# Guidance for Topic 6 – Practical 1

## *Investigating the conditions needed for the action of a protease enzyme*

### Safety

Although great care has been taken in checking the accuracy of the information provided in this guidance, Cambridge University Press shall not be responsible for any errors, omissions or inaccuracies.

Teachers and technicians should always follow their school and departmental safety policies. You must ensure that you consult your employer’s model risk assessments and modify them as appropriate to meet local circumstances before starting any practical work. Risk assessments will depend on your own skills and experience, the skills and experience of your students, and the facilities available to you. Everyone has a responsibility for his or her own safety and for the safety of others. The notes below should not be regarded as a risk assessment.

You should carry out the practical yourself before presenting it to students. Make sure you are comfortable with the procedures, and can anticipate any difficulties any of your students may encounter.

### Guidance

This straightforward practical, which investigates the effect of pH on the digestion of egg albumin by pepsin, is useful as a starting point for students to design their own enzyme investigations. It gives the opportunity to investigate denaturation of enzymes and the rate of an enzyme-catalysed reaction. It can also be used to revise the Biuret test for protein.

Note that this practical may be used in addition to Practical **2** in this topic, or as an alternative to it. It could instead be used during Topic **2**, *Molecular biology*.

### Apparatus and materials

Each student or pair will need:

• Biuret reagent • six test tubes and rack

• 10 cm3 egg albumin suspension • pen to mark test tubes

• 2 cm3 1% pepsin solution • water bath at 35–40 C

• 1 cm3 boiled 1% pepsin solution • four 2 cm3 syringes

• 1 mol dm-3 hydrochloric acid

### Setting up the practical

Egg albumin suspension should be made up according to the directions on the dried albumin package, or sprinkle 10 g dried albumin on 1000 cm3 3% sodium chloride solution, leave to stand for 1 hour, stir gently and store in the refrigerator. Alternatively, a beaten egg white can be mixed with 500 cm3 water and filtered through glass wool to produce the suspension.

1 mol dm-3 hydrochloric acid can be supplied in dropping bottles as only a few drops are needed per group.

Biuret reagent can be bought ready prepared to add directly to the test solution. If this is not available, the Biuret test can be carried out as follows.

• Solution A, 2 mol dm-3 NaOH (**corrosive**) – 80 g sodium hydroxide (wear eye protection and gloves) made up to 1000 cm³ with water

• Solution B, 0.2 mol dm-3 Cu2SO4 – 5 g copper(II) sulphate-5-water made up to 1000 cm³ with water

Mix solution A with the test solution in the ratio 1 : 1. Add solution B one drop at a time, shaking well after each addition. A purple or pink colour shows the presence of protein.

### Supporting the practical

Students may need reminding about the colour change produced by the Biuret test. It can be helpful to highlight the temperature of the water bath and ask why this temperature has been chosen (see Questions).

### Answers to questions

**1** Digestion breaks down large insoluble molecules to produce small soluble molecules. In this case protein is digested to amino acids by protease (pepsin).

**2** Digestion should occur in tube C, where the enzyme is present and the acid provides the optimum pH for it to work. There may be a little digestion in tube A, where the enzyme is present, but because there is no acid the pH is not optimum, so digestion cannot proceed at a fast rate.

**3** Digestion does not occur at all in tubes B and D. Tube B has no enzyme present, and in tube D the boiled enzyme is ineffective.

**4** Boiling denatures the protease enzyme, which is a protein, so that it can no longer catalyse the reaction.

**5** The temperature of the water bath is approximately human body temperature, the optimum temperature for the enzyme to work.

**6** Students should suggest that the pH of the mixture is measured and that a range of different pHs, from 1 to 7, is used. The time taken for the albumin solution to become clear should be noted at each pH (to obtain a rate of enzyme reaction).

# Guidance for Topic 6 – Practical 2

## *Investigating the effect of temperature on enzyme activity*

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### Guidance

This relatively straightforward practical introduces students to the idea that temperature has an effect on enzyme activity. Just four temperatures are suggested but this can be modified to include a fuller range. The experiment described has no control tube and students should recognise this failure and understand why a control is important.

This practical can be used as the basis for a range of practical work using other enzymes and variables such as substrate concentration or pH.

Note that this practical may be used in addition to Practical **1** in this topic, or as an alternative to it. It could instead be used during Topic **2**, *Molecular biology*.

### Apparatus and materials

Each student or pair will need:

• 30 cm3 5% amylase solution • water baths at 0 C, 25 C, 35 C and 60 C

• iodine solution • two 5 cm3 syringes

• 30 cm3 1% starch solution • dropping pipette

• eight test tubes and rack • four stopwatches

• pen to mark test tubes

### Setting up the practical

Amylase can be obtained from a biological supplier; alternatively, students’ own saliva can be used. If using saliva as a source of amylase, ensure good hygiene as it is a potential (if unlikely) source of infection. Students should supply saliva for their own investigation only.

### Supporting the practical

As they work, students should consider why the tubes are placed in the water baths for 10 minutes before the contents are mixed.

### Clearing up

If using saliva as a source of amylase, ensure good hygiene to minimise the small risk of infection. Students should dispose of their saliva after the investigation by rinsing it down a sink and placing the equipment in bleach.

Solutions should be washed down the sink with plenty of water.

### Answers to questions

**1** In this experiment, the fastest breakdown of starch should occur at 35 C, which suggests that the optimum temperature for amylase is close to this value.

**2** No digestion of starch should occur at 0 C or 60 C. At 0 C, the temperature is too cold for enzyme and substrate molecules to meet frequently enough to produce a reaction that occurs at an observable rate, while at 65 C the enzyme will have been denatured and therefore unable to function.

**3** A control tube should contain the substrate but no enzyme, at 35 C. This is important to show that the presence of enzyme is necessary for the digestion of starch.

**4** Suggestions should propose a similar investigation using temperatures close to the rough optimum suggested by this experiment. Temperatures at intervals between 25 C and 45 C would provide a good range.

# Guidance for Topic 6 – Practical 3

## *Heart dissection and examination of arteries*

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### Guidance

This dissection provides the opportunity to examine the structures of a mammalian heart and draw comparisons between the muscle of atria and ventricles, as well as the construction of valves and blood vessels associated with the heart.

The second part of the practical enables student to observe the strength and elasticity of the arterial walls. The practical is best conducted individually if sufficient hearts can be obtained from a local butcher. Some students may prefer to observe rather than handle animal tissue.

### Apparatus and materials

Each student or pair will need:

• fresh sheep or pig heart • clamp stand

• dissection board • simple mass holder

• scalpel • masses from 100 g to 1000 g

• dissecting scissors • 50 cm3 saline solution in a glass beaker

• ruler • antiseptic wipes

• drawing paper and pencil • soap and hot water

### Setting up the practical

Each student will need 50 cm3 physiological saline to store their cut rings of artery until required. This is made with 0.9% w/v sodium chloride.

### Supporting the practical

An artery ring may support masses up to 1000 g so care should be taken that the artery rings are not held high above the work surface. Students should be warned not to overload smaller sections of artery, which may break and cause the masses to fall. A cloth can be placed under the masses to prevent harm.

### Clearing up

Animal tissue should be wrapped and disposed of in the approved manner for food waste.

Gloves, aprons and other materials can be separated.

Students should separate sharp dissecting instruments from other washing up to prevent risk of injury to technicians. All materials should be washed in hot water and detergent.

Everyone who handles animal tissue should wash hands thoroughly with hot soapy water afterwards.

### Answers to questions

**1** The graphs should follow Hooke’s law and show that the artery tissue has elastic properties until the elastic limit is reached, after which no recoil is possible.

**2** Students should relate the elastic properties of the artery tissue to the presence of elastic fibres in the walls and the strength of the artery to the presence of collagen.

**3** Improvements to the procedure might include a more accurate way of measuring length, and a method of stretching the artery that does not require a metal holder and separate masses. Students might suggest a strain gauge attached to a data-logger.

# Guidance for Topic 6 – Practical 4

## *Modelling digestion and absorption using Visking tubing*

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### Guidance

The first part of this practical enables students to familiarise themselves with Visking tubing as a ‘model’ gut. Only small molecules will pass through the tubing in the same way that only small molecules are able to diffuse through the wall of the small intestine. There is also the opportunity to revise the tests for starch and glucose, which may be useful in other practical work.

The second part of the practical considers the additional concept of digestion. A series of timed tests of the water surrounding the Visking tubing will enable students to follow the progress of the digestion of starch by the enzyme amylase. Only when the large molecules have been digested can they pass through the tubing.

### Apparatus and materials

Each student or pair will need:

• 10 cm length of Visking (dialysis) tubing • test tubes

• 10 cm3 syringe • test tube rack

• 10% glucose solution • spotting tile

• 1% starch suspension • dropping pipettes

• thread to tie the tubing • iodine solution

• boiling tube • 250 cm3 beaker

• rubber band or sticky tape • boiling water

• distilled water • Benedict’s reagent

• stopwatch

For part 2:

• 5% starch suspension

• 1% amylase

### Setting up the practical

1% starch suspension is prepared using 5 g soluble starch in 500 cm3 water; 5% starch suspension is prepared using 25 g soluble starch in 500 cm3 water. In each case, boil the solution then cool to room temperature.

10% glucose solution is prepared using 10 g glucose in 100 cm3 water.

### Supporting the practical

Visking tubing can be difficult to knot securely; students may need help with this. It is important that if the same piece of tubing is used in the second part of the experiment that it is washed carefully as contamination will spoil the results. Two-minute intervals are suggested for the glucose and starch tests but this may be varied according to lab conditions such as temperature which may make the reaction proceed more quickly.

### Answers to questions

**1** Similarities: only small molecules can pass through it the tubing; molecules move through it by diffusion down their concentration gradient.

Differences: in the gut active transport and facilitated diffusion are also important; the gut is made of living tissue; the surface area of the gut is massively increased by the presence of villi and microvilli; a concentration gradient is maintained in the gut because blood transports absorbed products away.

**2** Speeded up: increase in temperature, increase the concentration of enzyme.

Slowed down: cool the apparatus, decrease the concentration of enzyme