Philip Pearson **AS Biology Practical Assessment**

An investigation to find out how temperature affects membrane permeability.

Problem

What we will hopefully try and find out in this investigation is if temperature affects the permeability of a beetroot membrane

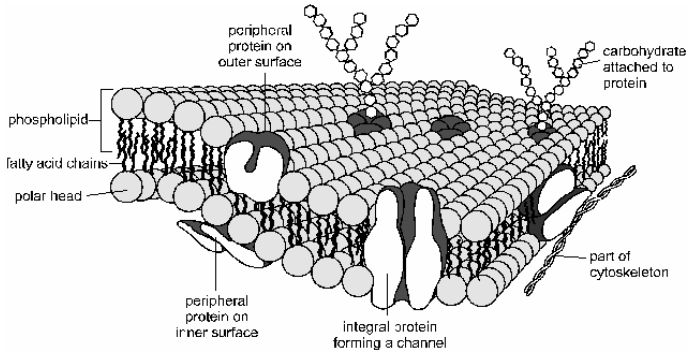
Hypothesis

As the temperature in which the beetroot is put in increases there will be more red dye diffusing out of the beetroot due to the denaturing of the proteins in the cell membrane as a result of the high temperatures.

Background Knowledge

The cell membrane can be represented as the fluid mosaic model as shown below. It is selectively permeable and controls what enters and exits the cell. It does this by proteins, however small lipid molecules, non-polar molecules and small water molecules can enter and exit the cell straight across the membrane through the phospholipids, due to the properties of the molecules enabling them to do so. Extrinsic and intrinsic proteins in the cell membrane help other the molecules enter or leave the cell by either facilitated diffusion or active diffusion. Different proteins are specific to certain molecules hence the cell membrane being selectively permeable.

Here is a diagram of the cell membrane:



As you can see the cell membrane is made up of a phospholipid bilayer which the extrinsic and intrinsic proteins span through. Some of the extrinsic proteins act as antigens for cell recognition with a carbohydrate attaching to then forming a glycoprotein. Intrinsic proteins are usually associated with the movement of molecules in and out of the cell.

Proteins are enzymes and are made up of specific amino acids and only work in certain conditions, for example the pH has to stay constant and the

temperature also has to stay constant. If these two factors are not controlled e.g. the temperature around the cell becomes higher or lower than the optimum temperature for that cell, then the hydrogen bonds that keep the proteins tertiary structure (overall 3D shape) will be broken (denaturing the protein). Consequently the proteins in the cell membrane are unable to function correctly. Therefore a higher temperature will denature the proteins and diffusion will be able to take place easily due to the proteins being unable to manage what enters or leaves the cell.

The optimum temperature of the beetroots normal working conditions should be around 40 C as the optimum temperature for animal cells is 40 C, therefore I assume that the beetroot should be around that temperature due to similar living conditions of both organisms. Therefore if the temperature goes above this optimum temperature then the proteins in cell membrane of the beetroot will become denatured and anything can enter or leave the cell. A good indication of this easy diffusion, due to denatured proteins, would be the amount of red dye that diffuses out of beetroot. This is what the experiment is investigating.

Equipment list and why using it:

NAME OF EQUIPMENT	REASONS FOR EQUIPMENT
Cork borer	Used to cut out pieces of beetroot. It keeps the surface area of each beetroot piece the same
Ruler	Is used to measure each piece of beetroot and make sure each piece is the same length
Blade	Used to cut the beetroot pieces
Heat proof mat	To mount other equipment on and to protect table surface
Tripod	Used to put glass beaker on. Has the bunson burner placed under it
Gauge	Is placed on tripod. This is what the glass beaker is put on
Bunson Burner	Used to heat the water
250 ml glass beaker	Used for the water bath. Is glass so it does not easily melt under the higher temperatures
Thermometer	Used to see what the temperature of the water bath is in C
3 x boiling tubes	Are what the beetroot pieces and distilled water are place in
Distilled water	20 ml placed in each boiling tube. Distilled water is used because it has a constant pH and no impurities,

	which may affect the reading.
100 ml measuring cylinder	Used to make sure we keep a constant amount of distilled water in the boiling tubes
Stopwatch	To time how long the beetroot is in the water bath for
4 x Cuvettes	1 of the cuvettes is filled with distilled water to set the colourimeter to 0, while the other 3 are used to place the solution of the three red pigments in
Pipettes	Are used to put the red pigment from each boiling tube into each cuvette
Colorimeter	This is a piece of digital equipment that measures optical density (light absorbency) in Arbitrary units. This is what tells how much red pigment has been lost by the beetroot
Test tube rack	Used to put the boiling tubes in when using the colourimeter

Plan

The variable that will be changed in this experiment will be the temperature of each water bath. The steps of each measurement will be around 30, 40, 50, 60, 70, and 80 degrees centigrade. I have chosen these steps because I have predicted that 40 C will be the optimum temperature for the beetroot and will indicate little red pigment diffusing out of the beetroot therefore showing if the cell membrane has kept its integrity. Referring to my prediction 30 C should show the temperature being to low for the proteins to work properly, and as a result show little permeability in the cell membrane. The other temperatures beyond 40C will show the beetroot being denatured, with more red pigment as the temperature increases, diffusing into the distilled water. I will change the temperature by heating up the glass beaker, which contains water acting as the water bath and the 3 boiling tubes containing distilled water, to the desired temperature using a Bunsen burner. I will use a thermometer to check what the temperature of the water is; making sure the temperature of the water bath is accurate. When the water has reached the specific temperature I will then remove the Bunson burner, add the three beetroot pieces to the boiling tubes then start the stopwatch. The factors that will have to stay constant throughout the experiment are:

- pH of the water in which the beetroot is in. This is because pH is a factor that can affect proteins in a similar way to what temperature can, it breaks the hydrogen bonds in the proteins if too alkaline or acidic, denaturing the

proteins. Therefore this has to stay constant to make sure it does not denature the proteins in anyway, consequently we will use distilled water, which has a constant pH of 7.

- Surface area of the beetroot, as a difference in surface area of the beetroot pieces will cause different beetroot pieces to have more membrane exposed to the water in the water bath causing easier diffusion of the red pigment out of the beetroot. Each beetroot piece will be 1 cm long, measured by a ruler, and have the same diameter using the same cork borer.
- Amount of water in the boiling tubes, 20 mm of distilled water will be in every boiling tube. This has to be the same in every boiling tube otherwise if there was more distilled water in one boiling tube compared to another one then that red pigment in the boiling tube with more distilled water in would be more diluted.
- Time in which the beetroot is placed in the water bath for. If the time that each beetroot piece is in the water bath for varied then more or less red pigment will be lost by the beetroot just because it has been in a certain temperature for longer not just because of the temperature itself, which is what we are measuring. Therefore we will keep each beetroot piece in the water bath for exactly 5 minutes.

The way in which I will collect this data is by using a colorimeter. This is a digital piece of equipment, which measures the optical density of a substance, which has been placed in a cuvette. This means how much light the substance absorbs and is measured in arbitrary units. It comprises of a lamp, filament that will be blue, a photosensitive element and a digital display. The substance is added from one of the boiling tubes into a cuvette, holding the cuvette on the 'grooved' side so that no finger marks will affect the reading, the cuvette is then placed into the colourimeter with the notch facing forwards. The light should therefore shine through the clear sides on the cuvette. As the colorimeter is measuring how much light the substance in the cuvette is absorbing it obvious that the more red pigment that is in the cuvette the more light it will absorb as the substance is of a higher density because there is more pigment. To make sure the colorimeter is set up the same for each cuvette we put into it for a reading I will put a cuvette filled with distilled water in, in first and set the colorimeter to zero.

When cutting up the pieces of beetroot in the first place the membrane of part of the beetroot will be broken resulting is red pigment being lost. This acts as excess pigment, which we do not want to measure and therefore we would need to get rid of it. The best way of doing this is the rinse each piece of beetroot that you have cut under distilled water, this will get rid of that excess pigment and therefore you will only measure the red pigment that has been lost in the boiling tubes. Keeping an eye on how long the beetroot is in the water bath for is also important, as already stated earlier in the plan. Making sure that you don't get any finger marks on the cuvettes when using the colourimeter is also important because if any marks get on the clear sides

of the cuvettes the cuvettes will be darker and therefore more light will be absorbed. Also making sure that you read the reading of the thermometer at eye level will make sure that you record the reading correctly.

I will do 3 replicates for each measurement i.e. there will be three boiling tubes in a specific water bath. Doing this will enable me to take and average of all replicates and therefore discounting any anomalous results.

Here is what my result table looks like:

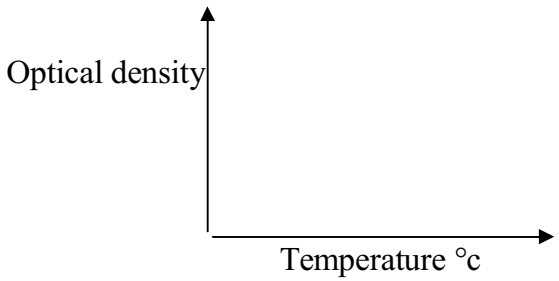
	Optical density		
Temperature of water bath (C)	1	2	3
27	0.10	0.05	0.12
42	0.19	0.17	0.19
51	0.24	0.25	0.25
62	0.84	0.55	0.73
68	0.97	1.10	1.05
83	1.37	1.49	1.43

Here is a results table for the averages in the experiment:

	Optical density			
Temperature of water bath (C)	1	2	3	average
27	0.1	0.05	0.12	0.09
42	0.19	0.17	0.19	0.183333
51	0.24	0.25	0.25	0.246667
62	0.84	0.55	0.73	0.706667
68	0.97	1.1	1.05	1.04
83	1.37	1.49	1.43	1.43

The graph for my results will look like this:

Graph to show to the effect of temperature
on the average amount of light the water absorbs



The real graphs are on the next page:

Conclusion

By referring to the graph for this investigation you can see that the curve goes from the bottom left to the top right starting off slowly and increasing fairly rapidly, indicating that as the temperature around the beetroot increases the amount of red pigment that diffuses out increases. Between temperatures 30 and 51C the amount of red pigment diffusing out of the beetroot is very low, showing that the proteins in the beetroots membrane have not been denatured. However, when the temperature of the water bath goes beyond 51 C the optical density increases rapidly meaning that more red pigment is diffusing out of the beetroot, therefore proteins in the beetroot membrane are being denatured.

This denaturing of the proteins in the beetroot cell membrane is a result of the higher temperatures causing an increase kinetic energy. As the kinetic energy increases due to an increase in temperature the protein's hydrogen bonds are broken in the proteins tertiary structure, which are there to keep the proteins shape. This denaturing enables the red pigment to easily diffuse out of the beetroot, so therefore as more proteins in the cell membrane denature more red pigment can diffuse out of the cell membrane into the surrounding water.

Evaluation

There are a few different looking points on the graphs. For instance if you look at 62^{o}C the 3 results are a long way apart and when it gets to 68^{o}C the results have all switched round with run 1 going from highest to lowest optical density. Run 3 goes from lowest to highest and the points are still quite a distance away from each other.

There are many different reasons for why these anomalies may have occurred. One of these could have been that when the beetroot was cut into pieces to be then placed in the boiling tubes, the cell membrane of the beetroot may have been broken resulting in red pigment leaking out of it. This would have caused excess red pigment on the beetroot. We tried to wash this excess pigment off the beetroot using distilled water, but you can't say that you washed the same amount of red pigment off each beetroot piece; therefore more excess red pigment may have been on some pieces than others. This excess pigment can easily get into the water in the boiling tube and as a result cause a higher reading on the colorimeter, and make our results less reliable. You have to cut the beetroot anyway so there is always going to be excess pigment on the beetroot but devising a method to reduce or control this excess pigment would have to be used to make the results become more accurate and reliable. Another reason for the anomalies could have been that when we touched the cuvettes we may have put a finger mark on the clear side by accident thus causing a higher reading on the colourimeter as more

light will be absorbed. Maybe using tweezers to pick up the cuvettes would have reduced the chances of getting fingerprints on the cuvettes making our results become more reliable. A reason for the anomalous results could also be down to the fact that we used a bunson burner to gain the temperature for the water baths. A bunson burner cannot be very precise therefore the water baths may not have been to their specific temperatures as it is easy for the water bath to get warmer. If we had used electronic equipment to measure the temperature of the water then it would have made the results a lot more accurate and reliable as it would measure the temperature of the water bath a lot more accurately. Also another reason for the anomalies could be the fact that the beetroot pieces were cut out of different parts of the beetroot. This could mean that different parts of the beetroot could have more red pigment than others, resulting in those beetroot pieces with more red pigment diffusing out more red pigment when heated. Making sure each beetroot piece was cut out of the same place in the beetroot would cause all the beetroot pieces to hopefully contain the same amount of red pigment to start off with.

All these things that may have caused the couple of anomalous results may cause my conclusion to change, but the significance of the anomalies is not very high therefore I still stand by my conclusion.

Overall I think that the results have been fairly reliable, shown by the results from working out the standard deviation of each measurement, which show a small number therefore high reliability. Also the results do back up my prediction strongly; therefore the results must have been accurate and reliable.