A Continuous Dilution Method for Phase Diagram Determination

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A relatively simple continuous dilution method with on-line spectrophotometric measurement has been used to determine the phase transition boundaries of aqueous two-phase polymer systems. The observed range of compositions over which the phase transition occurs is dominated by the polydispersity of the polymers, but also is a function of the dilution path followed. The binodal curve obtained by the continuous dilution method agrees well with the results obtained by the traditional method of phase equilibration, separation and analysis of liquid chromatography, which is slower and requires more expensive equipment.

Introduction

In aqueous two-phase polymer systems, the distribution of the polymers between the phases is governed to a large extent by their molecular weight. For economic reasons, the polymers used for biological separations in aqueous two-phase systems are usually polydisperse. Koningsveld and Staverman (1968) and later Kang and Sandler (1988) showed that the phase behavior of aqueous two-phase systems is affected by the degree of the polydispersity of the polymers. One effect of polydispersity in the phase-forming polymers in aqueous two-phase systems is that the transition from a two-phase dispersion to a one-phase solution is not sharp (Albertsson, 1986).

The binodal curve for aqueous two-phase polymer systems can be constructed by the titration method (Albertsson, 1986). In this method, a turbid two-phase mixture with a known concentration of the two polymers is quantitatively diluted with the solvent (water) until a homogeneous solution is obtained. The final compositions of the two polymers calculated correspond to one point on the binodal curve. After obtaining the first point, a concentrated solution of one of the polymers is quantitatively added to the solution to again obtain a turbid dispersion, the new concentrations of the two phase-forming polymers are determined, and dilution with solvent (water) is repeated to obtain a second point on the binodal curve. This procedure is continued until a sufficient number of points for the construction of the binodal curve are obtained. This method is useful when the turbid-clear transition is relatively sharp, as happens when the polymers are monodisperse. When the polymers are polydisperse, however, the turbid-to-clear transition is gradual and difficult to determine precisely.

Here, using a relatively simple spectrophotometric measurement of the change of turbidity due to the transition from two phases to one phase, the shape of the binodal curve for the aqueous system of PEG 8000 and Dextran 500 was obtained using a continuous flow dilution method. The results of these experiments are compared with the binodal curve obtained from direct two-phase equilibration and analysis by liquid chromatography, and with model predictions.

Experimental Method

Dextran (with average molecular weight of 487,000) and PEG (with average molecular weight of 8000) were obtained from the Sigma Chemical Co. The binodal curve for the two-phase system of Dextran 500 and PEG 8000 was determined using a continuous dilution technique suggested by Larsson and Mattiasson (1988, 1990) based on spectrophotometric measurement of the turbidity. In all, 20 different feed compositions of the two polymers were studied. The samples were made with concentrations varying from 11% PEG 8000 and 1% Dextran to 1.5% PEG 8000 and 16.0% Dextran. After each sample was prepared, it was separately introduced into the experimental equipment of Figure 1, which consists of recirculation and dilution loops. The recirculation loop includes a beaker containing the sample, a variable speed pump,

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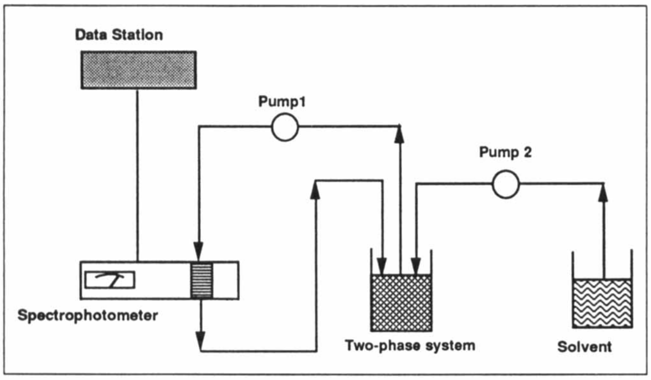

Figure 1. Continuous dilution experiment.

and the flow cell of a computer-interfaced Bausch and Lomb Spectronic 20 spectrophotometer. The dilution portion of the equipment consisted of a 200-mL beaker of deionized water, another variable speed pump, and 0.14-mm-dia. connecting tubing. The spectrophotometer was adjusted to accommodate the full range of absorbances at 600-nm wavelength. The results are unaffected by the calibration since, as will be explained shortly, only the difference in time between the beginning and end of the turbidity change is of interest, not the absolute values of the transmittance at any time.

A measurement was begun by introducing a 25-g sample of the prepared feed into the sample beaker and stirring continuously. The recirculation pump was then set at a flow rate of 1.3 mL/s and the mixture circulated through the spectrophotometer until a sufficient number of points on the upper, straight line portion of the curve were obtained (Figure 2). The dilution pump was then started and kept at a constant flow rate throughout the run (dilution rates between 0.0374 and 0.116 g/s were used, with the higher flow rates being used for the more concentrated polymer solutions), and spectrophotometer readings were taken every 1 or 3 s depending on the dilution rate. The run was completed when a large change in the absorbance of the sample occurred, followed by a leveling off to a new plateau as seen in Figure 2. Separate measurements showed that the holdup time for the system was 3 s compared to a run time of more than 300 s, and a phase transition time for the polymer systems of from 100 to 250 s.

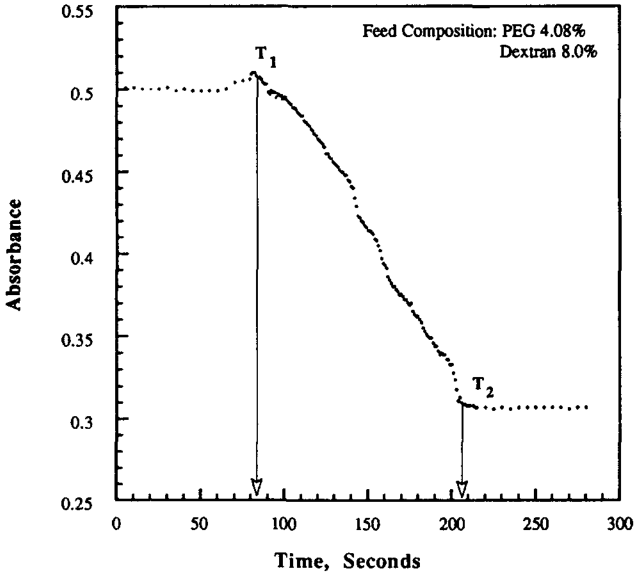

Figure 2. Absorbance of the two-phase system during a continuous dilution experiment.

For comparison, we also obtained phase boundaries by the more traditional analytical method. Five different aqueous two-phase systems were prepared from varying amounts of the 20% (w/w) PEG 8000 and 20% (w/w) dextran 500 stock solutions. These solutions were well mixed and then allowed to separate. After 24 hours, the two phases were carefully withdrawn, and the concentrations of the PEG 8000 and dextran 500 in the upper and bottom phases were determined using a Waters HPLC system (model 600 solvent delivery system and model 410 differential refractometer) with four gel permeation columns in series (two ultrahydrogel linear, one ultrahydrogel 250 and one ultrahydrogel 120). The mobile phase was a 0.1-N sodium nitrate solution flowing at rate of 1 mL/min.

Results and Discussion

The change in absorbance found in the continuous dilution measurements corresponds to the transition from a well mixed two-phase dispersion (high absorbance) to a one-phase (minimum absorbance) solution (Figure 2). The time T_1 indicates when enough solvent has been added to begin the transition from two phases to one. Additional dilution leads to the steady reduction of absorbance between times T_1 and T_2. At T_2 the original turbid two-phase dispersion has become a homogeneous one-phase solution. The concentrations at this point correspond to the true phase boundary. The difference between the concentrations at times T_1 and T_2 is, in part, a measure of the polydispersity of the phase forming polymers as will be discussed below. Multiple runs of the continuous dilution experiments show that T_1 and T_2 have errors of about 3%.

To relate the polymer concentrations of interest to the observed times T_1 and T_2, an unsteady-state mass balance was used to account for the holdup in the flow system. The mass balance for any component i is:

$$\frac{d}{dt}[C_i(t)V(t)] = Q_D C_{iD} + Q_R C_i(t - t_{\text{holdup}}) - Q_R C_i(t) \quad (1)$$

and the overall mass balance is:

$$\frac{dV}{dt} = Q_D \text{ or } V = V_o + Q_D t \quad (2)$$

where

$C_i(t)$ = concentration of component i in the two-phase system, g/cm^3
$C_{iD}(t)$ = concentration of component i in the diluting solvent, g/cm^3
$V(t)$ = volume of the sample, cm^3
t = time from when dilution is started, s
t_{holdup} = holdup time in the equipment, s
Q_D = flow rate of dilution liquid, cm^3/s
Q_R = recirculation flow rate, cm^3/s
V_o = initial volume of the two-phase system

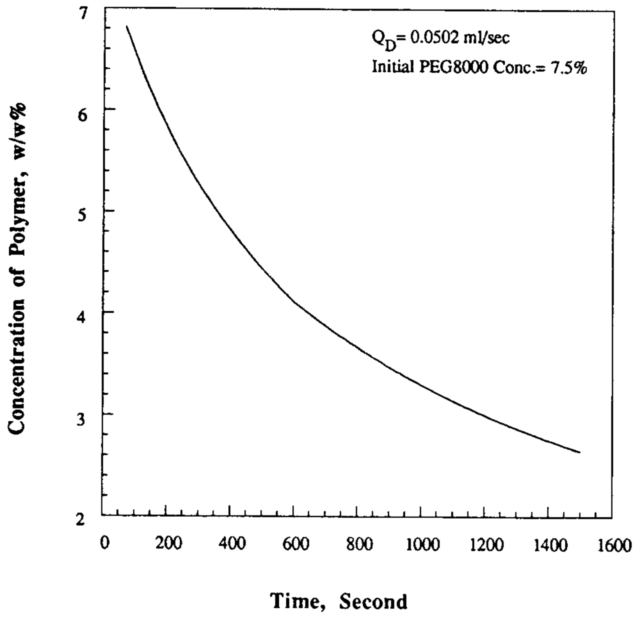

Figure 3. Calculated concentration history for a polymer in the continuous dilution experiment.

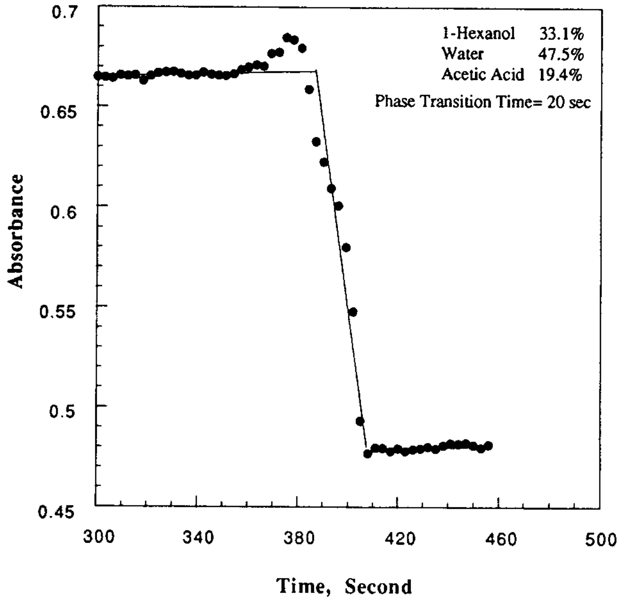

Figure 5. Change in absorbance with time for a mixture of 1-hexanol, water, and acetic acid.

Combining Eqs. 1 and 2 gives:

$$\frac{dC_i(t)}{dt}=\frac{Q_D C_{iD}}{V_o+Q_D t}-\frac{Q_D+Q_R}{V_o+Q_D t}C_i(t)+\frac{Q_R}{V_o+Q_D t}C_i(t-t_{\text{holdup}}) \tag{3}$$

This equation was solved numerically, and a sample result is presented in Figure 3. Using the values of T_1 and T_2 obtained from the spectrophotometric measurements and concentrations of each polymer as a function of time obtained from the mass balance, binodal curves were constructed in which each polymer has two concentrations, one at the beginning of the two-phase transition (T_1) and another at the end (T_2). The results of these calculations are plotted in Figure 4.

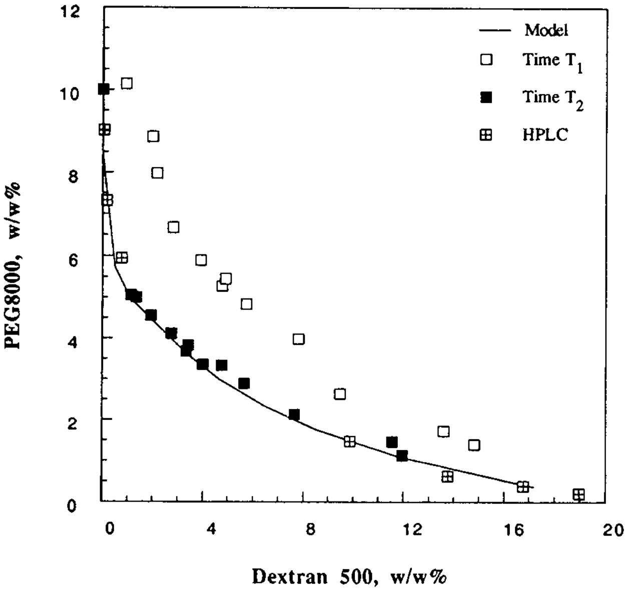

Figure 4. Experimental and predicted binodal curves for the aqueous PEG 8000-Dextran 500 system.

To compare the results obtained by the continuous dilution method for polydisperse systems with those for a mixture of two monodisperse materials in water, a model two-phase system consisting of water, 1-hexanol, and acetic acid was prepared and a similar continuous dilution experiment performed. However, since water and n-hexanol are the partially miscible components, acetic acid was added in this case to the initial alcohol-water mixture. The results of the measurement are shown in Figure 5 and compared with the results of direct two-phase equilibration (Esquivel and Bernardo-Gil, 1991) in Table 1. The time for transition from a two-phase dispersion to a one-phase solution in this case is relatively short (20 s) compared to the 200 to 250 s needed for the transition in the aqueous two-phase polymer systems.

While the system of water, 1-hexanol, and acetic acid is composed of completely monodisperse components, the transition time $\Delta T = T_2 - T_1$ is almost 20 s. The duration of this transition is a function of the locations of the feed and diluent

Table 1. Experimental Results for the Liquid-Liquid-Phase Boundary for 1-Hexanol, Water, and Acetic Acid

	Feed Comp. w/w %	Continuous Dilution Exp. Comp., w/w % T1	T2	Exp.* w/w %
Water	40.0	35.0	34.0	34.0
Acetic Acid	20.0	31.0	32.0	34.0
n-Hexanol	40.0	34.0	34.0	32.0
Water	47.5	41.0	40.0	39.0
Acetic Acid	19.4	32.0	33.0	35.0
n-Hexanol	33.1	28.0	27.0	26.0

*From Esquivel and Bernardo-Gil (1991).

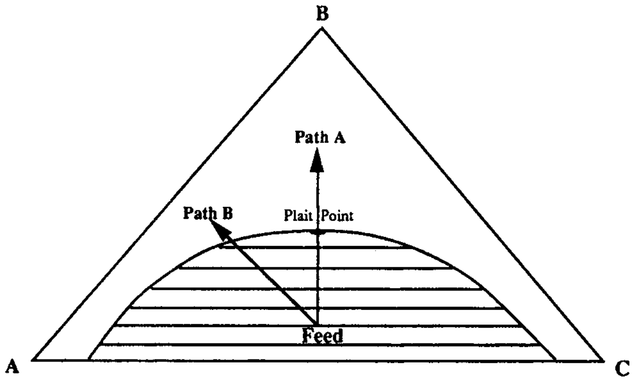

Figure 6. Different dilution paths in a ternary diagram.

compositions in the two-phase region (a thermodynamic effect) and the dynamics of the phase coalescence process (a kinetic effect). To understand the thermodynamic effect, consider the ternary diagram of Figure 6. If one proceeds along the dilution path A which passes through the plait point, the two phases will have approximately equal volumes and the turbidity of the system will remain constant with dilution until near the plait point. Consequently, the phase transition and turbidity change would be rather sharp. In contrast, along dilution path B, where the relative amounts of the two phases are changing, the phase transition and turbidity change will be less sharp due to the gradual disappearance of one of the phases. A conclusion then is that ΔT is not only a function of the polydispersity of the components, but is also a function of the dilution path. The time for phase separation or coalescence also depends on the viscosity of the two phases (Albertsson, 1986), which contributes to the larger ΔT observed with polymer solutions.

During the dilution, the absorbance first increases slightly from a constant value just before the phase transition begins (see Figures 2 and 5). This increase is possibly due to the low interfacial tension between the phases in the vicinity of the plait point which results in a smaller dispersed droplet size and an increase in the scattered light. As the dilution is continued, these small droplets dissolve and the absorbance of the system decreases.

For the continuous dilution method to be useful, the compositions determined should be independent of the dilution rate. The effect of the rate of addition of solvent, Q_D, on the value of ΔT and the corresponding polymer concentrations at times T_1 and T_2 can be estimated by examining a simplified form of Eq. 3 obtained by assuming that t_{holdup} is zero. If C_{iD} is also zero, Eq. 3 becomes:

$$\frac{dC_i(t)}{dt} = -\frac{Q_D}{V_o + Q_D t} C_i(t) \tag{4}$$

and so

$$\frac{C_i^o}{C_i(t)} = 1 + \frac{Q_D t}{V_o} \tag{5}$$

Equation 5 can also be written in terms of the weight percent of polymer i $[w_i(t)]$ as:

$$w_i(t) = \frac{M_o w_i^o}{M_o + Q_D t} \tag{6}$$

where M_o is the initial mass of the two-phase system, w_i^o is initial weight percentage of polymer i, and the density is assumed to be 1 g/cm^3. Thus, the concentration of polymer i at time T_1 is:

$$w_i(T_1) = \frac{M_o w_i^o}{M_o + Q_D T_1} \tag{7}$$

and the ratio of w_i at T_1 and w_i at T_2 is:

$$\frac{w_i(T_1)}{w_i(T_2)} = 1 + \frac{Q_D \Delta T}{M_o + Q_D T_2} \tag{8}$$

Clearly if $Q_D \Delta T$ and $Q_D T_2$ or $Q_D T_1$ remain constant regardless of the dilution rate, the individual concentrations of the polymers at T_1 or T_2 also will not depend on the dilution rate.

To find the relationship of Q_D to ΔT and T_2, the phase transition times were measured as a function of the dilution rate for a feed solution consisting of 4 wt. % PEG and 8 wt. % dextran (Figures 7 and 8). The time for phase transition monotonically decreases with increasing flow rate. The solid lines in Figures 7 and 8 correspond to $Q_D \Delta T = 0.076$ and $Q_D T_2 = 0.066$, respectively. Thus, to an excellent approximation $Q_D \Delta T$ and $Q_D T_2$ are independent of Q_D and the concentrations corresponding to times T_1 and T_2 do not depend on the dilution rate.

The binodal points obtained by two-phase equilibration and HPLC analysis are also plotted in Figure 4. Since clear phases were obtained in the equilibration experiment, the HPLC results should agree with the continuous dilution method concentrations at time T_2, and this is the case. In fact, the agreement between the two sets of data is remarkable, especially when one considers that one set is determined from simple turbidity measurements and the other using HPLC analysis with expensive equipment.

The UNIQUAC solution thermodynamic model (Abrams and Prausnitz, 1975) with predetermined parameters was used to describe the solution nonidealities. It was assumed that UNIQUAC interaction parameters are independent of the chain length of the polymers and could be used for phase equilibrium calculations with different molecular weight monodisperse polymers. This assumption is justified partly by the fact that the interaction parameters of the UNIQUAC model are based on a unit of area of interacting surface and should be constant with a change in the molecular weight of the polymers. The method of calculation is as follows. First, a molecular weight distribution is chosen for each polydisperse polymer. Then, using the Gauss quadrature method, pseudocomponents with discrete molecular weights and weight fractions representing the continuous distribution are obtained. Using these pseudocomponents and the experimental concentrations as initial guesses, the total Gibbs free energy of the system is calculated using the UNIQUAC model and its parameters. The concentrations of the components in each of the phases are varied until a global minimum of the Gibbs free energy is obtained using the Nelder-Mead (1964) simplex method (Kang and Sandler, 1988).

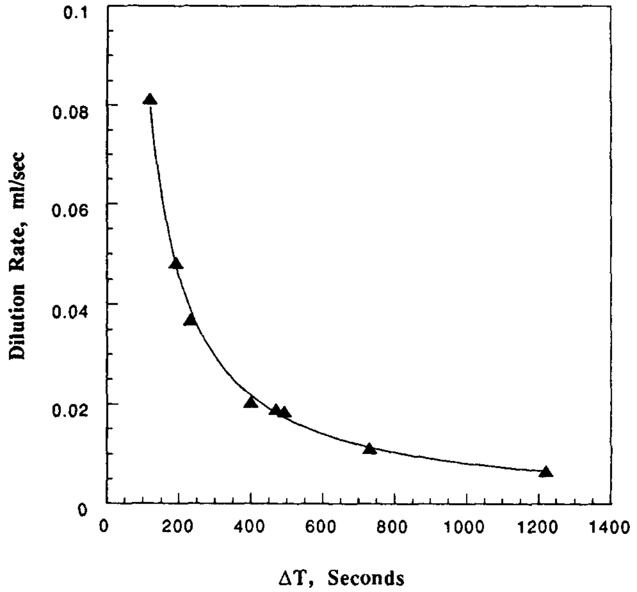

Figure 7. Phase transition time as a function of dilution rate Q_D for a feed of 4 wt. % PEG and 8 wt. % dextran.

The results of these phase equilibrium calculations are also presented in Figure 4. In these calculations, PEG (with the polydispersity parameter defined as $b = \overline{MW}_w/\overline{MW}_n = 1.161$) was considered to be monodisperse, while Dextran 500 ($b = 2.75$) was considered to be polydisperse. Number average molecular weights of 177,090 for Dextran and 8,920 for PEG were used in the calculations, and the pseudocomponents obtained from the Gauss quadrature method for Dextran are 133,500 and 528,900. The phase equilibrium calculations are in good agreement with the experimental results for compositions measured at time T_2 (Figure 4).

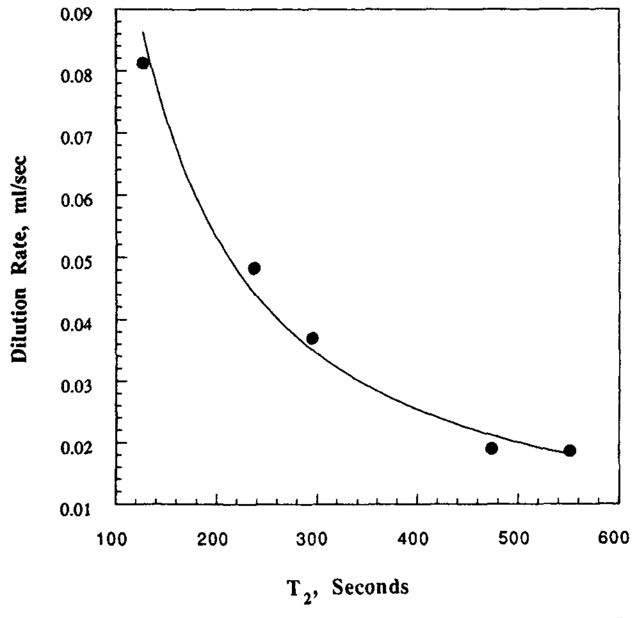

Figure 8. Time T_2 as a function of the dilution rate Q_D for a feed of 4 wt. % PEG and 8 wt. % dextran.

Finally, a possible explanation of the phase transition starting at time T_1 and ending at time T_2 is by analogy with bubble and dew points in vapor-liquid equilibrium systems. Consider a binary gas mixture compressed isothermally at a temperature below the critical point of the mixture. The pressure of the gas is increased until the first drop of liquid is formed. This pressure defines the dew point for that temperature. On further compression, the pressure changes until the last trace of gas disappears at the bubble point. Thus, the gas-liquid-phase transition takes place over a range of pressures and a period of time for the compression. In the dilution experiment, the transition takes place over a range of solvent concentrations, which is equivalent here to a period of time for the dilution. However, because of the polydisperse nature of the polymers (and the unknown tails of the molecular weight distribution function), the time T_1 is not calculable.

Summary

We have shown that the binodal curve for aqueous two-phase polymer mixtures can be determined using a relatively simple continuous dilution method with an on-line spectrophotometric measurement of the change of turbidity during the phase transition. To compare the observed transition from a two-phase to a one-phase system for polydisperse and monodisperse ternary systems, a mixture of 1-hexanol, water, and acetic acid was also studied. In the latter case, a much sharper change in the transmittance was observed compared to the gradual change in the polydisperse two-phase polymer system. The time lag for the phase transition of this monodisperse system shows that the difference between the times T_1 and T_2 is not only a function of the polydispersity of the polymers, but also depends on the path that one selects in the continuous dilution experiment and on the dynamics of phase coalescence. The sharpness of the phase transition can be increased by choosing a dilution path through the plait point that gives equal volumes of the two phases.

To check the accuracy of the binodal curve obtained from spectrophotometric measurement, a binodal curve was also constructed using the more traditional method of phase separation and subsequent analysis by HPLC. Both sets of results are in good agreement, though the continuous dilution experiment is much easier and cheaper to perform. Finally, the model predictions are also in good agreement with the experimental results for compositions corresponding to time T_2. An important, but not the only, contribution to the overall breadth of the phase transition is the polydispersity of the polymers.

Acknowledgment

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