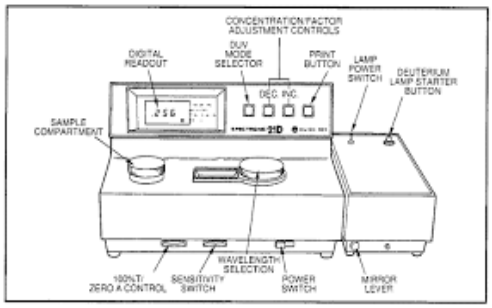
Research Question:

How does changes in temperatures (0-5,20,40,60,80 °C) of universal solvent (Water) and concentrations (0,10,20,30,40,50%) of Organic solvent (Acetone) affect the Rate of Diffusion of pigment known as Betanin? Present in Beetroots.

When my mother was not well, the doctors had recommended her to include Beetroot in her meals, in the form of boiled beetroots or beetroot juice as shown in the picture[1] on the right hand side. She was told to follow this for around two to three weeks, everyday. Due to the illness, her hemoglobin level had decreased, which was quite below the normal level required for her age. When she started having it as a staple in her diet, nearly just a few days after, her red blood cells started to increase again. This was due to the rich content in Iron that the beetroot had. In a way this vegetable appealed to me as a "magic vegetable" and so I started to research more about it. I came across articles and personal blogs that stated benefits of it, but the fact that astonished me was the experiences people had with this vegetable. They stated that sometimes when people had consumed the vegetable, their urine had a pinkish color to it. This was especially surprising to me, and so when I found more about the reason behind it, it was due to the Betanin pigment. I got to know that there was so much more to this vegetable. Therefore, I decided that I wanted to carry out an experiment related to it so I could learn more about Beetroots, both literally and practically as well.

Introduction:

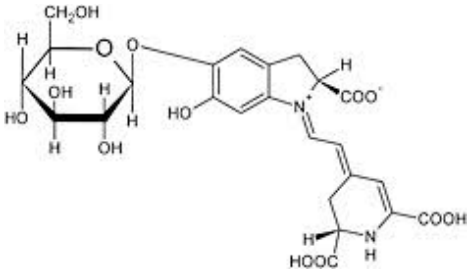


Visible spectrophotometer is a device that measures the absorbance of different kinds of solutions. Some wavelengths of light can easily pass through these, while other wavelengths tend to reflect the light back. It is obviously red in the case of the pigment found in beetroots. Thus, the pigment represents wavelengths that are related to it and absorbs all other wavelengths in various colors. The sensors in the equipment then record the readings of the reflected light. This is then displayed on the computer screen, making it easier to capture readings. The diagram[2] would give a clearer idea about the different mechanisms of the spectrophotometer.

[1] Melão, Alice. "Researchers Using Natural Compound to 'Beet' Alzheimer's Progression." *Alzheimer's News Today*, 11 Apr. 2018, alzheimersnewstoday.com/2018/04/11/researchers-using-natural-compound-betanin-beet-alzheimers/.

[2] "CHAPTER XVII: LIGHT-SCATTERING AND MOLECULAR SPECTROPHOTOMETRY." *CHAPTER XVII:* www.ecs.umass.edu/cee/reckhow/courses/572/572bk17/572BK17.html.

Betanin is the red pigment present in beetroots. It is basically a glycoside colored portion composed of sugars. It is also soluble in water, which allows diffusion to occur in aqueous conditions. This pigment is normally found in the vacuoles of the beetroot cells. When different temperatures of Water and concentrations of Acetone are exposed to the plasma membrane of the plant's cell, they start to denature it. As a result, the betanin starts to flow out of the cells, through a concentration gradient. The diagram represents the basic structure of the Betanin pigment.[3]



The aim of this experiment is to test the rate of absorbance of the pigment in different concentrations and at different temperatures, and henceforward find a relationship between the diffusion rate and the changes in variables. The beetroot that are cut in cube forms after they are washed, contain plasma membranes around its cells to help prevent its red pigment from leaking. However, when temperature and concentrations of the solvents are changed, this causes the plasma membranes to get destroyed and eventually make the red pigment leak out from the solution. Therefore, the relationship between temperature and concentration of different solvents and its effects on the rate of reaction, represented by the absorbency of the solution, will be tested.

There is a law that explains the relation between absorbance and other aspects present in the spectrophotometer. This law is called the Beer-Lambert Law. It states that $A=ebc$, where A is the absorbance, e is the molar absorptivity of a particular solution, b is the path length of the cuvette (which is the container in which the solution is poured) and c is the concentration of the particular solution. In this particular test, the molar absorptivity and cuvette length is constant because only the beetroot solution is being tested and is poured into the same cuvette used in the photo spectrometer. Thus when two of these variables are constant, the law shows that the absorbance will be directly proportional to concentration of the solution and the other variable which is the temperature of the solutions.

Hypothesis:

Normally, the rate of diffusion is represented by change in absorbance in a particular solution. The acetone used as a solvent, has an ability to destroy the plasma membrane of the cells present in the beetroot. The increase in the concentration of this solvent will affect the plasma membrane even more, which will result in greater rate of diffusion, secreting more betanin. When the beetroot pieces are put in different concentrations, the lowest concentration would not have any much of an effect and the solution will remain somewhat transparent and have an absorbance value closer to 0, while the highest concentrated solution will have the greatest absorbance value. Same goes for the temperature factor, for the lowest temperature the absorbance rate will also be low, and for the highest possible temperature the value will be high. This is because as temperature increases, the kinetic energy of the particles has increased. The increased motion of the particles causes them to diffuse faster. Therefore, at higher temperatures, the rate at which fluid particles will diffuse is faster than at lower temperatures. Hence, the relationships between the two factors and the absorbency of the solution should be directly proportional.

[3] https://www.researchgate.net/figure/Chemical-structure-of-betanin_fig2_51792699

Variables:

Variables	Description	Method of Measure
Independent	*C_3H_6O (Acetone) Concentration (%)*	• 100% Acetone was diluted to different concentrations [20%,40%,60%,80%] using distilled water. • The distilled water was used for control (0% Acetone) • Double trials were performed on each of the concentration
	Temperature of H_2O (Water)	• Water was boiled to raise the temperature [10°C,20°C,30°C,40°C,50°C] • 0°C - 5°C was maintained by collecting ice water
Dependent	*Rate of diffusion of the pigment betanin from beetroot cells* $r = \frac{\Delta \text{ Absorbance}}{\text{Time}} / \text{h}^{-1}$	• Here, rate of diffusion is represented by change of absorbency in a given time. • The Absorbance was measured using a visible spectrophotometer at λ_{max} = 545nm. • The cuvette used was same, keeping the parameters same.
Controlled	*Type & Size of beetroot*	• Beetroot was cut into identical cubes and 1x2 cm in size. Since the beetroot was circular, only the middle part of the beetroot was used to achieve the cube shape needed.
	Type of Cuvette	• The same type of cuvette present in the spectrophotometer kit was used
	Volume of Acetone and Water	• Equal volume of the solutions was used for all the trials. (10ml) A Measuring Cylinder was used for accurate measurement
	Time	• 5 minutes were given at the start of the diffusion process, along with 2 minutes' intervals between each temperature, concentrations solutions. Trials were then halted by removing the beetroot pieces from the container.
	Temperature	• The experiments were conducted in the Biology Lab, at a room temperature. Approximately at 25°C - 30°C
	λ_{max}	• λ_{max} at 545 nm was used as the pigment absorbs this wavelength the best.

Apparatus:

- Photo spectrometer
- Small sized beakers (x80)
- Cuvette
- 250 ml Measuring cylinder (x1) **(± 2cm^3)**
- 100 ml measuring cylinder (x1)
- Knife
- Tweezers
- Ruler **(± 0.5mm)**
- Refrigerator
- Water boiler
- Gas Stove
- Tissue papers
- Labels
- Rubber Gloves

Materials:

- Beetroots
- C_3H_6O (Acetone)
- Distilled Water

Procedures:

1. First off, wash and peal off all the beetroots. Be careful to not expose the inside section to water.
2. Using a knife, cut the beetroot into cubical pieces (1x2cms).
3. After cutting the beetroot, put the pieces into a bucket of water to get rid of any pigments that have already seeped out during the cutting process.

Safety Precautions:

- ✓ I wore a mask, hand gloves, and maintained social distancing, which were mandatory requirements to be followed during the COVID-19 Pandemic
- ✓ I wore glasses to avoid contact with my eyes
- ✓ I washed all equipment with a detergent solution
- ✓ I cleaned the area with water and tissues after the experiment
- ✓ I washed my hands with hand soap and cleaned the surface used for the experiment

Acetone Concentration Experiment:

1. Dilute the Acetone into different concentrations using the measuring cylinder and distilled water.
2. Label the small beakers for each of the concentrations
3. To prepare the solution, pour each of the concentrated solution into 5 different small beakers labeled for each of the concentrations.
4. Place the beetroot cube pieces into each of the solution using tweezers and wait for 5 (+2) minutes to allow the diffusion to take place.
5. Remove the cubes and keep the solution
6. Calibrate the cuvette using acetone and set the spectrophotometer at λ_{max} = 545nm.
7. Place the solution nearly to the top of the cuvette and measure the absorbance.
8. Record the value displayed on the display board.
9. Repeat the steps for each of the concentrations

Water Temperature Experiment:

1. Take 50ml of distilled water into a container, and boil the water to the desired temperature
2. Label the small beakers for each of the concentrations
3. To prepare the solution, pour the water into 5 different small beakers labeled for each of the temperature
4. Place the beetroot cube pieces into each of the solution using tweezers and wait for 5 (+2) minutes to allow the diffusion to take place.
5. Remove the cubes and keep the solution
6. Calibrate the cuvette using acetone and set the spectrophotometer at λ_{max} = 545nm.
7. Place the solution nearly to the top of the cuvette and measure the absorbance.
8. Record the value displayed on the display board.
9. Repeat the steps for each of the temperatures
10. *To obtain 0°C - 5°C, a freezing solution (Mixture of Ice & Salt) was prepared.

Data Collection and Processing:

Concentration of Acetone	Table 2: Shows Results from the Changes in Concentration Experiment: (Absorbance at λ_{max} = 545nm.)									
Trials	**1**		**2**		**3**		**4**		**5**	
0%	0.105	0.114	0.102	0.092	0.067	0.066	0.164	0.163	0.106	0.097
10%	0.090	0.081	0.057	0.055	0.067	0.068	0.213	0.216	0.226	0.228
20%	0.259	0.246	0.260	0.266	0.295	0.285	0.234	0.250	0.230	0.226
30%	0.721	0.719	0.646	0.661	0.709	0.708	0.434	0.425	0.670	0.692
40%	0.902	0.889	1.090	1.127	1.109	1.192	1.013	1.004	1.460	1.472
50%	1.600	1.615	1.026	1.127	1.001	0.991	1.109	1.197	1.313	1.318

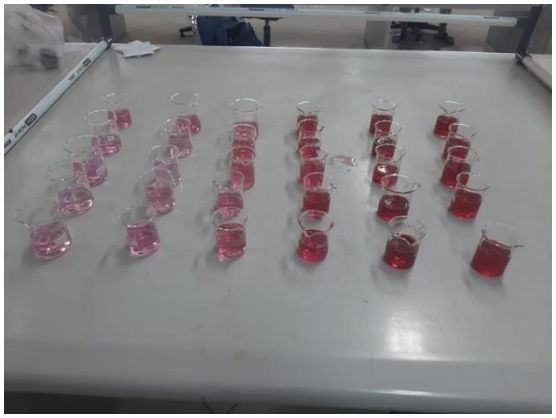


Figure 2. Shows Results of the Experiment for Effect of Different Acetone Concentrations on Absorbed Betanin

Temperature of Water	Table 3: Shows Results from the Changes in Temperature Experiment: (Absorbance at λ_{max} = 545nm.)									
Trials	**1**		**2**		**3**		**4**		**5**	
0-5°C	0.099	0.105	0.077	0.126	0.092	0.090	0.047	0.045	0.064	0.062
20°C	0.085	0.092	0.044	0.040	0.051	0.053	0.104	0.109	0.013	0.004
40°C	0.161	0.163	0.118	0.132	0.122	0.105	0.060	0.057	0.163	0.169
60°C	0.307	0.306	0.216	0.215	0.589	0.611	0.333	0.312	0.198	0.202
80°C	0.515	0.547	0.444	0.440	0.655	0.664	0.654	0.635	0.394	0.386

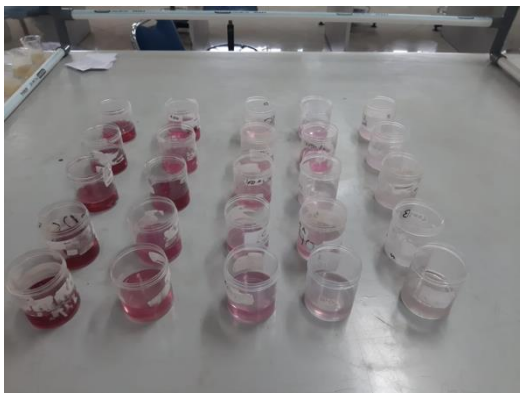


Figure 3. Shows Results of the Experiment for Effect of Different Temperatures of Water on Absorbed Betanin

Qualitative Data:

Temperature of Water	Observation	Color Shade	Concentration of Acetone	Observation	Color Shade
0°C	*clear*		10%	*very pale pink*	
20°C	*very pale pink*		20%	*pale pink*	
40°C	*pale pink*		30%	*darker pink*	
60°C	*darker pink*		40%	*dark maroon*	
80°C	*dark maroon*		50%	*dark red*	

Data Processing

Sample Calculations:

** Note that the same all methods of calculations were used for the temperature data set as well*

- **Calculation for 50% Acetone concentration (max) for the mean of 5 trials, with 2 sub-trials each**

$$\text{Mean} = \frac{\frac{(\text{Trial } 1{,}1+1{,}2)}{2} + \frac{(\text{Trial } 2{,}1+2{,}2)}{2} + \frac{(\text{Trial } 3{,}1+3{,}2)}{2} + \frac{(\text{Trial } 4{,}1+4{,}2)}{2} + \frac{(\text{Trial } 5{,}1+5{,}2)}{2}}{5}$$

$$\text{Mean} = \frac{\frac{(1.600+1.615)}{2} + \frac{(1.026+1.127)}{2} + \frac{(1.001+0.991)}{2} + \frac{(1.109+1.197)}{2} + \frac{(1.313+1.318)}{2}}{5}$$

$$\text{Mean} = \frac{1.6075 + 1.0765 + 0.9960 + 1.1530 + 1.3155}{5} = 1.2297$$

∴ Mean [$\bar{x}$] = **1.2297**

- **Calculation for 50% Acetone concentration (max) for the standard deviation of 5 trials, with 2 sub- trials each**

$$\text{Standard Deviation} = \frac{\sqrt{(\Sigma X_{trials} - \bar{x})}}{5}$$

$$\frac{\Sigma (1.6075 - 1.2297)^2 + (1.0765 - 1.2297)^2 + (0.9960 - 1.2297)^2 + (1.1530 - 1.2297)^2 + (1.3155 - 1.2297)^2}{5}$$

$$= \frac{0.1427 + 0.0234 + 0.0546 + 0.0058 + 0.0073}{5} = 0.244$$

∴ Standard Deviation = ± 0.2

Data Presentation:

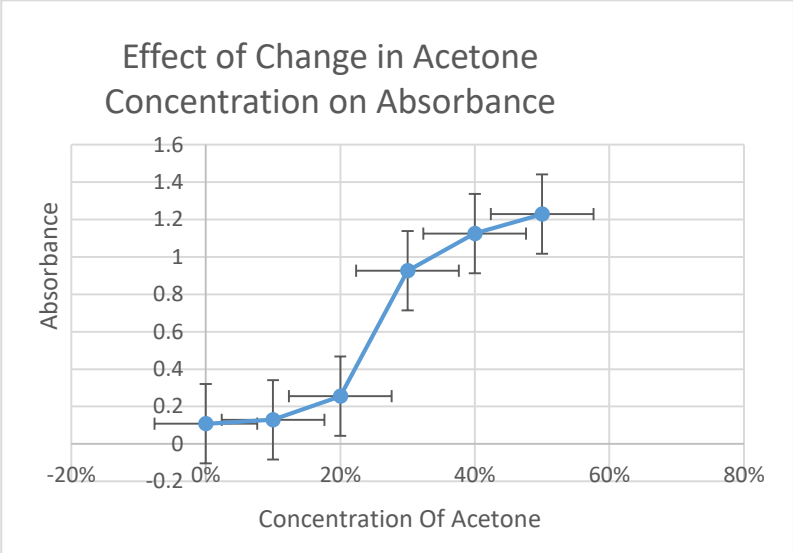
Table 4. Shows Averaged Values for result of experiment on Effect of Concentration of Acetone on the Betanin Pigment

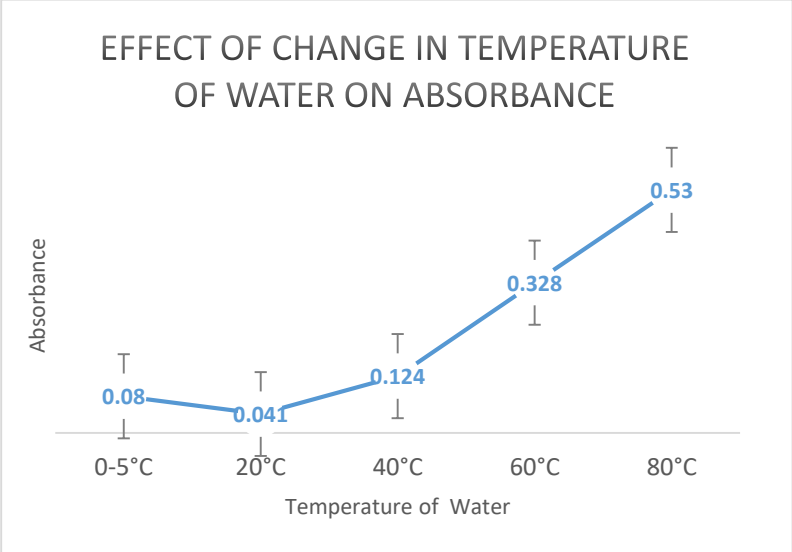
Table 4 : ***Absorbance at λ_{max} = 545nm (Concentration of Acetone Averaged Values)***							
Trials	**1**	**2**	**3**	**4**	**5**	**Average**	**Std. Dev**
0%	0.109	0.097	0.066	0.163	0.105	0.108	± 0.10
10%	0.085	0.056	0.067	0.214	0.227	0.129	± 0.10
20%	0.252	0.263	0.290	0.242	0.228	0.255	± 0.10
30%	0.720	0.653	0.708	0.429	0.681	0.926	± 0.30
40%	0.895	1.108	1.150	1.008	1.466	1.125	± 0.10
50%	1.607	1.076	0.996	1.153	1.315	1.229	± 0.20

Table 5. Shows Averaged Values for result of experiment on Effect of Changes of Temperature of Water on the Betanin Pigment.

Table 5 : ***Absorbance at λ_{max} = 545nm (Temperature Averaged Values)***							
Trials	**1**	**2**	**3**	**4**	**5**	**Average**	**Std. Dev**
0-5°C	0.102	0.101	0.091	0.046	0.063	0.080	± 0.02
20°C	0.088	0.042	0.052	0.016	0.008	0.041	± 0.06
40°C	0.162	0.125	0.113	0.058	0.166	0.124	± 0.03
60°C	0.306	0.215	0.600	0.322	0.200	0.328	± 0.10
80°C	0.531	0.442	0.659	0.644	0.390	0.530	± 0.10

Graphical Representation of Averaged Value of Results for Both the Experiments :





Notes on Changes observed in the graphs:

- Data points representing 0%,10%,20% Acetone concentration gradually increase.
- Greater change in absorbance rate from data points of 20% to 30% acetone concentration
- Gradual increase again after 30% acetone concentration
- Negligible decrease from 0-20°C due to drastic temperature changes.
- Gradual increase of data points of water after temperature of 20°C
- 40 °C there is even more increase compared to that of the previous data points

ONE WAY ANOVA TEST

	Treatments	
Variables	CONCENTRATION	TEMPERATURE
N	6	5
ΣX	3.772	1.103
Mean	0.6287	0.2206
ΣX^2	3.7269	0.4119
Standard Deviation	0.5207	0.2053

Result Details				
Source	*SS*	*df*	*MS*	
Between-treatments	0.4541	1	0.4541	*F = 2.68165*
Within Treatments	1.5242	9	0.1694	
Total	1.9783	10		
The *f*-ratio value is 2.681. The *p*-value is 0.135. The result is *not* significant at $p < 0.05$.				

** The F value (2.681) obtained from this test is more than that of the degrees of freedom value (df =1.0) for the between treatments source. We can say that the result obtained is significant. The reason behind this significance may be due to the differences in the absorbency value of the Betanin pigment.*

Limitations and Improvements:

Limitations
• When the beetroot pieces were cut, it was made out that the pieces did not come out to be identical, due to a large number of beetroot pieces that needed to be cut to be used in the experiment. Only the middle section of the beetroot was used, but the pieces may have not been exactly same. This may add some kind of uncertainties to the results later on.
• The beetroot pieces were kept in a bucket of water. This made it unsure whether the pigments on the surface of the piece was washed out or not. There is no such technique to make sure of this and therefore remains an unsure point
• While pouring the liquids were poured in the container, as temperature increases, the rate of evaporation of liquid may increase too. This may reduce some quantity of water in the container, therefore all the containers may not contain equal quantities of liquid.
Improvements
• Pour the liquids slowly, so that some amount of liquid doesn't spill. Thus enabling an equal amount of liquid throughout all the containers during the experiments.
• Use a vegetable cutting tool to help make the beetroot cubes more accurately, to enabling all the cubes to be similar in size, to prevent uncertainties in the results.
• Carry out the experiment quickly in a short time interval, so that the temperature of the water stays intact and the results obtain stay accurate.

Conclusion:

The data and results indicate that as the Acetone concentration and Water temperature increases, the rate of diffusion of the betanin pigment increases. This proves that the hypothesis I proposed is valid. The graph also shows that there is a positive regression that is observed between the Absorbance and the concentration of Acetone and temperature of Water. Although there is a positive correlation, we can make out that the results are not directly proportional.

For the changes in concentration of Acetone, while observing the first three data sets on 0%, 10%, 20% there were only some gradual increases. This might be due to low concentrations of Acetone initially. This low concentration may not be able to destroy the membrane of the beetroot totally, therefore less betanin pigment was excreted. The graph then increases significantly, showing a huge change in Absorbance at 20-30%. Then the graph starts to increase gradually again till the end. The result therefore shows that as the concentration of Acetone increases, this causes even more damage to the membranes of the beetroot, thus causing more excretion of the betanin pigment and finally increasing Absorbance as well.

For the changes in temperature of Water, while observing the first three data sets on 0 – 20 °C there was an increase then some gradual decrease. This may indicate that the temperature might have not been that effective to break down the membranes in the beetroot in order for the pigment to be secreted. From 40 °C onwards we can see that there is a significant increase in the rate of absorbance. This proves that the high temperatures were successful in damaging the membranes of the cells, thus allowing the betanin to be secreted. The result therefore shows that as the Temperature of Water increases causes more excretion of the betanin pigment and finally increasing Absorbance too.

Extension:

A potential extension to this investigation would be to further experiment and study on how other types of solvents along with changes in its temperatures may affect the rate of diffusion of the betanin pigment ad eventually the absorbance as well. This would allow me to gain more information on which solvent causes the pigment to seep out the most and which solvent causes the pigment to seep out the least along with allowing me to find out which temperatures have the greatest impact on the inner membranes of the vegetable and which ones have the least impact on them, without fully denaturing or degrading the vegetable. A further interesting experiment for the investigation would be an experiment, where I can also use different types of vegetables that contain some kind of colored pigment to compare which vegetable excretes out the least and the most pigment and which one gets affected most by the different parameters mentioned earlier.

Bibliography:

- *Anna Gliszczyńska-Świglo, Henryk Szymusiak, Paulina Malinowska. Betanin, the main pigment of red beet - molecular origin of its exceptionally high free radical scavenging activity. Food Additives and Contaminants, 2006, 23 (11), pp.1079-1087. ff10.1080/02652030600986032ff. ffhal-00577387f*

- *Libretexts. "2.1.5: Spectrophotometry." Chemistry LibreTexts, Libretexts, 15 Aug. 2020, chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Supplemental_Modules_(Physical_and_Theoretical_Chemistry)/Kinetics/02%3A_Reaction_Rates/2.01%3A_Experimental_Determination_of_Kinetics/2.1.05%3A_Spectrophotometry#:~:text=A%20spectrophotometer%20is%20an%20instrument,the%20intensity%20of%20light%20detected.*

- "Membrane Permeability Beetroot Practical." *YouTube*, YouTube, 27 May 2016, www.youtube.com/watch?v=Hc3Mg0Yc7kI.

- "Uncertainties in Biology - Mr. Hurwitz IBHL Biology." *Google Sites*, sites.google.com/site/mrhurwitzscienceclass/uncertainties-in-biology#:~:text=When%20using%20an%20apparatus%20with,bigger%20than%20any%20scale%20uncertainty.

- *Mean,Standard Deviation and Standard Error*. www.nausetschools.org/cms/lib/MA02212418/Centricity/Domain/204/Mean%20Standard%20Deviation%20and%20Standard%20Error%20Notes%202017.pdf.