**Abstract**

**"Effect of medium on enzymatic reactions and the importance of the activity coefficient "**

Metabolism and industrially relevant enzymatic reactions seldom take place under neat conditions that only contain the reacting agents dissolved in the reaction solvent (most often water). The reaction solutions usually also contain a large variety of species as e.g., buffer components, co-factors, stabilizers (e.g. natural solutes such as osmolytes), and solubilizers. The presence of all these components influences the thermo-physical properties of the system, the components in the solution as well as the biochemical reactions taking place. Thermodynamic activity takes into account the real-mixture behavior of multi-component solutions. The thermodynamic equilibrium constant, $K_{th}$, is the product of the thermodynamic activities, $a_i$, of all reaction partners $i$ exponentiated with the respective stoichiometric factor, $v_i$:     $K_{th}(T,p) = \prod_i a_i^{v_i} = \prod_i \left( x_i^{v_i} \cdot \gamma_i^{v_i} \right) = K_x K_\gamma$          (1)

In this work, PC-SAFT is used to access $K_\gamma$. The idea is to use phase equilibrium data in order to parameterize the reacting agents and to predict $K_\gamma$, i.e. these predictions are based on model parameters that are independent from reaction data. Activity-based equilibrium constants $K_{th}$ are of particular importance for the calculation of metabolic networks, which requires standard reaction data instead of apparent concentration-dependent reaction data such as standard biochemical Gibbs energies of reaction, $\Delta^R g^0 = -RT \ln K_{th}$. The observed reaction equilibrium position, $K_x$, yields observed (or apparent) Gibbs energies of reaction $\Delta^R g^{obs} = -RT \ln K_x$.

This work will show two major applications of the proposed approach. First, new experimental $K_x$ values were measured for glycolytic reactions, and it was found that $K_x$ data depended on the initial substrate concentration. Combining these data with PC-SAFT predicted $K_\gamma$ values yielded new consistent $\Delta^R g^0$ for glycolytic reactions. Second, the influence of cosolvents on kinetics and maximum yield was measured for aqueous and non-aqueous enzymatic reactions. An approach is shown that allows quantitatively predicting this behavior. Overall, evaluation of activity coefficients of the reacting agents are very important to determine activity-based standard data from observed apparent equilibrium constants and to predict yields or equilibrium positions of reactions at given standard data as well as the influence of cosolutes or cosolvents on reaction equilibria.