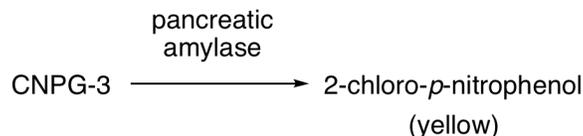


# Factors Affecting the Rate of an Enzyme-Catalyzed Reaction

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## Introduction

Amylase hydrolyzes starch into maltose, a disaccharide of glucose. To observe its activity level, 2-chloro-*p*-nitrophenyl- $\alpha$ -D-maltotrioside (CNP3) is used instead of starch:



The intensity of the yellow color produced is proportional to the product concentration which can be measured using a spectrophotometer, a device that measures the amount of light absorbed by a substance. The rate that absorbance increases is proportional to the increase in amylase activity (reaction rate) in a sample.

**Objective:** Use experimental results to determine how different factors affect the rate of a reaction catalyzed by pancreatic amylase and apply concepts from the enzyme unit to explain the results.

**Instructions:** Use the information from **Table 1** to compare the results shown in **Figure 1**. Complete the chart on page 3 and answer the questions on page 4.

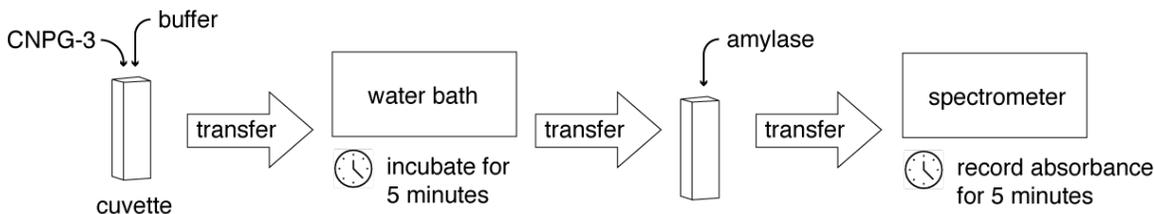
**Description of Experiment:** See **Table 1** for the volume of each reagent and temperature used.

Method used to collect results:

1. For each trial, CNP3 and buffer were transferred to a cuvette.
2. The cuvette was incubated for 5 minutes in a water bath at the appropriate temperature.
3. After the cuvette was removed from the water bath, pancreatic amylase was added and thoroughly mixed. In trial 9, Montbretin A, a flavonol extracted from *Crocsmia crocosmiiflora* plants, was added to the cuvette before adding the pancreatic amylase.
4. The cuvette was placed in the spectrophotometer and the absorbance was recorded every second for 5 minutes. The results were graphed with a best-fit line.
5. Steps 1 – 4 were repeated with the appropriate conditions for the next trial.

Note: 1 $\mu$ L = 0.001mL

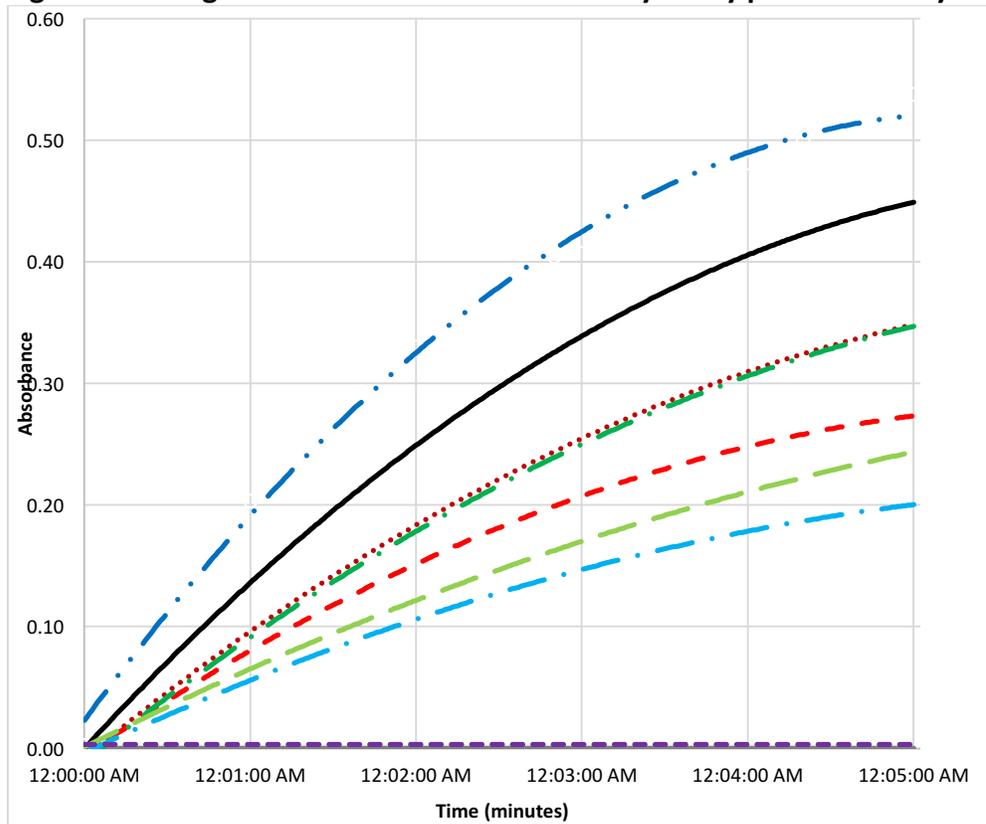
Flow chart:



**Table 1: Reagent volumes and temperature used for each trial**

Trial Conditions		Trial #								
		1	2	3	4	5	6	7	8	9
Volume of Each Reagent Added ( $\mu\text{L}$ )	CNPNG-3	1000	1000	750	500	1000	1000	1000	1000	1000
	buffer	200	100	350	600	125	150	100	1000	0
	Montbretin A	0	0	0	0	0	0	0	0	100
	pancreatic amylase	0	100	100	100	75	50	100	100	100
Initial Temperature ( $^{\circ}\text{C}$ )		21	21	21	21	21	21	4	30	21

**Figure 1: Change in absorbance of CNPG-3 catalyzed by pancreatic amylase**



<b>Claim:</b> For the following trials, identify which factor was tested. Describe how this factor affects the reaction rate.	<b>Evidence:</b> Describe the evidence that supports your claim.	<b>Reasoning:</b> Apply concepts from this unit to explain the connection between your claim & supporting evidence.
<b>Trials 1 &amp; 2</b> <i>e.g. Control trials: Negative control verifies that amylase is required to catalyze hydrolysis of substrate, CNPG-3. Positive control verifies that amylase catalyzes hydrolysis of substrate and provides baseline results for comparison with factors that affect the reaction rate.</i>	<i>e.g. No change in absorbance (remained colorless) indicates that no products were produced so no reaction occurred, while gradual increase in absorbance (increasing intensity of yellow) indicates increasing product concentration as reaction occurred.</i>	<i>e.g. Results verify that the substrate remains unreacted (stable) until it gets hydrolyzed by an enzyme. Hydrolysis of the substrate into products only occurs in the presence of the appropriate enzyme.</i>
<b>Trials 3 &amp; 4</b>		
<b>Trials 5 &amp; 6</b>		
<b>Trials 7 &amp; 8</b>		
<b>Trial 9</b>		

**Questions:**

For each of the following changes in trial condition, predict its effect on the reaction rate and expected results and explain your reasoning.

Condition	Effect on Reaction Rate & Expected Results	Reasoning for Expected Results
Increasing temperature to 60°C		
Doubling concentration of substrate		
Adding 100µL 1M HCl		

Explain why the slope decreases as the reaction proceeds.

The buffered, salt solution was used to dissolve and dilute the CNPG-3 and amylase. Explain why a buffer and salt are used instead of water.

Criteria	A – Exceeds Expectations	B – Fully Meets Expectations	C – Expectations Not Met
Claim	Makes logical & complete claim.	Makes logical but incomplete claim.	Makes illogical & incomplete claim.
Evidence	Provides appropriate & sufficient evidence that fully supports claim.	Provides appropriate evidence, but is insufficient or includes some inappropriate evidence that somewhat supports claim.	Provides inappropriate or insufficient evidence, such that evidence does not adequately support claim.
Reasoning	Provides reasoning that fully connects evidence to claim & demonstrates comprehensive understanding of enzymes.	Provides some reasoning that somewhat connects evidence to claim & demonstrates adequate understanding of enzymes.	Provides insufficient/incorrect reasoning that does not adequately connect evidence to claim, e.g. only repeats evidence. Only demonstrates minimal understanding of enzymes.

**Evaluation:**