Sensitivity and Non-Uniqueness in Determining Enzyme Kinetic Parameters

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Highlights

- Different kinetic parameter sets may produce nearly identical model fits.
- Parameter non-uniqueness increases with model complexity.
- Error surfaces exhibit elongated valleys and flat minima regions.
- Reliable reactor design requires sensitivity and identifiability analysis.

1. Introduction

Enzyme kinetic models are widely used for the design, optimization, and scale-up of biocatalytic reactors. However, the determination of kinetic parameters from experimental data often suffers from severe sensitivity and identifiability limitations. In practice, different parameter combinations may reproduce experimental observations with nearly identical accuracy, resulting in non-unique solutions of the inverse problem. Similar concerns regarding the reliability of kinetic parameter estimation were already discussed by Schnell and Maini [1].

The present work addresses the phenomenon of parameter non-uniqueness in enzyme kinetics, with emphasis on Michaelis–Menten-type models of increasing complexity. The implications for mechanistic reactor modeling and model-based process design are discussed.

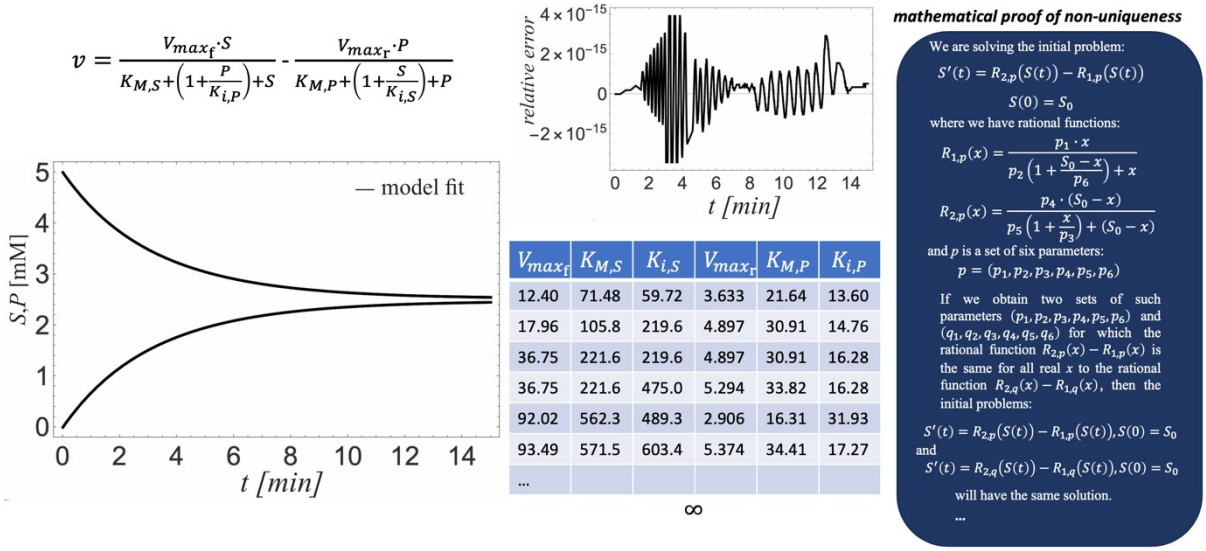
2. Methods

Several enzyme kinetic models were analyzed, ranging from the classical two-parameter Michaelis–Menten equation to reversible and inhibited reaction schemes involving up to six independent kinetic parameters. Parameter estimation was performed using nonlinear regression and numerical optimization.
The local sensitivity matrix and the Jacobian structure were analyzed to evaluate parameter identifiability. Error landscapes were investigated through multidimensional parameter scans and null-space exploration. The study included both synthetic datasets and experimentally relevant biocatalytic reaction systems, including a continuous microscale bioreactor previously described in the literature [2].

3. Results and discussion

The results demonstrate that multiple parameter sets can generate virtually indistinguishable model predictions despite large differences in individual parameter values. This effect becomes increasingly pronounced as the number of kinetic parameters increases.
For simple Michaelis–Menten kinetics, elongated valleys in the objective-function landscape already indicate strong parameter correlations. In reversible and inhibition models, entire regions of parameter space produce nearly identical fits within numerical accuracy. A recent mathematical analysis further demonstrated that non-uniqueness can represent an intrinsic structural property of enzyme kinetic models rather than merely a numerical artifact [3].

The analysis confirms that excellent agreement between model predictions and experimental data does not necessarily imply unique or physically reliable kinetic parameters. This has important consequences for reactor design, optimization, and scale-up of enzymatic processes, particularly in continuous-flow and microreactor systems where mechanistic models are extensively employed. Figure 1 illustrate representative error surfaces or identical model fits obtained with substantially different parameter sets.



V_{max_f}	$K_{M,S}$	$K_{i,S}$	V_{max_r}	$K_{M,P}$	$K_{i,P}$
12.40	71.48	59.72	3.633	21.64	13.60
17.96	105.8	219.6	4.897	30.91	14.76
36.75	221.6	219.6	4.897	30.91	16.28
36.75	221.6	475.0	5.294	33.82	16.28
92.02	562.3	489.3	2.906	16.31	31.93
93.49	571.5	603.4	5.374	34.41	17.27
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Figure 1. Addressing Non-Uniqueness in the Six-Parameter Reversible Michaelis–Menten Model with Competitive Product Inhibition

4. Conclusions

The study demonstrates that parameter non-uniqueness is an intrinsic feature of many enzyme kinetic models and not merely a numerical artifact. Increasing model complexity substantially reduces practical identifiability of kinetic parameters.

Sensitivity analysis and identifiability assessment should therefore accompany parameter estimation in model-based biocatalytic reactor design. The presented results highlight the importance of combining kinetic experiments with mechanistic transport and reactor modeling to improve parameter reliability.

References

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Keywords

Enzyme kinetics; Parameter estimation; Non-uniqueness; Sensitivity analysis