Mechanical Properties of Native and Crosslinked Gelatins in a Bending Deformation

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ABSTRACT: Gelatin samples, native and chemically crosslinked with three different diisocyanates, were studied by bending-creep measurements. These samples were characterized by the number-average molecular weight of a chain segment between two points of crosslinkage M_c. The chemical network was found to contribute to a marked extent to the mechanical behavior of the samples. The dependence of the creep compliance on the time for different loads was determined. The experimental results were compared with calculated ones according to a model, comprising four parameters, to obtain a better understanding of structure–property relationships for these materials. A very good agreement between the model and experimental data was found. Two of the fitting parameters, however—the relaxation time and η (which is connected with the viscosity)—were found to strongly depend on the time of the experiment. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 76: 2041–2048, 2000

Key words: crosslinked gelatin; isocyanates; bending; creep; relaxation time; viscosity

INTRODUCTION

In the past 50 years the plastic industry has worked on synthesizing and formulating durable materials that are more and more adapted to their particular uses. There is a permanent interest in the production of biodegradable polymers as a result of increased demand for environmentally friendly products. One such polymer is gelatin, a biodegradable and environmentally benign biopolymer. The main reason for the current interest in gelatin is its biodegradability from the action of naturally present microorganisms. It possesses the additional advantage of leaving no waste products.[1]

In order to transform gelatin into a plastic with desirable properties, its crosslink density must be controlled. Thus, instead of the existing physical network, the creation of more flexible chemical crosslinking is required.[2,3] Both the native and the chemically crosslinked gelatins possess attractive properties for commercial applications. The principal uses of gelatin are categorized as edible (55%), pharmaceutical (25%), photographic (15%), and other technical (5%).[4] Uses as technical grades are extensive and include adhesive applications,[5] such as gummed tape, abrasives, bookbindings, and boxes; coating and sizing of yarns; fabric sizing; dichromated gelatins for holography and photoresists; paper coatings and sizing; printing-press rollers; bacteriological culture media; colored filters; and protective colloids in emulsion polymerizations and cooper refining.

The mechanical properties of these gelatin products are of primary importance. All the above listed products are subject to various straining actions such as: (1) tension or compression, (2) shearing, (3) torsion, and (4) bending. Consequently, gelatin samples have to be tested in various ways in accordance with their possible application.

In the present work mechanical studies of native gelatin samples and of three crosslinked with various agents were carried out. They involve measurements of creep during bending, which is particularly well suited for hard materials and consequently for gelatin. This information is critically important with regard to its functioning and may serve as a guide for various applications, listed above. The results should also provide a better understanding of the relationship between the mechanical properties and the polymer structure of this widely used material.

EXPERIMENTAL

Materials

The gelatin was type A, bloom value 300 (Sigma, St. Louis, MO). 1,4-diisocyanatobutane (TMDIC); 1,6-diisocyanatohexane (HMDIC); 1,12-diisocyanatododecane (DDMDIC) and 2,2,2-trifluoroethanol (TFE) were purchased from Aldrich (Milwaukee, WI). Triethylamine was purchased from Fluka (Milwaukee, WI).

Preparation of Samples

Collagen folds in aqueous solutions of gelatin act as physical crosslinks, and for this reason the gelatin films become very brittle. In addition, the number of physical crosslinks is difficult to control. That is why as a solvent we chose TFE, in which gelatin displays excellent solubility at room temperature without forming collagen folds.[6]

Sample 1 (chemically uncrosslinked, native gelatin) was obtained by dissolving 2 g gelatin in 20 mL TFE at room temperature for 12 h. The solution was subsequently homogenized by a magnetic stirrer for 1 h at 25°C and poured in poly(ethylene terephthalate) Petri dish. After drying for 2 days at room temperature an isotropic film was obtained.

The chemically crosslinked samples 2–4 were obtained by dissolving at room temperature for 2 h 2 g gelatin in approximately 25 mL TFE (the exact amount TFE depended on the nature of the crosslinker) in order to obtain 5.5 wt % gelatin concentration. The resulting solutions were subsequently homogenized by a magnetic stirrer and crosslinked by three different diisocyanates with a concentration of 0.0128M. Triethylamine in an amount equal to $\frac{1}{3}$ of that of the crosslinker was used as a catalyst. The crosslinking was carried out at 25°C for 1 h, and the resulting mixture was subsequently poured in poly(ethylene terephthalate) Petri dishes and dried at room temperature.

Investigation of Bending Behavior

We generally followed our previous work on the bending behavior of some elastomers.[7] The thickness of the round film (as obtained from the Petri dish) was measured at several (10–20 points), and subsequently a beam-shaped specimen, relatively uniform in thickness (30–50 mm long, 7–9 mm wide and 0.3 mm thick), was cut from each sample. The length and width of each specimen were measured with a caliper gauge. Since the mechanical behavior of the specimen is specifically very sensitive to its thickness, it was measured very accurately with a micrometer in several points, and if the values obtained differed by more then 0.01 mm, another specimen was cut. The specimen was clamped so as to leave approximately 1 cm inside the clamp, which was attached to a stable vertical bar. This so-called cantilevered beam was loaded by applying constant loads to the other end, thus normally causing displacement to the originally unloaded position (deflection). The deflection, δ, depends on the load P through.

$$\delta = AP/E \qquad (1)$$

where

$$A = 4 \ L^3/bh^3 \qquad (2)$$

is a dimension-related constant; E, the Young's modulus; L, the length of the beam, measured from the fixed end to the point of application of the force; b, the width; and h, the thickness of the beam. To account for the real load, the contribution of the specimen weight itself, calculated as $3wL/8$,[7] where w is the weight per unit length, was added to the applied (external) load.

Generally, the deflection, δ, depends on the time, so according to eq. (1) the creep compliance $Y(t) = 1/E(t)$ might be written as

$$Y(t) = \delta(t)/AP \qquad (3)$$

In order to obtain $Y(t)$ through eq. (3) a small constant load, P, was applied, and a cathetometer was used to measure δ very accurately as a function of time. Since it changes with time very slowly, it took a couple of days to collect data for $\delta(t)$ for one constant load, that is, to obtain one creep curve. Later, the load was removed, the beam released and put for a few minutes at 30–35°C and a pressure of 6–8 kN/cm^2 between the plates of a heating press. Subsequently, the press was permitted to cool down to the temperature at which the experiment was conducted (19–21°C). Thus, the beam straightened and was ready for the next measurement with higher load. This procedure was repeated with a total of four loads in ascending order. Although some sample damage and accumulation of the plastic strain is possible, this procedure assures exactly the same sample dimensions for the next load. All measurements were conducted around the gelatin glass-transition temperature (43–50°C[8]) for the equilibrium moisture content of 15–17 wt %.[9]

Evaluation of Network Crosslink Density

Gelatin samples (squares of 10×10 mm) were swollen at 20°C for 48 h in distilled water in order to achieve an equilibrium swelling. The gelatin volume fraction in swollen samples, ν_G, was determined, assuming an additivity of a specific volume of gelatin and water in the sample, as

$$\nu_G = W_0\rho_w/[W\rho - W_0(\rho - \rho_w)] \qquad (4)$$

where W_0 is the initial weight of the sample; W is the weight of the swollen sample, which was measured after removing excess liquid on the surface of the sample by a filter paper; ρ_w is the density of the water at 20°C; and ρ is the density of the dry, chemically uncrosslinked gelatin. In the first approximation this value was also used for the crosslinked gelatin samples.

With ν_G thus obtained, to estimate the network crosslink density, the number-average molecular weight of a chain segment between two points of crosslinkage, M_c, was calculated according to the Flory–Rehner equation[10]

$$M_c = -\rho V_1\nu_G^{1/3}/[\chi\nu_G^2/2 + \ln(1 - \nu_G) + \nu_G] \quad (5)$$

where V_1 is the molar volume of the solvent and $\chi = 0.49 \pm 0.05$[11] is the polymer–solvent interaction parameter.
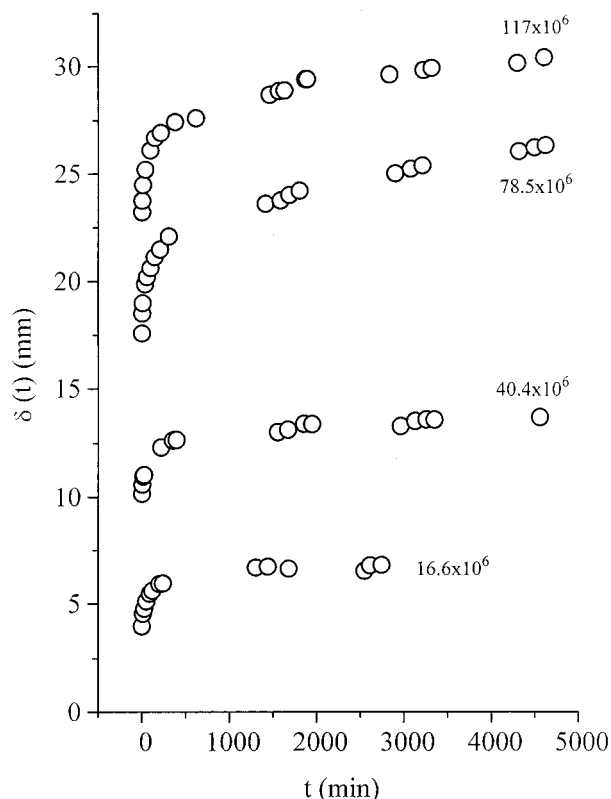

Figure 1 Typical deflection, δ, versus time, t, behavior (native gelatin sample taken as an example). Each curve is labeled with the corresponding normalized load, AP, in N/m. Sample length is 53 mm for this specimen.

RESULTS AND DISCUSSION

Typical curves of deflection versus time for sample 1 are presented in Figure 1, with each curve labeled by the normalized load, AP, in N/m. The corresponding figures for samples 2–4 look very similar and are not given here for the sake of clarity. Some of the points are vertically shifted off the regression line (not drawn here), due mainly to changes of the ambient temperature within 1–3°C and possibly to minor changes of the ambient moisture content in a long-term scale. Generally, the deflection increases relatively fast after the beginning of the experiment and continues to increase slowly for longer times without leveling off (Fig. 1). This continuous increase is better expressed for higher normalized loads.

Figure 2 shows the dependencies of the creep compliance, $Y(t)$, on time (t) for samples 1–4. The loads were taken so as to produce initial deflections of the same order of magnitude for every
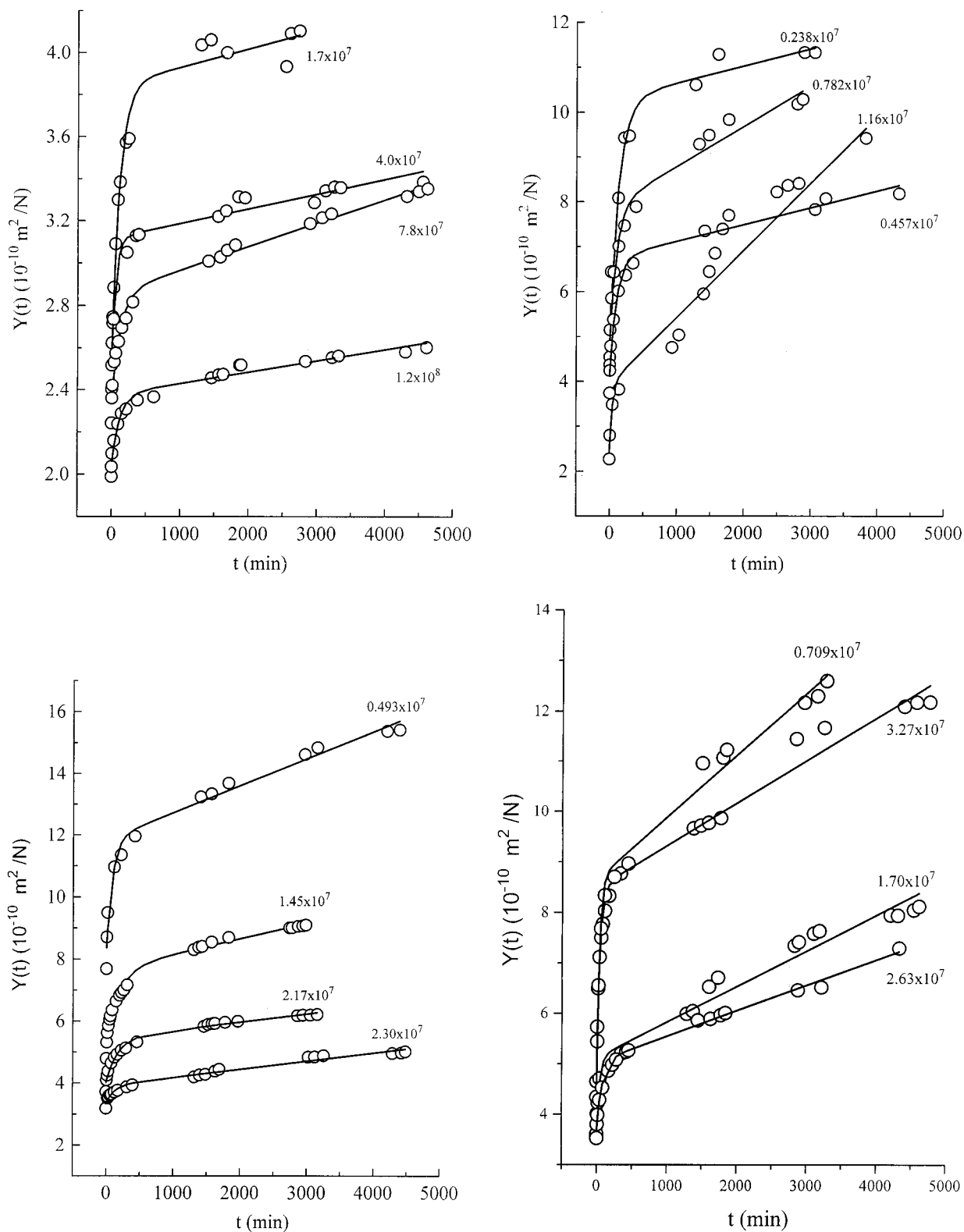
Figure 2   Dependence of creep compliance, $Y(t)$, on time. The circles are the experimental data, and the lines are the fitting curves. Each curve is labeled with the corresponding normalized load AP in N/m. The samples are: (a) native gelatin; and gelatin crosslinked with (b) 1,4-diisocyanatobutane; (c) 1,6-diisocyanatohexane; and (d) 1,12-diisocyanatododecane.

Table I   Normalized Load *AP*, Fitting Parameters *Y*(0), *Y*(∞), Their Difference *Y*(∞)–*Y*(0), Relaxation Time, τ, η, and Correlation Coefficient *R*² For The Sample of Native Gelatin and The Three Chemically Crosslinked Samples

AP, 10^6 (N/m)	$Y(0)$, 10^{-10} (m²/N)	$Y(\infty)$, 10^{-10} (m²/N)	$Y(0) - Y(\infty)$, 10^{-10} (m²/N)	τ, min	η, 10^{12} (N · min/m²)	R^2
			Native Gelatin			
16.6	2.55	3.84	1.29	113	115	0.98
40.4	2.56	3.12	0.56	59.1	142	0.98
78.5	2.34	2.85	0.51	122	89.8	0.99
117	2.04	2.38	0.34	118	186	0.98
			TMDIC Crosslinked Gelatin			
2.38	5.11	10.28	5.17	137	11.2	0.95
4.46	2.27	3.43	1.16	10.2	53.9	0.97
7.82	4.87	7.89	3.02	103	11.2	0.97
11.6	2.42	3.94	1.52	41.6	6.72	0.96
			HMDIC Crosslinked Gelatin			
4.93	6.21	8.02	1.81	59.5	11.5	0.93
14.5	5.30	7.66	2.35	193	20.2	0.98
21.7	4.02	5.44	1.42	174	38.0	0.98
23.0	3.33	3.92	0.59	124	38.2	0.97
			DDMDIC Crosslinked Gelatin			
7.09	4.58	8.66	4.08	35.6	8.12	0.99
17.0	3.72	5.12	1.40	42.2	14.1	0.97
26.3	3.66	5.04	1.38	77.2	19.7	0.99
32.7	5.15	8.48	3.33	46.0	11.8	0.98

sample. Because of the reasons already stated, some points are shifted vertically off the regression line (not drawn here) in these figures like those in Figure 1 [see eq. (3)]. Actually, the lines in Figure 2 represents best fits of the function $Y(t)$

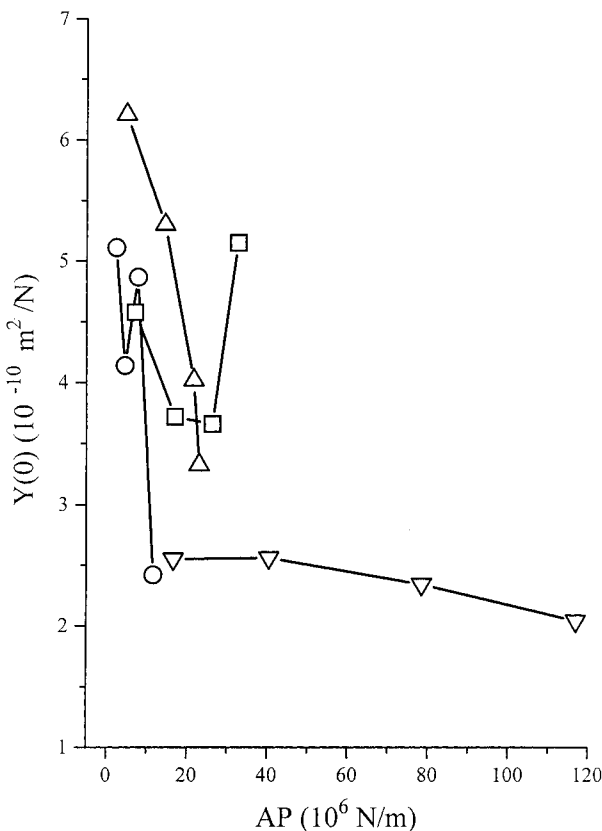$$Y(t) = Y(0) + [Y(\infty) - Y(0)][1 - \exp(-t/\tau)] + t/\eta \quad (6)$$

to the experimental data.[7] Here $Y(0)$ is the initial compliance, $Y(\infty)$ is the compliance after unlimited time, τ is the relaxation time, and η is a parameter connected with the viscosity. The three terms in eq. (6) are contributions from Hookean deformation, elastic relaxation, and Newtonian flow, respectively. The points in every curve in Figure 2 were fitted with eq. (6) and the fitting curves are shown in the same figures. The fitting parameters $Y(0)$, $Y(\infty)$, τ, and η were obtained for every sample and normalized load and their values are given in Table I together with the normalized loads AP.

In order to comment on the data in Table I, an estimation is needed of the crosslink density. For this reason the average molecular weight between crosslinks, M_c, was calculated according to eq. (5), and the results are presented in Table II. As seen from this table, the higher the crosslinker length, the lower the M_c value, that is, the denser

Table II   Number-Average Molecular Weight of a Chain Segment Between Two Points of Crosslinkage M_c for Chemically Uncrosslinked Sample 1 and Chemically Crosslinked Samples 2–4

Sample Number	Crosslinking Agent		M_c
1	Native Gelatin		6000
2	1,4-diisocyanatobutane	$OCN—(CH_2)_4—NCO$	3600
3	1,6-diisocyanatohexane	$OCN—(CH_2)_6—NCO$	3200
4	1,12-diisocyanatododecane	$OCN—(CH_2)_{12}—NCO$	1100

These figures are quite similar except for the higher values of $Y(\infty)$. Sample 1 has the lowest, with practically constant values of both $Y(0)$ and $Y(\infty)$, which correlates with its highest M_c value. $Y(0)$ and $Y(\infty)$ for samples 2–4 depend strongly on AP. Since samples 2–4 are crosslinked with long and flexible chains, containing 4, 6, and 12 single-bond carbon atoms, respectively (Table II), their flexibility is high, leading in turn to higher compliances of $Y(0)$ and $Y(\infty)$ (Figs. 3 and 4). These three samples are also characterized by a much denser network as can be seen from the respective values of M_c (Table II). It seems that for relatively low M_c values the flexibility of the chemically crosslinked samples is higher than the flexibility of the native gelatin—which remains to be clarified in further studies.

The values of the relaxation time, τ, characterize the elastic relaxation. They are too scattered both within one sample and between the samples, and it is impossible to discern any trend. Every single experiment on bending was done for a total time of 3000–5000 min (Fig. 2). According to eq. (6) the main relaxation takes place within a time

Figure 3 Initial compliance, $Y(0)$, versus the normalized load, AP, as follows: ($\triangledown$) native gelatin; and gelatin crosslinked with ($\bigcirc$) 1,4-diisocyanatobutane; ($\triangle$) 1,6-diisocyanatohexane; and ($\square$) 1,12-diisocyanatododecane.
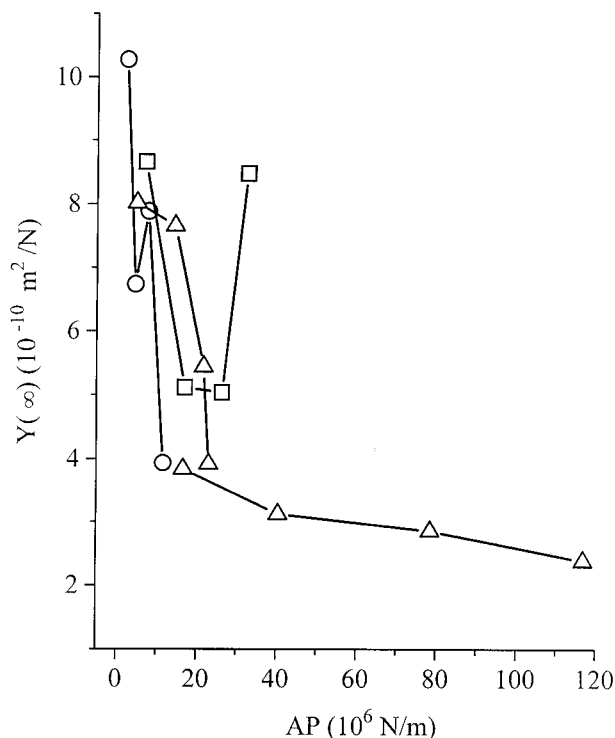
the network and consequently a different bending behavior is to be expected (Table I). Although sample 1 is not chemically crosslinked and TFE is believed to destroy the physical network, this sample also swelled without dissolving. The value of 6000 for M_c (Table II) points to some loose physical network existing in this sample.

What could be the reason for the decrease of M_c with the increase of the length of the crosslinker? Let us imagine that the crosslinker has reacted with one end to one gelatin molecule. In order to produce a network, the other end of the crosslinker should react with another gelatin molecule. It seems reasonable that with the increase of the crosslinker length, the number of the possible reaction partners (gelatin molecules) will increase. Hence, the longer the crosslinker, the denser the network. This is actually the case (Table II).
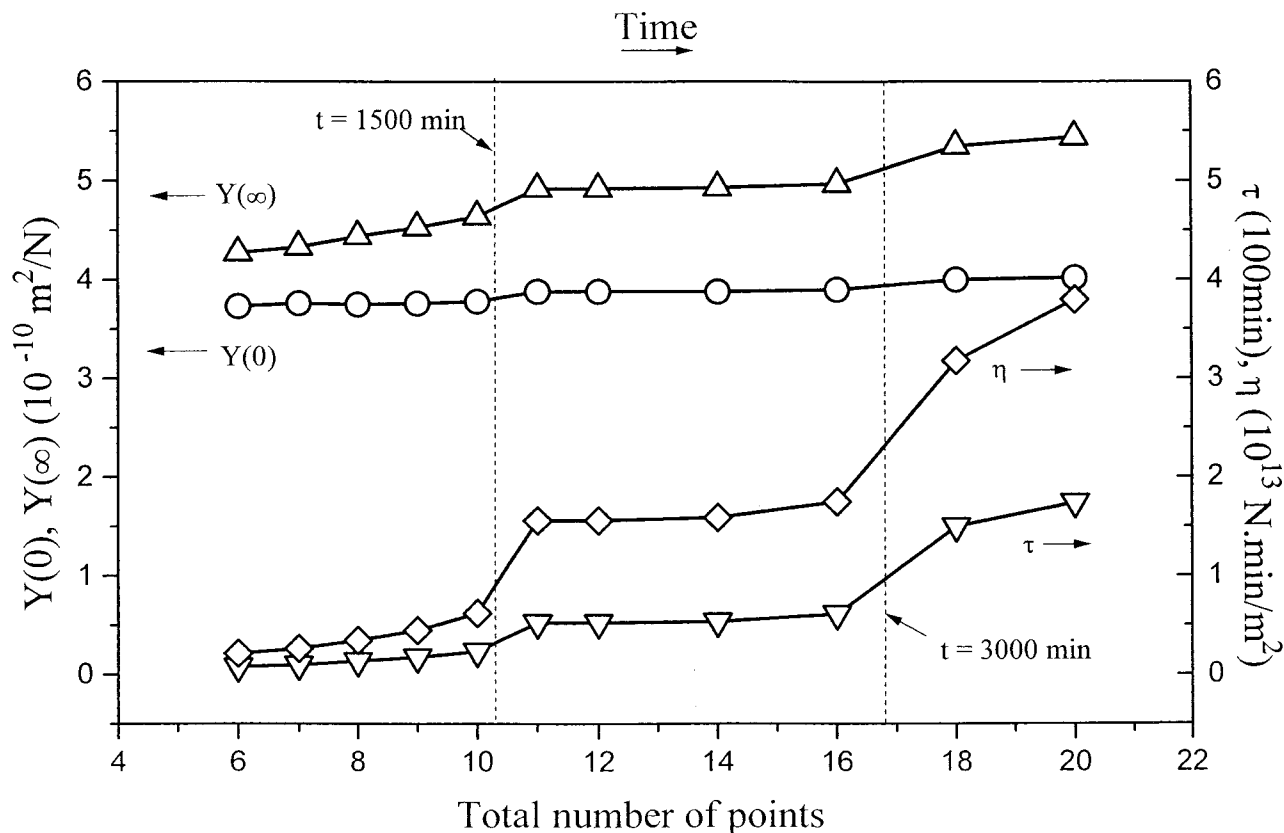
In Figures 3 and 4 the compliances $Y(0)$ and $Y(\infty)$ versus the normalized load, AP, are shown.

Figure 4 Compliance after unlimited time, $Y(\infty)$, versus the normalized load, AP, as follows: ($\triangledown$) native gelatin; and gelatin crosslinked with ($\bigcirc$) 1,4-diisocyanatobutane; ($\triangle$) 1,6-diisocyanatohexane; and ($\square$) 1,12-diisocyanatododecane.

Figure 5 Dependencies of the fitting parameters $Y(0)$, $Y(\infty)$, τ, and η on both total number of points, taken to obtain the fit, and on the time.

lag of 3τ, so it is interesting to follow the dependencies of the fitting parameters on the various total times of the experiment, that is, for the various total number of points taken consecutively to fit eq. (6). These dependencies are shown in Figure 5 for one sample only (Table I, sample 3, *PA* = 21.7).

Let us first clarify the kinks in all four parameters, occurring approximately at times of 1500 and 3000 min (Figure 5, dashed lines). Since the data in any single bending experiment are collected for a couple of days, points are normally taken during the day, and they are not spread uniformly with the time but rather are gathered in groups [Fig. 2(c), *PA* = 21.7]. The first group is situated between 0 and 500; the second, between 1500 and 2000; and the third, between 2800 and 3200 min. It can easily be seen in Figure 5 that the kinks correspond to the times when the first point of the next group has been included in the total number of points η. Although τ and η change significantly with *n*, the fit holds very well (correlation coefficient *R* > 0.98 for every *n*).

According to Figure 5 the initial compliance $Y(0)$ does not depend practically on the number of points taken to obtain it. $Y(\infty)$ changes moderately with this number, while τ and η change significantly. In addition, τ is under the exp sign; hence $Y(t)$ is very sensitive to its changes. The time for reading the 20th point in Figure 2(c) (*AP* = 21.7) is about 3200 min—much higher then the corresponding τ value of 174 min (Table I, sample 3, *AP* = 21.7). But the value of τ has not yet leveled off.

The η value changes more then one order of magnitude in relation to the number of the points taken to obtain it. Obviously τ and η are not real but are a mathematical result of fitting a certain number of points with a certain equation. In our case this is eq. (6), comprising four fitting parameters, and the values obtained reflect this particular model, which must be considered as only an approach to the system under investigation. It seems an easy step to increase the number of the fitting parameters, for instance, to consider several different relaxation times, but this will com-

plicate the model and eventually cloud its physical meaning.

The quantity η from the Newtonian-flow region plays a dominant role in the large time scales and generally is much smaller for the chemically crosslinked samples than for the native one. However, there is no steady trend of η, neither with the length of the crosslinker nor with the normalized load within any of the samples (Table I). As already stated, it should be noted that with the glass transition temperature for room-conditioned gelatin, containing 15–17 wt %, significant Newtonian flow, observed for the sample temperature of 19–21°C, may be a result of the transition taking place in a wide temperature interval around 40°C, which probably expands to 19–21°C. On the other hand, the values of η (Table I) are quite uncertain since η changes more then an order of magnitude with the number of points to be fitted (Fig. 5). A possibly unambiguous way to obtain η might be to consider the flow as non-Newtonian, that is, to regard η as a function of the load and/or deformation (deflection) of the specimen.

Generally, eq. (6) fits all the data adequately; therefore the model chosen to describe the observed behavior works quite well. The values of the parameters, though, depend on the number of data points. In some cases [Figs. 2(b, d)] curves for different loads do not show a systematic trend with an increase of the load, and, in addition, in Figure 2(b) curves for different loads cross over with each other. Therefore, the scatter of the fitted values given in Table I and Figures 3–5 does not seem to be connected with the number of data points but is possibly a result of the original data. As was explained in "Preparation of samples," above, the specimens are cut from circular films that are more or less nonuniform in thickness. Since the dimension-related constant, A, depends on the third power of h, the deflection, δ, and subsequently the creep compliance, Y, are very sensitive to h. On the other hand, some cracks and voids may be formed in the film during the process of evaporation of the solvent (see "Preparation of Samples," above). This is one possible way to explain the scatter of the fitted values given in Table I and Figures 3–5. It is possible to decrease the scatter of the data points by making the specimens more homogeneous and more uniform in thickness by decreasing the rate of evaporation of the solvent from the gelatin in the process of obtaining the films in the Petri dish.

CONCLUSIONS

A model, comprising Hookean deformation, elastic relaxation, and Newtonian flow, has been successfully applied to native and to three chemically crosslinked gelatins, which differ in the nature of the crosslinker. All samples are characterized by the number-average molecular weight of a chain segment between two points of crosslinkage. Four fitting parameters—namely the initial compliance, the compliance after unlimited time, the relaxation time, and η—were obtained for every sample and for four different loads. In the present case, the first three parameters of the four different gelatins have a clear physical meaning, while η may be connected with the viscosity only in a general sense.

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