

Topic 2 – Practical 1

Investigating the conditions needed for the action of a protease enzyme

Safety

- Care should be taken to check that no students are allergic to eggs.
- 1 mol dm^{-3} hydrochloric acid, Biuret reagent and 1% pepsin are irritants.
- Wear eye protection and laboratory aprons when handling enzymes, Biuret reagent or acid.
- If chemicals come in contact with skin or eyes, rinse with plenty of water.

Apparatus and materials

- Biuret reagent
- 10 cm^3 egg albumin suspension
- 2 cm^3 1% pepsin solution
- 1 cm^3 boiled 1% pepsin solution
- 1 mol dm^{-3} hydrochloric acid
- six test tubes and rack
- pen to mark test tubes
- water bath at $35\text{--}40^\circ\text{C}$
- four 2 cm^3 syringes

Introduction

Pepsin is a protease enzyme found in the stomach. It digests protein into polypeptides and amino acids. Egg white (albumin) contains protein. When it is boiled the protein is denatured so that the egg albumin becomes cloudy. In this experiment you will investigate the effect of pepsin on egg albumin (a suspension made up from egg albumin powder), with and without 1 mol dm^{-3} hydrochloric acid.

Procedure

- 1 Using a syringe, place approximately 2 cm^3 albumin suspension in a test tube and add an equal volume of Biuret reagent. A purple colour indicates that protein is present.
- 2 Label four test tubes – A, B, C, D – and put about 2 cm^3 of the albumin suspension into each one using a syringe.
- 3 Using clean syringes for each of the pepsin solutions, add the following to each test tube:
A – 1 cm^3 1% pepsin solution
B – three drops 1 mol dm^{-3} hydrochloric acid
C – 1 cm^3 1% pepsin solution and three drops 1 mol dm^{-3} hydrochloric acid
D – 1 cm^3 boiled 1% pepsin solution and three drops 1 mol dm^{-3} hydrochloric acid
- 4 Place all four tubes in a water bath at $35\text{--}40^\circ\text{C}$ and leave for approximately ten minutes.
- 5 Observe and record the results. If the mixture has become clear, this is an indication that digestion of protein has occurred.
- 6 Using a clean syringe, take a sample of the liquid from a clear tube, place in a clean test tube and add an equal volume of Biuret reagent. Note any colour change.

Questions and further work

- 1 Why does a clear solution indicate that digestion has occurred?

- 2 In which tube(s) was the protein digested?

- 3 In which tube(s) was protein not digested?

- 4 What effect does boiling have on pepsin solution?

- 5 Why was the water bath used in this experiment?

- 6 Design a similar experiment that you could use to determine the optimum pH for pepsin activity.