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Rheological studies of the kinetics of gelation.

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RHEOLOGICAL STUDIES OF THE KINETICS OF GELATION

A Thesis Presented

By

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DECEMBER 1973

DEDICATION

To The One Creator Who Created This Creation

To my parents, for their never ending love and confidence

To Professor Middleman, for his guidance and understanding

EK ONG KAR SAT NAM SIRI WHA GURU

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ABSTRACT

The rheological characteristics of the kinetics of gelation of a single extraction calf skin derived gelatin were studied as a function of shear rate, shear rate history, concentration, pH, and temperature. Kinetic as well as molecular interpretations are provided. Changes in any of these variables alters the rate as well as the structure formed during gelation under shear.

Optical rotatory dispersion studies failed to indicate an increase in the chain helix content during gelation, however, it is suspected that interference may be masking this rotation.

I. INTRODUCTION

The complex process of gelatin gel formation has been widely studied under varied conditions. However, a great deal of uncertainty still plagues the research being done on gelatin, principally due to the inherent complexity of the collagen-gelatin system. An area of research which has not been extensively studied is that of gelatin network formation under shear. Here rheology offers an effective tool for observing and classifying structural changes which occur during the time dependent gelation process.

Several levels of structural complexity have to be considered when dealing with the protein gelatin: the order or sequence of the amino acids in the long polypeptide chain (primary structure), the orderly twisting or bending of the chain (secondary structure), the compacting and folding back of secondary structure (tertiary structure), and finally the formation of larger and more complex units between two or more polypeptide chains (quaternary structure). While the primary structure is examined chiefly by chemical methods, physical methods, including rheology and optical rotatory dispersion, play a decisive role in the study of the higher order structures.[17] During gelatin network formation in solution, these interactions as well as other participating mechanisms occur, all of which can be influenced by shear. In this study the several principal molecular interactions studied would include: single chain helical coil formation, helical winding of two or more molecules, chain segment-water molecule interactions, and interchain interactions to form cross linked networks.

Some of the important rheological variables involved in determining the nature of the thixotrope gelatin are shear stress, shear strain, shear rate, rate of change of shear stress, and rate of change of shear rate. This study evaluates the relationship of gelatin viscosity and rate of change of viscosity to changes in time, shear rate, shear rate history, concentration, pH, and temperature. These relationships are considered

from both a rheological as well as molecular level of understanding; an understanding based on the many theories and observations of the previous research done in this area, as well as from observations of this study.

More specifically, the following questions were considered in this work:

(a). How are the gelation kinetics affected by the thermodynamic, hydrodynamic, and physical parameters such as temperature, shear rate, pH, concentration, etc.?

(b). Can kinetic changes from (a) be interpreted qualitatively from a mechanistic-structural view point, based on what is reported or what can be extrapolated from the literature?

(c). Will rest (zero shear) and/or shear rate history influence the gelation kinetics and structure formed during gelation?

It was assumed that little primary gelatin chain degradation occurs during shearing. This is a valid assumption since low temperatures were used, the gelatin was prepared in dilute solution which resulted in low viscosities, and relatively low rates of shear were used for measurements.

II. CHEMISTRY OF GELATIN

In order to understand gelatin, one must first have some knowledge of the parent substance collagen. Collagen is the most important constituent of skin, tendon, connective tissue, and most of the organic portion of bone.

The basic collagen macro-molecular unit appears to be a three chain helical rod like structure (3000 Å x 14 Å) in which the individual chains are wound in a super helix. Chemical and physical analyses have shown that these fibers or chains are polymers of a number of different amino acid residues. Table II-1 lists typical amino acid compositions for a calf skin derived gelatin. Collagen owes its distinctive structure to its high content of of the cyclic imino acids proline and hydroxyproline, along with the non-polar amino acids with short chains, glycine and alanine. These acidic, along with other basic, dibasic, or dicarboxylic functional groups are distributed nonuniformly along the collagen chains. The triple helical structure is stabilized in part by the formation of hydrogen bonds between the NH groups on the backbone of one chain and a C=O group on the backbone of a neighboring chain. The existence of either one, two, or three hydrogen bonds per three amino acid residues has been reported.[21]

Gelatin is the water soluble product derived from the parent collagen by any of a number of procedures usually involving the dissolution, disorganization, or degradation of the primary, secondary, tertiary, and quaternary structure of collagen (denaturation). During the gelatin forming process, the coils comprising the collagen are split apart, both laterally and longitudinally, into separate strands (α gelatin) and groups of strands (β and γ gelatin). In general the degradation is not completely random and most gelatins are not homogeneous with respect to molecular weight or weight distribution.

Two processes[36] are used commercially for obtaining gelatin from collagen; the alkaline process, in which the collagen receives a lengthy low temperature pretreatment with mild alkali before being extracted with warm water at a pH near neutrality, and the acid process, in which little or no pre-

TABLE II-1

AMINO ACID COMPOSITION OF ALKALINE EXTRACTED CALF SKIN GELATIN[6]

	Percent (%)
Alanine	9.3 - 11.0
Arginine	8.5 - 8.8
Aspartic Acid	6.6 - 6.9
Cystine	trace
Glycine	26.9 - 27.5
Glutamic Acid	11.1 - 11.4
Histidine	0.74 - 0.78
Hydroxylysine	0.91 - 1.2
Hydrxyproline	14.0 - 14.5
Isoleucine	1.7 - 1.8
Leucine	3.1 - 3.4
Lysine	4.5 - 4.6
Methionine	0.8 - 0.9
Phenylalanine	2.2 - 2.5
Proline	14.8 - 16.4
Serine	3.2 - 4.2
Threonine	2.2
Tyrosine	0.2 - 1.0
Valine	2.6 - 3.4

treatment is used other than that necessary to give the raw material an acid pH (3.5 to 4.0), and extraction with warm water takes place at this pH.

Analyses reveal that the reactive amino acid residues vary in number between the acid and alkaline derived gelatins. This variation in chemical structure produces different characteristics and properties in the two types of gelatin. An important difference in structure is described by the physical properties known as isoelectric and isoionic points. The isoelectric point is the pH of a buffer in which an electric current produces no migration, i.e., the net charge on the gelatin is zero. Isoionic point is the pH of isoionic gelatin in water. Isoionic gelatin is material that contains no noncolloidal ions other than hydrogen or hydroxyl. Generally the isoionic and isoelectric points of acid processed gelatin lie within a pH of 7.0 to 9.0 and within a pH of 4.7 to 5.1 for alkaline processed gelatin.[30]

Veis and Cohen[32] have reported on the molecular configuration of commercial acid and alkaline extracted gelatin with molecular weights near 3 x 10^5. They have suggested: (1) that the acid precursor gelatins are more compact structures than the alkaline precursor gelatins; (2) that the configuration of the acid-gelatin is less readily altered by environmental conditions; and (3) that differences may arise because the peptide chains in the acid-gelatin structure are arranged so that the internal charge compensation is great, i.e., many of the acidic and basic functional grcups must be constrained, by interchain cross-linkages, to reside in nearby regions within the molecular domain of the random gelatin network.

Due to the absence of appreciable internal order, gelatin chains take up a random configuration in aqueous solutions at high temperatures. Polyelectolytic characteristics are confered on the gelatin chains by the acidic and basic functional groups, governing to some extent the interactions between gelatin molecules and water. These characteristics are altered considerably by changes in any number of factors such as pH[15],

temperature, ionic structure, or aging resulting in marked changes in hydrodynamic properties, thermodynamic properties[14,24,26] , light scattering characteristics, etc..[30]

III. MOLECULAR INTERPRETATION OF GEL FORMATION

Although the occurence of network gel formation in gelatin solutions has long been recognized, it still remains a poorly understood phenomenon. For the most part gelatin characterization studies have been done in undisturbed systems; the structural changes and kinetic changes which occur during gelation under shear have not been extensively studied. However, to provide a background for understanding the molecular processes which may be invoked during shearing, some of the classic and contemporary theories and studies dealing with collagen fold formation and gelation will be summarized.

The one indispensable event in gelation is the development of the collagen fold involving portions of each gelatin molecule.[30] Collagen fold formation is that process in which peptide chain segments of uncertain and probably varying length, assume a stable helical configuration similiar to the helical configuration of the peptide chains in native collagen.

Harrington and Von Hippel[16] suggest that the collagen fold is developed along single peptide strands which then attain, through the cooperative hydrogen bonding of water bridges at every available site on the backbone, a stability and finite life. Crystallites or compound helices are then formed by a slower process involving the release of some of the backbone water to form hydrogen bonds between peptide strands.

Flory and Weaver[12] however propose that the single strand helix intermediate, formed by the locking in of the poly-L-proline II segments, would have only a transitory existence. The collagen fold becomes stabilized only when favorably situated intermediates, created by statistical mechanical configurational fluctuations, interact rapidly to form intersegment hydrogen bonds.

These studies assume gelatin concentrations which favor fold formation. Network gelation mechanisms favored at higher concentrations would be considerably more complex. Veis and Schnell[35] suggest that the first

step in the process involves the interaction of chain segments via hydrogen bonding to form aggregates, followed by collagen fold formation within the aggregates.

Coopes[8] feels that single chain helices cannot exist as stable entites, but require interaction with other chain segments from either the same or different molecules. The more stable crosslinks would consist of lengths of chain in the collagen fold formation stabilized by interchain hydrogen bonds. Helical structure would grow slowly along the chain, resulting in greater rigidity in the gel. He suggests that two chain helicies predominate since the probability of three chains becoming correctly aligned is low.

Some basic conclusions can be drawn from these studies concerning the molecular processes which are occuring during gelation. The first event on cooling a gelatin solution appears to involve a combined intramolecular reorganization of parts of the gelatin peptide chains to a configuration which has been described above as the collagen fold and a non-specific interaction of the ordered segments of different intimately entangled molecules - reactions involving rotations about specific peptide bonds, random collisions, and ordering and stabilization of individual chain elements - to form weak intermolecular crosslinks formed by neighboring hydrogen bonding, hydrophobic bonding, or electrostatic bonding. The chain segments primarily involved in this interaction are the non-polar regions rich in proline and hydroxyproline. Slowly, additional collagen folding and rigid gel networks form at these junction points which are joined by flexible unstructured individual peptide chains. Both aggregates and the free chain segments in hydrated systems are subject to aging or tempering resulting in the development of stable crystallites.

There is a substantial amount of motion of the chain segments in highly hydrated gelatin systems, even at low temperatures. This is in agreement with the progressive increase in crystallinity of gels upon maturation, but further suggests that water plays an important role in stabilizing the collagen fold.[30]

The terms "gelation" and "renaturation" are not synonyms. The collagen molecule can be considered as having two levels of structural order. At the first level is the helical winding of the individual peptide strands into their characteristic collagen fold configuration. The second level involves the very precise alignment of these three peptide strands to provide complimentary interaction of both side chain functional groups and nonpolar chain backbone segments. The term renaturation applies only to the process whereby gelatins may be induced to attain both levels of structural organization. Gelation as mentioned previously involves the formation of isotropic three dimensional gel networks. Renaturation and gelation are thus competing processes. Network gel formation via random segment interactions is concentration dependent and is favored at high concentrations. Conversely, renaturation is completely inhibited by random collagen fold formation and network formation, so that renaturation proceeds most effeciently at gelatin concentrations less than 0.2 percent.[30]

IV. INSTRUMENTATION

Viscosities were measured with an A. G. Epprecht, Ltd., Rhecmeter - Rheomat 15 (Figure IV-1), which consists of two main components - a measuring head mounted on a stand, and a control cabinet. The control cabinet houses electrical components including a 15 position frequency selector which inputs 15 possible angular velocity settings to the measuring head from 5.6 to 352 RPM. A precision spring assembly housed in the measuring head measures 388 dyne-cm of torque per scale division or 38,800 for full scale deflection.

A great number of different designs of measuring systems has been described in the literature. A special double cup and bob used with the Epprecht designated by the letters MS-O consists of a hollow rotor with holes in the top to allow air to escape when the rotor is inserted into an annular cup channel. A schematic diagram of this unit is shown in Figure IV-2. The system can be considered as two simple viscometers having the same angular velocity while the measuring torque is the sum of the two individual torques. With the MS-O unit and Epprecht drive, shear rates from 27 to 1717 sec^{-1} can be obtained with shear stresses varying from 2.23 to 223 dyne cm^{-2}. Hence viscosities as low as 0.0013 poise and as high as 8.4 poise may be measured.

Appendix A presents a profile of the angular velocities, shear rates, and Newtonian conversion factors which are used with the MS-O viscometer. Appendix B compares viscosity data which have been corrected for non-Newtonian effects and those which have not. Differences between the corrected and uncorrected viscosities are minimal, and since the results presented in this study are treated comparatively, no further corrections were made to any data taken.

In order to ensure that the gelatin viscosity data reflect the results of stable laminar flow within the annular region of the MS-O unit, the regions of secondary or turbulent flow as reflected by the critical

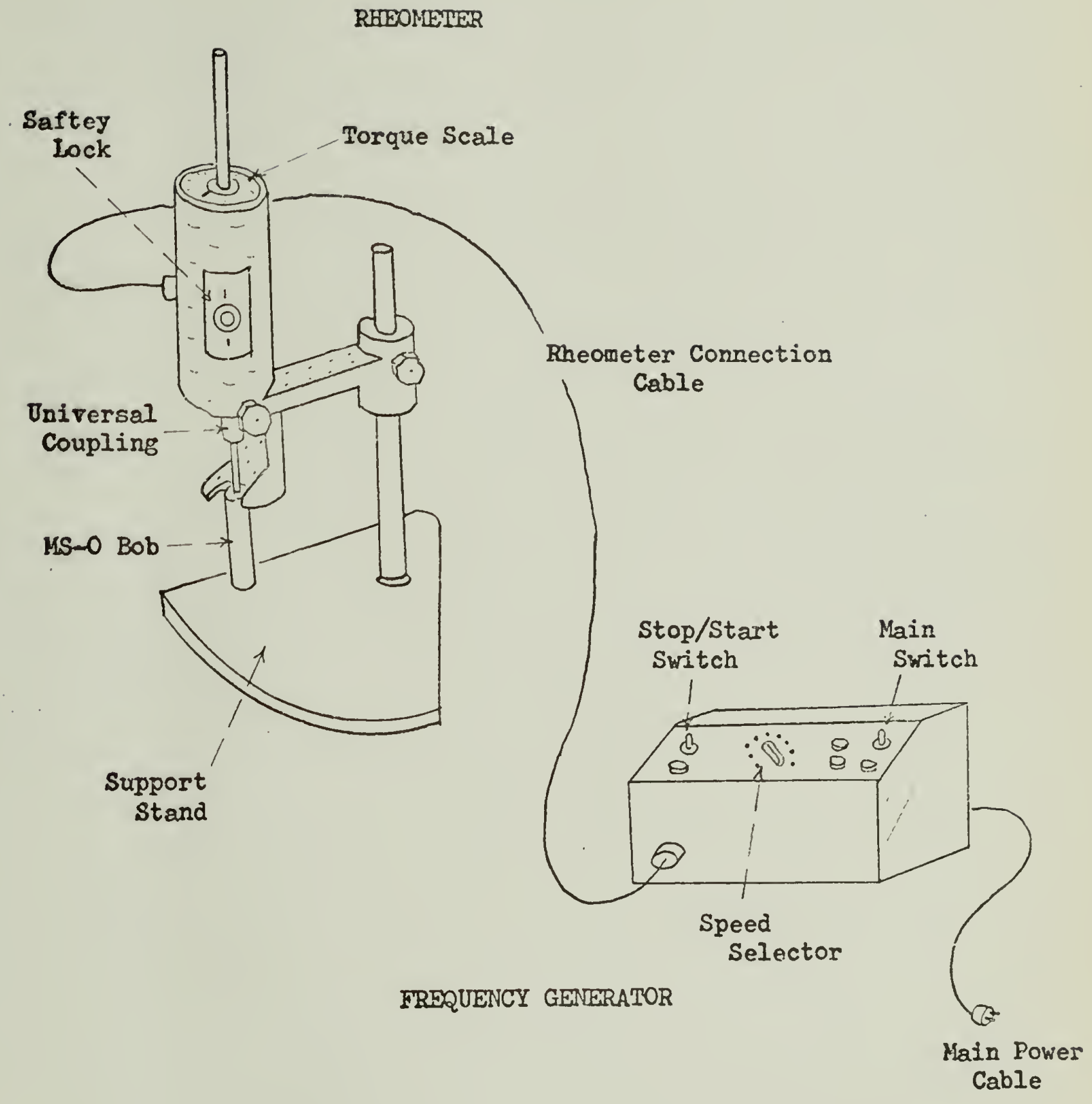
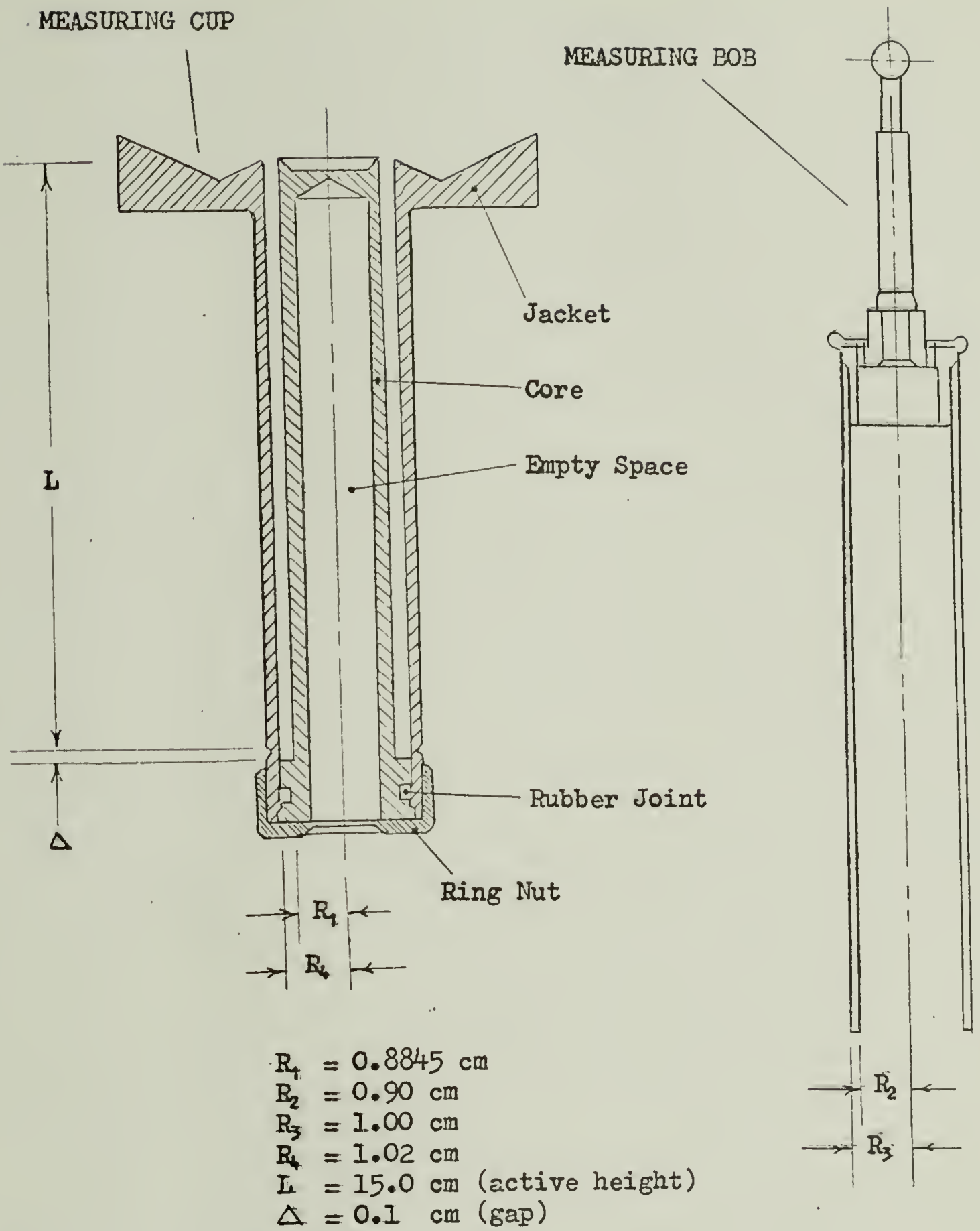


FIGURE IV-1. EPPRECHT RHEOMAT 15 WITH MS-O VISCOMETER



R_1 = 0.8845 cm
R_2 = 0.90 cm
R_3 = 1.00 cm
R_4 = 1.02 cm
L = 15.0 cm (active height)
Δ = 0.1 cm (gap)

FIGURE IV-2. SCHEMATIC DIAGRAM OF MS-O UNIT[7]

Reynolds numbers were examined (Appendix C). Generally the critical Reynolds numbers range from 10 (inner wall [R_3] rotating, outer [R_4] stationary) to 80 (inner wall [R_1] stationary, outer [R_2] rotating) orders of magnitude greater than the maximum Reynolds number the system could possibly generate with gelatin. The transition to turbulent flow takes place at a much higher Reynolds number in the case where the inner wall is stationary and the outer is rotating. This is because the transition is influenced by centrifugal forces. When the outer wall is rotated, inertial forces have a high stabilizing effect on flow, however, when the inner wall is rotated, centrifugal forces tend to introduce instability.[2]

The MS-O double cup and bob viscometer can be used in such a way that practically the whole of the test material is subjected to similiar shearing conditions. Moore and Davies[23] have shown that the shearing stress distributions in both gaps are equal (i.e., $T_1 = T_3$ and $T_2 = T_4$) what ever the type of material involved provided that $R_1 / R_2 = R_3 / R_4$. Appendix D presents a summary of this derivation.

Rotary viscometers which shear a thin layer of material in a repeated path and which provide measurement in time of the shear stress and shear rate have been most suitable for the study of non-Newtonian and especially thixotropic materials such as gelatin. Also these viscometers allow for measurements made on non-Newtonian materials to be interpreted in absolute units.

The MS-O unit is capable of holding 13.66 ml of fluid, 3.66 ml in the annular region which is undergoing shear and 10 ml in the cup shaped storage area which experiences insignificant shearing. The unit was maintained at constant temperature with the use of a water circulating temperature controller. Generally, consistent initial readings were obtainable within a minute after filling the viscometer with the heated fluid. Because of the small gap clearances, the solution reaches the equilibrium temperature of the MS-O unit within seconds after filling. Appendix E presents a typical unsteady state heat conduction problem which demon-

strates the rapid rate at which the fluid reaches the equilibrium temperature of the viscometer and bath.

V. MATERIALS

Ten commercial suppliers provided samples of single extraction calf and pig skin derived gelatin. Of these samples, one pig skin and three calf skin gelatins were briefly evaluated with respect to molecular weight and kinetic behavior during gelation inorder to select one material for intensive examination. Based on these results, an alkaline processed gelatin supplied by United States Gelatin, Division of Peter Cooper Corporation was chosen for further investigation. This gelatin (Lot 320) was extracted from an alkaline treated precursor. Specifically, the calf skin stock was initially washed and then treated with lime water until cured. The stock was then washed and treated with dilute sulfuric acid for neutralization of residual alkalinity and washed again prior to gelatin extraction. This sample represents gelatin from a single extraction and is not a blend of various lots.

A viscosity average molecular weight of 100,000 was determined for the Peter Cooper gelatin (which will subsequently be refered to as PC) using intrinsic viscosity techniques and the Mark-Houwink relationship, assuming a linear randomly coiled polymer. Equation constants were obtained from the Polymer Handbook[5]. The molecular weight discussed above was calculated from plots of reduced viscosity versus concentration for two gelatin PC solutions (Figure V-1). Intrinsic viscosity and molecular weight were also determined for three other gelatins during the initial screening studies; Appendix F presents reduced viscosity-concentration plots for these samples.

Molecular weight and other characteristic data for the PC gelatin along with the three other gelatins are summarized in Table V-1 for comparative purposes. The molecular weights are quite diverse ranging from 45,000 to 170,000. Generally the molecular weight of the parent collagen is close to 300,000; thus each gelatin chain should have a value close to 100,000. The low value of 45,000 may reflect some chain degradation caused during extraction. The higher value of 170,000 on the other hand may indicate

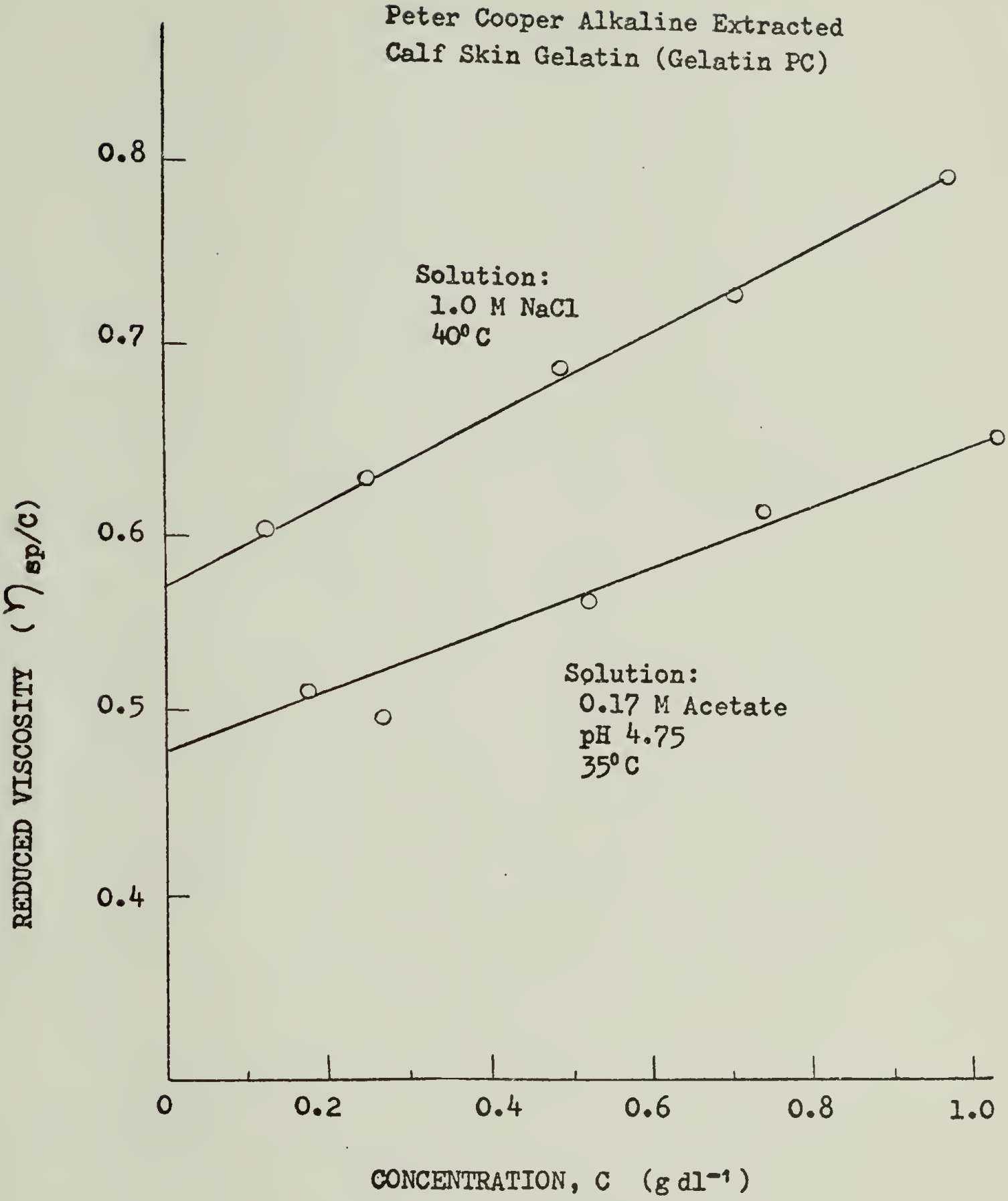


FIGURE V-1. REDUCED VISCOSITY AS A FUNCTION OF CONCENTRATION

TABLE V-1

COMPARATIVE GELATIN PROPERTIES

Supplier	Animal Source	Treatment	pH	Intrinsic Viscosity*	Viscosity Average Molecular Weight***
United States Gelatin Division of Peter Cooper Corporation Gowanda, New York	calf skin	alkaline	7.0	0.57 (0.48)**	100,000
Atlantic Gelatin Division of General Foods Corporation Woburn, Massachusetts	calf skin	?	6.5	0.66	170,000
Swift Chemical Company Division of Swift and Company Winchester, Massachusetts	pig skin	acid	5.2	0.51	70,000
Kind and Knox Camden, New Jersey	calf skin	alkaline	6.2	0.29	45,000

* Solution properties:[5] 1.0 M NaCl, 40° C, $K = 2.69 \times 10^{-5}$ dl/g, a = 0.88

** Solution properties:[5] 0.17 M Acetate, 35° C, $K = 1.66 \times 10^{-5}$ dl/g, a = 0.885

*** Obtained using the Mark - Houwink relation: $[\eta] = KM^a$

incomplete denaturation or a higher concentration of β and γ gelatin (i.e., crosslinked α gelatin chains).

Although other methods for molecular weight characterization have been successfully applied to gelatin (i.e., osmotic pressure techniques, analytical ultracentrifugation methods[29,38], fractionation procedures[25], and light scattering techniques[3,9,13,31,32,33,34]), this viscometric study provided a convenient and rapid way to differentiate and characterize the species of gelatin which were available.

It is interesting to note some of the observations based on light scattering studies:[30]

- Gelatins are molecularly dispersed in aqueous solutions at temperatures of 40° C.
- Molecular weight may range up to values greater than 10^6.
- Even after fractionation, gelatins are extremely heterogeneous.
- The same configurational model cannot be used for all gelatins.

Thus through out this study it must be continually remembered that the gelatin under evaluation, even though from a single extraction source, is still a crude material as to molecular weight distribution, chain sequence uniformity, chain branching, chain crosslinking, and chain aggregation.

VI. PROCEDURE

Solutions were prepared by agitating granulated gelatin in 40° C water for three hours. After this period, the temperature was raised to 70° C and the solution was filtered through a Whatman no. 1 qualitative filter paper. The filtrate was cooled to 40° C again, stirred, and alloted to several small flasks which were sealed and refrigerated. The solution concentrations were determined by measuring the weight of solute remaining after evaporating the solvent from a standard volume of solution.

Basically two forms of viscometric data are reported in this text: static data during which no time - shear stress changes occur as a function of a single shear rate, and kinetic data during which the shear stress measured varies as a function of time (thixotropic behavior). Both studies involved essentially identical start-up and operative procedures, however collection of kinetic data required a longer time.

Concentrated hydrochloric acid and pelletized sodium hydroxide were used to change the pH of the solutions. pH adjustments were made one half hour prior to initiating an experimental run.

Each gelatin solution was maintained at 40° C for one half hour prior to beginning the run to insure that the chains were completely randomized in solution. The experimental time began at the instant 3.66 ml of 40° C gelatin solution was poured into the annular cup region of the MS-O viscometer. Readings were obtained within one minute of experimental time. As described earlier (section IV), the time required for the solution to achieve the equilibrium temperature of the viscometer and bath is very short. A one minute reading is thus believed to reflect a close approximation of the viscosity of the randomly coiled chains (no structure) at a temperature below the gel melting zone. Most of the viscosity growth curves presented in the next chapter substantiate this claim since very little viscosity change occurs during the first three to five minutes.

VII. RHEOLOGY OF GELATION

A. Introduction

B. Kinetics and Shear Rate

C. Shear Rate History

D. Kinetics and Concentration

E. Kinetics and pH

F. Kinetics and Temperature

VII-A. Introduction

There are basically two forms of viscometric data reported in this chapter exhibiting marked differences in performance. Viscosity data taken above the melting zone ($>25^{0}C$) of gelatin exhibit typical time independent shear thinning behavior as a function of increasing shear rate, whereas data taken below the melting zone show characteristic thixotropic behavior. The melting zone refers to the temperature range where structured gelatin networks and randomly coiled gelatin chains coexist in solution. Above this zone randomly coiled chains in solution are thermodynamically favored, whereas network formation occurs below the zone.

Although the figures in this chapter have been described sufficiently in their accompanying text, a few additional comments will serve to clarify any remaining ambiguities. Plots of viscosity versus time (viscosity growth curves) are on full logarithmic coordinates because of the extended ranges encompassed by the data. Plots of normalized viscosity ([equilibrium viscosity - viscosity at time t] / [equilibrium viscosity - initial viscosity]) versus time are on semi-logarithmic coordinates inorder to attain an understanding of the rate and mechanism of the gelation process.

Viscosity growth, normalized / kinetic curves, and several other variations are presented only for the Peter Cooper calf skin gelatin (gelatin PC) as functions of shear rate, concentration, pH, and temperature. As briefly mentioned in section V, kinetic growth curves were also constructed for three other species of gelatin during the screening studies. These plots are included in Appendix G. For a better understanding of these curves and the molecular mechanisms involved, it is advisable to review section VII-B first. Remember that these data are of gelatin originating from different animal and extraction processes, and as such only general comparisons can be made between the results. The equilibrium viscosities (Appendix G) demonstrate a typical viscosity molecular weight

interdependence. The short term kinetic curves show a slight dependence on molecular weight of the rate of equilibrium attainment for the three calf skin derived gelatins, however, this difference becomes insignificant as gelation continues.

VII-B. Kinetics and Shear Rate

The most convenient type of data for studying the kinetics of gelation is that obtained by observing viscosity change as a function of either shear rate or shear stress. The former approach was chosen as it lended itself suitable to the Epprecht Rheometer.

Although the occurance of thixotropic non-Newtonian behavior has long been recognized, it still remains a poorly understood phenomenon. It is generally accepted that thixotropy results from structural changes that are functions of the shear rate, shear stress, and time.

A type of experiment which can provide information on the solid like structure that develops during gelation is that obtained by shearing gelatin solutions at temperatures below the melting zone for periods of time at various constant shear rates and observing the viscosity growth. In the case of gelation the time to attain an equilibrium viscosity or constant structure ranges from several hundred minutes at high rates of shear to one or two thousand minutes at very low rates of shear. These results are presented in Figures VII-B1 and B2 where viscosity versus time plots are presented as a function of shear rate. These equilibrium times are considered extremely long (some thixotropes have been observed to achieve equilibrium within seconds); however, due to the complex nature of the gelatin molecule this is expected.

There appears to be a broad range of strengths of particle to particle associations or structures that can exist depending on the shear level, in fact the equilibrium viscosity at the lowest shear rate (27.3 sec^{-1}) is almost 16 times larger in magnitude than that at the highest shear rate of 1717 sec^{-1}. Perhaps at low rates of shear weak structure can exist because the disruptive forces are low, while at high rates of shear only stronger structures can exist.

The results shown on Figures VII-B3 and B4 have been normalized with respect to the initial and equilibrium viscosities to provide a kinetic

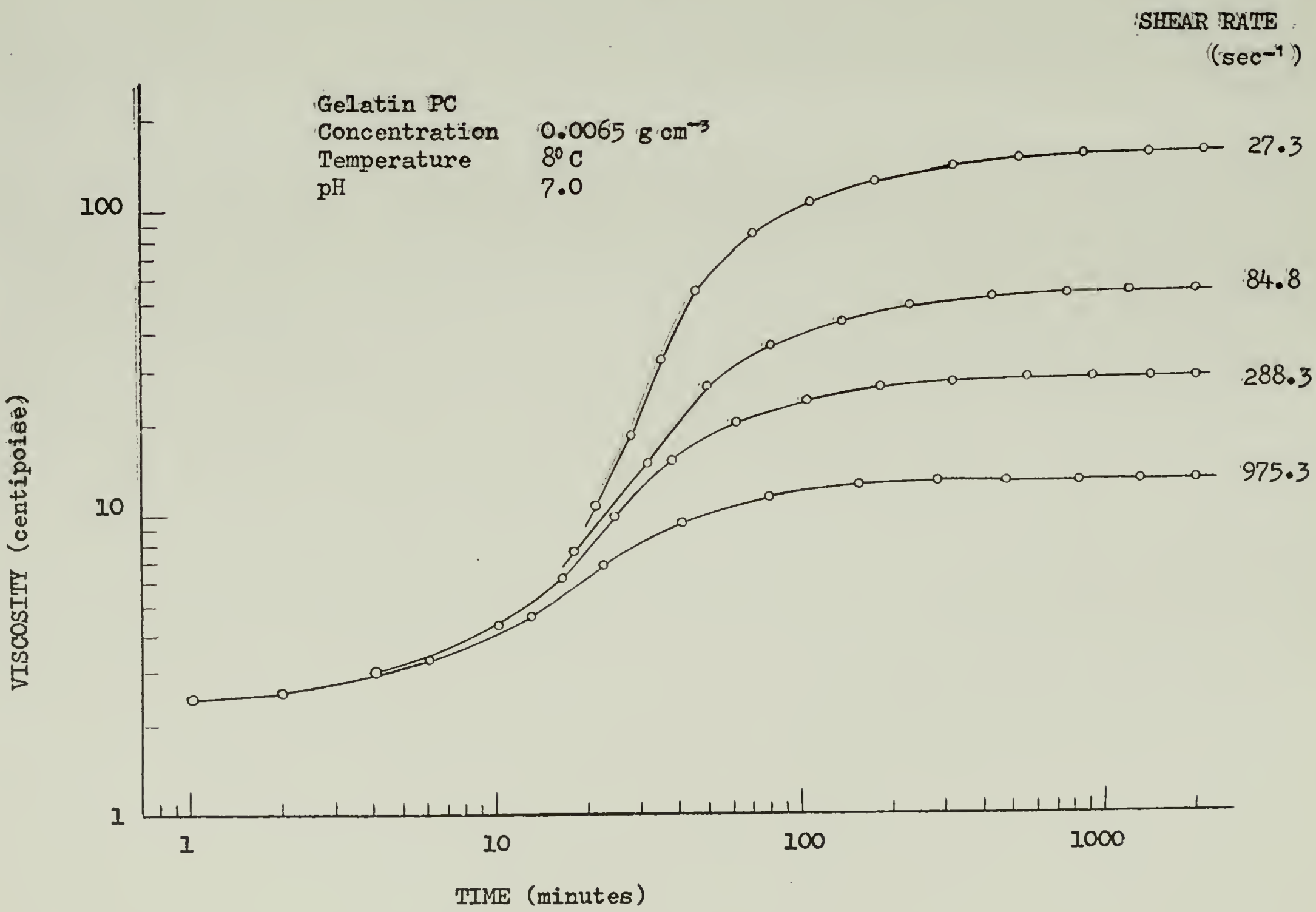


FIGURE VII-B1. VISCOSITY vs. TIME - EFFECT OF SHEAR RATE

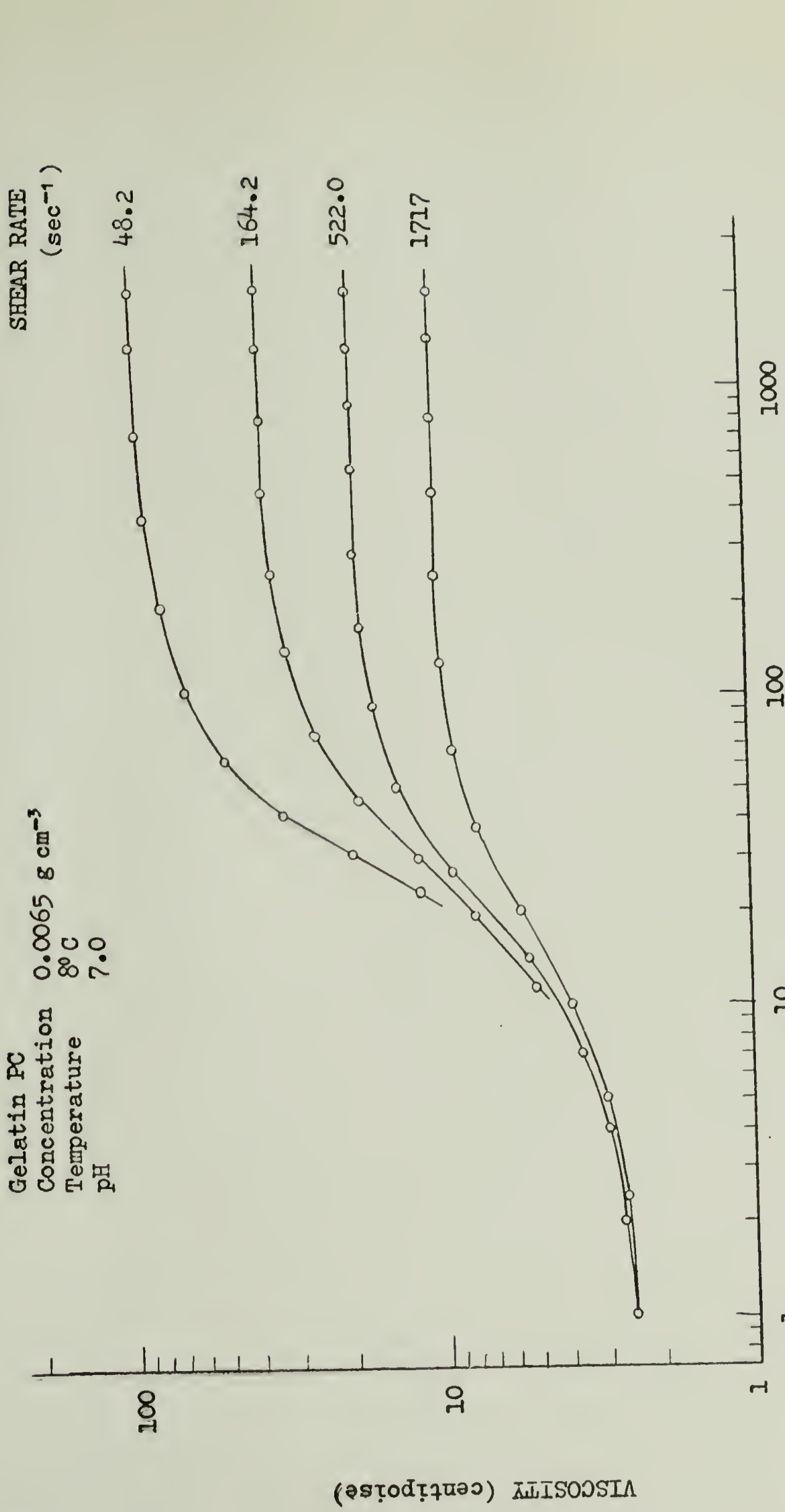


FIGURE VII-B2. VISCOSITY vs. TIME - EFFECT OF SHEAR RATE

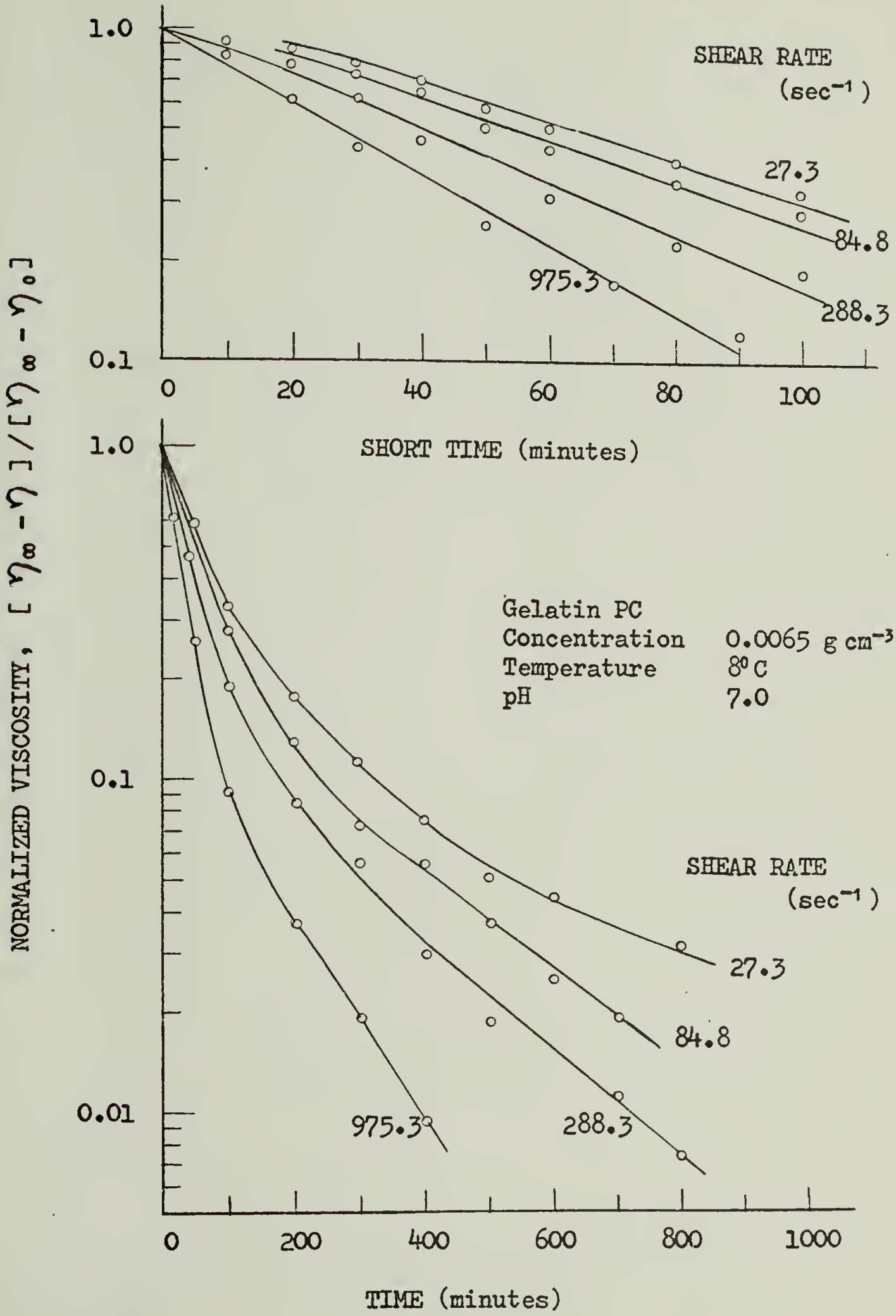


FIGURE VII-B3. NORMALIZED VISCOSITY vs. TIME - EFFECT OF SHEAR RATE

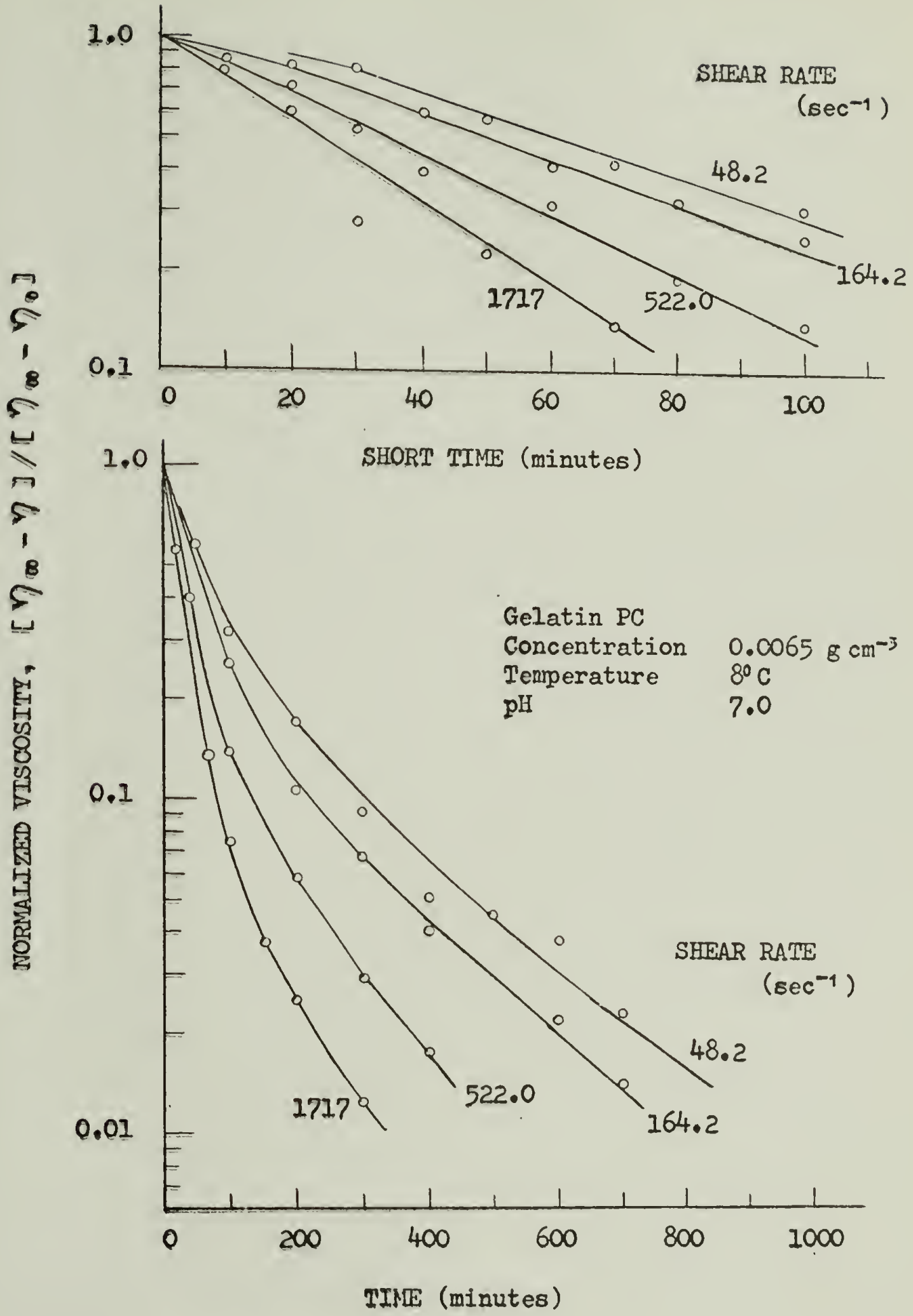


FIGURE VII-B4. NORMALIZED VISCOSITY vs. TIME - EFFECT OF SHEAR RATE

analysis. Due to limitations of the viscometer, initial viscosities at several of the lower shear rates were not obtainable. However a similiar initial viscosity was assumed for all shear rates based on the following observations and assumptions:

- The initial structures at 8° C are identical regardless of the rate of shear (i.e., the gelatin chains exist as random coils).
- Shear thinning is minimal as demonstrated in Figure VII-D1.
- The initial viscosity is small compared to the equilibrium viscosity.
- The initial viscosities were essentially identical at those shear rates at which readings were obtained.

As observed in these figures the rate of gelation increases as a function of increasing shear rate, both in a short time and long time consideration. The observed increase in viscosity growth rate with shear rate is to be expected if the build up of structure is controlled by the rate at which particles are brought into contact with each other and with the already existing aggregates. The higher the shear rate, the more particles of the proper orientation and size are brought into the gel aggregate zone per second. As the shear rate is lowered, the contribution of the shear rate to particle orientation becomes smaller when compared to that of the molecular (Brownian) motion.

Based on the two seemingly linear regions of these rate curves, it appears as though two overall stages or mechanisms are involved during gelation under shear. These might be described as an initiation stage and a long term stage. The inflection or divergence of these two stages appears to fall within 100 to 200 minutes depending on the rate of shear. The first scheme probably includes such molecular processes as the intramolecular reorganization of parts of the gelatin molecule into a configuration described as the collagen fold and the non specific interaction of the ordered segments of the different intimately entangled molecules to form weak intermolecular crosslinks. The second stage probably includes the slower additional collagen folding and gel aggregate formation at the junction points and the slower aging or tempering process resulting in the develop-

ment of stable crystallites. The lack of a sharp transition indicates a process in which all or some of the mechanisms described above are occuring simultaneously, but with increasing or decreasing influence.

Figure VII-B5 shows equilibrium and constant structure curves obtained from the preceding shear rate data. The object of this form of presentation is to demonstrate the behavior of the gelled system at various "structural" levels, with the level being determined by shearing the gelatin solution until equilibrium was established at the selected shear rate. The slightly hyperbolic shaped curve reflects the equilibrium shear stress at each shear rate. After attaining an equilibrium shear stress (or viscosity), the constant structure curves were determined (shown as dashed lines emanating from the equilibrium flow curve) by lowering the shear rate to a new level rapidly enough so that no structural change could take place during the time required to change the shear rate. After the stress was measured at the new lower level, the shear rate was changed back to the original level until equilibrium was once more attained(usually no deviation was observed) after which another measurement could be made. The fact that no deviation was observed is good evidence that a "constant structure" was maintained.

The slopes of the constant structure curves increase as a function of an increasing shear rate on the equilibrium flow curve. This demonstrates that the equilibrium gel structure or network formed at each shear rate is different, different in the respect of containing some unique structural composition.

Unfortunately it was experimentally unfeasible to extend shear rate beyond 27 or 1717 sec^{-1} so as to observe any discontinuities in the equilibrium flow curve. Joye and Poehlein[19] have presented data (although not commenting upon it) which appear to indicate that an inflection or transition point on an equilibrium flow curve for a clay water suspension may exist around 100 sec^{-1} shear rate. The data of Figure VII-B5 also allow for this speculation, although certainly more confirming work is needed. An inflection as described might indicate a transition zone where in-

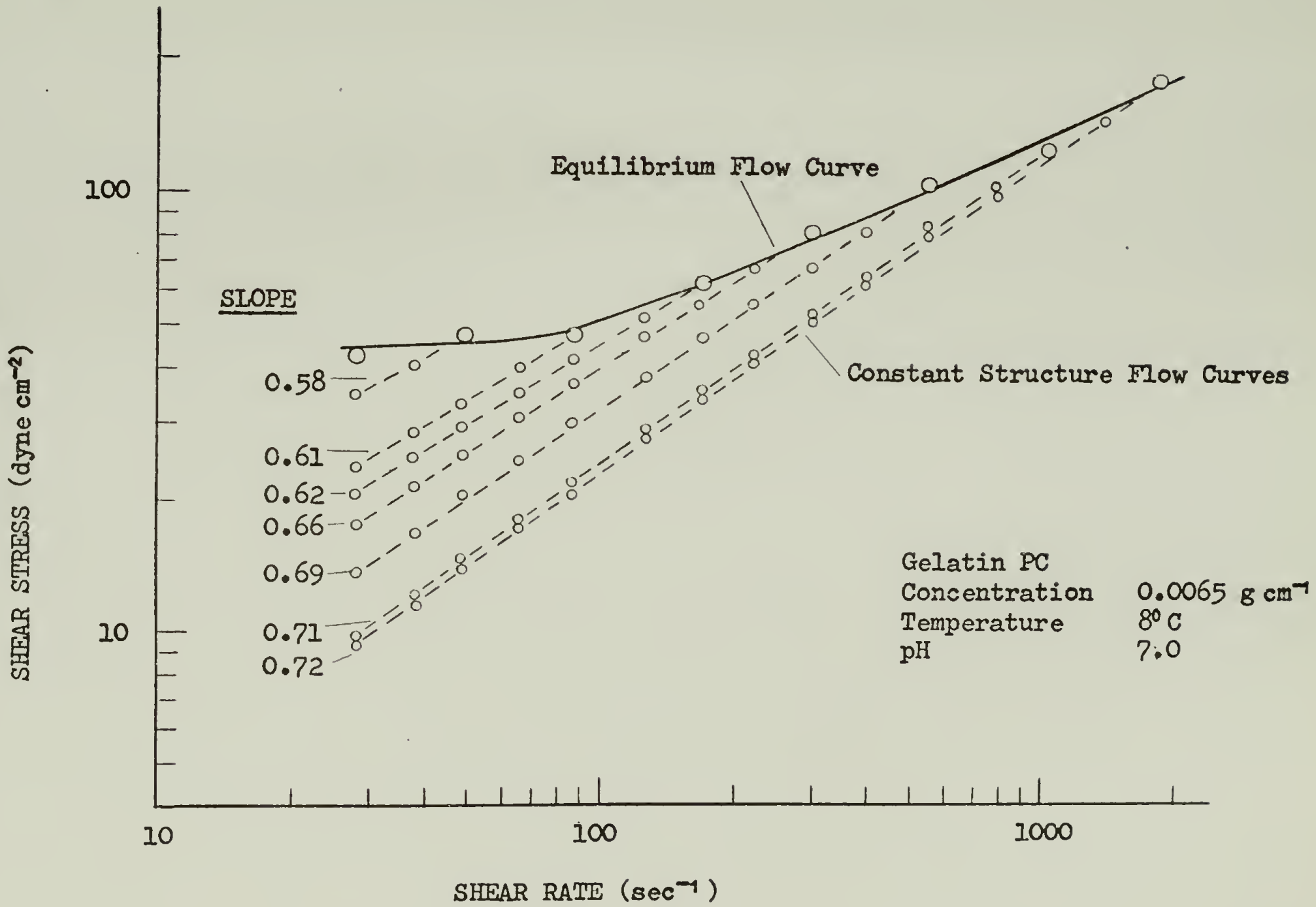


FIGURE VII-B5. EQUILIBRIUM FLOW CURVE AND CONSTANT STRUCTURE LEVELS

creases or decreases in shear rate will influence the structure formed to a greater or lesser extent.

VII-C. Shear Rate History

The objective of this study was to determine the behavior of the gelation process and structure formed as a function of shear rate history. Figures VII-C1, C2, and C3 illustrate the results.

The viscosity growth curve of Figure VII-C1 (curve A) illustrate a gelatin solution which was continuously sheared at a uniform shear rate of 288.3 sec^{-1} until an equilibrium viscosity was achieved (2000 minutes). On attaining equilibrium, the shear rate was decreased to 0 sec^{-1} for 2000 minutes. After this second 2000 minute period, the shear rate was again raised to the initial 288.3 sec^{-1} level and data were recorded (curve B) as the viscosity decreased slowly to the equilibrium level attained previously by a continuous 288.3 sec^{-1} shear program. From these plots it appears as though additional structure forms during the 2000 minute 0 sec^{-1} shear period as evidenced by the higher immediate viscosity, however, this new or additional structure is shear degradable and gradually breaks down. Thus it would seem unlikely that a rearrangement of the original structure formed at 288.3 sec^{-1} occured during the 0 sec^{-1} shear period, and perhaps only previously unattached or peripheral gelatin chains are involved in forming new interchain bonds which are weakly connected to the main structure.

A second experiment, also shown in Figure VII-C1, examines differences in structure which form at rest and at a finite shear rate. Here a system, with no prior shear history, was allowed to gel for 2000 minutes with 0 sec^{-1} shear. After this 2000 minute period, a 288.3 sec^{-1} shear rate was imposed on the system (curve C). Note that even after 5000 minutes of shearing, the decreased level of viscosity is considerably greater than the equilibrium viscosity shown for the continuous viscosity growth curve and shows little hope of even approaching this level within a practical length of time. It appears that gelation at zero shear, with no prior shear history, produces a structure which cannot be rearranged or decomposed very easily thru shearing to yield a structure characteristic for

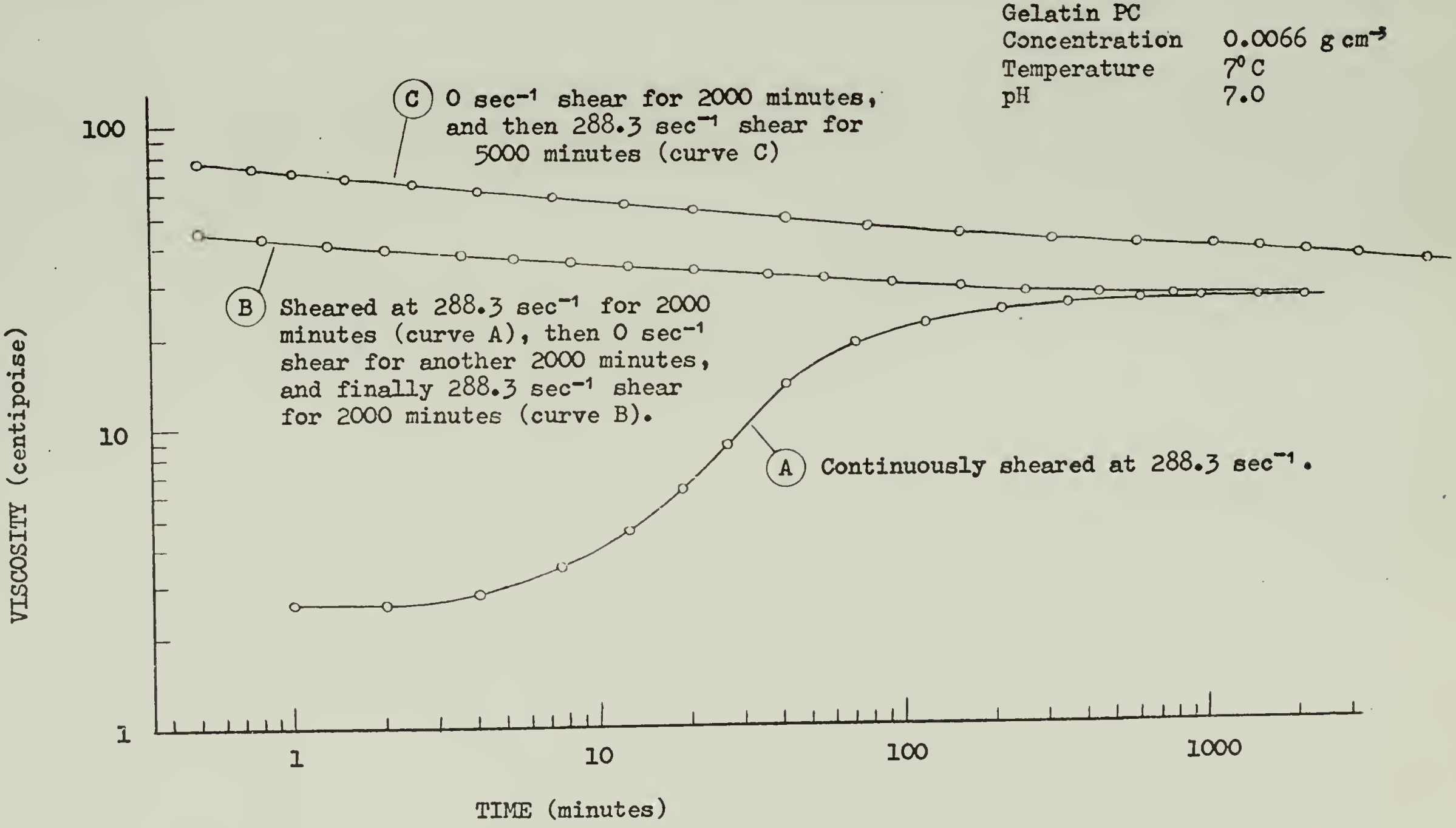


FIGURE VII-C1. VISCOSITY vs. TIME - EFFECT OF SHEAR DEFORMATION AND REST

that shear rate. The structure formed under 0 sec^{-1} shear must then be quite different in interchain bonding and network arrangement.

To determine if a shear rate one order of magnitude lower than 288.3 sec^{-1} would also produce a seemingly irreversible structure, the data of Figure VII-C2 were obtained. After observing a typical viscosity growth pattern for 2000 minutes at 27.3 sec^{-1} (curve B), the shear rate was increased to 288.3 sec^{-1} (curve C). The viscosity decays slowly to the viscosity characteristic of 288.3 sec^{-1} continuous shearing (curve A) suggesting an interchangeability of structure for systems which form under shear, at least for gelatin networks formed at a 27.3 sec^{-1} shear rate or above.

A third figure (Figure VII-C3) shows a gelatin solution which experienced a sequence of a short 0 sec^{-1} shear period for 80 minutes and then a 288.3 sec^{-1} shear until achieving equilibrium (curve B). A yield peak is observed which decays to a level very near the correct time - viscosity position for a 288.3 sec^{-1} continuously sheared system (curve A). The viscosity growth continues on almost a parallel course with the continuously sheared solution. Apparently the structure formed during these 80 minutes is not sufficiently developed to retain its strength under shear as was the case of a 2000 minute 0 sec^{-1} shear developed gel network (Figure VII-C1 [C]). Even though the gel network has attained approximately 50 percent of its final strenth after 80 minutes of zero shear as measured by viscosity, the additional bonds which continue to form with time at zero shear are apparently the more influential in providing the highest order of interchain bond strength.

All of these facts demonstrate quite strongly that different quantitative and qualitative structure form at different shear rates and depending on the shear rate history may or may not be easily reversible processes.

Joye and Poehlein[19] have presented an intuitive argument describing the overall different molecular processes involved in gelation under zero shear and gelation during a finite shear rate. They feel that only weak cross links or bonds between "quasi-elements" are responsible for the growth or increase of structure under constant shear rate. Because of these weak structural units, it is unlikely that stronger inter-unit bonds

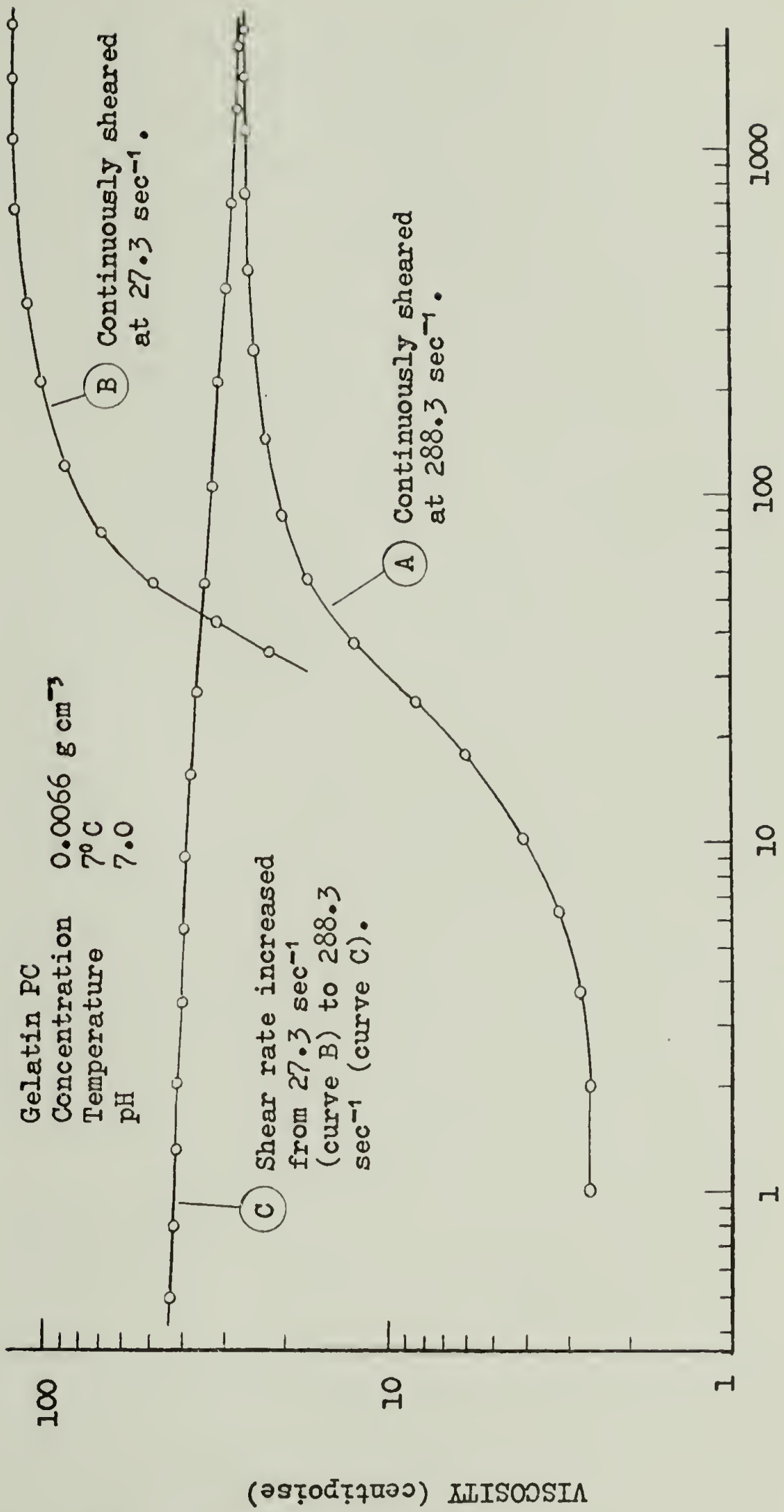


FIGURE VII-C2. VISCOSITY vs. TIME - EFFECT OF SHEAR DEFORMATION HISTORY

Gelatin PC
Concentration 0.0066 g cm^{-3}
Temperature 7° C
pH 7.0

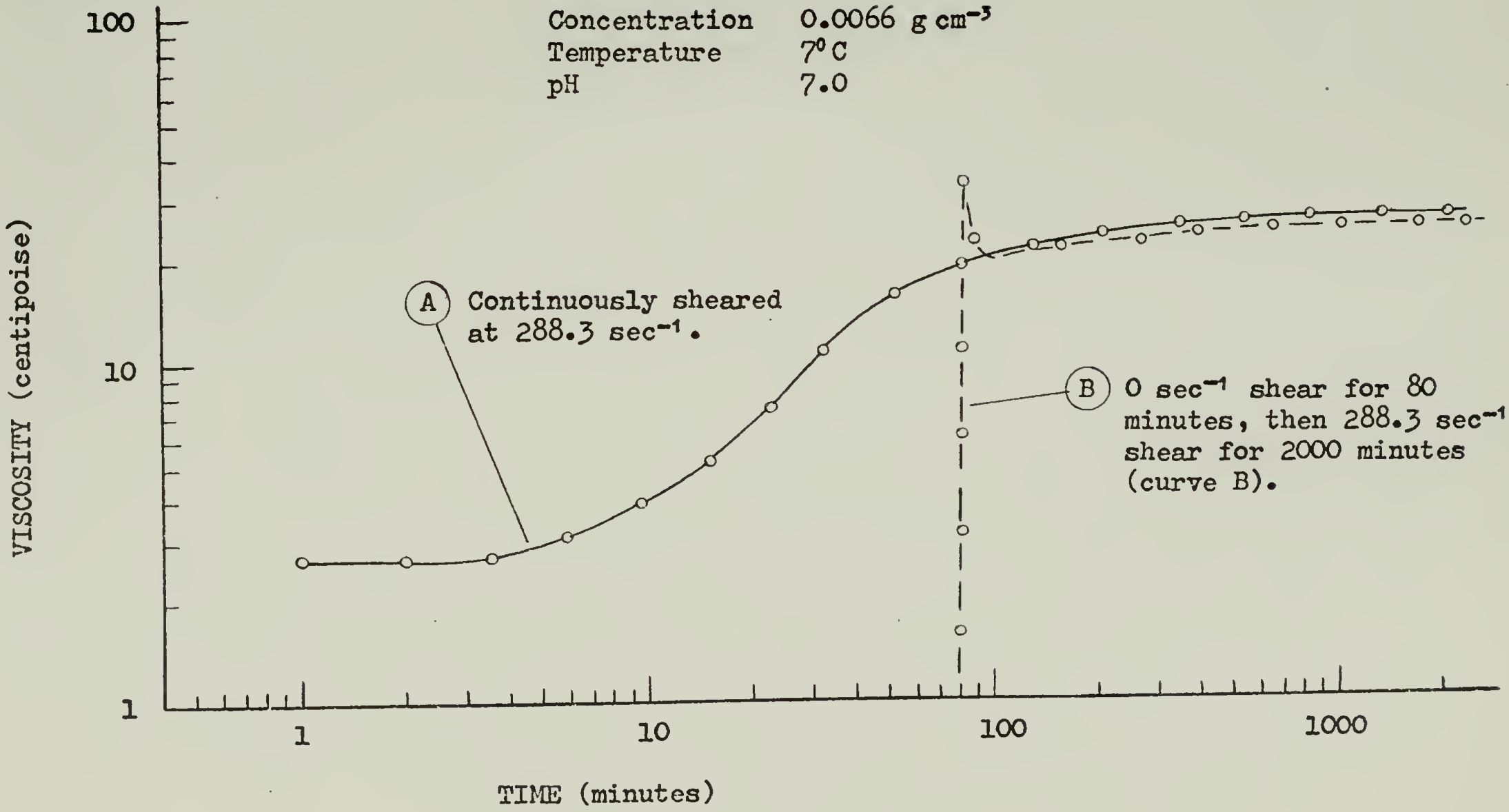


FIGURE VII-C3. VISCOSITY vs. TIME - EFFECT OF SHEAR DEFORMATION AND REST

can form during shear. During zero shear inter-unit bonds can form easily. They propose that a loose gel network forms rather quickly during rest, after which additional network crosslinks complete a strong gelled system.

Portions of this argument could be reinforced with the data just presented, however, the distinction between the zero shear and sheared structure may not be as clear as Joye assumes. It is entirely possible that stronger "inter-unit" bonds form during shearing as well as during rest.

VII-D. Kinetics and Concentration

The following experiments present information concerned with the influence of concentration on the rheology and kinetics of the gelation mechanism. Figure VII-D1 depicts viscosity/shear rate data for λ gelatin in solution at 40° C as a function of concentration. These results, which show a slight non-Newtonian or shear thinning behavior, are time independent since the formation of network gels generally will not occur above 25° C.

The viscosity growth curves of Figure VII-D2 are data illustrating gelation at several concentrations ranging from 0.4 to 0.7 percent. At these concentrations the network gelation forming mechanism is favored over renaturation. A dilute solution exists when the volume of solution is larger than the volume encompassed by the sum of all the unperturbed volumes of the molecular domains. A solution may be thought of as concentrated when the molecular domains overlap, and contacts between chain elements of different chains becomes as probable as contacts between elements of the same chain.[30] Apparently even at high dilutions the chain segment density within the domain of a single random coil is relatively high. Boedtker and Doty[3] examined an α gelatin of a molecular weight of 90,000 at infinite dilution and determined a weight concentration within the polymer domain of 2.1×10^{-3} g cm^{-3}. Thus a gelatin chain segment is never in an environment less concentrated than about 0.2 percent. At total gelatin concentrations greater than 0.2 percent the molecular domains must overlap so that intermolecular contacts are equally probable as intramolecular contacts.

The rates of structure formation as a function of concentration are considerably different as shown in Figure VII-D3. Perhaps at lower concentrations the gelatin chains retain some mobility for longer periods of time. At higher concentrations the chain mobility will be restricted much sooner and thus not allow for the slow rate of development of stronger or more ordered bonds.

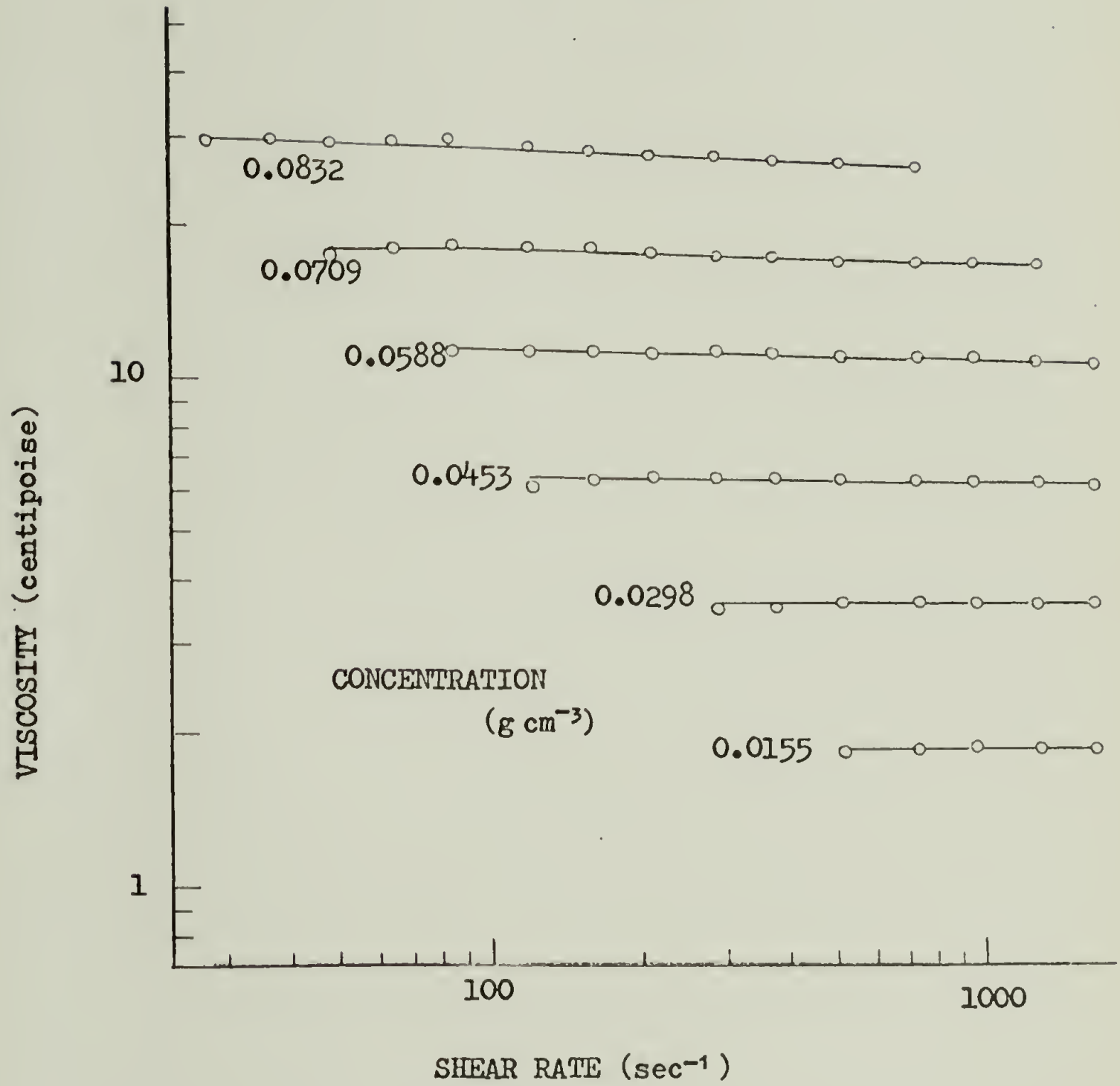


FIGURE VII-D1. VISCOSITY vs. SHEAR RATE - EFFECT OF CONCENTRATION

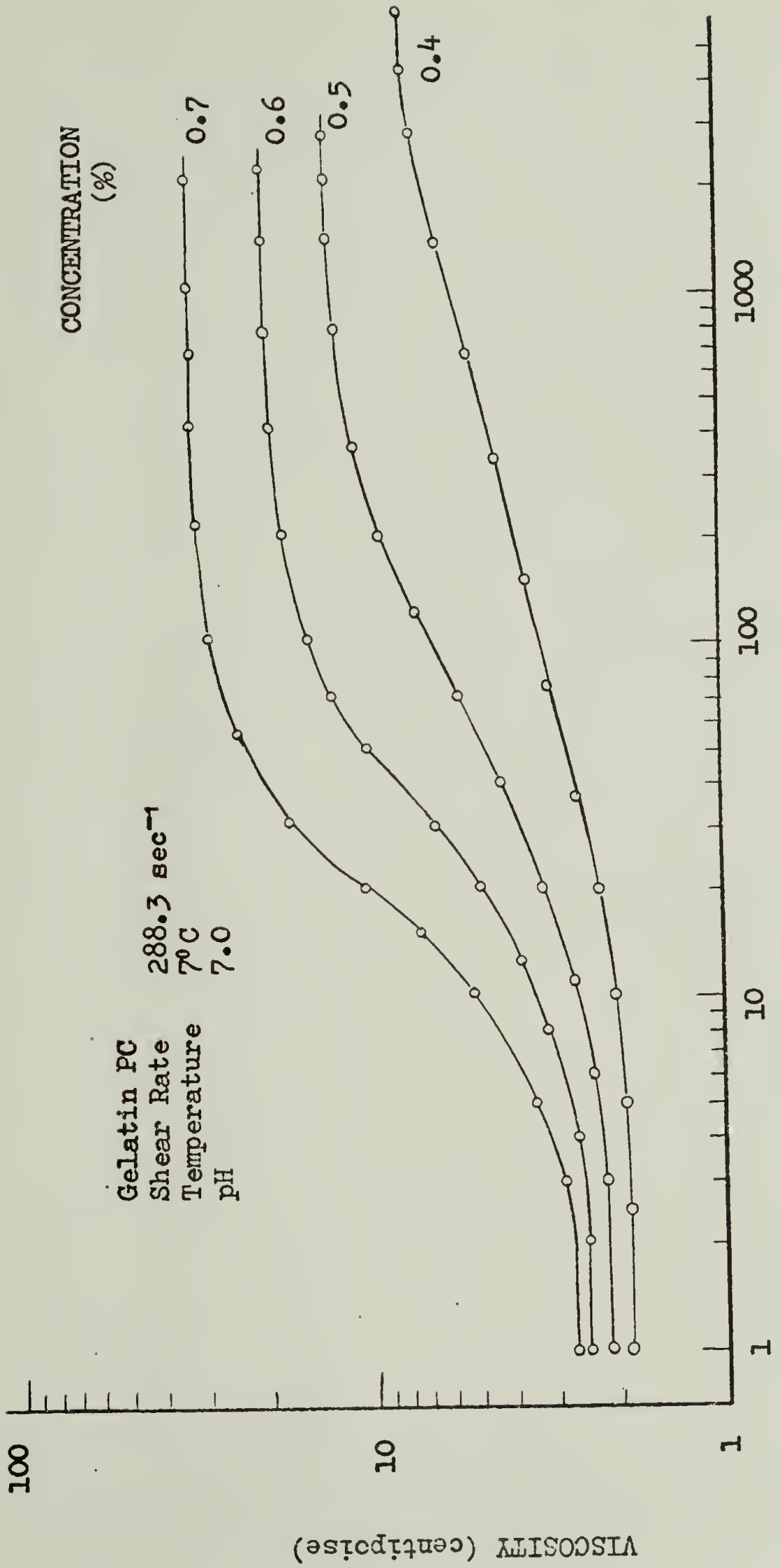


FIGURE VII-D2. VISCOSITY vs. TIME - EFFECT OF CONCENTRATION

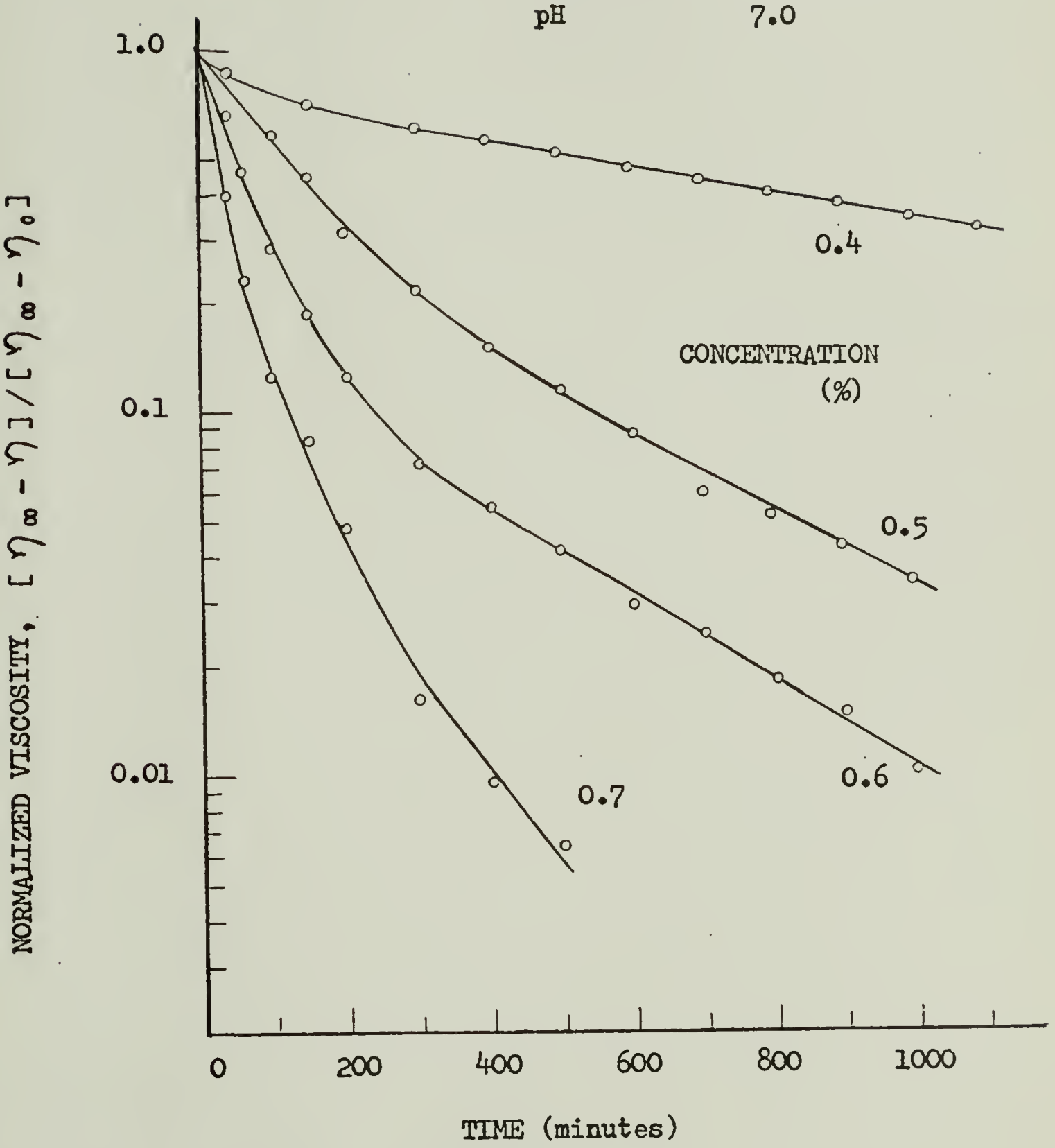


FIGURE VII-D3. NORMALIZED VISCOSITY vs. TIME - EFFECT OF CONCENTRATION

Again as presented in section VII-B, these curves appear to exhibit long and short term slopes suggesting two overall mechanisms for network growth.

VII-E. Kinetics and pH

The following study demonstrates the behavior of gelation over a range of pH. The expansions and contractions of the gelatin molecule with varying pH have been extensively examined in previous work.[30] The viscosity has been found to have a minimum value at the isoelectric pH and increases as the net molecular charge increases. The isoelectric point is the pH of a buffer on which no net migration of the protein is produced by application of an electric field.

Viscosity growth curves for the alkaline derived gelatin PC are shown in Figures VII-E1 and E2 as a function of pH. The pH is observed to have a considerable influence on the final equilibrium structure of the gelled network as well as the rate at which it is attained. The normalized kinetic plots of these growth curvesare shown in Figures VII-E3 and E4.

Figure VII-E5 presents a plot of the equilibrium viscosities shown in figures E1 and E2 as a function of pH. At pH values above and below those shown on this plot, studies[30] have observed that viscosities fall rapidly, and at the extreme acid end, take on values even less than the viscosity at the isoelectric pH. At a pH of 5.25, corresponding to a value near the isoelectric point, the equilibrium viscosity reaches a minimum. Correspondingly its rate of growth to equilibrium is very fast. Visually the "gelled" system at this pH is turbid. The gelatin molecules have probably assumed intramolecularly compacted structures due to the absence of repulsive charges on the segments of the chain. The expansion that accompanies the charging of the gelatin molecule thru altering the pH from the isoelectric point increases viscosity and reduces the turbidity.

These equilibrium viscosities, which are a reflection of inter- and intramolecular arrangements, apparently correlate well with the viscosities of randomly coiled gelatin molecules in solution (i.e., above 25° C) as shown in Figure VII-E6. Here two shear rates (288.3 and 1717 sec^{-1}) were used to obtain viscosity as a function of pH for a 4.71 percent gelatin solu-

tion

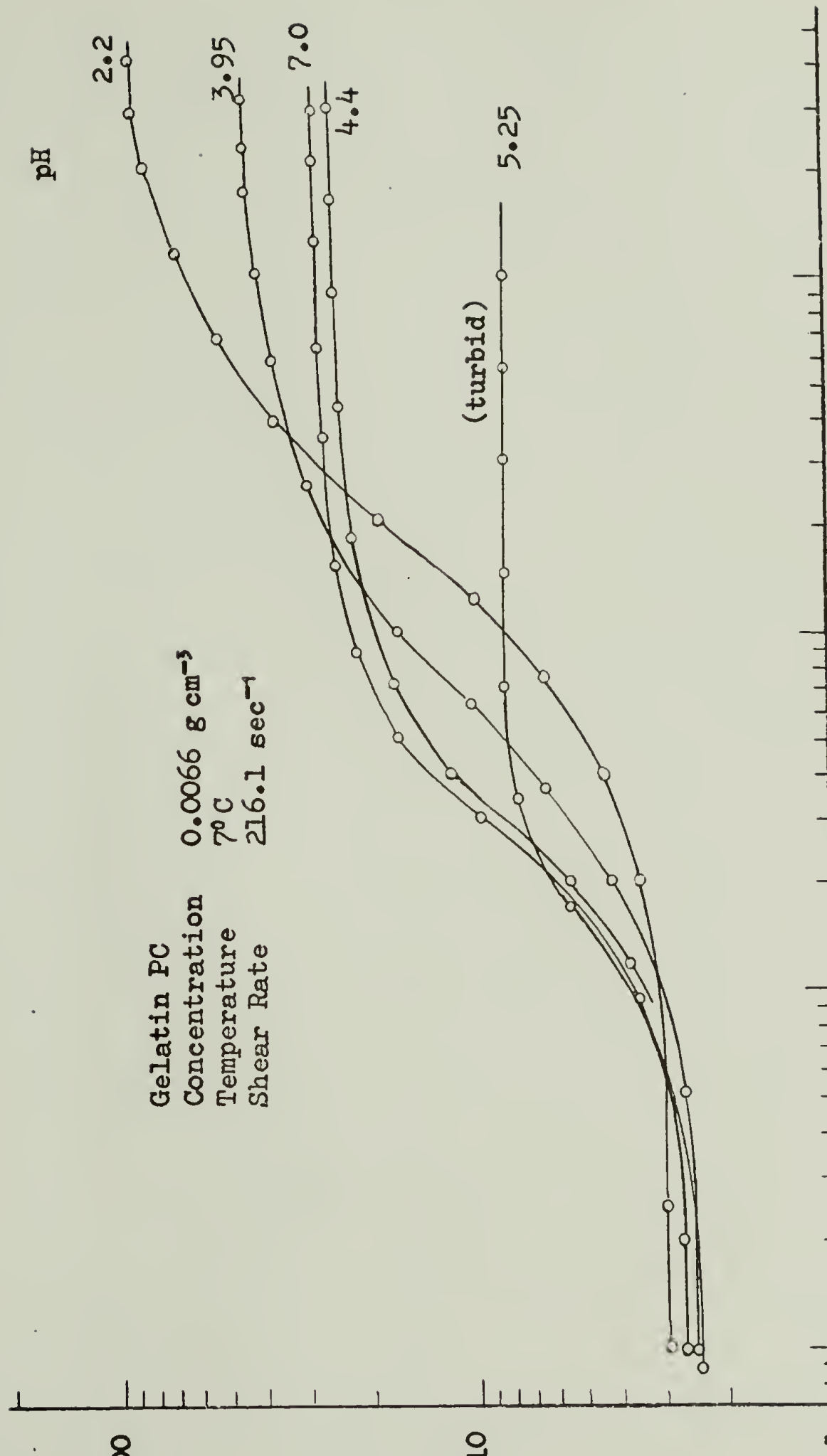


FIGURE VII-E1. VISCOSITY vs. TIME - EFFECT OF pH

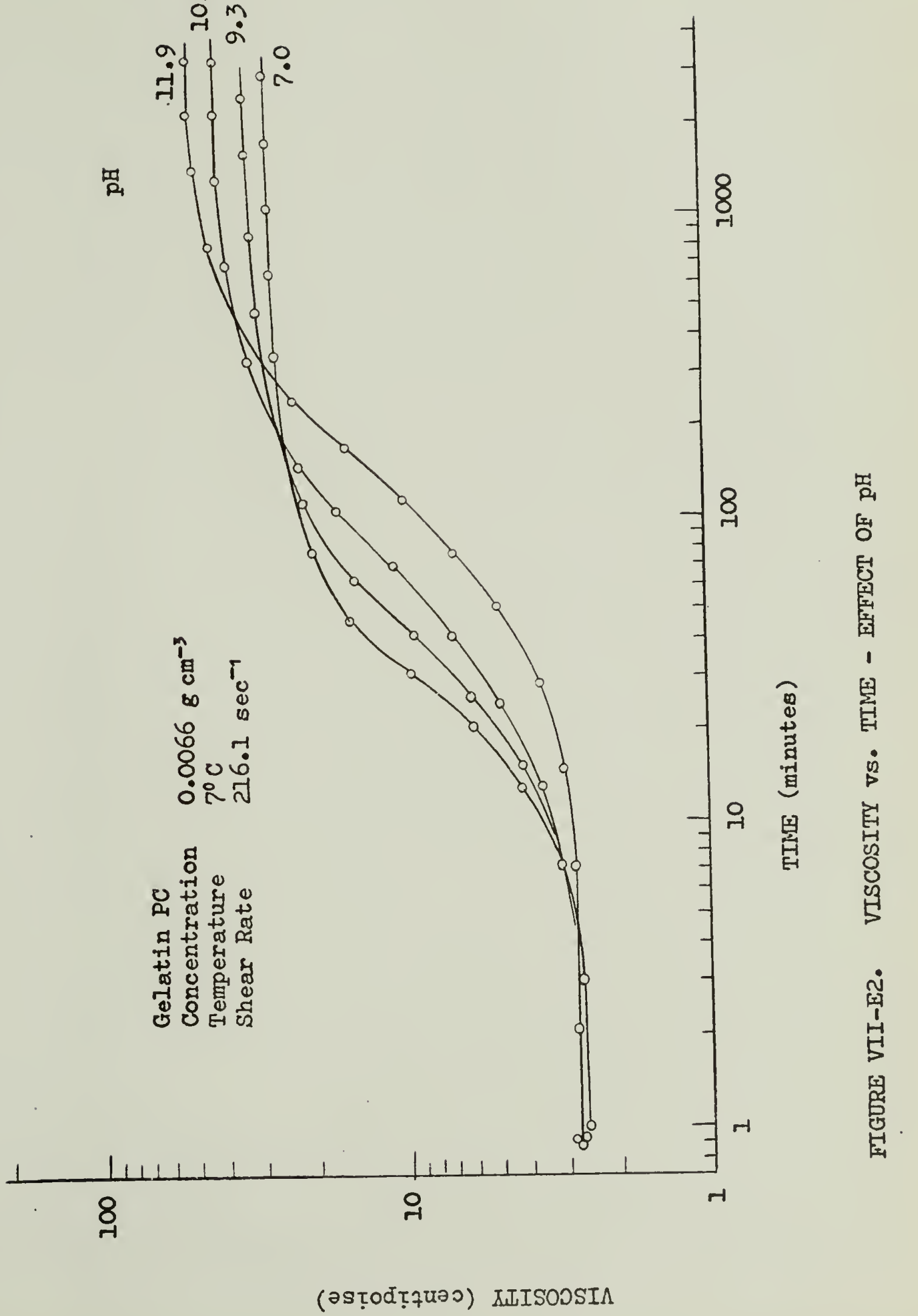


FIGURE VII-E2. VISCOSITY vs. TIME - EFFECT OF pH

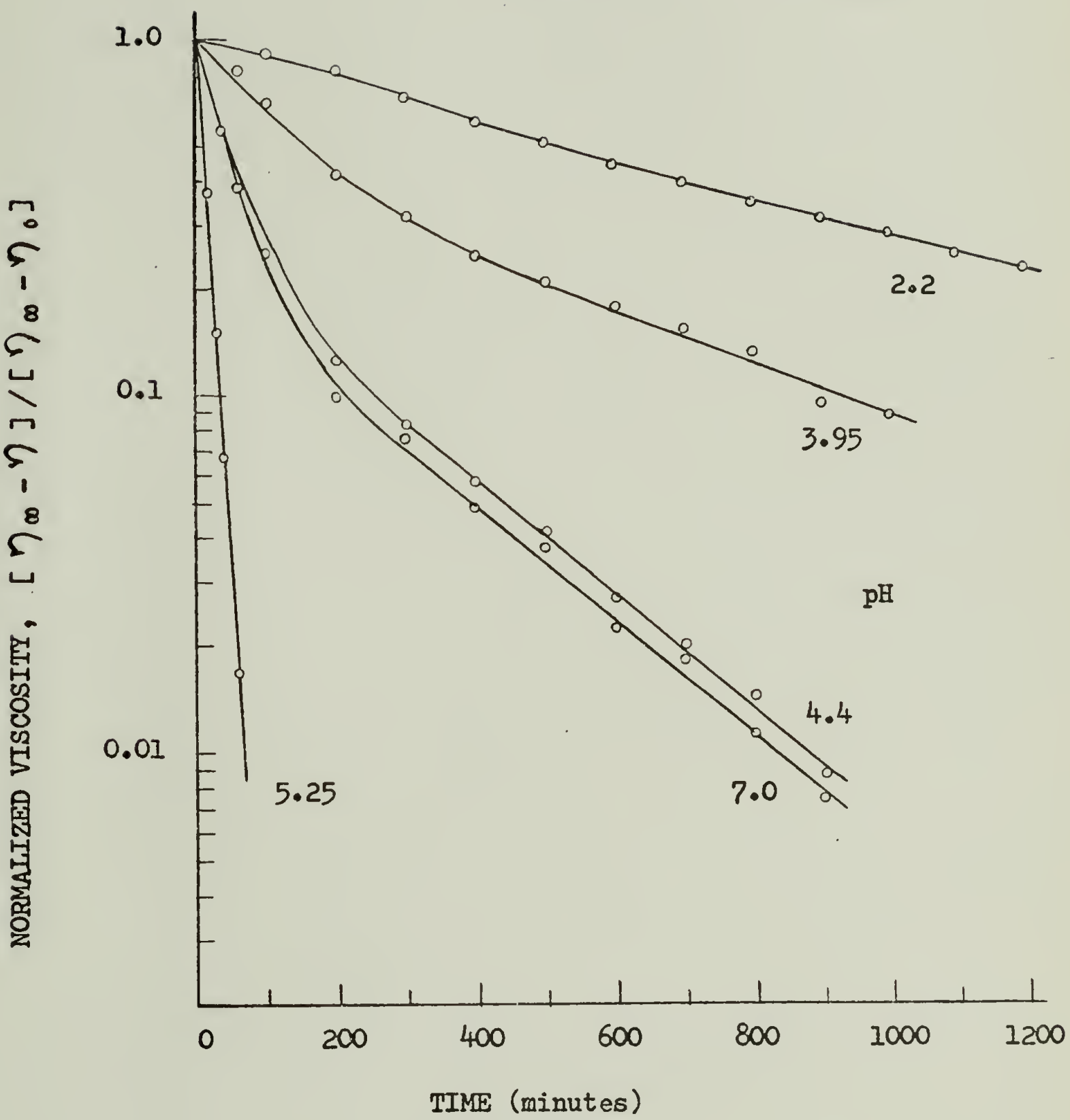


FIGURE VII-E3. NORMALIZED VISCOSITY vs. TIME - EFFECT OF pH

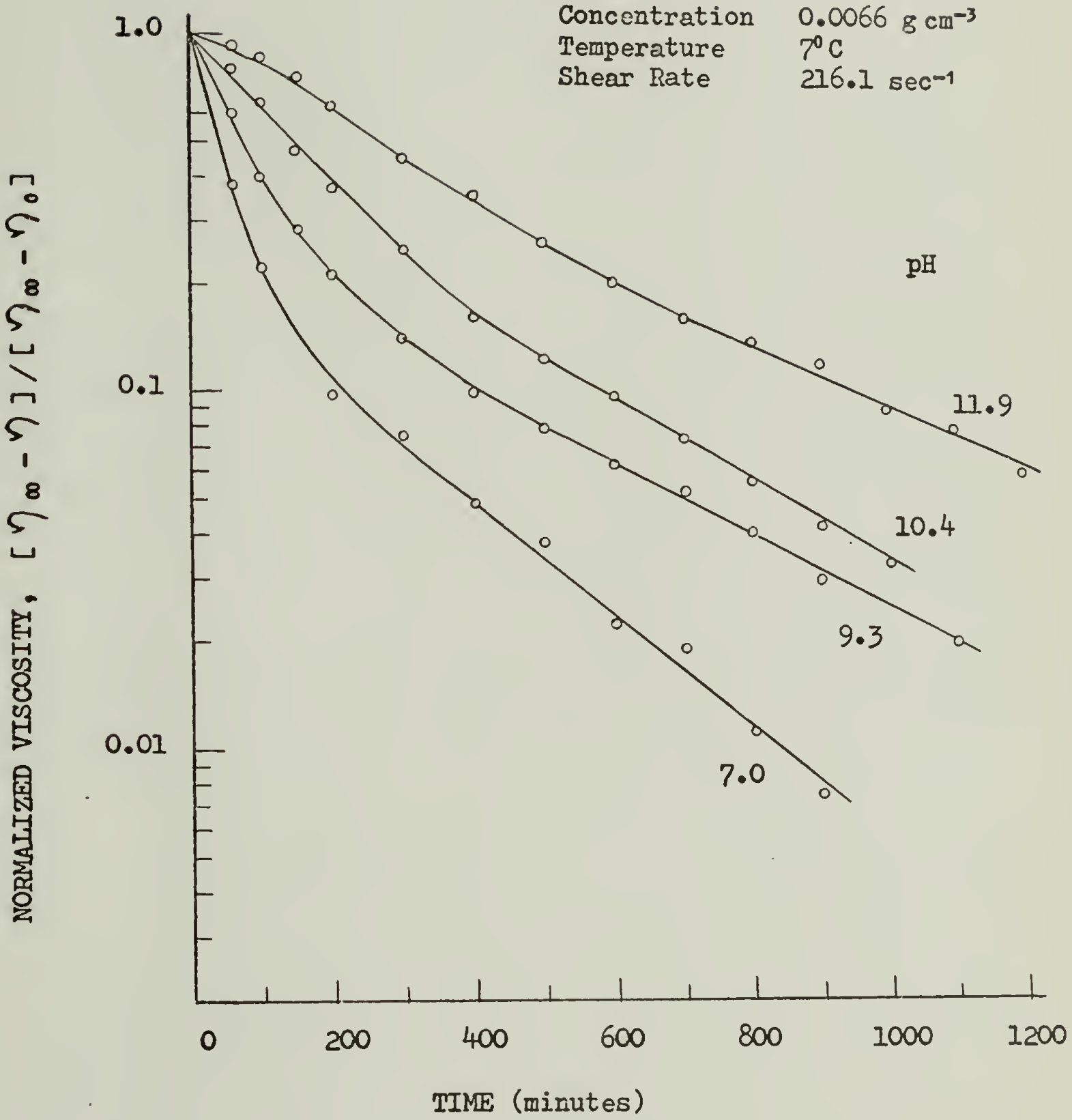


FIGURE VII-E4. NORMALIZED VISCOSITY vs. TIME - EFFECT OF pH

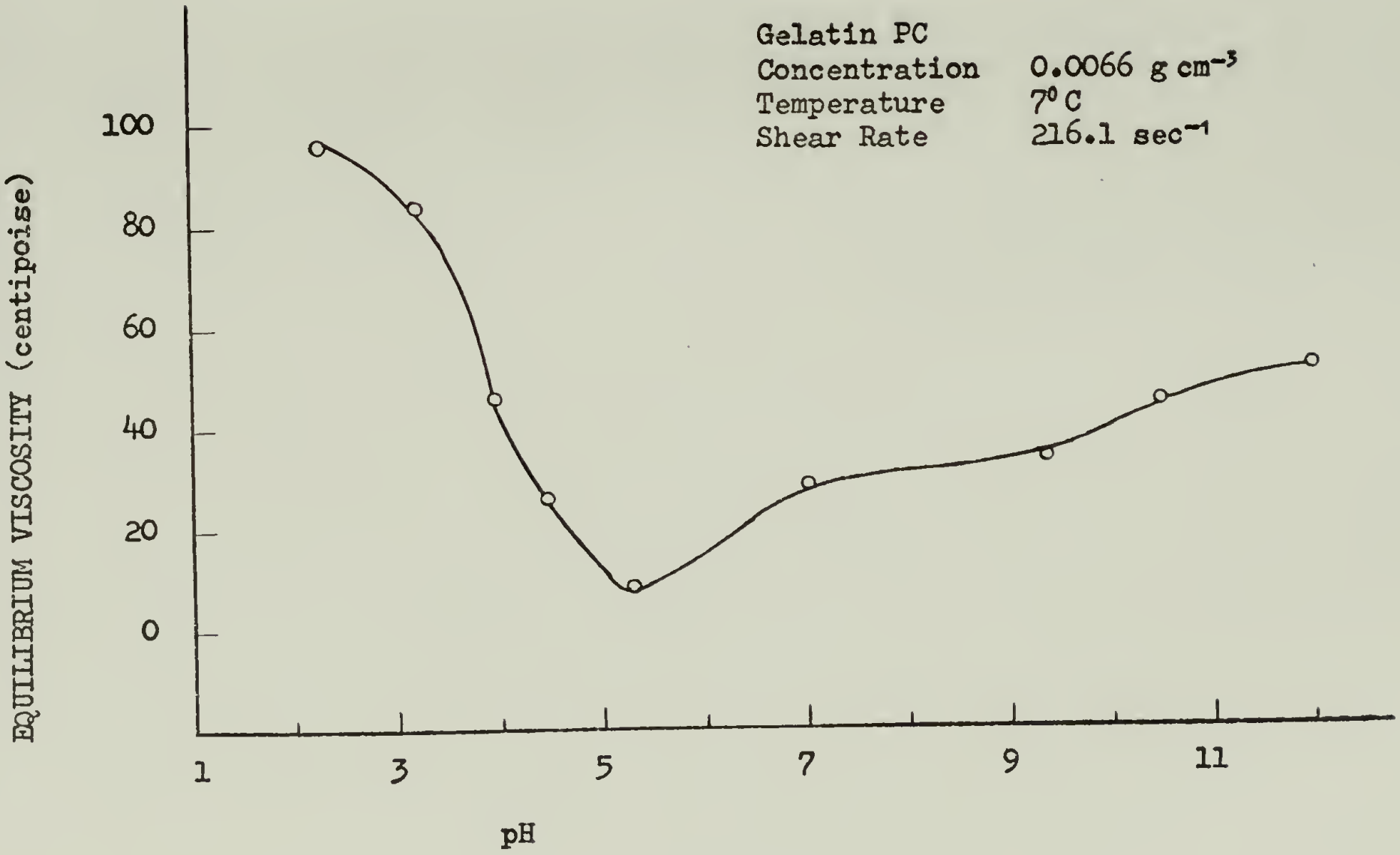


FIGURE VII-E5. EQUILIBRIUM VISCOSITY vs. pH

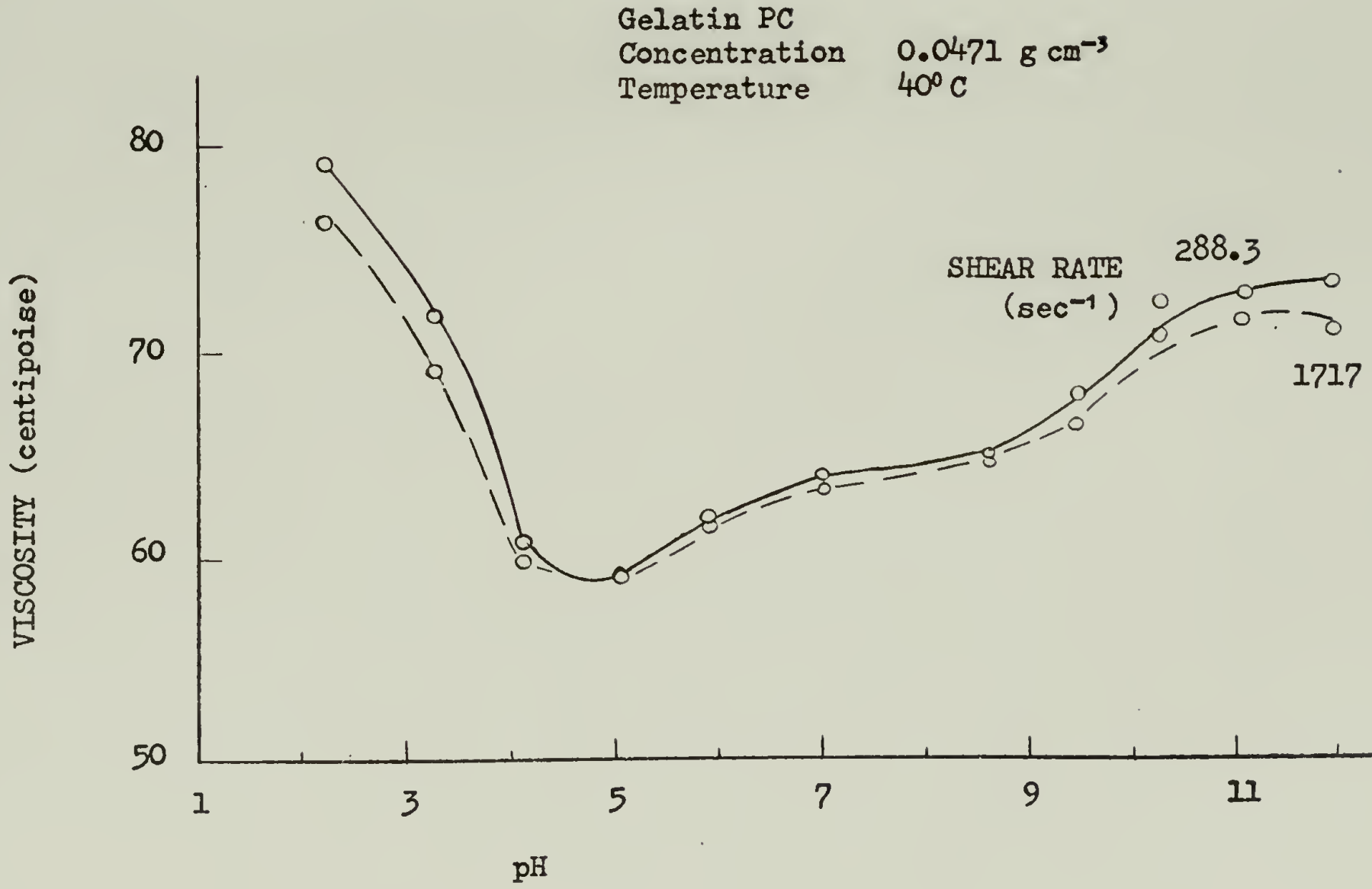


FIGURE VII-E6. SOLUTION VISCOSITY vs. pH

tion at 40° C. Based on the similarities in these curves it appears that the gelled network structure is influenced considerably by the initial chain configuration.

VII-F. Kinetics and Temperature

Temperature plays an important role in determining the nature and potential for gelation. Network systems such as gelatin which are stabilized by secondary forces such as hydrogen bonds, rather than by primary covalent bonds, exhibit a strong dependence upon the solvent environment and the system temperature. These bonds represent a delicate balance between solute-solvent and solute-solute interactions.

As demonstrated in the preceding studies, the structure of a network gel is determined by the mode of gel formation. For example, if network formation is brought about at high gelatin concentration, random chain contacts lead to the formation of network structures composed of non-aligned small chain segments. Another way for altering structure is to change the rate by means of temperature. Gelation occuring near the melting zone will permit a greater ordering of the interacting segments, leading to the development of large crystallites comprised of uniformly aligned chains. Tempering a gel formed as randomly aligned small chains will also permit a gradual rearrangement to a more ordered structure.[30]

Figures VII-F1 and F2 present viscosity growth and normalized kinetic rate curves for gelation as a function of temperature. Although differences in initial viscosities are noticeable over the temperature range of 2.5 to 20° C, the final equilibrium viscosities are not significantly different (excluding the system gelled at 20° C), thus indicating the final equilibrium viscosity to be independent of temperature. Unfortunately these data do not differentiate the type of structure formed at each temperature. Since the rates are much slower at the higher temperatures, more ordered and stronger structures may have formed, thus attaining the strengths characteristic of gelatin solutions gelled at lower temperatures. Perhaps temperatures near 20° C may impose a limit for forming network structures comparable to those gelled below this temperature. This

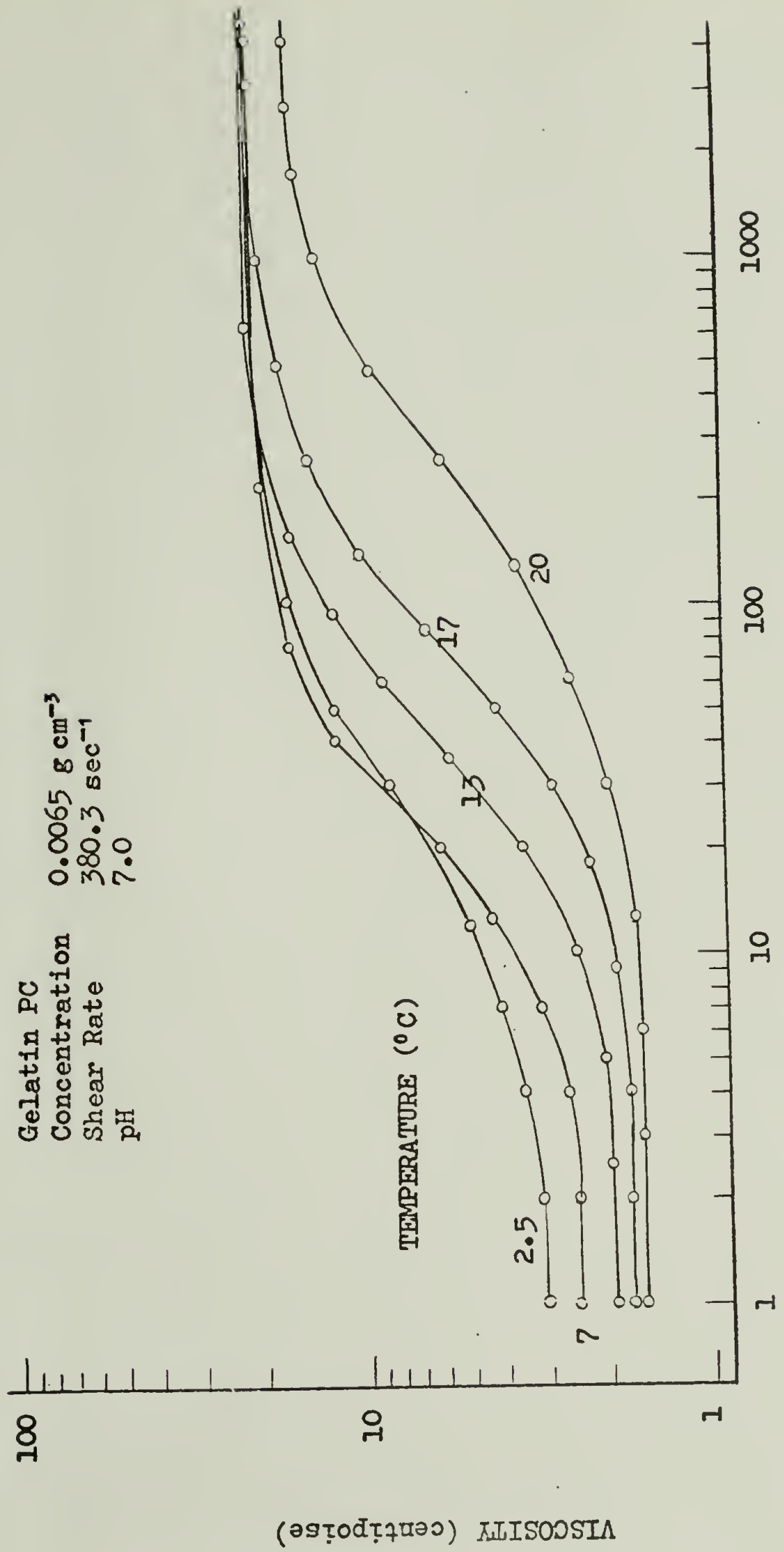


FIGURE VII-TI • VISCOSITY vs. TIME - EFFECT OF TEMPERATURE

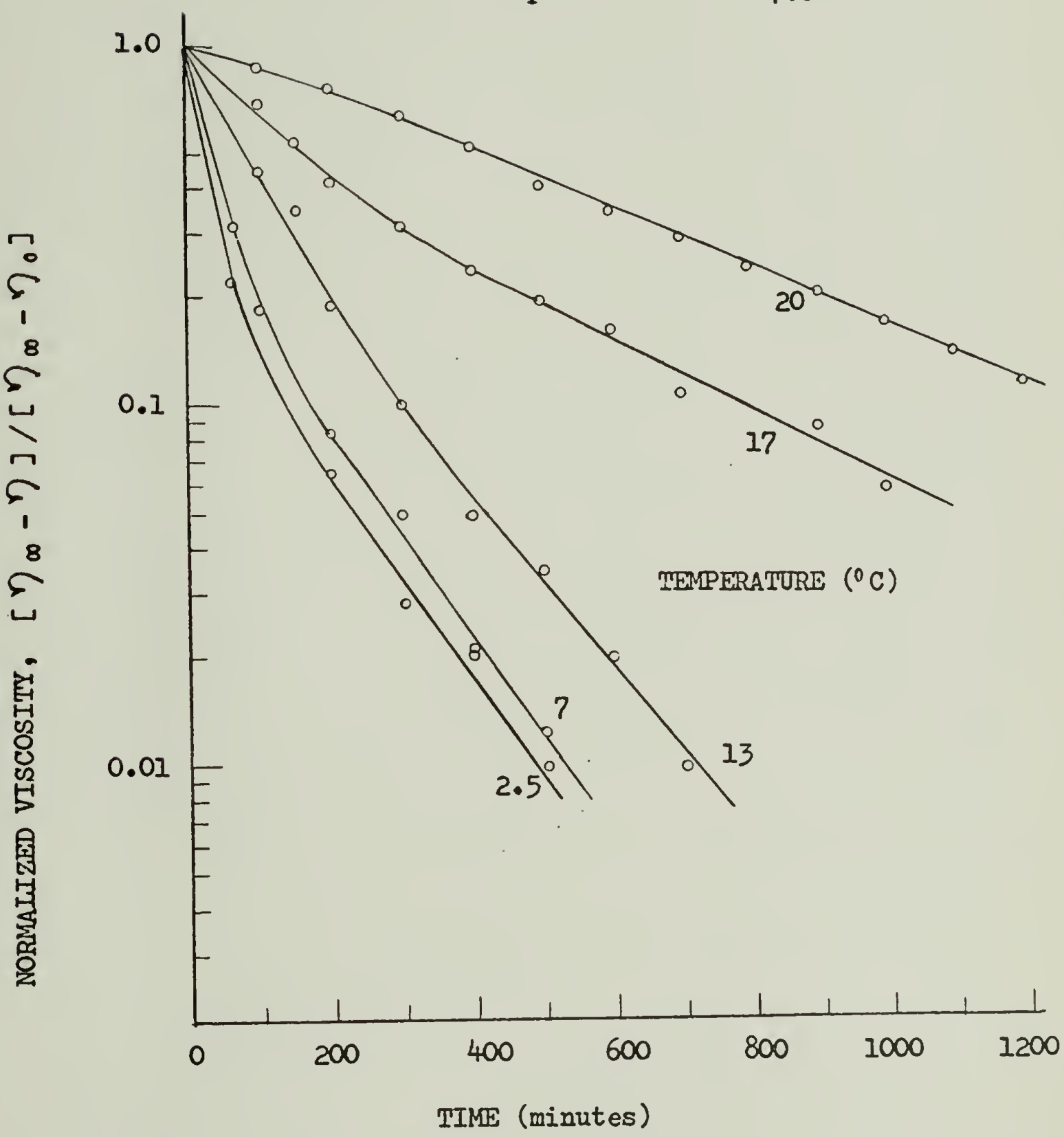


FIGURE VII-F2. NORMALIZED VISCOSITY vs. TIME - EFFECT OF TEMPERATURE

is entirely acceptable since temperature above 25° C will permit no gelation whatsoever.

Figure VII-F3 demonstrates the complete reversibility of the gelatin network. After attaining equilibrium (curve A) the system was rapidly brought to 40° C (curve B). The viscosity decreases quickly returning to the original viscosity characteristic of the 40° C solution. Because of the time lag in heat conduction thru the measuring unit, these data should not be analyzed kinetically.

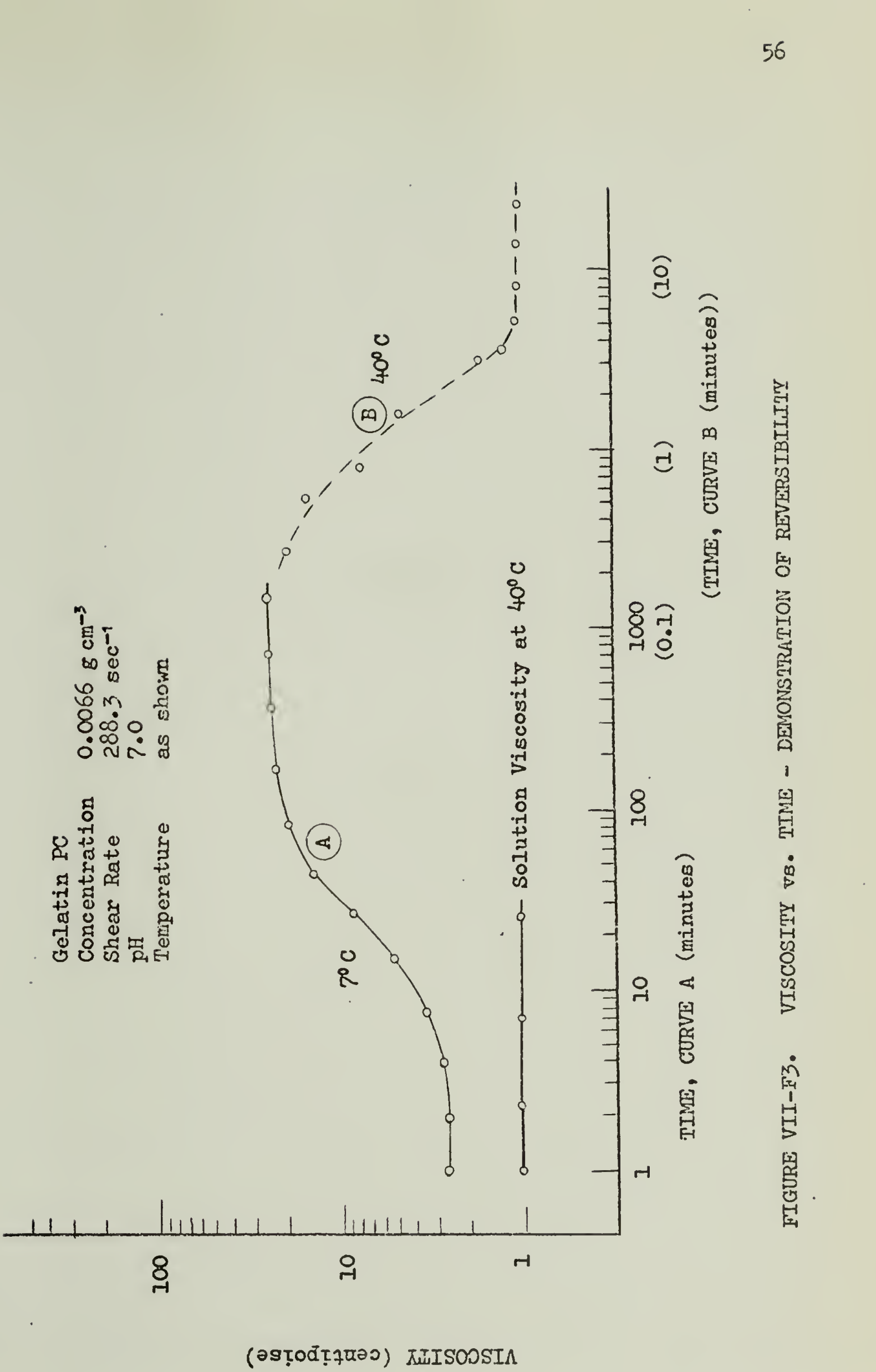


FIGURE VII-F3. VISCOSITY vs. TIME - DEMONSTRATION OF REVERSIBILITY

VIII. OPTICAL ROTATORY DISPERSION STUDIES

Optical rotation has been used for many years[17] as a sensitive probe to follow conformational transitions in proteins such as denaturation or renaturation. The basis of this rotatory power of polypeptides and proteins lies first in the presence of an asymmetric carbon in the majority of the amino acids, and second in the asymmetric spatial arrangement of the peptide backbone or conformation of the protein.[11]

Analysis of helical conformations can be made by use of the Moffitt equation. Moffitt considered the α-helix as a rigid array of identical chromophores which interact to form a cooperative unit acting as a single exciton system.

The phenomenologically developed Moffitt equation is

$$[\alpha]_\lambda = \frac{100}{MRW}\,\frac{[n^2+2]}{3}\,\frac{a_0\lambda^2}{\lambda^2-\lambda_0^2} + \frac{b_0\lambda_0^4}{[\lambda^2-\lambda_0^2]^2}$$

which can be rearranged to form

$$[M_f]\left[\frac{\lambda^2}{\lambda_0^2} - 1\right] = a_0 + b_0\left[\frac{\lambda^2}{\lambda_0^2} - 1\right]^{-1}$$

where $[\alpha]_\lambda$ = Specific rotation $= \frac{100}{LC}$

α_λ = Observed rotation in degrees

L = Path length in decimeters

C = Concentration of optically active solute in grams per 100 ml

MRW = Mean residue molecular weight

n = Refractive index of solvent at wavelength

λ_0 = 2120 Å

a_0 = Constant

b_0 = Constant

Mf = Reduced mean residue rotation = $\frac{MRW}{100} \frac{3}{[n^2+2]} [\alpha]_\lambda$

When $[Mf][\lambda^2 / \lambda_0^2 - 1]$ is plotted against $[\lambda^2 / \lambda_0^2 - 1]^{-1}$, b_0 is obtained from the slope and a_0 from the intercept.

b_0 and λ_0 are principally functions of the helical backbone, independent of both the side chains and the environment, and a_0 represents both intrinsic and residue rotations, present irrespective of the helix, and rotations due to interactions within the helix. The sign of b_0 is indicative of the sense of helix. Generally a value of -630 is associated with the right handed helix of the L-amino acids, whereas a +630 is associated with the left handed helix of the D-amino acids.[11]

Figure VIII-1 shows Moffitt plots using wavelengths of 3663 to 5461 Å for Gelatin PC in solution at 40° C and after achieving a gel equilibrium at 7° C. The value of b_0 for a random chain is usually zero but can be much less. The non-zero value of -160 shown for both 0.2 and 0.5 percent concentrations at 40° C may actually reflect a random configuration or perhaps a mixture of random and helical chains, or interference.

Helically wound collagen generally has a b_0 value close to -630. The b_0 values for the 0.2 and 0.5 percent concentrations which were gelled at 7° C for 3000 minutes were each determined to be -165, essentially no different from the original b_0. As seen from this plot, the data at the high wave length (5461 Å) are unexplainably high. The higher wave lengths would normally be expected to give the more accurate results. In any event, inorder to compare the results, these data were disregarded and a straight line was fit to the remaining data.

Based on the unchanged b_0 results, it might be concluded that no (additional ?) helical structure forms during gelation. However other factors may be influencing the observed rotatory dispersion such as interference from the growing gel network or chirality which compensates for

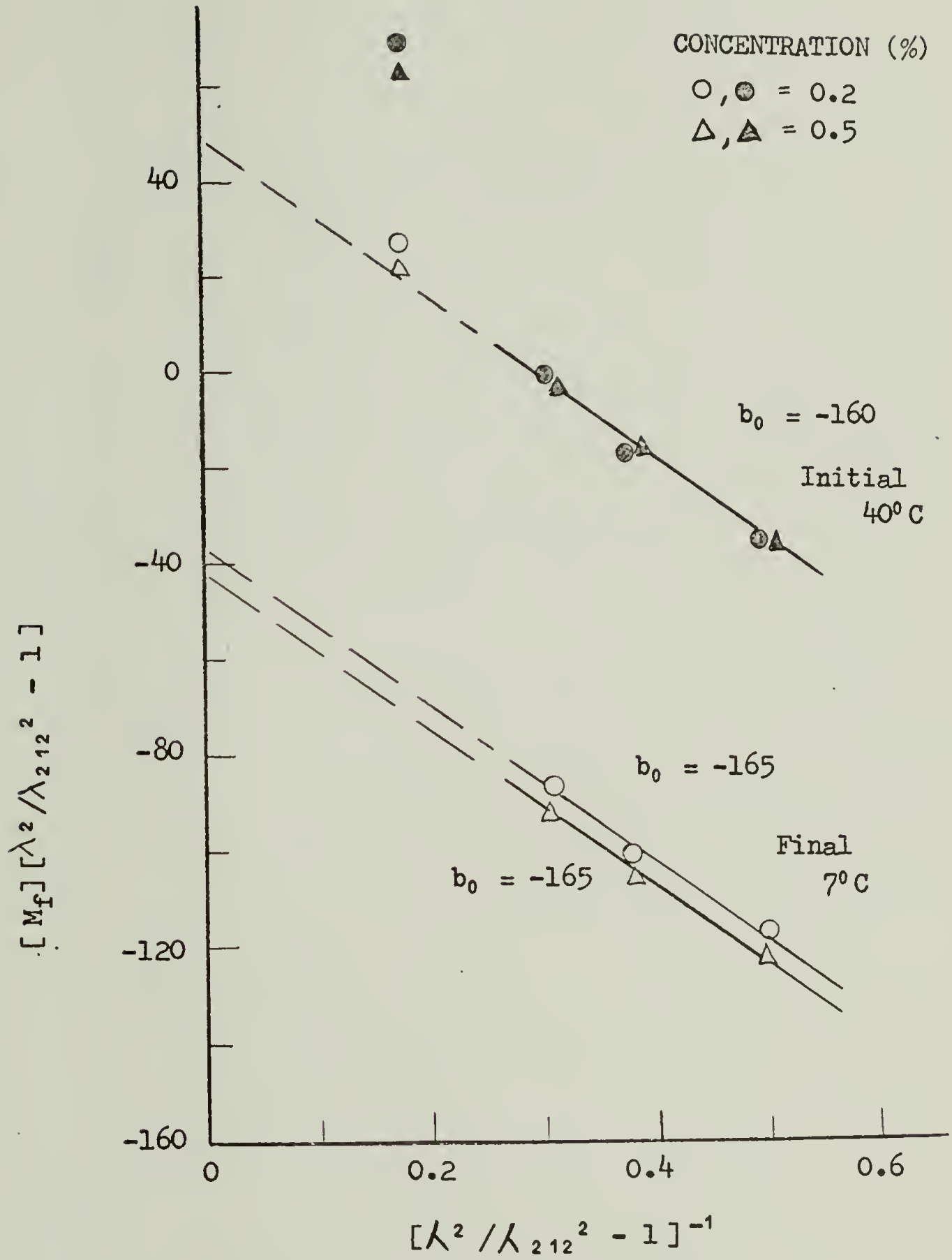


FIGURE VIII-1. MOFFITT PLOTS OF GELATIN PC IN SOLUTION AT 40° C AND AFTER ACHIEVING A GEL EQUILIBRIUM AT 7° C

any helicity formed.

The automatic feature of the polarimeter allowed rotation measurements to be taken as a function of time. These are plotted as specific rotation versus time in Figure VIII-2. An increasing growth curve results, similar to the viscosity growth curves, which achieves an equilibrium level with time. Based on the unchanged b_0 values and a practical consideration of what may be occuring during gelation, helix generation could not be solely responsible for this change.

The optical rotatory data and experimental specifics are listed in Appendix H. Due to obvious limitations, neither samples of sheared gelatin gel nor gelatin under going shear were studied with the polarimeter. Introducing shear formed gel into the polarimeter cell would disrupt the equilibrium yielding uninterpretable results.

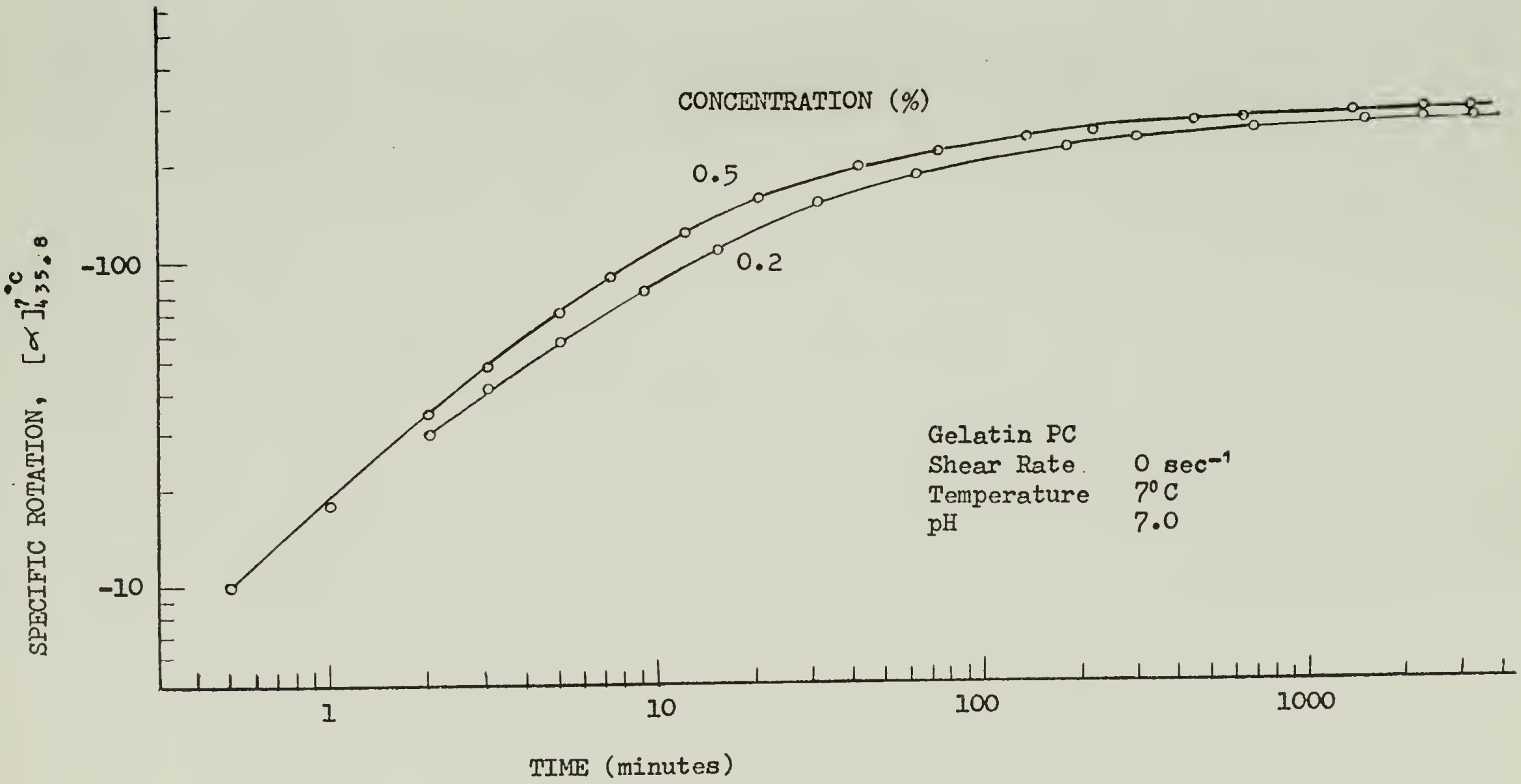


FIGURE VIII-2. SPECIFIC ROTATION vs. TIME - EFFECT OF CONCENTRATION

IX. SUMMARY

The gelation kinetics are influenced considerably by shear rate, concentration, temperature, and pH. The rate of gelation increases as shear rate and concentration increase, as temperature decreases, and as the pH converges on the isoelectric point (pH~5) from either a basic or acidic system. Each of these variables, with the possible exception of temperature, appear to alter the type of structure formed during gelation under shear.

As observed by numerous other investigations, the mechanism of gelation is considerably complex. Viscometric techniques are capable of classifying the general transformations occuring during gelation, however, viscometry is not usually used to differentiate subtle molecular interactions, especially when these conformational or configurational changes occur simultaneously or consecutively. Single chain helical coil formation, helical winding of two or more molecules, chain segment-water molecule interactions, and interchain interactions to form cross linked networks are probably the major gelation transformation phenomenon.

Most of the viscosity data of this study appear to indicate a two stage mechanism for gelation, the inflection or divergence of these mechanisms generally occuring around 100 to 200 minutes. The first molecular scheme is felt to involve single and interchain helical generation and the formation of weak intermolecular cross links thru the non specific interaction of the ordered segments of the different intimately entangled molecules. The second scheme probably includes the slower additional collagen folding and gel aggregate formation at the junction points and the slower aging or tempering process resulting in the development of stable crystallites.

The rate of gelation increases as a function of increasing shear rate (over the range of 27.3 to 1717 sec^{-1}) in both a short time and long time

consideration. This observed increase in viscosity growth rate with shear rate is to be expected if the build up of structure is controlled by the rate at which particles are brought into contact with each other and with the already existing aggregates. The slopes of constant structure curves (Figure VII-B5) increase as a function of an increasing shear rate on the equilibrium flow curve, demonstrating an equilibrium gel structure which is different in structural composition.

The shear rate history has quite an impact on the nature of the geiled network. Gelatin gel structure formed under zero shear is more resistant to shear network degradation than gel formed during shear. Even at very low shear rates, the structure formed will decay with time under a higher shear, achieving an equilibrium viscosity which is characteristic for the higher rates.

The increase in rate of structure formation as a function of increasing concentration may reflect the relative chain mobility. At higher concentrations the chain mobility will be restricted much sooner and thus not allow for the slower rate of development of stronger or more ordered bonds.

At a pH of 5.25 corresponding to a value near the isoelectric point, the equilibrium viscosity reaches a minimum, while its rate of growth to equilibrium is very fast. The expansion that accompanies the charging of the gelatin chain thru altering the pH from the isoelectric point increases viscosity as well as time to gel equilibrium formation. Based on the similarities in equilibrium viscosity at 7°C and solution viscosity above 25° C, it appears that the gelled network structure is influenced considerably by the initial chain configuration.

Although differences in initial viscosities are noticeable over the temperature range of 2.5 to 20°C, the final equilibrium viscosities are not significantly different, thus indicating the final equilibrium viscosity to be independent of temperature for systems gelled below 20°C. These

data however do not differentiate the type of structure formed at each temperature.

Optical rotatory dispersion studies failed to indicate an increase in the helical content of gelled gelatin over gelatin in a randomly coiled conformation at 40° C. However, this data is highly suspect. Interference from the growing molecular network may be influencing the results and consequently masking any rotation which would be created thru helix formation.

X. REFERENCES

1. Bauer, W.A., and Collins, E.A., "Thixotropy and Dilatancy", in Rheology, (Eirich, F.R., ed.), 4, Academic Press, New York, 423-459, (1967).

2. Bird, R.B., Stewart, W.E., and Lightfoot, E.N., Transport Phenomena, John Wiley and Sons, Inc., New York, 95-96, 356, (1960).

3. Boedtker, H., and Doty, P., "A Study of Gelatin Molecules, Aggregates and Gels", Journal of Physical Chemistry, 58, 968-983, (1954).

4. Boedtker, H., and Doty, P., "The Native and Denatured States of Soluble Collagen", Journal of the American Chemical Society, 78, 4267-4280, (1956).

5. Brandrup, J., and Immergut, E.H., Polymer Hand Book, Interscience, New York, IV-43, IV-62, (1966).

6. Burtonite Company Sales Publication, "Gelatin: Chemical and Physical Properties", 14, The Burtonite Company, P.O. Box 7, Nutley, New Jersey.

7. Contraves AG Zurich, Instruction Manual for Measuring System MS-O 4 ml, Publication 4211 E 350 BLe.

8. Coopes, I.H., "Helix Formation and Formaldehyde Crosslinking in Gelatin Solutions", Journal of Polymer Science, A-1, 8, 1793-1811, (1970).

9. Courts, A., and Stainsby, G., "Evidence for Multi-Chain Gelatin Molecules", in Recent Advances in Gelatin and Glue Research, (Stainsby, G., ed.), Pergamon Press, New York, 100-105, (1958).

10. Drake, D.A., and Veis, A., "Interchain Interactions in Collagen-Fold Formation. I. The Kinetics of Renaturation of -Gelatin", Biochemistry, 3, 135-145, (1964).

11. Fasman, G.D., "Optical Rotatory Dispersion", in Methods in Enzymology, (Colowick, S.P.,and Kaplan, N.O., ed.), 6, Academic Press, New York, 928-957, (1963).

12. Flory, P.J., and Weaver, E.S., "Helix Coil Transitions in Dilute Aqueous Collagen Solutions", Journal of the American Chemical Society, 82, 4518-4525, (1960).

13. Gouinlock, E.V., Flory, P.J., and Scheraga, H.A., "Molecular Configuration of Gelatin", Journal of Polymer Science, 16, 383-395, (1955).

14. Haas, H.C., Manning, M.J., and Hollander, S.A., "Differential Thermal Analysis of Thermally Reversible Gels", in Analytical Calorimetry, (Porter, R.S., and Johnson, J.F., ed.), 2, Plenum Press, New York, 211, (1970).

15. Halberstadt, E.S., Henisch, H.K., Nickl, J., and White, E.W., "Gel Structure and Crystal Nucleation", Journal of Colloid and Interface Science, 29, 469-471, (1969).

16. Harrington, W.F., and Von Hippel, P.H., "The Structure of Collagen and Gelatin", in Advances in Protein Chemistry, (Anfinsen, C.B., et. al., ed.), 16, Academic Press, New York, 1-138, (1961).

17. Jirgensons, B., "Optical Rotatory Dispersion of Proteins and Other Macromolecules", in Molecular Biology Biochemistry and Biophysics, (Kleinzeller, A., Springer, G.F., and Wittmann, H.G., ed.), Springer-Verlag, New York, (1969).

18. Jones, L.G., and Brodkey, R.S., "Thixotropic Behavior of a Colloidal Suspension", in Proceedings of the Fifth International Congress on Rheology, (Onogi, S., ed.), 2, 267-280, (1970).

19. Joye, D.D., and Poehlein, C.W., "Characterization of Thixotropic Behavior", Transactions of the Society of Rheology, 15 (1), 51-61, (1971).

20. Lee, K.H., and Brodkey, R.S., "Time Dependent Polymer Rheology Under Constant Stress and Under Constant Shear Conditions", Transactions of the Society of Rheology, 15 (4), 627-646, (1971).

21. Macsuga, D.D., "Thermal Transitions in Gelatin: Optical Rotation and Enthalpy Changes", Biopolymers, 11, 2521-2532, (1972).

22. Middleman, S., The Flow of High Polymers, Interscience, New York (1968).

23. Moore, F., and Davies L.J., "The Consistency of Ceramic Slips", Transactions of the British Ceramic Society, 55, 313-338, (1956).

24. Petrie, S.E.B., and Becker, R., "Thermal Behavior of Aqueous Gelatin Solutions", in Analytical Calorimetry, (Porter, R.S., and Johnson, J.F., ed.), 2, Plenum Press, New York, 225-238, (1970).

25. Piez, K.A., "Molecular Weight Determination of Random Coil Polypeptides from Collagen by Molecular Sieve Chromatography", Analytical Biochemistry, 26, 305-312, (1968).

26. Pouradier, J. "Structural Studies of Gelatin. IX. Rigid Gel Bond Energy", Journal de Chimie Physique, 64, 1616-1620, (1967).

27. Pouradier, J., Roman, J., and Venet, A.M., "Structural Studies of Gelatin. III. Variations of Chemical Properties with Molecular Weight", Journal de Chimie Physique, 47, 887-891, (1950).

28. Pouradier, J., and Venet, A.M., "Structural Studies of Gelatin. V. Degradation of Gelatin in Isoelectric Solution", Journal de Chimie Physique, 49, 239-244, (1952).

29. Scatchard, G., Oncley, J.L., Williams, J.V., and Brown, A., "Size Distribution in Gelatin Solutions. Preliminary Report", Journal of the American Chemical Society, 66, 1980-1981, (1944).

30. Veis, A., The Macromolecular Chemistry of Gelatin, Academic Press, New York, (1964).

31. Veis, A., Anesey, J., and Cohen, J., "Acid-Precursor Gelatin Structure and Significance in the Collagen-Gelatin Transition Process", in Recent Advances in Gelatin and Glue Research, (Stainsby, G., ed.), Pergamon Press, New York, 155-163, (1958).

32. Veis, A., and Cohen, J., "On the Molecular Configuration of Gelatin", Journal of Polymer Science, 26, 113-116, (1957).

33. Veis, A., and Cohen, J., "A Non-Random Disaggregation of Intact Skin Collagen", Journal of the American Chemical Society, 78, 6238-6244, (1956).

34. Veis, A., Eggenberger, D.N., and Cohen, J., "The Degradation of Collagen. III. Characterization of Soluble Products of Mild Acid Degradation", Journal of the American Chemical Society, 77, 2368-2374, (1955).

35. Veis, A., and Schnell, J., Symposium on Fibrous Proteins - Australia 1967, (Crewther, W.G., ed.), Butterworths, Australia, 193, (1968).

36. Ward, A.G., and Saunders, P.R., "The Rheology of Gelatin", in Rheology, (Eirich, F.R., ed.), 2, Academic Press, New York, 313-362, (1958).

37. Watase, M., and Arakawa, K., "Rheological Properties of Concentrated Gelatin Gels - The Effect of Urea on Rheological Properties of Gelatin Gels", Nippon Kagaku Zasshi, 90, 658-663, (1969).

38. Williams, J.W., Saunders, W.M., and Cicirelli, J.S., "Size Distribution Analysis in Plasma Extender Systems. I. Gelatin", Journal of Physical Chemistry, 58, 774-782, (1954).

ADDITIONAL REFERENCES (UNCITED)

- Ananthanarayanan, S., and Veis, A., "The Molecular Properties of Monomeric and Acid-Soluble Collagens. Low Shear Gradient Viscosity and Electric Birefringence", Biopolymers, 11, 1365-1377, (1972).

- Beier, G., and Engel, J., "The Renaturation of Soluble Collagen. Products Formed at Different Temperatures", Biochemistry, 5, 2744-2755, (1966).

- Billington, E.W., and Huxley, A.S., "Some Measurements of the Un-Steady Motion of a Thixotropic Fluid Confined Between Rotating Coaxial Cylinders", Journal of Colloid and Interface Science, 22, 257-268, (1966).

- Bradbury, E.M., and Martin, C., "The Effect of the Temperature of preparation on the Mechanical Properties and Structure of Gelatin Films", Proceedings of the Royal Society of London, A-214, 183-192, (1952).

- Burdygina, G.I., Fridman, I.M., Kozlov, P.V., and Kargin, V.A., Features of Structural Transitions in Gelatin over a Wide Range of Temperatures", Vysokomol. Soyed., All, No. 1, 118-131, (1969), (English Translation).

- Cerny, L.C. Grauz, J.D., and James, H., "A Viscometric Study of Dilute Collagen Solutions", Biorheology, 6, 161-168, (1970).

- Denny, D.A., and Brodkey, R.S., "kinetic Interpretation of Non-Newtonian Flow", Journal of Applied Physics, 33, 2269-2274, (1962).

- Dinsdale, A., and Moore, F., Viscosity and Its Measurement, Reinhold, New York, (1962).

- Engle, J., "Investigation of the Denaturation and Renaturation of Soluble Collagen by Light Scattering", Archives of Biochemistry and Biophysics, 97, 150-158, (1962).

- Ferry, J.D., and Eldridge, J.E., "Studies of the Cross-Linking Process in Gelatin Gels. I.", Journal of Physical Colloid Chemistry, 53, 184-196, (1949).

- Fukada, E., and Date, M., "Viscoelastic Properties of Collagen Solutions in Dilute Hydrochloric Acid", Biorheology, 1, 101-109, (1963).

- Gary-Bobo, C.M., and Lindenberg, A.B., "The Behavior of Non-Electrolytes in Gelatin Gels", Journal of Colloid Science, 29, 702-709, (1969).

- Haas, H.C., Chiklis, C.K., and Moreau, R.D., "Synthetic Thermally Reversible Gel Systems. III.", Journal of Polymer Science, A-1, 8, 1131-1145, (1970).

- Harrington, W.F., and Karr, G.M., "Collagen Structure in Solution. II. Analysis of Reforming Kinetics in Terms of Nucleation and Growth Processes", Biochemistry, 9, 3725-3733, (1970).

- Harrison, M.A., Morgan, P.H., and Park, G.S., "Thermoreversible Gelation in Polymer Systems. I. The Sol-Gel Transition in Dilute Poly-(Vinyl Chloride) Gels", European Polymer Journal, 8, 1361-1373, (1972).

- Harrington, W.F., and Rao, N.V., "Collagen Structure in Solution. I. Kinetics of Helix Regeneration in Single-Chain Gelatins", Biochemistry, 9, 3714-3724, (1970).

- Hauschka, P.V., and Harrington, W.F., "Collagen Structure in Solution. III. Effect of Cross-Links on Thermal Stability and Refolding Kinetics", Biochemistry, 3734-3745, (1970).

- Hauschka, P.V., and Harrington, W.F., "Collagen Structure in Solution. IV. Conformational Properties of Refolded Cross-Linked Chains", Biochemistry, 9, 3745-3754, (1970).

- Hauschka, P.V., and Harrington, W.F., "Collagen Structure in Solution. V. Kinetic Mechanism of Refolding of Cross-Linked Chains", Biochemistry, 9, 3754-3763, (1970).

- Hermans, J., "The Viscosity of Concentrated Solutions of Rigid Rodlike Molecules (Poly- -Benzyl-L-Glutamate in m-Cresol)", Journal of Colloid Science, 17, 638-648, (1962).

- Joly, M., A Physico-Chemical Approach to the Denaturation of Proteins, Academic Press, New York, (1965).

- McBride, O.W., and Harrington, W.F., "Helix-Coil Transition in Collagen. Evidence for a Single-Stranded Triple Helix", Biochemistry, 9, 1499-1514, (1967).

- Mueller, W.S., "Effect of Thermal Treatments on Physico-Chemical Properties of Gelatin in Connection with its use in Ice Cream", P.H.D. Thesis, University of Massachusetts, (1939).

- Piez, K.A., and Carrillo, A.L., "Helix Formation by Single and Double Chain Gelatins from Rat Skin Collagen", Biochemistry, 3, 908-914, (1964).

- Piez, K.A., Eigner, E.A., and Lewis, M.S., "The Chromatographic Separation and Amino Acid Compositon of the Subunits of Several Collagens", Biochemistry, 2, 58-66, (1963).

- Pouradier, J., and Venet, A.M., "Structural Studies of Gelatin. II. Variation of Mechanical Properties with Molecular Weight", Journal de Chimie Physique, 47, 391-398, (1950).

- Ramachandran, G.N., and Chandrasekharan, R., "Interchain Hydrogen Bonds Via Bound Water Molecules in the Collagen Triple Helix", Biopolymers, 6, 1649-1658, (1968).

- Stamberger, P., "The Mechanical Stability of Colloidal Dispersions", Journal of Colloid Science, 17, 146-154, (1962).

- Swift, D.L., and Friedlander, S.K., "The Coagulation of Hydrosols by Brownian Motion and Laminar Shear Flow", Journal of Colloid Science, 19, 621-647, (1964).

- Traub, W., and Piez, K.A., "The Chemistry and Structure of Collagen", in Advances in Protein Chemistry, (Anfinsen, C.B., et. al., ed.), 25, Academic Press, New York, 243-352, (1971).

- Van Wazer, J.R., Lyons, J.W., Kun, K.Y., and Colwell, R.E., Viscosity and Flow Measurement, Interscience, New York, (1963).

- Whitmore, R., Jones, H., Windus, W., and Naghski, J., "Preparation and Visco-Elastic Properties of Fibrous Collagen Dispersions from Limed Cattle-hide Splits", Journal of Food Science, 37, 302-305, (1972).

- Williams, J.W., "Sedimentation Analysis of Some Plasma Extender Gelatins", in Recent Advances in Gelatin and Glue Research, (Stainsby, G., ed.), Pergamon Press, New York, 106-113, (1958).

- Yannas, I.V., "Stress Relaxation of Anhydrous Gelatin Rubber", Journal of Applied Polymer Science, 12, 1-8, (1968).

- Yannas, I.V., and Huang, C., "Viscoelastic Distinction Between Helical and Coiled Macromolecules", Macromolecules, 5, 99-100, (1972).

- Yannas, I.V., and Tobolsky, A.V., "Crosslinking of Gelatin by Dehydration", Nature, 509-510, (1967).

XI. APPENDICES

APPENDIX A

EPPRECHT RHEOMAT 15 MS-O SPECIFICATIONS[7]

Rheomat 15 Machine Setting	Angular Velocity (radians sec^{-1})	Shear Rate (sec^{-1})	Viscosity/Scale Reading * (centipose/scale)
1	0.585	27.28	8.425
2	0.786	36.64	6.276
3	1.034	48.21	4.770
4	1.38	64.32	3.575
5	1.822	84.85	2.710
6	2.625	122.3	1.880
7	3.521	164.2	1.400
8	4.64	216.1	1.064
9	6.19	288.3	0.798
10	8.16	380.3	0.605
11	11.86	522.0	0.417
12	15.92	741.2	0.310
13	20.94	975.3	0.236
14	28.0	1301	0.177
15	36.83	1717	0.134

Shear Stress = 2.22994 / Scale Reading *

* Assuming Newtonian behavior.

APPENDIX B

CORRECTIONS FOR NON-NEWTONIAN EFFECTS

The Epprecht Instruction Manual[7] provides shear rate calculated from the equation:

$$\dot{\gamma} = 2\Omega/[1 - S^2]$$

where Ω is the angular velocity and S is the radius ratio

$$S = R(\text{inner wall})/R(\text{outer wall})$$

This equation, however, is only exact for a Newtonian fluid.

The gelatin PC fluid obeys power law behavior as shown by the essentially constant slopes ($m = d[\log \Omega]/d[\log \tau]$) of a plot of shear stress (τ) versus angular velocity for several concentrations (Figure B-1).

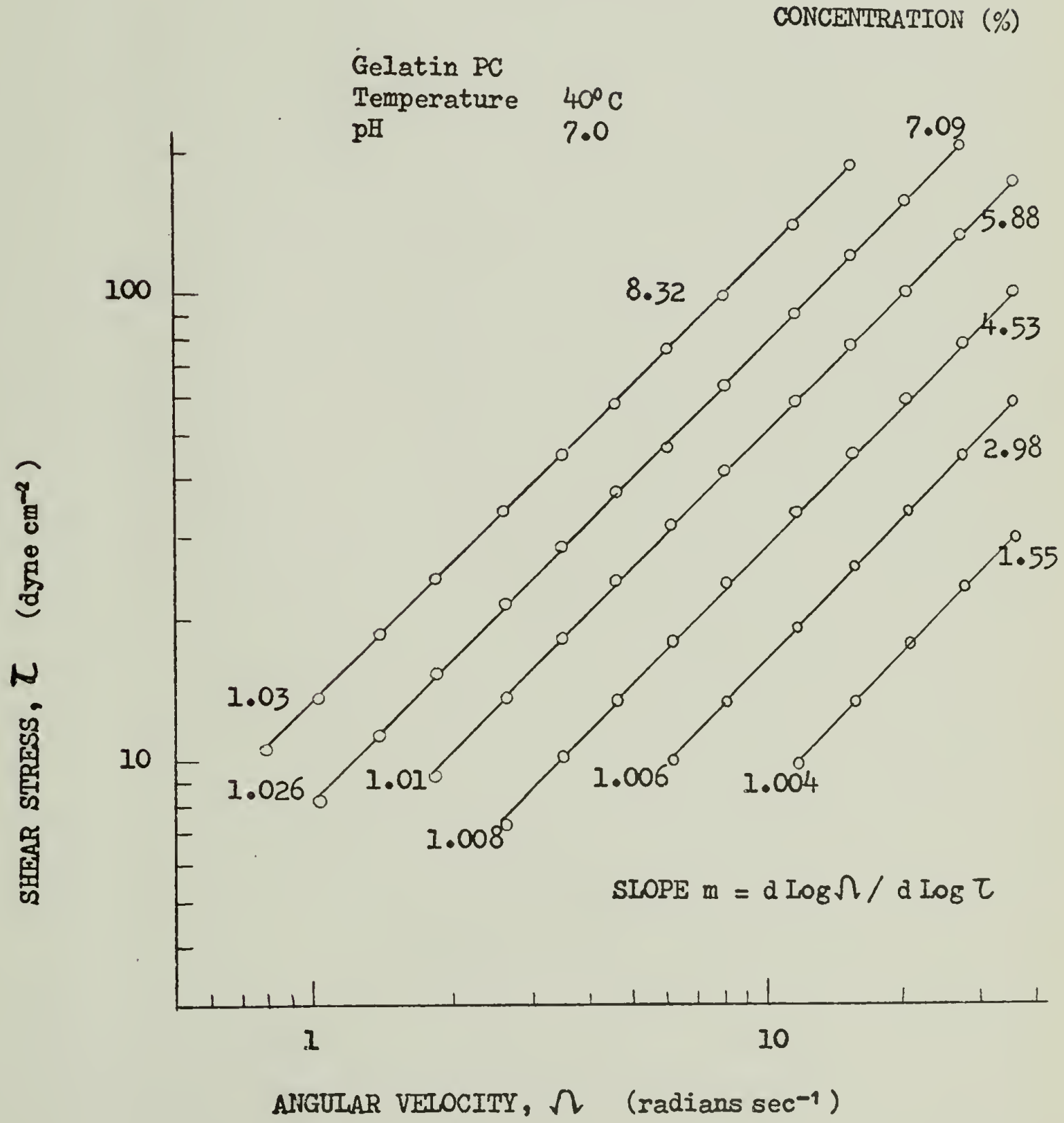
Hence the power law fluid equation for shear rate can be used to obtain the corrected shear rate.

$$\dot{\gamma} = 2m\Omega/[1 - S^{2m}]$$

Since the slopes (m) are very close to unity, the non-Newtonian viscosity correction is insignificant as shown in Figure B-2 where viscosity is plotted against shear rate for a few representative concentrations.

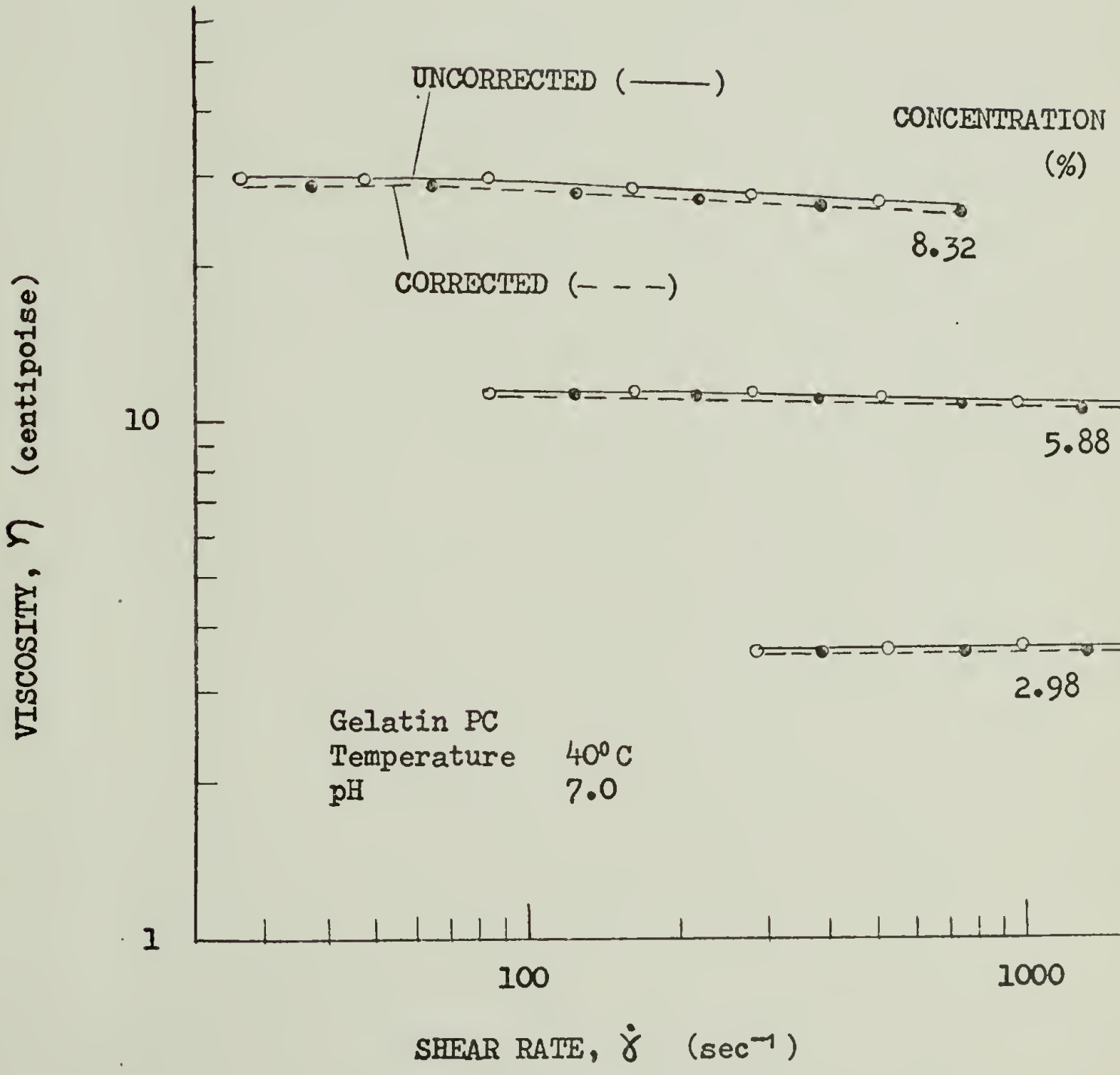
APPENDIX B cont.

FIGURE B-1. SHEAR STRESS vs. ANGULAR VELOCITY - CALCULATION OF SLOPE $d\log\tau / d\log\Omega$



APPENDIX B cont.

FIGURE B-2. VISCOSITY vs. SHEAR RATE - NON-NEWTONIAN CORRECTIONS



APPENDIX C

LAMINAR FLOW CRITERIA IN THE MS-O VISCOMETER[2]

Parameters:

Maximum Angular Velocity (Ω)	37 radians sec^{-1}
Solution Density (d)	1 g cm^{-3}
Minimum Viscosity (u)	0.025 g cm^{-1} sec^{-1}

CASE I. INNER CYLINDER WALL (R_3) ROTATING, OUTER (R_4) STATIONARY

Maximum Reynolds number (Re) obtainable with the specified gelatin solution:

$$Re = \Omega k R_4^2 d/u$$

where $k = R_3 / R_4 = 1.00 / 1.02 = 0.982$

$$Re(max) = (37)(0.982)(1.02)^2 (1) / (0.025)$$

$$Re(max) = 1510$$

Reynolds number at which transition from laminar to turbulent flow occurs:

$$Re(trans) = 41.3 / (1-k)^{3/2}$$

$$Re(trans) = 17{,}100$$

CASE II. INNER CYLINDER WALL (R_1) STATIONARY, OUTER (R_2) ROTATING

Maximum Reynolds number obtainable with the specified gelatin solution:

$$Re = \Omega k R_2^2 d/u$$

where $k = R_1 / R_2 = 0.8845 / 0.90 = 0.982$

$$Re(max) = (37)(0.982)(0.9)^2 (1) / (0.025)$$

$$Re(max) = 1200$$

Reynolds number at which transition from laminar to turbulent flow occurs:

From figure 3.5-2, Bird, Stewart, and Lightfoot[2]

at $1 - k = 0.02$

$$Re(trans) = 100,000$$

Therefore laminar flow will prevail in the MS-0 viscometer with these gelatin solutions.

APPENDIX D

SHEARING STRESS DISTRIBUTIONS IN THE DOUBLE CUP AND BOB SYSTEM[23]

The measured torque T is the sum of the torques, T_{12}, produced between the cylinders R_1 and R_2, and the torque, T_{34}, produced between the cylinders R_3 and R_4 (see Figure IV-2).

The shear stresses (τ) exerted by the fluid on each cylinder face are:

$$\tau_1 = T_{12} / (2\pi L R_1^2) \qquad \tau_2 = T_{12} / (2\pi L R_2^2)$$

$$\tau_3 = T_{34} / (2\pi L R_3^2) \qquad \tau_4 = T_{34} / (2\pi L R_4^2)$$

where L is the active cylinder height.

If the cup and bob are designed, such as the MS-O viscometer, to satisfy the radius relation,

$$R_1 / R_2 = R_3 / R_4 \qquad (1).$$

then,

$$\tau_1 / \tau_2 = R_2^2 / R_1^2 = R_4^2 / R_3^2 = \tau_3 / \tau_4$$

For simple shear flow, it can be demonstrated[22] with the dynamics equations that

$$\Omega = 0.5 \int f(\tau)\, d\tau / \tau$$

where Ω is the angular velocity and $f(\tau)$ is the rate of shear.

The angular velocity of the rotating cylinder is common to both gaps,

$$2\Omega = \int_{\tau_2}^{\tau_1} f(\tau)\, d\tau / \tau = \int_{\tau_4}^{\tau_3} f(\tau)\, d\tau / \tau \qquad (2).$$

Differentiating with respect to τ_2,

$$2\, d\Omega / d\tau_2 = [f(\tau_1)/\tau_1]\, d\tau_1 / d\tau_2 - f(\tau_2)/\tau_2$$

Since $\tau_1 R_1^2 = \tau_2 R_2^2$,then

$$d\tau_1 / d\tau_2 = [R_2 / R_1]^2 = 1 / S^2$$

Substituting,

$$2\tau_2 \, d\Omega / d\tau_2 = f(\tau_1) / S^2 - f(\tau_2)$$

which is positive since $\tau_1 > \tau_2$ and $d[f(\tau)] / d\tau$ is always positive in practical consistency curves.

Since $d\Omega / d\tau_2$ is always positive, the graph of Ω against τ_2 can have no maxima or minima, and therefore, for a given value of , there can be only one value of τ_2 to satisfy equation (2).

Therefore,

$$\tau_1 = \tau_3 \quad \text{and}$$

$$\tau_2 = \tau_4$$

Thus, providing the cup and bob are designed according to equation (1), shearing conditions in the inner and outer gaps are alike.

By equating the expressions for τ_1 and τ_3 and for τ_2 and τ_4,

$$T_{12} / T_{34} = R_1^2 / R_3^2 = R_2^2 / R_4^2$$

from which

$$\tau_1 = \tau_3 = T / [2\pi L (R_1^2 + R_3^2)]$$

$$\tau_2 = \tau_4 = T / [2\pi L (R_2^2 + R_4^2)]$$

APPENDIX E

HEAT CONDUCTION IN THE MS-O VISCOMETER[2]

Problem:

To determine the time required for a gelatin solution initially at 40° C in the MS-O viscometer to decrease to within 3 degrees (91% decrease) of its final temperature of 7° C.

Assume:

- Unsteady state heat conduction in a slab of finite thickness.
- Annular wall temperatures are constant.

Parameters:

Bath temperature (T_1)	7° C
Original Solution Temperature (T_0)	40° C
Temperature at the Center of the Fluid at Time t (T)	10° C
Solution Slab Thickness (b) (i.e., $R_4 - R_1 = 1.02 - 0.8845$) *	0.1355 cm

$\Theta = (T_1 - T) / (T_1 - T_0) = 0.91$ (dimensionless temperature)

Using the temperature profiles for unsteady state heat conduction in a slab of finite thickness (figure 11.1-1, Bird, Stewart, and Lightfoot)[2],

at the center of the solution, $\alpha t / b^2 = 1.0$ (dimensionless time)

where $\alpha = k / d C_p = 1.5 \times 10^{-3}$ cm^2 sec^{-1} (thermal diffusivity of the solvent water)

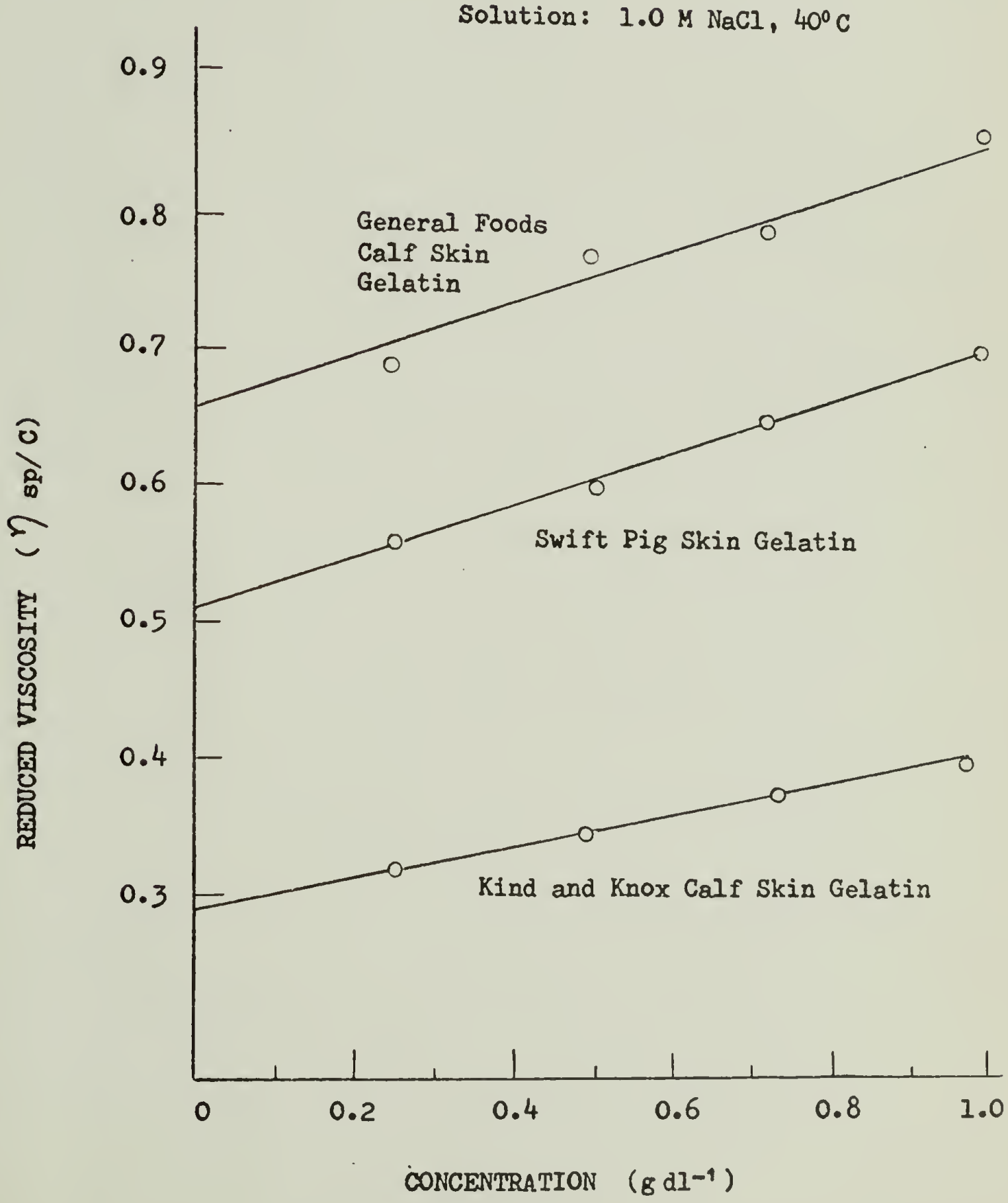
Therefore, $t = (1.0)(0.1355)^2 / (1.5 \times 10^{-3})$

$t = 12$ seconds

* Note that the cooling influence of the annular bob has been neglected.

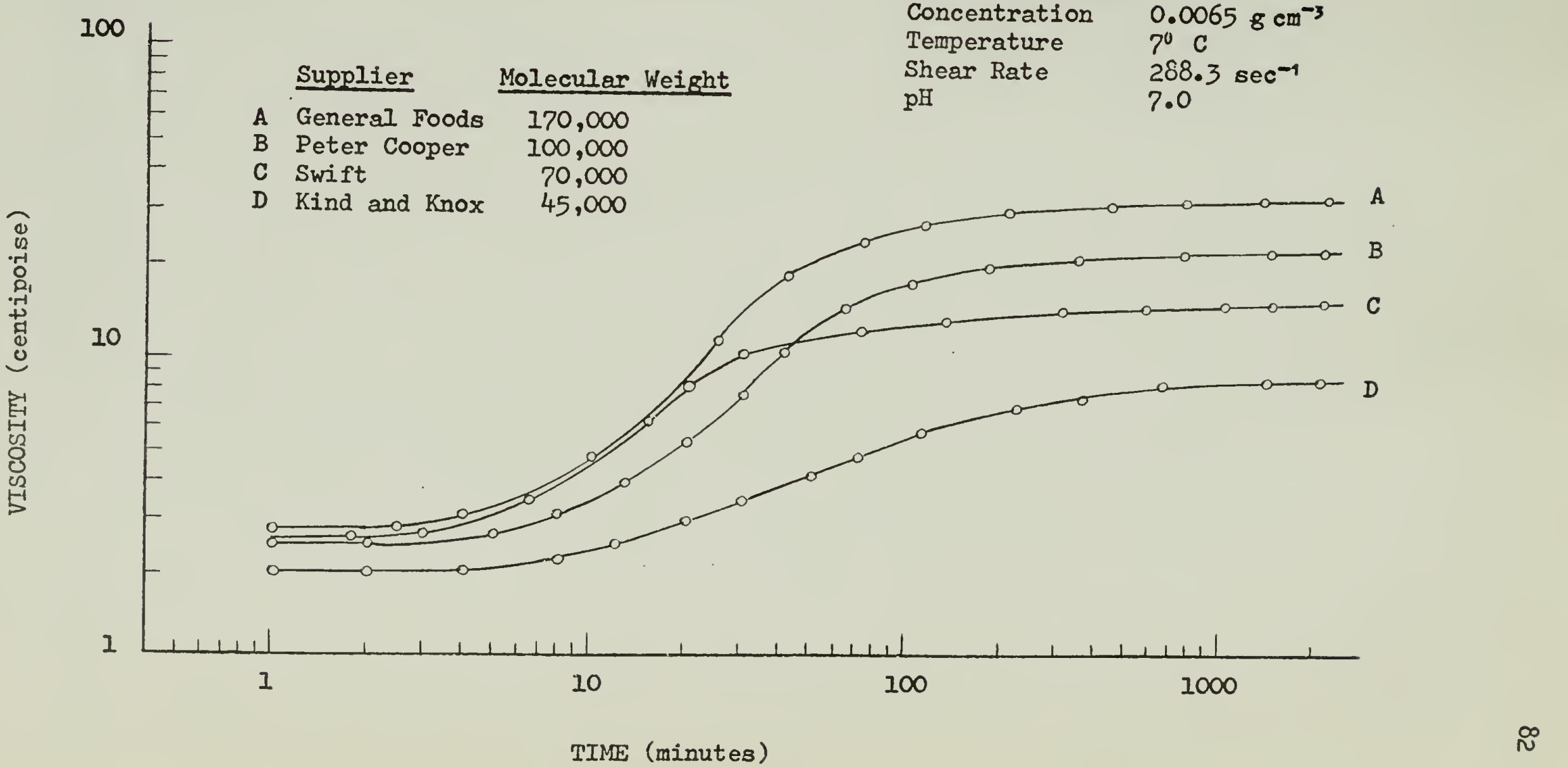
APPENDIX F

REDUCED VISCOSITY AS A FUNCTION OF CONCENTRATION



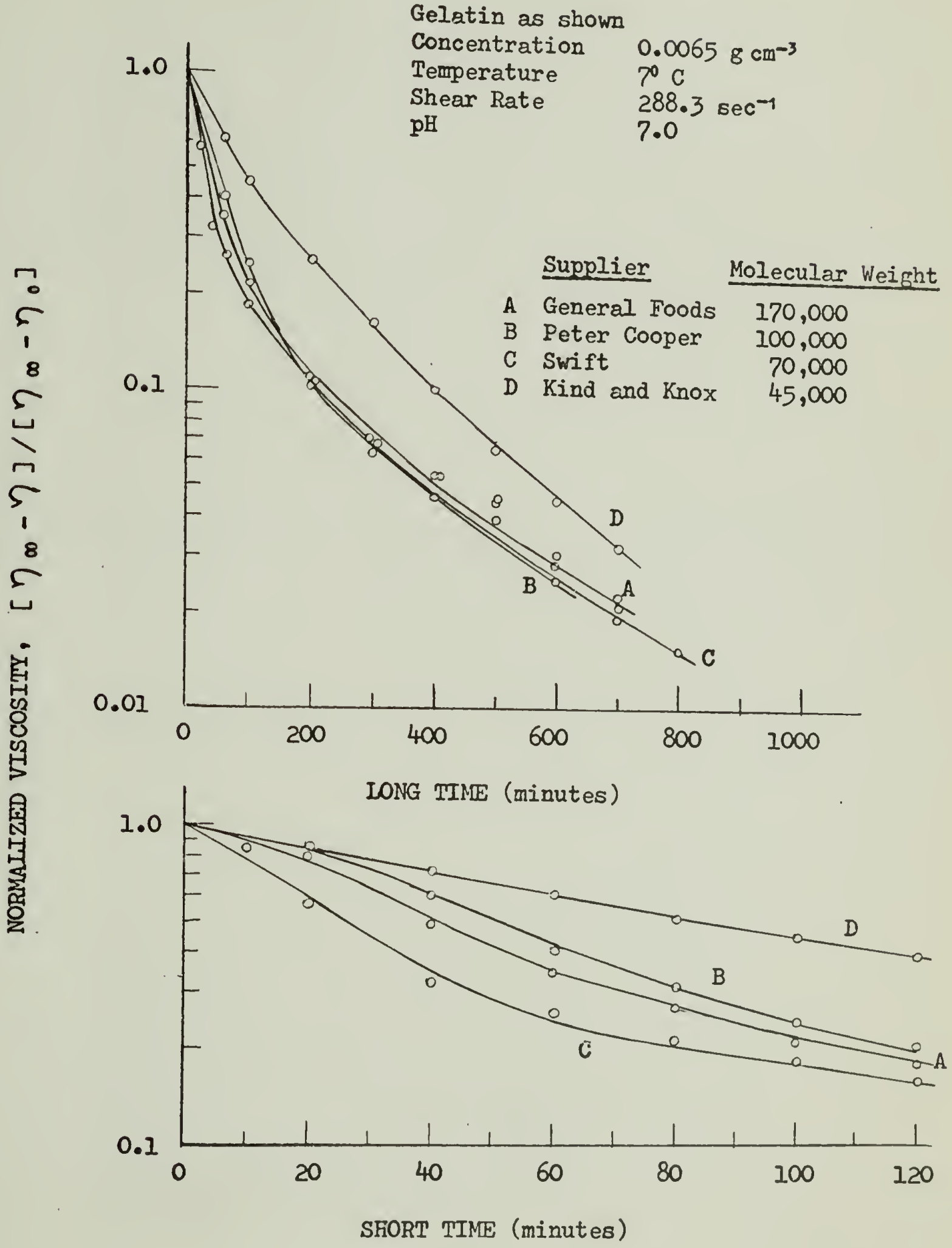
APPENDIX G-1

VISCOSITY vs. TIME - INFLUENCE OF OTHER SPECIES OF GELATIN



APPENDIX G-2

NORMALIZED VISCOSITY vs. TIME - INFLUENCE OF OTHER SPECIES OF GELATIN



APPENDIX H

OPTICAL ROTATORY DISPERSION DETAILS[11]

Perkin-Elmer 141 Polarimeter
Cell Number = 3178
Lamp Source = Mercury
Path Length = 10 cm
MRW = 115 (used for comparative purposes)
λ_0 = 2120 A^0

Wave Length λ, (mu)	Refractive Index Water, n^{20}	$\frac{3}{n^2+2}$	$\frac{\lambda^2}{\lambda_0^2} - 1$	$\left[\frac{\lambda^2}{\lambda_0^2} - 1\right]^{-1}$
366.3	1.3468	0.7866	1.99	0.50
404.7	1.3428	0.7888	2.64	0.379
435.8	1.3403	0.7902	3.22	0.311
546.1	1.3345	0.7935	5.62	0.178