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8073
GCE A' Level CourseWork

Auntie Val's Beetroot Problem

When preparing her beetroot crop for pickling Auntie Val peeled the fresh roots and placed them into cold water. Once all the roots had been peeled the water was brought to the boil and simmered for 25 minutes.

Auntie Val noticed that before cooking the water was a pale pink colour but after cooking the water had turned a very strong pink / purple.

Hypothesis

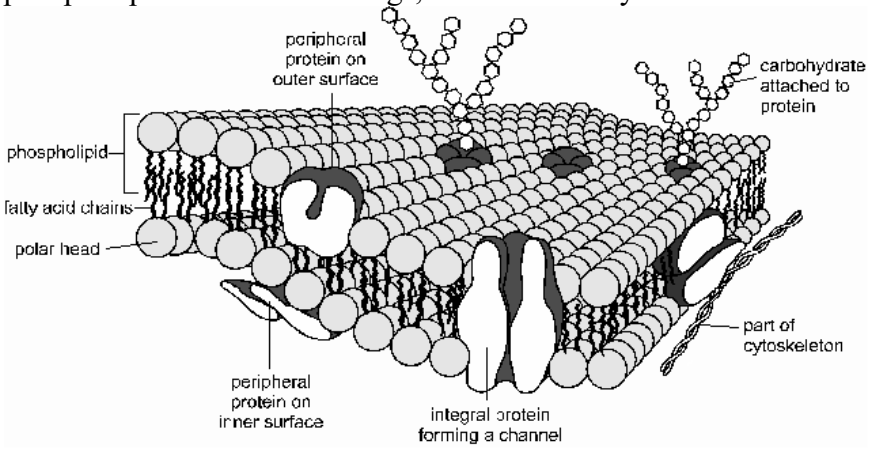
My Hypothesis for the reason that the water being boiled containing the beetroot turned a strong pink / purple from a pale pink colour; is because the variable for example the temperature affects the proteins in the cell membranes of the beetroot, which affects the permeability of the pigment and the cytoplasm across the membrane to leak out; resulting in faster diffusion. Similarly, people have also found the same hypothsis I have for example a person; James; who has done the experiment has also said "As the temperature increases, the permeability of the beetroot also increases." The person also gave a null hypothesis (invalid) saying "as we increase the temperature the permeability does not increase" this is because if this were to happen then it would go against Auntie Val's observations of the water turning from a pale pink to a strong pink / purple.

Background Information

Membranes

The plasma membrane surrounds all living cells. It controls how substances can move in and out of the cell and is responsible for many other properties of the cell as well. The membranes that surround the nucleus and other organelles are almost identical to the cell membrane. Membranes are composed of phospholipids, proteins, cholesterol and carbohydrates arranged in a fluid mosaic structure, as shown in the diagram.

The phospholipids form a thin, flexible sheet, while the proteins "float" in the phospholipid sheet like icebergs, and the carbohydrates extend out from the proteins.



The phospholipid tails point inwards, facing each other and forming a non-polar hydrophobic interior. The phospholipid heads face aqueous (water containing) medium that surrounds the membrane. Some of the phospholipid tails are saturated and some are unsaturated. The more unsaturated they are the more fluid the membrane. This is because the unsaturated fatty acids tails are bent and therefore fit together more loosely. As temperatures decreases membranes become less fluid.

The **proteins** usually span from one side of the phospholipid bilayer to the other (intrinsic proteins), but can also sit on one of the surfaces (extrinsic proteins). They can slide around the membrane very quickly and collide with each other, but can never flip from one side to the other. The proteins have hydrophilic amino acids in contact with the water on the outside of membranes, and hydrophobic amino acids in contact with the fatty chains inside the membrane. Proteins comprise about 50% of the mass of membranes, and are responsible for most of the membrane's properties.

- Proteins that span the membrane are usually involved in transporting substances across the membrane.
- Proteins on the inside surface of cell membranes are often attached to the cytoskeleton and are involved in maintaining the cell's shape, or in cell motility. They may also be enzymes, catalyzing reactions in the cytoplasm.
- Proteins on the outside surface of cell membranes can act as receptors by having a specific binding site where hormones or other chemicals can bind. This binding then triggers other events in the cell. They may also be involved in cell signaling and cell recognition, or they may be enzymes, such as maltase in the small intestine.

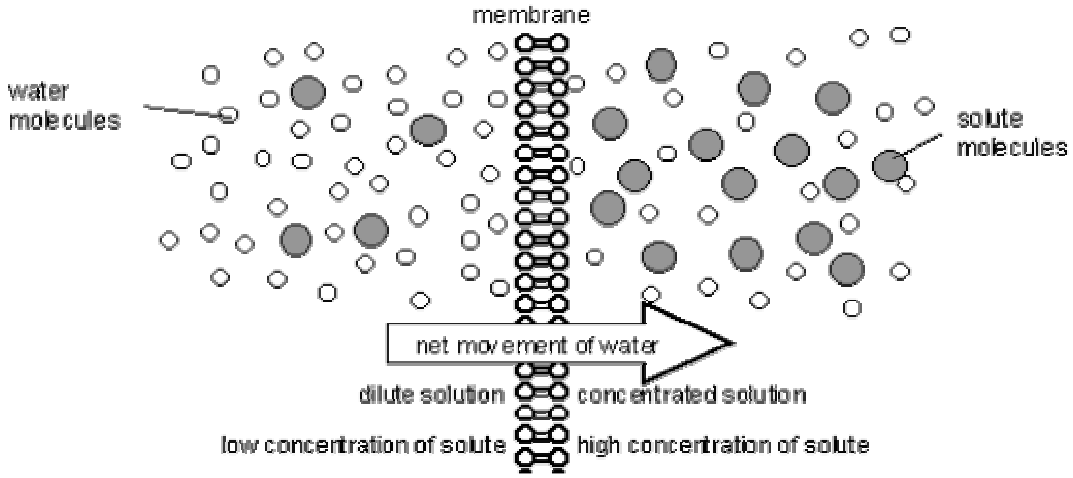
Cell membranes are a barrier to most substances, and this property allows materials to be concentrated inside cells, excluded from cells, or simply separated from the outside environment. This is compartmentalization is essential for life, as it enables reactions to take place that would otherwise be impossible. Eukaryotic cells can also compartmentalize materials inside organelles. Obviously materials need to be able to enter and leave cells, and the main methods by which substances can move across a cell membrane:

- 1. Osmosis
- 2. Simple Diffusion
- 3. Facilitated Diffusion

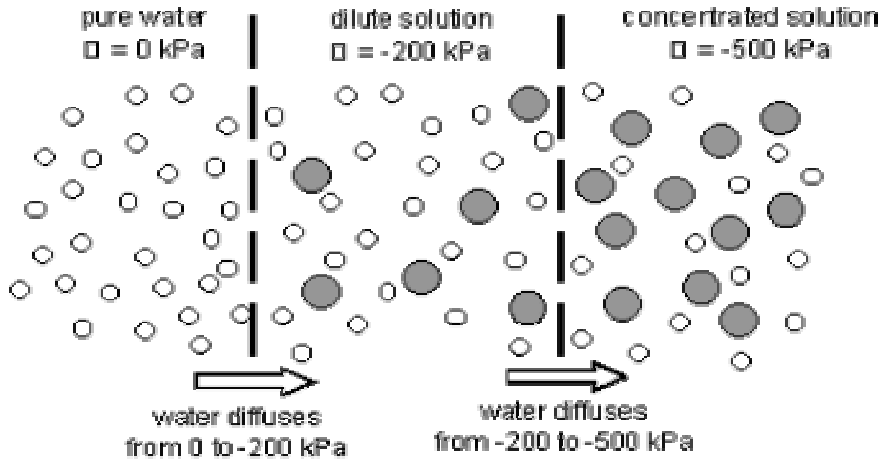
Osmosis

Osmosis is the diffusion of water across a membrane. It is in fact just normal lipid diffusion, but since water is so important and so abundant in cells, the diffusion of water has its own name- osmosis. The contents of cells are essentially solutions of numerous different solutes, and the more concentrated the solution, the more solute molecules there are in a given volume, so the fewer water molecules there are. Water molecules can diffuse freely across a membrane, but always down their concentration gradient, so water therefore diffuses from a dilute to a concentrated solution. This is why when Auntie Val

saw the water at first the water was a pale pink this is because Osmosis occurred which is the net movement of water molecules from a region of high water potential to a region of low water potential, through a partially permeable membrane, as a result of random motion.

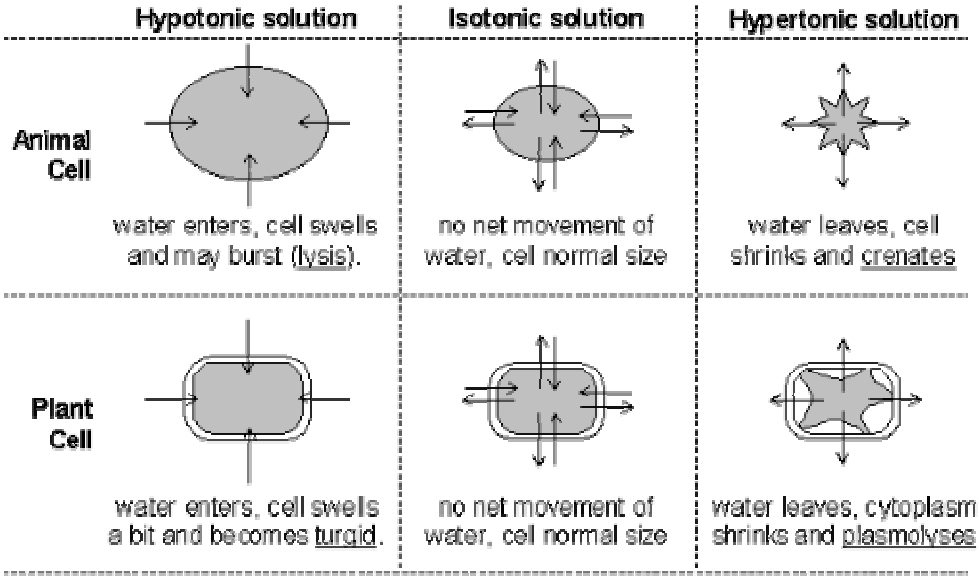


Osmosis can be quantified using water potential, so we can calculate which way water will move, and how fast. Water potential is a measure of the water molecule potential for movement in a solution. It is measured in units of pressure and the rule is that water always moves by osmosis from less negative to more negative water potential. 100% pure water has □ = 0, which is the highest possible water potential, so all solutions have □ < 0.



Cells and Osmosis. The concentration of the solution that surrounds a cell will affect the state of the cell, due to osmosis. There are three possible concentrations of solution to consider:

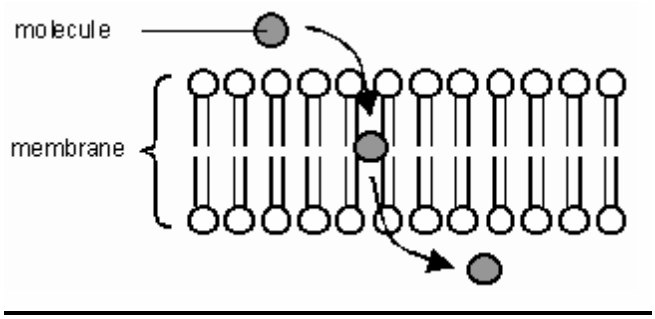
- Isotonic solution a solution of equal concentration to a cell
- Hypertonic solution a solution of higher concentration than a cell
- Hypotonic solution a solution of lower concentration than a cell
- The effects of these solutions on cells are shown in this diagram:



These are problems that living cells face all the time. For example:

- Simple animal cells in fresh water habitats are surrounded by a hypotonic solution and constantly need to expel water using contractile vacuoles to prevent swelling and lysis.
- Cells in marine environments are surrounded by a hypertonic solution, and must actively pump ions into their cells to reduce their water potential and so reduce water loss by osmosis.
- Young non-woody plants rely on cell turgor pressure for their support, and without enough water they wilt. Plants take up water through their root hair cells by osmosis, and must actively pump ions into their cells to keep them hypertonic compared to the soil. This is particularly difficult for plants rooted in salt water.

Simple Diffusion

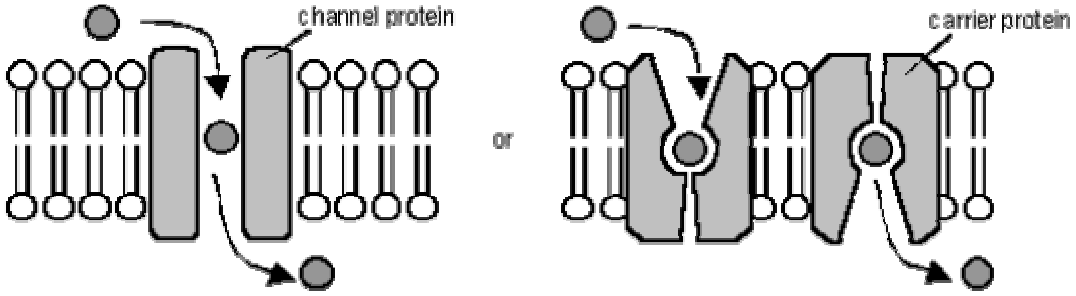


A few substances can diffuse directly through the lipid bilayer part of the membrane. The only substances that can do this are lipid-soluble molecules such as steroids, or very small molecules, such as H_2O, O_2 and CO_2. For these molecules the membrane is no barrier at all. Since lipid diffusion is (obviously) a passive diffusion process, no energy is involved and substances can only move down their concentration gradient.

At very high temperatures, molecules and ions have much more kinetic energy than at lower temperatures. They move around faster, thus diffusion takes place faster.

This is why when Auntie Val saw the water turned a very strong pink/ purple the reason being is that diffusion occurred which is the net movement, as a result of random motion, of its molecules of ions, of an substance (pigment) of an area of relatively high concentration to an area of relatively low concentration. This is because the proteins have been denatured and there was no barrier between the pigment and the water. Therefore diffusion was the final result.

Facilitated Diffusion



Facilitated diffusion is the transport of substances across a membrane by a trans-membrane protein molecule. The transport proteins tend to be specific for one molecule (a bit like enzymes), so substances can only cross a membrane if it contains the appropriate protein. As the name suggests, this is a passive diffusion process, so no energy is involved and substances can only move down their concentration gradient. There are two kinds of transport protein:

- Channel Proteins form a water-filled pore or channel in the membrane. This allows charged substances (usually ions) to diffuse across membranes. Most channels can be gated (opened or closed), allowing the cell to control the entry and exit of ions.
- Carrier Proteins have a binding site for a specific solute and constantly flip between two states so that the site is alternately open to opposite sides of the membrane. The substance will bind on the side where it at a high concentration and be released where it is at a low concentration.

The rate of diffusion of a substance across a membrane increases as its concentration gradient increases, but whereas lipid diffusion shows a linear relationship, facilitated diffusion has a curved relationship with a maximum rate. This is due to the rate being limited by the number of transport proteins.

METHOD	USES ENERGY	USES PROTEINS	SPECIFIC	CONTROLLABLE
Simple Diffusion	**N**	**N**	**N**	**N**
Osmosis	**N**	**N**	**Y**	**N**
Facilitated Diffusion	**N**	**Y**	**Y**	**Y**
Active Transport	**Y**	**Y**	**Y**	**Y**

Pigments

The betacyanin pigment of beet roots is normally sequestered in the vacuole and, by means of the properties of the tonoplast and cell membrane, does not leak into the cytosol or the extra-cellular sap of the beet root. Of course if the beet root is cut cells are sliced open and the pigment spills out, but if the membrane is altered (phospholipid bilayer + proteins) more subtly leakage (diffusion) of betacyanin is induced.

Betalains: What are betalains?

- Betalains are alkaloid pigments that are found in some families of plants belonging to the order Caryophyllales, but in no other plants.
- Betalains are not found in plants containing anthocyanin pigments. Structurally they are unrelated.
- They can be divided into betacyanins and betaxanthins based upon their molecular structure.
- betacyanins generally appear red to red violet in colour
- betaxanthins generally appear yellow in colour
- They cause colour in both flowers, fruits and sometimes vegetative organs
- They are found in the vacuole
- They are aqueous in solubility
- Beetroot contains 2 Betacyanins Betanin and a derivative
- Little is known about the role of betalains but it is thought they may protect against pathogens
- Unlike anthocyanin, betalains are not pH indicators, i.e. they do not change colour when the pH is lowered.
- Beet pigments are unstable at high temperatures, but the chemistry depends on the pH and composition of the solution oxygen concentration, how long the solution is boiled etc/

The basic structure of betacyanins

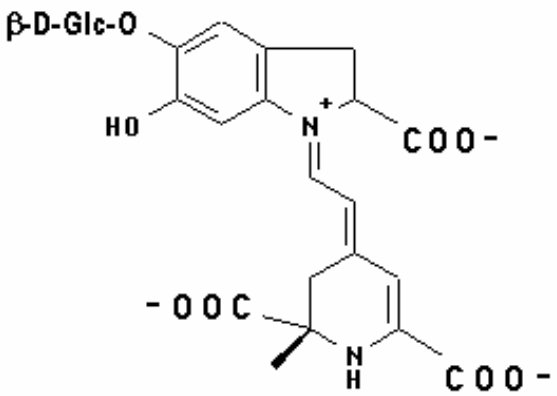


Fig.#1
Betanin a betacyanin

(http://www.mrothery.co.uk/cells/resources/rec82/htm)

Colorimeter

A colorimeter is used to test out the pigment intensity of the envirnmental solution. Bluie light form the the LED light source of the colorimeter will pass through the solution and strike a photocell. The test solutions made in the experiment are clear. If the beetroot pigment leaks into the solution, it will color the solution red. A higher concentration of the pigment in the solution absorbs more light and transmits less light than a solution of less pigment. The computer interfaced colorimeter monitors the light received by the photocell as either a absorbance or a percentage transmittance value. The outout of the data recorders is theabsorbance value. A higher absorbance value indicates more light has been absorbed by the environmental solution and thus indicates more membrane damage.

The Factors Which Might Affect The Phenomenon

The factors which might affect the permeability of the membrane are:

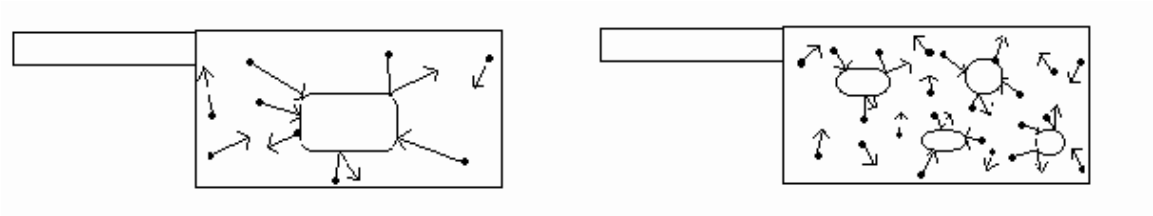
- Surface Area (thickness etc)
- Temperature
- Time
- Concentration of the water
- Environmental aspects for example light intensity
- Physical aspects for example degree and frequency of shaking the tube contents.
- Tissue of beetroot (pigment)

Scientific Knowledge Of The Factors

Surface Area

The surface area of a solid is the area of its which is exposed. If the solid is cut up into smaller pieces, more surface area is expoesd and this means it can react faster. This

explains why for example cut of beetroots cook faster than whole beetroot. With more surface area exposed, the smaller pieces of beetroots cook more quickly. Reactions occur when particles collide. When the beetroot is cut up into smaller pieces, hot water particles collide more frequently with particles on the surface of the beetroot.This causes more particles to react per minute. So the beetroots cook more quickly. (As shown below)



In addition the surface area is affected elsewhere in the experiment, in this case diffusion is taking place. I know this because at the start Auntie Val noticed in cold water the water already turned a pale pink therefore diffusion must have already occurred. When there is a greater surface area in diffusion, then there are more molecules or ions that can cross the membrane at any one moment, and therefore the faster diffusion can occur. Therefore, resulting in a deeper colour at the start in cold water then before.

Temperature (Independent)

At higher temperatures, particles are moving around faster (An increase in random movement of the water). Therefore they collide more frequently and with more energy. This means the reaction rate increases when the frequency or energy of collsion between particles increase. Plus the more collisions there are the greater the chance of the particles colliding with the solid (beetroot) In addtion to the water particles moving faster, diffusion also takes place faster due to the high kinetic energy.

Temperature not only affects the liquid it also affects solid. For this experiment it is the membrane of the beetroot that is affected, which contains proteins for example carrier proteins, channel proteins and recognition proteins; that are used for specific functions. The proteins at high temperatures breaks the hydrogen bonds formed between the stongly polar groups; therefore denatures.(the protein loses shape and activity.)

The overall trend seems to be that as the temperature increases, the average percentage of transmission decreases. This is suggesting that membrane permeability increases with temperature and therefore more pigment leaks out. The darker the colour means that more pigments have leaked out and therefore the lower percentage of transmission.

From 0ºC to 20ºC, there's a slight increase in the average percentage of transmission. This is because when water freezes, it expands and this makes the cell membrane bursts. The bursting results in more pigments leaking out and the darker the colour of the liquid obtained.

Between 20ºC and 60ºC, proteins are denaturing more and more as the temperature increases. This is lowering the percentage of transmission as more pigments are leaking out due to the denatured proteins and the lower the transmission (the darker the colour).

By 80ºC most of the proteins have already denatured and this explains the steep slope on the graph. By that temperature, most colour s has already leaked out and therefore resulting in the highest transmission.

Tissue of beetroot (pigment)

The dependant variable is the intactness of the call membrane is measured by this permeability to the red pigment, (or to the number of cells of which the cell membrane is damaged by the organic substance.)

To be exact, the amount of the red pigment diffused out does not depend on the extent to which the cell membrane is being destroyed. Rather it is due to the number of cells being damaged. In other words, the cell membrane once destroyed would become completely permeable to the red pigment.

The dependant variable can be measured by the amount of red pigments retained inside the cells or the amount of red pigments diffused out into the surroundings solution over a fixed period of time. (Using a colorimeter)

Time

This is a controlled variable which need to be considered very important, because the more time given during the experiment the more pigment and strong the colour of the water. Therefore the time needs to be checked precisely.

Concentration of the water

The concentration of the water will need to be kept the same this can be done by getting the water from a controlled source e.g. tap water etc Distilled or de-ionized water would produce more accurate results but there is insufficient supply. Tap water often contains Chlorine, Fluoride, Sulphur, Minerals and Salts, especially Calcium and Magnesium Minerals that make the water hard. Heavy metals such as Copper, Lead and Iron also often contaminate tap water. However the majority of the contaminants in tap water are large, and often insoluble, molecules which have a lesser effect on □ w than small soluble molecules, meaning the inaccuracy caused by using tap water should not be very large.

Environmental Aspect

The environmental variable is the hardest variable to control this is due to the fact of how the temperature can be varied. This is because:

- Light Intensity- increases temperature from the light bulbs around the room and the sunlight shining through the windows. This can be controlled by keeping the blinds shut at all times. The light bulbs need to be kept on at all times to keep the temperature the same in the air.
- Windows- letting cold air in and effects the temperature on the beetroot therefore this can be controlled by keeping the windows all closed.
- Door- left open can cause a draft of air (cold) therefore this can be kept closed at all times so that temperature is not affected.
- Heaters- in room effect the room temperature rapidly therefore needs to be controlled. Either on or off. Preferably off this is because of the bunsen burners giving off enough heat.
- Bunsen Burners- all around the room at all times, this will be another factor that affects temperature. But because it is needed at all times then this will have to be left on. Preferably the heaters will be turned off.

Physical Aspects

The physical aspect of the experiment is to do the experiment with great accuracy going around the problems therefore this has to be kept controlled for example if the water is shaken while the beetroot was in there then this would effect the permeability of the membrane. Therefore, to keep this controlled as much as possible is to not have an effect like shaking. (handle the beetroot with care at all times) The beetroot is the main aspect of this variable.

Which Factor Has The Greater Effect?

Each factor has an effect that contributes to the change in the pigmentation of the water in there own way. The more the surface area the faster the diffusion starts off other than less surface area, but over a period of time the effect will slow down as diffusion reaches equilibrium and the pigmentation will not be as strong (slow reaction). Whereas the temperature may start slow, but once it gets past the optimum temperature of the protein, which will start to denature the beetroot will show a dramatic effect on the pigmentation (fast reaction).

The environmental aspects of the experiment can cause a affect in the temperature and can play a part on the experiments results however, this is hard to control well therefore this will have to be taken with great care.

Time is also an effect on the amount of pigmentation released into the water during a scientific process for example diffusion this can also play a large part on the results and can affect the overall pattern of the results for example the higher the temperature the more pigmentation released, over a certain period of time. If time alters the experiment is altered; more time, more pigmentation; less time, less pigmentation.

The concentration of water is not a large affect on the experiment as long as the same type of water is used, because the pigmentation will still move through the partially permeable membrane of the beetroot.

The physical aspect of the experiment is seen to be controlled easy and has quite a large affect on the pigmentation. Therefore this is seen as a great factor which needs to be considered.

The tissue of the beetroot does cause affects of the pigmentation released, and needs to be the same type of beetroot. This can cause a great affect on the type of heat the beetroot can withstand as it is dependant on what part of temperature the beetroot has came from.

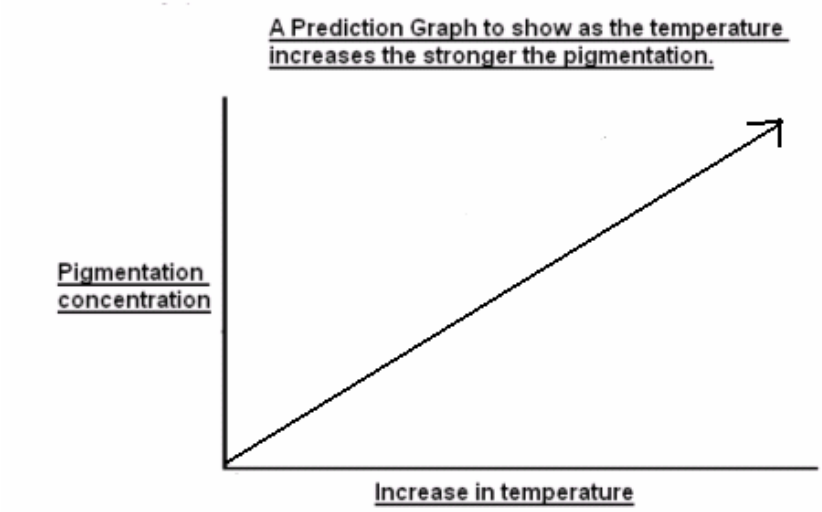
Therefore, I think the factors that increase the temperature will have a greater affect on the overall pigmentation than the change in the factors that don't, with the exception of the beetroot type and the physical aspects of the experiment.

Aim

The aim of this experiment is to measure effect of a certain variable on the beetroots membrane and see how it effects the release of the pigmentation in the water. To see why in Auntie Val's cooking the water had turned colour after being heated for a period of time.

Prediction

I think that increasing the temperature of the water the faster the rate of the reaction. I think that this will happen because the membrane in the cell denatures faster as the temperature rises past the optimum level for the protein in the membranes. Therefore more pigmentation will leak out and therefore the stronger the colour.



About the prediction Graph

This graph shows that at the beginning of the experiment the temperature around 0°C to 20°C shows a slight increase transmission of the pigment moving into the water from the beetroot; this is because of factors occurring such as osmosis, the amount collisions and temperature. To begin with, osmosis is occurring, which is the diffusion of the water molecules moving across the beetroots from high water potential to low water potential through a partially permeable membrane. Plus, the random motion of the water particles colliding with the beetroots surface area placed into the cold water results in there being a pigmentation released into the cold water. Due to the temperature of the water being cold during the start of the experiment means that the molecules are expanding and some of the pigment is leaking which results in the being a slight increase percentage of transmission.

In the middle of the experiment this is when the temperature is at 20°C to 60°C the temperature is ensuing the proteins in the membrane of the beetroot are denaturing; Hence, the lowering of the percentage of the transmission as more pigment should be leaking out due to the denaturing of the enzymes this explains why the slope on the graph is still increasing steadily. Furthermore because of the denaturing of the membrane this resulting in there being an effect on facilitated diffusion, which involves proteins and the number of collisions. In facilitated diffusion, which is mainly involved on the protein molecules to transport controlled substances to pass through the membrane; but, because of the increase in the temperature the proteins become denatured around the 40°C mark, there is a point where there is a loss of control for the proteins in the membrane; resulting in the membrane to become slightly fluid as some of the molecules are becoming bend and are becoming loosely fit together. Demonstrating there to be more pigment being

released into the water, which in colour is becoming darker. The line on the graph is still straight not curved at any point because of the proteins because at 20°C to 40°C, before the denaturing of protein molecules there should be collisions between the water molecules and the surface of the beetroot producing more pigment to be released with diffusion (osmosis) as the increase temperature results in there being more random movement (kinetic energy) which is therefore increasing the rate of diffusion. Consequently there should be an increase in the colour of the pigment.

At the end of the experiment this is when the temperature is about 60°C to 90°C, this is where all of the proteins should be denatured, and resulting in there being no proteins to affect what goes in and out of the beetroot membrane. Because of the lack of proteins in the membrane, there is an increase in the fluidity of the membrane because of more molecules within it are loosely fit together and are bent, which can cause a high percentage of the pigment to be released. Therefore, most of the pigment should be already released resulting in a dark pigment colour in the water.

In conclusion to the whole of the prediction graph, I consider that as the temperature increases the pigmentation being released is also increasing, representing the graph to have a strong positive correlation (going diagonally).

Plan

I am planning to keep the temperature as an independent variable as this will be constantly getting higher in each experiment. And the dependant variable is the pigmentation released by the beetroot which will be measure using a colorimeter. The other variables are all going to be kept constant as these will affect the experiment, for example the beetroot should be kept of the same type, if not then could affect what temperatures the membranes denature because different types of beetroot are brought up in different environments. Therefore such controlled variables must be kept controlled at all times. Because other variables are hard to keep controlled as the room temperature varies at all times because Bunsen burners will be constantly on in the room, and the light intensity giving off heat. Therefore there needs to be enough experiments done so that results can show some clear pattern of the temperature against the pigment.

As a result there are going to be 7 experiments of the beetroot against different temperatures. This is done by the temperature rising every 10 Celsius for example the 1st experiment is starting at a temperature of 30C and is rising so that the 2nd is 40C , 3rd is 50C, 4th is 60C, 5th is 70C, 6th is 80C and 7th is 90C

Apparatus

Choice	Reason
Beetroot	Preferably same type, to make the experiment a fair test.
White tile	Provides hard surface onto which the celeriac can be cut. Suitable background to observe variations in the tissue.
Forceps	Enables beetroot to be held securely without skin contact, increasing accuracy of results.

Scalpel	Enables the making of the length more precise and the surface uniform.	
Mounted razor	Cuts a straight line vertically through the beetroot giving it a straight edge, ensuring the length of beetroot is uniform and precise.	
Cork borer	Enable us to take part of the beetroot with same radius.	
Distilled Water	Enable a more accurate result as normal tap water has chemicals added which can affect the experiment.	
A beaker	Enables a large enough to place the water and the beetroot as it is getting heated	
Gauge	Enables a place needed for the beaker on top of the Bunsen burner as it is getting heated	
Tripod	Acts as a stand to place the gauge on.	
Stop-watch-	Enables to measure the time. How long each experiment goes on for etc as this affects the results of the colorimeter.	
Thermometer	Enables us to take readings and precautions of the water, important during experiment as this determines it to be a fair test	
Colorimeter	Enables us to measure the readings of the pigment	
Heat proof mat	Enables us to keep the Bunsen burner on something safe.	
Ruler	Measures the length of the beetroot	
Safety spectacles	To keep eyes safe from the beetroot.	

Safety

Procedure	Risk	Precaution
Transferring liquids into and out of glass ware	Glassware is easily smashed and the shards can cut people	Care must be taken in the handling of all glassware and of broken glass
Using syringes	Solution squirting out of syringe	Goggles worn when using syringe
Using scalpel to cut beetroot and carrying it to and from the bench	Cutting hands	Use with caution, holding beetroot steady with forceps. Carry with blade pointing towards the floor.
Using cork borer to cut chips of beetroot	The cork borer must be used with considerable force to cut the chips, risk of injuring hands	Ensure hands are well away and always cut down onto a white tile
Use of beetroot	Do no eat	Constantly wash hands and handling take care
Heating using a Bunsen Burner	Fire and burn of skin or environment	Tie hair back, no running and take precautions on using equipment.

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Method

- Cut the beetroot into three large sections. Using a cork borer, size 3, pushing down onto a white tile, cut out 7 chips each a 1 center-meter long. Push the chips out of the cork borer using a knitting needle, preventing handling of the chips. It is important to have so many replicates so that three chips can be placed in each solution and an average result can be obtained for each solution. This reduces the risk of anomalous results and produces a more accurate and reliable result.
- It is important to take the chips from a similar region of the beetroot. The tissue of the beetroot is very variable, some of it is very pithy and there are small spots dense in starch, starch grains, demonstrated by the food tests. This will affect the water potential of the cells. Each type of tissue will have a different water potential. This makes it important to use the same tissue when calculating the pigmentation moved across the membranes with a colorimeter.
- Use a scalpel to remove the skin, ensuring all is removed, as it is less permeable than the inner tissue. Cut the beetroot chips using a scalpel on a white tile to an exact and equal length of one centimeter. A scalpel should be used as this ensures a straight vertical cut. Handle the beetroot with forceps, as grease from hands is non-polar and therefore makes the surface of the beetroot less permeable to water, which is polar.
- Begin to set up the heating equipment like the tripod, gauge, and Bunsen burner heat proof mat the appropriate way and begin the heat.
- Transfer the distilled water to beakers (make sure clean thoroughly). And ensure that the same amount is always added each time in each beaker so there is an ensured controlled variable. To make the chips identifiable (when placed into the beaker one at a time), place a coloured paper near the beaker or label the beaker of the chip and record which chip is which colour.
- Take the temperature of the water before adding the beetroot, and make sure that the temperature is correct to what each beetroot should undergo.
- When the right temperature of the water is exceeded place the beetroot inside and make sure that the beetroot is handled with care at all times, plus to make sure that the stop watch is started.
- Keep checking the temperature at regular intervals and increase or decrease the temperature if too high or low. This can be done by adding the Bunsen burner or by taking the Bunsen burner away. (The temperature needs to be seen with great care and great accuracy at all times if necessary)
- After the 2 minutes are over for each beetroot experiment, take the heat off the water, take a sample of the pigment of the water and make sure that the colorimeter is ready; to test out the pigmentation of the water.
- Do this experiment at least 7 times to get enough results to see is the prediction is correct.
- Remember to record each result in a table at the end of each experiment, and make sure that the results are put down to the nearest measurement.

Preliminary Experiment

Here I am testing out if my plan, method and apparatus is suitable in the actual experiment. I have found out that the amount of water needs to be over the 'immerse to water' on a thermometer to receive accurate results therefore instead of putting 150mls as intended, I will need to put something around 200mls.

In addition I have found out to receive more accurate results on the temperature; I have concluded that as the water is being heated by the Bunsen burner, the temperature continues to rise, past the temperature needed to place the beetroot. Instead I have found out that the Bunsen needs to be taken off 5 Celsius before the temperature needed is obtained or instead, wait till the temperature has started to cool and place the beetroot in the test- tube when the temperature has been obtained

Furthermore I have found in the preliminary experiment that the amount of water affects the pigment; as the pigment diffuses through the whole of the beaker making the concentration of the pigment differ. Instead placing the beetroot in a test tube (with water) will prevent this and then place the test tube in a beaker (with water). To make sure that the temperature of the water is obtained right in the test tube. The thermometer can be placed in the test tube. This will keep the concentration of the pigment the same and more accurate.

A Results Table to Show How the Temperature Affects the Permeability of a Membrane of a Beetroot Membrane, Measured By a Colorimeter in Optical Density

	Optical Density Of the Pigment			
Temperature in Celsius (x)	**1**	**2**	**3**	**Average (y)**
85	8.40	8.20	8.00	8.20
80	6.90	7.50	**6.00**	7.20
75	7.20	6.70	7.00	6.97
70	5.90	5.70	5.75	5.78
65	4.50	4.10	4.50	4.37
63	4.30	4.00	4.00	4.10
60	4.00	3.90	3.80	3.90
55	3.50	2.80	3.50	3.27
50	2.70	2.50	3.00	2.73
45	2.50	2.40	2.70	2.53
648				49.05

Analysis

The Product-Moment Correlation Coefficient

This is done to see the measure of the strength of the correlation of the two variables; temperature and optical density.

Key $\sum$	Sum Of
n	Number Of Data

$$r = \frac{\sum x y - \frac{\sum x \sum y}{n}}{\sqrt{\left(\sum x - \frac{(\sum x)}{n}\right)\left(\sum y - \frac{(\sum y)}{n}\right)}}$$

$$r = \frac{\sum 3406.9 - \frac{\sum 648 \sum 49.05}{10}}{\sqrt{\left(\sum 43494 - \frac{(\sum 648)}{10}\right)\left(\sum 276.7329 - \frac{(\sum 49.05)}{10}\right)}}$$

R = 0.98

Interpreting the Product-Moment Correlation Coefficient

The values of **R** between 0 and 1, the nearer the value of r is to 1 the stronger the positive correlation between the two variables. This is shown on the graph, as I can see that there is a strong positive correlation (R= 0.98). If R = 1 there is a perfect positive linear correlation between the two variables (all points fit a straight line with positive gradient) In this case they do not, but they do follow the straight line, showing the relationship is strong. Id R = zero or close to Zero there is no linear correlation; meaning that there is no relationship between the two variables. If this were the case then the temperature does not affect the permeability of the beetroot membrane. However, the experiment that I have prepared clearly shows that there is an affect of temperature on a beetroot membrane.

Linear Regression

Is a way to show that the relationship between two variables temperature, x and the beetroot tissue (pigment), y. Linear Regression is to find the law connecting the two variables x and y that allows me to predict the value of y, for a given value of x. Here I am trying to find a linear relationship.

In the linear regression model, the experimental results are used to find a regression line, which is the equation $y = a + bx$ where a and b are estimates. The regression line is

known as the regression line of y (Optical Density Reading) on x (Temperature) since y is the response for a given value of x.

The least square regression line is drawn through the points representing the observations in such a way that the sum of the squares (points plotted on scatter graph) of the vertical distances between the line and the points plotted is minimized.

The equation of the regression line of y on x is:

Key ∑	**Sum Of**
N	**Number Of Data**
Ў	**The mean of y**
Ж	**The mean of x**

y = a + b * x	**A = Ў – b * Ж**
B = ∑(x - Ж)(y - Ў) / ∑(x - Ж)²	

Same ↔ Sxy / Sxx

x	**y**	**xy**	**x squared**
85	8.2	697	7225
80	7.2	576	6400
75	6.97	522.75	5625
70	5.78	404.6	4900
65	4.37	284.05	4225
63	4.1	258.3	3969
60	3.9	234	3600
55	3.27	179.85	3025
50	2.73	136.5	2500
45	2.53	113.85	2025
648	**49.05**	**3406.9**	**43494**

Placing the numbers into the least square regression formula to find B

Sxy = 3406.9 – ((648 * 49.05) / 10) = 228.46

Sxx = 43494 – ((648 * 648) / 10) = 1503.6

B = 228.46 / 1503.6

B = 0.152

Placing the numbers into the least square regression for straight line to find A

A = Ў – b * Ж

A = (49.05 / 10) – (0.152 * 648) / 10

A = -4.941

Then the regression line of y on x is

Y = .4.941 + 0.152 x

Therefore if x (temperature) = 40 C

Y = .4.941 + 0.152 * 40

Y = 1.139 (should have been the approximate value of the optical density at 40 C)

Graph

The graph of how the temperature affects the permeability of a beetroot membrane, measured by a colorimeter in the form of optical density shows me a strong positive correlation with the results; plus it shows that as the temperature rises so does the colorimeter reading this is because after a set temperature the cell membrane breaks down and the pigment is realized.

Between 45ºC and 65ºC, proteins are denaturing more and more as the temperature increases. More pigments are leaking out due to the denatured proteins and reading of the colorimeter increases reads 2 - 3 at around 45ºC and at around 65ºC they read 4 - 5.

By 80ºC most of the proteins have already denatured and the colorimeter reads 8 - 9.

In addition I have done a product moment correlation co-efficient to see how correlated the graph is from this the result was 0.98 therefore this backs up that the relationship between the two variables are very strong.

The overall trend seems to be that as the temperature increases; the pigmentation is permeability of the membrane increases the pigmentation. This is suggesting that membrane permeability increases with temperature and therefore more colour leaks out. The darker the colour means that more pigments has leaked out the lighter the colour the less pigment has leaked out.

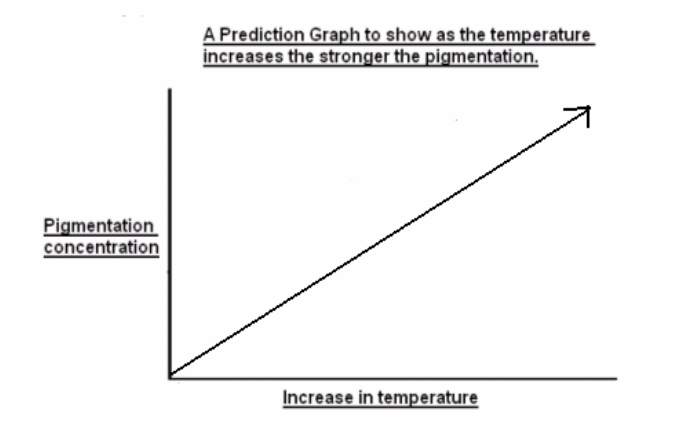
STATISTICAL ANALYSIS

Comparing Temperatures	Null Hypothesis of Results (probability)	Significance In Optical Density
45 - 45	1.000	No
45 – 50	0.305	No
45 – 55	0.042	No
45 – 60	0.000	Yes
45 – 63	0.000	Yes
45 – 65	0.000	Yes
45 – 70	0.000	Yes
45 – 75	0.000	Yes
45 – 80	0.000	Yes
45 - 85	0.000	Yes

The Statistical Analysis

The statistical knowledge that I have further gathered from my results shows: that my hypothesis-which is the first step made to make assumptions about the population under consideration. This involves stating a statistical hypothesis about the population. The basis of this hypothesis is that there is no significant difference between observed and expected results. It is known as a Null Hypothesis.

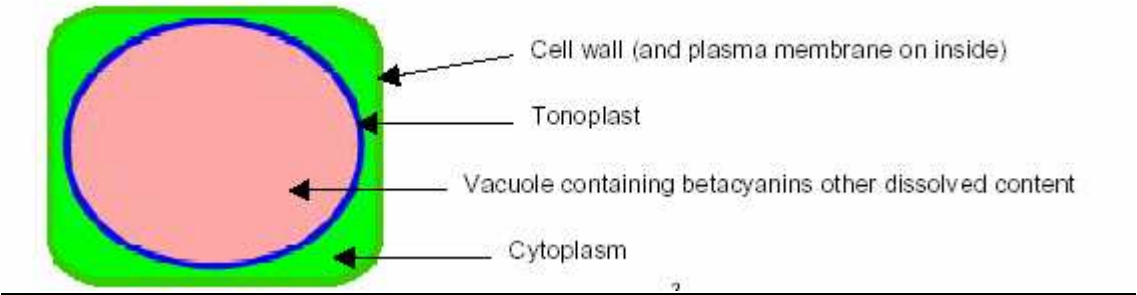
In the experiment there is a degree of freedom of 4 with a 0.05 (5%) confidence level. There is a show that the null hypothesis is a range from 1.000 to 0.000, if the result is closer to 1.000 then there is no significant difference in optical density (membrane permeability) of the range of temperatures. However because most of results are 0.000 then it shows that the there is a significant difference in the optical density; which gives a strong indication that the hypothesis concluded is true and accurate



My results support my prediction as the results graph shows that as the temperature increases the betacyanin of the water is stronger. Both of the graphs have a positive correlation (going diagonally). Increasing the temperature of the water; the faster the membrane of the cell denatures as the temperature rises past the optimum level for the protein in the membranes making it more fluid, (the stronger the colour.).

The Scientific Reason

The scientific reason to explain why the beetroot pigment leaks out with increasing temperature, we must consider the location of the red betacyanin in a beetroot cell.



The red betacyanin is located within the cell vacuole. We can assume that when the cell is functioning normally, the tonoplast keeps the betacyanin in. For the betacyanin to leak out, we can see at once from the diagram that it must pass through the tonoplast, the cytoplasm, the plasma membrane and the cell wall. Of these four barriers, only the tonoplast and the plasma membrane are selectively permeable and would pose any problems to the passage of the pigment. The cell wall is fully permeable because it is not a regulatory structure like the plasma membrane (in fact its function is provide support and to stop the cell from bursting when it is turgid). The cytoplasm is mostly water and so would allow free passage of betacyanin through it. Both the tonoplast and the plasma membrane control passage of complex molecules like Betacyanin by transport proteins embedded in them.

In this experiment we used water of varying temperatures to heat the beetroot pieces, the temperatures kinetic energy plays a part; as the higher the energy the more the molecules have higher random motion, and the betacyanin escapes the membrane to its surroundings, there is also an increase in fluidity of the membrane as the molecules are bent and loosely fit together. The varying temperatures causes the hydrogen bonds to break and the transport proteins were no longer able to contain the betacyanin in the vacuole or even in the cytoplasm as they lost control in the membrane. The sudden increase in betacyanin concentration in the beetroot solution between 50º C and 60º C was due to lots of the bonds breaking around that temperature range. This would make sense if we assumed most of the bonds breaking were of the same type and so had the same sort of tolerance to temperature.

The hydrogen, the ionic bonds and the hydrophobic interactions in the tertiary structure, and hydrogen bonds in the secondary structure of the protein were broken, because the and disulphide bonds which were also holding together the tertiary structure are very strong but were likely to break at fairly high temperatures.

Evaluation

The results that were collected follow the same pattern as results collected by similar studies carried out within our class, so therefore it is safe to say that the results can be repeated reliably and the methods can be used universally. The results obtained follow a strong positive correlation on the graph, except for the result obtained at 80 C had a reading of 6 on the colorimeter, which was anomalous.

There are many possible outcomes for this:

The equipment used places limits on both precision and accuracy. The lack of precision of the instruments is reflected in the number of decimal places the data can be recorded to.

Apparatus	Limit on Precision/Accuracy
Ruler	It is hard to read a ruler more accurately than to the nearest mm. This is quite a large error in terms of the length of the beetroot chips and is imprecise
Colorimeter	The colorimeter reads to no decimal places or 1g. This is not precise and the number never remaining constant precision. This meant that every time the reading was read the accuracy was poor and the precision of each reading needed to be improved
Cork borer	It is impossible to ensure that the cork borer enters the beetroot tissue at exactly the same angle. This means that if the cells are considered to be running in a similar direction a different number will be damaged each time. The more diagonally the cork borer enters the beetroot the more cell walls and membranes will be damaged making the results more inaccurate. This can be seen highly variable.

It would have been beneficial to have repeated the experiment more times than 3 to make certain that the results were not gained through chance or by an external factor. The control experiment used was highly accurate, using distilled water, which is the clearest possible liquid, meant that even the slightest deviation in colour could be detected by the colorimeter.

The tissue in a beetroot is highly variable. It was visibly different in colour, some of it appearing brown and more fibrous. As was stated in the plan the beetroot tissue acts as a store of substances useful for the growth of the plant and has many different regions. It has a relatively thick, hard outer layer of skin, which is waterproof. The water potential of each region is different; however it also varies within each region. Therefore the release of betacyanin was always different dependant on where the tissue was from. This could have rendered the results

The variation in temperature of the samples will have reduced the accuracy of the results; optimally they would have been kept in a constant temperature environment. However the change in temperature, and its effect, will have been the same for all the samples and it should only be a large factor of the temperature caused the result to be individually anomalous.

The experiment could have been many errors of which some I could change if I had more time:

- The beetroot may not contain the normal amount of dye due to a lack of minerals when it was grown this passable error could be removed or reduced by using several beetroots
- Pigment could have been lost in the preparation of the beetroot by the cell being damaged when the cylinders were handled. This could have been avoided by holding the beetroots ends and only using the centre parts of the whole beetroot tissue in the experiment.

Source of error	Remedy / Improvement
Subjective judgment of the colour intensities by the human eye on the colorimeter as the readings on the colorimeter where not as clear as they should be.	Colour intensities to be measured by means of a colorimeter (measuring % transmission). Plus the colorimeter could have been a digital colorimeter which could show the readings at an appropriate degree of accuracy for example 3 decimal places. This would have made the results more accurate, plus the results would have been true, where the before there could have been human error. The colorimeter was also difficult to use in that it gave unstable readings – the time between the calibration of the colorimeter and the when the reading was taken for the beetroot solution mattered when trying to determine the absorbance of the beetroot solution. Therefore this could have been

	looked at further during the experiment.
Physical damage done to the beetroot cells when handling them, say, during the transfer from one tube to another.	Handle the beetroot discs as carefully as possible.
Sampling errors i.e. the beetroot discs may have come from different parts of a beetroot or even from different beetroot. There is a possibility of biological variations.	Try cutting the discs from the same beetroot and to use tissue of comparable colour as far as could be identified by the naked eye.
Beetroot discs not cut to the same thickness (because of free hand sectioning). This would affect the surface area to volume ratio which would, in turn, affect the treatment effect as well as the diffusion effect.	Cut discs by means of using a device in which two razor blades are fixed permanently at a distance of say 2 mm apart.
The temperature of each beetroot experiment could have been checked more to have accurate temperatures all the time.	Digital thermometers which feed information on temperature continuously to a computer system would be ideal.

Further experimentation

High temperature is not the only factor that causes transport proteins to denature. Salt concentration and pH can also have the same effect, and an experiment which varies those factors will probably be easier to carry out, since salt concentration does not drop after time like temperature does.
The aim of the investigation would be to find out whether the alkalinity of the environment of the Beetroot cells denatures the transport proteins to the same extent as the acidity of the environment of the beetroot cells.

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