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Phase separation in binary polymer solution: Gelatin/Poly (ethylene glycol) system

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ABSTRACT

We systematically investigated the phase behavior of gelatin and poly(ethylene glycol) (PEG) aqueous solution. With a decrease in temperature, the gelatin/PEG solution became opaque and underwent phase separation. The phase separation point (T_p) was increased with an increase in concentration of gelatin concentration (C_g). However, when C_g was lower than a critical value (C^*_g), T_p was slightly increased with decreasing C_g. In addition, the phase separation mechanism was changed from spinodal decomposition type to nucleation growth type at around C^*_g. The increases in PEG concentration (C_{peg}) and in the molecular weight of PEG (M_{peg}) raised the T_p curve. A further decrease in temperature resulted in a sol–gel transition for gelatin. In the case of a gelatin solution without PEG, the melting temperature of the gelatin gel (T_m) increased with an increase in C_g and approached a saturation point. The addition of PEG raised the T_m curve closer to the saturation point, implying that T_m for the sample with a larger C_{peg} reached the saturation point at a lower C_g. Furthermore, melting enthalpy for the gelatin gel was increased with increases in C_{peg} and M_{peg}. These results indicate that the addition of PEG increases the strength of hydrogen bonding between gelatin chains. The appearance of minimum in the T_p curve might be originated from the interplay between self-aggregation via intra-chain concentration at a lower C_g and phase separation via inter-chain association at a higher C_g.

1. Introduction

Phase separation of polymer gels has been established with a great variety of de-solvating agents. This kind of phase separation, named "*coacervation*" in 1929 by Bungenberg de Jong and Kruyt [1], has attracted a great deal of attention in recent decades owing to its application to microencapsulation, drug delivery, and protein purification [2–4]. When de-solvating agents such as methanol and ethanol are mixed with water, they become poor solvents for polymer gels such as gelatin [5–11] and poly(*N*-isopropylacrylamide) (PNIPAM) [12–15]. In addition, it is reported that gelatin or PNIPAM in a mixed solvent of methanol/water has upper or lower critical solution temperatures (UCST or LCST), respectively [7,11–14]. For PNIPAM in a methanol/water solution, the phase diagram showing LCST behavior is explained by the competitive hydrogen bonding of water and methanol with the polymer chain [16–19].

Phase separation of polymer gels can also be triggered by the addition of different polymers. When gelatin is mixed with a poly(ethylene glycol) (PEG) solution that has both a UCST and an LCST [20,21], phase separation occurs with a decrease in temperature [22–25]. The phase separation is observed at a temperature above and also below a sol–gel transition for gelatin. Therefore, temperature history strongly affects the phase behavior. In addition, depletion-induced phase separation above and below the gelation temperature is reported for other gelatin in mixed solvents, such as gelatin/dextran and gelatin/LBG (locust bean gum) systems [26–28]. Although the mixture of gelatin/PEG in acetate buffer has been shown to have a phase diagram like UCST solutions [25], the precise phase behavior of a gelatin/PEG/water system and the phase separation mechanism are still unclear.

In this study, we systematically investigated the phase behavior of a gelatin/PEG aqueous solution. When the temperature was gradually decreased, the homogeneous phase underwent phase separation and a sol–gel transition of gelatin was observed. We discuss the effects of PEG addition on phase separation temperature and the melting temperature of the gelatin gel.

2. Experimental section

2.1. Materials

Alkali-treated (type-B) gelatin was obtained from Merck (No. 1040781000; Darmstadt, Germany). The average molecular weight (M_g) determined by gel permeation chromatography was 69,100 and the polydispersity index was 3.1. The isoelectric point of the sample was 4.9. Poly(ethylene glycol) (PEG) was obtained from Wako Pure Chemical Industries (Osaka, Japan). The average molecular weights of

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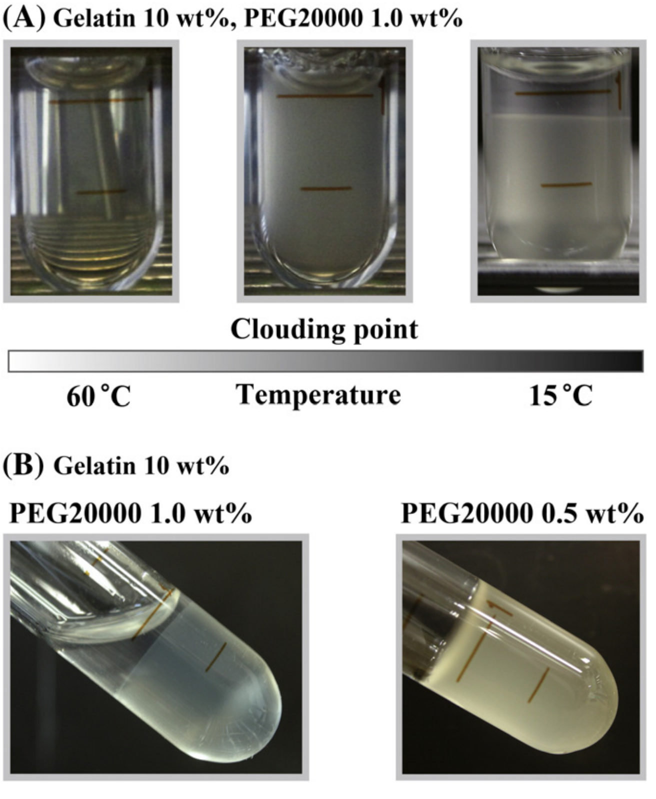

Fig. 1. (color online) (A) Phase behavior of gelatin/PEG aqueous solution at different temperatures and (B) phase-separated samples below gelation temperature.

PEG (M_{peg}) were 6000 and 20,000. These materials were used without further purification. FITC (fluorescein isothiocyanate isomer; Sigma-Aldrich, St. Louis, MO) tagged gelatin was prepared as reported previously [29], and added to gelatin/PEG samples 0.5 wt% of the gelatin for fluorescent observation. To prepare gelatin/PEG aqueous solutions, PEG was first dissolved in distilled water and, subsequently, gelatin was added at 60 °C, thus forming a homogeneous phase. The concentrations of gelatin and PEG were varied from 5.0 to 20 wt% and from 0 to 2.5 wt%, respectively.

2.2. Methods

To observe the phase separation, the temperature of the gelatin/PEG aqueous solution was increased to 60 °C and then gradually decreased to 15 °C at a rate of 5 ± 0.2 °C/h using a temperature-controlled bath. The phase-separation point (T_p) is defined as the point at which the sample is observed to become opaque. T_p was confirmed using 90° light scattering, and a temperature control device was used to fix the temperature of each sample for ~30 min before measurement. In addition, phase behavior was observed using a phase-contrast and fluorescence microscope (Carl Zeiss; Jena, Germany) with a temperature-control stage (Tokai Hit; Sizuoka, Japan). An aliquot of the sample was placed on a glass slide for observing the homogeneous phase at 60 °C and subsequently below T_p. The gelatin-rich phase demonstrated a sol–gel transition below 30 °C [30–32]. The melting temperature of the gelatin gel (T_m) was monitored by the falling-ball method using a ~16 mg Teflon ball with a 2.4 mm diameter. To avoid the effects of temperature history on the measurement of T_m, the samples were maintained at 15 °C for

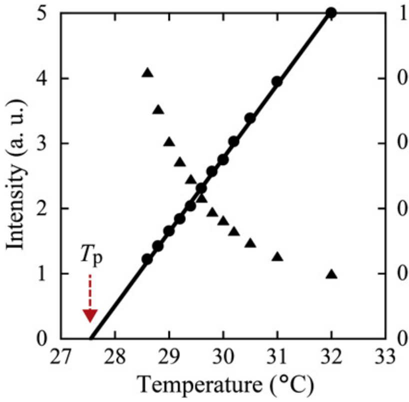

Fig. 2. (color online) Temperature dependence of light scattering intensity in gelatin 8.0 wt% and PEG20000 1.0 wt% solution. The scattering intensity (triangle), normalized to the value at 32 °C, is diverged at the phase separation point T_p, where the inverse of the intensity (circle) intersects with the x-axis.

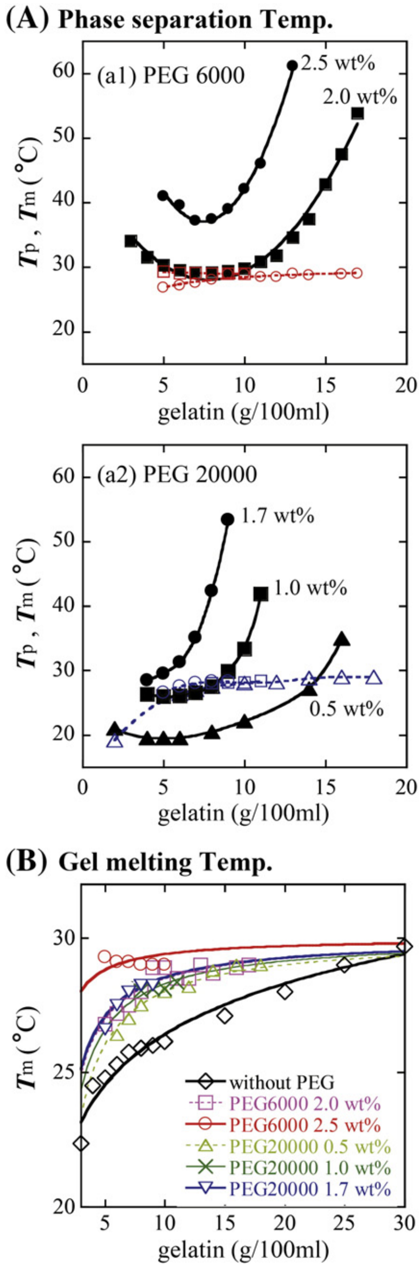

Fig. 3. (color online) Dependence of phase transition temperature (T_p, solid symbols) and melting temperature of gelatin-rich gel phase (T_m, open symbols) on concentration of gelatin in gelatin/PEG solution under fixed concentration of PEG6000 or PEG20000.

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