Liquid–Liquid Extraction Using the Composition-Induced Phase Separation Process

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Liquid−Liquid Extraction Using the Composition-Induced Phase Separation Process

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This paper describes a new separation process of liquid−liquid extraction. It consists of first mixing the system to be extracted with a primary solvent, which is soluble with the native solvent, and subsequently adding a modifier, which is insoluble with either the native or the primary solvent. This process is similar to the phase transition extraction, which was described in a previous paper, where the liquid mixture, together with the solute to be separated, is first heated above its critical temperature, where it forms a uniform solution, and then cooled to the region below the miscibility curve, where it separates. Both processes have the advantage that the resulting separation of the solvents is very rapid, even in the presence of emulsion-forming impurities. In addition, the extraction efficiency of the new process may be 10 times higher than that of the traditional liquid−liquid extraction. The new process is thought of having significant advantages in the extraction of products from fermentation broths, plants, and other natural sources.

1. Introduction

In a previous paper [Ullmann *et al.* (1995)], one of the authors developed a novel liquid extraction process, named PTE (phase transition extraction), which is based on using solvents that, together with the liquid to be extracted, form a solution with a critical point of miscibility. In the new process, the mixture is heated and cooled across its coexistence curve: in the heating stage the solvents form a single phase, while in the cooling stage the solution separates into two phases, with the desired solute dissolved in the solvent phase. In Ullmann *et al.* (1995) the new process was applied to extract an antibiotic, i.e. ephretomycin, from a fermentation broth containing fractured cells, obtaining the two startling and unexpected following results.

(1) Phase separation was very fast (it was completed within few minutes), whereas conventional extraction produced a stable emulsion that required a centrifuge to separate.

(2) The extraction yield was very high and could not be duplicated even when conventional extraction in multiple stages was performed. This was surprising, as the partition coefficient for the conventional solvent was higher.

More recently, Eliyahu and Ludmer (1995) have confirmed these results using different systems, and in particular they achieved very high yields when extracting compounds from microbial cells that had not been fractured. These results are potentially of great relevance, especially in the extraction of biological compounds from fermentation broths, mammalian cells, and plants.

The present paper has two purposes. The first is to explain the two results that are mentioned above, providing a scientific basis for the absence of emulsion formation and the higher yields observed in the PTE process. The second objective is to propose and investigate another process, in which the phase transition of partially miscible mixtures is achieved by adding a modifier, i.e. changing the mixture composition instead of its temperature. In the following, we will refer to this

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process, as to the one described in Ullmann *et al.* (1995), as PTE, since they both involve phase transition, and shall distinguish one from the other by denoting the first process as temperature-induced phase separation (TIPS) and the second as composition-induced phase separation (CIPS). CIPS greatly simplifies the separation process of TIPS, as it can be carried out isothermally, and in addition, it allows a better control of the final composition of the two phases, thereby improving the separation yield even further.

To better understand the physical reasons of the higher yields obtained using PTE, we developed a model system, consisting of solid particles whose size is similar to the cells of a typical fermentation broth, using a dye as our solute. This allowed us to perform a series of controlled and reproducible experiments and to compare our results with the theoretical predictions. Our work on the model system provides a physical explanation of the experimental results obtained by Ullmann *et al.* (1995) and shows under which conditions PTE can lead to higher extraction yields. This is of special relevance in the extraction of large and sensitive molecules from broths, mammalian cells, or plants, where the high shear required to break the cells could deteriorate the solute molecules.

The performances of the composition-induced phase separation process were compared in detail with those of both conventional liquid extraction and the temperature-induced phase separation process. From our results it appears that the new, CIPS process could lead to substantially higher yields and provides a useful tool for dealing with difficult separation processes, which are hard to handle using currently-used methods. Patents for the new processes have been applied for [Ludmer *et al.* (1990); Shinnar and Mauri (1995)], and interested parties should approach the authors.

2. The PTE Process Using the Temperature-Induced Phase Separation (TIPS)

As mentioned above, TIPS, like all phase transition extraction (PTE) processes, capitalizes on the properties of partially miscible solvents with a critical point of solubility. In this process the liquid mixture, together with the solute to be separated, is first heated above
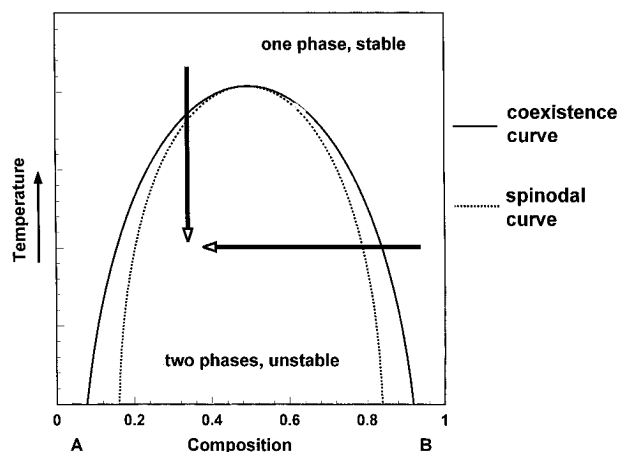
Figure 1. Coexistence and spinodal curves of a liquid mixture, giving the ratio between the temperature of the system and its critical temperature, as a function of the primary solvent concentration. The vertical arrow describes the temperature quench of the PTE process; the horizontal arrow describes the addition of a modifier (which results in a decrease of the primary solvent concentration) in the CIPS process.

the critical temperature, where it forms a uniform solution, and then cooled to the thermodynamically unstable region below the miscibility curve (see Figure 1). Ullmann (1993) described how this process can be used to design and operate a multistage counter current extraction column composed of successive heating and cooling units. More recently, Ullmann *et al.* (1995) described an important application of this process to extract an antibiotic from a fermentation broth, finding, surprisingly, that the new process had higher yields than its conventional counterpart. In addition, coalescence was very rapid, and phase separation was completed within a few minutes, despite the fact that the broth was not filtered and that the suspended cells normally form a stable emulsion that need a centrifuge to separate. While the reasons for having a higher yield will be described in the next section, here we will concentrate on the phenomenon of rapid coalescence, presenting some additional observations, which complement those of Ullmann *et al.* (1995).

The rapid coalescence of critical mixtures was studied in detail by Ullmann *et al.* (1995) using as a model system a dye (i.e. crystal violet) dissolved in water. The dye allowed an easy visual observation of the coalescence process; in addition, since the dye acted as an emulsion stabilizer, the timescale of the hindered coalescence during conventional liquid−liquid extraction (LLE) could be varied from minutes to hours at will, by simply increasing the dye concentration from 2 to 50 ppm. As the main solvent, Ullmann *et al.* used acetonitrile, to which they added 4% (molar) of a modifier, i.e. toluene, to raise the critical temperature of the solution from about 0 to 40 °C. The water−acetonitrile−toluene solution was put in a vial, heated to 50 °C, where it became homogeneous, and then cooled back to ambient temperature, at which point the system phase separated very quickly. On the other hand, when the system was agitated isothermally, separation occurred very slowly (up to several hours), as crystal violet had caused the formation of a stable emulsion. Very similar results were obtained for the fermentation broth, where MIBK, instead of toluene, was used as a modifier.

Ullmann *et al.*'s results, although sufficient to clearly show the rapid coalescence phenomenon, had one limitation, namely the cooling was too slow (about 1 min)
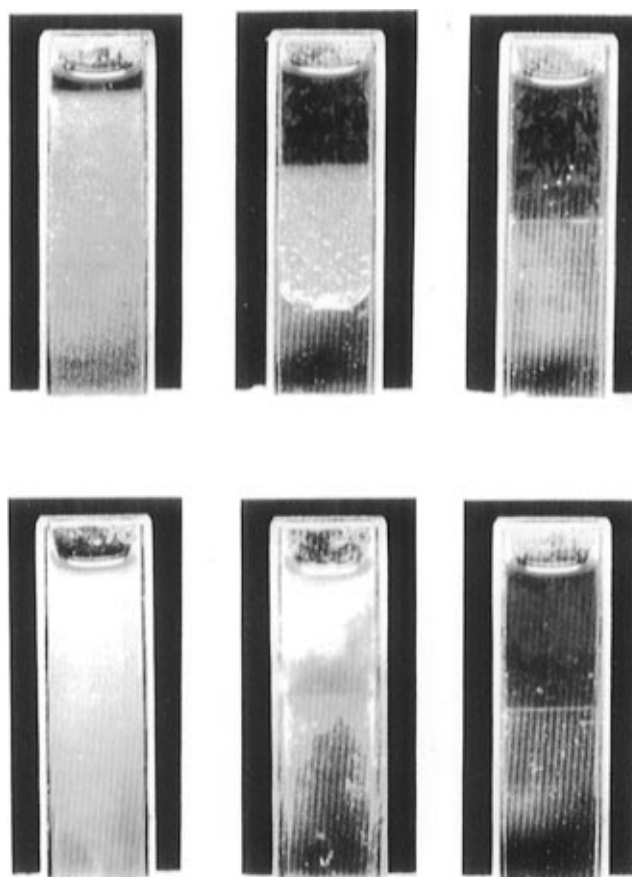

Figure 2. Direct observation of phase separation of a water/ACN/MIBK mixture using LLE (pictures at the top of the figure) and TIPS (pictures at the bottom of the figure). For each process, pictures were taken with a 3 s interval between each other. We see that, while in LLE there are two interfaces moving toward the center of the sample, during TIPS a single interface forms rapidly and moves only slightly in time.

to allow observations at short timescales. This is important because, when we perform TIPS, 1 min after cooling the main phase separation has already been completed, and the small changes that are observed at later times are due to secondary nucleation. Therefore, we repeated Ullmann *et al.*'s separation experiments using a much thinner (i.e. 1.2 mm thick) glass cell, where cooling could be completed within 2 s. A mixture of 52 cc of acetonitrile, 38 cc of water, and 10 cc of MIBK was used, with a small quantity, 2 ppm, of crystal violet dye to facilitate visualization and retard coalescence. Finally, the phase separation resulting after isothermally shaking the system (i.e. performing LLE) was compared with that after heating and cooling (i.e. performing TIPS). As one can see from Figure 2, there is an inherent difference between the two processes. In LLE we see two, more or less defined, interfaces, which move toward the center of the cell until they merge. This behavior was observed also when the composition of the system was varied, and the time required to complete the process increased from 5 to 10 s, in the absence of the dye, to 2 h, with 20 ppm of dye. On the other hand, during TIPS the interface appears almost immediately at its final position, and the solution clears up rapidly, with no emulsion formation, even in the presence of larger quantities of dye. This fast phase separation in the presence of emulsion-forming compounds is a special property of PTE.

These observations are clearly at odds with the explanation of the phenomenon that was put forward
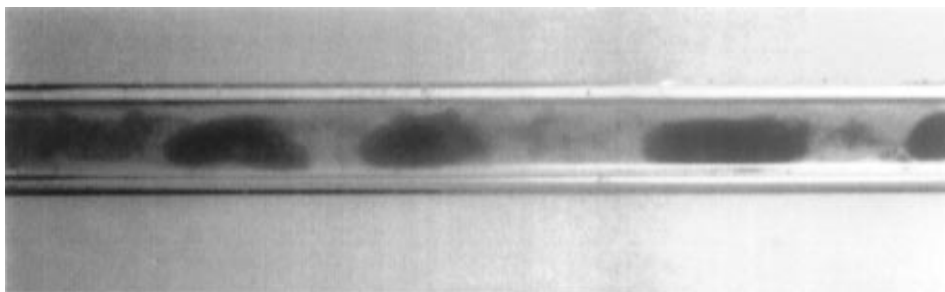
Figure 3. Phase separation of a water/ACN/MIBK mixture in a long tube using TIPS. The picture was taken 2 s after quenching and shows the formation of large drops. The tube inner diameter was 4 mm.
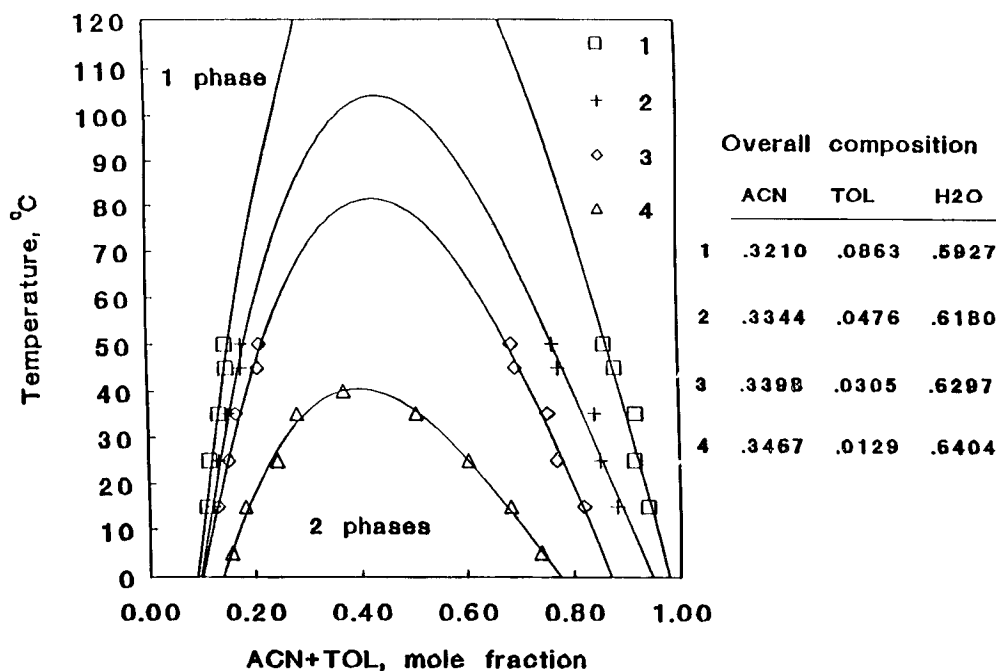

Figure 4. Phase diagram of the water/ACN/toluene system with four different toluene concentrations.

by Ullmann *et al.* (1995), who assumed (as most people do) that at the beginning of the process very small nuclei are formed, which subsequently grow by both diffusion and coalescence. The fact that coalescence is evidently not slowed down by the presence of emulsion-forming compounds was explained by the conjecture that there are no stable interfaces to which such compounds can adhere. However, it is well known [Gunton *et al.* (1983); Binder (1986); Mauri *et al.* (1996)] that, following a temperature quenching, a process, generally referred to as spinodal decomposition, takes place which can lead to phase separation without nucleation. In fact, the process begins with the appearance of a uniformly dispersed phase that subsequently develops into distinct regions of coexisting equilibrium phases. To further investigate the initial stage of phase separation, the quenching experiment described above was repeated in a 40 in. long cylindrical tube, with a 4 mm inner diameter. After a few seconds, large, centimeter-size drops were formed, as shown in Figure 3, indicating that during spinodal decomposition large drops can form very rapidly, without nucleation. This is the reason why separation during the PTE process is so fast, irrespective of the presence of emulsion-forming compounds.

3. CIPS: Composition-Induced Phase Separation

When using the TIPS process, we realized that very few solvents, when mixed with the native solvent, form

a solvent mixture with a critical temperature that is suitable for simple laboratory experiments. This problem is even more acute when TIPS is used for extractions of biological compounds, as in these cases strong constraints about the temperature range apply. In Ullmann *et al.* (1995) this problem was overcome by using solvent mixtures with a low critical temperature and then adding a modifier to change the miscibility curve. For example, as shown in Figure 4, taken from Ullmann (1993), the critical temperature of the water—acetonitrile mixture can be raised from 0 to 120 °C by adding 5% (molar) of toluene. In the same way, the critical temperature of any solvent mixture can be changed almost at will (obviously within the limitations of the boiling and freezing points) by adding a suitable modifier.

This simple observation suggested to us a new way to perform spinodal decomposition. Let us suppose, for example, that our system has a 1.3% molar concentration of toluene and is quenched from an initial 50 °C temperature, where it forms a single phase, to 25 °C, where it separates (see Figure 4). Now, the same separation can be achieved isothermally, at 25 °C, by raising the toluene molar concentration from 0%, where the system forms a single phase, to 1.3%, where it separates. In other words, the key of success of the PTE process is to bring the system to a point of thermodynamic instability below the miscibility curve. As shown in Figure 1, this can be achieved either by changing the
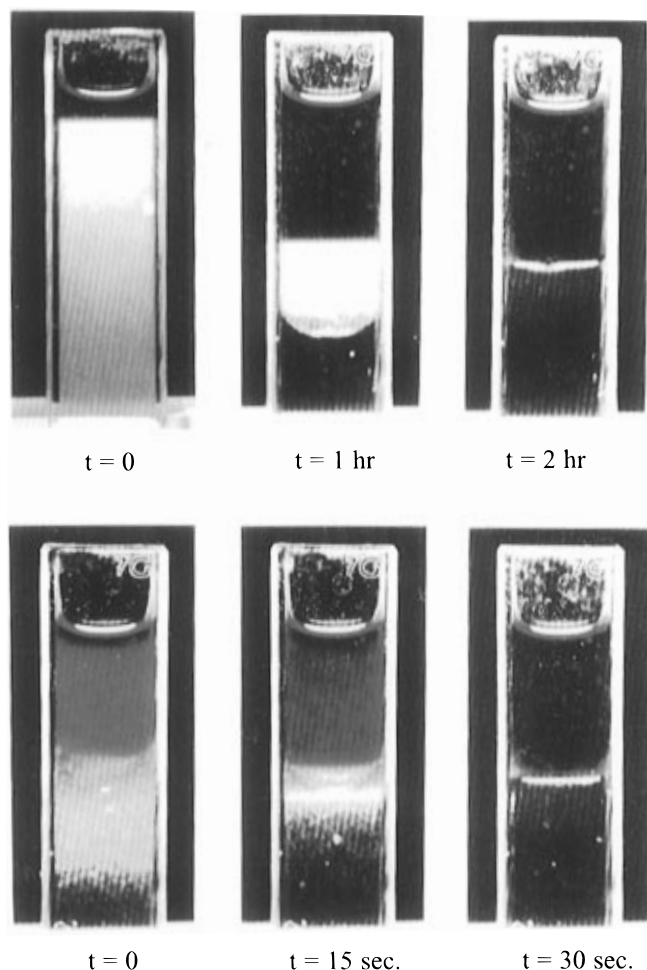
Figure 5. Direct observation of isothermal phase separation using LLE (pictures at the top of the figure, taken within 1 h from each other) and CIPS (pictures at the bottom of the figure, taken within 15 s from each other). The system consists of a water/ACN/MIBK mixture in the presence of 20 mg/L of surface-active crystal violet dye. We see that while CIPS was completed after 30 s, LLE was still in process after 2 h.

temperature of the system, as in the TIPS process, or by changing its composition, as in the new process that we propose here. In this so-called CIPS process [see Shinnar and Mauri (1995)], the system to be extracted is first mixed with a primary solvent, which is soluble with the native solvent, and subsequently a modifier is added, which is insoluble with either the native or the primary solvent.

The "equivalence" between TIPS and CIPS is substantiated in the series of pictures of Figure 5, where we used the same water−acetonitrile−MIBK mixture as in Figure 2, only in the presence of a larger amount, 20 ppm, of the crystal violet dye. We see that while after isothermal mixing a stable emulsion was obtained, with small ≈10 μm drops that remained suspended for about 2 h, when we used CIPS, within 30 s the mixture had completely separated. As in the case of temperature-induced phase separation, during CIPS we do not observe any moving interfaces, but instead a very fast phase separation takes place, which is almost unaffected by the presence of emulsion-forming compounds, with subsequent cleaning of the cloudy regions around the interface.

At the moment, we do not have a completely satisfactory theory that can generalize our results to all cases. So, we do not claim that adding any modifier to an otherwise single-phase binary mixture will always

result in spinodal decomposition and fast coalescence, just as we cannot claim that cooling a mixture below its miscibility curve will always result in fast phase separation, independent of the rate of cooling and of the composition of the mixture. However, we have considered a significant number of cases, using different solvents and modifiers, under conditions in which isothermal intense mixing would form stable emulsions, and in all these cases our concentration-induced phase separation process worked. In fact, separation was fast even when inorganic salts, instead of liquid solvents, were used as modifiers, and results identical to those of Figure 5 were obtained, when 5 g of sodium chloride was added to a 50 cc water−50 cc acetonitrile mixture. Clearly, this is not surprising, as salts too modify the critical temperature of liquid mixtures.

It is important to note that the addition of the modifier must result in a mixture composition which is in the unstable, not the metastable, region below the miscibility curve. However, the miscibility curve is not essential to perform the CIPS process, while it is so for TIPS. For example, if we add 9% molar toluene to a water−acetonitrile mixture, the critical temperature would raise to 160 °C (see Figure 4), so that TIPS could be performed only by pressurizing the system. On the other hand, CIPS could be performed easily, even without knowing how high the critical temperature is.

One difference between CIPS and TIPS is that TIPS can be performed, at least in principle, by heating and cooling the binary mixture composed of the native and primary solvents alone, i.e. without modifiers, provided that the critical temperature of the system is acceptable. A modifier is used in TIPS only to bring the critical temperature up or down to a convenient value and is not essential to the process, while, on the other hand, adding a modifier is essential to CIPS. However, in practice it is very unlikely that one could find a primary solvent that, together with the native solvents, forms a mixture with the right critical temperature; in fact, modifiers were used in most of the experiments performed by Ullmann (1993) and Ullmann *et al.* (1995). On the other hand, adding a modifier as in CIPS leaves us free to choose the operating temperature according to the process needs (such as low temperatures for sensitive materials). More importantly, in CIPS we are free to add the amount of modifier that is necessary to maximize the extraction efficiency of the process. In fact, increasing the relative amount of modifier generally means improving the separation between the two phases, and therefore it increases the partition coefficient of the solute between the two phases. Instead, in TIPS the amount of modifier is limited by the fact that the critical temperature of the mixture increases as more modifier is added. Therefore, the partition coefficient here can be improved only at the cost of increasing the temperature excursion of the heating/cooling process, which is not only expensive (as in some cases pressurization would be required) but often not even feasible. For example, in most biological extractions the samples cannot be heated above 60 °C. So, in conclusion, CIPS is equivalent to a TIPS process with a large temperature differential and rapid cooling. Its advantage is that it achieves the same results (i.e. high yield and no emulsion formation) and yet it is performed isothermally.

Finally, it should be stressed that for modifiers which are themselves good solvents the operating conditions can be predicted using the large amount of available
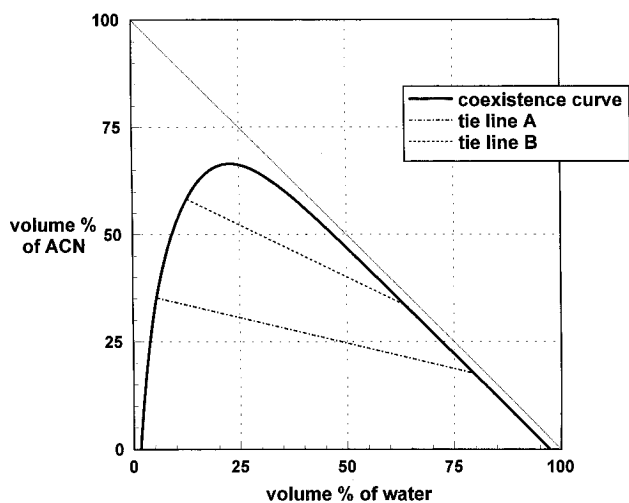
Figure 6. Triangular diagram for the ternary mixture composed of water, acetonitrile, and MIBK, where the horizontal and vertical axes represent the volumetric concentrations of water and acetonitrile, respectively. The curves have been calculated using the UNIQUAC equations, with parameters taken from Sorensen and Arlt (1980).

data. For example, one can use the triangular diagrams given in Sorensen and Arlt (1990) or generate them using thermodynamic data (see Figure 6 as an example), to predict the compositions of the phases after separation, and also check whether the operating point is in the unstable region, therefore ensuring that the separation process is a spinodal decomposition.

4. Extraction from Porous Solids

In the previous Sections we have seen how the PTE processes (both TIPS and CIPS) work, with rapid phase separation even in the presence of emulsion-forming compounds. However, the most important characteristic of the PTE processes is their ability to obtain higher yields when applied to the extraction of compounds from porous solid particles or membranes. This was first shown by Ullmann *et al.* (1995) for the extraction of efrotomycin from a fermentation broth containing fractured cells. However, quantitative scientific studies of the extraction of compounds from fermentation broths, plants, and other systems containing a solid phase have one difficulty, namely it is hard to get a completely reproducible system and in addition, due to the complexity of the system, it is difficult to know how the extraction process takes place microscopically. Therefore, we decided to develop a well-defined system of solute and solvents, so that we could perform a series of controlled experiments.

4.1. The Model System. This model system was intended to mimic a "real" fermentation broth, which typically consists of an aqueous solution of cell debris containing the solute to be extracted, such as the active compounds resulting from fermentation. Our model system consisted of water, a dye (2 mg/L of crystal violet), playing the role of the solute, and 2% (dry weight) of silica gel particles, which were intended to model the cell debris. These particles are S-662 silica gel macroporous beads, with sizes ranging from 70 to 250 μm, purchased from Fisher and commonly used in gas chromatography.

Using a dye as our solute had the advantage that its concentration could be readily measured with a spectrophotometer. In fact, using a Lambda 2 Perkin Elmer UV/vis spectrophotometer, one can see [Ullmann (1993)]

that the absorption spectra of crystal violet show a maximum at a wavelength of 590 nm and that this value is unchanged whether the dye is dissolved in water or in a solvent. Therefore a calibration line was determined, finding that the crystal violet concentration is linearly related to the absorbance of the sample at 590 nm, provided that the concentration is smaller than 12 mg/L.

The most interesting aspect of our system (and the reason why it is a good model of fermentation broths) is that when the silica gel particles were added to the water/dye system, after 10 min of stirring 97% of the dye was adsorbed on the particles. In fact, the absorbance of the water/dye system decreased when the silica gel particles were added, until, after stirring for 2 and 10 min, it became 15% and 3% of its initial value (i.e. without particles), respectively. This value did not change when we stirred the system for longer times. Now, considering that the diffusion time of the dye in the particle pores is about 30 s (see section 5.4), these measurements show that the adsorption/desorption time of the dye is no longer than a few minutes.

In the following we will denote by "model system" the water/particles/dye suspension, with 97% of the dye adsorbed on the particles. This system is a good model of "real" fermentation broths, where the active compounds are often, "imprisoned" within $\approx$100 μm-size cell debris.

4.2. The Solvents. Three solvents were used in our experiments, namely pure MIBK (methyl isobutyl ketone) and two mixtures of MIBK and ACN (acetonitrile) in 1:1 and 1:4 volumetric ratios, respectively. In all cases, the solvents were added to equal volumes of our model system, composed of 98% water and 2% dye-saturated particles. Now, MIBK is almost insoluble with water, and when 50 cc of MIBK was added to 50 cc of water containing 2 ppm of crystal violet, then after agitation, the dye was found to distribute between the two phases, with a $p_{sw} = 5$ partition coefficient. Here and in the following the partition coefficient p_{sw} is defined as the ratio between the dye concentration in the upper, solvent-rich phase and that in the water-rich phase.

Studying the second solvent system, we must consider that the ACN/MIBK mixture is partially soluble with water. In fact, when a mixture of 25 cc of ACN and 25 cc of MIBK was added to 50 cc of pure water, then after agitation, the mixture separated immediately into 55 cc of a water-rich phase (raffinate) and 45 cc of a solvent-rich phase (extract). The compositions of the two phases were measured using an HP gas chromatograph, finding 79% water, 18% ACN, and 3% MIBK volume composition for the water-rich phase and 6% water, 35% ACN, and 59% MIBK volume composition for the solvent-rich phase. As shown in Figure 6 (see tie line A), these values were within a few percent of the equilibrium compositions calculated using the UNIQUAC equation, with parameters given by Sorensen and Arlt (1980). In addition, when 2 ppm dye was added to the system, the dye was found to partition between the two phases, so that at the end of the separation 96% of it was dissolved in the upper, solvent-rich phase, which corresponds to a partition coefficient $p_{sw} = 25$. Comparing this result with that obtained using pure MIBK, we see that acetonitrile is a far better solvent of the dye than MIBK.

Finally, we analyzed the third solvent system, by adding a mixture of 40 cc of ACN and 10 cc of MIBK to 50 cc of water, finding 25 cc of an upper phase with 13%
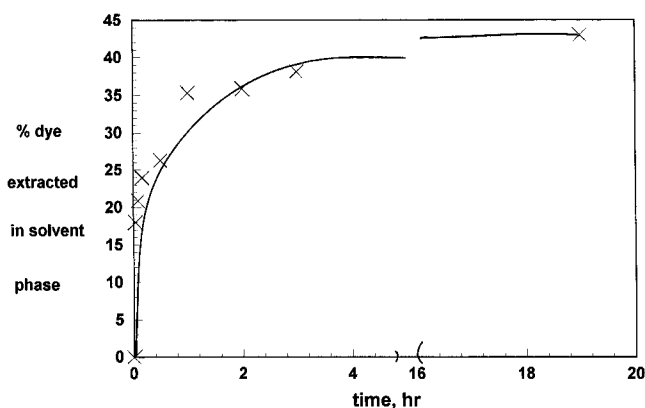
Figure 7. Recovery of the dye as a function of time, with the vertical axis representing the percent of the dye originally dissolved in the model system that is extracted using LLE. The solvent is pure MIBK, mixed with an equal amount of our model system.

water, 58% ACN, and 29% MIBK volume composition and 75 cc of a lower phase with a 64% water, 33% ACN, and 3% MIBK volume composition, in agreement, within a few percent, with the equilibrium compositions calculated using the UNIQUAC equation (see tie line B in Figure 6). In addition, adding the dye, we found that 42% of its original amount was dissolved in the upper phase and 58% in the lower phase, which corresponds to a partition coefficient $p_{sw} = 2.2$.

5. Comparison between CIPS, LLE, and PTE

5.1. The LLE Process. Having saturated the solid particles with crystal violet dye, we performed various extraction experiments, to compare the different processes. First, we performed conventional liquid−liquid extraction (LLE), where the two solvents described in the previous section were added and stirred isothermally. During this process, at prescribed intervals, the batch was centrifuged, to break the stable emulsion, and the dye concentration in the upper phase was measured. The results are given in Figure 7 for pure MIBK (50% MIBK and 50% of our model system) and in Figure 8a for the ACN/MIBK mixture (25% acetonitrile, 25% MIBK, and 50% of our model system).

Comparing Figures 7 and 8a, we see that at the end of the extraction using the ACN/MIBK mixture, 95% of the dye initially embedded in the particles was dissolved in the upper phase, while with MIBK that amounted to only 45%. This behavior was expected, since acetonitrile is a far better solvent of the dye than MIBK (see previous section), and is typical of most processes, where water-soluble solvents extract far better than non-water-soluble solvents. What was not expected in these experiments is that the timescale of the extraction using the ACN/MIBK mixture was much shorter than that with pure MIBK, i.e. 10 versus 40 min, where by timescale we refer to the time nedded to extract 63% of the dye dissolved in the solvent phase at equilibrium. As discussed in section 5.4, this is due to the higher solubility of the dye in the water-rich phase when we use the ACN/MIBK mixture, so that there is more dye dissolved inside the pores, and the extraction can be completed faster.

5.2. The CIPS Process. 5.2.1. First Example. At this point we performed our composition-induced phase separation process, using the same ACN/MIBK solvent mixture that we used for LLE. To do that, our model system (i.e. water, containing 2% of dye-saturated
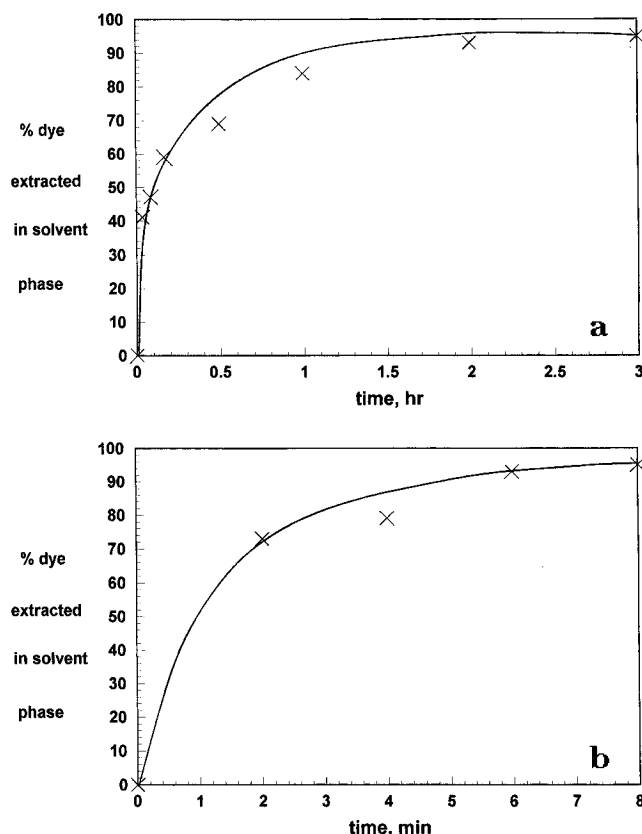

Figure 8. Recovery of the dye as a function of time, with the vertical axis representing the percent of the dye originally dissolved in the model system that is extracted using (a) LLE and (b) CIPS. The solvent is a 1:1 mixture of ACN and MIBK, mixed with an equal amount of our model system.
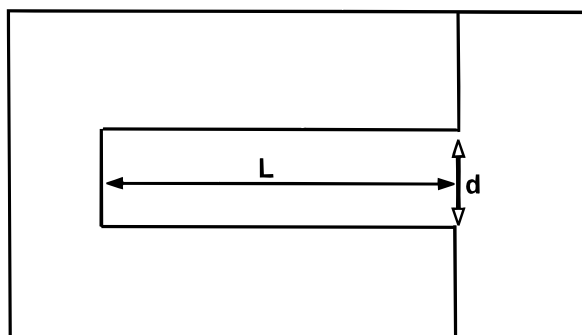

Figure 9. Schematics of the pore geometry.

silica gel particles) was first mixed with acetonitrile (25 cc of ACN to 50 cc of water), forming a homogeneous mixture that was agitated for 10 min. Then we added the appropriate amount of MIBK (1 part to 3 parts solution), stirred mildly, and let settle for 2 min, obtaining two phases that separated without the help of a centrifuge. (Actually, for sake of precision, before analyzing the samples we centrifuged them, to eliminate any residual traces of suspended particles that might disrupt the reading of the spectrophotometer. However, the centrifuge was in no way necessary to separate the two phases.) When the two phases were analyzed, we obtained the results of Figure 8b, showing that the same equilibrium state as that using LLE was reached, but within a much faster timescale. In fact, as the dye concentration in the water/ACN mixture is about 30 times larger than that in the water phase of the water/MIBK mixture, a reduction of the timescale from 40 to 2 min was expected.

Table 1. Equilibrium Data of LLE and CIPS Extraction Processes[a]

	global composition	solvent phase composition	water phase composition	ratio, solvent phase to water phase	% dye			p_{pw}[b]	p_{sw}[c]
					solvent phase	water phase	solid phase		
LLE	50% MIBK, 50% water	98% MIBK, 2% water	3% MIBK, 97% water	1	44	9	47	263	5
LLE/CIPS	25% MIBK, 25% ACN, 50% water	59% MIBK, 35% ACN, 6% water	3% MIBK, 18% ACN, 79% water	0.82	95	4	1	22	25
CIPS[d]	33.3% ACN, 66.6% water				99	1		.75	

[a] All concentrations are based on unit weight. All partition coefficients were measured separately. [b] p_{pw} = concentration of dye in particle phase/concentration of dye in water-rich phase. [c] p_{sw} = concentration of dye in solvent-rich phase/concentration of dye in water-rich phase. [d] Before modifier addition to homogeneous phase; solvent phase = water phase.

Table 1 summarizes our experimental results, giving the compositions of the phases after separation, the amount of dye in the particles and in the water-rich and solvent-rich phases, and the equilibrium partition coefficients.

5.2.2. Second Example. CIPS can be performed using a wide variety of solvents. In the first example described above we have used as modifier a solvent, i.e. MIBK, which is insoluble with the native solvent, i.e. water, and soluble with the primary solvent, i.e. ACN. However, we could use a modifier which is soluble with water and insoluble with ACN, such as sodium chloride. To show that, we added 50 cc of ACN to 50 cc of our model system, agitated for 2 min, and then added 5 g of sodium chloride. As a result, the system separated immediately into 40 cc of an upper phase, with a 33% water, 67% ACN, and 0% salt volume composition, and 65 cc of a lower phase, with a 58% water, 39% ACN, and 3% salt volume composition. In this case we found that the dye would partition between the two phases with a 1:6 ratio, and 85% of the total quantity of dye was dissolved in the upper phase.

5.3. CIPS versus TIPS. At this point, a direct comparison between TIPS and CIPS was in order. To this purpose, we performed the TIPS process, adding 50 cc of the third solvent system described in section 4.2 to 50 cc of our model system, heating the resulting mixture above its 40 °C critical temperature, and cooling it down to ambient (25 °C) temperature. We saw that, as expected, two phases formed very rapidly, with 43% of the original amount of dye dissolved in the upper phase and 57% in the lower phase, in agreement with the partition coefficient measurement of section 4.2.

We then performed the same extraction using CIPS and found the same results; namely we were able to extract 98% of the original amount of dye from the silica gel particles, but only 43% of it was dissolved in the upper phase, due to the relatively low partition coefficient and the fact that the upper phase volume was only 25% of the total volume. As a comparison, in the extraction described in section 5.2.1, we were also able to extract 98% of the original amount of dye from the particles, but due to the very large partition coefficient, most of it was dissolved in the upper phase. However, this is not a direct comparison between CIPS and TIPS, as we did not optimize the conditions of TIPS. In fact, we could have increased the amount of dye in the upper phase by increasing the quantity of solvents and/or the critical temperature.

Therefore, we may conclude that when we use the same solvent system, CIPS and TIPS have the same extraction efficiency. However, comparing the results described in this section with those of section 5.2.1, we see that by applying CIPS we can achieve a higher efficiency than when using TIPS, as we are allowed to use a larger amount of modifier, so that the solvent content in the water-rich phase is reduced. Clearly, we could have achieved the same result by heating the mixture of section 5.2.1 to about 150 °C under pressure (to prevent evaporation). However, apart from the obvious cost of heating and cooling the mixture across such a large temperature gap, many organic compounds, such as the products of fermentation broths, cannot be heated to such high temperatures. Therefore, as in single-stage extractions TIPS has no apparent advantages over CIPS; the added expense has no justification.

5.4. Theoretical Model. The large difference between the extraction efficiencies of LLE and CIPS is due solely to a difference in the transfer rates: eventually, if we wait long enough, the same equilibrium state must be reached. A simple theoretical model can help us understand this phenomenon.

Consider a mass M_p of porous spherical particles of radius a immersed in a binary mixture composed of a water-rich phase of mass M_w and a solvent-rich phase of mass M_s. A thin film of the water-rich phase surrounds the particles, so that the system can be schematized as in Figure 8, showing a single capillary pore of length $L \approx a$, in contact at the mouth with a thin film of a well-stirred water-rich liquid phase followed by a well-stirred solvent-rich phase. Initially, a mass m_{tot} of dye is adsorbed on the capillary walls. Then, as time progresses, the water-rich phase removes the dye from the wall, and in turn the solvent-rich phase removes the dye from the water-rich phase. In general, the concentration profile of the dye will depend on the partition coefficients $p_{pw} = c_p/c_w$, $p_{sw} = c_s/c_w$, and $p_{ps} = c_p/c_s = p_{pw}/p_{sw}$, expressing the ratios between the concentrations of dye at equilibrium in the water-rich phase, c_w, in the solvent phase, c_s, and in the particles, c_p. At equilibrium, the fraction of dye extracted, $\epsilon = m_s/m_{tot}$, where $m_s = c_s M_s$ is the mass of the dye dissolved in the solvent phase, can be easily found to be

$$\epsilon = \frac{P_{sw}}{1 + P_{sw} + P_{pw}} \qquad (1)$$

where the coefficients P_{sw} and P_{pw} are defined as

$$P_{sw} = \frac{m_s}{m_w} = p_{sw}\frac{M_s}{M_w}; \quad P_{pw} = \frac{m_p}{m_w} = p_{pw}\frac{M_p}{M_w} \qquad (2)$$

From this expression one may conclude that the larger P_{sw} becomes the better extraction we get. However, this does not take into account the fact that if it takes the characteristic time τ too long to reach equilibrium, the extraction process becomes inefficient. In fact, this time

Table 2. Experimental (τ) and Predicted (τ') Values of the Extraction Relaxation Time

	global composition	τ	$p_{pw}(1 - m_p/m_{total})$	τ'
LLE	50% MIBK, 50% water	40 min	140	70 min
LLE	25% MIBK, 25% ACN, 50% water	10 min	22	11 min
CIPS[a]	33.3% ACN, 66.6% water	<2 min	0.75	45 s

[a] Before modifier addition to homogeneous phase; solvent phase = water phase.

is inversely proportional to the mean flux in the pores and so to the amount of dye dissolved in the water phase, and therefore it is proportional to $(1 + p_{pw})$. In addition, τ is proportional to the amount of the dye that leaves the pores, $(1 - m_p/m_{tot})$, and to the diffusion time $\tau_d = \theta a^2/D$ that it takes a soluble molecule to exit a pore, where a is the particle radius, D the diffusion coefficient of the solute molecules, and θ the tortuosity factor. This analysis can be performed rigorously following Froment and Bischoff (1979), finding that the ratio between the dye extracted and the total amount of dye as a function of time is equal to $\epsilon[1 - \exp(-t/\tau)]$, where

$$\tau = \tau_d(1 + p_{pw})\left(1 - \frac{m_p}{m_{tot}}\right) \quad (3)$$

From this equation, after easy manipulations we find

$$\tau = \frac{\theta a^2}{D}(1 + p_{pw})\frac{1 + P_{sw}}{1 + P_{sw} + P_{pw}} \quad (4)$$

This result is valid, provided that the diffusive time τ_d is much smaller than τ, which means assuming that the quasi-steady-state approximation can be applied. In our case, since τ_d is about 30 s, this approximation is correct (see Figures 7 and 8). In addition, in eq 4 we have neglected the desorption time. This approximation, although appropriate in our case, as we saw in section 4.1, might not be valid in some applications. However, we will assume that eq 4 is correct, since our goal here is not to exactly predict the value of the extraction time but to understand the role that the different physical parameters play in the diffusion of the dye out of the particle pores.

In Table 2 we give the measured values of τ, together with their predicted values. Clearly, in predicting the relaxation time, we have assumed a certain value of the diffusive time, $\tau_d \approx 30$ s, which is consistent with the particle size $a \approx 100\,\mu$m, a diffusion coefficient $D \approx 10^{-5}$ cm^2/s, and a tortuosity coefficient $\theta \approx 3$. As we can see, the theoretical predictions, in particular the ratios between the relaxation times obtained using different solvents, are in good agreement with our experimental results. Note that in the CIPS process the relaxation time was too short to be measured accurately, and in addition, the quasi-steady-state approximation cannot be applied, so that both measurements and predictions should be taken *cum grano salis*.

The most interesting aspect of our results is that in LLE the extraction timescale is several orders of magnitude larger than the diffusion timescale, despite the fact that the process is actually diffusion controlled. This analysis can be applied also, for example, to a fermentation broth, even if the transport process in this case is clearly more complex and may involve diffusion across membranes. However, if the desorption process which

in our model has been assumed to be instantaneous (or at least of the same order as τ_d) is slow, with its rate dependent on the solvent used, then our simple model should be modified accordingly. In addition, it should be pointed out that even when the cells are fractured, the fracturing may be incomplete and uneven, and cells can form loose aggregates, unless intense agitation continues during the extraction. Such aggregates will behave like a porous solid and will hinder extraction, as the solvent cannot wet the particles easily.

Thus our results explain why in some cases the yield for PTE (both CIPS and TIPS) can be much higher than that for conventional LLE (see Ullmann *et al.* (1995) and Eliyahu and Ludmer (1995)), especially as the extraction times are normally limited to a few minutes. They also explain the results of Eliyahu and Ludmer, who showed that intense fracturing improves the yield of conventional extractions. CIPS has the additional advantage over TIPS that one can reduce the solvent concentration in the water-rich phase and thereby increase p_{sw} and improve the yield of a single extraction stage, while TIPS would need additional stages to achieve the same result. Our results also indicate another potential advantage of PTE, especially CIPS, as the ability to choose better, water-soluble solvents for the extraction. Now, in our case, ACN is a much better solvent for the dye than MIBK. We know that this is accidental, as there is no inherent reason why we could not find a better solvent that is not water soluble. However, although this would improve the yield of the process, it would not affect its long timescale. Now, for natural materials, very often water-soluble solvents are superior to immiscible solvents. In that case, CIPS would have a very large advantage, in addition to the other advantages of a short extraction timescale and the absence of stable emulsions formed. It should be pointed out that CIPS can also be performed using centrifuges, without loosing its advantages of higher yields.

In conclusion, CIPS has clear advantages over both LLE and TIPS, since, unlike LLE, it reaches equilibrium very quickly and, unlike TIPS, it allows us to select solvents that give large extraction yields.

6. Summary and Conclusions

In this work we have presented a novel separation method named composition-induced phase separation or CIPS. The CIPS process is composed of two mixing stages: first, the system to be extracted is mixed with a primary solvent, which is soluble with the native solvent; second, a modifier is added, which is insoluble with either the native or the primary solvent. Immediately after the addition of the modifier, the system separates rapidly into two coexisting phases, even in the presence of emulsion-forming impurities.

CIPS is conceptually similar to the temperature-induced phase separation, or TIPS, described in Ullmann *et al.* (1995), where a mixture of native and primary solvents is heated and cooled across its coexistence curve. In fact, when the same solvent system is used, CIPS gives the same results as TIPS, provided that the temperature differential of TIPS is workable. The similarity of the two methods was demonstrated experimentally.

The advantages of CIPS over the conventional liquid—liquid extraction, or LLE, process can be summarized as follows.

(1) Improved extraction yield. When the solute is adsorbed on solid particles, CIPS has a clear advantage over LLE, since, by using primary solvents that are miscible with the native solvent, it does not have the wetting problems that are encountered in LLE, where insoluble or partially miscible solvents are used. This is particularly important in the extraction of natural products and fermentation broths.

(2) Ability to handle emulsion-forming systems. Unlike LLE, CIPS is almost unaffected by the presence of surface active agents, and no stable emulsions are formed.

(3) Equipment savings. Since we do not need to use centrifuges to break stable emulsions, as we do when using the traditional LLE process, the equipment required to perform CIPS is only a tank. Even when a distillation equipment is required to separate the primary solvents from the modifier, CIPS is still significantly cheaper to perform than LLE.

(4) Lower product degradation. Any possible shear stress damage to large solute molecules is prevented in the CIPS process, where only a mild mixing is required, as opposed to the high centrifugation of traditional LLE. In addition, the fact that in the CIPS process a small amount of the native solvent is contained in the extract will help prevent the unfolding of large solute molecules, such as proteins.

(5) Use of water-soluble solvents. The CIPS process allows the use of water-soluble solvents, which, despite being in general good solvents of biological materials, cannot be used in conventional extraction processes.

Now, most of these advantages over traditional LLE are also shared by TIPS. Like the CIPS process, TIPS takes place rapidly and is not much affected by the presence of surface active agents in the native system. In addition, since during the heating stage the native and primary solvents are miscible, the resistance to extraction of the solute through the cells interface is greatly reduced; in fact, we found that when we used the same solvents the extraction efficiency of the CIPS and TIPS processes was the same and consistently larger than that of the traditional LLE. Therefore it appears that TIPS, like the CIPS process, has many significant advantages over the traditional LLE process, at least for cases in which either stable emulsions are formed or the solute is sensitive to shear. Yet, comparing CIPS with TIPS, we may conclude that CIPS has the following advantages:

(1) Improved extraction yield. Since there is no concern that high temperatures may be required to reach the single-phase region, the final compositions of the water-rich and solvent-rich phases can be adjusted over a wide range. Consequently, the partition coefficient of the solute to be extracted between the two phases can be higher, to achieve the desired separation.

(2) No need for separate heat transfer equipments. The extraction can be carried out in a single vessel, e.g, a fermentation vessel. Alternatively, low-cost settlers may be used for large volume production. In addition, the difficulty of rapidly cooling large amounts of liquid is eliminated. In fact, since mixing is always much faster than cooling, the reemulsification that takes place during slow cooling cannot occur here.

(3) Larger flexibility in the choice of solvents. The range of primary and secondary solvents that can be used is much greater due to the elimination of the temperature constraint.

Conversely, TIPS has the following advantage over CIPS:

(1) Possibility of multistage processes. Since the same system of solvents is alternately heated and cooled, it is simpler to have multistage extractions. However, this advantage must be weighted against the much higher single-stage efficiency of CIPS.

To conclude, we list the areas of applications where the CIPS process may bring significant advantages. They include the following:

(1) The extraction of a fermentation broth both in batch and continuous processes. This avoids the need to centrifuge and gives better yields due to better contact during the mixing stage.

(2) The extraction of different compounds from natural plant or animal materials. Due to the presence of a single water-rich phase, improved contacting between the solvent and the material to be extracted is achieved. Here there is no water−solvent interface and no wetting problems, and the solvent can easily penetrate the cell walls, thereby increasing the yield of the process.

(3) Replacement of solvents which are environmentally objectionable, e.g. in extraction processes using chlorinated solvents. The CIPS process is more powerful and versatile and also permits the use of solvents which are environment friendly.

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