Phase Separation Coupled with Gelation in Polyethylene Glycol–Gelatin–Aqueous Buffer System

Takashi NEZU and Hiroshi MAEDA*,†
Department of Chemistry, Faculty of Science, Nagoya University, Nagoya 464-01
† Department of Chemistry, Faculty of Science, Kyushu University, Fukuoka 812
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Phase behavior of a pseudoternary system, gelatin–polyethylene glycol–acetate buffer, was examined. Phase diagram was constructed by determining the compositions of separated phases. One phase contained mostly gelatin but a trace amount of polyethylene glycol. When temperature was lower than the gelation temperature corresponding to the composition of the separated phase, diverse outlooks of the whole system were found. Observation of solutions under an optical microscope revealed microgels or network structure when gelatin was labeled with sirius red. Two different types of microgel formation were noted: nucleation-growth and spinodal decomposition. Size of the microgels formed by spinodal decomposition decreased with quench temperature.

Multicomponent polymer solutions often undergo phase separation either due to mutual incompatibility[1,2] or due to difference between their interactions with solvent ($\Delta\chi$-effect).[3] When one of separated phases gels, it is anticipated that phase separation process becomes involved and diverse outlooks are observed. Furthermore, if gelation takes place before the completion of the phase separation, we can expect to fix intermediate stages of phase separation by gelation. In the present study, we examine phase behavior of aqueous mixtures of gelatin and polyethylene glycol (PEG). Gelatin–water two component system undergoes gelation rather than phase separation when temperature is lowered in contrast to another popular aqueous two component system agarose–water where microgels are produced as a result of phase separation.[4] Microgels of gelatin has been prepared through simple coacervation induced by the addition of a poor solvent such as alcohol.[5] Later, microgels of gelatin was obtained in aqueous media by the addition of polyethylene glycol.[6] This type of phase separation will be examined in the present study.

Experimental

Gelatin (Sigma type B from bovine skin) was dialyzed against distilled water and freeze-dried subsequently. Weight-average molecular weights of polyethylene glycol (PEG) (Wako Pure Chemical Industries, Ltd.) and purified gelatin were $(2.6\pm0.1)\times10^4$ and $(9\pm1)\times10^4$, respectively as determined from light scattering. Both PEG and gelatin samples showed single peaks on GPC. Acetate buffer (pH 5.0; 5×10^{-2} M, 1 M= 1 mol dm^{-3}) was used as solvent unless otherwise stated.

Concentrations of gelatin (C_g) and PEG (C_{PEG}) of separated phases were determined with HPLC (TSK G4000PW gel). Gelatin concentration was determined from absorption at 260 nm where absorption of PEG was negligible (molar extinction of PEG was about 1/20 of that of gelatin). Refractive index increment consisted of contributions from both polymers and their additivity was confirmed from preliminary measurements. PEG concentration was then obtained by subtracting gelatin contribution from the total refractive index increment. Polymer concentrations were expressed in weight percent; grams of one polymer component in 100 g solution. For microscopic observation an Olympus model BHA microscope equipped with a photographing unit PM-10A was used. Stage was thermostatted within ±0.5 °C.

Result

Phase Separation and Gelation. Two examples of various states accompanying the phase separation are shown in Fig. 1(A) and (B) for polyethylene glycol concentration C_{PEG} of 1.5% and 2% respectively. Gelling process taking place in a gelatin-rich phase gives rise to diverse appearance of the whole system. A solid curve in Fig. 1(A) or (B) represents critical solution temperatures, a kind of binodal line. At temperatures higher than this line a single solution phase is stable, while two phases are expected to appear at temperatures below this line. Actually, two solution phases (⊖) appeared at temperatures not far from the line. Sometimes one of the two phases was dispersed in another phase and the whole system looked like a single turbid solution (□). When temperatures were low, sol–gel (◒) or rarely gel–gel (▲) type phase separation took place. When temperature was lowered further, gelation occurred rapidly before separated microdomains merged into a macroscopic phase. In this case, single turbid gel resulted (●). Under certain conditions, flocculates accumulated at the interface between upper sol and lower gel and hence exhibited three phase behavior (sol–flocculates–gel). All these results were reproducible and irrespective of the direction to reach the final temperature either from higher or lower temperature side. Essentially similar results were obtained at other PEG concentrations.

These results are schematically summarized in Fig. 2. A solid curve represents critical solution temperatures (binodal). At temperatures just below the line, liquid–liquid phase separation usually takes place (region II). Region IV, which is below the gelation curve of gelatin, is always associated with gelation. Complex behaviors appear in region III.

In Fig. 3, critical solution temperatures (c—g) or gelation temperatures (a,b) are shown for different PEG concentrations. At C_{PEG}=0.55%, gelation instead of

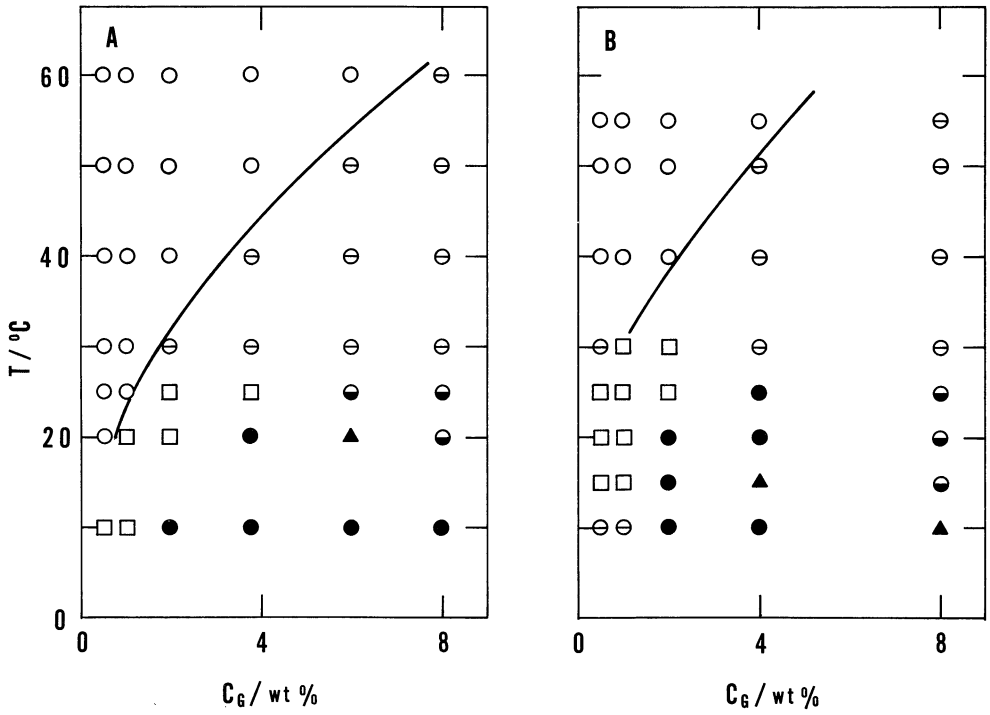

Fig. 1. Phase diagram of gelatin–PEG–aqueous buffer. (A) C_{PEG}=1.5%, (B) C_{PEG}=2.0%. Various symbols representing various apparently different states: (○) single solution, (⊖) two solutions, (◒) solution–gel, (●) gel(turbid), (▲) gel–gel, and (□) emulsion (turbid).

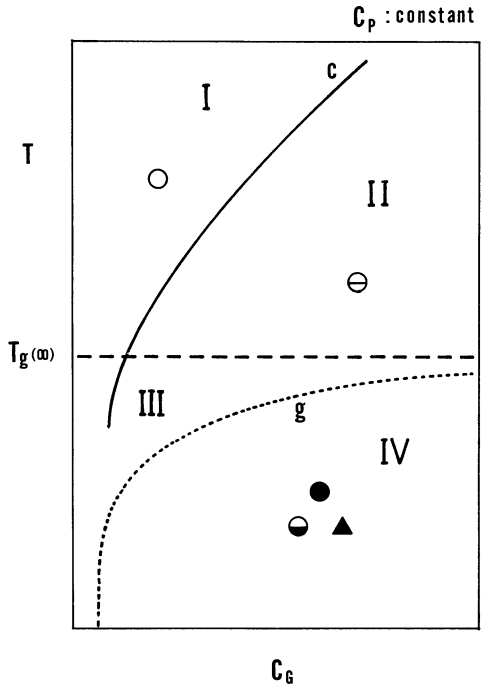

Fig. 2. Schematic presentation of four regions encountered in gelatin–PEG–aqueous buffer. A solid curve c represents critical solution temperatures. Dotted curve g represents gelation temperatures T_g of gelatin solution. $T_g(\infty)$ is the gelation temperature at infinite gelatin concentration.

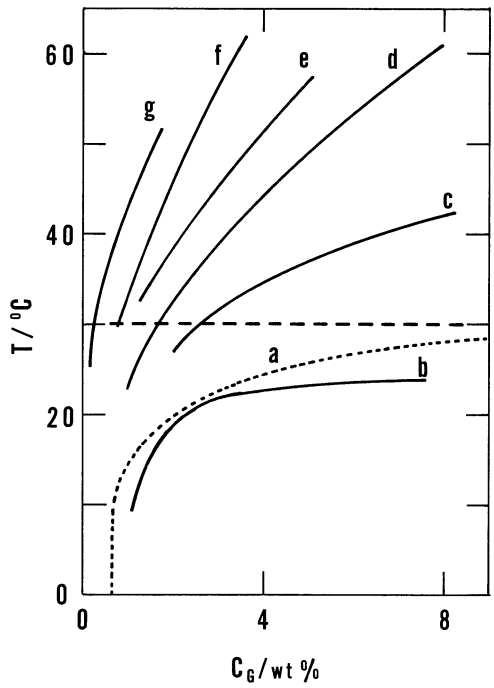

Fig. 3. Effect of PEG concentration on phase behavior. Solid curves represent critical solution temperatures (c—g) or gelation temperatures (b). Dotted curve (a) and dashed curve represent gelation temperatures of gelatin solution and $T_g(\infty)$, respectively. C_{PEG}(%): (a) 0, (b) 0.55, (c) 1.0, (d) 1.5, (e) 2.0, (f) 3.0, and (g) 4.0.

phase separation occurred. Gelation curve (b) at C_{PEG}=0.55% lies at lower temperatures than that of gelatin (a). This indicates that PEG molecules are excluded from gelatin gel and remain in solution phase; 'gel point depression' is expepcted to occur.

Effect of pH was examined at an ionic strength of 0.05 M for pH 5.0, 6.0, and 7.0. Little difference was observed with respect to phase behavior among these pHs. Effect of ionic strength was examined at a pH of 5.1 (10 mM acetate buffer). The solution was turbid in the absence of 0.2 M NaCl but it was clear in the presence of 0.2 M NaCl.

Compositions of Separated Phases. Concentrations of gelatin and PEG in two separated phases were determined with HPLC as described in Experimental section. Results obtained at 40 °C and 25 °C are shown in Fig. 4(A) and (B), respectively. In constructing phase diagrams shown in Fig. 4, we approximated aqueous acetate buffer as a single component. We found that two separated phases showed identical pH which coincided with that of original solution before phase separation. Preferential binding of one of two buffer components to either of the polymer components was thus ruled out. We assumed that buffer concentrations did not differ much on phase separation. As shown in Fig. 4(B), two solutions characterized with different initial compositions give approximately identical compositions of two separated phases, in so far as initial compositions lie on a common tie line. This supports that phase equilibrium was attained in the present study. Binodal lines are close to both axes indicating that separated phases were rich in either of the two components. In particular, PEG concentrations in gel phase were zero within experimental error in all cases examined. This suggests that repulsive interaction between the two polymer components plays an important role to induce the phase separation. Compositions of flocculates in two apparently three-phase systems were examined at 25 °C: C_g=4.0%–C_{PEG}=3.0% and C_g=3.0%–C_{PEG}=2.0%. The compositions were found to be identical to those of gel phase. This has provided a good example indicating that in the present study we dealt with two phase system in thermodynamic sence in spite of three-phase appearance.

All these diverse phase behaviors were expected to originate from inhibited growth or merge of microscopic domains (enriched with gelatin) formed at the initial stage of the phase separation. If gelation had not occurred, these microdomains could have grown and could have merged into three dimensional network (modulated structure in spinodal decomposition) which eventually could have changed into another bulk liquid phase. It is expepcted, therefore, that translational movement of gelatin molecules becomes inhibited by gelation and that the whole process of phase separation is kinetically frozen. If this is to be the case, intermediate stages of phase separation can be visuallized, for example, by staining gelatin with sirius red as was done in the present study.

Observation of Phase Separation under a Microscope. We observed under a microscope solutions containing labeled gelatin. With dilute solutions, a large number of red spherical microgels were observed after phase separation. However, time intervals prior to their emergence differed greatly depending on the compositions of initial solutions. For a solution (C_g=1.25% and C_{PEG}=1.0%) microdroplets first appeared about 8 h after it was brought to the final temperature (25 °C) and sizes of these microbeads did not change appreciably for 24 h. These beads were considered to be produced through nucleation-growth process (NG regime). For another solution (C_g=2.0% and C_{PEG}=1.3%) under the same conditions, on the other hand, microgels appeared immediately after quenching. Phase separation in this case is considered to occur through spinodal decomposition (SD regime). Microdroplets appeared immediately after quenching were not gel-like but of liquid nature. They grew up and merged

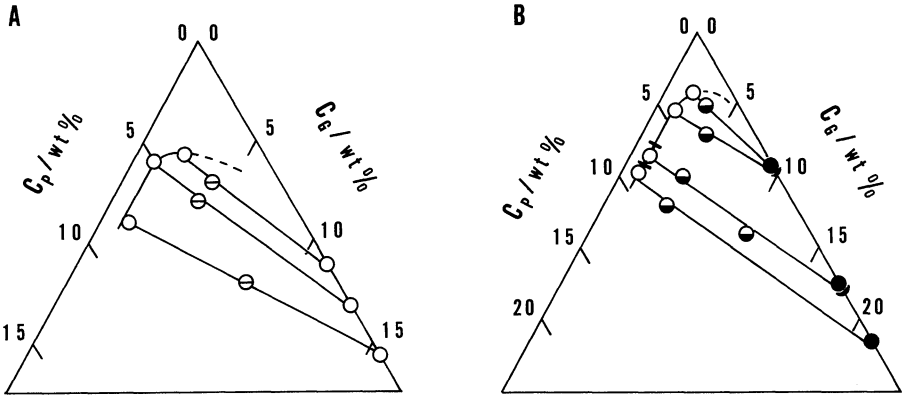

Fig. 4. Phase diagram of gelatin–PEG–aqueous buffer (A) 40 °C and (B) 25 °C. Symbols are used in the same sense as in Fig. 1 and correspond to the final state.

with each other and their size became bigger with time but reached eventually a constant level at 50—60 min after quenching, probably as a result of loss of fluidity due to advancement of gelation. Their average diameters (d) measured at 55 min. (or later) after quenching were 1.70, 2.01, and 2.76 μm at quench temperatures of 11.7, 16.2, and 20.5 °C, respectively. Calibration of dimension was carried out with polystyrene latex (d=5.854 μm; SD=0.21). Distributions of d about the average value were rather narrow at three temperatures examined. Spinodal temperature (T_S) of the system was determined to be 23.8±1.0 °C from the maximum light scattering intensity. In Fig. 5, values of $\log d$ are plotted against $\log \Delta T$, where $\Delta T(=T_s-T)$ represents a quench depth. The result can be expressed as $d \propto (\Delta T)^{-n}$ with n=0.4±0.1. That is, we get smaller beads as quench depth increases.

When solutions were more concentrated and quenched to low temperatures, we observed network structure (as shown in Fig. 6) rather than dispersed microbeads. This

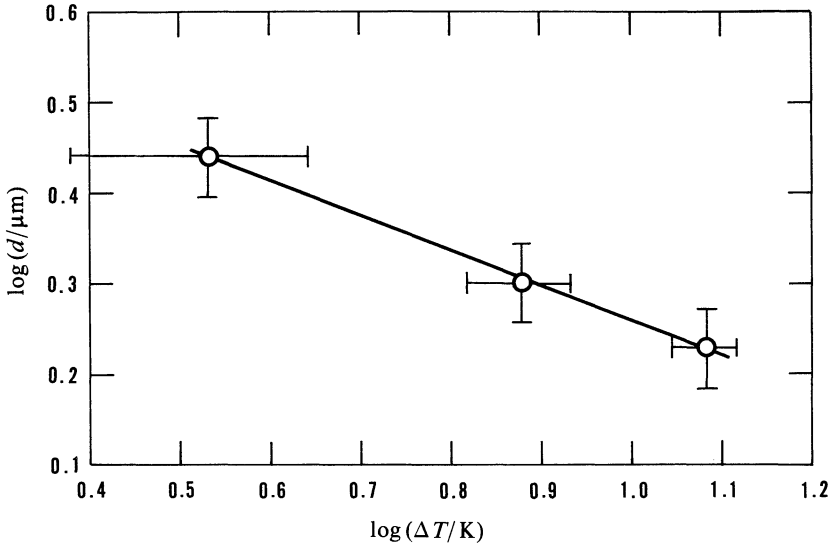

Fig. 5. Dependence of particle diameters d on quench depth ΔT. C_g= 2.0%, C_{PEG}=1.3%, T_s=296.9 K (23.8 °C). Three points correspond to quench temperatures of 11.7, 16.2, and 20.5 °C. Slope of the straight line is −0.4±0.1.

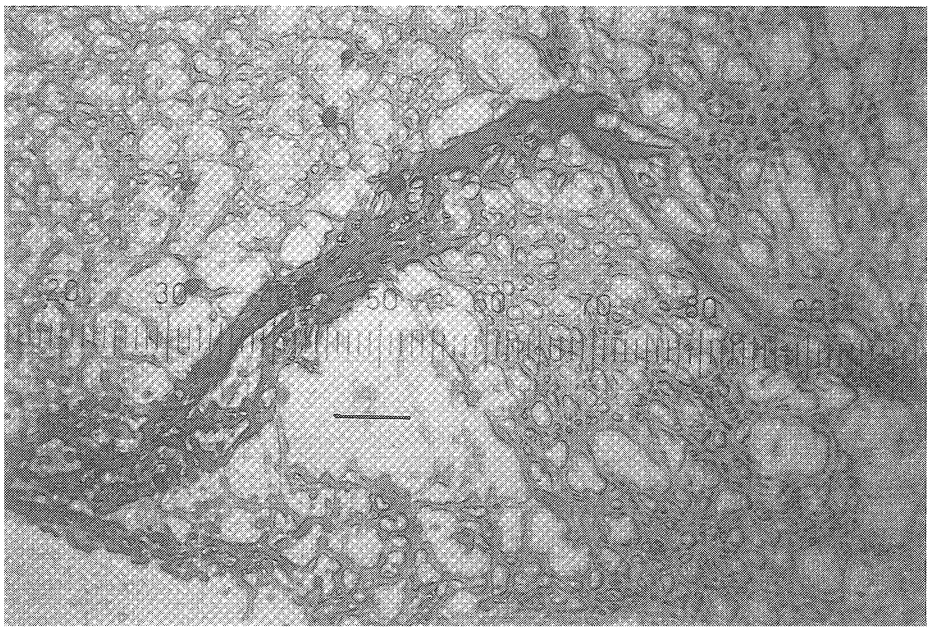

Fig. 6. Network structure consisting of gel (observed at 25 °C) formed at intermediate stage of phase separation at −10 °C. Solution (C_g=2.5% and C_{PEG}= 2.0%) was incubated at −10 °C for 3 h. A horizontal bar corresponds to 100 μm.

picture (Fig. 6) was obtained when a solution (C_g=2.5% and C_{PEG}=2.0%) at 60 °C was quenched at −10 °C for 3 h and then brought to 25 °C for observation. The network structure was stable at 25 °C for more than 30 min indicating it was gel. When the same solution was quenched at −10 °C for 30 min, thin branches melted and only thick branches remained during observation. When quenching duration was further reduced to 10 min, no network structure was seen any more when brought to 25 °C for observation. The dependence of strength of network structure on quenching duration suggests that gelation became predominant over phase separation with decreasing temperature, although both processes became slow at low temperatures. Generally, we can expect this situation from the following consideration. Aggregation of gelatin chains occurs prior to gelation. These aggregates make the phase separation process slower and hence enhance the aggregation further. It is reasonable, therefore, that gelation predominates over phase separation due to this autocatalytic effect.

In Fig. 6, both thin and thick branches consist of gelatin gel. Gelatin concentration in these gels is consistent with phase equilibrium at 25 °C rather than at −10 °C where they were formed. This is because gel composition changed to the equilibrium value at 25 °C during observation primarily through solvent movement. However, observed structural pattern itself reflects the intermediate stages of phase separation at the quench temperature of −10 °C.

Modulated structure has been observed under a microscope for other multicomponent polymer systems.[9-11]

Discussion

In the present study, microgels or network structures were observed in SD regime. It is pertinent to examine whether or not these observations are consistent with, at least qualitatively, the prediction of general theory of SD.

According to Cahn's theory,[7] concentration fluctuation taking place at initial stages of SD is characterized with a particular wavelength λ_m, which is given as follows.[8]

$$\lambda_m = 2\pi\, l\, [3\Delta T/T_s]^{-1/2} \quad (1)$$

Here l is related to some characteristic dimension of poymer coils. It is to be noted that Eq. 1 has been derived for two component systems,[7,8] either polymer–polymer or polymer–solvent. Dependence of λ_m on ΔT originates from a term related to the mixing free energy, which is a function of single concentration variable in the theory.[7,8] On the other hand, mixing free energy depends on two concentration variables in the present pseudoternary system. However, essential step of the phase separation undoubtedly involves translational movement of two kinds of polymers toward opposite directions. Consequently, phase separation in the present study is expected to be approximated with that occurs in a polymer–polymer two component system. If microgels are formed through spinodal decomposition, then their size d should be closely related to the characteristic wavelength λ_m. According to Eq. 1, $\log \lambda_m$ decreases with $\log \Delta T$ with a slope of −0.5. Experimentally, a slope of about −0.4 was observed (Fig. 5). We are not in a position, however, to compare the experimental and the theoretical values of the slope, since there are many uncertain factors to be clarified in both theoretical and experimental aspects.

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