The Effect of Shear Rate on the Kinetics of Gelation

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ABSTRACT: A viscometric technique has been used to follow the kinetics of gelation. A two-stage mechanism is indicated, and the rate of gelation of each stage is found to be an increasing function of shear rate. The shear history of the gelatin strongly affects the nature of the gelled network. Gel structure formed at rest is much more resistant to shear degradation than gel formed during shear. Altering the pH from the isoelectric point increases the viscosity as well as the time required to reach equilibrium gel formation, at a given shear rate.

In recent years there have been several studies which indicate that kinetic processes which occur in macromolecular systems can be affected significantly by the presence of a flow field. Among the phenomena studied one can cite crystallization in both shear[1] and stretching[2] flows and protein denaturation in shear.[3] The effect of the flow field may be to alter the *rate* of the kinetic process, or to modify the *morphology* of the product, or both.

Gelatin is an example of a macromolecular system which can undergo a relatively slow transformation in structure in dilute solution. In this paper we report a study of the effect of shear rate on the kinetics of gelation and on the structure of the gelled system.

Molecular Interpretation of Gelatin

Although the occurrence 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 changes that occur during gelation under shear have not been 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.[4] Collagen fold formation is that process in which peptide chain segments of uncertain and probably varying length assume a stable helical configuration similar to the helical configuration of the peptide chains in native collagen.

Harrington and Von Hippel[5] 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,[6] 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[7] 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 entities 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 occurring 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 nonspecific 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 nonpolar 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.[4]

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 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 concentra-

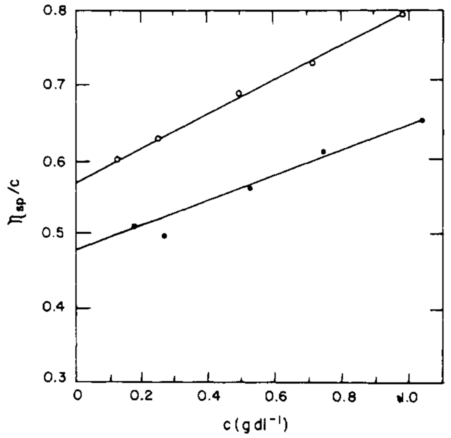

Figure 1. Reduced viscosity as a function of concentration for Peter Cooper Alkaline extracted calf skin gelatin in (○) 1.0 *M* NaCl at 40° and (●) 0.17 *M* acetate buffer.

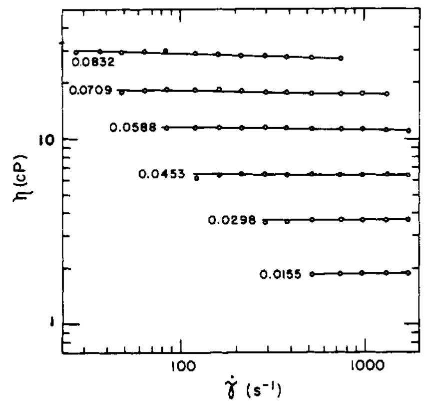

Figure 2. Effect of concentration of gelatin on viscosity at 40° and pH 7.0. Curves labeled with concentration in g cm^{-3}.

tions. Conversely, renaturation is completely inhibited by random collagen fold formation and network formation, so that renaturation proceeds most efficiently at gelatin concentrations less than 0.2%.[4]

Materials and Experimental Procedure

Basically, the experiments reported here involve the preparation of gelatin solutions and the continuous monitoring of solution viscosity as gelation proceeds with the passage of time.

An alkaline processed calf skin gelatin supplied by United States Gelatin, Division of Peter Cooper Corp. was chosen. 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."[9] The molecular weight was calculated from plots of reduced viscosity *vs.* concentration for two gelatin PC solutions (Figure 1).

Viscosity was measured using an Epprecht RM-15 coaxial cylinder viscometer with an MS-O cup and bob system. The MS-O system holds approximately 4 ml of solution in a narrow (0.02 cm) annular region. The shear rate is uniform across this narrow gap. A discrete set of 15 rotational speeds produces shear rates in the range 27 to 1717 sec^{-1}. The cup is immersed in a constant temperature bath, and because of the narrow gap design excellent isothermal temperature control of the sheared fluid is achieved.

Solutions were prepared by agitating granulated gelatin in 40° water for 3 hr. After this period, the temperature was raised to 70° and the solution was filtered through Whatman no. 1 qualitative filter paper. The filtrate was cooled to 40° 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 paper: "static" data during which no shear stress changes occur as a function of time at 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.

Concentrated hydrochloric acid and pelletized sodium hydroxide were used to change the pH of the solutions. pH adjustments were made 0.5 hr prior to initiating an experimental run.

Each gelatin solution was maintained at 40° for 0.5 hr prior to beginning the run to ensure that the chains were completely randomized in solution. The experimental time began at the instant 3.66 ml of 40° gelatin solution was poured into the annular cup region of the MS-O viscometer. Readings were obtained within 1 min of experimental time. The time required for the solution to achieve the equilibrium temperature of the viscometer and bath is very short. A 1-min 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. The viscosity growth curves obtained substantiate this claim since very little viscosity change occurs during the first 3 to 5 min.

Static Data

Figure 2 shows data for viscosity as a function of shear rate for gelatin solutions at 40°, a temperature too high to promote gel formation. Hence no time-dependent effects were observed. Only the three highest concentrations show measurable non-Newtonian effects at these shear rates. In Figure 3 these data are replotted to show the effect of concentration on viscosity. The strongly increasing slope in the neighborhood of 3% gelatin suggests that entanglements, intermolecular interactions, dominate the viscous behavior at that concentration and higher. At lower concentrations, and at these high temperatures, entanglement effects disappear.

Data to be shown subsequently are at relatively low concentrations (<1%), but the role of concentration in gelatin kinetics is more complex than in its effect on viscosity through entanglements, and one must be careful about using the terms "dilute" and "concentrated" when discussing gelatin. A solution may be thought of as concentrated when the solute molecular domains overlap, and contacts between chain elements of different chains become as probable as contacts between elements of the same chain. Apparently even at high dilutions the chain segment density within the domain of a single random coil of gelatin is relatively high. Boedtker and Doty[10] examined an α gelatin of a molecular weight of 90,000 at "infinite dilution"

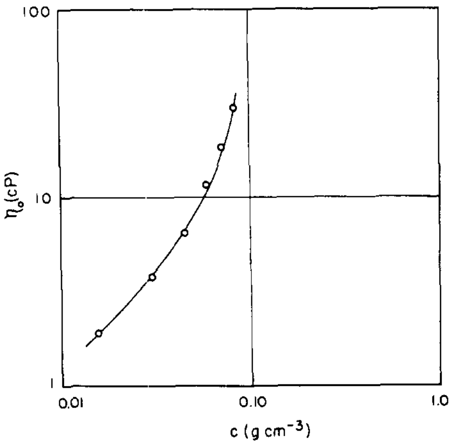

Figure 3. Data of Figure 2, replotted as zero-shear viscosity *vs.* concentration.

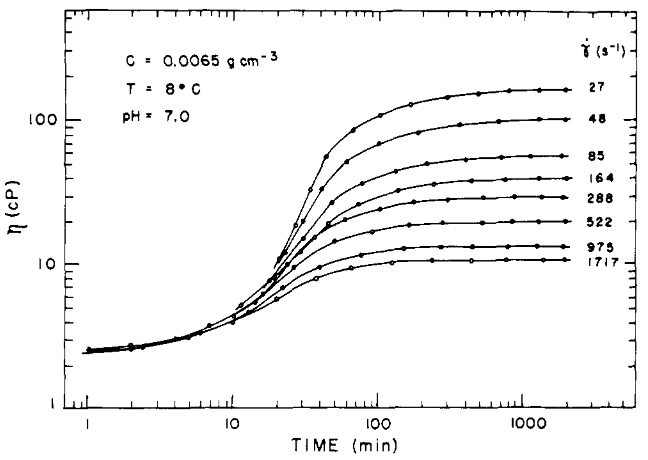

Figure 4. Viscosity as a function of time of shearing at different shear rates.

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%. At total gelatin concentrations greater than 0.2% the molecular domains must overlap so that intermolecular contacts are equally as probable as intramolecular contacts. Nevertheless, entanglements in the rheological sense apparently do not occur until considerably higher concentrations.

Kinetic Data

The most convenient type of data for studying the *kinetics* of gelation are those obtained by observing the viscosity change with time as a function of shear rate, at temperatures below the melting zone.

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 Figure 4 where viscosity–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 sec^{-1}) is almost 16 times larger in magnitude than that at the highest shear rate of 1717 sec^{-1}. At low rates of shear weak structures can exist because the disruptive forces are low, while at high rates of shear only stronger structures can exist.

The results shown on Figure 4 have been normalized with respect to the initial and equilibrium viscosities and replotted in Figures 5a and 5b to provide a kinetic analysis.

As observed in these figures the rate of gelation increases as a function of increasing shear rate, both in a short-time and a 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 small 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 min depending on the rate of shear. The first stage 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 nonspecific 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 development of stable crystallites.

Figure 6 shows equilibrium and "constant structure" curves obtained from the preceding data. The object of this form of presentation is to demonstrate the behavior of the gelled system at various "constant structural" levels, with the level being determined by shearing the gelatin solution until equilibrium is established at the selected shear rate. The "equilibrium flow 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 of shear rate, the shear rate was changed back to the original level until equilibrium was once more attained (usually no deviation of shear stress from the original level 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 during the shift from high to low shear rate, and back.

Joye and Poehlein[11] have presented data in a form similar to Figure 6 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}. The data of Figure 6 also allow for this speculation, although certainly more confirming work is needed. An inflection such as described might indicate a transition zone where increases or decreases in shear rate will influence the structure formed to a greater or lesser extent.

Effect of Shear Rate History

Curve A of Figure 7 illustrates a gelatin solution which was continuously sheared at a uniform shear rate of 288

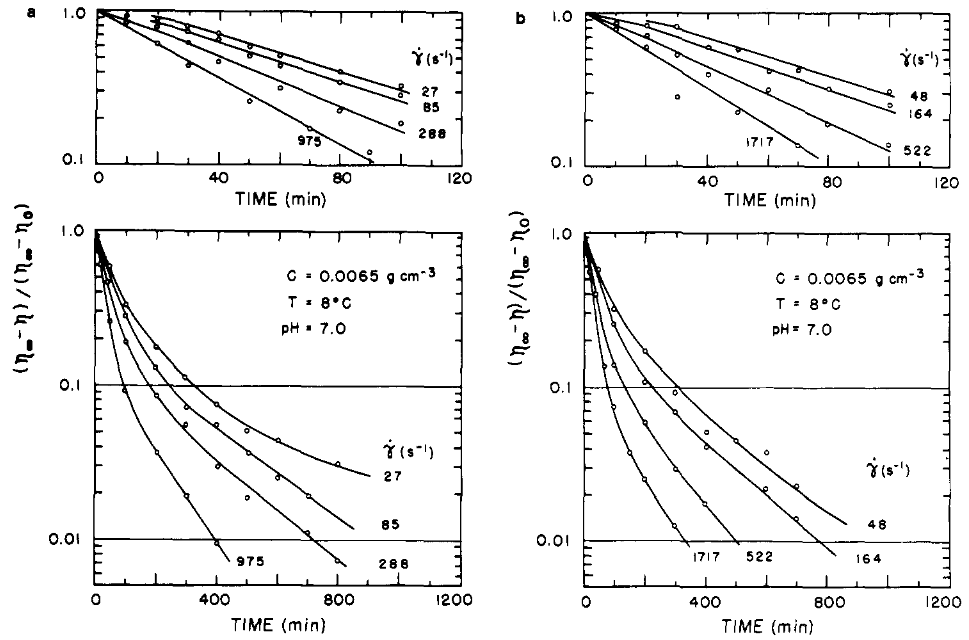

Figure 5. Normalized viscosity of gelatin solutions as a function of time of shearing at different shear rates. The upper plot gives data on an expanded time scale at short times.

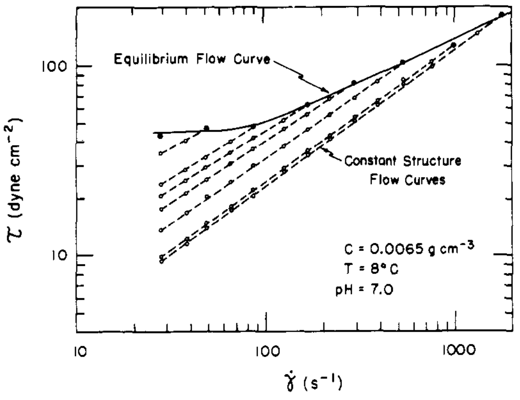

Figure 6. Equilibrium flow curves (shear stress *vs.* shear rate) and "constant structure" flow curves.

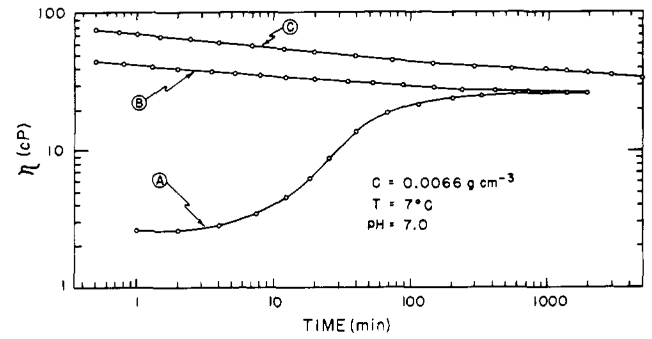

Figure 7. Effect of shearing "history" on viscosity as a function of time. See text for discussion of curves A, B, and C.

sec^{-1} until an equilibrium viscosity was achieved (2000 min). On attaining equilibrium, the shear rate was decreased to 0 sec^{-1} for 2000 min. After this 2000-min period, the shear rate was again raised to the initial 288 sec^{-1} level and data were recorded (curve B) as the viscosity decreased slowly to the equilibrium level attained previously by a continuous 288-sec^{-1} shear program. From these plots it appears as though additional structure forms during the 2000-min zero-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 sec^{-1} occurred during the zero-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 7, 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 min at rest. After this 2000-min period, a 288-sec^{-1} shear rate was imposed on the system (curve C). Note that even after 5000 min of shearing, the 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 through shearing to yield a structure characteristic for 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 sec^{-1} would also produce a seemingly irreversible structure, the data of Figure 8 were obtained. After observing a typical viscosity growth pattern for 2000 min at 27 sec^{-1} (curve B), the shear rate was increased to 288 sec^{-1} (curve C). The viscosity decays slowly to the viscosity characteristic of 288 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 sec^{-1} shear rate or above.

A third figure of this series (Figure 9) shows a gelatin solution which experienced a sequence of a short 0-sec^{-1} shear period for 80 min and then a 288-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 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 min is not sufficiently

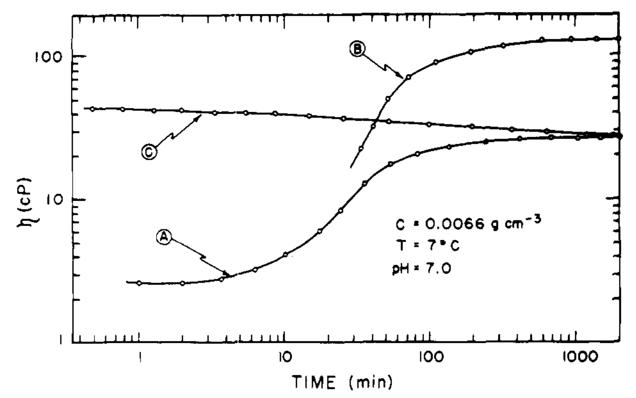

Figure 8. Effect of shearing "history" on viscosity as a function of time. See text for discussion of curves A, B, and C.

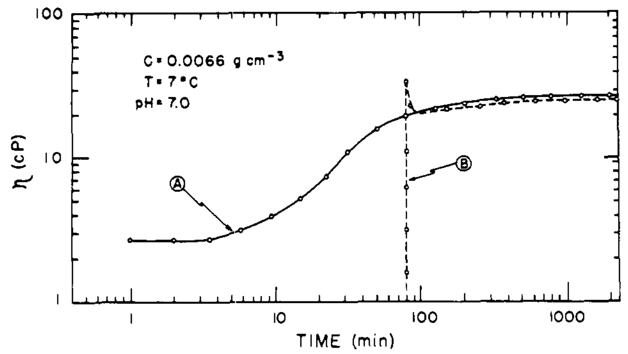

Figure 9. Effect of shearing "history" on viscosity as a function of time. See text for discussion of curves A and B.

developed to retain its strength under shear as was the case of a 2000-min 0-sec^{-1} shear developed gel network (Figure 7 (curve C)). Even though the gel network has attained approximately 50% of its final strength after 80 min 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 structures form at different shear rates and, depending on the shear rate history, may or may not be easily reversible.

Joye and Poehlein[11] have presented an intuitive argument describing the different molecular processes involved in gelation under zero shear and gelation at a finite shear rate. They feel that only weak crosslinks 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 interunit bonds can form during shear. During zero shear interunit 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 possibly that stronger "interunit" bonds form during shearing as well as during rest.

The Effect of pH on Gelation

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.[4] 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 in which no net migration of the protein is produced by application of an electric field.

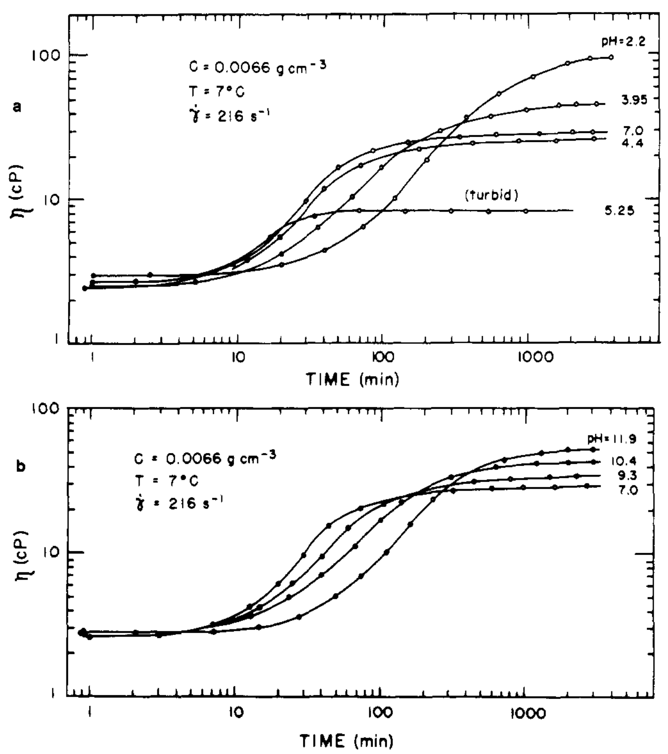

Figure 10. Effect of pH on viscosity *vs.* time for pH (a) <7.0 and (b) ≥7.0.

Viscosity growth curves for the alkaline derived gelatin PC are shown in Figures 10a and 10b 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 on the rate at which it is attained. The normalized kinetic plots of these growth curves are shown in Figures 11a and 11b.

Figure 12 presents a plot of the equilibrium viscosities shown in Figures 10a and 10b as a function of pH. At pH values above and below those shown on this plot, studies[4] 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 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 through 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°), as shown in Figure 13. Here two shear rates (288 and 1717 sec^{-1}) were used to obtain viscosity as a function of pH for a 4.71% gelatin solution at 40°. Based on the similarities in these curves it appears that the gelled network structure is influenced considerably by the initial chain configuration.

Summary

As observed by numerous other investigations, the mechanism of gelation is very complex. Viscometric techniques

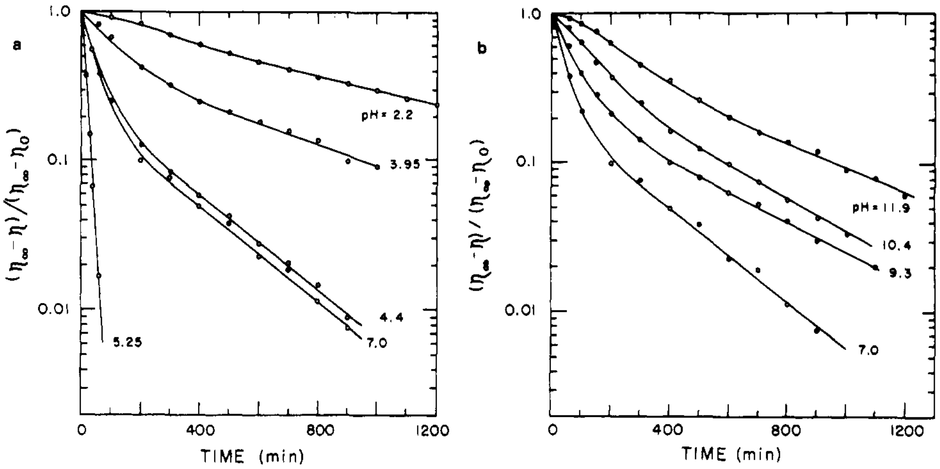

Figure 11. (a) Data of Figure 10a, normalized. (b) Data of Figure 10b, normalized.

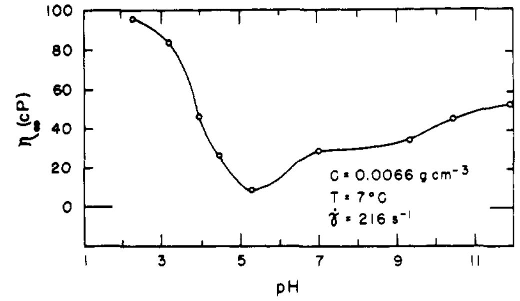

Figure 12. *Equilibrium viscosity, at 216 sec^{-1}, as a function of pH.*

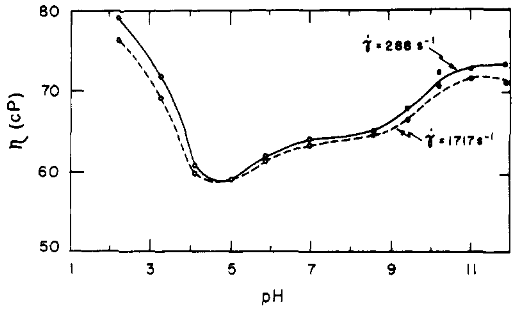

Figure 13. Effect of pH on viscosity of ungelled solutions of gelatin at 40°. Concentration is 0.047 g cm^{-3}.

are capable of classifying the general transformations occurring during gelation; however, viscometry is not usually used to differentiate subtle molecular interactions. 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 phenomena.

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 occurring around 100 to 200 min. The first molecular scheme is felt to involve single and interchain helical generation and the formation of weak intermolecular cross-links through the nonspecific 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 to 1717 sec^{-1}) in both the short-time and long-time stage. 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 molecular segments are brought into contact with each other and with the already existing aggregates.

The shear rate history has quite an impact on the nature of the gelled 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 shear rate.

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 through altering the pH from the isoelectric point increases the viscosity as well as the time to reach equilibrium gel formation. Based on the similarities in equilibrium viscosity at 7° and solution viscosity above 25°, it appears that the gelled network structure is influenced considerably by the initial chain configuration.

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