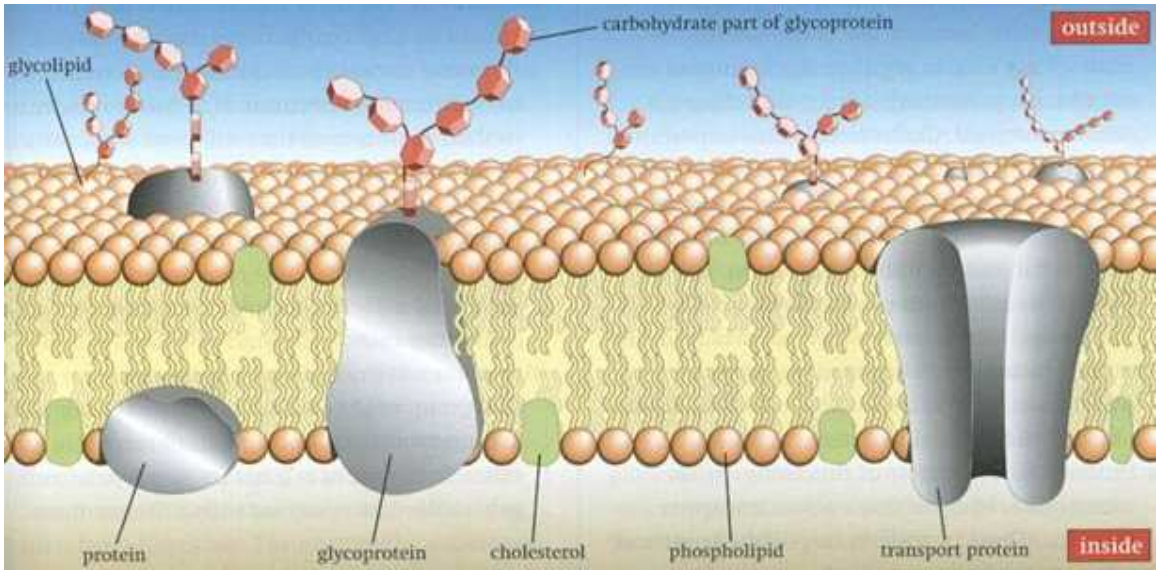
Beetroot Experiment

Beetroot cells are coloured red because they contain a red dye called anthocyanin. In beetroot cells there red anthocyanin pigment occurs in the vacuoles. A membrane called the tonoplast surrounds each vacuole. The cytoplasm and vacuole is surrounded by the plasma membrane.

The function of a cell membrane is to control the movement of material into and out of the cell. The tonoplast does the same job for the vacuole.



The two main components of any membrane are proteins and fats. The anthocyanin can only be released and leak out of the cell if the membrane are broken or damaged. The anthocyanin diffuses out of cells.
Diffusion – gases move about at random and will move about at random and will move from where they are in high concentration to where they are in lower concentration.
Temperature has an affect on membranes. If the temperature is too hot the membrane changes shape, and makes holes it is damaged and the anthocyanin leaks out, the more damage the more leakage. If the temperature is too hot it denatures the proteins and enzymes.
The higher the temperature the more kinetic energy, the energy is given to the atoms and they begin to vibrate. They vibrate faster and faster and bang off each other, causing the structure to break. This allows the anthocyanin to leak out.

I predict that the higher the temperature the more damage done to the beetroot membranes. Therefore more anthocyanin will diffuse out of the beetroot cells therefore less transmission of light.

Factors we are planning to keep constant are:

- Surface area of beetroot by using cork borer, measuring the diameter and length and keeping them the same.
- Time spent in water bath. 1minute timed using stop watch.
- Time spent in test-tube of distilled water. 20minutes timed using stop watch.
- Volume of distilled water. 10mm measured using cylinder.
- Use same colorimeter each time.
- Use same filter in colorimeter.
- Calibrated using same blank.

Factors that will change:

- Temperature. Range, 30, 40, 50, 60, 70, 80 ˚

- This gives a good range of temperatures for me to plot my graph from.
- Repeat each temperature 3 times to increase reliability of results. Allow I would have liked to do more to make my results more reliable.
- As we had limited and equipment we will use results from other members of the class to increase the reliability of the results.

I will measure the percentage transmission of light using a colorimeter.
Distilled water gives 100% transmission of light, the more dye there is in the water the lower the percentage of transmission of light. The more dye there is in the water the darker and redder it will be therefore giving a lower % of transmission of light.

Temperature (℃)		% transmission of light	
	1st	2nd	3rd
80			
70			
60			
50			
40			
30			

I will plot a graph with the average of the class results for the percentage of the transmission of light at each temperature.

Safety is a high priority you must be careful as there are many hazards in the laboratory.

- When using the Bunsen, make sure the gas is off when you are not using the Bunsen.
- Do not pass body parts or clothing over the flame.
- Be careful with the hot water, as it can burn skin.
- When cutting be careful to keep your fingers away from the blade.
- When using the forceps be careful to hold the cork borers tight and away from your body.
- When walking with the knife point it downwards or away from you and other pupils.
- As we are using glass beakers and test-tubes there are obvious hazards. Be careful when carrying them so that you don't trip and keep them in the middle of the desk so they don't fall off.

Method

Prepare beetroot take out of tuber and cut into 18 cylinders each 3.5cm long using a ruler and a knife. Remove excess dye by running water over the cylinders.

Set up apparatus (as shown in diagram)

1) Place water bath containing 200ml³ of water on heat until the temperature of the water reaches 80℃
2) While the water is heating arrange 18 test-tubes in test-tube racks. Labelled for distinction of each set of temperatures 80℃, 70℃, 60℃ etc.
3) Fill each test-tube with 10cm³ of distilled water measured using a measuring cylinder.
4) Set up colorimeter, leave for five minutes to warm up. Use a sample of distilled water to set the colorimeter.

Results

Temperature		% of transmission of light	
(℃)	1st	2nd	3rd
30	99	94	99
40	89	94	98
50	98	96	97
60	76	70	60
70	84	92	27
80	4	4	2

My graph firstly is high in percentage transmission of light with low temperature, and then it drops dramatically to a low percentage transmission of light with a high temperature. High temperatures can make the membrane change shape and be damaged this is proved by my results and graph. The higher the temperature the more kinetic energy, the energy is given to the atoms and they begin to vibrate. They vibrate faster and faster and bang off each other, causing the structure to break. This allows the anthocyanin to leak out this has happened in my experiment. The dip at the end is because the beetroot membrane has been damaged severely so no more anthocyanin could be released or leak out of the cell and diffuse. The shape of the graph tells me that the proteins and enzymes are denatured by the temperature at point 4 (60℃).

Looking at the graph point two (40℃) looks to be anomalous as it does not follow the trend of the rest of the graph, if it was to follow the trend the point would have been approximately 95.5%. However, it can be accounted for, as 40℃ like lower temperatures, does not affect the proteins. The bonds are still stable and intact therefore it won't have as much variation in the percentage of light transmission as the sample. Other than that average, the other average results follow the predicted pattern of

increasing the percentage of light transmission with the decreasing of temperatures.
These results, both in the tables and on the graph show that as the temperature increased the percentage transmission of light went further and further down. This means that as the temperature less transparent and the more pigment the water had. So each time the temperature was increased the plasma membrane's permeability worsened and became less selective.

My results back up my prediction of the higher the temperature the less the percentage of light transmission.

The individual readings were as expected, the percentage of light transmission increased with decreasing temperature. Many inaccuracies could have occurred during the course of the experiment. One being the uneven spread of pigmentation in the beetroot, some areas was much darker than in other areas, this could have an effect on the amount of pigment which was released by the cell. This factor is not counted as human error as there is nothing we can do to prevent this from happening. Also if different pieces of beetroot were used, it could affect the results obtained. This could be overcome by using the same beetroot through out the experiment, that way we know that if the pieces are from the same beetroot, then the colouration of the pigment will be similar in all the pieces. If they were from different pieces of beetroot then the intensity of the pigment released may cause inaccuracies to occur. The beetroot may not have rinsed properly therefore the pigment may have leaked out prematurely in effect causing the percentage transmission to be less than would have been otherwise. Rinsing the beetroot thoroughly would make the first reading more accurate. We could prevent this inaccuracy from happening by blotting the beetroot before transferring it into the test tubes. The surface area of the beetroot may also play a part in the accuracy of the results, as varying sizes in area would have produce a varying amount of pigment leakage. The larger the surface area, the more pigment will leak out. This could be due to free hand

sectioning. A way to avoid the variation could be to use a device which has cutting utensils at a set distance which would produce pieces that were of equal length.

Temperature was another aspect that could have caused results to be inaccurate as the temperature was very hard to keep the same, as it was very hard to stop the fluctuation from occurring. The baths may have been heated too much, or they may have had time to cool down, in effect, affecting the amount of pigment leaking out of the cell due to the increased fluidity of the membrane. Inconsistent stirring could have lead to uneven spread of the pigment, therefore when a sample was taken out for testing; the percentage transmission was greater than would have been. otherwise. One way of overcoming this would be to stir regularly to ensure even spread of pigment.

The apparatus was fine for a small experiment but it could make our results unreliable and inaccurate. The apparatus was not the most accurate we could have used. For example we could have used a cutting device to cut the beetroot as there could be possible human error if doing so by hand. The water baths were not kept constant at one temperature so it could make the results inaccurate. As we had to take the cork borers out one by one so there was slight time differences between each sample. As we were using stop watches it made it more reliable although there was lots of room for human error. The colorimeter's screen was very difficult to read so perhaps a bigger screen or a digital colorimeter would have been more accurate.

I would use up to date apparatus if I was to change things or if I was to do this experiment again.

I feel my results are very accurate and reliable. The results do vary slightly but not very much there is an obvious pattern between them. I have one anomaly at point 3 but it didn't up set my results too much.
If I was to do this again I would change the amount of time we had to do it and keep the samples in longer for better results and

not be limited on the equipment by having up to date machines and apparatus.

I am very sure that my results are reliable as they followed my previous theory.