Protease and Casein Reaction Using a Colorimeter

Technician and Teacher Sheet

Apparatus

Colorimeter set to Transmission and Blue.

2 x Cuvettes

1% Protease solution (v/v)

1% Milk powder solution (w/v)

2 Syringes (10ml) or calibrated pipettes

EasySense software App

Data Recording setup.

Default settings

Setup: Continuous (if sample *in situ* constantly), Snapshot of time based sample aliquots are used

Run for 5 minutes

Introduction

This practical represents a generic protocol for protease and protein activities, to study simple enzyme kinetics. This experiment can form the basis for several other investigations into enzyme activity: the study of the effect temperature, pH, Group 1 and 2 metals, inhibitors can also be performed by simple adaptation of these detailed methods.

The enzyme uses a digestion process that is easy to relate to the students experiences. Most students will be aware of milk based drinks, for example. The digestion of cloudy milk powder suspension offers a simple reaction that is very reproducible.

Milk powder suspension (from dried milk powder) contains mainly casein, which is weakly soluble in water. The suspension has a milky appearance. A non-specific protease enzyme will digest the casein present and create a straw coloured to colourless solution of amino acids. The rate at which the opacity of the solution declines is proportional to the rate of the reaction for the consumption of the starting material.

The liquid protease enzyme system offers several advantages over the traditional amylase and starch alternative: the enzyme is very stable and can be kept under ambient conditions, without losing activity.

A powdered protease enzyme such as Trypsin can be used as an alternative to the one detailed here. Timings and concentrations of the enzyme will need to be altered though as the dynamics are different.

Practical Notes

The Casein Substrate

Add 1 g of fat free dried milk powder to 100 cm^3 of water (1% w/v casein to water). Scaling up the volume does not present any problems. This stock is stable and can be kept for at least 24 hours if chilled (after this time decomposition will start to take place). If dried milk powder containing fat is used, there is no significant difference to the experiment timings, but the resultant digested solution will not be as defined.

Protease Enzyme Solution

The experiments described in these notes use bacterial liquid protease purchased from NCBE*. Its trade name is Neutrease. It is a stable form of the enzyme, and its activity is not greatly affected by storage or in overnight preparations: refrigeration at 4°C is adequate for such storage.

The enzyme is made up as a 1.0 % v/v solution with distilled water and allowed to reach room temp (circa 21°C). The stability of this product means that buffer is not required, neither is an acid activator. The colour of the enzyme does influence the final values shown in the colorimeter, if this concentration is varied. Other proteases can be used, but the best concentration will need to be "fine-tuned" by testing. Our experience has shown that a 1.0 cm^3 to 100 cm^3 stock is a good starting point (alternatively move to 0.1 cm^3 to 100 cm^3). The activity of the enzyme needs to be modified to give completed digestion at room temperature in 3 minutes, pH 7.

Milk powder suspension (milk made from dried milk powder) contains mainly a casein protein, which is weakly soluble in water. A non-specific protease enzyme will digest the casein present and create a solution of colourless amino acids. The rate at which the opacity of the solution declines is proportional to the rate of the reaction.

Adding the casein substrate to the enzyme gives a clear start point for the experiment and helps mix the reagents in the cuvette.

The cuvettes have nominal capacity of 4.0 cm^3, some have a volume of 4.5 cm^3. All instructions here are aimed at a final volume of 4.0 cm^3. A smaller volume in a larger (taller) cuvette has no effect, as the colorimeter uses a light path through the cuvette about 1/3rd of the total distance up from the base of the cuvette to its top.

If care is taken solutions can be added to the cuvette while it is in the colorimeter: this will help to reduce errors in overall determined timings.

If you are using powdered protease take care when handing the powder (regarding allergies).

Data Harvest's colorimeter does not use optical filters, and the control of the light is through software (EasySense). In older colorimeters, the experiment could have been conducted without the coloured filter. The use of additional wavelength filtering does allow the teacher to introduce the idea of the need to control the wavelength of the light used in colorimetry. In this experiment the wavelength of the light is not critical.

The reaction should be complete within 3 minutes, assuming a temperature of the solutions is at 20 to 22°C.

*NCBE: The National Centre for Biotechnology Education at the University of Reading.

A Note About Temperature

If you need to work away from room temperature, then the following should be considered.

Use a large reaction mixture and set the software (EasySense) to give a digital readout from the CHART option.

Place the reaction mixture in a temperature-controlled water bath, and sample 4 cm^3 aliquots, at timed intervals noting the data to a standard results table.

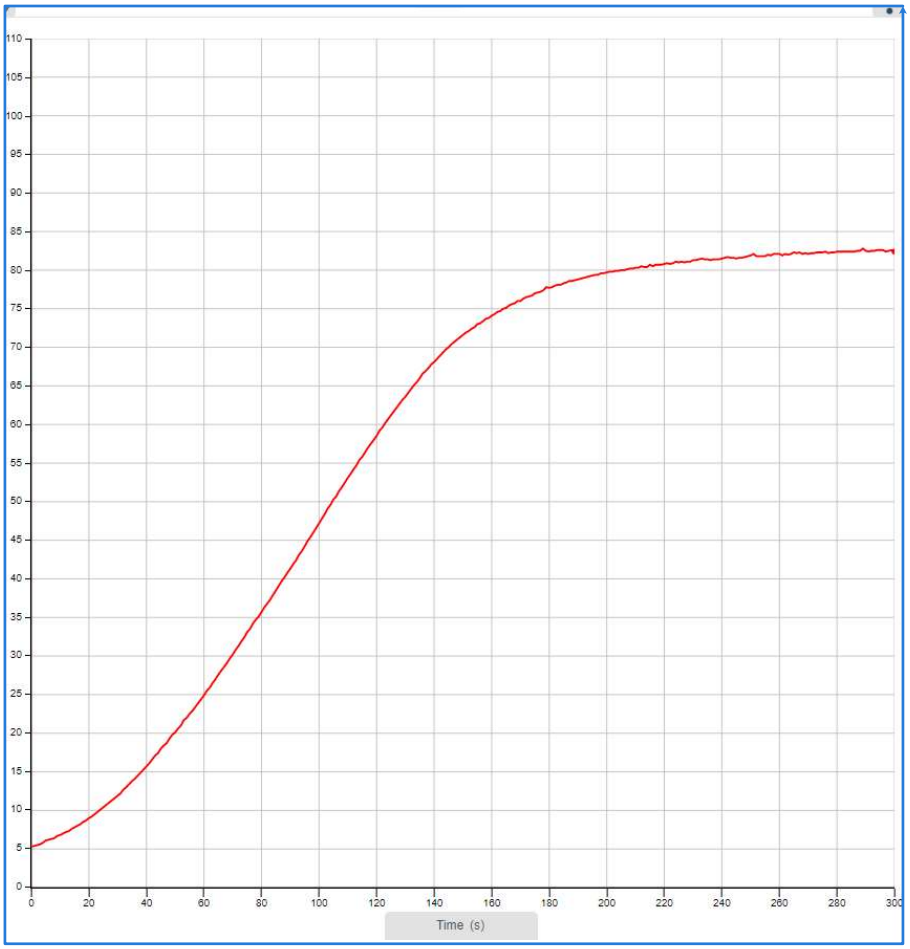
or

Set up a Snapshot recording (in EasySense) and let the software place the time stamp, as each value is recorded.

With Snapshot, you can impose a "Prompt for value on each sample" from the Setup, which can be the seconds for the sample time.

A graph of % transmission vs. time can then be prepared on graph paper or in the software.

You will know your class! if samples are being taken at timed intervals a weaker enzyme concentration and a stronger protein concentration will probably be needed to give the time control required.



Example of a simple digestion of milk proteins by a non-specific protease.

Change in transmission against time is plotted.

Note the transmission may not reach 100%; the protease enzyme has a dark brown colour which gives some colouring to the solution even when diluted. Additionally, not all the opacity of the milk powder solution is due to the suspended protein.

In this experiment the powdered milk contained fatty material to improve the flavour of the reconstituted milk!

If a concentration vs. time is required, the data should be collected as absorbance over time.

Lambert Beer Law

The amount of light that penetrates a solution, relative to an incident reference, is known as the transmittance.

The transmittance of a solution varies the following factors:

- The molar absorptivity of the solution, E
- The molar concentration, C
- The cell or cuvette dimension (path length), l

E and l are constants in this practical.

If we take the incident intensity to be I_0, the intensity after the beam has passed through material as I_l, the Transmittance (%), T, is defined as:

$$T = I_l / I_0$$

Absorbance, A, is defined as

$$A = \text{Log}(I_0 / I_l)$$

Therefore

$$A = \log(T^{-1})$$

Absorbance is also equal to

$$A = ECl$$

And so

$$\log(T^{-1}) = ECl$$

Software Knowledge Required.

1. Connecting the sensor to the software
2. Change the measurement scale of the sensor
3. Configure the correct colour wavelength to use
4. Configure a continuous data collection