NEATH PORT TALBOT COLLEGE
COLEG CASTELL NEDD PORT TALBOT

School of Maths & Science
Science Practical

In this practical you will be assessed on skills C, D, F, G and H

The Effect of pH on Trypsin Activity

◆ Aim

To investigate the effect of pH on Trypsin activity.

◆ Introduction

Principle
Trypsin is a protease which catalyses the hydrolysis of proteins to shorter chain polypeptides. One method by which its action can be detected is to use strips of film negative in which silver grains are held in place by gelatin, a protein. As the gelatin is digested, the silver grains fall off leaving a clear film.

◆ Safety

Control Measures

- The wearing of **safety glasses** and a **laboratory coat** at all times will be sufficient to take account of most hazards and significant risks.
- Some enzymes cause allergic reactions in people handling them. All enzymes and enzyme solutions should be handled carefully.
- Avoid airborne dust from powdered enzymes, wear eye protection and mop up any spills immediately.
- Keep stoppers on bottles as much as possible.
- All waste is to be placed in the labelled container immediately after use.
- You are reminded of the need for good laboratory practice in order to maintain a safe working environment.

Hazards

Harmful Trypsin

Irritant Buffer solutions

◆ Materials

Buffer solutions at pH 4.4, 6.0, 7.6, 9.2 and 10.0.
Strips of film negative (uniform size).
2% Trypsin solution.

◆ Apparatus

Water bath at 37^0C.
Test tubes.
Graduated pipettes and pipette fillers.
Stop watch.

◆ Procedure

1. Take 15 test tubes. Set up 3 test tubes at each pH as shown below:

pH	Volume of buffer / cm^3	Number of film strips
4.4	5	1
6.0	5	1
7.6	5	1
9.2	5	1
10.0	5	1

2. Place all tubes in a water bath at 37^0 C for 5 minutes to equilibrate. Simultaneously, place 100 cm^3 of enzyme solution in a beaker / measuring cylinder and place in the water bath for 5 minutes to equilibrate.

3. Add 5 cm^3 of enzyme solution to each of the test tubes in the water bath.

4. Set up suitable controls.

5. Observe the film closely and record the time taken for the film to clear (as the gelatin disappears the silver grains will sink to the bottom of the tube).

6. Present the results in a suitable table (calculate mean values) and use them to plot a suitable graph.

7. Interpret the results and evaluate the practical work.