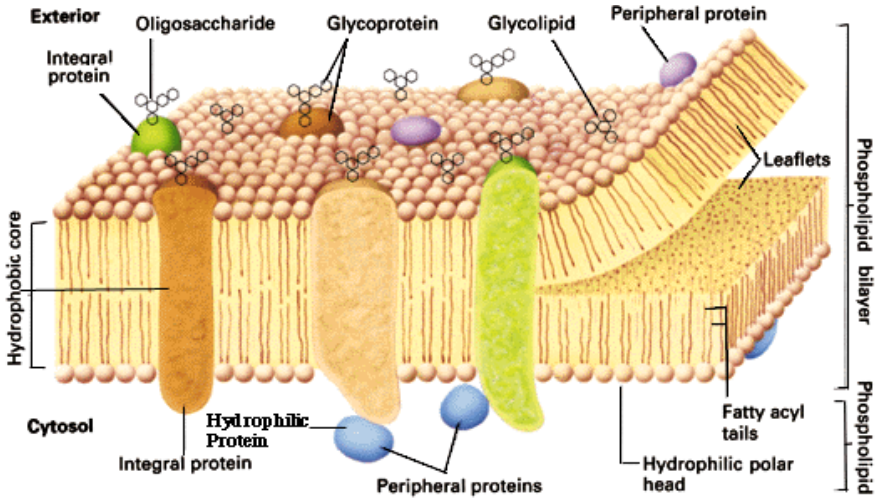
# How does temperature affect the permeability of a cell membrane in a beetroot.

**Introduction:**

The purpose of the cell membrane is to control the transport of substances moving into and out of the cell. It is made of a phospholipid bilayer that has proteins floating in it. The proteins span the membrane and touch both the inside and outside of the cell. The cell membrane is between 6-8 nanometers (nm) thick and made up of many different molecules. These are shown in the diagram below.



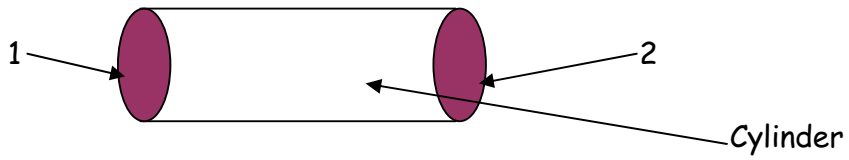

The phospholipid bilayer is the basic structure of the membrane. The fatty acid tails are non-polar; this means it is difficult for polar molecules or ions to pass through them as they act as a barrier to most water-soluble substances. The molecules move in and out of the cell through diffusion, osmosis and active transport. Diffusion is the net movement of molecules from a high concentration to a low concentration.

In the cells of a beetroot plant a substance called anthocyanin is contained in the plasma membrane. The anthocyanin gives the beetroot its purple appearance. If the cell membrane becomes damaged then the anthocyanin "bleeds" from the cell like a dye. This charact eristic of beetroot will be used in this experiment. The experiment is to test the

effects of temperature on the cell membrane. The molecules in the membrane move using kinetic energy. As the temperature increases the molecules gain more kinetic energy and diffuse faster. Temperature also causes the lipids of the membrane to be more fluid. It also causes the proteins to denature. However as the proteins denature and the lipids become liquid like, bigger gaps emerge so larger molecules can diffuse more freely. As the proteins denature the protein channels will increase in size so that more hydrophilic molecules can diffuse through in a shorter space of time.

**Surface area of beetroot:**

The surface area of the beetroot is to be the same for each of the segments. The diagram below shows the shape of the segments:




Surface area is worked out using the following formula:

Area of end 1 + Area of end 2 + area of sides (cylinder shape)

$$\pi r^2+\pi r^2+(2\pi r)h$$

Height = 1cm

Radius = 0.5

$$\pi 0.5^2+\pi 0.5^2+(2\pi 0.5)\ 1$$

$$= 2(\pi 0.5^2)+2(2\pi 0.5)$$

$$= 1.570796327\ldots+6.283185307\ldots$$

$$=7.853981643\ldots$$

$$=\mathbf{7.85cm^2}\ (3\ \text{significant figures})$$

**Variables:**

| Variable | Why it must be controlled? | How will it be controlled? |
|---|---|---|
| Size of beetroot | This affects its surface area, if more is exposed on one then when the membranes are being denatured more dye will be released, as there are more exposed surfaces. | I will be controlling this by using a cork borer so that all the cylinders will be perfectly round as well as measuring the length accurately with a ruler. |
| Amount of water | The amount of water the beetroot is placed in will affect the end result of % of light transmitted. | I will be place the beetroot in 10ml of water. I have decided on this amount from my preliminary work in which I used 25ml which I found to be considerably too much water. I will be using the syringe to measure the water. |
| Beetroot | The beetroot must be controlled as different beetroots may have different anthocyanin levels. Which would affect the colour that the water turned. | I will be using the same beetroot for the entire experiment. This will hopefully reduce the anomalous results caused by different beetroots. |
| Time | The time that the beetroot is in the water will affect the amount of pigment released. If one piece is left in for 2 minutes and another for 15 this may affect the result. | I will be leaving each piece of beetroot in the water for 10 minutes I discovered this to be an effective time from my preliminary work. |

| | | |
|---|---|---|
| Treatment of beetroot | The way that the beetroot is treated after being removed from the cork borer will affect the pigment released from the beetroot. | I will aim to control the way that the beetroot is treated by cutting it into 1cm long cylinders then washing them and rolling them dry on paper towelling. Before placing them in the water. |
| Temperature | Temperature is the variable that we will be changing. In the hope of denaturing the cell membrane. | This will be controlled using water baths set at certain temperatures, observed constantly, using the bunsen burner to maintain a constant heat. |
| Surface area of beetroot. | The surface area of the beetroot must be controlled, as this will affect how much beetroot is exposed to the water. A higher surface area will allow a higher % of pigment out of the cells, as more can be denatured at a quicker rate as more will be exposed. | The surface area will be controlled using the cork borer to make sure a perfect cylinder is cut, and a scalpel is used to cut the cylinders 1cm long measured with a ruler. This will give them the same surface area. |

**Apparatus:**

| Apparatus | Used for |
|---|---|
| Fresh beetroot | The pigment anthocyanin. |
| Scalpel | Cutting of the beetroot. Into 1cm long cylinders. |
| White tile | Cutting the beetroot on. |
| 15 test tubes | Filling with water and placing beetroot in. |
| 2 water baths | To heat the water in the test tubes. |

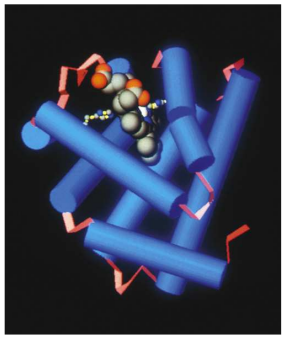
| | |
|---|---|
| Bunsen burner | Heat the water in beaker. |
| Tripod | Hold the gauze and beaker. |
| Gauze | Place on top of tripod, to stand the beaker on. |
| 250ml beaker | To make water bath. 150 ml of water placed in and heated. |
| Pipette | Remove liquid from test tube. 3mls to place in cuvette |
| Syringe | Measure water out in test tube. 10mls of water |
| Ruler | Measuring of 1cm for beetroot cylinders. |
| Cork borer | Removing beetroot in cylinders the same size. |
| Heat proof mat | Rest tripod and bunsen burner on for water bath. |
| Distilled water | To heat the beetroot in. |
| Stop clock | To measure time. 10 minutes from when beetroot in heated test tube. |
| Safety goggles | To protect my eyes. |
| Results table and pen | To record my results. |
| Cuvette | To place the solution in to put in the colorimeter. |
| Colorimeter / Blue filter | To test the light % through the solution. In the colorimeter I would use a blue filter, as it is the closest to the pigment anthocyanin found in the beetroot. This will test the light able to get through the pigment. The blue filter allows colours other than blue to through the pigment testing how much blue light passes through the solution, indicates the concentration of pigment. |

**Risk assessment:**

During the experiment I must be careful when handling the hot water as it may scald. When using a bunsen burner I must be careful not to burn myself on the flame and leave it on the safety flame or off when not using it.
I must push the cork borer into the beetroot away from the body, holding the beetroot at an angle where the cork borer will not touch.
When using the scalpel to cut the beetroot I must be careful to avoid cutting my fingers or hands.
Safety goggles should be worn at all times. To pro tect my eyes from flying objects or splashes.
I shall also tie back my hair and not wear lose clothing which might catch on fire.

**Prediction:**

I expect the concentration of anthocyanin to increase as the temperature of the water is increased. This is because an increase in temperature will damage and denature the proteins in the plasma membrane this will cause the substances inside the membrane to be released and leak into the surrounding liquid. The proteins in the cell membrane are at a tertiary level, which means that there are globular in shape as, in the picture below.



When the proteins are heated the hydrogen bonds (in red) are broken. This allows the anthocyanin and other substances from inside the membrane to escape. The higher the temperature the quicker the proteins will denature so the more anthocyanin will be able to escape.

**Method:**

1. Collect equipment
2. Set up the bunsen burner, tripod, gauze and beaker with 150ml of water.
3. Place 3 test tubes filled with 10ml of water in the water baths to heat, using the thermometer to measure the heat of the water. I am using 3 test tubes at once so the experiment will be quicker as I can do three results at once.
4. Using cork borer remove a cylinder out of the beetroot.
5. Place on white tile and cut into 1cm long cylinders.
6. Rinse the beetroot and roll dry on paper towel.
7. Place the cylinders in the test tubes, one per test tube.
8. Leave for 10 minutes
9. After 10 minutes, remove the beetroot from the test tube. And shake the mixture. This will distribute the colour equally.
10. Remove 3ml of the liquid using a pipette and place in cuvette.
11. Place the cuvette in the colorimeter and test the light transmission. Using a blue filter.
12. Record the result in table.
13. Repeat steps 3-12 at each temperature.

Results to show how temperature affects the permeability of a cell membrane in a beetroot.
All my results are given to 1 decimal place.

| Temperature in °C | Transmission % Experiment 1 | Transmission % Experiment 2 | Transmission % Experiment 3 | Transmission % Average | Pigment % |
|---|---|---|---|---|---|
| 30 | 98.4 | 99.6 | 100.0 | 99.3 | 0.1 |
| 40 | 86.2 | 90.3 | 76.1 | 84.2 | 1.6 |
| 50 | 85.2 | 77.5 | 70.1 | 77.6 | 2.1 |
| 60 | 39.6 | 42.5 | 44.8 | 42.3 | 9.5 |
| 70 | 22.3 | 39.3 | 25.3 | 28.9 | 15 |

**Analysis:**

As you can see from this table I have a few anomalous results. I have highlighted the inconsistent results in red. These are the results that are the extremes from the averages. The reasons for these extremes will be explained later in my evaluation.

My secondary results from the lab tech. are as follows these are to two significant figures:

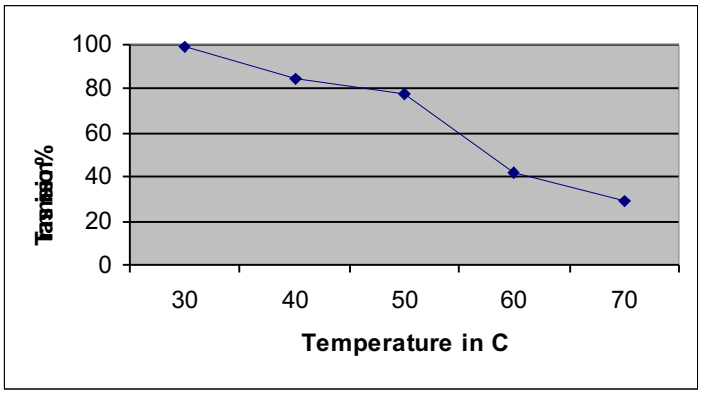
| Temperature in °C | Transmission % |
|---|---|
| 10 | 86 |
| 17 | 78 |
| 19 | 70 |
| 35 | 57 |
| 40 | 48 |
| 50 | 28 |
| 70 | 4 |

**Graphs:**

On graph 1 I have plotted my results.
On graph 2 I have plotted my secondary results.
On graph 3 I have plotted a calibration curve.
On graph 4 I have plotted pigment and temperature.

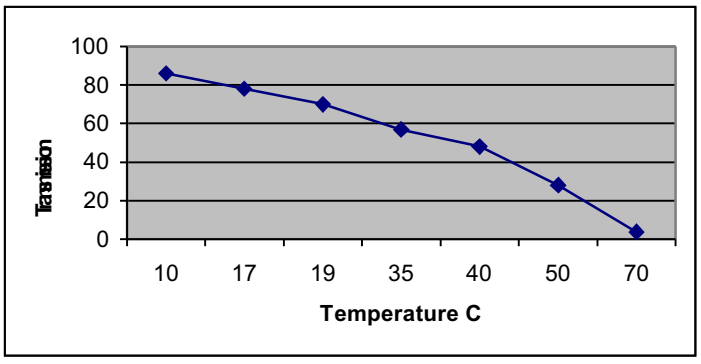
The experiment has confirmed my prediction that the higher the temperature the more pigment will be released. As when the heat from the water denatured the proteins in the cell membrane the hydrogen bonds are broken changing the shape of the protein allowing the anthocyanin to diffuse into the surrounding water. This caused the colour of the water to darken as the pigment was released. As the temperature increased more anthocyanin could be released in the 10 minutes allowed to release the pigment into the water.

A copy of the graph using my results is below.

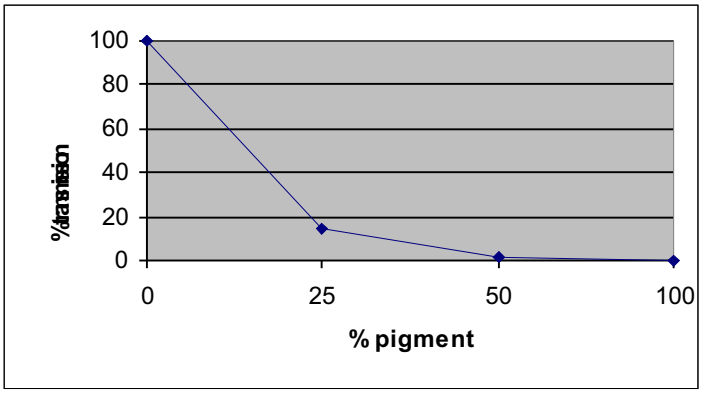



As you can see from this graph my results give a definite negative correlation this means that the percentage of transmission becomes lower as the temperature increases. This backwards "S" shape means that at a certain temperature (between 50 and 60) there is a sharp increase in the speed at which the proteins denature. This is because between the temperatures of 30 and 40 °C the diffusion increases as the beetroot reaches it's optimum diffusion levels. However at 45 °C the proteins in the membranes denature causing the holes in the membrane to expand. This allows more pigment out. So between 50 and 60 °C the membrane becomes more fluid with more gaps in the membrane allowing more pigment to escape. While still diffusing at a fast rate. Between 60 and 70 °C the membrane is still being denatured but not with such a sharp increase in speed. The pigment is still diffusing rapidly and there are more spaces in the membrane.

My secondary set of results came from the schools lab. technicians experiment. These have a different range than my results as they go up in unequal steps. These results start at 17°C but range up to 70 °C. As you can see from graph 2 there is one point in which a s teep decrease is seen. Many of the results in this are very close together.



Calibration is the process of determining the absolute values. The calibration curve is to see the proportion of pigment that has been released from beetroot. This is used so that we can relate the figures to known standards. This calibration curve is showing the lower the % pigment the higher the transmission.




**Evaluation:**

My experiment gave me good results over all as they gave me a set of results that confirmed my prediction. There were however a few anomalous results. This could have been caused by inaccuracies in my method.

| Inaccuracy | How inaccuracy was caused. | Improvement | Justification | Rank |
|---|---|---|---|---|
| Temperature | When heating the water bath with a bunsen burner in it very difficult to accurately keep to the specified temperatures. This means that the temperatures could have fluctuated a few degrees. | To improve this I would use electric water baths for all the experiments. | By using electric water baths for all of the experiments the water temperature that the beetroot was in would be more constant. This would give more accurate results. | 1 |

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|---|---|---|---|---|
| Surface area of beetroot | The surface area could have differed, as the angle that the cut was made to make the cylinders the same size could have been marginally different. This could cause the surface area to be different. Also the length although measured using a ruler could be slightly out by up to a couple of mm. | To improve this I would use a more accurate cutting instrument. | By using a more accurate cutting device this would mean that the margin of error would be reduced. So the surface area would be more constant. | 4 |
| pH of water | Although intending to use distilled water the whole way through the experiment I was forced to use tap water for some temperatures. This is slightly acidic. This could have had an affect on the membrane of the cell. This could have caused it to start to denature more rapidly. Allowing pigment out of the cell. | To improve this I would use distilled water throughout the experiment. | By using distilled water throughout the experiment this would mean it was the temperature denaturing the cell membrane and could not be acidic water. | 6 |

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| Beetroot | In the experiment it was necessary to use more than one beetroot. This was due to the beetroot being quite small so I quickly ran out of places to use the cork borer, meaning I had to change beetroots. This meant that the different beetroots could have had different pigment concentrations. | To improve this I would try to use the same beetroot throughout the experiment. | By using the same beetroot throughout the experiment it keeps the concentration of pigment more constant, and means that the cells have been exposed to the same things so they will react in the same way. | 5 |
| Area of beetroot segment taken from | The area that the segment of beetroot is taken from will determine the concentration of the pigment. As there is a higher concentration of pigment in the centre of the beetroot than on the outer layers. This means that if you remove the segment from the inner of the beetroot when the proteins denature there will be more pigment to be released. | To improve this I would remove segments of beetroot from the same area. | By removing segments from the same area of the beetroot, I will be removing cells with similar concentrations of anthocyanin. | 3 |

| | | | | |
|---|---|---|---|---|
| Time | Inaccuracies in time that the beetroot was in water were caused by not being able to remove them all after exactly 10 minutes or place all in at the exact same second and start the stop clock. This slight increase in time for some of the beetroot sections will have meant that were exposed to the heat for longer than the 10 minutes this means that the beetroot has had more time to diffuse the pigment, this would have caused a decrease in the transmission of light. | To improve this experiment I would use only one test tube at a time heating one test tube for 10 minutes then removing it and doing the next. | By using only one test tube at a time this will allow me to concentrate on it and remove it after exactly 10 minutes. | 2 |