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**Research Question:** What is the time-course of enzyme to catalyze the breakdown of a protein, into its smaller units, amino acids?

**Hypothesis:** Adding an enzyme to the protein concentration will not only speed up the reaction but will also decrease the activ ation energy. The enzyme will have a fast rate of reaction initially and then will gradually start reaching a stop, when there is no more protein to be broken dow n, in which case the biuret solution will not change colour.

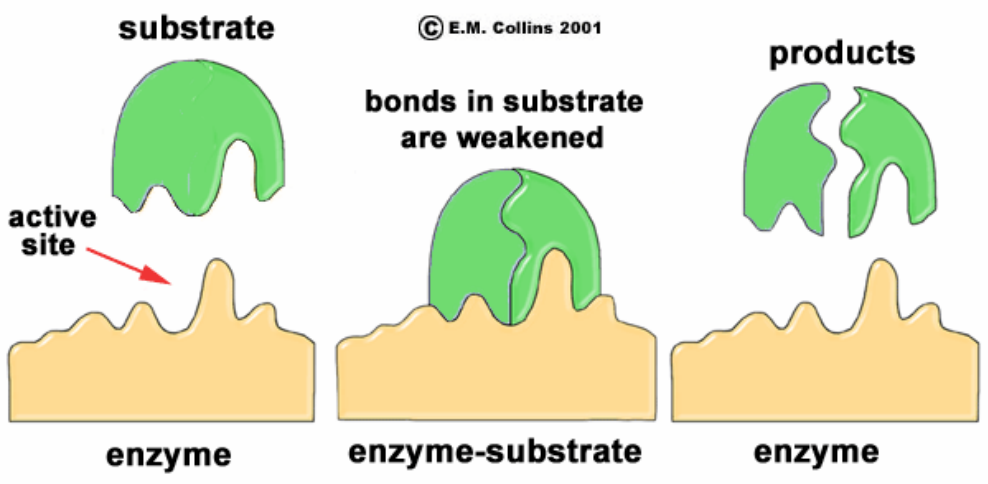


**Figure 1 : Lock and Key Theory**[1]

Here is one of the proposed theories of how enzymes catalyze reactions, lock and key theory, suggests that the substrate (Albumin) will bind to an active site in the enzyme. The enzyme (Protease) will then hydrolyse the peptide bonds (Hydrogen bonds) and the product will be 2 amino acids.

Using protein solutions at different concentrations (1%, 0.8%, 0.6%, 0.4%, 0.2% and 0%), and a mixture of biuret, I will calibrate the colorimeter. I will take the amount of green light absorbed by the solution; this will tell me the colour of purple that is present in the solution. The higher the protein concentration, the darker the purple or the solution and thus the more green light was absorbed.

[1] http://www.tutorvista.com/biology/lock-and-key-hypothesis

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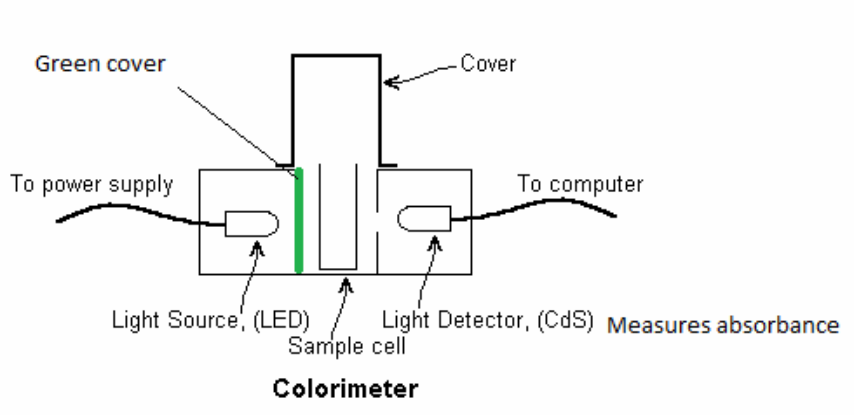


**Figure 2: Colorimeter** [2]

To measure the amount of protein present in the samples, I will compare the absorbance of the samples given to the calibration results. For example, if the samples give an absorbance (AU) of 0.0478, then I will compare this result to the graphical representation of the calibration results and plot to see the concentration of the sample.

**Data Collection and Processing:**

Table 1: Calibration of Colorimeter

| **Protein Concentration (%)** | **Absorbance AU)** |
|---|---|
| 1 | 0.607 |
| 0.8 | 0.565 |
| 0.6 | 0.489 |
| 0.4 | 0.380 |
| 0.2 | 0.234 |
| 0.0 | 0.061 |

Observations: When I placed the enzyme/substrate complex into the colorimeter the Absorbance value kept decreasing. (Due to the fact that there are still reactions happening).

[2] http://wwwchem.csustan.edu/chem1112/DyeKinetics.htm

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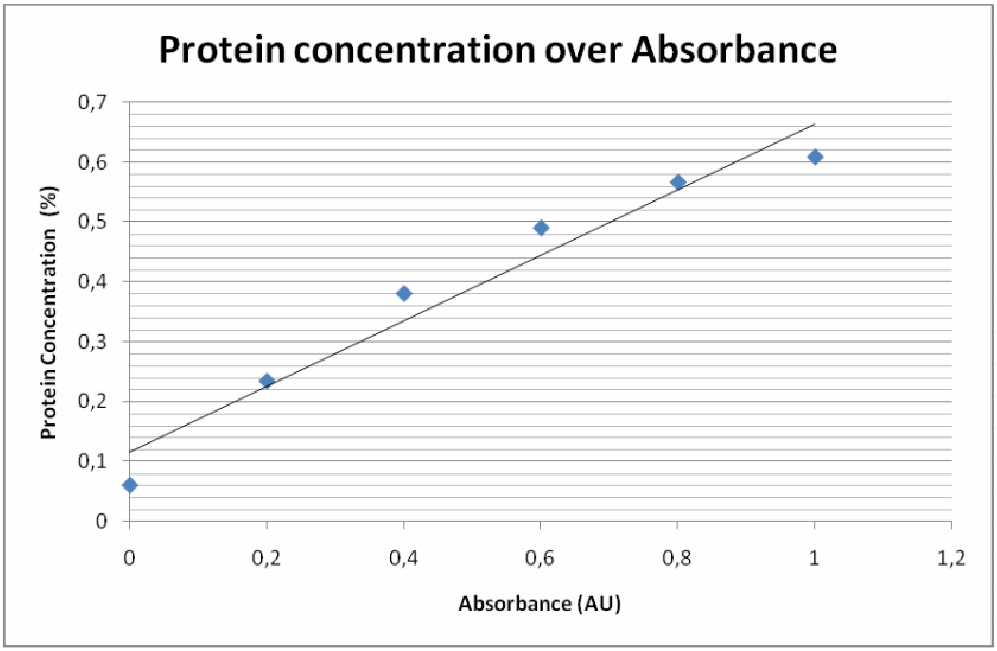


Table 2: Time-course of enzyme catalyzed bread down of Albumin

| Time (sec) ±10 | Trial 1(AU) | Trial 2(AU) | Trial 3(AU) | Mean (AU) ±0,003 | Reliable Mean (AU) |
|---|---|---|---|---|---|
| 0 | 0,448 | 0,440 | 0,448 | 0,445 | 0,445 ±0,003 |
| 4 | 0,446 | 0,461 | 0,436 | 0,447 | 0,434 ±0,002 |
| 8 | 0,432 | 0,494 | 0,423 | 0,449 | 0,427 ±0,002 |
| 12 | 0,418 | 0,464 | 0,411 | 0,431 | 0,418 ±0,001 |
| 16 | 0,414 | 0,437 | 0,417 | 0,422 | 0,415 ±0,002 |
| 20 | 0,314 | 0,485 | 0,399 | 0,399 | 0,356 ±0,002 |
| 24 | 0,400 | 0,413 | 0,376 | 0,396 | 0,394 ±0,002 |
| 28 | 0,354 | 0,449 | 0,327 | 0,376 | 0,340 ±0,002 |

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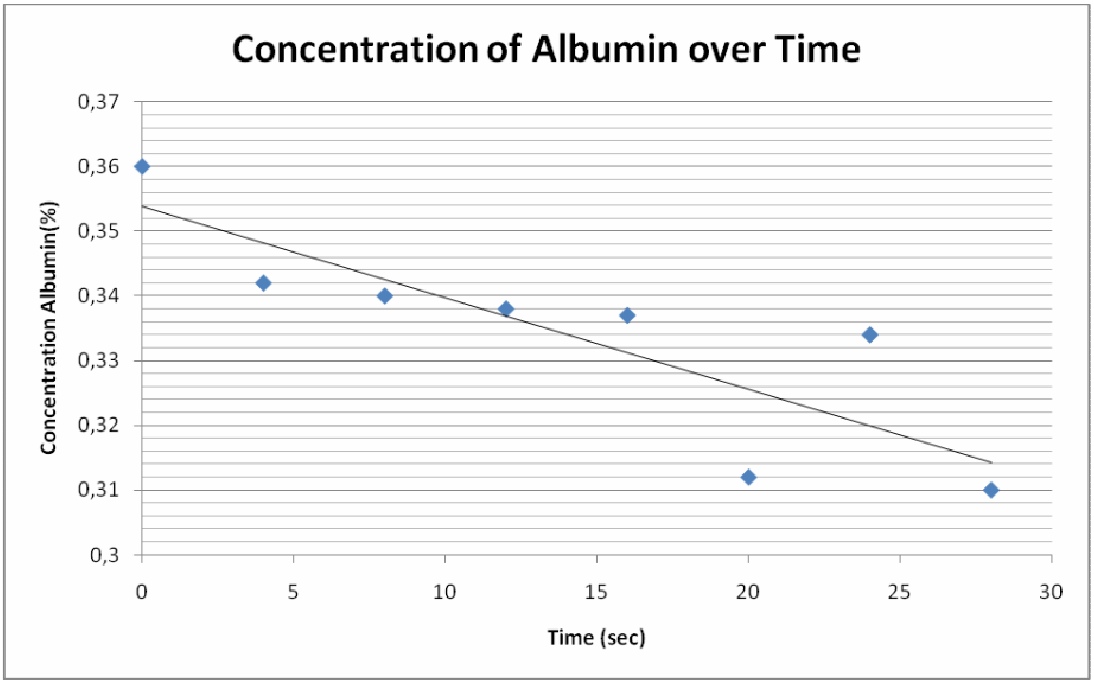
Table 3: Concentration of Albumin over Time

| Time (Sec) ±10 | Reliable Mean Albumin Concentration (%) |
|---|---|
| 0 | 0,360 |
| 4 | 0,342 |
| 8 | 0,340 |
| 12 | 0,338 |
| 16 | 0,337 |
| 20 | 0,312 |
| 24 | 0,334 |
| 28 | 0,310 |




The rate of reaction seems to increase in the beginning (even though it is not very clear) and is it proceeds the reaction slow down. If the 20 and 25 seconds are excluded, then it is possible to see that to the end of the react ion the line is already reaching zero rate of reaction. Initial rate of reaction is faster than the rate of reaction at the middle or at the end, this is because as the reaction proceeds the Albumin will become more dilute and reach a point where there is no more Albumin for the Protease to catalyse, thus the limiting reactant of this reaction being Albumin.

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**Conclusion:**

The results establish that the hypothesis is accurate (even though the results are not very accurate); the curve is steep at the beginni ng but then starts to level out in general. As the reaction starts the rate of reaction is higher then as the reaction proceeds over time, as the reaction reaches completion the rate of reaction reaches zero, where the concentration of Albumin has reached to zero (when no more Albumin is present for the enzymes to catalyse). Other scientific experiments confirm this conclusion and thus seem to be a reliable conclusion. [3]

**Evaluation:**

This experiment as of Biology in and of its self comes with a lot of anomali es, the results are not very reliable the numerous amounts of errors may account for the anomalies but there are certain errors that are not possible to control. The best way to lower the errors and find the anomalies with more precision, is to have more trials, this will lower the amount of effect that the anomalies have on the final results and thus produce more reliable and precise results. This will also produce a more accurate line of best fit.

To further improve the accuracy of the testing, it would be necessary to take the protein concentration every other minute (instead of every 5 minutes). This would improve the results and decrease the number of anomalies, further more it would also be more accurate if the measurements were taken for as long as the reaction finishes. This would allow for us to deduce, when the reaction has stopped taking place and would mean that the all the Albumin molecules have been used up.

One of the bigger weaknesses is the calibration; the colorimeter uses light to measure the intensity of the colour present. However this maybe misleading, because other particles are also present in the solution tricking the machine into believing that there is less light going through the solution, thus increasing the value of the colour (w hich then translates to the concentration of the solution). An even better idea to increase the accuracy of the results would be to increase the percentage of concentration present, for example increase the concentration to 5%, 10%, 15%, 20% etc… This woul d allow for overall accuracy increase and the anomalies would be spotted with more facility.

A smaller limitation of this experiment is the fact that only one protein and enzyme was used, therefore the conclusion is only based on the results obtained. To improve this factor it would be effective to take several different proteins and enzymes; then it is possible to analyse the effect that enzymes have of the time course of the protein break down into amino acids.

[3] http://en.wikipedia.org/wiki/File:Michaelis-Menten_saturation_curve_of_an_enzyme_reaction.svg

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Biology Lab Report

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