

*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

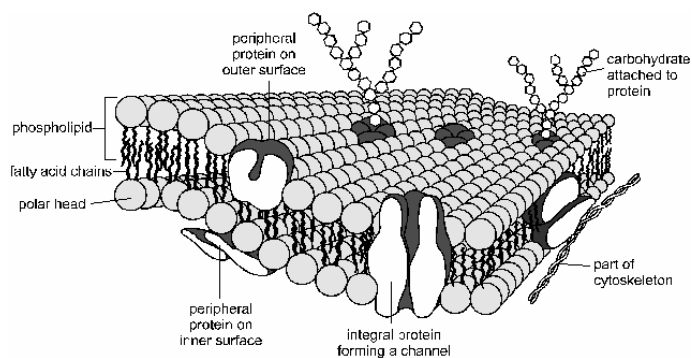
Hypothesis

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.

### Prediction

Referring to background knowledge, I can predict that an increase in temperature beyond 40 C will increase the permeability of the beetroot cell membrane. Therefore I predict that more red dye should be present outside the beetroot at a temperature of 70 C than there would be at a temperature of say 50 C.

### Equipment list and why using it:

<b>NAME OF EQUIPMENT</b>	<b>REASONS FOR EQUIPMENT</b>
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 bunsen burner placed under it
Gauge	Is placed on tripod. This is what the glass beaker is put on
Bunsen 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 placed 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

Diagram of equipment

## Plan

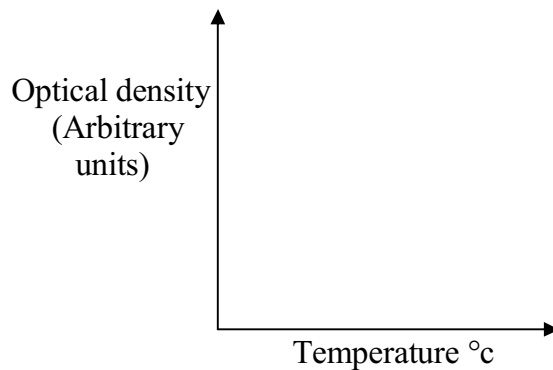
The variable that will be changed in this experiment will be the temperature of each water bath. The steps of each measurement will be 30, 40, 50, 60, 70, 80, 90 and 100 degrees centigrade. I have chosen these steps because I have predicted that 40 C will be the optimum temperature for the beetroot and 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 too 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 Bunsen 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 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



Then I will work out the standard deviation and mark it on the graph. This enables me to analyse the reliability of my evidence. Standard deviation tells us how far a set of results deviates from the mean. The higher it's standard deviation the more unreliable it is.

The formula for working out standard deviation is:

$$SD = \sqrt{\frac{\sum (x-\bar{x})^2}{n}}$$

$\Sigma$  = sum of     $x$  = result     $\bar{x}$  = mean     $n$  = number of replicates

$\sqrt{\quad}$  = Square root

## 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 60°C the amount of red pigment diffusing out of the beetroot is very low, showing that the proteins in the beetroot's membrane have not been denatured. However, when the temperature of the water bath goes beyond 60°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 in kinetic energy. As the kinetic energy increases due to an increase in temperature the protein's hydrogen bonds are broken in the protein's tertiary structure, which are there to keep the protein's 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 anomalies in my results and you can see these by referring to the graph. Firstly the first anomaly is for 30°C; this is because the overall trend for the graph shows that as the temperature increases so does the permeability of the beetroot. However, the permeability of the beetroot at 30°C is actually more than at 40°C. Therefore, more red pigment was allowed to diffuse out of the beetroot at a temperature of 30°C than at a temperature of 40°C. The other two anomalies occurred at the temperatures of 70 and 80°C. By referring to the standard deviation of 70°C you can see that the reliability of the result is much lower than any of the other results obtained. Indicating that something was done wrong or differently when doing this measurement.

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 bunsen burner to gain the temperature for the water baths. A bunsen 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.