Investigating effect of concentration on the activity of trypsin

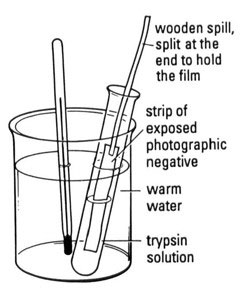
In this practical you will learn to:

* investigate how trypsin activity changes with concentration
* evaluate this method of following a chemical reaction

### Procedure

SAFETY: Wear eye protection and quickly rinse any splashes of enzyme solution from the skin.

Make sure you know what to do if a thermometer is broken.



Investigation

1. Label a test tube with the concentration of trypsin to be investigated.
2. Use a measuring cylinder to measure volumes of 5% trypsin solution and distilled water into as shown in the table below. Make up each concentration in a clean test tube.

|  |  |  |  |
| --- | --- | --- | --- |
| Concentration of trypsin in final solution (%) | Volume of 5% trypsin needed to make 10 cm3 of solution (cm3) | Volume of water needed to make 10 cm3 of final solution  (cm3) | Final volume of trypsin solution made up  (cm3) |
| 0 | 0 | 10 | 10 |
| 1 | 2 | 8 | 10 |
| 2 | 4 | 6 | 10 |
| 3 | 6 | 4 | 10 |
| 4 | 8 | 2 | 10 |
| 5 | 10 | 0 | 10 |

1. Place the test tubes and the thermometer in a beaker of warm water at close to 35ºC. Maintain the temperature within two degrees of this during the course of the investigation by adding more hot water as necessary.
2. Make a note of the temperature of the water.
3. Cut pieces of photographic film to fit in the test tube as shown. (This may have been done for you.)
4. Attach wooden spills to each piece of film to be used (see diagram), or tie a piece of cotton thread through a sprocket hole and suspend the film from a spill placed across the tube.
5. Place the pieces of film in the test tubes as shown in the diagram and start the clock.
6. As each piece of film clears, make a note of the time on the stopclock/ stopwatch. Do not turn off the stopclock/stopwatch.
7. Plot a suitable graph of the results. This could be a graph of time taken for the reaction to occur against temperature. Or, you can convert this to a rate of reaction graph by calculating 1 ÷ time for each of the temperatures. (If any tubes have not reacted in the time taken, this is a rate of zero.)

**QUESTIONS**

1. When trypsin breaks down gelatine (a protein), what is formed?
2. Describe the effect of trypsin concentration on the breakdown of gelatine.
3. Explain why changing the concentration of trypsin has this effect on the rate of this reaction.
4. How could you improve this investigation?
5. Design another investigation using this apparatus to look at a different factor that could affect the action of trypsin.

**ANSWERS**

1. When trypsin breaks down gelatine (a protein), amino acids are formed.
2. Increasing trypsin concentration increases the rate of breakdown of gelatine.
3. Changing the concentration of trypsin has this effect on the rate of the reaction because increasing the concentration increases the number of particles that can react each second as more enzyme molecules are available to collide with the protein molecules.
4. Students may be able to see how to control variables more effectively or how to establish the end-point more accurately.
5. Students may have an idea for using a similar apparatus to investigate the effects of temperature or pH.