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Maximise Equilibrium Conversion in Biphasic Catalysed Reactions: How to Obtain Reliable Data for Equilibrium Constants?

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Abstract: For the prediction and optimisation of the equilibrium conversion in biphasic catalysed reactions, the equilibrium constant of the desired reaction and the partition coefficients of all reactants have to be known. Within this contribution we have examined the alcohol dehydrogenase-catalysed reduction of several linear and aromatic ketones in biphasic reaction media with respect to equilibrium conversion. In this example, the equilibrium constant can be expressed in terms of differences in oxidation-reduction potentials ΔE^0. However, for a large variety of organic compounds, these data are quite rare in the literature. To overcome this lack of data, we have utilised methods of computational chemistry to calculate data for the Gibbs free energy ΔG_R leading to the equilibrium constants of a homologous series of linear ketones. To obtain comparable data for the reduction of substituted acetophenone derivatives, the Hammett relation leads to the necessary equilibrium constants. Furthermore, we compare the equilibrium conversions of a set of cofactor regeneration methods for the alcohol dehydrogenase-catalysed reductions. These results lead to a time-saving experimental design for the enantioselective reduction of 2-octanone to (*R*)-2-octanol on a preparative scale utilising biphasic reaction conditions.

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

Introduction

Biphasic reaction media are a promising tool to optimise process conditions of homogeneously catalysed reactions. So far, biphasic media are used for a broad variety of reactions and solvents.[1] Next to water and organic solvents,[2–4] fluorinated solvents[5], ionic liquids[6–8] and supercritical solvents like supercritical carbon dioxide[9] are applied in multiphase catalysis with homogeneous catalysts as well as with biocatalysts.

From a reaction engineering point of view, the maximisation of equilibrium conversion and product yield are main targets for optimisation, next to catalyst activity, selectivity and stability. These factors are influenced by the thermodynamic driving force of the reaction, e.g., the difference in oxidation-reduction potentials ΔE^0 and by the partition behaviour of all reactants given for a set of solvents, while free parameters of optimisation are given by the phase volume ratio and the initial substrate ratio.[10] To obtain reliable data for a suitable set of biphasic systems, these parameters were often examined experimentally for a large number of solvents.[11] This strategy is a time-consuming and cost-intensive step of process development, and may quite often be an unattractive perspective for the utilisation of biphasic reaction media in industry.

To overcome the effort of numerous experiments and to offer a time-saving and effective method of experimental design, we have recently derived a mathematical expression that enables the chemist to predict the equilibrium conversion and yield of catalysed biphasic reactions.[10] This method could successfully be applied on the alcohol dehydrogenase-catalysed reduction of acetophenone in several biphasic reaction

media, using organic solvents as well as ionic liquids combined with an aqueous buffer phase.

Here, we use the previously derived equation to predict the equilibrium conversion of alcohol dehydrogenase-catalysed reductions of several linear and aromatic ketones under biphasic reaction conditions. The mathematical expression requires reliable data for the equilibrium constant K as given by the oxidation-reduction potentials of the reactants. For a variety of ketones and corresponding alcohols, the oxidation-reduction potentials ΔE^0 are available in the literature.[12,13] However, these data are limited, especially regarding ketones with low water solubilities, restricting the practical applicability of the developed mathematical expression. To overcome this lack of data, we investigated alternative ways to obtain the equilibrium constants K. To allow a concrete comparison of the different calculation methods, monophasic as well as biphasic reaction conditions are compared with respect to equilibrium conversions.

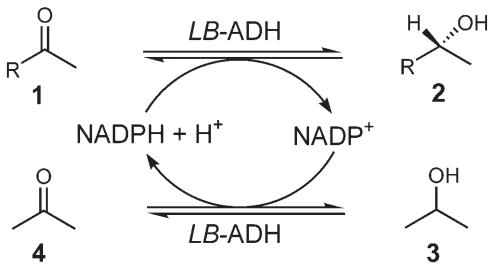
Finally, a comparison of cofactor regeneration methods leads to an application of practical relevance. Therein, the alcohol dehydrogenase-catalysed reduction of 2-octanone to (*R*)-2-octanol is performed with high chemical purity and high enantioselectivity under biphasic reaction conditions on a preparative scale.

Results and Discussion

Alcohol Dehydrogenase-Catalysed Reductions

The enantioselective reduction of prochiral ketones is a reaction of synthetic relevance leading to important intermediates for pharmaceutical compounds and food additives.[14] The application of a large pool of biocatalytic reactions allows the synthesis of these chiral intermediates with high selectivities.[15,16] Here, we investigated the alcohol dehydrogenase-catalysed reduction of several linear and aromatic ketones. All of these substrates have low water solubilities in common ($<0.5\ \mathrm{mol\,L^{-1}}$). Thus, the application of a biphasic reaction medium is a necessity to obtain preparative yields and sufficiently high space-time yields. The NADPH-dependent alcohol dehydrogenase from *Lactobacillus brevis* (*LB*-ADH) is a good biocatalyst with high selectivity and stability under these reaction conditions.[3,7] Cofactor regeneration was carried out in a substrate-coupled approach, by oxidation of excess 2-propanol as co-substrate (Scheme 1).

A general description of any biphasic catalysed reaction system[17] includes a reactive phase, in which the catalyst is dissolved and in which the reaction takes place. In biocatalytic applications, this phase usually is an aqueous buffer phase, containing the dissolved biocatalyst and the cofactor, if required. The



Scheme 1. Reduction of prochiral ketones (**1**) to chiral alcohols (**2**) catalysed by the (*R*)-selective alcohol dehydrogenase from *Lactobacillus brevis* (*LB*-ADH); substrate-coupled cofactor regeneration with 2-propanol (**3**) yielding acetone (**4**).

second phase is defined as a non-reactive phase, representing a reservoir for all reactants. This phase may, for example, be represented by non-miscible organic solvents or ionic liquids.[7] The reactants show a certain affinity to each phase, expressed by their specific partition coefficients. It is important to mention that if the catalyst is soluble in both phases, the equilibrium conversion in biphasic systems does not differ from the monophasic case.

Recently, we have shown that the equilibrium conversion X of a catalysed bimolecular reaction under biphasic reaction conditions, can be calculated using the following expression [Eq. (1)]:[10,18,]

$$X = \frac{mK\left((S+1) - \sqrt{(1-S^2) + 4S\cdot(mK)^{-1}}\right)}{2(mK-1)}.^{[19]} \quad (1)$$

Thus, X depends on the equilibrium constant K of the reaction and on the initial substrate ratio S. Furthermore, X depends on the factor m that includes the partition coefficients of all reactants α, β, γ, δ and the phase volume ratio V to relate molar amounts with effective concentrations in the biphasic system [Eq. (2)]:[20]

$$m = \frac{(\gamma V+1)(\delta V+1)}{(\alpha V+1)(\beta V+1)}. \quad (2)$$

Similar expressions were obtained by Martinek et al.[21] If the catalyst is soluble in both phases or monophasic reaction conditions are applied, m equals 1.

To use Eq. (1) to predict the equilibrium conversion X for the investigated reductions, we have measured the partition coefficients of all reactants in the presence of methyl-*tert*-butyl ether (MTBE) as non-reactive phase (Table 1). In previous works, MTBE turned out to be the non-reactive phase of choice for the *LB*-ADH reduction of acetophenone with respect to maximum equilibrium conversion and yield.[10] Additionally, the utilised *LB*-ADH has shown high stabilities in the presence of MTBE in earlier studies.[3,7]

Table 1. Measured partition coefficients of reactants between MTBE and aqueous buffer solution (30 °C).

Substrate	MTBE	
	Ketone	Alcohol
Acetone	1.1 ± 0.2	1.1 ± 0.2
2-Pentanone	10.47 ± 0.41	10.54 ± 0.52
2-Hexanone	33.6 ± 11.2	41.9 ± 1.7
2-Heptanone	170 ± 28	198 ± 28
2-Octanone	457 ± 66	600 ± 122
2-Nonanone	-[a]	-[a]
Acetophenone	66.9 ± 1.5	32.1 ± 2.4
p-Nitroacetophenone	107 ± 4.8	73.1 ± 4.7
p-Chloroacetophenone	345 ± 52	356 ± 77
p-Methoxyacetophenone	63.3 ± 4.0	64.8 ± 3.7
m-Methoxyacetophenone	79.4 ± 9.7	5.26 ± 0.12
o-Methoxyacetophenone	424 ± 56	9.40 ± 1.2

[a] For 2-nonanone and 2-nonanol, concentrations in the aqueous phase were too low to obtain reliable data.

As substrates, we have chosen linear ketones in the range from 2-pentanone to 2-nonanone and acetophenone with some of its derivatives.

For the linear reactants, the partition coefficients increase with chain length. The linear alcohols show higher partition coefficients compared to their corresponding ketones. In the case of 2-nonanone and 2-nonanol, concentrations in the aqueous phase were extremely low and could not be determined reliably. For acetophenone derivatives, the position of the substituent strongly influences the partition behaviour, e.g., in the case of the different methoxyacetophenones the partition coefficient of the ketone differs from 63.3 up to 424. In contrast, the alcohols corresponding to *meta*- and *ortho*-methoxyacetophenone show a much lower affinity to the organic solvent compared to the ketones (Table 1).

Obtain *K via* ΔE^0

As we consider an oxidation-reduction reaction, the difference in oxidation-reduction potentials ΔE^0 can be used to calculate the equilibrium constant $K(\Delta E^0)$ and thus to predict the equilibrium conversion $X(\Delta E^0)$. The results were compared to experimental data using a monophasic approach ($m = 1$) as well as biphasic reaction conditions (Table 2). In the monophasic approach, acetonitrile was added as co-solvent with a mole fraction of $x_{acetonitrile} = 0.05$ [~13% (v/v) acetonitrile], to realise a substrate solubility of at least 10 mM in the aqueous solution.

Except for the reduction of *n*-hexanone, the experimental results for the monophasic reaction are in good agreement with the calculated data, while the experimental results obtained under biphasic reaction conditions are higher than the calculated values. This difference may be caused by the neglected activity coefficients during the measurement of the partition coefficients. However, in the case of acetophenone, X can be predicted with very high accuracy for the monophasic as well as for the biphasic case. As a general result we can conclude that the addition of MTBE as non-reactive phase does not have a high impact on the calculated equilibrium conversion $X(\Delta E^0)$ of the reduction of lower linear ketones compared to monophasic conditions, due to similar partition coefficients of substrates and products.

Table 2. Calculated equilibrium conversion $X(\Delta E^0)$ compared to experimental data X_{exp} obtained under monophasic (aqueous buffer; $x_{acetonitrile} = 0.05$) and biphasic (aqueous buffer/MTBE; $V = 1$) reaction conditions ($S = 20$; 30 °C). X is given in percent.

Substrate	$X(\Delta E^0)$ monophasic	X_{exp} monophasic	$X(\Delta E^0)$ biphasic	X_{exp} biphasic
2-Pentanone	92.3	89.7	92.3	98.7
2-Hexanone	91.8	85.2	93.6	84.6
2-Heptanone	91.8	90.0	92.8	87.9
2-Octanone	-	90.0	-	87.6
2-Nonanone	-	89.2	-	77.8
Acetophenone	90.0	90.0	82.2	83.6

For most of the results shown so far (Table 2), K could be calculated using ΔE^0 from the literature.[12,13] However, investigating the reduction of 2-octanone and several aromatic ketones like *p*-chloroacetophenone or *p*-nitroacetophenone, no comparable data were available. To realise the prediction of the equilibrium conversion X without available data for ΔE^0, we tried to fill this lack of data by calculating K *via* computational chemistry or by using the Hammett sigma constant for the reduction of acetophenone derivatives.

Obtain K *via* Computational Chemistry

One possibility to obtain K is the calculation of the Gibbs free energy of reaction $\Delta G_{a,R}$ in aqueous solution that is defined as the stoichiometric sum of Gibbs free energies of formation of all reactants in aqueous solution $\Delta G_{a,f,i}$. The latter can also be expressed in terms of the standard Gibbs free energy of formation in aqueous solution $\Delta G^0_{a,f,i}$ or the standard Gibbs free energy of reaction in aqueous solution $\Delta G^0_{a,R}$ [Eq. (3)]:

$$\Delta G_{a,R} = \sum_i v_i \cdot \Delta G_{a,f,i} = \sum_i v_i \cdot \Delta G^0_{a,f,i} + RT \ln K = \Delta G^0_{a,R} + RT \ln K \quad (3)$$

The reaction equilibrium in aqueous solution is given if $\Delta G_{a,R}=0$. In this case the equilibrium constant K can be calculated from $\Delta G^0_{a,R}$ with x_i being the mole fraction of reactant i in the solution and γ_i being its activity coefficient [Eq. (4)]:

$$K=\prod_i (x_i^{eq}\gamma_i^{eq})^{v_i}=\exp\left(\frac{-\Delta G^0_{a,R}}{RT}\right)=\exp\left(\frac{-\sum_i v_i\cdot\Delta G^0_{a,f,i}}{RT}\right) \quad (4)$$

Several values for $\Delta G^0_{a,f,i}$ are tabulated.[22] However, regarding the large variety of molecules treated in (bio-)catalysis, only sparse data are available. In this case, predictive methods, like group contribution methods or continuum solvation models can be applied.

Mavrovouniotis et al. introduced a group contribution method to obtain $\Delta G^0_{a,f,i}$ in aqueous solution.[23,24] However, this method could not be applied for the investigated set of reactions, since the group contributions on the substrate and product side are the same, yielding a standard $\Delta G^0_{a,R}=0$, which is physically not reasonable.

A more sophisticated way to describe solute solvent systems is the COSMO-RS method based on the COSMO continuum solvation model.[25,26] COSMO-RS combines quantum-chemical methods on the DFT level with those of statistical thermodynamics and allows a genuine prediction of VLE and LLE data.[20] Besides the prediction of interaction effects between solute and solvents, COSMO-RS also gives the total Gibbs free energy of a molecule $G_{a,i}$. The Gibbs free energy of formation $\Delta G_{a,f,i}$ is defined by the sum of the energies of molecules minus the sum of atom energies. For a reaction, where amount and type of atoms equal one another on the substrate and product side, the atom information cancels out in the Gibbs free energy of formation $\Delta G_{a,f,i}$. Consequently, in this case the Gibbs free energy of reaction $\Delta G_{a,R}$ equals the sum of the total Gibbs free energies of molecules $G_{a,i}$ or the sum of the Gibbs free energies of formation $\Delta G_{a,f,I}$ [Eq. (5):

$$G_{a,R}=\sum_i v_i\cdot G_{a,i}=\sum_i v_i\cdot\Delta G_{a,i}=\Delta G_{a,f,i} \quad (5)$$

COSMO-RS does not only calculate the total Gibbs free energies in aqueous solution $G_{a,i}$, but gives the same in any real solvent. In our work, calculations were done for a homologous series of reductions, keeping one substrate (2-propanol) and one product (acetone) constant, while raising the number of carbon atoms in the second substrate and product (Table 3). Although the results obtained for $K(\Delta G)$ differ by a factor of 2 to 4.6 from the values calculated *via* the oxidation-reduction potentials $K(\Delta E^0)$, the decreasing trend in K with increasing number of carbon atoms is well returned in comparison to $K(\Delta E^0)$.

Table 3. Equilibrium constants K calculated using oxidation-reduction potentials[12] (ΔE_0) and the COSMO-RS model (ΔG) and the predicted conversions $X(\Delta E^0)$ and $X(\Delta G)$ for a monophasic system ($S=20$; 25 °C). X is given in percent.

Substrate	K (ΔE^0)	K (ΔG)	X (ΔE^0)	X (ΔG)
2-Butanone	0.627	2.87	92.8	98.2
2-Pentanone	0.580	2.48	92.3	98.0
2-Hexanone	0.536	2.03	91.8	97.5
2-Heptanone	0.536	1.61	91.8	96.9
2-Octanone	-	1.59	-	96.9

On the one hand, these results show that, where no comparable data are available from the literature, COSMO-RS can be used to predict trends in K for different reactions quite well. On the other hand, the higher the complexity of the molecules the more effort has to be done with respect to conformer choice and geometry optimisation.

Obtain *K via* the Hammett Relation

For the conversion of substituted aromatic compounds, the Hammett sigma constant σ_X can be used to calculate the equilibrium constant $K(\sigma_X)$, if an equilibrium constant $K_{unsubstituted}$ is available for the conversion of the unsubstituted compound [Eq. (6)]:[27]

$$K(\sigma_X)=K_{unsubstituted}\cdot 10^{\sigma_X} \quad (6)$$

Acetophenone with $\sigma_H=0.0$ is the origin of the Hammett relation. Thus, using the equilibrium constant $K(\Delta E^0)=K_{unsubstituted}$ for acetophenone, Eq. (6) gives $K(\sigma_X)$ for acetophenone derivatives substituted in the *para* and *meta* positions. Due to steric effects, no Hammett sigma constants σ_X are available for derivatives substituted in the *ortho* position.[27] $K(\sigma_X)$ can be used to predict the equilibrium conversion $X(\sigma_X)$. To show the applicability of this concept, we have chosen a series of substituted acetophenone derivatives. Where available, we compared $X(\sigma_X)$ to data calculated for $X(\Delta E^0)$ and to experimental results X_{exp} obtained for the monophasic and biphasic system (Table 4). In most cases, the calculated equilibrium conversions $X(\Delta E^0)$ and $X(\sigma_X)$ are in very good agreement. However, in the case of *p*-methoxyacetophenone, the calculated equilibrium conversions differ up to 14%. One reason may be the accuracy of the literature data. Unfortunately, using the *LB*-ADH as catalyst, no reliable experimental results could be obtained for the methoxyacetophenones. The reaction is far from equilibrium, which is probably due to a co-

Table 4. Calculated equilibrium conversions X using oxidation-reduction potentials (ΔE_0)[12], and the Hammett sigma constant (σ_X)[27] compared to experimental data X_{exp} for the monophasic (buffer; $x_{acetonitrile} = 0.05$) and biphasic (buffer/MTBE; $V = 1$) system ($S = 20$; 30 °C).[28] X is given in percent.

Substrates	Monophasic system			Biphasic system		
	X (ΔE^0)	X (σ_X)	X_{exp}	X (ΔE^0)	X (σ_X)	X_{exp}
Acetophenone	90.0	-	90.0	83.2	-	83.6
p-Chloroacetophenone	-	93.6	94.1	-	89.8	83.4
p-Bromoacetophenone	95.2	93.6	-	-	-	-
p-Nitroacetophenone	-	98.0	99.4	-	97.2	99.5
m-Nitroacetophenone	99.1	97.7	-	-	-	
p-Methoxyacetophenone	72.1	83.9	-	74.2	88.2	-[a]
m-Methoxyacetophenone	91.2	92.1	-	59.0	57.0	-[a]
o-Methoxyacetophenone	98.0	-	-	65.1	-	-[a]

[a] Irreversible binding of substrate; no reliable data available.

valent binding of the substrates to the catalytic centre of the enzyme that leads to a deactivation of the enzyme. Currently other catalysts are under investigation to obtain experimental results that allow a further comparison and discussion of these data.

The experimental results X_{exp} obtained for *p*-chloroacetophenone and *p*-nitroacetophenone were predicted with high accuracy for the monophasic system, closing the gap of ΔE^0 availability. A difference between $X(\sigma_X)$ and X_{exp} of about 6% for the reduction of *p*-chloroacetophenone in the biphasic system may be due to large standard deviations for the measurement of the partition coefficients (Table 1).

Choice of Cofactor Regeneration Method

In contrast to whole cell processes, most enzyme-catalysed oxidation-reduction reactions require a regeneration of the cofactor within the catalytic process. Different methods can be applied to achive an efficient cofactor regeneration, even in organic sovents or biphasic systems.[29,30] Using Eq. (1), it is now possible to predict and compare equilibrium conversions $X(\Delta E^0)$ of processes with different regeneration methods and thus to choose a suitable regeneration method for the desired process to maximise $X(\Delta E^0)$.

We have calculated $X(\Delta E^0)$ for the catalysed reduction of 2-heptanone, comparing different regeneration methods (Table 5). Utilising a substrate-coupled approach, where one enzyme catalyses the desired reduction as well as the regeneration reaction of NADPH, leads to $X(\Delta E^0) = 42.3\%$ with 2-propanol as co-substrate in a monophasic system ($S = 1$). To obtain $X > 99.0\%$ under these conditions, the sustrate ratio has to be increased to at least $S > 180$.[10] In contrast, utilising different enzyme-coupled regeneration methods, where a second enzyme is used to catalyse the regeneration reaction, leads to $X(\Delta E^0) > 90\%$ in the case of glucose as co-substrate and even reaches $X(\Delta E^0) > 99.9\%$, if phosphite, formate or hydrogen are used as co-substrates. Similar results could be calculated for an electrochemical regeneration method. In comparison, results obtained for the biphasic system (MTBE/buffer; $V = 1$; $S = 1$), $X(\Delta E^0)$ values differ only slightly for the co-substrates 2-propanol (44.1%) and glucose (92.3%), due to the similar partition behaviour of 2-heptanone and 2-heptanol (Table 1).

Table 5. Comparison of cofactor regeneration methods with respect to calculated data of the thermodynamic equilibrium constant $K(\Delta E^0)$ and conversion $X(\Delta E^0)$ for the reduction of 2-heptanone ($E^0 = 121$ mV) in a monophasic system (buffer, $x_{acetonitrile} = 0.05$; $S = 1$;). E^0 is given in *mV*; $X(\Delta E_0)$ is given in percent.

Co-substrate	E^0	$K(\Delta E^0)$	$X(\Delta E^0)$
Substrate-coupled:			
2-Propanol[12]	+ 29	0.536	42.3
Enzyme-coupled:			
Glucose (GDH)[31]	+54	125	91.8
Phosphite (PTDH)[32]	−650	$1.2 \cdot 10^{26}$	> 99.9
Formate (FDH)[33]	−199	$6.7 \cdot 10^{10}$	> 99.9
Hydrogen (hydrogenase)[34,35]	−413	$1.5 \cdot 10^{18}$	> 99.9
Electrochemical:			
$RhMed_{Ox}$[36]	−430	$4.4 \cdot 10^{18}$	> 99.9

These calculations underline that the driving force of the reaction ΔE^0 can be increased by the right choice of co-substrate and regeneration method. However, next to the regeneration method and solvent combination for biphasic reaction media, enzyme stability, activity and inhibitory effects influence the choice of reaction conditions applied on preparative scale.

We investigated the *LB*-ADH-catalysed reduction of 2-octanone to (*R*)-2-octanol under biphasic reaction conditions. The main advantage of this approach is an easy work-up of the product (*R*)-2-octanol. Due to a satisfactory half-life time of 60 h under reaction conditions (MTBE/buffer), cofactor regeneration was realised with a glucose dehydrogenase from *Pseudomonas* species (*PS*-GDH) with glucose as co-substrate. In a batch reactor (MTBE/buffer; $V = 0.25$; $S = 1.25$), 2-octanone could be converted to (*R*)-2-octanol with $X_{exp} > 99\%$ [$X(\Delta E^0) = 97.9\%$; reduction of 2-heptanone] and an $ee > 99.5\%$. After 2 days of reac-

tion, this lead to an STY = 20 g L^{-1} d^{-1} and a ttn (NADPH) = 7920.[20] While the *LB*-ADH, NADP(H), glucose and gluconate stay in the aqueous buffer phase, the product stays in the organic phase and can easily be removed by phase separation (isolated yield after distillation 71%). In contrast, utilising 2-propanol as co-substrate with a substrate-coupled cofactor regeneration, the co-substrate would be needed in high excess (S > 460) to obtain similar results. Additionally, this large amount of co-substrate would influence the phase separation and partition behaviour and thus, work-up to obtain product with high chemical purities is more difficult.

Conclusions

In conclusion, the lack of data for the prediction of equilibrium conversion in catalysed biphasic and monophasic reactions can partly be overcome by ab initio calculations and by empirical calculations. While ab initio methods like COSMO-RS are valuable to predict relative trends in equilibrium constants and conversions, the Hammett relation is a suitable method to calculate equilibrium constants for the conversion of substituted aromatic substrates. Next to oxidation-reduction reactions, this method is also applicable for other reactions types. However, due to reasons of steric hindrance, no Hammett sigma constants are available for derivatives substituted in the *ortho* position.[27]

Furthermore, it was possible to predict and compare equilibrium conversions for 6 different cofactor regeneration methods. If enzyme availability and stability allow their use under the reaction conditions, enzyme-coupled cofactor regeneration should be employed for the preparative production by enzymatic means.

Experimental Section

Materials

Acetophenone, 1-phenylethanol, 4-chloroacetophenone, 1-(4-chlorophenyl)-ethanol, 4-methoxyacetophenone and, all linear ketones and alcohols, *n*-dodecane and MTBE were purchased from Merck/Germany. 3-Methoxyacetophenone and 1-(2-methoxyphenyl)-ethanol were supplied by Acros Organics/Germany, 2-methoxyacetophenone was obtained from Fluka/Germany. 2-Propanol and acetone were purchased from J.T. Baker/Germany. All chemicals were obtained in *p.a.* quality.

The alcohol dehydrogenase from *Lactobacillus brevis* (EC 1.1.1.2), the glucose dehydrogenase from *Pseudomonas* species (E.C. 1.1.1.47) and the NADP$^+$ (disodium salt) were supplied by Jülich Chiral Solutions/Germany. Aqueous solutions were buffered using 50 mM potassium phosphate buffer to pH 7 containing 1 mM magnesium chloride if not mentioned otherwise.

Analysis

Ketones and alcohols 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 a FS-Cyclodex-beta-I/P column Macherey&Nagel/Germany (He 2.0 mL min^{-1}). To measure concentrations of acetone and 2-propanol an HPLC method was used. Therefore, the HPLC was equipped with an RI detector and a BIORAD Aminex HDK-87 H Ion Exclusion column (300 × 7.8) 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.[37,38] All measurements were carried out at least 7-fold. Standard deviations are given as errors. Substrate solutions containing the ketone and alcohol were prepared in MTBE. In screw capped vials (8 mL), the aqueous phase (4 mL) was covered with the 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[20] is then calculated according to ε = [(starting concentration)/(residual concentration)] − 1. In this method, activity coefficients describing the deviations from dilute behaviour were neglected. Measurements at different substrate concentrations assured that partition coefficients were not concentration depended in this concentration range.

Equilibrium Conversion (*LB*-ADH), Monophasic

Acetonitrile was added to the buffered aqueous solution in a mole fraction $x = 0.05$ (13% v/v). *LB*-ADH, NADP$^+$ (0.1 mmol L^{-1}), 2-propanol and the ketone were dissolved in this mixture (2.5 mL) and stored at 30 °C. Samples were withdrawn, diluted with ethyl acetate as appropriate and analysed by GC.

Equilibrium Conversion (*LB*-ADH), Biphasic

An aqueous solution (2.5 mL) of *LB*-ADH, NADP$^+$ (0.1 mmol L^{-1}) and 2-propanol was covered with MTBE

(2.5 mL) containing the ketone (10 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 the ketone and alcohol were corrected by their partition coefficients:

total concentration of E = [(concentration of E in non-reactive phase)/ε] + concentration of E in non-reactive phase.

Conversion was calculated according to:

X = (total product concentration)/[(total substrate concentration) + (total product concentration)].

The same reaction conditions were applied to obtain 1-(4-methoxyphenyl)-ethanol, 1-(3-methoxyphenyl)-ethanol and 1-(4-nitrophenyl)-ethanol for the investigation of partition coefficients.

For the reduction of 2-octanone to 2-octanol on a preparative scale, *LB*-ADH (300 mg), *PS*-GDH (18 mg), $NADP^+$ (40 mg) and glucose (90 g) were dissolved in the aqueous phase and covered by MTBE (200 mL) and 2-octanone (51 g). Samples were withdrawn from the organic phase and analysed by GC-FID. After the reaction, the organic phase was separated and dried over sodium sulfate. MTBE was evaporated and the remaining (*R*)-2-octanol was distilled for further purification (isolated yield: 71.2%).

Calculations with COSMO-RS

The DFT/COSMO calculations with the B-P density functional and the TZVP basis set combination were performed using Turbomole 5.7.1. For the following statistical thermodynamics, COSMO*therm*-C2.1–01.054 was used. The free energy of a molecule in the solvent provided from COSMO*therm* was calculated for 25 °C and corrected by adding $RT\mathrm{Ln}(x_i)$. The mixture composition was iterated until the free energy of reaction $G_{s,R}$ was zero [Eq. (5)]. With this mixture composition and the referring activity coefficients, also provided from COSMO*therm*, the equilibrium constant $K(\Delta G)$ was calculated using Eq. (4).

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References

[1] B. Cornils, W. A. Herrmann, I. T. Hovat, W. Leitner, S. Mecking, H. Olivier-Bourbigou, D. Vogt, *Multiphase homogeneous catalysis*, Wiley-VCH, Weinheim, **2005**.
[2] H. Gröger, H. Hummel, C. Rollmann, F. Chamouleau, H. Hüsken, H. Werner, C. Wunderlich, K. Abokitse, K. Drauz, S. Buchholz, *Tetrahedron* **2004**, *60*, 633–640.
[3] M. Villela Filho, T. Stillger, M. Müller, A. Liese, C. Wandrey, *Angew. Chem. Int. Ed.* **2003**, *42*, 2993–2996.
[4] H. Klein, R. Jackstell, M. Beller, *Chem. Commun.* **2005**, 2283–2285.
[5] D. J. Adams, D. J. Cole-Hamilton, E. G. Hope, P. J. Pogorzelec, A. M. Stuart, *J. Org. Chem.* **2004**, *68*, 1413–1417.
[6] P. J. Dyson, *Chimia* **2005**, *59*, 66–71.
[7] M. Eckstein, M. Villela Filho, A. Liese, U. Kragl, *Chem. Commun.* **2004**, 1084–1085.
[8] H. Pfründer, M. Amidjojo, U. Kragl, D. Weuster-Botz, *Angew. Chem. Int. Ed.* **2004**, *43*, 4529–4531.
[9] W. Leitner, *Pure Appl. Chem.* **2004**, *76*, 635–644.
[10] M. Eckstein, M. Peters, J. Lembrecht, A. Spieß, L. Greiner, *Adv. Synth. Catal.* **2006**, *348*, 1591–1596.
[11] W. Keim, *Green Chem.* **2003**, *5*, 105–111.
[12] H. Adkins, R. M. Elofson, A. G. Rossow, C. C. Robinson, *J. Am. Chem. Soc.* **1949**, *71*, 3622–3629.
[13] S. Gladiali, G. Mestroni, in: *Transition metals for organic synthesis – Building blocks and fine chemicals*, (Eds.: M. Beller, C. Bolm), Wiley VCH, Weinheim, **1998**, Vol. 2.
[14] A. J. J. Straathof, S. Panke, A. Schmid, *Curr. Opinion Biotechnol.* **2002**, *13*, 548–556.
[15] W. Kroutil, H. Mang, K. Edegger, K. Faber, *Curr. Opinion Biotechnol.* **2004**, *8*, 120–126.
[16] M. Breuer, K. Ditrich, T. Habicher, B. Hauer, M. Keßler, R. Stürmer, T. Zelinski, *Angew. Chem. Int. Ed.* **2004**, *43*, 788–824.
[17] For a general scheme of biphasic reactions see Supporting Information.
[18] L. Greiner, S. Laue, A. Liese, C. Wandrey, *Chem. Eur. J.* **2006**, *12*, 1818–1823.
[19] For mK = 1, it can be shown that $X = S(S + 1)^{-1}$.
[20] For a comprehensive lists of all mathematical definitions, abbreviations and symbols used, see the Supporting Information.
[21] K. Martinek, A. M. Klibanov, G. P. Samokhin, A. N. Semenov, I. V. Berezin, *Bioorg. Khimiya* **1977**, *3*, 696–702.
[22] D. D. Wagmann, W. H. Evans, V. B. Parker, R. H. Schumm, I. Halow, S. M. Bailey, K. L. Churney, R. L. Nuttall, *J. Phys. Chem. Ref. Data* **1982**, *11*, suppl.2.
[23] M. L. Mavrovouniotis, *Biotech. Bioeng.* **1990**, *36*, 1070–1082.
[24] R. G. Forsythe, P. D. Karp, M. L. Mavrovouniotis, *CABIOS – Computer applications in the biosciences* **1997**, *13*, 537–543.
[25] A. Klamt, *J. Phys. Chem.* **1995**, *99*, 2224–2235.
[26] W. Arlt, O. Spuhl, A. Klamt, *Chem. Eng. Process.* **2004**, *43*, 221–238.
[27] P. Sykes, *Reaktionsmechanismen der organischen Chemie*, 9th edn., VCH, Weinheim, **1988**.
[28] For values of ΔE^0 and σ_X see Supporting Information.
[29] P. Adlercreutz, *Biocatal. Biotrans.* **1996**, *14*, 1–30.
[30] M. Eckstein, T. Daußmann, U. Kragl, *Biocatal. Biotrans.* **2004**, *22*, 89–96.

[31] W. M. Clark, *Oxidation-reduction potentials of organic systems*, R. E. Krieger Publishing Company, Huntington, N. Y., **1972**.
[32] J. M. Vrtis, A. K. White, W. M. Metcalf, W. A. van der Donk, *Angew. Chem. Int. Ed.* **2002**, *114*, 3391–3393.
[33] R. P. S. Chaplin, A. A. Wragg, *J. Appl. Electrochemistry* **2003**, *33*, 1107–1123.
[34] L. Greiner, D. H. Müller, E. C. D. van den Ban, J. Wöltinger, C. Wandrey, A. Liese, *Adv. Synth. Catal.* **2003**, *345*, 679–683.
[35] R. Mertens, L. Greiner, E. C. D. van den Ban, H. B. C. M. Haaker, A. Liese, *J. Mol. Catal. B* **2003**, *24–25*, 39–52.
[36] K. Vuorilehto, S. Lütz, C. Wandrey, *Bioelectrochemistry* **2004**, *65*, 1–7.
[37] K. Fan, P. Ouyang, X. Wu, Z. Lu, *J. Chem. Technol. Biotechnol.* **2001**, *76*, 851–856.
[38] J. Bruijn *de*, F. Busser, W. Seinen, J. Hermens, *Environmental Toxicology Chemistry* **1989**, 8, 499–512.

