# Phase Transition Extraction Using Solvent Mixtures with Critical Point of Miscibility

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*A novel separation process, phase transition extraction, proposed in this article uses solvents that have a critical point of miscibility with the feed liquid to be extracted. Alternate heating and cooling across the coexistence curve leads alternatively to a homogeneous phase or two separate phases. The formation of a homogeneous phase in the mixing stage eliminates the need for intense agitation. Coalescence on cooling in the settling stage is rapid in the presence of impurities that in conventional extraction lead to emulsions. A fermentation broth is extracted without a centrifuge with improved recovery of the antibiotic extracted. The process may have a significant advantage for cases in which extraction is difficult either due to formation of stable emulsions and dispersions or shear sensitivity.*

## Introduction

Formation of emulsions and slow coalescence due to impurities is a severe problem in a number of liquid-liquid extraction processes. In some cases the problem can be ameliorated by adding chemical emulsion breakers. In other cases, such as in the extraction of fermentation broth, more extreme measures are required. The presence of cells, finely divided solids and impurities in the broth causes the formation of stable dispersions with most solvents. The common practice today is to break the stable dispersions by using centrifuges. This is not only expensive but can also damage some of the desired products as high molecular weight compounds are often degraded by shear. Alternatively, the broth has to be filtered which is often difficult prior to the extraction process. Furthermore, the filtration can reduce the product yield as part of the products remains in the solids' cake.

This article describes an alternative type of separation process which we call phase transition extraction (PTE). The main idea underlying the PTE process is based on the use of solvents that have a critical point of miscibility. By manipulating the temperature of such solvents across the coexistence curve, one can alternately create regions where the solvents either form a homogeneous solution or they separate into two distinct phases. Hence, the mixing and settling sections of the conventional extraction equipment are replaced in the PTE process by heated and cooled sections.

The formation of a single phase in the mixing section results in a substantially superior contact between the solvents, eliminating the need for intense agitation.

The continuous change in the composition of the phases during the phase separation process prevents the formation of stable interfaces on which solids or emulsion-forming impurities can adhere. Consequently, the coalescence process is very fast, and the process is not sensitive to the presence of impurities or emulsifiers. The method was applied to the extraction of a typical fermentation broth, which in conventional extraction formed completely stable dispersion. Application of the PTE process led to complete coalescence in one minute when the PTE process was applied. Extraction efficiency was simultaneously improved.

PTE could have many interesting industrial applications such as extractions of fermentation-broth or breaking of stable emulsions. It could extend the applicability of solvent extraction to systems where until now it was not viable due to one of the problems mentioned above.

While PTE can be applied in conventional mixer-settlers by adding heaters and coolers, it also allows some novel design concepts for extraction columns with a large number of effective stages which is not practical in present columns. It also allows the design of multistage columns with no mechan-

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ical mixers (Ullmann, 1993). This will be discussed in a separate article. Some features of the PTE process are patented (Ludmer et al., U.S. Patent No. 4,954,260 and Foreign application pending).

This article deals with the investigation of the phenomena that make PTE feasible and presents some practical examples.

### *Liquid-liquid extraction*

Liquid-liquid extraction (LLE) is a crucial step in the manufacturing of a wide range of products and has been extensively studied (Treybal, 1963; Akel and King, 1984; Alegert, 1988).

The LLE process procedure is simple in concept and usually requires contacting of a feed containing the solute to be extracted with a solvent. A basic requirement of conventional LLE processes is a minimal mutual miscibility of the feed and the extracting solvents.

It is important at this stage to recognize certain features of LLE that the present work will seek to improve. The LLE involves liquid-liquid contacting in which the droplets of one phase are dispersed in a second phase and mass transfer has to take place across liquid-liquid boundary. There are several types of contact equipment in industrial use, and their detailed descriptions are given in general works and handbooks (Lo et al., 1983; Treybal, 1963; Schweiter, 1979). Basically, there are two classes of equipments. Those in which each individual stage comprises separate mixer and settler units, and those in which several stages are integrated into one column. The latter can be a simple spray or packed column, or can have stages equipped with various types of mixing devices separated by coalescence sections. The efficiency and the throughput of LLE equipments are, obviously, a strong function of the mass-transfer and coalescence rates.

Extraction of slow mass-transfer systems is commonly facilitated by intense mixing that forms small drops and good contact between the phases. However, the shear stress induced by such a mixing can in many cases damage high molecular weight molecules (Hsien-Wen, 1980). In addition, the intense mixing forms fine dispersions that reduce the coalescence rate, or in the presence of surface active impurities may even cause a stable dispersion (emulsion).

For all practical purposes, the coalescence rate and the breakup time of the dispersions limit the throughput of the extraction equipment. Systems that form stable dispersions cannot be handled by conventional extractors.

Emulsion formation is a common problem in the pharmaceutical industry where the desired products are extracted from the fermentation broth by organic solvents. However, it is also common in many other extraction processes where mechanical agitation is used.

### *Properties of solvent systems with a critical point of miscibility*

As our whole approach in the present work is based on using partially miscible liquid solvents, a short review of their properties is in order.

Consider a system composed of two partially miscible liquids. For a substantial range of the system composition at a given temperature, two liquid phases of constant composition will coexist. The variation in the composition of these saturated solutions with temperature is conveniently shown graphically in the so-called miscibility (or coexistence) curves. Composition and temperature usually are the only variables considered since the effect of pressure on liquid phases is negligible (Treybal, 1963).

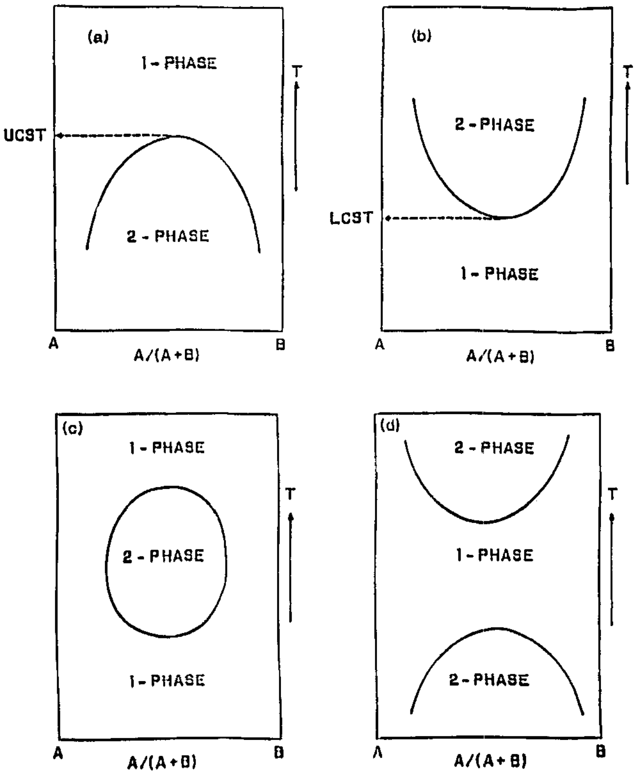

**Figure 1. Equilibrium composition in a two-component system.**

(a) Upper critical solution temperature; (b) lower critical solution temperature; (c and d) both upper and lower critical solution temperature.

Figure 1 describes four types of miscibility curves, where "1-phase" denotes regions of complete miscibility, while "2-phase" denotes regions in which the solution is separated into two mutually saturating liquid phases. The maximum and the minimum temperature $T_c$ of the miscibility curve are called upper critical solution temperature (UCST) and lower critical solution temperature (LCST), respectively.

Liquid-liquid equilibrium data for solvent systems with CST are available in several comprehensive data collections such as Stephen et al. (1979) and Sorensen and Arlt (DECHEMA Chem. Data Series V/1, 2, 3, 1979–1980). More than 6,000 critical solution temperature observations are listed by Francis (1961, 1963) and more than 300 involve water as one of the solvents.

The addition of even a small amount of a third component to a two-component liquid system may alter the CST considerably. Typically, when the third component is much more soluble in one of the binary mixture components, its addition raises the UCST and lowers the LCST (Hales et al., 1966). When the third component distributes roughly equal propor-

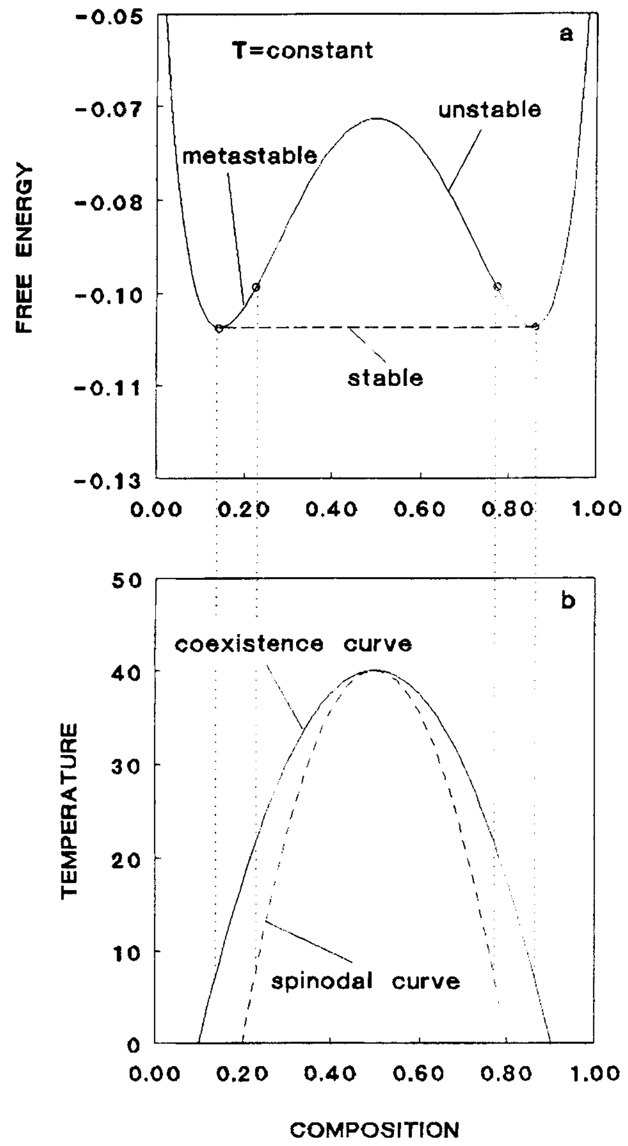

**Figure 2. (a) Gibbs energy of binary mixtures as function of composition; (b) coexistence curve and spinodal line of binary mixture.**

tions between the two components, its addition tends to lower the UCST and to raise the LCST (Snyder and Eckert, 1973).

The mutual solubility of solvents and the critical solution temperature phenomena can be examined by using the classical phase-equilibria thermodynamics and performing thermodynamic stability analysis. A state of equilibrium is characterized as having a minimum Gibbs energy at a given temperature, pressure and composition. Thus, a mixture of solvents will split to separated phases whenever the total Gibbs energy of the heterogeneous solution is less than that of the homogeneous solution (see Figure 2). The point of critical solution (the CST) is mathematically characterized as having $\partial^2 G/\partial X^2 = \partial^3 G/\partial X^3 = 0$ (where $G$ is the Gibbs free energy and $X$ is the system composition). This behavior of binary mixtures, as well as the generalization to multicomponent mixtures, was described by Lupis (1983).

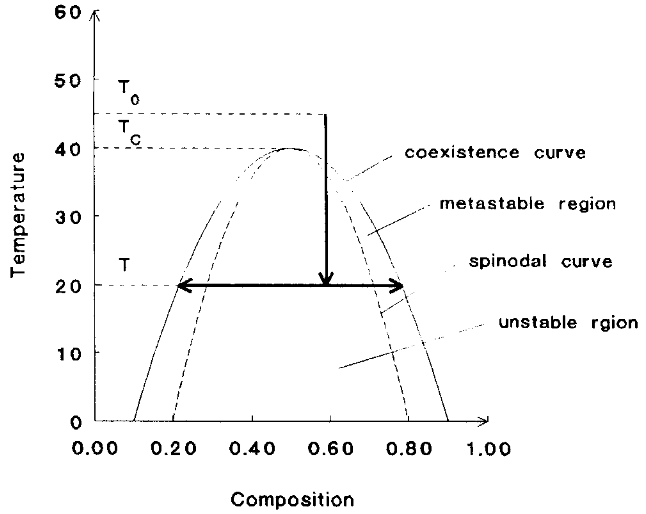

**Figure 3. Temperature quench into the metastable region of CST forming mixture.**

### *Dynamics of phase transition*

Quenching of a binary mixture from a state at temperature $T_0$ in the one-phase region to a state at temperature $T$ inside its coexistence curve (see Figure 3) will eventually result in the formation of two phases at thermodynamic equilibrium. The dynamic process in which the system evolves from its initial nonequilibrium and unstable state to its final equilibrium state is highly nonlinear. In spite of extensive experimental and theoretical studies, a first principle understanding of this process does not yet exist.

According to the classical theory of first-order phase transition, the region inside the coexistence curve is divided into two distinct types of instability. The first is the metastable region in which $\partial^2 G/\partial x^2 > 0$ and the second is the unstable region where $\partial^2 G/\partial x^2 < 0$. In the metastable region only finite concentration fluctuations larger than a critical radius are able to grow (nucleation). In the second region, where the system is thermodynamically unstable, nonlocalized concentration fluctuations of infinitesimal amplitude can grow. With this distinction, two different phase-transition mechanisms were considered (Binder, 1980): the "spinodal decomposition" in the unstable region is the growth of long-wavelength (delocalized) concentration fluctuations with time; in the metastable region "nucleation" of (localized) microdomains of the other phase starts the phase transition. According to the classical theory, the metastable and the unstable regions are sharply separated by the spinodal curve that is the locus of the points inside the coexistence curve for which $\partial^2 G/\partial x^2 = 0$. However, recent research suggests that there is in fact no sharp distinction between such states (Gunton et al., 1983). A smooth transition zone between the two states (a spinodal nucleation zone) has been suggested by Binder (1984) and supported by the experimental findings of Jayalakshmi et al. (1992).

Many attempts have been made to develop theories of the various stages of the phase separation. Some of the studies were summarized by Gunton and collaborators (1983).

First-order phase transitions were first studied in alloys using X-ray techniques (Rundman and Hilliard, 1967), and only more recently, experiments have been conducted on binary liquid mixtures (Huang et al., 1974; Chou and Goldburg, 1979, 1981; Wong and Knobler, 1978, 1981; Beysens et al. 1987, 1988, 1991). The interdiffusion constant $D$ in fluids is normally several orders of magnitude larger than in alloys. Thus, the time scale for the spinodal decomposition is very small. However, near the critical point the diffusion rate is very low and the spinodal decomposition occurs on an experimentally observable time scale. Similar time scales were achieved using various high viscosity polymer blends (Cumming et al., 1992; Katzen and Reich, 1993).

Most investigations of coalescence have dealt with binary mixtures very near the critical point (few mK below the CST), while we are dealing here in temperature quenches of 5–10K. Thus, most of the published theory does not apply to our case. Moreover, there are no published data on the influence of surface-active solutes on the coalescence rate that is of main interest in our study of the phase transition extraction process.

As the coalescence rates in the presence of stabilizing compounds cannot be predicted based on data or studies of clean solvent systems, we are not bringing here a detailed description of the previous studies. For our purposes it is sufficient to note that, as reported above, the spinodal decomposition far from the critical point is a fast process.

The phase transition approach was previously tested by Murata et al. (1972) and Hong et al. (1979) for batch extraction of metals (molybdenum and iron). However, the authors have not tested, and therefore not considered, the coalescence rates in the presence of emulsifying substances. Thus, no significant advantage was noted.

## Phase Transition Extraction Concept

Based on the properties of mixtures with critical point of miscibility discussed in the previous sections, the PTE novel separation process is proposed.

To illustrate the PTE process, one can consider a conventional countercurrent solvent extraction process using mixer settlers. Instead of an immiscible solvent system, PTE uses a solvent pair with a critical point of solubility. Multicomponent systems that form two phases and have a similar critical point can also be applied. The basic concept of the proposed approach is to induce a phase transition cycle on the solvents by means of temperature change in each stage (see Figure 4).

In order to do so (for example, using UCST system), the equipment is modified in a way that each mixing section is equipped with a heater and each settling section with a cooler. Such modifications can be introduced in a mixer-settler type device (Figure 5) or in a column (Figure 6). Systems with a lower critical point can be used as well by simply exchanging the positions of heaters and coolers. The mixing section will now contain the cooler and the settler will be heated. In the hot (that is, mixing) section, the mixture is heated above the coexistence curve into the single-phase region. In the absence of interface barriers, the formation of a perfectly mixed homogeneous mixture is easy and fast. The rate of this process is much higher than the rate of mass transfer between dispersed droplets and a continuous phase which is the governing mechanism in the mixing section of conventional extractors.

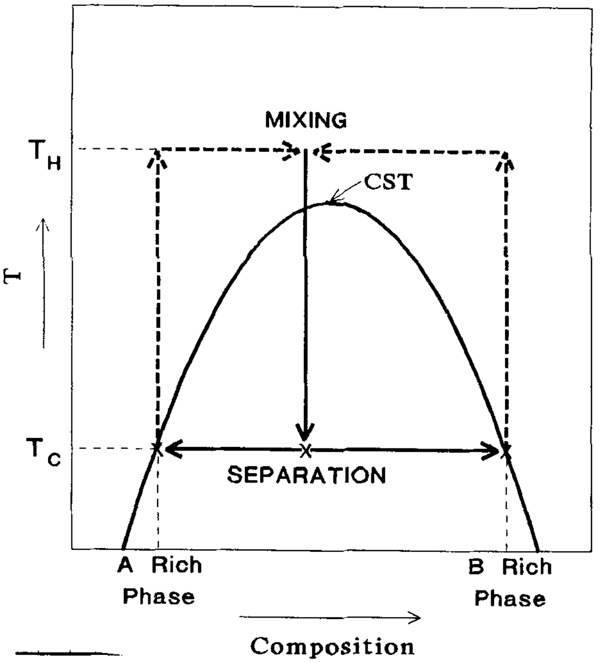

**Figure 4. Temperature-induced phase transition cycle in the PTE process.**

- - -→: heating into the single phase region in the mixing stage; →: cooling and phase separation in the settling stage.

In the cold (that is, settling) section, the mixture is cooled below the coexistence curve into a state of thermodynamic instability leading to coalescence and phase separation. The phases thus separated contain solute and solvents at thermodynamic equilibrium composition corresponding to the system temperature.

For all practical purposes the time scale and therefore the throughput is governed by the phase separation rate in the settler. As will be shown in the next section, in the PTE pro-

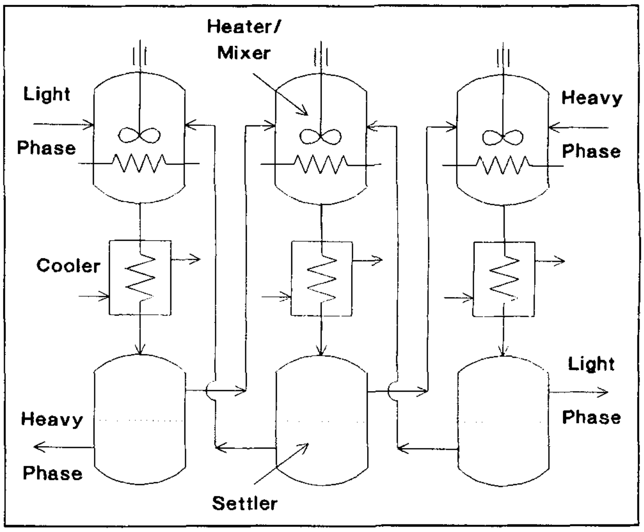

**Figure 5. PTE mixer-settler extractor.**

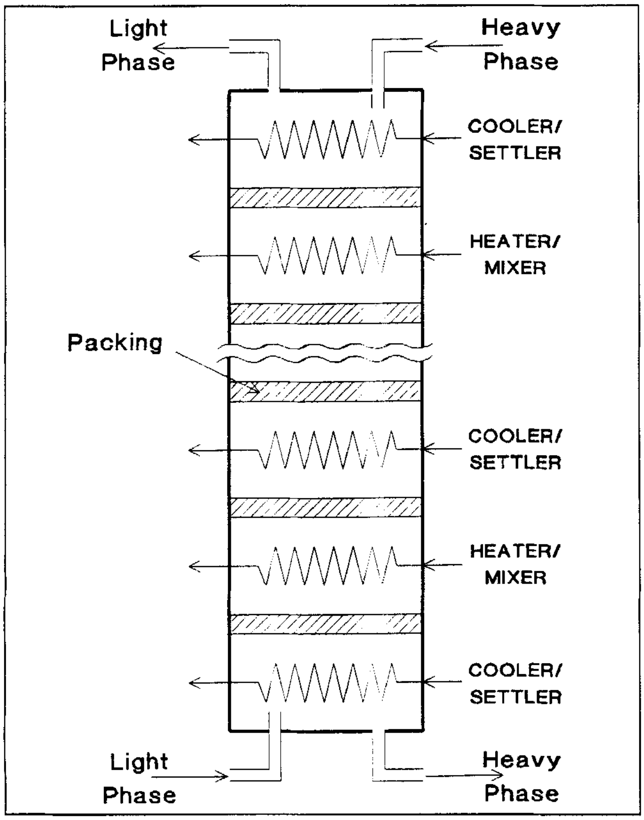

**Figure 6. PTE column.**

cess the rate of coalescence is high even in the presence of impurities that hinder coalescence in conventional extractions.

The selection of solvents for the PTE process involves standard criteria such as capacity, selectivity, availability, physical properties, safety and cost. However, several additional features are important. First, the deviation of the critical temperature of the mixture from the ambient temperature should be small, since a large deviation would make the PTE process energy intensive. Secondly, it is advantageous to have as low a solvent concentration in the feed-rich phase as possible; this will minimize the need for solvent separation by distillation, and will also increase the distribution coefficient of the solute. Thirdly, the temperature change required to achieve significant phase separation should be small.

## Testing Feasibility of PTE Process

In order to demonstrate the feasibility of the PTE process, two critical subjects required testing: (a) whether following the phase transition the solutes are distributed between the two separated phases according to the equilibrium state; (b) whether the rate of the phase separation process is sufficiently fast.

## Experimental Studies

### *Solvents and solutes*

Water was selected as one of the components in most of our experimental solvent systems, since it is frequently used as a feed solvent in many commercial extraction processes especially in biotechnology.

The selection of the solvents and solutes was mainly determined by our need for accurate measurements of the concentrations.

All the solvents were of HPLC (high-pressure liquid chromatography) grade and purchased from Aldrich. Water was distilled twice. Dyes were also purchased from Aldrich and used without any further treatment.

### *Measurement of solute and solvent concentrations*

Dye concentrations were determined by means of a Perkin Elmer UV/VIS spectrophotometer model "Lambda 2" hooked up to a PC computer.

The analysis of the solvent composition was conducted with a "Carl Series 400" gas chromatograph (GC) with a thermoconductivity detector, connected to a Spectra Physics model SP4270 integrator.

### *Determination of phase diagram*

The phase diagrams (the binodal curve and tie lines) of the binary and ternary solvent systems were determined by analyzing the solvents composition in each of the coexisting phases with the gas chromatograph. By varying the overall composition and the system temperature, the entire phase diagram was measured. The equilibrium state of each data point was confirmed by repeating the procedure of mixing the solvents and analyzing the separated phase until undeviating results were obtained. The binodal curves obtained in this way were further validated by employing the cloud point method (a detailed description of this method is given by Treybal, 1963).

### *Microscopic observation*

An experimental setup was designed and built to allow the observation of droplets in the sizes range of 5 to 1,000 $\mu$m. The experimental setup consists of a temperature-regulated sample cell, an optical microscope (Nicon SMZ-2T), and a video (Panasonic CCTV Model WV-BD400) or still camera (Nicon N2020) mounted on the microscope. The temperature in the sample cell is regulated by placing it in a water jacket and circulating water from temperature controlled reservoirs through the water jacket. This arrangement allows us to quench the temperature at the sample cell up to 30°C within a few seconds.

## Results and Discussion

### *Solvents and solute distribution following phase transition*

We studied the solvents and solute distribution following phase transition for two-component (hexane-methanol), three-component (water-acetonitrile-toluene) and four-component (water-acetonitrile-toluene as solvents and crystal-violet dye as solute) systems. In these experiments we first obtained the equilibrium distributions of the components and then compared the solvents and solute distributions following phase transition with the corresponding equilibrium results.

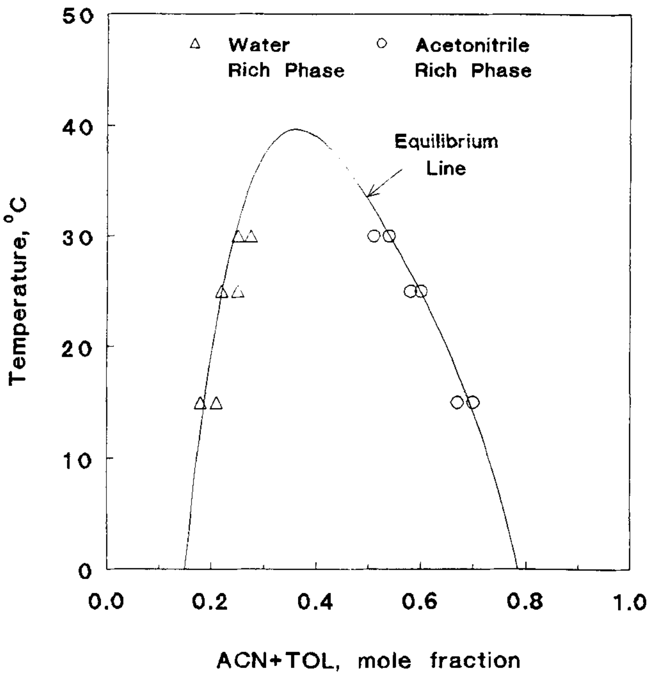

**Figure 7. Solvents composition following a heating and cooling cycle vs. equilibrium phase of acetonitrile-water-toluene system.**

The diagram is based on an overall composition of 0.585 acetonitrile, 0.3711 water and 0.044 toluene (volumetric fraction).

### *Equilibrium solvents distribution — phase diagrams*

*Hexane-Methanol.* The phase diagram and the critical solution temperature, 34°C, obtained in our experiments is in agreement with the data reported by Francis (1963).

*Acetonitrile-Water-Toluene.* Four different toluene concentrations were examined in order to determine the effect of the toluene concentration on the critical temperature. For low concentration of toluene, the change in the CST is nearly linear, about 30°C for each mole percent of toluene dissolved.

For the following experiments, we chose an overall composition of 0.585 acetonitrile, 0.371 water, and 0.044 toluene (volumetric fraction), which is equivalent to molar fraction of 0.347, 0.640 and 0.013, respectively. This composition of the solvents system gives a convenient UCST at 40°C and a sufficient phase separation at ambient temperature. The composition of the organic component (toluene + acetonitrile) as a function of temperature of the above mixture is plotted in Figure 7. For the simplicity of the graphic representation and since the toluene is mainly dissolved in the acetonitrile, the system is considered in this figure as a pseudo two-component, aqueous and organic system (Cohn and Jacobs, 1984). Figure 7 is accurate only for the initial composition mentioned above.

### *Equilibrium solute distribution*

We studied the distribution of the crystal violet dye between the two phases of the acetonitrile-water-toluene system with the above compositions. The solvents were mixed to reach equilibrium at room temperature and then separated into acetonitrile-rich and water-rich phases. A weighted amount of dye was dissolved in one of the phases, and the two phases were mixed again in a funnel to reach equilibrium. The concentration of the dye in each phase was measured by the spectrophotometric method.

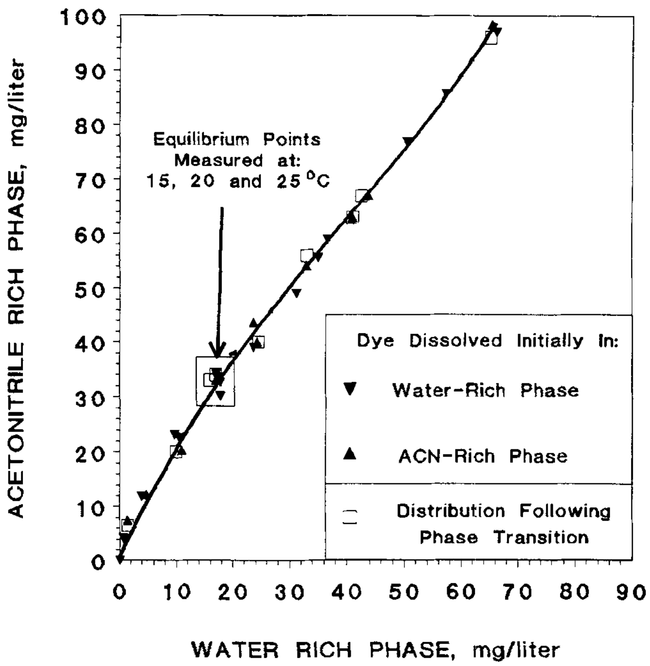

**Figure 8. Solute distribution: by isothermal mixing of solvents (at 20°C) vs. following a heating and cooling cycle.**

The total amount of the dye was calculated, based on the concentrations and the phase volumes, and the result was compared to the amount of dye dissolved. A discrepancy of less than 2% between the measured and the calculated values assured the accuracy of the concentration measurements. The experiment was repeated with increasing quantities of dye, and intermittently once in the water-rich and once in the acetonitrile-rich phases. The results presented in Figure 8 indicate that an equilibrium state was really achieved.

The effect of temperature on the dye distribution was tested by repeating the measurements of one data point at temperatures of 15, 20 and 25°C. Figure 8 shows that the temperature effect is very small, and within the limits of the experimental error.

In addition, no observable effects on either the CST or the phases' composition of the solvent system was detected up to crystal violet concentration of 500 mg/L, which is more than five times higher than the concentration used in our experiments.

### *Solvents and solute distributions following phase transition*

The experimental procedure was similar to that employed to determine the equilibrium distribution data. However, in the present experiments, the conventional isothermal mixing of the phases was replaced by performing a heating and cooling cycle. The results obtained in this way for the hexane-methanol and the acetonitrile-water-toluene systems were compared with their corresponding equilibrium phase diagrams. This comparison showed that within limits of experimental error the separated phases have reached a thermodynamic equilibrium composition corresponding to their temperature. Similar results were obtained for the two systems studied, and, as an example, the results of the experiments with the acetonitrile-water-toluene system are given in Figure 7.

The results for the distribution of the crystal violet dye in the acetonitrile-water-toluene system summarized in Figure 8 demonstrate that the solute concentrations following phase transition are in a good agreement with the equilibrium distribution data obtained by isothermal mechanical mixing of the solvents.

### *Phase separation rate during phase transition*

The rate of phase separation during phase transition was evaluated by measuring the time needed to complete a full-phase separation of CST forming mixtures following a temperature quench. This time was compared with the separation time of the same mixture when isothermally-mixed and separated.

For all the two-component as well as three-component solvent systems studied in clean systems, virtually the same time was required to complete the phase separation following either temperature quench and isothermal mixing. In both cases a complete separation was obtained in less than 1 min.

However, a significant difference in the separation rates was observed when the crystal violet dye was added as a solute to the acetonitrile-water-toluene system. This dye was found to have a strong emulsifying effect on the solvents' system. When the solvents were mechanically mixed with the solute at ambient temperature, a dispersion of very fine droplets was formed, and the phase separation was completed after only seven hours. In comparison, when the heated homogeneous mixture of the same system was cooled to the ambient temperature, the primary phase separation was completed within one minute. However, a haze of fine droplets remained and cleared up only after 20 min. The total amount of solvent droplets in this haze was less then 0.5%, and for a practical separation it would have no importance. This haze was caused by a secondary (late) nucleation, a phenomenon that we will discuss further.

We repeated the experiment with a different dye, that is, violamine R, using the same solvent system. The violamine R was found to have a weaker emulsifying effect on the solvent system, but still sufficient to demonstrate the rapid coalescence phenomenon. Coalescence under isothermal condi-

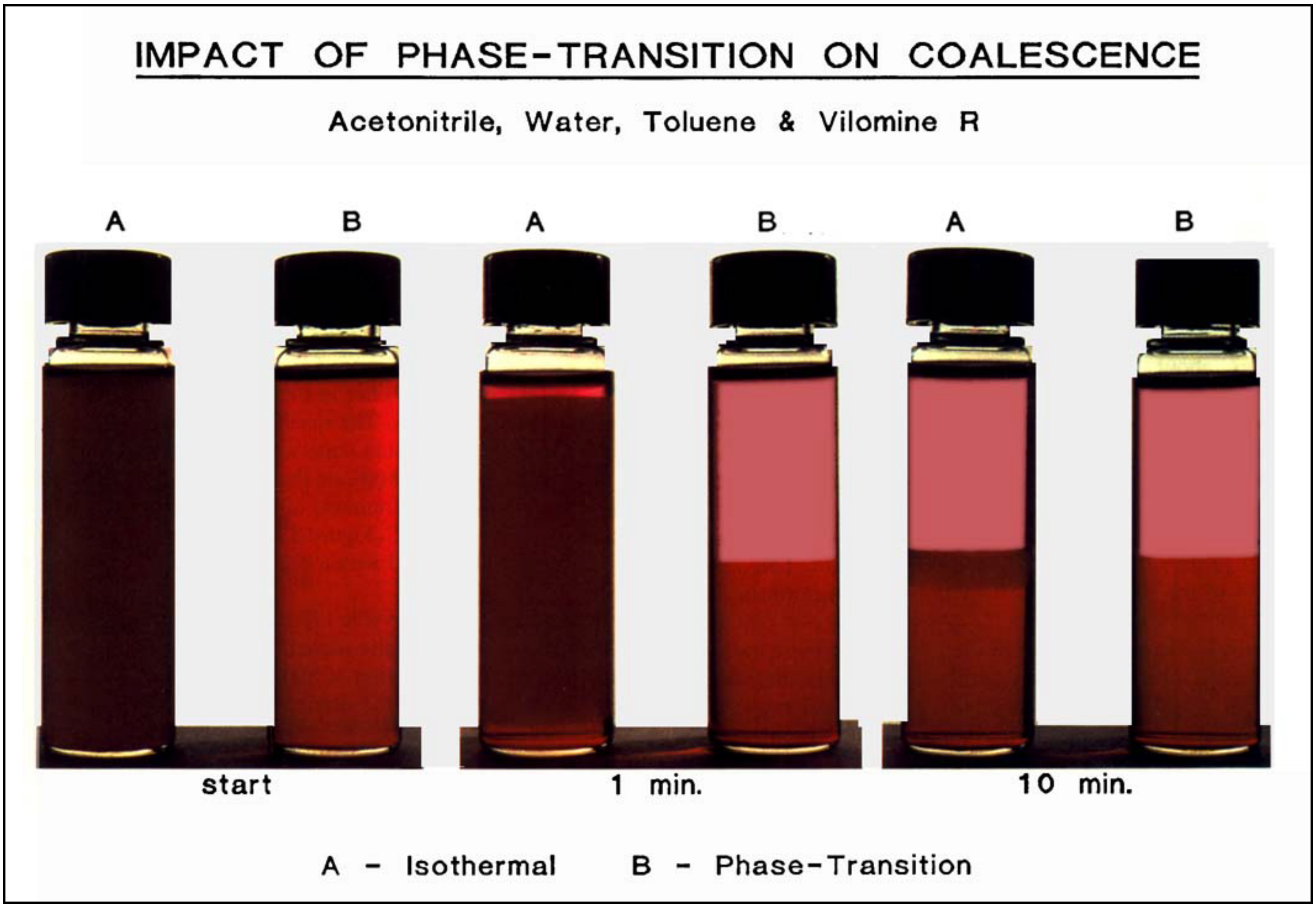

**Figure 9. Phase separation by temperature quench vs. isothermal phase separation in the presence of emulsifying impurities and Violamine-R dye.**

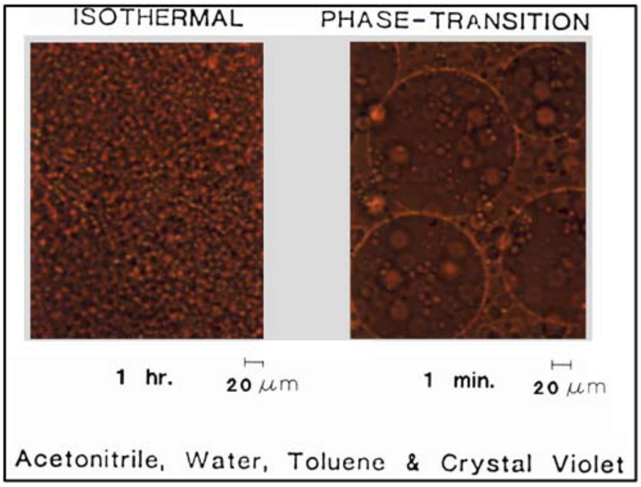

**Figure 10. Direct observation of isothermal phase separation vs. phase separation during phase transition process (acetonitrile-water-toluene system with crystal violet dye).**

tions (25°C) lasted 10 min vs. only 1 min for the phase transition case with no haze formation. This experiment is shown in Figure 9. The vial on the left contains the agitated mixture and the one on the right contains the same mixture that was first heated above the critical temperature, and then cooled down to the room temperature.

### *Direct microscopic observation*

A microscopic observation of the phase transition process provides the ability to follow the morphology of the separating phases, and to observe the motion of the droplets and the droplets interfaces throughout the various stages of the phase separation process.

We have used the microscope setup, described in the "Experimental Studies" section, to follow the phase separation of the acetonitrile-water-toluene system with crystal violet as an emulsifying substance under both isothermal and phase-transition conditions.

In Figure 10 we show microphotographs of the morphologies of the two phase separation processes photographed by a still camera. The left picture in the figure refers to the isothermal phase separation, whereas the right picture refers to the phase transition induced by a temperature quench of 15°C below the CST.

In this figure one can clearly observe the following:

(a) The rate of the droplets growth during the phase transition process is significantly faster than in the case of the isothermal separation.

(b) In the isothermal separation the distribution of the droplets size is relatively narrow, whereas in the phase transition case the droplets vary considerably in size.

The phase transition process was also followed by means of a video camera mounted on the microscope. Following the dynamics of the phase transition revealed a very rapid process of simultaneous growth and coalescence of the droplets (from 10 to 700 $\mu$m within 20 s). This process was accompanied by a continuous formation of new tiny droplets that could explain the wide distribution in size of the droplets (shown also in Figure 10).

### *Extraction of fermentation broth*

One interesting application of the PTE process could be found in the biotechnology and the pharmaceutical industries, namely the extraction of products from fermentation broth.

A common problem in such extractions is the formation of stable dispersions or emulsions due to the presence of impurities, finely divided solids and cells in the broth. Since the phase separation is a crucial step in any extraction process, the rapid-phase separation during phase transition could play an important role.

In order for the PTE process to be applicable for the extraction of fermentation broth the following three criteria have to be met:

(a) Having appropriate solvents that will form a CST mixture with the broth.

(b) A rapid-phase separation.

(c) Efficient extraction of the product from the broth.

In the following section we describe experiments that prove the above criteria can be met demonstrating the advantages of the PTE process for extraction of fermentation broth.

The industrial fermentation broth containing water, cells, and an antibiotic was supplied by "Merck & Co. Inc." The nature and the concentration of the antibiotic, as well as the broth composition, are confidential and will not be described here. In the original process the mixing of the broth with the extracting solvent methyl isobutyl ketone (MIBK) produces a very stable emulsion and the MIBK-broth slurry has to be separated using a centrifugal decanter.

Two solvent systems were studied: the first was based on the MIBK, and the second on propylene carbonate (PC).

The MIBK and the broth (which is mainly composed of water) have very low mutual solubility at ambient temperature. Practically, they can be considered as immiscible solvents, and therefore cannot be used for the PTE process. We modified this system by adding acetonitrile, which has good solubility in both water and MIBK to form a CST-forming mixture. A system composed of 39.6% broth, 12% MIBK and 48.4% acetonitrile (volumetric composition) with upper CST at 40°C was selected for the experiments.

The second solvent studied, the propylene carbonate (PC), was not chosen to be a better extracting solvent, since the modified MIBK was adequate. Our sole intention here was to study the coalescence process of an additional solvent. From this aspect the PC has two interesting properties: (a) PC forms a CST mixture with water without modifiers unlike the MIBK; (b), it has a higher density than water.

The PC possesses an upper CST with water (or broth) at 71°C. The phase transition temperature of the broth-PC system can easily be adjusted to any desired temperature. We reduced the phase transition temperature to 44°C by adding methanol and used a system with the following volumetric composition: 43.1% broth, 50% PC, and 6.9% methanol. Experiments were performed with both the broth-PC and the broth-PC-methanol systems. This allowed us to examine whether the addition of a third component has another effect

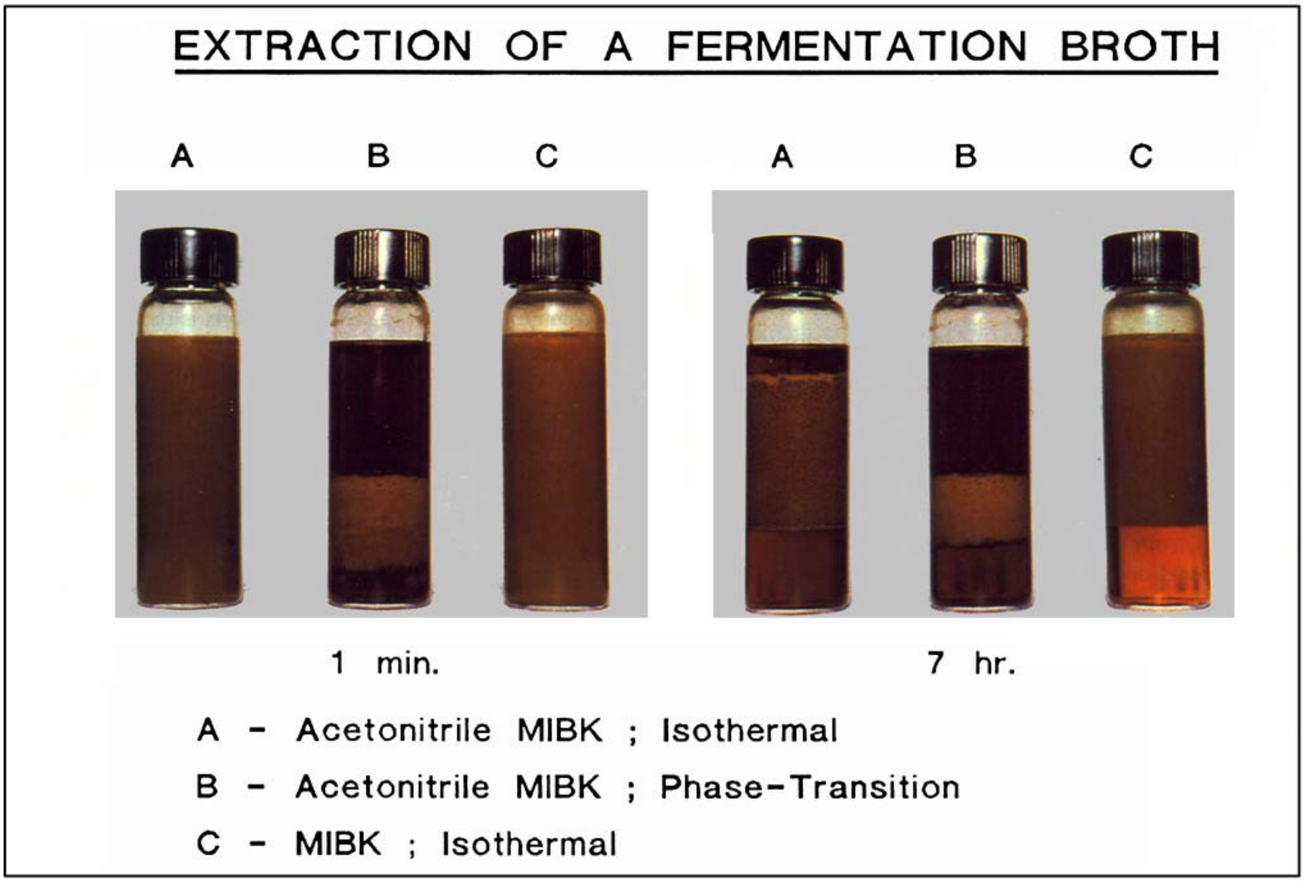

**Figure 11. Phase separation in solvent extraction process (broth-MIBK and broth-MIBK-acetonitrile systems).**

on the process besides the change of the phase transition temperature.

### *Phase separation rate*

*Broth-MIBK and Broth-MIBK-Acetonitrile.* Three operational procedures were examined and compared, and the results are summarized in Figure 11. Vials A and B contain the critical mixture of broth-MIBK-acetonitrile where vial C contains the broth with the MIBK alone—a noncritical mixture. The volume ratio of the organic to aqueous phases is the same in the three vials, 1:1, and the organic phase in all cases is the lighter one. The mixtures in vials A and C were mixed vigorously for 15 min. at ambient temperature (20°C), and then allowed to settle, where the mixture in vial B was heated above its critical temperature, and then cooled down to ambient temperature.

The isothermal mixing of the broth with the MIBK alone (Vial C) led to a dispersion that was very hard to separate. In fact, the separation of the sample in vial C was incomplete after 30 h. Furthermore on separation, both the emulsion and the solids concentrate in this case in the MIBK phase that also contains the desired products. To separate the cells from the extracting solvent and to break the emulsion for any downstream processing (such as precipitation and crystallization), a centrifuge separation is required.

The isothermal mixing of MIBK-acetonitrile with the broth resulted also in the formation of very stable dispersion that separated only after 30 h (Vial A).

In stark contrast, heating the mixture (broth-MIBK-acetonitrile) above its CST and cooling it down to the ambient temperature resulted in a very fast coalescence. A complete separation of the phases was achieved within one min (Vial B). This means that the rate of coalescence increased by more than three orders of magnitude.

The rapid-phase separation is clearly the result of the phase transition process, since an isothermal mixing of the same solvent mixture resulted in a stable emulsion (Vial A). In addition, the rapid-phase separation is not a result of the temperature variation alone since a similar heating and cooling cycle enforced on the emulsified broth-MIBK mixture (Vial C) did not bring any appreciable change in the stability of the emulsion.

The above experiments demonstrated another advantage in performing the extraction via phase transition. After the coalescence is completed, the solids concentrate in the aqueous phase (see Figure 11 Vial B). This allows to perform a multistage extraction when a single-stage extraction is not sufficient, and simplifies the downstream processing of the products.

*Broth-PC and Broth-PC-Methanol.* The experiments with these solvent systems were conducted in a similar way to the experiments with the first system, and the results are shown in Figure 12 for the two-component (PC-broth) system. Vials

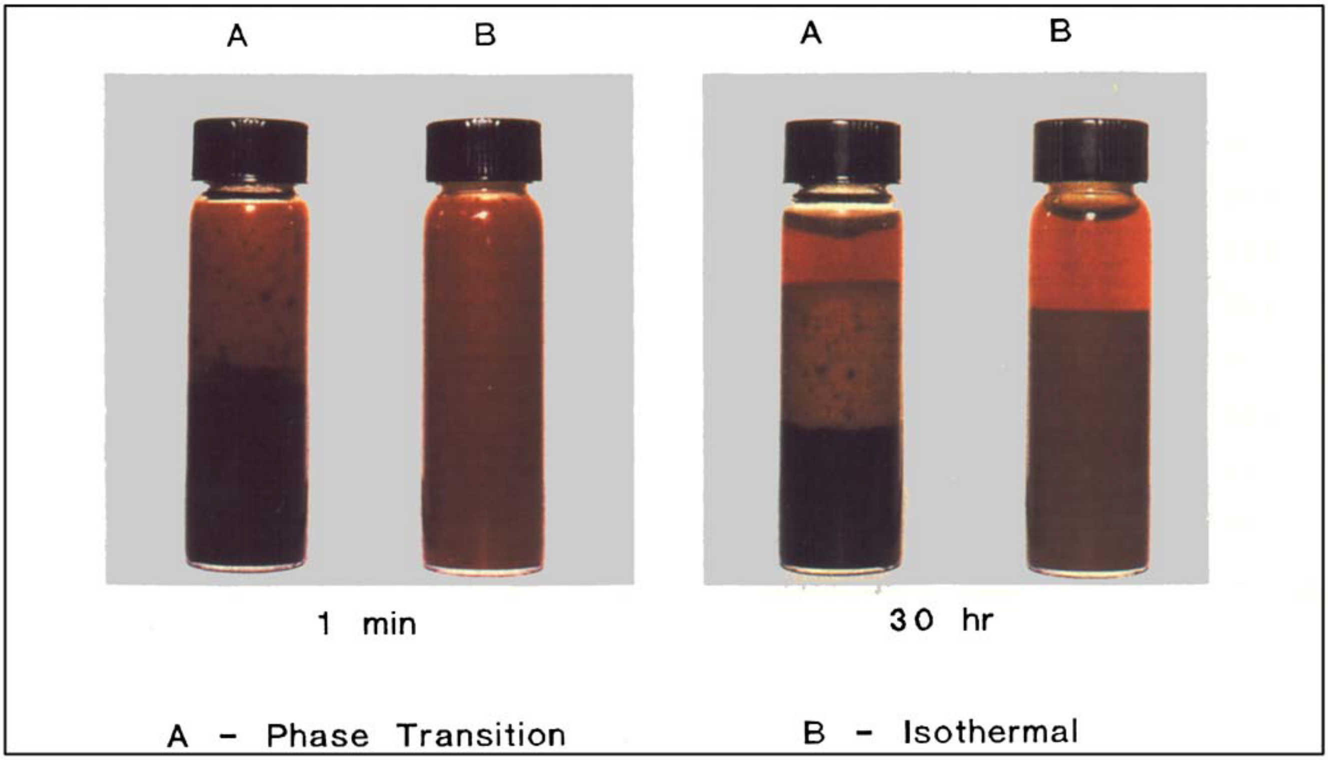

**Figure 12. Phase separation in solvent extraction process (broth-propylene carbonate system).**

A and B contain the same mixture with 1:1 volume ratio of organic to aqueous phase. The mixture in vial A was heated above the CST and then cooled down to the ambient temperature, whereas the mixture in vial B was mixed at the ambient temperature and then allowed to settle.

Isothermal mixing of the solvents (Vial B) led to a very stable emulsion that did not separate even after 30 h. Again, the temperature induced phase-transition (Vial A) resulted in a very rapid phase separation that was completed within one min. Moreover, all the solids concentrated at the aqueous phase (the light phase in this case).

No appreciable differences were observed between the results of the experiments with the two-component and those with the three-component solvent systems. This confirms again that the rapid-phase separation is a result of the phase-transition process and not of the addition of the third component to the solvents system. The only effect of the third component is the adjustment of the CST of the system to a desired value.

### *Extraction efficiency*

The efficiency of the PTE process was studied by extracting the products from the broth by two methods: (a) Isothermal mixing with MIBK (a solvent used by "Merck" in their original process); (b) the PTE process using the MIBK and acetonitrile solvent system.

The product concentrations in the organic (extracting) phases were analyzed by standard procedures using HPLC. In both cases the broth was extracted three times by fresh organic solvents. This was a simple procedure in the case of the PTE, since, as we mentioned above, the solids concentrated in the aqueous phase after every extraction step. However, in the case of extraction by the MIBK following the isothermal mixing the solids had to be filtered in order to facilitate the phase separation. In both cases the extraction was completed after two extraction cycles, and no traceable amount of products was found in the organic phase after the third extraction cycle. However, the extraction by phase transition was evidently significantly more efficient than the conventional extraction by the MIBK (see Figure 13).

The higher products' yield of the phase transition process may be explained by the higher capability of this process to extract the products from the interior of the organism cells. By achieving a complete miscibility of the extracting solvent with water during the single-phase stage of the process, the extraction of the products from the cells containing mostly water may encounter much less resistance at the cell interface than in the case of two immiscible solvents.

### *Rapid phase separation — interpretation of phenomenon*

One of the most important findings of this study is the dramatic high rate of phase separation accomplished by the PTE process even in the presence of emulsifying substances. The rapid phase separation phenomenon has not been previously reported, and has not received a thorough theoretical consideration. A detailed study will have to be conducted in order to fully understand the mechanism of coalescence in the presence of impurities. However, some interpretation of the phenomenon can be suggested based on the dynamics of the phase transition process as presented above.

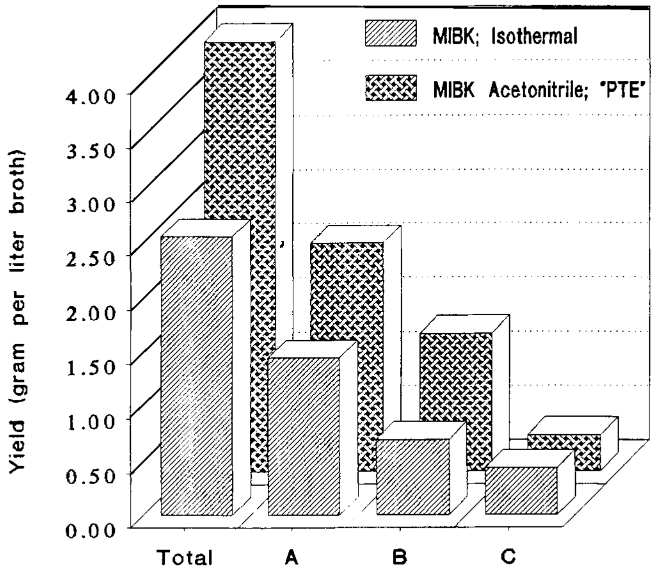

**Figure 13. Amount of components extracted from the fermentation broth: PTE vs. conventional process.**

A, B, and C, on three different products.

An essential condition to the stabilization of dispersions or the emulsion formation is the presence of emulsifiers, such as impurities, surface-active agents (surfactants) or finely divided solids, in the liquid-liquid system. Basically the dispersion can be stabilized by the formation of either electrical double layers (charge stabilization) or protective layers on the droplet surface (stearic stabilization). The stabilizing mechanism is determined by the emulsifier type (see a review by Becher, 1983).

Charge stabilization is caused by the adsorption of ionic surfactants or polyelectrolytes at the droplets interface. The increase in the coalescence time is caused by the formation of electrical double layers on the surface of the droplets. The electrical double layers tend to retard the draining of the liquid film due to the attraction force of the opposite charges at the interface and the flowing film (electroviscous effect). Furthermore, the repulsion of the equally-charged droplets prevents the flocculation of the droplets (Tadros and Vincent, 1983).

In the presence of nonionic emulsifiers, polymers or finely divided solids, the stearic mechanism of stabilization dominates. Polymer molecules that adsorb on the liquid-liquid interface can form a protective layer (Shinnar, 1961). These protective layers generate a repulsive force between the droplets and prevent the flocculation (Heeler and Pugh, 1954). Moreover, the macromolecular film formed at the interface in view of its viscoelastic properties can provide a "mechanical" barrier to coalescence (Bikerman, 1958). In a similar way, solid particles adsorbed on the droplet interface can form a continuous monoparticulate film, thus making the coalescence more difficult, essentially by keeping the droplets from coming into close contact (Tadros and Vincent, 1983).

Regardless of the emulsifier type or stabilization mechanism, a basic requirement for the stabilization is the adsorption of the emulsifier on the droplet interface. Effective stabilization requires a full coverage of the droplet's interface by the adsorbed substances. It will, therefore, be reasonable to assume that if the adsorption of emulsifiers on the droplet's interface is prevented, or if the rate of droplet growth is faster than the rate of emulsifier adsorption, the stabilizing effect will be negligible.

We can now try to determine the effect of emulsifiers on the rate of phase separation of critical mixtures during a phase-transition process.

In the first stage of the spinodal decomposition, the unstable mixture is separated via a very fast exponential growth of nonlocalized concentration fluctuations (Gunton et al., 1987). In this stage there are no stable or well-defined boundaries between the different phases at which emulsifiers can adsorb.

As described by Gunton (1987) and also observed by us, in the later stage of the phase transition process, the initially very diffused boundaries between the phases are sharpening with time until well-defined regions are formed. The growth mechanism of the separated domains combines coalescence of droplets and growth of individual droplets by mass transfer. Here, the mass transfer of the solvents into the growing droplets, if fast enough, may prevent formation of a stable boundary and thereby reduce the chances of stabilization by the emulsifiers.

The rate of the droplet's interface coverage by the emulsifiers is determined in this stage by the ratio between two competing mechanisms, namely the rate of the emulsifier adsorption, and the rate of the droplet's growth by mass transfer that causes an expansion of the droplet surface area. If the rate of droplet growth is sufficiently higher than the rate of the surfactant adsorption, then the coverage of the droplet's surface by emulsifiers will be prevented, and consequently the coalescence and the phase separation processes will be rapid.

The fact that solids (or cells) do not adhere to the droplet's interface during the initial stages of the phase transition separation is well demonstrated in the experiments conducted with the fermentation broth. The sequence of the pictures in Figure 14 displays the start of the phase separation of the broth-PC-methanol system. The first picture (Figure 14A) shows the critical mixture heated to a temperature above the CST. The organism-cells and solids from the fermentation-broth float in the single-phase solvents mixture that has a specific density $\rho \approx 1.11$. Following the phase separation, the solids become heavier than the light, aqueous phase ($\rho \approx 1.04$), but lighter than the organic phase ($\rho \approx 1.19$). They sink and concentrate on the main interface (Figure 14C). However, in Figure 14B, which was taken a short time after the mixture was cooled to ambient temperature, it can be clearly seen that the main phase separation is completed without any appreciable change in the bulk of the solids at the top of the vials. Since the phase separation during the phase-transition is not localized and occurs all over the solvent bulk, any adherence of solids to the droplets' interface during the phase separation will result in sweeping of solids to the bulk of the lower (organic) phase or at least to the main interface. The undisturbed solids bulk therefore serves as evidence that during phase transition the adherence of the cells to the interface and the consequent stabilizing effect are prevented.

As we have mentioned already, the primary phase separation during the phase transition process was fast for all the systems studied in our experiments. However, in the acetoni-

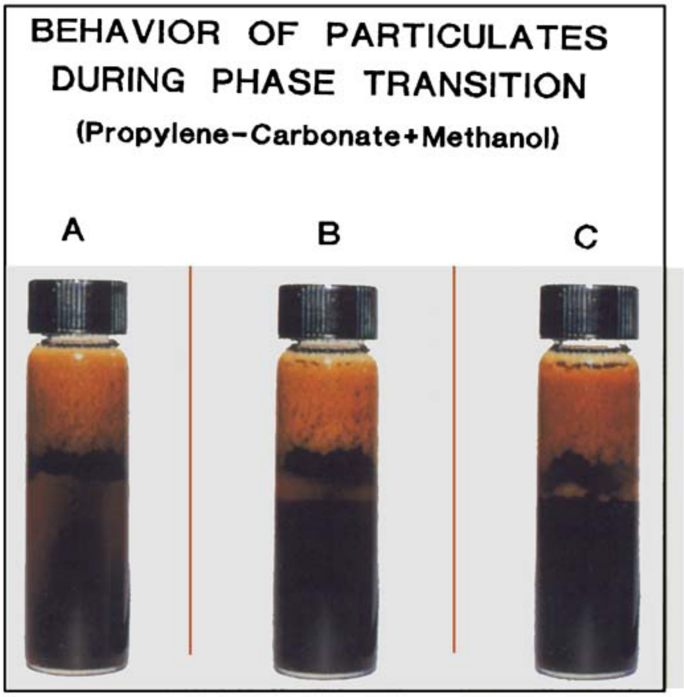

**Figure 14. Solid-phase behavior during PTE of fermentation broth (broth-propylene carbonate-methanol system).**

trile-water-toluene system with the crystal violet dye following the primary separation some fog-like dispersions (secondary dispersions) remained in the bulk of the separated phases and settled down after 20 min. The secondary dispersions have a minor effect on the extraction efficiency since they contain a very small amount (less than 0.5%) of the main phase volume.

An appearance of secondary dispersions was also reported for isothermal mixing of immiscible pure solvents (Treybal, 1963). However, in our experiments the secondary dispersions should be related to the presence of emulsifiers, in this case the crystal violet, since it was not observed during the phase separation of the pure solvents.

One question that arises is how would our results scale up to larger systems. In our small samples cooling was rapid. In a large tank cooling would be slower. However, if the homogeneous mixture is pumped through a heat exchanger to a settler, cooling occurs within several seconds. Other arrangements would require further study.

A better understanding of all the aspects of the rapid phase separation phenomenon requires a thorough experimental and theoretical work. Such work is now in progress, but is outside the scope of the present study.

## Conclusions

The validity of the basic concepts underlying the PTE process has been proven through a series of batch experiments, which showed that following the completion of a heating and cooling cycle, the separated phases established a thermodynamic equilibrium composition corresponding to their temperature. The experiments have also demonstrated that low mixing intensity is sufficient to obtain a homogeneous solution in the single-phase region.

The rapid coalescence and phase separation of solvent systems undergoing a temperature induced phase transition have been demonstrated for several two-component and three-component systems. It has also been shown that such a phase separation has very low sensitivity to any presence of emulsifying substances. The absence of well-defined interfaces between the separating phases during the phase-transition process seems to prevent the adsorption of emulsifiers on the droplets' interface. Consequently, the stabilizing effect of the impurities is neutralized. This rapid-phase separation is of a great importance in performing extraction of systems that are sensitive to the formation of stable emulsions.

A possible application of the PTE process was demonstrated by applying it to the extraction of a fermentation broth. Here, the extraction of the broth by phase transition process has some considerable advantages over conventional extraction processes, these are:

(a) The formation of stable emulsions, one of the crucial problems of broth extraction, is prevented without the need to either filter the broth prior to the extraction process, or to use expensive and complicated centrifuges.

(b) The rapid-phase separation and the concentration of the solids in the aqueous phase allow a convenient multistage extraction and also simplifies any downstream processing of the products concentrated in the organic phase.

(c) Bringing the solution to conditions of full miscibility between water and solvent substantially reduces the interface resistance to extraction of the product through the cell's interface. Consequently, a high product yield is obtained without the need for a prior breaking of the cells and a severe mixing of the broth with the extracting solvent.

(d) Eliminating both the need for intense mixing in the contacting stage of the process and the use of centrifuges in the settling stage prevents any possible shear-stress damage to large molecules.

At this point, it is important to present answers to problems that may rise regarding the use of PTE for the extraction of fermentation broth:

(1) The need for solvents that form a critical mixture with the broth (or water) may seemingly restrict the number of available solvents for the PTE process.

However, as previously mentioned, there is a considerable number of solvents that form a critical mixture with water, and therefore can be used for the PTE process. In addition, as we demonstrated for the broth-MIBK system, one can add an appropriate solvent to modify the original solvent system. Thus, almost all solvents that are currently used in conventional (or centrifuge-based extractions) and only slightly soluble in water can be integrated into mixtures with a critical point of miscibility.

(2) The heating of the mixture, while performing the PTE process, may damage heat-sensitive compounds.

Here again by the addition of an appropriate solvent the phase-transition temperature of the solvent system can be adjusted to any desired level so as to eliminate the problem. Furthermore, for very sensitive systems, one could even adjust the critical point to a level lower than the ambient temperature and use refrigeration.

(3) The variation of the solvents' temperature across the

miscibility curve requires a higher energy consumption for heating as compared to the energy required for mixing in conventional extraction processes.

The energy requirement is indeed larger than for conventional extraction. However, for the heating and cooling, only low grade energy is required. The portion that is relevant is the energy consumed for solvent recovery by distillation. That portion is modestly larger than the corresponding amount required in conventional extraction. This added energy cost will have to be weighted by the designer against the advantages the PTE process offers.

We may conclude here that the rapid-phase separation, along with the high efficiency of the PTE process, provides a flexible and powerful tool for performing difficult separations in the biotechnological and pharmaceutical industries. It offers exciting opportunities to perform complex separations that are at present neither economical nor feasible. Since these properties of PTE, especially rapid separation, have not been known until now, we hope that our work will stimulate new applications.

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