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Maximise Equilibrium Conversion in Biphasic Catalysed Reactions: Mathematical Description and Practical Guideline

Marrit F. Eckstein,[a, b] Martina Peters,[b] Julia Lembrecht,[a] Antje C. Spiess,[c] and Lasse Greiner[b,*]

[a] Lehrstuhl für Technische Chemie, Rostock University, A.-Einstein-Str. 3a, 18059 Rostock, Germany
[b] Institut für Technische und Makromolekulare Chemie, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany
Fax: (+49)-241-802-2177; e-mail: greiner@itmc.rwth-aachen.de
[c] Lehrstuhl für Bioverfahrenstechnik, RWTH Aachen University, 52074 Aachen, Germany

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Abstract: What is the best solvent combination for a catalytic biphasic reaction system to maximise equilibrium conversion? And which phase volume ratio and reaction stoichiometry maximises the conversion? Which product yield can be obtained using a distinct biphasic reaction mixture? These questions are essential, as optimisation can only be carried out within the thermodynamically imposed boundaries. Within this paper we introduce a mathematical expression to calculate and predict equilibrium conversion and product yield in biphasic reaction mixtures with only one reactive phase. This mathematical expression allows a time-saving design of biphasic experiments with respect to maximum conversion and product yield. The calculations were verified by experimental data obtained from a substrate-coupled alcohol dehydrogenase-catalysed reduction under different biphasic reaction conditions.

Keywords: biotransformation; biphasic catalysis; equilibria; oxidoreductases; prediction

Introduction

While heterogeneous catalysts are widely applied in industrial processes, homogeneous catalysis often requires high technical and financial effort for catalyst recycling and product separation. Similar problems arise when dissolved biocatalysts are applied in organic synthesis. A promising strategy to push the approach of homogeneous catalysts even on an industrial scale is the utilisation of multiphase reaction media. Important examples for industrial processes on a ton-scale using the advantages of liquid/liquid biphasic metal-catalysis are the oligomerisation in the Shell higher olefin process (SHOP) and the Ruhrchemie/Rhône–Poulenc process.[1] Recent developments have also lead to various processes in the pharmaceutical industry that are based on multiphase catalysis.[2]

Focusing on biphasic reaction media, a so-called reactive phase can be defined in which the catalyst is dissolved and in which the reaction takes place. The second phase is the so-called non-reactive phase representing a reservoir for all reactants. The reactants show a certain affinity to each of the two phases, expressed by their partition coefficients.

So what are the promising advantages, biphasic reaction media can offer for homogeneous catalysis and biocatalysis? First, the product may easily be removed and purified by phase separation. Second, the retention of the dissolved catalyst in the reactive phase allows a re-use of the catalyst. If conversion is sufficiently high, additional separation steps after the reaction can be avoided.[3–5] Third, possible inhibitory effects on the catalyst can be suppressed if substrate and product concentrations can be kept low in the reactive phase. And fourth, being of importance for limited substrate solubility in the reactive phase, e.g., aqueous solution for biocatalysis, the second phase acts as a reservoir for the reactants allowing one to obtain preparative quantities in reasonable concentrations.

These advantages of biphasic reaction media are used for a broad variety of reactions and solvents.[5] Next to water and organic solvents,[6–8] fluorinated solvents,[9] ionic liquids,[10–12] and supercritical solvents like supercritical carbon dioxide[13] are applied

in multiphase catalysis with homogeneous catalysts as well as with biocatalysts.

However, a general problem of various reactions of preparative and industrial interest is their disadvantageous equilibrium position. Usually thermodynamic driving forces can be enhanced by using powerful reactants like acid chlorides for esterifications. But these reactions very often lead to numerous coupled products and dissipation of chemical energy as heat. Furthermore, highly reactive agents may not be compatible with conditions required for the catalyst, especially having biocatalysis in mind. The challenge is to exploit equilibrium reactions with lower driving forces by means of careful optimisation of reaction conditions. Therefore, the application of biphasic reaction conditions is an important target for the sustainable optimisation of catalytic reactions.

To use the tool of biphasic reaction conditions effectively for reaction engineering purposes, a distinct knowledge of the equilibrium thermodynamic boundaries is of great value. First of all, one needs information about the driving forces of the reaction, e.g., the difference in oxidation-reduction potentials ΔE^0. Furthermore, the partition behaviour of all reactants, phase volume ratio and substrate ratio are essential experimental data that have to be estimated to optimise the equilibrium position and conversion, respectively, in biphasic media. The necessity of these data was impressively reported by Keim describing the development of SHOP.[1] Before a pilot plant could be built, numerous solvents had to be tested and evaluated with respect to substrate and product solubility, partition behaviour of all reactants and catalyst stability. To obtain reliable data for all these parameters, a large number of time-consuming and expensive experiments is necessary, which quite often is an unattractive perspective for process optimisation.

To overcome the effort of numerous experiments for reaction optimisation in biphasic systems, we have developed a mathematical description to predict the equilibrium conversion for reactions in biphasic media. This description allows us to answer the questions, which solvent combination and which phase volume ratio lead to high equilibrium conversions and to high product yields. To verify the mathematical expression, we have applied it to alcohol dehydrogenase-catalysed enantioselective reductions of a prochiral ketone in several biphasic systems.

Results and Discussion

Analytical Solution

There have been several approaches to characterise reactions in biphasic reaction media. Especially in biocatalysis, not only the kinetic behaviour,[14] but also thermodynamic characterisations[3,15–19] have been discussed by various authors. However, many approaches are of an empirical character, especially if choice of solvent and general reaction conditions are investigated. Beyond the equilibrium position of biphasic reactions, the equilibrium conversion and the product yield that are available in one phase of the system are of major interest from a preparative and commercial point of view. Here, reliable mathematical methods for synthesis, design, and optimisation of biphasic reactions are rare in the literature, so far.

Characterising non-catalysed reactions in biphasic media, the expression for the equilibrium conversion does not differ from monophasic cases.[20] However, the majority of biphasic reactions is catalysed and is represented by the general reaction Scheme of a bimolecular reaction with partitioning of all reactants (Figure 1). If the catalyst is insoluble in the non-reactive phase (index *N*) predicting the equilibrium conversion in this system is not trivial. Even for the simplified case where activity coefficients are neglected, selectivity is set to unity, and independency of solubility for each reactant is assumed, the influence of the parameters is complex.

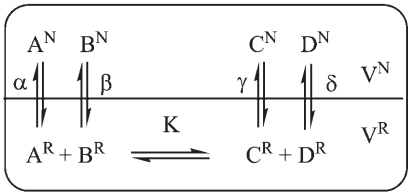

Figure 1. General Scheme of the equilibrium catalysed biphasic bimolecular reaction system with the reaction taking place only in the reactive phase.

In contrast to numerical approaches based on simulation, we derived an analytical expression to describe the equilibrium conversion in biphasic reactions. Following the simplified case stated above the overall conversion X is defined as

$$X = 1 - \frac{n(A)}{n_0(A)} \tag{1}$$

The reaction takes place in the reactive phase (index R), with A being the limiting substrate and C regarded as the product of interest. The equilibrium conversion X depends on the equilibrium constant K, which can be written as

$$K = \frac{[C]_R[D]_R}{[A]_R[B]_R} \tag{2}$$

with equilibrium concentrations throughout. Hence, X depends on the phase volume ratio, the initial ratio

$$V = \frac{V_N}{V_R} \geq 0$$

of starting materialsand the partition coefficients, β, γ, and δ, accordingly.

$$S = \frac{n_0(B)}{n_0(A)} \geq 1$$

$$\alpha = \frac{[A]_N}{[A]_R}$$

Martinek et al. derived an effective equilibrium constant for biphasic reaction media which includes the equilibrium constant K_W of an aqueous phase, the phase volume ratio and the partition coefficients of all reactants.[17] The result of our analytical derivation shows similar influences of the phase volume ratio and of the partition coefficients on the equilibrium conversion. But in contrast to Martinek et al., we consider K as intrinsic property of the reaction. Thus, we defined m to relate molar amounts with effective concentrations in the biphasic system.

$$m = \frac{(\gamma V + 1)(\delta V + 1)}{(\alpha V + 1)(\beta V + 1)} \tag{3}$$

Substitution of the equilibrium concentrations with the starting conditions using the mass balance expressions leads to the analytical expression for the equilibrium conversion:[21]

$$X = mK(S+1) - \sqrt{\frac{((1-S)^2 + 4S(mK)^{-1}}{2(mK-1)}} \tag{4}$$

The equation is of similar form as the solution for the monophasic case extended by the correction factor m.[20] Noteworthy, partial partitioning and the monophasic case are included in this expression as the respective partitioning coefficients are zero and $m = 1$, respectively.

For preparative purposes equilibrium yield η of the desired product being present in the non-reactive phase is of main interest. Equilibrium yield of the desired product C in the non-reactive phase is related to X by

$$n = \frac{n(C)_N}{n(A)_0} = \gamma(\gamma+1)V \cdot X \tag{5}$$

These equations enable the experimentalist to calculate the equilibrium conversion and equilibrium yield directly if the equilibrium constant K and the partition coefficients are known. Rearrangement with respect to S leads to

$$S = X\left(\frac{1+X}{mK(1-X)}\right) \tag{6}$$

It allows the direct calculation of S as a function of X and the direct optimisation of the cosubstrate concentration. Unfortunately, a solution for V as function of X cannot be derived.

By means of the analytical solution, general trends of reaction behaviour in biphasic systems can be predicted. Unsurprisingly, X generally rises with increasing S. The influence of V on the equilibrium conversion turned out to be more complex. Three extreme cases will be considered which are nonetheless practically relevant to examplify the trends:

A
- $\alpha \approx \beta > \gamma \approx \delta$
- $\alpha > \gamma;\ \beta \approx \delta$
- $\alpha \approx \gamma;\ \beta > \delta$

max. X for $S\uparrow$ and $V\downarrow$

B
- $\alpha \approx \beta \approx \gamma \approx \delta$
- $\alpha \approx \gamma;\ \beta \approx \delta$

max. X for $S\uparrow$; no influence of V

C
- $\alpha \approx \beta < \gamma \approx \delta$
- $\alpha < \gamma;\ \beta \approx \delta$
- $\alpha \approx \gamma;\ \beta < \delta$

max. X for $S\uparrow$ and $V\downarrow$

Experimental Verification

To show the accuracy of the developed mathematical expression, the reduction of acetophenone was examined with respect to its equilibrium conversion X and equilibrium yield η in several biphasic reaction media. We have chosen the (*R*)-selective alcohol dehydrogenase from *Lactobacillus brevis* (LB-ADH) as biocatalyst, because this enzyme has shown a good stability towards organic solvents and ionic liquids as non-reactive phases in previous works.[7,11] As non-reactive phase alkanes, ethers, toluene, cyclohexanone, and ionic liquids were combined with water as reactive phase. NADPH was regenerated by substrate-coupled cofactor regeneration with 2-propanol catalysed by the same enzyme.

To use Eq. (4) to predict X, the partition coefficients of the reactants were determined experimentally (Table 1). While the choice of non-reactive phase does not have a big impact on the partition behaviour of the co-substrate 2-propanol and the co-product acetone, the partition coefficients for acetophenone and 1-phenylethanol vary over up to two orders of magnitude.

K is related to the free energy change $\Delta G = -RT\ln K$. For the reduction of acetophenone using 2-propanol as co-substrate K is given by the difference in oxidation-reduction potentials ΔE_0. At 30 °C this

Table 1. Partition coefficients of reactants between aqueous buffer solution and solvent.

Solvent	Acetophenone	2-Propanol	1-Phenylethanol	Acetone
n-hexane	17.5 ± 1.6	0.35 ± 0.26	1.10 ± 0.10	0.56 ± 0.22
n-heptane	17.9 ± 1.3	0.2 ± 0.07	0.96 ± 0.04	0.42 ± 0.12
MTBE	66.9 ± 1.5	1.1 ± 0.2	32.1 ± 2.4	1.1 ± 0.2
DIPE	53.9 ± 3.9	0.54 ± 0.08	15.7 ± 0.9	0.68 ± 0.13
toluene	144.6 ± 1.5	0.31 ± 0.03	9.2 ± 1.0	1.1 ± 0.08
cyclohexanone	70 ± 10.6	1.4 ± 0.2	35.5 ± 4.0	1.5 ± 0.4
[BMIM][$(CF_3SO_2)_2N$]	144 ± 48	0.6 ± 0.4	4.0 ± 0.5	2.0 ± 0.2
[BMIM][PF_6]	186 ± 15	0.7 ± 0.5	14.5 ± 2.8	2.2 ± 0.3

results into $K = 0.426$ which was derived from literature values.[22,23] This leaves S and V as variables.

The reaction system can be classified as case A. Thus, if X has to be maximised, low V and high S are favourable. The smaller V is chosen, the smaller S is necessary for the desired conversion. This prediction matches the results obtained experimentally, investigating the equilibrium conversion X with changing V at a fixed S (Table 2). To obtain clear data for demonstration issues a low value of $S = 2$ was chosen. As expected, in *n*-hexane the influence of V is more pronounced as the partition coefficients of acetophenone and 1-phenylethanol differ more strongly compared to methyl *tert*-butyl ether (MTBE).

Table 2. Equilibrium conversion X as function of V and experimentally obtained values X_{exp} for $S = 2$ (values given in percent).

Solvent	V	X	X_{exp}
MTBE	0.1	44.7	48.6
	0.5	43.1	46.4
	1	42.8	45.9
	10	42.6	44.6
hexane	0.1	40.4	41.0
	0.5	28.7	30.1
	1	25.4	27.1
	10	24.1	28.2

Increasing the substrate ratio to $S = 20$ with a fixed phase volume ratio of $V = 1$, X increases from 27% to 66% in the presence of *n*-hexane and from 46% to 84% when MTBE is used (Table 2 and Table 3). A further increase in substrate ratio S leads to preparative useful equilibrium conversions (e.g., $X = 97.7\%$ $X_{exp} = 98.0\%$; $S = 200$, $V = 1$, MTBE/aqueous buffer).

To find a suitable non-reactive phase, the equilibrium conversion and the product yield were compared for different solvents (Table 3). For $S = 20$ the highest equilibrium conversions are achieved in the presence of MTBE and diisopropyl ether (DIPE). The equilibrium product yield η is only slightly lower than X, because the partition coefficients of the product 1-phenylethanol are high for both systems. In contrast, using *n*-hexane or *n*-heptane, the partition coefficients of 1-phenylethanol are close to unity. Not only conversion is lower, but η is only half of X for $V = 1$.

Table 3. Equilibrium conversion X, equilibrium yield η, and experimentally obtained values (index *exp*) for $S = 20$ and $V = 1$ (values given in percent).

Solvent	X	X_{exp}	η	η_{exp}
MTBE	82.2	83.6	79.7	81.0
hexane	63.1	66.3	33.1	34.5
n-heptane	62.0	65.4	30.4	32.1
DIPE	77.8	79.4	73.1	74.6
toluene	60.5	61.2	54.6	55.2
cyclohexanone[a]	83.9	-	81.6	-
[BMIM][$(CF_3SO_2)_2N$]	51.2	64.9	41.0	51.7
[BMIM][PF_6]	66.1	66.2	61.8	61.9

[a] Experimental result not accessible due to rapid enzyme deactivation (see text).

For the application of cyclohexanone the calculation promises $X = 84\%$. However, no experimental data could be obtained for this system, due to the lack of enzyme stability with a half-life time of $t_{1/2} = 0.03$ h. This example demonstrates that the mathematical expression cannot cover the influence of factors like (bio-)catalyst stability in the biphasic mixture.

Finally, we can compare X and η obtained by calculation to experimental data X_{exp} and η_{exp}. In general, both values are in good agreement (Figure 2). The observation that experimental results are slightly lower than calculated data may be due to the neglected activity coefficients. The differences between the calculated and experimentally determined conversion and product yield are highest for the ionic liquids where experimental error for the partition coefficients is highest. This shows that the data base is of course critical for correct predictions.

Conclusions

We presented an analytical expression for the calculation of the thermodynamic equilibrium conversion X

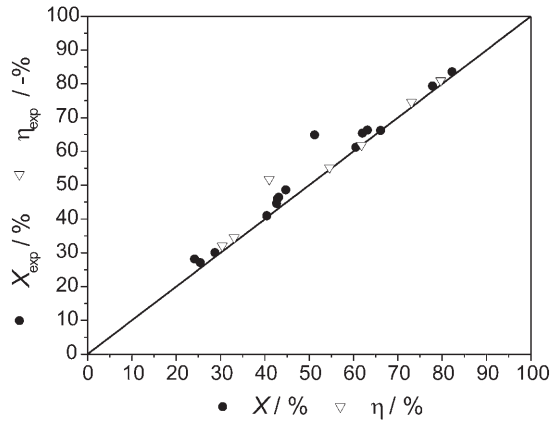

Figure 2. Parity plot of calculated and experimental results for conversion X and yield η.

and yield η in biphasic catalysed reactions. With the knowledge of the equilibrium constant K of the desired reaction and of the partition coefficients for all reactants in the biphasic medium, it is possible to predict a suitable solvent combination and to maximise the equilibrium conversion. Wheras the substrate ratio S has to be increased in all cases to maximise conversion, influence of the phase volume ratio V is more complex and depends on the partition coefficients of all reactants.

Furthermore, the mathematical expression was successfully applied on the substrate-coupled alcohol dehydrogenase-catalysed reduction of acetophenone. It could be verified for 8 different biphasic reaction mixtures. Large differences between experimental and calculated results hint to the dominating influence of other factors, e.g., (bio-)catalyst stability.

In conclusion, we can offer a mathematical expression and a practical guideline that enable the preparative chemist to estimate suitable reaction conditions for biphasic catalysed reactions. This is the basis for a time-saving and effective experimental design of catalytic biphasic reactions.

Experimental Section

Algebraic transformations were carried out with Maple 10 (Maplesoft). Acetophenone and 1-phenylethanol were purchased from Merck, Germany. All other chemicals were obtained in *p. a.* quality from J. T. Baker, Germany. All solvents were of HPLC grade and supplied by Merck and J. T. Baker. The alcohol dehydrogenase from *Lactobacillus brevis* (EC 1.1.1.2) and the $NADP^+$ (disodium salt) were supplied by Jülich Fine Chemicals, Germany. Ionic liquids were supplied by Solvent Innovation, Germany. Aqueous solutions were buffered using 50 mM phosphate buffer to pH 7 containing 1 mM magnesium chloride if not mentioned otherwise.

Analysis

Acetophenone and 1-phenylethanol were analysed by gas chromatography, using a Varian CP 3800 equipped with DB-1701 capillary column Alltech, Germany (dodecane as internal standard, flow rate He 2.0 mL min^{-1}). Samples were diluted with ethyl acetate prior to measurement. Enantiomeric excess was determined by GC-FID with an FS-Cyclodex-beta-I/P column Macherey&Nagel, Germany (He 2.0 mL min^{-1}). Concentrations of acetone and 2-propanol were determined by HPLC equipped with RI detector and a BIORAD Aminex HDK-87 H Ion Exclusion column (300 × 7.8 mm) with (sulfuric acid 6 mM; 0.8 mL min^{-1}). Samples were diluted with water as appropriate.

Partition Coefficients

Partition coefficients were measured adopting methods reported previously.[24,25] All measurements were carried out at least 7 times. Standard deviations are given as errors. Substrate solutions containing acetophenone and 1-phenylethanol were prepared in the desired solvents. In screw-capped vials (8 mL), the aqueous phase (4 mL) was covered with organic phase (4 mL). The samples were shaken (30 °C; 400 rpm). The mixtures were stored in a water bath (30 °C; 3 days). Samples were taken from each phase and analysed. Partition coefficients of acetone and 2-propanol were determined following the decline of concentration in the aqueous phase before and after the addition of the organic phase. Samples were taken from the aqueous phase containing acetone and 2-propanol prior to contacting with solvents. Following the same procedure as above, samples were analysed by HPLC. The partition coefficient [ε] is then calculated according to [ε] = (starting concentration)/(residual concentration) – 1.

Equilibrium Conversion/Yield

An aqueous solution (2.5 mL) of LB-ADH, $NADP^+$ (0.1 mmol L^{-1}) and 2-propanol was covered with the organic solvent (2.5 mL) containing acetophenone (20 to 80 mM) and the mixture was shaken in a vertical shaker (30 °C; 200 rpm). Samples were withdrawn from the non-reactive phase and analysed by GC. Mass balance of acetophenone and 1-phenylethanol was corrected by their partition coefficients.

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