PHASE DEMIXING STUDIES IN AQUEOUS TWO-PHASE SYSTEM WITH POLYETHYLENE GLYCOL (PEG) AND SODIUM CITRATE

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Phase Demixing Studies in Aqueous Two-Phase System with Polyethylene Glycol (PEG) and Sodium Citrate

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The kinetics of phase demixing in an aqueous two-phase system (ATPS) consisting of PEG/sodium citrate, with various PEG molecular weights (2000, 4000, and 6000) was investigated at 25°C. The rate of demixing of the phases (top/bottom) is essential to scale up the aqueous two-phase extraction (ATPE) process. The individual phase physical properties like density, viscosity, and interfacial tension are highly dynamic with the phase composition, and are responsible for the demixing of phases. Hence, in the present study, the variation of physical properties with phase compositions in terms of tie line length (TLL) was considered for the analysis of the rate of demixing of phases. The effect of the volume ratio of the phases (top/bottom) on the kinetics of phase separation also studied since the dispersed phase holdup in the mixture influences the rate of demixing. The higher TLL with a salt-rich phase as continuous phase showed higher reduction in demixing time when than low TLL at the volume ratio of <1. Based on the analysis, empirical correlations were proposed for top and bottom phase demixing rate in terms of Morton's number, volume ratio, and TLL. The coefficients and constants from the literature and the present correlations were estimated through regression analysis. The developed correlation predicted the demixing rate with lower AARD (±7%) for both the phases. The proposed correlation was accurate and simple in predicting the demixing rate of PEG/sodium citrate ATPS at 25°C.

Keywords Aqueous two-phase system; Demixing kinetics; Gravity separation; Tie line length

Introduction

In the past six decades, aqueous two-phase extraction (ATPE) has been widely exploited for the recovery and purification of various molecules such as proteins/enzymes, metal ions, antibodies, biosynthesized nanoparticles, and dyes from different sources such as wastewater, microbial fermentation broth, and animal and cell organelles (Raja et al., 2011). The recent research on ATPE confirms that it has potential to purify valuable compounds on an industrial scale. Studies conducted on ATPE revealed that it is suitable for separation of various biomolecules

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by utilizing several properties of the molecules, like sources/category, thermodynamic behavior, and physico-chemical characteristics. Recently, researchers have also found that polymer-salt ATPE systems are efficient and cost-effective in comparison to conventional methods for the recovery of macromolecules from fermentation broth and biological extracts (Rosa et al., 2010).

To perform scaleup studies, polymer-salt systems are more preferred than polymer-polymer aqueous two-phase systems (ATPS) as they offer lower viscosity, low chemical cost, and high phase separation rate (Raghavarao et al., 1995, 1998). Generally, ATPS was prepared with polyethylene glycol (PEG) with a high concentration of selective salts having cations, namely, ammonium, potassium, or sodium, and anions such as phosphate, sulfate, or carbonates. These salts, however, led to high concentrations of sulfate and phosphate salts in effluent streams, causing environmental problems. Recently, Zafarani-Moattar et al. (2004, 2008) reported that citrate salts have been used due to their biodegradability and nontoxicity. The efficacy of ATPE was analyzed through the partition coefficient, which is influenced by several factors such as concentration and molecular weight of the phase-forming polymer, charge (anionic or cationic) of various salts and their concentrations, temperature, and pH (Ragahava Rao and Nair, 2011; Saravanan et al., 2006, 2007). Unlike conventional solvent extraction, the mass transfer rate is not a limiting factor in ATPE, since the interphase mass transport does not follow the diffusion-controlled mechanism as long as mixing is ensured (Kaul et al., 1995). However, the limiting step is the phase formation/demixing in the ATPE process. Limited fundamental studies have been reported in the literature on phase formation kinetics to date. In general, if the phase components are added as concentrated solutions, the equilibrium with respect to phase composition and partition (separation) can be reached rapidly within 2 min under mild agitation/mixing (Narayan et al., 2011).

In general, the combination of mechanically agitated contactors followed by a gravity settler is employed for ATPE. The major hurdle to scale up the ATPE process is the slow demixing nature of the equilibrated mixture. Slow demixing of the thoroughly mixed phases was exhibited due to lower density and viscosity differences between the phases and interfacial tension (Salamanca et al., 1998). For the design of large-scale gravity settlers and also continuous column contactors, it is essential to have knowledge of demixing kinetics of phases. The kinetics of phase separation in ATPS has been studied in terms of the physico-chemical properties of the phases by measuring dispersion height as a function of separation time (Mistry et al., 1996). Asenjo et al. (2002) were the first to investigate the kinetics of phase separation on polymer/salt (PEG 4000/phosphate)-based ATPS. They studied the separation rate at various conditions and concluded that the physical properties of the phases had a strong effect on the rate of phase separation. Narayan et al. (2011) studied the kinetics of demixing of organic-aqueous salt systems (t-butanol/ammonium sulfate) and ATPS (PEG 4000/potassium phosphate). Both the systems were compared for the rate of demixing and the demixing rates were correlated with their physical properties (Equation (1)), which was initially developed by Asenjo et al. (2002):

$$\frac{dh}{dt}\left(\frac{\mu_d}{\gamma}\right) = a\left(\frac{\mu_d}{\mu_c}\right)^b \left(\frac{\Delta\rho}{\rho_d}\right)^c \left(\frac{\gamma}{\sigma_w}\right)^d \tag{1}$$

The rate of phase separation was analyzed at different TLL values, and it was found that the demixing rate increased exponentially with an increase in TLL for

t-butanol/ammonium sulfate systems. In the case of PEG 4000/potassium phosphate systems, the separation rate was higher at high TLL (Narayan et al., 2011). Salamanca et al. (1998) studied the TLL effect on kinetics of phase separation in batch and continuous systems with PEG 1500/sodium sulfate ATPS in the presence and absence of cell extract. Both Asenjo et al. (2002) and Salamanca et al. (1998) represented the profile of dispersion height as a function of initial height. The demixing profile was found to be independent of geometrical dimension of the separator, which is of prime importance during the design of large-scale separators. However, phase dispersion height and separation time depends on physico-chemical properties of the phases such as the density difference between the phases, viscosity, and interfacial tension (Merchuk et al., 1980). During ATPE, the phase volume ratio also plays an important role in the kinetics of demixing. Merchuk et al. (1980) also found that the kinetic behavior of the phases depends on the volume of the PEG or salt phase present in the mixture. The coalescence of the dispersed PEG or salt phase in the other phase was highly influenced by the continuous phase properties. The total recovery/yield calculation of the target molecules through the partitioning coefficient also depends on the volume ratio.

Phase separation in the polymer-salt system is of great technical interest and consequently has received widespread application in the recovery of biomolecules. Accordingly, the present study was formulated to understand the kinetics of demixing in ATPE under gravity force with different ATPSs like PEG molar masses of 2000, 4000, and 6000/sodium citrate at different volume ratios (0.66, 1, and 1.5) and TLL values (26.4–43.9). The experimental value of the rate of phase demixing was analyzed and correlated as a function of Morton's number, volume ratio, the ratio of interfacial tension to surface tension, and TLLs for both the PEG-rich top phase and salt-rich bottom phase regions.

Experimental Section

Materials and Methods

Tri-sodium citrate dehydrate (purity $> 99\%$) was obtained from Merck (Mumbai, India). Analytical grade PEG 2000 (average molar mass: 1900), PEG 4000 (average molar mass: 3800), and PEG 6000 (average molar mass: 5700) were obtained from Sigma-Aldrich (USA). Deionized distilled water (Technik, Karlsruhe, Germany) was used for all the experiments.

Preparation of the Phase System and Equilibrium Characteristics

The equilibrated PEG-rich top and salt-rich bottom phases were prepared at 25°C according to the available binodal curves in the literature for PEG 2000/sodium citrate (Murugesan and Perumalsamy, 2005), PEG 4000/sodium citrate (Oliveira et al., 2008), and PEG 6000/sodium citrate (Zafarani-Moattar et al., 2004). The phase equilibrium characteristics of the ATPS may be demonstrated by the tie lines. Since the tie line data at the required composition of the phase components for the selected systems were not available in the open literature except for PEG 2000/sodium citrate (Murugesan and Perumalsamy, 2005), tie line data were generated in the present work (Table I). To understand the effect on demixing of concentration of PEG and salt in the ATPS, the tie line lengths were calculated for the selected ATPSs.

Table I. Physical properties of PEG 2000, 4000, and 6000 with sodium citrate system

System	Top phase						Bottom phase							
	Wp (w/w%)	Ws (w/w%)	ρ (kg/m³)	μ (mPas)	σ (mN/m)	Mo	Wp (w/w%)	Ws (w/w%)	ρ (kg/m³)	μ (mPas)	σ (mN/m)	Mo	TLL (%)	γ (mN/m)
PEG 2000/ sodium citrate (Murugesan and Perumalsamy, 2005)	26.76	6.77	1086.3	7.4858	57.819	334457	4.17	20.5	1132.1	2.1690	49.466	2361.1	26.46	0.1615
	30.78	5.66	1086.6	11.6874	58.153	297652	3.23	23.25	1147.2	2.2479	50.488	407.38	32.68	0.3342
	34.43	4.89	1088.2	13.9607	56.570	173394	2.58	25.4	1158.3	2.3958	50.712	150.39	37.88	0.5321
	38.32	3.89	1088.8	17.0189	58.010	262075	1.7	28.11	1175.5	2.7897	51.840	97.20	43.90	0.7824
PEG 4000/sodium citrate	30.51	3.33	1084.6	23.2601	58.294	1116730	3.21	18.84	1128.9	2.0476	51.088	67.06	31.35	0.4847
	33.64	3.02	1085.5	28.6021	57.538	1081496	3.52	20.12	1145.1	2.1901	54.028	37.19	34.82	0.7125
	37.36	2.75	1086.3	35.1463	57.838	1258065	3.79	21.50	1156.8	2.3297	55.603	24.29	38.88	0.9431
	42.32	2.45	1090.1	43.8159	58.967	1398365	4.08	22.55	1171.8	2.5323	57.864	15.60	43.94	1.2827
PEG 6000/sodium citrate	31.93	3.04	1081.5	28.7994	58.793	753875	0.30	17.99	1127.1	1.7300	48.170	06.44	34.98	0.8534
	33.80	2.96	1082.4	30.5332	58.056	769211	0.31	19.16	1128.7	1.8432	53.219	05.40	37.20	0.98981
	36.76	2.61	1085.1	53.7952	62.109	1892913	0.32	21.38	1137.1	2.0549	55.866	04.03	40.99	1.3121
	38.85	2.50	1088.7	62.3688	57.499	2120868	0.52	23.58	1154.9	2.2253	57.163	0.43	43.74	1.6154

The phase equilibrium studies were carried out in glass separators (200 cm³). The feed samples (100 g basis) were prepared by mixing appropriate amounts of polymer, salt, and water in the vessel. The samples were centrifuged at 3500 rpm and then the mixtures were allowed to settle for 24 h at constant temperature (25°C) using a thermostat with an uncertainty of ±0.05°C (Lab Companion, Model RW-0525 G, South Korea). The two phases were then separated. The separated individual phase concentrations of PEG and salt were determined by refractive index measurement (RX-500, ATAGO Co. Ltd., Japan) and flame photometry (Elico Ltd., CL-378, India) respectively. The precision of the mass fraction of sodium citrate and PEG measurements was better than ±0.002 and ±0.001 respectively (Murugesan and Perumalsamy, 2005).

The tie line length of the ATPS was defined as

$$TLL\ (\%) = \left[(W_{P(t)} - W_{P(b)})^2 + (W_{S(t)} - W_{S(b)})^2 \right]^{1/2} \tag{2}$$

where $W_{P(t)}$ and $W_{P(b)}$ represent the weight percentages of polymer composition in the top and bottom phases and $W_{S(t)}$ and $W_{S(b)}$ represent the weight percentages of salt composition in the top and bottom phases respectively. In each system, four different TLLs with three different volume ratios for individual TLLs were considered for the present study (Table I). Volume ratio is defined as

$$Volume\ ratio\ (VR) = \frac{Top\ phase\ volume\ (ml)}{Bottom\ phase\ volume\ (ml)} \tag{3}$$

Measurements of Physical Properties

Densities of the distinctive phases (top and bottom) were measured by a digital density meter (DDM 2911, Rudolph, USA) with an accuracy of ±0.00005 g/cm³. Viscosity of all the individual phases was measured using an Ostwald-U-tube viscometer with proper calibration and an uncertainty of ±0.002 mPas. Interfacial tension between the phases was measured by a spinning drop tension meter with an uncertainty of ±0.001 mN/m. Surface tension was measured by a DST 30 series surface tension meter (SEO Corporation, South Korea) with an uncertainty of ±0.001 mN/m. All these properties were measured for both PEG-rich top and salt-rich bottom phases of all the tie lines of the individual ATPSs (Table I) at 25°C in triplicate.

Demixing Kinetics

The demixing experiments were conducted in a 50 mL jacketed cylinder with a total graduation of 13.3 cm. Externally cooled water was circulated through the jacket to maintain the temperature at 25°C with an uncertainty of ±0.05°C (Lab Companion, Model RW-0525 G, South Korea). The desired phase volume ratios (top phase, mL/bottom phase, mL) along the tie lies for the individual systems were prepared by taking the required volume of equilibrated stock solution of PEG-rich top and salt-rich bottom phases. The top and bottom phases were thoroughly mixed to obtain sufficient dispersion. The prepared solution was then allowed to separate into two

phases. The reduction in the dispersion height and height increase in the clear phases (top and bottom) were noted with respect to time. The experiment was continued until the disappearance of the dispersion region. All the experiments were carried out in triplicate, and average values are reported. The error in the analysis was within ±2%. In total, 36 demixing kinetic experiments were conducted in the present work. Detailed information on the procedure is also available elsewhere (Narayan et al., 2011).

Results and Discussion

Effect of Physical Properties on Demixing

Under gravitational force, the separation/demixing kinetics of the immiscible/ partially miscible phase is mainly dependent on physical properties like density and viscosity of the phases and interfacial tension of the system. In the ATPS, both the top and bottom phases were formed with three major components as PEG, salt, and water. The concentrations of each molecule in the phases are responsible for the overall physical properties/change of physical properties in the phases at constant temperature. Hence, the physical properties of both top and bottom phases were highly dependent on the phase constituent's concentration, which is accounted for in the form of TLL in the ATPS phase diagram. The physical properties of the top and bottom phases comprising different molecular weights of PEG and sodium salt at various concentrations (TLL) were measured at 25°C and are given in Table I.

Density

Among all the physical properties, density of the individual phases and resulting differences are the important criteria for the gravitational demixing process. In the current work, four different TLLs were considered for an individual ATPS (PEG 2000, 4000, 6000/sodium citrate). The lengths of the tie lines were varied depending on the concentration of the individual components (PEG, salt, water) present in the equilibrated phases according to the phase diagram. The resulting densities of the individual phases were measured and are given in Table I. It is observed that the density variation of the top phase was less than that of the bottom phase for the tie lines considered. The effect of TLL on the equilibrated phase density difference for different PEG molar mass systems is illustrated in Figure 1. The density difference between the phases was found to increase with increasing PEG concentration and decrease with increasing PEG molecular weight (Murugesan and Perumalsamy, 2005). PEG 2000 system's bottom phase was found to be densest of all the systems. As the TLL increases the density difference between the phases also increased, since the TLL becomes longer, which enforces the higher composition of salt and PEG in the bottom and top phases respectively at equilibrium conditions. As a result, the demixing rate increased with increasing TLL length.

Viscosity

The viscosity of the individual equilibrated phases (top and bottom) and the difference in viscosity between the equilibrated phases were measured at four different TLLs for different ATPSs. It was observed from Table I that both phase viscosities
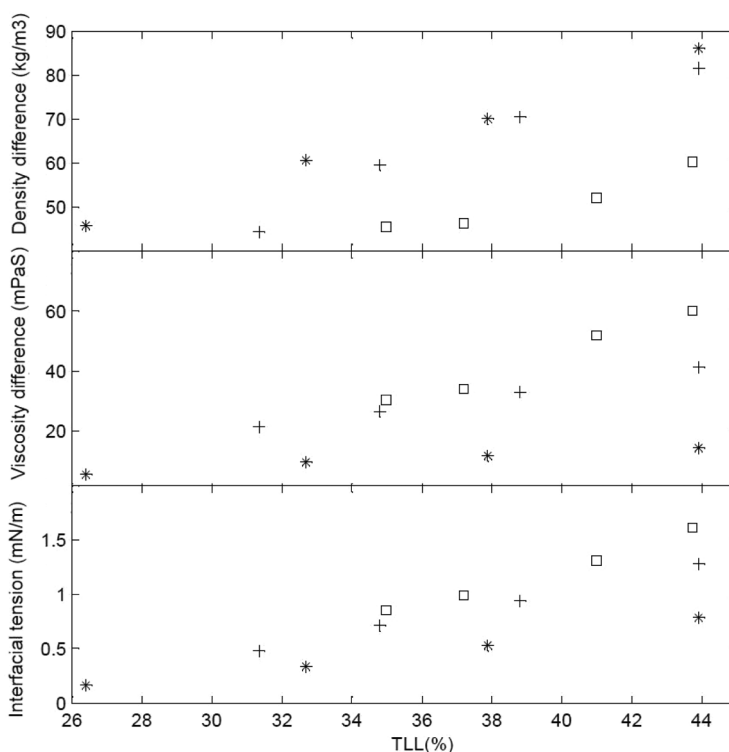
Figure 1. Effect on physical properties of PEG/sodium citrate system: *, PEG 2000; +, PEG 4000; □, PEG 6000.

increased with an increase in concentration of either PEG or salt in the equilibrated phases and TLL. But the effect of PEG concentration on viscosity was higher than that of salt concentration. Since the top phase predominantly contains PEG and is low in salt, the top phase viscosity drastically increased with PEG concentration. The ratio of the viscosities of the top to bottom phases varied in a large range of 3.5 to 30 mPas with TLL, and the bottom phase was found to be less viscous than the top phase. Hence, viscosity difference was also found to increase in accordance with TLL (Figure 1). The same trend was observed for ammonium carbamate in combination with PEG (2000, 4000, and 10000) systems at a temperature of 25°C by Berlo et al. (1998) and for PEG 2000/sodium citrate system by Murugesan and Perumalsamy (2005). As the polymer phase had higher viscosity, the friction between the drops and phase was high, hence the demixing rate was slower. When the bottom salt phase was continuous, PEG drops of the top phase moved through the bottom lower viscosity salt phase, favoring coalescence, hence the demixing rate was faster (Salamanca et al., 1998). At the higher volume ratio, the amount of PEG-rich top phase was greater than the salt-rich bottom phase, which offers more restrictions for phase separation (Figure 2). At higher PEG concentrations (larger TLL), even though the higher density difference was observed between the phases, the coalescence of the fine PEG droplets and the bigger PEG drop formation in the dispersion zone were reduced due to the higher viscosity of the phases. As a result, the separation rate was further reduced.
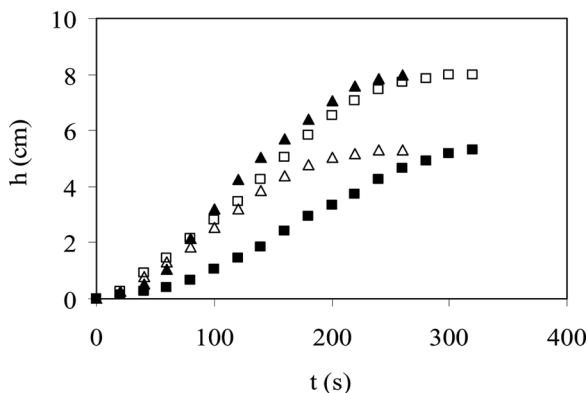
Figure 2. Kinetics of clear phase formation in PEG 2000/sodium citrate system at 37.88% TLL with different phase volume ratios: top phase: ■, 0.66; ▲, 1.5; and bottom phase: □, 0.66; △, 1.5.

Interfacial Tension

Interfacial tension increased with increasing TLL or concentration difference of salt and PEG between the phases and increased with increasing polymer molecular weight (Figure 1). It was also observed that at low PEG molecular weights the interfacial tension was greatly affected, but at high molecular weights the change of interfacial tension was less with concentration change (Wu et al., 1996). In general, the total salt concentration and the unsymmetrical partitioning of salt between the two bulk phases are the primary factors that strongly influence interfacial tension (Kim and Rha, 2000). From Figures 2 and 3, it was observed that the rate of demixing of the salt-rich bottom phase increased with increasing volume ratio for a constant TLL. This observed fact suggests that the coalescence of PEG droplets was faster in the dispersion region at higher salt concentration as a result of lower viscosity difference and higher interfacial tension between the phases. But the demixing rate of the PEG phase was comparatively lower at the same condition, since the
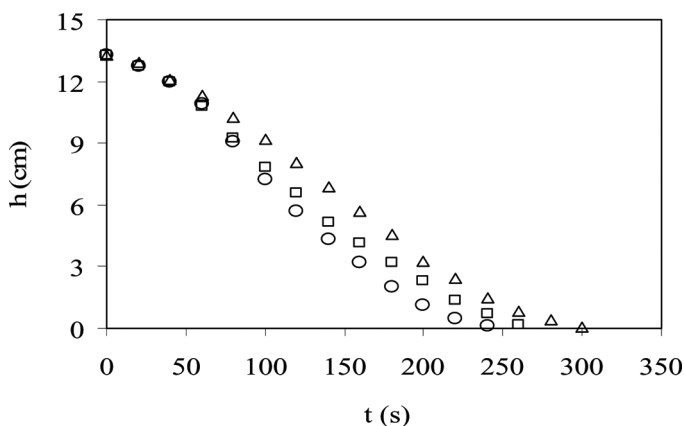

Figure 3. Kinetics of phase demixing in PEG 2000/sodium citrate system at TLL 37.88% (w/w) at various phase volume ratios: ○, 1.5; □, 1; △, 0.66.

viscosity force, due to high PEG concentration, was dominant over the interfacial tension force. At lower interfacial tension condition, the PEG drops formed were stable and small in size when compared to the higher interfacial tension systems (Narayan et al., 2011), hence the PEG drop formation/separation rate was found to be lower.

Kinetics of Demixing

During the demixing process, one interphase was formed at the top between the clear PEG-rich phase (top phase) and the dispersed region, and another interphase was formed at the bottom between the clear salt-rich phase (bottom phase) and the dispersed region. As demixing proceeds, both interfaces move towards each other and merge at a particular point, resulting in two phases at the end of the demixing process. The kinetics of demixing was highly dependent on the chemical and thermo-physical conditions of the ATPS (Kaul et al., 1995). In the present work, various phase components and their compositions and physical properties were considered (Table I) to study the demixing rate of equilibrated ATPS phases. Figure 3 shows the demixing profile of the dispersed region at constant TLL with three different volume ratios (0.66, 1, and 1.5).

Effect of Equilibrium Properties on Demixing

The phase equilibrium that exists between the top and bottom phases of the ATPS is a complex phenomenon. To explain the nature of the equilibrium, several parameters like tie line length, volume ratio, and phase-forming component properties were considered in the literature. In this section, the effect of some of these parameters was considered to understand the demixing phenomenon of the ATPS.

PEG Molar Mass and TLL

The kinetics of phase demixing was measured using dispersion height as a function of separation time. Figure 3 shows the demixing characteristics of the PEG 2000/sodium citrate system at a TLL of 37.88% (w/w) with different phase volume ratios. In addition, the individual phase formation rate was also calculated and is represented in Figure 4 for the volume ratio of 0.66. Four different tie lines were considered for each ATP system, and the corresponding TLL is presented in Table I. From Figure 4, it was observed that the rate of formation of the clear salt solution is higher than the rate of formation of the PEG phase for all the molar mass of PEG at 0.66 volume ratio and the rate of demixing decreases with PEG molar mass (Figure 5) and increases with increasing TLL (Figure 6). At higher tie line length, PEG and salt concentrations were high in the top and bottom phases respectively, resulting in high physical property differences between the phases. Hence, the separation/demixing rate was found to be high for higher TLL. Overall, the demixing rate increases for both phases with increasing TLL due to the higher amount of PEG and salt present in the total system. A similar trend was observed for the entire range of volume ratios considered in the present study.

The TLL also depends on the molar mass of PEG, while the binodal curve is shifted towards the single-phase region in the phase diagram with higher molar mass of PEG. At a constant salt concentration, the amount of PEG required to form the two phases is reduced at high molar mass of PEG. As a result, the equilibrium
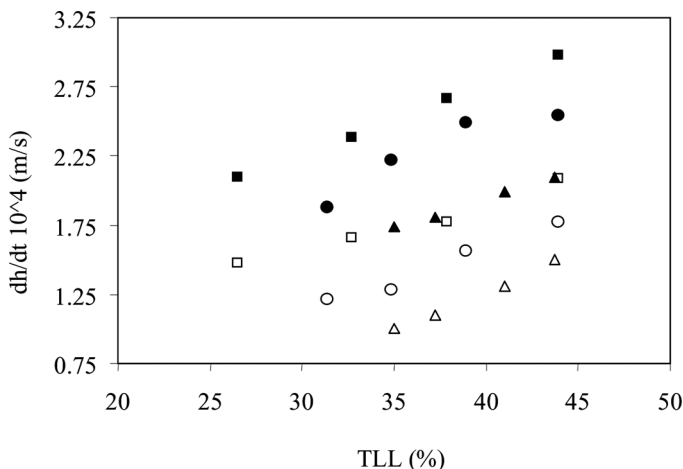
Figure 4. Rate of individual phase separation in PEG/sodium citrate system with varying molar mass of PEG. Bottom phase: ■, PEG 2000; ●, PEG 4000; ▲, PEG 6000; top phase: □, PEG 2000; ○, PEG 4000; Δ, PEG 6000.

composition of the phases may vary and result in a change in phase properties. The equilibrated phase demixing kinetics in PEG 2000, 4000, and 6000 with sodium citrate systems of higher TLL at the volume ratio of 1 is shown in Figure 5, and it is observed that kinetics of phase demixing decreased with increasing PEG molecular weight. Even though enough density difference was noticed between the phases at higher PEG molar mass system and higher TLL, the demixing rate was reduced due to the drastic increase in PEG phase viscosity.

Effect of Volume Ratio
The rate of demixing at constant TLL for different volume ratios is shown in Figure 3. A maximum rate of phase separation was observed at a volume ratio of
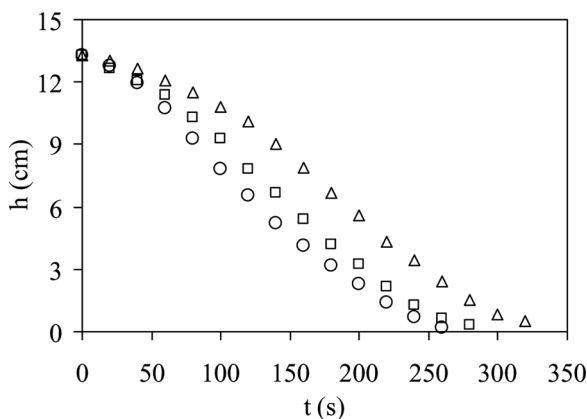

Figure 5. Kinetics of phase demixing in PEG/sodium citrate system with varying molar masses of PEG at higher TLL % (w/w) with phase volume ratio of 1: ○, PEG 2000; □, PEG 4000; Δ, PEG 6000.
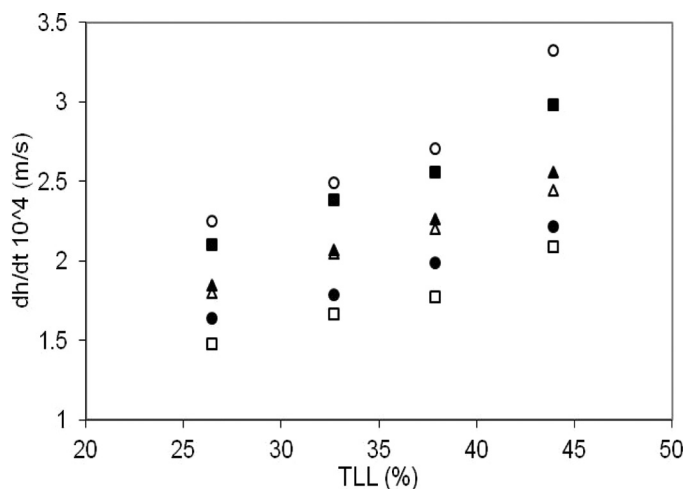
Figure 6. Rate of individual phase separation in PEG 2000/sodium citrate system with varying volume ratios. salt-rich bottom phase: ●, volume ratio 1.5; ▲, volume ratio 1; ■, volume ratio 0.66; PEG-rich top phase: ○, volume ratio 1.5; △, volume ratio 1; □, volume ratio 0.66.

1.5. However, a lower rate of phase separation was observed at lower volume ratios of 0.66 and 1. To determine the effect of volume ratio on the PEG/salt phase separation rate, Figure 6 was plotted. The PEG phase separation/formation rate was slower than that of the salt phase at the volume ratio of 0.66 and higher than that of the salt phase at a higher volume ratio (=1.5). At an equal volume ratio both phase formation rates approached each other or were almost equal for all tie line lengths. Based on this observation, it was understood that a higher volume of PEG presence in the dispersion region led to a higher demixing rate, since the PEG phase acts as a continuous phase with high viscosity due to the larger number of PEG droplets present in the lower volume of the salt phase. The higher droplet concentration led to more droplet-droplet interactions, which provide a faster rate of droplet coalescence, resulting in an increase in droplet size. Bigger droplets traveled faster to the interface, eventually resulting in lower demixing time or higher demixing rate (Merchuk et al., 1998). A similar type of phase separation behavior was also observed and reported for an acoustic field–assisted demixing of ATPS (Srinivas et al., 2000, 2002). At a lower volume ratio, the salt phase volume is higher in the dispersion region and acts as a continuous phase, which restricts the coalescence of the fine PEG droplets to bigger PEG droplets.

Empirical Correlation Development for ATPS
Asenjo et al. (2002) and Salamanca et al. (1998) considered physical properties and their difference between the top and bottom phases of aqueous-organic dispersions and correlated them with the rate of phase separation under gravitational force (Equation (1)). The correlation was also successfully extended to a PEG/phosphate ATPS. Later, Narayan et al. (2011) studied the kinetics of demixing and compared an ATPS (PEG 4000/potassium phosphate) and an organic-aqueous system (t-butonol/ammonium sulfate). The results were correlated using Equation (1) for PEG-rich top and salt-rich bottom phases. In the present study, the demixing

rates of both top and bottom phases were experimentally measured for PEG (2000, 4000, 6000)/sodium citrate systems at four different tie lines and three volume ratios at each tie line (36 experiments). The experimental data were correlated using the dimensionless groups involved in Equation (1), and coefficients and constants of the equations were determined through regression analysis and given as follows.

For the salt-rich bottom phase:

$$\left(\frac{dh}{dt}\right)_t \left(\frac{\mu_d}{\gamma}\right) = 0.00128 \left(\frac{\mu_t}{\mu_b}\right)^{0.4596} \left(\frac{\Delta\rho}{\rho_t}\right)^{0.8589} \left(\frac{\gamma}{\sigma_w}\right)^{-0.6252} \qquad (4)$$

For the PEG-rich top phase:

$$\left(\frac{dh}{dt}\right)_b \left(\frac{\mu_b}{\gamma}\right) = 0.07628 \left(\frac{\mu_b}{\mu_t}\right)^{-0.4548} \left(\frac{\Delta\rho}{\rho_b}\right)^{-0.0794} \left(\frac{\gamma}{\sigma_w}\right)^{0.3449} \qquad (5)$$

The data were fitted in Equations (4) and (5) with average absolute relative deviation (AARD) values of 11.73% and 20.16% of the salt-rich bottom and PEG-rich top phase respectively. Based on the analysis, it was found that the rate of separation of the top phase was much slower than that of the bottom phase at low volume ratio, and it was higher at a higher volume ratio. It was revealed by the experiments that the demixing rate of the phases depends not only on their physical properties but also on the phase composition (in the form of TLL) and their volume ratio. Further, the molar mass of PEG also has an effect on the phase separation rate. The physical properties are more highly dependent on the molar mass of PEG than the salt molar mass. In a polymer/salt system, the demixing phenomenon was achieved through bubble/drop formation in the dispersion region. The volume ratio decides which phase is going to be the dispersed phase in the dispersion region, i.e., at a higher volume ratio the salt phase is transformed as the dispersed phase and the PEG phase becomes the dispersed phase at a lower volume ratio. The Morton number was considered in the present correlation to explain the combined effect of physical properties like phase density, viscosity, and interfacial tension, which was highly exploited in aqueous-organic extraction systems (Murugesan and Regupathi, 2004). Even though interfacial tension was considered in the Morton number, the ratio of interfacial tension to surface tension was incorporated in the correlation to consider the relatively stable dispersion due to lower interfacial tension. Hence, for the rate of demixing of both phases, a generalized correlation was developed for all the ATPSs with different PEG molar masses and sodium citrate by including Morton's number (*Mo*), the ratio of interfacial tension to surface tension, volume ratio, and TLL.

The modified equation is

$$\frac{dh}{dt}\left(\frac{\mu_C}{\gamma}\right) = a \; M_O^b \left(\frac{\gamma}{\sigma_C}\right)^c \left(\frac{V_{tp}}{V_{bp}}\right)^d (TLL)^e \qquad (6)$$

Parameters of the proposed correlation (Equation (6)) were used to fit the experimental values, and the coefficients of the equation were determined through regression analysis for both PEG-dispersed salt continuous phase and salt-dispersed PEG continuous phase.

For the salt-rich bottom phase region, the correlation found was

$$\frac{dh}{dt}\left(\frac{\mu_b}{\gamma}\right) = 1.10 \times 10^{-5} M_O^{0.3623} \left(\frac{\gamma}{\sigma_b}\right)^{-0.4149} \left(\frac{V_{tp}}{V_{bp}}\right)^{-0.3303} (TLL)^{0.2292} \qquad (7)$$

For the PEG-rich top phase region, the correlation found was

$$\frac{dh}{dt}\left(\frac{\mu_t}{\gamma}\right) = 1.19 \times 10^{-5} M_O^{0.3479} \left(\frac{\gamma}{\sigma_t}\right)^{-0.9709} \left(\frac{V_{tp}}{V_{bp}}\right)^{0.5332} (TLL)^{2.6699} \qquad (8)$$

The correlations predicted the demixing rate with an AARD value of 1.72% for PEG-dispersed salt continuous bottom phase and 1.54% for salt-dispersed PEG continuous top phase. Equation (7) was used to predict the demixing rate in salt continuous phase (bottom) and was compared with the experimental values; it was found that all experimental points were predicted with $\pm 5\%$ error. Similarly, the experimental points for the PEG continuous phase (top) values (Equation (8)) were predicted with $\pm 10\%$ error. The values of the constants indicate the difference of the demixing kinetics between the two phases. The Morton number has an almost similar effect on the demixing rate of both phases. Even though the density effect was greater for the lower molar mass of PEG, the viscosity difference caused a slower demixing rate. As a result, the effect of Morton's number was the same for the higher density difference system (PEG 2000) and the higher viscosity difference system (PEG 6000). Further, the combination of viscosity and density forces on the demixing rate was identical for the phases, resulting in a similar constant for Morton's number. The interfacial tension terms have greater effect on the PEG phase (top) demixing rate than on the salt phase (bottom), since the degree of variation of interfacial tension with PEG concentration was higher than that with salt concentration. The top phase contains greater amounts of PEG, resulting in lower interfacial tension, leading to lower demixing rate. The same trend was also reflected in the correlation. The TLL also had a significant effect on phase separation, which was noticed in the correlation. The demixing rate of the PEG-rich phase was more highly dependent on TLL than that of the salt-rich phase, since higher TLL offered higher concentration of PEG in the top phase and the physical properties were more dynamic with PEG concentration.

Conclusions

The physical properties of polyethylene glycol with molecular weights ranging from 2000 to 6000 with sodium citrate aqueous two-phase systems were determined experimentally. The differences of density, viscosity, and interfacial tension between the phases increase with increasing TLL. The rate of demixing increases with increases in TLL, and a higher rate of phase separation was observed for the bottom phase at a lower volume ratio and for the top phase at a higher volume ratio. The molar mass of PEG also influences the rate of demixing of the phases, and it was observed that the salt-rich bottom phases were demixed at a higher rate for all the molar mass of PEG with lower volume ratio, and PEG 2000 systems show faster demixing than the PEG 6000 systems. Further, the demixing rate was correlated using a literature-obtained empirical correlation, and the coefficients and constants were found for the PEG-rich top phase and the salt-rich bottom phase with the AARD values of 20.16%

and 11.73% respectively. Since the existing literature correlation terms failed to represent the demixing rate of the present systems, a new correlation was proposed with three new dimensionless terms, Morton's number, volume ratio, and TLL, to estimate demixing rate for both phases. The modified correlation was fitted with the experimental demixing rate and the coefficients and constants were found. The new correlations predict the experimental points with an average AARD value of 1.52% and 1.72% for top and bottom phases respectively. The experimental result and the proposed correlations are very useful for the design of the continuous extraction process as well as the design of the gravity phase separators for ATPS. The current research and results may be useful for the scaleup of the ATPS process.

Nomenclature

AARD   average absolute relative deviation

$$AARD = \left(\sum_{i=1}^{n} \left| \frac{y_{\exp,i} - y_{cal,i}}{y_{\exp,i}} \times 100 \right| \right) \times \left(\frac{1}{n} \right)$$

where $y_{exp,i}$, $y_{cal,i}$, and n are experimental, calculated, and number of data points, respectively

a, b, c, d, e   coefficient of the equations.
H height of the interphase, cm
Mo Morton number $\left(\frac{\mu^4 g}{\gamma^3 \Delta\rho} \right)$
t time of phase separation, s
TLL   tie line length, %
V_{bp} volume of bottom phase, mL
V_{tp} volume of top phase, mL
Wp weight fraction of polymer, w/w%
Ws weight fraction of salt, w/w%

Greek letters

γ interfacial tension, mN/m
μ_b viscosity of bottom phase, mPas
μ_t viscosity of top phase, mPas
$\Delta\mu$ viscosity difference between the phases
ρ_b density of bottom phase, kg/m^3
ρ_t density of top phase, kg/m^3
$\Delta\rho$ density difference between the phases
σ_c surface tension between air-continuous phase interface, mN/m
σ_d surface tension between air-dispersed phase interface, mN/m
σ_w surface tension between air-water interface, 72 mN/m at 25°C

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