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Prediction of partition coefficients using COSMO-RS: Solvent screening for maximum conversion in biocatalytic two-phase reaction systems

Antje C. Spieß [a,*], Werner Eberhard [a], Martina Peters [b], Marrit F. Eckstein [b], Lasse Greiner [b], Jochen Büchs [a]

[a] *RWTH Aachen University, Biochemical Engineering, Worringerweg 1, 52074 Aachen, Germany*
[b] *RWTH Aachen University, Institute of Technical and Macromolecular Chemistry, Worringerweg 1, 52074 Aachen, Germany*

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Abstract

Many biocatalytic reaction systems are biphasic with a reactive and an immiscible non-reactive phase. The reactive, mostly aqueous phase provides a natural enzyme environment and the non-reactive phase serves for delivery of dissolved substrates at high concentrations and for extraction of products. The proper choice of the non-reactive phase will have manifold influence on the catalytic parameters, such as activity, selectivity, and stability, but also on the maximum obtainable conversion or yield.

Conversion or yield constitutes a concise target of practical relevance for rational solvent screening which requires thermodynamic information on coupled reaction and phase equilibria as input information. As long as the reactive phase is kept constant, only the partition equilibrium of each solute in any solvent combination has to be determined. The experimental determination of these data requires a considerable laboratory effort. Therefore, an in-silico screening of solvents for maximal conversion of alcohol dehydrogenase-catalysed oxidoreductions of prochiral ketones was evaluated. COSMO-RS was used for the prediction of solute partitioning between organic solvents and aqueous reaction medium.

Although significant absolute deviations were found, COSMO-RS still predicted the correct trends for the partition coefficients of solutes in different solvents. The calculated overall reaction equilibrium using these partition coefficients again resulted in the prediction of the correct best solvent regarding conversion.

Keywords: Thermodynamics; COSMO-RS prediction; Two-phase systems

1. Introduction

Biphasic reaction systems have many beneficial properties for poorly soluble, instable, or inhibitory substrates and products targeted by industrial biocatalysis. They may increase volumetric productivity by applying a high substrate concentration in the non-reactive phase and by reducing enzyme inhibition in the reactive phase. Aqueous degradation reactions may be suppressed and product recovery is facilitated by separation of the organic phase, thus eventually providing the transfer medium to a further chemical reaction step. Finally, the overall reaction equilibrium may be shifted [1]. Therefore, the biocatalytic production of fine chemicals and pharmaceuticals using various enzymes has been performed in biphasic systems ranging from aqueous [2] over aqueous-organic [3,4], aqueous-ionic liquid [5] to ionic liquid-supercritical carbon dioxide systems [6].

One task during process design is the screening or engineering of a suitable medium [7]. A multitude of screening targets exist, since the choice of solvent influences the enzyme stability, which is sometimes correlated with solvent hydrophobicity and functionality [8,9], the enzyme activity and selectivity, which may at least partially be described by substrate solvation [10,11], and last but not least the overall thermodynamic reaction equilibrium. To describe substrate solvation, Halling suggests the use of classical thermodynamic approaches since solvation may already explain the major part of the phenomena observed in non-conventional biocatalysis [12]. For biphasic media, analytical expressions of varying complexity for the overall reaction equilibrium or equilibrium conversion have been derived, both for simple systems [13–15] and for those taking changes in

* Corresponding author. Tel.: +49 241 80 28111; fax: +49 241 80 22265.
E-mail address: spiess@biovt.rwth-aachen.de (A.C. Spieß).

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phase volume during reaction into account [16]. Using estimation methods such as UNIFAC, even concentration-dependent partitioning can be considered [17,18].

The replacement of time-consuming screening experiments by computational approaches is considered a pre-requisite for the establishment of biocatalytic routes to fine chemicals and pharmaceuticals on the market since they may reduce the development time [19,20]. A sound molecular thermodynamic basis, including the correct description of large biomolecules in electrolyte systems, needs to form the core of future biochemical flow-sheeting tools for the description of biochemical reaction and downstream processing operations [21,22]. Here, the classical group contribution methods have the intrinsic disadvantage of being limited to interpolation and partially extrapolation, so they necessarily fail for structurally new compounds such as ionic liquids or complex pharmaceuticals [23]. Quantum mechanic based tools, among them COSMO-RS, are currently suggested at least for semi-quantitative tasks like solvent screening for fine chemicals, pharmaceuticals and biotechnology [24,25].

Within this publication we describe the evaluation of COSMO-RS performance in predicting equilibrium partitioning for enzyme-catalysed oxidoreductions in biphasic media. At first, the quantitative performance for a specific reaction and solvent and then for a set of substrates and solvents is evaluated. Then, the results are used for solvent screening for a defined reaction based on equilibrium conversion as optimisation target. Part of the experimental data has been published previously [13,26,27].

2. Experimental and computational methods

2.1. Materials

Acetophenone, 1-phenylethanol, 4-chloroacetophenone, 1-(4-chlorophenyl)-ethanol, 4-nitroacetophenone, 1-(4-nitrophenyl)-ethanol, and all linear ketones and alcohols, dodecane, MTBE, and formic acid were purchased from Merck, Darmstadt, Germany. Acetophenone, and 2-chloroacetophenone were obtained from Fluka, Seelze, Germany. 2-Propanol and acetone were purchased from J.T. Baker, Lohmar, Germany. All chemicals were obtained in p.a. quality. All solvents were of HPLC grade and were supplied by Merck, Darmstadt, Germany, J.T. Baker, Lohmar, Germany, or Roth, Karlsruhe, Germany, respectively.

The alcohol dehydrogenase from *Lactobacillus brevis* (*LB*-ADH, E.C. 1.1.1.2) and the $NADP^+$ (disodium salt) were supplied by Jülich Fine Chemicals (now Julich Chiral Solutions), Jülich, Germany. Aqueous solutions were buffered using 50 mM phosphate buffer to pH 7 containing 1 mM magnesium chloride if not mentioned otherwise.

2.2. GC and HPLC analysis

The concentrations of acetophenone and 1-phenylethanol were determined using Varian CP-3800 using a FS-FFAP-CB-0.25 column by CS-Chromatographie Service, Langerwehe, Germany (decane as internal standard, flow rate N_2 0.8 mL min^{-1}, temperature from 40 °C to 180 °C). 2-chloroacetophenone was determined using the same GC, with Varian CP-7717 column (again flow rate N_2 0.8 mL min^{-1}, temperature from 40 °C to 185 °C). All other ketones and alcohols were analysed by gas chromatography, using a Varian CP-3800 equipped with DB-1701 capillary column Alltech, Unterhaching, Germany (dodecane as internal standard, flow rate He 2.0 mL min^{-1}). Samples were diluted with ethyl acetate prior to measurement.

Formic acid was analysed by HPLC using RI detection with CS Organic Acid Resin column (300 mm × 8 mm at 60 °C) with 2 mM sulphuric acid (flow rate 0.6 mL min^{-1}) as eluent. 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) with sulphuric acid (6 mM, 0.8 mL min^{-1}). Samples were diluted with water as appropriate.

2.3. Measurement of equilibrium partitioning

For the measurement of concentration dependent (measurement range between 1.5 mM and 350 mM) equilibrium partitioning at 25 °C, quantities of water, solvent, and solute (acetophenone, 1-phenylethanol, 2-chloroacetophenone, formic acid) were defined to well exceed the analytical detection limit, and filled into 4 mL screw capped glass vials. The headspace was minimized so as to reduce the loss of volatile material. The vials were repetitively shaken manually and left for one week. Previously it was verified by sampling after different time periods that the phase equilibrium was achieved within that period. One 200 μL sample per vial was taken from the organic phase and analysed by GC, except for formic acid, where the aqueous phase was sampled from an upside-down vial in order not to penetrate the organic phase during sampling and the equilibrium concentration was determined using HPLC.

Constant partition coefficients, i.e. concentration-independent partition coefficients at finite dilution [13], were measured adopting methods reported previously [28,29]. All measurements were carried out at least 7fold. Substrate solutions containing the ketone and alcohol in the concentration range between 10 and 100 mM 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. To avoid traces of the organic phase in the aqueous sample, the GC syringe needle was cautiously rinsed in the aqueous phase. In the case of acetone and 2-propanol, samples were taken only from the aqueous phase both prior to contacting with solvents and after adding the organic phase and equilibrating subsequently. Following the same procedure as above, samples were analysed by HPLC. The partition coefficient P is then calculated according to:

$$P = \frac{c_0}{c_{\text{equilibrium}}} - 1 \quad (1)$$

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2.4. Measurement of reaction equilibria [13]

An aqueous solution (2.5 mL) of *LB*-ADH, $NADP^+$, 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 horizontal shaker (30 °C, 200 rpm). Samples were withdrawn from the non-reactive phase and analysed by GC. The mass balance of acetophenone and 1-phenylethanol was checked using the respective partition coefficients.

2.5. Calculation of partition coefficients with COSMO-RS

The software GaussView (Version 3.09, Gaussian Inc., Wallingford, US) was used to assemble all molecules. The DFT/COSMO (COnductor-like Screening MOdel [30],) calculation was performed using TURBOMOLE (Version 5.7.1, COSMO*logic*, Leverkusen, Germany) with the BP86 density functional and a TZVP basis set combination [31]. For the following statistical thermodynamics, the software COSMO*therm* (Version C2.1-0104/C2.1-0105, COSMO*logic*, Leverkusen, Germany) was used as implementation of COSMO for real solvents (COSMO-RS) [32,33]. The partition coefficients at infinite dilution are direct result of COSMO*therm* calculations:

$$P_i^{org-aq} = \frac{\exp(\mu_i^{aq} - \mu_i^{org})}{RT} \times \frac{v^{aq}}{v^{org}} \quad (2)$$

The mutual solubility of the solvents was taken into account using an experimental data collection [34]. The quotient of the molar volumes of the phases (v^{aq}/v^{org}) was estimated from the COSMO volumes since only ambient temperatures and pressures are considered here.

The equilibrium partitioning at finite concentrations, however, was determined iteratively using the activity coefficients in both phases calculated by COSMO*therm*. In contrast to the calculation at infinite dilution, no experimental data on phase composition could be provided here. Components (water, organic solvent and solute) were exchanged between the aqueous and the organic phase, until the equilibrium condition for all three components held true:

$$\gamma_i^{aq} x^{aq} = \gamma^{org} x^{org} \quad (3)$$

The resulting molar fractions x_i^{org} and x_i^{aq} of both phases could then be used to calculate the volumetric equilibrium concentrations.

2.6. Calculation of equilibrium conversion for biphasic systems

The equilibrium conversion X for the bimolecular catalysed reaction was calculated according to the following recently derived [13] algebraic equation:

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

with K being the equilibrium constant in the reactive phase, S the initial substrate ratio and m a factor taking the ideal partitioning of all reactants between the reactive and the non-reactive phase into account:

$$m = \frac{(P_C V + 1)(P_D V + 1)}{(P_A V + 1)(P_B V + 1)} \quad (5)$$

Here, P_i denote the concentration-independent partition coefficient of compound i and V the phase volume ratio. Similar expressions were obtained previously by Martinek et al. [14]. The calculation of the equilibrium conversion was performed both with the COSMO-RS calculated partition coefficients at infinite dilution and with the experimentally determined constant partition coefficients.

3. Results and discussion

3.1. Alcohol dehydrogenase catalysed reductions

The biocatalytic asymmetric reduction of acetophenone and derivatives is a typical example for a reaction where the advantages of biphasic systems pay out. The value-creating reduction reaction needs to be coupled with an oxidation reaction to regenerate the costly cofactor NAD(P)H. Interesting substrates typically exhibit a low solubility in water, whereas the alcohol dehydrogenases prefer an aqueous working environment. Therefore, aqueous-organic [35–37], and aqueous–ionic liquid systems [38,39] are in use. The reaction equilibrium K of the coupled oxidoreduction is significantly influenced by the choice of the regeneration method for the cofactor, but considered constant in the aqueous solution. The addition of an immiscible solvent to the reaction system will influence the overall reaction equilibrium by partitioning of all substrates and products between the non-reactive organic and the reactive aqueous phase (Fig. 1).

The design and optimisation of the reaction system requires the knowledge of the partitioning of all substrates and products

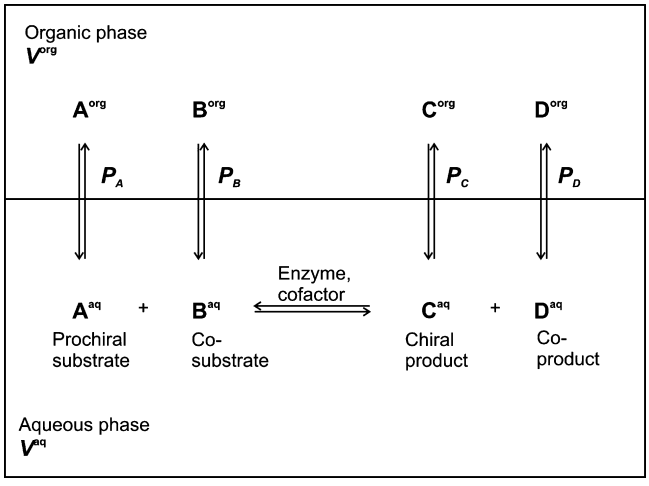

Fig. 1. Alcohol dehydrogenase catalysed reduction of prochiral substrate A (ketone) to chiral product C (alcohol). Cofactor regeneration with co-substrate B (alcohol) to co-product D (ketone). All substrates and products partition between the organic and the aqueous phase. The enzyme is considered to be insoluble in the organic solvent.

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between the both phases. The intent here is a technical application; therefore concentrations of the solutes in the order of magnitude of 100 mM are envisaged. For a biocatalytic esterification in aqueous-organic two-phase systems, it was previously found that non-ideal partitioning occurs [40]. Therefore, the calculation and measurement of concentration-dependent partitioning equilibrium was performed.

3.2. Prediction of concentration-dependent partitioning equilibrium

The first system investigated was the reduction of acetophenone and a functionalized derivative (2-chloroacetophenone) to the corresponding alcohols in biphasic reaction medium hexane-water. The formate dehydrogenase (FDH) coupled cofactor regeneration with formic acid as co-substrate yielded volatile carbon dioxide as co-product, thus driving the reaction equilibrium to the desired product. The predicted concentration-dependent phase equilibria, and those at infinite dilution as well as experimental data points for the distribution of acetophenone, 1-phenylethanol, 2-chloroacetophenone and formic acid in water/hexane are given in Fig. 2.

As indicated by the experimental values, the solutes show a non-linear concentration-dependent distribution between the organic and the aqueous phase. For acetophenone (Fig. 2A), COSMO-RS predictions for finite concentrations are both qualitatively and quantitatively in good agreement. At concentrations of 300 mM in the organic phase, the solubility limit in the aqueous phase seems to be reached at around 15 mM. Solubility of acetophenone in pure water at 20 °C is 0.55 mM [41]. For the corresponding reaction product, 1-phenylethanol (Fig. 2B), however, larger deviations between the prediction and the experimental data occur. The same, even stronger effect can be observed for the chloro-substituted acetophenone (Fig. 2C). In both cases, a more hydrophobic character of the substances is predicted than determined experimentally. A similar overestimation of octanol–water partition coefficients for alcohols and chloro-substituted compounds has been observed previously [42]. Even the deviation of up to factor 10 lies within the previously published prediction accuracy. For formic acid, however, the predicted and experimental values deviate within two orders of magnitude. In the same plot (Fig. 2D), the prediction of the formic acid concentration is not distinguishable from the ordinate. Only in the insert in Fig. 2D, it can be seen that the aqueous formic acid concentration differs from infinity. The deviations here can be attributed to the formation of dimers in the organic solvent at higher concentrations of the acid in the organic phase [40,43]. The mere statistical approach of COSMO-RS is not capable of representing this associating behaviour of a chemical compound. The consideration of conformers in case of alcohols and of dimers in case of carboxylic acids during the quantum mechanical calculation of the molecule surface will most probably lead to better quantitative results.

Some error is introduced to the prediction of equilibrium partitioning at finite concentration because in this ternary system the phase composition has to be calculated from the activity coefficients, wherease in the infinite dilution case, experimental binary

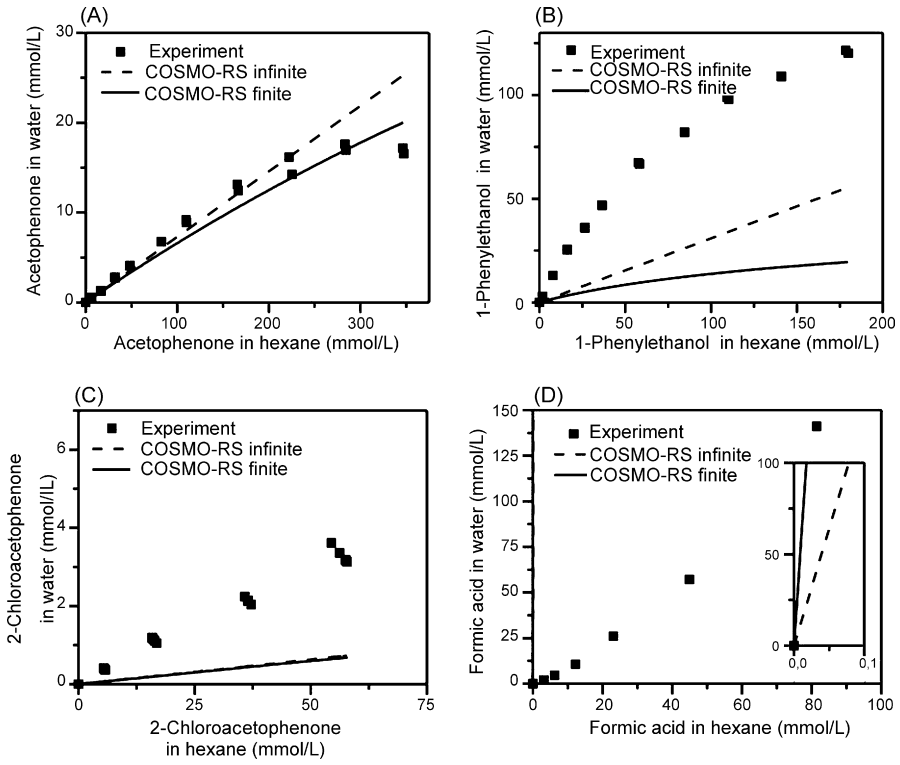

Fig. 2. Concentration-dependent equilibrium partitioning in hexane–water biphasic systems. (A) acetophenone; (B) 1-phenylethanol; (C) 2-chloroacetophenone; (D) formic acid. Squares indicate experimental data points. Solid lines refer to COSMO-RS calculations for finite dilution and dashed lines refer to infinite dilution.

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liquid-liquid-equilibrium data may be used. For infinite dilution both approaches can be compared: For acetophenone and 1-phenylethanol in hexane/water at 30 °C, the partition coefficients at infinite dilution calculated using COSMO*therm*'s phase composition calculation are slightly lower (17.232 and 6.842) than those calculated using the experimental LLE (19.231 and 8.621). Transferring those results qualitatively to the finite concentrations, the observed deviation in Fig. 2B between experimental data and the predicted curves would even increase.

Although the prediction of partitioning at finite dilution better represents the qualitative behaviour of the solute partitioning between the aqueous and hexane phase, the prediction of partitioning at infinite dilution is equal (for 2-chloroacetophenone) or superior (in all other cases) in quantitative terms. Therefore, only infinite dilution partition coefficients were predicted for further studies. A similar approach was chosen previously for the prediction of partitioning using UNIFAC [44].

3.3. Prediction of infinite dilution partition coefficients

A set of experimentally determined partition coefficients was compared to the COSMO-RS predicted partition coefficients at infinite dilution. In order to eliminate the systematic error of formic acid, the chosen solutes now cover substances used in the reduction of aliphatic ketones and acetophenone-derivatives with a substrate-coupled cofactor regeneration using isopropanol as co-substrate in several biphasic solvent combinations (hexane, heptane, MTBE, toluene, cyclohexane–water). In the experimentally covered concentration range of 10 to 100 mM, the partition coefficients dit not vary, but remained constant. Therefore they are considered a good approximation to infinite dilution. The numerical values for the experimental data can be found in [13,26,27].

Fig. 3 shows a logarithmic parity plot of the predicted infinite dilution partition coefficients over three sets of experimentally determined data. The first data set covers substrates and products of the acetophenone reduction using 2-propanol as co-substrate in several solvent systems [13]. The second set covers linear ketones and some acetophenone derivatives and their corresponding alcohols in MTBE/water biphasic systems [26]; the third data set the same compounds like the second, however in hexane/water biphasic system [27]. A certain bias towards overestimation of the partition coefficients towards hydrophobicity as mentioned above can be observed. The lower 95% boundary corresponds to factor 2.5 units (0.4 $\log(P^{\infty})$) deviation, and the upper 95% boundary to factor 8 (0.9 $\log(P^{\infty})$ units). A calculation of the root mean square (rms) deviation results in 0.25 $\log(P^{\infty})$ units. Even the COSMO-RS parameterisation data set has a higher rms deviation of 0.34 $\log(P^{\infty})$ units [45]. The prediction of the octanol-water partition coefficient $\log P_{O/W,i}$ for more than 60 compounds resulted in a slightly inferior deviation of 0.43 $\log P_{O/W,i}$ units (rms) [42]. In comparison to the prediction accuracy (0.64–0.83 $\log P_{O/W,i}$ units rms) of two UNIFAC versions [42], the predictive power of COSMO-RS becomes apparent. An analysis of data subsets indicates a significantly better prediction of partitioning of the ketones in comparison to the alcohols. In both groups MTBE represents the solvent with the largest scatter.

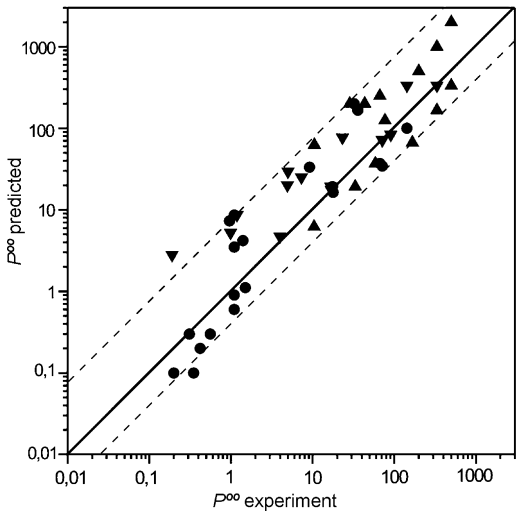

Fig. 3. Comparison of experimental and COSMO-RS predicted infinite dilution partition coefficients for selected substrates and products in organic/aqueous biphasic reaction systems. Circles indicate the data points corresponding to reference [13], triangles up those of [26], and triangles down those of [27]. The thick solid line indicates parity. The thin dashed lines enclose 95% of the data points.

3.4. Solvent screening – partition coefficients

Considering this encouraging data quality, the screening of solvent combinations for one specific oxidoreduction reaction is now carried out by again predicting infinite dilution partition coefficients using COSMO-RS. The investigated reaction is the *Lactobacillus brevis* alcohol dehydrogenase catalysed reduction of acetophenone to 1-phenylethanol using the 2-propanol-coupled regeneration of NADP with acetone as co-product. First, an overview of partition coefficient prediction for all solutes across several solvents is given in Fig. 4. The solvents cover a representative range of organic solvents which are often used for enzyme catalysed biphasic reaction systems.

Fig. 4 indicates that the prediction of partition coefficients of ketones in several solvent combinations is more successful than that of the corresponding alcohols. The predicted partition coefficients at infinite dilution of acetophenone (Fig. 4A) and acetone (Fig. 4D) have a relative error of less than 30% with respect to the measured partition coefficients. Nearly quantitative prediction is obtained for acetophenone in linear alkane/water solvent combination. A bias to underestimate the infinite dilution partition coefficients of ketones can be observed for all solvent combinations. In contrast to the ketones, the infinite dilution partition coefficients of 1-phenylethanol are overestimated for all solvent combinations (Fig. 4B). Only the partitioning of the very small 2-propanol molecule is good agreement in toluene and even underestimated in linear alkanes (Fig. 4C). The prediction accuracy for our reaction system is within one order of magnitude in linear scale. Looking at the sequence of partition coefficients for one solute across several solvent combinations, COSMO-RS is capable of predicting the correct trends.

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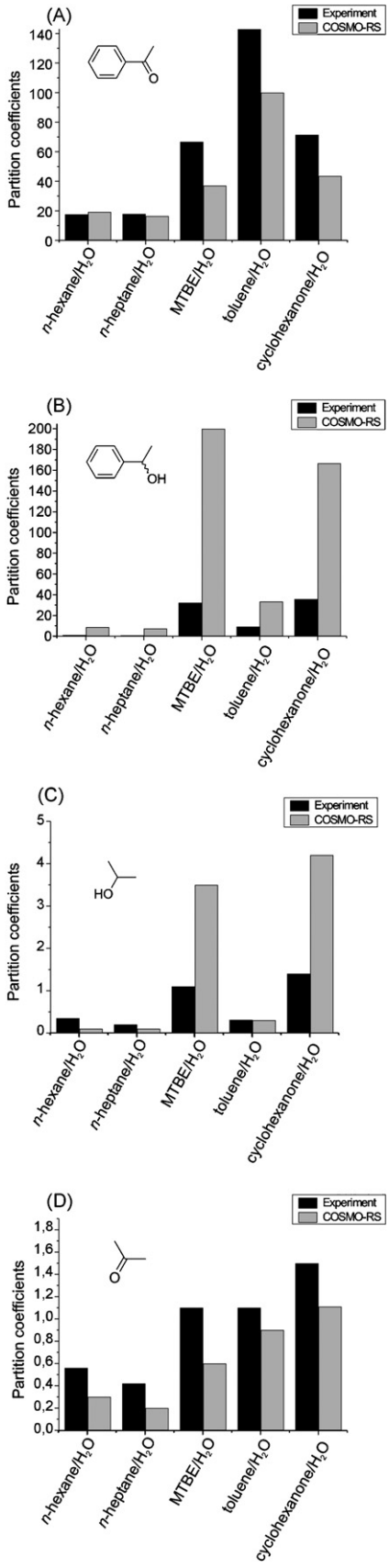

Fig. 4. Experimental and COSMO-RS predicted values for infinite dilution partition coefficients of acetophenone (A); 1-phenylethanol (B); 2-propanol (C); and acetone (D) in various organic solvent/water systems.

3.5. Solvent screening with maximum conversion as target

Having identified correct trends for infinite dilution partition coefficients for all reactants, the solvent combinations may now be compared with respect to the target function of maximum conversion (Equation (4)). The reaction equilibrium constant K (= 0.426 at 30 °C) is intrinsically defined by the aqueous reaction environment. The substrate ratio $S = 20$ and the phase volume ratio $V = 1$ are kept constant across all solvent combinations. Thus, the maximum conversion may be calculated using the predicted partition coefficients at infinite dilution, and compared with both the calculated conversion using the experimentally determined partition coefficients and the experimentally determined equilibrium conversion (Fig. 5).

The screening target theoretical conversion as calculated based on predicted infinite dilution partition coefficients shows a systematic deviation of roughly 20% above the corresponding experimental data (Fig. 5). The analogously calculated conversion using experimentally determined partition coefficients, however, is in good agreement with the directly measured maximum conversion. Therefore, the error contribution of the underlying ideality assumption in equation (4) does not account too much. Although this assumption is in contrast to the non-ideal partitioning of the investigated reaction systems (Fig. 2), the influence on the maximum conversion is negligible in comparison to the deviation in the predicted partition coefficients. The error contribution may be explained by analysing equation (5). The overall reaction equilibrium constant is corrected by an expression containing the phase volume ratio and the partition coefficients. The overestimation of 1-phenylethanol partition coefficients contributes strongest to the factor m, thus resulting in an overestimated overall reaction equilibrium constant mK. This again leads to a higher calculated equilibrium conversion.

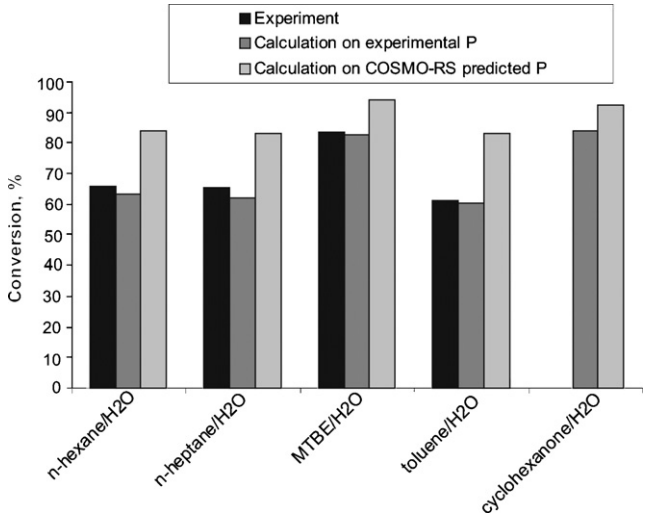

Fig. 5. Equilibrium conversion of acetophenone to 1-phenylethanol in various organic solvent/water systems using *LB*-ADH with 2-propanol as co-substrate. Black bars indicate experimental values; dark grey bars represent the calculated conversion based on experimentally determined partition coefficients. Light grey bars show the calculated conversion based on the COSMO-RS predicted partition coefficients at infinite dilution. $K = 0.426$; $S = 20$; $V = 1$.

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Even if the absolute values for predicted conversions deviate similar to the prediction of individual partition coefficients, the trends are again represented well. One reason for this is that errors may cancel out in a reaction situation where functional groups such as ketones and alcohols are retained during the reaction. The highest experimentally determined conversion was successfully identified by the highest predicted conversion.

Looking at the solvent combination cyclohexanone/water, however, the second highest conversion was predicted both using predicted and experimentally determined partition coefficients, but was not reached experimentally since the enzyme did not retain sufficient activity in this solvent combination. This is an often observed effect for solvents of moderate hydrophobicity [8], also for oxidoreduction reactions [35]. This brings the alternative solvent screening targets back into mind: Enzyme activity, selectivity, and stability. Thus, the thermodynamic screening may indeed be performed in-silico, but an experimental verification of the top solvent candidates is mandatory. Even with a final confirmation run, the in-silico-screening approach will save considerable laboratory effort.

4. Conclusion

For the design of biocatalytic biphasic reaction systems, the knowledge of partition equilibria for all reactants in suitable solvent systems is helpful for process modelling as well as for the targeted solvent screening. The quantum mechanical and statistical thermodynamics-based tool COSMO-RS was evaluated with respect to above quantitative task, i.e. prediction of finite and infinite dilution partition coefficients for modelling purpose and the qualitative task of solvent screening for maximum conversion.

Although non-ideal equilibrium partitioning was well predicted, the infinite dilution partition coefficients still lead to smaller overall deviations from the experimental values. Therefore, more sophisticated approaches to overall reaction equilibrium calculations, taking the changing reactant partitioning during the reaction into account, seem overdone at the current stage. For the selected components the experimental data were systematically better represented than by other tools, but an improvement of the quantitative performance would still be highly desirable for a more in-depth process design.

For qualitative tasks like solvent screening, however, the quality of prediction seems sufficient, since even the order of solvents regarding their infinite dilution partition coefficients was predicted correctly. Based on these data, the theoretical conversion could be calculated, resulting in the selection of suitable solvents for biphasic reductions of acetophenone to 1-phenylethanol. The correct order of solvents regarding their theoretical conversion was achieved, because systematic deviations in predicted partition coefficients for individual compounds neutralise in the reaction context. The experimental verification of this choice will remain necessary with respect to solvent tolerance of the catalyst and solvation effects that influence the catalytic performance, such as activity, specificity, and selectivity.

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Appendix A. Nomenclature

Symbols

c	concentration (mol L^{-1})
K	equilibrium constant (–)
m	factor accounting for partitioning in biphasic media (–)
P	partition coefficient (–)
R	gas constant (8.314 J mol^{-1} K^{-1})
S	substrate ratio ($=c_{B,0}/c_{A,0}$) (–)
T	temperature (K)
v	molar volume (L mol^{-1})
V	phase volume ratio ($=V^{org}/V^{aq}$) (–)
x	mole fraction (–)
X	conversion (–)

Greek letters

γ	activity coefficient (–)
μ	chemical potential (J mol^{-1})

Subcripts

A, B, C, D	compounds
eq	equilibrium
i	arbitrary compound
O	octanol
W	water
0	initial

Superscripts

aq	aqueous
org	organic
∞	infinite dilution

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