Fractal Morphogenesis and Interacting Processes in Gelation

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In the self-assembly of a hydrogel, we observe the simultaneous development of a self-similar structure of cross-linked molecules and of a larger scale structure of domains resulting from spinodal demixing. The fractal dimension of the smaller scale structure grows initially, then it freezes-in at the gel point, which can thus be operationally defined in terms of geometric (fractal), not only of rheological properties. These observations highlight novel aspects of kinetic competition and of multiple path interactions of processes in supramolecular self-assembly. [S0031-9007(97)04580-8]

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Gels represent a unique state of matter characterized by space-filling ramified structures responsible for solidlike properties of macroscopic samples. The process of gelation is of high relevance to fields ranging from critical phenomena and statistical mechanics [1,2] to polymer science, biology, and technology [3]. Gelation of biopolymeric solutions can occur even at very low concentrations. In many such cases, it has been shown that the thermodynamic phase transition of demixing is the significant symmetry breaking step which promotes *correlated* cross-linking and its progress up to the topological phase transition of gelation [4,5]. Critically divergent fluctuations can serve the same purpose [6]. This paper concerns the case of gelation in conditions such that the distinct processes of sol demixing and molecular cross-linking can both start independently and kinetically interact. Our experiments show the simultaneous development of a self-similar structure of cross-linked molecules and of a larger scale structure of domains resulting from demixing. Self-similarity is observed across the entire scale interval falling between the size of single polymers and that of demixed regions. The fractal dimension of the smaller scale structure grows initially and then it freezes-in at the gel point, which can thus be operationally defined in terms of geometric (fractal) and not only of rheological properties. These observations illustrate novel aspects of kinetic competition and of multiple path interactions of processes leading to a final two-scale structure.

Transition from a uniform solution (sol) to the gel state was in origin viewed in terms of Flory-Stockmayer-Gordon infinite cluster [7] and, later, of percolation [8] and critical phenomena [2,8]. Thermoreversible gelation of biopolymeric systems was soon understood to involve, in many cases (or to be accompanied by), demixing [9]. Clear evidence for a spinodal process in an early stage of gelation was provided by Feke and Prins [10]. This and a variety of other experiments focused, however, on the final equilibrium state with kinetic observations covering no more than the early stages [9–11]. The "important ingredient of gelation reality, namely dynamics, is missing" [2] also in further, valuable experimental and theoretical [12] studies taking explicitly into account the suggested role of solubility [1]. Complete kinetic studies related to gelation started in 1978 in a conceptually simple case of entanglement caused by the single process of spinodal demixing and mimicking cross-linking [13]. Since 1985 such studies have been extended to systems allowing both demixing and cross-linking [4], two processes occurring on different length and energy scales. Solute-solute correlations due to spinodal demixing or even to critically divergent fluctuations of sufficient lifetime and amplitude have been found in several cases to promote gelation at *average* concentrations substantially below the random cross-linking percolation threshold [4,5,14,15]. In demixing-promoted gels, gelation was found to freeze-in the mesoscopic structure caused by demixing [4,14–16]. Further, at even lower concentration, gelation was found to occur on a mesoscopic scale within disconnected, demixed domains freely drifting in the mesoscopically fluid specimen [7]. Interesting features are revealed by simulations, even using one single scale of interaction energy. In this case, percolation could be demonstrated on a transient basis only [18]. Further simulation work, however, has covered the case of systems with two different interaction energies and ranges [19].

The present experiments concern self-assembly of hydrogels of agarose, an uncharged biostructural polysaccharide [Seakem HGT (P), from BioProducts, Marine Colloids Division, Rockland, Maine]. Powder was dissolved in millipore water for 20 min at 100 °C and filtered (0.22 μm filter) directly in measuring cells [15]. The sol was quenched from 90 °C to the region encompassed by both the gelation line and the spinodal line of the sol as such. Kinetics of structural evolution were quantitatively monitored by both small and large angle elastic light scattering. Argon (514.5 nm) or helium-neon (632.8 nm) lasers were used. Data were collected at a wide angle using a Brookhaven BI-200SM goniometer. For small angle measurements, a Panasonic CCD (Matsushita Electric Industries Co. Ltd., Osaka, Japan) camera

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was used, similar to Ref. [20]. Gelation was monitored by the plain drop-ball method, having previously checked by viscoelastic measurements the appropriateness of this method to the present purpose. The four chosen quenching points are labeled A, B, C, and D in Fig. 1(a) and they correspond to the same concentration (2% wt. %) and different temperatures. Changing the sole quenching temperature allowed selecting within an ample variety of gel self-assembly conditions. In A [Fig. 1(a)], the determinant initial symmetry break leading to gelation occurs on the micrometer scale and it is due to the thermodynamic phase transition of spinodal demixing. In D, the determinant initial symmetry break occurs on a scale of up to 0.1 μm and is due to the formation and further progression of polymer cross-linking [4,14,15]. Within this ample span, the relative role of the two processes is dictated by their kinetics. In general, a twofold percolation can be expected in the gel: that of domains resulting from demixing and, on a shorter scale within and across such domains, that of cross-linked polymers.

After quenching to points A, B, or C, concentration fluctuations leading to spinodal demixing are observed to grow around a wavelength L_m. Cahn's plot in Fig. 1(b) evidences the spinodal mechanism in the early stage of demixing [21]. In simple cases, this stage is followed by coarsening, revealed by a shift of the peak of the structure function $q_m = 2\pi/L_m$ towards lower values and characterized by a typical scaling regime [22]. In the present case, however, the correlation length L_m of demixed domains is not observed to increase, because cross-linking "freezes" it even before coarsening [23]. This is shown in Fig. 1(c), where the position of the peak of the structure function $S(q)$ does not shift to the left, notwithstanding a marked increase of the peak itself. When the quenching temperature is decreased in the order A, B, C [Fig. 1(a)], the structure function peak becomes less and less pronounced and it occurs at increasing q values. Further, both demixing and cross-linking kinetics become faster. Numerical values of durations of the early stages of demixing t_C (Cahn's linear regime) and of gelation times t_g for the different quenchings are in Table I. The table shows that, at decreasing quenching temperatures, cross-linking and related gelation tend to shorten the duration of demixing. Consistently with that and with the fact that the shorter the value of t_C, the less pronounced the peak of the structure function due to demixing, for quenching to point D, cross-linking and gelation totally override and inhibit demixing and no peak is observed in $S(q)$ [Fig. 1(d)].

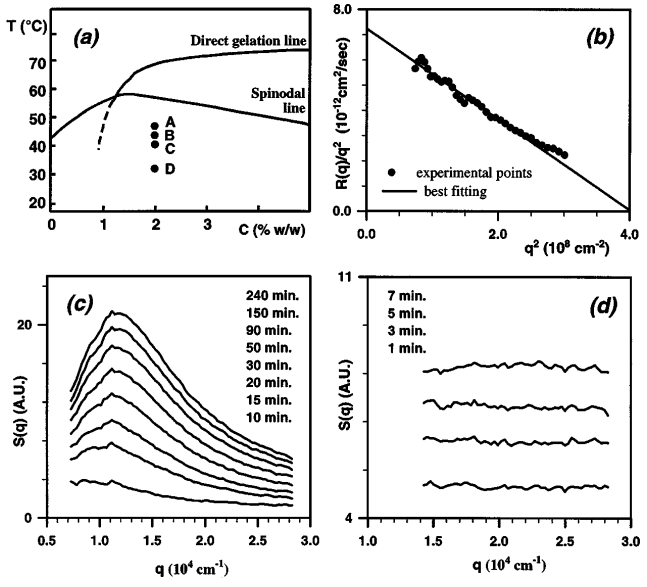

FIG. 1. (a) Phase diagram of agarose-water systems (redrawn from Ref. [15]). Quenching points used in the present work are labeled A, B, C, and D. (b) Cahn's plot relative to quenching to point B. Amplification factors $R(q)$ are measured from the exponential growth of scattered intensity $\log S(q) \sim R(q)t$, where t is the time elapsed after quenching (Ref. [21]). (c) Structure functions $S(q)$ at different times after quenching relative to quenching to point B. (d) Same as (c), relative to quenching to point D.

Novel and relevant information comes from the kinetics of self-assembly on the scale window $L_p < 2\pi q^{-1} < L_m$. Here, L_m is the correlation length of the pattern of domains of different concentrations resulting from demixing. As stated above, and visible from Fig. 1(c), L_m is in the micrometer range. The lower limit of the window, L_p, is the size of the individual "building blocks" of the gel. One could consider such building blocks to be individual polymers, on the grounds that gelation is in this case due to physical, thermoreversible cross-linking rather than to further, chemical polymerization. However, it is well known that in the present case the gel building blocks consist in bundles of double helices made of several individual polymers [4,24]. This raises the size of L_p from that of a single polymer coil ($\sim$0.01 μm) to $\sim$0.1 μm.

Within the $L_p < 2\pi q^{-1} < L_m$ interval, no characteristic length is observed and the structure remains self-similar during the entire kinetics, as shown by log-log plots of the structure function [Fig. 2(a)] evidencing a relation of the type $S(q) \sim q^{-d_f}$. The d_f parameter, obtainable from slopes, corresponds to the mass fractal dimension, defined by the relation $M \sim r^{d_f}$, where M is the mass of objects within a radius r [25]. A typical

TABLE I. Characteristic times of kinetics relative to quenchings to points A, B, C, and D in Fig. 1(a): T_Q, quenching temperature; t_C, duration of Cahn's linear regime; t_g, time of macroscopic gelation.

	T_Q (°C)	t_C (min)	t_g (min)
A	46.5	50	60
B	43.0	12	22
C	40.0	7	17
D	31.5	No measurable demixing	9

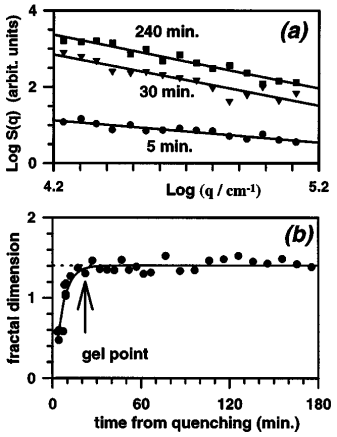

FIG. 2. (a) Log-log plot of structure functions at different times relative to quenching to point B in Fig. 1(a). Lines are obtained by best fittings. (b) Time course of fractal dimension relative to quenching to point B in Fig. 1(a).

example of the kinetics of growth of the fractal dimension is in Fig. 2(b), relative to quenching to point B in Fig. 1(a). The fractal dimension of the solution immediately after quenching is about 0.5. A fractal dimension $d_f < 1$ stands for a topological dimension $d_T = 0$, since it must be $d_T < d_f$. Therefore, at the very beginning of demixing, the scattering objects in the solution are not connected ($d_T = 0$). At longer times, the d_f value rises, goes past the unitary value, and finally "saturates" to the stationary value d_g at a time coincident with the time t_g of macroscopic gel point. Analogous fractal dimension kinetics are observed for quenchings to A, C, and D points. Note that fractal dimensions are derived from data such as in Fig. 2(a) (which refer to the appropriate scale window) and not from data such as in Figs. 1(c) and 1(d) (which refer to the L_m range). Their characteristic times are shown in Table I. In all those cases and just as in the case relative to point B [Fig. 2(b)], the fractal dimension stops growing at the time t_g of macroscopic gelation. Remarkably, the stationary final value is essentially the same for all quenching points, falling in the narrow interval from 1.2 (C, D) to 1.4 (A, B). Although rather unusual, such low values are well consistent with the overall loose packing of molecules, warranting cross-link percolation at such low average concentrations. They are also endorsed by the spanning fiberlike structure of double helices in these gels [24] as well as by analogous findings in other systems [26]. Indeed, fractal dimensions close to unit speak for sparse ramified structures. The latter are allowed and expanded in the present case by the polymer cross-linking mechanism provided by "kink sites" existing along the polymer chains [24]. The well known filtration properties of Agarose gels depend, in fact, upon the existence of such loose structures.

In summary, we have reported for the first time the kinetic observation of fractal dimension growth in the course of gel self-assembly and its relation to the simultaneously observed kinetics of mesoscopic demixing. This allows drawing significant conclusions on the relation between the two kinetics and on its relevance to the final gel structure. Quantitative measurements of the time evolution and of kinetic competition of self-assembly on the two scale lengths show that "freezing-in" of both structures occurs when d_f reaches its steady value of 1.2–1.4. Notwithstanding the role of spinodal demixing in the progress of d_f towards this steady value, the latter is observed to be independent of the stage reached by demixing itself. This shows that freezing-in of both structures is dictated by those geometric and topological hindrances which correspond to the final d_f value. Two processes concur and compete in generating the final fractal structure: the longer range diffusion involved in demixing and the shorter range diffusion (intradomain or even internal molecular mobility) involved in cross-linking and in related [24] conformational change. Therefore, we deal here with an extension of cases of aggregation dominated by one single process (diffusion-limited aggregation, reaction-limited aggregation, etc. [27]). Also, the present data illustrate how the plurality of the final gel states depends upon the plurality, comparison, and multiple-path interaction of kinetics, at least as strongly as upon the plurality of interaction energies and ranges. The huge variety of kinetically controlled final gel states mirrors the known complexity of configurational energy landscapes of gels and emphasizes the need for theoretical nonequilibrium treatments of gelation. In passing, the present results offer a new operational definition of "gel point" in the kinetic terms of freezing-in of fractal dimensions, that is, of geometric rather than rheological or topological properties.

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