tion is due exclusively to its directly bonded proton, which is not strictly correct. Nevertheless, the qualitative features of the calculation, and the resulting conclusions are still, we feel, correct.

Acknowledgments. The author thanks Dr. E. O. Stejskal (Monsanto Co., St. Louis) for many helpful discussions of various kinds of distributions of correlation times, and Dr. Allan W. Dickinson (Monsanto Co., St. Louis) for computer evaluations of the integrals resulting from the use of the log-χ^2 distribution of correlation times.

Structure–Property Relations of Thermoreversible Macromolecular Hydrogels

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ABSTRACT: Thermoreversible hydrogels of agarose and gelatin have been studied as models for more complex polysaccharide and protein biogels. In addition a study is made of the gelation of poly(vinyl alcohol) (PVA) in ethylene glycol–water mixtures. The structures are characterized by means of optical rotation and low-angle light scattering. The time-dependent and equilibrium mechanical behavior is measured in unilateral compression. Gelatin gels are found to be randomly cross-linked by the formation of "collage-fold like" junction zones. The viscoelastic relaxation and equilibrium stress are both rubberlike, provided junction breakdown and bacterial degradation is eliminated by a suitable extrapolation procedure. Agarose gels are not rubberlike in their mechanical behavior. Their structure consists of a regular array of micron-sized spherical polymer-rich regions probably formed by a nucleation free- (spinodal) phase separation. Hysteresis in the optical rotation indicates the existence of a wide spectrum of junction zones, present in agarose but absent in gelatin gels. The PVA gels are formed *via* an initial liquid–liquid, nucleated phase separation, followed by syneresis caused by the formation of small crystallites in the polymer-rich regions. The findings show that the structural makeup of hydrogels is strongly reflected in their time-dependent and equilibrium mechanical behavior, and that a structural model based on randomly cross-linked Gaussian chains is often times inadequate.

Macromolecular gels that occur in nature are frequently reversible, *i.e.*, the chains are not chemically cross-linked but exhibit certain junction zones, depending on pH, temperature, and electrolyte concentration. It is the purpose of the present article to investigate the relation between the structure and mechanical properties of such hydrogels, by considering three model systems, *viz.*, gelatin, agarose, and poly(vinyl alcohol). In all three systems an aqueous polymer solution is brought to gelation by lowering the temperature. We will show that the resulting gels vary widely in structure and properties.

Gelatin is prepared by partial hydrolytic degradation and disorganization of collagen.[2a] In the native state collagen occurs in organized fibrils of triple helices of collagen molecules, each molecule being about 100,000 in molecular weight.[2b] The molecules are rich in glycine, proline, and hydroxyproline sequences and are twisted together in the triple-helical "collagen fold" in which the individual molecules occur in the poly(L-proline II) (trans) helical conformation, stabilized by interchain hydrogen bonding provided by the glycine, which occurs at every third residue along the chain.

In gelatin solutions (3–20 wt %) above the gelling temperature the single-strand molecules occur in the random-coil conformation and upon cooling, some re-formation of the collagen fold is thought to occur either as double or as triple helices,[2a] giving rise to junction zones. Only at high concentrations (>30 wt %) does X-ray diffraction reveal some crystallinity.

Agarose is a naturally occurring, alternating copolymer of (1→4)-linked 3,6-anhydro-α-L-galactose and (1→3)-linked β-D-galactose. According to Rees[3] the energetically favored conformational agreement is an extended 3_1 double helix. Upon cooling, agarose solutions above about 0.3 wt % in concentration will set into a gel in which chain segments are thought to form double-helical regions in which stabilization is enhanced by interchain hydrogen bonding. Simultaneously aggregation of these double-helical regions takes place into larger domains.[3]

Poly(vinyl alcohol) (PVA) is a water-soluble synthetic macromolecule which forms a thermoreversible gel in mixed solvents, as, for example, water–ethylene glycol. After gelation, the gel exhibits a pronounced exudation of diluent (syneresis) and develops crystallinity.[4-6] Because of the smallness of the hydroxyl group the extended chains fit into a crystal lattice similar to that of polyethylene even when the macromolecules are atactic.[7]

Conformational changes occurring during gelation can be conveniently monitored by measuring the optical rotatory power during the sol $\rightleftarrows$ gel transition, provided, of course, that the macromolecules contain asymmetric carbon atoms. This is the case for gelatin and agarose, but not for PVA. Conformational restraints (whether intra- or intermolecular) caused by the cooling and gelation enhance the intrinsic rotatory power of a molecule. This method thus provides structural information about the

(1) Based upon part of a thesis submitted by E. P. in partial fulfillment of the requirements for the Ph.D. in Biophysics at Syracuse University, 1972.

(2) (a) A. Veis, "Macromolecular Chemistry of Gelatin," Academic Press, New York, N. Y., 1964, p 127 ff. (b) A. Rich and F. Crick, *J. Mol. Biol.*, 3, 483 (1961); see also G. N. Ramachandran, "Treatise on Collagen," Vol. I, Academic Press, New York, N. Y., 1962.

(3) D. A. Rees, *Advan. Carbohyd. Chem. Biochem.*, 24, 267 (1969); see also *J. Chem. Soc. B*, 217 (1969).

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(5) M. Donkersloot, J. H. Gouda, J. J. Van Aartsen, and W. Prins, *Recl. Trav. Chim. Pays-Bas*, 86, 321 (1967).

(6) S. P. Papkov, S. G. Yefimova, M. V. Shablygin, and N. V. Mikhailov, *Vysokomol. Soyed.*, 8, 1035 (1966).

(7) C. Bunn, *Nature (London)*, 161, 929 (1948); see also J. G. Pritchard, "Poly(Vinyl Alcohol), Basic Properties and Uses," Gordon and Breach, New York, N. Y., 1970.

gelation at a molecular level. The specific optical rotation at a wavelength λ as defined by

$$[\alpha]_\lambda = \alpha/lc$$

where α is the measured rotation in degrees, l the optical path in decimeters, and c the concentration in grams per milliliter.

In order to probe the supramolecular ordering in gels, the light-scattering technique was employed. Since we want to relate the structures of our gels to the mechanical properties, it is useful to recall that the classical theory of elasticity of polymer gels is based upon a model for the structure which stipulates that the gel contain a collection of randomly coiling, randomly cross-linked macromolecules. Experimental evidence has been accumulating over the past years from light scattering, X-ray diffraction, photoelasticity, and electron microscopy,[8] indicating that even "rubber-like" gels can be structured to some extent. A nonrandom structure can arise, for example, as a result of inhomogeneous cross-linking or because of microphase separations. The dimensions of the heterogeneities can be of the same order of magnitude as the wavelength of light.

For a scattering medium of spatially varying but isotropic polarizability, exhibiting spherical symmetry in its spatial correlation, one has for the Rayleigh ratio[9]

$$R^{V_v}(\theta) = (64\pi^5/\lambda_0^4)\langle\eta^2\rangle\int_0^\infty \gamma(r)\frac{\sin\ hr}{hr}r^2dr \qquad (1)$$

In this equation V_v stands for polarization of the scattered and incident light vertical to the plane defined by the incident and scattered wave vector. Furthermore $h = (4\pi/\lambda)\sin\theta/2$, where θ is the scattering angle and λ the wavelength of the light in the gel; λ_0 is the wavelength *in vacuo*. The correlation function $\gamma(r)$ is defined by

$$\gamma(r) = \frac{\langle\eta_i\eta_j\rangle_r}{\langle\eta^2\rangle}$$

where η_i ($= \alpha_i - \bar{\alpha}$) represents the polarizability fluctuation at position r_i. The spherical symmetry in $\gamma(r)$ can be verified by rotating both polaroids through arbitrary angles ψ after setting their transmittance axes parallel at $\theta = 0$.[9,10]

Equation 1 allows the determination of $\langle\eta^2\rangle$ if a suitable analytical form of $\gamma(r)$ can be inferred from the measured angular dependence of the light scattering. Considering $\gamma(r)$ as a sum of various Gaussian functions

$$\gamma(r) = \sum_{i=1}^{N} X_i e^{-r^2/a^2_i}$$

Equation 1 can be integrated to yield

$$R^{V_v}(\theta) = (16\pi^5/\lambda_0^4)\langle\eta^2\rangle\sum_{i=1}^{N} X_i a_i^3\pi^{1/2}\ \exp(-h^2a_i^2/4) \qquad (2)$$

where the set $\{a_i\}$ characterizes the extent of the correlations responsible for the angular dependence of the scattering and X_i is the fraction of correlation distance a_i. If a plot of log $R^{V_v}(\theta)$ *vs.* h^2 can be resolved in N straight lines then $\{a_i\}$ follows from the slopes. From the intercepts and slopes $\langle\eta^2\rangle$ and $\{X_i\}$ can be calculated. This procedure can be applied to the sol as well as the gel state.[9]

For a randomly cross-linked gel obeying the classical theory for a rubber elastic swollen polymer network the quantity $\langle\eta^2\rangle_{gel}$ can be calculated and compared to the $\langle\eta^2\rangle_{sol}$ obtained from the scattering of a solution of the same concentration prior to gelation.[11] The calculation predicts that the ratio $\langle\eta^2\rangle_{gel}/\langle\eta^2\rangle_{sol}$ decreases monotonically from a value of 2 at 2 vol % to a value of 1.1 at 20 vol % polymer concentration. Larger ratios are a measure of the deviation of the actual gel structure from that of a randomly cross-linked rubbery gel.

Since the gels under consideration are very low in polymer content, and thus in modulus, the mechanical behavior is most conveniently measured in unilateral compression. If rubbery gels are de-formed without allowing an adjustment in the degree of swelling, the equilibrium stress σ_e is related to the de-formation ratio Λ as follows[8]

$$\sigma_e = G(\Lambda^2 - \Lambda^{-1}) \qquad (3)$$

The modulus G equals $q^{-1/3}q_0^{-2/3}\nu_e^*RT$, if q is the volume degree of swelling, q_0 the reference degree of swelling at which the polymer chains have their unstrained mean-square end-to-end dimensions and ν_e^* is the number of elastically effective network chains per cm^3 dry network. In the case of thermally reversible gels which do not undergo syneresis it is reasonable to put $q_0 = q$ so that one has $G = q^{-1}\nu_e^*RT$. An estimate of the average molecular weight between cross-links, $\overline{M}_c$, can be obtained employing the "dangling end" correction (see, *e.g.*, ref 8) $\nu_e^* = \nu^*(1-2\overline{M}_c/\overline{M}_n)$, where $\overline{M}_n$ is the number-average molecular weight of the primary chains before gelation. Inserting $\nu^* = \rho_d/\overline{M}_c$ and choosing a value of unity for the dry density (ρ_d) of the network, one has

$$\overline{M}_c = [2\overline{M}_n^{-1} + Gq/RT]^{-1} \qquad (4)$$

Upon the application of a given de-formation, rubbery gels often exhibit an extremely long-term stress relaxation, thus making the determination of the equilibrium stress experimentally not feasible. Thirion and Chasset[12] have pointed out that in chemically cross-linked rubbery gels the de-formation and time dependences of the stress are separable: $\sigma(\Lambda,t) = \sigma_e(\Lambda)\sigma_2(t)$, and that

$$\sigma_2(t) = 1 + (t/t_0)^{-m} \qquad (5)$$

is as adequate analytical representation of the stress relaxation over four decades of time. The parameter m can be determined by plotting

$$\log\ [-d\sigma_2/d\ \log\ t] = \text{const} - m\ \log\ t \qquad (6)$$

and t_0 as well as σ_e are obtained from

$$\sigma(\Lambda,t) = \sigma_e(\Lambda)[1 + (t/t_0)^{-m}] \qquad (7)$$

The time dependence of the stress is caused by internal relaxation modes of motion extending over several chains linked together by cross-links, as well as by relaxation modes derived from the slippage of entanglement couplings between molecules.[13] In thermally reversible gels there are no chemical cross-links but only physically linked junction zones. If these are not sufficiently strong to resist slippage under an imposed de-formation, no true equilibrium stress will exist. Analysis by recourse to the procedure outlined in eq 5-7 may sometimes still be made in a more restricted sense if such junction zone slippages have much larger relaxation times than the other modes of motion of the network.

(8) K. Dušek and W. Prins, *Fortschr. Hochpolym. Forsch.*, **6**, 1 (1969).
(9) See, *e.g.*, W. Prins in "Polymer Networks, Structural and Mechanical Properties," A. J. Chompff and S. Newman, Ed., Plenum Press, New York, N. Y., 1971.
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(12) P. Thirion and R. Chasset in "Physics of Non Crystalline Solids," J. A. Prins, Ed., North Holland Publishing, Amsterdam, 1965; see also J. D. Ferry, "Viscoelastic Properties of Polymers," John Wiley and Sons, New York, N. Y., 1970, p 437 ff.
(13) A. J. Chompff and J. A. Duiser, *J. Chem. Phys.*, **45**, 1505, (1966); see also J. D. Ferry in ref 12, p 263 ff.

Experimental Section

Materials and Methods. Sample Preparation. A de-ashed lime-processed calf skin gelatin sample with an isoelectric point of 4.7 was kindly provided by Dr. H. Curme, Kodak Research Laboratories, Rochester, N. Y. (Eastman No. 1099, Batch 70-5120). Its amino acid composition is that of a typical gelatin[14] except for a 0.18 mmol/g of ornithine and 0.318 mmol/g of arginine content. It has a broad molecular weight distribution with $\bar{M}_w/\bar{M}_n \geq 3$ and $\bar{M}_n = 105{,}000$. Solutions were made by first soaking the gelatin in doubly distilled deionized water for 2 hr, followed by heating at 50° for 15 min. The warm solution was centrifuged for approximately 10 min at 30,000*g* to remove debris and dust, and after reheating to erase possible aggregation effects, pipetted into the ORD and light-scattering cells. For the unilateral compression measurements, gels of 3-cm height and 2-cm diameter were obtained by placing an open-ended aluminum cylindrical mold, thinly greased with silicone as releasing agent, in a beaker containing the hot gelatin solution. After 20-min equilibration at room temperature the beaker was placed in a refrigerator at 4° for 12 hr. A cylindrical piece of gel was obtained by cutting with a taut wire across the open top and bottom of the mold to ensure flat and parallel faces of the test piece. The cylindrical specimen was then placed in silicone oil. In order to obtain reproducible measurements of the unidirectional compressional modulus, the gels were given a uniform thermal history which consisted of two cycles of 2 hr at 24°, and 2 hr at 4°. Following the annealing procedure, each specimen was equilibrated at the temperature of the measurement (19 ± 1°) for 24 hr before the start of compression measurements. The gels were stored under silicone oil to prevent evaporation of the water and to reduce bacterial degradation.

Agarose solutions were made by first soaking agarose (Aldrich Chemical Co., Milwaukee, Wis., Batch 13704-9) for 30 min in doubly distilled deionized water and then dissolving at 85°. The hot solution was centrifuged for 16 min at 30,000*g* in a preheated rotor to prevent gelation (around 40°). After reheating to erase possible remaining aggregation, the sols were pipetted into the ORD and light-scattering cells. Gels of 2-cm height and 2-cm diameter were made as before and stored under silicone oil. Thermal conditioning as for the gelatin, proved to be unnecessary. Compression measurements were performed at 18 ± 1°.

A 5 wt % fully hydrolyzed PVA (Du Pont, Wilmington, Del., Product No. 72-60G, Batch 3-3930-1) solution in 45 wt % ethylene glycol and 50 wt % doubly distilled deionized water was obtained by soaking and then heating to 85° for 1 hr. The hot solution was hot filtered (0.8 μ Millipore filter) and, after reheating to 85°, pipetted into the light-scattering cell. Gels of 2-cm height and 2-cm diameter were made by first keeping the beaker with the mold at room temperature for 2 hr and then ageing the contents at 4° for 48 hr. This prolonged cold time was necessary to ensure sufficient rigidity for the cutting procedure. A hot taut wire was used to secure flat surfaces on the test piece. Samples were again stored under silicone oil prior to mechanical testing at 18 ± 1°.

Optical Rotation. The optical rotations at $\lambda_0 = 520$ nm of the agarose and the 3 wt % gelatin samples were measured on a Jasco J-20 spectropolarimeter using thermostated optical cells ranging in optical path from 2 cm to 1 mm. The cell temperature was established with a thermocouple to be constant within 0.05° in the range of 10–85°. For the higher gelatin concentrations a Perkin-Elmer Model 141 polarimeter was used at $\lambda_0 = 589$ nm.

Light Scattering. Absolute Rayleigh ratios at $\lambda_0 = 546$ nm (and occasionally also at 436 nm) were obtained on a small-angle light-scattering photometer[15] from 30° down to about 1° scattering angle so as to be able to reliably extrapolate the data to zero angle. A thermostated rectangular light-scattering cell of optical glass (5.0 × 2.5 × 0.2 cm) was used to measure the scattering of the sol or gel and the scattering of the solvent. The latter never amounted to more than 10% of the total scattering and was subtracted so as to yield the Rayleigh ratio due to the macromolecules. The cell thickness was chosen to be such that multiple scattering especially in high turbidity samples did not interfere with the interpretation. To this end Van de Hulst's criterion that the product of turbidity and thickness of the cell should not exceed 0.3 was employed.[16] The various corrections for reflection, refraction, and turbidity needed in the data reduction have been described in the literature.[17] In order to determine whether the samples exhibited a measurable amount of anisotropic scattering, the polaroids were crossed and the photocurrent measured. By keeping the polaroids truly crossed but varying the direction of polarization with respect to the scattering plane, one can establish the true anisotropic scattering under an azimuthal angle ϵ.[10] One has to be careful, however, to correct for any polaroid leakage. One establishes readily[18] that the true anisotropic scattering $R^{\perp}(\theta,\epsilon)$ is obtained from the measured Rayleigh ratio (labeled "m") as follows

$$R^{\perp}(\theta,\epsilon) = [R^{\perp}(\theta,\epsilon)]_m - \frac{I^{\perp}(\theta,\epsilon)}{2I_0^{\parallel}} \times [R^{\parallel}(\theta,90° + \epsilon) + R^{\parallel}(\theta,\epsilon)$$

The parallel bars indicate the parallel component of the scattered light[10] which reduces to H_h and V_v if $\epsilon = 90°$; the subscript "0" refers to the zero-scattering angle position, *i.e.*, to the transmitted intensity.

Although the leakage of the crossed polaroids, $I_0^{\perp}$, is quite small (typically 3×10^{-4}) the correction becomes important if the "parallel" scattering (which includes V_v) is much larger than the "perpendicular" scattering (which includes H_v). All our samples showed anisotropic scattering with an interesting azimuthal dependence *before* the correction, but *after* the correction this was reduced to a small amount not distinguishable from the anisotropic scattering of the diluent. It is thus appropriate to analyze the results in terms of eq 1, which assumes isotropic scattering only. In all cases $R^{Hh}(\theta) = R^{Vv}(\theta)\cos^2\theta$ obtained so that spherical symmetry in the correlation function $\gamma(r)$ was assured.[9]

Unilateral compression measurements were undertaken on the cylindrical specimens which were kept at constant diluent content by submersion in silicone oil. The silicone oil served at the same time as a lubricant to minimize the friction with the Teflon end plates, one of which was moved downward to compress the sample. The temperature of the silicone bath was lowered to a stationary state at 18 ± 1° by means of a thermoelectric Stir Kool Plate (Model SK-12, Thermoelectronics Unlimited, Wilmington, Del.). The top Teflon plate was driven downward by a motor with a gearbox allowing different deformation rates between 0.001 and 10 cm per hr. The compressive force was monitored by an inductive force transducer (Model Q1/5, Hottinger Messtechnik, Darmstadt, Germany) which formed one arm of an inductive bridge, fed by a carrier wave amplifier (Type KWS 5-II, Hottinger-Baldwin). The output was recorded as a function of time. The calibration was periodically checked by inverting the transducer and placing known weights on the Teflon covered end plate. The amount of compression could be determined with 0.005-cm accuracy by means of a cathetometer.

Results

Gelatin Gels. The specific rotation of a 3 wt % gelatin sample increases rapidly upon gelation from a value of $[\alpha]_{520} = -160$ (deg ml)/(dm g) at $T = 50°$ to -370 at 5°. The cooling and heating curves are coincident and independent of the rate of thermal treatment, if at temperatures below 40° the samples are maintained at each temperature for a sufficiently long period to reach equilibrium. This sometimes required periods of up to 15–30 hr. The transition is cooperative and the inflection point of the sigmoid $[\alpha]$–T curve at 21–22° will be taken as the characteristic temperature T_c. This temperature is the same as the conformational transition temperature reported for gelatin in the literature.[19]

The specific rotation of a series of gelatin samples with concentrations from 3 to 20 wt % was found to be a constant −209 (deg ml)/(dm g) at 589 nm and independent of the thermal history of the samples, provided enough time (20 hr) was allotted before the final data were recorded at 23°. The concentration independence of $[\alpha]_{589}$ is in agreement with previous data of Ferry and Eldridge.[20]

(14) See ref 2a, p 142.
(15) A. E. M. Keyzers, J. J. Van Aartsen, and W. Prins, *J. Appl. Phys.*, 36, 2874 (1965).
(16) H. C. Van de Hulst, "Light Scattering by Small Particles," John Wiley and Sons, New York, N. Y., 1957.
(17) R. Stein and J. Keane, *J. Polym. Sci.*, 17, 21 (1957).
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(20) J. D. Ferry and J. Eldridge, *J. Phys. Colloid Chem.*, 53, 184 (1949).

Table I
Parameters Quantitatively Describing the Absolute Light Scattering of Gelatin Sols and Gels

Wt %	cm $10^{+}a_1$	cm $10^{+}a_2$	cm $10^{+}a_3$	X_1	X_2	X_3	$10^5\langle\eta\rangle$	$\langle\eta^2\rangle_{gel}/\langle\eta^2\rangle_{sol}$
3% gel	0.59	1.43	3.16	0.67	0.28	0.05	25.3	1.67
3% sol	0.62	1.70	3.52	0.87	0.10	0.03	15.1	
10% gel	0.56	1.52	3.47	0.90	0.07	0.03	40.4	1.12
10% sol	0.53	1.55	3.70	0.91	0.07	0.01	36.5	

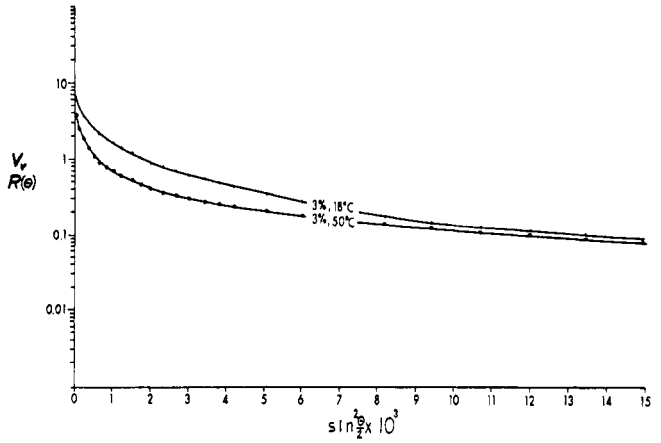

Figure 1. The logarithm of the Rayleigh ratio $R^{V_v}(\theta)$ *vs.* $\sin^2\theta/2$ for a 3% gelatin sol and gel.

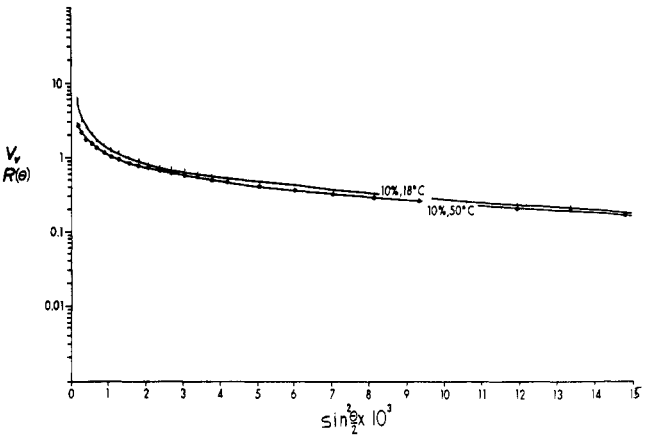

Figure 2. The logarithm of the Rayleigh ratio $R^{V_v}(\theta)$ *vs.* $\sin^2\theta/2$ for a 10% gelatin sol and gel.

Figures 1 and 2 show a typical set of light-scattering data at small angles. In both cases it is seen that upon gelation the level of scattering increases by only a small amount. The gel data were taken after quenching the sol to 4° for 12 hr, followed by 12-hr maturation at 18°. The same results are also obtained without quenching provided 70-hr equilibration time is allotted at 18°.

The results can be given a quantitative evaluation according to the procedure outlined in the first section. The curve can be decomposed into three linear portions. Hence the correlation function $\gamma(r)$ can be written as the sum of three Gaussians; the parameters X_i (relative amounts) and a_i (correlation distances) for each, as obtained from those data, are given in Table I. The dominant correlation range of the polarizability fluctuations is 5000–6000 Å. Since the isolated molecules have a radius of gyration of at most 250 Å in the randomly coiling state, the 5000–6000-Å correlation range is indicative of aggregation, known to be present from earlier light-scattering work at wider angles[21] as well as from recent quasi-elastic laser light-scattering work at lower concentrations.[22] Quasi-elastic light-scattering measurements on the gels undertaken by us are less easily resolved because of the presence of nontranslational factors in the self-beat spectrum[18] but the data do confirm the existence of aggregates of several thousands of ångströms in size.

The point to stress regarding Figure 1 and 2, as well as Table I, is that the absolute level of the scattering, $\langle\eta^2\rangle$, obtained by proper extrapolating of the data to zero scattering angle, changed relatively little upon gelation.

In a previous note[11] we have pointed out that the light scattering of a randomly cross-linked swollen polymer network should be within a factor of two of that of a solution containing the same polymer at the same concentration but not containing cross-links. The difference should be smaller the higher the polymer concentration. Both features are indeed exhibited by the gelatin gels, although the absolute levels of light scattering of both sol and gel are higher than calculated for a collection of randomly coiling chains[11] because of the aggregation exhibited by the gelatin. The state of aggregation does not change, however, upon gelation as evidenced by the very similar angular dependence of the light scattering as well as by the closeness of the $\langle\eta^2\rangle_{gel}$ and $\langle\eta^2\rangle_{sol}$ values listed in Table I.

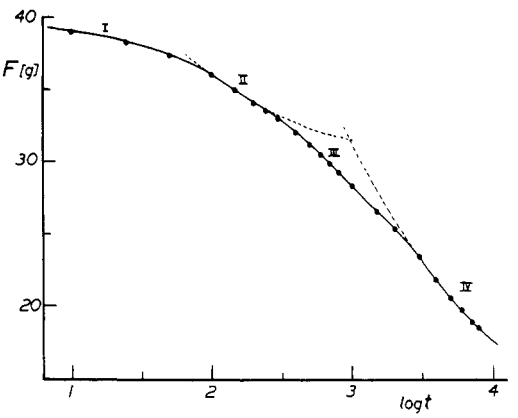

Figure 3. Example of the force relaxation regime of gelatin gels. The temperature is 19°, gelatin concentration 15 wt % under 7.4% unilateral compression.

Measurements of the stress under unilateral compression were undertaken on samples of 5, 10, and 15 wt % gelatin content. More dilute gels exhibited bulging during compression. Figure 3 shows a typical example of the long-term stress relaxation, eventually ending in complete loss of stress because of bacterial degradation.

It was found that only the first few decades of stress relaxation could be treated by the Thirion-Chasset (TC) procedure outlined in the first section. The first 50 sec (region I) are excluded because it takes some time to establish a de-formation. Region II is used for the TC proce-

(21) H. Boedtker and P. Doty, *J. Phys. Chem.*, 58, 968 (1954).
(22) M. French, J. Angus, and A. Walton, *Biochim. Biophys. Acta*, 251, 320 (1971).

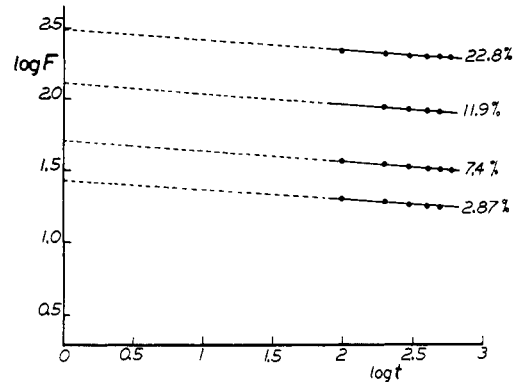

Figure 4. Demonstration of the separability of de-formation (2.87–22.8% compression) and time for a 15 wt % gelatin gel at 19°.

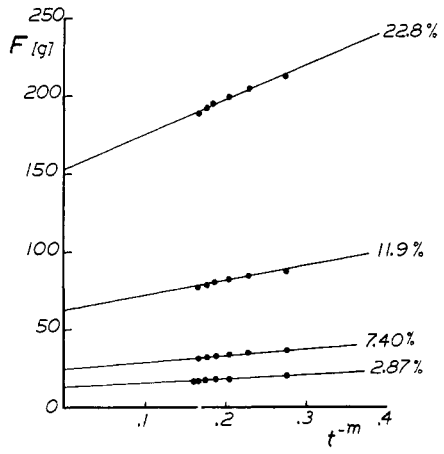

Figure 5. Determination of the quasi-equilibrium force by the Thirion-Chasset procedure (eq 7); 15 wt % gelatin gel; $m = -0.28 \pm 0.01$.

dure. The onset of increased relaxation (region III) is ascribed to junction zone breakdown leading to network structures of lower cross-link densities. In order to test whether gelatin gels behave rubber elastically one should ideally determine the equilibrium stress prior to the breakdown of junction zones. This was accomplished in an admittedly somewhat arbitrary fashion by following the procedure given in eq 5–7 for region II (Figure 4).

As an example the stress relaxation data in region II of a 15 wt % gelatin gel under various unilateral compressions are plotted logarithmically. The fact that the data plot as a series of parallel lines indicates the separability of time and de-formation, *i.e.*, $f(\Lambda,t) = f_e(\Lambda)f_2(t)$. For all gels the value of m was determined according to eq 5, which yielded $m = -0.28 \pm 0.015$. This value is somewhat larger than that found for chemically cross-linked hydrogels and other rubbery materials.[23] The quasi-equilibrium forces were subsequently obtained as illustrated in Figure 5, so that quasi-equilibrium stress–strain curves (Figure 6) could be constructed. From the slopes and the dimensions of the test pieces, the quasi-equilibrium moduli G_e were calculated (eq 3) with the results shown in Figure 7.

The moduli obtained in this way are, of course, substantially lower than the moduli calculated from the initial stress (labeled "0" in Figures 6 and 7). The G_0 values are in agreement with known values,[24] but the G_e values in Figure 7 are somewhat more meaningful characteristics of the network structure than the G_0 values. According to

(23) M. Ilavsky and W. Prins, *Macromolecules*, 3, 425 (1970).
(24) K. Scott, Private Communication, Kodak Research Laboratories.

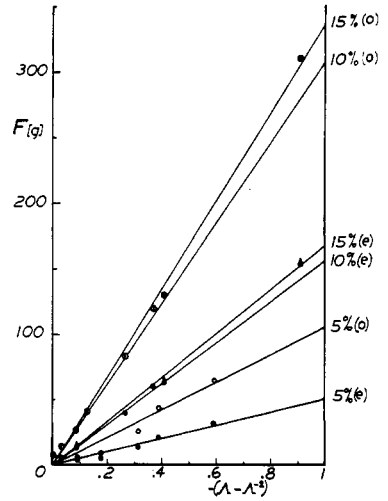

Figure 6. Rubber-elasticity plots based on initial force (0) and quasi-equilibrium force (e) for gelatin gels at 19° of 5, 10, and 15 wt % concentration.

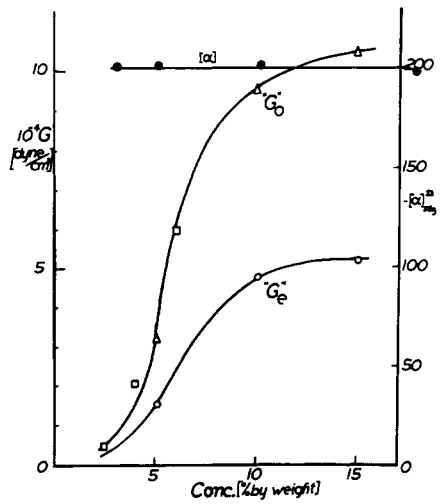

Figure 7. Initial (0) and quasi-equilibrium shear modulus of gelatin gels at 19° as a function of concentration. Also plotted is the specific rotation at 589 nm and 23°.

the relation $G_e = q^{-1}\nu_e^* RT$ the modulus should be proportional to q^{-1} (which is the same as requiring proportionality to the concentration of gelatin) as long as ν_e^* is constant. The data points in Figure 7 show that G_e (as well as G_0) at intermediate concentrations is larger than the proportionality which the concentration predicts. The most reasonable explanation for this phenomenon is that there are two competitive processes at work. With rising concentration more junction zones are formed as a result of intermolecular encounters (a bimolecular encounter would give a c^2 dependence of G_e) but at still higher concentrations growth and perfection of existing junction zones apparently predominate over the formation of new ones. Since the modulus is determined mainly by the concentration of junction zones and not their size or degree of perfection, the predominance of the perfection process would explain the very small increase in modulus observed when going from 10 to 15 wt % gelatin content. The average molecular weights between cross-links calculated from eq 4 are M_c = 31,000, 25,000, and 30,000, respectively, for the three concentrations investigated by us. The constancy of $[\alpha]$, also plotted in Figure 7, indicates that the conformational restraints are the same whether the collagen fold occurs in new junction zones or adds on to already existing ones.

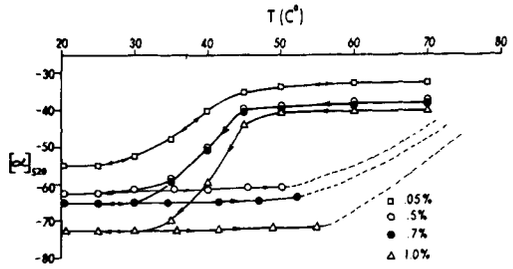

Figure 8. Specific rotation at 520 nm of agarose sols and gels as a function of temperature. The dashed lines indicate the eventual return to the high temperature value of $[\alpha]$.

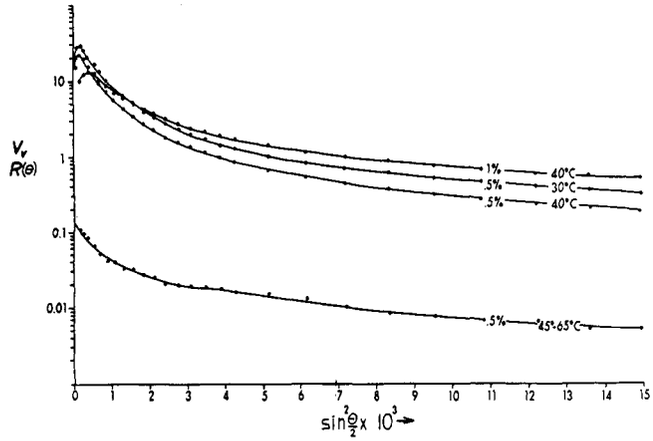

Figure 9. The logarithm of the Rayleigh ratio $R^{V_V}(\theta)$ *vs.* $\sin^2 \theta/2$ for agarose sols and gels.

Agarose Gels. The specific rotation of dilute agarose samples at 520 nm is shown in Figure 8 as a function of temperature and concentration. The cooling curves are independent of the rate of cooling as long as the rotation is measured after equilibrium at a given temperature is obtained (approximately 1.5 hr). The heating curves show a pronounced hysteresis effect as soon as the concentration is such that gels have formed during the cooling ($c > 0.3$ wt %). A characteristic temperature, T_c, can be quite accurately determined from the inflection point on the $[\alpha]$–T plots during cooling. It is somewhat concentration dependent, *viz.*, 41° for 1% and 39° for 0.5 and 0.7% samples. The 0.05% does not gel but gives a corresponding T_c of 37°. The concentration dependence of the specific rotation indicates that in the case of agarose the conformational restraints do not solely arise from the postulated double-helical agarose chain[3] but also from further interhelical packing effects.

The hysteresis loop in the optical rotation appears only if gels are formed. At very low agarose concentrations no gelation occurs but both the optical rotation and the light-scattering data to be reported below, indicate the existence of extensive aggregates of chains to at least partially ordered conformations (double helices most likely). The hysteresis at higher concentrations must therefore be ascribed to the existence of junction zones *between* the aggregates. These junction zones are very likely also double helical on a molecular level but they must exist in a wide variety of metastability; the most stable ones melt out at a much higher temperature than where the first ones form during cooling. Rees[25] has described a similar hysteresis phenomenon in the sol $\rightleftharpoons$ gel transition of the polysaccharide carrageenan and has stressed that in general, hysteresis is exhibited if junction zones of various types of order have been formed. The formation of an array of different types of junction zones is considered by Rees to be controlled by nucleation, with the free energy of activation possibly residing in the initiation of the double-helix conformation.

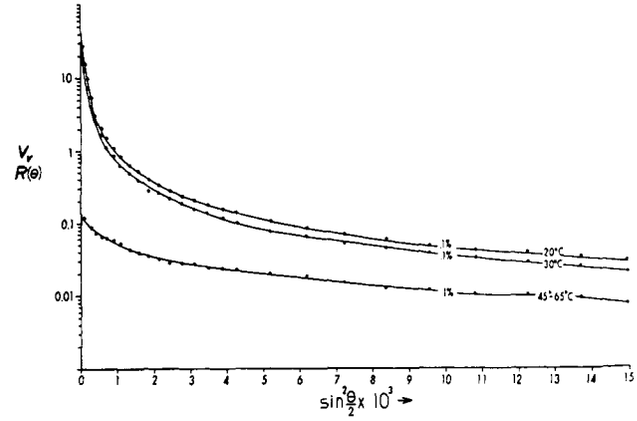

Figure 10. The logarithm of the Rayleigh ratio $R^{V_V}(\theta)$ *vs.* $\sin^2 \theta/2$ for a nongelling dilute agarose sol.

Table II
Parameters Quantitatively Describing the Absolute Light Scattering of Agarose Sols

Temp (°C)	cm $10^4 a_1$	cm $10^4 a_2$	cm $10^4 a_3$	X_1	X_2	X_3	$10^5 \langle \eta^2 \rangle$
45–65	0.45	1.0	2.5	0.89	0.09	0.02	2.5
20	0.60	1.5	3.1	0.56	0.21	0.23	9.9

Our light-scattering data provide some indication that at least the initial stage of the phase separation into domains (consisting of aggregates of double-helical chains) is not controlled by a nucleation process. Figure 9 shows that upon cooling a 0.5 or 1% solution, a very large increase in light scattering occurs and, most interestingly, that a maximum appears in the angular dependence. Figure 10 shows that a very similar increase in scattered intensity but no maximum is observed upon cooling a 0.1% solution, which does not form a gel. As was also the case for the optical rotation data, about 1.5 hr are necessary for the scattering level to become constant at 40°.

The 0.1% solution data can be analyzed in terms of eq 2. The results are shown in Table II. Three Gaussian functions are needed to describe the data. Above the transition temperature the first correlation distance of 4500 Å is predominant, but below the transition much larger distances of up to 30,000 Å become important. Larger aggregates are thus formed.

In the gel state, an analysis by means of Gaussian correlation functions is impossible because of the appearance of a maximum in the angular distribution of the scattering. Observation under the microscope suggests that the gel contains closely packed spherical regions of the order of a few times 10,000 Å. A fairly broad maximum will be exhibited by a liquid-type close packing of spherical domains according to the following Zernike–Prins equation[26] for the normalized intensity $I(h)$

$$I(h) = F^2\left[1 - \frac{4\pi N}{V}\int_V (1 - g(r))\frac{\sin hr}{hr} r^2 dr\right] \quad (8)$$

where $g(r)$ is the radial distribution function of the N do-

(25) D. A. Rees, I. W. Steele, and F. B. Williamson, *J. Polym. Sci., Part C*, 28, 261 (1969).

(26) G. Oster, *Rec. Trav. Chim. Pays-Bas*, 68, 1123 (1949).

mains in the volume V and F^2 is the scattering factor of a spherical region of radius R

$$F^2 = [(3/(hR)^3)(\sin\ (hR) - hR\ \cos\ (hR))]^2 \quad (9)$$

The maximum of eq 8 appears at

$$hR = (4\pi R/\lambda)\ \sin\ \theta/2 = 3.0 \quad (10)$$

Since the maxima in Figure 9 appear at $\theta = 2.25$ and 1.50° for the 1 and 0.5% gel, respectively, this yields $R = 49{,}000$ and 73,000 Å, respectively.[27] Oster[26] has pointed out that the maximum of eq 8 is very close to the apparent Bragg spacing between closely packed spheres that one calculates from

$$2d\ \sin\ \theta/2 = \lambda$$

with $d = 2R$.

The appearance of a predominant Bragg spacing in the light scattering would not be expected from a collection of spheres which have grown from randomly positioned nuclei. We suggest, therefore, that the agarose phase separation has taken place through the nonnucleated, spinodal decomposition mechanism described by Cahn.[29] In Cahn's theory spontaneous concentration waves in which one wavelength predominates are formed in the unstable (spinodal) region of the free-energy composition diagram, if the rate of cooling relative to the diffusion rate of the molecules is fast so that the regular nucleation stage is bypassed. Upon the subsequent formation of junction zones between the regularly disposed regions of high agarose concentrations, the structure is fixed and the permanent display of an apparent Bragg spacing thus explained. It should be repeated that the formation of junction zones between the phase-segregated domains does appear to be nucleation controlled, because of the observed hysteresis in the optical rotation data.

Evidence for a spinodal mechanism in the phase separation of a polymer solution, where diffusion rates are notoriously slow, has previously been noted by Van Aartsen and Smolders.[30] The reality of nonnucleated domain formation in agarose gels cannot be unequivocally established without a closer study of the phase decomposition over a wide range of cooling rates. Such experiments are now in progress in conjunction with a more detailed study of an audio frequency resonance in the quasi-elastic light scattering of agarose gels which was reported by one of us recently.[31]

The mechanical behavior of agarose gels as measured in unilateral compression is fundamentally different from that of gelatin gels. Figure 11 shows that the stress relaxation depends markedly on the imposed de-formation, Λ, so that the separation of de-formation and time which forms the basis of the TC treatment given in eq 5–7 is not feasible. The stress relaxation exhibits a more or less exponential time course, with the faster decay rates occur-

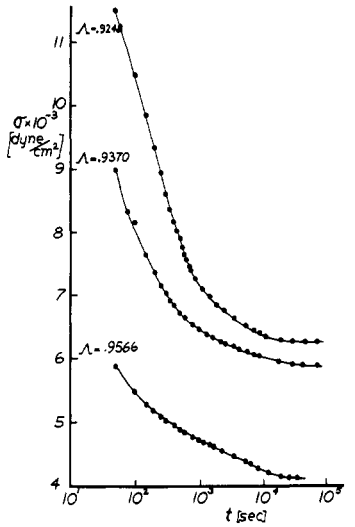

Figure 11. Stress relaxation of a 1% agarose gel at 18°. The time course is different for each compression ratio, demonstrating the inapplicability of the Thirion-Chasset procedure (eq 5–7).

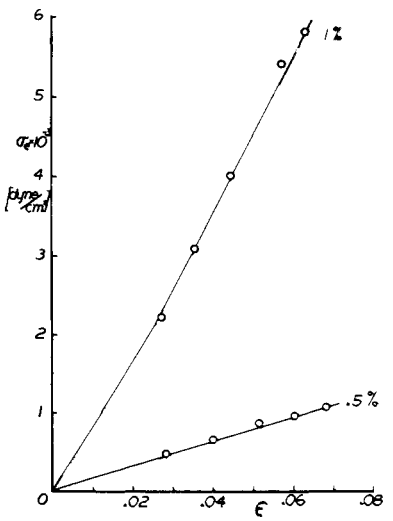

Figure 12. Equilibrium stress-strain curves for 1% and 5% agarose gels at 18°.

ring at the larger de-formations. The most likely, qualitative explanation, seems to be that we are not dealing with viscoelastic relaxation modes of rubbery chains (as in region II of gelatin, Figure 3), but with junction zone breakdown (as in region III and IV of gelatin, Figure 3). The higher the mechanical stress (the higher the de-formation) the more probable it will be that a junction zone will reach the critical energy required to break. The stronger junction zones are then responsible for the equilibrium stress, and they are also the reason that upon removal of the load the specimen elastically recovers 99.5% of its original height.

Figure 12 shows equilibrium stress-strain data for the 1 and 0.5% agarose gels. It is rather meaningless to even attempt a semiquantitative analysis of the moduli, which can be calculated from this figure. No theory exists at the present time for describing the equilibrium mechanical behavior of a structured network of spherical domains connected by a variety of junction zones of various degrees of perfection. Using eq 4, with an estimated[3] $\bar{M}_n$ of 120,000 yields $\bar{M}_c$ values of 7600 and 17,800 for the 1 and 0.5% agarose gels, respectively. Such a large concentration dependence of $\bar{M}_c$ again indicates the inapplicability of rubber elasticity theory for these gels.

(27) An alternative explanation of the maximum in the light scattering rests on the single sphere scattering function, F^2, which exhibits a maximum at $hR = 5.76$. The maxima of Figure 9 would then give calculated radii of 94,000 and 150,000 Å for the 1 and 0.5% agarose gels, respectively. Several arguments make this alternative explanation unlikely. In the first place, the maximum will only appear if the domains are extremely monodisperse.[28] Secondly, the microscopy indicates the existence of domains of at most a *few* times 10,000 Å. And, thirdly, eq 9 predicts that the intensity drops very rapidly to zero relative to the maximum. This is in contradistinction to an experiment which shows a quite substantial scattering beyond the maximum. Equation 8, which brings in the interparticle interference, exhibits a much broader maximum than eq 9, in line with the observations.

(28) R. Duplessix, C. Picot, and H. Benoit, *J. Polym. Sci., Part B*, **9**, 321 (1971).
(29) J. W. Cahn, *J. Chem. Phys.*, **42**, 93 (1965).
(30) J. J. Van Aartsen and C. Smolders, *Eur. Polym. J.*, **6**, 1106 (1970).
(31) W. Prins, L. Rimai, and A. J. Chompff, *Macromolecules*, **5**, 104 (1972).

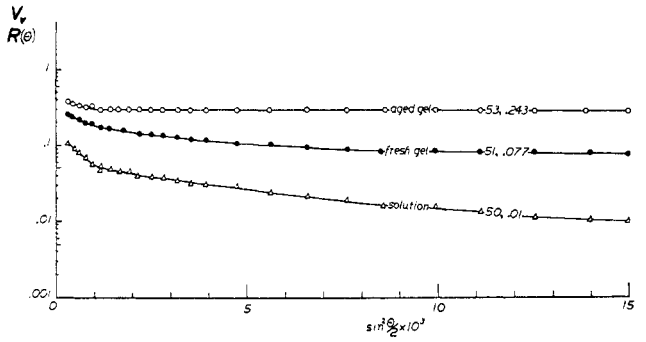

Figure 13. The logarithm of the Rayleigh ratio $R^{V_v}(\theta) \sin^2 \theta/2$, for PVA in 50 wt % water and 45 wt % ethylene glycol. The codes 50, 51, and 53 refer to a 5 wt % concentration at day 0, 1, and 3, respectively; the decimal numbers indicate the product of turbidity and sample thickness.

Poly(vinyl alcohol) Gels. A 5% PVA solution, prepared as described above, shows a low level of scattering upon cooling from 70 to 23°, with an angular dependence at small angles which is indicative of aggregation (Figure 13). Upon subsequently quenching the sol to 4° until the first sign of gelation appears after 4.5 hr, and then measuring the light scattering over various days at room temperature, one finds that the intensity goes up markedly and that the angular dependence gradually disappears (Figure 12). Beyond 3 days pronounced syneresis has occurred and the turbidity has become so large that multiple scattering no longer can be safely neglected. The data in Figure 13 are therefore restricted to the first 3 days.

Our interpretation of the gelation process is as follows. First, a liquid–liquid microphase separation occurs, with the polymer-rich regions connected through junction zones so that a gel with a finite modulus is formed. Upon aging, the centers of the polymer-rich regions develop into crystallites of high scattering power but of a size that is insufficient to give an angular dependence in the light scattering between $\theta = 1°$ and $\theta = 30°$. At larger angles some angular dependence has previously been observed,[5] so that the crystallite dimensions must be between 300 and 700 Å. Slow crystallization of PVA in the gel state receives independent support from the slow emergence of a characteristic crystalline ir band.[6] Support for our interpretation of the gelation and syneresis is also found in the quasi-elastic laser light scattering.[31] In the early stages of the gelation an audio frequency resonance appears, ascribed to underdamped oscillations of the very soft viscoelastic polymer-rich regions. Upon aging this phenomenon disappears, which can be rationalized as being due to the formation of small hard regions, the Stokes frictional coefficient of which is too large relative to their elastic suspension in the gel. They are, therefore, not able to undergo thermally excited underdamped oscillations.[31]

The syneresis is, of course, reflected in the stress relaxation under unidirectional compression. Figure 14 shows the results for a gel sample of original height 2.009 cm and $\Lambda = 0.951$ at 18°. The gel looses all stress in 7×10^5 sec; upon 2 days further aging without stress the height is

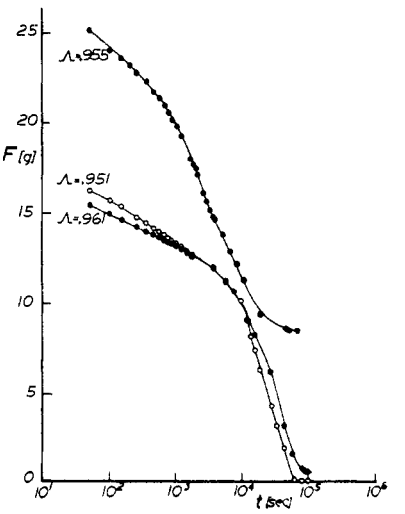

Figure 14. Force relaxation of a 5% PVA gel in water–ethylene glycol at 18°. The lowest curve shows a drop to zero force caused by syneresis; the second one shows the same after 2-days further aging and then imposing a new de-formation. The top curve is obtained after a total of 9 days after gel preparation and demonstrates the force relaxation after completion of the syneresis.

1.855 cm and under a newly imposed de-formation of $\Lambda = 0.961$ the gel looses again essentially all stress in 10^5 sec. Upon 7 days of further aging without stress the height is 1.581 and now exhibits upon de-formation ($\Lambda = 0.955$) a stress relaxation curve ending in a finite equilibrium stress. In this final experiment the crystallization is most likely complete. The final stress relaxation has an exponential tail as was also the case with agarose. It seems likely, therefore, that some breaking of weaker junction points (crystallites) takes place, perhaps leading to growth of the stronger ones through recrystallization. It is clear that here again an explanation on the basis of the molecular theory of rubbery viscoelasticty[13] has to fail.

Perspective. The three hydrogels investigated by us are found to be strikingly different in supramolecular organization. The gel (gelatin) which is least supramolecularly organized behaves as a soft rubber elastic material. However, the microphase separated gels (agarose, PVA) are quite different and will require the development of new predictive relations between structural makeup and mechanical properties.

A combination of various experimental techniques such as used in this study provides insight in the structure and dynamics of hydrogels as well as in the processes leading to gelation. It is hoped that the phenomenology, thus established, will be of use in the further development of structure–property relation in soft polymer system such as occur abundantly as coacervates in biological gels.

Acknowledgments. The authors gratefully acknowledge the instrumental assistance given by R. Wynaendts, formerly at Syracuse University, and the careful mechanical measurements on gelatin gels undertaken by A. Gourdenne at Syracuse, during his leave of absence from the Centre de Biophysique Moléculaire, Orléans, France.