Crosslinked Biopolymers: Experimental Evidence for Scalar Percolation Theory

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Rheological measurements have been performed on pectin biopolymers close to the sol-gel transition. From these measurements scaling exponents were determined independently for the viscosity, $s=0.82(5)$, for the elastic modulus, $t=1.93(8)$, for the frequency-dependent modulus, $\Delta=0.71(2)$, and for the relaxation times below and above the transition, $\nu z=2.67(12)$ and $\nu z'=2.65(9)$. The exponents satisfy the scaling relations predicted by the theory and their numerical values agree with those from scalar elasticity percolation. Universal scaling functions were constructed from the data for the complex modulus and the dynamic viscosity.

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Pectin is a major linear polysaccharide in plants. It is a widely used gelling agent in the food industry. Probably the most familiar example of a gelation process involving pectin is the making of fruit jellies with grandmother's favorite recipe. The rheological properties of these biopolymer systems have been studied extensively, both in the sol phase and in the gel phase.[1-4] Here we are interested in the viscoelastic properties of pectins *at the gel point* in order to test the material-independent predictions of gelation theory.[5-8]

The classical theory of the gelation transition has been developed by Flory[5] and by Stockmayer.[6] A detailed description of critical phenomena near the gel point was given much later in terms of percolation theory.[7,8] This theory predicts universal scaling behavior.[9] Let us denote by R the parameter that controls the degree of gelation (R may be the concentration of crosslinkers, the temperature, etc.) and by R_c its value at the gel point. For $\epsilon \equiv |(R-R_c)/R_c| \ll 1$, the gel structure is characterized by the correlation length ξ, which scales as $\xi \sim \epsilon^{-\nu}$, and the relaxation time τ, which scales as $\tau \sim \xi^{z} \sim \epsilon^{-\bar{z}}$. ν, z, and $\bar{z}=\nu z$ are critical exponents.[9]

The rheological properties of a viscoelastic system are characterized[10] by the dynamic viscosity η and by the complex shear modulus $G^* = G' + iG''$. η depends on the shear rate $\dot{\gamma}$ and G^* on the frequency ω. The exponents used to describe the viscoelastic singularities near R_c are s, t, and Δ in[7,8]

$$\eta_0 \sim \epsilon^{-s}, \tag{1a}$$

$$G_0 \sim \epsilon^{t}, \tag{1b}$$

$$G' \sim G'' \sim \omega^{\Delta}. \tag{1c}$$

η_0 is the static viscosity, G_0 is the static elastic modulus, and G' (G'') is the storage (loss) part of the complex shear modulus. G' and G'' are evaluated at the gel point. A general scaling form[11,12] has been postulated for G^*, in the limit when $\omega, \epsilon \to 0$,

$$G^*(\omega,\epsilon) = G_0 \phi^{\pm}(i\omega/\omega_0), \tag{2a}$$

$$\omega_0 \sim \xi^{-z} \sim \epsilon^{\bar{z}}. \tag{2b}$$

At frequencies $\omega \ll \omega_0$,

$$\phi^{-}(i\omega/\omega_0) = B^{-}(i\omega/\omega_0) + C^{-}(i\omega/\omega_0)^2 + \cdots$$

for $R<R_c$ and $\phi^{+}(i\omega/\omega_0) = A^{+} + B^{+}(i\omega/\omega_0) + \cdots$ for $R>R_c$; for $\omega_0 \ll \omega$, $\phi^{-}(i\omega/\omega_0) \sim \phi^{+}(i\omega/\omega_0)^{\Delta}$. This leads to the correct frequency dependence for the elastic (viscous) part G' (G'') for $\omega \to 0$: below the gel point $G' \sim \omega^2$ ($G'' \sim \omega$); above the gel point $G' \sim$ const ($G'' \sim \omega$). With $\eta_0 = \lim_{\omega \to 0} G^*(\omega)/\omega$, Eqs. (2) lead to the scaling laws[11]

$$\bar{z} = s + t, \tag{3a}$$

$$\Delta = t/\bar{z}. \tag{3b}$$

A remarkable consequence of the scaling hypothesis is the universal value of the ratio G''/G' for $\omega \gg \omega_0$: $G''/G' = \tan(\Delta\pi/2)$.[13] The scaling form for G^* also implies that G' and G'' cross in the vicinity of the gel point. However, finite-frequency estimates of the gel point converge in a singular way towards the true gel point. This fact will be used to calculate the scaling exponents.

The viscosity η is singular at the gel point. Hence, we propose the following scaling form in the limit when $\dot{\gamma}, \epsilon \to 0$,

$$\eta(\dot{\gamma},\epsilon) = \eta_0 \psi^{\pm}(\dot{\gamma}/\dot{\gamma}_0), \tag{4a}$$

$$\dot{\gamma}_0 \sim \xi^{-z} \sim \epsilon^{\bar{z}}. \tag{4b}$$

$\dot{\gamma}_0$ is the characteristic shear rate that separates the regime where the gel structure is unperturbed by the shear field ($\dot{\gamma} \ll \dot{\gamma}_0$) from the regime where the gel structure is dominated by the shear field ($\dot{\gamma} \gg \dot{\gamma}_0$). Writing $\dot{\gamma}_0 \sim \epsilon^{\bar{z}}$ anticipates that $\dot{\gamma}_0$ scales with the dynamic critical exponent $\bar{z}$. The scaling function has the limits $\psi^{\pm}(\dot{\gamma}/$

$\dot{\gamma}_0 \rightarrow 0) = \text{const}$ and $\psi^{\pm}(\dot{\gamma}/\dot{\gamma}_0 \rightarrow \infty) \sim (\dot{\gamma}/\dot{\gamma}_0)^{-s/\bar{z}}$. Hence $\eta \sim \dot{\gamma}^{-s/\bar{z}}$ for $\dot{\gamma} \gg \gamma_0$. We expect that this scaling form only applies to weak (reversible) gels; it is not appropriate for strong (irreversible) gels where the gel structure depends on the shear history.

The theory presented so far is general. Different universality classes for gelation are characterized by different sets of exponents. The most extensively studied model—which turns out to be relevant for our experiments—is scalar elasticity percolation.[12] The exponents s and t for this model have been calculated accurately by numerical methods: $s = 0.75(4)$ (Ref. 14) and $t = 1.94(10)$ (Ref. 15) were obtained for the equivalent random resistor network. Equations (3) then determine $\bar{z} = 2.69(14)$ and $\Delta = 0.72(4)$. The exponent $\bar{z}$ is consistent with the conjecture $\bar{z} = d\nu = 2.64$.[16]

To identify the dynamic model corresponding to a gelation experiment, *all* exponents must agree. To our knowledge, the first agreement between theory and experiment was found for radical copolymerization of polystyrene and for polycondensation of polyurethanes.[17] Both processes lead to chemical (strong) gels. For copolymerization, s and t agree with the scalar model; for polycondensation s, Δ, and the universal ratio G''/G' agree with the scalar model but t is too large. Various scaling exponents have been measured for several other strong gels: for polyurethanes $\Delta = 0.5$ (at balanced stoichiometry),[18] for epoxy resins $\Delta = 0.70$ and $s = 1.4$ (Ref. 19), and for branched polyesters $s \geq 1.3$.[20] The exponent values suggest that these gels are not described by scalar elasticity. On the other hand, the values $s = 0.75$ and $t = 1.9$ (Ref. 21) obtained for polyacrilamides crosslinked by chromium are consistent with the scalar model. There are two biological systems for which scaling properties near the gel point were determined: (i) For casein[22] (a component of milk protein that gels irreversibly) t was obtained from oscillatory measurements and found to lie in the range 1.8–2.6 (depending on frequency); (ii) for gelatin[23] which forms weak (thermoreversible) gels, dynamical measurements determined $s = 1.48(8)$ and $t = 1.82(15)$; t lies in the range of the exponents for scalar percolation, but s does not.

Pectin consists of randomly connected $\alpha(1\text{-}4)$ *D*-galacturonic acid units and their methyl esters.[2] Pectin with a low methyl ester content ($< 45\%$) forms thermoreversible gels upon addition of divalent cations (calcium). The extrinsic parameters which can be used to control the sol-gel transition are temperature, polymer concentration, and crosslinker density through the ratio $R = 2(\mathrm{Ca}^{2+})/(\mathrm{COO}^-)$. The generally accepted mechanism of Ca^{2+}-induced interchain association is dimerization of homogalacturonic acid chain sequences (at least fourteen residues). The occurrence of methyl ester groups in the primary backbone limits the extent of such junction zones.[4] The junction zones play the role of the points of crosslinking in the traditional picture of the sol-gel transition.

The prectin sample used for the measurements is a commercial apple pectin (618F) kindly provided by Unipectine (France). After purification the anhydrogalacturonic acid content was 78% and the fraction of galacturonic residues which were esterified was 28%. The size exclusion chromatography analysis of the aqueous pectin solution ($0.1M$ $\mathrm{NaNO_3}$) gave an average molecular weight $M_w = 350\,000$ and a polydispersity index of 3. Intrinsic viscosity at 25 °C, $p\mathrm{H} = 7$ in the same solvent was $[\eta] = 298$ $\mathrm{cm^3/g}$. Homogeneous pectin gels were prepared by mixing equal volumes of an aqueous pectin solution containing 11.7 g/L NaCl and a calcium chloride solution at 70 °C during 5 min. The final concentration in the gel was $C = 14.9 \times 10^{-3}$ $\mathrm{g/cm^3}$ ($C[\eta] = 4.5$) and the final ionic strength was $0.1M$. The ratio R was varied from 0.03 to 0.13 by adjusting the calcium chloride concentration before mixing. The hot mixture was put onto the rheometer and the gel was cured for about 24 h. The quenching temperature of the rheometer was kept at $20 \pm 0.1\,°\mathrm{C}$ throughout all the experiments.

The constant-shear-viscosity measurements were performed with a Low-Shear 30 Contraves Rheometer over a range of the shear rate between 0.017 and 128 $\mathrm{s^{-1}}$. The dynamical measurements were performed using a Carri-med CS-50 controlled stress rehometer with a cone-plate device (radius 2.5 cm, cone angle 4°). A low deformation of 0.04 was maintained throughout the frequency range explored, between 10^{-3} and 10 Hz. The kinetics of the gel formation was followed by measuring G^* at a fixed frequency until equilibrium was reached. The mechanical spectrum has been recorded (1) in equilibrium for different R and (2) during gel formation for fixed $R > R_c$. A constant strain imposed on the gel (for $R > R_c$) does not relax for days, indicating that during a measurement the gel can be considered irreversibly bonded. In contrast with other biological gels,[23] no hysteresis effects were observed during temperature-driven gelation under present experimental conditions.

Figures 1–5 present the scaling analysis of our data (from fully cured gels). First, the gel point R_c and the exponents s, t, $\bar{z}$, and $\bar{z}'$ ($\bar{z}$ and $\bar{z}'$ characterize the relaxation time below and above the gel point, respectively[9]) are determined using Eqs. (1), (2b), and (4b). Figure 1 summarizes the results: Plotting $\eta_0^{-1/s}$ vs R and adjusting s for the best straight-line fit determines $s = 0.82(5)$ and yields an estimate for R_c. Similarly, $t = 1.93(8)$ was obtained from G_0, $\bar{z} = 2.67(12)$ from $\dot{\gamma}_0$ below the gel point ($\dot{\gamma}_0$ is the shear rate where η begins to deviate from η_0), and $\bar{z}' = 2.65(9)$ from ω_0 above the gel point (ω_0 is the frequency where G' crosses G''). All methods yield the same $R_c = 0.086(2)$ to within 2%. The exponents $\bar{z}$ and $\bar{z}'$ are equal to within experimental errors, in agreement with the prediction of the theory of critical phe-

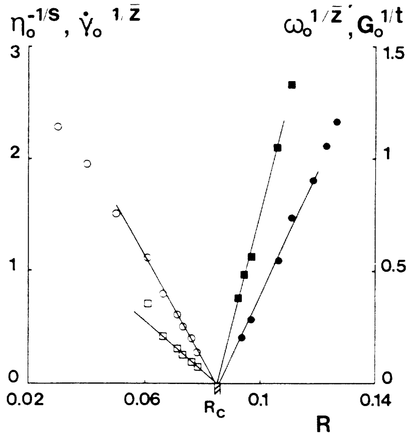

FIG. 1. Determination of the gel point R_c and the exponents s, t, $\bar{z}'$, and $\bar{z}$ from Eqs. (1a), (1b), (2b), and (4b). The static viscosity η_0 (Pas) determines $s=0.82(5)$ (○); the elastic constant G_0 (Pa) determines $t=1.93(8)$ (●); the characteristic shear rate $\dot{\gamma}_0$ (s^{-1}) determines $\bar{z}=2.67(12)$ (□) below the gel point and the characteristic frequency ω_0 (Hz) determines $\bar{z}'=2.65(9)$ (■) above the gel point. The common gel point is $R_c=0.086(2)$. The indicated error bars are obtained from varying the exponent estimates but conserving a common gel point R_c and the straight-line behavior near R_c.

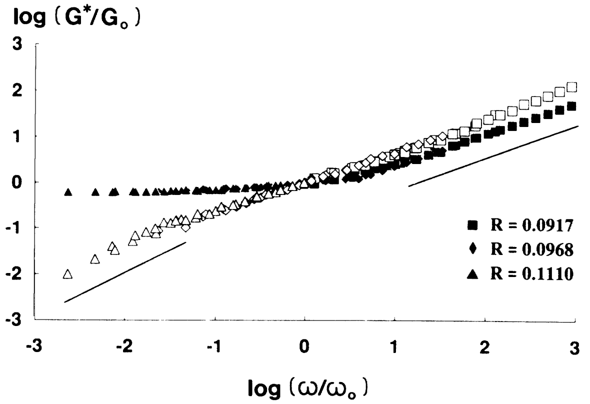

FIG. 3. Data collapse of G'/G_0 (solid symbols) and G''/G_0 (open symbols) vs ω/ω_0, from oscillatory measurements above the gel point ($\log_{10}$-$\log_{10}$). The straight line on the left has slope 1: the one on the right has slope 0.71 ($=\Delta$). The fluctuations of the data points are representative of the run to run variations of the rheological measurements.

nomena.[9] In addition, the exponents s, t, and $\bar{z}$ ($=\bar{z}'$) satisfy the scaling law of Eq. (3a).

The exponent Δ can be calculated *without* knowing R_c explicitly. If, for a given ϵ, G_x is the modulus and ω_0 is the frequency where the storage modulus crosses the loss modulus, $G_x(\omega_0)=G'(\omega_0)=G''(\omega_0)$, Eq. (2) predicts $G_x(\omega_0)\sim\omega_0^{\Delta}$. Figure 2 shows that this is an accurate method to calculate Δ. The measured value is $\Delta=0.71(2)$. The exponents Δ, s, and t satisfy the scaling law of Eq. (3b).

In Fig. 3 the master curves for G'/G_0 and G''/G_0 vs ω/ω_0 are presented, for $R>R_c$. For $\omega\ll\omega_0$, G'/G_0 is constant and $G''/G_0\sim\omega$; for $\omega\gg\omega_0$, both G' and G'' scale with the exponent $\Delta=0.71$. To construct a master curve for the ratio G''/G' only the variable ω has to be rescaled. Figure 4 shows G''/G' vs ω/ω_0. For $\omega\ll\omega_0$, one recovers the expected linear behavior in ω/ω_0, and for $\omega\gg\omega_0$, G''/G' is constant. The value of this constant, 1.99(8), agrees very well with the universal prediction $G''/G'=\tan(\Delta\pi/2)=2.04$.[13]

Figure 5 shows the scaled viscosity η/η_0 vs $\dot{\gamma}/\dot{\gamma}_0$ below the gel point. The data from different ϵ clearly fall on a single curve, confirming the scaling *Ansatz* of Eq. (4). For $\dot{\gamma}\gg\dot{\gamma}_0$, η/η_0 approaches the predicted slope $s/\bar{z}$.

Additional results corroborate our analysis: (i) Measurements of G^* for $R<R_c$ agree with the scaling hypothesis but they are less accurate than those $R>R_c$. (ii) Oscillatory measurements were performed at finite

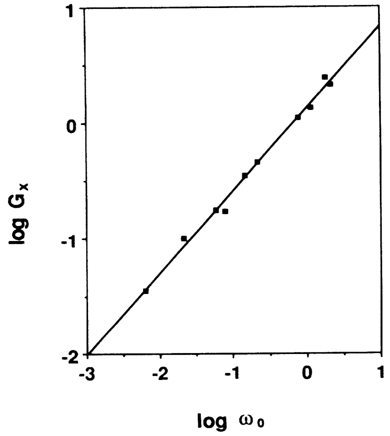

FIG. 2. Shear modulus G_x as a function of frequency ω_0 for $G'=G''$ ($\log_{10}$-$\log_{10}$). The straight line has slope $\Delta=0.71(2)$ [Eq. (1c)].

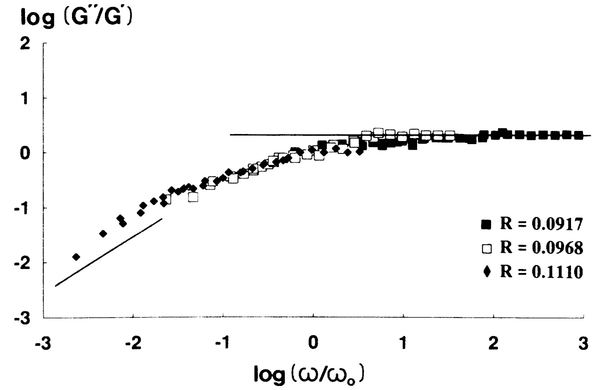

FIG. 4. The ratio G''/G' as a function of ω/ω_0 ($\log_{10}$-$\log_{10}$). The straight line on the left has slope 1; the horizontal line on the right has the value $G''/G'=1.99(8)$. With $\tan(\Delta\pi/2)=G''/G'$ this leads to the estimate $\Delta=0.70$.

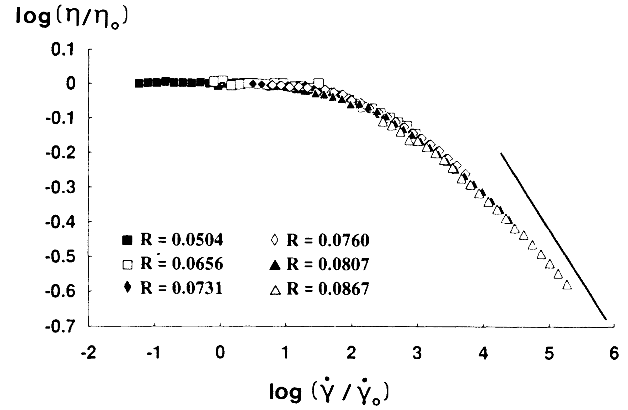

FIG. 5. Data collapse of the viscosity η/η_0 vs the shear rate $\dot{\gamma}/\dot{\gamma}_0$ below the gel point ($\log_{10}$-$\log_{10}$). The straight line has slope $s/\bar{z} = 0.31$.

time t for a fixed calcium concentration $R > R_c$. Defining $\epsilon \equiv |(t - t_g)/t_g|$ (t_g is the gel time) the results agree with those from fully cured gels. For example, Fig. 3 is reproduced to within the size of the symbols.

Our results show that scaling theory can describe the properties of the gelation transition accurately. The consistency of the exponents obtained independently in different measurements is a stringent test for the scaling *Ansatz* (the oscillatory measurements used in conjunction with the complex modulus perturb the gel in a fundamentally different way than the steady-state measurements used for the dynamic viscosity). It is strong evidence that the measurements were performed in the critical region of the sol-gel transition.

The identification of the relevant gelation model follows from comparing the dynamic exponents with theoretical predictions; we find excellent agreement with scalar elasticity percolation. This conclusion is supported by known structural properties of pectins:[3] (i) The polymer concentration used in the experiments places the system in the weakly entangled semidilute regime and (ii) from the calcium concentration, the size of the junction zones, and the polymer persistence length, the distance between two neighboring junction zones on a polymer is estimated to be ~15 persistence lengths.[3]

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