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DEVELOPMENT AND CHARACTERIZATION OF EDIBLE
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of the requirements for

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Department of Food Science
and Human Nutrition

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**DEVELOPMENT AND CHARACTERIZATION OF EDIBLE
AND/OR DEGRADABLE FILMS FROM WHEAT PROTEINS**

BY

Luis Martin Rayas

A DISSERTATION

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Michigan State University
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ABSTRACT

DEVELOPMENT AND TESTING OF EDIBLE AND/OR DEGRADABLE FILMS FROM WHEAT PROTEINS

By

Luis Martin Rayas

A new procedure for making degradable films was developed which resulted in the issue of U.S. patent No. 5,472,511 (issued December 5, 1995). This procedure consisted of bringing the proteins into solution using wheat flour as raw material. A centrifugation step yielded a supernatant, containing the solubilized wheat proteins, called the film-forming solution (FFS). Several films were produced by casting the FFS on a flat inert surface. Depending on the FFS characteristics (e.g., pH and cross-linker), films with different properties were fabricated.

Analyses of the films were performed with different techniques involving mass transfer, rheological, chemical, and physical studies. Mass transfer was studied by oxygen permeability, showing low values, similar to those of nylons, and influenced mainly by temperature and type of flour used to make the films. Rheological methods included tensile stress and tensile creep: Tensile stress demonstrated the effect of a cross-linker on the mechanical properties and tensile creep showed the importance of the environmental conditions on long-term behavior. Chemical studies involved the analysis of the films by Fourier-Transformed Infrared Spectrometry (FTIR) and swelling

behavior. FTIR showed important chemical groups or bonds present in the films. Swelling behavior was used to determine a solubility parameter for the protein films. Physical studies included the color analysis by the Commission Internationale de l'Eclairage Laboratory (CIE-Lab) technique. An increase in the yellow and red values upon cross-linking with glutaraldehyde was found while a reduction in the yellowness was observed when the films were cross-linked with cysteine or formaldehyde.

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DEDICATION

To my wife, Ana Teresa, with all my love

**To my sons and daughter, Luis Alberto, Anna Teresa,
and Diego Alejandro**

To my parents, Luis and Irma, for their infinite love and support

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NOMENCLATURE

<i>A</i>	film area, m^2
<i>a</i>	green-to-red [CIE-Lab system], - to +
<i>b</i>	blue-to-yellow [CIE-Lab system], - to +
<i>c</i>	velocity of light in vacuum, $2.9979 \times 10^8 \text{ m s}^{-1}$
<i>D</i>	diffusion constant, $\text{m}^2 \text{ s}^{-1}$
<i>E</i>	energy of photon, ergs or J
<i>E</i>	molecular quantized energy, ergs or J
<i>E_a</i>	activation energy, Kcal mol^{-1}
<i>E₀</i>	constant in Burgers model, Pa
<i>E₁</i>	constant in Burgers model, Pa
<i>G*</i>	complex modulus, Pa
<i>h</i>	Planck's constant, $6.6262 \times 10^{-34} \text{ J s}$
<i>J</i>	creep compliance (ϵ/σ), Pa^{-1}
<i>J</i>	oxygen transmission rate (STP = standard temperature and pressure = 273.15 K; $1.013 \times 10^5 \text{ Pa}$), $\text{m}^3 (\text{STP}) \text{ m}^{-2} \text{ s}^{-1}$
<i>J_A</i>	asymptotic creep compliance, Pa^{-1}
<i>J₀</i>	instantaneous compliance, Pa^{-1}
<i>J₁</i>	retarded compliance, Pa^{-1}
<i>k₁</i>	constant in Peleg model, Pa s
<i>k₂</i>	constant in Peleg model, Pa
<i>L</i>	lightness, %
<i>L</i>	length, m
<i>L₀</i>	initial length or length of undeformed sample, m
<i>ℓ</i>	film thickness (1 mil = 0.001 in), mil or m
<i>M</i>	desired moisture basis (wet basis), %
<i>M_L</i>	moisture loss, g
<i>m</i>	weight of the swollen sample, g
<i>m₀</i>	weight of the dry sample, g
<i>n</i>	rotor speed, rpm
<i>N</i>	moles of polymer chains per unit volume, mole m^{-3}

P	permeability (STP = standard temperature and pressure = 273.15 K; 1.013 x 10 ⁵ Pa), m ³ (STP) m m ⁻² s ⁻¹ Pa ⁻¹
P^0	Arrhenius constant, m ³ m m ⁻² s ⁻¹ Pa ⁻¹
P_1	partial water vapor pressure inside face (product) of film, mmHg or Pa
P_2	partial water vapor pressure outside face (environment) of film, mmHg or Pa
Q	amount of penetrant passing through a film, m ³
Q	swelling coefficient, ml g ⁻¹
r	radius from centerline of rotor to the point in the tube where relative centrifugal force (RCF) is required, cm
R	gas constant per mole, 1.987 cal mol ⁻¹ K ⁻¹
S	solubility coefficient (STP = standard temperature and pressure = 273.15 K; 1.013 x 10 ⁵ Pa), cm ³ (STP) cm ⁻³ Pa ⁻¹
S_w	original weight of sample, g
t	time, s
t_w	time for weight change, day
T	absolute temperature, K
T_g	glass transition temperature, °C or K
T_m	melting temperature, °C or K
$WVTR$	water vapor transmission rate, g m ⁻² day ⁻¹
W_w	weight of water absorbed in the cup (at t=t _w), g
W_1	weight fraction of component 1, dimensionless
W_2	weight fraction of component 2, dimensionless
α	extension ratio, dimensionless
δ	solubility parameter, MPa ^{1/2}
ΔG_M	change in Gibb's free energy, J
ΔH_M	enthalpy of mixing, J
ΔP	partial gas or vapor pressure differential across the film, Pa
ΔS_M	entropy of mixing, J K ⁻¹
ϵ	strain/elongation ($\alpha-1$), dimensionless
ϵ_A	asymptotic tensile strain, dimensionless
ϵ_h	Hencky strain, dimensionless
γ	shear strain, dimensionless

λ	wavelength, cm
λ_{ret}	retardation time, s
μ_0	constant in Burgers model, Pa s
μ_1	constant in Burgers model, Pa s
λ_{μ}	wavelength, μm
ρ_s	density of swelling agent, g ml^{-1}
σ	shear stress, Pa
σ	retractive force per unit area ($f A_0^{-1}$), where A_0 is original cross-sectional area, kg cm^{-2}
σ_0	constant stress, Pa
ν	wavenumber, cm^{-1}
ν'	frequency (cycles s^{-1}), Hz
ω	angular velocity, rad s^{-1}

1. INTRODUCTION

Most industries package their products to protect and contain them, thus preventing contamination, physical damage, chemical changes, etc. This practice is done so commodities reaching the consumer will have the expected quality and shelf life (especially in foodstuffs). As a result, capital losses due to the product-environment interaction is minimized. In this study, discussions will be focused on the possibilities of film usage in food markets, although other applications can be identified for these films.

In the last ten years, several new types of films have been developed that are degradable and/or edible. Knowledge of these materials, in terms of mechanical and barrier properties, among others, is increasingly being demanded by the packaging industry, government and the general public.

Several potential advantages for using degradable/edible films include: an improvement in the mechanical-handling properties or structural integrity of the packaged food; if disposed, quick degradation in the landfills; if consumed along with the food it may increase the food value (especially in protein-based films) of the product; different nutrients and additives can be incorporated thus enhancing organoleptic properties and preventing deterioration of the packaged foods; control of gases and moisture migration from and into the product can be achieved; and, if used as coatings (films that are formed directly on the surface of the foods) these films can be used to package small foods or to prevent

deterioration of larger food items (Donhowe and Fennema, 1994 and Gennadios and Weller, 1990).

Although degradable/edible films can be successfully formed using carbohydrates, lipids, and proteins, today there is limited information available on degradable packaging materials produced from these goods. Development of films utilizing wheat proteins present a good opportunity because in the United States, approximately 57 million metric tons of wheat are produced per annum. At the farm gate, this figure contributes more than \$ 7 billion to the US Gross Domestic Product. Given that edible/degradable films, if commercialized, could replace some synthetic packaging materials, their future seems assured. It is estimated that growth of 4% in the global consumption of plastic resins from about 101 million metric tons in 1993 to about 123 million metric tons in 1998 will be observed, with the US consumption estimated to rise from about 30.5 to about 36 million metric tons in these same period (Modern Plastics Encyclopedia, 1995). From these figures, packaging is the single largest market for plastics, and most processed foods are packaged. Therefore, the possibility of finding a market for edible/degradable films seems promising.

Growth in packaging will be stimulated by rebounding markets as the global economy emerges from the economic slowdown of the early 1990's. While the stronger global economy over the next few years will foster improved end-use markets for plastics, growth will also stem from the quality, versatility, durability, and light weight of plastics, as well as technological and resin improvements (Modern Plastics Encyclopedia, 1995).

Wheat proteins present unique characteristics for use as edible/degradable films. First, they account for 8-15% of the dry weight of wheat kernels and about 70% of the total protein is contained in the wheat endosperm, the major source of wheat flour (Gennadios *et al.*, 1994). Second, the presence of high molecular weight groups of proteins is highly advantageous in order to form a film with desirable rheological properties. Third, the barrier characteristics of films obtained from proteins in general has been determined to be low for gases (Donhowe and Fennema, 1994); therefore, good control of permeability to gases such as oxygen and carbon dioxide can be achieved using these films.

In this research, the main objective was to develop a new process to make films from wheat flour proteins. This resulted in the issue of U.S. patent No. 5,472,511 (issued December 5, 1995). The method consisted of separating proteins from wheat flour by solubilizing them, followed by separating the insoluble material, and casting the protein solution on an inert flat surface. Other objectives of the proposed study were to gather rheological, oxygen barrier, chemical, and physical information on films made using the above procedure and to compare some of these values with current synthetic films used in several food applications. Results should establish the potential for commercialization and utilization of these edible/ degradable films.

2. LITERATURE REVIEW

2.1 CHARACTERIZATION OF FLOUR

Different types of flours can be characterized on a compositional basis according to their moisture and protein content, and on a quality basis by checking the amylase content and the protein behavior under mixing. All of these characteristics are important to help identify a flour and recommend appropriate end uses for the flour. It is important to mention that even variation in factors (e.g. proteins) within cultivars for a single variety could affect the end-use property of the flours (McLendon *et al.*, 1993).

2.1.1 MOISTURE CONTENT

According to Kent (1983), the optimum flour moisture level is between 12 and 13 percent. At a moisture below 12%, there is a risk of increasing fat oxidation and rancidity, while at moisture above 13% mold growth may develop.

When flour is to be stored for long periods of time, it is generally recommended storage be in a closed atmosphere for the following reasons (Kent, 1983): a) flour acidity increases due to the lipolysis of linoleic and linolenic acids (which are slowly oxidized); b) slow reduction of disulfide groups (S-S) and therefore little increase in sulfhydryl groups (-SH); c) decrease in the solubility of gluten protein; and d) changes in processing strength, although this is minor.

2.1.2 PROTEIN CONTENT

The protein present in the wheat flour plays a very important role in the formation of a strong, cohesive dough capable of retaining gas. This unique characteristic is attributed to protein, and particularly to the gluten protein fraction (Hoseney, 1994).

Albumins and globulins in flour are commonly referred to as the soluble proteins (Kent, 1983). Albumin proteins of wheat, according to Kent (1983), have molecular weights of 17,000 to 28,000. They are responsible in part for the differences in baking characteristics among flours. This author also indicated that globulins are essential for proper baking performance. Both, albumin and globulins, have metabolic and structural functions.

The insoluble proteins (gliadin and glutenin) are regarded as the storage proteins of wheat and are contained primarily in the endosperm. The gluten fraction is composed of two main groups of proteins: a) gliadin, soluble in 70% ethanol, and b) glutenin, soluble in dilute acetic acid. According to Hoseney, (1994), the gliadins are a heterogeneous group of proteins which have an average molecular weight of about 40,000 and are single-chained. Kent (1983) mentioned reported values of 42,000 to 47,000 for the molecular weight of the gliadins. These proteins are extremely sticky when hydrated with little or no resistance to extension. Gliadins are thought to be responsible for dough cohesiveness.

The molecular weight of the peptide chains of the glutenin fraction is on the order of 20,000 (Kent, 1983). Hoseney (1994) indicated that the range of

the molecular weights of the glutenin subunits is from 16,000 to about 133,000. They are a heterogeneous group of proteins bonded together by disulfide bonds into macrounits with molecular weight from about 100,000 to several million (Hoseney, 1994 and Kent, 1983). Their average complex molecular weight is about 3 million. A physical characteristic of this group of proteins is that they are resilient but not cohesive, therefore these proteins are known to give dough its property of resistance to extension (Hoseney, 1994).

The determination of flour protein is important because it is recognized (Baik *et al.*, 1994; Hay, 1993; and Roels *et al.*, 1993) that two factors, protein quantity and protein quality, may produce significant differences in the end-product quality. The quantity of protein in a flour can be resolved with great accuracy by several techniques, however, the quality cannot be determined so easily (Hoseney, 1994).

2.1.3 FALLING NUMBER

The Falling Number method is an internationally standardized method for the determination of α -amylase in grain, flour and other starch containing products, particularly wheat and rye. It determines α -amylase activity using the starch in the sample as the substrate. The method is based upon the rapid gelatinization of a suspension of flour or meal in a boiling water bath and the subsequent measurement of the liquefaction of the starch slurry by α -amylase. Falling Number values bear a complex inverse relationship with the quantity of α -amylase in the sample. This relationship is known as the Perten Liquefaction equation.

The activity of α -amylase indicates the extent to which the viscosity of a flour/water paste is reduced by amylolytic hydrolysis of the starch. A flour/water suspension in a tube immersed in a boiling water bath is stirred for exactly 60 sec. A plunger is then allowed to fall freely through the suspension under standardized conditions (Kent, 1983). The Falling Number is defined as the total time in seconds from the immersion of the viscometer tube into the water bath until the viscometer stirrer has fallen the prescribed distance through the gelatinized suspension. Thus, the stirring time is included (Perten, 1988).

2.1.4 FARINOGRAPHY

Farinography is used by the cereal industry as a relative measure of the dough strength, that is, a farinograph records the power that is needed to mix dough at a constant speed (Pomeranz, 1987). A farinograph chart is composed of two parts: one in which an amount of water is added (start of mixing) and the point of maximum resistance (or minimum mobility), is identified; the second part consists of a moderate decrease in consistency and resistance to mixing. These parts were identified by Bloksma (1984) as the stages in which the farinograph mixes flour and water into a dough, develops it, and finally overmixes it.

The general practice has been to determine the standard absorption by "titration curve" with water added from a buret to the flour as it is mixed. This technique has been most useful in determining water absorption, although information on mixing time, mixing tolerance index and dough stability, among other values, can be also obtained from the same chart. The standard water

absorption is one of the most commonly used and widely accepted farinograph measurements. It is defined as “the amount of water required to center the farinograph curve on the 500-BU [Brabender Units of torque] line for a flour-water dough” (Shuey, 1984a).

The dough development time in a farinogram is defined as “the time, to the nearest half minute, between the first addition of the water and the development of the dough’s maximum consistency, or minimum mobility, i.e., the point immediately before the first indication of weakening” (Shuey, 1984b). Stability is defined as “the difference in time, to the nearest half minute, between the point at which the top of the curve first intercepts the 500-BU line (arrival time) and the point at which the top of the curve leaves the 500-BU line (departure time). The mixing tolerance index (MTI) is defined as “the difference, in Brabender units, between the top of the curve at the peak and the top of the curve measured 5 min after the peak is reached” (Shuey, 1984b).

2.2 CHARACTERISTICS OF WHEAT PROTEINS.

Until the eighteenth century, there was very little knowledge regarding the role of the proteins in the dough formation. It was first observed that the properties of the dough and wet gluten did not vary significantly, therefore, the water absorbing power was attributed mainly to the proteins of the system (Meredith, 1969). Various authors have reported the importance of the protein in the development of good pasta, bread, cake, cookies, and other flour-related products depending upon the percent protein present in the flour as well as the quality of it.

Wheat flour proteins are very unique and therefore could be studied apart from other cereal proteins. Krull and Wall (1969), reported the composition of wheat proteins based on solubility and identified the following types: albumin (water soluble), globulin (salt solution soluble), gliadin (70% ethanol soluble), and glutenin (dilute acid soluble) proteins. Similarly, Chen and Bushuk (1970) reported the components of hard red spring wheat using the classical protein fractionation procedure of Osborne (see Table 1), in which components were analyzed for protein content and the fraction that each one represented as compared to the total sample weight.

Each of these fractions have a different amino acid composition which in turn gives them their solubility and functional characteristics. Krull and Wall (1969) reported that concentrations of ionizable amino acids (acidic: glutamic, aspartic; and basic: lysine, histidine and arginine) are low for gliadin and glutenin but high for albumin and globulin. Glutamine, accounts for more than 35% of the total amino acids present in wheat gluten (Hoseney, 1994), corresponding to similar values reported by Wall and Beckwith (1969), who indicated that about 37% of the total amino acid content in wheat gluten proteins was glutamine and Krull and Inglett (1971) reported that about 37% of glutamine was present in gluten. This is in contrast with a more recent publication (Gontard *et al.*, 1993) which reported that about 45% of the amino acid present in wheat gluten proteins is glutamine. It is possible that this last value is higher than the actual glutamine value since most of the literature reports values similar to the first three citations. Another important amino acid in gluten is proline

Table 1. Solubility fractionation of the endosperm proteins of hard red spring wheat.¹

Component	Protein content (%)	Fraction of total protein (%)
Water-soluble fraction	53.3	11.9
Salt-soluble fraction	76.2	5.2
Alcohol-soluble fraction	89.7	28.5
Acetic-acid-soluble fraction	70.2	16.6
Residue	6.4	34.0

¹Adapted from Chen and Bushuk (1970)

because of its influence in the structural conformation of the proteins. This amino acid comprises 12.5% and 13.9% in glutenin and gliadin proteins, respectively. When a proline residue is present in an α -helix, it imparts a twist to the polypeptide chains. In addition, it is known that proline residues are frequently found in flexible regions of proteins (Branden and Tooze, 1991), which suggests that the gluten proteins be elastic.

According to Krull and Wall (1969), physical bonds (i.e. hydrogen bonding, electrostatic interaction, and hydrophobic interaction) can be broken by the use of specific solvents, changing the pH of the solution, increasing the temperature or altering the salt content. Also, using an acetic acid solution (pH 3.8) gliadin had approximately 23% helix as compared to 14% for glutenin. They also indicated that gliadin has a more stable structure as compared to glutenin due to internal disulfide bonds, and found that glutamine residues and non polar amino acids are responsible for the aggregation of gluten proteins in aqueous media. In this study, they also reported that amide groups are the primary sites of association of wheat flour proteins through hydrogen bond interaction, which in part give the dough cohesive and elastic characteristics.

Vital wheat gluten is the name used to indicate wheat gluten that, when hydrated, has the ability to form an elastic dough-like material, i.e., has not lost its ability to form a cohesive mass during its production. It is readily available today in the form of powder. It is, in general, a creamy-tan to light tan powder, produced from wheat flour by drying freshly washed gluten at a controlled temperature. Several companies (e.g., ADM-Arkady and Tenstar Aquitaine)

produce starch and gluten from wheat flour. Gluten is obtained as a co-product in this process. The approved method to obtain vital wheat gluten is AACC 38-20 (AACC, 1990a). This method is the basis for producing dried vital wheat gluten utilized by the refining industry.

Several authors (Gontard *et al.*, 1993; Gennadios *et al.*, 1993; Gontard *et al.*, 1992; Gennadios and Weller, 1990; and Krull and Inglett, 1971), among others have prepared films using vital wheat gluten obtained either from the industry (i.e. Pro-80™ and F 33000) or prepared in the laboratory according to AACC Method 38-10 (AACC, 1990b). Different properties and characteristics of films have been obtained depending of the process, but most gluten protein film producers agree that the commercial vital wheat gluten yields more opaque and granular films (Gennadios and Weller, 1990 and Krull and Inglett, 1971). Furthermore, the industrially prepared vital wheat gluten is made from hard wheat flours, and its application is typically in the bakery industry for flour fortification of bakery products (Magnuson, 1985). According to Magnuson (1985), other applications include meat, fish, and poultry products; pet foods; breakfast cereals, nutritional snacks; breading and batter mixes, coatings; pasta products; cheese analogues, pizza; aquaculture; and some nonfood applications.

One important characteristic that must be considered when a process requires the extraction of starch and gluten is the parameters that must be maintained for the operation to be successful. These requirements are usually based on the grain quality (i.e. wheat), and according to Jones (1987) the most

important process parameters to consider when extracting wheat starch and gluten from flour are: a) process economics, including raw material costs, yields, losses and waste treatment costs, b) final product quality, and c) trouble-free plant operation. In choosing which wheat to purchase, it is necessary to consider: a) wheat class in relation to price, b) wheat seed variety, c) growing conditions and agricultural treatment, and d) climatic variations. He concluded that some of these factors are beyond processor control, therefore flexibility in the operating conditions must exist to control minor changes due to wheat quality or varietal mix. This is important to consider since there are various reports (Jones, 1987; Miller, 1974) indicating that in the production of wheat starch, gluten is a commercial co-product.

Gontard *et al.* (1992) reported that proteins have been studied less extensively than lipids or polysaccharides in terms of their film-forming ability, especially those of wheat gluten. According to Gennadios *et al.* (1993) and Gennadios and Weller (1990), commercial film-forming preparations are limited and include mainly those prepared from collagen, gelatin, and zein.

2.3 SEPARATION OF GLUTEN FROM FLOUR.

2.3.1 INDUSTRY VS. LABORATORY

The aim of gluten production is, until today, that of improving the gluten quality as related to a higher bread making quality of the flour. One important difference between the commercial and the laboratory preparation of gluten powder is that laboratory-prepared gluten generally has less starch and impurities as compared to an industrially-prepared gluten powder (Godon *et al.*,

1983). These authors also reported that the Martin process (in which a dough is prepared before the extraction of gluten) is the most frequently used process in the world. They developed a laboratory-size machine to separate the gluten from the starch using flour as raw material. This produced material of comparable quality and with similar yields to those found in industry. Also, no relationship between particle size and performance of the gluten was found in this study.

McDermott (1985) reported that the simplest test for gluten quality (in terms of baking performance) is probably the lactic acid sedimentation test. This procedure requires that 1 ml of ethyl alcohol and 25 ml of lactic acid (0.03M) are combined with 100 mg of gluten and mixed every 2 minutes for 10 minutes. A volume is transferred to a colorimeter tube and the turbidity of the gluten dispersion is measured at 2 times (0 and 3 minutes). The sedimentation value is calculated as the percent reduction in turbidity after 3 minutes. Best glens have a lower sedimentation value.

2.3.2 WHEAT PROTEINS FOR FILM FORMATION

The above information does not necessarily indicate the production of better gluten quality for film formation is needed. Furthermore, details of manufacturing methods are not obtained easily when companies are asked whether they used the "dough" or "batter" method (McDermott, 1985), and what wheat varieties they use for the process.

There is evidence that different uses for gluten (i.e. wheat gluten) exist and could increase beyond the current use as bread flour improvers. It is

important to cite the production of gluten by companies that produce starch utilizing flours as the raw material (e.g. ADM Arkady, Olathe, KS among others). This type of gluten is the one that most researchers use (Krull and Inglett, 1971; Anker *et al.*, 1972; Kester and Fennema, 1986; Gennadios and Weller, 1990; and Gontard *et al.*, 1992) as raw material for the manufacture of protein-based films derived from cereal grains.

2.4 GENERAL PROCEDURES FOR MAKING EDIBLE/BIODEGRADABLE FILMS

There are three terms which could create confusion because only recently (1989) was a formal definition proposed for them. The terms are degradable, bio-degradable, and photo-degradable plastics. The definition of degradable plastics, according to Narayan (1989), is "plastic materials that undergo bond scission in the backbone of a polymer through chemical, biological and/or physical forces in the environment at a rate which is reasonably accelerated, as compared to a control, and which leads to fragmentation or disintegration of the plastic." Bio-degradable plastics are "those degradable plastics where the primary mechanism of degradation is through the action of microorganisms such as bacteria, fungi, algae, and yeast." Photo-degradable plastics are "those degradable plastics where the primary mechanism of degradation is through the action of sunlight." From these definitions, and because degradable plastics is a broader definition, protein films are going to be considered and referred to as degradable packaging materials hereafter. In addition, Gennadios and Weller (1990) defined the terms edible film and

coatings to mean "thin layers of edible material applied on (or even within) foods by wrapping, immersing, brushing, or spraying in order to offer a selective barrier against the transmission of gases, vapor, and solutes while also offering mechanical protection."

Because of the properties of a protein are generally related to its amino acid composition and sequence, it is recognized that the large percentage of glutamine in the gluten result in extensive intermolecular interactions (Gontard *et al.*, 1993), which in turn are in great part responsible for the formation of the protein film. Gontard *et al.* (1993) indicated that the disulfide bonds found in gliadin are only intramolecular, while glutenin contain both types (intramolecular and intermolecular) of disulfide bonds that link protein chains into high-molecular-weight polymers. This is very important when considering the overall properties of each of these fractions. In protein film formation, gluten can be used as the raw material. It is necessary, however, to add a plasticizer to prevent the film from becoming brittle (Krull and Inglett, 1971; Wall and Beckwith, 1969; Gennadios and Weller, 1990). The function of plasticizers is to reduce intermolecular forces, softening the film structure, increasing the mobility of the biopolymer chains, and thereby improving the mechanical properties of the films (Gennadios and Weller, 1990). According to Davies *et al.* (1991), plasticisation is responsible for the change of brittle to flexible behavior in a material. This is due to increased segmental mobility of chains in the amorphous regions of glassy and partially crystalline polymers. Since plasticizers loosen the protein structure, diffusion of various gases and vapors

through the film is faster; therefore, the type and quantity of plasticizer are very important aspects of film fabrication.

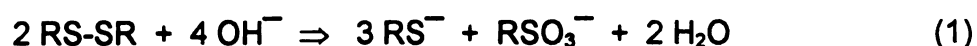
Examples of plasticizers, according to Gennadios and Weller (1990) are glycerol, diglycerol, polypropylene glycol, and polyethylene glycols. According to Kester and Fennema (1986), all of the preceding examples are food grade plasticizers. Gontard *et al.* (1993) reported that polyols and lactic acid were the only substances tested that plasticized gluten film (increased flexibility), and they determined that glycerol was the most effective plasticizer. They also indicated that the amphipolar substances tested (glycol monostearate, acetic ester of monoglyceride, sucrose ester of stearic acid and diacetyl tartaric ester of monoglyceride) had no substantial plasticizing effect while hydrophobic substances (i.e. beeswax and fatty acids such as lauric, stearic, and oleic acids) had an anti-plasticizing effect on gluten film, that reduced flexibility. Kester and Fennema (1986) stated that food-grade plasticizers are in general polyols, including also sorbitol, and mannitol. Magnuson (1985) reported that aqueous-alcoholic dispersions of wheat gluten have been prepared to form strippable, edible coatings, such as sausage casings. He also reported that vital wheat gluten, when hydrated, can be cast into films.

There are certain conditions that must be met for a protein film to form. Okamoto (1978) studied some of the main factors that affect the formation of a protein film. They used a protein film from soy milk called "yuba," a popular foodstuff in Japan, as well as films from wheat flour proteins, and films from gliadin/keratin mixture. The yuba film had approximately 55% protein and 25%

lipid. They found that protein contributes to the structure while lipids and carbohydrates contribute significantly to the flavor and physical properties of the film. Also, they reported that a yuba-like film can be formed from a solution containing soybean protein and no lipids or carbohydrates. Okamoto (1978), reported that the following conditions are required for a yuba film to form: a) a specific range of pH for the film-forming solution (for complete protein dissolution); b) possibly heating to a certain temperature and time interval (to produce a partial heat denaturalization of the proteins); c) free evaporating surface (no saturation with water vapor) and d) possibly a selective solvent (for complete protein dissolution). They determined that a very important condition to meet for film formation is the dissolution of the proteins in the solution, and this can be attained by a) or d) (adjusting pH or by selecting suitable solvents). Kamper and Fennema (1986) defined the process of film formation by this manner as "simple coacervation," where a single hydrocolloid is driven from aqueous suspension or caused to undergo a phase change by evaporation of the solvent, addition of a water-miscible non electrolyte in which the hydrocolloid is not soluble (e.g., alcohol), addition of an electrolyte to cause salting out or cross-linking, or alteration of pH.

The way in which the film was prepared in the Kamper and Fennema (1986) study was similar to the yuba film procedure. This process included the complete dissolution of gluten in water by increasing the pH beyond 10 and then heating the solution. Gennadios and Weller (1990), explained that the heating process facilitates the sulfhydryl-disulfide interchange through unfolding of the

polypeptide chains. One important difference with respect to pH that Okamoto (1978) found is that a wheat gluten film formed at pH 10, had rubber elasticity while at pH 11 the film was similar to yuba films (full of creases and translucent). This author attributed this fact to disulfide bonds, which contribute to the viscoelasticity of gluten may remain at pH 10 but are hydrolyzed at pH 11, as follows:



One important fact that Okamoto (1978) reported was that the addition of mercaptoethanol (ME) resulted in a significant increase in strength of the film formed.

Gennadios and Weller, (1990), explained how the cleavage of S-S bonds by reducing them into SH groups results in polypeptide chains with lower molecular weights, destroying elasticity and cohesiveness of gluten. Then, the dispersed gluten is reoxidized in the air and the reformation of S-S bonds yields the film structure. Okamoto (1978) also studied the development of films from a gliadin/keratin mixture and found that both proteins spread at random in the film and few available linkages that are built function property. Also, there is a limitation in making films using two or more proteins since a solvent suitable for dissolving them both in the same solution is necessary. An interesting observation by Anker *et al.* (1972) is that up to 20% and up to 50% of the gluten in a film-forming mixture can be successfully substituted with corn zein and soy protein isolate, respectively.

Gennadios and Weller (1990) reported various advantages of using edible films from wheat and corn proteins such as: 1) the film and product can be consumed simultaneously (environmentally ideal package); 2) since the films are made of a base of organic material (in the case a film is not consumed), it can degrade more easily compared to current synthetic materials; 3) additives can be incorporated into the film enhancing its general properties (i.e. flavor, color, antimicrobial and antioxidant agents, etc.); 4) nutritional value of the food is supplemented since the film contain proteins; 5) when films are used between two different structures in a food, they can reduce moisture and solute migration; 6) if an edible film is used along with non edible films (i.e., synthetic polymer) in a multilayer structure, the edible films could be the internal layers (in direct contact with the food). Similarly, some of the properties of these films as suggested by Kester and Fennema (1986) include retardation in moisture migration, gas diffusion (oxygen, carbon dioxide), oil and fat migration, solute migration, improvement in mechanical-handling properties of foods, added structural integrity to foods, retention of volatile flavor compounds, and incorporation of food additives.

Golding (1959) reported the use of some proteins as industrial plastics. Some of these include polyamides from milk, specifically casein (used in the fabrication of buttons, buckles, ornamental jewelry, and wool-like fibers); soy bean polyamides (used as extenders in phenolic molding powders and as fiber for staple-fiber form in automobile upholstery); polyamides from corn, particularly zein (used in coating of paper which provides scuff-resistant surface without high

gloss, plastics for manufacture of buttons, buckles, and other novelties); and polyamides from peanuts (used -currently discontinued- by combining equal mixtures of Ardil -peanut protein fiber- and wool, cotton or rayon to produce textile fabric products). In addition, there have been several reports on the preparation of films made from corn and soybean (Sian and Ishak, 1990; Gennadios and Weller, 1990; and Andres, 1984). It is important to realize that the properties of these films are less desirable compared to those obtained from wheat proteins. Gennadios *et al.* (1993), reported that film formation can be attained using wheat gluten, collagen, gelatin, casein, whey proteins, corn zein, soy protein, and peanut protein. A film based on soybean protein was discussed before (the yuba film). Sian and Ishak (1990) studied various factors influencing yuba film. They took a solution of soybean milk and adjusted the solution with NaOH or HCl to pH values of 2.0, 6.7, 7.5, 9.0, and 11.0. Samples of soy milk were preheated to 84-86 °C and poured into a tray fixed in a boiling water bath. The film which formed on the surface of the solution was picked up with an L-shaped wire every 20 minutes and numbered according to that sequence. From these films, they determined protein, fat, moisture, and ash contents as well as film yield (weight of the film after drying at 25 °C for 48 hours). They found that protein and carbohydrate composition increased as the sequence number of the film increased, while the lipid content decreased. Percent moisture and ash also increased with the film number. They attributed the lower carbohydrate content with film number to the presence of more fat (in the first films) which tends to bind less carbohydrate and minerals (ash). Also, they indicated that at higher

pH, the protein solubility was high, and thus more protein was incorporated into the film. This higher incorporation of negatively charged proteins at higher pH probably increased its capacity to hold more polar groups such as minerals and carbohydrates but reduced the tendency to hold the non polar lipids. An important consideration is that at elevated pH an increase in emulsifying capacity of the proteins was achieved. Kester and Fennema (1986) indicated that emulsifying agents may be incorporated when a stable oil-in-water, film-forming emulsion or micro emulsion, is desired.

In general, zein films are prepared and currently used as coating materials rather than film sheets (Gennadios and Weller, 1990 and Andres, 1984). Andres (1984) indicates that an increase in shelf life of food products can be obtained by using an edible coating composed of corn protein (zein) and vegetable oils. Torres *et al.* (1985) developed a zein film in which incorporation of preservatives was performed in order to control microbial growth at the surface of intermediate moisture-coated foods when exposed to temperature changes (emulating environmental changes during production, distribution and storage). Andres (1984) suggested that some applications could include: coating of nuts to prevent rancidity and maintain desirable texture; in confectionery products coating could be useful for maintaining desirable levels of moisture and texture; coating of fruit pieces or raisins to retain moisture when these food particles are incorporated as dry ingredients in breakfast cereals or mixes.

Andres (1984) depicted a commercial edible coating composed of alcohol, zein, special vegetable oils, glycerin, citric acid, BHA, and BHT. This is a good example of the incorporation of an antioxidant into a protein-based coating film. The mode of application of the coating includes dipping, misting, and spraying. In this type of process, when the coating is still in liquid form, zein interacts primarily with the alcohol. Once the alcohol evaporates, the zein hydrophobically interacts with the vegetable oil, and produces a non-greasy, transparent, shiny film (Andres, 1984).

Andres (1984) also studied storage tests with pecans. The pecans were held at room temperature (70 °F), 50% relative humidity, and in normal indoor light. Non-coated pecans developed abnormal flavor and chewiness after one month. On the other hand, coating with 1% zein prevented off flavors and chewiness to appear providing pecans that were fresh and felt crisp after three months. It was also found that uncoated pecans darken more rapidly than coated ones, and Andres (1984) attributed this to absorption of ultraviolet light by zein in the coating.

A different approach is given by Gennadios and Weller (1990) in which they report the use of zein coatings for pharmaceutical tablets along with foodstuffs. They also reported the use of colorants in the coatings. Other possible uses of zein coating films include application on candy products (sugar-burnt peanuts, candy corn and butter creams, chocolate-panned confections, hard gum candies, and sugar coated pan candies), on fortified rice, on egg shell (to improve quality), and micro encapsulation of food products (Gennadios and

Weller, 1990). These authors also explain that zein films can be formed in a similar way as that for wheat gluten films, that is, aqueous-alcoholic solutions of zein can be cast on flat, non reactive surfaces and then peeled off after drying. One problem with corn protein films formed in this way is that the resulting films are brittle so, the presence of a plasticizer is required to give some flexibility (Gennadios and Weller, 1990). These authors indicated that zein films prepared with and without plasticizers resulted in that: a) the unplasticized films were yellowish and transparent to translucent, and b) the plasticized films were yellow, opaque and had higher permeability to water vapors and oxygen. Also the mechanical properties of both type of films were inferior as compared to commercial synthetic polymer materials in use today.

Kester and Fennema (1986) indicated that edible films and coatings could never replace non edible, synthetic packaging materials when used in storage for prolonged periods of time. They suggested that the utility of these films is based primarily on their capacity of improving overall food quality and extending shelf life as well as improving the economic efficiency of packaging materials. One important point to consider is that synthetic polymer chains are very well controlled during polymerization (Sperling, 1992 and Birllet *et al.*, 1992). On the other hand, edible polymers are already formed in the material used for film preparation so the size cannot be controlled, except by cross-linking existing chains. The size of protein molecules could be increased by chemical and/or physical specific conditions (addition of reducing and oxidant agents to the film-forming solution, pH control, etc.). This is important since, as

polymer chain length and polarity increase, structural cohesion is enhanced, and this increase in cohesion generally reduces film flexibility, porosity, and permeability to gases, vapors, and solutes (Gennadios and Weller, 1990 and Rodriguez, 1989). Gennadios and Weller (1990) proposed some ways to increase cohesion: a) produce a uniform distribution of polar groups along the polymer chain which increases the likelihood of interchain hydrogen bonding and ionic interactions; b) produce maximum solvation and extension of the polymer molecules by using ethanol and water-ethanol combination as solvents; c) maintain good environmental control during film formation (i.e., application of warm film-forming solutions to a warm receiving surface yields) to avoid excessive temperatures since an excessive rate of evaporation during film drying could immobilize polymer molecules before they have a chance to interact to form a continuous, coherent film (i.e. defects such as pinholes or non uniform thickness). Pinholes have been reported as important permeability-increasing factors (Labuza and Contreras-Medellin, 1981, Kamper and Fennema, 1984b, and Gennadios and Weller, 1990).

Disulfide bonds play a very important role in the protein conformation of the gluten proteins. Beckwith *et al.* (1965) studied the effects of a reduction and reoxidation process when applied to gliadin. They found that gliadin can be reduced with β -mercaptoethanol at pH 7.4 in solution of 6M urea, and can also be reoxidized in acid solvents at 0.1% protein concentration. An important finding by these authors is that gliadin proteins can be reduced and then reoxidized to yield a product closely resembling the native protein. Similar

reports of the reduction-reoxidation capability of the gliadin molecules was reported by Schofield *et al.* (1983). Beckwith *et al.* (1965) assessed the restoration of native structure by immunological studies in which antibodies to gliadin present in the sera of individuals who have celiac disease were used. They found that gliadin proteins can be reduced and then reoxidized to yield a product closely resembling the native protein. It was suggested, however, that the conditions for reoxidation of the proteins must be controlled (mainly protein concentration in the solution) to get a high restoration of the native structure. For instance, Beckwith *et al.* (1965) indicated that previous studies involving microimmunodiffusion resulted with immunochemical properties of the original gliadin was restored at 0.1% protein concentration. On the other hand, the properties were not recovered when the reoxidation took place at a 5% protein concentration. This information could be useful in case disruption of the gluten protein is necessary to introduce functional groups to improve the general properties of the wheat protein films.

Stewart and Mauritzen (1966) studied the feasibility of adding cysteine to proteins of wheat dough. Their findings were that the incorporation of cysteine into dough was possible and increased in the absence of air. This suggested that a S-S exchange reaction was the formation mode rather than oxidation. They mixed a dough with cysteine in a Brabender farinograph in which nitrogen was flushed to maintain anaerobic conditions during the mixing process. In the case of gluten films, this information could be useful in an attempt to increase the molecular weight of the proteins so that tensile strength may be

increased. Rodriguez (1989), and Brown (1981) have reported that an increase in molecular weight of the polymer system will yield an increase in tensile strength. There have been few papers published related to direct measurement of the disulfide bond and its characterization as related to protein film performance.

2.5 CURRENT METHODOLOGY FOR PREPARATION OF GLUTEN-BASED FILMS

2.5.1 OVERVIEW

Krull and Inglett (1971) developed films from whole gluten obtained both in the laboratory and from the industry. These films were cast from a 20% solution of gluten in a solvent comprised 60% ethanol, 20% lactic acid, and 20% water. Lactic acid is a non-volatile acid (Gontard *et al.* 1992), which double functions as glutenin dissolving agent and plasticizer. All of these films (Krull and Inglett, 1971) were extremely brittle, not water resistant, nor did they compare favorably in tensile strength to some synthetic films.

Gennadios and Weller (1990) prepared films using commercial Pro-80™ vital wheat gluten. They put 15 g of the gluten in 72 ml of 95% ethanol. Then added 6 g of glycerol as plasticizer (instead of lactic acid used in the previous method) and boiled the solution to disperse the wheat gluten. They added 48 ml distilled water and increased the pH with 14 ml of 6N ammonium hydroxide to dissolve the glutenin fraction. The resulting films, after casting and drying, were strong, flexible, and translucent, but not transparent. Good barrier

properties to oxygen and carbon dioxide were obtained for the films, but they had a very high water permeability.

More recently, Gontard *et al.* (1992) developed films using commercial F 33000 vital gluten. These films were prepared from a solution of gluten in absolute ethanol, acetic acid, and water with glycerol added as plasticizer. The concentrations of gluten (g/100 ml solution), ethanol (ml/100 ml solution, and the pH of the solution (adjusted with acetic acid) were varied to test for the influence of these variables on film properties. They did not find an optimum condition for the formation of a film with "ideal" characteristics. The significant findings were that pH and ethanol concentration of the film-forming solution were the two most important factors influencing film opacity, water solubility and water vapor permeability. Also, mechanical properties appeared to be strongly influenced by the concentration of gluten and the pH. They suggested that depending on the film use, application technique or any other consideration, a particular film-formation combination may be chosen which could cover the basic properties to optimize.

In a more recent study, Gontard *et al.* (1993) investigated the influence of plasticizers and how they affect mechanical and water vapor barrier properties of gluten films. They prepared the films using commercial F 33000 vital wheat gluten in a concentration of 7.5/100 ml solution, 45 ml ethanol / 100 ml solution. The pH of the solution was adjusted to 4 with acetic acid. Glycerol was added as the variable to test in concentrations from 0 to 33.3 g/100 g dry film matter. Their findings were that polyols (glycerol, sorbitol, propanediol) and

lactic acid were the only substances that especially plasticized gluten film. Amphipolar substances (glycol monostearate, acetic ester of monoglyceride, sucrose ester of stearic acid and diacetyl tartaric ester of monoglyceride) had no real plasticizing effect. The hydrophobic substances (beeswax and fatty acids such as lauric, stearic, and oleic acids) had an anti-plasticizing effect on gluten film. The most effective plasticizer according to Gontard *et al.* (1993) was glycerol. Another significant finding was that water activity (a_w) and temperature were crucial parameters for establishing gluten films properties. Gennadios *et al.* (1993) fabricated gluten films by following the technique of Gennadios *et al.* (1990) in which 15 g wheat gluten, 72 ml 95% ethanol, and 6 g glycerol were mixed. This particular study showed the temperature effect on oxygen permeability of the gluten film. The result was that oxygen permeability values increased with an increase of the testing temperature. This was due to enhanced motion of the polymer segments and to increased energy levels of the permeating oxygen molecules. The oxygen permeability vs. temperature plot fitted the Arrhenius model. They proposed that probably both intermolecular and intramolecular disulfide bonds are significant in the complex formation. Furthermore, Gennadios *et al.* (1993) suggested that the extended random-coiled glutenin polypeptides provide the frame of the structure, and the smaller globular gliadin polypeptides are packed into the network.

2.5.2 CROSS-LINKING OF PROTEINS

The term "cross-linking" denotes stable association of generally large elements at specific places to create a new entity that has distinct properties as

a result of the juncture (Friedman, 1977). In the case of proteins, cross-linking promotes changes in the chemical, functional, nutritional, and biochemical properties, in addition to physical properties as related to the new molecular size and shape.

In synthetic polymerization reactions, ordered step-by-step progressions do not occur precisely. That is, each macromolecule will react at different rates, thus, the final product is represented by a mixture of macromolecules with different molecular weights. Usually a bimodal distribution for the molecular weight is obtained. In some cases, controlled rheology resins with a narrow molecular weight distribution are preferred (Gruenwald, 1993). More importantly, it is generally recognized (Gruenwald, 1993; Nicholson, 1991; Sperling, 1992; Mandelkern, 1993) that the molecular weight of a polymer greatly influences different properties, including the viscosity of the polymer melt and the mechanical properties of the films obtained.

Cross-linking of proteins has been reported to be successful by various authors (Lotan and Sharon, 1977; Uy and Wold, 1977; Harland and Fearheller, 1977; Jane *et al.*, 1993; Mahmoud and Savello, 1993). Protein polymers, in contrast to synthetic polymers, are already formed in the source material used to make the films. This is easily understood since the synthesis of the former occur inside an organism upon the expression of genetic information which starts the mechanism of protein synthesis. There are two points for comparing the complexity of protein and synthetic polymers. The first is that proteins are more complex than most linear polymers in that they can incorporate 20 different

monomers (amino acids) in their manufacture instead of one or two for synthetic polymers. On the other hand, proteins are structurally less complex since most chemical polymers are synthesized by polymerizing a mixture of monomers, thereby producing a distribution of chain lengths and (if more than one type of monomer is present) an approximately random sequence of monomers. Proteins are linear and unbranched and have precise lengths and exact sequences of amino acids. In fact, it is only the differences in length and sequence that distinguish one protein from any other, and make possible a diversity of structures and functions. The linear polymeric chain of almost every natural protein has the property of being able to assume a specific three-dimensional folded conformation (Creighton, 1993). In addition, these configurations, ranging from random coils to highly complex helices, are essential to the behavior and role of proteins in the life processes. This would make the chemical complexity of protein macromolecules essentially limitless (Battista, 1958).

Mahmoud and Savello (1993) used transglutaminase to cross-link covalently concentrated proteins solutions of a 1:1 (wt/wt) mixture of α -lactalbumin and β -lactoglobulin to form gels. These gels were dehydrated to produce transparent films. An important finding by these authors was that although the protein films were insoluble in aqueous buffers at various pH and heat treatments, these films were protease-digestible. Golding (1959) reported that when casein is immersed in a 4 to 5 percent formaldehyde solution, the ϵ -amino group (of lysine), as well as the unsubstituted α -amino group, can bind

one or two moles of formaldehyde, depending on the concentration of the latter. This author also reported that zein and casein plastics can be cured by immersing the plastics in formaldehyde solution. This reaction can be catalyzed by acids, being HCl the most effective.

Lim and Jane (1993) reported that mixtures of starch and zein with cross-linking agents including formaldehyde and glutaraldehyde yielded plastic-like materials when a compression-molding technique was used. They found that the effect of the cross-linking in the material was that of the enhanced physical strength and water-resistance. They indicated that their starch-zein plastics were economically feasible replacements for petroleum-based plastics.

Cross-linking of proteins with glutaraldehyde was reported successful by Richards and Knowles (1968), and indicated that the protein cross-linking is irreversible, surviving treatments with urea, semicarbazide, and wide ranges of pH, ionic strength and temperature. Richards and Knowles (1968) and Chatterji (1989) reported the formation of a Schiff base by the interaction between the aldehyde and the amino groups of the amino acids. Chatterji (1989) attributed the change in color from pale yellow to deep orange to the formation of the Schiff (aldimine) linkage between the free amino groups of the proteins and glutaraldehyde. This author also reported that when native gelatin is treated with glutaraldehyde, the reaction exclusively involves the lysines, to almost 100%. The schematic of the reaction is represented in Figure 1. Satyanarayana and Chatterji (1991) demonstrated that the cross-linking of gelatin granules with glutaraldehyde involves the ϵ -amino groups of the lysine residues of the protein

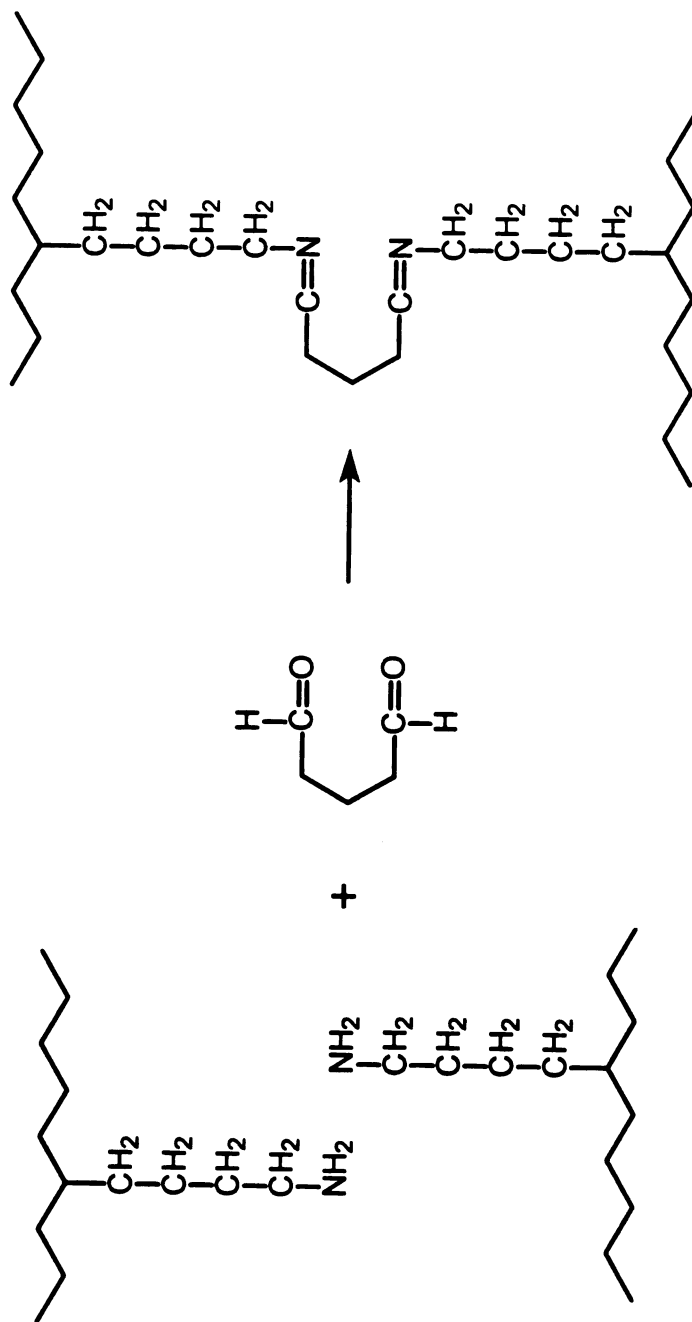


Figure 1. Scheme of reaction between the ϵ -amino group of lysine residues of two protein chains and glutaraldehyde.
[Adapted from Chatterji, 1989]

and the aldehyde functionality of the glutaraldehyde yielded a versatile cross-linked matrix.

2.6 MASS TRANSFER IN POLYMERIC FILMS FILMS

Gas and vapor transport in polymeric materials is of great importance to the packaging industry because this is one of the functions of a package in protecting contents. No polymer film is known to provide a complete barrier to the transport of a gas or vapor molecule (Brown, 1981). In the absence of cracks, pinholes, or other defects the mechanism for gas flow through a polymer film is by activated diffusion, driven by a concentration gradient. The penetrant dissolves in the film matrix at the high concentration side, and then diffuses through the film towards the low concentration side. The overall phenomenon is influenced primarily by the chemical composition, size, shape, and polarity of the penetrating molecule and chemical composition and polymer-chain segmental motion within the film matrix. When the concentration of the permeant at both sides of the film are kept constant, the system eventually reaches a steady state after an initial period of transient flow. The determination of the permeability of edible films is carried out in a similar fashion as for non-edible films using continuous flow and quasi-isostatic methods (Donhowe and Fennema, 1994).

The solution to the second Fick's law for a continuous flow permeation experiment for oxygen (Hernandez *et al.*, 1986) is given by the following equation:

$$\frac{F_t}{F_\infty} = \left(\frac{4}{\sqrt{\pi}}\right) \left(\sqrt{\frac{\ell^2}{4Dt}}\right) \sum_{n=1,3,5}^{\infty} \exp\left(\frac{-n^2 \ell^2}{4Dt}\right) \quad (2)$$

where F_t is the flow rate of oxygen permeating the films at time t , F_∞ is the oxygen flow rate under steady state conditions, ℓ is the film thickness, and D is the oxygen diffusion coefficient. By taking only the first term of the series, the permeation experiment up to a value of the flow ratio of 0.95 can be simplified to the following equation:

$$\phi = \frac{F_t}{F_\infty} = \left(\frac{4}{\sqrt{\pi}}\right) X^{1/2} \exp(-X) \quad (3)$$

where $X = \ell^2/(4Dt)$, and ϕ is the ratio between the flow rates at time t and at steady state. From a continuous flow permeability experiment, F_t values can be obtained as a function of time from $t=0$ to the steady state. The Newton-Raphson method can be used to evaluate X from the Eq. (3) as a function of time. The diffusion coefficient D is determined from the slope of the straight line of the plot X^{-1} vs. time for values within the range of $0.05 < \phi < 0.95$ (Hernandez *et al.*, 1986). The oxygen permeability constant, P , can be determined directly from the steady state value of each permeability experiment:

$$P = \frac{F_\infty \ell}{\Delta P} \quad (4)$$

where ΔP is the driving force given by the oxygen or permeant pressure gradient across the film.

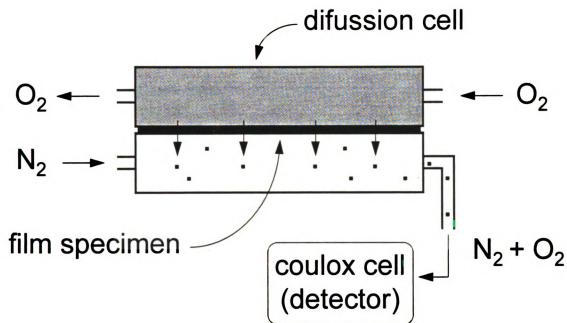
Finally, according with Hernandez (1994), Gavara and Hernandez (1994), and Kester and Fennema (1986), the solubility (S) of the permeant into the film can be calculated by applying Fick's and Henry's laws with the following equation:

$$S = \frac{P}{D} \quad (5)$$

It is assumed, in Eq. 5, that D is independent of the concentration of the penetrant. Figure 2 presents a diagram of a setup for oxygen permeability studies using a diffusion cell, with carrier gas (nitrogen) and test gas (oxygen) flushed on either side of the cell. The terminology of the units is also explained in this figure. Although this study focused only on measurement of permeability of oxygen (gas) through the films produced, both, gas and water vapor permeability on other films are discussed. It must remain understood that permeability is not a universal property of the film, but rather a characteristic of both film and penetrant under specific environmental conditions. Permeability of water vapor through a polar film matrix, for example, increases significantly as vapor pressure is increased (Kester and Fennema, 1986).

2.6.1 PERMEABILITY OF GASES

Brown (1981) explained the various methods which can be employed for gas permeability measurements: a) manometric method in which the quantity of gas that has permeated through a test piece in a given time is measured as a change in pressure and volume b) constant volume method in which the steady state portion of the plot of pressure versus time may be near linear, hence the



$$P = DS = \frac{Q\ell}{At(\Delta P)} = \frac{[\text{m}^3\text{O}_2(\text{stp})](\text{m})}{(\text{m}^2)(\text{s})(\text{Pa})}$$

where:

- P = Permeability
- D = Diffusion constant
- S = Solubility coefficient
- Q = Amount of oxygen passing through the film at standard temperature and pressure (stp)
- ℓ = Film thickness
- A = Film area
- t = Time
- ΔP = Oxygen gas partial differential pressure

Figure 2. Experimental setup for oxygen permeability studies on films.

calculation of the gas transmission rate can be carried out either at a single point or over an extended time interval, c) the constant pressure method in which pressure is kept constant in the low chamber of a cell and the volumetric change in permeated gas is measured, and d) carrier gas method in which the test gas flows at a constant rate through one chamber and a second gas, the carrier, flows through the other chamber at a constant rate. The test gas which permeates through the polymer is swept away to a detector which may be of the absorptiometric or thermal conductivity type.

Another important consideration in permeability of protein-based films (wheat gluten, corn zein, and wheat gluten/soy protein isolate) was discussed by Gennadios *et al.* (1993). According to Gennadios *et al.* (1993) not much information is available on the mechanical properties and barrier characteristics for protein films because much of the information on the preparation of these edible films derives from patents. These authors found that the differences among oxygen permeability in the three films at each temperature were significant. They also suggested that, as a general rule, permeability values follow a direct proportion effect meaning there is an increase in permeability with increase of temperature. This is attributed to enhanced motion of the polymer chains and to the greater energy level of the permeating molecules due to a more energetic system. Gennadios *et al.* (1993) reported that wheat gluten films had a lower permeability compared to corn zein films under the same conditions. This was attributed to the more complex structure and closer polymer segments in the gluten films. They concluded that oxygen molecules can permeate more readily

through a zein helical conformation (about 50%) than through the highly cross linked gluten structure, and the nature of interaction involves disulfide bonding. One of the major contributions of this study was that it gives practical comparisons between the three protein films with respect to other films. An important characteristic of these films was that the oxygen permeability values were lower compared to other polysaccharide and composite polysaccharide/lipid edible films. These values were also lower than some of the common plastic films used, such as low and high density polyethylene, polypropylene, polystyrene, and unplasticized polyvinyl chloride. They mention that the protein films were even less permeable to oxygen than polyamide-6 (Nylon-6) which is known as a good oxygen barrier. It is important to note, however, that the oxygen permeability values were obtained at 0% relative humidity. Oxygen permeability may be very different if tested at higher relative humidities because of the hydrophilic nature of the protein molecules (Gennadios *et al.*, 1993). Gontard *et al.* (1993), demonstrated that during hydration of a gluten film (increase of relative humidity), mechanical properties such as puncture strength and elasticity decrease while water vapor transmission rate and extensibility decreased. According to Gennadios *et al.* (1993) the response of protein films showing increased oxygen permeability with an increase in relative humidity is comparable to synthetic moisture-sensitive films such as polyvinyl alcohol, polyvinyl acetate, cellophane, cellulose acetate, polyamide, and ethylene-vinyl alcohol. A very interesting suggestion made by Gennadios *et al.* (1993) is that of the possible application of these types of films

in multilayer laminates. In this case, the good oxygen barrier properties of the protein films could be used in one of the layers of the laminates, while the other layers could protect the moisture sensitive characteristic of the protein films. This type of laminate is used in industry, i.e. ethylene-vinyl alcohol (EVOH). It has very good oxygen barrier properties but is moisture sensitive (water vapor reduces its oxygen barrier properties). Thus, EVOH is placed in a multilayer laminate structure in which the other materials protect it from moisture.

2.6.2 PERMEABILITY OF WATER VAPOR

Brown (1981) indicated that water vapor is generally measured by the gravimetric method. This method consist of introducing a desiccant or water into the bottom of a dish and the test piece is sealed onto the lip of the dish using wax. The assembly is then placed in an atmosphere of controlled temperature and humidity. The weight gain or loss is measured at regular intervals of time and, when this becomes approximately constant, the rate of weight change is used to calculate the quantity of water vapor which has permeated through a unit area of the test piece per unit time.

Kamper and Fennema (1984a) explained that water permeability through films varies significantly with film composition and with orientation of molecules in the film. They suggested that lipids appear to be the most effective barrier to the movement of water through an edible film.

Water vapor permeability of edible films was studied for a bilayer film by Kamper and Fennema (1984a). In this study, the film matrix was composed of hydroxypropyl methyl cellulose, a carbohydrate. This film was considered a

layer and additional lipid layers were tested for water vapor permeability using the test mentioned above (gravimetric method). They determined water vapor transmission rate (WVTR) and permeability (P) by using the following equations:

$$\text{WVTR} = \frac{W_w}{At_w} \quad (6)$$

and

$$P = \frac{W_w \ell}{At_w(P_2 - P_1)} \quad (7)$$

where: $P_2 - P_1$ = vapor pressure differential across the film (ΔP).

They found that water vapor transmission through the emulsion film was highly dependent on chain length and degree of saturation of the fatty acids. The introduction of one double bond to the fatty acid hydrocarbon chain increased the WVTR. Decreasing the chain length of the saturated fatty acid also increased the permeability of the emulsion film to water vapor.

In a similar work, Kamper and Fennema (1984b) evaluated the water vapor permeability of a bilayer film composed of stearic and palmitic acids as one layer and hydroxypropyl methyl cellulose as the other layer. They found that the amount of lipid added to the film forming solution influenced the WVTR, and this amount resulted in a significant reduction in permeability for additions of 0.46 mg/cm² or more. They also noted that when the hydrophilic side of the film was exposed to relative humidities other than 0%, the film would absorb water from the atmosphere, apparently resulting in an alteration of the lipid layer and discontinued resistance to the water vapor through the film. Kamper and

Fennema (1984b) also noted that the amount of water vapor transmission through the films increased as the temperature decreased. It is important to note, however, that this effect may vary significantly depending of the film, since Labuza and Contreras-Medellin (1981) showed that polyethylene had a greater permeability to water at -30 °C than at 35 °C. Also, a small disruption in film integrity could significantly diminish the ability of a film to delay the permeability of water vapor (Kamper and Fennema, 1984b).

A different approach was taken by Kamper and Fennema (1985) in which the water vapor gradient was retained for longer periods of time when an edible film composed of a layer of stearic-palmitic acid and a layer of hydroxypropyl methyl cellulose was placed between two food components. One had a high water activity (tomato paste) while the other had low water activity (ground crackers). The film significantly retarded the transfer of water from the tomato paste to the crackers in two of the three conditions studied: 14 days at 25 °C, and 21 days at 5°C. They also found that the film basically halted the transfer from tomato paste to the crackers during 70 days at -20 °C. All films, after storage for 5 or more weeks, were more elastic, due to hydration of the film. In this case, an increase on water permeability could be predicted (Kamper and Fennema, 1985).

2.7 RHEOLOGICAL METHODS

Rheology is "the science of the deformation and flow of matter; the study of the manner in which materials respond to applied stress or strain" (Steffe, 1992). A polymeric material possesses a wide range of material

properties and among these the most significant are hardness, deformability, toughness, and ultimate strength (Cowie, 1991). Alfrey (1948) raised a question that any polymer-material design engineer would like to answer: "If an object of some known shape is constructed from a polymer of known properties and is subjected to a known sequence of surface forces or constraints, what will be the detailed response of this object?." The answer has to be studied by different techniques involving different behavioral characteristics. This usually involves the knowledge of the properties of the polymer by solving differential equations of practical interest which can be formulated in terms of stress and strain.

According to Cowie (1991), most of the time one would expect certain desired characteristics for a material but, unfortunately, many times these would correspond to conflicting properties. A polymer, for example, with a high modulus and low creep response does not absorb energy by deforming easily, hence it has poor impact strength. This indicates that a compromise must be sought depending on the final use of the polymer, and this requires a detailed knowledge of the mechanical properties. There exist, in the broader sense, two main types of mechanical properties that can be analyzed based on the type of study performed: at small deformations, in which creep tests are included, and at large deformation, including the ultimate properties, in which tensile strength is determined.

2.7.1 TENSILE STRENGTH

The terms "ultimate strength" or "tensile strength" of a material is the stress at or near failure and is usually measured in tension. It is amongst the

most significant properties studied in polymers (Cowie, 1991). Tensile strength correlates with toughness, tear resistance, and fatigue, hence it has real merit.

Toughness can be defined as the energy absorbed at failure. The area under the stress-strain curve has the units of energy per unit volume, or joules per cubic meter (when stress has units of pascals and strain of meters per meter). The energy may be stored elastically or may be dissipated as heat causing a permanent deformation.

A common tensile test involves elongating a dumbbell-shaped sample held in jaws that separate at a constant rate (Figure 3). The stress is measured as a function of time. Despite the difficulty of measuring strain accurately, the dumbbell samples have the great advantage that ultimate failure will take place in the center of the sample and will not be affected by stress concentration at the jaws.

2.7.2 TENSILE CREEP

In order for an object made from a polymeric material be of any practical use, it must be able to retain its shape when subjected to tension or compression over long periods of time. This particular dimensional stability is an important consideration when choosing a polymer to use in the manufacture of an item.

Cowie (1991) defined creep as "a progressive increase in strain, observed over an extended time period, in a polymer subjected to a constant stress." Steffe (1992) indicated that in a creep test, an "instantaneous stress" is

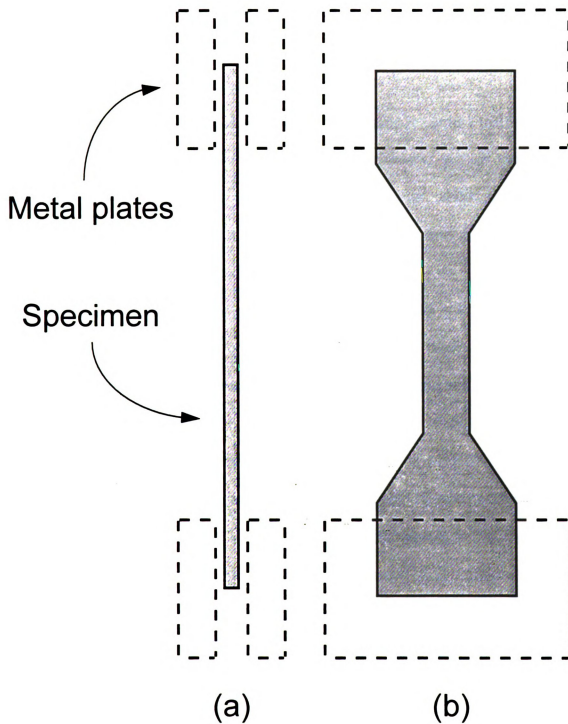


Figure 3. Example of dumbbell-shaped polymer sample specimen to evaluate tensile properties.
(a) side view, (b) front view.

applied to the sample and the strain (creep) is observed over time. Data are usually presented in terms of creep compliance:

$$J = f(t) = \frac{\varepsilon}{\sigma_0} \quad (8)$$

which is defined as the ratio of the relative elongation at time t to the stress. Ferry (1970) indicated that for the cross-linked rubbers, $J(t)$ at long times approaches a limiting value J_e , the equilibrium compliance, which according to the theory of rubber-like elasticity is proportional to the number-average molecular weight between cross-links in the network.

This idealized picture of creep behavior in a polymer has a simple mechanical equivalent constructed from springs and dashpots. Figure 4 represents an schematic representation of a creep curve, and in Figure 5 gives a series of diagrams (four-element Burgers model) which explain the simple, idealized creep behavior of a polymer. In diagram (i) the system is at rest. In diagram (ii), an instantaneous stress (σ_0) has been applied and the free spring extends by an amount σ_0/E_0 . This is followed by a decreasing rate of creep with a progressively increasing amount of stress being carried by E_1 . Given sufficient time, both dashpots are fully extended (iii). Such behavior is described by

$$\varepsilon = f(t) = \frac{\sigma_0}{E_1} \left[1 - \exp\left(\frac{-t}{\lambda_{ret}}\right) \right] \quad (9)$$

where the retardation time ($\lambda_{ret} = \mu_1/E_1$) is the time taken for the delayed strain (E_1 and μ_1) to reach $1-1/e$ or approximately 0.632 of the total deformation. A

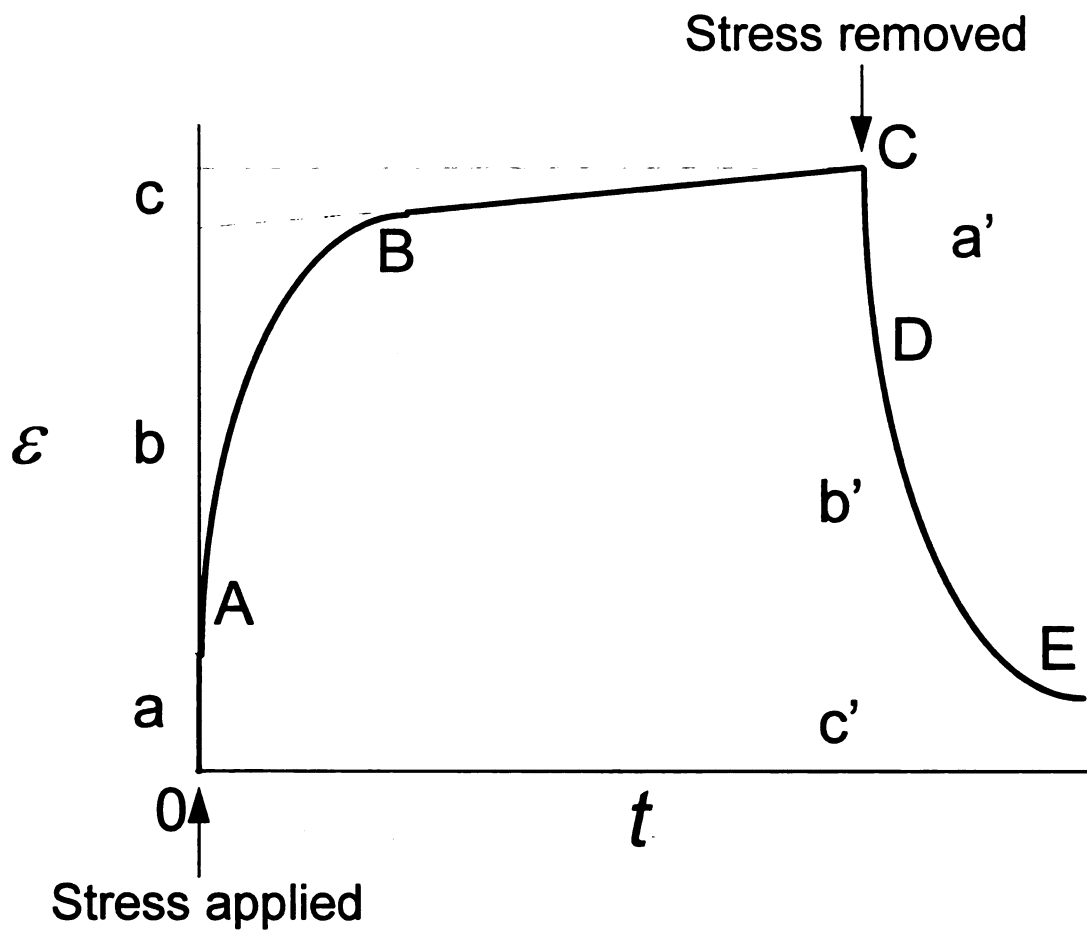


Figure 4. Schematic representation of a creep curve.

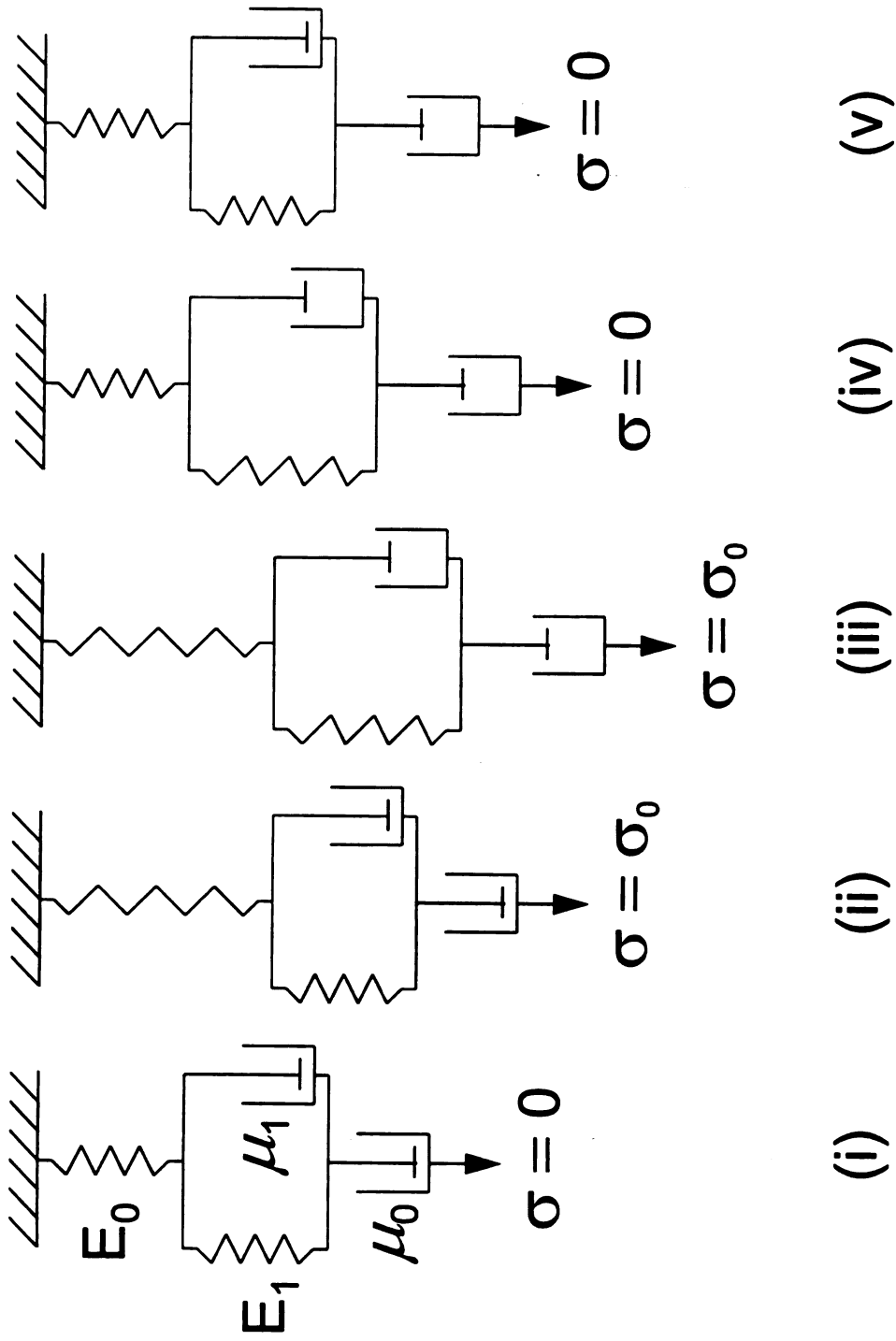


Figure 5. Burgers Model diagram of creep behavior of a polymer.

considerably longer time may be required for complete deformation to occur. When spring E_1 is fully extended the creep attains a constant rate corresponding to movement in the dashpot restricted by μ_0 . Viscous flow continues and the dashpot (μ_0) is deformed until the stress is removed.

When the stress is removed, the deformation of the free spring is recovered quickly, followed by a slow recovery in the parallel spring-dashpot system. At that time, E_0 retracts quickly (Figure 5) along section a' and a period of recovery results (b'). During this time spring E_1 forces the dashpot plunger in (μ_1) back to its original position (iv).

As no force acts on μ_0 it remains in the extended state, and corresponds to the non-recoverable viscous flow (v), defined by c' in Figure 4. The flow of the dashpot (μ_0) is retained as a permanent "set."

The complete four-element Burgers model is represented by

$$\varepsilon = f(t) = \frac{\sigma_0}{E_0} + \frac{\sigma_0}{E_1} \left[1 - \exp\left(\frac{-E_1 t}{\mu_1}\right) \right] + \frac{\sigma_0 t}{\mu_0} \quad (10)$$

in which the first part of the sum corresponds to the elastic behavior of the free spring (E_0), the second to the behavior of the combination of the spring and dashpot in parallel (E_1 and μ_1), and the third to the Newtonian behavior of the free dashpot (μ_0). Writing Eq. (10) in terms of creep compliance yields

$$J = f(t) = J_0 + J_1 \left[1 - \exp\left(\frac{-t}{\lambda_{\text{ret}}}\right) \right] + \frac{t}{\mu_0} \quad (11)$$

where $J_0 = \sigma_0/E_0$ and $J_1 = \sigma_0/E_1$.

Creep compliance data can also be modeled using an empirical equation proposed by Peleg (1980):

$$\frac{t}{J} = k_1 + k_2 t \quad (12)$$

Eq. (12) can be very useful in modeling complex biological systems.

2.7.3 THERMAL MECHANICAL ANALYSIS

Thermal mechanical analysis (TMA) deforms a sample under a static load as its temperature is changed. At very low loads, it measures the volume change of the sample with temperature (dilatometry). The applied load can be in tension, compression or in flexure. Oscillating load studies are performed by dynamic mechanical analyses (DMA).

Tension loads are used to study films and fibers (Gulke, 1994). According to this author, the equipment measures the linear expansion of the polymer and works with solid samples only. The volume change of a sample is an integral change, while the change in heat capacity is a differential change. The sensitivity of TMA, coupled with accurate temperature control, enables such factors as volume changes, melting properties and various rheological parameters to be measured (Ma *et al.*, 1990). According to these authors, volumetric changes in the substance measured can be obtained as a function of temperature or time.

Another advantage of using TMA, is that it can measure the amount of orientation of polymers, which can be used in turn to optimize the spinning or

drawing process (Gulke, 1994). Ma *et al.* (1990) indicated that in the tension mode configuration, samples are held in jaws that are attached to the probe, allowing the examination of films and fibers under tension load. According to these authors, this could be used to measure the tensile strength of films and other materials commonly used in food packaging.

2.7.4 DIFFERENTIAL SCANNING CALORIMETRY

Differential scanning calorimetry (DSC) is a technique in which the difference in heat flow between a sample and an inert reference can be measured as a function of time and temperature as both are subjected to a controlled environment of temperature, atmosphere, and pressure. This technique has become an essential tool in measuring and understanding thermal transitions in polymeric materials (Progelhof and Throne, 1993). A typical DSC heating curve is seen in Figure 6. The differential amount of energy needed to maintain the rate is plotted as the abscissa. Either time or temperature is plotted as the ordinate. These two parameters are related since the heating/cooling rate is predetermined. Points ② and ③ represent phase changes or first-order thermodynamic phase transitions (Progelhof and Throne, 1993 and Gruenwald, 1993). These transitions are identified by discontinuities in the curve. The polymer at point ② has enough molecular mobility that it continues to crystallize. At point ③, the formed crystallites now begin to melt. Point ① represents a second-order thermodynamic phase transition known as the glass transition (T_g). This transition is identified by a discontinuity in the slope of the curve.

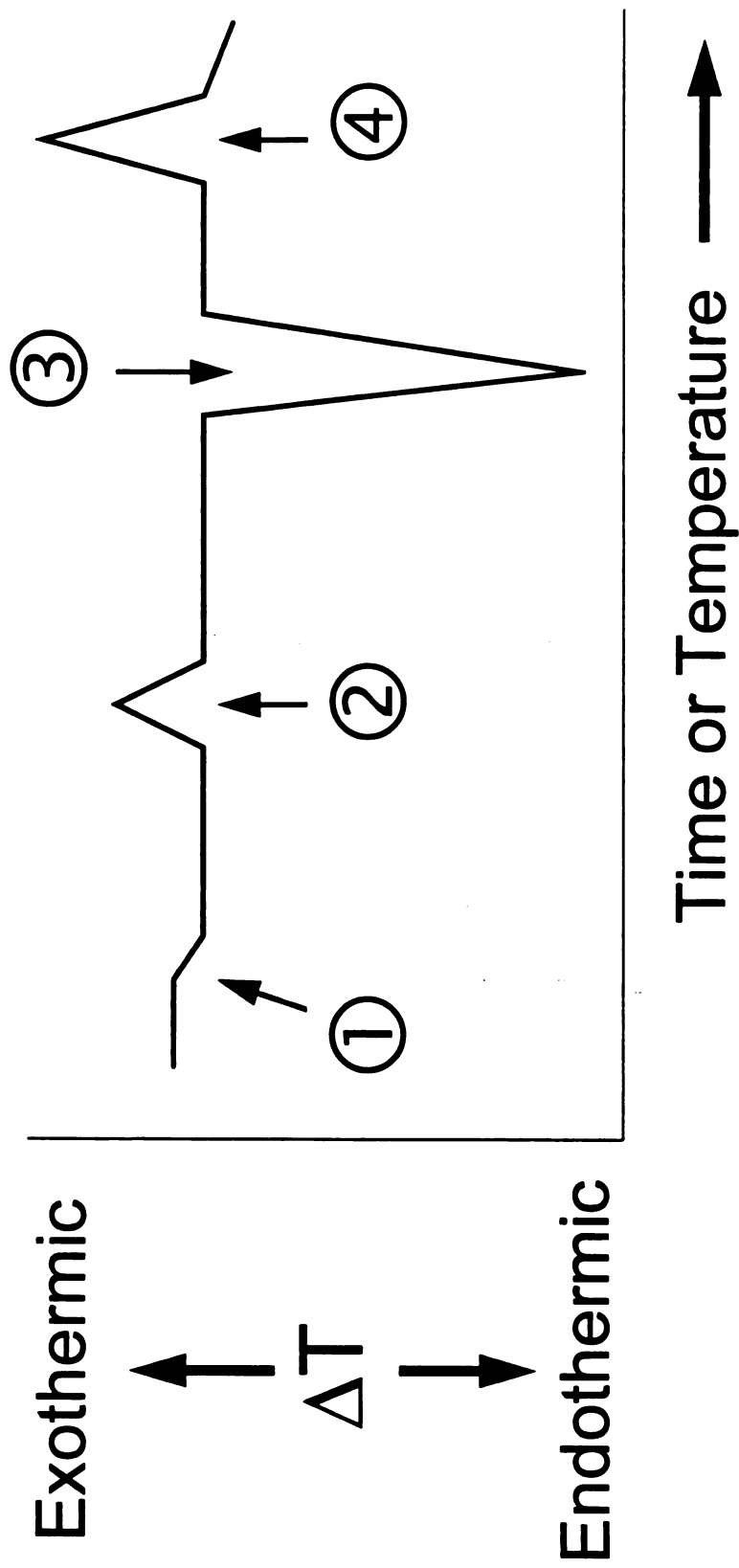


Figure 6. Schematic of a typical Differential Scanning Calorimetry (DSC) heating curve for a polymeric film.
 ①: Glass transition region, T_g ②: Cold crystallization region, T_c ③: Crystalline melting region, T_m
 ④: Chemical reaction.

[Adapted from Progelhof and Throne (1993) and Rodriguez (1989)]

The most important factor that determines the glass transition temperature of a polymer is backbone flexibility. The phenomenon is observed mainly in linear amorphous polymers (Nicholson, 1991). According to Gruenwald (1993), a viscosity of 10^{10} to 10^{12} Pa-s is generally regarded as a reasonable region to separate liquid from solid behavior, which represents the characteristic attribute for the glass transition temperature. It is important to realize that T_g is neither a thermodynamic transition such as the melting transition nor a sharp temperature point.

Polymers with flexible backbones have low glass transition temperature values and the opposite is true for polymers with rigid backbones (Eisenberg, 1993). Steric conformation, that is, hindrance caused by location or size of a bulky group, can also increase T_g . Important for proteins is the fact that an increase in factors such as molecular asymmetry and polarity of the polymer structure, are known to cause an increase in T_g values (Progelhof and Throne, 1993 and Painter and Coleman, 1994). Also, these authors indicated that additives and low molecular weight polymers can severely reduce the glass transition temperature of a polymer. This, based on free volume arguments, is explained by the increase of the free volume of the system due to these low molecular weight species, and is assumed that the relationship between the free volume of the mixture and the components are simply additive. The equation that relates the T_g of the mixture to that of its components, is

$$\frac{1}{T_g} = \frac{W_1}{T_{g1}} + \frac{W_2}{T_{g2}} \quad (13)$$

where T_{g1} and T_{g2} are the T_g 's of the pure components and W_1 and W_2 are the respective weight fractions present in the mixture.

Painter and Coleman (1994) discussed the effect of cross-linking polymeric structures on the T_g . They indicated that the effect of cross-linking can be explained on the basis of simple free volume reasoning. Since parts of the chain are tied more closely together because cross-linking decreases free volume, T_g increases. Since cross-linking is usually accomplished by the addition of a specific cross-linking agent (which can be considered a comonomer in addition to being a cross-linker), two different effects must be considered: a copolymer effect, resulting from the incorporation of a second unit, and a cross-linking effect (Eisenberg, 1993).

T_g is marked by a change in the polymer character from a brittle solid to a rubbery state. Typically the modulus or stiffness of the polymer drops by a factor of a thousand or more (Progelhof and Throne, 1993). Table 2 shows typical values of T_g for several polymers. Rosen (1982) categorizes polymer molecular motions as: ① vibrations of atoms about equilibrium positions (similar to crystal lattice vibration except that there is no precise location of the atoms in amorphous polymers and only an imperfect one in crystalline polymers); ② motion of a few (5 to 10) atoms along the main chain or rotation and vibration of side chains and pendant groups; ③ cooperative wriggling and jumping of segments of molecules approximately 40 to 50 carbon atoms in length which permits chain flexing and uncoiling (high temperature creep and second stage creep are also thought to be examples of this type of motion); and ④

Table 2. Typical glass transition temperature (T_g) values of some synthetic polymers.*

Polymer	Physical State at Room Temperature	T_g (°C)
Polystyrene	glassy	100
Polymethyl methacrylate	glassy	105
Amorphous polyethylene terephthalate	glassy	70
Silicone rubber	rubbery	-123
Polyisobutylene	rubbery	-75
Polyisoprene (natural rubber)	rubbery	-73
High-density polyethylene	rubbery-crystalline	-100
Isotactic polypropylene	rubbery-crystalline	-10
Crystalline polyethylene terephthalate	brittle-crystalline	70
Polyphenylene sulfide	brittle-crystalline	88

* Adapted from Progelhof and Throne (1993)

translational motion (sliding, reptation) of entire molecules, also called flow or permanent deformation. Below the glass transition temperature, only polymer motions of type ① and ② are found. Type ③ is important between T_g and melting temperature (T_m), and type ④ dominates in the liquid state.

Progelhof and Throne (1993) suggested that because of the nature of polymer macromolecules, "transitions are not as instantaneous as they are with simple molecules." Therefore, to obtain useful thermodynamic data, the DSC heating/cooling rate must be sufficiently slow to allow for near-isothermal transitions. Sperling (1992) suggests that both the sample and the reference material be heated at a uniform rate between 10 to 20 °C per minute. According to Progelhof and Throne (1993), the imperfect nature of the polymer structure allows some void (free volume) around the polymer chains. As the polymer is heated, the free volume increases and the increased space allows for more molecular motion. This is important because if an external stress is applied to the polymer in this state, the molecular motion will be such as to relieve the stress. Also, T_g determination in polymers is important because, as indicated by Nicholson (1991), the coefficient of thermal expansion, heat capacity, refractive index, mechanical damping, and electrical properties are among the many physical properties that change profoundly at the glass transition.

A new technique that can be used to determine the parameters discussed was recently developed and consists of a variation in the Standard DSC technique. It is referred as modulated differential scanning calorimetry (MDSC). This technique provides the same information as conventional DSC

plus additional benefits which significantly increase the understanding of material properties. A heater control and data analysis capability can make two simultaneous analyses on the same sample. In addition, (a) MDSC furnishes separation of complex transitions into more easily interpreted components; (b) has the ability to determine the initial (i.e., prior to heating) crystallinity of a material; (c) has increased sensitivity for detecting weak T_g and T_m values; (d) increases resolution without loss of sensitivity; (e) measures heat capacity and heat flow in a single experiment and; (f) can measure thermal conductivity.

2.8 CHEMICAL AND PHYSICAL CHARACTERIZATION OF POLYMERS

Chemically, two components with similar chemical groups or similar polarities will generally be compatible and soluble in each other. The free energy of mixing (Eq. 14) is used to quantitatively predict the solubility between two components,

$$\Delta G_M = \Delta H_M - T \Delta S_M \quad (14)$$

where ΔG_M is the change in Gibb's free energy, ΔH_M is the enthalpy of mixing, T is the absolute temperature, and ΔS_M is the entropy of mixing.

During mixing, there is always an increase in the entropy of mixing, so the term $T \Delta S_M$ is always positive. The sign of the term ΔG_M indicates whether or not the solution process will occur spontaneously. For instance, a negative value of ΔG_M indicates that the solution process will occur spontaneously. An ideal solution has a zero heat of mixing ($\Delta H_M = 0$), therefore since the entropy of mixing is always positive, mixing in all proportions will occur spontaneously.

However, in a regular solution (practical solution), the term ΔH_M is larger than zero, and if the value of ΔH_M is greater than $T \Delta S_M$, then the process will yield a phase separation. In practice, since the value of ΔH_M controls the sign of ΔG_M , a solvent may or may not dissolve a polymer in this type of solution. Rodriguez (1989) indicated that dissolving a polymer in a low-molecular-weight liquid causes the random coil to expand and occupy a greater volume than it would in the dry, amorphous state. If the polymer is composed of single molecules, viscous flow can occur, and the viscosity will be increased as the polymer expands. It is expected that when the polymer and solvent have the same solubility parameter (δ), the maximum expansion will occur and therefore the highest viscosity (for a given concentration) will be obtained. If the polymer comprises a cross-linked network, a solution cannot occur, but individual parts of the polymer chains (polymer segments) can solvate to give a swollen gel. Nicholson (1991) indicated the importance of the swelling experiments, since this behavior depends on the nature and extent of interchain covalent bonds. For instance, an non-cross-linked polymer will usually dissolve in an appropriate solvent, given appropriate polymer-solvent compatibility and sufficient time. By contrast, Nicholson (1991) also indicated that cross-linked polymers will not dissolve. However, these materials will swell and become softer by admitting solvent. Birley *et al.* (1992) suggested that, for uncross-linked polymers, the swelling effect is seen as a breakdown of intermolecular bond attraction due to the presence of the migrating species. However, these authors also indicated

for the cross-linked polymers this effect is reduced, so swelling experiments are a useful empirical tool for determining the degree of cross-linking. Such swelling is generally reversible Nicholson (1991) and, given appropriate conditions, solvent that has entered a cross-linked structure can be removed and the polymer will return to its original size.

2.8.1 SWELLING OF POLYMER NETWORKS

When a piece of a cross-linked polymer is put in contact with a compatible solvent, a swelling process occurs. Mark (1993) indicated that this process is a three-dimensional dilation in which the polymer absorbs solvent and reaches an equilibrium degree of swelling. At equilibrium, the free energy decrease due to the mixing of the solvent with the network chains is balanced by the free energy increase due to the stretching of the chains. The theoretical extent of swelling is predicted by the Flory-Rehner theory on the basis of the cross-link density and the attractive forces between the solvent and the polymer (Sperling, 1992). Forces considered arise from three sources: (a) the entropy change caused by mixing polymer and solvent (the entropy change from this source is positive and favors swelling); (b) the entropy change caused by reduction in numbers of possible chain conformations on swelling (the entropy change from this source is negative and opposes swelling); and (c) the heat of mixing of polymer and solvent, which may be positive, negative, or zero (usually, it is slightly positive, opposing mixing).

In a cross-linked polymer, the best solvent is defined for the purposes of the experiment as the one with the closest solubility parameter. This solvent

also swells the polymer the most. The swelling coefficient, Q (ml g^{-1}), is defined by

$$Q = \left(\frac{m - m_0}{m_0} \right) \left(\frac{1}{\rho_s} \right) \quad (15)$$

where m is the weight of the swollen sample, m_0 is the dry weight, and ρ_s is the density of the swelling agent.

According to Grulke (1994), mixed solvents can be treated as a single solvent by determining the solubility parameter of the solvent mixture. However, if both the solvents and the polymer interact, then the description becomes more complicated. Rodriguez (1989) and Sperling (1992), have indicated that liquids with like solubility parameters are apt to dissolve the same solutes and to be mutually compatible.

Solubility data are useful for determining chemical resistance of plastic materials, due to the fact that a solvent (i.e. organic media, polyelectrolytes, etc.) can penetrate the amorphous phase of the polymer investigated. Materials manufacturer's data sheets often define chemical resistance in an arbitrary manner (Birley *et al.*, 1992), which can be useful if the precise experimental constraints (reagent concentration, temperature, etc.) are defined. Yeo (1986) performed swelling experiments of perfluorosulfonic acid membranes using various solvents, with a range of solubility parameters of 7.4 (triethyl-amine solvent) to 23.4 [cal cm^{-3}]^{1/4} (water solvent). He found that both the sample pretreatment and the functional groups present in the membranes had a

significant effect on their swelling properties. He also observed an increase in the swelling of the membranes with an increase in the solvent temperature.

Chatterji (1989) studied the effect of swelling on gelatin networks. He also pointed out that swelling behavior is "entirely determined by the chemical nature of the chains." The gelatin macromolecules were formed by reacting gelatin chains with glutaraldehyde, which yielded an insoluble random protein network. This author indicated that swelling behavior can be used to differentiate cross-linked copolymers from one another. Among the gelatin samples, he reported that when gelatin was not cross-linked, it dissolved in water but did not observe any uptake when methanol or benzene/THF (tetrahydrofuran) were used. This same behavior was observed by Satyanarayana and Chatterji (1991).

2.8.2 FOURIER-TRANSFORM INFRARED SPECTROSCOPY

Fourier-Transform Infrared Spectroscopy (FTIR) has been recognized as an important tool for studying spatial chain conformation and structure of proteins (Wang and Smith, 1994 and Creighton, 1993). Garton (1992) published a comprehensive review on FTIR as applied to polymeric materials. This author explained the important operational parameters that a spectrometer must have to produce quality data. Among them, apodization (function introduced to truncate the data when spectra are to be compared); spectral range resolution; reproducibility; mirror velocity; phase correction; and Fourier deconvolution.

According to Garton (1992), the frequency of an absorption band in the infrared spectra is usually expressed in terms of wavenumbers (ν), whose unit is the reciprocal centimeter (cm^{-1}). The wavenumber is the reciprocal of the wavelength (λ) in centimeters, or when the wavelength is in microns, the wavenumber is expressed by Eq. 16

$$\nu = \frac{1 \times 10^4}{\lambda_{\mu}} \quad (16)$$

where wavenumber (ν) is in cm^{-1} and λ_{μ} is wavelength in microns.

According to quantum theory, the frequency (ν') in cycles/second of a photon is proportional to its energy (E) in so that

$$E = h \nu' \quad (17)$$

and the frequency (ν') is therefore related to the wavelength (λ) in cm and to the wavenumber (ν) in cm^{-1} as shown in Eq. 18:

$$\nu' = \frac{c}{\lambda} = \nu c \quad (18)$$

The useful area in the infrared spectrum for analyzing polymeric materials using FTIR is between 4000 cm^{-1} and 600 cm^{-1} ($2.5 - 16.6 \mu$). In addition, identification of polymer samples can be made by making use of the “finger-print” region, where it is least likely for one polymer to exhibit exactly the same spectrum as another (Cowie, 1991). This region lies within the range 1500 to 800 (6.6 to 12.5μ). Garton (1992) indicated that the energy involved in an infrared (IR) transition is insufficient to break most chemical bonds, but it is

enough to impart bond deformation associated with a vibrational transition. Giacin (1995) indicated that in molecular absorption of radiant energy, several components must be considered, which sum for the total quantized energy levels within the molecules:

$$E_{\text{total}} = E_{\text{electronic}} + E_{\text{vibrational}} + E_{\text{rotational}} + E_{\text{translational}} \quad (19)$$

Translational energies can be disregarded since radiation energy is not imparted through spectroscopy. Also, the energy difference between electronic energy levels is greater than that for vibrational energy, which are greater than those for rotational energy (Giacin, 1995). In addition, the energies associated with vibrational and rotational changes are comparable to those for infrared radiation, and it is the vibrational and rotational energy changes which are measured in the infrared region. Gruenwald (1993) indicated that, depending on their chemical makeup, each specific chemical group possesses characteristic absorption bands making it possible to readily identify the chemical composition of any plastic. It should be emphasized that motion is being described for the entire molecule, and not simply bending or stretching of one set of bonds. Therefore, what is observed on the atomic level (although quantum restrictions prevent from doing so) would be superposition of the many normal modes of vibration (Garton, 1992).

Giacin (1995) points out some criteria for obtaining an accurate interpretation of an infrared spectrum:

1. The spectrum must be adequately resolved and of adequate intensity;

2. The spectrum should be of a reasonably pure compound;
3. The spectrophotometer should be calibrated so that bands are observed at their proper frequencies or wavelengths;
4. The method of sample handling must be specified.

There are two important areas for examination of an infrared spectrum, the region above 1300 cm^{-1} , and the $909\text{-}650\text{ cm}^{-1}$ region. The higher frequency portion of the infrared spectrum is called the functional group region. The characteristic stretching frequencies for important functional groups such as OH, NH, CN and C=O occur in this region. The other region ($909\text{-}650\text{ cm}^{-1}$) provides with information about aromatic or heteroaromatic compounds. These low frequency bands are due to strong out-of-plane C-H bending and ring bending modes (Giacin, 1995).

The intermediate portion of the spectrum, $1300\text{-}909\text{ cm}^{-1}$, on the other hand, is usually referred to as the "fingerprint" region. Absorption in this intermediate region is unique for every molecular species (Giacin, 1995). Some typical functional groups with their absorption frequency ranges are presented in Table 3.

Satyanarayana and Chatterji (1991) observed that the IR spectra for three different polymeric materials: cross-linked gelatin (Gel-x), polymethyl acrylate (PMA), and cross-linked gelatin with polymethyl acrylate grafts (Gel-x/PMA). They observed an absorption band at 1640 cm^{-1} which they attributed to the amide and aldimine linkages. They also observed an absorption peak between $3200\text{-}3500\text{ cm}^{-1}$ which was attributed to N-H stretching and another for

Table 3. Some typical group stretching vibrational absorption frequencies for polymer analysis.*

Functional Group	Structure Found	Absorption Frequency (cm^{-1})
Hydroxyl (O-H)	1. Alcohols, ROH	3650-3584
	2. Phenols, ROH (where R = aromatic)	3546-3194
	3. Acids, R-COOH	3300-2500
Amino (N-H)	4. Amine, R-NH-	3500-3300 (two bands for primary, one band for secondary)
	5. Amide, R-CONH ₂	3521-2941
	6. Amide, R-CONH-	N-H @ 3450; amide I @ 1640; amide II @ 1570-1510
Carbon-hydrogen bond (C-H)	7. R-H (where R = aromatic)	> 3000
	8. R-CH ₂ -H (where R = aliphatic)	3000-2840
	9. methyl, -CH ₃	2962-2872
Nitrile (C≡N)	10. R-C≡N	2260-2240
Isocyanate (N=C=O)	11. R-N=C=O	
Carbonyl (C=O)	12. General Region	1870-1540
	13. Aldehyde, R-COH	1740-1720
	14. Ketone, R-CO-R aliphatic and saturated	1720
	15. Carboxylic acid, R-CO-OH Monomer	1760
		Dimer
	16. Ester, R-CO-OR saturated	1720-1706
	17. Amide, R-CO-NH ₂	1751-1736
Unsaturation	18. C=C	1650-1689
Halide	19. -C-F	C-H @ 3030; C=C @ 1640 (intensity varies with structure and substitution)
	20. -C-Cl	
	21. -C-Br	

* Modified from Giacin (1995) and Garton (1992)

N-H deformation at 1540 cm^{-1} . They also reported that the peak observed at about 2920 and 1440 cm^{-1} was due to the C-H stretching and deformation, respectively. They attributed the appearance of an absorption band at 1740 cm^{-1} to the grafting of the polymer during its formation. Chatterji (1989) analyzed these same polymeric materials and indicated that the characteristic absorption of the backbone peptide bond occurring at 1540 and 1650 cm^{-1} were the only distinguishing features of the gelatin. However, this author attributed the extra absorption peak observed for the cross-linked gelatin at 1450 cm^{-1} to the aldimine linkages, as opposed to 1640 cm^{-1} by Satyanarayana and Chatterji (1991).

Wang and Smith (1994) studied the effect of isothermal heating on spectra for chicken breast myosin. The IR spectra was useful to investigate unfolding of the proteins. They found that irregular structures were formed and were accounted for by irreversible unfolding of helices and β -structures which occur when the protein was heated at $55\text{ }^{\circ}\text{C}$. It is important to note that these authors worked with a very pure protein system, myosin from chicken breast, thus making the IR analysis very suitable according to the guidelines of Giacini (1995) explained above.

2.8.3 PLASTIC/FILM COLOR MEASUREMENTS

Color was defined by Francis (1985) as a term to denote the human eye's perception of colored materials. Another definition by Progelhof and Throne (1993), is "color is the property of radiant energy that permits a living organism to distinguish by eye between two uniform, structure-free patches of

identical size and shape. For instance, color of a protein-based film would have a significant effect on the acceptability of such film as "edible" by the population, due to several factors, including cultural, geographical, and sociological. According to Francis (1985), "certain food groups are acceptable only if they fall within a certain color gamut." This may be applicable to edible films because they could be used in the preparation of foods, especially when used as coatings or wrapping materials.

Gruenwald (1993) indicated that accurate measurement of color is important in plastic materials so the manufacturer can offer uniform colored products. For this purpose, he pointed out that there are several methods to physically describe any colored surface. In these systems, as Gruenwald (1993) indicated, three numbers must be designated: the hue (the dominant color relating to the whole visible spectrum); the chroma (indicating the intensity or purity of the color); and the value (representing the lightness or superimposed gray scale of the color, with pure black and white at opposite poles on the color sphere). Color matching involves establishing the X, Y, Z coordinates for two specimens, then determining the length of the vector between the two coordinate points. Progelhof and Throne (1993) emphasized that nowadays computers are used to determine the vector length in a chromaticity diagram. They indicated that the units are always listed as CIELab units ["CIELab" refers to Commission Internationale de l'Eclairage Laboratory, an international laboratory that established the unit system in 1931].

The importance of these values is of greatest interest when differences among plastics can be observed. Examples include those of GPPS and PMMA which are water-white transparent. Others such as polyethylene are translucent. Some are milky white and nearly opaque, like polyacetal and nylon. Others are off-white and opaque, like plasticized PVC and ABS. Some are hay- or straw-colored, such as polyimides and polyphenylene sulfide. Polysulfones are amber. And some, such as polyamide-imide, phenolic and resorcinol are cherry red when ultra-pure. Progelhof and Throne (1993) indicated that polymers are quite easily colored and so provide products that have the color "all the way through." Color measurements are very important for assuring a consistent color of the plastic products. In addition, Gruenwald (1993) also indicated the importance of the product lighting condition on acceptability. Lighting may simulate daylight, fluorescent, or incandescent light, and pigments can be applied for products to match a specific color.

More specifically, an example of the importance of color related to proteins include those changes observed by Chatterji (1989) in the color of gelatin granules from pale yellow to deep orange within minutes upon cross-linking treatment of the gelatin with glutaraldehyde. This author attributed the color change to the establishment of aldimine (Schiff) linkage between the free amino groups of the protein and the glutaraldehyde (Figure 1). The measurement of color in edible films is important since that will be the first contact of the consumer with the new film/coating material.

3. MATERIALS AND METHODS

3.1 FLOUR SAMPLES

The flours used in this study included three types:

- a) commercial bread flour (C), bought from a commercial store in Lansing. Label stated it was “Bakers and Chefs” brand, an “enriched, bleached bread flour, blend of hard spring and winter wheat class flours,” distributed by North Arkansas Wholesale Co. Inc. (Bentonville, Arkansas);
- b) hard red winter flour (H) class (King Milling Co., Lowell, Michigan);
- c) soft white flour (S) class (King Milling Co., Lowell, Michigan).

The basis for the use of these three flours was the difference that a commercial (usually blend of different classes of wheat flours and added vital wheat gluten to it) versus pure-class wheat flours may have on the film performance. Also, hard versus the soft classes represent unique groups since generally these classes are used in different food applications (Hoseney, 1994) due to their distinct physical and chemical characteristics. In addition, the soft wheat class is of particular interest since over 70 percent of Michigan's wheat is white grained. White wheat bran is in far greater demand than red wheat bran due to flavor and color problems associated with the red bran pigments after processing. White flour can be used in the production of edible and/or degradable films, among other industrial uses.

The flours used in this study were first characterized for protein content, moisture content, falling number, farinography, and by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE), under reduced and unreduced conditions. SDS-PAGE was done to confirm that there were differences between each flour so that the films produced from them may show differences in the properties to be measured.

3.2 FLOUR CHARACTERIZATION

The flours were characterized on the basis of protein content, percent moisture, falling number, farinography, and SDS-PAGE.

3.2.1 PROTEIN CONTENT

The protein of the flours was determined according to an American Association of Cereal Chemists approved micro-Kjeldahl standard method AACC 46-13. A Tecator 1008 Control Unit with a Digestion System 40 1006 Heating Unit (Silver Spring, Maryland) was used to digest the flour samples following distillation using a Kjeltec System 1002 Distilling Unit (Silver Spring, Maryland). Finally, a titration of the extracted samples was performed using hydrochloric acid. The % nitrogen was then calculated according to the following equation:

$$\% \text{ Nitrogen} = \frac{(\text{ml HCl sample} - \text{ml blank}) \times \text{N HCl} \times \text{equiv wt N} \times 100}{\text{wt of sample (mg)}} \quad (20)$$

where: ml HCl sample = milliliters of HCl used in titration of sample; ml blank = milliliters of HCl used in titration of a blank (no flour sample); N HCl = normality of HCl used for titration; and equiv wt N = number of equivalent

weights of nitrogen. From this, the crude protein (%) was calculated by the following expression:

$$\% \text{ Crude Protein} = (\% \text{ Nitrogen}) \times (5.7) \quad (21)$$

where: 5.7 represents the factor obtained by dividing 100 mg protein / 17.54 mg nitrogen (for wheat).

The percent crude protein was then adjusted to 14 % moisture basis (m.b.) for all the flour samples, since this basis is the most commonly used in the United States (Pomeranz, 1987). The adjustment was done according to the equation in AACC method 46-14A (Crude protein - Udy Dye Method):

$$\% \text{ protein (\% m.b.)} = \% \text{ prot. measured} \left[\frac{100 - M}{100\% - \% \text{moisture measured}} \right] \quad (22)$$

where M represents the desired moisture basis (wet basis).

3.2.2 FLOUR MOISTURE

The water content in the flour was determined according to AACC Method 44-15A. This method required a two to three gram portion with a tared moisture dish. Five tared moisture dishes (replications) for each flour were used in this determination. Once the flour samples were weighed, they were heated for exactly 60 minutes in an oven held at 130°C (±1°). The dishes were removed from the oven and transferred to a desiccator as quickly as possible. Weight of dishes was determined after they reached room temperature.

The % moisture was calculated by use of the following equation:

$$\% \text{ moisture} = \left(\frac{M_L}{S_W} \right) (100) \quad (23)$$

where M_L represents moisture loss and S_w is the original weight of the sample. The % moisture values were used as a basis for the adjustments in the quantities of flour used in the preparation of the films, to assume consistent dry weight values.

3.2.3 FALLING NUMBER

The Falling Number was determined using a Falling Number Apparatus Type 1400 (Perten Instruments, Huddinge, Sweden), which conforms with AACC Method 56-81-B. In general, Falling number determination on flour falls from 0-30 units higher than that for the corresponding grain. This methodology required that the weight of the flour samples be corrected to a 14% moisture content according to Table A1 in the Appendix A.

3.2.4 FARINOGRAPHY

A Farinograph (C.W. Brabender Instruments, Inc., South Hackensack, USA.) composed of a dynamometer type PL-2H and a measuring head type S-300 was used to evaluate the flow behavior of the flour.

A sample of 300 g for each type of flour used in this study was placed inside the chamber of the measuring head and water was added continuously so that mixing followed, and gave, a maximum peak value. Titration followed until a curve with a peak value of 500 ± 20 BU was obtained. A horizontal line crossing half way between the lowest and the highest value at the peak was drawn and calculations of the percent water absorption, dough development time (mixing time), mixing tolerance index (MTI), and stability were calculated for each farinogram obtained.

3.2.5 SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was done according to the technique described by Ng *et al.* (1986) to determine differences in the chemistry of the proteins present in the three experimental flours. This technique consisted of extracting the protein from the flour by adding extracting buffer solution to the flour sample. The extracting buffer solution contained mercaptoethanol (ME), which is known to reduce the disulfide bonds of wheat proteins. For the unreduced samples, no ME was added to the solution. These mixtures were heated in a boiling water bath for about 2.5 minutes. Polymerization of both, separating-gel and stacking-gel, were accomplished separately. Once the gels solidified and were ready for the sample application, eight μ l of the clear extract from the flour mixture were deposited into each slot of the stacking-gel. Also flour from Neepawa (pure variety), which is a Canadian cultivar, was prepared and included as a standard for the electrophoretic analysis. A d.c. electric current was passed through the gel and separation of the protein subunits was achieved after about 24 hours. After the current was stopped, and the electrophoregram was developed by rinsing the gel with a rinsing solution. Staining the gel followed by covering the gel with a staining solution which contained commasie brilliant blue and gently shaking it overnight by sitting the gel on a variable speed shaker. Destaining the gel was accomplished by rinsing the staining solution out and putting in a new rinsing solution for a few hours, after which a final rinse with water was done. A photograph of the gel was taken for later analysis.

3.3 FILM PREPARATION

A new procedure for film formation was developed based on utilizing the flour as the raw material and directly extracting the protein fraction into a solution. A schematic representation of the general process is depicted in Figure 7.

3.3.1 PROTEIN SOLUBILIZATION

The general procedure is described below, with variables that will be discussed for each particular film in the next section.

To approximately 150 g of wheat flour (actual quantity adjusted based on protein present with a 14% moisture content for the particular flour) 360 ml of a 70% ethanol solution was added and mixed using a magnetic stirrer (Fisher Scientific Co., Pittsburgh, USA.) to form a slurry. Agitation sufficient to form a vortex in the mixture continued under magnetic stirring for ½ hour at room temperature. Two different basic procedures, low and high pH, were used. For the low pH procedure, an adjustment of the pH to a value of 4.0 was done using acetic acid (glacial), and for the high pH procedure, an adjustment to a value of 11.0 was done using a 6N solution of sodium hydroxide (NaOH). The pH was measured using a Fisher Accumet pH Meter model 810 (Fisher Scientific, Pittsburgh, USA.). Mixing continued for one more hour at 40°C. Centrifugation of the slurry followed at 10,500 x gravity (g) using a Sorvall Refrigerated Superspeed Centrifuge model RC-5B (Du Pont Co., Newton, CT, USA.).

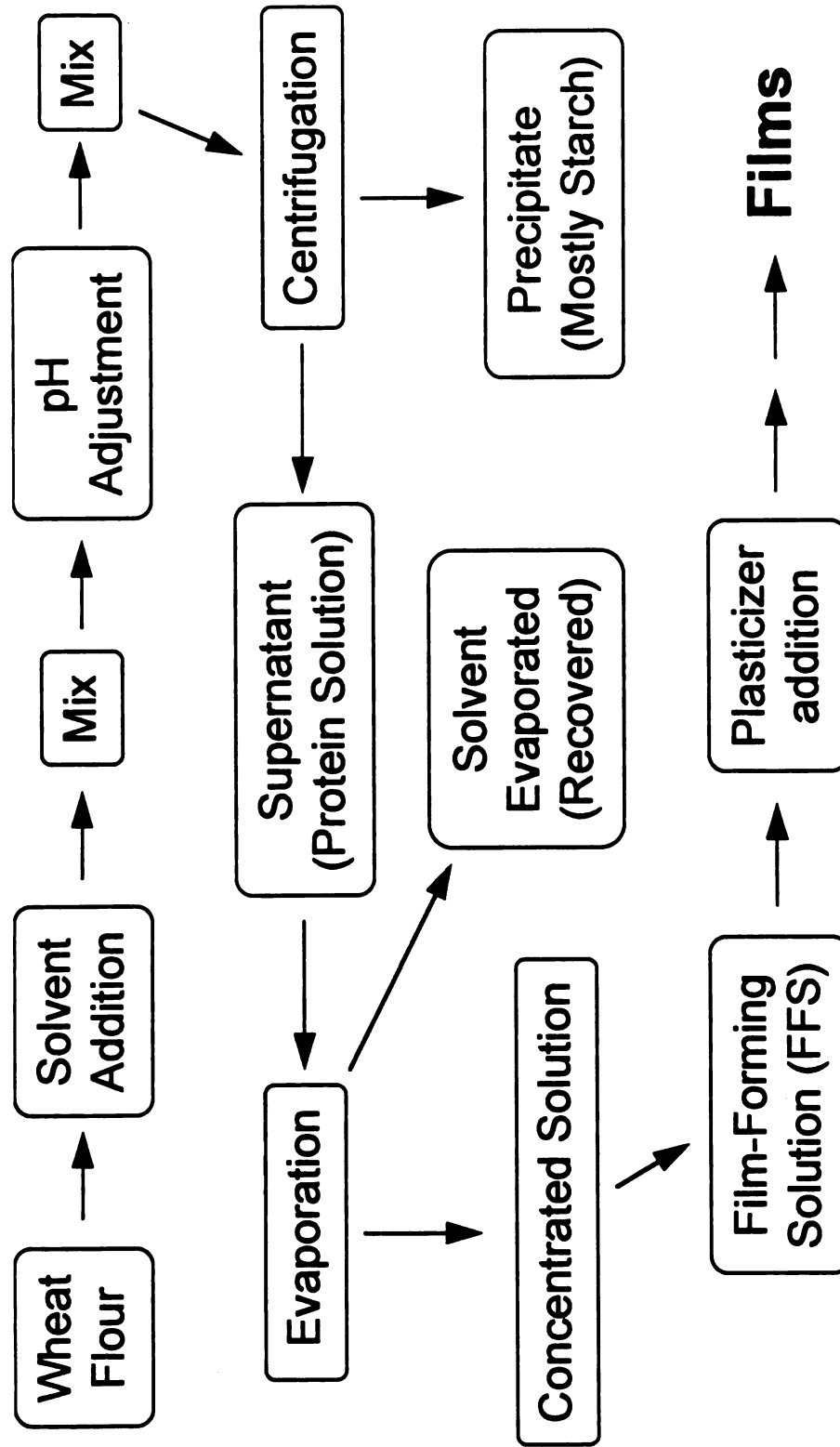


Figure 7. Schematic of film making process developed in this study.

The Relative Centrifugal Force (RCF), "g," was determined according to the following equation^{*} :

$$n = \left(\frac{(RCF)(1000^2)}{(1117)(r)} \right)^{1/2} \quad (24)$$

where n is the rotor speed (rpm) and r is the radius from the centerline of the rotor to the point in the tube where RCF value is required. The rotor used with the centrifuge was a Sorvall GSA superspeed fixed-angle rotor. For this rotor, the maximum radius has a value of 14.57 cm, and the calculated rotor speed was 7011 rpm (734 rad/s).

After the centrifugation step, the supernatant contained solubilized the proteins that were responsible for film formation. This supernatant was defined as the "film forming solution," FFS, since by just letting it evaporate on a flat surface it was capable of forming a film. The FFS was then decanted from the precipitate and poured into a 170 x 90 crystallizing dish (Fisher Scientific Co., Pittsburgh, USA.).

3.3.2 PROTEIN CROSS-LINKING

Films in which cross-linking of the proteins was required were produced by adding one of the following reagents to the FFS: a) 15 ml 0.1 M cysteine (Sigma Chemical Co., St. Louis, MO, USA); b) 0.75 ml of formaldehyde solution (Mallinckrodt Specialty Chemicals Co., Paris, KY, USA); c) 0.75 ml of

^{*} Modified from Du Pont (1980).

glutaraldehyde solution (Aldrich Chemical Co., Milwaukee, WI, USA). The addition of these reagents was done slowly after boiling the FFS for about 5 minutes, except for the glutaraldehyde solution which was added just prior to casting the film. This was done to avoid an excessive change in color from the typical light-yellow-brownish (obtained for all films produced without cross-linker and those produced using cysteine or formaldehyde) to brown-reddish of the films produced in using glutaraldehyde.

3.3.3 FILM FORMATION

Glycerol, the plasticizer, was added to each FFS. This polyalcohol was added in a concentration of 3.6 g/150 g (14% m.c.) flour. Evaporation of the solvent was permitted to continue. After about 75% of the original volume was evaporated, the solution was poured into a heated Pyrex™ glass funnel. This step was done to avoid bubbles from entering a thin-layer chromatography (TLC) spreader with layer thickness regulator model # 611 (Desaga Co., Heidelberg, Germany) when the solution was transferred to it via a Tygon® hose connected to the funnel (see Figure 8). The thickness in the TLC spreader was set at 0.75 mm. Spreading of the solution was done by moving the TLC spreader across the glass surface.

Films were dried for 48 hours in a Forced Air Isotemp Oven model 737-F (Fisher Scientific Co., Pittsburgh, USA.) set at 45°C. Films were then placed in a room with controlled temperature ($25 \pm 1^\circ\text{C}$) and relative humidity ($12 \pm 1\%$) for 24 hours. The relative humidity was measured using a Hygrocheck™ hygrometer (Hanna Instruments, Woon Socket, USA.). Films were then peeled

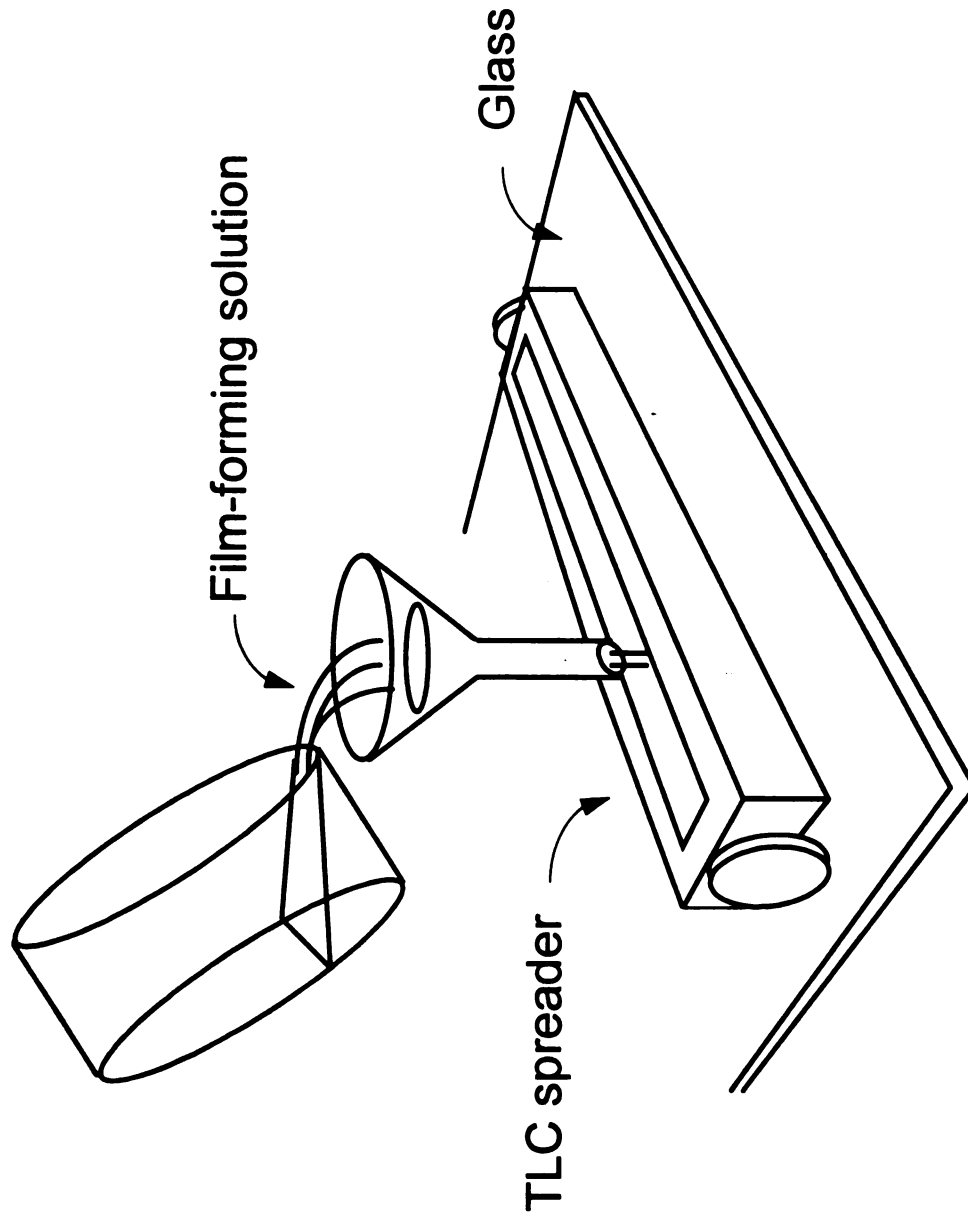


Figure 8. Transfer of film-forming solution to TLC spreader with thickness regulator.

off the glass surface and put, depending on the specifications of the film, into closed containers with controlled relative humidity for 72 hours so that the film equilibrated with this environment. The analytical tests were then performed on the films. Films based on the above procedure with different variables were produced and studied. The conditions and film fabrication processes for each test were selected based on the variable combinations considered most important for obtaining and influencing results.

3.4 ANALYTICAL TESTS OF FILMS

3.4.1 BARRIER PROPERTIES

3.4.1.1 Oxygen Permeability

Oxygen permeability was determined for selected films (Table 4) prepared according to the general procedure elucidated above. Two 11 x 11 cm samples of the film to be tested were cut and each masked with an aluminum foil mask (Mocon, Inc., Minneapolis, MN., USA.) so that a circular film area of 5 cm² was tested. The masked films were placed in a 50 cm² cell of an Oxtran 200 permeability tester apparatus (Modern Controls, Inc., Minneapolis, MN., USA.) equipped with an Endocal temperature control bath model RTE 100 (Neslab Instruments, Inc., Newington, USA.), which conforms with standard method ASTM D-3985.

The tests were run at 0% relative humidity at temperatures of 10, 25, and 40°C using nitrogen (containing 2% hydrogen) as carrier gas and air (21% oxygen) as test gas. The oxygen permeability (P) is reported in m³ m m⁻² s⁻¹ Pa⁻¹

Table 4. Films produced and tested for oxygen permeability and ultimate tensile properties in this study.

Film	Flour*	pH	Cross-linker
1	C	4.0	none
2	C	11.0	none
3	C	11.0	cysteine
4	H	4.0	none
5	H	4.0	cysteine
6	H	11.0	none
7	S	4.0	none
8	S	4.0	cysteine
9	S	11.0	none

* C = commercial bread flour; H = hard red winter flour;
S = soft white flour

for all films by converting the oxygen transmission rate values (J) obtained from the instrument ($\text{cc m}^{-2} \text{ day}^{-1}$) by the use of the appropriate conversion units and the following equation:

$$P = \frac{J\ell}{\Delta P} \quad (25)$$

3.4.2 RHEOLOGICAL METHODS

3.4.2.1 Mechanical Properties

Two techniques to determine mechanical properties of the films were used: ultimate properties (in tension mode) and tensile creep. Ultimate properties include tensile strength, or ultimate strength, which is the tensile stress at or near failure (Rodriguez, 1989) and elongation or engineering strain, also known as Cauchy strain (Steffe, 1992).

3.4.2.1.1 Tensile Strength

The films (Table 4) were analyzed using an Instron Model 4201 equipped with a 1 kN static load cell (Canton, USA.). This test was done in conformance to test method A (static weighing, constant-rate-of-grip separation test) of the standard ASTM method D882-91 (ASTM, 1991a). This method employs a constant rate of separation of the grips holding the ends of the test specimen. The extension of the specimens may be measured in these test methods by grip separation, extension indicators, or displacement of gage marks. The values stated in SI units are to be regarded as the standard. Tensile properties measured by this method may vary with specimen thickness, method of preparation, speed of testing, type of grips used, and manner of

measuring extension. Consequently, these factors were carefully controlled. Results may be utilized to provide data for research and development and engineering design as well as quality control and specification. The tensile modulus of elasticity (E) is an index of the stiffness of thin plastic sheeting. The method specifies that when different materials (i.e., film processes) are tested and compared, specimens of identical dimensions must be employed.

The width of the film samples were cut to 25.4 mm (1 in) using a JDC Precision Sample Cutter model 25 (Thwing Albert Instrument Co., Philadelphia, PA, USA) and the edges were cut with scissors to about 200 mm of length. The separation between the grips of the Instron was set to 50.8 mm (2 in). The cross head speed for the test was set to 508 mm/min (20 in/min). The method indicates that a width-thickness ratio of at least eight shall be used. This is because narrow specimens magnify effects of edge strains or flaws, or both. It is recommended that the utmost care be exercised in cutting specimens to prevent nicks and tears which are likely to cause premature failure. Also, the edges shall be parallel to within 5% of the width over the length of the specimen between the grips. All films were conditioned at $22 \pm 1^\circ\text{C}$ with a $50 \pm 2\%$ relative humidity for 48 hours, and testing was performed at these environmental conditions.

3.4.2.1.2 Tensile Creep

The films were analyzed using an apparatus fabricated in our laboratory in which films were gripped between two sets of aluminum plates so that the upper plates were immobilized and the lower plates had a known weight

attached to them. The measure of strain was determined over real time by mounting a video camera to the apparatus while having a constant stress applied on the film (see Figure 9).

The geometry of the film samples for the creep tests conformed to type M-II tension test specimen specifications (Figure 10), as described in the standard method ASTM D-638M-91a, "Standard Test Method for Tensile Properties of Plastics (Metric)" (ASTM, 1991b). The grip separation was set as indicated by the specimen specification standard at 80 mm. Creep tests were performed according to the standard method ASTM D-2990-91 (ASTM, 1991c). This method indicates that for measurements of creep-rupture, tension is the preferred stress mode, and the values stated in SI units are to be regarded as standard. This method consisted of measuring the extension as a function of time and time-to-rupture, or failure of the specimen subjected to constant tensile load under the specified environmental conditions. The method explains that data from creep and creep-rupture tests are necessary to predict the creep modulus and strength of materials under long-term loads and to predict dimensional changes that may occur as a result of such loads. These data can be used for many purposes: (1) to compare materials; (2) in the design of fabricated parts; (3) to characterize films for long-term performance under constant load; and (4) under certain conditions, for specification purposes.

Film specimens were put into a chamber maintained at the appropriate temperature and relative humidity for at least 40 hours so that the films equilibrated with that environment. Analytical tests were then performed on the

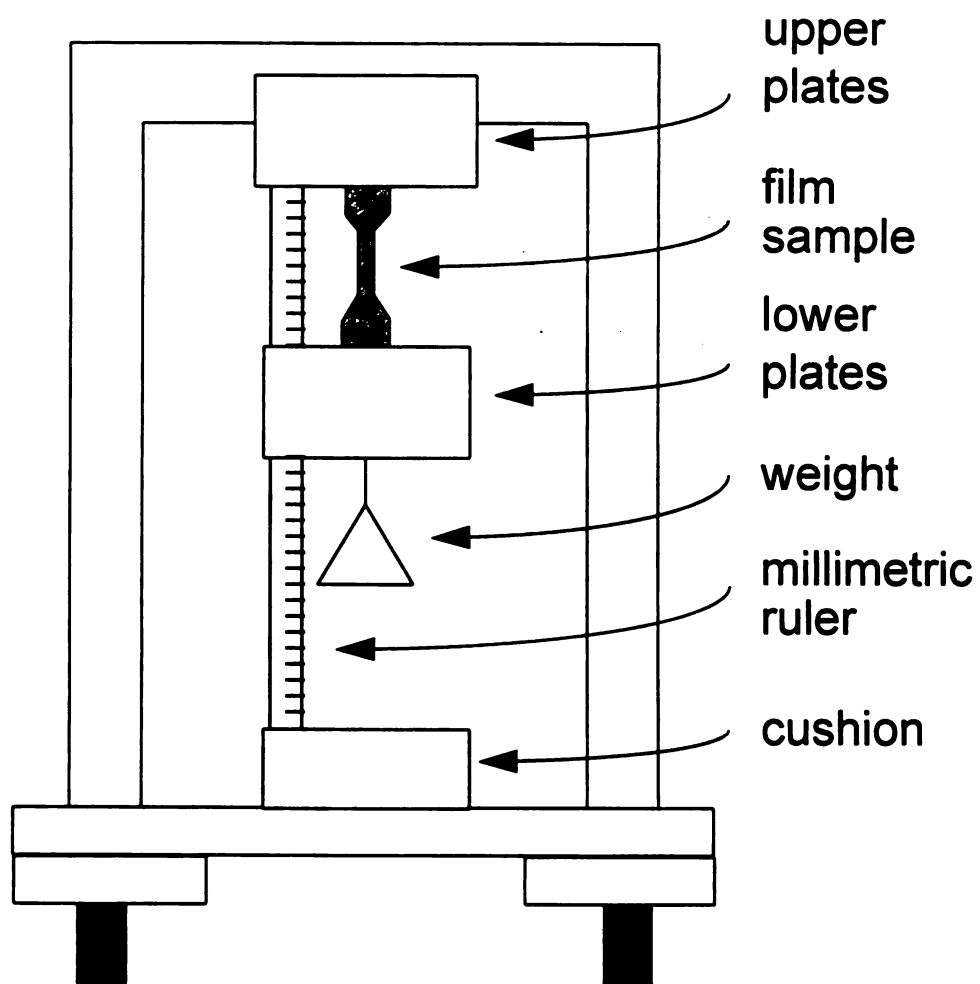
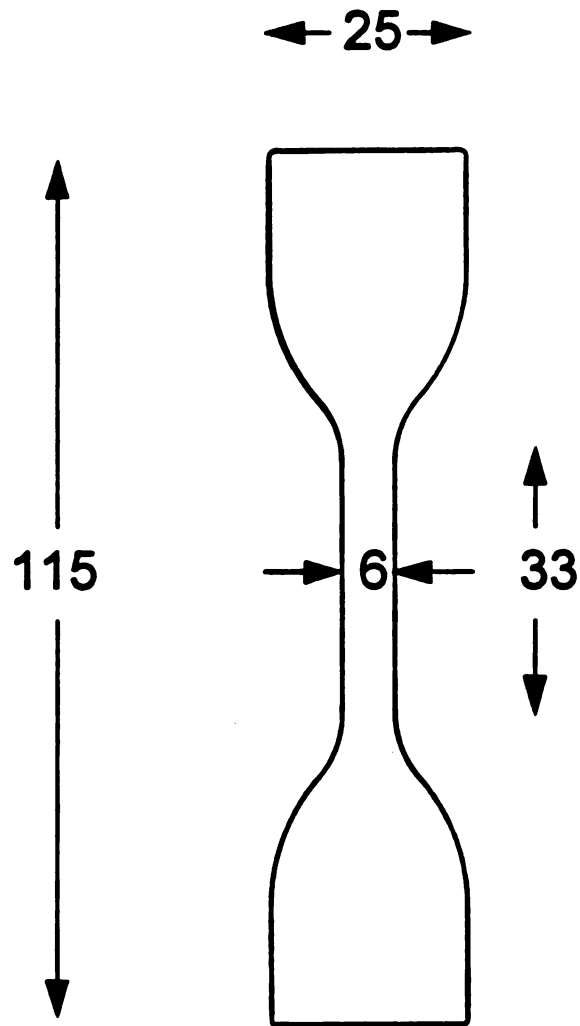


Figure 9. Diagram of tensile creep machine made for this study.



**Figure 10. Type M II tension test specimen dimensions (mm).
[shown to actual size]**

films using a creep apparatus located inside the environmental chamber. Different films, produced with their respective variables used for measuring creep, are presented in Table 5. The reduction in cross-sectional area during the creep experiment for all films was adjusted to obtain more accurate stress values. This adjustment was calculated on the basis of a linear relationship of the cross-sectional area as a function of the differential displacement (δL) observed. Figure B1 (Appendix) shows the linear relationship and the equation obtained for performing this adjustment.

3.4.2.2 Film Characterization

3.4.2.2.1 Thermal Mechanical Analysis

Thermal mechanical analysis (TMA) was used with the intention of determining the coefficient of expansion and T_g of selected films in Table 6. A thermo-mechanical analyzer model 943 (DuPont Instruments, Wilmington, DE., USA) located in the Composite Materials and Structures Center Laboratory at the Engineering Research Complex, Michigan State University, was used. The method used was as follows: (1) equilibrate at $-80\text{ }^{\circ}\text{C}$; (2) ramp $10.00\text{ }^{\circ}\text{C}/\text{min}$ to $100\text{ }^{\circ}\text{C}$.

3.4.2.2.2 Modulated Differential Scanning Calorimetry

Modulated differential scanning calorimetry (MDSC) technique was used to examine films. The apparatus was a Modulated DSC instrument model DSC 2920 (TA Instruments, Inc., New Castle, DE., USA) located in the Composite Materials and Structures Center Laboratory at the Engineering

Table 5. Film variables and conditions used to perform creep tests in this study.

Film #	Process Variable	Test Conditions	
		Temperature (°C)	Relative Humidity (%)
1	None	10	40
2	Glutaraldehyde	10	40
3	Formaldehyde	10	40
4	None	25	25
5	Glutaraldehyde	25	25
6	Formaldehyde	25	25
7	None	25	30
8	Glutaraldehyde	25	30
9	Formaldehyde	25	30
10	None	40	25
11	Glutaraldehyde	40	25
12	Formaldehyde	40	25

NOTE: Proces with FFS of pH 4.0 was used

Table 6. Films produced and examined by thermal mechanical analysis (TMA) and modulated differential scanning calorimetry (MDSC) techniques.*

Film #	pH	Cross-linker
1	4.0	None
2	4.0	Cysteine
3	4.0	Formaldehyde
4	4.0	Glutaraldehyde
5	11.0	None
6	11.0	Cysteine
7	11.0	Formaldehyde
8	11.0	Glutaraldehyde

* Commercial bread flour was used for their fabrication

Research Complex, Michigan State University. This apparatus was equipped with a refrigerated cooling system and a heater control. Data analysis capability made two simultaneous analyses on the same sample in an attempt to determine glass transition temperature (T_g) and melting temperature (T_m). Various film samples (Table 6) were analyzed.

Films were fabricated and the samples kept in a desiccator containing Drierite (W.A. Hammond Drierite Co., Xenia, OH. USA) until tested. The method used was as follows: (1) equilibrate at $-55\text{ }^{\circ}\text{C}$; (2) modulate $\pm 1.000\text{ }^{\circ}\text{C}$ for 5.00 min; (3) isothermal for 5.00 min; (4) ramp $5.00\text{ }^{\circ}\text{C}/\text{min}$ to $184.70\text{ }^{\circ}\text{C}$.

3.4.3 SWELLING STUDY

Determination of the swelling coefficient, Q , was performed for films shown on Table 7. Pieces of film weighing between 0.2 and 0.4 g were cut from the films studied. Three replications were made for each film. Five different solvents were used: (1) n-butyl alcohol (Mallinckrodt, Inc., Paris, KY); (2) hexane (EM Industries, Inc., Gibbstown, NJ); (3) distilled water (obtained from the laboratory); (4) 70% ethanol (prepared from 95% ethanol bulk-obtained from general stores, MSU), with a pH adjusted to 4 using glacial acetic acid; and (5) methanol (J.T. Baker, Inc., Phillipsburg, NJ).

The films were kept at $55\text{ }^{\circ}\text{C}$ for 24 hours in a forced-air isotemp oven model 737-F (Fisher Scientific, Pittsburgh, PA) prior to immersion in the solvents. Weight of the films (before immersion) were recorded. 250 ml of each solvent was placed in a 400 ml beaker and three pieces of film (replications)

Table 7. Films produced* for determination of swelling coefficient (Q), analysis by Fourier-Transformed Infrared Spectroscopy (FTIR), and measurements of color by CIE-Lab system.

Film #	Cross-linker
1	None
2	Cysteine
3	Formaldehyde
4	Glutaraldehyde

* Commercial bread flour was used for their fabrication; low pH process (4.0) was used

were added. The polymer-solvent system was gently-stirred continuously for 24 hours at 23 °C using a magnetic stirrer.

After this time, the films were removed from the solvent and placed in between two circles of Whatman paper #42 (ashless) and a book (about 600 g) was placed on top. This was done for 1 minute to remove the excess solvent from the surface of the samples. The weight of the film was recorded by placing the swollen films on previously tared aluminum plates. Films were then dried for 24 hours at 55 °C. The weight of the films were then recorded again. The swelling coefficient (Q) was calculated according to Eq. 15.

3.4.4 FOURIER-TRANSFORM INFRARED SPECTROSCOPY

The Fourier-Transform Infrared Spectra were obtained by using a Perkin-Elmer FT-IR Spectrophotometer model Paragon 1000 (Perkin-Elmer Limited., Beaconsfield Bucks, England) located in the Engineering Research Complex, MSU. The spectra were obtained for the wavenumber range between 4000 cm^{-1} to 600 cm^{-1} . Each spectrum represents the average of 16 runs and the data were collected at room temperature using a very thin film deposited on the surface of potassium bromide (KBr) tablets. The tablets were fabricated from approximately 300 mg of KBr by placing the powder into a metallic cylinder and holding it at nine tons of pressure for 3 minutes using a Carver Lab. Press model SP-F 6030 (Menomonee Falls, WI, USA). The tablets were prepared within 24 hours of using them in the FT-IR spectrophotometer. The dimensions of the tablets were approximately 12.8 mm diameter and 1 mm thickness. Two drops of FFS were deposited on the surface of a tablet for each film studied and

allowed to dry for 12 hr at room temperature. The films studied are presented in Table 7.

3.4.5 COLOR STUDY

The physical measurement of color for the films listed on Table 7 was done using a Color Hunter Lab instrument model 45/0 ColorQUEST (Hunter Associates Laboratory, Inc., Reston, VA. USA) located in the Permeation Laboratory in the School of Packaging, MSU. CIE-Lab [CIE are the French initials for "Commision Internationale de 'Eclairage"] values for "L", "a", and "b" were obtained. The films were conditioned overnight to 50% relative humidity prior to testing. Five replications were performed and both, values in the reflectance (with black background) and transmittance (with white background) modes, are reported.

3.5 STATISTICAL ANALYSIS

The numerical results were analyzed by either one-way or two-way analysis of variance, and the comparisons among different factors were investigated according to either of one of the Tukey's or Student-Neuman-Keul's multiple comparison tests. MSTAT-C statistical program version 2.10 (Michigan State University, East Lansing, MI., USA) and Minitab statistical software release 10.0 (Minitab Inc., State College, PA., USA) were used to perform all statistical calculations.

4. RESULTS AND DISCUSSION

4.1 FLOUR CHARACTERIZATION

4.1.1 MOISTURE CONTENT

Moisture content of the flours (wet basis) is reported in Table 8. Results indicate that these flours were in the lower recommended moisture level of 12 to 13% so that minimum oxidation or mold growth would occur. From this, it was expected and assumed that no significant detrimental changes that could vary the results would be found in the flours. As a preventive action, all three flours were stored at 4 °C in sealed containers while not in use.

4.1.2 PROTEIN CONTENT

The results for the protein tests of the three flours used in this study are presented in Table 9. From these results, it is possible to observe that a significant difference in the protein content existed among all three flour samples.

The end purpose of the C was to serve as a bread flour, and it was labeled as a blend of hard spring and winter wheat, therefore, it was expected that the C would have the highest protein content. This was assumed because flour companies usually add vital wheat gluten to the blend of wheat flours sold to adjust or replace the original protein content of the flours, depending on final use (McDermott, 1985 and Magnuson, 1985). As for the H, it is a blend of flours from a single class (hard red winter wheat), with the characteristic, when compared to soft wheat classes, of having a higher water absorption and tighter

Table 8. Moisture content of the flours used in this study.*

Flour	Moisture (%)
C	12.11 ± 0.08
H	11.80 ± 0.07
S	11.96 ± 0.07

*** Average ± standard value of 5 replications**

Table 9. Protein content of the flours used in this study.¹

Flour	Protein ² (%)	Protein ³ (%)
C	13.86 ± 0.09	13.56 ± 0.09
H	12.93 ± 0.06	12.61 ± 0.06
S	9.83 ± 0.20	9.60 ± 0.20

¹ Average ± standard deviation values of 5 replications

² Samples dried for 48 hours at 120 ± 1 °C

³ Adjusted values to a 14% moisture content

adherence (stronger interaction) of the protein and starch. The protein content of this flour falls in between the C and S. S is also a blend of flours within a class, but with the characteristic of being from all soft white wheat. This flour sample had the lowest protein content.

According to D'Appolonia (1994), H with a protein range of 10.0-11.0 percent would be used as an "all purpose" flour. On the other hand, S with a protein range of 8.0-10.0 percent could have typical applications in flat bread, noodles, and pastries. Finally, since C is a "mixture of hard spring and winter wheat class flours," it may be expected to be applicable in making white pan bread, which typically requires a protein range of 11.0-13.0 percent.

4.1.3 FALLING NUMBER

Results of the Falling Number determination are presented in Table 10. The values for the C and S fall within the range indicated by Perten (1988) for unsprouted wheat. The value corresponding to the H indicated low amylase activity with the value in the lower limit for a sound wheat. From these values, it was anticipated that possible problems associated with the α -amylase activity, such as presence of free sugars in the FFS (non-enzymatic browning, etc.), would have been minimum, or absent from this study. In addition, since flours milled from sprouted wheat may present not only α -amylase but protease activity (since both are enzymatic changes involved in the development of the new plant), it was assured that the flours used in this study contained the proteins with minimum, or no degradation. Overall, the determination of α -amylase as a variable is expected to play a minor role in the process since one of the

Table 10. Falling Number of the flours used in this study.

Flour	Falling Number * (s)
C	241 ± 11.20
H	351 ± 8.40
S	271 ± 2.70

* Average ± standard deviation of 5 replications

objectives of the preparation of the film is to separate the starch by centrifugation.

4.1.4 FARINOGRAPHY

Figure 11 show the farinograms obtained from the studies performed for the C, H, and S flours, respectively. Dough resistance to mixing during the successive stages of its development is measured with the farinograph. It was observed from these figures that a different shape among the three different flours was obtained (Figure 11). This indicated that there was a difference among individual flour constituents of the flours analyzed. A strengthening of the farinograph curve decreased from Figures 11-C, 11-H, and 11-S, respectively. This behavior agrees with a report by D'Appolonia (1984) in which the author indicated that, with an increase in protein content, a definite strengthening of the farinograph curve occurs. It was also observed that the gluten-flour bread of the C in Figure 11-C as compared to the H flour (no gluten added) showed a strengthened (or improved) flour curve. A similar report was made by Lorenz (1984). Also, by comparing the shapes from a study by Preston and Kilborn (1984), it was established that C and H could be considered strong flours. This was further validated since the values for MTI of 50 and 40 (Table 11), respectively, fell between the 30 (strong) to 80 (medium strength) according to the classification developed by these authors. On the other hand, S was considered a weak flour in agreement with Preston and Kilborn (1984) since the MTI value of 170 (Table 11) for the S was similar to the 180 value determined by these authors for a weak flour.

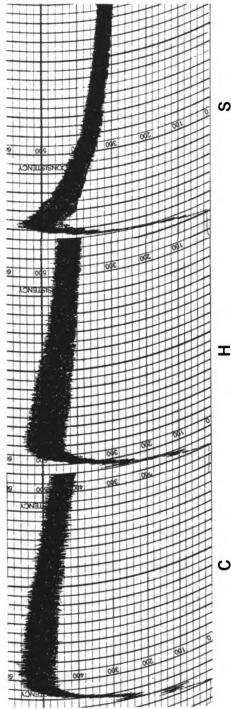


Figure 11. Farinograms of the flours used to make the films in this study.

C=Commercial bread flour, H=Hard red winter flour, S=Soft white flour.

The calculated values obtained from the curves in Figure 11 for water absorption, dough development time (mixing time), and stability are also reported in Table 11. The water absorption value of H fell within the typical absorption levels for bread flours of 58 to 66 percent (D'Appolonia, 1994). Since absorption is correlated positively by the protein content and the damaged starch (D'Appolonia, 1994), and C contained added gluten, it was expected that the higher protein level in C yielded a slightly higher value of the maximum typical value expected. The absorption values for the three types of flours studied indicated a resemblance with the observations made by Shuey (1984a) in which absorption generally increases, when all other variables are kept constant, with an increase in the protein content of the flour. This indicated that C, H, and S required, respectively, greater amounts of water to give the same consistency. The mixing time values showed that the weak flour (S), required about half the time to reach the maximum consistency as compared to the strong flours (C and H). The stability values showed a decrease from C, H, and S, respectively, which corresponded to an indication for a greater tolerance to mixing from values of 7.25 minutes to 1.50 minutes.

The above results indicated that differences in physical behavior existed among the C, H, and S flours. It is important to mention, however, that these differences were produced predominantly by the protein and starch interaction of the flours used (D'Appolonia, 1984).

Table 11. Farinograph results for the flours used in this study.*

Flour	Water absorption (%)	Mixing time (min)	Stability (min)	MTI (BU)
C	67.05	2.50	7.25	40
H	60.90	2.25	5.75	50
S	55.70	1.25	1.50	170

* Values obtained from Figure 11

4.1.5 SDS-PAGE

The one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoretic (SDS-PAGE) patterns of the C, H, and S flours, both under reduced and unreduced conditions are presented in Figure 12. Both C and H flours are mixtures of two or more varieties, evidenced by more than five high molecular weight glutenin subunits (HMW-GS) present in each flour on the SDS-PAGE gel. From the analysis of the different bands obtained for the high molecular weight (HMW) glutenin subunits region for each flour, different subunits were identified and are presented in Table 12. It was observed that both, C and H flours, contained the HMW-GS pair "5+10," whose presence has been suggested as an indicator of strong gluten (Gupta and MacRitchie, 1994). On the other hand, soft white flour contained the HMW glutenin subunit pair "2+12," whose presence has been suggested to be an indicator of weak gluten, by producing a smaller percentage of larger sized polymers as compared to those of flours containing the subunit pair "5+10" (Gupta and MacRitchie, 1994).

These observations on the differences in the HMW-GS composition found in the flours may in part explain the film behavior discussed later on. In addition, it was also noted that there were some differences in the regions corresponding to the low molecular weight glutenin subunits (LMW-GS). These variations in the types of LMW-GS, which have been studied less extensively than HMW-GS, may also be responsible for the differences in the film properties observed. Similarly, differences in the unreduced electrophoregram patterns are evident: Both the intensity and the number of bands present increased from S,

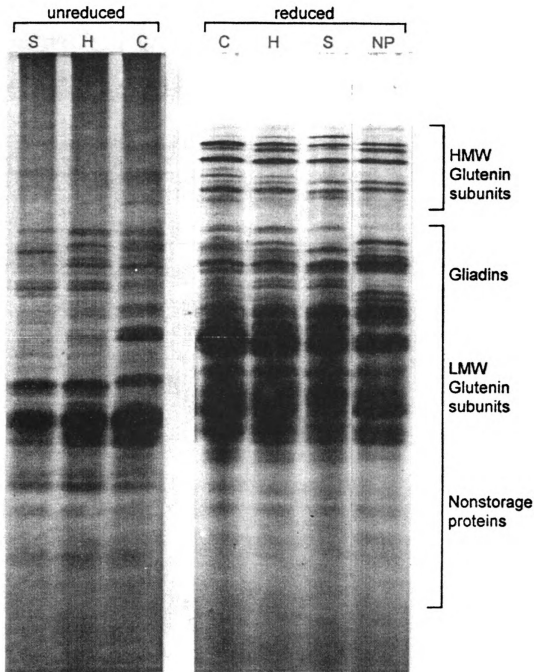


Figure 12. One-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of C, H, and S flours
 C = Commercial flour
 H = Hard red winter flour
 S = Soft white flour
 NP = Neepawa flour (Canadian hard wheat -pure variety-)

Table 12. High-molecular-weight glutenin (HMW-GS) subunits present in the flours used in this study.

Flour	HMW-GS identified from gel electrophoresis:							Subunits encoded by gene chromosome:	
								1A	1B 1D
NP ¹		2*	5	7	9	10		2*	7+9 5+10
C ²		2*	5	7	8(?) ⁵	9	10	2*	7+8(?)+9 5+10
H ³		2*	5	7	8(?)	9	10	2*	7+8(?)+9 5+10
S ⁴	1 2			7	9		12	1	7+9 2+12

¹ NP = Neepawa (a canadian hard wheat cultivar used as a reference for identification of HMW-GS)

² C = Commercial bread flour

³ H = Hard red winter flour

⁴ S = Soft white flour

⁵ Most probable subunit

H, and C flours. The films were formed using the unreduced flours except when a reducing agent (cross-linker) was used (i.e., tensile strength, tensile creep, FTIR and color studies). This is important because, as observed in Figure 12, a great number of polymeric protein molecules could not enter the separating gel, an indication of the great size that these proteins have before a reducing treatment is applied. Nonetheless, these results are preliminary and further confirmation of these observations is needed to present more conclusive statements.

4.2 FILM FORMATION PROCESS

Several advantages were found by using the methodology defined in this study as compared to previously published reports for making films using wheat proteins. These include: (a) the flour disperses more easily and does not form clumps while mixing it with solvents at room temperature; (b) after centrifugation, only soluble materials (proteins, etc.) are present, because the starch and other insoluble materials that may be found in the commercial wheat gluten have been removed; (c) the films are homogeneous with a smooth (not granular) texture, and; (d) a wider variety of raw materials can be used to prepare the films including flour and protein powder.

4.3 BARRIER PROPERTIES

4.3.1 OXYGEN PERMEABILITY

The oxygen permeability, determined for films 1, 2, 4, 5, 7, and 8 (Table 4) with the data at 10, 25, and 40 °C, are presented in Table 13. A plot of

Table 13. Oxygen permeability values^{1,2} for selected films at different temperatures.

Temperature (°C)	Film Number							
	1	2	4	5	7	8		
10	1.4 ± 0.3	2.9 ± 0.4	1.7 ± 0.2	6.0 ± 0.5	6.4 ± 0.6	6.5 ± 0.8		
25	5.9 ± 0.2	8.7 ± 0.4	6.6 ± 0.6	11.9 ± 0.7	14.4 ± 0.4	18.5 ± 0.8		
40	16.2 ± 0.2	18.0 ± 0.4	18.6 ± 0.4	28.2 ± 0.1	29.7 ± 0.8	32.8 ± 2.9		

¹ Value ± Standard Deviation of two replications

² Units in (m³ m m² s⁻¹ Pa⁻¹) x 10²⁰

permeability vs. temperature is shown in Figure 13. It was observed from these values that the temperature was found to be the most significant factor ($p < 0.05$) affecting oxygen permeability. The values showed that an increase in temperature corresponded an increase in the permeability values for films. This behavior is in agreement with that observed previously by Gennadios *et al.* (1993). The second important factor that influenced the oxygen permeability was the type of flour used to prepare the films: flour type was found highly significant ($p < 0.01$) with the effect of oxygen permeability. Finally, it was found that the pH of the solution did not have a significant effect ($p > 0.05$) on the oxygen permeability for the films prepared and analyzed in this study.

The oxygen permeability values were fitted to an Arrhenius model, obeying the following equation:

$$P = P^0 e^{-E_a/RT} \quad (26)$$

An Arrhenius plot was then generated by plotting the logarithmic form of the above equation:

$$\ln P = \ln P^0 - E_a/RT \quad (27)$$

and is presented in Figure 14. From this plot, the Arrhenius factor (P^0) and the Energy of Activation (E_a) were calculated and reported in Table 14. Note that E_a is expressed as $E_a R^{-1}$, thus the values of Table 14 can be multiplied by the gas constant, R ($0.001987 \text{ Kcal mol}^{-1} \text{ K}^{-1}$) to obtain E_a in Kcal mol^{-1} . There was an agreement between the oxygen permeability values at the different temperatures and the values of E_a found for these films. At higher temperatures, the term

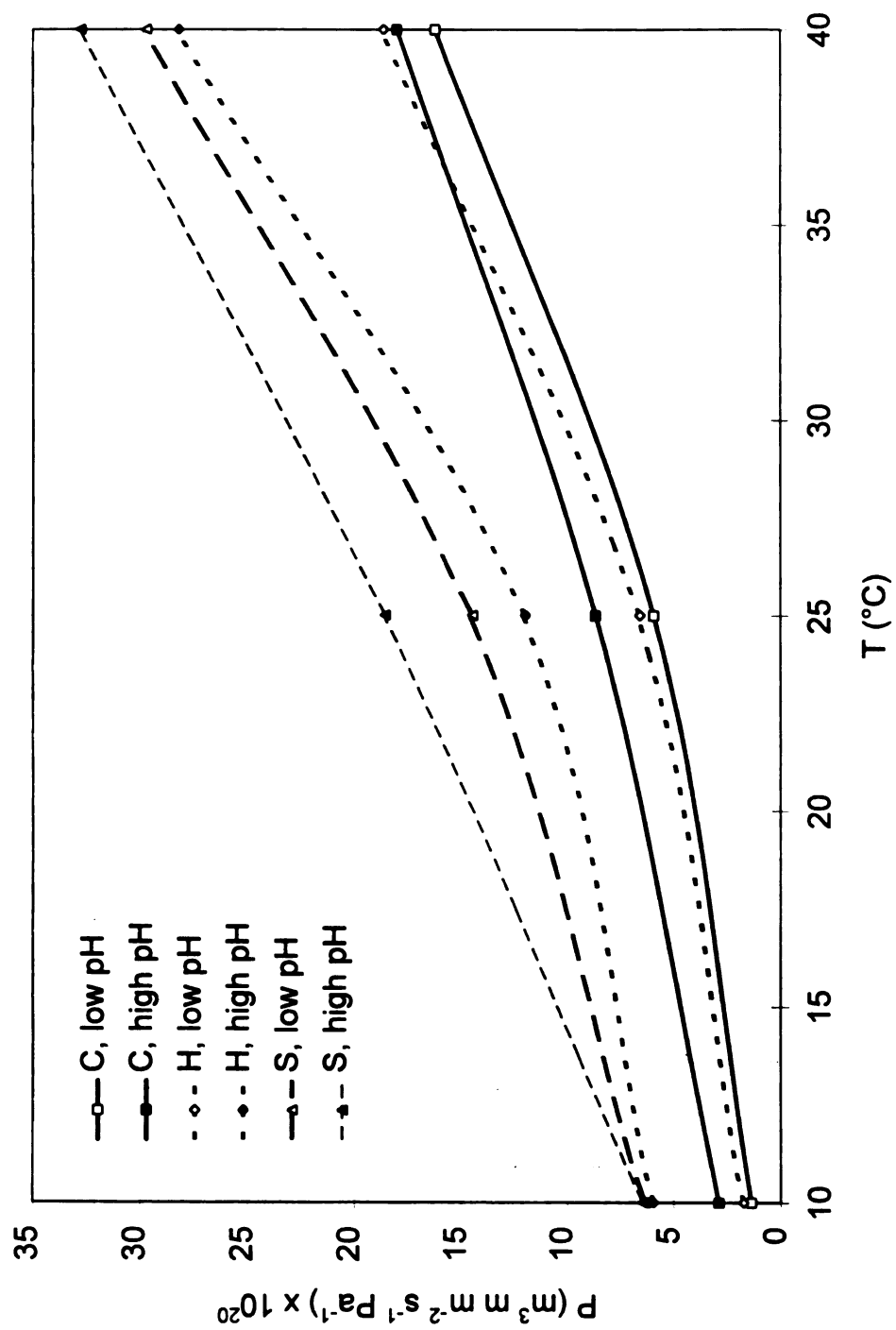


Figure 13. Oxygen permeability of selected films as a function of temperature.

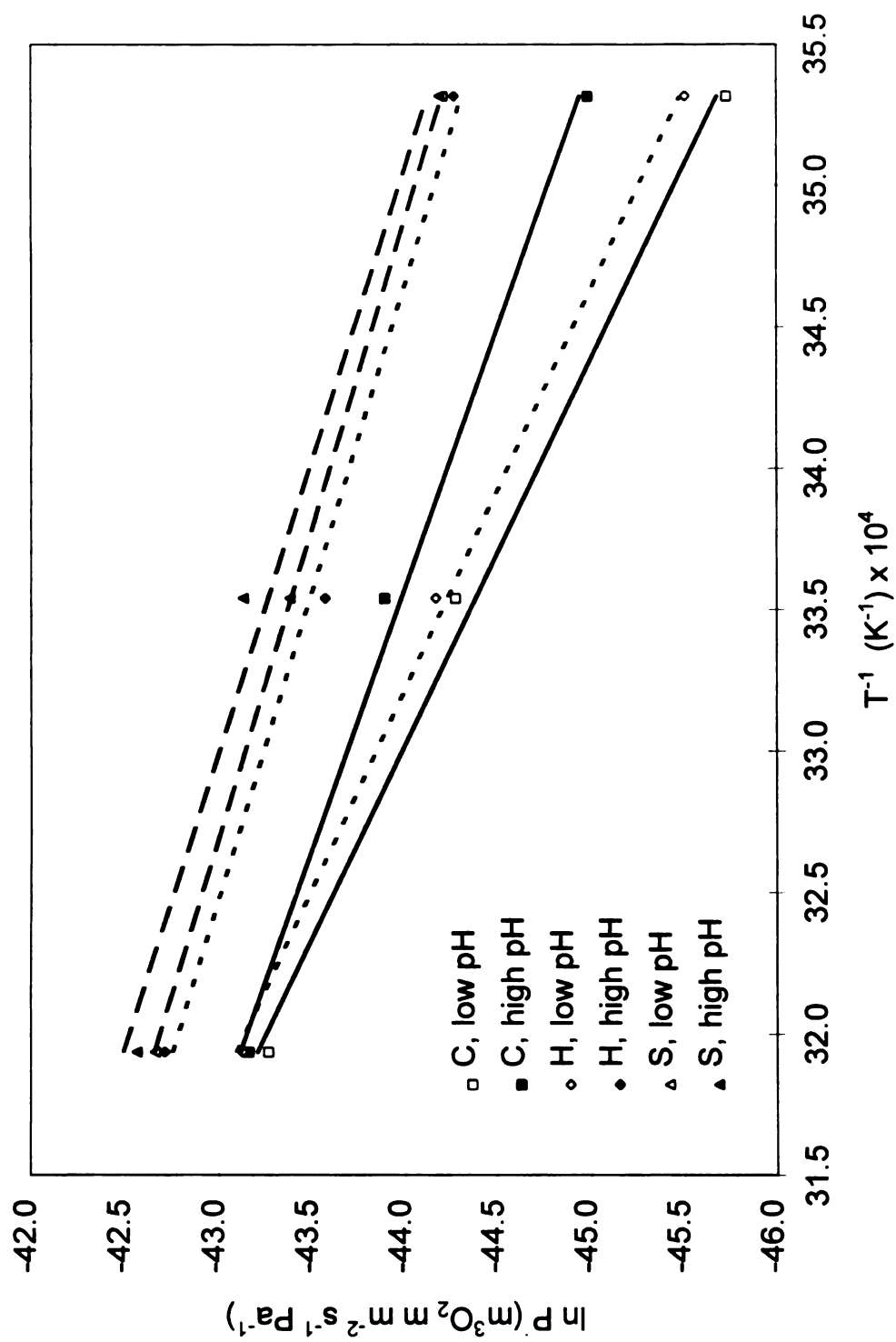


Figure 14. Arrhenius plot of selected films.

Table 14. Arrhenius constant (P^0) and Activation Energy (expressed as $E_a R^{-1}$) for selected films.

Film	P^0 ($\text{m}^3 \text{m}^{-2} \text{s}^{-1} \text{Pa}^{-1}$)	$E_a R^{-1}$ (K)
1	2.3×10^{-9}	7297.4
2	5.9×10^{-12}	5032.7
4	1.2×10^{-9}	7045.8
5	6.11×10^{-13}	4579.8
7	6.35×10^{-13}	4579.8
8	1.70×10^{-12}	4831.4

($E_a R^{-1} T^{-1}$) becomes smaller so that the exponential $e^{-[E_a/(RT)]}$ becomes larger. Therefore, as the temperature increases, the oxygen permeability (Eq. 26) increases meaning that oxygen molecules permeate faster through the films. Also the term P^0 , which in this case is the probability of an oxygen molecule colliding with a polymer (protein) molecule, was found to be larger in the films made from commercial flour and hard red winter flour using the low pH method (Table 14). This may suggest that a more compact structure is found in these films as compared to the other films studied.

The activation energy values of the oxygen permeability process between 9.1-14.5 kcal mol⁻¹ fell within the range of those found for synthetic polymers. It appeared that, at pH 4, C and H films showed higher values than S films. The activation energy of the permeation process is the addition of the activation energy of the diffusion process and the enthalpy of sorption of the permeant in the film. Unfortunately, there are no data regarding the enthalpy of sorption of oxygen in these wheat films. But in general it is less than the activation energy of the films. Nevertheless, assuming that the activation energy of the diffusion process is the dominating term, it can be suggested that a higher value of the activation energy of the permeation process implies a higher energy value for the oxygen molecule to diffuse through the polymeric matrix. This also implies that C and H films are produced with the characteristic of having a more tortuous path than S films.

No significant correlation between the protein content in the flour and oxygen permeability of the resulting films was found ($p > 0.05$) when measured at

dry conditions (moisture content < 2.5 % w/v for all films). Although this is not completely understood, it may indicate that the total protein content present in the flour is less important than the type of protein in the determination of the morphology of the polymers which ultimately determine the gas barrier characteristics. The oxygen permeability values of the wheat film measured at 25°C ranged from 5.9 to $18.5 \times 10^{-20} \text{ m}^3 \text{ O}_2 \text{ (STP) m m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$. These values were similar to those for the oxygen permeability of nylon-6, reported to have a value of $13.7 \times 10^{-20} \text{ m}^3 \text{ O}_2 \text{ (STP) m m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$ at 23°C and 0% RH (Gavara and Hernandez, 1994). The similarity values may be explained by the similar chemical structure of these materials based on the polyamide functionality.

The non-proportional protein content/oxygen permeability ratio for the different temperatures studied may indicate that it is not only the total protein content present in the film which is responsible for the total oxygen permeability, but also the type of protein (referred as “quality” in baking) present in the wheat flour.

4.4 RHEOLOGICAL MEASUREMENTS OF FILMS

4.4.1 MECHANICAL PROPERTIES

4.4.1.1 Tensile Strength

The ultimate properties were studied on films 1, 3, 4, 6, 7, and 9 (Table 4). These properties include elongation, tensile strength, and modulus of elasticity. Results are presented in Table 15. Measurements were made with films conditioned at 50% RH. Figures 15 and 16 represent the elongation and

Table 15. Ultimate tensile properties for selected films.

Film # ¹	elongation ^{2,3} (%)	tensile strength ² (Pa) x 10 ⁻⁴	modulus of elasticity (Pa) x 10 ⁻³
1	640.7 ± 0.6 a	23.5 ± 2.5 d	36.8 ± 4.4
3	623.3 ± 0.4 a	43.3 ± 1.7 a	69.7 ± 5.7
4	520.8 ± 0.2 b	24.1 ± 2.1 d	46.4 ± 3.7
6	485.8 ± 0.2 b	38.0 ± 0.4 b	78.3 ± 2.1
7	537.3 ± 0.1 b	25.3 ± 0.4 d	47.1 ± 1.6
9	514.6 ± 0.2 b	33.5 ± 1.2 c	65.1 ± 2.0

¹ See Table 3 for type of flour and conditions used

² Mean ± standard deviation values of three replications followed by the same letter are not significantly different (p>0.05) according to Student-Neuman-Keul's Test

³ Engineering strain at failure

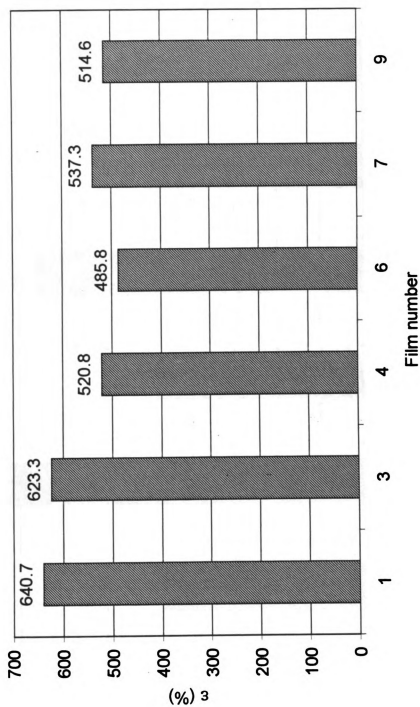


Figure 15. Engineering strain (elongation) at failure of selected films.

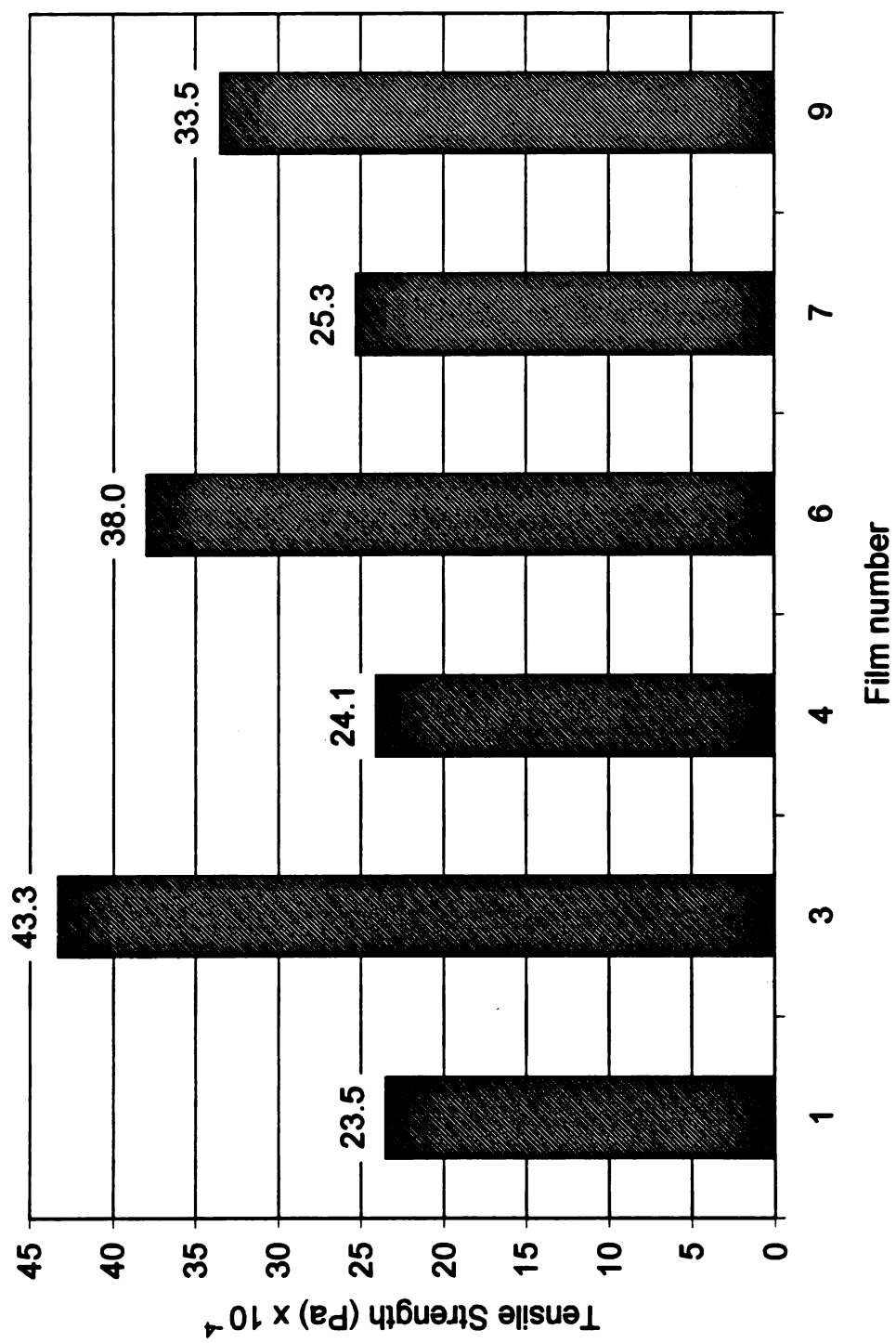


Figure 16. Tensile strength of selected films.

tensile strength values, respectively for the films studied. From these results it was observed that differences in elongation for the films were significant ($p < 0.05$) for the films prepared using the commercial bread flour and the others. The two classes of flour, hard red winter and soft white, did not present significant ($p > 0.05$) differences in the values obtained for them. On the other hand, differences observed using the reducing agent (cysteine) were not significant for films made of the same flour. Gennadios et al (1993) reported elongation at break values for a wheat gluten film ranging 216-260%, measured at 50% RH. These values were lower in magnitude than films made in this study having a range of 490 to 640%. Elongation at break values of films produced with reducing agent were not significantly different from films produced without it. This may be attributed to the fact that the measurements were made at 50% RH and because of the hydrophilic nature of the films, water served as a plasticizer. This may have softened the structure resulting in elongation values that were higher than if the tests were performed at a lower relative humidity. According to Modern Plastics Encyclopedia (1995), typical values of elongation at break for selected synthetic polymers are: low density polyethylene (LDPE), 300-1000%; high density polyethylene (HDPE), 100-1000%; ethylene-vinyl acetate (EVA), 600-900%; and polypropylene (PP), 20-800% (Progelhof and Throne 1993). These numbers indicate that elongation values for wheat films (485-640%) are similar to those found for synthetic polymers.

Tensile strength was observed to be significantly different ($p < 0.05$) among the films in which the reducing agent (cysteine) was added. The values

of the tensile strength among the films that did not have cysteine, although made with different flour, were not significantly different ($p>0.05$). This may support the suggestion by Guthrie and Gruen (1990), that the use of cysteine promotes inter-molecular cross-links. The cross-linking process involves the exchange of disulfide groups present in the molecules, acting as a catalyst for the “disulfide interchange” reaction. The result of this would be an increase in the film tensile strength. Values of the tensile stress at break of the wheat protein film ranged from $0.2\text{--}0.4 \times 10^4$ Pa and were more than one order of magnitude lower in than the commercial synthetic plastics such as LDPE, HDPE, EVA, and PP that showed a tensile stress at break from $8\text{--}37 \times 10^4$ Pa (Modern Plastics Encyclopedia 1995).

Values for modulus of elasticity (E) for the protein films were in the range of $3.7\text{--}7.8 \times 10^5$ Pa (Table 15). Literature reports values of 2.0×10^6 for soft rubber, 2.2×10^8 for LDPE, 1.08×10^9 for HDPE, 1.19×10^9 for Nylon-6, and 2.52×10^9 for Nylon-66 (Sperling 1992 and Modern Plastics Encyclopedia 1995). This indicates that the wheat protein films were from less than one to almost four orders of magnitude, less resistant to stretching compared to these commercial polymeric materials.

4.4.1.2 Tensile Creep

All the creep curves (Figures 17 to 23) were obtained for time values up to two hours, which resulted in reaching the point of failure (rupture) of the protein films for most of the samples. It was observed that, as the temperature and/or relative humidity increased, the time to reach failure was reduced

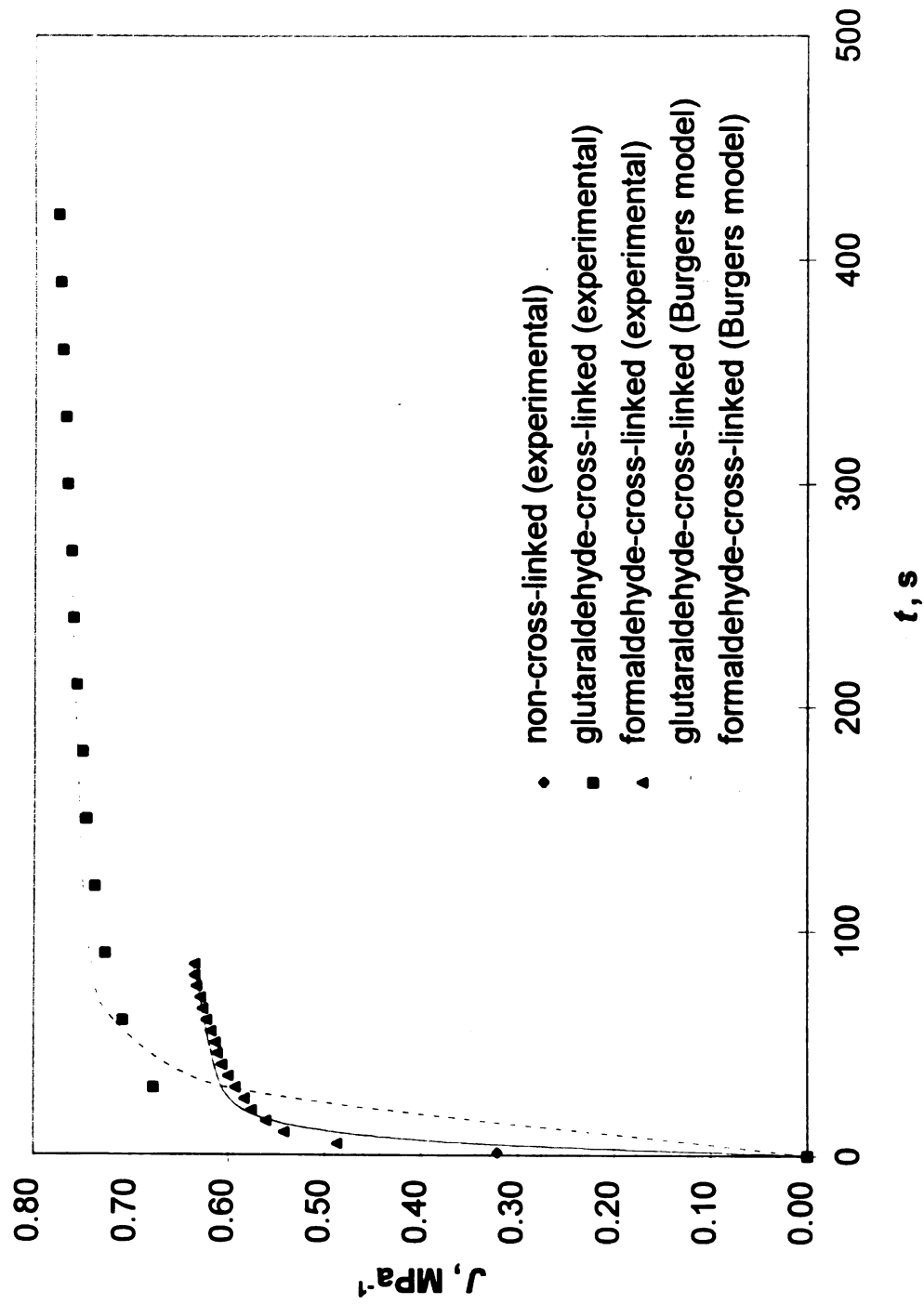


Figure 17. Creep compliance of films at 5°C and 85% RH.

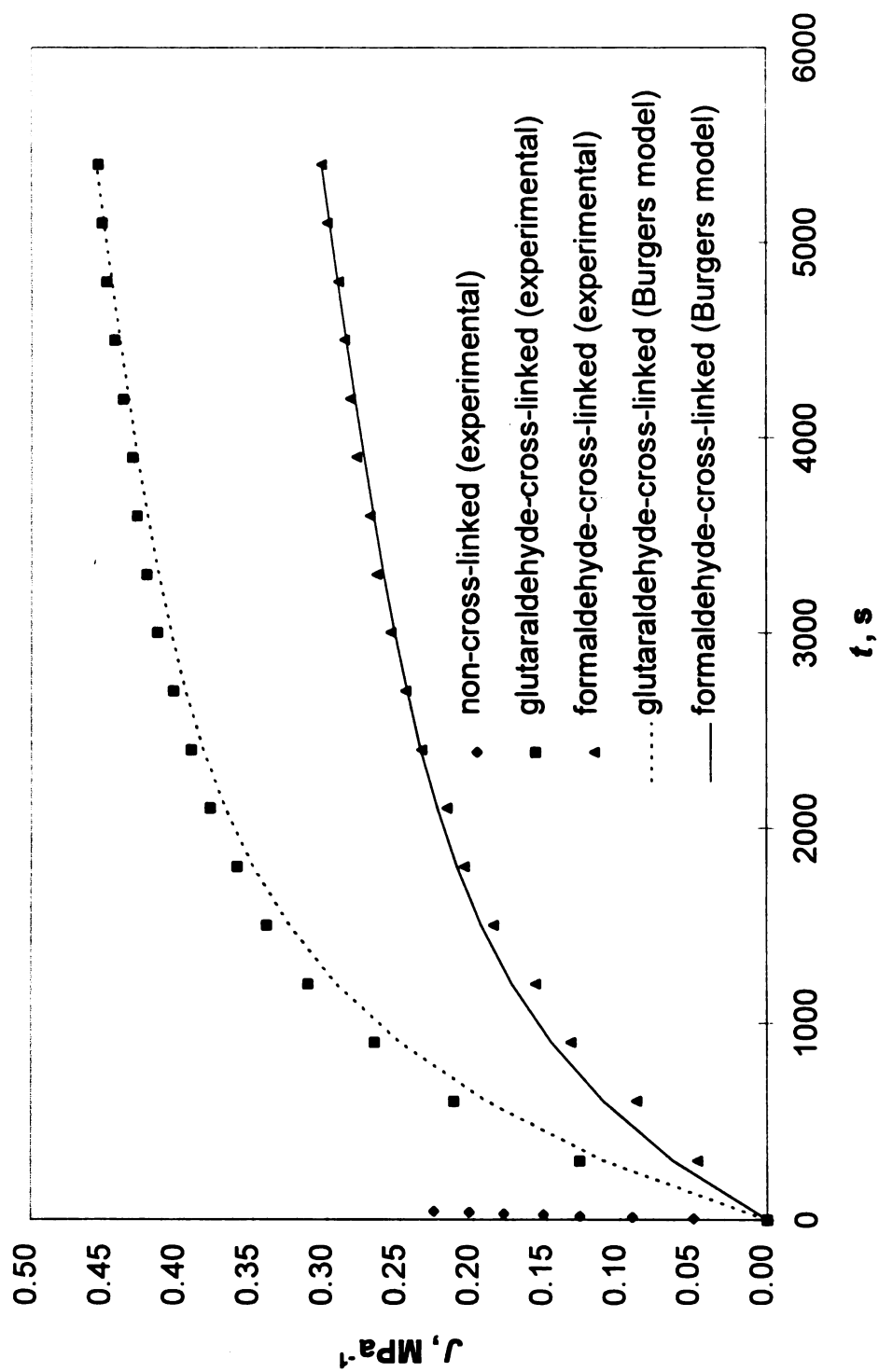


Figure 18. Creep compliance of films at 10°C and 40% RH.

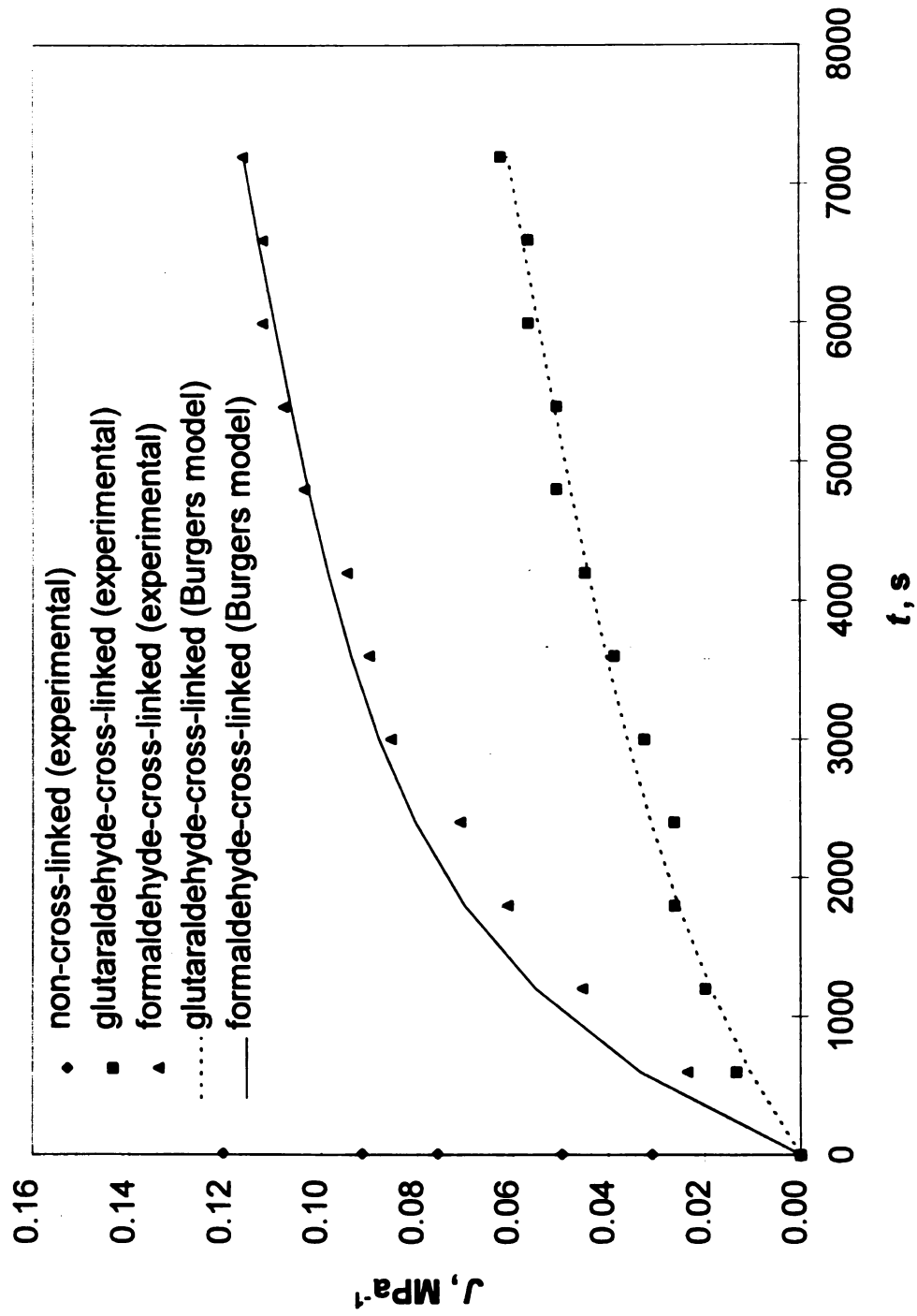


Figure 19. Creep compliance of films at 20°C and 35% RH.

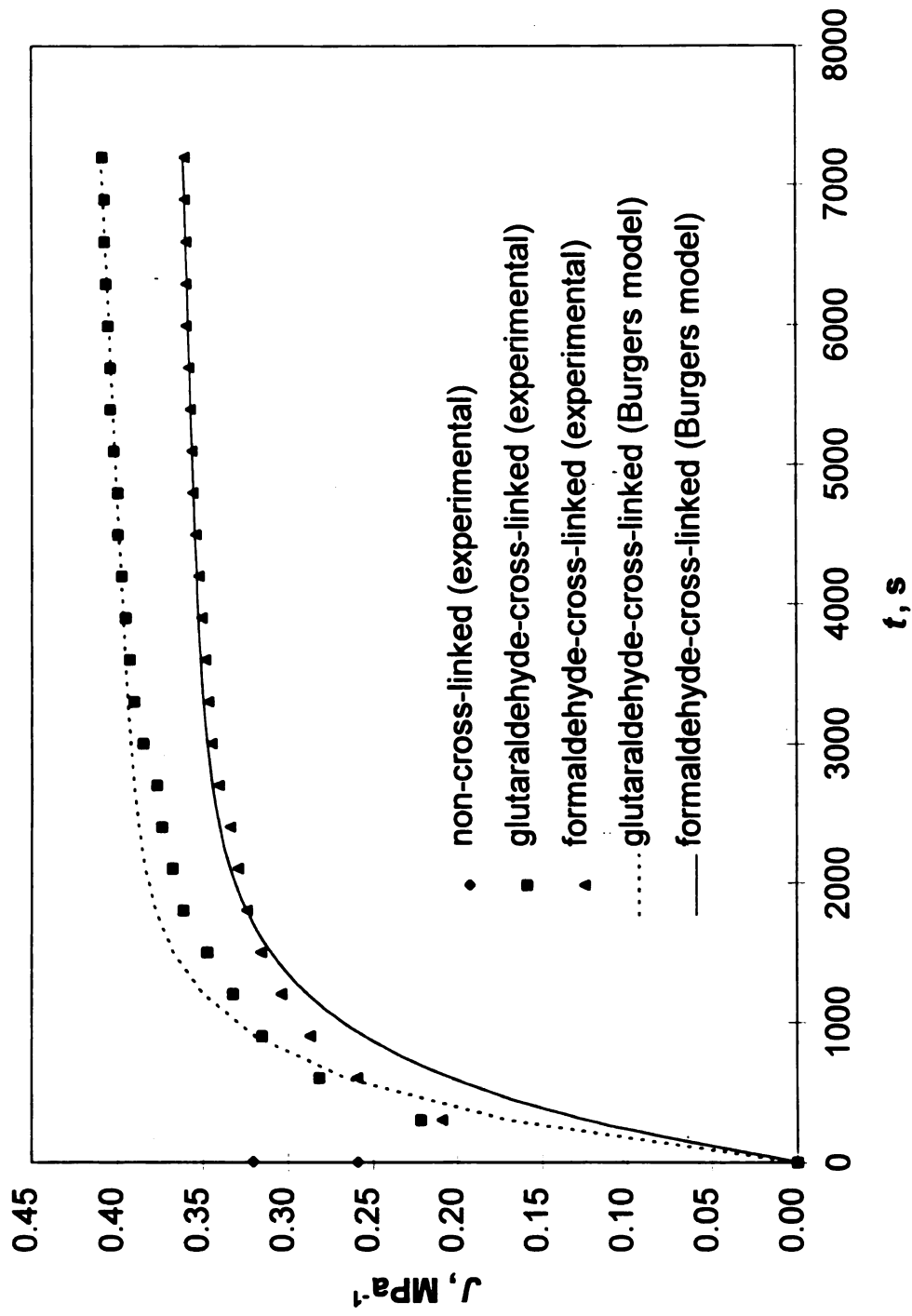


Figure 20. Creep compliance of films at 20°C and 55% RH.

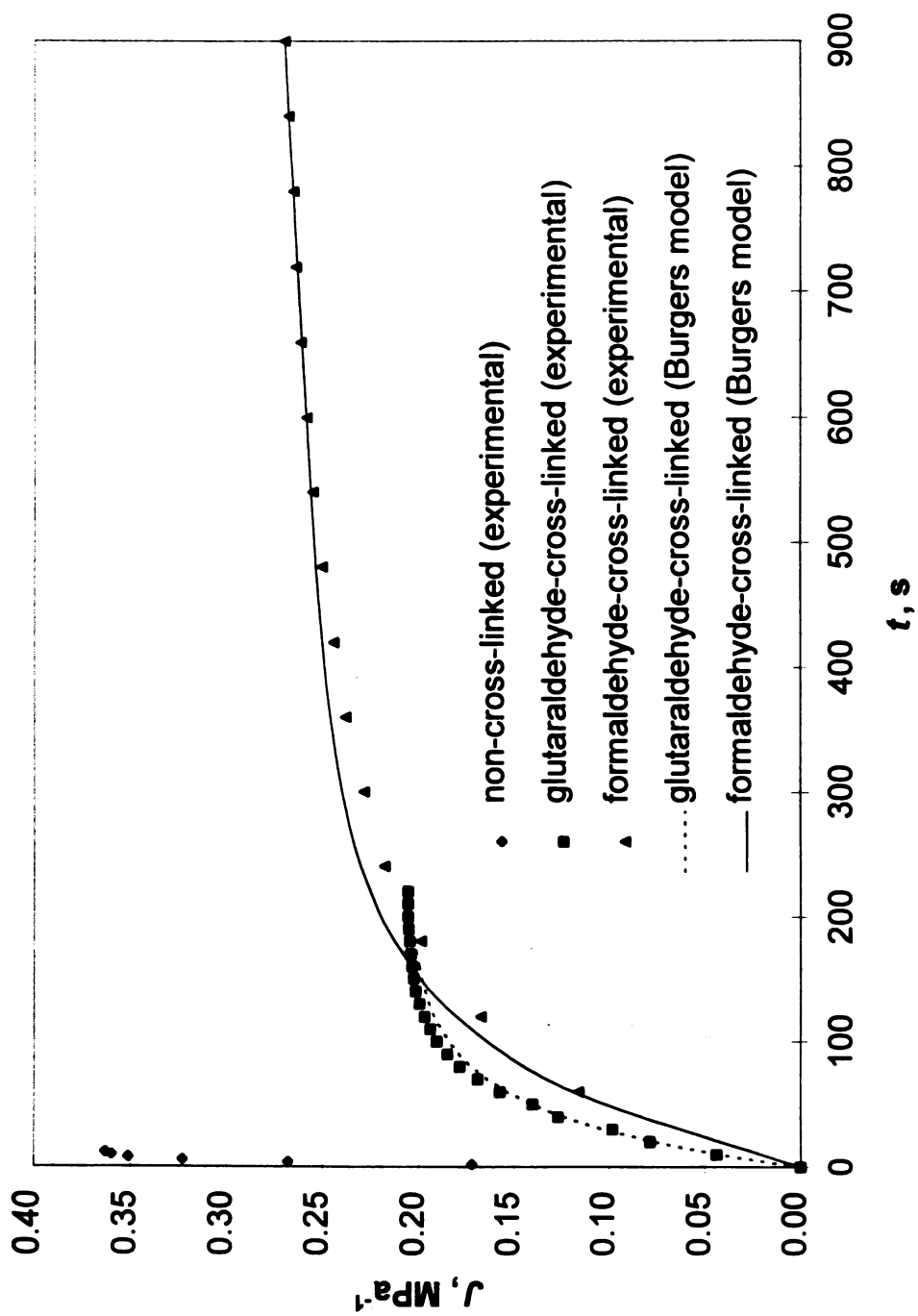


Figure 21. Creep compliance of films at 25°C and 25% RH.

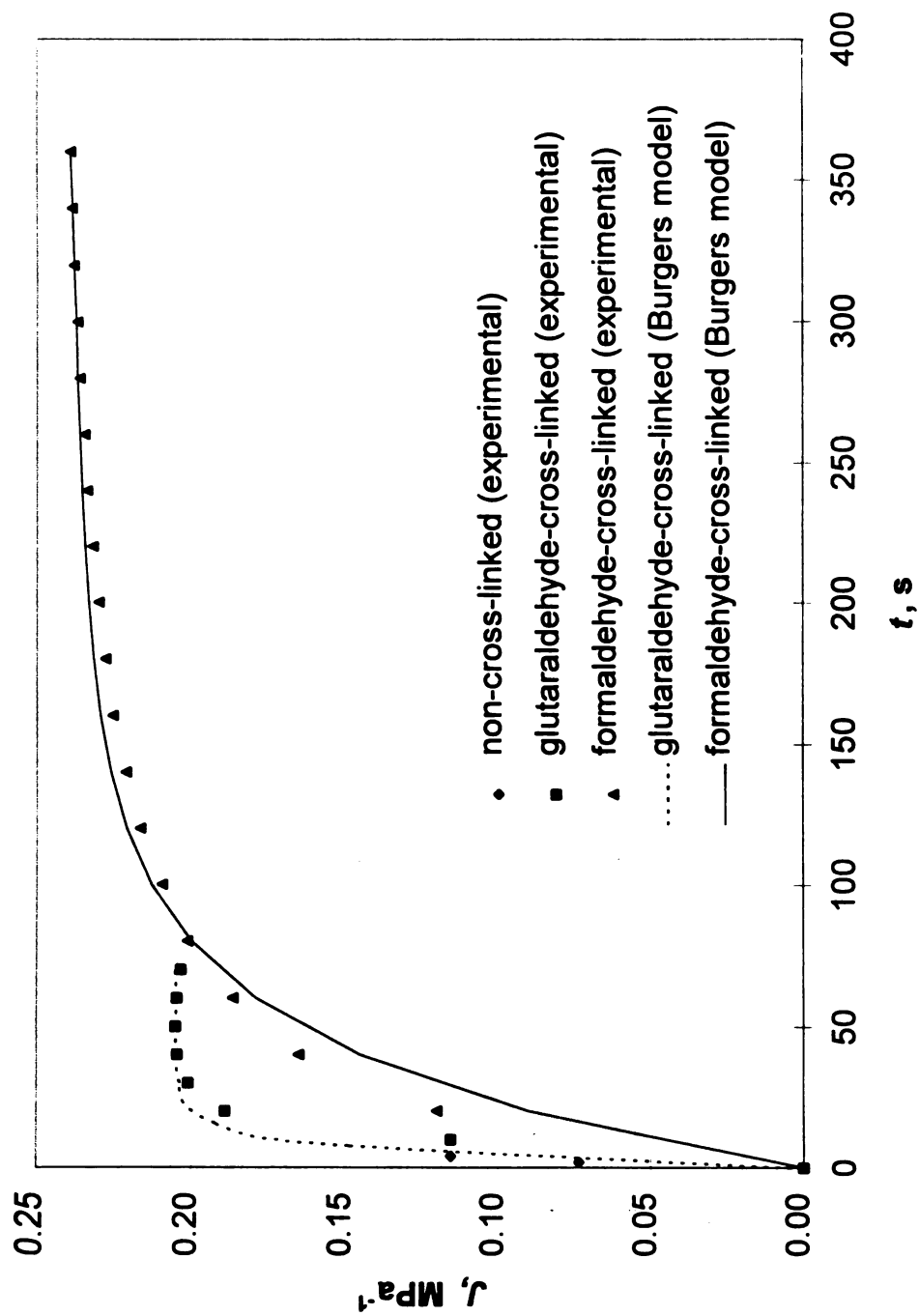


Figure 22. Creep compliance of films at 25°C and 30% RH.

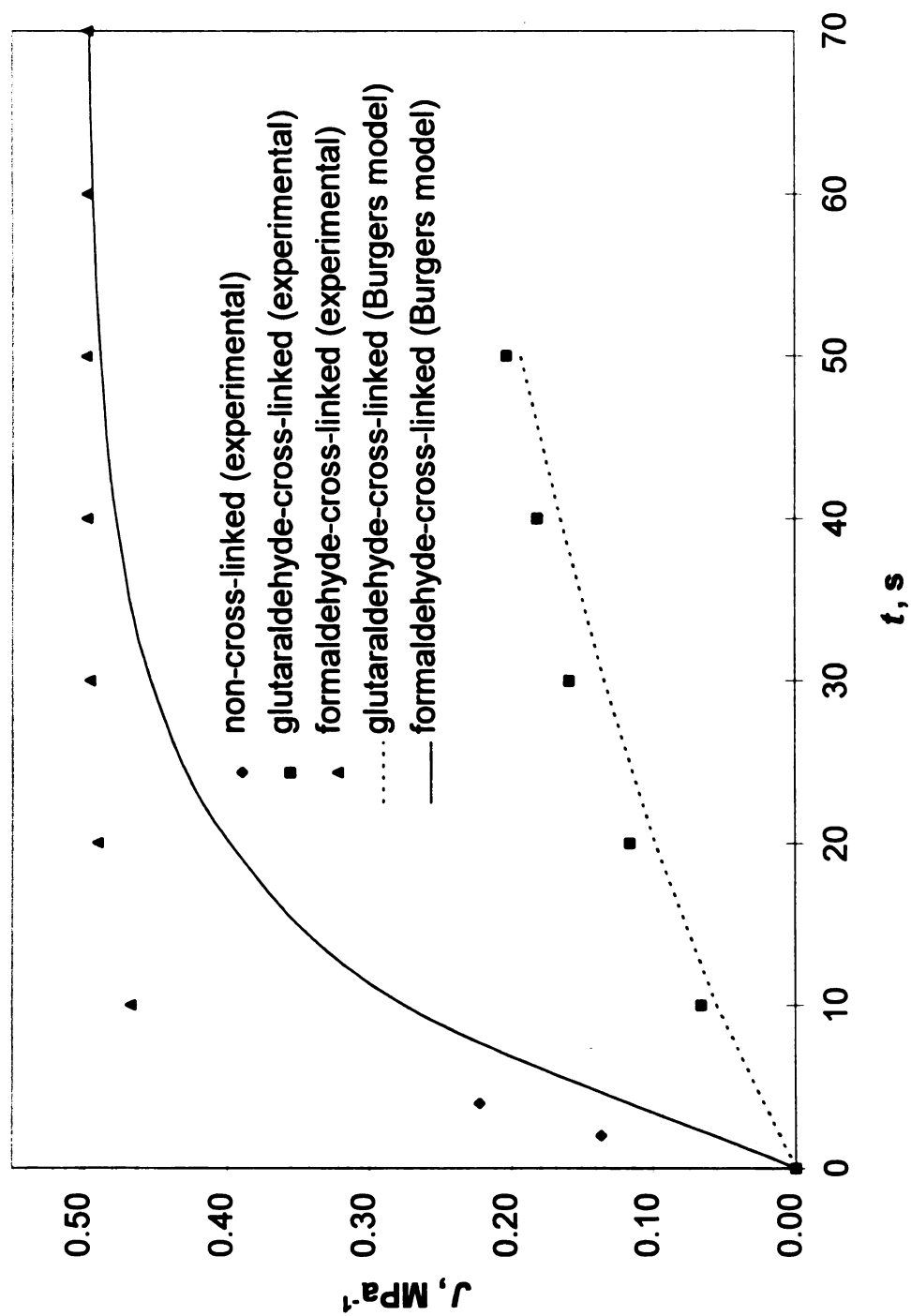


Figure 23. Creep compliance of films at 40°C and 25% RH.

considerably. It was also observed that temperature had a greater effect on the creep behavior of the films than the relative humidity.

By comparing the experimental curves of Figures 17 to 23, it was observed that creep compliance of films made with the cross-linking agents were smaller than those for films made without a cross-linking agent. Also, by comparing the experimental curves of films made with the same cross-linking agent (formaldehyde or glutaraldehyde) in Figures 21 and 23, it was found that an increase in temperature produced an increase in the creep compliance. This was expected since the creep compliance is proportional to strain and proteins, as well as many other polymers, increase their molecular mobility (i.e. become more rubbery) as temperature increases. This process may have promoted successive changes in the proteins so that the biopolymers changed from hard (brittle) to viscoelastic materials.

Similarly, an increase in the relative humidity yielded higher values of the creep compliance for both pair of conditions investigated (Figures 19 & 20 and 21 & 22). This was attributed to the plasticizing effect of the water in the film which is known to decrease the modulus of elasticity of the protein biopolymers. This behavior is explained by the action of water that reduces the intermolecular forces (dipole and dispersion forces and hydrogen bonding), loosening the bonding of polymer molecules with each other. Also at the same temperature, at low relative humidity the films were stiffer to the touch than the ones at higher relative humidity. This was in accordance with the thermodynamic theory of plasticization. This theory explains that in a stiff, brittle polymer the

intermolecular separations are small, compared with an elastomer, and every deformation causes internal stresses which the molecules cannot accommodate; consequently, the elasticity of the protein films was lower producing a hard rubber type consistency.

The plots in Figures 17 to 23 include curves for the four-element Burgers model obtained by calculating element values from experimental data. Table 16 shows the numerical values obtained for the Burgers Model as defined by Eq. (10). At lower temperatures, better curve fitting was achieved. This result may be interpreted on the basis of molecular theory. The increased temperature permitted assemblies of protein chains to move in a coordinated manner, being governed by the principles of reptation and diffusion. Since the protein molecules were cross-linked, this motion was thought to involve several chain segments which are bound together. This behavior may be predicted using more complicated mathematical models (e.g., introduce one or more additional Kelvin elements) which accounts for the new thermodynamic molecular behavior exhibited by the biopolymeric film with the increase of the temperature.

Analysis of the raw data using the model proposed by Peleg (1980) yielded a much better fitting. It was observed (Figures 24 to 30) that the greater the value of the intercept (k_1), the lower the creep compliance differential over time. This inverse of k_1 was indicative of an ability to exhibit a lower tensile deformation over longer periods of time. The value of the inverse of the slope

Table 16. Four-element Burgers Model constants for wheat protein films.

Film ¹	T-RH (°C-%)	J_0^2 MPa ⁻¹	J_1 MPa ⁻¹	λ_{ret} s	μ_0 MPa-s	σ_0^3 MPa
17 / N	05-85	--	--	--	--	4.44
17 / G	05-85	--	0.74	18.2	12155.6	2.34
17 / F	05-85	--	0.60	6.1	2191.8	2.59
18 / N	10-40	--	--	--	--	3.19
18 / G	10-40	--	0.32	888.0	62296.7	2.46
18 / F	10-40	--	0.21	905.2	55829.9	2.75
19 / N	20-35	--	--	--	--	2.94
19 / G	20-35	--	0.03	1776.0	205783.0	2.26
19 / F	20-35	--	0.08	1260.7	200483.8	2.57
20 / N	20-55	--	--	--	--	4.46
20 / G	20-55	--	0.38	510.4	260536.9	2.78
20 / F	20-55	--	0.34	678.9	434219.3	3.21
21 / N	25-25	--	--	--	--	3.84
21 / G	25-25	--	0.20	41.7	40230.6	5.92
21 / F	25-25	--	0.24	88.8	27677.6	4.12
22 / N	25-30	--	--	--	--	4.11
22 / G	25-30	--	0.20	5.5	284871.0	6.35
22 / F	25-30	--	0.23	90.9	37980.7	4.84
23 / N	40-25	--	--	--	--	3.92
23 / G	40-25	--	0.09	6.1	452.2	3.77
23 / F	40-25	--	0.50	12.34	61563.8	3.28

¹ Figure # / Letter (N=non-; G=glutaraldehyde-; and F=formaldehyde-cross-linked)² By limitations of the creep tester, this value could not be determined [When J_0 is 0, this is called the Jeffreys Model]³ Value at failure

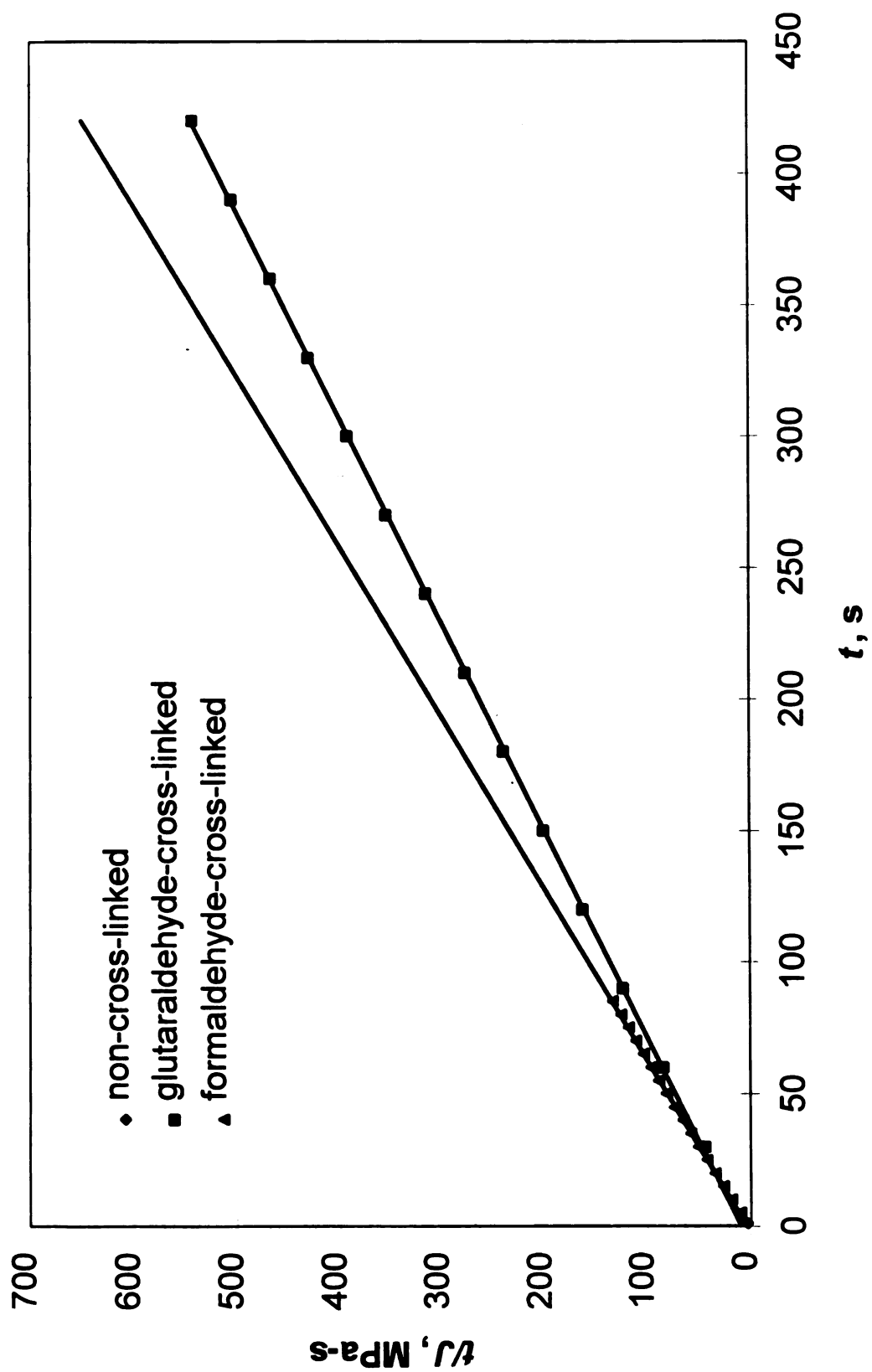


Figure 24. Linearized creep curves of films at 5°C and 85% RH.

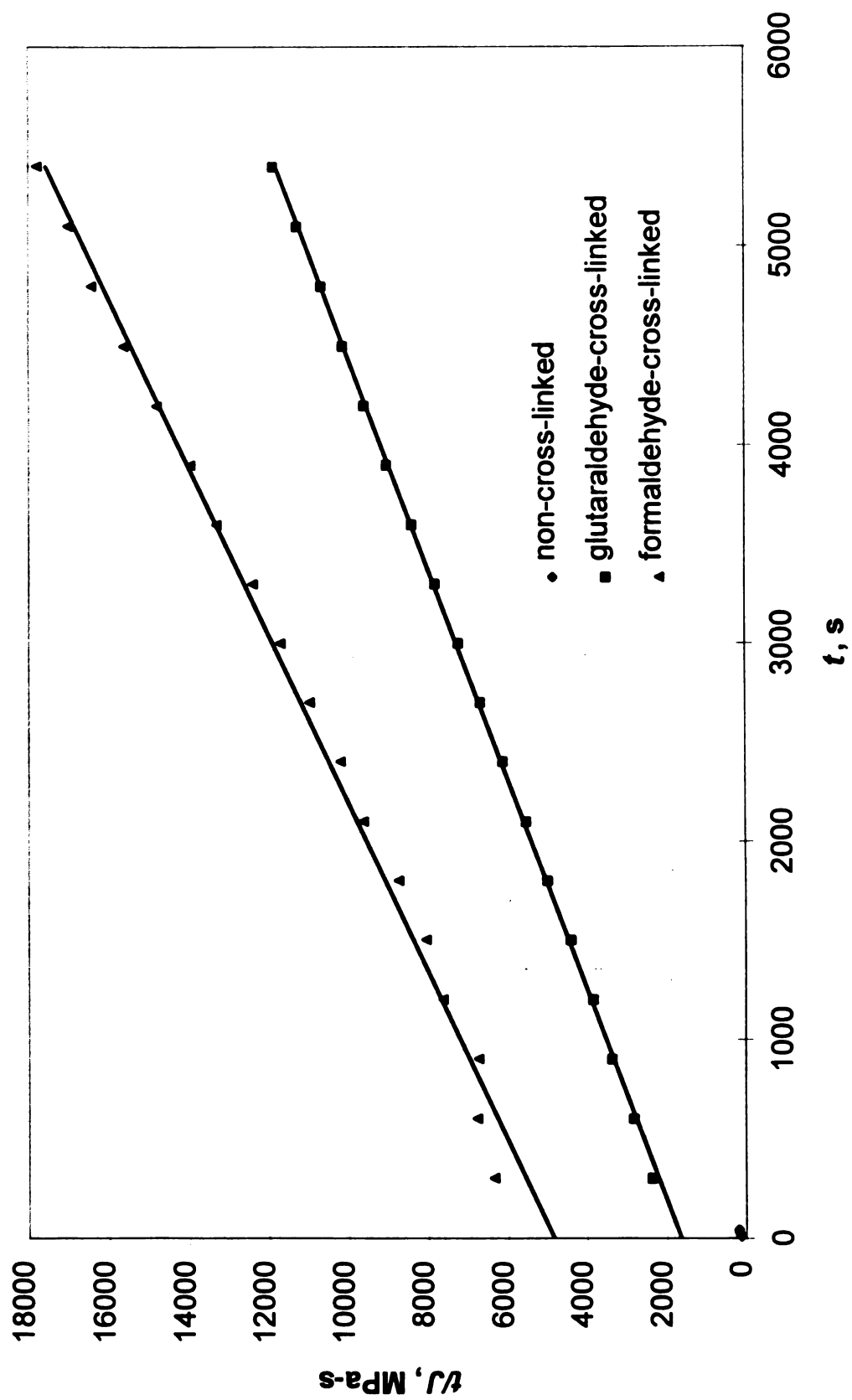


Figure 25. Linearized creep curves of films at 10°C and 40% RH.

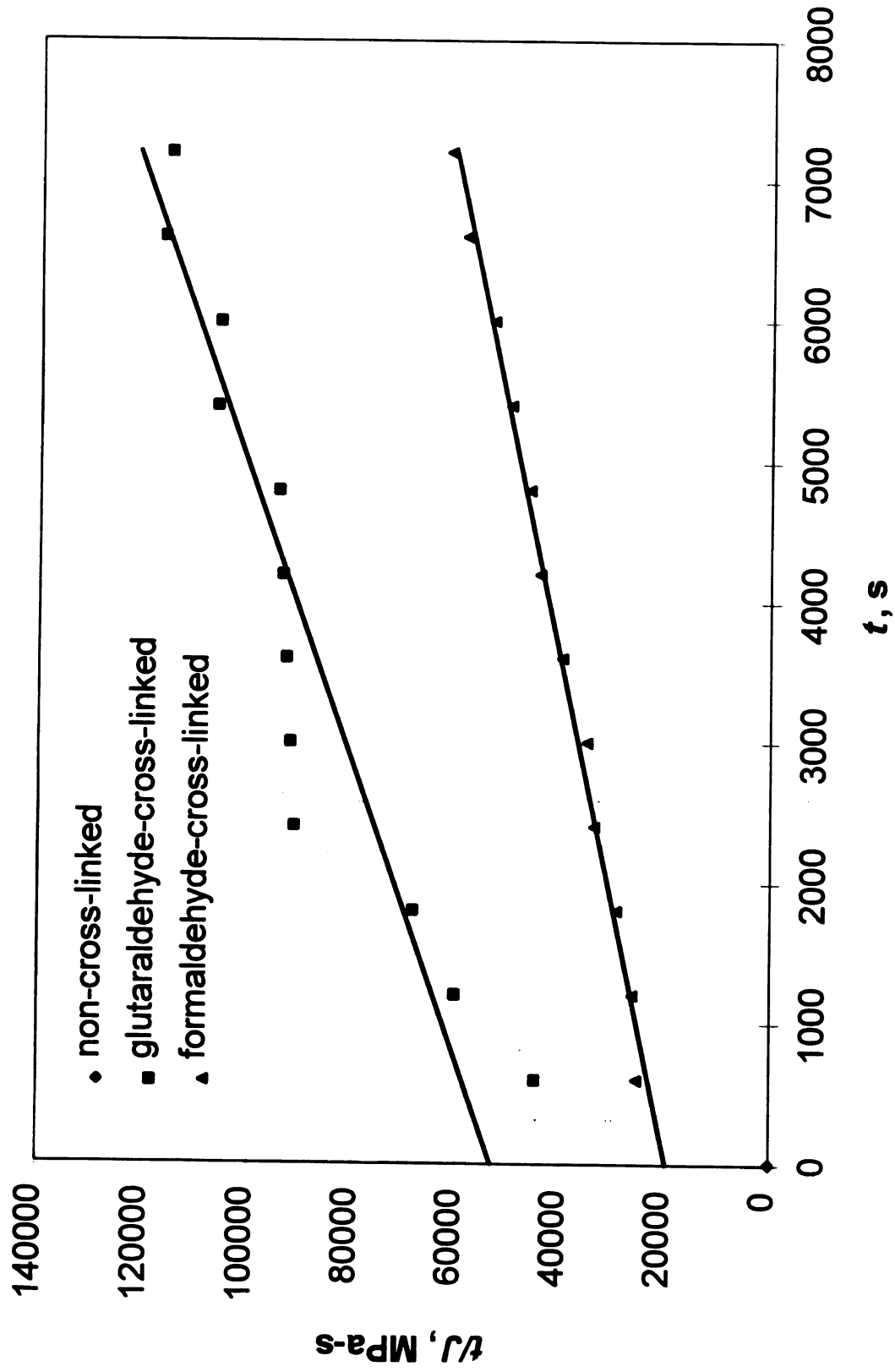


Figure 26. Linearized creep curves for films at 20°C and 35% RH.

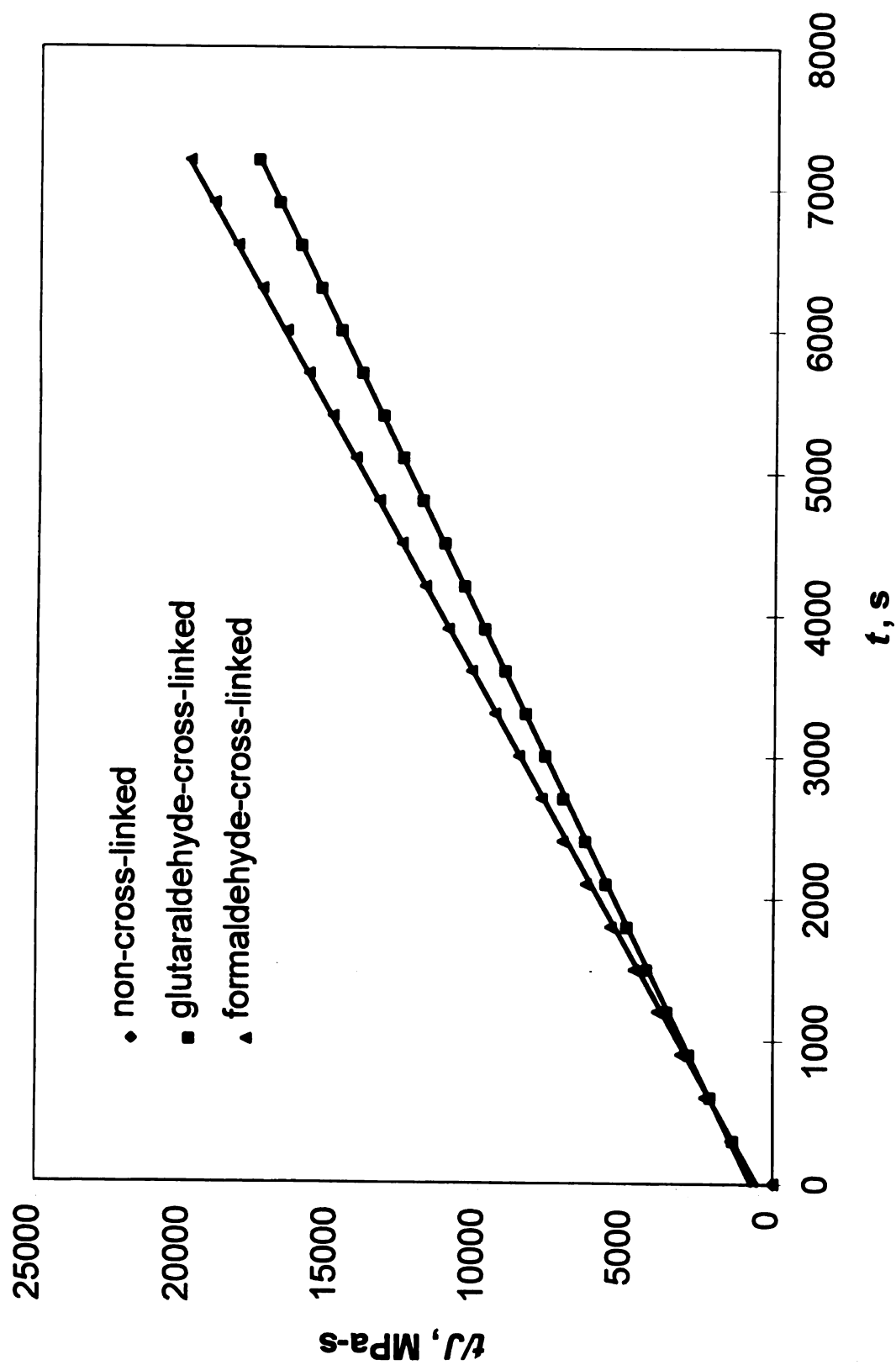


Figure 27. Linearized creep curves of films at 20°C and 55% RH.

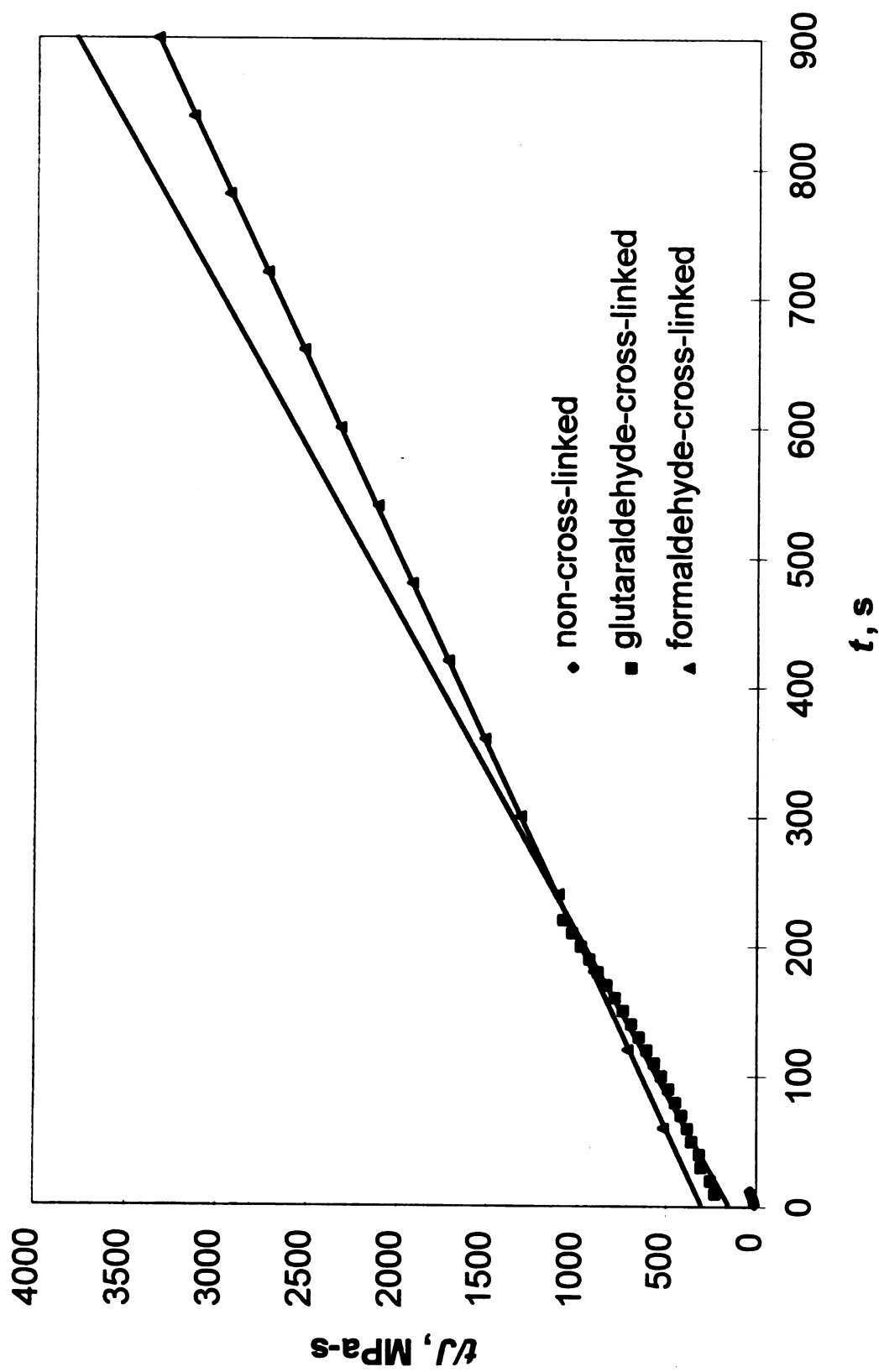


Figure 28. Linearized creep curves of films at 25°C and 25% RH.

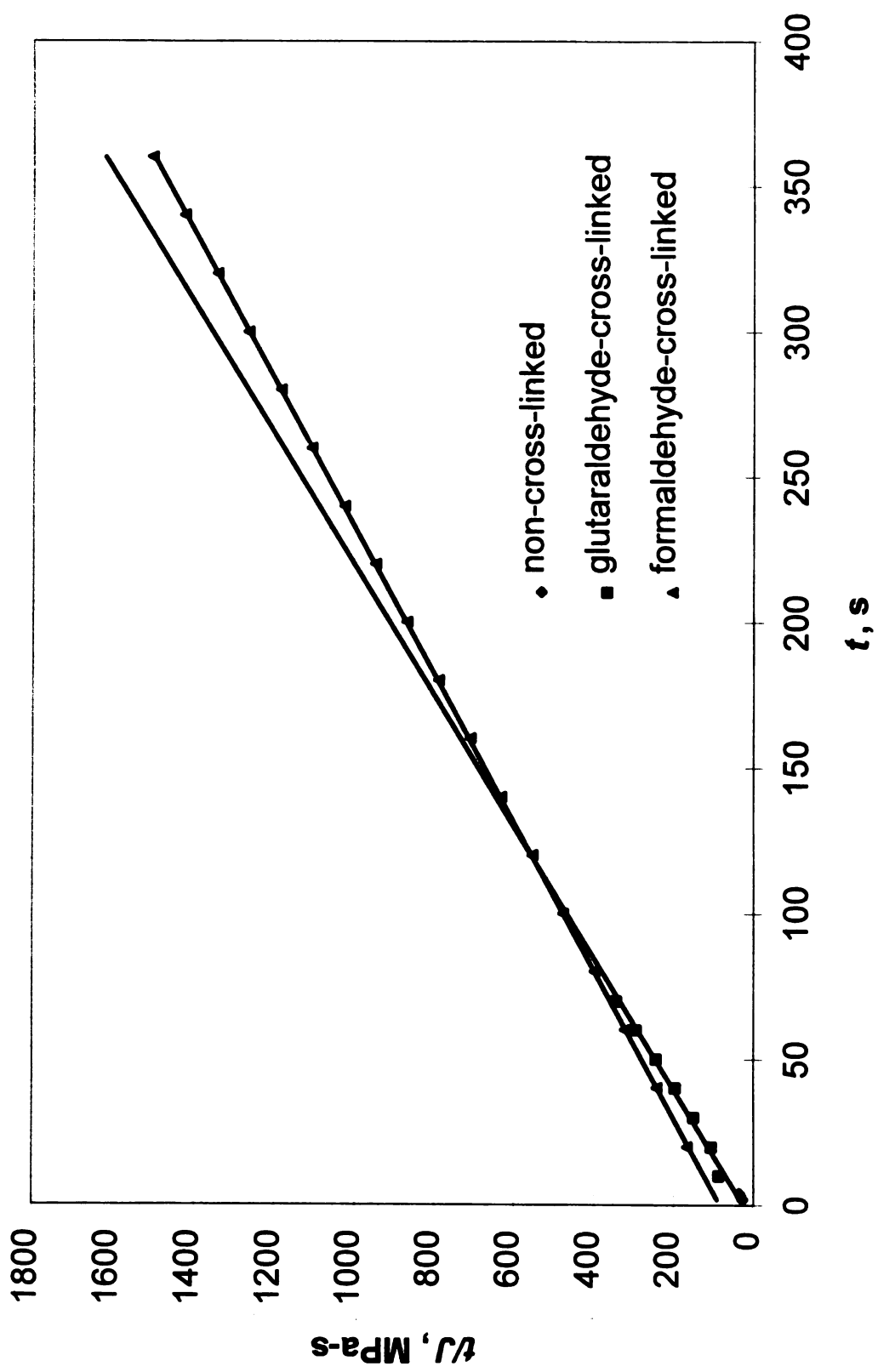


Figure 29. Linearized curves of films at 25°C and 30% RH.

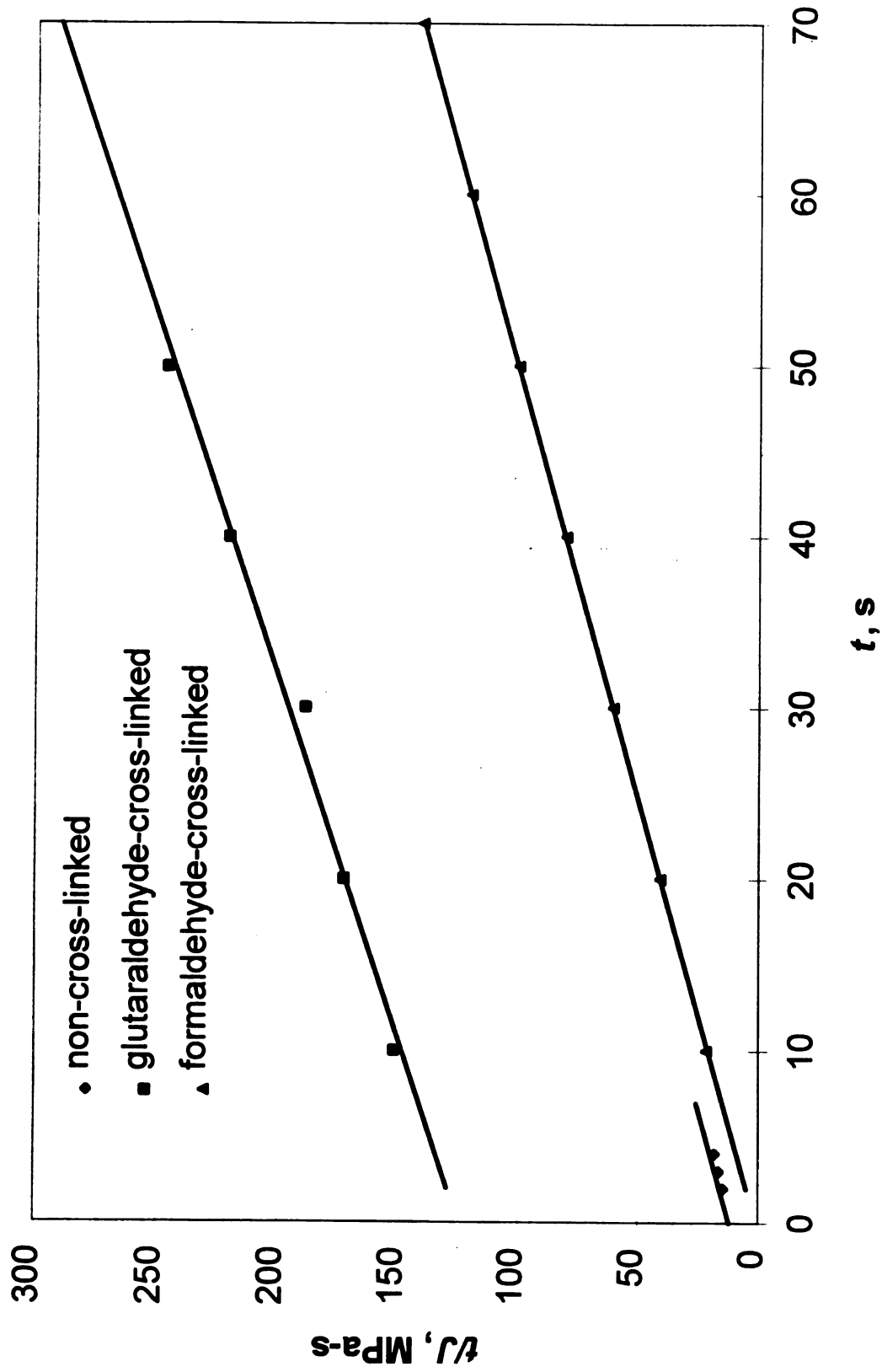


Figure 30. Linearized creep curves of films at 40°C and 25% RH.

$(1/k_2)$, on the other hand, represents a value of constant creep, that is, when the hypothetical equilibrium (if failure of the specimen did not occur) was reached. Such behavior is observed in the following relationship:

$$J_A = \frac{\varepsilon_A}{\sigma_0} = \frac{1}{k_2} \quad (28)$$

in which J_A = asymptotic creep compliance and ε_A = asymptotic tensile strain.

From this, the asymptotic tensile strain can be calculated:

$$\varepsilon_A = \frac{\sigma_0}{k_2} = J_A \sigma_0 \quad (29)$$

which may be a more useful parameter for determining the overall deformation rate of the protein film. It was observed that the lower the ε_A number, the lower the deformation rate for the curves studied. This value appears to account for the initial instantaneous deformation (J_0 in the Burgers Model) since the magnitude of the ε_A value was related to the free spring behavior of the creep curves. The limitation appears to be that it does not account for the total experimental time required for strain measurements for each film. These values of k_2 are also sensitive to the environmental conditions, which in biological materials would account particularly for the influence of temperature and relative humidity.

Therefore, for practical purposes, it is recommended that comparison for these values for different films: (1) are made on different films tested at the same environmental conditions (eg., temperature and relative humidity) to account for film differences; or (2) are made of the same films at the different

environmental conditions to account for the influence of these variables on the creep behavior.

Parameters for Peleg Model, given by Eq. 12, are summarized in Table 17. Unlike the Burgers equation, all curves could be plotted, with an $r^2 \geq 0.98$ in all cases except one using the Peleg relationship. The exception was for the glutaraldehyde-cross-linked film tested at 20°C and 35% RH, with an r^2 of 0.87, which was attributed to a very small strain presented by the sample (see Hencky strain in Table 17). Thus, a Hencky strain of about 0.30 (the next value up observed for the formaldehyde-cross-linked film in Table 17) was determined to be the limit for obtaining creep curves with good accuracy using the creep tester.

Results indicated that k_1 is related to a large initial change in strain. Values for k_1 were obtained for all films studied. By comparing the different films tested under the same environmental conditions, it was generally observed that the cross-linked films had higher values as compared to the uncross-linked films. This indicated that a long time passed without major change in creep compliance. By comparing groups of films tested at different conditions, it was observed that the films at lower temperatures and lower relative humidity, exhibited a smaller change in creep compliance, as noted in Eq. 28.

It is important to mention that there is possibility for a better fitting of the curves using a 6- or more-element Burgers model. However, this model becomes more complicated when compared to the Peleg model. Consequently, it was observed that in a comparison of both 4-element Burgers and Peleg models, the last was superior for examining the creep behavior of the films.

Table 17. Peleg Model constants (k_1 and k_2) for wheat protein films.

Film ¹	T-RH (°C-%)	k_1 MPa ⁻¹ -s	k_2 MPa ⁻¹	t^2 s	$\epsilon_h^{2,3}$
24 / N	05-85	-- ⁴	-- ⁴	1	1.42
24 / G	05-85	9.3	1.3	420	1.82
24 / F	05-85	3.9	1.5	85	1.65
25 / N	10-40	109.9	1.9	42	0.72
25 / G	10-40	1632.5	1.9	5400	1.12
25 / F	10-40	4840.1	2.4	5400	0.83
26 / N	20-35	66.0	2.2	10	0.35
26 / G	20-35	53361.0	9.5	∞^5	0.14
26 / F	20-35	19765.0	5.7	∞	0.30
27 / N	20-55	1.5	2.4	2	1.43
27 / G	20-55	755.7	2.3	∞^5	1.14
27 / F	20-55	720.2	2.7	∞	1.16
28 / N	25-25	6.5	2.1	12	1.39
28 / G	25-25	148.2	4.0	220	1.21
28 / F	25-25	302.9	3.4	900	1.11
29 / N	25-30	19.9	3.7	4	0.47
29 / G	25-30	25.2	4.4	70	1.29
29 / F	25-30	83.1	3.9	360	1.16
30 / N	40-25	11.3	1.7	4	0.87
30 / G	40-25	124.9	2.4	50	0.77
30 / F	40-25	1.0	2.0	70	1.63

¹ Figure # / Letter (N=non-; G=glutaraldehyde-; and F=formaldehyde-cross-linked)

² Value at failure

³ Hencky strain

⁴ Could not be determined due to insufficient data points

⁵ Did not break in the 2 hr. observation period

$$\frac{t}{J} = k_1 + k_2 t$$

4.4.2 THERMOMECHANICAL PROPERTIES

4.4.2.1 Thermal Mechanical Analysis

TMA was performed for the films but no useful results were obtained for these tests. The main limiting factor was the thickness of the films. It was not possible to produce a film thicker than 1.25 mm (approx. 0.05 in). The particular TMA instrument considered required samples at least 125 mm (approx. 0.5 in) in thickness. The intended thermal expansion coefficient was not obtained using this technique. A sample output of a glutaraldehyde-cross-linked film measured using this instrument is presented in the Appendix (Figure C1).

4.4.2.2 Modulated Differential Scanning Calorimetry

A couple of samples of MDSC curves obtained for the glutaraldehyde-cross-linked film are presented in Figures D1 and D2 in the Appendix. This technique although very useful for obtaining T_g and T_m values in polymeric materials, was not very useful for the protein films studied. It is suspected that no data were obtained primarily because of the process used for fabricating the film. The protein solution was heated to boiling temperature (measured to be between 82-83 °C) thus making the proteins denature.

Hoseney (1994) reported that a non-reversible thermal effect on the gluten-water system occurs between around 40 and 70 °C, which represents values below the boiling temperature of the FFS during film fabrication. The non-reversible thermal effect of the proteins in the FFS was demonstrated by the use of RheoStress[®] Measuring System model RS-100 (Haake Mess-Technik GmbH u. Co., Karlsruhe, Germany). FFS was taken and placed between two 60

mm diameter parallel plates using a solvent trap. The distance between the plates was set to 0.25 mm and an oscillatory test was performed. Results for this test can be seen in Figure E1 in Appendix E. In this figure, the values for G^* were observed to significantly increase when the temperature reached about 62°C from a value of about 0.007 Pa to a final value (in the ramping-up process) of just under 600 Pa which was attributed to the unfolding of the protein molecules. Once it reached the upper end temperature, the FFS was cooled down immediately and, as expected, a decrease of the G^* value was not observed. In the cooling process (ramping-down), the G^* value increased from around 600 to about 1000 Pa in the interval from about 80 to around 65°C, and remained constant at lower temperatures. Based on this experiment, for the actual process it was assumed that the structure opened and, when a cross-linker was added to the FFS, a macromolecule formed as the film formed. Similarly, in the FFS with no cross-linker added, the structure remained open, in a disordered structure (Hoseney, 1994) with no formation of a macromolecule. Since MDSC measures the denaturation as T_g , being a change in the baseline of the heat flow, and no change in the baseline was observed, it was assumed that the biopolymeric structure was opened. In addition, the protein films degraded before the T_m temperature was reached (especially for the non-cross-linked films, since the cross-linked film would not show a true melting point). This degradation temperature was evidenced by the sudden increase of the heat flow around 150 °C observed for all the films (see Figure D1 in Appendix).

4.5 CHEMICAL CHARACTERIZATION

4.5.1 FOURIER-TRANSFORM INFRARED SPECTROSCOPY

Spectra obtained experimentally for the wheat protein films (Table 7) by Fourier-Transform Infrared Spectroscopy (FTIR) are presented in Figure 31. The FTIR spectra obtained from the literature for some commercial polymers are shown in Figure 32. It is not coincidental that the overall shape and a number of the characteristic absorption bands observed in the nylon-6 spectrum (Figure 32), are similar to those obtained for the protein films (Figure 31), since all of these films have the polyamide functionality. For instance, proteins are sometimes referred as nylon-2 (Sperling, 1992), and have the following general structure:



The "R" group will depend on which of the 20 common types of amino acids are present in the protein structure. These amino acids follow very specific sequences, but, according to Sperling (1992), they are copolymers in the broad sense of the term.

The main features observed for the bands in Figure 31 are: a broad band around 3300 cm^{-1} attributed primarily to the hydroxyl (-OH) groups from the plasticizer (glycerol) and to presence of water molecules (Colthup *et al.*, 1964). According to Colthup *et al.* (1964) and Satyanarayana and Chatterji (1991), the

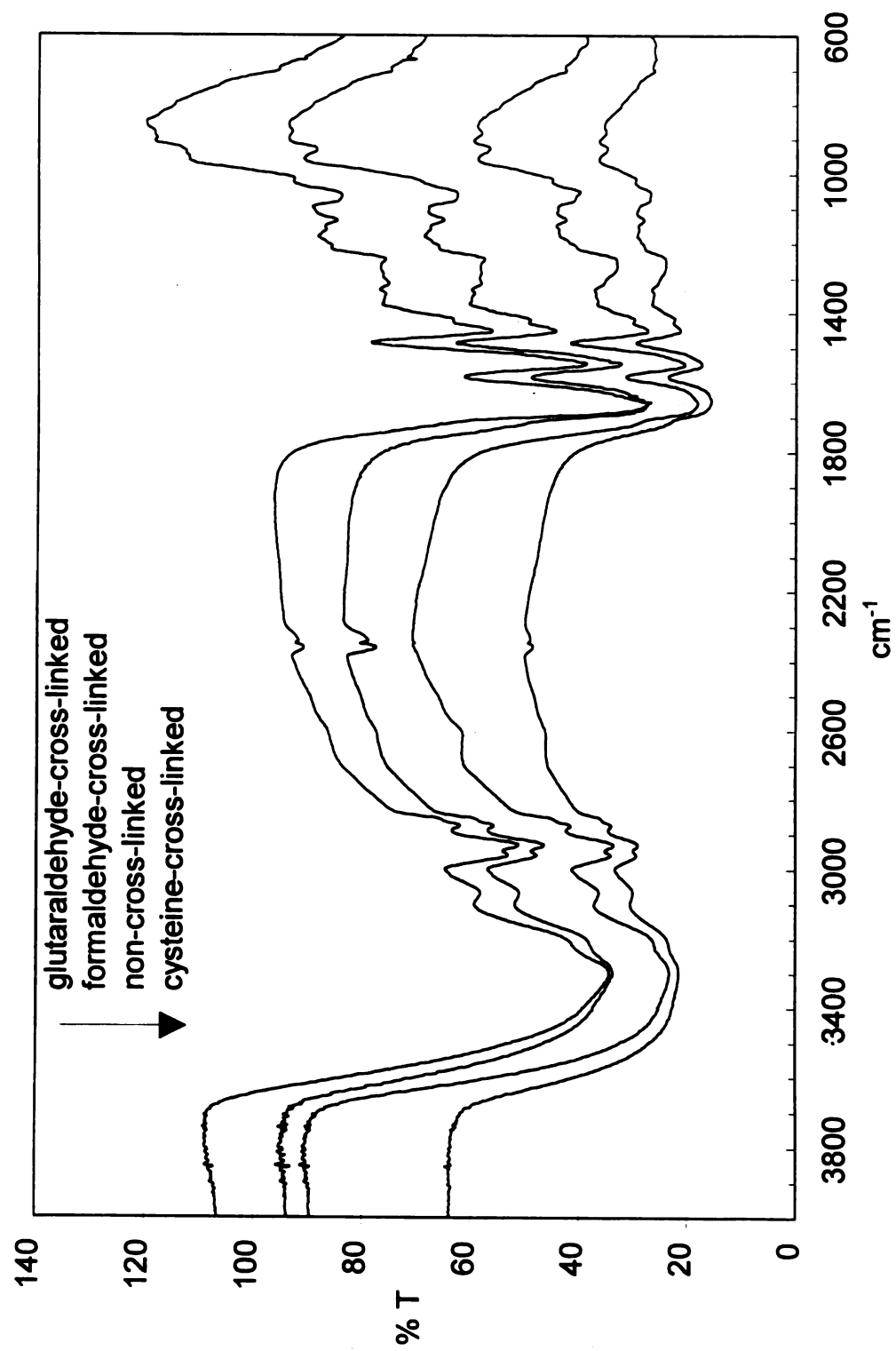


Figure 31. FTIR spectra obtained for selected wheat protein films.

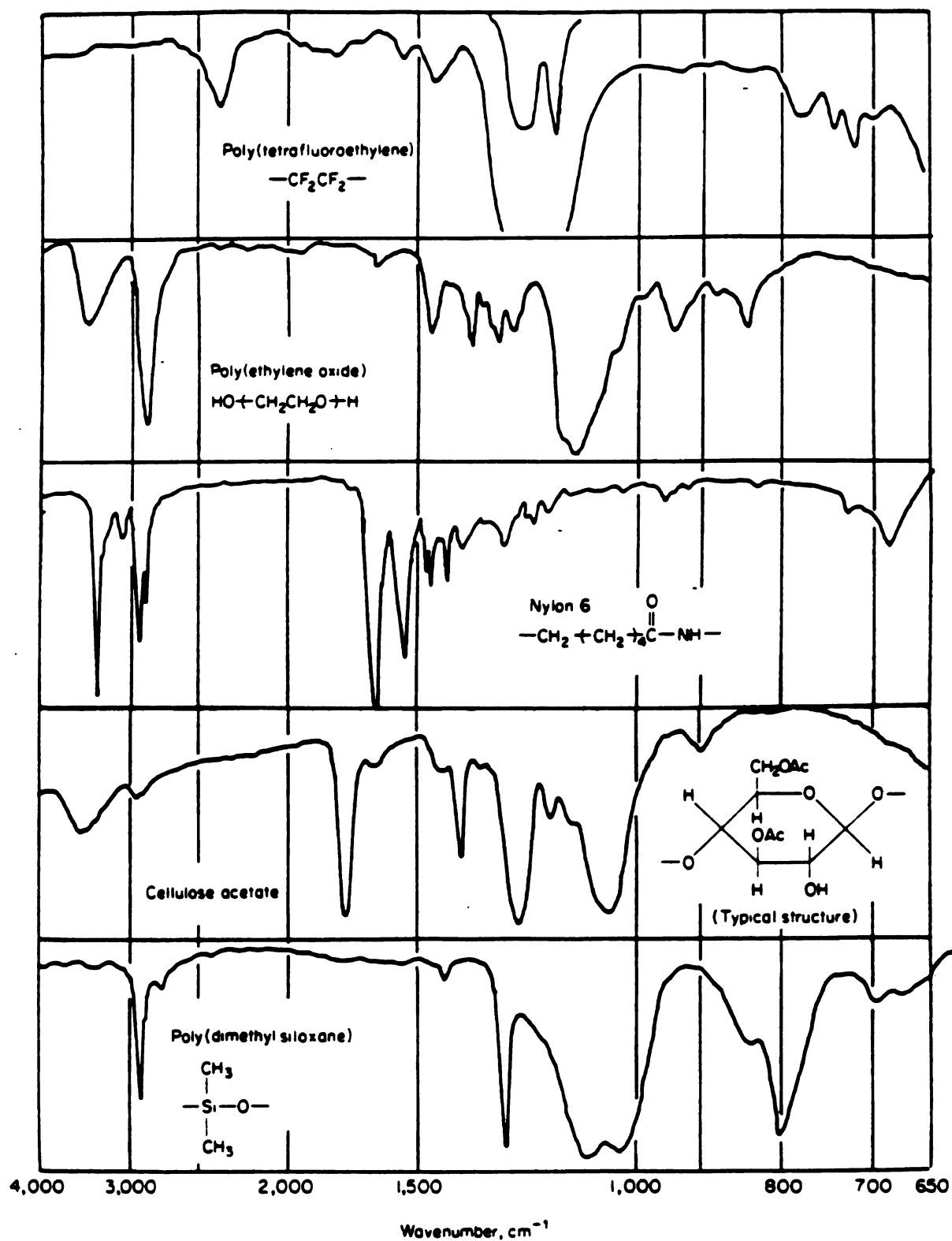


Figure 32. Typical transmission spectra in the infrared region for some commercial polymers. The ordinate in each diagram is % transmission (T).
[from Rodriguez, 1989]

close narrower bands observed at around 2950 and 2920 cm^{-1} are typical of the methyl group ($-\text{CH}_3$) and C-H bond stretching, respectively, which are typical groups found in the protein structure.

There was a small band present at around 2350 cm^{-1} , which is not found for many of the synthetic polymers. This is probably due to rotational vibration of free water molecules (Colthup *et al.*, 1964) which were present in the protein films. These films were very hydrophilic (see results of swelling studies), which made it easy for them to pick up water from the environment.

There were two very typical absorption bands found for the films, which are typical of polyamides. The first at around 1650-1640 cm^{-1} , which corresponded to the amide-I band (Giacin, 1995). This band was found due to the carbonyl ($\text{C}=\text{O}$) stretching in the peptidic bond (see Eq. 30). The second one, known as amide-II, due to the N-H bending, was observed at around 1540 cm^{-1} . This helped to confirm that polyamide functionality in the films was preserved after the casting process. In addition, it can also be concluded that the cross-linking process did not affect the peptide bond. Finally, a band found at 1450 cm^{-1} attributed to the aldimine absorption (Satyanarayana and Chatterji, 1991) was also observed, particularly for the glutaraldehyde-cross-linked film. The peaks for the disulfide ($-\text{S}-\text{S}-$) bonds are typically present between 525-509 cm^{-1} (Colthup *et al.*, 1964). Because data were collected only up to 600 cm^{-1} , this research could not account for the FTIR spectra.

4.5.2 SWELLING STUDY

The swelling coefficient (Q) values obtained for the different film samples calculated after immersion in different solvents are presented in Table 18. Comparisons for Q values for all the films in the solvents used did not show significant differences for hexane and n-butanol, with mean values of 0.063 and 0.069 ml g^{-1} , respectively. However, Q was found to be significantly different for methanol, 70% ethanol with a pH of 4.0 (to be referred as the “ethanol” solvent this point forward, which was intended to simulate the evaporated solvent when the films formed), and water ($p < 0.05$), with mean values of 0.803, 3.192, and 2.294, respectively. When the comparisons for all treatments were analyzed for the Q values on the films, it was found that significant differences were present for the uncross-linked films with the cysteine-cross-linked and glutaraldehyde-cross-linked but not with the formaldehyde cross-linked. Their mean Q values were 0.303, 1.109, 1.531, and 1.252, respectively. It is important here to note that the mean value for water is much smaller than what would theoretically be due to the dissolution of the film in the ethanol and water solvents. Figure 33 represents the mean values obtained for the films as calculated using Eq. 14 by using the dry weight values of the films before immersion in the solvents (m_0 in Eq. 14). In this figure, it is observed that very little uptake of hexane and butanol was performed by all of the films. As for methanol, the smallest molecule of the alcohol series ($\text{CH}_3\text{-OH}$) with a relatively bulky polar group, little uptake was observed for cross-linked films but not for water. This may be attributed to the

Table 18. Swelling coefficients* of selected protein films in several solvents.

Crosslinker	Solvent	Swelling coefficient (ml g⁻¹)
none	hexane	0.0652 ± 0.0072
cysteine	hexane	0.0347 ± 0.0023
formaldehyde	hexane	0.0848 ± 0.0352
glutaraldehyde	hexane	0.0682 ± 0.0167
none	n-butanol	0.0902 ± 0.0095
cysteine	n-butanol	0.0542 ± 0.0154
formaldehyde	n-butanol	0.0626 ± 0.0127
glutaraldehyde	n-butanol	0.0689 ± 0.0139
none	methanol	0.5829 ± 0.0043
cysteine	methanol	0.9441 ± 0.0536
formaldehyde	methanol	0.7552 ± 0.1022
glutaraldehyde	methanol	0.9296 ± 0.0405
none	70% ethanol, pH 4	film dissolves
cysteine	70% ethanol, pH 4	3.0131 ± 0.4222
formaldehyde	70% ethanol, pH 4	3.7796 ± 0.3088
glutaraldehyde	70% ethanol, pH 4	2.7830 ± 0.1296
none	water	film dissolves
cysteine	water	1.4998 ± 0.0433
formaldehyde	water	2.9729 ± 0.5326
glutaraldehyde	water	2.4086 ± 0.1819

* Average ± standard deviation of three replications

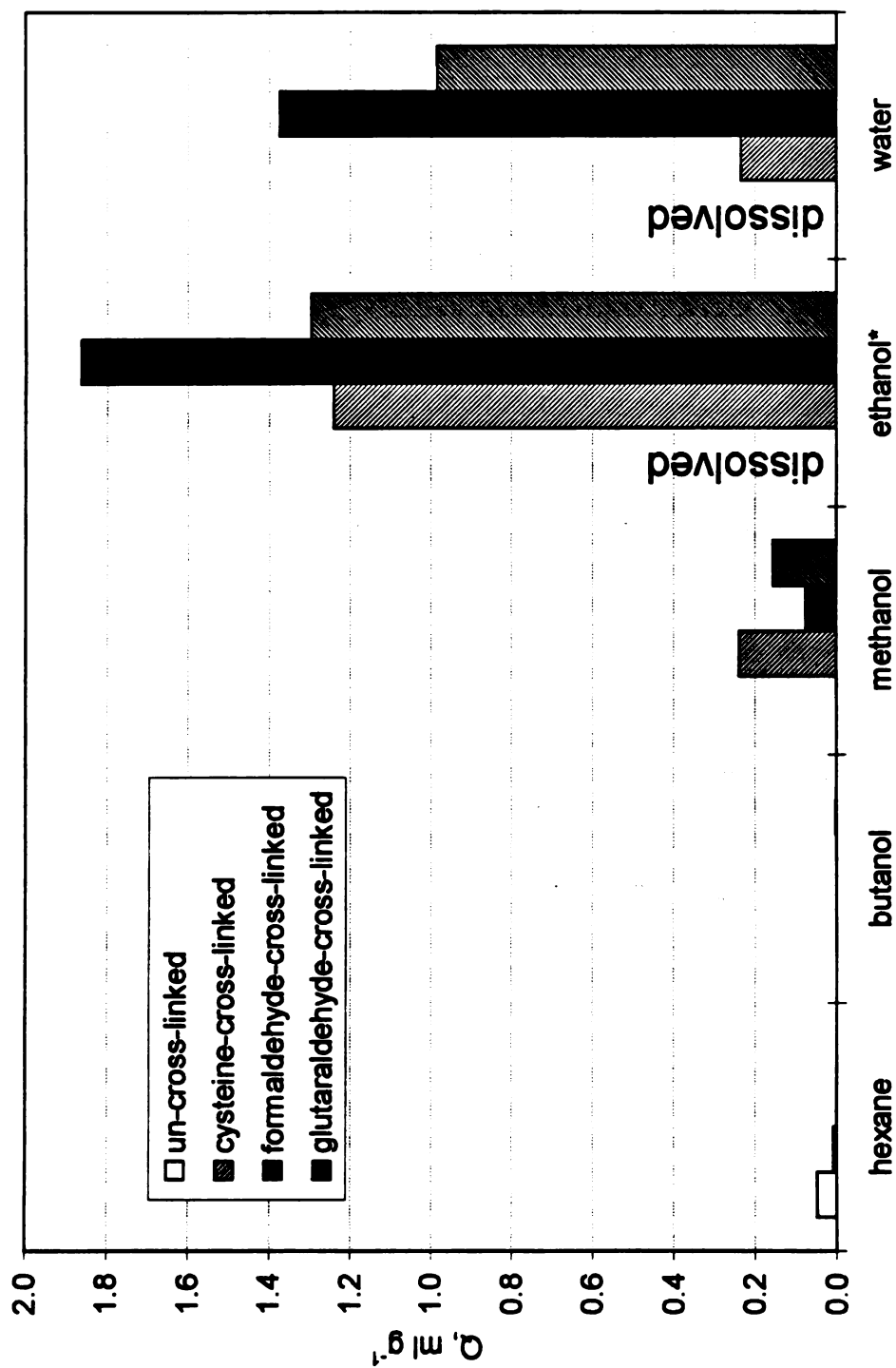


Figure 33. Swelling coefficient (Q) of different protein films, calculated using dry weight of film before immersion.
* 70% (v/v), pH 4.0

fact that some glycerol escaped to the solvent and, in the case of the cross-linked biopolymers, the stress created by the glycerol voids in the macromolecules provided the driving force for the introduction of some methanol molecules. A much greater uptake was observed by the use of both the ethanol and water solvents, with uptakes of more than 250% in weight for the solvents. The uncross-linked films dissolved in both the ethanol and water solvents, which was expected, as similar results were reported for uncross-linked gelatin proteins in a swelling study by Chatterji (1989). For this case, the dissolving of the biopolymer in the solvent causes the random coil to expand and occupy a greater volume than it would in the dry, amorphous state. In this case, viscous flow occurred, and the viscosity, although not measured, would increase as the polymer expands (Rodriguez, 1989). According to Rodriguez (1989), it is expected that when the polymer and solvent have the same solubility parameters, δ , the maximum expansion will occur and therefore the highest viscosity (for a given concentration) will be obtained.

Figure 34 represents the weigh loss observed on the films during the 24 hours of solvent immersion. These values were obtained by difference of the dry weight before after immersion in the solvents. The order of the solvents, in ascending order with respect to their solubility parameter, δ , (from hexane to water) have values of 14.9, 28.7, 29.7, 32.6, and 48.0 (Mpa)^{1/2}. This is important because an increase in the weight loss is observed as polarity is increased for the solvent. It is suspected that glycerol was extracted during the immersion time. This was partially confirmed by the greater flexibility retained by the films

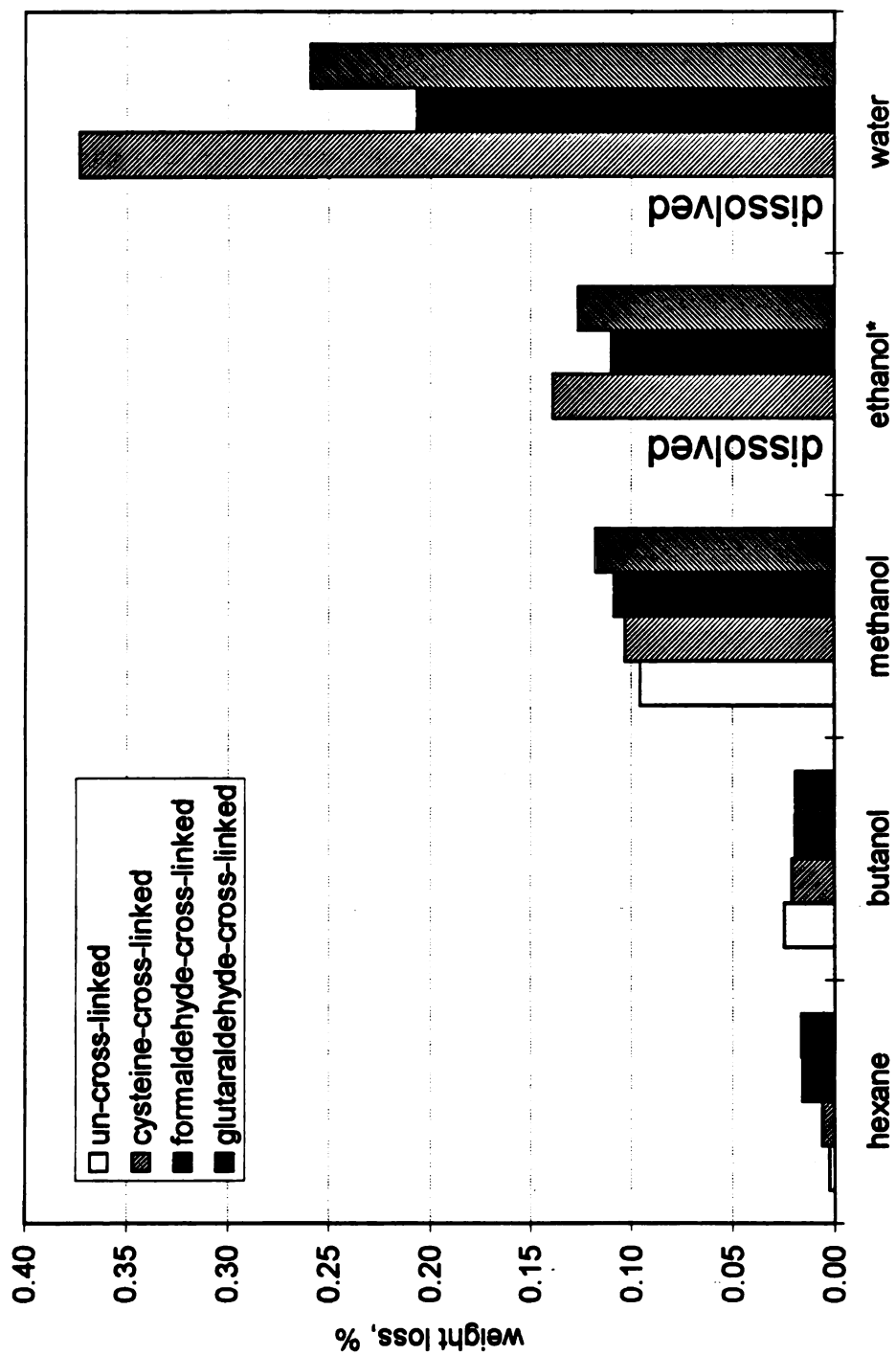


Figure 34. Weight loss of the films during time of solvent immersion.
* 70% (v/v), pH 4.0

immersed in the hexane and butanol solvents as compared to the ones immersed in the ethanol and water solvents (after they were dried in the oven). Glycerol is a very polar molecule, with three hydroxyl groups present in the molecule, thus very compatible to water solvent. There is the possibility that some loose proteins came into solution as well, especially with the ethanol and water solvents, although this was not confirmed in this study.

Figure 35 presents the swelling coefficient values of the protein films calculated from the dry weight of the film after immersion. This may be more representative of the actual solubility parameters (as compared to the values of Figure 33) because in the calculation of these values it was used the biopolymer's dry weight, which does not account for the weight loss (which is included in the dry weight before immersion). Thus, it is assumed that only the evaporated solvent occupied the rest of the volume in the swollen state. Very similar behavior was observed when compared to Figure 33 except that the values are greater (takes in account the weight lost during the immersion time). Comparison among the Q values for films immersed in hexane does not show a significant difference among them ($p>0.05$). Similar results were found for the films immersed in butanol, in which no significant differences on the Q values were observed ($p>0.05$). However, for the films immersed in methanol, the non-cross-linked value was found to have a smaller swelling coefficient as compared to the rest, followed by the formaldehyde-cross-linked film, with mean values of 0.5829 and 0.7552 ml g⁻¹, respectively. The cysteine- and glutaraldehyde-cross-linked films were found to have greater solubility values, different than the other

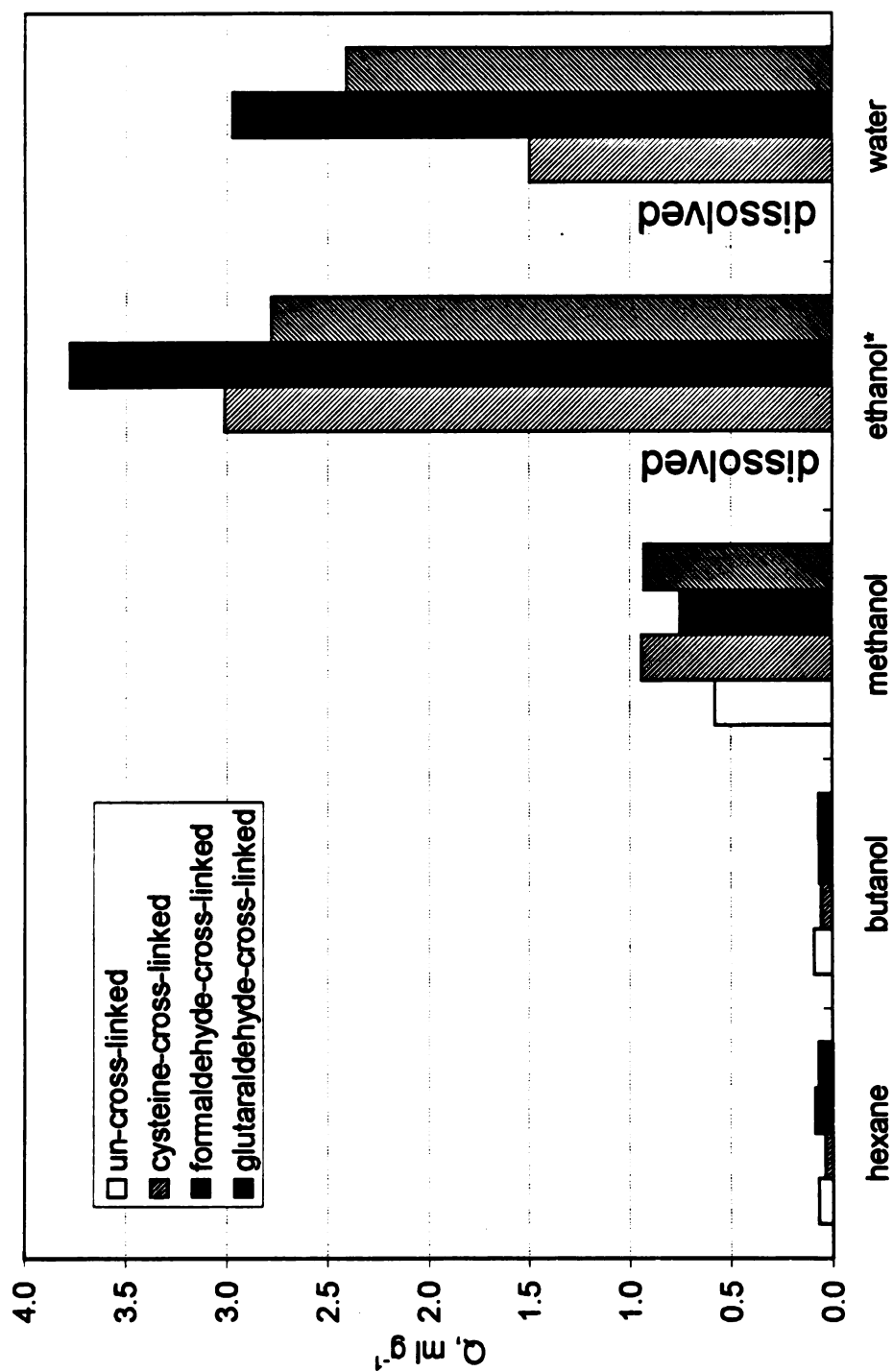


Figure 35. Swelling coefficient (Q) of different protein films, calculated using dry weight of film after immersion.
* 70% (v/v), pH 4.0

two described above but not different among them. Swelling coefficients for these films were determined to be 0.9441 and 0.9296 ml g⁻¹, respectively. Comparison of the swelling coefficients among the cysteine- and glutaraldehyde-cross-linked films show no significant difference among these two but were found to have lower values as compared to the formaldehyde-cross-linked films ($p < 0.05$), as shown in Figure 35. When water was used as the solvent, Q values were significantly smaller for the cysteine-cross-linked film as compared to the other two, formaldehyde- and glutaraldehyde-cross-linked films ($p < 0.05$).

Solubility parameters for the solvents used were obtained from the literature (Rodriguez, 1989). The swelling coefficients calculated during this study for each film and solvent are presented, as a function of the solubility parameter, in Figure 36. This representation is useful to determining the solubility parameter for any film since the swelling coefficient reaches a maximum when the solubility parameter of the solvent nearly matches that of the polymer (Rodriguez, 1989). Rodriguez (1989) indicated that the solubility parameter is very useful because when the hydrogen-bonding index for various solvents are plotted in a two-dimensional plot, along with the solubility parameters, a swell map is obtained. Such a map has great value in selecting solvents for a coating system or in judging probable resistance to swelling for a particular application (Rodriguez, 1989). In the case of these films, the curves for the non-cross-linked films follow only up to the third point [methanol, with a δ value of 29.7 (Mpa)^{1/2}] because they dissolved in the other solvents. Similar shaped-curves for polyurethane, polystyrene, and polyurethane-polystyrene

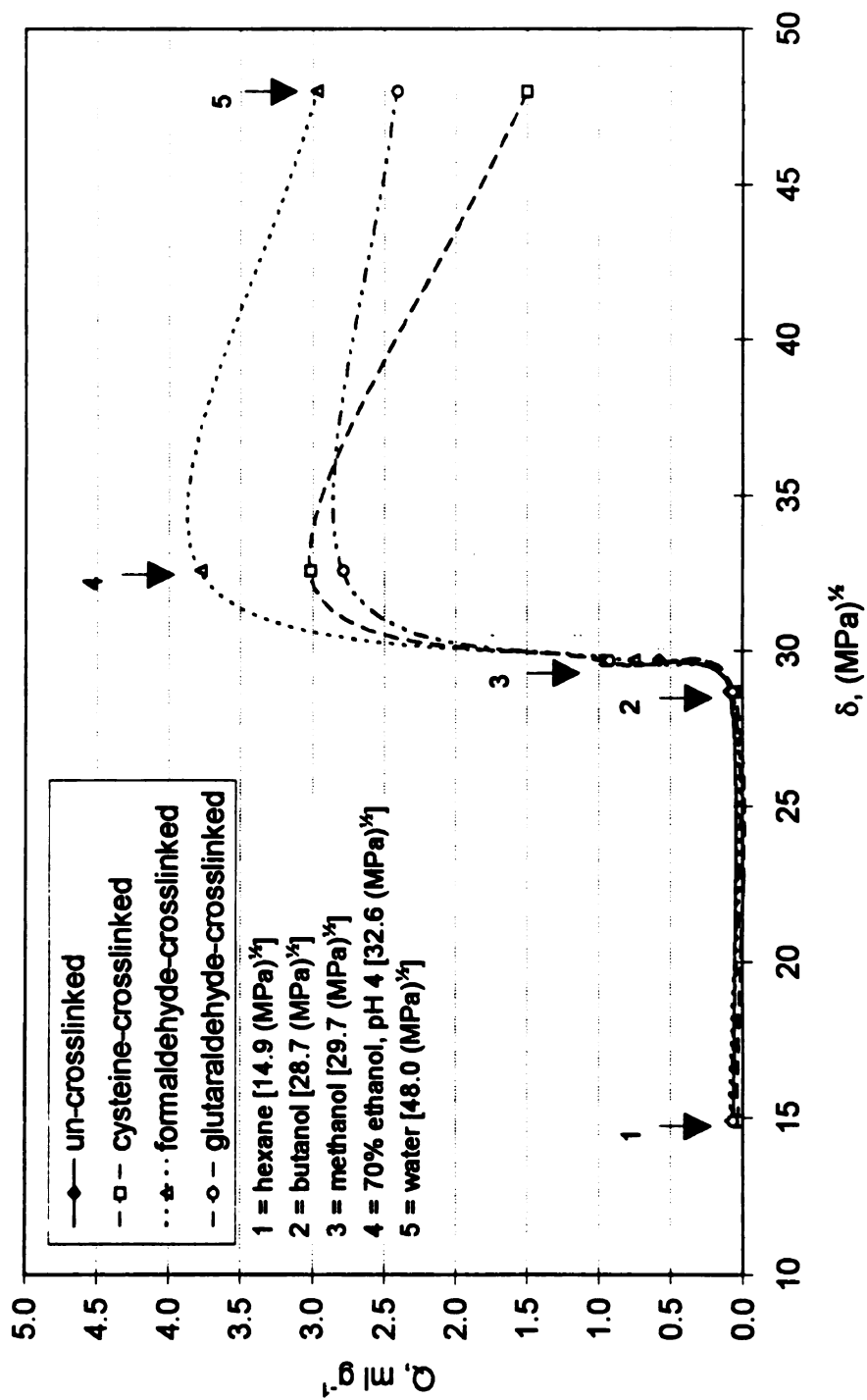


Figure 36. Swelling coefficient, Q , of the films as a function of solubility parameter, δ , of various solvents.

interpenetrating polymer networks are presented in a study by Sperling (1992) using different solvents for each polymer system. Since most synthetic polymers are formed by chemical reactions, and the polymer formed has an “unknown” chemical property, a common way to determine their solubility parameters is to do swelling studies. The curves as presented were expected because in the case of these particular protein films, they were cast directly from a solvent composed of 70% ethanol having a pH of 4.0 (referred to as the “ethanol” solvent throughout this discussion). There is an imminent advantage that the solvent (in which the proteins came into solution, thus being the “optimum” solvent) is known. Therefore, this makes it possible that the actual (if not very close) solubility parameter, δ , for the protein films is 32.6 (MPa)⁴.

4.5.3 COLOR STUDY

A diagram showing the CIE-Lab three-dimensional color evaluation parameters and significance is shown in Figure 37. Determination of color CIE-Lab parameters for the protein films are shown in Table 19. Results for the luminous transmittance mode (see schematic of Figure 38 for luminous mode explanation) showed the cysteine- and formaldehyde-cross-linked films have the lightest color (L values around 90%) among the films studied, with no significant difference among these two ($p>0.05$). They are closely followed by the uncross-linked film with a value in the lower eighties. The glutaraldehyde-cross-linked film, was significantly less light in color (L value of about 56%). The color change, due to the formation of the aldimine linkage between the free amino groups of the protein and the glutaraldehyde during the cross-linking reaction,

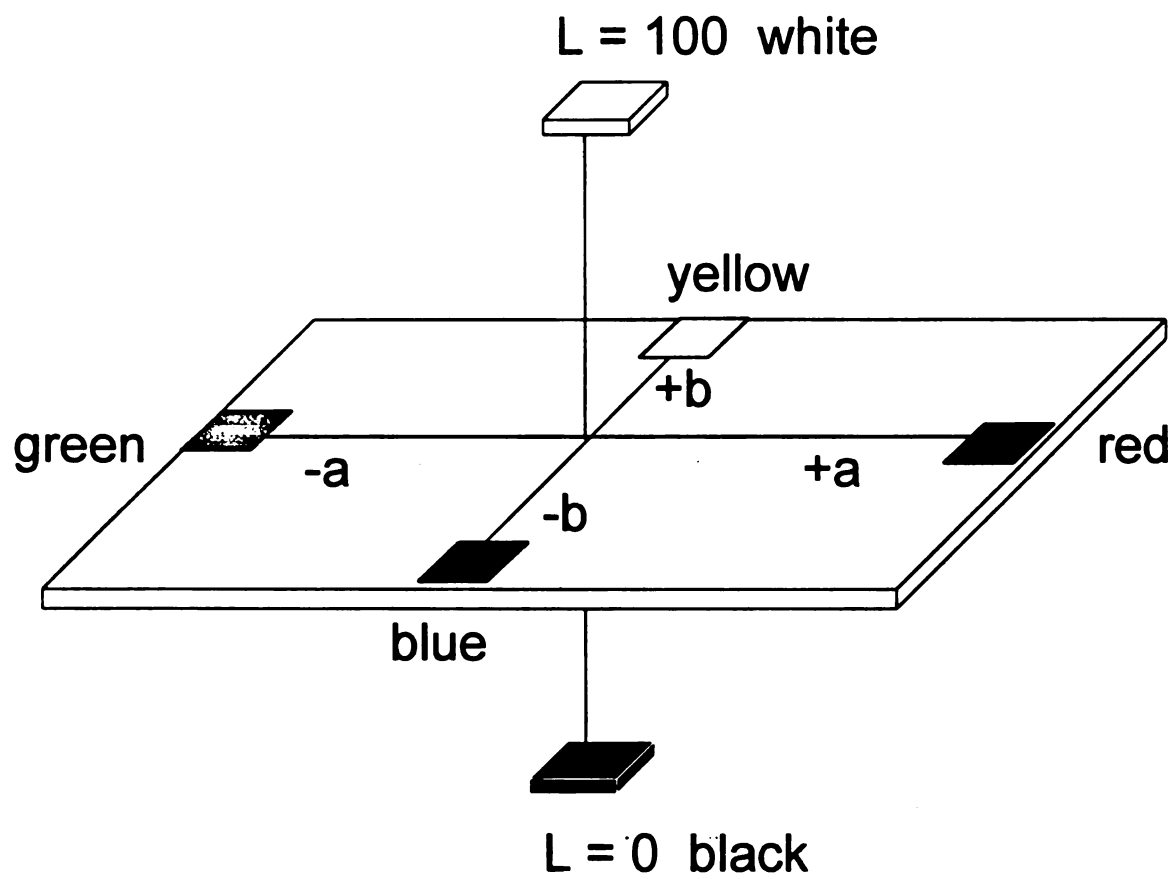
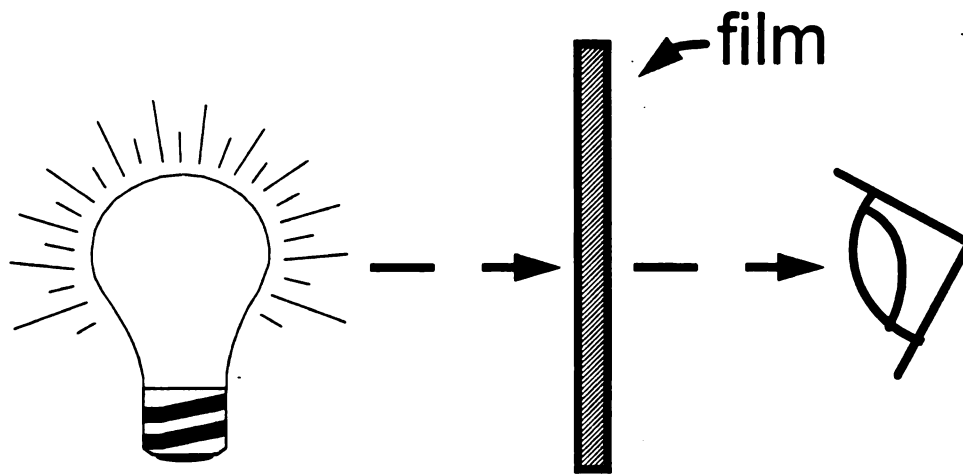


Figure 37. Three-dimensional color evaluation using the CIE-Lab system.
[Adapted from Birley *et al.*, 1992]

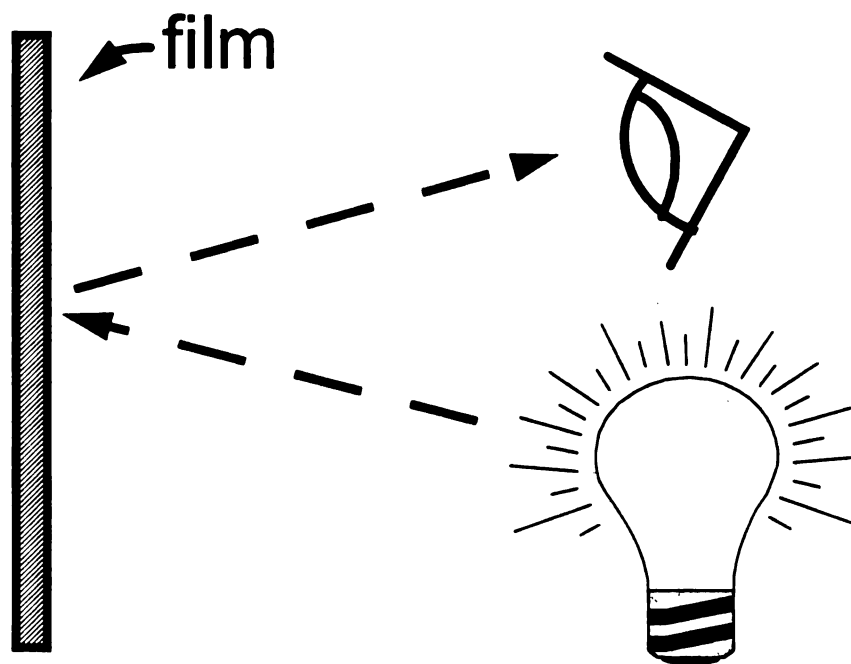
Table 19. Color analysis of selected wheat protein films.

Crosslinker	CIELab parameter	Value* $\pm$ std. dev.
Luminous Transmittance Mode		
none	L	83.33 $\pm$ 0.24
cysteine	L	89.71 $\pm$ 0.54
formaldehyde	L	89.28 $\pm$ 1.15
glutaraldehyde	L	56.59 $\pm$ 3.55
none	a	2.17 $\pm$ 0.08
cysteine	a	-1.82 $\pm$ 0.13
formaldehyde	a	-1.40 $\pm$ 0.23
glutaraldehyde	a	27.29 $\pm$ 3.05
none	b	39.29 $\pm$ 0.39
cysteine	b	29.79 $\pm$ 1.38
formaldehyde	b	29.50 $\pm$ 2.18
glutaraldehyde	b	61.79 $\pm$ 1.63
Luminous Reflectance Mode		
none	L	32.66 $\pm$ 0.42
cysteine	L	28.89 $\pm$ 0.37
formaldehyde	L	31.27 $\pm$ 0.23
glutaraldehyde	L	19.99 $\pm$ 0.66
none	a	-0.53 $\pm$ 0.12
cysteine	a	-2.47 $\pm$ 0.05
formaldehyde	a	-2.00 $\pm$ 0.06
glutaraldehyde	a	11.38 $\pm$ 1.35
none	b	17.56 $\pm$ 0.35
cysteine	b	10.32 $\pm$ 0.92
formaldehyde	b	11.19 $\pm$ 0.99
glutaraldehyde	b	25.83 $\pm$ 0.41

* Average of 5 replications. L is expressed as %, a & b as positive or negative values with color corresponding to scheme presented in Figure 37.



(a)



(b)

Figure 38. Transmittance (a) and reflectance (b) significance for color measurement.

was observed for the films. Chatterji (1989) reported this color change when the color of gelatin granules turned from pale yellow to deep orange within minutes upon cross-linking of the gelatin with glutaraldehyde. There is a significant increase in the "a" (red) and "b" (yellow) color components for these films. As observed in Table 19, the color of the films changed from light yellow, with values of about 40 for the non-cross-linked to over 60 for the glutaraldehyde-cross-linked films. The red-green component, "a", remained very close to 0, which in practice meant that the main color observed for all films (except for the glutaraldehyde cross-linked) had a yellow tone.

The same observations can be made for the films studied in the luminous reflectance mode. The values were lower since most of the incident light was absorbed by the black background and only the color reflected by the film was picked up by the machine. The main effect observed was related to lightness: It was reduced about three-fold, while approximately a two-fold reduction was observed for the yellowness. It was also observed that the yellowness in the films was reduced for the cysteine- and -formaldehyde-cross-linked films by a proportion of about 75% and 60% for the transmittance and reflectance mode, respectively.

In practice, the luminous reflectance mode color values may be more significant for the protein films studied. This is because if commercialized as films (with function of containing and protecting the food product), and since they are not transparent, the primary contact that the consumer would have prior to opening and consuming the items contained would be the films themselves.

On the other hand, if the FFS is cast directly on the food items (as a coating) then the luminous transmittance mode would be possibly of more value. This because the food product would be reflecting the transmitted light back to the eyes of the consumer, and the color of the products inside the coatings will look different than without them.

5. SUMMARY AND CONCLUSIONS

A new procedure for making films from wheat proteins was developed. Barrier, rheological, chemical, and physical properties were studied for different films prepared using this methodology. Three types of flours, two different pH film-forming solutions, three reducing (cross-linking) agents, and different test temperatures and relative humidities were considered for the preparation of the different wheat protein-based films. From the tests performed to the flours used in this study, both farinography and SDS-PAGE results were the best methods to aid in the selection of good flours in film making.

Oxygen permeability was affected primarily by the temperature and the type of flour used to form the film. The pH of the solutions tested did not significantly influence this factor. The non-proportional change in permeability for a specific temperature, with respect to the percent protein present in the flour sample, suggests that the permeability is affected not only by the quantity but also by the type of protein (referred to as "quality" in baking technology) present in the material used to make the films. The elongation deformation was found not to be affected by the use of cysteine, and the only significant difference found was the use of commercial bread flour as compared to the other two.

Tensile strength was found to be influenced by the cysteine and the type of flour (protein) used. Significant differences were found for the elongation values of the films based on the type of flour alone. Creep behavior of wheat protein films is very sensitive to differences in temperature and relative humidity.

Temperature was found to be the most significant factor affecting the tensile creep behavior, followed by relative humidity. Cross-linking of the biopolymers produced a stronger film capable of supporting higher loads, with formaldehyde-cross-linked films being able to stand smaller deformations than glutaraldehyde-cross-linked films. Similarly, cross-linked films were able to resist deformation at high stresses as compared to the non-cross-linked films.

Fourier-Transformed Infrared Spectrometry (FTIR) showed a pattern similar to that of the nylons. Appearance of an absorption peak for the cross-linked films compared to the uncross-linked ones indicated that chemical changes occurred during the cross-linking process.

Swelling Studies of films helped to find the value of the solubility parameter, δ , for the films studied. The films were observed to have a maximum swelling coefficient when immersed in a solvent whose composition was similar to that of the original FFS. This confirmed that the main chemical groups that aid in the interaction solvent-polymer molecule are kept and probably unaltered while only specific groups react forming the network. Further, this may indicate that the chemical properties of the cross-linked molecules are similar to that of the uncross-linked molecules. No swelling in solvents with low values of δ was observed on the protein films studied.

The color analysis of the films indicated that a reduction in the yellowness occur with cross-linking using cysteine or formaldehyde, as compared to the uncross-linked films. On the other hand, a significant increase

in yellowness and redness (whose combination yields orange) is observed for the films cross-linked with glutaraldehyde.

The development and analysis of edible/degradable films is relatively new and thus the area of study is broad as new techniques for the analysis of these films are introduced. In general, techniques used with synthetic polymeric materials can be applied towards the study of these new materials, because they are formed from biopolymers. Both areas, Food and Polymer Science converge when studies of these materials are required.

In general, there is interest by the food industry and general public towards the development of a successful, useful package that will serve as a nutrient if consumed, or will be environmentally-friendly if disposed. This possibility is offered by the development of films and coatings made from polymers that nature provides. However, pilot-plant studies and new techniques to produce these materials on a large scale are still required.

6. SUGGESTIONS FOR FUTURE RESEARCH

There is a need to looking for more quantitative methods to characterize flours for their film-making potential. This could lead to define good specific methods for flour selection in film making. Molecular weight determination for the uncrosslinked films by SDS-PAGE seems possible and different densities of the cross-linked SDS-PAGE gels may be experimented to obtain good separation and resolution. As for the cross-linked films, determination of the Flory-Huggins polymer-solvent dimensionless interaction term, χ_1 , and application of the Flory-Rehner theory principles could be used to determine the molecular weight between cross-links. This could be tried with different cross-linkers and at different concentration levels.

Further studies are needed to investigate the possible differences between the type of protein present in the material as how it influences the oxygen permeability of films made from wheat flour. There is also need to test for differences in color by using different variables (e.g., pH, solvents, pre-treatment of flour, etc.) on the initial flour or the solutions prepared since the pigments present in the FFS may be affected by this factor. This is supported by simple visual comparison of films that were made using the same procedure except for the pH of the solution. In addition, in order to estimate the shelf-life for specific applications, film aging studies could be considered given that, as suggested in the literature, association of protein chains via disulfide bonds slowly increases during aging.

Over the last ten years scientists have been linking the theory and analysis of polymer rheology, processing, engineering, and science as it is related to synthetic polymers and starting to apply this information to the study of the edible/degradable films. There is, however, need for more work on these converging areas so that less general and more conclusive statements can be drawn with respect to the relationship between film chemistry and film properties. Particularly important in this respect is the application of polymer rheology and processing to commercialization of wheat protein films.

Finally, this study opens the possibility of using different techniques to modify or test variables that could improve the overall performance of the films formed by using this new methodology. These could include improvements and/or studies on both the engineering of the film forming solutions (rheological methods) and the properties of the resulting films.

APPENDIX

APPENDIX A

Determination of weight sample for Falling Number determination.

The official method AACC 56-81A recommends correction of the sample weight used in the determination of the Falling Number. Table A1 shows the required sample weight, at different moisture contents, corresponding to 7 grams at 14% moisture (no change is made in the quantity of water used).

Table A1. Correction of sample weight to 14% moisture basis (AACC 56-81A).*

% moisture in sample	.0	.2	.4	.6	.8
8	6.54	6.56	6.57	6.59	6.60
9	6.62	6.63	6.64	6.66	6.67
10	6.69	6.70	6.72	6.73	6.75
11	6.76	6.78	6.80	6.81	6.83
12	6.84	6.86	6.87	6.89	6.90
13	6.92	6.94	6.95	6.97	6.98
14	7.00	7.02	7.03	7.04	7.07
15	7.08	7.10	7.12	7.13	7.15
16	7.17	7.18	7.20	7.22	7.24
17	7.25	7.27	7.297	7.31	7.32
grams needed for test					

* From Perten (1988)

APPENDIX B

Determination of equation used to correct for cross-sectional area in tensile creep studies.

An adjustment in the cross-sectional area obtained experimentally was necessary to get more accurate values of the stress measured at a given time, since stress, by definition, was calculated by dividing force over the cross-sectional area. Figure B1 shows the relationship of the cross-sectional area as a function of the displacement observed.

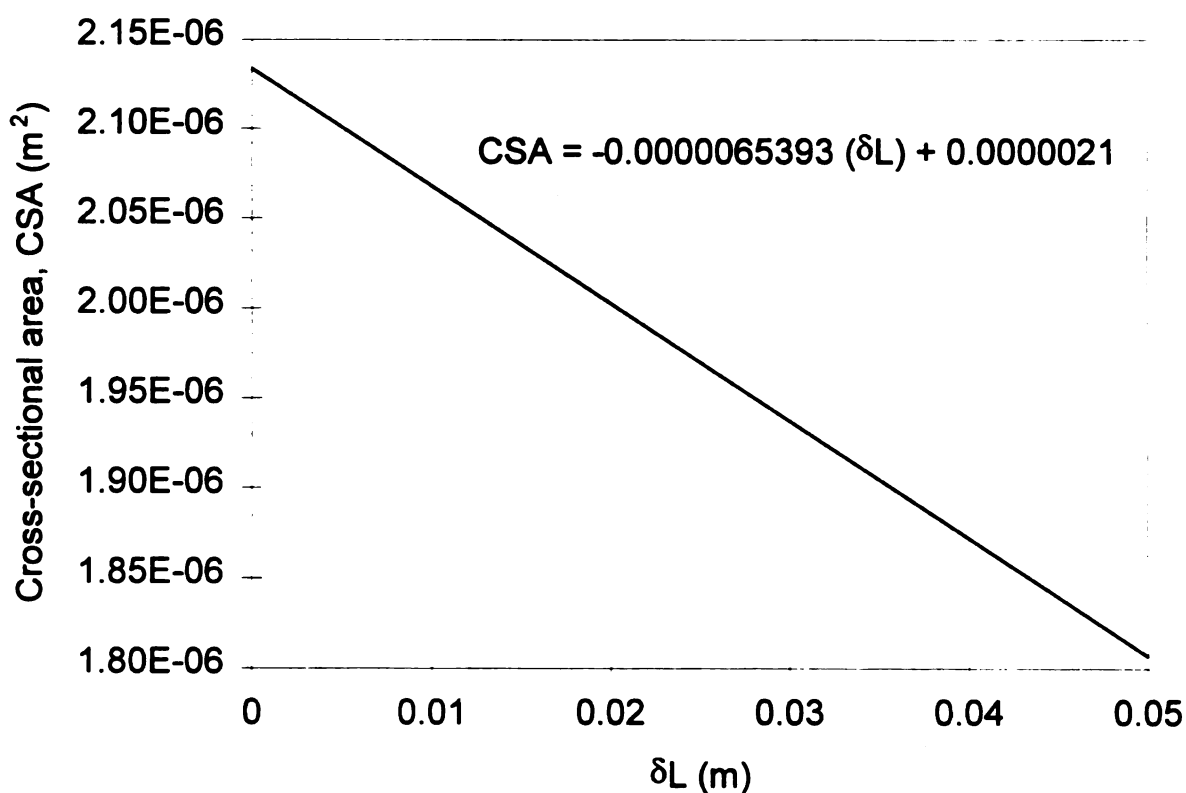


Figure B1. Cross-sectional area of films as a function of grip displacement for correction of tensile creep data.

APPENDIX C

Sample of TMA curve for a wheat protein film.

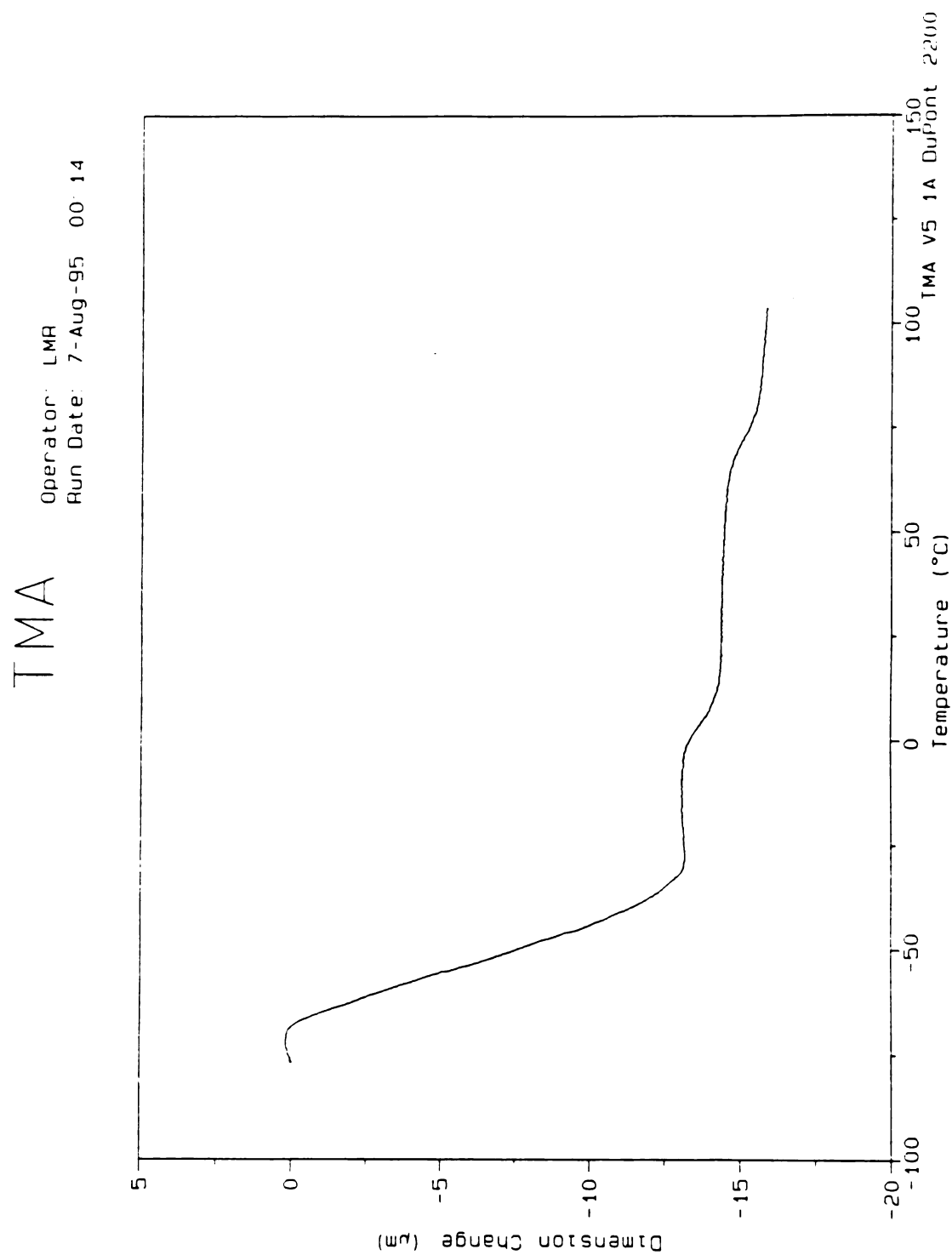


Figure C1. TMA curve for glutaraldehyde-cross-linked protein film. Similar results were observed for other film types.

APPENDIX D

Samples of MDSC curves for films. Curves for the other film types were similar.

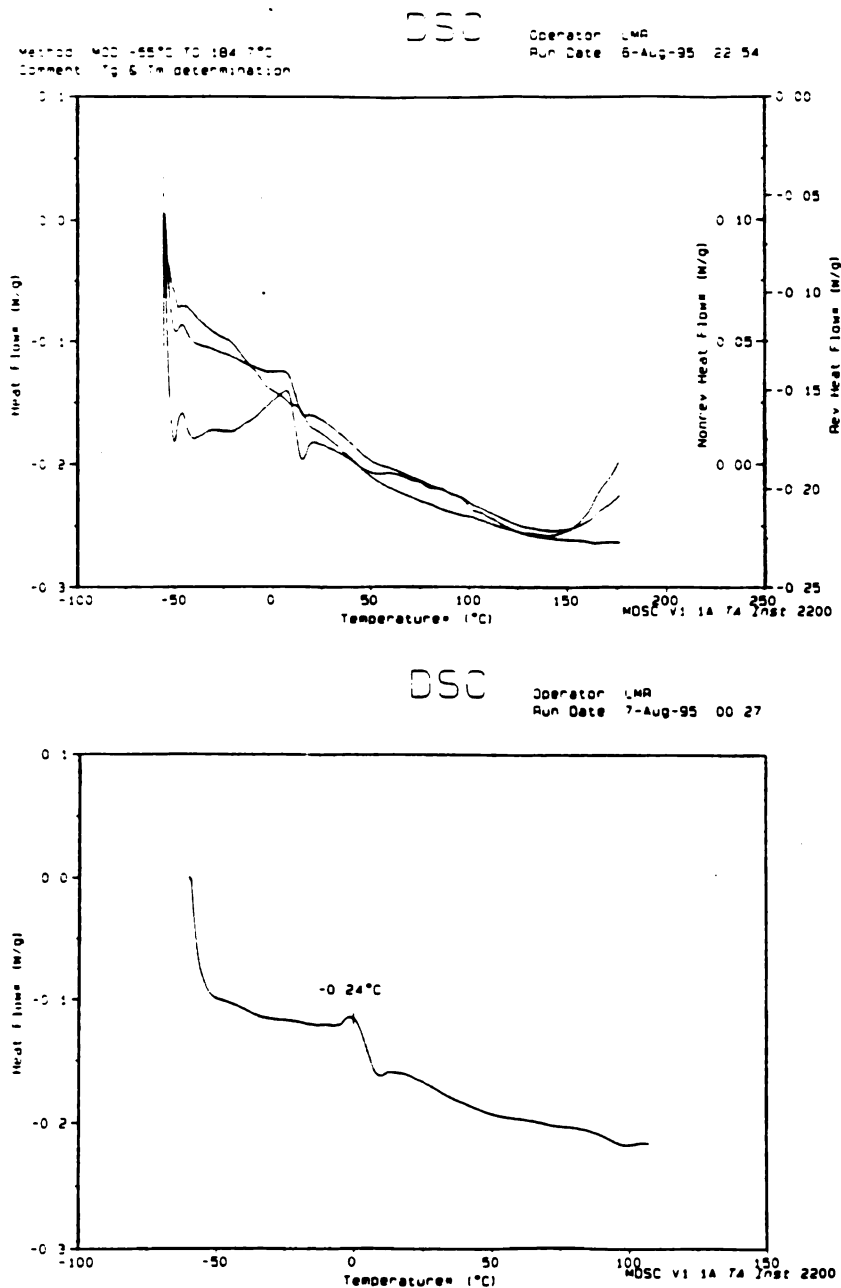


Figure D. MDSC curves for a glutaraldehyde-cross-linked wheat protein film. (1) shows total heat flow, along with reversible (lower line) and non-reversible (upper line) heat flow. (2) shows total heat flow with a thermal change around 0°C attributed to phase transition change of water.

APPENDIX E

Rheological measurement of the FFS in heating-cooling process simulation.

Figure E1 represents the output of the complex modulus (G^*) over a temperature ramp (heating curve by the triangular marker; cooling curve by rounded marker). Test settings for RheoStress® RS-100 were: $\sigma=0.5$ Pa; $\omega=0.100$ Hz; heating temperature ramp= $2^\circ\text{C}/\text{min}$ from 25 to about 80°C (simulating heating of the FFS during the actual process). The solution was immediately cooled down at $2^\circ\text{C}/\text{min}$. This showed an irreversible thermal change of the wheat proteins in the FFS, since G^* relates to the increase of the more elastic behavior provided by the unfolding of the protein molecules.

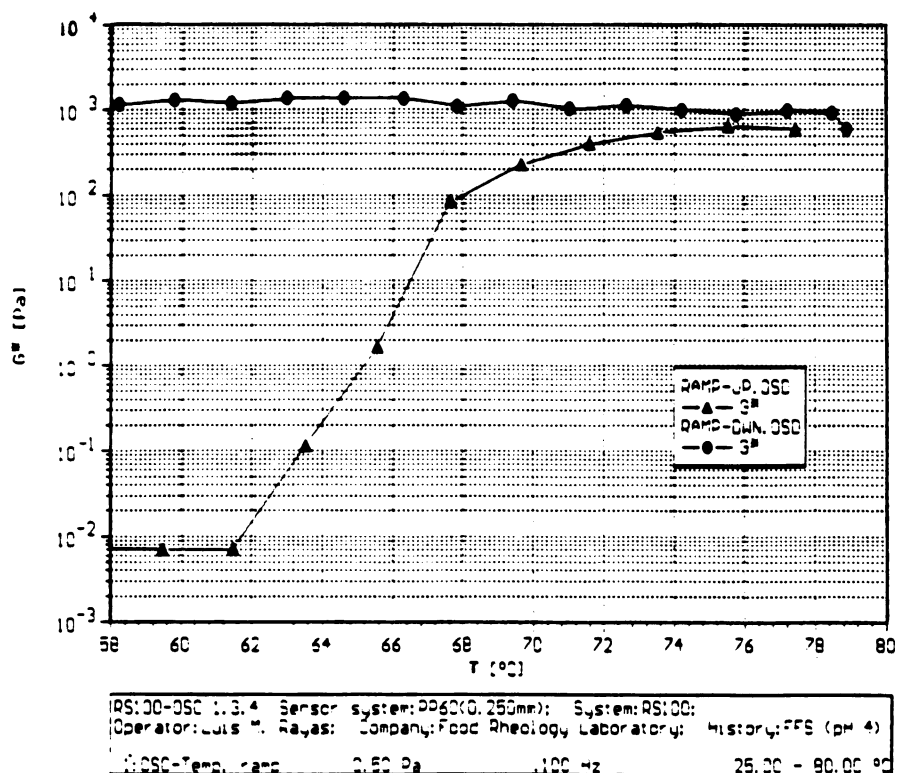


Figure E1. Thermal change of wheat proteins observed in the FFS.

APPENDIX F

The data points of Figures 17-30 were obtained from the raw experimental values of Table F1.

Note: N = non-cross-linked.

G = glutaraldehyde-cross-linked.

F = formaldehyde-cross-linked.

Table F1. Raw data for calculation of creep curves.

5°C; 85 %RH; N								
Time (min)	Time (s)	Distance (cm)	δL (m)	area (m ²)	σ (MPa)	L/Lo	ϵ_h	J (MPa ⁻¹)
0.00	0	8.10	0.00E+00	1.83E-06	2.78689	1.00000	0.00000	0.00000
0.02	1	18.50	1.04E-01	1.15E-06	4.43684	4.15152	1.42347	0.32083
5°C; 85 %RH; G								
Time (min)	Time (s)	Distance (cm)	δL (m)	area (m ²)	σ (MPa)	L/Lo	ϵ_h	J (MPa ⁻¹)
0.00	0	8.20	0.00E+00	2.29E-06	2.22951	1.00000	0.00000	0.00000
0.50	30	20.70	1.25E-01	2.20E-06	2.31219	4.78788	1.56609	0.67732
1.00	60	22.00	1.38E-01	2.20E-06	2.32114	5.18182	1.64516	0.70877
1.50	90	22.80	1.46E-01	2.19E-06	2.32668	5.42424	1.69088	0.72673
2.00	120	23.30	1.51E-01	2.19E-06	2.33016	5.57576	1.71843	0.73747
2.50	150	23.70	1.55E-01	2.18E-06	2.33295	5.69697	1.73993	0.74581
3.00	180	23.90	1.57E-01	2.18E-06	2.33435	5.75758	1.75052	0.74990
3.50	210	24.20	1.60E-01	2.18E-06	2.33645	5.84848	1.76618	0.75593
4.00	240	24.40	1.62E-01	2.18E-06	2.33785	5.90909	1.77649	0.75988
4.50	270	24.50	1.63E-01	2.18E-06	2.33855	5.93939	1.78161	0.76184
5.00	300	24.70	1.65E-01	2.18E-06	2.33995	6.00000	1.79176	0.76572
5.50	330	24.80	1.66E-01	2.18E-06	2.34066	6.03030	1.79680	0.76765
6.00	360	25.00	1.68E-01	2.18E-06	2.34206	6.09091	1.80680	0.77146
6.50	390	25.10	1.69E-01	2.18E-06	2.34277	6.12121	1.81176	0.77334
7.00	420	25.20	1.70E-01	2.17E-06	2.34347	6.15152	1.81670	0.77522
5°C; 85 %RH; F								
Time (min)	Time (s)	Distance (cm)	δL (m)	area (m ²)	σ (MPa)	L/Lo	ϵ_h	J (MPa ⁻¹)
0.00	0	8.10	0.00E+00	2.06E-06	2.47723	1.00000	0.00000	0.00000
0.08	5	16.20	8.10E-02	2.00E-06	2.54270	3.45455	1.23969	0.48755
0.17	10	18.00	9.90E-02	1.99E-06	2.55772	4.00000	1.38629	0.54200
0.25	15	18.70	1.06E-01	1.99E-06	2.56360	4.21212	1.43797	0.56092
0.33	20	19.30	1.12E-01	1.98E-06	2.56867	4.39394	1.48023	0.57626

0.42	25	19.60	1.15E-01	1.98E-06	2.57122	4.48485	1.50070	0.58366
0.50	30	20.00	1.19E-01	1.98E-06	2.57461	4.60606	1.52737	0.59324
0.58	35	20.30	1.22E-01	1.98E-06	2.57717	4.69697	1.54692	0.60024
0.67	40	20.60	1.25E-01	1.98E-06	2.57973	4.78788	1.56609	0.60707
0.75	45	20.80	1.27E-01	1.97E-06	2.58144	4.84848	1.57867	0.61155
0.83	50	20.90	1.28E-01	1.97E-06	2.58229	4.87879	1.58490	0.61376
0.92	55	21.10	1.30E-01	1.97E-06	2.58400	4.93939	1.59724	0.61813
1.00	60	21.30	1.32E-01	1.97E-06	2.58572	5.00000	1.60944	0.62243
1.08	65	21.50	1.34E-01	1.97E-06	2.58743	5.06061	1.62149	0.62668
1.17	70	21.60	1.35E-01	1.97E-06	2.58829	5.09091	1.62746	0.62878
1.25	75	21.80	1.37E-01	1.97E-06	2.59001	5.15152	1.63929	0.63293
1.33	80	21.90	1.38E-01	1.97E-06	2.59087	5.18182	1.64516	0.63498
1.42	85	21.90	1.38E-01	1.97E-06	2.59087	5.18182	1.64516	0.63498

10°C; 40 %RH; N

Time (min)	Time (s)	Distance (cm)	δL (m)	area (m ²)	σ (MPa)	L/Lo	ϵ_h	J (MPa ⁻¹)
0.00	0	8.10	0.00E+00	1.83E-06	2.78692	1.00000	0.00000	0.00000
0.10	6	8.60	5.00E-03	1.80E-06	2.83765	1.15152	0.14108	0.04972
0.20	12	9.10	1.00E-02	1.76E-06	2.89026	1.30303	0.26469	0.09158
0.30	18	9.60	1.50E-02	1.73E-06	2.94487	1.45455	0.37469	0.12724
0.40	24	10.00	1.90E-02	1.70E-06	2.99006	1.57576	0.45474	0.15208
0.50	30	10.50	2.40E-02	1.67E-06	3.04853	1.72727	0.54654	0.17928
0.60	36	11.00	2.90E-02	1.64E-06	3.10934	1.87879	0.63063	0.20282
0.70	42	11.60	3.50E-02	1.60E-06	3.18559	2.06061	0.72300	0.22696

10°C; 40 %RH; G

Time (min)	Time (s)	Distance (cm)	δL (m)	area (m ²)	σ (MPa)	L/Lo	ϵ_h	J (MPa ⁻¹)
0.00	0	7.90	0.00E+00	2.51E-06	2.02685	1.00000	0.00000	0.00000
5.00	300	8.90	1.00E-02	2.45E-06	2.08096	1.30303	0.26469	0.12720
10.00	600	9.80	1.90E-02	2.39E-06	2.13220	1.57576	0.45474	0.21327
15.00	900	10.50	2.60E-02	2.34E-06	2.17383	1.78788	0.58103	0.26728
20.00	1200	11.20	3.30E-02	2.30E-06	2.21711	2.00000	0.69315	0.31263
25.00	1500	11.70	3.80E-02	2.27E-06	2.24910	2.15152	0.76617	0.34066
30.00	1800	12.10	4.20E-02	2.24E-06	2.27537	2.27273	0.82098	0.36081
35.00	2100	12.50	4.60E-02	2.21E-06	2.30225	2.39394	0.87294	0.37917
40.00	2400	12.80	4.90E-02	2.19E-06	2.32284	2.48485	0.91021	0.39185
45.00	2700	13.10	5.20E-02	2.17E-06	2.34379	2.57576	0.94614	0.40368
50.00	3000	13.40	5.50E-02	2.15E-06	2.36513	2.66667	0.98083	0.41470
55.00	3300	13.60	5.70E-02	2.14E-06	2.37957	2.72727	1.00330	0.42163
60.00	3600	13.80	5.90E-02	2.13E-06	2.39419	2.78788	1.02528	0.42824
65.00	3900	13.90	6.00E-02	2.12E-06	2.40157	2.81818	1.03609	0.43142
70.00	4200	14.10	6.20E-02	2.11E-06	2.41646	2.87879	1.05737	0.43757

75.00	4500	14.30	6.40E-02	2.10E-06	2.43154	2.93939	1.07820	0.44342
80.00	4800	14.50	6.60E-02	2.08E-06	2.44680	3.00000	1.09861	0.44900
85.00	5100	14.60	6.70E-02	2.08E-06	2.45451	3.03030	1.10866	0.45168
90.00	5400	14.70	6.80E-02	2.07E-06	2.46226	3.06061	1.11861	0.45430

10 °C; 40 %RH; F

Time (min)	Time (s)	Distance (cm)	δL (m)	area (m ²)	σ (MPa)	L/Lo	ϵ_h	J (MPa ⁻¹)
0.00	0	7.90	0.00E+00	2.13E-06	2.38879	1.00000	0.00000	0.00000
5.00	300	8.30	4.00E-03	2.11E-06	2.41843	1.12121	0.11441	0.04731
10.00	600	8.70	8.00E-03	2.08E-06	2.44883	1.24242	0.21706	0.08864
15.00	900	9.20	1.30E-02	2.05E-06	2.48791	1.39394	0.33213	0.13350
20.00	1200	9.50	1.60E-02	2.03E-06	2.51197	1.48485	0.39531	0.15737
25.00	1500	9.90	2.00E-02	2.00E-06	2.54478	1.60606	0.47378	0.18618
30.00	1800	10.20	2.30E-02	1.98E-06	2.56995	1.69697	0.52884	0.20578
35.00	2100	10.40	2.50E-02	1.97E-06	2.58701	1.75758	0.56394	0.21799
40.00	2400	10.70	2.80E-02	1.95E-06	2.61303	1.84848	0.61437	0.23512
45.00	2700	10.90	3.00E-02	1.94E-06	2.63067	1.90909	0.64663	0.24580
50.00	3000	11.10	3.20E-02	1.92E-06	2.64855	1.96970	0.67788	0.25594
55.00	3300	11.30	3.40E-02	1.91E-06	2.66667	2.03030	0.70819	0.26557
60.00	3600	11.40	3.50E-02	1.90E-06	2.67583	2.06061	0.72300	0.27020
65.00	3900	11.60	3.70E-02	1.89E-06	2.69433	2.12121	0.75199	0.27910
70.00	4200	11.70	3.80E-02	1.89E-06	2.70367	2.15152	0.76617	0.28338
75.00	4500	11.80	3.90E-02	1.88E-06	2.71308	2.18182	0.78016	0.28755
80.00	4800	11.90	4.00E-02	1.87E-06	2.72256	2.21212	0.79395	0.29162
85.00	5100	12.10	4.20E-02	1.86E-06	2.74172	2.27273	0.82098	0.29944
90.00	5400	12.20	4.30E-02	1.85E-06	2.75139	2.30303	0.83423	0.30320

20°C; 35 %RH; N

Time (min)	Time (s)	Distance (cm)	δL (m)	area (m ²)	σ (MPa)	L/Lo	ϵ_h	J (MPa ⁻¹)
0.00	0	8.20	0.00E+00	1.83E-06	2.78689	1.00000	0.00000	0.00000
0.03	2	8.50	3.00E-03	1.81E-06	2.81711	1.09091	0.08701	0.03089
0.07	4	8.70	5.00E-03	1.80E-06	2.83762	1.15152	0.14108	0.04972
0.10	6	9.00	8.00E-03	1.78E-06	2.86896	1.24242	0.21706	0.07566
0.13	8	9.20	1.00E-02	1.76E-06	2.89023	1.30303	0.26469	0.09158
0.17	10	9.60	1.40E-02	1.74E-06	2.93375	1.42424	0.35364	0.12054

20°C; 35 %RH; G

Time (min)	Time (s)	Distance (cm)	δL (m)	area (m ²)	σ (MPa)	L/Lo	ϵ_h	J (MPa ⁻¹)
0.00	0	8.00	0.00E+00	2.29E-06	2.22951	1.00000	0.00000	0.00000
10.00	600	8.10	1.00E-03	2.28E-06	2.23591	1.03030	0.02985	0.01335
20.00	1200	8.15	1.50E-03	2.28E-06	2.23912	1.04545	0.04445	0.01985
30.00	1800	8.20	2.00E-03	2.27E-06	2.24234	1.06061	0.05884	0.02624

40.00	2400	8.20	2.00E-03	2.27E-06	2.24234	1.06061	0.05884	0.02624
50.00	3000	8.25	2.50E-03	2.27E-06	2.24557	1.07576	0.07303	0.03252
60.00	3600	8.30	3.00E-03	2.27E-06	2.24881	1.09091	0.08701	0.03869
70.00	4200	8.35	3.50E-03	2.26E-06	2.25206	1.10606	0.10080	0.04476
80.00	4800	8.40	4.00E-03	2.26E-06	2.25532	1.12121	0.11441	0.05073
90.00	5400	8.40	4.00E-03	2.26E-06	2.25532	1.12121	0.11441	0.05073
100.00	6000	8.45	4.50E-03	2.26E-06	2.25858	1.13636	0.12783	0.05660
110.00	6600	8.45	4.50E-03	2.26E-06	2.25858	1.13636	0.12783	0.05660
120.00	7200	8.50	5.00E-03	2.25E-06	2.26186	1.15152	0.14108	0.06237

20°C; 35 %RH; F

Time (min)	Time (s)	Distance (cm)	δL (m)	area (m ²)	σ (MPa)	L/Lo	ϵ_h	J (MPa ⁻¹)
0.00	0	8.10	0.00E+00	2.06E-06	2.47723	1.00000	0.00000	0.00000
10.00	600	8.30	2.00E-03	2.04E-06	2.49308	1.06061	0.05884	0.02360
20.00	1200	8.50	4.00E-03	2.03E-06	2.50913	1.12121	0.11441	0.04560
30.00	1800	8.65	5.50E-03	2.02E-06	2.52131	1.16667	0.15415	0.06114
40.00	2400	8.75	6.50E-03	2.01E-06	2.52949	1.19697	0.17979	0.07108
50.00	3000	8.90	8.00E-03	2.01E-06	2.54187	1.24242	0.21706	0.08540
60.00	3600	8.95	8.50E-03	2.00E-06	2.54602	1.25758	0.22919	0.09002
70.00	4200	9.00	9.00E-03	2.00E-06	2.55018	1.27273	0.24116	0.09457
80.00	4800	9.10	1.00E-02	1.99E-06	2.55856	1.30303	0.26469	0.10345
90.00	5400	9.15	1.05E-02	1.99E-06	2.56276	1.31818	0.27625	0.10780
100.00	6000	9.20	1.10E-02	1.99E-06	2.56698	1.33333	0.28768	0.11207
110.00	6600	9.20	1.10E-02	1.99E-06	2.56698	1.33333	0.28768	0.11207
120.00	7200	9.25	1.15E-02	1.98E-06	2.57122	1.34848	0.29898	0.11628

20°C; 55 %RH; N

Time (min)	Time (s)	Distance (cm)	δL (m)	area (m ²)	σ (MPa)	L/Lo	ϵ_h	J (MPa ⁻¹)
0.00	0	8.00	0.00E+00	1.83E-06	2.78689	1.00000	0.00000	0.00000
0.02	1	12.50	4.50E-02	1.53E-06	3.32131	2.36364	0.86020	0.25899
0.03	2	18.50	1.05E-01	1.14E-06	4.46224	4.18182	1.43075	0.32063

20°C; 55 %RH; G

Time (min)	Time (s)	Distance (cm)	δL (m)	area (m ²)	σ (MPa)	L/Lo	ϵ_h	J (MPa ⁻¹)
0.00	0	8.20	0.00E+00	2.29E-06	2.22951	1.00000	0.00000	0.00000
5.00	300	10.50	2.30E-02	2.14E-06	2.38653	1.69697	0.52884	0.22160
10.00	600	11.50	3.30E-02	2.07E-06	2.46191	2.00000	0.69315	0.28155
15.00	900	12.20	4.00E-02	2.02E-06	2.51758	2.21212	0.79395	0.31536
20.00	1200	12.60	4.40E-02	2.00E-06	2.55053	2.33333	0.84730	0.33220
25.00	1500	13.00	4.80E-02	1.97E-06	2.58436	2.45455	0.89794	0.34745
30.00	1800	13.40	5.20E-02	1.95E-06	2.61910	2.57576	0.94614	0.36125
35.00	2100	13.60	5.40E-02	1.93E-06	2.63682	2.63636	0.96940	0.36764

40.00	2400	13.80	5.60E-02	1.92E-06	2.65479	2.69697	0.99213	0.37371
45.00	2700	13.90	5.70E-02	1.91E-06	2.66386	2.72727	1.00330	0.37663
50.00	3000	14.20	6.00E-02	1.89E-06	2.69146	2.81818	1.03609	0.38496
55.00	3300	14.40	6.20E-02	1.88E-06	2.71018	2.87879	1.05737	0.39015
60.00	3600	14.50	6.30E-02	1.87E-06	2.71963	2.90909	1.06784	0.39264
65.00	3900	14.60	6.40E-02	1.87E-06	2.72916	2.93939	1.07820	0.39507
70.00	4200	14.70	6.50E-02	1.86E-06	2.73875	2.96970	1.08846	0.39743
75.00	4500	14.80	6.60E-02	1.85E-06	2.74840	3.00000	1.09861	0.39973
80.00	4800	14.80	6.60E-02	1.85E-06	2.74840	3.00000	1.09861	0.39973
85.00	5100	14.90	6.70E-02	1.85E-06	2.75813	3.03030	1.10866	0.40196
90.00	5400	15.00	6.80E-02	1.84E-06	2.76792	3.06061	1.11861	0.40413
95.00	5700	15.00	6.80E-02	1.84E-06	2.76792	3.06061	1.11861	0.40413
100.00	6000	15.05	6.85E-02	1.84E-06	2.77285	3.07576	1.12355	0.40520
105.00	6300	15.10	6.90E-02	1.83E-06	2.77779	3.09091	1.12847	0.40625
110.00	6600	15.15	6.95E-02	1.83E-06	2.78275	3.10606	1.13336	0.40728
115.00	6900	15.15	6.95E-02	1.83E-06	2.78275	3.10606	1.13336	0.40728
120.00	7200	15.20	7.00E-02	1.83E-06	2.78773	3.12121	1.13822	0.40830

20°C; 55 %RH; F

Time (min)	Time (s)	Distance (cm)	δL (m)	area (m ²)	σ (MPa)	L/Lo	ϵ_h	J (MPa ⁻¹)
0.00	0	8.20	0.00E+00	2.06E-06	2.47723	1.00000	0.00000	0.00000
5.00	300	10.70	2.50E-02	1.89E-06	2.69107	1.75758	0.56394	0.20956
10.00	600	11.70	3.50E-02	1.83E-06	2.78731	2.06061	0.72300	0.25939
15.00	900	12.40	4.20E-02	1.78E-06	2.85887	2.27273	0.82098	0.28717
20.00	1200	12.90	4.70E-02	1.75E-06	2.91229	2.42424	0.88552	0.30406
25.00	1500	13.30	5.10E-02	1.72E-06	2.95648	2.54545	0.93431	0.31602
30.00	1800	13.60	5.40E-02	1.70E-06	2.99051	2.63636	0.96940	0.32416
35.00	2100	13.80	5.60E-02	1.69E-06	3.01363	2.69697	0.99213	0.32921
40.00	2400	14.00	5.80E-02	1.68E-06	3.03712	2.75758	1.01435	0.33398
45.00	2700	14.30	6.10E-02	1.66E-06	3.07305	2.84848	1.04679	0.34063
50.00	3000	14.50	6.30E-02	1.65E-06	3.09747	2.90909	1.06784	0.34475
55.00	3300	14.60	6.40E-02	1.64E-06	3.10983	2.93939	1.07820	0.34671
60.00	3600	14.70	6.50E-02	1.63E-06	3.12229	2.96970	1.08846	0.34861
65.00	3900	14.80	6.60E-02	1.63E-06	3.13485	3.00000	1.09861	0.35045
70.00	4200	14.90	6.70E-02	1.62E-06	3.14751	3.03030	1.10866	0.35224
75.00	4500	15.00	6.80E-02	1.61E-06	3.16027	3.06061	1.11861	0.35396
80.00	4800	15.10	6.90E-02	1.61E-06	3.17314	3.09091	1.12847	0.35563
85.00	5100	15.15	6.95E-02	1.60E-06	3.17961	3.10606	1.13336	0.35644
90.00	5400	15.20	7.00E-02	1.60E-06	3.18611	3.12121	1.13822	0.35725
95.00	5700	15.25	7.05E-02	1.60E-06	3.19263	3.13636	1.14306	0.35803
100.00	6000	15.35	7.15E-02	1.59E-06	3.20577	3.16667	1.15268	0.35956
105.00	6300	15.35	7.15E-02	1.59E-06	3.20577	3.16667	1.15268	0.35956
110.00	6600	15.35	7.15E-02	1.59E-06	3.20577	3.16667	1.15268	0.35956

115.00	6900	15.40	7.20E-02	1.59E-06	3.21237	3.18182	1.15745	0.36031
120.00	7200	15.40	7.20E-02	1.59E-06	3.21237	3.18182	1.15745	0.36031

25°C; 25 %RH; N

Time (min)	Time (s)	Distance (cm)	δL (m)	area (m ²)	σ (MPa)	L/Lo	ϵ_h	J (MPa ⁻¹)
0.00	0	8.20	0.00E+00	1.98E-06	2.57251	1.00000	0.00000	0.00000
0.03	2	10.20	2.00E-02	1.85E-06	2.75433	1.60606	0.47378	0.17201
0.07	4	12.20	4.00E-02	1.72E-06	2.96381	2.21212	0.79395	0.26788
0.10	6	14.20	6.00E-02	1.59E-06	3.20778	2.81818	1.03609	0.32299
0.13	8	16.10	7.90E-02	1.46E-06	3.47991	3.39394	1.22199	0.35116
0.17	10	17.30	9.10E-02	1.39E-06	3.67691	3.75758	1.32377	0.36002
0.20	12	18.20	1.00E-01	1.33E-06	3.83995	4.03030	1.39384	0.36298

25°C; 25 %RH; G

Time (min)	Time (s)	Distance (cm)	δL (m)	area (m ²)	σ (MPa)	L/Lo	ϵ_h	J (MPa ⁻¹)
0.00	0	8.20	0.00E+00	1.37E-06	3.71585	1.00000	0.00000	0.00000
0.17	10	8.80	6.00E-03	1.33E-06	3.82528	1.18182	0.16705	0.04367
0.33	20	9.40	1.20E-02	1.29E-06	3.94134	1.36364	0.31015	0.07869
0.50	30	9.80	1.60E-02	1.27E-06	4.02271	1.48485	0.39531	0.09827
0.67	40	10.50	2.30E-02	1.22E-06	4.17350	1.69697	0.52884	0.12671
0.83	50	10.90	2.70E-02	1.20E-06	4.26485	1.81818	0.59784	0.14018
1.00	60	11.50	3.30E-02	1.16E-06	4.40962	2.00000	0.69315	0.15719
1.17	70	12.00	3.80E-02	1.12E-06	4.53800	2.15152	0.76617	0.16883
1.33	80	12.50	4.30E-02	1.09E-06	4.67407	2.30303	0.83423	0.17848
1.50	90	12.90	4.70E-02	1.06E-06	4.78895	2.42424	0.88552	0.18491
1.67	100	13.30	5.10E-02	1.04E-06	4.90962	2.54545	0.93431	0.19030
1.83	110	13.60	5.40E-02	1.02E-06	5.00419	2.63636	0.96940	0.19372
2.00	120	13.90	5.70E-02	9.99E-07	5.10247	2.72727	1.00330	0.19663
2.17	130	14.20	6.00E-02	9.79E-07	5.20469	2.81818	1.03609	0.19907
2.33	140	14.50	6.30E-02	9.60E-07	5.31109	2.90909	1.06784	0.20106
2.50	150	14.70	6.50E-02	9.47E-07	5.38448	2.96970	1.08846	0.20215
2.67	160	14.90	6.70E-02	9.33E-07	5.45992	3.03030	1.10866	0.20305
2.83	170	15.10	6.90E-02	9.20E-07	5.53750	3.09091	1.12847	0.20379
3.00	180	15.30	7.10E-02	9.07E-07	5.61732	3.15152	1.14788	0.20435
3.17	190	15.50	7.30E-02	8.94E-07	5.69948	3.21212	1.16693	0.20474
3.33	200	15.70	7.50E-02	8.81E-07	5.78407	3.27273	1.18562	0.20498
3.50	210	15.90	7.70E-02	8.68E-07	5.87122	3.33333	1.20397	0.20506
3.67	220	16.00	7.80E-02	8.62E-07	5.91578	3.36364	1.21302	0.20505

25°C; 25 %RH; F

Time (min)	Time (s)	Distance (cm)	δL (m)	area (m ²)	σ (MPa)	L/Lo	ϵ_h	J (MPa ⁻¹)
0.00	0	8.20	0.00E+00	1.68E-06	3.04027	1.00000	0.00000	0.00000

1.00	60	9.70	1.50E-02	1.58E-06	3.22922	1.45455	0.37469	0.11603
2.00	120	10.70	2.50E-02	1.51E-06	3.36880	1.75758	0.56394	0.16740
3.00	180	11.50	3.30E-02	1.46E-06	3.48946	2.00000	0.69315	0.19864
4.00	240	12.10	3.90E-02	1.42E-06	3.58578	2.18182	0.78016	0.21757
5.00	300	12.50	4.30E-02	1.40E-06	3.65300	2.30303	0.83423	0.22837
6.00	360	12.90	4.70E-02	1.37E-06	3.72280	2.42424	0.88552	0.23786
7.00	420	13.20	5.00E-02	1.35E-06	3.77692	2.51515	0.92233	0.24420
8.00	480	13.50	5.30E-02	1.33E-06	3.83264	2.60606	0.95784	0.24992
9.00	540	13.80	5.60E-02	1.31E-06	3.89002	2.69697	0.99213	0.25504
10.00	600	14.00	5.80E-02	1.30E-06	3.92925	2.75758	1.01435	0.25815
11.00	660	14.20	6.00E-02	1.28E-06	3.96927	2.81818	1.03609	0.26103
12.00	720	14.40	6.20E-02	1.27E-06	4.01011	2.87879	1.05737	0.26368
13.00	780	14.50	6.30E-02	1.26E-06	4.03085	2.90909	1.06784	0.26492
14.00	840	14.70	6.50E-02	1.25E-06	4.07298	2.96970	1.08846	0.26724
15.00	900	14.90	6.70E-02	1.24E-06	4.11600	3.03030	1.10866	0.26935

25°C; 30 %RH; N

Time (min)	Time (sec)	Distance (cm)	δL (m)	area (m ²)	σ (MPa)	L/Lo	ϵ_h	J (MPa ⁻¹)
0.00	0	8.20	0.00E+00	1.37E-06	3.71585	1.00000	0.00000	0.00000
0.03	2	9.30	1.10E-02	1.30E-06	3.92151	1.33333	0.28768	0.07336
0.07	4	10.20	2.00E-02	1.24E-06	4.10751	1.60606	0.47378	0.11535

25°C; 30 %RH; G

Time (min)	Time (s)	Distance (cm)	δL (m)	area (m ²)	σ (MPa)	L/Lo	ϵ_h	J (MPa ⁻¹)
0.00	0	8.50	0.00E+00	1.37E-06	3.71585	1.00000	0.00000	0.00000
0.17	10	10.50	2.00E-02	1.24E-06	4.10751	1.60606	0.47378	0.11535
0.33	20	13.50	5.00E-02	1.04E-06	4.87889	2.51515	0.92233	0.18905
0.50	30	14.80	6.30E-02	9.60E-07	5.31109	2.90909	1.06784	0.20106
0.67	40	15.70	7.20E-02	9.01E-07	5.65810	3.18182	1.15745	0.20457
0.83	50	16.20	7.70E-02	8.68E-07	5.87122	3.33333	1.20397	0.20506
1.00	60	16.70	8.20E-02	8.35E-07	6.10101	3.48485	1.24842	0.20464
1.17	70	17.20	8.70E-02	8.03E-07	6.34953	3.63636	1.29098	0.20334

25°C; 30 %RH; F

Time (min)	Time (s)	Distance (cm)	δL (m)	area (m ²)	σ (MPa)	L/Lo	ϵ_h	J (MPa ⁻¹)
0.00	0	8.50	0.00E+00	1.52E-06	3.34427	1.00000	0.00000	0.00000
0.33	20	10.30	1.80E-02	1.41E-06	3.62418	1.54545	0.43532	0.12011
0.67	40	11.40	2.90E-02	1.33E-06	3.81955	1.87879	0.63063	0.16510
1.00	60	12.10	3.60E-02	1.29E-06	3.95523	2.09091	0.73760	0.18649
1.33	80	12.70	4.20E-02	1.25E-06	4.07945	2.27273	0.82098	0.20125
1.67	100	13.10	4.60E-02	1.22E-06	4.16668	2.39394	0.87294	0.20950
2.00	120	13.50	5.00E-02	1.20E-06	4.25773	2.51515	0.92233	0.21663

2.33	140	13.80	5.30E-02	1.18E-06	4.32867	2.60606	0.95784	0.22128
2.67	160	14.10	5.60E-02	1.16E-06	4.40202	2.69697	0.99213	0.22538
3.00	180	14.30	5.80E-02	1.14E-06	4.45231	2.75758	1.01435	0.22783
3.33	200	14.50	6.00E-02	1.13E-06	4.50377	2.81818	1.03609	0.23005
3.67	220	14.70	6.20E-02	1.12E-06	4.55643	2.87879	1.05737	0.23206
4.00	240	14.90	6.40E-02	1.11E-06	4.61033	2.93939	1.07820	0.23387
4.33	260	15.00	6.50E-02	1.10E-06	4.63777	2.96970	1.08846	0.23469
4.67	280	15.20	6.70E-02	1.09E-06	4.69363	3.03030	1.10866	0.23621
5.00	300	15.30	6.80E-02	1.08E-06	4.72206	3.06061	1.11861	0.23689
5.33	320	15.50	7.00E-02	1.07E-06	4.77998	3.12121	1.13822	0.23812
5.67	340	15.60	7.10E-02	1.06E-06	4.80948	3.15152	1.14788	0.23867
6.00	360	15.70	7.20E-02	1.05E-06	4.83934	3.18182	1.15745	0.23918

40°C; 25 %RH; N

Time (min)	Time (sec)	Distance (cm)	δL (m)	area (m ²)	σ (MPa)	L/Lo	ϵ_h	J (MPa ⁻¹)
0.00	0	8.20	0.00E+00	1.60E-06	3.18501	1.00000	0.00000	0.00000
0.03	2	10.20	2.00E-02	1.47E-06	3.46850	1.60606	0.47378	0.13660
0.07	4	12.80	4.60E-02	1.30E-06	3.92234	2.39394	0.87294	0.22256

40°C; 25 %RH; G

Time (min)	Time (s)	Distance (cm)	δL (m)	area (m ²)	σ (MPa)	L/Lo	ϵ_h	J (MPa ⁻¹)
0.00	0	8.20	0.00E+00	1.60E-06	3.18501	1.00000	0.00000	0.00000
0.17	10	9.00	8.00E-03	1.55E-06	3.29266	1.24242	0.21706	0.06592
0.33	20	9.80	1.60E-02	1.50E-06	3.40783	1.48485	0.39531	0.11600
0.50	30	10.70	2.50E-02	1.44E-06	3.54743	1.75758	0.56394	0.15897
0.67	40	11.30	3.10E-02	1.40E-06	3.64703	1.93939	0.66238	0.18162
0.83	50	12.00	3.80E-02	1.35E-06	3.77054	2.15152	0.76617	0.20320

40°C; 25 %RH; F

Time (min)	Time (s)	Distance (cm)	δL (m)	area (m ²)	σ (MPa)	L/Lo	ϵ_h	J (MPa ⁻¹)
0.00	0	8.20	0.00E+00	2.44E-06	2.09017	1.00000	0.00000	0.00000
0.17	10	16.50	8.30E-02	1.90E-06	2.68862	3.51515	1.25708	0.46756
0.33	20	18.50	1.03E-01	1.76E-06	2.88786	4.12121	1.41615	0.49038
0.50	30	19.50	1.13E-01	1.70E-06	2.99899	4.42424	1.48710	0.49587
0.67	40	20.20	1.20E-01	1.65E-06	3.08200	4.63636	1.53393	0.49771
0.83	50	20.90	1.27E-01	1.61E-06	3.16974	4.84848	1.57867	0.49804
1.00	60	21.30	1.31E-01	1.58E-06	3.22216	4.96970	1.60336	0.49760
1.17	70	21.70	1.35E-01	1.56E-06	3.27634	5.09091	1.62746	0.49673

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