Tunable Solvents for Homogeneous Catalyst Recycle

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A novel class of tunable solvents facilitates recycle of homogeneous catalysts for both economic and environmental advantages. In a mixed organic−aqueous tunable solvent (OATS), reactions between water-soluble catalysts and moderately hydrophobic substrates can be run homogeneously, with subsequent phase separation for product purification and catalyst reuse. One example demonstrated is the dimethyl ether (DME)−water system, which has been employed as a benign alternative to organic solvents for alcohol dehydrogenase (ADH)-catalyzed reduction of hydrophobic ketones coupled with regeneration of the cofactor NADH. We also show the feasibility of a biphasic DME−water separation scheme to couple with the biocatalytic reaction. The subsequent downstream processing offers the advantages of easily isolating water-insoluble products from the aqueous phase and recycling enzyme−cofactor. Other OATS systems are discussed where the preferential dissolution of modest pressures of CO_2 causes phase separations, which result in very large distribution coefficients of target molecules in the biphasic organic−aqueous system, with substantial promise as tunable solvents for biocatalysis.

Introduction

Homogeneous catalysts are generally more active and more selective than heterogeneous catalysts, and further they preclude any need for mass transport of reactants and products to and from the site. They are especially useful for chiral synthesis because they almost always result in superior enantiomeric excesses. However, a major barrier to their use is that they are often very expensive and/or toxic: they must be separated from the products.

Many researchers have tried various strategies to ameliorate this limitation (for example, tethering the catalyst), and many of these are effective in certain situations.[1] What we relate here is a phase equilibrium approach: run the reaction homogeneously in a single phase and then cause a phase separation to give a heterogeneous system for product recovery and catalyst recycle. Such a phase change can be brought about in a variety of ways: by changes in composition or temperature or by addition of an antisolvent.

A further issue for many reactions is to choose a solvent system that permits dissolution of all reactants as well as catalysts. Often, one of these is hydrophobic, and one is hydrophilic. We and others have used tunable solvent systems to deal with these issues, such as phase-transfer catalysis and near-critical water.[2−4] Here we develop mixed-solvent systems of water with an organic for potential applications for enantioselective biocatalysis of hydrophobic substrates.

Pharmaceutical compound manufacture is often limited by the solubility of a substrate in aqueous solutions. Basically, we are working with hydrophilic enzymes, which are inactive without water present, and hydrophobic substrates, where neither the reactant nor product has sufficient solubility in water. Moreover, because enzymes are generally expensive, it is common to reuse them to achieve turnover numbers above 10^6.[5] Also, the difficulties of downstream separation and/or purification of the product and the lack of biocatalyst with suitable stability/activity in organic solutions pose a significant challenge for the commercial production of promising therapeutic agents. A benign solvent with some organic character would facilitate solubility of hydrophobic molecules such as drug candidates or their precursors while retaining an environment suitable for biocatalysis. In the context of bioprocessing, a further challenge is the recovery of the biocatalyst and separation of the substrate and product.

The desirable characteristics of a tunable solvent system include the following: (1) readily implemented phase change; (2) chemically inert, inexpensive, benign, and safe; (3) good solubility characteristics for the reaction and ready dissolution of nonpolar, polar, and ionic materials; (4) lopsided composition distribution after phase change; (5) facile downstream processing and recycle.

We discuss in this paper three possibilities of achieving a viable separation: first, a very volatile organic component, easily removed from water; second, a temperature-induced phase change; third, a phase change induced by an antisolvent, such as CO_2.

Here we show as an example of the dimethyl ether (DME)−water solvent system for the reduction of ketones with alcohol dehydrogenase (ADH) to yield enantiomerically pure alcohols as building blocks for pharmaceuticals.[6−8] Reactions with horse liver alcohol dehydrogenase (HL-ADH) in more hydrophobic organic solvents have been investigated by the groups of Jones[9] and Klibanov.[10]

Experimental Section

ADH [EC 1.1.1.1] from horse liver, formate dehydrogenase (FDH) [EC 1.2.1.2] from *Candida boidinii*,

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β-nicotinamide adenine dinucleotide (NAD^+), reduced disodium salt (NADH), and all other chemicals were purchased from Sigma-Aldrich and used as received. DME (99.5% purity) and SFC grade carbon dioxide (99.99% purity) were provided by Matheson Gas Products. The CO_2 was further purified to remove trace water using a Matheson (model 450B) gas purifier and filter cartridge (type 451). High-performance liquid chromatography grade tetrahydrofuran (THF; 99%), 1,4-dioxane (99%), acetonitrile (99%), and water (99%) were obtained from Aldrich and were used as received. UV−vis spectra were measured in a high-pressure titanium cell with sapphire windows. The temperature of the cell was controlled to ±0.1 °C (Omega Inc.), and the pressure was monitored with an uncertainty of 0.01% in the range of 0−20.7 MPa (Druck Inc.). A Hewlett-Packard 8453 diode-array UV−vis spectrophotometer (Agilent Technologies, Inc.) was used for spectral measurements and processing. Cofactor stability and reaction kinetics were investigated based on assays of NADH at a wavelength of 340 nm, following the disappearance/appearance of NADH as a function of time. Assuming zero-order kinetics at the initiation of the reaction, rate constants (k_{cat}) were calculated based on the initial rate, purity of the enzyme, and number of active sites per molecule ($n = 2$). Solubility and partitioning measurements were carried out in a stainless steel stirred autoclave with two quartz windows (Parr). The temperature in this cell was regulated using a Parr controller to within ±1 °C of the setpoint. Agitation was maintained at 500 rpm for at least 2 h prior to sampling. A dip tube attached to a sample loop of known volume was installed to take samples from a single liquid phase. For the measurements in biphasic systems, two dip tubes were applied for simultaneous sampling of both liquid phases. Analysis of solute concentrations was achieved using a Hewlett-Packard (model 6890) gas chromatograph with a flame ionization detector.

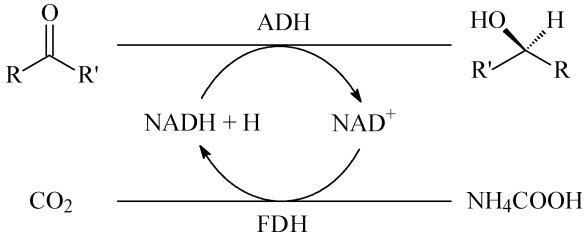
The ternary water−organic−carbon dioxide phase equilibria were measured using a variable-volume view cell. The procedure followed is similar to that of Laugier et al.[11] The volumes of all phases are measured, and with three components, three phases, and a fixed temperature and pressure, there are 0 degrees of freedom; thus, the concentration of each phase will be independent of the overall concentration. A minimum of three loadings of different composition is necessary to calculate the composition and molar volumes of the three phases from the measured volumes of each phase and the overall composition. In this experiment, five loadings were performed for greater precision.

Additionally, the composition and molar volume of the vapor phase were assumed from known data. Because one of the liquid phases is mostly water, the partial pressure of water in the vapor phase was assumed to be the vapor pressure, and the composition of the other two components was predicted from correlated binary data. The molar volume of the vapor phase was assumed to be that of pure CO_2 because the vapor composition is never less than 98% CO_2.

Results and Discussion

I. Highly Volatile Organic Component. One example of this class is the DME−water tunable solvent system. DME has a normal boiling point of −24.8 °C and is a compressed liquid at ambient temperature (e.g., vapor pressure is 0.69 MPa at 30 °C).[12] DME is an organic with polarity and basicity similar to those of acetone but is far easier to remove. DME and water have 12−14% mutual solubility at ambient temperature.[13] DME is relatively safe compared to most other ethers, which can form explosive peroxides. Therefore, we used DME−water mixtures for homogeneous biocatalysis involving a relatively water-insoluble substrate. DME−water may be superior to traditional organic solvents such as toluene or alcohols in terms of a compromise of hydrophilicity and hydrophobicity to optimize biocatalytic processes. After reaction, the DME-rich phase, containing the product, is separated from the aqueous phase, retaining the catalyst for reuse. Product recovery is facilitated by simple depressurization and vaporization of the DME, which can be subsequently recondensed for reuse.

Scheme 1. ADH-Catalyzed Reduction of Ketones with NADH Regeneration



Scheme 1 describes NADH-dependent reduction of ketones with HL-ADH, coupled with a regeneration of cofactor NADH catalyzed by formate dehydrogenase (FDH).[14] NADH−NAD^+ are cofactors functioning as a hydrogen transfer agent. Formate is the substrate in regeneration of NAD^+ to NADH; ammonium formate also acts as a buffer.

Solubility Enhancement in Monophasic DME−Water. Figure 1 gives solubility data for the water-insoluble model substrate 4-*tert*-butylcyclohexanone in pure water and in a monophasic system of water with different amounts of DME at 30 °C. The solubility of the hydrophobic component can be enhanced noticeably by adding a small amount of DME to water. For example, the solubility is increased by factors of 3.8 and 5.6 in the presence of 7 and 12 mol % DME in water, respectively.

Stabilization of NADH. To evaluate the feasibility of a biocatalysis process, we determined the solvent effects of DME on NADH stability. The decomposition of NADH follows first-order kinetics.[15] The half-life ($t_{1/2}$) of NADH was calculated based on the decomposition rate constant in water and in DME−water mixtures of

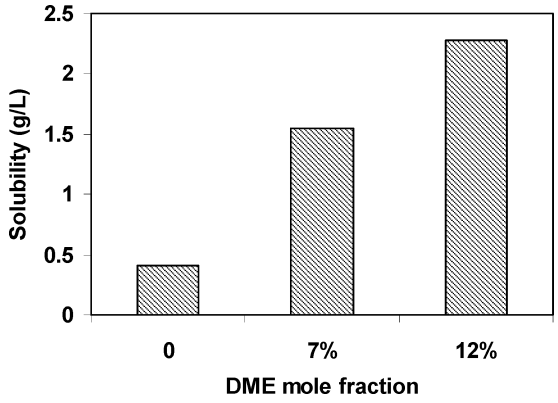


Figure 1. Solubility of 4-*tert*-butylcyclohexanone in monophasic DME−water mixtures at 30 °C.

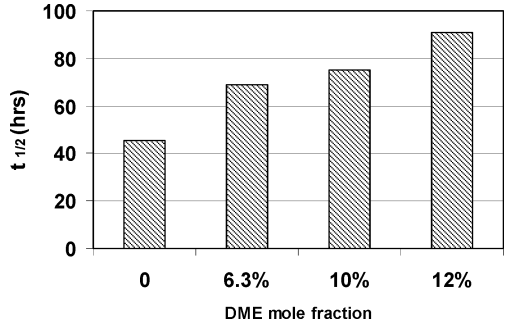


Figure 2. Half-life of NADH in DME−water mixtures at 30 °C (20 mM NH_4COOH; pH 6.5).

Table 1. Relative Rate Constants of HL-ADH-Catalyzed Ketone Reduction at 30 °C

	cyclohexanone	2-hexanone	acetone
water	1.0[a]	6.9×10^{-3}	7.7×10^{-3}
water−7.3% DME	6.9×10^{-2}	3.3×10^{-4}	4.3×10^{-4}
water−8.8% DME	7.4×10^{-2}		

[a] k_{cat} for cyclohexanone in water is 12.3 s^{-1} (pH 6.5). Initial concentrations (mmol/L): HL-ADH, (4−9) × 10^{-4} (~0.5 U/mg); NADH, 0.16; NH_4COOH, 100; acetone, 16; 2-hexanone, 11; cyclohexanone, 27.

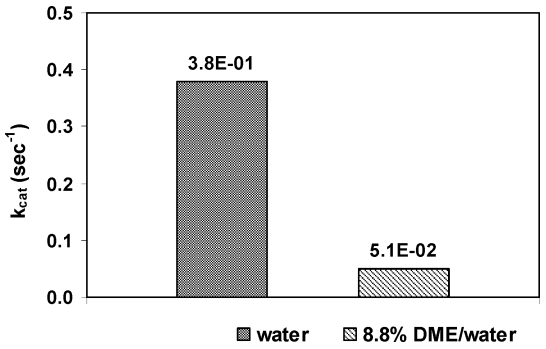


Figure 3. Enzymatic activity (k_{cat}) for FDH-catalyzed reduction at 30 °C (pH 6.5 in water). Initial concentrations (mmol/L): FDH, 1×10^{-3} (0.4 U/mg); NAD^+, 1.7; NH_4COOH, 100.

different compositions, as shown in Figure 2. As an example, in the presence of 12 mol % DME, a 2-fold increase in the half-life was observed compared to that in pure water. This suggests that the stability of NADH increases as the mole fraction of DME in the aqueous phase increases; DME may stabilize the valuable cofactor.

Activity of HL-ADH. The activity of HL-ADH in a monophasic DME−water system was investigated based on the reduction kinetics of ketones (acetone, 2-hexanone, and cyclohexanone). The calculated rate constants (k_{cat}) are expressed in relation to the rate constant in pure water for cyclohexanone, the best of the three substrates for HL-ADH (Table 1). This indicates that there is about a 10-fold reduction in the rate constant when the reaction is conducted in DME−water mixtures for all three substrates.

NADH Regeneration. We also investigated the regeneration of a cofactor catalyzed by FDH, by following the appearance of NADH under saturation conditions of NAD^+ and ammonium formate. As expected, zero-order kinetics in both concentrations of NAD^+ and NH_4COOH was observed, and the catalytic rate constants (k_{cat}) were calculated in water and in DME−water, as shown in Figure 3. The enzyme activity in a 8.8 mol % DME−water mixture is 7.5-fold reduced with respect to that in pure water. The reaction proceeds

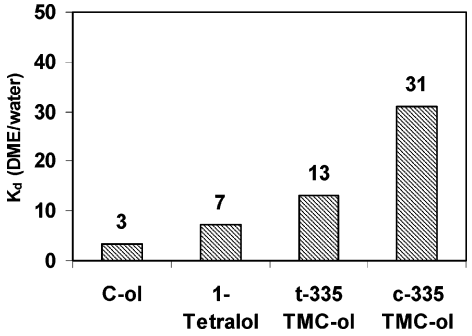
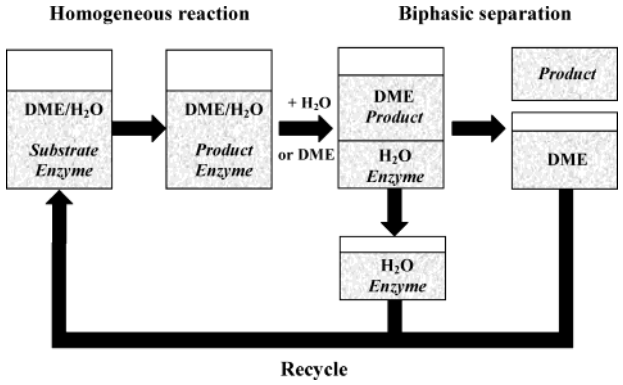


Figure 4. Distribution coefficients K_d (C_{DME}/C_{water}) of alcohols in a DME−water biphasic solution at $T = 30$ °C (C-o1, cyclohexanol; t,c-335-TMC-ol, *trans*,*cis*-3,3,5-trimethylcyclohexanol).

Scheme 2. Diagrammatic Sketch for Biocatalysis/Separation Processing in a DME−water System at Ambient Temperature



satisfactorily in the presence of DME with only moderate loss in activity of FDH.

Facilitated Separation in Biphasic DME−Water. In addition to a monophasic reaction medium, the DME−water system in its biphasic mode offers a new avenue for advantageous downstream separation. The organic phase can be used to extract organic products from the aqueous phase. Advantages include facile product isolation, elimination of the solvent residue, and recycling of biological catalysts. The partitioning behavior of the reaction products plays a key role for liquid−liquid extraction. Figure 4 gives the distribution ratio for a number of model products. The experimental data are each the average of at least three independent measurements. These results suggested that the less-water-soluble solutes partition rather favorably into the DME phase. Thus, DME has significant potential as an extraction agent for hydrophobic products.

We describe a biocatalytic reaction coupled with separation in DME−water, depicted in Scheme 2. The reaction is performed homogeneously in a DME−water single-phase mixture, where the less-water-soluble substrate has enhanced solubility. After the completion of the reaction, an additional amount of water or DME is added to create a second liquid phase and a biphasic separation follows. The reusable enzyme and cofactor remain in the aqueous phase; the product remains in the DME phase and is easily isolated by depressurizing DME. Meanwhile, DME, water (containing enzyme−cofactor), and substrate are reloaded into the reactor to start a new cycle.

II. Phase Change Using CO_2 as a Miscibility "Switch". Perhaps the simplest system to imagine would be a miscible mixture of water and a relatively

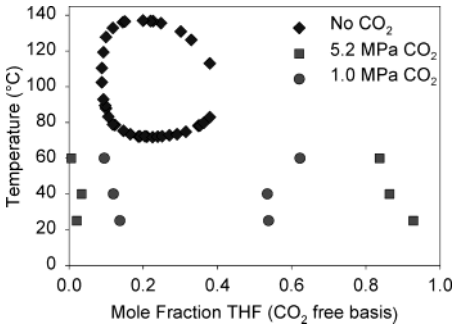


Figure 5. Liquid−liquid phase boundary of water−THF. The effect of 1.0 and 5.2 MPa of CO_2. (The binary data are reproduced from ref 16).

chemically inert organic, with a phase change coming about by changing temperature into a liquid−liquid region. For example, in Figure 5, the upper loop (solid diamonds) shows the binary phase behavior of THF−water.[16] To achieve phase separation, the required temperature is above the lower critical solution temperature of ∼72 °C. Yet, the effect of temperature is not great enough to offer an efficient separation, and the mutual miscibilities between water and THF are significant. Furthermore, it seems unlikely that most biocatalysts could survive at the two-phase-region temperatures.

A potential method for carrying out a heterogeneous separation subsequently to a homogeneous reaction is the preferential dissolution of CO_2 in organics relative to water−CO_2, as CO_2 is quite miscible with most organics and yet is relatively insoluble in water. As a result, when a miscible organic−aqueous tunable solvent (OATS) system is exposed to even modest pressures of CO_2, it will split into two phases, a water phase with little CO_2 in it and an organic phase rich in CO_2. Thus, reaction can occur in the initial homogeneous phase with a hydrophobic substrate and a water-soluble catalyst, yet after CO_2 addition, the catalyst will be in the aqueous phase and the products, along with the unreacted starting material, will be in the organic phase. Figure 5 shows the effect of only 1.0 MPa of CO_2 pressure on the water−THF system; even with this low pressure, the split is quite dramatic. An increase in the CO_2 pressure to 5.2 MPa further reduces the mutual solubility of THF and water in both liquid phases. This demonstrates that the solvent properties can be tuned simply by manipulating the CO_2 pressure. Additionally, comprehensive reviews are available that compile high-pressure fluid-phase equilibria investigated for the past 2 decades.[17−19]

Even more dramatic is the potential for separation as the antisolvent renders the organic phase a weaker solvent. Figure 6 shows the change at 3.0 MPa of CO_2 pressure for a water−THF system with a dilute, water-soluble catalyst analogue (dye) in it. The chromophore is soluble in the miscible OATS mixture at atmospheric pressure. At 3.0 MPa of CO_2 pressure, it is completely in the lower (aqueous) phase and could not even be detected in the organic phase. The resulting distribution coefficient is in excess of 10^4. Although many enzymes will denature in the CO_2 equilibrated water phase, as the pH goes down to around 3, in principle this could be used for enzyme catalysis because the reaction is run before CO_2 is introduced, exposure times to CO_2 are short, and the aqueous phase can be buffered. It is also worthwhile considering such tunable OATS systems for other homogeneously catalyzed syntheses.

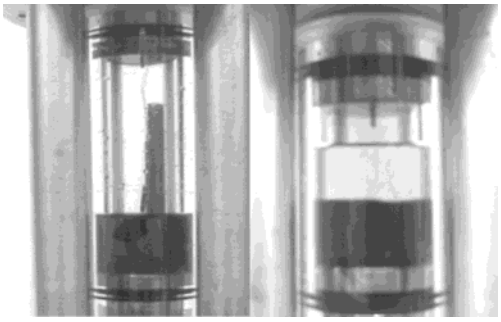
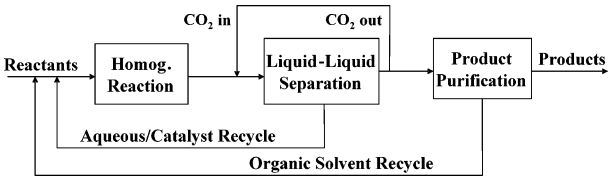


Figure 6. Water−THF mixture with dilute water-soluble dye: left side, ambient pressure; right side, 3.0 MPa of CO_2 pressure.

Scheme 3. Schematic of the OATS Reaction with a CO_2 Switch for Catalyst Recycle



For such applications, we envision a flowsheet similar to that shown in Scheme 3. First the reaction is run homogeneously at atmospheric pressure. Then the CO_2 is used as a "switch" to cause a phase separation, and the phases are decanted from each other under pressure. The aqueous phase, with the catalyst, is recycled, and the organic phase with the product is subsequently depressurized for product purification and solvent recycle. The choice of the organic solvent would depend on many obvious factors, such as cost, safety, and ease of removal, but perhaps the overriding consideration would be that the solvent is chemically inert for the chemistry being used in the synthesis. In this regard, in addition to water−THF, we have found similar effects of the CO_2 pressure with other OATS systems, such as water−dioxane and water−acetonitrile.[20] Comprehensive reviews on phase behaviors of organic + water + CO_2 systems are also available, offering a useful database for a wide variety of organic solvents, which makes it possible to tailor solutions for specific separations on demand.

Summary

We show the application of OATS for homogeneous catalysis of hydrophobic substrates. A reaction can be run homogeneously in a water−organic miscible mixture, followed by a phase separation, giving a product-containing organic phase and a catalyst-containing aqueous phase, for recycle and reuse of expensive or toxic homogeneous catalysts.

We report the results for HL-ADH reduction of ketones in a tunable DME−water solvent system, which offers opportunities for biocatalysis and downstream separation. DME−water is a benign alternative to toxic organic solvents. Monophasic DME−water possesses the ability to stabilize the cofactor and yet retain sufficient enzymatic activity. We also describe a biphasic DME−water separation scheme to be coupled with the biocatalytic process. The biphasic system can extract less-water-soluble organics from the aqueous phase and leave the enzyme−cofactor in water. The product can be easily isolated, and enzyme can be recovered.

In addition, we show the possibility of using CO_2 to give liquid−liquid-phase splitting of OATS systems. At

even modest pressures of only a few tens of bars, distribution coefficients become very large. The use of CO_2 as a miscibility "switch" makes a reaction and separation scheme with facile downstream processing and product purification possible.

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