Pattern Formation during Polymerization of Acrylamide in the Presence of Sulfide Ions

Miklós Orbán,*,[†] Krisztina Kurin-Csörgei,[†] Anatol M. Zhabotinsky,*,[‡] and Irving R. Epstein[‡]

Department of Inorganic and Analytical Chemistry, L. Eötvös University, H-1518 Budapest 112, P.O. Box 32, Hungary, and Department of Chemistry, Brandeis University, Waltham, Massachusetts 02254-9110

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When polyacrylamide gel is prepared from a mixture of acrylamide, *N*,*N*′-methylene-bisacrylamide and triethanolamine with a peroxodisulfate initiator in the presence of sulfide, spatial pattern formation occurs in the gel if water is spread on top of the gel after it solidifies. These patterns, typically arrays of spots or stripes, persist for many hours. Analysis of the effects of temperature, composition, stirring, and other factors suggests that the pattern formation occurs during polymerization due to convection driven by the highly exothermic polymerization reaction. The role of sulfide ion, which is essential for the pattern formation, appears to be to terminate the polymer chain elongation while maintaining the overall exothermic chain reaction.

Introduction

Since the publication of Turing's classic paper[1] nearly a half century ago, the reaction–diffusion patterns known as Turing structures (TS) have attracted considerable attention from chemists and biologists.[2] Turing structures were observed experimentally for the first time in a chemical system, the CIMA (ClO_2^-/ I^-/malonic acid) reaction, in a continuously fed gel reactor.[3,4] Prior to this discovery, there had been reports of spatial patterns analogous to TS that appeared in potentially oscillating liquid-phase chemical and biochemical reactions. These patterns, however, proved to be of convective origin.[5] The convective patterns that arise via Rayleigh or Marangoni instabilities strongly resemble Turing structures.[6] Therefore, prevention of convection is a necessary condition for observation of true TS, and gels have been employed in majority of the experiments on TS.

Recently, Turing-like structures have been observed in polyacrylamide gels formed in the presense of the components of the methylene blue oscillating reaction (MBO), methylene blue (Mblue), sulfide (S^{2-}), sulfite (SO_3^{2-}), and oxygen.[7-9] Here, we show that patterns similar but not identical to TS arise during the polymerization of acrylamide in the presence of sulfide alone.

Experimental Section

Chemicals. All reagents were of the highest grade commercially available. These were: acrylamide (AA), Sigma, electrophoresis reagent, ≥99%; *N*,*N*′-methylene-bisacrylamide (Bis), Sigma, electrophoresis reagent, ≥98%; triethanolamine (TEA), Fisher, 99%; ammonium peroxodisulfate ($S_2O_8^{2-}$), Sigma, electrophoresis reagent, ≥98%; sodium sulfide (S^{2-}), Merck, 35% Na_2S.

For preparation of stock solutions, the chemicals were dissolved in deionized water. All stock solutions but $(NH_4)_2S_2O_8$ and Na_2S could be stored for extended periods of time without decomposition. The following stock solutions were made: [AA] = 2.37 M, [Bis] = 0.13 M, [TEA] = 1.62 M, [$(NH_4)_2S_2O_8$] = 0.79 M, and [Na_2S] = 0.16 M.

[†] L. Eötvös University.
[‡] Brandeis University.

Procedure. The gel components, AA, Bis, and TEA, were mixed with S^{2-}, and then $(NH_4)_2S_2O_8$ was added to initiate the gelation process. The final volume, composed of the stock solutions and water, was 12.6 cm³, which formed a solution layer about 2 mm in depth in a 9 cm i.d. Petri dish. In most experiments, the concentration of the gel components was [AA] = 1.035 M (5.50 cm³ of stock solution), [Bis] = 4.85 × 10⁻³ M (0.47 cm³), (TEA) = 0.055 M (0.47 cm³). The polymerization time varied significantly with [S^{2-}] and [$S_2O_8^{2-}$]. For example, at [S^{2-}] = 1.5 × 10⁻² M (1.20 cm³), 3 × 10⁻² M (2.40 cm³), and 5 × 10⁻² M (3.94 cm³) and [$S_2O_8^{2-}$] = 0.113 M (1.80 cm³) or 0.226 M (3.60 cm³), gelation takes about 10 min, 20 min, and 3–4 h or 5, 10, and 40–50 min, respectively. After the mixture became solid, the gel layer was covered with water (6 cm³, at the ambient temperature of 21 or 25 °C). Observations were made visually from above using illumination (light box) from below. Images were recorded with a video camera, and the data were processed with an OPTIMAS image-analysis system (Bioscan).

The elastic modulus of the gel was measured with a homemade modulus balance instrument (manufactured by the Department of Colloid Chemistry, L. Eötvös University[10]). The method is based on measuring the penetration of a hard ball into the gel layer when known forces are imposed on the ball.[11]

Results

Pattern Formation in the AA/Bis/TEA/$S_2O_8^{2-}$/S^{2-} System. The gel was prepared in the presence of S^{2-}, and pure water was spread on the surface of the gel. Structures appeared in the gel 20–60 min after the water was added. The patterns were clearly visible in the milky opaque gel, especially if the Petri dish was placed on a light box. The first spots or stripes usually appeared at the wall of the Petri dish and then spread to occupy the entire gel surface. The structures were composed of white circles (1–2 mm i.d.), deformed spots, or stripes, depending on the composition of the system. The patterns persisted for many hours. During this time, the gel swelled significantly, eventually taking up the entire amount of water from the surface. As a result of the swelling, the structure evolved in time; the circular spots gradually took on an elipsoidal shape and then transformed into stripes, or, if stripes appeared initially, their

Pattern Formation during Polymerization of Acrylamide

J. Phys. Chem. B, Vol. 103, No. 1, 1999 **37**
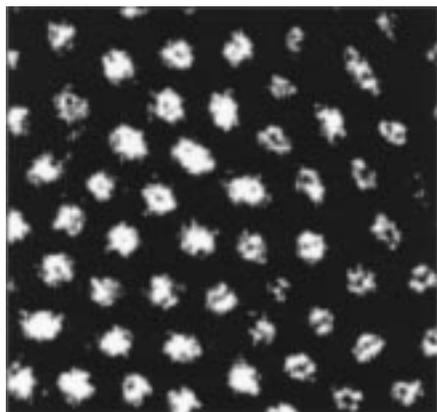
Figure 1. Spotted pattern in the AA/Bis/TEA/$S_2O_8^{2-}$/S^{2-} gel system. Composition of gel mixture: [AA] = 1.035 M; [Bis] = 4.85 × 10^{-3} M; [TEA] = 0.055 M; [$S_2O_8^{2-}$] = 0.112 M; [S^{2-}] = 3 × 10^{-2} M. Initial temperature: T_0 = 40 °C. Covering solution: water. The domain size is 27 mm × 25 mm. Average diameter of spots is about 1.5−2 mm.

Figure 2. Striped pattern in the AA/Bis/TEA/$S_2O_8^{2-}$/S^{2-} gel system. All conditions and concentrations, except [S^{2-}] = 5 × 10^{-2} M, as in Figure 1.

width increased with time. The structures can be seen more easily if methylene blue (Mblue) is present either in the gel or in the solution above the gel layer because the blue color gives better contrast. Other dyes such as chrysoidines, erioglaucine, or ferroin have a similar effect, offering the possibility of orange, green, or red patterns.

The presence of sulfide in the initial mixture is essential for the development of patterns in the gel. Without sulfide, no structure developed under any conditions we tried. The question remains whether sulfide can be replaced by other chemicals.

Figures 1 and 2 show spotted and striped patterns developed at the same gel matrix composition but different concentrations of sulfide. The surface of the structured gel is smooth initially but then becomes very uneven with tiny hills and valleys developing along the boundaries of the spots and stripes. In contrast, a layer of pure polyacrylamide gel, which is completely transparent, undergoes only a slight and uniform swelling, and its surface remains smooth indefinitely. If we allow the structured gel to dry out, which takes about a day, the thickness of the layer shrinks to about a tenth of a millimeter without cracking. This hard film retains the pattern, which can be regarded as a "fossil print" of the structure that existed in the swollen gel.

Factors Influencing Pattern Formation in the Polyacrylamide/Sulfide System. After preliminary experiments to assess the effect of various parameters on the evolution of patterns in
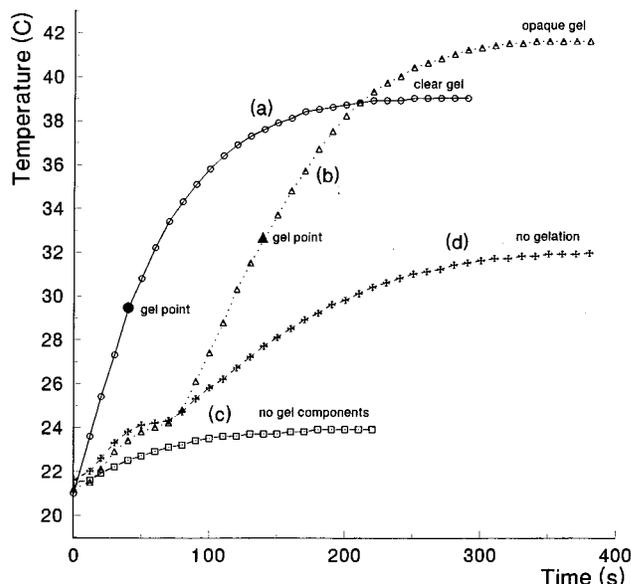

Figure 3. Temperature vs time in mixtures (total volume = 12.6 cm^3) at standard composition with mechanical stirring (600 rpm). Initial temperature T_0 = 21 °C. Gel point is defined as the time at which the stirring bar stops rotating in the mixture. Curves are as follows: (a) AA/Bis/TEA/$S_2O_8^{2-}$; (b) AA/Bis/TEA/$S_2O_8^{2-}$/S^{2-}; (c) $S_2O_8^{2-}$/S^{2-}; (d) composition as in system b, but with [AA] = half of standard value.

the gel, we selected the following concentrations as standard: [AA] = 1.035 M, [Bis] = 4.85 × 10^{-3} M, [TEA] = 5.5 × 10^{-2} M, [S^{2-}] = 3 × 10^{-2} M, and [$S_2O_8^{2-}$] = 0.112 M. At this composition, the time required for solidification of the mixture is about 5−6 min. We allowed the system to stand for about 10 min after the initiation of polymerization before covering the gel layer with water. When the gel components were mixed at room temperature (about 21 °C), a spot-type structure (see Figure 1) started to develop about 1 h after water was added to the gel. We describe below the effects of such factors as temperature, oxygen, thickness and surface area of the layer, light, stirring, and composition on the pattern formation using a gel of standard composition as a reference.

Effect of Temperature. The polymerization of acrylamide is highly exothermic. The temperature was monitored as a function of time using the standard concentrations in the following systems: (a) AA/Bis/TEA/$S_2O_8^{2-}$; (b) AA/Bis/TEA/$S_2O_8^{2-}$/S^{2-}; (c) $S_2O_8^{2-}$/S^{2-}; (d) 0.5 × AA/Bis/TEA/$S_2O_8^{2-}$/S^{2-}. The reactions were started by adding the initiator $S_2O_8^{2-}$ to a mixture of the other components. The reactions were run in a covered Dewar flask equipped with a magnetic stirrer to maintain quasi-adiabatic conditions. The results are shown in Figure 3.

During polymerization of pure acrylamide (Figure 3, curve a), the temperature in the solution increases linearly (~10 °C/min) until about 1 min, when the mixture becomes solid. This transition is referred to as the gel point. The polymerization continues after the gel point (the length of the polymer chains and the degree of cross-linking increase further), and the temperature approaches a final value of 39 °C about 4−5 min after the initiation of polymerization. In system b, the temperature first rises slowly, but after 90 s, it changes in a manner similar to system a. The gel point in system b occurs at a much longer time and at a somewhat higher temperature, with the constant final temperature of 42 °C being reached in 7−8 min (Figure 3, curve b). In the reaction between $S_2O_8^{2-}$ and S^{2-}, the increase in temperature is less than 3 °C (Figure 3, curve c). The sum of the final temperature increases in systems a and c is equal to that measured in system b, which suggests that the

overall heat production in system b is the sum of the heat of polymerization and the heat of reaction between $S_2O_8^{2-}$ and S^{2-}, since the heat capacities of all the systems are very close to each other. The heat production in system b was unchanged when [Bis] was either increased or decreased by a factor of 2, but halving the amount of AA present in the mixture reduced the heat measured by 50% (Figure 3, curve d), and no gelation occurred at this composition.

From the data shown in Figure 3, we conclude that the high exothermicity of polymerization is primarily associated with the formation of polyacrylamide chains, and the contribution of cross-linking processes to the total heat production is not significant. The observations in system b also suggest that, in experiments on pattern formation in polyacrylamide gel layers, the time that polymerization is allowed to proceed before spreading water on the top surface may be a factor that needs to be controlled in order to obtain reproducible results.

The initial temperature in the mixture of gel components (denoted T_0) influences significantly both the time (denoted t) required for the appearance of a pattern after layering water on the gel and the evolution of the pattern. As T_0 is increased from 21 to 50 °C, t decreases from 100 to 15 min. At $T_0 \leq 5$ °C or $T_0 \geq 50$ °C, no pattern formation was observed. We found that $T_0 = 40$ °C gave the most clearly defined patterns. At $T_0 = 40$ °C, t is about $15-20$ min and the structure evolves rapidly, occupying almost the entire surface area of the Petri dish.

We also tested whether structure formation occurs under quasi-isothermal conditions by carrying out the gelation process in a thermostated Petri dish. Although we were unable to maintain perfectly isothermal conditions, because of the long lasting highly exothermic nature of the reaction, we did observe that when all processes took place at nearly constant temperature pattern formation was suppressed.

System a is colorless in each phase of the process, while systems b and c undergo similar changes in color and turbidity. After the colorless components are mixed, systems b and c first become yellow then give rise to a yellowish-white precipitate, followed by formation of a white precipitate. The gel formed in system b shows a milky opacity.

Effect of Oxygen. The effect of oxygen was assessed by comparing experiments run in air and in an argon atmosphere. These experiments suggest that patterns develop much more easily in the presence of oxygen than when the polymerization is carried out in an argon atmosphere.

Effect of Stirring. It is possible to stir either the mixture of gel components prior to polymerization or the water layer above the gel after solidification. When the gel components were constantly stirred by tilting and rotating the Petri dish until the gel point was reached, no structures were formed in the resulting gel layer. When the water layer above a previously prepared gel was stirred, the structures developed in the same fashion as in the unstirred system.

Effects of Other Factors. The gel layer was illuminated from below the Petri dish because the pattern is more easily observed in transmitted light than in reflected light. The intensity of illumination did not appear to affect the pattern evolution.

The thickness (0.5, 1.0, and 2.0 mm) and the surface area (23.8, 56.7, and 113 cm^2) of the gel and the volume of water (6, 12, and 20 cm^3) above the gel did not have any noticeable effect on the pattern formation. We prepared gels in both plastic and glass Petri dishes. The only observable difference was that in several cases the patterns found in the glass dishes were sharper and more regular.

TABLE 1: Elastic Modulus (in kPa) of Pure Polyacrylamide Gel (G_p), Prepared at $T_0 = 21$ °C and of Gel Prepared with 3×10^{-2} M Sulfide at $T_0 = 21$ °C (G_{21}) and at $T_0 = 40$ °C (G_{40}) as a Function of the Applied Force (Weight)[a]

weight (g)	G_p	G_{21}	G_{40}
1	5.76	1.77	0.61
2	5.55	1.79	0.68
3	5.75	2.05	0.76
4	6.25	2.25	0.91

[a] Standard deviation in the value of G measured at a given weight is about $5-8\%$.

Effect of Gel Preparation Technique. We have noted that if the gel layer is prepared by spreading the appropriate mixture of gel components in an open Petri dish, patterns form and are observable after the gel is covered with water. We also carried out the polymerization of the same mixture under closed conditions—a 2 mm thick gel layer was made between two glass plates. The gel in this case showed no sign of being structured after one of the plates was removed and the free gel surface was exposed to water. This gel swelled to the same extent as the structured gel in the Petri dish, but the swelling was uniform.

Effect of Composition. We looked at the results of varying the concentration of each component in the system. If $[S_2O_8^{2-}]$ and $[S^{2-}]$ are kept at the standard levels, [AA], [Bis], and [TEA] in the gel matrix must be maintained in a narrow range between 0.75 and 1.5 times the concentrations present in our standard composition; otherwise, the result is not a solid gel but a jelly-like layer or a liquid. In the jelly, wide convective stripes may appear without speading water on the top (adding water usually destroys the jelly layer). The ratio of $[S^{2-}]$ to $[S_2O_8^{2-}]$ affects the time of gelation as described above. When a gel forms, the character of the pattern is determined by $[S^{2-}]$. For example, using the standard composition for all other components but varying $[S^{2-}]$, at $[S^{2-}] = 1 \times 10^{-2}$ M the gel is almost transparent, the structure is barely visible, whereas at $[S^{2-}] = 3 \times 10^{-2}$ M and $[S^{2-}] = 5 \times 10^{-2}$ M, the gel is opaque in the former case and well-developed spots and stripes (see Figures 1 and 2) appear in the gel layer in the latter case.

Elastic Moduli of Pure Polyacrylamide and the Sulfur-Containing Gel. The elastic modulus (G) is an indicator of the degree of polymerization and cross-linking in the gel. The higher the value of G, which is inversely proportional to the swelling capacity of the gel, the harder is the gel.

To obtain information about the physical properties of different polyacrylamide gels, 2 mm thick layers of standard composition were prepared at an initial temperature of $T_0 = 21$ °C in the absence of sulfide (G_P) and at temperatures of $T_0 = 21$ and 40 °C with sulfide ions (G_{21} and G_{40}) added to the mixture to be polymerized, and the elastic moduli of these layers were measured. The results are summarized in Table 1.

Comparing the average values of the elastic moduli shown in Table 1, we find G_p, the elastic modulus of the sulfide-free gel, to be about 3 times larger than G_{21}. Increasing the temperature to 40 °C lowers G by about a factor of 2.7. These results clearly demonstrate that the physical properties of the gel depend very sensitively on the way it is prepared. The sulfur-containing gel is much softer than the pure gel. A higher initial temperature also results in a softer gel.

Discussion

The pattern formation observed in sulfur-containing polyacrylamide gel layers results from a combination of chemical and physical processes that occur during polymerization. The predominant driving force of the pattern formation is the heat

Pattern Formation during Polymerization of Acrylamide

*J. Phys. Chem. B, Vol. 103, No. 1, 1999* **39**

of polymerization. The high exothermicity, however, is only a necessary but not a sufficient condition for appearance of the structure. Additional chemical processes associated with the sulfide in the mixture to be polymerized also contribute to establishing conditions under which patterns can develop in the two-dimensional gel system.

Chemistry behind the Pattern Formation. The polymerization of the monomer AA in the presence of Bis is initiated by free monomer radicals, which are produced by the base TEA-catalyzed formation of oxygen free radicals from peroxodisulfate. Molecular oxygen and other free radical scavengers are known to inhibit or even prevent polymerization. Here, we discuss the effect of sulfide ions on the polymerization and on the properties of the gel.

The added sulfide reacts in a relatively fast reaction with the initiator peroxodisulfate. Under our experimental conditions, $[S_2O_8^{2-}] \gg [S^{2-}]$. The overall reaction between these species is therefore

$$S^{2-} + S_2O_8^{2-} \rightarrow S + 2SO_4^{2-} \quad (1)$$
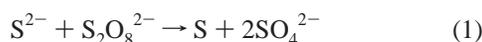
Polysulfides (S_x^{2-}) are formed as intermediates in reaction 2,

$$S^{2-} + (x-1)S \rightarrow S_x^{2-} \quad (2)$$
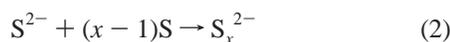
Reactions 1 and 2 represent the underlying chemistry taking place in system c of Figure 3. Curve c in Figure 3 suggests that the overall process terminates within about 2 min. We also followed the oxidation of S^{2-} by $S_2O_8^{2-}$ both in aqueous solution and in a gel mixture, using Pb^{2+} ions to monitor the sulfide concentration qualitatively in time. The product PbS is black, while all other products of the reaction are white. We added $Pb(NO_3)_2$ to 1 cm^3 aliquots of the reaction mixture at 30 s intervals. An intense black color appeared only in the first 30 s, a less intense coloration was observed after 60 s, and the sample mixture remained white after 120 s, which is somewhat shorter than the time required for gelation using the standard concentrations. The colors of the end product S and the intermediate S_x^{2-} are white and deep yellow, respectively. The changes in color and turbidity during the polymerization of acrylamide in the presence of initial sulfide are the same as in reaction 1 both in character and in time scale. From the time of reaction 1 and from the gel point in system b (Figure 3, curve b), we conclude that reaction 1 is complete when the AA/Bis/TEA/$S_2O_8^{2-}$/sulfur species mixture is still in the liquid phase.

Reaction 1 between S^{2-} and $S_2O_8^{2-}$ takes place according to a radical mechanism, producing radical intermediates both from S^{2-} (e.g., HS$^\bullet$ and HSS$^\bullet$) and $S_2O_8^{2-}$ (e.g., $SO_4^{\bullet-}$, HO$^\bullet$). The high concentration of radicals initiates the polymerization of more AA molecules but also increases the probability of chain termination owing to the reactions of polymer radicals with each other and with the inorganic radicals. Therefore, in the presence of sulfide, more but shorter polymer coils are formed in the gel mixture in a given time interval compared to the sulfide-free case.

The physical properties of the gel mixture before and after gelation are largely determined by the average length of the polymer chains in the system. A shorter average length is associated with a longer time for the liquid to reach the gel state. The sulfide ions present in the initial mixture and the higher initial temperature lengthen the gelation time, resulting in a gel that is much softer (cf. G_{21} with G_p and G_{21} with G_{40} in Table 1), has a higher penetrability for water, and shows a significantly higher swelling capacity than sulfur-free polyacrylamide prepared under the same conditions.

Physics behind the Pattern Formation. The polymerization of acrylamide is strongly exothermic. In the systems in which pattern formation is observed, the temperature rises significantly above the initial value (T_0) during the transformation of monomer AA to polyacrylamide. At $T_0 = 5, 21, 40$, and 50 °C, the temperature rises to 25, 42, 55, and 63 °C, respectively. The contributions of other processes, such as the oxidation of S^{2-} by $S_2O_8^{2-}$ or cross-linking, to the total heat production are not significant.

The large difference in temperature between the reaction mixture and its environment results in Benard convection in the layer during the time that the mixture exists in a liquid state. The relatively small effect of the layer thickness during polymerization suggests that the driving force for the convection is surface tension rather than buoyancy, that is, that convection is more likely of the Marangoni−Bénard than of the Rayleigh−Bénard type.[12] Oxygen is consumed when it terminates the polymerization chain propagation. Consequently, the highest oxygen concentration is found in the downward flow and the lowest [O$_2$] in the upward flow. As a result, a nonuniform polymerization takes place in the solution layer, which retains the pattern of convection and becomes "fossilized" when the liquid turns to a solid gel. The pattern in the gel layer cannot be seen with the naked eye. After water is spread on the top of the gel, a rapid, nonuniform swelling occurs, which makes the fossilized pattern visible.

The initial temperature of the mixture (T_0) has a significant influence on the time needed for the appearance and the development of the structure after adding water. This finding can be interpreted by analyzing how T_0 affects the rate and the degree of polymerization as well as the temperature gradient in the system. At $T_0 \leq 5$ °C, no pattern occurs. At this low temperature, relatively long polymer chains are formed owing to the low concentration of radicals. The viscosity of the mixture increases rapidly, the time of gelation is short, and the gel formed is hard. The heat generated drives the temperature of the gel mixture toward the ambient value. All of these effects work against formation and detection of the convective pattern. At 5 °C < T_0 < 50 °C, all physical properties of the system change with temperature. With increasing T_0, the polymer chains become shorter, the time of gelation is longer, the gel is softer, its swelling capacity is higher, and the temperature difference between the polymerizing system and its environment increases with time. The optimal initial temperature, that is, the optimal physical parameters for structure formation, come together at $T_0 \approx 40$ °C. At $T_0 \geq 50$ °C, no gel is formed and therefore no gel structures can occur.

Conclusion

In a thin gel layer prepared by polymerization of acrylamide, Benard-type convective patterns can be observed after swelling if sulfide ions are also present in the initial mixture of monomer AA/Bis/TEA and initiator $S_2O_8^{2-}$. The strong exothermicity of the polymerization and the chemical processes associated with the sulfide ions are both essential in bringing about the pattern formation. The steep temperature gradient induces and maintains convective heat and mass currents in the liquid layer until the gel point is reached. The role of sulfide appears to be to ensure optimal physical properties of the system (e.g., time of gelation, viscosity, mechanical strength of the gel, elasticity, swelling, ...) for effective convection and stucture formation and detection in the liquid and gel states.

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