Simultaneous Prediction of Co-Solvent Influences on Michaelis Constants and Reaction Equilibrium of Ketone Reductions

Experimental and Theoretical Study on Enzyme-Catalyzed Reactions

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Biotechnologists commonly apply co-solvents in order to improve enzyme-catalyzed reaction systems. The effect of such co-solvents on reaction kinetics and reaction equilibrium of enzyme-catalyzed reactions is mainly studied experimentally. However, this does neither allow explaining nor predicting the observed co-solvent effects on reaction kinetics or equilibrium compositions. In this work, the reaction equilibrium and the Michaelis constants of ketone reductions were predicted with ePC-SAFT. ePC-SAFT showed that adding 17 wt.-% of PEG 6000 is beneficial for reaction kinetics while shifting the reaction equilibrium backwards to the reactant side. Experimental validation showed that these predictions were in a very good agreement to the experimental data. As co-solvent – enzyme interactions were not considered for the predictions co-solvent – reacting agent interactions are decisive for the co-solvent influence on reaction equilibrium and the Michaelis constants for the considered reactions.

New developments in biotechnology have led to new processes in which enzymes pose an alternative to chemical catalysts. However, enzymes are usually stable and active in a small temperature and pH window. Thus, co-solvents have to be added to reaction mixtures to stabilize enzymes and improve reaction kinetics and equilibrium. In this work, the influence of 17 wt.-% of PEG 6000 on the reaction equilibrium and on the Michaelis constants of the reduction of butanone and 2-pentanone was predicted. This approach was based on predicting the influence of co-solvents on the thermodynamic activity coefficients of reacting agents using ePC-SAFT for the following studied reactions:
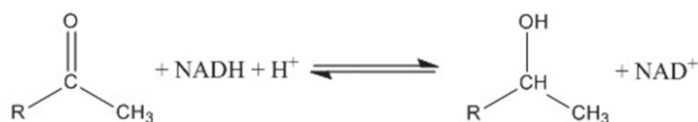

Figure 1: Reaction scheme of the reduction of a ketone to the respective alcohol, catalyzed by ADH270 (25°C, 1 bar, pH 7).

First, reaction equilibrium and kinetics of two reactions ($R_1 = C_2H_5$ and $R_2 = C_3H_7$) were measured in water, and activity-based thermodynamic equilibrium constants K_{th} and Michaelis constants K_M^a were determined using these data and ePC-SAFT. This finally allowed predicting the co-solvent influence of 17 wt.-% of PEG 6000 on the reaction equilibrium (expressed as the ratio of reacting agents X^{exp}) and reaction kinetics (characterized by the Michaelis constant K_M). Figure 2 and Figure 3 show results on the simultaneous prediction of the PEG influence on X^{exp} and K_M.

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Publications:
A. Wangler, D. Böttcher, A. Hüser, G. Sadowski, C. Held, Chem. Eur. J. 24, 16418-16425 (2018).

A. Wangler, R. Loll, T. Greinert, G. Sadowski, C. Held, J. Chem. Thermodynamics 128, 275-282 (2019).

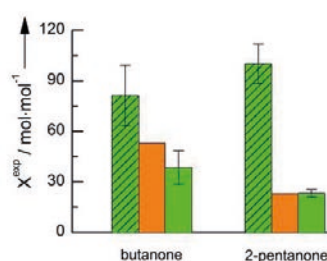A. Wangler, A. Hüser, G. Sadowski, C. Held, ACS Omega 4, 6264-6272 (2019).

Figure 2: Influence of 17 wt.-% of PEG 6000 on X^{exp} of reduction of butanone or 2-pentanone. Orange: ePC-SAFT, green: experimental data. Striped green bars represent the neat (co-solvent free) values used to determine K_{th}.
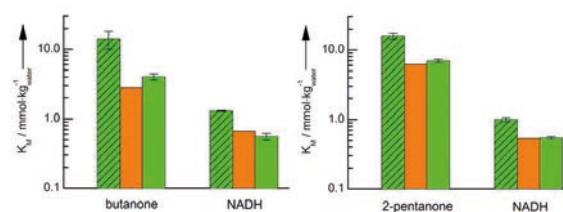

Figure 3: Comparison between the Michaelis constants K_M for neat reaction conditions (striped green bars) and under the influence of 17 wt.-% PEG 6000 (green bars) for the reduction of butanone (left diagram) and 2-pentanone (right diagram). Orange: ePC-SAFT predictions, green: experimental data.

As can be seen from Figure 2, adding PEG decreases the ratio of reacting agents' concentrations, i.e. the reaction equilibrium is shifted to the left-hand side. In Figure 3 it is illustrated that PEG decreases the K_M values, i.e. the kinetics of the reactions is improved upon PEG addition. These influences on the reaction equilibrium and Michaelis constants could be predicted quantitatively correct.

This new approach can serve as screening tool to simultaneously predict co-solvent influences on reaction equilibrium and Michaelis constants of enzyme-catalyzed reactions. As the enzyme was not considered in these predictions it can be concluded that co-solvent – enzyme interactions do not play a major role to understand co-solvent effects on reaction kinetics and equilibrium of the ketone reductions. Rather, co-solvent – reacting agent interactions decisively influence the reaction behavior of the enzyme-catalyzed reactions under investigation.