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Understanding of hydrogel network formation and its application in the architecture of significantly enhanced hydrogel

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An understanding of the physical hydrogel network formation has been obtained by dynamic rheological experiments. The evidence shows that the network formation turns out to be a nucleation-controlled process. It was found that there exists a critical temperature T^c; fiber branching is greatly enhanced when the network formation is performed in the regime of $T<T^c$ (T, the final setting temperature). This finding enables the authors to build significantly enhanced gel networks. So far G' (elastic modulus) of the hydrogel network has been enhanced by 187% while the formation period can be greatly shortened to only 1/20 of the previous process. © *2006 American Institute of Physics*. [DOI: 10.1063/1.2338007]

Physical hydrogels with three-dimensional (3D) fibrous network structures find numerous applications in many fields such as crystallization, drug delivery, pharmacy, porous materials in the novel separation for macromolecules, etc.[1,2] As a typical type of low density material, the microstructure of a hydrogel network becomes the dominant feature that controls its mechanical/rheological behavior.[3] Unfortunately, although this topic has been extensively studied in the past few decades,[4] the achieved progress is far from satisfactory. The formation mechanism of hydrogel networks still remains very elusive.[5] Although many mechanisms have been published,[4] most of them focus on the early gelation stages and therefore, to date, people are still not well in a position to manipulate the micro-/nanonetwork structure to create significantly enhanced gel networks. In this work, utilizing small-amplitude oscillation measurements, we aim at obtaining an in-depth understanding of hydrogel network formation with an emphasis on its structural aspect, so that we can build significantly enhanced gel networks accordingly.

An aqueous agarose solution was selected as the model gelling system as it is a repetitive, essentially uncharged, marine polysaccharide (Fig. 1). The agarose used in this experiment was Bio-Rad certified low-melt (LM) agarose. Agarose solutions were prepared by dispersing agarose powder in de-ionized water at room temperature. Solutions were prepared by heating mixtures in a pressure vessel to 90 °C for 1 h. The hot solutions ("sol") were then transferred between the measuring plates of an advanced rheological expansion system (ARES-LS, Rheometric Scientific) for small-amplitude oscillation measurements.[5] The sample was subjected to sinusoidal oscillations by loading it between circular plates with a diameter of 25 mm, the gap between the two plates being 1 mm. The frequency was set to 1 Hz and the amplitude of the oscillations was controlled to obtain a 0.1% strain in the sample. Under this strain limit, the structure of the gel is not destroyed by the measurements.[6] Samples were covered with mineral oil to prevent evaporation during the measurements.

To verify the formation mechanism, a series of dynamic rheological experiments was performed. As shown in Fig. 2(a), with the network establishment, the elastic modulus (G') will start to grow very fast. Therefore we utilize the development curve of G' to measure the induction time (t_i) [Fig. 2(a)].[5–7] If the network formation is essentially controlled by a nucleation kinetics, network junctions [refer to the inset of Fig. 2(a)] should be generated through nucleation and its propagation kinetics should follow a linear relationship between $\ln t_i$ and $(kT)^3(\Delta\mu/kT)^2$ (Refs. 6 and 7) ($\Delta\mu$, the chemical potential difference between agarose molecules in the junction and in the liquid phase; k, Boltzmann's constant; and T, the final setting temperature). To obtain $\Delta\mu/kT$, see Eq. (2). According to the generic 3D nucleation model, the nucleation rate J (the number of critical nuclei generated per unit time volume) can be expressed as[6–8]

$$J = f''[f]^{1/2} B \exp\left(-\frac{\Delta G^*}{kT} f\right), \tag{1}$$

with

$$\Delta G^* = -\frac{16\pi\gamma_{cf}^3\Omega^2}{3[(kT)\Delta\mu/kT]^2} \quad \text{and} \quad \frac{\Delta\mu}{kT} \cong \frac{\Delta H_{diss}}{kT_{eq}T}$$

$$\Delta T, \quad \Delta T = T_{eq} - T, \tag{2}$$

where Ω is the volume of growth units, B is a constant for a given system, f'' and f are the factors describing the structural correlation between the primary fiber (substrate) and the oncoming agarose chain (nucleating phase),[6–8] ΔH_{diss} de-

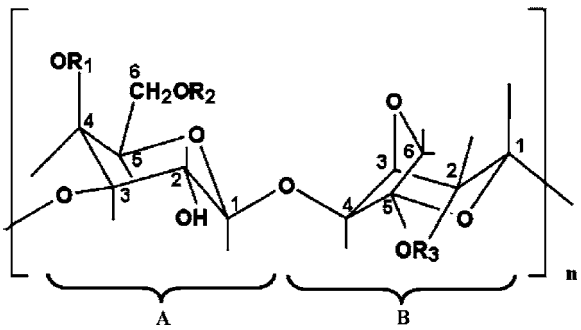

FIG. 1. Idealized AB repeat unit of agarose polymer. A—1,3 linked β-D-galactose residue; and B—1,4 linked 3,6-anhydro-α-L-galactose residue. Native agarose: $R_1=R_2=R_3=$H.

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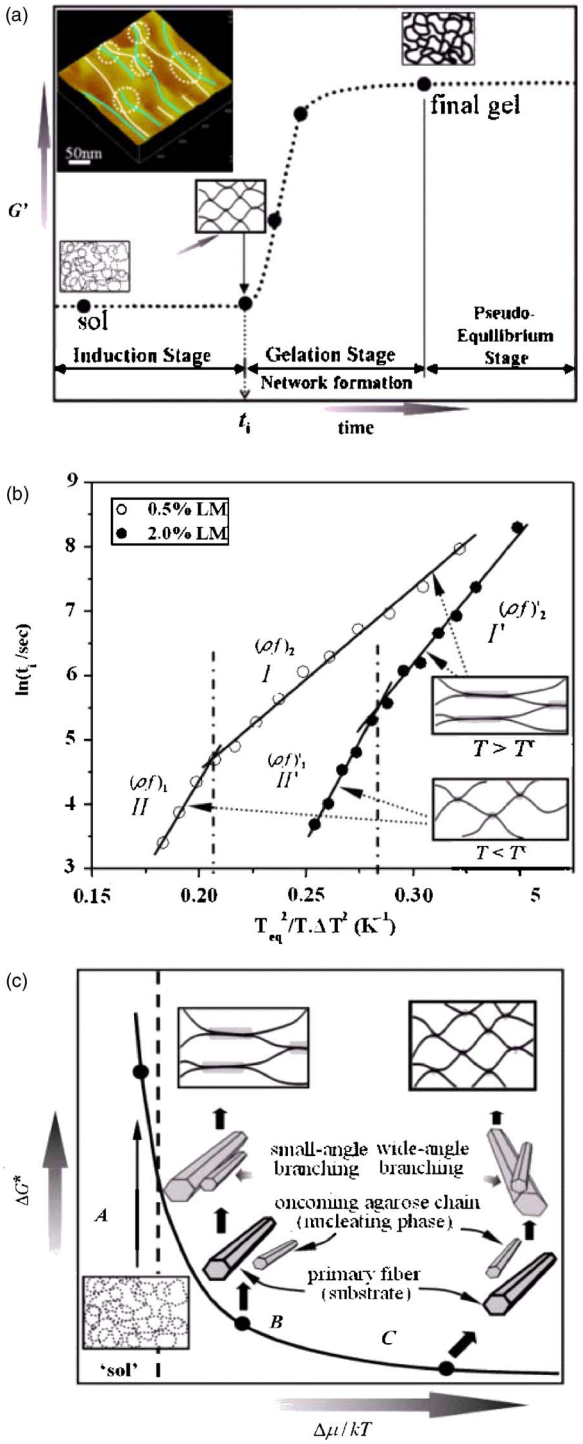

FIG. 2. (Color online) (a) Schematic illustration of agarose gelation process. Inset: Atomic force microscopy micrograph image of 2% w/v agarose gel network formed under 22 °C (network junctions are highlighted by dotted circles). (b) The plot of $\ln(t_i)$ vs $T_{eq}^2/T\Delta T^2$. The solid lines are obtained by linear fitting. (c) Illustration of gel network formation through nucleation. As for $\Delta\mu/kT$ and ΔG^*, see Eq. (2).

notes the molar dissolution enthalpy of the nucleating phase, and T_{eq} is the melting point. For the 0.5% w/v and 2% w/v LM agarose gels, T_{eq} are 334.2 and 338.0 K, respectively. Considering $t_i \sim 1/J$,[8] according to Eqs. (1) and (2), we have

$$\ln(t_i) = \rho f \frac{T_{eq}^2}{T\Delta T^2} - \ln(ff''B), \tag{3}$$

with

$$\rho = \frac{16\pi\gamma_{cf}^3\Omega^2 k\Delta H_{diss}^2}{3}. \tag{4}$$

As shown by the linear segments *I* and *I'* in Fig. 2(b), we indeed obtain such a linear relationship between $\ln t_i$ and $(kT)^3(\Delta\mu/kT)^2$. In addition, we found that the slope of segment *I'* [2% w/v agarose $(\rho f)'_2 \approx 43.0$] is much larger than that of segment *I* [0.5% w/v agarose $(\rho f)_2 \approx 28.0$]. If ρ can be treated as a constant, a larger slope will give a larger f, which corresponds to a higher degree of mismatch between the oncoming agarose chain and the primary fiber [i.e., wide-angle branching, Fig. 2(c)].[7–9] It turns out that the stronger gel prepared from more concentrated agarose solutions (i.e., larger nucleation driving force) is not only due to more agarose molecules incorporated into the network but also due to the higher degree of mismatch between the oncoming agarose chain and the primary fiber (i.e., so-called mismatch nucleation). This finding inspired us to extend the gelation into the regime of much lower T (T, the final setting temperature) for a gelling system with *fixed* concentration: if the sufficiently large nucleation driving force resulting from higher concentration can promote wide-angle branching, then for the gelling system with fixed concentration, the large nucleation driving force resulting from sufficiently lower T should have the similar effect. If the nucleation driving force does play a key role in the mismatch nucleation, when T is sufficiently low, we should also observe the abrupt slope variations similar to the evident slope difference between segments *I* and *I'* [Fig. 2(b)]. Some additional measurements extended to much lower T's were thus conducted. As shown in Fig. 2(b), we did obtain another two linear segments, segments *II* and *II'*. For both gelling systems with the fixed concentration (0.5% w/v or 2% w/v), straight segments with different slopes intersect each other indicating the existence of two regimes. In the regime of $T > T^c$ (i.e., small nucleation driving force), a loose network with small-angle branching (i.e., small f) formed due to small-angle branching [point *B*, Fig. 2(c)]. However, in the regime of $T < T^c$ (i.e., large nucleation driving force), a dense network with wide-angle branching (i.e., large f) formed [point *C*, Fig. 2(c)]. The existence of a critical temperature may also be applied for other hydrogels. For example, te Nijenhuis studied the temperature dependence of the induction time for 2% w/v mammalian gelatin hydrogel.[10] By reprocessing the data published, we obtained similar results as for agarose gel. The critical temperature of the gelatin hydrogel is found to be 19.4 °C.

This understanding of the network formation enables us to create highly enhanced hydrogel networks. It is well known that a loose network with small-angle branching is very weak whereas a dense network with wide-angle branching is quite strong.[7,8] As illustrated in Fig. 3, in both the "weak-gel" regime ($T > T^c$) and the "strong-gel" regime ($T < T^c$), a gradually decreasing T will only give a gradually increasing nucleation rate; therefore, only a limited network enhancement can be achieved due to the junction density that increases only gradually. However, when the network formation is shifted from the weak-gel regime to the strong-gel regime, on the one hand, decreasing T will give a higher nucleation rate so as to generate more junctions, leading to a denser network; and on the other hand, decreasing T will also give an abruptly increased f. In this case, as the epitaxial nucleation occurs more freely, one agarose chain is allowed to join in more junctions. Both these two aspects facilitate

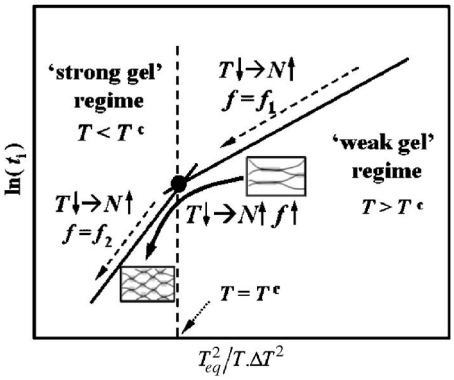

FIG. 3. Illustration of the protocol used to fabricate significantly enhanced gel networks. T, the final setting temperature; T^c, the critical final setting temperature; N, the junction number of the hydrogel network; and f, the factors describing the structural correlation between the primary fiber (substrate) and the oncoming agarose chain (nucleating phase), $f_2 > f_1$.

the formation of a much stronger gel. As any large scale network reorganization for an already formed gel is energetically highly infeasible, to create significantly enhanced gels in the weak-gel regime ($T > T^c$), we can first perform the gelation in the regime of $T \ll T^c$ to establish a dense and highly branched "skeleton" of a strong gel, and then shift the system back to the weak-gel regime. As shown in Fig. 4, this protocol does allow us to create highly enhanced gel networks. A gel formed by directly quenching the gelling system (2% w/v agarose solution) to 30 °C is extremely weak ($G'_\infty \approx 8700$ Pa) and requires an extremely long time ($>6.0 \times 10^4$ s) to achieve its quasiequilibrium state. However, when we first quench the gelling system under the condition of $T = 5\ ^\circ\text{C} \ll T^c = 28.0\ ^\circ\text{C}$ [$T^c = 28.0\ ^\circ\text{C}$; see Fig. 2(b)] and then heat it back to 30 °C, the system obtains its quasiequilibrium state much faster (<3000 s) and G'_∞ is much higher ($G'_\infty \approx 2.5 \times 10^4$ Pa).

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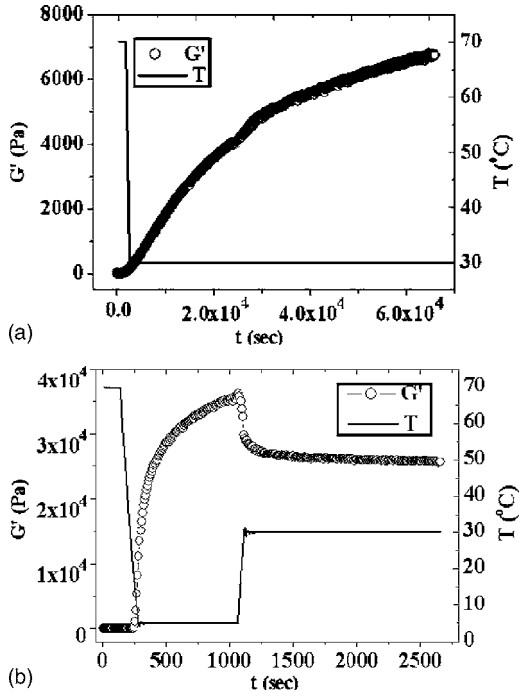

FIG. 4. Rheological tests of 2% w/v agarose systems. (a) Elastic modulus evolution curve for the gel formed by directly quenching the gelling system to 30 °C. $G'_\infty \approx 8700$ Pa is obtained by data extrapolation using the plot of G' vs $1/t$ (not shown). (b) Elastic modulus evolution curve for the gel formed by first quenching the system to 5 °C and then heating the system back to 30 °C.