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Predicting the high concentration co-solvent influence on the reaction equilibria of the ADH-catalyzed reduction of acetophenone

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ABSTRACT

The use of co-solvents for the enhancement of the reaction parameters reaction rate, yield and enantioselectivity is an established optimization strategy in biotechnology. To determine the influence of co-solvents on even one of these reaction parameters requires a great amount of experimental data. Thus, predictive and physically sound models are desired to decrease the amount of experimental effort. This work aims at providing such a framework, which was applied to the ADH (alcohol dehydrogenase)-catalyzed reduction of acetophenone at 303.15 K and 1 bar in water (neat) and under the influence of up to 20 wt-% of polyethylene glycol (PEG) and 15 wt-% trisodium citrate (Na_3Cit). In a first step, the equilibrium composition was measured at constant pH. It was then shown that high concentration of PEG or Na_3Cit changed the equilibrium position significantly (up to a factor of 13) compared to neat reaction mixtures. To be able to predict this strong co-solvent influence on the reaction equilibrium, the experimentally determined equilibrium compositions of the neat reaction were converted into a thermodynamic equilibrium constant K_{th} using the activity coefficients γ_i of the reacting agents. The latter were predicted by electrolyte Perturbed-Chain Statistical Associating Fluid Theory (ePC–SAFT). These finally allowed quantitatively predicting the high concentration co-solvent influence on the equilibrium position.

1. Introduction

The application of co-solvents to optimize enzyme-catalyzed reactions is well described in literature. The addition of co-solvents can significantly change the reaction rate, yield and enantioselectivity of these reactions. Zhao et al. [1] described the significant influence of the ionic liquid 1-ethyl-3-methylimidazolium acetate on the selectivity and the enantiomeric excess of DL-phenylalanine methyl ester catalyzed by lyophilized *Bacillus licheniformis* protease. Kitagawa et al. [2] described the selective esterification of galactose catalyzed by an alkaline protease from *Streptomyces sp.* induced by the co-solvent dimethyl sulfoxide and Okochi et al. [3] investigated the increased reaction velocity of 3α-hydrosteroid dehydrogenase under the addition of the co-solvent 1-butyl-3-methylimidazolium (l)-lactate. These are only a few examples of the different diverse co-solvent influences studied in literature. This work focuses on the prediction of co-solvent influences on the reaction equilibrium of the reduction of acetophenone (ACP) to 1-phenylethanol (1-PE) catalyzed by a modified alcohol dehydrogenase (ADH) extracted from *E. Coli* (evo-1.1.270). The reaction was chosen due to its model character [4–7] and a previous work related to the reaction equilibria [8]. The reaction mechanism is shown in Fig. 1 in accordance to the recommendation [9] and to other works [4,8,10,11] based on the so-called chemical reference reaction. Please note, that $[NAD^+]^{-1}$ and $[NADH]^{-2}$ will be further denoted NAD^+ and NADH according to biochemical textbook knowledge [12].

In this work especially the influence of high co-solvent concentration on the reaction equilibrium of the ACP reduction was studied using up to 15 wt-% of trisodium citrate (Na_3Cit) and even up to 20 wt–% of polyethylene glycol (PEG). This level of co-solvent concentration is of interest as these co-solvents are either used as enhancer for reaction kinetics [13–16] or such co-solvents are present in biocompatible extraction systems, and thus are used in product purification steps (e.g. in-situ product removal) [17–22]. A framework to predict these influences is given in this work by

Abbreviations: ACP, acetophenone; ADH, alcohol dehydrogenase; ARD, average relative deviation; NADH, nicotinamide adenine dinucleotide in its protonated form; NAD+, nicotinamide adenine dinucleotide in its deprotonated form; 1-PE, 1-phenylethanol; PEG, polyethylene glycol; ePC-SAFT, electrolyte Perturbed-Chain Statistical Associating Fluid Theory.

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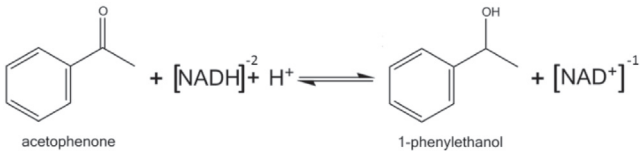

Fig. 1. Reaction scheme of the reduction of acetophenone to 1-phenylethanol with the required co-factor nicotinamide adenine dinucleotide in its protonated form ($NADH + H^+$) and deprotonated form (NAD^+) catalyzed by a modified alcohol dehydrogenase from *E. Coli* (evo-1.1.270) in aqueous medium.

predicting the activity coefficients of the reacting agents using the electrolyte Perturbed-Chain Statistical Associating Fluid Theory (ePC-SAFT) equation of state. Among the many different electrolyte thermodynamic models that have been proposed in the literature [23–26] ePC-SAFT was used in this work. The reason behind was that ePC-SAFT allows prediction of activity coefficients in multi-component electrolyte systems, which has already been shown in previous works [8,27–30].

The approach proposed in this work provides a screening tool for possible co-solvents that might be present during reaction steps or which are seen as promising in reactive media for a more efficient product separation.

2. Theoretical background

The driving force for every (bio)reaction is the Gibbs free energy of reaction $\Delta^R g$, which is defined as shown in Eq. (1):

$$\Delta^R g = \sum_i \nu_i \mu_i \tag{1}$$

$\Delta^R g$ is calculated as product of ν_i (the stoichiometric coefficient of the reacting agent i) and μ_i (the chemical potential of the reacting agent i) summed over all reacting agents i for a constant temperature T and pressure p. The chemical potential of component i in the liquid phase can be calculated with Eq. (2) based on the chemical potential of the pure component μ_{0i}, the universal gas constant R, the mole fraction x_i of the component i and the activity coefficient γ_i of the component i.

$$\mu_i(T,p) = \mu_{0i}(T,p) + RTln(x_i\gamma_i) \tag{2}$$

The chemical potential of the pure component μ_{0i} can be related to the standard Gibbs free energy of reaction $\Delta^R g^0$ and the thermodynamic chemical equilibrium constant K_{th} as shown in Eqs. (3)–(5).

$$\mu_{0i}(T,p) = g^0_{0i}(T,p) \tag{3}$$

$$\Delta^R g = \sum_i \nu_i g^0_{0i}(T,p) + RTln\prod_i a_i^{\nu_i} \tag{4}$$

$$\Delta^R g = \Delta^R g^0 + RTlnK_{th} \tag{5}$$

Based on the chemical reference reaction in Fig. 1, the chemical equilibrium constant can be expressed according to the recommendation [10]. Then, the chemical equilibrium constant is based on the activities of all present reacting species. In contrast to this recommendation, species-averaged activity coefficients of NAD^+ and NADH were used in this work. This yields the thermodynamic biochemical equilibrium constant K'_{th} of the ACP reduction as shown in Eq. (6).

$$K'_{th} = K'_{exp}\cdot K'_{\gamma} = \frac{x_{1-PE}\cdot x_{NAD^+}}{x_{ACP}\cdot x_{NADH}}\cdot\frac{\gamma_{1-PE}\cdot\gamma_{NAD^+}}{\gamma_{ACP}\cdot\gamma_{NADH}}\cdot\frac{1}{a_{H^+}} \tag{6}$$

$$K'_{th}\cdot a_{H^+} = X^{exp}\cdot\Gamma = \frac{x_{1-PE}\cdot x_{NAD^+}}{x_{ACP}\cdot x_{NADH}}\cdot\frac{\gamma_{1-PE}\cdot\gamma_{NAD^+}}{\gamma_{ACP}\cdot\gamma_{NADH}} \tag{7}$$

Eq. (6) is the classical law-of-mass-action-based expression of equilibrium constant that considers the activity of all reacting agents. In Eq. (7), the activity of H^+ was transferred to the side of K'_{th} as the pH and thus the activity of H^+ was constant in this work, leading to a constant value of the product $K'_{th}\cdot a_{H^+}$. Although H^+ is a reacting agent, the mole-fraction ratio X^{exp} does not contain H^+. Thus, X^{exp} is a direct and suitable indicator for the location of the equilibrium position. Further, the activity-coefficient ratio Γ of the reacting agents were required to determine $K'_{th}\cdot a_{H^+}$. These activity coefficients were predicted using the ePC-SAFT equation of state.

The activity coefficients of all reacting agents were calculated based on the pure-component reference state indicated by the subscript *0i*. The activity coefficients were calculated with the respective fugacity coefficients φ_i as shown in Eqs. (8)–(10).

$$\gamma_i = \frac{\varphi_i}{\varphi_{0i}} \tag{8}$$

$$ln(\varphi_i) = \frac{\mu_i^{res}(T,V,x)}{k_B\cdot T} - ln\left(1 + \left(\frac{\partial\left(\frac{a^{res}}{k_B\cdot T}\right)}{\partial\rho}\right)_{T,x_i}\right) \tag{9}$$

$$\frac{\mu_i^{res}}{k_B\cdot T} = \frac{a^{res}}{k_B\cdot T} + Z - 1 + \left(\frac{\partial\left(\frac{a^{res}}{k_B\cdot T}\right)}{\partial x_i}\right)_{T,V,x_{k\neq i}} - \sum_{j=1}^{N}\left(x_j\left(\frac{\partial\left(\frac{a^{res}}{k_B\cdot T}\right)}{\partial x_j}\right)_{T,V,x_{k\neq j}}\right) \tag{10}$$

The fugacity coefficient was calculated with the residual chemical potential $\boldsymbol{\mu_i^{res}}$, the Boltzmann constant k_B, the temperature T, the number density ρ and the residual Helmholtz energy a^{res}. In Eq. (10) Z denotes the compressibility factor and x_i the mole fraction of the component i. The residual Helmholtz energy a^{res} required for calculations was predicted by the ePC-SAFT equation of state [31].

$$a^{res} = a^{hc} + a^{disp} + a^{assoc} + a^{ion} \tag{11}$$

In this work four contributions to a^{res} were taken into account, namely a^{hc} (representing hard-chain forces), a^{disp} (representing dispersion interactions), a^{assoc} (describing hydrogen bonding) and the Debye-Hückel contribution a^{ion} for interactions of charged species. ePC-SAFT uses five pure-component parameters for the description of uncharged components that form hydrogen bonds: The segment number m_i^{seg}, the segment diameter σ_i, the dispersion-energy parameter u_i/k_B, the association-energy parameter $\epsilon^{A_iB_i}/k_B$ and the association-volume parameter $\kappa^{A_iB_i}$. In contrast to uncharged components, ions are commonly modeled as spherical non-associating components [31]. Pure-component parameters of ACP, 1-PE and water were taken from a previous work of Voges et al. [8], while parameters for the ion Na^+ were taken from Held et al. [32]. The parameters for citrate were fitted to osmotic-coefficient data and density data of aqueous sodium citrate and potassium citrate solutions. Homopolymer PEG parameters were taken from Stoychev et al. [33]. Pure-component parameters for NADH and NAD^+ were fit to experimental density data and osmotic-coefficient data of these components in water. Both components were assumed to form strong hydrogen bonds (HB) similar as the example of ATP/ADP pure-component parameters published by Meurer et al. [28]. This was accounted for by the association scheme N_i^{assoc} of 8 HB donor and 8 HB acceptor sites in agreement