# Derivation and Analysis of the Michaelis-Menten Theory

Kelly Black, Ph.D.
Department of Mathematics
Union College
Schenectady, NY 12308
black@union.edu

Kristin M. Fox, Ph.D.
Department of Chemistry
Union College
Schenectady, NY 12308
foxk@union.edu

## Summary of Activity:

In this activity students examine Michaelis-Menten kinetics from two perspectives, both biochemical and mathematical. First, they use differential equations to derive the Michaelis-Menten relationship. Secondly they collect experimental data using the enzyme tyrosinase that verifies this relationship and use non-linear curve fitting to fit their data to the equation they have derived. As an extension of this experiment they examine the transition that tyrosinase undergoes during a pH change and the effect that this has on the kinetics. Again they derive an equation that potentially explains this phenomenon. They collect data at a variety of pH values and attempt to fit their data to this equation. This time things are not as straightforward, and they must think about what is going on in this more complex situation.

## Integration of Disciplines:

In this set of laboratory exercises, we ask the students to explore one facet of kinetics, Michaelis-Menton theory. This is one of the most basic and important aspects of kinetics in biochemistry, and it is commonly examined in biochemistry classrooms. In this laboratory, though, we have developed one activity and a laboratory exercise to place the development of enzyme kinetics in the context of systems of differential equations.

The exercises have been developed for students who have a background in calculus but not differential equations. We focus on how the basic rate equations can be transformed into the equations from the Michaelis-Menton theory when the chemical systems are near equilibrium. The first part is an activity that prompts the students to simplify the system of nonlinear equations and explore the resulting nonlinear terms. The laboratory exercise is based on a paper that explores the kinetics of an enzyme that has a protonated form. The laboratory part requires the students to explore the kinetics of a similar enzyme, and accompanying worksheets have been developed that require the students to develop the resulting system of equations. The laboratory and worksheet activities are brought together by asking the students to compare the two results and show that they are consistent in one case and not consistent in the other.

## Learning Objectives:

The goal is for the students to start with a conceptual model of an enzyme/substrate system and ask if the laboratory results are consistent with the conceptual model. The students must develop a system of nonlinear differential equations that approximate the behavior of the conceptual model and make a qualitative prediction as to how the system should behave in solutions of varying pH.

The students are also asked to take part in a set of laboratory activities that determine the kinetics of the enzyme/substrate system. They are required to observe the concentrations of the product with solutions of varying pH. The students are then asked to determine if their results are consistent with the predicted results that arise from the conceptual model. In this case the results are exactly the opposite of what is expected, and the students are required to recognize that there is a problem with either the conceptual model or the experimental design.

The students are required to take part in an exercise in which the proposed chemical properties of an enzyme are given to them. They are then asked to take part in predicting the resulting chemical kinetics, and then demonstrating in the laboratory that the prediction does not match the laboratory results. The mathematical analysis of the conceptual system is used to demonstrate how the basic conceptual model of the system can be tested in a laboratory setting.

## Target Level:

The target level for this is upper-level undergraduates. Students should have had introductory calculus and organic chemistry as pre-requisites for understanding the material in this activity. Students should currently be enrolled in a biochemistry course in which enzyme kinetics is discussed.

## Tools and Materials:

Chemicals:

- 4-methyl catechol (Sigma-Aldrich #M34200))
- mushroom tyrosinase (Sigma-Aldrich #T3824)
- potassium phosphate monobasic (Fisher Scientific)
- potassium phosphate dibasic (Fisher Scientific)

Equipment:

- spectrophotometer measuring in the visible wavelengths using a kinetics program
- pH meter
- 3-mL cuvets
- glass test tubes
- tank of compressed air
- pasteur pipets

Student Handout:
http://bioengineering.union.edu//FoxBlackModule/FoxBlackStudent.pdf

Worksheets:

- Michaelis-Menten Kinetics Worksheet and Answer Sheet
  - http://bioengineering.union.edu/FoxBlackModule/MMKinetics.pdf
  - http://bioengineering.union.edu/FoxBlackModule/MMKineticsSolutions.pdf
- pH-Mediated Transition Worksheet and Answer Sheet
  - http://bioengineering.union.edu/FoxBlackModule/pHTransition.pdf
  - http://bioengineering.union.edu/FoxBlackModule/pHTransitionSolutions.pdf

## Theory and Background:

The kinetics of chemical reactions are rarely explored beyond the most basic levels in undergraduate biochemistry courses. The analysis of the systems of differential equations is a difficult task for the most basic systems. The role of kinetics in biochemistry, though, is vital to understanding the interactions in biological systems. The simplest nonlinear systems can give rise to remarkably complex phenomena, but such interactions are rarely explored by undergraduate students in biology or mathematics beyond the most rudimentary aspects.

In the set of activities that have been developed we explore the Michaelis-Menton theory in the context of differential equations. The rate equations for any reaction are found, and the students are asked to modify the equations when assuming that the systems are near steady-state. The first part of the exercises are similar to the way the basic Michaelis-Menton theory is often explored in an undergraduate course, but the students are required to develop the systems of nonlinear differential equations themselves.

The second part of the set of activities is more involved. The students are asked to explore the results of a paper in which an enzyme is examined which has a protonated form and an isomerization of the deprotonated form, "Kinetics of the Slow pH-Mediated Transition of Polyphenol Oxidase" Archives of Biochemistry and Biophysics, Vol. 331, No. 1, July 1, pp. 15-22, 1996. The article offers a detailed explanation of the chemical system and includes an appendix that offers a detailed derivation of the system of differential equations describing the kinetics.

The students are asked to explore the kinetics of an enzyme that is expected to offer similar kinetics in a laboratory setting. Outside of the laboratory the students are required to explore the system of nonlinear equations used to describe the kinetics of the resulting system and make a prediction. The two activities are brought together when the students are asked if the two results are consistent. The laboratory results for the enzyme in the article show that the reaction rates decrease as the pH increases, but the system examined in the student's laboratory demonstrates the opposite trend. The students are required to recognize that their laboratory results do not match the qualitative behavior predicted through the mathematical analysis of the conceptual chemical model.

## Safety Precautions:

Use standard lab safety procedures.
Monitor student use of the nitrogen tank carefully and ensure that the tank is well-secured before use.