Inhibition of the Reaction Kinetics of the Enzyme *o*-Diphenol Oxidase

by Kieran F. Lim

Experiment Overview

Chemical kinetics is a fundamental component of chemistry. Traditional chemistry-laboratory exercises have concentrated on the study of non-biological organic or inorganic reaction kinetics. Historically, these experiments are chosen for their perceived didactic value – they usually exhibit simple kinetics, without complicating factors – rather than for any "relevance" to the world outside the chemistry laboratory. The same lack-of-relevance is applicable to many "traditional" experiments in other areas of chemistry. Therefore, the fact that students are avoiding the study of chemistry[1] should not be surprising.

The aim of this experiment is to investigate the kinetics of an enzyme-catalysed reaction, and the kinetics in the presence of an inhibitor[2-15]. This relates physical chemistry to a "real world" application – the action of a biological catalyst, *o*-diphenol oxidase (oDPO). Better students need to be challenged[16] by extensions to the experiment, which is easily achieved since the complexity of biological systems offers many avenues for exploration.

Enzyme kinetics is usually described by the Michaelis-Menten model, which can be used to illustrate several concepts in the curriculum. The behaviour of enzyme-catalysed reactions is not simply proportional to the power of the reactant (substrate) concentration [S]. The kinetics changes from being first-order with respect to [S] at low [S], to being zeroth-order at high [S], with non-integer order at intermediate [S][3-10]. The derivation of the Michaelis-Menten kinetic equations involves use of the quasi-steady-state approximation[3-6]. Furthermore, the laboratory is an opportunity to learn how to handle photo-sensitive reagents.

In this laboratory exercise, *p*-nitrophenol and cyanide anion inhibitors are used because they were used in the experiment at University of New England. Other inhibitors of oDPO activity[15], which pose smaller health and safety risks, can also be used.

This exercise has the potential to be further developed as an affordable high-school experiment using the browning of mashed raw fruit or potatoes as the enzyme-catalysed reaction and a home-made colorimeter for under $15[17]. Table salt (NaCl) can be used as the inhibitor[15]. In this configuration, the most expensive items of equipment or reagents would be a sturdy kitchen knife and food processor!

Aims and Objectives

Chemical kinetics is a fundamental component of physical chemistry. The aim of this experiment is to investigate the kinetics of an enzyme-catalysed reaction, and the kinetics when there is a competing reaction due to the presence of an inhibitor[2-15]. This relates physical chemistry to a "real world" application – the oxidation of an organic compound through the action of a biological catalyst.

Level of Experiment

Second year undergraduate

Keyword Descriptions of the Experiment

Domain
physical chemistry, biological chemistry

Specific Descriptors
reaction kinetics, enzyme catalysis, Michaelis-Menten model, enzyme inhibition

Course Context and Prerequisite Knowledge and Skills

The School of Biological and Chemical Sciences at Deakin University has students enrolled in the biology, biotechnology, forensic and wine science streams as well as the chemistry stream: the physical chemistry unit has to cater for a wide range of student interests and mathematical skills.

One of the foci of the School is the promotion of the interdisciplinary nature of modern chemistry (via "biologically relevant" chemistry) and of modern biology (via molecular biology). Instead of doing a "traditional" kinetics experiment, an enzyme kinetics reaction is studied, also with inhibition via a competing equilibrium (here "competing equilibrium" is used in the usual chemical sense, meaning alternative pathway).

This kinetics experiment is the third experimental exercise (total of five such experimental exercises) done during the semester-long second year physical chemistry laboratory.

- Students are expected to have knowledge of first year kinetics and first year equilibrium topics.
- Prior to the start of this laboratory exercise, students have been introduced to the use of a spreadsheet package. Nevertheless, students are told that use of a spreadsheet is part of the computer laboratory component of the exercise and should consult their demonstrator if they encounter difficulties.
- Students are not expected to have knowledge of kinetics or equilibrium topics at second year level. An appendix introducing enzyme kinetics is included in the student notes for those students who are unfamiliar with the topic.

Some students may have encountered enzyme kinetics in Biochemistry during the first semester of second year. Those students would have done a biochemistry laboratory exercise on enzyme kinetics (Michaelis-Menten kinetics), but not an exercise on *inhibited* enzyme kinetics. Over half the students attempting *this*laboratory exercise would *not* have attempted the biochemistry laboratory exercise.

Time Required to Complete

Prior to Lab: 1 h reading
In Laboratory: 2 h
After Laboratory: 2-3 h report writing

Experiment History

The original source of this experiment is unknown.

Kieran Fergus Lim (Deakin University) and Robert Learmouth (University of Southern Queensland) both have versions of this experiment derived from an experiment run by Robert Learmouth when he was at University of New England (c. 1990). Robert Learmouth has given permission for the experiment to be adapted and used.

Verbal tradition at the University of New England suggests that the experiment came from the University of Sydney (c. 1960s? 1970s?), but current Sydney staff (c. 2000) have no knowledge of the experimental exercise.

Many similar experiments (mostly on un-inhibited Michaelis-Menten kinetics) have been published in the literature: see, for examples, references 14, 15, and 18-23.

Comments

J.R.L. Walker (retired, University of Canterbury, New Zealand) has run a similar experiment using the same enzyme extracted from mushrooms and bananas. Extraction from other fruit is also possible. The University of Canterbury experiment usually used the kinetics to determine comparative concentrations of *o*-diphenol oxidase enzyme (oDPO or tyrosinase) in different parts of the mushroom, and hence was not a kinetics experiment as such. Walker has published a paper on the inhibition of o-diphenol oxidase by phenolic acids[14] and has given a list of inhibitors of *o*-diphenol oxidase activity[15].

In this laboratory exercise, *p*-nitrophenol and cyanide anion inhibitors were used for historics reasons (ie used at the University of New England). One of the referees has pointed out that use of these inhibitors poses health and safety risks: we will investigate other inhibitors of *o*-diphenol oxidase activity[15] in this laboratory exercise with future cohorts of students.

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