BIODEGRADABLE ADHESIVES FOR ORTHOPEDIC SURGERY

by

DENNIS P. ORGILL

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Certified by Signature redacted

Professor I.V. Yannas
Thesis Supervisor

Accepted by Signature redacted

Warren M. Rohsenow
Chairman, Departmental Graduate Committee

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ABSTRACT

An experimental investigation was carried out to see if certain protein adhesive systems could be used in orthopedic surgery. Several proteins were tested including albumin, gelatin, and collagen. Common to all these systems are low wet strengths when conventional cross-linking procedures are used. Dehydrothermal cross-linking, photo-initiated cross-linking, and glutaraldehyde cross-linking in an acetone bath were investigated as alternative cross-linking procedures with gelatin and collagen. Other methods of strengthening the polymer system such as orientation crystallinity, and adding a reinforcing material were investigated.

A gelatin-collagen composite was found to have an ultimate tensile wet strength of over 1,000 p.s.i. and is recommended for further study as a biodegradable orthopedic adhesive.

Thesis Supervisor: I.V. Yannas
Title: Professor of Polymer Science and Engineering

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Chapter 1 INTRODUCTION

Treatment of bone fractures is one of the major problems which the medical profession faces. A broken bone typically restricts an individual for six to twelve weeks. As medical progress has developed, several methods of treating fractures have evolved. There are many methods of treating fractures used today, each having certain advantages and disadvantages.

The treatment of a fracture depends on the location of the fracture, type of fracture, age and health of the individual, and treatment alternatives available (1). To illustrate the complexity of the decision process, the treatment alternatives of a tibial fracture will be discussed.

Non-invasive treatments generally involve external immobilization by the use of a cast. The fact that these methods do not involve any of the risks of surgery makes this method of treatment attractive to many. Advocates of this method often claim that the weight bearing which can be applied while the leg is in the cast is a stimulant to bone regeneration and healing. External fixation carries risks which also must be considered. Immobilizing the leg often does not sufficiently immobilize the bone, and misalignment, and non-union can occur. This method of treatment also has frequent complications of pulmonary

emboli, stiffness and edema.

Invasive methods most commonly use one of three stainless steal prostheses: fixation plates, compression plates or intramedullary nails (2). These devices provide much better immobilization of the bone, and the patient is able to ambulate sooner. Because the moduli of elasticity is much greater for steal than for bone, most of the load bearing is transferred across the prosthesis. This seems to be of benefit in the early stages of healing, but has detrimental effects in later stages. It is thought that the removal of physiologic stress acting on the bone causes resorption of bone around the prostheses in later stages of healing. Often these devices are surgically removed following the initial stages of healing to avoid the bone weakening.

1.1 Fracture Healing

Two types of healing mechanisms have been described in the literature (3,4). One is referred to as contact healing where the osteoid grows directly across the fracture surface. The second and clinically most common mechanism involves the formation of a callus around the outside of the bone. Depending upon the conditions of healing, this callus consists of different combinations of fibrous tissue, calcified cartilage, woven bone, and lamellar bone. In a fracture where movement is restricted, fibroblasts

lay down a fibrous tissue which is composed mainly of collagen. This tissue is replaced with woven bone which is in turn replaced with lamellar bone. As time proceeds, this callus is gradually remodeled and resorbed, and the bone returns to its normal configuration.

1.2 Adhesives in Orthopedic Surgery

The use of adhesives in fracture healing is an idea which has long intrigued physicians. For someone to come with a "super glue", which would give bone its original strength, has been the dream of many orthopedists. Past attempts at finding such an adhesive have been relatively unsuccessful.

1.2.1 Polyurethane

The best documented case of an attempt to glue bones together in patients was reported by Redler (5). In this study, the fractures of 42 patients were treated with a polyurethane adhesive system. The adhesive was found to have a compressive strength of 2,000 p.s.i., a shear strength of 500 p.s.i., and a tensile strength of 800 p.s.i. Of the 42 treatments with polyurethane, 37 were considered failures. Of the patients which success was claimed, many had problems with infection, and some used intramedullary rods as adjuncts. Although the results of this study are discouraging, they point to areas of future research for

bone glues.

Failure was claimed to occur due to either a fracture of the intramedullary polymer column, or a failure at the bone-polymer interface. Histologic evidence showed no resorption of the polymer even up to three years post-operatively. Even though polyurethane is a strong engineering material, its use in a biological system was unsuccessful.

1.2.2 Polymethylmethacrylate (PMMA)

PMMA is the bone cement currently used by orthopedic surgeons. The main applications of this material are the implantation of joint prostheses, as an adjunct to stainless steel prostheses in fracture healing, and the replacement of skeletal parts. This adhesive is appealing to orthopedists because of its high strength and easy applicability, and has been fairly successful in many applications. In fracture healing, this cement has shown the greatest success in the treatment of pathologic fractures due to metastatic bone disease (6,7). In this application, the patient is able to mobilize soon after the operation making the last few months of life much less painful.

PMMA has also claimed to be effective in reducing mechanical stresses between implants and bone due differences in moduli of elastiscity. Charnley (8) reports a

reduction in fretting in a biomechanical analysis of femoral head prostheses when PMMA is used.

PMMA has several limitations. It forms a mechanical bond with bone, by polymerizing within bone irregularities, and trabeculae. The fact that a chemical bond is not formed often results in a weak interface. The polymerization of PMMA is exothermic, and the resulting high temperatures have been implicated in causing local bone necrosis. The methylmethacrylate monomer is also toxic. Not only is it responsible for local tissue damage, but cardiovascular and systemic effects have been reported (9,10). After time, the interface can loosen, and the mechanical bond fails. Yablan (11), in a study of fracture healing using methylmethacrylate, observed that bone in the area of the cement was necrotic and failed to uptake tetracycline or Ca-45. PMMA is also not biodegradable.

Because of the good strength and application properties of PMMA, many investigations have looked at ways of improving PMMA. Iida (12) has added tri-n-butylborane to PMMA, and claims a chemical bond with collagen. Hennig (13) has added bioactive glass ceramic particles to PMMA to improve the adherance. Several different types of additives have also been investigated to reduce the temperature generated (14).

PMMA will undoubtedly be further improved as a bone adhesive as investigators better understand the effects of different additives which are being tested. It is felt, however, that there are too many inherent limitations in this system for it to be an ideal bone cement. It is difficult to imagine this system ever being non-toxic or biodegradable.

1.3 Specifications of an Ideal Bone Cement

At the beginning of this investigation, five basic specifications for an ideal bone cement were outlined.

1. Fluidity
2. Initial tack to hold joint together.
3. Pot life of greater than five minutes, but less than thirty.
4. Wet strength comparable to rat tail tendon, and chemical bonding at adhesive-bone interface.
5. Non-toxic, non antigenic
6. Biodegradable

It was originally proposed to use a water soluble, protein, and cross-link this protein with glutaraldehyde. The glutaraldehyde is thought to act by connecting together amino groups of protein molecules such as lysine residues. Since bone is composed of collagen, which is fairly rich in lysive residues, it was thought that protein molecules adjacent to the bone could be bound to the

bone by glutaraldehyde. It was hoped that the cross-link density of the protein polymer would help control the biodegradability time constant. Two biodegradable adhesive systems using proteins have been well described in the literature, and are briefly described below.

1.4 Biodegradable Tissue Adhesives

1.4.1 Gelatin-Formaldehyde-Resorcinol (GFR)

This adhesive system was developed by the Batelle Memorial Institute and tested extensively in animals. The adhesive mixture contains 45% gelatin, 15% resorcinol, and 40% water. The resorcinol acts as a gelation suppressant and forms a polymer network within the system. The formaldehyde can either be placed directly on the tissue and allowed to diffuse through the GFR, or added to the adhesive. When the formaldehyde is added to the glue, the pH is adjusted to pH 5 yielding a pot life of about five minutes. Many animal experiments with this formulation have shown it to be effective as a hemostatic agent (15,16), and a soft tissue adhesive (17,18). It has been used in dentistry as a hemostatic agent in a few hemophiliacs (19).

Despite the success reported in the animal studies, this adhesive has not been used much clinically. The adhesive is fairly difficult to work with because of its short pot life, and the temperature control required. Maximum

in vitro bond strength reported by Matsumoto (20) are of the order of 10 p.s.i. This low strength makes this adhesive system unsuitable as a hard tissue adhesive. The system also contains a high percentage of resorcinol which can be potentially toxic. Resorcinol is currently used in some topical skin ointments for the control of acne. Small amounts absorbed into the skin on a chronic basis have been shown to induce hypothyroidism in some patients by inhibiting thyroid peroxidase (21). It has also been shown to have a cilliostatic effect (22).

1.4.2 Clotting System Proteins

One method of gluing tissue together is by making use of the clotting pathway. This system is attractive because it is the physiologic method by which wounds are held together. A mixture of fibrinogen, thrombin, and platelet factor XIII has been shown to be effective as alternatives to sutures in many applications including nerve anastamosis, blood vessel anastamosis, and soft tissue reconstruction (23,24,25,26,27). Because of the method of preparation of this system, there is always a finite risk of Hepatitis B transmission.

The mechanical properties of this material can be inferred from the literature. Matras (27) reports a maximum tensile strength for an anastamosed rabbit sciatic nerve of 22 grams. Assuming a sciatic nerve has a

typical diameter of 2 mm, this gives a tensile strength of the order of 10 p.s.i.

This adhesive system has also been applied to bone. Although this adhesive did give some small strength after surgery, its benefit was shown to be in a speeding up of the fracture healing process (28). In experimental rabbits, the healing process was judged to be faster especially during the first, fourteen days of surgery as judged by histology and strength tests. Most of these studies were done with stainless steel implants as adjuncts.

The biodegradable adhesives in use at the present time are only useful in low strength applications. The low wet strength of these materials seems to be due to the high water content which these adhesives achieve in an aqueous environment. This investigation looks at several protein adhesive systems, including blood proteins, gelatin, and collagen. In each system, the adhesive system is characterized, and its limitations investigated.

Chapter 2 BLOOD PROTEINS

Blood has been the source of many of the adhesives used in industry today (29). This investigation began with a dried blood sample from Hormel, Austin, Minn. (Product No. 1456). The manufacturer specified this sample to be water soluble, but under the many conditions tested in our lab, it was not observed to dissolve in water. Because much of the information regarding the processing of a particular dried blood product is proprietary, it was decided to try a simpler system. The adhesive qualities of blood are attributed to its high protein content. The two protein classes in blood which are in high concentrations are albumin and the globulins. Because of its availability, albumin was chosen for the investigation.

2.1 Handling Properties of Albumin

Bovine albumin (Cohn Fraction V Powder, Sigma Chemical Company) comes in a fine light brown powder. A solution is made by placing the powder on top of water and letting it dissolve slowly. Because of the negative charge associated with albumin, stirring causes clumping and bubble formation. The time required to dissolve the powder is dependent on the surface area and temperature. It takes about four hours to dissolve 5 grams of albumin in 7 ml of water in a 100 ml beaker. Upon drying, the

albumin becomes a clear, dark brown brittle solid.

2.2 Peel Strength Experiments

Four dilutions of albumin were used in measuring the peel strength of two strips of paper glued together with albumin. A first order estimate of the adhesive quality was obtained by noting the maximum peel force developed during a 180° peel test (ASTM Standard 0773-47). In these experiments, one drop (0.04 ml) was spread on one end of one strip of paper (1.3 cm x 4 cm). Another strip of paper was applied to the wetted surface, and the two strips were pressed together by hand. One of the paper strips was attached to a clamp connected to a Type B load cell, and the other strip was clamped to the cross-head of a Model TM Instron Testing machine. These experiments were done with a cross-head speed of ten inches per minute.

Since approximately equal volumes of solution were used in each test, it would be expected that as the concentration of albumin rises, a corresponding increase in strength would be observed. Figure 2.1 plots the maximum peel strength of observed one hour after the adhesive application as a function of concentration. The peel strength experiment was repeated with a 42% albumin solution at different times after the application of albumin. Figure 2.2 indicates that a maximum strength is achieved

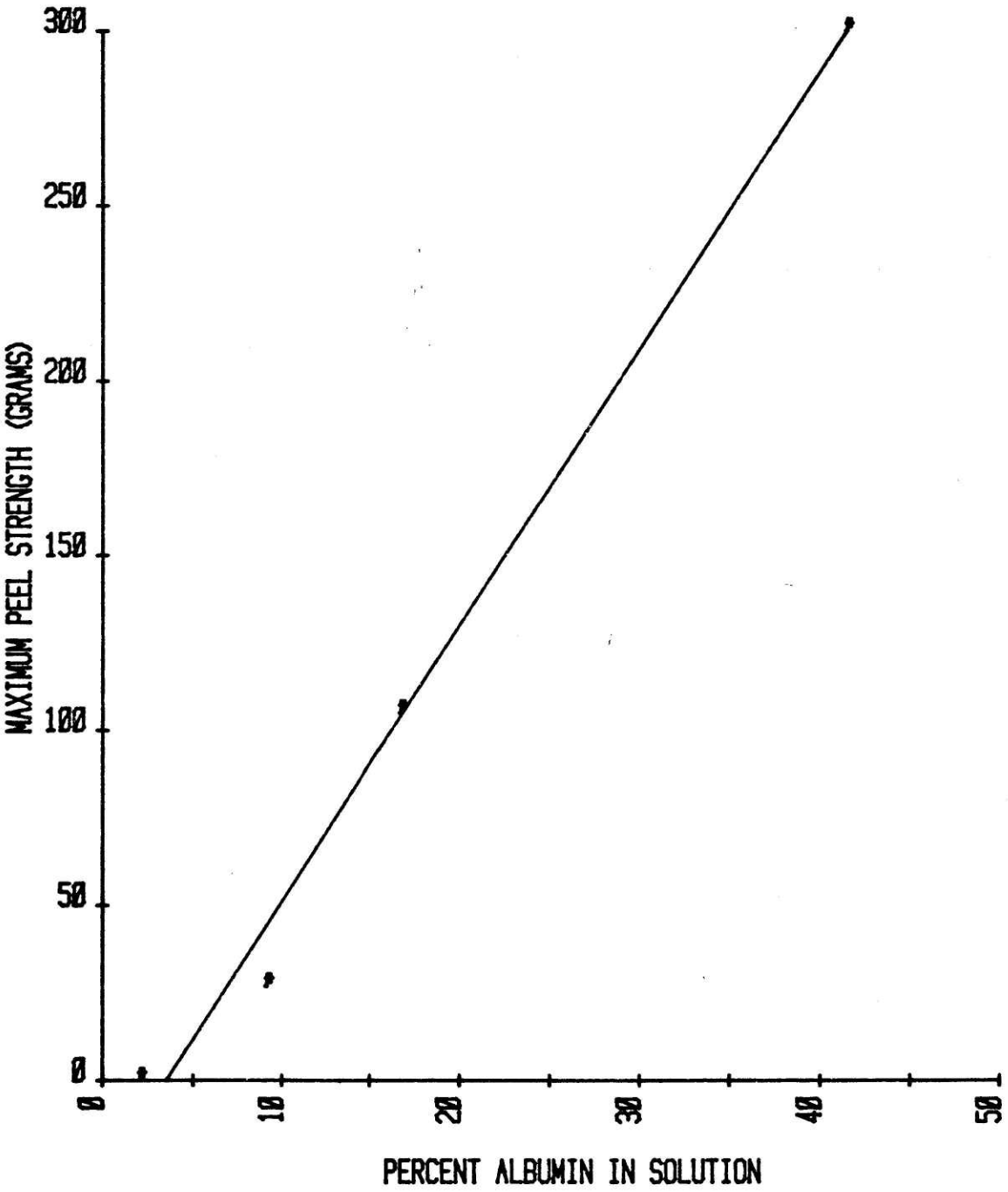

Figure 2.1 The maximum force recorded in a 180° Peel Strength Test is plotted as a function of the percentage of albumin in a solution applied as an adhesive to two paper strips. Least squares fit parameters: Slope = 7.81, Y Intercept = -27, R = 0.90.

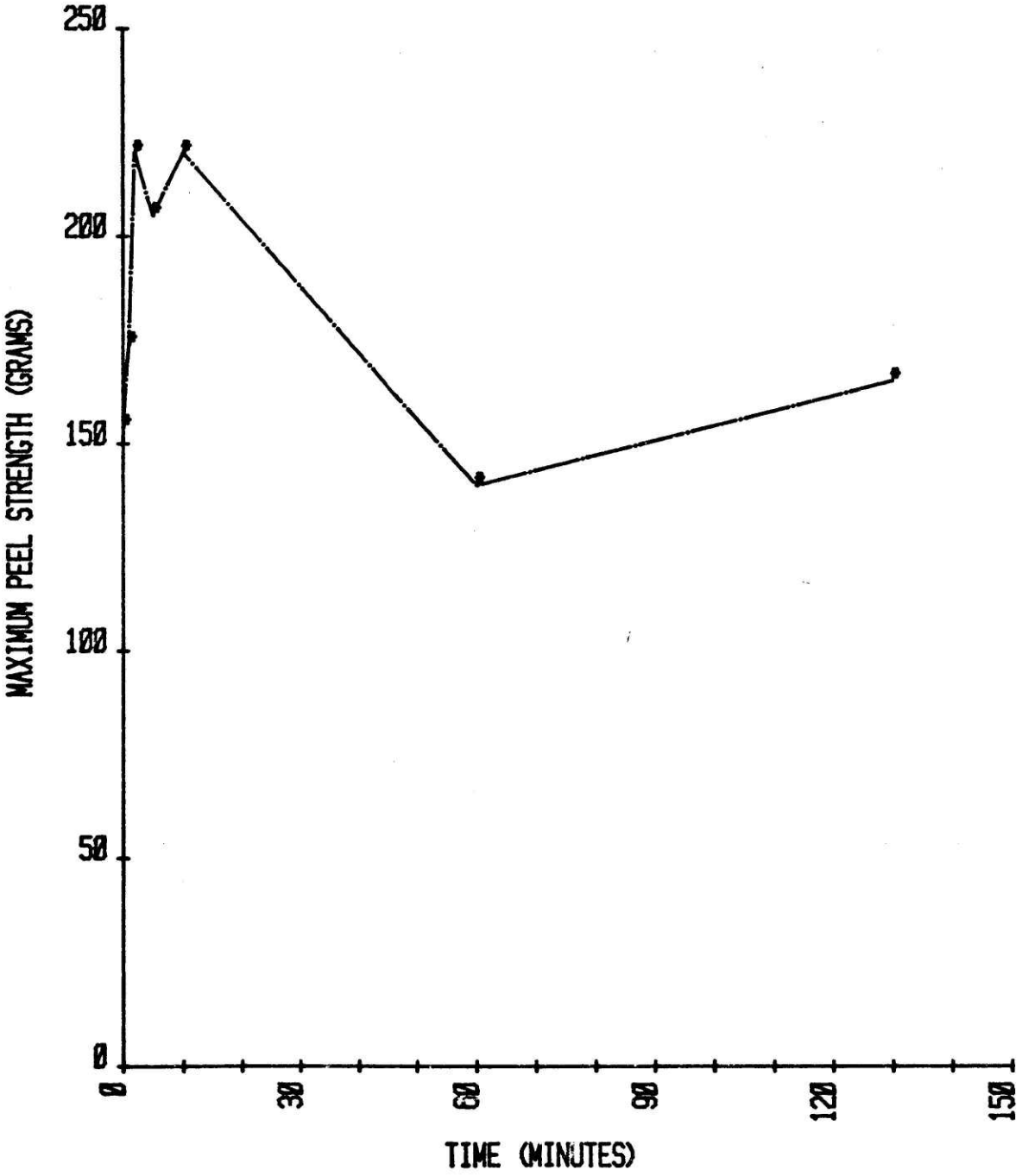

Figure 2.2 The maximum force recorded in a 180° Peel Strength Test is plotted as a function of the time after one drop of a 42% albumin solution is applied to two paper strips.

between two and ten minutes after application.

2.3 Glutaraldehyde as a Cross-Linking Agent

A cross-linking agent is needed to keep the albumin from redissolving when in an aqueous medium as well as bind the protein to the organic bone matrix. Glutaraldehyde was selected as a cross-linking agent because of its ability to cross-link proteins in an aqueous media. Different volumes of 2.5% glutaraldehyde (Practical Grade, J.T. Baker Chemical Company), were added to 0.5 ml of a 42% albumin solution, and peel strengths were measured after different time intervals. The adhesive which was not placed on the paper strips was stirred, and a time was recorded when the mixture could no longer be stirred. Except for one solution where only 0.05 ml of 2.5% glutaraldehyde was added, all of the mixtures hardened very fast. A pot life of about five minutes was estimated from the time of addition of glutaraldehyde until the mixture could no longer be stirred. After one hour, half of the residual hardened adhesive was taken from its mixing dish and placed in water for three days. The other half of the residual adhesive was left exposed to air. The samples which were immersed all absorbed water, were much softer, and had very weak strength characteristics. The mixture with only 0.05 ml of glutaraldehyde added redissolved in the water. Similarly, the samples left to the air all

hardened. Increasing amounts of glutaraldehyde decreased both the hydration, and the dehydration of the samples.

2.4 Summary

The initial albumin concentration, the amount of cross-linking agent, and the degree of hydration are three important parameters in determining the adhesive characteristics of albumin. Initial experiments with albumin indicated the use of a saturated albumin solution to obtain maximum peel strength. In addition, a slightly hydrated adhesive yields higher strengths. This is due to the fact that the albumin becomes brittle when too much water is lost.

The peel strength vs. time experiment, when performed four minutes after application, is a good index of the initial tack of the substance. Figure 2.3 shows that the tack of the albumin decreases as increasing amounts of cross-linking agent are added. The peel strength experiment performed at one hour is a good index of the final adhesive strength. The addition of too much glutaraldehyde causes the albumin to become overly cross-linked resulting in decreased peel strengths.

The kinetics of glutaraldehyde cross-linking were quite fast with observed hardening times on the order of five minutes. These times can be lengthened substantially by lowering the pH or the temperature. The fact that

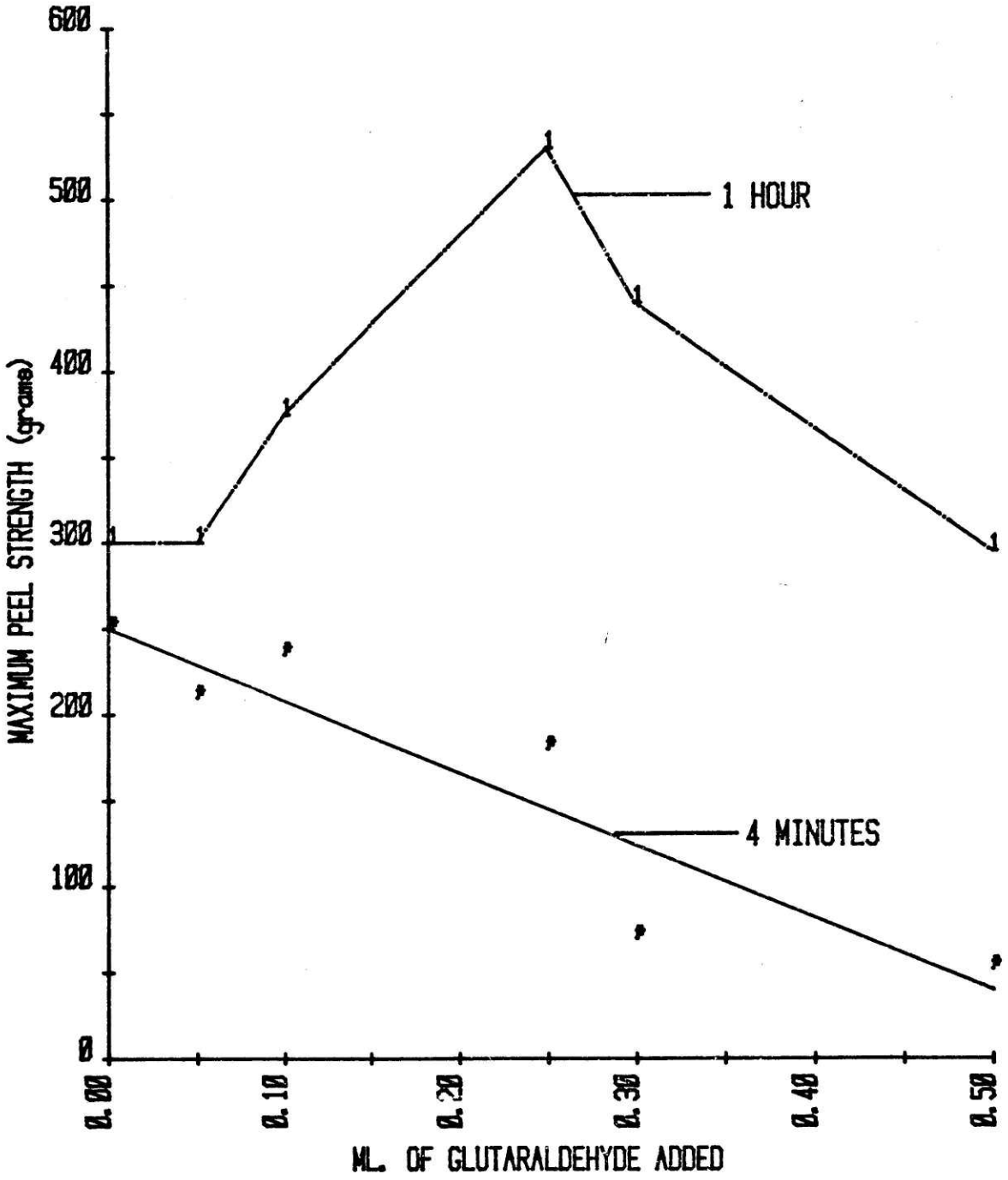

Figure 2.3 The maximum force recorded in a 180° Peel Strength Test is plotted as a function of the number of ml of 2.5% glutaraldehyde added to 0.5 ml of a 42% albumin solution used as an adhesive. Top curve: 1 hour after addition. Bottom curve: 4 minutes after addition. Least square parameters for the lower curve are: Slope = -4.20, Y Intercept = 250, R = 0.90.

albumin exhibited very low wet strengths, redirected the research focus to other proteins.

Chapter 3 GELATIN

Gelatin is formed from collagen when it is denatured. Since bone contains a large fraction of collagen, a gelatin based adhesive system would replace the bone deficit with a derivative of a bone constituent. This would solve the problems of toxicity and biodegradability inherent in synthetic adhesive systems. Experience with artificial skin and collagen sutures indicate that collagen and its derivatives are only weakly antigenic (30). There is also some evidence that a gelatin based adhesive would stimulate bone regeneration (31). It was hoped that a gelatin system appropriately cross-linked would provide a strong adhesive for orthopedic application. Addition of synthetic polymers was avoided because of their inherent toxicity.

3.1 Gelation

Perhaps the most interesting property of gelatin is its ability to set into a gel state. Gelation has been described as a formation of reversible cross-links between protein molecules. The melting temperature of gelatin is that temperature at which a gelatin solution forms a gel. This melting temperature is a function of many variables including molecular weight, pH, concentration, chemical modifications, and the addition of various chemicals in solution.

In industry, gelatin is used extensively as a hot melt adhesive. To be effective as an adhesive, it must possess good flow characteristics as well as a high solids content. Skiest (29) lists glue concentrations and viscosities for various grades of animal glue. In industrial applications, a concentrated gelatin solution is heated to 60° C. so that it has good flow characteristics. After application to a room temperature joint, the heat is quickly dissipated, and the polymer rapidly gels to form a bond. The strength of this bond further increases as water is lost by evaporation. In specialized adhesive applications, gelation depressants are added to the solution such that the adhesive can be stored and used at room temperature. The most effective non-toxic gelation depressant reported in the literature is sodium salicylate (32,33,34). In the lab, it was found that the addition of 2.5% sodium salicylate to a 33% gelatin solution would depress gelation for over an hour. Higher concentrations of sodium salicylate would depress gelation indefinitely.

3.2 Cross-Linking

The reversible cross-links formed in the gel state are easily broken when gelatin is immersed in water. Because of the aqueous environment inside the body, covalent cross-links are essential for an effective gelatin adhesive system. The cross-linking of gelatin has been

extensively studied, however, much of the literature is in patent form (33,35). Many tanning agents have been reported including formaldehyde, glutaraldehyde, acid halides, and a mixture of aluminum sulfate, sodium acetate and borax.

3.2.1 Kinetics of Glutaraldehyde Cross-Linking

The kinetics of glutaraldehyde hardening are dependent on temperature, concentration and pH. To illustrate the effect of pH, a solution prepared with 50 grams of distilled water, 25 grams of gelatin (Kind and Knox Gelatin Type 3049), and 10 grams of sodium salicylate (Sigma Chemical Company) was used. Using dilute NaOH and HCl, the pH of the various solutions was altered and measured with a calibrated glass pH electrode connected to a Beckman pH meter. To 10 ml of each adhesive mixture, was added 1.0 ml of 5% glutaraldehyde solution. The pot life was estimated as the time elapsed until the solution could not be stirred.

pH of solution	pot life
4.2	10 minutes
4.7	4 minutes
5.7	2 minutes
6.5	1 minute
9.7	20 seconds

3.2.2 Wet Strength of Gelatin Films

Data from the gelatin-resorcinol-formaldehyde literature (13-15), indicates that failure of the adhesive bond most often occurred within the gelatin itself. Cross-linked cast gelatin films were studied to quantitate wet strength characteristics.

A 30% gelatin solution (Kind and Knox Gelatin Company, Type 3049) was prepared and poured into plastic trays which were air dried. Each film contained about 1 gram of gelatin and had the approximate dimensions of 2" x 2" x 0.013". These films were soaked in 0.25% and 0.5% glutaraldehyde for various lengths of time and allowed to dry. The films were subsequently immersed in 0.9% saline for 36 hours and cut into strips 0.4" in width. Samples were tested in tension on the Instron testing machine (Model TM, Load Cell B) within two minutes of removal from the saline. Samples were tested under a strain rate of 100% per minute. Percent solids determinations were made by finding the ratio of dry weight to the wet weight. Films were considered dry after 24 hours in an oven at 105^{o} C.

Exposure to glutaraldehyde, for prolonged periods of time, results in asymtotic values of Young's Modulus and Percent Solids values. If these quantities are indexes of the cross-linking which has occurred, the data shows that most of the cross-linking is completed by one hour.

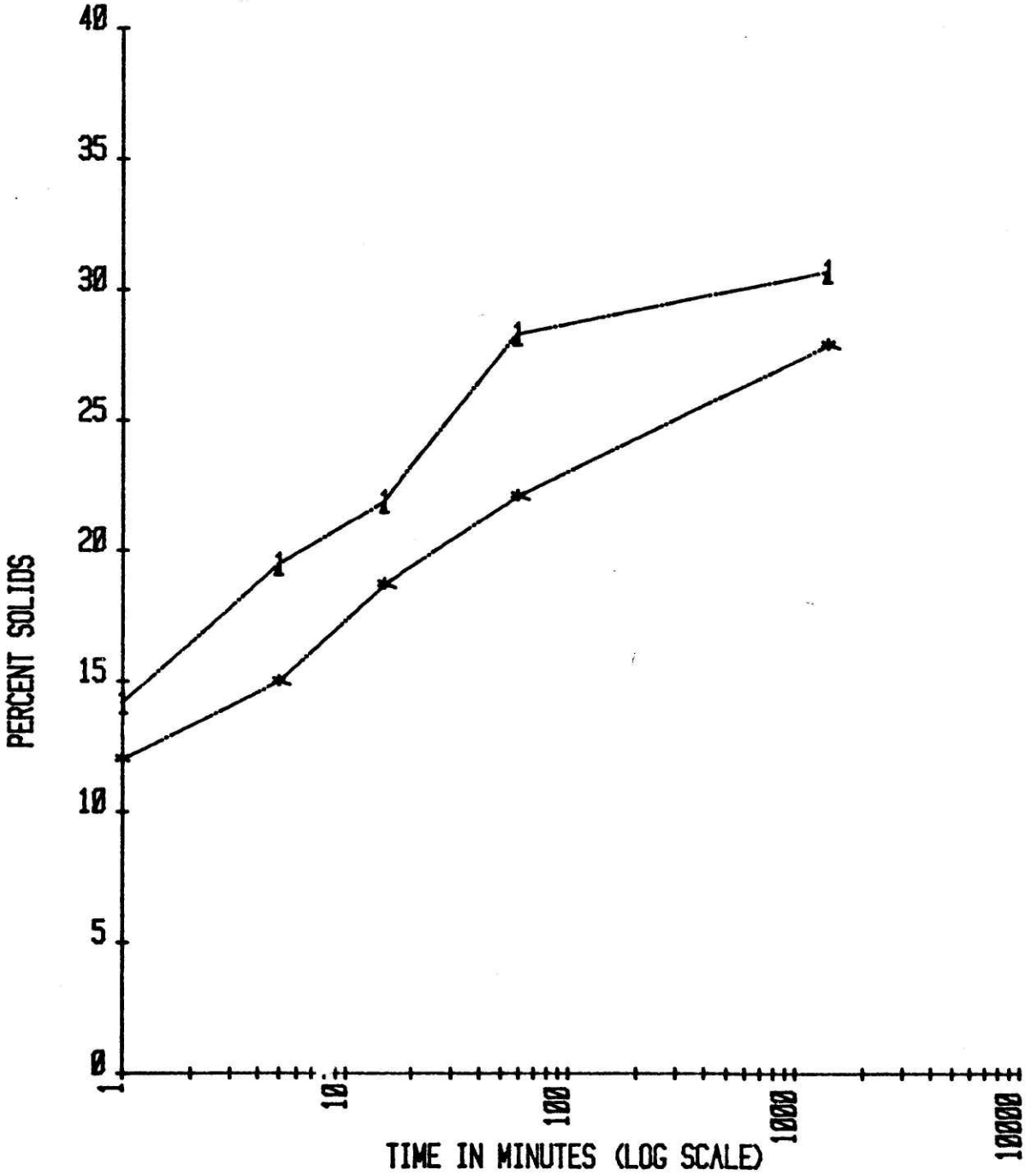

Figure 3.1 The percent solids content of cross-linked gelatin films equilibrated in saline is plotted as a function of the time that the films were exposed to the cross-linking agent. The upper curve denotes cross-linking in a 0.5% glutaraldehyde solution. The lower curve is cross-linking with 0.25% glutaraldehyde.

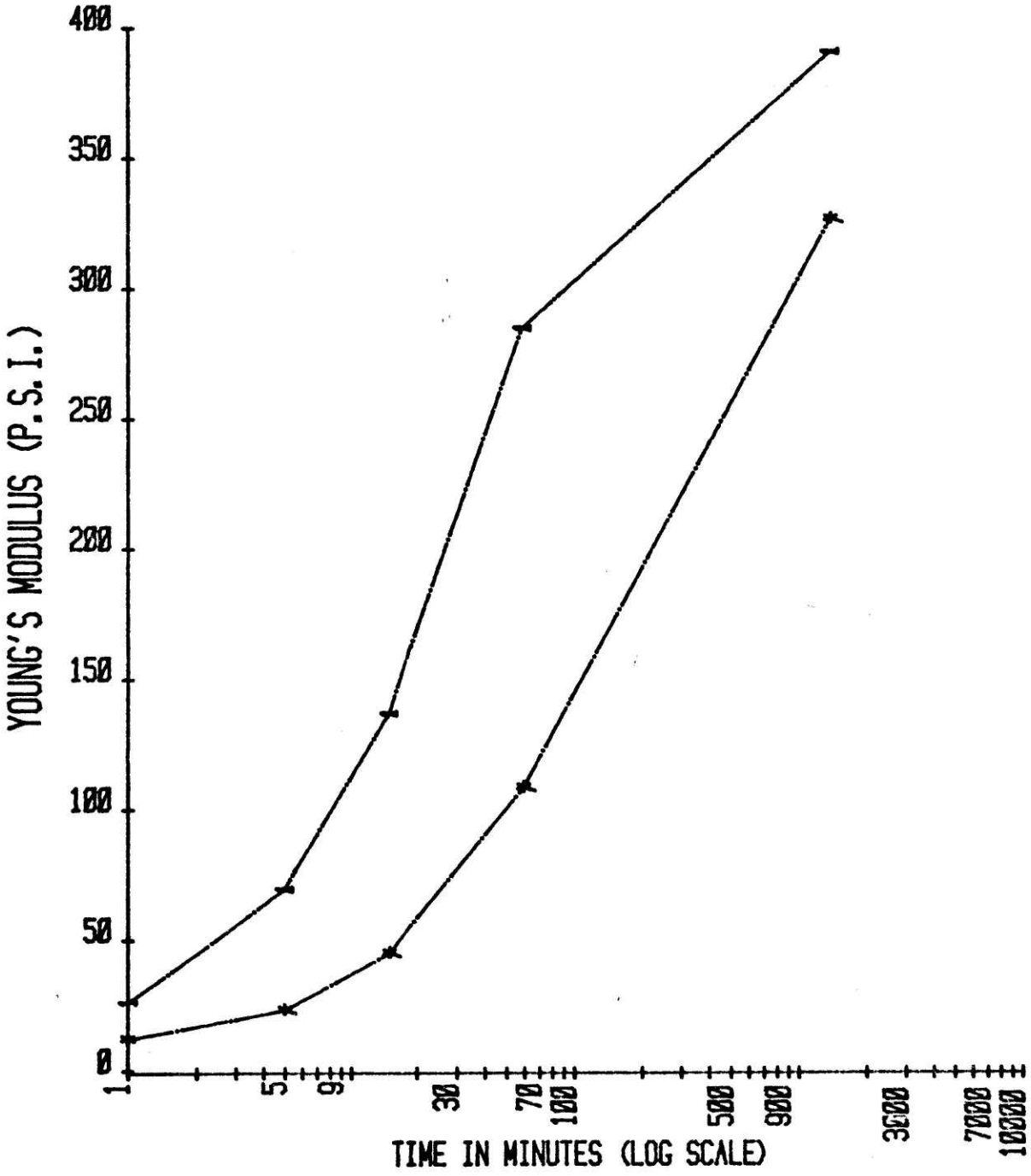

Figure 3.2 Young's Modulus of the equilibrated gelatin films is plotted as a function of time of immersion in the glutaraldehyde bath. Top curve: 0.5% glutaraldehyde. Bottom curve: 0.25% glutaraldehyde.

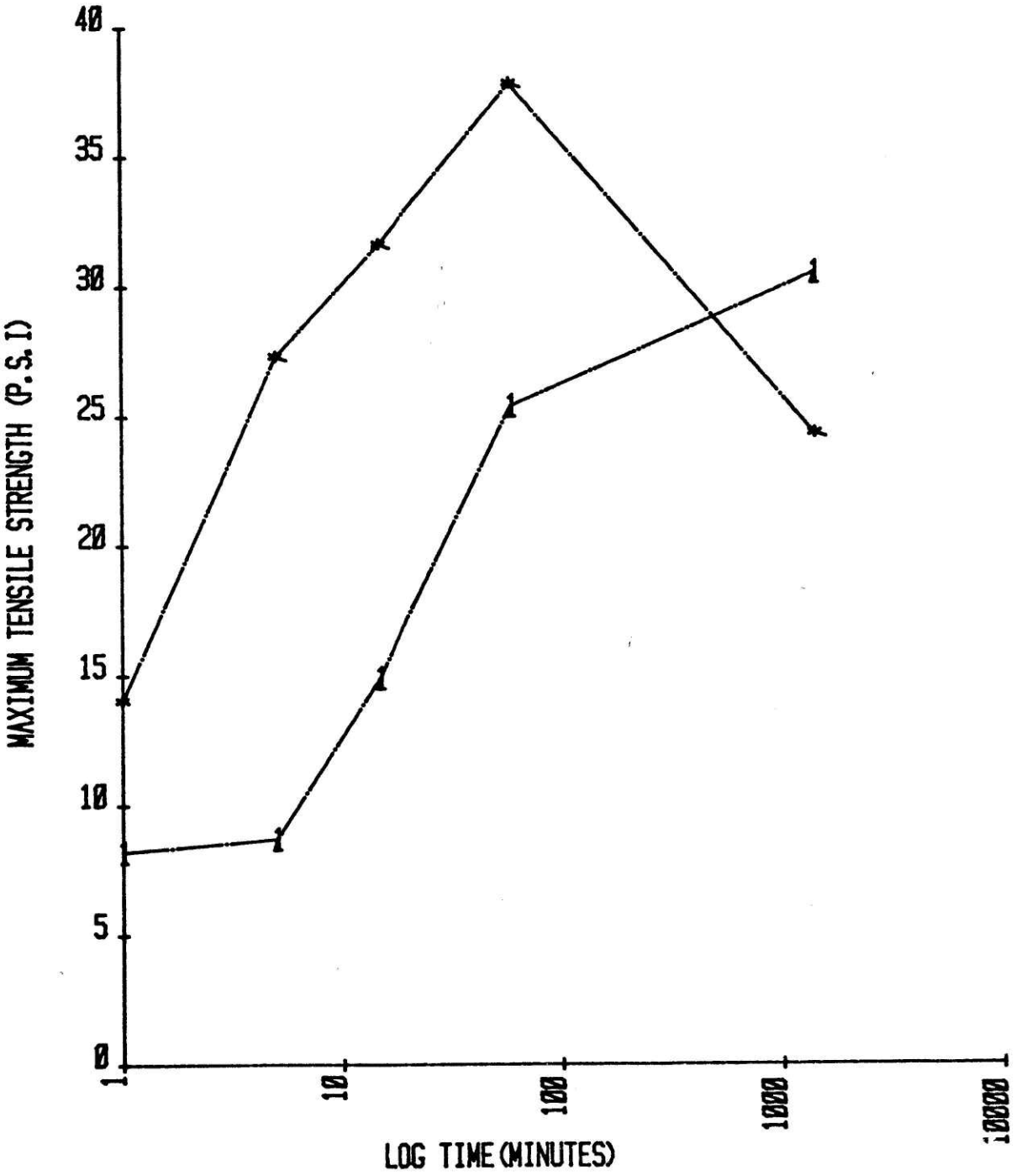

Figure 3.3 The maximum tensile strength of the equilibrated gelatin films is plotted as a function of time of immersion in the glutaraldehyde bath. Top curve: 0.5% glutaraldehyde. Bottom Curve: 0.25% glutaraldehyde.

Ultimate tensile strength generally increases with time of exposure to glutaraldehyde. The exception to this is in the film, cross-linked for 24 hours in 0.5% glutaraldehyde. This film was observe to be quite brittle or overly cross-linked. The effects of different glutaraldehyde concentrations can be observed in Figures 3.1, 3.2, and 3.3. The data indicates that cross-linking in a 0.5% solution of glutaraldehyde for one hour is an appropriate cross-linking protocol for gelatin films.

3.2.3 Cross-Linking with Glutaraldehyde in Acetone

Cross-linking a gelatin film in a glutaraldehyde bath exposes the film to a high water concentration. Even when glutaraldehyde is used from the reagent bottle without dilution (25% glutaraldehyde in water), most of the solution is water. The water is absorbed by the gelatin which causes it to swell. Because the gelatin is cross-linked in the swollen state, the strength of the material is reduced.

By using a substance which is a solvent for the glutaraldehyde solution but not for gelatin, it was hoped that the wet strength of the gelatin films would improve. Acetone is a substance which does not dissolve gelatin, but is miscible with water. Several 1.5 gram gelatin films were prepared and placed in 50 ml baths containing a mixture of acetone and glutaraldehyde (25% solution) for one hour. After removal from the acetone-glutaraldehyde bath, samples were placed in water baths for one hour, and then

dehydrated in a vacuum oven (-28 p.s.i.g., 105° C.) for 24 hours. Weights of the films obtained during the process were used to determine the percent solids.

Figure 3.4 shows the percent solids of the films one hour after exposure to the cross-linking bath, and after they had been placed in the water baths for one hour. Very little swelling occurred in films which were immersed in baths containing high concentrations of acetone. These films appeared very much like they did before they were immersed. The films exposed just to glutaraldehyde swelled significantly, and became much softer and compliant. Placement in water caused all films to swell. Those which had low concentrations of glutaraldehyde present were not heavily cross-linked and swelled greatly.

In another set of experiments, gelatin films were placed in a bath of 80% acetone and 20% glutaraldehyde (25% in water) and removed at various time intervals. After removal, these films were equilibrated in water for 24 hours; they were tested for mechanical strength as described earlier. Percent solid determinations after immersion in the cross-linking bath, and after equilibration with water are plotted in Figure 3.5. The films, while exposed to the cross-linking bath, swell as time proceeds and reach an asymtotic percent solids value. After the films are removed from the cross-linking bath

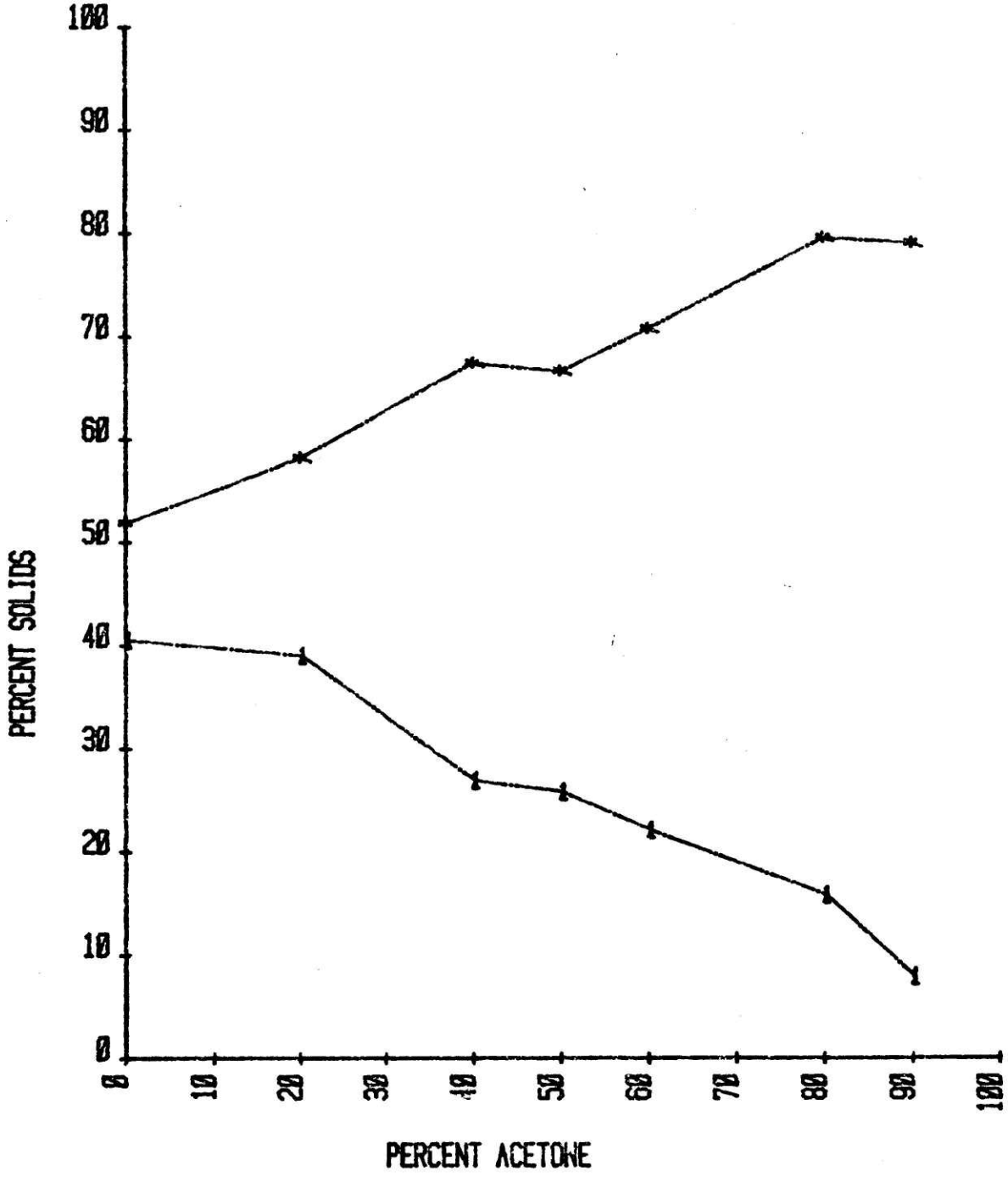

Figure 3.4 Percent solids is plotted as a function percent acetone in the cross-linking bath containing glutaraldehyde and acetone. The upper curve is the percent solids after one hour in the bath. The lower curve is the percent solids following one hour in water.

and placed in water, further swelling occurs. Those which were exposed for longer periods to the cross-linking agent swelled less during the water equilibration step and ultimately achieved a higher percent solids value.

The percent solids value obtained after equilibration provides a good index of the degree of cross-linking. Figure 3.6 plots the Young's Modulus as a function of time of immersion in the cross-linking bath. A trend similar to that observed for percent solids is observed. Since the Young's Modulus is difficult to measure in many of the low solids films, the percent solids determination provides a more accessible index to cross-linking.

The ultimate tensile strength data is plotted in Figure 3.7. In this series, the maximum tensile strength of 88 p.s.i. was observed for a film immersed in the bath for six days. The film immersed for seven days had a decreased tensile strength (63 p.s.i.) due to over cross-linking.

Maximal tensile strength in this system depends on several factors. A high acetone concentration is essential to minimize swelling during cross-linking. When acetone concentrations are too high, diffusion of glutaraldehyde through the film is limited, and cross-linking

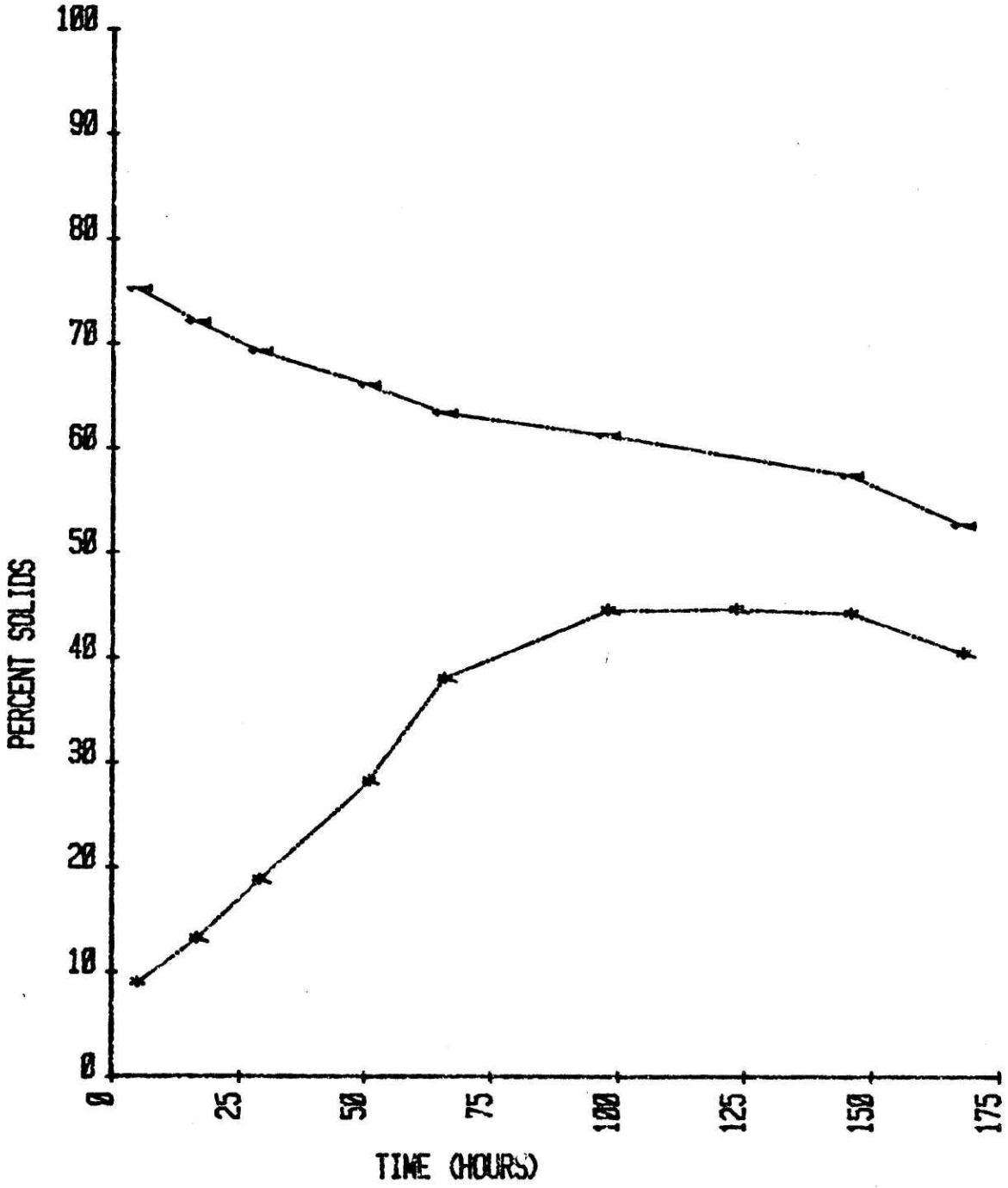

Figure 3.5 Percent solids is plotted as a function of time of exposure of gelatin films to a 80% acetone, 20% glutaraldehyde bath. The upper curve is the percent solids after removal from the bath. The lower curve is the percent solids after subsequent equilibration with water.

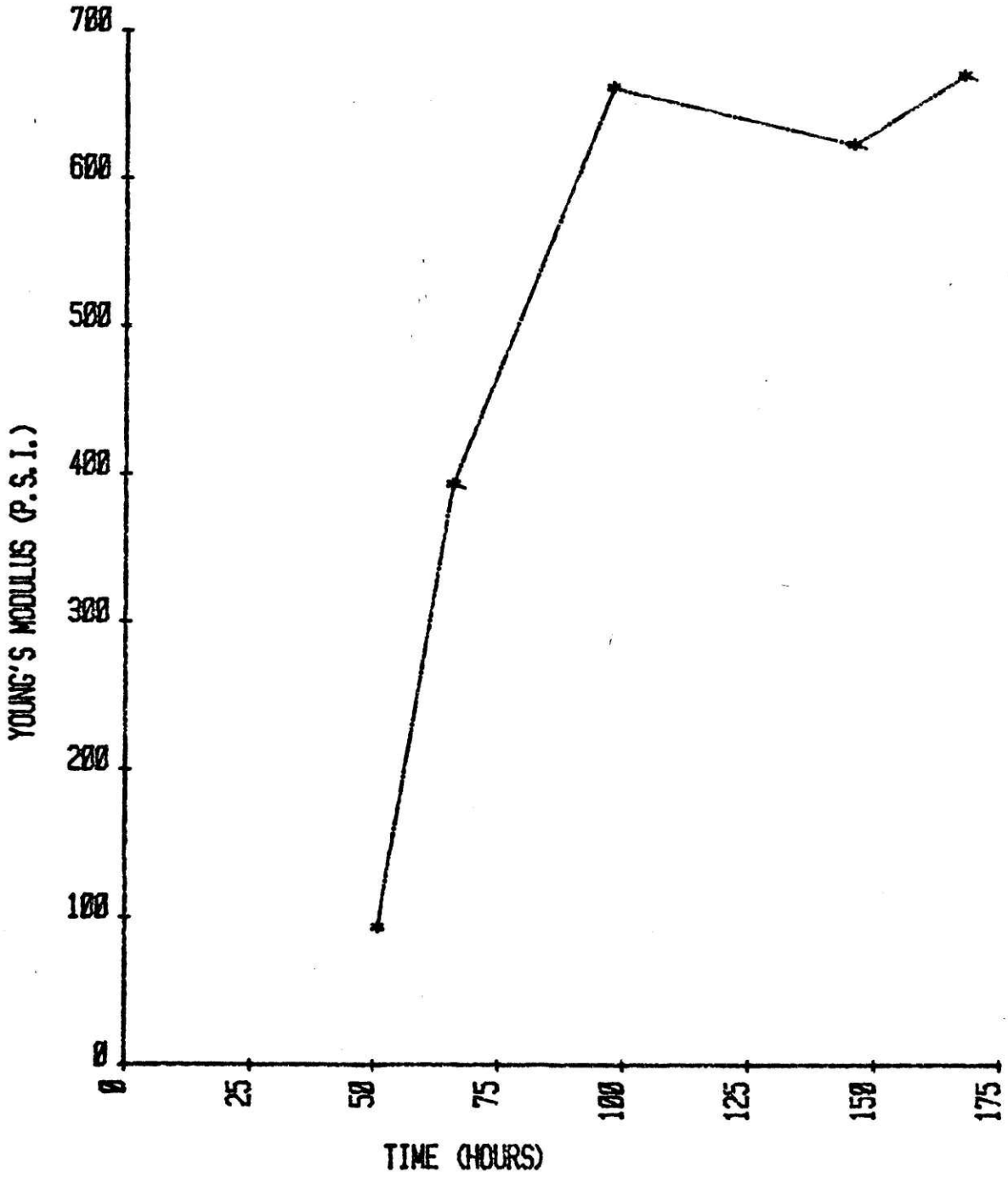

Figure 3.6 Young's Modulus is plotted as a function of time of exposure of gelatin films to a 80% acetone, 20% glutaraldehyde bath. The mechanical tests were performed after equilibration with water for 24 hours.

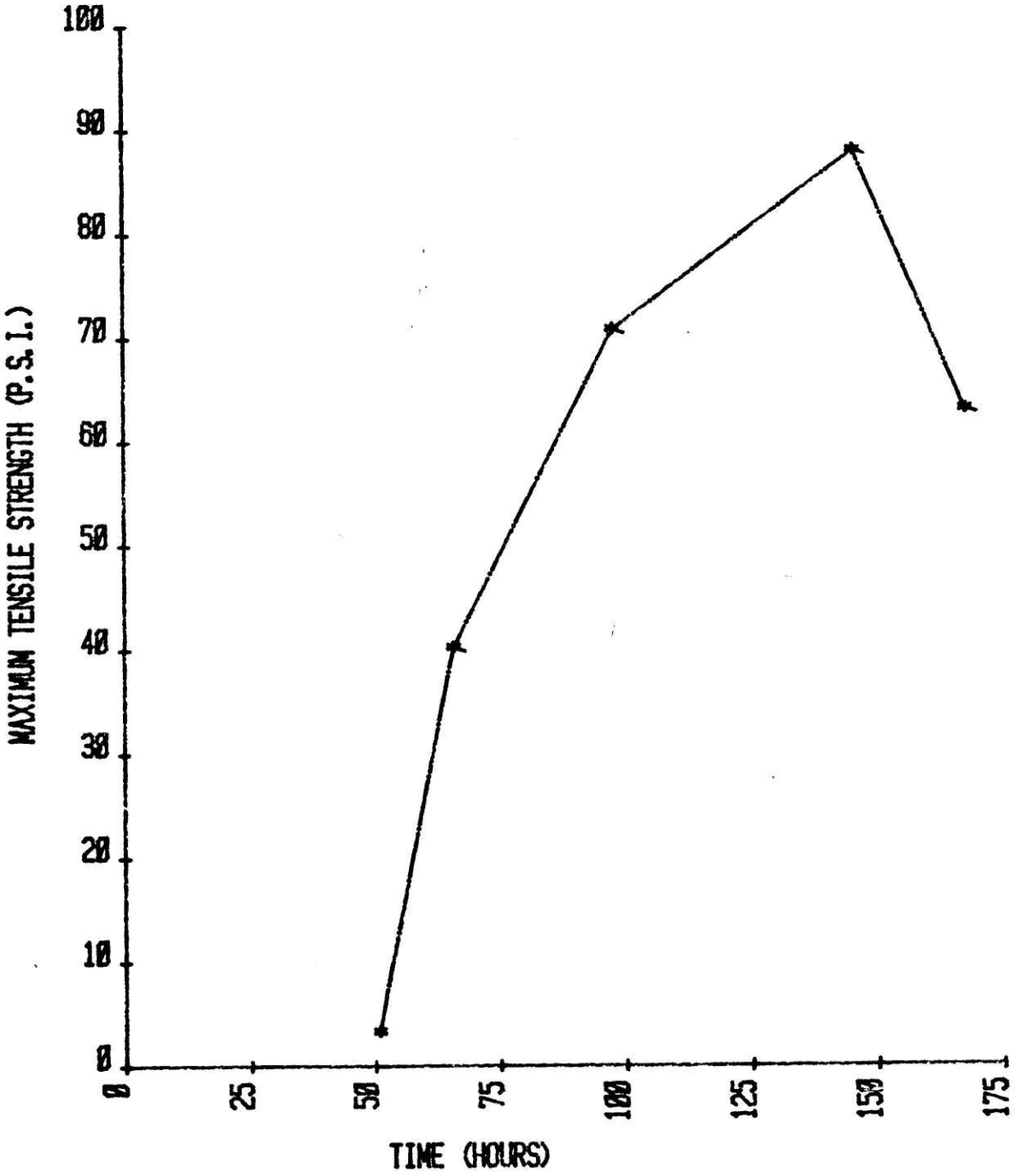

Figure 3.7 The maximum tensile strength is plotted as a function of time of exposure of gelatin films to a 80% acetone, 20% glutaraldehyde bath. The mechanical tests were performed after equilibration with water for 24 hours.

impaired. Although water swells gelatin, it is important as a carrier for the glutaraldehyde molecules within the gelatin film. In films where the acetone concentration was high, it was noted that the surfaces, but not the centers of the films were cross-linked indicating a diffusionally limited cross-linking process.

To further improve the strength of gelatin films, a thinner film of gelatin was cast (0.015"). This film was exposed to a 98% acetone, 2% glutaraldehyde (25% in water) for four days. After equilibration in water, the tensile strength was measure as 131 p.s.i. The percent solids of this film were determined to be 49%.

3.2.4 Dehydrothermal Cross-Linking

Dehydrothermal Cross-Linking was investigated as an alternative protocol for cross-linking gelatin films. The aim of this study was to investigate the possibility of obtaining a high wet strength gelatin film by cross-linking the protein in the solid state. Yannas (36) observed that gelatin films placed in a vacuum oven at 105° C. for five days did not dissolve in water after two hours. At a very high solids content, apparently a solid state cross-linking reaction takes place.

In a series of experiments, two sets of gelatin films were cast. One set weighed about 6 grams and had the dimensions of 2" x 2" x 0.09". The other set weighed 1.5

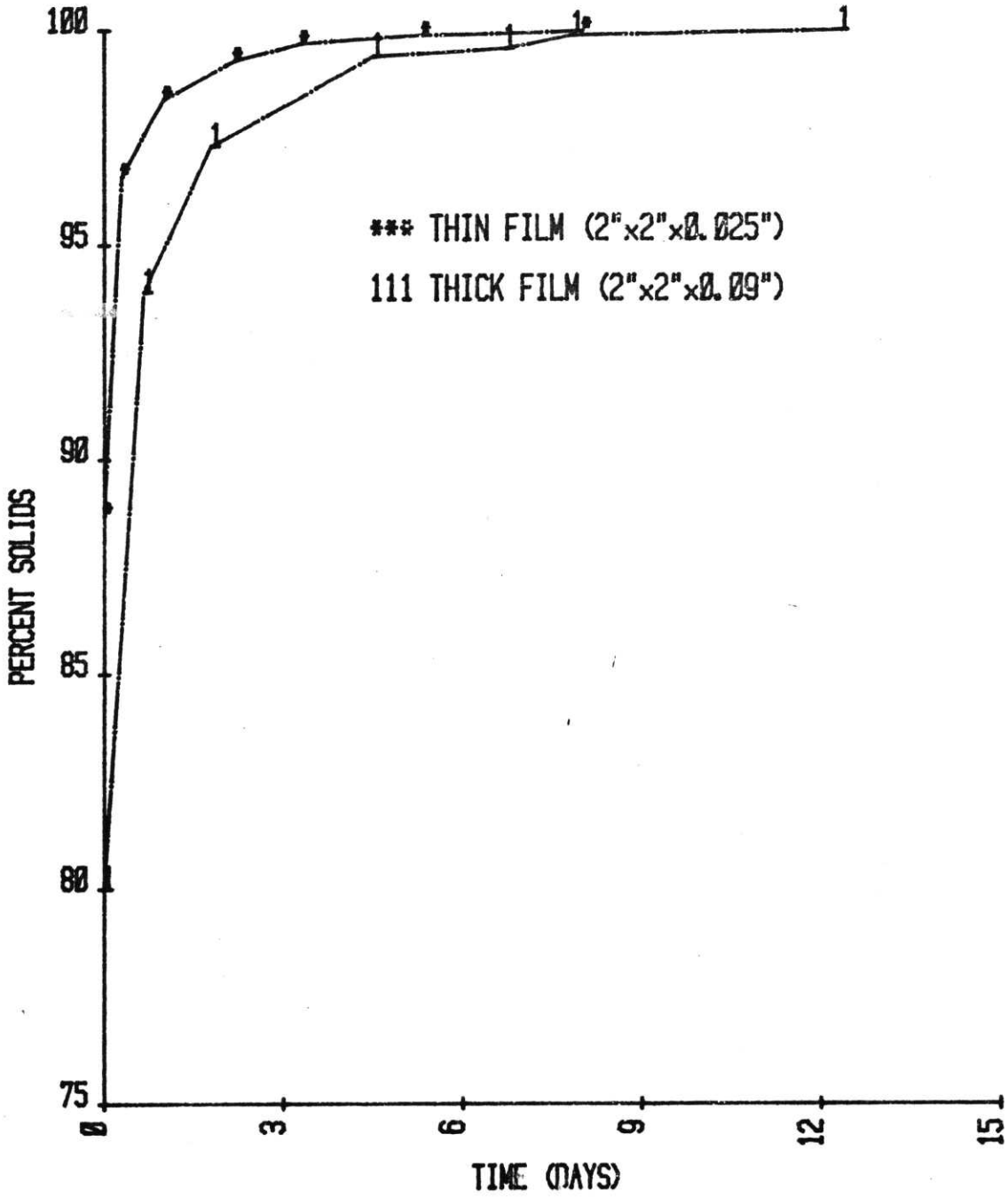

Figure 3.8 Percent solids of films placed in a 28 p.s.i. vacuum oven at 105° C. is plotted as a function of time. The thin film has a time constant of 6.3 hours. The thick film's time constant is 14.5 hours.

grams and had dimensions of 2" x 2" x 0.025". One film from each set was placed in a vacuum oven at 105° C. with a pressure of -28 p.s.i.g. These films were weighed on a daily basis and returned to the oven. When the weight of the films stabilized, they were assumed to be dry. Figure 3.8 plots the percent solids of the films as a function of time in the oven. If the data is fitted with an exponential curve, it is found that the thin film has a time constant of 6.3 hours, while the thick film has a time constant of 14.5 hours.

Additional films were placed in the vacuum oven to measure swelling ratios as a function of time in the oven. It was found that by placing the thick films in a 45° C. oven for one day, followed by a 65° C. for one day, reduced cracking and bubbling which occurred in the vacuum oven. Samples were removed from the vacuum oven and placed in a water bath for 24 hours. Although films did not dissolve as easily as cold cast gelatin films, even films which had been in the oven for 14 days almost completely dissolved in the water over a period of 24 hours.

3.2.5 Photoinitiated Cross-Linking

In designing an adhesive system which requires a cross-linking agent, the pot life of the adhesive is an important consideration. Pasts attempts of using gelatin as a biologic adhesive have been troubled by the fact that after the cross-linking agent was added, the pot life of the adhesive has typically been less than 5 minutes (20). By using an agent which could be activated by an external stimulus such as light or pH, greater control over the cross-linking process could be achieved.

Several photoinitiated cross-linking agents have been described in the literature as devices to identify or label submicroscopic structures (34,35,36). These agents are bifunctional molecules having one group which will non-specifically bind when stimulated by light. The two agents chosen for this investigation were methyl 4-azidobenzoimidate, and N-hydroxysuccinimide ester of 4-azidobenzoic acid (Pierce Chemical Company). In both of these molecules the azido group is the photo-sensitive moiety. The other functional group, methyl or N-hydroxysuccinimide, binds to amino groups. The binding to the amino groups is ph dependent, achieving maximum kinetics above a pH of 9.3.

These experiments were carried out in a well ventilated dark room, and manipulations were made under a minimum of light. In a preliminary experiment, 50 mg of methyl 4-azidobenzoic acid was dissolved in 30 ml of 0.1 molar

Na_2HPO_4 buffer solution. To this solution was added 20 ml of 1% gelatin solution. The amount of gelatin added was calculated so that if the cross-linking reaction was complete, the average molecular weight between cross-links would be about 1000. By titrating the solution with NaOH, the pH was raised to 9.3. To avoid hydrolysis of the gelatin, the pH was lowered to pH 7 by addition of acetic acid after one hour. Yip (37) indicates that the reaction of the methyl group is complete after a one hour exposure to pH 9.3. This solution was poured into a plastic tray along with a control without the agent, and placed near a fan in the dark room to speed evaporation. The weight and tack of the sample and control were noted periodically throughout the drying sequence. After 18 hours in the dark room, the films had achieved a state where they were dry along the edges and tacky in the middle. The films were removed from the dark room and placed 10 cm from a 200 watt incandescent bulb for 30 minutes. It was noted that the film became less translucent during the photolysis process as compared to the control. The film was weighed after light exposure, and placed in water for 24 hours. The film was observed to be very weak, and mechanical tests were not performed. Percent solids was determined to be 5.2%. The film had a 30% solids value before photolysis. In the film, Na_2HPO_4 crystals were observed. The experiment was performed again without a buffer and a similar film was achieved. Many crystals were observed in the film without

the buffer, and it was concluded that some of the cross-linking agent crystallized within the gelatin. This film also appeared to be weakly cross-linked.

The N-hydroxysuccinate ester of 4-azidobenzoic acid was insoluble in water, but soluble in N,N-dimethyl formamide. 50 mg of N-hydroxysuccinate ester of 4-azidobenzoic acid was dissolved in 10 ml of N,N-dimethyl formamide (Fisher Scientific Company). To this was added, 20 ml of a 1% gelatin solution, and the pH was adjusted to 9.3. After one hour, the pH was readjusted to pH 7 and the solution was air dried in a ventilated dark room for 18 hours. Exposure to an incandescent light for 30 minutes resulted in the film turning a light brown. This color is similar to a film lightly cross-linked in glutaraldehyde. After light exposure, the film was equilibrated in a water bath for 24 hours and weighed. The result was a very weak film with a percent solids determined to be 9.2%.

Although strong films were not obtained using the procedures described above, it was demonstrated that a photo-initiated cross-linking agent can lightly cross-link gelatin films. Controlling the cross-linking reaction by an externally modifiable parameter is an important concept. In a surgical environment this capability would be very useful because of the many time constraints present in the operating room.

3.3 Summary

Several methods of cross-linking gelatin films were investigated. Glutaraldehyde cross-linking was found to give maximum tensile strengths of the order of 40 p.s.i. with a percent solids of 30%. Other methods of cross-linking were investigated, including dehydrothermal cross-linking, photoinitiated cross-linking and cross-linking with glutaraldehyde in acetone to improve the wet strength of gelatin film.

One of the reasons which explains the low wet strength values of cross-linked gelatin with glutaraldehyde is that glutaraldehyde cross-links the material when it is swollen. The high water content in the film weakens the material. By cross-linking in acetone, a non-solvent for gelatin, swelling ratios were observed to be much smaller. A film cross-linked in a bath containing 80% acetone, and 20% of a 25% glutaraldehyde solution was found to have a wet strength of 131 p.s.i. and a solids content of 49%. This data gives us an idea of the maximum wet strength of a gelatin film as well as directing research to different cross-linking protocols.

Chapter 4 COLLAGEN

Collagen is a major structural building block. As a composite with hydroxyapatite it forms bone, and it is the major constituent of tendon. Measurements of rat tail tendon (40) give an ultimate tensile strength of 3300 p.s.i. Yamada (41) reports the strength of human tendons in a range of 6,000 to 10,000 p.s.i.

4.1 Rheology of Collagen

Exposure of collagen to an acidic solution causes it to swell and loose much of its triple helical structure. To quantitate this phenomena, collagen was dispersed at various ph values and the rheology observed.

Bovine collagen prepared by the U. S. Department of Agriculture (42) was the source of all the collagen used in this investigation. The collagen was Wiley milled (Arthur Thomas Co.) using a 20 wire per inch grid. 0.55 grams of milled collagen were added to a 200 ml mixture of 0.05 molar acetic acid and distilled water. This mixture was placed in an ice jacketed Eberbach blendor for one hour. The ice jacket maintained the temperature of the slurry at about 4^{o} C. throughout the process. The blendor was run at 60% of full speed voltage by connection to a variable transformer. After blending, the pH of the slurries was determined using a calibrated pH glass electrode connected to a Beckman pH meter. These dispersions were centrifuged for

15 minutes at 2300 r.p.m., and the supernatant discarded. Percent solids determinations were taken of the dispersions.

pH of dispersion	percent solids	rheology
3.2	0.28%	Swollen
3.4	0.35%	Swollen
4.3	0.47%	Swollen
4.5	0.54%	Swollen
4.7	5.9%	Unswollen
4.8	4.9%	Unswollen

A marked rheologic change was observed between the pH values of 4.5 and 4.7.

Because the percent solids of the unswollen collagen is an order of magnitude higher than that of swollen collagen, it was hoped that this material could be used as an adhesive paste. By drying this material on a filter paper, a thick white paste was obtained with a solids content estimated to be about 30%. Reaction with glutaraldehyde caused the material to thicken, but negligible cohesion was observed even after 48 hours.

4.2 Properties of Reconstituted Collagen Products

Reconstituted collagen cross-linked with formaldehyde is used as a degradable suture in surgery. A sample of surgical suture was immersed in 0.9% saline for 24 hours and its tensile strength was measured at 9300 p.s.i. Its

transverse tensile strength was not measured, but it was noted that the fibers could be pulled apart easily. A sample of collagen tape was obtained which was also observed to be highly anisotropic. After equilibration for 24 hours in 0.9% saline, its tensile strength was calculated to be 3400 p.s.i. Silver (43) reports the wet strength of a randomly oriented collagen film to be on the order of 400 p.s.i.

4.3 Acetone Precipitation of Collagen Films

To speed the formation of collagen films acetone precipitation was investigated. A collagen dispersion prepared from 0.55 grams of milled collagen was prepared as described in section 4.1. After blending for one hour the pH was measured to be 3.2. The ph of the solution was gradually raised to pH 7 by titrating with dilute NaOH. This slurry was mixed with 92 ml of distilled water, 8 ml of 25% glutaraldehyde and 100 ml of acetone and then filtered using an 11 cm filter paper (Schleicher and Schuel, No. 589, Keene, New Hampshire) in a Buchner Funnel under aspiration. About an hour later, a thin collagen film formed. Its wet strength was measured as described previously to be 595 p.s.i. with a percent solds content of 45.2%. Percent solids were also determined during the precipitation process. After the Buchner Funnel had stopped dripping, the solids were found to be 20.7%.

Acetone precipitation was also attempted with swollen collagen. It was found that the Buchner Funnel drained very

slowly. Final formation of the film was done by evaporation. The film adhered to the filter paper and couldn't be peeled off.

4.4 Summary

Collagen is a strong biological polymer for several reasons. Its triple helical structure give it intrinsic strength due to crystallinity. Fiber orientation gives additional strength in tendons and surgical suture. Cross-linking collagen physiologically or with an agent such as glutaraldehyde is another important factor contributing to its strength. Because of the crystallinity of collagen, cross-linking with glutaraldehyde yields a higher percent solids value than corresponding crosslinking of gelatin in glutaraldehyde.

In the reconstituted state collagen has two phases which are ph dependent. At a ph value below 4.7, the collagen fibrils swell and loose part of their triple helical structure. In this state collagen forms a very viscous low solids fluid. After evaporation the swollen collagen adheres well to materials such as filter paper and aluminum trays. Reconstituted collagen at ph values above 5 tends to form a particulate slurry. Since this slurry has a higher solids content than swollen collagen, it would be easier to work with as an adhesive. It also forms strong films by acetone precipitation under suction.

Chapter 5 GELATIN-COLLAGEN COMPOSITES

Gelatin possesses the desirable adhesive properties of fluidity and tack. In the wet state however, it does not have mechanical properties suitable for a hard tissue adhesive. Collagen possesses better mechanical properties but does not have the adhesive qualities of fluidity and tack. A series of experiments indicate the effect of mixing gelatin and collagen on the strength properties and solids content of the composites.

5.1 Unswollen collagen added to gelatin

One way to strengthen the gelatin system is by adding a fiber reinforcing agent. Collagen fibers which were milled as previously described had a fiber length of about 1 mm. These fibers were added to a gelatin solution such that the resultant mixture contained equal amounts of gelatin and collagen by weight. This mixture was cast into a film and crosslinked in 0.5% glutaraldehyde solution for one hour, and prepared for tensile strength testing as described in Section 3.2.2. Ultimate tensile strength was measured at 20 p.s.i. This decrease in observed strength is due in part to the shortness of fiber used. Surgical collagen suture cut in longer pieces (6mm-10mm) may provide suitable reinforcement for a gelatin adhesive used as a filler.

5.2 Swollen Gelatin-Collagen Composites

Swollen collagen solutions were prepared by blending

0.55 grams of milled collagen in 200 ml of 0.5 molar acetic acid as described in section 4.1. A gelatin solution was prepared by dissolving 0.55 grams of gelatin (Type 3049) in 200 ml of distilled water at 90° C. After the gelatin had cooled to room temperature, composites were cast by mixing different volumes of gelatin and swollen collagen in plastic trays. These composites were allowed to air dry at room temperature. After being dried they were immersed in 0.5% glutaraldehyde solution for one hour and air dried. They were subsequently immersed in 0.9% saline for 24 hours at which time tensile strength and percent solids determinations were initiated. Films were cut into strips with typical dimensions of 2" x 0.4" x 0.05" and tested in the Instron Testing Machine as described in Section 3.2.2. Surface water droplets were blotted from the surface of the films immediately before weighing. After weighing, films were dried at 105° in a 28 p.s.i. vacuum oven for 24 hours and reweighed to determine a percent solids content.

Figure 5.1 indicates the maximum tensile strength observed for the different composites. Where possible, three strips from each sample were tested and a mean was calculated. A mean maximum tensile strength observed for a composite of 66.7% collagen, 33.3% gelatin was 1010 p.s.i. This strength is significantly higher than the strength of gelatin or collagen films alone.

The percent solids of each of these films is plotted

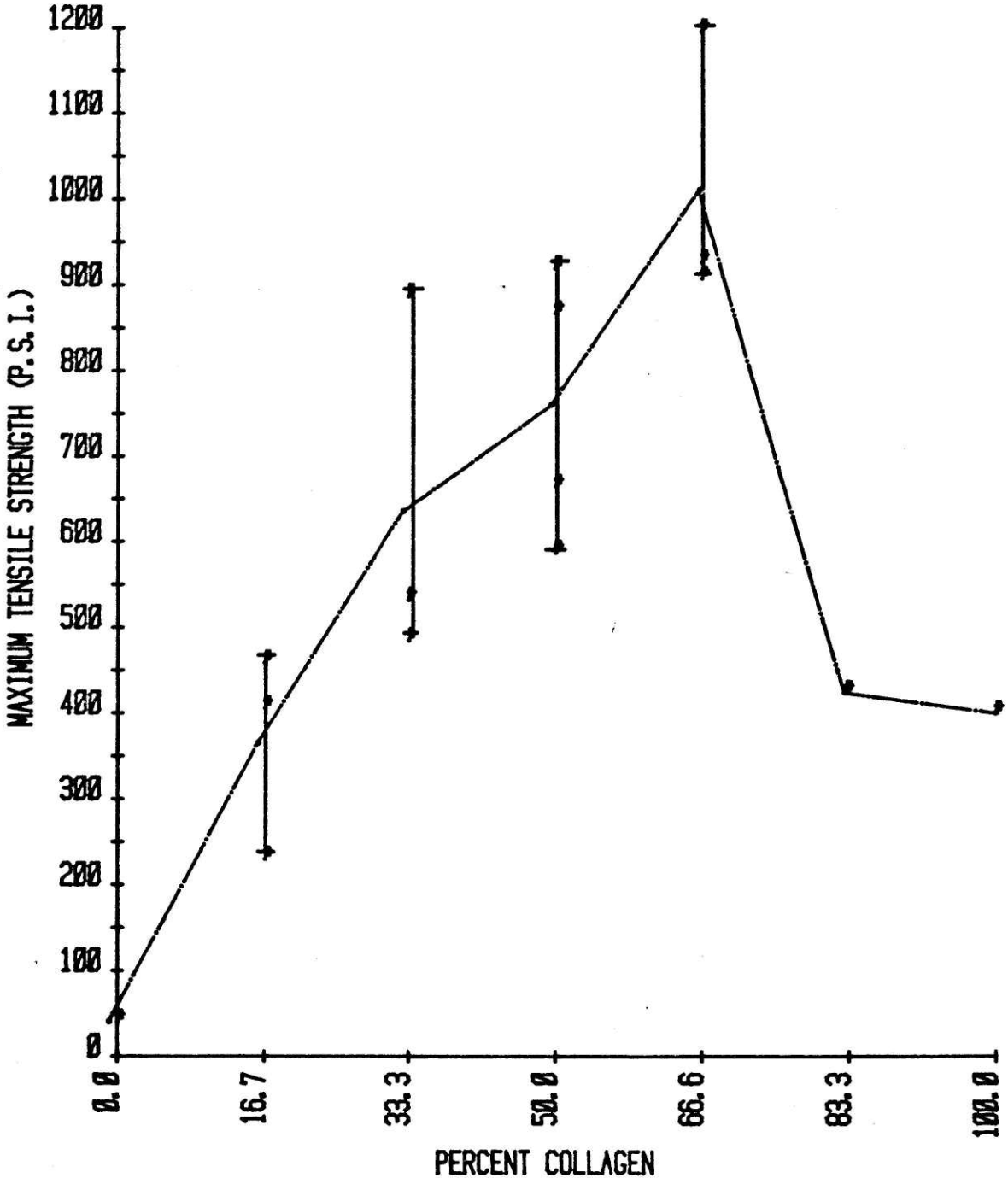

Figure 5.1 The maximum tensile strength of gelatin-collagen composites is plotted as a function of the percent collagen in the composite. Note that the percent gelatin = 100 - % collagen.

in Figure 5.2. A monotonic increases is observed as the percentage of gelatin in the films decreases. This increase in solids content can explain much of the strength increase observed in the films containing higher collagen concentrations. Gelatin also plays an important role in strengthening the composites. Figure 5.3 plots typical stress-strain curves for for many of the composites. The data indicate that gelatin permits greater elongations before breaking. In certain concentrations, gelatin may even stiffen the composite.

The 100% collagen film and the 83% collagen film formed a significant amount of bubbles in the drying process. Only one of the strips tested of the 83% collagen film was felt to represent the strength of the film. The 100% collagen film contained so many bubbles that its measured strength (120 p.s.i.) was not felt to represent its true strength. A value of 400 p.s.i. was plotted on figure 5.1 based on Silver's data (43) and that obtained in our experiments by preparing collagen films in Buchner Funnels. The stress-strain curve plotted in Figure 5.3 for the 100% collagen film is based on a collagen film evaporated on a filter paper in a Buchner Funnel. The cross-linking and testing of this film followed the same protocol of the other films in the series.

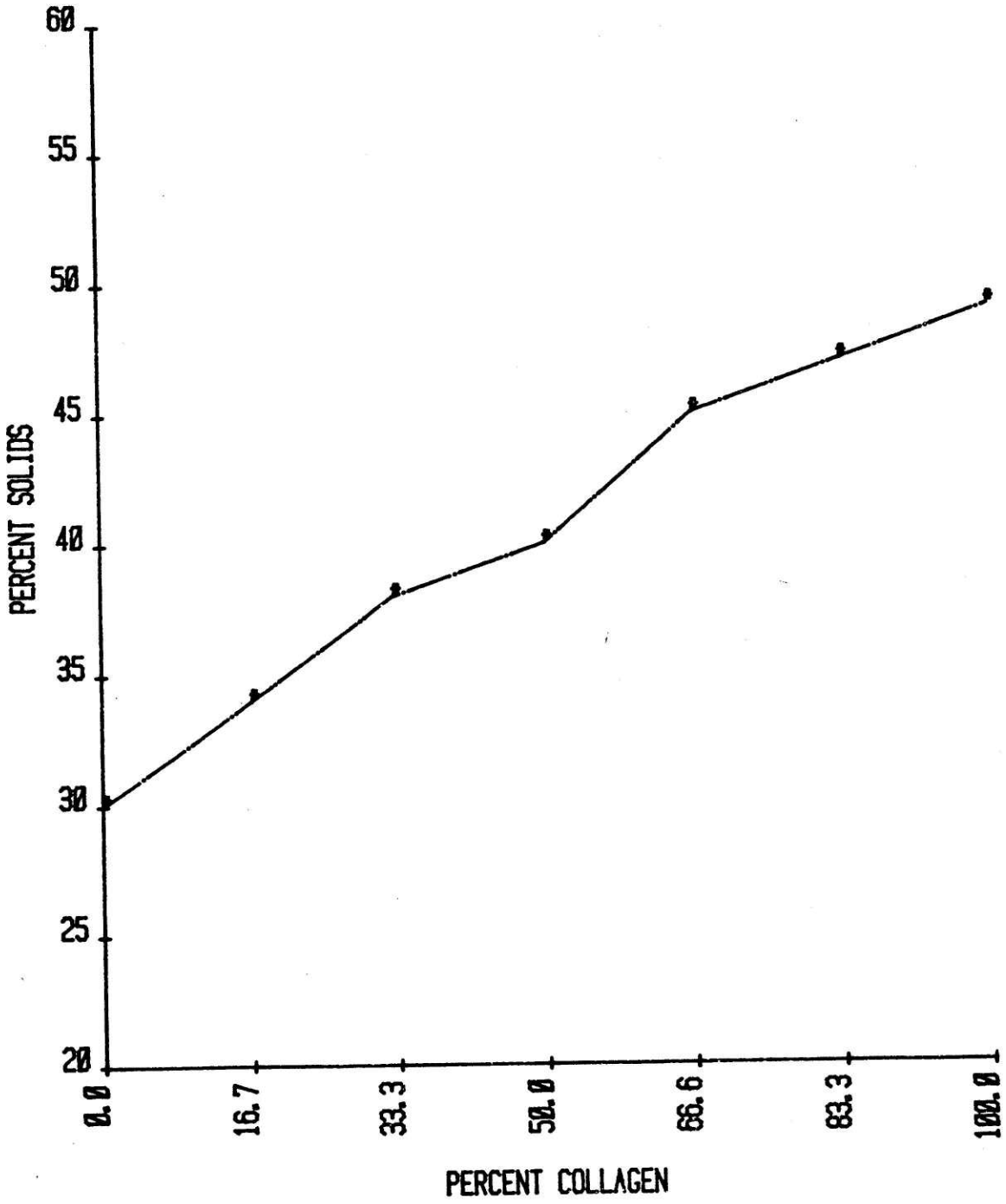

Figure 5.2 The percent solids of the equilibrated composite films is plotted as a function of percent collagen in the composite. % Gelatin = 100 - % Collagen.

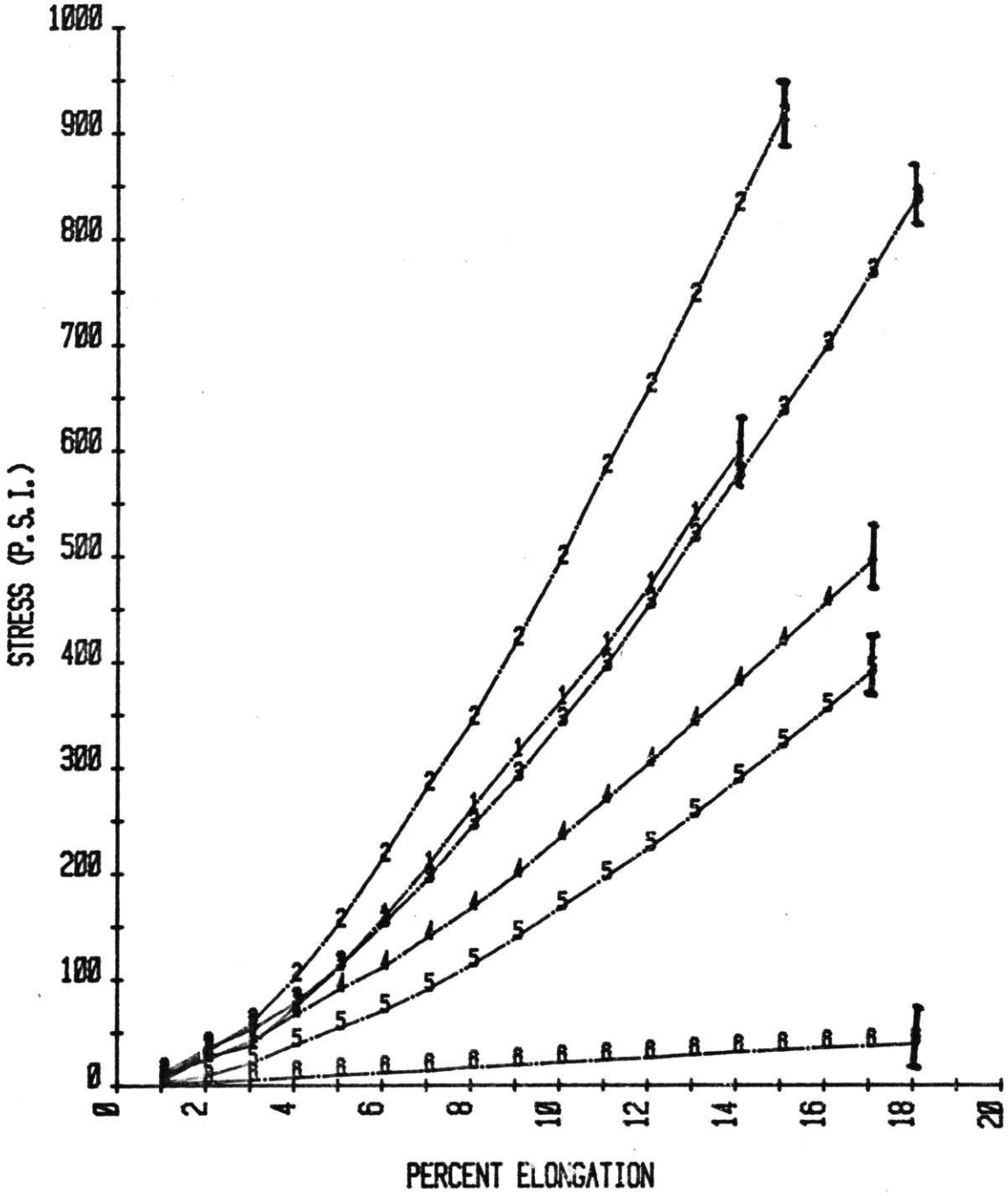

Figure 5.3 Typical Stress-Strain curves for Gelatin-Collagen composites. 1: 100% Collagen. 2: 66.6% Collagen. 3: 50% Collagen. 4: 33% Collagen. 5: 16.7% Collagen. 6: 100% Gelatin.
Note: % Gelatin + % Collagen = 100.

Gelatin-Collagen composites were also prepared by acetone precipitation. 0.18 grams of swollen collagen in 75 ml of 0.5 molar acetic acid was brought to pH 7 by titration with NaOH. 100 ml of acetone was added to the collagen, and was filtered through the Buchner funnel for 2 minutes. At this time a solution containing 100 ml acetone, 98 ml water, 2 ml of 25% glutaraldehyde, and 30 ml of 5% gelatin was added. The resulting film adhered to the paper and couldn't be removed. The film appeared to have a high wet strength by Instron testing, but the data are difficult to interpret because the paper and the film were glued together. Time limitations precluded a more detailed analysis of the film.

5.3 Summary

Films cast with a mixture of gelatin and swollen collagen were cross-linked in glutaraldehyde and equilibrated in saline. These films showed that many of the composite films have a higher tensile strength than either of the constituent films. Acetone precipitation of these composites has proven to be a rapid method of obtaining these films and may be important in the designing a method for applying these proteins as adhesives in a biological environment. Further experiments will be required to better understand the mechanism of reinforcement which accounts for the increase in strength in these composites.

Chapter 6 DISCUSSION

This investigation has consisted of many experiments to try to improve the wet strength of protein-based adhesive systems. In order to review the experiments, a table is listed below with the materials, their ultimate tensile strengths, and percent solids values.

TABLE 6.1

Material	Tensile Strength (wet)	Percent Solids
Tissue Adhesives (GFR and Clotting System Adhesives)	10	25-65
Gelatin film cross-linked in glutaraldehyde	40	30
Gelatin film cross-linked in glutaraldehyde in acetone	130	49
Gelatin film cross-linked with a photoinitiated agent	low	9
Gelatin-Collagen composite cross-linked with glutaraldehyde	1000	45
Randomly oriented Collagen film cross-linked with glut.	400	49
Surgical Suture	9300	42
Collagen Tape	3400	42
Tendon	3,000-10,000	40
Bone	10,000-18,000	96 (41)

These data show many important properties of polymer physics. Bone achieves its wet strength through mechanisms of anisotropy, crystallinity, cross-linking, and the addition

of a composite material. In addition, the high solids content of bone adds to its strength. Polymethylmethacrylate is the current adhesive used in orthopedic surgery. PMMA is a strong engineering material and has been successful in many applications in orthopedic surgery. As a cement, PMMA has several limitations including its toxicity, heat of reaction, and the fact that it is not biodegradable. Two soft tissue adhesives, gelatin-formaldehyde-resorcinol and fibrinogen-thrombin-platelet factor XIII, have been well described in the literature (15-28). These adhesives are biodegradable, but their mechanical properties are only suited to low strength applications.

This investigation focused on the issue of wet strength in protein polymer systems. It was found that the wet strength of gelatin could be increased from 40 p.s.i. to 130 p.s.i, when the cross-linking was appropriately performed in acetone. The acetone reduced the swelling of the gelatin with water which occurs when conventional methods are used. Gelatin-Collagen composites were found to have ultimate tensile strengths of over 1000 p.s.i. A composite material enables one to combine the good adhesive properties of gelatin, fluidity and tack, with the mechanical properties of collagen.

These data indicate that biodegradable tissue adhesives have the potential to be one or two orders of magnitude stronger than those currently being used (Table 6.1). An adhesive which achieves these potential strengths

would have possible hard tissue applications.

One goal of this investigation was to evaluate the possibility of using a biodegradable adhesive in bone fracture healing. In making this evaluation, it is essential that a relevant criteria is applied. Applying the constraint that the cement must have the strength of intact bone is probably an unrealistic criteria. Because of the structure and anisotropy in bone, requiring a bone derivative to possess mechanical properties similar to bone, would over-constrain the problem. A more realistic criteria would require the adhesive to have strength properties comparable to bone at a stage of fracture healing when the limb is clinically usable in a limited way. The mechanical properties of bone at different stages of fracture healing have been reported in the literature (45,46). In rabbits, the tensile strength of an osteotimized radius increases linearly over a period of four weeks and then reaches a constant level by about six weeks (2800 p.s.i.). At three weeks, an externally mobilized rabbit radius has an ultimate tensile strength of about 700 p.s.i. It is about this stage of healing when restricted use of a limb might be allowed clinically. Therefore, if a gelatin-collagen composite could be applied as an adhesive, it would meet this strength criteria. The polyurethane adhesive system (5) which was tested on human subjects had comparable mechanical properties to the gelatin-collagen composites discussed in this paper. Failure seemed to

occur because of fatigue, and the fact that it is not biodegradable. A biodegradable adhesive with similar mechanical properties may produce superior results.

In studying the wet strength of various protein films very little attention was given to the problem of adhesive application. It was felt that the initial strength data provided by these experiments was necessary before a second evaluation could be made to the potential of proteins as adhesives. Further work will be required to solve the problems involved in applying these protein systems in orthopedic surgery.

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