An experiment to test the effect of different temperatures on the permeability of cell membrane.

Aim

In this experiment, I am trying to find out how, why, and by how much does the difference in temperature affects the permeability of cell membrane of plant cells.

Prediction

Having done a pilot experiment and by using the scientific knowledge gathered, some predictions can be made. The permeability of cell membrane should increase as the temperature increases, and this is shown by the decrease in the percentage transmission of light on the colorimeter.
As guidance, the results of the pilot experiment could be used:

Theory of prediction

There are five possible ways that the dye could leak out of the cell in normal condition without the aiding of heat, and these are simple diffusion through the lipid bi-layer, facilitated diffusion by protein carriers, and facilitated diffusion via ion channels, active transport, and exocytosis.

- The dye would have no trouble passing through the cell wall by diffusion as it is fully permeable, but the tonoplast and the cell membrane are only partially permeable, and would only allow non-polar substances to pass through. As anthocyanin is a polar substance, it cannot leave the cell by diffusion. Active transport is the next possible way for the dye to leak out, but this process requires energy, and so it is not likely to occur. Exocytosis is possible through the cell membrane, but it is a process involving bulk transport, and this is not likely to take place through the tonoplast.

- After looking at all the possible ways in which the dye could leak out of the cell, it can be certain that the dye would not leak out of the cell in normal conditions, and this means that the cell membrane is quite impermeable at room temperature.
- By increasing the temperature, it is predicted that the cell membrane would become more permeable, and this is due to several reasons:
- The phospholipids bi-layer of the cell membrane is similar to a fluid layer as the phospholipids can move around and change places with each other, and when this happens, sometimes spaces appear between the phospholipids, and the anthocyanin particles could pass through. Although this is possible, but these spaces rarely appear as the movement of phospholipids are limited in normal conditions. But if the temperature increases, the lipids would have more kinetic energy, they would move more quickly and violently, resulting in more spaces to occur, and thus the cell membrane would become more permeable.

- The cell membrane is often seen as a fluid mosaic model, and it is mosaic due to all the different proteins and cholesterol among the phospholipids. In normal condition the dye would not be able to pass through this complex membrane layer, but when temperature rises and gets above 40°C, the transport protein, receptor protein, glycoprotein and all other carrier proteins would start to denature. When the proteins denature, they would change shape or rupture, and no longer fit in the membrane, allowing spaces for leakage to appear and the membrane becomes more permeable. The anthocyanin particles can pass through these gaps created, and this shows that the membrane gets more permeable with increasing temperature.
- Although the actual cell membrane is much more complex than the fluid mosaic model, the basic theory would still works, and the main reason for the membrane to get more permeable with increasing temperature is that the protein would get denatured. This means that temperature and permeability are not in direct proportion, but the ratio would change when it gets above 40°C when permeability increase more per amount of temperature increased.

Justification of apparatus

- The test tubes are used to contain the beetroot cells in the fixed amount of water so that the anthocyanin dye only go into the water in the test tube and not into the

water bath. They can fit the beetroot cells in and have them totally emerged in the amount of water used.
- Thermometers are used to check the temperature of the water baths to ensure that they are constant and correct. These thermometers are accurate to the nearest °C.
- A digital colorimeter is used to record the % transmission of light through the water that contained the beetroot cells. This is used instead of an analogue one as digital would be more accurate than the human eye, and produce the required level of accuracy. This digital colorimeter is accurate to the nearest 1% while the analogue one is accurate to the nearest 5%.
- The cuvettes are used to hold the water that was in the test tubes when the water needs to be read by the colorimeter. Cuvettes have unique and accurate shape, size and volume, and their sides are very transparent, reducing the amount of light being absorbed by the cuvettes to be a minimum and have a good accuracy.
- Scalpel is used to cut the ends of the beetroot so that they are even and that each pieces are the same. The scalpel is very sharp, and so can cut the beetroot cells to be the same shape, size and have the same surface area every time, giving a high level of accuracy.
- The timer is to make sure each test tube is placed in the hot water for the same amount of time and a fixed amount of time. The timer is definitely accurate enough as the accuracy is limited to the reaction speed of the human hand. It is also more accurate than having the time counted by a human.
- The white tile is where we cut the beetroot so that we do not contaminate them with the things on the bench, and also to keep the bench clean from dye.
- The syringe is used to measure the exact same amount of water in each test tube. A syringe is used as it is much more accurate than the measuring cylinder in the level of measuring 5ml of water, and that they are measured to the nearest 0.2ml, while the measuring cylinder is accurate to the nearest 2ml, showing that the syringe is 10 times more accurate.
- The cork borer is used to cut circular tubes of beetroot out so that each piece has the same volume, shape and surface area as any other piece. This is chosen instead of other cutting instruments as most of the sides are already of fixed area, and do not need further measuring.

Variables
- The input variable of this experiment is the temperature of the water baths, and this will be between 20°C and 80°C.
- The output variable is the % transmission of light through the water dyed by different amount of anthocyanin.

The fixed variables of this experiment are as follows:
- Length of time the test tubes are in the water bath.
- Surface area and volume of beetroot in each test tube.
- Each piece of beetroot is cut from the same beetroot. Amount of water added to each test tube.
- Same colorimeter with the settings unchanged is used.

Ranges
The input variables would be between 20°C and 80°C, and results would be taken in intervals of 10°C, resulting in input temperatures of 20°C, 30°C, 40°C, 50°C, 60°C, 70°C, and 80°C.
The output variables are in terms of a percentage, having a range from 0% to 100%. Each reading of the experiment would be carried out 4 times, which means that there will be 3 repeats taken for each reading.

Method
- Firstly the beetroot must be cut using the cork borer on the white tile, the cork borer need to be pushed into and through the beetroot, and then it could be pulled out. By doing this, a cylinder of beetroot could be collected.
- The above process is done a few times on the beetroot until enough cylinders of beetroot are collected.

- Now there are cylinders of beetroot with uniform diameter, they are of different lengths, and so their ends need to be cut using the scalpel, making each piece to be exactly 3cm long.
- The pieces of beetroot could now be washed by placing all of them under cold running water for few hours until all the anthocyanin on the surface are washed off.
- One piece of cylindrical beetroot is now placed in each of four dry test tubes.
- 5ml of distilled water is added to each test tube, using the syringe.
- 7 water baths of different temperatures are now being set up using the electrical water baths, having temperatures of 20°C, 30°C, 40°C, 50°C, 60°C, 70°C, and 80°C.
- When the water baths are at the correct temperature, checked by using the thermometer, each of the 4 test tubes could be placed into a different water baths for 20 minutes, counted using the timer.
- At the end of the 20 minutes, the test tubes are taken out of the water baths immediately, and the dyed water in each would now be poured into a different curvette, prepared to be placed into the colorimeter.
- One by one the 4 cuvettes with the dyed water can be placed into the colorimeter and the % transmission of light passing through the dyed water can then be recorded.

Risk assessment
Normal lab safety precautions must always be taken, but apart from those, there are few other precautions that are needed for this experiment. The water bath can get up to 80°C, and might burn the skin, and so caution is needed when putting the test tubes in. When cutting the beetroot with the scalpel, safety spectacles are needed to be worn as the blade could break off and damage the eye.

When the temperature range was between 20°C and 60°C, the drop in light transmission, from 95% at 20°C to about 80% at 60°C, was in proportion to the increase in temperature, creating a steady group of results, lying on a straight line on the graph. The calculated gradient of this part of the graph is -0.5%°C^{-1}, meaning that for every °C increased the percentage light transmission would drop by 0.5%. This was most likely caused by the increased in the fluidity of the phospholipid bi-layer. When temperature increased, the particles in the membrane got more kinetic energy and so moved more at greater magnitude; this created gaps more often than usual in the plasma membrane to allow the anthocyanin leaking out of the plant cells. At the same time, when temperature increased, the dye got more kinetic energy as well, leading to an increased rate of diffusion. When the anthocyanin leaked out of the beetroot cells, it went into the water, which was tested in the experiment, and this dye, when passed through the colorimeter, absorbed some of the light, therefore reduced the % light transmission. This part of the graph has a moderately gently gradient compare to other parts, showing there is another reason in the reduction of % light transmission at other temperature ranges.

When the temperature range was between 60°C and 80°C, the drop in light transmission, from 80% at 60ºC to about 20% at 80°C was in proportion to the increase in temperature. The calculated gradient of this part of the graph is -3.0%°C^{-1}, meaning for every °C increased the percentage light transmission would drop by 3.0%, which is of a much greater magnitude in gradient. The graph at this part has a much steeper gradient than the earlier part of the graph, this means that for the same amount of change in temperature, there is a greater change in the % light transmission that before. This was because apart from the increase in fluidity of the phospholipid bi-layer, there was another factor affecting the permeability of the plasma membrane. Apart from the phospholipids bi-layer, the plasma membrane also contains many protein molecules useful for cellular processes. At this high temperature, the proteins on the plasma membrane denatured. The different bonds would break in order of weakness, and so the hydrogen bonds would break first, then the hydrophobic interaction, then the ionic and disulphide bonds, and finally the peptide bonds. First the quaternary structure broke down when the disulphide bonds, ionic bonds, hydrophobic interaction, and hydrogen bonds were broken. As the tertiary structure has the same bonds as the quaternary structure, these bonds broke as well. Hydrogen bonds in the secondary structure and the peptide bonds in the primary structure were also broken at slightly higher temperatures. In the above ways, the plasma membrane broke down, which created gaps where the proteins were before. Due to this, the red dye,

anthocyanin, was able to leak out of the plasma membrane, getting out of the cells and into the water and therefore reduced the % light transmission of blue light through the water as most of the light got absorbed.

After doing the experiment and having done some calculation using the results, I can conclude that my prediction was correct. Increasing temperature would reduce the % light transmission through the dyed water. Although I predicted that the relationship shown on the graph would be linear at low temperatures, and that the gradient would be greater at higher temperatures, I thought the temperature where the gradient changed would have been near 40°C; instead it was at 60°C. This was possibly because that the length of time the beetroot cells stayed in the water baths was only 5 minutes, and the plasma membrane did not have enough time to heat up to the same temperature as the water baths.

As we have found out from the experiment, increasing the temperature would reduce the amount of light passing through the dyed water, and this could be related back to the aim of the experiment, which is how the change in temperature have on the permeability of the plasma membrane. The reason that the % light transmission through the dyed water reduces with the increase in temperature is because as temperature increases, more anthocyanin leak out of the beetroot cells via the plasma membrane, and this is possible only because the permeability of the plasma membrane increases.

My conclusion is that an increase in temperature would increase the permeability of the plasma membrane of plant cells, due to the increase in fluidity of the phospholipids bi-layer, and the denaturing of protein molecules at increasing temperature.

Evaluating evidence and procedures - Was the method suited to the aim of the experiment?
Evaluating the conclusion of the whole experiment, I think that this experiment using beetroot cells gives a good representation of how the permeability of the plasma membrane of a plant cell get affected by different external temperatures, however this does not represent the effects of temperature on the plasma membrane of other type of cells, for example an animal cell. Neither does it give a good representation of the effect of temperature on other types of membrane in a cell.

Sources of errors
The method used for this experiment had many sources of errors, leading to the anomalous results obtained. The main problem in the experiment was to keep the variables constant throughout the whole experiment. Firstly the surface area of the beetroot pieces were quite inconsistent; although each pieces of beetroots were approximately the same size, their surface area varied due to their small size, also that they were pre-cut, and so this factor was not controlled totally by me. There was another problem with having the beetroots pre-cut, as it was impossible to tell which pieces were cut from the same beetroot; and as the concentration of dye varied greatly between different beetroots, this was a possible source of error. Keeping the water baths at the desired temperature for a period of time was not easy, as heat was constantly lost to the environment, and this was another source of error.

Accuracy of results
The accuracy of most of the apparatus used was at a sufficient level as the result could only be as accurate as the method of the experiment, and it was not necessary to have a level of accuracy higher that the errors produced. For example the digital colorimeter was accurate to the nearest percent, while the standard deviation was about 5%.

Anomalous results + Reliability of results
Out of all the readings obtained, 4 out of 93 readings were anomalous as they were more than two standard deviation away from the mean values; these were 89% for 20°C, 97% for 40°C, 73% for 50°C, and 31% for 80°C. 4 anomalous out of 93 readings taken was quite a small amount of error, and this showed that the results, and thus the experiment, were reliable.
Overall this experiment was quite successful as most of the results collected and used followed a similar pattern. Although on the graph some of the error bars overlapped each other, a clear pattern could still be seen.

Relative magnitudes of error sources

- The greatest error on any measurements measured was the temperature of the water bath. As its temperature was constantly loosing, it started off with having the temperature 2°C higher than the recorded value, and at the end the temperature was 2 °C lower than recorded value, making the error on the temperature to be plus or minus 2°C.
- The next variable with the greatest source of error is the surface area of the beetroot pieces. Although each piece of beetroot has a similar surface area, but at such a small size, a small difference would be a great error, and I think that the error on the surface area of beetroot pieces is about 5%.
- All the other variables have very little amount of errors, for example the amount of water added to each test tube, and the length of time the test tubes are in the water bath would both have an error of less than 1%.
- Although the colorimeter is only accurate to the nearest one % of light transmission, it should have a minimal amount of error as it is digital.

Improvements

To improve on the experimental procedures, several things could be done. The reliability of the experiment could be improved by trying to use the beetroot pieces cut out from the same beetroot, or at least from the same type of beetroot, instead of using beetroot of different sizes. For most experiments, doing more repeats would increase the reliability, but this would not apply to this experiment as over 90 readings were taken and there were at least 10 repeats done for each set of conditions. However doing the same experiment at more temperature conditions would increase the reliability as a smoother trend could be seen. To minimise sources of error, the first way to improve the experiment will be to cut the beetroot personally, instead of having them pre-cut, and also that a more accurate template could be used, which would keep a consistent surface area for different pieces of beetroot. Another way is to use proper electronic water baths to maintain the exact temperature for each of the different temperatures; although this is not practical as it would take time, however errors would be reduced greatly if this could be done.

How safe is the conclusion

Although there are noticeable amount of errors in the experiment, shown by the size of the error bars on the graph, the conclusion is still very valid and reliable. This is because although the possible amount of error is great, the line on the graph being not erratic shows that these possible errors did not occur in this experiment. Even if we try to plot the worst line on the graph within the range of the error bars, a line leading to the same conclusion would still appear.