# Topic 8 – Practical 2

## *Using a redox indicator to show the activity of dehydrogenase enzyme*

### Safety

• Triphenyl tetrazolium chloride is harmful.

• Wear a lab coat to protect skin and clothes.

• Wear eye protection.

• Rinse away any solution that contacts the skin and mop up any spilled solutions immediately.

### Apparatus and materials

• a suspension of live, respiring yeast • 5 cm3 syringes (or graduated pipettes)

• triphenyl tetrazolium chloride (TTC) solution • stirring rod

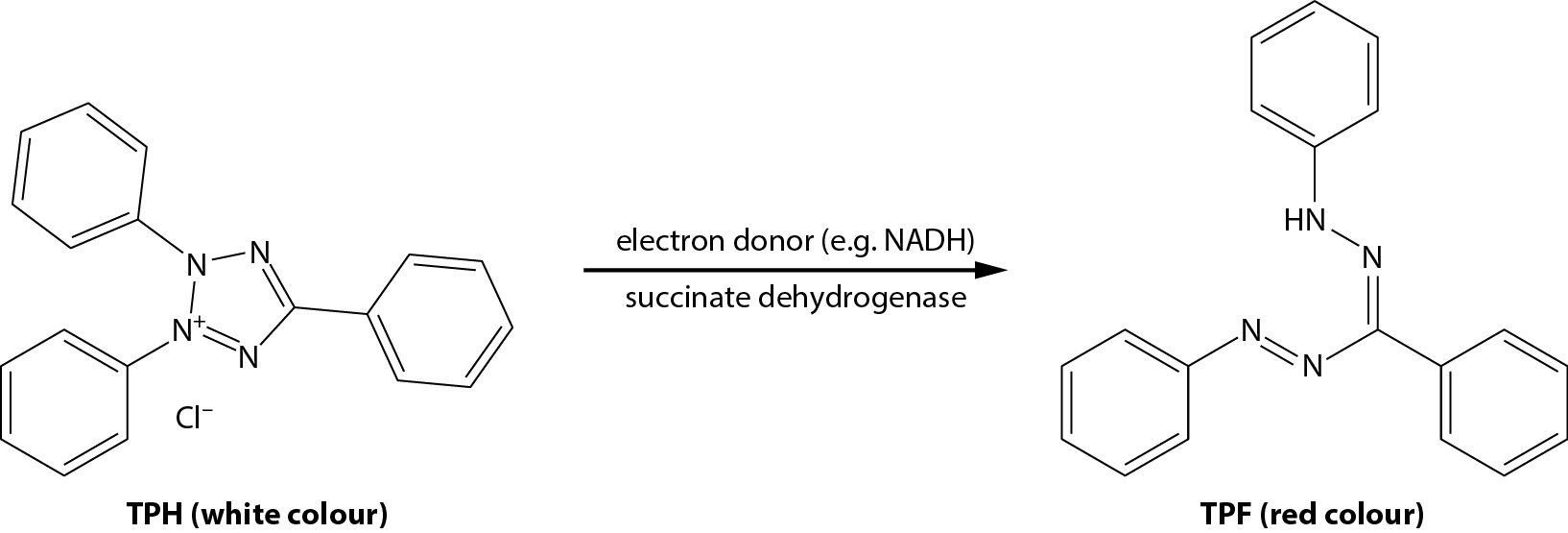
• distilled water • thermometer

• test tubes • stopwatch

• water baths at 5 °C, 30 °C, 35 °C, 40 °C  
and 50 °C

### Introduction

Triphenyl tetrazolium chloride (TTC or tetrazolium chloride) is a redox indicator that is used in biochemical experiments to indicate cell respiration. It is an example of an artificial hydrogen acceptor. TTC is colourless when oxidised but forms insoluble red compounds called formazans when it is reduced.



This experiment investigates the effect of temperature on the activity of dehydrogenase enzymes. The source of dehydrogenase is respiring yeast cells.

### Procedure

**1** Use a syringe to measure 5 cm3 of yeast suspension into one test tube and 0.5 cm3 of TTC solution into another test tube.

**2** Chose one temperature for your first experiment and place both test tubes in the appropriate water bath. Leave the tubes for 5–10 min until their contents have reached the temperature of the water.

**3** Check the temperature of both tubes with a thermometer, then pour the TTC solution into the yeast suspension, return the tube to the water bath and immediately start the stopwatch.

**4** Stir the contents of the tube from time to time and observe it carefully. Note the time taken for any colour change to develop.

**5** Stop the stopwatch and record the time when you see a stable red colour.

**6** Carry out two further trials at this temperature (steps **1**–**5**) and calculate the mean value of the time taken to establish a stable red colour. (If there is insufficient time to do this, collect data from other students in your class.)

**7** Repeat the whole procedure (steps **1**–**6**) at each of the other temperatures in the different water baths.

**8** Record your results in a suitable table, such as the one below.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Time taken for colour change \ s (±30 s) | | | |
| Temp \ °C | Test 1 | Test 2 | Test 3 | Average |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |

**9** On separate graph paper, plot a suitable graph of your data.

### Questions and further work

**1** What conclusions can you draw about the effect of temperature on the activity of dehydrogenase in yeast?

**2** Why is step **6** in the procedure important to your conclusion?

**3** Why is a margin of error of ±30 s shown in the sample table above? Is this is suitable margin?

**4** What procedure could you adopt to confirm the optimum temperature for dehydrogenase activity?