NETWORK HYDROGEL POLYMERS FOR APPLICATION TO HEMODIALYSIS

by

Jeffrey Earl Silliman

B.S., Ch.E., Lehigh University (1968)

Submitted in Partial Fulfillment
of the Requirements for the Degree
of Doctor of Science

at the

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

June 1972

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ABSTRACT

Network hydrogel polymers prepared by simultaneously cross-linking polyvinyl alcohol (PVA) and grafting the anticoagulant heparin to the matrix with a mixture of formaldehyde and glutaraldehyde by the acid-catalyzed acetal reaction have previously demonstrated excellent blood compatibility. To study the effect on network properties of the reactants in the hydrogel synthesis, equilibrium polymer contents in saline solution as a function of temperature and stress-strain behavior were measured for a range of specimens. Rubber elasticity theory, modified for application to a network formed in the presence of solvent and subsequently swollen, was used to interpret the stress-strain data.

The results indicated that glutaraldehyde functions as the cross-linking agent and formaldehyde functions to modify the network by the introduction of cyclic formals on the PVA chains. The formation of cyclic formals, favored by higher concentrations of acid and formaldehyde and longer reaction times, tends to increase network polymer concentrations, to reduce the amount of cross-linking per original PVA chain, and to accentuate the shrinkage of the network with temperature.

Hydrogels prepared with heparin exhibited lower polymer contents, higher amounts of cross-linking, and reduced thermal response relative to controls without heparin. This was attributed to a buffering effect of heparin on the reaction mixture, which resulted in the introduction of less cyclic formal.

Permeability studies on heparinized hydrogel membranes revealed improved permeabilities relative to conventional hemodialysis membranes on an equivalent thickness basis. This was attributed to the high water content of the hydrogel.

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Department of Chemical Engineering
Massachusetts Institute of Technology
June 23, 1972

Professor David B. Ralston
Secretary of the Faculty
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Dear Professor Ralston:

In accordance with the regulations of the Faculty, I herewith submit a thesis, entitled "Network Hydrogel Polymers for Application to Hemodialysis," in partial fulfillment of the requirements for the degree of Doctor of Science in Chemical Engineering at the Massachusetts Institute of Technology.

Respectfully submitted,

Jeffrey E. Silliman

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Finally, the author wishes to express his debt of gratitude to his wife Julie for her love and understanding, to his son Jonathan who has helped keep things in their proper perspective, and to his parents whose love, encouragement, and sacrifices made his education possible.

To

Julie, Jonathan, and My Parents

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CHAPTER 1

SUMMARY

A. INTRODUCTION

Whenever blood contacts a foreign surface, such as that of an artificial kidney circuit, a series of biochemical reactions are initiated which ultimately lead to blood coagulation (18). In the application of hemodialysis as a clinical technique for the treatment of chronic renal failure, there are numerous hemorrhagic complications that arise from the necessity to administer an anticoagulant to the patient (21,24,25,26). If nonthrombogenic materials were available to construct the blood-contacting surfaces of the dialysis circuit, a significant improvement in hemodialysis therapy could be achieved.

Since the membrane represents by far the largest area contacted by blood during the hemodialysis therapy, any attack on the problem must improve the blood compatibility of the membrane surface. Few of the various biomaterials now under study for their nonthrombogenic properties could be used as a hemodialysis membrane. A significant exception is the network hydrogel materials of Merrill et al. (59) to which a naturally occurring anticoagulant called heparin has been covalently bonded. Recently reported *in vivo* test results indicate the superior blood compatibility of these materials (61). Owing to their high water content, these materials are feasible for application as hemodialysis membranes as well as for construction of the tubing and cannulae of the artificial kidney circuit.

The principal objective of this thesis was to study the effect of the various reaction variables in the network synthesis on the properties of the hydrogel polymers of Merrill et al. To accomplish this objective, the theory of rubber elasticity was modified for application to a hydrogel system. This analysis was used to interpret experimentally measured moduli of a range of hydrogel compositions synthesized under carefully controlled reaction conditions. Along with experimentally measured volume fractions of polymer in the gels as a function of temperature, these data were used to explain the effect of various reaction variables in the system.

A procedure was developed to cast membranes of the hydrogel material with a suitable support. Permeability measurements were made on these membranes.

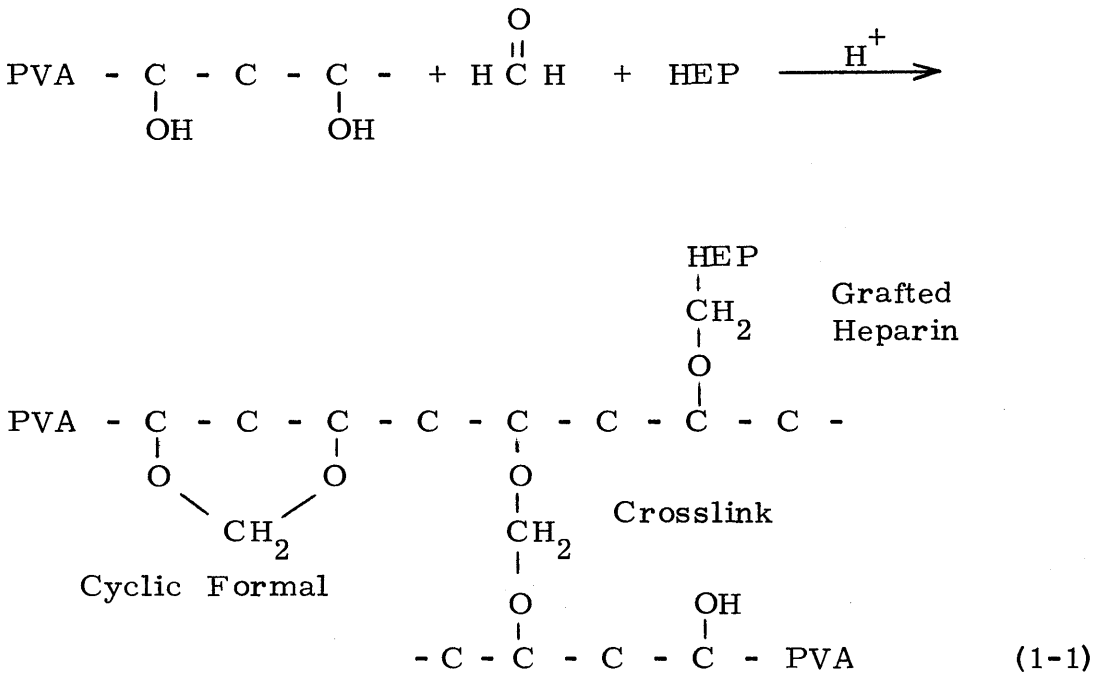
B. BACKGROUND

1. Hydrogel Chemistry

In the synthesis of the nonthrombogenic network hydrogels, polyvinyl alcohol $\left\{C\text{-}\underset{\underset{OH}{|}}{C}\right\}$ (PVA) is cross-linked by a mixture of aldehydes (formaldehyde and glutaraldehyde) via acid-catalyzed acetal formation with the secondary hydroxyls on the polymer "backbone." Heparin (Figure 2-4) is coupled to the matrix by acetalization with the secondary hydroxyls on the 3-carbon of the anhydroglucose unit.

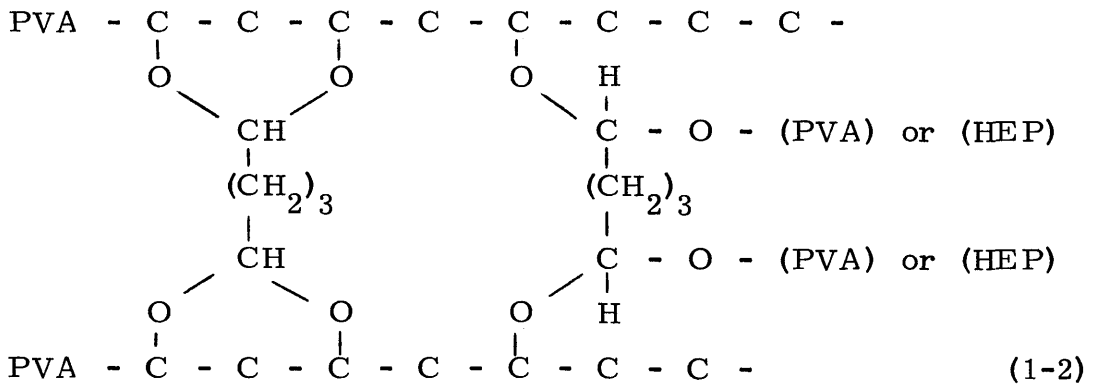
For formaldehyde as the specific aldehyde the reaction schemes shown in Equation (1-1) are envisioned. With glutaraldehyde (pentanedial) each end of the molecule can participate in an acetal reaction and the following network structures can be proposed.

Reactions with Formaldehyde (1-1)



(1-1)

Network Structures with Glutaraldehyde (1-2)



(1-2)

Two facts from studies on the general acetal reaction shed light on the probable network structures formed. The acetal reaction is an equilibrium reaction and forcing conditions for the removal of water are often employed (62). Also, the equilibrium of acetal formation does depend upon the structure of the alcohol, six-membered ring acetals being highly favored (66).

Inasmuch as the hydrogel synthesis is carried out in an approximately 85% aqueous medium, it would suggest that the principal structure formed is the cyclic acetal. Therefore, glutaraldehyde functions as the key cross-linking agent by forming two cyclic acetal structures with different PVA chains. Formaldehyde, able to form only a single cyclic formal, serves to modify the character of the network polymer chains.

2. Modulus Evaluation

a. Study of Cross-Linked Networks

Parameters relevant to a discussion of cross-linked polymer networks may be defined with the aid of Figure 1-1. The polymer network is comprised of N polymer chains of

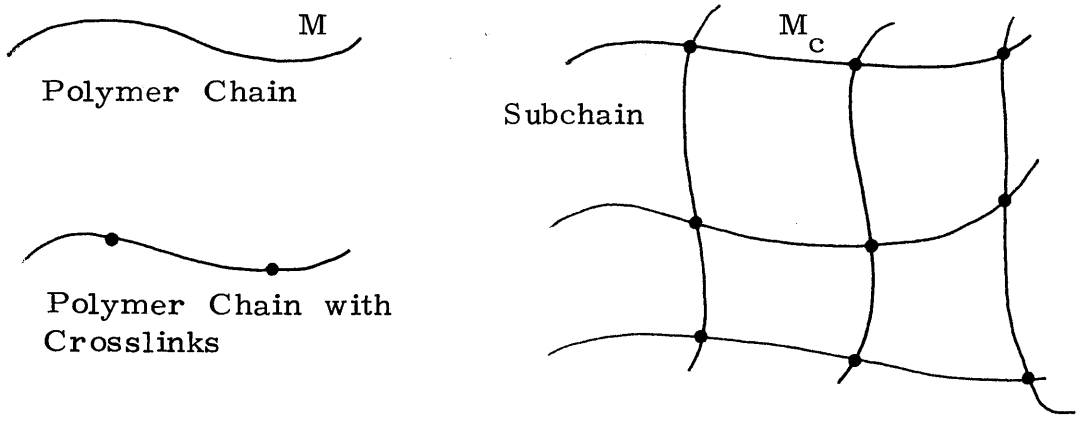


Figure 1-1. Idealized Network.

molecular weight M ($\bar{M}_n$ for polydisperse polymer) before cross-linking. In the cross-linked network there are $\nu/2$ cross-linked points or a total of ν cross-linked subchains of molecular weight M_c (number average value), if each cross-link is taken as a tetrafunctional crossover point. Since real polymer networks are formed from macromolecules of finite molecular weight, correction factors are employed to relate ν_e, the number of subchains effective in network deformations, to ν, the total number of subchains.

The objective of this thesis is therefore achieved by appropriate theoretical analysis of experimental data to find ν or the analogous parameter M_c as function of reaction conditions. For solvated networks the theory of network deformation by isotropic swelling (76), as expressed by Equation 1-3, might be employed.

$$\ln(1-v_2) + v_2 + \chi_1 v_2^2 = V_1 \left(\frac{\nu_e}{V}\right)\left(v_2^{1/3} - \frac{v_2}{2}\right) \qquad (1\text{-}3)$$

Unfortunately application of the theory requires a knowledge of the polymer-solvent interaction parameter χ_1, which is not accurately known for PVA in water, and which is unknown for the hydrogel network consisting of at least two polymer segments (vinyl alcohol and cyclic formal), not to mention grafted heparin when present.

Since the hydrogels described herein exhibit characteristic rubber-like elasticity, the application of the theory of network elasticity, which does not require a knowledge of thermodynamic parameter χ_1, is a viable alternative. Application of the theory to deformations of bulk elastomers in which the

cross-links were introduced in the unswollen, amorphous state has received considerable attention in the literature (103, 107,112). With the most recent refinements (102), the theory leads to the following equation for the isothermal, unidirectional deformation of an elastomer:

$$\tau = \frac{f}{A_o} = RT \left(\frac{\nu_e}{V}\right) \left(\frac{\overline{r_i^2}}{\overline{r_o^2}}\right) \left(\alpha - \frac{1}{\alpha^2}\right) \qquad (1\text{-}4)$$

Stress τ in force per unit initial cross-sectional area is a function of $(\alpha - 1/\alpha^2)$ with $\alpha = L/L_o$, the elongation in the x-direction relative to a gauge length L_o. The parameter (ν_e/V) is the number of effective subchains[1] expressed in moles per unit sample volume. The parameter $(\overline{r_i^2}/\overline{r_o^2})$ enters the derivation in the development of the expression for the Helmholtz free energy of deformation from the statistics of a single chain and is frequently called the "front factor." For loosely cross-linked networks the parameter is essentially equal to one (120).

With the aid of the following definitions:

$$\nu = \frac{V}{\bar{v}M_c} \qquad N = \frac{V}{\bar{v}M_n} \qquad (1\text{-}5)$$

and the network correction factor proposed by Flory (105):

$$\nu_e = \nu - 2N \qquad (1\text{-}6)$$

the equation may be written in terms of M_c as follows:

$$\tau = \frac{RT}{\bar{v}M_c} \left(\frac{\overline{r_i^2}}{\overline{r_o^2}}\right) \left(1 - \frac{2M_c}{\bar{M}_n}\right) \left(\alpha - \frac{1}{\alpha^2}\right) \qquad (1\text{-}7)$$

---

1. "Effective" in the sense that they contribute to the elastic restoring force f.

Careful experimentation (108,109,111) has indicated that under ideal conditions, i.e., equilibrium between the tensile and retractive force at each elongation, the relationship between force and elongation is correctly represented by the theoretical equation (1-7).

b. Application to Hydrogel Networks

To apply the theory of rubber elasticity to a study of the polyvinyl alcohol hydrogel system, there are two important modifications which must be made. In the conventional theory the parameters ν and N are defined assuming that the cross-linking is carried out in bulk amorphous polymer, which is not the case for the hydrogels synthesized from reaction mixtures containing from 6-10 wt % PVA. The definitions are appropriately modified by the inclusion of the term $v_{2,i}$ representing the volume fraction of polymer (cc polymer/cc solution) at the cross-linking volume V_i. Hence,

$$\nu = \frac{V_i}{\bar{v}M_c} v_{2,i} = \frac{V_i}{M_c} C_{2,i} \qquad (1\text{-}8)$$

$$N = \frac{V_i}{\bar{v}M} v_{2,i} = \frac{V_i}{M} C_{2,i} \qquad (1\text{-}9)$$

For the purposes of this study the volume V_i, which represents the relaxed state of the network, was taken as the volume of the system at the end of the reaction period characterized by $v_{2,i}$. By a series of experiments it was found that the volume fraction of polymer at 70°C in the original reaction environment was the same within 10% as the volume fraction at 70°C measured in buffered saline.[1]

---

1. 0.3 N ions, pH = 7.3.

Since the hydrogels synthesized at 70°C do undergo a significant volume change (swelling) when equilibrated with saline at room temperature (25°C), the isotropic deformation introduced into the network by this volume change must be taken into account to correctly evaluate stress-strain data for M_c. A swelling factor δ was defined in terms of the sample volume as reacted (70°C) V_i and the swollen equilibrium volume of the sample at 25°C, V_s.

$$\delta = \frac{V_i}{V_s} = \frac{v_{2,25°C}}{v_{2,70°C}} \tag{1-10}$$

Since the sample is now deformed by both isotropic swelling and unidirectional elongation, the parameter δ enters the derivation as a correction for the extension parameter α. The final form of the equation used in the study of the hydrogel materials is as follows:

$$\tau_s = \frac{f}{A_{o,s}} = RT\left(\frac{C_{2,i}}{M_c}\right)\delta^{1/3}\left(1 - \frac{2M_c}{\bar{M}_n}\right)\left(\alpha - \frac{1}{\alpha^2}\right) \tag{1-11}$$

The subscript s on $A_{o,s}$ denotes the swollen, unstretched specimen cross section, and the front factor $(\overline{r_i^2}/\overline{r_o^2})$ has been taken equal to one, as explained earlier.

In the application of this theory to the study of a swollen cross-linked network, one must remain cognizant of those factors which would cause non-Gaussian behavior of the subchains and thus violate one of the basic assumptions of the theoretical development. Among these are finite extensibility of the chains, stress crystallization, and the presence of microcrystalline regions or inhomogeneities in the network

structure.

In this work only small sample elongations were used ($\alpha < 1.5$), and this precluded any problem with finite extensibility of the chains as well as assuring the validity of the constant volume deformation assumption, which is used to derive the $(\alpha - 1/\alpha^2)$ dependence of τ. Stress crystallization normally occurs only at higher elongations and is unlikely in a highly swollen network. Extreme reaction conditions such as would lead to inhomogeneous structures, e.g., by a phase separation of the cyclic formal portion of the network, were scrupulously avoided. Indeed, hydrogels of this character would have little value in the proposed biomedical applications.

Microcrystallization of the PVA component of the gel cannot be completely ruled out. Solutions of PVA and water containing 6 wt % have been observed to become slightly turbid upon standing, suggesting a phase separation (possibly crystallization), but hydrogels of the same polymer content will maintain their clarity indefinitely on storage in buffered saline. It is to be supposed that the presence of cyclic acetals along the backbone of the polymer as well as grafted heparin in some compositions should significantly reduce the level of crystallization, if any, as compared to a PVA solution of equal concentration.

C. EXPERIMENTAL WORK

1. Hydrogel Synthesis

Because of the complexity of the hydrogel reaction, the decision was made in this work to begin with a hydrogel

composition that had previously demonstrated adequate biocompatible properties and to synthesize various compositions, varying one reactant at a time. This control composition and a summary of the compositions prepared in this work are presented in Table 4-1. Except as noted, reaction conditions were 70°C for 60 minutes.

The choice of feasible reaction conditions is limited primarily by the necessity to maintain heparin activity. In earlier work Merrill et al. (59) demonstrated that the heparin would remain viable for reaction conditions of one hour at 70°C with acid concentrations (H^+) less than 0.2 N. The solubility properties of heparin prevent the addition of any significant amount of miscible, nonaqueous solvents such as alcohol, methyl acetate, or acetone to affect the reaction equilibrium.

All compositions studied in this work were prepared by careful weighing or dilution from analyzed stock solutions. The two grades of PVA used were Du Pont Elvanol (R) 73-125G ($\bar{M}_n$ = 100,000) and Elvanol (R) 71-30 ($\bar{M}_n$ = 60,000). After the reactants had been homogeneously mixed, the casting solution was degassed under vacuum and the hydrogel was cast between two 1/4-inch polished glass plates (6" x 6") with an aluminum shim (0.040 inches) to fix the reaction volume. The glass plates were clamped with a specially fabricated aluminum holder and were suspended in a thermostatted, circulating water bath (70.0 $\pm$ 0.2°C) for reaction. At the end of the reaction time the hydrogel was removed from between the glass plates and quenched in room temperature buffered saline.

2. Volume Fraction Determination

For each hydrogel specimen four volume fraction determinations were done at 25°C, two at 37°C, and two at 70°C. All determinations were done with the sample in equilibrium with buffered saline. For each determination the weight of the sample in air and the weight of the sample suspended in buffered saline (25°C) were recorded and used to calculate the sample volume. The sample was then thoroughly washed in distilled water, dried in a circulating air oven, and the solids weight noted. With the known density of PVA the volume fraction at temperature of interest (cc polymer/cc hydrogel) could be calculated.

3. Modulus Determination

Three samples for each hydrogel specimen were cut using a standard steel die (0.250 inch width) and their thickness measured with an Ames Dial Comparator. Stress-strain data were measured with an Instron Universal Testing Machine (Table Model) using a 0-50 gram load cell and the lowest rate of extension (0.2 inch/minute) to assure equilibrium between the tensile force and the elastic retractive force. To prevent loss of fluid from the sample during the experiment either by evaporation or by exudation, all runs were carried out with the sample immersed in a room temperature bath of buffered saline.

4. Permeability Studies

Hydrogel membranes (6.0 $\pm$ 1.0 mils) were cast with a calendered, nonwoven nylon fabric (0.4 oz/yd^2, 1.3 $\pm$ 0.3 mils

thickness) as support using the synthesis apparatus described above. Permeability data for a range of solutes were obtained using the batch dialyzer apparatus and analytic techniques developed by Colton (9). Stagnant diffusion studies with myoglobin and albumin were performed using commercially available lucite test cells (transport area approximately 10 cm^2) and the Micro-Biuret analysis for proteins (141). All measurements were performed with the solutes in buffered saline.

D. RESULTS AND DISCUSSION

1. Results for Control Composition

Figure 5-1 presents a plot of the tensile stress in force per unit swollen cross section as a function of the extension parameter $(\alpha - 1/\alpha^2)$ for one sample of the control composition measured in buffered saline at 25°C. Except for slight deviations at very low values of the extension parameter, which are caused by experimental error in measuring small values of force and extension, the points fall almost perfectly on a straight line, as predicted by the theory of rubber elasticity. These data are representative of all modulus determinations performed in this work.

Table 5-1 presents a summary of the data for five hydrogel specimens, all prepared according to the control composition. Elastic modulus as reported is the slope of the plot of stress τ as a function of $(\alpha - 1/\alpha^2)$, i.e., $\partial\tau/\partial(\alpha - 1/\alpha^2)$, and is not to be confused with Young's modulus E, defined as $\partial\tau/\partial(\alpha - 1)$. Values of M_c are calculated according to Equation (1-11). The values of modulus and M_c reported are the average

of three determinations on each specimen, and the volume fraction data are the average of all determinations on the five specimens.

Throughout this discussion the units for elastic modulus (E.M.) are dynes per square centimeter, M_c is given in grams per gram-mole, and concentrations are expressed in gmoles reactant per gmole PVA mer $\{CH_2 - CHOH\}$.

2. Effect of Glutaraldehyde

With glutaraldehyde as the independent variable the results shown in Figure 5-2 were obtained. The sample containing zero glutaraldehyde proved to be a viscous mass insoluble in water but impossible to study, indicating that formaldehyde contributes little to the cross-linking of the network. Since the experimentally measured elastic modulus will be a function of both the amount of cross-linking (M_c) and the polymer content of the specimen ($v_{2,25°C}$), both of which vary with the amount of cross-linking, the M_c data must be considered to interpret the effect of the reaction variable.

While the number of cross-links per chain (proportional to $1/M_c$) does increase with higher concentrations of glutaraldehyde, the values of M_c appear to approach an asymptote, though additional data at higher levels of glutaraldehyde would be required to confirm this point. What this suggests is that some equilibrium limitation exists in the chemical cross-linking of the PVA. While such an equilibrium undoubtedly depends on the water present in the system, certainly the other components such as formaldehyde, methanol, and possibly PVA hydroxyls also

affect it.

3. Effect of Acid Concentration, Reaction Time, and Formaldehyde Concentration

The effect of increasing the concentration of sulfuric acid in the reaction mixture is presented in Figures 5-4 and 5-5. As the concentration of acid increases, the value of the elastic modulus rises but M_c also increases, indicating fewer cross-links per initial PVA macromolecule. Figure 5-5 shows that the increasing values of the elastic modulus are, in fact, caused by the higher volume fraction of polymer in the test specimen. Higher sulfuric acid concentrations favor the introduction of increasing amounts of cyclic formal groups in the hydrogel matrix to produce the drastic thermal response seen in Figure 5-5. Since the amount of cross-linking drops (higher M_c) as the cyclic formal content of the network increases, these data suggest a competitive reaction between formaldehyde and glutaraldehyde.

Studies with longer reaction times and varying formaldehyde concentrations indicated, in summary, that, as formaldehyde concentration is increased or as time of reaction is increased, the cyclic formal content of the network chains rises and the amount of cross-linking per initial polymer chain falls (higher M_c). These trends are similar to those seen when hydrogen ion concentration was increased and lend support to the concept of a competitive reaction between formaldehyde and glutaraldehyde.

4. Higher Polymer Concentration

Figure 5-11 presents the effect on M_c of the weight

fraction of PVA in the initial reaction mixture ($w_{2,0}$) for the two PVA starting materials. The near superposition of the two curves for the two molecular weight species offers encouragement that the network correction factor (eqn. 1-6) is a reasonable approximation.

The decreasing value of M_c as polymer concentration rises represents another aspect of the competitive reaction between the two aldehydes. Since the formaldehyde concentration was held constant (6.0 wt %), the molal ratio of formaldehyde to PVA was, in fact, decreasing. At the lowered F/PVA molal ratio fewer PVA hydroxyls were blocked by unstable formaldehyde hemiacetals and were thus available for cross-linking by the glutaraldehyde.

5. Heparinized Hydrogels

Hydrogel specimens prepared with 1.0 wt % heparin at PVA contents of 0.06 and 0.10 weight fraction exhibited slightly lower elastic moduli, lower values of M_c, and a notably less pronounced thermal response when compared with controls of the same PVA content. While the lower shrinkage of the heparin-containing specimens might be ascribed to the countervailing influence of the highly charged polyanion heparin on the network contraction, the reason for lower values of M_c is not immediately obvious. Since the grafted heparin content of the hydrogel network (0.7 mg/g PVA dry basis) is far too low to contribute any significant number of cross-links to the network, one must presume that the heparin has affected the reaction in some other manner.

Quantitative chemical analysis of the stock heparin revealed that at least one of the carboxylic acid groups per tetramer is in the sodium form. If, in addition, one allows for some buffering effect from the two sulfamate groups on the heparin tetramer, the final hydrogen ion concentration of the reaction mixture (0.06 N initially) is in the range of 0.035 to 0.590 N. Comparison of the results for a heparinized hydrogel with an unheparinized control synthesized with 0.04 N H^+ as shown in Table 5-9 lends support for the proposed explanation.

6. Permeability Studies

Permeability studies on heparinized hydrogel membranes synthesized according to the control composition in Table 4-1 with 1.0 wt % heparin showed that the hydrogel membranes have improved permeabilities relative to conventional hemodialysis membranes when compared on the basis of equivalent thickness. The improvement is especially significant for the so-called middle molecular weight solutes of current interest in hemodialysis research and can be attributed to the high water content of the hydrogels. Protein permeation studies indicate that the transport of these solutes is higher than desirable for the heparinized hydrogels of this study with a water content of $v_{H_2O} = 0.95$.

E. CONCLUSIONS

1. The theory of rubber elasticity proposed in this work for application to networks formed in a solvated state proved to be a viable experimental method to study the effect of reaction variables on the hydrogel networks.

2. The importance of the two processes occurring during the hydrogel synthesis--cross-linking and chain modification--were demonstrated. Glutaraldehyde functions as the cross-linking agent by formation of two cyclic acetals with PVA hydroxyls, and formaldehyde functions primarily to modify the network chains by formation of cyclic formals.

3. Higher amounts of cyclic formal in the network tend to increase the equilibrium volume fraction of polymer at any temperature and to accentuate the thermal response of the network.

4. Conditions which favor the introduction of more cyclic formal--higher acid concentration, longer reaction times, and higher molal ratios of formaldehyde to PVA--tend to decrease the amount of glutaraldehyde cross-linking per PVA chain.

5. Heparin exerts a buffering effect on the reaction mixture, and this accounts for the properties observed with heparinized hydrogels.

6. The improved permeability characteristics of heparinized hydrogel membranes relative to conventional hemodialysis membranes is attributed to their high water content.

CHAPTER 2

INTRODUCTION

A. MOTIVATION AND PURPOSE

For the past two decades considerable research has been devoted to the improvement of hemodialysis as a clinical treatment for chronic renal failure. The most significant advances have come from cooperation among numerous disciplines including physicians, engineers, chemists, and hematologists.

According to the Report of the Committee on Chronic Kidney Disease, 35,000 patients per year die of chronic renal failure and of this group 7,000 per year would be good candidates for treatment (1). Current statistics place the total number of patients receiving regular dialysis treatment at about 3,500, though these statistics are not complete (2). Based on statistics of the National Dialysis Registry (2), a five-year survival is an optimistic estimate for patients on dialysis.

Clearly there are still large numbers of patients who are unable to receive treatment. There is a real lack of medical resources to handle the problem. The cost of dialysis is often raised as a crucial element which discourages the expansion of available facilities. If one considers a five-year survival of 50% of the patients receiving treatment along with 7,000 new patients per year, the imagination staggers at the dimension confronting society ten years hence, if all were treated. The need for further research to understand and improve the dialysis treatment is clearly necessary.

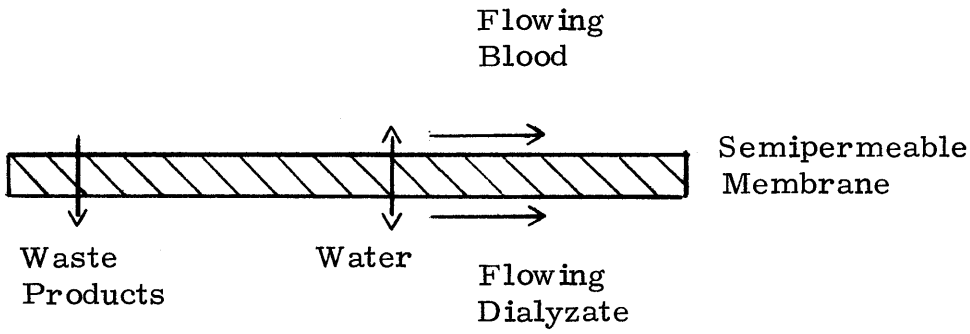
It is the purpose of this thesis to focus on one particu-

lar area of hemodialysis research, namely, membrane development. Though the motivation for this work lies in the biomedical area, the research has in fact been an application of the principles of polymer science to study a hydrogel system based on dialdehyde crosslinking of polyvinyl alcohol that has certain unique advantages for application as a hemodialysis membrane.

For the reader who desires some background in kidney physiology and types of kidney failure, a standard text of medical physiology (3,4,5) or the more detailed discussions by Smith (6) and Pitts (7) will be helpful. While this background is not essential to the understanding of the work in this thesis, it will be useful to the reader who has not been previously exposed to the physiological basis of the engineering problems in hemodialysis research.

B. HEMODIALYSIS

Hemodialysis as a replacement for kidney function, first successfully applied by Kolff in 1944 (8), is illustrated in Figure 2-1. Blood passes in laminar flow on one side of a semipermeable membrane; dialyzate, an osmotically balanced solution of electrolytes and glucose, flows on the other side. Solutes such as urea, creatinine, uric acid, excess electrolytes, and other trace materials diffuse across the membrane in response to the concentration driving force maintained between the blood side and the dialyzate bath. In normal operation water is removed from the blood to relieve excess edema in the patient either by decreasing the dialyzate osmolality



$$\text{Overall Mass Transfer Resistance} = \sum \text{Resistances}$$

$$= \text{Blood Phase} + \text{Membrane} + \text{Dialyzate Phase}$$

Figure 2-1. Conceptual Illustration of Hemodialysis.

or by increasing the hydrostatic pressure difference between blood and dialyzate.

Mass transfer from the blood stream to the dialysis bath occurs through three resistances in series--the blood side resistance, the membrane resistance, and the dialyzate side resistance. For small solutes such as urea a considerable amount of the transport resistance resides in the liquid boundary layers, but for larger solutes (MW 500 or more) the membrane resistance becomes the controlling one with current dialysis membranes (9).

Figure 2-2 illustrates a typical artificial kidney circuit. Access to the patient is gained through an arteriovenous (A-V) shunt. Many dialysis systems require the use of a blood pump since the normal A-V pressure drop (∿100 mm Hg) is not adequate to maintain the desired blood flow rates through the dialyzer. Illustrated in Figure 2-2 is a coil-type artificial kidney, though other geometries in common use are the flat plate configuration and the hollow fiber devices (10,11). A key feature to note is the regional heparinization of the patient's blood to prevent activation of the clotting mechanism when the blood contacts the foreign surfaces of the hemodialysis circuit.

C. DEVELOPMENT OF MEMBRANE CRITERIA

1. Improved Permeability to Middle Molecular Weight Solutes

For a number of years there has been speculation that so-called middle molecular weight molecules play an important

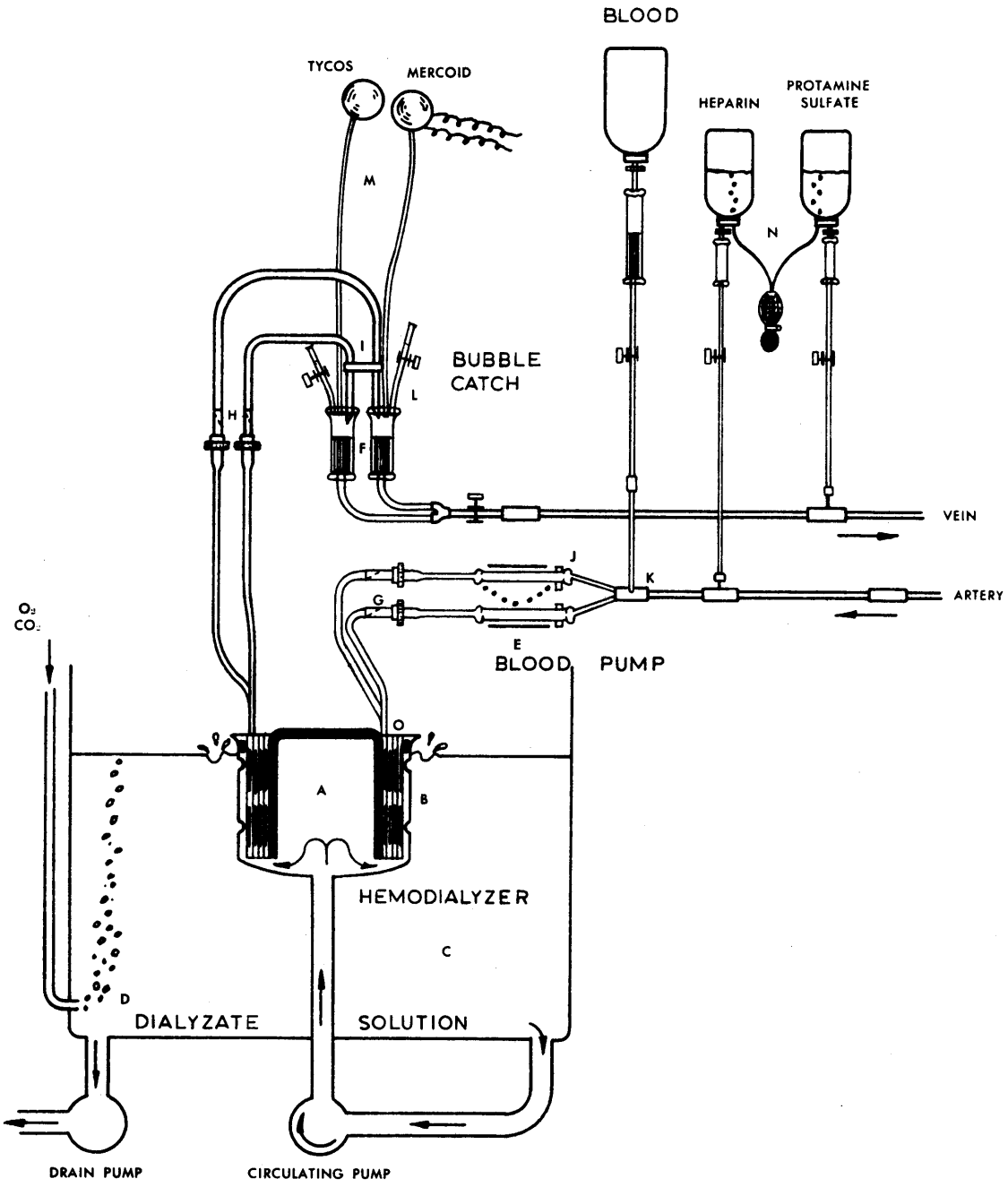


Figure 2-2. Typical Artificial Kidney Circuit

role in the toxicity of uremia (12). Middle molecules refer to as-yet-undefined substances in the molecular weight range 500-5000 which, because of their size, are removed slowly relative to urea during dialysis. The principal mass transfer resistance to these solutes resides in the dialyzer membrane.

Two clinical observations support this hypothesis. A comparison of the results of hemodialysis and peritoneal dialysis suggests that, since patients on the latter therapy remain well and free of uremic neuropathy in spite of higher urea and creatinine levels (13), the peritoneum must be passing some toxic substances more readily than cellophane. This can be interpreted as an increased permeability of the peritoneal membrane to middle molecules relative to cellophane. Secondly, the prevention of peripheral neuropathy is found to be dependent upon an adequate number of hours of dialysis per week rather than on maintaining certain pre-dialysis levels of serum urea and creatinine (14). Since the plasma concentration of small molecules falls rapidly during dialysis, longer dialysis times contribute little to the removal of these solutes. However, the concentration gradient for the more slowly diffusing middle molecules remains high throughout the dialysis, so increased dialysis time could significantly improve the removal of these substances.

Babb, Scribner, and colleagues (15) are currently investigating these hypotheses by a series of clinical strategies designed to selectively increase and decrease the pre-dialysis levels of solutes in the low molecular weight range and in the

middle molecular weight range. Though not conclusive, their results have been encouraging (16). Translated into a criterion for membrane development, the need for a membrane with permeabilities to low molecular weight solutes comparable to commercial cellulosic membranes and with improved permeabilities to middle molecular weight solutes is indicated.

2. Non-Thrombogenic Dialysis Membrane

The human body is protected from unwanted bleeding by the blood coagulation mechanism (Figure 2-3). Two semi-complementary pathways each involve a cascade series of activation of proteins resulting in the conversion of soluble fibrinogen to an insoluble fibrin clot. The roman numerals on the figure refer to specific clotting factors.

Living tissues, such as blood vessel walls, lungs, and brain, contain a lipid-protein complex (Factor III, tissue thromboplastin) capable of activating Factor X in the presence of Factor VII. In response to tissue damage Factor III is released and the cascade proceeds to a fibrin clot within seconds.

Even in the absence of tissue damage, contact with a foreign surface can produce clotting via the intrinsic pathway. In this case, the mechanism begins with conversion of Factor XII (Hageman) from an inactivated to an activated form. Activated Factor XII initiates a series of biochemical reactions culminating in the conversion of prothrombin to thrombin which catalyzes the conversion of fibrinogen to fibrin.

Briggs and MacFarlane have presented a good discussion of

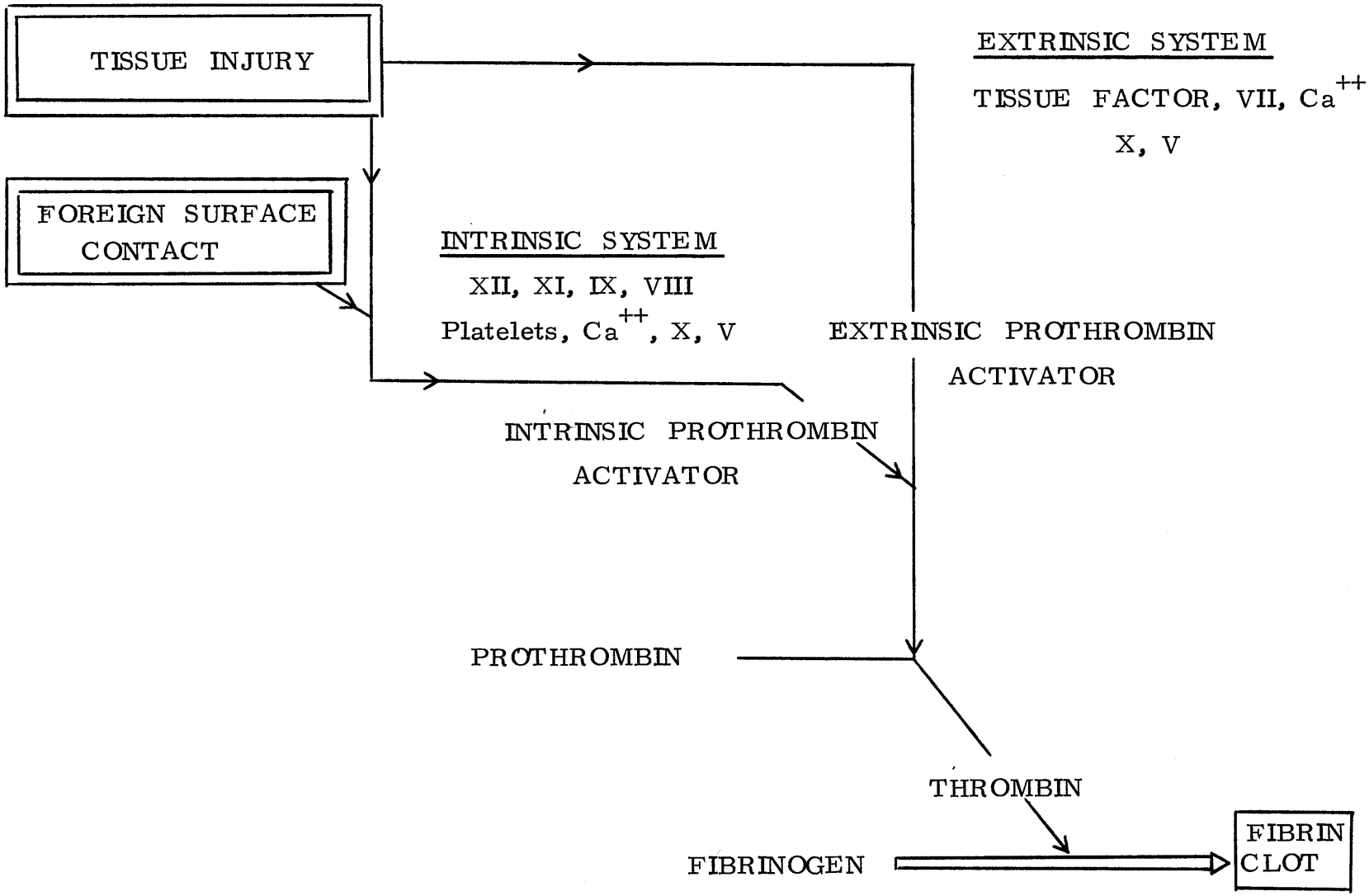


Figure 2-3. Human Blood Coagulation Mechanism.

blood coagulation (17). The article by Deykin (18) is an excellent review of the mechanism of thrombogenesis.

Any clinical situation requiring blood contact with a foreign surface has to overcome the intrinsic clotting mechanism. This is most frequently done by administering a naturally occurring anticoagulent called heparin, a highly sulfated α-1,4 linked, mucopolysaccharide (19), shown in Figure 2-4. Heparin interferes with the clotting mechanism by complexing activated Factor IX, and it also interferes with active thrombin, probably by complexing (20).

If suitable non-thrombogenic biomaterials were available for the construction of the blood-contacting surfaces of the dialysis circuit (tubing, cannulae, membrane), there are several disadvantages in the heparinization that could be overcome. The system for administering and back-titrating the heparin during dialysis (Figure 2-2) is complex and contributes to the capital cost of the equipment. Also associated with regional heparinization is a problem known as "heparin rebound." The protamine in the heparin-protamine complex is preferentially lysed such that free heparin occurs in the bloodstream several hours after dialysis, causing a hemophilic syndrome (21).

A simplified procedure would be advantageous in light of the trend toward home dialysis to reduce treatment cost (22,23). Heparin therapy has been indicated as the cause of bilateral adrenal hemorrhage (24). Additional hemorrhagic complications arising during long-term dialysis have been discussed in the

Heparin Tetramer

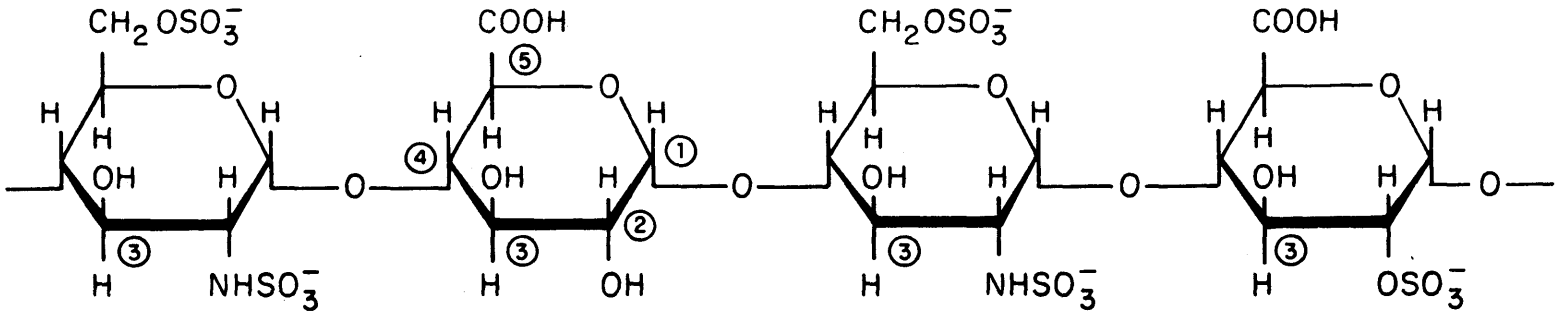


FIGURE 2-4. HEPARIN TETRAMER.

review by Pendras and Erickson (25) and in the article by Remmers et al. (26).

One of the major improvements in hemodialysis has been the sorbent-based, low-volume dialyzate regeneration system reported by Gorden and colleagues (27). Here the 200-liter dialysis bath has been successfully replaced by a six-liter system that regenerates the dialyzate by means of a sorbent cartridge containing urease, zirconium phosphate, zirconium oxide, and activated charcoal. While the advantages of this system are numerous and have been discussed in the above reference, one that should be noted here is the portability of the system and the fact that it gives the dialysis patient far greater freedom to travel. In this context a non-thrombogenic dialysis system could offer an additional advantage to the dialysis patient.

Since the dialysis membrane represents by far the largest surface area contacted by blood in the artificial kidney circuit, any effort to eliminate or reduce the amount of heparin used per dialysis must improve the blood compatibility of the membrane. Thus another requirement for membrane development is (ideally) that the membrane be non-thrombogenic.

3. Additional Membrane Requirements

Other requirements for a suitable dialysis membrane (28,29,30) will be summarized here.

(a) Good mechanical strength--the membrane should have sufficient mechanical strength and tear resistance to permit fabrication into existing dialysis devices.

(b) Physical stability--the membrane should not contain any elutable toxic substances that could leach into the bloodstream during dialysis. Also it should not undergo any change in physical properties (strength, tear resistance, permeability) while in storage or in use.

(c) Chemical stability--the membrane should not degrade either by chemical or biological attack when in contact with the blood or in storage.

(d) Retention of proteins--the membrane should have negligible transport ("cut off") of the plasma proteins, albumin (MW 69,000) being the smallest in the series.

D. CURRENT MEMBRANE RESEARCH

The most commonly used hemodialysis membrane is Cuprophane (R-Bemberg), a cellulosic membrane produced by the cuprammonium process. Because Cuprophane (R-Bemberg) can be produced commercially in thin (0.001 inch), uniform, pinhole-free sheets of reasonable physical strength, it has enjoyed wide application in hemodialyzers. The principal drawbacks of the membrane are that it will activate the intrinsic clotting mechanism, that its permeability to solutes in the middle molecular weight range is less than desired, and that it has poor resistance to tear propagation when water-swollen.

Current membrane research can be categorized as follows:

1. Efforts to Improve Cellulosic Membranes

Within this group falls the research on the production of ultra-thin membranes to improve transport properties (31), the variation of coating techniques and other treatments to affect membrane morphology and hence permeability (32,33,34), and the attempts to treat existing membranes to make them non-thrombogenic (35,36). Cellulose acetate membranes are also being studied for application in hemodialysis (37,38).

2. Insolubilization of Water-Soluble Polymers

Successful dialysis membranes have a reasonably high equilibrium water content, usually greater than 50%. Recognition of this fact has led various investigators to study the transport properties of membranes prepared by cross-linking polymers such as polyvinyl alcohol, polyethylene glycol, and polyvinylpyrrolidone and by hydrolyzing polyvinylbutyral (28,30). The cross-linked poly (2-hydroxyethyl-methacrylate) hydrogels of Wichterle and Lim have been considered for application as dialysis membranes (39).

3. Copolymers of Water-Soluble and Water-Insoluble Polymers

The logic here is that the water-soluble portion will impart "swellability" to the membrane and the insoluble portion will contribute to the strength of the material. Examples here are membranes prepared from Bisphenol A copolyether-carbonates (40), block copolymers of polyoxyethylene oxide and polyethylene terephthalate (41), and block copolyether-urethanes (42).

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4. Other Approaches

In this category fall efforts such as the collagen membranes of Stenzel (43) prepared by solubilization with enzymes and cross-linking with ultraviolet radiation, and the study of synthetic polypeptides as possible hemodialysis membranes (44). Also, the development of hollow fiber membranes for hemodialysis (45,46) represents a novel approach for membrane configuration in a hemodialysis device. Cross et al. have suggested their polyelectrolyte complexes for use as hemodialysis membranes (47).

While results on these various areas of hemodialysis membrane research have indicated some success, usually as improved permeabilities relative to Cuprophane (R-Bemberg), none has been superior enough in all aspects to replace it as the membrane for regular dialysis treatment. Looking into the future, one can envision sophisticated systems which discriminate on the basis of selected characteristics, such as stereospecific structure, electric polarizability, or solubility characteristics of the molecules, so that the efficiency of artificial kidneys would be much enhanced (48). Except for the very limited selectivity of some block copolymer membranes (49), it is likely that dialysis membranes will continue to select molecules primarily on the basis of size. Indeed, the best criteria available (29) for which solutes to remove and which to retain are scanty.

Few dialysis membranes under study are superior to Cuprophane (R-Bemberg) in blood compatibility and none are

non-thrombogenic. The development of a non-thrombogenic membrane with good transport properties and adequate physical strength would represent a significant contribution to the improvement of hemodialysis therapy.

E. NON-THROMBOGENIC MATERIALS

A logical approach to the problems presented above would be to select a material that has previously demonstrated non-thrombogenic character and adapt it for application as a hemodialysis membrane. With the excellent review of Salzman (50) and the summary of Bruck (51) in the literature, no attempt will be made here to discuss the entire field.

A good portion of the research on non-thrombogenic surfaces has been directed toward the study of rigid materials and hydrophobic materials for various applications such as heart valves, cannulae, and catheters. While these materials could be used in the artificial kidney circuit, they have no application as hemodialysis membranes. The work of Gerson (52) on modifying cellulose to make it non-thrombogenic attacked the problem but was not completely successful.

The hydrogels of Lim and Wichterle (53) and the polyelectrolyte complexes of Cross and Michaels (47) have demonstrated reasonable results as non-thrombogenic surfaces, probably because of their high water content (51). While high water content is an advantage for a blood-contacting surface (50), it is unlikely that that factor alone can impart long-term blood compatibility to a material (51).

Heparinization of various polymeric materials has received considerable study (54,55,56,57). Most investigators have

devised techniques of modifying the surface to contain cationic groups onto which the heparin is ionically linked. Lipps (35) and Britton (36) attempted to heparinize cellulose by ionic coupling, but were not completely successful. Martin et al. (37) are pursuing the heparinization of cellulose by another ionic bonding technique, but they have no conclusive results on blood compatibility. Lagergren and Erickson (58) have ionically bonded heparin to a rigid substrate and then have chemically cross-linked the heparin monolayer. Their results are quite impressive, but their technique could not be used to treat a dialysis membrane.

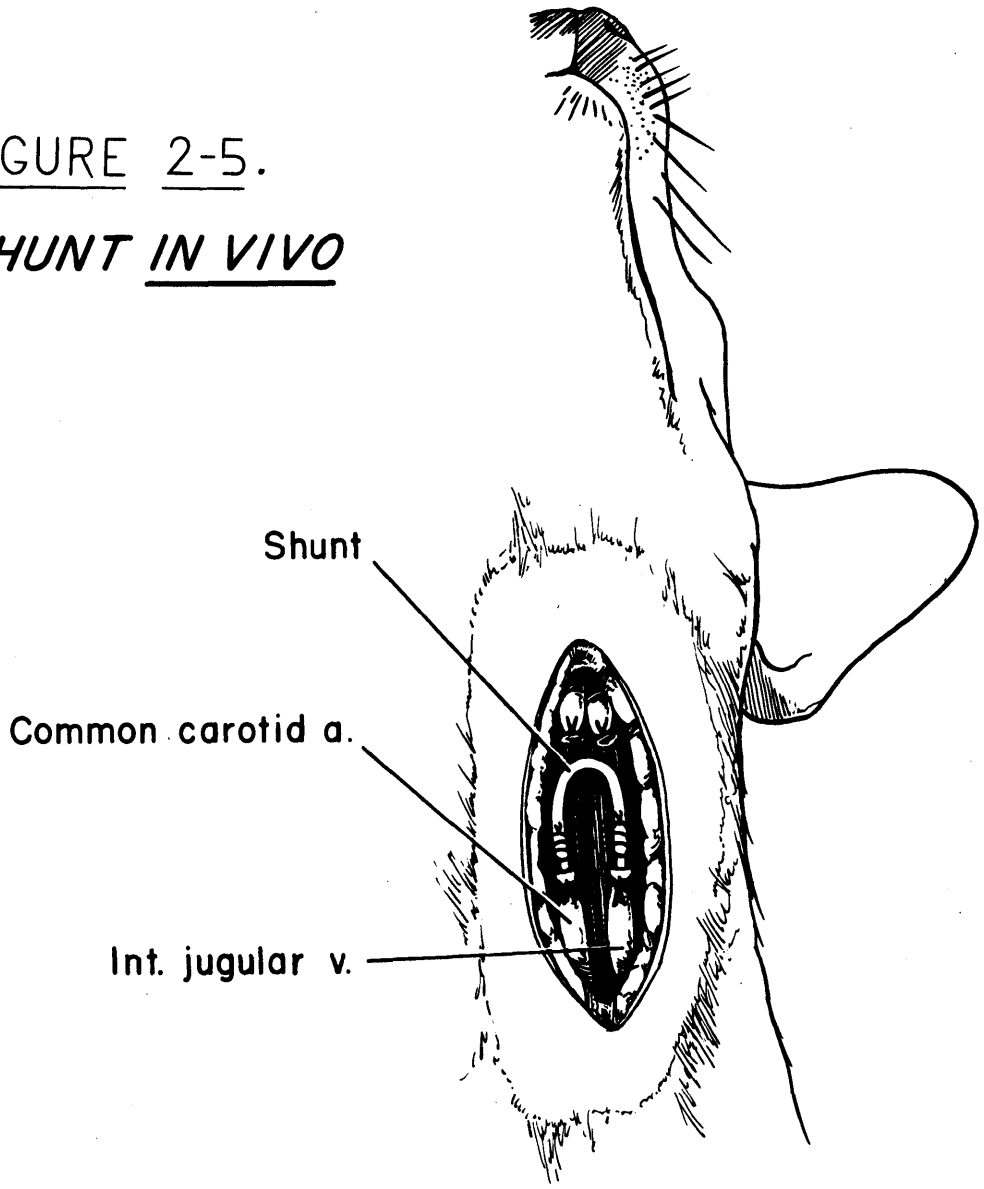
F. HEPARINIZED HYDROGELS AS NON-THROMBOGENIC MATERIALS

Merrill and colleagues were the first to covalently bond heparin to a polymer matrix without diminishing the biological activity of the heparin (59). Their material is a polyvinyl alcohol hydrogel and could be used as a dialysis membrane. *In vitro* assessment of the material indicated that the material does not activate the intrinsic clotting mechanism and, in particular, does not activate Factor XII, the first step in the intrinsic clotting cascade (59).

In vivo tests, the *sine qua non* for any non-thrombogenic material, had been hampered primarily by the physical properties of the material. Usually poor fluid mechanics for blood flow resulted in stagnation points near the vessel-hydrogel junction or the cannula-hydrogel junction. Clotting factors, activated by contact with the teflon cannula in an A-V shunt configuration (Figure 2-5) or by damaged endothelial lining (resulting from surgical difficulties) in the classic Gott

FIGURE 2-5.

A-V SHUNT IN VIVO

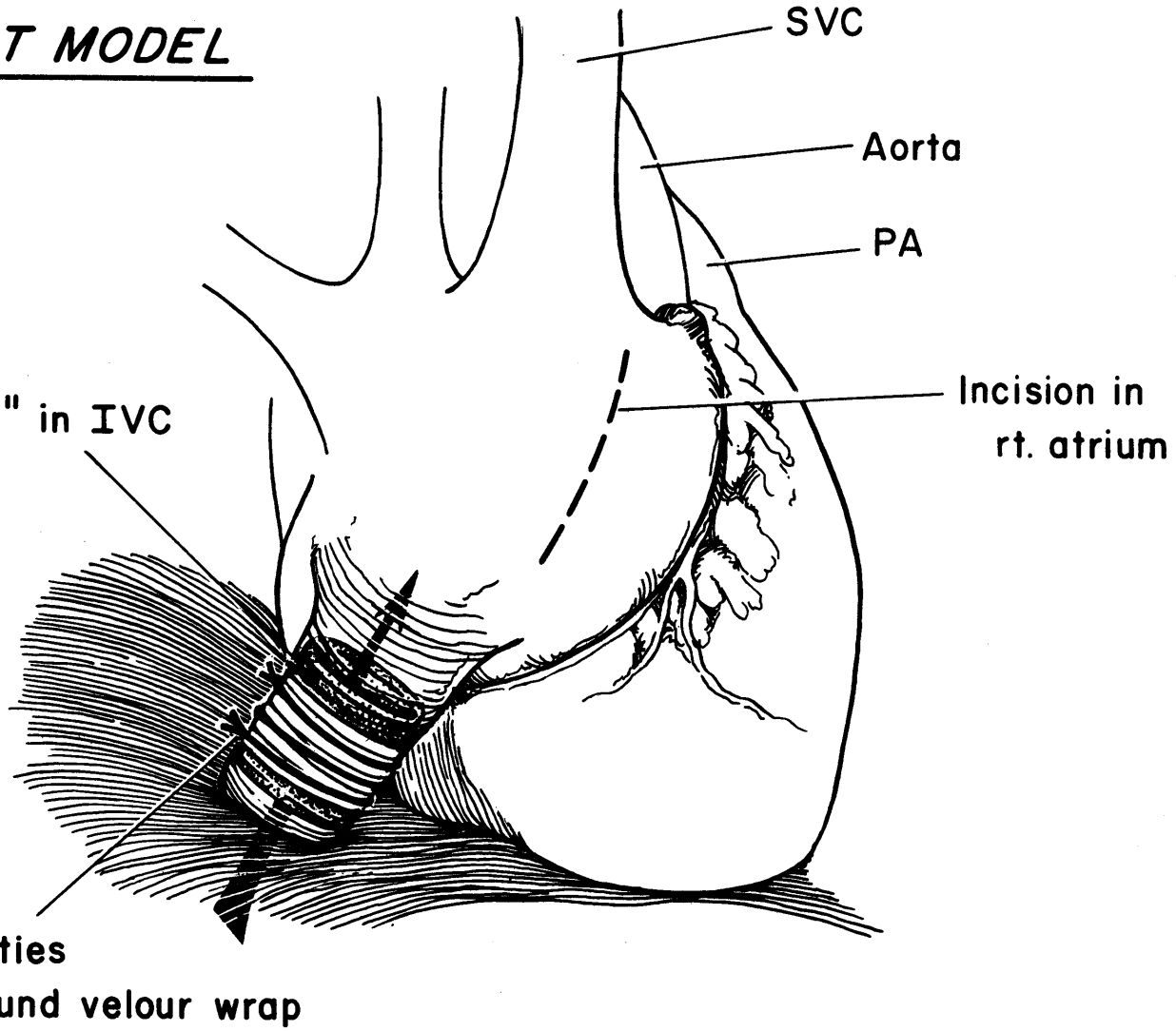


position (Figure 2-6), were not washed downstream to be dealt with by natural defenses. A thrombus adherent to the teflon cannula in the A-V shunt or adherent to vessel wall at the hydrogel-vessel junction in the Gott model would result, obscuring valid evaluation of the material.

Recent in vivo results presented in a report by Wong (60) and in a paper by Merrill et al. (61) have been most encouraging. Improved A-V shunts with good hemodynamics were implanted for periods of 5 minutes, 60 minutes, and 240 minutes. While both the heparinized hydrogel shunts and the unheparinized hydrogel controls remained patent for more than four hours, electron microscopic examination of the control shunts revealed numerous platelet thrombi adherent to the gel, having developed at some time between 5 and 60 minutes. Although the thrombi had disappeared after some four hours, a layer of degranulated platelet-protein-fibrin coating was deposited on the wall. In contrast, hydrogels with covalently bonded heparin showed no thrombi at any time, relatively few clusters of platelets on the surfaces in the interval between 5 and 60 minutes of implantation, and these platelets seemed not to be granulated. After four hours of implantation, the surface of the heparinized hydrogel was found to have no platelets on it whatever. Though further in vivo evaluation of these materials is necessary, the results indicate superior performance of the heparinized gels relative to the unheparinized controls. Study of this material for ultimate application in the artificial kidney circuit is needed.

FIGURE 2-6.

GOTT MODEL



G. STATEMENT OF OBJECTIVES

The objectives of this thesis are twofold:

1. To study the chemistry of the network hydrogel polymers of Merrill and Wong. In particular, to study the effect on physical and chemical properties of various reaction variables.
2. To develop techniques for preparing membranes with this material and to evaluate their transport properties.

H. THESIS PROGRAM

To accomplish the first objective of this thesis, the classic theory of rubber elasticity was modified for application to a hydrogel system. This analysis was used to interpret experimentally measured moduli of a range of hydrogel compositions synthesized under carefully controlled reaction conditions. Along with experimentally measured volume fractions of polymer in the gels as a function of temperature, these data are used to explain the effect of various reaction variables in the system.

A procedure was developed to cast membranes of the hydrogel material with a suitable support. Permeability measurements were made on these membranes.

I. PREVIOUS WORK

Previous studies on these hydrogels have been done by Merrill and Wong and will be appropriately referenced in the body of the thesis.

Odian and Leonard (30) studied polyvinyl alcohol membranes

crossed with allyl methracrylate under radiation. Markle et al. (28) cross-linked polyvinyl alcohol films with dialdehyde, but their reaction conditions were considerably different than those employed here.

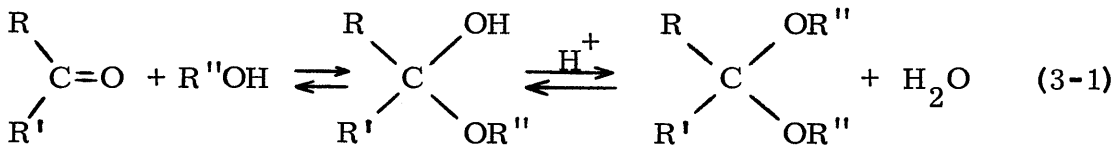
CHAPTER 3

THEORETICAL BACKGROUND

SECTION I. HYDROGEL CHEMISTRY

A. GENERAL ACETAL REACTION

The key reaction in the hydrogel synthesis is the acid catalyzed acetalization of a carbonyl group and an alcohol. The general reaction may be written:

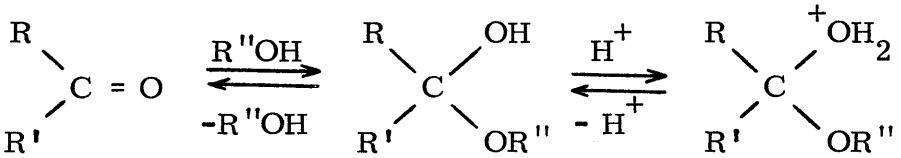


(3-1)

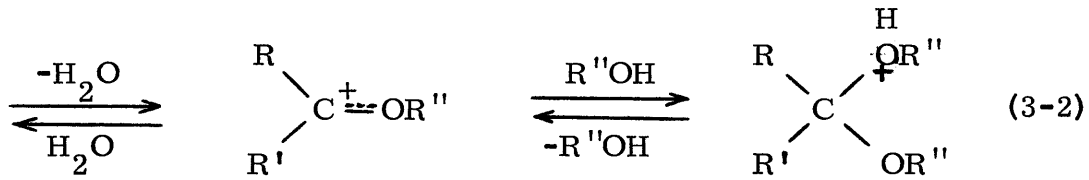
Hemiacetal Acetal

It proceeds through the unstable hemiacetal form and requires the acid catalyst for conversion to the acetal.

At present the reaction mechanism shown in (3-2) is accepted for acetal formation (62). Although acetal cleavage was thoroughly examined, kinetic data on acetal formation are poor because the reaction starts from the hemiacetal for which precise concentration measurements are difficult. Qualitative conformation for the reaction mechanism may be found in its being the exact reverse of the thoroughly studied mechanism of hydrolysis; all steps of the reaction are reversible. Since the rate-determining step of acetalization is the formation of cation II from the protonated hemiacetal I, it is to be expected that substituents will have effects analogous to those

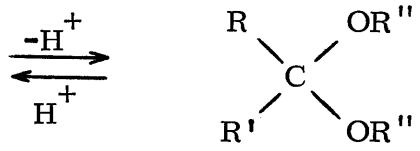


Hemiacetal I



(3-2)

II III



Acetal

they exert in the rate-determining step of acetal cleavage, involving cation formation from the protonated acetal III.

The following influences of the alcoholic component are evident from the mechanism of acetal formation. Branching in the alcohol component decreases the concentration of hemiacetal, from which the acetal formation starts (63). The equilibrium in the protonation step of the hemiacetal should depend somewhat on the structure. The rate of acetalization will be influenced by the alkoxy group of the hemiacetal, but will be

independent of the entering alcohol molecule, since reaction with the latter takes place after the rate-determining cation formation.

The acetal reaction is an equilibrium reaction and, as a rule, does not go to completion. Branching of the alkyl group either in the aldehyde or in the alcohol decreases the tendency to form acetals (64). The equilibrium being essentially unfavorable for the formation of acetals, the main problem in their preparation is to shift the equilibrium by reducing the concentration of water. In favorable cases it is sufficient to keep the concentration of water low by addition of a large excess of alcohol. Another method is the removal of water by azeotropic distillation (65).

The equilibrium of acetal formation does depend on the structure of the alcohol. For example, the pentaerythritol acetal of formaldehyde hydrolyzes 10^4 times more slowly than formaldehyde diethyl acetal (66). Assuming similar rates of formation, it follows that the six-membered ring acetal has an equilibrium constant greater by a factor of about 10^4. Therefore, the preferred form in water solution (unfavorable equilibrium) is the cyclic acetal.

Studies on acetal hydrolysis established a linear dependence of the reaction rate on the acidity function of the solution (67).[1] This is probably valid for acetal formation as

---

1. For acid concentrations $[H^+]$ of less than 1.0 N the acidity function $H_o \simeq$ pH. A complete discussion of the acidity function and data on acetal hydrolysis are in reference (67).

well, the anion of the acid being unimportant.

B. SYNTHESIS OF HYDROGEL NETWORK

In the synthesis of the non-thrombogenic network hydrogels polyvinyl alcohol $\left\{C\text{-}C\right\}$ (PVA) is cross-linked by a mixture of aldehydes (formaldehyde and glutaraldehyde) via acetal formation with the secondary hydroxyls on the polymer backbone. Heparin (Figure 2-4) is coupled to the matrix by acetalization with the secondary hydroxyls on the 3-carbon of the anhydroglucose unit.

While the acetalization of PVA has been well studied because of its commercial importance in the production of resins such as polyvinyl butyral and polyvinyl formal (68), the reaction conditions for the hydrogel system differ significantly.

1. Instead of reaction as bulk polymer, the PVA is cross-linked in a 6-10 wt.% aqueous solution.

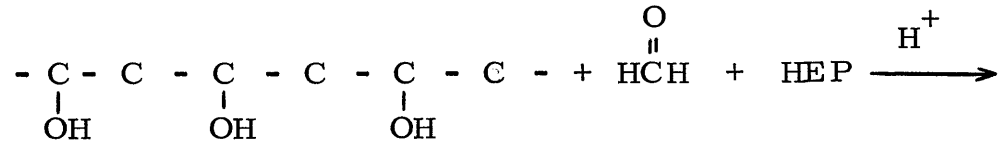
2. The dialdehyde glutaraldehyde (pentanedial) was used as well as formaldehyde to effect cross-linking.

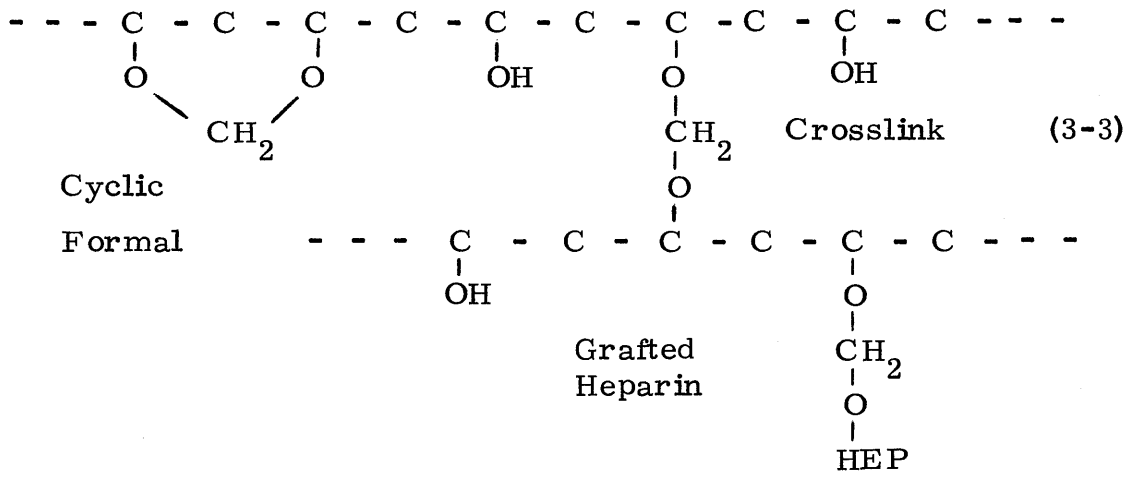
3. Heparin initially present in the reaction mixture with the PVA is grafted to the matrix.

4. Milder reaction conditions are used to avoid degradation of the biological activity of heparin.

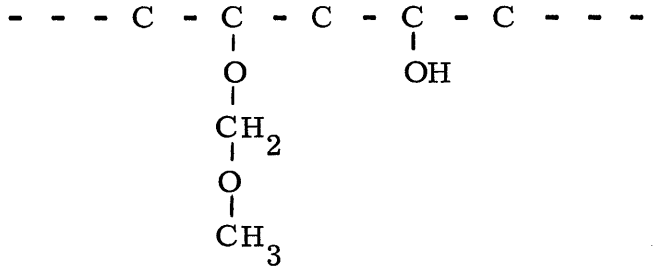
With formaldehyde as the specific aldehyde the reaction schemes shown in (3-3) below are envisioned.

HEP represents a heparin molecule grafted to the matrix. Since the primary alcohol methanol is also present in the reaction mixture, structures such as shown in (3-4) may also be present. However, based on the discussion of the acetal mechanism (Section 3-I-A), the cyclic acetal is the most stable





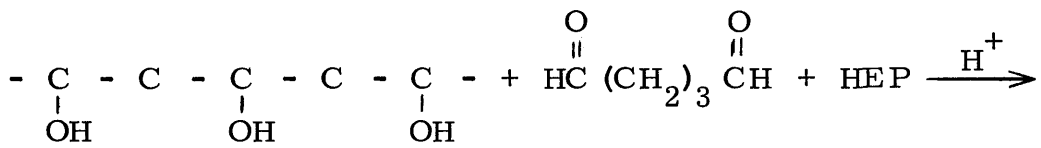
(3-3)

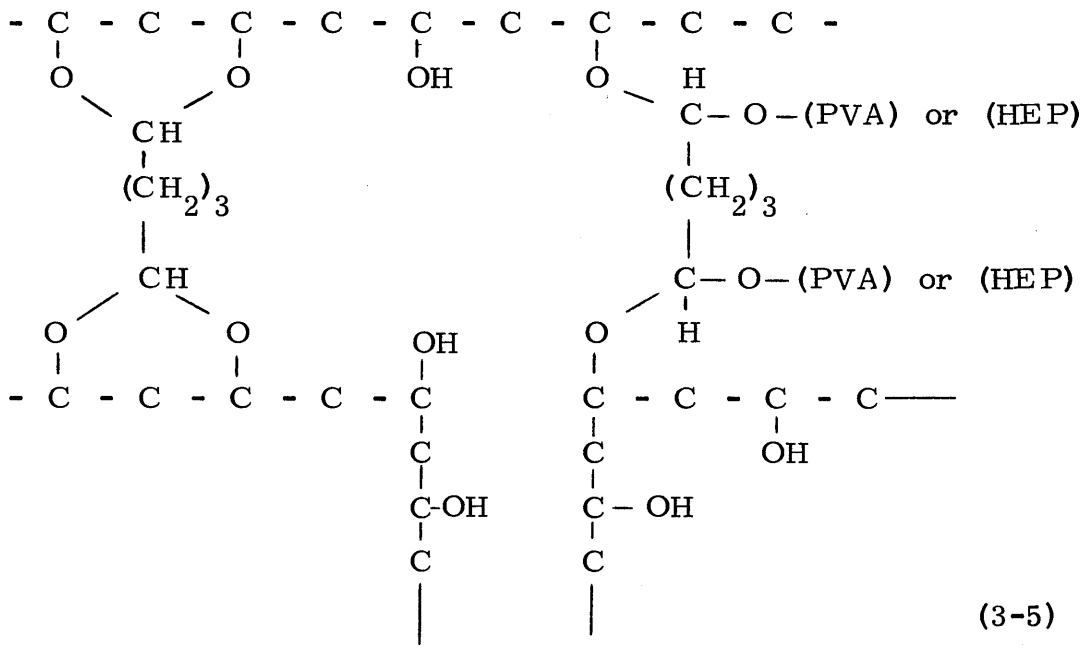


(3-4)

form. The principal effect of the formaldehyde is probably a modification of the polymer backbone by the formation of cyclic acetals.

Since each end of a glutaraldehyde can participate in a reaction, it functions as the key cross-linking agent. The following reactions can be proposed.

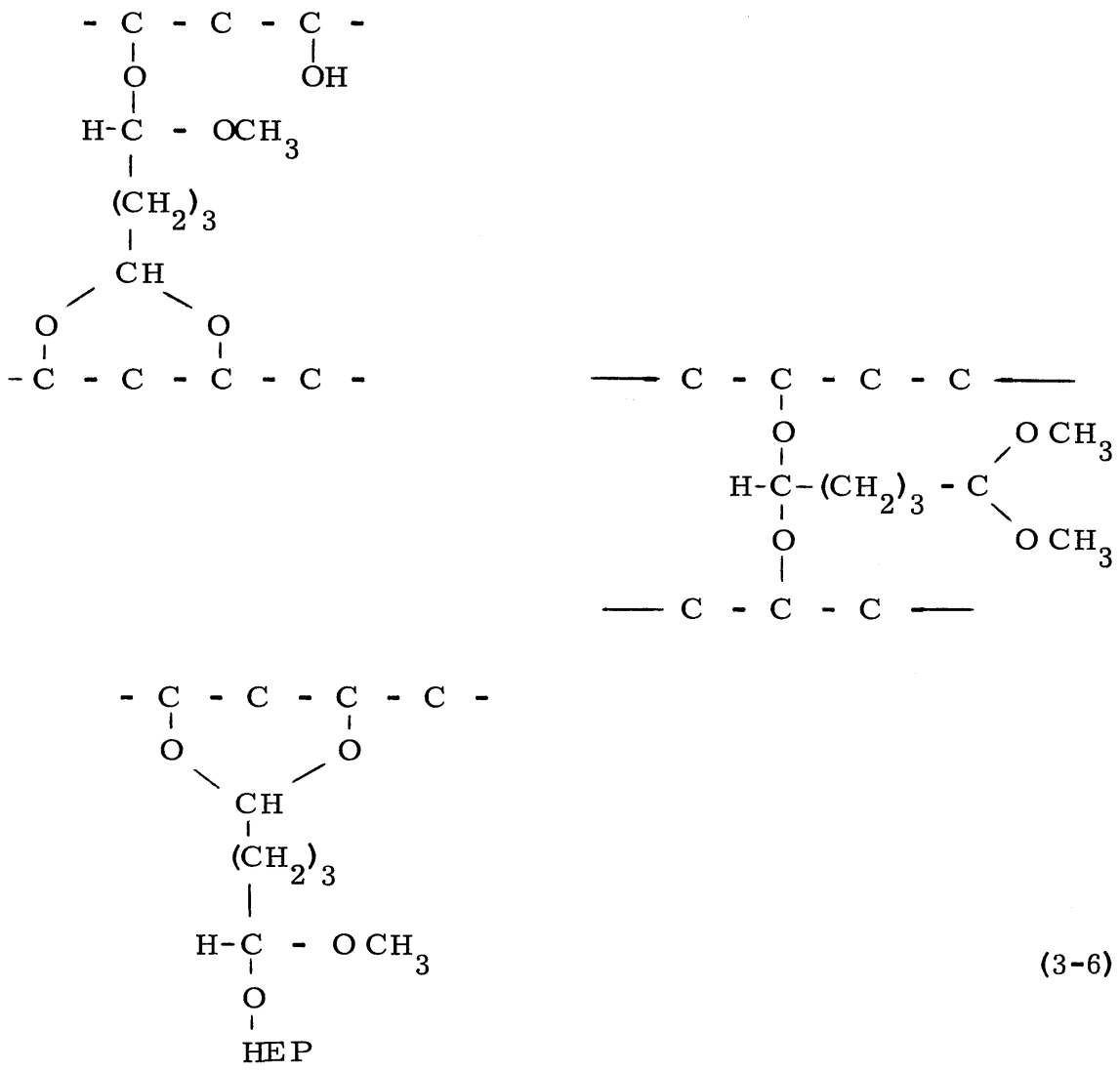




(3-5)

Ultimately one cannot rule out a reaction of glutaraldehyde with four different PVA chains, but the probability of this would seem low. Again recalling the inherent stability of the six-membered ring, the participation of one glutaraldehyde in two cyclic acetals is probably favored.

Methanol could function here also as a "capping" reagent. A few potential structures are as shown in (3-6). Clearly other variations are possible.



(3-6)

Clearly other variations are possible.

C. PREFERRED REACTION COMPOSITION

Previous work by Wong (69) has led to a preferred reaction mixture for synthesis of the hydrogels. The casting solution contains 0.060 weight fraction polyvinyl alcohol (volume fraction 0.047) with other components listed in Table 3-1. Concentrations are expressed directly and as a molal ratio of the component to a repeat unit $\{CH_2 - CHOH\}$ of polyvinyl alcohol (mer weight 44 grams).

TABLE 3-1. STANDARD (CONTROL) HYDROGEL

Reaction Mixture

	concentration	mole ratio
Polyvinyl alcohol $\{CH_2CHOH\}$	6.0 wt %	1.00
Formaldehyde (HCHO)	6.0 wt %	1.47
*Methanol (CH_3OH)	ca. 2.0 wt %	0.50
Glutaraldehyde ($OCH(CH_2)_3CHO$)	0.076 wt %	0.0056
Sulfuric acid (H_2SO_4)	0.06 N	0.0440
Acetic acid (CH_3COOH)	1.0 N	0.73

*Methanol enters the reaction mixture because it is present as a stabilizer (ca. 12 wt %) in stock formalin solution (ca. 37 wt % HCHO).

TABLE 3-2. HEPARIN-HYDROGEL REACTION

Reaction Mixture

	concentration	mole ratio
Polyvinyl alcohol $\{CH_2CHOH\}$	6.0 wt %	1.00
Formaldehyde (HCHO)	6.0 wt %	1.47
*Methanol (CH_3OH)	ca. 2.0 wt %	0.50
Glutaraldehyde ($OCH(CH_2)_3CHO$)	0.076 wt %	0.0056
Sulfuric acid (H_2SO_4)	0.06 N	0.0440
Acetic acid (CH_3COOH)	1.0 N	0.73
Heparin, sodium	1.0 wt %	0.03**

**0.03 mole ratio of heparin hydroxyls (taken as 5 per tetramer) to polyvinyl alcohol repeat units.

The mixture is normally reacted at 70°C for 60 minutes followed by quenching at room temperature in buffered saline (pH = 7.4, 0.30 N in ions).

D. LIMITATIONS ON REACTION CONDITIONS

The choice of feasible reaction conditions is limited primarily by the heparin. Since heparin is insoluble in all solvents except water, many of the classical coupling reactions such as diisocyanate reactions cannot be used. In addition, there are numerous references in the literature documenting the deactivation of heparin when exposed to acid in aqueous media (70,71,72). Since there is some indication that higher molecular weight species of heparin are biologically more active (73), it could be postulated that the exposure to acid causes a cleavage of the glucosidic linkages. More likely, however, the heparin undergoes a change in conformation that affects its activity (74). Heparin normally exists in solution in a helical conformation held together by internal hydrogen bonds. The acid media could interfere with the hydrogen bonding and thereby permit a change in conformation.

In their earlier work, Merrill et al. (59) demonstrated that for a certain range of reaction conditions the heparin would remain viable. Their data appear in Figure 3-1, where the ordinate "(Protamine/Heparin) Weight Ratio" can be interpreted as a measure of the activity of heparin remaining after reaction relative to the control value at 0.0 N acid. Reaction conditions are therefore limited to one hour at 70°C for acid concentrations less than 0.2 N. Somewhat longer exposures at lower temperatures could be tolerated.

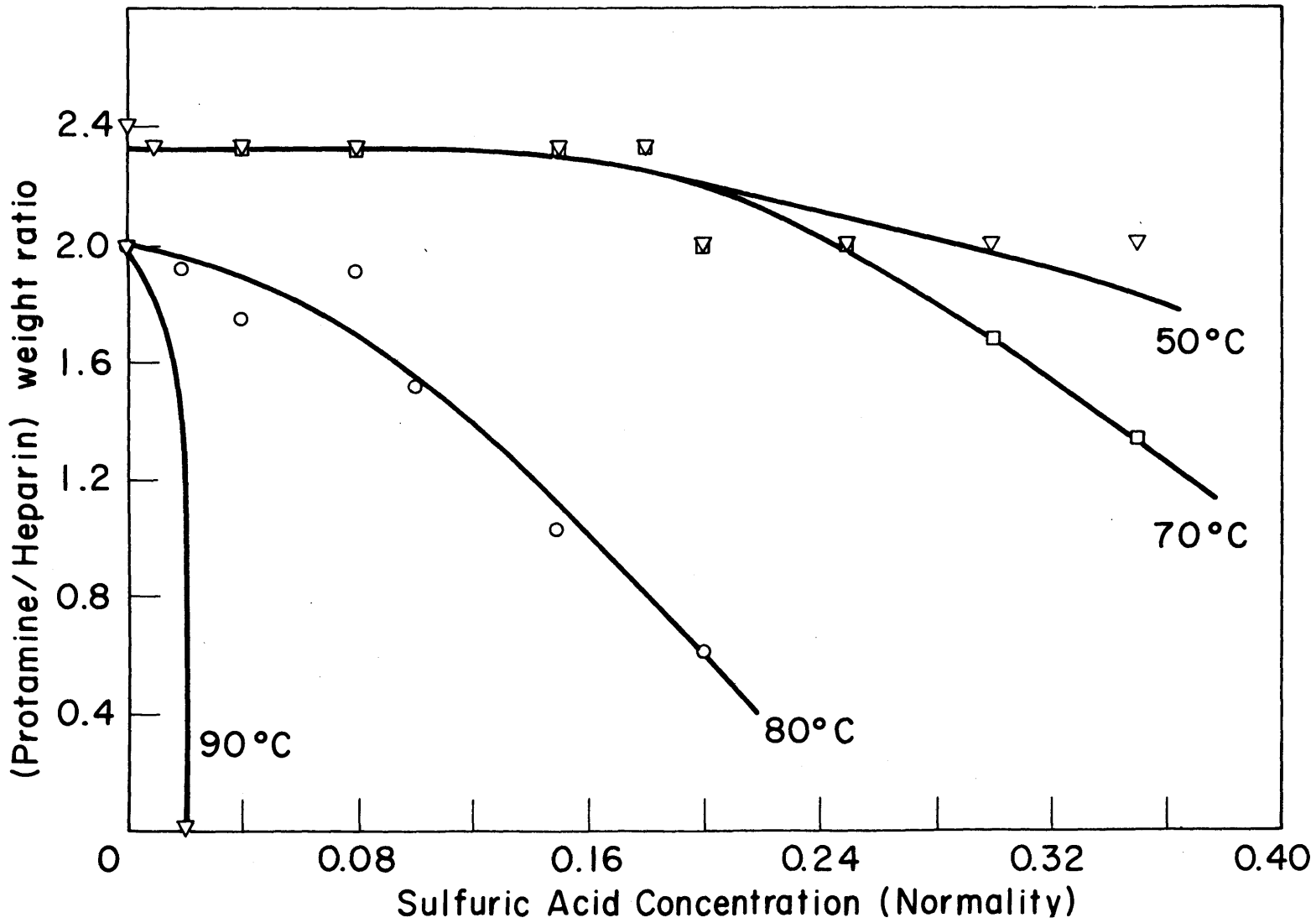


FIGURE 3-1. INACTIVATION OF HEPARIN FOR ONE HOUR EXPOSURES.

In the discussion of the general acetal reaction (Section 3-I-A) it was noted that acetal reactions are usually equilibrium-limited and that forcing conditions for removal of water are often employed. Unfortunately the solubility properties of heparin prevent the addition of any significant amount of miscible, nonaqueous solvents such as alcohols, methyl acetate, or acetone.

Besides the limitations imposed on the reaction conditions by the necessity for preserving heparin activity, experimental realities also preclude the synthesis of certain compositions. This will be noted in the experimental section.

E. SOLUTION PROPERTIES OF POLYVINYL ALCOHOL

Polyvinyl alcohol, the "backbone" of the hydrogel matrix, exhibits some curious behavior in aqueous solution. On the basis of viscosity data Dieu (75) assigned a theta temperature[1] of 97°C to the PVA-H_2O system and negative values for Flory's thermodynamic solution parameters κ_1 and ψ_1 (76). Upon heating from room temperature the intramolecular expansion factor α decreases toward unity and the osmotic second virial coefficient toward zero. These results were confirmed by the work of Matsuo (77).

In the recent literature Tager et al. (78) have claimed the existence of both a LCMT and a UCMT. Their data indicate

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1. This is a lower critical mixing temperature (LCMT). Phase separation occurs when the temperature is increased to the LCMT. The term "theta temperature" as defined in Flory (76) is usually applied to a system with an upper critical mixing temperature (UCMT), i.e., phase separation occurs as the solution is cooled to the UCMT. See reference (82).

that the LCMT is approximately 240°C in contradiction with Dieu's value of 97°C, but their identification of a UCMT at higher PVA concentrations appears to be a misinterpretation of the onset of gelation due to the formation of a semicrystalline structure (79). It is interesting to note that their data on the variation of intrinsic viscosity and second virial coefficient with temperature do indicate the same trends as Dieu (75) and Matsuo (77).

The recent paper of Klenina et al. (80) clearly demonstrates the complexity of the PVA-H_2O system and possibly indicates the source of some of the discrepancies in the literature. Klenina presents a diagram showing the change in supermolecular order in PVA-H_2O solutions (Figure 3-2). He identifies four regions:

1. A region where visual solution of the PVA does not occur;
2. A region where visual solution occurs, supermolecular order at a colloidal degree of dispersion being present in the system, the level of this order progressively increasing as the temperature increases. The upper boundary of region 2 is found from the point corresponding to the start of the melting process in the main part of the supermolecular formations; as a rule, the start of the melting corresponds to the maximum value of turbidity at each concentration of the solution;
3. Supermolecular order in region 3 breaks down to

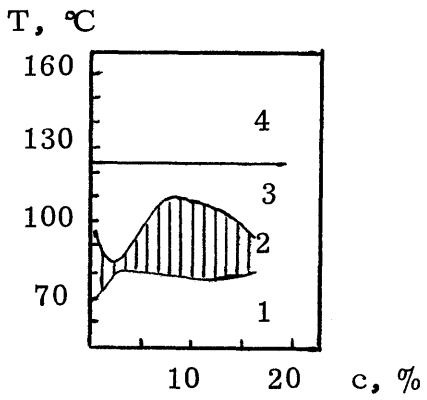

Figure 3-2. Diagram Showing the Change in Supermolecular Order in Aqueous Solutions of Polyvinyl Alcohol. From Reference (80).

its minimum level characteristic of each concentration which corresponds approximately to the line $T = 125°C$;

4. The region above 125°C. No data was taken in this region.

Fuji (81) has suggested that many of the properties of PVA, both as a solid and in solution, are highly dependent on the stereoregularity of the polymer chains. These are determined by the conditions of polymerization and the starting monomer. In addition, small variations in the amount of residual acetate on the PVA chain could significantly affect the behavior.

It appears that any interpretation of the PVA-H_2O phase behavior must contend with several competing phenomena. Classical polymer characterization techniques such as light scattering, osmometry, and intrinsic viscosity are dilute solution[1] measurements, and these are the basis of the data of Dieu, Matsuo, and Tager. For a polymer (PVA) that can hydrogen bond with a solvent (H_2O) it is expected that lower temperatures will favor more interaction and hence an expanded coil. As temperature rises, the interaction is less favorable and the coil in dilute solution shrinks, which is what the data of

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1. A dilute polymer solution is one in which the expanded polymer coils are separated from one another by solvent. The upper limit of a dilute solution is the concentration where the coils just touch, and above this limit the solution is termed concentrated. Since the size of a polymer coil in solution is a function of its molecular weight, one can only approximately estimate this limit. The result is in the range of 0.6% PVA in water. See Appendix C.

Dieu, Matsuo and Tager indicate.

For concentrated PVA solutions the existence of supermolecular order in the solution[1] is well documented (80,83,84). Concentrated PVA-H_2O systems appear to be metastable below $T \cong 125°C$ and with sufficient time will form gel particles (Klenina's supermolecular order) of equal refractive index in the mixture. It has been suggested that these gel particles are of a paracrystalline nature (86). As temperature is increased, the crystalline regions slowly melt which permits a swelling of the particle in the mixture, hence the increase in the size of particles noted by Klenina (80).

Further clarification of the PVA-H_2O phase behavior is needed. The importance of PVA-H_2O phase behavior to this work can be stated in two general categories.

1. Determination of the Molecular Weight of Polyvinyl Alcohol

PVA is soluble in only a limited number of solvents, and water is the only one for which data using the classical methods of polymer characterization are reported in the literature. Klenina's data indicate that method of preparing the aqueous PVA solution could influence the results. In addition, differences in residual acetate content and in stereoregularity could affect the data. Even dilute solutions of PVA will show some phase separation upon aging at room temperature (87). Indeed, the discordant results given by five different Mark-

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1. "Solution" is used loosely here since the supermolecular order could really be a separate phase (85).

Houwink relationships for PVA in water at 25°C (75,88,89,90,91) demonstrate the problems cited above. For this work the correlation by Beresniewicz (90) gave results that were most in line with data supplied by the manufacturer.

2. Interpretation of Experimental Results on Various Hydrogels Synthesized for Study

The preferred composition for hydrogels described earlier resulted from experimental efforts to prepare a homogeneous, heparinized hydrogel with adequate physical properties for *in vivo* blood compatibility evaluation. Since the composition had shown reasonable blood compatibility, it is the most feasible point to begin the work of this thesis. However, it is clear that any component in the reaction could have several effects.

a. It is directly involved in the chemical reactions;

b. It has affected the equilibrium of the reactions;

c. It has affected the complex phase behavior of the PVA-H_2O system.

These various factors must be considered in analyzing experimental results.

CHAPTER 3

THEORETICAL BACKGROUND

SECTION II. RUBBER ELASTICITY THEORY

A. DEFINITION OF NETWORK PARAMETERS

It will be helpful at this point to define the parameters normally used in the discussion of a polymer network.

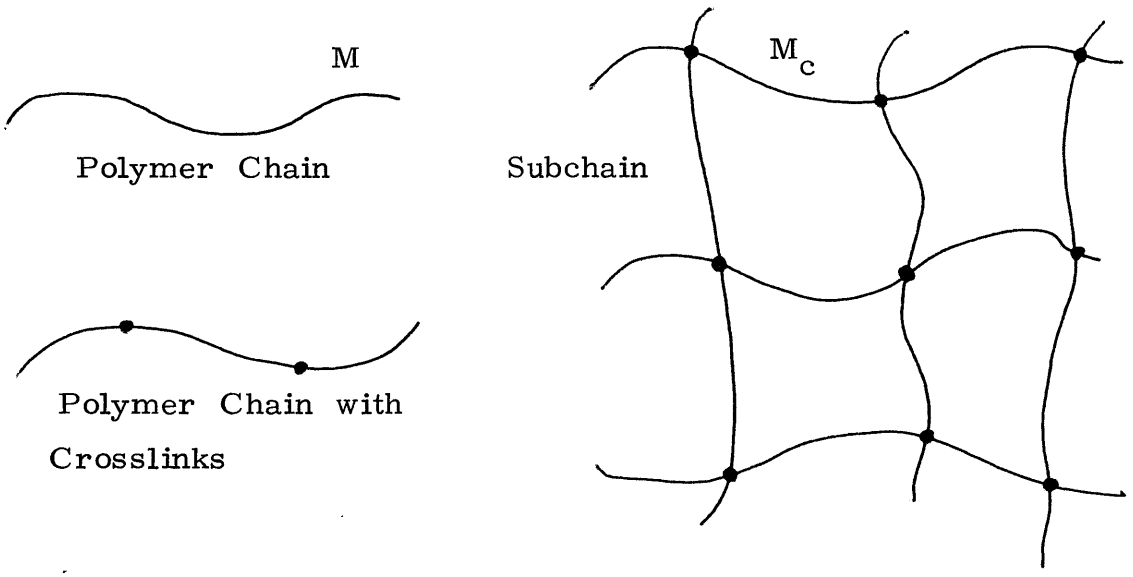

Figure 3-3. Idealized Network.

The polymer network is comprised of N polymer chains of molecular weight M before cross-linking. Although illustrated here as rather extended chains, the real polymer coils follow a very tortuous and winding path throughout the bulk material. In the cross-linked network there are $\nu/2$ cross-linked points or a total of ν cross-linked subunits of molecular weight M_c, if each cross-link is taken as a tetrafunctional crossover. Since the theories are developed in terms of the number of subunits cross-linked, rather than in the number of cross-link-

ages, the terms chosen are immediately applicable to networks of other functionalities. Because the network is formed from chains of finite molecular weight, it is clear that the network will contain some number of subunits cross-linked at only one end. These subunits are inactive in deformations of the network and appropriate network corrections are employed to relate ν_e, the effective number of subunits determined by an appropriate experiment, to ν, the total number of subunits.

B. STUDY OF HYDROGEL NETWORKS

In the study of the hydrogel networks there are principally two questions to be answered.

1. What are the physical properties of the material as a function of the various components in the reaction mixture?
2. Are the various components contributing to cross-linking of the network, to modification of the polymer backbone, or are they affecting the course of the reaction in some other manner?

These questions can be studied by direct experiment and by appropriate theoretical analysis of experimental data to give ν_e and the analogous parameter M_c as a function of reaction conditions.

Neilson (92) has discussed the various methods used in the study of cross-linked polymer networks. For highly swollen materials such as the hydrogels, the applicable methods are the swelling experiment and the tensile experiment.

1. Swelling Theory

When a cross-linked polymer is immersed in a liquid, which in the absence of cross-links would be a solvent, the gel swells until the osmotic forces tending to swell the network are just balanced by the elastic forces of the network. The free energy change ΔF involved in the mixing of pure solvent with the initially pure, amorphous, unstrained network is conveniently considered to consist of two parts: the ordinary free energy of mixing ΔF_M, and the elastic free energy ΔF_{el} consequential to the expansion of the network structure, which may be written:

$$\Delta F = \Delta F_M + \Delta F_{el} \tag{3-7}$$

A suitable expression for ΔF_M may be obtained from the Flory-Huggins theory of polymer solutions (76), bearing in mind that the number of polymer molecules is taken as zero, since no individual polymer molecules exist in the structure. The rubber elasticity theory, based on an assumption of no internal energy change on swelling except as accounted for in ΔF_M, provides the substitution for ΔF_{el} (76). The final equation is:

$$-[\ln(1-v_2) + v_2 + \chi_1 v_2^2] = V_1\left(\frac{\nu_e}{V_o}\right)\left(v_2^{1/3} - \frac{v_2}{2}\right) \tag{3-8}$$

where v_2 represents the equilibrium volume fraction of polymer, V_1 is solvent molar volume, and (ν_e/V_o) is the number of effective subchains per unit unswollen volume.

Unfortunately, the calculation of (ν_e/V_o) from the swelling experiment is rather sensitive to the value of Flory's

Chi Factor, χ_1, which is a parameter characterizing the interaction of a given polymer and solvent at a given temperature. It can be calculated from values of the second virial coefficient as measured by osmometry or light scattering in dilute solution. Equilibrium vapor pressures of polymer-solvent solutions can also be used to determine values of χ_1.

For a system with the complex phase behavior of PVA, any experimental technique to measure χ_1 will be plagued with the difficulties discussed earlier (Section 3-I-E). Extrapolation of a χ_1 value from dilute solution measurements to a concentrated PVA-H_2O mixture are dubious. The tacticity of the PVA molecule could be expected to affect the value of χ_1. Literature values of χ_1 for PVA-H_2O solutions (93,94) are in disagreement, probably for the reasons cited above.

There is an additional problem in the application of swelling theory to analysis of the hydrogel material, again associated with the χ_1 factor. During the course of the reaction both acetal cross-linking and acetalization of the polymer backbone are occurring. What was initially PVA will be a modified polymer with vinyl alcohol units, formal units, and some grafted heparin. The χ-factor would thus vary with each reaction composition, making analysis of the hydrogels by this technique a hopeless quandary.

2. Tensile Experiments

Fortunately, calculation of ν_e/V_o and M_c from elastic modulus theory does not require a knowledge of thermodynamic parameters for the system. Since the network hydrogels do

exhibit characteristic rubber-like elasticity, the application of elastic modulus theory is a viable alternative. In the following sections the modulus theory for application to a swollen hydrogel system will be developed.

C. RUBBER ELASTICITY THEORY

To study a cross-linked elastomer by means of its rubber-like elastic behavior, one performs a standard tensile experiment. A sample of the material is cut, usually in a dumbbell shape, and the force-elongation behavior is measured on appropriate apparatus, such as an Instron Universal Test Machine. The parameters (ν_e/V) and M_c can be determined from the force-elongation curve at low extensions.

The first theory giving a direct and logical explanation of the behavior of cross-linked elastomers in terms of molecular structure was suggested in 1932 (95). Further refinements of this theory (96-102) have produced quantitative relationships among the elastic properties of cross-linked high polymers for any kind of deformation. Experimental results on various polymer systems have indicated that, as a result of recent refinements (102), the theory represents the experimental behavior to a satisfactory degree.

Appendix A presents the derivation of the theory of rubber elasticity for isothermal deformations of the sample. The following assumptions are important in the development of the theory:

1. The cross-links are introduced into an amorphous polymer unswollen by any diluents.

2. The ν subunits of the network are long enough, the cross-linking degree not excessive, and volume effects negligible in order to assure the validity of the Gaussian equation for the conformations of the subunits.
3. The deformation of the chains must be affine to the deformation of the sample.
4. The deformation of the sample occurs at constant volume.
5. The contributions made by the effective subunits to the total retractive force must be additive.
6. The deformation of the sample is a completely reversible process in the thermodynamic sense.

A corollary of assumption 2 is that the theory is restricted to low deformations (i.e., $\alpha = L/L_o < 3$). Gaussian statistics do not apply if stress crystallization occurs during deformation nor do they describe the subunit conformations at large deformations. Langevin statistics can be used to predict elastic behavior at larger deformations (103).

With these assumptions and restrictions the following equation for unidirectional elongation of the sample at constant temperature may be derived (Appendix A):

$$\tau = \frac{f}{A_o} = RT \left(\frac{\nu_e}{V}\right) \left(\frac{\overline{r_i^2}}{\overline{r_o^2}}\right) \left(\alpha - \frac{1}{\alpha^2}\right) \qquad (3\text{-}9)$$

Stress τ in force per unit initial cross-sectional area is a

function of $(\alpha - 1/\alpha^2)$ with $\alpha = L/L_o$, the elongation in the x-direction relative to a gauge length L_o. (ν_e/V) is the number of effective subunits expressed in moles per unit sample volume.

The parameter $(\overline{r_i^2}/\overline{r_o^2})$ enters the derivation in the development of the expression (eqn. A-21) for the Helmholtz free energy of deformation from the statistics of a single chain and is frequently called the "front factor." The term $\overline{r_i^2}$ is the mean square end-to-end distance for the network subunits[1] in the undeformed isotropic state, and $\overline{r_o^2}$ is the corresponding mean-square end-to-end distance for the undeformed subunits in the absence of cross-links. In general, the two quantities will not be equal. In fact, $\overline{r_i^2}$ depends upon the volume of the network while $\overline{r_o^2}$, which is characteristic of the polymer, depends upon bond lengths, bond angles, potential hindering rotations, and, accordingly, upon temperature. Earlier theories such as those of Kuhn (96), Wall (97), Flory and Rehner (99), Treloar (100), and Hermans (101) utilized the constant internal energy assumption (i.e., $(\partial E/\partial L)_{T,V} = 0$) and differ from the result presented in equation (3-9) only by the absence of the front factor, which was considered equal to one.

D. NETWORK STRUCTURE AND DEFECTS

In the literature experimental results are frequently reported in terms of M_c, the molecular weight of a subunit, rather than as the analogous parameter (ν_e/V). These parameters

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1. The network contains (ν_e/V) effective subunits per unit sample volume and each subunit has a characteristic molecular weight M_c. Refer to Figure 3-3.

may be related by the following definitions. With reference to Figure 3-3 let M_c be the number average molecular weight of the subchains[1] between cross-links and $\bar{v} = 1/\rho$ be the specific volume of the polymer (amorphous density ρ). The total number of subchains expressed in moles is

$$\nu = \frac{V}{\bar{v}M_c} \tag{3-10}$$

where V is the sample volume.

As was discussed in Section 3-II-A and in Appendix A, it is necessary to distinguish between ν_e, the number of subchains cross-linked at two points and hence actively contributing to the retractive force, and ν, the total number of subchains as defined above. Suppose that the material consisted originally of N individual polymer chains of molecular weight M ($\bar{M}_n$, number average molecular weight, for a polydisperse polymer). The minimum number of cross-links which would be required to convert this material into a continuous network is N, i.e., one cross-link per molecule. The chemical reactions which link the pairs of chains in the unstrained and entangled mass occur at random. Thus the cross-links are not shared in any regular way between the chains and, when N cross-links have been introduced, many chains will remain unconnected to the network. Generally, cross-linking in excess of 3N (104) is sufficient to assure that nearly all of the original macromolecules are tied to the network.

Two ways in which sections of chains can be connected to

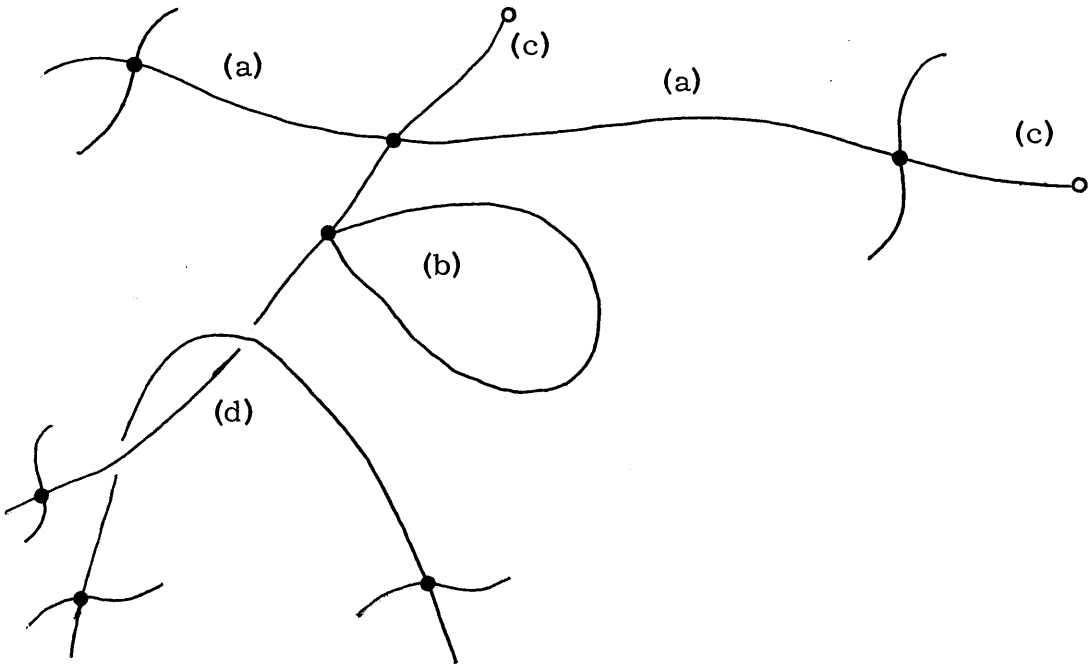
---

1. Subchains of the network are equivalent to subunits in the statistical theory.

the network at only a single point are shown in Figure 3-4. The existence of loose ends (designated (c)) remaining from the original polymer molecules is an inevitable feature of all networks formed from polymers of finite molecular weight. There are 2N such loose ends, and a quantitative allowance can be made for their presence (105). Short loops such as (b) are wasted except insofar as the loop may be involved in entanglements not otherwise operative. The proportion of these short path cross-linkages cannot be quantitatively discounted, but their number should be small unless the cross-linking is carried out in a dilute polymer solution, a case of no practical interest.

Entanglements such as (d) become permanent only in the presence of definite cross-linkages. Linear polymer chains of high molecular weight normally are almost hopelessly entangled with their neighbors. This type of entanglement restricts the movement of the chains to a lesser extent than the introduction of an extra cross-link, but a number of entanglements must increase the effective number of junction points and hence the tension for a particular value of the extension. Bueche (106) has suggested an empirical correction for entanglements involving an extrapolation of the modulus to zero cross-links, but no one has presented a theoretical treatment of the problem.

If it is assumed that there will be no completely unconnected chains in the final network, then at least one cross-link will occur somewhere along the length of the original molecules. Thus the first (N-1) cross-links may be imagined



- • cross-links
- o chain ends

(a) active subchains

(b) inactive loops

(c) inactive loose ends

(d) entanglements

FIGURE 3-4. Network Structures (from reference 103).

to be introduced at the rate of one per chain so that the N molecules are just connected into a continuous system. It will not, however, be a network and it may dissipate all orientations imposed by a deformation if given sufficient time for rearrangement of the system. The remaining $[\nu/2 - (N-1)]$ cross-links can now be introduced at random, each additional one imparting one closed circuit and hence two active subchains to the structure.

Hence taking $N >> 1$,

$$\nu_e = \nu - 2N \tag{3-11}$$

Now N may be expressed in moles as

$$N = \frac{V}{\bar{\nu} M} \tag{3-12}$$

Combining Equations (3-10), (3-11), and (3-12) gives the network correction

$$\nu_e = \nu\left(1 - \frac{2M_c}{M}\right) \tag{3-13}$$

or

$$\frac{\nu_e}{V} = \frac{1}{\bar{\nu} M_c}\left(1 - \frac{2M_c}{M}\right) \tag{3-14}$$

Substitution into Equation (3-9) gives the following relation for stress τ as a function of $(\alpha - 1/\alpha^2)$:

$$\tau = \frac{RT}{\bar{\nu} M_c}\left(\frac{\overline{r_i^2}}{\overline{r_o^2}}\right)\left(1 - \frac{2M_c}{M}\right)\left(\alpha - \frac{1}{\alpha^2}\right) \tag{3-15}$$

E. EXPERIMENTAL VERIFICATION OF THE THEORY OF RUBBER ELASTICITY

Considerable research activity has been centered on the problem of the dependence of the force on elongation at con-

stant temperature. Certain experimentally observed deviations from the theoretical predictions were unexplained and cast serious doubt on the correctness of the theory (107). Cifferi and Flory (108) indicate that the deviations arise from the difficulty of performing the force-elongation measurements under true equilibrium conditions, which is a principal assumption in the development of the theory (Appendix A).

From Equation (3-15) for a given extent of cross-linking (M_c) the value of $[\tau/(\alpha - 1/\alpha^2)]$ should be constant. Gumbrell, Mullins, and Rivlin (109) illustrate the kind of deviations observed in their experimental results on samples of natural rubber. Instead of $[\tau/(\alpha - 1/\alpha^2)]$ equal to a constant, they found that their data fit an equation, originally proposed by Mooney (110), having the form:

$$[\tau/(\alpha - 1/\alpha^2)] = 2C_1 + 2C_2\ (1/\alpha) \qquad (3\text{-}16)$$

where C_1 and C_2 are constants. The constant C_1 was shown to increase with the degree of cross-linking, as predicted by the rubber elasticity theory, while the constant C_2 was shown to decrease when the sample was swollen with solvent. On this basis it was suggested that the theory was valid for highly plasticized elastomers.

Performing experiments on natural rubber and polydimethylsiloxane similar to those of Gumbrell et al. (109), Ciferri and Flory (108) found that the curves of $[\tau/(\alpha - 1/\alpha^2)]$ versus $1/\alpha$ obtained during elongation differed from those obtained during retraction from the maximum elongation for the same sample. Furthermore, the slope of the curves obtained during

the elongation, identified with the parameter $2C_2$, decreases with the hysteresis for various polymers all having the same degree of cross-linking. A similar and even more striking decrease of C_2 with the decrease of the hysteresis was observed when the same sample was plasticized with a solvent, or when the temperature or the time during which the sample was maintained at each elongation was increased. Dynamic experiments reported by Ferry (111) also support the view that the C_2 term is associated with time-dependent effects arising from the failure to obtain true mechanical equilibrium. These results are interpreted to mean that under ideal conditions, i.e., equilibrium between the tensile and retractive force at each elongation, the relationship between force and elongation is correctly represented by the theoretical equation (112,103).

F. EXPERIMENTAL STUDIES ON THE EFFECT OF MOLECULAR WEIGHT

There are two aspects of molecular weight which enter into the stress-strain equation (3-15). The first of these is concerned with the network imperfections which are taken into account by the correction factor $(1-2M_c/M)$ as discussed in Section 3-II-D). Flory (104) has studied the problem by preparing a set of samples with constant M_c but varying M. This was accomplished by copolymerizing isobutylene with small but variable amounts of isoprene, which gave copolymers containing different amounts of isoprene units distributed randomly along the chains and available for vulcanization. After fractionation of the polydisperse copolymers, their molecular weight was determined. Samples were then vulcanized and the elastic

retractive force measured.

Theory (eqn. 3-15) predicts that at constant T, α, and M_c a plot of stress against 1/M should be linear. Flory's data (104) showed that for each degree of unsaturation in the uncross-linked polymer, and hence for constant M_c, the stresses gave good straight lines when plotted as a function of (1/M). In addition, the values of M_c for each group of samples, obtained from the slope of the plot of stress versus (1/M), agreed well with values of M_c predicted from the theory of gelation. In view of the many experimental difficulties involved the results are not sufficiently precise to allow a clear differentiation between Flory's form of the correction factor $(1-2M_c/M)$ and other modifications such as the one proposed by Scanlan (113).

The second important effect of molecular weight is the dependence of the modulus on M_c or on the equivalent parameter ν_e. In order to verify experimentally the theory's prediction of the dependence of the modulus on the degree of cross-linking, the number of effective cross-links must be known with precision and, as discussed in Section (3-II-E), the modulus must be determined under equilibrium conditions.

Flory, Rabjohn, and Schaffer (114) have taken advantage of the quantitative nature of the cross-linking reaction of disazodicarboxylates to prepare a series of samples of natural rubber and GR-S (butadiene-styrene) rubber with known values of M_c from linear polymers of constant M. Their results showed that the observed and predicted tensions were only in rough agreement as to magnitude. At high degrees of cross-linking

the stress was less than predicted, while at lower extents of cross-linking the stress was higher than predicted. In addition, the stress did not extrapolate to zero at zero degree of cross-linking.

Mullins (115) performed a similar series of experiments using di-tertbutyl peroxide to cross-link samples of natural rubber. As a result of stress-strain measurements on these samples, swollen to assist the attainment of equilibrium, it appeared that a plot of stress against $1/M_c$ was linear, somewhat steeper than predicted by theory (eqn. 3-15), and extrapolated to a finite value at zero cross-linking. Moore and Watson (116) compared the experimental value of the modulus determined by Mullins (115) with that calculated on the basis of an independent chemical estimation of the number of cross-links. Their results also indicated that the measured modulus was consistently greater than the one calculated according to the theory. The discrepancy with the theory has been attributed to the effect of chain entanglements which, by restricting the chain motions, function as additional cross-links.

Yet a different result was found by Bueche (106) who studied polydimethylsiloxane crossed by high energy electrons. Estimating the degree of cross-linking in his samples by a calibration of the effect which the radiation induced on low molecular weight silicones, Bueche found that the modulus from stress-strain measurements was only one-half of that predicted by Equation (3-15). This result agrees with the prediction of the theory of James and Guth (117).

Certainly, an exact chemical estimation of the number of active cross-links is extremely difficult when one considers the myriad of possible reactions occurring. Also, the complexity involved in the detailed theoretical treatment of a real network with entanglements, closed loops, and other structures further complicates a quantitative interpretation of these experimental results. Apart from the problem of experimentally verifying this quantitative dependence of modulus on the degree of cross-linking, the theory has met with considerable success in predicting the behavior of elastic networks for isothermal deformations.

G. MODIFICATIONS OF RUBBER ELASTICITY THEORY FOR APPLICATION TO PVA HYDROGELS

To apply the theory of rubber elasticity to a study of the polyvinyl alcohol hydrogel system, there are two important modifications which must be made. For one, the parameter ν, the total number of subchains in the network defined by Equation (3-10), and the parameter N, the number of polymer chains present before cross-linking (eqn. 3-12), are defined in the conventional theory, assuming that the cross-linking is carried out in bulk amorphous polymer. Clearly, this is not the case for the hydrogels synthesized from reaction mixtures containing from 6-10% PVA by weight. The definitions are appropriately modified by the inclusion of the term $v_{2,i}$, representing the volume fraction of polymer (cc polymer/cc solution) at the cross-linking volume denoted by subscript i. Hence,

$$\nu = \frac{V_i}{\bar{v}M_c} v_{2,i} \qquad (3\text{-}17)$$

$$N = \frac{V_i}{\bar{v}M} v_{2,i} \tag{3-18}$$

By use of the expression from Flory (76)

$$v_{2,i} = \bar{v} C_{2,i} \tag{3-19}$$

the definitions may be written in terms of $C_{2,i}$, the concentration of polymer (g/cc) in the reaction volume:

$$\nu = \frac{V_i}{M_c} C_{2,i} \tag{3-20}$$

$$N = \frac{V_i}{M} C_{2,i} \tag{3-21}$$

The meaning of the reaction volume denoted V_i deserves some comment. During the course of a hydrogel synthesis both cross-linking and chain modification are occurring. As might be predicted from the fact that the polymer polyvinyl formal is completely insoluble in water, the conversion of a small portion of the PVA backbone to cyclic formal makes it less hydrophilic and hence the structure tends to contract during the synthesis reaction. Since the acetal reaction is reversible in acid media, it is assumed in this work that the system volume at the end of the reaction time (usually one hour) represents the relaxed state of the sample. An equivalent statement is that the cross-links are unstressed at volume V_i.

Another important consideration is the validity of the statistical treatment for cross-linking in a system swollen with solvent. From literature values of unperturbed polymer coil dimensions (118), it is possible to estimate the concentration of polymer at which the polymer coils just touch one another ($C_{2,critical}$). As shown in Appendix C, this value for

PVA in water is 0.006 g/cc, or approximately 0.6 wt %. Since the concentrations of interest in this work are tenfold greater than $C_{2,critical}$, an equal segment density distribution, as discussed in Flory (76), may be safely assumed. This means there is an equal probability of finding polymer segments in all volume elements of the system, and thus the assumption of uniform random cross-linking should be valid.

In this work the network correction factor proposed by Flory (105) will be employed (eqn. 3-11). Combining Equations (3-20), (3-21), and (3-11), it can be seen that the form of the network correction is identical to the bulk polymer case.

$$\frac{\nu_e}{V} = \frac{C_{2,i}}{M_c}\left(1 - \frac{2M_c}{M}\right) \qquad (3\text{-}21)$$

Comparison of Equation (3-21) above with Equation (3-14) for the bulk polymer shows that they differ only by the replacement of $1/\bar{v}(=\rho_2)$ with $C_{2,i}$.

While the possibility exists for more network imperfections of the type not discounted by the theory (as discussed in Section (3-II-D)) when the cross-linking is carried out in the presence of solvent, no theoretical basis exists for their evaluation. Consistent application of the Flory correction factor should permit valid comparisons among various hydrogel compositions, though the absolute values of M_c may be somewhat in error.

Figure 3-5 illustrates the need for the second important modification of the rubber elasticity theory for application to the PVA hydrogel system. Buffered saline (Appendix D) is used

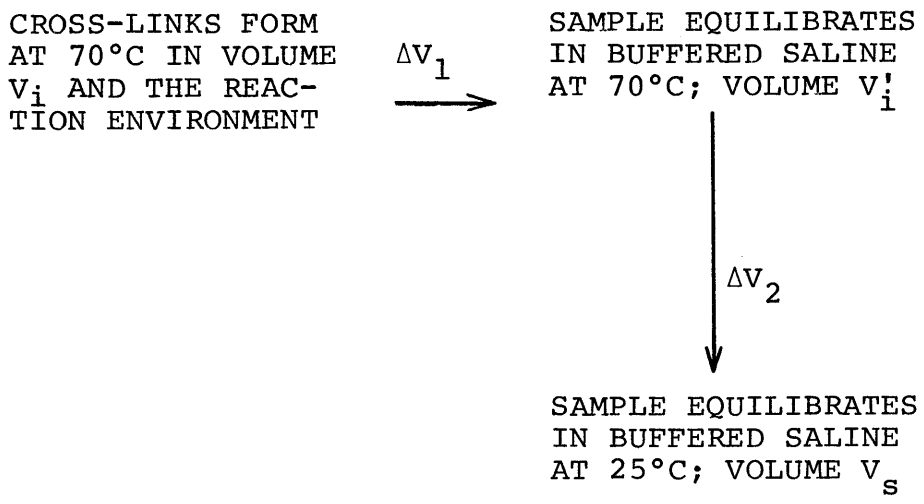


FIGURE 3-5. Volume Changes of Hydrogel Sample

for all equilibrations because of its physiological significance (3) and because of the inherent stability of the acetal bond in neutral or basic media. At the end of the reaction time the cross-links are in a relaxed state at volume V_i, the reaction temperature (70°C), and the reaction environment characterized by the presence of aldehydes, acids, and water more or less as specified in the synthesis composition (Section 3-I-C). Volume change ΔV_1 corresponds to a change of volume of the hydrogel sample on equilibration with buffered saline at 70°C, and ΔV_2 represents the volume change of the sample associated with equilibration in buffered saline at room temperature where the force-elongation data is taken.

Experimentally it is not possible to determine the exact value of ΔV_1, but in the work of Lee (119) it was demonstrated to be at most 10% relative to V_i', which is insignificant compared to ΔV_2 as will be shown in the results. For all intents and purposes a negligible approximation is involved in letting

$V_i = V_i'$. However, the isotropic deformation introduced into the network by the volume change ΔV_2 associated with equilibration of the sample in room temperature buffered saline must be taken into account to correctly evaluate stress-strain data for M_c or (ν_e/V_i).

Appendix B presents the derivation of the correction for swelling. The earlier analysis (Appendix A) must be modified since the sample is now deformed by both isotropic swelling and unidirectional elongation. By introduction of a swelling factor $\delta = V_i/V_s$, where V_i is the reaction volume and V_s is the swollen volume, the final expression can be used to analyze force-elongation data obtained on the swollen samples for M_c and (ν_e/V). In terms of (ν_e/V_i), moles of effective subchains per unit reaction volume, the equation for stress expressed as force per unit area of the swollen, unstretched sample reads:

$$\frac{f}{A_{o,s}} = \tau = RT \left(\frac{\nu_e}{V_i}\right) \delta^{1/3} \left[\frac{\overline{r_i^2}}{\overline{r_o^2}}\right]_o \left(\alpha - \frac{1}{\alpha^2}\right) \qquad (3\text{-}22)$$

The parameter $\alpha = L_s/L_{o,s}$, both lengths being measured in the swollen condition. Similarly, the moles of effective subchains per unit swollen volume can be calculated as:

$$\tau = RT\left(\frac{\nu_e}{V_s}\right) \delta^{-2/3} \left[\frac{\overline{r_i^2}}{\overline{r_o^2}}\right]_o \left(\alpha - \frac{1}{\alpha^2}\right) \qquad (3\text{-}23)$$

Finally, by use of Equation (3-21) the results can be expressed in terms of M_c as:

$$\tau = RT \frac{C_{2,i}}{M_c} \delta^{1/3} \left[\frac{\overline{r_i^2}}{\overline{r_o^2}}\right]_o \left(1 - \frac{2M_c}{M}\right)\left(\alpha - \frac{1}{\alpha^2}\right) \qquad (3\text{-}24)$$

The subscript o on the parameter $(\overline{r_i^2}/\overline{r_o^2})_o$ denotes the value of the parameter at the temperature of interest in the absence of additional diluent. For loosely cross-linked networks, which is the case in this study, the parameter may be taken equal to one with negligible error (120).

H. EXPERIMENTAL CONSIDERATIONS IN APPLICATION OF THE THEORY

In the application of this theory to the study of a swollen cross-linked network, one must remain cognizant of those factors which would cause non-Gaussian behavior of the subchains and thus violate one of the basic assumptions of the theoretical development. Among these are finite extensibility of the chains, stress crystallization, and the presence of microcrystalline regions or inhomogeneities in the network structure. Meares (103) has presented a good discussion of these phenomena and attempts at theoretical treatment of the problems.

Mullins (121) has pointed out that swollen samples tend to reach the limit of finite extensibility at lower elongations then dry samples. In this work only small deformations are considered ($\alpha < 1.5$) so this should present no problem. This restriction to low elongations also benefits the constant volume deformation assumption. Stress crystallization normally occurs only at higher extensions and is unlikely in a highly swollen network.

Earlier work by Wong (69) has shown that reaction at higher

levels of acid and temperature produces grossly microporous material. Electron microscopy revealed "vacuoles" connected by dense material, presumed to be cyclic acetal. Such hydrogels cannot be studied by the theory presented, and in this work extreme reaction conditions such as would lead to inhomogeneous structures were avoided. Indeed, hydrogels of this type would have little value for application as hemodialysis membranes.

Microcrystallization of the PVA component of the gel as suggested in Section 3-I-E cannot be completely ruled out. Solutions of PVA and water containing 6 wt % have been observed to become turbid upon standing, suggesting a phase separation (possibly crystallization), but hydrogels of the same polymer content will maintain their clarity indefinitely on storage in buffered saline. It is to be supposed that the presence of cyclic acetals along the backbone of the polymer as well as grafted heparin in some compositions should reduce the level of crystallization, if any, as compared to a PVA solution of equal concentration.

CHAPTER 3

THEORETICAL BACKGROUND

SECTION III. MEMBRANE PERMEABILITY MEASUREMENT

Measurement of the transport properties of hemodialysis membranes has been the subject of an extensive theoretical and experimental study by Smith, Colton, Merrill, and others (9,122). In this work the interest is in the measurement of the permeabilities of hydrogel membranes for comparison with conventional cellulosic membranes for which an excellent data base exists (123,124). The objective in this section is to summarize the appropriate equations, principally from reference 9 that will be used in this work.

A. ANALYSIS OF EXPERIMENTAL DATA

Similar to the situation existing in a hemodialyzer, the experiment to measure the transport properties of a membrane may be visualized, as shown in Figure 3-6. In the absence of a hydrostatic pressure gradient, the transport of a solute from the side of high concentration 1 to the side of low concentration 2 occurs by a diffusive mechanism through the membrane of thickness Δx. Transport through the liquid boundary layers adjacent to the membrane is characterized by the transport coefficient k_f, and transport through the membrane is described by the membrane permeability P_m. Any accompanying volume flow or osmotic flow is assumed negligible, which appears justified for the condition of constant chamber volumes V_1 and V_2 and dilute solutions (9).

In terms of solute concentrations at the membrane surface

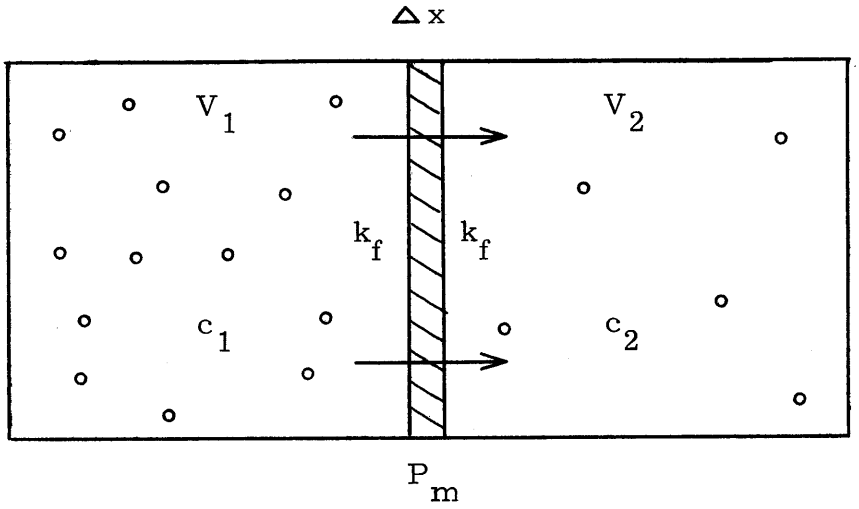


Figure 3-6. The Dialysis Experiment.

$C_{1,s}$ and $C_{2,s}$, the membrane permeability is defined as

$$\dot{N} = P_m A (C_{1,s} - C_{2,s}) \tag{3-25}$$

where A represents the membrane surface area and $\dot{N}$ the mass transfer rate. Experimentally the overall mass transfer coefficient K_o is measured, and this is given by:

$$\dot{N} = K_o A(C_1 - C_2) \tag{3-26}$$

where C_1 and C_2 are taken to be the well-mixed bulk concentration. A material balance may be written for each chamber of the dialysis cell:

$$\dot{N}_j = \frac{d(V_j C_j)}{dt} \tag{3-27}$$

Combining Equations (3-26) and (3-27) and integrating from t = 0 to t = t gives the following expression which may be used to analyze experimental data for K_o:

$$\ln \frac{(C_1 - C_2)_t}{(C_1 - C_2)_o} = -K_o \, At \, \left(\frac{1}{V_1} + \frac{1}{V_2}\right) \tag{3-28}$$

With an appropriate correlation to estimate k_f, the true membrane permeability is obtained by the following relation:

$$\frac{1}{P_m} = \frac{1}{K_o} - \frac{2}{k_f} \tag{3-29}$$

in which k_f is assumed equal on both sides of the membrane. For the case of larger solutes the liquid phase resistance is negligible relative to the membrane resistance, and the approximation $P_m \simeq K_o$ is used.

Experimentally, it is most accurate to follow concentration as a function of time and to evaluate K_o from the slope of plot of $\ln[(C_1-C_2)_t/(C_1-C_2)_o]$ as a function of t. For the lower molecular weight solutes studied on a batch dialyzer, discrete samples were withdrawn from one chamber of the dialyzer and were simultaneously replaced with isotonic saline. It is necessary to correct for this effect, and Equation (3-30) may be modified as shown in reference 9:

$$\sum_{m=0}^{m=n} \ln \frac{(C_1 - C_2)_{t_{m+1}}}{C_1 - C_2')_{t_m}} = -K_o A t_n \left(\frac{1}{V_1} + \frac{1}{V_2}\right) \tag{3-30}$$

where

$$C_{2,m}' = C_{2,m} - \frac{V_r}{V_2} C_{2,m}$$

$$C_{1,m+1} = C_{1,m} + \frac{V_2}{V_1} (C_{2,m}' - C_{2,m+1})$$

$C_{2,m}$ = concentration of m^{th} sample from chamber 2

V_r = volume of sample removed and replaced; solute concentration in V_r taken as zero

The mass transfer correlation used to estimate k_f was developed by Smith et al. (122).

B. SOLUTES FOR MEMBRANE EVALUATION

At the present time the total family of solutes requiring removal by an artificial kidney is not defined. A listing of possible test solutes for in vitro membrane evaluation appears in reference 29.

The solutes selected for study in this work are given in Table 3-3. Urea, creatinine, and uric acid are relatively low molecular weight compounds that are known to be of importance in the chronic uremia state. While the remaining solutes represent somewhat of an arbitrary set, they were chosen to span the middle molecular weight range of current interest and to study the "cut-off" point of the membranes to higher molecular weight species. Data on the permeability of these solutes with Cuprophane PT-150 (R-Bemberg), the commonly used hemodialysis membrane, is available in the literature and will be presented for comparison purposes in the results section.

Table 3-3 also lists estimates of the characteristic radius of each solute for permeation and the values of the diffusivity in water at 37°C for each solute. These values are taken from reference 9, and the reader is referred there for a complete discussion of the correlations used in their estimation.

TABLE 3-3. SOLUTES FOR MEMBRANE EVALUATION

Solute	Molecular Weight (g/gmole)	Solute Radius for Permeation (Å)	Diffusivity* in Water at 37°C (cm^2/sec)x10^5
Urea	60	2.5	1.81
Creatinine	113	3.1	1.29
Uric acid	168	3.3	1.16
Sucrose	342	4.7	0.697
Inulin	∿ 5,200	6.7	0.215
Dextran	∿ 16,000	10.1	0.130
PEG	∿ 4,000	16.3	0.210
Myoglobin	∿ 17,000	17.5	0.171
Albumin	∿ 66,000	22.5	0.0909

*Where applicable, values refer to infinite dilution.

CHAPTER 4

EXPERIMENTAL WORK

A. OVERVIEW

One approach to the development of a hemodialysis membrane from the hydrogel materials would be to synthesize a particular composition, measure the permeability characteristics, and then to speculate on possible improvements as required. In addition to being a very time-consuming and frequently frustrating process, little or no fundamental understanding of the reaction chemistry could be gained from such an approach. Indeed, the problems of reproducing a pinhole-free membrane sufficiently thin (i.e., 0.001-0.005 mils) to be a possible candidate for hemodialysis applications could well obscure the interpretation of any permeability data as a function of the reaction conditions for the membrane. It was for these reasons that this work concentrated on evaluating the hydrogels by means of the rubber elasticity theory. With these results in hand, the development of a hydrogel membrane for hemodialysis could be undertaken in a more fundamental manner than the trial-and-error technique of casting various hydrogel compositions for permeability evaluation.

Because of the complexity of the hydrogel reaction, the decision was made in this work to begin with the standard hydrogel composition (Table 3-1) and to synthesize various compositions, varying one variable at a time. While this is not the most efficient approach from the point of view of statistical design, it is the only way to proceed in a system where the re-

sponse as a function of the variables is unknown. Subsequent sections will present the various experimental procedures used in this work.

B. REAGENTS

All compositions studied in this work were prepared by careful weighing or dilution from more concentrated stock solutions. Typical stock solutions and their preparation are described below.

1. Sulfuric Acid

Stock sulfuric acid solutions of 2.00 N and 4.00 N were prepared by diluting commercially available standards. The stock bottles were checked by titration with 1.00 N NaOH using phenolphthalein as an indicator and were found to be accurate to ±0.1%.

2. Acetic Acid

The acetic acid stock was reagent grade glacial acetic acid (17.4 N) obtained from Mallinkrodt Chemical.

3. Formaldehyde

Reagent grade formalin solution (Merck and Co., Inc.) containing 36-38 wt % HCHO and about 12 wt % methanol as a stabilizer was used as the stock formaldehyde solution. For each stock bottle the actual formaldehyde concentration was determined by titration using the sodium sulfite procedure (Appendix F) and these results (generally 36.2-36.4 wt %) were used in calculating the necessary dilutions for the preparation of a particular reaction composition.

4. Glutaraldehyde

Biological grade glutaraldehyde (pentanedial) was

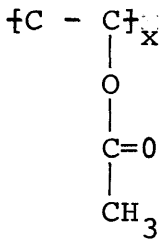
obtained as a 50.0 wt % aqueous concentrate from Fisher Scientific. Since the concentrated solution must be refrigerated to prevent polymerization, small quantities of 2-3 wt % aqueous solutions, which are stable at room temperature, were prepared as reagent stock by careful weighing of the concentrate into a volumetric flask followed by dilution with distilled water. The concentration of glutaraldehyde in the concentrated solution was verified by titration using the hydroxylamine hydrochloride procedure (Appendix F).

5. Heparin

Sodium heparin (B grade) with a biological activity of 160 USP units/mg was obtained from Calbiochem, Los Angeles. It was found to have a $\bar{M}_v$ = 13,800 (69) and was analyzed to contain 11.3% Na and 0.25% Fe (125). Using a heparin tetramer molecular weight without counter ions of 1069 g/gmole, this indicates six sodium ions per tetramer. Referring to the diagram of the heparin tetramer (Figure 2-4), it would appear that on the average one of the two carboxylic acid groups per tetramer must be in the sodium form.

6. Polyvinyl Alcohol

Polyvinyl alcohol is derived from polyvinyl acetate:



by hydrolysis that results in removal of the acetate groups and creation of secondary hydroxyl groups on the main polymer chain.

Since the number of network imperfections in the synthesized hydrogel at any given degree of cross-linking varies inversely with the molecular weight of the original polymer, the highest molecular weight grade commercially available--Du Pont Elvanol (R) 73-125G--was used in most of this work. A few hydrogels were also synthesized using a lower molecular weight material--Du Pont Elvanol (R) 71-30.

Qualitative spectrographic analysis of the Elvanol (R) 73-125G indicated that the only ion present in any significant amount was sodium (0.01-0.1 wt % dry basis)(125), probably from the surfactant used in the emulsion polymerization of the original polyvinyl acetate. Infrared spectrographic analysis of the polymer showed no indication of acetal, peroxide, or carboxylic acid and only a small amount (less than 0.1 wt % dry basis) of carbonyl (126). The degree of hydrolysis in mole percent is reported by the manufacturer to be 99.3-100% for Elvanol (R) 73-125G and 99.0-99.8% for Elvanol (R) 71-30.

The characterization of PVA in aqueous solution is indeed complicated by the phase behavior of the PVA-water system (Section 3-I-E). Working with only freshly-prepared, filtered solutions of PVA in water, Burke (87) obtained intrinsic viscosities at 25°C of [n] = 1.27 dl/g for Elvanol (R) 73-125G and [n] = 0.857 dl/g for Elvanol (R) 71-30. By use of the Mark-Houwink relation:

$$[n] = K' \bar{M}_v^a$$

with the constants being $K' = 5.95 \times 10^{-4}$ and $a = 0.63$ as reported by Beresniewicz (90), the viscosity average molecular

weight $\bar{M}_v$ is estimated to be 193,000 for Elvanol (R) 73-125G and $\bar{M}_v$ = 103,000 for Elvanol (R) 71-30. For the purpose of estimating $\bar{M}_n$ as required in elastic network theory, the value of 100,000 for Elvanol (R) 73-125G and 60,000 for Elvanol (R) 71-30 will be used in this work. These estimates are based on the assumption of a Gaussian distribution of molecular weights in the polymer sample,[1] which seems justified by the value $\bar{M}_w/\bar{M}_n$ = 2.0 reported by Wu (127).

Stock solutions of PVA in water were prepared in 1500g batches containing anywhere from 9-17 wt % PVA as desired. Generally, it was found most convenient to work with as low a polymer content in the stock as was feasible to prepare the desired reaction compositions. Approximate quantities of PVA and distilled water were weighed into a large volumetric flask and were heated overnight in a circulating air oven at 90-95°C, as suggested by the manufacturer to achieve complete solution. The material was then centrifuged at 1100 rpm for about 40 minutes in a Servall Refrigerated Centrifuge to separate out particulate contamination. After centrifugation the purified polymer solution was heated to reduce its viscosity and was well mixed with a mechanical stirrer to assure a homogeneous solution. This stock solution was then analyzed for the exact polymer content by a solids determination (Appendix G), and the results of the analysis were used in calculating the desired reaction compositions.

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1. See reference (76), p. 313.

C. PREPARATION OF THE CASTING SOLUTION

With the stock reagents described in Section 4-B, the exact amounts of each required to prepare a casting solution of a given composition could be calculated. Usually 50 grams of casting solution were used in the preparation of hydrogel samples for modulus evaluation and 75 grams were used in the preparation of dialysis membranes. The various compositions studied are summarized in Table 4-1. As emphasized earlier, one reactant was varied at a time and Table 4-1 records that variable, all others fixed at their control values. Five membranes of the control composition were prepared to test the reproducibility of the casting and evaluation techniques. Needless to say, quite a few samples were prepared while developing the experimental procedures.

The necessary amount of the PVA stock and heparin, when used, were weighed into a beaker and all other reagents were pipetted. As soon as the aldehydes are added to the acidified PVA solution, they begin to form hemiacetals with the secondary hydroxyls on the polymer backbone. With formaldehyde this presents no problem, since a formaldehyde hemiacetal is not a cross-link. Acidified PVA-formaldehyde solutions will stay fluid for hours at room temperature. However, with glutaraldehyde the formation of two hemiacetals represents a cross-link. Depending upon the concentrations of PVA and glutaraldehyde in the solution, gelation of the casting solution would occur at room temperature in a matter of five to twenty minutes after

TABLE 4-1. COMPOSITIONS STUDIED

Control Composition

	Concentration	Mole Ratio[b]
Elvanol (R) 73-125G	6.0 wt %	1.00
Formaldehyde	6.0 wt %	1.47
Methanol	ca. 2.0 wt %	ca. 0.50
Glutaraldehyde	0.076 wt %	0.00557
Sulfuric acid (H^+)	0.06 N	0.0440
Acetic acid	1.0 N	0.73

Variable	Concentration[a]	Mole Ratio[b]
Control	(5)[c]	(5)
Glutaraldehyde	0, 0.04%, 0.08%, 0.12%, 0.16%	0.00293, 0.00587, 0.00880, 0.0117
Formaldehyde[d]	0(2), 2.0%, 4.0%	0(2), 0.490, 0.980
Sulfuric acid	0.04N(2), 0.09N(2), 0.12N(2), 0.15N(2)	0.0293(2), 0.0660(2), 0.0880(2), 0.1100(2)
Acetic acid	0	0
Heparin	1.0%(3)	0.0303[f]
Reaction Time	90 min, 120 min	--
Elvanol(R)73-125G	8.0%, 10.0%	--
Elvanol(R)71-30[e]	6.0%, 13.0%	--
Elvanol 73-125G + Heparin	10.0% PVA, 1.0% heparin	--, 0.0303[f]

a. Concentrations in wt % except as noted.

b. Gmole Reactant/Gmole PVA mer.

c. Number in parentheses denotes number of specimens prepared if other than one.

d. Methanol concentration varied with formaldehyde concentration as determined by stock formalin (i.e., 3:1 formaldehyde to methanol weight ratio).

e. Used in place of Elvanol (R) 73-125G.

f. Mole ratio heparin hydroxyls to PVA mer.

addition of the glutaraldehyde. To allow adequate time for mixing the reactants and casting the sample, glutaraldehyde was not added until the other reagents had been homogeneously mixed.

For casting solutions containing 6 wt % PVA mixing could be done with a magnetic stirring apparatus. Higher polymer content solutions required the use of a motor-driven impeller. In all cases, care was taken to introduce as small an amount of air into the mixtures as possible, since this prolonged the degassing procedure.

After the addition and mixing of the glutaraldehyde into the casting solution, the beaker was quickly transferred to a glass dessicator and put under vacuum to remove the air bubbles. By carefully controlling the vacuum, the suspended air bubbles could be removed in a few minutes without causing the mixture to "boil over." A check on the weight of the contents of the beaker indicated a weight loss of less than 0.5% from this procedure, which was probably a small amount of water, methanol, or formaldehyde. This degassed solution was then ready to be cast.

D. MEMBRANE SYNTHESIS APPARATUS

Earlier work in the production of hydrogel samples in a flat configuration that would be suitable to provide test specimens for modulus evaluation or for permeability studies attempted to react the materials on glass plates in covered aluminum pans placed in a circulating air oven at the temperature of interest. This approach was undesirable in this work for

several reasons. Temperature control with the air oven was inaccurate (±3°C). Poor heat transfer to the reaction mixture resulted in a transient warm-up period of as long as 25 minutes, which was 40% of the total reaction time. Unavoidable evaporation of the reactants (probably formaldehyde, methanol, and water) during the reaction resulted in a concentrating of both the polymer and the acid catalyst. Hence the actual reaction conditions were controlled not by the initial reaction composition, but rather by the unpredictable evaporation process. A closed reaction system with better temperature control was clearly desirable.

Figure 4-1 illustrates the apparatus used in this work for the production of samples for both modulus evaluation and permeability studies. Outer aluminum plates of dimension 8" x 8" x 1/8" were used to tightly clamp two glass plates of 6" x 6" x 1/4". In the preparation of samples for modulus evaluation an aluminum shim of 6" x 6" x (0.040" ± 0.001") with an open center portion of 4-1/2" x 4-1/2" was placed between the glass plates and determined the dimensions of the hydrogel sample. For membrane casting, either no shim or else a stainless steel shim of (0.005" ± 0.0002") in thickness and the same square dimensions was used.

The outer sponge rubber gasket prevented leakage of excess reactants into the water bath. "Windows" cut in the aluminum clamping plates were of the same size as the inside dimensions of the shims, i.e., 4-1/2" x 4-1/2". Rubber shims placed at the glass plate-aluminum clamp interface served the dual pur-

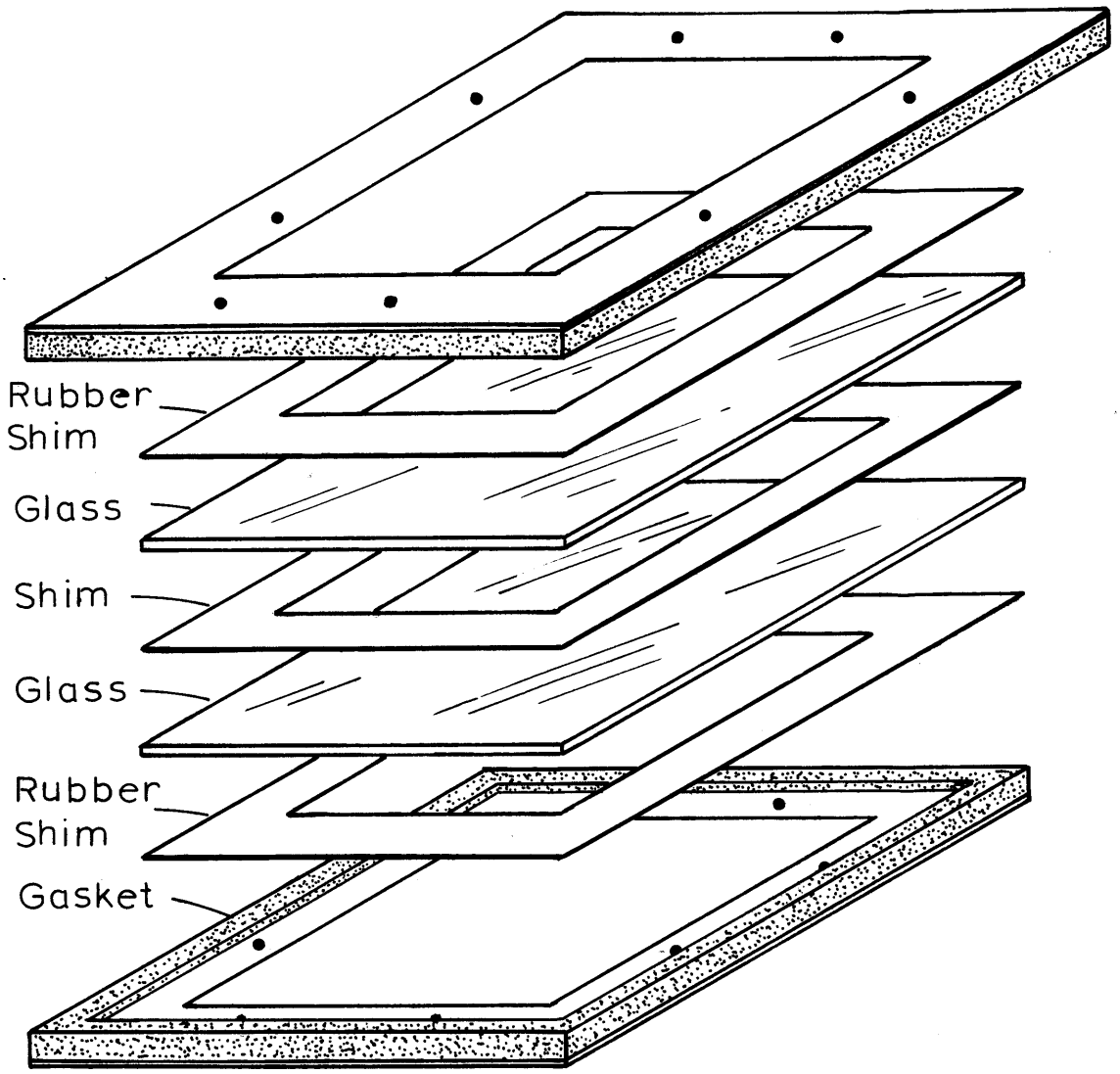


FIGURE 4-1 MEMBRANE SYNTHESIS APPARATUS

pose of allowing for expansion of the two materials during heating and insulating the glass plate from the aluminum clamp. Heat transfer to the hydrogel sample being synthesized was essentially one-dimensional through the glass plates exposed to the external environment.

With an interest in minimizing the transient warm-up period, a number of metal materials, including ferrotype plate, aluminum plate and nickel plate, were evaluated for use in place of the glass. In all cases, the combined mixture of dilute sulfuric acid and acetic acid was sufficient to attack the metal surface. This was noted both as visible corrosion on the metal plate and, more dramatically, by discoloration of the hydrogel sample, most likely from the formation of colored complexes by the ionized metal species and phosphate from the quenching bath.

Rather than risk contamination of the hydrogel sample by adventitious metal ions, polished plate glass was chosen to contain the reacting hydrogel material. While thinner glass sheets would seem desirable, they are not readily available with the tolerances ($\pm$0.0002") and heat stability of the 1/4" polished plate glass. For the case of 1/4" glass plate, an approximate transient heat transfer analysis indicated that the warm-up period would be around three minutes, and this was verified by following the temperature between the glass plates with a thermocouple during a typical run.

E. CASTING OF A HYDROGEL SAMPLE FOR TENSILE EVALUATION

The glass plates were carefully cleaned in acid-chromate

cleaning solution, washed in distilled water, and air-dried. One plate was placed on a flat surface, usually a 12" x 24" x 1/4" piece of polished plate glass, and a thin (0.001") sheet of water-swollen cellophane was placed on top of the glass plate to serve as a liner. The cellophane permitted easy removal of one glass plate after reaction, and was found not to affect the reaction in any way except by a very minor dilution of the reactants. The aluminum shim was placed on top of the cellophane liner, and the degassed reaction mixture was slowly poured into the space without entraining air bubbles. After covering the reaction mixture with the second glass plate and forcing out the excess material, the glass plates were clamped with the aluminum device described earlier (Figure 4-1).

For the reaction, the whole device was suspended in a circulating water bath at 70°C, and the reaction time was followed by means of a stop watch. The water bath was a Precision Scientific (Model No. 66648) with approximate inside dimensions of 10-1/2" x 12-1/2" x 8". Circulation was provided with two impellers driven by standard laboratory motors, and precise temperature control (70.0 $\pm$ 0.1°C) was achieved by use of a Bronwill Scientific mercury contact thermoregulator (sensitivity $\pm$0.005°C) and a Precision Scientific Electronic Relay (Model No. 62690). The 1000-watt heating element of the Precision Scientific water bath was only used in heating the bath from room temperature. The relay controlled the heat input from two 450-watt immersion heaters. A probe of the bath with a thermocouple on the most sensitive potentiometer scale ($\pm$0.05°C) revealed no measurable temperature variations or

gradients.

At the end of the reaction time the casting device was removed from the water bath, and the glass plates containing the sample were placed in a large container of buffered saline (composition given in Appendix D). Usually the glass plate on the side with the cellophane liner was removed first and the sample was allowed to soak before removing it from the second glass plate. Except in the case of samples that had a high cyclic acetal content, removal of the sample was fairly easy. The sample was then placed in a fresh container of buffered saline and was allowed to equilibrate for at least 24 hours before further study. Longer equilibration times did not affect the experimental results, and shorter times were not considered due to the scheduling of the experimental work.

F. CASTING OF A HYDROGEL MEMBRANE FOR PERMEABILITY STUDIES

There were two key problems that had to be overcome in this phase of the work. Because of their high water content, the hydrogels lack sufficient physical strength to be cast in thin sheets (of order 0.005 inches) that could be feasible hemodialysis membranes. Hence, a suitable support material had to be found. Secondly, there was the ever-present problem of casting a uniform, pinhole-free membrane for study.

1. Materials for Membrane Support

In the early stages of this work hydrogel membranes were cast using a high wet-strength paper of 1.5 mils dry thickness supplied by the P. J. Schweitzer Division of the Kimberly-Clark Corporation. While the backing was adequate to permit

evaluation of the material on the batch dialyzer, there were several drawbacks to this support material.

a. The paper tended to swell when wet, making it difficult to keep the backing flat while knife-coating a layer of the hydrogel reaction mixture.

b. Qualitative observation revealed that the paper support lacked sufficient strength and tear-resistance to be fabricated into an existing dialysis device.

c. It was practically impossible by any coating technique to assure that no paper fibers (cellulose) protruded through the hydrogel layer. Since cellulose is inherently thrombogenic, the fibers could serve as sites for thrombogenesis when in contact with blood, thereby hampering the performance of the hydrogels as nonthrombogenic dialysis membranes. In addition, the protruding fibers could cause membrane pinholes permitting the leakage of blood plasma proteins.

d. Efforts to coat a hydrogel layer on the paper material were always hampered by de-wetting of small areas of the paper. Attributed to higher concentrations of the wet-strength resin, this phenomenon made reproducibility of the membranes difficult.

A spun-bound nylon fabric called Cerex (R-Monsanto Co.)

proved to be a far superior material for membrane support purposes. In the commercial production of the material molten nylon filaments are dribbled onto a continuously moving belt. The randomly-oriented fibers are then calendered and briefly exposed to an acid vapor, which is believed to cause strong hydrogen bonding between the filaments at each random cross-over point (128,129).

Because of the continuous nature of the nylon filaments, there is no problem of fibers protruding through a hydrogel coating. According to information supplied by the manufacturer (130), Cerex (R-Monsanto) fabric is made from 100% nylon 6-6 polymer and has no added binders, finishes, or surface coatings. However, very small amounts (PPM range) of heat and light stabilizers are added. While these stabilizers presented no problem in this work, the manufacturer has available an unstabilized fabric which should be used for materials that will be tested in *in vivo* or *ex vivo* situations. This fabric did not swell when exposed to the casting solution. In addition, the casting solution wet the nylon filaments, permitting uniform coating of the web with hydrogel.

2. Evaluation of Nylon Fabric as a Membrane Support

There were two criteria considered in the evaluation of the support fabric. Since the thickness of the finished membrane can be no less than the backing thickness, only fabrics in the 1.0-5.0 mil range were considered. This limited consideration to fabrics of weight 0.3 oz/yd^2, 0.4 oz/yd^2, and 0.5 oz/yd^2. With an interest in producing a uniform membrane,

the more important consideration was not the actual fabric thickness but rather the thickness variations. Secondly, the tensile properties of Cuprophane (R-Bemberg) as reported by Colton (9) were taken as an approximate guideline for the necessary strength of the support material. Since the principal problem with Cuprophane (R-Bemberg) is not its tensile properties but rather its poor resistance to tear propagations, using these data as a guideline represents a conservative estimate. Cerex (R-Monsanto) is far superior in tear resistance because the random web is inherently resistant to the propagation of a tear.

Samples of the fabric of weight 0.3, 0.4, and 0.5 oz/yd^2 were supplied by the manufacturer. Since the commercially available materials proved to have wide variations in thickness, e.g., 2-5 mils for 0.3 oz/yd^2 material, the manufacturer also provided samples of the same initial fabric weights calendered to 1.3 mils $\pm$ 0.3 mils.

In this work the tensile properties were determined by experiment for calendered and noncalendered samples of 0.3 oz/yd^2 fabric weight and for calendered samples of 0.4 and 0.5 oz/yd^2. To evaluate the effect of the hydrogel reaction conditions on the material, samples of the calendered 0.3 oz/yd^2 were placed in a solution of composition 0.2 N H_2SO_4, 1.3 N acetic acid, and 6.0 wt % formaldehyde (including 2.0 wt % CH_3OH) at 70°C for two hours, which represented more stringent reaction conditions than would be employed in membrane production. After washing in a bicarbonate buffered

solution, the tensile properties were determined.

Although Cerex (R-Monsanto) is a uniform material, in the lighter fabric weights considered in this work a somewhat preferred alignment of the nylon fibers could be noted and this direction is called the machine direction (M.D.), the cross direction being the transverse direction (T.D.). For each fabric sample three tensile samples were cut in both the machine direction and the transverse direction, using a die of standard dumbbell shape with a neck width of 0.250". The thickness of the fabric samples was measured, using an Ames Dial Comparator (Model 412) on which readings may be made directly to 0.1 mil with the next decimal place estimated to $\pm$0.01 mil.

3. Casting Procedure

Hydrogel membranes for study were prepared using calendered fabrics of 0.3 oz/yd^2 and 0.4 oz/yd^2 in weight. The nylon backing was carefully cut in a square shape of 6-1/2" x 6-1/2", the exact dimensions being recorded, and the weight and thickness were measured. These data, along with the density of nylon, were used to calculate approximate values for the open area of the membrane. Casting solutions were prepared as described in Section 4-C, and the casting procedure was the same as in the preparation of tensile specimens, except as will be noted.

Both the cellophane liner and the nylon backing were presoaked in a solution of identical composition with the casting solution, except that it contained no PVA. This was done to prevent any dilution of the reactants by the water contained

in the cellophane and to condition the support material. The cellophane liner was placed on top of the glass plate as before, and a 5-mil layer of casting solution was spread on top using a Gardner casting knife. Nylon fabric was laid on top of the knife-coated layer by starting at one end of the glass and slowly allowing the fabric to progressively contact the viscous layer. This procedure was found best to minimize entrapment of air bubbles.

The fabric was pulled taut by the edges and the shim, if used, was put in place. A second 5-mil layer of casting solution was knife-coated on top of the support, the cover glass was put in place forcing out excess solution, and the glass plates were clamped in the aluminum holder. By carefully tightening the wing nuts on the aluminum clamping device, a reasonably uniform membrane ($\pm$0.001 inch) could be made. Remaining bubbles could sometimes be forced out while tightening the clamps. If a visual observation revealed no bubbles inside the reaction space, the membrane was reacted and quenched in the same manner as the tensile specimens. About 50% of the time a few bubbles would remain and the effort had to be abandoned, since these would be pinholes in the structure.

G. EXPERIMENTAL LIMITATIONS OF THE CASTING PROCEDURES

These limitations were imposed by the problems of assuring homogeneous mixing of reagents, of degassing the reaction mixtures, and by too rapid gelation of the casting solution. Both higher polymer content and particularly higher levels of glutaraldehyde tended to gel rapidly. Mixtures containing 0.2 wt %

and higher of glutaraldehyde gelled before they could be degassed and cast. In addition to a more rapid gelation at higher polymer contents (e.g., 10% Elvanol (R) 73-125G, 13% Elvanol (R) 71-30), the solution viscosities hampered mixing of the reagents. In fact, the reaction compositions containing 10% Elvanol (R) 73-125G could only be prepared by mixing the stock PVA and formalin in the correct proportions at higher temperature, cooling to room temperature, and then adding the other reagents. Higher polymer content solutions tended to form stable foams under vacuum and hence could not be degassed. By minimizing the air introduced in the mixing process, satisfactory tensile specimens could be produced without degassing. However, pinhole-free dialysis membranes could not be prepared at these concentrations.

H. VOLUME FRACTION DETERMINATION

The volume fraction of polymer in a swollen specimen, represented by v_2 where the subscript 2 denotes polymer following Flory's notation (76), is defined as:

$$v_2 = V_p/V_s \tag{4-1}$$

with V_p being the volume of polymer present and V_s the total volume of the swollen specimen. In this work the volume fraction of polymer in hydrogel specimens was measured in equilibrium with buffered saline (Appendix D) at 25°C, 37°C, and 70°C for all compositions synthesized. Volume fraction results at 25°C ($v_{2,25°C}$) and at 70°C ($v_{2,70°C}$) are used in computing values of M_c from the stress-strain data using the rubber elasticity theory (eqn. 3-24), specifically to evaluate the concen-

tration of polymer $C_{2,i}$ and the swelling factor δ. The volume fraction at 37°C was determined because of its relevance to the biomedical application of the material.

To determine the swollen volume of a hydrogel sample, the sample (about 0.3-0.5 grams) is placed in a covered aluminum weighing pan and its weight (denoted W_{air}) determined on a precision balance (Sartorius Model No. 2662). A fine stainless steel wire (0.020") is attached to the balance arm and the tare is adjusted to zero with the end of the wire suspended in a beaker of buffered saline. The same sample is then hooked on the end of the wire such that it is completely immersed in the buffered saline, and the weight (W_{saline}) is recorded. Using a density for the buffered saline at 25°C of 1.004 g/cc, the volume of the sample V_s is calculated from the simple expression:

$$V_s = \frac{W_{air} - W_{saline}}{\rho_{saline}} \qquad (4\text{-}2)$$

After washing of the sample for about one hour in two large volumes of distilled water to remove the buffered saline solution, the sample is placed in an aluminum weighing pan and dried to constant weight in a circulating air oven at 90°C. The dry weight of polymer (W_p) is recorded and the volume of polymer (V_p) calculated using the amorphous density of polyvinyl alcohol, ρ = 1.27 g/cc (131). Since the polymer is highly solvated in the original hydrogel specimen, the amorphous density should apply. The volume fraction is then calculated according to Equation (4-1).

Usually the swollen volume was measured at 25°C for four samples of each composition. The samples were then put in covered jars of buffered saline, and two were equilibrated at 37°C and two at 70°C. Temperatures were maintained at 70°C using the same water bath described in Section 4-E, and temperature control at 37°C was achieved with an identical experimental setup. In the work of Lee (119) it was found that four hours at the temperature of interest was adequate to assure equilibrium. Since it was found impossible to maintain a constant tare value for the wire in saline at 37°C and 70°C, probably because of thermal gradients or evaporation, all W_{saline} readings were taken in 25°C saline. With samples equilibrated at 37°C and 70°C, constant W_{saline} readings could be obtained up to ten minutes after immersing the sample in room temperature saline. This procedure does not introduce any approximation into the volume fraction determination. For samples from one hydrogel specimen the results would vary $\pm$5%, and the same variation was found in comparing results for different specimens of the same composition.

I. DETERMINATION OF THE ELASTIC BEHAVIOR OF THE HYDROGELS

To determine the elastic modulus of a hydrogel specimen, an Instron Universal Testing Machine (Table Model TM) was used with a 0-50-gram load cell (load cell A). Usually three samples of each hydrogel were cut for study using a standard dumbbell-shaped die (neck width 0.250 inches). To prevent loss of fluid from the sample during the experiment either by evaporation or by exudation, all runs were carried out with the sam-

ple immersed in a room temperature bath of buffered saline. The exact bath temperature was recorded for calculation of M_c from the data.

The standard Instron manual sample jaw was used to clamp the bottom of the hydrogel sample. In order to run with the saline bath a special holder was fabricated to clamp the sample on the top. A 17-gauge stainless steel tube with electrical eyelets soldered on the ends (overall length about 19 inches) was used to suspend a small lucite clamp from the load cell. The lucite clamp was made from two pieces of lucite (1/8" x 1-1/2" x 1-1/4") covered on the inside face with a fine nylon mesh. Two brass wing nuts were used to tighten the clamp and a rigid steel wire was used to attach the clamp to the stainless steel tube. The device was light enough to permit zeroing of the weight with the Instron controls.

After calibration of the Instron, the two jaws were adjusted so that they just touched, and the gauge length dial was set to zero at this point. The sample was then carefully clamped at the neck with pieces of damp filter paper between the hydrogel and the jaws to prevent tearing of the sample, and the inital length L_o was read from the gauge length indicator on the Instron. The buffered saline bath was raised with a laboratory jack so that the water level was just below the bottom edge of the lucite clamp which remained stationary during the run.

To minimize viscoelastic contributions to the measured force, the lowest rate of extension (0.2 inches/minute) was

used. Force as a function of elongation was recorded on a continuous strip chart recorder with a chart speed of 2.00 inches per minute. By stopping the crosshead during a run and observing the force at a fixed elongation with time, Lee (119) demonstrated that there was no measurable viscoelastic relaxation, and therefore that at the extension rate used true equilibrium forces were being measured.

The 50-gram load cell was sufficiently sensitive so that the sample weight registered on the strip chart recorder. Rather than complicate the zero adjustment procedure, this zero force was taken as a constant correction, which it was, and was subtracted from the total force in the data analysis. Since the sample was not completely stressed at the start of the experiment, the value of L_o from the gauge length indicator was corrected by adding the small incremental extension Δx as determined on the chart from the point where the elongation was begun (corresponding to L_o gauge) to the point where the force clearly rose from the zero force value. Prior to the elongation experiment, the thickness of the hydrogel sample was measured in the neck portion using the Ames Dial Comparator described earlier (Section 4-F).

J. SOLUTE PERMEATION STUDIES WITH THE BATCH DIALYZER

The batch dialyzer used in this work was previously used in the thesis work of Colton (9), and a detailed description of the apparatus and procedures may be found there. Briefly, the membrane was securely clamped between two identical cylindrical lucite chambers of diameter 2.482 inches and length 3.0 inches,

and an axially-mounted, four-blade impeller, 2.235 inches in diameter, 1/4 inch wide, and 1/16 inch thick, was positioned 1/8 inch from each face of the membrane. The lucite chambers and the seal and bearing housings were water-jacketed for temperature control (37.0 $\pm$ 0.1°C). Both impeller shafts were connected by timing belts to a common drive shaft, and their speed was controlled by a tachometer-feedback, variable speed motor coupled to a step function speed reducer. A calibration curve from the work of Colton (9) was used to set the impeller speed at 200 rpm for runs with low molecular weight solutes and at 150 rpm or 100 rpm for larger solutes.

Permeabilities of all solutes listed in Table 3-3, except for myoglobin and albumin, were measured on the batch dialyzer for hydrogel membranes of the standard composition with heparin (Table 3-2). All these solutes were obtained with a C^{14}-label (Appendix H), and the concentrations of test samples were measured by liquid scintillation-counting techniques, as described by Colton (9).

In a typical experimental run a solution of buffered saline with a tracer amount of the radioactive labeled solute[1] was charged to chamber one of the batch dialyzer (V_1 = 231.4cc) and buffered saline was charged to chamber two (V_2 = 234.2cc). The solutions preheated to 37°C, and they were charged simultaneously to prevent bowing of the membrane and to prevent any flow through the membrane due to a hydrostatic pressure gradient. The impellers were started, and as soon as thermistor

1. About 10-20 u curies/liter.

readings indicated that the temperatures were stabilized, samples (2.5 ml) were withdrawn from both chambers and the timer started. Additional samples (2.5 ml) were withdrawn as a function of time from chamber two (low concentration side) and the volume was simultaneously replaced with fresh buffered saline.

Since the equilibrium volume fraction of polymer in hydrogel membranes varies with temperature, the membrane was equilibrated in 37°C buffered saline before clamping it in the dialyzer. To prevent damage to the membrane, membrane thicknesses were measured after the permeation studies, using the Ames Dial Comparator.

In his work Colton noted a problem of adsorption of some of the larger solutes (inulin, heparin, dextran) on the lucite walls of the batch dialyzer. He recommended that 10 grams/liter of unlabeled solute be used in the solutions to swamp out adsorptive effects, and the suggestion was followed in this work for inulin, dextran, and polyethylene glycol (PEG).

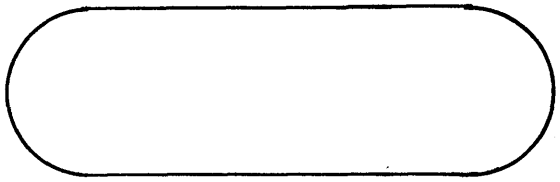
K. STAGNANT DIFFUSION STUDIES

The permeability of the proteins myoglobin and albumin (Appendix H) through several hydrogel membranes of different composition were studied. Three stagnant diffusion cells made of lucite were obtained from the Cole Parmer Instrument Company. Designated A, B, and C by etching into the lucite, the precise cross-sectional areas and approximate total volumes are listed in Table 4-2.

The accuracy of the stagnant diffusion experiment is

TABLE 4-2

STAGNANT DIFFUSION CELLS



Cell Cross Section

Cell	Approximate Total Volume (ml)	Transport Area (cm^2)
A	10	10.31
B	10	11.28
C	20	11.13

compromised by two factors. Since radiolabeled proteins tend to fragment in solution, the experiment is done with unlabeled solutes and the assay technique measures the total protein present. Therefore, a finite concentration difference exists across the membrane and a small flux (ca. 0.5 ml) of saline into the concentrated side in response to the osmotic gradient cannot be prevented. Because the diffusivities of the protein species are low, long times (ca. 24 hours) are required to perform the experiment and small chamber volumes are used. Concentration measurements are performed at only one time, which permits experimental error to enter the result, but using larger volumes would extend the time required and the stability of the proteins would be questionable.

In this work the hydrogel membrane was pre-equilibrated at 37°C in buffered saline and was then clamped in the stagnant diffusion cell. Using a syringe, the concentrated side was

charged with the protein in a buffered saline solution (ca. 4% albumin or ca. 1% myoglobin), the precise volume being noted. Then the dilute side was charged with a measured volume of buffered saline and the dialysis cell was incubated in an air oven at 37°C for periods of 12 to 36 hours. At the end of the experiment the contents of each chamber of the cell were removed, the volumes noted, and the protein concentrations determined, using the Micro-Biuret technique (Appendix I). The remainder of the original stock solution, which was stored under refrigeration, was also analyzed at this time. As a bacteriostat, 5×10^{-4} M KCN was used in all solutions. Membrane thicknesses were measured with the Ames Dial Comparator after the diffusion experiment.

CHAPTER 5

RESULTS AND DISCUSSION

In this chapter the results of the volume fraction determinations and the elastic modulus studies will be presented together to permit a unified discussion of the effect of the various reaction variables. Again, it should be emphasized that one component in the reaction mixture was varied at a time, all others being held constant at their control values (Table 3-1). Table 4-1 presented a complete summary of the compositions studied. Data from the evaluation of the Cerex (R-Monsanto) fabric as a membrane support material and the results from permeability studies on hydrogel membranes will follow in separate sections.

Throughout this discussion the term "elastic modulus" will be used and this refers to the slope of a plot of τ as a function of $(\alpha - 1/\alpha^2)$, according to Equation (3-24). Elastic modulus in this work is $\partial\tau/\partial(\alpha - 1/\alpha^2)$, and should not be confused with Young's Modulus E which is defined as $\partial\tau/\partial(\alpha - 1)$. In the limit as α goes to 1.0, the elastic modulus as defined in the work is equal to E/3.

A. RESULTS ON THE CONTROL COMPOSITIONS

Figure 5-1 presents a plot of the tensile stress in force per unit swollen cross section as a function of the extension parameter $(\alpha - 1/\alpha^2)$ for one sample of the control composition (Table 3-1) measured in buffered saline at 25°C (Section 4-I). Except for slight deviations at very low values of the extension parameter, which are caused by experimental error in mea-

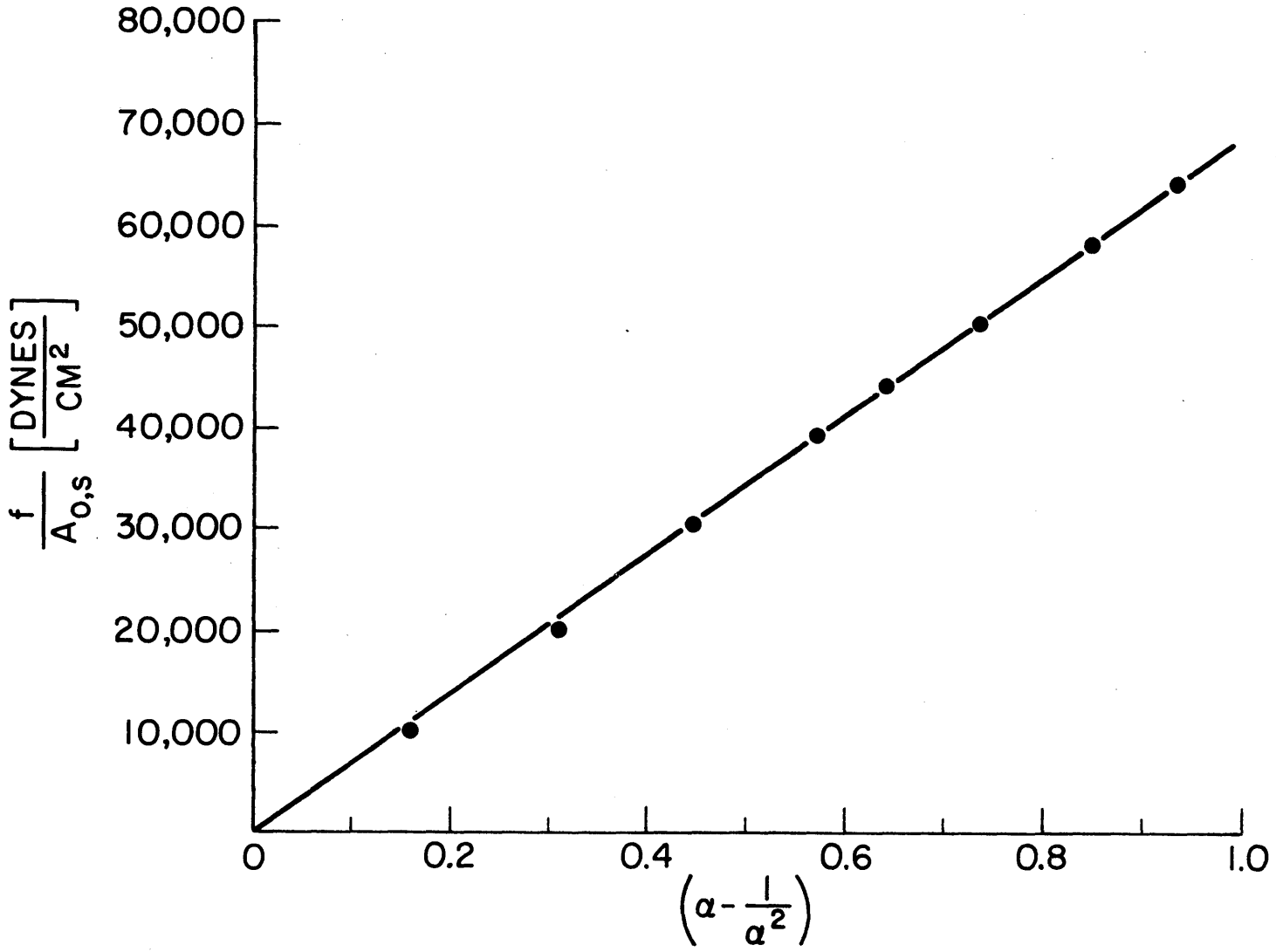


FIGURE 5-1. TYPICAL TENSILE DATA FOR HYDROGEL SPECIMEN.

suring small values of force and extension, the points fall almost perfectly on a straight line, as predicted by the theory of rubber elasticity (eqn. 3-22). These data are representative of all modulus determinations performed in this work and should be contrasted with the substantial variation observed in much of the reported work on bulk elastomers (76,107).

Table 5-1 presents a summary of the data for five hydrogel specimens, all prepared according to the control composition (Table 3-1). Elastic modulus as reported is the slope of the plot of stress τ as a function of $(\alpha - 1/\alpha^2)$, i.e., $\partial\tau/\partial(\alpha - 1/\alpha^2)$, and M_c is calculated according to Equation (3-24). The values of modulus and M_c reported are the average of three determinations on each specimen. Volume fraction data as shown are the average of all determinations on the five specimens, or a total of eighteen at 25°C, nine at 37°C, and nine at 70°C. Inasmuch as deviations of the same percentage ($\pm$5%) are noted in the results for each specimen as well as in the results for the five specimens, it is felt that the reproducibility of the synthesis technique is within the limits of experimental error on the characterization techniques.

B. EFFECT OF GLUTARALDEHYDE

Figures 5-2 and 5-3 present results from the studies on hydrogels with glutaraldehyde as the independent variable. In both figures the concentration axis (G/PVA) is expressed in moles of glutaraldehyde per mole of PVA mer. For Figure 5-2, the units of M_c are g/gmole, and elastic modulus (E.M.), as defined in Section 5-A, is expressed in dynes per square centi-

TABLE 5-1. SUMMARY OF DATA FOR SPECIMENS OF THE CONTROL COMPOSITION

Specimen	Elastic Modulus ($dynes/cm^2$) x 10^{-5}	M_c
A	0.628	24,640
B	0.654	23,470
C	0.637	24,000
D	0.697	23,250
E	0.670	23,610
Average	0.657 (±5%)	23,820 (±5%)
	Volume Fraction Determinations	
25°C	37°C	70°C
0.0503(±5%)	0.0733(±5%)	0.1316(±5%)

meter. Volume fraction of polymer (v_2) is a dimensionless quantity.

Specimens at five levels of glutaraldehyde were synthesized, but the sample containing zero glutaraldehyde proved to be a viscous mass insoluble in water but impossible to study. This suggests that the formaldehyde in the absence of glutaraldehyde contributes on the average about one cross-link per weight average polymer chain, which is essentially the point of gelation.

As the experimentally measured modulus (E.M.) increases, the value of M_c drops (Figure 5-2), as one would predict from the theory of rubber elasticity. Yet, given that for a four-fold increase in glutaraldehyde concentration the modulus in-

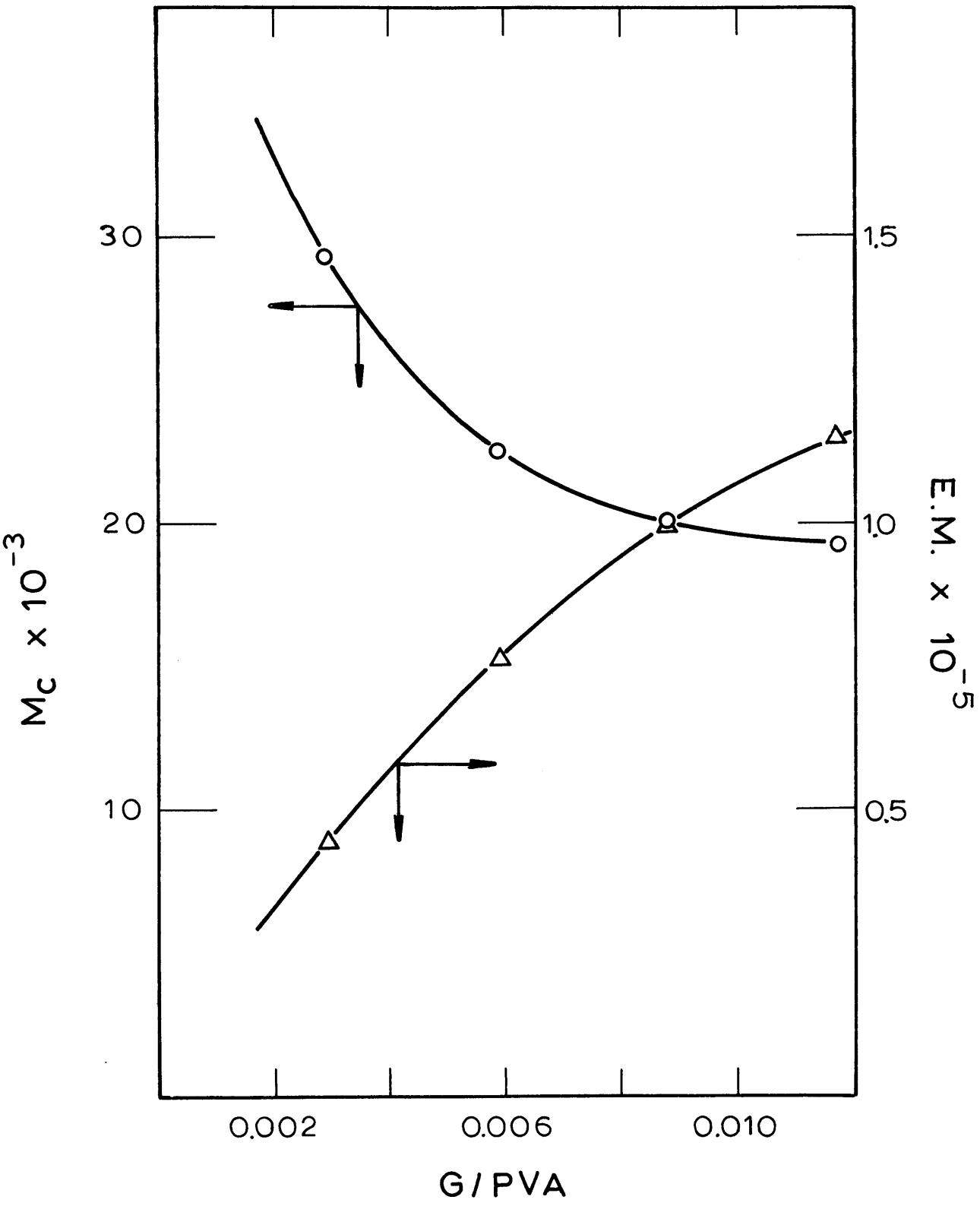


FIGURE 5-2 RESULTS FOR GLUTARALDEHYDE

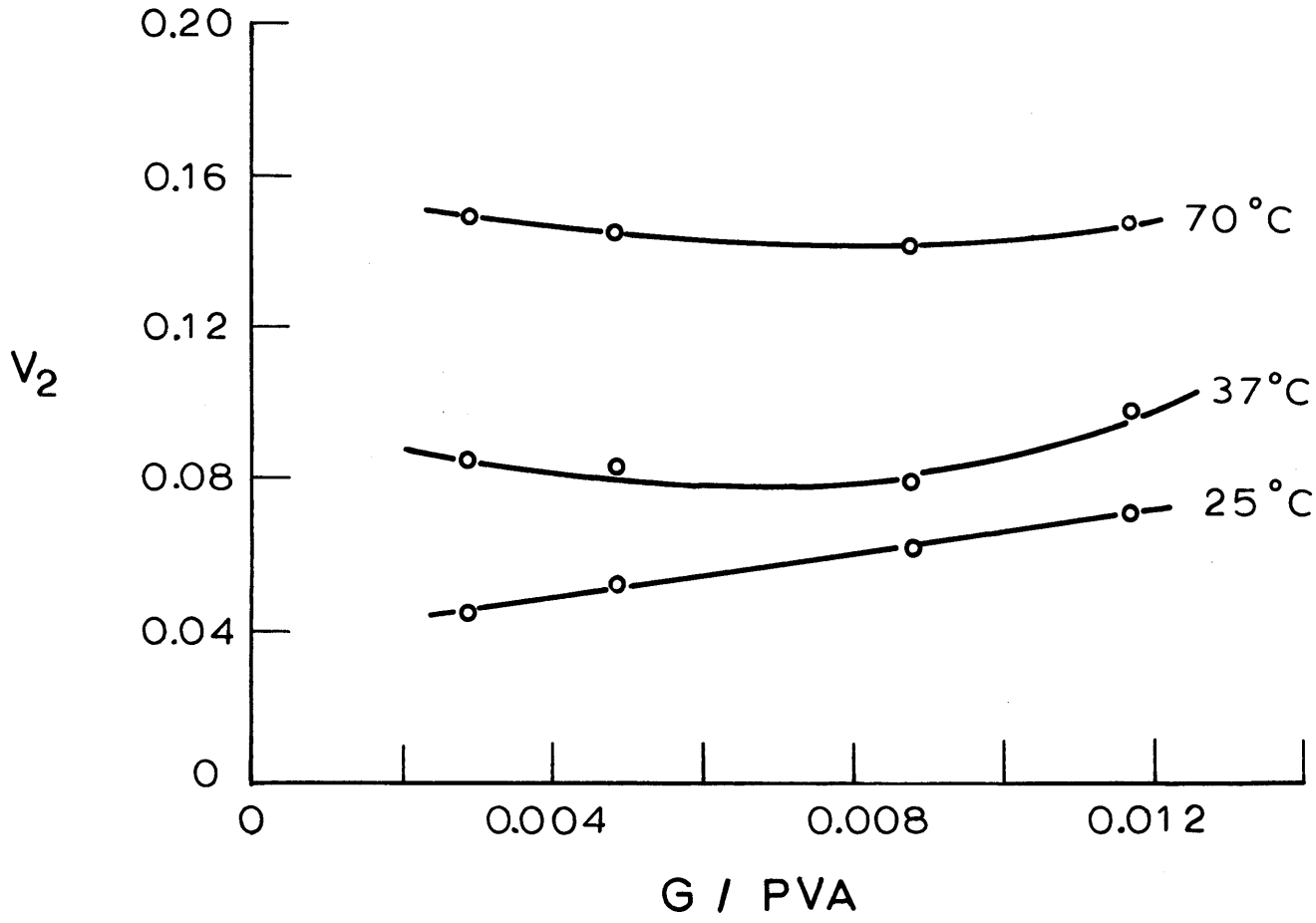


FIGURE 5-3 v_2 AS A FUNCTION OF T AND G/PVA

creases by a factor of 2.6 while the value of M_c drops by a factor of 1.5, these results might appear to be in contradiction.

In fact, they are not. The experimentally measured modulus (E.M.) is a function both of the amount of cross-linking in the specimen and of the amount of polymer in the swollen specimen (v_2). Consider Equation 3-24 and ignore for the moment the swelling parameter δ. If there are two swollen samples designated A and B in which the elastic modulus of B is twice that of A, sample B could have an M_c one half that of A (i.e., twice as many cross-links per chain), or sample B could simply have twice as much polymer present ($C_{2,i}$). Since all hydrogel samples are studied at their swollen equilibrium polymer contents (v_2) in buffered saline, the two parameters M_c and v_2 do not necessarily vary independently of one another. Volume fraction data at 25°C in Figure 5-3 illustrate the increase of v_2 with higher amounts of cross-linking, as should be expected from network swelling theory (Section 3-II-B).

The point of this discussion is that a consideration of elastic modulus (E.M.) values without proper attention to the factors involved could lead to erroneous conclusions. Since the derivation of the rubber elasticity theory for swollen systems accounts for the variations in polymer content and for isotropic swelling, M_c, as calculated according to Equation 3-24, is the valid parameter to indicate the effect of the reaction variable under study in the hydrogel synthesis. While a precise measure of the cross-linking should not be expected from the theory (Section 3-F), the changes in M_c do reflect the

characteristics of the synthesized network.

In this context, the data in Figure 5-2 indicate that, while the number of cross-links per chain (proportional to $1/M_c$) does increase with higher concentrations of glutaraldehyde, the increase is not proportional. Indeed, the values of M_c appear to approach an asymptote, though additional data at higher levels of glutaraldehyde would be required to confirm this point.[1] What this suggests is that some equilibrium limitation exists in the chemical cross-linking of the PVA. While such an equilibrium undoubtably depends on the water present in the system, certainly the other components such as formaldehyde, methanol, and possibly PVA hydroxyls also affect it. This consideration will be discussed in later sections.

C. EFFECT OF ACID CONCENTRATION

Figures 5-4 and 5-5 present an interesting aspect of the hydrogel chemistry, namely, the effect of increasing the concentration of sulfuric acid in the reaction mixture. As before, M_c is given in g/gmole, elastic modulus (E.M.) in dynes/sq cm, and the abscissae are expressed in gmoles of hydrogen ion[2] (H_s^+) per gmole of PVA mer. Note in Figure 5-4 that the E.M. axis is discontinuous to permit plotting of both curves on the figure.

In this series sulfuric acid concentration was varied from 0.04 N to 0.15 N, all other reactants fixed at their control values (Table 3-1). Since the acid serves as a catalyst for

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1. It was noted in Section 4-G that reaction mixtures of these compositions gelled too rapidly to be cast.

2. From sulfuric acid only. The acetic acid is not dissociated at reaction pH.

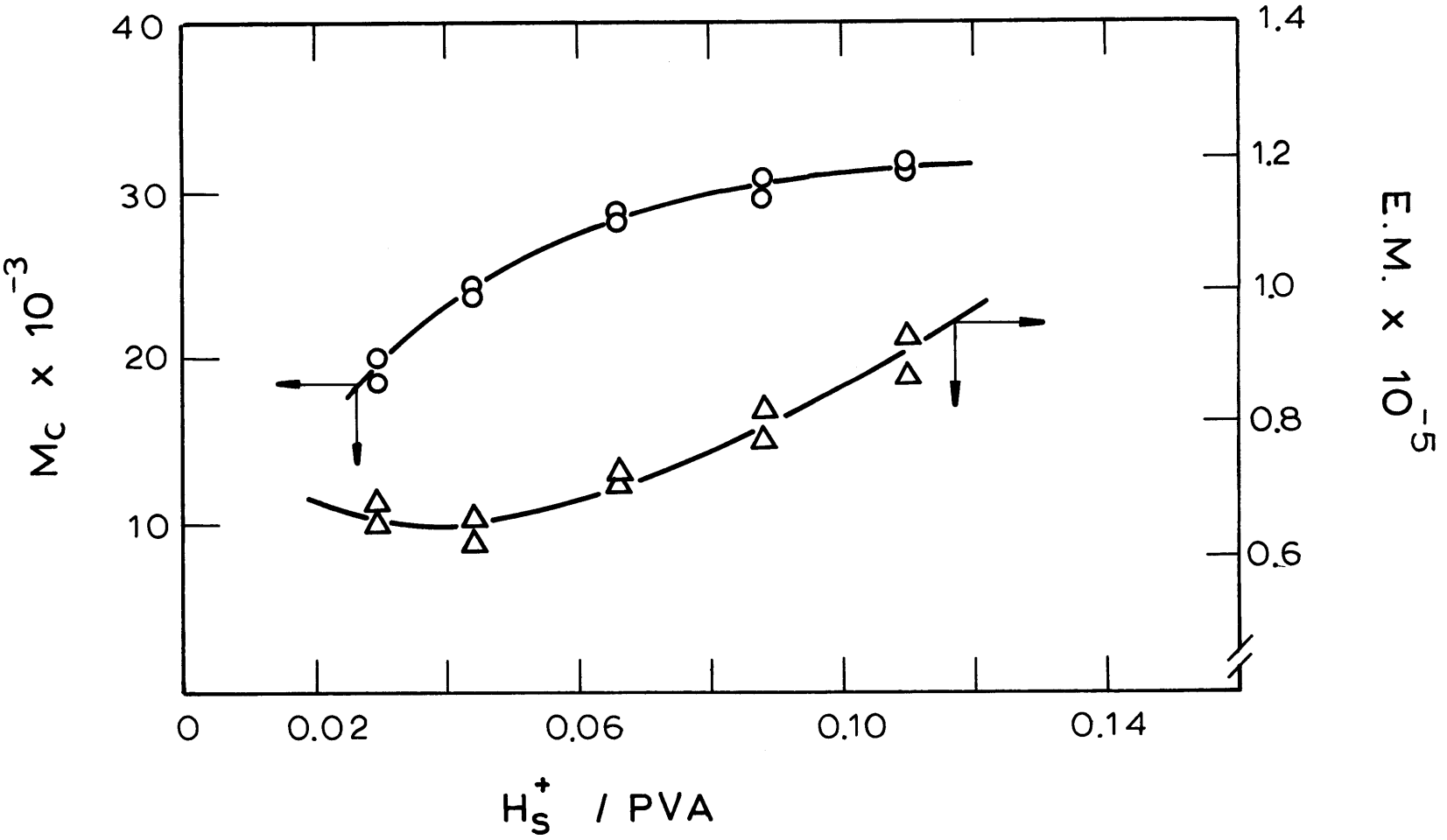


FIGURE 5-4 EFFECT OF ACID CONCENTRATION

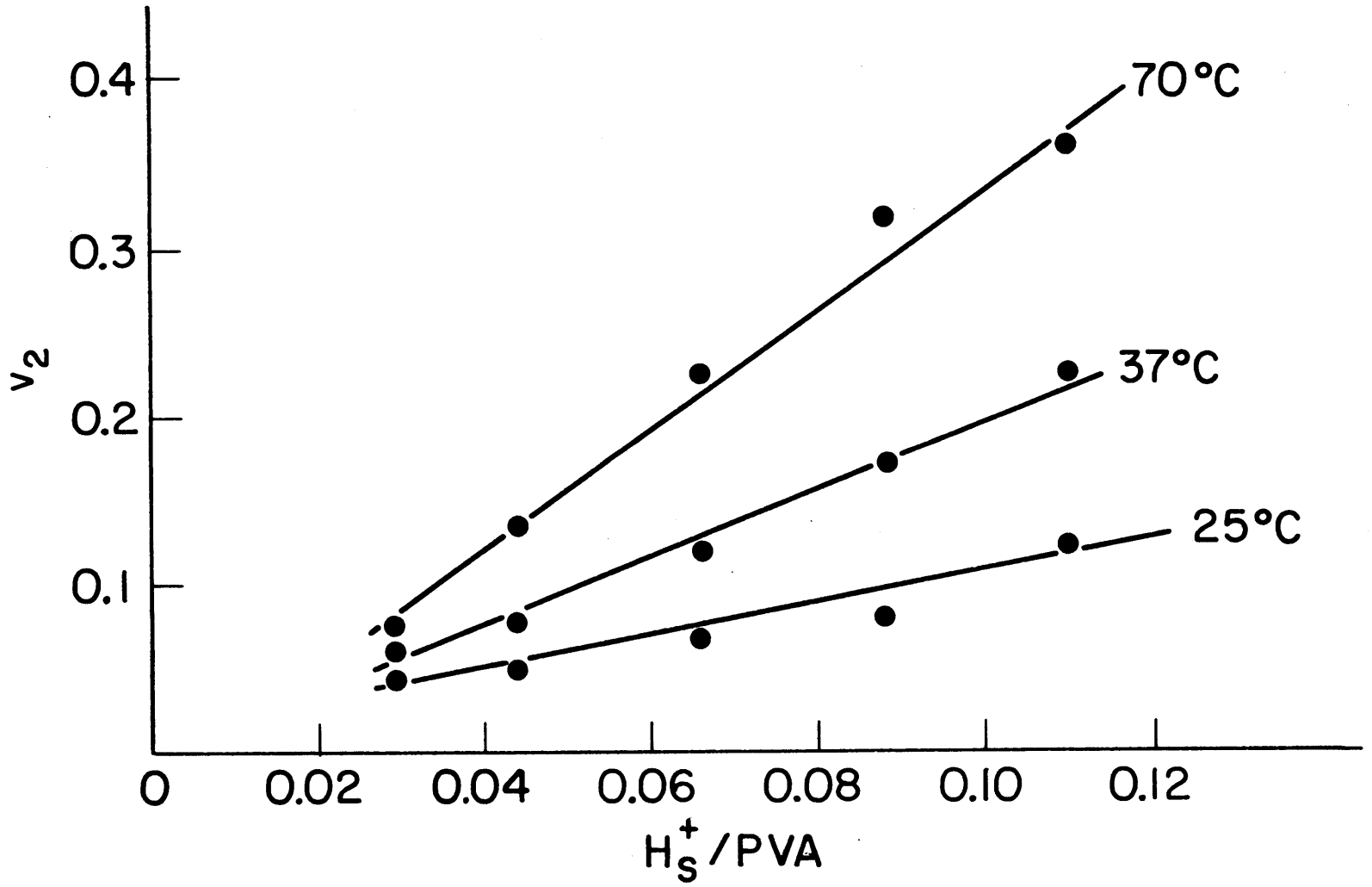


Figure 5-5. Polymer Volume Fraction as a Function of T and H_s^+/PVA.

the conversion of the hemiacetal to the final acetal form, it might be expected for fixed reaction time (60 minutes) that higher levels of acid should promote further cross-linking of the PVA.

The results are most interesting. As the concentration of acid increases, the value of E.M. rises but M_c also increases (Figure 5-4), indicating fewer cross-links per initial PVA macromolecule. Referring to Figure 5-5, it is seen that the increase in the elastic modulus is due to the higher volume fraction of polymer in the test specimen. Particularly impressive is the drastic effect on the thermal response of the hydrogels as acid concentration is increased (Figure 5-5), indicating increasing numbers of cyclic formal groups on the polymer chains in the hydrogel matrix.

These data indicate the importance of the two processes occurring during the hydrogel reaction, cross-linking and chain modification. Since higher levels of acid increase both the elastic modulus and the volume fraction of polymer, particularly as observed in the thermal response, but contribute less cross-linking (higher M_c), it appears that the principal effect is the introduction of cyclic formals. Furthermore, since the lowest M_c corresponds to the lowest acid level, these data indicate a competitive reaction between formaldehyde and glutaraldehyde.

Cross-linking of the PVA chains by glutaraldehyde appears to be a rapid process relative to cyclic formalization of the PVA by formaldehyde. Since the rate-limiting step in the

acetal reaction is the conversion of the hemiacetal to the acetal (Section 3-I-A), each glutaraldehyde quickly forms two hemiacetals which then either react with a PVA hydroxyl or a hydroxyl from methanol, the so-called "capping reaction." However, the cyclic formals, while forming at a slower rate, have a more favorable equilibrium. During the course of reaction, cross-links formed by glutaraldehyde back down (the reactions are completely reversible) in favor of cyclic formals.

D. EFFECT OF FORMALDEHYDE

Results for studies in which the formaldehyde was reduced from the control value of 6.0 wt % to lower levels are presented in Figures 5-6 and 5-7. Units on the ordinates are the same as in the previous figures and the abscissae are given in gmoles formaldehyde per gmole PVA mer. Since the formaldehyde was added from stock formalin solution containing methanol as a stabilizer (1:3 weight ratio of methanol to formaldehyde), methanol also varies as shown in Table 5-2.

TABLE 5-2. VARIATION OF METHANOL WITH FORMALDEHYDE IN THE MIXTURES

F/PVA	CH_3OH/PVA (approx.)
0	0
0.490	0.17
0.980	0.33
1.47	0.50

units: (moles reactant/mole PVA mer)

The data indicate trends similar to those seen when sulfuric acid concentration was varied (Section 5-C), though the variations are nowhere near as pronounced. As the concentra-

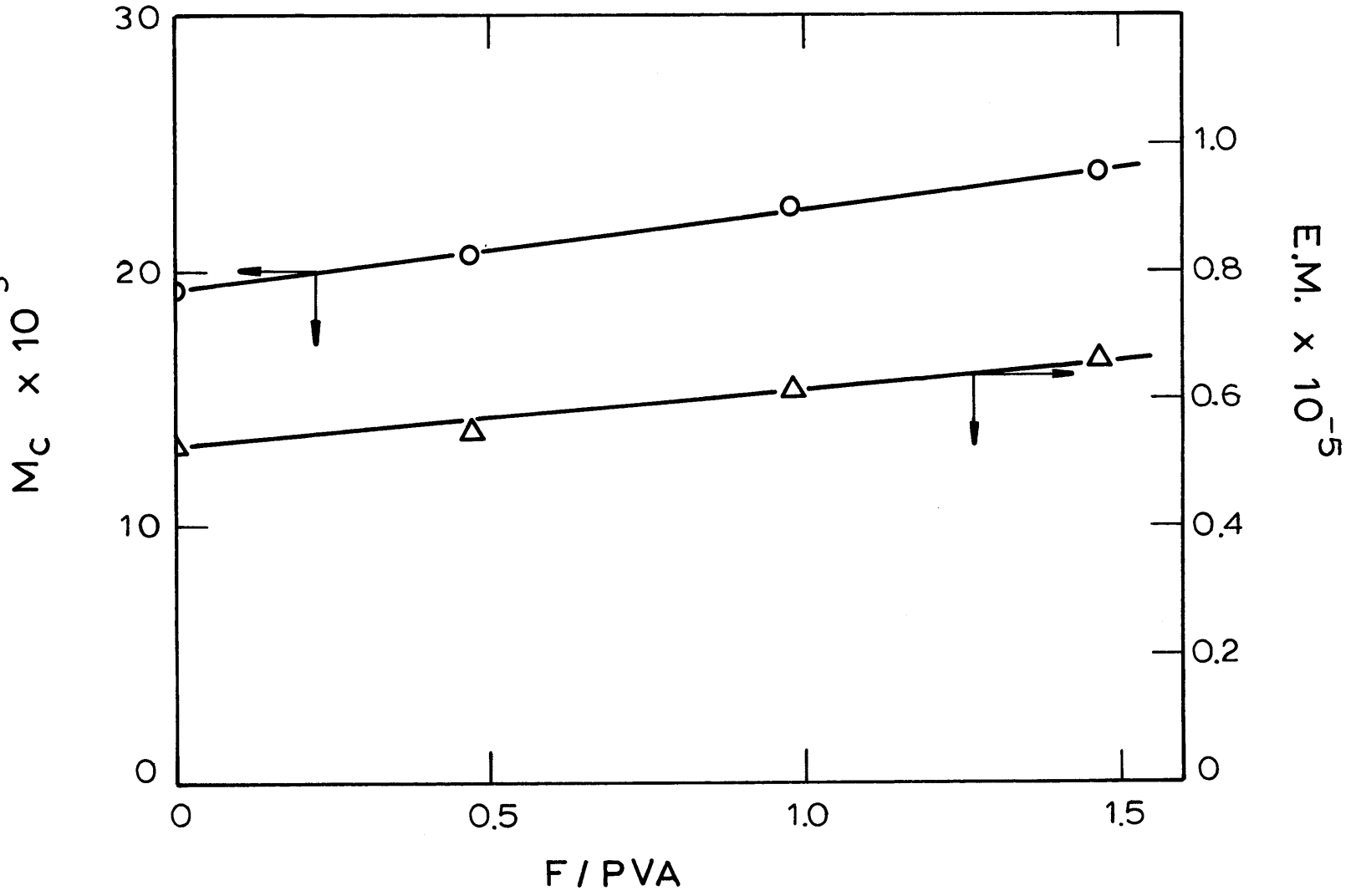


FIGURE 5-6 EFFECT OF FORMALDEHYDE

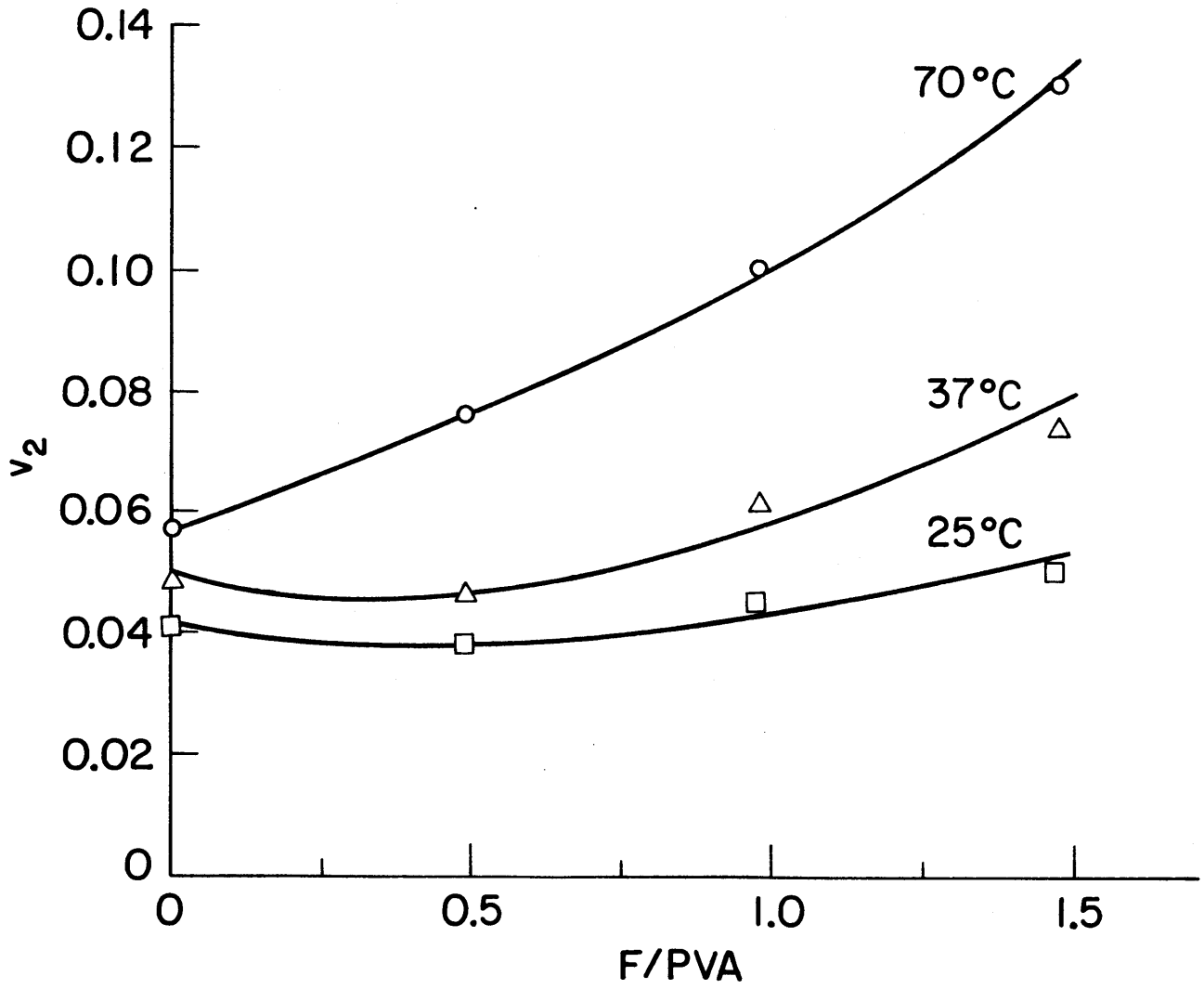


Figure 5-7. Polymer Volume Fraction as a Function of T and F/PVA.

tion of formaldehyde in the reaction mixture is increased, the elastic modulus (E.M.) rises but the amount of cross-linking (proportional to $1/M_c$) drops. Volume fraction data in Figure 5-7, particularly the variations with temperature, further support the view that increasing amounts of cyclic formal enter the network structure at the expense of glutaraldehyde cross-links.

The volume fraction data for the first composition presented in Figure 5-7 deserve some comment. Since this specimen was prepared with only glutaraldehyde (control value 0.076 wt %) and no formaldehyde or methanol present, the data should represent the maximum volume change with temperature that could be ascribed to the hydrogel containing only intact PVA subchains and the cyclic acetals or other network structures resulting from the reaction with glutaraldehyde. The data are presented for comparison with the control composition in Table 5-3.

TABLE 5-3. EFFECT OF CYCLIC FORMALS ON THE EQUILIBRIUM POLYMER CONTENT OF THE HYDROGEL NETWORKS

	Values of v_2*	
Temperature	G only	Control
25°C	0.041	0.050
37°C	0.049	0.073
70°C	0.058	0.132

*All values of v_2 measured in equilibrium with buffered saline (Appendix D).

Two conclusions can be drawn from these data. First, at any temperature of interest the presence of formaldehyde in the reaction mixture increases the volume fraction of polymer

present in the finished hydrogel. Since the basic chemistry involved (Section 3-I-A), the data for the glutaraldehyde series (especially at 0.0 wt % glutaraldehyde), and the data presented in this section all lend support for the idea that formaldehyde contributes little or nothing to the cross-linking of the network, this observation can only be attributed to the hydrophobic character of the cyclic formals. Secondly, the pronounced thermal response of all hydrogels prepared with formaldehyde present is due to the formation of cyclic formals on the network chains during the hydrogel synthesis.

E. EFFECT OF REACTION TIME

Reaction times were increased to study the effect on the cross-linking of the network. If the hypothesis of a competitive reaction between glutaraldehyde cross-linking and formaldehyde cyclicization is correct, then presumably longer reaction times should indicate similar trends to those observed with higher levels of sulfuric acid. The data are presented in Figures 5-8 and 5-9 with the reaction time axis (t) given in hours. Note that the axis is discontinuous, since times less than one hour were not considered. Elastic modulus (E.M.) and polymer content (v_2) both increase with longer reaction times but the amount of cross-linking decreases. These data do support the competitive reaction hypothesis. Volume fraction data again prove to be a sensitive indicator of increased cyclic formal content. Because of the problems of heparin deactivation (Section 3-I-D), longer reactions would not normally be considered in the production of a hydrogel for biomaterial applications.

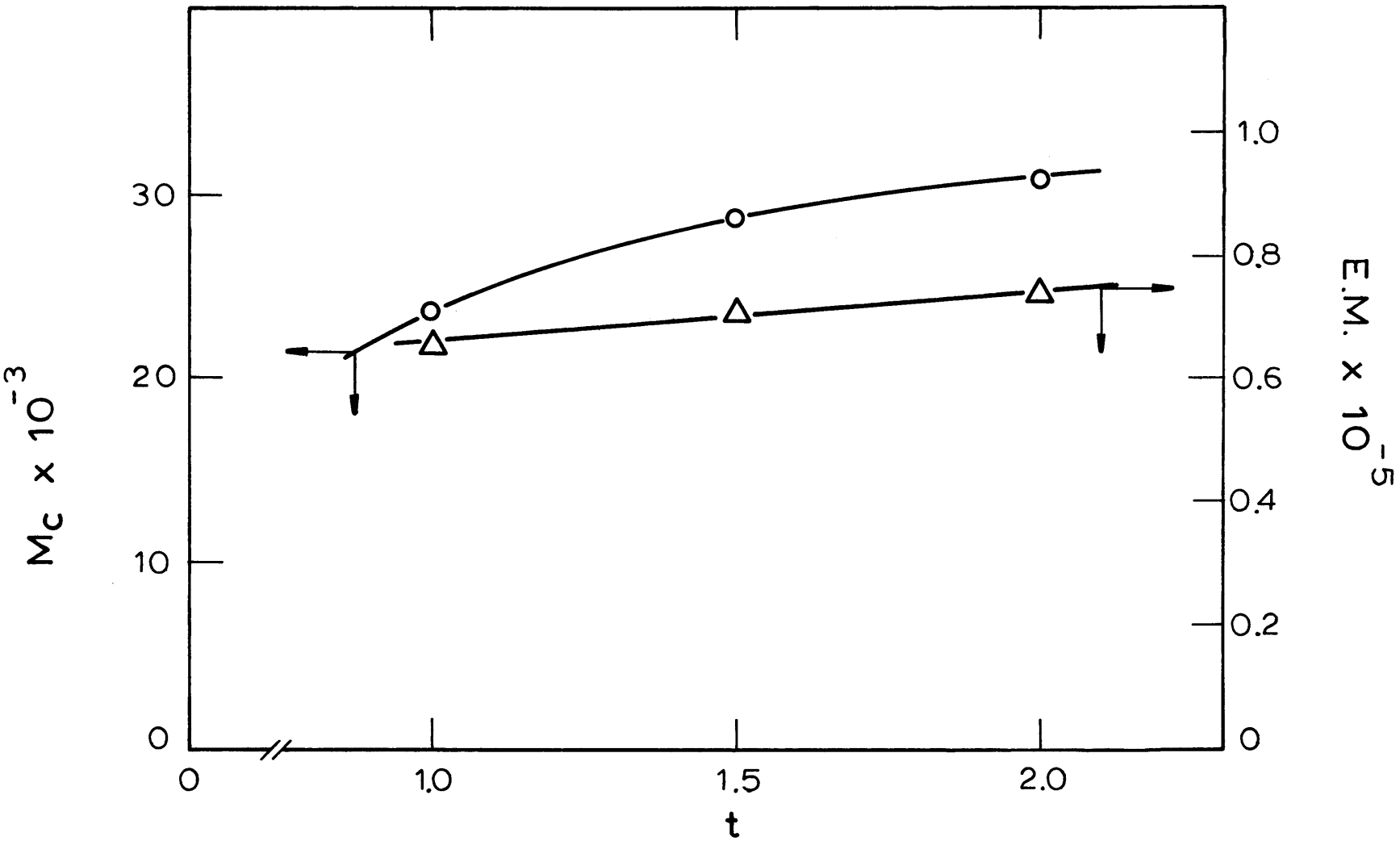


FIGURE 5-8 EFFECT OF REACTION TIME

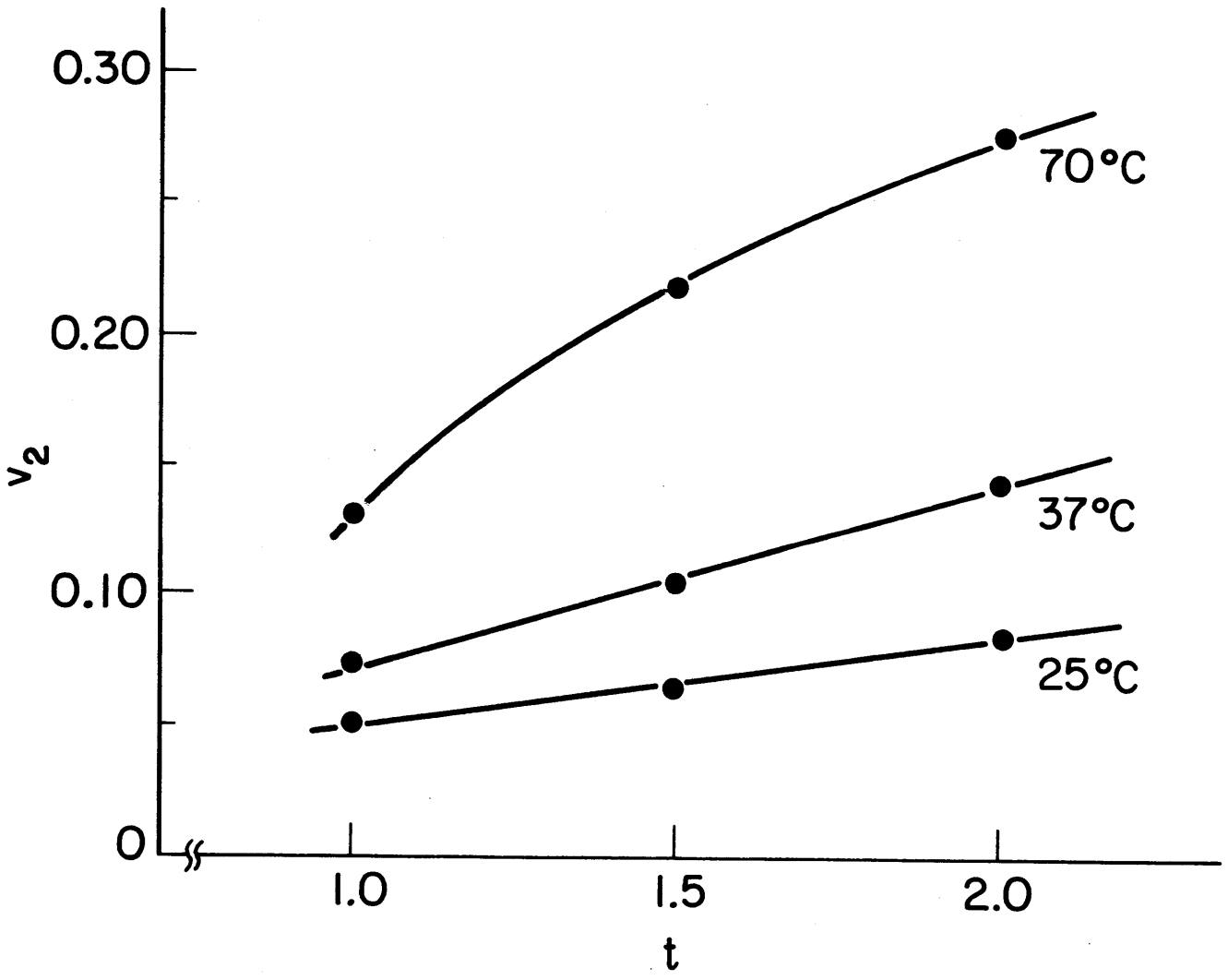


Figure 5-9. Polymer Volume Fraction as a Function of T and Reaction Time.

F. EFFECT OF ACETIC ACID

In the earlier research that led to the control composition presented in Table 3-1, hydrogels prepared without acetic acid were observed to contain a faint cloudiness when in equilibrium with buffered saline at room temperature. This was taken as an indication of some sort of phase separation resulting during the course of the hydrogel synthesis. Addition of acetic acid to the casting solution was found to produce a completely transparent hydrogel, which presumably was more homogeneous.

Data taken in this work on a hydrogel specimen prepared without acetic acid present are reported in Table 5-4. The exact effect of the acetic acid is not clear from these data. Values of M_c are slightly higher, indicating less cross-linking, but the difference is barely significant for typical experimental errors. Although volume fraction data as a function of temperature have proven to be a sensitive indicator of cyclic formal content, the data here indicate only slightly less cyclic formal. However, since acetic acid is not dissociated at

TABLE 5-4. COMPARISON OF HYDROGELS SYNTHESIZED WITH AND WITHOUT ACETIC ACID

	Control Composition	Control without Acetic Acid
Elastic Modulus (dynes/cm^2) x 10^{-5}	0.657	0.587
M_c (g/gmole)	23,820	24,638
Volume Fraction (v_2)		
25°C	0.050	0.050
37°C	0.073	0.068
70°C	0.132	0.123

the pH of the reaction mixture (pH = 1.2), which is determined by the sulfuric acid, it is unlikely that it functions as a catalyst. In any case these facts could not explain the effect acetic acid has on the visual homogeneity of the gel.

Normally, the presence of a separate phase in a tensile specimen would be expected to appear as a lower M_c (higher cross-linking), inasmuch as the precipitated phase contributes multifunctional junctions to the network structure. The data in Table 5-4 contradict this idea. Since network junctions contributed by a precipitated phase need not be permanent in the sense of covalently-bonded cross-links, it is possible that at the low extension rates used, the chains involved in the separate phase could relax in response to the deformation.

The most plausible explanantion for the effect of acetic acid is that it acts as a common solvent during the reaction for both PVA and for the PVA chains modified by cyclic formal. Earlier data presented in this work (especially Section 5-C) have documented the marked shrinkage of the hydrogel specimens in response to temperature, particularly at higher levels of cyclic formal, indicating less favorable solvent (water)--polymer (poly(vinyl alcohol-co-formal)) interaction. In the reaction environment of the hydrogel synthesis cyclic formals that form tend to aggregate, essentially forming a dispersed phase. The acetic acid apparently affects the solvent character in some manner to prevent the buildup of these aggregates.

That this phenomenon is associated with the cyclic formals is qualitatively supported by the author's observation in

earlier work that the cloudiness of a hydrogel specimen was more pronounced when higher levels of sulfuric acid were used. It should be stressed here that, except for the results cited in this section, all other hydrogel specimens studied in this work were prepared with acetic acid and were not observed to have any evidence of a phase separation.

G. EFFECT OF POLYMER CONCENTRATION AND MOLECULAR WEIGHT

Both the effect of increasing polymer concentration and the effect of polymer molecular weight were studied in this work. The two PVA grades used had molecular weights ($\overline{M}_n$) of 100,000 (Du Pont Elvanol 73-125G) and 60,000 (Du Pont Elvanol 71-30). Their characterization was discussed in Section 4-A.

Elastic modulus ($\partial\tau/\partial(\alpha - 1/\alpha^2)$) data are presented in Figure 5-10 for hydrogels synthesized from the two materials, the units being dynes/cm^2 as before. The abscissa gives the weight fraction of polymer in the casting solution ($w_{2,0}$), and the axis is discontinuous since only $w_{2,0}$ values in the range of 0.06 to 0.13 were studied. Experimental difficulties that precluded casting of specimens with higher amounts of polymer were discussed in Section 4-G.

In Figure 5-10 it can be seen that at a given value of $w_{2,0}$, the elastic modulus (E.M.) of specimens prepared with the higher molecular weight PVA is greater than that of the lower molecular weight material. This is the effect of the higher number of chain ends (network imperfections) in the sample prepared with the lower molecular weight PVA.

Volume fraction data for hydrogels prepared from the two

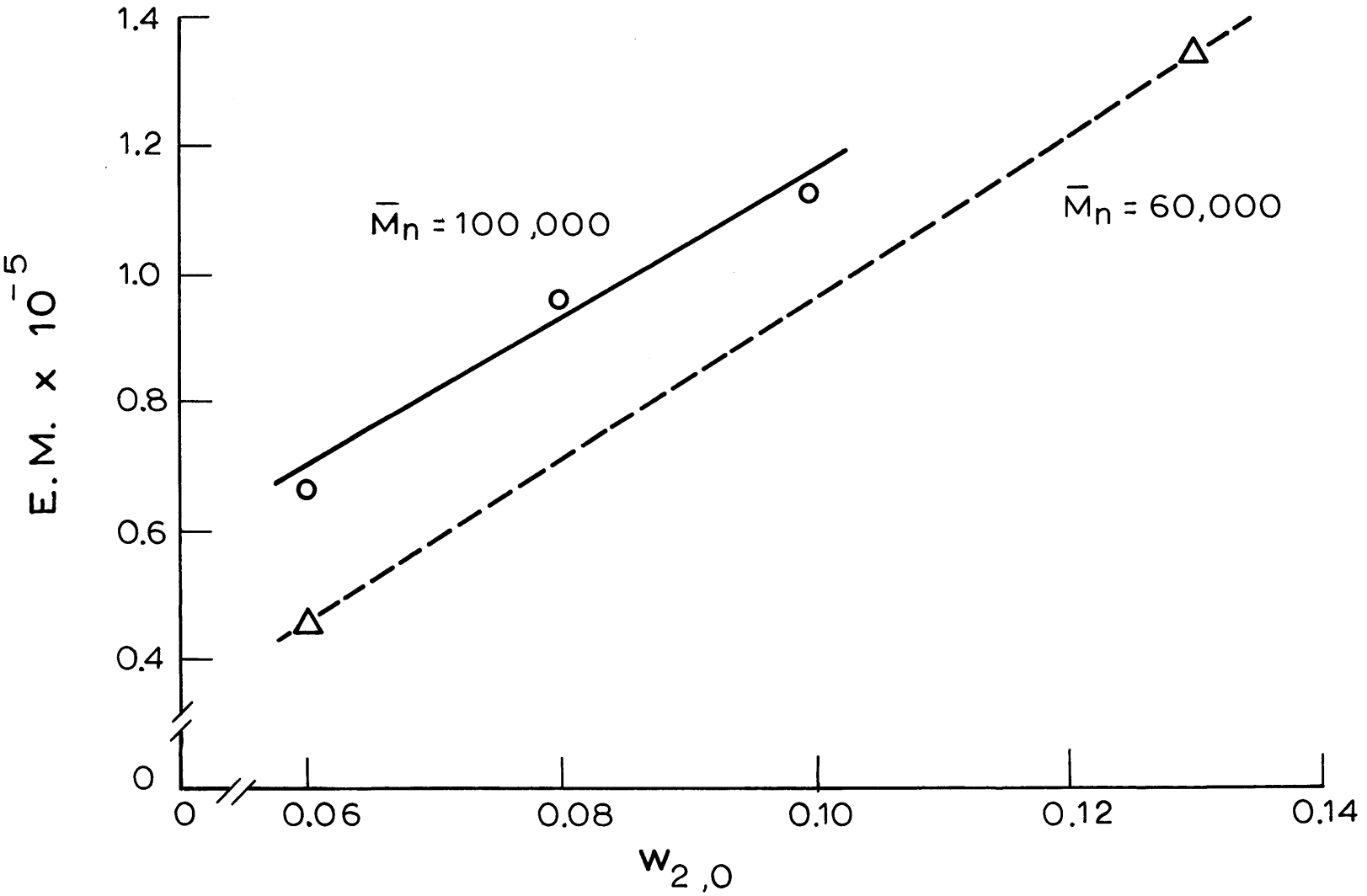


FIGURE 5-10 ELASTIC MODULUS AS A FUNCTION OF POLYMER CONTENT

grades of PVA with the same amount of polymer in the casting solution ($w_{2,0}$ = 0.06) are compared in Table 5-5. It is interesting to note for the data at 25°C that, in spite of the larger number of network imperfections in the $\bar{M}_n$ = 60,000 specimen, the volume fractions differ only slightly. Furthermore, the thermal response of the $\bar{M}_n$ = 60,000 specimen is more pronounced. Since there is no reason to expect the molecular weight of the PVA to affect the acetalization reaction, these data would appear to reflect some difference in the two PVA grades, possibly in the amount of residual acetate.

TABLE 5-5. VOLUME FRACTION DATA FOR HYDROGELS OF THE CONTROL COMPOSITION WITH DIFFERENT MOLECULAR WEIGHT PVA

	Volume Fraction (v_2)	
Temperature	$\bar{M}_n$ = 100,000	$\bar{M}_n$ = 60,000
25°C	0.050	0.046
37°C	0.073	0.076
70°C	0.132	0.157

$w_{2,0}$ = 0.06

Values of M_c (g/gmole) calculated from the modulus data according to Equation 3-24 are presented in Figure 5-11 as a function of $w_{2,0}$. The near superposition of the curves for the different PVA starting materials offers encouragement that the network correction factor used is valid and, indirectly, that the values of $\bar{M}_n$ used for the two samples are reasonable estimates (see Section 4-B).

In Figure 5-11 it can be seen that, as the weight fraction of PVA in the reaction mixture ($w_{2,0}$) is increased, the

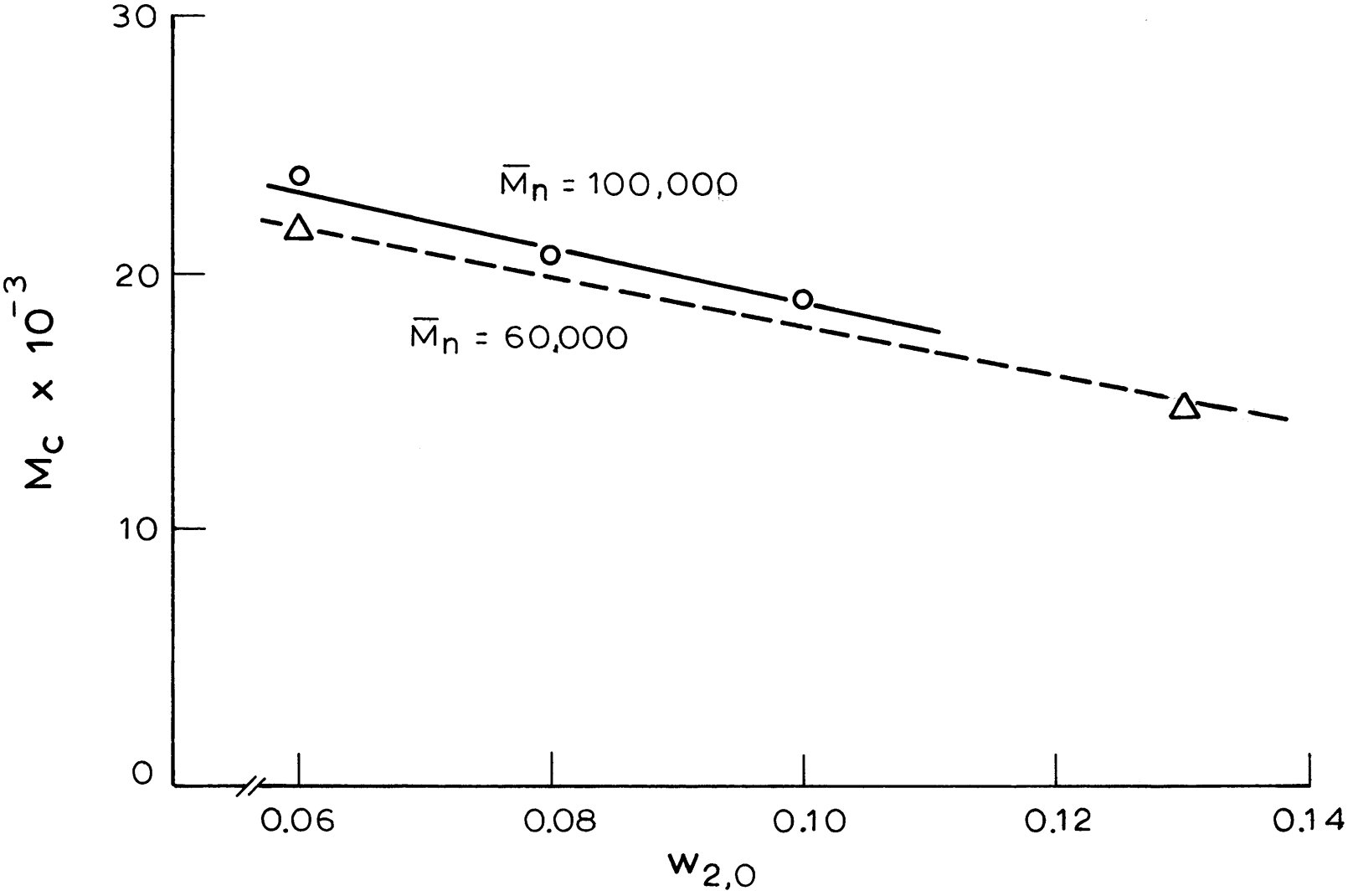


FIGURE 5-11 EFFECT OF POLYMER CONCENTRATION ON M_c

value of M_c for the finished hydrogel drops. Since there is no a priori reason to expect more cross-linking per PVA macromolecule, this merits discussion.

Certainly one possibility is that at higher PVA contents the polymer chains are more completely entangled with one another and the probability of forming intrachain cross-links is reduced. However, since even the control PVA concentration ($w_{2,0} = 0.06$) is a factor of ten greater than the critical polymer concentration (Appendix C), this explanation does not seem plausible. In addition, it should be noted that intrachain cross-links are not necessarily wasted in the sense that they do not contribute to the elastic retractive force of the network. Except for ineffective short loops (see Section 3-II-D), which should only result for cross-linking in dilute solution (76), intrachain cross-links will contribute to the elastic modulus, providing that they occur on the average with subchains of length M_c and that the original PVA macromolecule is connected to the network structure.

A more plausible explanation and one that is consistent with the hypothesis in this work that the cross-links form at an equilibrium reaction volume V_i (characterized by $v_{2,i}$) can be put forth as follows. Table 5-6 summarizes the volume fraction data for three hydrogel specimens prepared at different values of $w_{2,0}$ with PVA of $\bar{M}_n = 100{,}000$. The important point is that within experimental error ($\pm 5\%$) the volume fractions at 70°C, which represent the reaction volume V_i, are approximately equal. These volume fractions ($v_{2,i}$) represent the

shrinkage of the system that has occurred during reaction in response to the introduction of cyclic formal along the polymer backbone.

Recall that the introduction of cyclic formal to the network structure is a relatively slow kinetic process and is affected principally by the concentration of acid catalyst (H_2SO_4) as shown in Section 5-C. In the present series acid concentration was fixed at the control value (0.06N), so presumably the same amount of cyclic formal was introduced along the polymer chains in each specimen. The network contracted to a volume essentially determined by the thermodynamics of the polymer-solvent interaction, and the data in Table 5-6 support this concept.

TABLE 5-6. SUMMARY OF RESULTS FOR HYDROGELS PREPARED WITH DIFFERENT INITIAL PVA CONTENTS

Weight Fraction PVA* ($w_{2,0}$)	M_c (g/gmole)	Volume Fraction Data		
		25°C	37°C	70°C
0.06	23,820	0.050	0.073	0.132
0.08	20,690	0.063	0.082	0.144
0.10	18,570	0.062	0.086	0.139

*PVA ($\bar{M}_n$ = 100,000)

While it is likely that only a small portion (∿10%) of the PVA "backbone" has been converted to cyclic formal during the reaction, it should be recalled that the rate-limiting step is the conversion of the unstable hemiacetal to the finished acetal form. The formation of the hemiacetal does not require

the acid catalyst nor does the concentration of water present limit the reaction. It is probable at the molal ratio of formaldehyde present in the control reaction mixture that a significant amount of the PVA hydroxyls exist in a hemiacetal form. Data presented in reference 62 on the equilibrium amounts of hemiacetal present in alcoholic solutions would seem to support this view.

Since all variables including formaldehyde were held constant at their control values (Table 3-1) while the PVA content was varied ($w_{2,0}$), the molal ratio of formaldehyde to PVA (F/PVA) changed with the PVA content as shown in Table 5-7.

TABLE 5-7. VARIATION OF F/PVA WITH PVA CONCENTRATION

Weight Fraction PVA* ($w_{2,0}$)	Molal Ratio F/PVA
0.06	1.47
0.08	1.10
0.10	0.88

*PVA ($\overline{M}_n$ = 100,000)

The competitive reaction between formaldehyde (cyclic formalization) and glutaraldehyde (cross-linking by formation of two cyclic acetals) was demonstrated in Sections 5-B, 5-C, and 5-D. In this context the increased cross-linking (lower M_c) observed with higher amounts of PVA can be interpreted as the effect of the lower molal ratio of formaldehyde essentially permitting the formation of more glutaraldehyde cross-links.

When the hydrogel specimen is quenched in buffered saline at 25°C following synthesis, it swells to an equilibrium volume

fraction ($v_{2,25°C}$) which depends on:

1. The network-solvent interaction characterized by the thermodynamic parameter χ (76). This parameter is affected primarily by the amount of cyclic formal present.
2. The amount of cross-linking, characterized by M_c.
3. The relaxed volume of the specimen, characterized by $v_{2,i}$.

These factors are important and can be used to explain many of the phenomena observed in this work.

For example, referring to Figure 5-3 which presents the volume fraction data for the glutaraldehyde series, it can be seen that the volume fraction data at 70°C vary slightly but are approximately equal, as would be predicted for the same amounts of cyclic formal (i.e., formaldehyde fixed, H_s^+ fixed). The amount of cross-linking should not affect the relaxed state of the sample (70°C), but when the samples are equilibrated in 25°C buffered saline they swell to different volume fractions, indicative of the amounts of cross-linking introduced. In the sulfuric acid (H_s^+) series the volume fraction trends are controlled principally by the amounts of cyclic formal introduced (effect on χ factor).

H. EFFECT OF HEPARIN

A total of three hydrogel specimens containing heparin (0.01 wt fraction sodium heparin) were prepared according to the composition given in Table 3-2, and two specimens were prepared with higher polymer content (0.10 wt fraction PVA)

and heparin as above. The results are summarized in Table 5-8 along with data for unheparinized hydrogels of the same composition. Average values are reported where applicable. At both levels of PVA the samples containing heparin have a slightly reduced elastic modulus, lower values of M_c (indicating more cross-linking per initial PVA chain), and a notably less pronounced thermal response as shown in the volume fraction data.

Precise interpretation of the results is difficult because of the various effects heparin could have on the network. Heparin is, after all, a biological polymer ($\bar{M}_v$ = 13,800) and, if grafted to the network in at least two points per heparin macromolecule, would contribute effective cross-links to the network. However, Merrill et al. (61) have reported the grafted heparin content for a hydrogel prepared according to the composition in Table 3-2 at 0.7 milligram per gram of network polymer (i.e., dry basis). Inasmuch as the initial ratio in the reaction mixture is 170 mg/g PVA, the efficiency of grafting is poor.

If the value of M_c is taken as a quantitative measure of the amount of cross-linking per PVA chain, then the glutaraldehyde contributes an average of four cross-links per number average chain.[1] If each heparin macromolecule is tied to the network at two points, which may be an optimistic estimate in

---

1. If M_c be taken as 20,000, then the original PVA macromolecule of $\bar{M}_n$ = 100,000 is divided into five subchains of M_c = 20,000, which requires four cross-link points.

	PVA Content			
	$w_{2,0} = 0.06$		$w_{2.0} = 0.10$	
	Control[b] (5)[a]	Heparin[c] (3)	Control[d] (1)	Heparin[e] (2)
Elastic Modulus (dynes/sq. cm.) $\times 10^{-5}$	0.657	0.623	1.12	1.10
M_c (g/g-mole)	23,820	19,630	18,570	15,039
Volume Fraction (v_2)				
25°C	0.050	0.042	0.062	0.053
37°C	0.073	0.053	0.086	0.067
70°C	0.132	0.080	0.139	0.092

a Number in parentheses indicates number of specimens.

b Composition in Table 3-1.

c Composition in Table 3-2.

d 0.10 weight fraction PVA; all other variables fixed at control values (Table 3-1).

e Same as (d) and containing in addition 0.01 weight fraction sodium heparin.

Table 5-8. Summary of Results for Hydrogels Prepared with Heparin.

view of the poor grafting efficiency, then it would contribute two cross-link points to the network. On the basis of the reported heparin content (61), this indicates a maximum contribution of 0.01 cross-links per PVA chain, which is negligible.

Since heparin is a polyelectrolyte, it undoubtedly functions to increase the equilibrium volume fraction of the network at any temperature. Also, it opposes the shrinkage of the network structure as temperature is increased. However, the low residual heparin content of the hydrogel would cast a doubt as to the significance of this effect.

The most plausible explanation of the data is obtained from consideration of the buffering effect heparin can exert on the reaction mixture. In Section 4-B the results of a quantitative analysis on the heparin material used indicated six sodium ions (Na^+) per heparin tetramer. With reference to the diagram of the heparin tetramer (Figure 2-4) this indicated that, on the average, one of two carboxylic acid groups on the tetramer must be in the sodium form. Undoubtedly this group would accept a hydrogen ion in the reaction mixture. In addition, the two sulfamate groups per heparin tetramer might exert a buffering effect.

If the buffering effect of heparin is computed on the basis of 1, 2, and 3 gmole of hydrogen ion (H_s^+) consumed per tetramer for a reaction mixture containing 0.01 weight fraction sodium heparin, the results in gmoles of H^+ per liter are 0.0083, 0.017, and 0.025, respectively. Although this is in a less sensitive range on the pH meter, measurements on a control

(0.06 N H^+) and on a heparin-containing solution (0.06 N H^+, 0.01 wt fraction heparin) indicated that the results are in the correct range. The final hydrogen ion concentration of the reaction mixture is in the range of 0.035 to 0.590 N H^+.

Data for the specimen prepared at the lowest level of sulfuric acid as reported in Section 5-C are presented in Table 5-9 for comparison with the heparinized hydrogel synthesized according to the composition in Table 3-2. Both samples had 0.06 weight fraction PVA in the initial reaction mixture.

With the assumptions made in comparing these two specimens, the excellent agreement for the results in Table 5-9 may be more good fortune than experimental fact. However, the data certainly appear to support the proposed explanation and this explanation is definitely more feasible than one based on the low residual heparin content of the heparinized hydrogel.

TABLE 5-9. COMPARISON OF RESULTS FOR HEPARINIZED HYDROGEL AND CONTROL PREPARED AT 0.04 N ACID

	Heparinized Hydrogel	Control with[a] 0.04 N H^+
Elastic Modulus (dynes/sq cm) x 10^{-5}	0.623	0.669
M_c (g/gmole)	19,630	19,380
Volume Fraction (v_2)		
25°C	0.042	0.043
37°C	0.053	0.059
70°C	0.080	0.085

[a]From Section 5-C.
Average values for two specimens.

While the effect of the residual heparin content on the thermal response of the hydrogel cannot be ruled out, it seems

that the properties of the heparinized hydrogels are due to the reduced acid concentration in the reaction mixture. As shown in Section 5-C, lower acid levels introduce less cyclic formal to the network structure in a fixed reaction time and also permit more cross-linking by the glutaraldehyde. For the higher PVA content material, both the buffering effect of heparin and the reduced molal ratio of formaldehyde to PVA mer promote the lowest value of M_c (highest amount of cross-linking per chain) achieved by the reaction conditions studied in this work.

I. EVALUATION OF THE MEMBRANE SUPPORT MATERIAL

Table 5-10 summarizes the tensile data from the evaluation of the Cerex (R-Monsanto) material as a membrane support. Data of Colton (9) for Cuprophane (R-Bemberg) PT-150, the most commonly used hemodialysis membrane, are presented for comparison. Since the Cerex (R-Monsanto) fabric does not exhibit a true tensile break, the point where the material began to tear was recorded as the ultimate tensile strength and ultimate break elongation ($(\Delta L/L_o)\%$). Young's modulus E is defined in the usual manner, i.e., $\tau = E(\Delta L/L_o)$. Data for the uncalendered support material considered are not presented because the wide variations in fabric thickness made it unsuitable for use as a membrane support. The calendered samples are 1.3 ± 0.3 mils in thickness.

For the calendered fabrics studied, examination of the data in Table 5-10 indicates that the 0.4 oz/yd^2 material has the best tensile properties and is superior to Cuprophane (R-Bemberg) in its ultimate properties. Since "a chain is no

Table 5-10. Summary of Tensile Data for Membrane Support Material.

Material and Test Direction	Ultimate Tensile Strength ($psi \times 10^{-3}$)	Young's Modulus ($psi \times 10^{-3}$)	Ultimate Break Elongation (%)
[a]Cuprophane PT-150			
M.D.	3.28 ± 0.47 (14%)	18.2 ± 1.9 (11%)	17.3 ± 3.2 (18%)
T.D.	0.73 ± 0.23 (31%)	3.8 ± 0.60 (15%)	48.2 ± 14.3 (30%)
[b]Calendered Cerex ($0.3\ oz/yd^2$)			
M.D.	1.31 ± 0.46 (35%)	5.74 ± 2.0 (34%)	23.0 ± 3.0 (13%)
T.D.	0.22 ± 0.04 (20%)	0.85 ± 0.17 (20%)	27.0 ± 7.0 (26%)
Calendered Cerex ($0.3\ oz/yd^2$) after exposure[c]			
M.D.	2.26 ± 0.12 (5%)	9.17 ± 1.8 (20%)	26.0 ± 5.0 (19%)
T.D.	0.49 ± 0.10 (21%)	1.33 ± 0.13 (10%)	35.0 ± 7.0 (20%)
Calendered Cerex ($0.4\ oz/yd^2$)			
M.D.	4.75 ± 0.34 (7%)	12.6 ± 0.70 (6%)	39.0 ± 1.0 (3%)
T.D.	1.36 ± 0.03 (2%)	3.5 ± 0.04 (1%)	39.0 ± 1.0 (3%)
Calendered Cerex ($0.5\ oz/yd^2$)			
M.D.	2.47 ± 0.15 (6%)	4.90 ± 0.12 (3%)	52.0 ± 4.0 (8%)
T.D.	0.68 ± 0.13 (19%)	1.65 ± 0.56 (30%)	45.0 ± 7.0 (16%)

M.D. Machine Direction
T.D. Transverse Direction (Perpendicular to M.D.)
a Cuprophane (R-Bemberg). Data of Colton (9).
b Cerex (R-Monsanto).
c Samples soaked for 2 hours at 70°C in 0.2 N H_2SO_4, 1.3 N acetic acid, 6.0 wt. % formaldehyde (Section 4-F).

stronger than its weakest link," properties determined in the transverse direction, being generally poorer than in the machine direction, must be taken as the minimal criteria. The 0.4 oz/yd^2 fabric compares favorably with the Cuprophane (R-Bemberg).

Data for the 0.3 oz/yd^2 fabric indicate poorer properties than the 0.4 oz/yd^2 material, as should be expected. The inferior properties of the 0.5 oz/yd^2 fabric relative to the 0.4 oz/yd^2 material are most likely the result of the higher denier fiber used in the manufacture of the 0.5 oz/yd^2 fabric.

Calendered 0.3 oz/yd^2 fabric was soaked in a solution of acids and aldehyde representing the most stringent reaction conditions to be employed in membrane synthesis (Section 4-F). While the purpose of the experiment was to determine if any deterioration of the fabric would occur, in fact the properties improved considerably (Table 5-10), a most gratifying result. If it is recalled that the spun-bonded fabric is held together by hydrogen bonding at the random crossover points, the data suggest that the acid media caused a slight swelling of the nylon fiber and further fusion of the fibers at the crossover points.

Cerex (R-Monsanto) fabric of weight 0.4 oz/yd^2 is ideally suited for use as a hydrogel membrane support material. Additional advantages of the fabric were discussed in Section 4-F.

J. PERMEABILITY STUDIES ON HYDROGEL MEMBRANES

A total of twenty-one hydrogel membranes for permeation studies were cast on calendered Cerex (R-Monsanto) during the

course of this work. Careful examination of the finished membranes revealed that six of these contained bubbles ("pinholes") and could not be tested. This group unfortunately included all attempts to cast membranes with higher PVA contents. Of the remainder seven were prepared with heparin according to the composition in Table 3-2, six were prepared at the control composition (Table 3-1), and two were prepared with higher levels of acid (0.15 $\underline{N}$ H^+, other reactants as given in Table 3-1).

A complete set of permeability measurements were performed only on the heparin containing hydrogel membranes. The remaining membranes were used in stagnant diffusion studies and in developing the experimental techniques. All measurements were performed at 37°C.

The calendered support fabric used in the membrane production had a fabric weight of 0.4 oz/yd^2 (1.36 x 10^{-3}g/sq cm) and varied from piece to piece by $\pm$3%. Fabric thickness was 1.3 $\pm$ 0.3 mils, and the open area of the fabric is estimated at 65%.

Microscopic examination of the hydrogel membranes used revealed minor surface imperfections but no structures that appeared to penetrate into the hydrogel coating. The membranes appeared to have a continuous, uniform coating tightly adherent to the nylon fibers embedded within the hydrogel material.

Table 5-11 presents the results for studies on the heparin containing hydrogels (Table 3-2). Solute permeabilities from urea through polyethylene glycol (PEG) were measured using the

Table 5-11. Summary of Permeability Measurements.

Solutes	Membrane Resistance (min/cm) at 37°C: Cuprophane PT-150 (1.10 mil) [a]	Avisco Wet Gel (2.59 mil) [b]	Heparinized Hydrogel R_m (min/cm)	Heparinized Hydrogel Δx (mils)
Urea	17.0	18.2	22.4	5.60
Creatinine	30.8	30.6	32.6	
Uric Acid	32.8	33.6	39.6	
Sucrose	98.7	56.8	79.3	
Inulin (10 g/ℓ unlabelled)	522.	194.	324.	6.17
Dextran (10 g/ℓ unlabelled)	843.	288.	427.	
Peg (10 g/ℓ unlabelled)	7310.	1020.	956.	
Myoglobin	1.9×10^5 [c]	3.3×10^3 [c]	3.3×10^3	7.60
Albumin	4.2×10^5 [c]	1.2×10^5 [c]	1.5×10^4	

a R-Bemberg. Data from reference (9).

b R-FMC Corporation. Data from (9).

c Data from reference (9) corrected to 37°C.

batch dialyzer (Section 4-J), and the overall mass transfer coefficient was corrected for liquid phase resistance as discussed in Section 3-III-A using the correlation of Smith et al. (122). Values reported in the table are membrane resistance ($R_m = P_m^{-1}$ (min/cm)).

Data from the work of Colton (9) for Cuprophane PT-150 (R-Bemberg) are presented for comparison. Colton's data on the commercial cellulosis membrane showed a standard deviation in the range of ±5%, but the variation in the hydrogel permeability data of this work is estimated on limited data to be ±10%. This is most likely caused by the less uniform membrane (±0.9 mil) and the inherent reproducibility of the synthesis technique.

The data reported in Table 5-11 for myoglobin and albumin were taken using the stagnant diffusion cells (Section 4-K). On the basis of multiple determinations on both the control membrane (Table 3-1) and the high cyclic acetal membrane (0.15 N H^+) the accuracy of these data is in the range of ±40%. Colton reported similar deviations in his permeability data with the protein solutes. Additional permeability data taken with the stagnant diffusion cells is reported in Table 5-12.

Since it was found impossible to exactly reproduce the membrane thickness or to perform all measurements on one membrane specimen, the data are reported with the measured membrane thickness. A more relevant comparison of the data can be made by calculating the effective diffusivity of the solute in the membrane by

$$D_{eff} = P_m \Delta x \qquad (5\text{-}1)$$

Table 5-12. Summary of Protein Permeability Studies at 37°C.

Membrane	Myoglobin		Albumin	
	R_m (min/cm)	D_{eff} (cm^2/sec)	R_m (min/cm)	D_{eff} (cm^2/sec)
[a]Cuprophane PT-150 (1.10 mil)	1.9×10^5	2.5×10^{-10}	4.2×10^5	1.1×10^{-10}
[a]Avisco Wet Gel (2.59 mil)	3.3×10^3	3.3×10^{-8}	1.2×10^5	9.2×10^{-10}
Heparinized Hydrogel (7.60 mil)	3.3×10^3	9.8×10^{-8}	1.5×10^4	2.1×10^{-8}
Control Hydrogel				
7.0 mil	--	--	1.8×10^4	1.6×10^{-8}
7.8 mil	4.3×10^3	7.7×10^{-8}	--	--
8.2 mil	3.3×10^3	$11. \times 10^{-8}$	2.5×10^4	1.4×10^{-8}
High Cyclic Acetal Hydrogel				
5.1 mil	--	--	2.4×10^4	9.0×10^{-9}
6.4 mil	--	--	3.2×10^4	8.3×10^{-9}
4.0 mil	3.0×10^3	5.5×10^{-8}	--	--
4.8 mil	7.6×10^3	2.6×10^{-8}	--	--

a Data for Cuprophane (R-Bemberg) and Avisco Wet Gel (R-FMC) from reference (9). Values of R_m corrected to 37°C.

where Δx is the membrane thickness. Values of D_{eff} are reported along with membrane resistances in Table 5-12 for the protein studies.

Referring to Table 5-11 it can be seen that the permeabilities measured for the heparinized hydrogel membranes are nearly equivalent to Cuprophane (R-Bemberg) in the low molecular weight range and are significantly superior in the middle molecular weight range in spite of a fivefold difference in membrane thickness. The hydrogel membrane is also superior to Avisco Wet Gel (R-FMC) when compared on the basis of equivalent thickness. However, the wet gel cellophane is not widely used in hemodialysis because of its poor physical properties.

In Figure 5-12 the effective diffusivities for all solutes calculated according to Equation 5-1 are plotted as a ratio to the solute diffusivity in water (D_{H_2O}, Table 3-3), both values being at 37°C. This diffusivity reduction (D_{eff}/D_{H_2O}) is plotted as a function of the solute radius for permeation (Table 3-3). Curve A is for the heparinized hydrogel and curves C and B are for Cuprophane (R-Bemberg) and Avisco Wet Gel (R-FMC), respectively, from reference (9).

Figure 5-12 illustrates in graphical form the improved permeabilities of hydrogel membranes relative to the commercial cellulosic membranes. For all three membranes no clear "cutoff" exists, but rather a continuous decrease of the effective diffusivity as solute radius increases. This should be expected for any polymeric membrane with randomly oriented polymer chains and randomly distributed "tie points" (i.e., cross-links or crystallites).

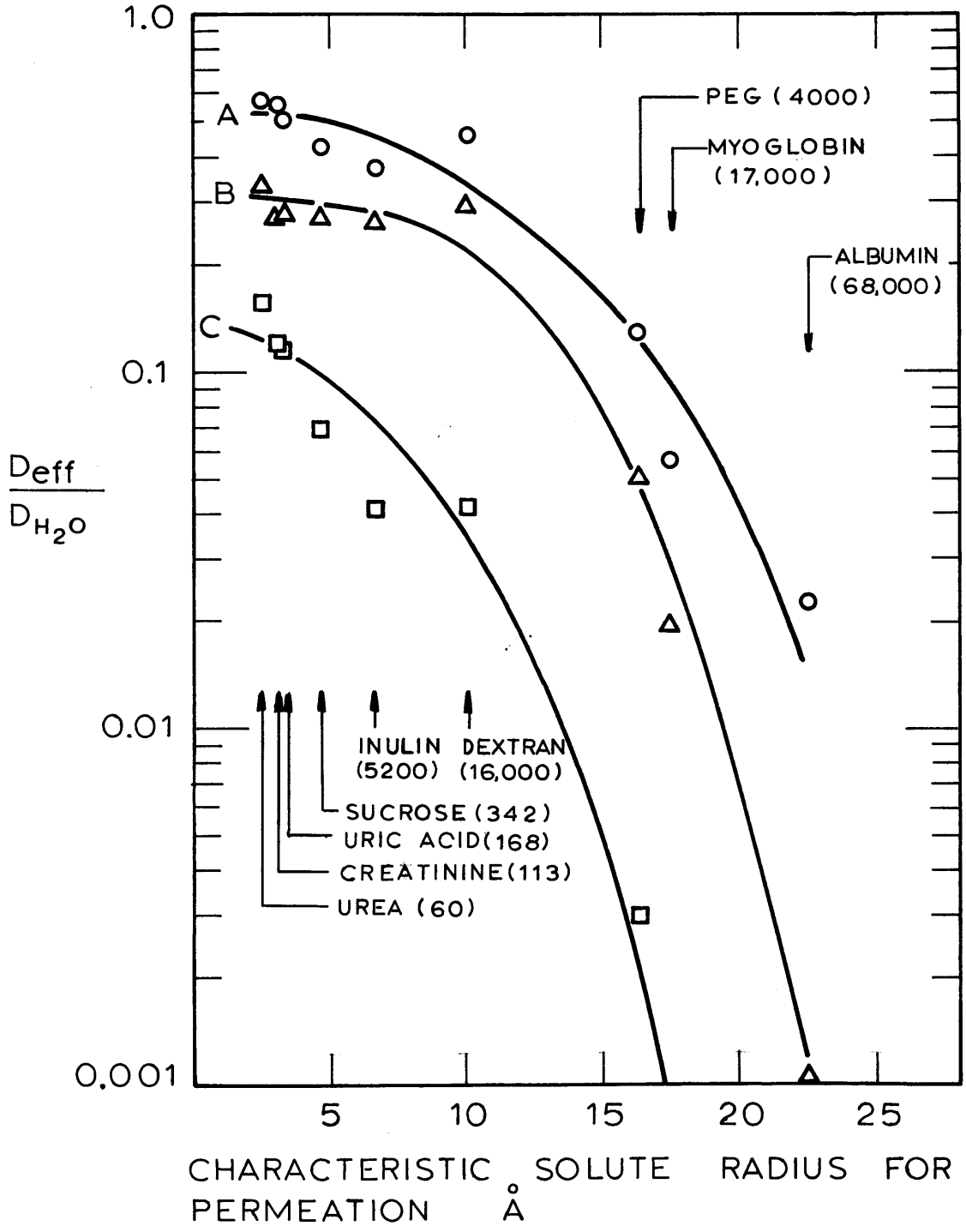


FIGURE 5-12 DIFFUSIVITY REDUCTION AS A FUNCTION OF MOLECULAR SIZE

It has been demonstrated in studies of the permeability characteristics of cellulosic membranes (123,132) that improved performance of a dialysis membrane can be attributed to a higher water content (i.e., lower volume fraction of polymer) in the membrane. The improved permeability characteristics of the heparinized hydrogel membranes relative to the commercial cellulosic membranes are undoubtedly due to their high water content. In volume fraction of water the values for the three membranes compared in Figure 5-12 and Table 5-11 are 0.645 for Cuprophane PT-150 (R-Bemberg), 0.818 for Avisco wet gel (R-FMC), and 0.947 (37°C) for the hydrogel phase of the fabric supported membrane.

The principal drawback to the application of the heparinized hydrogel membrane in hemodialysis is the inadequate "cut-off" of albumin, as shown in the data in Tables 5-11 and 5-12. For the standard criterion of a maximum protein loss per dialysis of one gram, which is about 1% of the patient's albumin, a treatment time of eight hours, and an effective membrane area of 0.5 square-meters, the maximum allowable albumin permeability is in the range of 10^{-5} cm/min. This is approximately an order of magnitude less than the values found for the hydrogel membranes.

Data in Table 5-12 shows that the effective diffusivity of albumin in the control hydrogel membrane is slightly less than in the heparinized material, which can be attributed to a slightly lower volume fraction of water (v_{H_2O} = 0.927 for hydrogel at 37°C). If the sole criterion to improve the albu-

min "cutoff" be a higher volume fraction of polymer, then the best way might be to increase the amount of cyclic acetal by using higher levels of acid catalyst. However, the data for the high cyclic acetal membrane (v_{H_2O} = 0.773 for hydrogel at 37°C) indicate only slight improvement. This observation suggests that at 37°C some association of the cyclic formals not detected in the modulus determinations has occurred to cause a nonhomogeneous material. It should be noted that this membrane was prepared at the highest level of acid used in any synthesis in this work. The point of the discussion is that a satisfactory hydrogel membrane must have a higher polymer content and must be homogeneous on a molecular level, i.e., no microphase separations.

K. IMPLICATIONS FOR FURTHER WORK

There are two important criteria to be met in further improvement of the supported heparinized hydrogels for application in hemodialysis.

1. The equilibrium polymer content of the membrane must be increased.
2. The hydrogel phase of the supported membrane must be homogeneous on a molecular level.

Unfortunately, many logical approaches to achieve one of the criteria tend to thwart the other. In this work the concept of the network shrinking during reaction to a relaxed state V_i at which the cross-links are unstressed was put forth. In general, the higher the polymer fraction at V_i, the higher will be the equilibrium polymer content of the finished mem-

brane, but the effect is compounded by the chain modification that occurs.

Variables that were found best to achieve this, namely, longer reaction times, higher formaldehyde concentrations, and most notably higher acid concentrations, accomplish the concentrating of the network by introduction of cyclic formals. It was demonstrated in this work that at least at the highest acid levels the cyclic formalization of network chains results in a nonhomogeneous structure on the molecular level. While it should be possible to optimize the two effects, one is still faced with the unappealing aspect of a material whose permeation properties would depend on temperature and which would undergo dimensional changes with temperature within a hemodialysis unit.

The alternative is to take advantage of those factors which will preserve network homogeneity and still increase equilibrium polymer content. Lower levels of acid and formaldehyde and higher concentration of glutaraldehyde and PVA could be used. While simply removing formaldehyde from the reaction mixture might appear as the ultimate solution, this is probably not feasible. By competing with glutaraldehyde in the formation of hemiacetals on the PVA in the room temperature reaction mixture, formaldehyde undoubtedly serves as a moderator in the reaction, preventing rapid gelation during the casting process. A rapid gelation could both introduce nonhomogeneities into the finished material and would tend to entrap air bubbles in the membrane structure.

CHAPTER 6

CONCLUSIONS

A. RUBBER ELASTICITY THEORY

1. The theory of rubber elasticity proposed in this work for application to networks formed in a solvated state proved to be a viable experimental method to study the effect of reaction variables on the hydrogel networks. Experimental tensile measurements showed excellent agreement with the theory at low sample extensions.

2. The concept of the relaxed state of the network represented by the system volume at the end of the reaction time was justified by the experimental data.

B. STUDY OF THE HYDROGEL CHEMISTRY

1. The importance of the two processes occurring during the hydrogel synthesis--cross-linking and chain modification--was demonstrated. Glutaraldehyde functions as the cross-linking agent by forming two cyclic acetals with PVA hydroxyls. While contributing little to the cross-linking of the PVA, formaldehyde modifies the network chains by formation of cyclic formals.

2. Higher amounts of cyclic formal in the network tend to increase the equilibrium volume fraction of polymer at any temperature. The thermal response of the hydrogels becomes more pronounced as cyclic formal content increases.

3. Glutaraldehyde cross-linking is a fast process relative to cyclic formalization by formaldehyde, but cyclic formalization has a more favorable equilibrium. Conditions which

favor the introduction of more cyclic formal--higher acid concentration, longer reaction time, higher molal ratios of formaldehyde to PVA--tend to decrease the amount of glutaraldehyde cross-linking per PVA chain.

4. Acetic acid serves as a solvent in the reaction mixture for cyclic formals, preventing association and possible phase separation.

5. Higher elastic modulus values achieved with higher PVA concentrations in the reaction mixture were explained on the basis of reduced molal ratios of formaldehyde to PVA, which permitted more glutaraldehyde cross-linking per PVA chain.

6. Heparin exerts a buffering effect on the reaction mixture, and this accounts for the properties observed with heparinized hydrogels. The residual heparin content may slightly affect the thermal shrinkage of the hydrogels.

C. MEMBRANE SUPPORT EVALUATION

Calendered nonwoven nylon fabric (0.4 oz/yd^2) was found to have superior tensile and tear-resistant properties relative to commercial cellulosic hemodialysis membranes. The material is stable in the conditions of the hydrogel synthesis and can be satisfactorily coated with hydrogel to serve as a potential hemodialysis membrane.

D. PERMEATION STUDIES

1. Heparinized hydrogel membranes cast with a nylon fabric support are equivalent to the standard cellulose hemodialysis membrane in the transport of low molecular weight solutes and superior in the transport of middle molecular weight

solutes.

2. The improved permeability characteristics of heparinized hydrogel membranes is attributed to their high water content.

3. The albumin "cutoff" of heparinized hydrogel membranes is insufficient to permit immediate application in hemodialysis therapy.

CHAPTER 7

RECOMMENDATIONS

1. The validity of the theory of rubber elasticity for application to networks formed in the presence of diluent proposed in this work should be tested over a wider range of polymer-solvent mixtures and amounts of cross-linking. Preferably a well-characterized polymer-solvent system should be chosen, as opposed to one with the complicated phase behavior of PVA-water.
2. Further study of the hydrogel system with the methods presented in this thesis should be pursued for various combinations of the reaction variables. Of particular interest are higher PVA concentrations, higher glutaraldehyde concentrations, and lower concentrations of acid.
3. Other acids such as p-toulene sulfonic acid should be substituted for the sulfuric acid. Being a more efficient catalyst for the acetal reaction, equivalent hydrogel properties might be achieved at higher pH and therefore with less heparin deactivation.
4. Studies such as proposed in (2) above should lead to an optimum composition for a hydrogel hemodialysis membrane with adequate retention of the plasma proteins.
5. The nonwoven nylon fabric evaluated in this work merits further consideration as a membrane support and in other biomaterial applications. Fabric prepared with nylon fiber containing no heat and light stabilizers should be used for <u>in vivo</u> applications.

APPENDIX A

DERIVATION OF THE THEORY OF RUBBER ELASTICITY FOR ISOTHERMAL DEFORMATIONS

The derivation presented in this section is based primarily on the discussion by Meares (103), the review paper by Ciferri (112), and the text of Flory (76). Specific references are cited in the text.

A. THERMODYNAMICS OF STRESS AND STRAIN FOR CROSS-LINKED ELASTOMERS

When a cross-linked elastomer is subjected to a sudden stress, it reaches a state of equilibrium strain almost instantaneously and, similarly, the stress remains constant for an indefinite time in a sample maintained in a state of constant strain. Such equilibrium situations naturally lend themselves to a thermodynamic treatment and this has provided an invaluable basis for the study of highly elastic behavior. Actually, the experimental attainment of a true state of equilibrium is not quite as simple as was at one time supposed. Practical difficulties, however, once recognized, can usually be overcome and in the best studies of the deformation of rubber and synthetic elastomers it may confidently be accepted that reversible processes have been examined.

For a reversible process the first and second laws of thermodynamics may be combined to give the following relationship among internal energy E, entropy S, absolute temperature T, and the work W done by the system:

$$dE = TdS - dW \tag{A-1}$$

If a linear force f acts upon a sample of length L, the work

done by the system during a small extension dL is -fdL. If the volume of the system increases by dV as a result of this strain, then

$$dW = -fdL + PdV \tag{A-2}$$

where P is the external pressure.

The Helmholtz free energy A is defined by:

$$A = E - TS \tag{A-3}$$

Differentiating and combining with Equations (A-1) and (A-2) gives:

$$dA = -PdV + fdL - SdT \tag{A-4}$$

from which it follows that

$$f = \left(\frac{\partial A}{\partial L}\right)_{T,V} \tag{A-5}$$

For an amorphous polymer deformed by an external force f, the accompanying volume change is extremely small, which results in the PdV term being negligible at ordinary pressures (107).

B. STATISTICS OF A SINGLE CHAIN

The first step in the theory of rubber-like elasticity is the derivation of the statistical properties of a single chain. It is necessary to know quantitatively the free energy of the molecule as a function of its end-to-end distance r and a quantitative relation between this distance and the force necessary to maintain it. Because of Brownian motion of the chain, the distance r must be considered an average value of the end-to-end distances during the period of application of the force f.

For real chains with hindered rotations, the configurations which are consistent with normal bond lengths and angles,

and which give rise to a particular chain-end displacement vector $\underline{r}$, do not all have the same internal energy and hence do not have equal probabilities. A configurational integral $Z(r,T)$ may be set down (133) for a chain of n bonds which are represented by the vectors $\underline{\ell}_1, \underline{\ell}_2, \ldots \underline{\ell}_n$, where

$$\underline{r} = \sum_{i=1}^{n} \underline{\ell}_i \qquad \text{(A-6)}$$

If $\varepsilon(\underline{\ell})$ is the internal energy associated with a particular set of values of $\underline{\ell}_i$, then

$$Z(r,T) = \int \cdots \int \exp[-\varepsilon(\underline{\ell})/_{kT}] \; d\underline{\ell}_1 \; d\underline{\ell}_2 \cdots d\underline{\ell}_n \qquad \text{(A-7)}$$

where the integration is taken over all possible values of the bond vectors $\underline{\ell}_i$ which are consistent with the constant displacement vector $\underline{r}$.

The Helmholtz free energy A of the chain with displacement vector $\underline{r}$ (134) is

$$A = -kT \ln Z(r,T) + \text{constant} \qquad \text{(A-8)}$$

where the constant involves all the nonconfigurational degrees of freedom of the chain which are unaltered by changes of configuration. Replacing L by r in the relation between A and tension (Section A) gives

$$f = \left(\frac{\partial A}{\partial r}\right)_{T,V} = -kT \left(\frac{\partial \ln Z(r,t)}{\partial r}\right)_{T,V} \qquad \text{(A-9)}$$

in which only the magnitude of r and not its direction is important.

For a chain without constraints, the probability that $\underline{r}$ lies between $\underline{r}$ and $(\underline{r} + dr)$ is given by

$$W(\underline{r})dr = \frac{Z(r,T)dr}{\int_0^\infty Z(r,T)dr} \tag{A-10}$$

Multiplying by $4\pi r^2$ removes the directional restriction on $\underline{r}$, hence

$$W(r)dr = \frac{Z(r,T)dr}{\int_0^\infty Z(r,T)dr}\, 4\pi r^2 \tag{A-11}$$

Rearranging the equation and taking logarithms gives

$$\ln Z(r,T) = \ln W(r) + \ln \int_0^\infty Z(r,T)dr - \ln 4\pi - \ln r^2 \tag{A-12}$$

and on differentiation

$$\left(\frac{\partial \ln Z(r,T)}{\partial r}\right)_T = \left(\frac{\partial \ln W(r)}{\partial r}\right)_T - \frac{2}{r} \tag{A-13}$$

since $\int_0^\infty Z(r,T)dr$ is independent of r.

The distribution function W(r) can be approximated by the Gaussian function (102)

$$W(r)dr = (\beta/\sqrt{\pi})^3 \exp\{-\beta^2 r^2\}\, 4\pi r^2\, dr \tag{A-14}$$

Hence
$$\left(\frac{\partial \ln Z(r,T)}{\partial r}\right)_T = -2\beta^2 r \tag{A-15}$$

where for real chains β is a parameter of the equivalent random chain. Using the concept of the equivalent chain (96,135) permits expression of β in terms of $\overline{r_o^2}$, the mean square displacement length of the equivalent random chain.

$$\beta^2 = \frac{3}{2\overline{r_o^2}} \tag{A-16}$$

Substituting Equation (A-15) and Equation (A-16) into Equation (A-9) gives the equation of state

$$f = \frac{3kTr}{\overline{r_o^2}} \tag{A-17}$$

which is valid over the range for which the Gaussian distribution is valid for the equivalent random chain. Since it has not been assumed that all chain configurations have the same internal energy or a priori probability, the derivation of this equation of state is general in the statistical thermodynamic sense.

C. ELASTICITY OF THE NETWORK

The second step in the theory consists in calculating the free energy A_{el} of the network as a function of the macroscopic parameters which characterize the deformation. Since the network is formed by connecting single polymeric chains through the cross-links, the derivation ought to begin from the expression for the elastic retractive force f of a single chain (eqn. A-17). Moreover, the presence of these links eliminates any distinction among the initial macromolecules; consequently, the network can be considered an ensemble of subunits, each subunit being that portion of an original macromolecule extending from one cross-link to the next. It has to be assumed that the degree of cross-linking of the network is sufficiently low for the mean lengths of these subunits between cross-links to be long in the sense that a statistical treatment can be applied to enumerate their configurations. Treloar (136) has shown that twenty-five or more freely-jointed links or their equivalent between cross-links is sufficient to justify the statistical treatment.

Only those subunits which extend between cross-links will be effective in determining the retractive forces of the cross-

linked sample, and these will be denoted as ν_e. In an actual elastomer inactive material is also present, such as loose ends of chains, rings which are connected to the active network at only one point, and some macromolecules not joined to the network at all. This inactive material is effective only in determining the volume occupied by the system. The configurations of these inactive subunits are not altered when the fixed points move relative to one another when the network is strained. Hence they do not contribute to the entropy of deformation. In the theory it is assumed that the behavior of the system is the sum of the contributions of the ν_e subunits.

To develop the theory, it is necessary to assume some correlation between macroscopic dimensions of the sample and the set of r values. Since the material is initially isotropic, the simplest assumption is that the components of the vectorial length of each subunit are changed by the deformation in the same ratio as the corresponding dimensions of the sample. This is the so-called "affine" deformation (107,76). Consider a subunit having one end at the origin of a rectangular coordinate system and the other end at a distance r_i away, with coordinates (x_i,y_i,z_i). A deformation modifies the conformation of the subunit in such a way that the distance between the two ends is r, with one end having coordinates (x,y,z) while the other remains fixed at the origin. The initial macroscopic sample, assumed to be a unit cube, is changed by the deformation (at constant volume) into a parallelepiped having edges α_x, α_y, α_z in the directions of the x, y, and z axes respec-

tively. That deformation for which $x = x_i \alpha_x$, $y = y_i \alpha_y$, and $z = z_i \alpha_z$ is considered affine.

If the network consists of ν_e active subunits of mean square length $\overline{r_i^2}$ in the isotropic unstrained state and of mean length $\overline{r^2}$ in the unstrained state, then for such an affine deformation,

$$\overline{r^2} = \frac{1}{3} (\alpha_x^2 + \alpha_y^2 + \alpha_z^2)\overline{r_i^2} \tag{A-18}$$

Recalling that for an isothermal process

$$\Delta A = -W \tag{A-19}$$

the total work done by the subunits is given by (using Equation A-16)

$$-W = 3\nu_e \left[\frac{kT}{\overline{r_o^2}}\right] \int_{(\overline{r_i^2})^{1/2}}^{(\overline{r^2})^{1/2}} r \, dr = \Delta A_{el} \tag{A-20}$$

ΔA_{el} is the change in the Helmholtz free energy of the network due to the deformation. Hence,

$$\Delta A_{el} = \nu_e \frac{kT}{2} \left(\frac{\overline{r_i^2}}{\overline{r_o^2}}\right) \left(\alpha_x^2 + \alpha_y^2 + \alpha_z^2 - 3\right) \tag{A-21}$$

Consider a unidirectional elongation in which the sample, originally of length L_o, is elongated to L. Let $\alpha = L/L_o = \alpha_x$. For this deformation at constant volume $\alpha_x \alpha_y \alpha_z = 1$; consequently $\alpha_y = \alpha_z = \alpha^{-1/2}$. Therefore,

$$\Delta A_{el} = \nu_e \frac{kT}{2} \left(\frac{\overline{r_i^2}}{\overline{r_o^2}}\right) \left(\alpha^2 - \frac{2}{\alpha} - 3\right) \tag{A-22}$$

$$f = -\left(\frac{\partial A}{\partial L}\right)_{T,V} = -\frac{1}{L_o}\left(\frac{\partial A}{\partial \alpha}\right)_{T,V} \qquad \text{(A-23)}^*$$

Differentiating Equation (A-22) gives the retractive force for the case of simple elongation:

$$f = \frac{\nu_e kT}{L_o}\left(\frac{\overline{r_i^2}}{\overline{r_o^2}}\right)\left(\alpha - \frac{1}{\alpha^2}\right) \qquad \text{(A-24)}$$

The force can be converted to stress by dividing by the initial cross-sectional area V/L_o where V is the volume of the sample.

$$\frac{f}{A_o} = \tau = R\,T\left(\frac{\nu_e}{V}\right)\left(\frac{\overline{r_i^2}}{\overline{r_o^2}}\right)\left(\alpha - \frac{1}{\alpha^2}\right) \qquad \text{(A-25)}$$

Replacing Boltzmann's constant k by the gas constant R simply means the number of effective subunits ν_e will be expressed in moles.

---

*An approximation, which actually is quite negligible, is involved in letting $(\partial A/\partial L)_{T,V} = (\partial A/\partial \alpha)_{T,V}/L_o$, since α is defined as L/L_o where both lengths are measured at the same temperature and pressure (137).

APPENDIX B

RUBBER ELASTICITY THEORY APPLIED TO SWOLLEN HYDROGEL SAMPLES

The derivation presented here parallels the one presented by Flory (76), but has been modified by the author for swelling of a sample initially cross-linked in the presence of solvent. Assume that the network formed at volume V_i and volume fraction polymer $v_{2,i}$ is subsequently swollen isotropically be a diluent to a volume V_s.

$$v_{2,s} = v_{2,i} \left(\frac{V_i}{V_s}\right) \tag{B-1}$$

For deformation due to elongation the constant volume assumption is used, i.e.,

$$V_s = \text{constant} \tag{B-2}$$

during deformation. Let α_x, α_y, and α_z represent the changes in dimensions x, y, and z resulting from the combination of swelling and deformation. Therefore,

$$\alpha_x \alpha_y \alpha_z = \frac{1}{(V_i/V_s)} \tag{B-3}$$

which is constant during deformation due to elongation. Define a swelling factor

$$\delta = (V_i/V_s) \tag{B-4}$$

hence

$$\alpha_x \alpha_y \alpha_z = \frac{1}{\delta} \tag{B-5}$$

Considering the case of a simple elongation in the direction of the x-axis, let α represent the length in this direction relative to the swollen, unstretched length $L_{o,s}$, i.e.,

$$\alpha = \frac{L_{f,s}}{L_{o,s}} \tag{B-6}$$

Since swelling is isotropic,

$$L_{o,s} = (V_s/V_i)^{1/3} L_o \tag{B-7}$$

where L_o represents the unswollen, unstretched gauge length.

Then $$\alpha_x = \alpha(V_s/V_i)^{1/3} = \alpha/\delta^{1/3} \tag{B-8}$$

and, since $\alpha_x \alpha_y \alpha_z = \delta^{-1}$,

$$\alpha_y = \alpha_z = \left(\frac{1}{\alpha_x \delta}\right)^{1/2} = \frac{1}{\alpha^{1/2} \delta^{1/3}} \tag{B-9}$$

Recall equation (A-21) which gives the change in the Helmholtz free energy of the network due to the deformation:

$$\Delta A_{el} = \frac{\nu_e kT}{2} \left[\frac{\overline{r_i^2}}{\overline{r_o^2}}\right]_o \left(\alpha_x^2 + \alpha_y^2 + \alpha_z^2 - 3\right) \tag{A-21}$$

The subscript o denotes the value of $\left(\overline{r_i^2}/\overline{r_o^2}\right)$ at the temperature of interest in the absence of diluent. Introducing relations (B-8) and (B-9) into equation (A-21) gives

$$\Delta A_{el} = \frac{kT\nu_e}{2\delta^{2/3}} \left[\frac{\overline{r_i^2}}{\overline{r_o^2}}\right]_o \left(\alpha^2 + \frac{2}{\alpha} - 3\delta^{2/3}\right) \tag{B-10}$$

The elastic retractive force for the swollen network is

$$f = -\frac{1}{L_{o,s}} \left(\frac{\partial A}{\partial \alpha}\right)_{T,V} \tag{B-11}$$

Differentiating equation (B-10) gives

$$f = \frac{kT\nu_e}{\delta^{2/3} L_{o,s}} \left[\frac{\overline{r_i^2}}{\overline{r_o^2}}\right]_o \left(\alpha - \frac{1}{\alpha^2}\right) \tag{B-12}$$

since δ is constant during elongation. The initial swollen, unstretched cross-sectional area is $A_{o,s} = V_s/L_{o,s}$. The stress

τ expressed as force per unit area of the swollen, unstretched sample is:

$$\frac{f}{A_{o,s}} = \tau = RT\left(\frac{\nu_e}{V_s}\right)\delta^{-2/3}\left[\frac{\overline{r_i^2}}{\overline{r_o^2}}\right]_o\left(\alpha - \frac{1}{\alpha^2}\right) \qquad \text{(B-13)}$$

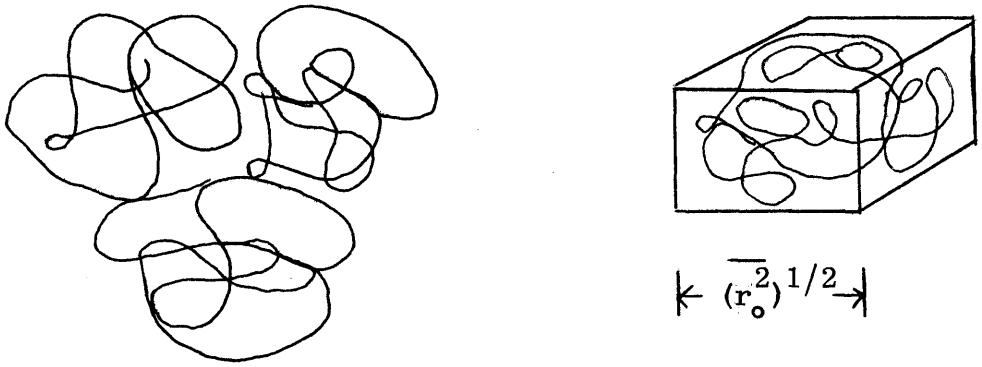
where (ν_e/V_s) is moles of effective chains per unit swollen volume. Similarly the equation can be written for ν_e/V_i, moles of effective chains per unit of initial volume:

$$\tau = RT\left(\frac{\nu_e}{V_i}\right)\delta^{1/3}\left[\frac{\overline{r_i^2}}{\overline{r_o^2}}\right]_o\left(\alpha - \frac{1}{\alpha^2}\right) \qquad \text{(B-14)}$$

APPENDIX C

CALCULATION OF THE CRITICAL CONCENTRATION FOR POLYVINYL ALCOHOL IN WATER

The quantity $C_{2,\ critical}$ is defined as the concentration at which the polymer coils in solution just touch one another.



Brandrup and Immergut (118) report the following value for the parameter $(\overline{r_o^2}/M)^{1/2}$ for PVA in water at 30°C with $(\overline{r_o^2})^{1/2}$ in Å and M in g/gmole.

$$(\overline{r_o^2}/M)^{1/2} = 0.95$$

For a molecular weight $\overline{M}_n = 100{,}000$,

$$(\overline{r_o^2})^{1/2} = 300\text{Å}$$

This value may be used as approximation for the edge of a cube of solvent that would just contain the coil as diagramed above. Hence, $C_{2,critical}$ may be calculated:

$$C_{2,crit} = \frac{\overline{M}_n/\text{Navagadro}}{(\overline{r_o^2})^{3/2}}$$

$$= \frac{(10^5\ \text{g/gmole})/(6.02\text{x}10^{23}\ \text{molecules/gmole})}{(300\text{Å})^3\ (\text{cm}^3/10^{24}\ \text{Å}^3)}$$

$$C_{2,crit} = 0.006\ \text{g/cm}^3$$

or approximately 0.6% weight if density is taken as $\rho = 1.0$.

APPENDIX D

COMPOSITION OF BUFFERED SALINE

0.08 M NaCl

0.03 M Na_2HPO_4

0.01 M KH_2PO_4

200 PPM formaldehyde added as a bacteriostat

pH ≃ 7.3

For permeation studies with proteins 5×10^{-4} M KCN was used as a bacteriostat in place of the formaldehyde.

APPENDIX E

LOCATION OF ORIGINAL DATA

The data and calculations of this thesis are contained in laboratory notebooks and computer printout on file with Professor E. W. Merrill, Room 12-108, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

APPENDIX F

ANALYSIS OF STOCK ALDEHYDES

Some amount of time was spent in finding suitable analytic techniques to use on the stock solutions of formaldehyde and glutaraldehyde. The general reviews of Mitchell (138) and Reynolds and Irwin (139) were helpful in the pursuit, but unfortunately both the reviews and the original papers on specific techniques did not contain sufficient detail to clearly assess the reliability of one technique relative to another. The sodium sulfite procedure (140) was found best for formaldehyde and the hydroxylamine hydrochloride technique (140) proved most satisfactory for glutaraldehyde.

A. SODIUM SULFITE PROCEDURE FOR FORMALDEHYDE

Because of its accuracy, simplicity, and rapidity, the sodium sulfite procedure is probably the best procedure for the determination of formaldehyde in commercial formalin solutions. The method is based on the quantitative liberation of sodium hydroxide when formaldehyde reacts with sodium sulfite to form the formaldehyde-bisulfite addition product:

$$CH_2O + Na_2SO_3 + H_2O \rightarrow NaOH + CH_2(Na_2SO_3)OH$$

Procedure

From a fresh stock bottle of formalin three samples of 5-6 grams each were precisely weighed into 100 ml volumetric flasks and diluted to the mark with distilled water. After careful mixing on a magnetic stirrer, the following procedure was followed for each volumetric flask (designated I, II, III).

1. Neutralize the 100 ml to pH = 7.0 using pH meter

and a few drops of acid or base as required.

2. Three titrations of each sample flask. For each:
   a. 50 ml of 1 M Na_2SO_3 plus 4 drops thymolphthalein indicator neutralized with acid (one or two drops) to colorless.
   b. Add exactly 25.0 ml of sample from the volumetric flask.
   c. Stir ten minutes, then add distilled water ice to cool the solution.
   d. Titrate with 1.00 N H_2SO_4.
3. Computation

$$\text{wt \% } CH_2O = \frac{(\text{net ml})(1 \text{ gmole/liter}) \times (30.03\text{g } CH_2O/\text{gmole})}{(10^3 \text{ml/liter})(\text{g sample})(\frac{\text{sample aliquot}}{100.0 \text{ ml}})} \times 100\%$$

Ten minutes was found sufficient to assure equilibrium in the formation of the formaldehyde-bisulfite addition product. Longer mixing times did not affect the results. For nine titrations performed on samples from one stock formalin bottle (three volumetric sample flasks times three titrations on each) the results were found to have a percentage error of ±0.2%, e.g., (36.44 ± 0.09)% error ±0.2%.

B. HYDROXYLAMINE HYDROCHLORIDE METHOD FOR GLUTARALDEHYDE

Hydroxylamine hydrochloride reacts with the carbonyl groups of aldehydes and ketones forming an oxime and liberating hydrochloride acid. With glutaraldehyde the reaction may be written:

$$OHC\cdot(CH_2)_3\cdot CHO + 2NH_2OH\cdot HCl \rightarrow$$
$$HON{:}HC\cdot(CH_2)_3\cdot CH{:}NOH + 2H_2O + 2HCl$$

Procedure

1. Ten milliliters of a 10 wt % aqueous solution of $NH_2OH \cdot HCl$ are added to two 125 ml Erlenmeyer flasks. One is a blank and the other will be the sample flask.
2. Into the sample flask precisely weigh one gram of the concentrated glutaraldehyde solution to be analyzed.
3. Stir both flasks for 20 minutes and then titrate with 1.00 N NaOH with three drops bromophenol blue as indicator. The end-point is marked by a color change from yellow to light purple.
4. Computation

net ml = (sample ml) - (blank ml)

$$\text{wt \% G} = \frac{(\text{net ml})(1.00 \frac{\text{gmole}}{\text{liter}} \text{NaOH})(\frac{100.12\text{g}}{\text{gmole}}) \times 100\%}{(10^3 \text{ml/liter})(\text{sample wt})(2 \text{ aldehydes/G})}$$

For three samples from one bottle of the concentrated glutaraldehyde solution, the results were found to have an error of $\pm 0.04\%$, e.g.,

$(50.40 \pm 0.02)\%$ error $\pm 0.04\%$

APPENDIX G

SOLIDS DETERMINATION

Approximately 10g of the PVA-water solution to be analyzed was precisely weighed into an aluminum weighing pan of known tare weight. Usually three samples were run on a given 1500g batch of PVA stock solution. The weighing pans containing the polymer solution were placed in a thermostatted vacuum dessicator at 100°C and dried to constant weight (usually 24 hours was sufficient). Final weights were recorded and the percent solids calculated in a straightforward manner:

$$\% \text{ solids} = \left[\frac{\text{final wt - tare wt}}{\text{initial wt - tare wt}}\right] \times 100\%$$

Percent polymer was assumed equal to the percent solid, which should be justified in light of the analytic chemistry results cited in Section (4-B-6). Typical results on three samples were:

$$(8.80 \pm 0.02)\% \quad \text{error} \pm 0.2\%$$

APPENDIX H

SOLUTES USED IN PERMEATION STUDIES

SOLUTE	SUPPLIER	CATALOGUE NO.
Urea-C^{14}	NEN	NEC-108
Creatinine-4-C^{14}	ICN	17126
Uric acid-2-C^{14}	ICN	13057
Sucrose-C^{14}	NEN	NEC-100
Inulin-carboxyl-C^{14}	NEN	NEC-164P
Dextran-carboxyl-C^{14}	NEN	NEC-218A
Polyethylene-1, 2-C^{14}-glycol	NEN	NEC-473
Myoglobin	S/M	1155, horse heart, 2X crystallized
Albumin	CBC	126658, human, crystallized, electrophoretically pure

NEN New England Nuclear, Boston, Massachusetts.
ICN International Chemical and Nuclear Corporation, Irvine, California.
S/M Schwarz/Mann, Orangeburg, New York.
CBC Calbiochem, Los Angeles, California

APPENDIX I

MICRO-BIURET ANALYSIS FOR PROTEINS

The micro-Biuret technique described here is based on the paper of Itzhaki and Gill (141) as modified by Bellantoni (142).

The micro-Biuret reagent was prepared by adding one part of a 1.05 wt % solution of ($CuSO_4 \cdot 10H_2O$) in saline[1] to four parts of a 30 wt % solution of NaOH in saline. This solution was designated working solution A, and working solution B was 30 wt % NaOH in saline.

Reference solutions were prepared for each analytic run as follows:

A_1 2.0 ml saline + 1.0 ml working solution A.

B_1 2.0 ml saline + 1.0 ml working solution B.

In the analysis of albumin the sample should contain between 0.05 to 0.10 wt % of albumin, and for myoglobin the sample concentration should be in the range of 0.005-0.05 wt %. Dilutions, if necessary, are done with saline.

With the concentrations in the ranges specified above, sample solutions for analysis were prepared as follows:

A_2 2.0 ml sample + 1.0 ml working solution A.

B_2 2.0 ml sample + 1.0 ml working solution B.

Absorbence was measured on a Coleman Junior II Spectrophotometer (Model 6/35) at 325 mμ as follows:

---

1. Saline refers to physiological saline which is an aqueous solution of 0.85 wt % NaCl.

Read A_2 standardized against A_1.

Read B_2 standardized against B_1.

Optical density (O.D.) = $A_2 - B_2$, and was found linearly proportional to the wt % protein present in the ranges specified. If the B_2 reading was greater than 0.05, ultraviolet absorbers other than protein were present in the sample, and the results were considered invalid.

Standard working curves were prepared for both the albumin and the myoglobin and were linear in the ranges specified. Since they are not of general utility, being a function of the spectrophotometer used, they will not be included here.

APPENDIX J

NOMENCLATURE

This appendix lists the more important variables in the body of this thesis. Additional variables which were used only in specific derivations are defined in the text.

A	transport area (cm^2)
A_o	initial cross-sectional area (cm^2)
$A_{o,s}$	initial cross-sectional area (sq cm), swollen specimen
$C_{2,i}$	concentration of polymer (g/cc) in reaction volume
C_1, C_2	concentration of solute in permeability studies for the side of high concentration 1 and the side of low concentration 2
D	solute diffusivity in water (cm^2/sec)
D_{eff}	effective diffusivity
E	Young's Modulus
f	elastic retractive force (dynes)
ΔF	Gibbs free energy change
k_f	liquid phase mass transfer coefficient
K_o	overall mass transfer coefficient
L	length (cm)

L_o	gauge length (cm)
M	molecular weight (g/g-mole)
M_c	molecular weight of network subchain
$\overline{M}_n$	number average molecular weight
N	number of primary polymer chains of molecular weight M in the network
$\dot{N}$	mass transport rate
P_m	membrane permeability
R	gas constant
R_m	membrane resistance (P_m^{-1})
$(\overline{r_i^2}/\overline{r_o^2})$	"front factor"; ratio of the mean square end-to-end distance for the network subchains in the undeformed, isotropic state ($\overline{r_i^2}$) to the corresponding mean square end-to-end distance for the undeformed subchains in the absence of cross-links ($\overline{r_o^2}$).
t	time
T	temperature (°K)
$\overline{v}$	specific volume of polymer (cc/g)
v_2	volume fraction of polymer in swollen network (cc polymer/cc hydrogel)
V	specimen volume (cc)
V_i	hydrogel reaction volume; relaxed volume of network

V_r volume of replacement solution (permeability studies)

V_p volume of polymer (unswollen)

V_1 solvent molar volume

V_1, V_2 chamber volumes for batch dialyzer

$w_{2,0}$ weight fraction of polymer in initial reaction mixture

W weight

Δx membrane thickness

x,y,z axial coordinates

Greek Symbols

α extension parameter ($=L/L_o$)

δ swelling factor ($=V_i/V_s$)

ν number of cross-linked subchains of molecular weight M_c in the network

ν_e number of effective subchains

ρ density

τ stress (dynes/sq cm)

χ_1 polymer-solvent interaction parameter

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BIOGRAPHICAL NOTE

Jeffrey Earl Silliman, son of Mr. and Mrs. Earl Silliman, was born and reared in and about a small town in Pennsylvania named Palmerton. He entered Lehigh University in Bethlehem, Pennsylvania, in September 1964 and received his Bachelor of Science degree in chemical engineering with honors in June 1968.

Entering M.I.T. in the fall of 1968, he passed the doctoral qualifying examinations in May 1969. While at M.I.T. he has held appointments both as teaching and research assistant. His minor field of study was management.

During his first year at M.I.T. the author met the former Julie Ann Barter whom he married in the summer of 1970. Since January 20 of this year, their household has been expanded to include Jonathan Andrew with whom the author has since shared many a midnight bottle after an evening in the lab.

The author has accepted a position as Research Engineer with the Deering Milliken Research Corporation in Spartanburg, South Carolina.