JOURNAL OF APPLIED POLYMER SCIENCE VOL. 19, 3299–3313 (1975)

Gelation of Dilute Collagen Solutions by Ultraviolet Light*

GARY HAMED† and FERDINAND RODRIGUEZ, *School of Chemical Engineering, Olin Hall, Cornell University, Ithaca, New York 14853*

Synopsis

Dilute collagen and collagen–polymer solutions were irradiated at 4°C with UV light (254 nm) under a nitrogen atmosphere. The collagen had been recovered from native tissue by a process of simultaneous acid solubilization together with proctase treatment to remove telopeptides. In the range of concentrations studied (0.1% to 0.9% collagen), gelation occurs after a few minutes, and the dynamic mechanical properties are followed thereafter using a freely oscillating torsion pendulum. During the irradiation, crosslinking and scission reactions compete. Initially crosslinking reactions dominate and the storage modulus increases. However, with extensive irradiation, scission reactions become important, and the storage modulus passes through a maximum and finally decreases. Furthermore, the time for incipient gelation decreases with concentration, and the maximum storage modulus increases with concentration to the 2.5 power for collagen solutions. As an example, the maximum storage modulus for a 0.5% solution is 1.1×10^4 dynes/cm^2. The modulus–dose behavior can be reduced to a single relationship in which the ratio of modulus to maximum modulus is a function of dose divided by dose to reach the maximum modulus.

INTRODUCTION

Collagen is a structural protein which constitutes about 30% by weight of all protein in the mammalian body.[1] In vivo it exists as a relatively insoluble, inert matrix of fibrils (for example, it is the major component of tendon, cartilage, and skin), but under the proper conditions of pH and temperature, a certain part of the collagen can be solubilized.[2] Much research has been performed in an attempt to find various ways in which this fraction of collagen can be reconstituted, treated, and subsequently used as a biomaterial.

Collagen membranes have been used in corneal surgery[3] and for dialysis membranes in artificial kidneys.[4] Diseased vitreous in the eye has been effectively replaced by collagen gels,[5] which have also been used for burn dressings.[6] In addition, blood vessel and heart valve prostheses have been fabricated from collagen.[6]

In many cases, the biomaterial is strengthened by the introduction of covalent crosslinks by ultraviolet (UV) or gamma irradiation.[7] This means of toughening collagen is "ideal" since toxic residues are not introduced, and the low antigenicity of collagen is unaffected.

* Presented before the American Institute of Chemical Engineers, 77th National Meeting, Pittsburgh, June 1974.

† Present address: Department of Polymer Science, University of Akron, Akron, Ohio 43420.

There are three categories of soluble collagens[2] that have been used in the past for UV irradiation studies: (1) those extracted from collagenous tissue with sodium chloride solutions, (2) those extracted with slightly acidic solutions after prior removal of neutral salt-soluble collagen (acid-soluble collagen, ASC), and (3) collagen recovered from native tissue by a simultaneous process of acid solubilization together with proctase treatment to remove telopeptides (telopeptide-poor or enzyme-solubilized collagen, ESC). The collagen used in this study was ESC.

EXPERIMENTAL

Apparatus

The essential parts of the apparatus consist of an ultraviolet irradiation chamber, a torsion pendulum with recorder, and a cooling system.

The irradiation chamber used here is constructed of plywood partially lined with aluminum sheet metal and contains eight 15-watt germicidal lamps which radiate primarily at 254 nm. It is also equipped with a fan so that warm air in the chamber can be exhausted.

In the torsion pendulum, a gas bearing is used to provide nearly frictionless rotation, and the moment arm consists of a horizontal aluminum shaft (6 in. long and ⅜ in. in diameter) with an aluminum block at each end.[8] Frequencies range from 0.2 to 5 hertz.

The actual shearing of the sample takes place in a cup-bob geometry (Fig. 1). The bob is made of aluminum and is 1.5 in. long and 0.875 in. in diameter. It is connected to the gas bearing by means of a 0.25-in.-diameter stainless steel shaft which fits tightly into a collet that is screwed onto the lower

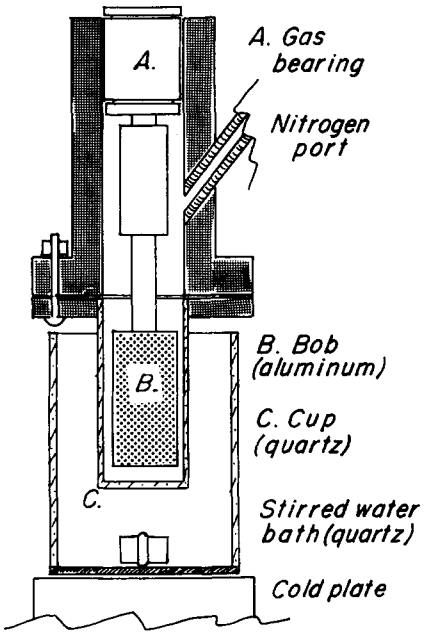

Fig. 1. Torsion pendulum.

end of the gas bearing. The cup is made of 1.0-in.-I.D. precision-bore quartz tubing, giving a clearance of 0.0625 in. between the cup and the bob. There is a 0.25-in. clearance between the bottom of the bob and the bottom of the cup. The contribution that the material at the bottom of the cup makes to the modulus can be neglected because of this relatively wide gap and since the ratio of the surface area of the end of the bob to that of the sides is small (about 0.1). The cup is made of quartz rather than glass since quartz is transparent to UV light, whereas ordinary glass is not.

Prepurified nitrogen at about 15 psig is supplied to the gas bearing and the apparatus is designed so that a nitrogen seal is maintained over the sample during the entire irradiation. The detection and recording of the response of the torsion pendulum is achieved by means of a Bentley-Nevada Proximitor probe (driven by an 18-V power supply) which sends out a voltage signal proportional to the distance between itself and a conducting object. The signal is monitored on a Clevite-Brush Mark 250 recorder.

A cold plate equipped with a magnetic stirrer (Thermoelectrics Unlimited) is used to cool a temperature bath in which the cup and bob are placed during irradiation. The bath vessel has a stainless steel bottom to facilitate heat transfer between the bath and the cold plate.

Procedure

Solutions

A 1.9% solution of enzyme-treated collagen in 0.01*N* HCl obtained from the Cornell Medical School Rogosin Laboratory was the masterbatch for all preparations. The collagen used in this study is prepared in the following manner: A swollen fiber dispersion in acid is produced by grinding dehaired steer hide which has been soaked in lime water to remove soluble proteins and mucopolysaccharides followed by washing with sodium chloride solution, washing with water, then sonification in acid. The dispersion is treated with proctase, a proteolytic enzyme. The now-solubilized collagen is precipitated by raising the pH to 8 and washed with water. The precipitation and washing is repeated twice. The washed precipitate is defatted six times by washing with an ether-ethanol mixture. The dried fibers are solubilized in 0.01*N* HCl, dialyzed exhaustively against 0.01*N* HCl to remove salts, and passed through a Millipore filter (pore size 0.65 μ).[9]

Collagen sample solutions were prepared by diluting with the appropriate amount of 0.01*N* HCl and stored at 3° to 4°C to prevent thermal denaturation. Five other solutions (all 0.50 weight per cent) were also prepared. PVP, PAM, PEO, PVA, and MBS (see Table II) solutions were made by diluting the monomer or polymer with 0.01*N* HCl and shaking overnight. All polymers dissolved after this treatment except the PVA in which case it was necessary to heat the solution to boiling to hasten dissolution. These solutions were also stored at 3° to 4°C.

Typical Run

A sample is poured into the cup and placed in an ice bath. Prepurified nitrogen at a pressure of two inches of water is bubbled through the solution for

½ hr to eliminate dissolved oxygen. The quartz cup is then fitted into place, immersed in the constant temperature bath, and the space above the solution is purged with prepurified nitrogen for ½ hr to remove oxygen there. The cold plate maintains the temperature at 4° ± ½°C.

The sample is located in the center of the square arrangement of the lamps, approximately 9 in. from the center of each lamp. When the sample is first exposed to the light, readings are taken every 2 or 3 min, then later every half-hour or hour depending on the concentration of the solution. To take a reading, the detector is placed approximately 0.07 in. from one of the aluminum blocks. The moment arm is given a very slight displacement with a gentle tap. If the solution has gelled, it acts as a restoring force and the pendulum oscillates. The output is a damped sinusoidal curve from which the frequency of oscillation is determined by knowing the chart speed, and the log decrement is calculated from the degree of damping. Readings are continued until the storage modulus (or frequency) passes through a maximum.

RESULTS AND DISCUSSION

Calculations

When a viscoelastic material (e.g., a gel) is periodically sheared, part of the energy is stored (represented by a storage modulus) and part is dissipated as heat (represented by a loss modulus). For free-oscillatory shearing between a cup and bob the storage and loss modulus are given by[10]

$$G' = (\omega^2 I/b)(1 + (\Delta^2/4\pi^2)) \tag{1}$$

and

$$G'' = (\omega^2 I/b)(\Delta/\pi) \tag{2}$$

where

$$b = \frac{4\pi L}{(1/R_1^2) - (1/R_2^2)} \tag{3}$$

and G' = storage modulus (dynes/cm^2); G'' = loss modulus (dynes/cm^2); Δ = logarithmic decrement; ω = frequency of oscillation (rad/sec); L = length of bob, 3.81 cm; R_1 = radius of bob, 1.11 cm; R_2 = radius of cup, 1.27 cm; and I = moment of inertia (g-cm^2), 3205 gm-cm^2 (in most runs).

Collagen Solutions

Time to Gel

When there are sufficient intermolecular crosslinks, the entire solution sets to a wall-to-wall gel. In a monodisperse polymeric solution that is randomly crosslinked, incipient gelation occurs when there is one crosslink for every two molecules.[11] Even at the gel point, however, some molecules contain no crosslinks at all. There is, in fact, a large amount of sol present at the gel point. The sol consists of single molecules and all combinations of multiple molecules not attached to the rigid gel structure.

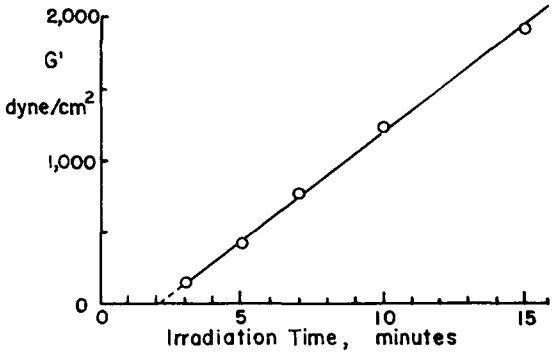

Fig. 2. Storage modulus vs. irradiation time for a 0.50% collagen solution.

The time to gel, t_g, in the present study was determined by extrapolating the storage modulus versus time of irradiation curve to zero modulus (Fig. 2). Concentrations ranged from 0.306 to 0.900 wt-% collagen. (A 0.101% solution was also gelled but was not considered sufficiently rigid to obtain good results, especially at low irradiation times.) This method was convenient since the plots were linear. Also, it did not involve a subjective observation to determine whether the solution had gelled or not.

As the UV irradiation of a collagen solution proceeds, there are two opposing effects taking place via a free-radical mechanism—chain scission and crosslinking. Under nitrogen, the crosslinking reactions dominate initially. Both intramolecular and intermolecular crosslinks are possible. However, intramolecular crosslinks in collagen are different than those induced by gamma irradiation of single-stranded polymer molecules in solution. Because of the rigid rodlike helical structure of collagen, this macromolecule is unable to bend upon itself (backbite) to form an intramolecular crosslink; rather, intramolecular crosslinks are formed between the three polypeptide strands that make up the triple helix. Thus, the free radicals within a collagen molecule must be in close proximity before they can combine to form an intramolecular crosslink, whereas two radicals on a single-stranded freely twisting molecule can intramolecularly crosslink by backbiting.

It is probable that chain scission results in shorter collagen molecules that still retain the helical structure. In order for this to occur, three polypeptide chains must be broken in the same part of the molecule. Apparently, when one of the chains is severed, the other two become stressed and more easily broken.

The linearity of the plots suggests that the crosslinking and scission reactions are initially both directly proportional to the irradiation time. This is reasonable and has been observed by other authors working with gamma irradiation of polymer solutions.[13,14] The gel time decreases with increasing concentration (Fig. 3), indicating that the efficiency of crosslinking is greater the more concentrated the solution. At low concentrations, one would expect the time to gel to increase rapidly as the critical concentration for gelation is approached; at high enough concentrations, molecules will associate enough that very little irradiation would be needed for gelation. The data can be fitted with the expression $t_g = 0.65/c^{1.5}$, where c is the concentration in weight per cent collagen.

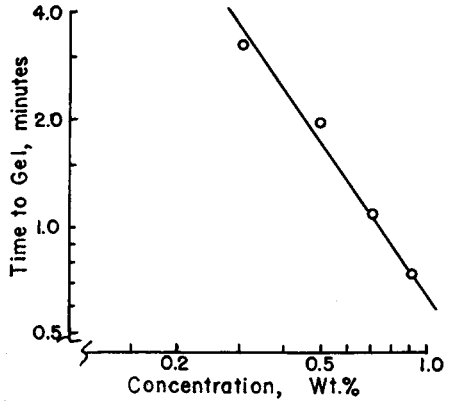

Fig. 3. Gelation time as a function of collagen concentration.

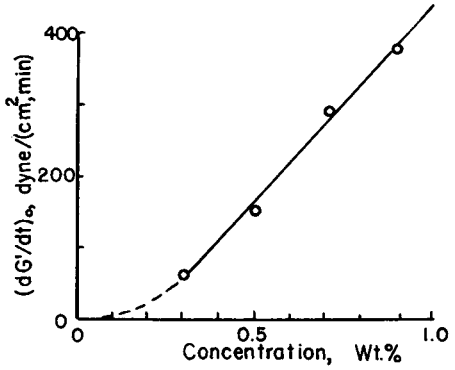

Fig. 4. Initial rate of modulus increase as a function of concentrating.

Figure 4 shows the *initial* rate of modulus increase as a function of concentration. This again shows the increased efficiency of crosslinking at higher concentrations.

Storage Modulus

As the irradiation proceeds and more molecules are being added to the gel network from the sol phase and crosslinking continues within the established gel, the storage modulus continues to increase but deviates markedly from linearity. Typical results are shown in Figure 5. It is reasonable to assume that the number of chain scissions is directly proportional to the time of irradiation since (as long as similar chains exist to be broken) a given quanta of absorbed light would be expected to cause a certain number of scissions. However, the number of crosslinks may be expected to deviate from linearity. Initially, the molecules are not highly restricted, and the gel is a loose, pituitous network. A macroradical can easily diffuse to a given site and crosslink. The sol phase molecules are relatively free to move in the gel network. As the network tightens and the sol phase molecules become more restricted, they cannot as easily diffuse to an active crosslinking site. The effect would be for crosslinking to be dependent on the irradiation time to a power somewhat less than the first.

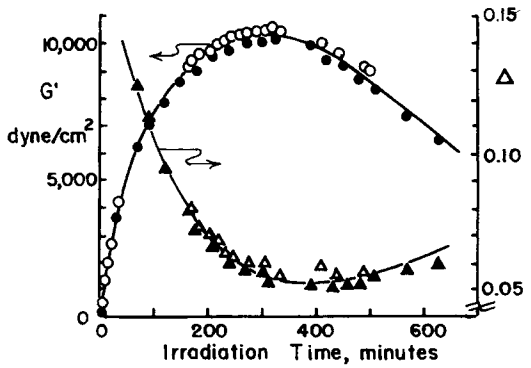

Fig. 5. Storage modulus and log decrement vs. irradiation time for a 0.500% collagen solution.

TABLE I
Maximum Storage Modulus and Corresponding Irradiation Time

Concentration, wt-% collagen	G'_{max}, dynes/cm²	t_{max}, min
0.101	109	60
0.306	2840	225
0.500	10300	300
0.709	23500	375
0.900	37500	450

All concentrations of gels are characterized by a maximum in the storage modulus denoting the point at which chain scissions become dominant over crosslinking. Table I gives the time to reach the maximum storage modulus (t_{max}) and the maximum storage modulus (G'_{max}). Behavior of (t_{max}) is linear ($t_{max} = 387c + 100$) for concentrations above 0.300% and deviates from linearity as the critical concentration is approached. The linearity indicates that there are a given number of sites in the molecule that are capable of reacting. If the concentration and hence the number of these sites is increased, proportionately more quanta of light must be absorbed to initiate these reactions. For storage moduli above $c = 0.3\%$, $G'_{max} = 52000c^{2.5}$. Van Brederode[12] has found that the maximum modulus for poly(ethylene oxide) gels formed by gamma irradiation varies with concentration to the 2.5 power.

Figure 6 represents a mastercurve of all concentrations of collagen from 0.306% to 0.900%. It is a dimensionless plot of the fraction of the maximum storage modulus attained ($\gamma = G'/G'_{max}$) versus the difference in the irradiation time and the time to gel divided by the difference in the time to reach the maximum modulus and the time to gel ($\tau = (t - t_g)/(t_{max} - t_g)$). Data for all four concentrations fit on the same curve. An empirical equation to fit the data was found after making several assumptions.

First, it was assumed that there were two competing reactions, one tending to increase G'(crosslinking) and the other tending to decrease G'(scission). Furthermore, it was assumed that the number of chain scissions was directly proportional to the irradiation time. This led to a semiempirical equation of the form $\gamma = k_1\tau^a - k_2\tau$, where k_1, k_2, and a are constants. Since it was desired that the plot pass through a maximum at the point (1,1), the derivative

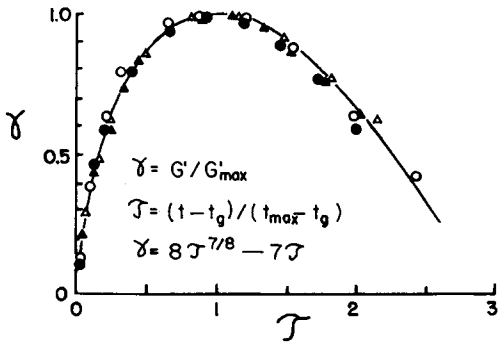

Fig. 6. Storage modulus, time mastercurve with eq. (4): (○) 0.306%; (△) 0.500%; (●) 0.709%; (▲) 0.900%.

of γ was taken with respect to τ and set equal to zero. This determined the condition that $a = k_2/k_1$. A value of $k_2 = 7$ and $k_1 = 8$ was chosen and fit the data well.

Several comments are warranted. First of all, although by only a slight amount, the exponent on the crosslinking portion of the equation (7/8) is less than one. This would be the expected behavior if, as each crosslink occurs, the formation of the next crosslink is less likely to occur. In addition, although the exponent in the scission portion of the equation is one and the crosslinking portion very nearly one, the behavior markedly deviates from linearity. To be sure, this is a consequence of the mathematics; but, nonetheless, it is interesting that it is *possible* to have two opposing reactions, one varying to the first power and the other very nearly to the first power of irradiation time and still obtain the highly curved behavior observed here. It is likely, however, that the exponents as well as the coefficients in the scission and crosslinking portion are changing with irradiation time as the gel is continually changing its internal structure. Independent of what an actual theoretical equation would reveal, it can be stated, nonetheless, that the effective ratio of the coefficient for scission and crosslinking is 7:8 and the *effective* dependence of crosslinking on the irradiation time is to the seven-eights power.

There is another explanation that could explain the highly curved modulus-versus-irradiation time curve. Suppose that there are two types of sites in the collagen molecule; one type that will engage predominantly in crosslinking and the other that will engage predominantly in degradation under UV light. Further, suppose that the crosslinking sites require less energy than the degradation sites to be activated. The result would be that after a short irradiation time, sufficient energy would have been supplied to initiate crosslinking but not enough to cause significant scission. But, when enough quanta had been absorbed, the degradation sites would be activated and the gel structure would be broken down.

Other authors[12,13] have found for gamma irradiation of dilute solutions of synthetic polymers that the modulus increased linearly to a plateau value and that further irradiation caused syneresis of the gels. No syneresis is observed in the present study since chain scission prevents stresses from building up within the network and, hence, the gel does not become so tight that solvent is "squeezed out."

Combining all results, the following expression for G' as a function of concentration and irradiation time is found:

$$\left[\frac{G'}{52000c^{2.5}}\right] = 8\left[\frac{t - (0.65/c^{1.5})}{387c + 100 - (0.65/c^{1.5})}\right]^{7/8} - 7\left[\frac{t - (0.65/c^{1.5})}{387c + 100 - (0.65/c^{1.5})}\right]. \quad (4)$$

This equation is valid for 0.300% to 0.900% collagen solutions.

Logarithmic Decrement

The logarithmic decrement (Δ) is defined as the natural logarithm of the ratio of the amplitude of two successive oscillations.[10] Since this property is very nearly independent of amplitude (for small amplitudes), it may be calculated by the equation

$$\Delta = \frac{\ln (A_i/A_n)}{n - i} \quad (5)$$

where A_i and A_n are the amplitudes of the ith and nth peaks, respectively. The log decrement gives a measure of the damping of the gel. Just after gelation, the gel is loose and pituitous, and there are many loose chain ends as reflected by a large Δ. As the gel tightens with further irradiation, the gel becomes more resilient and the Δ decreases rapidly. In Figure 5, the log decrement is plotted with the storage modulus. The Δ continues to decrease until the storage modulus passes through a maximum. As chain scission becomes predominant and the gel structure is being broken down, Δ increases. However, for a given value of the storage modulus, the Δ corresponding to this value on the rising portion of the modulus curve is much larger than the Δ corresponding to the same value of the modulus on the decreasing portion of the modulus curve. This can be easily understood since, when the gel network is being built up, there is a large sol fraction not attached to the network that contributes to the damping; whereas, when the network has been "perfected" to its greatest extent and is then broken down, the chains are still attached to the gel lattice even though they may be severed.

The minimum value of the Δ decreases with increasing concentration (0.05 for 0.3%, 0.035 for 0.9%). Van Brederode[12] has found similar behavior with PEO solutions gelled with gamma irradiation. The more concentrated solutions are presumed to form a tighter more resilient network because of increased efficiency of crosslinking.

Loss Modulus

The loss modulus G'' (Fig. 7) is directly proportional to the Δ and to the square of the frequency of oscillation. For all concentrations, the loss modulus passes through a maximum after a short irradiation time and then decreases with further irradiation. Just after incipient gelation, not only is the Δ large but the frequency of oscillation is also very small. The frequency effect dominates, and initial values of the loss modulus are small but increasing. As the frequency increases at a decreasing rate, finally a point is reached

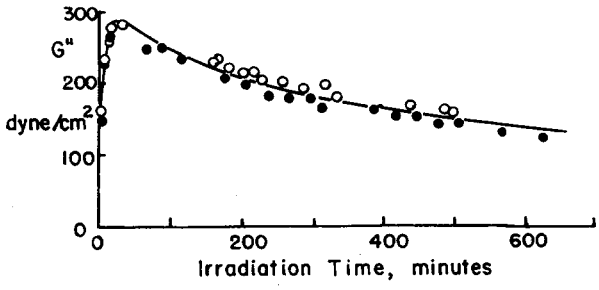

Fig. 7. Loss modulus vs. irradiation time for a 0.500% collagen solution.

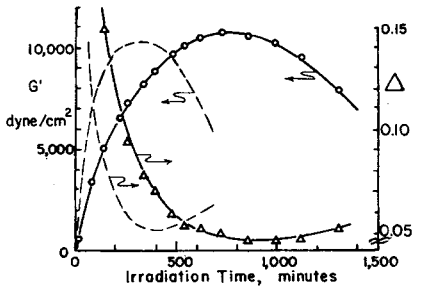

Fig. 8. Effect of dose rate on storage modulus and log decrement of a 0.500% collagen solution. Solid curve represents data for 4 lamps; dotted curve, 8 lamps.

where the declining Δ becomes predominate. Beyond this point, the loss modulus continues to decline smoothly even as the storage modulus passes through a maximum.

Dose Rate

The effect of dose rate was estimated by using all eight lamps and then just four of the lamps (Fig. 8). Within experimental error, the effect is about the same whether eight lamps are used for a given time or whether four lamps are used for twice this time. These results indicate that dose rate does not affect gelation properties.

Temperature of Irradiation

The time for incipient gelation is not affected for a change in irradiation temperature from 4°C to 20°C (Fig. 9). However, upon further irradiation, chain scissions are greatly enhanced, as can be readily discerned from the rapid decrease in storage modulus after it peaks. The rate at which the modulus decreases at 20°C is about three times that at 4°C. If an expression of the Arhennius type is assumed,

$$k = A \exp(-E_{act}/RT) \tag{6}$$

where k = rate of decreasing modulus, R = gas constant, T = temperature, E_{act} = activation energy, and A = constant coefficient, then an activation energy for chain scission may be calculated:

$$\ln(k_{20C}/k_{4C}) = (E_{act}/R)[(1/277) - (1/293)]. \tag{7}$$

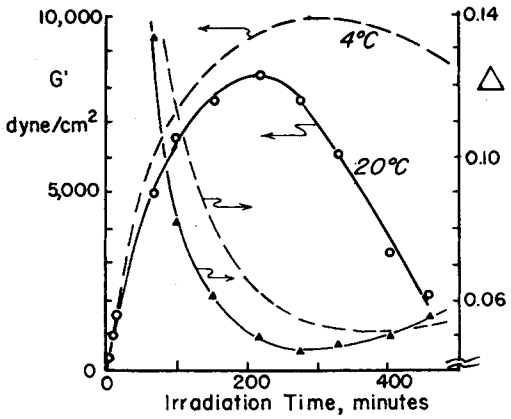

Fig. 9. Effect of irradiation temperature on storage modulus and log decrement of a 0.500% collagen solution.

An activation energy of approximately 11.1 kcal/mole is calculated from the preceding equation. This value may be compared to those found by other authors for the oxidative degradation of crosslinked rubber networks. Tobolsky[14] reported an activation energy for oxidative chain scission of 30 kcal/mole for synthetic rubber valcanizates. Similarly, Ore[15] gave a value of 22 kcal/mole for natural rubber crosslinked with di-*tert*-butyl peroxide. From this, it can be concluded that chain scission on collagen is not a simple oxidative scission; however, results do indicate it is an activated process.

There may also be thermal denaturation effects that contribute to the lower moduli at the higher irradiation temperature.

Temperature After Irradiation

A 0.306% collagen solution was irradiated for 220 min (time to reach maximum modulus). Then, the irradiation was stopped and the temperature of the gel was varied to determine the effect on the storage modulus. One hour was allowed for the gel to equilibrate at the new temperature before measuring the modulus (Fig. 10). The modulus is relatively constant up to and including 29°C; then there is a drastic drop in the modulus as the temperature is raised to 31°C.

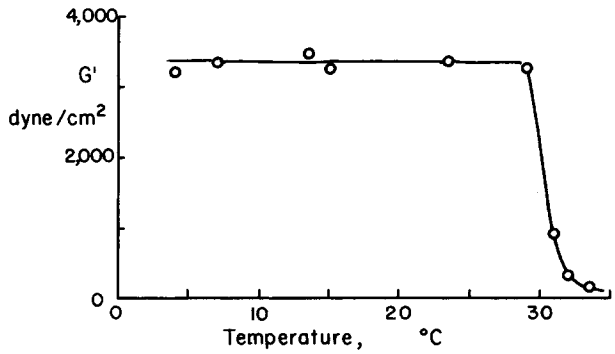

Fig. 10. Effect of temperature after irradiation on storage modulus of a 0.306% collagen solution (irradiation stopped after 220 min).

The normal denaturation temperature of unirradiated acid-soluble collegen is about 40°C. UV irradiation of collagen, although strengthening the material by introducing crosslinks, also disrupts the stable hydrogen-bonded structure of the triple helix. As a result, the melting temperature of the collagen declines.[7] Although the network is held together by covalent crosslinks, once the melting temperature is reached, the whole collagen structure "unravels."

Collagen–Polymer Mixtures

All collagen–polymer mixtures discussed in this section contain 0.25% collagen and 0.25% polymer (Table II).

Time to Gel

The time for incipient gelation of the mixtures was determined by extrapolation of the storage modulus versus irradiation time plot (Table IV). The expected irradiation time to cause gelation of a 0.25% collagen solution containing no polymer is 3.7 min. The onset of incipient gelation was prolonged in all mixtures, although none of the polymers completely inhibited gelation. The PVP–collagen and PVA–collagen mixtures gelled at about the same irradiation time as did the PEO–collagen and PAM–collagen mixtures. The MBA–collagen solution inhibited gel formation for over ½ hr before the onset of incipient gelation. Also, the expected initial rate of increase of the storage modulus for a 0.25% collagen solution is 40 dyne/cm^2-min. None of the mixtures had an initial rate even half this value (Table III).

The polymers may inhibit the gelation by interfering with the diffusion of collagen macroradicals that must come together to form crosslinks. Another possibility is that the polymers combine with the macroradicals to form radicals that are no longer of the crosslinking type. However, if this existed to a great extent, gelation would be completely inhibited.

Storage Modulus

As further irradiation occurs the collagen–polymer mixtures show behavior qualitatively similar to the irradiation of collagen solutions containing no

TABLE II
Polymers Used

Abbreviation	Identification
PVP	poly(vinyl pyrrolidone): Type NP-K90 supplied by General Aniline and Film Corporation
MBA	N,N'-methylenebisacrylamide: supplied by American Cyanamid Company
PEO	poly(ethylene oxide): friction-reducing agent, supplied by Union Carbide Corporation
PAM	polyacrylamide: Type M-352B, supplied by American Cyanamid Company
PVA	poly(vinyl alcohol): Elvanol Grade 71-306, supplied by E.I. du Pont de Nemours and Company

TABLE III
Properties of Collagen–Polymer Gels[a]

Solution[b]	Time to gel t_g, min	Initial rate $(dG'/dt)_0$, dyne/cm²-min	G'_{max}, dyne/cm²	t_{max}, min
PVP–Collagen	6.2	13.0	1675	370
MBA–Collagen	31	14.5	9200	850
PEO–Collagen	4.6	15.9	2000	370
PVA–Collagen	6.0	8.0	790	370
PAM–Collagen	4.3	17.8	2170	370
Control[c]	3.7	40.0	1600	185

[a] 0.25 wt-% of each component.
[b] Measured at 4°C in 0.01*N* HCl.
[c] 0.25 wt-% collagen alone.

polymer. Crosslinking in the gel continues to dominate up to a certain point (G'_{max}), then degradative reactions become predominate and the storage modulus declines. Again, a comparison of the collagen–polymer mixtures will be made with a solution containing 0.25% collagen and no polymer. A 0.25% collagen solution would attain a maximum modulus of about 1600 dynes/cm² after 185 min of irradiation. The results for the mixtures also are given in Table III. Within experimental error, the PVP has no effect on the maximum modulus attained, but twice the irradiation time is required to reach this peak. This suggests that the polymer absorbs as much light as the collagen, thus twice the irradiation time is required to initiate the available reaction sites on the collagen. In fact, in four out of five of the mixtures (PVP–, PEO–, PVA–, and PAM–collagen), the modulus peaked after about 370 min of irradiation. In the PEO– and PAM–collagen mixtures, the polymers may have been incorporated into the gel structure to a slight extent since higher maximum moduli of 2000 and 2170 dyne/cm² were observed for these gels. Of all the mixtures, the PVA–collagen solution was the only one that showed a marked inhibition of crosslinking by the synthetic polymer. For this sample, the storage modulus peaked at a value (790 dynes/cm²) about half that had the PVA not been present. MBA was found to have the most marked affect on the gelation of collagen. After a long inhibition period, incipient gelation occurred, and then the modulus increased for 850 min reaching a value of 9200 dynes/cm², very nearly as high as the value a solution containing 0.500% collagen attains upon irradiation. Apparently, MBA is incorporated into the gel effectively crosslinks with the collagen in the network. It should be pointed out, however, that this gel had a white hazy color whereas all the other gels (collagen–polymer as well as collagen alone) were optically clear.

Logarithmic Decrement

In contrast to the collagen solutions with no polymer, the Δ continues to decrease well after the storage modulus has passed through a maximum and does not begin to increase again until the storage modulus has decreased to a significant degree. The minimum Δ for the PEO– and PVA–collagen mixtures is about 0.065, whereas the PVP– and PAM–collagen mixtures at-

tain a minimum of about 0.04. The MBA–collagen mixture has the lowest minimum Δ of all the solutions ($\Delta = 0.025$).

CONCLUSIONS

1. UV irradiation of dilute deoxygenated collagen solutions under a nitrogen atmosphere induces gelation. Two opposing reactions, crosslinking and scission, take place simultaneously. Initially, crosslinking reactions predominate (increasing G'); but with prolonged irradiation, chain scission becomes the more important factor (decreasing G').
2. The time for incipient gelation decreases with increasing concentration.
3. The initial rate of change of the storage modulus increases linearly with concentration.
4. G'_{max} increases with concentration to about the 2.5 power in the concentration range from 0.300% to 0.90 collagen.
5. The time to reach the maximum modulus is a linearly increasing function of concentration.
6. The log decrement decreases rapidly as G' is increasing, and its minimum value decreases with increasing concentration.
7. The G'' passes through a maximum at an early stage of irradiation, then decreases monotonically with further irradiation.
8. Dose rate does not affect gel properties.
9. Increasing the temperature of irradiation from 4° to 20°C significantly enhances chain scission.
10. The denaturation temperatures of collagen is lowered after exposure to UV light.
11. The presence of PVA, PVP, PEO, PAM, or MBA in a collagen solution prolongs the time for incipient gelation and lowers the initial rate of modulus increase.

The authors wish to thank the National Science Foundation for partial support under Grant No. GH-34387X

References

1. G. N. Ramachandran, Ed., *Treatise on Collagen,* Academic Press, New York, 1968.
2. H. R. Elden, Ed., *Biophysical Properties of the Skin,* Wiley-Interscience, New York, 1971
3. M. W. Dunn, K. H. Stenzel, L. E. Hopkins, M. J. Powers, A. L. Rubin, and T. Miyata, *J. Ophth. Surg.,* **2,** 20 (1971).
4. A. L. Rubin, R. R. Riggio, R. L. Nachman, G. H. Schwartz, T. Miyata, and K. H. Stenzel, *Trans. Amer. Soc. Artif. Int. Org.,* **14,** 169 (1968).
5. M. W. Dunn, K. H. Stenzel, L. E. Hopkins, M. J. Powers, A. L Rubin, and T. Miyata, *Arch. Ophthal.,* **82,** 840 (1969).
6. A. L. Rubin, T. Miyata, and K. H. Stenzel, *J. Macromol. Sci.-Chem.,* **A3,** 113 (1969).
7. T. Miyata, T. Sohde, A. L. Rubin, and K. H. Stenzel, *Biochim. Biophys. Acta,* **229,** 672 (1971).
8. F. Rodriguez, R. A. van Brederode, and G. G. Cocks, *J. Appl. Polym. Sci.,* **12,** 2415 (1968).
9. A. L. Rubin, Rogosin Laboratories, private communication, June 15, 1972.
10. J. D. Ferry, *Viscoelastic Properties of Polymers,* Wiley, New York, 1961.
11. P. J. Flory, *Principles of Polymer Chemistry,* Cornell University Press, Ithaca, N.Y., 1969.
12. R. A. van Brederode, F. Rodriguez, and G. G. Cocks, *J. Appl. Polym. Sci.,* **12,** 2097 (1968).

13. E. Kiran and F. Rodriguez, *J. Macromol. Sci.-Phys.*, **B7**, 209 (1973).
14. A. V. Tobolsky, *Properties and Structure of Polymers*, Wiley, New York, 1960.
15. S. Ore, *On the Oxidative Stress Relaxation of Natural Rubber Vulcanized with Di-tert-butyl Peroxide*, Oslo University Press, Oslo, 1958.

Received April 18, 1975