Biology Coursework – Skill P

Title:
Design an experiment to determine the water potential of celeriac.

Aim:
To calculate the water potential of celeriac. In other words, to determine the net tendency of celeriac to donate water to its surroundings.

Input variables:
The following factors are known to influence the water potential of solutions:

i) concentration of solute in solution

ii) pressure acting on solution

Note that how these variables affect water potential will be discussed in the scientific background.

Scientific Background:

The plasma membrane:
All cells are separated from their external environment by a membrane, called the **plasma membrane**. Its functions include facilitating the transport of substances in and out of the cell, preventing the loss of substances and the entrance of other harmful substances, such as toxins.
The structure of the plasma membrane is explained in the fluid mosaic model. The membrane consists of two layers of phospholipid molecules arranged so that the hydrophobic tail points inwards. **Extrinsic** protein molecules are located on the outside and inside of the lipid bilayer, and other **intrinsic** proteins run across the membrane. These are able to move freely through the membrane, resulting in its 'fluidity'.
The plasma membrane is **partially permeable** – it only allows certain substances through. Water molecules can easily pass through any partially permeable membrane, as they are small enough to pass through tiny gaps called pores.

The net movement of water molecules down a concentration gradient, i.e. from a high concentration to a low concentration, across a partially permeable membrane, such as the plasma membrane, is known as **osmosis**.
Consider two solutions separated by a partially permeable membrane as in figure1. One can see that solution B has a higher concentration of solute molecules than solution A. The solute molecules are too large to pass through the membrane, but the water molecules can easily fit through the pores of the partially permeable membrane, as they are small enough. The water molecules will therefore diffuse from solution A to

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solution B down the concentration gradient, until equilibrium is reached in which the concentration of both solutions is the same.

Figure 1; the mechanism of osmosis:

As mentioned before, the tendency of a solution to donate water to its surroundings is known as its **water potential** (symbol: ❸ - Greek letter psi). Water potential can also be defined as the potential energy per unit volume of a solution. The units would therefore be joules per cubic metre, which is equivalent to a pascal:

$J = N \times m$

$$\frac{J}{m^3} = \frac{N \times m}{m^3} = \frac{N}{m^2} = Pa$$

Water potential can therefore be thought of as a 'pressure' as the pascal is the SI unit for pressure.

In the previous example, solution A has a higher water potential than solution B, as water molecules diffused from solution A to solution B. Therefore, regions of high water concentration have higher values for water potential than regions of lower water concentration. Osmosis can thus also be defined as the net movement of water molecules from a region of high water potential to a region of low water potential across a partially permeable membrane.

(input variables:)

One can also see from figure 1 that the presence of solute molecules lowers the water potential so that the maximum value for ❸ possible is that of pure water, which is by convention zero. The amount by which the solute molecules decrease the water potential is called the **solute potential** (symbol: $❸_s$), which therefore always has a

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negative value. If more solute molecules are present in a solution, then more water molecules will become associated with the solute molecules. Therefore, less water molecules will be able to diffuse resulting in a decrease in water potential. Note that a higher concentration of solute in solution, results in a lower water potential and a higher solute potential.

There is another factor that affects the water potential of a solution. When pressure is applied to a solution in the following way, see below, net movement of water molecules from A to B can be prevented:

The greater the pressure applied to solution B, the greater the tendency for water molecules to be forced back into solution A. Therefore, it is clear that increasing the pressure on solution B, increases its water potential, as water is now moving back to solution A. This kind of pressure on a solution is known as the **pressure potential** (symbol: ψ_p), and has positive values.

Osmosis in plant cells:

It is important to determine the affect of solute potential and pressure potential on water potential in a plant cell, as celeriac is an underground storage organ, similar to the potato, and thus has the typical characteristics of a plant cell.

Unlike animal cells, plant cells have a fully permeable cell wall. When the external water potential, i.e. the ψ of the solution in which a plant cell is bathed, is higher than the intracellular solution, water molecules enter the cell through the cell wall and the plasma membrane. Therefore, the cell starts to expand as normal. However, due to the cell wall, the cell ceases to expand and thus starts to exert a pressure on the cell wall. This pressure acts as a pressure potential, and the water potential in the cell starts to increase until it equals the external water potential and equilibrium is reached. The cell wall thus prevents the cell from bursting and provides support for the plant as its cells are now said to be **turgid**.

Therefore, for plant cells only, the water potential inside the cell is equal to the solute potential and the pressure potential as exerted on the cell wall:

$$\psi = \psi_s + \psi_p$$

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However, when the external water potential is lower than the water potential of the intracellular solution in a plant cell, water leaves the cell by osmosis so that less pressure is exerted upon the cell wall, as the contents of the cell shrink. As the contents of the cell continue to shrink, they pull away from the cell wall. This is called **plasmolysis**. Eventually, when no more pressure is exerted on the cell wall, known as **incipient plasmolysis**, the pressure potential reaches zero and the water potential is equal to the solute potential. The water potential continues to decrease as water molecules leave the cell, until equilibrium is reached when the external water potential is equal to the water potential of the cell.

Note that when an external solution has a higher water potential than the internal solution, it is said to be **hypotonic**, and when it is lower than the internal water potential, it is said to be **hypertonic**. In the case of equal water potentials on each side of a separating membrane, the solutions are said to be **isotonic**.

Output variables:

The most practical way of determining the water potential of celeriac, is by finding out when it is equal to an external water potential, as can be controlled by the concentration of bathing solution. When the water potential of the intracellular solution is equal to the external water potential, there would be no net movement of water molecules between celeriac and the bathing solution, as indicated by either a change in mass or in length.

Therefore, by measuring the mass, or length, of the celeriac cylinders before and after they are placed in the bathing solution, and thus calculating the percentage change in mass/length, one can plot a graph of percentage change (y-axis) against concentration of bathing solution (x-axis). The point at which the line touches the x-axis, i.e. no percentage change in mass or length, shows the concentration of bathing solution that has a water potential equal to that of celeriac.

Hypothesis:

In this investigation, we will try to determine the water potential of celeriac, i.e. its tendency to donate water molecules to its surroundings, by finding out when it is equal to the external water potential, as controlled by the concentration of sucrose solution.

Although a value for the water potential of celeriac cannot be predicted, one can say that the percentage change in mass will be positive at lower concentrations of bathing solution, and negative at higher concentrations of bathing solution.

Preliminary work:

Before any experimental work was done, a rough method was established. In the pilot experiment, the rough method was improved upon and a final procedure was ascertained, see final procedure.

In the pilot experiment, potato was used instead of celeriac; though this made little difference to any results obtained, as the two are very similar. Firstly, cylindrical pieces of potato, the heights of which were kept constant so that the surface area was also constant, were obtained using a cork borer. We were able to accurately measure the

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heights using a vernier calliper. Next, using a sensitive electronic balance, the masses of each cylinder were measured. Six cylinders of potato were prepared. These were then placed in six test tubes containing different concentrations of 1 molar, i.e. 1 mole of substance in 1 cm^3, sodium chloride (salt) solution ranging from 100% to 0%. Note that in the final procedure sucrose solution will be used rather than sodium chloride (salt). To prepare different concentrations of sodium chloride, distilled water was added, using measuring cylinders. The ratio of distilled water added to sodium chloride enabled us to quantify the concentrations. We decided that the volume of salt solution in each test tube would be $30cm^3$. Therefore for a concentration of 100%, $30cm^3$ was placed into one test tube. To make a concentration of 20% or 0.2 mol/dm^3, $24cm^3$ water was added to $6cm^3$ of sodium chloride. The following concentrations of salt solution were made in this way: 0%, 20%, 40%, 60%, 80% and 100%.
Once each test tube had been filled with $30cm^3$ salt solution of different concentrations, one cylinder was placed in each test tube and the timer was started. The pieces of potato were left to bathe in solution for as long as possible. After 20 minutes, the cylinders of potato were removed and the mass of each measured using an electronic balance. The results of the pilot experiment are recorded in table 1, see page 6.

In order to determine whether the method used in the pilot experiment was suitable, further calculations were done using the results obtained in table 1. The percentage change in mass was calculated using the following formula:

$$\text{Percentage change in mass} = \frac{\text{change in mass}}{\text{original mass}} \times 100$$

A graph was then drawn of percentage change in mass against concentration of salt solution, see page 7. Using this graph, we were able to determine the concentration of salt solution that has an equal water potential to that of potato, as explained in the output variables. The value obtained was 0.265 mol/dm^3. As well as enabling a prediction to be made, the graph showed us that the general method used in the pilot experiment was suitable enough to obtain a set of readings from which the water potential of celeriac can be determined.

However, it was realised that in the final procedure, it would be better to leave the cylinders in for a longer time period so that a greater change in mass could be recorded thereby increasing the accuracy of the data.

Method:

Apparatus:
Celeriac, cork borer, 1 molar sucrose solution, measuring cylinders – 10ml, 50ml, electronic balance, white tile, razor, six test tubes, test tube rack, timer, $250cm^3$ beaker, distilled water and vernier callipers.

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Safety equipment:
Safety spectacles and apron.

Diagram:

Final procedure:
The apparatus previously mentioned should be set up as in the diagram above, then the following procedure should be carried out.

Firstly, obtain six cylindrical pieces of celeriac using a cork borer and make sure that their lengths are all approximately equal by using a razor and a vernier calliper. Next, record the mass of each piece using an electronic balance accurate to 0.005g. A greater accuracy in the instruments used decreases the uncertainties associated with any measurements taken, thereby making them more reliable.

Next, six solutions of sucrose of the following concentrations: 0% (0mol/dm^3), 20% (0.2mol/dm^3), 40% (0.4mol/dm^3), 60% (0.6mol/dm^3), 80% (0.8mol/dm^3) and 100% (1mol/dm^3) are prepared by adding distilled water in a certain volume ratio. As the chosen volume of sucrose solution will always be 30cm^3, to make a sucrose solution of 0.2mol/dm^3 or 20%, 24ml water is added to 6ml sucrose solution. Note that a solution of concentration 0% is basically 30ml of distilled water. These volumes of distilled water and sucrose solution should be measured using the 10ml and 50ml measuring cylinders. Each sucrose solution is then poured into six test tubes, each one labelled with the appropriate concentration.

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Once there are six different concentrations of sucrose solution in six test tubes, one celeriac cylinder is placed in each test tube and the timer is started. The apparatus is then left overnight, each cylinder of known mass immersed fully in a bathing solution of known concentration. After one night, the cylinders are reweighed, again using the electronic balance, and the measurements are recorded.

Analysis:

Using the values for the original mass and the mass after the cylinders were placed in the sucrose solution, one can calculate the percentage change in mass using the following formula:

$$\text{Percentage change in mass} = \frac{\text{change in mass}}{\text{original mass}} \times 100$$

Next, a graph should be plotted of percentage change in mass (y-axis) against concentration of sucrose solution (x-axis). As explained earlier, the point at which the line crosses the x-axis, indicates the concentration of sucrose solution that has a water potential equal to the water potential of celeriac. Once this concentration has been determined, a value for the water potential of the sucrose solution, and therefore of the celeriac as well, can be determined using a graph of water potential against concentration of sucrose solution.

Safety:

Before any laboratory work is carried out, one must be aware of the safety measures that need to be taken when undergoing this procedure.

As well as all the usual safety rules such as no obstruction of the corridors, no eating or drinking in the laboratory, extra care must be taken when handling the measuring cylinders as they are made of glass. It could be potentially dangerous if these were broken and there were pieces of glass. This is one of the reasons why safety spectacles should be worn throughout the course of the experiment.

Predictions:

Using our scientific knowledge of osmosis, one can make a set of predictions concerning the results of the final procedure outlined above.

Although there is no way of predicting the value for the water potential of celeriac, one can however, predict the graph of percentage change in mass against concentration. We know that the water potential of the bathing solution is lowest when the sucrose concentration is 100%. At this concentration, water molecules will diffuse from the celeriac, a region of higher water potential, to the bathing solution by osmosis. The percentage change in mass will therefore be a negative one, as the mass of water that was originally in the celeriac cells is lost. However, when there is no sucrose in solution, i.e. pure water, the water potential is high, and water molecules diffuse into the celeriac, which is now comparatively a region of lower water potential. As a result, the mass of

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the celeriac increases, and the percentage change in mass is a positive value. So we can predict the graph of percentage change in mass against sucrose concentration, see below.

Note that the graph drawn above is similar to the graph on page 7; the predictions based on scientific knowledge, correspond to earlier predictions made that were based on preliminary work.

Pre-evaluation:

One can also spot some flaws within the final procedure, even before it is carried out.

As with all experiments, the reliability of any measurements taken is restricted by the accuracy of the instruments used. The electronic balance was especially chosen due to its high degree of accuracy. However, the measuring cylinders are less accurate, and result in a systematic error associated with any volumes measured using this apparatus. In other words, we can only be sure of the volume obtained to a certain degree. This reduces the reliability of, in this case, the concentration values of the sucrose solutions.

The lengths of the cylinders will most likely not all be equal in magnitude, as was the case in the pilot experiment. As the length governs the surface area, it may have an affect upon the osmotic mechanism. However, when the vernier callipers are used to keep length constant, the difference in surface area is so minimal that the affect of this variable would be negligible.

Bibliography:

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