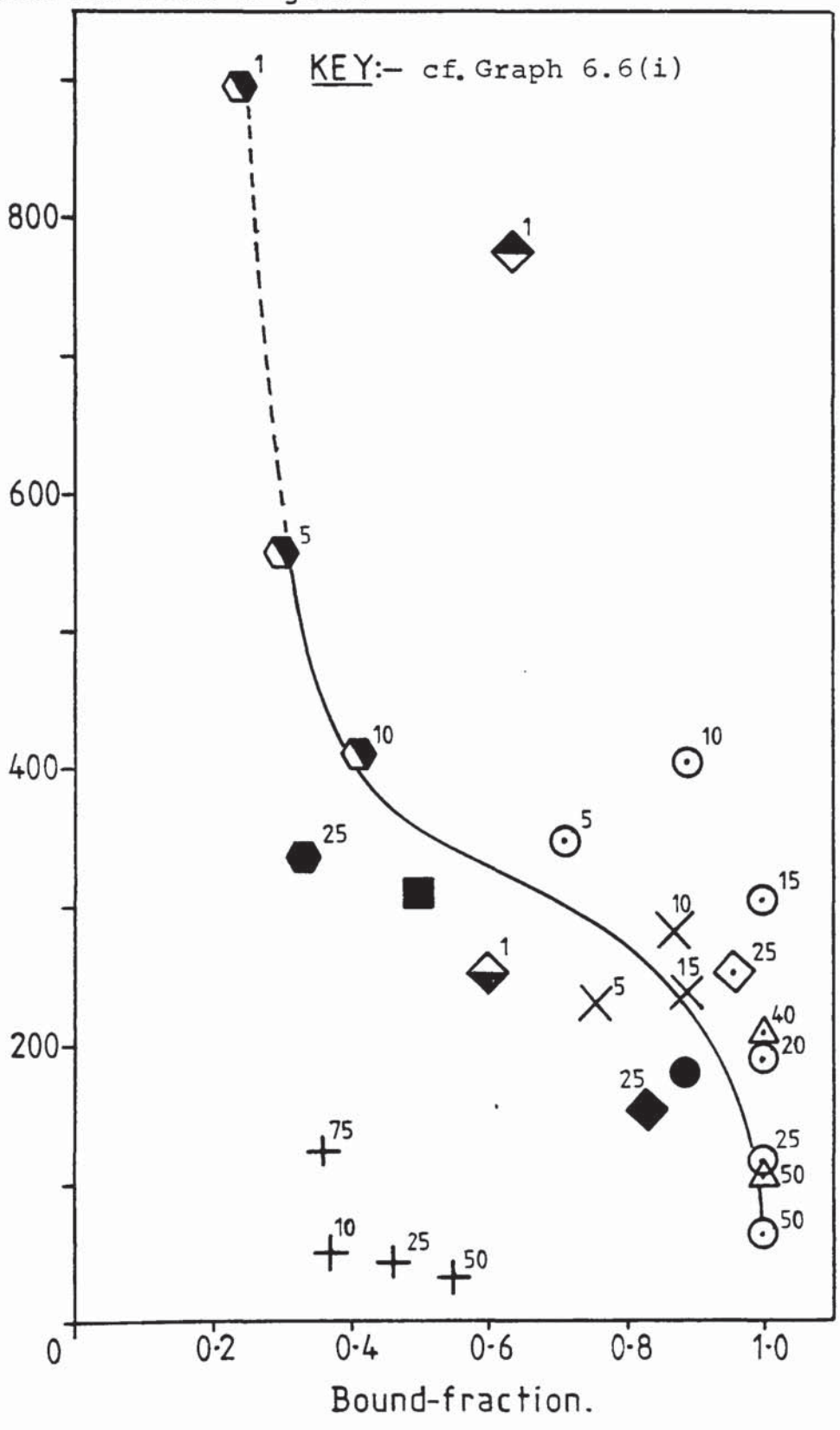
$$\text{Bound fraction} = \frac{\text{non-freezing (bound) water content}}{\text{E.W.C.}}$$

Although a relationship between bulk structuring and adsorption does exist, it is a very crude and somewhat tenuous correlation. It is the surface structured water that influences the adsorption process and although bulk structuring effects will influence the surface structuring they will not be necessarily identical in nature. Thus DSC measurement of the total freezing and non-freezing water contents is heavily weighted towards the predominant bulk structured water and will overshadow the very much smaller surface structured water. Until techniques exist to solely measure this surface

Graph 6.7(i): Relationship of Bound fraction to Adsorption



Graph 6.7(ii): Relationship of Bound fraction to Adsorption



structured water, precise correlations with adsorbed proteins will not be feasible.

From Graph 6.7(i), adsorption of HFb in relation to bound fraction values can be seen to be comparable to the EWC trends, and as would be expected, Region 1 type 'gels' have a very high bound fraction value and low adsorbed HFb levels; Region 2-type gels tend towards high bound fraction values and high adsorbed HFb levels, and Region 3-type high water content gels as expected have low bound fraction values and low adsorbed HFb.

Similarly the general trends exhibited in Graph 6.7(ii) for HSA adsorption in relation to bound fraction values tends to be comparable with their EWC trends.

Individual variations in trends for the different hydrogel copolymer subsets may be attributed to the inherent low sensitivity of the experimental DSC technique to surface structured water.

Taking all the above bulk features into consideration, it would seem that although hydrophilicity (EWC) is an important parameter, essentially it is the inherent water structuring of the constituent chemical groups, particularly those of the surface, which will directly influence both adsorption and absorption of proteins.

2. Surface Effects

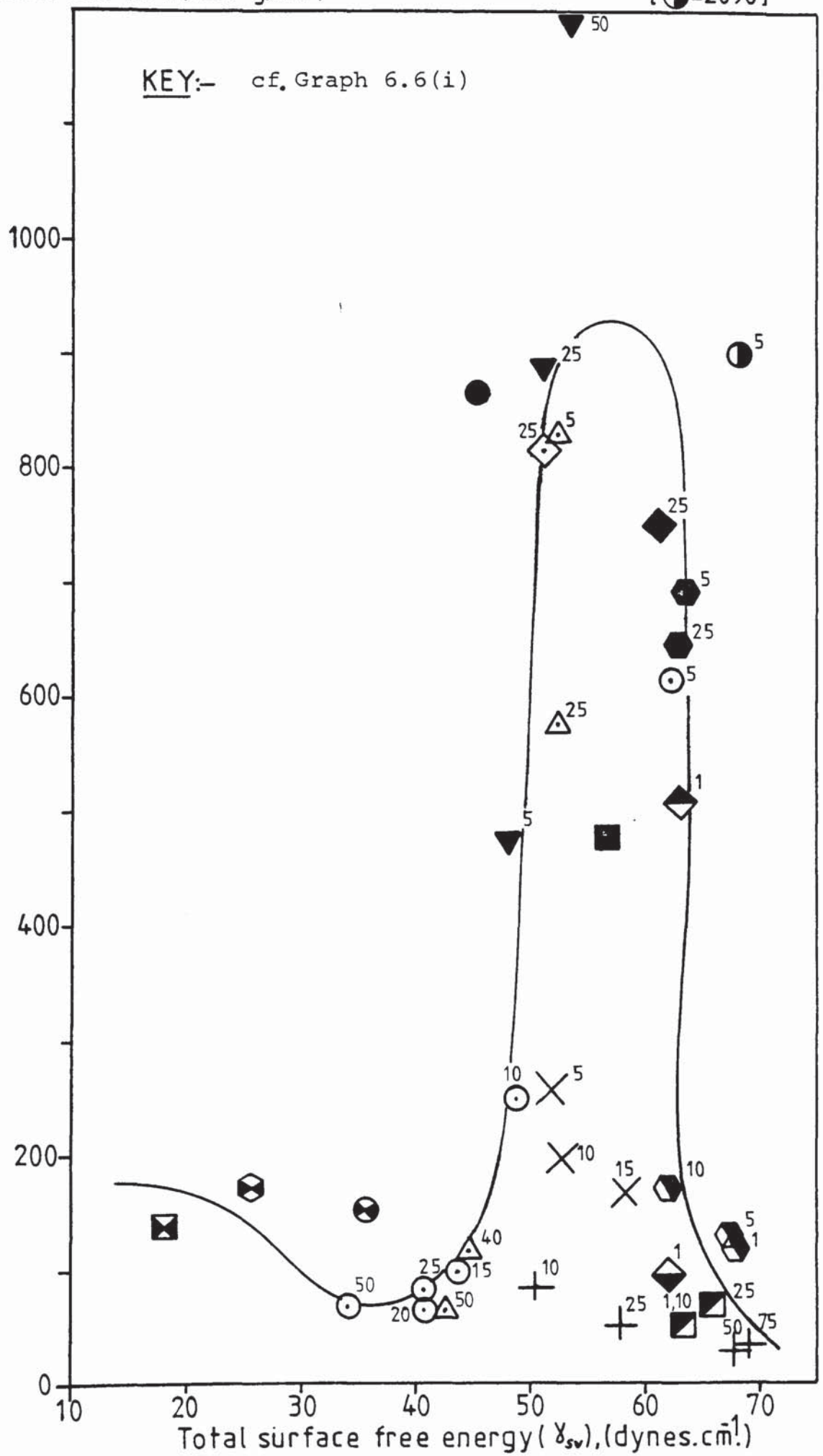
Because no known techniques exist to directly quantify total surface free energy (γ_{sv}) and interfacial free energy (γ_{sw}), (for hydrated materials) the mathematical derivation of their data produces values which are somewhat artificial in nature. Direct use of the air bubble contact

angle data can in its own right be a useful technique for following trends in the relationship between surface hydrophilicity (wettability) and adsorption. Although from the scatter diagrams, Graphs 6.8 to 6.10, individual trends for the copolymer subsets are evident, a unifying trend for all hydrogels is somewhat more difficult to postulate due to these same individual variations. Accordingly, these graphs for the most part can give only an impression of the relationships between surface properties and adsorbed HFb and HSA levels. Due to surface hydrophilicity, γ_{sv} and γ_{sw} being so closely interrelated, the scatter graphs are also of a similar nature and as such will be considered together.

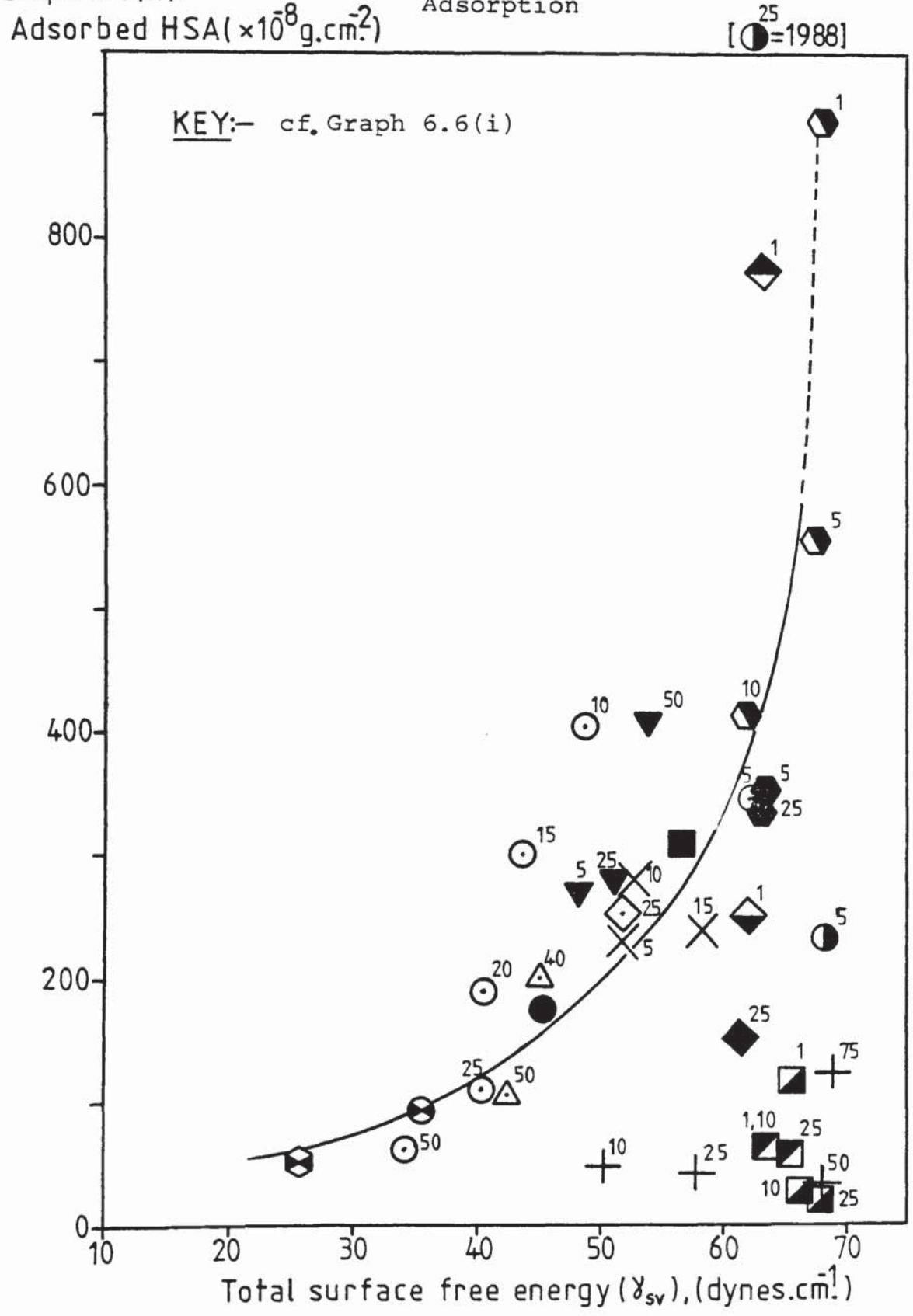
In accordance with the bulk structure-property effects on surface chemistry parameters as discussed in the text of Chapter 5, it is not too surprising that both the HFb and HSA adsorption relationships to surface hydrophilicity, γ_{sv} and γ_{sw}, shows virtually identical trends to those observed for the bulk properties of EWC and bound fraction. The three hydrogel categories (regions) of HFb adsorption types are given below but are not now so clearly demarcated as observed with the EWC data, (See Graphs 6.8(i), 6.9(i) and 6.10(i)).

Region 1 - low adsorption: total surface free energies (γ_{sv}) are below $\sim$ 45 dynes. cm.$^{-1}$; low surface wettability ($> 55^{o}$ air contact angle) and tend towards high interfacial free energies (tensions) and accordingly high conformational changes are expected.

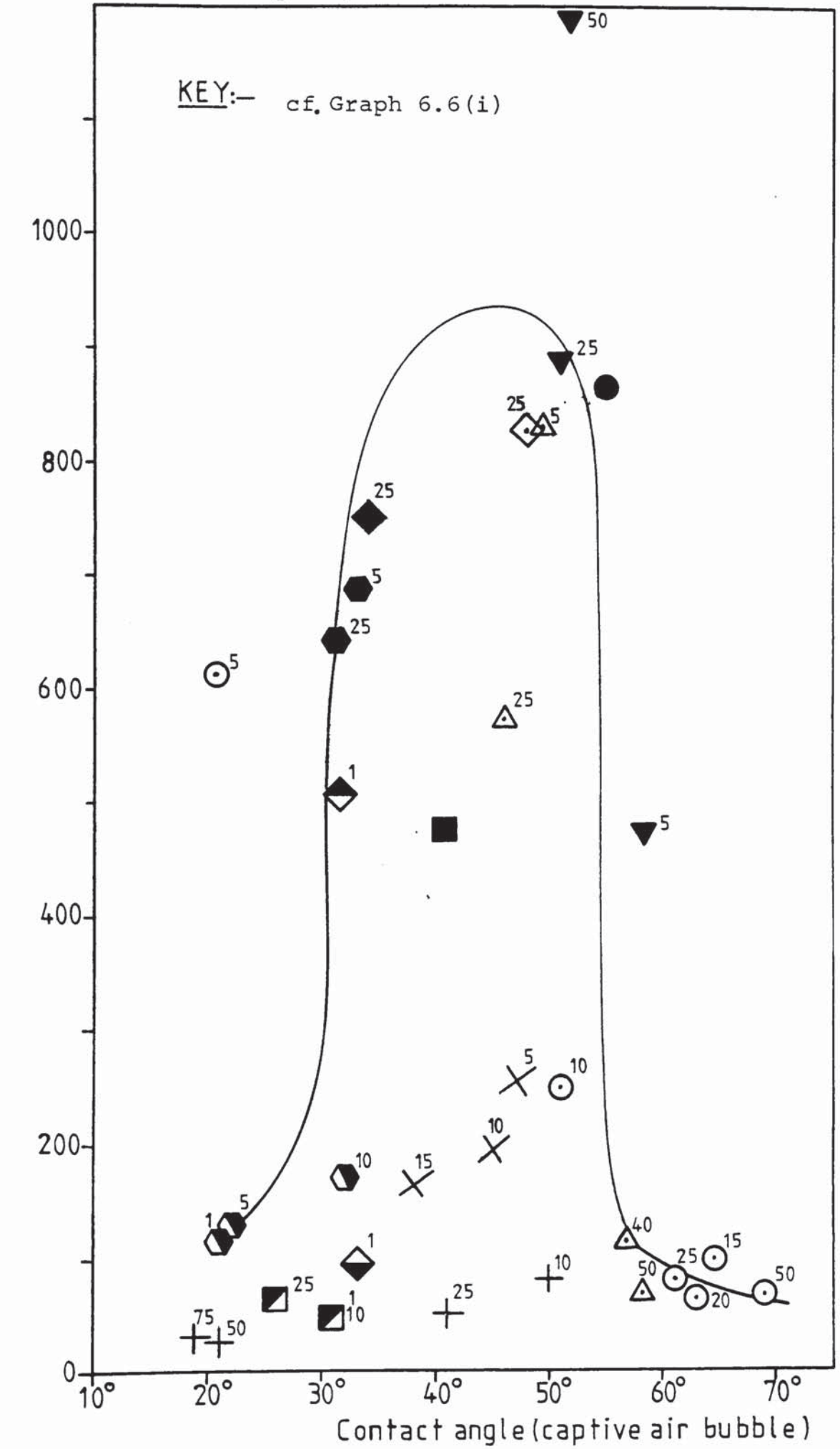
Graph 6.8(i): Relationship of γ_{sv} to Adsorption



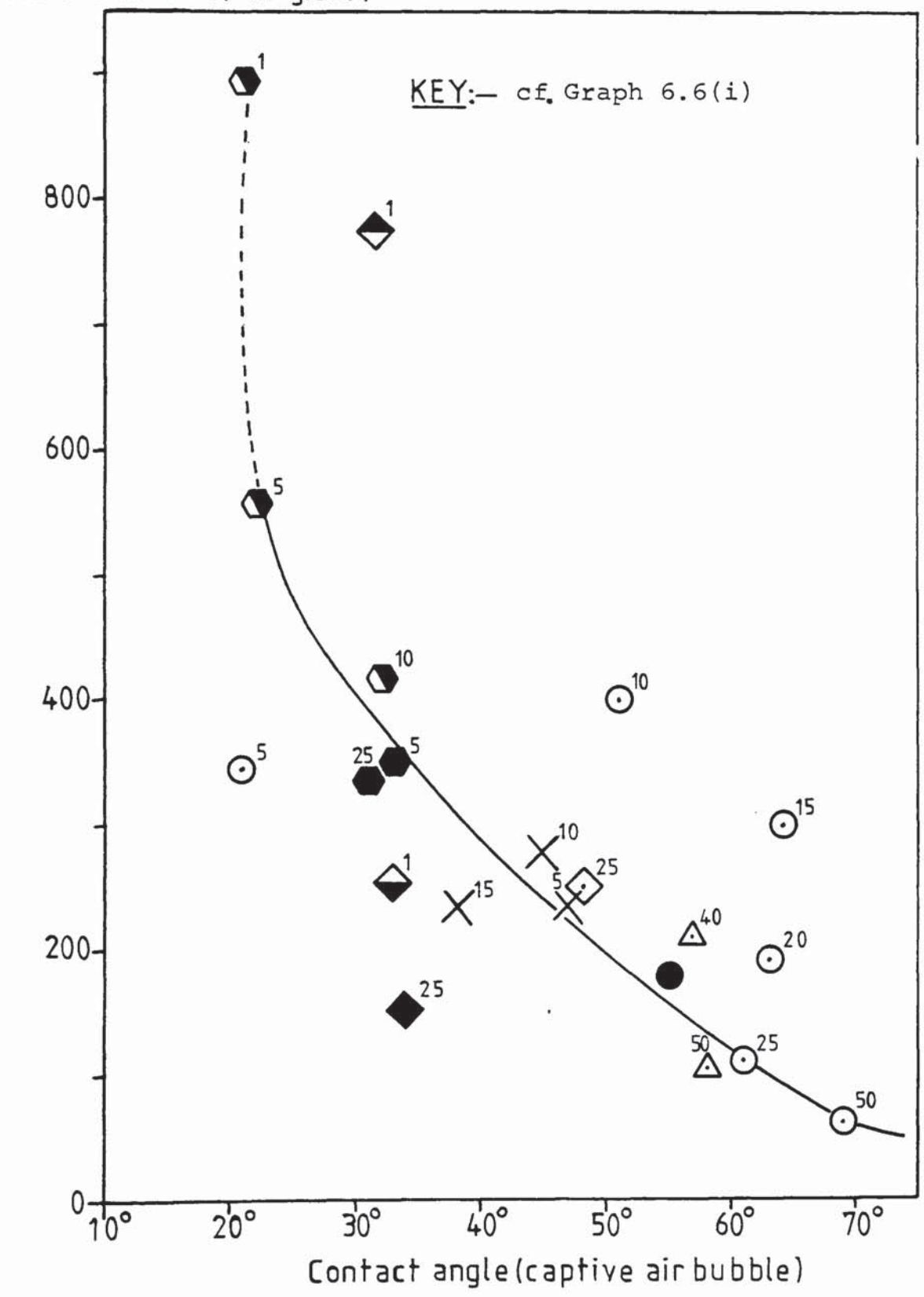
Graph 6.8(ii): Relationship of γ_{sv} to Adsorption



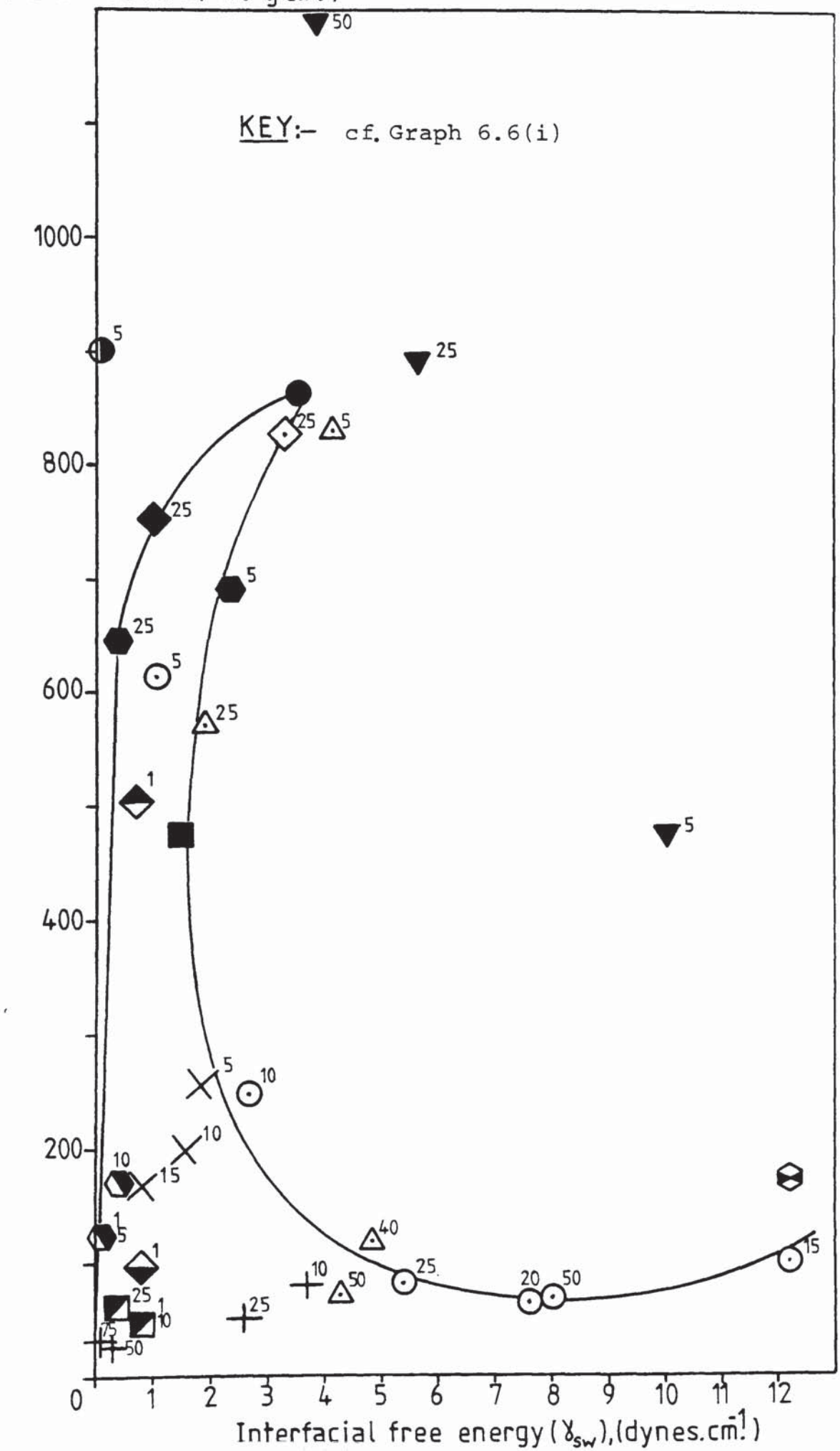
Graph 6.9(i): Relationship of Surface Wettability to Adsorption



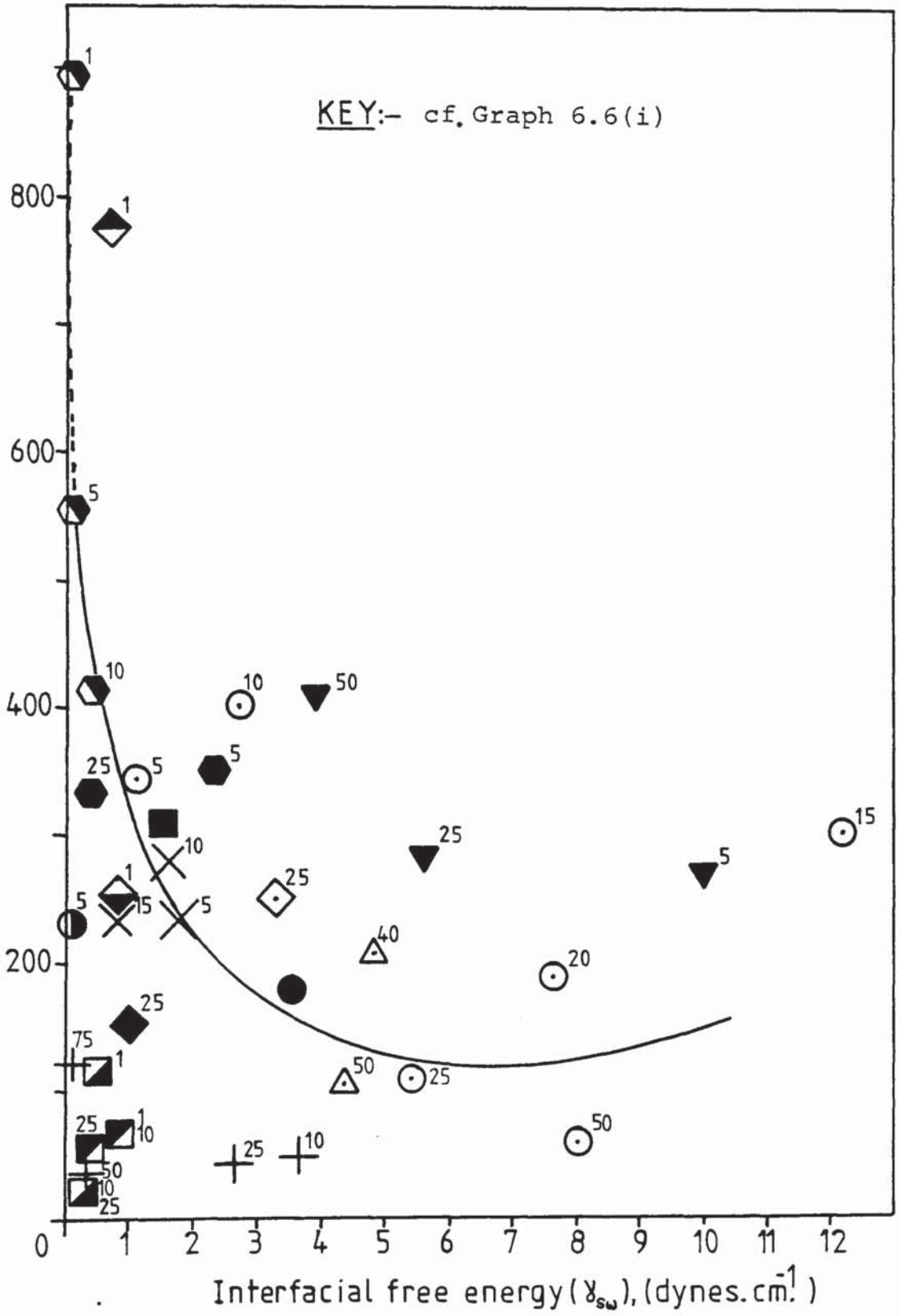
Graph 6.9(ii): Relationship of Surface Wettability to Adsorption



Graph 6.10(i): Relationship of γ_{sw} to Adsorption



Graph 6.10(ii): Relationship of γ_{sw} to Adsorption



Region 2 - high adsorption: moderate to high total surface free energy (45-63 dynes. cm.$^{-1}$); moderate to high surface wettability (55^{o}-30^{o}); and low interfacial free energies (< 4 dynes. cm.$^{-1}$).

Region 3 - low adsorption: high total surface free energy ($\sim$55-70 dynes. cm.$^{-1}$); high surface wettability ($\sim 40^{o}$-18^{o}); and very low interfacial free energies ($\ll$ 1 dynes. cm.$^{-1}$).

Similar wide overlap is evident for the HSA adsorption data given in Graphs 6.8(ii), 6.9(ii) and 6.10(ii), and again ignoring the ionic hydrogels, the general trend would appear to be that a low adsorption is observed for those polymers tending to be hydrophobic in nature (i.e., $\gamma_{sv} < 45$ dynes. cm.$^{-1}$) and having low surface wettability and high interfacial free energies (γ_{sw}): Progressive increase in total surface free energy and wettability, together with commensurate decrease in interfacial tension, then tends to progressively increase HSA adsorption levels, due to the postulated increase in hydrophilic-exothermic (enthalpy driven) surface interactions.

6.7.7 Dynamic "Flowing"-Adsorption System

The design and construction of this system, as outlined in Chapter 3, was intended to provide an adjunct to, or even supersede the previous static technique. However many problems were encountered with the practical implementation of this flowing system. Not least of these problems was due to the inherent mechanical weakness of the hydrogels. Clamping these very friable materials over the windows of the 'flow'-cells was a somewhat time consuming and laborious procedure, often requiring re-

petition due to leaks developing. This was found to occur when the clamping pressure was unevenly distributed and also tended to result in tearing of the hydrogels. To offset this latter problem hydrogels of increased thickness were synthesized. In order that data may be treated statistically, it was necessary to carry out 5 or more 'flow'-cells in series for each adsorption time for each material. As a consequence, more time must be spent synthesizing large amounts of hydrogel membranes. This makes large scale analysis of a series of copolymers unfeasible due to the time consuming nature of the experimental procedure, and also because this technique requires much greater quantities of the radiolabelled protein than the static technique, the technique is very much less cost effective.

Unfortunately due to the above problems with this technique, only one statistically viable set of results was achieved for poly (HEMA). This data cannot therefore be directly compared with the static data, but it does show very similar sigmoidal multilayer HFb adsorption as previously observed for poly (HEMA), See Appendix 5 for data.

Where this technique does score over the simpler static technique is that both static and dynamic studies may be carried out, together with more precise measurements of adsorption per unit area; and it is likely that dynamic adsorption studies will be of greater relevance to biomedical material applications than its static counterpart. This is particularly so, when considering the electrostatic charge effects of flowing aqueous solutions on materials, particularly ionic hydrogels, for blood contact applications.

CHAPTER 7

TOWARDS A CONTINUAL WEAR CONTACT LENS: PROBLEMS OF BIOCOMPATIBILITY

7.1 Introduction

The advent of hydrophilic polymeric materials for contact lens wear, brought not only the inherent advantages of this type of material over the conventional hydrophobic 'hard' lens material (such as wearer comfort and high gas permeability), but also certain disadvantages. One such disadvantage which is associated with the prolonged contact of a synthetic material with biological systems, is the formation of deposits on and within the expanded gel network. This can of course present a serious problem to contact lens wearers, because the deposits build up with time and a loss in visual acuity occurs, together with increasing wearer discomfort.[174] These deposits have been quite widely studied and found to be essentially proteinaceous in nature, but mucopolysaccharides, lipid and ionic species are also known to be present.[7] Although this problem has only been highlighted during recent years in the contact lens field, the deposition of protein is a well known interfacial phenomenon which seems to encompass all situations where foreign bodies of both natural and synthetic origin are in contact with a proteinaceous solution. Thus the deposition of tear fluid constituents on contact lenses, the clotting of blood at foreign surfaces, the formation of dental plaque and marine fouling are all examples that illustrate the same interfacial phenomenon: the adsorption of protein at solid surfaces.

In order to obtain a clearer insight into the

adsorption phenomena taking place at the tear film/ hydrophilic contact lens interface, background and information regarding both the historical development and design characteristics of daily wear contact lenses are given, together with the physico-chemical parameters of the tear film and cornea.

The very latest innovative programmes of contact lens research and development have been devoted to the formulation of continual wear contact lenses. With these lenses it is essential that the above problem of protein adsorption is either eliminated or, at the very least, minimised. In the text some consideration of the problem of protein adsorption/deposition is given to a number of continual wear contact lens formulations now undergoing clinical trials by Kelvin Lenses Ltd., and also to a number of currently available commercial hydrogel contact lenses. Possible surface modifications of hydrogels to meet the requirements for a continuous wear contact lens are also discussed.

7.2 Function and Historical Development of Contact Lenses

The actual concept of a contact lens may be traced back to Leonardo da Vinci almost 500 years ago,[288] however, it was not until 1887 that the first examples of lenses being fitted to a human eye can be found. The primary application of a contact lens is to correct visual defects such as: myopia (short sightedness) in which light from an object focusses in front of the retina due to the focal length of the eye being too short since the eye-ball is too long, or conversely, hypermetropia (long sightedness) for the opposite reasons given for

myopia, and astigmatism in which the image is focussed on the retina but in differing planes due to widely differing curvatures of the cornea. In practice however, these primary applications may be a secondary consideration to their more desirable aesthetic cosmetic appearance and functional convenience (i.e., for sports purposes) as compared with spectacle-wear.

All early lenses were individually ground from glass and it was not until the 1940's that poly (methyl-methacrylate) began to gain favour as a contact lens material, first in scleral form and finally as corneal lenses in 1948.[289]

The choice of poly (methyl methacrylate) as the thermoplastic to supercede glass, was somewhat empirically based upon its properties of toughness, 'apparent' physiological inactivity and ease of processability.[169]

Both the rigidity and impermeability to oxygen of this polymer make it necessary for contact lenses fabricated from this material to be worn on a daily wear basis and to be removed prior to sleep. In view of these disadvantages poly (methyl methacrylate) is known as a hydrophobic 'hard' contact lens material and must be fitted in such a way as to promote the flow of oxygen to the cornea, by rocking on a pre-corneal layer of tear fluid which transports dissolved oxygen via a pumping motion.[290,291]

This problem has encouraged the development of rigid thermoplastics such as poly (4-methyl pent-1-ene) and cellulose acetate butyrate having a far greater oxygen permeability than that of poly (methyl methacrylate).

These materials have failed to supercede poly (methyl methacrylate) as the most widely used hard contact lens material due to other complications arising from their structures.[288] The surface characteristics of poly (methyl methacrylate) are such that a coherent layer of tear fluid may be sustained on the lens giving rise to a greater degree of physiological compatibility than that of poly (4-methyl pent-1-ene) which is not wettable by tear fluid unless first subjected to a surface treatment which raises its surface hydrophilicity. This problem does not arise with certain varieties of cellulose acetate butyrate, but, due to the dimensional instability of this material, difficulties arise in obtaining correctly fitted lenses.[292]

With time epithelial erosion of the cornea is often a feature with all types of hydrophobic 'hard' contact lens wear and is a direct consequence of the inherent rigidity of the materials. Although the corneal epithelium is capable of rapid regeneration, prolonged irritation by a rigid 'hard' lens material may lead to permanent damage to the Bowman's membrane (See Fig. 7.1, Section 7.3). As a consequence of this, often certain patients find it necessary to resort to spectacle wear after having been fitted with hard lenses.

This problem has led to the development of polymers for potential 'soft' contact lens materials having a far greater degree of comfort. Two types of polymer that have been widely investigated as potential 'soft' contact lens materials are synthetic elastomers, of which the most studied example is silicone rubber (poly(dimethyl-siloxane)), and hydrogels, namely poly (2-hydroxy ethyl methacrylate).

Table 7.1 shows a comparison of the mechanical properties of these materials with those of poly (methyl methacrylate) and the cornea itself.[173] The main drawback to the use of silicone rubber and other synthetic elastomers as 'soft' contact lens materials is that, like poly (4-methyl pent-1-ene), a surface treatment is required to render the material wettable by tear fluid. This problem does not arise with hydrogel polymers due to their inherent surface hydrophilicity however, as discussed in the text this inherent characteristic presents the further problem of lens deposits. Since the earliest patent by Wichterle[293] on the application of poly (2-hydroxy ethyl methacrylate) as a contact lens material in 1961, a vast number of claims have been filed citing numerous hydrogel compositions and methods of preparation. An extensive review of the patent literature relating to the use of hydrogels as contact lens materials has recently been compiled by Pedley, Skelly and Tighe.[294] The majority of 'soft' contact lens materials have been based on poly (2-hydroxy ethyl methacrylate) and have a water content of approximately 40 per cent. More recently however a number of commercial concerns throughout the world have been marketing 'soft' contact lenses manufactured from high water content hydrogel polymers, which offer suitable compatibility for extended periods of wear. A summary of some of these materials that are currently 'on the market' are illustrated in Table 7.2. The much improved comfort and tissue compatibility offered by these materials encourages the prospect of continuous wear contact lenses.

Table 7.1

MECHANICAL PROPERTIES OF POTENTIAL CONTACT LENS MATERIALS COMPARED TO THAT OF THE CORNEA[173]

Material	Modulus of rigidity (Nm^{-2})	Tensile strength (Nm^{-2})	Tear strength (Kg/mm)
Cornea	1×10^{7}	5×10^{6}	1.5
Poly (methyl methacrylate)	1×10^{9}	5×10^{7}	Strong, but brittle
Poly (2-hydroxy ethyl methacrylate)	0.5×10^{7}	5×10^{5}	1×10^{-2}
Silicone rubber	0.8×10^{7}	1×10^{7}	2.0

Table 7.2

COMMERCIAL HIGH WATER CONTENT HYDROGEL CONTACT LENS MATERIALS

Product Name	Manufacturer	Component Monomers	% Water content	Reference
Duragel	Cooper-Vision optics	Amido-Amino copolymer	73.5	(295,296)
Flexol	Burton Parsons	HEMA, AMA, MAA	72	(297,298)
Permalens	Cooper-Vision optics	NVP, HEMA, MAA	72	(299)
SP-77	Contact Lens Manufacturing	undisclosed	77	-
Scanlens	Scanlens	Amido-Amino copolymer	73.5	(295,296)
Yumecon	Tokyo Contact Lens Research	undisclosed	70	(300)

7.3 Towards a Continuous Wear Contact Lens

The popularity of contact lenses has shown a definite upsurgence over the past decade or so, even so it is expected that spectacles will remain for sometime as the major optical appliance for correction of visual refractive defects.

The past problems of wearer discomfort associated with the hydrophobic 'hard' contact lens materials together with the friability and deposition problems of current daily and extended wear hydrophilic 'soft' contact lens preparations, has not endeared the product to the general public. In addition both the troublesome removal and application procedures of daily wear contact lenses before and after sleep periods, is not the most conducive aspect of contact lens wear.

The majority of these above problems may be overcome to a certain extent by the use of a suitable hydrophilic 'soft' contact lens material in a continual-wear regime.

Such a continual wear lens would not only have a mandatory high degree of tissue compatibility (i.e., biocompatibility) and hence minimal wearer discomfort, but also the continual wear regime ensures that the risks of bacterial contamination, rupture and loss due to handling procedures would be considerably reduced if not eliminated.

As with any biomedical applicable material, continual wear contact lenses should meet the requirements as listed in Chapter 1. In addition a continuous wear lens should meet the following functional criteria for

'the ideal contact lens':-[301]

1) Comfort and safety

2) Visual acuity

3) Ease of wearing

4) Dimensional and optical stability

5) Simple care procedure

Since a continuous wear lens may be considered as an extension of the cornea any material used for this application should possess three other properties of major importance in addition to the fundamental feature of optical clarity:-

a) oxygen permeability in excess of 200×10^{-10} cm.^{3} mm. cm.$^{-2}$ sec.$^{-1}$ cm,Hg.$^{-1}$[302]

b) surface properties which discourage the accumulation of proteinaceous deposits[288], but allow wetting by tears.

c) sufficient mechanical stability to resist the deforming forces of the eyelid during the blink cycle, and yet be easily elastically deformable and rapidly recoverable in order to minimise mechanical pressure on the eye and avoid visual instability.[173]

The cornea is avascular and in the main acquires oxygen directly from the atmosphere and the minimum oxygen tension to maintain normal optical clarity and corneal respiration is of the order of 12 - 18 mm Hg.[303] This criteria may be met by the requisite oxygen permeability given in a) above. In addition to direct permeation through the lens, oxygen may also be transported to the cornea via an air saturated reservoir of tear fluid which lies between the lens and the eye. Therefore it is necessary that the lens material is sufficiently hydrophilic (i.e., wettable) in order that the lachrymal fluid may wet the lens

sufficiently to maintain a continuous tear film.

As was discussed in Chapter 4, the oxygen permeability of hydrogels increases exponentially with increasing water content,[166,304] and it has been concluded from these studies that in the case where no tear fluid transports oxygen behind the lens, a hydrogel suitable as a continuous wear material should have an equilibrium water content of 70 per cent or more. At such high water contents the mechanical strength of the majority of hydrogels falls far below that required for use as a contact lens material, however, recent advances in the field of continual wear contact lenses by I. P. Middleton[155] has succeeded in eliminating many of the inherent weaknesses of previous high water content contact lens formulations.

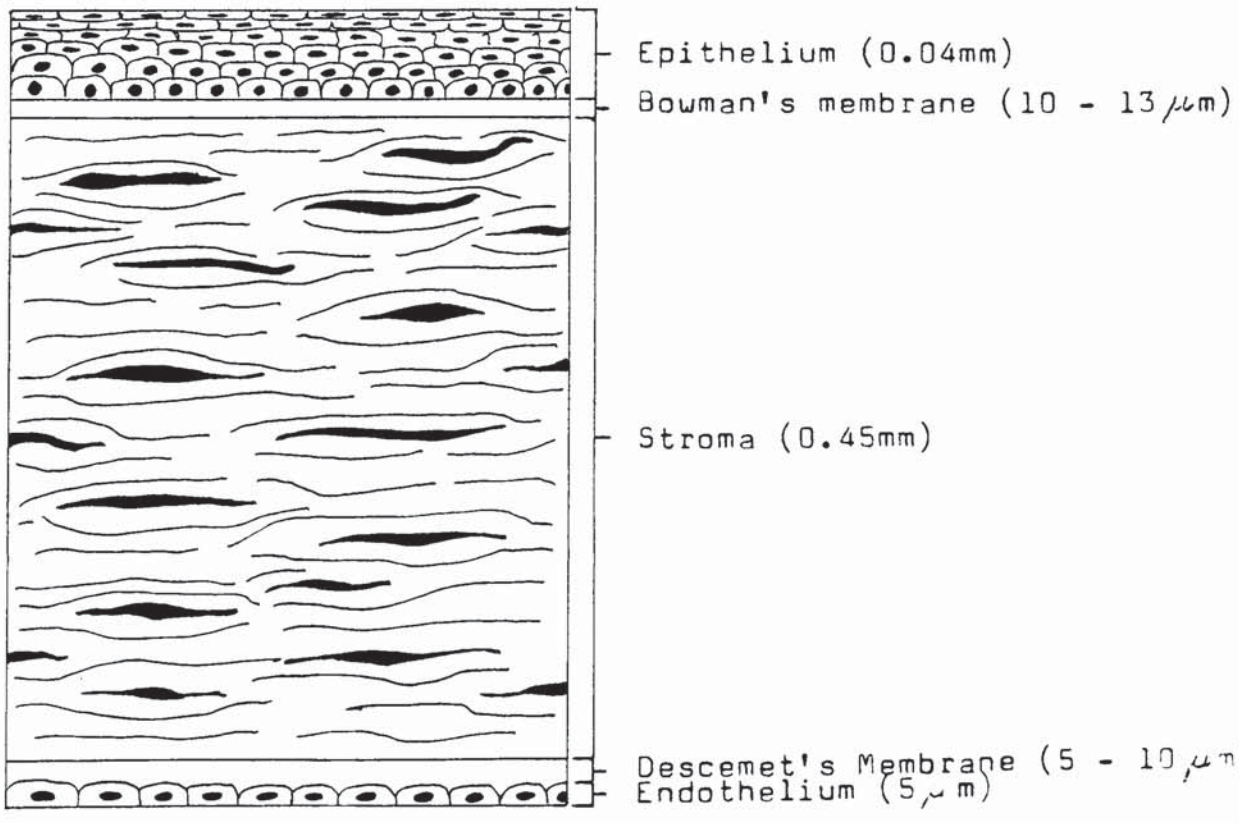
The concept of a continuous wear contact lens as an extension of the cornea is in practise very difficult to achieve in that the cornea is comprised of a complex array of structural elements which govern its properties. The bulk properties are generally governed by the stroma whereas the epithelium is responsible for the characteristics of the corneal surface, as is evident from the sectional view of the cornea, Fig. 7.1.[305] Some of the more relevant features of thc cornea that would be desirable to model a continuous wear lens material are summarised in Table 7.3.[169]

Table 7.3

PROPERTIES OF THE CORNEA TO BE APPROACHED BY AN EXTENDED WEAR CONTACT LENS[169]

Water content	~ 81% (17% free, 65% bound)
Critical Surface Tension	~ 30 dynes. cm.$^{-1}$
Refractive index	1.37
Mechanical Properties	Linear viscoelastic material at low stress modulus of rigidity. ~ 1.3×10^{8} dynes. cm.$^{-2}$
Oxygen Permeability	~ 300×10^{-10} cm.^{3} mm. cm.$^{-2}$ s^{-1} cm. Hg.$^{-1}$
Oxygen Consumption	~ $(3.5 - 7) \times 10^{6}$ l. cm.$^{-2}$ h.$^{-1}$
Minimal Oxygen-Tension	12 - 18mm. Hg.
Normal Oxygen—	155mm. Hg. (open eye)
Tension in tear fluid	55mm. Hg. (closed eye)

Figure 7.1 SECTIONAL VIEW OF THE CORNEA



7.4 Chemistry and Function of the Pre-corneal Tear Film[306-314]

The pre-corneal tear film may be thought of as being composed of three layers; the superficial lipid layer, intermediate aqueous layer and a deep mucoidal layer, as illustrated in Fig. 7.2.

7.4.1 The superficial lipid layer[306]

The presence of large 'tree-like' glands in the human eyelid was noted by Meibomuis in 1666. The upper lid commonly has from 30 to 40 of these glands, whereas the lower lid contains only 20 to 30 glands which are somewhat smaller. Each gland has an orifice at the lid margin on the inner aspect of the intermarginal sulcus. Because the meibomian glands are located between the tarsal plates in each lid, the simple act of blinking is sufficient to spread their secreted lipid material onto the tear film. The function of this secreted lipid are thought to be:

1) Lubrication between the lid margin and the cornea

2) Provision of a limiting hydrophobic barrier at the lid margins for the tear film

3) Reduction of the rate of water loss by evaporation from the underlying aqueous phase.

In addition, an increase in the optical properties of the tear film by producing a smooth anterior surface, has been cited.

The composition of these lipid meibomian secretions consist of principally cholesterol ester and lecithin with minor amounts of fatty acids, cholesterol and some

Figure 7.2

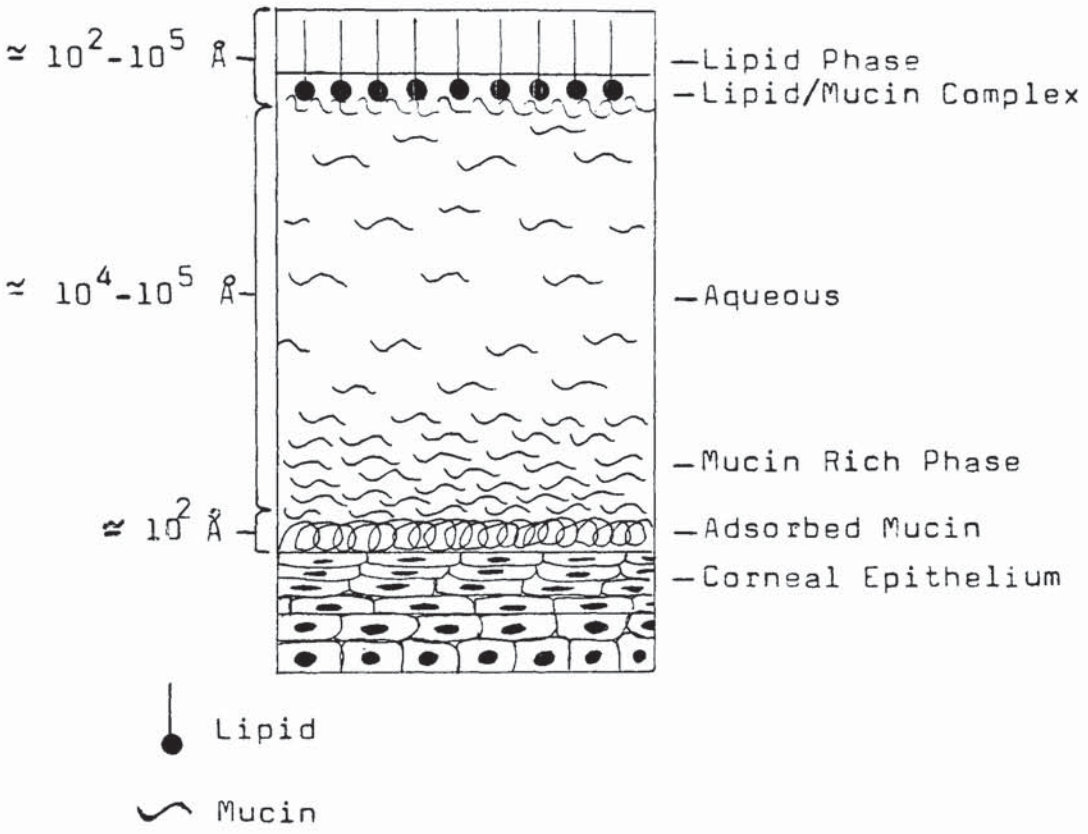


Figure 7.2 Schematic Representation of Tear Film

unidentified phospholipids. Dispersal of this lipid phase over the intermediate aqueous phase is believed to be due to the surfactant properties of mucin (muco-polysaccharides), or possibly the proteins (i.e., albumin) in solution.

7.4.2 The Intermediate Aqueous Phase[307-313]

Normal secretion of tears by the lacrimal gland situated in the upper lid is necessary for nutrition of the cornea and for the formation of an optically stable, continuous fluid film over the cornea. The fluid layer of the pre-corneal tear film (mainly aqueous) was found to be 6 - 7 μm thick between blinks. Both the secretion rate and composition of human tears is variable depending on age, sex and health,[312] and to this list must now also be added the wearing of contact lenses.

Tears contain 98.2% water and only 1.8% solids in solution. The osmotic pressure of tears is approximately the same as for blood plasma, being isotonic with a 0.9% saline solution and having a pH value of 7.4 to 7.5. Proteins are the major constituents of the solids and are comprised mainly of three types: albumin (serum albumin and tear albumin), globulins (α, β and γ, including IgA, IgG and IgM) and lysozyme. The ratio of these principal protein fractions in stimulated and unstimulated (normal) human tears is given below in Table 7.4.

Table 7.4[311]

Protein Fraction	Tears	
	Normal (%)	Stimulated (%)
Albumin	58.2	20.2
Globulin	23.9	56.9
Lysozyme	17.9	22.9

In addition appreciable amounts of unidentified glycoproteins have also been found, together with mucopolysaccharides (mucin). The residual dissolved solid material of the tear film (aqueous) consists of lipids, glucose, amino acids and inorganic electrolytes such as sodium and potassium chloride.

The cornea is maintained in an optically stable deturgescent state by an ATPase Na^+/K^+ pump mechanism which actively extrudes water from the corneal stroma and thus one of the more important functions of the precorneal tear film is to supply nutrient oxygen and glucose to maintain corneal-metabolic homeostasis. Another important function of the tear fluid is its lubricating effect which allows the eyelid to sweep across the cornea, removing any unwanted 'foreign' debris.

The tear film is also responsible for the natural immune defenses of the eye.[313] This immunity is both of the acquired humoral type due to immunoglobulins, Ig, (i.e., antibodies), in solution or by the non-specific bacteriocidal action of the enzyme lysozyme, which acts by way of a β (1-4) hydrolysis of mucopolysaccharide portions of bacterial cell walls.

7.4.3 The Deep Mucoidal Layer[311,314]

The majority of the adsorbed mucins of this layer are derived from the goblet cells of the conjunctiva, although some may be secreted by the corneal epithelial cells themselves. These same mucins are also found in the aqueous phase and also in complex with the surface lipids of the tear film. They range from mucopoly-

saccharides (high in carbohydrate content and low protein content), through to glycoproteins (low carbohydrate content and high protein content). Often the terminal carbohydrate groups are sialic acid (i.e., N-acetyl neuraminic acid), which is completely ionised at a physiological pH, and contributes a high negative charge density and hydrophilic nature. The main role of mucins was always assumed to be the protection of the delicate membrane surfaces in the body. This function is achieved by formation of a protective viscous aqueous layer over the epithelial surfaces of mucous membranes, such as is seen with the corneal epithelium. More recently the function of mucins as surface active agents (surfactants) has also been investigated, and their mechanism of action as regards the tear film/cornea interface is discussed in the following section.

7.5 The Tear Film/Cornea Interface[315]

In order to understand the interactions taking place at the tear film/contact lens interface, it is necessary to first consider the nature of the tear film/cornea interface.

In the normal open eye situation, a stable thin tear film (6-10 μm), is established over the cornea and conjunctival epithelium. This film is not homogeneous but consists of various phases (i.e., lipid, aqueous, mucoidal), as given previously. The corneal epithelium is relatively hydrophobic in nature with a critical surface tension of approximately 30 dynes. cm.$^{-1}$.

In order that a stable tear film should be maintained the liquid film must completely wet the corneal surface. This would normally mean that it must have a surface tension not less than the critical surface tension of the cornea. The main constituent of tear fluid is water which has a high surface tension (72.8 dynes. $cm.^{-1}$). Synthetic wetting agents which are capable of reducing the surface tension of water to 30 dynes. $cm.^{-1}$, also have the inherent disadvantage of lowering the interfacial tension of oil and water to zero, which would lead to spontaneous emulsification and solubilisation of cell membrane lipids. The natural wetting agents found in tear fluid, the mucins (namely mucopolysaccharides), cannot lower the surface tension so dramatically, but neither do they cause this undesirable lipid solubilisation at their normal physiological levels. In simplistic terms, they may be regarded as acting in two combined ways to bring about epithelial wetting:

(i) by reduction of surface tension of the water component of tears $\simeq$ 40 - 46 dynes. $cm.^{-1}$, and

(ii) by increasing the critical surface tension of the cornea to $\simeq$40 dynes. $cm.^{-1}$ by adsorption on to its surface.

Once the tear film is established, the anterior superficial lipid layer is believed to play a large part in stabilising the film, and this itself is thought to be stabilised by the mucins in the aqueous phase. (Fig. 7.2).

7.6 Protein Adsorption and the Hydrophilic 'Soft' Contact Lens

7.6.1 General Considerations

Attempts to deal with the problems of deposition on hydrophilic 'soft' contact lenses have been centred on the use of the detergent effect of surfactants to clean the lenses, and also more recently, oxidative and enzymic cleaning preparations. The cleaning properties of these polymeric surfactants depend on their ability to lower the surface tension of the oil-water or solid-water interface. Ideally a surfactant should be of a viscosity which will protect the lens surface from rubbing and friction during cleaning, and it should not penetrate the lens matrix and therefore must be of a high molecular weight. This latter point is of particular concern with regard to the new formulations of high water content contact lenses, and probably to date the varied range of commercial surfactants do not meet this requirement. Surfactants have been found to have very little effect when the deposits have become firmly established and their role is now primarily seen as a prophylactic preventative measure. Although chemically oxidative cleaning regimes have had some success in dealing with the problem, they also tend to produce detrimental changes in the polymer structure and therefore their use in the long term usually produces problems. Their use has in fact been cited as producing even greater problems with deposit formation.[316] Some success has been achieved with enzymic preparations, based on the peptide hydrolysing

action of papain, but again it alleviates and does not eliminate the deposition problem. Ocular sensitization due to the enzymic cleaning preparations or the hydrolised protein fragments, is the greatest drawback to this mode of cleaning. Coupled with this the absorption and adsorption of the hydrolysed protein fragments may in fact generate still further problems in deposit formation with repetitive use of these enzymic cleaning preparations.

Although poly (HEMA), (like most synthetic hydrogels), is mechanically weak, it does possess properties that are valuable in a biomedical material, in that it is chemically stable at extremes of pH and temperature. One of the procedures that a prosthetic device should normally undergo is steam sterilization (autoclaving). The problem of protein deposition on 'soft' lenses is often compounded by this type of sterilization procedure. This is discussed by Wedler,[317] who cites a possible hypothesis for the deposition, involving two steps:

(1) Proteins and lipids deposit on the hydrophilic lens, via polar lipid-head groups or the ionic amino acid side chains of proteins.

(2) Routine heat sterilization denatures the layer of proteins exposing apolar interior groups to lipids, and this then allows disulphide linkages to occur between peptide chains, thus producing cross-linked denatured protein subunits.

As stated previously, the methods to eradicate the problem of deposits on lenses, have relied mainly on action after the event. It would therefore seem logical to tackle the problem at its outset, which in

this case involves both the constituent monomeric components and fabrication techniques used in contact lens manufacture. It is these factors that influence the surface properties of lenses and it is these properties that are ultimately responsible for deposition problems. The surface characterisation of various polymer systems by protein adsorption studies is therefore of prime importance in the search for improved 'soft' lens materials for daily and extended wear.

The most common technique of 'soft' lens fabrication is by the lathe cutting of dehydrated xerogel in rod form, to give the necessary optical radii, followed by a polishing procedure to remove surface irregularities. Both the lathe cutting and the polishing procedure are liable to contaminate the lens surface with lipid like materials. During the polishing process the 'lens' is affixed to a lath-chuck by wax and polished by way of particulate polishing compounds based on aluminium oxide lubricated with paraffin or silicone oil. Both the wax and oil are normally removed by washing with an appropriate solvent, however, depending on the efficiency of this washing procedure, variable degrees of surface contamination can lead to lens fitting problems and wearer discomfort[318] and ultimately these contaminants may serve to enhance the deposition problem.

One other common mode of surface contamination of contact lenses worthy of mention occurs during the routine handling of the lens by the wearer or his/her optician. Sebaceous lipid secretions from the skin are very likely to contaminate the lens surface if a poor handling procedure

is adopted, (i.e., unwashed hands). The more polar lipid components of the sebaceous secretions are liable to bind irreversibly to the hydrophilic lens surface in a somewhat random distribution[319], and in so doing may potentiate the deposition problem.

A very limited test was carried out in support of these latter views, whereby three hydrogel compositions; poly (HEMA), poly (ACM), and NVP/EMA, 75:25 (Moles %) copolymer, previously characterised by protein adsorption studies in Chapter 6, were deliberately contaminated by sebaceous skin secretions prior to subjecting them to similar HFb adsorption studies. The results of this test are given in Table 7.5

Table 7.5

THE AFFECT OF LIPID CONTAMINATION

(Sebaceous Secretions) ON FIBRINOGEN ADSORPTION

Hydrogel Composition	Monomer Ratio (Moles %)	EWC % (Krebs)	HFb - Adsorption*	
			Uncontaminated ($\times 10^{-8}$g cm.$^{-2}$) $\bar{x} \pm \sigma^{n-1}$	Lipid Contaminated ($\times 10^{-8}$g cm.$^{-2}$ $\bar{x} \pm \sigma^{n-1}$
HEMA	100:0	41.6	864.5 ± 72.8	588.9 ± 32.5
NVP:EMA	75:25	71.6	2090.6 ± 321.3	3769.9 ± 272.9
ACM	100:0	93.3	117.4 ± 13.0	101.1 ± 10.0

* - As at the plateau time of ~ 70 hours.

These polymers were chosen simply based on both their water content distribution and common commercial usage of their monomers. However, quite obviously from these results

the effects of lipid surface contamination are related to the surface interactions of the constituent monomers (as discussed in Chapter 5), and not simply to the bulk hydrophilicity of the polymers. Although detailed analysis of these results is not feasible they do go some way to supporting the previous view that surface contamination by lipids does influence protein adsorption and should therefore be considered as playing a possible role in the multifactorial deposition problem.

It can be appreciated therefore that continual (extended) wear contact lenses would go some way to minimising many of the previously discussed problems; simply based on the decreased need to clean/sterilize/ handle the lenses, should both improve the life expectancy of the 'soft' lens and the associated deposition problem.

7.6.2 Continual Wear Contact Lenses

In this section the surface characterisation and protein adsorption studies of two potential continual wear contact lens formulations are discussed. Both the surface characterisation and protein adsorption studies are based on the relevant techniques previously outlined in Chapter 5 and 6, respectively. The monomeric compositions of these two formulations (i.e., Kelvin-1 and Kelvin-2) are tabulated overleaf in Table 7.6, together with their respective equilibrium water contents in Kreb's solution.

Samples of these compositions were provided by Dr I. P. Middleton and were formulated as part of a collaborative continual wear research and development programme between the aforementioned and Kelvin Lenses Ltd. The

relevant functional criteria for these contact lens formulations (i.e., optical stability, mechanical strength etc.) have been previously covered by Dr I. P. Middleton.[155]

Normally these formulations would be fabricated in a dehydrated (Xerogel)-rod form in order that they may be easily lathe-cut into lenses, however, in this study the compositions were fabricated as for the previously studied bulk-membrane polymerised hydrogels of this project. It is likely therefore that some slight differences in surface characters will exist between the surfaces of the 'membrane' and 'rod'-bulk polymerised forms. For practical purposes however, the differences cannot be measured simply because of the surface treatment necessary to process the rod-form into lenses.

The surface data for these two formulations are given in Table 7.7. Quite obviously from the very complex compositions of these two continual wear formulations, attributing the surface characters to particular monomer structure-property relationships is somewhat unrealistic. However, from the data in Table 7.7 it can be seen that Kelvin-1 possesses a higher surface wettability than its counterpart, Kelvin-2, and that in accordance with this Kelvin-1 also possesses a higher total surface free energy (γ_{sv}), and a lower interfacial free energy (γ_{sw}) than Kelvin-2. These changes in surface characters may be attributed to the partial substitution of ACM and NVP by the less hydrophilic monomers of MACM and DAACM, thereby making the Kelvin-2 formulation more 'hydrophobic' in character. A comparison of the individual polar and

Table 7.6

Kelvin-1		Kelvin-2	
Composition (EWC = 77.2%) (Krebs)	Monomer ratio (Moles %)	Composition (EWC = 76.3%) (Krebs)	Monomer ratio (Moles %)
ACM	33	ACM	20
NVP	47	MACM	10
HPA	10	DAACM	10
STY	10	NVP	40
MAA	1% by weight	HPA	10
		STY	10
		MAA	2% by weight

Table 7.7

Continual wear formulation	Air^{o} contact angle	n-Octane^{o} contact angle	γ^{d}_{sv} dynes. cm.$^{-1}$	γ^{p}_{sv} dynes. cm.$^{-1}$	γ_{sv} dynes. cm.$^{-1}$	γ_{sw} dynes. cm.$^{-1}$
Kelvin-1	42.2 ± 3.8	148.2 ± 1.3	12.0	43.6	55.7	1.7
Kelvin-2	54.2 ± 2.8	150.3 ± 2.2	4.6	44.5	49.2	6.5

Table 7.8

Continual Wear formulation	Adsorbed HFb		Adsorbed HSA	
	Time (Hours)	(x 10^{-8} g.cm.$^{-2}$) $\bar{x} \pm \sigma_{n-1}$	Time (Hours)	(x 10^{-8} g.cm.$^{-2}$ $\bar{x} \pm \sigma_{n-1}$
Kelvin-1	3.17 6.83 42.08 69.33	10.1 ± 1.2 18.4 ± 2.1 663.1 ± 95.1 830.2 ± 89.7	69.33	191.4 ± 32.3
Kelvin-2	69.4	419.2 ± 49.5	72.9	534.2 ± 170.4

dispersive parameters of surface free energy for Kelvin-1 and 2, would seem to indicate that these more 'hydrophobic' monomers of MACM and DAMCM undergo bulk interactions, thereby reducing the surface dispersive component (γ^{d}_{sv}) of the Kelvin-2 formulation. Bulk hydrophilicity in the Kelvin-2 forumlation would seem to be apparently maintained by the additional 1% by weight increase in the MAA content.

The data for the HFb and HSA adsorption studies performed on these two compositions are given in Table 7.8. From the data for the HFb adsorption onto the Kelvin-1 formulation it is evident that a time dependent, multiple-layered (sigmoidal) type adsorption is occurring, as found with other hydrophilic materials. For comparison purposes adsorbed protein levels will be taken at approximately 70 hours, which for all intents and purposes can be regarded as the steady state plateau value. From Table 7.8 it is evident that the adsorbed HFb for the Kelvin-1 formulation is very much greater than for the Kelvin-2 formulation, whereas quite the reverse is true for the adsorbed HSA levels with Kelvin-2 showing very high HSA adsorption.

From the surface data given in Table 7.7 it is apparent that the above adsorption results for these two materials are not in total agreement with the previous gross relationships between surface characteristics and adsorbed HFb and HSA levels, as discussed in Chapter 6. Compared to the majority of the hydrogels previously considered these materials do show markedly lower surface free energies (γ_{sv}) and surface wettability, not in agreement with their highly hydrophilic bulk characteristics

(i.e., high water contents). The fall in HFb-adsorption coupled with the seeming preferential adsorption of HSA by the Kelvin-2 formulation, would seem to go someway towards the ideal blood contact material: with regard to the contact lens materials the above adsorption criteria should also be ideal, because fibrinogen, although not present in normal tear fluid, can be used as a model to represent the less stable proteinaceous components (i.e., lysozyme, γ-globulins, glycoproteins etc.), of the tears which give rise to the deposition problem. Thus to minimise the HFb interactions whilst maximising the conformationally stable HSA interactions should in theory help to minimise if not eliminate the problem of deposition on 'soft' lenses.

Both the Kelvin-1 and Kelvin-2 continual wear formulations have been the subject of extended wear clinical trials conducted by Kelvin Lenses Ltd.; they are not yet however, intended for commercial marketing. The adsorption of HFb onto these lenses and other commercially available contact lenses is given in Table 7.9. It is apparent from these results that both the Kelvin-1 and Kelvin-2 formulations in contact lens form, show reduced adsorption levels as compared with their bulk-membrane polymerised forms.

As previously discussed, this can be attributed to the surface treatment(s) necessary to fabricate lenses, and therefore it is to be assumed that the other commercial contact lenses will be equally affected.

With this in mind a comparison of these results does seem to show that both Kelvin-1 and Kelvin-2 should

fair favourably with the majority of the market opposition, with regard to the problem of protein deposition. In fact from the clinical trials on these two formulations, Kelvin-1 appeared to have a greater incidence of deposits than the earlier marketed daily-wear Eurolens composition, whereas the Kelvin-2 faired much better than either.[318] These clinical findings would seem to be in very close agreement with the observed relationship between the HFb adsorption for these lenses as given in Table 7.9, and would seem to indicate the usefulness of the protein adsorption technique for invitro assessment of a material's "bio-potential", (biocompatibility).

The Kelvin-2 formulation although showing a marked improvement over comparable commercial formulations, does not however completely resolve the problem of protein deposition on 'soft' lenses. It is believed that the introduction of 'hydrophilic' lenses effectively converts the surface chemistry of the cornea from hydrophobic to hydrophilic and in so doing, disturbs the normal chemistry and functions of the tear-film and thereby contributes to the random deposit formation on lenses. This concept is more fully discussed in the following section.

Table 7.9

COMMERCIAL 'SOFT' CONTACT LENSES

Product Name	Manufacturer or supplier	Component Monomers	EWC (%)	Adsorbed Hfb* (x 10^{-8}g.cm.$^{-2}$
Duragel	Duralens/Cooper Vision optics	Amido-amino copolymer	73.5	1757.2±249.2
Eurolens	Kelvin Lens Ltd	HEMA	40.0	370.8±47.3
Kelvin-1	Kelvin Lens Ltd	As given in text	73.6	506.9±95.0
Kelvin-2	Kelvin Lens Ltd	As given in text	76.3	332.3±54.2
Permalens	Cooper vision optics	HEMA, NVP, MAA	72.0	479.5±43.7
Sauflon-75	Contact lens manufacturing	NVP, MMA	50.0	1461±84.5
Sauflon-85	Contact lens manufacturing	NVP, MMA	50.0	1323±88.2

* Adsorption study times ~ 70 hours

7.6.3 A Possible Hypothesis for Deposit Formation on Lenses[315]

One of the problems in relating the general proposition that protein adsorption is the precursor to the formation of deposits on contact lens surfaces, lies in producing an explanation for the fact that such deposits are not uniformly distributed over the lens surface. It is with this aspect of the problem that the present hypothesis is concerned.

It would seem less than speculative, after considering the composition and structuring of the normal tear film, to assume that the introduction of a hydrophilic material into this environment, may produce a disruption in the tear film stability. Work by Zisman et al,[319] in another context has shown that a thin aqueous film coating a hydrophilic surface can be made to rupture and recede by the migration of polar lipid-like compounds placed on the water surface. Even if these compounds are relatively immiscible with water, eventually sufficient migrate to the interface and adsorb there, giving localised hydrophobic areas and increasing the interfacial tension. On the basis of this model it would seem likely that the more polar components of the lipid phase of tear fluid, would migrate to the surface of the hydrophilic 'soft' lens.

Elevated dissolved mucin levels are likely to occur due to the anterior hydrophilic surface now presented to the tear film, as opposed to the normal hydrophobic surface of the cornea. Such increase in the dissolved mucin levels may tend to favour the formation of mucin-lipid complexes, and these together with albumin-lipid complexes may enhance the above lipid migration phenomenon.

Maintenance of a stable film over a surface is known to be dependent on the film thickness and the nature of the surface and tear film thickness is much less than the critical film thickness of liquids over hydrophobic surfaces.[314] It is therefore feasible that 'dry spot' formation on hydrophilic 'soft' lenses could follow from a combination of the above factors. Moreover, it is also likely that the blink-reflex will re-establish the tear film and these abnormal areas will either be washed off or rewetted by mucin. Repeated cycles of the above process may, however, eventually give rise to stable hydrophobic areas on the lens surface. If this interfacial phenomenon of lipid migration and adsorption does take place, the interfacial tension would be locally raised producing an increased likelihood of protein adsorption and conformational change and the formation of strongly and irreversibly bound deposits. In conjunction with this repeated 'dry spot' cycles will produce the profound conformational changes in the adsorbed protein species as has been reported for protein adsorption at air/solution interfaces and this will serve to merely compound the problem.[220]

A schematic representation of this hypothesis based on earlier depictions of 'dry spot' formation on the cornea[320] is shown in Fig. 7.3.

A mechanism of this type is not in conflict with the previously discussed phenomenon of protein adsorption at polymer surfaces. The rapid initial monolayer formation observed for hydrophobic materials, will begin the process of surface modification and the subsequent competitive protein deposition would be expected to affect the rate at which lipid interactions, by the mechanism described in

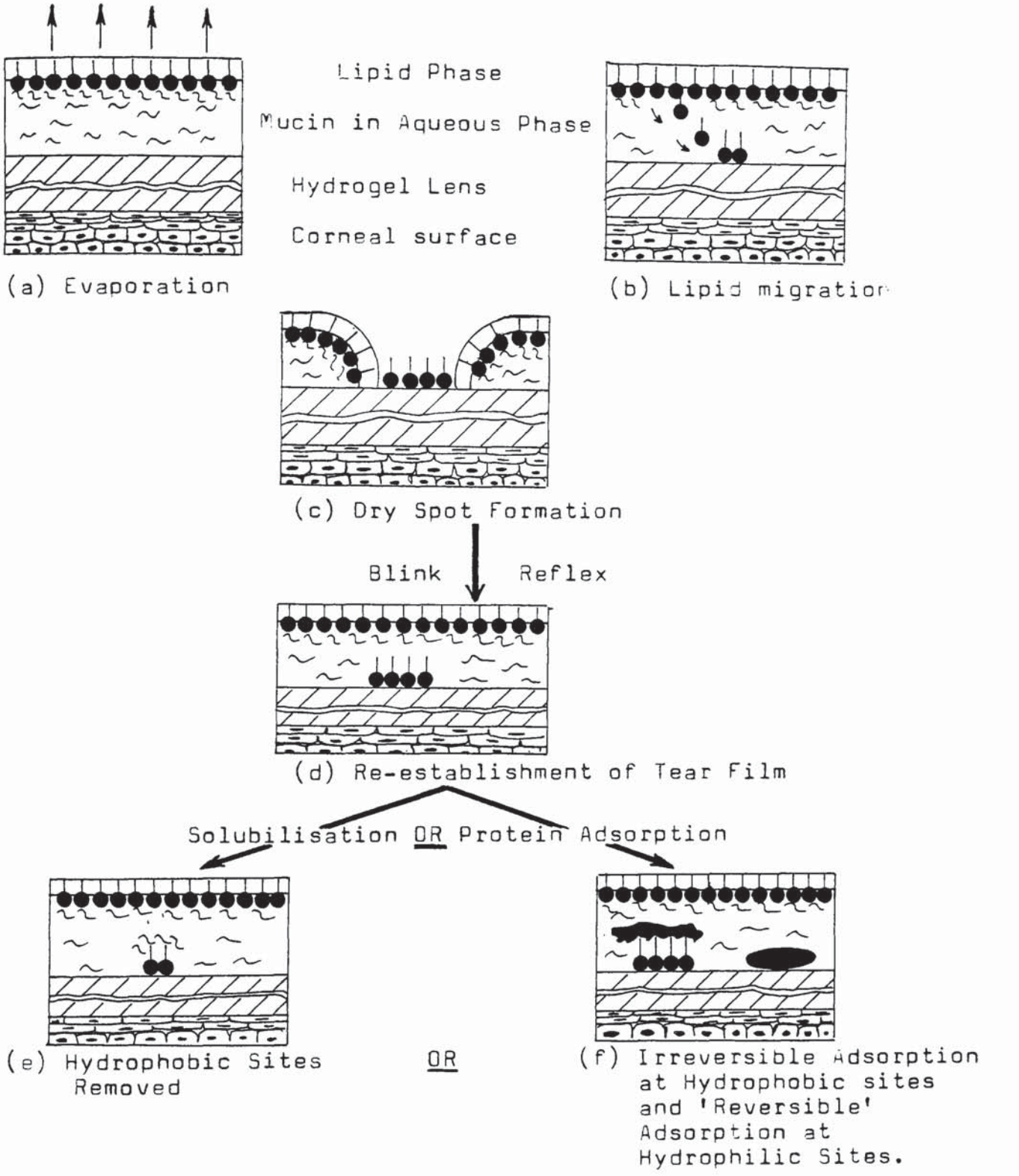


Fig. 7.3 Schematic Protein Adsorption Hypothesis

the above model, could take place. Equally, lipid and protein accumulation will continue as competitive processes, the individual rates of which will depend both on the nature of the modified surface that has been built up and upon various factors associated with the tear film. These latter factors will, of course, encompass the tear film chemistry of the individual wearer and any changes that it may undergo. Although a variety of species (for example lysozyme, glycoproteins, mucopolysaccharides and lipids) have been identified in lens deposits, it is in the nature of the initial interaction of the material with tear proteins that the origin of these deposits lies. Furthermore, despite the fact that any attempt (such as the one discussed here) to describe the mechanism of local accumulation of deposits must, at present be speculative, it is apparent that careful physico-chemical design of the lens surface is an important factor in minimising deposit formation.

7.6.4 Incorporation of Fluorinated Monomers to Produce Hydrophilic 'soft' Lenses of Reduced Surface Energy

With regard to the previous discussion concerning the problems of 'dry spot' formation on hydrophilic 'soft' lenses,[315] attempts to alleviate this problem have centred on the modification of the continual wear formulations to ellicit a reduced surface energy more in keeping with the natural corneal surface chemistry.

Polymers containing a number of fluoro substituents are known to exhibit low intermolecular forces at the air-solid interface and hence give rise to reduced surface adhesive characteristics.[168,321] This has encouraged the

study of copolymers of hydrogels and fluorinated monomers, carried out in these laboratories by Barnes.[201] From these studies it was found that the inclusion of fluorinated species within a hydrogel (Xerogel)-structure does indeed yield a material of lower surface energy, with respect to that of the corresponding homopolymer containing no fluoro groups, due to the reduction produced in the polar component. This would indicate that an appreciable number of fluorinated groups are positioned within the surface structural layers of the polymer. Measurement of the surface polar components (by the Hamilton Contact Angle technique) of these polymers in hydrated form, determined that the surface polar components of the fluoro-derivatives had substantially increased upon hydration, as opposed to only slight increase with the non-fluorinated homopolymer.[154,201] These findings have been explained as being due to the chain rotation of the polymer back-bone, leading to a re-orientation of the fluorinated substituents from the surface layers into the bulk of the hydrated gel and their replacement by hydrophilic groups.

The above studies were unable to ascertain the surface dispersive components (γ^{d}_{sv}) of the hydrated gels and accordingly the total surface free energy (γ_{sv}) could not be derived, and further comment on the subject was not attempted. The effects of the hydrophobic fluoro-monomers on the bulk hydrophilicity of continual wear formulations has been studied[155] and from these studies it was determined that up to 3% by weight of the fluoro-monomer could be incorporated, without too adverse a reduction in the water content criteria necessary for a con-

tinual wear formulation (i.e., > 70% EWC at room temperature).

Both the Kelvin-1 and Kelvin-2 continual wear formulations were modified in these studies by the addition of two types of fluorinated-monomer; hexafluoro isopropyl methacrylate (HFIPMA) and 1,1,3 Trihydroperfluoro propyl methacrylate (1,1,3THPFPMA). The structural formulae of these fluoro-monomers are given in Fig. 7.4. Clinical trials of these fluorinated versions of the Kelvin-1 and Kelvin-2 contact lenses are now in progress and to date problems with deposit formation have been minimal.[318]

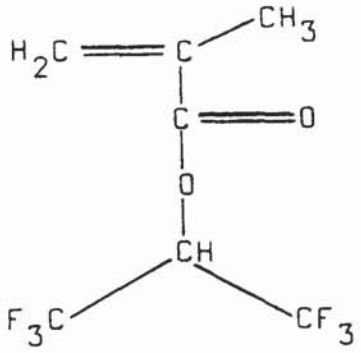
Surface characterisation and HFb adsorption studies have been carried out on fluoro-derivatives of the Kelvin-1 formulation and the pertinent results, together with the water contents of these compositions, are given in Table 7.10 and 7.11 respectively.

From a comparison of the water content and surface data given in Table 7.10, it can be observed that the addition of these fluoro-monomers to the Kelvin-1 formulation produces some very interesting and significant surface-bulk structure-property effects.

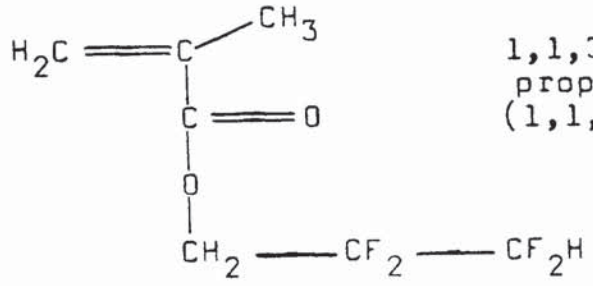
Incorporation of 1,1,3 THPFPMA (1% by weight) can be seen to unexpectedly raise the water content, however the corresponding rise in the surface dispersive component (γ^{d}_{sv}), seems to corroborate this finding. It appears that from the structural formulae of 1,1,3 THPFPMA the flexible side-chain, fluoro-group is capable of concentrating at the hydrogel surface, and as a consequence the more hydrophilic groups are forced into the bulk, producing a concomitant rise in water content, together with a raised surface wettability and total surface free energy (γ_{sv}).

Fig. 7.4

STRUCTURES OF FLUORINATED MONOMERS



Hexafluoro isopropyl methacrylate (HFIPMA)



1,1,3 Trihydroperfluoro propyl methacrylate (1,1,3 THPFPMA).

Table 7.10

WATER CONTENTS AND SURFACE CHARACTERS

OF THE FLUORINATED KELVIN-1 FORMULATIONS

Fluoro-Monomers	% EWC ('Krebs')	Air° Contact angle	Hamilton° contact angle	γ^d_{sv} dynes. cm.$^{-1}$	γ^p_{sv} dynes. cm.$^{-1}$	γ_{sv} dynes. cm.$^{-1}$	γ_{sw} dynes. cm.$^{-1}$
None	77.2	42.2±3.8	148.2±1.3	12.0	43.6	55.7	1.7
1,1,3THPFPMA 1% by weight	82.3	37.9±2.7	147.3±1.4	15.1	43.2	58.4	0.9
1,1,3THPFPMA 2% by weight	77.2	49.7±2.9	136.8±2.6	11.5	38.1	49.6	2.6
HFIPMA 1% by weight	76.3	44.5±1.8	151.0±0.5	9.7	44.8	54.5	2.6
HFIPMA 2% by weight	72.4	61.8±3.6	152.7±0.3	1.4	45.5	46.8	12.4

Further increase in the content of this fluoro-monomer to 2% by weight, can be observed to reverse this trend with restoration of the water content (i.e., 77.2%) to that of the non-fluorinated formulation. Reduction of both surface wettability and polar component (γ^{p}_{sv}), together with the total surface free energy (γ_{sv}) can be attributed to the fluoro-monomer acting both at the surface and within the bulk of the hydrogel.

In contrast to 1,1,3 THPFPMA the addition of HFIPMA produces the unwanted raising of the surface polar components (γ^{p}_{sv}), as reported in previous studies.[154,201]

From the depression of water content observed with HFIPMA, it would seem likely that the less flexible side-chain fluoro group of this fluoromonomer (as compared with 1,1,3 THPFPMA), undergoes bulk hydrophobic interactions and this would appear to be corroborated by the marked reduction in the surface dispersive components (γ^{d}_{sv}). The overall effect of this fluoromonomer however, can be seen to be a reduction in both surface wettability and total surface free energy (γ_{sv}), as observed for the HFIPMA 2% by weight composition. This latter composition can be observed to exhibit a higher interfacian tension (i.e., 12.4 dynes. cm.$^{-1}$) than any of the hydrogels so far studied by these surface chemistry techniques.

Generally the lower total surface free energy materials normally designated as hydrophobic, have high interfacial tensions (i.e., P.T.F.E., Melinex, Polyethylene, silicone rubber - see Appendix 4), whereas for high total free energy materials (i.e., hydrophilic) approximating towards 70 dynes. cm.$^{-1}$ the converse is true. Previously

such high water content gels would have been assumed to exhibit very low interfacial tensions however, in Chapter 5 it was determined that this was only true for a surface when both polar (γ^{p}_{sv}) and dispersive (γ^{d}_{sv}) surface free energy components approached or approximated to the respective surface free energy components of water, and not simply to the total surface free energy. From the HFb-adsorption data for these fluoro-compositions given in Table 7.11, it can be seen that no one surface parameter as discussed above, appears to be influencing the protein adsorption phenomenon. Moreover, it is apparent that the inclusion of the fluoro-monomers appears to produce a substantial non-specific reduction in HFb adsorption, seemingly unrelated to fluoro-monomer type, concentration or surface activity. Quite obviously the complex array of monomers incorporated into the Kelvin-1 formulation makes it very difficult to establish the structure-property effects of the material and therefore, further comment on the protein-polymer interactions would be somewhat speculative in nature. However, one possible argument which may lend itself to the nature of the non-specific reduction in adsorption, is that the incorporation of the fluoro-substituent groups effectively displaces the negatively charged methacrylic acid groups towards the hydrogel surface. This then sets up an electrical double layer effect at the hydrogel surface and produces a concomitant electrostatic barrier to HFb adsorption, as previously discussed in Chapter 6.

Clinical trials with these fluorinated continual wear formulations have shown reduced deposit formation in accordance

Table 7.11

HFb ADSORPTION DATA FOR THE FLUORINATED KELVIN-1 FORMULATIONS

Fluoro-Monomers	HFb - Time (Hours	Adsorption ($\times 10^{8}$ g.cm.$^{-2}$ $\bar{x} \pm \sigma_{n-1}$
None	6.83 69.33	18.4 ± 2.1 830.2 ± 89.7
1,1,3THPFPMA 1% by weight	6.6 69.2	7.5 ± 1.9 276.0 ± 46.5
1,1,3THPFPMA 2% by weight	69.3	267.4 ± 48.4
HFIPMA 1% by weight	6.0 69.5	7.5 ± 0.8 274.5 ± 36.6
HFIPMA 2% by weight	69.4	298.5 ± 49.5

with their above invitro HFb adsorption levels.[318] It would seem therefore that the incorporation of low concentrations of fluoro-monomers is justified but, whether their influence on the surface properties of a material is by a direct action or whether its effects are mediated by other monomers within the formulation (i.e., MAA), has still to be determined and this will govern their future usage.

CHAPTER 8

CONCLUDING DISCUSSION AND SUGGESTIONS FOR FURTHER WORK

8.1 Concluding Discussion

The aims of this thesis have been primarily to examine and characterise the bulk and surface structure-property effects of hydrophilic polymeric materials (hydrogels), and in particular to relate these said effects to the previously discussed interfacial phenomenon of protein adsorption.

Since the development of hydrogels in the early 1960's, many biomedical applications (and proposed applications) of hydrogels have relied somewhat theoretically on the assumption that the inherent characteristics of these materials, (such as similarity to soft tissues and permeability to small molecules) are such that they are endowed with the requisite properties necessary for a biocompatible material.

Based on the interfacial adsorption of the plasma proteins, albumin (HSA) and fibrinogen (HFb), and their relationship to biocompatibility, (as previously outlined in Chapter one) the study of HSA and HFb adsorption was envisaged as being a somewhat novel approach to the characterisation of hydrogel surfaces. To date no previous study known to the author has attempted to correlate the adsorption behaviour of these proteins, based on their known physico-chemistry, to the surface structure-property effects of the adsorbent material.

The material properties of hydrogels may be considered as lying somewhere between those properties

exhibited by both solids and liquids. Therefore when considering the surface/interfacial properties of hydrogels as with liquids, the bulk structure-property effects greatly influence the surface chemistry and must be taken into account.

Although hydrogel polymers have been the subject of many studies, in the main such studies have been restricted to individual characteristic properties of empirically derived hydrogel compositions. The work described in this thesis has attempted to approach the problem of characterisation by investigating the bulk and surface properties of the gel(s) as a whole. Previously no one study has attempted to correlate the relationships between the monomeric structural chemistry of hydrogel compositions and both their bulk and surface properties. In particular the measurement of surface/interfacial free energies of hydrated hydrophilic materials has presented a considerable stumbling block to many previous studies in this area.

Many of the above objectives in this thesis have been successfully completed. However, because of the comprehensive nature of these studies and the sometimes diverse complexities associated with each area of study, it is perhaps warranted that this discussion should reiterate some of the more salient features evident in each of the relevant chapters concerning bulk, surface and protein adsorption studies. Moreover, this should help to clarify the bulk and surface structure-property effects and their interrelationships with the protein adsorption studies.

The investigations carried out in Chapter 4 on the

bulk properties of hydrogels were restricted to the characterisation by both hydration studies and water-binding (structuring) studies as measured by differential scanning calorimetry (D.S.C.). In these studies it was found that the individual structural chemistry of the constituent monomeric components of hydrogels directly influenced both the equilibrium water contents (E.W.C.) of the gel(s) and their relative amounts of free (freezing) and bound (non-freezing) water contents.

The progressive introduction of the hydrophobic monomers into the purified poly (2-hydroxy ethyl methacrylate) (poly (HEMA)) gel structure, to form the hydrophilic/hydrophobic copolymer series was observed to produce an equally progressive depression in the water contents of these gels. This depression of water content was observed to be directly related to the relative hydrophobicity of the pendant side-chain groups of the adjuvant hydrophobic monomers, with the bulky hydrophobic phenyl group of styrene (STY) producing a more drastic depression in water content, than the less hydrophobic ethyl side-chain group of ethyl methacrylate (EMA). From the discussion on the hydration studies carried out on the copolymers of both HEMA and its structural isomer 2-hydroxy propyl acrylate (HPA), it became clear that not only were the type(s) of chemical groups present in the monomer structure important in relation to hydration, but also the intramolecular locations of the said groups. For example, whereas the flexible side-chain methyl group (in the 2-position) of HPA could easily undergo hydrophobic, Van der Waals interactions with adjacent hydrophobic groups, the α-methyl backbone group of HEMA produced a restriction to both chain rotation and formation of hydrophobic

interactions. Thus, additions of hydrophobic monomers to HPA produce a greater depression in EWC than its HEMA counterparts, even though for the same structural considerations given above, poly (HPA) possesses a higher water content (~ 50% EWC) than its structural isomer poly (HEMA), (~ 40% EWC).

With regard to the water-binding data for the hydrophilic/hydrophobic copolymers, the hydration trends were in the main believed to be attributable to the exclusion of free water. Some increase however, in the bound water content was observed for the HEMA/STY copolymers, unfortunately these earliers water-binding studies in our laboratories were not performed using the purified HEMA monomer, as utilised in this work. Therefore the above water structuring attributable to STY, may be a consequence of the interactions between the STY and impurities present in the HEMA, rather than as a direct result of the STY molecule.

The influence of the monomeric impurities present in the commercial 2-hydroxy ethyl methacrylate (HEMA), (i.e., methacrylic acid (MAA) and ethylene glycol dimethacrylate (EDMA)), were shown to profoundly affect the water-binding data of poly (HEMA) gels, and that following the removal of these impurities substantial increase in the bound (non-freezing) water content was possible.

Because of the raised bound water fraction of the purified poly (HEMA), the incorporation of EMA was seen to produce the much desired properties of high bound/low free water content deemed necessary for a reverse osmosis membrane (as used in desalination treatment). It was further postulated that perhaps with a more hydrophilic

monomer than HEMA, the addition of EMA may produce a copolymer with a much improved bound/free ration. To some extent this was borne out by the high water content NVP/EMA (~71% EWC) copolymer, which exhibited an anomalously greater strength than poly (HEMA), (~40% EWC), and which was taken as being indicative of a very high bound to low free water content. Unfortunately however, no water-binding data is available to support this view.

Somewhat unexpectedly the initial inclusion of the hydrophobic monomers into the poly (HEMA) structure produced a rise in the total surface free energy (γ_{sv}) and a commensurate decrease in the interfacial free energy (tension), (γ_{sw}). These surface effects were ascribed to the disruption of the bulk hydrophobic, Van der Waals and dipole-dipole interactions of poly (HEMA), and which resulted in a reduction in the bulk constraints to chain rotation, thereby allowing the interfacial free energy to minimise. From the abrupt increase in the interfacial tension, together with a corresponding abrupt change to a completely bound water content, for the HEMA/STY (85:15 Mole %) composition, it was summised that for the above chain rotation to occur, free (freezing) water is necessary to plasticise the chain segments. Moreover, the increase in interfacial free energy (tension) could be shown to correspond with high bound water contents and which correspondingly signified increased bulk constraints to chain rotation.

Predictably the progressive introduction of hydrophilic monomers into the poly (HEMA) structure, to produce the hydrophilic/hydrophilic cooolymer series, was in general

accompanied by an elevation in EWC together with substantial increase in free (freezing) water content. However, as observed with the hydrophobic adjuvant monomers, superimposed on the more general trends were hydration patterns specific to the individual structural chemistry of the adjuvant hydrophilic monomer. For example, the monomer acrylamide (ACM) was found to produce a near linear rise in water content due to a preponderance of polymer-water interactions rather than polymer-polymer interactions. However, for the ACM derivatives, methacrylamide (MACM) and diacetone acrylamide (DAACM), polymer-polymer interactions were postulated to be occurring in the form of hydrophobic, Van der Waals, interactions between the α-methyl group of HEMA and the respective α-methyl and 1,1 dimethyl 3-oxy-butyl groups of MACM and DAACM, and these accounted for their initial depressions in water content. Moreover, the extent of this depression and the ultimate gel bulk hydrophilicity and water structuring, were seen to be dependent both on the location and type of these hydrophobic groupings within the monomer structure and their resultant influence on the very hydrophilic side-chain amine group. In some accord with the above bulk structural chemistry considerations predictably, the surface data for these hydrophilic compositions showed general increases in surface wettability, total surface free energy (γ_{sv}) and reduction in interfacial free energy (γ_{sw}).

In order to ascertain more accurately the potential invivo performance of the aforementioned hydrogels, comparative hydration studies were carried out using both 'physiological' Kreb's solution and the more traditional hydration medium of distilled-water. Only those hydrogel

compositions containing appreciable ionic content, were found to show significant variations in E.W.C.

The best studied example of the above was conducted on the HEMA/MAA copolymers, which in distilled-water (and at a preferential ratio of 60:40 Mole %) were believed to undergo polymer-polymer interactions (hydrogen-bonding) between adjacent carbonyl and hydroxyl groups, producing a depression in E.W.C. When however, these same copolymers were hydrated in Kreb's solution, vast increases in E.W.C. were observed, which were inversely proportional to those in distilled-water. These findings were ascribed to disruption of the above polymer-polymer interactions by the formation of methacrylic carboxylate anions (due to ionisation) and the subsequent interaction of these groups with the very hydrophilic sodium cations in solution.

The bound (non-freezing) water contents for these copolymers also followed the same parabolic pattern as observed for the above hydration studies (in Kreb's solution) whereas, the free (freezing) water contents were observed to progressively increase in accordance with the increasing gel MAA content. In some agreement with this free-water bulk trend, the surface measurements of these copolymers indicated that total surface free energy (γ_{sv}), and surface wettability also progressively increased, whereas interfacial free energy (γ_{sw}) was inversely related to the bound-water bulk trend. This latter relationship would seem to support the view that the surface chemistry of hydrogels is inherently dependent on the bulk interactions and that it is the degree of bulk interactions or constraints (as indicated by the bound-water content levels) that are

imposed on chain rotation, which determine the ability of hydrogel surface chain segments to minimise their interfacial free energy (γ_{sw}).

Further evidence in support of this view was provided by the surface data for the HEMA/EDMA copolymers, whereby somewhat paradoxically the increasing content of the hydrophobic cross-linking agent EDMA was actually observed to produce an increase in both total surface free energy (γ_{sv}) and surface wettability and a decrease in the interfacial free energy (γ_{sw}).

This phenomenon was believed to result from the non-isotropic cross-link distribution of the EDMA molecules. Such effects, understandably wouldbe expected to favour an increase in chain segment length and accordingly greater freedom of chain mobility, which would then allow the surface chain segments to orientate to minimise the interfacial free energy (γ_{sw}). Although some contention exists as to the exact water structuring of these copolymers (due to monomer impurities), it is believed likely that a reduction in bulk interactions (as indicated by a reduction in bound water content) accompanies these surface manifestations.

The choice of the plasma proteins, human serum albumin (HSA) and human fibrinogen (HFb), for the protein adsorption studies, was based on the premise for biocompatibility as outlined in Chapter one, that is high HSA and low HFb adsorption was indicative of a non-thrombogenic (biocompatible) material (and vice versa). From these studies, it was hoped, that not only would the surfaces in question be assessed for their possible bio-

compatibility, but that in conjunction with the surface-measurement techniques as outlined in Chapter five (i.e., the Hamilton and captive air bubble-contact angle techniques) the surface/interfacial chemistry of hydrogels in a 'physiological' environment, may be more fully elucidated.

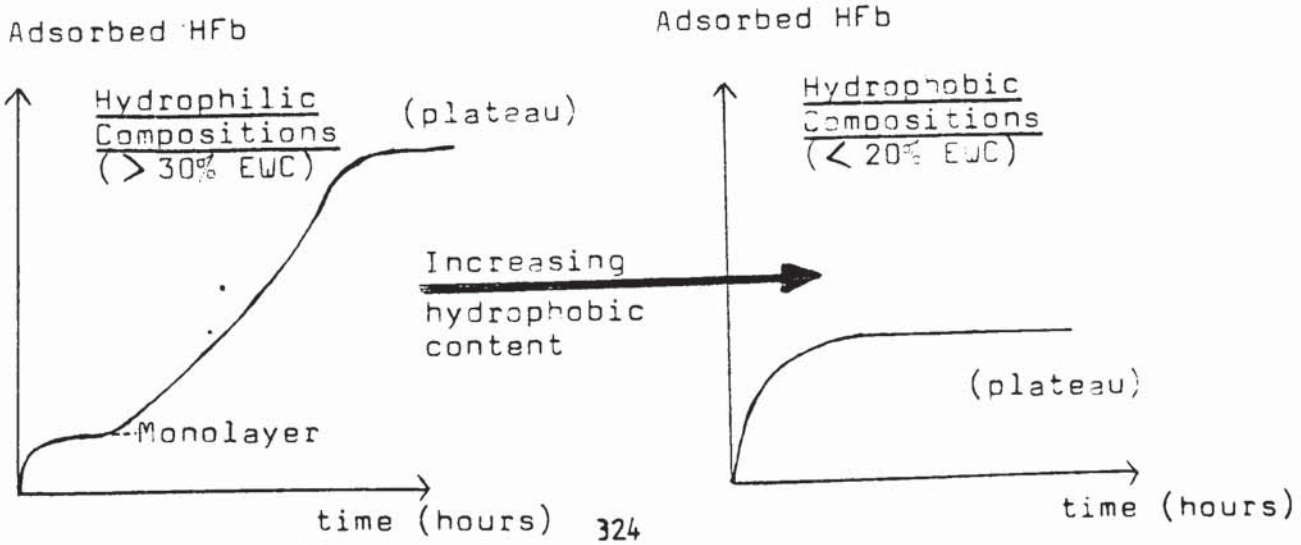
Previous research has shown that the conformational stability of HFb and HSA differed markedly. In some contrast to HSA, HFb did not appear to possess sufficient intramolecular stability to maintain its 'native' globular conformation when adsorbed on to hydrophobic materials. This instability of the HFb molecule did present problems in this work, in assessing both the value for monomolecular coverage, and also the type(s) of interactions that the molecule undergoes when it adsorbs. With regard to this latter aspect, significant variation in HFb adsorption type was observed between the low water content ('hydrophobic', low surface free energy) gels ($<$ 20% EWC), and the higher water content ('hydrophilic', high surface free energy) gels ($>$ 30% EWC).

The HFb adsorption-time studies onto the hydrophilic compositions($>$ 30% EWC) were observed to follow a multilayered sigmoidal pattern. In particular, a time dependant or rate limiting step was observed to be occurring, approximating to the monomolecular coverage value for the 'native' HFb molecule ($\sim$ within the range (13-21) $\times 10^{-8}$ g.cm.$^{-2}$). This was attributed to an increase in the interfacial pressure (π) which will accompany the developing HFb monolayer coverage, together with a time-dependent modification or restructuring which decreases the interfacial pressure (π) and thus enables successive

monolayers to adsorb. Alternatively, based on thermodynamic considerations, it was postulated that as the more energetic hydrophilic adsorption sites are depleted, so the enthalpy of adsorption becomes significantly less exothermic, and then following time-dependent lateral intermolecular interactions, a pseudo-fibrin polymer-like film is formed. This produces further adsorption sites, raising the enthalpy of adsorption and enabling a second monolayer to adsorb onto the first monolayer. Further successive monolayers are then layed down until finally a steady state plateau is attained.

Increasing the hydrophobic content of the hydrophilic gels was observed to markedly reduce the steady state plateau values. Also with the progressive increase in the hydrophobic content, so the sigmoidal hydrophilic adsorption-time curve was progressively converted into a more parabolic shape, with loss of the time dependent step, as illustrated in Figure 8.1 below.

Figure 8.1



The truth of this trend was reinforced by the similar HFb-adsorption patterns onto the very hydrophobic polymers of P.T.F.E., Melinex and silicone rubber.

With these hydrophobic polymers, low steady state plateau values ($\sim$(75-175) x 10^{-8}g.cm.$^{-2}$) were evident, together with increased rates of adsorption. This adsorption behaviour was ascribed to the disruption of the conformational stability of the HFb molecule, leading to the entropically driven adsorption of denatured molecules, and the formation of multiple low enthalpy hydrophobic, Van der Waals interactions between the protein and the hydrophobic surface. Due to the substantially lower value for monolayer coverage by denatured HFb molecules, multilayer formation (adsorption) for these hydrophobic surfaces is presumed to be commensurate with the hydrophilic compositions.

As a result of the hydrophilic characteristics and inherent conformational stability of the HSA molecule the interactions of this protein with surfaces tended to be predominantly polar (exothermic) in nature.

Thus an increase in the surface free energy of materials was observed in general to produce a corresponding increase in the level of multilayer HSA-adsorption. It should be remembered however, that the adsorption process for any protein (or macromolecule) will be a composite function of the many different interaction types (i.e., hydrophobic, Van der Waals, ionic, dipole-dipole (polar) etc) together with their population density and distribution and although a specific interaction type may predominate (as in the above example), the cumulative effects of the other interaction types must also be considered.

The somewhat empirical general relationships which exist between the aforementioned hydrogel(s) bulk and surface structure-property effects and their protein adsorption behaviour, are given below:

The predominantly high bound fraction, low water content hydrogels ($<30\%$ EWC), which possess very small 'pore' sizes (high chain segment density) and in general high surface concentrations of non-polar (hydrophobic) groups, tended to exhibit both low HFb and HSA adsorption, but for markedly different reasons. The low HFb adsorption was accountable to the previous entropic stability considerations for the molecule, whereas the low HSA adsorption may be ascribed to low enthalpy interactions.

Individual variations in the surface properties of these low water content gels have been attributed to the gel ability to undergo chain rotation and thus, minimise their interfacial free energy (γ_{sw}) and raise surface free energy (γ_{sv}). These surface manifestations are borne out by the corresponding, accompanying rise in HSA adsorption.

As the bulk hydrophilicity is increased to give hydrogels of 30 to 60% EWC, so accordingly, 'pore' size is increased ($\sim \leq 10$Å diam) and the chain segment density is decreased slightly. The surface chemistry of these gels is comprised of high concentrations of both polar and non-polar groups ($\gamma_{sv} \approx 45$-63 dynes.cm.$^{-1}$) and the interfacial free energies are reduced ($\gamma_{sw} < 4$ dynes. cm.$^{-1}$). As would be expected for these gels, HSA adsorption progressively increases due to increasing polar (hydrophilic) interactions over the range. In contrast HFb adsorption was observed to increase abruptly, attaining a maximum peak value (865×10^{-8} g.cm.$^{-2}$), for the poly (HEMA) composition

(~40% EWC), after which the adsorption levels were observed to fall-off over the range. This maximum can be attributed to high enthalpy hydrophilic interactions together with low enthalpy hydrophobic interactions. With the reduction in interfacial free energy (γ_{sw}), it is believed that adsorbing HFb molecules can maintain their 'native' conformation and accordingly the previously discussed entropic mechanism for the adsorption of HFb molecules will no longer be evident.

With still further increase in the bulk hydrophilicity to form the low bound fraction, high water content gels (> 70% EWC), moderate to large 'pore' sizes would be expected (20-200Å diam.), with the chain segment density and surface concentration of predominantly polar groups being inversely proportional to the 'pore' dimensions. Low HFb adsorption is a feature of these gels and results from low enthalpy hydrophilic interactions. In accordance with the progressive increase in bulk and surface hydrophilicity, so the HSA adsorption is observed to progressively increase.

Some contention exists however, as to whether 'adsorption' is occurring with high water content gels (> 90% EWC), or due to the small molecular size of HSA (38 x 150Å), absorption. With the exponential increase in 'pore' size and the corresponding decreasing chain segment density and surface concentration of polar groups, adsorption enthalpy similarly decreases; thus, in theory, true adsorption of HSA would decrease in a similar manner to that observed for HFb. The determination of the molecular cut-off point and differentiation between adsorption and absorption for these high water content gels was out-

side the scope of this project and further investigations will be necessary before the above issue can be resolved.

With regard to the effectiveness of these adsorption studies as a potential invitro index of biocompatibility, it is perhaps of significance to note that very low adsorption levels of HSA and HFb was a feature of the high water content ionic hydrogel compositions, and compared favourable to earlier Lee White blood clotting studies in our laboratories (in collaboration with the Liver Support Unit, King's College Hospital, London). In these studies it was found that although neutral hydrogels increased the coagulation times, by far the most significant increase in coagulation time was exhibited by ionic (acidic) gels.

The somewhat unexpected reduction in interfacial free energy (γ_{sw}) observed for the low water content gels, correlated with their lowered HFb adsorption and raised HSA adsorption (indicating thromboresistance). These findings would seem to support the 'Minimal Interfacial Energy' hypothesis (as discussed in Chapter 1 which postulated that a low interfacial free energy ($\gamma_{sw} < 5$ dynes. cm.$^{-1}$) was a prerequisite for a biocompatible material. Some corroboration for the above is given by previous research investigations on hydrophilic/hydrophobic copolymers (namely HEMA/EMA); where it was found that additions of the hydrophobic monomer (HEMA/EMA $\sim$ 75:25) produced both a maximum reduction in invitro cellular adhesion together with a maximum increase in invivo thromboresistance.

With reference to the contact lens field problem of deposits on hydrophilic lenses, a correlation was observed between the clinical incidence of lens deposits on the commercial and continual wear formulations (as discussed in Chapter 7) and the HSA/HFb adsorption studies. For those hydrophilic lens formulations showing a low HFb adsorption (with or without supporting high HSA adsorption) a corresponding low incidence of lens deposits was noted. The rather unusual bulk and surface properties together with the anomalously high protein adsorption exhibited by the N-vinyl pyrrolidone (NVP) containing copolymers (in particular NVP/EMA) has been discussed previously as being a function of the water-structuring due to the close proximity of the hydrophobic alkyl ring and the large dipole-moment which exists on the imide group. It is therefore not too surprising that the commercial lenses containing significant amounts of NVP monomer, particularly when in conjunction with hydrophobic monomers (i.e., Sauflon 75 and 85), tend to suffer from the problem of lens deposits.

In conclusion the studies carried out in this thesis have shown that both the bulk and surface structure-property effects of hydrogels, may be specifically related to the degree and type(s) of adsorption behaviour of plasma proteins (HSA and HFb) with known physico-chemical characteristics and that with this knowledge some indication of a material's biocompatibility may be derived. Further to the above, the protein adsorption studies performed in this work have indicated that the

adsorption process was of a multilayer adsorption type and did not follow Langmuir's monolayer type isotherms. Moreover, the maximum numerical level of multilayer adsorption was seen as a composite function of: (i) the physico-chemistry of the adsorbing protein specie; which determines both its rate of diffusion to the surface and its degree and type of interaction with a surface and which in itself is influenced by its own (ii) bulk protein concentration (iii) surface/interfacial chemistry of the adsorbent surface.

8.2 Suggestions for Further Work

This work has in itself raised many interesting issues which require further investigation. Several suggestions for further work are as follows:

(1) Further investigation and extension of the hydrophilic/hydrophobic compositions, in particular with regard to the influence of the length of side chain alkyl groups on the bulk properties (i.e., bound and free water content/mechanical strength/permeability etc), together with complementary surface characterisation as performed in this work. Moreover, special attention should be given to the unusual bulk and surface properties of N-Vinyl pyrrolidone (NVP) containing copolymers.

(2) In order to resolve the apparent anomalies in the observed water binding data of the HEMA/EDMA and HEMA/STY copolymers, further work should be performed using the purified HEMA systems.

(3) Permeability studies on high water content gel systems (> 70% EWC), with particular reference to the molecular cut-off points for various macromolecules, will enable the differentiation between adsorption and absorption to be made.

(4) The characterisation of new copolymer systems by further protein adosprtion studies (in conjunction with bulk and surface characterisation) and with regard to potential biomedical applications, the study of surface grafted hydrogel compositions.

(5) In accordance with the above investigations, complementary invivo animal testing of materials will be necessary to prove the usefulness of potential biomaterial compositions, as ascertained by the invitro protein adsorption technique advocated in this work.

(6) Water-binding studies on surface-grafted hydrogel formulations may enable the derivation of surface/interfacial structured water however, the success of such studies will be greatly influenced by the analytical sensitivity of differential scanning calorimetry.

(7) Refinement of the experimental equipment design for the dynamic adsorption 'flow-cell', followed by dynamic adsorption studies with particular reference to the flow charge characteristics of ionic and high dipole moment polymers.

APPENDICES

Appendix 1

Analysis - G.L.C. operating conditions
Instrument: Perkin-Elmer F11, G.L.C. with flame ionisation detector.
Column was packed with 10% Carbowax 20 M on Chromosorb G(60-80)
Detector temperature = 200°C; Injection port temperature = 170°C
Column temperature = 175°C. Sensitivity Range: (8×10^{-3}-16×10^{-2})
Flame ionisation detector gases: H_2 ~ 18 lb.□ "; Air ~ 8 lb.□ ".
Carrier gas: N_2 = 50 ml/min.
Sample size = 1 μl Chart speed = 120 mm/hr

Monomer resolution times are given below:

Monomer	Distance	Time (minutes)	Retention Volume (l)
H.E.M.A. (1 μl)	32.3 mm	~16.2 mins	0.810
EDGMA (1 μl)	40.5 mm	~20.3 mins	1.015
M.A.A. (1 μl)	21.0 mm	~10.5 mins	0.525
H.P.A. (1 μl)	22.0 mm	~11 mins	0.550
ACM/methanol (1 μl)	53.5 mm	~26.8 mins	1.340
A.A. (1 μl)	14.0 mm	~7 mins	0.350

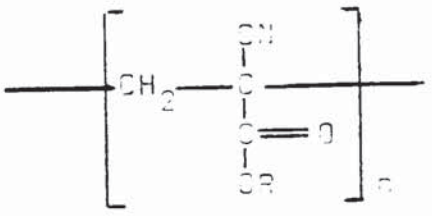
D.S.C. operating Conditions

Perkin Elmer D.S.C. (model DSC-2)
Sample size ~ 3 mg Cooling rate 20 °C/min to -40°C
Heating rate 1.25°C/min to +10°C
Cooling/heating cycle repeated 3 times at both 1.25°C/min & 5°C/min

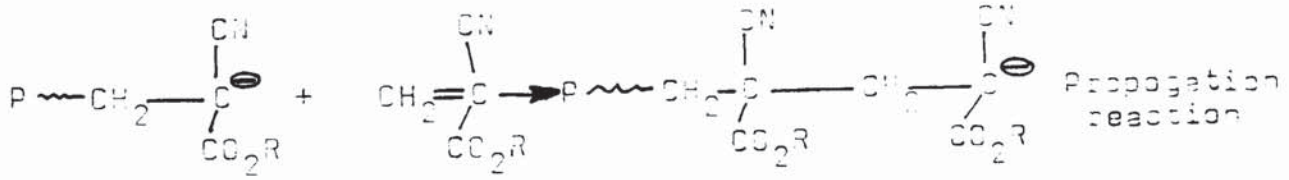
Hamilton's method: α-cyanoacrylate glue-adhesion mechanism:

This adhesive is often used in experimental surgery of soft tissue injuries, i.e., liver, spleen, kidneys etc., where normal sutures are invalid. The adhesion mechanism can take place even in an aqueous environment and is thus of use in bonding even very high water content hydrogels ($\leq$95%) to a substrate backing. The adhesive mechanism is given below.

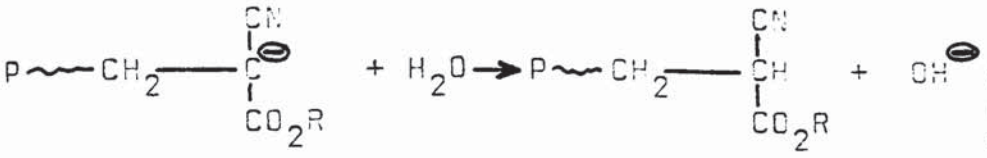
α-cyanoacrylate (R = alkyl group)



The polymerisation is notable in apparently involving anionic mechanism even in aqueous solution;



It seems that the combined presence of the cyano & ester groups on the α-carbon atom serves to delocalise the anionic charge from that atom and so inhibit the expected chain-terminating proton transfer reaction with water.



while these 2 groups on the monomer, **activate it** sufficiently to give a high rate of polymerisation

The strong polymer to tissue adhesion probably arises from the initiation of polymerisation by weakly basic groups on the tissue constituents (proteins, carbohydrates) so that polymer chains become covalently attached to tissues.

Appendix 2

'PHYSIOLOGICAL'-HEPES SOLUTION

This was made up and corrected to pH 7.4 as follows:

Reagent	Concentration (g/l)	Supplier
Sodium Chloride (A.R.)	6.9	Fison
Potassium Chloride (A.R.)	0.35	"
Magnesium Chloride (A.R.)	0.11	"
Sodium Hydrogen Carbonate (A.R.)	2.1	"
Sodium diHydrogen Phosphate (A.R.)	0.14	"
Glucose (A.R.)	2.0	"
*Calcium Chloride (A.R.)	0.28	"

* N.B. $CaCl_2$ must be added to the solution after all other reagents otherwise an insoluble precipitate will form.

Technical Information on the Iodinated (^{125}I)-Proteins

^{125}I-HSA injection B.P.: Sterile solution of human albumin, iodinated to ensure uniformity of labelling. Average iodine content not more than 1atom/mol of albumin. Solution contains 0.9% benzyl alcohol and is made isotonic with sodium chloride.

Protein concentration: 20mg/ml Purity: < 5% free iodide
Storage: store at 2-4°C.
Supplied in multidose vials of 500 μCi at ref. date.
Radioactive concentration - 50 μCi/ml;
Specific activity: 2.5 μCi/mg albumin.

^{125}I-HFb injection: Freeze-dried preparation of human fibrinogen iodinated to ensure uniformity of labelling. Average iodine content 0.5 atom/mol fibrinogen. Contains albumin together with sodium chloride and sodium citrate which when constituted according to directions will yield isotonic solution.

Specific activity: > 100 μCi/mg fibrinogen
Purity: Not less than 80% of ^{125}I can be coagulated with bovine thrombin in the presence of human plasma.
Note: fibrinogen used in this preparation is of a grade that is licenced for therapeutic use in the U.K. (i.e., Australian antigen -ve).
Storage: at 2 - 4°C in dark.
Supplied in vials of 110 μCi at ref. date.

Nuclear Enterprises Type - 8312 γ/β Counter Operating Conditions

(^{125}I decays with a half-life of 60 days and with the release of 35.5 keV γ or KX-rays).

^{125}I-Machine Counting Efficiency ~ 60%

Maximum Pulse Height Spectrum obtained with the window settings of $\mathcal{E}$ = 0.5 & $\Delta\mathcal{E}$ = 3.0.

Counter set to count for 600 seconds or 2×10^5 counts on γ-mode.

Surface Areas of Dynamic 'flow'-cell windows:-

AI = 4.13 AII = 4.59	DI = 4.52 DII = 4.44
BI = 3.96 BII = 3.96	EI = 3.65 EII = 4.15
CI = 3.55 CII = 3.41	

Appendix 3

I BULK-POLYMERISED COMPOSITIONS

Hydrogel Composition	Monomer ratio (Moles %)	EWC (%) (Distilled) (H_2O) at ~ 20°C	EWC (%) 'Krebs' at ~ 20°C	Non-Freezing Water %	Freezing Water %
HEMA (Commercial)	100:0	41.2±1.4	57.2±0.8	28.3	28.9
HEMA (Purified)	100:0	39.9±0.9	41.6±1.8	36.9	4.7
HEMA/STY	95:5	34.2±0.0	33.7±0.4	-	-
HEMA/STY	90:10	29.3±1.6	30.0±1.5	-	-
HEMA/STY	85:15	23.9±1.7	23.2±1.9	-	-
HEMA/STY	80:20	19.9±0.9	18.9±1.3	-	-
HEMA/STY	75:25	17.4±0.0	15.7±0.3	-	-
HEMA/STY	50:50	9.5±1.3	9.7±1.1	-	-
HEMA/EMA	95:5	36.1±0.7	35.9±0.9	32.7	3.2
HEMA/EMA	75:25	26.9±1.1	29.0±1.1	28.3	0.7
HEMA/EMA	60:40	17.3±0.5	17.9±1.3	-	-
HEMA/EMA	50:50	13.4±0.4	12.7±0.6	-	-
HEMA/EDMA	95:5	31.9±0.5	31.1±1.0	-	-
HEMA/EDMA	90:10	26.8±1.1	26.2±0.8	-	-
HEMA/EDMA	85:15	21.9±1.5	22.0±1.9	-	-
HEMA/MAA	90:10	32.3±1.1	74.5±1.5	37.6	36.9
HEMA/MAA	75:25	27.9±0.7	86.3±0.9	40.5	45.8
HEMA/MAA	50:50	27.4±0.6	86.9±0.8	39.7	47.2
HEMA/MAA	25:75	41.0±1.1	80.6±2.6	28.7	51.9
HEMA/ACM	95:5	41.2±1.0	42.4±1.8	-	-
HEMA/ACM	75:25	50.0±0.6	49.7±1.4	33.6	16.1
HEMA/MACM	75:25	42.1±0.2	40.9±1.8	34.0	6.9
HEMA/DAACM	75:25	36.0±0.7	35.4±0.4	33.9	1.5
HEMA/NVP	95:5	44.5±1.0	46.0±0.5	-	-
HEMA/NVP	75:25	50.6±0.8	52.0±1.0	-	-
HEMA/NVP	50:50	64.7±0.9	64.2±0.7	-	-
NVP/EMA	95:5	91.2±1.0	90.1±1.8	-	-
NVP/EMA	75:25	70.9±0.8	71.6±1.1	-	-

Appendix 3

I SOLUTION-POLYMERISED COMPOSITIONS

Hydrogel Composition	Monomer Ratio (Moles %)	EWC (%) (Distilled) (H_2O) at ~ 20°C	EWC (%) 'Krebs' at ~ 20°C	Non-Freezing Water %	Freezing Water %
ACM/MBACM	99:1	92.4±0.5	93.3±0.3	22.0	71.3
ACM/MBACM	95:5	80.5±0.1	82.3±0.9	24.6	57.7
ACM/MBACM	90:10	74.5±0.6	75.7±1.0	30.7	45.0
MACM/MBACM	99:1	69.0±0.4	69.5±0.9	41.9	27.6
DAACM/MBACM	99:1	-	35.1±1.8	22.4	12.7
ACM/MAA	99:1	89.9±0.1	94.4±0.5	-	-
ACM/MAA	90:10	92.3±0.2	94.5±0.3	-	-
ACM/MAA	75:25	90.8±0.1	93.7±0.3	-	-
ACM/AEMA	99:1	93.8±0.2	96.5±0.6	-	-
ACM/AEMA	90:10	89.8±0.4	94.7±0.4	-	-
ACM/AEMA	75:25	95.9±0.2	95.2±1.0	-	-
ACM/MAA/AEMA	90:2.5:7.5	91.7±0.2	95.1±0.7	-	-
ACM/MAA/AEMA	90:5:5	88.9±0.3	95.5±0.9	-	-
ACM/MAA/AEMA	90:7.5:2.5	91.1±0.2	96.5±0.9	-	-

N.B. All Solution polymerised copolymers were cross-linked by 1 Mole % of NN' Methylene bis-acrylamide except for the ACM/MBACM copolymers.

N.B. All Bulk Polymerised copolymers were cross-linked by 1 Mole % of Ethylene glycol dimethacrylate except for the HEMA/EDMA Copolymers.

Appendix 3

II BULK-POLYMERISED COMPOSITIONS

Hydrogel Composition	Monomer Ratio (Moles %)	EWC (%) 'Krebs' at ≈ 20°C	Calculated Pore Size (°A)	Relative Mechanical Strength
HEMA (Commercial)	100:0	57.2	≈ 12	Moderate
HEMA (Purified)	100:0	41.6	≈ 8	Moderate
HEMA/STY	95:5	33.7	≈ 7	Strong
HEMA/STY	90:10	30.0	≈ 7	Very strong
HEMA/STY	85:15	23.2	≈ 5.5	Very strong
HEMA/STY	80:20	18.9	≈ 5.0	Very strong
HEMA/STY	75:25	15.7	≈ 4.5	Very strong
HEMA/STY	50:50	9.7	≈ 4.0	Very strong
HEMA/EMA	95:5	35.9	≈ 7	Strong
HEMA/EMA	75:25	29.0	≈ 7	Very strong
HEMA/EMA	60:40	17.9	≈ 5.0	Very strong
HEMA/EMA	50:50	12.7	≈ 4.0	Very strong
HEMA/EDMA	95:5	31.1	≈ 7	Strong
HEMA/EDMA	90:10	26.2	≈ 6	Very strong
HEMA/EDMA	85:15	22.0	≈ 5.5	Very strong
HEMA/MAA	90:10	74.5	≈ 25	Weak
HEMA/MAA	75:25	86.3	≈ 55	Very weak
HEMA/MAA	50:50	86.9	≈ 60	Very weak
HEMA/MAA	25:75	80.6	≈ 35	Weak
HEMA/ACM	95:5	42.4	≈ 8	Strong
HEMA/ACM	75:25	49.7	≈ 10	Moderate
HEMA/MACM	75:25	40.9	≈ 8	Strong
HEMA/DAACM	75:25	35.4	≈ 7	Strong
HEMA/NVP	95:5	46.0	≈ 9	Moderate
HEMA/NVP	75:25	52.0	≈ 10	Moderate
HEMA/NVP	50:50	64.2	≈ 15	Weak
NVP/EMA	95:5	90.1	≈ 120	Weak
NVP/EMA	75:25	71.6	≈ 20	Strong

Appendix 3

II SOLUTION-POLYMERISED COMPOSITIONS

Hydrogel Composition	Monomer Ratio (Moles %)	EUC (%) 'Krebs' at ~20°C	Calculated Pore Size (Å)	Relative Mechanical Strength
ACM/MBACM	99:1	93.3	≈ 160	Weak
ACM/MBACM	95:5	82.3	≈ 40	Moderate
ACM/MBACM	90:10	75.7	≈ 30	Moderate
MACM/MBACM	99:1	69.5	≈ 20	Moderate
DAACM/MBACM	99:1	35.1	≈ 7	Weak
ACM/MAA	99:1	94.4	≈ 200	Very Weak
ACM/MAA	90:10	94.5	≈ 200	Very Weak
ACM/MAA	75:25	93.7	≈ 180	Very Weak
ACM/AEMA	99:1	96.5	≈ 200	Very Weak
ACM/AEMA	90:10	94.7	≈ 200	Very Weak
ACM/AEMA	75:25	95.2	≈ 200	Very Weak
ACM/MAA/ AEMA	90:2.5: 7.5	95.1	≈ 200	Very Weak
ACM/MAA/ AEMA	90:5:5	95.5	≈ 200	Very Weak
ACM/MAA AEMA	90:7.5: 2.5	96.5	≈ 200	Very Weak

Appendix 4

Hydrogel Composition	% EWC 'Krebs' at ~20°C	Air°	n-Octane°	$\gamma_{sv} - \gamma_{s\omega}$	$I_{s\omega}$	γ^d_{sv}	γ^p_{sv}	γ_{sv}	$\gamma_{s\omega}$	$\frac{\gamma^p_{sv}.\gamma^d_{sv}}{\gamma_{sv}}$
						(dynes.cm.$^{-1}$)				
(HEMA) (Commercial)	57.2±0.8	40.8±2.6	148.5±2.2	55.11	94.5	12.8	43.8	56.6	1.5	9.92
(HEMA) (Purified)	41.6±1.8	55.0±2.2	130.3±2.0	41.8	84.0	10.7	34.6	45.3	3.5	8.18
HEMA/EDMA 95:5	31.1±1.0	46.8±1.3	130.3±5.8	49.9	84.0	17.1	34.6	51.7	1.8	11.46
HEMA/EDMA 90:10	26.2±0.8	45.2±1.3	133.1±3.2	51.3	85.9	16.8	36.1	52.9	1.6	11.46
HEMA/EDMA 85:15	22.0±1.9	37.8±0.6	140.0±0	57.5	90.1	18.6	39.8	58.4	0.8	12.66
HEMA/STY 95:5	33.7±0.4	32.6±2.3	136±0.5	61.3	87.7	24.7	37.7	62.4	1.1	14.94
HEMA/STY 90:10	30.0±1.5	50.7±3.3	123.7±4.7	46.1	79.3	18.0	30.8	48.8	2.7	11.36

Appendix 4 (cont'd)

Hydrogel Composition	% EWC 'Krebs' at ~ 20°C	Air°	n-Octane°	$\gamma_{sv} - \gamma_{sw}$	I_{sw}	γ_{sv}^{d}	γ_{sv}^{p}	γ_{sv}	γ_{sw}	$\frac{\gamma_{sv}^{p}.\gamma_{sv}^{d}}{\gamma_{sv}}$
HEMA/STY 85:15	23.2±1.9	64.5±4.6	144.7±3.6	31.3	92.6	1.5	42.1	43.6	12.2	1.47
HEMA/STY 80:20	18.9±1.3	63.0±1.3	132.8±0.3	33.1	85.7	4.7	36.0	40.6	7.6	4.14
HEMA/STY 75:25	15.7±0.3	61.0±2.7	112.0±3.0	35.3	70.1	16.6	24.1	40.7	5.4	9.81
HEMA/STY 50:50	9.7±1.1	68.8±1.0	118.3±1.0	26.3	75.2	6.6	27.7	34.3	8.0	5.32
HEMA/EMA 95:5	35.9±0.9	48.7±1.6	151.3±2.8	48.1	95.7	7.2	44.9	52.2	4.1	6.23
HEMA/EMA 75:25	29.0±1.1	46.0±1.7	138.8±3.8	50.6	89.4	13.3	39.2	52.4	1.8	9.91
HEMA/EMA 60:40	17.9±1.3	56.7±1.5	135.5±5.1	40.0	87.4	7.4	37.4	44.8	4.8	6.17

Appendix 4 (cont'd)

Hydrogel Composition	% EWC 'Krebs' at ≈ 20°C	Air°	n-Octane°	$\gamma_{sv} - \gamma_{sw}$	I_{sw}	γ_{sv}^{d}	γ_{sv}^{p}	γ_{sv}	γ_{sw}	$\frac{\gamma_{sv}^{p} \cdot \gamma_{sv}^{d}}{\gamma_{sv}}$
HEMA/EMA 50:50	12.7±0.6	58.3±1.3	126.8±0.3	38.3	81.6	10.0	32.6	42.6	4.3	7.64
HEMA/MAA 90:10	74.5±1.5	50.0±1.3	145.5±0.5	46.8	93.0	8.1	42.4	50.5	3.7	6.80
HEMA/MAA 75:25	86.3±0.9	40.7±0.3	161.3±1.0	55.2	99.3	9.4	48.3	57.8	2.6	7.90
HEMA/MAA 50:50	86.9±0.8	21.3±1.3	149.5±2.2	67.8	94.9	23.9	44.2	68.1	0.3	15.53
HEMA/MAA 25:75	80.6±2.6	18.7±0.6	154.8±0.3	69.0	97.2	22.8	46.3	69.1	0.1	15.29
HEMA/NVP 95:5	46.0±0.5	58.3±1.0	153.8±0.8	38.3	96.8	2.3	45.9	48.2	10.0	2.23
HEMA/NVP 75:25	52.0±1.0	51.2±0.6	153.5±2.0	45.6	96.6	5.4	45.8	51.2	5.6	4.86

Appendix 4 (cont'd)

Hydrogel Composition	% EWC 'Krebs' at ~ 20°C	Air°	n-Octane°	$\gamma_{sv} - \gamma_{sv}$	I_{sw}	γ^d_{sv}	γ^p_{sv}	γ_{sv}	γ_{sw}	$\frac{\gamma^p_{sv} \cdot \gamma^d_{sv}}{\gamma_{sv}}$
HEMA/NVP 50:50	64.2±0.7	46.7±4.2	155.5±1.3	49.9	97.4	7.4	46.5	53.9	3.9	6.35
HEMA/ACM 95:5	42.4±1.8	32.9±3.4	129.3±0.6	61.1	83.3	29.4	34.0	63.4	2.3	15.77
HEMA/ACM 75:25	49.7±1.4	30.8±0.6	150±3.0	62.5	95.2	18.5	44.4	62.9	0.4	13.06
HEMA/MACM 75:25	40.9±1.8	34±2.9	158.2±0.8	60.35	98.4	13.9	47.4	61.3	1.0	10.74
HEMA/DAACM 75:25	35.4±0.4	48.2±1.0	147±0.6	48.5	93.8	8.7	43.1	51.8	3.3	7.24
NVP/EMA 95:5	90.1±1.8	20.5±0.5	156±0.5	68.2	97.6	21.6	46.7	68.3	0.1	14.77
NVP/EMA 75:25	71.6±1.1	35.9±3.9	158.7±2.5	59.0	98.5	12.7	47.6	60.3	1.3	10.01

Appendix 4 (cont'd)

Hydrogel Composition	% EWC 'Krebs' at 20°C	Air°	n-Octane°	$\gamma_{sv} - \gamma_{s\omega}$	$I_{s\omega}$	γ^d_{sv}	γ^p_{sv}	γ_{sv}	$\gamma_{s\omega}$	$\frac{\gamma^p_{sv} \cdot \gamma^d_{sv}}{\gamma_{sv}}$
ACM/MBACM 99:1	93.3±0.3	20.8±1.0	159.3±0.8	68.1	97.7	20.4	47.8	68.1	0.1	14.32
ACM/MBACM 95:5	82.3±0.9	22.2±1.5	154.3±1.6	67.4	97.0	21.4	46.1	67.5	0.1	14.64
ACM/MBACM 90:10	75.7±1.0	32.1±3.0	149.3±0.3	61.7	94.9	18.0	44.1	62.1	0.4	12.78
MACM/MBACM 99:1	69.5±0.9	32.7±0.6	158.5±1.8	61.3	98.5	14.5	47.5	62.1	0.8	11.13
DAACM/MBACM 99:1	35.1±1.8	31.0±1.0	160.5±0.5	62.4	99.1	15.0	48.1	63.1	0.7	11.42
ACM/MAA 99:1	94.4±0.5	30.6±1.1	162±1.8	62.7	99.5	14.8	48.6	63.3	0.7	11.36
ACM/MAA 90:10	94.5±0.3	30.6±0.5	166.2±1.9	62.7	100.5	14.0	49.5	63.5	0.9	10.91

Appendix 4 (cont'd)

Hydrogel Composition	% EWC 'Krebs' at ~20°C	Air°	n-Octane°	$\gamma_{sv} - \gamma_{sw}$	I_{sw}	γ^d_{sv}	γ^p_{sv}	γ_{sv}	γ_{sw}	$\frac{\gamma^p_{sv} \cdot \gamma^d_{sv}}{\gamma_{sv}}$
ACM/MAA 75:25	93.7±0.3	26.0±0.7	167.3±1.3	65.4	100.8	16.1	49.8	65.9	0.4	12.17
ACM/AEMA 99:1	96.5±0.6	26.9±0.9	167±0.8	64.9	100.7	15.7	49.7	65.4	0.5	11.95
ACM/AEMA 90:10	94.7±0.4	25.0±0.6	169±0.5	66.0	101.1	16.3	50.1	66.4	0.4	12.31
ACM/AEMA 75:25	95.2±1.0	21.5±0.4	169±0.6	67.7	101.1	17.9	50.1	67.9	0.2	13.17
KELVIN 1 (See Ch. 7)	77.2±0.6	42.2±3.8	148.±1.3	53.9	94.4	12.0	43.6	55.7	1.7	9.43
KELVIN 1 + 1st THPFPMA	82.3±2.1	37.9±2.7	147.3±1.4	57.5	93.9	15.1	43.2	58.4	0.9	11.21
KELVIN 1 + 2nd THPFPMA	77.2±0.6	49.7±2.9	136.8±2.6	47.1	88.2	11.5	38.1	49.6	2.6	8.85

Appendix 4 (cont'd)

Hydrogel Composition	% EWC 'Krebs' at ~ 20°C	Air°	n-Octane	$\gamma_{sv} - \gamma_{sw}$	I_{sw}	γ^d_{sv}	γ^p_{sv}	γ_{sv}	γ_{sw}	$\frac{\gamma^p_{sv}.\ \gamma^d_{sv}}{\gamma_{sv}}$
KELVIN 1 + 1% HFIPMA	76.3±0.9	44.5±1.8	151±0.5	51.9	95.6	9.7	44.8	54.5	2.6	7.99
KELVIN 1 + 2% HFIPMA	72.4±1.2	61.8±3.6	152.7±0.3	34.4	96.3	1.4	45.5	46.8	12.4	1.32
KELVIN 2 (See Ch 7)	76.3	54.2±2.8	150.3±2.2	42.6	95.3	4.6	44.5	49.2	6.5	4.19
P.T.F.E.	0	90.3±2.1	49.5±0.5	0	17.9	34.1	1.6	35.7	36	1.50
Melinex (ethylene-terephth-alate)	0	79.3±5.2	98.0±3.3	13.5	58.1	9.1	16.6	25.7	12.2	5.88
Polyethy-lene	0	73.3±4.9	69.2±1.8	20.9	32.9	42.4	5.3	47.7	26.8	4.71
Silicone Rubber	0	90±1.5	86.3±2.0	0	47.7	7.2	11.2	18.4	18.4	4.38

Appendix 5

THEORETICAL DERIVATION OF HSA AND HFb ADSORPTION LEVELS FOR COMPLETE MONOLAYER COVERAGE

The derivation of both HSA and HFb values for monolayer coverage is based on the following assumptions: that

(i) the proteins adsorb in their 'native'-conformation.

(ii) the proteins adsorb in a non-random closely packed monolayer.

These assumptions ensure that complete monolayer coverage is attained. However in practise it is likely that they would not be fulfilled.

HSA: Molecular weight = 66,248 daltons
Molecular dimensions = $38 \times 150 \text{Å}^2$

1 mole of HSA (i.e., 66,248g) = 6.02217×10^{23} molecules (Avagadros number)

therefore 1 HSA molecule = 1.10006×10^{-19} g

From the above dimensions of HSA, the rectilinear area for 1 HSA molecule = 5,700 Å^2.

and therefore there are 1.75438×10^{12} molecules $cm.^{-2}$ ($1cm.^2 = 1 \times 10^{16} \text{Å}^2$)

and accordingly the adsorption value for complete monomolecular HSA coverage = 19.3×10^{-8} g.$cm.^{-2}$

Unless the molecular packing density is very high, which is unlikely, the prolate ellipsoid area may be equated with the rectilinear area and for all practical purposes the maximum value for complete monolayer coverage may be taken as being

$\sim 20 \times 10^{-8}$ g.$cm.^{-2}$

HFb: Molecular weight = $340,000 \pm 20,000$
Molecular dimensions = between $30 \times 90 \text{Å}^2$ to $450 \times 90 \text{Å}^2$

1 mole of HFb (i.e., 340,000g) = 6.02217×10^{23} molecules (Avagadros number)

therefore 1 HFb molecule = 5.6458×10^{-19} g.

From the above range of dimensions of HFb molecules the rectilinear area will be within the range

$27,000 \text{Å}^2$ ——— $40,500 \text{Å}^2$ ($1cm. = 1 \times 10^{16} \text{Å}^2$)

and therefore there will be between 2.46913×10^{11} and 3.7037×10^{11} molecules $cm.^{-2}$

thus for complete monolayer coverage the adsorption value for HFb will lie within the range

13.0×10^{-8} g.$cm.^{-2}$ to 21.0×10^{-8} g.$cm.^{-2}$

APPENDIX 5: PROTEIN ADSORPTION STUDY DATA

N.B. Except where otherwise indicated all bulk polymerised compositions contain 1 Mole % of EDMA and similarly all solution polymerised compositions contain 1 Mole % of MBACM.

(†† - All other hydrogel compositions are based on purified HEMA).

(All adsorption studies were carried out at room temp. ($\sim 20^{o}C$) except where indicated *).

(Bulk HFb concentration = 10mg % and Bulk HSA concentration = 75mg %).

Hydrogel Composition	Monomer ratio (Moles %)	EWC % 'Krebs'	Adsorbed - HFb (10mg %) Time (hours)	Level ($\times 10^{-8}$ g.cm.$^{-2}$) $\bar{x}$	$\pm \sigma_{n-1}$	Adsorbed - HSA (75mg %) Time (hours)	Level ($\times 10^{-8}$ g.cm.$^{-2}$) $\bar{x}$	$\pm \sigma_{n-1}$
HEMA (Commercial)	100:0	57.2	2.17	28.7	±3.2	6.50	147.7	±13.5
			6.67	11.6	±4.1	69.00	308.4	±65.8
			46.50	312.7	±35.5			
			69.58	474.3	±44.8			
†† HEMA (purified)	100:0	41.6	2.33	23.1	±6.6	2.58	68.8	±4.5
			6.83	14.7	±3.1	6.25	114.8	±11.0
			46.67	588.9	±76.2	46.33	185.8	±27.9
			69.75	864.5	±72.8	69.58	167.5	±31.5
HEMA *($0-4^{o}C$)	100:0	41.6	2.42	20.9	±7.6			
			6.92	13.3	±4.7			
			46.75	53.5	±16.0			
			69.83	82.9	±17.8			
HEMA *($\sim 37^{o}C$)	100:0	41.6	2.25	20.6	±7.1			
			6.25	22.1	± 3.1			
			44.92	971.8	±54.4			
			66.58	1055.9	±77.6			

Hydrogel Composition	Monomer ratio (Moles %)	EWC % 'Krebs'	Adsorbed - HFb (10mg %) Time (hours)	Level ($\times 10^{-8}$ g cm.$^{-2}$) $\bar{x}$	$\pm\sigma_{n-1}$	Adsorbed - HSA (75mg %) Time (hours)	Level ($\times 10^{-8}$ g cm.$^{-2}$) $\bar{x}$	$\pm\sigma_{n-1}$
HEMA:EDMA	95:5	31.1	6.3 69.8	16.5 256.7	±5.2 ±70.4	69.9	231.9	±15.7
HEMA:EDMA	90:10	26.2	6.4 69.8	16.0 195.9	±4.0 ±16.2	70.0	279.2	±12.2
HEMA:EDMA	85:15	22.0	69.9	167.0	±25.8	70.1	236.9	±18.7
HEMA:STY	95:5	33.7	2.75 6.75 43.5 67.67	29.1 21.9 384.9 614.4	±16.4 ±11.5 ±23.5 ±60.4	5.92 68.17	208.5 346.6	±14.2 ±32.4
HEMA:STY	90:10	30.0	6.1 69.3	21.9 248.8	±13.9 ±25.5	69.5	403.4	±52.4
HEMA:STY	85:15	23.3	6.2 69.5	30.4 99.4	±6.4 ±13.7	69.7	301.4	±31.5
"	80:20	18.9	6.3 69.5	34.6 64.2	±8.6 ±9.5	69.7	190.9	±23.9
"	75:25	15.7	2.92 6.96 43.75 67.83	93.8 84.8 69.9 83.8	±24.5 ±26.6 ±16.0 ±13.3	2.17 6.00 47.00 68.5	18.0 28.3 88.1 110.8	±3.0 ±2.3 ±8.3 ±14.0
"	50:50	9.7	69.7	68.4	±12.3	69.8	61.1	±4.0
HEMA:EMA	95:5	35.9	72.92	831.4	±88.8			
	75:25	29.0	72.83	574.3	±45.2			

Hydrogel Composition	Monomer ratio (Moles %)	EWC % 'Krebs'	Adsorbed - HFb (10mg %) Time (Hours)	Level ($\times 10^{-8}$ g cm.$^{-2}$) $\bar{x}$	$\pm \sigma_{n-1}$	Adsorbed - HSA (75mg %) Time (hours)	Level ($\times 10^{-8}$ g cm.$^{-2}$) $\bar{x}$	$\pm \sigma_{n-1}$
HEMA:EMA	60:40	17.9	69.00	117.5	±16.8	72.3	200.8	±29.4
	50:50	12.7	69.1	67.3	±10.7	72.5	107.0	±16.8
NVP:EMA	95:5	90.1	5.5 68.8	10.8 899.9	±1.4 ±145.0	68.9	230.7	±21.3
	75:25	71.6	5.6 68.9	13.5 2090.6	±3.2 ±321.3	69.1	1988.1	±80.6
P.T.F.E.	-	~ 0.0	6.8 69.4	157.7 151.2	±18.0 ±13.9	72.6	92.8	±21.3
Melinex	-	~ 0.0	6.9 69.9	141.0 173.2	±18.1 ±28.2	72.7	53.5	±16.5
Silicone rubber	-	~ 0.0	6.2 69.8	152.0 123.5	±14.3 ±20.8			
HEMA:ACM	95:5	42.4	2.17 6.17 47.75 69.0	10.4 23.2 600.2 690.1	±2.7 ±7.3 ±65.9 ±45.1	6.33 68.75	134.1 352.5	±11.6 ±44.8
	75:25	49.7	2.0 6.0 47.58 68.92	9.0 14.6 616.6 644.4	±2.3 ±2.7 ±60.4 ±53.0	2.33 6.17 47.17 68.67	103.9 163.3 232.8 335.8	±12.1 ±21.2 ±34.8 ±34.1
HEMA:MACM	75:25	40.9	73.08	751.9	±42.3	70.42	150.5	±13.3

Hydrogel Composition	Monomer ratio (Moles %)	EWC % 'Krebs'	Adsorbed - HFb (10mg %) Time (Hours)	Level ($\times 10^{-8}$ g cm.$^{-2}$) $\bar{x} \pm \sigma_{n-1}$		Adsorbed - HSA (75mg %) Time (Hours)	Level ($\times 10^{-8}$ g cm.$^{-2}$) $\bar{x} \pm \sigma_{n-1}$	
HEMA:DAACM	75:25	35.4	2.33 6.33 47.83 69.17	7.5 13.6 565.2 826.9	±1.2 ±3.1 ±78.1 ±56.3	70.58	250.7	±25.5
HEMA:NVP	95:5	46.0	6.0 69.2	12.5 474.6	±3.3 ±54.1	69.2	270.2	±22.6
	75:25	52.0	6.0 69.2	13.5 890.9	±2.2 ±109.7	69.3	280.0	±30.8
	50:50	64.2	5.9 69.2	20.3 1192.0	±5.0 ±197.0	69.3	469.0	±43.3
HEMA:MAA	90:10	74.5	71.0	81.8	±8.1	67.92	48.8	±14.5
	75:25	86.3	70.83	51.7	±4.7	67.83	41.9	±8.7
	50:50	86.9	70.67	28.5	±3.6	67.67	32.1	± 6.1
	25:75	80.6	71.08	32.8	±4.6	68.08	123.1	±39.2
ACM:MBACM	99:1	93.3	2.25 6.25 42.5 67.08	4.9 8.8 99.6 117.4	±0.5 ±1.5 ±14.0 ±13.0	2.0 6.58 47.25 68.75	17.8 21.2 531.0 895.1	±7.0 ±4.7 ±89.2 ±165.0
	95:5	82.3	2.42 6.33 42.67 67.25	4.3 6.6 97.7 128.6	±1.3 ±1.5 ±25.3 ± 23.9	6.67 68.92	3.5 556.0	±0.4 ±209.3

Hydrogel Composition	Monomer ratio (Moles %)	EWC % 'Krebs'	Adsorbed Time (Hours)	HFb (10mg %) Level ($\times 10^{-8}$ g cm.$^{-2}$) $\bar{x}$	$\pm \sigma_{n-1}$	Adsorbed Time (Hours)	HSA (75mg %) Level ($\times 10^{-8}$ g cm.$^{-2}$) $\bar{x}$	$\pm \sigma_{n-1}$
ACM:MBACM	90:10	75.7	2.58	3.2	±0.9	7.0	3.1	±0.8
			6.50	6.1	±1.6	69.0	413.2	±173.8
			42.83	127.2	±21.8			
			67.33	169.8	±44.2			
MACM:MBACM	99:1	69.5	3.08	4.7	±1.7	69.5	251.4	±75.4
			7.0	6.6	±1.0			
			43.92	56.3	±16.6			
			67.92	93.5	±4.0			
DAACM:MBACM	99:1	35.1	71.7	507.8	±52.6	72.8	773.2	±71.2
ACM:MAA	99:1	94.4	2.25	6.7	±2.2	5.92	3.9	±0.6
			5.83	10.0	±3.9	69.08	62.0	±18.8
			18.67	25.8	±3.4			
			44.75	41.8	±8.2			
			71.67	30.8	±2.9			
			103.75	55.8	±6.1			
	90:10	94.5	2.08	16.6	±3.9	6.0	4.8	±1.1
			5.92	16.0	±3.9	69.17	66.5	±7.2
			18.75	27.8	±5.2			
			44.58	53.6	±6.2			
			71.75	47.0	±7.4			
			103.9	56.7	±12.6			
	75:25	93.7	2.5	7.8	±3.0	6.17	3.4	±0.9
			6.5	18.5	±2.5	69.33	58.8	±8.4
			18.5	34.8	±4.5			
			40.33	68.1	±14.4			

Hydrogel Composition	Monomer ratio (Moles %)	EWC % 'Krebs'	Adsorbed - HFb (10mg %) Time (Hours)	Level ($\times 10^{-8}$ g cm.$^{-2}$) $\bar{x}$	$\pm \sigma_{n-1}$	Adsorbed - HSA (75mg %) Time (Hours)	Level ($\times 10^{-8}$ g cm.$^{-2}$) $\bar{x}$	$\pm \sigma_{n-1}$
ACM:MAA	75:25	93.7	69.08	55.1	±18.7			
			92.83	58.3	±15.8			
ACM:AEMA	99:1	96.5	2.58	4.9	±0.8	6.42	119.7	±36.7
			6.67	11.0	±4.2	70.08	118.2	±17.1
			18.25	26.8	±5.8			
			40.42	52.5	±8.6			
			69.33	56.4	±10.1			
			93.00	61.4	±7.0			
	90:10	94.7	2.83	7.7	±2.5	6.58	2.6	±1.1
			6.5	13.1	±1.6	70.25	27.2	±10.3
			41.83	76.2	±12.2			
			68.83	46.8	±10.1			
	75:25	95.2	3.0	14.9	±4.8	6.67	3.8	±1.4
			6.67	23.4	±2.8	70.33	24.1	±7.5
			41.92	93.3	±14.7			
			69.17	49.2	±9.4			
ACM:AEMA:MAA	90:2.5:7.5	95.1	2.83	20.4	±3.2			
			6.5	20.1	±2.2			
			43.0	69.3	±21.6			
			69.67	57.7	±11.8			
	90:5:5	95.5	3.0	25.3	±5.0			
			6.67	23.0	±2.7			
			43.17	73.9	±6.2			
			69.83	63.7	±19.1			
	90:7.5:2.5	96.5	3.17	21.8	±2.4			
			6.83	18.0	±2.0			
			43.3	78.1	±22.7			
			70.0	63.5	±22.1			

Appendix 5

MISCELLANEOUS ADSORPTION DATA

Hydrogel Composition	Monomer ratio Mole %	EWC % 'Krebs'	Bulk protein concentration (mg %)	Adsorbed Protein Time (hrs)	Level (10^{-8} g cm.$^{-2}$) $\bar{x}$	$\pm s_{n-1}$
HEMA	100:0	41.6	50mg % HSA	8.0	18.5	±1.4
				16.0	18.6	±1.2
				32.0	25.8	±1.2
				54.0	40.7	±4.6
HEMA:EMA	95:5	35.9	1mg % HFb	3.33	1.2	±0.3
				6.17	1.8	±0.4
				44.5	40.2	±3.9
				67.83	59.0	±4.9
	75:25	29.0	1mg % HFb	3.17	3.5	±0.8
				6.0	2.5	±0.4
				44.3	20.4	±3.2
				67.67	37.6	±5.2
	95:5	35.9	50mg % HSA	2.0	6.3	±0.9
				8.0	17.4	±2.3
				16.5	23.8	±3.1
				25.0	31.4	±4.2
				71.0	37.3	±2.0
	75:25	29.0	50mg % HSA	8.0	3.6	±0.3
				16.5	23.5	±4.6
				25.0	32.3	±4.2
				71.0	61.2	±9.6
Silicone Rubber	-	~0.0	50mg % HSA	17.0	17.0	±1.9
				27.0	20.5	±3.6
				71.0	28.7	±2.1
ACM:MBACM	99:1	93.3	50mg % HSA	6.0	3.1	±1.0
				16.0	4.4	±1.0
				47.5	21.0	±4.7
				71.5	20.8	±2.5
	95:5	82.3	50mg % HSA	17.0	2.3	±0.8
				27.0	3.9	±1.0
				71.0	12.3	±1.4
Dynamic 'Flowing' Adsorption Data:						
HEMA	100:0	41.6		1.0	14.6	±2.0
				4.0	27.6	±2.6
				16.0	101.2	±7.3
				40.0	363.4	±26.5
Flow rate ~ 10mls/min. Bulk protein conc. ~ 10mg % HFb.						

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