

Investigation into the effect of temperature or pH on enzyme activity

Specification reference: 1.4

Biological reactions are regulated by enzymes

Introduction

Phenolphthalein is an indicator that is pink in alkaline solutions of about pH10, but turns colourless in pH conditions less than 8.3. In this investigation, an alkaline solution of milk, lipase and phenolphthalein will change from pink to colourless as the fat in milk is broken down to form fatty acids (and glycerol) thus reducing the pH to below 8.3. The time taken for this reaction to occur is affected by temperature.

Apparatus

Milk, full-fat or semi-skimmed
Phenolphthalein in a dropper bottle
lipase solution (5g/ 100cm³)
Sodium carbonate solution (0.05 mol dm⁻³)
5x Test tubes and rack
2 x 10cm³ syringes/measuring cylinders
2cm³ syringe
Stirring rod
Thermometer
Water baths set to 15°C, 25 °C, 35°C, 45°C and 55°C.
Ice

Method

1. Place a beaker of lipase solution in the 25 °C water bath.
2. Place 5 cm³ milk, in a test tube.
3. Add 5 drops of phenolphthalein to the test tube.
4. Add 7 cm³ of sodium carbonate solution.
5. Place the test tube in the 25°C water bath for 10 minutes to equilibrate.
6. Add 1 cm³ of lipase from the beaker in the water bath and start the stop clock.
7. Stir the contents of the test tube until the solution loses its pink colour, record the time taken.
8. Repeat steps 1 – 7 for 15°C, 35°C, 45°C and 55°C.

Risk Assessment

Hazard	Risk	Control measure
Sodium carbonate is irritant at high concentrations	May splash or transfer into eye when placing into test tube	Use low concentrations and wear safety glasses
Phenolphthalein indicator contains ethanol which is flammable	If using Bunsen burners for water baths, could ignite the ethanol	Do not use ethanol near Bunsen burners

Teacher/ Technicians notes

Sodium carbonate solution, 0.05 M. Make with 5.2 g of anhydrous solid, or 14.2 g of washing soda per litre of water. See CLEAPSS Hazcard; it is an IRRITANT at concentrations over 1.8 M.

Ethanol (IDA) in the phenolphthalein indicator is described as HIGHLY FLAMMABLE on the CLEAPSS Hazcard (flash point 13 °C) and HARMFUL (because of presence of methanol).

Lipase solution (5%) is best freshly made, but it will keep for a day or two in a refrigerator.

Phenolphthalein is described as low hazard on CLEAPSS Hazcard 32. Refer to Recipe card 33 (acid-base indicators): Dissolve 1 g in 600 cm³ of IDA then make up to 1 litre with water. Label the bottle highly flammable. Suppliers of phenolphthalein solution may not use IDA; it also may be diluted. Follow any hazard warning on supplier's bottles.

Thermostatically controlled water baths could be set up at the required temperatures or students could be given equipment to enable them to make their own water baths (tripod, gauze, beaker, Bunsen burner and thermometer).

More details available from

<http://www.nuffieldfoundation.org/practical-biology/investigating-effect-temperature-activity-lipase>

Sample results

The quantities used should take approximately 4 minutes to change from pink to white at normal laboratory temperature. If this is not the case, change the concentration of enzyme to alter the speed of the reaction (more enzyme will reduce the time or increase the speed). Students will need to use the same volume at each temperature.

Further work

- Use a pH probe or data logger to give quantitative results
- It would be possible to vary the concentration of the lipase and look at the effect of enzyme concentration on the breakdown of fat in milk.
- Different types of milk could be used Jersey, full cream, semi-skimmed and skimmed, to explore the effect on the reaction of changing fat concentration.

Practical Techniques

- use appropriate apparatus to record a range of quantitative measurements (to include mass, time, volume, temperature, length and pH)
- use laboratory glassware apparatus for a variety of experimental techniques to include serial dilutions
- use ICT such as computer modelling, or data logger to collect data, or use software to process data