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# Acoustic field assisted demixing of aqueous two-phase systems

Naveen Nagaraj, N.D. Srinivas<sup>1</sup>, K.S.M.S. Raghavarao<sup>\*</sup>

Department of Food Engineering, Central Food Technological Research Institute, Mysore 13, India

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### Abstract

Acoustic field assisted demixing was employed to decrease the demixing time in aqueous two-phase systems (polyethylene glycol-maltodextrin and polyethylene glycol-potassium phosphate). Application of acoustic field has decreased the demixing time in polyethylene glycol-maltodextrin by around twofold and up to about 3.2-fold in polyethylene glycol-potassium phosphate systems. Ultrasonication has induced mild circulation currents in the phase dispersion, which has enhanced the rate of droplet coalescence, eventually resulting in decreased demixing time. In the polyethylene glycol-maltodextrin system, phase demixing was found to depend greatly on which of the phases is continuous and viscosity of the continuous phase was observed to have a strong influence on the movement of the droplets and hence controlling the phase demixing rate. In case of the polyethylene glycol-potassium phosphate system, droplet coalescence was found to play a critical role in phase demixing. Addition of NaCl increased the demixing time and presence of *Escherichia coli* cells did not seem to have any influence on phase demixing.

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# 1. Introduction

Aqueous two-phase extraction (ATPE) is finding applications in the area of biotechnology and developments of this technique have been discussed in detail [1-4]. Major hindrances for the large-scale applications of this technique are high cost of the phase forming polymers and slow demixing of the equilibrated phases. Temperature induced phase separation for recovery and recycling of the poly-

\*Corresponding author. Fax: +91-821-517-233.

mers has shown a solution for the high cost problem of the phase forming polymers [5].

On the other hand, relatively less attention has been paid to the slow demixing rate in ATPE. Slow demixing rate of the thoroughly mixed phases is due to small difference in densities between the phases, high viscosity of the individual phases and low interfacial tension. In general, polymer–salt systems have lower demixing times (in the range of 5–15 min) when compared to polymer–polymer systems (which will be in the range of 5 min–12 h) [1]. Kaul et al. [6] studied in detail the phase separation kinetics in polymer–salt [polyethylene glycol (PEG-4000)–potassium phosphate] under gravity. Their investigations provided a background for the design of large-scale gravitational separators for aqueous two-phase systems (ATPSs).

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*E-mail address:* raghava@cscftri.ren.nic.in (K.S.M.S. Raghavarao).

<sup>&</sup>lt;sup>1</sup>Present address: Mycology Laboratory, David Axelrod Institute, Albany, NY, USA.

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Attempts have been made to enhance the demixing rates in ATPSs by the application of external fields such as magnetic and electric fields. Wikrostrom et al. [7] introduced magnetic field assisted demixing process which significantly enhanced the demixing rates in polyethylene glycol (PEG)-dextran system, by the addition of iron particles or ferro-fluids to the system. However, this technique was not found useful when the PEG phase was dispersed. Raghavarao et al. [4] have extensively studied electrokinetic demixing process which resulted in the enhancement of demixing rate markedly in polymerpolymer systems, and is also without limitations. In this case, there is need for fabrication of special equipment and addition of chemicals such as salts to the system and furthermore, the technique is not applicable for PEG-salt systems.

Srinivas et al. [8] have recently reported the reduction in demixing times in ATPSs by the application of acoustic field. Conventional wisdom predicts that application of acoustic field causes mixing rather than demixing in the system, since it imparts energy to the system in order to achieve dynamic agitation, shear, cavitation, heating, etc. This is the reason seen behind the application of acoustics for cleaning of surfaces and disruption of microbial cell walls. However, we have found that this is not the case with respect to acoustic fields of higher frequency (MHz range) [9] and in contrast it has resulted in mild circulation currents in the phase dispersion, which has increased the droplet coalescence, eventually resulting in lower demixing time. The method appears to be simple, easy to scale-up and economical to operate (the acoustic transducer being high voltage and low current devices). Furthermore, readily available ultrasonic transducers could be employed.

For large-scale extraction of proteins, polymer– salt systems are well suited, whereas, for affinity partitioning polymer–polymer systems are preferred. This is due to the interference caused by the high salt concentration of polymer–salt systems in majority of the protein–ligand affinity interactions, which are of electrostatic in nature [10]. Demixing rates of polymer–polymer systems are much slower than polymer–salt systems, due to high phase viscosities, much lower interfacial tension and similar phase densities. Hence, there is a need to enhance the demixing rates in polymer–polymer systems, which has been attempted in the present study by the application of acoustic field.

PEG-dextran is the most popular polymer-polymer system studied for research purpose. Purified dextran being very expensive (US\$ 500 per kg) severely limits its use on a large scale. In order to improve the economics, crude dextran and partially hydrolyzed dextran were explored as phase forming polymers along with PEG and detailed partition studies were conducted for many enzymes/proteins to demonstrate their suitability [11]. A PEG-hydroxypropyl starch system (cost of hydroxypropyl starch being US\$20 per kg), which showed similar characteristics to the PEG-dextran system was also successfully employed for the large-scale extraction and purification of enzymes from pig muscle [12]. On similar lines, Szlag and Guiliano have developed an inexpensive phase system comprising of PEGmaltodextrin (MDX) (cost of MDX US\$ 1.0 per kg) which appears to have a good scope on an industrial scale [13]. Hence, in the present work the PEG-MDX system is selected to study the influence of acoustic field on phase demixing rate. The study in detail includes, the effect of various parameters such as phase composition, time duration of acoustic field application, salt concentration, and microbial cell concentration on demixing time, in contrast to our previous study [8] which just demonstrates the applicability of acoustics to enhance the demixing rate in the PEG-MDX system in only one phase composition.

From a practical point of view, it was thought desirable to reduce the demixing time further. Hence, we have studied the effect of varying the axial distance from the transducer on demixing time. The present study undertaken is in contrary with the conventional understanding of acoustic effect with varying the axial distance from the transducer. Since, conventional wisdom predicts that with increase in axial distance from the source of energy (acoustics in the present case) its effect (intensity) decreases, in other words, rate of demixing is expected to decrease with increase in axial distance. However, this was not the case in the present study. Therefore, we have studied the effect of varying the axial distance from the transducer on demixing time with respect to polymer-salt (PEG-potassium phosphate) and polymer-polymer (PEG-MDX) systems.

# 1.1. Theoretical aspects of phase demixing

Some of the researchers have assumed that approximately the Stoke's law (which was developed for a rigid sphere) can describe phase demixing under unit gravity:

$$V_{\rm s} = \frac{D^2 \Delta \rho g}{18\mu_{\rm C}} \tag{1}$$

where *D* is the droplet diameter,  $\Delta \rho$  is the density difference between the phases,  $\mu_{\rm C}$  is the dynamic viscosity of the continuous phase, *g* is the acceleration due to gravity and *V*<sub>s</sub> is the droplet rise/fall velocity.

It is rightly indicated that for a swarm of droplets considerable deviations from the Stoke's law can be expected [8]. As the droplets are not rigid the circulation inside them (induced by the drag of the continuous phase) has to be taken into account as given by the Hadamard–Rybzcynski equation:

$$V_{\rm s} = \frac{D^2 \Delta \rho g}{18\mu_{\rm C}} \cdot \left(\frac{3\mu_{\rm D} + 3\mu_{\rm C}}{3\mu_{\rm D} + 2\mu_{\rm C}}\right) \tag{2}$$

where  $\mu_{\rm C}$  and  $\mu_{\rm D}$  are the viscosities of the continuous and dispersed phases, respectively.

Phase demixing can be seen as a combined effect of droplet rise/fall and droplet coalescence. If a single droplet is considered then the two steps are clearly in series. The droplet has to rise/fall to the interface and there it coalesce with the interface [6]. In this situation, droplet migration will be the controlling step in the overall demixing process. In ATPSs, this situation can be seen when the phase volume ratios are either very high or very low. The time required for the separation of the two phases at this situation can be represented by Eq. (2). However, this may not be the case generally. The presence of multiple droplets leads to considerable droplet-droplet interaction, which leads to coalescence as they rise/fall. This will increase the droplets size and in turn alters their rise/fall velocities (proportional to the square of the droplet diameter).

In the present study, retardation of the drop rise velocity, due to high viscosity of continuous phase was found to play a major role on the demixing behavior.

### 2. Materials and methods

# 2.1. Chemicals

PEG ( $M_r$  6000) was purchased from Sisco Research Labs. Mumbai, India and MDX ( $M_r$  105 000) was procured from Laxmi Starch, Mumbai, India. Potassium phosphate was procured from S d Fine Chemicals, Mumbai, India and sodium chloride was purchased from Qualigens Fine Chemicals, Mumbai, India.

For the present study, Escherichia coli D21 obtained through the courtesy of Dr. M.A. Linggood, Uniliver Research, UK, which is maintained as stock culture at the Department of Food Microbiology, CFTRI, Mysore was used. The culture was maintained at 4 °C on brain heart infusion (BHI) agar slants and subcultured at 30-day interval. Prior to use in experimental trials, the culture was propagated twice in BHI broth at 37 °C. BHI broth in aliquots of 3 l was sterilized, inoculated with active culture of E. coli, mixed well and incubated at 37 °C for 48 h. Cells were harvested by centrifuging the medium for 30 min at 5000 rpm. The pellet obtained was washed with distilled water thoroughly and again centrifuged for 30 min at 5000 rpm. The washed pellet was dried at 50 °C for 2 h and then used for experimentation.

## 2.2. Phase system preparation

Phase systems were prepared by adding predetermined weighed quantities of phase forming polymers and polymer–salt to distilled water, allowing them to dissolve for 2 h and then mixed well for 1 h using a magnetic stirrer. The well-mixed phases were allowed to separate into two phases in a separating funnel kept overnight. The equilibrated and separated phases were collected and used as stock for demixing experiments. In this way 500 g amounts of systems were prepared in each case.



Fig. 1. Schematic diagram of the acoustically assisted demixing process.

### 2.3. Phase demixing experiments

For the PEG-MDX system, phase demixing experiments were carried out at three different phase compositions (7:30, 10:30, 10:40) for the following three phase volume ratios of top to bottom phase; 30:70, 50:50, and 70:30. The details of the experimentation are given elsewhere [8]. For the studies with NaCl and cells, required quantities of either salt or cells were added to 100 ml dispersion, mixed for 10 min and then poured back into the contactor. Fig. 1 shows the schematic diagram of the acoustically assisted demixing process. In case of polymer-polymer system, for all the experiments ultrasonication was provided from the bottom in repeated cycles of 5 min sonication and 5 min gravity. This was done to avoid excess of heat generation in the system due to continuous application of acoustics. Further, demixing experiments were carried out at intermediate phase compositions of polymer-salt (PEG-potassium phosphate; phase

Table 1

Viscosity and density values at various phase compositions of PEG-MDX system

composition 15:11) and polymer-polymer (PEG-MDX; phase composition 10:30) systems at all the three volume ratios mentioned as above by varying the axial distance from the transducer (0-5.2 cm). For these experiments demixing contactor made of polycarbonate (PC) and polypropylene (PP) (L/D)ratio 1.46:1) were employed along with glass contactor. For the polymer-salt system ultrasonication was provided continuously from the bottom, since the demixing time being less resulted in no heat generation in the dispersion. All the experiments were carried out with traveling wave mode of ultrasonication. The dispersion height was defined as the height of the non-separated dispersion (cloudy region). The time for complete phase demixing was taken, as the time required for clear horizontal interface to be formed. All the experiments were repeated three times and average values reported. The readings were found well within  $\pm 5\%$  of error.

Phase density and viscosity measurements were carried out as mentioned elsewhere [8].

# 3. Results and discussion

# 3.1. Effect of phase composition

Variation in phase composition changes the physical properties such as viscosity, density and interfacial tension of the system. As kinetics of phase demixing is greatly dependent on these physical properties, the effect of phase composition on demixing was studied. Various compositions selected are given in Table 1 along with their viscosity and density values for polymer–polymer and polymer– salt systems. Kinetics of phase demixing (dispersion

Phase composition	Viscosity (mPa s)		Density (kg $m^{-3}$ )		Density difference
	Тор	Bottom	Тор	Bottom	$({\rm kg} {\rm m}^{-3})$
PEG-MDX (%, w/w)					
7:30*	10.4	33.5	1106.3	1187.3	81.0
10:30*	13.5	85.1	1107.0	1226.4	119.4
10:40*	33.4	202.5	1123.0	1326.0	203.0
PEG-potassium phosphate (%, w/w)					
15:11*	34.8	2.2	1080	1130	50

\* Rest water.



Fig. 2. Kinetics of phase demixing in PEG 10% and MDX 30% two-phase system. Open symbols: demixing under ultrasonication alone; closed symbols: demixing under gravity;  $\bullet$  70:30 volume ratio;  $\bigstar$  50:50 volume ratio;  $\bigstar$  30:70 volume ratio.

height versus time) for an intermediate composition (PEG–MDX system) is shown in Fig. 2. Demixing times at all the three phase compositions selected in the present study are presented in Table 2. At each phase composition, the demixing studies were conducted at 30:70, 50:50 and 70:30 volume ratios. This study was conducted by realizing the fact that, from process point of view, it is highly desirable to concentrate the product by reducing the volume of the phase to which it preferentially partitions.

Application of acoustic field has resulted in a notable decrease in demixing time in these systems. At low phase composition (7:30 corresponding to the tie line closest to the binodal) the intensity of decrease in demixing time was insignificant. How-

ever, at intermediate and high phase compositions it has resulted in significant decrease in demixing time, particularly at the 30:70 volume ratio where the bottom phase is continuous. In these systems up to twofold decrease in demixing time was observed as indicated in Table 2.

Ultrasonication has caused mild circulation currents in the phase dispersion thereby increasing the droplet–droplet interaction (collision), enhancing the coalescence as the droplets rise/fall, which in turn enhanced the demixing rates. This could be observed in the form of enhanced slopes in Fig. 2.

In general, it was observed that for the phase compositions selected in the present study, demixing time remained practically similar or slightly varied at the 70:30 and 50:50 volume ratios, where the top PEG light phase is continuous. The demixing time increased dramatically at the 30:70 volume ratio where bottom MDX heavy phase is continuous (Table 2). This indicates that demixing time is largely dependent on which of the phase is continuous, precisely on the viscosity of the continuous phase. Interfacial tension, being very low, can be neglected in polymer-polymer systems. In case of polymer-salt system, rate of droplet coalescence is the controlling factor, which depends on interfacial tension of dispersed/continuous phases [8]. Density difference remains same at any volume ratio of a given phase composition. Hence, we could say that it is only the viscosity, which largely determines the demixing rate in polymer-polymer systems. At the 30:70 volume ratio, high viscosity of the continuous

Table 2

Effect of phase volume ratio on demixing time in various PEG-MDX two-phase systems

Phase composition	Volume ratio	Demixing time under gravity alone (min)	Demixing time under ultrasonication (min)
7:30	30:70	180	155
	50:50	65	55
	70:30	73	65
10:30	30:70	245	150
	50:50	58	45
	70:30	65	56
10:40	30:70	540	255
	50:50	164	90
	70:30	172	94

Mode of application of acoustic field: 5 min acoustics followed by 5 min gravity.

bottom phase retards the migration (rise/fall) velocity of the dispersed phase droplets, which eventually results in lengthening the demixing time as can be seen from Eq. (2). In contrast, at the 70:30 and 50:50 volume ratios, due to the lower viscosity of the continuous top phase, the average droplet migration velocity will be significantly higher when compared to the 30:70 volume ratio. This higher droplet velocity eventually resulted in lower demixing time.

It has been reported that near the critical point the density difference is small and therefore the demixing time will be high [1]. Far away from the critical point polymer concentration and the viscosity are high, hence the demixing time will be high. Thus, at intermediate compositions demixing time is the shortest. Similar behavior was observed in these studies with an increase in phase composition only at volume ratios where top phase was continuous (70:30 and 50:50), but not at volume ratio where bottom phase was continuous (30:70). At this volume ratio (30:70), demixing time increased markedly with an increase in phase composition (Table 2). The reason being that is the high viscosity of the continuous bottom phase. Further, viscosity of the continuous phase increases with an increase in phase composition, which dominates the density effect causing significant retardation in migration velocity of the droplets and hence, results in higher demixing time.

Another noteworthy observation from Table 2 was that at any given phase composition for polymer– polymer systems, demixing time was shortest at the 50:50 volume ratio. Continuous phase (PEG) being the same for the 70:30 and 50:50 volume ratios (top phase), the lower demixing time observed in the latter case is due to higher volume of the dispersed phase, which provides higher concentration of droplets. The higher droplet concentration has lead to higher droplet–droplet interaction, which hastened the rate of droplet coalescence, resulting in an increase in droplet size. Bigger droplets traveled faster to the interface, as  $V_s$  is proportional to  $D^2$ (Eq. (1)), eventually resulting in lower demixing time.

# 3.2. Effect of time duration of acoustic field application

Demixing experiments were carried out to determine the optimum condition at various modes of repeated interval of acoustic field followed by gravity field as listed below.

Mode (1)—5 min acoustics field followed by 5 min gravity.

Mode (2)—10 min acoustics field followed by 10 min gravity.

Mode (3)—10 min acoustics field followed by 20 min gravity.

Mode (4)—20 min acoustics field followed by 10 min gravity.

Experiments were carried out at low and intermediate phase compositions for the 30:70 volume ratio and the results are presented in Table 3. This is in view of the fact that acoustic field effect was prominent only at the 30:70 volume ratio (Table 2). In both phase compositions the mode 4 method produced best results in terms of decrease in demixing time (about 1.7- to 2.2-fold). However, temperature rise of the dispersion measured immediately after 20 min exposure to acoustic field was found to be around 10 °C. From the practical point of view, this may not be suitable for processing of biomolecules especially for enzymes/proteins, which are heat sensitive. Hence, the mode 1 method, with practically similar results (about 1.5- to 2.1-fold

Table 3

Demixing times at the 30:70 volume ratio of two-phase compositions at various duration of application of acoustic field/gravity in cycles

Phase composition (PEG-MDX, %, w/w)	Gravity demixing (min)	Acoustics/gravity (min)				
		5/5	10/10	20/10	10/20	
7:30	178	120	150	110	170	
10:30	250	140	160	115	200	
Rise in temperature (°C)	-	1.5-2	3.5-4	8-9	3.5-4	

decrease in demixing time and with 1.5-2.0 °C rise in temperature), was found to be suitable.

# 3.3. Effect of NaCl concentration

Addition of neutral salt is known to influence the protein partition behavior. Hence it was, thought desirable to study the effect of NaCl on phase demixing. As very low demixing times were observed at the 50:50 volume ratio of any studied phase composition, study was restricted to the effect of NaCl concentration on phase demixing only at this volume ratio in an intermediate composition. Density and viscosity values at different NaCl concentrations are tabulated in Table 4. Effect of addition of NaCl on demixing time at various concentrations is presented in Table 5. It was observed that demixing time increased gradually with an increase in NaCl concentration of up to 4%. However, any further increase in salt concentration (above 4%) has resulted in a dramatic increase in demixing time (Table 5). The gradual increase in demixing time up to 4% salt concentration is mainly due to a decrease in density difference between the individual phases with an increase in salt concentration (Table 4). The lower the density difference, the lower is the buoyant motion of dispersed phase droplets, eventually resulting in increased demixing time as can be seen from Eq. (1). However, the dramatic increase in demixing time above 4% NaCl concentration is mainly due to extent of decrease in volume ratio of the system (Table 4), which has changed the lower viscous dispersed phase to high viscous continuous phase. This resulted in significant increase in demixing time. Nevertheless, acoustically assisted process has shown a decrease in demixing time of about Table 5

Effect of NaCl concentration on demixing time in PEG 10% and MDX 30% two-phase system at the 50:50 volume ratio

NaCl concentration (%, w/v)	Demixing time under gravity (min)	Demixing time under ultrasonication (min)
0	58	45
1	63	50.5
2	66	52
4	68.5	53
5	72	54.5
6	256	175
10	327	224

Mode of application of acoustic field: 5 min acoustics followed by 5 min gravity.

1.3-fold when PEG was continuous (up to 4% salt) and about 1.5-fold decrease in demixing time when even more viscous MDX phase was continuous (above 4% salt) as shown in Table 5.

#### 3.4. Effect of microbial cell concentration

It was reported that addition of microbial cells to the system results in formation of stabilized emulsion, which will discourage the droplet coalescence thereby increasing the demixing time considerably [14]. However, in our experiments it was observed that addition of *E. coli* cells to the system at the 50:50 volume ratio results only in slight increase in demixing time. This clearly indicates the absence of materials, which are responsible for formation of stabilized emulsion. The demixing time increased slightly up to certain concentration (3%, w/v) and there after (4-6%, w/v) it decreased slightly to values lower than that of the pure system (Table 6).

Table 4

Viscosity and density values in presence of NaCl at the 50:50 volume ratio of 10% PEG and 30% MDX phase composition

NaCl (%, w/v)	Change in	Viscosity (mPa s)		Density (kg $m^{-3}$ )		ΔDensity
	volume ratio	Тор	Bottom	Тор	Bottom	$({\rm kg} {\rm m}^{-3})$
0	50:50	13.5	85.1	1107.0	1226.4	119.4
1	49:51	14.0	85.8	1114.8	1227.4	112.6
2	48:52	14.2	86.4	1121.2	1230.5	109.3
4	46:54	16.0	87.2	1133.8	1237.8	104.0
5	45:55	17.2	87.5	1137.6	1239.9	101.2
6	43:57	18.9	87.7	1142.9	1242.5	99.6
10	37:63	20.1	89.8	1158.6	1248.4	90.83

E. coli concentration	Demixing time under	Demixing time under		
(%, w/v)	gravity (min)	ultrasonication (min)		
)	60	44		
1	64	45		
2	67	46.5		
3	68	47		
4	58	42		
6	53	39		

Effect of E. coli cell concentration on demixing time at the 50:50 volume ratio of 10% PEG and 30% MDX phase composition

Mode of application of acoustic field: 5 min acoustics followed by 5 min gravity.

The slight decrease in density difference with the addition of cells up to 3% (w/v) and an increase in density difference after 3% as indicated in Table 7 are the probable reasons for an increase and a decrease in demixing times, respectively. Density difference being directly related to droplet migration velocity (Eq. (1)), higher the density difference lower will be the demixing time and vice versa.

In all systems application of acoustic field has decreased the demixing time by about 1.4-fold. Visual observations of the contactor after demixing showed a clear top phase and somewhat a yellowish turbid bottom phase indicating that *E. coli* cells predominantly partitioned to the bottom phase. In addition, a slight increase in the volume of the bottom phase after demixing also supported the statement that cells predominantly partitioned to the bottom phase.

# 3.5. Effect of varying the axial distance from the transducer on demixing time

In the PEG-potassium phosphate system, results of demixing experiments performed by varying the axial distance from the transducer are presented in Table 8. It was observed that at this condition demixing rates enhanced by up to 1.1–3.2-fold (only about twofold increase in demixing time observed in case of PEG-potassium phosphate system without varying the axial distance [8]). In this present case, the acoustic intensity has increased with increase in axial distance, which is in contrary to the conventional understanding. The enhanced demixing rate could be attributed to the following factors. First, the less dense dispersion in case of PEG-potassium phosphate facilitates faster propagation of ultrasonic waves. Secondly, with increase in axial distance there is less reflection of ultrasonic energy from the contactor bottom. Hence, these factors act synergistically towards hastening rate of coalescence between the droplets, increasing the buoyant velocity of the droplets, eventually enhancing demixing rate.

For the PEG–MDX system, there was no appreciable enhancement in demixing rates under otherwise similar conditions. In this case, velocity of ultrasonic waves is reduced when the wave propagates through more denser/viscous dispersion. This results in attenuation of acoustic intensity in polymer–polymer

Table 7

Viscosity and density values in presence of E. coli at the 50:50 volume ratio of 10% PEG and 30% MDX phase composition

E. coli (%, w/v)	Change in volume ratio	Viscosity	Viscosity (mPa s)		$g m^{-3}$ )	Density difference
		Тор	Bottom	Тор	Bottom	$(\text{kg m}^{-3})$
0	50.0:50.0	13.5	85.1	1107.0	1226.4	119.4
1	49.5:50.5	14.4	89.1	1112.2	1229.8	117.6
2	49.0:51.0	15.3	97.2	1113.4	1231.5	118.1
3	48.5:51.5	16.2	105.9	1113.9	1232.7	118.8
4	48.0:52.0	17.8	113.4	1114.5	1240.0	125.5
6	47.0:53.0	19.7	121.9	1116.1	1260.7	144.6

Table 6

Table 8

Material of demixing contactor	Gravity (min)	Demixing tim	Demixing time with increase in distance from the transducer (min)				
		0 cm	3 cm	3.8 cm	5.2 cm		
30:70 (v/v)							
Glass	8	5.1	_	-	_		
Polycarbonate	2.45	2.34	2.28	2.13	1.34		
Polypropylene	2.27	2.27	2.14	2.03	1.34		
50:50 (v/v)							
Glass	10.29	5.32	_	_	_		
Polycarbonate	3.24	2.5	2.4	2.29	2.29		
Polypropylene	4.32	2.5	2.35	2.32	2.32		
70:30 (v/v)							
Glass	5.22	3.29	_	-	_		
Polycarbonate	2.0	1.6	1.56	1.5	1.23		
Polypropylene	2.52	2.2	2.1	2.09	1.11		

Effect of varying the axial distance from the transducer on demixing time phase system: PEG-potassium phosphate: phase composition 15:11

Mode of application of acoustic field: continuous.

system, thereby no further enhancement (above twofold) in demixing rate is observed with increase in axial distance from the transducer.

# 4. Conclusions

Acoustic demixing, a method for decreasing phase demixing time in a polymer–polymer (PEG–MDX) system is reported. In these systems phase demixing depends greatly on which of the phases is continuous and viscosity of the continuous phase strongly influences the movement of the droplets and hence the phase demixing. Addition of NaCl (>4%, w/v) at a 50:50 volume ratio, has changed the bottom dispersed phase to continuous phase thereby increasing the demixing time.

Further enhancement in demixing rates has been achieved in a polymer–salt system by varying the axial distance from the transducer. Rate of droplet coalescence, played a critical role in phase demixing in this type of phase systems. Efforts to enhance the demixing rates further in polymer–polymer systems did not yield desired results.

Acoustically assisted process employed here significantly decreased the demixing time. Ultrasonication has induced mild circulation currents in the phase dispersion, which has enhanced the rate of droplet coalescence, eventually resulting in decreased demixing time. The method appears to be simple, economical (low current and high voltage device), easy to scale-up and readily available ultrasonic transducers could be employed.

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# References

- P.A. Albertsson, Partitioning of Cell Particles and Macromolecules, 2nd ed., Wiley, New York, 1986.
- [2] H. Walter, D.E. Brooks, D. Fisher (Eds.), Partitioning in Aqueous Two-Phase Systems: Theory, Methods, Uses, And Applications To Biotechnology, Academic Press, Orlando, FL, 1985.
- [3] B.Y. Zaslavasky, Aqueous Two-Phase Partitioning: Physical Chemistry and Bioanalytical Applications, Academic Press, 1995.
- [4] K.S.M.S. Raghavarao, R.M. Stewart, S.R. Rudge, P. Todd, Biotechnol. Prog. 14 (1998) 922.
- [5] I. Galaev, B. Mattiasson, Enzyme Microb. Technol. 15 (1993) 354.

- [6] A. Kaul, R.A.M. Pereira, J.A. Asenjo, J.C. Merchuk, Biotechnol. Bioeng. 48 (1995) 246.
- [11] K.H. Kroner, M.R. Kula, H. Hustedt, Adv. Biochem. Eng./ Biotechnol. 37 (1982) 73.
- [7] P. Wikrostrom, S. Flygare, A. Grondalen, P.O. Larsson, Anal. Biochem. 167 (1987) 331.
- [8] N.D. Srinivas, R.S. Barhate, K.S.M.S. Raghavarao, P. Todd, Appl. Microbiol. Biotechnol. 53 (2000) 650.
- [9] K.S.M.S. Raghavarao, P. Todd, US Pat. 6 090 295 (2000).
- [10] H. Walter, G. Johansson, D.E. Brooks, Anal. Biochem. 191 (1991) 1.
- [12] F. Tjernald, G. Johansson, M. Joelsson, Biotechnol. Bioeng. 30 (1987) 809.
- [13] D.C. Szlag, K.A. Guiliano, Biotechnol. Technol. 2 (1988) 277.
- [14] P.O. Larsson, Methods Enzymol. 228 (1994) 112.