Spinodal Phase Separation in a Macromolecular Sol → Gel Transition

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ABSTRACT: The supramolecular structure of thermoreversible agarose gels is found to be governed by the thermal history of the sol → gel transition. It is shown that the appearance of a Bragg maximum in the angular dependence of the scattered light intensity is due to spinodal decomposition. In this nucleation-free mechanism, spontaneous fluctuations in polymer segment concentration of a particular wave number K_m grow much more rapidly than any other fluctuations. The growth rates $r(K)$ were obtained from the time evolution of the light scattered from a series of gelling agarose solutions. The K dependence of the growth rates obeys the prediction of Cahn's theory of spinodal decomposition. The observed broadening of the Bragg maximum with lower quenching temperature is a consequence of the temperature dependence of K_m. If the quenching temperature lies above the spinodal temperature, the time evolution of the scattering as well as the supramolecular structure are determined by a nucleation and growth mechanism. In this case no Bragg maximum will occur, in accordance with observation.

Measurements of the angular dependence of the intensity of light scattered from a macromolecular gel provide information about the supramolecular structure of the gel. In a recent article Pines and Prins[1] have drawn attention to the fact that a Bragg-like diffraction maximum appears in the angular dependence of the light scattered from agarose gels. It is the purpose of this note to examine the pronounced influence of thermal history on the supramolecular structure of agarose gels and to explain the findings in terms of the phase separation mechanisms taking place during the process of gelation.

Agarose is a naturally occurring, alternating copolymer of (1→4)-linked 3,6-anhydro-α-L-galactose and (1→3)-linked β-D-galactose of about 100,000 in molecular weight. The angular dependence of the scattered light from aqueous agarose solutions[1] indicates that at temperatures above the sol → gel transition temperature, the agarose chains are somewhat aggregated to a characteristic size of 4500 Å, a size which exceeds the expected dimensions of the more or less randomly coiling, single agarose chains. The sudden increase in optical rotation which is observed upon cooling[1] indicates that the agarose molecules undergo a conformational transition, presumably to the double helix arrangement calculated by Rees[2] to be energetically most favored. Above a concentration of about 0.2 wt % the solutions set into a thermoreversible gel. Below 0.2 wt % no macroscopic gel formation occurs. Light-scattering data[1] indicate that in this case large microgel particles are formed with characteristic sizes up to 30,000 Å.

Figure 1 shows the results of our measurements of the angular dependence of the absolute scattered intensity, $I(\theta)$ expressed as Rayleigh ratios, $R_{V_V}(\theta)$. The symbol V_V stands for incident and scattered light polarized perpendicularly to the plane defined by the incident and scattered wave vector. The Rayleigh ratio is defined by

$$R(\theta) = I(\theta)R_0^2/I_0V \quad (1)$$

where R_0 is the distance from the scattering volume, V, to the detector and I_0 is the incident light intensity. We may write $I_0 = P(0)/S$ where $P(0)$ is the power in the incident beam of cross-sectional area S. We may also write $I(\theta) = P(\theta)/R_0^2\Omega$, where $P(\theta)$ is the power in the scattered beam and Ω is the solid angle viewed by the detector. Equation 1 thus becomes

$$R(\theta) = P(\theta)/P(0)\Omega d \quad (2)$$

where d is the thickness of the sample. We obtained Rayleigh ratios using a Spectra Physics 124A helium–neon laser as a light source and an RCA 7265 photomultiplier as a detector. The data were acquired by means of a pulse-counting technique that utilizes a digital autocorrelator in conjunction with a minicomputer.[3] The number of pulses per 10-μsec channel are collected at angle θ, $n(\theta)$, and at $\theta = 0$, $n(0)$, for about 10–30 sec, stored, and averaged by the minicomputer. Since $\langle n(\theta)\rangle$ and $\langle n(0)\rangle$ are proportional to the power in the beam falling on the photomultiplier, the Rayleigh ratio is readily obtained. Neutral density filters were used to reduce the $\langle n(0)\rangle$ count and corrections for turbidity, reflection, and refraction were applied in the usual manner.[4]

Gels were prepared by quenching 1 wt % aqueous agarose solutions from 82° to the indicated temperatures. The gelation temperature is approximately 41.5° at this concentration. Rectangular light-scattering cells of 2-mm optical path were mounted inside a copper jacket through which water of constant temperature was circulated. A rapid quench was achieved in about 5 sec by switching the connections from the high-temperature bath to a bath at a temperature below the gelation temperature.

Figure 1 shows that the Rayleigh ratios differ as much as 1000-fold depending on the quenching temperature. The systematic increase in the $R_{V_V}(\theta)$ values at higher quenching temperatures indicates that regions of high agarose concentration of increasingly larger dimensions are present in those gels.

Figure 1 also shows a maximum which shifts to larger scattering angles at lower quenching temperatures. Such a maximum can be due either (1) to the formation of extremely monodisperse aggregates which become suspended in the gel matrix in a random array[5] or (2) to a predominant spacing between the regions of high agarose concentration. From the previous study[1] it was concluded that the first possibility is unlikely because no maximum is observed if very dilute, nongelling solutions are measured at a temperature below that at which more concentrated solutions gel: the microgel particles formed under these circumstances are apparently not very monodisperse.

The occurrence of a predominant spacing between polymer-rich regions would not be expected from phase separation based on nucleation and growth at random points in the sample. It was suggested therefore[1] that a nucleation-free, so-called spinodal decomposition mechanism is responsible for the occurrence of the maximum.

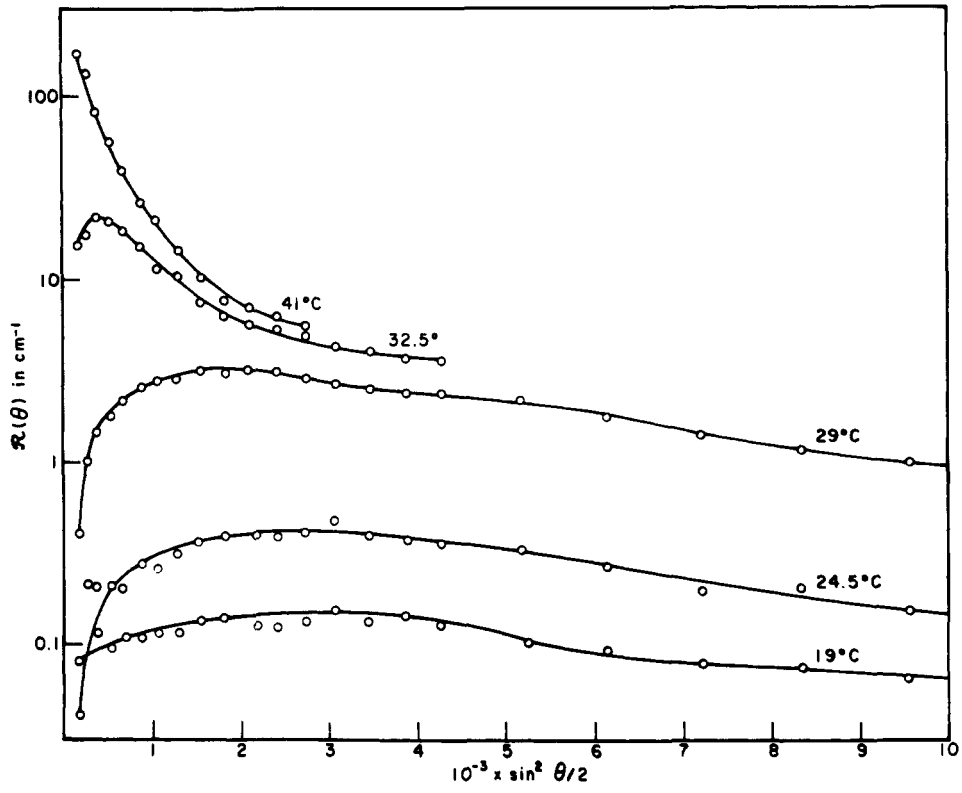

Figure 1. The logarithm of the equilibrium Rayleigh ratios (V_v polarization) as a function of the scattering angle for 1 wt % aqueous agarose gels quenched to the various indicated temperatures from the sol state at 82°. Note the 1000-fold range in $R(\theta)$ and the appearance of a maximum.

Phase separation of a polymer solution into polymer-rich and polymer-poor regions occurs upon cooling when the free energy of the homogeneous solution is greater than the sum of the free energies of the two separated phases. The Gibbs criterion for the metastability of a phase with respect to small fluctuations[6] is $\partial^2 F/\partial n_0^2 \geq 0$, where F denotes the free energy of the homogeneous solution of average polymer segment concentration n_0. On a T–n_0 phase diagram the boundary of the unstable region is defined by the locus of the so-called spinodal, *i.e.*, by the locus of $\partial^2 F/\partial n_0^2 = 0$. Phase separation that occurs within the unstable region is known as spinodal decomposition.

The study of the kinetics of spinodal decomposition starts with an expression for the free energy of an inhomogeneous solution[7,8]

$$F = \int [f(n) + \kappa(\nabla n)^2] dV \tag{3}$$

Here $f(n)$ is the free-energy density of the homogeneous portion of the solution containing polymer segment concentration n; $\kappa(\nabla n)^2$ is the additional free-energy density arising from the segment density gradient present in the inhomogeneous portion; κ is a positive interaction parameter. Following Cahn's procedure[9] we may obtain the kinetics of the initial stages of phase separation in a mixture of solvent (component 1) and polymer (component 2) by solving the diffusion equation. We first define a mobility, M, as the ratio of the diffusional flux of polymer segments, $\mathbf{J}$, to the gradient of the chemical potential difference

$$\mathbf{J} = M\nabla(\mu_1 - \mu_2) \tag{4}$$

We may obtain $\mu_1 - \mu_2$ by minimizing the free-energy integral in eq 3 with respect to n, subject to the condition that $\int (n - n_0) dV = 0$. This yields[10]

$$\mu_1 - \mu_2 = \partial f/\partial n - 2\kappa\nabla^2 n + \ldots \tag{5}$$

During the early stages of the phase separation the higher order terms can be neglected. Inserting eq 5 into eq 4 and taking the divergence then yields the following diffusion equation

$$\partial n/\partial t = M(\partial^2 f/\partial n^2)\nabla^2 n - 2M\kappa\nabla^4 n \tag{6}$$

The solution is a three-dimensional Fourier series

$$n(\tau, t) - n_0 = \sum_{\mathbf{K}} \exp[r(\mathbf{K})t]\{A(\mathbf{K}) \cos(\mathbf{K}\cdot\mathbf{r}) + B(\mathbf{K}) \sin(\mathbf{K}\cdot\mathbf{r})\}] \tag{7}$$

where $r(\mathbf{K})$ is given by

$$r(\mathbf{K}) = -[M(\partial^2 f/\partial n^2)(\mathbf{K}\cdot\mathbf{K}) + 2M\kappa(\mathbf{K}\cdot\mathbf{K})^2] \tag{8}$$

and $\mathbf{K}$ is the wave vector associated with the fluctuations. In the stable and metastable region $r(\mathbf{K})$ is negative so that concentration fluctuations decay. In the unstable region, however, $\partial^2 f/\partial n^2 < 0$ so that $r(\mathbf{K})$ is positive for all wave numbers $K < K_c$ where the critical wave number K_c is given by

$$K_c = [-(\partial^2 f/\partial n^2)/2\kappa]^{1/2} \tag{9}$$

Fluctuations with wave numbers $K < K_c$ will therefore grow rather than decay. We may rewrite the growth rate, $r(K)$, as

$$r(K) = 2M\kappa K^2(K_c^2 - K^2) \tag{10}$$

The function $r(K)$ has a sharp maximum at $K_m = K_c/(2)^{1/2}$. Because $r(K)$ occurs in an exponent in the amplitude of each Fourier component (eq 7), we expect that components with wave numbers clustered around K_m will, after a very short time, dominate the decomposition. If this pattern of concentration waves of random orientation

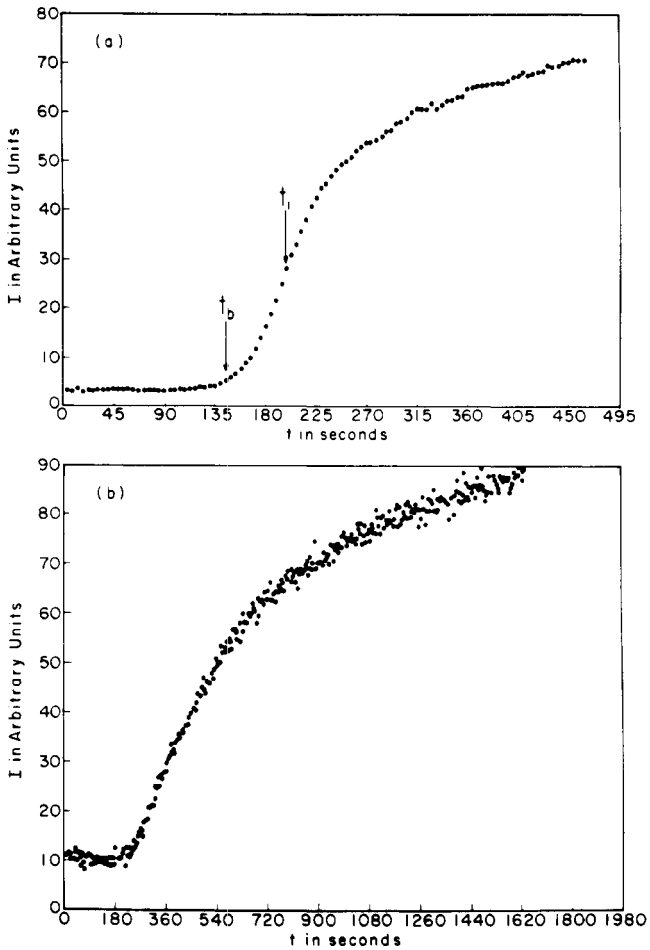

Figure 2. Time evolution of the scattered intensity at an external scattering angle $\theta_e = 12°$ (corresponding to $K = 2.069 \times 10^4$ cm^{-1}) of a 1 wt % aqueous agarose solution (buffered to pH 4.9) upon quenching: (a) from 82 to 25.5° and (b) from 82 to 40°. Note the exponential growth up to the inflection point t_i in (a) and the absence of such a growth in (b).

and phase is conserved in the later stages of phase separation, then a characteristic length, $\Lambda_m = 2\pi/K_m$, will occur most frequently as the spacing between polymer-rich regions. Indeed, if a gelling agarose solution is cooled sufficiently rapidly into the unstable region so that the usual nucleation and growth mechanism is bypassed, then spinodal decomposition is likely to occur and to be conserved because the subsequent three-dimensional gelation involves the formation of junction zones between agarose-rich regions.

So as to confirm the occurrence of spinodal decomposition in agarose solutions we will show experimentally that the Fourier components of the concentration fluctuations do, in fact, grow exponentially (eq 7) and that the growth rate does follow the K dependence predicted by eq 10.

The intensity of the light scattered at an angle θ by a medium containing concentration fluctuations is proportional to the square of the amplitude of that Fourier component of the fluctuations whose wave vector **K** equals the scattering vector of magnitude $(4\pi/\lambda) \sin \theta/2$, where λ is the wavelength of the light in the medium. The time evolution of the scattered light intensity at a given **K** value after a temperature jump into the unstable region has been imposed, follows from eq 7

$$I(K, t) = I(K, 0) \exp[2r(K)t] \qquad (11)$$

where $I(K,0)$ is the initially observed scattered intensity.

Figure 2a shows an example of such a time evolution

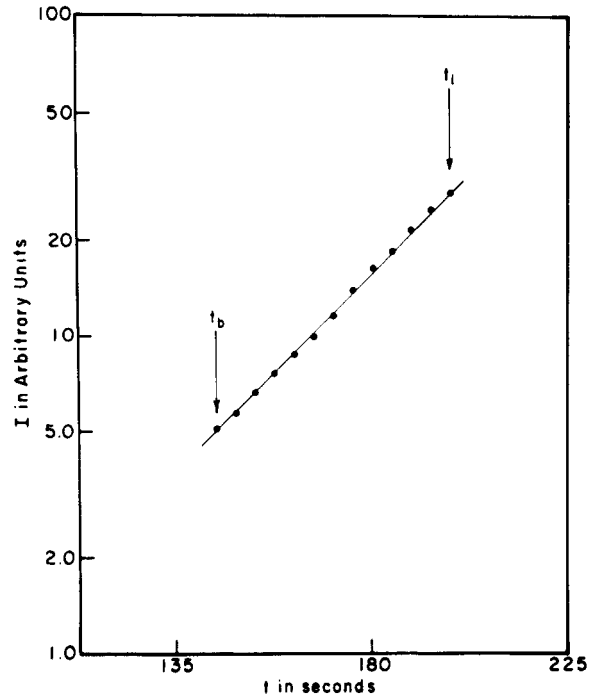

Figure 3. Demonstration of the exponential character of $I(K,t)$ in the time interval $t_b < t < t_i$. The logarithmic plot yields twice the growth rate $r(K)$ as slope. Data from Figure 2a.

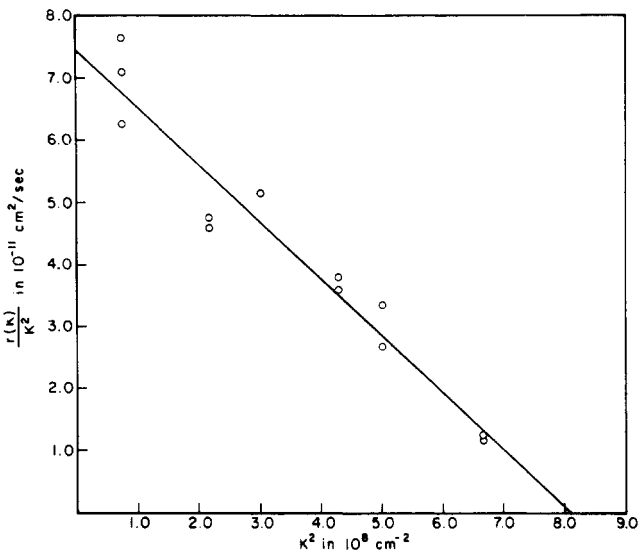

Figure 4. Test of eq 10 derived for the initial stages of spinodal phase separation. The points refer to twelve different 1% buffered agarose solutions all quenched from 82 to 25.5°. Growth rates $r(K)$ were determined as in Figure 3. Solid line is the linear least-squares fit to the data.

obtained experimentally by pulse counting at 4.5-sec intervals beginning immediately after the bath connections were switched. All samples were buffered to pH 4.9 in order to suppress any pH dependence of the gelation process.

According to eq 11 a plot of ln $[I(K,t)]$ *vs.* time should be linear with a slope of $2r(K)$. Figure 3 shows that after a response time $0 < t < t_b$, such a plot is indeed linear for $t_b < t < t_i$. In Figure 2b there is no time interval during which exponential growth occurs. In this case a sample was quenched to just below the gelation temperature so that phase separation took place by nucleation and growth. The observed increase in scattering must then be explained as arising from the growth of agarose-rich re-

gions from nuclei which are randomly positioned in space and time.

A plot of $[r(K)/K^2]$ *vs.* K^2 should be linear with a slope of $-2M_K$ and an intercept at $K^2 = 0$ of $2M_K K_c^2$ (see eq 10). Figure 4 shows that experiment confirms this prediction. The scatter in the points is due to unavoidable variations in the twelve different 1% agarose solutions which were used to collect the data.

The least-squares fit shown in Figure 4 yields $M_K = 4.62 \times 10^{-20}$ cm^4/sec and $K_c^2 = 8.08 \times 10^8$ cm^{-2}. Van Aartsen *et al.*[11-13] have confirmed the occurrence of spinodal decomposition during a liquid–liquid phase separation in a polymer system. They show that K_c is an increasing function of $T_s - T$ where T_s is the spinodal temperature for a given concentration and T the quenching temperature. If we associate the locations of the maxima in the Rayleigh ratios in Figure 1 with the magnitudes of $K_m = K_c/(2)^{1/2}$ we are thus able to account for the observed temperature shift in terms of the spinodal mechanism. We can then also conclude that the absence of a maximum in the upper curve in Figure 1 is the result of quenching to a temperature above T_s. This conclusion is supported by the absence of an exponential growth region in Figure 2b.

The increased broadening of the maxima in Figure 1 at lower quenching temperatures is most likely a result of spinodal decomposition occurring while the sample is cooling down to the final quench temperature. Since the wave number of the fastest growing Fourier component of the fluctuations increases with decreasing temperature, the resultant maxima will be broadened at lower quench temperatures.

Finally we note that, while we have confirmed the occurrence of spinodal decomposition in the agarose system by observing its initial stages, it is the entire mechanism that is responsible for the final structure observed in Figure 1. Studies of the later stages of spinodal decomposition[14] indicate that a coarsening reaction sets in resulting in an increase in the spacing between regions of high concentration. And indeed, we find that the maxima in Figure 1 do occur at lower K values than the K_m values obtained from the exponential growth during the initial stages.

We have seen that in order for spinodal decomposition to occur, the stage of phase separation proceeding by nucleation and growth must be bypassed. This is possible if the rate of material diffusion is slow relative to the rate at which the quenching temperature is established in a sample. This condition is likely to prevail in polymer systems where the viscosity is high. Our study of the agarose system may thus be viewed as an example of the significant control which the mechanism of spinodal decomposition can exert over the final morphology of polymer systems.

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Thermodynamic Interactions in Polymer Systems by Gas–Liquid Chromatography. IV. Interactions between Components in a Mixed Stationary Phase

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ABSTRACT: The glc method of determining specific retention times of a vapor-phase probe in a polymeric stationary phase is used to measure the thermodynamic interaction between the components (2,3) in a binary stationary phase. The Flory–Huggins interaction parameter χ_{23} may be obtained. An application of the more recent Prigogine–Flory theory is made to the ternary probe–stationary-phase system, leading to the X_{23} parameter. A variety of probes, mainly aliphatic hydrocarbons, are used with pure *n*-tetracosane (*n*-C_{24}), di-*n*-octyl phthalate (DOP), and poly(dimethylsiloxane) (PDMS) stationary phases. Binary stationary phases are represented by *n*-C_2DDOP and *n*-C_{24}–PDMS. Self-consistent χ and X parameters are obtained for interactions between the probes and the pure stationary phases, and also those within the binary stationary phases.

The application of gas–liquid chromatography (glc) to the determination of thermodynamic interactions in systems with a polymeric component in the stationary phase has been the subject of several recent publications.[3-5] In our earlier work, we have obtained specific retention volumes (V_g^0) for hydrocarbon gas-phase components (probes) at high dilution in the polymer. This led to the evaluation of thermodynamic interaction parameters (χ, χ^*) at the limit of the concentration range. The work of Brockmeier and coworkers[6] has extended the capability of the glc approach to finite concentration of the volatile phase, so that useful thermodynamic information may now be obtained over a broad range of polymer–probe concentrations. To date, limits of accuracy of thermodynamic data obtained from the glc results have not been established unequivocally.[7] It is clear, however, that glc is a most rapid and convenient method to obtain thermodynamic data for polymer systems. In the work being presented here, we have extended the glc method, using probes to measure the interaction between two nonvolatile