Protease Enzymes and Jello

Materials

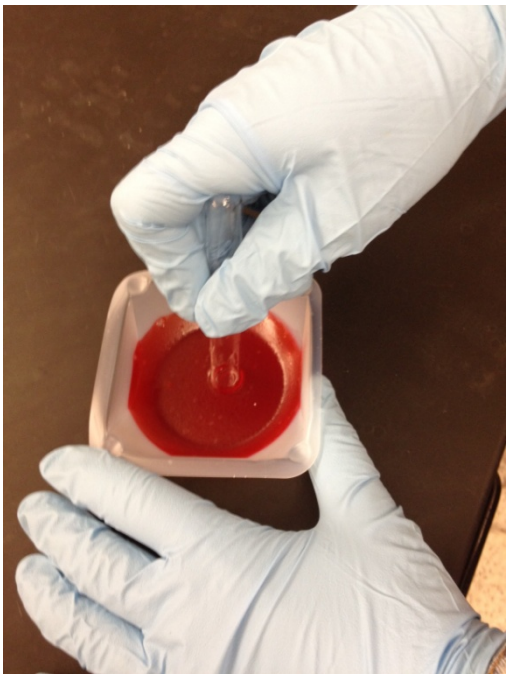
- 1 packet of Jello powder
- Deionized water
- Weigh Boats
- 250-mL Erlenmyer Flask
- 10-mL Graduated Cylinder
- Pasteur Pipettes and bulbs
- Spectrophotometer test tubes
- Spectrophotomer
- Heating Block
- Papain enzyme solution

Procedure

1. Jello Preparation
    a. Stir one packet of Jello into 350 mL of hot deionized water
    b. When Jello is completely dissolved, allow to cool for 10 minutes
    c. Pour Jello into weigh boats approximately 1 cm deep
    d. Allow to thicken and dry overnight
2. Jello Absorption Spectrum
    a. Use a moistened test tube to cut out one piece of Jello (as pictured below)
    b. Place the piece into a 250-mL Erlenmeyer flask
    c. Add 3mL deionized water
    d. Swirl the contents for 3 minutes
    e. Zero the spectrophotometer at wavelength=460 nm as described in the standard operating procedure
    f. Pipette the liquid into a clean test tube being careful not draw up Jello particles (having particles in solution will cause error in absorbance measurements)
    g. Insert the tube in the spectrophotometer. Measure and record the absorbance in table 1 in the data sheets
    h. Repeat steps e.-g. for wavelengths 470 nm, 480 nm, 490 nm, 500 nm, 510 nm, 520 nm, and 530 nm using the same test tube of solution
    i. Make a graph of Absorbance vs. wavelength and determine the wavelength of maximal absorbance (λ_{max})
3. Spectrophotometer Measurements
    a. Use a moistened test tube to cut out one piece of Jello
    b. Place into 250-mL Erlenmeyer flask
    c. Add 3 mL deionized water
    d. Swirl contents for 1 minute timed and record observations
    e. Pipette the liquid into a test tube, being careful not to draw up Jello particles (as shown below)
    f. Zero the spectrophotometer for the λ_{max} as described in the standard operating procedure
    g. Insert the test tube into the spectrophotometer. Measure and record its absorbance
    h. Remove the test tube and pour the solution back into the Erlenmeyer flask
    i. Add 3 mL of the Papain solution to the Erlenmeyer

j. Repeat steps d.-h. 10 times, recording all measurements and observations in table 2 in the data section
k. Make a graph of Absorbance vs. time swirled and describe the trend

4. Measurements with Heat
    a. Use a moistened test tube to cut out another piece of Jello
    b. Pour 3 mL of deionized water into the test tube
    c. Place in the heating block and wait for 1 minute
    d. Pipette the solution into another test tube, being careful once again to not take up any Jello particles
    e. Be sure the spectrophotometer is zeroed
    f. Measure the absorbance of the solution
    g. Return the solution to the test tube with Jello
    h. Repeat steps c.-g. 10 times, recording measurements and observations in table 3
    i. Make a graph of Absorbance vs. time heated and describe the trend



Data Sheet

Table 1 Absorption Spectrum

Wavelength (nm)	**Absorbance**
460	
470	
480	
490	
500	
510	
520	
530	

λ_{max}:_____

Table 2 Spectrophotometer Measurements:

Measurement #	**Time swirled (min:sec)**	**Absorbance**	**Appearance**
0 (no Papain)			
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

Data trend:

Table 3 Measurements with Heating

Measurement #	Time heated (min:sec)	Absorbance	Appearance
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

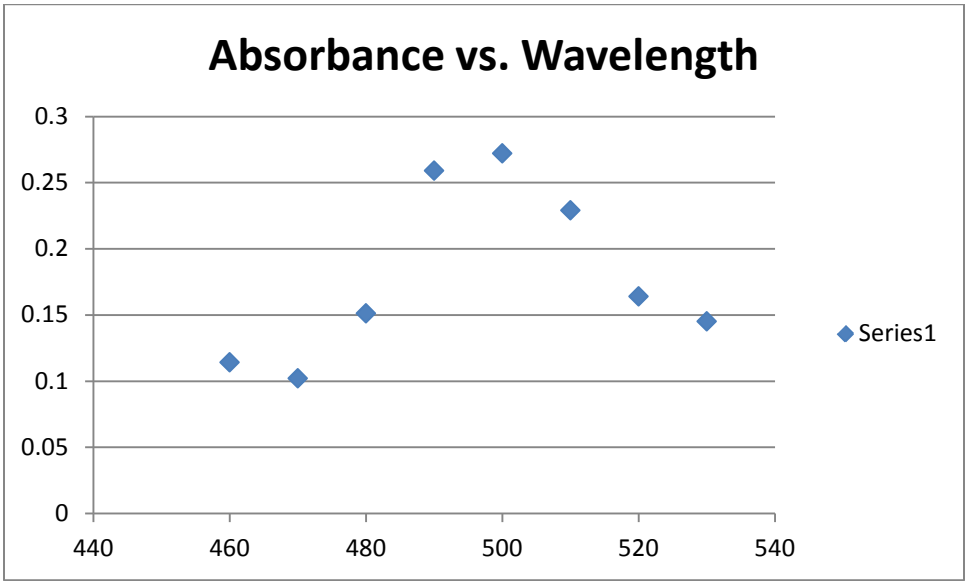
Data Trend:

Jello and Papain Supplement

Here is some example data from when I ran the experiment:

Part 1)

Wavelength (nm)	Absorbance
460	0.114
470	0.102
480	0.151
490	0.259
500	0.272
510	0.229
520	0.164
530	0.145

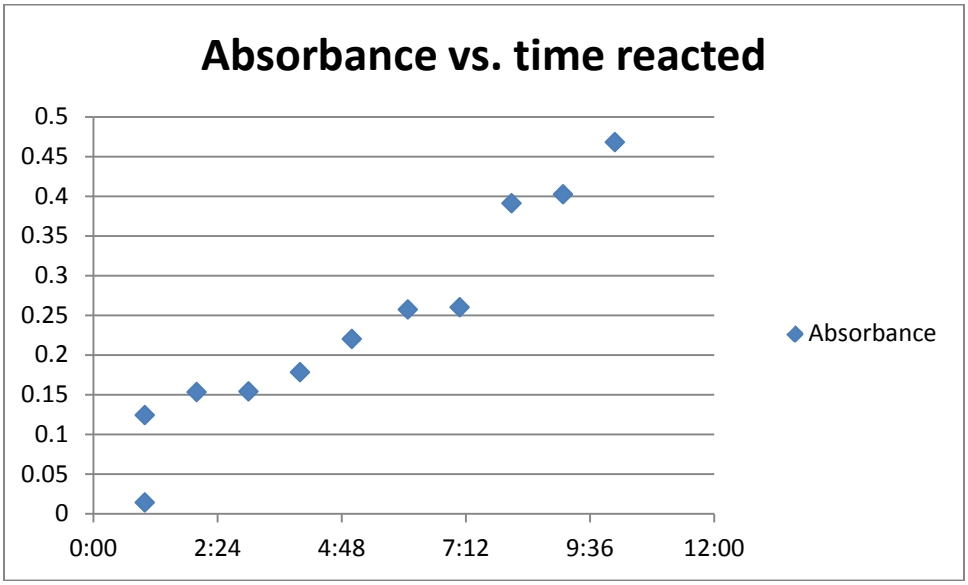


λ_{max}=500 nm

Part 2)

Measurement #	Time swirled (min:sec)	Absorbance	Appearance
0 (no Papain)	1:00	0.014	
1	1:00	0.124	

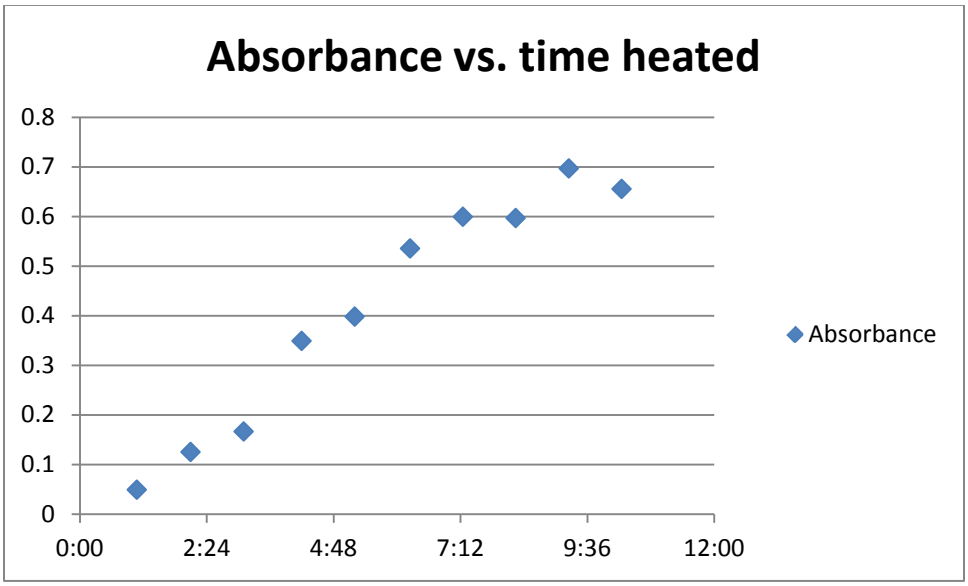
2	2:00	0.153	
3	3:00	0.154	
4	4:00	0.178	
5	5:00	0.22	
6	6:05	0.257	
7	7:05	0.26	
8	8:05	0.391	
9	9:05	0.402	
10	10:05	0.468	



Part 3)

Measurement #	Time heated (min:sec)	Absorbance	Appearance
1	1:05	0.049	
2	2:06	0.125	
3	3:06	0.166	
4	4:12	0.349	
5	5:12	0.398	
6	6:15	0.535	
7	7:15	0.599	

8	8:15	0.596	
9	9:15	0.696	
10	10:15	0.655	



Note: Another variation of part 3 could be to use an acid or base to change the pH and cause protein or enzyme denaturation. We did not investigate this yet because of time constraints.

VWR Product List

Package 6 (Papain):

10 mL graduated cylinders	65000-000
13x100 mm test tubes	47729-572
PTFE stir rods	89026-280
Papain carica papaya, 50 g	80057-240

This experiment was adapted from:

Hagar, William G. and Bullerwell, Lornie D. Supermarket Proteases. *The Science Teacher.* October, 2003 pp. 26-30.