

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

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## **Practical:**

### **Abstract**

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. Many students feel that such studies bear little relationship to the world outside the chemistry laboratory. The aim of this experiment is to investigate the kinetics of a reaction catalysed by the enzyme o-diphenol oxidase (oDPO or tyrosinase) and the kinetics in the presence of an inhibitor. This relates physical chemistry to a “real world” application — the oxidation of an organic compound through the action of a biological catalyst.

### **Intended academic level**

Undergraduate 2

### **Duration**

1 hr reading, 2 hr laboratory, 2 hr analysis of results

### **Outcomes**

Students should appreciate that reaction kinetics is applicable to real-life systems, not just systems involving methyl isocyanide and other “textbook” systems.

Students should appreciate that complicated reaction mechanisms (like the Michaelis-Menten mechanism) will give rise to non-integer reaction orders.

Students will learn to operate a simple spectrophotometer.

Students will learn to handle light-sensitive reagents.

Students must understand and use the relationship between the transmitted light intensity of a “blank” and the transmitted light intensity of the sample, in order to determine the absorbance of the sample using a single-beam spectrophotometer.

Students must understand and apply the Beer-Lambert Law in order to use absorbance to measure concentration (or at least concentration in arbitrary units).

Students must understand the definition of reaction rate in order to measure rate (in arbitrary units) by the change in concentration (in arbitrary units) over time.

### **Materials**

potato, 200 g

Phosphate Buffer 0.01 M, pH 7.0, 1 litre

Catechol (substrate) 2.5 mM, (0.138 g in 500 ml)

Potassium cyanide 2.5 mM, (0.0814 g in 500 ml)

p-Nitrophenol 25 mM, (1.74 g in 500 ml)

Sodium chloride, 250 mL volumetric flask(s) required to make up solution(s). Twelve 15 cm long (2 cm diameter) test tubes and test tube rack for each group of two students

Spectrophotometer, one per group of two students. At Deakin University, we use a Spectronic 20 Spectrometer, but any single-beam instrument is sufficient. The experiment monitors only one wavelength (500 nm) so scanning is not important. Spectrometer tubes/cuvettes. Minimum number is one tube/cuvette per spectrometer per group of two students.

Blender/ macerator

(kitchen) knife + cutting board

ice, approx 2 litres per session

Stopwatch, one per group of two students

Beakers, assorted sizes. These are normally part of the standard equipment in our laboratories so we do not keep note. In principle, they are used only to hold ice, collect "waste" solution, etc.

Graduated pipettes (0.5 mL, 1 mL, 2 mL, 5 mL and 10 mL) and pipette fillers. These were used in the original version of the exercise. We have now replaced these with P1000 micro-pipettes (ideally two micro-pipettes per group of two students).

vacuum filter funnels + flasks + filter paper, one per group

aluminium foil

500 mL bottles

### **Costs**

Major cost is any single-beam visible Spectrometer of between USD\$100-500. ongoing costs are small.

### **Further comments**

Detailed student notes, technical notes, demonstrator notes, evaluation of teaching and learning outcomes are available.

### **Reading**

See K. F. Lim, "Inhibition of the reaction kinetics of the enzyme o-diphenol oxidase: An APCELL experiment", Australian Journal of Education in Chemistry, 2002, 59, 11-16.

### **Contact details**

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