Modulus jump and degradation of collagen gels: dependence on concentration and pH*

C. W. Zvanut and F. Rodriguez
School of Chemical Engineering, Cornell University, Ithaca, New York 14853, USA
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The gels that result when acidic solutions of telopeptide-free collagen are exposed to u.v. light at temperatures below 25°C can be characterized by a storage modulus and a log decrement measured in a torsion pendulum. When the temperature of the previously formed gel is raised above 25°–30°C, the collagen denatures, the modulus increases rapidly (jumps) and then decays with time. The maximum modulus during irradiation increases with the collagen concentration to about the second power except at low concentrations where the power is higher. The maximum modulus also increases by a factor of 3 to 5 when the pH during irradiation is increased from 2.5 up to the solubility limit of about 4.2. The modulus jump, the log decrement, and the rate of degradation of the gels also have been characterized as functions of concentration and pH.

INTRODUCTION

Dilute collagen gels can be made by irradiating collagen solutions with u.v. light under an inert atmosphere[1,2]. When the temperature of the gel is raised above the denaturation temperature of the protein, a 'modulus jump' occurs[3]. These gels have potential biomedical applications[4]. Since a strong gel is desired, a detailed study of the maximum storage modulus and modulus jump has been conducted. The theory of rubber elasticity can be used to explain the results.

For biomedical applications, the gels must be stable at body temperature. The gels degrade, however, even at room temperature[3]. The stability of collagen gels at 40°C was studied as a function of pH, collagen concentration, and NaCl concentration. A parallel study of solution viscosity was conducted. From these results, a better understanding of the degradation process can be derived.

The reason for the dependence of properties of the collagen solutions and gels on pH is that several amino-acids in collagen have charged side groups. An analysis of a titration curve for an insoluble collagen shows that between pH 3 and 5, the carboxyl groups become protonated[5]. The solutions become cloudy around pH 4.5, and lose their high viscosity near pH 5.

Addition of salts to a collagen solution allows ions to bind to the protein chain or shield the charges on it. Besides the phenomenon of 'salting in' or 'salting out' the protein, the salts,also effect the collagen denaturation temperature. Addition of most salts, including sodium chloride, lowers the denaturation temperature of dilute collagen solutions[6–8]. The reasons for this are not clear, especially since the ions, by neutralizing or shielding the charges on the protein, should stabilize the molecule.

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† Present address: Photo Products Laboratory, E.I. du Pont de Nemours & Co., Parlin, New Jersey 08859, USA.

EXPERIMENTAL

The collagen used in this study was extracted from cow hide by an enxyme treatment as described previously[2]. The pH of the solutions was adjusted by careful addition of NaOH, and monitored on a Corning Model 5 pH meter. The concentration was adjusted by diluting a 5% stock solution with 0.01 N HCL. The pH study was limited by insolubility of the collagen above pH 4.

The gels were prepared by pouring a solution into a quartz cup, bubbling with nitrogen to remove oxygen, and then placing the solution in a water bath maintained at 10°C. The water bath is centred in a chamber with eight 15 W germicidal lamps which radiate primarily at 254 nm. The modulus was measured by a torsion pendulum which uses the gel as a restoring element. Most of the measurements were made with the air-bearing pendulum used by Hamed[2]. The rest of the work was carried out with the less elegant test-tube pendulums described previously[3].

The modulus was measured until it reached a maximum value under the u.v. lights. In order to study the modulus jump, the sample was placed in a 40°C bath after reaching a maximum modulus. Since the gels sometimes degrade quickly at 40°C [3], the modulus jump was obtained by measuring the modulus at increasing times and then extrapolating back to time zero on a semi-log plot. The slope of this plot served as a measure of the rate of degradation. Viscosity of the collagen solutions was measured in Ubbelohde viscometers.

RESULTS AND DISCUSSION

Theory of rubber elasticity

The modulus behaviour of collagen gels can be better understood in light of the theory of rubber elasticity. The theory of gels crosslinked in solution is presented by Ferry[9]

and only a brief review is presented here. The theory relates the modulus to crosslink density and some assumptions are made here to relate the crosslink density to the collagen concentration. Since the theory is derived for polymers that do not have long range interactions and that assume a random coil conformation, it would not be expected to work as well with collagen. The theory has been applied, however, to swelling of insoluble collagen with some success[10,11]. A good qualitative agreement is hoped for.

The storage modulus obtained by the torsion pendulum is closely related, if not equal, to the 'equilibrium shear modulus' mentioned in Ferry. Often, in studying gels, the modulus increases steadily with time, forcing the establishment of a 'pseudo-equilibrium' shear modulus. This is one problem not encountered in studying collagen gels.

The storage modulus should be given by:

$$G' = A\frac{\overline{r_E^2}}{r_0^2}\nu RT \tag{1}$$

where A is the front factor, a dimensionless constant; $\overline{r_E^2}$ is the mean square end-to-end distance of a strand, or the distance between two crosslinks; r_0^2 is the mean square end-to-end distance of a strand if not constrained by crosslinks; R is the gas constant (8.31×10^7 erg/mol K); T is the absolute temperature (K); ν is the moles of network strand per cm^3. The factor A may deviate far from unity for gels, particularly for dilute solutions[9].

The ratio $\overline{r_E^2}/r_0^2$ should be near one for collagen gels, since the rigid-rod structure should be unchanged by crosslinks. However, for denatured gels this ratio will deviate far from unity. The network strands will tend toward a much more coiled conformation than the crosslinks will permit. An increase in this ratio signifies an increase in the number of conformations to which the stretched polymer can relax.

An expression for the moles of network strands, which includes network defects, is:

$$\nu = 2\nu_c(1 - b/2\nu_c\bar{M}_n) + 2\epsilon T_e \tag{2}$$

where ν_c is the moles of chemical crosslinks per cm^3; b is a constant, equal to twice the polymer density; $\bar{M}_n$ is the number-average molecular weight (=30 000 g/mol); ϵ is the moles of entanglement loci per cm^3; T_e is the probability that such an entanglement has been trapped in the crosslinking process so that it acts like an extra crosslink. The rod-like structure of the collagen and the low densities of the gels would almost prohibit entanglements, so the entanglement factor is taken to be negligible. The term $(1 - b/2\nu_c\bar{M}_n)$ is a correction term for the strands that are not involved in the crosslinked network.

The problem now is in determining ν_c. It can be expressed as:

$$\nu_c = \frac{nC}{\bar{M}_n(100)} \tag{3}$$

where C is the concentration of collagen (g/dl), and n is the number of crosslinks per collagen molecule. This merely transforms the problem to determining n. Since a network cannot be established without at least two crosslinks per molecule, there must be a minimum concentration, C_{min}, below which no gel is formed. Also, n is expected to increase with increasing concentration. Combining these two ideas, an expression for n could be:

$$n = 2\left(\frac{C}{C_{min}}\right)^a \tag{4}$$

where a is an empirical constant greater than 0. This expression was chosen because it is simple, it is realistic, and it reduces the expression for the modulus to that observed experimentally at high concentrations.

The expression for the storage modulus becomes:

$$G' = \frac{4A\frac{\overline{r_E^2}}{r_0^2}C^{a+1}}{100\bar{M}_n C_{min}^a}\left[1 - 1/2\left(\frac{C_{min}}{C}\right)^a\right]RT \tag{5}$$

Maximum modulus

The effect of concentration on the maximum modulus of the gels is shown in *Figure 1*. The points connected by a solid line were obtained using the air-bearing torsion pendulum. The log of the maximum modulus is directly proportional to the log of the concentration at high concentrations, with deviations becoming large as C_{min} is approached. The slope of the straight-line portion is 1.8, which is close to that found for gelatin gels[9].

The calculated values in *Figure 1* were obtained using equation (5). The constants in equation (5) were fitted from the experimental results. The value for a was determined from the slope of the line at high concentrations. The minimum concentration to gel was obtained by plotting G'_{max} vs. C and extrapolating to zero modulus. The constant A was then fitted by using the experimental modulus at 0.6 g/dl. The values for the constants are shown in *Table 1*.

The value for A is generally quite low for gels, and should never be greater than one. The low values generally ob-

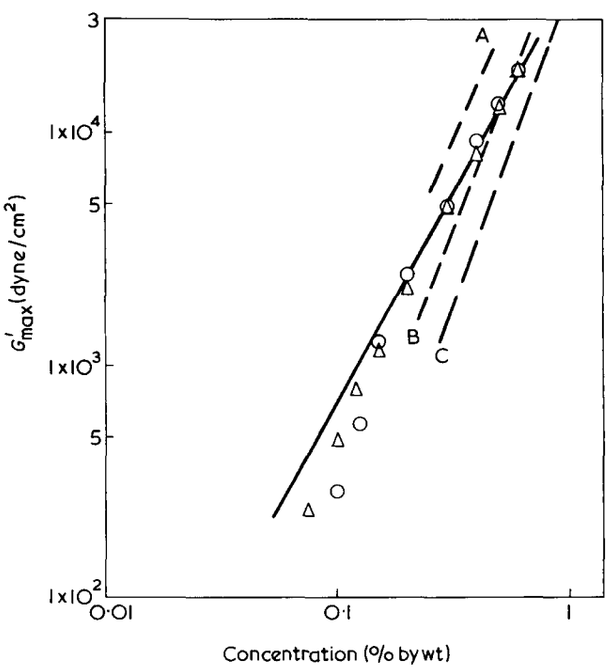

Figure 1 Dependence of maximum modulus on concentration (pH = 3.2). – – –, obtained using a different sample of collagen at pH: A, 4.0; B, 3.2; C, 2.5. △, Calculated from equation (5)

Table 1 Calculated values of the constants in equation (13)

Constant	Calculated value (g/dl)
A	1.93
a	0.81
C_{min}	0.075

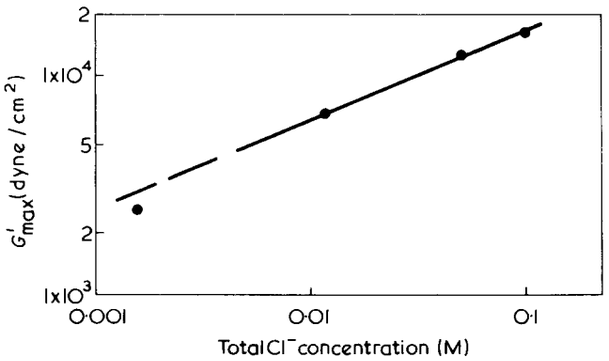

Figure 2 Dependence of maximum modulus on chloride ion concentration of 0.5% collagen gels (pH = 2.8)

tained may be due to 'incomplete overlapping of the molecular domains as well as a substantial sol fraction'[9]. However, since the collagen is a rigid rod and has an extremely narrow molecular-weight distribution, it would not be surprising for A to be close to one. The high value reported here reflects the inadequacy of the theory and equation (4). It is not so large, however, as to be grounds for discarding the theory.

The calculated results from equation (5) fit the experimental modulus values well at high concentrations, but are too high at low concentrations. It is interesting to note that deviations from the straight-line behaviour are predicted from the theory. The decreased number of crosslinks at low concentrations leaves proportionately more strands not included in the gel network. This is reflected in the increased importance of the $(1 - b/2\nu_c\bar{M}_n)$ term.

Changing the pH of the solution changes the modulus obtained at a given concentration (points connected by broken lines in *Figure 1*). These data were obtained using the test-tube pendulums and a different collagen sample from that used previously. Increasing the pH of the solution decreases the repulsion between the protein molecules by neutralizing somewhat the charge on the molecule. This allows the molecules to move closer together, and increases the number of crosslinks per molecule. The slope of the lines is essentially unchanged by pH. The lines are shifted upward, however, indicating that C_{min} increases with increasing pH. This is quite reasonable.

The modulus behaviour of the collagen gels at various salt concentrations was measured during u.v. irradiation using the air-bearing torsion pendulum. These gels were made from dry, salt-free collagen. The time to gel decreased and the time to reach maximum modulus increased with increasing salt concentration. These changes are probably due to the screening of the charge on the collagen by the anions. This will decrease intermolecular repulsion and make crosslinks easier to form. This would also explain the increase in modulus, which is proportional to the chloride ion concentration to the 0.4 power (*Figure 2*). This total Cl^- concentration includes the contribution from the hydrochloric acid. The additional shielding at increasing salt concentrations is not as effective as the initial increment, which is to be expected. The shape of the irradiation curves for all salt concentrations is similar, indicating that the crosslinking mechanism has not changed. The cationic sites along the collagen chain must not be important in the crosslinking process. If they were, the bound ions would undoubtedly interfere with the reaction.

Modulus jump

The modulus jump is due primarily to an increase in the ratio $\overline{r_E^2}/\overline{r_0^2}$ in equation (5). The magnitude of this ratio will depend heavily on the length of the strand. The length of the entire collagen molecule will change greatly after denaturation, but a small segment of the molecule may remain relatively unchanged. The greater the strand length, then, the larger the modulus jump. If the number of crosslinks per molecule increases, then the length of a strand will decrease. The ratio $\overline{r_E^2}/\overline{r_0^2}$, and the modulus jump, will depend on the crosslink density. Since the crosslinks per molecule decrease with concentration, the modulus jump should increase. This is verified experimentally.

Experimental values for $\overline{r_E^2}/\overline{r_0^2}$ would be extremely difficult to obtain. A ratio of the end-to-end distance of the collagen to that of denatured collagen, or gelatin, is easily measured, however. This ratio will be closest to the modulus jump at C_{min}. At this concentration, there are only two crosslinks per molecule, and the distance between the crosslinks will be a maximum. The crosslinks are probably near the ends of the protein, since this would create a stable network at the lowest possible concentration.

In order to determine the modulus jump at C_{min}, it was necessary to extrapolate to this concentration. This is difficult to do when jump is plotted against concentration since the modulus jump increases greatly at low concentrations. The concentration times the modulus jump is fairly constant at low concentrations (*Figure 3*). The modulus jump at C_{min} is then found to be 8.7. This can be related to the ratio of the length of the collagen to the gelatin molecule, using equation (5).

$$\frac{G'(40)}{G'(10)} = \frac{\left(\dfrac{313\text{K}}{\overline{r_0^2}(40)}\right)}{\left(\dfrac{293\text{K}}{\overline{r_0^2}(10)}\right)} \approx \frac{\overline{r_0^2}(10)}{\overline{r_0^2}(40)} = \frac{\overline{r_0^2}(\text{collagen})}{\overline{r_0^2}(\text{gelatin})} \qquad (6)$$

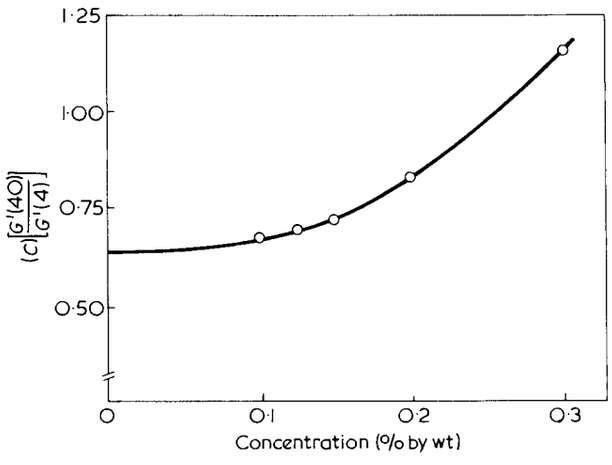

Figure 3 Determination of modulus jump ratio at C_{min} (pH = 3.2) from the product of concentration and jump ratio

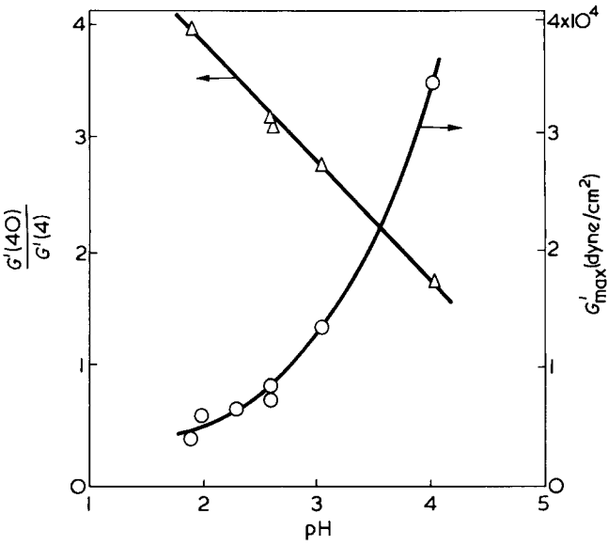

Figure 4 Effect of pH on modulus and modulus jump of 0.5% collagen gels

Table 2 Effect of crosslink density on modulus jump, 0.3% collagen, pH = 3.2

G' (10°C) (dyne/cm^2)	G' (40°C)/ G' (10°C)
4850	3.8
2350	5.5

Table 3 Effect of NaCl concentration on the modulus jump ratio

NaCl concentration (N)	G'_{40}/G'_{10}
0.01	3.55
0.05	2.68
0.10	3.10

All of the other terms should be relatively independent of temperature.

The intrinsic viscosity, $[\eta]$, can be used to measure the root-mean-square end-to-end distance of a polymer if the molecular weight is known[12]. Again, this theory cannot strictly be applied to collagen. However, it should give a good indication of the ratio of lengths. This ratio can be written in terms of intrinsic viscosities as:

$$\frac{\overline{r^2}(10)}{\overline{r^2}(40)} = \left(\frac{3[\eta_{40}]}{[\eta_{10}]}\right)^{2/3} \tag{7}$$

The factor 3 appears because of the molecular weight change after the unfolding of the triple helix.

The intrinsic viscosity of the collagen is 22 dl/g and that of the denatured collagen 2 dl/g. These were determined from intercepts on Huggins' plots. From these values, the ratio of the end-to-end distances was calculated to be 10.3. This is close to the value for the modulus jump at C_{min}. A literature value for the ratio of the z-average radius of gyrations[13] is 14.2. It is not surprising that these values are higher than that for the modulus jump, since even at C_{min} the crosslinks would not occur solely at the ends of the collagen chain.

Increasing the pH of the gel decreases the modulus jump significantly (*Figure 4*). This may be due to an increase in the length of the gelatin molecule with pH, as is evident from intrinsic viscosity data[14]. The increase in maximum modulus denotes an increase in crosslink density. Since the crosslinks are closer together on the molecule at high pH, the modulus jump decreases. The fact that crosslink density alone has an effect on the modulus jump can easily be demonstrated. Two identical collagen solutions irradiated for different times gave different moduli. These two gels also have different modulus jumps (*Table 2*). So a change in crosslink density alone has changed the modulus jump.

The modulus jump of the gels was relatively independent of salt concentration (*Table 3*). The modulus jump was not measured for the case of zero salt, owing to the rapid initial decrease in modulus. There was probably some syneresis of the gel which would account for the initial rapid decay and the disparity between the samples. It would be expected that the modulus jump would decrease with increasing salt concentration, because of the increased crosslink density. This may be true, since an estimate obtained by extrapolating the degradation curves back to zero time, for the jump at zero salt concentration, would place it around 4. Another factor influencing the jump, however, is the decreased end-to-end distance of the denatured collagen with increasing ionic shielding. This would tend to increase the modulus jump at higher salt concentrations, and may cancel the effect of increasing crosslink density.

Log decrement

All the data for the log decrement were obtained using the air-bearing torsion pendulum. The log decrement corresponding to the maximum modulus increases greatly as the concentration approaches C_{min} (10°C data, *Figure 5*). This is realistic, since the log decrement will increase with the number of loose chain ends and long polymer segments[15,16]. As the crosslink density decreases, the free ends and polymer segments can become quite long. There may even be free molecules in solution. The viscous damping in the gels will then increase. The log decrement is constant at high concentrations. This indicates that even though the number of free strands and polymer segments increases, their length

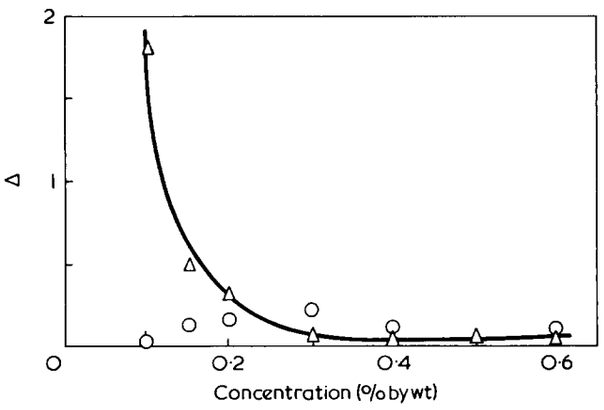

Figure 5 Minimum log decrement (△) for pH = 3.2 collagen gels (△, 10°C; ○, 40°C)

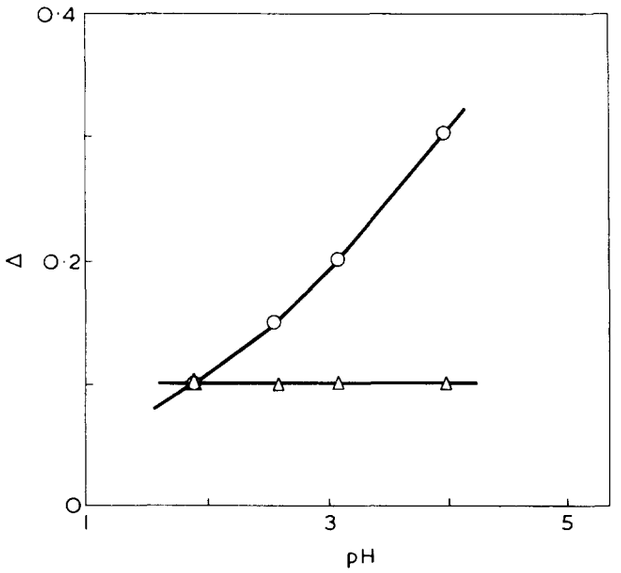

Figure 6 Minimum log decrement for 0.5% collagen gels (△, 10°C; ○, 40°C)

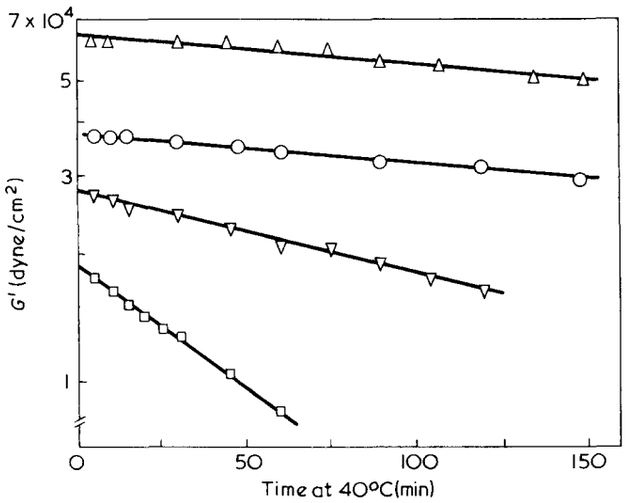

Figure 7 Effect of pH on degradation of 0.5% collagen gels: □, pH 1.9, slope 0.014 min^{-1}; ▽, pH 2.6, slope 0.0046 min^{-1}; ○, pH 3.1, slope 0.0016 min^{-1}; △, pH 4.0, slope 0.0017 min^{-1}

decreases. Apparently these two compensating effects keep the log decrement constant.

After denaturing the gels, dependence of the log decrement on the concentration changes (40°C data, *Figure 5*). There is an extraordinary decrease in the log decrement at low concentrations, and a slight increase at higher concentrations. Denaturation allows the free strands to change from rigid rods to a random-coil conformation. When the collagen concentration is low, the free ends will be further apart in the coil state, and the log decrement will decrease. Also, any free molecules will add less to the damping forces in the denatured state. This is evident by the decrease in viscosity after denaturation. At higher concentrations, the distance between the free ends will be small. There will be more entanglements in the denatured state, thereby increasing the log decrement.

Before denaturation, the log decrement is independent of pH (10°C data, *Figure 6*). The lengths of the free strands and polymer segments decrease with increasing pH, while the attractive forces between the molecules increase. These two effects could compensate each other and keep the log decrement constant. After denaturation, the log decrement increases with pH (40°C, data, *Figure 6*). This can be attributed to increasing the attractive forces. The free strands move closer together, and entanglements are likely. This increases the viscous damping of the gels.

Degradation of gels

The pH has a marked influence on degradation of the gels (*Figure 7*). Below pH 3 the stability decreases rapidly. This could be due to acid catalysis of the peptide bond cleavage[17]. Another explanation for the stability at pH 4 may be the increase in crosslink density. As will be discussed later, an increase in concentration also increases the crosslink density and the stability.

The rate of degradation at pH 4 seems to be slightly lower than that at 3.1. The data are not good enough, however, to distinguish between these two rates. One would expect the pH 4 gel to be much more stable, since the acidity is lower and the crosslink density is much higher. There may be additional effects caused by the change in ionic character around pH 4. This will be discussed in more detail with the stability of collagen solutions.

If the samples at pH 3–4 are maintained at 40°C for 4–5 h, they begin to disintegrate. The continuous gel structure breaks down, and the result is clumps of gel entirely surrounded by solvent. The directly proportional dependence of (log) G' on time vanishes. The lower pH gels maintain the straight-line dependence for a few more hours. The gels are completely destroyed if kept at 40°C overnight. There is apparently some sort of autocatalytic reaction occurring. When a certain number of bonds break in the gel, the strain on the rest of the system may increase, thereby increasing the rate of degradation. The amount of time required to reach this point is apparently dictated by the pH. This could actually be due to a slight change in NaCl concentration, as will be discussed later.

The rate of degradation depends strongly on the concentration. All these tests were conducted in test-tube torsion pendulums, except for the 0.125% sample. The dependence of the log rate on concentration (*Figure 8*) is linear.

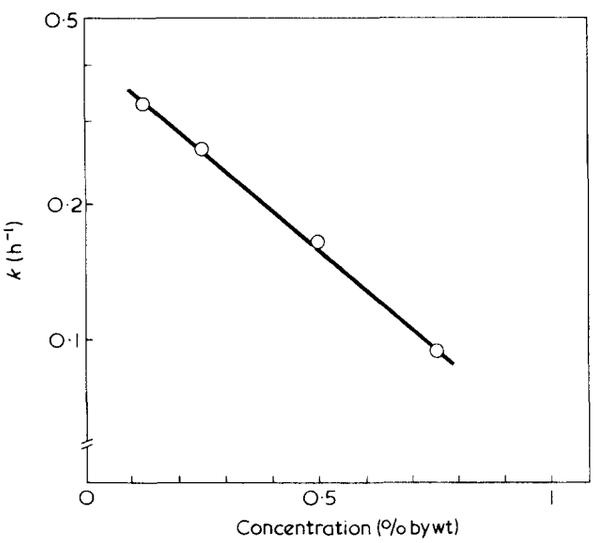

Figure 8 Concentration dependence of the rate constant for collagen gels (pH = 2.5)

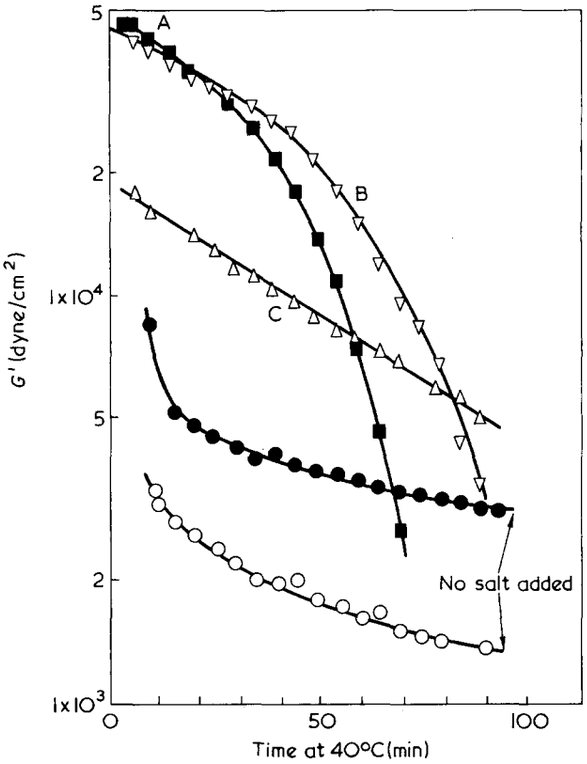

Figure 9 Effect of NaCl normality on degradation of 0.5% collagen gels (pH = 2.8): A, 0.1 N; B, 0.05 N; C, 0.01 N

Although the linearity of this plot is fortuitous and not the result of any theory, it does demonstrate the great increase in stability with concentration. This is not uncommon, as most protein systems are more stable at higher concentrations[18]. The crosslink density also increases with concentration and the protein chains are closer together. This parallels, then, the increase in stability with pH. As the pH and concentration increase, the collagen molecules may have a tendency to line up in a fibril formation. This would increase the stability, since the native fibril form is quite stable. This is evident by the very harsh treatment that is required to convert native collagen to gelatin[19].

The stability of the gels decreased greatly with increasing salt concentration (*Figure 9*). The rate of degradation was extremely rapid and apparently autocatalytic for concentrations above 0.01 N NaCl. This degradation is different from the thermal instability, or denaturation, of collagen that is usually studied. It is assumed here that covalent bonds, either in the crosslinks or in the protein chain, are being broken. The rate at which these bonds are broken, however, could be increased by the same phenomenon that increases the denaturation of the protein. The rate could be influenced by a change in the structure of water surrounding the protein, interactions of the anions at peptide bonds or at the crosslinking sites, or the disruption of hydrogen bonds by the ions. This rate of degradation has already been shown to be dependent on the helical state of the collagen. It is likely, then, that anything influencing the denaturation could also change the rate of degradation of the gels.

The screening of the positive charges on the collagen would allow the free molecule to assume a random-coil conformation in the denatured state. The chloride ions are not large enough to interfere with the random-coil formation. The average distance between the free amines on a collagen molecule is approximately 16.6 Å, or 50 Å on an individual helix. This is calculated using 3000 Å as the length of the collagen molecule, and obtaining the number of free amines per collagen molecule from an amino-acid analysis. The diameter of a chloride molecule is only 1.8 Å. This would allow many anions to surround the free amines, and leave plenty of room for the molecule to assume a random-coil conformation in the denatured state. The strands in the gel network are fixed in length, however, by the crosslinks, so they are far from their equilibrium position. This would place additional stress on the crosslinks. As one crosslink breaks, more stress would be placed on the remaining ones. This could account for the autocatalytic effect at the higher salt concentrations.

Degradation of collagen solutions

In order to better understand the degradation of collagen gels, a parallel study was conducted on collagen solutions. The pH again showed a marked effect on the stability of the collagen (*Figure 10*). The reciprocal of the specific viscosity is used, since this can be related to the number average chain length, as discussed previously. As the pH is raised from 2 to 3, the rate of degradation decreases. This is the same trend as observed in the gels. When the pH is raised to 4, however, the rate increases greatly. The same result is obtained with a 0.2% solution.

The greater stability with increasing pH is expected since the acidity is decreasing and the attractive forces are increasing. The increase in attractive forces is evident by the increase in viscosity, especially at pH 4. There are two large changes that occur at pH 4: the charge on the collagen and the protein–solvent interaction. The charge is greatly neutralized at pH 4, and segments of the collagen are hydrophobic. The orientation of water surrounding proteins is different for the ionic and hydrophobic segments[20–22]. Water is more ordered around hydrocarbons. It is not clear how this would affect the hydrolysis reaction, however. It has been suggested, though, that structurally involved water might be very important in the stabilization of the collagen helix[23].

The great increase in rate at pH 3, however, is quite surprising. Woodlock and Harrap[8] report a maximum in the denaturation temperature at pH 3. One would expect that

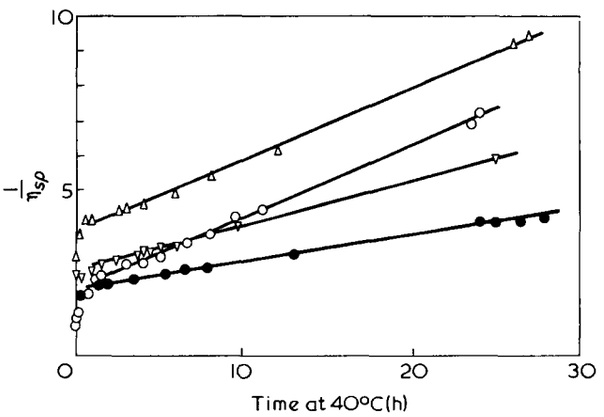

Figure 10 Effect of pH on degradation of 0.1% collagen solutions: △, pH 2.0, slope 0.21 h^{-1}; ▽, pH 2.2, slope 0.135 h^{-1}; ●, pH 3.0, slope 0.081 h^{-1}; ○, pH 4.0, slope 0.22 h^{-1}

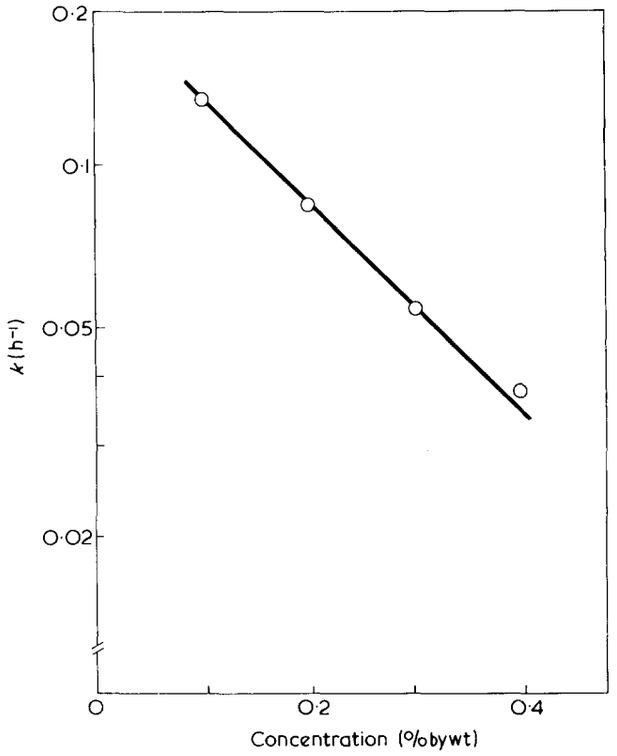

Figure 11 Concentration dependence of the rate constant for degradation of collagen solutions (pH = 2.2)

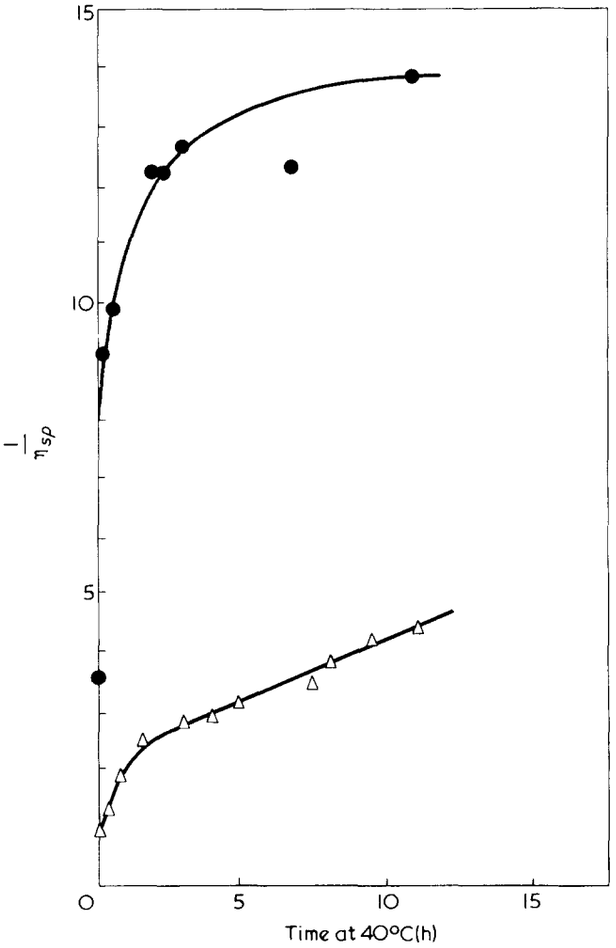

Figure 12 Effect of NaCl concentration on degradation of 0.1% collagen solutions (pH = 4.0): ●, 0.1 N; △, 0.01 N

by increasing the charge on the protein, the thermal instability would increase. There must be some other interaction occurring near pH 3 that overrides this electrostatic effect.

Although there is not this sharp decrease in stability at pH 4 for the gel, there is also no increase. The increased crosslink density may serve to stabilize the system, and this would compensate for the effects encountered in the solutions. The most stable gel, then, should be one between pH 3 and 4.

Two more solutions at pH 2.2 were studied in order to see the effect of concentration on degradation. Again, a semi-log plot of the rate constant vs. concentration is linear (*Figure 11*). This shows that the concentration influence remains without the increase in crosslink density. The increase in stability of the collagen seems again to be due to its surroundings in solution. More collagen molecules are present to surround other collagen chains and stabilize the system.

The viscosity of collagen solutions at 40°C was greatly reduced by increasing the salt concentration (*Figure 12*). This is to be expected, since the ionic shielding decreases the intramolecular repulsions, and a random coil has a much lower viscosity than an extended chain. The salts may also increase the rate of degradation of the collagen, but this is hard to determine. The viscosity of the 0.1 N NaCl in collagen is very close to that of water, making the measurement of the degradation rate extremely difficult.

CONCLUSIONS

The theory of rubber elasticity can be used to explain several phenomena of collagen gels. The effects of pH and concentration of the maximum modulus and modulus jump are much better understood in light of the theory.

Increasing the pH, collagen concentration, and NaCl concentration increases the modulus of the gels. The stability of the gels increases with collagen concentration and pH, but decreases with NaCl concentration. The pH increase is limited to the insolubility of the collagen above pH 4. Collagen gels of concentrations greater than 1% can be made, but the high viscosity of the solutions presents some problems. Also, the clarity of the gels decreases with increasing pH and concentration. This makes uniform irradiation difficult, and is undesirable for some uses.

The primary influence on the stability of the collagen gels and solutions seems to be the immediate surroundings of the protein. The collagen is apparently most resistant to hydrolysis when it is ionically charged and surrounded by other collagen molecules, and not in contact with ions of sodium chloride.

The crosslinks in the gels have a stabilizing influence. This is apparent since the gels are relatively stable at pH 4, while the solutions are not.

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